Trace Element Levels of Human Fluids and Tissues for Iraqi Individuals

By

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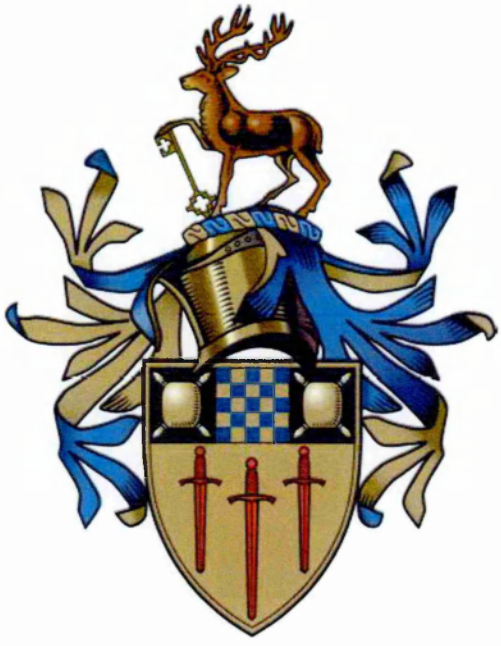

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For Imam Hussein

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

In the Name of God, the Beneficent, the Merciful

God will raise the position of the believers and of those who have received knowledge. God is Well-Aware of what you do (11).

Abstract

Trace element levels (B, V, Cr, Mn, Fe, Cu, Zn, As, Sr and Cd) in environmental (water and cigarette tobacco) and biological (tear drop, saliva, scalp hair and fingernail) samples collected from Iraqi individuals resident in Karbala (Iraq) and London (UK) were determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Multi-element analysis was carried out on drinking (commercial, domestic bottled and tap) and irrigation (river, artesian and well) water samples. It was found that most trace element levels (μg/l) were lower than the permissible guidelines for drinking water recommended by the World Health Organisation (WHO) and Iraqi government. The only exceptions were for B in artesian and well waters; and Cd in river, artesian and well waters. The highest levels in drinking and irrigation waters were found for Sr when compared with other trace elements. Trace element levels in tap water from Karbala were higher than those from London. The levels of trace elements in cigarette tobacco were found to be at higher to lower levels through the following sequence: Fe > Mn > Sr > Zn > Cu > As > Cd > Cr > V. Moreover, multi-element analysis was undertaken for tear drop, saliva, washed scalp hair and fingernail samples for Iraqi individuals from Karbala (Iraq) and London (UK). Discriminant analysis suggested that Sr, Mn, B, V, As, Cd and Zn levels could be used to discriminate between healthy and diabetic populations (83% of cases correctly classified). Higher trace element levels were found in the tear drop, saliva, scalp hair and fingernail samples from Karbala than those from London. The influence of various factors (health status, gender and smoking activity) and covariates (individual's age and drinking water) on elemental levels in tear drops was investigated using the analysis of covariance (ANCOVA). Significant differences (at $P < 0.05$) were found between the healthy and diabetic individuals for B, Mn and Sr; males and females in terms of the levels of Fe; and smokers and non-smokers for Cd. Trace element levels in drinking water were found to have significant effects on the levels of V, Mn and Sr, whilst an individual's age has a significant effect in terms of Zn and As. Inter-element interactions were evaluated for each pair of trace elements in tear drops, and between tear drops and saliva, washed scalp hair and fingernails. There were 32 and 27 statistically significant correlations of the total 45 tested using tear drops from healthy and diabetic individuals, respectively. Similar results were observed for saliva, washed scalp hair and fingernails in terms of residential location, health status and inter-element interactions. The use of tear drops as a potential biomarker for assessing human health status has been evaluated using several studies in this research, namely; drinking water analysis, smoking activity and type 2 diabetes.

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Abbreviations

AAS	Atomic Absorption Spectrometry
amu	Atomic Mass Units
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
atm	Atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BOD	Biochemical Oxygen Demand
CRM	Certified Reference Material
CV (%)	Coefficient of Variation
Cyst	Cysteine
D	Diabetic
d.w.	Dry weight
DFA	Discriminant Function Analysis
dc	Direct Current
DDW	Distilled Deionised Water
df	Degrees of Freedom
EC	Electrical Conductivity
EPA	Environmental Protection Agency
ETAAS	Electrothermal atomic absorption spectrometry
ETS	Environmental Tobacco Smoke
EU	European Union
F	Female
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agriculture Organisation of the United Nation
F_{calc}	Calculated F value
F_{crit}	Critical F value
GF	Graphite Furnace
H	Healthy
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
In	Indium
IS	Internal Standard
IUPAC	International Union of Pure and Applied Chemistry
K	Karbala
K_e	Correlation factor
L	London
LOD	Limit of Detection
LOQ	Limit of Quantification

M	Male
MS	Mass Spectrometry
NCCLS	National Committee for Clinical Laboratory Standards
NIST	National Institute of Standards and Technology
NS	Non-smoker
QC	Quality Control
R	Pearson Product Moment Correlation Coefficient
R^2	Linear Regression
rpm	Revolution Per Minute
RSD (%)	Relative Standard Deviation
S	Smoker
SD	Standard Deviation
SE-HPLC	Size Exclusion High-Performance Liquid Chromatography
ELISA	Enzyme-Linked Immunosorbent Assay
Sig	Significance
T	Temperature
t_{calc}	Calculated t value
t_{crit}	Critical t value
TDS	Total Dissolved Solid
UK	United Kingdom
UN	United Nation
USA	United States of America
USEPA	United States Environmental Protection Agency
v/v	volume/volume
WHO	World Health Organization
WTW	Water Treatment Works

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Chapter One

General Introduction

1.0 Introduction

The effect of inorganic elements on human health has long been recognised, particularly when in the 17th century it was discovered that iron (Fe) was essential for human health (Iyengar, 1989). In nature, there are 90 elements that exist between environmental, geological, biological or marine systems (Ward, 2000). On the other hand, 23 elements are recognised to relate to specific physiological activities in human and animal life (Fraga, 2005; Patriarea *et al.,* 1998). There are many studies that have discussed the essentiality of some of these elements in animal and human systems (Manso *et al.,* 2007; Villanueva & Bustamante, 2006). Values outside of "normal" levels can lead to a number of health disorders (Fido & Al-Saad, 2005). It is well known that these elements enter the human body via different ways: namely the respiratory tract, the digestive system (GI tract) and in some cases through the skin from different media (air, water, foods and drugs). They are then transported and distributed through blood into the organs, such as the liver and kidney, and are removed from the organism through different pathways: sweat, hair, nails, urine, saliva, tear drops and faeces, as shown in Figure 1.1 (Chojnacka *et al.,* 2005; Apostoli, 2002). Trace element transportation, storage and regulation in the human body are 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 and tissues (Adair, 2002).

Human and other living organisms are exposed to "toxic" elements that are introduced into the environment from natural sources, as well as a result of anthropogenic (or man-made) activities. In order to monitor human exposure to essential, non-essential and toxic elements, an invasive (blood) or non-invasive matrixes (such as hair, nails, saliva, urine, and semen) have been used (Esteban & Castano, 2009).

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 capability, such as inductively coupled plasma mass spectrometry (ICP-MS) (Millos *et al.,* 2008). 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).

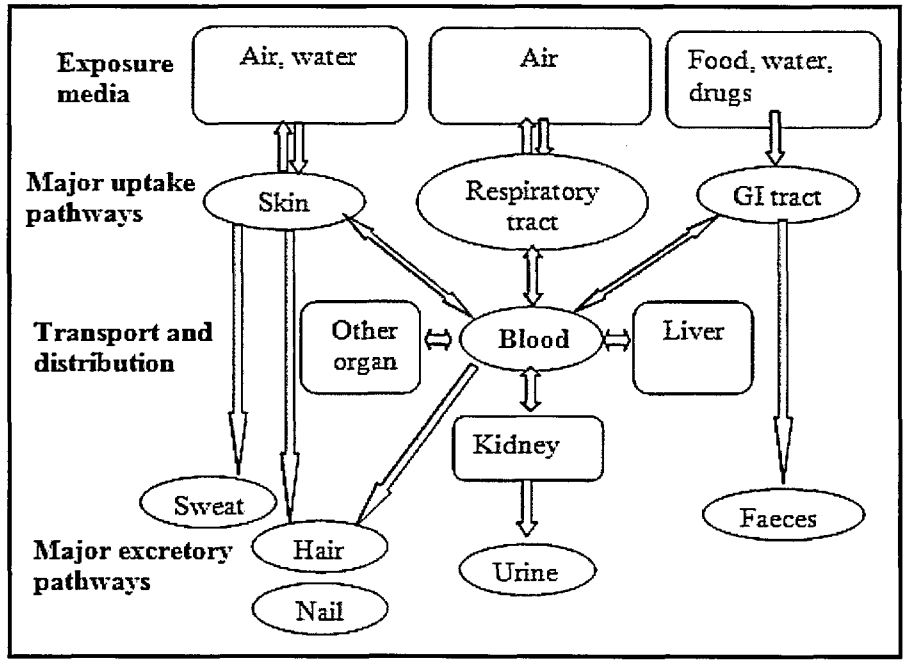

Figure 1.1: Exposure and metabolic pathways for elements in the human body (after Apostoli, 2002), (GI = Gastrointestinal).

1.1 Classification of Elements

The classification of elements in biological systems can be grouped into three categories: *major* elements (95%) consisting of C, H, N, O; *minor* elements (3.6%) including Ca, Cl, Mg, P, K, Na; and *trace* or *ultra-trace* elements (1%) (Ward, 2000). This classification depends upon the elemental levels in tissues and fluids of "normal", control or healthy individuals. The actual elemental levels vary throughout the human body, such that some are classified as minor in human tissues and trace or ultra-trace in fluids (Parsons & Barbosa, 2007). In fact, 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 (Ward, 2000). Elemental abundance varies for the different categories of environmental or human media, for example, the abundance of Ca in the earth's crust is 3.6% (or 36000 mg/kg) whilst in the human body it is 1.4% (or 14000 mg/kg).

1.1.1 Essentiality and Toxicity of Elements

The classification of elements as major, minor, trace or ultra-trace provides a broad picture of the total concentrations that are expected to be inside the body, but it is fairly inexact and gives no real indication of the role or activity of a specific element. As a result, there is the need for further classification in relation to a biological system. In this classification, elements can be grouped as being essential, non-essential or toxic in terms of human health, as shown in Figure 1.2. Human and other living organisms require essential elements to maintain their normal physiological functions. Furthermore, it is difficult for an organism to maintain the normal life cycle, or achieve healthy growth, without the presence of essential elements (Parsons & Barbosa, 2007). Moreover, an element can be considered essential 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 (Goldhaber, 2003). There are many studies that have discussed the potential essentiality of some of these elements in animal and human systems (Manso *et al.,* 2007; Villanueva & Bustamante, 2006; Goldhaber, 2003; Patriarca *et al.,* 1998).

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 (Partriarca *et al.,* 1998).

Trace elements are considered to be risk elements to an organism if they are (i) associated with intakes that are too high, resulting in toxic levels or effects; and (ii) associated with intakes that are too low that are linked with nutritional problems (Goldhaber, 2003). For example, selenium is essential and found at typical levels of 0.1 μg/l in urine and 40 μg/l in serum, but it is toxic if in excess (Akl *el al.*, 2006). All elements, including those considered essential, can become toxic if the concentration in the human body is higher than the optimal concentration threshold. Furthermore, others are quite toxic even at low concentrations, such as Cd, Hg and Pb (Savory & Wills, 1992). Any deficiency in the concentration of an essential element below that required for normal growth will lead to a number of health disorders (Parsons & Barbosa, 2007). Medical treatments can contribute to an increase in the levels of elements inside the human

body such as: dental fillings (Hg) (Drexler & Schaller, 1998); and implantation of orthopedic and orthodontic prostheses (Co, Cr, Ni, and others). In general, the rate of toxicity for any element depends on its concentration, duration, route of exposure, and the chemical form (Parsons & Barbosa, 2007): As an example, chromium is essential in its Cr(III) form and toxic if found as Cr(VI) (Hosseini & Belador, 2009). In addition, toxicity can include those considered to be non-essential elements if they are present above a critical concentration (Fraga, 2005).

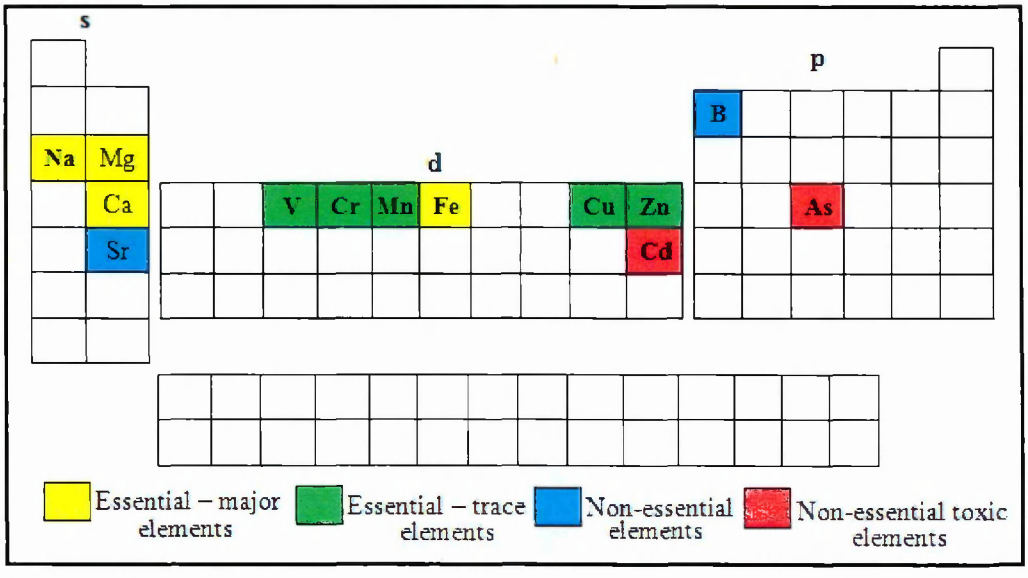

Figure 1.2: Modified periodic table showing selected elements in this study. Essential elements are subdivided into major and trace based on the NCCLS classification (Parsons & Barbosa, 2007).

1.1.2 Dose Response Curve

Many elements can be considered essential to life when their concentrations are highly variable and in some cases are extremely small (i.e. below 0.1 μg/l). The level of an essential trace element in a human follows a dose response curve (Figure 1.3). In this curve, there are three parts. Firstly, the deficiency range, in which the concentration of the trace element is below the optimal level for normal physiological requirements. In this situation, an individual will survive but they will have a heavily impaired physiological response. The concentration of a trace element in human tissues and fluids can gradually increase, but may not be at the level required to produce normal biological functions. Secondly, the normal range where biological functions are optimal, usually results in the individual having

"normal" health. Finally, the toxicity range, which arises through the further increase in the concentration of an element, can lead to inhibited metabolic functions. This may lead to the death of the individual (Stone, 2006).

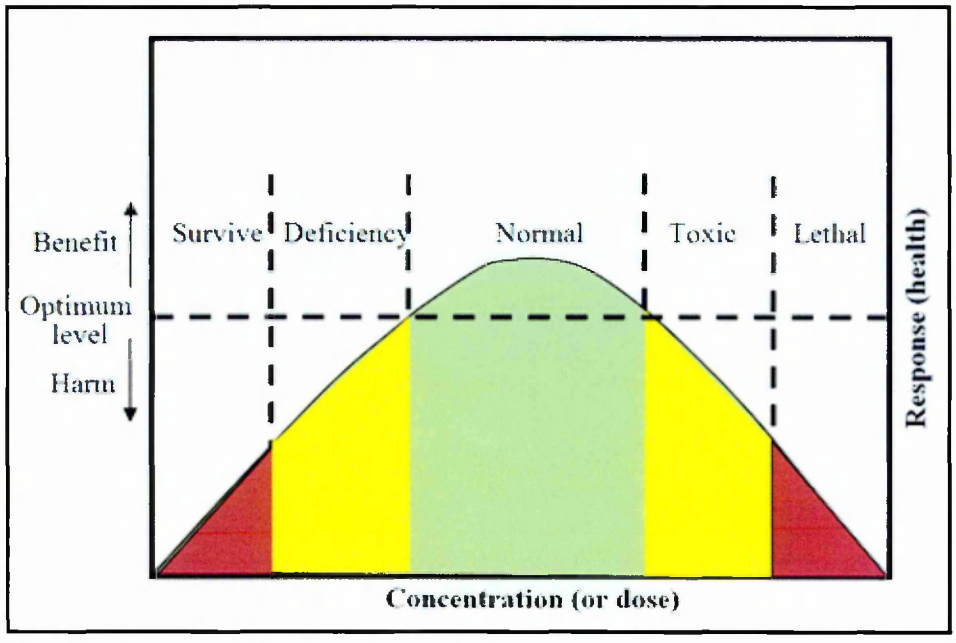

Figure 1.3: Typical dose-response relationship for essential trace elements in the human body (after Underwood & Mertz, 1987).

1.1.3 Role of Trace Elements in Human Health

The optimum balance of essential trace elements in the human body may be a prominent key to maintaining a healthy existence. The role of essential elements in the human body is continually being investigated. As such, it has been found that their main physiological function is associated with enzymes (Stovell, 1999). For example, metalloenzymes (metal-enzyme complexes) exist through the strong bond between a metal and an enzyme molecule (Schutte, 1964). There are many biological processes in the human body that depend on the action of these enzymes such as respiration, muscle contraction, digestion, growth, the oxidation–reduction reaction, transport processes and the synthesis and stabilisation of proteins and nucleic acids (Versieck & Cornelis, 1989).

The significance of trace elements in human health and disease has been discussed by several reviewers, such as Thomson (2004) and Patriarca *et al.* (1998).

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 (Fraga, 2005). In addition to these officially recognised trace elements, there are other elements which are not essential, but they are needed by the body to successfully process or metabolise essential elements successfully. For example, there are many positive correlations between the essential and non-essential elements in different human tissues and fluids (Manso *et al.,* 2007; Chojnacka *et al.,* 2005). In addition, the determination of trace elements is important in assessing environmental (including contaminated water and food) or occupational exposure (Hussein *et al.,* 2008; Bjorkman *et al.,* 2007). The following sections review the trace and ultra-trace elements that were selected for this research.

1.1.3.1 Boron

Boron is a non-essential element and required by the human body in very small amounts for good health (Nielsen, 1997). Boron can enter the human body in a variety of ways. It is naturally present in many foods and drinking water. Boron compounds can be used in different industrial processes, such as the production of fibreglass, borosilicate glass and detergents (Power & Woods, 1997). One study in the USA has reported a link between boron levels, fertility in males and exposure to inorganic boron from the environment (Woods, 1994). It was found that high boron levels in ground waters and soil were to blame. Therefore, the contamination of ground water (including drinking water supplies) with boric acid has become a serious environmental problem, especially in regions of low rainfall such as Turkey and Iraq (U.S. EPA, 2003; Nable *et al.,* 1997). The World Health Organisation (WHO) recommends a maximum drinking water level of 0.3 mg/L B (WHO, 2006). A recent study in Japan has developed a new method to remove boron from ground water by using bacteria that can absorb high levels of the element (Miwa & Fujiwara, 2009).

In terms of human health, boron is important in the metabolism and utilisation of calcium and magnesium (Hill, 2009; Usuda *et al.,* 2007). Boron is also necessary to allow the brain to function properly; a low boron intake by humans causes decreased brain activity (Nielsen, 1994).

Despite the fact that calcium builds strong bones, there is evidence that boron also plays a significant role in healthy bones and joints (Newnham, 1994). The bones become brittle and easy to break without small amounts of boron. Many studies have also mentioned that boron can be used to treat and prevent various forms of arthritis (Havercroft & Ward, 1991; Shah & Vohora, 1990; Travers *et al.,* 1990).

1.1.3.2 Vanadium

Vanadium is an essential trace element for humans (Fernandes *et al.,* 2007). It was found that high levels of vanadium in the human tissues and fluids may be due to an occupational and/or environmental exposure, especially near steelmaking or oil-burning power production plants. As a result, the determination of vanadium levels in environmental and biological samples becomes very important (Romero & Granadillo, 1993). It is believed that diabetics may benefit from vanadium as it can improve insulin status in healthy people and stabilise blood sugar levels in a diabetic patient (Seko *et al.,* 2006; Srivastava & Mahdi, 2005; Wang *et al.,* 2001). In healthy individuals, the accepted reference interval for vanadium in plasma or serum is 0.016 – 1.3 µg/l (Versieck & Cornelis, 1989) and for whole blood 2 – 5 µg/l (Ekmekcloglu *et al.,* 2001; Hamilton *et al.,* 1994).

1.1.3.3 Chromium

Chromium is an essential element and is a component of the low molecular weight protein chromodulin, otherwise known as the glucose tolerance factor. This important factor is known to potentiate the effect of insulin, presumably by allowing it to bind to cell receptor sites (Devlin, 2002). Individuals who are chromium deficient are known to have impaired glucose tolerance and decreased insulin effectiveness (Skalnaya & Demidov, 2007; Wrobel *et al.*, 1999; Anderson *et al.*, 1990). Many researchers have reported lower chromium levels in type 2

diabetic patients compared with healthy individuals (Rukgauer *et al.,* 2002; Ravina *et al.,* 1995).

Toxicity of Cr is mainly due to Cr^{5+} that can be absorbed by the respiratory tract, and also to a certain extent by skin. In serum Cr occurs as Cr^{3+} and is bound to serum proteins, especially transferrin and albumin (Lauwerys & Hoet, 1993). The levels of chromium in air and drinking water are usually low, but contaminated well water may contain dangerous levels of the chromium (VI) ion (Kumar & Riyazuddin, 2009). Human fluid (blood, urine and saliva) and tissue (scalp hair and nail) samples have been used as biomarkers for chromium levels in the human body (Olmedo *et al.,* 2010; Sukmar & Subramanian, 2007; Stone, 2006).

1.1.3.4 Manganese

Manganese is an extremely important element that the human body uses for a variety of vital processes. For instance, it is involved in different enzymes and plays a significant role in normal metabolic processes, for example, carbohydrate metabolism (Devlin, 2002). On the other hand, exposure to high levels of Mn from industrial sources, such as Mn alloy production, iron and steel production plants, ferromanganese refineries, battery production and welding, can cause a number of health problems. These include neurobehavioral dysfunction and changes in mood (Haynes *et al.,* 2010). In addition, people exposed to manganese via contaminated drinking water may suffer from neurotoxic effects. Several materials, including hair, nail, blood, urine and saliva, have been used as biomarkers of manganese exposure to environmental and occupational sources (Olmedo *et al.,* 2010; Wei *et al.,* 2010; Heitland & Koster, *et al.,* 2006).

1.1.3.5 Iron

Iron has been recognised as an integral part of haemoglobin and myoglobin which carry oxygen and carbon dioxide, respectively (Devlin, 2002). It also plays a key role in the regulation of many metabolic processes. Iron deficiency anaemia will arise if the human body has a lack of iron (Harris, 2007). This is probably the most common nutritional disease in the world, particularly in women due to blood losses during menstruation, and through the increased iron demands of pregnancy

and blood loss during childbirth (Jian *et al.,* 2010). In addition, iron deficiency can cause insulin deficiency. Some researchers have reported that high iron levels can cause insulin deficiency, but usually this is insufficient to result in diabetes (Cooksey *et al.,* 2010; Rajpathak *et al.,* 2009).

1.1.3.6 Copper

Many studies have reported that copper is necessary for good human health, as it has many physiological functions, especially associated with enzymes. For example, ferroxidase uses copper to regulate the oxidation state of iron to absorb only Fe^{2+}, whilst Fe^{3+} will connect to the plasma protein transferrin (Devlin, 2002). This important element also contributes to the development of diabetes (Tanaka *et al.,* 2009). Higher levels of Cu have been reported in diabetic rather than non-diabetic individuals (Hussein *et al.,* 2009). Copper deficiency has also been associated with reproductive failure (Davis & Mertz, 1987).

1.1.3.7 Zinc

Zinc is an essential part of more than 300 human enzymes participating, in various vital processes, such as digestion and metabolism (Devlin, 2002). High levels of zinc are associated with the onset of Parkinson's disease, which leads to nervous system diseases. This causes the destruction of specific nerve cells inside the brain (Forte *et al.,* 2005). Previous studies have reported a negative correlation between the levels of zinc in the human body and various disorders, such as obesity, insulin resistance and type 2 diabetes (Skalnaya & Demidov, 2007). For instance, a recent study found significantly low levels of zinc in a diabetic group when compared with a control group (Hussein *et al.,* 2009). Zinc deficiency has also been reported to cause impaired glucose metabolism and taste bud development (Devlin, 2002).

1.1.3.8 Arsenic

Arsenic, similar to other elements, can become toxic if its concentration in the human body is too high. A level of 1 - 3 mg/kg As is enough to be lethal in a

human adult (Ellenhorn, 1996). The toxicity of arsenic is strongly related to its oxidation state and chemical form (B'Hymer & Caruso, 2004). It was found that inorganic arsenic is suggested to be more toxic than organic forms in terms of human health (Chen *et al.,* 2009). Most cases of arsenic-induced toxicity in humans are due to natural exposure to inorganic arsenic via air, water, soil, dust and food (Brima *et al.*, 2006; Mandal *et al.*, 2004). In recent decades many studies have reported that arsenic plays a significant role in a number of diseases, such as cancer and diabetes (Wang *et al.,* 2009). Chronic arsenic exposure has been suggested to have an etiologic role in diabetes development (Navas-Acien *et al.,* 2006) with more than one study in the USA reporting that arsenic in drinking water is associated with the onset of diabetes (Kile *et al.,* 2008; Navas-Acien *et al.,* 2008). Another study in Bangladesh has shown that the risk of diabetes is increasing among people exposed to high levels (more than 100 µg/l) of arsenic through drinking water (Rahman *et al.* 1998).

1.1.3.9 Strontium

Strontium has been reported in the literature as a non-essential element (Parsons & Barbosa, 2007). It has the same properties as calcium and accumulates at high levels in bones, thereby displacing calcium in hard tissue metabolic processes. Therefore, strontium interferes with normal bone development at high concentrations (Verberckmoes *et al.,* 2007; Krefting *et al.,* 1993). A previous study has suggested that Sr can be used in new drugs to prevent postmenopausal osteoporosis (Malaise *et al.,* 2007). Adults and children are both exposed to strontium via drinking water and food, but young children have more hand-to-mouth activity or may eat soil accidentally and thus consume more strontium. This may increase the prevalence of rickets in a Sr-rich soil area due to calcium displacement (Usuda *et al.,* 2007).

1.1.3.10 Cadmium

Some trace elements are known to be toxic to humans and animals, even at very low concentrations, especially cadmium, lead and mercury (Ozden *et al.,* 2007). In general, cadmium is considered a toxic element as it causes adverse effects in

human biology (Bernard, 2008). It accumulates in the kidney cortex and the concentration increases with age (Skrzydlewska *et al.,* 2003). Cadmium exposure is mainly from industrial sources. However, cigarette smoking can significantly increase body levels, for both active and passive (non-smoking) individuals (Vahter *et al.,* 2002). Cadmium has been a serious health concern in recent years (Kazi *et al.,* 2008). Children born to mothers who smoked cigarettes whilst pregnant may be at an increased risk later in life from developing certain types of childhood cancers, asthma, type 2 diabetes, hypertension, obesity, and/or behavioral disorders (Ng & Zelikoff, 2007). Long-term exposure is linked to hypertension, kidney problems, infertility and possible birth defects (Ozden *et al.,* 2007; Goldhaber *et al.,* 2003; Vahter *et al.,* 2002).

The above section describes the different diseases that can arise as a result of both an excess and deficiency of the essential and non-essential elementals in the human body. Chronic exposures to some inorganic elements have been associated with the onset of different diseases, such as diabetes, anemia, cancer, asthma and heart disease. Diabetes is one of the most common chronic diseases in the world (Wang *et al.,* 2009).

1.2 Diabetes Mellitus

Diabetes is a Greek word that means "excessive urine" and Mellitus is a Latin name for "honey". Normally, the amount of sugar in the blood is controlled by a hormone called insulin. Insulin is produced in sufficient quantities in the Beta (β) cells of the islets of Langerhans in the pancreas, a glandular organ located behind the stomach. Insulin helps to move glucose out of the blood into the cells in order to produce energy. In people with diabetes the level of glucose builds up in the blood stream because the body does not produce enough insulin, or the cells do not respond to the insulin that is produced (Raju *et al.*, 2006).

There are three types of diabetes: (i) insulin-dependent (type 1) which is caused by destruction of β-cells in the pancreas. In this case the body does not produce enough insulin to carry glucose from blood into cells throughout the body. This type can be treated only by daily insulin injections; (ii) insulin resistance (type 2) which usually results due to aging, obesity and other environmental factors. In this type, the body's cells do not use insulin properly; therefore, type 2 can be treated

by using several types of synthetic therapeutic substances together with a controlled diet and physical exercises; and (iii) gestational diabetes, which affects pregnant women who have never had diabetes before, this may develop into type 2 diabetes. This study will focus upon type 2 diabetes as it is more widespread in the Middle East area, including the Iraqi population (Mansour *et al.,* 2008).

1.2.1 Type 2 Diabetes

A study has reported that type 2 diabetes has become a major challenge to public health and affects more than 200 million individuals worldwide (Kamal *et al.,* 2009). The main risk factors for a "diabetic epidemic" include: population growth, older age, urbanisation, obesity and physical inactivity (Aspray, *et al.,* 2000). In general, diabetes develops largely in people above 40 years of age (Wild *et al.,* 2004). A previous study has reported that type 2 diabetes can be diagnosed in people over 20 years old (Taormina *et al.,* 2007). One study in the south of Iraq (Basra), has found that the prevalence of diabetes was 7.43 % of the population, and about 28.81% of 3176 subjects were previously undiagnosed (Mansour *et al.,* 2008). The prevalence of diabetes differs among ethnic groups, for example, some immigrant groups have a higher prevalence in European countries, such as south Asian immigrants in the UK (Wändell *et al.,* 2008). Diabetes can be identified through the analysis of trace elements and glucose (Skalnaya & Demidov, 2007).

1.2.2 Trace Elements and Type 2 Diabetes

Many studies have observed that trace elements are associated with type 2 diabetes through the relative deficiency of insulin and insulin resistance (Navas-Acien *et al.,* 2006; Nurmohammadi *et al.,* 2000; Anderson, 1997; Kimura, 1996). Some of the essential elements might have a significant role to develop and progress diabetes based on the metabolism of several trace elements in the human body (Hussein *et al.,* 2009). Obesity has been associated with an increased risk for diabetes. Previous studies have shown that the trace element levels in the human fluids and tissues are associated with the symptoms of type 2 diabetes (Skalnaya & Demidov, 2007; Rajpathak *et al.,* 2005; Rajpathak *et al.,* 2004). For example, diabetes has been linked with elevated hair K, Na, and Hg and decreases in Ca,

Mg, Zn, and Co (Skalnaya & Demidov, 2007). Table 1.1 show the level of trace elements for different human tissues and fluids in the literature.

Table 1.1: Elemental levels in human tissues and fluids for healthy individuals (controls) and diabetes mellitus patients.

Element	Human sample	Unit	Concentration	
			Healthy	Diabetes
B	Hair	mg/kg	5*	nv
	Fingernails	mg/kg	15.2	nv
V	Serum	µg/l	5.91 ± 1.23**	1.94 ± 1.05
	Urine	µg/l	4.39 ± 2.92	2.74 ± 1.81
Cr	Scalp hair	mg/kg	2.2	2.3
	Fingernail	mg/kg	1.0	0.7
	Serum	µg/l	1.44 ± 0.7	0.66 ± 0.58
	Urine	µg/l	1.92 ± 1.37	2.09 ± 1.51
Mn	Serum	µg/l	1.44 ± 0.69	2.83 ± 1.25
	Urine	µg/l	1.52	1.39
	Scalp hair	mg/kg	3.05 – 4.55+	1.82 – 3.67
Fe	Scalp hair	mg/kg	30.5 – 33.3	35.7 – 41.3
	Blood	mg/l	705	655
	Urine	mg/l	2.4	1.83
Cu	Scalp hair	mg/kg	10.5 – 13.3	10.9 – 14.5
	Fingernail	mg/kg	50.5	75.3
	Serum	µg/l	915 ± 194	1221 ± 299
	Urine	µg/l	14.4 ± 12.9	15.2 ± 15.4
Zn	Serum	µg/l	606 ± 87	612 ± 148
	Scalp hair	mg/kg	183.7	124.8
	Fingernail	mg/kg	206	133.8
	Urine	µg/l	279 ± 167	455 ± 373
As	Serum	µg/l	1.33 ± 0.41	0.83 ± 0.59
	Urine	µg/l	21.2 ± 14.8	27.0 ± 12.6
Cd	Serum	µg/l	0.04 ± 0.01	0.13 ± 0.48
	Scalp hair	mg/kg	0.5	0.8
	Fingernail	mg/kg	1.1	0.9
	Urine	µg/l	0.32 ± 0.21	0.13 ± 0.21

* mean, ** mean ± standard deviation, + range, nv = no value.

Source: Flores *et al.*, 2011; Kazi *et al.*, 2008; Sukumar & Subramanian, 2007; Batista *et al.*, 2006; Nourmohammadi *et al.*, 2005; Abou-Shakra *et al.*, 1989; Bowen, 1979.

1.3 Trace Element Measurements

Trace and ultra-trace element levels can be measured in different human tissues and fluids (Esteban & Castano, 2009). In general, the levels of these elements vary from one tissue or fluid to another due to multiple factors including lifestyle, age, gender, environmental exposure, diet, alcohol consumption and cigarette smoking (Chojnacka, 2005; Partriarca *et al.,* 1998). The main reason for the selection of human tissues (scalp hair and fingernails) and fluids (tear drops and saliva) in this study is that they can be used to biomonitor human health (Madej, 2010; Esteban & Castano, 2009). A long-term growth material, such as scalp hair and nails, may provide some useful data, especially if the subject's results are compared with a corresponding reference concentration range for a well defined "healthy or control" population (Sukumar & Subramanian, 2007).

In the case of tear drops, the main reason for inclusion in this study is that this media can be considered a new area of research, as there is no published data about the elemental levels of this fluid. This may be because it is difficult to collect enough tear drop volume for trace element analysis as analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS), usually need at least 3 ml of sample. Previous studies have determined the levels of some elements in saliva (Costa *et al.,* 2010; Olmedo *et al.,* 2010; Menegario *et al.,* 2001). In addition, environmental samples (water and cigarette tobacco) were chosen in order to assess whether these media make any significant contribution to the specific elements in the human tissues and fluids under investigation.

1.4 Human Fluids

1.4.1 Tear Drops

This section describes the chemical composition, main functions and the relationship between tear drops and human diseases with respect to trace element levels.

1.4.1.1 Types and Chemical Composition of Tear drops

Human tear fluid consists of three main layers, proposed by Zhao *et al.* (2010). Firstly, the lower layer, which has a mucous and hydrophilic coating, is produced by the conjunctiva goblet cells. It can increase the stability of the aqueous layer over the eyeball (Lemp & Wolfley, 1992). Secondly, the middle layer which is the aqueous layer is secreted by the lachrymal gland. Finally, the upper layer, also known as the oil or lipid layer is produced by the meibomian glands at the rim of the eyelid which is responsible for this layer (Zhao *et al.,* 2010; Filik & Stone, 2008; Davidson & Kuonen, 2004). This is shown in Figure 1.4.

There are three types of tear drops: (i) basal tear, (unstimulated tear), which occurs in healthy human eyes to keep the cornea continuously moistened. The secretion rate will significantly increase based on physical and emotional stimulation. The volume range is 6 – 7 µl/min with a maximum capacity 30 µl/min and basic flow about 1.2 µl/min (Madej, 2010); (ii) reflex tear (stimulated tear), the secretion rate of this tear usually depends on different factors, such as foreign particles, onion vapour, tear gas, pepper spray, bright light and vomiting; and (iii) crying tear, in which the subject would be in a strong emotional state of stress suffering, mourning or physical pain.

The amount of tear drops can increase in specific situations, such as interpersonal relationships; such as loss, conflict, reunions, marriage and deaths. In other cases, it can result from social factors, such as culture, gender, age and socialisation. It is extremely useful to report that there is a significant difference between the composition of stimulated and unstimulated tear fluids. A previous study reported a significantly higher range of values of glucose (211 - 256 µM) in tears induced by onions of non-diabetic subjects when compared with glucose levels (13 – 51 µM) of unstimulated tears collected from the same non-diabetic subjects (Taormina, 2007). The main question one can ask is what the mechanism of tear drop formation in the human eye is? A possible explanation for this process is that tear drops are secreted on the surface of the cornea from different glands. It was found that a tear drop will break during 1 – 30 seconds due to the combined effects of evaporation and surface tension. When a drop is released, the formation of a new one will start immediately. The occurrence of dry spots is prevented by reforming tear drops through frequent blinking (Jossic *et al.,* 2009).

Human tear fluid has a complex structure including, water, proteins, electrolytes, metabolites and lipids (Filik & Stone, 2008). Previous studies have suggested that about 500 proteins (in low abundance) are present in tear fluid (Li *et al.*, 2008; de Souza *et al.*, 2006). The main proteins are lysozyme, tear lipocalin, secretory immunoglobulin A and lactoferrin (Zhao *et al.*, 2010). This enables tear fluid to carry out various functions in terms of the ocular system (Ohashi *et al.*, 2006). Healthy functioning of the eyes is strongly associated with the formation of tear fluid (Filik & Stone, 2008). Therefore, any changes in the chemical composition of tear fluid can lead to more disorders such as ocular pathology (Davidson & Kuonent, 2004). Previous studies have reported that the transparency of the cornea will be fundamentally affected by quantitative or qualitative changes in the composition of tears (Ohashi *et al.*, 2006; Grus *et al.*, 2005).

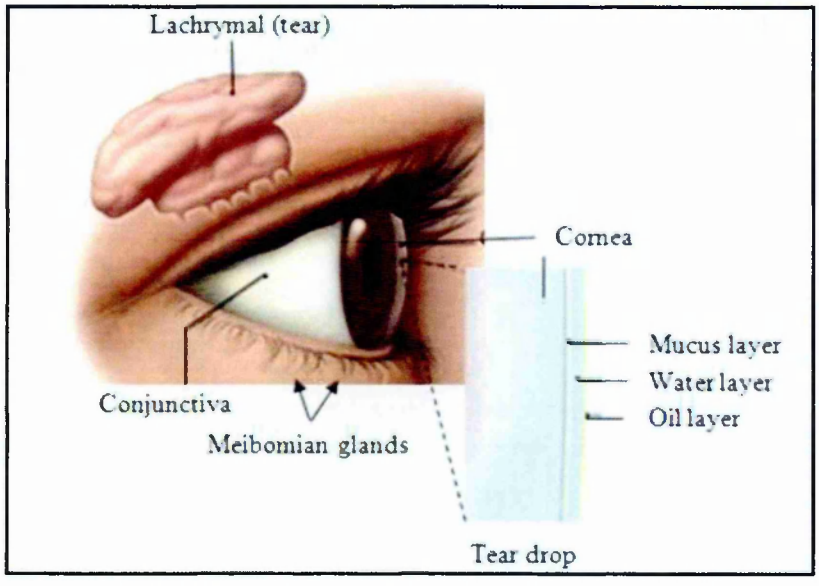

Figure 1.4: The human eye and tear drops (www ref.1)

1.4.1.2 Major Functions of Human Tear Fluid

Knowledge of the functions of tear fluid, and the specific interactions with human eyes, will lead to an improved understanding of tear fluid (Jossic *et al.*, 2010; Filik & Stone, 2008; Ohashi *et al.*, 2006; Albarran *et al.*, 1997). There are many different functions that are provided by tear fluid, but the fundamental roles are:

- keeping the surface of the cornea smooth in order to maintain clear vision. In this case, tear fluid will be the first refractive surface encountered by light in order to protect the surface of the cornea, which is the second refractive component (Lemp & Blackman, 1981);
- using the oil layer to lubricate the conjunctiva, cornea and the eyelids. This will protect the eye surface from any mechanical damage resulting from increased pressure caused by each blink (Lamberts, 1994);
- transporting oxygen and nutrients to the cornea (nutrition source) and regulating the electrolyte composition and pH (Lamberts, 1994);
- provides the cornea and conjunctiva with white blood cells (Lamberts, 1994);
- removes foreign materials from the surface of the cornea and conjunctiva (Lamberts, 1994);
- protects the ocular surface from pathogens and infection via defensive proteins and antibodies (Lemp & Blackman, 1981); and
- maintains the viscosity and prevents tear fluid evaporation (Zhao *et al.,* 2010; Lemp & Wolfley, 1992).

In addition, tear fluid can also protect the surface of the cornea from the effects of the external environment, such as desiccation, bright light, cold, mechanical stimulation, physical injury, noxious chemicals, bacteria, viral and parasitic infection (Ohashi *et al.,* 2006). It was also found that protein components can play significant roles to provide continued health and protection of the cornea (Sariri & Ghafoori, 2008). A recent study has shown that various protein components perform an important role in tears (Zhao *et al.,* 2010). Another fundamental function of human tear fluid is the protection of the eye from different diseases, for example, dry eye disease (Flanagan & Willcox, 2009).

1.4.1.3 Human Tear Fluid in Health and Disease

There are two categories responsible for many ocular diseases: systemic and local (Seal, 1985). The first reason can arise from in-born disorders of metabolism. For instance, Tay-Sachs disease leads to high levels of glycosidase in the tear fluid

and reduces the subject's ability to produce sufficient tear fluid. The second reason results from infections or injuries which can lead to the accumulation of various materials in tears. In these conditions, some quantities of blood components can be transported into the tears and vice versa. This process will mean only a small volume of tears is available. As a result, the levels of toxic products will increase, which lead to "toxic tears" (Tiffany, 2003). Several studies have detected a series of changes in the composition of tear fluid during many health disorders and diseases, such as diabetes, renal disease (Ozdemir *et al.,* 2004; Grus *et al.,* 2002) and Parkinson's disease (Tamer *et al.,* 2005*).* Moreover, one study in Australia has found a number of differences between the composition of healthy dog tears and those with various cancers. The authors suggested that it is possible to use tear fluid as a non-invasive test in order to diagnose canine cancers (Campos *et al.,* 2008).

The use of pharmaceuticals in the treatment of eye diseases may lead to more effects on tear fluid, for example, tarsorrhaphy, which is used for severe dry eye. This disease is a worldwide problem for elderly individuals (Gharaee *et al.,* 2009). Furthermore, contact lenses may lead to decreases in the volume of tears, and the amount of lysozyme protein (Flanagan & Willcox, 2009).

Cigarette smoking provides a significant risk in terms of several eye diseases, for example, macular degeneration, glaucoma, and cataract formation. It was found that toxic and oxidative effects of tobacco lead to damage of the eye tissue, including the onset of dry eye disease (Grus *et al.,* 2002).

Human tears can be used as a new non-invasive approach in the early diagnosis and analysis of the pathogenesis of diabetes, including ocular surface disease. A previous study reported a significant increase in the concentration of tear drop protein for diabetic patients who have dry eye disease compared with diabetic patients who do not suffer from this disease (Grus *et al.,* 2002). However, trace elements in tear fluid may play a role in the conditions of these diseases, as many trace elements are reported to be an important biomarker for different diseases, as shown through using other biological samples, such as hair, nail and saliva (Skalnaya & Demidov, 2007; Rajpathak *et al.,* 2004).

1.4.1.4 Trace Elements in Tear Drops

In recent decades, the use of unconventional biological materials as biomarkers in trace element studies has increased in terms of published research studies, for example, scalp hair, fingernails and saliva (Esteban & Castaño, 2009; Rodrigues *et al.*, 2008). Human tear drops can also be used as a useful tool to evaluate the health status of an individual (Zhao *et al.*, 2010). This fluid was used to assess the levels of glucose in diabetic individuals (Taormina *et al.*, 2007; Jin *et al.*, 2004). Several studies have reported that the concentration of glucose in blood can be correlated with the level in tear fluid (Baca *et al.*, 2007). In contrast, the analysis of trace elements in tear fluid has not been established so far. The main challenge in analysing this fluid is insufficient amounts of sample available for multi-element determination using most analytical techniques (Madej, 2010). One study has reported the levels of Na^+, K^+, Cl^- and total Ca in tear fluid for normal subjects (Lew *et al.*, 2004).

In terms of considering tear fluid as a possible biomarker, a review of the use of other tissues or fluids is presented.

1.4.2 Saliva

1.4.2.1 Chemical Composition of Saliva

Human saliva is a complex fluid which is secreted into the mouth by the various salivary glands including: parotid glands located behind the jaw in front of the ear; submandibular and sublingual glands that lie under the jaw and tongue (Wang *et al.*, 2008). These are illustrated in Figure 1.5. It was found that salivary fluid differs from one gland to another, for example, the parotid gland produces saliva with a watery (serous) consistency, whilst the sublingual glands produce a more viscous (mucous) fluid. A mixture of serous and mucous saliva can be produced by the submandibular glands (Wang *et al.*, 2008; Whelton, 1996). Normally, human salivary fluid contains 98 % water, dissolved inorganic electrolytes, antibacterial constituents, protein, mucus, carbohydrate, and various enzymes (Shigemi *et al.*, 2008; Reznick *et al.*, 2006). The total daily secretion of saliva from all the glands ranges between 800 and 1500 ml/day (Wang *et al.*, 2008). The

salivary flow rate is lowest during sleep and highest when eating (5 ml/min). The main reason leading to a reduction in this flow rate is dehydration and after significant blood loss this can lead to the sensation of thirst (Whelton, 1996). In this case there are many oral functions which can be affected, such as chewing and swallowing, and speaking will become uncomfortable and sometimes difficult to perform. In addition, dental diseases (namely dental caries and periodontal disease) can result when salivary flow is significantly reduced. Saliva helps to dissolve food inside the human mouth (part of the digestive process) due to the many enzymes found in this fluid. Saliva also plays role in the tasting process.

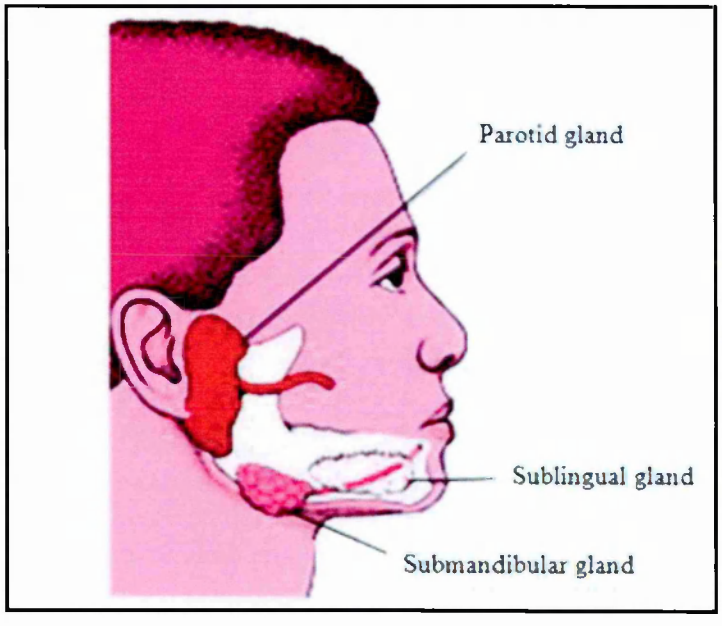

Figure 1.5: Location of the major human salivary glands (after Lawler *et al.*, 2004; www ref.2).

1.4.2.2. Trace Elements in Saliva

Many studies in the literature use blood and urine for studying heavy metal exposure in occupational and environmental areas (Olivero-Verbel *et al.*, 2007; Heitland, *et al.*, 2006). Saliva has also been recognised to play a significant role in terms of providing a reliable index of environmental and occupational exposure (Costa *et al.*, 2010; Olmedo, *et al.*, 2010; Barbosa *et al.*, 2006). The use of saliva as an alternative matrix for risk assessment is beneficial, as it is readily accessible and is a non-invasive sampling technique for the general population (Wang *et al.*,

2008). In this respect, manganese levels in saliva have been used as a biomarker for manganese exposure among career welders. Such exposure also leads to changes in the levels of some other trace elements, such as, Mn, Cu, Zn, Cd, and Pb in saliva. Recent studies have shown that the relationship between Pb-saliva and environmental contamination is significant. Therefore, saliva has been used as a biomarker of Pb exposure (Costa *et al.*, 2010; Barbosa *et al.*, 2006; Wilhelm *et al.*, 2002).

As mentioned, there are three different types of saliva: whole; submandibular/sublingual (sub) and parotid saliva. The elemental levels are found to be different between types of saliva, for example, the lead levels of the whole, sub, and parotid saliva were found to be 1.7, 1.4 and 1.3 μg/l Pb, respectively for children exposed to lead in Brazil (Costa *et al.*, 2010). The authors found a significant correlation between Pb-serum and Pb-parotid saliva, as the concentration of lead in saliva reflects the level in plasma. This may be because the active transport media is water, and the ions in saliva came from plasma fluid.

Some trace elements in saliva have a significant positive correlation between each other, for example, manganese positively correlates with copper and zinc (Wang *et al.*, 2008). Previous studies have shown that some of the trace elements, such as strontium, could play a significant role in the development of dental caries (Curzon, 1985; Athanassouli *et al.*, 1983). Although there is little information about strontium in terms of human health, it was found that high strontium levels in saliva and human scalp hair is associated with skeletal problems and dental caries (Shigemi *et al.*, 2008; Curzon, 1985). One study in Japan has shown that the strontium levels in saliva collected from school children were significantly increased in those with caries. In contrast, the concentration of fluoride in toothpaste inhibits strontium dissolution from teeth which leads to the protection of the teeth (Shigemi *et al.*, 2008). Saliva may also be used to evaluate whether an orthodontic appliance releases any metal ions, such as nickel, into the oral cavity (Fors & Persson, 2006).

Trace element levels in saliva may change in relation to diseases, for example, copper levels in saliva increased in taste disorder patients compared to control subjects. However, the levels of other elements, such as zinc and manganese, also decreased (Watanabe *et al.*, 2005). Another study has determined the levels of trace elements in saliva in order to assess whether factors like sample collection

procedures, dental prostheses, and amalgam fillings may affect the elemental levels in saliva fluid (Monaci *et al.,* 2002). Inductively coupled plasma mass spectrometry (ICP-MS) has been used to determine the elemental levels in unstimulated and stimulated saliva samples (Costa *et al.*, 2010; Wang *et al.,* 2008; Yuan *et al.,* 2008; Watanabe *et al.,* 2005). Table 1.2 reports the elemental levels in saliva.

Table 1.2: Control elemental concentrations (µg/l) of human saliva.

Element	Concentration (µg/l)
B	$0.6 - 20.5^{+}$
V	nv
Cr	0.41 – 1.64
Mn	0.47 – 7.23
Fe	32 – 270
Cu	$19.6 \pm 13.6^{++}$
Zn	11 - 158
As	0.19 – 3.3
Sr	2.16 ± 0.96
Cd	0.02 – 1.90

nv = no value, $^{+}$ range, $^{++}$mean ± standard deviation.
Source: Gil *et al.,* 2011; Y- Kim *et al.,* 2010; Wang *et al.,* 2008; Yuan *et al.,* 2008; Ward, 1993; Ward & Ward, 1991.

1.5 Human Tissues

1.5.1 Scalp Hair

Scalp hair is a fibrous material derived from skin which has two main parts; the shaft, which protrudes out from the skin, and the root, which lies below the surface of the skin (de Antonio *et al.,* 1982). The matrix cells grow in the root, and during their formation are exposed to circulating blood, lymph and extracellular fluids. When the hair grows, it hardens to form the shaft in the process called keratinisation (Valkovic, 2000; de Antonio *et al.,* 1982). The hair root comprises three layers, namely the hair fibre, inner root sheath and outer root

sheath. The hair shaft includs three main parts; the cuticle on the outside, the medulla in the centre and the cortex in between, as shown in Figure 1.6 (Dunnett, 2001). The main constituents in human hair are protein, namely (keratin) (80 – 85 %), water (< 15 %), lipids (1 – 9 %), melanins (0.3 – 1.5 %) and inorganic minerals (0.25 – 0.95 %) (Dunnett, 2001). The rate of growth of scalp hair is slower in males than females and is about 0.3 to 0.5 mm/day in a human adult and 0.2 mm/day in a newborn (Valkovic, 2000).

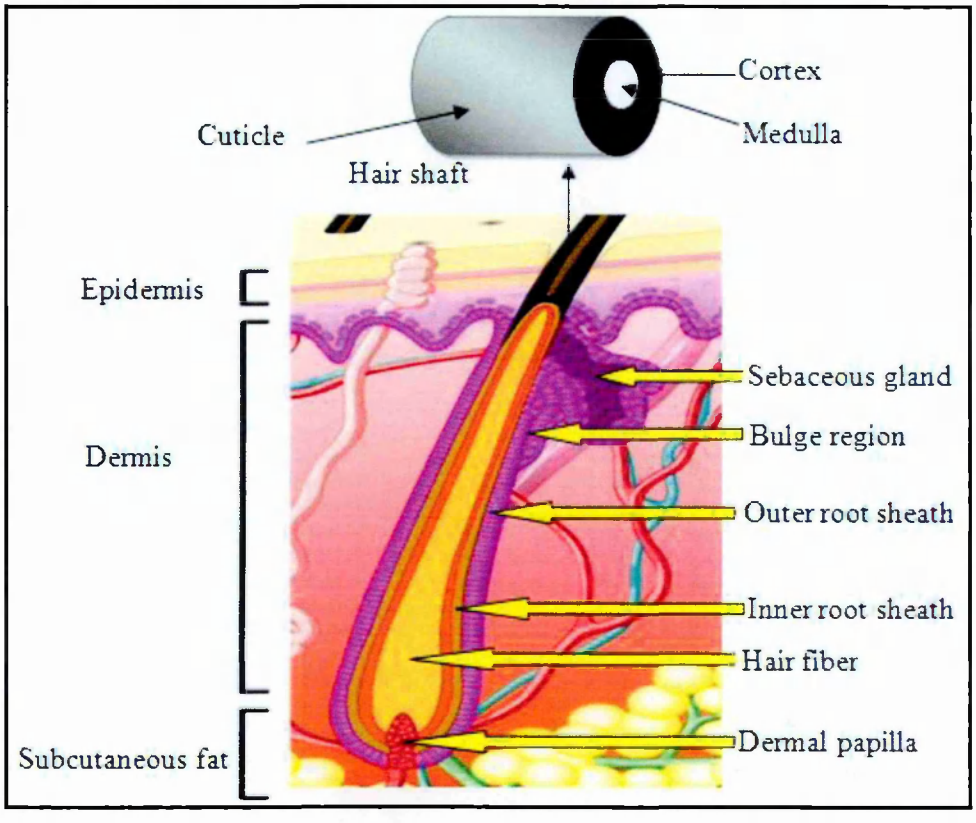

Figure 1.6: Hair structure (after Dunnett, 2001; www ref. 3).

1.5.2 Fingernails

Fingernails are a material formed by living skin cells. The structure of nails contains several parts; (i) matrix (nail root) which is located under the cuticle where new cells are produced and continually pushed towards the nail plate to produce the nail plate; (ii) cuticle, the tissue that is surrounding the nail plate and defends the matrix from attacking bacterial and physical damage (Freinkel & Woodley, 2001); (iii) lunula (half-moon), the base of the nail and meeting point

between the matrix and nail bed; and (iv) nail plate which represents visible nail that rests on the nail bed up to the free edge (Freinkel & Woodley, 2001). These components are shown in Figure 1.7.

As the new cells grow in the matrix the older cells are pushed out from the matrix. There are several factors that can influence the growth of nails such as, age, diet and health status. The rate of growth ranges from 0.03 to 0.05 mm/day in toenails to 0.1 mm/day in fingernails (Slotnick & Nriagu, 2006). This rate usually is faster in young people than older individuals, in the summer rather than winter and during pregnancy (Batista *et al.*, 2008). If a fingernail is lost or injured, new nail will always grow. The only exception is if the matrix is damaged, then the nail will grow back deformed (Figure 1.7).

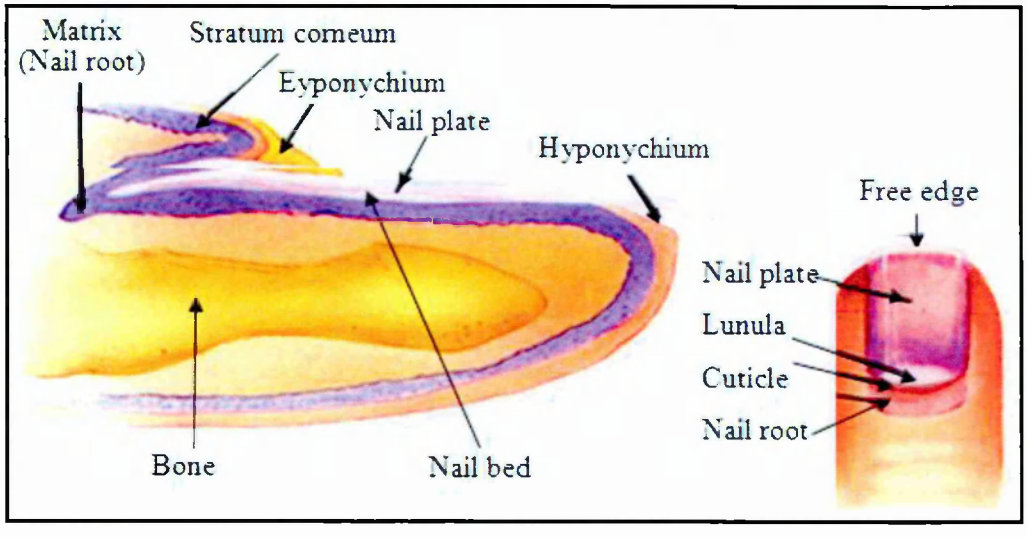

Figure 1.7: Fingernail structure (after Freinkel & Woodley, 2001).

Although there are a lack of studies discussing the use of nails, nail material is considered a suitable tissue to determine the levels of minor and trace elements in the human body (Silvera & Rohan, 2007).

1.5.3 Human Scalp Hair and Nails as a Biomarker

In the last few decades, human scalp hair and nails (finger & toe) have widely been used as a good biomarker in the assessment of exposure to various pollutants in an occupational and/or environmental setting, and in terms of assessing the

metabolic state of humans, for essential and toxic trace elements (Olmedo *et al.,* 2010; Esteban & Castano, 2009; Li *et al.,* 2008; Ohno *et al.,* 2007; Ashraf *et al.,* 1995). Hair and nail tissues have several advantages over blood and urine, including: non-invasive materials and easily sampled; potentially represent a long-term growth material; and several trace elements may accumulate in hair and nail tissues over a time frame of 2 to 18 months. These advantages may provide useful data in determining the health status of an individual over long periods, as the tissues remain isolated from other metabolic activities in the human body (Wang *et al.,* 2009; Batista *et al.,* 2008; Sukumar & Subramanian, 2007; Slotnick & Nriagu; 2006; Kales & Christiani, 2005; Bermejo-Barrera *et al.,* 2002; Sera *et al.,* 2002; Bass, 2001; Chłopicka *et al.,* 1995).

In contrast, the analysis of human fluids, such as blood and urine is 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 (Hannigan, 2005; Dong, 1998). In terms of these facts, the concentration of trace elements can be used to investigate: (1) the dietary intake of trace elements, especially for non-essential or "toxic" elements; (2) environmental exposure from anthropogenic sources, including chemical pollutants that are released into the environment (Esteban & Castano, 2009); (3) the relationship with smoking activity (non, passive and active); and (4) any possible link between specific trace elements and diseases, such as diabetes (type 2) (Sukumar & Subramanian, 2007; Senofonte *et al.,* 2001).

Hair analysis also has some challenges associated with it, which include external contamination, differentiating between endogenous and exogenous deposition and the difficulties in establishing normal or reference ranges (Bass, 2001). In addition, there are various factors which have been found to affect the level of elements in hair and nails, such as age, gender, lifestyle, environmental exposure, smoking activity and general health status (Ozden *et al.,* 2007; Sukumar & Subramanian, 2007; Chojnacka *et al.,* 2006; Rodushkin & Axelsson, 2000; Garland *et al.,* 1996).

Table 1.3: Reported normal or control levels of trace elements in washed scalp hair and fingernail.

Element	Country	Concentration, Mean (range) (mg/kg)			
		n	Scalp hair	n	Fingernail
B	Sweden	114	0.13 – 3.30[+]	96	0.12 – 3.33[+]
	Rio de Janeiro	83	1.0 – 3.0		nv
V	Rio de Janeiro	83	0.35 – 0.80		nv
	Sweden	114	0.005 – 0.134	96	0.018 – 0.476
Cr	Rio de Janeiro	83	0.78 – 1.0		nv
	India	113	2.2 ± 0.5[++]	113	1.0 ± 0.2[++]
	Sweden	114	0.046 – 0.527	96	0.224 – 3.20
Mn	Rio de Janeiro	83	0.26 – 0.75		nv
	Sweden	114	0.08 – 2.41	96	0.19 – 3.30
	Italy	18	0.28 ± 0.19		nv
	Bangladesh	44	1.85 – 43.56	33	3.51 – 91.33
Fe	Rio de Janeiro	83	6.0 – 15		nv
	Sweden	114	4.9 – 23	96	12 – 189
	Italy	18	13.1 ± 7.1		nv
	Bangladesh	44	16.53 – 304.49	33	39.76 – 1967.46
Cu	Rio de Janeiro	83	13 – 35		nv
	India	113	44.4 ± 6	113	50.5 ± 7
	Sweden	114	8.5 – 96	96	4.2 – 17
	Italy	18	7.49 ± 3.41		nv
	Bangladesh	44	4.2 – 55.29	33	4.6 – 28.78
	Pakistan	150	15 – 21.8		nv
Zn	Rio de Janeiro	83	125 – 165		nv
	Sweden	114	68 – 198	96	80 – 191
	India	113	183.7 ± 24	113	206 ± 27
	Pakistan	150	227.2 – 262.8		nv
	Italy	18	131 ± 47		nv
	Bangladesh	44	82.52 – 339.64	33	72.77 – 130.39
As	Kuwait	40	0.11 – 0.16		nv
	Sweden	114	0.034 – 0.319	96	0.065 – 1.09
	Pakistan	150	0.73 – 0.94		nv
Sr	Rio de Janeiro	83	1.0 – 7.6		Nv
	Sweden	114	(0.14 – 5.54)	96	0.17 – 1.39
Cd	Rio de Janeiro	83	< 1.0		nv
	India	113	0.5 ± 0.08	113	1.1 ± 0.3
	Bangladesh	44	0.008 – 2.14	33	0.017–1.93
	Kuwait	40	0.13 – 0.18		nv
	Sweden	114	0.010 – 0.356	96	0.013 – 0.438
	Pakistan	150	2.51 – 3.89		nv

[+] range, [++] mean ± standard deviation, nv = no value, n = the number of sample.

Source: Sukumar & Subramanian, 2007; Bocca *et al.*, 2006; Kazi *et al.*, 2006; Chojnacka *et al.*, 2005; Fido & Al- Saad, 2005; Forte *et al.*, 2005; Samanta, *et al.*, 2004; Rodushkin & Axelsson, 2000; Miekeley *et al.*, 1998.

1.6 Environmental Sources of Trace Elements

The significant roles of trace elements in a living organism affect the direct interactions with surrounding systems, namely; environmental, geological, biological and marine (Ward, 2000). Therefore, the levels of elements in environmental (soil, sediment, water, etc), biological and foodstuff samples may be a potential risk factor in assessing the quality of human health (Arain *et al.,* 2009). Trace elements are transported from aquatic media to the human body through food chain and drinking water, and then are stored in different tissues and fluids (Arain *et al.,* 2009). The monitoring of trace elements, especially toxic elements in the environment, maintains the attention of many scientific researchers, who consider it is necessary to understand the long-term health effects of chronic exposure to low concentrations of toxic elements.

The assessment of human exposure to pollutants from an environmental media can be monitored by using non-invasive tissues and fluids, such as hair, nail, blood, urine and saliva samples (Button *et al.,* 2011; Costa *et al.,* 2010; Olmedo *et al.,* 2010; Kazi *et al.,* 2008; Rodrigues *et al.,* 2008). The high levels of trace elements in these tissues and fluids may be due to exposure from the consumption of drinking water and food. For example, the concentration of arsenic and other elements (Pb, Ni, Cd, Mn, Fe, Zn, Se and Hg) can be measured in scalp hair and nails of people who are drinking arsenic contaminated water (Sthiannopkao *et al.,* 2010; Samanta *et al.,* 2004; Skrzydlewska *et al.,* 2003).

1.6.1 Water

Normally, drinking water (DW) is taken from rivers, lakes, reservoirs, springs and wells. During the flow of water over the surface of land, some components of rocks and soil may dissolve and be carried to the final consumer (Mandal & Suzuki, 2002). Organic and inorganic contaminations can be released into drinking water from different industrial processes, such as petroleum production, chemical fertilizers, iron, leather, pharmaceutical and refining, as well as domestic activities (Majumder, 2009). In recent years, the chemical, biological and physical quality of the aquatic environment has been found to be a main factor controlling the state of health and disease for both humans and animals. Therefore, many studies have used water samples to assess the effect of trace elements on human

health (Chen *et al.*, 2009; Oymak *et al.*, 2009; Navas-Acien *et al.*, 2008; Karadede *et al.*, 2004; Karadede & Unlu, 2000). As a result of these studies, the World Health Organisation (WHO) modified the permissible levels for some risk elements based on their findings; for example, the guideline level for arsenic in drinking water was reduced from 50 to 10 µg/l As, as shown in Table 1.4 (WHO, 2008). It is, therefore, necessary to establish the natural elemental levels of the different water types in order to evaluate the impact of trace element contamination. Typical values are reported in Table 1.5.

Table 1.4: Water quality guidelines for drinking, irrigation and livestock consumption.

Trace element	WHO Drinking water limits (µg/l)	FAO Irrigational water limits (µg/l)	FAO Livestock drinking water limits (µg/l)
B	500	nv	5000
V	15	nv	100
Cr	50	100	1000
Mn	400	200	50
Fe	nv	nv	Nv
Cu	2000	200	500
Zn	3000	2000	24000
As	10	100	200
Sr	nv	nv	Nv
Cd	3	nv	50

FAO – Food and Agriculture Organisation of the United Nations, WHO - World Health Organisation, nv = no value.
Source: WHO, 2008; FAO, 1994.

Table 1.5: Typical natural trace element concentrations for fresh-, river- and seawater.

Trace elements	Concentration (µg/l)		
	Fresh	River	Sea
B	10	10	5000
V	0.5	1	2.5
Cr	1	1	0.05
Mn	10	7	0.2
Fe	500	40	2
Cu	3	5	2
Zn	15	20	10
As	0.5	2	3
Sr	70	60	8000
Cd	0.03	0.02	0.1

Source: Ward, 2000.

1.6.2 Cigarette Tobacco

Tobacco contains a complex mixture of more than 4000 components (Ng & Zelikoff, 2007). They include the stimulant nicotine; along with benzo-pyrene, benzene, lead, chlorinated dioxins and furans. In addition, cigarettes also contain hydrogen cyanide, arsenic, acrolein, acetaldehyde, 1,3-butadien, toluene and phenol which can cause adverse effects on vital human processes, such as the cardiovascular, respiratory, reproductive and nervous systems (IARC, 2004; Ward, 1993). Furthermore, high levels of heavy toxic elements have also been reported in tobacco (Verma *et al.,* 2010). Therefore, cigarette tobacco becomes a high risk source for various diseases, such as mouth cancer (Kazi *et al.,* 2010). In recent years cigarette smoking has become a major health issue, especially in terms of active and passive smoking linked exposure to chemicals released from the combustion of tobacco (WHO, 2008). The World Health Organisation reported the smoking rates for males, females and total population in different regions in the world: Africa (36.2, 9.4 and 22.9%); Americas (34.7, 23 and 28.7%); Eastern Mediterranean (34.2, 8.7 and 21.8%); Europe (43.5, 23.4 and 33%); South East Asia (48.2, 8.2 and 28.6%); and the Western Pacific (62.3, 5.8 and 34.4%) (WHO, 2003). Although, there is no data for smokers in Karbala, Iraq, the majority of smokers are men, with more than 50% of them being heavy smokers (smoked >1 pack/day) (Figure 1.8). This finding is based on the questionnaire that was used during the collection of biological samples from Karbala (refer to Appendix A). Amongst pregnant, women in the USA between 13 - 20% smoke during pregnancy (Ng & Zelikoff, 2007). Previous studies have shown that exposure to tobacco can lead to health disorders for children, such as childhood cancer (Stavrou *et al.,* 2009).

The World Health Organization reported that many diseases can be caused by smoking, such as cancers (namely; larynx, oropharynx, lung, leukemia, stomach, pancreas, kidney, colon, cervix and bladder) and chronic diseases, for example, stroke, periodontitis, coronary heart disease, asthma, and reproductive effects in women (including reduced fertility). In addition, there are some diseases caused by second-hand smoke, especially in children, for example, brain tumours, asthma, lymphoma, leukemia and lower respiratory disease. Adults can also suffer from diseases caused by second-hand smoke, such as, stroke, breast cancer, lung

cancer, asthma and reproductive effects in women (including reduced fertility) (WHO, 2008). Smoking has been recognised to be an important risk factor in diabetes (Ng & Zelikoff, 2007; Montgomery & Ekbom, 2002), with one study in the USA reporting a link between cigarette tobacco and the onset of this disease (Will *et al.*, 2001; Meliker *et al.*, 2007).

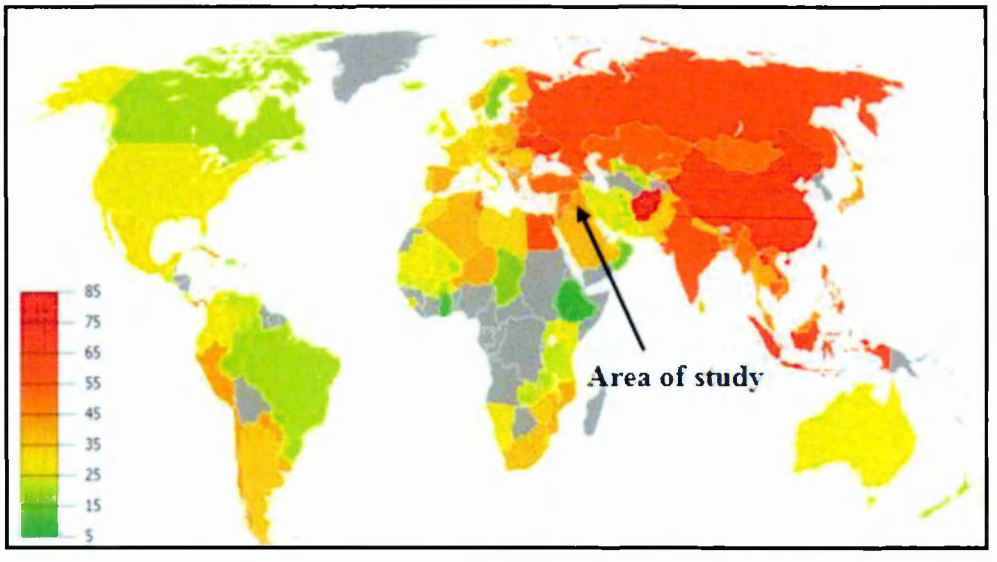

Figure 1.8: Percentage of males smoking any tobacco product in the world (WHO, 2003).

1.6.2.1 Elemental Composition of Tobacco

Tobacco plants absorb many of the essential, non-essential and toxic elements from soils. Fertilizers and pesticide treatments can influence the elements of tobacco, including levels during cigarette harvesting, storage, processing and packing (Martinez *et al.*, 2008). In addition to food sources, tobacco smoking is a major source of environmental trace element exposure to Cr, Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Cd and Pb. (Kazi *et al.*, 2010; Verma *et al.*, 2010; Hamidatou *et al.*, 2009; Vahter *et al.*, 2002). Many studies have reported the levels of trace elements in tobacco in different countries, including Brazil, India, Mexico, Turkey, Iran, Egypt and Japan. This data is summarised in Table 1.6 (Long-Moulin *et al.*, 2006; Cevik *et al.*, 2003; Vega-Carrillo *et al.*, 1995).

Table 1.6: Reported trace element levels of commercial cigarette tobacco (mg/kg).

Element	Mexican (n =9)	Algeria range (n = 5)	USA range (n = 4)
B	nv	nv	nv
V	nv	nv	nv
Cr	nv	4.44 – 29.3	<0.1 – 3.45
Mn	81 - 148	nd	155 – 400
Fe	359 - 564	656 - 823	325 – 520
Cu	9 - 17	nv	nv
Zn	38 - 48	42.80 – 68.06	16.8 – 30.5
As	nv	4.05 – 6.4	<1
Sr	111 - 150	136.88 – 203.20	29.7 – 49.5
Cd	nv	nv	nv

nv = no value.

Source: Hamidatou *et al.*, 2009; Martinez *et al.*, 2008; Oliveira *et al.*, 2000.

1.7 Overview of the Study Area

The key region investigated in this study is the province of Karbala, Iraq. This country is in western Asia, as shown in Figure 1.9. The majority of the Iraqi population use the Tigris and Euphrates rivers for drinking water, domestic use and irrigation (Heyvaer & Baeteman, 2008; Spotts, 2003).

During the past few years, continued release of untreated waste from domestic, industrial and agricultural sources or other human activities into the rivers has lead to an increase in the concentration of many elements in the water (Al-Bedri & Al-Jobori, 1991). One of the most significant sources of pollution in Iraq is from military weapons that were used in the wars, along with oil spills and scrap metal from destroyed military vehicles. According to reports from the United Nations Environment Programme (UNEP), there are 300 sites in Iraq that are considered to be contaminated by various pollutants (UNEP, 2003). As a result, air, water and soil environments have been chemically contaminated. Eventually, these chemicals pass into vegetables, fruit, plants and livestock. The population of Iraq has suffered from many diseases, such as cancer, diabetes, asthma, heart disease, leukaemia and various unknown diseases (Phelps, 2005).

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A major problem now in Iraq is extreme birth deformities, possibly caused by depleted uranium ammunition and other toxic elements. In addition, most children have been exposed to cigarette smoke, as there is no anti-smoking law. Smokers can light up wherever and whenever they choose, for example, 29.2% of students are exposed to second-hand smoke in public places (WHO, 2003). A recent study in northern Iraq has reported that the overall prevalence of current cigarette smoking was 15.3%, 25.1% and 2.7% in adults, boys and girls, respectively (Siziya *et al.*, 2007).

1.7.1 Karbala

Karbala is a city in Iraq located about 60 miles south west of Baghdad at 32.61°N, 44.08°E with approximately one million inhabitants, as shown in Figure 1.10. Unfortunately, in Karbala there is no information available regarding the reference levels of trace and ultra-trace elements in human scalp hair, nails, saliva and tear drops (see confirmation letters from different universities from Iraq in Appendix A). It is, therefore, necessary to establish a database of "normal" or non-contaminated levels of trace elements in this region. This can be used for comparison with other countries, and for the evaluation of future environmental pollution and possible human health disease studies in Iraq.

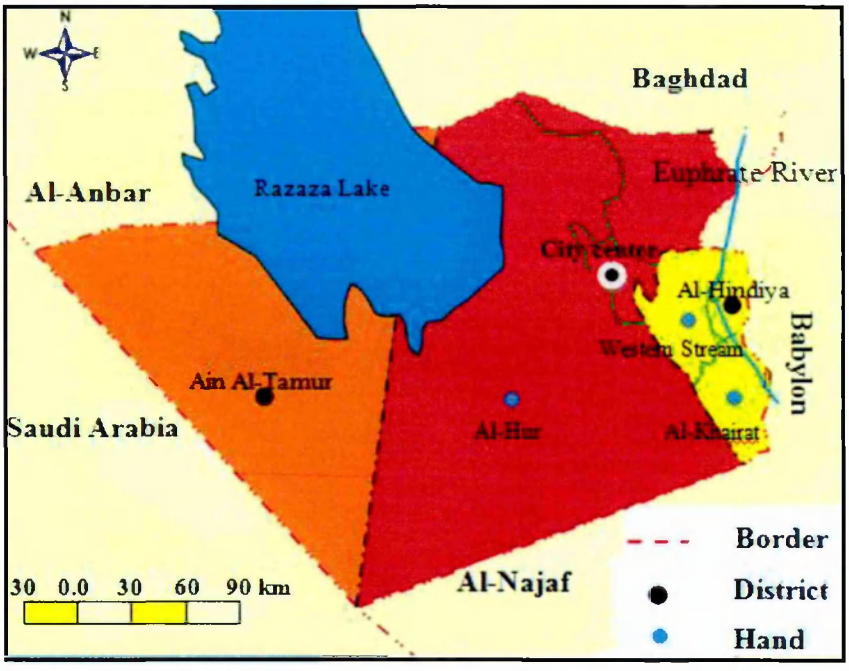

Figure 1.10: Map of Karbala, Iraq (taken from the local Karbala government, www ref. 6).

1.8 Aim and Objectives

1.8.1 Aim

Recent studies have shown that trace and ultra-trace elements play an important role in terms of human health. In the last few decades, blood, urine, saliva, scalp hair and nails have been used as good biomarkers in the assessment of exposure to various pollutants in occupational and/or environmental settings, and in terms of assessing the metabolic state of essential and toxic trace elements in humans (Olmedo *et al.,* 2010; Esteban & Castano, 2009). In recent decades many researchers have seen that the use of unconventional biological materials, such as human tear drops can be used as a tool in determining the health status of an individual (Zhao *et al.,* 2010). So far, no studies have been published on the use of tear drops as a biomarker for trace and ultra-trace elements, as the main problem is the small amount of sample available. There is also a lack of reviews on the use of new analytical techniques for studies using tear drops. In Iraq, no studies have

been published on the use of hair, nails, saliva or tear drops as biomarkers for trace elements and ultra-trace elements in the human body.

The main aim of this study is to develop and validate the use of tear drops as a new biomarker for determining trace element levels in the human body. Other biological samples (scalp hair, fingernails and saliva) were used in order to provide comparative data for evaluating the potential of using tear drops. This methodology was then applied to evaluate if any possible trace element relationships exist between healthy individuals and those with human health conditions, such as smoking activity and type 2 diabetes. Environmental samples (water and cigarette tobacco) were also collected in order to evaluate whether these media make any significant contribution to the elemental levels of the selected tissues and fluids in this study.

1.8.2 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 tear drops;
- develop analytical methods for the determination of elemental levels in washed scalp hair, fingernail, saliva, various water sources (tap, river and ground) and cigarette tobacco;
- validate the developed methods through the use of certified reference materials in order to establish quality control (precision and accuracy) values;
- assess the elemental composition of human scalp hair, fingernail, saliva and tear drops as a useful tool in determining the health status of an individual;
- investigate whether human scalp hair, fingernail, saliva and teardrops can be used as biomarkers in the assessment of exposure to pollutants in an occupational and/or environmental setting in Iraq;
- investigate using above media whether there is any possible link between specific trace elements and the onset of type 2 diabetes;

- investigate whether environmental samples (water and tobacco) make any significant contribution to the elemental levels of tissues and fluids under investigation;
- evaluate the levels of trace elements, especially 'toxic' or non-essential elements in relation to smoking activity of an individual; and to
- investigate whether factors like gender, age and residential location may affect the elemental concentrations in tear drops of the individuals under study.

The next chapter describes the analytical methodology and the instrumentation that were used to achieve the aim and objectives of this work. Chapter 3 (water and tobacco), Chapter 4 (human tear drops), Chapter 5 (saliva, washed scalp hair and fingernails) report the results of the human health and environmental trace elements studies, with the conclusion reported in chapter 6.

Chapter Two

Analytical Methodology, Instrumentation and Statistical Methods

2.0 Introduction

This Chapter describes the sampling, storage and preparation methods that were carried out on the environmental and biological matrices, as shown in Section 2.1. The fundamental theory for each method, along with any development procedures used for the determination of trace element levels are reported in Sections 2.1.1 – 2.2.6. The main technique used for the determination of trace and ultra-trace elements was inductively coupled plasma mass spectrometry (ICP-MS), as outlined in Section 2.6. Further analysis was also performed using inductively coupled plasma atomic emission spectrometry (ICP-AES) and flame atomic absorption spectrometry (FAAS). The use of certified reference materials (CRMs) and replicate analysis ensured accuracy and precision throughout the analysis, as presented in Section 2.8.

2.1 Demographic Characteristics of Study Populations

Environmental (water and cigarette tobacco), biological fluids (tear drops and saliva) and tissues (scalp hair and fingernails) were collected from Iraqi individuals resident in Karbala (Iraq), as shown in Figure 2.1. As part of a comparative study, the same samples were also collected from Iraqi individuals who have lived for more than five years in London (UK). In this study, the biological samples were classified into various groups, namely healthy, diabetics, smoker and non-smoker individuals covering both genders and different ages.

2.1.1 Environmental Samples

Water samples (n = 190) (commercial bottled, domestic bottled, tap, river, spring and well water) were collected from Karbala, as reported in Table 2.1. Commercial bottled water is used for drinking purposes, whereas domestic bottled and tap waters are used for drinking and domestic purposes. Surface (river) and ground water (spring and well) are usually used for irrigation and domestic purposes. In order to evaluate the possible health effects associated with water samples in Karbala, comparative tap water samples were collected from the residences of Iraqi individuals in London (UK). Cigarette tobacco samples were

purchased from various markets in Karbala, as outlined in Table 2.2. The codebook for environmental samples can be found in Appendix D.

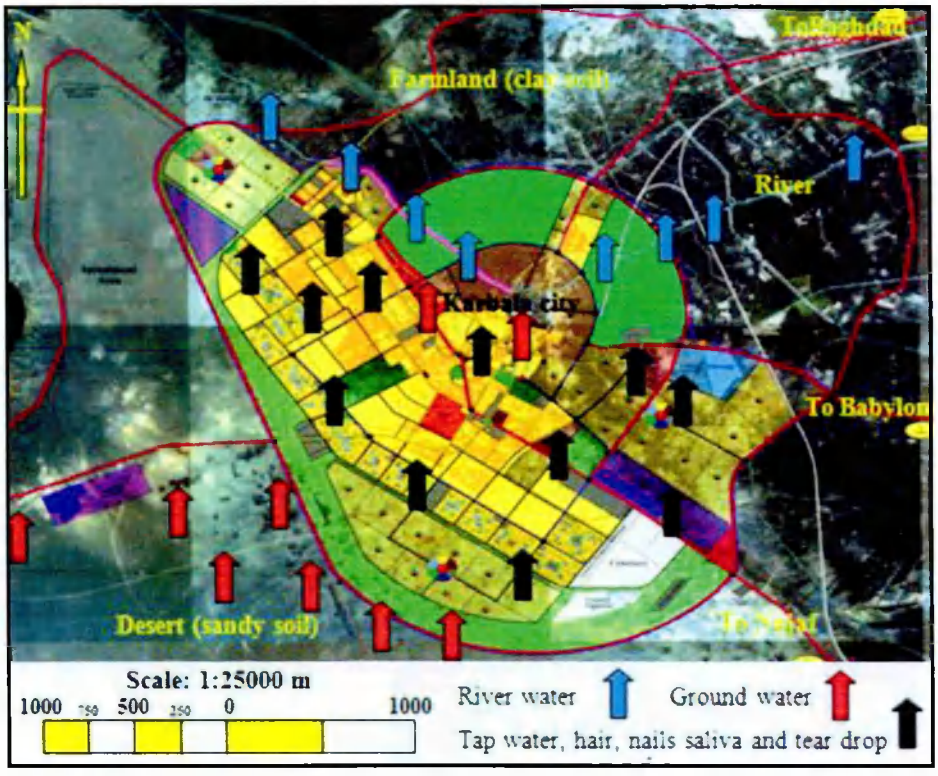

Figure 2.1: Map highlighting the location of samples as collected from Karbala (taken from the local Karbala government, www ref. 6).

Table 2.1: Water samples collected from Karbala (n = 174) and London (n = 16).

Water type	Water samples
Tap	Karbala (n = 50), London (n = 16)
Commercial	Karbala (n = 3)
Domestic bottled	Karbala (n = 33)
River	Karbala (n = 33)
Well	Karbala (n = 47)
Spring (artesian)	Karbala (n = 8)

Table 2.2: Commercial cigarette tobacco samples used in this study (n = 16).		
ID code	Brand	Manufacture (country)
KCT-1	Kent	USA
GCT-2	Ghamdan	Yemen
RCT-3	Royale	France
RCT-4	Roseman	United Arab Emirate
GCT-5	Gauloises	European Union
BCT-6	Brilliant	European Union
GCT-7	Gold seal	Germany
ECT-8	Elegance	Germany
CCT-9	Craven	Switzerland
ICT-10	Ishtar	Jordan
DCT-11	Dunhill	London
ACT-12	Affair	USA
FCT-13	Five stars	Hong Kong
BCT-14	Bon	USA
MCT-15	Miami	Germany
PCT-16	Pine	South Korea
(KCT-1) where K corresponds to the Kent brand, and is replaced by (G) Ghamdan and so on; C corresponds to cigarette; T corresponds to tobacco; and 1 corresponds to the sample code number.		

2.1.2 Biological Samples

This study was approved by the Ethics Committee of the University of Surrey under the University's Ethical Guidelines for Teaching and Research (approval ref. EC/2009/15/FHMS), as shown in Appendix A. The participants were clearly informed of all the study procedures before signing the consent form. All subjects completed the Study Questionnaires so as to provide personal details and information about health, diet, smoking activity and lifestyle at the time of sample collection, as shown in Appendix A. The codebooks that were developed for these questionnaires can be found in Appendices E & F. All the questionnaires were labelled with the code - which was laid out in the following format K-SH-H-010209-1, where K corresponds to the province in Iraq (K) Karbala, and may be replaced by (L) London in the UK; SH corresponds to scalp hair and may be replaced by FN (fingernails), TD (tear drops) and S (saliva); H corresponds to healthy and may be replaced by D (diabetes); 010209 corresponds to the date (DDMMYY); and 1 corresponds to the participant code number.

Generally, the study population followed a similar dietary programme comprising of rice, bread, cereals, vegetables, fruit, meat, oils, cheese, butter, cream and milk; and the main drinks being soft drinks, fruit juice and tea; prepared with household tap water.

The subjects were classified into two main groups, namely healthy and diabetic, as shown in Table 2.3 and Section 2.2.3. At the time of sample collection data on gender, age, smoking activity, residential location, factors relating to having type 2 diabetes and consumption of drinking water were collected by questionnaire. The main reason to collect samples from London was to provide a database for comparative purposes with Karbala (Iraq) samples. In addition, some individuals provided either two or four types of samples, namely tear drops/saliva (n = 42); tear drops/scalp hair (n = 50); tear drops/fingernails (n = 51); and tear drops/saliva/hair/fingernails (n = 30). This then enabled an examination of any significant differences and whether a possible relationship between the levels of trace elements between these media existed.

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

Human sample	Number of samples		
	Healthy		Diabetic
	Karbala (Iraq)	London (UK)	Karbala (Iraq)
Tear drops	111	18	44
Saliva	43	25	29
Scalp hair	171	50	44
Fingernails	127	45	87

2.2 Sample Collection and Preparation

The environmental and biological material samples were either solid (cigarette, scalp hair and fingernails) or liquid (water, tear drops and saliva), and homogenous or heterogeneous in terms of physical and chemical composition. The accuracy of an analysis depends significantly on the conditions under which the sample is collected (Christian, 1994). For example, heterogeneous samples require further care during sampling and will need special pre-analysis treatment before storage and analysis (Ebdon *et al.*, 1998). However, certain precautions

should be taken in order to prevent or minimize contamination, loss, decomposition, or matrix change.

2.2.1 Water

Water samples, namely, commercial, domestic bottled, tap, surface (river), and ground (well and spring or artesian) were collected from Karbala (Iraq) using 50 ml Sterilin® containers (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK), as shown in Table 2.1. All collection containers were rinsed three times with water from the site being sampled, to minimise potential elemental contamination from the surface of the container during storage. In order to prevent any possible oxidation of the sample by air being present in the filled container, all containers were filled with a sufficient amount of water, capped and kept tightly closed (Arain *et al.*, 2008).

Tap water samples were collected from Karbala and London after allowing the tap to run for more than two minutes through the pipes, in order to obtain a "real" water sample from the main pipeline, and to minimise any possible contamination from the pipe and tap materials.

River and spring waters were sampled from Karbala at a range of depths (0 – 30 cm below the water level). Surface water samples were collected using 50 ml Sterilin® containers (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK).

Ground waters (untreated well water) were taken from Karbala (approximately at a depth 10 – 12 m) which are located in the desert region (sandy soil) of west Karbala up to the international border with Syria and Saudi Arabia. This region is called Pliocene (Iraqi Ministry of Water Resources, 2010). Water flow-rates were dependent on pumps that are used to draw up the groundwater into untreated open-air storage tanks.

The important water quality parameters (pH; total dissolved solid (TDS), mg/l; and electrical conductivity, (EC), µS/cm) were measured immediately at the time of sampling (Arain *et al.*, 2008) using a fully calibrated Hanna HI 98129 Digital Combo Meter (Hanna Instruments Ltd, Bedfordshire, UK), as outlined in Table 2.4.

2.2.1.1 Sample Storage, Method of Transfer and Preparation

Water samples were stored in a cool environment of 4°C during field sampling using a Tropicool 14 litre Thermoelectric cool box TC-14 (Waeco©, Dorset, UK) to prevent vaporization and biodegradation, as described in Section 2.2.3.1 (Atta & Abdul Razzak, 2008). On return to the laboratory the samples were transferred to a refrigerator (4°C), and sub-samples were taken for analysis by inductively coupled plasma mass spectrometry (ICP-MS) (Section 2.6). Certified reference materials (CRMs), namely; NIST SRM® 1643e Trace Elements in Water (National Institute of Standards and Technology, Maryland, USA); and TMDA 54.4 Trace Elements in Fortified Lake Ontario Water (National Water Research Institute, Ontario, Canada), reagent blanks (field blanks) and "pooled" samples were prepared in the same manner in order to undertake quality control measurements, as outlined in Section 2.8.3.

All water samples were removed from the fridge prior to any analysis and allowed to equilibrate to room temperature. Multi-elemental analysis was carried out for all water samples by ICP-MS within 2 weeks of sample collection.

2.2.1.2 pH, Conductivity and Total Dissolved Solid (TDS)

pH is a measure of the hydrogen ion activity (a_H^+) in a solution, expressed as its negative logarithm: pH = - log a_H^+. Basically, pH values range from 0 to 14. Natural waters have a range of pH from 4 to 9, and usually are slightly basic because of the presence of naturally occurring carbonates and bicarbonates (Skoog *et al.*, 1998). The recommended pH range for potable water (drinking water) is set at 6.5 – 8.5 by the World Health Organisation (WHO, 2008).

Electrical conductivity (EC) is a measure of the ability of an aqueous solution to carry an electrical current based on the concentration, mobility, and the valence state of the ionised species in a solution (Siosemarde *et al.*, 2010). The conductivity value is increased when the concentrations of the ions increase. Some ions have a major effect on the conductivity of water, such as H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^- (Radojevic & Bashkin, 2006). Conductivity is temperature-dependent, for example, an elevated temperature makes water less

viscous and increases dissociation which leads to changes in the speed with which different size and charge ions move (Artiola, 2004).

The main ions found in most natural waters include Ca^{2+}, Mg^{2+}, K^{+}, CO_3^{2-}, HCO_3^{-}, Cl^{-}, SO_4^{2-}, and NO_3^{-}, resulting from natural contact with rocks and soil (Jain & Singh, 2003). These species represent the solid residue that remains after water evaporates. Therefore, the total concentration of these species is commonly referred to as the total dissolved solid value in water. If these dissolved species exist as ions, this leads to an increase in the electrical conductivity of solutions (Atekwana *et al.,* 2004). The conductivity of water varies depending on the concentration of such dissolved solids and the relationship between EC and TDS can be described in the following equation.

$$TDS = K_e\, EC \text{ ---------- Equation 2.1}$$

where K_e, is a correlation factor (Atekwana *et al.,* 2004). The value of K_e is often high for chloride-rich water and low for sulphate-rich water (Atekwana *et al.,* 2004). The total quantity of dissolved solids in water has been used as a common indicator to evaluate the quality and freshness of drinking, irrigation and domestic water (Pernitsky & Meucci, 2002). The World Health Organisation has reported a permissible TDS and EC limit for drinking water of 600 – 1000 (mg/l) and 250 (µS/cm), respectively (WHO, 2008).

The Hanna HI 98129 Digital Combo Meter is limited to the measurement of specific detectable ranges, as shown in Table 2.4. Therefore, for any reading of conductivity and TDS outside these ranges (0 to 3999 µS/cm) and (0 to 2000 mg/l) respectively, the water sample must be diluted (at the time of sample collection) with deionised water. However, this was not possible so values are reported to have been above the upper limit of the probe.

Table 2.4: Calibration and specification of the Hanna HI 98129 Digital Combo Meter.

Parameter	Range at 20°C		Calibration
pH	0.00 – 14.00	(± 0.05)	Buffer solution at pH 4.01, 7.01 and 10.01
EC (µS/cm)	0 to 3999	(± 2%)	0.01 M of KCl (1413 µS/cm)
TDS (mg/l)	0 to 2000	(± 2%)	Solution at 1382 mg/l

Source: Hanna, 2008

2.2.2 Cigarette Tobacco

Sixteen of the most consumed cigarette brands sold in the Karbala market were randomly purchased from local grocery stores, as shown in Table 2.2. All cigarette brands have a filter. Tobacco material was extracted from 10 cigarettes of each brand after the wrapping paper was carefully separated (Martinez *et al.*, 2008). Tobacco was placed on filter paper and allowed to dry at ambient temperature (18°C) in the laboratory. Two digestion methods were used to digest cigarette tobacco samples, as described below.

2.2.2.1 Dry Ashing

Tobacco digestions for this study were carried out using dry ashing and wet digestion methods. In dry ashing, 0.500 ± 0.001 g of dried cigarette tobacco was weighed out into a clean/dried porcelain crucible (VWR, Leicestershire, UK). The crucible was then covered and placed into a Gallenhamp muffle furnace (Vindon Scientific, Oldham, UK) and set at 200°C for a minimum 2 hours. The temperature was then raised to 500°C and the samples were left at this temperature overnight. The crucibles were then placed in desiccators for cooling. One milliliter of concentrated nitric acid (Aristar® 65%) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) was added to each crucible, and the solutions were then diluted to 50 ml with distilled de-ionised water (DDW; 18.2 MΩ) using a polyethylene volumetric flask (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK). Solutions were centrifuged at 3000 rpm for 10 minutes and filtered through Millex filter units with MF-Millipore (0.45 µm). Then, the solutions were transferred to clean, labelled 50 ml Sterilin® centrifuge tubes (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) and stored at 4°C in a fridge.

2.2.2.2 Wet Digestion - Kjeldahl™ Tube

The dried cigarette tobacco samples were accurately weighed to 0.500 ± 0.001 g (Hamidatou *et al.*, 2009). Samples were transferred into Kjeldahl™ tubes in a

fume cupboard and 1 ml of concentrated nitric acid (Aristar® 65%) was added to each tube. All tubes were covered with P.V.C. Clingfilm and placed on the digestor block at 165 ± 10°C (~ half hour). The digested solutions were transferred into a polyethylene volumetric flask (50 ml) and made up with de-ionised water (18.2 MΩ) (Hamidatou *et al.*, 2009). Sample solutions were centrifuged for 10 minutes at 3000 rpm (MSE Mistral 2000 Thermo Life Sciences) and filtered through a Millex filter, MF-Millipore (0.45 μm) (Millipore, Carrigtwohill, Co. Cork, Ireland). The samples were stored in the fridge at 4°C prior to ICP-MS analysis. Multi-elemental analysis was performed for cigarette tobacco samples by inductively coupled plasma atomic emission spectrometry (ICP-AES) within one month of sample collection, as shown in Section 2.7.
The reagent blanks and "pooled" samples were also prepared using the same two digestion methods. In addition, three sub samples of standard reference materials (SRM, NIST 1573a "Tomato leaves" and NIST 1572a "Citrus leaves"), were provided by the National Institute of Standards and Technology, Maryland, USA, and were subjected to acidic digestion and ICP-AES analysis for QC purposes, as outlined in Section 2.8.

2.2.3 Tear drops

The three main methods of sample collection reported in the literature for tear drops that have been used include: filter papers; capillary tubes (Baca *et al.*, 2007); and cotton swabs (Baeyens & Gurny, 1997), as shown in Table 2.5. In this study, tear drop samples were obtained from 155 subjects (healthy and those with diabetes) resident in Karbala (Iraq), as described in Table 2.3. The individuals ranged in age from 2 to 75 years. For comparative purposes, 18 samples were collected from healthy Iraqi individuals who have been living in London (UK) for more than five years. At least 300 μl of unstimulated tear drops (crying tear) were collected from the left and/or right eye using micro-centrifuge polypropylene tubes (1.5 ml) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK). Samples were collected from the outer canthus, where the upper and lower eyelids meet. In this case, the micro-centrifuge tube was gently touched with any drops released from the eye and the tear fluid was drawn in by the micro-centrifuge tube. The tear volume ranged

from 300 to 600 µl, which was collected over a 15 to 30 minutes period (Kuizenga *et al.*, 1991). Ten ml of a "pooled" tear drop sample was also collected from six individuals who were living in the same family residence, for quality control measurement purposes.

Table 2.5: Sample collection, sample amount and analytical technique reported in the literature for tear drop analysis.

Study	Sampling technique	Sample amount (µl)	n	Analytical technique
Effect of sample treatment on protein in tear drops	Glass capillary tube	25 – 100	7	Electrophoresis
Determination of anions in human and animal tear fluid and blood serum by ion chromatography	Micropipette	10 - 100	10	Ion chromatography
Changes in human tear protein levels with progressively increasing stimulus	Capillary tube	nf	10	SE-HPLC and ELISA
Analysis of human tear fluid by Raman spectroscopy	Capillary tube	1.5	3	Raman spectroscopy
Sialic acid in normal human tear fluid	Saline and micropipette	20	31	HPLC

n is the number of subjects, nf = not found, SE-HPLC is size exclusion high-performance liquid chromatography, ELISA is enzyme-linked immunosorbent assay.

Source: Filik & Stone, 2008; Nakamura *et al.*, 2001; Salas-Auvert *et al.*, 1995; Meijer & Van Haeringen, 1994; Kuizenga *et al.*, 1991.

2.2.3.1 Sample Storage, Method of Transfer and Preparation

Tear drop samples were stored in a cool environment of 4°C during field sampling using a Tropicool 14 litre Thermoelectric cool box TC-14 (Waeco©, Dorset, UK), connected to a battery powered car cigarette lighter socket. Tear drop samples were then kept at −20°C. All samples were safely transferred from Karbala (Iraq) to Guildford (UK) in a fully charged Tropicool cool box (when fully charged the Tropicool cool box can maintain a temperature of 4°C for ~ 12 hours when unopened). Disposable ice packs were also added to the samples in storage to help

maintain/prolong a temperature of 4°C. On return to the laboratory, tear drop samples were directly diluted with distilled de-ionised water (DDW), resulting in a dilution factor of 10 fold. Solutions were centrifuged at 3000 rpm for 10 minutes and filtered through Millex filter units with MF-Millipore (0.45 µm) to remove protein and cellular debris. Samples were then decanted off into a clean, labelled 15 ml Sterilin® centrifuge tube (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) and subsequently transferred to a refrigerator (4°C). 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); and TMDA 54.4 Trace Elements in Fortified Lake Ontario Water (National Water Research Institute, Ontario, Canada) were utilised for Quality Control (QC) measurements, as outlined in Section 2.8. Samples were removed from the fridge prior to any analysis and allowed to equilibrate at room temperature. All samples were analysed within two weeks of collection time by using an Agilent 7700 Series ICP-MS instrument (Section 2.6). A recovery test and regression time plot were used to check whether any analyte was lost between sample collection and analysis, as determined in Section 2.2.3.2. Figure 2.2 shows the methodology procedure that was used to prepare tear drop samples in this study.

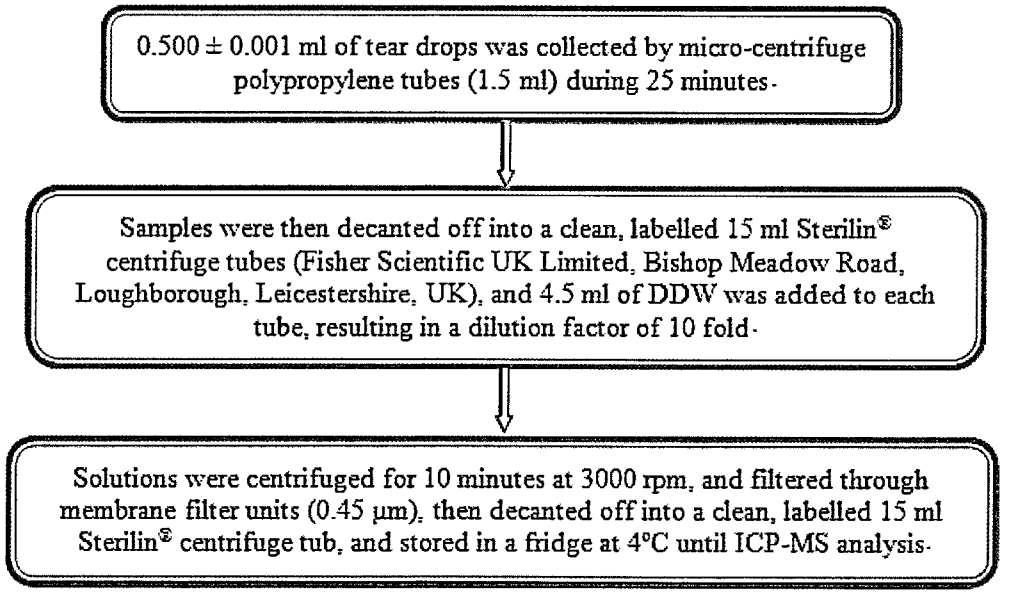

Figure 2.2: Methodology for the pre-analysis preparation of tear drops.

2.2.3.2 Testing of Sample Pre-treatment Procedures

In the analysis of trace elements in biological matrices the following essential requirements should be performed: (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. In terms of the above, in this study, the subtraction of the blank signal and the use of internal standards (ISs) covering all of the mass range were sufficient for controlling the above problems, and for reducing reagent impurities, instrumental drifts and matrix effects (Bocca *et al.*, 2006; Ward, 2000).

In addition, ultra-high-purity grade reagents were used for the digestion or dilution of samples so as 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 (Ebdon *et al.*, 1998).

A major feature associated with analysis by inductively coupled plasma mass spectrometry is the possibility of detecting any sources of contamination during the analytical process. For example, collection and storage containers may increase the contamination or losses of the sample through (i) surface desorption or leaching and (ii) adsorption on surfaces, respectively (Ebdon *et al.*, 1998). 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) (Bocca *et al.*, 2006).

In order to test whether any analyte was lost between the sample collection and analysis, the variation of elemental levels was determined as a function of storage time for replicate analysis of a "pooled" tear drop sample, as shown in Table 2.6 and Figure 2.3. A recovery value (%R) was calculated as 100 x measured concentration after four weeks/measured concentration after one week and an acceptance limit between 90 and 110% was considered as the desired range according to the criteria described by the Commission Decision 2002/657/EC

(Olmedo *et al.*, 2010). A stability study of 10 elements in a "pooled" tear drop sample over 4 weeks storage at 4°C revealed no significant differences for B, V, Cr, Fe, Mn, Cu, Zn, As, Sr and Cd (see Appendix E).

Table 2.6: Elemental levels (mean and standard deviation (µg/l)) and percentage recovery values for replicate analysis of a "pooled" tear drop sample stored in a fridge at 4°C and a repeatedly analysed (n = 6) over a 4 week period.

Element	Mean value and standard deviation (µg/l)				% R
	Week (1)	Week (2)	Week (3)	Week (4)	
B	509 ± 23	511 ± 21	510 ± 19	514 ± 21	101
V	2.76 ± 0.05	2.69 ± 0.08	2.68 ± 0.08	2.60 ± 0.06	94
Cr	3.95 ±0.09	3.73 ± 0.17	3.81 ± 0.17	3.77 ± 0.15	96
Mn	19.44 ± 1.80	19.38 ± 1.10	18.14 ± 0.94	18.72 ± 1.01	96
Fe	283 ± 13	284 ± 14	284 ± 11	286 ± 11	101
Cu	207 ± 7	204 ± 8	209 ± 6	208 ± 7	100
Zn	758 ± 14	754 ± 13	751 ± 11	750 ± 12	99
As	0.48 ± 0.06	0.50 ± 0.09	0.48 ± 0.10	0.48 ± 0.09	98
Sr	598 ± 27	591 ± 22	589 ± 25	594 ± 25	99
Cd	0.28 ± 0.01	0.26 ± 0.02	0.28 ± 0.02	0.27 ± 0.02	97

% Recovery is calculated for first and last mean values (%R = mean value for week (4) x 100/ mean value for week (1)), R = recovery.

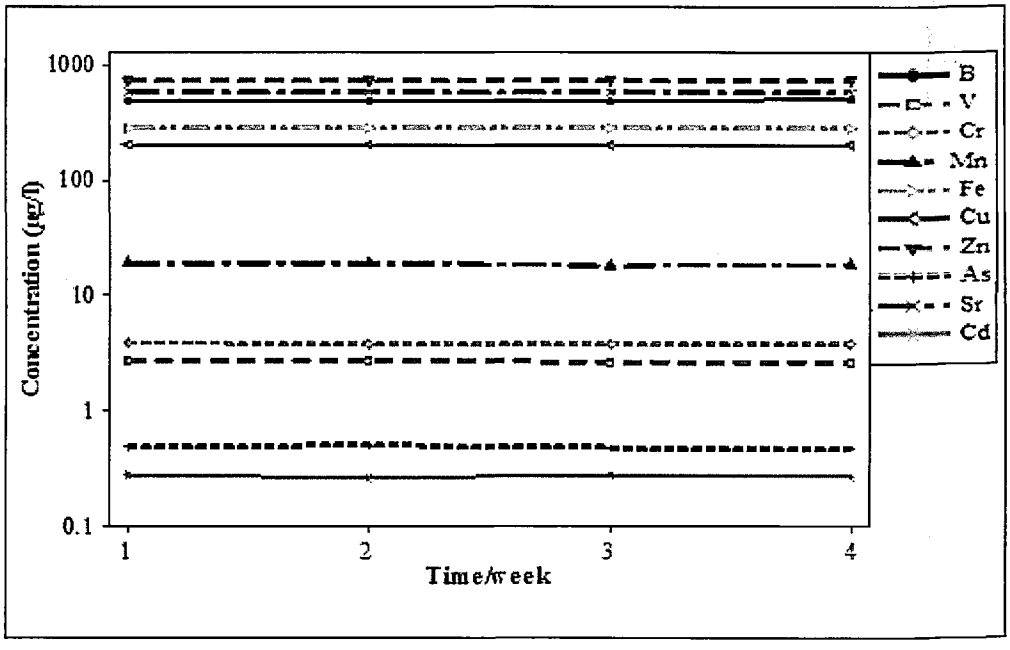

Figure 2.3: Variation of elemental mean values (µg/l) of a "pooled" tear drop sample as a function of storage time.

In general, for most of the trace elements investigated there was good agreement between the measured values throughout the storage period and the percentage (%) recovery values, which were found to be between 94 – 101%. The effect of storage time on the elemental levels in tear drops is presented in Figure 2.3.
Overall the various storage times produce consistent elemental mean values for the replicate analysis of a "pooled" tear drop sample over a four week storage period from the time of sample collection.

2.2.4 Saliva

Saliva samples were taken from the mouth after 15 hours of fasting, as shown in Table 2.3. All subjects were requested to rinse their mouth three times with distilled water (Gil *et al.*, 2011). After discarding the first 1 ml, 5 to 10 ml were collected in Sterilin® containers (25 ml) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) over a period of 10 to 20 minutes (Kim *et al.*, 2010). A pooled sample (30 ml) was collected from six individuals from the same family for analytical development purposes and quality control measurements (Section 2.8). The samples were stored and transported to the laboratory, as described in Section 2.2.3.1. Any sample that contained blood was directly discarded. All saliva samples were centrifuged for 10 minutes at 3000 rpm, and filtered through Millex filter units with MF-Millipore (0.45 μm) in order to remove cellular debris, foam and protein. A 1 ml portion of saliva supernatant was transferred into a clean, labelled 15 ml Sterilin® centrifuge tube (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK), and 9 ml of DDW was added to each solution, resulting in a dilution factor of 10 fold. A reagent blank was prepared using the same procedure. Certified Reference Materials (SRM 1643e and TDMA 54.4) were also prepared for QC measurements, as outlined in Section 2.8.3. All samples were stored at 4°C in a fridge until ICP-MS analysis (Section 2.6). The preparation procedure for saliva is highlighted in Figure 2.4.

