University of Kerbala
College of Science
Department of Chemistry

Determination of Trace Element Levels in Serum and Fingernails for Hemodialysis Patients and Healthy Individuals in Iraq By Using ICP-OES

A thesis

Submitted to the Council of the College of Science, University of Kerbala as a Partial Fulfilment of the Requirements for the Degree of
Doctor of Philosophy in Chemistry

By

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

[قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (25) وَيَسِّرْ لِي أَمْرِي (26) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (27) يَفْقَهُوا قَوْلِي (28)]

صدق الله العلي العظيم

(سورة طه : الاية 25 - 28)

In the Name of God, the Beneficent, the Merciful

My Lord! Uplift my heart for me (25), and make my task easy (26), and remove the impediment from my tongue (27), so people may understand my speech (28).

Dedication

Thanks Almighty, to Allah, the most gracious and merciful. I would like to extend to my thoughts and gratitude to my father and mother, who carry the true meaning of love, kindness, and tenderness…….

I greatly appreciate and respect them, and I consider them as my teachers, Dr. Baker A. Joda and Dr. Rana M. Hameed. For their help.

To my lovely husband Ali, who encouraged and supported me all that time and still is, I also thank his love, care, and thank his patience…..

To my loved ones, the secret of my laughter and smile, to my daughter Ola and my son Ahmed ……

To all my friends who support me, brothers and sisters, I am very grateful…..

Rusul Azeez Jaafar Al-Mayali

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Rusul Azeez Jaafar Al-Mayali

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We Certify that the thesis entitled "**Determination of Trace Element Levels in Serum and Fingernails for Hemodialysis Patients and Healthy Individuals in Iraq By Using ICP-OES"** was conducted under our supervision at the Department of Chemistry, College of Science, University of Kerbala, as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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Abstract

The levels of interested trace elements, namely Al, Cd, Cu, Mn, Pb, Se, and Zn in various biological samples (fingernail and serum) were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Analytical methods for determining the elemental levels were developed and validated using quality control and significance tests. Multi-element analysis was undertaken for serum and washed fingernails collected from Iraqi individuals. The influence of health status, gender, smoking activity, and age on elemental levels in serum and fingernails was investigated. In general, significantly higher serum levels of Mn and Zn are found in healthy individuals (0.166 ± 0.296 μg/mL of Mn and 0.302 ± 0.068 μg/mL of Zn) when compared with hemodialysis patients (0.128 ± 0.036 μg/mL of Mn and 0.259 ± 0.057 μg/mL of Zn). On the other hand, significant differences were identified between healthy individuals and patients in fingernails for all elements under investigation. The effect of gender on the levels of elements in serum and fingernails was investigated using a two-tailed t-test. It was found that gender has a significant effect on the levels of Mn and pb in serum samples, while this significant effect was found for Al, Cd, Mn, Pb, and Se for fingernail samples. In addition, there are no significant effects of all elements between smokers and non-smokers for both fingernails and serum samples. The only exception was found for Mn in fingernails which is significantly higher in non-smokers compared to smokers.

The results indicate that the effect of the individual's age on the levels of Cd, Cu, and Mn was significant in fingernail samples at $P = 0.05$, while only one significant effect of age was reported for Zn in the case of serum samples. The two interaction factors, namely between factors (health status, gender, and smoking activity) and inter-element correlations for all elements in both media have also been investigated. The use of blood serum and fingernails as a potential biomarker for assessing human health status has been evaluated using several studies in this research, namely, smoking activity, Age, Gender, and hemodialysis.

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((Abbreviation))

ANOVA	Analysis of Variance
CRM	Certified Reference Material
CV (%)	Coefficient of Variation
Sd	Standard Deviation
DDW	Distilled Deionized Water
F calc	Calculated F value
F crit	Critical F value
H	Healthy
P	Patient
ICP	Inductively Coupled Plasma
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LOD	Limit of Detection
QC	Quality Control
T calc	Calculated t value
Tcri	Critical t value
BDH	British Drug House
CT	Computed Tomography

Chapter One

General Introduction

1.0 Introduction

There are ninety elements in nature, divided into environmental, geological, biological, and marine systems [1, 2]. Nonetheless, 23 elements have been connected to specific physiological functions in the lives of humans and other animals [3,4]. Numerous studies have examined these components' roles in both human and animal systems [5,6]. A wide range of health issues could result from values that go outside of "normal" ranges of these elements [7]. It is well recognized that these substances can enter the human body through several different channels, such as the Gastrointestinal tract, the respiratory system, and occasionally the skin from different media (foods, drinks, air, and medications). As shown in Figure 1.1, they are eventually eliminated from the body through a variety of pathways, including perspiration, hair, nails, urine, saliva, teardrops, and feces. They are then carried and distributed by blood into organs like the liver and kidney [8]. Trace elements transported, storage, and regulation in the body is controlled by homeostasis. This is an important biological process which maintains a relatively constant concentration of ions and other constituents in the various body fluids [9].

Exposure to "toxic" elements occurs when they enter the environment through both natural and anthropogenic (or man-made) sources for humans and other living animals. Human exposure to necessary, non-essential, and dangerous components has been tracked using a matrix [10]. In recent years, an increasing need to determine trace elements (mg/L or part per million, ppm) and ultra-trace elements (μg/L or part per billion, ppb) in human tissues and fluids has resulted in the development of sensitive analytical techniques with multi-element capabilities, such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [11]. However, before considering any analytical requirements for the measurement of elements, it is necessary to

understand the classification (based on concentration levels in tissues and fluids) and the possible relationship of each element in terms of human health (essentiality and toxicity).

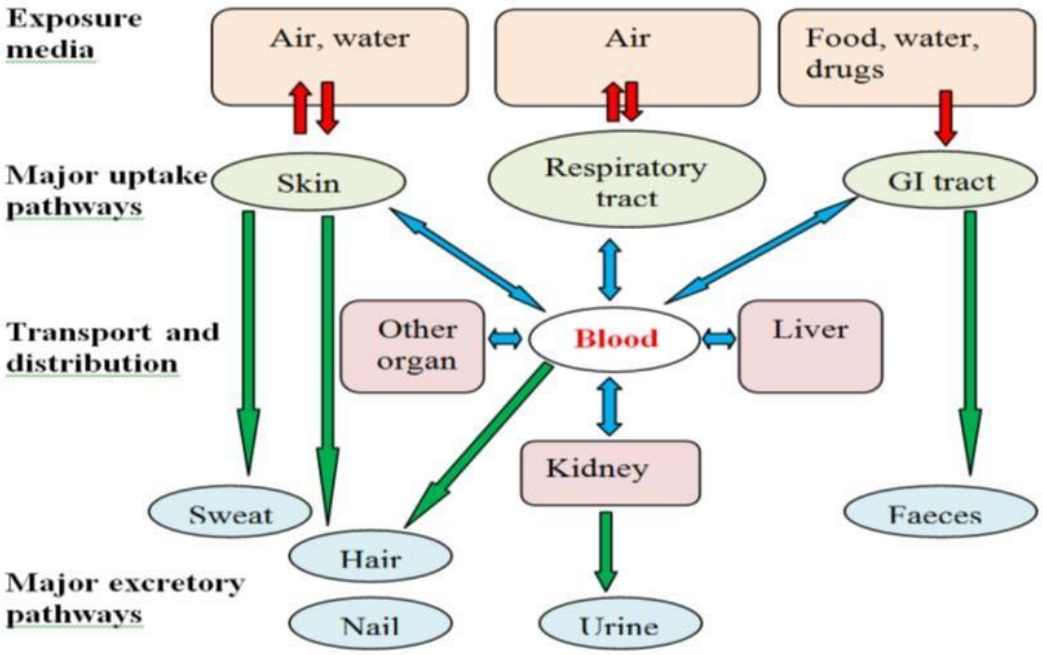


Figure 1.1: Exposure and metabolic pathways for elements in the human body [9], (GI = Gastrointestinal).

1.1 Classification of Elements

Three categories can be used to classify elements in biological systems: *major* elements (95%) which are C, H, N, and O; *minor* elements (3.6%) including Ca, Cl, Mg, P, K, and Na; and *trace* or *ultra-trace* elements (1%) such as Cu, Co, Mn, Se, ..etc. [12]. This classification is based on the element levels found in the tissues and fluids of "normal," control, or healthy individuals. The real elemental levels vary throughout the human body, such that some are classified as minor in human tissues and trace or ultra-trace in fluid [13]. There is no acceptable range of what the concentration intervals should be, although major levels are mainly > 1000 mg/kg;

minor levels < 1000 mg/kg; trace levels < 100 to 0.01 mg/kg; and ultra-trace levels < 0.01 mg/kg [12]. For instance, the calcium content of the earth's crust is 3.6 percent (or 36000 mg/kg), while the calcium content of the human body is 1.4 percent (or 14000 mg/kg).

1.1.1 Essentiality and Toxicity of Elements

Although the major, minor, trace, and ultra-trace element classifications give an approximate picture of the overall concentrations that are expected inside the body, they do not provide the role of a specific element. Consequently, additional classification of a biological system is needed. In this classification, elements can be categorized as essential, non-essential, or toxic based on their potential impact on human health, as shown in Figure 1.2. Humans and other living things need essential components to sustain proper physiological functions. Furthermore, an organism finds it challenging to maintain a normal life cycle or achieve healthy growth without the presence of essential elements [14]. Moreover, an element is considered an essential element to an organism if it is present in living matter, interacts with a living system, and is present in the human diet to maintain a normal physiological function [15]. Many studies have examined the possible significance of some of these components in animal and human systems [16]. Therapeutic elements have been used as medical treatment for different diseases, for example, platinum is used in anti-cancer drugs; gold is used for the treatment of rheumatoid arthritis; lithium is used for the treatment of manic depression; and zinc and molybdenum are used to treat Wilson’s disease [17]. If trace elements are associated with either (i) excessively high intakes that lead to toxic levels or consequences or (ii) excessively low intakes that are linked to nutritional issues, then they are considered dangerous elements for an organism [15]. For example, selenium is essential and present in

urine at 0.1 µg/L and in serum at 40 µg/L; nevertheless, excessive use of selenium can be harmful [18]. Each element can become toxic if the concentration in the human body is more than the normal concentration threshold, but other elements such as Pb, Hg, and Cd are quite harmful even in small doses [19]. Numerous health disorders will arise from any deficiency in the concentration of essential elements below what is necessary for the best possible growth [20]. The installation of orthopedic and orthodontic prostheses, dental fillings (Hg) [21], and other procedures can all lead to an increase in the levels of elements present in the human body (Co, Cr, Ni, and others). Any element's rate of toxicity depends on its concentration, exposure duration, route of exposure, and chemical form [22]. For instance, chromium is hazardous in its Cr(IV) form but required in its Cr(III) form [23]. Moreover, non-essential elements may also be harmful if their concentration exceeds a threshold [24].

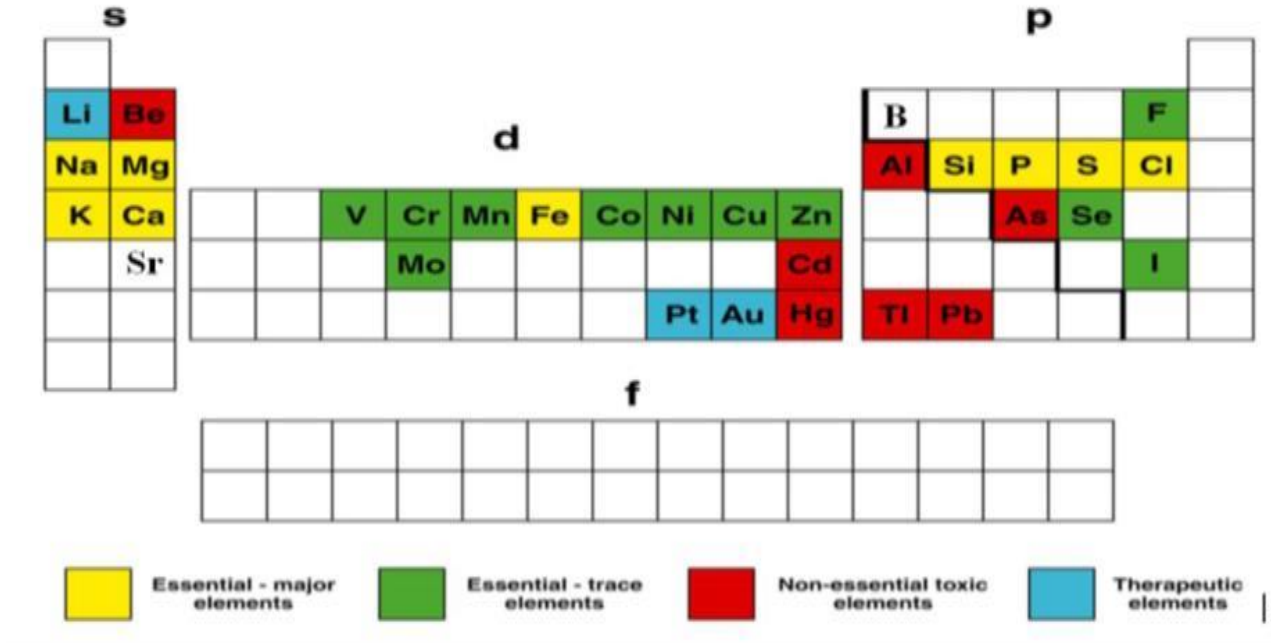


Figure 1.2: Essential, non-essential, poisonous, and therapeutic elements are displayed in a modified periodic table (B and Sr are non-essential elements).

1.2 Dose Response Curve

The concentration of an essential trace element in a human follows a dose-response curve, as presented in Figure 1.3. In this curve, there are three ranges, firstly, the deficiency range (the concentration of elements is below the normal range). In this range of concentration, humans will live, but their ability to respond physiologically will be greatly impaired. The concentration of a trace element can gradually increase in human tissues and bodily fluids, but it might not reach the level required to sustain regular biological processes. Secondly, the normal range (The biological processes are operating at peak efficiency, and the individual's health is typically considered "normal"). Finally, toxicity range, the individual may die as a result of this blocking metabolic functions that may occur when an element's concentration increases [25].

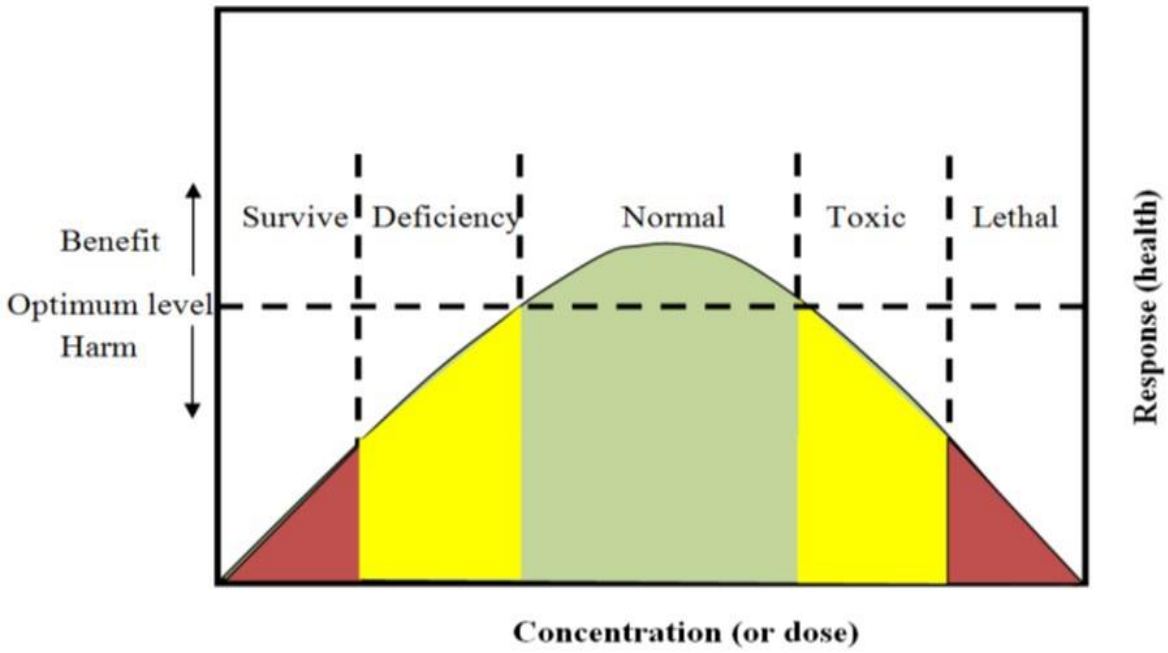


Figure 1.3: The representative curve for vital trace elements in the human body (adapted from [26]).

1.3 Role of Trace Elements in Human Health

The maintenance of good health may be significantly influenced by the human body's optimal balance of essential trace elements. Research on the function of essential elements in the human body is still in progress, and their main physiological role is associated with enzymes [27]. For instance, a strong bond between an enzyme molecule and a metal leads to the form of metal-enzyme complexes [3]. The oxidation-reduction reaction, growth, respiration, muscle contraction, digestion, transport systems, and stability are just a few of the biological processes in the human body that depend on the action of these enzyme [28]. Several reviewers have highlighted the significance of trace elements in human health and disease [29]. Although many of these elements are found or used in very small quantities within the human body, they can have significant roles in terms of essential body processes. Specific elements are found to be bioconcentrated in human scalp hair and nails, thus it is advantageous to use these media in population monitoring studies. Some of these elements are more essential for the human body than others, such as V, Cr, Mn, Fe, Co, Cu, Zn, Se, and Mo [4]. In addition to these officially recognized trace elements, other elements are not essential, but they are needed by the body to successfully process or metabolize essential elements successfully. For example, there are many positive correlations between the essential and non-essential elements in different human tissues and fluids [6,8]. Overall, the determination of trace elements is important in assessing environmental (including contaminated water and food) or occupational exposure [30,31]. The following sections review the trace and ultratrace elements that were selected for this research.

1.1.3.1 Selenium

In the body, selenium is a vital, essential trace element, and the optimum levels are much lower [32]. Glutathione peroxidase, a protein containing selenium, is involved

in thyroid metabolism, immune response modulation, and regulating the physiological antioxidant status [32]. Reduced glutathione peroxidase activity may be linked to the production of harmful lipid peroxides, which can cause endothelial dysfunction and arterial stiffness s[33,34].

1.1.3.2 Aluminum

In the crust of the earth, aluminum ranks third in abundance. Although it has been utilized for centuries in the forms of alum, glass, and clay, commercial use of it dates to the late 1800s. Despite being widely distributed, Al is not necessary for any physiological functions that are fundamental to life. Because of its affinity for phosphates, aluminum has been shown to block over 300 biologically significant kinase or phosphatase processes [35]. According to [36], it mediates the additional mitochondrial release of free oxygen radicals, which causes Fe-induced lipid peroxidation and protein denaturation.

1.1.3.3 Manganese

Manganese is an essential element which are needed by the human body for several vital processes. It participates in several enzyme activities and play an important role in normal metabolic processes such as the metabolism of carbohydrates [37]. On the other side, excessive exposure to Mn from industrial sources, such as the production of manganese alloys, iron and steel manufacture, ferromanganese refineries, battery manufacturing, and welding, can lead to a variety of health disorders. For examples, depressive and neurobehavioral dysfunction [38]. Moreover, one of the most important sources of Mn in human is contaminated water which may be caused neurotoxic effects [38]. In recent century, materials of hair, nails, blood, urine, and saliva have been used as biomarkers for manganese exposure from occupational and environmental sources [39-41].

1.1.3.4 Lead

Pb is found everywhere in our surroundings. However, according to [42], no physiological function in biological systems has yet been identified. Pb directly disrupts a few specific enzyme functions, deactivates sulfhydryl antioxidant pools, inhibits the absorption of vital trace elements in a competitive manner, and increases arterial stiffness. Lead is a prime example of an environmental contaminant that has a high level of toxicity on a variety of exposed species' tissues and organs. It can disrupt vital physiological functions because it can substitute zinc and calcium in proteins [43]. According to [44] the primary consequences of lead exposure include cardiovascular, pulmonary, and neurological diseases. These are linked to various illnesses and are typically predicated on causing disruptions in immune regulation and oxidative and inflammatory processes [45]. A previous study has explained the impact of Pb on cardiovascular disease [46]. Furthermore, the high levels of Pb in the human body lead to adverse effects on the metabolism of vitamin D [47].

1.1.3.5 Copper

Numerous studies have shown that copper is necessary for optimal human health because it supports a wide range of physiological functions, especially those connected to enzymes. For instance, ferroxidase uses copper to regulate the iron's oxidation state such that only Fe 2+ is absorbed and Fe 3+ binds to the transferrin plasma protein [37]. This essential element also contributes to the onset of several diseases [28]. For example, diabetes has been linked to greater levels of Cu compared to non-diabetics [49]. Women's infertility has also been connected to copper deficiency [26]. In addition, Cu is a key element in metal-enzyme complexes

(metalloenzymes) that catalyze or regulate many vital body processes such as respiration, muscle contraction, nerve conduction, digestion, and growth [49].

1.1.3.6 Zinc

Zinc is necessary for the efficient operation of over 300 human enzymes, including those involved in digestion and metabolism. After iron, Zn is the most abundant trace element in the human body, an average of 70 Kg of adult humans contains 2.3 g of Zn [37]. In most cases, the Zn ion is an essential cofactor for the observed biological function of these metalloenzymes. The biological function of Zn is found in many tissues are most often associated with proteins [37]. Elevated zinc levels have been connected to the onset of Parkinson's disease, which results in problems with the nervous system by causing damage to the brain nerve cells [50]. Previous studies have discovered a connection between the body's zinc levels and several illnesses [49, 51].

1.1.3.9 Cadmium

Even at very low concentrations, certain trace metals—most notably cadmium, lead, and mercury are hazardous to both people and animals [52]. Due to its detrimental effects on human biology, cadmium is categorized as a hazardous element [53]. As you age, the concentration of it increases and accumulates in the renal cortex [54]. The majority of cadmium intake occurs during industrial activities. Contrarily, cigarette smoking can significantly increase body levels in both active and passive people [55]. Lately, cadmium has emerged as a serious health concern [56]. Mothers who smoked during their pregnancies may increase their child's risk of developing asthma, type 2 diabetes, hypertension, obesity, pediatric cancers, and/or behavioral problems in later life [57]. Long-term exposure has been linked to birth abnormalities, hypertension, kidney problems, and infertility [52,55,15]. The

previous section describes the illnesses that might arise in the human body due to an excess or a lack of necessary and non-essential elements. Prolonged exposure to inorganic elements emergence of illnesses such as diabetes, anemia, cancer, asthma, and heart disease [40].

1.2 Chronic Kidney Disease (CKD)

The kidneys are two bean-shaped, reddish-brown organs found in vertebrates. In adult individuals, they are located on the left and right sides of the retroperitoneal region and have a length of around 12 centimeters (4+12 inches) [60,61]. The paired renal veins allow blood to exit the body after entering through the paired renal arteries. The kidney is involved in the regulation of different electrolyte concentrations, acid-base balance, fluid osmolality, bodily fluid volume, and toxin removal. The glomerulus, where filtration occurs, filters one-fifth of the blood volume that enters the kidneys. Amino acids, salt, bicarbonate, glucose, and solutefree water are a few examples of substances that are reabsorbed. Among the substances emitted are uric acid, hydrogen, ammonium, and potassium. Additionally, Additionally, the kidneys carry out other tasks such as producing the hormones erythropoietin and renin, for instance, and transforming a precursor of vitamin D into its active form, calcitriol. Globally, chronic kidney disease (CKD) has been recognized as a significant public health concern. Between 5 and 7 million people worldwide suffer from kidney failure and need renal replacement therapy; the estimated prevalence of CKD is 13.4% [62]. Procedures used in the management of kidney disease include kidney biopsy and CT scan to assess for abnormal anatomy, measurement of kidney function by calculating the estimated glomerular filtration rate (eGFR) using serum creatinine, and chemical and microscopic examination of the urine (urinalysis). Kidney failure is treated with dialysis or kidney

transplantation; when renal function is less than 15%, one (or both) of these treatments is almost always performed. Nephrectomy is the standard treatment for renal cell carcinoma.

1.2.1 The reasons for CKD

Usually, other illnesses that strain the kidneys are the cause of chronic renal disease. It frequently results from a confluence of various issues [62]. The following reasons can cause CKD:

high blood pressure: over time, this can cause the kidneys' tiny blood capillaries to become overworked and cease to function correctly;

The little filters in the kidneys can be harmed by an excess of glucose in the blood;

elevated cholesterol Fatty deposits may accumulate in the blood vessels supplying your kidneys as a result, which may impair their ability to function normally;

kidney infections;

glomerulonephritis – kidney inflammation .

Cyst formation in the kidneys is a hereditary illness known as polycystic kidney disease;

obstructions in the urine's flow, such as an enlarged prostate or kidney stones that recur; and

prolonged, regular use of some medications, such as non-steroidal antiinflammatory drugs (NSAIDs) and lithium.

1.2.2 Trace Elements and Chronic Kidney Disease

The levels of trace elements were used as a biomarker to evaluate if there were any significant differences between the two groups (healthy and people with different

diseases) [64-66, 73]. Based on the metabolism of many trace elements in the human body, some critical elements may play a major role in the development and progression of hemodialysis, as shown in Table 1.1 [74,75].

Table 1.1: Elemental levels, mean ± standard deviation, and (range) (µg/L, $P <$ 0.001) using the whole blood of haemodialysis patients, and healthy individuals (controls) from the literature [74, 75*].

Elements	Hemodialysis	Healthy
Al*	43.5 ± 22.4	9.6 ± 6.23
Cd	0.038 ± 0.016, (0.008-0.080)	0.013 ± 0.005, (0.008-0.018)
Cu	671 ± 150, (341-1046)	812 ± 139, (621-1070)
Mn	0.40 ± 0.10, (0.22-0.65)	0.52 ± 0.11, (0.36-0.76)
Pb	0.287 ± 0.149, (0.025-0.755)	0.096 ± 0.083, (0.021-0.317)
Se	62 ± 13, (32-90)	82 ± 15, (58-103)
Zn	448 ± 84 (271-658)	681 ± 114, (475-876)

1.3 Trace Element Measurements

It is possible to test for the presence of trace and ultra-trace elements in various human tissues and fluids [74]. In general, these components' concentrations vary from tissue to fluid because of a range of factors, such as lifestyle, age, gender, exposure to the environment, dietary habits, alcohol consumption, and cigarette smoking [8]. Because they can be used for biomonitoring human health, blood serum, and fingernails were selected as the main human tissue and fluid for this study [76-78]. Long-term growth materials such as nails can yield some useful information

if the results are compared with a comparable reference concentration range for a well-defined "healthy or control" population [67].

1.4 Human Tissues and Fluids

1.4.1 Fingernails

The material that comprises fingernails is created by living skin cells. The cuticle is the tissue that surrounds the nail plate and shields the matrix from bacterial and physical attack. The nail plate itself, along with the lunula (half-moon), the base of the nail, and the point where the nail bed and matrix converge, are the areas beneath the cuticle that contain the matrix (also known as the nail root); the nail plate, which is a visible nail that extends to the free border of the nail bed [79]. While newer cells proliferate within the matrix, older cells are forced out, as shown in Figure 1.4. A few factors that could influence nail growth are age, food, and overall health. In general, fingernails grow 0.1 mm daily, but toenails grow between 0.03 and 0.05 mm daily [80]. This rate is often higher in young people than in senior people, during the summer as opposed to the winter, and pregnancy [81]. If a fingernail is broken or lost, a new one will always grow in its place. The only exception is that if the matrix is removed, the nail will grow back deformed (Figure 1.4). Despite the dearth of research on the subject, nail material is believed to be a useful tissue to estimate the levels of minor and trace elements in the human body [82]. Several factors have been shown to affect the number of elements in nails, including age, gender, lifestyle, exposure to the environment, smoking habits, and general health [67, 83].

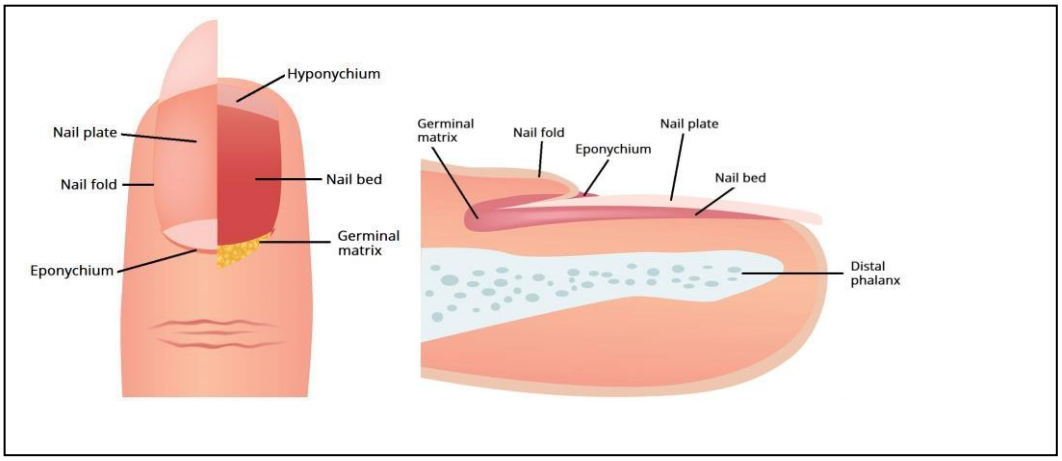


Figure 1.4: Nail structure [Re-Produced].

Human scalp hair and nails (finger and toe) have been utilized extensively in the past few years as reliable biomarkers for assessing human metabolic state for both essential and toxic trace elements, as well as for assessing exposure to various contaminants in occupational and/or environmental settings [39,84-87]. Compared to blood and urine, hair and nail tissues have several advantages, including the ability to accumulate various trace elements throughout 2 to 18 months, being non-invasive and easy to collect, and possibly acting as a long-term growth medium. These advantages might make it feasible to gather information that can be utilized over an extended period to evaluate an individual's health status [40,67,80,81,88-90]. In contrast, the analysis of human fluids, such as blood and urine are accompanied by several problems, including the composition at the time of sampling and the fact that many trace element levels are regulated by homeostatic processes [91,92]. In light of these facts, the following can be investigated using the concentration of trace elements: (1) the amount of trace elements consumed through nutrition, especially non-essential or "toxic" elements; (2) exposure to environmental pollutants released into the environment by humans; (3) the association with any kind of smoking,

including passive, active, and non-passive; and (4) any potential correlation between particular trace elements and diseases [67,93]. Furthermore, defining normal or reference ranges, differentiating between endogenous and exogenous deposition, and external contamination are some of the challenges associated with hair analysis [94]. Other factors that may impact the concentration of components in the hair and nail tissue include age, gender, smoking habits, and residential location [52,95,96].

1.4.2 Serum

The chemical composition of blood serum fluid includes various proteins in different concentrations. In addition, to several small molecules such as sugars, amino acids, lipids, and salts, serum, contains 60–80 mg of protein/ml (97). For example, transferrin, albumin, lipoproteins, haptoglobin, and immunoglobulins (98). Moreover, the serum also includes other proteins created or lost from cells and tissues inside the body (99). The total number of proteins in the serum is estimated up to 10,000 proteins which are presented in serum at very low relative abundances (100). Human serum is preferred for certain applications such as DNA research, cell cultures, and cancer therapy studies where animal serum could result in findings that can't be applied to people [97]. Studying serum from individuals who live with certain diseases is one way to uncover novel targets for the development of new drugs. Further, human cells often need human serum to grow properly, so any research involving human cell lines will often require serum from a human source [98]. Serum is also used for epidemiological surveillance studies to identify people within a population who have protective immunity to specific diseases, or to measure past exposures [99].

Comparing serological profiles from the same population over time can provide insights into changes in the prevalence of diseases and changes in health or

nutritional status. For example, since 1985 the US Department of Defense has curated a serum depository for health protection goals. Samples are collected before and after military deployments [101]. Human serum is also useful in therapeutic applications. When people recover from an infectious illness, their serum contains disease-fighting antibodies specific to what infected them. This can be collected and transferred to other individuals who are ill with the same disease, to boost their immune system's response. While it's playing catchup compared to vaccination, serum therapy — also called convalescent plasma therapy — can help save lives while a vaccine is being developed or distributed [100].

1.5 Aim and Objectives

1.5.1 Aim

The main goal of this research is to investigate whether there is any significant relationship between the levels of trace elements with the onset of hemodialysis disease.

1.5.2 Objectives

The main objectives of this work were to:

establish a new method for the sample collection and subsequent analysis of trace and ultra-trace elements in human fingernails and serum;

develop analytical methods for the determination of elemental levels in washed fingernail and serum;

validate the developed methods through the use of certified reference materials to establish quality control (precision and accuracy) values;

assess the elemental composition of human fingernail and serum as a useful tool in determining the health status of an individual; and

investigate whether factors like gender, smoking activity, and age may affect the elemental concentrations in the fingernails and serum of the individuals under study.

Chapter Two

Analytical Methodology, Instrumentation & Statistical Methods

2.0 Introduction

This Chapter describes the sampling, storage, and preparation methods that were carried out on the biological matrices. The fundamental theory for each method and any development procedures used to determine trace element levels are also reported. The main technique used for the determination of trace and ultra-trace elements was inductively coupled plasma Optical Emission spectrometry (ICP- OES).

The use of certified reference materials (CRMs) and replicate analysis ensured accuracy and precision throughout the analysis. Demographic Characteristics of Study tissue (fingernails) was collected from Iraqi individuals' resident in Karbala (Iraq). The biological samples were classified into various groups, namely healthy, hemodialysis, smoker, and non-smoker individuals covering both genders and different ages.

2.1 Materials and Instruments

In this research, ultra-high-purity grade reagents were used for the digestion or dilution of samples so as to avoid contamination at trace element levels, as shown in Table 2.1. Moreover, instruments and tools were also used to determine the levels of trace elements in fingernails and serum, as presented in Table 2.2.

Table 2.1: Materials used in this study.

No.	Materials	Company
1	Distilled Deionised Water (DDW)	Jawhara, Iraq
2	Millex Filter Millipore (0.45µm)	Cork, Ireland
3	Nitric Acid (Aristar® 65%)	BDH
4	Aceton	BDH, POOLE, UK
5	Hydrogen Peroxide	BDH, POOLE, UK

Table 2.2: Instrument used in this study

No.	Equipment	Company	Place
1	Balance, ABP 200-5AM	KERN	Kerbala University, College of Science
2	Hot Plate	Luxell, Turkey	Kerbala University, College of Science
3	ICP-OES	Shimadzu, Japan	Agricultures Research Centre, Technology and Science Ministry
4	Oven	Memert, Germany	Kerbala University, College of Science
5	Ultrasonic Cleaner	Sunshine, China	Kerbala University, College of Science
6	Centrifuge	ESAW, India	Kerbala University, College of Science

2.2 Demographic Characteristics of Study Populations

In this study, the biological fluids (blood serum) and tissue (fingernails) samples were collected from Iraqi individuals' resident in Karbala (Iraq), as reported in the following Sections.

The participants were informed of all the study procedures before signing the consent form. In addition, the subjects completed the study questionnaires to provide personal details and information about health, diet, smoking activity, and lifestyle at the time of sample collection. All the questionnaires were labeled with the code - which was laid out in the following format BS-H-011222-1, where BS corresponds to blood serum and may be replaced by FN (fingernails); H corresponds to healthy and may be replaced by Hem (Hemodialysis); 011222 corresponds to the date (DDMMYY); and 1 corresponds to the participant code number.

The results from the questionnaires show that the participants used a similar dietary program including vegetables, rice, cereals, fruit, bread, meat, butter, oils, cheese, cream, and milk; and the main drinks being fruit juice, soft drinks, and tea; prepared with household tap water or domestic water. In this study, two main groups, namely healthy and hemodialysis were used to classify the subjects. Moreover, data on health status, smoking activity, age, and gender have been reported at the time of sample collection. Generally, the individuals have provided two types of samples, namely fingernails/blood serum for healthy (n = 59) and patients (n = 50), as shown in Table 2.1. This then allowed this study to check whether there are any possible significant differences and relationships in terms of the levels of trace elements between these media.

Table 2.3: Study populations for different human samples collected from Karbala/(Iraq).

Human sample	Number of Samples	
	Healthy	Patients
Fingernails	59	50
Blood Serum	59	50

2.3 Sample Collection and Preparation

The biological samples were either solid (fingernails) and liquid (blood serum), and homogenous or heterogeneous. However, the heterogeneous samples need extra care during sampling and pre-treatment before storage and analysis [102]. Therefore, various steps should be taken to minimize loss, contamination, decomposition, and matrix change.

2.3.1 Fingernails

Fingernail samples were collected from all 10 fingers using acetone-distilled deionized water-washed clippers [80]. The majority of studies have used this method to obtain nail samples, but in some cases, only thumbnails have been collected [103]. The main advantages to collecting all fingers rather than one big finger are sufficient sample mass, and an estimate of the complete hand of

exposure [104]. Nail samples were cleaned manually of any visible dirt (e.g. soil) on the surface of nails prior to applying the washing procedure [105].

In brief, the fingernail samples were cut and washed (three times with DDW (~ 5 ml) and one time with enough volume of acetone) then the samples were sonicated for 10 minutes at 35 MHz and separated by centrifugation (5 minutes, 1000 rpm). Then, the samples were dried overnight at 60ºC using an oven. A Kjeldhal tubes was utilized to digest the samples. The labeled polyethylene tubes were used to store the samples at room temperature.

2.3.1.2 Washing Procedure

The purpose of a washing procedure is to remove exogenous contaminants from the surface of the fingernails to provide true levels of endogenous elements [106]. Many methods have been used in the literature to wash the fingernail samples [106,9]. The useful techniques reported in the literature are using acetone water water water-acetone; ether-Triton X-100-water-water; and ether-acetone water ether, respectively [106,107,108]. The first method was used in this study; therefore, a sufficient volume of acetone was added to each sample to cover the fingernail materials. Then, samples were sonicated for 10 minutes (35 MHz) at room temperature and separated by centrifugation (1000 rpm for 5 minutes). Then, samples were dried at 60ºC and stored for digestion.

2.3.1.3 Digestion Methods

All the samples were digested to destroy the organic material and leave behind an inorganic residue in the sample. In general, several methods can be used for

this purpose, for example, dry ashing and wet digestion [56,50]. In this study, wet digestion has been used to digest the fingernail samples.

2.3.1.3.1 Wet digestion

In this method, 1 ml of nitric acid was added to the (0.500 ± 0.001 g) of a fingernail sample and moved into a clean/dried Kjeldhal™ Tube, which is placed in the digester and heated at 165°C (± 10°C) for a half hour. The volume was diluted with DDW using a polyethylene volumetric flask, resulting in a dilution factor of 100fold. Sample solutions were centrifuged for 10 minutes at 3000 rpm and filtered through a Millex filter, MF-Millipore (0.45 µm). The digested fingernail solutions were stored in a labeled 25 ml Sterilin® centrifuge tube and stored in the fridge at 4ºC prior to ICP-OES analysis. Along with the fingernail samples, a reagent blank and certified reference human hair material (GBW09101), provided by the National Research Centre for Certified Reference Materials, China, were treated in the same manner according to the procedures using wet digestion in order to check the precision and accuracy for each method.

2.3.2 Blood Serum

The Samples were digested using 100 µl of blood serum with 200 µl of approximately 65% nitric acid (HNO_3) and 100 µl of hydrogen peroxide (H_2O_2) in a 15 ml polypropylene tube with a screw cap (VWR laboratories) and the mixture vortexed briefly [109]. Then the tubes were set for 90 min at 60˚C in a heating system. After incubation, samples were cooled down by adding 2100 µl deionised water (18 MΩ cm^{-1}). After vortexing and centrifugation (3 min/ 2500 rpm), samples were ready to measure. Ten ml of a "pooled" serum sample was

also collected from six individuals, who were living in the same family residence, for quality control measurement purposes.

2.3.2.1 Sample Storage, Method of Transfer and Preparation

Blood serum samples were collected at Al-Hussein hospital in a special laboratory, and then kept at −20°C. All samples were safely transferred from hospital to the chemical laboratory in a fully charged Tropicool cool box to maintain a temperature of 4ºC for ~ 12 hours when unopened). Disposable ice packs were also added to the samples in storage to help maintain/prolong a temperature of 4ºC. On return to the laboratory, serum samples were directly prepared. Reagent blank (field blank) and "pooled" samples were also prepared using the same procedure. Certified reference materials (CRMs), namely, NIST SRM® 1643e Trace Elements in Water (National Institute of Standards and Technology, Maryland, USA) were utilised for Quality Control (QC) measurements. All samples were analysed within two weeks of collection time by using the ICP-OES instrument (Shimadzu, Japan).

2.3.2.2 Testing of Sample Pre-treatment Procedures

In this study, the following requirements processes were used before to determine the levels of trace elements in biological matrices, namely: (i) the reduction of sample preparation time; (ii) the lowering of blank values; (iii) the control of the deposition of solids in the sample introduction devices of an instrument; (iv) the minimization of saline matrix influences on the analytical signals; (v) and the capability of detecting elements present at ultra-trace levels in the collected samples. The subtraction of the blank signal and the use of internal standards (ISs) were sufficient for controlling the above problems, and for

reducing reagent impurities, instrumental drifts, and matrix effects [102,110]. Moreover, ultra-high-purity grade reagents were used for the digestion or dilution of samples to avoid contamination at trace element levels. Reagent blanks that test exposure to any contamination during the whole process (sampling, transport, preparation, and analysis) were run for all analyses, even if high-purity reagents were used, in order to confirm that the instrument was clean, and the reagent solvents were of good quality [102]. In order to minimize the contamination from collection devices, all containers were thoroughly soaked overnight with a mixture of 10% (v/v) of HNO_3 (65% Aristar®) followed by final rinses with distilled de-ionised water (DDW) [110].

2.4 Analytical Instrumentation

There is a wide range of analytical techniques that have been used for trace element analysis, such as flame atomic absorption spectrometry (FAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) [112]. The ideal analytical technique for measuring trace elements in environmental and human samples must offer (i) very low detection limits; (ii) a wide linear dynamic range; (iii) simple interference-free data; (iv) qualitative and quantitative analysis; (v) simple sample preparation; and high throughput per determination [111]. The following subsections describe in detail the analytical instrumentation employed throughout this work. The fundamentals, instrument configuration, interferences, and methods of calibration are reported.

2.4.1 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Plasma sources were developed for emission spectrometry in the 1960s and became commercially available in the mid-1970s [112,113]. Inductively coupled plasma optical emission spectrometry (ICP-OES) is a technique that has been commonplace in analytical laboratories for many decades.

2.4.1.1 Fundamentals

ICP-OES is a multi-element analysis technique that uses an inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. A detector measures the intensity of the emitted light and calculates the concentration of that element in the sample [113-115]. The main advantages of this method are the large dynamic range, autosampler, high-throughput sample introduction system, accepts samples with a matrix of 1% dissolved solids content, good detection limits, and the ability to detect most elements of the periodic table [116]. The basic aim of this technique is to identify elements (qualitative analysis) and quantify their concentrations in various media (quantitative analysis) by the measurement of light emitted from plasmas by atoms after the absorption of energy as heat [102,115,116]. ICP-OES instruments generally have four main parts: the sample introduction system (nebuliser and spray chamber); ICP torch; transfer optics; and spectrometer, as shown in Figure 2.1 [117]. In brief, the sample is usually transported into the instrument as a stream of liquid samples. Inside the spray chamber, the liquid is converted into an aerosol through a process known as nébulisation. The sample aerosol is then transported to the plasma where it is desolvated, vaporised, atomised, and excited and/or ionised by the plasma. The excited atoms and ions emit their characteristic radiation which is collected by a

device that sorts the radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into concentration information for the analyst [113]. The wavelength range of the plasma radiation is extended from 200 to 800 nm [115].

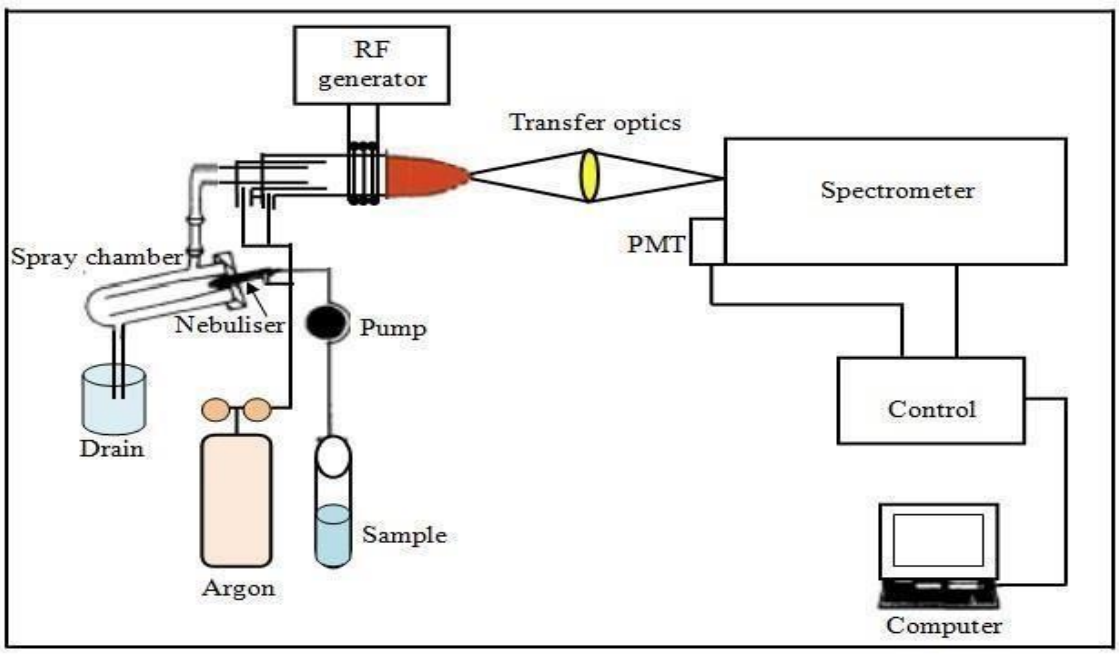


Figure 2.1: Typical configuration for ICP-OES instrument (axial viewing of the ICP) [118].

