



University of Aden
Faculty Of Education-Aden
Chemistry Department

Trace-Element Concentrations In Hair Of Students From Two Primary Schools In Aden-Yemen - A comparison study-

Presented By

ADEL AHMED MOHAMMED SAEED

Under Supervision

Associate professor

Dr. MANSOUR MOHAMMED HASSAN

Aden University

This dissertation is presented in a partially fulfillment of the requirements
of the Master degree in Chemistry Science field awarded by
University of Aden, Yemen

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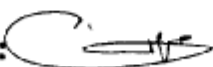
Certificate

This is to certify that this thesis has been done under my supervision and I forward it to the Faculty of Education, University of Aden, for the debate of Master Degree in Chemistry.

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Thesis Approval Sheet

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Dedication

In memory of my father

To my family, teachers & friends

To all people who helped me

Acknowledgments

In the name of Almighty Allah, the Merciful the Sustainer of the worlds and may peace and blessing upon the prophet Mohammed, his family, all his companions and those who follow his religious teachings to hereafter.

I would like to express my special thanks to my supervisor Associate professor Dr. Mansour Mohammed Hassan, who helped and guided me with great patience to accomplish and understanding this to make work.

Special thanks to the directors of both the fish (Al-Tawahi-Aden) and Al-Areesh laboratories (Al-Areesh-Aden) for their help and their teams for cooperation and help in the experimental work.

Many thanks to the team of Aden Central laboratory for their cooperation in the analysis.

I cannot find words to thank both teams of Micro Analytical Center (Cairo University) and Central unit of analysis and scientific services- Atomic Absorption unit (National Research Center- Cairo).

I appreciate the cooperation and understanding of the heads and teachers of the two primary schools: Al-Areesh and 30 November, where hair samples are collected.

My sincere thanks and respect to the dean of my college and chemistry department staff.

My deepest gratitude to Dr. Raby Abdo Ahmed (psychology department / Qina University-Egypt) for helping on statistic part.

I owe special thanks to my family for their support, patience and love.

Abstract

The present work was conducted to study the levels of the concentrations of some trace elements (essential and toxic elements) in hair samples taken from healthy children (male; aged 9-13 yrs) .The children were students of two primary schools (from level 3 to level 7) of Aden governorate. The two schools are Al-Areesh school, which is located in Al-Areesh region-Khormaksar, and The 30 November school, which located in Al-Shaikh Othman region. Both of them experience and exist under different environments (see Map2 and Map3; Chapter 2).

Hair samples were collected during September and March 2004-2005 from Al-Areesh school ($n \approx 100$ samples; ~ 20 samples per level) and Al-Shaikh Othman school ($n \approx 100$ samples; ~ 20 samples per level).

Hair samples were cut close to the skin behind the head at the back of the head (the nap of the neck), put in clean labeled polyethylene bags and taken to the laboratory for analysis.

The samples were washed, digested and prepared for analysis as described in the experimental section (Chapter 2).

Two method were selected to determine the concentrations of the essential and toxic elements zinc (Zn), copper (Cu), cobalt (Co), nickel (Ni), arsenic (As), cadmium (Cd), lead (Pb) and aluminum (Al) : (a) Molecular absorption spectrometry; using a professional spectrophotometer (HACH model DR/RD/2010;US instrument) and (b) flame atomic absorption spectrometry using four different FAAS instruments at four different places two of them here in Yemen that were the Central Research laboratory (University of Aden) using Vairo 6 AAS; Germany instrument and the Fish Wealth laboratory At-Tawahi, Aden using UNICAM VP90 AAS;UK instrument. The other two places were in Egypt and were: The

Central unit of Analysis and Scientific Services -Atomic Absorption unit, National Research Center- Cairo using Varian spectrAA 220 AAS; US instrument and Micro Analytical Center, Cairo University using Perkin Elmer AAnalyst 100 AAS;US instrument.

Additional method was a graphite furnace atomic absorption spectrometry (GFAAS) which was used specifically to determine arsenic by using Varian spectrAA 220 AAS equipped with graphite furnace Auto-sampler (GTA-110); US instrument.

The reason behind using different techniques and several atomic absorption spectrometers is two fold: (1) to check for both measurement precision and accuracy for data obtained for those elements analyzed repeatedly with different techniques and instruments, (2) Inability of measuring the concentrations of some of the investigated elements using home AAS due to shortage in the corresponds hollow cathode lamps for these elements.