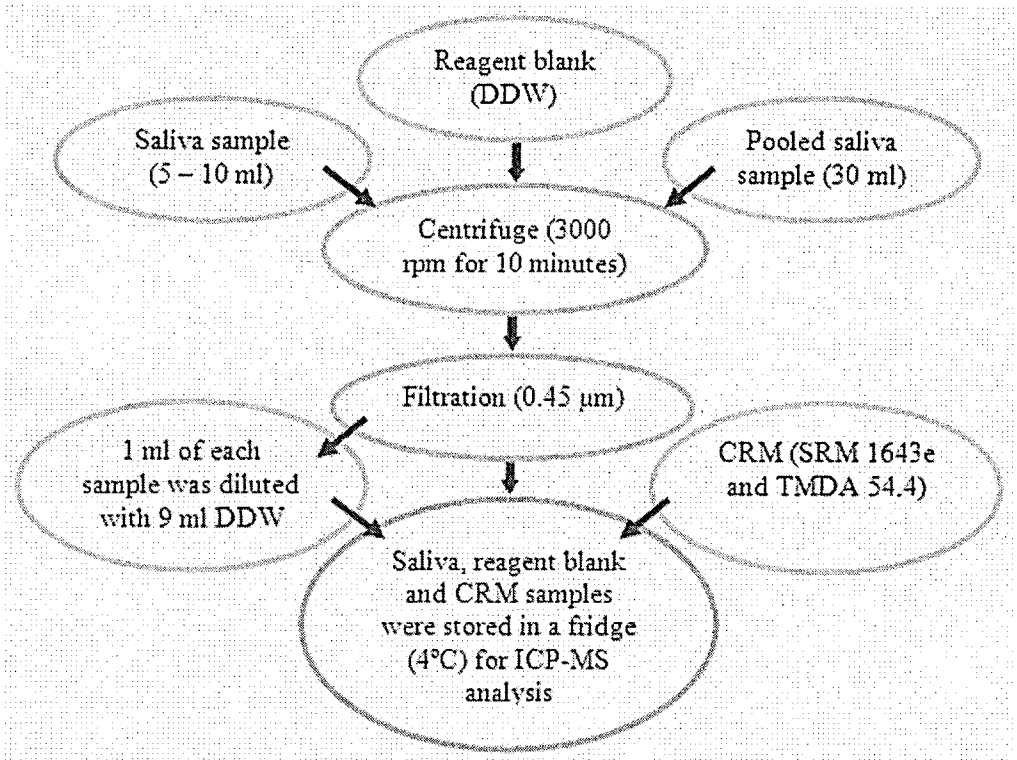

Figure 2.4: The development method for saliva analysis.

2.2.5 Scalp Hair

Scalp hair samples were collected from the same site of the head for all individuals, namely, from the back of the head, less than 1 cm from the scalp using acetone/distilled deionised water washed scissors. This pre-treatment was undertaken to prevent contamination introduced by the tool during sample collection. Generally, a sample (mass > 0.5 g) was collected and stored in a polyethylene bag at room temperature until the time of analysis (Rodrigues *et al.*, 2008; Hartman, 2006; Senofonte *et al.*, 2001). Hair samples were cut into small pieces (~ 5 mm) using acetone/distilled deionised water washed scissors so as to make the sample more homogenous (Hartman, 2006). The cut hair samples were transferred into a labelled 50 ml Sterilin® centrifuge tube (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK) for the washing procedure.

2.2.5.1 Effect of Sample Mass and Dilution Factor

The dilution factor (mean of sample and digest/dilution volume) used to prepare the sample for analysis is an important feature of the analysis procedure. If too little a sample is available, the resulting large dilution factor which is linked to the need to have a certain volume for solution uptake by the instrument, results in a possible over-estimate of the calculated concentration.

The trace element levels of a "pooled" scalp hair sample using different masses (0.15, 0.20, 0.25, 0.40 and 0.50 g) are summarised in Table 2.7 (for a constant dilution factor) and Table 2.8 (for a different dilution factor).

The results for the constant dilution factor method show a consistently low RSD of $\leq$ 12%, (Table 2.7). In contrast, the data for variable dilution factor method produces high RSD's ranging from 6 to 70% (Table 2.8). It is suggested that this is mainly due to the small analyte signal relative to the background which for small sample masses is over corrected using a large dilution factor (Stovell, 1999). Thus, the constant dilution factor method provides the best results for most of the elements under investigation, resulting in acceptable levels of precision.

Table 2.7: Elemental levels (mg/kg) for "pooled" scalp hair sample – unwashed (n = 3) ranging from 0.15 to 0.50 g mass digested in different volumes (constant dilution factor, 100 fold).

	Elemental level^{+} (mg/kg)					
DF / *Element	100	100	100	100	100	%RSD
Na	159	154	156	154	150	2
Mg	39.5	43.6	42.2	42.6	44.6	4
Ca	241	245	246	242	246	1
V	0.35	0.33	0.32	0.33	0.35	4
Cr	0.40	0.35	0.38	0.36	0.39	5
Mn	3.74	3.05	3.39	3.33	3.13	8
Fe	22.6	22.9	22.3	22.9	23.3	2
Cu	19.82	20.28	18.68	21.18	19.63	5
Zn	172.56	149.03	149.03	169.66	196.79	12
As	0.68	0.63	0.74	0.73	0.76	7
Sr	7.71	6.97	7.19	7.60	7.18	4
Cd	0.07	0.08	0.09	0.07	0.08	11

DF = dilution factor, RSD is relative standard deviation, $^{+}$ n = 3 replicates, * all the elements were determined by a Finnigan MAT Sola ICP-MS instrument except Na, Mg, Ca and Fe by FAAS.

Table 2.8: Elemental levels (mg/kg) for "pooled" scalp hair sample – unwashed (n = 3) ranging from 0.15 to 0.50 g mass digested in a constant volume 50 ml (variable dilution factor ranging from 100 – to 333 fold).

	Elemental level^{+} (mg/kg)					
DF / *Element	333	250	200	125	100	%RSD
Na	175	161	157	155	149	6
Mg	65.2	58.1	53.1	48.5	44.7	9
Ca	297	276	255	244	242	7
V	0.10	0.14	0.22	0.19	0.38	52
Cr	0.19	0.11	0.14	0.11	0.39	63
Mn	11.57	5.69	7.75	4.56	3.17	50
Fe	126	93	50	32	22.5	70
Cu	37.87	36.84	35.49	21.77	20.23	28
Zn	286.73	189.20	293.17	181.75	202.29	24
As	3.21	1.68	1.45	0.73	0.80	64
Sr	2.84	2.29	4.68	6.87	7.16	47
Cd	0.04	0.06	0.07	0.09	0.09	30

DF = dilution factor, RSD is relative standard deviation, $^{+}$ n = 3 replicates, * all the elements were determined by a Finnigan MAT Sola ICP-MS instrument except Na, Mg, Ca and Fe by FAAS.

The effect of using a variable and constant dilution factor on the elemental concentration can be demonstrated in Figures 2.5 & 2.6. In general, the constant dilution factor method produces a consistent calculated elemental level for the "pooled" scalp hair samples, whereas the variable dilution factor method provides typically variable estimates of the final calculated concentration.

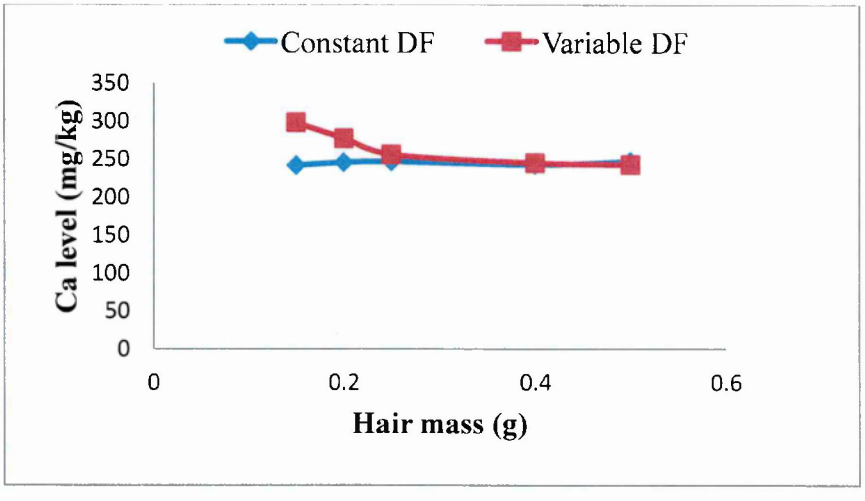

Figure 2.5: Effect of the dilution factor (constant and variable) on the analysis of calcium in "pooled" scalp hair (unwashed) sample.

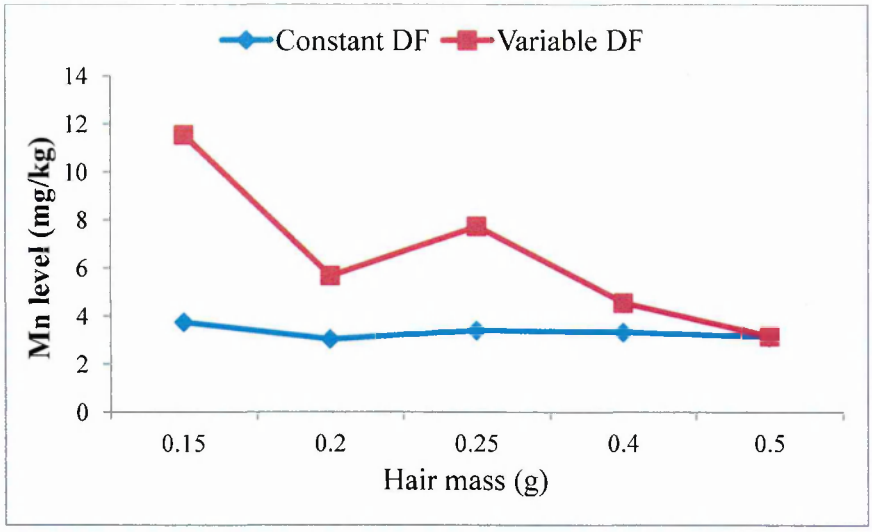

Figure 2.6: Effect of the dilution factor (constant and variable) on the analysis of manganese in "pooled" scalp hair (unwashed) sample.

This finding is very important as most researchers only use a "fixed" sample volume for digesting/diluting the sample during pre-analysis preparation, irrespective of the available sample mass. Furthermore, the "best practice" would be to set a minimum sample mass of 0.25 or 0.50 g, but this is not always possible practice for many study populations, for example, children or males that tend have a limited amount of scalp hair available for sampling and analysis.

2.2.5.2 Washing Procedure

The main function of a washing procedure is to remove exogenous contaminants from the surface of the scalp hair in order to provide true levels for endogenous elements. Several washing procedures have been proposed in the literature for scalp hair samples. Many authors believe that an ideal washing procedure would remove only external contaminants and leave endogenous elements (Apostoli, 2002). In this study, three washing procedures were undertaken to determine the effect of washing human scalp hair relative to an unwashed portion, namely, for sequential washing in an ultrasonic bath with: (i) Method A: acetone-water-water-water-acetone; (ii) Method B: ether-Triton X-100-water-water; and (iii) Method C: ether-acetone-water-ether (Gault *et al.*, 2008; Rodrigues *et al.*, 2008). For the

method development, a "pooled" scalp hair sample was washed using these methods to investigate which of the methods could be used as the appropriate method for scalp hair analysis. Method A [the International Atomic Energy Agency (IAEA) procedure, (IAEA, 1978)] was found to be preferred in this study based on the validation data, as reported in Section 2.3.2.1. In this method, a sufficient volume of acetone was added to each tube to cover the hair sample. All tubes were sonicated for 10 minutes (35 MHz) at room temperature and then separated by centrifugation (1000 rpm for 5 minutes). The same procedure was repeated three times with DDW, and finally with acetone. The washed samples were dried in an oven overnight at 60°C and subsequently stored in a labelled polyethylene bag until pre-analysis digestion.

2.2.5.3 Digestion Methods

In general, digestion methods are used to destroy the organic matter of a sample leaving behind only the inorganic residue. Three digestion methods were used in this study to digest a "pooled" scalp hair sample, namely: (i) Method X: dry ashing without nitric acid addition (muffle furnace); (ii) Method Y: dry ashing pre-nitric acid digestion in a water bath within the fume cupboard; and (iii) method Z: wet digestion using nitric acid in a Kjeldahl™ tube (Kazi *et al.*, 2008; Forte *et al.*, 2005). In dry ashing the sample is slowly decomposed in a muffle furnace over a ramped temperature range of 200 to 500°C, leaving behind an inorganic residue that is soluble in dilute acid. In the case of wet digestion the organic matter can be destroyed by heating with an oxidizing acid, such as nitric acid.

Dry ashing (Method X)

In this method, 0.500 ± 0.001 g of a "pooled" unwashed scalp hair was weighed out in a clean/dried 100 ml Pyrex™ beaker (n = 5 replicates), dry ashed for 2 hours at 200°C in a muffle furnace, before raising the temperature to 500°C overnight. The ash was dissolved in 1 ml of concentrated nitric acid (Aristar® 65%) and then diluted to 50 ml with de-ionised water in a polyethylene volumetric flask. Hair samples were centrifuged at 3000 rpm for 10 minutes and subsequently filtered

through a Millex filter, MF-Millipore (0.45 μm)\. Then, samples were transferred into a labelled 50 ml Sterilin® centrifuge tube and stored in the fridge at 4°C prior to ICP-MS analysis (Dombovari & Papp, 1998).

Dry ashing - pre-nitric acid (Method Y)

The same mass of a "pooled" scalp hair was used in this method (0.500 ± 0.001 g), then 1 ml of concentrated nitric acid (Aristar® 65%) was added to each hair sample (n = 5 replicates) using a Pyrex™ beaker in a fume cupboard and left overnight. The same steps (as described in Method X) were used to digest these samples using the muffle furnace, Method Z (Friel & Ngyuen, 1986).

Wet digestion (Method Z)

The same mass (0.500 ± 0.001 g) of a "pooled" scalp hair was utilised and transferred into a clean/dried Kjeldahl™ Tube for method development. 1 ml of nitric acid (Aristar® 65%) was added to each tube, then the digestion tubes were placed on a digestion block for heating at 165°C (± 10°C) until the hair sample was digested, namely ~ half hour. All digested samples were diluted with DDW using a polyethylene volumetric flask, resulting in a dilution factor of 100 fold. Sample solutions were centrifuged for 10 minutes at 3000 rpm (MSE Mistral 2000 Thermo Life Sciences) and filtered through a Millex filter, MF-Millipore (0.45 μm) (Millipore, Carrigtwohill, Co. Cork, Ireland). The digested hair solutions were stored in a labelled 50 ml Sterilin® centrifuge tube and stored in the fridge at 4°C prior to ICP-MS analysis. Along with the scalp hair samples, a reagent blank and certified reference human hair materials (GBW09101 and GBW07601 Human Hair), provided by the National Research Centre for Certified Reference Materials, China, and were treated in the same manner according to the procedures using dry and wet digestion in order to check the precision and accuracy for each method. The preparation procedure for hair and nail samples is highlighted in Figure 2.7.

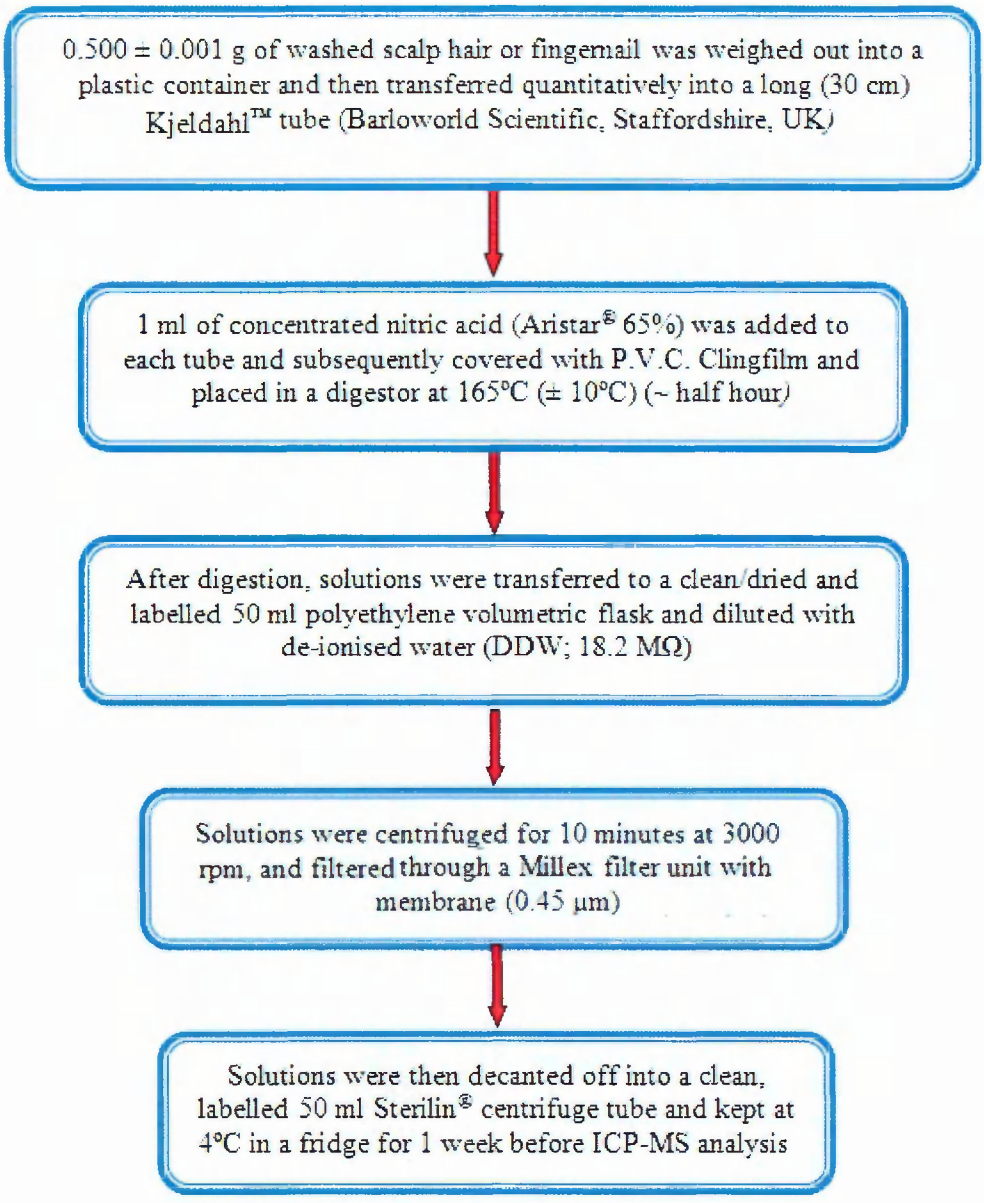

Figure 2.7: Digestion procedure using Kjeldahl™ tube for the pre-analysis preparation of washed scalp hair and fingernails.

2.2.6 Fingernails

Fingernail samples were collected from all 10 fingers using acetone distilled de-ionised water washed clippers (Slotnick *et al.*, 2006). The majority of studies have used this method to obtain nail samples, but in some cases only thumb nails have been collected (Helzlsouer *et al.*, 2000). The main advantages to collect all fingers rather than one big finger are: sufficient sample mass, and an estimate of the

complete hand of exposure (Longnecker *et al.,* 1993). Fingernail samples were cleaned manually of any visible dirt (e.g. soil) on the surface of nails prior to application of the washing procedure (Samanta *et al.,* 2004).

The effect of sample mass and dilution factor; washing procedure and digestion method were determined and validated in similar manner as described in scalp hair, the results are reported in Appendix F. In brief, the cut fingernail samples were washed using a sufficient volume of acetone to cover the fingernails in a 25 ml Sterilin® centrifuge tube (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK), sonicated for 10 minutes at 35 MHz at room temperature and subsequently separated by centrifugation (5 minutes, 1000 rpm). The fingernails were washed a further three times with DDW (~ 5 ml) then a final acetone wash. On each occasion the fingernails were sonicated (10 minutes, 35 MHz) and centrifuged (5 minutes, 1000 rpm). Samples were dried in an oven overnight at 60°C then stored at room temperature in labelled polyethylene bags. Samples were digested using Kjeldahl™ tubes in the same manner as described in scalp hair (Section 2.2.5.3), as shown in Figure 2.7.

2.3 Validation of Analytical Methods

2.3.1 Cigarette Tobacco

In this study, two digestion methods were used to prepare cigarette tobacco samples for multi-element analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). Table 2.9 shows the results of determining trace element levels (mg/kg) by the two digestion methods. In order to evaluate whether there is any significant difference between the two methods for determining the levels of trace elements in cigarette tobacco, a paired t-test was used to determine the difference between each of the paired measurements on each sample. An average difference is calculated and the individual deviations of each from average difference are used to calculate the standard deviation (further information can be found in Appendix D for data and Appendix C for equations). If t_{calc} is greater than t_{crit} for n-1 degrees of freedom, then a statistically significant difference is observed (Table 2.9). In general, there are no significant differences

(at $P < 0.05$) between the levels of trace elements (mg/kg dry weight) measured by the dry ashing and Kjeldahl™ tube methods with the only exception being Sr.

Table 2.9: Comparison of the elemental levels (mg/kg) in commercial tobacco samples (n = 16) from Karbala, Iraq using two digestion methods along with a paired t-test results.

Element	Kjeldahl™ tube	Dry ashing	t_{calc}
B	nd	nd	nd
V	0.42 ± 0.12	0.45 ± 0.12	0.52
Cr	0.62 ± 0.17	0.69 ± 0.18	0.74
Mn	99 ± 24	102 ± 21	0.19
Fe	257 ± 52	264 ± 50	0.23
Cu	5.36 ± 2.54	7.53 ± 1.66	0.81
Zn	26.8 ± 5.2	23.2 ± 4.1	1.09
As	1.7 ± 1.1	0.5 ± 0.2	1.03
Sr	75 ± 14	57 ± 11	3.37*
Cd	0.90 ± 0.47	0.79 ± 0.38	0.56

t_{calc} = calculated value, the critical value (t_{crit}) at the 95% confidence level for 15 degrees of freedom is 2.13, * indicate significant difference level at $P < 0.05$ (i.e. $t_{calc} > t_{crit}$), nd = not determined.

The accuracy for both digestion methods was evaluated by replicate analysis (n = 3) of a certified reference material NIST SRM® 1573_a Tomato leaves and reporting the results by determining the percentage (%) recovery, as shown in Table 2.10. The dry ashing method provides poorer levels of accuracy than the Kjeldahl™ tube method in terms of the % recovery data. The high temperature in dry ashing leads to loss of volatile elements, such as cadmium and zinc (Sardans *et al.*, 2010: Kubova *et al.*, 1997). The findings show that the %R measured by the Kjeldahl™ tube method are at an acceptable range between 90 and 110%. Moreover, the trend is observed for the CRM (NIST SRM® 1573 Tomato Leaves) analysis with the higher elemental levels being found using the Kjeldahl™ tube. As such, the Kjeldahl™ method was accepted as the preferred digestion procedure for cigarette tobacco. The precision of the Kjeldahl™ method was calculated by replicate analysis (n = 10) of a "pooled" tobacco brand, as outlined in Section 2.8.3.

Table 2.10: Accuracy levels as attained through the analysis of the certified reference material, NIST SRM® 1573 Tomato leaves using different digestion methods, presented as mean ± SD and %R for measured values and mean ± SD for certified values.

Element (n = 3)	Elemental level (mg/kg)				
		Digestion method			
	Certified value	Dry ashing		Kjeldahl™tube	
		Measured value mean ± SD	%R	Measured value mean ± SD	%R
B	nd	nd	nd	nd	nd
V	0.835	0.772 ± 0.01	92	0.90 ± 0.03	108
Cr	1.99	1.90 ± 0.02	96	1.84 ± 0.01	93
Mn	246	204 ± 6	83	222.7 ± 0.7	91
Fe	368	247.19 ± 1.25	67	332.7 ± 0.6	90
Cu	4.7	4.1 ± 0.02	85	5.16 ± 0.03	110
Zn*	29 ± 2	22.23 ± 0.14	77	27.9 ± 0.2	96
As*	3.1 ± 0.3	2.82 ± 0.1	91	2.82 ± 0.05	91
Sr*	100 ± 2	89.9 ± 0.99	90	90 ± 1	90
Cd	1.52	1.31 ± 0.31	86	1.49 ± 0.01	98

%R = percentage recovery = (measured value / certified value) x 100, * Citrus leaves SRM 1572 has been used for Zn, As and Sr due to there are no certified values were found for these elements in NIST SRM® 1573 Tomato leaves, NIST is National Institute of Standards and Technology, nd = not determined.

2.3.2 Scalp Hair

2.3.2.1 Washed Procedure

Table 2.11 shows data reported using different washing procedures for "pooled" human scalp hair samples. The mean elemental values decreased as a result of the various washing procedures relative to an unwashed "pooled" scalp hair sample (Hawkins & Ragnarsdottir, 2009). The effectiveness of the various washing procedures was evaluated by determining the percentage analyte removal level, compared with the unwashed hair. The percentage removal for most elements was significantly higher for washing methods A and B than method C. However, method B was more problematic than method A as frothing from the detergent increased the washing time. Interestingly, the highest % levels of elemental removal was for Na > Ca ~ Mg ~ Sr ~ Cr > As ~ Cd > Mn > V ~ Fe ~ Cu > Zn. Therefore, these elements associated with "soil / dust" contribution and body

secretion / sweat seem to produce the higher exogenous levels on the scalp hair surface (Forte *et al.*, 2005). As a result, based on these findings, the sequential washing procedure (method A: acetone-water-water-water-acetone) was adopted in this study (Rodrigues *et al.*, 2008).

Table 2.11: Elemental concentrations (mg/kg dry weight) and in brackets the percentage removal for "pooled" scalp hair sample (using a 0.50 g, constant dilution factor 100 fold dilution, volume of 50 ml) using different washing procedures* (n = 3).

Element	Elemental level (mg/kg) (% removed)			
	Unwashed	Washing procedures*		
		A	B	C
Na	299	39 (87)	33 (89)	52 (83)
Mg	21.7	11.5 (47)	12.6 (42)	11.8 (46)
Ca	793	401 (49)	413 (48)	373 (53)
V	0.21	0.19 (10)	0.19 (10)	0.21 (0)
Cr	0.15	0.08 (46)	0.10 (33)	0.11 (27)
Mn	2.51	2.14 (15)	2.10 (16)	2.33 (7)
Fe	25.6	23.1 (10)	16.7 (35)	18.1 (29)
Cu	26.00	23.59 (9)	25.58 (1.6)	24.49 (6)
Zn	138.98	129.78 (7)	133.50 (4)	126.34 (9)
As	0.26	0.20 (23)	0.15 (42)	0.24 (8)
Sr	4.96	2.61 (47)	3.04 (39)	3.31 (33)
Cd	0.25	0.20 (20)	0.10 (60)	0.21 (16)

*A: sequential washing in ultrasonic bath with acetone-water-water-water-acetone, B: sequential washing in ultrasonic bath with ether-Triton x-100-water-water, C: sequential washing in ultrasonic bath with ether-acetone-water-ether, values in brackets were calculated using this equation, Removed % = {(unwashed value – washed value)/unwashed value} x 100.

2.3.2.2 Digestion Method

The accuracy of digestion procedures was tested by using Certified Reference Material GBW 09101 Human Scalp Hair (Bass, 2001). In the case of the Kjeldahl™ tube method, there is a good agreement between the measured and certified values, and the recovery values were between 90-109 % with exceptions being Ca, Cr and As (Table 2.12). The measured value for Ca was lower than the certified value due to the formation of a less volatile compound between Ca and phosphate in the flame at 422 nm. This compound is less volatile when compared

with calcium chloride and then prevents the formation of Ca atoms (Ebdon *et al.*, 1998). Chromium can be lost during the wet digestion as volatile chloride (Stovell, 1999). The polyatomic interference $^{40}Ar^{35}Cl^{+}$ can overlap with $^{75}As^{+}$ and reduce the accuracy of As in ICP-MS analysis (Broekaert, 2005).

Table 2.12: Accuracy and precision assessment for human scalp hair CRM GBW 09101 using Kjeldahl™ tube method, presented as mean, %RSD and %R for measured values and mean for certified values.

Element^{+} (n = 3)	Elemental levels (mg/kg)			
	Accuracy			Precision
	Measured value	Certified value	(%R)	%RSD
Na	289	266	109	4.6
Mg	96	105	92	5.8
Ca	859	1090	79	3.6
V	0.062	0.069	90	4.1
Cr	3.97	4.77	83	3.1
Mn	2.69	2.94	92	2.9
Fe	64.56	71.2	91	3.6
Cu	22.5	23	98	2.7
Zn	177	189	94	0.4
As	0.78	0.59	132	2.4
Sr	4.54	4.19	108	1.5
Cd	0.089	0.095	94	7.8

RSD is relative standard deviation, %R is percentage recovery, $^{+}$ all the elements were determined by a Finnigan MAT Sola ICP-MS instrument except Na, Mg, Ca and Fe by FAAS.

The precision of the digestion method, based on triplicate analysis of the GBW 09101 material produced acceptable levels of relative standard deviation (RSD) between 0.4 – 7.8% for all elements (Table 2.12). The wet digestion method using a Kjeldahl™ tube provided the best data in this work, and as a result was employed for the complete digestion of washed human scalp hair in this research.

Overall, the washing and digestion methods validated above were used for scalp hair and fingernail preparation with respect to the available sample mass for each material. The results for fingernails are reported in Appendix F based on a similar procedure discussion as reported for scalp hair.

2.4 Analytical Instrumentation

There are a wide range of analytical techniques that have been used for trace element analysis, such as flame atomic absorption spectrometry (FAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) (Harris, 2007). 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 (Ward, 2000). The following sub-sections describe in detail the analytical instrumentation employed throughout this work. The fundamentals, instrument configuration, interferences and methods of calibration are reported.

2.5 Flame Atomic Absorption Spectrometry

Flame atomic absorption spectrometry (often abbreviated FAAS) was used throughout this work to determine elemental concentrations in human scalp hair and was particularly suited for the analysis of concentrations at the mg/kg or ppm level, which would be unsuitable for ICP-MS determination without vast dilutions. This technique has been widely used for the determination of major, minor and trace elements in water and biological samples, including, tissues and fluids (Batista *et al.*, 2008; Sukumar & Subramanian, 2007; Kazi *et al.,* 2006; Lorenzo *et al.*, 2005; Das *et al.*, 2004; Bustamante *et al.*, 2000; Nowak & Chmielnicka, 2000).

2.5.1 Fundamentals

Flame AAS follows an exponential relationship between the intensity I of transmitted light and the absorption path length b (Lambert's law), as shown below:

$$I = I_0 \, exp\,(-K_v b) \text{---------- Equation 2.2}$$

where I_0 is the intensity of the incident light beam and K, is the absorption coefficient at the frequency v.

In quantitative spectroscopy, absorbance A is defined by

$$A = \log(I_0/I)$$ ---------- Equation 2.3

Absorbance is so important because it is directly proportional to the concentration, c, of the light absorbing species in the sample (Beer-Lambert law):

$$A = \varepsilon bc$$ ---------- Equation 2.4

The concentration of the sample, c, is usually given in units of moles per litre (M) or mg/l (ppm) and µg/l (ppb) (Ebdon *et al.*, 1998; Skoog *et al.*, 1998). The quantity ε (epsilon) is called the molar absorptivity (or extinction coefficient) and has the units M^{-1} cm^{-1} to make the product εbc dimensionless. The path length, b, is commonly expressed in centimetres. A simple schematic of a typical FAAS instrument is shown in Figure 2.8.

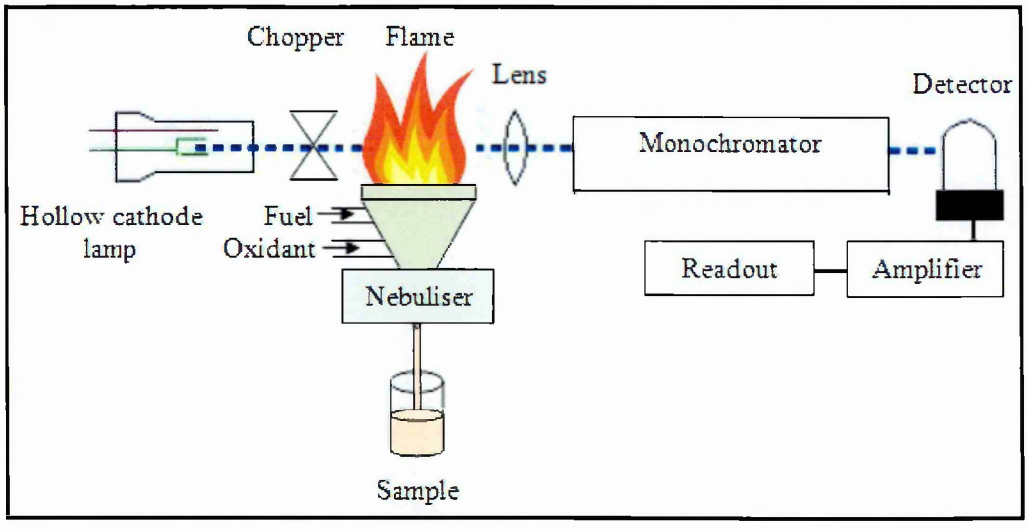

Figure 2.8: Simple schematic diagram of flame atomic absorption instrument (adapted from Vandecasteele & Block, 1993).

In flame AAS, the sample solution is typically aspirated into a flame by a pneumatic nebuliser. The sample is drawn up through a capillary tube by decreased pressure, created by an expanding oxidant gas at the end of the capillary; when the liquid meets with the gas, fine droplets of the sample liquid are formed. These are then mixed turbulently with additional oxidant and fuel gases, before passing into the burner head and flame. Approximately 85 – 90% of the droplets are removed from the aerosol as they deposit in the burner head and

drain away to waste. A flame is used as an atomisation source to produce free atoms. In the flame, the aerosol is desolvated, vaporised and finally atomised.
The energy transferred to the atoms is directly proportional to the flame temperature. If it is too low, the sample is not atomised and if it is too high, the atoms are excited further to a state of ionisation. In the latter situation, the atoms are no longer in the ground state and are unable to absorb energy from the light source. In this work an air/acetylene flame was used, which has a combustion temperature of approximately 1540 K. Light at a characteristic wavelength, which is dependent on the element of interest is passed through the flame and in the presence of the analyte atoms, a portion of this light is absorbed. The unabsorbed light passes through a monochromator and is detected. The absorption of light is directly proportional to the concentration of the element of interest, as described by the Beer-Lambert law (Equation 2.4) (Harris, 2007; Skoog *et al.*, 1998; Vandecasteele & Block, 1993). The light source used in this work was a hollow cathode lamp (HCL) which comprised of a glass container with a quartz window. The cathode is inside a hollow cylinder covered with the element of interest or an alloy of the elements and the container is filled with an inert gas, either argon or neon. When a potential of 300 V (1 – 50 mA) is applied, the inert gas ionises and the positive ions accelerate towards the cathode. Upon striking it, some of the atoms of the cathode material are transformed into the gaseous state. These are then excited by collisions with the electrons and ions, causing them to emit their characteristic atomic emission line (Skoog *et al.*, 1998; 1993; Christian, 1994).

2.5.2 Interferences

Interferences are effects that cause a systematic deviation in the measurement of the signal whilst the concentration of the analyte remains unchanged. Many of the interferences caused by concomitants are quite similar with all atomic spectrometric techniques (Ebdon *et al.*, 1998; Vandecasteele & Block, 1993).

2.5.2.1 Spectral Interferences

Spectral interferences occur when the absorption line of interfering element/species overlaps or is close to the wavelength of the element of interest

(i.e. be within 0.01 nm). In order to minimise the effect of spectral interferences, wavelengths have been selected which are the least susceptible to interference (Elsaied *et al.,* 2009; Dockery *et al.,* 2008). Interferences from the matrix can be physical or chemical in nature (Skoog *et al.*, 1998).

2.5.2.2 Physical Interferences

Typical physical interferences can arise from dissolved or suspended solids in the sample or have a different viscosity to the calibration standards which affects the rate of sample uptake as well as the nebulisation process. The best way to correct for physical interferences is to matrix-match the calibration standards to that of the samples (Vandecasteele & Block, 1993).

2.5.2.3 Chemical Interferences

Chemical interferences are the biggest source of problems in FAAS. Chemical matrix effects occur when compounds of low volatility are formed in the flame, typically by anions combining with the element of interest, and hence eliminating the proportion of free atoms of the sample in the flame and subsequent detection (Vandecasteele & Block, 1993). Some elements, such as calcium, readily form compounds with a low volatility, such as oxides, phosphates or sulphates (Fifield, 2000). In flames where oxygen is readily available, refractory metal oxides are formed making these metals (e.g. iron) highly susceptible to this type of interference (Dockery *et al.,* 2008). This effect can be minimised by using several approaches such as a releasing agent; hotter flame; and adjustment of the nebuliser to produce a smaller particle size (Ebdon *et al.*, 1998). In this study, 1 ml of 2% $SrCl_2$ was used as a 'releasing agent', which binds preferentially to the anions present, releasing the element of interest, namely Ca (Nkono & Asubiojo, 1998).

2.5.2.4 Ionisation Interferences

Ionisation interferences occur when a sample contains easily ionised elements of interest. Alkali and alkaline earth elements are especially susceptible to this type

of interference. Ionisation decreases the concentration of free atoms in the flame and, therefore, must be minimised. This can be achieved through using an ionization suppressor to all samples and standards (Vandecasteele & Block, 1993).

2.5.3 Limitations of FAAS

There are several limitations of FAAS, aside from the affecting interferences. It is a mono-elemental technique which is very useful if only one or two elements are to be determined per sample. Analyses are also fairly rapid (~ 15 second / sample). However, the samples volume requirement for solution nebulisation is quite high – requiring 2 to 3 ml per element. The elemental detection limits are also higher when compared to other atomic spectrometric techniques (Harris, 2007; Vandecasteele & Block, 1993).

2.5.4 Instrumentation

A Perkin Elmer Model AAnalyst 400 spectrometer (Perkin Elmer, Beaconsfield, UK) was used, which is a computer controlled spectrometer, operating Winlab 32 for AA^{TM} software. It has a double beam echelle monochromator optical system with a segmented solid state detector. An air-acetylene flame was used throughout with a 10 cm burner head. Table 2.13 summarises the operating parameters. Three readings were taken per sample then averaged, with a separation time of 2 seconds between each measurement.

Table 2.13: Typical operation conditions for elements analysed by a Perkin Elmer AAnalyst^{TM} 400.

Parameter	Na	Mg	Ca	Fe
Wavelength (nm)	589	285.21	422.67	248.33
Slit Width/height (mm)	1.8/0.6	2.7/1.05	2.7/0.6	1.8/1.35
Current (mA)	8	6	10	30
Acetylene flow (l/min)	2.5	2.5	2.5	2.5
Air flow rate (l/min)	10	10	10	10
Limit of Detection (mg/l)	0.01	0.03	0.04	0.01

2.5.5 FAAS - Calibration

Flame AAS was calibrated before sample analysis by serial dilution of standards from 1000 mg/l single element calibration standards (Aristar®, BDH, UK). A calibration blank was also prepared from 1% (v/v) nitric acid (Aristar® 65%) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK). The calibration range comprised of at least 6 standards, including the blank. The calibration curves were obtained using Microsoft® Excel™ by plotting absorbance of the calibration standards against the concentration. The resultant curves were used to determine the amount of analyte in each sample. The least squares regression line and the coefficient of determination, R^2, were also calculated, as described in Appendix C (Miller & Miller, 2010). Figure 2.9 shows a typical calibration graph for iron produced by the Perkin Elmer AAnalyst™ 400 FAAS software package.

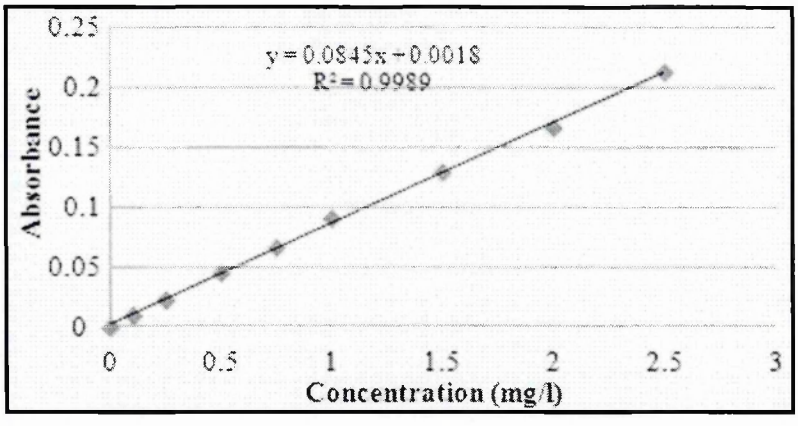

Figure 2.9: Typical calibration graph for iron as determined by Perkin Elmer AAnalyst™ 400 FAAS.

2.6 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) has been continually developed since the 1980s by combining the ease of sample introduction and rapid analysis of ICP technology with the accurate and low detection limits of MS. The resulting technique is capable of trace multi-elemental analysis, often at the ng/l level (Thomson, 2004; Pin & Le Fevre, 2002).

2.6.1 Fundamentals

Inductively coupled plasma mass spectrometry (ICP-MS) is the most widely used method for trace element analysis. In ICP-MS, the sample is introduced into the plasma as an aerosol, where it is desolvated, vaporised and ionised. A small proportion of the sample is then extracted into the mass analyser system, which is under a vacuum of 10^{-5} mbar, through differentially pressurised vacuum stages. Within the system, the quadrupole mass analyser separates elemental ions based on their mass-to-charge ratio (*m/z*). The ion counts are then acquired by the Channel Electron Multiplier. In general, the different ICP-MS instruments have many similar components, such as a nebuliser, spray chamber, plasma torch and detector, but can deviate quite significantly in the design of the interface, ion focusing system, mass separation and vacuum chamber (Vandecasteele & Block, 1993). Figure 2.10 shows a schematic of the Agilent 7700 Series ICP-MS instrument.

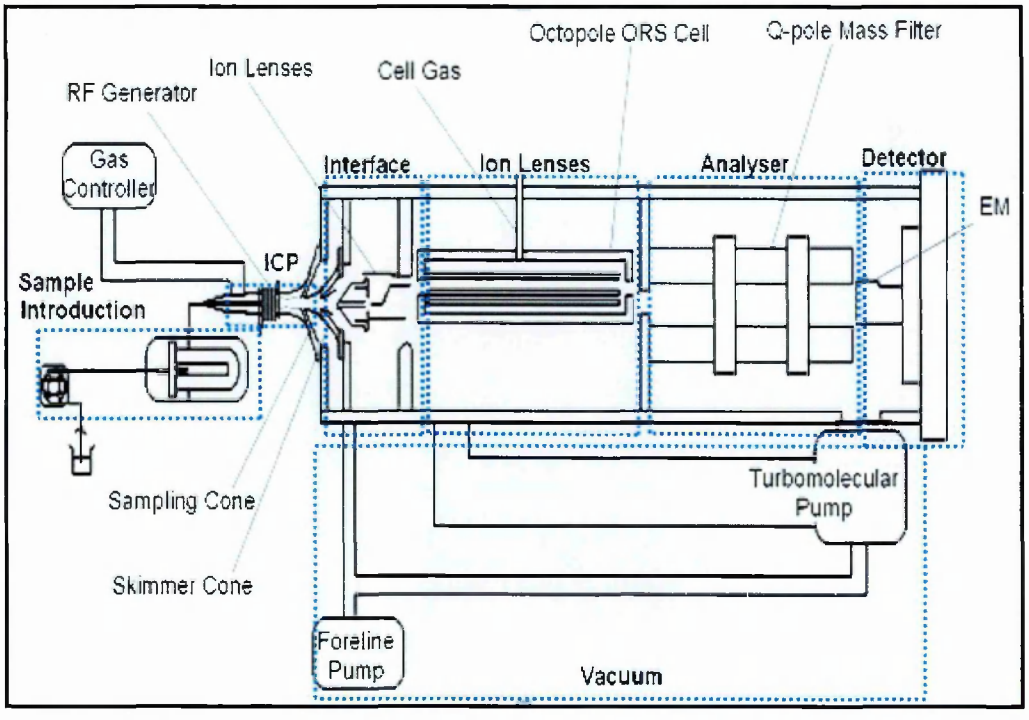

Figure 2.10: Schematic of Agilent 7700 Series ICP-MS instrument, EM is electon multiplier (adapted from Agilent, 2010).

2.6.2 Sample Introduction

There are several modes of sample introduction into the inductively coupled plasma (ICP), where ionisation takes place. The most common form of sample injection is by means of aerosol generated using a pneumatic nebulisation. Other methods can also be used, such as ultrasonic nebulisation, laser ablation, hydride generation, and electrothermal volatilisation (O'Connor & Evans, 2007; Rodushkin & Axelsson, 2003; Vandecasteele & Block, 1993). In general, there are numerous types of nebulisers available including concentric, Babington, cross-flow and glass frit, each having their own benefits and disadvantages. The nature of sample introduction technique is dependent on several factors: (i) the nature of of sample specimen; (ii) the analytical concentration levels; (iii) scope and chemical form of the analytes to be determined; and (iv) the quantity of sample available for analysis (Taylor, 2000).

The sample solution is continuously pumped at ~ 1ml/min into a nebuliser via a peristaltic pump (Figure 2.10), where it is converted into a fine aerosol (< 10 μm diameter). A sample aerosol then passes through a double-pass spray chamber (at a temperature of 4°C) where the larger sample droplets are removed by collision with the spray chamber wall and drain off into a waste bottle. This process improves the signal stability. In addition, cooling the sample aerosol in the spray chamber via a thermoelectric device gives the instrument a very stable ion signal, removes some of the water from the sample and reduces the level of polyatomic oxide species formed. As a result the spray chamber enables the remaining sample aerosol to continue via the gas flow into the injector, that is the centre tube of the torch. In this process, only 1 - 2% of the sample solution reaches the plasma (Taylor, 2000; Montaser, 1998).

2.6.3 Inductively Coupled Plasma (ICP)

The ICP is an electrically neutral gas that is made up of positive ions and electrons (Harris, 2007). The plasma has sufficiently high energy to atomise and ionise virtually all elements in the periodic table, which are intentionally introduced into it for the purpose of elemental chemical analysis (Thomas, 2008; O'Connor & Evans, 2007; Nelms, 2005).

The plasma is formed by coupling energy produced by a Radio Frequency (RF) generator into the plasma support gas via an electromagnetic field, which is induced through the induction coil (also called a load coil – usually copper) (Kenkel, 2003; Ebdon *et al.*, 1998). This coil is wrapped two or three times around the ICP torch and has water flowing through it for cooling purposes (Thomas, 2008). The ICP torch is comprised of three concentric quartz tubes through which streams of argon pass, as shown in Figure 2.11. Between the outer and inner tube of a quartz torch, plasma gas flows tangentially (spiral) to the orifice of the torch. At the end of the torch, radio frequency power between 750 and 1700 W is applied via an induction coil forming an oscillating magnetic field. The plasma then forms when a spark from a Tesla coil is applied to argon gas; electrons are stripped from some of the argon atoms. These electrons trapped in the magnetic field are accelerated in the closed circular paths to reach energies sufficient to ionise gaseous atoms in the field (Nelms, 2005; Thomas, 2003; Taylor, 2000). This sustaining process is known as inductive coupling and the plasma formed is referred to as an inductively coupled plasma (ICP). The collision of these rapidly moving electrons with neutral argon atoms causes further electrons to be stripped from the atoms, creating a chain reaction. The formed annular plasma fireball consists of neutral argon atoms, positively charged argon ions and electrons (Jakubowski, 2008; Nelms, 2005). The plasma will exist for as long as the RF power is supplied to the induction coil. In the centre of the plasma, temperatures range from 8000 to 10000 K. The sample aerosol is instantaneously desolvated, vaporised, thermally atomised and ionised in the ICP. Thermal ionisation is induced by collisions among ions, atoms and free electrons in the plasma (Equations 2.5 – 2.7) (Thomas, 2003; Taylor, 2000; Vandecasteele & Block, 1993).

Electron impact: $M + e^- \rightarrow M^+ + 2e^-$ ---------- Equation 2.5

Charge transfer: $M + Ar^+ + \rightarrow M^+ + Ar$ ---------- Equation 2.6

Penning ionisation: $M + Ar^{m+} + \rightarrow M^+ + Ar + e^-$ ---------- Equation 2.7

where M is the analyte and Ar is the argon plasma gas (Ar^{m+} is the metastable species). If an electron absorbs sufficient energy equal to the first ionisation energy, it escapes the atomic nucleus and an ion is formed.

In the ICP the major mechanism by which ionisation occurs is thermal ionisation. When a system is in thermal equilibrium the degree of ionisation of an atom is given by the Saha equation (Equation 2.8) (Jakubowski, 2008; Ebdon *et al.*, 1998).

$$\frac{n_i n_e}{n_a} = 2\,\frac{Z_i}{Z_a}\left(2\pi mk\,\frac{T}{h^2}\right)^{3/2}\exp\left(-E_i/_{kT}\right) \text{ ---------- Equation 2.8}$$

where n_i, n_e and n_a are the number of densities of ions, free electrons and atoms, respectively, Z_i and Z_a are the ionic and atomic partition functions, respectively, m is the electron mass, k is the Boltzmann constant (1.380650 x 10^{-23} m^2 $kg/s^2/K$), T is the plasma temperature (6000 – 10000 K), h is Plank's constant (6.626068 x 10^{-34} m^2 kg/s) and E_i is the first ionisation potential (O'Connor & Evans, 2007; Ebdon *et al.*, 1998).

The extent of the ionisation, which is primarily a function of the first ionisation potential of the element relative to that of argon (15.76 eV), influences a number of factors including sensitivity and susceptibility to certain sample matrix effects. In argon plasmas, at a temperature of 7500 to 8000 K, most of the elements in the periodic table produce predominantly singly charged ions at yields ranging from 5 - 100% (Zhang, 2007; Nelms, 2005).

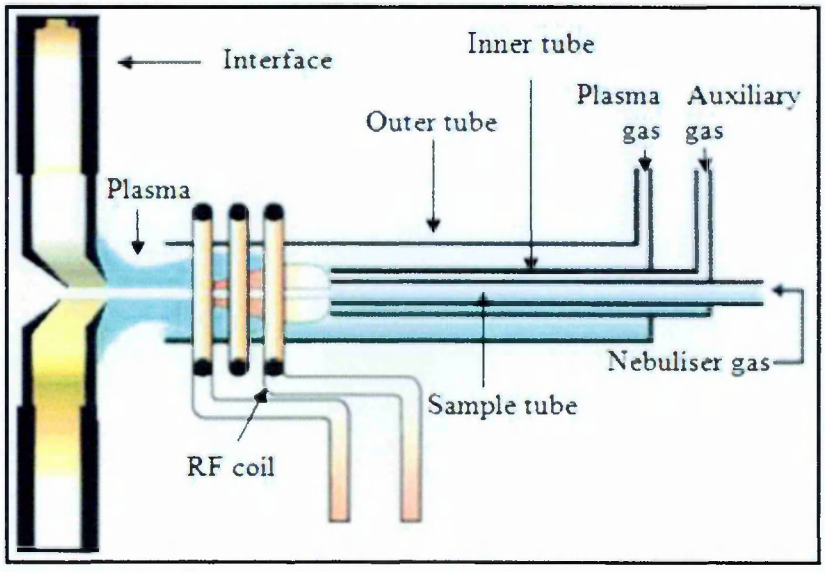

Figure 2.11: Schematic of the plasma torch and RF coil relative to the ICP-MS interface (after Thomas, 2008).

2.6.4 Sampling Interface

The aim of the interface region is to extract sample ions from the high-temperature atmospheric pressure argon plasma into the mass spectrometer, whereby they are isolated and their concentrations in the ion beam are measured (Figure 2.12). This is achieved by using two concentric water-cooled cones fabricated of metal (commonly nickel or platinum) and a series of differentially pumped vacuum chambers held at consecutively lower pressure (Jakubowski, 2008; Taylor, 2000; Ebdon *et al.*, 1998). The outside cone, called the sampling cone, is required to be in direct contact with the plasma, such that the orifice is immersed in the normal analytical zone. The diameter of the orifice is ~ 1 mm. On passing through this orifice the plasma gases, together with analyte ions expand adiabatically (without the gain or loss of heat), causing a decrease in gas density and kinetic temperature. The enthalpy (internal energy) of the source gas is converted into directional flow and the gas temperature drops (O'Connor & Evans, 2007). Ions pass through the sampling cone orifice into the interface, which is an expansion region evacuated by a mechanical vacuum pump to a pressure of about 2 mbar (~ 2 $x10^{-3}$ atmospheres, atm). The ion beam then passes through a second orifice called the skimmer, located immediately behind the sampling cone at a distance of a few millimetres (Figure 2.12). The skimmer cone has a much smaller orifice at its apex (~ 0.7 mm in diameter). This orifice samples the supersonic gas jet expanding through the sampling cone orifice, directing ions into the mass spectrometer (Nelms, 2005; Boss & Fredeen, 1997).

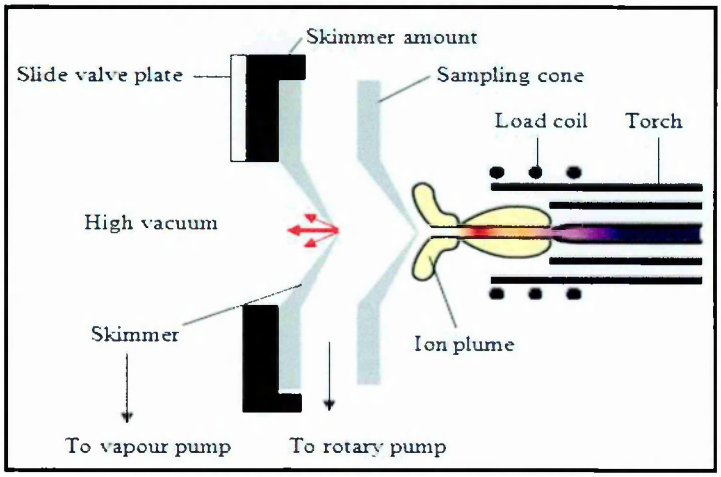

Figure 2.12: Schematic of the ICP-MS interface (after Thomas, 2008).

Ions are extracted from the interface stage and collimated by two conical extraction lenses prior to focusing by the ion optics. The intermediate stage contains the ion optic system and is evacuated by a turbo-molecular pump to a pressure of about 10^{-5} mbar (~ 10^{-8} atm), the normal operation pressure of the mass spectrometer, with an oil diffusion or turbo-molecular pump (Taylor, 2000; Mantaser *et al.*, 1998).

2.6.5 Ion Beam Focusing Unit

The ion beam must be focused before entering the quadrupole mass analyser in order to achieve high signal sensitivity. This can be achieved by subjecting charged ions to constant electric fields. These electric fields have an accelerating effect on the ions. Electrostatic plates, known as ion "lenses", are located within the intermediate stage through which the ion beam passes. The ion lenses perform the dual role of focusing the ion beam and preventing photons and neutral species (arising from the plasma) from reaching the detector. Although electron multipliers are very sensitive ion detectors, they are also sensitive to photons. The first component of an ion lens set often consists of a metal disk called a photon stop. This is mounted in direct alignment with the ion beam behind the skimmer cone of the interface. The purpose of the photon stop is to intercept photons and energetic neutral species produced by the ICP, thereby preventing them from entering the mass analyser. The positive analyte ions in the ion beam are directed by positively charged lenses to deflect around the photon stop, and as a result recombine on the opposite side (Taylor, 2000).

2.6.6 Collision / Reaction Cell (CRC)

In ICP-MS, there are a small number of elements renowned for having poor detection limits. These are predominantly elements that suffer from a lower first ionisation potential than that of the plasma gas – typically argon (15.76 eV) as determined by the Saha equation (Equation 2.8), with the result that few ions are produced. This causes major spectral interferences from ions generated from the argon gas, solvent, or sample matrix. For example, the interferences of $^{40}Ar^{16}O^{+}$ on the determination of $^{56}Fe^{+}$; $^{38}ArH^{+}$ on the determination of $^{39}K^{+}$; $^{40}Ar^{+}$ on the

determination of $^{40}Ca^{+}$; $^{40}Ar\ ^{12}C^{+}$ on the determination of $^{52}Cr^{+}$; and $^{40}Ar^{35}Cl^{+}$ on the determination of $^{75}As^{+}$ (Broekaert, 2005; Taylor, 2000). In order to help deal with these interference problems, the Octopole Reaction System (ORS) was developed for ICP-MS (Thomas, 2008). The ORS is an octopole ion guide contained within a stainless steel vessel and pressurised with a gas, most often He or H_2. The ORS is located between the ion lens assembly and the quadrupole mass filter. The use of 8 rods in the octopole has greater ion transmission efficiency compared to 6 rod (hexapole) or 4 rod (quadrupole) systems, usually operated in the RF-only mode. The RF-only field does not separate the masses like a traditional quadrupole, but instead has the effect of focusing the ions, which then collide and react with molecules of the collision/reaction gas cell in the ORS and in so doing lose kinetic energy, a process referred to as thermalisation (O'Connor & Evans, 2007; B'Hymer & Caruso, 2006; Nelms, 2005; Thomas, 2003). In this technology, ions extract from the interface under vacuum conditions into a collision/reaction cell in the ORS. The gas interacts with the ion beam to remove polyatomic interfering ions like $^{38}ArH^{+}$, $^{39}K^{+}$, $^{40}Ar^{+}$, $^{40}Ar^{16}O^{+}$ and $^{40}Ar^{+}Cl^{+}$ by one of two ways: (i) the gas reacts with an interfering ion to convert it to a different species (i.e. harmless non-interfering species), as shown in Equation 2.9; (ii) the gas collides with the polyatomic interfering ion, causing it to lose energy. Since polyatomic species are large, they undergo more collisions than do analytes, and so lose more energy. The lower energy (polyatomic ion) is then separate from the higher energy (analyte) by energy discrimination (i.e. the cell acts as a molecular filter) (Thomas, 2008).

$$^{38}ArH^{+} + H_2 \leftrightharpoons H_3^{+} + Ar \quad \text{---------- Equation 2.9}$$

The advantage of using this system for interference reduction (instead of employing a high-resolution mass spectrometer) is that in many cases reactions proceed without the loss of sensitivity (O'Connor & Evans, 2007; Yip & Sham, 2007; Broekaert, 2005).

2.6.7 Mass Analysis

Ions pass from the ion lens system through the collision/reaction cell into the analyser vacuum stage, where they are separated by the use of a mass

spectrometer. The mass spectrometer is essentially a mass filter designed to isolate a specific mass-to-charge ratio (*m/z*) ion from the multi-ion beam (Olesik, 2000). After separation, the specific charged isotopic or molecular species are directed to a detector devised to measure their individual ion currents. The magnitude of these ion currents is proportional to the population of the analyte ion species in the multi-component ion beam sampled from the ICP (O'Connor & Evans, 2007; Nelms, 2005; Taylor, 2000). There are two types of mass filter frequently used for ICP-MS, namely, the quadrupole and the magnetic sector (Boss & Fredeen, 1997). The common type in a routine analytical instrumentation is a quadrupole.

A quadrupole mass spectrometer is comprised of four precisely machined cylindrical rods (diameter ~ 1 cm and length of about 15 – 20 cm) aligned parallel to each other in a symmetrical configuration (Figure 2.13). These rods are manufactured of highly polished or metal-plated (gold) ceramic. The centre space contained between the rods is aligned concentric with the ion beam passing through and configured by the electrostatic ion lenses (Nelms, 2005). When a mixture of varying *m/z* ions pass through this centre space, travelling parallel to the length of the rods, only a single *m/z* ion species is permitted to traverse unimpeded and exit at the opposite end. All other masses are rejected by the quadrupole (Taylor, 2000). This process involves the application of both a direct current (dc) potential (E) and an RF alternating current potential (V cos(ωt)) to pairs of the rods. A combined electrical potential of (E + V cos(ωt)) is applied to two oppositely positioned rods, while simultaneously an applied combined potential of – (E + V cos(ωt)) is applied to the other two opposing rods such that they oscillate 180° out of phase (O'Connor & Evans, 2007; Nelms, 2005). By varying these voltages on the rods, an electrostatic field is established which combines with the beam of mixed ion species. Each ion will be deflected into a spiral path, the magnitude of which is related to the fields created by the applied potentials (Becker, 2008). All ions, with the exception of those with a specific unique *m/z*, will be deflected in such a way as to cause them to travel in a wide spiral and collide with the quadrupole rods. Those ions with a unique *m/z*, will continue in a stable path through the central axis of the rods, exiting at the opposite end for eventual interaction with the ion detector positioned behind the quadrupole rods (Bacon *et al.*, 2000).

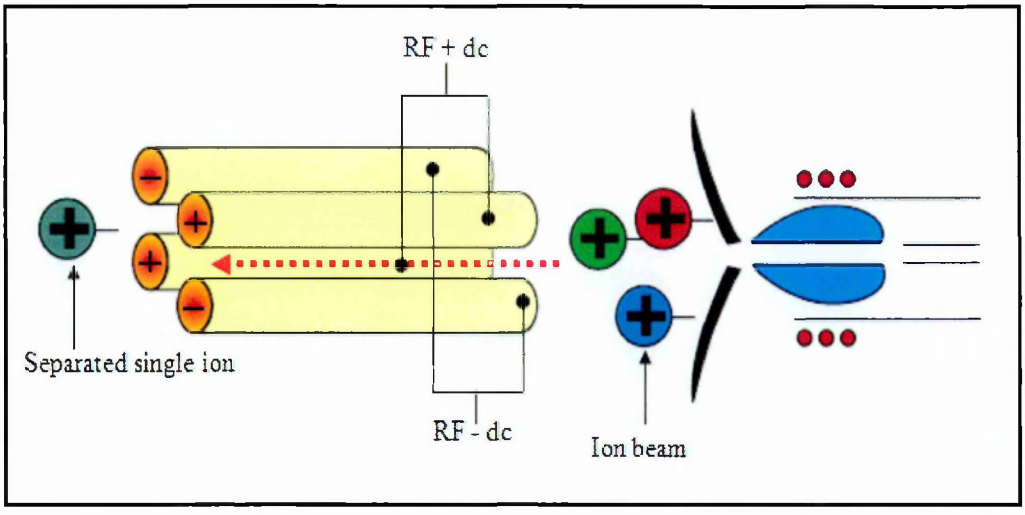

Figure 2.13: Schematic of quadrupole mass filter (Thomas, 2008).

2.6.8 Ion Detection and Signal Handling

Detection of ions can be carried out by a variety of methods, such as electron multiplier, channel electron multiplier and Faraday cup detector, but the commonest by far is the channel electron multiplier (Ebdon *et al.*, 1998). After passing through the quadrupole, ion signals are measured by the channel electron multiplier detector. Both the quadrupole and detector are located in the analyser stage, which is evacuated by a second turbo-molecular pump. The channel electron multiplier detector consists of a horn-shaped glass tube of approximately 1 mm internal diameter, coated on the inside with a lead oxide semi-conducting material (Kebbekus & Mitra, 1998). A voltage difference is applied to the cone, with the wide end being held at about – 3000 V and the back at near ground (Krems *et al.*, 2005). As ions strike the oxide near the entrance, electron ejection occurs. These electrons bounce down the tube, in turn producing more electrons at each encounter with the walls. The resulting pulse of electrons at the end of the tube is amplified by a factor of 10^8 over the original ion collision. The advantage of these detectors over electron multipliers is that exposure to air will not damage them if the voltage is not on at the time. However, these multipliers have a limited lifetime and must be replaced when the sensitivity begins to decline, and higher

voltages must be applied to keep the response at the same level (Kebbrkus & Mitra, 1998).

2.6.9 Limitations of ICP-MS

ICP-MS has become a widely used technique. The main advantages that ICP-MS has over other techniques are low detection limits (1-10 pg/ml) range for quadrupole instruments, a wide dynamic range and rapid multi-element analysis (Ebdon *et al.*, 1998). However, ICP-MS also suffers from various interference effects. There are two main types of interferences which must be considered when using ICP-MS, namely spectroscopic and non-spectroscopic interferences (Thomas, 2003; Ebdon *et al.*, 1998).

2.6.9.1 Spectroscopic Interferences

There are two spectroscopic problems, isobaric and polyatomic, which occur when the interfering species has the same *m/z* as the isotope of interest (Vanheacke & Moens, 2004).

Isobaric interferences arise when the same mass isotopes of different elements overlap between each other. For example, $^{54}Fe^{+}$ overlaps with $^{54}Cr^{+}$; $^{87}Rb^{+}$ with $^{87}Sr^{+}$; $^{58}Ni^{+}$ with $^{58}Fe^{+}$ and $^{113}Cd^{+}$ with $^{113}Sn^{+}$ (Becker, 2008; Krouse, 2000; Vandecasteele & Block, 1993). In order to overcome this problem, other lower abundance isotopes can be selected, for example $^{66}Zn^{+}$(27.8%), $^{67}Zn^{+}$(4.11%), $^{68}Zn^{+}$(18.6%) can be used as appropriate to prevent isobaric interference between $^{64}Zn^{+}$(48.9%) and $^{64}Ni^{+}$(1.16%).

Polyatomic interferences result from the presence of molecular ions overlapping with the isotope analysed. They arise either in the high-temperature plasma or in the interface region between plasma and the mass filter. These polyatomic ions may then overlap with isotopes of the same nominal mass (O'Connor & Evans, 2007; Beauchemin, 2006; May & Wiedmeyer, 1998). The polyatomic species commonly results from different sources, namely the plasma support gas (argon); entrained atmospheric gases; water, acids used for dissolution; oxides formation; doubly charged ions; and the sample matrix (Becker, 2008; Nelms, 2005; Prichard *et al.*, 1996). Methods to overcome these problems include choosing an alternative

isotope of the analyte which is free from interference. The only exceptions are for monoisotopic elements such as As and Mn. In ICP-MS, the determination of As in most biological and environmental samples has problems due to the spectroscopic interference by the high levels of chloride. Arsenic only has one isotope at *m/z* 75 and the chloride matrix causes interference at *m/z* 75 due to $^{40}Ar^{35}Cl^{+}$ (Ebdon *et al.*, 1998). In addition, alternative sample preparation methods, alternative sample introduction, instrumental and other methods were also used to overcome spectroscopic interferences (Evan & Giglio, 1993).

The Agilent 7700 Series ICP-MS instrument utilised in this study was equipped with collision/reaction cell technology (refer to Section 2.6.6). It was found that the use of the collision cell mode reduces the interference effect when compared with those instruments without this technology such as a Finnigan MAT Sola ICP-MS instrument; for example, $^{40}Ar^{35}Cl^{+}$ (see Table 2.12, 23 & 2.26). The use of collision/reaction cell technology to overcome spectroscopic interferences was investigated by another researcher (Watts *et al.*, 2010). Table 2.14 summarises the typical spectroscopic interferences affecting the elements of interest, as well as the internal standard.

2.6.9.2 Non-spectroscopic Interferences

Non-spectroscopic interferences are caused by species present in the sample matrix which affect the signal intensity, and are particularly prevalent when analysing high concentrations of dissolved solids (Stone, 2006). The effect may cause either suppression or enhancement of the analyte signal (Ebdon *et al.*, 1998). The most common example is when the matrix contains a high level of salts with a low volatility. In most cases, salt may be deposited on the apertures of the cones resulting in a reduction of the ion signal. Even if deposition does not occur, the analysis of samples containing high levels of salts causes many other effects, particularly ionisation suppression (Olivares & Houk, 1986). The introduction of easily ionised elements to the plasma contributes strongly to the electron density of the plasma, i.e. depletes the plasma of available electrons to ionise elements of interest. This shifts ionisation equilibrium, causing the analyte ions to be ionised to a lesser extent.

Table 2.14: Isobaric and polyatomic interferences on elements of interest in ICP-MS analysis, where the selected isotopes are shown in **bold** (Vandecasteel & Block, 1997; Evan & Giglio, 1993).

Isotope (% abundance)	Isobaric interferences (%abundance)	Poly atomic interferences
$^{10}B^+$ (19.9)		
$^{11}B^+$ (80.1)		
$^{50}V^+$ (0.25)	$^{50}Ti^+$ (5.4), $^{50}Cr^+$ (4.35)	$^{34}S^{16}O^+$, $^{36}Ar^{14}N^+$, $^{35}Cl^{15}N^+$, $^{36}S^{14}N^+$, $^{32}S^{18}O^+$, $^{33}S^{17}O^+$
$^{51}V^+$ (99.75)		$S^{34}S^{16}OH^+$, $^{35}Cl^{16}O^+$, $^{38}Ar^{13}C^+$, $^{36}Ar^{15}N^+$, $^{36}Ar^{14}NH^+$, $^{37}Cl^{14}N^+$, $^{36}S^{15}N^+$, $^{33}S^{18}O^+$
$^{52}Cr^+$ (83.8)		$^{35}Cl^{16}OH^+$, $^{40}Ar^{12}C^+$, $^{36}Ar^{16}O^+$, $^{34}S^{18}O^+$, $^{36}S^{16}O^+$, $^{38}Ar^{14}N^+$, $^{36}Ar^{15}NH^+$
$^{53}Cr^+$ (9.5)		$^{37}Cl^{16}O^+$, $^{38}Ar^{15}N^+$, $^{38}Ar^{14}NH^+$, $^{36}Ar^{17}O^+$, $^{36}Ar^{16}OH^+$, $^{35}Cl^{17}OH^+$, $^{35}Cl^{18}O^+$, $^{36}S^{17}O^+$, $^{40}Ar^{13}C^+$
$^{55}Mn^+$ (100)		$^{40}Ar^{14}NH^+$, $^{39}K^{16}O^+$, $^{37}Cl^{18}O^+$, $^{40}Ar^{15}N^+$, $^{38}Ar^{17}O^+$, $^{36}Ar^{18}OH^+$, $^{38}Ar^{16}OH^+$, $^{37}Cl^{17}OH^+$, $^{23}Na^{32}S^+$
$^{54}Fe^+$ (5.8)	$^{54}Cr^+$ (2.37)	$^{37}Cl^{16}OH^+$, $^{40}Ar^{14}N^+$, $^{38}Ar^{15}NH^+$, $^{36}Ar^{18}O^+$, $^{38}Ar^{16}O^+$, $^{36}Ar^{17}OH^+$, $^{37}Cl^{17}O^+$
$^{56}Fe^+$ (91.8)		$^{40}Ar^{16}O^+$
$^{58}Fe^+$ (0.28)	$^{58}Ni^+$ (68.3)	$^{23}Na^{35}Cl^+$, $^{40}Ar^{18}O^+$, $^{40}Ca^{18}O^+$, $^{40}Ca^{17}OH^+$, $^{42}Ca^{16}O^+$, $^{40}Ar^{17}OH^+$
$^{63}Cu^+$ (69.2)		$^{31}P^{16}O_2^+$, $^{40}Ar^{23}Na^+$, $^{23}Na^{40}Ca^+$, $^{46}Ca^{16}OH^+$, $^{46}Ca^{16}OH^+$, $^{36}Ar^{12}C^{14}NH^+$, $^{14}N^{12}C^{37}Cl^+$, $^{16}O^{12}C^{35}Cl^+$
$^{65}Cu^+$ (30.8)		$^{32}S^{16}O_2H^+$, $^{40}Ar^{25}Mg^+$, $^{36}Ar^{14}N_2H^+$, $^{32}S^{33}S^+$, $^{32}S^{16}O^{17}O^+$, $^{33}S^{16}O_2^+$, $^{12}C^{16}O^{37}Cl^+$, $^{12}C^{18}O^{35}Cl^+$
$^{64}Zn^+$ (48.6)	$^{64}Ni^+$ (0.91)	$^{32}S^{16}O_2^+$, $^{31}P^{16}O_2H^+$, $^{32}S_2^+$, $^{31}P^{16}O^{17}O^+$, $^{36}Ar^{14}N_2^+$
$^{66}Zn^+$ (27.9)		$^{34}S^{16}O_2^+$, $^{33}S^{16}O_2H^+$, $^{32}S^{16}O^{18}O^+$, $^{32}S^{17}O_2^+$, $^{33}S^{16}O^{17}O^+$, $^{32}S^{34}S^+$, $^{33}S_2^+$,
$^{67}Zn^+$ (4.1)		$^{35}Cl^{16}O_2^+$, $^{33}S^{34}S^+$, $^{34}S^{16}O_2H^+$, $^{32}S^{16}O^{18}OH^+$, $^{34}S^{16}O^{17}O^+$, $^{33}S^{16}O^{18}O^+$, $^{32}S^{17}O^{18}O^+$, $^{33}S^{17}O_2^+$, $^{35}Cl^{16}O_2^+$
$^{68}Zn^+$ (18.8)		$^{36}S^{16}O_2^+$, $^{34}S^{16}O^{18}O^+$, $^{40}Ar^{14}N_2^+$, $^{35}Cl^{16}O^{17}O^+$, $^{34}S_2^+$, $^{36}Ar^{32}S^+$, $^{34}S^{17}O_2^+$, $^{33}S^{17}O^{18}O^+$, $^{32}S^{18}O_2^+$, $^{32}S^{36}S^+$
$^{75}As^+$ (100)		$^{40}Ar^{35}Cl^+$
$^{86}Sr^+$ (9.86)		
$^{87}Sr^+$ (7.00)		
$^{88}Sr^+$ (82.6)		$^{176}Lu^{+2}$, $^{176}Yb^{+2}$
$^{111}Cd^+$ (12.80)		
$^{113}Cd^+$ (12.22)	$^{113}In^+$ (4.3)	
$^{114}Cd^+$ (28.73)	$^{114}Sn^+$ (0.65)	$^{98}Mo^{16}O^+$

Such interferences are usually corrected for by the following approaches.

- Sample dilution (Ebdon *et al.*, 1998);
- The method of internal standardisation. In this method, a non-endogenous element of known concentration is added to all standards and samples and is monitored. The internal standard elements are affected by the matrix in the same way as the analyte elements. It is therefore necessary that the mass range of the internal standards covers the same as the analyte range. Correction is applied using the ratio of the internal standard signal with the isotopes of interest (see Section 2.6.13 for further information) (Adair, 2002); and
- Standard addition method – a known concentration of analyte is added to the sample (Vandecasteel & Block, 1997).

2.6.10 Instrumentation

The instrument used in this study was a quadrupole Agilent 7700 Series ICP-MS (Agilent, Cheshire, UK) with ASX-500 Autosampler controlled through the use of dedicated Agilent software (ChemStation). A Finnigan MAT Sola ICP-MS (Finnigan Corp., Hemel Hempstead, UK) was also used in the preliminary research until as a result of instrument failure the Finnigan ICP-MS was replaced with the new Agilent 7700. As such this thesis will report the instrumental operating conditions of the latter instrument which was used for the analysis of most of the Karbala samples.

2.6.11 Operating Conditions

Optimisation of the Agilent 7700 Series ICP-MS instrument was performed daily before the calibration, validation stages and prior to any samples being analysed. The instrument operating parameters were optimised using an Agilent standard tuning solution (1 μg/l mixed solution of Li, Co, Y, Ce and Tl in 2% HNO_3). The adjustments for each parameter such as the forward power, nebuliser gas flow rates and ion lens positions were made in order to achieve the maximum sensitivity for the signal values of trace elements under investigation. Parameter settings were then saved and used in the corresponding sample analysis. The

typical operating conditions for the Agilent 7700 ICP-MS instrument are shown in Table 2.15.

Table 2.15: Typical operating conditions for the Agilent 7700 Series ICP-MS instrument.

Parameter	Typical operating conditions
Forward power	1550 W
Plasma gas flow rate	15 l/min
Auxiliary gas flow rate	0.8 l/min
Nebuliser carrier gas flow	0.8 l/min
Nebuliser make up gas flow	0.3 l/min
Cooling water temperature	15 – 40°C
Cooling water minimum flow rate	5.0 l /min
He gas (CCT conditions)	4.8 ml/min
Acquisition time	120 – 240 seconds
Integration time	0.1 seconds
Nebuliser	Micromist concentric
Spray chamber	PTFE Scott-type
Spray chamber temperature	4°C
Torch	Quartz 1 – 2.5 mm
Mass range	6 – 260 amu
Type of detector	simultaneous
Sample uptake time	50 seconds
Sample stabilisation time	30 seconds
Wash time between samples	90 seconds

2.6.12 ICP-MS - Calibration

Calibration standards for ICP-MS were prepared by serial dilution of a 1000 mg/l single element standard solution (Aristar®, BDH, Primar®, Fisher Scientific). The calibration concentrations ranged from 1 – 500 µg/l. A calibration blank was also prepared from 1% (v/v) nitric acid (Aristar® 65%) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, UK). A calibration plot was constructed, based on the measured signal for elements of interest against their concentration in a known solution. Figure 2.14 shows a typical calibration graph for iron produced by the Agilent 7700 Series ICP-MS software package in which the internal standard (IS) corrected signal was plotted against the calibration standard concentration. The least squared regression line and the linear regression coefficient, R^2, were calculated, as described in Appendix C.

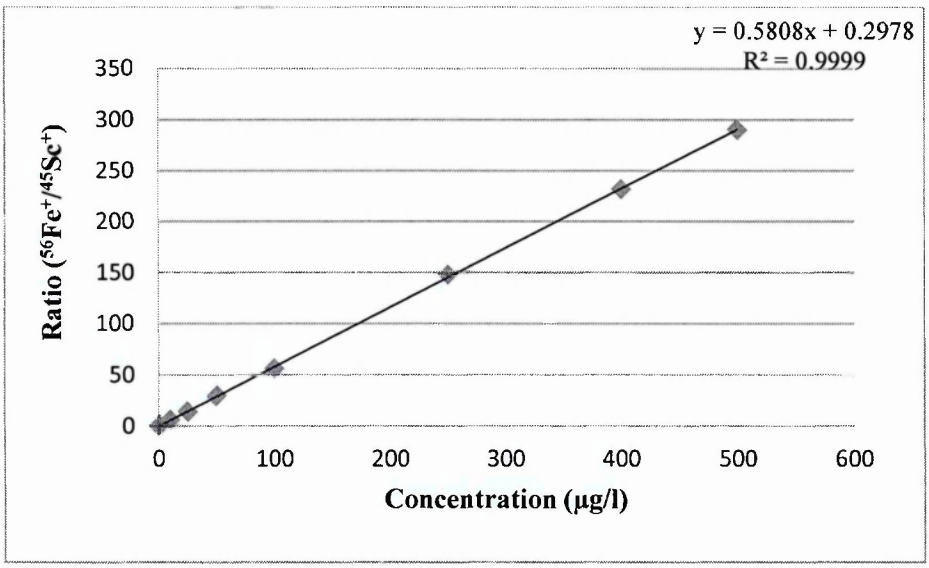

Figure 2.14: Typical calibration graph for iron by the Agilent 7700s ICP-MS instrument.

2.6.13 Internal Standard (IS)

The use of a multi-element internal standard (IS) solution helped to monitor the performance of the Agilent 7700s ICP-MS instrument through the detection of any instrumental drift during analysis (Figure 2.15). The data was used for correcting the effects of enhancement/suppression in the ICP signal. In general, the IS solution contains elements that are not present in the sample. All internal standards should have an atomic mass and a first ionization potential that is near to that of the elements to be measured. Suitable internal standards were selected to cover the wide range of masses in the periodic table. Internal standards of $^{9}Be^{+}$, $^{45}Sc^{+}$, $^{72}Ge^{+}$, $^{103}Rh^{+}$, $^{115}In^{+}$ and $^{209}Bi^{+}$ 100 µg/l were used for multi-element analysis using the Agilent 7700s ICP-MS instrument.

It was found that the stability of the internal standards measured by Agilent 7700s ICP-MS (Figure 2.15) was more stable than those measured by the Finnigan MAT Sola ICP-MS (Appendix F). Possible explanations are that the Agilent has a better interface and stable vacuum system, and the modern technology (collision/reaction gas cell) reduces potential interferences in the Agilent 7700s ICP-MS rather than the Finnigan MAT Sola ICP-MS. Internal standard (IS) correction was carried out automatically through the Agilent ChemStation software according to Equation 2.10.

$$\frac{\text{Analyte intensity(cps)}}{\text{IS intensity(cps)}} \text{ ---------- Equation 2.10}$$

The raw count signals reported by the Agilent 7700 Series ICP-MS instrument were utilised to manually monitor for the IS correction.

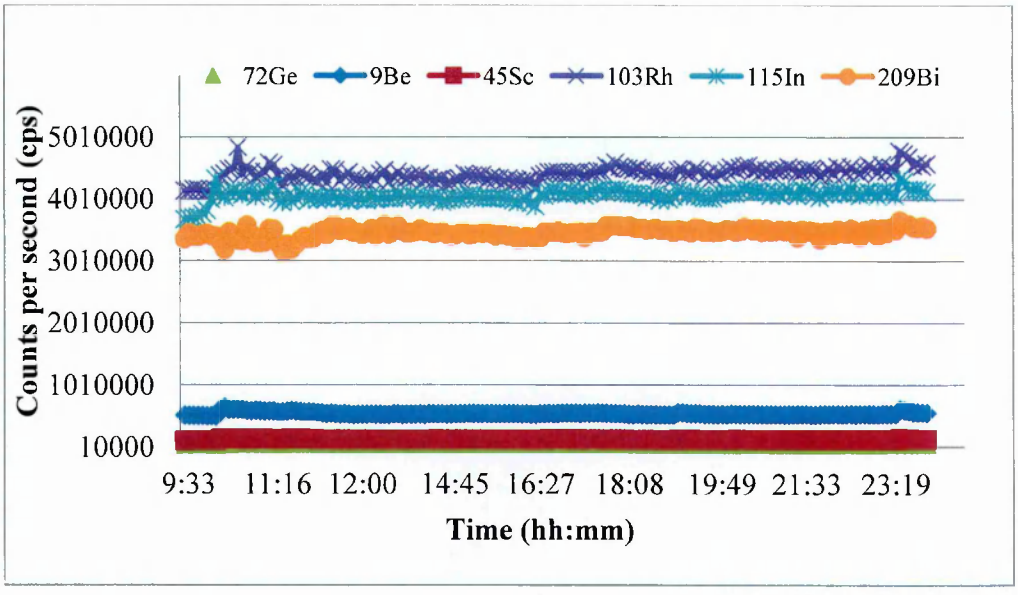

Figure 2.15: Typical long-term stability during the analysis of tear drops using a 100 µg/l of ^{9}Be, ^{45}Sc, ^{72}Ge, ^{103}Rh, ^{115}In and ^{209}Bi as an internal standard solution for multi-element analysis by the Agilent 7700 Series ICP-MS instrument.

2.7 Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

Atomic emission spectrometry (AES) is the oldest atomic spectrometric multi-element technique using classical sources (e.g. flame, arc and spark) to atomise the sample and to excite (and possibly ionise) the atoms of the sample (Vandecasteele & Block, 1993). Plasma sources were developed for emission spectrometry in the 1960s and have become commercially available in the mid 1970s (Harris, 2007). Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a technique which has been in common place in analytical laboratories for many decades. In this work, it was used to determine the elemental composition of cigarette tobacco.

2.7.1 Fundamentals

ICP-AES 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 particular element in the sample (Lehn & Hieftje, 2003; Skoog *et al.*, 1998; Vandecasteele & Block, 1993). The main advantages of this method are the large dynamic range, auto sampler, 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 (Hou & Jones, 2000). 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 (Ebdon *et al.*, 1998; Skoog *et al.*, 1998; Manning & Grow, 1997).

In general, ICP-AES instruments have four main parts, including: the sample introduction system (nebuliser and spray chamber); ICP torch; transfer optics; and spectrometer, as shown in Figure 2.16 (Selinus *et al.*, 2009). The first two parts, namely sample introduction systems and radiofrequency generators, and the nature of ICP itself were found to be the same for ICP-AES and ICP-MS systems, with the usual differences between the manufacturers (Ebdon *et al.*, 1998). In brief, the sample is usually transported into the instrument as a stream of liquid sample. Inside the spray chamber, the liquid is converted into an aerosol through a process known as nebulisation. 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 (Vandecasteele & Block, 1993). The wavelength range of the plasma radiation is extended from 200 to 800 nm (Skoog *et al.*, 1998).

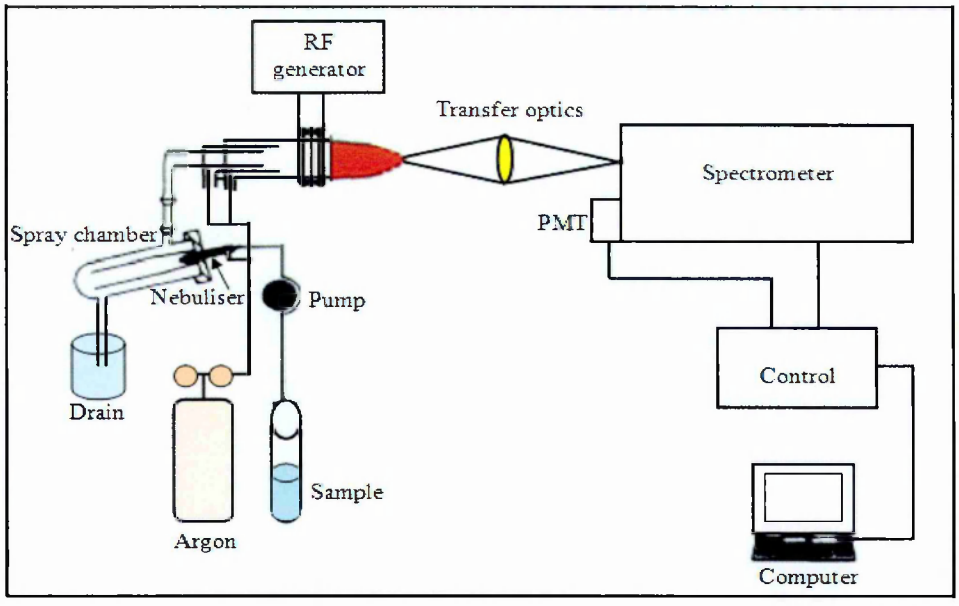

Figure 2.16: Typical configuration for ICP-AES instrument (axial viewing of the ICP) (adapted from Boss & Fredeen, 1997).

2.7.2 Excitation, Ionisation and Emission

Once the sample aerosol has been desolvated, vaporised and atomised, the plasma has one, or possibly two, functions remaining. These functions are excitation and ionisation. Typically, the atoms are preferred to be in stable or ground state (i.e. the electrons of an atom are in the orbitals closest to the nucleus and lowest in energy). When an atom absorbs energy, one of its electrons must be excited to a higher energy level (excited state) through an excitation process. In an excited state, an atom is less stable and will thus fall back to a less excited state by losing energy through a collision with another particle or by emission of a particle of electromagnetic radiation, known as a photon, which is characteristic for that particular transition (Hou & Jones, 2000; Ebdon *et al.*, 1998; Boss & Fredeen, 1997). Since many elements have their strongest emission lines emitted from the ICP by excited ions, the ionisation process may also be necessary for some elements. This process occurs when the energy absorbed by an atom is sufficient, equal to the first ionisation energy, an electron may be completely dissociated from the atom, leaving an ion with positive charge, and another electron can be excited (Ebdon *et al.*, 1998). This is the most important advantage of using ICP-

AES products from the excitation properties of the high temperature source utilised in this method. This thermal excitation source can provide a large number of different energy levels for several different elements at the same time. All of the excited atoms and ions can then emit their characteristic radiation at nearly the same time. This provides high level of flexibility to choose from several different emission wavelengths for an element and the ability to measure emission from several different elements concurrently (Hou & Jones, 2000; Boss & Fredeen, 1997).

Figure 2.17 shows the excitation, ionisation and emission processes schematically. The horizontal lines of this simplified diagram represent the energy levels of an atom. The vertical arrows represent energy transitions, or changes in the amount of energy of an electron. The energy transitions in an atom or ion can be either radiational (involving absorption or emission of electromagnetic radiation) or thermal (involving energy transfer through collisions with other particles). The difference in energy between the upper and lower energy levels of a radiative transition defines the wavelength of the radiation that is involved in that transition.

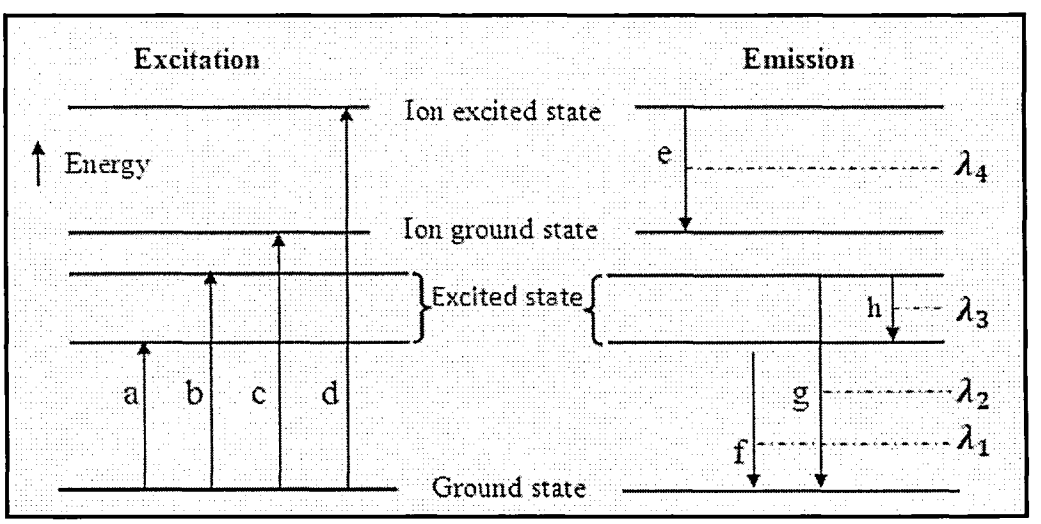

Figure 2.17: Energy level diagram showing energy transitions where a and b represent excitation, c is ionisation, d is ionisation/excitation, e is ion emission, and f, g and h are atom emissions (Boss & Fredeen, 1997).

2.7.3 Collection and Detection of Emission (Spectrometer)

In ICP-AES, the light emitted by the excited atoms and ions in the plasma is measured to obtain information about the sample. Since the excited species in the plasma emit light at several different wavelengths, the emission from the plasma is polychromatic. This polychromatic radiation must be separated into individual wavelengths so the emission from each excited species can be identified and its intensity can be measured without interference from emission at other wavelengths. The separation of light according to wavelength is generally done using a monochromator, which is used to measure light at one wavelength at a time, or a polychromator, which can be used to measure light at several different wavelengths at once. The actual detection of the light, once it has been separated from other wavelengths, is done using a photosensitive detector such as a photo-multiplier tube (PMT) or advanced detector techniques such as a charge-coupled device (CCD) (Harris, 2007; Hou & Jones, 2000; Ebdon *et al.*, 1998; Skoog *et al.*, 1998). The combination of focusing optics, monochromator and detector is generally referred to as a spectrometer.

2.7.3.1 Focusing Optics

The emission radiation from the plasma is sampled on to the entrance slit of the monochromator by a focusing optic such as a convex lens or a concave mirror (Ebdon *et al.*, 1998). There are two ways of viewing the light emitted from an ICP, namely radial and axial. In the classical ICP-AES configuration, the light across the plasma is viewed radially (side-on), resulting in the maximum signal intensity and least interferences. By viewing the light emitted by the sample looking down the centre of the torch or axial (end-on), the background signal from the ICP itself is reduced, the sample path is maximized. Axial viewing provides better detection limits than those obtained via radial viewing by as much as a factor of 10. Recently, instruments that combine both radial and axial viewing, called dual view, have been introduced (Boss & Fredeen, 1997).

2.7.3.2 Monochromator

The next step in ICP-AES is the separation of the emission radiation of the element of interest from the radiation emitted by other elements and molecules by using a monochromator device. A monochromator is defined as an instrument that can be used to separate a narrow range of wavelengths (e.g. 1 - 0.01 nm) anywhere in a wide spectral range. A diffraction grating was commonly used with most modern instruments. It is a mirror that has a line or density from 600 to 4200 lines per millimetre etched into it. There are several ways to mount a grating in a monochromator such as the Ebert mounting which uses a large spherical mirror and the Czerny/Turner mounting using two small, spherical mirrors (Ebdon *et al.*, 1998). When light strikes such a grating, it is diffracted at an angle that is dependent on the wavelength of the light and the density of the grating. The use of a spectrometer with high resolution (~ 0.01 nm) is practical in order to differentiate between wavelengths. This can be achieved, either by increasing the number of lines per millimetre on the grating or by increasing the focal length of the monochromator. There is an additional wavelength dispersive device, called an echelle grating, which can achieve greater resolution (i.e. typically 100 lines per millimetre). The echelle grating separates the polychromatic radiation by wavelengths and produces multiple, overlapping spectral orders (Ebdon *et al.*, 1998).

2.7.3.3 Detector

The traditional types of ICP-AES system used a series of photo-multiplier tubes (PMT), which converts the photo signal into electron signal (Ebdon *et al.*, 1998). In recent decades, advanced solid-state detectors with high sensitivity and resolution have been developed; for example, the charge-injection device (CID) and the charge-coupled device (CCD). These detectors are based on the light-sensitive properties of solid-state silicon (Boss & Fredeen, 1997). In this study of ICP-AES systems, solid-state detectors based on a charge-coupled device (CCD) were used. The CCD is an extremely sensitive detector in which light creates electrons and holes in a semiconductor material. It is comprised of 224 linear photodetector arrays on a silicon chip with a surface area of 13 x 18 mm. For each

subarray there are several pixels, which are photosensitive areas of silicon. In the CCD, photons falling on a silicon substrate produce electron-hole pairs. The electrons are attracted to regions near positive electrodes, where the electrons in each are "stored" until they are ready to be counted. The number of electrons in each pixel (picture element) is proportional to the number of photons striking the pixel (Harris, 2007; Hou & Jones, 2000; Ebdon *et al.*, 1998; Skoog *et al.*, 1998). The main advantage of CCD is that it makes available as many as ten lines for each element in the sample. Therefore, lines which suffer from interferences can be identified and removed from the analysis (Ebdon *et al.*, 1998). The signal output from the detector is usually amplified, converted into a digital signal that can be read by computer.

2.7.4 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, modern trace elemental analysis instruments have been designed to minimize the interferences. Interferences in ICP-AES may start in the sample preparation stage and extend to the plasma operating conditions. In general, ICP-AES probably has fewest interferences when compared with commonly used analytical atomic spectrometry techniques (Hou & Jones, 2000). The technique suffers from three types of interferences, namely chemical, spectral and ionisation 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 (Hou & Jones, 2000; Ebdon *et al.*, 1998).

The most common interference problem in ICP-AES is spectral interference due to the line-rich spectra produced by the hot plasma source. The spectra are likely to be rich particularly for a highly complex and concentrated sample due to the ICP being capable of exciting almost any element that is introduced into the plasma. They can be minimized by using high-resolution spectrometers. In some cases, the spectral overlap may even exist with the best commercial system. In these cases advanced background correction techniques can be employed or a different analytical wavelength for the element(s) of interest is chosen (Hou &

Jones, 2000; Ebdon *et al.*, 1998; Manning *et al.*, 1997). In this study, background correction was used in order to overcome spectral interferences.

The ionisation interferences arise from easily ionised elements, such as the alkali or alkaline earth elements, in the sample matrix. These types of interference can be overcome by matrix matching the samples and standards or by using standard additions method (Ebdon *et al.*, 1998).

2.7.5 Instrumentation

A Perkin Elmer Optima™ 5300 DV ICP-AES (Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA) with WinLab32™ software and a PerkinElmer S10 autosampler (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) was used in this study. This technique was used to analyse different commercial cigarette tobacco samples. The typical operation parameters for this instrument are displayed in Table 2.16. An echelle grating and the charge-coupled device (CCD) were used in the ICP-AES instrument.

Table 2.16: Typical operating conditions for the Perkin Elmer Optima™ 5300 DV ICP-AES 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

2.7.6 ICP-AES – Calibration

Calibration for ICP-AES 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 V, Cr, Mn, Cu, Zn, As and Sr 1 – 10 mg/l and for Fe, Cr and Cd 1 – 5 mg/l. 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 2.18 shows the typical calibration curve for iron by Optima 5300 DV ICP-AES.

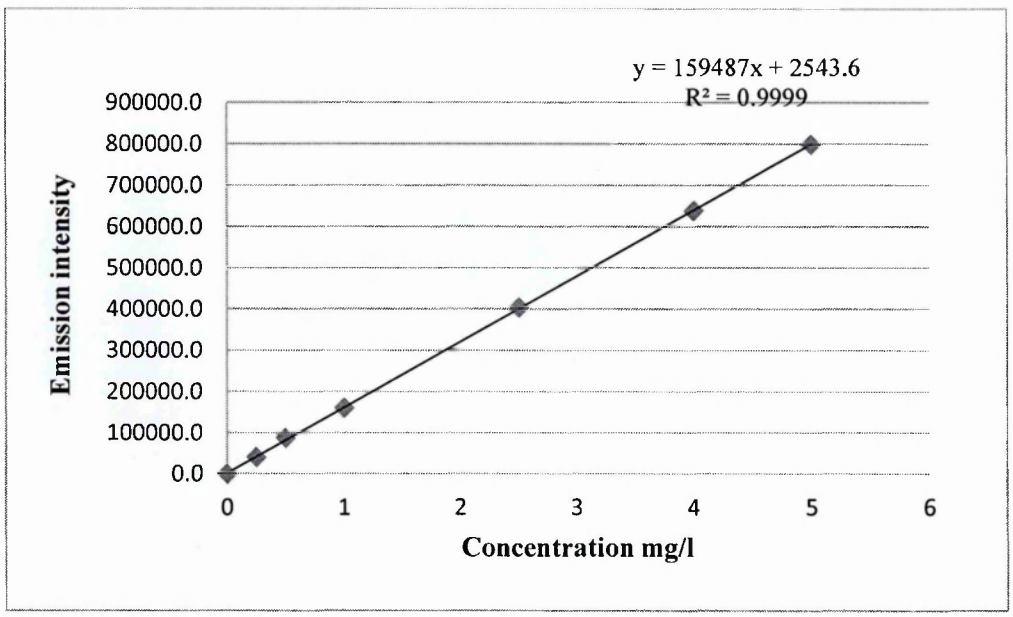

Figure 2.18: Typical calibration curve for iron as determined by Perkin Elmer Optima™ 5300 DV instrument.

2.8 Quality Control (QC)

There are many QC tests that can be used to evaluate the performance, precision and accuracy throughout the study. These evaluations are typically examined before any analysis of real samples in order to assess whether the method has the correct levels of precision and accuracy. Precision can be verified by using the

replicate analysis of samples and replicate portions of the same sample (with the level reported as the relative standard deviation (%RSD)). Accuracy was examined by using calibration checks, Certified Reference Materials, quality control samples and a blank. In this study, the values of intensity were corrected with a reagent blank by subtracting the response of the reagent blank from the response of the real samples. The next section describes the quality control analysis for this study.

2.8.1 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 but not necessarily quantified as an exact value'. In the Guidelines for Achieving Quality in Trace Analysis, the LOD is expressed as 'the concentration C_L or quantity q_L derived from the smallest measure X_L that can be detected with reasonable certainty for a given procedure. The value X_L is given by equation 2.11 (O'Connor & Evans, 2007).

$$X_L = X_{bl} + KS_{bl} \text{ ---------- Equation 2.11}$$

where X_{bl} is the mean of the blank measures, S_{bl} is the standard deviation (SD) of the blank measures and K is a numerical factor chosen according to the confidence interval required (typically 3) (O'Connor & Evans, 2007).

The instrumental LOD may be defined as that quantity of the element which gives rise to a reading equal to three times the SD of a series of at least ten determinations (n = 10) at near the blank level (Nelms, 2005; Ebdon *et al.*, 1998). The LODs for Agilent 7700 Series ICP-MS and Perkin Elmer Optima™ 5300 DV ICP-AES instruments were determined for a range of elements in this study. The LODs were calculated for a total of 15 blank solutions (1% HNO_3). The resulting LOD data, based on a mean blank (n = 15) signal + 3SD (Equation 2.11) is shown in Tables 2.17 & 2.18.

Table 2.17: Elemental limit of detection (LOD) values for the Agilent 7700 Series ICP-MS instrument (µg/l) and typical collision cell conditions.

Element	Isotope	Relative isotopic abundance (%)	Internal Standard	Collision cell gas	LOD
B	$^{11}B^+$	80.1	$^{9}Be^+$	No gas	7
V	$^{51}V^+$	99.8	$^{45}Sc^+$	He	0.001
Cr	$^{52}Cr^+$	83.8	$^{45}Sc^+$	He	0.01
Mn	$^{55}Mn^+$	100	$^{45}Sc^+$	He	0.01
Fe	$^{56}Fe^+$	91.8	$^{45}Sc^+$	He	0.05
Cu	$^{63}Cu^+$	69.2	$^{72}Ge^+$	He	0.03
Zn	$^{66}Zn^+$	27.9	$^{72}Ge^+$	He	0.1
As	$^{75}As^+$	100	$^{74}Ge^+$	He	0.01
Sr	$^{88}Sr^+$	82.6	$^{74}Ge^+$	He	0.2
Cd	$^{111}Cd^+$	12.8	$^{115}In^+$	No gas	0.01

Table 2.18: Elemental limit of detection (LOD) values for the Perkin Elmer Optima™ 5300 DV ICP-AES instrument (µg/l) and selected wavelength.

Element	Wavelength (nm)	LOD
V	292.402	1.01
Cr	205.560	0.81
Mn	257.610	0.2
Fe	238.204	1
Cu	324.700	0.81
Zn	213.857	1.3
As	188.979	5.3
Sr	232.235	5
Cd	228.802	0.63

2.8.2 Quality Control Chart

A control chart is a time plot of a measured concentration (QC standard), that is usually used to identify any instrument drift throughout the analysis run. In general, there are three different lines in this chart, the central line (green line) representing the mean value from the whole day, and the two pairs of limit lines (blue and red) demonstrating the control limits. It was found that the standard deviation of the procedure can be used as a useful tool in establishing the control lines (Christian, 1994). When all of the points are set above or below the central line it is possible to estimate any systematic error in the instrument. On the other

hand, if the points lie outside the control lines this indicates that one or more measurements are determined to be in error (Harris, 2007; Christian, 1994).

In this study, two control solutions (blank and a standard solution from the middle of the calibration range) were analysed after every 20 samples throughout the whole analysis run. This was carried out for the determination of every element using the Agilent 7700 Series ICP-MS. Figure 2.19 shows a typical instrumental drift chart for arsenic from the repeat analysis of 100 µg/l As calibration standard for the Agilent 7700 Series ICP-MS. If the instrumental drift was more than ± 5% RSD, correction was undertaken, as described in Equation 2.12.

$$\textit{Drift correction} = \frac{\textit{Unknown sample concentration}}{\textit{(Mean calibration standard / known calibration concentration)}} \quad \text{-----Equation 2.12}$$

Figures 2.20 & 2.21 show a typical instrument drift chart for 1 mg/l arsenic and 1.25 mg/l sodium that was used as a calibration standard for the Perkin Elmer Optima™ 3500 DV ICP-AES and AAnalyst™ 400 FAAS, respectively.

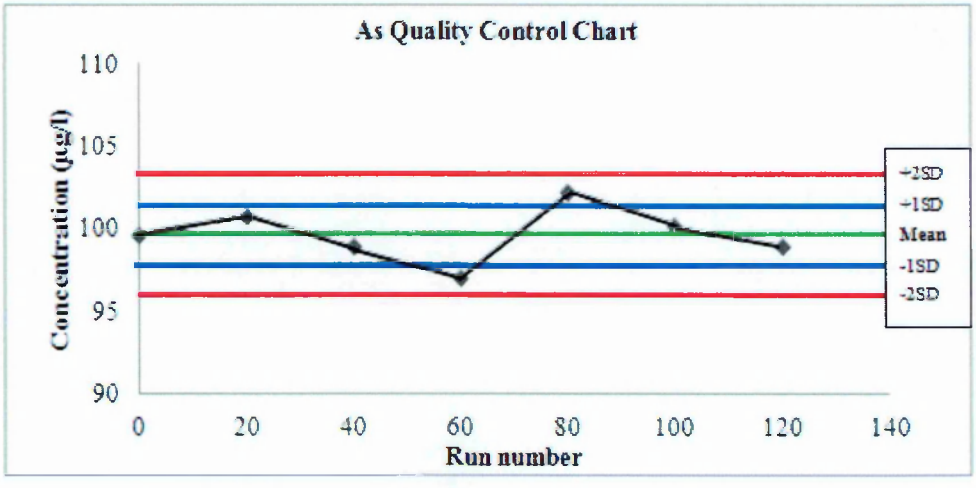

Figure 2.19: Instrumental drift chart for a 100 µg/l arsenic solution by Agilent 7700 Series ICP-MS.

Figure 2.20: Instrumental drift chart for a 1mg/l arsenic solution by Optima 3500 DV ICP-AES.

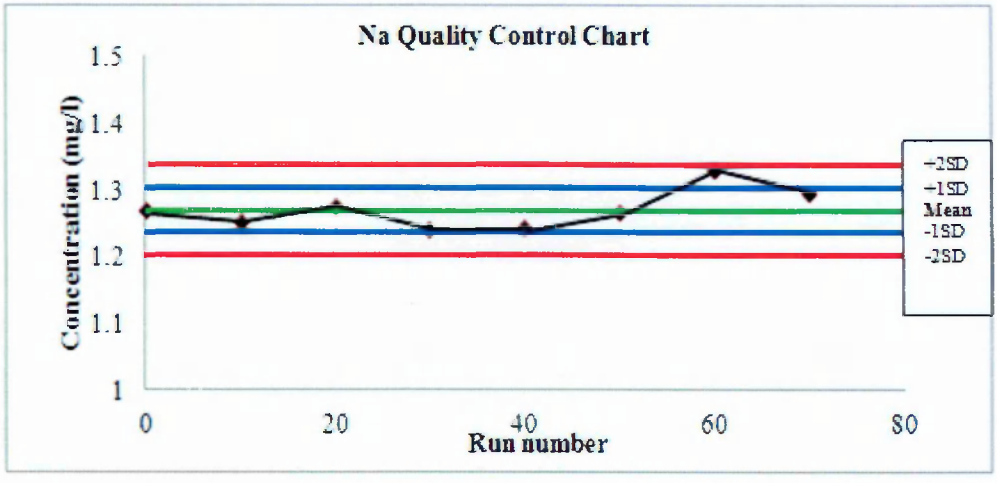

Figure 2.21: Instrumental drift chart for a 1.25 mg/l sodium solution by AAnalyst^{TM} 400 FAAS.

Table 2.19 reports the results of the quality control study for different analytical techniques. A student's t-test was used to compare the measured and true values. It was found that no significant difference was observed between the true concentrations of standard solutions and the measured values during the whole day analysis run for ICP-MS, ICP-AES and FAAS at a probability level, $P < 0.05$, as shown in Table 2.19. The %RSD value was used to compare the data between these techniques. The Agilent 7700 Series ICP-MS (%RSD = 2) has a higher level of precision than the Optima 5300 DV ICP-MS (%RSD = 5) for the arsenic analysis of different media (Table 2.19). A possible explanation may be due to the

effect of the matrix, since tobacco material has more than 4000 components (Ward, 1993). Another reason is that the ICP-MS is known to be more sensitive and selective than atomic emission, particularly the Agilent 7700 Series ICP-MS, which includes the collision / reaction cell technology (see Section 2.6.6).

Table 2.19: Statistic analysis of quality control data for the different analytical techniques.

Parameter	Instrument		
	Agilent 7700 Series ICP-MS	Optima 3500 DV ICP-AES	AAnalyst™400 FAAS
Standard concentration	100 µg/l As	1 mg/l As	1.25 mg/l Na
n	6 (one reading for every 20 samples)	10 (one reading for every 10 samples)	7 (one reading for every 10 samples)
Analysis	Tear drops	Tobacco	Scalp hair
Measured value $\overline{x} \pm$ SD	99.6 ± 2	0.98 ± 0.05	1.27 ± 0.03
%RSD	2	5	3
df = n-1	5	9	6
t_{calc}	0.5	1.4	1.77
t_{crit}	2.57	2.26	2.45
Result at $P <$ 0.05	No Sig?	No Sig?	No Sig?

n = number of samples, $\overline{x}$ = mean value, SD = standard deviation, RSD = relative standard deviation, df = degrees of freedom, t_{calc} and t_{crit} are calculated and critical values for student's t-test, P = probability, and Sig? = significant ($P < 0.05$).

2.8.3 Precision and Accuracy

Precision can be defined as "the degree of agreement between replicate measurements of the same quantity" and it does not necessary imply accuracy (Miller & Miller, 2010). Random errors cause the individual results to lie on both sides of the average value and this affects the level of precision (Miller & Miller, 2010; Harris, 2007; Christian, 1994). There are two terms that can be used to describe the level of precision, namely repeatability and reproducibility. Repeatability (*within-run* precision) is the degree of agreement between the consecutive results carried out under the same conditions and method. Reproducibility (*between-run* precision) is the degree of agreement between the individual results carried out under the same conditions and method (Miller &

Miller, 2010). Typically, the precision level of an instrument is described by using the coefficient of variation (CV (%)), which is also known as the relative standard deviation (%RSD). The best level of precision relates to a calculated value of 1% to 5% (%RSD); the acceptable range is between 1 - 10 %RSD) (Miller & Miller, 2010; Adair, 2002).