2.4.1.2 Interferences

Although, the presence of interferences can affect the accuracy of a determination, there is no analytical technique that is completely free from interferences. However, modem trace elemental analysis instruments have been designed to minimize the interferences. Interferences in ICP-OES may start in the sample preparation stage and extend to the plasma operating conditions. In general, ICP-OES probably has the fewest interferences compared to commonly

used analytical atomic spectrometry techniques [119]. The technique suffers from the main two types of interferences, namely chemical, and spectral interferences. The high temperature of the plasma helps to reduce chemical interferences due to this temperature being sufficient to break down most species into atoms or ions for excitation and subsequent emission [102,119]. [119,102,116] The most common interference problem in ICP-OES is spectral interference due to the line-rich spectra produced by the hot plasma source.

They can be minimized using high-resolution spectrometers, advanced background correction techniques, and different analytical wavelengths for the chosen element(s) of interest [119,116,102]. In this study, background correction was used in order to overcome spectral interferences.

2.4.1.3 Instrumentation

A Shimadzu ICPE- 9000 (Japan) was used in this study. The typical operation parameters for this instrument are displayed in Table 2.4. An echelle grating and the charge-coupled device (CCD) were used in the ICP-OES instrument.

Table 2.4: Typical operating conditions for the Shimadzu ICPE- 9000 instrument.

Parameter	Typical operating condition
RF Power	1300 W
Plasma gas flow	15 l/min
Auxiliary gas flow	0.2 l/min
Nebulizer gas flow	0.8 l/min
Plasma view	Axial View
Pump flow	1.5 ml/min
Peak processing	Peak area
Points per peak	3
Integration time	50 ms
Auto integration	5 sec min-20 sec max

Read delay	60 sec
Equilibration delay	15 sec
Rinse	30 sec
Replicates	3
Background correction	one or two points
Spray chamber	Double-pass Scott-type
Nebulizer	GemTip cross-flow pneumatic

2.4.1.4 ICP-OES - Calibration

Calibration for ICP-OES was achieved by serial dilution of a 1000 mg/L single element standard solution (Aristar®, BDH, Poole, UK). The calibration range for each element has at least 6 standards, including the blank, and a range of calibration standards for Mn, Cu, Zn, and Cd 0.1 - 1 µg/mL; for Pb and Se 0.1 - 15 µg/mL; and 0.5 – 10 µg/mL for Al. Calibration data was evaluated using WinLab32™ software, where the calibration graphs were automatically drawn by plotting the value of intensity against the concentration of each element. The linearity range was evaluated by inspection of the linear regression coefficient (R^2) for each calibration curve. Figure 2.2 shows the typical calibration curve for cadmium by the Shimadzu 9000 ICP-OES instrument. The Calibration Curves of other elements can be found in Appendix D.

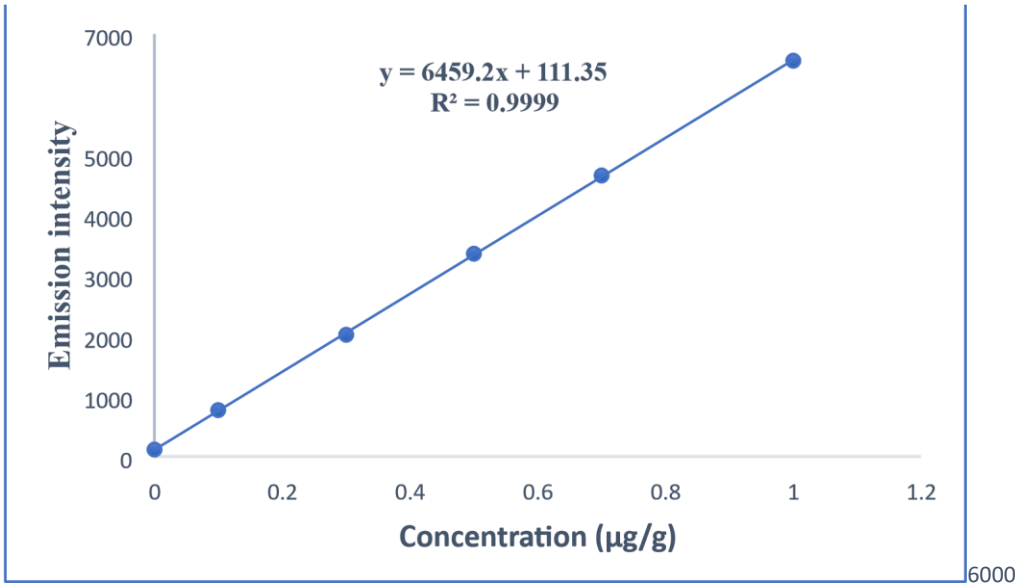


Figure 2.2: Typical calibration curve for Cadmium as determined by Shimadzu 9000 ICP-OES instrument.

2.5 Quality Control (QC)

Several evaluation methods can be used to determine the quality of this analysis, namely, the limit of detection, precision, and accuracy.

2.5.1 Limit of Detection (LOD)

The LOD of an individual analytical procedure is 'the lowest amount of an analyte in a sample that can be detected by the instrument', as given by equation 2.1 [120].

$$X_L = LOD = X_{bl} + KS_{bl} \text{ ---------- Equation 2.1}$$

where $\mathbf{X_{bl}}$ is the mean of the blank measures, $\mathbf{S_{bl}}$ is the standard deviation of the blank measures and **K** is a numerical factor chosen according to the confidence interval required (typically 3) [120]. Furthermore, the value of LOD can also be

based on a mean blank (n = 15, 1% HNO3 or DDW) signal + 3Sd [102,121], as mentioned in

Tables 2.5.

Table 2.5: Elemental limit of detection (LOD) values for Shimadzu 9000 ICP-OES instrument (µg/L) and selected wavelength.

Element	Wavelength (nm)	LOD
Al	394.401	22.284
Cd	226.502	5.006
Cu	324.700	11.693
Mn	257.610	8.723
pb	220.353	3.581
Se	196.026	2.463
Zn	213.857	2.336

2.5.2 Precision and Accuracy

Precision can also be known as random error which is the degree of agreement between replicate measurements of the same quantity of sample [122, 123]. Typically, the precision level of an instrument can be determined by using the values of relative standard deviation (%RSD) [122,124].

%RSD = (Standard deviation / Mean value) x 100 --- Equation 2.2

Accuracy can also be known as systematic errors which is the degree of agreement between a measured value and a true value" [112,123]. The value of accuracy can be measured by calculating the measured value of Certified Reference Materials (CRMs) compared with their certified value, as shown in

Table 2.6 [125]. This can be determined as the percentage recovery (%R), which can be calculated by the following equation:

%R = (Measured value)/(Certified value) x 100 ----- Equation 2.3

In general, good levels of precision were obtained for most elements with range of 3.647 (Se) - 9.975 (Cd) % RSD for fingernails, and 5.138 (Pb) – 9.981 (Cd) % RSD for the remaining elements in terms of the pooled sample of fingernails, while acceptable range of 5.138 – 9.981% RSD for all elements regarding the pooled sample of blood serum by using ICP-OES.

Table 2.6: Precision levels for selected trace elements in different pooled samples (n = 10) determined by the Shimadzu 9000 ICP-AES instrument; presented as mean, ± Sd and %RSD values, mg/l for blood serum and mg/kg for fingernail.

Element	Fingernail mean ± Sd (%RSD)	Blood Serum mean ± Sd (%RSD)
Al	4.283 ± 0.383 (8.942)	1.488 ± 0.130 (8.736)
Cd	0.042 ± 0.004 (9.523)	0.031 ± 3.003 (9.687)
Cu	0.137 ± 0.012 (8.759)	0.114 ± 0.010 (8.771)
Mn	0.406 ± 0.024 (5.911)	0.152 ± 0.015 (9.868)
pb	9.302 ± 0.426 (4.579)	3.133 ± 0.161 (5.138)
Se	3.851 ± 0.140 (3.635)	3.367 ± 0.221 (6.563)
Zn	3.735 ± 0.294 (7.871)	0.285 ± 0.019 (6.666)

Sd is standard deviation; RSD is a relative standard deviation (quoted as a % in brackets).

In this study, Standard Reference Material® 1643e Trace Elements in Water was used to evaluate the level of certificate for interested elements in terms of

blood serum samples, whereas CRM GBW 09101 was used in the case of fingernail samples. Measured CRM values obtained for the analysis of trace elements by ICPOES, were highly comparative to certified levels, as shown in Tables 2.7 and 2.8. Analytical recoveries ranged from 95.757% for Cd to 102.623% for Se regarding the blood serum and from 91.228% for Cd to 99.172 for Zn in the case of fingernails.

Table 2.7: Accuracy levels for blood serum using Standard Reference Material® 1643e Trace Elements in Water, presented as mean ± Sd, and %R for measured values and mean ± Sd for certified values.

Element (n = 103)	Elemental level (µg/l)		
	Accuracy		
	Measured value mean ± Sd	Certified value mean ± Sd	Percentage recovery (%R)
Al	137.512 ± 0.426	138.330 ± 8.400	99.408
Cd	6.139 ± 0.394	6.410 ± 0.071	95.772
Cu	22.060 ± 0.385	22.200 ± 0.310	99.369
Mn	37.430 ± 0.268	38.020 ± 0.440	98.447
pb	18.616 ± 0.133	19.150 ± 0.200	97.211
Se	11.986 ± 0.645	11.680 ± 0.130	102.619
Zn	75.606 ± 0.247	76.500 ± 2.100	98.831

Sd is standard deviation; (%R) the Percentage recovery (quoted as a % in brackets).

Table 2.8: Accuracy levels for fingernails using CRM GBW 09101, presented as mean ± Sd, and % R for measured values and mean ± Sd for certified values.

Element (n= 103)	Elemental level (µg/kg)		
	Accuracy		
	Measured value mean ± Sd	Certified value mean	Percentage recovery (%R)
Al	12.820 ± 0.114	13.3	96.391
Cd	0.087 ± 0.002	0.095	91.578
Cu	22.599 ± 0.511	23	98.256
Mn	2.768 ± 0.104	2.94	94.149
pb	6.830 ± 0.111	7.2	94.861
Se	0.533 ± 0.031	0.58	91.896
Zn	187.436 ± 1.780	189	99.172

Sd is standard deviation; (%R) the Percentage recovery (quoted as a % in brackets).

2.6 Significance Tests

In this study, significant tests have been used to evaluate the results and the influence of different factors. Therefore, Grubb's outliers, F-test, t-test, one-way analysis of variance (ANOVA), and Pearson's correlation analysis (r), were then undertaken. Regression analysis was also utilized to determine the linearity of the calibration curve for each trace element by the ICP-OES investigated in this research.

Chapter Three

Trace Element Levels in Serum

3.0 Introduction

In this chapter, the level of trace elements in human serum has been reported. The inductively coupled plasma optical emission spectrometry (ICP-AES) instrument was used for multi-element analysis. The results for aluminum (Al), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), selenium (Se), and zinc (Zn) in serum samples have been determined. The influence of health status, smoking activity, gender, age, and statistical interactions on the elemental levels was also investigated. The results were compared with published literature values. This chapter aimed to develop serum as a potential biomarker for monitoring trace element levels in the human body for short periods, in terms of the evaluation of human health.

3.1 Statistical Methods of Analysis

Significance tests were used to assess the level of trace elements in serum samples. For example, arithmetic mean, standard deviation, outliers, F-test, and a two-tailed t-test. A one-way analysis of virions ANOVA was also used. The Pearson correlation coefficient (r) was performed to evaluate associations between trace element levels in serum [122].

3.2 Elemental Composition of Serum

Many studies have determined the levels of trace elements in human fluids such as blood serum, plasma, urine, teardrops, saliva [126], and tissues (scalp hair and fingernails) [127,111,128,129]. In general, elemental levels in human biological samples vary from one country to another because of geographical differences; nutritional status; and the method of analysis [105]. Therefore, it is difficult to establish reference ranges for trace elements in human fluids and tissues because of

the effects of said factors, as they impose restrictions on the interpretation of the results. The results can be used to evaluate the possible relationship between serum and human health status as well as concerning trace elements. The main descriptive statistics of elemental levels in serum (arithmetic mean, standard deviation (Sd), range, median, 95% confidence interval for mean and the number of samples) for populations in comparison with their levels in the literature, as 36ummarized in Table 3.1. Figure 3.1 shows the box plots for trace element levels in the populations under investigation. The levels of trace elements (µg/L) in serum samples are increased according to the following sequence Al > Pb > Se > Zn > Mn > Cu > Cd. The main reasons for these differences are the effect of factors such as environmental exposure; diet; smoking activity; drinking water; gender; age; and health status, which all play a significant role in the evaluation of the metabolism of trace elements in the human body leading to various health disorders and diseases [107].

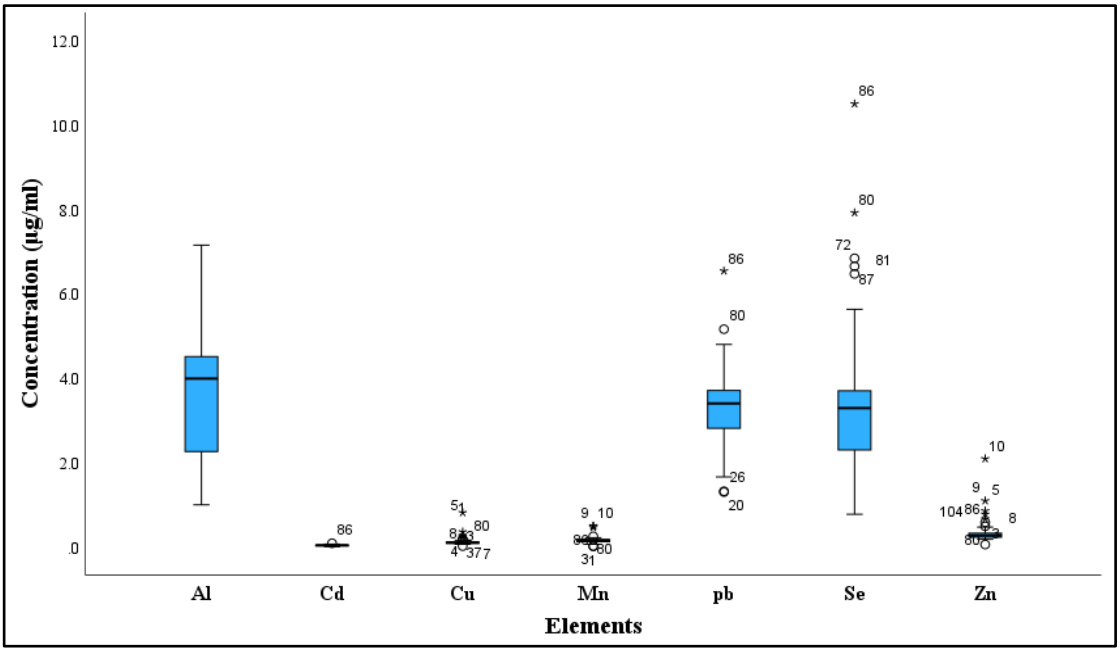


Figure 3.1: Trace element levels (µg/mL) in serum with outlier values for different elements; middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represents extreme values.

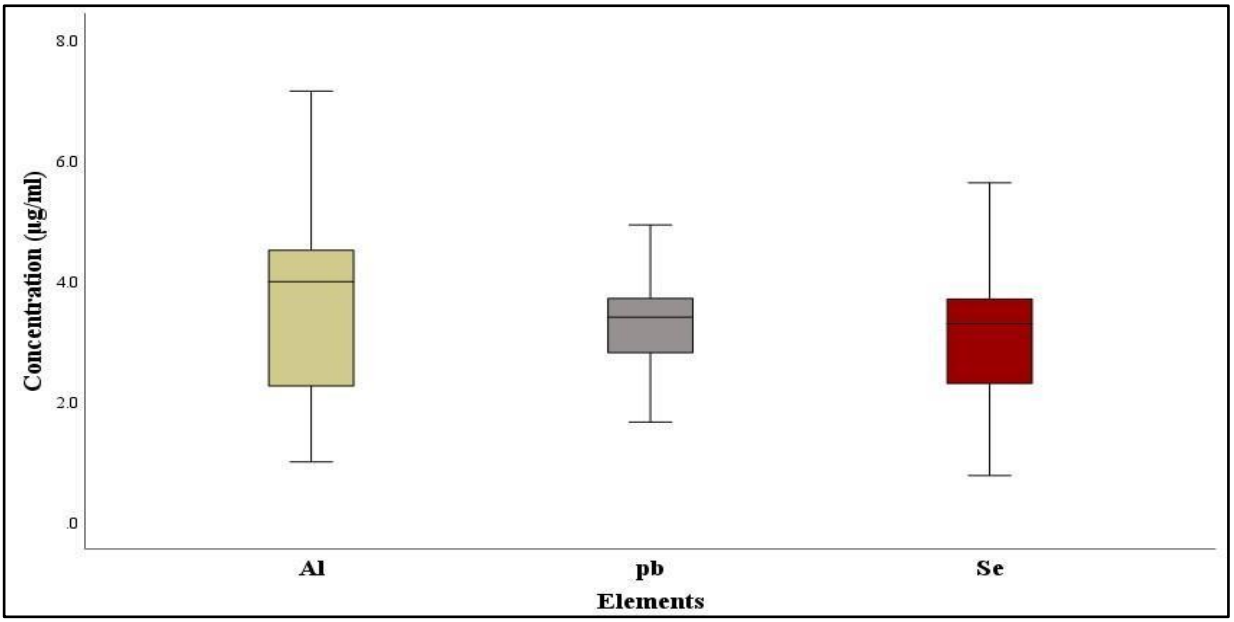
Table 3.1: Descriptive statistics for element concentrations in serum for 109 subjects, along with literature range.

Trace Elements	Variables	Concentration (µg/mL)	Literature range* (µg/mL)
Al (109)	Mean ± Sd	3.499 ± 1.441	12 -137
	Range	0.999 – 7.150	
	Median	3.990	
	95% confidence interval	(3.226- 3.773)	
Cd (109)	Mean ± Sd	0.035 ± 0.015	0.013 – 0.438
	Range	0.006 – 0.068	
	Median	0.044	
	95% confidence interval	(0.033-0.038)	
Cu (109)	Mean ± Sd	0.102 ± 0.024	4.2 - 17
	Range	0.052 – 0.154	
	Median	0.100	
	95% confidence interval	(0.097-0.107)	
Mn (109)	Mean ± Sd	0.149 ± 0.038	0.19 – 3.30
	Range	0.064 – 0.231	
	Median	0.156	
	95% confidence interval	(0.142-0.156)	
Pb (109)	Mean ± Sd	3.290 ± 0.784	0.27- 4.75
	Range	1.660 – 4.932	
	Median	3.400	
	95% confidence interval	(3.142-3.439)	
Se (109)	Mean ± Sd	3.003 ± 1.158	0.62 – 1.53
	Range	0.773 – 5.630	
	Median	3.290	
	95% confidence interval	(2.783-3.222)	
Zn (109)	Mean ± Sd	0.269 ± 0.067	80 - 191
	Range	0.143 – 0.455	
	Median	0.273	
	95% confidence interval	(0.269-0.295)	

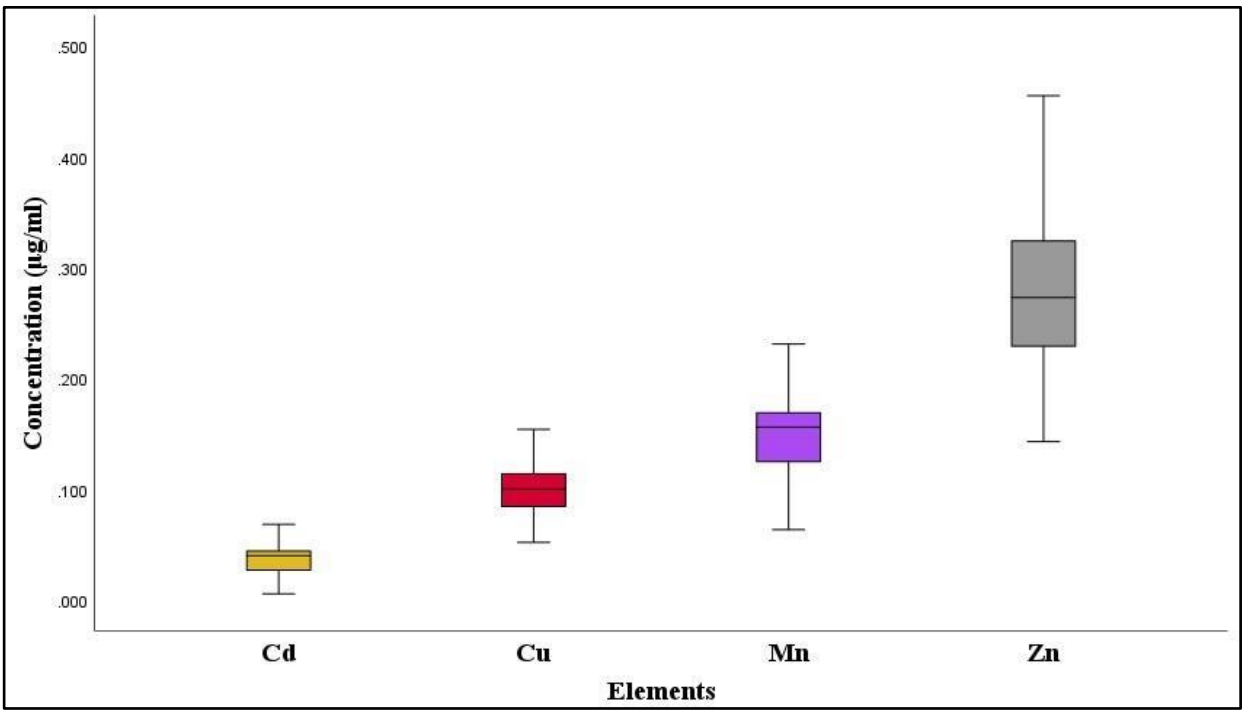
Sd is standard deviation, * [85].

3.3 Checking for Outliers

Many of the statistical tests covered in this study are sensitive to outliers [122]. The results of some trace elements were found to contain one (or possibly more) value/s that appear to differ unreasonably from the others in the study data set. These cases can have a disproportionate influence on statistical results such as the mean, which can result in misleading interpretations. Therefore, the data was inspected for statistical outliers using a Grubb's test (G). If the calculated value, G_{cal}, exceeds the critical value, G_{crit}, the suspect value is rejected so that it will not affect the accuracy of comparison studies between the various population groups [122]. No statistical outliers were found for the data of the total populations for Al, while the Cu and Zn were found to have 8 outliers. Mn and Se have 5 outliers, whereas Pb and Cd have 4 and 1 outliers, respectively. The results of Grubb's tests are summarised in Figure 3.2. In most cases, the effect of removal of the outliers can improve the histogram of trace elements in serum because the degree of positive skew (indicates the symmetry of the distribution) from the mean is decreased [130].



(a)



(b)

Figure 3.2 (a and b): Trace element levels (µg/mL) in serum without outlier values for different elements (n = 109); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively.

3.4 Results and Discussion

3.4.1 Factors Influencing Elemental Data (Factorial Analysis)

The mean values of the trace elements were categorized according to different parameters (factors) obtained from the questionnaire, as shown in Table 3.2. Multiple effects must be studied in research rather than the single effect for each factor [131]. The mean and standard deviation for each group is presented in Table 3.3. The effect of each factor was investigated.

Table 3.2: Demographic characteristics of participants according to different factors.

Factor	Code number *	Group	Number of subjects
Health status	1	hemodialysis	50 (male = 25, female = 25)
	2	healthy	59 (male = 33, female = 26)
Gender	1	male	58 (healthy = 33, diabetic = 25)
	2	female	51 (healthy = 26, diabetic = 25)
Smoking activity	1	smoker	24 (male = 23, female = 1)
	2	non-smoker	85 (male = 35, female = 50)
Total			109

* Each factor must be assigned a numerical code before it can be entered into SPSS.

Table 3.3: Descriptive Statistics (mean ± Sd µg/mL) for trace elements in serum samples with different factors.

Health status	Gender	Smoking activity	(n)	Elemental Level, mean ± Sd (µg/mL)						
				Al	**Cd**	**Cu**	**Mn**	**Pb**	**Se**	**Zn**
Hemodialysis	Male	Smoker	(8)	3.188 ± 0.842	0.035 ± 0.008	0.106 ± 0.031	0.124 ± 0.036	3.101 ± 0.882	2.864 ± 0.571	0.251 ± 0.071
		non-smoker	(17)	2.879 ± 1.102	0.032 ± 0.007	0.104 ± 0.021	0.119 ± 0.033	2.936 ± 0.917	2.629 ± 0.684	0.258 ± 0.059
		Total	(25)	**2.978 ± 1.102**	**0.033 ± 0.007**	**0.104 ± 0.024**	**0.121 ± 0.033**	**2.989 ± 0.891**	**2.704 ± 0.648**	**0.256 ± 0.061**
	Female	Smoker	(1)	3.700 ± 0.001	0.034 ± 0.001	0.154 ± 0.001	0.079 ± 0.001	3.740 ± 0.001	3.230 ± 0.001	0.254 ± 0.001
		non-smoker	(24)	3.603 ± 1.120	0.038 ± 0.01	0.102 ± 0.024	0.138 ± 0.036	3.299 ± 0.914	3.226 ± 0.908	0.262 ± 0.055
		Total	(25)	**3.607 ± 1.097**	**0.038 ± 0.009**	**0.104 ± 0.026**	**0.136 ± 0.037**	**3.317 ± 0.899**	**3.226 ± 0.889**	**0.262 ± 0.054**
	Total	Smoker	(9)	3.244 ± 0.806	0.035 ± 0.007	0.111 ± 0.033	0.119 ± 0.37	3.172 ± 0.852	2.904 ± 0.548	0.251 ± 0.066
		non-smoker	(41)	3.303 ± 1.157	0.036 ± 0.009	0.103 ± 0.023	0.131 ± 0.037	3.149 ± 0.922	2.978 ± 0.866	0.260 ± 0.056
		Total	(50)	**3.293 ± 1.095**	**0.035 ± 0.008**	**0.104 ± 0.025**	**0.128 ± 0.036**	**3.153 ± 0.901**	**2.965 ± 0.814**	**0.259 ± 0.057**
Healthy	Male	Smoker	(15)	3.773 ± 1.808	0.037 ± 0.020	0.104 ± 0.024	0.165 ± 0.026	3.280 ± 0.641	3.057 ± 1.477	0.319 ± 0.054
		non-smoker	(18)	4.123 ± 0.882	0.041 ± 0.010	0.085 ± 0.017	0.147 ± 0.020	3.027 ± 0.635	3.536 ± 0.917	0.259 ± 0.047
		Total	(33)	**3.964 ± 1.369**	**0.039 ± 0.016**	**0.093 ± 0.022**	**0.156 ± 0.025**	**3.142 ± 0.641**	**3.318 ± 1.208**	**0.286 ± 0.058**
	Female	non-smoker	(26)	3.309 ± 1.954	0.031 ± 0.022	0.108 ± 0.024	0.181 ± 0.029	3.745 ± 0.508	2.674 ± 1.542	0.322 ± 0.075
		Total	(26)	**3.309 ± 1.954**	**0.031 ± 0.022**	**0.108 ± 0.024**	**0.181 ± 0.029**	**3.745 ± 0.508**	**2.674 ± 1.542**	**0.322 ± 0.075**
	Total	Smoker	(15)	3.773 ± 1.808	0.037 ± 0.020	0.104 ± 0.024	0.166 ± 0.026	3.280 ± 0.641	3.057 ± 1.477	0.319 ± 0.054
		non-smoker	(44)	3.642 ± 1.640	0.035 ± 0.019	0.098 ± 0.024	0.167 ± 0.031	3.451 ± 0.661	3.027 ± 1.378	0.297 ± 0.072
		Total	(59)	**3.676 ± 1.669**	**0.035 ± 0.019**	**0.099 ± 0.024**	**0.167 ± 0.029**	**3.408 ± 0.655**	**3.034 ± 1.391**	**0.302 ± 0.068**
Total	Male	Smoker	(23)	3.569 ± 1.545	0.036 ± 0.017	0.104 ± 0.026	0.151 ± 0.036	3.218 ± 0.718	2.989 ± 1.225	0.295 ± 0.068
		non-smoker	(35)	3.519 ± 1.165	0.036 ± 0.010	0.094 ± 0.021	0.134 ± 0.030	2.983 ± 0.774	3.095 ± 0.923	0.259 ± 0.052
		Total	(58)	**3.539 ± 1.316**	**0.04 ± 0.013**	**0.098 ± 0.024**	**0.141 ± 0.033**	**3.076 ± 0.755**	**3.053 ± 1.044**	**0.273 ± 0.061**
	Female	Smoker	(1)	3.700 ± 0.001	0.034 ± 0.001	0.154 ± 0.001	0.079 ± 0.001	3.740 ± 0.001	3.230 ± 0.001	0.254 ± 0.001
		non-smoker	(50)	3.451 ± 1.599	0.034 ± 0.017	0.105 ± 0.024	0.160 ± 0.039	3.531 ± 0.758	2.939 ± 1.296	0.293 ± 0.072
		Total	(51)	**3.455 ± 1.584**	**0.034 ± 0.017**	**0.106± 0.024**	**0.159 ± 0.040**	**3.536 ± 0.751**	**2.945 ± 1.283**	**0.293 ± 0.072**
	Total	Smoker	(24)	3.575 ± 1.511	0.036 ± 0.016	0.107 ± 0.027	0.148 ± 0.038	3.239 ± 0.711	2.999 ± 1.199	0.293 ± 0.067
		non-smoker	(85)	3.479 ± 1.429	0.035 ± 0.015	0.101 ± 0.023	0.149 ± 0.038	3.305 ± 0.807	3.003 ± 1.153	0.279± 0.067
		Total	(109)	**3.499 ± 1.442**	**0.035 ± 0.015**	**0.102 ± 0.024**	**0.149 ± 0.038**	**3.291 ± 0.784**	**3.003 ± 1.158**	**0.282± 0.067**

n is the number of samples.

3.4.2 Influence of Hemodialysis - Link to Human Health

The elemental levels depend on the health status of individuals which is under normal conditions (healthy individuals) [124]. This is due to the metabolism of several trace elements which is changed in different diseases and may play significant roles in the pathogenesis and progress of these diseases [126,132-136]. The hemodialysis patients have a mean age of 51.08 ± 13.85 years (range 21 -75 years) as reported in the questionnaires of participants. The values of mean and standard deviation for elements in serum of the healthy and hemodialysis populations were compared by using an F-test and a two-tailed t-test, and the results are listed in Table 3.4. If the value of "Sig" for each factor is less than the level of significance (P = 0.05), then there is a significant effect for this factor. In general, significantly higher serum levels of Mn and Zn are found in healthy individuals (0.166 ± 0.296 µg/mL), and (0.302 ± 0.068) when compared with hemodialysis patients (0.128 ± 0.0.036 µg/mL) at (P = 0.05). Although the levels of Pb, and Se are slightly higher in healthy than patients, the differences are not statistically significant at P = 0.05. Cd are found in approximately similar levels in both population groups.

It was found that there is a good agreement between the results of this study and those reported by another study in the literature for Zn [136-139] and Cu [136] in blood serum in terms of the comparison between healthy and hemodialysis populations. Similar observations have been reported in the literature when the levels of Se in serum were compared for healthy individuals and hemodialysis patients [136,138]. These results are also in agreement with those published in the literature for Mn and pb in blood serum [140]. The high level of lead in serum may be related to an increase in the duration of maintenance haemodialysis for patients. Despite the concentration of Pb at a low level, chronic kidney disease will appear by causing hypertension, interstitial nephritis, an increased incidence of hypertension,

hyperuricaemia, cardiovascular disease, and cerebrovascular disease [135,141]. In spite of, the levels of Al being higher in healthy individuals (3.676 ± 1.669 μg/mL) when compared with those for patients (3.293 ± 1.095 μg/mL), there are no significant differences between the two groups at $P = 0.05$. This result agrees with other results reported in the literature [142]. Trace elements status in chronic kidney disease patients is influenced by a renal function residual, size, and dialyzer membrane surface. The water nature also is used for dialysis fluid preparation and composition. Trace elements in hemodialysis patients differed from healthy individuals. So, this issue requires accurate studies on trace elements and clinical aspects in hemodialysis patients [142]. In addition, the elemental levels may also be associated with several factors, such as environmental and/or lifestyle, smoking activity, age, gender, and medication dose [143,144]. This study confirms that patients on hemodialysis tend to present significant trace element imbalances. The concentration of trace elements in serum shows that chronic hemodialysis may affect intra- and extracellular blood compartments differently [145]. The separate boxplots for healthy and hemodialysis are shown in Figures 3.3 and 3.4. These Figures are used to identify any outlier from the histogram. The important thing to note is that the outlier detected in the histogram is shown up or down as an asterisk (*) on the boxplot [146]. The results indicate that there are no outliers that can be detected for healthy individuals and hemodialysis patients on boxplots. In general, boxplots provide useful details about the minimum value (the bottom horizontal line on each plot) and the maximum value (the top horizontal line of each plot). The lowest edge of the coloured box is the lower quartile, therefore, the distance between the lowest horizontal line and the lowest edge of the coloured box has involved 25% of values. The top edge of the coloured box is the upper quartile, therefore, the distance between the top edge of the shaded box and the top horizontal line includes 25% of values.

Table 3.4: Serum elemental levels found in the groups of healthy individuals and hemodialysis (n = 109).

Element (n_1, n_2)	Concentration (µg/mL) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Healthy	Hemodialysis	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	3.676 ± 1.669	3.293 ± 1.095	Equal variances assumed	10.749	0.001	1.388	107	0.168
(59,50)	(0.999-7.150)	(1.310-5.720)	Equal variances not assumed			1.435	101	0.154
Cd	0.035 ± 0.018	0.035 ± 0.008	Equal variances assumed	30.352	< 0.001	0.010	107	0.992
(59,50)	(0.006-0.068)	(0.020-0.054)	Equal variances not assumed			0.011	83	0.992
Cu	0.099 ± 0.024	0.104 ± 0.023	Equal variances assumed	0.184	0.669	0.928	107	0.355
(59,50)	(0.062-0.154)	(0.052-0.154)	Equal variances not assumed				103	0.357
Mn	0.166 ± 0.296	0.128 ± 0.036	Equal variances assumed	7.848	0.006	6.091	107	**< 0.001**
(59,50)	(0.097-0.231)	(0.064-0.185)	Equal variances not assumed			5.997	95	**< 0.001**
Pb	3.408 ± 0.655	3.153 ± 0.901	Equal variances assumed	15.239	< 0.001	1.704	107	0.091
(59,50)	(1.880-4.932)	(1.660-4.770)	Equal variances not assumed			1.661	88	0.100
Se	3.034 ± 1.391	2.965 ± 0.814	Equal variances assumed	16.585	< 0.001	0.310	107	0.757
(59,50)	(0.773-5.320)	(1.480-5.630)	Equal variances not assumed			0.323	96	0.757
Zn	0.302 ± 0.068	0.259 ± 0.057	Equal variances assumed	2.880	0.093	3.577	107	**< 0.001**
(59,50)	(0.180-0.455)	(0.143-0.423)	Equal variances not assumed			3.629	107	**< 0.001**

Sd is the standard deviation, n_1, n_2 are the number of samples for healthy and hemodialysis, respectively, df = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance P = 0.05, Sig. = level of significance. $t_{crit.}$ = 1.96 at P=0.05.

The box shows the interquartile range that is 50% of the values are bigger than the lowest part of the coloured area but smaller than the top part of the coloured area. These boxes are in different sizes for healthy, and patients. In the middle of the coloured box is a slightly thicker horizontal line. This represents the value of the median. The median values for healthy are higher than for patients in terms of Cd, Mn, Zn, Al, and Se, respectively, while the values of the median of both Cu and Pb are similar, as shown in Figures 3.3 and 3.4. Interestingly, like histograms, boxplots tell us whether the distribution is symmetrical or asymmetrical. If the whiskers (*the whiskers are the two lines outside the box, that go from the minimum to the lower quartile (the start of the box) and then from the upper quartile (the end of the box) to the maximum*) [146] are the same length then the distribution is symmetrical (the range of the top and bottom 25% of values is the same), however, if the top or bottom whisker is much longer than the opposite whisker then the distribution is asymmetrical (the range of the top and bottom 25% of values is different).

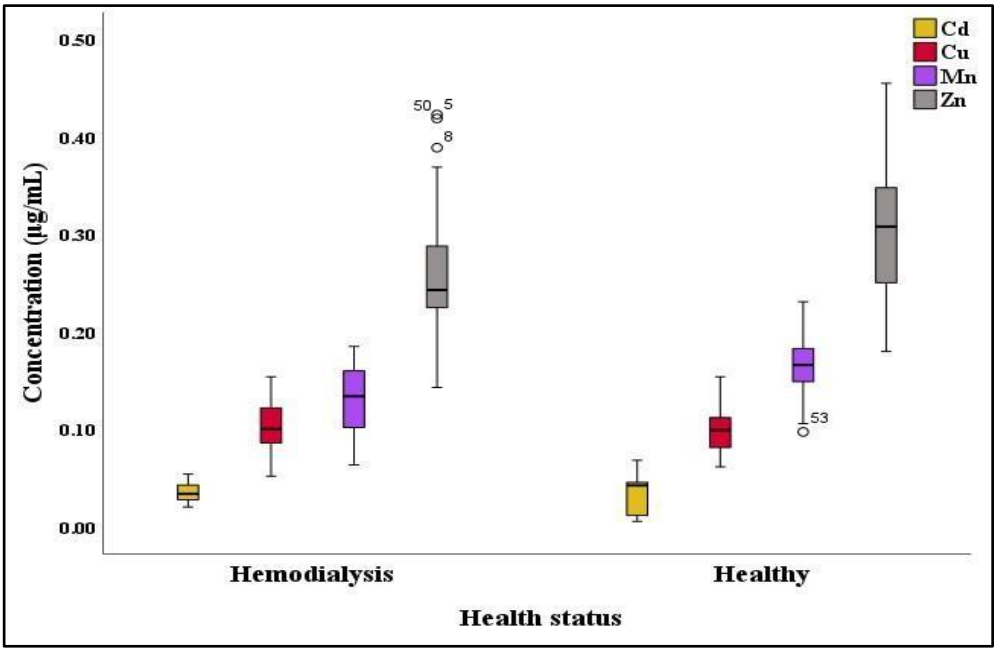


Figure 3.3: Trace element levels (Cd, Cu, Mn, and Zn µg/mL) in serum for different population groups (healthy and patients); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

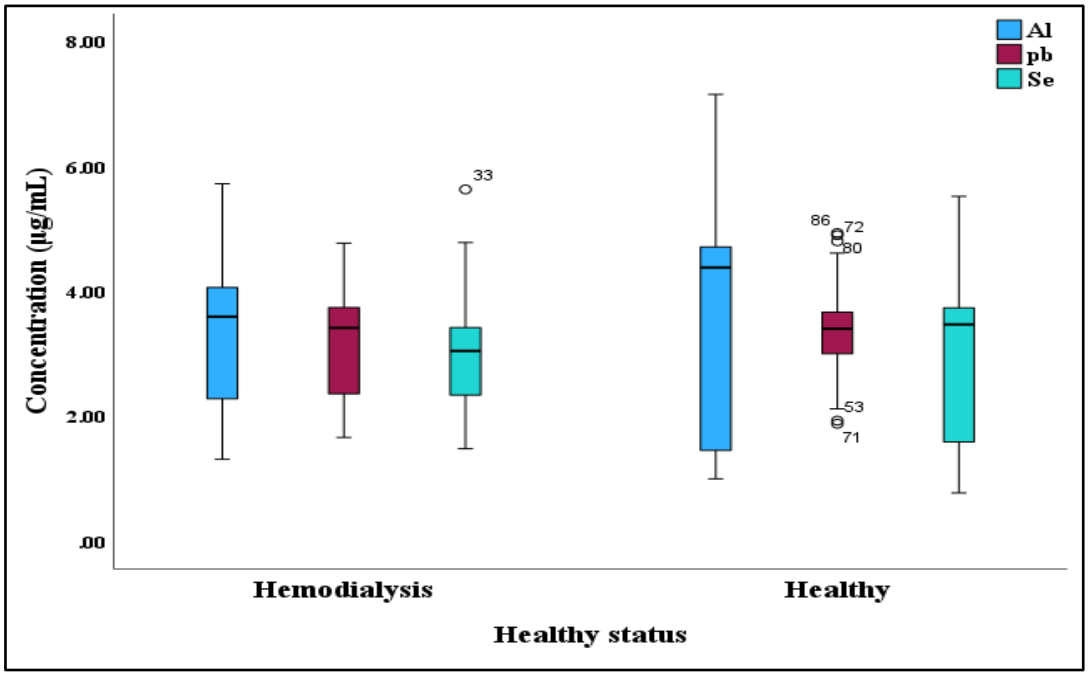


Figure 3.4: Trace element levels (Al, Pb, and Se µg/ml) in serum for different population groups (healthy and patients); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

3.4.3 Influence of Gender

The effect of gender on the levels of trace elements in human fluids and tissue samples was investigated by other researchers [128,147,148]. It was found that the gender effect can play a significant role in changing the levels of trace elements in the human body. The total population (n = 109) was divided into two gender groups, males and females. The mean and standard deviation (±Sd) for each gender group is summarised in Table 3.5. The highest mean values in the two gender groups are found for Al (males: 3.538 ± 1.316; and females: 3.455 ± 1.584 µg/mL Al) followed by lead for males (3.076 ± 0.755 µg/mL Pb) and females (3.535 ± 0.751 µg/mL pb). Cd showed the lowest concentration for both gender groups (males: 0.036 ± 0.013 µg/mL Cd) and (females: 0.034 ± 0.017 µg/mL Cd). The order of increasing trace element levels in the serum for males and females is Cd < Cu < Mn < Zn < Se < Pb < Al.

The significant effect of gender on the elemental levels in serum was investigated using a two-tailed t-test, and the results are listed in Table 3.5. The findings show that there is a significant effect of gender on the levels of Mn ($t_{(107)} = 2.563$, $t_{cit.}$=1.96 at $P = 0.05$) and Pb ($t_{(107)} = 3.179$, $t_{cit.}$=1.96 at $P = 0.05$). The higher level of Mn was also reported in the literature for the whole blood of females 10.0 (5.7–17.6) when compared with males 9.6 (4.5–19.5) [149-151]. The results of lead in serum disagree with those reported by other researchers for males and females in saliva [49-151]. No significant effect at ($P = 0.05$) was found for either gender for other trace elements such as Al, Cd, Cu, Se, and Zn. Similar results were also found by other researchers in terms of Zn, Cu, and Cd by using different human fluids and tissues such as serum, saliva, teardrops, scalp hair, and fingernails [126,143,152-154]. Selenium has similar findings in the literature in terms of the effect of gender on its level in human fluids and tissue [152].

The separate boxplots for males and females are shown in Figures 3.5 and 3.6. The results show that the boxplots of all elements are in different sizes for males and females. The median values for females are higher than males in terms of Cu, Mn, Zn, and Pb, while the values of the median of Cd, Al, and Se are similar for both groups. The results from Figures 3.5 and 3.6 show that the distribution of Cd and Se for males; Cd and pb for females are symmetrical, whereas the distributions for other elements in both groups are asymmetrical. In addition, some circles were identified above the male boxplots (Zn, 1; Se, 1). On the other hand, Pb has one circle above the female boxplot, while Mn and Pb have four and five circles below the female boxplots, respectively. These cases can consider to be also outliers.

Table 3.5: Serum elemental levels found in the groups of males and females, (n = 109).

Element (n_1, n_2)	Concentration (µg/mL) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Male	Female	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	3.538 ± 1.316	3.455 ± 1.584	Equal variances assumed	5.239	0.024	0.300	107	0.765
(58,51)	(0.999-7.150)	(1.110-6.070)	Equal variances not assumed			0.297	98	0.767
Cd	0.036 ± 0.013	0.034 ± 0.017	Equal variances assumed	7.224	0.008	0.610	107	0.543
(58,51)	(0.006-0.065)	(0.007-0.068)	Equal variances not assumed			0.599	92	0.551
Cu	0.098 ± 0.024	0.106 ± 0.024	Equal variances assumed	0.314	0.576	1.746	107	0.084
(58,51)	(0.052-0.154)	(0.066-0.154)	Equal variances not assumed			1.742	104	0.084
Mn	0.140 ± 0.033	0.158 ± 0.040	Equal variances assumed	0.476	0.492	2.563	107	**0.012**
(58,51)	(0.064-0.231)	(0.068-0.230)	Equal variances not assumed			2.533	97	**0.013**
Pb	3.076 ± 0.755	3.535 ± 0.751	Equal variances assumed	1.268	0.263	3.179	107	**0.002**
(58,51)	(1.88-4.932)	(1.660-4.898)	Equal variances not assumed			3.180	105	**0.002**
Se	3.053 ± 1.044	2.944 ± 1.283	Equal variances assumed	4.296	0.041	0.487	107	0.627
(58,51)	(0.773-5.520)	(0.786-5.630)	Equal variances not assumed			0.481	97	0.632
Zn	0.273 ± 0.061	0.292 ± 0.072	Equal variances assumed	3.565	0.062	1.533	107	0.128
(58,51)	(0.143-0.455)	(0.183-0.439)	Equal variances not assumed			1.517	99	0.132

Sd is the standard deviation, n_1, n_2 are the number of samples for males and females, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance. $t_{crit.} = 1.96$ at P=0.05.

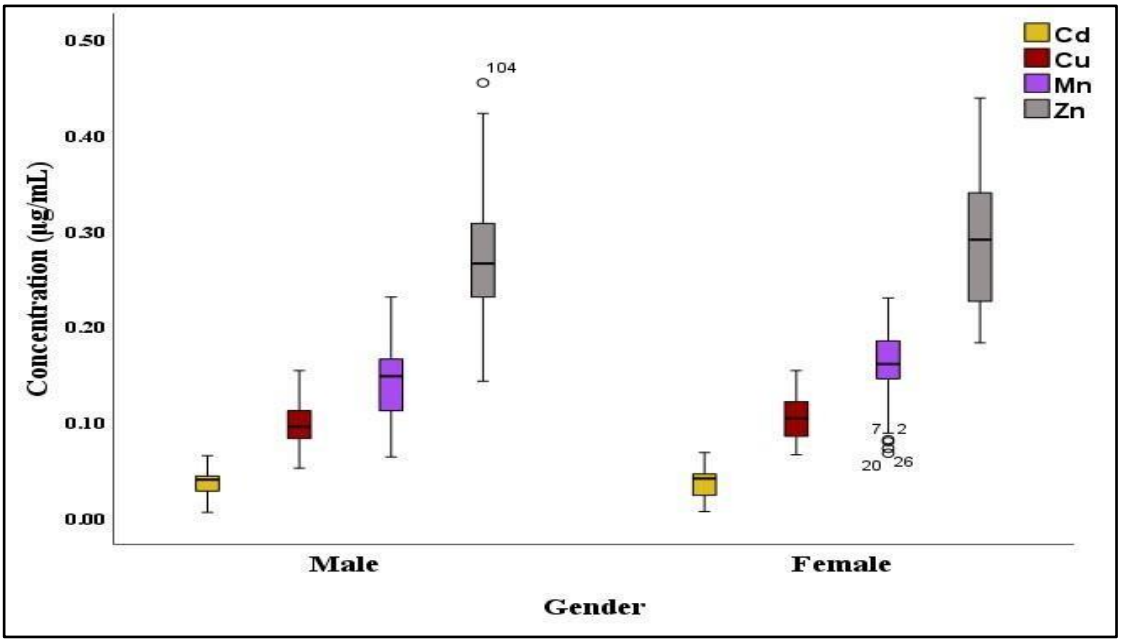


Figure 3.5: Trace element levels (Cd, Cu, Mn, and Zn µg/mL) in serum for different population groups (male and female); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

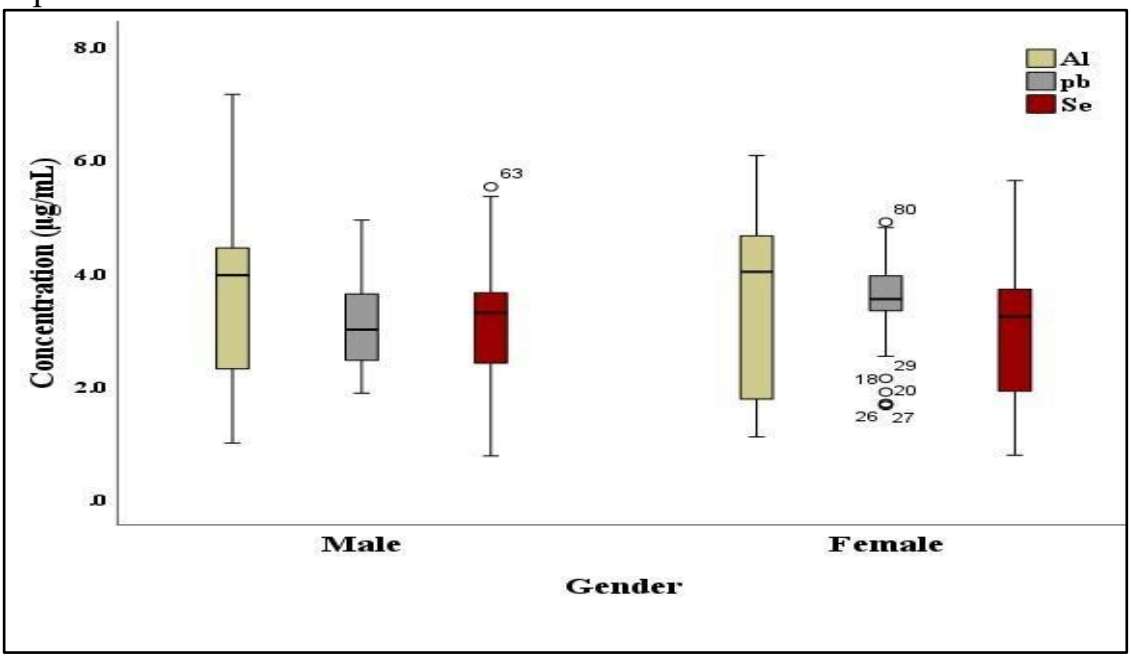


Figure 3.6: Trace element levels (Al, pb, and Se µg/ml) in serum for different population groups (male and female); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

3.4.4 Influence of Smoking Activity

The effect of smoking activity on trace element levels has been studied by several other researchers [128,129,143,151,154,155]. The population in this study was divided into smokers and non-smokers. The influence of smoking activity on the levels of trace elements in serum was investigated by using a two-tailed t-test, and the results are summarised in Table 3.6. Although the levels of Al, Cu, and Zn are slightly higher in smokers than non-smokers, the differences are not statistically significant ($P = 0.05$). Mn and Cd are found in approximately similar levels in both population groups. In addition, the levels of Pb and Se were slightly higher in nonsmokers than smokers, but there are no significant differences between the groups. Previous studies have reported high levels of Cd in the human scalp hair, nails, and blood of smokers compared with non-smokers [95,154-157]. Cadmium and lead are known to be toxic elements, and both have disorders for the human body. The main concern of cadmium is classified as a carcinogen by the International Agency for Research on Cancer (IARC). This metal plays a significant role in disrupting the metabolism of several micronutrients such as copper, iron, magnesium, and zinc [158-160]. Human exposure to lead through the environment and diet has become dramatic. It is counted to be one of the hazardous environmental exposures. The potential sources of lead include paints, occupational exposures, air, water, soil, dust, toys, dried herbs/herbal medicine, makeup products, old metal pipes, adulterated opium, and smoking [161,162]. The separate boxplots for smokers and non-smokers are shown in Figures 3.7 and 3.8. The results show that the boxplots of all elements are in different sizes for smokers and non-smokers. The only exception is for Cd. The median values for smokers are higher than non-smokers in terms of Cu, Mn, Zn, and Se. In contrast, the median value of pb was found to be higher in non-smokers than in smokers. The median values of Cd and Al are similar for both groups.

Table 3.6: Serum elemental levels found in the groups of smokers and non-smokers, (n = 109).

Element (n_1, n_2)	Concentration (µg/mL) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Smoker	Non-smoker	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	3.575 ± 1.511	3.479 ± 1.429	Equal variances assumed	0.095	0.759	0.288	107	0.774
(24,85)	(1.020-7.150)	(0.999-6.070)	Equal variances not assumed			0.279	36	0.782
Cd	0.036 ± 0.016	0.035 ± 0.015	Equal variances assumed	0.223	0.637	0.200	107	0.842
(24,85)	(0.006-0.065)	(0.006-0.068)	Equal variances not assumed			0.187	34	0.853
Cu	0.107 ± 0.027	0.101 ± 0.023	Equal variances assumed	0.226	0.635	1.067	107	0.288
(24,85)	(0.052-0.154)	(0.062-0.154)	Equal variances not assumed			0.977	33	0.336
Mn	0.147 ± 0.038	0.149 ± 0.038	Equal variances assumed	0.045	0.833	0.156	107	0.877
(24,85)	(0.068-0.231)	(0.064-0.230)	Equal variances not assumed			0.156	37	0.877
Pb	3.239 ± 0.711	3.305 ± 0.807	Equal variances assumed	0.657	0.419	0.361	107	0.718
(24,85)	(2.120-4.932)	(1.660-4.898)	Equal variances not assumed			0.388	41	0.700
Se	2.999 ± 1.199	3.003 ± 1.153	Equal variances assumed	0.007	0.933	0.013	107	0.989
(24,85)	(0.823-5.348)	(0.773-5.630)	Equal variances not assumed			0.013	36	0.990
Zn	0.293 ± 0.066	0.279 ± 0.067	Equal variances assumed	0.261	0.610	0.928	107	0.355
(24,85)	(0.143-0.455)	(0.180-0.439)	Equal variances not assumed			0.930	37	0.358

Sd is the standard deviation, n_1, n_2 are the number of samples for smokers and non-smokers, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance. $t_{crit.} = 1.96$ at P=0.05.

The results from Figures 3.7 and 3.8 show that the distribution of Cd, Se, and approximately Cu for smokers; Cd and Mn for non-smokers are symmetrical, whereas the distribution for other elements in both groups is asymmetrical. In addition, some circles were identified above (Zn, 1) for the smoker boxplot, and Cd has one circle below the smoker boxplot. The remaining elements did not have any outliers in the cases of smokers' and non-smokers' boxplots. These cases can consider to be also outliers.

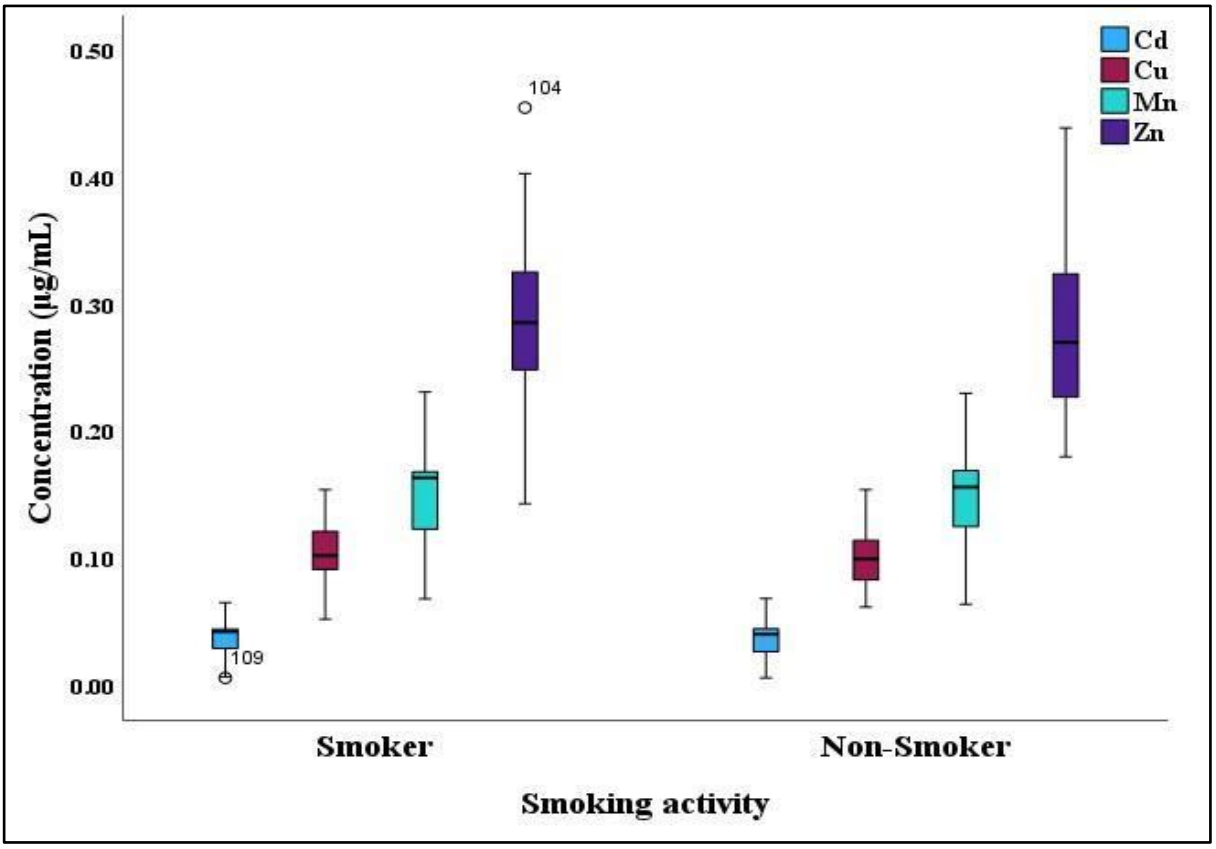


Figure 3.7: Trace element levels (Cd, Cu, Mn, and Zn µg/mL) in serum for different population groups (smokers and non-smokers); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

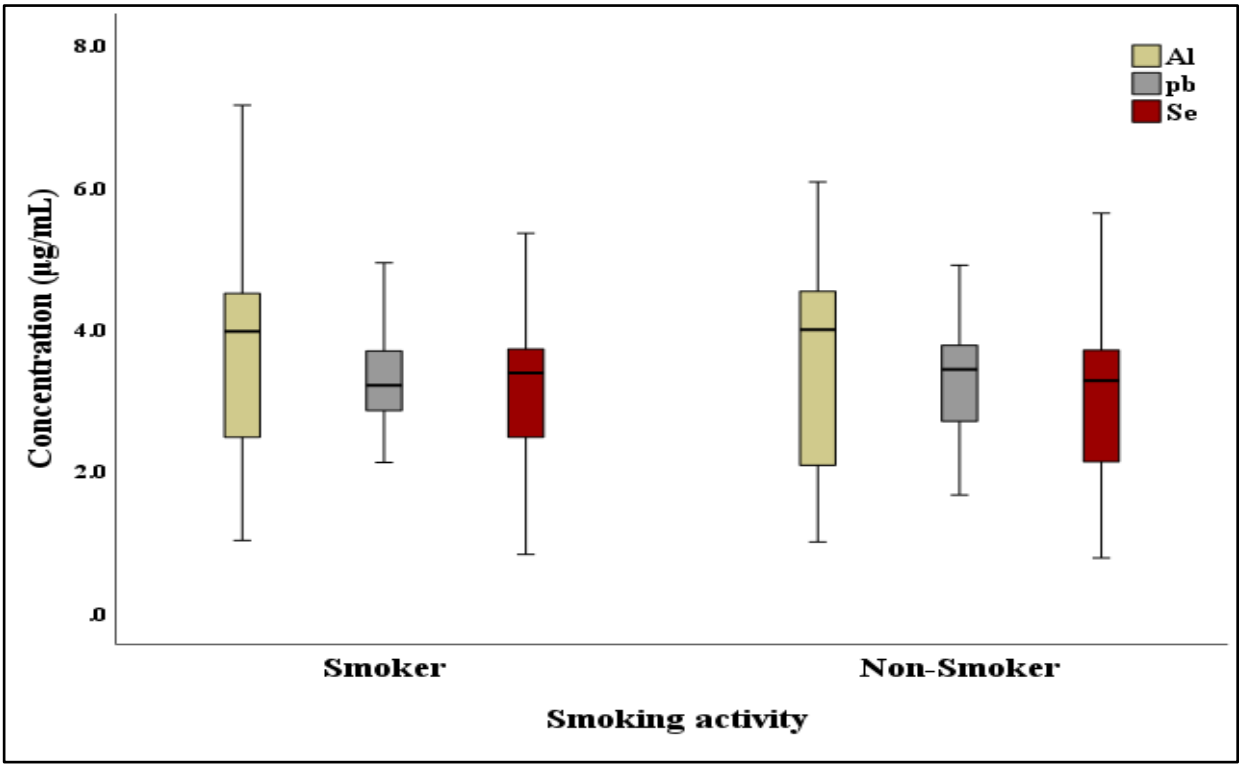


Figure 3.8: Trace element levels (Al, pb, and Se µg/ml) in serum for different population groups (smokers and non-smokers); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

3.4.5 Influence of Age

The effect of age on the levels of elements in serum samples has been investigated. The results show that the effect of an individual's age on the levels of Zn was significant, while this effect on the other elements was not significant at $P = 0.05$, as shown in Figures 3.9 and 3.10. The main reasons for this result may be due to levels of these elements depending on the environmental and diet factors.

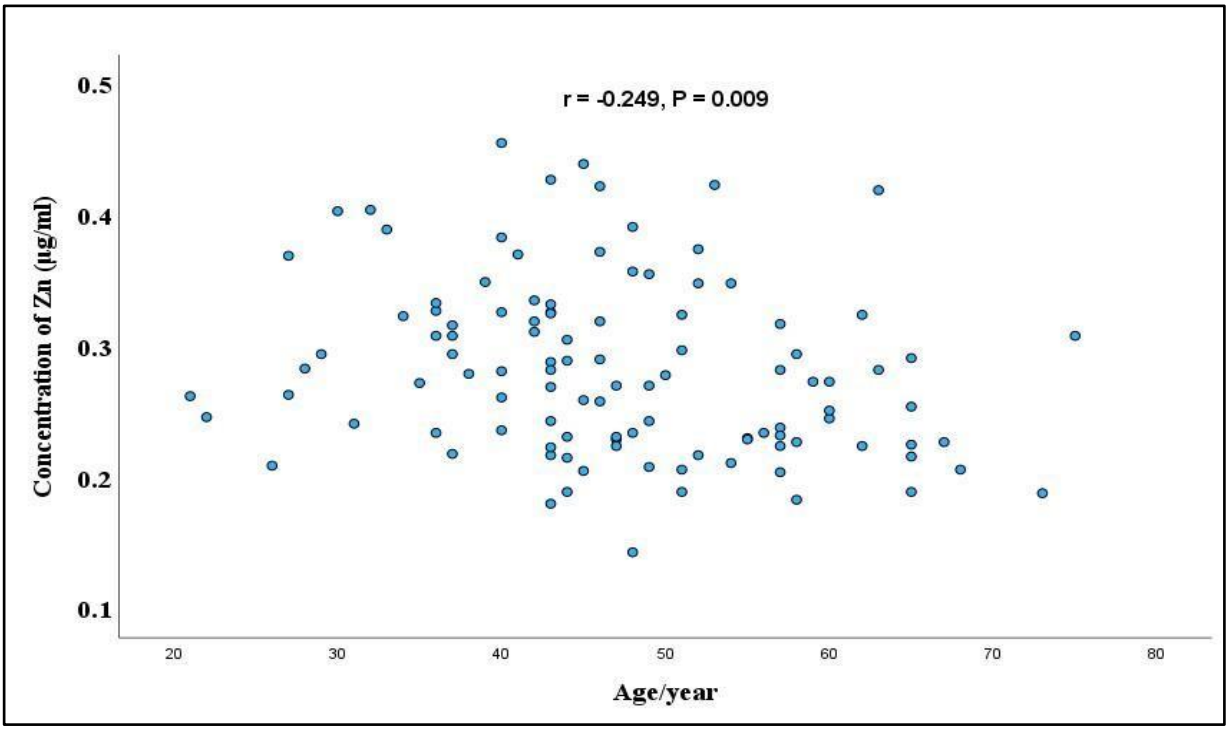


Figure 3.9: Correlation between Zinc levels (µg/mL) and age in serum for total individuals at $P = 0.05$.

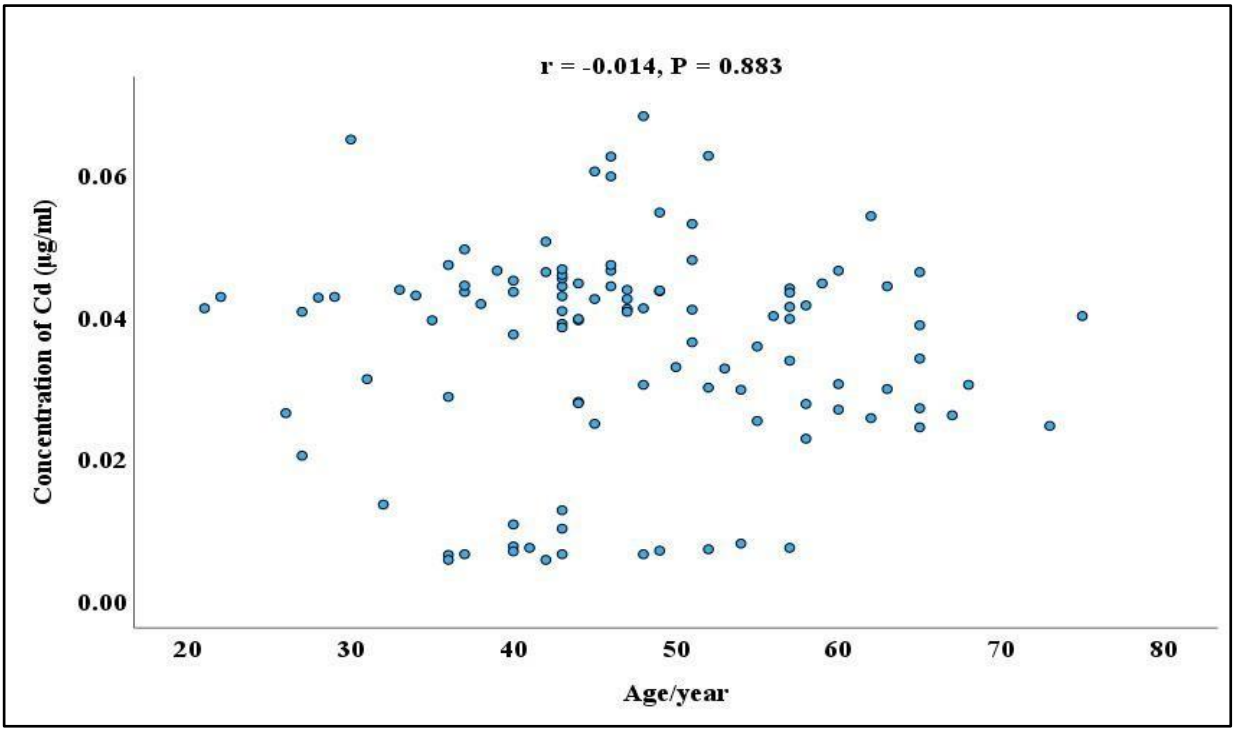


Figure 3.10: Correlation between cadmium levels (µg/mL) and age in serum for total individuals at $P = 0.05$.

3.5 Interaction Effect

The levels of elements correlate with two types of interactions, as reported in the following Sections.

3.5.1 Interaction Effects Between Factors

The interaction between different factors occurs when the effect(s) of one factor varies over the levels (groups) of another factor [146]. In general, parallel lines indicate that there is no interaction between factors, whilst non-parallel lines mostly mean that the interaction is significant [146]. The interaction results between different factors such as (health x smoking); (health x gender); and (gender x smoking) are shown in the following Sections.

3.5.1.1 Interaction Between Health Status and Gender

The interaction of health status and gender is presented in Figures 3.11-3.17. A plot was performed for each effect of a factor on the elemental levels in serum using the adjusted means. Figure 3.11 shows the interaction between health status and gender for Al. For hemodialysis patients, the Al mean value was lowest for males, while for healthy individuals, however, the lowest Al mean value occurs for females. This suggests that healthy and hemodialysis subjects appear to respond differently to gender, and that to explore the effect of gender on the levels of Al in serum, one must consider the health status of participants. Figures 3.12 and 3.13 explain the interaction between health status and gender for Cd and Se, respectively. In these Figures, males and females have a very different effect on health status. Therefore, both effects destroy each other, but cross-over interactions are found to be significant. Furthermore, there was no significant interaction between gender and health status found for other elements, as shown in Figures 3.14-3.18.

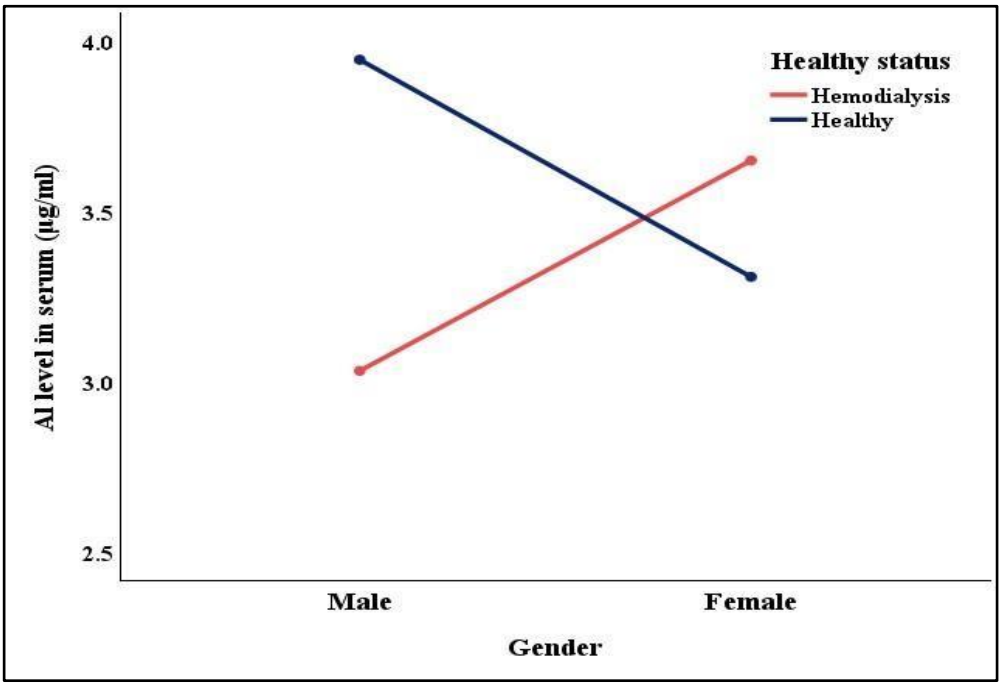


Figure 3.11: Interaction between health status and gender for Al levels (µg/mL) in serum samples.

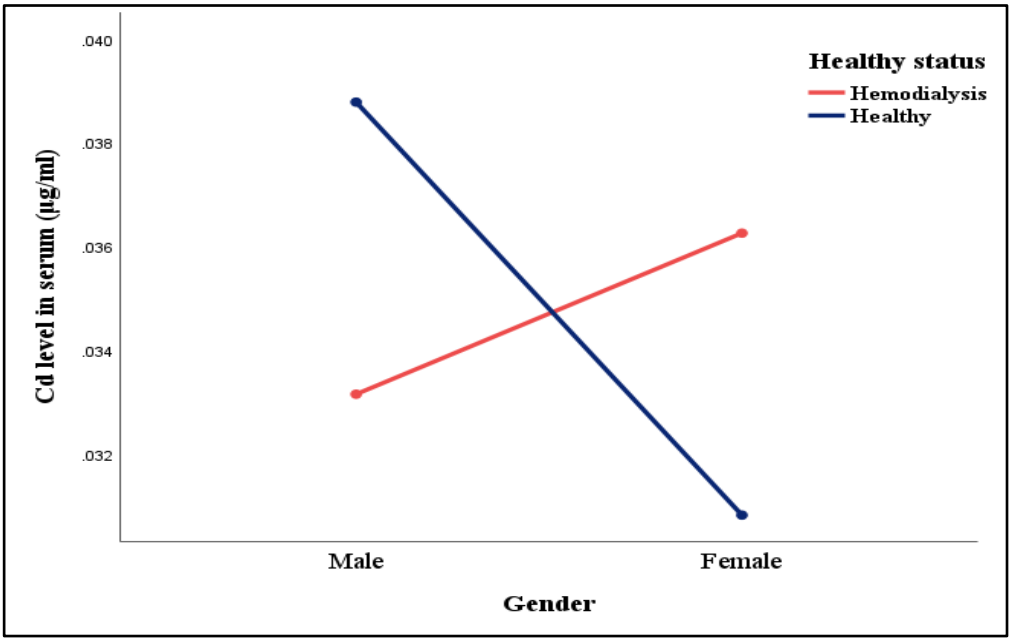


Figure 3.12: Interaction between health status and gender for Cd levels (µg/mL) in serum samples.

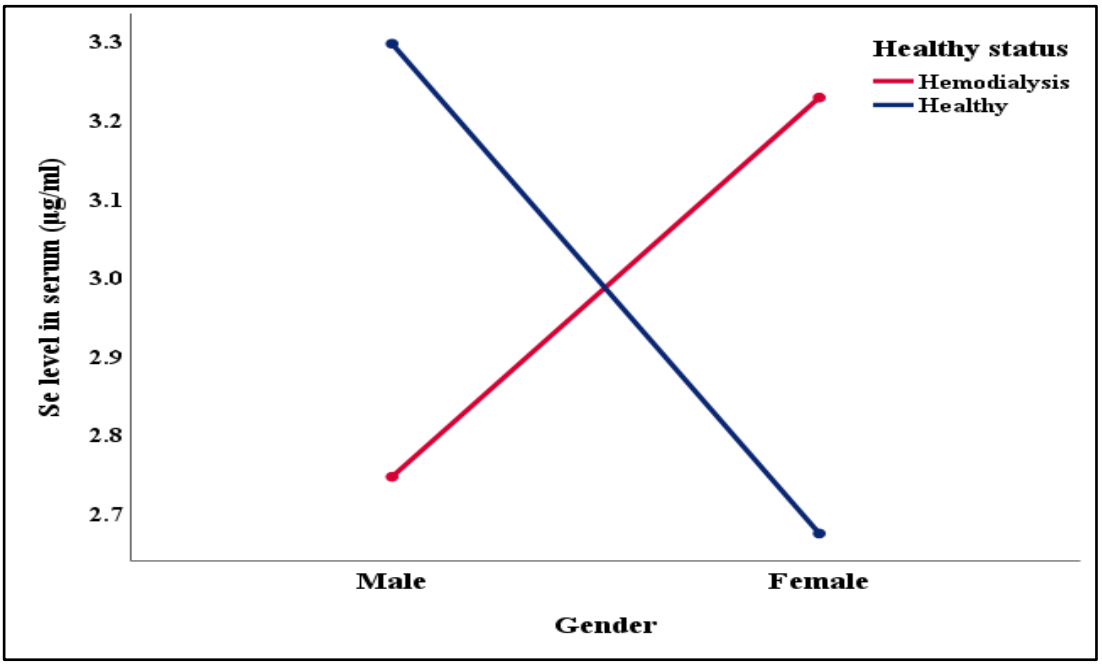


Figure 3.13: Interaction between health status and gender for Se levels (µg/mL) in serum samples.

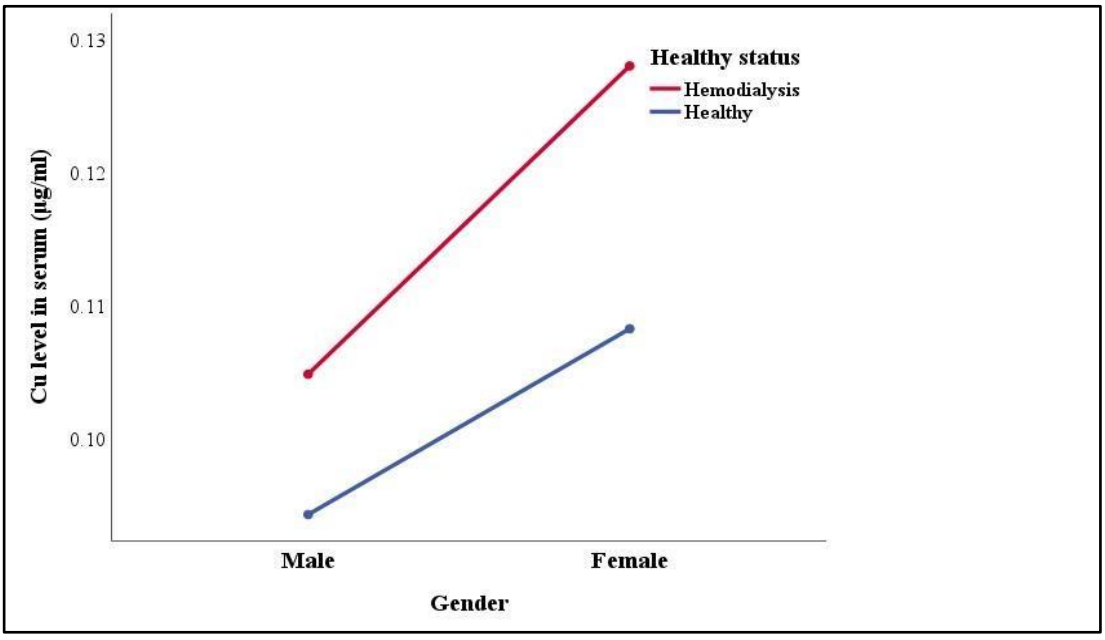


Figure 3.14: Interaction between health status and gender for Cu levels (µg/mL) in serum samples.

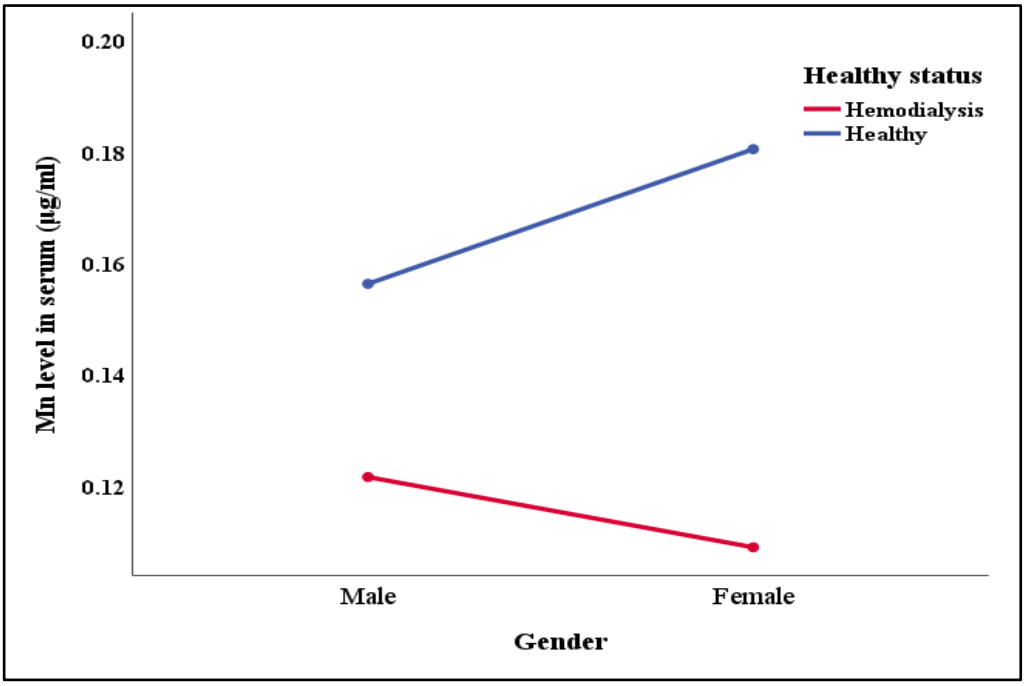


Figure 3.15: Interaction between health status and gender for Mn levels (µg/mL) in serum samples.

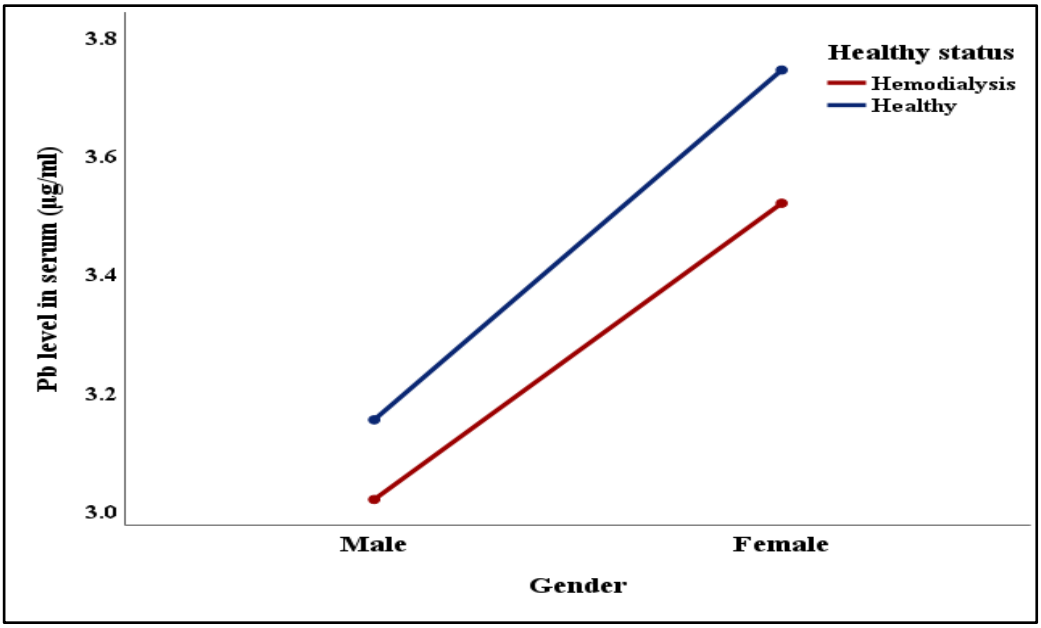


Figure 3.16: Interaction between health status and gender for Pb levels (µg/mL) in serum samples.

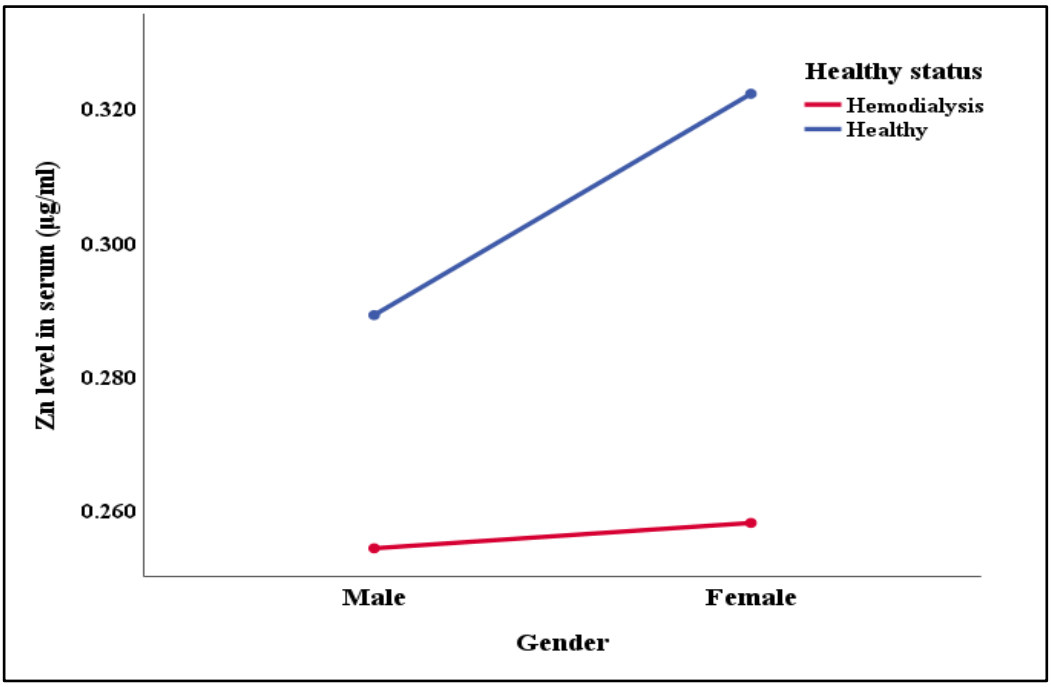


Figure 3.17: Interaction between health status and gender for Zn levels (µg/mL) in serum samples.

3.5.1.2 Interaction Between Health Status and Smoking Activity

The interaction of health status and smoking activity is presented in Figure 3.18. This Figure shows the interaction between health status and smoking activity for Pb. For hemodialysis patients, the Pb mean value was lowest for non-smokers, while for healthy individuals, however, the lowest Pb mean value occurs for smokers. This suggests that healthy and hemodialysis subjects appear to respond differently to smoking activities, and that to explore the effect of smoking activity on the levels of Pb in serum, one must consider the health status of participants. The interactions between smoking activity and health status were not significant for Al, Cu, Cd, Mn, Se, and Zn, as shown in Figures 3.19-3.24.

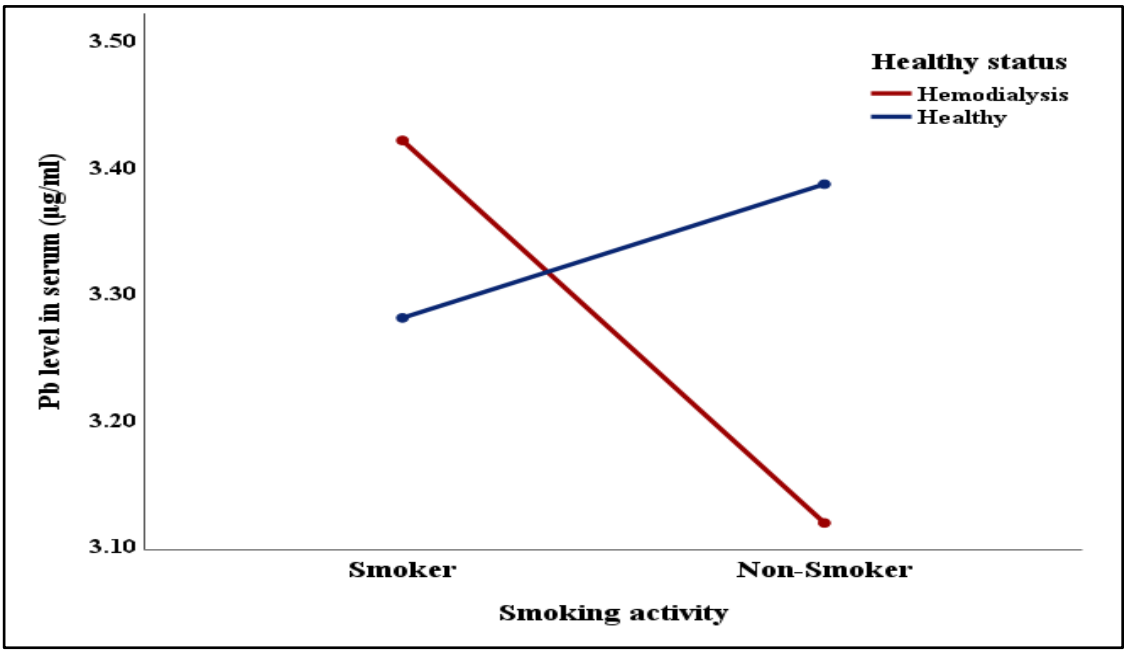


Figure 3.18: Interaction between health status and smoking activity for Pb levels (µg/mL) in serum samples.

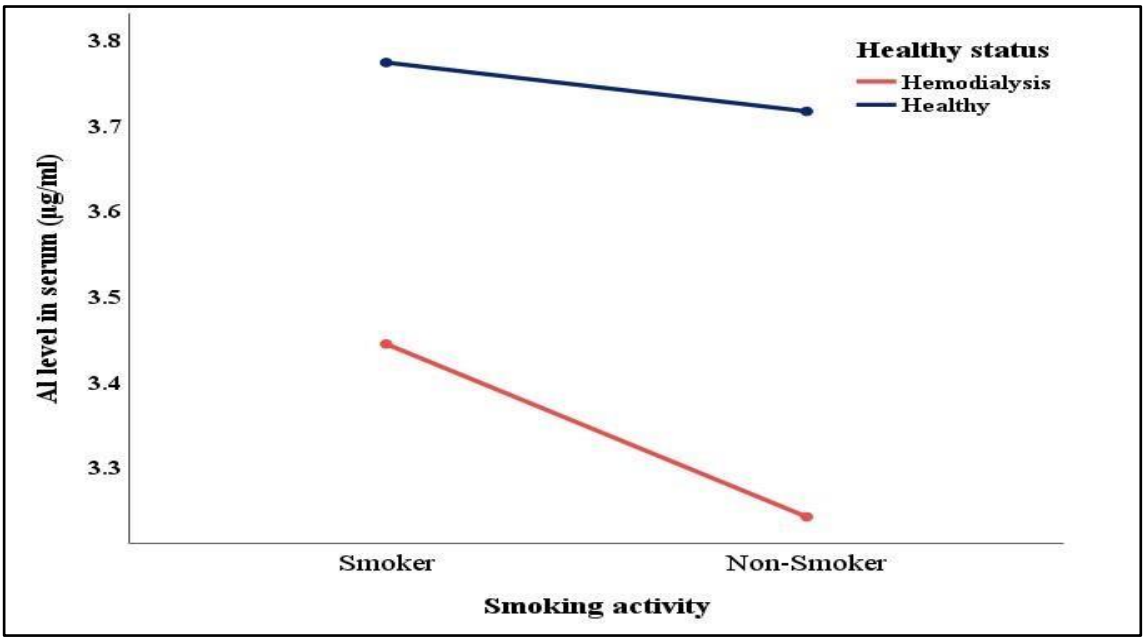


Figure 3.19: Interaction between health status and smoking activity for Al levels (µg/mL) in serum samples.

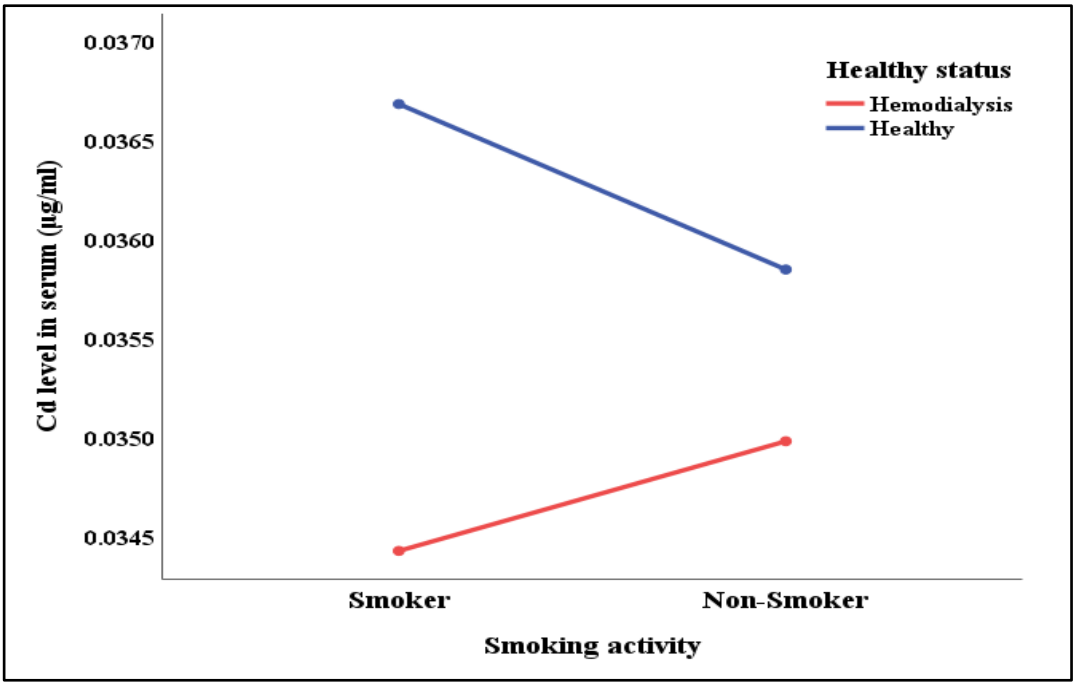


Figure 3.20: Interaction between health status and smoking activity for Cd levels (µg/mL) in serum samples.