Accuracy can be defined as "the degree of agreement between a measured value and a true value" (Harris, 2007; Christian, 1994). Systematic errors cause all results to be in error in the same sense and, therefore, affect accuracy (Miller & Miller, 2010). The use of Certified Reference Materials (CRMs) was employed in each analytical procedure to determine the validity and accuracy of methods. CRMs were chosen based on their similarity to the matrix involved and their certified chemical composition, as shown in Table 2.20 (Massart, *et al.,* 1996). This can be determined as the percentage recovery (%R), which can be calculated by the following equation:

$$\%R = (\text{Measured value})/(\text{Certified value}) \times 100 \text{ ------- Equation 2.13}$$

Table 2.20: Certified Reference Materials (CRMs) for Quality Control (QC) evaluation in this study.

Media	CRM	Reference
Water, tear drops and saliva	NIST SRM® 1643e Trace Elements in Water	National Institute of Standards and Technology, Maryland, USA
	TMDA 54.4 Trace Elements in Fortified Lake Ontario Water	National Water Research Institute, Ontario, Canada
Scalp hair and fingernails	GBW 07601 Human Hair	China National Analysis Centre for Iron and Steel, Beijing, China
	GBW 09101 Human Hair	China National Analysis Centre for Iron and Steel, Beijing, China
Tobacco	NIST SRM® 1573a Tomato leaves	National Institute of Standards and Technology, Maryland, USA
	NIST SRM® 1572a Citrus leaves	National Institute of Standards and Technology, Maryland, USA

In this study, precision levels were evaluated for any matrix effects by replicate analysis (n = 10) of a "pooled" sample that was prepared from at least 6 samples of water, tobacco, tear drops, saliva, scalp hair and fingernails. Mean, standard deviation (SD) and relative standard deviation (%RSD) values are summarized in Tables 2.21 & 2.22.

The precision of the preparation and analytical methods was also checked based on the triplicate analysis of the various CRMs analysed in this work, as shown in Tables 2.23 – 2.28. In general, good levels of precision were obtained for most elements with an acceptable range of 0.1 – 8.5% RSD, with exceptions being B in Tables 2.24 & 2.26 and As in Table 2.22 by ICP-MS and ICP-AES, respectively. The analysis of B suffers from a type of spectral interference. As boron is next to $^{12}C^{+}$, the presence of large quantities of carbon can cause a very large peak which can overlap on the $^{11}B^{+}$ peak area and even $^{10}B^{+}$ in extreme cases (Ward, 1993). The problem cannot be rectified through internal standardization as it does not affect the $^{9}Be^{+}$ peak. Another major problem concern with boron analysis is contamination; many collection devices contain traceable levels of boron (particularly glass), the acids and solvents used in sample preparation can contain as much as 20 μg/l and the basic components of many instruments, for example, the sample uptake tubing, spray chamber, ICP torch and ion optics of an ICP-MS instrument are prone to significantly enhance the boron levels as a result of memory problems (Hill, 2009).

In the case of arsenic, the ionisation interferences can influence the determination of As by ICP-AES. These interferences are caused by a large excess of easily ionised elements, such as the alkali or alkaline earth elements, in the sample matrix (Ebdon *et al.*, 1998). The effect of plant matrices on the determination of As have previously been investigated. It was found that the greatest changes in the arsenic emission intensity occurred in the presence of Ca and Mg matrices (Vassileva & Hoenig, 2001). The determination of arsenic by ICP-MS also has problems due to the polyatomic interference, $^{40}Ar^{35}Cl^{+}$ which overlaps with $^{75}As^{+}$ (Broekaert, 2005; Taylor, 2000). This was minimised by using collision/reaction cell technology (Section 2.6.6).

Measured CRM values obtained for the analysis of trace elements by ICP-MS and ICP-AES, were highly comparative to certified levels (Tables 2.23 to 2.28). Analytical recoveries ranged from 90 to 110% for all elements determined.

Table 2.21: Precision levels for selected trace elements in different pooled human samples (n = 10) determined by the Agilent 7700 Series ICP-MS; presented as mean, ± SD and %RSD values, µg/l and µg/kg for human fluids and tissues, respectively.

Element	Tear drops mean ± SD (%RSD)	Saliva mean ± SD (%RSD)	Scalp hair mean ± SD (%RSD)	Fingernails mean ± SD (%RSD)
B	506 ± 22 (4)	< 70	3382 ± 106 (3)	162 ± 19 (12)
V	2.7 ± 0.1 (3.7)	0.4 ± 0.02 (5)	2158 ± 17 (0.8)	350 ± 4 (1.1)
Cr	3.8 ± 0.1 (2.6)	< 0.1	1375 ± 9 (0.6)	747.4 ± 4.9 (0.7)
Mn	18.4 ± 0.9 (4.9)	1.3 ± 0.1 (7.7)	3656 ± 39 (1.1)	2440 ± 29 (1)
Fe	288 ± 14 (4.9)	8.3 ± 0.5 (6)	236363 ± 1567 (0.7)	2420 ± 28 (1.2)
Cu	209 ± 9 (4.3)	8.4 ± 0.3 (3.6)	15981 ± 181 (1.1)	3745 ± 48 (1.3)
Zn	773 ± 33 (4.3)	12.9 ± 0.5 (2.9)	6807883 ± 84572 (1.2)	171507 ± 2395 (1)
As	0.47 ± 0.02 (3.41)	0.76 ± 0.04 (5.2)	205 ± 2 (0.9)	201 ± 17 (8.5)
Sr	12535 ± 597 (4.8)	299 ± 11 (3.7)	194993 ± 230 (0.1)	8077 ± 101 (1.3)
Cd	0.29 ± 0.02 (6.9)	< 0.1	4638 ± 27 (0.6)	88 ± 3 (3.4)

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

Table 2.22: Precision levels for selected trace elements in different pooled environmental samples (n = 10), water and tobacco determined by the Agilent 7700 Series ICP-MS and Optima 3500 DV ICP-AES, respectively, presented as mean, ± SD and %RSD values, µg/l and µg/kg for water and tobacco, respectively.

Element	Water, mean ± SD (%RSD)	Tobacco, mean ± SD (%RSD)
B	1210 ± 11 (0.9)	nd
V	8.5 ± 0.2 (2.4)	15882 ± 62 (0.4)
Cr	7.5 ± 0.4 (5.3)	0.42 ± 0.02 (4.8)
Mn	32.8 ± 0.8 (2.2)	0.40 ± 0.03 (7.5)
Fe	32.1 ± 0.7 (2.3)	258 ± 3 (1.2)
Cu	18.1 ± 0.7 (3.9)	3.41 ± 0.04 (1.2)
Zn	212 ± 6 (2.8)	23.7 ± 0.2 (0.8)
As	44 ± 3 (6.8)	1.2 ± 0.4 (33)
Sr	5363 ± 103 (2)	69 ± 1 (1.4)
Cd	0.55 ± 0.01 (1.8)	0.89 ± 0.02 (2.2)

nd = not determined, SD is standard deviation, RSD is a relative standard deviation (quoted as a % in brackets).

Table 2.23: Accuracy and precision levels for tear drops and saliva CRM NIST SRM® 1643e, presented as mean ± SD, %RSD and %R for measured values and mean ± SD for certified values.

Element (n = 3)	Elemental level (µg/l)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean ± SD	Percentage recovery (%R)	%RSD
B	164.8 ± 3.6	157.9 ± 3.9	104	2.2
V	37.79 ± 1.8	37.86 ± 0.59	100	4.8
Cr	20.19 ± 0.41	20.40 ± 24	99	2.0
Mn	38.40 ± 2.73	38.97 ± 0.45	99	7.1
Fe	97.7 ± 5.5	98.1 ± 1.4	100	5.6
Cu	22.02 ± 0.76	22.76 ± 0.31	97	3.3
Zn	78.6 ± 2.8	78.5 ± 2.2	100	3.5
As	58.99 ± 2.07	60.45 ± 0.72	98	3.5
Sr	202.8 ± 7.5	223.1 ± 3.6	91	3.7
Cd	6.312 ± 0.465	6.568 ± 0.073	96	7.4

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets).

Table 2.24: Accuracy and precision levels for water CRM NIST SRM® TMDA 54.4, presented as mean ± SD, %RSD and %R for measured values and mean ± SD for certified values.

Element (n = 3)	Elemental level (µg/l)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean ± SD	Percentage recovery (%R)	%RSD
B	62.5 ± 11.7	60.6 ± 1.5	103	18.7
V	354 ± 3	340 ± 4	104	0.8
Cr	411 ± 2	438 ± 4	94	0.5
Mn	258 ± 5	275 ± 2	94	1.9
Fe	405 ± 12	382 ± 5	106	2.9
Cu	406 ± 1	443 ± 4	92	0.2
Zn	505 ± 44	537 ± 6	94	8.7
As	42.6 ± 2.3	43.6 ± 0.8	98	5.4
Sr	558 ± 24	589 ± 6	95	4.3
Cd	149 ± 13	158 ± 2	94	8.7

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets).

Table 2.25: Accuracy and precision levels for human scalp hair and fingernail CRM GBW 09101, presented as mean ± SD, %RSD and %R for measured values and mean for certified values.

Element (n = 3)	Elemental level (mg/kg)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean	Percentage recovery (%R)	%RSD
V	0.067 ± 0.01	0.069	97	7.5
Cr	4.429 ± 0.157	4.770	93	3.5
Mn	2.91 ± 0.06	2.94	99	2.1
Fe	65.9 ± 1.9	71.2	93	2.9
Cu	22.7 ± 0.5	23.0	99	2.2
Zn	193 ± 5	189	102	2.5
Sr	3.84 ± 0.03	4.19	92	0.8
Cd	0.089 ±0.01	0.095	94	0.9

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets).

Table 2.26: Accuracy and precision levels for human scalp hair and fingernail CRM GBW 07601, presented as mean ± SD, %RSD and R% for measured values and mean for certified values.

Element (n = 3)	Elemental level (mg/kg)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean	Percentage recovery (%R)	%RSD
B	1.2 ± 0.2	(1.3)	92	16
As	0.26 ± 0.01	0.28	93	1.1

Values in brackets are not certified, SD is standard deviation, and RSD is relative standard deviation (quoted as a % in brackets).

Table 2.27: Accuracy and precision levels for tobacco, NIST SRM® 1573a Tomato leaves, presented as mean ± SD, %RSD and R% for measured values and mean for certified values.

Element (n = 3)	Elemental level (mg/kg)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean	Percentage recovery (%R)	%RSD
B	nd	33.3	nd	nd
V	0.90 ± 0.03	0.835	108	3.3
Cr	1.84 ± 0.01	1.99	92	0.5
Mn	222.7 ± 0.7	246	91	0.3
Fe	332.7 ± 0.6	368	90	0.2
Cu	5.16 ± 0.03	4.7	110	0.6
Cd	1.49 ± 0.01	1.52	98	0.7

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets).

Table 2.28: Accuracy and precision levels for tobacco, NIST SRM® 1573a Citrus Leave, presented as mean ± SD, %RSD and R% for measured values and mean ± SD for certified values.

Element (n = 3)	Elemental level (mg/kg)			
	Accuracy			Precision
	Measured value mean ± SD	Certified value mean ± SD	Percentage recovery (%R)	%RSD
Zn	27.9 ± 0.3	29 ± 2	96	0.9
As	2.82 ± 0.05	3.1 ± 0.3	91	1.8
Sr	90 ± 1	100 ± 2	90	1.1

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets).

2.9 Significance Tests

The raw data obtained by atomic absorption spectrometry, inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission were entered into and processed using Microsoft Excel®. The concentration determined from the calibration curve were corrected where necessary for instrumental drift followed by any dilution factors applied, such as the initial sample mass and final digest mass. The final results for the sample location or population were then subjected to calculation of descriptive statistics such as arithmetic mean, standard deviation, relative standard deviation, median, geometric mean, 95% confidence interval and range as appropriate.

Suitable significance testing, namely Grubb's outliers, F-test, t-test, one-way analysis of variance (ANOVA), analysis of covariance (ANCOVA), Pearson's correlation analysis (*r*) and discriminant function analysis (DFA), were then undertaken. Regression analysis was also utilised to determine the linearity of the calibration curve for each trace element by the different techniques investigated in this research. Figure 2.22 summarises the statistical approach used in this work. A probability level of 5% was considered to be statistically significant. The calculations were performed using statistical packages Minitab® version 16, Excel® - QI Macros 2011, and IBM SPSS Statistics version 19 (SPSS Inc., Chicago, 2010). The following section describes the significance tests used in this work. The equations used to calculate these tests are reported in Appendix C.

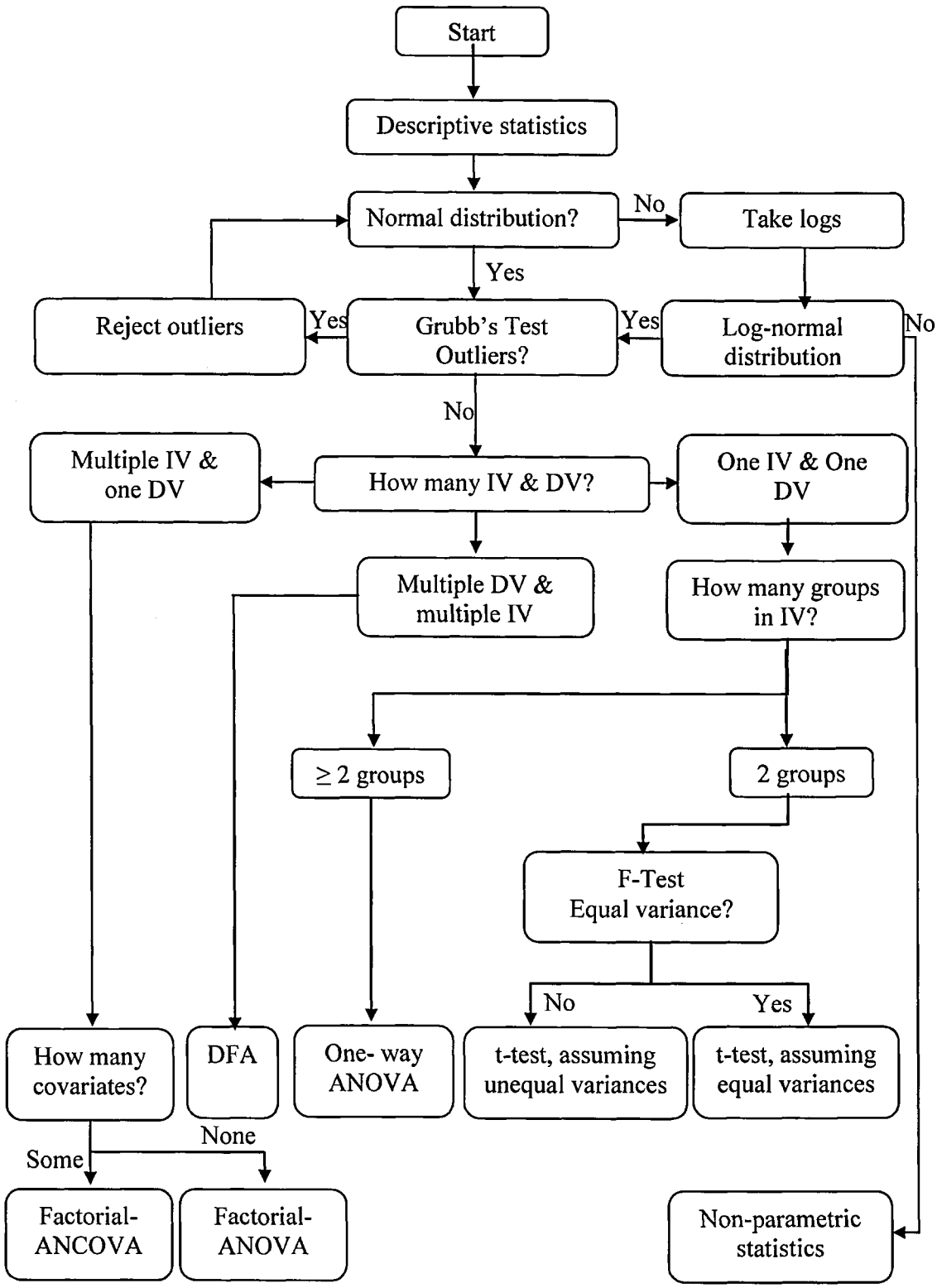

Figure 2.22: Statistical methodology flow chart used in this study, IV and DV are independent and dependent variables, ANOVA is "analysis of variance", ANCOVA is "analysis of covariance", DFA is "discriminant function analysis", and the DV is the category while the IV is trace element (Tabachnick & Fidell, 2007).

2.9.1 Grubb's Test

The Grubb's test was used to check whether any outliers were present in the data set. The tested data are the minimum and maximum values. If the calculated value G_{calc} exceeds the critical value G_{crit}, the suspect value is rejected, as reported in Chapter 4, Table 4.3 (Miller & Miller, 2010).

2.9.2 t-test

There are a number of different tests based on the t distribution used in this study, such as the paired t-test (paired-samples t-test), student's t-test, t-test for linear regression and two-tailed t-test (independent-samples t-test).

- Paired t-test was used to compare pairs of data such as the concentrations determined by two methods, for example two digestion methods. The calculated value (t_{calc}) is compared with the critical value (t_{crit}) for n-1 degrees of freedom at the 95% confidence interval, as reported in Table 2.9.
- Student's t-test was used to calculate the significance of a difference between a certified value (t_{calc}) and mean value measured for a reference material. The calculated value is compared with critical value (t_{crit}) for n-1 degrees of freedom at the 95% confidence interval, as reported in Table 2.19.
- Linear regression test based on the t distribution was used to determine the significance of a correlation from the product moment correlation coefficient (r) of n measurements. The calculated value (t_{calc}) is compared to the critical value (t_{crit}) for a t distribution with n-2 degrees of freedom at the 95% confidence interval, as reported in Chapter 4, Tables 4.17 & 4.18.
- Two tailed t-test was used to compare the mean values of two different groups of population such as healthy and diabetic; smokers and non-smokers; etc. There are two values of t-test provided; one is for equal variance and the other for unequal variance. The correct t-test value depends on the result of an F-test. If a $P > 0.05$ for the F-test, the result which refers to equal variances assumed was used, whereas, the result for unequal variances was used when the variances for the two groups are not

the same ($P < 0.05$), as reported in Chapter 4, Tables 4.4 & 4.5. If the value of Sig. (2-tailed t-test) is less than 0.05, then there is a significant difference in the mean values of trace elements between the two groups. Conversely, when the *P*-value of the t-test is > 0.05, there is no significant difference between the two groups (Miller & Miller, 2010; Field, 2009; Tabachnick & Fidell, 2007).

2.9.3 One-way Analysis of Variance (ANOVA)

A one-way ANOVA was used to compare the group categories of a specific variable (e.g. biological samples), for the mean values of continuous variables (e.g. trace element levels). The main purpose of using one-way ANOVA is to examine whether there are significant differences in the mean values of trace elements across groups. In general, an *F*-value test is calculated which represents the variance between the groups divided by the variance within the group at the level of significance ($P < 0.05$) (Hair *et al.*, 2010). The values of Sig. and *F* are used to evaluate whether the differences between the levels of trace elements over study groups are significant. If the Sig. value is less than 0.05, then there is a significant difference among the mean values of the trace elements across the groups (Field, 2009).

2.9.4 Analysis of Covariance (ANCOVA)

Analysis of covariance is an extension of analysis of variance, which was conducted to explore the effect of different factors on the levels of trace elements in tear drop samples (Tabachnick & Fidell, 2001). It involved three independent variables (health status, gender and smoking activity), one dependent variable (trace elements in tear drops) and two covariates (the level of trace elements in drinking water and an individual's age). The main advantage of using ANCOVA is to determine the differences between groups whilst statistically controlling additional variables. These additional variables (called the covariates) are the variables that are expected to influence the trace element levels in tear drops (Field, 2009). By removing the influence of these additional variables, ANCOVA can reduce the error and increase the power of the F-value. The question in

ANCOVA as ANOVA is whether mean differences in the dependent variable between groups are significant at $P < 0.05$ with respect to the interaction among factors (i.e. is one factor associated with the effectiveness of two groups for other factors and vice versa) (Sthiannopkao *et al.*, 2009; Tabachnick & Fidell, 2001). For the purpose of this analysis, health status, smoking activity and gender are represented by numeric expressions, for example, 1 for male and 2 for female and so on, as shown in Chapter 4, Table 4.9.

The ANCOVA summary table is useful to know whether there is a significant effect and interactions for each factor (health status, smoking and gender). In addition, the results in the ANCOVA table can be used to evaluate whether there is a significant relationship between the covariates and the trace element levels. The effects and interactions for each factor along with covariate effect are listed under the "Source" column, as shown in Appendix E. The values under the "Sig." column are important to determine whether there is a significant effect for each factor, covariate and interaction. If the value of "Sig" for each factor is less than the level of significant ($P < 0.05$), then there is a significant effect for this factor. The *P*-value could thus determine the most highly associated factors with the level of trace elements in tear drops, as shown in Chapter 4, Table 4.11.

The ANCOVA table also provides the value of Partial *eta*-squared (η^2), which can be used to determine the strength of significant effect for each factor on the level of trace element (i.e. strength of relationship), as shown in Chapter 4, Table 4.16. The partial *eta* squared statistic reports the "practical" significance for each factor, based upon the ratio of the variation (sum of squares) accounted for by the factor, to the sum of the variation accounted for by the factor and the variation left to error (Field, 2009), as shown in the following equation:

$$Partial\ \eta 2 = \frac{SS_{effect}}{SS_{effect} + SS_{error}} \quad \text{---------- Equation 2.14}$$

where SS_{effect} and SS_{error} are the variance attributable to the effect of interest and the variance of error, respectively (Field, 2009; Tabachnick & Fidell, 2007). The value of partial η^2 can range from 0 to 1. In order to interpret the strength of η^2 values, the following guidelines can be used (Cohen, 1988), as shown in Table 2.29.

Table 2.29: The range values for partial *eta* squared.	
η^2	Effect
0.01	Small
0.09	Medium
0.25	Large
Source: Cohen's, 1988.	

2.9.5 Correlation Analysis

Correlation analysis was used to describe the strength and direction of the linear relationship between trace element levels in tear drops and other biological samples. The significance t-test distribution was used to check the level of significance for these relationships at the 95% confidence level, as shown in Chapter 4, Tables 4.17 & 4.18, and Chapter 5, Tables 5.2, 5.3, 5.5, 5.6, 5.8 & 5.9. Cohen (1988) suggested guidelines for this purpose, as shown in Table 2.30. Further information about the equations and the degrees of freedom for each study tests can be found in Appendix C (Miller & Miller 2010).

Table 2.30: Correlation coefficient guidelines.	
Correlation coefficient value (*r*)	Strength of correlation
± 0.0 to ± 0.29	Small
± 0.3 to ± 0.49	Medium
± 0.5 to ± 1.0	Large
Source: Pallant (2005); Cohen (1988).	

2.9.6 Multivariate Discriminant Function Analysis (DFA)

Multivariate data analysis has been used widely by other authors (Pino *et al.*, 2005; Shah *et al.*, 2006). The use of multivariate methods such as principal component analysis (PCA) and discriminant function analysis has drastically increased in recent years for analysing environmental and biological data (Saadia, *et al.*, 2005; Charpentier *et al.*, 2000).

The main purpose of using DFA is description of group separation in which linear functions of the several variables (discriminant functions (DFs)) are used to describe or clarify the differences between two or more groups and identifying the relative contribution of all variables to separation of the groups. In addition, it is the prediction of observations to group in which linear functions of the variable

(classification functions (CFs)) are used to assign an observation to one of the groups (Johnson & Wichern, 2002). In DFA, the independent variables are the predictors (trace elements) and the dependent variables are the groups (e.g. healthy and diabetic), as outlined in Chapter 4, Section 4.5.3. Generally, several variables (such as trace elements) are included in a study to see which ones contribute to the discrimination between groups (e.g. healthy and diabetic). The method extracts n-1 discriminant functions, n being the number of groups to discriminate among, which are linear combinations of the original quantitative variables selected. The model parameters are Wilks' Lambda, an index of the discriminating power ranging between 0 and 1 (the lower the value the higher its discriminating power); eigenvalues, a measure of the variance in the dependent variable for each function; canonical correlations, a measure of the association between the groups formed by the dependent variable and the given discriminant function (the larger this value, the higher is the correlation between the discriminant functions and the groups). The first discriminant function (DF1) maximizes the differences between the values of the dependent variables. The second function (DF2), orthogonal to the first, maximizes the residual differences between values of this variable, and so on. The DF1 will be the most powerful differentiating dimension, but later functions may also represent additional significant dimensions of differentiation, as shown in Chapter 4, Table 4.7. Since the different size of the groups under study, the predictions were accordingly adjusted using a priori probabilities classification. The predictive validity of the model has been assessed by using cross validation method (Chojnacka *et al.*, 2010; Field, 2009; Tabachnick & Fidell, 2007).

2.10 Summary

The analytical methodology and instrumentation for the determination of trace elements in different biological and environmental samples has been described in this chapter. Sample collection, storage, methods of transfer and preparation procedures including different washing and digestion strategies are reported in Sections 2.1 – 2.4 in order to prepare for the analysis of water, tobacco, tear drops, saliva, scalp hair and fingernail samples by various spectrometric techniques. Two digestion methods were utilised in this study in order to develop a useful method

for cigarette tobacco analysis. A statistical test (paired t-test) was used to evaluate whether there is any significant difference between the dry ashing and Kjeldahl™ tube digestion methods (Table 2.9). The relative standard deviation %RSD and recovery test %R for trace elements confirmed that the wet digestion method (Kjeldahl™ tube) is the preferred digestion procedure for tobacco with acceptable analytical recoveries ranging from 90 to 110 %, as shown in Tables 2.27 & 2.28. Good levels of precision were obtained with acceptable RSD values from 0 to 7.5% (Table 2.22) for most elements with the exception being As (Section 2.8.3).

A new method for the sample collection and subsequent analysis of trace and ultra-trace elements in human tear drops was developed. Several washing and digestion procedures were developed for determining trace element levels in human scalp hair and fingernails (Section 2.2.3). The sequential washing procedure (acetone-water-water-water-acetone) was adopted in this study (Table 2.11). The wet digestion method using a Kjeldahl™ tube provided the best data in this work, and as a result was employed for the complete digestion of washed human scalp hair and fingernails (Table 2.12).

The principles, instrumentation, operating conditions, advantages and limitations for each technique are discussed in Sections 2.5 – 2.7. Schematics for the Agilent 7700 Series ICP-MS, Perkin Elmer Optima 5300 DV ICP-AES and Perkin Elmer AAnalyst™ 400 FAAS are presented with respective calibration curves, operating parameters and the calculated limit of detection. A long-term stability chart is described in Section 2.6.13 for a 100 μg/l solution of the selected internal standards in order to check for any instrumental drift during the total trace element analysis by ICP-MS. Quality control charts are reported in Section 2.8.2 for the identification of instrument drift throughout the whole analysis run for each material by various techniques. Precision and accuracy levels are presented in Section 2.8.3 through the determination of the standard deviation (SD), relative standard deviation (RSD) and percentage recoveries (%R). The data for these studies are shown in Tables 2.21 – 2.28. Several significance tests used in this study are reported in Section 2.9.

The developed methods and described techniques outlined in this chapter are now used for the elemental analysis of environmental samples (water and tobacco) and biological samples (tear drops, saliva, washed scalp hair and fingernails) and the results are reported in Chapters 3, 4 and 5.

Chapter Three

Environmental Analysis - Trace Element Levels in Water and Cigarette Tobacco

3.0 Introduction

Data for the trace element analysis of different water resources and commercial cigarettes tobacco are reported in this chapter. Trace element (B, V, Cr, Mn, Fe, Cu, Zn, As, Sr and Cd) levels in commercial, domestic bottled, tap, river, well and artesian waters are presented and discussed in Sections 3.1.1 – 3.1.4. All of the results are compared with the guideline values for drinking and irrigation waters as recommended by the World Health Organisation (WHO); and Iraqi specifications and the Food and Agriculture Organisation (FAO). Furthermore, the results are also compared with published literature values, particularly from other regions within Iraq and other locations near Iraq, such as Turkey and Middle East countries. Trace element levels were also determined in various commercial cigarette tobacco samples (since a significant proportion of the population in Iraq are active smokers) and the results are compared with those reported from other regions of the world.

3.1 Water Analysis

3.1.1 Water Location and Sampling

In total, 190 water samples were collected from Karbala (Iraq) and London (UK), as described in Sections 2.1.1. Tap waters were collected from Karbala and London, whilst commercial, domestic bottled, surface (river) and ground water (well and artesian) were collected from Karbala only. In general, the population resident in Karbala use tap and bottled waters for drinking and domestic activities (washing, cooking and cattle), whilst surface and ground waters are used for irrigation, livestock, and in some cases use for drinking purposes. Water samples were obtained from Karbala in order to assess whether this media makes any significant contribution to the levels of trace elements for the human tissues and fluids under investigation, whereas London water samples were used for comparative purposes with Karbala samples.

3.1.2 Instrumentation

An Agilent 7700 Series ICP-MS instrument equipped with collision/reaction cell technology was used for multi-element analysis of water samples. The optimisation and operation conditions of the ICP-MS instrument are reported in Section 2.6.11. The instrument was calibrated by using multi-elemental standard solutions, as shown in Section 2.6.12. The stability of the ICP-MS instrument throughout the water analysis run was checked by a long-term stability chart, as explained in Sections 2.6.13. The levels of precision and accuracy for the ICP-MS instrument were confirmed by calculation of the relative standard deviation (%RSD) and percentage recoveries (%R) using ten replicate measurements of a "pooled" water sample, and certified reference materials (CRMs), as shown in Section 2.8.3.

3.1.3 Results

The results for water analysis are divided into two parts, chemo-physical properties and multi-trace elemental analysis.

3.1.3.1 Chemo-Physical Properties

The pH values for water samples from commercial, domestic bottled, tap, river, well and artesian (spring) sources are reported as mean ± SD (range). The maximum pH values were found for commercial, tap (Karbala) and river waters (Table 3.1). Conductivity levels (µS/cm) ranged from 223 ± 5 (218 – 228) for commercial to (2505 - > 3999) for the well waters. The total dissolved solid content ranged from 112 ± 2 (111 – 114 mg/l) for commercial to (1254 - > 2000 mg/l) for well waters. The results show that the highest values of TDS (> 2000 mg/l) and EC (> 3999 µS/cm) were found in ground waters. A large difference for TDS and EC ranges were observed between the tap water and both of the commercial and London waters (Table 3.1).

3.1.3.2 Trace Elements

Trace element levels for drinking (commercial, domestic bottled and tap), irrigation and livestock (river, artesian and well) waters are shown in Tables 3.2 & 3.3 as mean, standard deviation (± SD) and range values along with the WHO, Iraqi and FAO guidelines for drinking, irrigation and livestock waters. In drinking water, the highest level for most trace elements was found in tap water, with the only exception being Zn (105 µg/l) which was higher in domestic bottled waters (Table 3.2). Commercial waters used primarily for drinking in Karbala, exhibited the lowest levels for all the trace elements in terms of the calculated mean and range values.

The trace element levels in surface (river) and ground waters (artesian and well) are presented in Table 3.3, as a mean, ± SD and range (µg/l). The highest level of trace elements in irrigation waters was found in well waters when compared with river and artesian waters.

The results in this study were also compared with another study carried out in Baghdad, Iraq for drinking (tap) and irrigation (river) water samples, as shown in Table 3.4. Furthermore, the mean, standard deviation and range values for trace elements in tap water from Karbala are compared with those reported for tap water samples from London (Table 3.4).

3.1.4 Discussion

In Karbala town, responsibility for the production and delivery of drinking water is by order of the municipality's office. During the last three decades, the quality of drinking water in Iraq has deteriorated due to the wars that took place at that time. Various industrial and man-made activities have dramatically decreased the quality of water in Iraq. Water treatment adds different new chemical compounds, especially during chlorination processes that can enhance the levels of contamination for water, such as trihalomethanes (Ward, 1989).

Table 3.1: Mean, standard deviation and range for pH, total dissolved solid (TDS) and conductivity (EC) values for commercial, domestic bottled, tap, river, well and artesian waters from Karbala and tap water from London relative to the WHO guideline values for drinking water quality.

Water resource	n	pH	EC (μS/cm)	TDS (mg/l)
Commercial	3			
Mean ± SD range		8.3 ± 0.2 8.1 - 8.5	223 ± 5 218 – 228	112 ± 2 111 - 114
Domestic bottled	33			
Mean ± SD range		7.9 ± 0.3 7.4 - 8.4	998 ± 472 216 – 1553	510 ± 238 108 – 778
Tap	50			
Mean ± SD range		8.0 ± 0.2 7.7 - 8.4	1134 ± 184 275 – 1294	566 ± 92 137 – 647
River	33			
Mean ± SD range		8.0 ± 0.2 7.8 - 8.5	1343 ± 40 1275 – 1442	675 ± 20 644 – 723
Well	47			
Mean ± SD range		7.5 ± 0.5 4.9 - 8.5	2505 - > 3999	1254 - > 2000
Artesian (spring)	8			
Mean ± SD range		7.7 ± 0.2 7.5 - 7.9	1172 – > 3999	583 – > 2000
London, Tap	16			
Mean ± SD range		7.6 ± 0.6 6.1 - 8.3	454 ± 136 189 – 582	227 ± 68 94 – 291
WHO, Guideline for drinking water		6.5 – 9.0	250	1000

n is the number of samples, SD is standard deviation.
Source: WHO, 2008.

3.1.4.1 Chemo-Physical Properties

The water quality constituents of Karbala and London water samples are reported in Table 3.1, along with the WHO guideline for drinking water. The pH values for all water samples are predominantly neutral to slightly alkaline, which are within the WHO guideline, as shown in Table 3.1 (WHO, 2008).

The conductivity and TDS values in various drinking and irrigation water samples from Karbala fluctuate due to the high levels of dissolved salts, such as chlorides and sulphates, which were observed during sampling (Barbooti *et al.*, 2010). A significant correlation ($R^2 = 0.9999$, $P < 0.05$) was observed between the TDS and

conductivity levels in different water resources, as presented in Figure 3.1. Similar observations have been reported in the literature in terms of the positive correlation between the levels of EC and TDS in water samples that contain higher levels of dissolved ions (Atekwana *et al.*, 2004). A previous study has provided a detailed analysis and discussion on the relationship of conductivity versus TDS data that ranged from 500 to 3000 mg/L TDS ($R^2 = 0.59$) (Howard & Statham, 1993). The highest levels of TDS and EC were reported in ground and surface waters when compared with tap and bottled waters (Table 3.1). Conductivity (μS/cm) levels for domestic bottled (216 – 1553) and tap (275 – 1294) waters from Karbala are higher than for commercial (218 – 228) and tap waters from London (189 – 582). These results indicate that Karbala waters are characterised by relatively high conductivity levels, which are not in agreement with the WHO and European recommended values for EC (250 μS/cm) for drinking water. The main reason may be related to the high temperature in the summer season, typically ~ 51°C, which increases the evaporation of water, and hence the higher levels of dissolved solids (Arain *et al.*, 2009; Yogendra & Puttaiah, 2008). In Iraq, high levels of hypochlorite are used in water treatment in order to destroy any organic matter. The high levels of chloride may lead to an increase in the conductivity of a water body (Barbooti *et al.*, 2010).

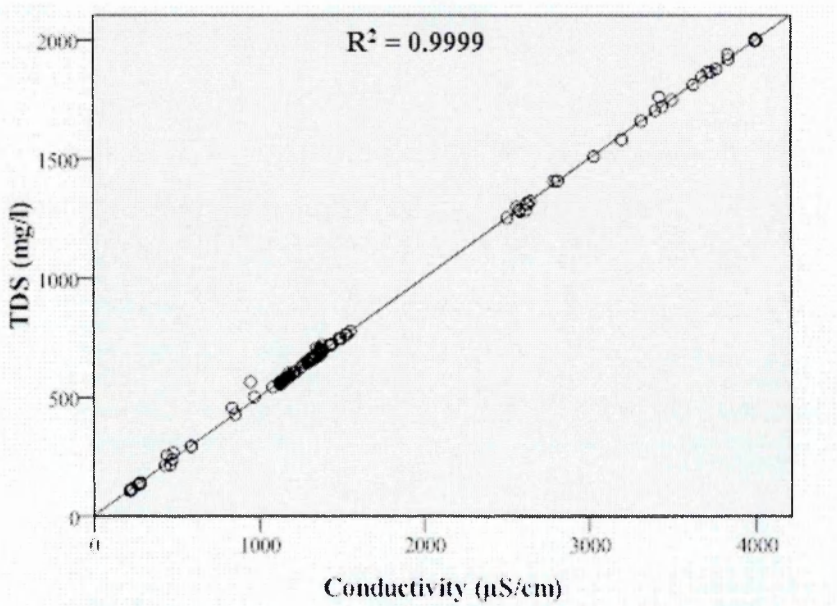

Figure 3.1: Correlation between the TDS and EC level of the waters for Karbala (n = 174).

3.1.4.2 Trace Elements

The mean and range values for most of the trace elements are lower than the permissible limits for drinking water recommended by the WHO and Iraqi guidelines. The only exceptions are for B in artesian (1049 ± 746 µg/l) and well waters (1569 ± 844 µg/l); Cd in river (8.71 ± 3.65 µg/l), artesian (5.28 ± 4.86 µg/l) and well waters (9.98 ± 0.31 µg/l). The highest trace element level was found in well water for Sr (7096 ± 2923 µg/l), whilst the lowest level was for Cd (< 0.01 µg/l) in commercial water from Karbala (Tables 3.2 & 3.3).

Table 3.2: Elemental levels in commercial (n = 3), domestic bottled (n = 33) and tap (n = 50) waters from Karbala relative to the WHO guideline for drinking water quality.

Element	Elemental level (µg/l)				
	Commercial[+]	Domestic bottled[++]	Tap	WHO, Guideline for drinking water	Iraqi specification
	Mean ± SD range	Mean ± SD range	Mean ± SD range		
B	160 ± 99 63 - 260	258 ± 70 75 - 350	354 ± 107 237 - 588	500	nf
V	0.3 ± 0.2 0.1 – 0.5	2.3 ± 1.6 0.1 - 4.1	4.0 ± 1.7 0.4 - 7.4	15	nf
Cr	0.07 ± 0.04 0.03 – 0.11	0.48 ± 0.80 0.06 – 4.85	0.46 ± 0.12 0.32 - 0.88	50	50
Mn	0.12 ± 0.07 0.06 – 0.19	1.2 ± 0.9 0.3 - 3.9	4.3 ± 8.0 0.1 – 42.0	400	100
Fe	0.8 ± 0.2 0.7 – 0.9	9.6 ± 7.2 0.8 – 35.6	9.4 ± 1.5 6.5 – 12.7	nf	300
Cu	0.5 ± 0.3 0.3 – 0.9	5.8 ± 5.7 0.4 – 30.4	5.4 ± 3.5 1.3 – 18.5	2000	1000
Zn	2 ± 1 1 – 2	105 ± 146 2 – 715	52 ± 58 3 – 197	3000	3000
As	0.12 ± 0.09 0.03 – 0.20	0.88 ± 0.51 0.05 - 1.57	1.56 ± 0.59 0.20 - 2.74	10	10
Sr	70 ± 49 22 – 120	817 ± 588 15 - 1535	1113 ± 425 78 - 2110	nf	nf
Cd	< 0.01 – 0.01	0.74 ± 0.41 0.02 – 1.17	0.90 ± 0.44 0.09 - 2.05	3	3

n (in brackets) is the number of samples, SD = standard deviation, [+] commercial samples are a potable water usually imported from abroad with high quality, [++] domestic bottled samples are made in Karbala for drinking and domestic uses, usually cheaper and with variable quality, nf = not found.
Source: Barbooti *et al.*, 2010; WHO, 2008.

In the case of Sr, there is no guideline value reported by the WHO or Iraqi standards that can be used to check whether the level of this element in drinking water is acceptable; however the Sr levels were compared with the literature values. Furthermore, the level of trace elements in the irrigation waters was below the FAO guideline for irrigation and livestock waters (Table 3.3). Comparison between Karbala and London tap water showed the levels for all elements are higher in Karbala samples, as presented in Table 3.4.

Table 3.3: Elemental levels in the river (n = 33), well (n = 47) and artesian (n = 8) waters from Karbala relative to the Food and Agriculture Organisation (FAO) guideline water quality for irrigation and the watering of livestock.

Element	Elemental level (µg/l)				
	Surface water	Ground water		FAO Guideline	
	River	Well	Artesian (spring)	Irrigation water	Watering of livestock
	Mean ± SD range	Mean ± SD range	Mean ± SD range		
B	445 ± 97 246 - 779	1569 ± 844 705 - 3941	1049 ± 746 411 - 2277	nf	5000
V	4.4 ± 1.5 3.1 - 8.2	6.5 ± 4.9 0.4 - 17.8	1.2 ± 0.7 0.3 - 2.4	nf	100
Cr	2.9 ± 1.3 0.3 - 7.1	16.8 ± 12.9 2.8 - 42.9	2.1 ± 1.2 0.9 - 3.5	100	1000
Mn	3.9 ± 2.5 1.5 – 12.8	17.6 ± 36.2 1.6 – 134.8	1.9 ± 0.9 1.1 - 3.6	200	50
Fe	84 ± 33 7 – 116	98 ± 8 92 – 132	65 ± 34 33 – 99	nf	nf
Cu	30.8 ± 14.6 1.1 – 77.0	34.7 ± 2.0 32.3 – 41.4	18.4 ± 16.8 1.9 – 37.3	200	1000
Zn	123 ± 48 96 – 377	131 ± 31 105 - 253	82 ± 56 14 - 140	2000	24000
As	2.6 ± 0.9 1.4 – 6.6	2.6 ± 2.1 1.3 - 13.1	1.5 ± 0.8 0.7 - 2.5	100	200
Sr	1321 ± 409 335 - 2755	7096 ± 2823 1512 - 14375	3448 ± 2998 1157 - 8308	nf	nf
Cd	8.71 ± 3.65 1.02 - 13.55	9.98 ± 0.31 9.67 - 11.41	5.28 ± 4.86 0.68 - 10.00	nf	50

n (in brackets) is the number of samples, SD = standard deviation' FAO – Food and Agriculture Organisation, nf = not found.
Source: FAO, 1994.

Table 3.4: Elemental levels (μg/l) reported in this study and another study in Baghdad, Iraq for drinking and irrigation waters.

Element	*Elemental levels (μg/l)				
	Drinking water (tap)			Irrigation water (river)	
	This study		Baghdad+ (n = 21)	This study (n = 33)	Baghdad+ (n = 6)
	London (n = 16)	Karbala (n = 50)			
B	45 ± 22 5 - 84	354 ± 107 237 - 588	< 100 – 230	445 ± 97 246 - 779	< 100
V	0.44 ± 0.32 0.04 – 0.95	4.0 ± 1.7 0.4 - 7.4	nd	4.4 ± 1.5 3.1 - 8.2	nd
Cr	0.11 ± 0.06 0.04 – 0.27	0.46 ± 0.12 0.32 - 0.88	< 5	2.9 ± 1.3 0.3 - 7.1	< 5
Mn	1.07 ± 2.96 0.04 – 12.16	4.3 ± 8.0 0.1 – 42.0	< 1 - < 10	3.9 ± 2.5 1.5 – 12.8	< 1 - 10
Fe	0.8 ± 0.1 0.7 – 1.0	9.4 ± 1.5 6.5 – 12.7	< 20 - 76	84 ± 33 7 – 116	< 20 - 624
Cu	4.1 ± 4.6 0.6 – 19.2	5.4 ± 3.5 1.3 – 18.5	< 5	30.8 ± 14.6 1.1 – 77.0	< 5
Zn	8.9 ± 14.2 0.7 – 45.8	52 ± 58 3 – 197	< 20 - 963	123 ± 48 96 – 377	< 20 - 40
As	0.70 ± 0.47 0.02 – 1.26	1.56 ± 0.59 0.20 - 2.74	< 10	2.6 ± 0.9 1.4 – 6.6	< 10
Sr	168 ± 94 6 – 357	1113 ± 425 78 - 2110	nd	1321 ± 409 335 - 2755	nd
Cd	0.03 ± 0.01 0.01 – 0.07	0.90 ± 0.44 0.09 - 2.05	< 1	8.71 ± 3.65 1.02 - 13.55	< 1

* Karbala data was taken from Tables 3.2 & 3.3, +this study was carried out in Baghdad (Iraq) by other researchers (Barbooti *et al.*, 2010).

Boron

Boron levels increase through the following sequence (well > artesian > river > tap > bottled > commercial > London), ranged from 705 - 3941, 411 - 2277, 246 - 779, 237 - 588, 75 – 350, 63 – 260 to 5 – 84 μg/l, respectively, as shown in Figure 3.2. These levels are higher than a typical mean value (10 μg/l) for fresh and river waters which have been reported in Table 1.5. The B levels in commercial (160 ± 99 μg/l) and bottled waters (258 ± 70 μg/l) are lower than the levels in bottled mineral water (360 μg/l) (Coughline, 1998) and higher than a typical mean value for fresh water (10 μg/l) (Ward, 2000); in tap water (354 ± 107 μg/l) are lower than the WHO guideline (500 μ/l B) for drinking water (WHO, 2008) and higher

than a typical mean value for fresh water (10 µg/l); and in river (445 ± 97 µg/l), artesian (1049 ± 746 µg/l) and well (1569 ± 844 µg/l) are higher than the WHO guideline (500 µ/l B) for drinking water and typical values for river waters (10 µg/l) (Table 1.5).

The highest elemental levels in irrigation waters were found to be at a level which could possibly cause toxicity symptoms and damage to plants (Hill, 2009). As such, irrigation waters play an important role in increasing the levels of B in soils (Nable, *et al.,* 1997). The high levels of B found in ground water were related to soil levels, where the B was added to the soil by irrigation waters and fertilisers (Nable, *et al.,* 1997).

Boron compounds are used in several industries as boric acid, such as glass, porcelain manufacture, carpets, photographic chemicals, and fertilisers. Moreover, the levels of B are also dependent on the geology conditions and waste water discharges that are released into the environment from detergents (production and end use). This process leads to increase in the levels of B in the waste effluent, and then in ground water (Vengosh *et al.,* 1994).

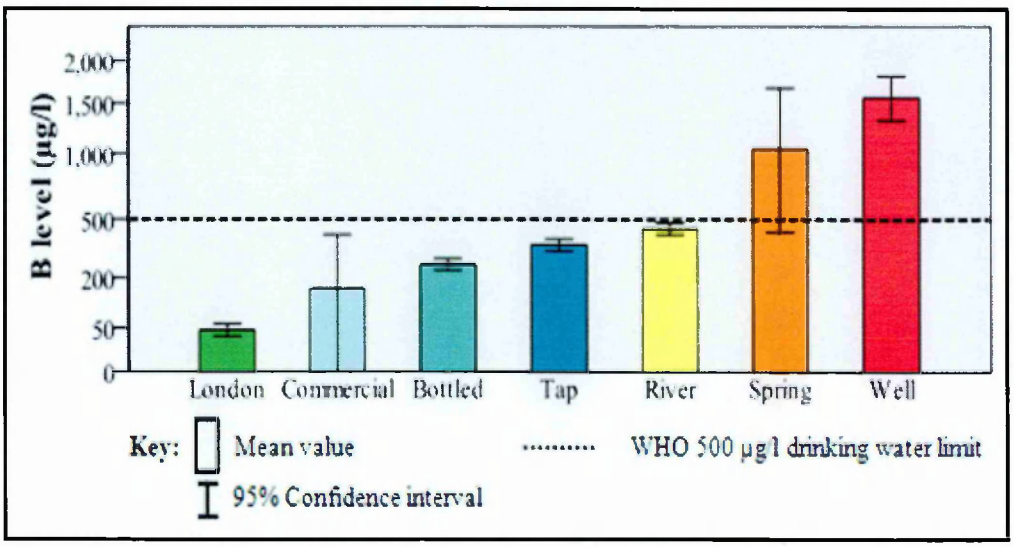

Figure 3.2: Level of boron (µg/l) reported in different water samples (see Appendix D for sample codes).

In Iraq, B compounds are typically used in the production of detergents and glass, and usually the waste water for these industries is released into the rivers (Barbooti *et al.*, 2010). Previous studies in Iraq have reported higher levels of B

(10 mg/l) in Karbala for ground water (Al-Dbbas, 2006), and lower levels in Baghdad (< 0.1 – 0.23 mg/l) for tap and (< 0.1 mg/l) for river water (Barbooti *et al.*, 2010). Boron levels reported in this study were also compared with the literature values for those reported in other countries. In general, B concentrations vary widely and depend on the surrounding geology and waste water discharges. For most of the world, the concentration range of boron in drinking water is judged to be between 100 and 300 µg/l (Hill, 2006). However, B ranged in tap (237 – 588 µg/l) and well water (705 – 3941 µg/l) in this study and are therefore higher than those reported in the UK (4.2 – 62.3 µg/l) and (1.5 – 55.8 µg/l), respectively (Ward, 1989). Ground water samples were collected from 47 wells distributed in the desert of Karbala, which are arid soils. A previous study has found that B can be very high in arid or semi-arid areas where leaching is limited (Gupta *et al.*, 1985). These regions are often characterised by high levels of salinity and, therefore, higher levels of B (Gupta *et al.*, 1985). Figure 3.3 shows the positive correlation between B and the TDS levels in Karbala waters (R^2 = 0.687, $P < 0.05$). Boron was reported in the literature to be a significant factor that can affect the metabolism of Ca and Mg (Usuda *et al.*, 2007). In the light of these results, water from Karbala may require chemical treatment at the municipal water plant in order to reduce B levels, and thereby improve the quality of drinking water.

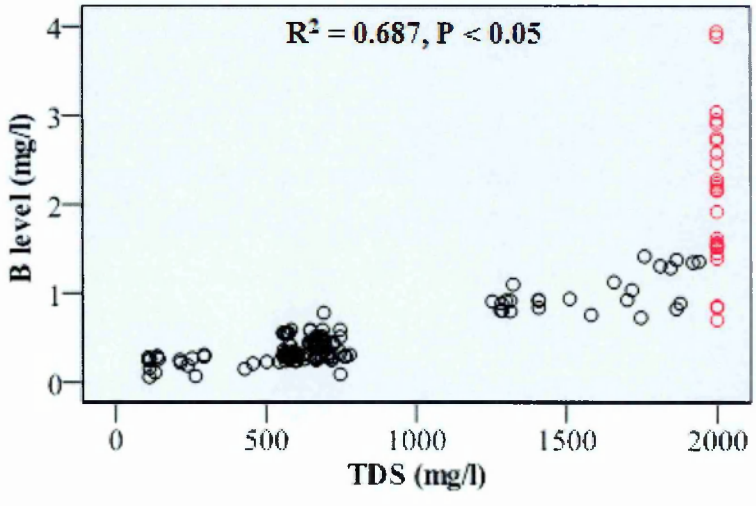

Figure 3.3: Correlation between boron and the TDS levels for water samples from Karbala (n = 174), the circles in red colour represent the values > 2000 as the Hanna HI 98129 Digital Combo Meter is limited by the detectable ranges (0 – 2000 mg/l TDS) (Hanna, 2008).

Vanadium

Vanadium levels for water samples increase through the following sequence (well > river > tap > bottled > artesian > London > commercial, as shown in Figure 3.4. The results in Tables 3.2 & 3.3 show that the mean values for water samples are below the guideline value for drinking water recommended by the WHO (15 µg/l V). There is no guideline value reported by the FAO for V in irrigation water (Table 3.3). However, V levels in irrigation water were lower than the guideline for livestock water (100 µg/l V). The highest levels of V in this study were found in ground water (well) (6.5 ± 4.9 µg/l), whilst the lowest levels were in commercial waters (0.3 ± 0.2 µg/l). In general, the levels (mean ± standard deviation µg/l) measured in commercial waters (0.3 ± 0.2) are lower, and in bottled (2.3 ± 1.6), tap (4.0 ± 1.7), river (4.4 ± 1.5), artesian (1.2 ± 0.7) and well (6.5 ± 4.9) are higher than the levels typically found in fresh (0.5 µg/l) and river (1 µg/l) waters (Ward, 2000).

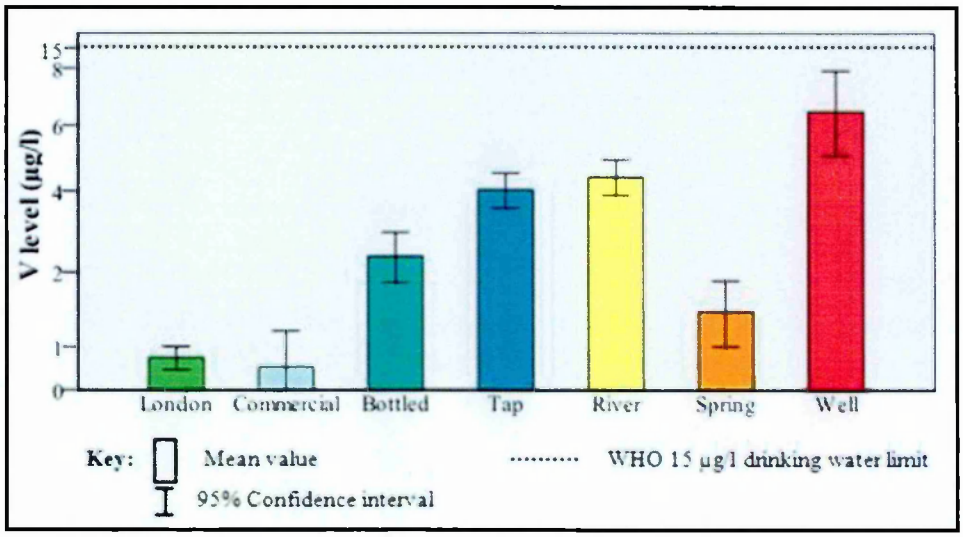

Figure 3.4: Level of vanadium (µg/l) reported in different water samples (see Appendix D for sample codes).

In comparison with the literature, the levels of V are within the ranges reported by other authors for drinking water (0.093 – 235 µg/l) (Reimann *et al.*, 2003), and lower than others (10 – 200 µg/l) (Ikem *et al.*, 2003). Several well waters in Karbala have levels of V (17.8 µg/l) exceeding the guideline value reported by the WHO for drinking water (Table 3.3). The main source of V in Iraq may be from

oil. Therefore, elements can seep from oil into the aqueous environment during the weathering, oil spill, oil combustion and the emissions from power plants (Baird & Cann, 2005). Interestingly, a previous study in Japan has suggested that possible beneficial health effects can arise from drinking water with a high level of V. In one study, drinking such water could lower blood glucose levels in diabetic patients, and improve the insulin-resistant status of healthy women (Seko *et al.*, 2006).

Chromium

Chromium is found at high levels in well waters (2.8 - 42.9 µg/l), and lowest levels in commercial waters (0.03 – 0.11 µg/l), as shown in Figure 3.5. In general, the levels of Cr in drinking and irrigation water samples are lower than the WHO guideline and Iraqi specification for drinking water (50 µg/l Cr) (Table 3.2), and FAO for irrigation and livestock (100 and 1000 µg/l Cr) (Table 3.3), respectively. The Cr levels for drinking water, reported as mean ± standard deviation (commercial, 0.07 ± 0.04 µg/l) (bottled, 0.48 ± 0.80 µg/l) and (tap, 0.46 ± 0.12 µg/l) are lower than the typical values in fresh and river water (1 µg/l Cr), respectively (Ward, 2000).

The results of Cr levels in the irrigation (river, 2.9 ± 1.3 µg/l) and drinking (tap, 0.46 ± 0.12 µg/l) waters in this study are in agreement with those reported in other places in Iraq (river and tap < 5 µg/l Cr) (Barbooti *et al.*, 2010). The levels of Cr in the water samples reported in this study are within the ranges published in the literature. Chromium was reported in the literature as ranging over (< 0.01 – 21.3 µg/l Cr) for ground water (Reimann *et al.*, 2003), (0.8 – 1.48 µg/l Cr) (Nkono & Asubiojo, 1998) and (0.4 – 1.50 µg/l Cr) (Ward, 1989) for tap waters. Figure 3.6 shows the relationship between Cr and the TDS levels in Karbala waters (R^2 = 0.564, $P < 0.05$). In the light of these results, Cr levels in Karbala waters are considered to be acceptable in terms of quality as they were below the acceptable value by the WHO.

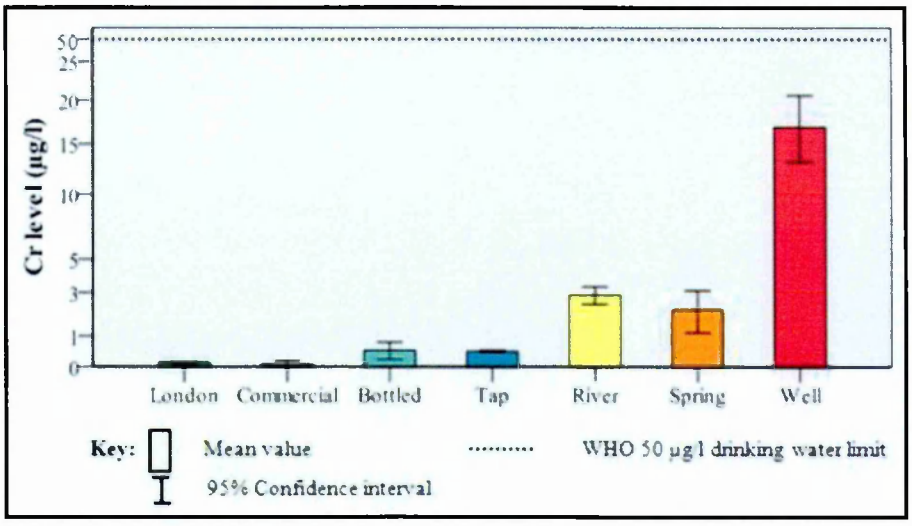

Figure 3.5: Level of chromium (µg/l) reported in different water samples (see Appendix D for sample codes).

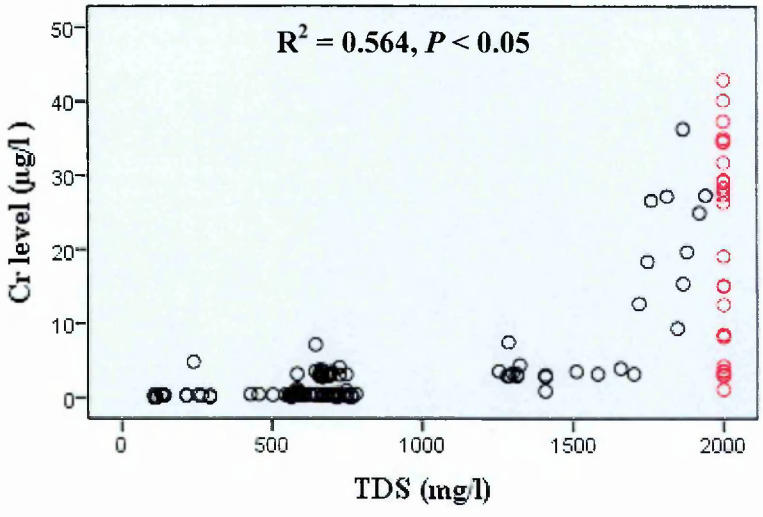

Figure 3.6: Correlation between chromium and the TDS levels for water samples from Karbala (n = 174). The circles in red colour represent the values > 2000 as the Hanna HI 98129 Digital Combo Meter is limited by the detectable ranges (0 – 2000 mg/l TDS) (Hanna, 2008).

Manganese

The lowest levels were found to be in commercial water (0.12 ± 0.07 µg/l) from Karbala, whilst the highest levels were in ground waters (well) (17.6 ± 36.2 µg/l), as shown in Figure 3.7. All of the results confirm that the mean Mn values for all types of drinking (commercial, bottled and tap) and irrigation waters (river, artesian and well) waters are lower than the permissible limits recommended by the WHO for drinking water (400 µg/l Mn) and FAO for irrigation (200 µg/l Mn)

and livestock (50 µg/l Mn), as reported in Tables 3.2 & 3.3. The levels of Mn (mean ± standard deviation) in tap water (4.3 ± 8.0 µg/l) are within the literature ranges reported in Iraq (< 1 – < 10 µg/l) (Barbooti *et al.*, 2010) and for other countries (1.40 – 4.54 µg/l) (Ward, 1989), (2.3 – 9.20 µg/l) (Nkono & Asubiojo, 1998). Manganese levels in river and ground waters are also in agreement with those reported in Iraq for river water, < 1 – 10 µg/l Mn (Barbooti *et al.*, 2010) and in another country for well water, < 0.1 – 2440 µg/l Mn (Reimann *et al.*, 2003). In addition, the typical levels reported in the literature for fresh (10 µg/l Mn) and river waters (7 µg/l Mn) (Ward, 2000) are higher than the Mn levels measured in this study for drinking and irrigation waters, respectively. The only exception is for well water (17.6 ± 36.2 µg/l). In summary, there appears to be no concerns over the levels of Mn in Karbala waters as the mean values are in agreement with the guideline reported by the WHO.

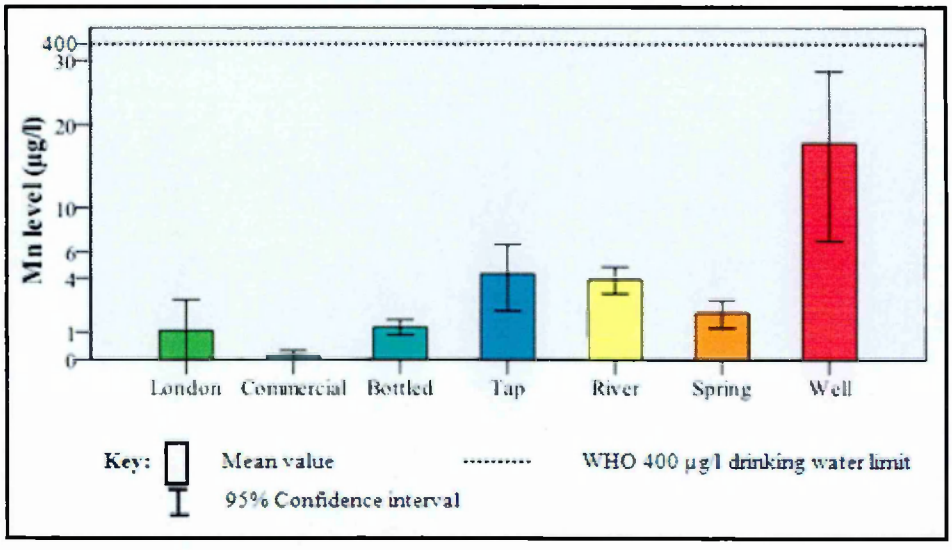

Figure 3.7: Level of manganese (µg/l) reported in different water samples (see Appendix D for sample codes).

Iron

The World Health Organization does not recommend a guideline value for Fe in drinking water (WHO, 2008). However, the levels of Fe in both drinking and irrigation water are significantly lower than the Iraqi specification (300 µg/l Fe) (Barbooti *et al.*, 2010). Levels of Fe in the water samples increase through the following sequence (well > river > artesian > bottled ~ tap > London ~

commercial, as shown in Figure 3.8. Iron in drinking water was found to be in the range of (0.7 – 0.9 µg/l) for commercial, (0.8 – 35.6 µg/l) for bottled and (6.5 – 12.7 µg/l) for tap water. It was found that Fe could be present in drinking water due to the coagulation process, where several Fe salts are used as coagulating agents in water treatment. In addition, the corrosion of steel, cast and galvanised iron pipes during water distribution can also increase the concentration of Fe in drinking water (Ilyas & Sarwar, 2003). Typical values for Fe in fresh water are reported in Table 1.5 (500 µg/l); Fe levels in drinking water samples (commercial, bottled and tap) are slightly lower than this typical levels and within the reported range in the literature (4.2 – 15.3 µg/l Fe) (Nkono & Asubiojo, 1998). Moreover, the levels of Fe in tap water are lower than those reported in Baghdad, Iraq (< 20 – 76 µg/l) (Table 3.4).

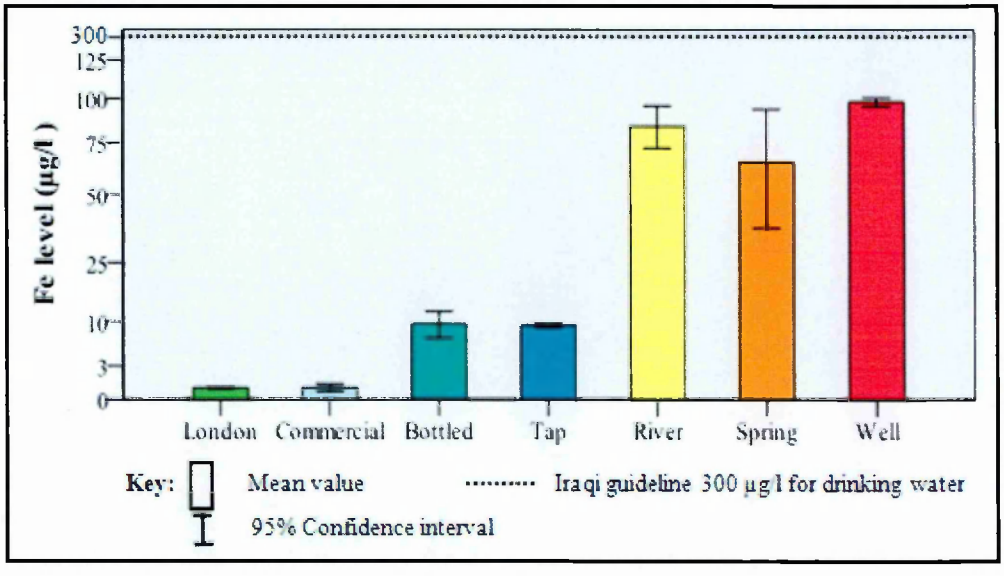

Figure 3.8: Level of iron (µg/l) reported in different water samples (see Appendix D for sample codes).

The levels of Fe in river (84 ± 33 µg/l); artesian (65 ± 34 µg/l); well (98 ± 8 µg/l) waters are higher than those reported as typical values for river (40 µg/l Fe) samples in Table 1.5, and in the literature for other countries (40 µg/l) (Khan *et al.*, 2005). The high levels of Fe in rivers arises from the waste water discharged by industrial activities, such as thermal power plants and a urea plant, which are located adjacent to nearby local rivers. The results obtained in this study are within the reported range for irrigation water in Iraq, Baghdad (< 20 - < 624 µg/l)

(Table 3.4). The level of Fe correlates with the TDS for Karbala waters, as shown in Figure 3.9. The results indicate that Fe levels in Karbala water are below the acceptable limit according to the Iraqi standard limit for drinking water.

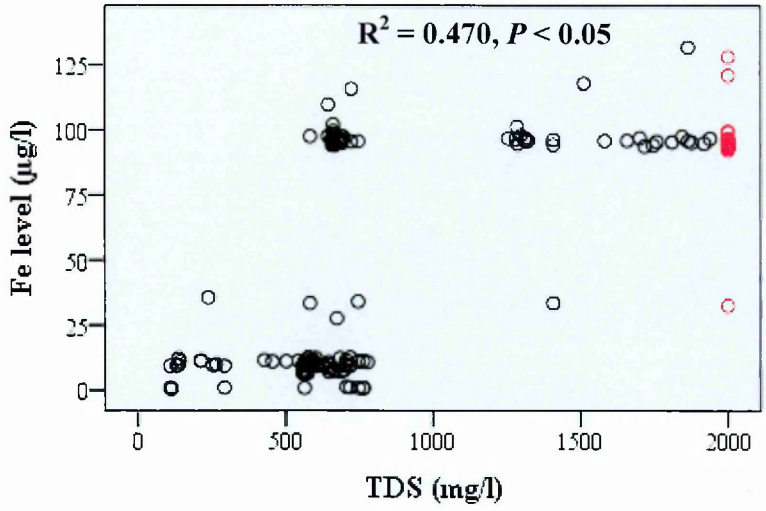

Figure 3.9: Correlation between iron and the TDS levels for water samples from Karbala (n = 174). The circles in red colour represent the values > 2000 as the Hanna HI 98129 Digital Combo Meter is limited by the detectable ranges (0 – 2000 mg/l TDS) (Hanna, 2008).

Copper

The distribution of Cu in various water samples is shown in Figure 3.10. Copper levels were found to be in commercial (0.3 – 0.9 µg/l), bottled (0.4 – 30.4 µg/l), tap (1.3 – 18.5 µg/l), river (1.1 - 77.0 µg/l), artesian (1.9 – 37.3 µg/l) and well (32.3 - 41.4 µg/l) waters at levels lower than the WHO and Iraqi guideline for drinking water, (2000 and 1000 µg/l), respectively. These values are higher than typical levels for fresh (3 µg/l) and river water (5 µg/l) (Ward, 2000). The only exception is for commercial water, which is lower than this typical level. In comparison with another study reported in Baghdad, the levels of Cu in drinking water (tap) are in disagreement with the Baghdad study (< 5 µg/l), and the levels in river water are correspondingly higher (< 5 µg/l) (Table 3.4). The Cu levels in drinking water reported in the literature cover the range, (5 – 18000 µg/l) (Ilyas & Sarwar, 2003). It is well known that copper is used in several commercial processes, such as copper pipes, valves, alloys and coatings (WHO, 2008). Copper levels can increase in drinking water due to the corrosion of plumbing (USEPA,

1991). Overall, the levels of copper in Karbala water are under the levels set by the WHO for drinking water.

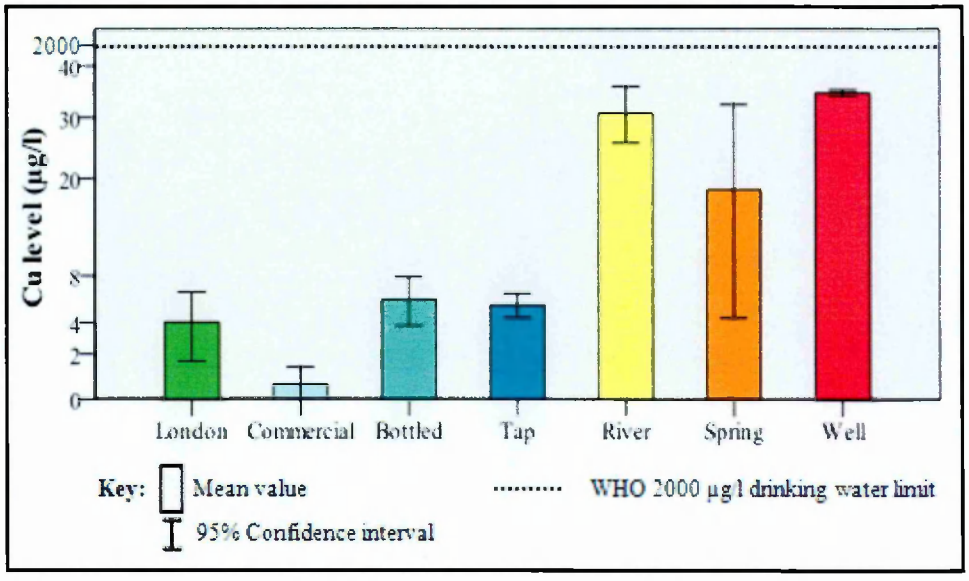

Figure 3.10: Level of copper (µg/l) reported in different water samples (see Appendix D for sample codes).

Zinc

Zinc levels in Karbala water samples are summarised in Tables 3.2 & 3.3 and presented in Figure 3.11. In general, the levels of Zn in both drinking and irrigation waters are in accordance with the WHO, Iraq and FAO guidelines. The highest Zn levels are observed in well and river waters, as mean ± standard deviation (131 ± 31 and 123 ± 48 µg/l), respectively, whilst the lowest level is measured in commercial water (1 – 2 µg/l). Typical zinc levels in fresh and river waters are reported in Table 1.5 (15 and 20 µg/l), respectively. The majority of drinking water samples (except commercial water) are higher than the values for fresh water. Furthermore, most irrigation waters are also higher than the published value for typical river water. Zinc values in the literature fluctuate, for example, Ilyas & Sarwar (2003) reported Zn levels in drinking waters of 0 – 3600 µg/l, Kabata-Pendias & Mukherjee (2007), 1.1 – 24000 µg/l.

The WHO states that the levels of Zn in drinking water could be increased through the dissolution of zinc from pipes (WHO, 2008). In this study, the levels

of Zn in drinking water are lower than literature values although the pipes used for water distribution in Karbala include Zn materials. A possible explanation for this phenomenon is that the pH of water samples was slightly alkaline, which can lead to a decrease in the solubility of Zn (Ilyas & Sarwar, 2003). On the other hand, the levels of Zn in domestic bottled water (105 ± 146 µg/l) are higher than tap water (52 ± 58 µg/l). The relatively high value of Zn in the bottled water is possibly due to the fact that these waters are stored in galvanised tanks which corrode rapidly in the tropical environment and leach the elements into the water supplies (Nkono & Asubiojo, 1998). The Zn levels in drinking water are in agreement with a study in Baghdad (Table 3.4). However, the Zn levels of irrigation water are higher than those reported in Baghdad (< 20 – 40 µg/l). In conclusion, the drinking and irrigation water data in this study provides a level of confidence that there are no zinc contamination problems in Karbala water samples.

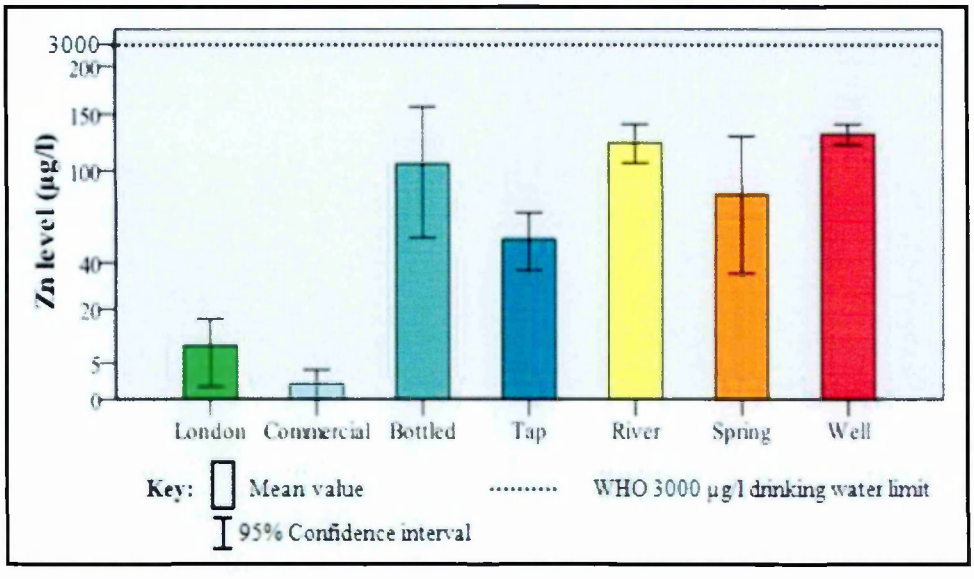

Figure 3.11: Level of zinc (µg/l) reported in different water samples (see Appendix D for sample codes).

Arsenic

Arsenic levels (µg/l) for commercial (0.12 ± 0.09), bottled (0.88 ± 0.51), tap (1.56 ± 0.59), river (2.6 ± 0.9), artesian (1.5 ± 0.8) and well waters (2.6 ± 2.1) are within the WHO guideline (10 µg/l) for drinking water, as shown in Figure 3.12. Arsenic levels (µg/l) for most drinking and irrigation samples are higher than

values for fresh and river waters, as reported in Table 1.5 (0.5 µg/l As) and (2 µg/l As), respectively. The only exception is for As levels (µg/l) in commercial waters, which are lower than fresh waters. Moreover, As values in this study are in agreement with those reported in Baghdad (< 10 µg/l As) for tap and river waters (Barbooti *et al.*, 2010). However, tap water values (1.56 ± 0.59 µg/l As) are lower than those reported in other countries, such as Nigeria (13 µg/l As) (Nkono, & Asubiojo, 1998), and higher than British tap water levels (0.04 – 0.45 µg/l As) (Ward, 1989). It should be noted that high natural levels of arsenic have been reported in different countries, such as Bangladesh or Thailand (> 1000 µg/l As) and Finland (about 50 mg/l As) (Mandal & Suzuki, 2002). In addition, a high arsenic concentration has also been reported in the USA (ground water), and La Pampa, Argentina (< 4 to 530 µg/l As) (Smedley *et al.*, 2002). The long term use of contaminated waters with high levels of As may cause an accumulation of As in soils and crops (Heikens *et al.*, 2007). According to the WHO and other authors, high levels of As in water can be a possible cause of adverse health effects and/or diseases (Arain *et al.*, 2009; Arain *et al.*, 2008; WHO, 2008).

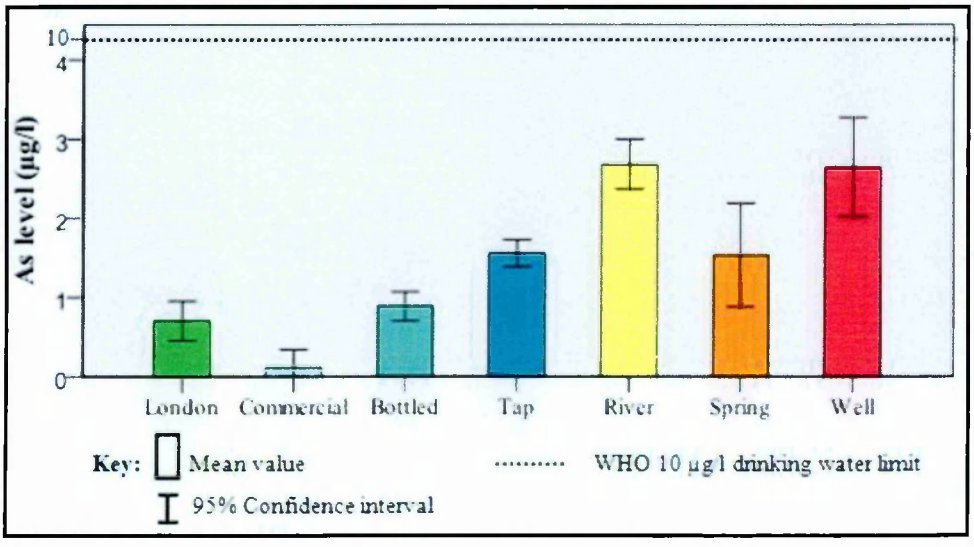

Figure 3.12: Level of arsenic (µg/l) reported in different water samples (see Appendix D for sample codes).

Furthermore, various studies in the USA have reported that As in drinking water has been associated with the onset of type 2 diabetes (Navas-Acien *et al.*, 2008; Kile & Christiani, 2008; Meliker *et al.*, 2007). According to the WHO guideline

for drinking water, the values for water samples in Karbala are at acceptable levels in terms of As.

Strontium

Strontium may be considered to be one of the important elements to be determined in this study. The level of Sr is higher in all water samples; the mean, standard deviation and range values in different water samples are summarised in Tables 3.2 & 3.3. The WHO do not recommend any guideline value for Sr in water samples. Another problem is that there is a lack of literature on Sr in environmental samples.

The results show that the levels of Sr (μg/l) increase according to the following trend (well > artesian > river > tap > bottled > London > commercial), with ranges from 1512 – 14375, 1157 – 8308, 335 – 2755, 78 – 2110, 15 – 1535, 6 - 357 to 22 - 120 μg/l Sr, respectively, as shown in Figure 3.13. The U.S. Environmental Protection Agency (USEPA) recommended that the acceptable level of Sr in drinking water should not exceed (4000 μg/l Sr) (Usuda *et al.*, 2007). The mean values for commercial, domestic bottled, tap, river and artesian waters are lower than the standard value (4000 μg/l Sr) of the USEPA (Tables 3.2 & 3.3), whilst the Sr levels in well waters (7096 ± 2823 μg/l) exceed this value. Thus, Sr levels have a strong correlation with the TDS ($R^2 = 0.850$, $P < 0.05$), as shown in Figure 3.14.

The levels of Sr in drinking water, namely commercial; domestic bottle; and tap are equal to, ~ 12 times higher than and 16 times higher than those reported in fresh water (70 μg/l Sr), respectively. Similar results were reported for irrigation waters when compared with typical values for river samples. The levels of Sr for river, artesian and well waters are equal to ~ 19 times higher, 49 times and 101 times higher than typical river value (60 μg/l Sr), respectively (Table 3.3). The results were also compared with data reported in the literature. The Sr levels in drinking water (domestic bottled and tap) are higher than those reported in other countries such as Dhaka (127.6 μg/L Sr); Karanikong (217.98 μg/L Sr); Japan (81.88 μg/L Sr) and Saudi-Arabia (376.46 μg/l Sr) (Chiba *et al.*, 2006; Al-Saleh, 1996). In addition, Sr levels in surface (river) and ground (artesian and well) waters are higher than those reported in the literature for surface (24.9 - 30.6 μg/l

Sr) water (Reimann *et al.*, 2003); (8.8 – 0850 µg/l Sr) and (800 µg/l Sr) for ground water (Azparren *et al.*, 2000; Kikuchi *et al.*, 1999). A previous study in Denmark has reported a high level of Sr in ground water – up to 53 mg/l Sr, (Greve *et al.*, 2007).

In the light of these results, the highest levels of Sr were found in ground water. The presence of Sr may be due to natural distribution throughout rocks, soil, dust, coal and oil in this region. Eventually, it is moved to the ground water through the natural re-crystallisation or weathering of rocks and soils (Greve *et al.*, 2007). The soil in Iraq includes high levels of oil and, therefore, might be a reasonable source for Sr. In addition, human activities could also increase the levels of Sr in the environment, where Sr is used to produce ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, medicines, colour television picture tubes and a red colour in fireworks (Usuda *et al.*, 2007). In general, the population are exposed to high levels of Sr via food and drinking water, where some Sr compounds are dissolved in such waters (Spector & Curzon, 1978). Previous studies have shown that Sr in drinking water can enter the bloodstream from the intestine and through the skin during bathing / swimming (Ozden *et al.*, 2007).

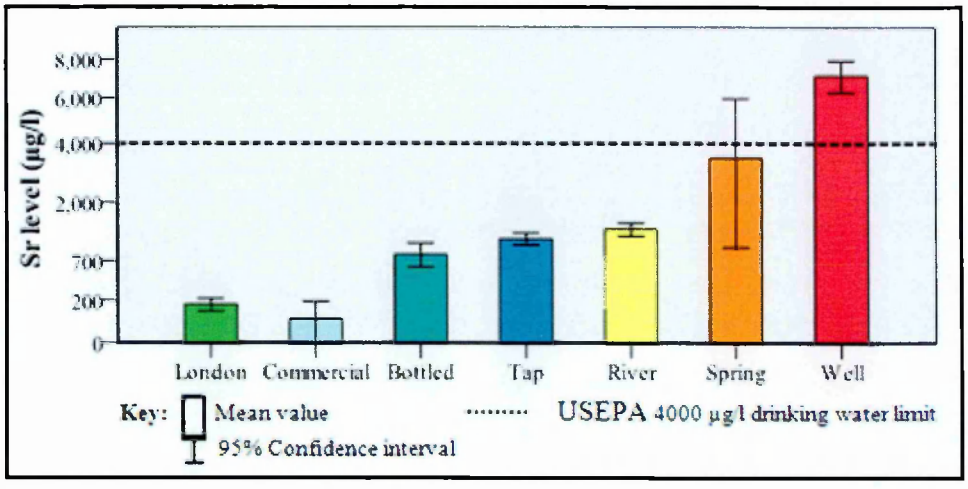

Figure 3.13: Level of strontium (µg/l) reported in different water samples (see Appendix D for sample codes).

The biological effects of strontium (as discussed in Section 1.1.3.9) are linked with bone disease due to the Sr being accumulated in bones as a "look-a-like" to Ca (Usuda *et al.*, 2007; Verberckmoes *et al.*, 2003). However, Sr could be used as

a drug for treatment of osteoporosis (Malaise *et al.*, 2007), and to cause rickets disease particularly in a strontium-rich soil, such as Turkey (Ozgur *et al.*, 1996).

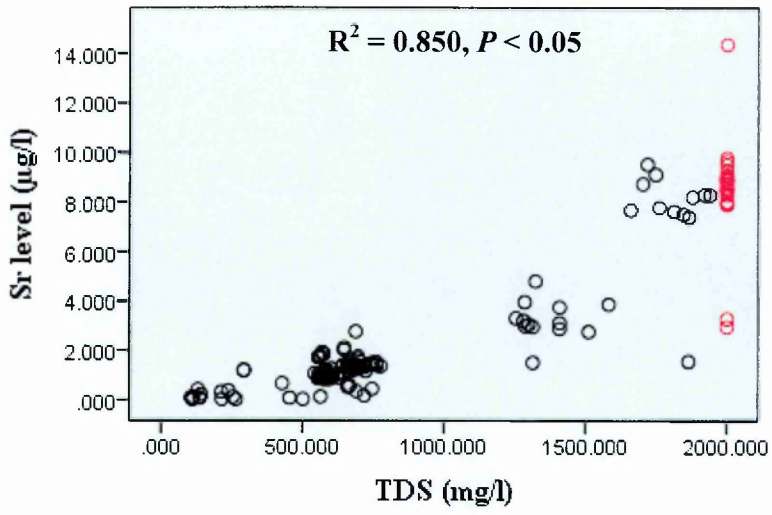

Figure 3.14: Correlation between strontium and the TDS levels for water samples from Karbala (n = 174). The circles in red colour represent the values > 2000 as the Hanna HI 98129 Digital Combo Meter is limited by the detectable ranges (0 – 2000 mg/l TDS) (Hanna, 2008).

Cadmium

Cadmium levels in Karbala water samples (drinking and irrigation) are presented in Tables 3.2 & 3.3. It was found that Cd levels (µg/l) in drinking water (commercial, < 0.01 – 0.01; bottled, < 0.02 - 1.17; and tap, 0.09 - 2.05) are lower than the WHO and Iraqi guidelines (3 µg/l Cd). The irrigation water samples (river, 1.02 - 13.55; artesian, 0.68 - 10.00; and well, 9.67 - 11.41 µg/l) also include Cd levels lower than the Permissible Limit reported by the FAO for Livestock (50 µg/l Cd), and higher than the WHO and Iraqi guidelines for drinking water, as shown in Figure 3.15. A positive relationship was found between Cd concentration and the TDS levels in the water samples from Karbala ($R^2 = 0.450$, $P < 0.05$), as shown in Figure 3.16.

The highest levels of Cd were found in ground water (9.98 ± 0.31 µg/l Cd), and the lowest in commercial water. Typical mean values reported in the literature for fresh and river water are, 0.03 and 0.02 µg/l Cd, respectively (Ward, 2000). The mean value (µg/l) of Cd in tap (0.90 ± 0.44) and river (8.71 ± 3.65) water in this study are 31 times and 400 times higher than typical values for fresh and river

waters, respectively. The reported values in the literature for Cd in drinking water are: 0.07 – 0.62 µg/l Cd (Ward, 1983) and 0.32 – 1.08 µg/l Cd (Nkono & Asubiojo, 1998) for tap water and 0.018 – 0.056 µg/l Cd (Ilyas & Sarwar, 2003) and < 0.002 – 6.41 µg/l Cd (Reimann *et al.*, 2003) for ground water. The results in this study show that the values of Cd in drinking water are within the literature range, whilst in ground water are higher than the literature ranges. In addition, these results are in agreement with those reported in Baghdad, Iraq by Barbooti *et al.* (2010) for drinking water (< 1 µg/l Cd) and higher than Baghdad levels (< 1 µg/l Cd) in terms of river water.

Cadmium is a toxic trace element (Skrzydlewska *et al.*, 2003; Jarup *et al.*, 1998). In general, besides cigarette smoking, people are usually exposed to Cd levels from industrial sources, such as steel, plastic, Zn smelting and battery manufacturers (e.g. electrode in rechargeable nickel-cadmium batteries used in calculators and smaller devices) (Baird & Cann, 2005). Cadmium is released to the environment through the wastewaters and fertilisers (WHO, 2008). Overall, the drinking water (commercial, bottled and tap) in Karbala can be used directly, whereas irrigation water (river, artesian and well) water may need chemical treatment prior to use as drinking water by the population.

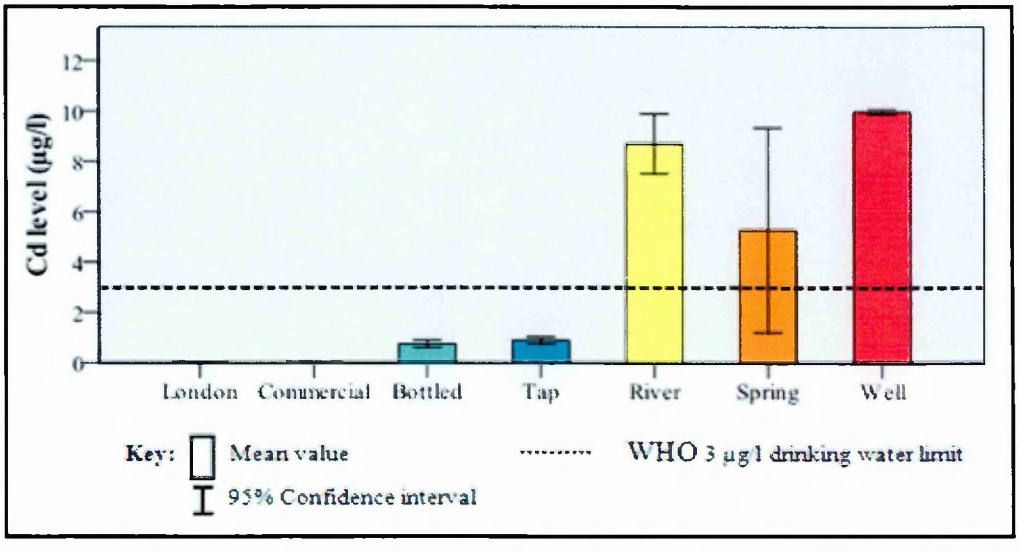

Figure 3.15: Level of cadmium (µg/l) reported in different water samples (see Appendix D for sample codes).

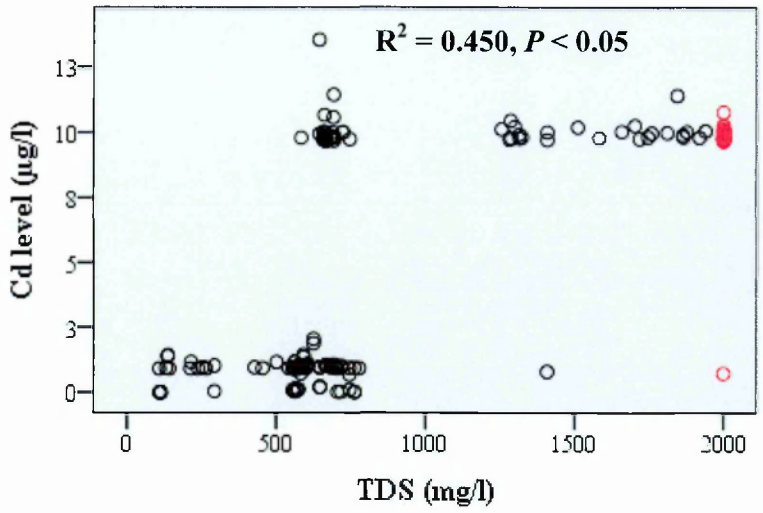

Figure 3.16: Correlation between cadmium and the TDS levels for water samples from Karbala (n = 174).

3.2 Cigarette Tobacco

Smoking is considered to be a major environmental risk factor associated with many serious systemic diseases, including respiratory diseases, heart diseases and cancers (Varela-Lema *et al*, 2009; Pappas *et al*., 2006; Fowles & Dybing, 2003; Tomar & Asma, 2000). One study in the USA reported that there is a link between smoking tobacco and the onset of type 2 diabetes (Will *et al*., 2001). Many toxic trace elements are found in cigarette tobacco which can cause more health problems and disorders (Kim *et al*., 2010; Chiba & Masironi, 1992).

Tobacco samples were collected from Karbala (n = 16), as described in Section 2.1.1. The tobacco samples under investigation cover the commercial materials available to the individuals who make-up the study group in Chapter 4. The aim of this study was to develop an analytical method for the determination of trace elements in cigarette tobacco, and to investigate whether cigarette tobacco may contribute to the elevation of elemental levels in the tissues and fluids of cigarette smoking individuals living in Karbala.

3.2.1 Instrumentation

The inductively coupled plasma atomic emission spectrometry (ICP-AES) operating conditions used in this study are reported in Table 2.16. The instrument was calibrated by using multi-elemental standard solutions, as shown in Section 2.7.6. The limit of detection (LOD) for the instrument was determined prior to any tobacco sample analysis and the results are reported in Table 2.18. The stability for the ICP-AES instrument was confirmed by using a control quality chart, which is reported in Section 2.8.2. The calculated relative standard deviation (%RSD) values were used to measure the precision of the ICP-AES instrument through the replicate analysis of pooled samples. Certified reference materials (CRMs) were used to determine the levels of accuracy (Section 2.8.3).

3.2.2 Results and Discussion

Soil is the main source for trace elements in plants. The mobility of trace elements from the soil solution into the plant as free ionic or complex forms which occurs either by ion exchange or adsorption between the root and soil (Baird & Cann, 2005). The processes of mobility and availability of trace elements in plants are based on several factors, namely pH, redox reactions, geochemical, biological, external weathering and condition and the internal bond to various compounds (Baird & Cann, 2005). Tobacco leaves are widely used in manufacturing smoking materials (Mench, 1998). Because of the possible transfer of certain elements from the tobacco to tobacco smoke during the combustion process, it is desirable to study the concentration of various elements present in cigarette tobacco (Martinez *et al.*, 2008). Multi-trace element analysis by ICP-AES was performed for cigarette tobacco samples; the elemental mean, standard deviation (±SD) and range values are summarised in Table 3.5. The highest mean trace elements values were found in tobacco samples as reported for Fe (257 ± 52 mg/kg Fe, dry weight, d.w.), whilst the lowest mean values are observed for V (0.42 ± 0.12 mg/kg V, d.w.). The overall order of the trace elements levels in all cigarette tobacco samples is Fe > Mn > Sr > Zn > Cu > As > Cd > Cr > V. Iron and Mn are found in higher levels when compared with other elements as they are widely spread in the soil ((Kabata-Pendias, 2000).

Table 3.5: Comparison of the elemental levels for commercial tobacco (n = 16) used in this study and those reported in the literature (mg/kg, dry weight).

Element	Elemental level (mg/kg, dry weight), mean or range		
	This study, Mean ± SD (range)	Literature range	Cigarette type
V	0.42 ± 0.12 (0.26 – 0.67)	0.49 – 5.33^{+}	nf
Cr	0.62 ± 0.17 (0.40 – 0.99)	< 0.1 – 3.45 4.44 – 29.3	USA Algerian
Mn	99 ± 24 (59 – 158)	81 – 148 155 – 400	Mexican USA
Fe	257 ± 52 (166 – 349)	359 – 564 656 – 823 325 – 520 449 ± 6*	Mexican Algerian USA Iran
Cu	5.36 ± 2.54 (2.45 – 9.88)	9 – 17 9.01 – 19.18	Mexican India
Zn	26.8 ± 5.2 (18.1 – 34.9)	16.8 – 30.5 35** 12.6 ± 0.4	USA Turkey Iran
As	1.7 ± 1.1 (0.7 – 4.2)	< 0.55 – 3.24 4.05 – 6.4 1**	nf Algerian Turkey
Sr	75 ± 14 (53 – 102)	74.2 – 151.2 136.88 – 203.20 29.7 – 49.5	Jordanian Algerian USA
Cd	0.90 ± 0.47 (0.24 – 2.03)	0.23 – 5.8 0.28 – 0.87	nf India

nf = not found, $^{+}$ range, * mean ± SD, ** mean value.

Source: Verma *et al.*, 2010; Hamidatou *et al.*, 2009; Martinez *et al.*, 2008; Oliveira *et al.*, 2000; Adachi, *et al.*, 1998; Vega-Carrillo *et al.*, 1995; Ward, 1993; Chiba & Masironi, 1992; Gulovali & Gunduz, 1983; Abedinzadeh *et al.*, 1997.

A great number of articles have reported the chemical levels of tobacco with data focusing on cigarette tobacco from different countries such as Turkey, Iran, Brazil, Mexico, etc. It is interesting to compare the analytical results in this study with those obtained in the literature, as presented in Table 3.5. In general, the mean values for most elements are in agreement with the literature ranges. On the other hand, there are differences between the reported ranges for all elements in different countries. The tobacco plant absorbs many essential, non-essential and toxic elements from the soil, irrigation water, pesticide treatments and contamination from the storage and packing processes, which varies between the

countries that produce tobacco (Rickert & Kalserman, 1994). Thus, the levels of trace elements in tobacco are higher when grown in soil contaminated with these elements. Other environmental factors may influence the trace element uptake by tobacco plants including soil pH and fertilizers applied to crops (Martinez *et al.*, 2008; Adamu *et al.*, 1989). The leaf age can also affect the level of these elements (i.e. the older leaves having higher elemental levels when compared to younger leaves) (Chiba & Masironia, 1992).

Many studies have investigated the elemental levels of cigarette tobacco and associated health/pollution implications. It has been known for a few decades that tobacco combustion has the potential to deliver dangerous quantities of heavy metals to the blood and various organs (Landsberger *et al.*, 1993; Chiba & Masironi, 1992). Cadmium in particular is regarded as one of the "strong carcinogens" in tobacco smoke (Hecht, 2003). Tobacco plants have a special ability to absorb Cd from soil and to accumulate it in unusually high concentrations in the leaves (ranging from 0.77 to 7.02 mg/kg) (Stavrides, 2006). In cigarettes, Cd concentrations range in this study from 0.24 to 2.03 mg/kg, with a mean level of 0.90 ± 0.47 mg/kg (dry weight). These are very high levels compared with those in food which are normally below 0.05 mg/kg (Landsberger *et al.*, 1993). A large proportion of the Cd contained in the cigarette passes into the smoke. Since Cd concentration in the ash is practically constant (about 16% of that present in the unsmoked cigarette and a further 15% is retained by the filter), the greater part (nearly 70%) passes into the smoke (Mussalo-Rauhamaa *et al.*, 1986; Schenker, 1984). Furthermore, the boiling point of trace elements can play a significant role in increasing or decreasing the levels of trace elements in cigarette smoke, and hence their effects on smoker health (Adachi *et al.*, 1998). For example, the boiling points of Cd and V are 767°C and 3000°C, respectively; the temperature of a cigarette could exceed 800°C at the end when ignited (Adachi *et al.*, 1998). Therefore, the concentration of Cd in cigarette smoke could potentially be higher than V due to the fact that the boiling point of Cd is lower than that of V. In contrast, the concentration of V in filter and ash is higher than Cd (Landsberger *et al.*, 1993). As a result, the impact of Cd on the smoker health will increase.

3.3 Summary

This chapter has reported the results from the environmental study (water and cigarette tobacco) of this research. The water quality measurements showed that the pH for water samples were slightly alkaline (7.5 – 8.3). Conductivity levels ranged from 223 µS/cm in commercial water to > 3999 µS/cm in ground water; and total dissolved solid (TDS) ranged from 112 mg/l in commercial water to > 2000 in ground water, as reported in Table 3.1. A significant correlation (R^2 = 0.9999, $P < 0.05$) was found between the TDS and conductivity levels in the water samples from Karbala, as presented in Figure 3.1. Conductivity values were higher for most water samples when compared with the guideline reported by the WHO (250 µS/cm); therefore, the high levels of EC require further investigation in order to link the EC values with human health, and to establish a guideline value for the EC limits within the Iraq Standard Specifications.

Multi-trace element analysis by ICP-MS was performed for drinking (commercial, bottled and tap) and irrigation (river, well and artesian) waters and tap water from London. In general, the highest level for all elements were found in ground water when compared with other types of water tested in this study, whereas the lowest level was found in commercial water (Tables 3.2 & 3.3). The trace element levels measured in tap water from Karbala were higher when compared with those from London (Table 3.4). According to trace element levels and water parameter values, the quality for water samples collected from London is higher when compared with those from Karbala.

The results were compared with the guideline value for drinking and irrigation water recommended by the WHO and FAO in order to evaluate the quality and freshness of drinking, irrigation and domestic uses. In general, most trace elements are lower than the permissible limits for drinking water recommended by the WHO and Iraqi standard. The only exceptions are for B in artesian (1049 ± 746 µg/l B) and well waters (1569 ± 844 µg/l B), Cd in river (8.71 ± 3.65 µg/l Cd), artesian (5.28 ± 4.86 µg/l Cd) and well waters (9.98 ± 0.31 µg/l Cd). Moreover, the high levels of Sr reported in this study for drinking and irrigation waters suggest that a follow-up study be undertaken to establish whether a possible link can be found through the analysis of soils and main foodstuffs in this region.

The results were also compared with the literature ranges reported in Baghdad (Iraq) and other regions in the world. In general, the level of trace elements is in agreement with Baghdad study for most elements. In addition, the results are comparable with those reported in different countries with the only exceptions found for B, Sr and Cd in ground water.

Generally, the samples of water analysed may be considered of good quality. Only the levels of B, Sr and Cd may require chemical treatment at the municipal water plant in order to improve the quality of drinking water. The results also confirmed that other trace elements that exist in drinking water are found to be at acceptable levels in terms of water quality.

The levels of trace elements in cigarette tobacco are reported in Table 3.5. It was found that all the elements are found in cigarette tobacco according to the following order: Fe > Mn > Sr > Zn > Cu > As > Cd > Cr > V. The findings confirm that the levels of trace elements in cigarette tobacco are in general agreement with the reported data for other countries. The highest trace element levels in tobacco were found to be for Fe (257 ± 52 mg/kg Fe d.w.), whilst the lowest levels were for V (0.42 ± 0.12 mg/kg V d.w.).

Chapter Four

Trace Element Levels in Tear Drops

4.0 Introduction

Trace element levels in human tear drops are reported in this chapter, as shown in Sections 4.4 - 4.5. Samples were collected and prepared, as outlined in Section 2.2.3. Methods were developed and validated, as described in Sections 2.2.3.1 & 2.2.3.2. An Agilent 7700 Series ICP-MS instrument was used for multi-element analysis, as described in Section 2.6. The results for boron (B), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), arsenic (As), strontium (Sr) and cadmium (Cd) in tear drop samples are reported in Tables 4.2 – 4.18. Residents from Karbala (Iraq) were recruited for the main study, whereas residents from London (UK) were used for a comparative study, as described in Section 2.1.2. The influence of health status, gender, age, drinking water, residential location, smoking activity and statistical interactions on the elemental levels were also investigated. The results were compared with published literature values, as described in Tables 4.5 & 4.6. The aim of this chapter was to develop tear drops as a potential new non-invasive biomarker for monitoring trace element levels in the human body for short periods, in terms of the evaluation of human health and possible use to identify the impact of environmental pollution.

4.1 Statistical Methods of Analysis

Statistical analysis used to evaluate the level of trace elements in tear drops samples starts with an examination of the results of a study population to evaluate the relationships between trace element levels and health status, gender, age, smoking activity, residential location and drinking water. The tests range from simple descriptive statistics, such as arithmetic mean and standard deviation, through to tests for statistical outliers and the comparison of data sets using an F-test and a two tailed t-test (refer to Appendix C). Multivariate Discriminant Analysis (MDA) was used to determine the set of variables (trace elements) that discriminated between healthy and diabetic groups, as shown in Section 2.9.6. Analysis of covariance (ANCOVA) was used to explore the effect and interactions of different factors (such as health status, smoking activity and gender) and covariates (age and drinking water) on the level of trace elements in the tear drop samples, as outlined in Section 2.9.4. The Pearson correlation

coefficient (*r*) was performed to evaluate associations between trace element levels in tear drops (Miller & Miller, 2010). The statistical analysis was carried out using the statistical package IBM SPSS, version 19 (SPSS, Chicago, IL, USA). Table 4.1 describes the statistic plan used in this study.

Table 4.1: Statistical plan used to evaluate the significance of the trace element leves in tear drop samples.

Step	Task	Analytic strategy	Section
Step 1	Sample data were divided into the three population groups, namely healthy and diabetic from Karbala and healthy from London.	Preliminary analysis	4.3
Step 2	Determine the value of arithmetic mean, standard deviation, median, geometric mean, range, 95% confidence interval for mean and box-plots.	Descriptive statistics	4.3
Step 3	Checking for outliers	Grubb's test	4.4.1
Step 4	Determine reliability of mean group differences	F-test and two tailed t-test	4.5.1 & 4.5.2
Step 5	Create a linear combination of IVs to maximize group differences.	DFA	4.5.3
Step 6	Effects and interactions for different factors and covariate variables.	ANCOVA	4.5.4 & 4.5.5
Step 7	Significance effect of factors on elemental data.	Partial *eta* squared (η^2)	4.5.6
Step 8	Degree of relationship among trace element levels in tear drops	Pearson's correlation coefficient (*r*)	4.5.7

ANCOVA is "analysis of covariance", IV = independent variable, DFA is "Discriminant Function Analysis".

4.2 Use of Tear Drops as a Biomarker

Human biological monitoring has become an important tool in the assessment of exposure to various pollutants in an occupational and/or environmental setting, and to evaluate the metabolic state in populations exposed to essential, non-essential and toxic elements (Nunes *et al.*, 2010; Olmedo *et al.*, 2010; Wang *et al.*, 2009; Amaral *et al.*, 2008; Gault *et al.*, 2008; Ozden *et al.*, 2007; Schuhmacher *et al.*, 2002; Bass, 2001; Ashraf *et al.*, 1995). In addition, biomonitoring has played

significant roles in terms of the establishment of occupational and environmental limits of exposure of trace elements, and then contributed to reduce exposure and prevent adverse health effects (Gil & Hernández, 2009).

4.3 Elemental Composition of Tear Drops

The major problem associated with tear drop analysis is the variable volume that is produced by the glands (Kuizenga *et al.*, 1991). Therefore, trace element analysis of tear drops has not yet been established due to the insufficient amounts of sample available for multi-element determination using most analytical techniques (Madej, 2010; Baeyens & Gurny, 1997). Many studies have determined the levels of trace elements in other human fluids (blood serum, plasma, urine and saliva) and tissues (scalp hair and fingernails) (Flores *et al.*, 2011; Menegario *et al.*, 2001, Stovell, 1999). 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 (Samatha *et al.*, 2004). 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. This study is the first to describe the detection of trace element levels in human tear drops. Therefore, the ranges obtained can be used as a valuable database for future studies. The results can be used to evaluate the possible relationship between tear drops and human health status as well as environmental exposure with respect to trace elements.

The main descriptive statistics of elemental levels in tear drops (arithmetic mean, standard deviation (SD), range, median, geometric mean, 95% confidence interval for mean and the number of samples) for Karbala (healthy and diabetic) and London (healthy) populations are summarised in Table 4.2. Figure 4.1 shows the box-plots for trace element levels in the populations under investigation.

The levels of trace elements (μg/l) for tear drops of healthy individuals from Karbala increase according to the following sequence Zn > Fe > Sr > Cu > Mn > Cr > As > V > Cd. In the case of diabetic patients from Karbala, the sequence is Zn > Sr > Fe > Cu > Mn > Cr > V > As > Cd, whereas for healthy individuals from London, the sequence is Cu > B > Zn > Fe > Sr > Mn > Cr > Cd > As > V. 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 problems, disorders and diseases (Gault *et al.*, 2008; Hill, 2006).

Table 4.2: Population data for trace element levels (µg/l) in tear drops from Iraqi individuals resident in Karbala (Iraq) and London (UK).

Element	Group	Mean ± SD	GM	Median	Range	95% CI
B*	HK	389 ± 158	355	383	< 70 - 898	(356, 421)
	DK	606 ± 415	494	479	< 70 - 2020	(466, 747)
	HL	216 ± 127	184	203	83 - 498	(330, 443)
V	HK	5.6 ± 5.3	3.7	3.4	0.5 - 21.2	(4.6, 6.6)
	DK	4.1 ± 2.6	3.1	3.7	0.1 - 10.8	(3.4, 4.9)
	HL	0.7 ± 0.4	0.5	0.6	0.1 - 1.3	(0.5, 0.8)
Cr	HK	13.4 ± 15.8	8.2	8.2	0.7 – 92.8	(10.5, 16.4)
	DK	11.3 ± 10.4	6.5	7.3	0.2 - 40.9	(8.2, 14.4)
	HL	4.6 ± 1.7	4.3	4.3	2.4 - 8.1	(3.8, 5.4)
Mn	HK	60.6 ± 100.5	32.1	32.8	1.9 – 822.7	(41.9, 79.3)
	DK	111.5 ± 113.8	54.2	68.6	0.8 – 445.5	(77.8, 145.1)
	HL	6.8 ± 2.2	6.3	6.4	3.4 - 11.1	(5.8, 7.9)
Fe	HK	734 ± 1198	346	370	7 - 9300	(512, 957)
	DK	577 ± 516	302	442	3 - 2003	(425, 730)
	HL	159 ± 68	143	157	64 - 269	127, 190)
Cu	HK	268 ± 156	222	223	35 - 741	(234, 294)
	DK	204 ± 145	128	190	1 - 594	(161, 247)
	HL	227 ± 62	217	242	90 - 335	(199, 256)
Zn	HK	1369 ± 1764	741	753	149 - 10562	(1041, 1697)
	DK	2122 ± 2638	995	1009	47 - 10434	(1320, 2924)
	HL	188 ± 58	179	186	79 - 324	(161, 215)
As	HK	8.3 ± 11.1	3.9	2.9	0.1 - 44.8	(6.3, 10.4)
	DK	2.7 ± 2.4	1.9	2.1	0.2 – 11.1	(2.0, 3.5)
	HL	1.4 ± 0.7	1.2	1.3	0.2 - 2.9	(1.1, 1.7)
Sr	HK	459 ± 255	382	431	49 - 1183	(411, 506)
	DK	1230 ± 1524	637	619	7 - 6552	(780, 1681)
	HL	62 ± 19	58	65	26 - 98	(53, 70)
Cd	HK	2.3 ± 3.5	1.4	1.4	0.1 – 13.1	(1.9, 2.8)
	DK	2.2 ± 2.1	1.4	1.5	0.1 - 8.4	(1.6, 2.8)
	HL	3.8 ± 2.7	3.0	3.0	1.3 - 9.0	(1.3, 6.3)

CI is confidence interval for mean, SD = standard deviation, GM = geometric mean; HK = healthy Karbala (n = 111); DK = diabetic Karbala (n = 44); HL = healthy London (n = 18), * the levels of boron in 19 samples of healthy and 8 of diabetic subjects from Karbala are below the limit of detection (70 µg/l).

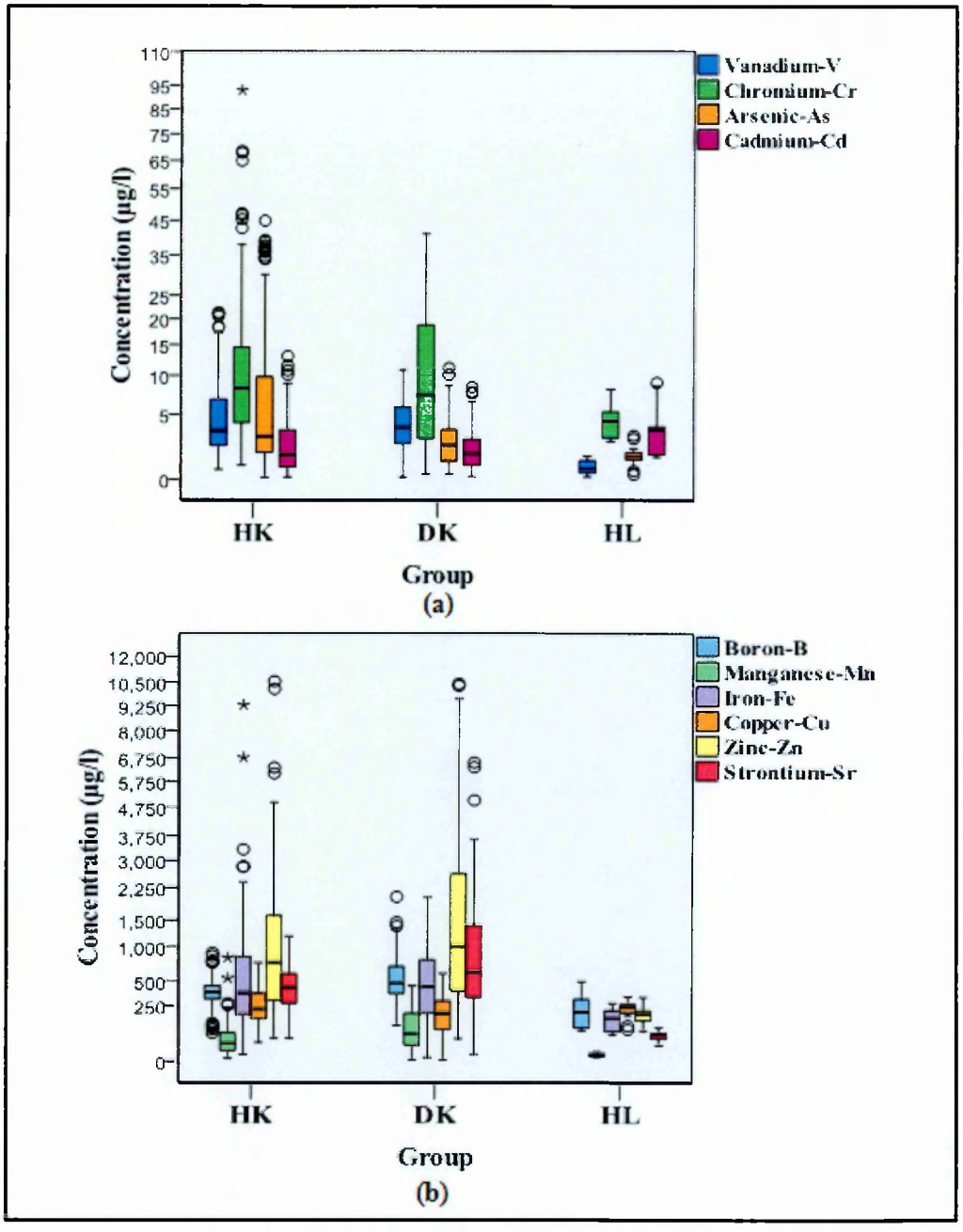

Figure 4.1: Trace element levels (µg/l) in tear drops (a & b) for different population groups: HK is "healthy Karbala" (n = 111) and DK is "diabetic Karbala" (n = 44) individuals from Karbala (Iraq); HL is "healthy London" (n = 18) individuals from London (UK), middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represents extreme values.

4.4 Study Population

A total number of 173 Iraqi individuals resident in Karbala (Iraq) (healthy, n = 111 and diabetic, n = 44) and London (UK) (healthy, n = 18) were selected to participate in this study, as described in Section 2.2.3.

4.4.1 Checking for Outliers

Many of the statistical tests covered in this study are sensitive to outliers (Miller & Miller, 2010; Field, 2009). 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. The data was inspected for statistical outliers by means of a Grubb's test (G), as described in Appendix C. If the calculated value, G_{calc}, 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 (Miller & Miller, 2010). No statistical outliers were found for the data of the healthy populations from London for all trace elements under investigation suggesting a link to normal distribution. The elemental patterns for healthy individuals from the London population were found to be normally distributed with the only exception being Cd (Adair, 2002), as shown in Appendix E. For the healthy population from Karbala, Cr, Mn, Fe, Zn and Cd were found to have 4, 6, 7, 5, and 5 outliers, respectively. In the case of diabetic patients, one value for Mn, three values for Zn and As and five values for Sr were detected as outliers with a Grubb's test. The results of the Grubb's tests are summarised in Table 4.3. In most cases the effect of removal of the outliers can improve the histogram of trace elements in tear drops because the degree of positive skew from the mean is decreased (Usuda *et al.*, 2007), as shown in Appendix E. The skew value provides an indication of the symmetry of the distribution. If the distribution is perfectly normal, then the value of skew is zero (Tabachnick & Fidell, 2007).

Table 4.3: Summary of Grubb's outlier testing on the healthy and diabetic population from Karbala (Iraq).

Parameter	Healthy (µg/l)					Diabetes (µg/l)			
	Cr	Mn	Fe	Zn	Cd	Mn	Zn	As	Sr
Before G-test									
Mean	13.4	60.6	734	1369	2.3	111	2122	2.7	1230
SD	15.8	100.5	1198	1764	2.5	114	2638	2.4	1524
Median	8.2	32.8	370	753	1.4	69	1009	2.1	619
95% CI	10.5 - 16.4	41.9 - 79.3	512 - 957	1041 - 1697	1.9 - 2.8	79 - 145	1320 - 2924	2.0 - 3.5	780 - 1681
Max	92.8	822.7	9300	10562	13.1	446	10434	11.1	6552
Skewness*	2.6	5.2	4.8	3.0	2.1	1.3	2.1	1.9	2.4
n	111	111	111	111	111	44	44	44	44
After G-test									
Mean	11.2	41.7	499	1075	1.9	104	1536	2.2	757
SD	10.6	35.4	460	1032	1.7	103	1520	1.4	589
Median	8.2	30.4	339	717	1.3	59	966	2.1	510
95% CI	9.2 - 13.2	34.9 - 48.6	409 - 588	876 - 1273	1.6 - 2.3	72 - 135	1056 - 2016	1.8 - 2.8	565 - 947
Max	47.1	158.9	2060	4164	6.7	381	5726	5.5	2361
Skewness*	1.8	1.1	1.3	1.4	1.1	1.1	1.3	0.6	0.9
n	107	105	104	106	106	43	41	41	39

CI = confidence interval, SD = standard deviation, n = number of samples, * Positive skewness values indicate positive skew (scores clustered to the left at the low value in histogram), whilst negative skewness values indicate a clustering of scores at the high end (right-hand side of a graph), as shown in Appendix E.

4.5 Results and Discussion

4.5.1 Influence of Residential Location - Link to Environmental Factors

In this study, tear drop samples were collected from Iraqi healthy individuals living in Karbala (Iraq) and London (UK) in order to compare the elemental levels between the two residential location subgroups (Appendix E). It is possible to use these results to evaluate whether food, lifestyle and drinking water can affect the elemental levels. The mean and standard deviation for trace elements between the two population groups were compared using an F-test and a two-tailed t-test and the results are summarised in Table 4.4. Further information regarding F-test, t-test and the degrees of freedom can be found in Appendix C.

Table 4.4: Elemental mean and standard deviation values in human tear drops for healthy individuals from Karbala (Iraq) and London (UK) (outliers omitted).

Element (n_1, n_2)	Mean ± SD (µg/l)		F-test			Two-tailed t-test			
	Karbala	London	Variance	F_{calc}	Sig.	t_{calc}	*df*	Sig.	t_{crit}
B (92, 18)	389 ± 158	216 ± 127	Equal variances assumed	0.058	0.810	**4.347**	108[+]	< 0.001	1.98
			Unequal variances assumed			5.044	28[++]	< 0.001	
V (111, 18)	5.6 ± 5.3	0.7 ± 0.4	Equal variances assumed	19.048	< 0.001	3.929	127	< 0.001	1.98
			Unequal variances assumed			**9.643**	116	< 0.001	
Cr (107, 18)	11.2 ± 10.6	4.6 ± 1.7	Equal variances assumed	12.434	0.001	2.633	123	0.010	198
			Unequal variances assumed			**6.001**	123	< 0.001	
Mn (105, 18)	41.7 ± 35.4	6.8 ± 2.2	Equal variances assumed	29.574	< 0.001	4.169	121	< 0.001	1.98
			Unequal variances assumed			**9.992**	108	< 0.001	
Fe (104, 18)	499 ± 460	159 ± 68	Equal variances assumed	21.421	< 0.001	3.115	120	0.002	1.98
			Unequal variances assumed			**7.094**	119	< 0.001	
Cu (111, 18)	268 ± 156	227 ± 62	Equal variances assumed	12.430	0.001	1.084	127	0.280	2.01
			Unequal variances assumed			1.950	60	0.056	
Zn (106, 18)	1075 ± 1032	188 ± 58	Equal variances assumed	23.332	< 0.001	3.634	122	< 0.001	1.98
			Unequal variances assumed			**8.770**	109	< 0.001	
As (111, 18)	8.3 ± 11.1	1.4 ± 0.7	Equal variances assumed	18.482	< 0.001	2.654	127	0.009	1.98
			Unequal variances assumed			**6.541**	115	< 0.001	
Sr (111, 18)	459 ± 255	62 ± 19	Equal variances assumed	24.345	< 0.001	6.591	127	< 0.001	1.98
			Unequal variances assumed			**16.154**	117	< 0.001	
Cd (106, 18)	1.9 ± 1.7	3.8 ± 2.7	Equal variances assumed	4.899	0.029	3.906	122	< 0.001	2.09
			Unequal variances assumed			**2.844**	19	0.010	

SD is standard deviation, n_1, n_2 are the number of samples for healthy individuals from Karbala and London, respectively, *df* = degrees of freedom at n_1 - 1 and n_2 - 1 for F-test, [+] degrees of freedom for t-test (n_1 + n_2 - 2), [++] degrees of freedom for t-test determined, as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

The results show significantly higher levels of B, V, Cr, Mn, Fe, Zn, As and Sr in the tear drop samples from the Karbala population (at $P < 0.05$) when compared to those from London. In contrast, a significantly higher level of Cd was found in the tear drop samples from London compared to Karbala individuals. No statistical difference was found for Cu between the two healthy groups at the probability level of $P < 0.05$.

This study has shown a wide variation in the total concentration for most trace elements between the two populations. The elevated levels of trace elements in Karbala healthy samples rather than London (except Cd) are probably due to the influence of trace element exposures and nutritional factors caused by different environmental settings (Samanta *et al.*, 2004).

The individuals from Karbala live close to sites of pollution such as from military-weapons that were used in the wars, along with oil spills and scrap metal from destroyed military vehicles. It is fairly well documented that many regions of Iraq have a high concentration of pollution (UNEP, 2003). Reports from UNEP have revealed 300 sites in Iraq that are considered to be contaminated by various pollutants. Furthermore, the presence of human activities has directly influenced the chemical balance of the Iraqi environment. This includes a wide range of metals (Cd, As, Cr, Cu and Zn) associated with commercial or industrial (smelters, power stations and mining drainage), transportational (petroleum related pollutants, combustion products) and agricultural factors (fertilizers, sewage, sludge, pesticides). Further information can be found in Chapter 3.

These findings reflect the content of trace elements in drinking water for these regions (Greve *et al.*, 2007). It was found that the trace element levels in drinking water from Karbala were higher than those collected from London (refer to Chapter 3). The high level of trace elements in drinking water may have caused elevated levels in tear drops of Karbala individuals when compared with London, as outlined in Section 4.5.4.5.

The higher Cd level in tear drop samples was found in London samples (3.8 ± 2.7 μg/l) rather than those from Karbala (1.9 ± 1.7 μg/l). This could have originated from industrial discharges such as electroplating, PVC and the production of batteries. Similarly, higher levels of Cd are found in the subjects living in the areas of greatest industrial contamination compared to those living in areas with lower industrial contamination (Bernard, 2008; Onyari *et al.*, 1991). A previous

study has shown that the elemental concentrations of scalp hair for Pakistani and Libyan populations were dependent on geographic location, environmental exposure and dietary habits (Shah *et al.*, 2006).

Overall, the results indicate that the environment and any factors that interfere with the environment have more influence on the levels of trace elements than other parameters as described above (further information can be found in Chapter 3).

4.5.2 Influence of Type 2 Diabetes - Link to Human Health

The concentration of essential trace elements are homeostatically regulated when the health status of individuals is under normal conditions (healthy individuals) (Adair, 2002). There is accumulating evidence that the metabolism of several trace elements is altered in type 2 diabetes mellitus, and may play significant roles in the pathogenesis and progress of this disease (Afridi *et al.*, 2009; Hussain *et al.*, 2009; Afridi *et al.*, 2008). Many studies have previously discussed the relationship between trace elements and type 2 diabetes for diabetic patients by comparing them with healthy individuals. These studies have used different human fluids and tissues such as blood (whole, plasma and serum), saliva, hair and nails (Flores *et al.*, 2011; Edwards *et al.*, 2009; Navas-Acien *et al.*, 2009; Sukumar & Subramanian, 2007; Stone, 2006; Wrobel *et al.*, 1999).

In this work, the results of healthy individuals and diabetic patients resident in Karbala have been compared in order to evaluate whether there are any significant differences in the elemental levels between the two groups (Appendix E). This can be used to describe whether type 2 diabetes plays any significant role in these differences by increasing or decreasing the elemental levels inside the human body through the effect on the metabolism of essential elements. The diabetic patients have a mean age of 53.93 ± 7.85 years (range 40 -75 years) with no other chronic or infectious diseases as reported in the questionnaires of participants in this study. The mean and standard deviation values for trace element levels in tear drops of the healthy and diabetic populations were compared by using an F-test and a two-tailed t-test, and the results obtained are listed in Table 4.5.

Table 4.5: Elemental mean and standard deviation values in human tear drops (this study) and blood serum (literature) for healthy individuals and diabetic patients from Karbala, Iraq (outliers omitted).

Element (n_1, n_2)	Published value, serum*		This study, tear drops								
	Mean ± SD (µg/l)		Mean ± SD (µg/l)		F-test			Two-tailed t-test			
	Healthy (n = 12)	Diabetes (n = 76)	Healthy	Diabetes	Variance	F_{calc}	Sig.	t_{calc}	df	Sig.	t_{crit}
B (92, 36)	nd	nd	389 ± 158	606 ± 415	EVA UVA	28.192	< 0.001	4.315 **3.063**	126+ 39++	< 0.001 0.004	2.02
V (111, 44)	5.91 ± 1.23	1.94 ± 1.05	5.6 ± 5.3	4.1 ± 2.6	EVA UVA	11.453	0.001	1.714 **2.255**	153 147	0.088 0.026	1.98
Cr (107, 44)	1.44 ± 0.70	0.66 ± 0.58	11.2 ± 10.6	11.3 ± 10.4	EVA UVA	0.426	0.515	0.051 0.052	149 82	0.959 0.959	1.98
Mn (105, 43)	1.44 ± 0.69	2.83 ± 1.25	41.7 ± 35.4	103.7 ± 102.7	EVA UVA	66.518	< 0.001	5.463 **3.864**	146 46	< 0.001 < 0.001	2.01
Fe (104, 44)	nd	nd	499 ± 460	577 ± 516	EVA UVA	0.554	0.458	0.914 0.873	146 73	0.362 0.386	1.98
Cu (111, 44)	915 ± 194	1221 ± 299	268 ± 156	204 ± 145	EVA UVA	0.719	0.398	**2.340** 2.414	153 84	0.021 0.018	1.98
Zn (106, 41)	606 ± 87	612 ± 148	1075 ± 1032	1536 ± 1520	EVA UVA	9.085	0.003	2.116 1.792	145 55	0.036 0.079	2.01
As (111, 41)	1.33 ± 0.41	0.83 ± 0.59	8.3 ± 11.1	2.2 ± 1.4	EVA UVA	34.735	< 0.001	3.521 **5.695**	150 120	0.001 < 0.001	1.98
Sr (111, 39)	nd	nd	459 ± 255	757 ± 589	EVA UVA	53.397	< 0.001	4.318 **3.059**	148 43	< 0.001 0.004	2.02
Cd (106, 44)	0.04 ± 0.01	0.13 ± 0.48	1.9 ± 1.7	2.2 ± 2.1	EVA UVA	0.610	0.436	0.812 0.736	148 66	0.465 0.465	1.98

SD is standard deviation, n_1, n_2 are the number of samples for healthy and diabetic, respectively, df = degrees of freedom at n_1 - 1 and n_2 - 1 for F-test, + degrees of freedom for t-test ($n_1 + n_2 - 2$), ++ degrees of freedom for t-test determined, as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance, EVA = equal variances assumed, UVA = unequal variances assumed.

Source: * Flores *et al.*, 2011.

In general, significantly higher tear drop levels of B, Mn and Sr are found in diabetic patients when compared with healthy individuals ($P < 0.05$). In contrast, the levels of V, Cu, and As are observed to be significantly higher in healthy individuals than diabetic patients. Although the levels of Fe and Zn are slightly higher in diabetic patients than healthy, the differences are not statistically significant ($P < 0.05$). Cr and Cd are found in approximately similar levels in both population groups. It is clear that there is a good agreement for most elements between the results of this study and those reported by Flores *et al.* (2011) for blood serum in terms of the comparison between healthy and diabetic populations, as shown in Table 4.5.

The results of healthy individuals and diabetic patients in this study were compared with the literature range reported for saliva and blood serum, as shown in Table 4.6. In terms of the results of healthy individuals, the mean value of Zn (1075 μg/l) is in agreement with the reference range (700 - 1600 μg/l Zn) for blood serum. Cu mean value (268 μg/l Cu) lies below the minimum values of the reference range (700 - 1300 μg/l Cu). The mean values for B (389 μg/l), V (5.6 μg/l), Cr (11.2 μg/l), Mn (41.7 μg/l), As (8.3 μg/l), Sr (459 μg/l) and Cd (1.9 μg/l) were above the maximum values of the reference range (39 - 365 μg/l B; 0.03 – 5.00 μg/l V; 0.1 - 0.5 μg/l Cr; 0.6 - 1.3 μg/l Mn; 0.5 - 1.8 μg/l As; and ~ 30 μg/l Sr; and 0.2 - 1.0 μg/l Cd).

The elemental ranges for tear drops overlap the literature ranges reported in saliva for most trace elements, the only exceptions are for Cu and Cd, which were within the ranges (Table 4.6).

The results for healthy individuals were also compared with the reference ranges reported for serum and plasma of European populations, as reported in Table 4.6 (Stone, 2006). The mean value of Zn (1075 μg/l) in tear drops was found to be in agreement with the reference range (120 – 2760 μg/l Zn) for two blood fractions (serum and plasma). Fe mean value (499 μg/l) was within the reference range reported for plasma (200 – 4455 μg/l Fe) and below the range reported for serum (1100 – 1377 μg/l Fe). The mean value for Cu (268 μg/l) lies below the minimum value of the reference ranges for serum and plasma (560 – 1850 μg/l Cu). The mean values for Cr (11.2 μg/l), Mn (41.7 μg/l) and V (5.6 μg/l) were found to be higher than the maximum values of the reference ranges for serum (0.14 - 0.43

µg/l Cr; 0.54 - 34.50 µg/l Mn; and 0.016 - 1.300 µg/l V) and plasma (0.03 - 0.39 µg/l Cr; 0.54 - 34.50 µg/l Mn; and 0.016 - 1.300 µg/l V), as shown in Table 4.6. These results are also in agreement with those published in the report by Muniz *et al.* (2001) for Zn in blood serum; and Gil *et al.* (2011) for Cd, Cr and Mn in blood and saliva.

The results of diabetic patients were compared with the reference ranges reported for serum and plasma of diabetic European populations, as shown in Table 4.6 (Stone, 2006). The mean value for V (4.1 µg/l) falls within the reference range of (2 – 11.4 µg/l V) in plasma samples. The mean values of Cu (204 µg/l Cu) and Fe (577 µg/l Fe) were found to be below the reference range of serum (565 – 1461 µg/l Cu; 690 – 1240 µg/l Fe) and plasma (1070 – 1226 µg/l Cu; 1430 – 4690 µg/l Fe). In contrast, the maximum values of Mn and Zn in diabetic serum (1.1 µg/l Mn; 1503 µg/l Zn;) and plasma (2.7 µg/l Mn; 1150 µg/l Zn) were found to be below the mean value in tear drops for diabetic patients (104 µg/l Mn; 1536 µg/l Zn) (Stone, 2006). Cr mean value (11.3 µg/l) was above the maximum value of the reference range of plasma (0.75 - 6.8 µg/l Cr). The mean values for As (2.2 µg/l) and Cd (2.2 µg/l) were higher compared with those reported by other researchers in serum (0.83 µg/l As and 0.13 µg/l Cd) (Flores *et al.*, 2011).

Table 4.6: Reported literature concentration for trace elements in biological fluids for healthy individuals and diabetic patients.

Element	Elemental level (µg/l)					
	Saliva	Blood serum			Blood plasma	
	Healthy[1]	Healthy[2]	Healthy[3]	Diabetes[3]	Healthy[3]	Diabetes[3]
B	0.6-20.5	39-365*	nd	nd	nd	nd
V	nv	0.03-5.00	0.016-1.3	nd	0.016-1.3	2-11.4
Cr	0.41-1.64	0.1-0.5	0.14-0.43	nd	0.03-0.39	0.75-6.8
Mn	0.47-7.23	0.6-1.3	0.54-34.5	0.0-1.1	0.54-34.5	0.6-2.7
Fe	32-270	-	1100-1377	690-1240	200-4455	1430-4690
Cu	23-387	700-1300	560-1850	565-1461	560-1850	1070-1226
Zn	11-158	700-1600	120-2760	523-1503	120-2760	499-1150
As	0.19-3.3	0.5-1.8	nd	nd	nd	nd
Sr	2.16 ± 0.96	~ 30*	nd	nd	nd	nd
Cd	0.33-2.35	0.2-1.0	nd	nd	nd	nd

nd is not determined.

Source: [1] Kim *et al.*, 2010; Yuan *et al.*, 2008; Ward, 1993; and Ward & Ward, 1991; [2] Flores *et al.*, 2011; [3] Stone, 2006; * Azparren *et al.*, 2000.

One study in Egypt also found that the Cr mean values did not differ in blood between healthy individuals (0.20 µg/l Cr) and type 2 diabetic patients (0.19 µg/l Cr) (Kamal, *et al.*, 2009). Cr is required for normal carbohydrate metabolism as a critical cofactor for insulin action (Kimura, 1996).

The results from this study are in agreement with several researchers who have reported that diabetics may benefit from V. It was found that V salts (such as $NaVO_3$ and $VOSO_4$) could lower blood glucose in diabetic patients, and improve insulin-resistant status in healthy women (Seko *et al.*, 2006; Srivastava & Mahdi, 2005; Wang *et al.*, 2001).

In recent decades, As has been suggested as being essential in the human body (Wang *et al.*, 2009). In general, people are exposed to inorganic As via drinking water and cigarette smoking (Navas-Acien *et al.*, 2008; Meliker *et al.*, 2007; Will *et al.*, 2001). According to World Health Organisation (WHO) instructors and previous studies, high levels of As in water can be a possible cause of adverse effect on human health (Arain *et al.*, 2009; Arain *et al.*, 2008; WHO, 2008). In this study, the As levels in drinking water were within the recommended guideline set by the World Health Organisation (WHO) (10 µg/l As) (see chapter 3, Table 3.2). However, the participants could be exposed to As by food and environmental sources. Significant exposure to As occurs through both anthropogenic and natural sources. Occupational exposure to As is common in the smelting, mining and microelectronic industries and the production of iron and steel (Baird & Cann, 2005). Inorganic As compounds are also used in common products such as wood preservatives, paints, pesticides and herbicides used in local or home gardens (Baird & Cann, 2005).

Arsenic exposure through food poses a substantial risk to humans in certain parts of the world, particularly in Asia from the consumption of staple foods such as rice, which have been irrigated with As-rich groundwater (Mondal & Polya, 2008; Meharg & Rahman, 2003). The population of Karbala followed a similar diet as found in the Study Questionnaires (refer to Appendix A). The diet included bread and rice as part of daily main meals. The Iraqi government usually imports these grains from various Asian countries such as India, Bangladesh and Thailand, where high (> 1000 µg/l) natural levels of As have been reported in irrigation water (Mandal & Suzuki, 2002).

Zinc has been suggested in the literature to play significant roles in terms of the activity of insulin; the ability of glucose to enter cells; and glucose metabolism (Hussain *et al.*, 2009; Kamal *et al.*, 2009).

There are a few reports on the reference values for B and Sr in human biological samples, and previous studies have been shown that they have specific biological effects. For example, B affects Ca absorption (Hegsted *et al.*, 1991), and Sr can reduce the risk of vertebral fractures in postmenopausal women with osteoporosis (Meunier *et al.*, 2004). The available data on human health effects following exposure to strontium is very limited. The excess of Sr could cause disturbance in the metabolism of Ca (Chojnacka *et al.*, 2010). Animal studies have indicated that the critical target after oral exposure to stable strontium is the skeleton (Greve *et al.*, 2007).

The literature indicates that there are conflicting results with reporting elevated and declined manganese concentrations in diabetes mellitus patients (Flores *et al.*, 2011; Hussain *et al.*, 2009). Manganese is a cofactor for the antioxidant enzyme, MnSOD (SOD is superoxide dismutase). In spite of the role of Mn not having been thoroughly presented in terms of the pathology of type 2 diabetes, Mn is known to be essential for glucose metabolism (Hussain *et al.*, 2009).

The results for Cu in tear drops disagree with those reported by other researchers for blood serum: they found high levels of Cu in diabetic patients rather than healthy individuals ($P < 0.05$) (Flores *et al.*, 2011; Hussain *et al.*, 2009). Another study in Iran has reported that the level of Cu in serum was significantly higher in diabetics when compared to the non-diabetic individuals (Nasli-Esfahani *et al.*, 2011). At this time no information is available to explain this finding. In general, the redox chemistry of Cu makes for a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radicals. Cells in the human body require Cu to drive important biochemical reactions; therefore, abnormal Cu metabolism can lead to several chronic conditions, such as diabetes (Thiele, 2003).

4.5.3 Discriminant Function Analysis

Variation in the level of trace elements in tear drops of healthy individuals and diabetic patients was evaluated through discriminant function analysis (DFA) (see Section 2.9.6). The DFA applied on raw data consisted of ten trace elements in

order to determine which discriminate between healthy and diabetic groups. Only two discriminant functions (DFs) were found to discriminate the three population groups (healthy Karbala (HK), diabetic Karbala (DK) and healthy London (HL)), as shown in Table 4.7. Wilk's Lambda test showed that DF is statistically significant at $P < 0.001$. Furthermore, 100% of the total variance between the three population groups was explained by only two DFs. It can be seen that, Sr, Mn, B, Zn, V, As and Cd exhibited a strong contribution in discriminating the three population groups and account for most of the expected variations in tear drops, while other trace elements showed less contribution in explaining the variation between the HK, HL and DK population (Table 4.7).

The DF1 explained 71.5% of the total variance with a good correlation value (0.706). In Table 4.7, the matrix structure coefficients, showing the correlations of each trace element in the model with each discriminant function, are also reported. The DF1 mostly discriminated the HK and HL groups (showing negative score values) from the DK group (high positive score values), as shown in Figure 4.2. The DF1 was mainly correlated to high concentrations of Sr (0.551), Mn (0.539), B (0.536) and Zn (0.313), as shown in Table 4.7. This means the cases with a positive score on DF1 (diabetic group in this case) tended to have higher concentrations of these elements (Figure 4.2) (see also Table 4.5). The high B, Mn, Zn and Sr levels associated with type 2 diabetes could be also connected with environmental and/or lifestyle factors such as drinking water quality and smoking (Gil *et al.*, 2011).

The DF2 value explained 28.5% of the total variance (DF1 + DF2 = 100%) with a correlation value equal to 0.532, and resulted in giving a useful contribution to the discrimination. The DF2 separated the HL and DK groups (negative values) from the HK group (positive values), as shown in Figure 4.2. In this case, the DF2 appeared mainly associated with high concentrations of V (0.533), As (0.504) and a low concentration of Cd (- 0.428). Therefore, cases with a positive score on DF2 (HK group in this case) tended to have higher levels of the former elements and lower levels of the latter element, as shown in Figure 4.2. The results show that cases with a negative score on the DF1 and DF2 (healthy individuals from London) tended to have higher levels of Cd and lower levels of Sr, Mn, B, Zn, V and As. This was discussed in Section 4.5.1.

Table 4.7: Matrix structure coefficients[+], percentage of variance, eigenvalues, correlations, cumulative% and Wilks' Lambda of the final model for tear drops.

Element	Discriminant function	
	DF1	DF2
Sr	0.551*	< 0.3
Mn	0.539*	< 0.3
B	0.536*	< 0.3
Zn	0.313*	< 0.3
V	< 0.3	0.533*
As	< 0.3	0.504*
Cd	< 0.3	- 0.428*
Cr	< 0.3	< 0.3
Fe	< 0.3	< 0.3
Cu	< 0.3	< 0.3
% of variance	71.5	28.5
Eigenvalues	0.991	0.395
Correlation	0.706	0.532
Cumulative%	71.5	100
Wilks' Lambda	0.360 at $P < 0.001$	0.717 at $P < 0.001$

* Largest absolute correlation between trace element and discriminant function, [+] the structure coefficients are similar to correlation coefficients, and reflect the uncontrolled association of the discriminating variables (trace elements) with the categorical variable (population groups).

Table 4.8 summarizes the degree of success of the classification of each group. The number of cases correctly classified and misclassified is displayed. Two results of the classification of the samples are shown: original and cross-validation. In the original results each case in the analysis is classified by the functions derived from all cases. With cross-validation, each case in the analysis is classified by the functions derived from all cases other than that case. This last procedure ascertains the efficiency of this model in classifying new samples. Rate errors of classification for each group are the proportion of cases not classified in this group. The discriminant functions appeared to have a good classification with 85% of original cases correctly classified and 83% of cases using the cross-validation procedure. The results show that the DK group was classified (75%) with 9/44 cases misclassified into the HK group. The HK group (84.7%) was also misclassified in 10/111 and 7/111 cases into the DK and HL group, respectively. The HL group was classified with high accuracy (94.4%). These classifications are represented in Figure 4.2 for the two most important discriminant functions.

Table 4.8: Classification results for tear drops of the three population groups.

Type of classification	Group		Predicted Group Membership			Total
			HK	DK	HL	
Original 85%	Count	HK	95	9	7	111
		DK	8	34	2	44
		HL	0.0	0.0	18	18
	%	HK	85.6	8.1	6.3	100
		DK	18.2	77.3	4.5	100
		HL	0.0	0.0	100	100
Cross-validated 83%	Count	HK	94	10	7	111
		DK	9	33	2	44
		HL	1	0.0	17	18
	%	HK	84.7	9.0	6.3	100
		DK	20.5	75.0	4.5	100
		HL	5.6	0.0	94.4	100

HK = healthy Karbala; DK = diabetic Karbala; and HL = healthy London.

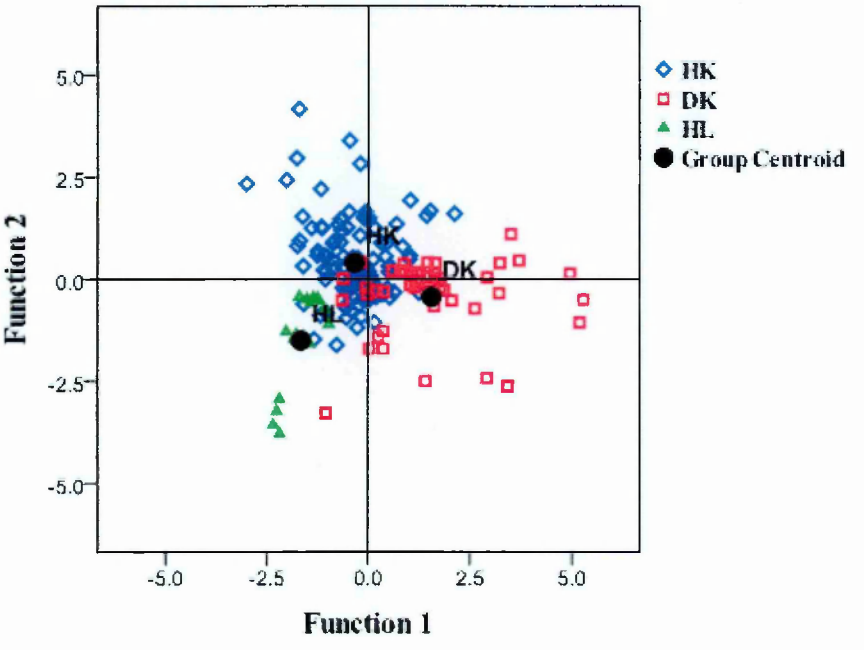

Figure 4.2: Plot of DF1 vs DF2 for tear drops. DF: discriminant function (see Appendix E for discriminant data).

In order to evaluate whether the differences in the elemental levels are caused by type 2 diabetes and not by other factors, the factorial analysis for the data of populations from Karbala was carried out. This analysis was used to determine the

effect for each factor, namely health status, smoking activity and gender, and covariates (age and drinking water) on the level of trace elements in the total tear drop samples (healthy and diabetic). In addition, the interaction between each pair of these factors can also be determined. The next section describes the analysis of covariance.

4.5.4 Factors Influencing Elemental Data (Factorial Analysis)

The mean values of the trace elements were categorised according to different parameters (factors) obtained from the questionnaire, as shown in Table 4.9. It is important that multiple effects should be studied in research rather than the single effect for each factor (Hair *et al.*, 2010). Analysis of covariance (ANCOVA) was used to investigate the effects and interactions of independent variables (factors) on the level of trace elements (dependent variables) in tear drops for individuals resident in Karbala (n = 155) (further information about ANCOVA can be found in Chapter 2, Section 2.9.4). The mean and standard deviation for each group are presented in Table 4.10. The results of ANCOVA for each element are reported in Appendix E, and the summary results for all elements are shown in Table 4.11.
The effect for each factor and covariate was investigated. The *P*-values can be used to determine whether there is a significant effect. 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.

Table 4.9: Demographic characteristics of participants according to different factors.

Factor	Code number *	Group	Number of subjects
Health status	1	diabetic	44 (male = 18, female = 26)
	2	healthy	111 (male = 42, female = 69)
Gender	1	male	60 (healthy = 42, diabetic = 18)
	2	female	95 (healthy = 69, diabetic = 26)
Smoking activity	1	smoker	30 (male = 15, female = 15)
	2	non-smoker	125 (male = 45, female = 80)
Total			155

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

Table 4.10: Descriptive statistics (mean ± SD µg/l) for trace elements in tear drop samples of all individuals from Karbala (Iraq) in relation to different factors.

Health status	Gender	Smoking	Elemental level, mean ± SD (µg/l)				
			B	V	Cr	Mn	Fe
Healthy	male	smoker	489 ± 199	6.9 ± 6.1	10.1 ± 3.4	49.9 ± 30.7	776 ± 414
		non-smoker	426 ± 164	5.7 ± 4.3	15.4 ± 12.1	46.8 ± 38.3	696 ± 571
		total	433 ± 166	5.8 ± 4.5	14.9 ± 11.7	47.2 ± 37.1	706 ± 549
	female	smoker	360 ± 127	6.6 ± 7.7	9.4 ± 9.2	45.8 ± 38.8	444 ± 431
		non-smoker	363 ± 152	5.3 ± 5.6	9.0 ± 9.4	37.8 ± 34.1	368 ± 342
		total	363 ± 149	5.4 ± 5.8	9.0 ± 9.3	38.4 ± 34.2	374 ± 347
	total	smoker	417 ± 166	6.7 ± 6.7	9.7 ± 6.8	47.8 ± 33.0	610 ± 435
		non-smoker	385 ± 158	5.4 ± 5.1	11.3 ± 10.9	41.1 ± 35.7	487 ± 464
		total	389 ± 158	5.6 ± 5.3	11.2 ± 10.6	41.7 ± 35.4	499 ± 460
Diabetic	male	smoker	519 ± 230	4.4 ± 2.6	10.3 ± 12.9	101.2 ± 127.9	535 ± 323
		non-smoker	713 ± 374	3.8 ± 1.9	12.7 ± 9.2	109.1 ± 85.6	917 ± 748
		total	609 ± 308	4.1 ± 2.3	11.4 ± 11.2	104.7 ± 108.1	705 ± 569
	female	smoker	821 ± 579	4.5 ± 2.9	17.5 ± 11.6	153.5 ± 92.3	658 ± 524
		non-smoker	490 ± 375	4.0 ± 2.9	8.0 ± 7.4	79.2 ± 98.2	399 ± 423
		total	605 ± 471	4.1 ± 2.8	11.3 ± 10.0	103.0 ± 100.9	489 ± 467
	total	smoker	680 ± 463	4.4 ± 2.6	13.7 ± 12.5	124.5 ± 113.5	593 ± 422
		non-smoker	554 ± 380	3.9 ± 2.6	9.5 ± 8.2	88.8 ± 93.7	565 ± 586
		total	606 ± 415	4.1 ± 2.6	11.3 ± 10.4	103.7 ± 102.7	577 ± 516
Total	male	smoker	508 ± 209	5.2 ± 4.0	10.2 ± 10.9	84.1 ± 106.8	616 ± 360
		non-smoker	473 ± 232	5.3 ± 4.0	14.9 ± 11.6	58.4 ± 54.9	738 ± 605
		total	482 ± 225	5.3 ± 4.0	13.8 ± 11.5	65.1 ± 71.8	706 ± 550
	female	smoker	643 ± 505	5.3 ± 5.2	14.6 ± 11.1	112.1 ± 91.9	581 ± 488
		non-smoker	391 ± 224	5.0 ± 5.2	8.8 ± 9.0	46.9 ± 56.8	375 ± 359
		total	431 ± 298	5.1 ± 5.1	9.6 ± 9.50	56.3 ± 66.6	407 ± 386
	total	smoker	581 ± 396	5.3 ± 4.6	12.4 ± 11.0	97.1 ± 99.4	599 ± 419
		non-smoker	419 ± 229	5.1 ± 4.8	11.0 ± 10.4	51.1 ± 56.2	503 ± 490
		total	450 ± 274	5.2 ± 4.7	11.2 ± 10.5	59.7 ± 68.5	522 ± 477

Table 4.10 continued

Health status	Gender	Smoking	Elemental level, mean ± SD (µg/l)				
			Cu	Zn	As	Sr	Cd
Healthy	male	smoker	342 ± 173	2113 ± 2099	7.5.1 ± 4.7	680 ± 444	3.6 ± 1.9
		non-smoker	285 ± 146	1014 ± 813	9.6 ± 12.6	455 ± 234	1.8 ± 1.6
		total	292 ± 148	1124 ± 1021	9.3 ± 11.9	482 ± 270	2.0 ± 1.7
	female	smoker	324 ± 236	1703 ± 1452	5.9 ± 4.9	604 ± 322	2.8 ± 1.9
		non-smoker	246 ± 151	979 ± 987	7.9 ± 11.0	430 ± 235	1.8 ± 1.6
		total	253 ± 160	1045 ± 1044	7.7 ± 10.6	445 ± 246	1.9 ± 1.7
	total	smoker	332 ± 200	1867 ± 1638	6.6 ± 4.6	638 ± 364	3.1 ± 1.9
		non-smoker	260 ± 150	992 ± 921	8.5 ± 11.6	439 ± 234	1.8 ± 1.6
		total	268 ± 156	1075 ± 1032	8.3 ± 11.1	459 ± 255	1.9 ± 1.7
Diabetic	male	smoker	118 ± 105	980 ± 1787	2.3 ± 1.6	311 ± 198	3.4 ± 3.3
		non-smoker	239 ± 138	1355 ± 1244	2.5 ± 1.4	1027 ± 728	2.4 ± 2.3
		total	172 ± 132	1168 ± 1500	2.4 ± 1.5	721 ± 660	2.9 ± 2.9
	female	smoker	252 ± 147	1451 ± 1069	3.3 ± 1.2	864 ± 550	2.2 ± 1.8
		non-smoker	212 ± 157	1923 ± 1693	1.6 ± 1.2	736 ± 575	1.4 ± 0.9
		total	226 ± 152	1772 ± 1515	2.1 ± 1.4	777 ± 559	1.7 ± 1.3
	total	smoker	182 ± 141	1216 ± 1443	2.7 ± 1.5	627 ± 509	2.8 ± 2.7
		non-smoker	221 ± 149	1741 ± 1561	1.9 ± 1.3	829 ± 628	1.7 ± 1.5
		total	204 ± 145	1536 ± 1520	2.2 ± 1.4	757 ± 589	2.2 ± 2.1
Total	male	smoker	193 ± 166	1358 ± 1883	4.0 ± 3.8	479 ± 368	3.5 ± 2.8
		non-smoker	277 ± 144	1076 ± 899	8.3 ± 11.7	557 ± 422	1.9 ± 1.7
		total	256 ± 153	1136 ± 1164	7.2 ± 10.5	542 ± 410	2.3 ± 2.2
	female	smoker	280 ± 184	1559 ± 1201	4.5 ± 3.5	752 ± 470	2.4 ± 1.8
		non-smoker	239 ± 152	1187 ± 1231	6.6 ± 10.2	495 ± 355	1.7 ± 1.5
		total	246 ± 157	1245 ± 1227	6.3 ± 9.5	533 ± 383	1.8 ± 1.6
	total	smoker	237 ± 178	1466 ± 1523	4.2 ± 3.6	632 ± 442	2.9 ± 2.4
		non-smoker	253 ± 150	1147 ± 1119	7.2 ± 10.7	517 ± 380	1.8 ± 1.6
		total	249 ± 155	1203 ± 1201	6.7 ± 9.9	536 ± 392	2.0 ± 1.8

SD = standard deviation.

Table 4.11: Influence of different factors and covariant variables on the trace element levels in tear drop samples for all individuals from Karbala, Iraq (outliers omitted).

Element	n	ANCOVA results, $F_{(df1,df2)}$, P-value				
		Health status	Gender	Smoking activity	Age	Drinking water
B	128	**$F_{(1,118)} = 12.573$, $P = 0.001^{**}$**	$F_{(1,118)} = 0.044$, $P = 0.835$	$F_{(1,118)} = 0.816$, $P = 0.368$	$F_{(1,118)} = 0.755$, $P = 0.387$	$F_{(1,118)} = 1.310$, $P = 0.255$
V	155	$F_{(1,145)} = 1.313$, $P = 0.254$	$F_{(1,145)} = 0.002$, $P = 0.968$	$F_{(1,145)} = 1.554$, $P = 0.215$	$F_{(1,145)} = 3.186$, $P = 0.076$	**$F_{(1,145)} = 13.305$, $P < 0.001^{***}$**
Cr	151	$F_{(1,141)} = 1.478$, $P = 0.226$	$F_{(1,141)} = 0.047$, $P = 0.828$	$F_{(1,141)} = 0.305$, $P = 0.582$	$F_{(1,141)} = 2.962$, $P = 0.087$	$F_{(1,141)} = 0.155$, $P = 0.694$
Mn	148	**$F_{(1,138)} = 16.286$, $P < 0.001^{***}$**	$F_{(1,138)} = 0.116$, $P = 0.734$	$F_{(1,138)} = 3.417$, $P = 0.067$	$F_{(1,138)} = 0.652$, $P = 0.421$	**$F_{(1,138)} = 8.240$, $P = 0.005^{**}$**
Fe	148	$F_{(1,138)} = 0.777$, $P = 0.380$	**$F_{(1,138)} = 5.626$, $P = 0.019^{*}$**	$F_{(1,138)} = 0.045$, $P = 0.833$	$F_{(1,138)} = 0.432$, $P = 0.512$	$F_{(1,138)} = 2.118$, $P = 0.148$
Cu	155	$F_{(1,145)} = 2.268$, $P = 0.134$	$F_{(1,145)} = 0.352$, $P = 0.554$	$F_{(1,145)} = 0.625$, $P = 0.430$	$F_{(1,145)} = 3.771$, $P = 0.054$	$F_{(1,145)} = 0.819$, $P = 0.367$
Zn	147	$F_{(1,137)} = 1.263$, $P = 0.263$	$F_{(1,137)} = 0.955$, $P = 0.330$	$F_{(1,137)} = 1.368$, $P = 0.244$	**$F_{(1,137)} = 6.373$, $P = 0.013^{*}$**	$F_{(1,137)} = 2.292$, $P = 0.132$
As	152	$F_{(1,142)} = 0.099$, $P < 0.754$	$F_{(1,142)} = 0.117$, $P = 0.732$	$F_{(1,142)} = 0.205$, $P < 0.652$	**$F_{(1,142)} = 17.176$, $P < 0.001^{***}$**	$F_{(1,142)} = 1.889$, $P = 0.171$
Sr	150	**$F_{(1,140)} = 5.388$, $P = 0.022^{*}$**	$F_{(1,140)} = 0.411$, $P = 0.522$	$F_{(1,140)} = 0.041$, $P = 0.841$	$F_{(1,140)} = 0.554$, $P = 0.458$	**$F_{(1,140)} = 175.783$, $P < 0.001^{***}$**
Cd	150	$F_{(1,140)} = 0.053$, $P = 0.819$	$F_{(1,140)} = 3.325$, $P = 0.070$	**$F_{(1,140)} = 9.681$, $P = 0.002^{**}$**	$F_{(1,140)} = 1.540$, $P = 0.217$	$F_{(1,140)} = 3.717$, $P = 0.056$

ANCOVA is "analysis of covariance", *df* = degrees of freedom, F = calculated value of F-test, P = probability, the **bold** values indicate significant differences at the level of significance $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, further information can be found in Appendix E.

4.5.4.1 Influence of Health Status

The effects of health status on the trace element levels in tear drops are reported in Table 4.11 using analysis of covariance (ANCOVA). In general, significant effects are found for B ($F_{(1,118)} = 12.573$, $P < 0.01$), Mn ($F_{(1,138)} = 16.286$, $P < 0.001$) and Sr ($F_{(1,140)} = 5.388$, $P < 0.05$). No significant effects found in the health status by the levels of V, Cr, Fe, Cu, Zn, As and Cd at $P < 0.05$. The results are in agreement with those reported in Table 4.5 for most trace elements. The only exceptions are found for V, Cu and As, which show significant differences between healthy individuals and diabetic patients using a two-tailed t-test procedure to compare means for two groups of cases. The possible explanation is that the levels for these elements are influenced by the individual's age (in the case of As ($P < 0.001$) and Cu ($P < 0.1$)) and their levels in drinking water (in terms of V ($P < 0.001$). However, when the covariates (age and drinking water) are removed from the model, the effect of health status becomes significant for Cu ($F_{(1,146)} = 7.733$, $P < 0.01$) and As ($F_{(1,143)} = 6.416$, $P < 0.05$), whilst approaching significant for V at $P < 0.05$ ($F_{(1,147)} = 3.316$, $P = 0.071$) (See Appendix E). A previous study in the UK has found that the distribution of trace elements may be attributed to the weighting caused by age bias (Stone, 2006).

4.5.4.2 Influence of Gender

The effect of gender on the levels of trace elements in tear drop samples was investigated. The total population from Karbala (n = 155) was divided into two gender groups, males and females. The mean and standard deviation (±SD) for each gender group are summarised in Table 4.10. The highest mean values in the two gender groups are found for Zn (males: 1136; and females: 1245 µg/l Zn) followed by iron for males (706 µg/l Fe) and strontium for females (533 µg/l Sr). Cd showed the lowest concentration for both gender groups (males: 2.3 µg/l Cd) and (females: 1.8 µg/l Cd). The order of increasing trace element levels in the tear drops for males is: Cd < V < As < Cr < Mn < Cu < Sr < Fe < Zn, whilst for females is: Cd < V < As < Cr < Mn < Cu < Fe < Sr < Zn.

The effect of gender on the level of trace elements was investigated using analysis of covariance, and the results are listed in Table 4.11. The findings show that

there is a significant effect of gender on the levels of Fe ($F_{(1,138)} = 5.626$, $P < 0.05$). Similar results were also reported by other researchers in blood (Stone, 2006; Devlin, 2002) and scalp hair (Forte *et al.*, 2005). In these studies, the researchers have observed the higher levels of Fe in males when compared to females for healthy individuals and diabetic patients. The lower levels of iron in females may be due to blood losses during menstruation (Jian *et al.*, 2011) and the difference in outdoor activities, difference of urine excretion or kidney activities (Ozden *et al.*, 2007).

No significant effect ($P < 0.05$) was found for either gender for other trace elements such as B, V, Cr, Mn, Cu, Zn, As, Sr and Cd, as shown in Table 4.11. Similar results were also found by Gil *et al.* (2011) and Shigemi *et al.* (2008) in terms of Sr and Cd. They found that Sr and Cd have similar levels for saliva samples in males (mean ± SD: 7.44 ± 3.54 µg/l Sr), (mean ± SD: 0.14 ± 0.23 µg/l Cd) and (mean ± SD: 7.97 ± 3.70 µg/l Sr), (mean ± SD: 0.23 ± 0.34 µg/l Cd) for females. According to Sukumar & Subramanian (2007), there were no significant differences found between males and females for Cd, Cu and Zn in the human scalp hair and fingernails.

The results of chromium in tear drops are in disagreement with those reported by other researchers for males (mean ± SD: 3.02 ± 8.87 µg/l Cr) and females (5.10 ± 9.54 µg/l) in saliva (Gil *et al.*, 2011).

The significant effect of gender on the elemental levels in tear drops was also determined using a two tailed t-test. Similar results to ANCOVA test were found for Fe ($t_{(90)} = 3.585$, $P < 0.01$) and all trace elements with the exception of Cr (refer to Appendix E). A significant interaction was found between smoking and gender for Cr ($F_{(1,141)} = 4.244$, $P < 0.05$), as shown in Section 4.5.5.3. This interaction leads to a change of effect of gender on the levels of Cr. Thus the interpretation of the effect may be incomplete or misleading (Field, 2009).

4.5.4.3 Influence of Smoking Activity

The effect of smoking activity on trace element levels in various invasive and non-invasive human fluids and tissues has been studied by several other researchers (Gil *et al.*, 2011; kim *et al.*, 2010; Sukmar & Subramanian, 2007; Chojnacka *et al.*, 2006).

Multi-element analysis of various brands of imported cigarette tobacco collected from Karbala is presented in Chapter 3 (Table 3.5) in order to evaluate whether any relationship exists between their levels in cigarette tobacco and human health. The population of Karbala was divided into smokers and non-smokers, as shown in Table 4.10. The influence of smoking activity on the trace element levels in tear drops was examined by using ANCOVA, and the results are summarised in Table 4.11. It was found that there is no significant effect of smoking activity for most trace elements in tear drops at $P < 0.05$; the only exception is for Cd ($F_{(1,140)} = 9.681$, $P < 0.01$). The results show higher levels of Cd in tear drops for smokers when compared to non-smokers.

The majority of studies in the literature reported high levels of Cd in the human scalp hair and nails (Sukumar & Subramanian, 2007; Chojnacka *et al.*, 2006) and blood (Gill *et al.*, 2011) of smokers when compared with those of non-smokers. Chojnacka *et al.* (2006) Hoffmann *et al.* (2000), Frery *et al.* (1993), and Ellis *et al.* (1981) also found that smokers have elevated blood and scalp hair Cd levels when compared to non smokers. Similar results were also reported when the Cd mean value for smokers was compared with non-smokers by using a two tailed t-test ($t_{(148)} = 2.527$, $P < 0.01$) (refer to Appendix E). On the other hand, significant differences reported for Mn at $P < 0.05$ using a two-tailed t-test ($t_{(148)} = 2.367$, $P < 0.05$) were also found by using ANCOVA test but at $P < 0.1$ ($F_{(1,138)} = 3.417$, $P = 0.067$). Arsenic levels were found to be significantly different between smokers and non-smokers by using a two tailed t-test ($t_{(148)} = 2.544$, $P < 0.05$), whilst there is no significant effect observed using ANCOVA ($F_{(1,142)} = 0.205$, $P < 0.652$). This may be due to the significant effects of individual's age and drinking water on the level of As ($F_{(1,142)} = 17.176$, $P < 0.001$) and Mn ($F_{(1,142)} = 8.240$, $P < 0.01$), respectively (Table 4.11).

4.5.4.4 Influence of Age

Additionally, age and drinking water were selected to be covariant variables in order to evaluate whether these parameters provide any significant effects on the levels of trace elements in tear drops along with other factors. The results show that the effect of an individual's age was significant for Zn ($F_{(1,137)} = 6.373$, $P < 0.05$) and As ($F_{(1,142)} = 17.176$, $P < 0.001$) levels. No significant effects at ($P <$

0.05) were caused by the individual's age for other elements such as B ($F_{(1,118)}$ = 0.755, P = 0.387), V ($F_{(1,145)}$ = 3.186, P = 0.076), etc., as presented in Table 4.11.

4.5.4.5 Influence of Drinking Water

The relationship between the level of trace elements in drinking water and tear drops was investigated. The effect of drinking water elemental levels was significant for several trace elements in tear drops such as Sr ($F_{(1,140)}$ = 175.783, P < 0.001); V ($F_{(1,145)}$ = 13.305, P < 0.001) and Mn ($F_{(1,138)}$ = 8.240, P < 0.01). The strength and direction of these relationships were evaluated using correlation coefficient (r) analysis; the value of r was calculated and then subjected to a significance test. A strongly positive significant correlation is found for Sr levels between drinking water and tear drops (r = 0.760, $t_{(153)}$ = 14.224, P < 0.001), as shown in Figure 4.3. Higher levels of Sr were found in drinking water (tap water; n = 50; range: 0.078 – 2.110; mean: 1.113 mg/l Sr) as compared with those from London (tap water; n = 16; range: 0.006 – 0.357; mean: 0.168 mg/l Sr). Thus, higher levels of Sr were found in tear drop samples of healthy Karbala individuals (459 ± 255 µg/l Sr) when compared to London (62 ± 19 µg/l Sr). The possible explanation is that the population living in Karbala are exposed to high levels of strontium via drinking water. Strontium values are generally very high reflecting a vast number of possible industrial discharges (such as ceramic, glass products and paint pigments) as described in Chapter 3. Similar strong linear relationship was found by other researches between the Sr levels in drinking water and surface enamel (r = 0.97, P = 0.001) (Spector & Curzon, 1978). A previous study has reported that the highest levels of Sr in saliva were found in the areas where Sr in drinking waters was highest (Spector & Curzon, 1978). Vanadium (r = 0.30, $t_{(153)}$ = 3.160, P < 0.01) and Mn (r = 0.30, $t_{(146)}$ = 3.377, P < 0.01) are found to have a weakly positive significant correlation. In addition, no significant correlations observed for other elements, namely B, Cr, Fe, Cy, Zn, As and Cd.

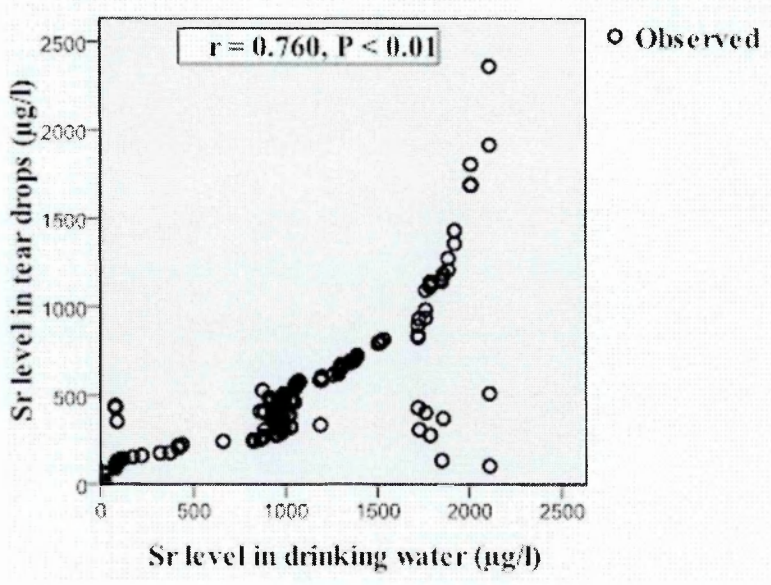

Figure 4.3: Correlation between strontium levels in tear drops and drinking water.

4.5.5 Interaction Effects

Generally, the interaction between different factors occurs when the effect(s) of one factor varies over the levels (groups) of another factor (Field, 2009). Part of the power of the analysis of variance and covariance is the ability to estimate the interaction effects. The interactions are very important, and the key to understanding them is being able to interpret interaction plots (Tabachnick & Fidell, 2007). A plot was performed for each significant effect of a factor on the trace element levels in tear drops using the adjusted means, as shown in Tables 4.13 – 4.15. In general, parallel lines indicate that there is no interaction between factors, whilst non-parallel lines mostly mean that the interaction is significant (Field, 2009). The interaction results between different factors such as health × smoking; health × gender; gender × smoking; and health × gender × smoking are reported in Table 4.12. Further information about the results of interaction can be found from ANCOVA summary tables in Appendix E.

4.5.5.1 Interaction Between Health Status and Smoking Activity

The results in Table 4.12 indicate that there are two significant interactions between health status and smoking activity for Zn ($F_{(1,137)} = 7.654$, $P < 0.01$) and Sr ($F_{(1,140)} = 8.165$, $P < 0.01$).

Table 4.12: Interaction effects between different factors for trace element levels in tear drops (outliers omitted).

Element	n	HS × G	HS × SA	G × SA	HS × SA× G
B	128	$F_{(1,118)+} = 0.841$, $P = 0.361$	$F_{(1,118)} = 0.209$, $P = 0.648$	$F_{(1,118)} = 3.200$, $P = 0.076$	$F_{(1,118)} = 3.328$, $P = 0.071$
V	155	$F_{(1,145)} = 0.001$, $P = 0.975$	$F_{(1,145)} = 0.027$, $P = 0.871$	$F_{(1,145)} = 0.234$, $P = 0.629$	$F_{(1,145)} = 0.425$, $P = 0.516$
Cr	151	$F_{(1,141)} = 0.812$ $P = 0.369$	$F_{(1,141)} = 0.920$, $P = 0.339$	**$F_{(1,141)} = 4.244$, $P = 0.041$***	$F_{(1,141)} = 0.107$, $P = 0.745$
Mn	148	$F_{(1,138)} = 0.407$, $P = 0.524$	$F_{(1,138)} = 0.986$, $P = 0.322$	$F_{(1,138)} = 1.891$, $P = 0.171$	$F_{(1,138)} = 0.888$, $P = 0.348$
Fe	148	$F_{(1,138)} = 0.486$, $P = 0.487$	$F_{(1,138)} = 0.642$, $P = 0.424$	$F_{(1,138)} = 3.011$, $P = 0.085$	$F_{(1,138)} = 2.174$, $P = 0.143$
Cu	155	$F_{(1,145)} = 1.108$, $P = 0.294$	$F_{(1,145)} = 3.084$, $P = 0.081$	$F_{(1,145)} = 1.998$, $P = 0.160$	$F_{(1,145)} = 0.392$, $P = 0.532$
Zn	147	$F_{(1,137)} = 1.285$, $P = 0.259$	**$F_{(1,137)} = 7.654$, $P = 0.006$****	$F_{(1,137)} = 0.024$, $P = 0.878$	$F_{(1,137)} = 0.081$, $P = 0.776$
As	152	$F_{(1,142)} = 0.052$, $P = 0.820$	$F_{(1,137)} = 0.035$, $P = 0.852$	$F_{(1,137)} = 0.429$, $P = 0.513$	$F_{(1,137)} = 0.429$, $P = 0.513$
Sr	150	$F_{(1,140)} = 0.719$, $P = 0.398$	**$F_{(1,140)} = 8.165$, $P = 0.005$****	$F_{(1,140)} = 0.344$, $P = 0.558$	**$F_{(1,140)} = 6.039$, $P = 0.015$***
Cd	150	$F_{(1,140)} = 1.242$, $P = 0.267$	$F_{(1,140)} = 0.285$, $P = 0.594$	$F_{(1,140)} = 0.734$, $P = 0.393$	$F_{(1,140)} = 0.005$, $P = 0.942$

n = number of samples, $^{+}$ *df* = degrees of freedom, *F* = calculated value of F-test, *P* = probability, HS = health status, G = gender, SA = smoking activity, the **bold** values indicate significant differences at the level of significance $^{*}P < 0.05$ and $^{**}P < 0.01$ (see Appendix E)

Table 4.13 and Figure 4.4 show the significant interaction between health status and smoking activity for Sr levels in tear drops. Although, the concentrations of Sr in the healthy and diabetic tear drops are similar for smokers, there is a big

difference for non-smokers. Therefore, the effect of health status was significant ($F_{(1,140)} = 5.388$, $P < 0.05$) in Table 4.11, as the mean value of Sr for diabetic cases (red line) is generally higher than healthy cases (blue line). This suggests that diabetic cases lead to higher Sr levels than healthy (Figure 4.4). On the other hand, the Sr mean values for smokers and non-smokers over the health status levels are roughly the same for Karbala population. Thus, the effect of smoking activity in Table 4.11 was not significant at $P < 0.05$ ($F_{(1,140)} = 0.041$, $P = 0.841$).

Table 4.13: The mean values of healthy individuals and diabetic patients across smoking activity groups for Sr levels in tear drop samples from Karbala (n = 150).

Health status	Smoking activity	Mean*	95% Confidence interval	
			Lower	Upper
healthy	smoker	613.868	470.351	757.386
	non-smoker	462.655	408.709	516.601
diabetic	smoker	600.499	459.254	741.744
	non-smoker	774.787	658.917	890.658

* Adjusted mean value which is determined at the arithmetic mean value for age = 36 years and Sr level in drinking water = 1069 µg/l.

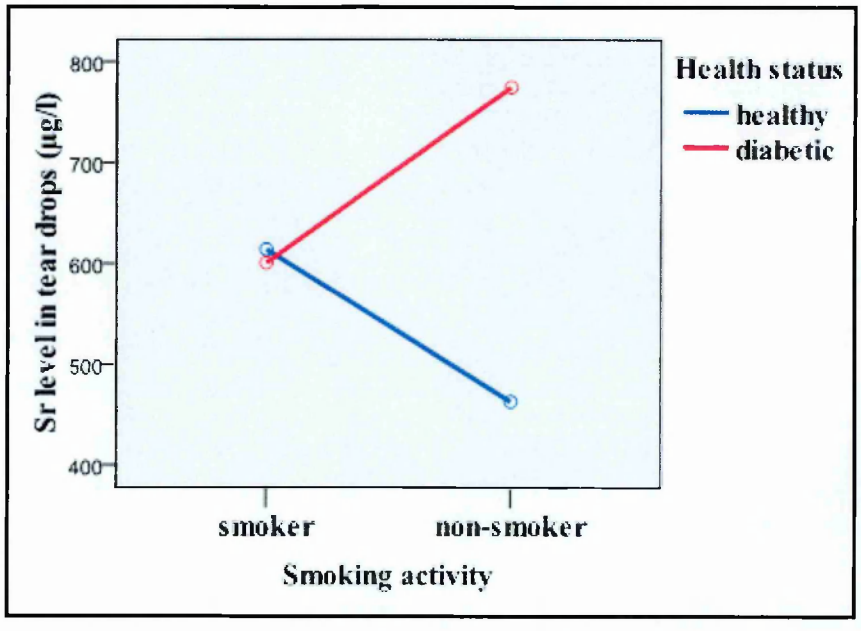

Figure 4.4: Interaction between health status and smoking activity for Sr levels (µg/l) in tear drop samples from Karbala (the data was taken from Table 4.13).

In the case of zinc, the results show that there are no effects for both factors on the levels of Zn in tear drops (Table 4.11), but cross-over interactions are found to be significant (Appendix E). No significant interactions ($P < 0.05$) were found between the health status and smoking activity for other elements, namely B, V, Cr, Mn, Fe, Cu, As and Cd, as presented in Table 4.12.

4.5.5.2 Interaction Between Health Status and Gender

In general, there was no significant interaction ($P < 0.05$) found between the health status and participants gender for all the trace elements in tear drops (Table 4.12). In other words, there is no change in the effect of health status over the levels of gender (male and female) and vice versa.

4.5.5.3 Interaction Between Gender and Smoking Activity

The results in Table 4.12 show that there is a significant interaction between gender and smoking activity for Cr ($F_{(1,141)} = 4.244$, $P < 0.05$), as shown in Table 4.14 & Figure 4.5. No significant effects ($P < 0.05$) were found in Table 4.11 for both factors on the levels of Cr in tear drops. In other words, both smokers and non-smokers have a very different effect on gender levels. Therefore, both effects destroy each other, but cross-over interactions are found to be significant. Furthermore, there was no significant interaction between gender and smoking activity found for other elements, as shown in Table 4.12.

Table 4.14: The mean values of males and females across smoking activity groups for Cr levels in tear drop samples from Karbala (n = 151).

Gender	Smoking activity	Mean*	95% Confidence Interval	
			Lower	Upper
male	smoker	10.874	4.860	16.887
	non-smoker	14.524	10.543	18.505
female	smoker	15.347	9.311	21.383
	non-smoker	8.994	6.172	11.816

*Adjusted mean value which is determined at the arithmetic mean value for age = 36 years and Cr level in drinking water = 0.5 µg/l.

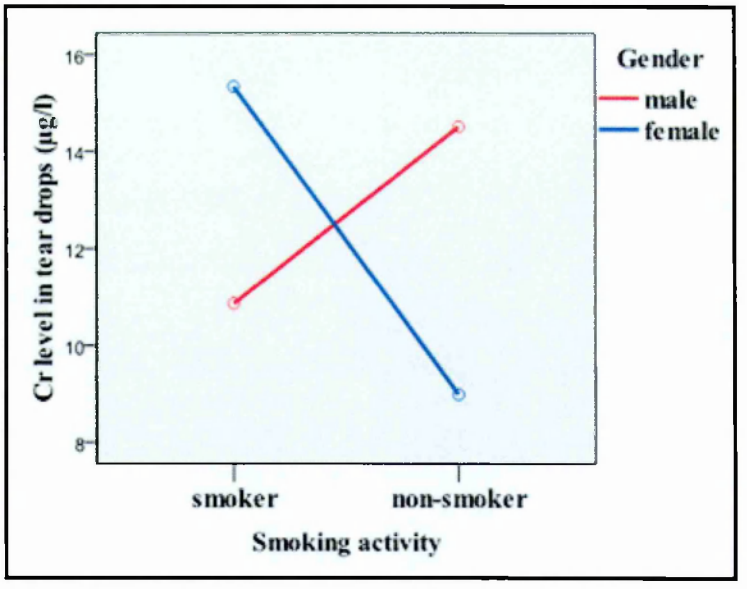

Figure 4.5: Interaction between gender and smoking activity for Cr levels (µg/l) in tear drop samples from Karbala (the data was taken from Table 4.14).

4.5.5.4 Interaction Between Health Status, Smoking Activity and Gender

The three-way interaction shows whether the health status × smoking activity interaction described above is the same for males and females. There is a significant three-way interaction between health status × smoking activity × gender for Sr levels ($F_{(1,140)}$ = 6.039, P < 0.05), as shown in Table 4.12. The nature of this interaction is presented in Table 4.15.

Figures 4.6 & 4.7 show the interaction between health status and smoking activity for males and females, respectively. The graph for male data shows the interaction between health status and smoking activity. For diabetic patients, the Sr mean value was lowest for smokers. For healthy individuals, however, the lowest Sr mean value occurs for non-smokers. This clearly suggests that healthy and diabetic subjects appear to respond differently to smoking activities, and that to explore the effect of smoking on the levels of Sr in tear drops, one must consider the health status of participants. The picture for females is quite different. For diabetic patients, there is no difference between smokers and non-smokers in terms of Sr levels (i.e. the Sr mean values are generally the same). In the case of healthy individuals, the levels of Sr are slightly lower for non-smokers than smokers. The data in Figure 4.7 suggests that there is unlikely to be a significant

interaction because the effect of smoking which is the same for healthy individuals and diabetic patients. Moreover, there are no significant three-factor interactions ($P < 0.05$) found for other elements in tear drops, as shown in Table 4.12.

Table 4.15: The mean values of healthy and diabetic for males and female across smoking activity groups for Sr levels in tear drop samples from Karbala (n = 150).

Health status	Gender	Smoking activity	Mean*	95% Confidence Interval	
				Lower	Upper
healthy	male	smoker	709.641	497.941	921.341
		non-smoker	451.234	372.250	530.218
	female	smoker	518.096	319.653	716.539
		non-smoker	474.076	407.054	541.099
diabetic	male	smoker	507.180	304.015	710.345
		non-smoker	856.926	681.732	1032.121
	female	smoker	693.818	513.415	874.221
		non-smoker	692.648	562.784	822.512

* Adjusted mean value which is determined at the arithmetic mean value for age = 36 years and Sr level in drinking water = 1069µg/l.

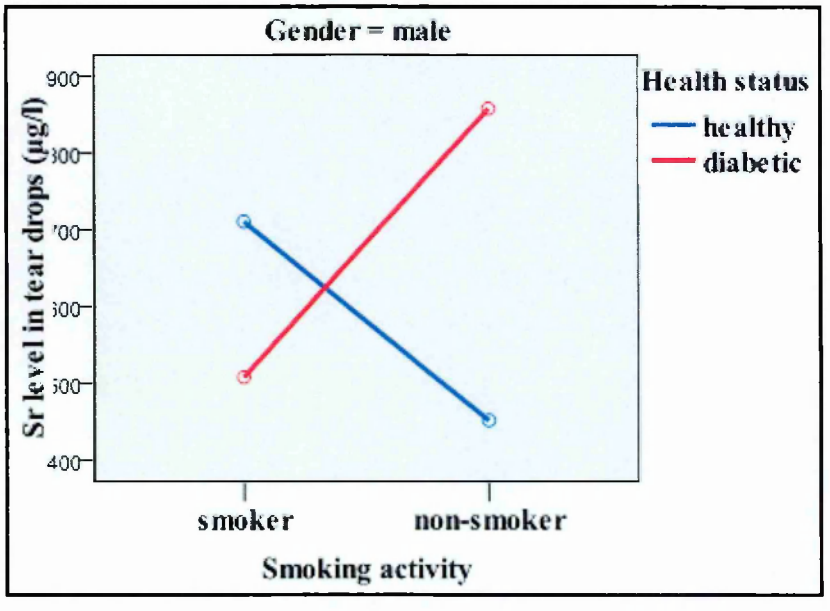

Figure 4.6: Interaction between health status and smoking activity for Sr levels (µg/l) in tear drop samples of males from Karbala (the data was taken from Table 4.15).

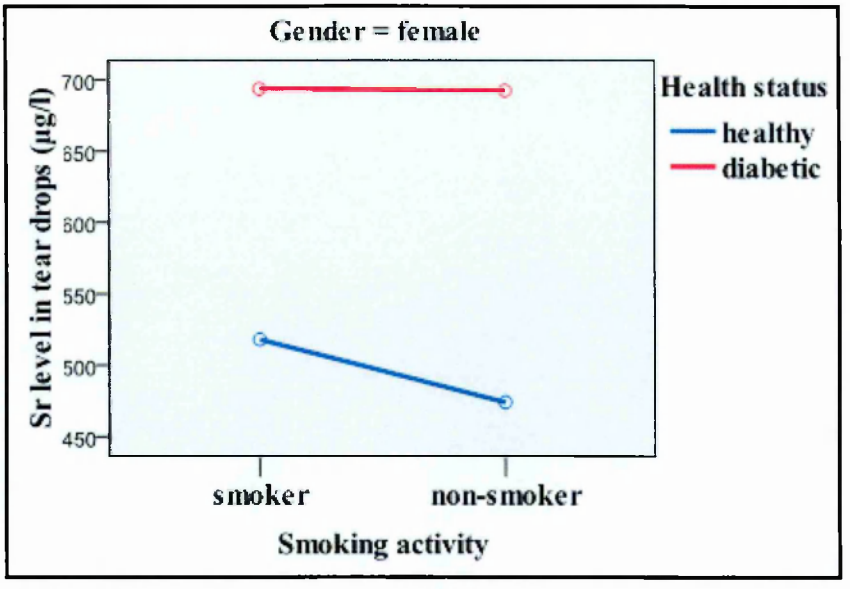

Figure 4.7: Interaction between health status and smoking activity for Sr levels (µg/l) in tear drop samples of females from Karbala (the data was taken from Table 4.15).

4.5.6 Significant Effect of Factors on Elemental Data

The results investigated above provide an indication of whether the difference between groups is statistically significant. The aim for most researchers is to find their results are significant (i.e. the factor effect on trace element levels is significant). This does not mean that the difference has any practical or theoretical significance; for example, with large samples, even very small differences between groups can become statistically significant (Field, 2009; Tabachnick & Fidell, 2007). Partial *eta* squared (η^2) was used to determine the strength of the significant effect for each factor on the level of trace elements (i.e. strength of relationship), as shown in Section 2.9.4.

Table 4.16 shows the η^2 values for the significant effects and interactions for health status, gender and smoking activity, and the effect of covariates (age and drinking water). There are four significant effects related to the levels of Sr in tear drops, namely drinking water: $\eta^2 = 0.557$ (55.7%); health status: $\eta^2 = 0.037$ (3.7%); interaction between health status and smoking activity: $\eta^2 = 0.055$ (5.5%); and interaction between health status, smoking activity and gender: $\eta^2 = 0.041$ (4.1%). Larger values of η^2 indicate a greater amount of variation caused by the

factor (Tabachnick & Fidell, 2007). The values of η^2 show that drinking water has a higher effect on the levels of Sr in tear drops when compared to other factors.

The results for other elements in Table 4.16 confirm that the major factors affecting the levels of trace elements in tear drops ($P < 0.05$) are: health status for B (9.6%), Mn (10.6%) and Sr (3.7%; drinking water for V (8.4%), Mn (5.6%) and Sr (55.7%); age for Zn (4.4%) and As (10.8%); gender for Fe (3.9%); and smoking for Cd (6.5%). Furthermore, some interactions between two or three factors can also make a major effect for trace elements, namely health status × smoking activity for Zn (5.3%) and Sr (5.5%); gender × smoking activity for Cr (2.9%); and health status × smoking activity × gender for Sr (4.1%). The η^2 value of other factors is approximately 0 for specific trace elements as they have a negligible amount of variation compared to the error term.

In the light of these results, health status and drinking water can be considered as the important factors for trace element levels in tear drops when compared to other factors, as shown in Table 4.16.

Table 4.16: Partial *eta* squared values for significant effects and interactions ($P <$ 0.05) of factors and **covariates** on the level of trace elements in tear drops from Karbala.

Effect	Partial *eta* squared (η^2)									
	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
Age	NS	NS	NS	NS	NS	NS	0.044	0.108	NS	NS
DW	NS	0.084	NS	0.056	NS	NS	NS	NS	0.557	NS
Health	0.096	NS	NS	0.106	NS	NS	NS	NS	0.037	NS
Gender	NS	NS	NS	NS	0.039	NS	NS	NS	NS	NS
Smoking	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.065
H * G	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
H * S	NS	NS	NS	NS	NS	NS	0.053	NS	0.055	NS
G * S	NS	NS	0.029	NS	NS	NS	NS	NS	NS	NS
H * G * S	NS	NS	NS	NS	NS	NS	NS	NS	0.041	NS

H = healthy, S = smoking activity, G = gender, DW = drinking water, * indication an interaction term, NS is not significant effect at $P < 0.05$ (refer to Appendix E).

4.5.7 Inter-Element Correlations

In the human body, the biological processes depend on the six major nutrient elements carbon, hydrogen, nitrogen, oxygen, sulphur, and phosphorus and are complemented by a selected group of other elements, usually metals or metalloids

present in trace quantities that serve critical cellular functions, such as enzyme co-factors (Berg *et al.*, 2007). Many biological processes are especially dependent on the essential trace elements to function correctly (Mertz, 1981). 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 (Hille, 2002; Lane & Morel, 2000); Cu and Mn (Gropper *et al.*, 2000) or Cu and Fe (Jameson & Ibers, 2007); Sr and Ca (Verberckmoes *et al.*, 2003); and As and P (Schoepp-Cothenet *et al.*, 2011; Wolfe-Simon *et al.*, 2011). Therefore, an investigation for any correlations between the elements analysed in this work was performed.

Outliers can have a dramatic effect on the correlation coefficient and make the *r* value much smaller than it should be, causing misleading results (Field, 2009; Tabachnick & Fidell, 2007); therefore, the outliers are removed from the data set, as described in Table 4.3. 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 particular pair of trace elements.

Pearson's Product Correlation Coefficient (*r*) was used to investigate the relationship between the trace element levels in tear drops for healthy individuals and diabetic patients from Karbala (Chojnacka *et al.*, 2005). This was investigated in order to evaluate which elements are correlated in tear drops and whether type 2 diabetes can be affecting inter-element relationships through a breakdown in metabolism or homeostatic regulations (Flores *et al.*, 2011). Different interpretations were suggested by researchers in terms of the values of *r* between 0 and 1 (further information see Section 2.9.6). 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 tear drops associated with healthy individuals and diabetic patients resident in Karbala are summarised in Tables 4.17 & 4.18, respectively.

4.5.7.1 Healthy Individuals

A total of 111-tear drop samples of healthy individuals were analysed for the trace elements under study using correlation analysis. Thirty-two of the examined 45

possible correlations were statistically significant after correlation for multiplicity, as shown in Table 4.17.

Table 4.17: Inter-element Pearson Correlation Coefficient (r) values for tear drops of healthy individuals from Karbala.

Element		B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	r	1.000									
	n	92									
V	r	NS	1.000								
	n	92	111								
Cr	r	NS	0.538**	1.000							
	n	89	107	107							
Mn	r	NS	0.488**	0.513**	1.000						
	n	87	105	103	105						
Fe	r	0.226*	0.514**	0.574**	0.638**	1.000					
	n	86	104	102	104	104					
Cu	r	NS	0.499**	0.581**	0.585**	0.496**	1.000				
	n	92	111	107	105	104	111				
Zn	r	NS	0.640**	0.478**	0.622**	0.384**	0.560**	1.000			
	n	88	106	102	101	100	106	106			
As	r	NS	0.244**	NS	NS	NS	NS	0.302**	1.000		
	n	92	111	107	105	104	111	106	111		
Sr	r	NS	0.453**	0.451**	0.606**	0.378**	0.483**	0.667**	NS	1.000	
	n	92	111	107	105	104	111	106	111	111	
Cd	r	NS	0.502**	0.401**	0.496**	0.408**	0.572**	0.650**	0.203*	0.404**	1.000
	n	88	106	102	102	101	106	102	106	106	106

** Correlation is significant at $P < 0.01$ level, * correlation is significant at $P < 0.05$ level, NS = no significant correlation at $P < 0.05$, n is the number of samples.

The highest correlation coefficient was found in tear drops between Zn-Sr (r = 0.667; $P < 0.01$) (Figure 4.8); Zn-Cd (r = 0.650; $P < 0.01$); V-Zn (r = 0.640; $P <$ 0.01); Mn-Fe (r = 0.638; $P < 0.01$); Mn-Zn (r = 0.622; $P < 0.01$); and Mn-Sr (r = 0.606; $P < 0.01$). Vanadium, Fe, Zn and Cd were statistically significantly correlated with the largest number of other elements (8 correlations), followed by Cr, Mn, Cu and Sr (7 correlations). Arsenic was statistically significantly correlated with three correlations with others (V, Zn and Cd), and B was correlated with Fe.

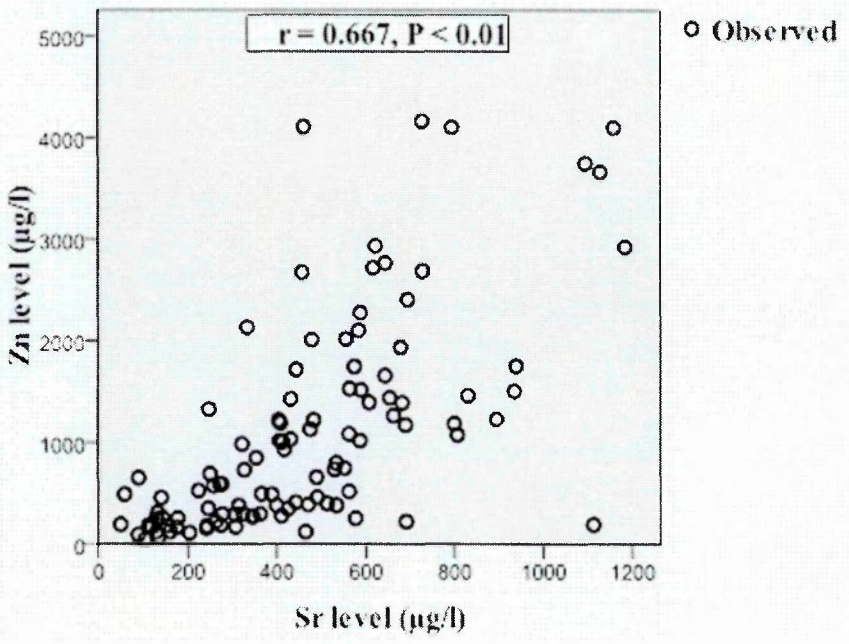

Figure 4.8: Correlation between zinc and strontium in tear drop samples from healthy individuals from Karbala (n = 106).

4.5.7.2 Diabetic Patients

A total of 44-tear drop samples from diabetic patients in Karbala were analysed for the trace elements under study using correlation analysis. Table 4.18 shows the correlations between trace element levels in tear drop samples for diabetic patients. There were 27 statistically significant correlations of the total 45 tested in tear drops. The strongest correlation, as indicated by the magnitude of r, is found between Cr-Mn ($r = 0.840$; $P < 0.01$), as shown in Figure 4.9. The elements with the most frequently statistically significant correlations were Cr and Mn (8 correlations), followed by Cu and Sr (7 correlations). All elements were statistically significantly correlated with at least three others.

4.5.7.3 Comparison Study

Comparison of correlation coefficients between healthy individuals and diabetic patients shows the following significant positive correlations ($P < 0.01$ or < 0.05) were found: V-Mn, V-Fe, V-As, Cr-Mn, Cr-Fe, Cr-Cu, Cr-Zn, Cr-Sr, Cr-Cd, Mn-

Fe, Mn-Cu, Mn-Zn, Mn-Sr, Mn-Cd, Fe-Cu, Fe-Sr, Cu-Zn, Cu-Cd, Cu-Sr, Zn-Sr and Zn-Cd, as shown in Tables 4.17 & 4.18. However, there is no difference between these correlations in both of the healthy individuals and diabetic subjects. Vanadium was correlated significantly with Cr, Cu, Zn, Sr and Cd; Fe with Zn and Cd; As with Zn and Cd; Sr with Cd and B with Fe in healthy populations rather than in diabetic patients. In contrast, there are several statistically significant correlations that were observed in tear drops of diabetic patients rather than healthy individuals, namely: B-Cr, B-Cu, B-Sr, As-Cr, and As-Mn. Figures 4.10 & 4.11 show the linear regression relationship between V-Zn in both population groups.

Table 4.18: Inter-element Pearson Correlation Coefficient (*r*) values for tear drops of diabetic patients.

Element		B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	r	1.000									
	n	36									
V	r	NS	1.000								
	n	36	44								
Cr	r	0.371*	NS	1.000							
	n	36	44	44							
Mn	r	NS	0.332*	0.840**	1.000						
	n	35	43	43	43						
Fe	r	NS	0.581**	0.393**	0.402**	1.000					
	n	36	44	44	43	44					
Cu	r	0.394*	NS	0.631**	0.432**	0.348*	1.000				
	n	36	44	44	43	44	44				
Zn	r	NS	NS	0.630**	0.592**	NS	0.611**	1.000			
	n	34	41	41	40	41	41	41			
As	r	NS	0.327*	0.547**	0.667**	NS	NS	NS	1.000		
	n	34	41	41	40	41	41	38	39		
Sr	r	0.431*	NS	0.574**	0.535**	0.329*	0.330*	0.337*	0.339*	1.000	
	n	32	39	39	38	39	39	38	36	39	
Cd	r	NS	NS	0.558**	0.457**	NS	0.428**	0.442**	NS	NS	1.000
	n	36	44	44	43	44	44	41	41	39	41

** Correlation is significant at $P < 0.01$ level, * correlation is significant at $P < 0.05$ level, NS = no significant correlation at $P < 0.05$, n is the number of samples.

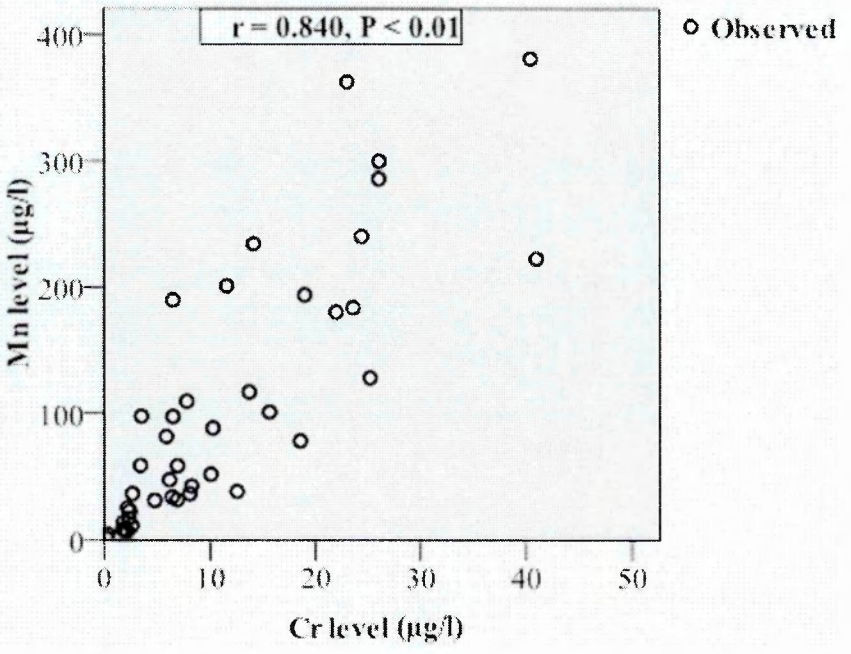

Figure 4.9: Correlation between manganese and chromium in diabetic tear drop samples (n = 43).

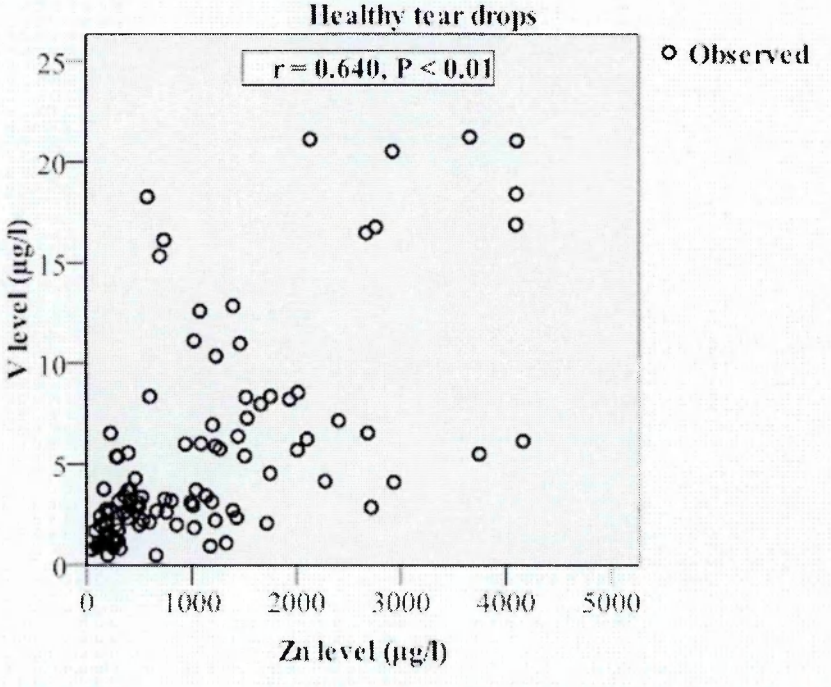

Figure 4.10: Correlation between vanadium and zinc in tear drop samples from healthy individuals in Karbala (n = 106).

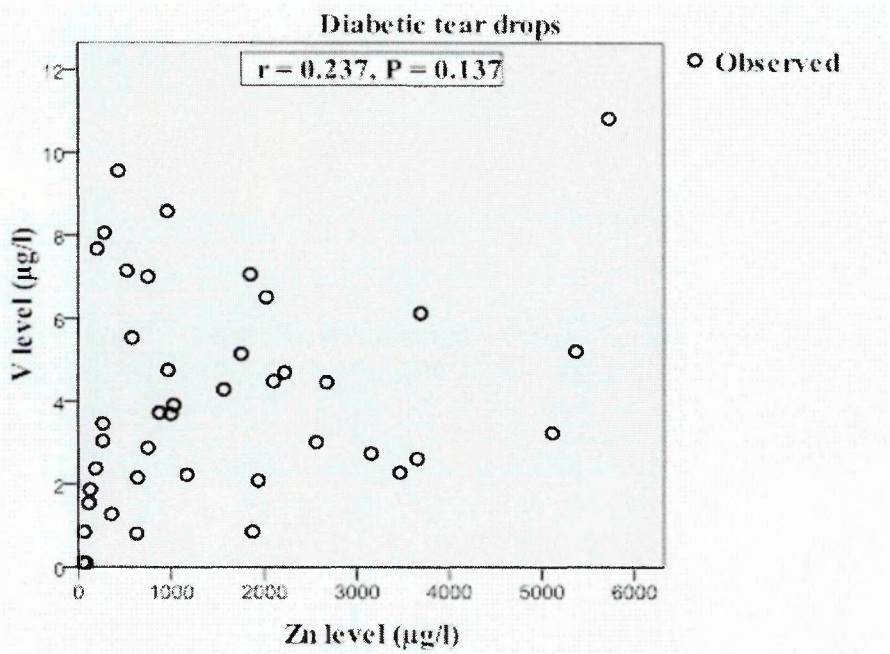

Figure 4.11: Correlation between vanadium and zinc in tear drop samples from diabetic patients in Karbala (n = 41).

Generally, inter-element interactions were discussed only in a few papers in the available literature (e.g. Flores *et al.*, 2011; Chojnacka *et al.*, 2010; Wang *et al.*, 2009; Shah, *et al.*, 2006; Chojnacka *et al.*, 2005; Forte *et al.*, 2005; Barany *et al.*, 2002; Faghihian & Rahbarnia, 2002; Vishwanathan *et al.*, 2002; Rodushkin & Axelsson, 2000; Georgescu *et al.*, 1998). For the group of healthy individuals, the following correlations have been found in blood serum between Zn-Cu ($r = 0.14$, $P < 0.01$) (Barany *et al.*, 2002), and in scalp hair between Zn-Mn ($r > 0.4$, $P < 0.05$) (Shah *et al.*, 2006). Chojnacka *et al.* (2010) reported the following statistically significant correlations between the elements in hair: V with Mn, Fe, Cu and As; Cr with Mn, Zn and Cd; Mn with Fe, Cu, Zn, Sr and Cd; Fe and Zn; Cu with Zn, Sr and Cd (Chojnacka *et al.*, 2010). Significant correlation was found between Mn–Sr ($r = 0.530$) in human scalp hair for healthy populations (Shah, *et al.*, 2006). Similar results were also found in scalp hair for Cu with B, V and As; Zn with Cd; Fe with Mn and Cr; Mn with Cr, V, B and Sr; Cr with B and As; and B with V and Cd (Chojnacka *et al.*, 2005).

In the case of diabetic patients, other researchers have found similar significant correlations in blood serum between: Mn-Cu ($r = 0.310$, $P < 0.05$), Zn-Cu ($r = 0.557$, $P < 0.01$) and Mn-Zn ($r = 0.394$, $P < 0.05$) (Flores *et al.*, 2011).

In this study, a large number of correlations were found between elements in tear drops for healthy individuals and diabetic patients, some of which have not been previously reported. Similar results were found by another study that reported 34 significant correlations between the trace element levels in blood serum (Barany *et al.*, 2002). The correlation between trace elements occurs according to their similarity of properties as well as co-occurrence in nature or common exposure sources (Rodushkin & Axelsson, 2000). However, to assess the implications of these correlations, several possible routes were investigated whereby such correlations could have arisen. Firstly, pre-analytical factors (e.g. contamination by two elements at the same time) and instrumental shortcomings (isotopic or polyatomic mass interferences, variations in instrument sensitivity, or blank signal) were considered (Barany *et al.*, 2002). Secondly, some trace elements are released into the environment by common sources that contribute to environmental pollution. These elements were significantly correlated (Chojnacka *et al.*, 2005). Finally, the correlation between two elements might be due to the first element affecting another or an additional variable affecting both elements. In addition, the majority of correlations between trace element levels in tear drops for healthy and diabetic populations were positive, as shown in Tables 4.18 & 19. There are two factors which could cause the positive correlations, namely exposure from the same source, or metabolic interactions such as binding to the same proteins (Barany *et al.*, 2002). At this time, there is no biological or mechanistical information available to explain many of the reported correlations, and more studies are needed in this area. This research therefore proposes a strong interdependence of various element levels in the tear drops matrix.

4.6 Summary

The results presented in this Chapter for elemental levels in human tear drop samples are summarised in Table 4.19, showing the descriptive statistics, namely, mean, standard deviation, geometric mean, median, range and the number of samples in each population group. The highest elemental level reported in the tear drops of healthy individuals and diabetic patients from Karbala was for Zn, (1075 ± 1032 µg/l) and (1536 ± 1520 µg/l), respectively. Similar results were found for

whole blood, serum and plasma as reported by other studies (Flores *et al.*, 2011; Stone, 2006).

Table 4.19: Summary of descriptive statistics of the elements measured in human tear drops for healthy individuals and diabetic patients from Karbala (Iraq) and healthy individuals from London (UK) (value in µg/l).

Element	n	Group	Mean ± SD	GM	Median	Range
B	92	HK	389 ± 158	355	383	< 70 - 898
	36	DK	606 ± 415	494	479	< 70 – 2020
	18	HL	216 ± 127	184	203	83 – 498
V	111	HK	5.6 ± 5.3	3.7	3.4	0.5 – 21.2
	44	DK	4.1 ± 2.6	3.1	3.7	0.1 – 10.8
	18	HL	0.7 ± 0.4	0.5	0.6	0.1 – 1.3
Cr	107	HK	11.2 ± 10.6	7.5	8.2	0.7 – 47.1
	43	DK	11.3 ± 10.4	6.5	7.3	0.2 – 40.9
	18	HL	4.6 ± 1.7	4.3	4.3	2.4 – 8.1
Mn	105	HK	41.7 ± 35.4	28.0	30.4	1.9 – 159
	43	DK	104 ± 103	51.9	58.8	0.8 – 381
	18	HL	6.8 ± 2.2	6.3	6.4	3.4 – 11.1
Fe	104	HK	499 ± 460	295	339	7 – 2060
	44	DK	577 ± 516	302	442	3 – 2003
	18	HL	159 ± 68	143	157	64 – 269
Cu	111	HK	268 ± 156	222	223	35 – 741
	44	DK	204 ± 145	128	190	1 – 594
	18	HL	227 ± 62	217	242	90 - 335
Zn	106	HK	1075 ± 1032	665	717	149 – 4164
	41	DK	1536 ± 1520	839	966	47 – 5726
	18	HL	188 ± 58	179	186	79 – 324
As	111	HK	8.3 ± 11.1	3.9	2.9	0.1 – 44.8
	41	DK	2.2 ± 1.4	1.7	2.1	0.2 – 5.5
	18	HL	1.4 ± 0.7	1.2	1.3	0.2 – 2.9
Sr	111	HK	459 ± 255	382	431	49 – 1183
	39	DK	757 ± 589	493	510	7 – 2361
	18	HL	62 ± 19	58	65	26 – 98
Cd	106	HK	1.9 ± 1.7	1.2	1.3	0.1 – 6.7
	44	DK	2.2 ± 2.1	1.4	1.5	0.1 – 8.4
	18	HL	3.8 ± 2.7	3.0	3.5	1.3 – 9.0

n = number of samples; SD = standard deviation; GM = geometric mean; HK = healthy Karbala; DK = diabetic Karbala; HL = healthy London.

The elemental levels were determined in healthy individuals from Karbala and were found to be significantly higher ($P < 0.05$) when compared with those from

London. There was no significant difference found for Cu between the two healthy groups, whilst the level of Cd was higher in London than Karbala (Table 4.19). The observed variations in elemental concentrations in tear drops of the two donor groups reflected different food habits, drinking water and geographic location as causatives that collectively affected individual variability and metabolic activity. The present work showed a marked trace element level in tear drop samples which is dependent on geographic location, environmental exposure and dietary habits of the donors.

The relationship between elemental level and health status is very strongly linked for many of the elements determined in this research. Discriminant function analysis was applied between three population groups (healthy and diabetic from Karbala and healthy from London) in order to find whether there were any differences between these groups and which elements could be used to discriminate the study populations. The results provided evidence that Sr, Mn, B, Zn, V, As and Cd in tear drops can be used to best discriminate (standardised coefficient > 0.3) between healthy individuals and diabetic patients. The model was able to correctly classify the 85% of cases and the 83% of cases after cross-validation. Thus, these findings suggest that DFA could be correctly applied to the type 2 diabetes as a diagnostic statistical test.

The results were compared with literature ranges for other human fluids such as blood serum and saliva, as described in Tables 4.5 & 4.6. In general, the results of healthy individuals from Karbala reported in this work are in agreement with those published by Flores *et al.* (2011) for V and Zn in blood serum; Muniz *et al.* (2001) for Zn in blood serum; Gil *et al.* (2011) for Cd, Cr and Mn in blood and saliva; Ward & Ward (1991) for Cu and Cd in saliva; and Stone (2006) for Fe in plasma and Zn in plasma and serum. The mean values for B, As and Sr are in disagreement with the reported literature range in serum (Flores *et al.*, 2011; Ward, 1993) and saliva (Kim *et al.*, 2010; Yuan *et al.*, 2008; Ward, 1993).

In comparison with diabetic results reported in the literature for blood serum, B, Cr, Zn, As, Sr and Cd levels were higher, whilst Fe and Cu were below the ranges reported in blood serum by Flores *et al.* (2011) and Stone (2006). The mean value of V falls within the reference range reported in plasma by Stone (2006).

The influence of various factors (health status, gender and smoking activity) and covariates (individual's age and drinking water) on elemental levels was

determined. The results were then subjected to ANCOVA and a two tailed t-test in order to check whether the effects of these factors and covariates are statistically significant. A statistical evaluation of the results are summarised in Table 4.20. The results of ANCOVA show that the influence of health status was significant on the level of B, Mn and Sr, whereas there were no significant effects for V, Cr, Fe, Cu, Zn, As and Cd (Table 4.20).

The effect of gender on the level of trace elements in the two populations from Karbala (healthy and diabetic) was investigated. It is known that trace element requirements and levels can differ between the genders. This may therefore also influence the trace element content of tear drops. This has been reported for other human fluids (Gil *et al.*, 2011) and tissues (Sukumar & Subramanian, 2007). Therefore, the study populations of Karbala were split into male and female sub-groups and the data was subjected to significance testing, as shown in Table 4.20. Of the elements under investigation in this research, the Fe levels were distinctly higher in males than females in the total population (healthy and diabetic) at $P < 0.05$. The results were in agreement with the findings of Gil *et al.* (2011), Sukumar & Subramanian (2007), Stone (2006) and Forte *et al.* (2005).

The influence of smoking activity on the trace element levels of human tear drops was examined and the results are reported in Table 4.10. The study population was split into smoker and non-smoker groups, and the significant differences were examined by using a two tailed t-test and ANCOVA, as shown in Table 4.20. It was found that Cd levels were significantly higher ($P < 0.05$) in tear drops for smokers when compared to non-smokers. The results for Cd are in full agreement with the majority of studies reported in the literature (Gil *et al.*, 2011; Sukumar & Subramanian, 2007; Chojnacka *et al.*, 2006; Hoffmann *et al.*, 2000; Ward, 1993).

The effect of age was evaluated to determine whether time played a role in the elemental levels present in human tear drops. A significant correlation was found between As and Zn levels and an individual's age using ANCOVA at $P < 0.05$. The remaining elements did not appear to have any significant relationship with age at $P < 0.05$. Similar results were also reported in the literature by other researchers (Sthiannopkao *et al.*, 2010; Sarah, 2009; Chojnacka *et al.*, 2006; Shah *et al.*, 2006).

The results of ANCOVA analysis found three significant relationships between drinking water and tear drops in terms of V, Mn and Sr at $P < 0.05$.

Table 4.20: Summary of reported significance results for trace element levels in tear drops of all populations from Karbala, (outliers omitted).

Element	n	Significant effect and interactions												
		Two-tailed t-test				ANCOVA								
		RL	HS	S	G	HS	G	S	Age	DW	H×S	H×G	S×G	H×S×G
B	146	Sig.	Sig.	NO^{+}	NO	Sig.	NO	NO	NO	NO	NO	NO	NO^{+}	NO^{+}
V	173	Sig.	Sig.	NO	NO	NO	NO	NO	NO^{+}	Sig.	NO	NO	NO	NO
Cr	169	Sig.	NO	NO	Sig.	NO	NO	NO	NO^{+}	NO	NO	NO	Sig.	NO
Mn	166	Sig.	Sig.	Sig.	NO	Sig.	NO	NO^{+}	NO	Sig.	NO	NO	NO	NO
Fe	166	Sig.	NO	NO	Sig.	NO	Sig.	NO	NO	NO	NO	NO	NO^{+}	NO
Cu	173	NO^{+}	Sig.	NO	NO	NO	NO	NO	NO^{+}	NO	NO^{+}	NO	NO	NO
Zn	165	Sig.	NO^{+}	NO	NO	NO	NO	NO	Sig.	NO	Sig.	NO	NO	NO
As	170	Sig.	Sig.	Sig.	NO	NO	NO	NO	Sig.	NO	NO	NO	NO	NO
Sr	168	Sig.	Sig.	NO	NO	Sig.	NO	NO	NO	Sig.	Sig.	NO	NO	Sig.
Cd	168	Sig.	NO	Sig.	NO	NO	NO^{+}	Sig.	NO	NO^{+}	NO	NO	NO	NO

n = total number of samples, ANCOVA = analysis of covariance, RL = residential location, HS = health status, S = smoking activity, G = gender, DW = drinking water, × = interaction, Sig. = significant effect/interaction at $P < 0.05$, NO = no significant effect/interaction at $P < 0.05$, $^{+}$ significant effect at $P < 0.1$.

The interactions between different factors such as health status, gender and smoking activity were determined by using ANCOVA. The results of Karbala population show that there are two significant interactions between health status and smoking activity for Zn and Sr at $P < 0.05$. There is a significant interaction between gender and smoking activity for Cr at $P < 0.05$. The interaction between health status, smoking activity and gender was significance for Sr at $P < 0.05$. The results are summarised in Table 4.20.

The correlation coefficient (r) was determined for each pair of elements in the two populations (healthy individuals and diabetic patients from Karbala). The results are presented in Tables 4.17 & 4.18 for healthy and diabetic individuals, respectively. Strong correlations, as indicated by the magnitude of r, were found between Zn-Sr ($r = 0.667$, $P < 0.01$) for healthy individuals and Cr-Mn ($r = 0.84$, $P < 0.01$) for diabetic patients. There were 32 and 27 statistically significant correlations of the total 45 tested in tear drops for healthy and diabetic cases, respectively. The elements with the most frequently statistically significant correlations were V, Fe, Zn and Cd for healthy individuals and Cr and Mn for diabetic patients. Similar results were also found for other biological media, namely blood serum, saliva and scalp hair (Flores *et al.*, 2011; Gill *et al.*, 2011; Barany *et al.*, 2002; Shah *et al.,* 2006).

In the light of these results, the present data can be used to establish a data base of normal levels for Iraqi individuals resident in Karbala as no study has been previously published in this region. This could act as baseline information for comparison with other countries and for the evaluation of future environmental pollution and possible human health studies in Iraq. Furthermore, tear drops could be used to determine the potential influence for health status, gender, age, smoking activity and residential location on the elemental levels in the human body. This can be used to investigate whether human tear drops can be used as a biomarker in the assessment of exposure to pollutants in an occupational and/or environmental setting in Iraq. In addition, the results can be used to evaluate or confirm previous data from published studies in order to asses whether there is any possible link between specific trace elements and type 2 diabetes.

Chapter Five

Trace Element Levels in Saliva, Washed Scalp Hair and Fingernails

5.0 Introduction

The level of trace elements in human saliva, washed scalp hair and fingernails are reported in this chapter, as shown in Sections 5.2 - 5.4. Samples were collected and prepared, as described in Sections 2.2.4 – 2.2.6. Methods were developed and validated, as outlined in Sections 2.3.2. Multi-elemental analysis was performed for boron (B), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), arsenic (As), strontium (Sr) and cadmium (Cd) in different media using an Agilent 7700 Series ICP-MS instrument with collision cell technology (CCT), as described in Section 2.6. The aim of this chapter was; to investigate whether human saliva, scalp hair, and fingernails could be used as a biomarker in the assessment of exposure to pollutants in an occupational and/or environmental setting; to compare the results with those obtained for tear drops collected earlier in this study; and to establish normal levels for Iraqi individuals which can be used for comparison with other countries and for evaluation of future environmental pollution and possible human health/disease studies in Iraq. The results are reported in Tables 5.1- 5.11. The main study was carried out on participants from Karbala (Iraq), whereas the subjects from London were used for a comparative study.

5.1 Statistical Methods of Analysis

Descriptive data analysis (arithmetic mean, standard deviation, range and 95% confidence interval) was performed on concentration values obtained for saliva, washed scalp hair and fingernails. The F-test and two tailed t-test were used to assess the significance of the variations in saliva, washed scalp hair and fingernails trace element levels between Karbala (Iraq) vs. London (UK) and healthy vs. diabetic populations (both from Karbala). The Pearson product correlation coefficient (r) calculation was carried out to evaluate associations between trace elements in different media, namely saliva, scalp hair and fingernails. A one-way ANOVA calculation was used when testing for differences between the four biological samples, as described in Section 2.9.3.

All statistical analysis was undertaken using the statistical package SPSS - version 19 (SPSS Inc., Chicago, IL, USA) (Miller & Miller, 2010; Field, 2009; Tabachnick & Fidell, 2007). See Appendix C for all equations.

5.2 Saliva

The main reasons for using saliva in this study are: (i) tear drops and saliva have similar properties, (namely non-invasive fluids) and have the same relative composition in terms of protein (lysozyme) and antibody (immunoglobulin) content; (ii) the transport media is water; and (iii) the nutritional source is the blood (Zhao *et al.*, 2010; Wang *et al.*, 2008). In addition, the use of saliva provides a data base of normal levels of trace elements for Iraqi individuals resident in Karbala (Iraq). This was established as no study has been published in the literature regarding Iraqi individuals living in Karbala.

Saliva has been used in the literature for multi-elemental analysis because of the ready access and non-invasive sampling nature (Wang *et al.*, 2008).

There are several limitations which have been reported in the literature in terms of the use of saliva as a biomarker for trace element levels in the human body: (i) variation in salivary flow-rates; (ii) potential blood contamination during sampling collection; (iii) lack of standard or certified reference materials (CRMs); (iv) the absence of reliable reference values for the human population; and (v) the presence of low concentrations of metal compounds (or trace elements) in saliva (Kim *et al.*, 2010; Esteban & Castaño, 2009; Barbosa *et al.*, 2006).