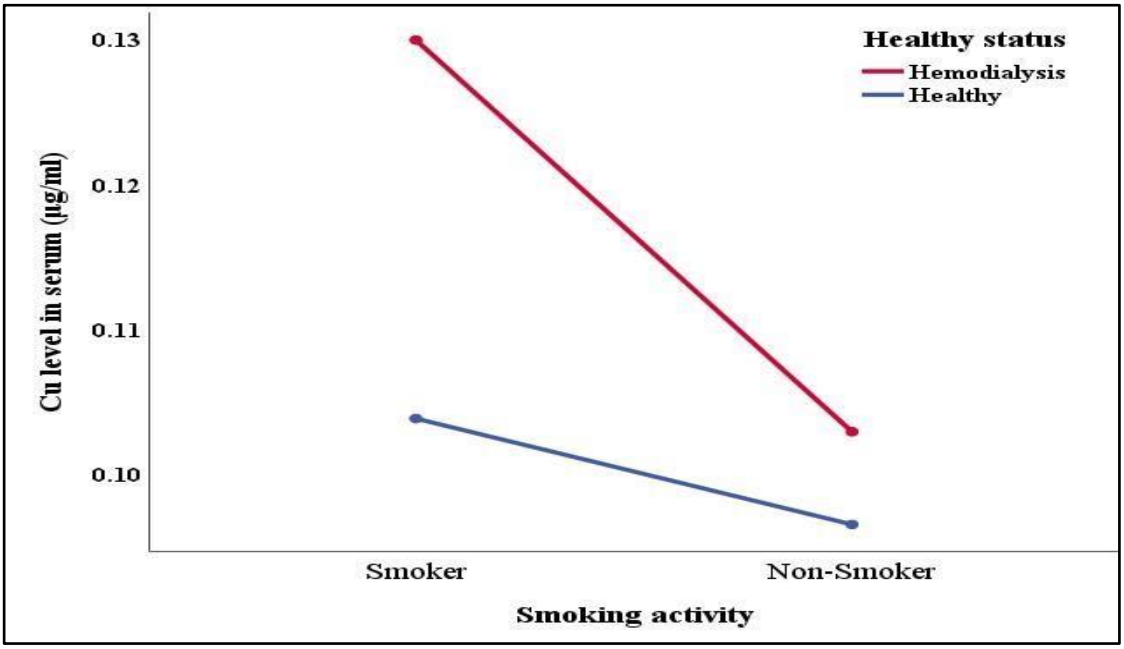


Figure 3.21: Interaction between health status and smoking activity for Cu levels (µg/mL) in serum samples.

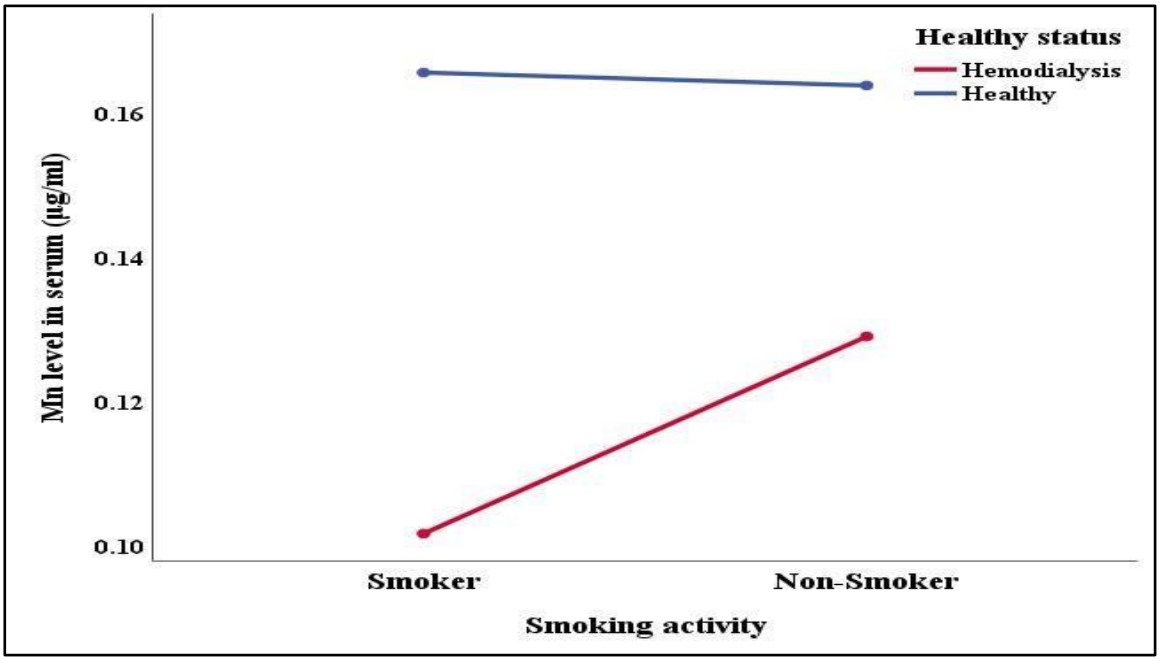


Figure 3.22: Interaction between health status and smoking activity for Mn levels (µg/mL) in serum samples.

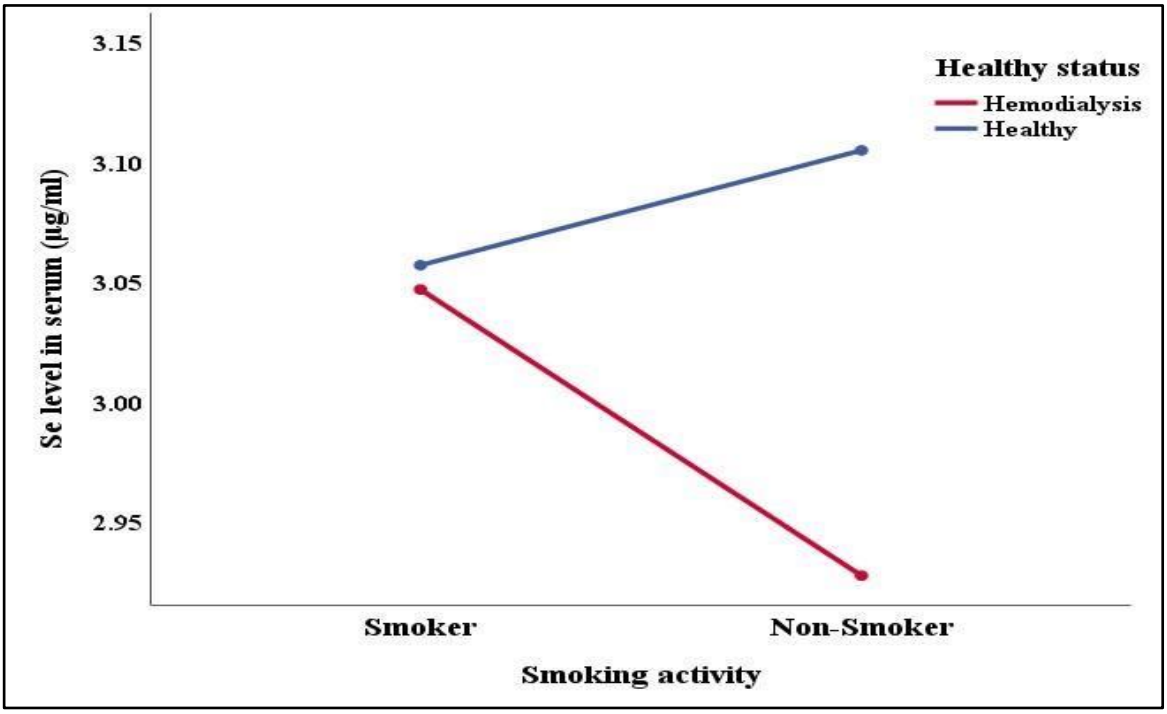


Figure 3.23: Interaction between health status and smoking activity for Se levels (µg/mL) in serum samples.

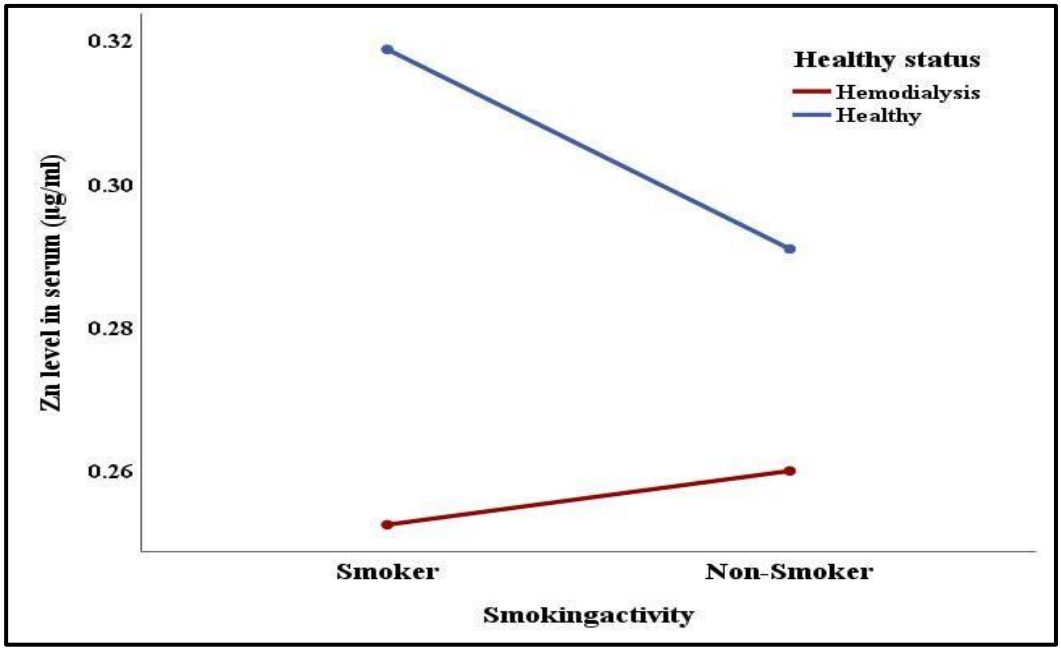


Figure 3.24: Interaction between health status and smoking activity for Zn levels (µg/mL) in serum samples.

3.5.1.3 Interaction Between Gender and Smoking Activity

The interaction of smoking activity with gender for Al, Mn, Se, and Zn is shown in Figures 3.25-3.28. In the case of smokers, the Mn and Zn mean values were highest for males, while for non-smokers, however, the highest Mn and Zn mean values occur for females, as identified in Figures 3.25 and 3.26. This suggests that smokers and non-smokers subjects appear to respond differently to gender, and that to explore the effect of gender on the levels of Mn and Zn in serum, one must consider the smoking activities of participants. The picture for Al and Se is quite different. For smokers, the Al and Se mean values were lowest for males, while for non-smokers, however, the lowest Al and Se mean values occur for females, as presented in Figures 3.27 and 3.28. Furthermore, there was no significant interaction between gender and smoking activity found for other elements, as shown in Figures 3.29-3.31.

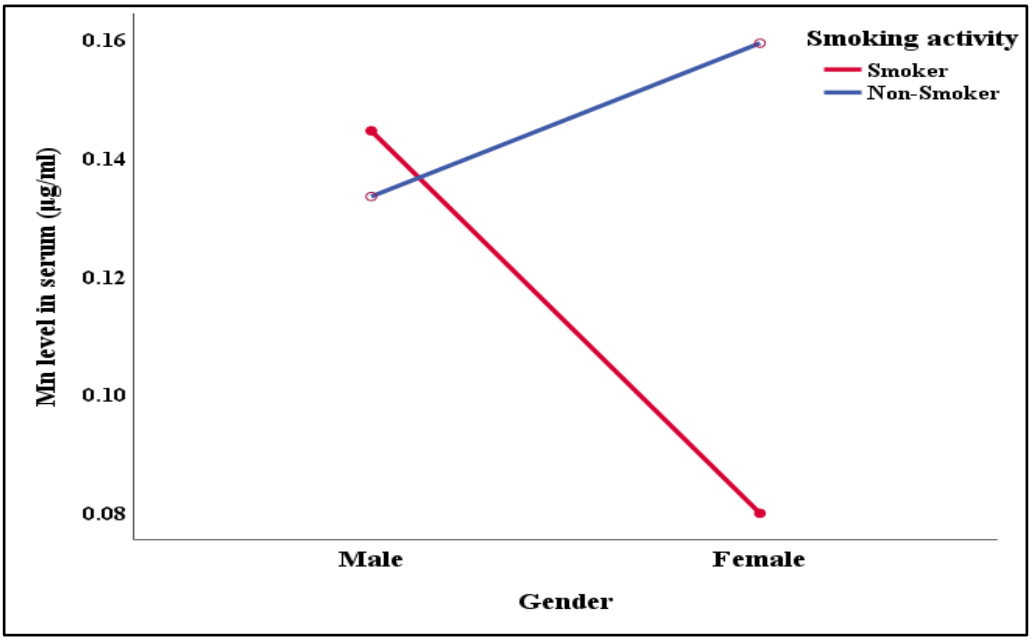


Figure 3.25: Interaction between gender and smoking activity for Mn levels (µg/mL) in serum samples.

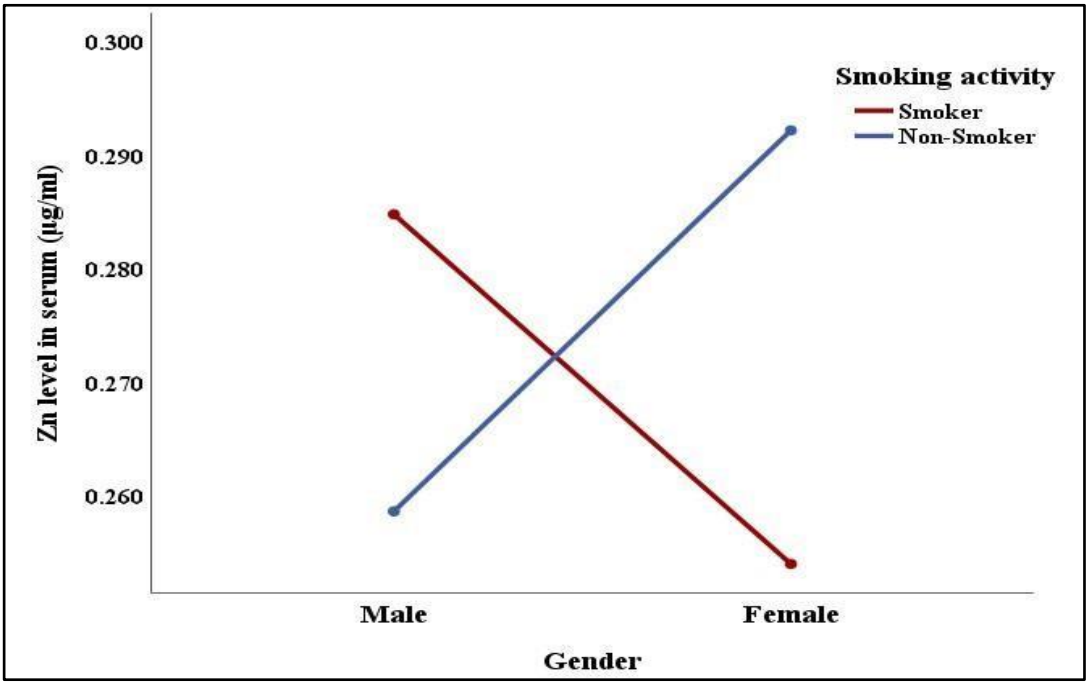


Figure 3.26: Interaction between gender and smoking activity for Zn levels (µg/mL) in serum samples.

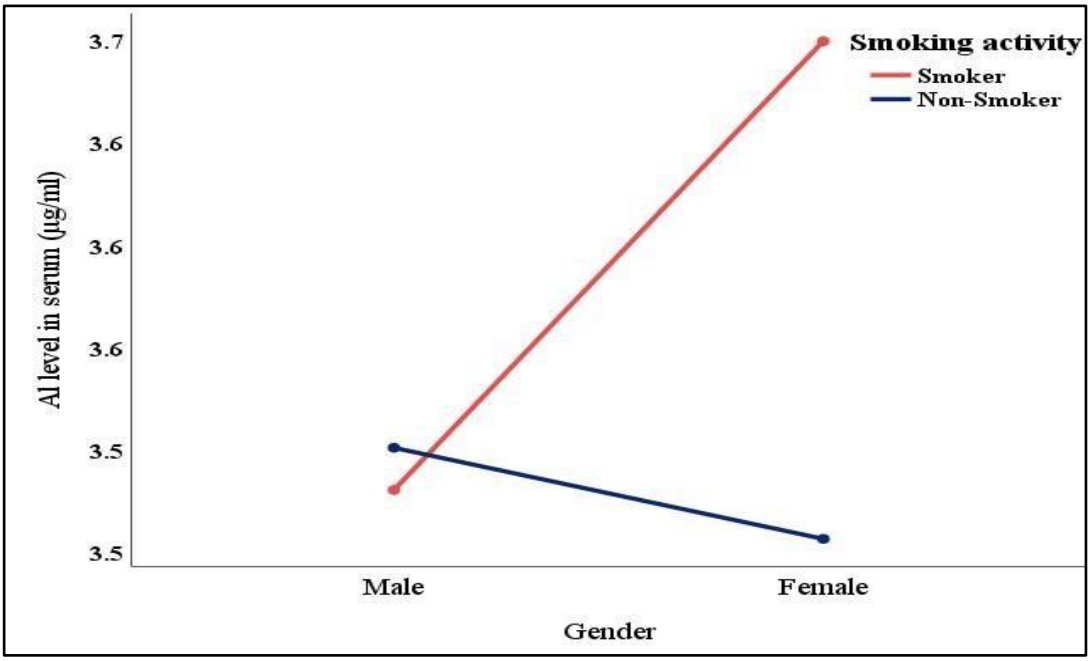


Figure 3.27: Interaction between gender and smoking activity for Al levels (µg/mL) in serum samples.

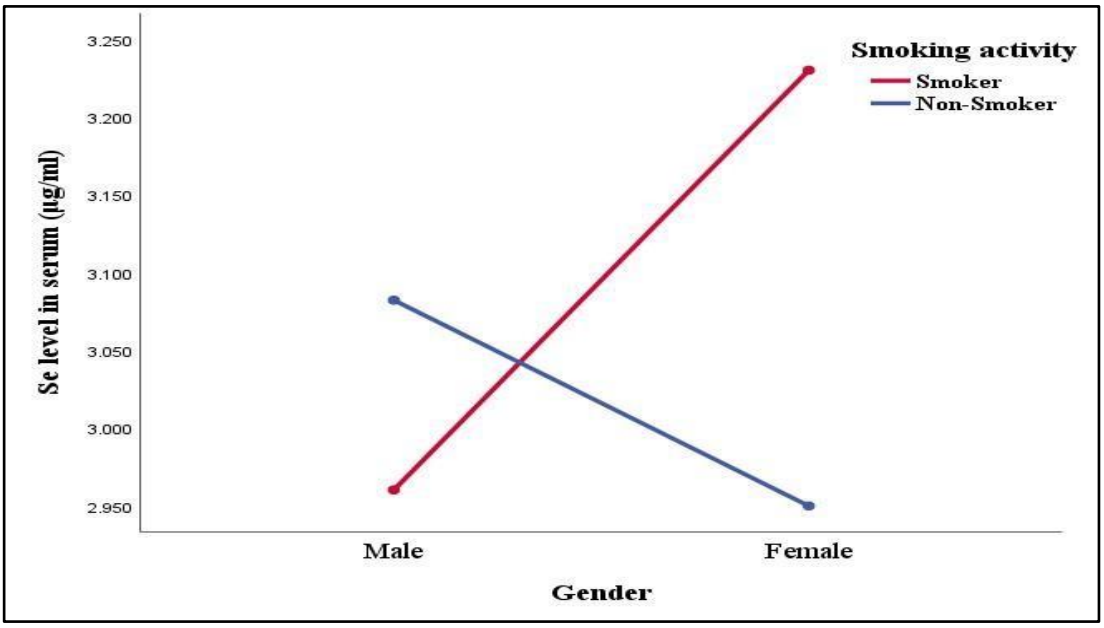


Figure 3.28: Interaction between gender and smoking activity for Se levels (µg/mL) in serum samples.

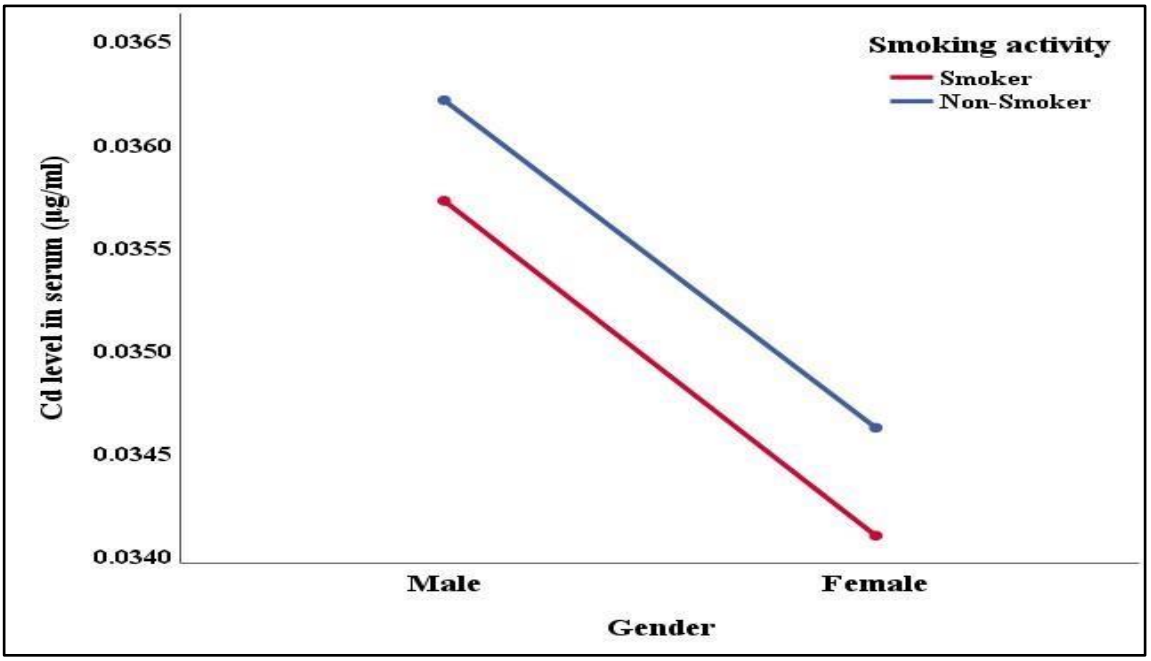


Figure 3.29: Interaction between gender and smoking activity for Cd levels (µg/mL) in serum samples.

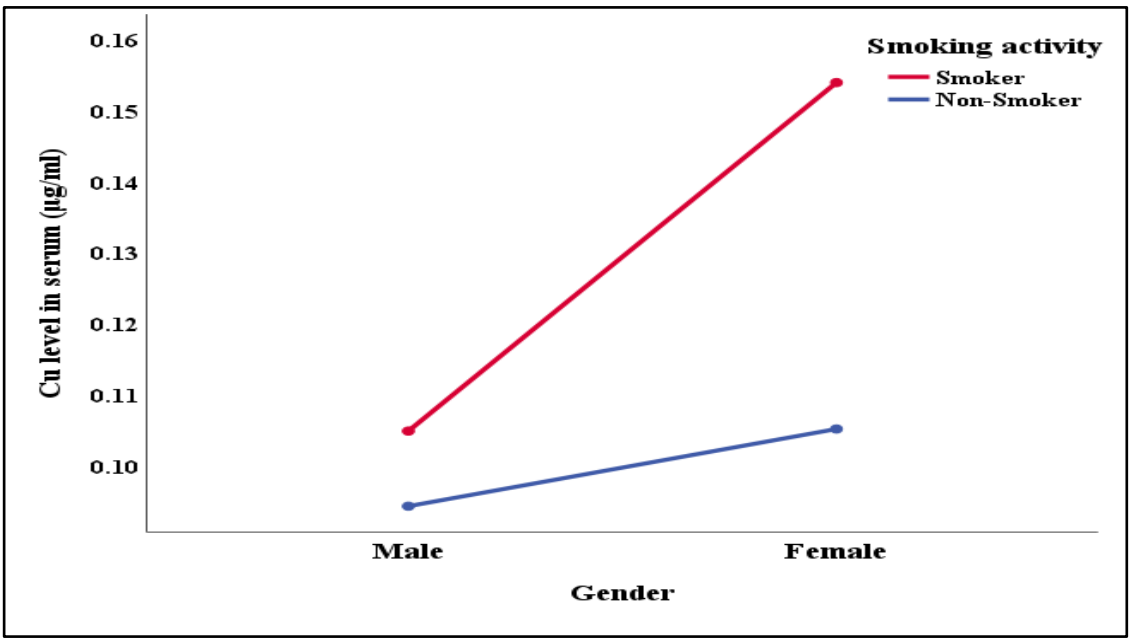


Figure 3.30: Interaction between gender and smoking activity for Cu levels (µg/mL) in serum samples.

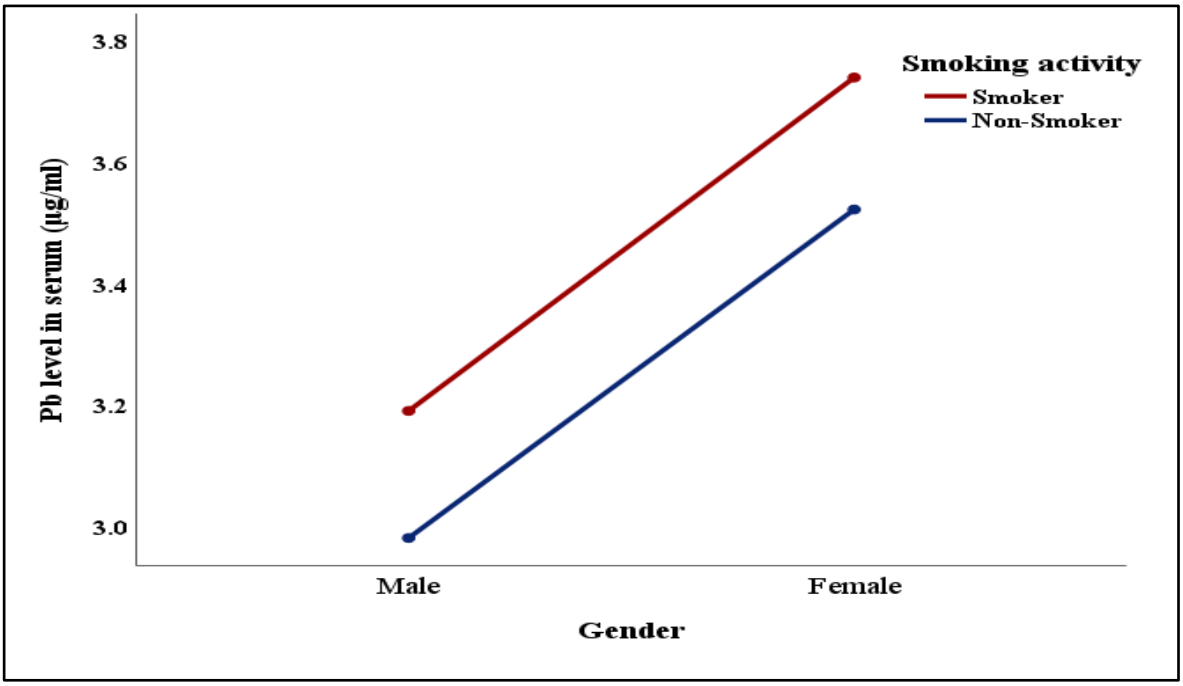


Figure 3.31: Interaction between gender and smoking activity for Pb levels (µg/mL) in serum samples.

3.5.2 Inter-Element Correlations

In the human body, several biological processes are especially dependent on the essential trace elements to function correctly [163]. However, these processes can be impaired by the presence of other elements which may have synergistic or antagonistic effects. Some elements are known to exhibit these relationships, such as the antagonism between Zn and Cd [164,165]; Cu and Mn [166] or Cu and Fe [167]; Sr and Ca [168]; and As and P [169]. Therefore, the correlations between study elements have been clarified. Outliers can have a dramatic effect on the correlation coefficient and make the *r* value much smaller than it should be, causing misleading results [146,170]. Moreover, any cases with missing values for one or both of a pair of trace elements for a correlation coefficient were excluded from the analysis

(excluding cases pairwise) as each coefficient is based on all cases that have valid codes on that pair of trace elements.

Pearson's Product Correlation Coefficient (r) was used to investigate the relationship between the trace element levels in serum [8]. This was investigated to evaluate which elements are correlated in serum and whether hemodialysis can affect inter-element relationships through a breakdown in metabolism or homeostatic regulations [171]. Different interpretations were suggested by researchers in terms of the values of r between 0 and 1. Therefore, the value of r was subjected to a significance test to examine whether r is significantly different at the 95% confidence interval (P = 0.05). The correlation coefficient results for serum associated with study elements are summarised in Table 3.7.

A total of 109 serum samples were analysed for the trace elements under study using correlation analysis. Fourteen of the examined 21 possible correlations were statistically significant after correlation for multiplicity, as shown in Table 3.7. The highest correlation coefficient was found in serum between Cd-Se (r = 0.971; P < 0.001) (Figure 3.32); Al-Cd (r = 0.948; P < 0.001) (Figure 3.33); Al-Se (r = 0.945; P < 0.001) (Figure 3.34). In addition to the above elements, the correlations between Al-Mn, Cd-Mn, Al-Pb, Cd-Pb, Cu-Pb, Mn-Pb, Mn-Se, Pb-Se, Cu-Zn, MnZn, and Pb-Zn have been determined, as shown in Table 3.7.

Lead was statistically significantly correlated with the largest number of other elements (6 correlations), followed by Mn (5 correlations). Aluminium, Cd, and Se were statistically significantly correlated with four correlations, and Zn and Cu were correlated significantly with three and two elements, respectively.

Table 3.7: Inter-element Person Correlation Coefficient (r) values for serum of subjects.

Element		Al	Cd	Cu	Mn	Pb	Se	Zn
Al	r	1						
	n	109						
	P							
Cd	r	0.948**	1					
	n	109	109					
	P	<0.001						
Cu	r	**-0.111**	**-0.095**	1				
	n	109	109	109				
	P	0.251	0.324					
Mn	r	0.422**	0.311**	0.185	1			
	n	109	109	109	109			
	P	<0.001	<0.001	0.055				
Pb	r	0.468**	0.385**	0.289**	0.668**	1		
	n	109	109	109	109	109		
	P	<0.001	<0.001	<0.001	<0.001			
Se	r	0.945**	0.971**	**-0.101**	0.311**	0.434**	1	
	n	109	109	109	109	109	109	
	P	<0.001	<0.001	0.294	0.001	<0.001		
Zn	r	0.063	**-0.019**	0.524**	0.662**	0.583**	0.024	1
	n	109	109	109	109	109	109	109
	P	0.514	0.842	<0.001	<0.001	<0.001	0.806	

** Correlation is significant at $P < 0.001$ level; **bold** values represent that there is no significant correlation at $P = 0.05$; n is the number of samples.

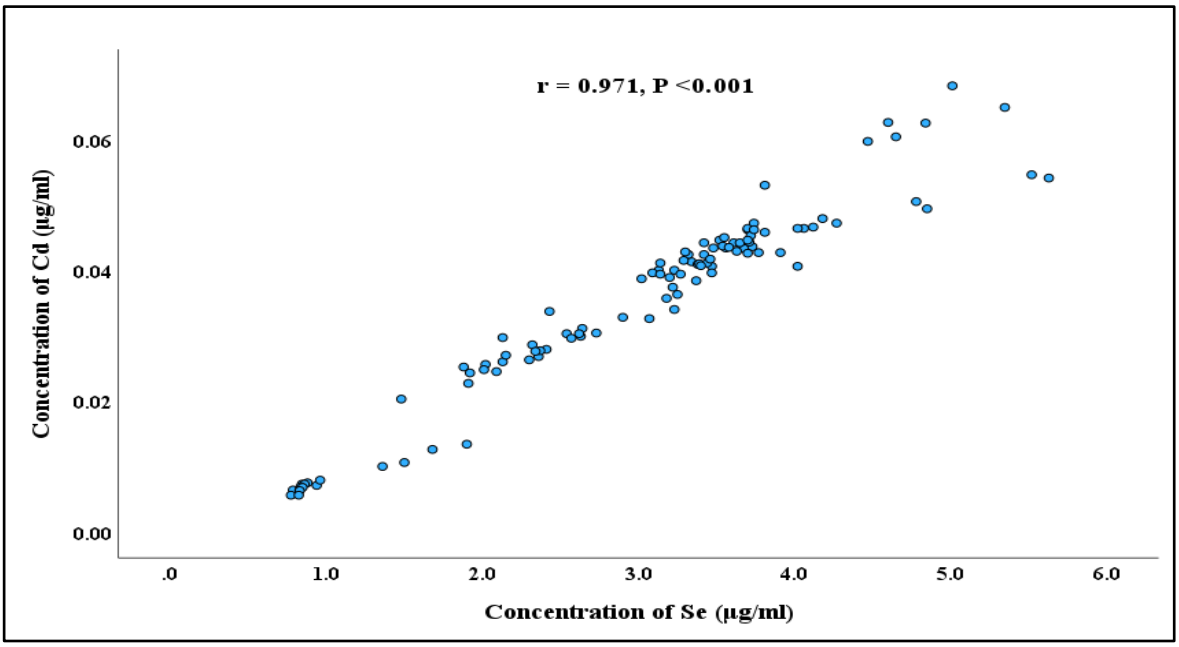


Figure 3.32: Correlation between Cd and Se in serum samples for subjects (n = 109).

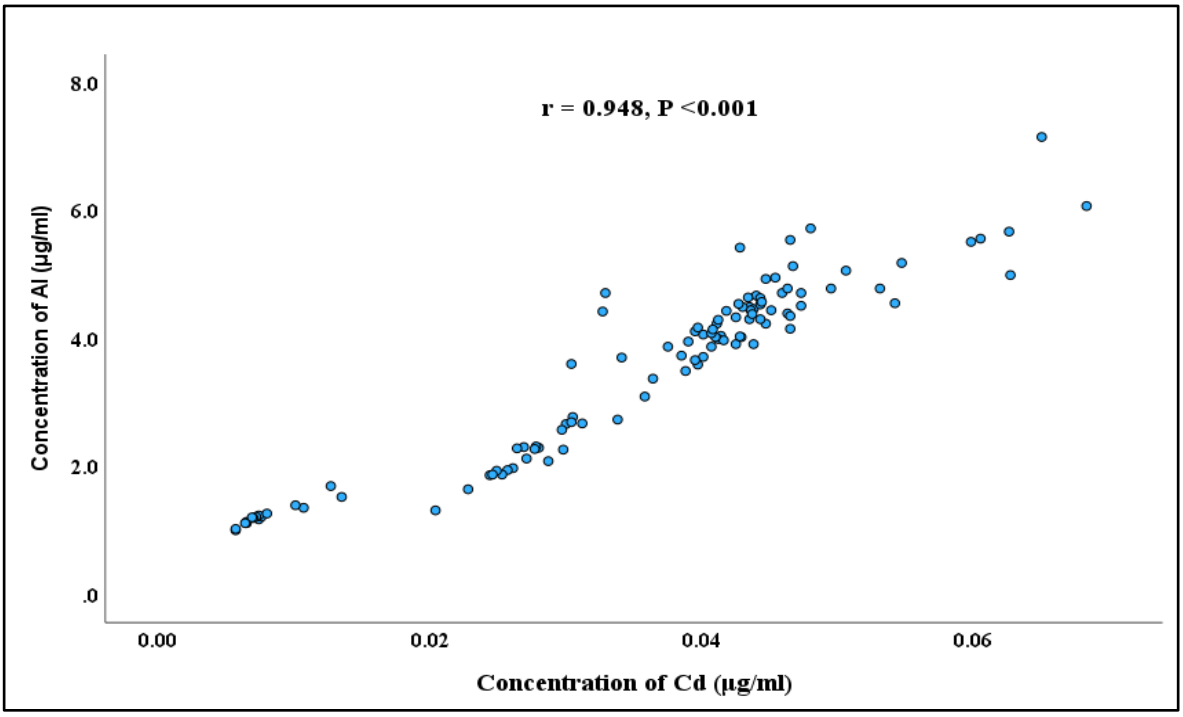


Figure 3.33: Correlation between Al and Cd in serum samples for subjects (n = 109).

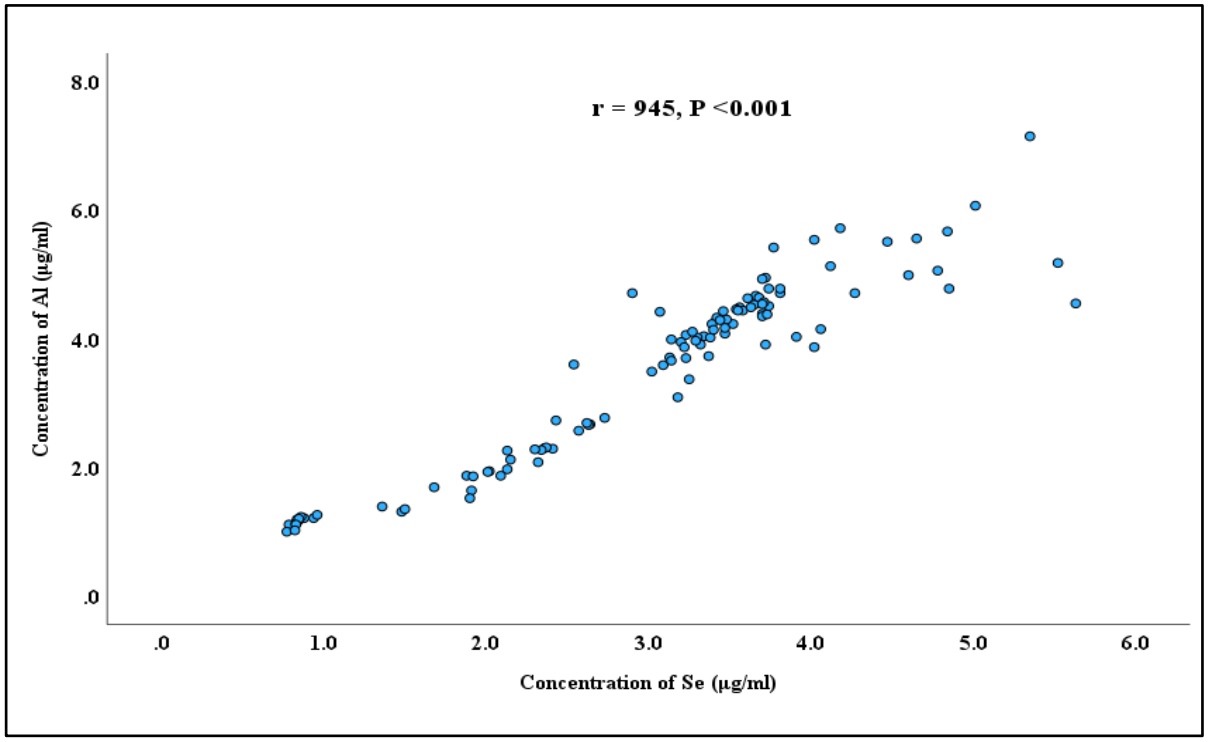


Figure 3.34: Correlation between Al and Se in serum samples for subjects (n = 109).

Chapter Four

Trace Element Levels in Fingernails

4.0 Introduction

In this Chapter, the concentrations of trace elements in human fingernails have been determined. Multi-elemental analysis was performed for aluminum (Al), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), selenium (Se), and zinc (Zn) in fingernails by using ICP-OES. The effect of various factors such as health status, smoking activity, gender, and age on the levels of trace elements have been investigated. A comparison study between the elemental levels in serum and fingernails was also investigated.

4.1 Statistical Methods of Analysis

Descriptive statistics, such as standard deviation, mean values, and outliers were determined for all the samples. The influence of different factors on the elemental levels was evaluated by using significant tests, for example, an F-test, a twotailed t-test, and ANOVA at $P = 0.05$. In addition, the Pearson correlation coefficient (r) was performed to evaluate the correlations between trace element levels in fingernail samples [1]. Statistical analysis was performed using the statistical package SPSS, version 29 (SPSS, IBM, Chicago, IL, USA).

4.2 Elemental Composition of Fingernails

Recently, human fingernail tissue has been known as a useful material for the evaluation of exposure to various pollutants in an occupational and/or environmental setting due to industrial activities, car engine outputs, etc. [172174]. Many previous studies have indicated that these elements have a major and direct role in kidney disease, given the sensitivity of this part to these pollutants that may affect the process of filtration and reabsorption, leaving the filtrate concentrated in the tubules and the collecting duct. These pollutants are important in developing kidney stones and chronic diseases [175,176]. The advantages of fingernail tissue analysis over other biological samples are that trace metal concentrations in this tissue are not subjected to rapid fluctuation due to diet, air, and water hence a long term stable nutritional status [177]. The mineral content of fingernails can provide a means to evaluate the level of nutrition in humans, as it has been noted that these elements have several metabolic roles through which diagnosis can be made between individuals and patients [178]. The levels of trace elements in fingernail

samples as arithmetic mean, standard deviation (Sd), range, median, 95% confidence interval for mean, and the number of samples for populations in comparison with their levels in the literature are summarized in Table 4.1. The levels of trace elements (µg/g) in fingernail samples are increased according to the following sequence Pb > Al > Se> Zn > Mn > Cu > Cd.

Table 4.1: Descriptive statistics for element concentrations in washed fingernails for 103 subjects along with literature range.

Trace Elements	Variables	Concentration (µg/g)	Literature range* (µg/g)
Al	Mean ± Sd	4.348 ± 1.387	12 -137
	Range	1.580 – 7.158	
	Median	3.990	
	95% confidence interval	(4.080- 4.616)	
Cd	Mean ± Sd	0.047 ± 0.019	0.013 – 0.438
	Range	0.012 – 0.092	
	Median	0.044	
	95% confidence interval	(0.043-0.051)	
Cu	Mean ± Sd	0.134 ± 0.051	4.2 - 17
	Range	0.072 – 0.255	
	Median	0.119	
	95% confidence interval	(0.124-0.144)	
Mn	Mean ± Sd	0.415 ± 0.168	0.19 – 3.30
	Range	0.126 – 0.890	
	Median	0.424	
	95% confidence interval	(0.383-0.448)	
Pb	Mean ± Sd	9.761 ± 3.337	0.27- 4.75
	Range	4.190 – 16.822	
	Median	9.050	
	95% confidence interval	(9.116-10.405)	
Se	Mean	3.498 ± 1.044	0.62 – 1.53
	Range	1.740 – 5.700	
	Median	3.280	
	95% confidence interval	(3.296-3.700)	
Zn	Mean ± Sd	2.568 ± 0.835	80 - 191
	Range	1.094 – 4.524	
	Median	2.370	
	95% confidence interval	(2.407-2.729)	

Sd is standard deviation, * [83].

Figure 4.1 indicates the box plots for trace element levels in the fingernails under investigation.

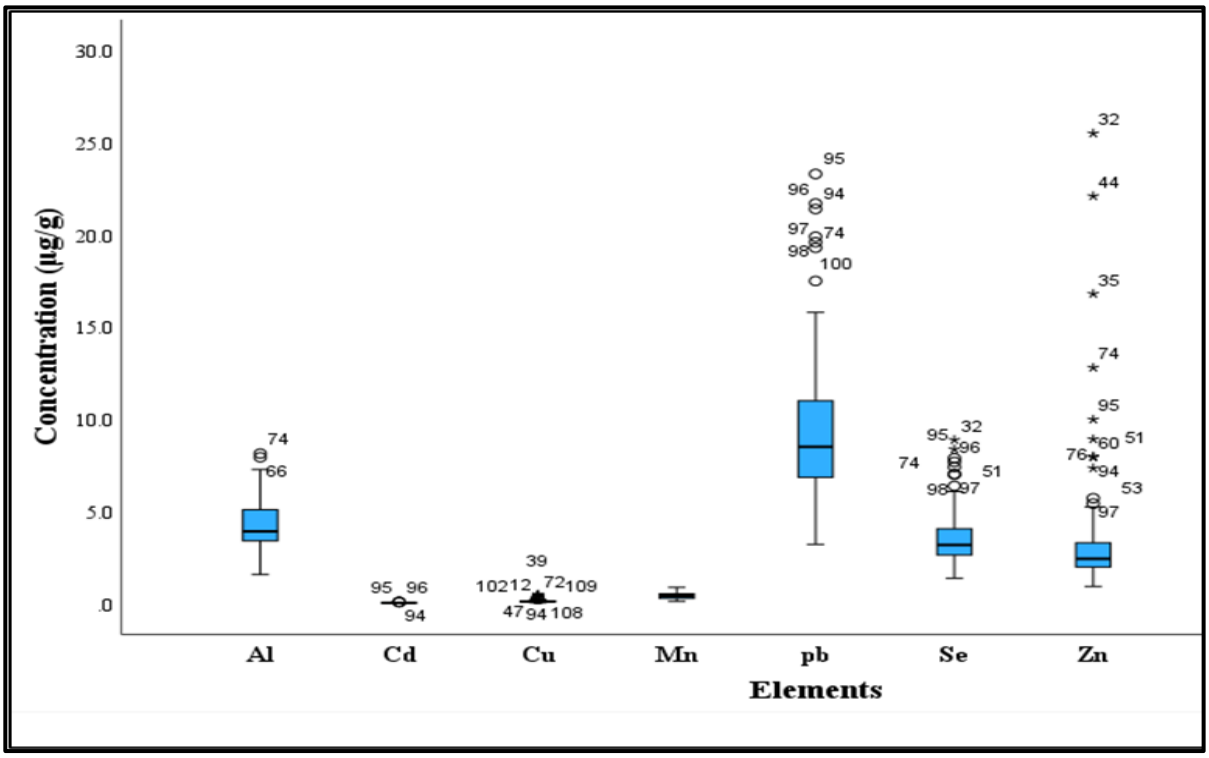
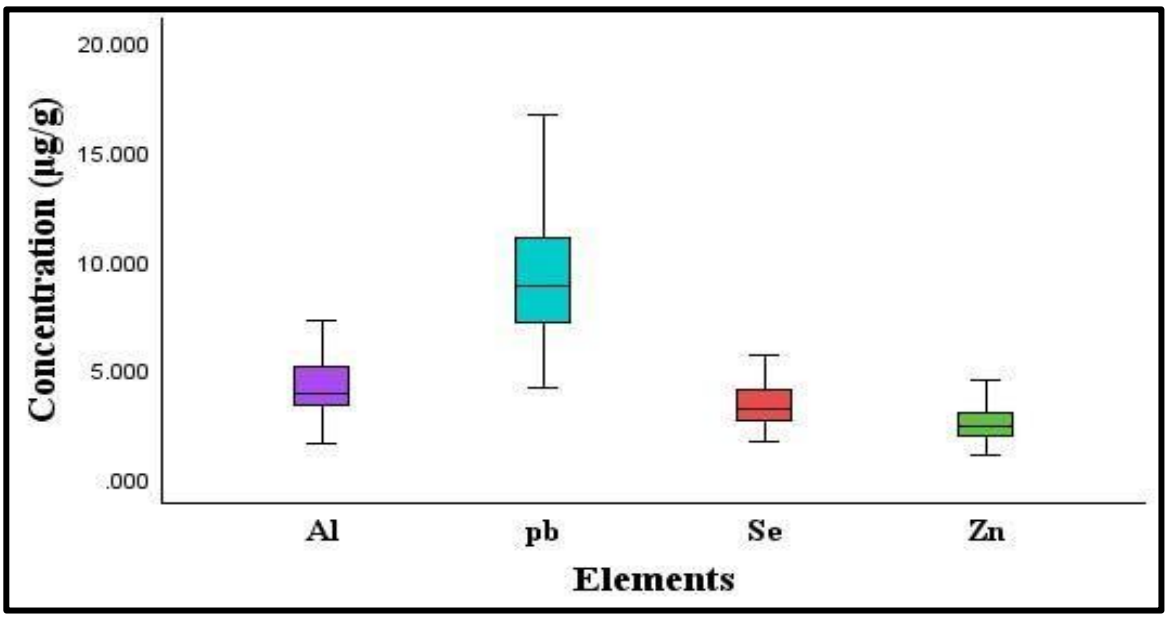


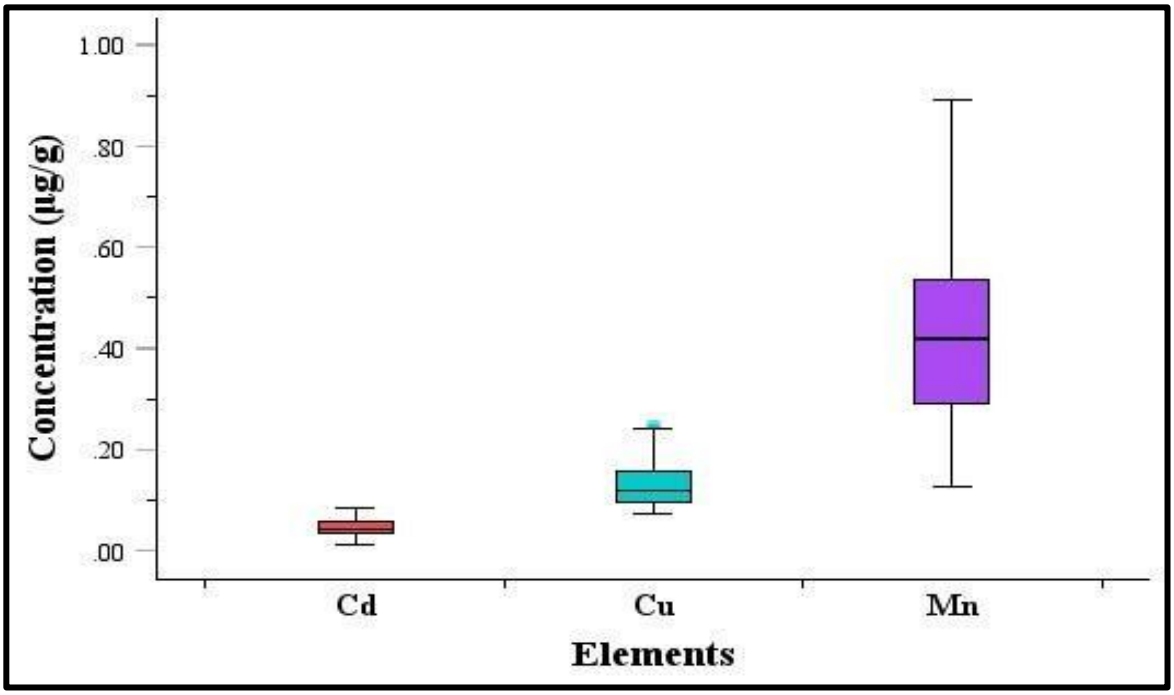
Figure 4.1: Trace element levels (µg/g) in fingernails with outlier values for different elements; middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represents extreme values.

4.3 Checking for Outliers

The results of Al, Cd, Cu, Mn, Pb, Se, and Zn were found to have one (or perhaps more) value(s) that appeared unreasonably different from other values in the obtained study data set. Grubb's test (G) was used to determine if there were any statistical outliers. If the calculated value (G_{cal}) exceeded the critical value (G_{crit}), the suspected value was rejected so that it did not affect the accuracy of the comparison between studies representing the populations differently [122]. No statistical outliers were found for the total population data for Mn, while the trace elements Zn and Cu were found to have 11 and 8 outliers, respectively. Pb and Se had 7 outliers; Cd and Al had 3 and 1 outliers, respectively. The results of Grubb's tests are summarized in Figure 4.2. In many cases, the effect of removing outliers from the investigated study samples can improve the fingernail trace element graph because the degree of positive skewness (which indicates the symmetry of the distribution) is lower than the mean [130].



(a)



(b)

Figure 4.2 (a and b): Trace element levels (µg/g) in fingernails without outlier values for different elements (n = 103); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively.

4.4 Results and Discussion

In recent years, fingernail materials have been known as useful samples for evaluating exposure to numerous contaminants in an environmental setting [39,87,105,179]. The levels of trace elements in fingernail tissue were found to be higher than in human fluids [154,180]. Interestingly, these materials can be used as a biomarker to determine the elemental levels and assess the role of different diseases on human health status. On the other hand, other factors such as gender, smoking activity, and age can play a significant effect on the increasing and decreasing of elemental levels inside the human body. Therefore, this study has investigated the influence of all these factors on the level of interested elements.

4.4.1 Factors Influencing Elemental Data (Factorial Analysis)

The mean values of the trace elements were categorized according to different parameters (factors) obtained from the questionnaire, as shown in Table 4.2. Multiple effects must be studied in research rather than the single effect for each factor [131]. The mean and standard deviation for each group is presented in Table 4.3. The effect of each factor was investigated.

Table 4.2: Demographic characteristics of participants according to different factors.

Factor	Code number *	Group	Number of subjects
Health status	1	hemodialysis	44 (male = 24, female = 20)
	2	healthy	59 (male = 33, female = 26)
Gender	1	male	57 (healthy = 33, haemodialysis = 24)
	2	female	46 (healthy = 26, haemodialysis = 20)
Smoking activity	1	smoker	24 (male = 23, female = 1)
	2	non-smoker	79 (male = 34, female = 45)
Total			103

* Each factor must be assigned a numerical code before it can be entered into SPSS.

Table 4.3: Descriptive Statistics (mean ± Sd µg/g) for trace elements in fingernail samples in relation to different factors.

Health status	Gender	Smoking activity	(n)	**Elemental Level, mean ± Sd (µg/g)**						
				Al	Cd	Cu	Mn	pb	Se	Zn
Hemodialysis	Male	Smoker	(8)	3.92 ± 1.10	0.03 ± 0.01	0.13 ± 0.03	0.49 ± 0.14	8.39 ± 1.39	3.48 ± 0.76	2.21 ± 0.71
		non-smoker	(16)	3.76 ± 1.14	0.03 ± 0.01	0.16 ± 0.05	0.48 ± 0.16	7.48 ± 1.82	2.91 ± 0.79	2.26 ± 0.63
		Total	(24)	**3.81 ± 1.11**	**0.03 ± 0.01**	**0.15 ± 0.04**	**0.48 ± 0.15**	**7.78 ± 1.71**	**3.10 ± 0.81**	**2.24 ± 0.64**
	Female	Smoker	(1)	3.00 ± 0.01	0.02 ± 0.001	0.09 ± 0.001	0.31 ± 0.001	4.91 ± 0.001	2.20 ± 0.001	2.18 ± 0.001
		non-smoker	(19)	3.98 ± 0.68	0.04 ± 0.01	0.15 ± 0.04	0.51 ± 0.17	7.91 ± 1.35	2.98 ± 0.71	2.27 ± 0.69
		Total	(20)	**3.93 ± 0.69**	**0.03 ± 0.01**	**0.15 ± 0.04**	**0.50 ± 0.17**	**7.75 ± 1.47**	**2.94 ± 0.71**	**2.27 ± 0.68**
	Total	Smoker	(9)	3.45 ± 1.08	0.03 ± 0.01	0.13 ± 0.03	0.47 ± 0.14	8.00 ± 1.74	3.34 ± 0.83	2.21 ± 0.66
		non-smoker	(35)	3.87 ± 0.91	0.03 ± 0.01	0.15 ± 0.04	0.49 ± 0.16	7.71 ± 1.57	2.94 ± 0.74	2.27 ± 0.67
		Total	(44)	**3.87 ± 0.93**	**0.03 ± 0.01**	**0.15 ± 0.04**	**0.49 ± 0.16**	**7.77 ± 1.59**	**3.03 ± 0.76**	**2.26 ± 0.65**
Healthy	Male	Smoker	(15)	4.32 ± 1.59	0.05 ± 0.01	0.12 ± 0.06	0.27 ± 0.10	9.69 ± 2.69	3.44 ± 1.00	2.64 ± 0.79
		non-smoker	(18)	4.24 ± 1.60	0.05 ± 0.01	0.11 ± 0.04	0.30 ± 0.17	9.39 ± 3.03	3.32 ± 0.98	2.79 ± 0.90
		Total	(33)	**4.28 ± 1.58**	**0.05 ± 0.01**	**0.11 ± 0.05**	**0.30 ± 0.14**	**9.53 ± 2.84**	**3.37 ± 0.98**	**2.72 ± 0.84**
	Female	non-smoker	(26)	5.25 ± 1.39	0.07 ± 0.02	0.13 ± 0.06	0.41 ± 0.13	13.42 ± 3.13	4.46 ± 0.93	3.04 ± 0.93
		Total	(26)	**5.25 ± 1.39**	**0.07 ± 0.02**	**0.13 ± 0.06**	**0.41 ± 0.13**	**13.42 ± 3.13**	**4.46 ± 0.93**	**3.04 ± 0.93**
	Total	Smoker	(15,15)	4.32 ± 1.59	0.05 ± 0.01	0.12 ± 0.06	0.27 ± 0.10	9.69 ± 2.69	3.44 ± 1.00	2.64 ± 0.79
		non-smoker	(44,39)	4.74 ± 1.55	0.06 ± 0.02	0.12 ± 0.05	0.38 ± 0.15	11.77 ± 3.64	3.99 ± 1.09	2.93 ± 0.92
		Total	(59)	**4.71 ± 1.56**	**0.06 ± 0.02**	**0.12 ± 0.05**	**0.25 ± 0.15**	**11.25 ± 3.35**	**3.85 ± 1.09**	**2.85 ± 0.89**
Total	Male	Smoker	(23)	4.18 ± 1.43	0.04 ± 0.01	0.12 ± 0.05	0.34 ± 0.15	9.24 ± 2.37	3.45 ± 0.91	2.49 ± 0.78
		non-smoker	(34)	4.02 ± 1.41	0.04 ± 0.02	0.14 ± 0.05	0.40 ± 0.18	8.49 ± 2.68	3.13 ± 0.91	2.52 ± 0.81
		Total	(57)	**4.08 ± 1.41**	**0.04 ± 0.01**	**0.13 ± 0.05**	**0.38 ± 0.17**	**8.79 ± 2.57**	**3.26 ± 0.91**	**2.51 ± 0.79**
	Female	Smoker	(1)	3.00 ± 0.01	0.02 ± 0.001	0.09 ± 0.001	0.31 ± 0.001	4.91 ± 0.001	2.20 ± 0.001	2.18 ± 0.001
		non-smoker	(45)	4.72 ± 1.29	0.05 ± 0.02	0.14 ± 0.05	0.469 ± 0.16	11.09 ± 3.72	3.83 ± 1.11	2.65 ± 0.90
		Total	(46)	**4.68 ± 1.31**	**0.05 ± 0.02**	**0.14 ± 0.05**	**0.46 ± 0.16**	**10.96 ± 3.79**	**3.79 ± 1.12**	**2.64 ± 0.89**
	Total	Smoker	(24)	4.13 ± 1.42	0.04 ± 0.01	0.12 ± 0.05	0.321 ± 0.15	9.06 ± 2.48	3.40 ± 0.92	2.48 ± 0.76
		non-smoker	(79)	4.42 ± 1.38	0.05 ± 0.02	0.14 ± 0.05	0.44 ± 0.17	9.97 ± 3.54	3.53 ± 1.08	2.59 ± 0.86
		Total	(103)	**4.35 ± 1.39**	**0.05 ± 0.02**	**0.13 ± 0.05**	**0.42 ± 0.17**	**9.76 ± 3.34**	**3.50 ± 1.04**	**2.57± 0.84**

n is the number of samples for Al, Cd, pb, Se and Cu, Mn, Zn, respectively.

4.4.1.1 Influence of Hemodialysis - Link to Human Health

In this section, the results of healthy individuals and hemodialysis patients have been compared to evaluate whether there are any significant differences in the elemental levels between the two groups. Moreover, this can be used to investigate whether these elements play a role in the onset of hemodialysis disease. The participants who provided the serum samples were also used to collect the fingernail samples with the same conditions. The mean and standard deviation values for trace element levels in fingernail samples of healthy individuals and hemodialysis patients were compared by using an F-test and a two-tailed t-test, and the results are presented in Table 4.4. In general, significantly higher fingernail levels of Cu (0.149 ± 0.080 μg/g) and Mn (0.492 ± 0.158 μg/g) are found in hemodialysis patients when compared with healthy individuals (0.120 ± 0.054 μg/g of Cu and 0.346 ± 0.147 μg/g of Mn) at (P = 0.05). On the other hand, the levels of Al (4.708 ± 1.562 μg/g), Cd (0.057 ± 0.016 μg/g), Pb (11.245 ± 3.530 μg/g), Se (3.851 ± 1.090 μg/g), and Zn (2.851 ± 0.886 μg/g) in healthy individuals are found to be significantly higher when compared with hemodialysis patients (3.868 ± 0.934 μg/g of Al; 0.033 ± 0.011 μg/g of Cd; 7.769 ± 1.589 μg/g of Pb; 3.025 ± 0.764 μg/g of Se and 2.255 ± 0.652 μg/g of Zn) at P = 0.05. It was found that there is a good agreement between the results of fingernails and those reported in serum for Zn and Cu. Similar observations have been reported in both materials for the levels of the remaining elements, the only exceptions are for Mn and Cd, as shown in Table 4.4. In general, the trace element levels in washed fingernails are found to be higher than those reported in serum for all elements. This is because fingernails are a longterm growth material, therefore most trace elements accumulate in this tissue [89]. These results agree with those reported in the literature between fingernails and other fluids [89,181]. The results in this study are in agreement with those published in the literature for Mn and Pb in blood serum [109].

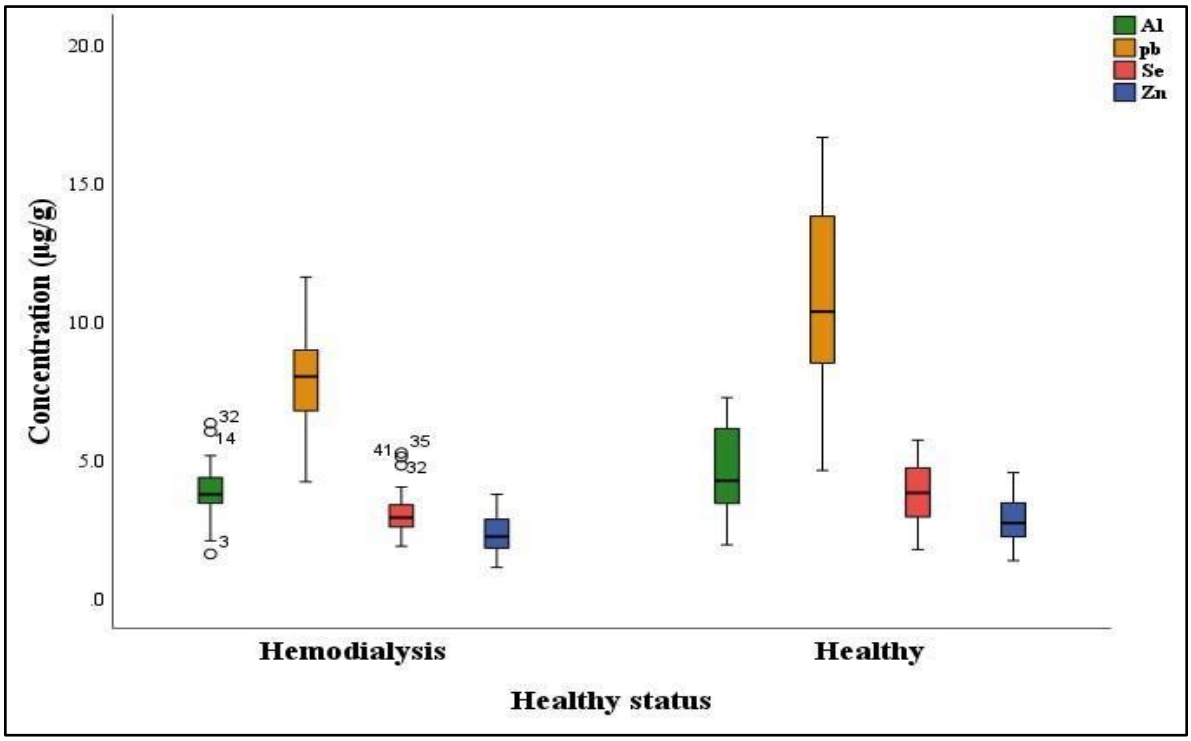
Table 4.4: Fingernail elemental levels found in the groups of healthy individuals and hemodialysis (n = 103).

Element (n_1, n_2)	Concentration (µg/g) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Healthy	Hemodialysis	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	4.708 ± 1.562	3.868 ± 0.934	Equal variances assumed	23.952	< 0.001	3.164	101	**0.002**
(59,44)	(1.910-7.236)	(1.580-6.310)	Equal variances not assumed			3.392	97	**0.001**
Cd	0.057 ± 0.016	0.033 ± 0.011	Equal variances assumed	10.372	0.002	8.520	101	**< 0.001**
(59,44)	(0.030-0.092)	(0.012-0.069)	Equal variances not assumed			8.979	100	**< 0.001**
Cu	0.120 ± 0.054	0.149 ± 0.080	Equal variances assumed	1.382	0.243	2.973	101	**0.004**
(54,49)	(0.072-0.255)	(0.080-0.252)	Equal variances not assumed			3.007	99	**0.003**
Mn	0.346 ± 0.147	0.492 ± 0.158	Equal variances assumed	0.001	0.980	4.828	101	**< 0.001**
(54,49)	(0.127-0.697)	(0.126-0.890)	Equal variances not assumed			4.810	98	**< 0.001**
pb	11.245 ± 3.530	7.769 ± 1.589	Equal variances assumed	29.091	< 0.001	6.083	101	**< 0.001**
(59,44)	(4.600-16.822)	(4.190-11.600)	Equal variances not assumed			6.707	85	**< 0.001**
Se	3.851 ± 1.090	3.025 ± 0.764	Equal variances assumed	10.098	0.002	4.293	101	**< 0.001**
(59,44)	(1.740-5.700)	(1.860-5.235)	Equal variances not assumed			4.515	101	**< 0.001**
Zn	2.851 ± 0.886	2.255 ± 0.652	Equal variances assumed	5.214	0.024	3.847	101	**< 0.001**
(54,49)	(1.340-4.527)	(1.094-3.740)	Equal variances not assumed			3.903	97	**< 0.001**

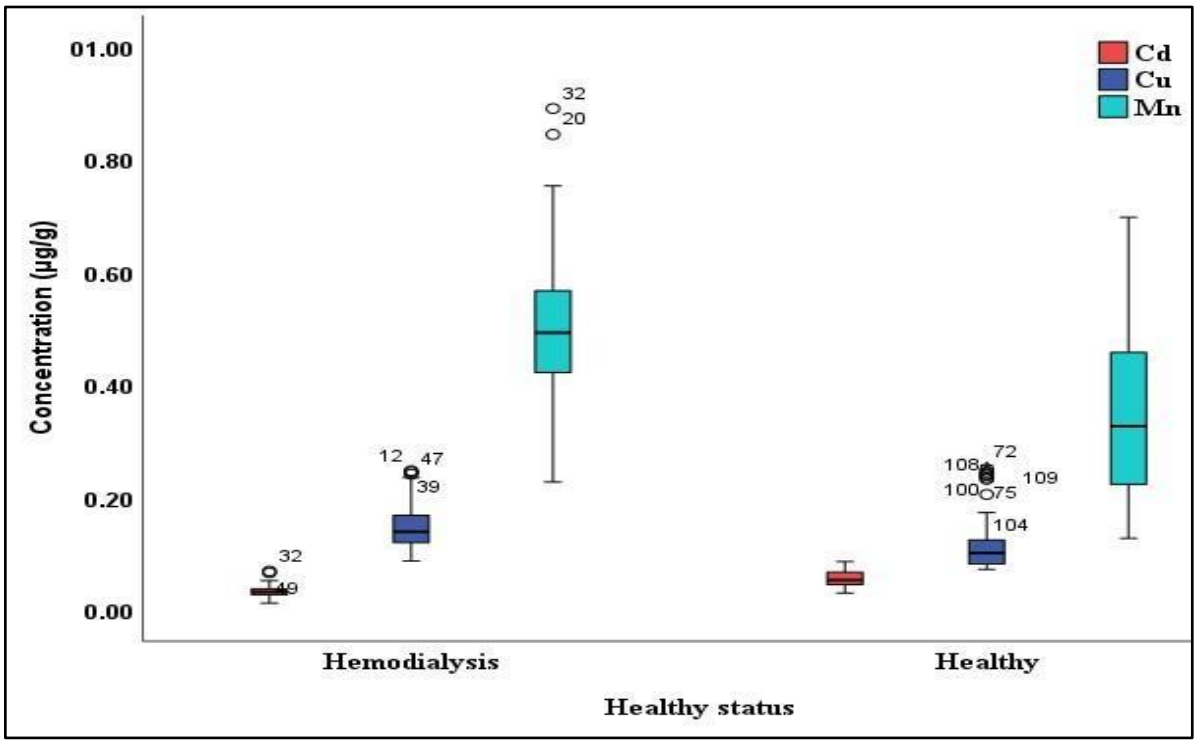
Sd is standard deviation, n_1, n_2 are the number of samples for healthy and hemodialysis, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance. $t_{crit.} = 1.96$ at P=0.05.

There are other factors such as the size of the kidney and the surface of the dialysis membrane can affect the function of the Kidney [142]. As well as, the environment, geography and/or lifestyle (quality of drinking water and smoking activity), age, sex, and dose of the drug taken can also affect the progress of this disease [143-145].

The separate boxplots for healthy and hemodialysis are used to identify any outliers from the histogram, as shown in Figure 4.3. In general, the outliers detected in the histogram are shown up or down as an asterisk (*) on the boxplots [146]. The results indicate that there are no outliers can be detected for healthy individuals in terms of Al, Cd, Mn, Pb, Se, and Zn; and hemodialysis patients for Pb and Zn on boxplots. In contrast, Al, Cd, Cu, and Se boxplots have 3, 2, 2, 3, and 3 outliers in the patients, while Cu has 6 outliers for healthy individuals. The boxes are in different sizes for healthy and patients, and the median values for healthy are higher than for patients in terms of Al, Cd, Se, Pb, and Zn. In contrast, the values of the median of both Cu and Mn are higher in patients than healthy, as shown in Figure 4.3.



(a)



(b)

Figure 4.3: Trace element levels (µg/g) in fingernails (a & b) for different population groups (healthy and patients); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

4.4.1.2 Influence of Gender

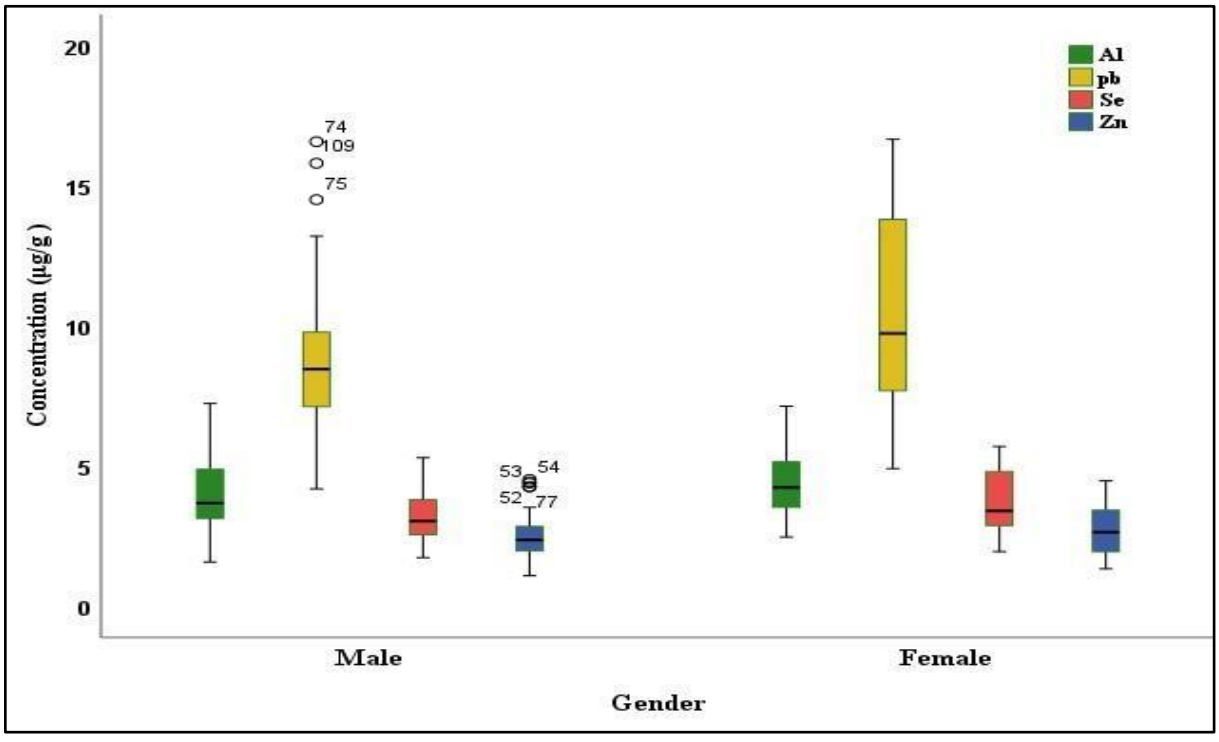
The influence of gender can play a significant role in changing the levels of trace elements in the human body [147]. The total samples (n = 103) were divided into two gender groups, males (n= 57) and females (n= 46). The mean and standard deviation (±Sd) for each group is reported in Table 4.5. The highest mean values in the two gender groups are found for Pb (males: 8.747 ± 2.563; and females: 10.519 ± 3.626.584 µg/g Pb) followed by aluminum for males (4.079 ± 1.418 µg/g Al) and females (4.520 ± 1.239 µg/g Al). Cd showed the lowest concentration for both gender groups (males: 0.042 ± 0.014 µg/g Cd) and (females: 0.055 ± 0.021 µg/g Cd). The order of increasing trace element levels in the fingernails of males and females is Cd < Cu < Mn < Zn < Se < Al < Pb. The significant effect of gender on the elemental levels in fingernails was investigated using a two-tailed t-test, and the results are presented in Table 4.5.

Table 4.5: Fingernail elemental levels found in the group of males and females (n = 103).

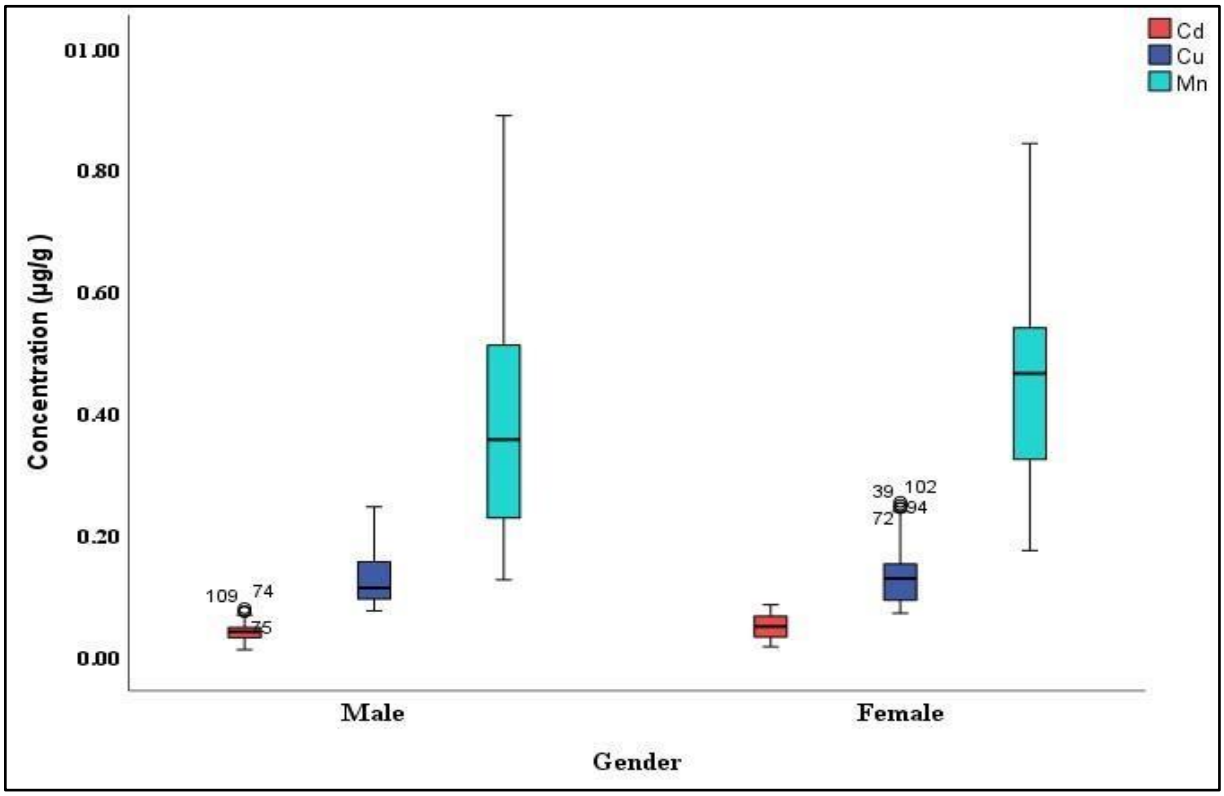
Element (n_1, n_2)	Concentration (µg/g) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Male	Female	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	4.079 ± 1.418	4.520 ± 1.239	Equal variances assumed	0.003	0.959	2.207	101	**0.031**
(57,46)	(1.580-7.236)	(2.470-7.137)	Equal variances not assumed			2.224	100	**0.028**
Cd	0.042 ± 0.014	0.055 ± 0.021	Equal variances assumed	14.675	< .001	3.041	101	**0.003**
(57,46)	(0.012-0.092)	(0.017-0.086)	Equal variances not assumed			2.912	74	**0.005**
Cu	0.129 ± 0.049	0.137 ± 0.052	Equal variances assumed	0.188	0.666	0.847	101	0.399
(57,46)	(0.076-0.247)	(0.072-0.255)	Equal variances not assumed			0.838	92	0.404
Mn	0.381 ± 0.172	0.459 ± 0.149	Equal variances assumed	1.038	0.311	2.424	101	**0.017**
(57,46)	(0.127-0.890)	(0.157-0.844)	Equal variances not assumed			2.447	99	**0.016**
pb	8.747 ± 2.563	10.519 ± 3.626	Equal variances assumed	14.958	< 0.001	3.442	101	**< 0.001**
(57,46)	(4.190-16.568)	(4.910-16.652)	Equal variances not assumed				76	**0.001**
Se	3.249 ± 0.921	3.674 ± 1.099	Equal variances assumed	4.765	0.031	2.676	101	**0.009**
(57,46)	(1.740-5.300)	(1.950-5.700)	Equal variances not assumed			2.618	86	**0.010**
Zn	2.515 ± 0.797	2.714 ± 0.887	Equal variances assumed	2.615	0.109	0.773	101	0.441
(57,46)	(1.094-4.524)	(1.340-4.476)	Equal variances not assumed			0.763	91	0.447

Sd is the standard deviation, n_1, n_2 are the number of samples for males and females, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance *P* = 0.05, Sig. = level of significance. t_{crit}. = 1.96 at *P*=0.05.

The results indicate that there are significant effects of gender on the levels of Al ($t(103) = 2.207$, $t_{cit.}$=1.96 at $P = 0.05$), Cd ($t(103) = 3.041$, $t_{cit.}$=1.96 at $P = 0.05$), Mn ($t(103) = 2.424$, $t_{cit.}$=1.96 at $P = 0.05$), Pb ($t(103) = 3.442$, $t_{cit.}$=1.96 at $P = 0.05$), and Se ($t(103) = 2.676$, $t_{cit.}$=1.96 at $P = 0.05$). No significant effect ($P = 0.05$) was found for either gender for Cu, and Zn, as shown in Table 4.5. Similar results of higher levels of Cd and Se in males than females; and higher levels of Cu and Mn in females compared to males were also found in the serum samples. The separate boxplots for males and females are shown in Figure 4.4. The results show that the boxplots of all elements are in different sizes for males and females. The median values for females are higher than males in terms of Al, Cd, Cu, Mn, Pb, Se, and Zn. The results show that the distribution of Cd for females; and Pb for males is symmetrical, whereas the distributions for other elements in both groups are asymmetrical. In addition, some circles were identified above the male boxplots (Cd, 3; Pb, 3; and Zn, 4). On the other hand, Cu has four circles above the female boxplot. These cases can consider to be also outliers. The remaining elements did not have any outlier values.



(a)



(b)

Figure 4.4: Trace element levels (µg g^{-1}) in fingernails (a and b) for different population groups (males and females); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

4.4.1.3 Influence of Smoking Activity

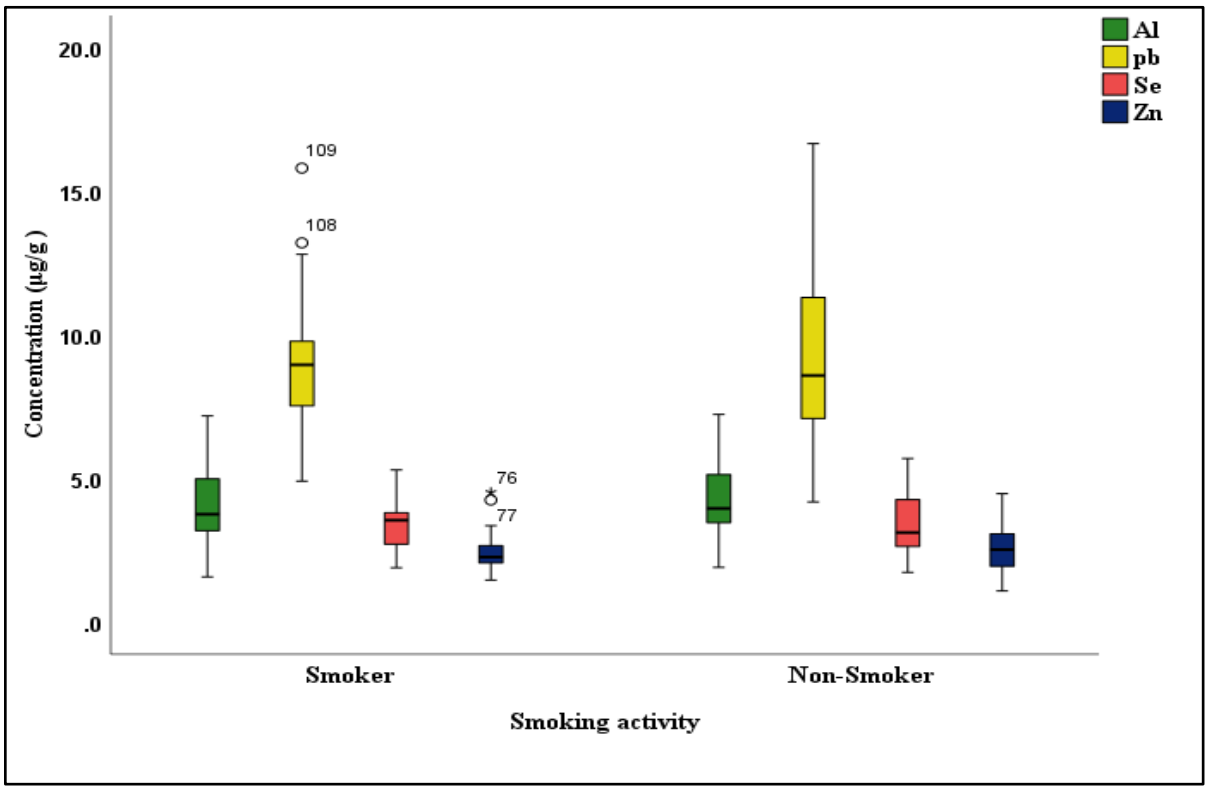
The influence of smoking activity upon the elemental levels in several invasive and non-invasive human fluids and tissues has been studied in the literature [128,129,143,151,154,155]. The population in this study was divided into smokers and non-smokers. The significant differences between the levels of trace elements in smokers and non-smokers in fingernails were investigated by using a two-tailed t-test, and the results are reported in Table 4.6.

Table 4.6: Fingernail elemental levels found in the groups of smokers and non-smokers (n = 103).

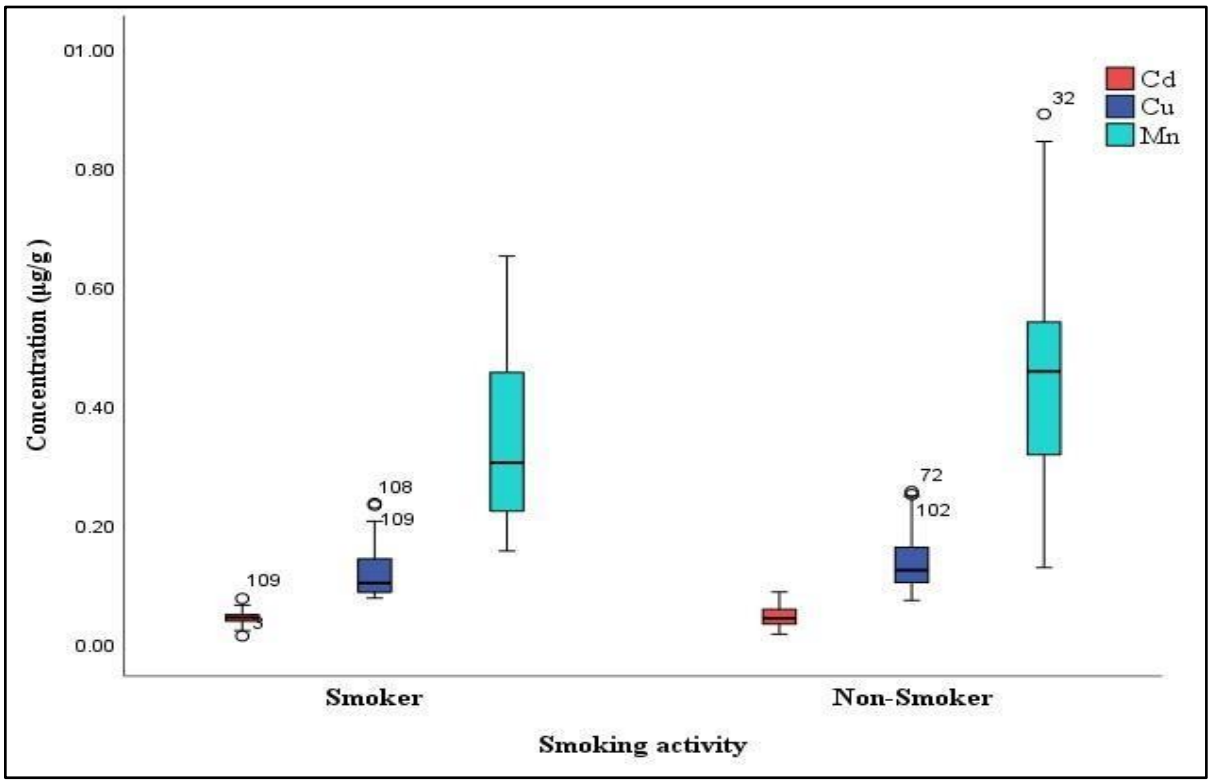
Element (n_1, n_2)	Concentration (µg/g) Mean ± Sd (range)		F-test			Two-tailed t-test		
	Smoker	Non-smoker	Variance	*F*	Sig.	*t*	*df*	Sig.
Al	4.132 ± 1.421	4.312 ± 1.341	Equal variances assumed	0.000	0.992	0.872	101	0.385
(24,79)	(1.580-7.185)	(1.910-7.236)	Equal variances not assumed			0.859	37	0.396
Cd	0.043 ± 0.013	0.046 ± 0.019	Equal variances assumed	9.776	0.002	1.183	101	0.240
(24,79)	(0.012-0.085)	(0.015-0.086)	Equal variances not assumed			1.469	58	0.147
Cu	0.120 ± 0.049	0.137 ± 0.080	Equal variances assumed	0.054	0.817	1.457	101	0.148
(24,79)	(0.076-0.235)	(0.072-0.255)	Equal variances not assumed			2.625	39	0.146
Mn	0.343 ± 0.150	0.438 ± 0.165	Equal variances assumed	0.115	0.736	2.478	101	**0.015**
(24,79)	(0.155-0.651)	(0.127-0.890)	Equal variances not assumed			2.625	42	**0.012**
pb	9.059 ± 2.483	9.652 ± 3.367	Equal variances assumed	6.362	0.013	1.178	101	0.242
(24,79)	(4.910-15.800)	(4.190-16.652)	Equal variances not assumed			1.418	54	0.162
Se	3.401 ± 0.923	3.442 ± 1.052	Equal variances assumed	2.185	0.142	0.517	101	0.606
(24,79)	(1.900-5.300)	(1.740-5.700)	Equal variances not assumed			0.563	44	0.576
Zn	2.478 ± 0.764	2.639 ± 0.862	Equal variances assumed	1.791	0.184	0.595	101	0.553
(24,79)	(1.470-4.524)	(1.094-4.476)	Equal variances not assumed			0.634	42	0.529

Sd is the standard deviation, n_1, n_2 are the number of samples for smokers and non-smokers, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), F and t are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance *P* = 0.05, Sig. = level of significance. $t_{crit.}$ = 1.96 at *P*=0.05.

It was found that there is a significant difference between the levels of Mn in smokers (0.343 ± 0.150) and non-smokers (0.438 ± 0.165) at the value of P = 0.05. In contrast, there are no significant differences between both groups in terms of Al, Cd, Cu, Pb, Se, and Zn. The separate boxplots for smokers and nonsmokers are shown in Figure 4.5. The results show that the boxplots of all elements are in different sizes for smokers and non-smokers. The median values for smokers are higher than non-smokers regarding Cd, Pb, and Se. In contrast, the median values of Al, Cu, Mn, and Zn were higher in non-smokers than in smokers. The median value of Cd is similar for both groups. The distributions of all elements are asymmetrical for both groups (smoker and non-smoker). In addition, some circles were identified above (Pb, 2); (Zn, 2); (Cd,1); and (Cu, 2) for the smoker boxplot, and Cd has one circle below the smoker boxplot. Cu has two circles, and Mn has one circle above the boxplot for non-smokers. The remaining elements did not have any outliers in the cases of smokers' and nonsmokers' boxplots. These cases can consider to be outliers.



(a)



(b)

Figure 4.5: Trace element levels (µg g^{-1}) in fingernails (a and b) for different population groups (smokers and non-smokers); middle band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers.

4.4.1.4 Influence of Age

The effect of human age on the elemental levels of fingernail samples has been determined in this section. The results indicate that the effect of the individual's age upon the levels of Cd, Cu, and Mn was significant at $P = 0.05$, as shown in Figure 4.6-4.8, respectively. On the other hand, there are no significant effects were found for age on the remaining elements, as shown in Figure 3.9 for Al.