5.2.1 Elemental Composition of Saliva

The level of trace elements in unstimulated saliva samples are summarised in Table 5.1. The results are presented as an arithmetic mean, standard deviation, range, 95% confidence limit for mean value and the number of samples in the three populations (healthy Karbala, diabetic Karbala and healthy London). Some trace elements, namely B, Cr and Cd, were found to have several values below the reported limit of detection. Therefore, the mean values for these elements were not calculated.

Table 5.1: Population data for trace element levels (µg/l) in saliva from individuals resident in Karbala (Iraq) and London (UK).

Element	Variable	Concentration (µg/l)		
		Karbala		London
		Healthy (n = 43)	Diabetes (n = 29)	Healthy (n = 25)
B^+	Mean ± SD	nd	nd	nd
	Range	< 70 - 1254	< 70 – 332	< 70 - 575
	95% CI	nd	nd	nd
	n	39	27	14
V	Mean ± SD	0.43 ± 0.47	0.35 ± 0.31	0.16 ± 0.20
	Range	0.02 – 1.79	0.02 – 1.21	0.03 – 0.94
	95% CI	(0.28, 0.57)	(0.23, 0.47)	(0.07, 0.24)
	n	43	29	25
Cr^+	Mean ± SD	nd	nd	nd
	Range	< 0.1 – 0.86	< 0.1 – 0.86	< 0.1 – 0.53
	95% CI	nd	nd	nd
	n	34	23	6
Mn	Mean ± SD	3.72 ± 5.09	8.12 ± 9.09	1.38 ± 1.72
	Range	0.19 – 23.64	0.51 – 39.01	0.10 – 7.38
	95% CI	(2.15, 5.28)	(4.66, 11.57)	(0.67, 2.09)
	n	43	29	25
Fe	Mean ± SD	29.39 ± 31.73	22.84 ± 27.58	9.40 ± 8.26
	Range	1.80 – 110.50	1.30 – 131.40	0.70 – 34.70
	95% CI	(19.62, 39.15)	(12.35, 33.33)	(5.99, 12.81)
	n	43	29	25
Cu	Mean	14.49 ± 14.72	12.34 ± 9.29	24.43 ± 18.20
	Range	1.40 – 68.50	1.20 – 41.20	1.80 – 171.03
	95% CI	(9.96, 19.02)	(8.80, 15.87)	(8.68, 40.22)
	n	43	29	25
Zn	Mean ± SD	74 ± 82	73 ± 70	37 ± 41
	Range	7 – 402	4 – 288	1 – 178
	95% CI	(48, 99)	(46, 99)	(20, 54)
	n	43	29	25
As	Mean ± SD	3.03 ± 3.96	1.09 ± 0.64	0.36 ± 0.55
	Range	0.11 – 23.19	0.15 – 2.74	0.11 – 2.47
	95% CI	(1.81, 4.25)	(0.84, 1.33)	(0.13, 0.60)
	n	43	29	25
Sr	Mean ± SD	109.28 ± 213.89	190.34 ± 464.22	29.64 ± 26.01
	Range	2.24 – 1324.35	4.43 – 2545.12	2.34 – 114.13
	95% CI	(43.45, 175.11)	(13.84, 366.84)	(14.77, 44.51)
	n	43	29	25
Cd^+	Mean ± SD	nd	nd	nd
	Range	< 0.1 – 1.03	< 0.1 – 0.37	< 0.1 – 1.01
	95% CI	nd	nd	nd
	n	25	9	11

SD is standard deviation, CI is confidence interval for mean, n is the number of samples, $^+$ the levels of B, Cr and Cd in several samples were found below the limit of detection (B < 70, Cr and Cd < 0.1 µg/l), nd is not determined.

Although the elemental concentrations in biological samples vary considerably due to geographical differences, nutritional status, and environmental factors, the results for different elements have been compared with other results reported by several researchers, and the range of worldwide mean values determined by other researchers (Samanta *et al.*, 2004). The results are in general agreement with values reported by other authors, as described in Table 4.6. The only exceptions are for B and Sr as their levels in the majority of samples were above the literature values. A possible explanation is that the drinking and irrigation waters in Karbala (Iraq) have higher levels of these elements which could be attributed to the industrial environment, as reported in Chapter 3, Tables 3.1 – 3.4.

5.2.2 Results and Discussion

The comparative results of trace element levels in saliva for various populations were investigated, as presented in Table 5.1. In order to determine whether there are any significant differences that can be attributed to diabetic status and residential location, an F-test and a two-tailed t-test were undertaken on the saliva data from healthy and diabetic individuals, as reported in Appendix F. The effect of residential location on the distribution of trace elements in the two healthy populations (Karbala and London) was investigated in order to evaluate whether this factor may affect the elemental levels in saliva samples. In this study, saliva samples were collected from Iraqi individuals resident in Karbala (n = 43) and London (n = 25). In general, using a two-tailed t-test, the levels of V ($t_{(62)} = 3.26$, $t_{crit} = 1.99$, $P < 0.01$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at the probability level of $P = 0.05$), Mn ($t_{(56)} = 2.75$, $t_{crit} = 2.0$, $P < 0.01$), Fe ($t_{(51)} = 3.91$, $t_{crit} = 2.01$, $P < 0.001$), Zn ($t_{(65)} = 2.45$, $t_{crit} = 1.99$, $P < 0.05$), As ($t_{(45)} = 4.34$, $t_{crit} = 3.52$, $P < 0.001$) and Sr ($t_{(46)} = 2.38$, $t_{crit} = 2.01$, $P < 0.05$) of healthy individuals from Karbala were significantly higher than those reported for London, as shown in Appendix F (Table F1.4). On the other hand, there is no significant difference found for Cu between the two healthy populations at the level of significance $P < 0.05$. Similar results were reported for tear drops when the healthy individuals from Karbala were compared with those from London, as reported in Table 4.4.

As can be seen, the levels for most of the trace elements are higher in saliva samples from Karbala than those collected from London. This may be due to diet as found from the questionnaire information collected during sampling. Furthermore, the risk of environmental input has to be seriously considered in terms of the Karbala samples (UNEP, 2003); the population of Karbala use drinking water with higher levels of trace elements when compared with London drinking water, as described in Chapter 3.

There are many studies which have used other non-invasive media to evaluate whether there is any possible relationship between the elemental levels and several disease conditions (Esteban & Castano, 2009; Gellein *et al.,* 2008).

The study population from Karbala (Iraq) was divided into healthy and diabetic sub-groups and the data was subjected to significance testing. The results show that the As levels in saliva samples for healthy individuals were significantly higher than those for diabetic patients using a two-tailed t-test (As $t_{(45)} = 3.145$, $t_{crit} = 2.01$, $P < 0.01$). In contrast, Mn levels for healthy were lower than diabetic individuals (Mn $t_{(40)} = 2.37$, $t_{crit} = 2.02$, $P < 0.05$). No statistically significant differences were observed for V, Fe, Cu, Zn and Sr ($P < 0.05$) between healthy and diabetic, as shown in Appendix F (Table F1.3). Interestingly, similar results were reported for V, Mn, Cu, As and Sr in diabetic tear drops; although the differences between healthy and diabetic saliva samples did not reach the significance level of $P < 0.05$ for some trace elements. Similar findings have been reported by other authors (Flores *et al.,* 2011; Kamal *et al.,* 2009).

The range of trace elements (μg/l) for the saliva of healthy individuals from Karbala increases through the following sequence (Sr > B > Zn > Fe > Cu > Mn > As > V > Cd > Cr). In the case of diabetic patients from Karbala, the sequence is (Sr > B > Zn > Fe > Cu > Mn > As > V > Cr > Cd), whereas for healthy individuals from London, the sequence is (B > Zn > Cu > Sr > Fe > Mn > As > Cd > V > Cr). A box-plot was used to visually inspect the differences among the three populations (namely, healthy and diabetic individuals from Karbala and healthy from London), as shown in Figure 5.1.

Overall, the results show that factors such as lifestyle and type 2 diabetes could affect element levels in saliva. However, this media can be used as a biomarker for human health and environmental exposure with respect to trace element levels.

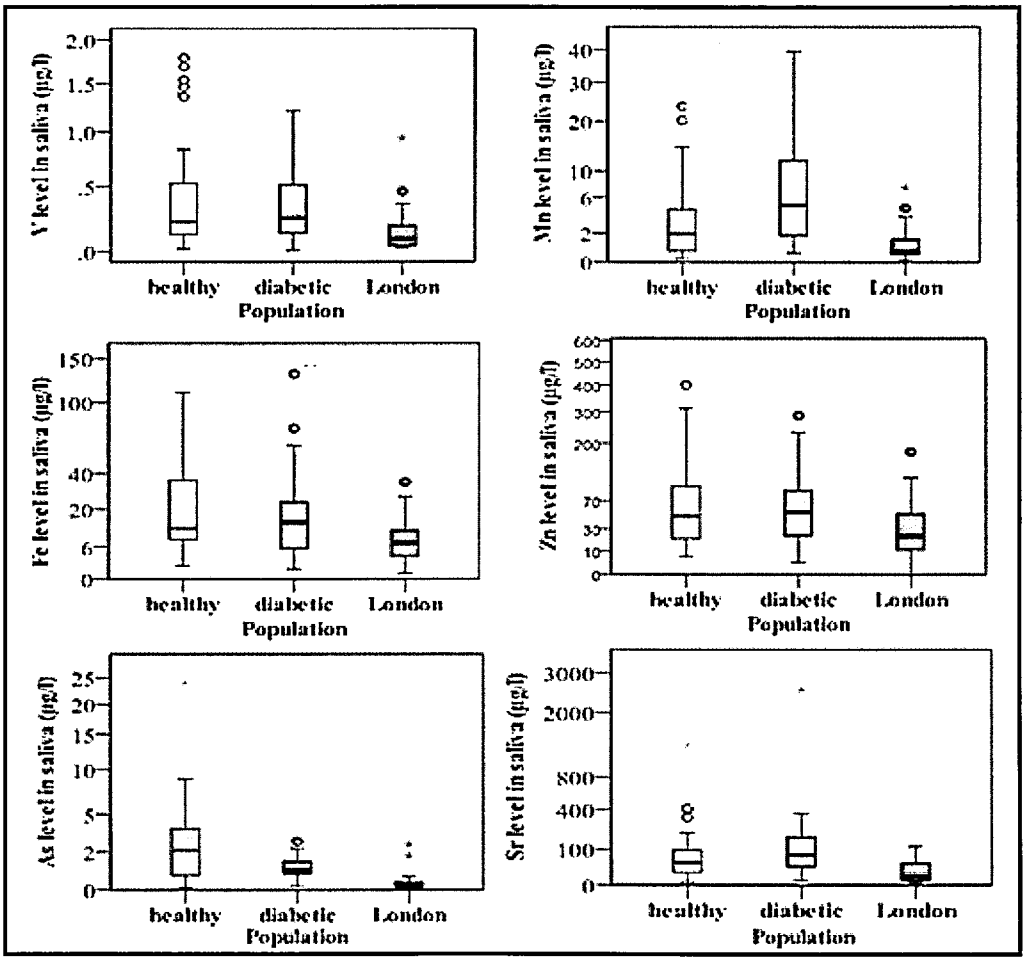

Figure 5.1: Box-plots for V, Mn, Fe, Zn, As and Sr levels in saliva samples for healthy individuals (n = 43) and diabetic patients (n = 29) from Karbala and healthy individuals (n = 25) from London. Middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represent extreme values (some extreme values were excluded from the figure in order to enlarge the scale; this did not change the relationship).

5.2.3 Inter-element Correlation of Saliva

A series of calculations were undertaken to evaluate the possible existence of significant ($P < 0.01$ or 0.05) inter-element correlations in saliva and tear drop samples from healthy and diabetic individuals resident in Karbala (Iraq). There were 32 and 27 statistically significant correlations of the total 45 tested in tear

drops for healthy individuals (refer to Table 4.17) and diabetic patients (refer to Table 4.18), respectively, whilst 37 and 11 statistically significant correlations of the total 45 tested in saliva samples for healthy individuals (Table 5.2) and diabetic patients (Table 5.3), respectively. Strong correlations, as indicated by the magnitude of r, are found between Zn-Sr ($r = 0.67$; $P < 0.01$) for tear drops (refer to Figure 4.17) and Zn-B ($r = 0.90$; $P < 0.01$) for saliva (Figure 5.2) of healthy individuals. For diabetic patients, strong correlations were found between Cr-Mn ($r = 0.84$; $P < 0.01$) for tear drops (refer to Figure 4.18) and Fe-Cr ($r = 0.70$; $P < 0.01$) for saliva, as shown in Figure 5.2. The elements with the most frequently statistically significant correlations were V, Fe, Zn and Cd (8 correlations) in tear drops of healthy individuals and Cr and Mn (8 correlations) in diabetic patients. In the case of saliva samples, Mn and Zn (9 correlations) and B, V, Fe and Sr (8 correlations) have the most frequently statistically significant correlation for healthy individuals, whilst Fe (4 correlations) was observed for diabetic patients. Such correlations were found in the biological samples from healthy individuals in Karbala, Iraq; namely, for saliva and tear drops, between:

- B-Fe;
- V with Cr, Mn, Fe, Cu, Zn, As and Sr;
- Cr with Mn, Fe, Cu, Zn and Sr;
- Mn with Fe, Cu, Zn, Sr and Cd;
- Fe with Cu, Zn and Sr;
- Cu with both Zn and Sr;
- Zn with As, Sr and Cd; and
- As-Cd.

Significant correlations were also found in both diabetic groups for tear drops and saliva between:

- B-Sr;
- V with Mn and Fe; and
- Cr with Fe and Cu.

Similar results were reported for Mn, Cu and Zn in saliva by another study (Wang *et al.*, 2008).

Table 5.2: Statistically significant correlations (r) between elements for saliva of healthy individuals (n = 43[+]).

TE	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.0									
V	0.699	1.0								
Cr	0.583	0.796	1.0							
Mn	0.376	0.487	0.410*	1.0						
Fe	0.755	0.727	0.586	0.493	1.0					
Cu	0.430	0.527	0.387*	0.637	0.598	1.0				
Zn	0.900	0.723	0.648	0.600	0.786	0.531	1.0			
As	0.508	0.325*	NS	0.388*	0.489	NS	0.602	1.0		
Sr	0.89	0.694	0.561	0.461	0.657	0.425	0.817	0.387*	1.0	
Cd	NS	NS	NS	0.420*	NS	NS	0.452*	0.595*	NS	1.0

[+] B (n = 39), Cr (n = 34), Cd (n = 25), NS = no significant correlation at $P < 0.05$, * correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$, TE is trace element.

Table 5.3: Statistically significant correlations (r) between elements for saliva of diabetic patients (n = 29[+]).

TE	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.0									
V	NS	1.0								
Cr	NS	0.677	1.0							
Mn	0.557	0.543	NS	1.0						
Fe	NS	0.527	0.701	NS	1.0					
Cu	NS	NS	0.497*	0.460*	0.373*	1.0				
Zn	NS	NS	NS	NS	NS	NS	1.0			
As	NS	NS	NS	NS	0.437*	NS	0.3980	1.0		
Sr	0.495	NS	NS	NS	NS	NS	NS	NS	1.0	
Cd	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0

[+] B (n = 27), Cr (n = 23), Cd (n = 9), NS = no significant correlation at $P < 0.05$, * correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$, TE is trace element.

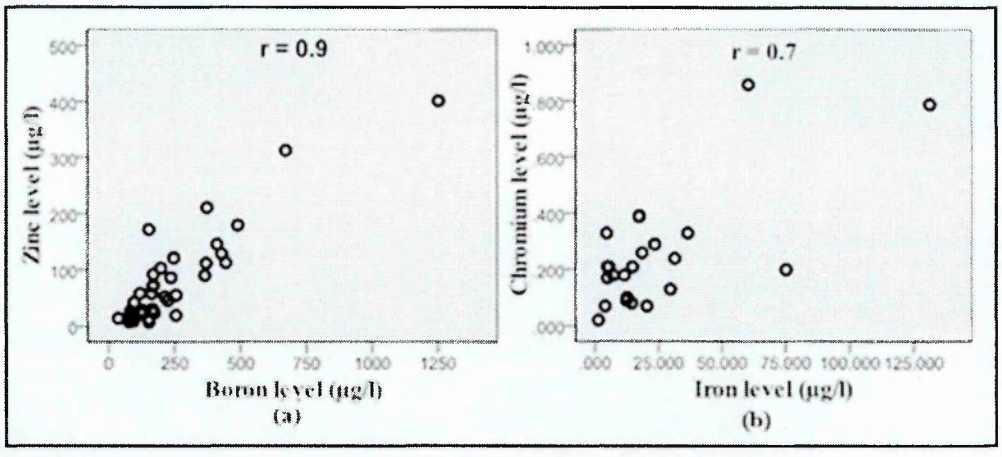

Figure 5.2: Correlation between (a) Zn and B for healthy individuals (n = 39) and (b) Cr and Fe for diabetic patients (n = 23) in saliva samples.

5.2.4 Comparison of Saliva and Tear drops

In this study 42 samples of tear drops and saliva were collected from the same healthy individuals. This sub-population was investigated to check whether any significant differences exist between the levels of trace elements in these media. An F-test and a two-tailed t-test were used to compare the two mean values for each element, as shown in Appendix F. In general, there are significant differences for all trace elements between tear drops and saliva. It was found that the levels of B ($t_{(66)} = 4.24$, $t_{crit} = 1.99$, $P < 0.001$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at $P = 0.05$), V ($t_{(42)} = 6.09$, $t_{crit} = 2.02$, $P < 0.001$), Cr ($t_{(41)} = 5.51$, $t_{crit} = 2.02$, $P < 0.001$), Mn ($t_{(41)} = 5.02$, $t_{crit} = 2.02$, $P < 0.001$), Fe ($t_{(41)} = 5.79$, $t_{crit} = 2.02$, $P < 0.001$), Cu ($t_{(42)} = 11.01$, $t_{crit} = 2.02$, $P < 0.001$), Zn ($t_{(41)} = 5.02$, $t_{crit} = 2.02$, $P < 0.001$), As ($t_{(52)} = 2.43$, $t_{crit} = 2.01$, $P < 0.05$), Sr ($t_{(78)} = 6.26$, $t_{crit} = 1.99$, $P < 0.001$) and Cd ($t_{(42)} = 4.24$, $t_{crit} = 2.02$, $P < 0.001$) were found significantly higher in tear drops when compared to saliva, as shown in Appendix F (Table F1.3).

In the light of these results, it can be seen that the trace element levels in saliva are far lower than those reported in tear drops. This result confirmed that the use of tear drops as a biomarker may be more meaningful than saliva, as several trace elements are elevated in tear drops. This is due to several limitations associated with the use of saliva as a biomarker, such as the potential blood contamination during sampling collection, very low concentration of analyte, and the

concentration of fluoride in toothpaste and amalgam fillings which may affect the elemental levels in saliva fluids (Wang *et al.*, 2008; Monaci *et al.*, 2002). In addition, the presence of metallic orthodontic appliances has the potential capability to increase the amount of elements in the saliva (Olmedo *et al.*, 2010). Figure 5.3 shows the mean and 95% confident interval of the mean for each element level in the tear drops and saliva for similar healthy individuals resident in Karbala.

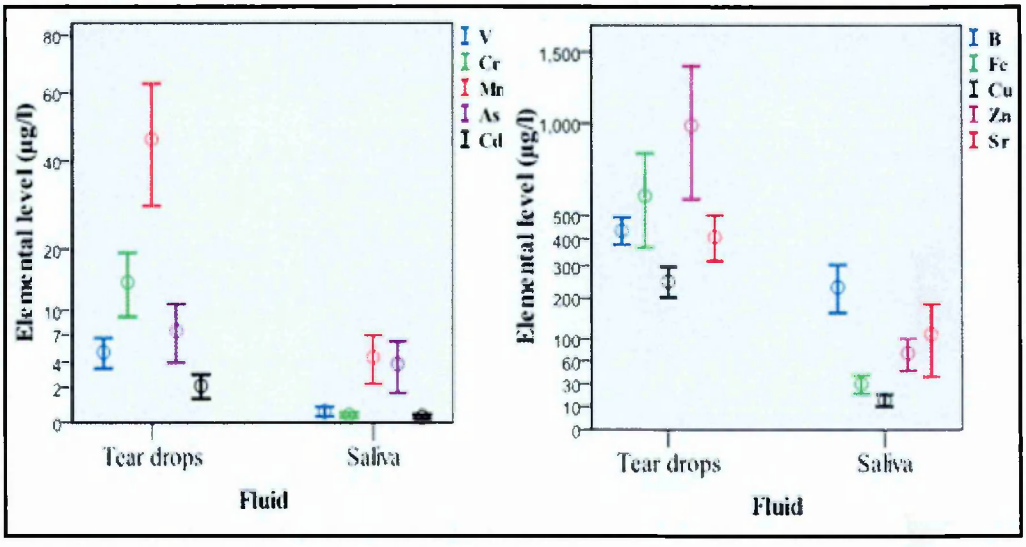

Figure 5.3: Elemental levels in tear drops and saliva for individuals from the healthy population of Karbala who provided both media. Circles represent mean value, whereas I represents 95% confidence interval.

5.3 Washed Scalp Hair

In the last two decades, human scalp hair has been used widely as a biomarker in the assessment of exposure to various pollutants in an occupational and/or environmental setting (Olmedo *et al.*, 2010; Esteban & Castano, 2009; Li *et al.*, 2008; Ohno *et al.*, 2007; Ashraf *et al.*, 1995) (as discussed in Chapter 1, Section 1.1.3). The main advantages of using scalp hair as a biomarker for trace element levels in the human body are: (i) it is a stable matrix; (ii) it does not show storage changes from the period between sampling and analysis; and (iii) it has long term potential for monitoring of past exposure (i.e. reflects the past exposure). On the other hand, the potential for external contamination and the failure to remove it completely by using different washing procedures can be considered the main

concerns associated with using scalp hair as a medium for assessing trace element status (Gil *et al.*, 2011).

5.3.1 Elemental Composition of Washed Scalp Hair

In total, 265 human scalp hair samples (refer to Appendix F) were collected from Iraqi individuals resident in Karbala (Iraq) (healthy individuals, n = 171 and diabetic patients, n = 44) and London (UK) (healthy individuals, n = 50) in order to determine the elemental composition of scalp hair. This can be used to investigate whether human scalp hair can play a significant role as a biomarker in the assessment of human health and environmental chemical exposure. Trace element levels (mg/kg dry weight, d.w.) in washed scalp hair samples for healthy individuals (HK) and diabetic patients (DK) resident in Karbala and healthy individuals (HL) from London are summarised in Tables 5.4. The data is reported as the mean, standard deviation, range, 95% confidence interval for mean and the number of samples. The highest elemental values in washed scalp hair were found for Zn (HK: 138 ± 87; DK: 86 ± 51; HL: 41 ± 4 mg/kg d.w. Zn), whilst the lowest was for As, as shown in Table 5.4. It was found that some of the trace elements in washed scalp hair samples were below the limit of detection, namely for B, Cr, Mn, As and Cd. A possible explanation is that the level of Zn, Fe, Cu and Sr are found at much higher levels in scalp hair when compared with the other elements under investigation. Therefore, the concentration of these elements cannot be detected by ICP-MS unless they were diluted. This dilution reduced the levels of other elements to below the limit of detection. As a result of the dilution factor used the mean values for Cr and As in the three populations; Mn for healthy London; and Cd for diabetic and healthy London were not determined. The results in this study are in general agreement with the literature ranges reported in Table 5.4 (Rodushkin & Axelsson, 2000). The only exception is for B, as the maximum value was higher when compared to the literature range. Similar findings have been previously reported by other researchers (Sukumar and Subramanian, 2007; Forte *et al.*, 2005).

Table 5.4: Population data for trace element levels (mg/kg) in washed scalp hair from individuals resident in Karbala (Iraq) and London (UK), along with literature range.

TE	Variable	Concentration (mg/kg, dry weight)			
		Karbala		London	Literature range* (n = 114)
		Healthy	Diabetes	Healthy	
B	Mean ± SD	nd	30 ± 30	10 ± 6	0.88 – 8.0
	Range	< 3.5 - 242	6 – 165	4 – 32	
	95% CI	nd	(21, 40)	(8, 12)	
	n	16+	44	50	
V	Mean ± SD	0.165 ± 0.129	0.005 ± 0.003	0.002 ± 0.001	0.005 – 160
	Range	0.010 – 0.740	0.001 – 0.012	0.001 – 0.006	
	95% CI	(0.146, 0.185)	(0.004, 0.006)	0.002 – 0.003	
	n	171	44	50	
Cr	Mean ± SD	nd	nd	nd	0.03 – 33
	Range	< 0.005 – 1.27	< 0.005 – 0.06	< 0.005 – 0.01	
	95% CI	nd	nd	nd	
	n	148+	21+	4+	
Mn	Mean ± SD	0.83 ± 0.66	0.02 ± 0.01	nd	0.03 – 50
	Range	0.13 – 3.85	0.01 – 0.07	< 0.005 – 0.08	
	95% CI	(0.73, 0.93)	(0.02, 0.03)	nd	
	n	171	44	8+	
Fe	Mean ± SD	13.58 ± 14.50	0.30 ± 0.22	0.07 ± 0.05	3 – 900
	Range	1.80 – 92.60	0.05 – 0.82	0.04 – 0.34	
	95% CI	(10.69, 16.47)	(0.23 – 0.37)	(0.06, 0.09)	
	n	171	44	50	
Cu	Mean	6.15 ± 3.26	0.57 ± 0.26	1.14 ± 1.29	0.3 – 293
	Range	1.80 – 27.90	0.17 – 1.31	0.36 – 6.41	
	95% CI	(5.50, 6.80)	(0.49, 0.65)	(0.65, 1.63)	
	n	171	44	50	
Zn	Mean ± SD	138 ± 87	86 ± 51	41 ± 4	40 – 327
	Range	36 - 602	12 – 148	29 – 50	
	95% CI	(125, 151)	(71, 102)	(40, 43)	
	n	171	44	50	
As	Mean ± SD	nd	nd	nd	0.015 - 26
	Range	< 0.005 – 0.19	< 0.005 – 0.06	< 0.005	
	95% CI	nd	nd	nd	
	n	119+	6+	0.0+	
Sr	Mean ± SD	6.45 ± 8.32	1.14 ± 1.09	0.38 ± 0.28	0.2 – 860
	Range	0.64 – 49.05	0.10 – 4.15	0.11 – 0.96	
	95% CI	(5.19, 7.70)	(0.80, 1.47)	0.30 – 0.46	
	n	171	44	50	
Cd	Mean ± SD	0.22 ± 0.34	nd	nd	0.02 – 16
	Range	0.02 – 3.12	< 0.005 – 2.06	< 0.005 – 0.70	
	95% CI	(0.15, 0.29)	nd	nd	
	n	171	11+	7+	

SD is standard deviation, CI is confidence interval for mean, + element has several samples were below the reported detection limit, n is the number of samples, nd is not determined, TE is trace element, * (Rodushkin & Axelsson, 2000).

The results of washed scalp hair were also compared with those reported in this study for tear drops and saliva. In general, the elemental levels for all of the trace elements in washed scalp hair are found to be far higher than those reported in tear drops and saliva; scalp hair is a long-term growth material, therefore most trace elements accumulate in the hair (Bermejo-Barrera *et al.*, 2002). As a result, scalp hair can provide some useful data, and reflect the body status over a long period of time (Sukumar and Subramanian, 2007).

5.3.2 Results and Discussion

The results for the three population groups, namely healthy Karbala (HK), diabetic Karbala (DK) and healthy London (HL) were compared using an F-test and a two-tailed t-test in order to investigate whether there are any significant differences between the different populations, as presented in Table 5.4. In general, the levels of V ($t_{(170)} = 16.55$, $t_{crit} = 1.97$, $P < 0.001$), Fe ($t_{(170)} = 12.18$, $t_{crit} = 1.97$, $P < 0.001$), Cu ($t_{(201)} = 16.21$, $t_{crit} = 1.97$, $P < 0.001$), Zn ($t_{(173)} = 14.45$, $t_{crit} = 1.97$, $P < 0.001$) and Sr ($t_{(171)} = 9.52$, $t_{crit} = 1.97$, $P < 0.001$) of healthy individuals resident in Karbala were significantly higher than those from London, as shown in Appendix F (Table F2.5). Similar results were reported for tear drops and saliva samples, as reported in Tables 4.4 & 5.1.

There were statistically significant differences in the levels of V ($t_{(170)} = 16.21$, $t_{crit} = 1.97$, $P < 0.001$), Mn ($t_{(171)} = 15.87$, $t_{crit} = 1.97$, $P < 0.001$), Fe ($t_{(170)} = 11.96$, $t_{crit} = 1.97$, $P < 0.001$), Cu ($t_{(178)} = 22.13$, $t_{crit} = 1.97$, $P < 0.001$), Zn ($t_{(213)} = 3.77$, $t_{crit} = 1.97$, $P < 0.001$) and Sr ($t_{(190)} = 8.08$, $t_{crit} = 1.97$, $P < 0.001$) between the healthy individuals and diabetic patients resident in Karbala, as shown in Appendix F (Table F2.4). Similar results were reported for V and Cu in tear drops (refer to Table 4.5). The results show that the level for most trace elements is higher in scalp hair samples of healthy individuals when compared to diabetic patients. The bioaccumulation of trace elements in human hair is a complicated process influenced by several factors during hair growth, namely metabolic changes, age, gender and living environment quality (Samanta *et al.*, 2004; Wolf-sperger *et al.*, 1994).

5.3.3 Inter-element Correlation of Washed Scalp Hair

Correlation analysis was performed on washed scalp hair data of healthy individuals and diabetic patients in order to describe the strength and direction of possible linear relationships between the trace element levels. A Pearson product correlation coefficient (r) was used for this purpose and the results are summarised in Tables 5.5 & 5.6. There was found to be 22 statistically significant correlations between trace elements for the washed scalp hair of healthy individuals, whilst 10 significant correlations were found in diabetic patients. The most highly significant correlations, as indicated by the magnitude of r, were found to exist between Mn-Cr ($r = 0.584$; $P < 0.01$) for healthy individuals and Mn-Sr ($r = 0.677$; $P < 0.01$) for diabetic patients, as shown in Figure 5.4.

Similar correlations were found in the biological samples from healthy individuals in Karbala, Iraq, namely for washed scalp hair and tear drops, between:

- V with Cr, Mn, Fe, Cu, Zn, As, Sr and Cd;
- Cr with Mn, Fe and Cu;
- Mn with Fe, Cu, Zn and Sr;
- Fe-Cu;
- Zn-Sr; and
- Sr-Cd.

Significant correlations were also found in both diabetic groups for tear drops and washed scalp hair between:

- B-Cu;
- V-Mn;
- Cr-Cu;
- Mn with Cu and Sr; and
- Zn-Sr.

In comparison with the literature values, similar correlations were found in scalp hair between Fe-Mn and Fe-Cu (Hill, 2009); Mn-Sr, V-Cu, V-Mn, Cr-Fe, Cr-Mn, Mn-Cu, Zn-Sr and Sr-Cd (Chojnacka *et al.*, 2005); Mn-Sr (Shah *et al.*, 2006); Cu with B and As, Fe-Mn and Cr-As (Chojnacka *et al.*, 2010).

Table 5.5: Statistically significant correlations (r) between elements for washed scalp hair of healthy individuals (n = 171[+]).

TE	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.00									
V	NS	1.00								
Cr	NS	0.437*	1.00							
Mn	NS	0.562	0.584	1.00						
Fe	NS	0.343	0.526	0.498	1.00					
Cu	NS	0.281	0.387	0.209	0.212	1.00				
Zn	NS	0.508	NS	0.284	NS	NS	1.00			
As	NS	0.289	0.318	0.474	0.566	0.242	NS	1.00		
Sr	NS	0.453	NS	0.379	NS	NS	0.481	NS	1.00	
Cd	NS	0.200*	NS	NS	NS	NS	NS	NS	0.329	1.00

[+] B (n = 16), Cr (n = 148) and As (n = 119), NS = no significant correlation at $P < 0.05$, *correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$ level, TE is trace element.

Table 5.6: Statistically significant correlations (r) between elements for washed scalp hair of diabetic patients (n = 44[+]).

Element	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.00									
V	NS	1.00								
Cr	NS	NS	1.00							
Mn	NS	0.507	NS	1.00						
Fe	NS	NS	NS	NS	1.00					
Cu	0.309*	0.454	0.496*	0.306*	NS	1.00				
Zn	NS	0.609	NS	NS	0.427*	NS	1.00			
As	NS	NS	NS	NS	NS	NS	NS	1.00		
Sr	NS	0.657	NS	0.677	NS	NS	0.62	NS	1.00	
Cd	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0

[+] Cr (n = 21), As (n = 6) and Cd (n = 11), NS = no significant correlation at $P < 0.05$, *correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$ level.

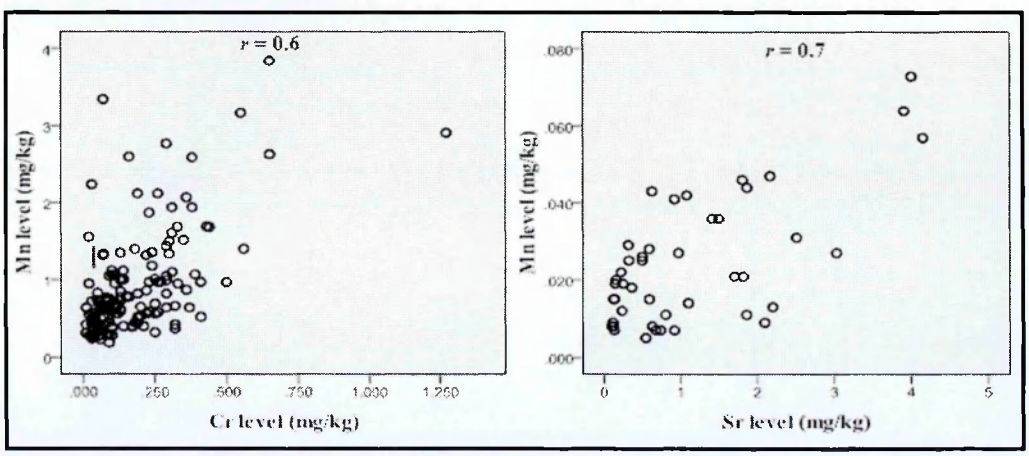

Figure 5.4: Correlation between (a) Mn and Cr for healthy individuals (n = 148), (b) Mn and Sr for diabetic patients (n = 44) in the washed scalp hair samples.

5.3.4 Comparison of Trace Element Levels of Tear Drops and washed Scalp Hair.

In total 50 tear drop and scalp hair samples were collected from the same healthy individuals in order to test whether there are any significant differences between the trace element levels in both media. An F-test and a two-tailed t-test were used to compare the two mean values for each element in the two media. It was found that there are significant differences for all trace elements between tear drops and washed scalp hair. The levels of V ($t_{(49)} = 9.28$, $t_{crit} = 2.01$, $P < 0.001$), Cr ($t_{(48)} = 7.68$, $t_{crit} = 2.01$, $P < 0.001$), Mn ($t_{(50)} = 11.75$, $t_{crit} = 2.01$, $P < 0.001$), Fe ($t_{(50)} = 9.31$, $t_{crit} = 2.01$, $P < 0.001$), Cu ($t_{(49)} = 17.44$, $t_{crit} = 2.01$, $P < 0.001$), Zn ($t_{(49)} = 10.05$, $t_{crit} = 2.01$, $P < 0.001$), As ($t_{(32)} = 4.50$, $t_{crit} = 2.04$, $P < 0.001$), Sr ($t_{(49)} = 6.37$, $t_{crit} = 2.01$, $P < 0.001$) and Cd ($t_{(49)} = 4.37$, $t_{crit} = 2.01$, $P < 0.001$) were found to be significantly higher in washed scalp hair than tear drops, as shown in Appendix F (Table F2.6). The levels of B for most hair samples were below the limit of detection; therefore, the comparison was not established for B between tear drops and scalp hair.

In the light of these results, it can be seen that the trace element levels in tear drops are far lower than those reported in washed scalp hair. Figure 5.5 shows the mean and 95% confidence interval for mean (lower-upper limits) for each element level in the tear drops and washed scalp hair for similar healthy individuals resident in Karbala.

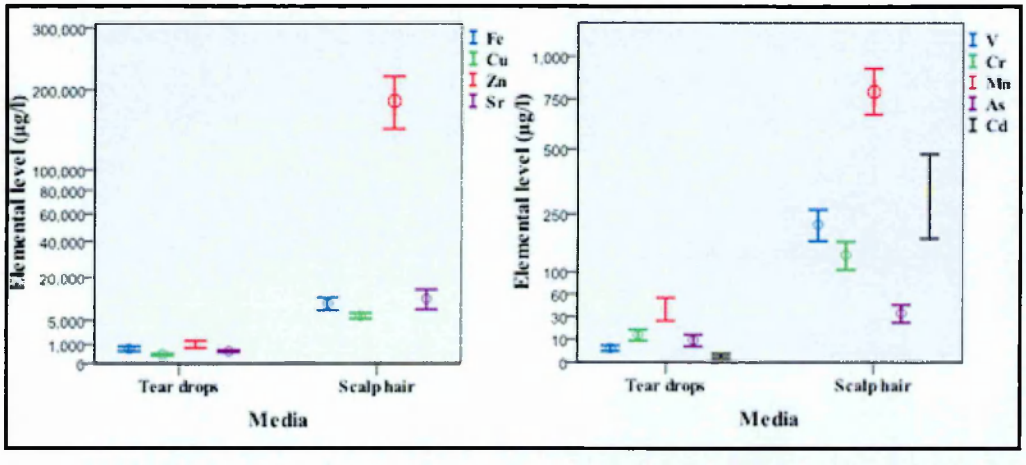

Figure 5.5: Elemental level in tear drops and washed scalp hair for individuals from the healthy population of Karbala who provided both media. Circles represent mean value, whereas I represents 95% confidence interval.

5.4 Washed Fingernails

Recently, human fingernail tissue has been recognised as an invaluable tissue for the assessment of exposure to various pollutants in an occupational and/or environmental setting. It provides a useful indication of exposure to many toxic and essential trace elements over a long period of time, as this material remains isolated from any metabolic activity in the human body. Thus, they are considered to be a finger-print of the body's trace element levels over a period of time, which is not possible with materials such as blood (Olmedo *et al.*, 2010; Batista *et al.*, 2008; Samanta *et al.*, 2004; Nowak & Chmielnicka, 2000). Previous studies have reported that the levels of trace elements in fingernail tissue were found to be higher than those of body fluids and other accessible tissues (Sukumar & Subramanian, 2007; Rodushkin & Axelsson, 2000).

Fingernail material has many useful advantages for trace element research than other biological media, namely: is a stable matrix; does not show storage changes from the period between sampling and analysis; and the potential for external contamination is lower when compared with scalp hair (Gil *et al.*, 2011).

5.4.1 Elemental Composition of Washed Fingernail

In this study, 259 fingernail samples (refer to Appendix F) were provided by Iraqi individuals (healthy (n = 127) and diabetic (n = 87)) resident in Karbala (Iraq) and healthy (n = 45) from London (UK). A major problem involves the limited sample mass provided by conventional collection methods, particularly children's samples. Table 5.7 compares the trace element levels (mean, standard deviation, range and 95% confidence interval) in the fingernail samples of healthy individuals (FHK) and diabetic patients (FDK) from Karbala, and healthy individuals (FHL) from London. The highest mean values in washed fingernails are reported for Zn (FHK: 89 ± 54; FDK: 73 ± 42; FHL: 78 ± 25 mg/kg d.w. Zn). Similar results were reported for tear drops, washed scalp hair and fingernails in this study. The levels of B and Cd in the three populations, Fe in the two healthy populations, and Cr, Mn and As in healthy individuals from London were found to have several samples under the limit of detection for these elements (Table 5.7), as described in scalp hair results (see Section 5.3.1).

Table 5.7: Population data for trace element levels in washed fingernails from individuals resident in Karbala (Iraq) and London (UK), along with literature range.

TE	Variable	Concentration (mg/kg)			
		Karbala		London	Literature range*
		Healthy	Diabetes	Healthy	
B	Mean ± SD	nd	nd	nd	0.12 – 3.33
	Range	< 3.5 – 44	< 3.5 – 16	< 3.5	
	95% CI	nd	nd	nd	
	n	10+	6+	0.0+	
V	Mean ± SD	0.164 ± 0.175	0.141 ± 0.086	0.026 ± 0.033	0.018 – 0.476
	Range	0.010 – 0.900	0.010 – 0.400	0.001 – 0.169	
	95% CI	(0.133, 0.195)	(0.122, 0.159)	0.016 – 0.036	
	n	127	87	45	
Cr	Mean ± SD	0.40 ± 0.48	0.53 ± 0.59	nd	0.224 – 3.20
	Range	0.01 – 3.45	0.02 – 4.12	< 0.005 – 0.85	
	95% CI	(0.32, 0.49)	(0.40, 0.66)	nd	
	n	127	87	17+	
Mn	Mean ± SD	2.27 ± 3.50	1.29 ± 1.18	nd	0.19 – 3.30
	Range	0.05 – 19.08	0.05 – 7.11	< 0.005 – 1.24	
	95% CI	(1.65, 2.88)	(1.04, 1.55)	nd	
	n	127	87	21+	
Fe	Mean ± SD	nd	73.1 ± 55.7	nd	12 - 189
	Range	< 0.025 – 326.94	2.8 – 263.71	< 0.025 – 37.18	
	95% CI	nd	(61.7, 85.5)	nd	
	n	103+	87	2+	
Cu	Mean	5.13 ± 6.25	0.69 ± 0.69	3.84 ± 1.23	4.2 - 17
	Range	0.44 – 61.31	0.01 – 4.29	2.36 – 7.63	
	95% CI	(4.04, 6.23)	(0.54, 0.84)	(3.47, 4.21)	
	n	127	87	45	
Zn	Mean ± SD	89 ± 54	73 ± 42	78 ± 25	80 - 191
	Range	8 – 427	1 – 277	38 – 155	
	95% CI	(80, 98)	(64, 82)	(70, 85)	
	n	127	87	45	
As	Mean ± SD	0.10 ± 0.13	0.14 ± 0.19	nd	0.065 – 1.09
	Range	0.01 – 1.16	0.02 – 1.69	< 0.005 – 1.26	
	95% CI	(0.08, 0.12)	(0.10, 0.18)	nd	
	n	127	87	35+	
Sr	Mean ± SD	4.38 ± 3.93	5.33 ± 4.79	0.82 ± 0.61	0.17 – 1.39
	Range	0.29 – 23.46	0.16 – 17.43	0.10 – 9.56	
	95% CI	(3.69, 5.07)	(4.30, 6.35)	0.34 – 1.29	
	n	127	87	45	
Cd	Mean ± SD	nd	nd	nd	0.013 – 0.438
	Range	< 0.005 – 1.71	< 0.005 – 1.42	< 0.005 – 0.33	
	95% CI	nd	nd	nd	
	n	94+	62+	28+	

SD is standard deviation, CI is confidence interval for mean, + element has several samples were below the reported detection limit, n is the number of samples, nd is not determined, TE is trace element, * (Rodushkin & Axelsson, 2000).

The mean values for most trace elements are within the literature ranges reported by other researchers, the only exception was for Sr (Rodushkin & Axelsson, 2000), as shown in Table 5.7. The high level of Sr for fingernail samples was also reported for tear drops and saliva from healthy individuals when compared with the literature values, as discussed in Section 5.2.1. In addition, the maximum values for B, Fe and Cd were found to be higher than the maximum values of the literature ranges (Table 5.7).

As described in the scalp hair discussion, the results of the trace element levels in washed fingernails are found to be far higher than those reported in tear drops and saliva (Bermejo-Barrera *et al.*, 2002). Thus, fingernail tissue can provide good data, and reflect the body status over a period of time (Sukumar & Subramanian, 2007).

5.4.2 Results and Discussion

Washed fingernails of healthy Karbala (FHK), diabetic Karbala (FDK) and healthy London (FHL) residents were compared to investigate whether there were any significant differences between the trace element levels of the three populations. In general, the levels for most trace elements in several fingernail samples collected from London were found to be below the limit of detection for these elements (except V, Cu, Zn and Sr) (Table 5.7). Thus, the comparison using an F-test and a two-tailed t-test was not established for these elements between the two healthy populations from Karbala and London. The levels of V ($t_{(148)} = 8.43$, $t_{crit} = 2.01$, $P < 0.001$) Cu ($t_{(150)} = 2.23$, $t_{crit} = 1.97$, $P < 0.05$), and Sr ($t_{(167)} = 8.44$, $t_{crit} = 1.97$, $P < 0.05$) of healthy individuals resident in Karbala were significantly higher than those from London ($P < 0.05$), as shown in Appendix F (Table F3.8). Similar results were reported for tear drops saliva and washed scalp hair samples, as reported in Tables 4.2, 5.1 & 5.4, respectively. In the light of this result, it would appear that factors like environmental exposure, food program and drinking water can affect the distribution of trace elements in various biological samples, namely: fingernails.

In comparison to diabetic patients, there are statistically significant differences in the levels of Mn ($t_{(165)} = 2.90$ $t_{crit} = 1.97$, $P < 0.01$), Cu ($t_{(130)} = 7.94$, $t_{crit} = 1.98$, $P < 0.001$) and Zn ($t_{(212)} = 2.29$, $t_{crit} = 1.97$, $P < 0.05$). Similar results have also been

reported in this study for tear drops in terms of Cu and washed scalp hair for Mn, Cu and Zn. No statistically significant differences are observed between the washed fingernails of healthy individuals and diabetic patients for other elements ($P < 0.05$), namely V ($t_{(196)} = 0.1.22$, $P = 0.22$), Cr ($t_{(212)} = 1.78$, $P = 0.33$), As ($t_{(212)} = 1.81$, $P = 0.07$) and Sr ($t_{(160)} = 1.53$, $P = 0.129$), as shown in Appendix F (Table F3.7). The results are in agreement with the literature values reported by other authors (Kazi *et al.*, 2008; Sukumar & Subramanian, 2007; Fort, 2005).

5.4.3 Inter-element correlation of Washed Fingernails

The Pearson product correlation coefficient (r) was used to evaluate the strength and direction of a linear relationship between the trace element levels for fingernail samples of healthy individuals and diabetic patients resident in Karbala, and the results are summarised in Tables 5.8 & 5.9. The number of significant correlations for trace elements in the washed fingernails of healthy individuals (24 correlations) was larger than those reported for diabetic patients (16 correlations). Strong correlations are found between Fe-V ($r = 0.912$; $P < 0.001$) and V-Sr ($r = 0.789$; $P < 0.001$) for healthy individuals and Fe-V ($r = 0.764$; $P < 0.001$) and Sr-Zn ($r = 0.683$; $P < 0.001$) for diabetic patients, as shown in Figure 5.6. Similar correlations were found in the biological samples from healthy individuals in Karbala, Iraq, namely for washed fingernails and tear drops, between:

- V with Cr, Mn, Fe, Cu, Zn, As and Sr;
- Cr with Fe, Cu, Zn and Sr;
- Mn with Fe and Sr;
- Fe with Cu, Zn and Sr;
- Cu with Zn and Sr; and
- Zn with As and Sr.

For diabetic patients, significant correlations were also observed in both diabetic groups for tear drops and washed fingernails between:

- V with Mn and Fe;
- Cr with Mn, Fe, Cu, Zn and Sr;
- Mn with Fe and Sr;

- Fe-Sr;
- Cu with Zn and Sr;
- Zn-Sr; and
- As-Sr.

Table 5.8: Statistically significant correlations (r) between elements for washed fingernails of healthy individuals (n = 127[+]).

TE	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.00									
V	NS	1.00								
Cr	NS	0.749	1.00							
Mn	NS	0.248	NS	1.00						
Fe	NS	0.912	0.735	0.346	1.00					
Cu	NS	0.276	0.304	NS	0.346	1.00				
Zn	NS	0.342	0.468	NS	0.369	0.331	1.00			
As	NS	0.301	0.262	0.348	0.227	NS	0.249	1.00		
Sr	NS	0.789	0.678	0.218*	0.675	0.231*	0.353	0.319	1.00	
Cd	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.00

[+] B (n = 10), Fe (n = 103), Cd (n = 94), NS = no significant correlation at $P < 0.05$, *correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$ level, TE is trace element.

Table 5.9: Statistically significant correlations (r) between elements for washed fingernails of diabetic patients (n = 87[+]).

TE	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
B	1.00									
V	NS	1.00								
Cr	NS	0.405	1.00							
Mn	NS	0.404	0.631	1.00						
Fe	NS	0.764	0.518	0.628	1.00					
Cu	NS	0.221*	0.216*	NS	NS	1.00				
Zn	NS	NS	0.310	NS	NS	0.290	1.00			
As	NS	NS	NS	NS	NS	NS	NS	1.00		
Sr	NS	NS	0.510	0.413	0.259*	0.259*	0.683	0.214*	1.00	
Cd	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.00

[+] B (n = 6), Cd (n = 62), NS = no significant correlation at $P < 0.05$, *correlation is significant at $P < 0.05$ level, otherwise correlation is significant at $P < 0.01$ level, TE is trace element.

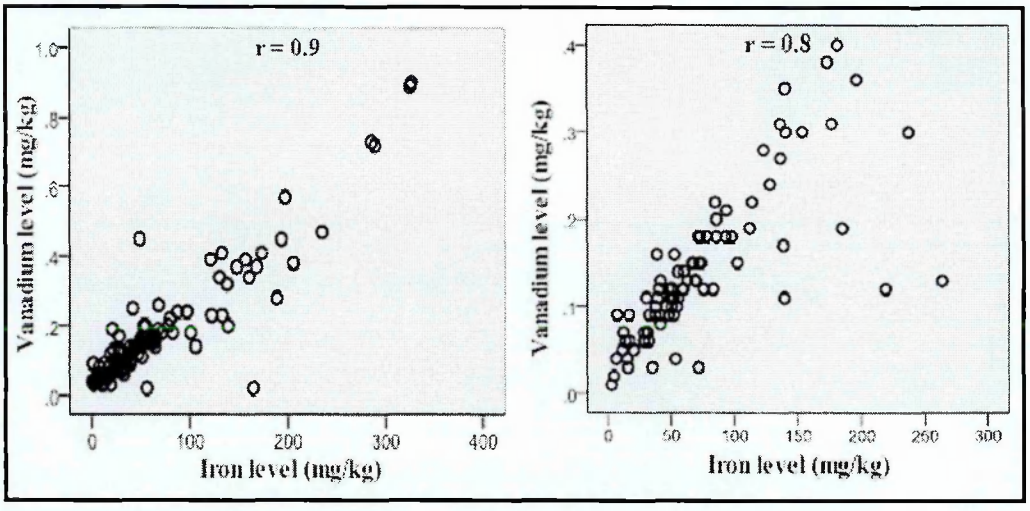

Figure 5.6: Correlation between V and Fe for (a) healthy individuals (n = 103), and (b) for diabetic patients (n = 87) in the washed fingernail samples.

5.3.4 Comparison of Trace Element Levels of Tear Drops and washed Fingernails.

In total 51 samples of both tear drops and fingernails were provided by Iraqi individuals in order to test whether there were any significant differences between the trace element levels in both media from an individual. An F-test and a two-tailed t-test were used to compare the two mean values for each element, as shown in Appendix F (Table F3.9).

The results show that there are significant differences for all trace element levels between tear drops and fingernails. The most significant levels were for V ($t_{(50)}$ = 5.97, t_{crit} = 2.01, $P < 0.001$), Cr ($t_{(50)}$ = 4.86, t_{crit} = 2.01, $P < 0.001$), Mn ($t_{(50)}$ = 4.30, t_{crit} = 2.01, $P < 0.001$), Fe ($t_{(39)}$ = 5.33, t_{crit} = 2.01, $P < 0.001$), Cu ($t_{(50)}$ = 8.91, t_{crit} = 2.00, $P < 0.001$), Zn ($t_{(50)}$ = 14.11, t_{crit} = 2.01, $P < 0.001$), As ($t_{(53)}$ = 8.94, t_{crit} = 2.01, $P < 0.05$), Sr ($t_{(51)}$ = 7.57, t_{crit} = 2.01, $P < 0.001$) and Cd ($t_{(40)}$ = 3.13, t_{crit} = 2.02, $P < 0.01$) in the washed fingernails rather than tear drops. The levels of B for most of the washed fingernails samples were below the limit of detection; therefore, there is no comparison for the B level between tear drops and fingernails. Figure 5.7 shows the mean and 95% confident interval for mean (lower-upper limits) for each element level in the tear drops and washed fingernails for similar healthy individuals resident in Karbala.

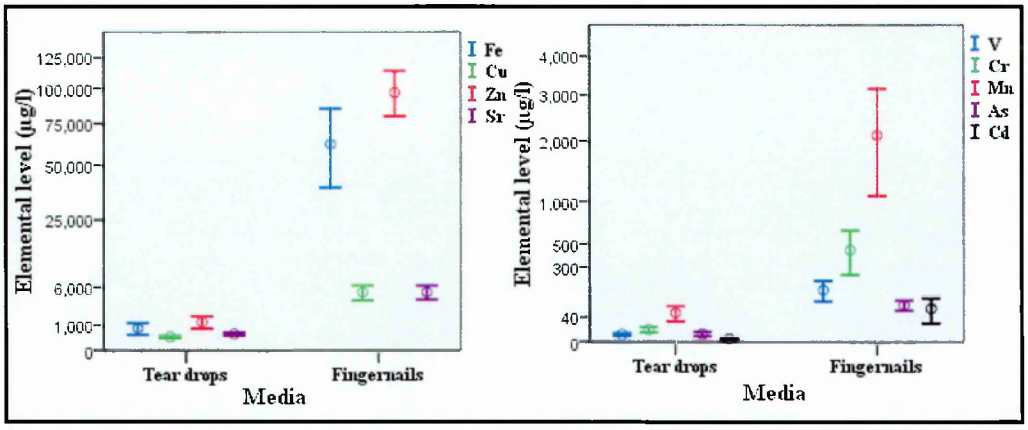

Figure 5.7: Elemental levels in tear drops and washed fingernails for individuals from the healthy population of Karbala who provided both media. Circles represent mean value, whereas I represents 95% confidence interval.

5.5 Comparison Study Between the Four Biological Media

In this study, human fluids (tear drops and saliva) and tissues (scalp hair and fingernails) were obtained in a few cases from the same healthy individuals from Karbala for comparison studies, as shown in Appendix F (Table F4.1). In total 30 samples of each biological sample was collected in order to determine whether there were any significant differences between the trace element levels of these media and the correlation for trace element levels between these media at $P < 0.05$. The highest mean values for most trace elements were found in human tissues (washed scalp hair and fingernails) when compared to human fluids (saliva and tear drops). A possible explanation is that scalp hair and fingernails are long-term growth materials; therefore, several trace elements accumulate in hair and nails (Sukumar & Subranian, 2007). The highest elemental level reported in this study was for Zn in the washed scalp hair of healthy individuals (mean ± SD: 157 ± 114 mg/kg d.w. Zn). In general, the lowest levels for most trace elements were measured in saliva samples and then in tear drops. Figure 5.8 shows the trace element levels in different biological samples used in this study. Human scalp hair was found to have higher mean values for V, Cu, Zn, Sr and Cd, whilst higher levels of Cr, Mn, Fe and As were observed for fingernail samples. The trace element levels in tear drops, saliva, washed scalp hair and fingernails are reported in Table 5.10.

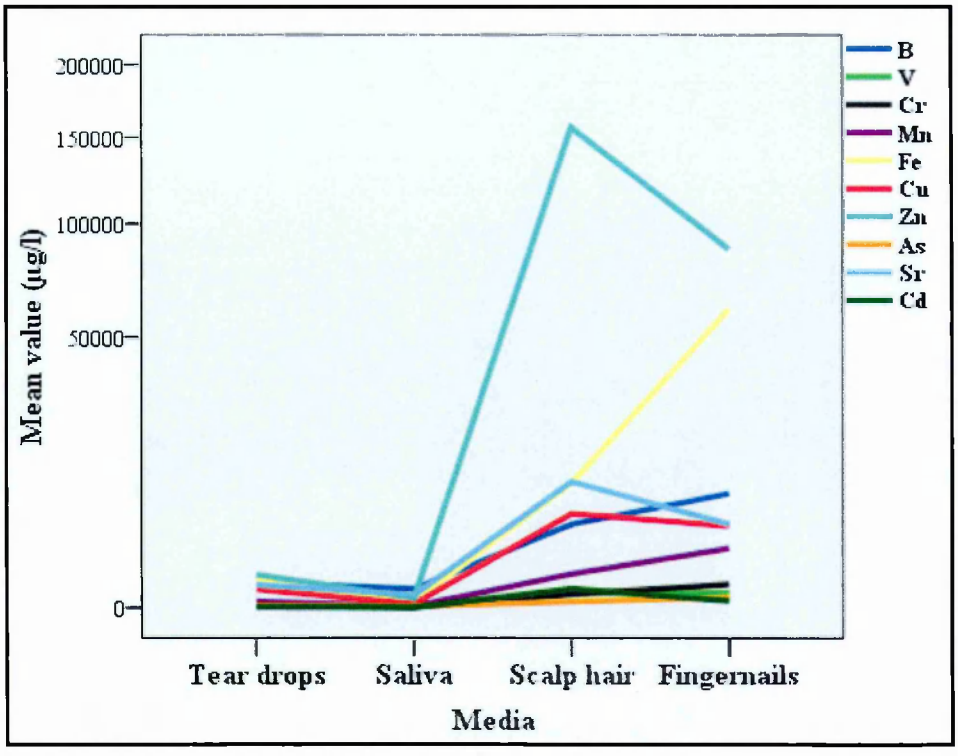

Figure 5.8: Elemental levels in different media for healthy individuals (n = 30) from Karbala who provided all four tissues and fluids.

One-way ANOVA was used to check whether there were any significant differences exist between groups of trace element levels at the probability level of $P < 0.05$. The results show that there are significant differences for all trace elements, as shown in Table 5.11. Figure 5.9 shows the box-plots for trace element levels in the four biological samples and the data is presented as the distribution of trace elements in tear drops, saliva, washed scalp hair and fingernails through the value of median, first (25%) and third quartile (75%), and lower/upper whiskers.

The Pearson product correlation coefficient (r) was determined for each element, as described in Appendix C, and the value of r was subjected to a significance test to evaluate if there was any significant correlation. Significant positive correlations were found between tear drops and fingernails for Mn ($r = 0.56$, $P < 0.01$) and Fe ($r = 0.47$, $P < 0.01$) and scalp hair for As ($r = 0.39$, $P < 0.05$), whilst negative significant correlations were found for B ($r = - 0.43$, $P < 0.05$) and Fe ($r = - 0.36$, $P < 0.05$) between tear drops and saliva. No statistically significant correlations for the remaining trace elements were found between tear drops and other media, namely saliva, washed scalp hair and fingernails.

Table 5.10: Mean, standard deviation, range and 95% confidence interval for mean of trace element levels in tear drops, saliva, washed scalp hair and fingernails for healthy individuals from Karbala, Iraq.

TE	Media	n	Mean ± SD (µg/l or µg/kg)	95% CI		Range (µg/l or µg/kg)
				Lower	Upper	
B*	Tear drops	24	472 ± 167	401	542	< 70 - 853
	Saliva	28	268 ± 238	175	360	< 70 - 1254
	Scalp hair	2	nd	nd	nd	< 3500 - 6077
	Fingernails	4	nd	nd	nd	< 3500 - 13472
V	Tear drops	30	4.5 ± 4.7	2.8	6.3	0.8 - 21.1
	Saliva	30	0.4 ± 0.4	0.3	0.6	0.1 - 1.8
	Scalp hair	30	179.9 ± 137.6	128.6	231.3	32.2 – 614.3
	Fingernails	30	173.6 ± 174.1	108.6	238.6	22.3 - 888.4
Cr	Tear drops	30	14.68 ± 17.29	8.23	21.14	0.73 - 68.39
	Saliva	26	0.28 ± 0.22	0.19	0.36	< 70 - 0.82
	Scalp hair	30	147.2 ± 108.9	106.5	187.9	20.3 – 390.2
	Fingernails	30	418.8 ± 400.4	269.3	568.3	9.4 – 1810.1
Mn	Tear drops	30	37.1 ± 50.3	18.3	55.8	5.2 - 270.1
	Saliva	30	3.2 ± 3.3	2.0	4.4	0.4 - 14.5
	Scalp hair	30	843.7 ± 654.5	599.3	1088.1	142.0 – 3350.0
	Fingernails	30	2531.0 ± 3741.1	1134.1	3927.9	192.0 - 15842.4
Fe	Tear drops	30	580 ± 613	352	809	13 – 2816
	Saliva	30	34 ± 33	22	47	2 – 110
	Scalp hair	30	10678 ± 7085	8032	13323	1875 – 31503
	Fingernails	26	60921 ± 76912	32201	89641	< 25 – 325194
Cu	Tear drops	30	257± 143	204	311	26 – 589
	Saliva	30	16 ± 15	10	21	3 – 69
	Scalp hair	30	6179 ± 1856	5486	6872	2822 – 10741
	Fingernails	30	4693 ± 3994	2157	3415	976 – 23027
Zn	Tear drops	30	791 ± 1009	414	1167	49 - 4109
	Saliva	30	78 ± 89	45	111	7 - 402
	Scalp hair	30	156682 ± 113805	114186	199177	44693 - 434110
	Fingernails	30	87513 ± 39310	72834	102191	30717 - 172970
As	Tear drops	30	6.0 ± 10.2	2.2	9.8	0.1 - 44.8
	Saliva	30	3.5 ± 4.4	1.8	5.2	0.2 - 23.2
	Scalp hair	20	33.9 ± 25.2	22.1	45.7	< 5 – 86.0
	Fingernails	30	92.4 ± 49.9	73.7	111.1	21.1 – 277.2
Sr	Tear drops	30	397± 249	304	409	58 – 1159
	Saliva	30	126 ± 245	34	217	5 – 1324
	Scalp hair	30	10970 ± 14526	5546	16394	710 – 49050
	Fingernails	30	4857 ± 3390	3591	6122	757 – 14811
Cd	Tear drops	30	1.5 ± 1.7	0.9	2.1	0.2 - 6.1
	Saliva	18	0.2 ± 0.2	0.1	0.3	< 0.1 - 1.0
	Scalp hair	30	276.3 ± 400.0	126.9	425.6	16.1 – 2050.2
	Fingernails	25	43.7 ± 35.3	29.2	58.3	< 5 – 154.1

SD = standard deviation, n = number of samples, * the level of B for most scalp hair and fingernail samples was below the limit of detection of B (< 3500 µg/kg), nd = not determined, CI is confidence interval for mean, TE is trace element.

Table 5.11: Analysis of variance ANOVA for trace element levels in the tear drops, saliva, washed scalp hair and fingernails for healthy individuals from Karbala, Iraq.

TE	Source of variance	Sum of Squares	*df*	Mean Square	*F*	Sig.
B	Between Groups	nd	nd	nd	nd	nd
	Within Groups	nd	nd	nd		
	Total	nd	nd			
V	Between Groups	912521	3	304173.7	24.7	< 0.001
	Within Groups	1428637	116	12315.8		
	Total	2341158	119			
Cr	Between Groups	3299993	3	1099998	24.6	< 0.001
	Within Groups	5002503	112	44665		
	Total	8302496	115			
Mn	Between Groups	126110533	3	42036844	11.6	< 0.001
	Within Groups	418370700	116	3606644		
	Total	544481233	119			
Fe	Between Groups	75660531935	3	2522017731 2	16.9	< 0.001
	Within Groups	173016229029	116	1491519216		
	Total	248676760964	119			
Cu	Between Groups	876596675	3	292198892	60.2	< 0.001
	Within Groups	563262306	116	4855709		
	Total	1439858981	119			
Zn	Between Groups	515830582903	3	1719435276 34	47.4	< 0.001
	Within Groups	420439848994	116	3624481457		
	Total	936270431897	119			
As	Between Groups	153693	3	51231	61.7	< 0.001
	Within Groups	88042	106	831		
	Total	241735	109			
Sr	Between Groups	2318257514	3	772752505	13.9	< 0.001
	Within Groups	6455507821	116	55650929		
	Total	8773765335	119			
Cd	Between Groups	1474216	3	491405	10.4	< 0.001
	Within Groups	4670312	99	47175		
	Total	6144528	102			

df = degrees of freedom, for between-groups (df_B) = number of groups – 1; within-group (df_W) = $df_T - df_B$; Total number of degrees of freedom (df_T) = number of observations – 1, mean square = (SS/*df*), *F* is the calculated value for F-test, $F = MS_B/MS_W$,* Sig. is the significance level, TE is trace element.

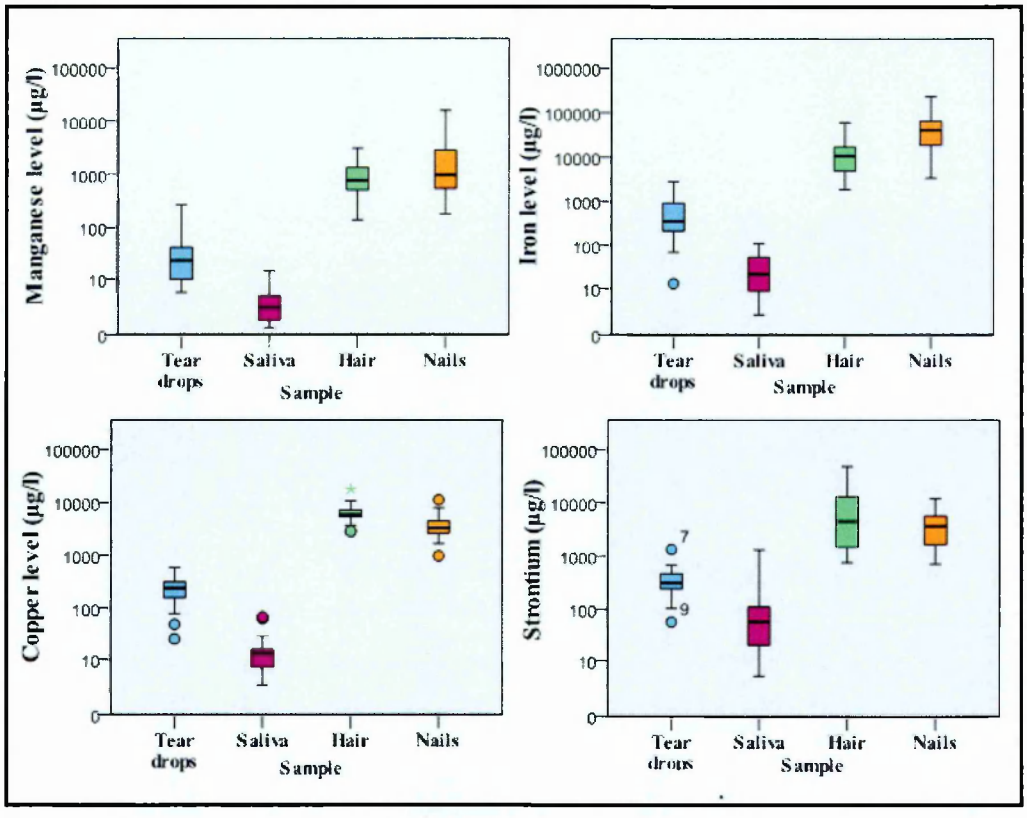

Figure 5.9: Manganese, Fe, Cu and Sr levels in tear drops, saliva, washed scalp hair and fingernails for healthy individuals (n = 30), Middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represents extreme values (other box-plots are reported in Appendix F (Figure F4.1), (some extreme values were excluded from the figure in order to enlarge the scale; this did not change the relationship).

5.6 Summary

This chapter has presented the trace element levels in human saliva, washed scalp hair and fingernails for healthy individuals and diabetic patients from two research sites (Karbala and London) which not only differ in terms of environmental factors, but also individuals life style, water consumption, etc. The results are summarised in Table 5.12 showing the statistical evaluation of the study populations for the trace elements determined in tear drops, saliva, scalp hair and fingernails. Higher levels were found for most of the trace elements for Iraqi individuals resident in Karbala (Iraq) when compared to those residents in London (United Kingdom). A significant difference ($P < 0.05$) was found for B, V, Cr, Mn, Fe, Zn, As, Sr and Cd in tear drops; V, Mn, Fe, Zn, As and Sr in

saliva; V, Fe, Cu, Zn and Sr in scalp hair and V, Cu and Sr in terms of fingernails. This is due to environmental exposure and diet (as found from questionnaire information collected during sampling). In Karbala, the risk of chemical input from environmental sources has to be seriously considered because of the Gulf wars during the last thirty years. In addition, the higher levels for most trace elements in drinking water from Karbala compared to London may be another possible source of these elements, as described in Chapter 3.

The effect of health state was investigated by determining the elemental levels in healthy individuals and diabetic patients. The results show that there are significant differences between healthy individuals and diabetic patients in terms of the level of B, V, Mn, Cu, As and Sr in tear drops; Mn and As in saliva; V, Mn, Fe, Cu, Zn and Sr in scalp hair; and Mn, Cu and Zn in fingernails, as shown in Table 5.12.

Table 5.12: Summary of the statistical comparison ($P < 0.05$) of study populations involving Iraqi individuals resident in Karbala (Iraq) and London (UK) for different biological media for all elements investigated.

Element	Group	Human sample			
		Tear drops	Saliva	Scalp hair	Fingernails
B	HK & HL	HK > HL	NC	NC	NC
	HK & DK	HK < DK	NC	NC	NC
V	HK & HL	HK > HL	HK > HL	HK > HL	HK > HL
	HK & DK	HK > DK	NS	HK > DK	NS
Cr	HK & HL	HK > HL	NC	NC	NC
	HK & DK	NS	NC	NC	NS
Mn	HK & HL	HK > HL	HK > HL	NC	NC
	HK & DK	HK < DK	HK < DK	HK > DK	HK > DK
Fe	HK & HL	HK > HL	HK > HL	HK > HL	NC
	HK & DK	NS	NS	HK > DK	NC
Cu	HK & HL	NS	NS	HK > HL	HK > HL
	HK & DK	HK > DK	NS	HK > DK	HK > DK
Zn	HK & HL	HK > HL	HK > HL	HK > HL	NS
	HK & DK	NS	NS	HK > DK	HK > DK
As	HK & HL	HK > HL	HK > HL	NC	NC
	HK & DK	HK > DK	HK > DK	NC	NS
Sr	HK & HL	HK > HL	HK > HL	HK > HL	HK > HL
	HK & DK	HK < DK	NS	HK > DK	NS
Cd	HK & HL	HK < HL	NC	NC	NC
	HK & DK	NS	NC	NC	NC

HK, DK are healthy and diabetic samples from Karbala, HL = healthy samples from London, > and < represent the significant difference, using a two tailed t-test, NS = no significance, NC = no comparison due to one or both groups having elemental levels below the limit of detection.

The results in this study are in general agreement with values reported by other researchers (Yuan *et al.*, 2008; Rodushkin & Axelsson, 2000; Ward & Ward, 1993). The mean values for most trace elements are found to be within the literature ranges reported for saliva (Samanta *et al.*, 2004). The only exceptions are for B and Sr as their levels in the majority of samples are above the literature values. The mean values for trace elements in washed scalp hair were comparable with those reported in the literature (Sukumar and Subramanian, 2007; Forte *et al.*, 2005; Rodushkin & Axelsson, 2000; Miekeley *et al.*, 1998). Similar findings have previously been reported by other researchers for most trace elements in fingernails (Kazi *et al.*, 2007; Sukumar & Subramanian, 2007; Fort, 2005; Rodushkin & Axelsson, 2000).

In order to compare the results of tear drops with other biological samples, paired samples, namely tear drops/saliva, tear drops/scalp hair and tear drops/fingernails were collected from the same healthy individuals. The highest elemental level reported in this study was for Zn in scalp hair (138 ± 87 mg/kg d.w.). The highest mean values for most elements were found in human tissues (hair and nail), whilst the lowest levels were in human fluid (tear drops and saliva) (Table 5.13). Furthermore, the trace element levels in saliva in this study are far lower than those reported for tear drops. Thus, it can be proposed that tear drops can be used as a useful matrix in occupational biomonitoring when compared with saliva (Table 5.13), as described in Section 5.2.4.

A Pearson product correlation coefficient (r) was used to describe the strength and direction of possible linear relationships between the trace element levels in saliva, washed scalp hair and fingernail samples collected from healthy and diabetic individuals, and the results were compared with tear drops. Positive significant inter-correlations were noted for healthy and diabetic individuals (at a probability P level of < 0.05 and < 0.01) for different media, as reported in Table 5.14. Similar correlations were found between most trace elements for tear drops and other human samples. Such correlations were found in the literature by other researchers (Chojnacka *et al.*, 2010; Kamal *et al.*, 2009; Hill, 2009; Shah *et al.*, 2006; Stone, 2006; Chojnacka *et al.*, 2005).

Table 5.13: Summary of statistical comparison ($P < 0.05$) between tear drops and other biological samples in the same healthy individuals from Karbala for all trace elements investigated.

Element	Comparison*	n	Significant difference ($P < 0.05$)
B	Tear drops/saliva	35/38	Tear drops > Saliva
	Tear drops/scalp hair	42/2	NC
	Tear drops/fingernails	44/4	NC
V	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/50	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Cr	Tear drops/saliva	42/34	Tear drops > Saliva
	Tear drops/scalp hair	50/45	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Mn	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/50	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Fe	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/40	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Cu	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/50	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Zn	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/50	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
As	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/26	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Sr	Tear drops/saliva	42/42	Tear drops > Saliva
	Tear drops/scalp hair	50/50	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails
Cd	Tear drops/saliva	42/24	Tear drops > Saliva
	Tear drops/scalp hair	50/41	Tear drops < Scalp hair
	Tear drops/fingernails	51/51	Tear drops < Fingernails

n is the number of samples, NC = no comparison due to the levels of B for most scalp hair and fingernails samples were below the limit of detection (< 3.5 mg/kg), * using an F-test and a two tailed t-test, further information can be found in Appendix F (Tables F1.5, F2.6 and F3.9).

Another statistical comparison was performed on the trace element levels in the four different types of biological media provided by a healthy individual, in order to evaluate the differences between different media. The results of one-way ANOVA show that there are significant differences in all trace elements between tear drops and other biological samples, as described in Table 5.11.

The Pearson product correlation coefficient (r) was determined for the trace element levels between tear drops and other media. Two significant correlations were reported. Positive correlations were found between tear drops and fingernails for Mn ($r = 0.56$, $P < 0.01$) and Fe ($r = 0.47$, $P < 0.01$), and between tear drops and scalp hair for As ($r = 0.39$, $P < 0.05$), whilst negative significant correlations were found between tear drops and saliva for B ($r = -0.43$, $P < 0.05$) and Fe ($r = -0.36$, $P = 0.05$).

Table 5.14: Summary of statistical correlations ($P < 0.05$) between trace element levels in different biological samples for healthy individuals and diabetic patients resident in Karbala, Iraq.

Correlation	Group	TD	S	SH	FN
V-Cr, V-Mn, V-Fe, V-Cu, V-Zn, V-As, V-Sr, Cr-Fe, Cr-Cu, Mn-Fe, Mn-Sr, Fe-Cu, Zn-Sr.	Healthy	Sig.	Sig.	Sig	Sig.
Cr-Mn, Mn-Cu, Mn-Zn.	Healthy	Sig.	Sig.	Sig	NS
Cr-Zn, Cr-Sr, Fe-Zn, Cu-Zn, Zn-As, Cu-Sr, Fe-Sr, Cu-Sr.	Healthy	Sig.	Sig.	NS	Sig.
Mn-As, Fe-As.	Healthy	NS	Sig.	Sig.	Sig.
B-Fe, Mn-Cd, Zn-Cd, As-Cd.	Healthy	Sig.	Sig.	NS	NS
V-Cd.	Healthy	Sig.	NS	Sig.	NS
Cr-As.	Healthy	NS	NS	Sig.	Sig.
Cr-Cd, Fe-Cd, Cu-Cd, Sr-Cd.	Healthy	Sig.	NS	NS	NS
B-V, B-Cr, B-Mn, B-Cu, B-Zn, B-As, B-Sr.	Healthy	NS	Sig.	NS	NS
Cu-As.	Healthy	NS	NS	Sig	NS
As-Sr.	Healthy	NS	NS	NS	Sig.
V-Mn, Cr-Cu.	Diabetic	Sig.	Sig.	Sig	Sig.
Mn-Cu.	Diabetic	Sig.	Sig.	Sig	NS
V-Fe.	Diabetic	Sig.	Sig.	NS	Sig.
Zn-Sr.	Diabetic	Sig.	NS	Sig.	Sig.
B-Sr, Fe-Cu.	Diabetic	Sig.	Sig.	NS	NS
B-Cu.	Diabetic	Sig.	NS	Sig.	NS
Cr-Mn, Cr-Zn, Mn-Fe, Cu-Zn, Cu-Sr, As-Sr	Diabetic	Sig.	NS	NS	Sig.
V-Cr.	Diabetic	NS	Sig.	Sig.	Sig.
V-Cu.	Diabetic	NS	NS	Sig.	Sig.
B-Cr, V-As, Cr-As, Cr-Cd, Mn-Zn, Mn-As, Mn-Cd, Cu-Cd, Zn-Cd.	Diabetic	Sig.	NS	NS	NS
B-Mn, Fe-As, Zn-As.	Diabetic	NS	Sig.	NS	NS
V-Zn, V-Sr, Fe-Zn.	Diabetic	NS	NS	Sig.	NS

TD, S, SH and FN represent tear drops, saliva, scalp hair and fingernails, Sig. = significant correlation at $P < 0.05$, NS = no significant correlation at $P < 0.05$, the data taken from Tables 4.17, 4.18, 5.2,5.3,5.5,5.6, 5.8 & 5.9.

Chapter Six

Conclusion and Further Work

6.0 Introduction

The use of human tissues (scalp hair and fingernails) and fluids (blood, saliva, and urine) as biomarkers for trace elements in the human body and environment have recently been investigated by several studies (Olmedo *et al.*, 2010; Sthiannopkao *et al.*, 2010; Esteban *et al.*, 2009; Rodrigues *et al.*, 2008). Human biomonitoring is used in several different situations to (Flores *et al.*, 2011; Sardans *et al.*, 2010; Wang *et al.*, 2009; Shah *et al.*, 2006; Wilhelm *et al.*, 2002; Jin *et al.*, 2000; Paulsen *et al.*, 1996; Schuhmacher *et al.*, 1996).

- identify and eliminate the potential environmental exposure sources;
- detect time trends in chemical variations;
- show the effectiveness of bans or restrictions;
- discover relationships between chemical exposure and diseases;
- map the geographical distribution of contaminated regions; and to
- identify relationships between chemical body burden and dietary system or an occupational exposure.

In general, there is no ideal matrix that can be used to monitor human health in every situation. The ideal biomarkers must have several characteristics, namely: collection does not cause a health risk to the individual; include chemical levels detectable by the techniques available; provide sufficient amounts for the analysis; easily accessible for sampling; and reflect the body problem (Esteban *et al.*, 2009).

Clinical methods are mainly used to analyse trace element deficiencies or to evaluate occupational and/or environmental exposure to toxic elements based on the analysis of blood (whole, serum, and plasma) specimens. The main disadvantage of using blood in human biomonitoring is that it is an invasive matrix and thus can have an adverse effect on the participant response in volunteer epidemiological studies (Rockett *et al.*, 2004). However, non-invasive matrices, such as tear drops, saliva, hair and fingernails were preferred by Iraqi individuals as they are easily accessible for collection, and more acceptable to the population than blood sampling, allowing for repeated determinations over time.

In consideration of the above, it is apparent that there is a need for further biomarkers with significant potential to monitor, in a non-invasive fashion, the required trace elements associated with health assessment. The main aim of this

study was to develop and validate a new biomarker for evaluation of the elemental levels of Iraqi individuals resident in Karbala (Iraq) and London (UK). Tear drop fluid was selected as a possible new biomarker in this research as there has been no previously published studies in this area. In addition, other biological samples, such as saliva, scalp hair and fingernails were also used in order to develop the analytical methods for the determination of trace elements in these media, and to establish a data base of normal levels for Iraqi individuals. This data can also be used to provide values for comparative analysis with the tear drop results. Furthermore, environmental samples (water and cigarette tobacco) were also collected from the areas of study in order to evaluate whether these media make any significant contribution to the elemental levels in the human tissues and fluids under investigation.

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 environmental samples, namely water and tobacco. The elemental results for tear drops were reported in Chapter 4. The comparative study data between tear drops and saliva, washed scalp hair and fingernails were outlined in Chapter 5.

6.1 Environmental Analysis

During the last century, Iraq's industry has suffered from a decade of economic sanctions and lack of investment. This has led to chronic environmental problems, such as discharges of untreated effluent into surface waters, spillages and discharges of chemicals into soils and ground water, and widespread uncontrolled emission of particulates and gases from stacks. The recent wars have undoubtedly exacerbated the chronic environmental stresses that have accumulated in Iraq over the past three decades. An important part of the environmental damage associated directly with the war arises from the looting and pillaging of key infrastructures and the ransacking of equipment and supplies, including hazardous and radioactive materials. According to reports from the United Nations Environment Programme (UNEP), there are 300 sites in Iraq that are considered to be contaminated by various pollutants (UNEP, 2003: Al-Bedri & Al-Jobori, 1991).

Environmental samples, namely water and cigarette tobacco were collected from Karbala, Iraq. Tap water was also collected from London (UK) so as to evaluate the relationship between the trace element levels in Iraqi residents in Iraq and London (UK) and whether different chemical levels in water may provide information on the trace element levels in various human tissues and fluids. The values of water parameters (pH, total dissolved solid (TDS) and electrical conductivity (EC)) for commercial, domestic bottled, tap, river and ground (well and artesian) waters were measured directly at the time of sampling, as they can change with storage time and temperature (Arain *et al.,* 2008), as shown in Table 3.1.

Results showed that the pH levels for all water samples were predominantly neutral to slightly alkaline (6.1 – 8.5), which are within the WHO guidelines for drinking water (WHO, 2008). Electrical conductivity on the other hand was higher in domestic bottled (998 ± 472 µS/cm), tap (1134 ± 184 µS/cm), river (1343 ± 40 µS/cm), artesian (1172 - > 3999 µS/cm) and well waters (2505 - > 3999 µS/cm) when compared with the WHO and European recommended values for EC (250 µS/cm) for drinking water. High levels of EC can be associated with salinity; ions that have a major influence on the EC are H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} and HCO_3^- (Radojevic & Bashkin, 2006). Therefore, EC increases with the mineral content of a water sample - thus its use in the measure of mineral contents. A significant correlation was observed between the TDS levels in different water resources and conductivity (R^2 = 0.9999) (Figure 3.1). Thus, higher levels of TDS were found in artesian (583 - > 2000 mg/l) and well waters (1254 - > 2000 mg/l) when compared with the recommended guidelines by the WHO for drinking water (1000 mg/l). The results showed significant correlations (at $P < 0.05$) between TDS and B (R^2 = 0.687), Cr (R^2 = 0.564), Fe (R^2 = 0.470), Sr (R^2 = 0.850) and Cd (R^2 = 0.450).

Multi-elemental analysis was performed for commercial, domestic bottled, tap, river, artesian and well waters collected from Karbala and tap water from London (Tables 3.1 – 3.4). In general, the highest trace element levels in drinking and irrigation waters were found for Sr in tap (1113 ± 425 µg/l) and well water (7096 ± 2823 µg/l) respectively, whilst the lowest levels were for Cd (< 0.01 – 0.01 µg/l) and V (1.2 ± 0.7 µg/l) in commercial and artesian waters, respectively.

Highly elevated trace element levels were reported in tap water from Karbala compared to the London samples, as presented in Table 3.4.

The findings were compared with the guideline values for drinking and irrigation waters as recommended by the World Health Organisation (WHO), Iraqi Specifications and the Food and Agriculture Organisation (FAO). In addition, the results were also compared with published literature values. In general, the mean and range values (μg/l) for most trace elements are lower than the permissible limits for drinking water recommended by the WHO and Iraqi guidelines. The only exceptions are for B in artesian (1049 ± 746 μg/l) and well waters (1569 ± 844 μg/l); and Cd in river (8.71 ± 3.65 μg/l), artesian (5.28 ± 4.86 μg/l) and well waters (9.98 ± 0.31 μg/l). In the majority of cases, levels of B and Cd greatly exceeded the WHO guideline limit of 500 μg/l B and 3 μg/l Cd, recommended as a maximum allowable level in potable waters (refer to Table 3.3). Total cadmium concentrations in Karbala were in good agreement with other literature sources reported for drinking (Barbooti *et al.*, 2010; Nkono & Asubiojo, 1998; Ward, 1983) and irrigation waters (Barbooti *et al.*, 2010; Reimann *et al.*, 2003).

The levels of B were in disagreement with other literature ranges reported for drinking water in Baghdad (Iraq) (Barbooti *et al.*, 2010) and other countries (Hill, 2006). On the other hand, the total B reported in ground water showed lower levels than another reported study in Karbala (Al-Dbbas, 2006), which also reported a maximum of 10 mg/l B in ground water supplies throughout the west of Karbala province, compared to a maximum of 3.9 mg/l B determined in this study (carried out over a more localised sampling area). These differences may be based on the natural geology of the province, the time of sampling and the different wells visited.

The levels of Sr were relatively high in different water samples; concentration of Sr in these waters were generally of the order well > artesian > river > tap > bottled > commercial. The WHO and Iraqi government do not recommend guideline values for Sr. Thus, the mean values of Sr were compared with the United States Environmental Protection Agency (USEPA) guideline limit of 4000 μg/l, recommended as an acceptable level in drinking water (Usuda *et al.*, 2007). According to the USEPA, the levels of Sr in ground water wells (1512 - 14375 μg/l) and artesian bores (1157 – 8308 μg/l)) were higher and require further investigation. There is a relative lack of data on Sr occurrence in water.

Commonly, Sr is present in water as carbonates and sulphates, which are slightly soluble. However, the concentration of Sr can differ depending on local conditions (Kabata-Pendias & Mukherjee, 2007). Sr levels in river waters ranged from 3 to 238 µg/l Sr, whilst the world average is 60 µg/l Sr (Gaillardet *et al.*, 2003). The level of Sr can be higher in industrial regions such as the river waters of Poland (> 300 µg/l Sr). Thus, the levels of Sr in river water from Karbala (335 – 2755 µg/l Sr) are higher comparing with those reported in Poland; in uncontaminated rivers Sr ranges from 10 to 35 µg/l Sr (Kabata-Pendias & Mukherjee, 2007). The levels of Sr were also higher when compared with other studies reported by other researchers, such as Ikem *et al.*, (2003); Reimann *et al.*, (2003); Azparren *et al.*, (2000); and Kikuchi *et al.*, (1999) for drinking and irrigation waters.

In summary, boron was found to be at higher levels in Karbala waters, which could possibly cause toxicity symptoms and damage to plants (Hill, 2009). Therefore, attention should be paid to using chemical treatment for Karbala water to reduce B levels, and thereby improve the quality of water. The higher levels of Sr found in all water samples requires further investigation. The drinking water (commercial, bottled and tap) in Karbala can be used directly, whereas irrigation water (river, artesian and well) may need chemical treatment prior to the use as drinking water by the population. The levels of Cr, Mn, Fe, Cu, Zn and As in Karbala water were under the levels set by the WHO for drinking water.

Multi-elemental analysis was also performed on cigarette tobacco from Karbala. This was established due to a significant proportion of the population in Iraq being active smokers, as shown in Section 3.2. The highest mean (and standard deviation) trace element value found in tobacco samples was reported for iron 257 ± 52 mg/kg Fe (dry weight or d.w.), whilst the lowest mean and standard deviation was observed for vanadium 0.42 ± 0.12 mg/kg V (d.w.) (Table 3.5). The findings were compared with those reported in the literature. In general, the levels of trace elements in cigarette tobacco are in agreement with the reported ranges for other countries. The results of different commercial brands of cigarette tobacco showed that the mean value for toxic elements particularly for Cd (0.90 mg/kg d.w.) was higher when compared to those reported for normal plant material (typically < 0.4 mg/kg Cd), as described in Section 3.2.2 (Ward, 1993).

6.2 Human Exposure Analysis

An evaluation of the trace elements (B, V, Cr, Mn, Fe, Cu, Zn, As, Sr and Cd) on the inhabitants of Karbala (healthy and diabetic) and London (healthy) was carried out, using tear drops, saliva, washed scalp hair and fingernails as potential biomarkers. The results showed elevated trace element levels in drinking water from Karbala compared to London (refer to Table 3.4). Elevated total trace element levels were also seen in tear drops, saliva, washed scalp hair and fingernails from Karbala compared to a comparative region (London) (refer to Tables 4.2, 5.1, 5.4 & 5.7). Differences in trace element levels in different human tissues and fluids were significant ($P < 0.05$) between Karbala and London, suggestive of potentially different environmental and dietary exposure to trace elements. These differences may be based on the natural environment of the two sites, as described above in Section 5.2 (UNEP, 2003).

Comparison between the elemental levels in the human tissues and fluids under investigation shows the highest elemental level reported in tear drops, washed scalp hair and fingernails of healthy individuals and diabetic patients was for Zn, whilst in saliva was for Sr (refer to Tables 4.2, 5.1, 5.4 & 5.7). Similar results were found in the literature for whole blood, serum and plasma (Flores *et al.*, 2011; Stone, 2006).

The trace element levels in saliva were lower than those reported in tear drops, suggesting tear drops may have an advantage of being a better biomarker for trace elements when compared with saliva fluid in terms of the capability of the analytical technique being able to determine more accurately the higher elemental levels (refer to Section 5.2.4). The higher mean values for all trace elements were found in human tissues (hair and fingernails) compared to human fluids (tear drops and saliva) (refer to Figure 5.8). A possible explanation is that tissues are long-term growth materials; therefore, several trace elements accumulate in hair and nails (refer to Sections 5.3.4 & 5.4.4) (Sukumar & Subranian, 2007).

The results for tear drops, saliva, washed scalp hair and fingernails were compared with literature ranges. In general, tear drop results are in agreement with those reported by Flores *et al.* (2011) for V and Zn in blood serum; Muniz *et al.* (2001) for Zn in blood serum; Gill *et al.* (2011) for Cd, Cr and Mn in blood and saliva; Ward & Ward (1991) for Cu and Cd in saliva; and Stone (2006) for Fe

in plasma, and Zn in plasma and serum. The mean values for B, As and Sr are in disagreement with the reported literature values in serum and saliva (Flores *et al.*, 2011; Gill *et al.*, 2011) (refer to Tables 4.5 & 4.6). Saliva results are in general agreement with values reported by other authors for most trace elements except B and Sr, as described in Tables 4.6 & 5.1. The mean values for most trace elements in washed scalp hair and fingernails were within the literature ranges reported by other researchers with the only exception being B (Sukumar and Subramanian, 2007; Forte *et al.*, 2005; Rodushkin & Axelsson, 2000). The majority of human tissue and fluid samples have B and Sr levels above the literature values, as the higher level of B and Sr in water samples may have elevated these elements for the individuals under investigation.

The levels of these elements have been suggested, in terms of deficiency or excess, to be a probable reason for the on-set of type 2 diabetes. This was evaluated by determining the trace element levels in the tear drops of both healthy individuals and diabetic patients with subsequent statistical evaluation using significance testing. Significantly higher tear drop levels of B, Mn and Sr, and lower levels of V, Cu and As were found in diabetic patients when compared with healthy individuals ($P < 0.05$). No significant differences were found for other elements between healthy and diabetic groups using a two-tailed t-test. These results are in agreement with those reported by Flores (2011) in Table 4.6 for V, Mn, Cu and As. Interestingly, similar results were reported for Mn and As in saliva; V and Cu in washed scalp hair; and Cu in fingernails.

Multivariate discriminant function analysis (DFA) was applied to evaluate which of the trace elements discriminates between healthy individuals (Karbala and London) and diabetic patients (Karbala). Only two discriminant functions (DF1 and DF2) were found to discriminate the three population groups, as shown in Table 4.7. It can be seen that, Sr, Mn, B, Zn, V, As and Cd exhibited a strong contribution in discriminating the three populations and accounts for most of the expected variations in tear drops (100%), whilst other trace elements showed a less contribution (< 0.3) in explaining the variation between healthy and diabetic populations. These results were in agreement with the results determined by a two tailed t-test (Tables 4.4 & 4.5) and ANCOVA results (Table 4.11).

The influence of various factors (gender, smoking activity, health status, individual's age and drinking water) on elemental levels was determined, using

the analysis of covariance (ANCOVA). Significant effects were found for health status, gender, smoking activity, age and drinking water on the levels of B ($F_{(1,118)}$ = 12.573, P = 0.001), Mn ($F_{(1,138)}$ = 16.286, P < 0.001) and Sr ($F_{(1,140)}$ = 5.388, P = 0.022); Fe ($F_{(1,138)}$ = 5.626, P = 0.019); Cd ($F_{(1,140)}$ = 9.681, P = 0.002); Zn ($F_{(1,137)}$ = 6.373, P = 0.013) and As ($F_{(1,142)}$ = 17.176, P < 0.001); and V ($F_{(1,145)}$ = 13.305, P < 0.001), Mn ($F_{(1,138)}$ = 8.240, P = 0.005); and Sr ($F_{(1,140)}$ = 175.783, P < 0.001), respectively. These results were consistent with other studies reported in the literature by Flores *et al.* (2011) for Mn, Forte *et al.* (2005); Kamakura (1983); Stone (2006), and Jian *et al.* (2010) for Fe; and Sukumar & Subramanian (2007); Chojnacka *et al.* (2006), and Gill *et al.* (2011) for Cd by using other biological samples. A Pearson product moment correlation was used to describe the strength and direction of the relationship between the trace element levels in tear drops and drinking water. A strongly positive significant correlation was seen between tear drop strontium and drinking water strontium (r = 0.760, $t_{(153)}$ = 14.224, P < 0.001), as shown in Figure 4.3.

6.3 Inter-Element Correlations

Inter-element interactions were investigated in this study by the calculation of correlation coefficients for healthy individuals and diabetic patients (refer to Tables 4.19 & 4.20). The Pearson correlation coefficient (r) was verified for each pair of trace elements for tear drop, saliva, washed scalp hair and fingernail samples in order to check if any significant correlations could be found between the trace elements in the matrix, and whether the effect of type 2 diabetes changes this, as described in Section 4.5.7. For tear drops from healthy individuals, strong correlations, as indicated by the magnitude of r, were found between Zn-Sr (r = 0.667, P < 0.01), whilst for diabetic patients they were between Cr-Mn (r = 0.84, P < 0.01). There were 32 and 27 statistically significant correlations of the total 45 tested in tear drops for healthy and diabetic individuals, respectively (refer to Tables 4.17 & 4.18). Similar correlations were reported in the literature for other biological samples (Flores *et al.* (2011); Gill *et al.* (2011); Barany *et al.* (2002), and Shah *et al.* (2006).

Comparison between tear drops and saliva showed similar correlations exist between B-Fe; V with Cr, Mn, Fe, Cu, Zn, As and Sr; Cr with Mn, Fe, Cu, Zn and

Sr; Mn with Fe, Cu, Zn, Sr and Cd; Fe with Cu, Zn and Sr; Cu with both Zn and Sr; Zn with As, Sr and Cd; and As-Cd for healthy individuals (Tables 4.17 & 5.2) and B-Sr; V with Mn and Fe; Cr with Fe and Cu; and Mn-Cu for diabetic patients (Tables 4.18 & 5.3).

In the case of washed scalp hair, the following correlations were also found in tear drops: V with Cr, Fe, Cu, Zn, As, Sr, and Cd; Cr with Mn, Fe and Cu; Mn with Fe, Cu, Zn and Sr; Fe-Cu; Zn-Sr; and Sr-Cd for healthy (Tables 4.17 & 5.5); and B-Cu; V-Mn; Cr-Cu; Mn with Cu and Sr; and Sr-Zn for diabetic (Table 4.18 & 5.6). Several correlations in washed scalp hair were also reported by other researchers in the literature, such as Fe-Mn and Fe-Cu (Hill, 2009), Mn-Sr, V-Cu, V-Mn, Cr-Fe, Cr-Mn, Mn-Cu, Zn-Sr and Sr-Cd (Chojnacka *et al.*, 2005).

Similar correlations were also found for tear drops and fingernails: namely for healthy individuals: V with Cr, Mn, Fe, Cu, Zn, As and Sr; Cr with Fe, Cu, Zn and Sr; Mn with Fe and Sr; Fe with Cu, Zn, and Sr; Cu with Zn and Sr; and Zn with As and Sr (Tables 4.17 & 5.8), whereas for diabetic are between V with Mn and Fe; Cr with Mn, Fe, Cu, Zn and Sr; Mn with Fe and Sr; Fe-Sr; Cu with Zn and Sr; Zn-Sr; and As-Sr (Tables 4.18 & 5.9).

A Correlation Coefficient (r) was also calculated to evaluate whether there were any significant correlations between the level of trace elements in tear drops and each of saliva, washed scalp hair and fingernails. In general, significant positive correlations were found between tear drops/fingernails for Mn ($r = 0.56$, $P < 0.01$) and Fe ($r = 0.47$, $P < 0.01$) and tear drops/scalp hair for As ($r = 0.39$, $P < 0.05$), whilst negative significant correlations were found for B ($r = - 0.43$, $P < 0.05$) and Fe ($r = - 0.36$, $P < 0.05$) between tear drops and saliva.

In conclusion:

The present study is the first full study, to my knowledge to highlight the use of tear drop fluid as a biomarker for the level of trace elements in the human body. This study provides a preliminary assessment of the determination of trace element levels in saliva, washed scalp hair and fingernails for Iraqi individuals in the province of Karbala, Iraq. The results show that both the aim and main objectives of this study have been achieved. Firstly, an assessment of the trace element exposure of the inhabitants of Karbala was carried out, using tear drops, saliva, washed scalp hair and fingernails as a potential biomarkers. Karbala samples showed elevated trace element levels in drinking water over the range of

London. Elevation of most of the trace elements were seen in tear drops, saliva, washed scalp hair and fingernails collected from Karbala when compared to those from London, suggestive of deleterious exposure to some trace elements such as B, Sr and Cd. Secondly, the data for most elements in tear drops is in agreement with those reported in saliva, scalp hair and fingernails for diabetic and healthy subjects. This provides evidence that tear drops can potentially be used as a new biomarker for determining the health status of an individual. Finally, significant differences were found in the levels of most of the trace elements throughout this study between females and males; Karbala and London; and smokers and non-smokers. These results can confirm that factors like gender, residential location and smoking activity can affect the elemental levels in the human body.

6.4 Further Work

Further research could be designed from this study as there are no previously published studies about the levels of trace elements in biological samples collected from Karbala (Iraq):

- The higher levels of Sr found in all water and biological samples require a follow-up study to establish whether a possible link exists with regard to soils and main foods in this region;
- The major problem associated with tear drop analysis is the variable volume that is produced by the glands. In most cases, the amount of tear drop fluid required for analysis using the techniques in this study is > 2.5 ml. As such, the potential of electrothermal sample introduction (ETV-ICP-MS), which requires smaller volumes for analysis (5 – 10 µl), needs to be investigated;
- As described in this study, tear drops provide data about human processes over short periods similar to that for blood and urine. However, more research is needed to evaluate whether or not this fluid provides any advantages over the traditional biological fluids (blood and urine);
- The data from this study confirms that the deficiency and excess of some trace elements may play a role in the development of diabetes mellitus. However, further clinical studies are required using larger numbers of

diabetic patients. In addition, blood and urine need to also be collected and analysed to enable a clearer picture of the trace elements of diabetics;

- Further studies are needed to explain many of the reported correlations of various elemental levels in the tear drops fluid;
- The levels (μg/l) of most trace elements in irrigation water are lower than the permissible limits for drinking water recommended by the WHO and Iraqi guidelines. The only exceptions are for B, Cd and Sr. However, water from Karbala may require chemical treatment at the municipal water plant in order to reduce B, Cd and Sr levels, and thereby improve the quality of drinking water;
- It was found that Sr has been associated with different diseases such as dental caries. However, new studies are needed to evaluate whether the high levels of Sr could be associated with local diseases or health conditions in Iraq; and
- The higher levels of electrical conductivity (EC) in the water samples from Karbala needs further investigation to link the EC values with human health, and to establish a guideline value for the EC limits within the Iraq Standard Specifications.

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Appendix A

Documentation and Clinical Study

Appendix A1

A1.1: Ethical Approval Documentation:

03 March 2009

Baker A Jada
Chemical Sciences
FHMS

Dear Baker

The Impact of Trace and Minor Elements on the Health Status of Iraqi Individuals and the Relationship with Smoking Activity and Diabetes
EC/2009/15/FHMS Fast-Track

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 2 march 2009.

The list of documents reviewed and approved by the Committee under its Fast Track procedure is as follows:

Document	Date
Protocol cover sheet	2 mar 09
Summary of the project	2 Mar 09
Detailed protocol	2 Mar 09
Evidence of agreement of collaborators	2 Mar 09
Information sheet for participants – English version	2 Mar 09
Information sheet for participants – Arabic version	2 Mar 09
Consent form – English version	2 Mar 09
Consent form – Arabic version	2 Mar 09
Questionnaire – English version	2 Mar 09
Questionnaire – Arabic version	2 Mar 09
Risk assessment form	2 Mar 09
Sample preparation procedures	2 Mar 09
Confirmation letters from Sponsors	2 Mar 09
Hazard checklist for Travellers	2 Mar 09
Checklist for Travellers	2 Mar 09

This opinion is given on the understanding that you will comply with the University's Ethical Guidelines for Teaching and Research.

The Committee should be notified of any amendments to the protocol, any adverse reactions suffered by research participants, and if the study is terminated earlier than expected with reasons.

You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely

Aimee Cox (Miss)
Secretary, University Ethics Committee
Registry

cc Professor T Desombre, Chairman, Ethics Committee
Professor Neil I Ward, PI
Professor David C Povey

A1.2: ATAS Certificate:

ATAS CERTIFICATE

Foreign & Commonwealth Office

You applied for ATAS clearance on 14/12/2007 to study PhD Research (JACS F1) at University of Surrey.

You stated that your thesis, or area of research, or the title of your taught Masters course would be:

The title of project"Chemical Fingerprinting Drugs of Abuse Using Chromatographic and Spectroscopic Analytical Techniques. The project will be study Development of chromatographic methods (GC-MS with solid phase extraction) of plants, drugs and human hair/nails for the identification of drugs of abuse (cannabinoids opiates, etc). Investigation of washing procedures of human samples to screen for false positive exposure cases and the effects of passive smoking exposure. Furthermore, to use trace elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS) to establish a possible screening method, relating the elemental fingerprint of the geochemistry of the plant/drug at source (for particular production regions), to the levels in drugs and human material of consumers. Possible trace elements of interest are Sr, Mo, V, Zn, Rb, Ni, etc.
The project provides specific analytical and instrumental training in major methods of chromatography and atomic spectroscopy, the latter in an internationally known facility for ICP-MS. Furthermore, data analysis and interpretation will require computational training such that the trainee will be able to establish a laboratory using the above techniques and sample preparation and validation methodologies. Transferable skills will be developed through research interactions with other projects in the ICP-MS Facility, the attendance of PG and Chemistry research lectures and RSC Analytical meetings.As you can see from the abstract above the project has excellent opportunities to become familiar with three major analytical techniques, one of which, ICP-MS, the University of Surrey are the birthplace of this technology.

I am pleased to inform you that your ATAS application was successful.

You should now apply to your nearest visa issuing post for a Visa / Entry Clearance / to undertake these studies, or, if you need to extend your existing stay in the United Kingdom you should apply to the Border and Immigration Agency.

Please remember to print off and present this certificate, along with your University offer letter to the Entry Clearance Officer. You will also need to present all of the supporting documentation needed for a student Visa / Entry Clearance / Extension of stay application. A full list of requirements for a student Visa / Entry Clearance can be found at www.ukvisas.gov.uk, or for an extension of stay, at www.bia.homeoffice.gov.uk

This ATAS certificate is only valid for the University / Higher Education Institution and course stated above. If you wish to study at another University / Higher Education Institution and / or do a different course / area of research you will need to apply for another ATAS certificate. You can do this via the ATAS website www.fco.gov.uk/atas.

The ATAS Team.

Foreign & Commonwealth Office
22/12/2007

A1.3: Confirmation letters from Iraq about the innovation in this research**:**

Prof. Neil I. Ward
University of Surrey
Faculty of Medical Sciences
Division of Chemical Sciences

24-8-2008

Dear . Prof.

It is of a great chance communicating you during this period of time. I do appreciate to be a co-adviser under your supervision on the postgraduate student "Baker Abid Alzahra Joda" for his task about trace elements comparing between Iraqi subjects living home and abroad .

According to your schedule, the following samples would be collected from Iraqi residents under my supervision :

A- Biological samples :
1- Human scalp hair.
2- Human nail (finger and toe).
3- Human tear drops.

B- Environmental samples :
1- Drinking water.
2- river and well water.

C-Any further suggestions.

According to my experiences, this search has not be done in Iraq. Though saliva, serum and tissue extract were used to evaluate only some of these trace elements in some diseases in Iraq.

Finally, we hope God would guide us to the best that " Thee Thoughts".

Yours faithfully,

Alaa .K.Mohammed , Ph.D
Assistant Prof .
University of Baghdad
College of Education, Ibn Al-Hathiam
Chemistry Department, Adamia
Baghdad-Iraq

*
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جمهورية العراق
REPUBLIC OF IRAQ

Ministry of Higher Education & Scientific Research
University of Karbala
College of Science
Department of Chemistry

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية العلوم
قسم الكيمياء

Prof. Neil I. Ward
University of Surrey,
Faculty of Health and Medical Sciences
Division of Chemical Sciences

Dear Prof Neil

I am pleased to inform you that Mr. Baker Joda has collected biological samples from Karbala residents. According to his study, the following samples were collected:

1- Scalp hair.
2- Nails (Finger and Toe).
3- Tear drops.
4- Saliva.

Furthermore, I would like to confirm that study has not been done in Karbala to date. Therefore, It considers one of the important studies for human health, environment and chemical researchers in Karbala.

Best Regards

Chemistry department,
College of Science,
University of Karbala, Karbala, Iraq.
E-mail: [illegible]@yahoo.com
Tel. [illegible]
[illegible]

A1.4: World Health Organisation document for water quality in Iraq:

WORLD HEALTH ORGANIZATION
Regional Office for the Eastern Mediterranean
ORGANISATION MONDIAL DE LASANTE
Bureau regional de la Mediterranee Orientale
Baghdad - Iraq

منظمة الصحة العالمية
المكتب الإقليمي لشرق البحر المتوسط
بغداد - العراق

OFFICE OF THE WHO REPRESENTATIVE

مكتب ممثل المنظمة

Press Release

WHO and Iraq Agree to Implement a Water Quality Control and Surveillance Project in Iraq and Rehabilitate the Central Water Quality Control Lab in Baghdad.

Water Quality Regulations and Policies in Iraq under Review

28 September 2004 | Amman -- The World Health Organization (WHO) and Iraq's Ministry of Environment (MOE) agreed to review and update the current regulations and policies for water quality in Iraq with participation of different parties from other ministries and universities. The two sides also agreed on the operational plan to rehabilitate the Central Water Quality Control (WQC) Lab in Baghdad.

"Access to safe water is a right to all people and has major implications on the protection of the health of people." said Dr. Naeema Al-Gasseer. WHO Representative in Iraq, adding that "Unsafe water leads to many diseases and threats of public health risks."

The agreement which was signed on 25 September 2004 in Amman by Dr. Al-Gasseer on behalf of WHO in Iraq, and Dr. Mishkat Mumin, Iraq's Minister of Environment, came at the end of a two-day working session in which delegations from both sides discussed the current environmental situation in Iraq and ways to improve its conditions. To that effect, the MOE and WHO agreed to start the implementation of an Iraqi proposal on Water Quality Control and Surveillance in Iraq that aims at designing a comprehensive water quality monitoring system. This system entails establishing 15 central labs and 30 district labs in the center and south of Iraq, funded by the European Commission through the UNDG Trust Fund.

The agreement also stipulates the rehabilitation of the Central WQC Lab in Baghdad beginning with the establishment of a joint committee to assess the conditions of the building and the financial and administrative preparations.

In addition, the agreement provides for WHO-supported capacity building for the MOE staff, with training courses to be held in and outside Iraq, and the conducting of researches in the field of environment with special emphasis on water quality.

The Iraqi Minister praised the efforts of WHO to organize the working session and underlined the significance of the agreed upon projects and their positive impact on the future of the Iraqi people.

Iraq-Baghdad Office: Tel 0047241372230, Jordan-Amman Office : P.O. Box 3044, Amman 11821 Tel 0096265510438, Fax 0096265510437, e-mail

A1.4: (continued) World Health Organisation document for water quality in Iraq:

WHO has been supporting the government of Iraq in improving the health situation in Iraq, upgrading health policies and strategies, and holding training workshops. WHO is also working in close collaboration with the Ministries of Health, Environment, Education, Higher Education, Planning and other ministries as well as UN partners and NGOs to help the Iraqi people enjoy the highest attainable standard of health as one of the fundamental rights of every Iraqi without distinction of race, religion, political belief, economic or social condition.