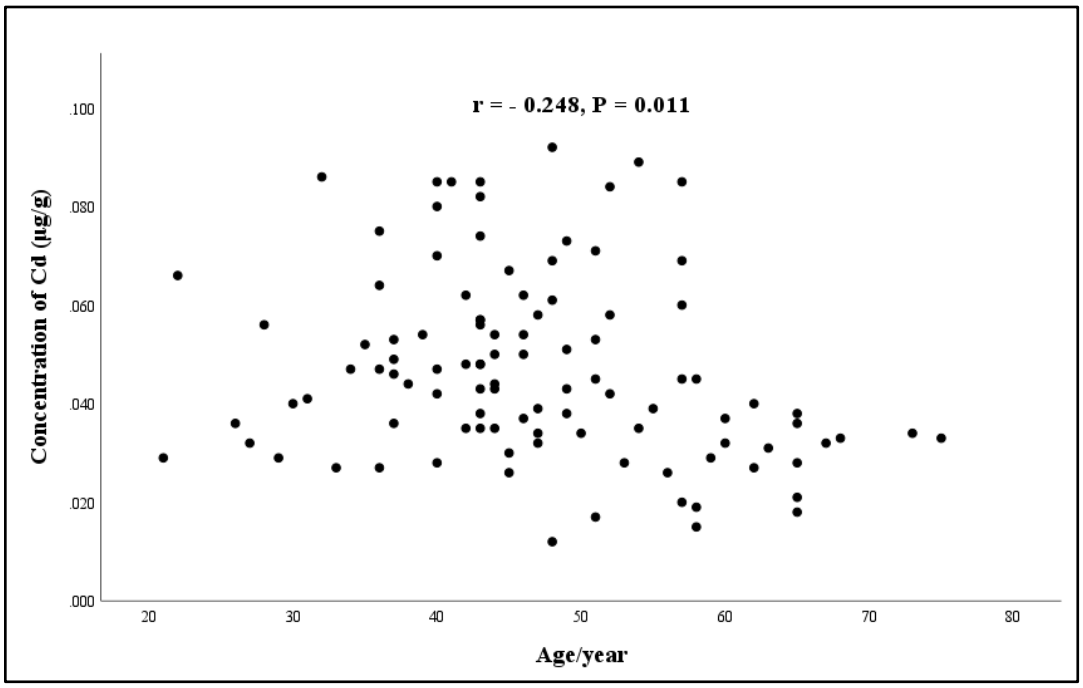


Figure 4.6: Correlation between cadmium levels (µg/g) and age in fingernails for total individuals at $P = 0.05$.

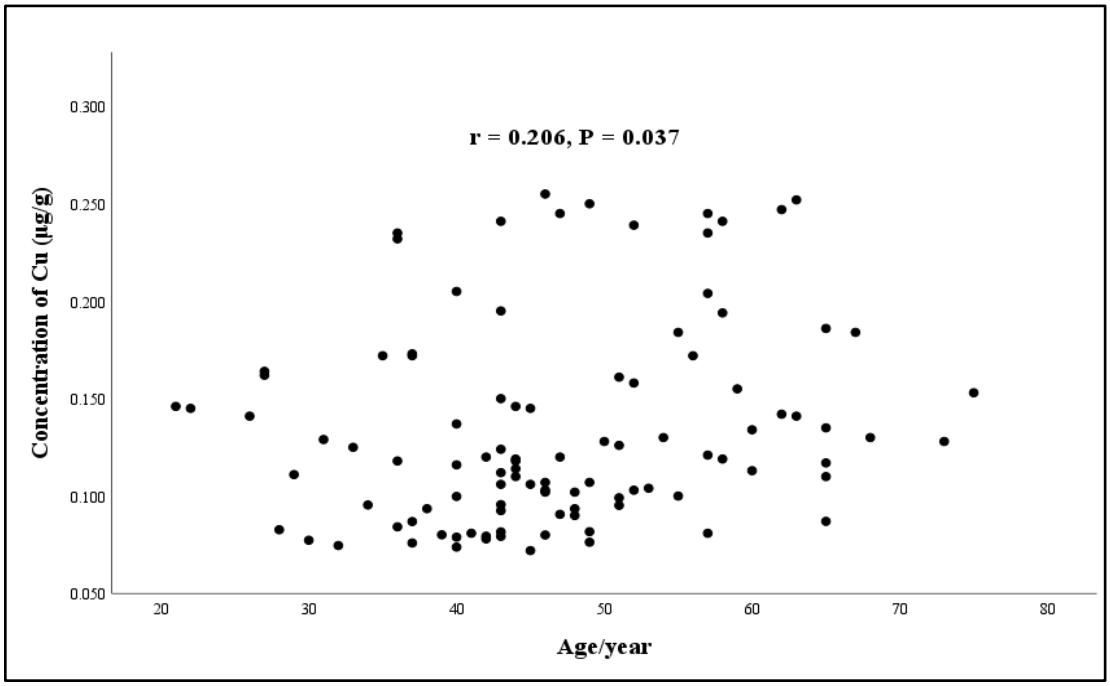


Figure 4.7: Correlation between cupper levels (µg/g) and age in fingernails for total individuals at $P = 0.05$.

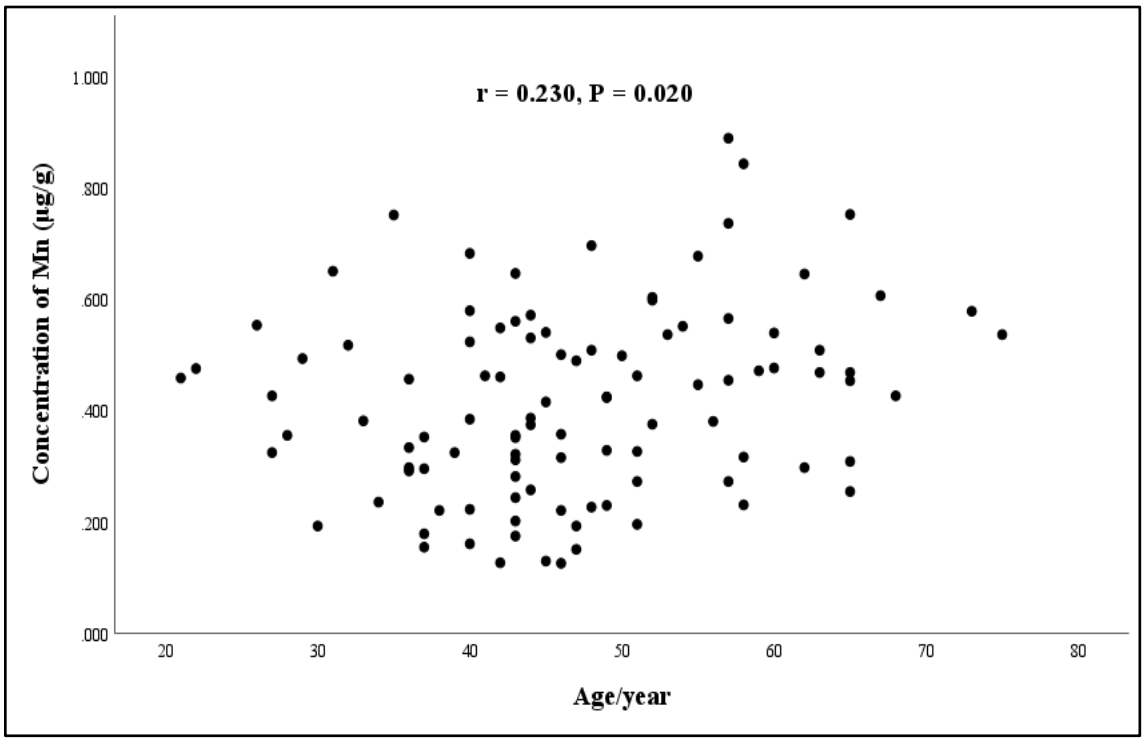


Figure 4.8: Correlation between manganese levels (µg/g) and age in fingernails for total individuals at $P = 0.05$.

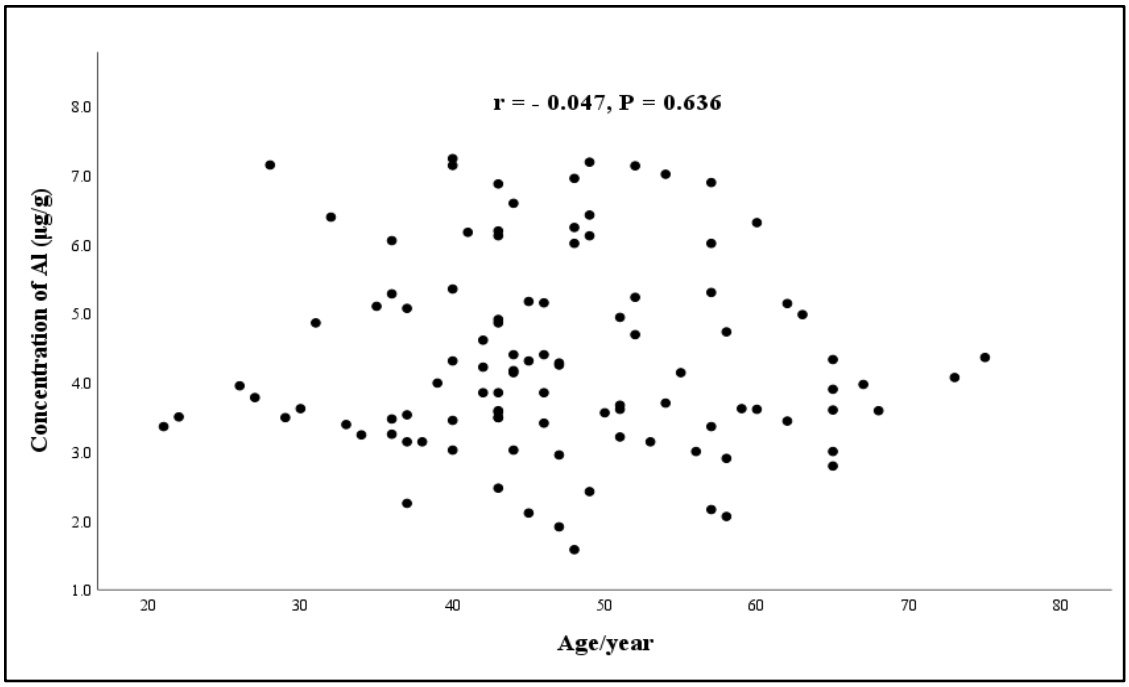


Figure 4.9: Correlation between aluminum levels (µg/g) and age in fingernails for total individuals at $P = 0.05$.

4.5 Interaction Effects

As described in Chapter Three, two interaction factors have been investigated for all the elements, as shown in the following sections:

4.5.1 Interaction Effects Between Factors

4.5.1.1 Interaction Between Health Status and Gender

Figures 4.10 – 4.16 show the significant interaction between health status and gender for Al, Cd, Cu, Mn, Pb, Se, and Zn. The results show that there are interactions between health status and gender for Cd, Cu, and Mn, as shown in Figures 4.10-4.12. Figures 4.10, 4.11, and 4.12 show the interaction between health status and gender for Cd, Cu, and Mn, respectively. For hemodialysis patients, the Cd, Cu, and Mn mean values were lowest for females, while for healthy individuals, the lowest Cd, Cu, and Mn mean values occurred for males. This suggests that healthy and hemodialysis subjects appear to respond differently to gender, and that to explore the effect of gender on the levels of Cd, Cu, and Mn in fingernails, one must consider the health status of participants.

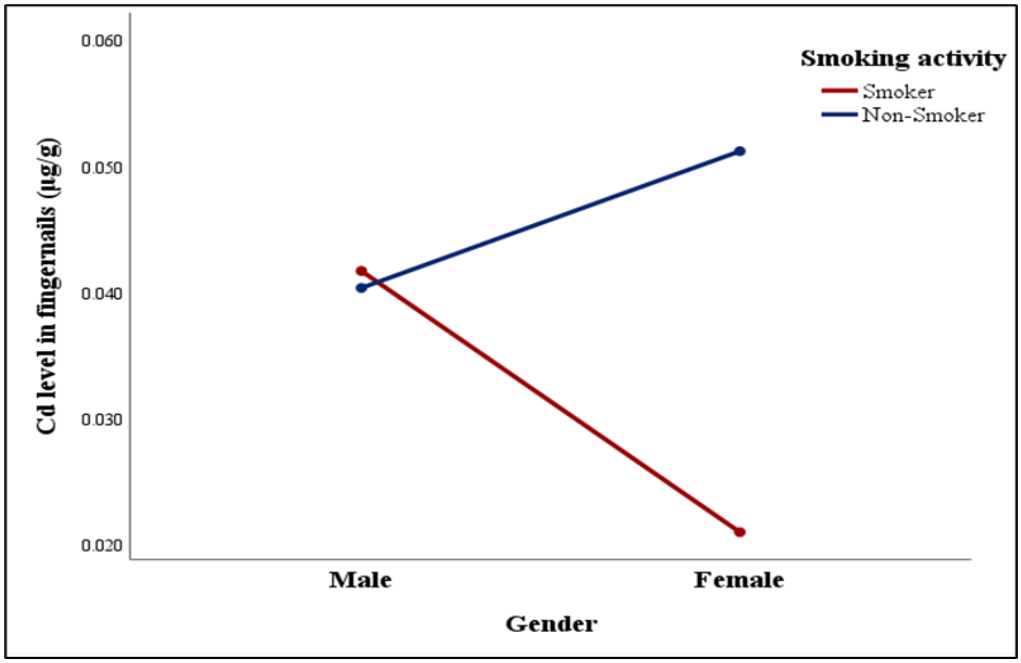


Figure 4.10: Interaction between health status and gender for Cd levels (µg/g) in fingernail samples.

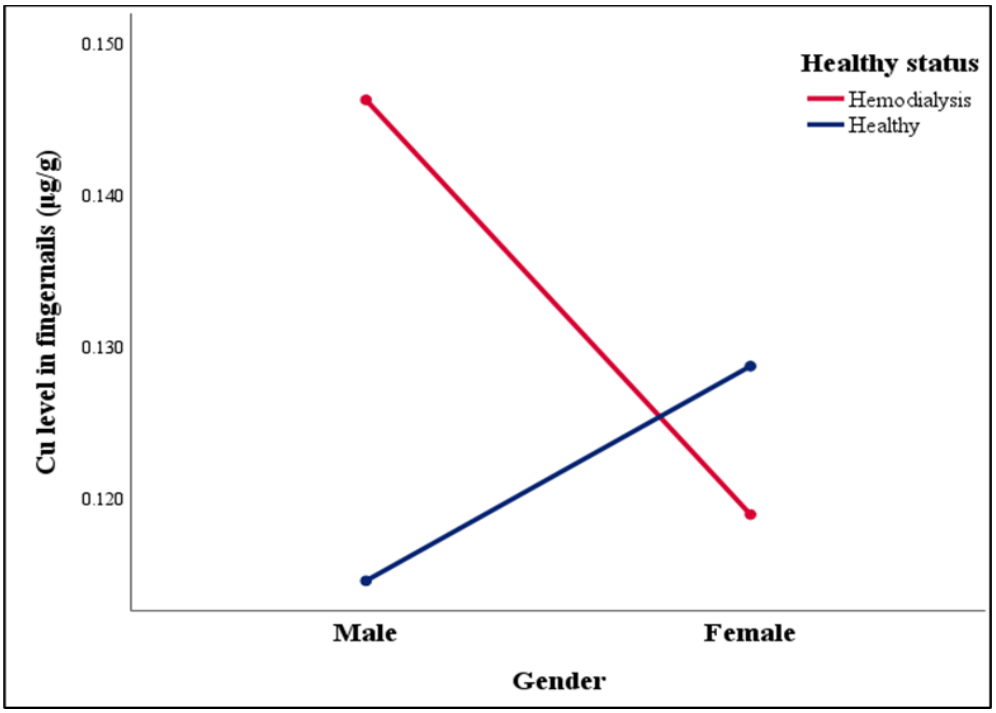


Figure 4.11: Interaction between health status and gender for Cu levels (µg/g) in fingernail samples.

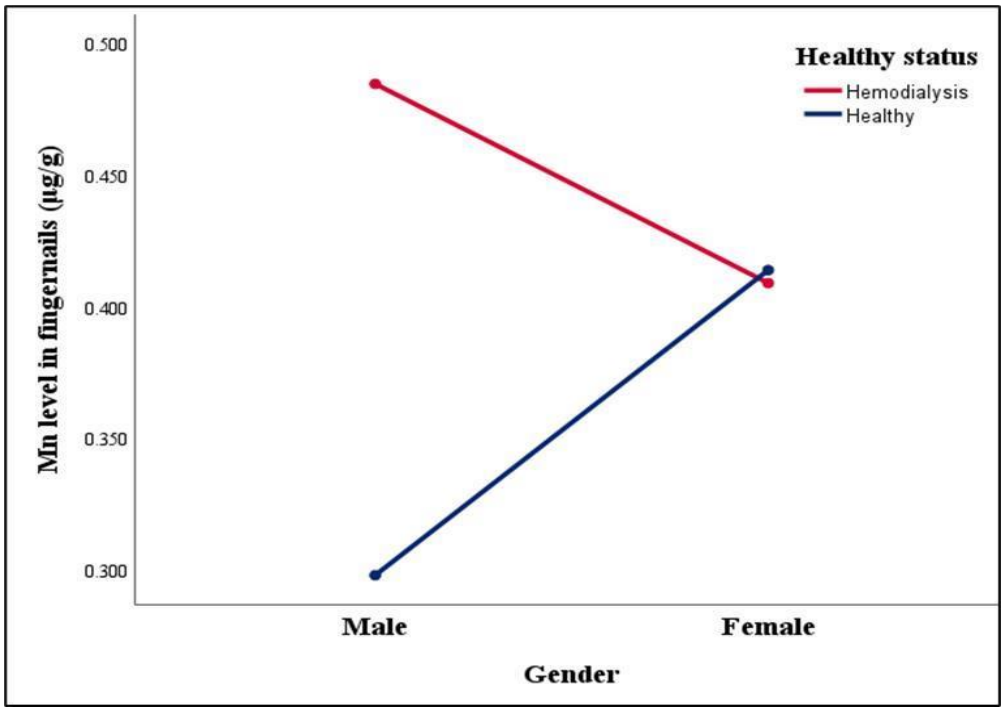


Figure 4.12: Interaction between health status and gender for Mn levels (µg/g) in fingernail samples.

Figures 4.13, 4.14, and 4.15 show the effect of health status (Al, Se, and Pb levels are reduced in hemodialysis patients), respectively, while the effect of gender (Al, Se, and Pb levels are reduced in healthy males and patient females than healthy females and patient males). In the case of Zn, the effect of health

status on the Zn levels is reduced in hemodialysis patients compared to healthy individuals of both genders. The effect of gender shows that the levels of Zn are similar for males and females in hemodialysis patients, while this effect makes the levels of Zn higher in healthy females than males, as shown in Figure 4.16.

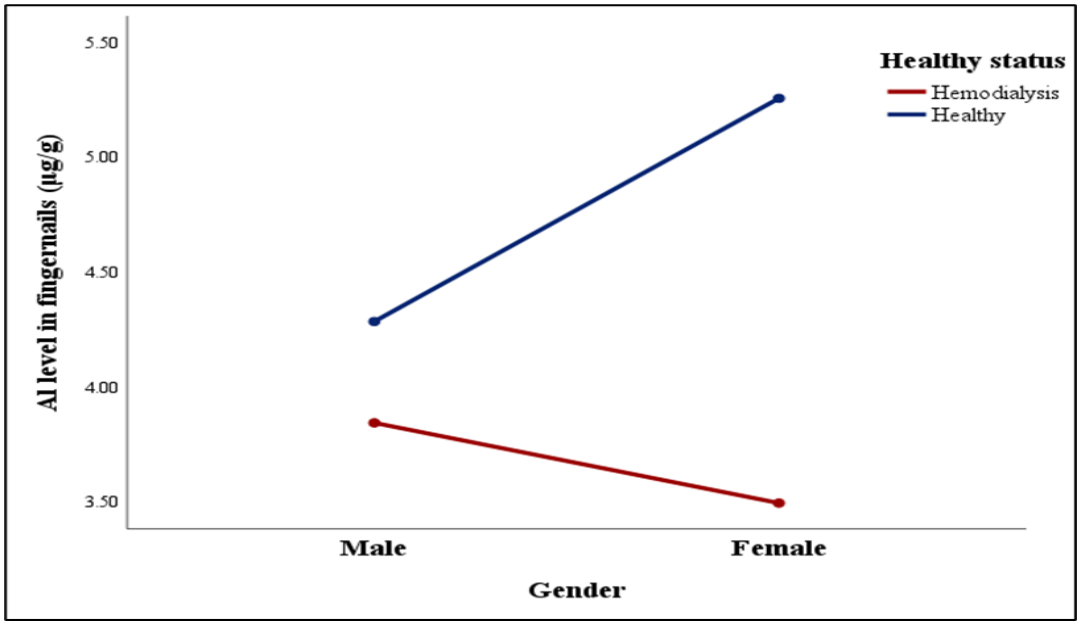


Figure 4.13: Interaction between health status and gender for Al levels (µg/g) in fingernail samples.

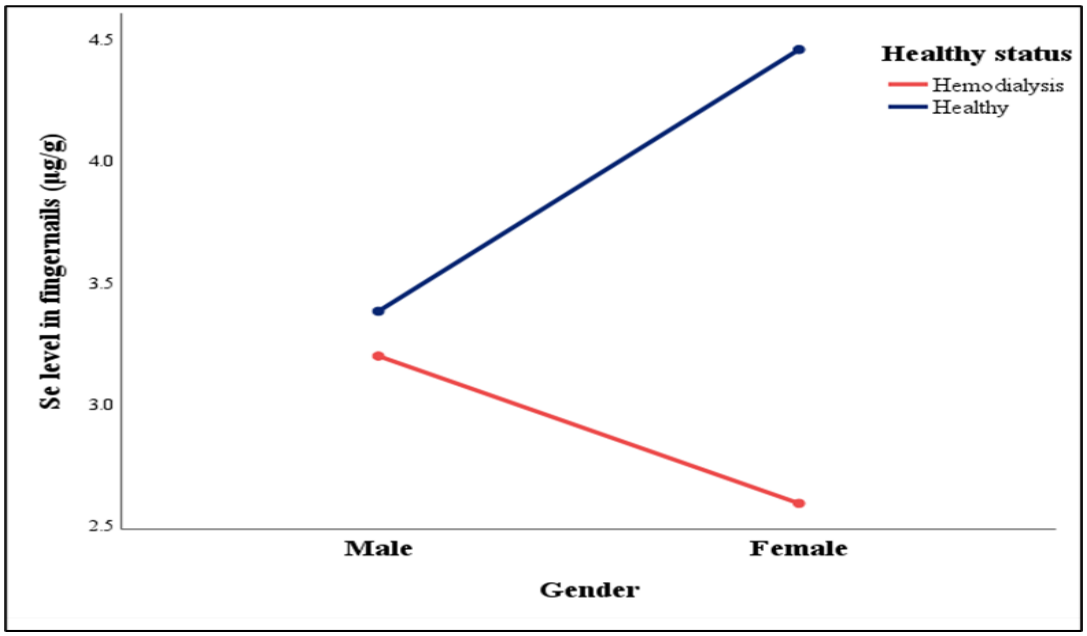


Figure 4.14: Interaction between health status and gender for Se levels (µg/g) in fingernail samples.

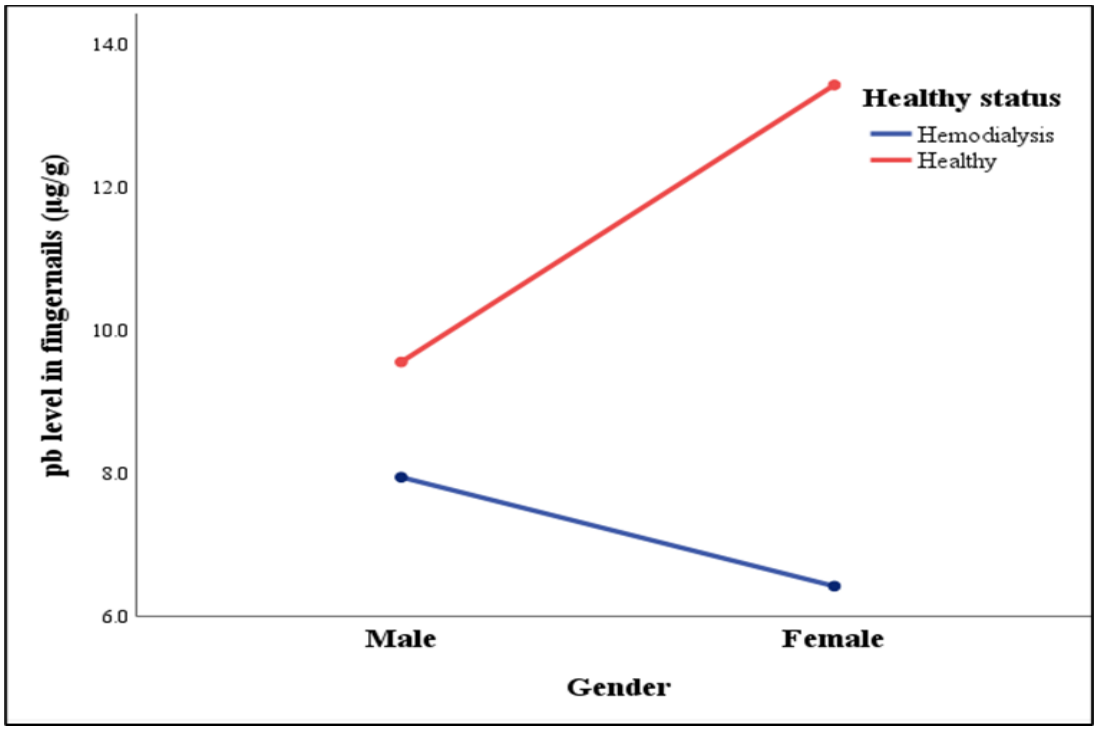


Figure 4.15: Interaction between health status and gender for pb levels (µg/g) in fingernail samples.

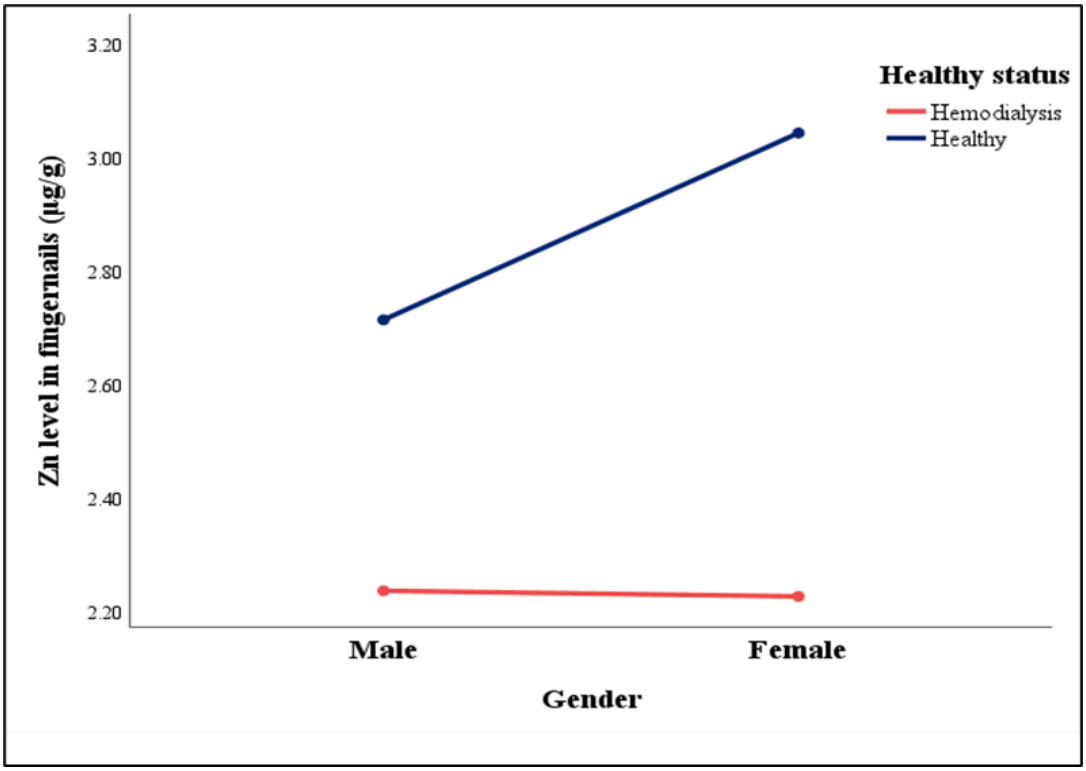


Figure 4.16: Interaction between health status and gender for Zn levels (µg/g) in fingernail samples.

4.5.1.2 Interaction Between Health Status and Smoking Activity

The interaction plots for Al, Cd, Mn, and Pb are similar, and there is no interaction between health status and gender due to the two lines of healthy and patient cases are parallel, as shown in Figures 4.17 -4.20. Se and Zn have similar plots with no interaction between smoking activity and healthy status, as presented in Figures 4.21-4.22. Figure 4.23 shows the interaction between health status and smoking activity for Cu. For hemodialysis patients and healthy individuals, the Cu mean value was lowest for smokers, while higher levels of Cu were found in non-smokers for both cases (healthy and patients).

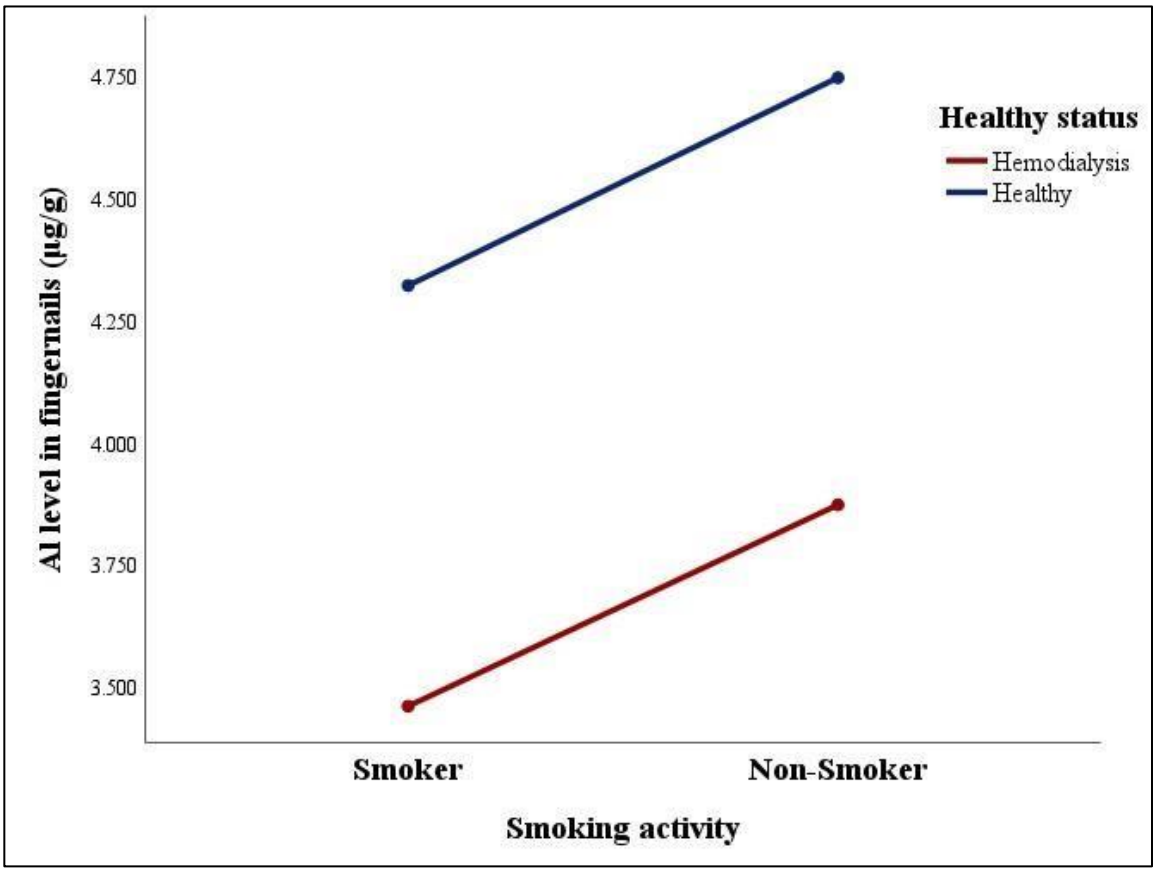


Figure 4.17: Interaction between health status and smoking activity for Al levels (µg/g) in fingernail samples.

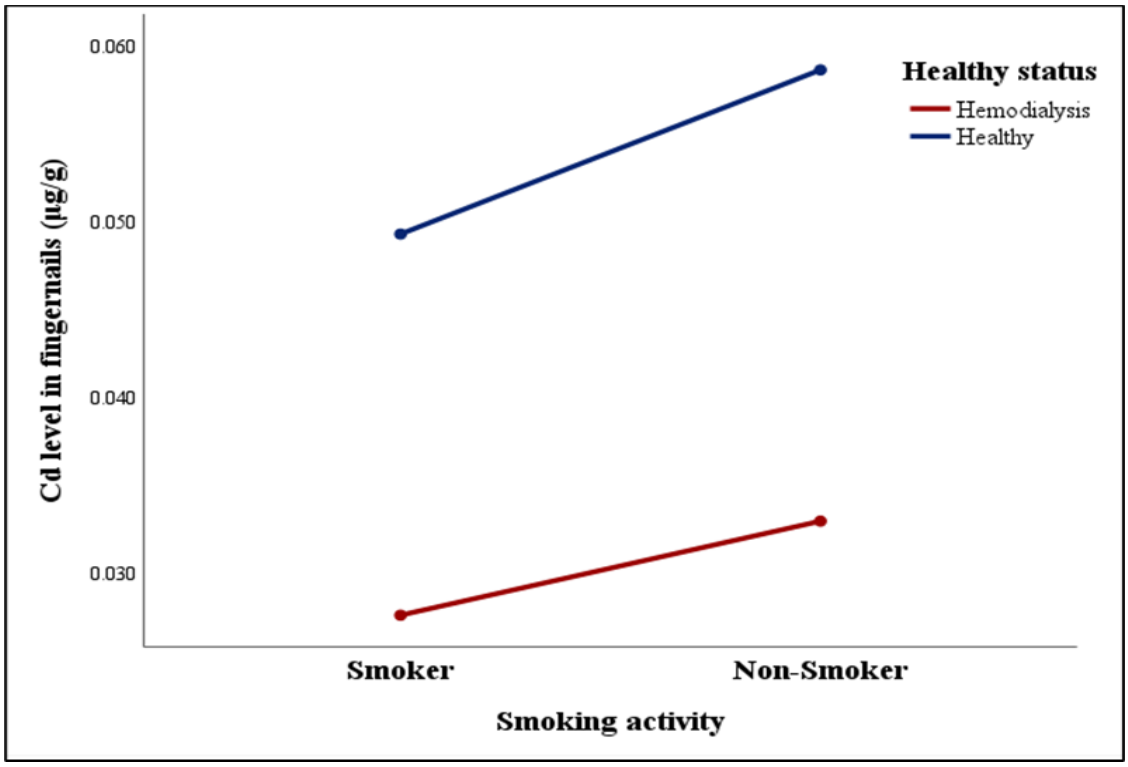


Figure 4.18: Interaction between health status and smoking activity for Cd levels (µg/g) in fingernail samples.

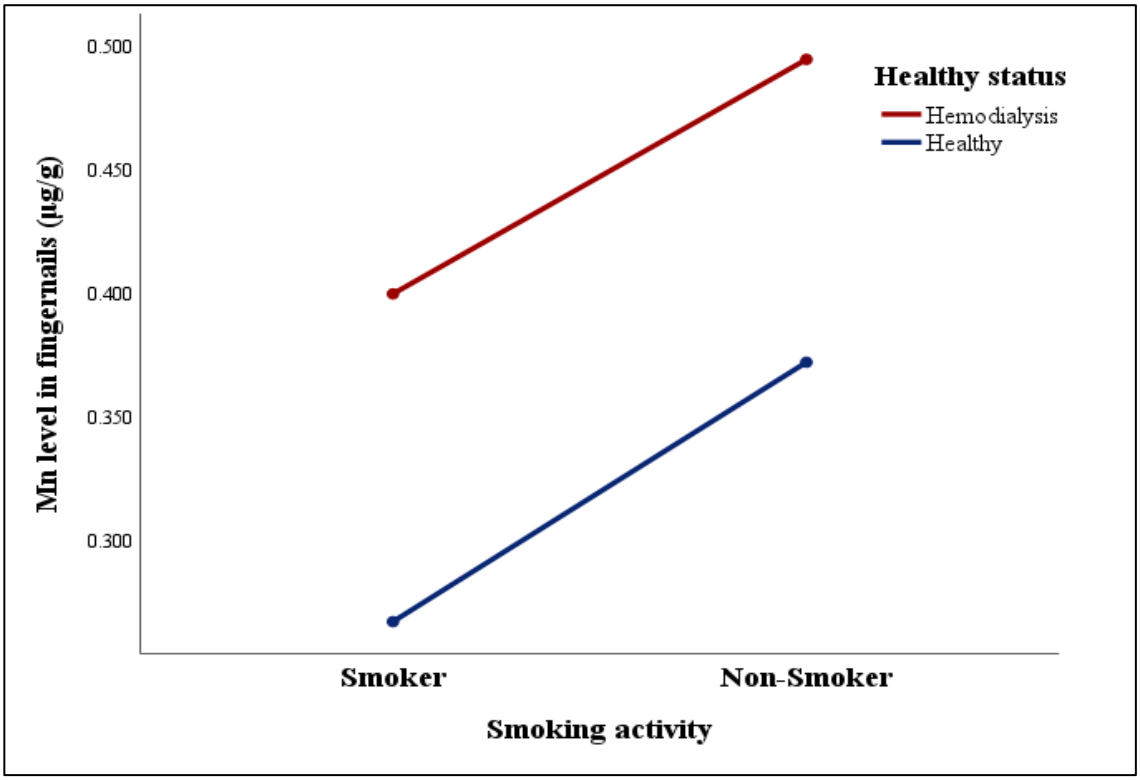


Figure 4.19: Interaction between health status and smoking activity for Mn levels (µg/g) in fingernail samples.

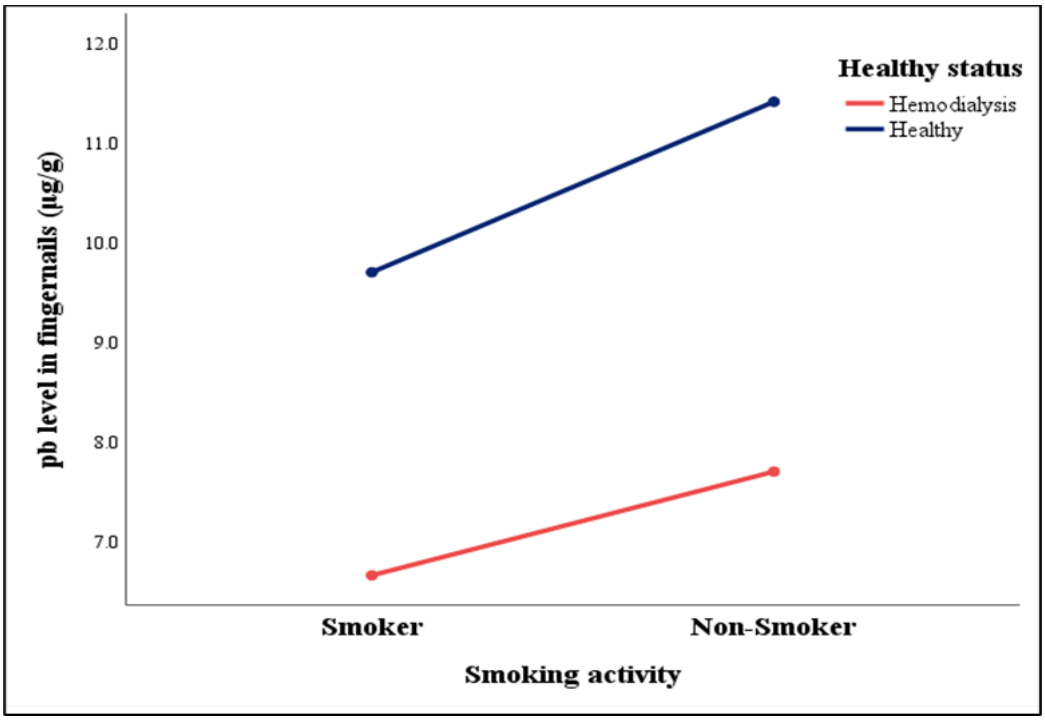


Figure 4.20: Interaction between health status and smoking activity for Pb levels (µg/g) in fingernail samples.

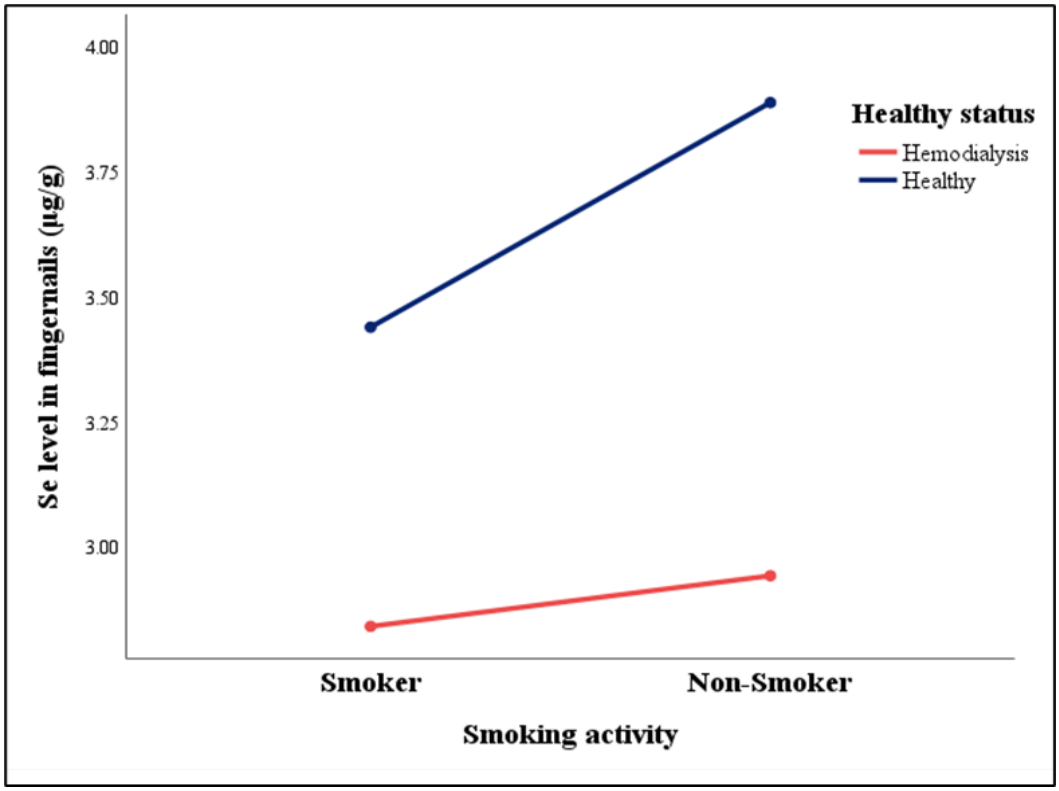


Figure 4.21: Interaction between health status and smoking activity for Se levels (µg/g) in fingernail samples.

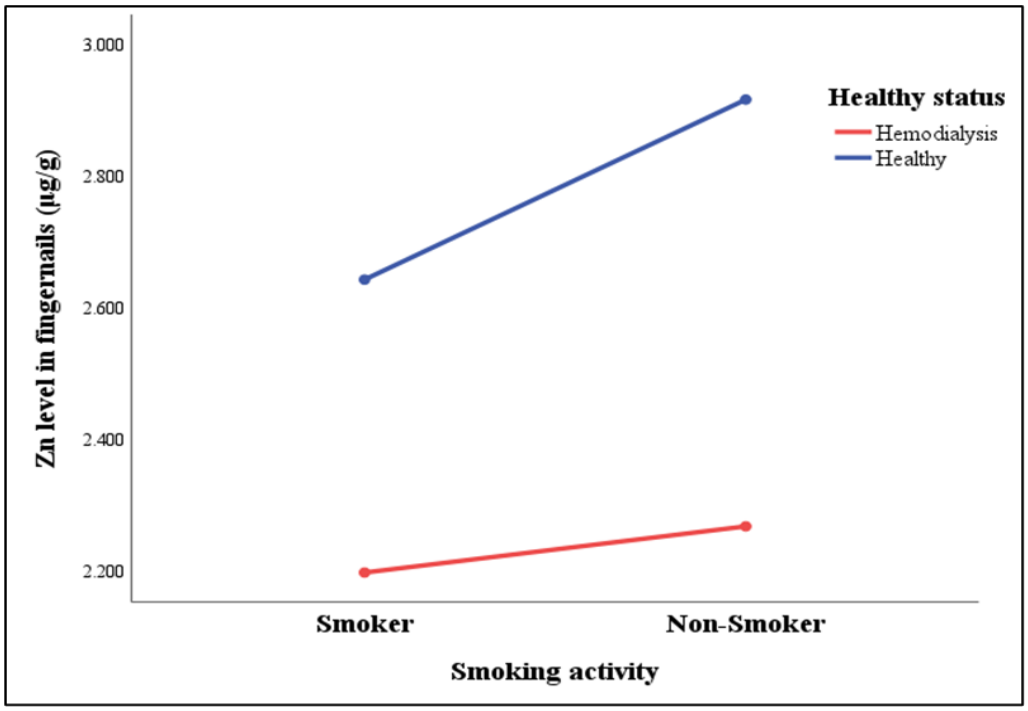


Figure 4.22: Interaction between health status and smoking activity for Zn levels (µg/g) in fingernail samples.