For more information contact:

Eng. Mohammad Hamasha
WHO/Iraq, Water and Sanitation Focal Person
Mobile: 00 96279 5043981
Thuraya: 00 88216 33330765
Email: hamasha1@yahoo.com

Ali Hamati
WHO/Iraq, Communication Officer
Mobile: 00 96279 5934876
Office: 00 9626 5510438 ext. 61024
Email: hamatia@irq.emro.who.int

A1.5: Field Sampling Questionnaire:

UNIVERSITY OF SURREY

Study of Human Biological Samples (Hair, Nails, Tear Drops and Saliva) in Karbala and London for Iraqi Individuals.

Code Number: ☐ **Collection Date:** ☐

Town/Province: _____

Type of sample:

Hair ☐ Finger Nails ☐ Toe Nails ☐ Tear Drops ☐ Saliva ☐

Residence: How long have you lived here? _____ years

Where did you live before? _____ town/city

Number of members of family _____ adults

_____ children

Personal Information: (For the sample code above)

Sex: ☐ Male ☐ Female

Age: ☐ Years ☐ Months

Height: ☐ Metres ☐ Centimetres

Weight: ☐ Kilograms

General Health: _____

_____

Do you have any permanent illness? ☐ Yes ☐ No

If yes: _____

_____

What temporary illness have you had in the last 12 months?

☐ None Or _____

Smoking history:

Do you smoke? Yes No

If yes: How many cigarettes per day?

Number of smoking years: Years Months

Type of cigarette:

Diet : How many meals do you eat a day?

☐ Number

What is your basic diet (sources and quantities)? List main foods for each meal*

Meal	Nutrition
	_____ _____
	_____ _____
	_____ _____
	_____ _____

***Note**: Include all sources of protein, carbohydrate, fruits/vegetables and fats.

Drinking water: What sources of drinking water do you use? Used filter Yes No

☐ Bottled ☐ Tap ☐ Other _____

Do you consume commercial beverages? If yes....

☐ Name ☐ Quantity per day/week

Do you apply any special treatments to you hair?

Special shampoos (dandruff, etc) ☐ Dyes ☐ Oxygenation ☐

Spray ☐ Hair gel ☐ Others ☐ _____

Do you apply any special treatments to you Nails? Yes or No:

Children only:

Does your child attend school? ☐ Yes ☐ No

If yes, School_____ Grade_____

How would you describe their academic achievement? _____

_____

Adults only:

Are you employed? ☐ Yes ☐ No

If yes: Where---------- Type of work----------

ALL INFORMATION WITHIN THIS QUESTIONAIRE IS CONFIDENTIAL TO THE PROJECT MANAGERS AND WILL NOT BE MADE AVAILABLE TO ANY OTHER INDIVIDUAL OR GROUP.

A1.6: Research Participant Consent Form:

Sample Consent Form

"Trace Element Levels in Human Tear Drops and Other Media"

- I have read and understood the **Information Sheet for Participants** provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

- I agree to comply with any instruction given to me during the study and to co-operate fully with the investigators.

- I agree to the investigators contacting my local medical practitioner about my participation in the study, and I authorise my local medical practitioner to disclose details of my relevant medical status, in confidence.

- I consent to put my personal data, as outlined in the accompanying information sheet, being used for the research project detailed in the information sheet, and agree that data collected may be shared with other researchers or interested parties.

- I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998).

- I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

Name of volunteer (BLOCK CAPITALS): .....

Signed: .....

Date: .....

Name of researcher/person taking consent (BLOCK CAPITALS):

.....

Signed: .....

Date: .....

A1.7: Information Sheet Form:

Information Sheet for Participants

The following information sheet is planned to be read by the participants in this research project.

"Trace Element Levels in Human Tear Drops and Other Media"

This study will focus on evaluating the effect of any excess or deficiency of trace and minor elements (**Na, Mg, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd and Hg**) on the health of the local population, particularly individuals with diabetes (type 2) by determining the elemental levels in human scalp hair, nails, tear drops and saliva samples. Further information will be obtained from environmental samples, such as, drinking water sources. In addition, information that will be obtained through the course of this study that will be available for other approved research studies. However, the researchers will not be given information that will identify any individual;

The main benefits that will result from this study include:

- a database of the local population about the levels of these elements in selected tissues and fluids of the body;
- to investigate the elemental quality of drinking water used by the local population and its impact on public health;
- to assess for the local population the potential causes that lead to an excess or deficiency of an essential elements in human body;
- to assess if environmental exposure has any effect on the health of participants through comparison with those living in another place of world;
- to strengthen the participants awareness and knowledge about the harmful effects of smoking and possible sources of environmental pollution in relation to the monitoring of essential and non-essential elements in selected tissues and fluids of the human body (through dissemination of the results via Iraqi conferences and to be incorporated into educational material at local universities);

- Participants for this study will be approached (based on their suitability) by a General Practitioner (GP) at the locations (Karbala and London) involved in the collaboration of this research. Sample consent forms will be issued and after approval, sample collection will then be carried out at these locations;
- The participant must cooperate fully with investigators, and to complete questionnaires to provide personal details and information about health, diet and lifestyle at the time the sample(s) are collected. The questionnaires will be administered by a trained interviewer or will be mailed to a participant for completion and returned in a reply-paid confidential labeled envelope;
- We cannot promise that you will benefit from being a participant. We do hope and expect that the outcomes of the research will be available via publications and presentations at conferences;
- It is probable, that information about the study might be published in scientific journals. However, no information that might identify participant will appear in these publications;
- The participants will need permission from their parents if their ages under 18 years. Therefore, investigators will carry out a parental interview. The purpose of this to obtain their permission, and information about their children;
- The participants are free to withdraw from the study at any time, without having to give a reason for withdrawing. All the information will be kept strictly confidential within the research team unless permission had been granted by the named participant to divulge certain information to a third party;
- Participants of the study may receive a report of the chemical results which will be obtained from a medical practitioner; and
- Any complaint or concerns about any aspects of the way you have been dealt with during the course of the study will be addressed; please contact Prof. Neil I. Ward, Principal Investigator on +44(0) 1483 68 93 or n.ward@surrey.ac.uk.

Appendix B

Publications

Appendix B1

B1.1

Use of Human Tear Fluid as a Potential New Biomarker for Trace Elements in Healthy Individuals and Diabetic Patients[+]

Baker A. Joda and Neil I. Ward
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Guildford, Surrey, GU2 7XH

Abstract: The use of unconventional biological materials as biomarkers in trace element studies has increased in terms of published research studies. In this study, human tear fluid was used to be a possible new biomarker for trace elements in the human body as no study has been published in this area yet. Samples were obtained from 111 healthy individuals and 44 diabetic patients resident in Karbala, Iraq, and 18 samples were also collected from healthy Iraqi individuals resident in London, UK, for comparative study. Saliva (n = 97) and water (n = 173) samples were also collected from the same regions. The level of V, Mn, Fe, Cu, Zn, As, Sr and Cd was determined by inductively coupled plasma mass spectrometry (ICP-MS). The validity, precision and accuracy of the methodology were evaluated using a "pooled" sample for each media and various certified reference materials. The validation methods provided acceptable levels of precision and accuracy with lower range of RSD (< 10%) and acceptable range of elemental recoveries (90 - 110 %), respectively. Significantly higher levels of V, Cr, Mn, Fe, Zn, As and Sr, and lower levels of Cd were found in tear drop samples from Karbala when compared with those from London ($P < 0.05$). Similar results were found in saliva for most trace elements. Discriminant analysis suggested that V, Mn, Zn, As, Sr and Cd levels could be used to discriminate between healthy and diabetic populations (83% of cases correctly classified).

Keywords: Tear drops, Saliva, Type 2 diabetes, Trace elements, Multivariate analysis, ICP-MS, Karbala.

[+] this paper was sent to the Journal of Trace Elements in Medicine and Biology

B1.2

Influence of Gender, Age and Smoking Habit on the Trace Elements Levels of Washed Scalp Hair of a Control Population from Karbala, Iraq[+]

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Abstract Hair samples (n=236) of healthy individuals were collected from of Karbala, a city in south-western Iraq. The study population consisted of males (n=196) and females (n=40), age: children (< 15 years, n=57); young (15 – 25, n=78); adults (25 – 45, n=76); and oldest (> 45 years, n=25). All cases were subdivided according to smoking habits (non, passive and active) so as to compare the levels of trace elements in scalp hair in relation to smoking habits. V, Mn, Co, Cu, Zn, Sr and Cd levels in washed scalp hair were measured by inductively coupled plasma mass spectrometry (ICP-MS). The validity and accuracy of the methodology were evaluated by using a certified reference material GBW 09101 Human Scalp Hair with an acceptable range for elemental recoveries ranging from 90 to 107 %. The results obtained showed significantly higher mean level (μg/g dry. weight) of Sr (11.58) in the scalp hair when compared with the reference range values for control or healthy individuals reported in different countries (0.06 – 6.31). It was found that the mean values of Sr and Co were significantly higher in females than males, whilst the levels of V, Mn, Cu, Zn and Cd were similar (at a probability level p = 0.05). Hair of the oldest group has more mean levels (μg/g d.w.) of V (0.42), Mn (2.75), Sr (12.24) and Cd (0.49) than the other age groups. The high of mean levels of Co (0.21 μg/g) were reported in the hair of individuals of age 15 – 25 years; whilst the high of mean levels (μg/g) of Cu (27.52) and Zn (249) were found in children (< 15 years). Hair of smokers contained significantly more V, Sr and Cd than the hair of non-smokers (at p = 0.05). The levels of Mn, Co, Cu and Zn were similar in both sub-groups of smoking activity (at p = 0.05).

Keywords Scalp hair analysis. Trace elements. Karbala, Iraq. ICP-MS. Smoking habits

[+] this paper was sent to Biological Trace Element Research Journal

Appendix C

Statistics

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

Arithmetic mean

The Arithmetic mean ($\bar{x}$) 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 $x_i =$ x value and $\bar{x} =$ 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 $\bar{x}$ = 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:

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

Drift Correction

The instrumental drift can be corrected by the following equation:

$$Drift\ correction = \frac{Unknown\ sample\ concentration}{Mean\ calibration\ standard / known\ calibration\ concentration}$$

Least Squares Regression Line fit

The least squares regression fit calculates a straight line in the form y = *m*x + c, that best fits the data. The regression line for the least squares line fit is calculated as follows:

$$Slope = m = \frac{\sum_i[(x_i - \bar{x})(y_i - \bar{y})]}{\sum_i(x_i - \bar{x})^2}$$

$$Intercept = c = \bar{y} - m\bar{x}$$

where x = value, $\bar{x}$ = mean of x values, y = value, $\bar{y}$ = mean of y values

Pearson Product Moment Correlation Coefficient

The Pearson product moment correlation coefficient (r) is a dimensionless index ranging from -1 to +1 inclusive, which reflects the extent of a linear relationship between two sets of data. It is calculated as follows:

$$r = \frac{\sum_i[(x_i - \bar{x})(y_i - \bar{y})]}{\sqrt{[\sum_i(x_i - \bar{x})^2][\sum_i(y_i - \bar{y})^2]}}$$

Coefficient of Determination (r^2 or R^2)

The coefficient of determination (r^2) is the square of the Pearson product moment correlation coefficient (r) for the purposes of linear calibration.

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 (μ) 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.

Anderson-Darling Test

The Anderson-Darling test is used to detect whether a sample of data came from a population with a specific distribution. The Anderson-Darling statistic (A^2) is defined as:

$$A^2 = -n - \frac{1}{n}\sum_{i=1}^{n}(2i-1).[\ln F(X_i) + \ln(1 - F(X_{n-i+1}))]$$

where n = the number of sample, F(X) = cumulative distribution function for the specified distribution and i = the i^{th} sample when the data is sorted in ascending order.

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:

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

F-Test

An F-test is used to compare the standard deviations of two populations (s_1^2 and s_2^2), whereby the ratio of two variances is calculated. The calculated value (F_{calc}) is compared to an F-critical value (F_{crit}) at the 95% confidence interval for $n_1 - 1$ and $n_2 - 1$ degrees of freedom. If the two variances are not significantly different, the F value will be close to 1. The value of F is calculated as follows:

$$F = \frac{s_1^2}{s_2^2}$$

Student t-test

A student's t-test is used to calculate the significance of a difference between a known value (μ), such as certified reference value and a measured mean ($\bar{x}$) and standard deviation (s). Then the calculated value (t_{calc}) is compared to the t-critical (t_{crit}) value for n-1 degrees of freedom at the 95% confidence interval ($P < 0.05$). The value is calculated as follows:

$$t = \frac{(\bar{x} - \mu)\sqrt{n}}{s}$$

Paired t-test

This test is used to compare pairs of data, such as when a single sample has been measured by the two analytical techniques or prepared by the two digestion methods. The difference between the data values for the two different methods is used to determine the calculated value (t_{calc}) value. This value is compared with a t-critical (t_{crit}) value for n-1 degrees of freedom at the 95% confidence interval ($P < 0.05$), as shown:

$$t_{calc} = \frac{\bar{D}\sqrt{n}}{s_d}$$

$$s_d = \sqrt{\frac{\sum(D_i - \bar{D})^2}{N - 1}}$$

Where s_d is standard deviation, D_i is the individual difference between the two methods for each sample, with regard to sign; and $\bar{D}$ is the mean of all the individual differences.

Significance test for Linear Regression

The significance of the Pearson product moment correlation coefficient (r) can be established using a t-test. The calculated (t_{calc}) value is compared to the t-critical (t_{crit}) 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 (t_{calc}) value is compared with the t-critical (t_{crit}) value for $n_1 + n_2 - 2$ 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 (df) 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 $\bar{x}_1$ and $\bar{x}_2$ are the means of populations 1 and 2, and s_1 and s_2 are the respective standard deviations.

Appendix D

Water and Tobacco Results

Appendix D1

Water Sample Results:

Table D1.1: Description and location of water samples with parameter measurements and total trace element levels.

PIN	Water parameter				Elemental level (µg/l) or (**mg/l**)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWCB1	Commercial	8.5	218	111	0.06	0.08	0.11	0.19	0.96	0.88	2.42	0.02	0.03	0.01
KWCB2	Commercial	8.3	228	114	0.26	0.53	0.03	0.06	0.72	0.26	0.77	0.20	0.12	0.01
KWCB3	Commercial	8.1	222	112	0.16	0.30	0.06	0.12	0.79	0.49	1.58	0.12	0.07	0.01
KWDB4	Domestic bottle	7.8	1184	591	0.35	3.61	0.63	1.73	11.92	6.22	260.61	0.71	0.96	0.95
KWDB5	Domestic bottle	8.1	279	142	0.26	0.57	0.37	0.41	11.14	8.40	38.92	0.45	0.22	0.92
KWDB6	Domestic bottle	7.6	1344	684	0.31	3.58	0.40	1.66	12.34	7.61	192.28	1.53	1.29	0.96
KWDB7	Domestic bottle	8.3	833	455	0.21	0.20	0.45	0.47	11.03	6.78	20.32	0.21	0.07	0.93
KWDB8	Domestic bottle	7.4	968	502	0.23	0.08	0.38	0.62	11.12	9.84	337.48	0.27	0.02	1.16
KWDB9	Domestic bottle	7.8	852	428	0.15	2.26	0.46	0.88	11.58	30.41	81.12	1.07	0.66	0.96
KWDB10	Domestic bottle	8.0	428	213	0.25	1.04	0.36	0.67	11.43	17.60	47.40	0.47	0.32	0.92
KWDB11	Domestic bottle	8.4	460	215	0.22	0.08	0.31	0.60	11.39	9.67	327.84	0.25	0.02	1.17
KWDB12	Domestic bottle	7.7	1080	543	0.23	2.70	0.40	0.47	11.29	7.60	102.27	1.15	1.07	0.93
KWDB13	Domestic bottle	7.7	1365	674	0.31	3.88	0.47	2.79	27.59	8.19	74.91	1.49	1.29	1.01
KWDB14	Domestic bottle	7.7	1377	719	0.33	3.36	0.55	1.60	12.52	8.36	715.46	1.19	1.35	1.02
KWDB15	Domestic bottle	7.7	1484	747	0.34	3.62	0.45	2.63	10.98	6.66	98.62	1.40	1.51	0.94
KWDB16	Domestic bottle	8.1	472	238	0.19	0.96	4.85	2.13	35.59	6.82	27.42	0.52	0.37	0.92
KWDB17	Domestic bottle	7.7	1343	705	0.32	3.99	0.47	0.78	11.47	7.31	19.94	1.57	1.30	0.94
KWDB18	Domestic bottle	7.8	216	108	0.28	0.45	0.44	0.90	9.38	3.88	22.29	0.21	0.09	0.92
KWDB19	Domestic bottle	8.2	476	265	0.07	0.08	0.31	0.99	10.03	3.98	97.34	0.13	0.02	0.92
KWDB20	Domestic bottle	8.0	585	293	0.31	3.67	0.35	0.59	9.46	4.73	53.98	1.22	1.20	1.03
KWDB21	Domestic bottle	8.2	262	131	0.11	1.90	0.47	0.50	9.48	4.18	85.30	0.75	0.42	0.95
KWDB22	Domestic bottle	7.7	1426	718	0.25	0.47	0.34	0.43	9.36	4.49	15.34	0.67	0.17	0.95
KWDB23	Domestic bottle	7.7	1528	763	0.30	3.71	0.39	0.78	11.17	4.31	163.74	1.22	1.38	0.94
KWDB24	Domestic bottle	7.7	1366	706	0.33	4.00	0.42	1.78	9.41	3.95	32.75	1.34	1.53	0.94
KWDB25	Domestic bottle	7.7	1370	702	0.31	3.97	0.44	3.86	10.15	4.20	68.36	1.39	1.39	0.93

Table D1.1 (continued)

PIN	Water parameter				Elemental level (µg/l) or (mg/l)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
KWDB26	Domestic bottle	7.7	1553	778	0.31	4.11	0.48	2.84	10.76	4.18	14.66	1.51	1.36	0.93
KWDB27	Domestic bottle	8.4	438	254	0.27	0.41	0.40	0.66	9.68	4.58	15.60	0.30	0.11	0.97
KWDB28	Domestic bottle	7.9	1175	602	0.24	2.85	0.40	1.52	9.47	4.23	313.05	0.74	1.26	1.06
KWDB29	Domestic bottle	7.7	1528	763	0.29	3.88	0.10	1.21	0.80	0.50	8.26	1.30	1.50	0.02
KWDB30	Domestic bottle	7.7	1484	747	0.09	1.92	0.20	0.35	0.88	0.41	46.57	0.69	0.44	0.11
KWDB31	Domestic bottle	8.4	945	564	0.24	0.38	0.06	0.46	0.86	0.82	1.69	0.13	0.12	0.03
KWDB32	Domestic bottle	7.7	1528	763	0.29	3.83	0.10	1.22	0.87	0.46	8.02	1.34	1.52	0.02
KWDB33	Domestic bottle	7.7	1426	718	0.28	3.78	0.10	0.63	1.07	0.55	130.94	1.27	1.38	0.03
KWDB34	Domestic bottle	7.8	216	108	0.25	0.43	0.06	0.75	0.76	0.42	2.49	0.05	0.10	0.02
KWDB35	Domestic bottle	7.7	1366	706	0.28	3.94	0.16	3.54	1.26	0.49	34.87	1.40	1.35	0.02
KWDB36	Domestic bottle	8.0	585	293	0.29	3.76	0.06	0.29	0.97	0.92	15.42	1.32	1.19	0.05
KWT37	Tap	7.7	1294	647	0.32	3.59	0.41	22.57	9.59	5.57	71.19	1.54	1.06	0.96
KWT38	Tap	7.7	1207	602	0.29	2.97	0.39	5.91	9.46	4.26	154.76	1.26	0.99	0.94
KWT39	Tap	7.9	1183	593	0.29	3.25	0.38	1.35	10.42	5.61	105.29	1.11	1.00	1.45
KWT40	Tap	8.4	275	137	0.29	0.42	0.32	3.24	9.75	9.71	30.47	0.20	0.08	1.39
KWT41	Tap	7.8	1151	574	0.31	3.65	0.53	0.44	9.57	4.27	30.90	1.13	0.99	1.00
KWT42	Tap	8.0	1147	574	0.27	2.87	0.41	2.40	9.30	4.10	160.67	0.97	0.83	0.99
KWT43	Tap	8.0	1128	564	0.34	3.86	0.42	0.30	9.32	5.37	29.67	1.49	0.98	1.16
KWT44	Tap	7.9	1185	592	0.29	2.85	0.42	6.25	9.28	6.86	38.50	1.29	1.04	0.97
KWT45	Tap	8.0	1177	588	0.29	3.34	0.38	4.08	9.60	18.47	19.77	1.52	0.92	0.96
KWT46	Tap	7.8	1251	626	0.31	2.86	0.48	15.03	9.31	6.92	184.66	1.62	1.07	2.05
KWT47	Tap	8.0	1218	607	0.31	3.80	0.39	5.23	9.29	4.65	35.63	1.42	0.93	1.07
KWT48	Tap	8.0	1123	558	0.30	3.59	0.41	0.27	9.34	4.47	17.49	1.40	0.91	0.97
KWT49	Tap	8.0	1158	578	0.28	3.06	0.43	3.91	12.66	4.63	65.78	1.08	1.02	1.04
KWT50	Tap	8.2	1157	577	0.30	3.61	0.52	1.49	9.33	4.36	185.99	1.19	0.96	1.00
KWT51	Tap	8.1	1168	583	0.32	3.77	0.88	0.33	9.60	9.52	24.74	1.95	0.98	0.98
KWT52	Tap	8.1	1155	576	0.30	3.36	0.45	0.49	9.41	4.06	41.90	1.29	0.94	1.01
KWT53	Tap	8.0	1124	561	0.29	3.40	0.40	0.36	9.61	4.04	28.68	1.31	0.89	0.94
KWT54	Tap	8.0	1141	570	0.30	3.71	0.52	0.75	9.71	4.39	17.37	1.42	0.96	0.99

Table D1.1 (continued)

PIN	Water parameter				Elemental level (µg/l) or (mg/l)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWT55	Tap	8.1	1126	557	0.31	3.78	0.39	0.61	9.28	3.98	13.65	1.44	0.88	0.93
KWT56	Tap	8.0	1150	574	0.29	3.63	0.53	0.32	9.53	3.79	20.66	1.28	0.95	0.94
KWT57	Tap	8.0	1150	574	0.55	7.09	0.60	0.41	6.55	1.33	5.49	2.57	1.85	0.12
KWT58	Tap	8.1	1126	557	0.55	7.36	0.34	0.80	6.65	1.96	3.43	2.61	1.73	0.10
KWT59	Tap	7.7	1294	647	0.59	7.30	0.40	25.38	7.07	4.21	31.23	2.45	2.11	0.20
KWT60	Tap	8.0	1141	570	0.55	7.25	0.44	1.09	6.80	2.26	4.82	2.74	1.89	0.15
KWT61	Tap	7.7	1294	647	0.31	3.68	0.41	21.97	10.81	5.77	69.89	1.38	1.05	0.97
KWT62	Tap	7.7	1207	602	0.30	3.07	0.42	5.55	12.34	6.65	170.69	1.23	1.03	0.98
KWT63	Tap	7.9	1183	593	0.24	2.61	0.36	0.91	10.92	6.59	89.69	0.93	0.83	1.35
KWT64	Tap	8.4	275	137	0.29	0.43	0.33	2.32	12.31	13.56	33.15	0.33	0.09	1.44
KWT65	Tap	7.8	1151	574	0.30	3.56	0.35	0.51	12.27	5.93	29.36	1.17	0.96	0.96
KWT66	Tap	8.0	1147	574	0.33	3.37	0.36	2.85	9.08	4.09	196.96	1.18	0.97	0.98
KWT67	Tap	8.0	1128	564	0.30	3.78	0.44	0.34	10.99	5.35	26.45	1.36	0.88	1.19
KWT68	Tap	7.9	1185	592	0.28	2.87	0.46	6.15	10.07	6.89	37.05	1.33	1.05	0.97
KWT69	Tap	8.0	1177	588	0.30	3.32	0.78	3.51	9.37	18.09	20.04	1.62	0.95	0.98
KWT70	Tap	7.8	1251	626	0.26	2.41	0.42	12.47	9.95	5.89	157.76	1.53	0.90	1.88
KWT71	Tap	8.0	1218	607	0.32	3.88	0.46	5.23	9.89	4.74	34.61	1.50	0.98	1.09
KWT72	Tap	8.0	1123	558	0.36	3.61	0.35	0.85	9.48	4.06	12.28	1.44	1.05	0.98
KWT73	Tap	8.0	1158	578	0.28	3.04	0.49	4.03	10.23	4.43	64.04	1.20	1.03	1.05
KWT74	Tap	8.2	1157	577	0.30	3.67	0.53	1.89	9.44	4.08	177.65	1.08	0.99	1.01
KWT75	Tap	8.1	1168	583	0.31	3.83	0.79	0.27	9.68	9.73	21.39	2.33	0.99	0.95
KWT76	Tap	8.1	1155	576	0.42	3.26	0.45	0.27	9.38	4.45	40.79	1.39	1.20	1.02
KWT77	Tap	8.0	1124	561	0.30	3.59	0.39	0.32	10.01	4.20	28.21	1.48	0.93	0.95
KWT78	Tap	8.0	1141	570	0.30	3.74	0.56	0.22	9.77	4.83	18.06	1.50	0.99	0.96
KWT79	Tap	8.1	1126	557	0.30	3.73	0.37	0.33	9.57	4.05	11.17	1.18	0.86	0.91
KWT80	Tap	8.0	1150	574	0.30	3.62	0.66	0.41	9.47	3.72	16.63	1.30	0.95	0.91
KWT81	Tap	8.0	1124	561	0.55	6.73	0.36	0.35	7.08	1.81	8.32	2.47	1.76	0.12
KWT82	Tap	8.0	1150	574	0.55	7.11	0.64	0.39	6.67	1.44	4.44	2.34	1.86	0.11
KWT83	Tap	8.1	1126	557	0.56	7.31	0.34	0.24	6.62	1.73	2.61	2.49	1.72	0.09

Table D1.1 (continued)

PIN	Water parameter				Elemental level (μg/l) or (**mg/l**)									
	Water type	pH	EC (μS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWT84	Tap	7.7	1294	647	0.58	6.85	0.48	42.04	8.39	4.26	37.53	2.57	2.01	0.21
KWT85	Tap	8.0	1141	570	0.56	7.38	0.53	0.17	7.14	2.58	5.05	2.73	1.92	0.15
KWT86	Tap	8.0	1124	561	0.56	6.90	0.35	0.11	7.64	2.21	7.79	2.52	1.79	0.11
LWT87	Tap	8.3	571	285	0.06	0.87	0.11	0.20	0.77	19.19	37.13	1.05	0.22	0.05
LWT88	Tap	8.1	579	290	0.06	0.95	0.11	0.35	0.98	6.50	2.80	1.26	0.22	0.03
LWT89	Tap	7.4	580	289	0.01	0.04	0.04	12.16	0.74	1.01	5.52	0.02	0.01	0.03
LWT90	Tap	7.8	572	285	0.02	0.44	0.07	0.23	0.72	2.35	0.66	0.40	0.09	0.01
LWT91	Tap	8.1	580	289	0.06	0.94	0.17	0.38	0.94	5.00	1.51	1.20	0.23	0.07
LWT92	Tap	7.0	414	206	0.05	0.45	0.09	0.24	0.75	3.04	2.73	0.95	0.15	0.03
LWT93	Tap	7.1	417	208	0.05	0.46	0.08	0.22	0.75	3.06	2.27	0.94	0.15	0.02
LWT94	Tap	8.2	582	291	0.06	0.91	0.10	0.35	1.00	5.43	1.63	1.23	0.23	0.02
LWT95	Tap	7.8	512	255	0.05	0.24	0.18	0.17	1.00	1.69	1.55	0.41	0.28	0.03
LWT96	Tap	7.8	329	165	0.04	0.10	0.08	0.66	0.80	8.40	5.04	0.25	0.20	0.02
LWT97	Tap	7.4	381	190	0.06	0.37	0.11	0.56	0.71	2.05	45.81	1.09	0.11	0.02
LWT98	Tap	7.5	383	191	0.06	0.41	0.16	0.70	0.71	1.82	26.23	1.08	0.12	0.02
LWT99	Tap	7.8	190	95	0.01	0.12	0.10	0.05	0.71	0.87	1.90	0.14	0.06	0.02
LWT100	Tap	7.8	189	94	0.01	0.12	0.06	0.04	0.72	0.61	1.54	0.14	0.05	0.02
LWT101	Tap	7.0	417	208	0.08	0.45	0.08	0.22	0.74	3.15	2.43	0.97	0.21	0.03
LWT102	Tap	6.1	566	284	0.06	0.17	0.27	0.68	0.69	0.79	1.85	0.12	0.36	0.04
KWR103	River	8.5	1373	692	0.44	4.84	3.13	7.96	97.45	38.05	136.79	3.07	1.37	11.44
KWR104	River	8.2	1379	697	0.46	5.14	3.44	7.03	97.14	35.79	118.88	2.74	1.38	9.79
KWR105	River	8.0	1381	691	0.48	4.58	3.06	7.13	96.20	35.74	116.52	2.29	1.23	10.57
KWR106	River	7.9	1375	688	0.51	4.83	3.13	5.78	95.71	35.67	113.41	3.32	1.43	9.98
KWR107	River	7.8	1362	682	0.49	4.88	2.93	4.29	95.11	34.93	110.67	3.07	1.36	9.75
KWR108	River	7.9	1442	723	0.45	4.39	4.08	4.71	115.65	34.59	115.47	2.70	1.36	10.00
KWR109	River	7.8	1311	662	0.48	5.15	3.12	4.09	97.07	34.74	120.65	2.80	1.39	9.78
KWR110	River	8.0	1320	667	0.52	4.64	3.04	4.92	94.88	33.89	111.99	2.31	1.35	9.97
KWR111	River	7.9	1316	660	0.49	5.23	2.90	7.49	96.57	35.00	121.15	3.11	1.42	9.80
KWR112	River	8.0	1305	658	0.47	4.49	2.89	2.36	96.66	38.47	123.64	2.80	1.38	9.82

Table D1.1 (continued)

PIN	Water parameter				Elemental level (µg/l) or (mg/l)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWR113	River	8.2	1331	666	0.48	4.51	2.82	2.76	97.88	34.34	105.50	3.26	1.33	9.68
KWR114	River	8.2	1323	667	0.49	6.21	3.62	7.12	98.48	35.27	134.97	3.36	1.50	9.97
KWR115	River	8.2	1322	661	0.46	4.88	3.78	6.25	102.06	36.22	169.49	3.14	1.38	10.66
KWR116	River	8.0	1323	658	0.48	4.59	2.96	2.85	96.91	34.11	113.92	2.44	1.36	10.02
KWR117	River	8.1	1275	644	0.46	7.72	7.14	12.78	109.60	77.02	377.50	6.63	1.39	13.55
KWR118	River	8.2	1379	697	0.40	4.82	0.34	3.96	7.69	1.77	115.03	1.77	1.65	1.03
KWR119	River	8.2	1323	667	0.41	5.09	0.36	2.87	7.58	1.63	129.09	1.73	1.65	1.05
KWR120	River	8.2	1379	697	0.41	4.70	0.46	2.73	7.91	2.27	130.52	1.68	1.75	1.06
KWR121	River	8.2	1323	667	0.27	3.15	0.31	3.21	7.74	1.54	125.43	1.45	1.14	1.02
KWR122	River	7.9	1375	688	0.59	8.15	0.40	1.47	7.16	1.11	135.60	2.69	2.76	1.06
KWR123	River	8.5	1373	692	0.78	4.17	3.33	2.14	96.00	34.83	108.02	2.55	1.38	9.67
KWR124	River	8.0	1381	691	0.34	1.12	3.01	1.86	94.98	34.49	112.10	1.56	0.34	9.81
KWR125	River	7.8	1362	682	0.52	4.07	3.07	1.82	95.57	34.82	105.78	3.06	1.38	9.81
KWR126	River	7.9	1442	723	0.43	3.39	3.07	3.05	95.51	33.31	99.02	3.03	1.16	9.99
KWR127	River	7.8	1311	662	0.29	1.81	3.60	2.04	99.58	33.34	105.53	2.12	0.59	9.87
KWR128	River	8.0	1320	667	0.46	4.20	2.89	2.02	94.02	32.84	95.66	2.60	1.36	9.65
KWR129	River	7.9	1316	660	0.27	1.65	3.30	1.94	95.38	32.76	100.55	2.41	0.52	9.68
KWR130	River	8.0	1305	658	0.45	4.36	2.92	2.22	94.17	32.83	102.91	2.75	1.40	9.96
KWR131	River	8.2	1331	666	0.43	4.34	3.10	1.96	95.15	33.02	101.27	2.71	1.35	9.69
KWR132	River	8.2	1323	667	0.25	1.64	2.81	1.72	94.58	33.42	98.18	1.76	0.56	9.73
KWR133	River	8.2	1322	661	0.39	3.96	2.91	2.64	98.38	33.20	100.79	2.46	1.24	9.80
KWR134	River	8.0	1323	658	0.41	4.26	3.01	2.92	96.10	32.27	106.52	2.51	1.36	9.73
KWR135	River	8.1	1275	644	0.43	4.32	3.56	2.12	97.55	33.86	105.12	2.61	1.40	9.93
KWW136	Well	7.2	> 3999	> 2000	0.86	4.94	3.10	121.09	96.31	33.80	116.38	2.37	9.09	9.76
KWW137	Well	7.6	> 3999	> 2000	1.45	6.27	29.44	4.02	128.27	38.43	149.76	1.88	8.61	10.10
KWW138	Well	7.6	2505	1254	0.91	0.58	3.56	4.48	96.82	34.33	112.36	2.91	3.31	10.12
KWW139	Well	7.7	2576	1287	0.81	0.65	3.00	3.00	94.70	34.38	108.31	1.80	2.99	9.77
KWW140	Well	7.6	> 3999	> 2000	2.91	5.09	31.74	3.59	93.38	35.15	120.04	2.24	8.90	10.02
KWW141	Well	7.7	2629	1314	0.92	1.15	3.19	3.32	96.90	35.09	111.42	2.06	2.96	9.91

Table D1.1 (continued)

PIN	Water parameter				Elemental level (µg/l) or (mg/l)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWW142	Well	7.7	3030	1511	0.94	0.43	3.54	4.75	117.94	34.39	118.04	2.70	2.76	10.19
KWW143	Well	7.6	2583	1281	0.81	0.96	2.95	5.04	96.60	35.52	121.51	2.83	3.21	9.72
KWW144	Well	7.7	2625	1314	0.80	1.80	2.92	3.36	96.02	34.51	127.96	1.41	1.51	9.76
KWW145	Well	7.7	2618	1285	0.89	2.89	7.48	17.58	101.36	38.56	192.12	5.74	3.96	10.45
KWW146	Well	7.6	> 3999	> 2000	1.54	1.74	12.54	3.48	96.28	33.76	119.13	1.62	8.69	9.70
KWW147	Well	8.5	3197	1582	0.76	0.78	3.20	4.76	95.88	32.71	122.46	3.37	3.87	9.77
KWW148	Well	7.5	> 3999	> 2000	1.39	11.56	29.19	2.24	96.47	36.08	122.77	1.78	8.78	10.23
KWW149	Well	7.6	2563	1299	0.92	0.81	3.32	3.04	97.98	34.01	123.35	1.88	3.05	10.21
KWW150	Well	7.8	> 3999	> 2000	2.72	3.81	28.08	5.61	96.20	34.60	214.18	2.08	9.26	10.15
KWW151	Well	7.5	3835	1940	1.36	14.95	27.34	3.83	96.85	41.39	139.87	1.84	8.28	10.05
KWW152	Well	7.5	3627	1812	1.31	14.82	27.23	2.61	95.52	36.54	129.60	2.14	7.63	9.98
KWW153	Well	7.5	3734	1866	1.38	14.39	36.29	1.87	95.95	33.14	119.31	1.72	7.39	9.82
KWW154	Well	7.6	> 3999	> 2000	1.57	6.26	8.54	12.04	96.67	32.99	193.15	2.62	8.55	10.79
KWW155	Well	7.5	3400	1703	0.93	14.07	3.22	114.80	97.04	34.80	115.12	4.60	8.74	10.25
KWW156	Well	7.6	> 3999	> 2000	1.62	5.42	27.40	3.26	95.83	34.61	131.85	1.84	8.50	9.88
KWW157	Well	7.5	3424	1760	1.42	9.71	26.63	2.29	95.63	33.97	120.66	2.56	7.78	9.96
KWW158	Well	7.3	> 3999	> 2000	0.70	5.18	3.64	134.76	95.38	34.41	195.77	2.03	9.53	9.79
KWW159	Well	7.3	> 3999	> 2000	2.75	5.89	26.31	5.03	121.16	34.91	124.94	1.76	7.91	9.94
KWW160	Well	7.4	3679	1846	1.29	1.62	9.39	3.84	97.60	35.36	123.89	2.41	7.50	11.41
KWW161	Well	7.4	> 3999	> 2000	2.58	4.50	8.20	27.92	95.10	33.95	122.46	2.21	9.80	10.10
KWW162	Well	7.5	> 3999	> 2000	2.20	17.78	19.10	2.04	94.67	33.08	109.99	1.88	9.06	9.73
KWW163	Well	7.8	> 3999	> 2000	1.52	8.22	34.51	3.41	93.92	35.63	138.42	1.80	8.53	10.29
KWW164	Well	7.7	> 3999	> 2000	2.95	7.97	42.86	3.76	94.18	33.90	134.46	2.14	8.04	10.04
KWW165	Well	7.6	3501	1748	0.73	13.17	18.40	1.98	94.25	33.60	114.96	1.79	9.12	9.81
KWW166	Well	7.6	2789	1408	0.84	0.39	2.85	2.29	94.33	32.75	109.20	1.32	3.75	9.70
KWW167	Well	7.6	> 3999	> 2000	2.24	6.04	35.02	1.81	95.71	32.25	104.72	1.38	8.96	9.83
KWW168	Well	7.5	3765	1878	0.89	8.28	19.70	2.93	95.38	40.46	253.27	1.71	8.20	10.02

Table D1.1 (continued)

PIN	Water parameter				Elemental level (µg/l) or (mg/l)									
	Water type	pH	EC (µS/cm)	TDS (mg/l)	**B**	V	Cr	Mn	Fe	Cu	Zn	As	**Sr**	Cd
KWW169	Well	7.4	> 3999	> 2000	3.94	1.62	2.95	44.65	92.49	32.76	110.01	1.84	14.38	9.95
KWW170	Well	7.5	> 3999	> 2000	2.47	8.63	34.66	3.02	96.86	32.46	109.34	1.63	8.43	9.79
KWW171	Well	7.6	3837	1920	1.35	5.40	24.93	1.99	94.99	34.01	121.26	1.51	8.29	9.80
KWW172	Well	7.5	> 3999	> 2000	2.16	7.49	28.44	2.00	94.84	33.12	116.66	2.39	9.01	9.89
KWW173	Well	7.6	> 3999	> 2000	0.84	2.18	15.00	3.94	99.68	36.61	139.31	3.80	2.96	10.09
KWW174	Well	7.6	> 3999	> 2000	1.52	6.28	40.10	4.22	93.50	32.33	178.10	1.72	7.93	9.78
KWW175	Well	7.6	> 3999	> 2000	1.92	11.50	37.31	1.80	93.55	33.20	131.64	2.01	8.91	9.67
KWW176	Well	7.5	3441	1719	1.04	17.08	12.71	1.63	93.87	34.45	110.73	2.02	9.53	9.74
KWW177	Well	7.6	> 3999	> 2000	1.56	2.21	15.15	4.61	95.71	34.39	114.69	1.74	8.89	9.77
KWW178	Well	7.5	> 3999	> 2000	3.89	9.96	8.47	1.85	93.66	33.17	108.22	2.30	9.67	9.70
KWW179	Well	7.1	2647	1323	1.10	4.29	4.40	101.07	96.02	34.90	111.85	10.52	4.80	9.81
KWW180	Well	6.6	3315	1659	1.13	12.00	3.95	4.93	96.05	39.13	121.84	2.98	7.67	10.02
KWW181	Well	4.9	3715	1865	0.83	7.95	15.40	8.27	131.84	34.28	118.95	2.43	1.56	9.90
KWW182	Well	7.4	> 3999	> 2000	3.04	5.92	4.25	122.43	96.77	35.42	107.74	13.07	3.29	9.77
KWS183	Spring	7.7	1172	583	0.41	0.64	3.17	3.64	97.52	33.01	108.84	2.52	1.24	9.78
KWS184	Spring	7.8	> 3999	> 2000	2.16	0.96	3.46	2.58	99.39	33.39	140.43	2.31	8.31	9.77
KWS185	Spring	7.5	1495	746	0.51	1.51	3.13	2.19	95.61	32.76	110.87	1.71	1.46	9.73
KWS186	Spring	7.9	2811	1408	0.92	2.39	3.06	1.69	96.33	37.27	132.04	2.37	3.12	10.00
KWS187	Spring	7.7	1172	583	0.59	0.33	1.15	1.98	33.41	1.89	14.53	0.75	1.16	0.72
KWS188	Spring	7.8	> 3999	> 2000	2.28	0.69	1.10	1.10	32.63	3.62	118.50	1.00	8.00	0.73
KWS189	Spring	7.5	1495	746	0.59	1.20	0.93	1.14	34.09	2.61	14.65	0.88	1.42	0.68
KWS190	Spring	7.9	2811	1408	0.93	2.02	0.90	1.23	33.53	2.99	15.34	0.75	2.88	0.80

EC = electrical conductivity, TDS = total dissolved solid, KWCB1, where K corresponds to the province in Iraq (K) Karbala, and may be replaced by (L) London in the UK; W corresponds to water, CB corresponds to commercial bottle and may be replaced by DB (domestic bottle), T (tap), R (river), W (well) and S (spring or artesian); and 1 corresponds to the sample code number.

Appendix D2

Cigarette Tobacco Results:

Table D2.1: Description of cigarette tobacco samples digested using dry and wet digestion methods (paired t-test data).

Kjeldahl™ tube	Elemental levels (mg/kg)*								
PIN	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
KCT-1	0.42	0.72	88.38	279.38	6.45	34.95	2.37	52.98	0.79
GCT-2	0.32	0.87	89.63	208.51	4.88	18.09	1.20	72.96	0.24
RCT-3	0.34	0.67	114.24	237.31	4.18	33.30	0.86	69.42	1.11
RCT-4	0.35	0.40	91.29	212.07	2.58	26.24	1.20	79.76	0.66
GCT-5	0.43	0.99	97.30	254.04	3.37	24.74	0.83	77.00	1.10
BCT-6	0.32	0.66	117.38	251.17	7.99	31.18	3.54	66.90	1.38
GCT-7	0.38	0.45	95.13	268.22	9.88	33.28	3.46	56.77	2.03
ECT-8	0.41	0.62	102.99	280.79	6.12	25.04	0.89	101.88	0.84
CCT-9	0.26	0.40	62.04	165.80	3.55	19.66	1.03	62.55	0.51
ICT-10	0.42	0.82	77.98	272.26	6.10	23.67	4.18	76.99	0.53
DCT-11	0.56	0.62	59.39	236.86	2.93	28.49	0.66	68.95	0.40
ACT-12	0.61	0.50	127.98	349.26	9.32	32.32	2.10	98.80	0.91
FCT-13	0.53	0.71	100.65	315.72	2.47	19.30	1.09	88.87	0.48
BCT-14	0.46	0.45	109.81	284.84	8.80	28.33	2.28	81.12	1.51
MCT-15	0.67	0.54	157.70	330.32	2.45	25.08	0.81	86.53	0.76
PCT-16	0.27	0.48	90.57	167.48	4.74	25.55	1.13	63.18	1.22
Dry ashing	Elemental levels (mg/kg)*								
PIN	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
KCT-1	0.42	0.69	82.95	256.56	6.61	26.56	0.62	37.78	0.61
GCT-2	0.41	0.99	80.61	240.80	8.60	19.60	0.44	57.08	0.20
RCT-3	0.35	0.74	106.92	247.19	9.60	28.62	0.75	54.86	1.15
RCT-4	0.49	0.52	102.70	271.66	9.33	24.59	0.81	59.18	0.74
GCT-5	0.37	0.90	121.46	230.79	6.98	22.67	0.72	55.71	1.00
BCT-6	0.37	0.81	95.60	241.66	7.15	23.73	0.47	50.40	1.03
GCT-7	0.39	0.53	101.99	238.12	9.74	33.47	0.45	42.02	1.84
ECT-8	0.42	0.59	91.04	258.70	7.43	21.70	0.17	66.74	0.79
CCT-9	0.27	0.50	72.71	166.94	7.28	18.37	0.38	46.94	0.51
ICT-10	0.58	0.98	96.81	327.02	5.91	20.76	0.48	60.46	0.33
DCT-11	0.55	0.89	69.29	297.27	9.85	26.75	0.64	54.75	0.68
ACT-12	0.70	0.56	128.38	359.62	7.76	24.28	0.13	73.73	0.76
FCT-13	0.56	0.68	127.49	306.41	4.94	20.19	0.35	74.48	0.51
BCT-14	0.51	0.54	112.72	286.69	8.10	20.71	0.75	65.59	0.91
MCT-15	0.58	0.51	143.72	313.19	4.18	18.08	0.63	68.87	0.70
PCT-16	0.32	0.56	91.26	184.78	6.95	21.91	0.52	44.49	0.94

* replicate value (n = 3), ID codes have been described in Section 2.1.1 (Table 2.2).

Appendix E

Human Tear Drop Results

Appendix E1

Human Tear Drop Results:

Table E1.1: Description of human tear fluid samples (n = 173) and elemental levels for Iraqi individuals from Karbala (Iraq) and London (UK).

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-1	H	M	33	S	Karbala	785	1.06	5.67	9.78	284	193	187	1.57	112	2.19
K-TD-H-9/2009-2	H	M	28	S	Karbala	360	7.24	13.27	84.49	1273	521	6334	10.01	592	3.53
K-TD-H-9/2009-3	H	M	28	S	Karbala	399	5.50	12.25	76.56	854	540	3748	12.64	1094	4.66
K-TD-H-9/2009-4	H	M	40	S	Karbala	< 70	3.70	9.13	35.83	427	203	416	3.71	442	1.32
K-TD-H-9/2009-5	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	255	4100	9.56	1159	6.13
K-TD-H-9/2009-6	H	M	44	NS	Karbala	504	4.28	7.33	9.90	344	196	460	0.48	140	0.75
K-TD-H-9/2009-7	H	M	46	NS	Karbala	417	2.07	5.37	8.03	271	159	186	0.96	112	0.76
K-TD-H-9/2009-8	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	427	494	1.34	58	0.34
K-TD-H-9/2009-9	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	313	598	1.83	271	1.17
K-TD-H-9/2009-10	H	M	3	NS	Karbala	761	12.61	35.56	96.17	2253	211	1079	2.75	806	0.71
K-TD-H-9/2009-11	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	431	1022	5.15	587	1.35
K-TD-H-9/2009-12	H	M	40	NS	Karbala	252	2.75	6.20	22.98	465	198	224	1.42	262	0.36
K-TD-H-9/2009-13	H	M	42	NS	Karbala	616	3.43	7.07	19.63	335	280	355	2.11	246	1.26
K-TD-H-9/2009-14	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	318	277	2.67	345	0.57
K-TD-H-9/2009-15	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	148	352	1.81	425	0.58
K-TD-H-9/2009-16	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	49	1175	33.94	689	0.16
K-TD-H-9/2009-17	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	218	295	44.82	306	2.41
K-TD-H-9/2009-18	H	M	2	NS	Karbala	256	5.99	17.91	13.63	1105	609	937	34.74	416	2.59
K-TD-H-9/2009-19	H	M	19	NS	Karbala	< 70	10.37	18.91	42.67	1610	209	1232	39.20	895	2.07
K-TD-H-9/2009-20	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	112	1327	14.89	248	0.31
K-TD-H-9/2009-21	H	M	20	NS	Karbala	146	1.68	3.62	1.95	251	75	78	37.73	134	0.61
K-TD-H-9/2009-22	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	356	173	2.16	307	0.64
K-TD-H-9/2009-23	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	79	49	0.69	104	0.15
K-TD-H-9/2009-24	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	91	263	0.12	143	0.15
K-TD-H-9/2009-25	H	M	45	NS	Karbala	373	12.66	37.86	227.30	2396	273	4933	18.82	469	11.53
K-TD-H-9/2009-26	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	589	1936	20.89	678	4.23
K-TD-H-9/2009-27	H	M	47	NS	Karbala	< 70	2.21	10.56	30.42	382	175	532	2.10	224	2.98
K-TD-H-9/2009-28	H	M	33	NS	Karbala	466	7.99	31.24	128.07	2060	367	1659	2.70	643	5.27

Table E1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-29	H	M	25	NS	Karbala	393	5.70	14.80	111.64	1023	291	2010	1.81	478	3.05
K-TD-H-9/2009-30	H	M	42	NS	Karbala	388	8.33	46.50	108.18	1484	532	1517	8.82	589	3.31
K-TD-H-9/2009-31	H	M	10	NS	Karbala	205	3.28	17.95	37.38	211	285	740	4.18	530	1.85
K-TD-H-9/2009-32	H	M	38	NS	Karbala	< 70	3.71	17.80	38.95	674	170	386	3.54	534	1.55
K-TD-H-9/2009-33	H	M	42	NS	Karbala	404	5.90	14.18	52.51	379	298	1223	8.96	482	13.05
K-TD-H-9/2009-34	H	M	11	NS	Karbala	368	6.24	11.44	58.08	1437	423	2103	11.56	583	3.52
K-TD-H-9/2009-35	H	M	41	NS	Karbala	265	10.99	14.29	92.13	399	324	1462	4.42	830	6.00
K-TD-H-9/2009-36	H	M	23	NS	Karbala	446	21.09	31.17	270.08	2816	547	4109	6.50	461	5.98
K-TD-H-9/2009-37	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	254	1134	1.74	475	1.51
K-TD-H-9/2009-38	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	244	753	2.67	552	1.43
K-TD-H-9/2009-39	H	M	43	NS	Karbala	440	5.40	42.60	62.94	152	421	1507	5.17	935	1.40
K-TD-H-9/2009-40	H	M	46	NS	Karbala	356	8.56	10.45	95.56	1115	371	2017	2.49	555	2.20
K-TD-H-9/2009-41	H	M	22	NS	Karbala	676	6.38	10.62	67.43	1199	367	1440	15.40	654	1.08
K-TD-H-9/2009-42	H	M	55	NS	Karbala	344	2.71	3.21	100.63	256	122	1391	3.92	682	1.27
K-TD-H-9/2009-43	H	F	53	S	Karbala	294	1.16	2.45	10.61	127	107	115	1.24	203	1.26
K-TD-H-9/2009-44	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	126	126	1.22	464	0.29
K-TD-H-9/2009-45	H	F	60	S	Karbala	376	2.21	6.24	38.82	276	220	1223	2.29	404	1.96
K-TD-H-9/2009-46	H	F	36	S	Karbala	367	7.16	15.43	95.86	973	741	2406	7.98	694	3.70
K-TD-H-9/2009-47	H	F	62	S	Karbala	< 70	6.54	22.24	74.80	828	413	2686	10.47	728	5.49
K-TD-H-9/2009-48	H	F	52	S	Karbala	552	21.26	92.76	822.70	6763	335	3663	12.00	1128	3.83
K-TD-H-9/2009-49	H	F	35	NS	Karbala	275	0.49	1.85	10.07	39	181	198	1.70	49	1.78
K-TD-H-9/2009-50	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	102	169	1.68	118	0.48
K-TD-H-9/2009-51	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	241	382	2.85	314	1.12
K-TD-H-9/2009-52	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	436	404	1.54	513	0.91
K-TD-H-9/2009-53	H	F	8	NS	Karbala	324	6.54	6.65	56.05	803	205	225	1.64	691	0.13
K-TD-H-9/2009-54	H	F	8	NS	Karbala	549	1.07	5.70	7.35	7	133	258	2.00	577	0.89
K-TD-H-9/2009-55	H	F	65	NS	Karbala	415	1.72	4.16	14.88	261	127	300	0.80	325	0.34
K-TD-H-9/2009-56	H	F	8	NS	Karbala	165	1.99	3.26	29.25	288	133	190	0.74	274	0.10
K-TD-H-9/2009-57	H	F	11	NS	Karbala	112	2.00	3.06	20.47	260	100	854	1.21	353	0.33
K-TD-H-9/2009-58	H	F	31	NS	Karbala	101	0.81	1.23	8.98	84	50	314	1.43	132	0.25
K-TD-H-9/2009-59	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	260	495	1.59	388	0.21
K-TD-H-9/2009-60	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	157	169	1.37	175	0.75

Table E1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-61	H	F	20	NS	Karbala	310	2.85	7.19	35.78	307	77	2718	7.09	616	0.40
K-TD-H-9/2009-62	H	F	8	NS	Karbala	336	2.42	8.24	16.15	370	117	125	38.70	129	1.60
K-TD-H-9/2009-63	H	F	23	NS	Karbala	71	1.98	8.21	5.09	264	98	125	0.62	160	0.64
K-TD-H-9/2009-64	H	F	20	NS	Karbala	184	2.75	11.55	7.28	371	133	181	1.44	243	0.56
K-TD-H-9/2009-65	H	F	7	NS	Karbala	898	6.15	13.95	86.05	586	185	4164	35.94	727	10.11
K-TD-H-9/2009-66	H	F	21	NS	Karbala	546	3.10	33.79	24.09	453	242	989	36.55	321	3.86
K-TD-H-9/2009-67	H	F	37	NS	Karbala	522	5.56	15.84	23.44	852	403	393	1.75	470	5.00
K-TD-H-9/2009-68	H	F	32	NS	Karbala	124	0.88	1.85	7.96	180	51	97	0.37	89	0.19
K-TD-H-9/2009-69	H	F	30	NS	Karbala	451	2.56	8.23	26.87	67	196	197	0.78	1113	0.47
K-TD-H-9/2009-70	H	F	35	NS	Karbala	< 70	4.15	11.15	58.35	418	292	2274	4.87	588	3.55
K-TD-H-9/2009-71	H	F	38	NS	Karbala	389	2.68	2.11	14.30	171	35	663	1.18	489	1.27
K-TD-H-9/2009-72	H	F	55	NS	Karbala	395	1.15	1.81	6.37	63	121	282	1.41	410	0.71
K-TD-H-9/2009-73	H	F	42	NS	Karbala	367	5.75	15.28	76.13	820	474	1267	15.33	662	1.30
K-TD-H-9/2009-74	H	F	34	NS	Karbala	400	4.43	9.15	58.37	683	285	10562	5.77	618	3.51
K-TD-H-9/2009-75	H	F	35	NS	Karbala	< 70	4.76	10.11	67.91	617	380	6088	13.55	371	6.75
K-TD-H-9/2009-76	H	F	37	NS	Karbala	384	2.35	4.80	31.03	293	254	1427	8.49	431	5.54
K-TD-H-9/2009-77	H	F	23	NS	Karbala	< 70	3.77	13.21	7.53	682	130	161	2.46	241	8.85
K-TD-H-9/2009-78	H	F	32	NS	Karbala	389	1.40	5.24	11.06	393	69	186	1.43	153	0.56
K-TD-H-9/2009-79	H	F	35	NS	Karbala	< 70	21.14	67.65	253.59	3323	499	2134	37.15	333	3.87
K-TD-H-9/2009-80	H	F	21	NS	Karbala	387	2.95	6.86	46.79	238	564	1011	3.67	413	0.95
K-TD-H-9/2009-81	H	F	40	NS	Karbala	406	16.14	2.87	14.11	88	204	736	4.02	327	0.88
K-TD-H-9/2009-82	H	F	13	NS	Karbala	233	7.29	9.61	77.28	764	222	1529	7.04	563	1.10
K-TD-H-9/2009-83	H	F	12	NS	Karbala	< 70	3.72	9.71	48.22	359	369	1038	7.65	431	1.18
K-TD-H-9/2009-84	H	F	19	NS	Karbala	507	16.48	18.67	158.94	1796	310	2672	14.12	457	2.65
K-TD-H-9/2009-85	H	F	20	NS	Karbala	335	18.28	2.87	7.62	177	126	580	2.48	258	2.04
K-TD-H-9/2009-86	H	F	16	NS	Karbala	303	17.17	13.01	26.14	507	402	10150	4.23	303	5.26
K-TD-H-9/2009-87	H	F	14	NS	Karbala	394	4.11	34.59	78.52	20	534	2934	3.02	622	1.61
K-TD-H-9/2009-88	H	F	24	NS	Karbala	451	12.87	11.96	153.67	982	539	1395	3.25	607	4.47
K-TD-H-9/2009-89	H	F	5	NS	Karbala	354	8.37	15.63	106.37	1247	338	1752	2.97	939	2.04
K-TD-H-9/2009-90	H	F	19	NS	Karbala	244	4.54	8.22	66.72	134	536	1747	7.61	574	4.71
K-TD-H-9/2009-91	H	F	19	NS	Karbala	366	2.87	5.24	32.83	211	182	466	1.84	491	0.63
K-TD-H-9/2009-92	H	F	10	NS	Karbala	377	2.32	4.19	24.97	155	248	385	1.89	399	0.55

Table E1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-93	H	F	19	NS	Karbala	419	1.19	3.49	16.01	35	216	299	1.41	278	1.81
K-TD-H-9/2009-94	H	F	32	NS	Karbala	231	1.99	3.14	29.44	36	164	499	1.45	365	1.95
K-TD-H-9/2009-95	H	F	45	NS	Karbala	412	1.99	2.69	23.10	150	155	276	1.38	346	0.29
K-TD-H-9/2009-96	H	F	44	NS	Karbala	294	6.02	10.52	87.13	714	273	1088	2.41	562	4.43
K-TD-H-9/2009-97	H	F	11	NS	Karbala	< 70	3.14	7.07	49.90	38	331	1190	1.30	799	3.03
K-TD-H-9/2009-98	H	F	45	NS	Karbala	89	3.18	7.91	17.53	546	223	301	1.67	362	0.40
K-TD-H-9/2009-99	H	F	17	NS	Karbala	< 70	1.29	2.68	16.78	75	190	259	1.33	177	0.40
K-TD-H-9/2009-100	H	F	35	NS	Karbala	279	2.07	4.22	24.93	55	164	1716	1.74	443	1.97
K-TD-H-9/2009-101	H	F	4	NS	Karbala	379	1.85	4.34	21.98	54	234	1021	30.03	405	2.43
K-TD-H-9/2009-102	H	F	17	NS	Karbala	382	0.48	1.36	7.42	46	37	657	35.79	89	0.28
K-TD-H-9/2009-103	H	F	30	NS	Karbala	< 70	3.36	7.11	41.67	414	248	522	24.30	562	0.84
K-TD-H-9/2009-104	H	F	54	NS	Karbala	592	0.84	1.83	8.23	8	113	250	9.08	128	2.93
K-TD-H-9/2009-105	H	F	21	NS	Karbala	472	15.34	1.47	8.08	58	130	697	12.01	249	1.06
K-TD-H-9/2009-106	H	F	11	NS	Karbala	< 70	18.43	8.89	55.74	471	526	4104	34.09	794	2.49
K-TD-H-9/2009-107	H	F	8	NS	Karbala	211	16.78	45.28	233.25	2811	450	2764	19.56	644	4.12
K-TD-H-9/2009-108	H	F	22	NS	Karbala	344	20.55	47.09	534.13	9300	690	2923	16.49	1183	10.77
K-TD-H-9/2009-109	H	F	58	NS	Karbala	310	2.13	4.14	29.36	322	223	593	2.86	276	1.34
K-TD-H-9/2009-110	H	F	43	NS	Karbala	< 70	3.19	5.83	47.35	817	194	804	1.08	533	0.77
K-TD-H-9/2009-111	H	F	23	NS	Karbala	297	6.96	10.18	100.52	743	210	1197	3.91	408	1.49
K-TD-D-9/2009-112	D	M	59	S	Karbala	< 70	0.10	0.23	1.02	4	12	82	1.25	7	1.55
K-TD-D-9/2009-113	D	M	45	S	Karbala	999	7.66	8.24	42.40	413	130	201	1.94	3056	2.04
K-TD-D-9/2009-114	D	M	45	S	Karbala	445	3.45	6.88	31.67	556	176	268	1.07	217	8.38
K-TD-D-9/2009-115	D	M	51	S	Karbala	490	6.88	10.25	88.41	787	368	10326	0.38	6552	6.55
K-TD-D-9/2009-116	D	M	51	S	Karbala	< 70	4.55	26.02	285.79	855	96	10434	4.91	6352	5.99
K-TD-D-9/2009-117	D	M	58	S	Karbala	< 70	2.87	2.17	25.22	235	58	747	4.20	510	0.54
K-TD-D-9/2009-118	D	M	46	S	Karbala	355	5.20	40.43	381.15	727	186	5369	3.87	3638	7.56
K-TD-D-9/2009-119	D	M	55	S	Karbala	399	8.05	3.50	97.91	1039	43	280	1.79	542	0.52
K-TD-D-9/2009-120	D	M	60	S	Karbala	607	3.04	2.65	36.44	508	47	261	0.77	266	0.27
K-TD-D-9/2009-121	D	M	55	S	Karbala	339	2.15	2.41	22.42	231	69	636	2.88	326	0.96
K-TD-D-9/2009-122	D	M	50	NS	Karbala	428	0.85	0.33	4.31	3	3	67	0.61	477	2.62
K-TD-D-9/2009-123	D	M	56	NS	Karbala	834	3.72	18.54	78.24	1827	346	872	1.55	1144	2.42
K-TD-D-9/2009-124	D	M	57	NS	Karbala	737	3.70	21.93	180.27	2003	288	994	3.17	2361	2.23

Table E1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-D-9/2009-125	D	M	54	NS	Karbala	1380	5.53	18.94	193.91	1257	201	578	5.28	1691	1.18
K-TD-D-9/2009-126	D	M	42	NS	Karbala	< 70	4.75	6.15	47.44	648	189	966	2.11	320	0.73
K-TD-D-9/2009-127	D	M	44	NS	Karbala	544	2.73	24.36	239.94	264	479	3152	3.24	1276	7.70
K-TD-D-9/2009-128	D	M	53	NS	Karbala	355	7.00	6.47	97.55	1077	198	749	2.14	481	1.09
K-TD-D-9/2009-129	D	M	48	NS	Karbala	< 70	2.27	4.75	31.11	256	206	3465	1.59	470	0.82
K-TD-D-9/2009-130	D	F	55	S	Karbala	1325	7.14	8.06	36.36	102	82	524	3.63	5012	0.70
K-TD-D-9/2009-131	D	F	65	S	Karbala	867	2.59	6.39	33.80	368	226	9626	1.98	697	1.45
K-TD-D-9/2009-132	D	F	50	S	Karbala	569	7.06	25.17	128.09	1101	314	1850	2.10	248	2.17
K-TD-D-9/2009-133	D	F	59	S	Karbala	2020	2.61	40.95	222.38	146	580	3651	2.72	1917	4.26
K-TD-D-9/2009-134	D	F	51	S	Karbala	576	3.91	18.87	445.53	305	223	1024	5.51	1210	1.18
K-TD-D-9/2009-135	D	F	46	S	Karbala	438	0.81	11.56	200.98	339	181	631	8.50	316	0.51
K-TD-D-9/2009-136	D	F	60	S	Karbala	370	4.28	26.04	299.57	781	269	1565	11.15	1142	2.80
K-TD-D-9/2009-137	D	F	46	S	Karbala	403	2.08	13.69	117.08	1260	294	1935	3.84	539	5.66
K-TD-D-9/2009-138	D	F	70	S	Karbala	< 70	9.55	6.49	189.78	1520	97	432	3.27	842	0.80
K-TD-D-9/2009-139	D	F	47	NS	Karbala	319	0.85	1.67	8.61	47	134	1877	0.62	134	0.86
K-TD-D-9/2009-140	D	F	48	NS	Karbala	1129	4.45	12.54	38.24	148	184	2675	1.97	290	1.94
K-TD-D-9/2009-141	D	F	60	NS	Karbala	384	1.86	1.86	7.27	20	16	129	0.69	1186	0.12
K-TD-D-9/2009-142	D	F	60	NS	Karbala	409	2.37	2.59	11.23	40	31	187	2.28	1432	0.66
K-TD-D-9/2009-143	D	F	60	NS	Karbala	468	1.54	2.15	6.70	43	21	114	1.13	984	0.48
K-TD-D-9/2009-144	D	F	60	NS	Karbala	109	0.12	0.26	0.83	20	1	47	0.23	21	0.37
K-TD-D-9/2009-145	D	F	46	NS	Karbala	142	5.14	10.07	51.82	608	293	1752	2.34	464	2.57
K-TD-D-9/2009-146	D	F	75	NS	Karbala	642	10.82	23.56	183.69	1701	397	5726	2.82	1359	2.76
K-TD-D-9/2009-147	D	F	54	NS	Karbala	558	6.12	7.78	109.75	789	343	3688	0.44	817	2.88
K-TD-D-9/2009-148	D	F	54	NS	Karbala	538	1.26	1.78	13.55	178	192	358	0.48	67	0.21
K-TD-D-9/2009-149	D	F	52	NS	Karbala	614	2.22	2.34	16.87	205	259	1165	0.73	479	1.34
K-TD-D-9/2009-150	D	F	41	NS	Karbala	1454	4.69	6.90	58.66	567	290	2221	0.94	709	1.52
K-TD-D-9/2009-151	D	F	40	NS	Karbala	< 70	3.23	22.98	362.66	343	329	5112	10.12	1807	2.16
K-TD-D-9/2009-152	D	F	70	NS	Karbala	173	8.58	14.10	234.20	576	229	954	4.69	378	1.32
K-TD-D-9/2009-153	D	F	59	NS	Karbala	< 70	6.51	15.62	101.13	684	594	2024	2.44	322	2.16
K-TD-D-9/2009-154	D	F	56	NS	Karbala	132	3.01	5.85	81.83	471	162	2561	2.52	376	0.72

Table E1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-TD-D-9/2009-155	D	F	60	NS	Karbala	277	4.48	3.41	58.86	340	132	2103	0.66	1690	2.11
L-TD-H-9/2009-156	H	F	10	NS	London	125	0.22	2.74	3.86	74	109	139	1.45	30	3.46
L-TD-H-9/2009-157	H	F	10	NS	London	321	0.57	6.8	10.65	189	304	195	0.24	71	1.45
L-TD-H-9/2009-158	H	M	8	NS	London	258	0.51	4.23	4.49	139	260	188	0.57	68	8.52
L-TD-H-9/2009-159	H	M	6	NS	London	441	1.33	7.71	5.31	200	335	205	2.67	72	8.39
L-TD-H-9/2009-160	H	M	13	NS	London	498	1.12	8.12	8.34	212	236	165	2.94	98	3.03
L-TD-H-9/2009-161	H	F	33	NS	London	210	0.36	2.59	5.77	79	222	184	1.86	72	3.44
L-TD-H-9/2009-162	H	M	42	NS	London	108	1.21	5.33	9.21	269	263	267	1.23	86	1.82
L-TD-H-9/2009-163	H	M	44	NS	London	97	1.03	4.11	7.51	240	204	211	1.25	62	1.64
L-TD-H-9/2009-164	H	M	41	NS	London	97	0.66	4.24	5.22	155	189	224	1.53	52	3.76
L-TD-H-9/2009-165	H	M	13	NS	London	259	0.18	2.61	6.56	64	268	136	1.09	40	1.42
L-TD-H-9/2009-166	H	M	42	NS	London	86	0.96	4.43	7.65	225	260	209	1.09	83	3.67
L-TD-H-9/2009-167	H	F	10	NS	London	309	0.33	5.83	9.05	158	267	258	1.25	64	1.38
L-TD-H-9/2009-168	H	M	44	NS	London	99	1.03	4.25	7.78	251	199	165	1.14	58	3.63
L-TD-H-9/2009-169	H	F	10	NS	London	139	0.12	2.44	3.42	65	90	79	1.44	26	1.37
L-TD-H-9/2009-170	H	M	6	NS	London	345	0.55	4.85	11.08	118	263	324	1.74	44	9.03
L-TD-H-9/2009-171	H	M	8	NS	London	225	0.5	4.67	4.86	154	248	137	0.43	67	7.06
L-TD-H-9/2009-172	H	M	41	NS	London	83	0.75	5.24	6.31	190	175	169	1.35	48	3.64
L-TD-H-9/2009-173	H	F	33	NS	London	196	0.29	2.52	5.71	76	196	126	1.58	69	1.27

HS = health status, G = gender, y = year, SA = smoking activity, K-TD-H-9/2009-1, K corresponds to the province in Iraq (K) Karbala and may be replaced by (L) London in the UK; TD corresponds to tear drops, H corresponds to healthy and may be replaced by D (diabetes); 9/2009 corresponds to the date (month/year); and 1 corresponds to the sample code number.

Table E1.2: Elemental data for "pooled" tear drop samples stored in a refrigerator at 4 °C and repeatedly analysed (n = 6) over a 4 week period.

Storage time	Concentration (µg/l)									
	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
Week 1										
1	480	2.75	4.02	19.14	299	211	780	0.52	648	0.30
2	508	2.79	3.96	19.19	281	209	767	0.48	589	0.29
3	550	2.83	4.00	23.02	287	206	753	0.45	580	0.28
4	497	2.74	3.94	18.09	281	218	757	0.48	609	0.27
5	512	2.68	3.77	18.59	291	200	751	0.56	584	0.28
6	509	2.77	4.02	18.60	260	199	741	0.41	575	0.26
Week 2										
1	487	2.68	3.66	17.81	269	209	734	0.50	599	0.29
2	508	2.79	3.68	18.77	268	203	771	0.60	596	0.26
3	511	2.76	3.46	19.03	281	189	766	0.53	587	0.24
4	512	2.68	3.92	19.47	291	210	753	0.53	554	0.24
5	503	2.59	3.81	20.81	296	202	755	0.34	621	0.25
6	550	2.62	3.85	20.42	302	214	749	0.51	592	0.28
Week 3										
1	515	2.70	3.49	18.14	294	203	740	0.44	591	0.27
2	518	2.79	3.94	18.64	286	210	755	0.53	552	0.29
3	526	2.69	3.91	18.57	273	211	754	0.38	595	0.31
4	490	2.69	3.89	17.02	292	218	748	0.62	629	0.28
5	485	2.65	3.87	17.07	294	209	742	0.53	577	0.27
6	530	2.53	3.77	19.40	268	202	769	0.38	590	0.27
Week 4										
1	500	2.67	3.66	19.21	276	203	754	0.49	608	0.29
2	508	2.58	3.98	20.20	273	208	758	0.48	595	0.30
3	518	2.60	3.67	18.66	296	207	753	0.37	604	0.26
4	486	2.67	3.67	18.52	302	202	756	0.62	614	0.25
5	546	2.59	3.96	17.09	282	221	755	0.50	602	0.27
6	523	2.52	3.70	18.66	289	208	725	0.40	545	0.28

Appendix E1
Trace Element Distribution

It is expected that essential elements follow a normal distribution in blood samples for healthy individuals. Log-normal distributions are typically found for non-essential and toxic elements, or in individuals where there is some breakdown in homeostatic regulation (Adair, 2002). The elemental patterns of healthy and diabetic populations from Karbala were found to be log-normally distributed for most trace elements. This is consistent with other findings indicating that the elemental levels in some biological samples are perhaps not subject to the same metabolic regulation as others (Stone, 2006). In contrast, the healthy individuals from London were found to have a normal distribution for most trace elements (except Cd which was log-normal). This difference is attributed to changes in various food habits, use of metal cookware and the environmental setting which could cause changes in elemental distributions in tear drops. In addition, factors like health status, gender, age, drinking water and the total number of the population may also affect the normality of distribution for trace elements in tear drops, as is a similar case for blood (Field, 2009; Stone, 2006; Sukumar & Subramanian, 2007).

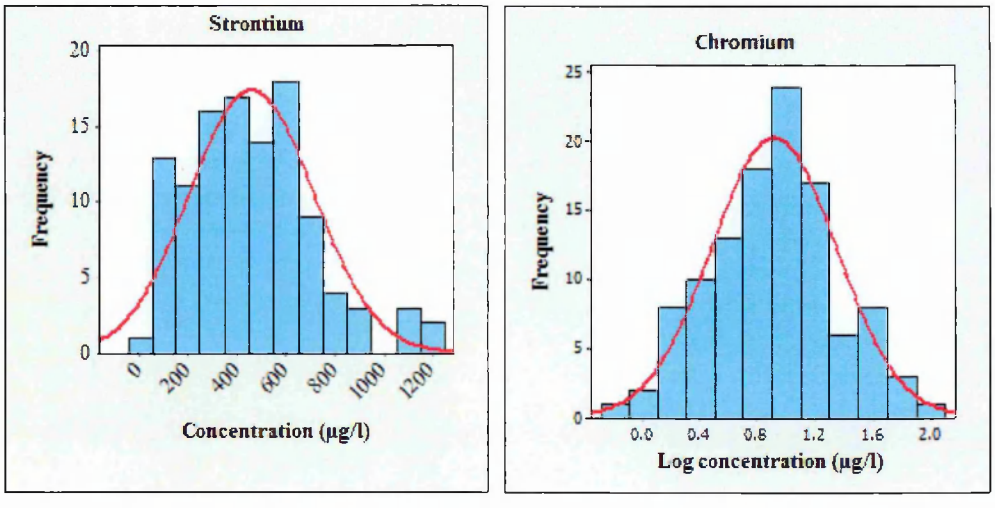

Figure E1.1: Normal distribution for strontium and log-normal for chromium in healthy Karbala population (n = 111) with normal curve (red line).

Table E1.3: Summary of the distribution and statistical comparison of Karbala (healthy and diabetes) and London (healthy) data sets for trace elements under investigation.

Element	Group	Distribution	Anderson-Darling test			Significant?
			A-squared value	A_{crit}	*P*-value	
B	HK	Normal	1.962	0.787	< 0.001	N
	DK	Log-normal	0.519	0.787	0.175	Y
	HL	Normal	0.649	0.787	0.075	Y
V	HK	Log-normal	0.473	0.787	0.239	Y
	DK	Normal	0.520	0.787	0.177	Y
	HL	Normal	0.402	0.787	0.323	Y
Cr	HK	Log-normal	0.262	0.787	0.699	Y
	DK	Log-normal	0.868	1.092	0.024	Y
	HL	Normal	0.515	0.787	0.166	Y
Mn	HK	Log-normal	0.537	0.787	0.165	Y
	DK	Log-normal	0.742	0.787	0.051	Y
	HL	Normal	0.234	0.787	0.761	Y
Fe	HK	Log-normal	0.705	0.787	0.064	Y
	DK	Normal	1.444	0.787	0.001	N
	HL	Normal	0.395	0.787	0.336	Y
Cu	HK	Log-normal	0.596	0.787	0.119	Y
	DK	Normal	0.535	0.787	0.162	Y
	HL	Normal	0.498	0.787	0.184	Y
Zn	HK	Log-normal	0.498	0.787	0.207	Y
	DK	Log-normal	0.281	0.787	0.624	Y
	HL	Normal	0.245	0.787	0.721	Y
As	HK	Normal	13.786	0.787	< 0.001	N
	DK	Log-normal	0.397	0.787	0.355	Y
	HL	Normal	0.617	0.787	0.091	Y
Sr	HK	Normal	0.978	1.092	0.013	Y
	DK	Log-normal	0.784	0.787	0.039	Y
	HL	Normal	0.247	0.787	0.716	Y
Cd	HK	Log-normal	0.348	0.787	0.472	Y
	DK	Log-normal	0.254	0.787	0.716	Y
	HL	Log-normal	0.765	0.038	0.787	Y

HK = Healthy Karbala (n = 111), DK = Diabetes Karbala (n = 44), HL = Healthy London (n = 18), Y = yes a significant result at $P < 0.05$ or $P > 0.01$ for Cr in diabetic group and Sr in healthy group, N = no significant difference at $P < 0.05$ or $P > 0.01$ for Cr in diabetic group and Sr in healthy group, P = probability (level of significance), A_{crit} = critical value.

Appendix E2

Paired Sample Results:

Table E2.1: Paired tear drops and drinking water samples from Karbala (Iraq) and London (UK).

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-H-9/2009-1	H	M	33	S	Karbala	785	1.06	5.67	9.78	284	292	0.43	0.33	2.32	12.31
K-TD-H-9/2009-2	H	M	28	S	Karbala	360	7.24	13.27	84.49	1273	425	3.26	0.45	0.27	9.38
K-TD-H-9/2009-3	H	M	28	S	Karbala	399	5.50	12.25	76.56	854	548	6.73	0.36	0.35	7.08
K-TD-H-9/2009-4	H	M	40	S	Karbala	< 70	3.70	9.13	35.83	427	294	0.42	0.32	3.24	9.76
K-TD-H-9/2009-5	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	549	7.09	0.60	0.41	6.55
K-TD-H-9/2009-6	H	M	44	NS	Karbala	504	4.28	7.33	9.90	344	274	0.41	0.40	0.66	9.68
K-TD-H-9/2009-7	H	M	46	NS	Karbala	417	2.07	5.37	8.03	271	294	0.42	0.32	3.24	9.76
K-TD-H-9/2009-8	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	217	0.08	0.31	0.60	11.39
K-TD-H-9/2009-9	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	305	3.78	0.39	0.61	9.28
K-TD-H-9/2009-10	H	M	3	NS	Karbala	761	12.61	35.56	96.17	2253	292	3.83	0.10	1.22	0.87
K-TD-H-9/2009-11	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	290	3.76	0.07	0.30	0.97
K-TD-H-9/2009-12	H	M	40	NS	Karbala	252	2.75	6.20	22.98	465	299	3.78	0.44	0.34	10.99
K-TD-H-9/2009-13	H	M	42	NS	Karbala	616	3.43	7.07	19.63	335	272	2.87	0.41	2.40	9.30
K-TD-H-9/2009-14	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	297	3.61	0.52	1.49	9.33
K-TD-H-9/2009-15	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	324	3.77	0.88	0.33	9.60
K-TD-H-9/2009-16	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	280	3.94	0.16	3.54	1.26
K-TD-H-9/2009-17	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	290	3.40	0.40	0.36	9.61
K-TD-H-9/2009-18	H	M	2	NS	Karbala	256	5.99	17.91	13.63	1105	296	3.71	0.52	0.75	9.71
K-TD-H-9/2009-19	H	M	19	NS	Karbala	< 70	10.37	18.91	42.67	1610	563	7.31	0.34	0.24	6.62
K-TD-H-9/2009-20	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	237	2.61	0.36	0.91	10.92
K-TD-H-9/2009-21	H	M	20	NS	Karbala	146	1.68	3.62	1.95	251	549	7.09	0.60	0.41	6.55
K-TD-H-9/2009-22	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	555	7.36	0.34	0.80	6.65
K-TD-H-9/2009-23	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	588	7.30	0.40	45.4	7.07
K-TD-H-9/2009-24	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	240	0.39	0.06	0.46	0.86
K-TD-H-9/2009-25	H	M	45	NS	Karbala	373	12.66	37.86	227.30	2396	312	3.68	0.41	21.9	10.82
K-TD-H-9/2009-26	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	323	3.99	0.47	0.78	11.47

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-H-9/2009-27	H	M	47	NS	Karbala	< 70	2.21	10.56	30.42	382	91	1.92	0.20	0.35	0.88
K-TD-H-9/2009-28	H	M	33	NS	Karbala	466	7.99	31.24	128.07	2060	308	3.88	0.47	2.79	27.59
K-TD-H-9/2009-29	H	M	25	NS	Karbala	393	5.70	14.80	111.64	1023	302	3.56	0.35	0.52	12.27
K-TD-H-9/2009-30	H	M	42	NS	Karbala	388	8.33	46.50	108.18	1484	311	3.67	0.35	0.59	9.46
K-TD-H-9/2009-31	H	M	10	NS	Karbala	205	3.28	17.95	37.38	211	299	3.78	0.44	0.34	10.99
K-TD-H-9/2009-32	H	M	38	NS	Karbala	< 70	3.71	17.80	38.95	674	312	3.68	0.41	21.97	10.82
K-TD-H-9/2009-33	H	M	42	NS	Karbala	404	5.90	14.18	52.51	379	296	3.32	0.78	3.51	9.37
K-TD-H-9/2009-34	H	M	11	NS	Karbala	368	6.24	11.44	58.08	1437	312	2.86	0.48	15.03	9.31
K-TD-H-9/2009-35	H	M	41	NS	Karbala	265	10.99	14.29	92.13	399	563	7.31	0.34	0.24	6.62
K-TD-H-9/2009-36	H	M	23	NS	Karbala	446	21.09	31.17	270.08	2816	361	3.61	0.35	0.85	9.48
K-TD-H-9/2009-37	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	282	3.04	0.49	4.03	10.23
K-TD-H-9/2009-38	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	361	3.61	0.35	0.85	9.48
K-TD-H-9/2009-39	H	M	43	NS	Karbala	440	5.40	42.60	62.94	152	555	7.36	0.34	0.80	6.65
K-TD-H-9/2009-40	H	M	46	NS	Karbala	356	8.56	10.45	95.56	1115	285	2.87	0.46	6.15	10.07
K-TD-H-9/2009-41	H	M	22	NS	Karbala	676	6.38	10.62	67.43	1199	308	3.88	0.47	2.79	27.59
K-TD-H-9/2009-42	H	M	55	NS	Karbala	344	2.71	3.21	100.63	256	280	3.94	0.16	3.54	1.26
K-TD-H-9/2009-43	H	F	53	S	Karbala	294	1.16	2.45	10.61	127	109	1.90	0.47	0.50	9.48
K-TD-H-9/2009-44	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	296	3.62	0.66	0.41	9.47
K-TD-H-9/2009-45	H	F	60	S	Karbala	376	2.21	6.24	38.82	276	548	6.73	0.36	0.35	7.08
K-TD-H-9/2009-46	H	F	36	S	Karbala	367	7.16	15.43	95.86	973	306	4.11	0.48	2.84	10.76
K-TD-H-9/2009-47	H	F	62	S	Karbala	< 70	6.54	22.24	74.80	828	314	3.97	0.44	3.86	10.15
K-TD-H-9/2009-48	H	F	52	S	Karbala	552	21.26	92.76	822.70	6763	560	6.90	0.36	0.11	7.64
K-TD-H-9/2009-49	H	F	35	NS	Karbala	275	0.49	1.85	10.07	39	217	0.08	0.31	0.60	11.39
K-TD-H-9/2009-50	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	292	0.43	0.33	2.32	12.31
K-TD-H-9/2009-51	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	309	3.83	0.79	0.27	9.68
K-TD-H-9/2009-52	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	588	7.30	0.40	45.38	7.07
K-TD-H-9/2009-53	H	F	8	NS	Karbala	324	6.54	6.65	56.05	803	335	3.36	0.55	1.60	12.52
K-TD-H-9/2009-54	H	F	8	NS	Karbala	549	1.07	5.70	7.35	7	312	2.86	0.48	15.03	9.31
K-TD-H-9/2009-55	H	F	65	NS	Karbala	415	1.72	4.16	14.88	261	302	3.07	0.42	5.55	12.34
K-TD-H-9/2009-56	H	F	8	NS	Karbala	165	1.99	3.26	29.25	288	291	3.63	0.53	0.32	9.53
K-TD-H-9/2009-57	H	F	11	NS	Karbala	112	2.00	3.06	20.47	260	292	0.43	0.33	2.32	12.31

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-H-9/2009-58	H	F	31	NS	Karbala	101	0.81	1.23	8.98	84	285	0.45	0.44	0.90	9.38
K-TD-H-9/2009-59	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	327	3.37	0.36	2.85	9.08
K-TD-H-9/2009-60	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	250	1.04	0.36	0.67	11.43
K-TD-H-9/2009-61	H	F	20	NS	Karbala	310	2.85	7.19	35.78	307	243	2.85	0.40	1.52	9.47
K-TD-H-9/2009-62	H	F	8	NS	Karbala	336	2.42	8.24	16.15	370	292	0.43	0.33	2.32	12.31
K-TD-H-9/2009-63	H	F	23	NS	Karbala	71	1.98	8.21	5.09	264	257	0.57	0.37	0.41	11.14
K-TD-H-9/2009-64	H	F	20	NS	Karbala	184	2.75	11.55	7.28	371	272	2.87	0.41	2.40	9.30
K-TD-H-9/2009-65	H	F	7	NS	Karbala	898	6.15	13.95	86.05	586	314	3.97	0.44	3.86	10.15
K-TD-H-9/2009-66	H	F	21	NS	Karbala	546	3.10	33.79	24.09	453	282	3.04	0.49	4.03	10.23
K-TD-H-9/2009-67	H	F	37	NS	Karbala	522	5.56	15.84	23.44	852	302	3.67	0.53	1.89	9.44
K-TD-H-9/2009-68	H	F	32	NS	Karbala	124	0.88	1.85	7.96	180	294	0.42	0.32	3.24	9.76
K-TD-H-9/2009-69	H	F	30	NS	Karbala	451	2.56	8.23	26.87	67	560	6.90	0.36	0.11	7.64
K-TD-H-9/2009-70	H	F	35	NS	Karbala	< 70	4.15	11.15	58.35	418	290	3.76	0.07	0.30	0.97
K-TD-H-9/2009-71	H	F	38	NS	Karbala	389	2.68	2.11	14.30	171	303	3.75	0.56	0.22	9.77
K-TD-H-9/2009-72	H	F	55	NS	Karbala	395	1.15	1.81	6.37	63	296	3.73	0.37	0.33	9.57
K-TD-H-9/2009-73	H	F	42	NS	Karbala	367	5.75	15.28	76.13	820	323	3.99	0.47	0.78	11.47
K-TD-H-9/2009-74	H	F	34	NS	Karbala	400	4.43	9.15	58.37	683	243	2.85	0.40	1.52	9.47
K-TD-H-9/2009-75	H	F	35	NS	Karbala	< 70	4.76	10.11	67.91	617	548	7.11	0.64	0.39	6.67
K-TD-H-9/2009-76	H	F	37	NS	Karbala	384	2.35	4.80	31.03	293	563	7.31	0.34	0.24	6.62
K-TD-H-9/2009-77	H	F	23	NS	Karbala	50	3.77	13.21	7.53	682	149	2.26	0.47	0.88	11.58
K-TD-H-9/2009-78	H	F	32	NS	Karbala	389	1.40	5.24	11.06	393	254	0.47	0.34	0.43	9.36
K-TD-H-9/2009-79	H	F	35	NS	Karbala	< 70	21.14	67.65	253.59	3323	290	3.76	0.07	0.30	0.97
K-TD-H-9/2009-80	H	F	21	NS	Karbala	387	2.95	6.86	46.79	238	288	3.25	0.38	1.35	10.42
K-TD-H-9/2009-81	H	F	40	NS	Karbala	406	16.14	2.87	14.11	88	294	2.97	0.39	5.91	9.46
K-TD-H-9/2009-82	H	F	13	NS	Karbala	233	7.29	9.61	77.28	764	317	3.60	0.41	22.58	9.59
K-TD-H-9/2009-83	H	F	12	NS	Karbala	< 70	3.72	9.71	48.22	359	294	0.42	0.32	3.24	9.76
K-TD-H-9/2009-84	H	F	19	NS	Karbala	507	16.48	18.67	158.94	1796	313	3.65	0.53	0.44	9.57
K-TD-H-9/2009-85	H	F	20	NS	Karbala	335	18.28	2.87	7.62	177	296	3.73	0.37	0.33	9.57
K-TD-H-9/2009-86	H	F	16	NS	Karbala	303	17.17	13.01	26.14	507	342	3.86	0.42	0.30	9.32
K-TD-H-9/2009-87	H	F	14	NS	Karbala	394	4.11	34.59	78.52	20	313	3.58	0.40	1.66	12.34
K-TD-H-9/2009-88	H	F	24	NS	Karbala	451	12.87	11.96	153.67	982	425	3.26	0.45	0.27	9.38

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-H-9/2009-89	H	F	5	NS	Karbala	354	8.37	15.63	106.37	1247	548	6.73	0.36	0.35	7.08
K-TD-H-9/2009-90	H	F	19	NS	Karbala	244	4.54	8.22	66.72	134	227	2.70	0.40	0.47	11.29
K-TD-H-9/2009-91	H	F	19	NS	Karbala	366	2.87	5.24	32.83	211	298	3.59	0.41	0.27	9.34
K-TD-H-9/2009-92	H	F	10	NS	Karbala	377	2.32	4.19	24.97	155	282	3.06	0.43	3.91	12.66
K-TD-H-9/2009-93	H	F	19	NS	Karbala	419	1.19	3.49	16.01	35	297	3.61	0.52	1.49	9.33
K-TD-H-9/2009-94	H	F	32	NS	Karbala	231	1.99	3.14	29.44	36	324	3.77	0.88	0.33	9.60
K-TD-H-9/2009-95	H	F	45	NS	Karbala	412	1.99	2.69	23.10	150	301	3.36	0.45	0.49	9.41
K-TD-H-9/2009-96	H	F	44	NS	Karbala	294	6.02	10.52	87.13	714	317	3.60	0.41	22.58	9.59
K-TD-H-9/2009-97	H	F	11	NS	Karbala	< 70	3.14	7.07	49.90	38	336	3.62	0.45	2.63	10.98
K-TD-H-9/2009-98	H	F	45	NS	Karbala	89	3.18	7.91	17.53	546	291	3.34	0.38	4.08	9.60
K-TD-H-9/2009-99	H	F	17	NS	Karbala	< 70	1.29	2.68	16.78	75	195	0.96	4.85	2.13	35.59
K-TD-H-9/2009-100	H	F	35	NS	Karbala	279	2.07	4.22	24.93	55	313	3.65	0.53	0.44	9.57
K-TD-H-9/2009-101	H	F	4	NS	Karbala	379	1.85	4.34	21.98	54	305	3.78	0.39	0.61	9.28
K-TD-H-9/2009-102	H	F	17	NS	Karbala	382	0.48	1.36	7.42	46	213	0.20	0.45	0.47	11.03
K-TD-H-9/2009-103	H	F	30	NS	Karbala	< 70	3.36	7.11	41.67	414	285	2.87	0.46	6.15	10.07
K-TD-H-9/2009-104	H	F	54	NS	Karbala	592	0.84	1.83	8.23	8	292	0.43	0.33	2.32	12.31
K-TD-H-9/2009-105	H	F	21	NS	Karbala	472	15.34	1.47	8.08	58	296	3.73	0.37	0.33	9.57
K-TD-H-9/2009-106	H	F	11	NS	Karbala	< 70	18.43	8.89	55.74	471	292	3.88	0.10	1.21	0.80
K-TD-H-9/2009-107	H	F	8	NS	Karbala	211	16.78	45.28	233.25	2811	308	3.88	0.47	2.79	27.59
K-TD-H-9/2009-108	H	F	22	NS	Karbala	344	20.55	47.09	534.13	9300	548	7.11	0.64	0.39	6.67
K-TD-H-9/2009-109	H	F	58	NS	Karbala	310	2.13	4.14	29.36	322	560	6.90	0.36	0.11	7.64
K-TD-H-9/2009-110	H	F	43	NS	Karbala	< 70	3.19	5.83	47.35	817	312	3.68	0.41	21.97	10.82
K-TD-H-9/2009-111	H	F	23	NS	Karbala	297	6.96	10.18	100.52	743	290	3.40	0.40	0.36	9.61
K-TD-D-9/2009-112	D	M	59	S	Karbala	< 70	0.10	0.23	1.02	4	75	0.08	0.31	0.99	10.03
K-TD-D-9/2009-113	D	M	45	S	Karbala	999	7.66	8.24	42.40	413	261	2.41	0.42	12.47	9.95
K-TD-D-9/2009-114	D	M	45	S	Karbala	445	3.45	6.88	31.67	556	109	1.90	0.47	0.50	9.48
K-TD-D-9/2009-115	D	M	51	S	Karbala	490	6.88	10.25	88.41	787	309	3.83	0.79	0.27	9.68
K-TD-D-9/2009-116	D	M	51	S	Karbala	< 70	4.55	26.02	285.79	855	350	3.61	0.63	1.73	11.92
K-TD-D-9/2009-117	D	M	58	S	Karbala	< 70	2.87	2.17	25.22	235	303	3.75	0.56	0.22	9.77
K-TD-D-9/2009-118	D	M	46	S	Karbala	355	5.20	40.43	381.15	727	291	3.34	0.38	4.08	9.60
K-TD-D-9/2009-119	D	M	55	S	Karbala	399	8.05	3.50	97.91	1039	361	3.61	0.35	0.85	9.48

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-D-9/2009-120	D	M	60	S	Karbala	607	3.04	2.65	36.44	508	299	3.78	0.44	0.34	10.99
K-TD-D-9/2009-121	D	M	55	S	Karbala	339	2.15	2.41	22.42	231	296	3.32	0.78	3.51	9.37
K-TD-D-9/2009-122	D	M	50	NS	Karbala	428	0.85	0.33	4.31	3	312	3.80	0.39	5.23	9.29
K-TD-D-9/2009-123	D	M	56	NS	Karbala	834	3.72	18.54	78.24	1827	549	7.09	0.60	0.41	6.55
K-TD-D-9/2009-124	D	M	57	NS	Karbala	737	3.70	21.93	180.27	2003	588	7.30	0.40	45.38	7.07
K-TD-D-9/2009-125	D	M	54	NS	Karbala	1380	5.53	18.94	193.91	1257	576	6.85	0.48	42.04	8.39
K-TD-D-9/2009-126	D	M	42	NS	Karbala	< 70	4.75	6.15	47.44	648	301	3.36	0.45	0.49	9.41
K-TD-D-9/2009-127	D	M	44	NS	Karbala	544	2.73	24.36	239.94	264	550	7.25	0.44	1.09	6.80
K-TD-D-9/2009-128	D	M	53	NS	Karbala	355	7.00	6.47	97.55	1077	327	3.37	0.36	2.85	9.08
K-TD-D-9/2009-129	D	M	48	NS	Karbala	< 70	2.27	4.75	31.11	256	298	3.59	0.41	0.27	9.34
K-TD-D-9/2009-130	D	F	55	S	Karbala	1325	7.14	8.06	36.36	102	291	2.85	0.42	6.25	9.28
K-TD-D-9/2009-131	D	F	65	S	Karbala	867	2.59	6.39	33.80	368	277	3.78	0.11	0.63	1.07
K-TD-D-9/2009-132	D	F	50	S	Karbala	569	7.06	25.17	128.09	1101	237	2.61	0.36	0.91	10.92
K-TD-D-9/2009-133	D	F	59	S	Karbala	2020	2.61	40.95	222.38	146	588	7.30	0.40	45.38	7.07
K-TD-D-9/2009-134	D	F	51	S	Karbala	576	3.91	18.87	445.53	305	550	7.25	0.44	1.09	6.80
K-TD-D-9/2009-135	D	F	46	S	Karbala	438	0.81	11.56	200.98	339	291	3.63	0.53	0.32	9.53
K-TD-D-9/2009-136	D	F	60	S	Karbala	370	4.28	26.04	299.57	781	560	6.90	0.36	0.11	7.64
K-TD-D-9/2009-137	D	F	46	S	Karbala	403	2.08	13.69	117.08	1260	361	3.61	0.35	0.85	9.48
K-TD-D-9/2009-138	D	F	70	S	Karbala	< 70	9.55	6.49	189.78	1520	563	7.31	0.34	0.24	6.62
K-TD-D-9/2009-139	D	F	47	NS	Karbala	319	0.85	1.67	8.61	47	252	0.43	0.06	0.75	0.76
K-TD-D-9/2009-140	D	F	48	NS	Karbala	1129	4.45	12.54	38.24	148	318	3.88	0.46	5.23	9.89
K-TD-D-9/2009-141	D	F	60	NS	Karbala	384	1.86	1.86	7.27	20	548	7.11	0.64	0.39	6.67
K-TD-D-9/2009-142	D	F	60	NS	Karbala	409	2.37	2.59	11.23	40	561	7.38	0.53	0.17	7.14
K-TD-D-9/2009-143	D	F	60	NS	Karbala	468	1.54	2.15	6.70	43	548	6.73	0.36	0.35	7.08
K-TD-D-9/2009-144	D	F	60	NS	Karbala	109	0.12	0.26	0.83	20	75	0.08	0.31	0.99	10.03
K-TD-D-9/2009-145	D	F	46	NS	Karbala	142	5.14	10.07	51.82	608	309	3.83	0.79	0.27	9.68
K-TD-D-9/2009-146	D	F	75	NS	Karbala	642	10.82	23.56	183.69	1701	561	7.38	0.53	0.17	7.14
K-TD-D-9/2009-147	D	F	54	NS	Karbala	558	6.12	7.78	109.75	789	331	4.00	0.42	1.78	9.41
K-TD-D-9/2009-148	D	F	54	NS	Karbala	538	1.26	1.78	13.55	178	231	0.08	0.38	0.62	11.12

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
K-TD-D-9/2009-149	D	F	52	NS	Karbala	614	2.22	2.34	16.87	205	288	3.25	0.38	1.35	10.42
K-TD-D-9/2009-150	D	F	41	NS	Karbala	1454	4.69	6.90	58.66	567	304	3.71	0.39	0.78	11.17
K-TD-D-9/2009-151	D	F	40	NS	Karbala	< 70	3.23	22.98	362.66	343	576	6.85	0.48	42.04	8.39
K-TD-D-9/2009-152	D	F	70	NS	Karbala	173	8.58	14.10	234.20	576	282	3.06	0.43	3.91	12.66
K-TD-D-9/2009-153	D	F	59	NS	Karbala	< 70	6.51	15.62	101.13	684	302	3.07	0.42	5.55	12.34
K-TD-D-9/2009-154	D	F	56	NS	Karbala	132	3.01	5.85	81.83	471	304	3.59	0.39	0.32	10.01
K-TD-D-9/2009-155	D	F	60	NS	Karbala	277	4.48	3.41	58.86	340	576	6.85	0.48	42.04	8.39
L-TD-H-9/2009-156	H	F	10	NS	London	125	0.22	2.74	3.86	74	56	0.87	0.11	0.20	0.77
L-TD-H-9/2009-157	H	F	10	NS	London	321	0.57	6.8	10.65	189	59	0.95	0.11	0.35	0.98
L-TD-H-9/2009-158	H	M	8	NS	London	258	0.51	4.23	4.49	139	5	0.04	0.04	12.16	0.74
L-TD-H-9/2009-159	H	M	6	NS	London	441	1.33	7.71	5.31	200	20	0.44	0.07	0.23	0.72
L-TD-H-9/2009-160	H	M	13	NS	London	498	1.12	8.12	8.34	212	61	0.94	0.17	0.38	0.94
L-TD-H-9/2009-161	H	F	33	NS	London	210	0.36	2.59	5.77	79	50	0.45	0.09	0.24	0.75
L-TD-H-9/2009-162	H	M	42	NS	London	108	1.2	5.33	9.24	269	50	0.46	0.08	0.22	0.75
L-TD-H-9/2009-163	H	M	44	NS	London	97	1.03	4.11	7.51	240	58	0.91	0.10	0.35	1.00
L-TD-H-9/2009-164	H	M	41	NS	London	97	0.66	4.24	5.22	155	49	0.24	0.18	0.17	1.00
L-TD-H-9/2009-165	H	M	13	NS	London	259	0.18	2.61	6.56	64	39	0.10	0.08	0.66	0.80
L-TD-H-9/2009-166	H	M	42	NS	London	86	0.96	4.43	7.65	225	56	0.37	0.11	0.56	0.71
L-TD-H-9/2009-167	H	F	10	NS	London	309	0.33	5.81	9.05	158	57	0.41	0.16	0.70	0.71
L-TD-H-9/2009-168	H	M	44	NS	London	99	1.03	4.25	7.78	251	10	0.12	0.10	0.05	0.71
L-TD-H-9/2009-169	H	F	10	NS	London	139	0.12	2.44	3.43	65	8	0.12	0.06	0.04	0.72
L-TD-H-9/2009-170	H	M	6	NS	London	345	0.55	4.85	11.08	118	84	0.45	0.08	0.22	0.74
L-TD-H-9/2009-171	H	M	8	NS	London	225	0.5	4.67	4.86	154	58	0.17	0.27	0.68	0.69
L-TD-H-9/2009-172	H	M	41	NS	London	83	0.75	5.2	6.31	190	59	0.95	0.11	0.35	0.98

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
L-TD-H-9/2009-173	H	F	33	NS	London	196	0.29	2.52	5.71	76	58	0.91	0.10	0.35	1.00
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-1	H	M	33	S	Karbala	284	193	187	1.57	112	13.6	33.15	0.33	86	1.44
K-TD-H-9/2009-2	H	M	28	S	Karbala	1273	521	6334	10.01	592	4.45	40.79	1.39	1198	1.02
K-TD-H-9/2009-3	H	M	28	S	Karbala	854	540	3748	12.64	1094	1.81	8.32	2.47	1762	0.12
K-TD-H-9/2009-4	H	M	40	S	Karbala	427	203	416	3.71	442	9.71	30.47	0.20	78	1.39
K-TD-H-9/2009-5	H	M	21	S	Karbala	1041	255	4100	9.56	1159	1.34	5.49	2.57	1850	0.12
K-TD-H-9/2009-6	H	M	44	NS	Karbala	344	196	460	0.48	140	4.58	15.60	0.30	113	0.97
K-TD-H-9/2009-7	H	M	46	NS	Karbala	271	159	186	0.96	112	9.71	30.47	0.20	78	1.39
K-TD-H-9/2009-8	H	M	42	NS	Karbala	325	427	494	1.34	58	9.67	327.8	0.26	16	1.17
K-TD-H-9/2009-9	H	M	8	NS	Karbala	1779	313	598	1.83	271	3.98	13.65	1.44	883	0.93
K-TD-H-9/2009-10	H	M	3	NS	Karbala	2253	211	1079	2.75	806	0.46	8.02	1.34	1515	0.02
K-TD-H-9/2009-11	H	M	37	NS	Karbala	1523	431	1022	5.15	587	0.92	15.42	1.32	1189	0.05
K-TD-H-9/2009-12	H	M	40	NS	Karbala	465	198	224	1.42	262	5.35	26.45	1.36	876	1.19
K-TD-H-9/2009-13	H	M	42	NS	Karbala	335	280	355	2.11	246	4.10	160.7	0.97	826	0.99
K-TD-H-9/2009-14	H	M	43	NS	Karbala	980	318	277	2.67	345	4.36	185.9	1.19	959	1.00
K-TD-H-9/2009-15	H	M	38	NS	Karbala	393	148	352	1.81	425	9.52	24.74	1.95	981	0.98
K-TD-H-9/2009-16	H	M	20	NS	Karbala	71	49	1175	33.94	689	0.49	34.88	1.40	1347	0.02
K-TD-H-9/2009-17	H	M	10	NS	Karbala	920	218	295	44.82	306	4.04	28.68	1.31	886	0.94
K-TD-H-9/2009-18	H	M	2	NS	Karbala	1105	609	937	34.74	416	4.39	17.37	1.42	957	0.99
K-TD-H-9/2009-19	H	M	19	NS	Karbala	1610	209	1232	39.20	895	1.73	2.61	2.49	1721	0.09
K-TD-H-9/2009-20	H	M	19	NS	Karbala	198	112	1327	14.89	248	6.59	89.69	0.93	829	1.35
K-TD-H-9/2009-21	H	M	20	NS	Karbala	251	75	78	37.73	134	1.34	5.49	2.57	1850	0.12
K-TD-H-9/2009-22	H	M	35	NS	Karbala	107	356	173	2.16	307	1.96	3.43	2.61	1727	0.10
K-TD-H-9/2009-23	H	M	45	NS	Karbala	202	79	49	0.69	104	4.21	31.23	2.45	2110	0.20
K-TD-H-9/2009-24	H	M	33	NS	Karbala	270	91	263	0.08	143	0.82	1.69	0.13	121	0.03
K-TD-H-9/2009-25	H	M	45	NS	Karbala	2396	273	4933	18.82	469	5.78	69.89	1.38	1046	0.97
K-TD-H-9/2009-26	H	M	33	NS	Karbala	1312	589	1936	20.89	678	7.31	19.94	1.57	1303	0.94

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-27	H	M	47	NS	Karbala	175	532	2.10	224	2.98	0.41	46.58	0.69	437	0.11
K-TD-H-9/2009-28	H	M	33	NS	Karbala	367	1659	2.70	643	5.27	8.19	74.91	1.49	1292	1.01
K-TD-H-9/2009-29	H	M	25	NS	Karbala	291	2010	1.81	478	3.05	5.93	29.37	1.17	959	0.96
K-TD-H-9/2009-30	H	M	42	NS	Karbala	532	1517	8.82	589	3.31	4.73	53.98	1.22	1195	1.03
K-TD-H-9/2009-31	H	M	10	NS	Karbala	285	740	4.18	530	1.85	5.35	26.45	1.36	876	1.19
K-TD-H-9/2009-32	H	M	38	NS	Karbala	170	386	3.54	534	1.55	5.78	69.89	1.38	1046	0.97
K-TD-H-9/2009-33	H	M	42	NS	Karbala	298	1223	8.96	482	13.05	18.1	20.05	1.62	954	0.98
K-TD-H-9/2009-34	H	M	11	NS	Karbala	423	2103	11.56	583	3.52	6.92	184.7	1.62	1073	2.05
K-TD-H-9/2009-35	H	M	41	NS	Karbala	324	1462	4.42	830	6.00	1.73	2.61	2.49	1721	0.09
K-TD-H-9/2009-36	H	M	23	NS	Karbala	547	4109	6.50	461	5.98	4.06	12.28	1.44	1048	0.98
K-TD-H-9/2009-37	H	M	20	NS	Karbala	254	1134	1.74	475	1.51	4.43	64.04	1.20	1026	1.05
K-TD-H-9/2009-38	H	M	20	NS	Karbala	244	753	2.67	552	1.43	4.06	12.28	1.44	1048	0.98
K-TD-H-9/2009-39	H	M	43	NS	Karbala	421	1507	5.17	935	1.40	1.96	3.43	2.61	1727	0.10
K-TD-H-9/2009-40	H	M	46	NS	Karbala	371	2017	2.49	555	2.20	6.89	37.05	1.33	1053	0.97
K-TD-H-9/2009-41	H	M	22	NS	Karbala	367	1440	15.40	654	1.08	8.19	74.91	1.49	1292	1.01
K-TD-H-9/2009-42	H	M	55	NS	Karbala	122	1391	3.92	682	1.27	0.49	34.88	1.40	1347	0.02
K-TD-H-9/2009-43	H	F	53	S	Karbala	107	115	1.24	203	1.26	4.18	85.30	0.75	416	0.95
K-TD-H-9/2009-44	H	F	40	S	Karbala	126	126	1.22	464	0.29	3.72	16.63	1.30	953	0.91
K-TD-H-9/2009-45	H	F	60	S	Karbala	220	1223	2.29	404	1.96	1.81	8.32	2.47	1762	0.12
K-TD-H-9/2009-46	H	F	36	S	Karbala	741	2406	7.98	694	3.70	4.18	14.66	1.51	1356	0.93
K-TD-H-9/2009-47	H	F	62	S	Karbala	413	2686	10.47	728	5.49	4.20	68.36	1.40	1390	0.93
K-TD-H-9/2009-48	H	F	52	S	Karbala	335	3663	12.00	1128	3.83	2.21	7.79	2.52	1787	0.11
K-TD-H-9/2009-49	H	F	35	NS	Karbala	181	198	1.70	49	1.78	9.67	327.8	0.26	16	1.17
K-TD-H-9/2009-50	H	F	33	NS	Karbala	102	169	1.68	118	0.48	13.6	33.15	0.33	86	1.44
K-TD-H-9/2009-51	H	F	12	NS	Karbala	241	382	2.85	314	1.12	9.73	21.39	2.33	989	0.95
K-TD-H-9/2009-52	H	F	14	NS	Karbala	436	404	1.54	513	0.91	4.21	31.23	2.45	2110	0.20
K-TD-H-9/2009-53	H	F	8	NS	Karbala	205	225	1.64	691	0.13	8.36	715.5	1.19	1349	1.03
K-TD-H-9/2009-54	H	F	8	NS	Karbala	133	258	2.00	577	0.89	6.92	184.7	1.62	1073	2.05
K-TD-H-9/2009-55	H	F	65	NS	Karbala	127	300	0.80	325	0.34	6.65	170.7	1.23	1027	0.98
K-TD-H-9/2009-56	H	F	8	NS	Karbala	133	190	0.74	274	0.10	3.79	20.66	1.28	946	0.94
K-TD-H-9/2009-57	H	F	11	NS	Karbala	100	854	1.21	353	0.33	13.6	33.15	0.33	86	1.44

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-58	H	F	31	NS	Karbala	50	314	1.43	132	0.25	3.88	22.29	0.21	91	0.92
K-TD-H-9/2009-59	H	F	45	NS	Karbala	260	495	1.59	388	0.21	4.09	196.9	1.18	969	0.98
K-TD-H-9/2009-60	H	F	42	NS	Karbala	157	169	1.37	175	0.75	17.6	47.40	0.47	319	0.92
K-TD-H-9/2009-61	H	F	20	NS	Karbala	77	2718	7.09	616	0.40	4.23	313.1	0.74	1258	1.06
K-TD-H-9/2009-62	H	F	8	NS	Karbala	117	125	38.70	129	1.60	13.6	33.15	0.33	86	1.44
K-TD-H-9/2009-63	H	F	23	NS	Karbala	98	125	0.62	160	0.64	8.40	38.92	0.45	219	0.92
K-TD-H-9/2009-64	H	F	20	NS	Karbala	133	181	1.44	243	0.56	4.10	160.7	0.97	826	0.99
K-TD-H-9/2009-65	H	F	7	NS	Karbala	185	4164	35.94	727	10.11	4.20	68.36	1.40	1390	0.93
K-TD-H-9/2009-66	H	F	21	NS	Karbala	242	989	36.55	321	3.86	4.43	64.04	1.20	1026	1.05
K-TD-H-9/2009-67	H	F	37	NS	Karbala	403	393	1.75	470	5.00	4.08	177.7	1.08	985	1.01
K-TD-H-9/2009-68	H	F	32	NS	Karbala	51	97	0.37	89	0.19	9.71	30.47	0.20	78	1.39
K-TD-H-9/2009-69	H	F	30	NS	Karbala	196	197	0.78	1113	0.47	2.21	7.79	2.52	1787	0.11
K-TD-H-9/2009-70	H	F	35	NS	Karbala	292	2274	4.87	588	3.55	0.92	15.42	1.32	1189	0.05
K-TD-H-9/2009-71	H	F	38	NS	Karbala	35	663	1.18	489	1.27	4.83	18.06	1.50	989	0.96
K-TD-H-9/2009-72	H	F	55	NS	Karbala	121	282	1.41	410	0.71	4.05	11.17	1.18	861	0.91
K-TD-H-9/2009-73	H	F	42	NS	Karbala	474	1267	15.33	662	1.30	7.31	19.94	1.57	1303	0.94
K-TD-H-9/2009-74	H	F	34	NS	Karbala	285	10562	5.77	618	3.51	4.23	313.1	0.74	1258	1.06
K-TD-H-9/2009-75	H	F	35	NS	Karbala	380	6088	13.55	371	6.75	1.44	4.44	2.34	1858	0.12
K-TD-H-9/2009-76	H	F	37	NS	Karbala	254	1427	8.49	431	5.54	1.73	2.61	2.49	1721	0.09
K-TD-H-9/2009-77	H	F	23	NS	Karbala	130	161	2.46	241	8.85	30.4	81.12	1.07	660	0.96
K-TD-H-9/2009-78	H	F	32	NS	Karbala	69	186	1.43	153	0.56	4.49	15.34	0.67	170	0.95
K-TD-H-9/2009-79	H	F	35	NS	Karbala	499	2134	37.15	333	3.87	0.92	15.42	1.32	1189	0.05
K-TD-H-9/2009-80	H	F	21	NS	Karbala	564	1011	3.67	413	0.95	5.61	105.3	1.11	1000	1.45
K-TD-H-9/2009-81	H	F	40	NS	Karbala	204	736	4.02	327	0.88	4.26	154.8	1.26	992	0.94
K-TD-H-9/2009-82	H	F	13	NS	Karbala	222	1529	7.04	563	1.10	5.57	71.19	1.54	1062	0.96
K-TD-H-9/2009-83	H	F	12	NS	Karbala	369	1038	7.65	431	1.18	9.71	30.47	0.20	78	1.39
K-TD-H-9/2009-84	H	F	19	NS	Karbala	310	2672	14.12	457	2.65	4.27	30.90	1.13	987	1.00
K-TD-H-9/2009-85	H	F	20	NS	Karbala	126	580	2.48	258	2.04	4.05	11.17	1.18	861	0.91
K-TD-H-9/2009-86	H	F	16	NS	Karbala	402	10150	4.23	303	5.26	5.37	29.67	1.49	985	1.16
K-TD-H-9/2009-87	H	F	14	NS	Karbala	534	2934	3.02	622	1.61	7.61	192.3	1.54	1289	0.96
K-TD-H-9/2009-88	H	F	24	NS	Karbala	539	1395	3.25	607	4.47	4.45	40.79	1.39	1198	1.02

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-H-9/2009-89	H	F	5	NS	Karbala	338	1752	2.97	939	2.04	1.81	8.32	2.47	1762	0.12
K-TD-H-9/2009-90	H	F	19	NS	Karbala	536	1747	7.61	574	4.71	7.60	102.3	1.15	1072	0.93
K-TD-H-9/2009-91	H	F	19	NS	Karbala	182	466	1.84	491	0.63	4.47	17.49	1.40	912	0.97
K-TD-H-9/2009-92	H	F	10	NS	Karbala	248	385	1.89	399	0.55	4.63	65.78	1.08	1022	1.04
K-TD-H-9/2009-93	H	F	19	NS	Karbala	216	299	1.41	278	1.81	4.36	186.0	1.19	959	1.00
K-TD-H-9/2009-94	H	F	32	NS	Karbala	164	499	1.45	365	1.95	9.52	24.74	1.95	981	0.98
K-TD-H-9/2009-95	H	F	45	NS	Karbala	155	276	1.38	346	0.29	4.06	41.90	1.29	944	1.01
K-TD-H-9/2009-96	H	F	44	NS	Karbala	273	1088	2.41	562	4.43	5.57	71.19	1.54	1062	0.96
K-TD-H-9/2009-97	H	F	11	NS	Karbala	331	1190	1.30	799	3.03	6.66	98.62	1.40	1512	0.94
K-TD-H-9/2009-98	H	F	45	NS	Karbala	223	301	1.67	362	0.40	18.5	19.77	1.52	920	0.96
K-TD-H-9/2009-99	H	F	17	NS	Karbala	190	259	1.33	177	0.40	6.82	27.42	0.52	372	0.92
K-TD-H-9/2009-100	H	F	35	NS	Karbala	164	1716	1.74	443	1.97	4.27	30.90	1.13	987	1.00
K-TD-H-9/2009-101	H	F	4	NS	Karbala	234	1021	30.03	405	2.43	3.98	13.65	1.44	883	0.93
K-TD-H-9/2009-102	H	F	17	NS	Karbala	37	657	35.79	89	0.28	6.78	20.32	0.21	72	0.93
K-TD-H-9/2009-103	H	F	30	NS	Karbala	248	522	24.30	562	0.84	6.89	37.05	1.33	1053	0.97
K-TD-H-9/2009-104	H	F	54	NS	Karbala	113	250	9.08	128	2.93	13.6	33.15	0.33	86	1.44
K-TD-H-9/2009-105	H	F	21	NS	Karbala	130	697	12.01	249	1.06	4.05	11.17	1.18	861	0.91
K-TD-H-9/2009-106	H	F	11	NS	Karbala	526	4104	34.09	794	2.49	0.50	8.26	1.30	1503	0.02
K-TD-H-9/2009-107	H	F	8	NS	Karbala	450	2764	19.56	644	4.12	8.19	74.91	1.49	1292	1.01
K-TD-H-9/2009-108	H	F	22	NS	Karbala	690	2923	16.49	1183	10.77	1.44	4.44	2.34	1858	0.12
K-TD-H-9/2009-109	H	F	58	NS	Karbala	223	593	2.86	276	1.34	2.21	7.79	2.52	1787	0.11
K-TD-H-9/2009-110	H	F	43	NS	Karbala	194	804	1.08	533	0.77	5.78	69.89	1.38	1046	0.97
K-TD-H-9/2009-111	H	F	23	NS	Karbala	210	1197	3.91	408	1.49	4.04	28.68	1.31	886	0.94
K-TD-D-9/2009-112	D	M	59	S	Karbala	12	82	1.25	7	1.55	3.98	97.34	0.13	15	0.92
K-TD-D-9/2009-113	D	M	45	S	Karbala	130	201	1.94	3056	2.04	5.89	157.8	1.53	903	1.88
K-TD-D-9/2009-114	D	M	45	S	Karbala	176	268	1.07	217	8.38	4.18	85.30	0.75	416	0.95
K-TD-D-9/2009-115	D	M	51	S	Karbala	368	10326	0.38	6552	6.55	9.73	21.39	2.33	989	0.95
K-TD-D-9/2009-116	D	M	51	S	Karbala	96	10434	4.91	6352	5.99	6.22	260.6	0.71	964	0.95
K-TD-D-9/2009-117	D	M	58	S	Karbala	58	747	4.20	510	0.54	4.83	18.06	1.50	989	0.96
K-TD-D-9/2009-118	D	M	46	S	Karbala	186	5369	3.87	3638	7.56	18.5	19.77	1.52	920	0.96
K-TD-D-9/2009-119	D	M	55	S	Karbala	43	280	1.79	542	0.52	4.06	12.28	1.44	1048	0.98

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-D-9/2009-120	D	M	60	S	Karbala	47	261	0.77	266	0.27	5.35	26.45	1.36	876	1.19
K-TD-D-9/2009-121	D	M	55	S	Karbala	69	636	2.88	326	0.96	18.1	20.05	1.62	954	0.98
K-TD-D-9/2009-122	D	M	50	NS	Karbala	3	67	0.61	477	2.62	4.65	35.63	1.42	927	1.07
K-TD-D-9/2009-123	D	M	56	NS	Karbala	346	872	1.55	1144	2.42	1.34	5.49	2.57	1850	0.12
K-TD-D-9/2009-124	D	M	57	NS	Karbala	288	994	3.17	2361	2.23	4.21	31.23	2.45	2110	0.20
K-TD-D-9/2009-125	D	M	54	NS	Karbala	201	578	5.28	1691	1.18	4.26	37.53	2.57	2008	0.21
K-TD-D-9/2009-126	D	M	42	NS	Karbala	189	966	2.11	320	0.73	4.06	41.90	1.29	944	1.01
K-TD-D-9/2009-127	D	M	44	NS	Karbala	479	3152	3.24	1276	7.70	2.26	4.82	2.74	1885	0.15
K-TD-D-9/2009-128	D	M	53	NS	Karbala	198	749	2.14	481	1.09	4.09	196.9	1.18	969	0.98
K-TD-D-9/2009-129	D	M	48	NS	Karbala	206	3465	1.59	470	0.82	4.47	17.49	1.40	912	0.97
K-TD-D-9/2009-130	D	F	55	S	Karbala	82	524	3.63	5012	0.70	6.87	38.50	1.29	1040	0.97
K-TD-D-9/2009-131	D	F	65	S	Karbala	226	9626	1.98	697	1.45	0.56	130.9	1.27	1375	0.03
K-TD-D-9/2009-132	D	F	50	S	Karbala	314	1850	2.10	248	2.17	6.59	89.69	0.93	829	1.35
K-TD-D-9/2009-133	D	F	59	S	Karbala	580	3651	2.72	1917	4.26	4.21	31.23	2.45	2110	0.20
K-TD-D-9/2009-134	D	F	51	S	Karbala	223	1024	5.51	1210	1.18	2.26	4.82	2.74	1885	0.15
K-TD-D-9/2009-135	D	F	46	S	Karbala	181	631	8.50	316	0.51	3.79	20.66	1.28	946	0.94
K-TD-D-9/2009-136	D	F	60	S	Karbala	269	1565	11.15	1142	2.80	2.21	7.79	2.52	1787	0.11
K-TD-D-9/2009-137	D	F	46	S	Karbala	294	1935	3.84	539	5.66	4.06	12.28	1.44	1048	0.98
K-TD-D-9/2009-138	D	F	70	S	Karbala	97	432	3.27	842	0.80	1.73	2.61	2.49	1721	0.09
K-TD-D-9/2009-139	D	F	47	NS	Karbala	134	1877	0.62	134	0.86	0.42	2.49	0.05	98	0.02
K-TD-D-9/2009-140	D	F	48	NS	Karbala	184	2675	1.97	290	1.94	4.75	34.61	1.50	978	1.09
K-TD-D-9/2009-141	D	F	60	NS	Karbala	16	129	0.69	1186	0.12	1.44	4.44	2.34	1858	0.12
K-TD-D-9/2009-142	D	F	60	NS	Karbala	31	187	2.28	1432	0.66	2.58	5.06	2.73	1920	0.15
K-TD-D-9/2009-143	D	F	60	NS	Karbala	21	114	1.13	984	0.48	1.81	8.32	2.47	1762	0.12
K-TD-D-9/2009-144	D	F	60	NS	Karbala	1	47	0.23	21	0.37	3.98	97.34	0.13	15	0.92
K-TD-D-9/2009-145	D	F	46	NS	Karbala	293	1752	2.34	464	2.57	9.73	21.39	2.33	989	0.95
K-TD-D-9/2009-146	D	F	75	NS	Karbala	397	5726	2.82	1359	2.76	2.58	5.06	2.73	1920	0.15
K-TD-D-9/2009-147	D	F	54	NS	Karbala	343	3688	0.44	817	2.88	3.95	32.75	1.34	1535	0.94
K-TD-D-9/2009-148	D	F	54	NS	Karbala	192	358	0.48	67	0.21	9.84	337.5	0.27	17	1.16

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
K-TD-D-9/2009-149	D	F	52	NS	Karbala	259	1165	0.73	479	1.34	5.61	105.3	1.11	1000	1.45
K-TD-D-9/2009-150	D	F	41	NS	Karbala	290	2221	0.94	709	1.52	4.31	163.7	1.22	1379	0.94
K-TD-D-9/2009-151	D	F	40	NS	Karbala	329	5112	10.12	1807	2.16	4.26	37.53	2.57	2008	0.21
K-TD-D-9/2009-152	D	F	70	NS	Karbala	229	954	4.69	378	1.32	4.63	65.78	1.08	1022	1.04
K-TD-D-9/2009-153	D	F	59	NS	Karbala	594	2024	2.44	322	2.16	6.65	170.6	1.23	1027	0.98
K-TD-D-9/2009-154	D	F	56	NS	Karbala	162	2561	2.52	376	0.72	4.20	28.21	1.48	928	0.95
K-TD-D-9/2009-155	D	F	60	NS	Karbala	132	2103	0.66	1690	2.11	4.26	37.53	2.57	2008	0.21
L-TD-H-9/2009-156	H	F	10	NS	London	109	139	1.45	30	3.46	19.2	37.13	1.05	225	0.05
L-TD-H-9/2009-157	H	F	10	NS	London	304	195	0.24	71	1.45	6.50	2.80	1.26	222	0.03
L-TD-H-9/2009-158	H	M	8	NS	London	260	188	0.57	68	8.52	1.01	5.52	0.02	6	0.03
L-TD-H-9/2009-159	H	M	6	NS	London	335	205	2.67	72	8.39	2.35	0.66	0.40	86	0.01
L-TD-H-9/2009-160	H	M	13	NS	London	236	165	2.94	98	3.03	5.00	1.51	1.20	235	0.06
L-TD-H-9/2009-161	H	F	33	NS	London	222	184	1.86	72	3.44	3.04	2.73	0.95	149	0.03
L-TD-H-9/2009-162	H	M	42	NS	London	263	267	1.23	86	1.82	3.06	2.27	0.94	148	0.02
L-TD-H-9/2009-163	H	M	44	NS	London	204	211	1.25	62	1.64	5.43	1.63	1.23	226	0.02
L-TD-H-9/2009-164	H	M	41	NS	London	189	224	1.53	52	3.76	1.69	1.55	0.41	280	0.03
L-TD-H-9/2009-165	H	M	13	NS	London	268	136	1.09	40	1.42	8.40	5.04	0.25	204	0.02
L-TD-H-9/2009-166	H	M	42	NS	London	260	209	1.09	83	3.67	2.05	45.81	1.09	115	0.02
L-TD-H-9/2009-167	H	F	10	NS	London	267	258	1.25	64	1.38	1.82	26.23	1.08	120	0.02
L-TD-H-9/2009-168	H	M	44	NS	London	199	165	1.14	58	3.63	0.87	1.90	0.14	55	0.02
L-TD-H-9/2009-169	H	F	10	NS	London	90	79	1.44	26	1.37	0.61	1.54	0.14	49	0.02
L-TD-H-9/2009-170	H	M	6	NS	London	263	324	1.74	44	9.03	3.15	2.43	0.97	209	0.03
L-TD-H-9/2009-171	H	M	8	NS	London	248	137	0.43	67	7.06	0.79	1.85	0.12	357	0.04
L-TD-H-9/2009-172	H	M	41	NS	London	175	169	1.35	48	3.64	6.50	2.80	1.26	222	0.03

Table E2.1 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Drinking water				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
L-TD-H-9/2009-173	H	F	33	NS	London	196	126	1.58	69	1.27	5.43	1.63	1.23	226	0.02
HS = health status, y = year, K-TD-H-9/2009-1, K corresponds to the province in Iraq (K) Karbala and may be replaced by (L) London in the UK; TD corresponds to tear drops, H corresponds to healthy and may be replaced by D (diabetes); 9/2009 corresponds to the date (month/year); and 1 corresponds to the sample code number.															