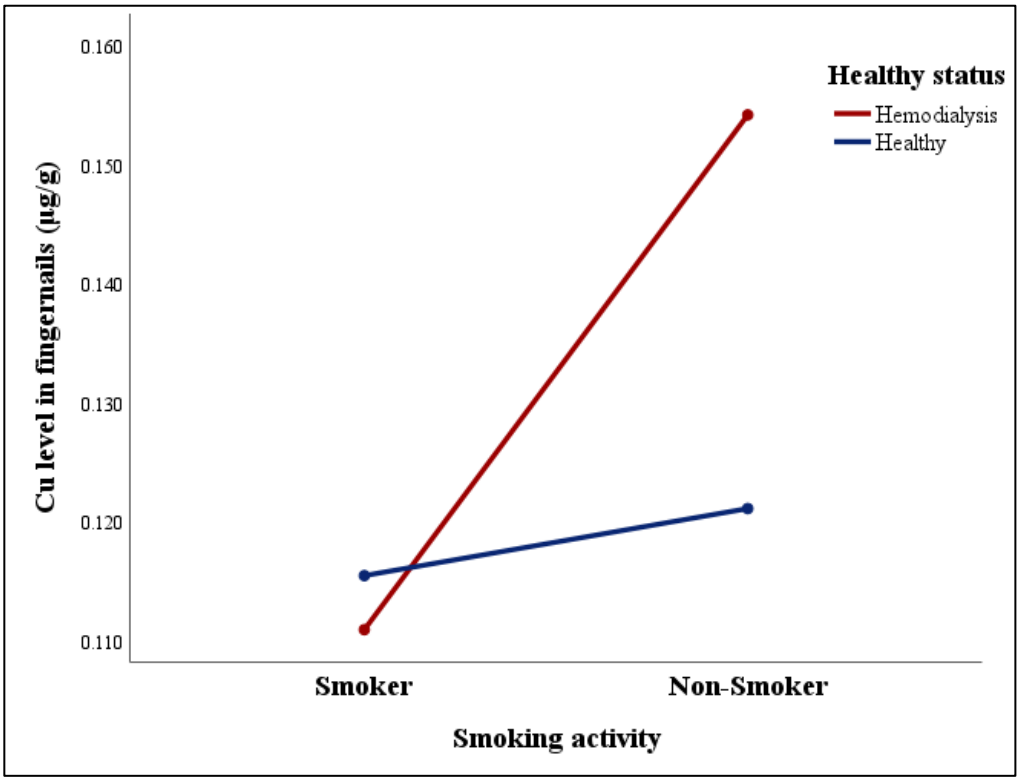


Figure 4.23: Interaction between health status and smoking activity for Cu levels (µg/g) in fingernail samples.

4.5.1.3 Interaction Between Gender and Smoking Activity

The interaction of smoking activity with gender was identified for Al, Cd, Cu, Mn, Pb, Se, and Zn. It was found that Al, Cd, Pb, and Se have similar significant interactions when compared with those for Cu, Mn, and Zn. Figures 4.24-4.27 show the interaction between smoking activity and gender for Al, Cd, Pb, and Se. In the case of smokers, the Al, Cd, Pb, and Se mean values were highest for males, while for non-smokers, however, the highest Al, Cd, Pb, and Se mean values occur for females. This suggests that smokers and non-smokers subjects appear to respond differently to gender, and that to explore the effect of gender on the levels of Al, Cd, Pb, and Se in fingernails, one must consider the smoking activities of participants. The remaining elements, namely Cu, Mn, and Zn did not have an interaction between smoking activity and gender, as shown in Figures 4.28-4.30.

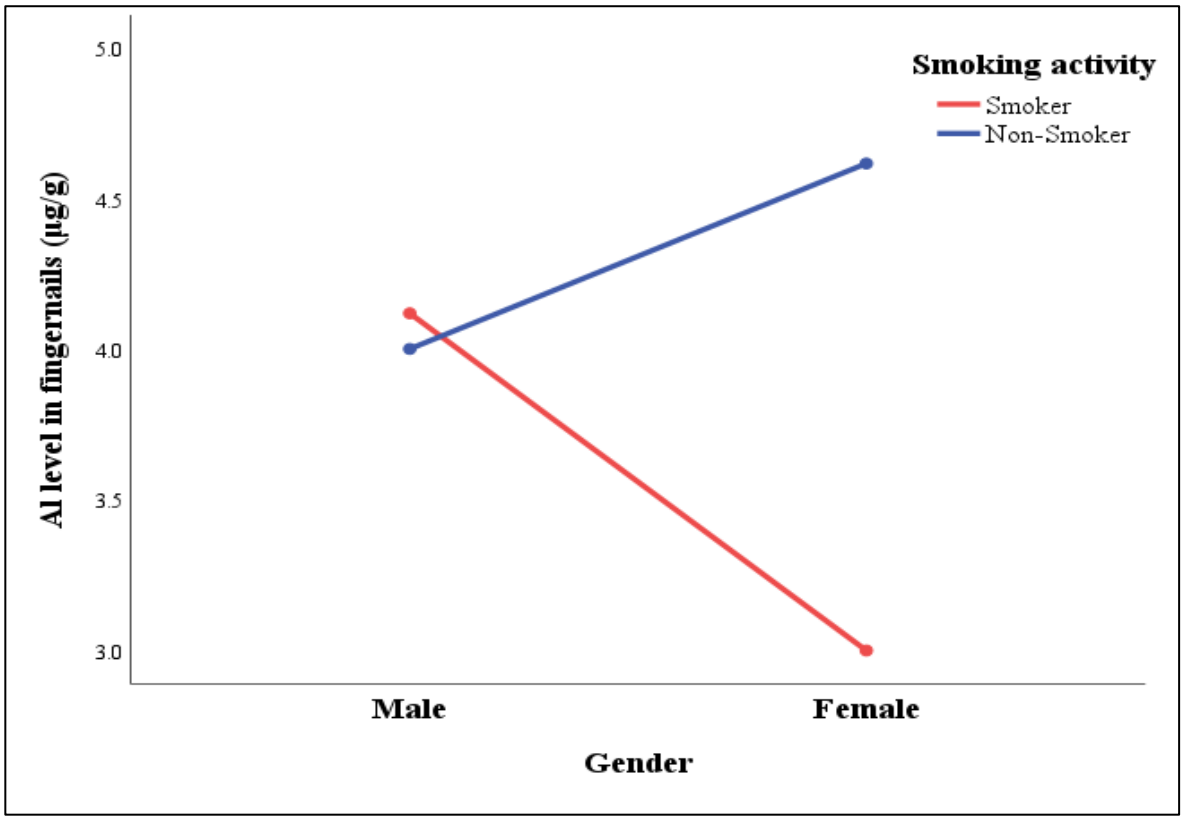


Figure 4.24: Interaction between gender and smoking activity for Al levels (µg/g) in serum samples.

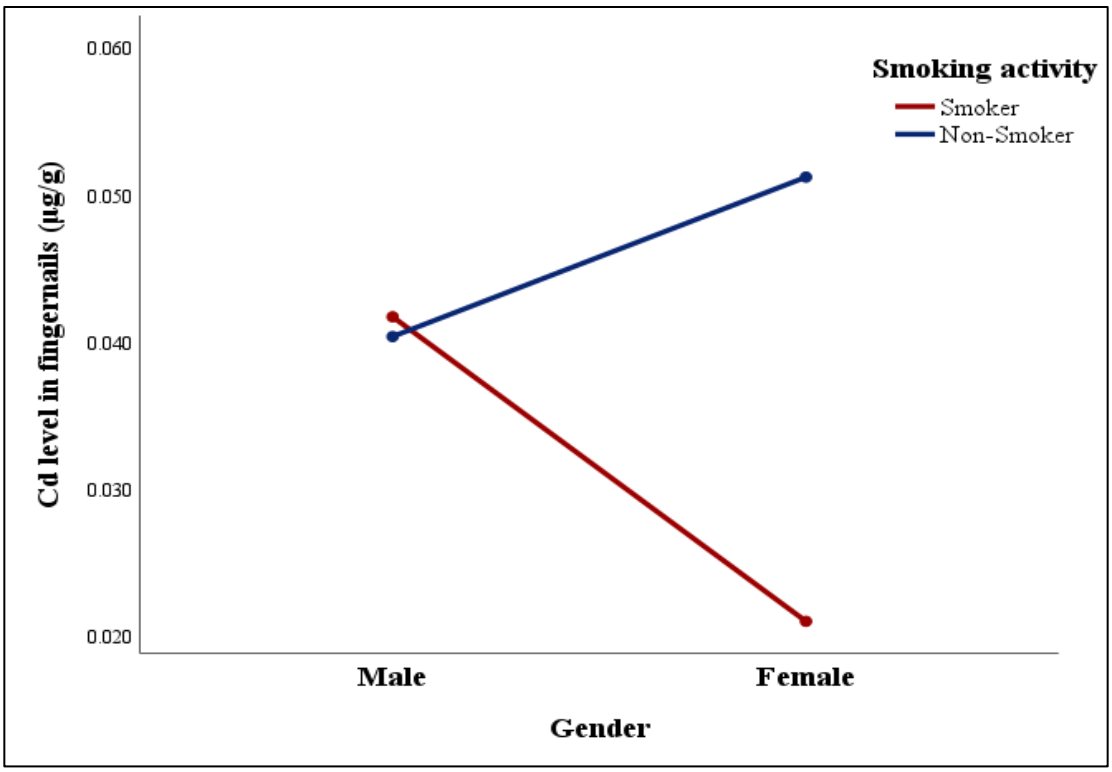


Figure 4.25: Interaction between gender and smoking activity for Cd levels (µg/g) in serum samples.

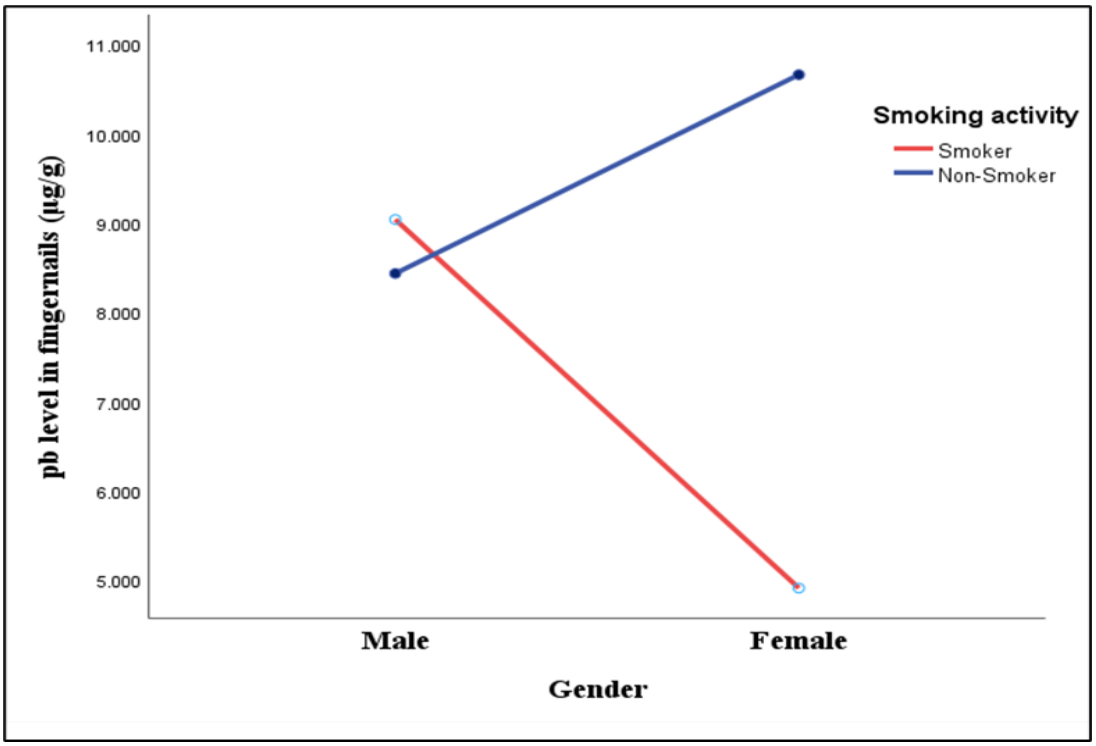


Figure 4.26: Interaction between smoking activity and gender for Pb levels (µg/g) in fingernail samples.

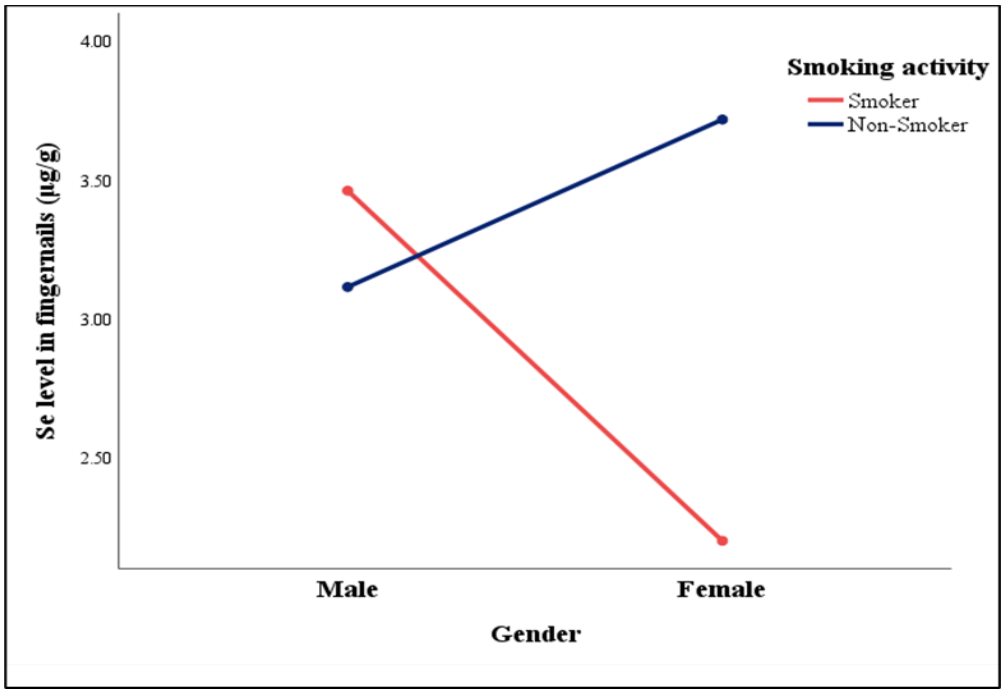


Figure 4.27: Interaction between smoking activity and gender for Se levels (µg/g) in fingernail samples.

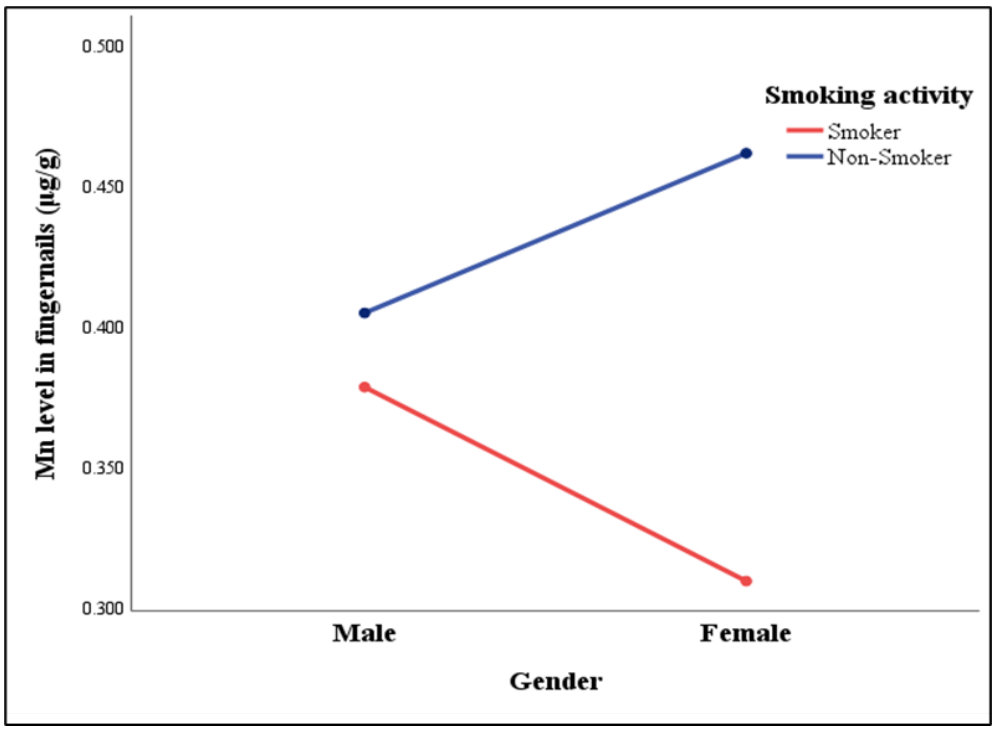


Figure 4.28: Interaction between smoking activity and gender for Mn levels (µg/g) in fingernail samples.

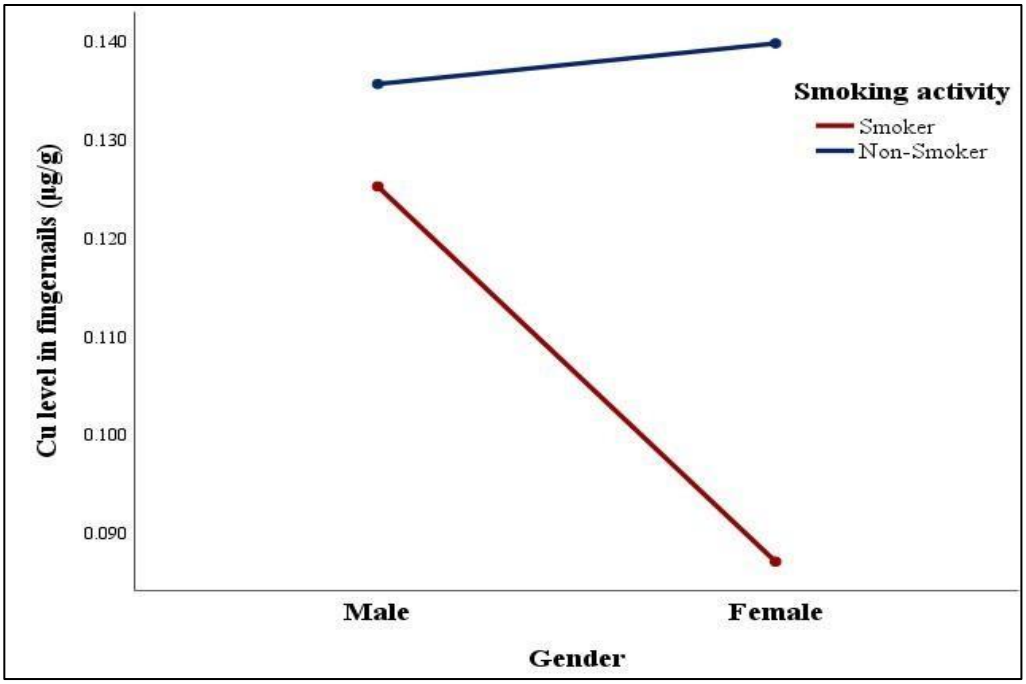


Figure 4.29: Interaction between smoking activity and gender for Cu levels (µg/g) in fingernail samples.

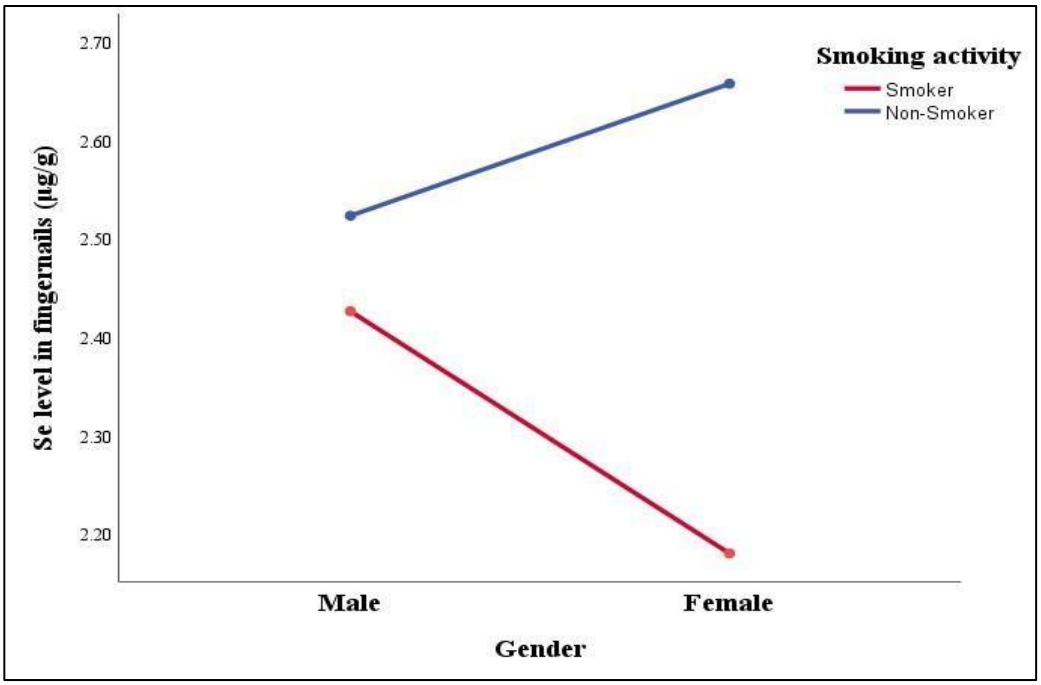


Figure 4.30: Interaction between smoking activity and gender for Zn levels (µg/g) in fingernail samples.

4.5.2 Inter-Element Correlations

Pearson's Product Correlation Coefficient (*r*) was used to investigate the relationship between the trace element levels in the fingernails of study subjects [54]. The r value was subjected to a significance test to examine whether r is significant at the 95% confidence interval ($P = 0.05$). The results of correlation coefficient trace elements in fingernails are reported in Table 4.7.

Significant correlations were found between the following elements at $P < 0.001$:

1- Al with Cd, Mn, Pb, and Se

2- Cd with Pb and Se

3- Cu with Mn

4- Mn with Pb and Se

5- Pb with Se

Table 4.7: Inter-element Person Correlation Coefficient (r) values for fingernails of subjects.

Element		Al	Cd	Cu	Mn	Pb	Se	Zn
Al	r	1						
	n	103						
	P							
Cd	r	0.774**	1					
	n	103	103					
	P	<0.001						
Cu	r	**0.192**	**0.073**	1				
	n	98	98	103				
	P	0.059	0.477					
Mn	r	0.495**	0.239*	0.422**	1			
	n	98	98	103	103			
	P	<0.001	0.018	<0.001				
Pb	r	0.766**	0.907**	**0.108**	0.283**	1		
	n	103	103	98	98	103		
	P	<0.001	<0.001	0.288	0.005			
Se	r	0.804**	0.858**	0.199*	0.406**	0.901**	1	
	n	103	103	98	98	103	103	
	P	<0.001	<0.001	0.049	<0.001	<0.001		
Zn	r	**0.079**	0.224*	**-0.007**	-0.187	**0.167**	0.253*	1
	n	98	98	103	103	98	98	103
	P	0.437	0.027	0.940	0.058	0.099	0.012	

** Correlation is significant at $P < 0.001$ level, * Correlation is significant at $P < 0.05$ level; **bold** values represent no significant correlation at $P < 0.05$; n is the number of samples.

Other correlation values were determined at the value of $P < 0.05$. In addition, fourteen values of correlation were reported with significant effects at both values of *P*. The highest correlation coefficient was found in fingernails between Cd-Pb ($r = 0.907$; $P < 0.001$) (Figure 4.31); Se-Pb ($r = 0.901$; $P < 0.001$) (Figure 4.32); followed by Cd-Se ($r = 0.858$; $P < 0.001$) (Figure 4.33); Al-Se ($r = 0.804$; $P < 0.001$) (Figure 4.34).

Selenium was statistically significantly correlated with the largest number of other elements (6 correlations), followed by Mn and Cd (5 correlations). Aluminum and Pb were statistically significantly associated with four correlations, and Zn and Cu were correlated significantly with two elements.

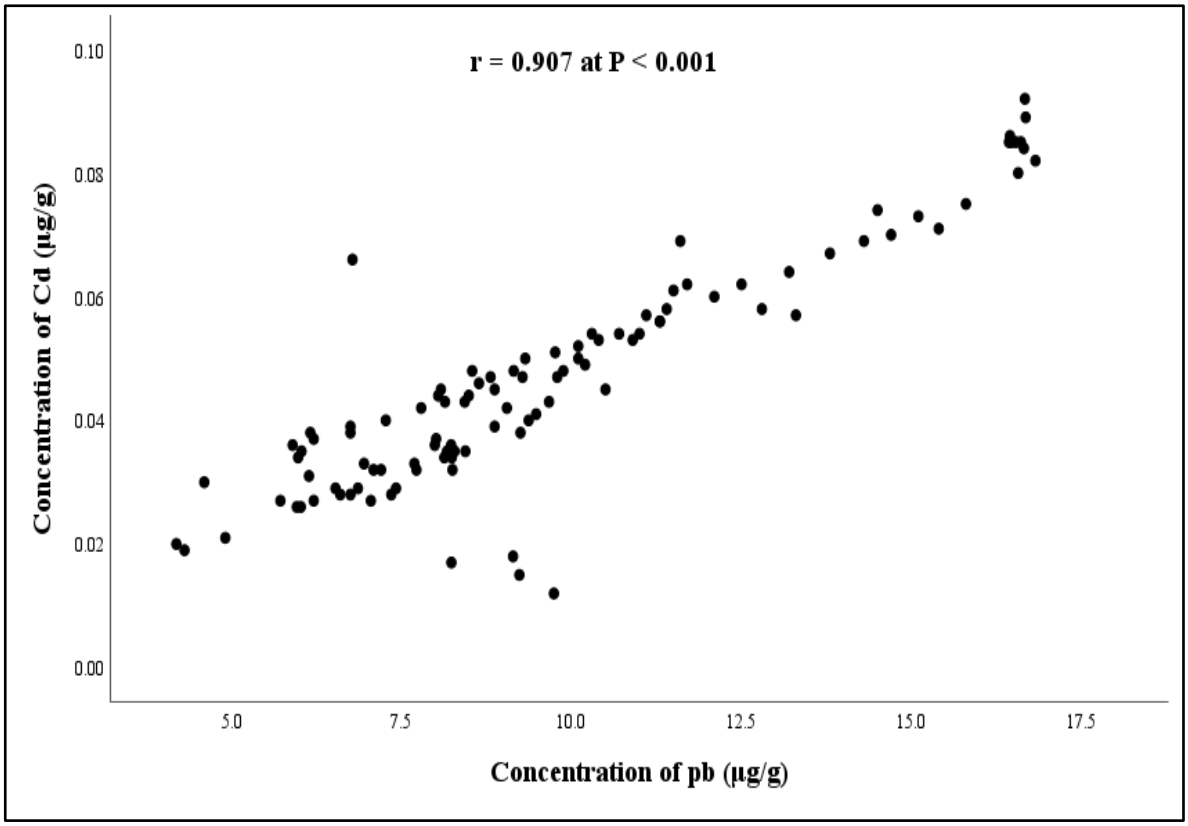


Figure 4.31: Correlation between Cd and Pb in fingernail samples (n = 103).

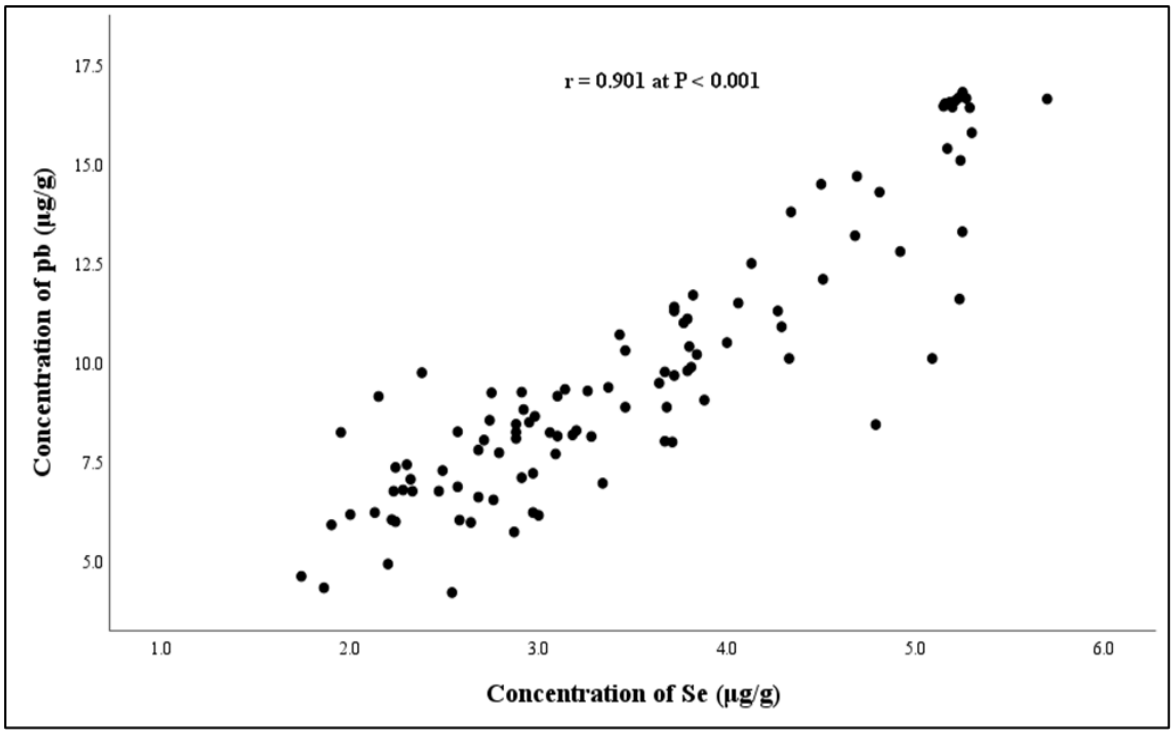


Figure 4.32: Correlation between Pb and Se in fingernail samples (n = 103).

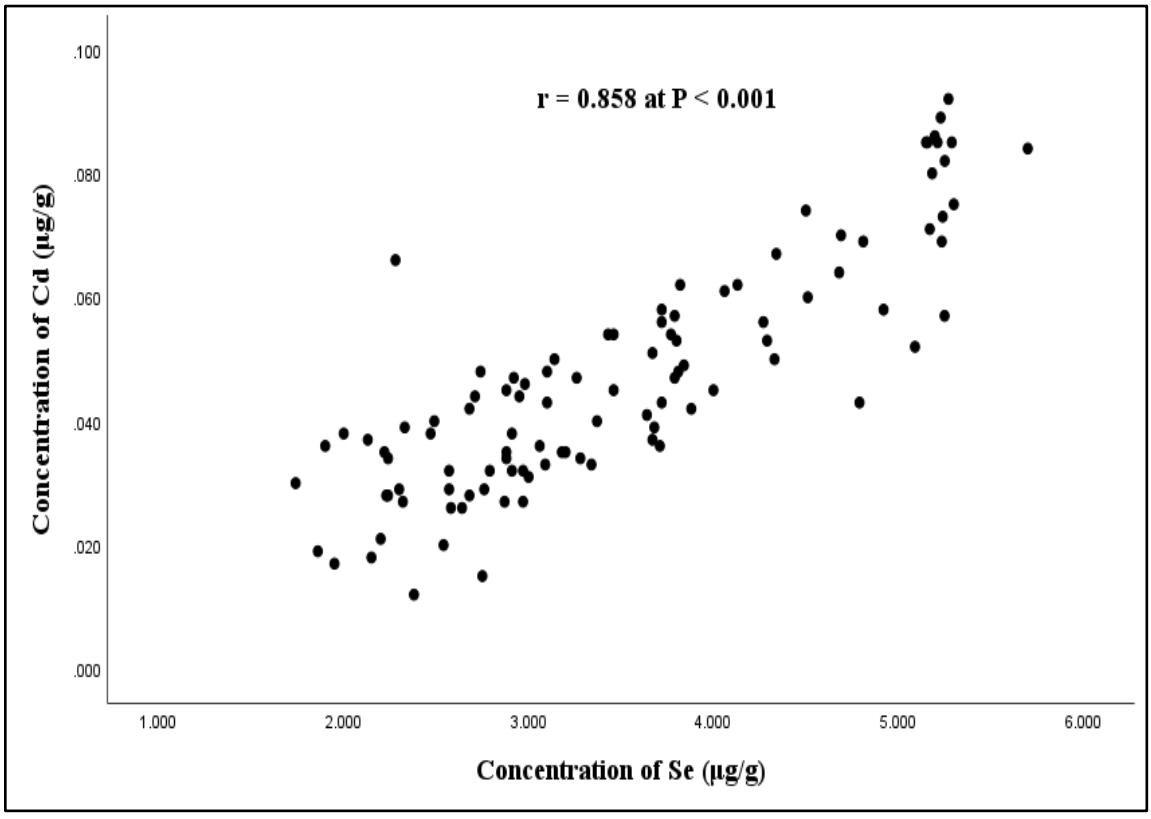


Figure 4.33: Correlation between Cd and Se in fingernail samples (n = 103).

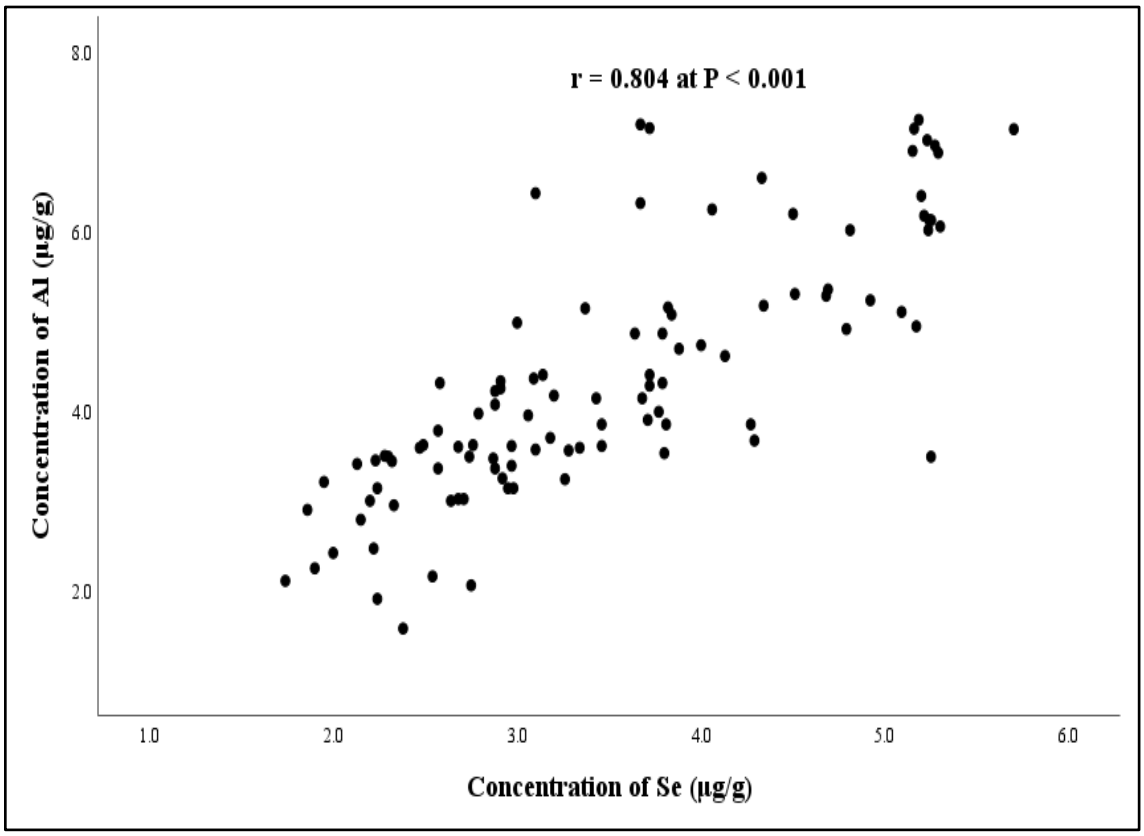


Figure 4.34: Correlation between Al and Se in fingernail samples (n = 103).

Chapter Five

Comparison Study, Conclusions, and Future Works

5.0 Introduction

In this chapter, the comparison study between the levels of trace elements in serum and fingernails for various groups, namely healthy/patients; smokers/non-smokers; males/females; and age. The relationships between the levels of trace elements in serum and fingernails have been determined using Pearson's Product Correlation Coefficient (r) along with t-test values.

5.1 Comparison of Trace Element Levels of Serum and Fingernails

The total samples of both serum and fingernails were provided by participants to identify whether there were any significant differences between the levels of trace elements in both media. The levels of trace elements in serum and fingernail samples as arithmetic mean, standard deviation (Sd), range, and 95% confidence interval for mean, and the number of samples for populations are summarized in Table 5.1. The results show that there are significant differences for all trace element levels between serum and fingernails using one-way ANOVA (Table 5.2) and two-tailed t-test (Table 5.3). Interestingly, similar results were found for both tests. The most significant levels were for Al ($t_{(110)} = 4.362$, $t_{crit} = 1.96$, $P < 0.001$), Cd ($t_{(110)} = 4.993$, $t_{crit} = 1.96$, $P < 0.001$), Cu ($t_{(110)} = 5.875$, $t_{crit} = 1.96$, $P < 0.001$), Mn ($t_{(110)} = 16.102$, $t_{crit} = 1.96$, $P < 0.001$), Pb ($t_{(110)} = 19.676$, $t_{crit} = 1.96$, $P < 0.001$), Se ($t_{(110)} = 3.266$, $t_{crit} = 1.96$, $P < 0.001$), and Zn ($t_{(110)} = 28.474$, $t_{crit} = 1.96$, $P < 0.001$), in the washed fingernails rather than serum. Figure 5.1 shows the mean and 95% confidence interval for the mean (lower-upper limits) for each element level in the serum and washed fingernail.

Table 5.1: Mean, standard deviation, range, and 95% confidence interval for the mean of trace element levels in serum and fingernails.

Element	Sample	n	Mean ± Sd (µg/ml or µg/g)	95% Confidence Interval for Mean		Range (µg/ml or µg/g)	
				Lower	Upper	Minimum	Maximum
Al	Fingernail	103	**4.349 ± 1.389**	4.077	4.621	1.580	7.236
	Serum	109	3.500 ± 1.442	3.226	3.774	0.999	7.150
Cd	Fingernail	103	**0.047 ± 0.019**	0.043	0.051	0.012	0.092
	Serum	109	0.035 ± 0.015	0.033	0.038	0.006	0.068
Cu	Fingernail	103	**0.134 ± 0.051**	0.124	0.144	0.072	0.255
	Serum	109	0.102 ± 0.024	0.097	0.107	0.052	0.154
Mn	Fingernail	103	**0.415 ± 0.168**	0.383	0.448	0.126	0.890
	Serum	109	0.149 ± 0.038	0.142	0.156	0.064	0.231
Pb	Fingernail	103	**9.761 ± 3.337**	9.109	10.413	4.190	16.822
	Serum	109	3.291 ± 0.784	3.142	3.440	1.660	4.932
Se	Fingernail	103	**3.498 ± 1.044**	3.294	3.702	1.740	5.700
	Serum	109	3.003 ± 1.158	2.783	3.222	0.773	5.630
Zn	Fingernail	103	**2.568 ± 0.835**	2.405	2.731	1.094	4.524
	Serum	109	0.282 ± 0.067	0.270	0.295	0.143	0.455

Sd = standard deviation, n = number of samples.

Table 5.2: Analysis of variance ANOVA for trace element levels in serum and fingernails for individuals.

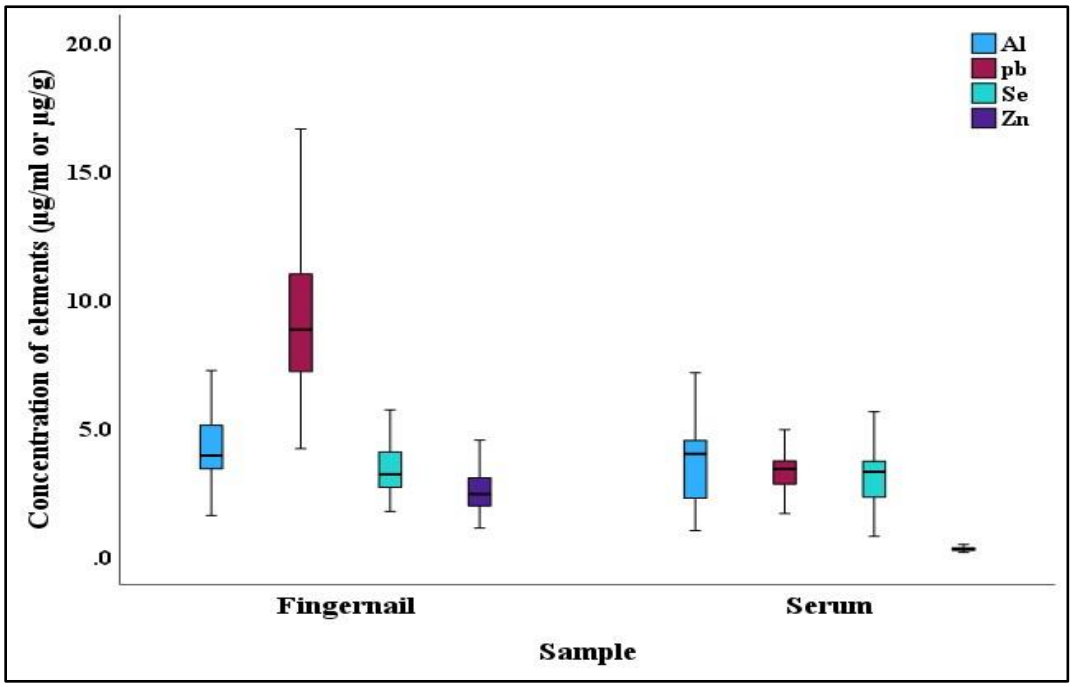
Element	Source of variance	Sum of Squares	*df*	Mean Square	*F*	Sig.
Al	Between Groups	38.178	1	38.178	19.028	**< 0.001**
	Within Groups	421.349	210	2.006		
	Total	459.528	211			
Cd	Between Groups	0.007	1	0.007	24.934	**< 0.001**
	Within Groups	0.060	210	0.000		
	Total	0.067	211			
Cu	Between Groups	0.055	1	0.055	34.516	**< 0.001**
	Within Groups	0.332	210	0.002		
	Total	0.387	211			
Mn	Between Groups	3.759	1	3.759	259.278	**< 0.001**
	Within Groups	3.045	210	0.014		
	Total	6.804	211			
Pb	Between Groups	2216.763	1	2216.763	387.149	**< 0.001**
	Within Groups	1202.432	210	5.726		
	Total	3419.195	211			
Se	Between Groups	13.005	1	13.005	10.665	**0.001**
	Within Groups	256.072	210	1.219		
	Total	269.076	211			
Zn	Between Groups	276.660	1	276.660	810.751	**< 0.001**
	Within Groups	71.660	210	.341		
	Total	348.320	211			

df = degrees of freedom, for between-groups (*dfB*) = number of groups – 1; within-group (*dfW*) = *dfT* – *dfB*; Total number of degrees of freedom (*dfT)* = number of observations – 1, mean square = (SS/*df*), *F* is the calculated value for F-test, *F* = MSB/MSW, Sig. is the significance level.

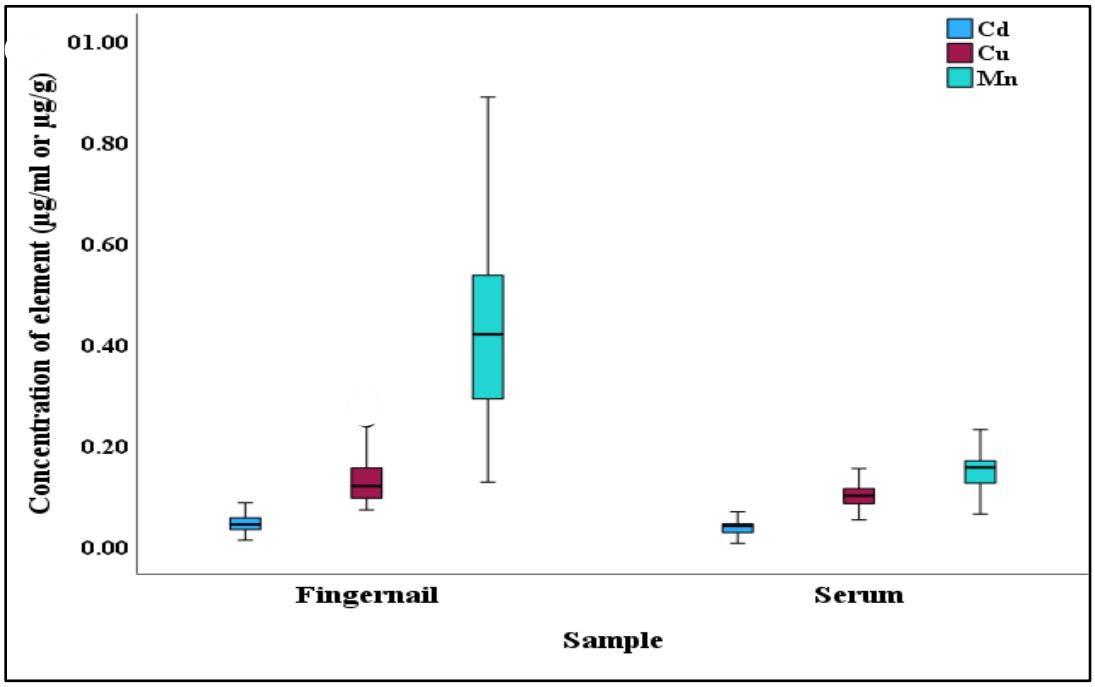
Table 5.3: Elemental levels of serum and fingernail samples.

Element (n_1, n_2)	Concentration Mean ± Sd		F-test			Two-tailed t-test		
	Serum (µg/mL)	Fingernail (µg/g)	Variance	*F*	Sig.	*t*	*df*	Sig.
Al (109,103)	3.499 ± 1.442	4.349 ± 1.389	Equal variances assumed Equal variances not assumed	1.100	0.296	4.362 4.367	210 210	**< 0.001** **< 0.001**
Cd (109,103)	0.035 ± 0.015	0.047 ± 0.019	Equal variances assumed Equal variances not assumed	5.273	0.023	4.993 4.961	210 195	**< 0.001** **< 0.001**
Cu (109,103)	0.102 ± 0.024	0.134 ± 0.051	Equal variances assumed Equal variances not assumed	44.153	< 0.001	5.875 5.770	210 144	**< 0.001** **< 0.001**
Mn (109,103)	0.149 ± 0.038	0.415 ± 0.168	Equal variances assumed Equal variances not assumed	137.892	< 0.001	16.102 15.693	210 112	**< 0.001** **< 0.001**
Pb (109,103)	3.291 ± 0.784	9.760 ± 3.337	Equal variances assumed Equal variances not assumed	106.104	< 0.001	19.676 19.181	210 113	**< 0.001** **< 0.001**
Se (109,103)	3.003 ± 1.158	3.498 ± 1.044	Equal variances assumed Equal variances not assumed	0.347	0.556	3.266 3.275	210 210	**< 0.001** **< 0.001**
Zn (109,103)	0.282 ± 0.067	2.568 ± 0.835	Equal variances assumed Equal variances not assumed	169.031	< 0.001	28.474 27.685	210 103	**< 0.001** **< 0.001**

n_1, n_2 are the number of samples for serum and fingernail samples, respectively, *df* = degree of freedom, n_1-1 and n_2-1 for F-test, degree of freedom for t-test (n_1+n_2-2), *F* and *t* are the calculated values for F-test and t-test, respectively, the bold values indicate significant differences at the level of significance *P* = 0.05, Sig. = level of significance. $t_{crit.}$ = 1.96 at *P*=0.05.