Appendix E3

Discriminant Analysis Results:

Table E3.1: Discriminant results for tear drops of 173 individuals from Karbala and London. (the original data were reported in Table E1.1)

G	Dis-1	Dis-2	G	Dis-1	Dis-2	G	Dis-1	Dis-2	G	Dis-1	Dis-2	G	Dis-1	Dis-2
DK	0.14307	-1.05792	DK	0.6275	-0.32228	HK	-0.27656	0.10887	HK	-0.20294	1.06547	DK	1.85959	-0.27572
HK	-0.89782	0.4793	HK	-1.16209	1.27131	HK	-0.60207	-0.20084	HK	-1.60214	0.31611	HL	-1.04062	-3.28223
HK	-0.0503	0.24152	HK	-1.09933	0.21738	HK	-0.26542	0.51261	HK	-0.83342	0.9302	HL	0.02297	-1.70566
HK	-0.16787	0.0777	HK	-0.09765	0.17488	HK	-0.48091	-0.19895	HK	0.07769	0.00404	DK	2.90954	-2.42513
DK	1.0046	0.48471	HK	-0.36967	0.51842	DK	1.01176	0.57393	HK	0.83224	0.79404	DK	1.21239	-0.08228
HK	-0.19307	0.09331	HK	-0.41953	0.48481	HK	-1.39904	1.24702	HL	-1.59044	-0.61045	DK	3.42246	-2.63138
HK	-0.40579	-0.20995	HK	-0.42007	-0.77601	HK	-1.05552	-0.00786	HK	0.04312	0.08667	DK	1.64883	0.39028
HK	-1.74865	0.80819	HK	-1.14658	1.24582	HK	-0.84782	0.25959	HK	-0.35995	0.11016	DK	1.139	-0.21143
HK	0.16149	1.23292	HK	0.17451	-0.18357	DK	2.11444	1.59542	HK	-0.55242	-0.70712	HK	0.20019	-0.29346
DK	1.43175	1.54959	HK	0.038	-0.21733	HK	-1.68991	0.93513	HK	-0.54053	-0.66433	DK	0.24546	-1.41162
HK	-0.07119	1.64347	HK	-0.73894	1.33274	HL	-1.1815	-0.89176	HK	0.06154	-0.04459	DK	1.63244	0.13572
HK	-0.56812	0.26723	HK	0.4148	0.55033	HK	-0.61318	-0.22345	HK	-0.17345	-0.92798	DK	3.69798	0.44898
HK	-0.20684	-0.09385	HK	0.68674	1.34441	HK	0.53803	0.31293	HK	-0.22999	-0.64164	DK	4.95269	0.13063
HK	-0.68748	0.90061	DK	1.24161	-0.33454	HK	0.01509	-0.50627	HK	-1.02115	0.55238	DK	0.90231	0.16371
HK	0.00278	0.18614	HK	-0.47715	-0.59426	DK	0.52194	-0.45157	HK	-0.39125	-0.16812	DK	1.40497	-2.49949
HK	-0.10959	1.52281	HK	-0.40029	-0.07297	HK	-0.02842	-0.30294	HK	-0.06522	-0.51152	DK	0.8739	0.37533
HK	-0.47386	1.63859	HK	-0.08093	-0.45052	HK	-0.6844	1.45737	HK	-1.09369	0.57161	DK	1.44034	0.04921
HK	-3.00101	2.32903	HK	-1.2438	0.68379	HK	-0.14634	-0.48916	HK	-0.65638	1.17762	DK	2.91672	0.0455
HK	-0.2019	2.8158	HK	-0.54628	-0.31499	HL	-1.33413	-1.47529	HK	-0.52449	1.25926	DK	1.48015	-0.28564
HK	0.11664	0.48079	HK	1.02154	1.92166	HL	-0.7792	-1.61824	HL	-0.27324	-1.19071	DK	0.78237	0.20755
HK	-1.62	1.52512	HL	-1.0302	-0.83694	HK	-0.49584	-0.18892	HK	0.01763	1.48899	DK	5.1862	-1.06211
HK	-1.03688	0.17955	HK	0.19119	-0.43907	HK	-0.01127	-0.25785	HK	-0.45816	3.38136	DK	1.46232	0.40034
HK	-0.21905	-0.25774	HK	0.45376	-0.23196	HK	-1.76494	2.95616	HK	-2.01661	2.42024	DK	1.61311	-0.66669
HK	-0.18415	-0.12675	HK	-0.62744	0.26915	HK	-1.25664	0.57643	HK	-1.70663	4.15963	DK	2.61093	-0.72107
HK	-1.17117	2.19963	HK	0.45314	0.88608	HK	-0.08631	1.47127	HK	-0.51272	-0.21629	DK	0.3637	-1.71244
HK	-0.87308	0.87075	HK	0.30061	-0.3056	HK	0.31561	0.8854	HK	0.49944	0.17698	DK	3.22639	0.39447
HL	-0.53579	-0.95286	HK	0.08721	-0.08221	HK	-0.61491	0.54983	HK	0.49786	0.31255	HK	-0.01995	-0.37452
HK	0.34088	-0.3732	HK	-0.4111	0.09541	DK	1.51609	1.66564	DK	0.37597	-1.26932	DK	2.04857	-0.52089

Table E3.1 (continued)

G	Dis-1	Dis-2	G	Dis-1	Dis-2									
DK	1.0986	0.12629	HL	-2.17867	-3.79158									
DK	1.25963	0.13321	HL	-2.18922	-2.93964									
DK	1.0288	-0.15002	HL	-1.79396	-1.41458									
HK	-0.64452	-0.52599	HL	-1.25766	-0.53559									
HK	-0.62543	0.00583												
DK	3.49629	1.09587												
DK	1.76657	-0.20761												
HK	-0.04064	-0.22												
HK	0.37088	-0.31136												
DK	3.20919	-0.34668												
DK	5.28479	-0.4986												
DK	1.20823	0.1744												
HK	-0.20271	0.40081												
HK	0.58882	0.19419												
DK	1.75664	0.02705												
HL	-1.4508	-1.58383												
HL	-1.35539	-0.4598												
HL	-2.34174	-3.56892												
HL	-2.25494	-3.23671												
HL	-0.97456	-1.10583												
HL	-1.60843	-1.41351												
HL	-1.69844	-0.44028												
HL	-1.51188	-0.49721												
HL	-1.81445	-1.4722												
HL	-1.41218	-0.58016												
HL	-2.02208	-1.29052												
HL	-1.24889	-0.4743												
HL	-1.77447	-1.36957												
HL	-1.07886	-0.74125												

G = population group, Dis = discriminant, H = healthy, D = diabetic, K = Karbala, L = London.

Appendix E4

Analysis of covariance (ANCOVA):

Table E4.1: ANCOVA results for B in tear drops (n = 128).

Source of variance	Sum of squares	*df*	MS	*F*	Sig.	η^2
Corrected Model	2155766.828	9	239529.648	3.835	0.000	0.226
Intercept	1420880.710	1	1420880.710	22.748	0.000	0.162
Age	47153.413	1	47153.413	0.755	0.387	0.006
DW	81800.316	1	81800.316	1.310	0.255	0.011
Health	785287.009	1	785287.01	12.573	0.001^{++}	0.096
Gender	2720.305	1	2720.305	0.044	0.835	0.000
Smoking	50945.837	1	50945.837	0.816	0.368	0.007
H * G	52524.896	1	52524.896	0.841	0.361	0.007
H * S	13045.937	1	13045.937	0.209	0.648	0.002
G * S	199892.460	1	199892.460	3.200	0.076^{+}	0.026
H * G * S	207882.412	1	207882.41	3.328	0.071^{+}	0.027
Error	7370339.598	118	62460.505			
Total	35421799.45	128				
Corrected Total	9526106.426	127				

H = healthy, G = gender, S = smoking, *df* = degrees of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, * indicates an interaction term, Sig. = level of significance, significant effect or interaction; $^{++}$ is significant at $P < 0.01$, $^{+}$ is significant at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.2: ANCOVA results for V in tear drops (n = 155).

Source of variance	SS	*df*	MS	*F*	Sig.	η^2
Corrected Model	421.772	9	46.864	2.264	0.021	0.123
Intercept	177.051	1	177.051	8.554	0.004	0.056
Age	65.945	1	65.945	3.186	0.076^{+}	0.022
DW	275.387	1	275.387	13.305	0.000^{++}	0.084
Health	27.182	1	27.182	1.313	0.254	0.009
Gender	0.033	1	0.033	0.002	0.968	0.000
Smoking	32.158	1	32.158	1.554	0.215	0.011
H * G	0.021	1	0.021	0.001	0.975	0.000
H * S	0.549	1	0.549	0.027	0.871	0.000
G * S	4.850	1	4.850	0.234	0.629	0.002
H * G * S	8.793	1	8.793	0.425	0.516	0.003
Error	3001.160	145	20.698			
Total	7543.614	155				
Corrected Total	3422.931	154				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water,*indicates an interaction term, Sig. = level of significance, $^{++}$ significant effect or interaction at $P < 0.001$, $^{+}$ significant at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.3: ANCOVA results for Cr in tear drops (n = 151).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	1842.140	9	204.682	1.971	0.047	0.112
Intercept	2605.908	1	2605.908	25.093	0.000	0.151
Age	307.558	1	307.558	2.962	0.087^{++}	0.021
DW	16.130	1	16.130	0.155	0.694	0.001
Health	153.468	1	153.468	1.478	0.226	0.010
Gender	4.914	1	4.914	0.047	0.828	0.000
Smoking	31.675	1	31.675	0.305	0.582	0.002
H * G	84.374	1	84.374	0.812	0.369	0.006
H * S	95.508	1	95.508	0.920	0.339	0.006
G * S	440.704	1	440.704	4.244	0.041^{+}	0.029
H * G * S	11.065	1	11.065	0.107	0.745	0.001
Error	14642.925	141	103.851			
Total	35512.641	151				
Corrected Total	16485.065	150				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction at $P < 0.05$, $^{++}$ significant at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.4: ANCOVA results for Mn in tear drops (n = 148).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	182083.342	9	20231.482	5.494	0.000	0.264
Intercept	72824.328	1	72824.328	19.775	0.000	0.125
Age	2401.711	1	2401.711	0.652	0.421	0.005
DW	30345.322	1	30345.322	8.240	0.005^{+}	0.056
Health	59974.369	1	59974.369	16.286	0.000^{++}	0.106
Gender	426.769	1	426.769	0.116	0.734	0.001
Smoking	12583.648	1	12583.648	3.417	0.067^{+++}	0.024
H * G	1499.985	1	1499.985	0.407	0.524	0.003
H * S	3631.583	1	3631.583	0.986	0.322	0.007
G * S	6965.341	1	6965.341	1.891	0.171	0.014
H * G * S	3269.306	1	3269.306	0.888	0.348	0.006
Error	508193.946	138	3682.565			
Total	1218423.933	148				
Corrected Total	690277.288	147				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{++}$ significant effect or interaction at $P < 0.001$, $^{+}$ is significant at $P < 0.01$, $^{+++}$ $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.5: ANCOVA results for Fe in tear drops (n = 148).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	5048460.351	9	560940.039	2.722	0.006	0.151
Intercept	2064113.423	1	2064113.423	10.017	0.002	0.068
Age	89110.069	1	89110.069	0.432	0.512	0.003
DW	436436.819	1	436436.819	2.118	0.148	0.015
Health	160001.161	1	160001.161	0.777	0.380	0.006
Gender	1159264.615	1	1159264.615	5.626	0.019^{+}	0.039
Smoking	9244.897	1	9244.897	0.045	0.833	0.000
H * G	100163.711	1	100163.711	0.486	0.487	0.004
H * S	132263.269	1	132263.269	0.642	0.424	0.005
G * S	620347.216	1	620347.216	3.011	0.085	0.021
H * G * S	447929.098	1	447929.098	2.174	0.143	0.016
Error	28435159.85	138	206051.883			
Total	73794721.11	148				
Corrected Total	33483620.20	147				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction at $P < 0.05$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.6: ANCOVA results for Cu in tear drops (n = 155).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	425055.163	9	47228.351	2.088	0.034	0.115
Intercept	1185011.746	1	1185011.746	52.387	0.000	0.265
Age	85309.540	1	85309.540	3.771	0.054^{+}	0.025
DW	18518.117	1	18518.117	0.819	0.367	0.006
Health	51296.824	1	51296.824	2.268	0.134	0.015
Gender	7971.983	1	7971.983	0.352	0.554	0.002
Smoking	14140.476	1	14140.476	0.625	0.430	0.004
H * G	25072.565	1	25072.565	1.108	0.294	0.008
H * S	69763.723	1	69763.723	3.084	0.081^{+}	0.021
G * S	45206.633	1	45206.633	1.998	0.160	0.014
H * G * S	8868.045	1	8868.045	0.392	0.532	0.003
Error	3279951.418	145	22620.355			
Total	13350029.34	155				
Corrected Total	3705006.581	154				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.7: ANCOVA results for Zn in tear drops (n = 147).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	29981961.1	9	3331329.0	2.529	0.010	0.142
Intercept	55022140.6	1	55022140.6	41.775	0.000	0.234
Age	8394559.3	1	8394559.3	6.373	0.013^{+}	0.044
DW	3018589.1	1	3018589.1	2.292	0.132	0.016
Health	1663286.1	1	1663286.1	1.263	0.263	0.009
Gender	1257753.5	1	1257753.5	0.955	0.330	0.007
Smoking	1801513.3	1	1801513.3	1.368	0.244	0.010
H * G	1692227.8	1	1692227.8	1.285	0.259	0.009
H * S	10080760.0	1	10080760.0	7.654	0.006^{++}	0.053
G * S	31207.6	1	31207.6	0.024	0.878	0.000
H * G * S	106701.0	1	106701.0	0.081	0.776	0.001
Error	1.804E8	137	1317119.3			
Total	4.233E8	147				
Corrected Total	2.104E8	146				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{++}$ significant effect or interaction at $P < 0.01$, $^{+}$ is significant at $P < 0.05$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.8: ANCOVA results for As in tear drops (n = 152).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	2844.709	9	316.079	3.773	0.000	0.193
Intercept	1437.011	1	1437.011	17.155	0.000	0.108
Age	1438.777	1	1438.777	17.176	0.000^{++}	0.108
DW	158.224	1	158.224	1.889	0.171	0.013
Health	8.272	1	8.272	0.099	0.754	0.001
Gender	9.835	1	9.835	0.117	0.732	0.001
Smoking	17.140	1	17.140	0.205	0.652	0.001
H * G	4.339	1	4.339	0.052	0.820	0.000
H * S	2.944	1	2.944	0.035	0.852	0.000
G * S	35.968	1	35.968	0.429	0.513	0.003
H * G * S	35.949	1	35.949	0.429	0.513	0.003
Error	11894.770	142	83.766			
Total	21543.089	152				
Corrected Total	14739.480	151				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{++}$ significant effect or interaction at $P < 0.001$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.9: ANCOVA results for Sr in tear drops (n = 150).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	14925165.043	9	1658351.671	29.156	0.000	0.652
Intercept	126951.378	1	126951.378	2.232	0.137	0.016
Age	31533.890	1	31533.890	0.554	0.458	0.004
DW	9998396.033	1	9998396.033	175.783	0.000^{+}	0.557
Health	306479.365	1	306479.365	5.388	0.022^{++}	0.037
Gender	23382.180	1	23382.180	0.411	0.522	0.003
Smoking	2311.539	1	2311.539	0.041	0.841	0.000
H * G	40923.593	1	40923.593	0.719	0.398	0.005
H * S	464395.290	1	464395.290	8.165	0.005^{+++}	0.055
G * S	19590.982	1	19590.982	0.344	0.558	0.002
H * G * S	343517.661	1	343517.661	6.039	0.015^{++}	0.041
Error	7963088.556	140	56879.204			
Total	66029062.247	150				
Corrected Total	22888253.600	149				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction $P < 0.001$, $^{++}$ significant at $P < 0.05$, $^{+++}$ significant at $P < 0.01$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.10: ANCOVA results for Cd in tear drops (n = 150).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	61.254	9	6.806	2.186	0.026	0.123
Intercept	93.718	1	93.718	30.107	0.000	0.177
Age	4.794	1	4.794	1.540	0.217	0.011
DW	11.572	1	11.572	3.717	0.056^{+}	0.026
Health	0.164	1	0.164	0.053	0.819	0.000
Gender	10.351	1	10.351	3.325	0.070^{+}	0.023
Smoking	30.136	1	30.136	9.681	0.002^{++}	0.065
H * G	3.866	1	3.866	1.242	0.267	0.009
H * S	0.887	1	.887	0.285	0.594	0.002
G * S	2.285	1	2.285	0.734	0.393	0.005
H * G * S	0.017	1	0.017	0.005	0.942	0.000
Error	435.800	140	3.113			
Total	1104.726	150				
Corrected Total	497.054	149				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{++}$ significant effect or interaction at $P < 0.01$, $^{+}$ is significant at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.11: ANCOVA results for Cu in tear drops (n = 155) without covariant variable (individual's age).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	339745.6	8	42468.203	1.842	0.074	0.092
Intercept	3104884.9	1	3104884.945	134.704	0.000	0.480
DW	21047.4	1	21047.376	0.913	0.341	0.006
Health	178235.7	1	178235.700	7.733	0.006+	0.050
Gender	1531.7	1	1531.707	0.066	0.797	0.000
Smoking	4426.1	1	4426.116	0.192	0.662	0.001
H * G	30322.3	1	30322.331	1.316	0.253	0.009
H * S	49177.6	1	49177.613	2.134	0.146	0.014
G * S	27226.9	1	27226.927	1.181	0.279	0.008
H * G * S	23326.8	1	23326.791	1.012	0.316	0.007
Error	3365260.9	146	23049.733			
Total	13350029.3	155				
Corrected Total	3705006.6	154				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction at $P < 0.01$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.12: ANCOVA results for As in tear drops (n = 152) without covariant variable (individual's age).

Source of variance	Sum of squares	*df*	Mean squares	*F*	Sig.	η^2
Corrected Model	1405.933	8	175.742	1.885	0.067	0.095
Intercept	159.110	1	159.110	1.706	0.194	0.012
DW	156.936	1	156.936	1.683	0.197	0.012
Health	598.247	1	598.247	6.416	0.012+	0.043
Gender	14.416	1	14.416	0.155	0.695	0.001
Smoking	7.672	1	7.672	0.082	0.775	0.001
H * G	13.463	1	13.463	0.144	0.705	0.001
H * S	56.035	1	56.035	0.601	0.439	0.004
G * S	.030	1	0.030	0.000	0.986	0.000
H * G * S	1.498	1	1.498	0.016	0.899	0.000
Error	13333.547	143	93.242			
Total	21543.089	152				
Corrected Total	14739.480	151				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, significant effect or interaction; $^{+}$ is highly significant at $P < 0.05$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Table E4.13: ANCOVA results for V in tear drops (n = 155) without covariant variables (age and drinking water).

Source of variance	Sum of squares	*df*	MS	*F*	Sig.	η^2
Corrected Model	86.546	7	12.364	.545	0.799	0.025
Intercept	2090.962	1	2090.962	92.127	0.000	0.385
Health	75.257	1	75.257	3.316	0.071+	0.022
Gender	0.314	1	0.314	0.014	0.906	0.000
Smoking	15.488	1	15.488	0.682	0.410	0.005
H * G	0.977	1	0.977	0.043	0.836	0.000
H * S	2.413	1	2.413	0.106	0.745	0.001
G * S	0.004	1	0.004	0.000	0.989	0.000
H * G * S	0.016	1	0.016	0.001	0.979	0.000
Error	3336.385	147	22.696			
Total	7543.614	155				
Corrected Total	3422.931	154				

H = healthy, G = gender, S = smoking, *df* = degree of freedom, *F* is the calculated value for F-test, $F_{critical}$ = 3.909, DW = drinking water, *indicates an interaction term, Sig. = level of significance, $^{+}$ significant effect or interaction at $P < 0.1$. Mean of square (MS) = sum of squares (SS)/df, $F = MS_{effect}/MS_{error}$, η^2 = partial *eta* squared = $SS_{effect}/SS_{effect} + SS_{error}$.

Appendix E5

The effect of Drinking Water on the Level of Trace Elements in Tear Drops

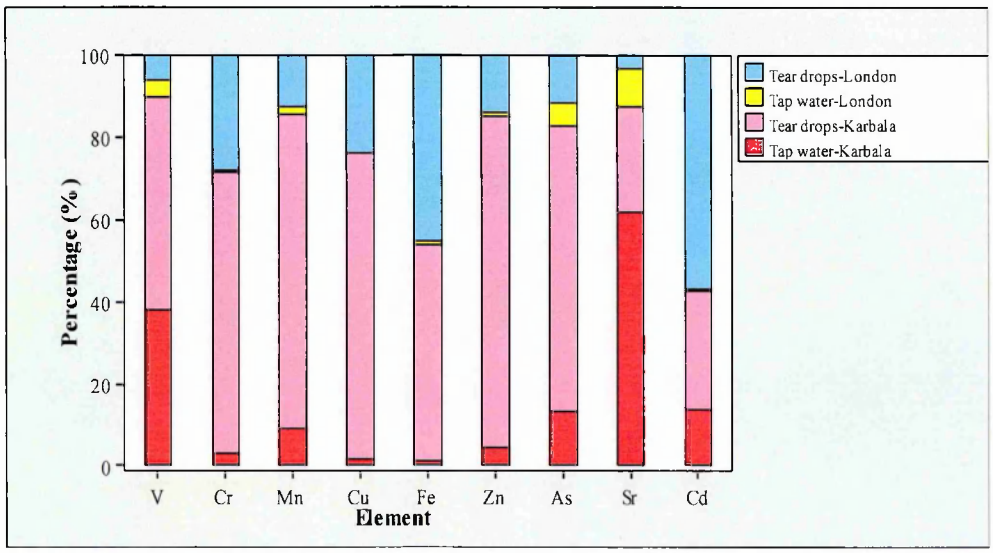

Figure E5.1: Comparative elemental levels in tear drops (µg/l) and corresponding tap water (µg/l) for two population groups (n = 155 for Karbala and 18 for London samples).

Appendix E6. Influence of Gender and Smoking Activity

Table E6.1: Elemental mean and standard deviation values in human tear drops for males and females from Karbala, Iraq, (outliers omitted).

Element (n_1, n_2)	Mean ± SD Range (µg/l)		F-teat			Two-tailed t-test			
	Male	Female	Variance	F_{calc}	Sig.	t_{calc}	*df*	Sig.	t_{crit}
B	482 ± 225	431 ± 298	Equal variances assumed	0.207	0.650	0.999	126^{+}	0.320	1.657
(47, 81)	< 146 - 1380	71 - 2020	Unequal variances assumed			1.075	117^{++}	0.285	
V	5.3 ± 4.0	5.1 ± 5.1	Equal variances assumed	1.941	0.166	0.318	153	0.751	1.976
(60, 95)	0.1 – 21.1	0.1 – 21.3	Unequal variances assumed			0.336	146	0.737	
Cr	13.8 ± 11.5	9.6 ± 9.5	Equal variances assumed	4.547	0.035	2.406	149	0.017	1.983
(58, 93)	0.2 – 46.5	0.3 – 47.1	Unequal variances assumed			**2.301**	104	0.023	
Mn	65.1 ± 71.8	56.3 ± 66.6	Equal variances assumed	0.188	0.665	0.757	146	0.450	1.976
(58, 90)	1.0 – 381.2	0.8 – 362.7	Unequal variances assumed			0.074	115	0.458	
Fe	706 ± 550	407 ± 386	Equal variances assumed	15.490	< 0.001	3.880	146	< 0.001	1.987
(57, 91)	3 - 2060	7 - 1796	Unequal variances assumed			**3.585**	90	0.001	
Cu	256 ± 153	246 ± 157	Equal variances assumed	0.027	0.869	0.394	153	0.694	1.978
(60, 95)	3 - 609	1 - 741	Unequal variances assumed			0.397	128	0.692	
Zn	1136 ± 1164	1245 ± 1227	Equal variances assumed	1.082	0.300	0.531	145	0.597	1.976
(56, 91)	49 - 5369	47 - 5726	Unequal variances assumed			0.537	121	0.592	
As	7.3 ± 10.5	6.3 ± 9.3	Equal variances assumed	0.326	0.569	0.557	150	0.578	1.976
(60, 92)	0.1 – 44.8	0.2 – 38.7	Unequal variances assumed			0.546	118	0.586	
Sr	542 ± 410	533 ± 383	Equal variances assumed	0.024	0.878	0.130	148	0.897	1.976
(56, 94)	7 - 2361	21 - 1917	Unequal variances assumed			0.127	109	0.899	
Cd	2.3 ± 2.2	1.8 ± 1.6	Equal variances assumed	5.110	0.025	1.641	148	0.103	1.976
(58, 92)	0.2 – 8.4	0.1 – 6.8	Unequal variances assumed			1.529	95	0.129	

SD is standard deviation, n_1, n_2 are the number of samples for males and females, respectively, *df* = degrees of freedom at n_1 - 1 and n_2 - 1 for F-test, $^{+}$ degrees of freedom for t-test ($n_1 + n_2 - 2$), $^{++}$ degrees of freedom for t-test determined, as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

Table E6.2: Elemental mean and standard deviation values in human tear drops for smokers and non-smokers from Karbala, Iraq, (outliers omitted).

Element (n_1, n_2)	Mean ± SD Range (µg/l)		F-test			Two-tailed t-test			
	Smokers	Non-smokers	Variance	F_{calc}	Sig.	t_{calc}	*df*	Sig.	t_{crit}
B	581 ± 396	419 ± 229	Equal variances assumed	5.842	0.017	2.675	126[+]	0.008	2.051
(24, 104)	< 70 - 2020	< 70 - 1454	Unequal variances assumed			1.930	27[++]	0.064	
V	5.3 ± 4.6	5.1 ± 4.8	Equal variances assumed	0.165	0.685	0.129	153	0.898	1.976
(30, 125)	0.1 – 21.3	0.1 – 21.1	Unequal variances assumed			0.132	45	0.896	
Cr	12.4 ± 11.0	11.0 ± 10.4	Equal variances assumed	0.349	0.556	0.660	149	0.511	1.976
(28, 123)	0.2 – 40.9	0.3 – 47.1	Unequal variances assumed			0.635	39	0.529	
Mn	97.1 ± 99.4	51.0 ± 56.2	Equal variances assumed	14.098	< 0.001	3.310	146	0.001	2.039
(28, 120)	1.0 – 381.2	0.8 – 362.7	Unequal variances assumed			**2.367**	31	0.024	
Fe	599 ± 419	503 ± 490	Equal variances assumed	0.068	0.794	0.970	146	0.333	1.976
(29, 119)	4 - 1520	3 - 2060	Unequal variances assumed			1.068	48	0.291	
Cu	237 ± 178	253 ± 150	Equal variances assumed	0.666	0.416	0.498	153	0.619	1.976
(30, 125)	12 - 741	1 - 690	Unequal variances assumed			0.449	39	0.656	
Zn	1466 ± 1523	1147 ± 1119	Equal variances assumed	6.521	0.012	1.233	145	0.219	2.039
(26, 121)	82 - 5369	47 - 5726	Unequal variances assumed			1.012	31	0.319	
As	4.2 ± 3.6	7.2 ± 10.7	Equal variances assumed	10.786	0.001	1.457	150	0.147	1.987
(28, 124)	0.4 – 12.4	0.1 – 44.8	Unequal variances assumed			**2.544**	130	0.012	
Sr	632 ± 442	517 ± 380	Equal variances assumed	1.852	0.176	1.341	148	0.182	1.976
(25, 125)	7 - 1917	21 - 2361	Unequal variances assumed			1.213	31	0.234	
Cd	2.9 ± 2.4	1.8 ± 1.6	Equal variances assumed	14.253	< 0.001	3.204	148	0.002	2.028
(30, 120)	0.3 – 8.4	0.1 – 7.7	Unequal variances assumed			**2.528**	36	0.016	

SD is standard deviation, n_1, n_2 are the number of samples for smokers and non-smokers, respectively, *df* = degrees of freedom, n_1-1 and n_2-1 for F-test, [+] degrees of freedom for t-test (n_1+n_2-2), [++] degrees of freedom for t-test determined as described in Appendix C , *F* and *t* are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

Appendix E7

Interaction effect

Table E7.1: The mean values of healthy individuals and diabetic patients across smoking activity groups for Zn levels in tear drop samples from Karbala (n = 147).

Health status	Smoking activity	Mean*	95% Confidence Interval	
			Lower	Upper
healthy	smoker	1938.493	1199.628	2677.359
	non-smoker	853.508	587.790	1119.226
diabetic	smoker	1522.981	895.759	2150.202
	non-smoker	1959.557	1410.792	2508.322

* Adjusted mean value which is determined at the arithmetic mean value for age = 36 years and Zn level in drinking water = 69 µg/l.

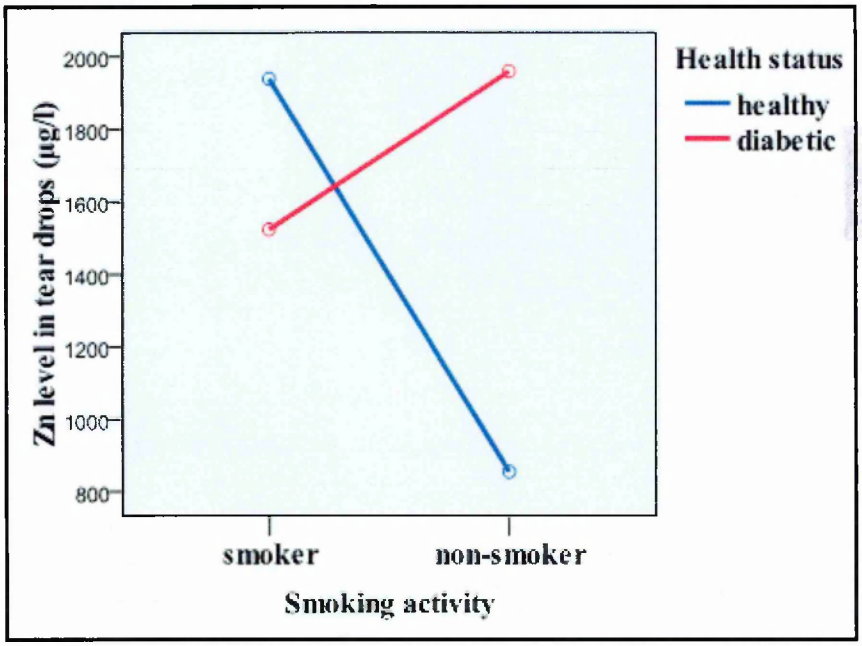

Figure E7.1: Interaction between health status and smoking activity for Zn levels (µg/l) in tear drop samples from Karbala (the data was taken from Table E7.1).

Appendix F

Human Saliva, Washed Scalp Hair and Fingernail Results

Appendix F1

Human Saliva Results:

Table F1.1: Description of human saliva samples (n = 97) and elemental levels for Iraqi individuals from Karbala (Iraq) and London (UK).

Sample description						Elemental level (µg/l)									
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-S-H-9/2009-1	H	M	38	NS	Karbala	163	0.20	0.60	2.15	24.9	14.1	58	0.51	66	0.12
K-S-H-9/2009-2	H	M	33	NS	Karbala	96	0.14	0.12	1.42	6.2	2.9	42	0.17	5	< 0.1
K-S-H-9/2009-3	H	M	33	S	Karbala	82	0.08	< 0.1	1.21	11.8	3.5	26	0.24	17	< 0.1
K-S-H-9/2009-4	H	M	19	NS	Karbala	489	1.47	0.78	11.45	110.5	62.9	180	2.16	415	0.32
K-S-H-9/2009-5	H	M	20	NS	Karbala	410	1.36	0.57	2.91	54.7	25.8	146	2.74	69	0.24
K-S-H-9/2009-6	H	M	45	NS	Karbala	167	0.25	0.33	20.24	9.9	26.3	70	2.33	154	0.47
K-S-H-9/2009-7	H	F	12	NS	Karbala	119	0.12	< 0.1	5.67	22.0	8.5	58	5.55	60	< 0.1
K-S-H-9/2009-8	H	F	14	NS	Karbala	213	0.48	0.17	4.35	28.5	11.5	52	4.01	86	0.14
K-S-H-9/2009-9	H	F	42	NS	Karbala	247	0.10	0.32	4.06	40.0	13.7	121	5.70	47	0.13
K-S-H-9/2009-10	H	M	35	NS	Karbala	369	0.58	< 0.1	2.72	66.1	11.8	112	6.00	203	0.16
K-S-H-9/2009-11	H	F	65	NS	Karbala	74	0.11	< 0.1	0.36	10.7	4.8	11	0.77	15	< 0.1
K-S-H-9/2009-12	H	M	40	NS	Karbala	169	0.14	< 0.1	4.67	20.2	13.6	92	1.40	39	0.11
K-S-H-9/2009-13	H	M	20	NS	Karbala	1254	1.79	0.82	7.79	107.9	27.6	402	7.61	1324	0.18
K-S-H-9/2009-14	H	M	23	NS	Karbala	37	0.17	< 0.1	0.43	8.6	9.7	14	0.17	11	0.12
K-S-H-9/2009-15	H	M	33	NS	Karbala	364	0.39	0.14	0.55	100.2	15.1	90	4.28	112	< 0.1
K-S-H-9/2009-16	H	M	41	NS	Karbala	153	0.34	0.33	7.69	31.3	28.9	172	0.56	46	0.76
K-S-H-9/2009-17	H	M	14	NS	Karbala	372	1.69	< 0.1	23.64	95.3	46.4	211	3.85	416	0.13
K-S-H-9/2009-18	H	F	37	NS	Karbala	< 70	0.06	0.01	1.60	9.7	8.0	28	2.67	17	< 0.1
K-S-H-9/2009-19	H	M	38	NS	Karbala	444	0.71	0.45	2.41	80.2	16.7	113	0.63	81	< 0.1
K-S-H-9/2009-20	H	F	33	NS	Karbala	124	0.09	0.11	2.48	8.8	14.5	29	0.37	27	< 0.1
K-S-H-9/2009-21	H	F	13	NS	Karbala	< 70	0.12	0.10	1.60	6.3	5.3	46	0.43	35	< 0.1
K-S-H-9/2009-22	H	M	42	N	Karbala	< 70	0.24	0.37	5.10	8.8	4.5	46	0.65	26	0.22
K-S-H-9/2009-23	H	M	37	NS	Karbala	236	0.48	0.39	2.48	32.0	13.7	86	2.15	14	< 0.1
K-S-H-9/2009-24	H	M	20	NS	Karbala	168	0.31	0.24	0.72	49.0	8.4	29	7.68	42	0.24
K-S-H-9/2009-25	H	M	42	NS	Karbala	198	0.83	0.20	6.08	70.3	13.4	103	8.85	163	0.22
K-S-H-9/2009-26	H	M	55	NS	Karbala	76	0.10	< 0.1	0.19	3.6	2.3	12	3.53	11	< 0.1
K-S-H-9/2009-27	H	M	43	NS	Karbala	173	0.22	0.08	1.22	8.9	4.9	23	3.26	69	< 0.1
K-S-H-9/2009-28	H	F	45	NS	Karbala	224	0.34	0.09	5.27	29.5	68.5	45	3.75	107	0.13

Table F1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-S-H-9/2009-29	H	M	44	NS	Karbala	256	0.80	0.24	1.44	7.4	6.0	55	1.80	171	< 0.1
K-S-H-9/2009-30	H	F	11	NS	Karbala	170	1.54	0.86	2.23	24.0	9.4	73	5.73	84	< 0.1
K-S-H-9/2009-31	H	F	60	NS	Karbala	126	0.38	0.08	0.47	3.1	2.4	28	1.83	44	< 0.1
K-S-H-9/2009-32	H	M	20	NS	Karbala	427	0.65	0.11	1.33	21.8	13.0	129	3.51	58	< 0.1
K-S-H-9/2009-33	H	F	40	S	Karbala	671	0.63	0.47	14.45	76.5	29.1	313	23.19	331	1.03
K-S-H-9/2009-34	H	M	40	S	Karbala	153	0.19	0.11	3.04	1.8	2.6	7	2.32	148	< 0.1
K-S-H-9/2009-35	H	M	43	NS	Karbala	153	0.25	0.15	0.45	9.3	4.8	13	2.97	18	0.14
K-S-H-9/2009-36	H	M	8	NS	Karbala	255	0.21	0.19	1.94	12.1	19.4	19	1.76	48	0.25
K-S-H-9/2009-37	H	F	19	NS	Karbala	90	0.15	0.17	0.52	4.7	7.0	15	0.40	16	0.21
K-S-H-9/2009-38	H	M	10	NS	Karbala	94	0.12	0.22	0.47	6.9	4.8	9	1.41	11	< 0.1
K-S-H-9/2009-39	H	M	42	NS	Karbala	125	0.10	0.16	0.59	4.9	6.8	21	0.70	37	0.18
K-S-H-9/2009-40	H	M	45	NS	Karbala	86	0.09	0.18	0.87	7.9	16.3	19	0.71	21	0.26
K-S-H-9/2009-41	H	F	34	NS	Karbala	77	0.12	0.18	0.33	9.3	6.4	8	0.64	15	0.19
K-S-H-9/2009-42	H	M	21	S	Karbala	166	0.13	0.10	1.08	14.3	6.5	30	0.47	18	0.26
K-S-H-9/2009-43	H	M	42	NS	Karbala	< 70	0.02	< 0.1	0.23	3.7	1.4	9	0.11	2	0.11
K-S-D-9/2009-44	D	F	30	NS	Karbala	310	0.52	0.24	13.51	31.5	20.4	233	2.12	164	0.31
K-S-D-9/2009-45	D	M	32	NS	Karbala	193	0.25	0.33	2.66	4.6	6.5	16	0.93	210	0.19
K-S-D-9/2009-46	D	F	51	S	Karbala	145	0.58	0.20	5.02	75.3	9.0	100	0.66	65	0.35
K-S-D-9/2009-47	D	M	46	NS	Karbala	241	0.35	0.21	7.97	5.5	8.4	10	0.15	176	< 0.1
K-S-D-9/2009-48	D	M	51	NS	Karbala	214	0.14	< 0.1	7.88	55.0	16.2	214	2.00	98	0.11
K-S-D-9/2009-49	D	F	52	S	Karbala	85	0.02	< 0.1	3.06	5.4	2.4	9	1.09	79	< 0.1
K-S-D-9/2009-50	D	M	43	S	Karbala	109	0.06	0.07	3.87	20.5	11.6	83	1.53	23	0.24
K-S-D-9/2009-51	D	F	50	NS	Karbala	309	0.16	< 0.1	13.03	6.6	12.6	45	1.51	354	< 0.1
K-S-D-9/2009-52	D	M	53	S	Karbala	99	0.13	< 0.1	1.97	20.9	41.2	65	0.73	58	< 0.1
K-S-D-9/2009-53	D	M	59	S	Karbala	130	0.15	0.10	1.81	12.7	9.9	130	1.12	65	< 0.1
K-S-D-9/2009-54	D	F	56	NS	Karbala	332	0.81	0.39	39.52	17.4	35.2	62	0.81	22	0.10
K-S-D-9/2009-55	D	M	45	NS	Karbala	272	1.06	0.79	20.20	131.4	28.1	89	2.74	272	< 0.1
K-S-D-9/2009-56	D	F	60	S	Karbala	223	0.74	0.86	11.94	60.5	15.8	108	0.80	87	< 0.1
K-S-D-9/2009-57	D	M	45	NS	Karbala	178	1.21	0.39	7.17	17.4	3.5	26	0.98	76	< 0.1
K-S-D-9/2009-58	D	F	55	S	Karbala	< 70	0.46	0.02	1.12	1.3	1.2	4	1.90	2545	< 0.1
K-S-D-9/2009-59	D	M	58	S	Karbala	321	0.08	0.09	3.41	12.4	5.8	52	0.30	12	0.37
K-S-D-9/2009-60	D	F	50	S	Karbala	322	0.53	0.17	25.00	4.9	12.1	288	1.42	368	< 0.1

Table F1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-S-D-9/2009-61	D	M	46	NS	Karbala	188	0.11	0.21	0.96	4.9	4.4	21	0.79	219	0.21
K-S-D-9/2009-62	D	F	52	S	Karbala	208	0.07	< 0.1	0.82	5.0	4.2	11	0.41	10	< 0.1
K-S-D-9/2009-63	D	F	74	S	Karbala	231	0.36	0.21	2.07	14.8	13.2	138	1.10	180	< 0.1
K-S-D-9/2009-64	D	M	57	NS	Karbala	244	0.54	0.26	8.44	18.6	13.8	78	0.77	34	< 0.1
K-S-D-9/2009-65	D	M	67	NS	Karbala	158	0.32	0.33	14.82	36.6	6.2	36	1.03	121	< 0.1
K-S-D-9/2009-66	D	M	54	NS	Karbala	114	0.04	0.18	5.90	11.6	10.2	23	0.87	44	< 0.1
K-S-D-9/2009-67	D	F	65	S	Karbala	218	0.39	0.13	22.62	29.8	9.4	47	1.41	150	< 0.1
K-S-D-9/2009-68	D	F	35	S	Karbala	260	0.52	0.29	5.50	23.6	12.5	54	0.90	37	< 0.1
K-S-D-9/2009-69	D	M	47	NS	Karbala	< 70	0.02	< 0.1	1.22	8.4	9.2	49	0.72	7	< 0.1
K-S-D-9/2009-70	D	M	43	NS	Karbala	90	0.25	0.07	0.51	4.0	6.0	20	0.21	4	0.16
K-S-D-9/2009-71	D	M	70	S	Karbala	291	0.14	0.18	1.73	7.2	20.4	38	2.24	9	< 0.1
K-S-D-9/2009-72	D	M	50	S	Karbala	138	0.13	0.08	1.69	14.5	8.4	54	0.38	31	< 0.1
L-S-H-9/2009-73	H	M	45	NS	London	78	0.46	0.53	0.45	17.9	60.0	1	0.51	44	1.01
L-S-H-9/2009-74	H	M	46	S	London	< 70	0.03	< 0.1	0.85	8.8	4.2	16	0.31	4	< 0.1
L-S-H-9/2009-75	H	F	42	NS	London	140	0.19	< 0.1	0.27	4.6	5.9	51	0.15	2	0.20
L-S-H-9/2009-76	H	M	39	NS	London	145	0.36	< 0.1	1.28	3.9	4.4	30	0.24	3	< 0.1
L-S-H-9/2009-77	H	F	34	NS	London	77	0.05	< 0.1	0.49	3.4	3.0	11	0.15	4	< 0.1
L-S-H-9/2009-78	H	M	45	NS	London	< 70	0.34	0.18	0.45	3.6	3.7	7	0.27	10	< 0.1
L-S-H-9/2009-79	H	F	16	NS	London	95	0.12	< 0.1	0.10	3.7	1.8	26	0.16	3	< 0.1
L-S-H-9/2009-80	H	F	40	NS	London	< 70	0.24	0.38	0.64	6.8	7.4	20	0.21	2	0.20
L-S-H-9/2009-81	H	M	41	NS	London	< 70	0.19	< 0.1	1.55	10.2	7.7	57	0.10	11	0.16
L-S-H-9/2009-82	H	M	23	NS	London	155	0.04	< 0.1	7.38	5.0	10.6	50	0.31	25	< 0.1
L-S-H-9/2009-83	H	F	56	NS	London	193	0.05	< 0.1	0.63	11.0	18.0	114	0.57	35	< 0.1
L-S-H-9/2009-84	H	M	38	NS	London	80	0.10	< 0.1	4.66	3.6	6.0	33	0.11	4	< 0.1
L-S-H-9/2009-85	H	M	3	NS	London	< 70	0.08	< 0.1	0.15	2.8	104.7	1	0.18	87	< 0.1
L-S-H-9/2009-86	H	F	40	NS	London	< 70	0.12	< 0.1	0.44	2.8	19.5	8	0.12	114	< 0.1
L-S-H-9/2009-87	H	M	3	NS	London	< 70	0.03	< 0.1	0.14	1.4	43.3	1	0.13	102	< 0.1
L-S-H-9/2009-88	H	M	6	NS	London	< 70	0.09	< 0.1	0.20	0.7	9.8	7	0.11	113	< 0.1
L-S-H-9/2009-89	H	M	9	NS	London	74	0.10	0.16	0.52	11.3	40.8	20	0.11	14	< 0.1
L-S-H-9/2009-90	H	F	11	NS	London	< 70	0.03	< 0.1	0.88	7.1	5.3	12	0.11	11	0.15
L-S-H-9/2009-91	H	M	42	NS	London	86	0.04	< 0.1	0.91	14.0	6.5	14	0.11	15	0.15
L-S-H-9/2009-92	H	F	10	NS	Karbala	69	0.03	< 0.1	2.33	21.9	11.0	23	0.11	10	0.15

Table F1.1 (continued)

Sample description						Elemental level (µg/l)									
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
L-S-H-9/2009-93	H	F	33	NS	London	< 70	0.04	< 0.1	0.52	11.5	6.4	23	0.11	11	0.15
L-S-H-9/2009-94	H	M	48	NS	London	134	0.05	< 0.1	0.67	10.1	17.9	72	0.21	46	0.22
L-S-H-9/2009-95	H	M	40	NS	London	575	0.94	0.14	2.13	34.7	19.4	178	2.47	45	< 0.1
L-S-H-9/2009-96	H	F	38	NS	London	< 70	0.03	< 0.1	3.15	8.0	23.0	48	0.51	17	0.21
L-S-H-9/2009-97	H	M	16	NS	London	160	0.18	0.36	3.68	26.3	171.0	95	1.72	9	0.13

HS = health status, H = healthy, M = male, F = female, S = smoker, NS = non-smoker, y = year, K-S-H-9/2009-1, K corresponds to the province in Iraq (K) Karbala and may be replaced by (L) London in the UK; S corresponds to saliva, H corresponds to healthy and may be replaced by D (diabetes); 9/2009 corresponds to the date (month/year); and 1 corresponds to the sample code number.

Appendix F1

Paired Sample Results:

Table F1.2: Paired tear drops and saliva samples (n = 42) for healthy individuals from Karbala (Iraq).

Sample description						Elemental level (µg/l)									
						Tear drop					Saliva				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TS1	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	364	0.39	0.14	0.55	100.2
TS2	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	410	1.36	0.57	2.91	54.7
TS3	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	168	0.31	0.24	0.72	49.1
TS4	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	489	1.47	0.78	11.45	110.5
TS5	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	86	0.09	0.18	0.87	7.9
TS6	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	163	0.23	0.63	2.15	24.9
TS7	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	166	0.13	0.10	1.08	14.3
TS8	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	671	0.63	0.47	14.45	76.5
TS9	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	125	0.12	0.16	0.59	4.9
TS10	H	M	20	NS	Karbala	146	1.68	3.62	1.95	251	427	0.65	0.11	1.33	21.8
TS11	H	M	42	NS	Karbala	616	3.43	7.07	19.62	335	198	0.83	0.24	6.08	70.3
TS12	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	1254	1.79	0.82	7.79	107.9
TS13	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	255	0.21	0.19	1.94	12.1
TS14	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	94	0.12	0.22	0.47	6.9
TS15	H	M	38	NS	Karbala	< 70	3.71	17.8	38.95	674	444	0.71	0.45	2.41	80.2
TS16	H	M	40	NS	Karbala	252	2.75	6.2	22.98	465	169	0.14	< 0.1	4.67	20.2
TS17	H	M	40	S	Karbala	< 70	3.7	9.13	35.83	427	153	0.19	0.11	3.04	1.8
TS18	H	M	23	NS	Karbala	446	21.08	31.17	270.08	2816	37	0.17	< 0.1	0.43	8.6
TS19	H	M	44	NS	Karbala	504	4.28	7.33	9.9	344	256	0.82	0.24	1.44	7.4
TS20	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	96	0.14	0.12	1.42	6.2
TS21	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	119	0.12	< 0.1	5.67	22.2
TS22	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	247	0.14	0.32	4.06	40.3
TS23	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	236	0.48	0.39	2.48	32.1
TS24	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	173	0.22	0.08	1.22	8.9
TS25	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	224	0.34	0.09	5.27	29.5
TS26	H	F	19	NS	Karbala	366	2.87	5.24	32.83	211	90	0.15	0.17	0.52	4.7

Table F1.2 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Saliva				
PIN	HS	Gender	Age (y)	Smoking	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TS27	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	213	0.48	0.17	4.35	28.5
TS28	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	124	0.09	0.11	2.48	8.8
TS29	H	M	33	S	Karbala	785	1.06	5.66	9.78	284	82	0.08	< 0.1	1.21	11.8
TS30	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	369	0.58	< 0.1	2.72	66.1
TS31	H	M	55	NS	Karbala	344	2.71	3.21	100.63	256	76	0.14	< 0.1	0.19	3.6
TS32	H	F	13	NS	Karbala	233	7.29	9.63	77.27	764	< 70	0.12	0.13	1.64	6.3
TS33	H	F	35	NS	Karbala	275	0.49	1.85	10.07	39	77	0.12	0.18	0.33	9.3
TS34	H	F	11	NS	Karbala	< 70	18.43	8.89	55.74	471	170	1.54	0.86	2.23	24
TS35	H	M	45	NS	Karbala	373	12.66	37.86	227.3	2396	167	0.25	0.33	20.24	9.9
TS36	H	F	37	NS	Karbala	522	5.55	15.84	23.44	852	< 70	0.06	0.01	1.63	9.7
TS37	H	F	65	NS	Karbala	415	1.72	4.16	14.88	261	74	0.11	< 0.1	0.36	10.7
TS38	H	F	60	S	Karbala	376	2.21	6.24	38.82	276	126	0.38	0.08	0.47	3.1
TS39	H	M	42	NS	Karbala	388	8.33	46.50	108.18	1484	< 70	0.02	< 0.1	0.23	3.7
TS40	H	M	42	NS	Karbala	404	5.90	14.18	52.51	379	< 70	0.24	0.37	5.12	8.8
TS41	H	M	41	NS	Karbala	265	10.98	14.29	92.13	399	153	0.34	0.33	7.69	31.3
TS42	H	M	43	NS	Karbala	440	5.40	42.59	62.94	152	153	0.25	0.15	0.45	9.3
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TS1	H	M	33	NS	Karbala	91	263	0.08	143	0.15	15.1	90	4.28	112	< 0.1
TS2	H	M	20	NS	Karbala	49	1175	33.94	689	0.16	25.8	146	2.74	69	0.24
TS3	H	M	20	NS	Karbala	254	1134	1.74	475	1.51	8.4	29	7.68	42	0.24
TS4	H	M	19	NS	Karbala	112	1327	14.89	248	0.31	62.9	180	2.16	415	0.32
TS5	H	M	45	NS	Karbala	79	49	0.69	104	0.15	16.3	19	0.71	21	0.26
TS6	H	M	38	NS	Karbala	148	352	1.82	425	0.58	14.1	58	0.51	66	0.12
TS7	H	M	21	S	Karbala	255	4100	9.56	1359	6.12	6.5	30	0.47	18	0.26
TS8	H	F	40	S	Karbala	26	126	1.22	464	0.29	29.1	313	23.79	331	1.03
TS9	H	M	42	NS	Karbala	427	494	1.34	58	0.34	6.8	21	0.70	37	0.18
TS10	H	M	20	NS	Karbala	75	78	37.73	134	0.61	13	129	3.51	58	< 0.1
TS11	H	M	42	NS	Karbala	280	355	2.11	246	1.26	13.4	103	8.85	163	0.22
TS12	H	M	20	NS	Karbala	244	753	2.67	552	1.43	27.6	402	7.61	1324	0.18
TS13	H	M	8	NS	Karbala	313	598	1.83	271	1.17	19.4	19	1.76	48	0.25
TS14	H	M	10	NS	Karbala	218	295	44.82	306	2.41	4.8	9	1.41	11	< 0.1

Table F1.2 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop					Saliva				
PIN	HS	Gender	Age (y)	Smoking	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TS15	H	M	38	NS	Karbala	170	386	3.54	534	1.55	16.7	113	0.63	81	< 0.1
TS16	H	M	40	NS	Karbala	198	224	1.42	262	0.36	13.6	92	1.44	39	0.11
TS17	H	M	40	S	Karbala	203	416	3.71	442	1.32	2.6	7	2.32	148	< 0.1
TS18	H	M	23	NS	Karbala	547	4109	6.52	461	5.98	9.7	14	0.17	11	0.12
TS19	H	M	44	NS	Karbala	196	460	0.48	140	0.75	6.3	55	1.81	171	< 0.1
TS20	H	M	33	NS	Karbala	589	1936	20.89	678	4.23	2.9	42	0.17	5	< 0.1
TS21	H	F	12	NS	Karbala	241	382	2.85	314	1.12	8.5	58	5.55	60	< 0.1
TS22	H	F	42	NS	Karbala	157	169	1.37	175	0.75	13.7	121	5.7	47	0.13
TS23	H	M	37	NS	Karbala	431	1022	5.15	587	1.35	13.7	86	2.15	14	< 0.1
TS24	H	M	43	NS	Karbala	318	277	2.67	345	0.57	4.9	23	3.26	69	< 0.1
TS25	H	F	45	NS	Karbala	260	495	1.59	388	0.21	68.5	45	3.75	107	0.13
TS26	H	F	19	NS	Karbala	182	466	1.84	491	0.63	7.4	15	0.4	16	0.21
TS27	H	F	14	NS	Karbala	436	404	1.54	513	0.91	11.5	52	4.01	86	0.14
TS28	H	F	33	NS	Karbala	102	169	1.68	118	0.48	14.5	29	0.37	27	< 0.1
TS29	H	M	33	S	Karbala	193	187	1.56	112	2.18	3.5	26	0.24	17	< 0.1
TS30	H	M	35	NS	Karbala	356	173	2.16	307	0.64	11.8	112	6.22	203	0.16
TS31	H	M	55	NS	Karbala	122	1391	3.91	682	1.27	2.3	12	3.53	11	< 0.1
TS32	H	F	13	NS	Karbala	222	1529	7.04	563	1.11	5.3	46	0.43	35	< 0.1
TS33	H	F	35	NS	Karbala	181	198	1.73	49	1.78	6.4	8	0.64	15	0.19
TS34	H	F	11	NS	Karbala	526	4104	34.09	794	2.49	9.4	73	5.73	84	< 0.1
TS35	H	M	45	NS	Karbala	273	4933	18.82	469	11.53	26.3	70	2.33	154	0.47
TS36	H	F	37	NS	Karbala	403	393	1.75	470	5.32	8.2	28	2.67	17	< 0.1
TS37	H	F	65	NS	Karbala	127	300	0.82	325	0.34	4.8	11	0.77	15	< 0.1
TS38	H	F	60	S	Karbala	220	1223	2.29	404	1.96	2.4	28	1.83	44	< 0.1
TS39	H	M	42	NS	Karbala	532	1517	8.82	589	3.31	1.4	9	0.11	2	0.11
TS40	H	M	42	NS	Karbala	298	1223	8.96	482	13.05	4.5	46	0.65	26	0.22
TS41	H	M	41	NS	Karbala	324	1462	4.42	830	6.21	28.9	172	0.56	46	0.76
TS42	H	M	43	NS	Karbala	421	1507	5.17	935	1.41	4.8	13	2.97	18	0.14

HS = health status, H = healthy, M = male, F = female, S = smoker, NS = non-smoker, y = year, TS1, T corresponds to tear drops, S corresponds to saliva; and 1 corresponds to the sample code number.

Appendix F1

Comparison Study:

Table F1.3: Summary of F-test and a two tailed t-test results for elemental levels in saliva samples of healthy and diabetic individuals from Karbala, Iraq.

Element (n_1, n_2)	F-test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	EVA	nd		nd			
(39, 27)	UVA						
V	EVA	2.602	0.111	0.764	70^{+}	0.448	
(43, 29)	UVA			0.826	70^{++}	0.412	
Cr	EVA	nd		nd			
(34, 23)	UVA						
Mn	EVA	7.861	0.007	2.626	70	0.011	
(43, 29)	UVA			2.368	40	**0.023**	2.02
Fe	EVA	1.983	0.164	0.904	70	0.369	
(43, 29)	UVA			0.929	66	0.356	
Cu	EVA	2.071	0.155	0.699	70	0.487	
(43, 29)	UVA			0.761	70	0.449	
Zn	EVA	0.283	0.596	0.058	70	0.954	
(43, 29)	UVA			0.060	66	0.952	
As	EVA	10.708	0.002	2.604	70	0.011	
(43, 29)	UVA			3.145	45	**0.003**	2.01
Sr	EVA	1.410	0.239	1.001	70	0.320	
(43, 29)	UVA			0.880	36	0.385	
Cd	EVA	nd		nd			
(25, 9)	UVA						

EVA and UVA are equal variances assumed and unequal variances assumed, nd = not determined due to there being several samples which were below the limit of detection (Table 2.17), n_1, n_2 are the number of samples for healthy individuals and diabetic patients, respectively, *df* = degrees of freedom, n_1-1 and n_2-1 for F-test, $^{+}$ degrees of freedom for t-test (n_1+n_2-2), $^{++}$ degrees of freedom for t-test determined as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

Table F1.4: Summary of F-test and a two tailed t-test results for elemental levels in saliva samples for individuals from the healthy population of Karbala and London.

Element (n_1, n_2)	F-Test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	EVA	nd		nd			
(39, 14)	UVA						
V	EVA	10.523	0.002	2.701	66^{+}	0.009	
(43, 25)	UVA			3.259	62^{++}	**0.002**	1.99
Cr	EVA	nd		nd			
(34, 6)	UVA						
Mn	EVA	7.449	0.008	2.219	66	0.030	
(43, 25)	UVA			2.754	56	**0.008**	2.00
Fe	EVA	22.001	0.000	3.080	66	0.003	
(43, 25)	UVA			3.908	51	**0.000**	2.01
Cu	EVA	7.991	0.006	1.532	66	0.130	
(43, 25)	UVA			1.251	28	0.221	
Zn	EVA	5.080	0.028	2.091	66	0.040	
(43, 25)	UVA			2.455	65	**0.017**	1.99
As	EVA	10.936	0.002	3.333	66	0.001	
(43, 25)	UVA			4.338	45	**0.000**	3.52
Sr	EVA	4.828	0.032	1.841	66	0.070	
(43, 25)	UVA			2.384	46	**0.021**	2.01
Cd	EVA	nd		nd			
(25, 11)	UVA						

n_1, n_2 are the number of samples for Karbala and London, respectively. Other key words can take from Table F1.3.

Table F1.5: Summary of F-test and a two tailed t-test results for elemental levels in tear drops and saliva for individuals from the healthy population of Karbala who provided both media.

TE	Mean* (µg/l) (T, S)	F-Test for equality of variances			t-test for equality of means			
		Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	(426, 234)	EVA	0.453	0.50	4.249	71	**0.000**	1.99
		UVA			4.303	67.711	0.000	
V	(5.0, 0.4)	EVA	41.409	0.00	6.091	82	0.000	
		UVA			6.091	41.637	**0.000**	2.02
Cr	(14.2, 0.3)	EVA	33.869	0.00	4.956	74	0.000	
		UVA			5.515	41.019	**0.000**	2.02
Mn	(45.81, 3.24)	EVA	26.496	0.00	5.019	82	0.000	
		UVA			5.019	41.454	**0.000**	2.02
Fe	(586, 28)	EVA	43.228	0.00	5.789	82	0.000	
		UVA			5.789	41.194	**0.000**	2.02
Cu	(257.1, 13.7)	EVA	60.222	0.00	11.005	82	0.000	
		UVA			11.005	41.792	**0.000**	2.02
Zn	(1004, 70)	EVA	35.709	0.00	5.023	82	0.000	
		UVA			5.023	41.367	**0.000**	2.02
As	(7.4, 3.0)	EVA	14.971	0.00	2.431	82	0.017	
		UVA			2.431	51.651	**0.019**	2.44
Sr	(427, 102)	EVA	5.878	0.02	6.265	82	0.000	
		UVA			6.265	78.465	**0.000**	1.99
Cd	(2.1, 0.3)	EVA	16.090	0.00	3.205	64	0.002	
		UVA			4.239	41.836	**0.000**	2.02

* n = 42, the only exception are for B in tear drops (n = 35) and saliva (n = 38); Cr in saliva (n = 34); and Cd in saliva (n = 24), TE is trace element.

Appendix F2

Washed Scalp Hair Results

Table F2.1: Typical operating conditions for a Finnigan MAT Sola ICP-MS instrument.

Parameter	Typical operating conditions
Plasma argon flow rate	16 l/min
Auxiliary argon flow rate	1.2 l/min
Nebuliser argon flow rate	0.8 l/min
Incident power	1400 W
Reflected power	5 W
Nebuliser pressure	2.0 bar
Sample orifice (nickel)	1.1 mm
Skimmer orifice (nickel)	0.7 mm
Spray chamber temp.	2 °C
Cooling water temp.	16 °C
Pump speed	10.2 rpm
Isotopes – internal standard solution (100 µg/L)	$^{9}Be^{+}$, $^{74}Ge^{+}$, $^{115}In^{+}$ and $^{209}Bi^{+}$

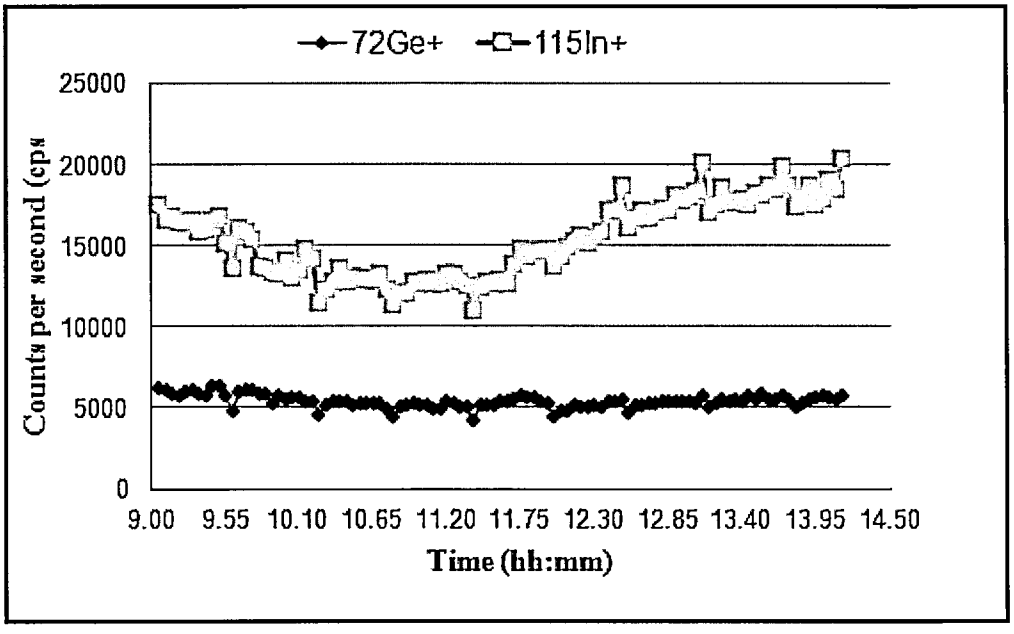

Figure F2.1: Typical long term-stability during the analysis of scalp hair using a 100 µg/l of ^{72}Ge and ^{115}In as an internal standard solution for multi-element analysis by the Finnigan MAT Sola ICP-MS instrument.

Washed Scalp Hair Results:

Table F2.2: Description of washed scalp samples (n = 265) and elemental levels for Iraqi individuals from Karbala (Iraq) and London (UK).

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-1	H	M	35	S	Karbala	4.54	0.050	0.100	0.280	5.00	8.90	143	< 0.005	5.77	0.150
K-H-H-9/2009-2	H	M	29	NS	Karbala	4.93	0.040	0.090	0.450	6.30	6.60	78	0.039	1.16	0.080
K-H-H-9/2009-3	H	M	29	S	Karbala	< 3.5	0.110	0.320	0.420	5.30	6.90	166	0.040	4.70	0.240
K-H-H-9/2009-4	H	M	32	S	Karbala	< 3.5	0.320	0.550	3.170	60.30	8.20	135	0.103	10.89	0.170
K-H-H-9/2009-5	H	M	20	NS	Karbala	< 3.5	0.170	0.310	1.100	21.20	9.80	117	0.059	3.79	0.380
K-H-H-9/2009-6	H	M	33	NS	Karbala	< 3.5	0.140	0.260	0.970	25.40	7.10	79	0.048	2.59	0.140
K-H-H-9/2009-7	H	M	62	S	Karbala	9.04	0.210	0.380	2.590	29.10	5.70	115	0.033	7.89	0.180
K-H-H-9/2009-8	H	M	24	S	Karbala	< 3.5	0.530	0.290	1.440	9.90	9.00	162	0.013	10.92	0.190
K-H-H-9/2009-9	H	M	23	S	Karbala	< 3.5	0.150	0.230	0.970	19.40	6.10	107	0.035	4.03	0.080
K-H-H-9/2009-10	H	M	20	NS	Karbala	6.08	0.090	0.040	0.420	5.00	10.10	102	0.052	2.72	0.070
K-H-H-9/2009-11	H	M	26	S	Karbala	3.12	0.080	0.100	0.400	5.60	6.50	133	0.012	2.57	0.090
K-H-H-9/2009-12	H	M	19	NS	Karbala	3.63	0.100	0.190	0.520	10.90	6.00	156	0.023	4.41	0.080
K-H-H-9/2009-13	H	M	37	S	Karbala	241.76	0.050	0.020	0.280	4.40	4.30	114	0.023	5.25	0.090
K-H-H-9/2009-14	H	M	22	NS	Karbala	< 3.5	0.180	0.190	0.820	37.00	27.90	99	0.033	3.08	0.570
K-H-H-9/2009-15	H	M	45	S	Karbala	< 3.5	0.180	0.300	1.500	27.60	8.80	148	0.055	6.62	0.160
K-H-H-9/2009-16	H	M	30	NS	Karbala	< 3.5	0.240	0.440	1.680	86.60	9.30	133	0.069	4.25	0.160
K-H-H-9/2009-17	H	M	45	NS	Karbala	< 3.5	0.120	0.160	0.780	20.00	10.70	128	0.029	3.14	0.620
K-H-H-9/2009-18	H	M	38	NS	Karbala	< 3.5	0.090	0.040	0.530	8.10	6.60	78	0.055	1.50	0.200
K-H-H-9/2009-19	H	M	26	NS	Karbala	4.34	0.130	0.290	1.050	16.80	7.70	110	0.051	4.68	0.170
K-H-H-9/2009-20	H	M	20	S	Karbala	< 3.5	0.210	0.130	0.620	3.20	4.00	76	< 0.005	15.21	0.090
K-H-H-9/2009-21	H	M	25	NS	Karbala	< 3.5	0.160	0.310	1.610	22.50	6.40	147	0.051	7.08	0.130
K-H-H-9/2009-22	H	M	27	NS	Karbala	< 3.5	0.260	0.430	1.690	28.70	12.70	156	0.046	5.33	0.490
K-H-H-9/2009-23	H	M	19	NS	Karbala	< 3.5	0.030	0.030	0.290	3.60	6.80	79	0.054	1.79	0.100
K-H-H-9/2009-24	H	M	40	S	Karbala	< 3.5	0.260	0.380	1.940	20.70	7.20	133	0.035	7.09	0.280
K-H-H-9/2009-25	H	M	51	NS	Karbala	< 3.5	0.130	0.290	2.770	6.70	5.40	275	< 0.005	13.02	0.350
K-H-H-9/2009-26	H	M	32	NS	Karbala	< 3.5	0.050	0.040	0.570	6.70	7.10	232	0.074	3.47	0.060
K-H-H-9/2009-27	H	M	21	S	Karbala	< 3.5	0.220	0.060	0.710	4.40	6.90	144	< 0.005	11.86	0.100
K-H-H-9/2009-28	H	M	25	NS	Karbala	3.91	0.100	0.110	0.950	15.40	6.30	111	0.050	3.49	0.100

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-29	H	M	42	NS	Karbala	< 3.5	0.060	0.050	0.380	7.40	6.60	45	0.032	1.16	0.070
K-H-H-9/2009-30	H	M	20	NS	Karbala	9.11	0.560	1.270	2.910	19.00	17.60	403	0.094	13.19	0.300
K-H-H-9/2009-31	H	M	29	NS	Karbala	< 3.5	0.180	0.350	1.520	46.60	5.90	97	0.042	4.76	0.070
K-H-H-9/2009-32	H	M	51	S	Karbala	6.33	0.210	0.500	0.970	16.00	26.60	87	< 0.005	8.22	0.150
K-H-H-9/2009-33	H	M	30	NS	Karbala	< 3.5	0.080	0.200	0.530	12.50	8.00	150	0.011	2.86	0.060
K-H-H-9/2009-34	H	M	18	NS	Karbala	< 3.5	0.040	0.080	0.290	5.00	7.00	104	0.004	1.01	0.050
K-H-H-9/2009-35	H	M	42	NS	Karbala	< 3.5	0.070	0.050	0.410	6.40	6.30	126	< 0.005	3.12	0.060
K-H-H-9/2009-36	H	M	20	NS	Karbala	< 3.5	0.090	0.090	0.540	12.10	5.40	67	0.010	2.52	0.020
K-H-H-9/2009-37	H	M	8	NS	Karbala	< 3.5	0.100	0.100	0.600	12.20	5.90	126	0.008	2.53	0.060
K-H-H-9/2009-38	H	M	51	S	Karbala	< 3.5	0.100	0.060	0.670	13.00	5.50	95	0.049	3.82	0.060
K-H-H-9/2009-39	H	M	44	NS	Karbala	< 3.5	0.080	0.170	0.400	12.80	5.80	136	0.046	2.56	0.070
K-H-H-9/2009-40	H	M	19	NS	Karbala	< 3.5	0.140	0.290	0.640	10.70	5.00	95	0.067	1.46	0.070
K-H-H-9/2009-41	H	M	10	NS	Karbala	< 3.5	0.180	0.290	0.820	13.30	5.70	141	0.079	0.95	0.750
K-H-H-9/2009-42	H	M	31	NS	Karbala	< 3.5	0.150	0.190	0.440	3.60	7.20	86	0.035	3.73	0.080
K-H-H-9/2009-43	H	M	28	NS	Karbala	< 3.5	0.060	0.130	0.730	10.90	6.50	127	0.004	5.46	1.780
K-H-H-9/2009-44	H	M	32	S	Karbala	< 3.5	0.270	0.360	2.070	23.60	6.30	137	< 0.005	18.51	0.160
K-H-H-9/2009-45	H	M	16	NS	Karbala	< 3.5	0.050	0.250	0.320	5.80	5.40	129	0.005	2.80	0.090
K-H-H-9/2009-46	H	M	26	S	Karbala	< 3.5	0.190	0.070	1.340	5.30	7.10	196	< 0.005	25.35	0.100
K-H-H-9/2009-47	H	M	29	NS	Karbala	< 3.5	0.100	0.110	0.560	13.20	6.20	121	< 0.005	4.50	0.070
K-H-H-9/2009-48	H	M	4	NS	Karbala	< 3.5	0.070	0.110	0.630	8.30	7.50	133	0.091	2.29	0.170
K-H-H-9/2009-49	H	M	29	NS	Karbala	< 3.5	0.230	0.130	1.350	18.60	8.00	133	0.018	2.22	0.090
K-H-H-9/2009-50	H	M	30	NS	Karbala	< 3.5	0.150	0.190	2.120	23.00	3.00	95	0.049	4.17	0.090
K-H-H-9/2009-51	H	M	38	NS	Karbala	< 3.5	0.030	< 0.005	0.160	2.90	5.50	138	0.010	1.31	0.140
K-H-H-9/2009-52	H	M	26	NS	Karbala	< 3.5	0.360	0.410	0.520	92.60	6.70	94	0.078	2.64	0.440
K-H-H-9/2009-53	H	M	32	NS	Karbala	< 3.5	0.060	< 0.005	0.400	2.50	5.60	50	< 0.005	1.83	0.180
K-H-H-9/2009-54	H	M	17	NS	Karbala	< 3.5	0.100	0.170	0.390	5.30	7.10	143	0.039	2.77	0.880
K-H-H-9/2009-55	H	M	58	S	Karbala	< 3.5	0.100	0.010	0.420	5.70	5.60	127	0.062	4.03	0.060
K-H-H-9/2009-56	H	M	5	NS	Karbala	< 3.5	0.150	0.140	1.120	13.50	6.80	158	0.003	14.67	0.090
K-H-H-9/2009-57	H	M	18	NS	Karbala	< 3.5	0.050	< 0.005	0.150	4.10	3.90	109	0.035	2.81	0.070
K-H-H-9/2009-58	H	M	5	NS	Karbala	6.49	0.180	0.360	0.870	18.30	15.90	176	0.112	2.04	0.770
K-H-H-9/2009-59	H	M	35	S	Karbala	< 3.5	0.030	< 0.005	0.350	1.90	2.00	76	< 0.005	2.61	0.050
K-H-H-9/2009-60	H	M	28	S	Karbala	< 3.5	0.100	0.070	0.420	8.80	4.10	123	0.054	2.86	0.080

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-61	H	M	25	S	Karbala	< 3.5	0.250	0.270	0.970	7.70	8.30	143	0.005	12.75	0.190
K-H-H-9/2009-62	H	M	14	NS	Karbala	< 3.5	0.170	0.180	0.450	9.00	7.90	150	0.030	0.99	0.080
K-H-H-9/2009-63	H	M	34	S	Karbala	< 3.5	0.060	0.130	0.600	6.40	8.20	133	0.012	3.93	0.160
K-H-H-9/2009-64	H	M	35	S	Karbala	< 3.5	0.030	< 0.005	0.190	3.00	5.60	109	< 0.005	1.81	0.350
K-H-H-9/2009-65	H	M	51	NS	Karbala	< 3.5	0.070	0.010	0.310	2.70	6.70	129	< 0.005	10.42	0.060
K-H-H-9/2009-66	H	M	30	S	Karbala	< 3.5	0.050	< 0.005	0.650	13.30	5.20	87	0.020	2.02	0.110
K-H-H-9/2009-67	H	M	17	NS	Karbala	< 3.5	0.330	0.160	2.600	78.70	5.10	102	0.186	1.96	0.180
K-H-H-9/2009-68	H	M	58	NS	Karbala	< 3.5	0.160	0.130	1.010	19.60	4.30	56	0.032	2.33	0.130
K-H-H-9/2009-69	H	M	9	NS	Karbala	< 3.5	0.060	< 0.005	0.310	4.80	6.90	102	0.005	2.09	0.240
K-H-H-9/2009-70	H	M	40	NS	Karbala	< 3.5	0.200	0.130	0.990	16.00	8.30	133	0.031	1.16	0.070
K-H-H-9/2009-71	H	M	13	NS	Karbala	< 3.5	0.070	0.210	0.400	5.70	4.50	91	0.008	2.09	0.470
K-H-H-9/2009-72	H	M	22	S	Karbala	< 3.5	0.200	0.220	1.320	20.10	3.30	68	0.038	3.48	0.170
K-H-H-9/2009-73	H	M	24	NS	Karbala	86.21	0.080	0.090	0.760	11.90	3.90	152	< 0.005	5.90	0.180
K-H-H-9/2009-74	H	M	40	S	Karbala	< 3.5	0.040	0.060	0.230	4.00	6.50	139	< 0.005	8.48	0.560
K-H-H-9/2009-75	H	M	19	NS	Karbala	< 3.5	0.050	0.030	0.440	4.10	4.20	88	0.005	1.91	0.200
K-H-H-9/2009-76	H	M	18	NS	Karbala	< 3.5	0.220	0.060	0.590	12.90	7.40	141	0.016	2.44	0.060
K-H-H-9/2009-77	H	M	4	NS	Karbala	< 3.5	0.170	0.120	0.680	17.00	5.10	82	0.015	1.61	0.050
K-H-H-9/2009-78	H	M	24	NS	Karbala	< 3.5	0.040	0.090	0.290	3.60	6.60	123	< 0.005	3.98	0.080
K-H-H-9/2009-79	H	M	28	S	Karbala	< 3.5	0.120	< 0.005	0.370	3.60	3.00	77	0.035	1.03	0.060
K-H-H-9/2009-80	H	M	7	NS	Karbala	< 3.5	0.100	0.060	0.340	7.90	3.70	140	0.013	2.26	0.040
K-H-H-9/2009-81	H	M	5	NS	Karbala	121.68	0.070	0.090	0.190	2.70	5.90	84	0.005	1.66	0.130
K-H-H-9/2009-82	H	M	37	S	Karbala	< 3.5	0.080	0.080	0.440	5.70	4.30	62	0.030	1.40	0.070
K-H-H-9/2009-83	H	M	51	NS	Karbala	5.67	0.190	0.250	0.690	25.20	4.70	78	< 0.005	2.04	0.120
K-H-H-9/2009-84	H	M	57	S	Karbala	< 3.5	0.080	0.370	0.640	10.40	5.00	72	0.050	1.71	0.910
K-H-H-9/2009-85	H	M	31	NS	Karbala	< 3.5	0.040	< 0.005	0.260	4.30	4.40	95	< 0.005	2.07	0.030
K-H-H-9/2009-86	H	M	30	NS	Karbala	< 3.5	0.130	0.130	0.850	26.80	6.60	133	0.021	1.34	0.050
K-H-H-9/2009-87	H	M	16	NS	Karbala	< 3.5	0.050	< 0.005	0.290	4.50	7.10	142	< 0.005	9.07	0.650
K-H-H-9/2009-88	H	M	19	NS	Karbala	< 3.5	0.130	0.080	0.620	16.80	4.70	115	0.032	1.92	0.060
K-H-H-9/2009-89	H	M	5	NS	Karbala	< 3.5	0.060	< 0.005	0.230	4.40	4.20	95	< 0.005	1.27	0.140
K-H-H-9/2009-90	H	M	5	NS	Karbala	< 3.5	0.040	< 0.005	0.380	5.10	4.40	128	< 0.005	5.29	0.100
K-H-H-9/2009-91	H	M	50	NS	Karbala	< 3.5	0.210	0.220	0.860	19.90	5.90	175	0.002	2.81	0.360
K-H-H-9/2009-92	H	M	4	NS	Karbala	< 3.5	0.110	0.070	0.520	13.80	4.50	86	0.011	1.37	0.060

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-93	H	M	3	NS	Karbala	< 3.5	0.050	< 0.005	0.430	4.20	2.90	89	0.006	3.30	0.060
K-H-H-9/2009-94	H	M	18	NS	Karbala	< 3.5	0.100	0.200	0.650	15.40	6.00	123	0.014	5.29	0.150
K-H-H-9/2009-95	H	M	34	NS	Karbala	< 3.5	0.200	0.190	0.510	14.10	4.50	36	0.050	1.29	0.070
K-H-H-9/2009-96	H	M	2	NS	Karbala	< 3.5	0.570	0.260	2.120	9.70	6.10	138	< 0.005	11.00	0.210
K-H-H-9/2009-97	H	M	25	S	Karbala	< 3.5	0.100	0.070	0.660	7.40	4.80	74	0.010	4.30	0.100
K-H-H-9/2009-98	H	M	17	NS	Karbala	< 3.5	0.200	0.180	1.400	32.30	4.00	93	0.022	3.17	0.080
K-H-H-9/2009-99	H	M	17	NS	Karbala	< 3.5	0.440	0.650	2.630	77.20	10.20	271	0.096	7.65	0.180
K-H-H-9/2009-100	H	M	15	NS	Karbala	< 3.5	0.230	0.240	1.180	34.30	5.80	115	0.026	2.07	0.090
K-H-H-9/2009-101	H	M	19	NS	Karbala	< 3.5	0.060	< 0.005	0.220	4.50	5.10	87	< 0.005	1.86	0.170
K-H-H-9/2009-102	H	M	30	NS	Karbala	< 3.5	0.090	0.030	0.400	7.60	4.40	64	0.004	3.82	0.240
K-H-H-9/2009-103	H	M	18	NS	Karbala	< 3.5	0.060	< 0.005	0.300	4.60	5.20	111	0.008	1.32	0.120
K-H-H-9/2009-104	H	M	12	NS	Karbala	< 3.5	0.040	< 0.005	0.250	4.80	3.30	125	0.008	2.26	0.090
K-H-H-9/2009-105	H	M	23	NS	Karbala	< 3.5	0.220	0.300	1.340	17.00	4.90	134	< 0.005	4.68	0.110
K-H-H-9/2009-106	H	M	17	NS	Karbala	< 3.5	0.040	< 0.005	0.220	4.60	3.40	135	0.009	2.56	0.080
K-H-H-9/2009-107	H	M	21	NS	Karbala	< 3.5	0.200	0.140	1.000	10.80	6.20	84	0.024	4.54	0.270
K-H-H-9/2009-108	H	M	20	NS	Karbala	< 3.5	0.070	0.060	0.630	12.90	2.60	98	< 0.005	3.66	0.120
K-H-H-9/2009-109	H	M	51	S	Karbala	< 3.5	0.040	< 0.005	0.140	3.10	2.30	76	< 0.005	0.64	0.050
K-H-H-9/2009-110	H	M	9	NS	Karbala	< 3.5	0.280	0.560	1.400	52.00	4.50	109	0.006	3.04	0.080
K-H-H-9/2009-111	H	M	12	NS	Karbala	< 3.5	0.150	0.320	0.660	24.60	6.20	65	0.017	1.51	0.080
K-H-H-9/2009-112	H	M	52	NS	Karbala	< 3.5	0.340	0.310	1.940	31.00	5.60	150	0.069	5.55	0.200
K-H-H-9/2009-113	H	M	54	S	Karbala	< 3.5	0.100	0.050	0.740	12.10	4.80	77	0.041	1.31	0.230
K-H-H-9/2009-114	H	M	5	NS	Karbala	8.08	0.130	0.120	0.660	4.10	4.30	140	0.053	4.04	0.220
K-H-H-9/2009-115	H	M	12	NS	Karbala	< 3.5	0.160	0.230	0.590	15.50	5.40	116	0.011	2.70	0.100
K-H-H-9/2009-116	H	M	10	NS	Karbala	< 3.5	0.150	0.110	1.060	15.60	4.70	83	0.004	4.06	0.090
K-H-H-9/2009-117	H	M	30	S	Karbala	< 3.5	0.040	0.060	0.280	5.60	5.40	80	0.019	0.82	0.050
K-H-H-9/2009-118	H	M	60	S	Karbala	< 3.5	0.120	0.320	0.370	10.00	4.40	84	0.010	1.76	0.040
K-H-H-9/2009-119	H	M	44	NS	Karbala	< 3.5	0.610	0.390	1.070	10.70	6.70	161	0.002	17.81	0.130
K-H-H-9/2009-120	H	M	20	NS	Karbala	< 3.5	0.100	0.010	0.310	6.40	4.70	71	0.035	1.78	0.330
K-H-H-9/2009-121	H	M	33	NS	Karbala	< 3.5	0.040	< 0.005	0.140	2.90	2.80	89	< 0.005	0.84	0.040
K-H-H-9/2009-122	H	M	6	NS	Karbala	< 3.5	0.090	< 0.005	0.310	6.40	9.40	56	0.053	0.65	0.040
K-H-H-9/2009-123	H	M	5	NS	Karbala	< 3.5	0.240	0.150	0.780	17.40	3.60	66	0.043	1.20	0.060
K-H-H-9/2009-124	H	M	2	NS	Karbala	< 3.5	0.180	0.250	0.560	11.10	5.00	140	0.035	2.72	0.110

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-125	H	M	6	NS	Karbala	< 3.5	0.110	0.010	0.640	6.50	5.90	87	0.079	1.66	0.090
K-H-H-9/2009-126	H	M	5	NS	Karbala	< 3.5	0.120	0.030	0.490	9.40	4.80	48	0.072	1.18	0.140
K-H-H-9/2009-127	H	F	13	NS	Karbala	< 3.5	0.210	0.100	0.590	3.90	8.20	205	0.075	28.38	0.320
K-H-H-9/2009-128	H	F	12	NS	Karbala	< 3.5	0.300	0.130	0.780	3.70	6.70	150	0.019	12.08	0.380
K-H-H-9/2009-129	H	F	42	NS	Karbala	< 3.5	0.190	0.030	2.240	1.90	5.80	140	< 0.005	39.56	0.790
K-H-H-9/2009-130	H	F	20	NS	Karbala	< 3.5	0.310	0.100	1.120	4.90	8.40	326	< 0.005	27.78	0.250
K-H-H-9/2009-131	H	F	21	NS	Karbala	< 3.5	0.530	0.140	1.010	3.90	8.90	298	0.072	15.84	0.160
K-H-H-9/2009-132	H	M	19	NS	Karbala	< 3.5	0.400	0.060	0.670	11.50	7.80	167	< 0.005	11.63	3.120
K-H-H-9/2009-133	H	F	20	NS	Karbala	< 3.5	0.180	< 0.005	0.410	1.80	6.00	77	< 0.005	23.17	0.300
K-H-H-9/2009-134	H	M	37	NS	Karbala	< 3.5	0.240	0.330	0.950	31.50	5.70	65	0.086	1.04	0.090
K-H-H-9/2009-135	H	M	15	NS	Karbala	< 3.5	0.230	0.250	1.010	29.40	7.20	74	0.070	1.93	0.120
K-H-H-9/2009-136	H	M	43	NS	Karbala	< 3.5	0.420	0.240	1.360	15.10	5.20	114	< 0.005	7.35	0.160
K-H-H-9/2009-137	H	F	8	NS	Karbala	< 3.5	0.230	0.080	0.770	4.40	4.20	411	< 0.005	7.34	0.580
K-H-H-9/2009-138	H	F	11	NS	Karbala	< 3.5	0.290	0.090	1.050	4.30	4.90	174	< 0.005	9.63	0.460
K-H-H-9/2009-139	H	F	40	S	Karbala	< 3.5	0.120	0.020	1.560	3.10	5.80	65	< 0.005	49.05	2.050
K-H-H-9/2009-140	H	M	31	S	Karbala	< 3.5	0.100	0.230	0.590	14.80	3.70	108	< 0.005	7.83	0.080
K-H-H-9/2009-141	H	F	8	NS	Karbala	< 3.5	0.570	0.230	1.870	24.60	7.80	303	< 0.005	9.71	0.220
K-H-H-9/2009-142	H	F	8	NS	Karbala	< 3.5	0.370	0.100	1.070	10.60	5.40	288	< 0.005	13.52	0.150
K-H-H-9/2009-143	H	F	7	NS	Karbala	< 3.5	0.300	0.080	0.720	5.60	3.10	602	< 0.005	14.85	0.300
K-H-H-9/2009-144	H	M	8	NS	Karbala	< 3.5	0.320	0.030	0.720	3.70	2.20	320	< 0.005	9.11	0.330
K-H-H-9/2009-145	H	F	14	NS	Karbala	< 3.5	0.330	0.080	0.760	11.40	2.90	434	< 0.005	14.00	0.550
K-H-H-9/2009-146	H	M	11	NS	Karbala	< 3.5	0.340	0.050	0.830	7.00	2.20	433	< 0.005	10.22	0.220
K-H-H-9/2009-147	H	F	4	NS	Karbala	< 3.5	0.230	0.040	0.460	6.10	8.60	159	0.004	4.10	0.110
K-H-H-9/2009-148	H	F	35	NS	Karbala	< 3.5	0.370	0.070	1.320	6.50	5.70	320	< 0.005	37.62	0.080
K-H-H-9/2009-149	H	F	33	NS	Karbala	< 3.5	0.240	0.020	0.950	5.30	3.70	432	< 0.005	24.91	0.130
K-H-H-9/2009-150	H	F	31	NS	Karbala	< 3.5	0.140	0.020	0.550	2.90	2.60	192	< 0.005	11.23	0.690
K-H-H-9/2009-151	H	F	8	NS	Karbala	< 3.5	0.320	0.290	0.990	16.70	5.80	372	< 0.005	14.94	0.260
K-H-H-9/2009-152	H	M	25	S	Karbala	< 3.5	0.150	0.050	0.730	7.10	6.50	124	< 0.005	6.47	0.140
K-H-H-9/2009-153	H	F	11	NS	Karbala	< 3.5	0.740	0.330	1.690	11.30	14.70	196	0.041	24.85	0.860
K-H-H-9/2009-154	H	M	45	NS	Karbala	< 3.5	0.090	0.100	0.370	12.30	4.90	86	0.039	1.11	0.040

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-H-9/2009-155	H	M	10	NS	Karbala	< 3.5	0.160	0.260	0.580	26.60	4.90	110	0.050	0.93	0.090
K-H-H-9/2009-156	H	F	12	NS	Karbala	< 3.5	0.060	0.030	0.570	4.20	4.90	149	0.003	4.99	0.100
K-H-H-9/2009-157	H	F	37	NS	Karbala	< 3.5	0.400	0.220	0.560	9.50	4.50	421	0.006	35.29	0.200
K-H-H-9/2009-158	H	M	38	S	Karbala	< 3.5	0.050	< 0.005	0.450	4.30	5.40	115	< 0.005	3.90	0.050
K-H-H-9/2009-159	H	M	46	NS	Karbala	< 3.5	0.010	< 0.005	0.130	4.20	1.80	71	< 0.005	1.09	0.040
K-H-H-9/2009-160	H	F	23	NS	Karbala	< 3.5	0.130	0.050	0.270	2.90	7.90	148	0.013	26.02	0.250
K-H-H-9/2009-161	H	M	8	NS	Karbala	< 3.5	0.060	0.050	0.440	3.20	4.30	131	0.005	4.51	0.060
K-H-H-9/2009-162	H	M	4	NS	Karbala	< 3.5	0.090	0.140	0.400	16.20	2.40	89	0.013	0.76	0.030
K-H-H-9/2009-163	H	M	54	S	Karbala	< 3.5	0.060	0.030	0.260	7.10	2.70	77	0.037	0.99	0.090
K-H-H-9/2009-164	H	M	10	NS	Karbala	< 3.5	0.110	0.650	3.850	20.70	3.60	234	< 0.005	1.06	0.300
K-H-H-9/2009-165	H	M	8	NS	Karbala	< 3.5	0.070	0.030	0.240	6.90	2.80	65	0.005	0.75	0.060
K-H-H-9/2009-166	H	M	8	NS	Karbala	< 3.5	0.120	0.120	0.560	19.70	5.50	112	0.018	1.34	0.080
K-H-H-9/2009-167	H	F	65	NS	Karbala	< 3.5	0.180	0.410	0.970	19.90	5.30	91	0.028	3.93	0.060
K-H-H-9/2009-168	H	M	9	NS	Karbala	< 3.5	0.080	0.100	0.390	14.00	4.10	82	0.024	1.02	0.050
K-H-H-9/2009-169	H	F	45	NS	Karbala	< 3.5	0.070	0.040	0.290	12.20	8.20	105	< 0.005	0.71	0.040
K-H-H-9/2009-170	H	M	35	NS	Karbala	< 3.5	0.090	0.180	0.440	5.70	5.80	47	0.055	0.77	0.070
K-H-H-9/2009-171	H	M	43	NS	Karbala	< 3.5	0.260	0.070	3.350	11.40	6.50	205	< 0.005	41.59	0.310
K-H-D-9/2009-172	D	M	41	S	Karbala	12.87	0.003	0.007	0.019	0.59	0.49	17	0.005	0.14	< 0.005
K-H-D-9/2009-173	D	M	33	S	Karbala	51.34	0.004	0.031	0.020	0.28	1.08	17	0.006	0.15	0.255
K-H-D-9/2009-174	D	M	47	NS	Karbala	6.55	0.003	< 0.005	0.015	0.37	0.25	12	< 0.005	0.13	< 0.005
K-H-D-9/2009-175	D	M	63	NS	Karbala	8.21	0.001	0.005	0.007	0.10	0.42	18	< 0.005	0.13	< 0.005
K-H-D-9/2009-176	D	F	73	NS	Karbala	19.29	0.007	< 0.005	0.047	0.13	0.44	115	< 0.005	2.17	< 0.005
K-H-D-9/2009-177	D	M	60	NS	Karbala	19.59	0.004	0.057	0.019	0.76	0.47	17	< 0.005	0.25	< 0.005
K-H-D-9/2009-178	D	F	52	NS	Karbala	12.79	0.004	< 0.005	0.041	0.16	0.45	20	< 0.005	0.92	< 0.005
K-H-D-9/2009-179	D	F	25	NS	Karbala	48.86	0.011	0.008	0.031	0.28	0.72	118	< 0.005	2.52	2.066

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-D-9/2009-180	D	F	19	NS	Karbala	10.69	0.006	< 0.005	0.015	0.12	0.61	117	< 0.005	0.59	< 0.005
K-H-D-9/2009-181	D	F	51	NS	Karbala	45.83	0.012	0.009	0.064	0.26	0.87	116	< 0.005	3.91	0.203
K-H-D-9/2009-182	D	F	43	S	Karbala	16.94	0.005	< 0.005	0.027	0.30	1.23	118	< 0.005	3.03	1.081
K-H-D-9/2009-183	D	F	63	NS	Karbala	24.43	0.006	0.011	0.042	0.20	0.66	121	< 0.005	1.08	0.058
K-H-D-9/2009-184	D	M	32	NS	Karbala	29.1	0.004	0.006	0.028	0.60	0.48	18	0.007	0.59	< 0.005
K-H-D-9/2009-185	D	F	52	S	Karbala	16.71	0.009	< 0.005	0.014	0.23	0.49	120	0.007	1.10	< 0.005
K-H-D-9/2009-186	D	F	50	S	Karbala	5.96	0.007	< 0.005	0.011	0.09	0.22	133	< 0.005	1.86	< 0.005
K-H-D-9/2009-187	D	F	60	S	Karbala	53.06	0.010	0.057	0.027	0.34	1.31	121	< 0.005	0.98	0.011
K-H-D-9/2009-188	D	F	55	S	Karbala	13.22	0.003	< 0.005	0.009	0.08	0.17	141	< 0.005	2.10	< 0.005
K-H-D-9/2009-189	D	M	69	NS	Karbala	10.37	0.001	< 0.005	0.008	0.21	0.30	20	< 0.005	0.10	< 0.005
K-H-D-9/2009-190	D	F	50	S	Karbala	11.6	0.006	< 0.005	0.007	0.10	0.70	120	< 0.005	0.74	< 0.005
K-H-D-9/2009-191	D	F	52	S	Karbala	26.56	0.004	< 0.005	0.007	0.05	0.41	114	< 0.005	0.92	< 0.005
K-H-D-9/2009-192	D	F	74	S	Karbala	21.75	0.005	0.005	0.044	0.12	0.42	112	< 0.005	1.87	< 0.005
K-H-D-9/2009-193	D	M	57	NS	Karbala	105.82	0.009	< 0.005	0.043	0.46	0.88	113	< 0.005	0.63	< 0.005
K-H-D-9/2009-194	D	M	67	NS	Karbala	86.64	0.006	0.010	0.026	0.37	0.71	111	< 0.005	0.50	0.137
K-H-D-9/2009-195	D	M	50	S	Karbala	15.74	0.002	< 0.005	0.012	0.28	0.44	17	< 0.005	0.23	< 0.005
K-H-D-9/2009-196	D	M	54	NS	Karbala	8.91	0.002	< 0.005	0.008	0.21	0.34	16	< 0.005	0.12	< 0.005
K-H-D-9/2009-197	D	F	35	S	Karbala	13.41	0.009	< 0.005	0.021	0.16	0.54	148	< 0.005	1.82	0.183
K-H-D-9/2009-198	D	M	32	NS	Karbala	32.26	0.004	0.007	0.025	0.60	0.49	20	0.006	0.50	< 0.005
K-H-D-9/2009-199	D	F	73	NS	Karbala	15.45	0.006	< 0.005	0.036	0.11	0.37	112	< 0.005	1.51	< 0.005
K-H-D-9/2009-200	D	F	50	S	Karbala	54.62	0.005	0.006	0.005	0.09	0.64	116	< 0.005	0.55	< 0.005
K-H-D-9/2009-201	D	F	52	S	Karbala	26.14	0.007	< 0.005	0.011	0.18	0.39	117	< 0.005	0.81	< 0.005
K-H-D-9/2009-202	D	F	73	NS	Karbala	14.42	0.006	< 0.005	0.036	0.11	0.35	111	< 0.005	1.41	< 0.005
K-H-D-9/2009-203	D	F	50	S	Karbala	15.22	0.005	< 0.005	0.007	0.11	0.72	120	< 0.005	0.68	< 0.005
K-H-D-9/2009-204	D	F	51	S	Karbala	20.51	0.005	< 0.005	0.008	0.15	0.76	121	< 0.005	0.63	< 0.005

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-H-D-9/2009-205	D	M	69	NS	Karbala	17.93	0.002	< 0.005	0.009	0.276	0.364	110	< 0.005	0.11	< 0.005
K-H-D-9/2009-206	D	M	41	S	Karbala	21.57	0.003	0.008	0.015	0.484	0.654	17	< 0.005	0.13	< 0.005
K-H-D-9/2009-207	D	M	32	NS	Karbala	11.59	0.005	0.010	0.029	0.820	0.541	112	< 0.005	0.32	< 0.005
K-H-D-9/2009-208	D	M	32	NS	Karbala	18.36	0.004	0.007	0.022	0.631	0.345	16	< 0.005	0.22	< 0.005
K-H-D-9/2009-209	D	F	50	S	Karbala	50.93	0.008	< 0.005	0.013	0.116	0.271	136	< 0.005	2.20	< 0.005
K-H-D-9/2009-210	D	M	50	S	Karbala	165.30	0.003	0.014	0.018	0.433	0.534	17	< 0.005	0.36	< 0.005
K-H-D-9/2009-211	D	F	73	NS	Karbala	26.33	0.006	< 0.005	0.046	0.133	0.453	114	< 0.005	1.81	< 0.005
K-H-D-9/2009-212	D	M	32	NS	Karbala	48.45	0.004	0.008	0.025	0.702	0.519	20	< 0.005	0.33	< 0.005
K-H-D-9/2009-213	D	F	35	S	Karbala	86.51	0.009	0.005	0.021	0.243	0.827	148	< 0.005	1.70	0.133
K-H-D-9/2009-214	D	F	34	NS	Karbala	7.47	0.008	0.018	0.073	0.401	0.700	148	< 0.005	4.01	0.210
K-H-D-9/2009-215	D	M	18	NS	Karbala	12.33	0.008	0.018	0.057	0.775	0.981	128	0.059	4.16	0.103
L-H-H-9/2009-216	H	F	35	NS	London	9.78	0.004	0.003	0.001	0.07	0.42	42	< 0.005	0.21	< 0.005
L-H-H-9/2009-217	H	F	40	NS	London	8.08	0.001	< 0.005	0.006	0.04	0.87	43	< 0.005	0.86	< 0.005
L-H-H-9/2009-218	H	F	26	NS	London	6.51	0.002	< 0.005	< 0.005	0.04	0.80	40	< 0.005	0.17	< 0.005
L-H-H-9/2009-219	H	F	43	NS	London	14.71	0.001	< 0.005	< 0.005	0.14	0.57	41	< 0.005	0.22	< 0.005
L-H-H-9/2009-220	H	F	44	NS	London	4.46	0.001	< 0.005	< 0.005	0.04	0.61	47	< 0.005	0.55	< 0.005
L-H-H-9/2009-221	H	M	23	NS	London	8.95	0.001	< 0.005	< 0.005	0.07	0.46	40	< 0.005	0.77	< 0.005
L-H-H-9/2009-222	H	F	39	NS	London	6.52	0.001	< 0.005	< 0.005	0.08	0.58	45	< 0.005	0.55	< 0.005
L-H-H-9/2009-223	H	F	27	NS	London	31.67	0.001	< 0.005	< 0.005	0.04	1.86	43	< 0.005	0.61	< 0.005
L-H-H-9/2009-224	H	F	36	NS	London	8.89	0.003	< 0.005	< 0.005	0.04	0.43	43	< 0.005	0.83	< 0.005
L-H-H-9/2009-225	H	F	40	NS	London	29.89	0.001	0.005	0.082	0.05	2.07	36	< 0.005	0.94	< 0.005
L-H-H-9/2009-226	H	F	48	NS	London	5.67	0.002	< 0.005	< 0.005	0.04	0.77	41	< 0.005	0.13	< 0.005
L-H-H-9/2009-227	H	F	50	NS	London	8.23	0.001	< 0.005	0.007	0.05	1.00	43	< 0.005	0.91	< 0.005
L-H-H-9/2009-228	H	M	6	NS	London	5.89	0.001	< 0.005	< 0.005	0.08	0.78	35	< 0.005	0.38	< 0.005
L-H-H-9/2009-229	H	M	9	NS	London	11.16	0.001	< 0.005	< 0.005	0.06	0.77	37	< 0.005	0.37	< 0.005

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
L-H-H-9/2009-230	H	F	11	NS	London	8.46	0.006	0.007	0.007	0.17	6.41	49	< 0.005	0.47	0.170
L-H-H-9/2009-231	H	M	42	NS	London	6.26	0.003	< 0.005	0.001	0.07	0.84	33	< 0.005	0.20	< 0.005
L-H-H-9/2009-232	H	M	14	NS	London	13.33	0.001	< 0.005	< 0.005	0.07	0.72	36	< 0.005	0.32	< 0.005
L-H-H-9/2009-233	H	F	33	NS	London	6.36	0.005	< 0.005	< 0.005	0.08	4.22	48	< 0.005	0.96	0.040
L-H-H-9/2009-234	H	M	48	NS	London	9.32	0.005	< 0.005	< 0.005	0.05	0.56	50	< 0.005	0.18	< 0.005
L-H-H-9/2009-235	H	M	45	NS	London	6.94	0.003	< 0.005	< 0.005	0.06	0.43	42	< 0.005	0.35	< 0.005
L-H-H-9/2009-236	H	F	2	NS	London	7.30	0.001	< 0.005	< 0.005	0.04	0.56	45	< 0.005	0.77	< 0.005
L-H-H-9/2009-237	H	M	41	NS	London	11.90	0.006	< 0.005	< 0.005	0.34	0.66	45	< 0.005	0.15	< 0.005
L-H-H-9/2009-238	H	M	47	NS	London	3.56	0.001	< 0.005	< 0.005	0.09	0.63	45	< 0.005	0.35	< 0.005
L-H-H-9/2009-239	H	F	4	NS	London	12.96	0.002	< 0.005	< 0.005	0.04	0.83	40	< 0.005	0.16	< 0.005
L-H-H-9/2009-240	H	M	41	NS	London	11.62	0.003	< 0.005	< 0.005	0.11	0.73	41	< 0.005	0.13	< 0.005
L-H-H-9/2009-241	H	F	2	NS	London	9.16	0.001	< 0.005	< 0.005	0.05	0.59	47	< 0.005	0.16	< 0.005
L-H-H-9/2009-242	H	F	4	NS	London	4.13	0.002	< 0.005	< 0.005	0.04	0.83	40	< 0.005	0.17	< 0.005
L-H-H-9/2009-243	H	M	45	NS	London	7.01	0.003	< 0.005	< 0.005	0.07	0.36	40	< 0.005	0.16	< 0.005
L-H-H-9/2009-244	H	F	4	NS	London	6.85	0.002	< 0.005	< 0.005	0.05	0.93	41	< 0.005	0.17	< 0.005
L-H-H-9/2009-245	H	M	41	NS	London	4.79	0.001	< 0.005	< 0.005	0.11	0.52	41	< 0.005	0.11	< 0.005
L-H-H-9/2009-246	H	F	2	NS	London	5.83	0.001	< 0.005	< 0.005	0.07	0.63	48	< 0.005	0.11	0.700
L-H-H-9/2009-247	H	M	47	NS	London	5.35	0.005	< 0.005	< 0.005	0.08	0.54	42	< 0.005	0.35	< 0.005
L-H-H-9/2009-248	H	F	42	NS	London	8.71	0.001	< 0.005	< 0.005	0.05	1.98	43	< 0.005	0.65	< 0.005
L-H-H-9/2009-249	H	F	2	NS	London	6.75	0.001	< 0.005	< 0.005	0.06	0.64	29	< 0.005	0.36	< 0.005
L-H-H-9/2009-250	H	M	45	NS	London	6.29	0.001	< 0.005	< 0.005	0.05	0.42	43	< 0.005	0.90	< 0.005
L-H-H-9/2009-251	H	M	47	NS	London	10.90	0.001	< 0.005	< 0.005	0.05	0.45	40	< 0.005	0.39	< 0.005
L-H-H-9/2009-252	H	M	3	NS	London	5.03	0.006	< 0.005	< 0.005	0.07	0.80	42	< 0.005	0.17	< 0.005
L-H-H-9/2009-253	H	M	5	NS	London	7.40	0.001	< 0.005	< 0.005	0.08	0.83	35	< 0.005	0.12	< 0.005
L-H-H-9/2009-254	H	M	45	NS	London	5.65	0.001	< 0.005	< 0.005	0.05	0.42	42	< 0.005	0.22	< 0.005

Table F2.2 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
L-H-H-9/2009-255	H	M	9	NS	London	4.85	0.003	< 0.005	< 0.005	0.05	0.73	37	< 0.005	0.18	< 0.005
L-H-H-9/2009-256	H	M	3	NS	London	8.37	0.003	< 0.005	< 0.005	0.15	1.00	41	< 0.005	0.18	< 0.005
L-H-H-9/2009-257	H	M	45	NS	London	7.57	0.001	< 0.005	< 0.005	0.06	0.51	46	< 0.005	0.11	0.080
L-H-H-9/2009-258	H	F	42	NS	London	16.22	0.001	< 0.005	0.006	0.06	2.05	44	< 0.005	0.71	< 0.005
L-H-H-9/2009-259	H	M	3	NS	London	10.57	0.004	< 0.005	< 0.005	0.04	0.73	41	< 0.005	0.19	< 0.005
L-H-H-9/2009-260	H	M	9	NS	London	5.98	0.001	< 0.005	< 0.005	0.06	0.77	37	< 0.005	0.27	< 0.005
L-H-H-9/2009-261	H	F	11	NS	London	9.01	0.006	0.006	0.007	0.12	5.74	48	< 0.005	0.46	0.210
L-H-H-9/2009-262	H	M	42	NS	London	15.81	0.001	< 0.005	< 0.005	0.09	1.20	34	< 0.005	0.18	0.210
L-H-H-9/2009-263	H	M	14	NS	London	10.49	0.001	< 0.005	< 0.005	0.05	0.91	36	< 0.005	0.11	< 0.005
L-H-H-9/2009-264	H	F	33	NS	London	19.64	0.004	< 0.005	< 0.005	0.07	4.29	47	< 0.005	0.92	0.050
L-H-H-9/2009-265	H	M	48	NS	London	26.55	0.003	< 0.005	< 0.005	0.06	0.66	40	< 0.005	0.17	< 0.005

HS = health status, G = gender, y = year, SA = smoking activity, K-H-H-9/2009-1, K corresponds to the province in Iraq (K) Karbala and may be replaced by (L) London in the UK; H corresponds to hair, H corresponds to healthy and may be replaced by D (diabetes); 9/2009 corresponds to the date (month/year); and 1 corresponds to the sample code number.