(a)



(b)

Figure 5.1: Trace element levels (µg/ml or µg/g) (a & b) for different elements (n = 109 for serum and 103 for fingernail) of individuals from Karbala (Iraq); middle

band, box, and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively.

Figures 5.2 and 5.3 show the comparison study between the levels of trace elements in serum and fingernail samples. The results show that the mean values for all elements are higher in fingernails than in serum.

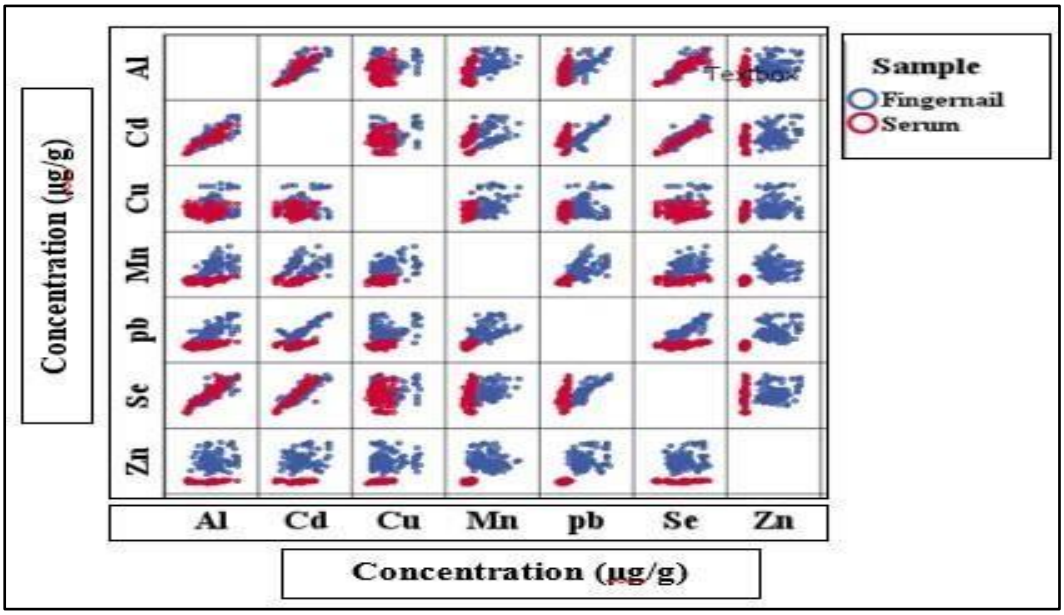


Figure 5.2: Comparison study for the correlation between the levels of elements in serum and fingernails.

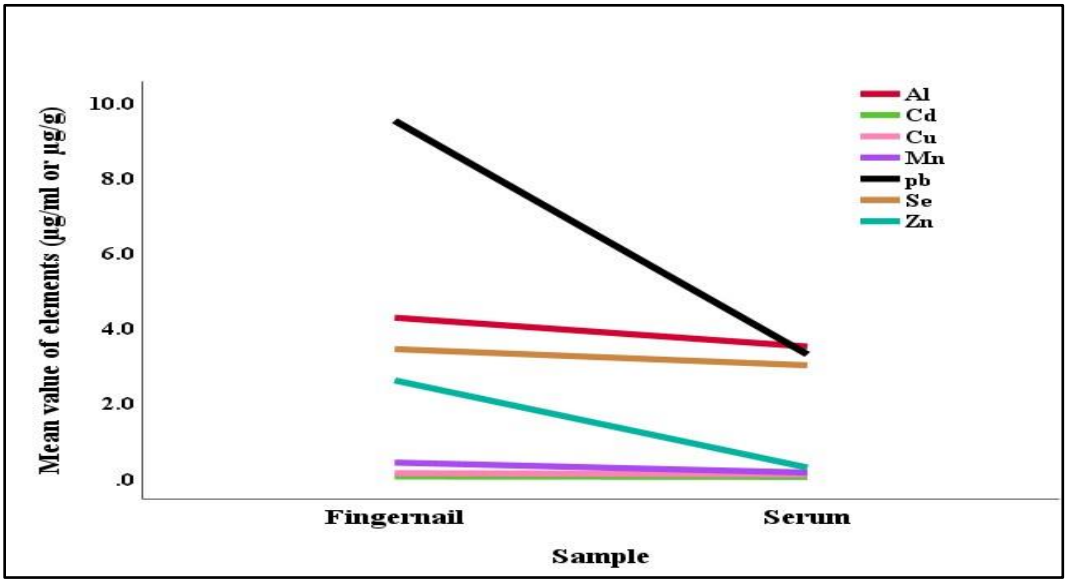


Figure 5.3: Elemental levels in different media for individuals who provided serum and fingernails.

Figures 5.4 - 5.6 show the strong linear relationships between the levels of trace elements in serum and fingernail.

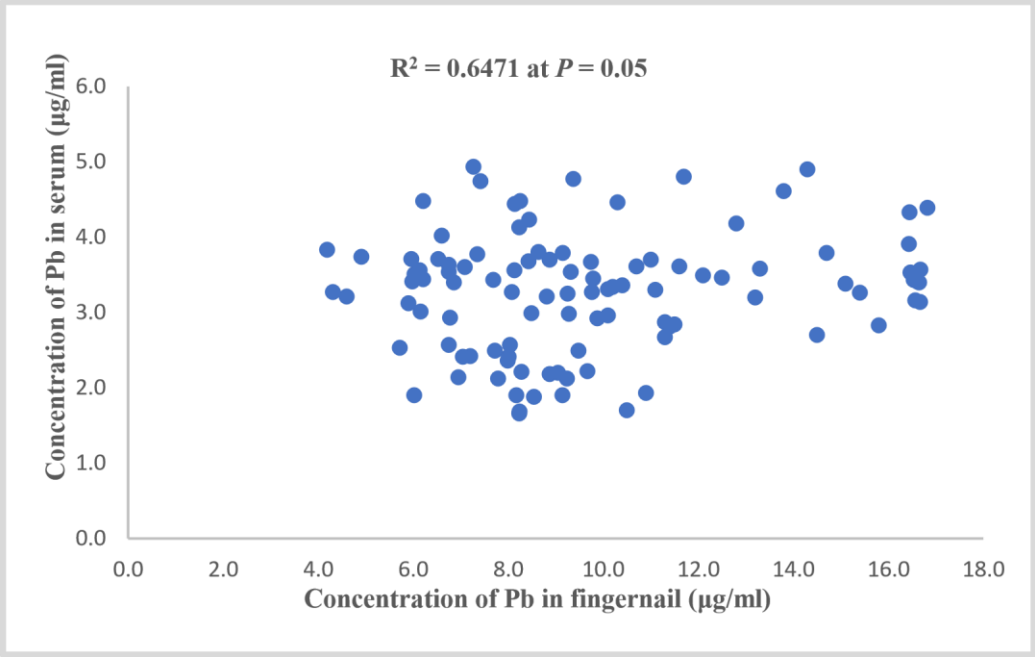


Figure 5.4: Correlation between Pb levels in serum and fingernail samples.

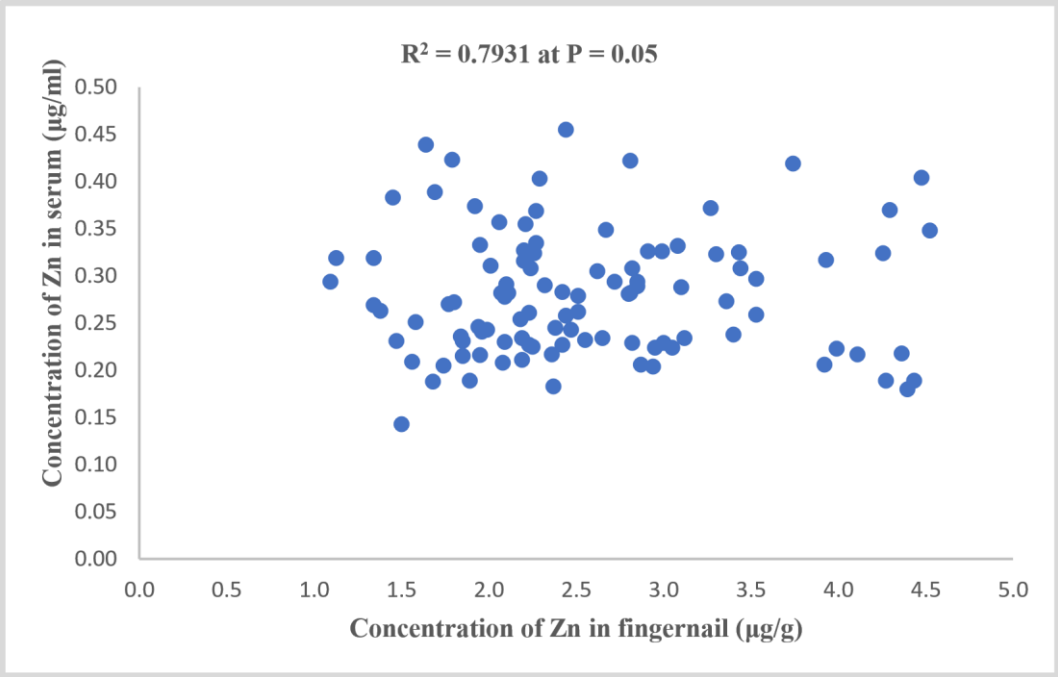


Figure 5.5: Correlation between Zn levels in serum and fingernail samples.

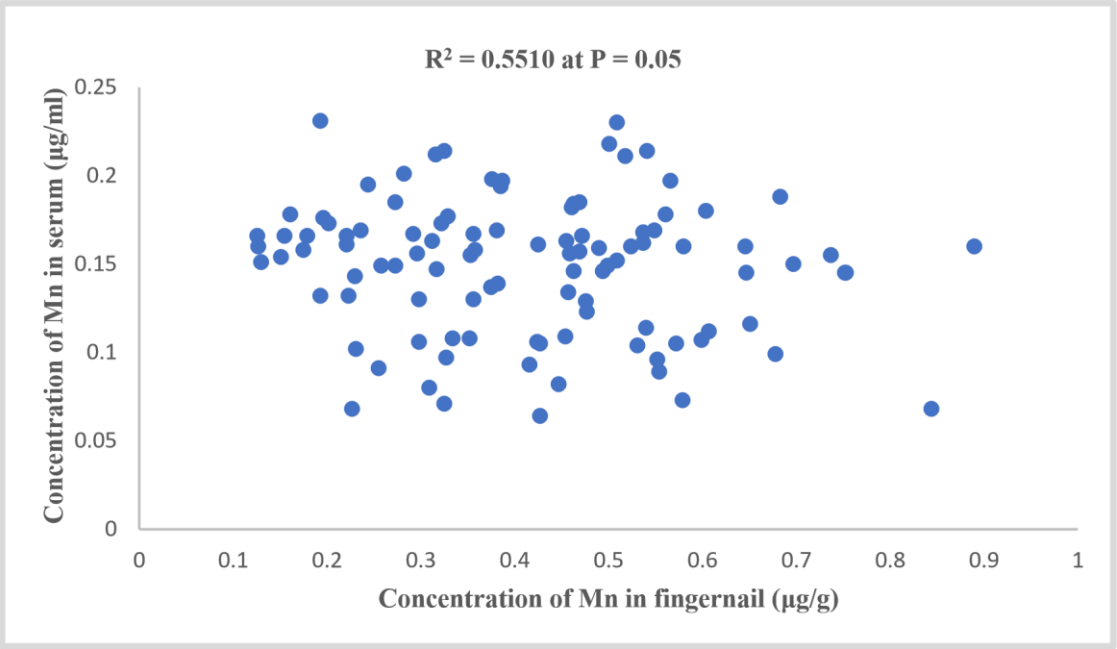


Figure 5.6: Correlation between Mn levels in serum and fingernail samples.

5.2 Conclusions

The use of human fluids (Serum) and tissues (fingernails) as biomarkers for trace elements (Al, Cd, Cu, Mn, Pb, Se, and Zn) in the human body have been investigated in this study. Fingernails has been selected to be a possible biomarker in this research. Serum fluid was also used for comparative analysis with fingernail results. The analytical methodological issues were described in Chapter 2, with appropriate dilution and digestion of samples and optimised instrumental conditions. Chapter 3 presented the results of the serum samples. The elemental results for fingernails were reported in Chapter 4. The comparative study data between fingernail and serum was outlined in Chapter 5.

5.2.1 Analytical Techniques and Methodology

The sampling process is the first step of any chemical analysis. Therefore, attention should be paid to handling and storing samples to minimize chemical contamination, loss, decomposition, and matrix change. Using a glass container to analyze trace elements leads to contamination as the sample matrix may be adsorbed on the surface of the container or the elements may be desorbed from the container surface into the sample solution. Therefore, all the samples must be collected using polyethylene containers. In addition, all the glassware was cleaned by soaking in 5% (v/v) nitric acid (Aristar® 65%) prior to use.

The sampling methodology was carried out using different procedures as described in Chapter 2. For the method development, “pooled” samples were used for each biological sample to investigate which methods could produce accurate and precise values and the range of trace elements under study. All the samples were digested before analysis. Multi-elemental analysis for serum and fingernails wre performed using an ICP-OES. Overall, the development and validation methods were used to

determine the concentration of interesting trace elements in serum and fingernail samples.

5.2.2 Serum and Fingernails Analysis

The following investigations were conducted for Al, Cd, Cu, Mn, Pb, Se, and Zn:

1. The levels of trace elements ($\mu g.mL^{-}$) for healthy individuals and hemodialysis patients were determined and compared with their levels in the literature.
2. The effects of health status, gender, smoking activity, and age have been clarified for elements under study.
3. It was found that there are several significant differences between study groups (healthy/patients; smokers/non-smokers; and males/females) in terms of the interested elements.
4. The effect of human age on the elemental levels of serum and fingernail samples has been determined. The results indicated that the effect of the individual's age on the levels of several elements was significant at $P = 0.05$.
5. The two interactions between factors (health status and gender; health status and smoking activity; and gender and smoking activity) have been investigated for all the elements in both serum and fingernails.
6. The relationship between the trace element levels in serum and fingernails of study subjects using Pearson's Product Correlation Coefficient (r). Significant correlations were found between several elements at $P < 0.001$ and $P < 0.05$.

5.2.3 Comparison Study

1. The comparison study between the levels of trace elements in serum and fingernails for various groups, namely healthy/patients; smokers/nonsmokers; males/females; and age have been investigated.
2. The levels of all elements were higher in fingernails when compared with serum.
3. The results show that there are significant differences for all trace element levels between serum and fingernails using one-way ANOVA and two-tailed t-test.

5.3 Future Works

Further research could be designed from this study about the levels of trace elements in biological samples, as shown below:

1- The high levels of Pb found in both biological samples require a follow-up study to establish whether a possible link exists concerning soils and main foods in this region;

2- The data from this study confirms that the deficiency and excess of some trace elements may play a role in the development of hemodialysis. However, further clinical studies are required using larger numbers of hemodialysis. In addition, urine need to also be collected and analysed to enable a clearer picture of the trace elements of hemodialysis;

3- Further studies are needed to explain many of the reported correlations of various elemental levels in the serum and fingernail; and

4- Further studies are needed to determine the levels of other elements in human serum and fingernails in order to evaluate whether there are any relationships between their levels and the onset of this disease.

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Appendix A

Documentation and Clinical Study

Appendix A1

A 1.1: Ethical Approval Documentation:

Republic of Iraq

Ministry of Health & Environment

Approval form of a Research Protocol/ Ministry of Health & Environment (Form number 02/2021)

استمارة المو افقة على مشروع بحث في وزارة الصحة والبيئة (استمارة رقم 2021/02)

- This form should be filled in electronically (Manually filled in form will not be accepted) by the researcher and submitted manually to the research unit at the relevant Health Directorate to be displayed at its Research committee. No research is allowed to be conducted at the institutions of Ministry of Health without having in advance the committee's approval.
- This form can be downloaded from the official website of Ministry of Health & Environment/ Iraq: ***https://moh.gov.iq/***

1. PERSONAL INFORMATION

1.1 Principal Investigator

Name	Specialty	Place of work	Phone number	E mail
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2. RESEARCH INFORMATION

2.1 Is the research title from the Ministry of Health's list of priorities:

☒ Yes ☐ No

2.1: The list is issued every year and found on MoH website.

2.2 The purpose of doing the research: Choose an item.

If the answer is others, please, specify: Click or tap here to enter text.

3. RESEARCH TOPIC

3.1 The Research Title:

Trace Element Levels of Human Tissues for Hemodialysis Patients and Healthy Individuals from Karbala city in Iraq

3.1: Write down the title of your research.

3.2 The Research Question:

3.2: Write down the smart research question.

A 1.2: Field Sampling Questionnaire:

استمارة جمع عينات بيولوجية

الرقم التسلسلي: تاريخ جمع النموذج: / /

العنوان: عدد السنوات التي قضاها في هذا المكان:

نوع النموذج(ضع دائرة حول النموذج الذي اعطيته):

شعر اضافر يد اضافر رجل دموع لعاب مصل دم

هل تعامل شعرك معاملة خاصة مثلا استخدام شامبو خاص للقشرة؟ اذا نعم اذكر أي نوع من أنواع المعاملة التالية؟

صبغ اوكسجين جل رذاذ أخرى

هل تستخدم صبغ اضافر؟

معلومات شخصية:

الجنس:

العمر:

الوزن:

الارتفاع:

الصحة العامة:

هل تحمل أي مرض ؟ نعم كلا

اذا نعم ما هو نوع المرض وكم سنة مصاب به (خصوصا مرض السكر):

ما هي الامراض غير المزمنة (حتى الانفلاونزه) التي تعرضت لها هذه السنة:

للكبار فقط التدخين:

هل تدخن ؟ اذا نعم كم سيكارة في اليوم وما نوع السيكارة؟

منذ متى بدأت التدخين؟

هل الزوج او الزوجة مدخن؟

اذا نعم تذكر فترة التدخين التي عاشاها سوية

كم سيكارة يدخن في اليوم

Appendix B

Publications

Appendix B1

Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **43** (special issue, Part 3): 873-80 (April 2024)

Article presented at the Fifth
International Scientific Conference
of Alkafeel University (ISCKU 2024)

Trace Elements in chronic kidney diseases: a key role or standby (review)

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SUMMARY. **Scientists scoured massive databases like Web of Science and MEDLINE to investigate the link between heavy metal exposure and advanced kidney disease (CKD), a growing global health concern. Despite their efforts, a recent review found scant evidence that arsenic, cadmium, lead, and other metals directly cause kidney problems. However, the researchers highlight the need for further studies to definitively confirm or disprove this connection.**

RESUMEN. Los científicos buscaron en bases de datos masivas como Web of Science y MEDLINE para investigar el vínculo entre la exposición a metales pesados y la enfermedad renal avanzada (ERC), un creciente problema de salud mundial. A pesar de sus esfuerzos, una revisión reciente encontró escasa evidencia de que el arsénico, el cadmio, el plomo y otros metales causen directamente problemas renales. Sin embargo, los investigadores destacan la necesidad de realizar más estudios para confirmar o refutar definitivamente esta conexión.

INTRODUCTION

Chronic kidney disease (CKD) is a circumstance in which the kidneys regularly lose their capacity to clear out waste merchandise and pollution from the blood. This can lead to the buildup of various substances in the body, including trace elements [1] Trace elements are minerals and metals needed by the body in small amounts for various functions, such as enzyme activity, antioxidant defense, and immune function. While some trace elements such as Cu, Zn and Se are essential and needed for good health, others can be harmful if they accumulate in excess [2] trace element accumulation might be contributing to Reduced kidney function. As CKD progresses, the kidneys become less efficient at filtering waste products, including trace elements. This can lead to the buildup of certain trace elements in the blood [3] consequences of trace element accumulation in CKD depend on the type and amount of element involved, for instance, in bone disease; Aluminum and fluoride accumulation can weaken bones and increase the risk of fractures. While in neurological problems, Aluminum accumulation can be associated with dementia and other neurological disorders. In case of cardiovascular disease, Iron overload can damage the heart and increase the risk of heart failure, and in Anemia cases deficiencies in iron, copper, and vitamin B12 were highly involved [4] The kidney is an important target organ for environmental toxins. Trace elements such as Cd, Pb and as are environmental toxins which are known to have nephrotoxic effects following acute and chronic exposures [5]. In Japan, cadmium (Cd) poisoning from consumption of Cd-contaminated rice was confirmed. It is characterized by several health effects, including severe kidney damage [6]. Homeostasis plays a crucial role in maintaining our internal chemistry. This intricate system delicately regulates the movement, storage, and utilization of trace elements, while also keeping the concentrations of ions and other vital substances in our fluids and tissues remarkably constant [7] Humans are uncovered to "toxic" factors that input the surroundings with the aid of using each herbal and anthropogenic source. An invasive (blood) or non-invasive (hair, nails, saliva, urine, and semen) samples has been applied to reveal human publicity to vital, non-vital, and unsafe components [6]. Many factors might effect: Minerals shape approximately 5% of the standard human food regimen however is vital for fitness and molecular functions. For the maximum vital factors, essentiality and toxicity are unrelated and toxicity is an issue of dose or publicity. Previously, to higher ap-

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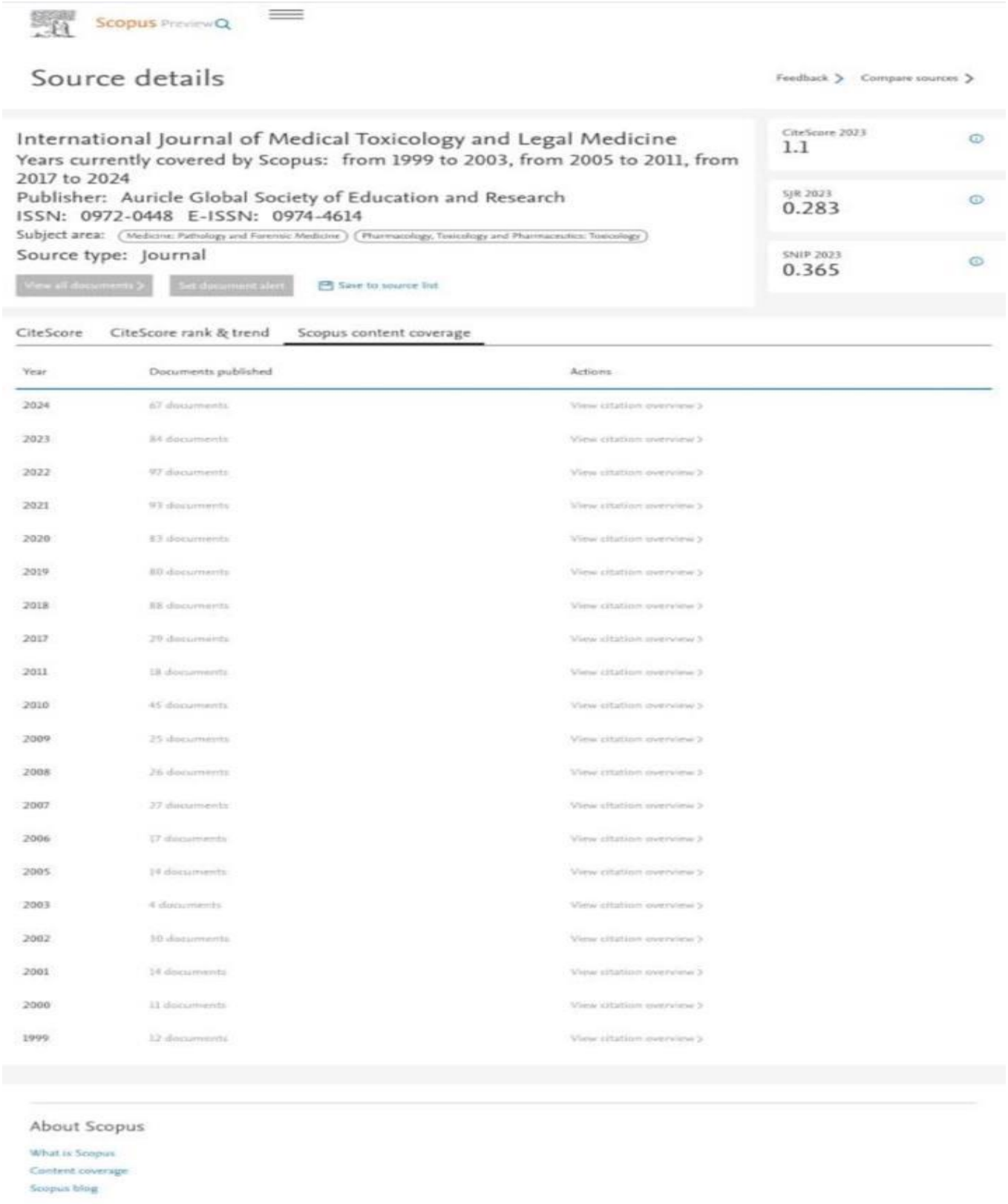
Appendix B2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International Journal of Medical Toxicology and Legal Medicine
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Publisher: Auricle Global Society of Education and Research
ISSN: 0972-0448 E-ISSN: 0974-4614
Subject area: Medicine: Pathology and Forensic Medicine Pharmacology, Toxicology and Pharmaceutics: Toxicology
Source type: Journal
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CiteScore 2023 1.1
SJR 2023 0.283
SNIP 2023 0.365
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Appendix C

Statistics

Appendix C1: Statistical Equations used in this Study. (Miller & Miller, 2010; Farrant,1997)

Arithmetic mean

The Arithmetic mean *(x)* is the sum of measured value divided by the number of measurements (n):

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

Standard deviation (s)

The standard deviation (s) is a measure of the agreement between a set of *n* data points; it is also the measure of random error. The following equation is used to calculate s:

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n-1}}$$

where = *x* value and *x* = arithmetic mean of *x* values.

Variance (S^2)

Variance is the square of the standard deviation and is a measure of the extent to which results in a set of data differ from one another. The larger the variance, the greater the difference between the results.

Relative Standard Deviation (%RSD)

Also known as the coefficient of variation, it is a measure of the relative error of a set of data. RSD enables comparison between the precision of results that may have different orders of magnitude or units, and can be determined as described below:

$$RSD = \frac{s}{\bar{x}} \times 100$$

where s = standard deviation and x = arithmetic mean of the data set.

Geometric Mean

The geometric mean is a measure of the average rate of change of values in a data set, given a varying rate of change. It is calculated as the nth root of the product of a set of data. It is more appropriate than the arithmetic mean when the population is log-normally distributed. The following equation can be used to calculate geometric mean;

$$Geometric\ Mean = \sqrt[n]{x_1 . x_2 \ldots . x_n}$$

Median

The median is the middle value in a set of data when the data is arranged in ascending order. It is another way of expressing the central tendency of dataset, and often gives a better approximation of the mean, particularly with small n and is independent of outliers.

Skewness

It is a measure of the degree of symmetry in a distribution. A symmetrical distribution has a skewness of zero and deviations from this are either positive or negative, depending upon the direction of the skew. It can be calculated by the following equation:

$$Skewness = \frac{\sum_i (x_i - \bar{x})^3}{s^3 . (n-1)}$$

Recovery

The recovery is used to identify any problems in the sample preparation process and the analytical measurement technique. The desired percentage recovery (%R) is 90 - 110 % and is calculated by the following formula:

$$\%R = \frac{Measured\ value}{Certified\ value} \times 100$$

Confident Interval

The confident interval (p) is the range of values within which there is a specified probability that the true value lies. It is used to evaluate whether there are any systematic errors throughout the analysis. The confidence limits for the mean are given as follows:

$$\mu = \pm zs/\sqrt{n}$$

where the value of z depends on the degree of confidence required, for 95% confidence limits z = 1.96, for 99% confidence limits, z = 2.58.

Outlier Identification - Grubb's Test

A Grubb's test is used to check whether one (or possibly more) value/s appears to differ from other values in the set of data. It is performed by calculating a value of G and comparing it to G-critical values at the 95% confidence interval. Any values where G calc > G crit maybe rejected as outliers. This test can be performed by calculation of a value of G and comparing it to G critical value (at P = 0.05). In order to use Grubbs' test for an outlier, the statistical G is calculated from:

$$G = \frac{|\bar{x} - suspect|}{s}$$

Significance test for Linear Regression

The significance of the Pearson product moment correlation coefficient (r) can be established using a t-test. The calculated (^caic) value is compared to the t-critical (fcrit) value for n-2 degrees of freedom at the 95% confidence interval *{P* < 0.05), the t value is calculated from:

$$t = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$$

T-test Assuming Equal Variance

This test is used to compare two experimentally determined means for which both

populations have equal standard deviations (pre-determined using an F-test). The calculation of t also requires the calculation of the pooled standard deviation (ps). The calculated (tcaic) value is compared with the t-critical (rent) value for U] + U**2** -
2 degrees of freedom at the 95% confidence interval (P < 0.05). The equations are as follows:

$$ps^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}$$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{ps\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

T-test Assuming Unequal Variance

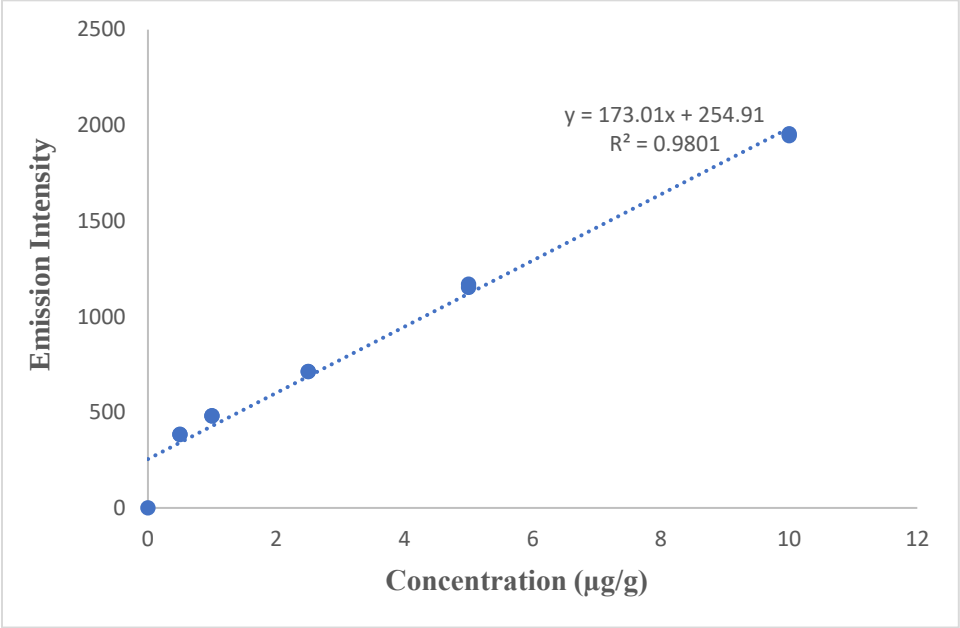
When the comparison of two experimentally determined means both have populations with significantly different standard deviations (pre-determined using an F-test), the following t-test is performed. A further calculation for the degrees of freedom *(d/)* is also required as it is not appropriate to use the pooled standard deviation.

$$df = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}{\frac{s_1^4}{n_1^2(n_1 - 1)} + \frac{s_2^4}{n_2^2(n_2 - 1)}} \qquad t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

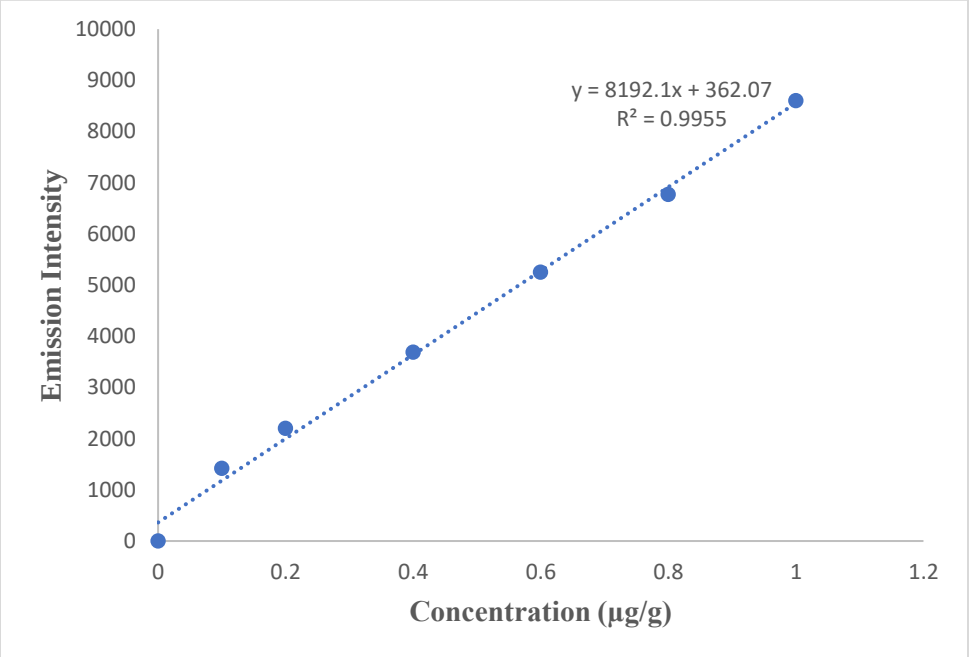
where *Xi* and **%2** are the means of populations 1 and 2, and Si and S2 are the respective standard deviations.

Appendix D
Calibration Curves

D1: Calibration Curve of Elements

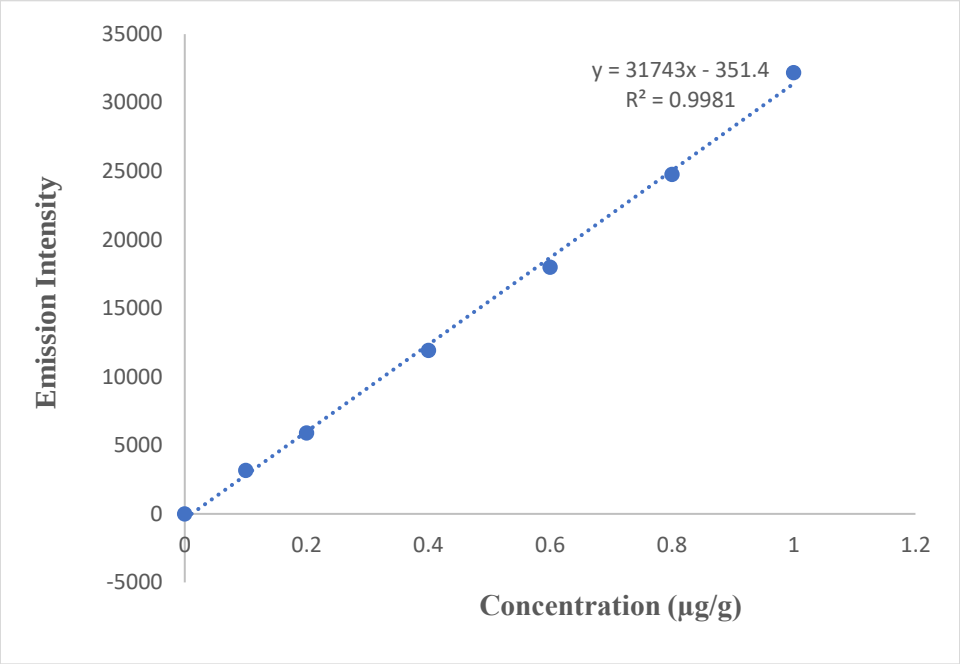


Typical Calibration Curve of Al

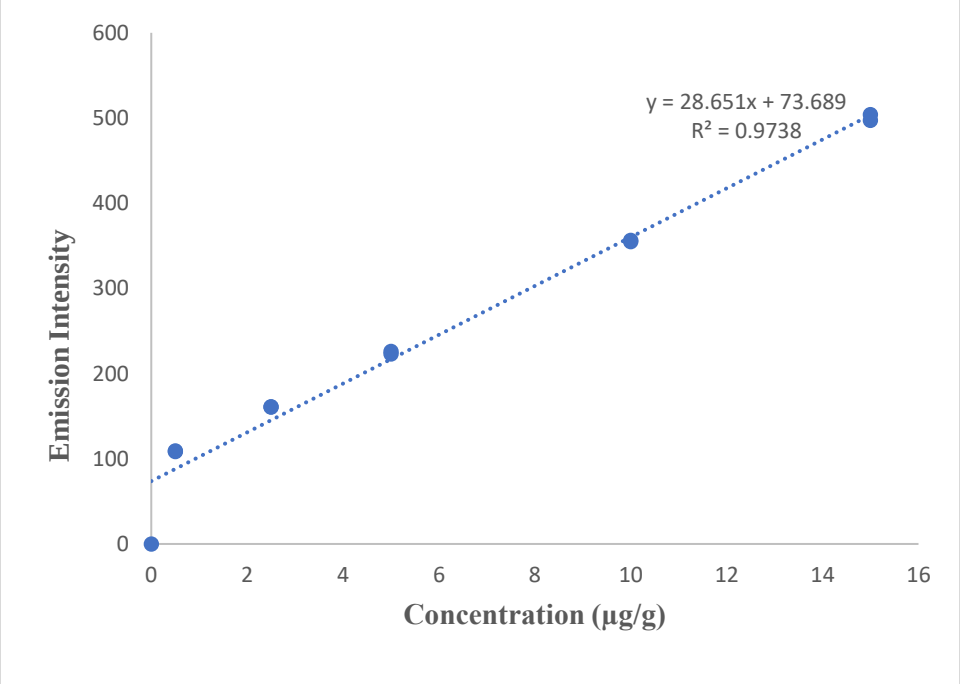


Typical Calibration Curve of Cu

D2: Calibration Curve of Elements

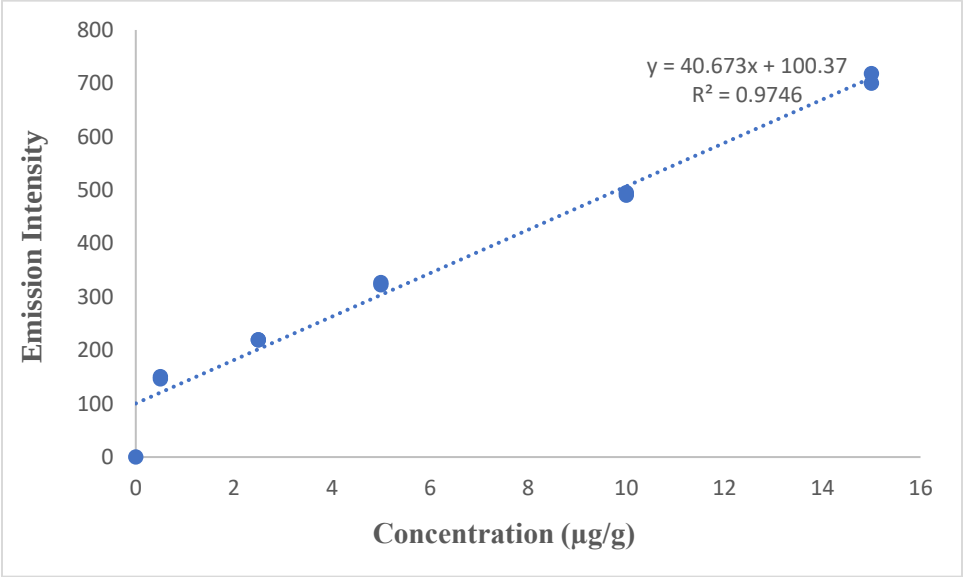


Typical Calibration Curve of Mn

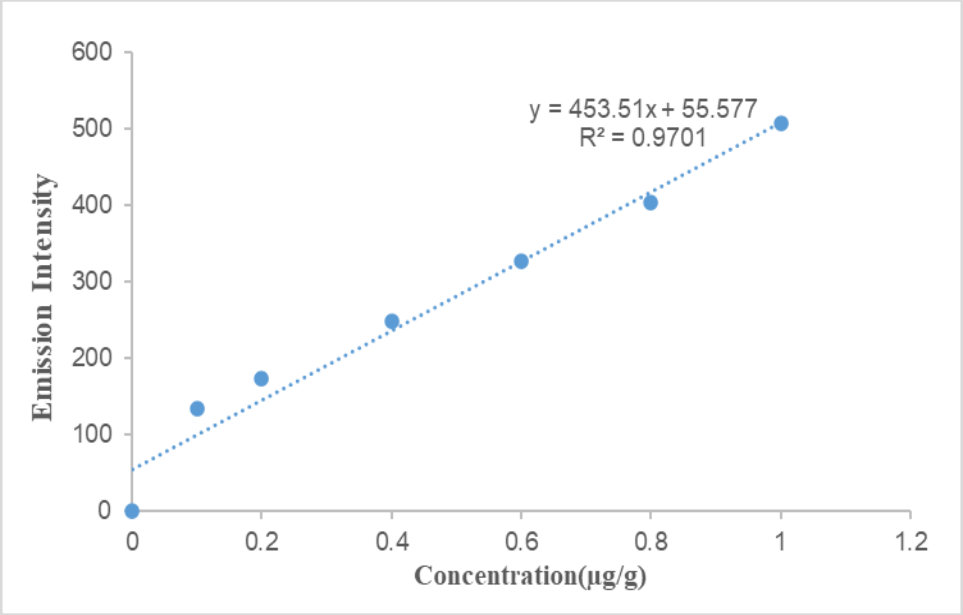


Typical Calibration Curve of Se

D3: Calibration Curve of Elements



Typical Calibration Curve of Pb



Typical Calibration Curve of Zn

الخلاصة

تم تقديرمستويات العناصر النزرة (Al, Cd, Cu, Mn, Pb, Se and Zn) في عينات بيولوجية مختلفة (مصل الدم والأظافر) بأستخدام مطيافية الانبعا ث الذري - البلازما المقترنة بالحث (ICP-OES)، حيث طورت وثبتت طرق تحليلية لتقديرمستويات هذه العناصر باستخدام تدابيرالجودة والاختبارا ت الأحصائية القياسية. أجريت تحاليل لكافة العناصر في عينات مصل الدم والاظافر المغسولة والتي جمعت من الافراد العراقيين (مدينة الامام الحسين (ع) الطبية ومركز الوارث لغسيل الكلى) .تم التحقق من تأثيرعوامل الحالة الصحية ونوع الجنس ونشاط التدخين والعمرعلى مستويات العناصرفي عينات كل من المصل والأظافر. بشكل عام، وجد أن مستويات عناصر المنغنيز والزنك في عينات مصل الأفراد الأصحاء (166.0 ± 296.0 ميكروغرام/مل من المنغنيز و302.0 ± 068.0 ميكروغرام/مل من الزنك) أعلى بشكل مؤثرمقارنة بتراكيزها في عينات مصل مرضى غسيل الكلى (128.0 ± 036.0 ميكروغرام/مل من المنغنيز و259.0 ± 057.0 ميكروغرام/مل من الزنك). من ناحية أخرى، تم تحديد فروقا ت ذات دلالة إحصائية في مستويات جميع العناصر المقدرة في عينا ت الأضافر للأفراد الأصحاء مقارنة بعينات مرضى غسيل الكلى.

تم التحقق من تأثير نوع الجنس على مستويات العناصر في عينات المصل والأظافر باستخدام اختبارt (two tailed t-test)، لقد وجد أن نوع لجنس له تأثير معنوي على مستويات المنغنيز والرصاص في عينات المصل، بينما وجد هذا التأثير المعنوي لنوع الجنس على مستويات عناصر الألمنيوم والكادميوم والمنغنيز والرصاص والسيلينيوم في عينا ت الأظافر. بالإضافة إلى ذلك، لا توجد تأثيرات ذات قيمة أحصائية لمستويات جميع العناصر بين الأفراد المدخنين وغير المدخنين لكل من عينات الأظافر والمصل بأستثناء عنصر المنغنيز الذي وجد بتراكيز عالية ومؤثرة في عينا ت الأضافر للأفراد غير المدخنين مقارنة بالمدخنين.

تشير النتائج إلى أن تأثيرعمر الأفراد على مستويا ت الكادميوم والنحاس والمنغنيز كان ذا قيمة مؤثرة في عينات الأظافر عند $P = 0.05$، في حين لوحظ هذا التأثير فقط على مستويات عنصرالزنك في حالة عينات المصل.

تمت دراسة التداخل بين عوامل (الحالة الصحية والجنس ونشاط التدخين) والترابط الداخلي بين العناصر في عينات الأضافر والمصل.

تم تقييم استخدام مصل الدم والأظافر كمؤشر حيوي يستخدم لتقييم الحالة الصحية للإنسان من خلال استخدام عدد من الدراسا ت في هذا البحث، مثل التدخين، سنوات العمر، نوع الجنس، وغسيل الكلى.

جامعة كربلاء

كلية العلوم

قسم الكيمياء

تقدير مستويات العناصر النزرة في المصل والاظافر لمرضى غسيل الكلى والافراد الاصحاء في العراق باستخدام بلازما الحث المقترن بمطيافية الانبعاث الذري

رسالة مقدمة الى كلية العلوم / جامعة كربلاء

وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الكيمياء

من قبل

رسل عزيز جعفر الميالي

بكالوريوس علوم كيمياء – جامعة كربلاء (2011)

ماجستير علوم كيمياء – جامعة كربلاء (2014)

بأشراف

أ.د باقر عبد الزهرة جودة

أ.م.د رنا مجيد حميد

2024 م **1446هـ**