Appendix F2

Paired Sample Results:

Table F2.3: Paired tear drops and washed scalp hair samples (n = 50) from Karbala (Iraq).

Sample description						Elemental level									
						Tear drop (µg/l)					Scalp hair (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TSH1	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	< 3500	144	259	971	25381
TSH2	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	< 3500	172	309	1103	21185
TSH3	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	6077	89	44	417	5009
TSH4	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	3627	102	187	518	10854
TSH5	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	< 3500	117	158	784	19973
TSH6	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	< 3500	85	37	532	8070
TSH7	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	< 3500	220	60	710	4400
TSH8	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	< 3500	121	21	1557	3060
TSH9	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	< 3500	64	49	375	7427
TSH10	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	< 3500	60	34	575	4164
TSH11	H	M	42	NS	Karbala	616	3.43	7.07	19.62	335	< 3500	67	55	415	6415
TSH12	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	< 3500	94	88	539	12127
TSH13	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	< 3500	101	102	601	12176
TSH14	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	< 3500	180	287	819	13264
TSH15	H	M	38	NS	Karbala	< 70	3.71	17.80	38.95	674	< 3500	32	< 5	160	2910
TSH16	H	M	40	NS	Karbala	252	2.75	6.20	22.98	465	< 3500	200	132	986	16009
TSH17	H	M	40	S	Karbala	< 70	3.71	9.13	35.83	427	< 3500	39	64	232	3979
TSH18	H	M	23	NS	Karbala	446	21.08	31.17	270.08	2816	< 3500	217	300	1342	16963
TSH19	H	M	44	NS	Karbala	504	4.28	7.33	9.90	344	< 3500	614	390	1066	10672
TSH20	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	< 3500	40	< 5	142	2941
TSH21	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	< 3500	189	28	2237	1875
TSH22	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	< 3500	240	331	953	31503
TSH23	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	< 3500	417	236	1358	15075
TSH24	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	< 3500	70	40	290	12200
TSH25	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	< 3500	334	84	762	11401
TSH26	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	< 3500	244	20	953	5269

Table F2.3 (continued)

Sample description						Elemental level									
						Tear drop (µg/l)					Scalp hair (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TSH27	H	M	33	S	Karbala	785	1.06	5.66	9.78	284	< 3500	396	221	556	9476
TSH28	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	< 3500	91	179	444	5657
TSH29	H	M	3	NS	Karbala	761	12.6	35.56	96.17	2253	< 3500	46	< 5	432	4168
TSH30	H	F	65	NS	Karbala	415	1.72	4.16	14.88	261	< 3500	181	410	969	19925
TSH31	H	M	10	NS	Karbala	205	3.28	17.95	37.38	211	< 3500	146	107	1065	15562
TSH32	H	F	13	NS	Karbala	233	7.29	9.60	77.27	764	< 3500	214	99	589	3939
TSH33	H	F	20	NS	Karbala	184	2.75	11.55	7.28	371	< 3500	314	99	1122	4867
TSH34	H	F	21	NS	Karbala	546	3.10	33.79	24.09	453	< 3500	532	137	1007	3894
TSH35	H	M	19	NS	Karbala	< 70	10.37	18.90	42.67	1610	< 3500	403	59	674	11456
TSH36	H	F	20	NS	Karbala	310	2.85	7.18	35.78	307	< 3500	175	< 5	411	1782
TSH37	H	F	8	NS	Karbala	336	2.42	8.24	16.15	370	< 3500	232	84	769	4389
TSH38	H	F	11	NS	Karbala	112	2.00	3.06	20.47	260	< 3500	288	92	1053	4347
TSH39	H	F	8	NS	Karbala	324	6.54	6.65	56.05	803	< 3500	366	104	1074	10595
TSH40	H	F	7	NS	Karbala	898	6.15	13.95	86.05	586	< 3500	299	79	721	5594
TSH41	H	M	11	NS	Karbala	368	6.24	11.44	58.08	1437	< 3500	341	47	829	7026
TSH42	H	F	4	NS	Karbala	379	1.85	4.34	21.97	54	< 3500	233	45	459	6112
TSH43	H	F	35	NS	Karbala	275	0.49	1.85	10.07	39	< 3500	373	69	1317	6529
TSH44	H	F	31	NS	Karbala	101	0.81	1.23	8.98	84	< 3500	137	23	553	2899
TSH45	H	F	8	NS	Karbala	549	1.07	5.70	7.35	7	< 3500	317	293	992	16664
TSH46	H	F	11	NS	Karbala	< 70	18.43	8.89	55.74	471	< 3500	738	328	1689	11349
TSH47	H	M	45	NS	Karbala	373	12.66	37.86	227.30	2396	< 3500	86	99	365	12339
TSH48	H	M	46	NS	Karbala	417	2.07	5.37	8.03	271	< 3500	14	< 5	128	4171
TSH49	H	F	23	NS	Karbala	71	1.98	8.21	5.09	264	< 3500	132	53	268	2926
TSH50	H	F	8	NS	Karbala	165	1.99	3.25	29.25	288	< 3500	569	226	1867	24633
PIN	HS	G	Age (y)	S	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TSH1	H	M	33	NS	Karbala	91	263	0.12	143	0.15	7059	78567	48	2592	138
TSH2	H	M	20	NS	Karbala	49	1175	33.94	689	0.16	9824	117027	59	3787	382
TSH3	H	M	20	NS	Karbala	254	1134	1.74	475	1.51	10065	101898	52	2715	73
TSH4	H	M	19	NS	Karbala	112	1327	14.89	248	0.31	5975	155678	23	4407	81
TSH5	H	M	45	NS	Karbala	79	49	0.69	104	0.15	10741	128015	29	3145	621
TSH6	H	M	38	NS	Karbala	148	352	1.80	425	0.58	6641	78314	55	1497	195

Table F2.3 (continued)

Sample description						Elemental level									
						Tear drop (µg/l)					Scalp hair (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TSH7	H	M	21	S	Karbala	255	4100	9.56	1359	6.12	6900	144000	< 5	11860	100
TSH8	H	F	40	S	Karbala	126	126	1.22	464	0.29	5762	65323	< 5	49050	2050
TSH9	H	M	42	NS	Karbala	427	494	1.34	58	0.34	6574	44693	32	1159	67
TSH10	H	F	12	NS	Karbala	241	382	2.85	314	1.12	4924	148833	5	4990	100
TSH11	H	M	42	NS	Karbala	280	355	2.11	246	1.26	6282	125824	< 5	3122	56
TSH12	H	M	20	NS	Karbala	244	753	2.67	552	1.43	5415	67059	10	2519	16
TSH13	H	M	8	NS	Karbala	313	598	1.83	271	1.17	5936	126291	8	2531	60
TSH14	H	M	10	NS	Karbala	218	295	44.82	306	2.41	5710	140812	79	947	750
TSH15	H	M	38	NS	Karbala	170	386	3.54	534	1.55	5520	137855	10	1314	135
TSH16	H	M	40	NS	Karbala	198	224	1.42	262	0.36	8256	133190	31	1158	75
TSH17	H	M	40	S	Karbala	203	416	3.70	442	1.32	6534	139055	< 5	8475	563
TSH18	H	M	23	NS	Karbala	547	4109	6.50	461	5.98	4949	134024	< 5	4681	110
TSH19	H	M	44	NS	Karbala	196	460	0.48	140	0.75	6737	161123	5	17807	133
TSH20	H	M	33	NS	Karbala	589	1936	20.89	678	4.23	2822	89405	< 5	836	44
TSH21	H	F	42	NS	Karbala	157	169	1.37	175	0.75	5797	139879	< 5	39559	789
TSH22	H	M	37	NS	Karbala	431	1022	5.15	587	1.35	5708	64529	86	1039	91
TSH23	H	M	43	NS	Karbala	318	277	2.67	345	0.57	5174	113666	< 5	7351	162
TSH24	H	F	45	NS	Karbala	260	495	1.59	388	0.21	8200	105000	< 5	710	40
TSH25	H	F	14	NS	Karbala	436	404	1.54	513	0.91	2871	434110	< 5	13999	553
TSH26	H	F	33	NS	Karbala	102	169	1.68	118	0.48	3668	432334	< 5	24912	130
TSH27	H	M	33	NS	Karbala	193	187	1.56	112	2.18	4547	420762	6	35288	196
TSH28	H	M	35	NS	Karbala	356	173	2.16	307	0.64	5784	46754	55	773	68
TSH29	H	M	3	NS	Karbala	211	1079	2.75	806	0.71	2927	89304	6	3303	64
TSH30	H	F	65	NS	Karbala	127	300	0.80	325	0.34	5277	90881	28	3930	60
TSH31	H	M	10	NS	Karbala	285	740	4.18	530	1.85	4689	83475	5	4060	89
TSH32	H	F	13	NS	Karbala	222	1529	7.04	563	1.13	8202	204636	75	28381	316
TSH33	H	F	20	NS	Karbala	133	181	1.43	243	0.55	8415	326117	< 5	27780	245
TSH34	H	F	21	NS	Karbala	242	989	36.55	321	3.86	8853	298486	72	15835	160
TSH35	H	M	19	NS	Karbala	209	1232	39.20	895	2.07	7837	166693	< 5	11632	3124
TSH36	H	F	20	NS	Karbala	77	2718	7.09	616	0.42	6007	77475	< 5	23174	301
TSH37	H	F	8	NS	Karbala	117	125	38.70	129	1.61	4192	410766	< 5	7336	576

Table F2.3 (continued)

Sample description						Elemental level									
						Tear drop (µg/l)					Scalp hair (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TSH38	H	F	11	NS	Karbala	100	854	1.21	353	0.33	4914	174240	< 5	9625	460
TSH39	H	F	8	NS	Karbala	205	225	1.64	691	0.13	5420	288189	< 5	13520	149
TSH40	H	F	7	NS	Karbala	185	4164	35.94	727	10.11	3140	602240	< 5	14846	295
TSH41	H	M	11	NS	Karbala	423	2103	11.56	583	3.52	2166	433104	< 5	10216	218
TSH42	H	F	4	NS	Karbala	234	1021	30.03	405	2.43	8583	158957	5	4098	112
TSH43	H	F	35	NS	Karbala	181	198	1.70	49	1.78	5671	319543	< 5	37617	82
TSH44	H	F	31	NS	Karbala	50	314	1.43	132	0.25	2641	191929	< 5	11225	691
TSH45	H	F	8	NS	Karbala	133	258	2.00	577	0.89	5773	372151	< 5	14942	258
TSH46	H	F	11	NS	Karbala	526	4104	34.09	794	2.49	14721	196168	41	24855	855
TSH47	H	M	45	NS	Karbala	273	4933	18.82	469	11.53	4920	86281	39	1109	43
TSH48	H	M	46	NS	Karbala	159	186	0.96	112	0.76	1769	71198	< 5	1087	40
TSH49	H	F	23	NS	Karbala	98	125	0.62	160	0.64	7942	148214	13	26024	246
TSH50	H	F	8	NS	Karbala	133	190	0.74	274	0.12	7837	303039	< 5	9707	215

HS = health status, G = gender, H = healthy, M = male, F = female, SA = smoking activity, S = smoker, NS = non-smoker, y = year, TSH1, T corresponds to tear drops, SH corresponds to scalp hair; and 1 corresponds to the sample code number.

Appendix F2

Comparison Study:

Table F2.4: Summary of F-test and a two tailed t-test results for elemental levels in washed scalp hair samples of healthy and diabetic individuals from Karbala, Iraq.

Element (n_1, n_2)	F-Test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	EVA	nd		nd			
(171, 44)	UVA						
V	EVA	52.140	0.000	8.217	213^{+}	0.000	
(171, 44)	UVA			16.214	170^{++}	**0.000**	1.97
Cr	EVA	nd		nd			
(148, 21)	UVA						
Mn	EVA	43.296	0.000	8.048	213	0.000	
(171, 44)	UVA			15.875	171	**0.000**	1.97
Fe	EVA	27.417	0.000	6.062	213	0.000	
(171, 44)	UVA			11.967	170	**0.000**	1.97
Cu	EVA	18.290	0.000	11.329	213	0.000	
(171, 44)	UVA			22.127	178	**0.000**	1.97
Zn	EVA	0.513	0.475	3.775	213	**0.000**	1.97
(171, 44)	UVA			5.078	115	0.000	
As	EVA	nd		nd			
(119, 6)	UVA						
Sr	EVA	24.598	0.000	4.218	213	0.000	
(171, 44)	UVA			8.080	190	**0.000**	1.97
Cd	EVA	nd		nd			
(171, 11)	UVA						

EVA and UVA are equal variances assumed and unequal variances assumed, nd = not determined due to there being several samples which were below the limit of detection (Table 2.17), n_1, n_2 are the number of samples for healthy individuals and diabetic patients, respectively, *df* = degrees of freedom, n_1-1 and n_2-1 for F-test, $^{+}$ degrees of freedom for t-test (n_1+n_2-2), $^{++}$ degrees of freedom for t-test determined as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $P = 0.05$, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

Table F2.5: Summary of F-test and a two tailed t-test results for elemental levels in washed scalp hair samples for individuals from the healthy population of Karbala and London.

Element (n_1, n_2)	F-Test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B (16, 50)	EVA	nd		nd			
	UVA						
V (171, 31)	EVA	60.192	0.000	8.938	219^{+}	0.000	
	UVA			16.551	170^{++}	**0.000**	1.97
Cr (171, 4)	EVA	nd		nd			
	UVA						
Mn (171, 8)	EVA	nd		nd			
	UVA						
Fe (171, 50)	EVA	32.197	0.000	6.574	219	0.000	
	UVA			12.177	170	**0.000**	1.97
Cu (171, 50)	EVA	8.671	0.004	10.607	219	0.000	
	UVA			16.208	201	**0.000**	1.97
Zn (171, 50)	EVA	28.456	0.000	7.834	219	0.000	
	UVA			14.454	173	**0.000**	1.97
As (171, 0)	EVA	nd		nd			
	UVA						
Sr (171, 26)	EVA	36.181	0.000	5.146	219	0.000	
	UVA			9.516	171	**0.000**	1.97
Cd (171, 7)	EVA	nd		nd			
	UVA						

n_1, n_2 are the number of samples for Karbala and London samples, respectively. Other key words can take from Table F2.4.

Table F2.6: Summary of F-test and a two tailed t-test results for elemental levels in tear drops and washed scalp hair for individuals from the healthy population of Karbala who provided both media.

TE	Mean* (µg/l) (T, H)	F-Test for equality of variances			t-test for equality of means			
		Variance	$F_{calc.}$	*Sig.*	$t_{calc.}$	*df*	Sig.	$t_{crit.}$
B	(414,nd)	EVA	nd		nd			
		UVA						
V	(4.7, 217.5)	EVA	70.956	0.000	9.283	98	0.00	
		UVA			9.283	49	**0.00**	2.01
Cr	(13, 137)	EVA	82.140	0.000	8.067	93	0.00	
		UVA			7.679	48	**0.00**	2.01
Mn	(39, 795)	EVA	66.923	0.000	11.756	98	0.00	
		UVA			11.756	50	**0.00**	2.01
Fe	(599, 9692)	EVA	78.053	0.000	9.312	98	0.00	
		UVA			9.312	50	**0.00**	2.01
Cu	(226, 6125)	EVA	52.551	0.000	17.439	98	0.00	
		UVA			17.439	49	**0.00**	2.01
Zn	(987, 183342)	EVA	75.964	0.000	10.053	98	0.00	
		UVA			10.053	49	**0.00**	2.01
As	(9.1, 33.7)	EVA	24.707	0.000	5.481	74	0.00	
		UVA			4.500	32	**0.00**	2.04
Sr	(409, 11131)	EVA	74.920	0.000	6.374	98	0.00	
		UVA			6.374	49	**0.00**	2.01
Cd	(1.7.1, 327.5)	EVA	23.875	0.000	4.375	98	0.00	
		UVA			4.375	49	**0.00**	2.01

T = tear drops, H = Scalp hair, * n = 50, the only exception are for B in tear drops (n = 42) and washed scalp hair (n = 2); Cr in washed scalp hair (n = 45) and As in washed scalp hair (n = 26), TE is trace element. Other key words can take from Table F2.4.

Appendix F3

Fingernail Results:

Table F3.1: Elemental levels (mg/kg) for a "pooled" fingernail sample – unwashed (n = 3) ranging from 0.05 to 0.20 g mass digested in different volumes (constant dilution factor, 100 fold).

Element	Elemental levels^{+} (mg/kg)				
	100*	100*	100*	100*	RSD%
V	0.06	0.06	0.08	0.08	16
Cr	0.24	0.28	0.19	0.26	16
Mn	0.77	0.79	0.88	0.68	11
Fe	28.02	28.66	30.55	28.89	4
Cu	5.01	4.74	5.79	5.14	9
Zn	190.23	231.56	212.96	203.64	8
As	6.05	6.17	6.24	6.77	5
Sr	4.98	6.39	4.13	5.63	18
Cd	0.34	0.36	0.37	0.38	4.7

*dilution factor, RSD is relative standard deviation, $^{+}$ n = 3 replicates.

Table F3.2: Elemental levels (mg/kg) for "pooled" fingernail samples – unwashed (n=3) ranging from 0.05 to 0.20 g mass digested in a constant volume 20 ml (variable dilution factor ranging from 100 – to 400 fold).

Element	Elemental levels^{+} (mg/kg)				
	400*	200*	133*	100*	RSD%
V	< LOD	0.63	0.19	0.12	-
Cr	< LOD	0.64	0.37	0.21	-
Mn	0.72	9.08	3.86	0.55	112
Fe	21.26	61.51	24.54	19.86	63
Cu	5.38	7.84	6.22	5.04	20
Zn	167.73	303.70	320.28	199.71	31
As	13.70	7.98	7.45	6.00	39
Sr	14.01	18.11	8.07	5.55	50
Cd	0.64	0.46	0.38	0.47	22

*dilution factor, RSD is relative standard deviation, $^{+}$ n = 3 replicates.

Table F3.3: Elemental levels (mg/kg dry weight) and percentage removal for "pooled" fingernail sample (using a 0.25 g, constant dilution factor 100 fold dilution volume of 25 ml) (using different washing procedure (n=3).

Element	Elemental levels (mg/kg) (% removed)		
	Unwashed	Washing procedures*	
		A	B
V	0.08	< LOD	< LOD
Cr	0.58	0.08 (86)	0.31 (47)
Mn	1.93	0.22 (89)	0.76 (61)
Fe	91.90	14.31 (84)	66.65 (27)
Cu	4.73	1.87 (60)	2.21 (53)
Zn	163.88	48.65 (70)	77.99 (52)
As	8.64	5.05 (42)	8.14 (6)
Sr	5.60	1.55 (72)	3.93 (30)
Cd	0.39	0.29 (26)	0.31 (21)

*A: sequential washing in ultrasonic bath with acetone-water-water-water-acetone, B: sequential washing in ultrasonic bath with ether-Triton x-100-water-water, values in brackets were calculated using this equation, Removed % = {(unwashed value – washed value)/unwashed value} x 100.

Table F3.4: Accuracy and precision levels for human scalp hair CRM GBW 09101, and matrix effect for fingernail analysis using a Kejldahl™ tube method.

Element (n = 3)	Elemental Levels (mg/kg)				
	Accuracy			Precision	
	Measured value mean ± SD	Certified value mean	%R	mean ± SD	%RSD
V	0.066 ± 0.011	0.069	96	0.14 ± 0.01	7
Cr	0.35 ± 0.04	0.37	95	0.32 ± 0.03	9.4
Mn	2.51 ± 0.014	2.94	85	2.96 ± 0.18	6.1
Fe	70.8 ± 0.84	71.2	99	176.1 ± 2.5	1.4
Cu	22 ± 0.11	23	96	3.57 ± 0.05	1.4
Zn	187 ± 0.32	189	99	85 ± 1	1.1
As	0.63 ± 0.41	0.59	107	4.22 ± 0.49	11.6
Sr	21.97 ± 9	24	92	13.8 ± 0.5	3.6
Cd	0.104 ± 0.01	0.11	95	0.17 ± 0.02	11.76

SD is standard deviation, RSD is relative standard deviation, R is percentage recovery.

Appendix F3

Washed Fingernail Results:

Table F3.5: Description of washed fingernail samples (n = 259) and elemental levels for Iraqi individuals from Karbala (Iraq) and London (UK).

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-H-9/2009-1	H	F	14	NS	Karbala	< 3.5	0.130	0.20	0.34	27.63	2.97	76	0.11	3.82	0.01
K-F-H-9/2009-2	H	F	16	NS	Karbala	< 3.5	0.570	3.45	2.50	197.12	4.79	349	0.31	18.06	0.01
K-F-H-9/2009-3	H	M	52	NS	Karbala	< 3.5	0.190	0.20	0.37	20.60	4.42	132	0.12	2.74	< 0.005
K-F-H-9/2009-4	H	M	44	NS	Karbala	< 3.5	0.340	0.82	2.80	129.97	11.15	146	0.16	12.12	0.15
K-F-H-9/2009-5	H	F	37	NS	Karbala	< 3.5	0.890	1.81	4.87	325.19	23.03	173	0.28	14.81	0.11
K-F-H-9/2009-6	H	F	12	NS	Karbala	< 3.5	0.240	0.95	1.43	96.89	5.69	80	0.13	9.24	0.02
K-F-H-9/2009-7	H	F	30	NS	Karbala	< 3.5	0.090	0.16	0.44	1.10	3.73	89	0.09	8.13	0.02
K-F-H-9/2009-8	H	F	22	NS	Karbala	< 3.5	0.730	1.96	2.79	285.25	23.04	427	0.43	14.54	0.19
K-F-H-9/2009-9	H	M	19	NS	Karbala	< 3.5	0.060	0.11	0.40	5.39	2.98	88	0.08	3.65	< 0.005
K-F-H-9/2009-10	H	M	43	NS	Karbala	12.31	0.100	0.49	0.72	28.56	3.53	87	0.11	7.97	0.02
K-F-H-9/2009-11	H	M	35	NS	Karbala	< 3.5	0.170	0.32	1.28	64.25	7.84	137	0.13	7.12	< 0.005
K-F-H-9/2009-12	H	M	45	NS	Karbala	10.09	0.250	0.53	2.28	41.37	0.98	33	0.05	7.12	< 0.005
K-F-H-9/2009-13	H	M	8	NS	Karbala	44.06	0.450	0.91	2.85	48.90	2.18	42	0.08	12.03	0.03
K-F-H-9/2009-14	H	M	67	NS	Karbala	< 3.5	0.050	0.06	0.51	7.51	2.35	73	0.11	5.11	0.01
K-F-H-9/2009-15	H	M	37	NS	Karbala	< 3.5	0.090	0.22	0.77	39.16	2.63	88	0.09	7.55	< 0.005
K-F-H-9/2009-16	H	M	33	NS	Karbala	< 3.5	0.050	0.28	0.61	16.16	6.34	133	0.13	2.79	< 0.005
K-F-H-9/2009-17	H	F	54	NS	Karbala	7.4	0.190	0.62	2.36	67.33	6.73	144	0.24	6.52	< 0.005
K-F-H-9/2009-18	H	M	35	NS	Karbala	< 3.5	0.090	0.14	0.66	12.02	2.09	61	0.08	4.99	0.02
K-F-H-9/2009-19	H	M	67	NS	Karbala	< 3.5	0.450	1.08	4.34	193.13	5.63	159	0.12	10.44	0.04
K-F-H-9/2009-20	H	M	33	NS	Karbala	< 3.5	0.130	0.40	0.64	27.36	3.72	123	0.11	4.78	0.06
K-F-H-9/2009-21	H	F	45	NS	Karbala	< 3.5	0.260	0.85	1.15	67.89	5.03	90	0.15	4.57	0.01
K-F-H-9/2009-22	H	F	42	NS	Karbala	< 3.5	0.140	0.33	0.99	48.32	4.28	50	0.05	3.38	0.09
K-F-H-9/2009-23	H	F	13	NS	Karbala	< 3.5	0.170	0.17	0.56	27.48	5.94	98	0.13	2.55	0.33
K-F-H-9/2009-24	H	M	31	NS	Karbala	< 3.5	0.170	0.34	1.20	51.10	20.10	159	0.10	7.54	0.03
K-F-H-9/2009-25	H	M	50	NS	Karbala	8.62	0.120	0.35	1.28	46.87	5.80	57	0.56	2.67	0.02
K-F-H-9/2009-26	H	M	54	NS	Karbala	< 3.5	0.240	0.73	1.30	87.14	6.00	80	0.13	4.09	0.01
K-F-H-9/2009-27	H	M	35	NS	Karbala	< 3.5	0.390	0.53	2.04	121.00	2.92	46	0.15	2.47	0.03
K-F-H-9/2009-28	H	M	41	NS	Karbala	7.06	0.720	1.77	5.50	289.07	13.91	50	0.14	6.63	0.04

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-H-9/2009-29	H	M	67	NS	Karbala	8.29	0.140	0.45	0.76	45.59	3.67	43	0.08	2.45	< 0.005
K-F-H-9/2009-30	H	M	38	NS	Karbala	13.47	0.140	0.47	1.36	105.61	1.68	31	0.06	4.17	0.03
K-F-H-9/2009-31	H	F	46	NS	Karbala	< 3.5	0.080	0.09	0.64	7.19	3.20	78	0.09	6.12	< 0.005
K-F-H-9/2009-32	H	M	58	NS	Karbala	< 3.5	0.370	0.81	2.82	167.84	3.33	65	0.15	3.70	0.04
K-F-H-9/2009-33	H	M	54	NS	Karbala	< 3.5	0.020	0.04	0.12	< 0.025	0.44	59	0.04	0.97	< 0.005
K-F-H-9/2009-34	H	M	62	NS	Karbala	< 3.5	0.100	0.20	0.86	28.65	2.34	49	0.06	2.10	< 0.005
K-F-H-9/2009-35	H	M	48	S	Karbala	< 3.5	0.120	0.29	0.88	27.13	2.40	63	0.10	5.71	0.04
K-F-H-9/2009-36	H	M	40	NS	Karbala	< 3.5	0.160	0.31	1.06	49.02	5.40	153	0.11	4.74	0.02
K-F-H-9/2009-37	H	M	10	NS	Karbala	< 3.5	0.147	0.07	0.55	< 0.025	4.45	91	0.07	2.01	0.05
K-F-H-9/2009-38	H	F	41	NS	Karbala	< 3.5	0.030	0.09	0.57	18.80	4.34	103	0.09	0.76	0.08
K-F-H-9/2009-39	H	M	50	NS	Karbala	< 3.5	0.070	0.16	0.81	30.15	5.15	106	0.17	0.92	0.20
K-F-H-9/2009-40	H	M	54	NS	Karbala	< 3.5	0.010	0.36	0.34	< 0.025	6.21	100	0.04	0.90	0.01
K-F-H-9/2009-41	H	M	33	S	Karbala	< 3.5	0.022	0.16	0.19	< 0.025	4.09	128	0.09	0.85	0.03
K-F-H-9/2009-42	H	M	42	NS	Karbala	< 3.5	0.120	0.34	0.98	37.71	2.87	50	0.08	4.01	0.05
K-F-H-9/2009-43	H	M	48	S	Karbala	< 3.5	0.180	0.34	1.57	71.11	3.25	117	0.49	3.36	< 0.005
K-F-H-9/2009-44	H	M	49	S	Karbala	< 3.5	0.160	0.29	1.33	60.81	2.82	100	0.44	2.94	< 0.005
K-F-H-9/2009-45	H	M	43	NS	Karbala	< 3.5	0.140	0.23	0.78	42.87	5.42	137	0.11	3.63	0.02
K-F-H-9/2009-46	H	F	45	NS	Karbala	< 3.5	0.220	0.47	2.36	79.64	3.75	54	0.16	5.36	< 0.005
K-F-H-9/2009-47	H	F	36	S	Karbala	< 3.5	0.150	0.28	1.26	51.52	3.12	75	0.09	3.34	0.84
K-F-H-9/2009-48	H	M	12	NS	Karbala	< 3.5	0.110	0.21	0.86	35.55	5.21	86	0.08	4.18	1.71
K-F-H-9/2009-49	H	M	70	S	Karbala	17.97	0.230	0.79	2.62	132.61	61.31	102	0.11	5.86	< 0.005
K-F-H-9/2009-50	H	M	54	NS	Karbala	< 3.5	0.100	0.20	0.95	39.83	3.11	69	0.09	3.63	0.50
K-F-H-9/2009-51	H	F	12	NS	Karbala	< 3.5	0.040	0.09	0.18	3.40	1.71	8	0.01	0.72	0.39
K-F-H-9/2009-52	H	M	61	S	Karbala	3.54	0.380	0.75	3.16	205.44	4.35	167	0.15	10.02	0.03
K-F-H-9/2009-53	H	F	65	NS	Karbala	< 3.5	0.150	0.29	1.43	48.84	5.34	144	0.09	6.69	0.01
K-F-H-9/2009-54	H	M	46	NS	Karbala	< 3.5	0.090	0.38	0.78	26.36	3.25	70	0.09	5.61	< 0.005
K-F-H-9/2009-55	H	M	65	NS	Karbala	< 3.5	0.030	0.01	0.17	< 0.025	2.87	72	0.05	1.69	< 0.005
K-F-H-9/2009-56	H	M	42	NS	Karbala	< 3.5	0.120	0.23	0.43	18.80	3.90	63	0.03	2.50	0.05
K-F-H-9/2009-57	H	M	45	S	Karbala	< 3.5	0.100	0.20	0.70	31.19	2.57	70	0.15	4.23	< 0.005
K-F-H-9/2009-58	H	M	73	NS	Karbala	< 3.5	0.340	0.98	2.52	160.28	16.40	100	0.18	12.05	0.20
K-F-H-9/2009-59	H	M	56	NS	Karbala	< 3.5	0.100	0.18	0.85	33.06	2.14	21	0.03	2.36	< 0.005
K-F-H-9/2009-60	H	M	38	NS	Karbala	< 3.5	0.080	0.15	0.53	16.77	2.12	32	0.02	3.08	0.03

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-H-9/2009-61	H	M	55	NS	Karbala	< 3.5	0.900	0.05	5.77	326.94	3.64	81	0.23	17.95	0.34
K-F-H-9/2009-62	H	M	61	NS	Karbala	< 3.5	0.130	0.55	0.37	22.01	2.81	43	0.04	3.98	0.07
K-F-H-9/2009-63	H	F	33	NS	Karbala	< 3.5	0.370	0.78	3.06	147.56	3.92	44	0.08	5.10	0.04
K-F-H-9/2009-64	H	M	8	NS	Karbala	< 3.5	0.390	0.88	2.94	156.65	2.92	31	0.08	5.28	0.02
K-F-H-9/2009-65	H	M	43	NS	Karbala	< 3.5	0.140	0.25	1.57	39.27	2.41	36	0.09	4.93	0.05
K-F-H-9/2009-66	H	M	26	NS	Karbala	< 3.5	0.200	0.50	1.66	54.42	6.86	142	0.08	6.73	< 0.005
K-F-H-9/2009-67	H	M	27	S	Karbala	< 3.5	0.770	1.99	8.43	< 0.025	2.88	78	0.17	23.46	0.01
K-F-H-9/2009-68	H	M	29	S	Karbala	< 3.5	0.230	0.47	1.95	121.89	7.15	71	0.07	2.84	0.03
K-F-H-9/2009-69	H	F	36	NS	Karbala	< 3.5	0.050	0.09	0.52	11.19	2.37	55	0.07	3.05	0.02
K-F-H-9/2009-70	H	F	31	NS	Karbala	< 3.5	0.200	0.41	1.39	77.71	6.14	169	0.10	6.75	0.02
K-F-H-9/2009-71	H	F	53	S	Karbala	< 3.5	0.090	0.51	0.61	23.38	5.98	144	0.06	2.25	0.10
K-F-H-9/2009-72	H	M	25	NS	Karbala	< 3.5	0.030	0.14	0.05	< 0.025	3.84	98	0.06	1.57	0.02
K-F-H-9/2009-73	H	M	48	NS	Karbala	< 3.5	0.410	1.13	3.07	132.29	4.49	115	0.15	5.93	0.15
K-F-H-9/2009-74	H	F	36	NS	Karbala	< 3.5	0.050	0.10	0.14	4.62	1.44	41	0.02	0.29	0.01
K-F-H-9/2009-75	H	M	36	S	Karbala	< 3.5	0.060	0.09	0.39	16.23	4.36	67	0.02	0.98	< 0.005
K-F-H-9/2009-76	H	M	60	NS	Karbala	< 3.5	0.200	0.44	1.82	52.55	3.02	105	0.07	4.37	< 0.005
K-F-H-9/2009-77	H	M	57	S	Karbala	< 3.5	0.150	0.34	1.08	57.77	4.72	72	0.08	2.87	0.03
K-F-H-9/2009-78	H	M	67	S	Karbala	< 3.5	0.150	0.32	1.08	61.69	2.37	81	0.03	6.00	0.01
K-F-H-9/2009-79	H	F	23	NS	Karbala	< 3.5	0.020	0.22	0.17	55.97	16.35	94	0.10	1.08	< 0.005
K-F-H-9/2009-80	H	F	45	NS	Karbala	< 3.5	0.020	1.27	1.27	164.67	4.64	94	0.01	1.44	0.02
K-F-H-9/2009-81	H	F	43	NS	Karbala	< 3.5	0.010	0.97	0.52	< 0.025	8.71	55	0.05	0.50	< 0.005
K-F-H-9/2009-82	H	F	25	NS	Karbala	< 3.5	0.100	0.27	0.45	28.69	12.77	76	0.05	1.47	< 0.005
K-F-H-9/2009-83	H	F	30	NS	Karbala	< 3.5	0.050	0.12	0.28	10.69	4.19	72	0.04	0.86	0.01
K-F-H-9/2009-84	H	M	27	S	Karbala	< 3.5	0.110	0.30	1.02	51.04	4.49	78	0.08	1.16	0.05
K-F-H-9/2009-85	H	M	40	S	Karbala	< 3.5	0.280	0.97	4.71	188.65	5.53	101	0.07	3.95	0.07
K-F-H-9/2009-86	H	M	34	NS	Karbala	< 3.5	0.200	0.50	2.31	138.83	9.02	74	0.08	4.99	< 0.005
K-F-H-9/2009-87	H	M	58	S	Karbala	< 3.5	0.160	0.34	1.07	65.24	4.53	68	0.05	1.75	0.01
K-F-H-9/2009-88	H	M	17	S	Karbala	< 3.5	0.320	0.71	2.99	137.80	4.30	45	0.06	4.30	0.03
K-F-H-9/2009-89	H	M	21	NS	Karbala	< 3.5	0.410	0.94	2.68	173.01	3.02	59	0.06	9.70	0.01
K-F-H-9/2009-90	H	M	48	S	Karbala	< 3.5	0.100	0.17	0.85	30.15	2.37	46	0.02	1.92	< 0.005
K-F-H-9/2009-91	H	F	40	S	Karbala	< 3.5	0.040	0.07	0.53	7.02	3.23	79	0.07	2.04	0.06
K-F-H-9/2009-92	H	M	28	S	Karbala	< 3.5	0.040	0.09	0.36	3.60	3.80	127	0.06	1.65	0.01

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-H-9/2009-93	H	F	19	NS	Karbala	< 3.5	0.110	0.09	0.15	< 0.025	5.60	120	0.03	0.74	< 0.005
K-F-H-9/2009-94	H	F	19	NS	Karbala	< 3.5	0.100	0.16	0.28	21.92	3.02	91	0.03	0.54	0.07
K-F-H-9/2009-95	H	F	22	NS	Karbala	< 3.5	0.010	0.02	0.48	< 0.025	3.72	107	0.04	0.49	0.04
K-F-H-9/2009-96	H	F	22	NS	Karbala	< 3.5	0.070	0.10	0.99	24.90	7.59	101	0.07	1.58	0.08
K-F-H-9/2009-97	H	F	20	NS	Karbala	< 3.5	0.020	0.05	0.97	< 0.025	5.71	95	0.01	3.37	0.02
K-F-H-9/2009-98	H	F	21	NS	Karbala	< 3.5	0.010	0.01	0.16	< 0.025	3.19	96	0.03	0.66	0.01
K-F-H-9/2009-99	H	F	19	NS	Karbala	< 3.5	0.010	0.05	4.21	< 0.025	2.86	95	0.01	4.44	0.03
K-F-H-9/2009-100	H	M	22	NS	Karbala	< 3.5	0.060	0.33	0.12	< 0.025	3.28	125	0.07	1.87	< 0.005
K-F-H-9/2009-101	H	F	22	NS	Karbala	< 3.5	0.050	0.08	0.09	< 0.025	3.80	103	0.02	0.57	0.02
K-F-H-9/2009-102	H	F	33	NS	Karbala	< 3.5	0.040	0.01	0.30	< 0.025	1.95	40	0.06	1.32	0.03
K-F-H-9/2009-103	H	F	21	NS	Karbala	< 3.5	0.210	0.17	0.10	< 0.025	5.83	260	0.08	0.38	0.10
K-F-H-9/2009-104	H	F	23	NS	Karbala	< 3.5	0.060	0.08	0.81	32.40	3.31	78	0.05	4.64	< 0.005
K-F-H-9/2009-105	H	F	23	NS	Karbala	< 3.5	0.020	0.07	0.24	< 0.025	3.19	59	0.08	3.93	0.01
K-F-H-9/2009-106	H	F	23	NS	Karbala	< 3.5	0.040	0.19	0.07	< 0.025	1.92	34	0.01	0.47	0.01
K-F-H-9/2009-107	H	F	21	NS	Karbala	< 3.5	0.030	0.04	1.06	11.76	1.65	44	0.01	0.99	0.01
K-F-H-9/2009-108	H	F	19	NS	Karbala	< 3.5	0.030	0.05	0.30	1.93	2.53	66	0.01	1.83	0.03
K-F-H-9/2009-109	H	F	21	NS	Karbala	< 3.5	0.070	0.27	2.03	29.29	3.97	80	0.04	6.18	0.16
K-F-H-9/2009-110	H	F	24	NS	Karbala	< 3.5	0.060	0.08	1.13	14.08	5.54	111	0.03	3.12	0.05
K-F-H-9/2009-111	H	M	30	NS	Karbala	< 3.5	0.050	0.05	0.20	< 0.025	2.64	10	0.01	8.75	< 0.005
K-F-H-9/2009-112	H	M	28	S	Karbala	< 3.5	0.070	0.16	1.66	22.00	4.22	113	0.04	2.75	< 0.005
K-F-H-9/2009-113	H	F	30	NS	Karbala	< 3.5	0.070	0.13	0.43	22.35	3.74	65	0.09	1.89	< 0.005
K-F-H-9/2009-114	H	M	20	NS	Karbala	< 3.5	0.080	0.22	0.85	31.21	2.77	70	0.03	5.75	0.03
K-F-H-9/2009-115	H	M	24	S	Karbala	< 3.5	0.040	0.31	0.35	0.20	3.66	40	0.08	2.19	0.02
K-F-H-9/2009-116	H	M	21	NS	Karbala	< 3.5	0.040	0.02	14.56	< 0.025	3.30	100	0.06	1.07	0.03
K-F-H-9/2009-117	H	M	42	S	Karbala	< 3.5	0.180	0.32	12.19	82.17	2.24	74	0.07	2.18	0.03
K-F-H-9/2009-118	H	M	20	NS	Karbala	< 3.5	0.050	0.09	6.06	10.87	4.59	64	0.05	0.76	0.01
K-F-H-9/2009-119	H	M	20	NS	Karbala	< 3.5	0.050	0.05	15.84	< 0.025	3.25	81	0.03	1.00	0.08
K-F-H-9/2009-120	H	M	21	S	Karbala	< 3.5	0.030	0.07	7.30	9.54	3.20	100	0.08	1.68	0.01
K-F-H-9/2009-121	H	M	27	S	Karbala	< 3.5	0.020	0.07	8.68	< 0.025	2.15	58	0.04	1.67	0.01
K-F-H-9/2009-122	H	M	22	S	Karbala	< 3.5	0.050	0.08	5.47	16.29	2.67	70	0.04	1.15	0.01
K-F-H-9/2009-123	H	M	22	NS	Karbala	< 3.5	0.180	0.63	10.92	100.69	3.08	92	0.10	9.09	0.10
K-F-H-9/2009-124	H	M	24	NS	Karbala	< 3.5	0.140	0.29	17.21	64.35	3.58	75	0.07	2.15	0.06

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-H-9/2009-125	H	M	23	NS	Karbala	< 3.5	0.470	0.95	13.22	234.76	3.25	71	0.11	10.00	0.02
K-F-H-9/2009-126	H	M	24	S	Karbala	< 3.5	0.020	0.04	4.35	< 0.025	3.31	60	0.06	1.04	0.01
K-F-H-9/2009-127	H	M	27	S	Karbala	< 3.5	0.090	0.18	19.08	35.44	2.16	47	1.16	6.60	0.11
K-F-D-9/2009-128	D	F	39	NS	Karbala	< 3.5	0.090	0.20	0.62	47.98	0.16	19	0.07	1.65	0.00
K-F-D-9/2009-129	D	M	41	NS	Karbala	< 3.5	0.120	1.01	3.74	219.04	0.85	71	0.20	14.46	0.04
K-F-D-9/2009-130	D	F	52	NS	Karbala	< 3.5	0.010	0.02	0.05	2.82	0.01	1	0.21	0.16	0.03
K-F-D-9/2009-131	D	M	36	NS	Karbala	< 3.5	0.150	0.30	1.19	102.17	0.22	45	0.09	2.12	0.01
K-F-D-9/2009-132	D	M	4	NS	Karbala	< 3.5	0.110	0.23	0.42	48.78	0.37	34	0.13	0.91	< 0.005
K-F-D-9/2009-133	D	F	29	NS	Karbala	< 3.5	0.090	0.11	0.23	7.27	0.58	125	0.10	13.85	0.04
K-F-D-9/2009-134	D	F	30	NS	Karbala	< 3.5	0.120	0.36	0.91	40.52	0.69	86	0.14	11.06	0.12
K-F-D-9/2009-135	D	F	22	NS	Karbala	< 3.5	0.060	0.30	0.23	13.73	3.40	71	0.06	8.74	< 0.005
K-F-D-9/2009-136	D	M	69	NS	Karbala	< 3.5	0.300	1.55	2.95	237.21	0.74	89	0.15	7.61	0.01
K-F-D-9/2009-137	D	F	50	S	Karbala	< 3.5	0.070	0.21	0.62	31.49	0.41	71	0.12	4.00	< 0.005
K-F-D-9/2009-138	D	F	58	NS	Karbala	< 3.5	0.140	1.52	0.96	55.33	3.75	94	0.07	14.34	0.01
K-F-D-9/2009-139	D	M	46	NS	Karbala	< 3.5	0.120	0.40	1.22	83.04	1.85	65	0.11	2.51	< 0.005
K-F-D-9/2009-140	D	M	57	S	Karbala	11.5	0.160	0.29	2.03	52.62	0.86	71	0.12	2.67	0.32
K-F-D-9/2009-141	D	M	67	S	Karbala	< 3.5	0.110	2.05	1.05	139.43	0.60	107	0.16	15.80	0.18
K-F-D-9/2009-142	D	M	50	S	Karbala	< 3.5	0.090	0.18	0.17	16.69	0.57	76	0.11	0.50	0.03
K-F-D-9/2009-143	D	M	42	NS	Karbala	< 3.5	0.310	0.89	1.30	176.30	4.29	78	0.17	4.27	< 0.005
K-F-D-9/2009-144	D	M	54	S	Karbala	< 3.5	0.130	0.49	1.24	69.30	0.54	70	0.13	2.81	0.05
K-F-D-9/2009-145	D	F	35	S	Karbala	< 3.5	0.080	0.23	0.67	41.47	0.59	47	0.09	2.19	< 0.005
K-F-D-9/2009-146	D	F	67	NS	Karbala	< 3.5	0.130	0.21	0.62	41.47	0.62	91	0.07	2.41	0.01
K-F-D-9/2009-147	D	M	69	NS	Karbala	< 3.5	0.090	0.13	0.98	37.35	0.68	68	1.69	8.14	< 0.005
K-F-D-9/2009-148	D	F	50	S	Karbala	< 3.5	0.120	0.35	1.15	50.88	0.92	80	0.12	4.23	0.07
K-F-D-9/2009-149	D	M	46	NS	Karbala	< 3.5	0.110	0.28	0.79	55.67	0.56	69	0.10	5.78	0.02
K-F-D-9/2009-150	D	M	13	NS	Karbala	< 3.5	0.030	1.70	7.11	71.79	0.50	63	0.27	15.86	0.09
K-F-D-9/2009-151	D	M	31	NS	Karbala	9.1	0.180	0.57	1.21	85.70	0.33	48	0.72	3.19	0.02
K-F-D-9/2009-152	D	M	50	S	Karbala	< 3.5	0.150	0.35	1.32	67.19	0.59	59	0.12	2.82	0.01
K-F-D-9/2009-153	D	M	54	NS	Karbala	< 3.5	0.220	0.69	2.20	113.60	0.43	76	0.12	3.83	0.06
K-F-D-9/2009-154	D	F	35	NS	Karbala	< 3.5	0.050	0.34	0.64	11.43	0.52	69	0.23	4.85	0.01

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-D-9/2009-155	D	F	41	NS	Karbala	< 3.5	0.030	0.08	0.28	35.25	0.11	3	0.03	0.64	< 0.005
K-F-D-9/2009-156	D	F	67	NS	Karbala	15.95	0.150	0.44	2.52	67.35	0.60	107	0.10	14.91	0.41
K-F-D-9/2009-157	D	F	23	NS	Karbala	< 3.5	0.070	0.14	0.35	28.40	0.62	73	0.09	9.91	0.04
K-F-D-9/2009-158	D	F	46	NS	Karbala	< 3.5	0.060	0.20	0.47	28.36	0.32	49	0.07	1.08	0.02
K-F-D-9/2009-159	D	F	58	NS	Karbala	5.23	0.210	0.51	1.29	93.55	0.73	70	0.11	1.91	0.04
K-F-D-9/2009-160	D	F	54	NS	Karbala	< 3.5	0.200	0.69	1.08	85.68	0.46	82	0.11	1.99	0.06
K-F-D-9/2009-161	D	M	62	S	Karbala	< 3.5	0.190	0.73	1.28	184.83	0.46	72	0.11	7.99	0.01
K-F-D-9/2009-162	D	F	48	NS	Karbala	< 3.5	0.180	0.69	1.18	73.03	0.47	95	0.15	2.59	0.05
K-F-D-9/2009-163	D	M	60	S	Karbala	< 3.5	0.050	0.15	0.38	20.47	0.50	69	0.10	1.73	0.02
K-F-D-9/2009-164	D	M	48	NS	Karbala	< 3.5	0.070	0.15	0.55	31.11	0.45	97	0.09	3.11	0.03
K-F-D-9/2009-165	D	F	41	NS	Karbala	< 3.5	0.090	0.99	0.74	41.22	2.06	277	0.22	15.72	0.37
K-F-D-9/2009-166	D	F	50	NS	Karbala	< 3.5	0.180	0.96	1.31	77.97	0.83	108	0.12	2.46	0.19
K-F-D-9/2009-167	D	M	54	NS	Karbala	< 3.5	0.060	0.20	0.48	28.89	0.30	43	0.06	1.57	0.19
K-F-D-9/2009-168	D	F	45	NS	Karbala	< 3.5	0.120	0.58	0.59	50.25	0.61	47	0.10	1.00	0.12
K-F-D-9/2009-169	D	F	60	NS	Karbala	< 3.5	0.100	0.35	0.82	54.65	0.98	66	0.08	2.08	< 0.005
K-F-D-9/2009-170	D	F	48	S	Karbala	< 3.5	0.130	0.28	1.23	69.19	0.74	104	0.10	12.25	0.02
K-F-D-9/2009-171	D	F	49	NS	Karbala	< 3.5	0.110	0.37	2.50	39.07	0.81	49	0.08	4.74	0.02
K-F-D-9/2009-172	D	M	57	NS	Karbala	< 3.5	0.190	0.45	1.68	112.01	0.51	55	0.10	4.16	0.04
K-F-D-9/2009-173	D	F	45	NS	Karbala	< 3.5	0.040	0.23	0.39	15.21	0.30	48	0.07	2.06	< 0.005
K-F-D-9/2009-174	D	F	62	NS	Karbala	< 3.5	0.090	0.25	0.74	52.19	0.81	74	0.08	2.91	0.08
K-F-D-9/2009-175	D	M	12	NS	Karbala	< 3.5	0.180	0.40	1.03	93.01	0.75	84	0.16	1.07	1.42
K-F-D-9/2009-176	D	M	70	NS	Karbala	< 3.5	0.100	0.25	0.58	50.17	0.70	100	0.10	13.52	0.13
K-F-D-9/2009-177	D	F	54	NS	Karbala	< 3.5	0.120	0.34	1.13	59.46	0.51	113	0.07	6.00	< 0.005
K-F-D-9/2009-178	D	F	58	NS	Karbala	< 3.5	0.110	0.29	0.75	50.53	0.55	80	0.46	11.61	0.02
K-F-D-9/2009-179	D	M	61	NS	Karbala	< 3.5	0.300	0.70	1.72	140.10	0.44	81	0.14	3.50	0.04

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-D-9/2009-180	D	M	39	NS	Karbala	< 3.5	0.150	0.31	0.96	71.93	0.46	74	0.11	2.18	0.02
K-F-D-9/2009-181	D	M	46	S	Karbala	< 3.5	0.240	0.59	1.50	127.69	0.53	49	0.08	1.82	0.06
K-F-D-9/2009-182	D	F	65	NS	Karbala	< 3.5	0.120	0.28	0.69	59.44	0.29	46	0.07	0.99	< 0.005
K-F-D-9/2009-183	D	F	46	NS	Karbala	< 3.5	0.360	0.99	2.85	196.14	1.10	172	0.21	15.67	0.13
K-F-D-9/2009-184	D	M	45	NS	Karbala	< 3.5	0.040	0.07	0.20	6.59	0.78	91	0.07	2.04	0.08
K-F-D-9/2009-185	D	F	73	NS	Karbala	< 3.5	0.020	0.08	0.33	4.71	0.45	55	0.09	1.42	< 0.005
K-F-D-9/2009-186	D	M	56	S	Karbala	< 3.5	0.170	0.35	1.30	138.53	0.40	155	0.08	12.53	0.03
K-F-D-9/2009-187	D	F	60	NS	Karbala	< 3.5	0.060	0.13	1.97	32.35	0.44	43	0.07	2.56	< 0.005
K-F-D-9/2009-188	D	F	55	NS	Karbala	< 3.5	0.040	0.09	0.31	53.54	0.16	13	0.04	0.74	< 0.005
K-F-D-9/2009-189	D	M	41	NS	Karbala	4.64	0.060	0.10	0.41	16.93	0.27	47	0.11	1.92	< 0.005
K-F-D-9/2009-190	D	M	51	NS	Karbala	< 3.5	0.270	0.54	2.20	136.21	0.36	42	0.12	5.64	0.02
K-F-D-9/2009-191	D	F	48	NS	Karbala	3.61	0.220	0.45	1.27	84.66	0.36	41	0.09	3.77	0.01
K-F-D-9/2009-192	D	M	43	NS	Karbala	< 3.5	0.030	0.14	0.24	15.82	0.08	13	0.02	1.12	0.01
K-F-D-9/2009-193	D	M	28	S	Karbala	< 3.5	0.090	0.18	0.41	32.59	0.42	52	0.05	1.09	0.06
K-F-D-9/2009-194	D	M	27	S	Karbala	< 3.5	0.300	0.70	2.02	153.21	0.56	61	0.10	4.43	0.21
K-F-D-9/2009-195	D	M	25	S	Karbala	< 3.5	0.280	0.88	2.37	122.80	0.32	37	0.11	4.57	< 0.005
K-F-D-9/2009-196	D	M	36	S	Karbala	< 3.5	0.140	0.26	0.75	60.04	0.56	58	0.10	2.03	< 0.005
K-F-D-9/2009-197	D	M	31	NS	Karbala	< 3.5	0.120	0.37	0.78	50.41	0.39	53	0.11	2.82	< 0.005
K-F-D-9/2009-198	D	M	35	NS	Karbala	< 3.5	0.130	0.46	0.81	62.78	0.34	46	0.10	4.50	< 0.005
K-F-D-9/2009-199	D	M	47	S	Karbala	< 3.5	0.180	0.77	4.40	71.32	0.37	62	0.13	5.86	0.02
K-F-D-9/2009-200	D	M	43	NS	Karbala	< 3.5	0.380	2.34	3.32	173.10	0.32	61	0.14	7.54	< 0.005
K-F-D-9/2009-201	D	M	36	S	Karbala	< 3.5	0.400	0.88	2.67	181.03	2.00	50	0.11	4.10	0.02
K-F-D-9/2009-202	D	M	36	S	Karbala	< 3.5	0.180	0.41	1.12	97.84	0.63	88	0.10	3.75	0.10
K-F-D-9/2009-203	D	F	60	S	Karbala	< 3.5	0.070	0.27	0.19	12.17	0.84	74	0.06	0.73	0.03
K-F-D-9/2009-204	D	M	57	S	Karbala	< 3.5	0.130	1.32	5.67	263.71	0.68	60	0.14	7.54	0.06

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
K-F-D-9/2009-205	D	M	67	S	Karbala	< 3.5	0.150	0.38	0.97	73.64	0.67	89	0.08	2.81	0.01
K-F-D-9/2009-206	D	F	73	S	Karbala	< 3.5	0.110	0.18	0.81	30.38	0.42	163	0.17	13.73	< 0.005
K-F-D-9/2009-207	D	M	35	NS	Karbala	< 3.5	0.310	0.65	1.60	135.79	1.27	100	0.13	3.33	0.03
K-F-D-9/2009-208	D	M	43	NS	Karbala	< 3.5	0.350	1.46	2.40	139.83	0.81	152	0.25	11.64	0.02
K-F-D-9/2009-209	D	F	25	NS	Karbala	< 3.5	0.100	0.30	0.70	37.80	0.37	54	0.09	2.70	0.01
K-F-D-9/2009-210	D	M	30	NS	Karbala	< 3.5	0.120	4.12	3.75	75.66	0.79	146	0.32	17.43	< 0.005
K-F-D-9/2009-211	D	M	27	NS	Karbala	< 3.5	0.110	0.25	0.89	52.25	0.46	12	0.04	1.87	< 0.005
K-F-D-9/2009-212	D	M	36	S	Karbala	< 3.5	0.120	0.28	0.74	47.71	0.23	17	0.10	1.07	0.03
K-F-D-9/2009-213	D	M	54	NS	Karbala	< 3.5	0.160	0.23	0.89	38.73	0.49	188	0.16	14.20	0.01
K-F-D-9/2009-214	D	F	58	NS	Karbala	< 3.5	0.100	0.25	0.67	45.79	0.29	30	0.07	2.04	< 0.005
L-F-H-9/2009-215	H	M	45	NS	London	< 3.5	0.013	< 0.005	< 0.005	< 0.025	2.63	56	0.01	0.55	0.04
L-F-H-9/2009-216	H	F	39	NS	London	< 3.5	0.002	< 0.005	< 0.005	< 0.025	3.53	94	0.01	0.22	< 0.005
L-F-H-9/2009-217	H	F	4	NS	London	< 3.5	0.033	< 0.005	< 0.005	< 0.025	4.16	40	< 0.005	0.96	0.33
L-F-H-9/2009-218	H	M	41	NS	London	< 3.5	0.013	0.84	0.03	< 0.025	3.64	73	0.01	0.37	0.03
L-F-H-9/2009-219	H	F	2	NS	London	< 3.5	0.011	< 0.005	< 0.005	< 0.025	7.63	66	< 0.005	0.51	0.04
L-F-H-9/2009-220	H	M	47	NS	London	< 3.5	0.005	< 0.005	0.12	< 0.025	3.29	101	0.02	0.56	0.01
L-F-H-9/2009-221	H	F	42	NS	London	< 3.5	0.010	< 0.005	< 0.005	< 0.025	2.40	61	0.02	0.45	0.00
L-F-H-9/2009-222	H	M	39	NS	London	< 3.5	0.035	< 0.005	< 0.005	< 0.025	3.05	63	< 0.005	0.74	< 0.005
L-F-H-9/2009-223	H	F	34	NS	London	< 3.5	0.022	< 0.005	0.11	< 0.025	4.15	71	< 0.005	0.16	< 0.005
L-F-H-9/2009-224	H	M	45	NS	London	< 3.5	0.003	< 0.005	< 0.005	< 0.025	3.76	55	< 0.005	0.39	0.01
L-F-H-9/2009-225	H	F	16	NS	London	< 3.5	0.008	< 0.005	< 0.005	< 0.025	3.42	50	1.26	0.15	< 0.005
L-F-H-9/2009-226	H	F	40	NS	London	< 3.5	0.016	0.01	0.05	< 0.025	3.24	77	0.09	0.17	0.00
L-F-H-9/2009-227	H	M	43	NS	London	< 3.5	0.037	< 0.005	< 0.005	< 0.025	2.57	83	0.04	0.10	< 0.005
L-F-H-9/2009-228	H	M	5	NS	London	< 3.5	0.010	0.03	< 0.005	< 0.025	3.84	38	< 0.005	0.12	0.02
L-F-H-9/2009-229	H	M	41	NS	London	< 3.5	0.048	< 0.005	< 0.005	< 0.025	3.02	63	0.02	0.14	< 0.005

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
L-F-H-9/2009-230	H	M	23	NS	London	< 3.5	0.004	< 0.005	< 0.005	< 0.025	3.75	98	0.02	0.21	< 0.005
L-F-H-9/2009-231	H	M	62	NS	London	< 3.5	0.012	0.85	0.05	< 0.025	3.79	75	0.01	0.40	0.04
L-F-H-9/2009-232	H	F	56	NS	London	< 3.5	0.028	0.00	0.12	< 0.025	3.45	134	0.03	0.59	0.01
L-F-H-9/2009-233	H	M	38	NS	London	< 3.5	0.009	0.08	< 0.0005	< 0.025	2.36	69	0.03	0.57	< 0.005
L-F-H-9/2009-234	H	M	3	NS	London	< 3.5	0.033	< 0.005	< 0.005	< 0.025	6.52	59	0.01	0.99	< 0.005
L-F-H-9/2009-235	H	F	40	NS	London	< 3.5	0.013	0.07	0.14	< 0.025	3.83	70	< 0.005	1.12	0.33
L-F-H-9/2009-236	H	F	3	NS	London	< 3.5	0.042	< 0.005	< 0.005	< 0.025	2.63	76	< 0.005	0.73	< 0.005
L-F-H-9/2009-237	H	M	6	NS	London	< 3.5	0.005	< 0.005	< 0.005	< 0.025	3.65	81	< 0.005	0.16	< 0.005
L-F-H-9/2009-238	H	M	6	NS	London	< 3.5	0.021	0.03	0.21	< 0.025	5.53	98	0.10	0.78	0.06
L-F-H-9/2009-239	H	M	9	NS	London	< 3.5	0.009	< 0.005	< 0.0005	< 0.025	2.46	70	0.03	0.59	< 0.005
L-F-H-9/2009-240	H	F	11	NS	London	< 3.5	0.014	0.07	0.14	< 0.025	3.69	67	0.02	1.09	0.30
L-F-H-9/2009-241	H	M	42	NS	London	< 3.5	0.001	< 0.005	< 0.0005	< 0.025	3.41	53	0.02	0.69	< 0.005
L-F-H-9/2009-242	H	M	14	NS	London	< 3.5	0.006	< 0.005	0.05	< 0.025	6.47	155	0.08	0.48	0.03
L-F-H-9/2009-243	H	F	33	NS	London	< 3.5	0.008	0.13	0.13	< 0.025	5.85	99	0.03	0.43	0.05
L-F-H-9/2009-244	H	M	48	NS	London	< 3.5	0.052	0.33	0.12	< 0.025	4.40	77	0.02	0.15	0.03
L-F-H-9/2009-245	H	M	45	NS	London	< 3.5	0.012	< 0.005	< 0.005	< 0.025	3.11	69	0.02	0.58	0.00
L-F-H-9/2009-246	H	M	47	NS	London	< 3.5	0.169	< 0.005	0.09	< 0.025	3.46	98	0.03	0.55	0.02
L-F-H-9/2009-247	H	M	41	NS	London	< 3.5	0.003	0.07	0.04	< 0.025	3.24	63	0.01	0.37	0.01
L-F-H-9/2009-248	H	M	38	NS	London	< 3.5	0.001	< 0.005	< 0.005	< 0.025	2.44	64	0.02	0.59	< 0.005
L-F-H-9/2009-249	H	M	45	NS	London	< 3.5	0.023	< 0.005	< 0.005	< 0.025	3.42	65	0.02	0.61	0.02
L-F-H-9/2009-250	H	M	47	NS	London	< 3.5	0.002	0.05	0.04	< 0.025	2.97	96	0.02	0.51	0.02
L-F-H-9/2009-251	H	M	45	NS	London	< 3.5	0.040	< 0.005	< 0.005	< 0.025	2.82	61	0.02	0.58	< 0.005
L-F-H-9/2009-252	H	M	45	NS	London	< 3.5	0.055	< 0.005	< 0.005	< 0.025	2.70	60	0.02	0.48	< 0.005
L-F-H-9/2009-253	H	M	42	NS	London	< 3.5	0.023	< 0.005	0.01	< 0.025	4.97	143	0.06	0.34	0.01
L-F-H-9/2009-254	H	M	42	NS	London	< 3.5	0.029	< 0.005	0.01	< 0.025	5.13	117	0.05	0.27	0.05

Table F3.5 (continued)

Sample description						Elemental level (mg/kg)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
L-F-H-9/2009-255	H	F	33	NS	London	< 3.5	0.004	0.33	0.14	< 0.025	4.82	67	< 0.005	0.42	0.06
L-F-H-9/2009-256	H	M	48	NS	London	< 3.5	0.039	0.05	< 0.005	< 0.025	3.79	68	0.01	0.17	< 0.005
L-F-H-9/2009-257	H	F	8	NS	London	< 3.5	0.099	0.12	1.24	14.26	3.99	91	0.09	9.56	0.05
L-F-H-9/2009-258	H	F	22	NS	London	< 3.5	0.039	< 0.005	0.18	< 0.025	3.24	96	0.08	6.13	0.01
L-F-H-9/2009-259	H	F	21	NS	London	< 3.5	0.125	0.14	0.42	37.18	6.45	75	0.04	0.97	0.00

HS = health status, G = gender, y = year, SA = smoking activity, K-F-H-9/2009-1, K corresponds to the province in Iraq (K) Karbala and may be replaced by (L) London in the UK; F corresponds to fingernails, H corresponds to healthy and may be replaced by D (diabetes); 9/2009 corresponds to the date (month/year); and 1 corresponds to the sample code number.

Appendix F3

Paired Sample Results:

Table F3.6: Paired tear drops and fingernail samples (n = 51) for healthy individuals from Karbala (Iraq).

Sample description						Elemental level									
						Tear drop (µg/l)					Fingernails (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TF1	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	< 3500	130.5	404	637	27356
TF2	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	< 3500	80.3	218	854	31213
TF3	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	< 3500	46.2	86	6064	10874
TF4	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	< 3500	56.3	107	396	5393
TF5	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	10087	252.8	525	2284	41372
TF6	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	< 3500	81.5	151	534	16770
TF7	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	< 3500	33.5	66	7295	9537
TF8	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	< 3500	44.8	65	531	7021
TF9	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	< 3500	120.2	230	434	18802
TF10	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	< 3500	241.0	948	1429	96891
TF11	H	M	42	NS	Karbala	616	3.43	7.07	19.62	335	< 3500	116.6	339	980	37712
TF12	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	< 3500	52.4	52	15842	< 25
TF13	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	< 3500	147.1	68	551	< 25
TF14	H	M	38	NS	Karbala	< 70	3.71	17.8	38.95	674	13472	135.1	472	1361	105608
TF15	H	M	40	NS	Karbala	252	2.75	6.2	22.98	465	420	156.3	314	1063	49022
TF16	H	M	40	S	Karbala	< 70	3.7	9.13	35.83	427	< 3500	277.4	966	4714	188652
TF17	H	M	23	NS	Karbala	446	21.08	31.17	270.08	2816	< 3500	468.0	946	13224	234761
TF18	H	M	44	NS	Karbala	504	4.28	7.33	9.9	344	< 3500	339.8	820	2803	129967
TF19	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	< 3500	54.5	285	613	16159
TF20	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	< 3500	138.2	333	992	48322
TF21	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	< 3500	89.1	215	765	39157
TF22	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	12310	103.9	491	716	28560
TF23	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	< 3500	257.3	853	1149	67888
TF24	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	< 3500	391.2	880	2937	156653
TF25	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	< 3500	133.5	200	342	27629
TF26	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	< 3500	43.3	9	297	< 25

Table F3.6 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop (µg/l)					Fingernails (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	B	V	Cr	Mn	Fe
TF27	H	M	33	S	Karbala	785	1.06	5.66	9.78	284	< 3500	22	161	192	< 25
TF28	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	< 3500	169.1	318	1282	64247
TF29	H	F	16	NS	Karbala	303	17.17	13.01	26.13	507	< 3500	572.2	3455	2496	197123
TF30	H	F	37	NS	Karbala	522	5.55	15.84	23.44	852	< 3500	888.4	1810	4867	325194
TF31	H	F	30	NS	Karbala	389	2.68	2.11	14.3	171	< 3500	85.5	159	439	1104
TF32	H	F	13	NS	Karbala	382	0.48	1.35	7.42	46	< 3500	173.7	170	564	27480
TF33	H	F	36	S	Karbala	367	7.16	15.43	95.86	973	< 3500	154.4	280	1261	51517
TF34	H	F	65	NS	Karbala	415	1.72	4.16	14.88	261	< 3500	154.0	288	1426	48841
TF35	H	F	53	S	Karbala	294	1.16	2.45	10.61	127	< 3500	88.7	509	613	23382
TF36	H	M	25	NS	Karbala	393	5.7	14.8	111.64	1023	< 3500	33.7	142	52	< 25
TF37	H	F	23	NS	Karbala	< 70	3.77	13.21	7.52	682	< 3500	22.2	219	167	55970
TF38	H	F	45	NS	Karbala	412	1.99	2.69	23.1	150	< 3500	20.0	1265	1272	164673
TF39	H	M	28	S	Karbala	399	5.5	12.25	76.56	854	< 3500	37.8	87	358	3602
TF40	H	F	19	NS	Karbala	507	16.48	18.67	158.94	1796	< 3500	106.0	85	153	< 25
TF41	H	F	19	NS	Karbala	419	1.19	3.49	16.01	35	< 3500	100.1	158	281	21924
TF42	H	F	20	NS	Karbala	335	18.28	2.87	7.62	177	< 3500	24.4	51	970	< 25
TF43	H	F	21	NS	Karbala	387	2.95	6.86	46.79	238	< 3500	6.8	13	162	< 25
TF44	H	F	19	NS	Karbala	244	4.54	8.22	66.72	134	< 3500	8.8	52	4212	< 25
TF45	H	M	22	NS	Karbala	676	6.38	10.62	67.43	1199	< 3500	55.7	329	120	< 25
TF46	H	F	22	NS	Karbala	344	20.55	47.09	534.13	9300	< 3500	51.2	79	92	< 25
TF47	H	F	23	NS	Karbala	297	6.96	10.18	100.52	743	< 3500	57.4	85	814	32430
TF48	H	F	19	NS	Karbala	366	2.87	5.24	32.83	211	< 3500	29.4	53	302	1929
TF49	H	F	21	NS	Karbala	472	15.34	1.47	8.08	58	< 3500	72.1	267	2032	29293
TF50	H	F	24	NS	Karbala	451	12.87	11.96	153.67	982	< 3500	64.2	82	1128	14085
TF51	H	M	28	S	Karbala	360	7.24	13.27	84.49	1273	< 3500	72.9	164	1658	21997
PIN	HS	G	Age (y)	S	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TF1	H	M	33	NS	Karbala	91	263	0.08	143	0.15	3715	122821	113	4780	60
TF2	H	M	20	NS	Karbala	49	1175	33.94	689	0.16	2774	70211	31	5753	33
TF3	H	M	20	NS	Karbala	254	1134	1.74	475	1.51	4592	64161	54	757	8

Table F3.6 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop (µg/l)					Fingernails (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TF4	H	M	19	NS	Karbala	112	1327	14.89	248	0.31	2981	87806	79	3648	< 5
TF5	H	M	45	NS	Karbala	79	49	0.69	104	0.15	976	32907	53	7124	< 5
TF6	H	M	38	NS	Karbala	148	352	1.8	425	0.58	2116	32057	21	3079	28
TF7	H	M	21	S	Karbala	255	4100	9.56	1359	6.12	3203	100173	79	1684	14
TF8	H	F	40	S	Karbala	26	126	1.22	464	0.29	3227	78509	74	2039	56
TF9	H	M	42	NS	Karbala	427	494	1.34	58	0.34	3896	63267	31	2500	46
TF10	H	F	12	NS	Karbala	241	382	2.85	314	1.12	5693	79764	128	9235	22
TF11	H	M	42	NS	Karbala	280	355	2.11	246	1.26	2869	50422	82	4013	53
TF12	H	M	20	NS	Karbala	244	753	2.67	552	1.43	3253	81353	33	998	82
TF13	H	M	10	NS	Karbala	218	295	44.82	306	2.41	4449	91370	65	2010	52
TF14	H	M	38	NS	Karbala	170	386	3.54	534	1.55	1681	31195	61	4173	32
TF15	H	M	40	NS	Karbala	198	224	1.42	262	0.36	5397	153490	110	4735	22
TF16	H	M	40	S	Karbala	203	416	3.71	442	1.32	5534	100541	72	3954	67
TF17	H	M	23	NS	Karbala	547	4109	6.5	461	5.98	3248	70733	114	9996	20
TF18	H	M	44	NS	Karbala	196	460	0.48	140	0.75	11149	145556	160	12116	154
TF19	H	M	33	NS	Karbala	589	1936	20.89	678	4.23	6337	133139	133	2789	< 5
TF20	H	F	42	NS	Karbala	157	169	1.37	175	0.75	4281	50010	47	3380	88
TF21	H	M	37	NS	Karbala	431	1022	5.15	587	1.35	2631	87678	87	7548	< 5
TF22	H	M	43	NS	Karbala	318	277	2.67	345	0.57	3526	86901	115	7971	22
TF23	H	F	45	NS	Karbala	260	495	1.59	388	0.21	5026	90349	147	4575	13
TF24	H	M	8	NS	Karbala	313	598	1.83	271	1.17	2918	30717	82	5283	15
TF25	H	F	14	NS	Karbala	436	404	1.54	513	0.91	2972	75510	108	3824	10
TF26	H	F	33	NS	Karbala	102	169	1.68	118	0.48	1955	39741	85	1324	26
TF27	H	M	33	S	Karbala	193	187	1.56	112	2.18	4089	127499	94	851	34
TF28	H	M	35	NS	Karbala	356	173	2.16	307	0.64	7845	137326	127	7123	< 5
TF29	H	F	16	NS	Karbala	402	10150	4.23	303	5.26	4788	348801	306	18056	11
TF30	H	F	37	NS	Karbala	403	393	1.75	470	5.00	23027	172970	277	14811	113
TF31	H	F	38	NS	Karbala	35	663	1.18	489	1.27	3730	89112	87	8128	21
TF32	H	F	17	NS	Karbala	37	657	35.79	89	0.28	5942	98313	134	2548	330
TF33	H	F	36	S	Karbala	741	2406	7.98	694	3.70	3116	75081	87	3339	836
TF34	H	F	65	NS	Karbala	127	300	0.8	325	0.34	5345	144223	90	6695	10

Table F3.6 (continued)

Sample description						Elemental level (µg/l)									
						Tear drop (µg/l)					Fingernails (µg/kg)				
PIN	HS	G	Age (y)	SA	Location	Cu	Zn	As	Sr	Cd	Cu	Zn	As	Sr	Cd
TF35	H	F	53	S	Karbala	107	115	1.24	203	1.26	5984	143998	56	2248	101
TF36	H	M	25	NS	Karbala	291	2010	1.81	478	3.05	3843	98278	63	1566	19
TF37	H	F	23	NS	Karbala	130	161	2.46	241	8.85	16354	93871	96	1077	< 5
TF38	H	F	45	NS	Karbala	155	276	1.38	346	0.29	4642	93536	14	1436	19
TF39	H	M	28	S	Karbala	540	3748	12.64	1094	4.66	3796	127291	58	1652	11
TF40	H	F	19	NS	Karbala	310	2672	14.12	457	2.65	5602	119843	32	741	< 5
TF41	H	F	19	NS	Karbala	216	299	1.41	278	1.81	3020	91075	30	542	68
TF42	H	F	20	NS	Karbala	126	580	2.48	258	2.04	5711	94893	9	3368	17
TF43	H	F	21	NS	Karbala	564	1011	3.67	413	0.95	3186	95527	29	664	6
TF44	H	F	19	NS	Karbala	536	1747	7.61	574	4.71	2864	94815	14	4443	27
TF45	H	M	22	NS	Karbala	367	1440	15.4	654	1.08	3281	124508	68	1871	< 5
TF46	H	F	22	NS	Karbala	690	2923	16.49	1183	10.77	3803	102684	16	572	22
TF47	H	F	23	NS	Karbala	210	1197	3.91	408	1.49	3306	78001	48	4637	< 5
TF48	H	F	19	NS	Karbala	182	466	1.84	491	0.63	2528	66319	12	1826	28
TF49	H	F	21	NS	Karbala	130	697	12.01	249	1.06	3966	80361	39	6179	162
TF50	H	F	24	NS	Karbala	539	1395	3.25	607	4.47	5541	111033	73	3118	52
TF51	H	M	28	S	Karbala	521	6334	10.01	592	3.52	4218	112503	42	2748	< 5

HS = health status, G = gender, H = healthy, M = male, F = female, SA = smoking activity, S = smoker, NS = non-smoker, y = year, TF1, T corresponds to tear drops, F corresponds to fingernails; and 1 corresponds to the sample code number.

Comparison Study:

Table F3.7: Summary of F-test and a two tailed t-test results for elemental levels in washed fingernail samples of healthy and diabetic individuals from Karbala, Iraq.

Element (n1, n2)	F-Test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	EVA	nd		nd			
(10, 8)	UVA						
V	EVA	13.515	0.000	1.092	212[+]	0.276	
(127, 87)	UVA			1.224	196[++]	0.222	
Cr	EVA	0.953	0.330	1.782	212	0.076	
(127, 87)	UVA			1.716	160	0.088	
Mn	EVA	19.930	0.000	2.496	212	0.013	
(127, 87)	UVA			2.900	165	**0.004**	1.97
Fe	EVA	nd		nd			
(103, 87)	UVA						
Cu	EVA	17.023	0.000	6.599	212	0.000	
(127, 87)	UVA			7.942	130	**0.000**	198
Zn	EVA	1.313	0.253	2.289	212	**0.023**	1.97
(127, 87)	UVA			2.398	209	0.017	
As	EVA	0.400	0.528	1.808	212	0.072	
(127, 87)	UVA			1.686	139	0.094	
Sr	EVA	8.450	0.004	1.584	212	0.115	
(127, 87)	UVA			1.526	160	0.129	
Cd	EVA	nd		nd			
(94, 62)	UVA						

EVA and UVA are equal variances assumed and unequal variances assumed, nd = not determined due to there being several samples which were below the limit of detection (Table 2.17), n_1, n_2 are the number of samples for healthy individuals and diabetic patients, respectively, *df* = degrees of freedom, n_1-1 and n_2-1 for F-test, [+] degrees of freedom for t-test (n_1+n_2-2), [++] degrees of freedom for t-test determined as described in Appendix C , F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at P = 0.05, reported for significant value, the **bold** values indicate significant differences at the level of significance $P < 0.05$, Sig. = level of significance.

Table F3.8: Summary of F-test and a two tailed t-test results for elemental levels in washed fingernail samples for individuals from the healthy population of Karbala and London.

Element (n1, n2)	F-Test for equality of variances			t-test for equality of means			
	Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B (10, 0)	EVA	nd		nd			
	UVA						
V (127, 32)	EVA	25.776	0.000	5.224	170^{+}	0.000	
	UVA			8.433	148^{++}	**0.000**	1.97
Cr (171, 17)	EVA	nd		nd			
	UVA						
Mn (127, 21)	EVA	nd		nd			
	UVA						
Fe (103, 2)	EVA	nd		nd			
	UVA						
Cu (127, 45)	EVA	5.433	0.021	1.385	170	0.168	
	UVA			2.228	150	**0.027**	1.97
Zn (127, 45)	EVA	6.094	0.015	1.339	170	0.182	
	UVA			1.852	160	0.066	1.97
As (127, 35)	EVA	nd		nd			
	UVA						
Sr (127, 45)	EVA	24.658	0.000	5.905	170	0.000	
	UVA			8.438	167	**0.000**	1.97
Cd (94, 28)	EVA	nd		nd			
	UVA						

n_1, n_2 are the number of samples for Karbala and London, respectively, nd = not determined due to there are several samples were below the limit of detection (Table 2.17). Other key words can take from Table F3.7.

Table F3.9: Summary of F-test and a two tailed t-test results for elemental levels in tear drops and washed fingernail for individuals from the healthy population of Karbala who provided both media

TE*	Mean (µg/l) (T, F)	F-Test for equality of variances			t-test for equality of means			
		Variance	F_{calc}	*Sig.*	t_{calc}	*df*	Sig.	t_{crit}
B	(nd,nd)	EVA	nd		nd			
		UVA						
V	(5.7, 138.4)	EVA	31.930	0.00	5.972	100^{+}	0.00	
		UVA			5.972	50^{++}	**0.00**	2.01
Cr	(12, 398)	EVA	31.319	0.00	4.859	100	0.00	
		UVA			4.859	50	**0.00**	2.01
Mn	(53, 1877)	EVA	27.362	0.00	4.301	104	0.00	
		UVA			4.301	50	**0.00**	2.01
Fe	(749, 62003)	EVA	55.994	0.00	6.030	89	0.00	
		UVA			5.332	39	**0.00**	2.02
Cu	(279, 4704)	EVA	19.422	0.00	8.906	100	0.00	
		UVA			8.906	50	**0.00**	2.01
Zn	(1244, 97495)	EVA	30.514	0.00	14.105	100	0.00	
		UVA			14.105	50	**0.00**	2.01
As	(6.6, 79.7)	EVA	31.558	0.00	8.937	100	0.00	
		UVA			8.937	53	**0.00**	2.01
Sr	(424, 4304)	EVA	49.191	0.00	7.567	100	0.00	
		UVA			7.567	51	**0.000**	2.01
Cd	(2.1, 68.5)	EVA	14.028	0.00	3.492	90	0.001	
		UVA			3.127	40	**0.002**	2.02

T = tear drops, F = fingernail, nd = not determined due to there are several samples were bellow the limit of detection (Table 2.17), * n = 51, the only exception are for B in tear drops (n = 44) and washed fingernails (n = 4); Fe in washed fingernails (n = 40) and Cd in washed fingernails (n = 41), TE is trace element. Other key words can take from Table F3.7.

Appendix F4

Comparison Study Between the Four Biological Samples:

Table F4.1: Paired tear drops, saliva, washed scalp hair and fingernail samples (n = 30) from Karbala (Iraq).

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
T1	H	M	33	NS	Karbala	314	1.34	4.65	12.88	270	91	263	0.1	143	0.15
T2	H	M	20	NS	Karbala	310	0.94	0.98	6.63	71	49	1175	33.9	689	0.16
T3	H	M	20	NS	Karbala	426	3.42	5.66	52.62	239	254	1134	1.7	475	1.51
T4	H	M	19	NS	Karbala	455	1.09	1.67	7.97	198	112	1327	14.9	248	0.31
T5	H	M	45	NS	Karbala	345	0.77	2.15	5.17	202	79	49	0.7	104	0.15
T6	H	M	38	NS	Karbala	< 70	2.55	14.31	35.67	393	148	352	1.8	425	0.58
T7	H	M	21	S	Karbala	412	16.91	68.39	42.58	1041	255	4100	9.6	1159	6.13
T8	H	F	40	S	Karbala	208	0.95	0.73	8.67	13	26	126	1.2	464	0.29
T9	H	M	42	NS	Karbala	< 70	2.89	21.79	9.43	325	427	494	1.3	58	0.34
T10	H	F	12	NS	Karbala	709	2.69	7.45	42.36	368	241	382	2.8	314	1.12
T11	H	M	42	NS	Karbala	616	3.43	7.07	19.63	335	280	355	2.1	246	1.26
T12	H	M	20	NS	Karbala	< 70	2.62	3.91	48.38	184	244	753	2.7	552	1.43
T13	H	M	8	NS	Karbala	748	8.37	30.79	31.82	1779	313	598	1.8	271	1.17
T14	H	M	10	NS	Karbala	853	5.37	10.73	12.65	920	218	295	44.8	306	2.41
T15	H	M	38	NS	Karbala	< 70	3.71	17.80	38.95	674	170	386	3.5	534	1.55
T16	H	M	40	NS	Karbala	252	2.75	6.20	22.98	465	198	224	1.4	262	0.36
T17	H	M	40	S	Karbala	< 70	3.70	9.13	35.83	427	203	416	3.7	442	1.32
T18	H	M	23	NS	Karbala	446	21.09	31.17	270.08	2816	547	4109	6.5	461	5.98
T19	H	M	44	NS	Karbala	504	4.28	7.33	9.90	344	196	460	0.5	140	0.75
T20	H	M	33	NS	Karbala	384	8.23	64.77	102.19	1312	589	1936	20.9	678	4.23
T21	H	M	43	NS	Karbala	440	5.40	42.60	62.94	152	421	1507	5.2	935	1.40
T22	H	F	42	NS	Karbala	316	2.11	2.84	15.41	219	157	169	1.4	175	0.75
T23	H	M	37	NS	Karbala	< 70	11.13	21.48	93.83	1523	431	1022	5.2	587	1.35
T24	H	M	43	NS	Karbala	398	5.37	9.41	6.78	980	318	277	2.7	345	0.57
T25	H	F	45	NS	Karbala	411	3.09	9.36	23.77	488	260	495	1.6	388	0.21
T26	H	F	37	NS	Karbala	522	5.56	15.84	23.44	852	403	393	1.7	470	5.00
T27	H	F	14	NS	Karbala	469	2.93	4.03	34.92	325	436	404	1.5	513	0.91
T28	H	F	33	NS	Karbala	546	1.05	1.86	10.53	108	102	169	1.7	118	0.48
T29	H	M	33	S	Karbala	785	1.06	5.67	9.78	284	193	187	1.6	112	2.19
T30	H	M	35	NS	Karbala	455	1.18	10.76	14.09	107	356	173	2.2	307	0.64

Appendix F4

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
S1	H	M	33	NS	Karbala	364	0.39	0.14	0.55	100	15	90	4.3	112	< 0.1
S2	H	M	20	NS	Karbala	410	1.36	0.57	2.91	55	26	146	2.7	69	0.24
S3	H	M	20	NS	Karbala	168	0.31	0.24	0.72	49	8	29	7.7	42	0.24
S4	H	M	19	NS	Karbala	489	1.47	0.78	11.45	110	63	180	2.2	415	0.32
S5	H	M	45	NS	Karbala	86	0.09	0.18	0.87	8	16	19	0.7	21	0.26
S6	H	M	38	NS	Karbala	163	0.20	0.60	2.15	25	14	58	0.5	66	0.12
S7	H	M	21	S	Karbala	166	0.13	0.10	1.08	14.3	6.5	30	0.5	18	0.26
S8	H	F	40	S	Karbala	671	0.63	0.47	14.45	77	29	313	23.2	331	1.03
S9	H	M	42	NS	Karbala	125	0.10	0.16	0.59	5	7	21	0.7	37	0.18
S10	H	F	12	NS	Karbala	119	0.12	< 0.1	5.67	22.0	8.5	58	5.5	60	< 0.1
S11	H	M	42	NS	Karbala	198	0.83	0.20	6.08	70	13	103	8.8	163	0.22
S12	H	M	20	NS	Karbala	1254	1.79	0.82	7.79	108	28	402	7.6	1324	0.18
S13	H	M	8	NS	Karbala	255	0.21	0.19	1.94	12	19	19	1.8	48	0.25
S14	H	M	10	NS	Karbala	94	0.12	0.22	0.47	7	5	9	1.4	11	< 0.1
S15	H	M	38	NS	Karbala	444	0.71	0.45	2.41	80	17	113	0.6	81	< 0.1
S16	H	M	40	NS	Karbala	169	0.14	< 0.1	4.67	20	14	92	1.4	39	0.11
S17	H	M	40	S	Karbala	153	0.19	0.11	3.04	2	3	7	2.3	148	< 0.1
S18	H	M	23	NS	Karbala	37	0.17	< 0.1	0.43	9	10	14	0.2	11	0.12
S19	H	M	44	NS	Karbala	256	0.80	0.24	1.44	7	6	55	1.8	171	< 0.1
S20	H	M	33	NS	Karbala	96	0.14	0.12	1.42	6	3	42	0.2	5	< 0.1
S21	H	M	43	NS	Karbala	153	0.25	0.15	0.45	9.3	4.8	13	3.0	18	0.14
S22	H	F	42	NS	Karbala	247	0.10	0.32	4.06	40	14	121	5.7	47	0.13
S23	H	M	37	NS	Karbala	236	0.48	0.39	2.48	32	14	86	2.1	14	< 0.1
S24	H	M	43	NS	Karbala	173	0.22	0.08	1.22	9	5	23	3.3	69	< 0.1
S25	H	F	45	NS	Karbala	224	0.34	0.09	5.27	29	69	45	3.7	107	0.13
S26	H	F	37	NS	Karbala	< 70	0.06	0.01	1.60	9.7	8.0	28	2.7	17	< 0.1
S27	H	F	14	NS	Karbala	213	0.48	0.17	4.35	29	12	52	4.0	86	0.14
S28	H	F	33	NS	Karbala	124	0.09	0.11	2.48	9	14	29	0.4	27	< 0.1
S29	H	M	33	S	Karbala	82	0.08	0.26	1.21	12	3	26	0.2	17	< 0.1
S30	H	M	35	NS	Karbala	369	0.58	< 0.1	2.72	66	12	112	6.0	203	0.16

Appendix F4

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
SH1	H	M	33	NS	Karbala	< 3500	144	259	971	25381	7059	78567	48	2592	138
SH2	H	M	20	NS	Karbala	< 3500	172	309	1103	21185	9824	117027	59	3787	382
SH3	H	M	20	NS	Karbala	6077	89	44	420	5009	10065	101898	52	2715	73
SH4	H	M	19	NS	Karbala	3627	102	187	518	10854	5975	155678	23	4407	81
SH5	H	M	45	NS	Karbala	< 3500	117	158	784	19973	10741	128015	29	3145	621
SH6	H	M	38	NS	Karbala	< 3500	85	37	532	8070	6641	78314	55	1497	195
SH7	H	M	21	S	Karbala	< 3500	220	60	710	4400	6900	144000	< 5	11860	100
SH8	H	F	40	S	Karbala	< 3500	121	21	1557	3060	5762	65323	< 5	49050	2050
SH9	H	M	42	NS	Karbala	< 3500	64	49	375	7427	6574	44693	32	1159	67
SH10	H	F	12	NS	Karbala	< 3500	60	34	575	4164	4924	148833	5	4990	100
SH11	H	M	42	NS	Karbala	< 3500	67	55	415	6415	6282	125824	40	3122	56
SH12	H	M	20	NS	Karbala	< 3500	94	88	539	12127	5415	67059	10	2519	16
SH13	H	M	8	NS	Karbala	< 3500	101	102	601	12176	5936	126291	8	2531	60
SH14	H	M	10	NS	Karbala	< 3500	180	287	819	13264	5710	140812	79	947	750
SH15	H	M	38	NS	Karbala	< 3500	32	204	160	2910	5520	137855	10	1314	135
SH16	H	M	40	NS	Karbala	< 3500	200	132	986	16009	8256	133190	31	1158	75
SH17	H	M	40	S	Karbala	< 3500	39	64	232	3979	6534	139055	< 5	8475	563
SH18	H	M	23	NS	Karbala	< 3500	217	300	1342	16963	4949	134024	< 5	4681	110
SH19	H	M	44	NS	Karbala	< 3500	614	390	1066	10672	6737	161123	5	17807	133
SH20	H	M	33	NS	Karbala	< 3500	40	207	142	2941	2822	89405	< 5	836	44
SH21	H	M	43	NS	Karbala	< 3500	260	70	3350	11401	6501	205212	< 5	41592	310
SH22	H	F	42	NS	Karbala	< 3500	189	28	2237	1875	5797	139879	< 5	39559	789
SH23	H	M	37	NS	Karbala	< 3500	240	331	953	31503	5708	64529	86	1039	91
SH24	H	M	43	NS	Karbala	< 3500	417	236	1358	15075	5174	113666	39	7351	162
SH25	H	F	45	NS	Karbala	< 3500	70	40	290	12200	8200	105000	< 5	710	40
SH26	H	F	37	NS	Karbala	< 3500	400	220	560	9501	4502	421231	6	35292	200
SH27	H	F	14	NS	Karbala	< 3500	334	84	762	11401	2871	434110	< 5	13999	553
SH28	H	F	33	NS	Karbala	< 3500	244	20	953	5269	3668	432334	< 5	24912	130
SH29	H	M	33	S	Karbala	< 3500	396	221	556	9476	4547	420762	6	35288	196
SH30	H	M	35	NS	Karbala	< 3500	91	179	444	5657	5784	46754	55	773	68

Appendix F4

Sample description						Elemental level (µg/l)									
PIN	HS	G	Age (y)	SA	Location	B	V	Cr	Mn	Fe	Cu	Zn	As	Sr	Cd
FN1	H	M	33	NS	Karbala	< 3500	131	404	637	27356	3715	122821	113	4780	60
FN2	H	M	20	NS	Karbala	< 3500	80	218	854	31213	2774	70211	31	5753	33
FN3	H	M	20	NS	Karbala	< 3500	46	86	6064	10874	4592	64161	54	757	8
FN4	H	M	19	NS	Karbala	< 3500	56	107	396	5393	2981	87806	79	3648	< 5
FN5	H	M	45	NS	Karbala	10087	253	526	2284	41372	976	32907	53	7124	< 5
FN6	H	M	38	NS	Karbala	< 3500	82	151	534	16770	2116	32057	21	3079	28
FN7	H	M	21	S	Karbala	< 3500	34	66	7295	9537	3203	100173	79	1684	14
FN8	H	F	40	S	Karbala	< 3500	45	65	531	7021	3227	78509	74	2039	56
FN9	H	M	42	NS	Karbala	< 3500	120	230	434	18802	3896	63267	31	2500	46
FN10	H	F	12	NS	Karbala	< 3500	241	948	1429	96891	5693	79764	128	9235	22
FN11	H	M	42	NS	Karbala	< 3500	117	339	980	37712	2869	50422	82	4013	53
FN12	H	M	20	NS	Karbala	< 3500	52	52	15842	< 25	3253	81353	33	998	82
FN13	H	M	8	NS	Karbala	< 3500	391	880	2937	156653	2918	30717	82	5283	15
FN14	H	M	10	NS	Karbala	< 3500	147	68	551	< 25	4449	91370	65	2010	52
FN15	H	M	38	NS	Karbala	13472	135	472	1361	105608	1681	31195	61	4173	32
FN16	H	M	40	NS	Karbala	420	156	314	1063	49022	5397	153490	110	4735	22
FN17	H	M	40	S	Karbala	< 3500	277	966	4714	188652	5534	100541	72	3954	67
FN18	H	M	23	NS	Karbala	< 3500	468	946	13224	234761	3248	70733	114	9996	20
FN19	H	M	44	NS	Karbala	< 3500	340	820	2803	129967	11149	145556	160	12116	154
FN20	H	M	33	NS	Karbala	< 3500	54	285	613	16159	6337	133139	133	2789	< 5
FN21	H	M	43	NS	Karbala	< 3.5	140	231	782	42870	5422	137213	110	3632	23
FN22	H	F	42	NS	Karbala	< 3500	138	333	992	48322	4281	50010	47	3380	88
FN23	H	M	37	NS	Karbala	< 3500	89	215	765	39157	2631	87678	87	7548	< 5
FN24	H	M	43	NS	Karbala	12310	104	491	716	28560	3526	86901	115	7971	22
FN25	H	F	45	NS	Karbala	< 3500	257	853	1149	67888	5026	90349	147	4575	13
FN26	H	F	37	NS	Karbala	< 3500	888	1810	4867	325194	23027	172970	277	14811	113
FN27	H	F	14	NS	Karbala	< 3500	134	200	342	27629	2972	75510	108	3824	10
FN28	H	F	33	NS	Karbala	< 3500	43	9	297	< 25	1955	39741	85	1324	26
FN29	H	M	33	S	Karbala	< 3500	22	161	192	< 25	4089	127499	94	851	34
FN30	H	M	35	NS	Karbala	< 3500	169	318	1282	64247	7845	137326	127	7123	< 5

H = health status, G = gender, H = healthy, M = male, F = female, SA = smoking activity, S = smoker, NS = non-smoker, y = year, T = tear drops, S = saliva, SH = scalp hair, FN = fingernails.

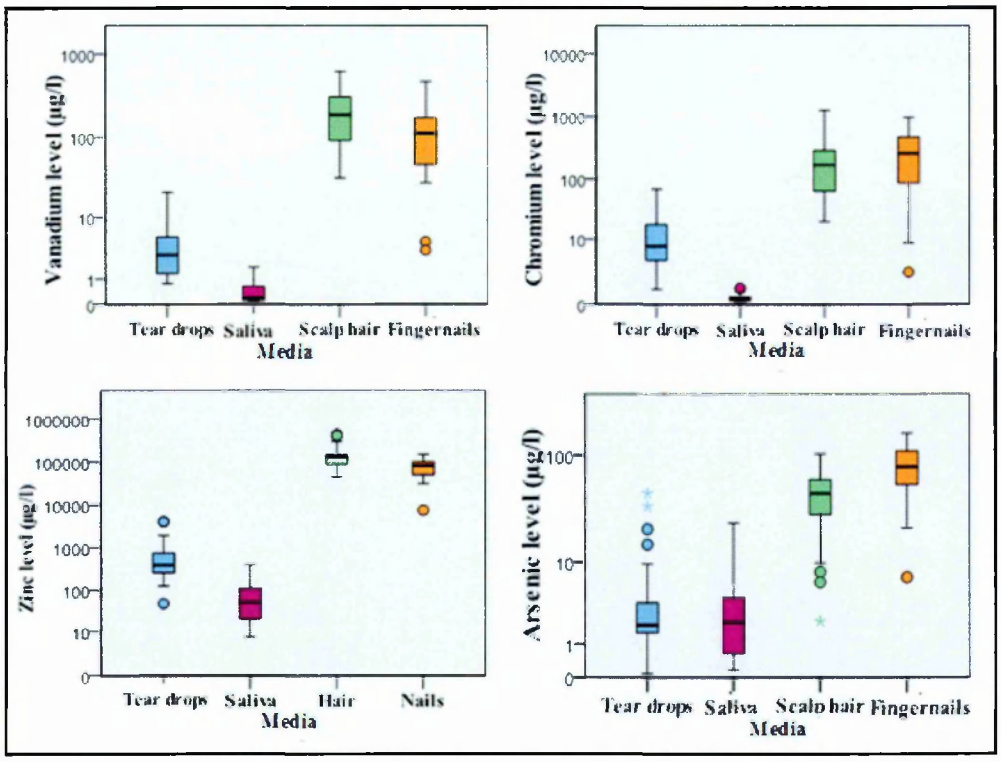

Figure F4.1: Vanadium, Cr, Zn and As levels in tear drops, saliva, washed scalp hair and fingernails for healthy individuals (n = 30), Middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively. Circles represent outliers, whereas "*" represents extreme values.