Analyses using different techniques /or between the different laboratories of the FAAS instruments were, in generally, gave close readings. However, in some few cases, the readings were substantially different (see Results and Discussion, Chapter 3).

The results obtained in the study showed that (with the exception of arsenic ,copper and zinc) there are consistent trends toward higher metal levels in Al-Shaikh Othman school with aluminum showing the highest concentration among the rest on the investigated elements. Copper, zinc and arsenic showed no consistent trends in theirs concentration levels between the two schools.

As a result, it can be said that Al-Shaikh Othman school students are more subjected to pollution than Al-Areesh students are.

This result was expected due to the bad environments situations surrounding Al-Shaikh Othman school.

Also, cadmium, nickel and cobalt revealed high concentrations in comparison with international reference values ([trace elements](#) and [doctor's data reports, 2006](#)).

Statistical Analysis such as t-test and one-way ANOVA analysis were applied to assess the difference in the studied element concentrations.

Pearson correlations showed that there exist proportional relationships between the investigated elements.

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List of Abbreviations and Symbols

AAS	Atomic Absorption Spectrometry
A	Absorbance
Alpha (α)	Significance level
Al	Aluminum
ANOVA	Analysis of Variance (F-test)
Ar	Argon
As	Arsenic
ATSDR	The Agency for Toxic Substances and Disease Registry
b	Cell length
Cd	Cadmium
Co	Cobalt
CoefVar	Coefficient of Variance
Conc.	Concentration
Cu	Copper
CIL	Lower 95% Confidence Interval of the Mean
CIU	Upper 95% Confidence Interval of the Mean
Dilf	Dilution factor
DL	Detection Limit
df	Degree of freedom
ϵ	Molar extinction coefficient or molar absorptivity
EPA	Environmental protection agency (US government)
FAAS	Flam Atomic Absorption Spectrophotometer
F	Fisher's least significant difference
5% TM	5% Trimmed Mean
GEMS	Global Environmental Monitoring System
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometer
HCL	Hollow Cathode Lamp
H₀	Null Hypothesis
H₁	Alternative Hypothesis
IAEA	International Atomic Energy Agency
IQR	Inter Quartile Range
IR Spectroscopy	Infrared Spectroscopy
JAMA	The Journal of the American Medical Association
λ	Wavelength
Lab	Laboratory
LSD	Least Significant Difference
μS	Micro Siemens
Min-Max	Minimum-Maximum
N	Number
N_v	Number of value
n.d	No date
Ni	Nickel

Pb	Lead
P25- P95	25th-95th Percentile
POPs	Persistent Organic Pollutants
ppb	Part per billion
ppm	Part per million
<i>p</i>-value	Probability for rejection Null Hypothesis
QA/QC	Quality Assurance and Quality Control
Q_c	Q Critical Value
Q_{exp}	Q Experimental Value
Q1	First Quarter
Q3	Third Quarter
r	Pearson Correlation Coefficient
RSD	Relative Standard Deviation
SE(±)	Standard Error
SD(±)	Standard Deviation
SPSS	Statistical Package for Social Science
SRA	Society for Risk Analysis
t	t-Test
TDS	Total Dissolve Solid
U	U-test or Mann-Whitney test
UV/Vis	Ultraviolet/Visible
Var	Variance
W	Shapiro-Wilk value
WHO	World Health Organization
YSMQCO	Yemen Standardization, Metrology and Quality Control Organization
Zn	Zinc

chapter One

INTRODUCTION

Chapter (1)

INTRODUCTION

1.1 Scope on Trace Elements Analysis

The expression "trace elements"^{*} is defined as “Metals normally found in trace amounts due to their insolubility or to their relative lack of abundance in the crust of the earth”. ([Society for Risk Analysis \(SRA\), 2005](#)) and those “chemical element(s) required by an organism in only trace amounts” ([Holmgren Lab , 2004](#)).

The determination of trace elements is necessitated by their importance in the chemical and pharmaceutical industries and in the environment. Although the needs of these fields may all be different, they all have a requirement to measure trace elements

[Soni \(1985\)](#) divided the trace elements into the following classes:

- 1- Those which are essential for life or health such as iron (Fe) and copper (Cu).*
- 2- Those which are inert to life processes .For example: silver (Ag) and lithium (Li).*
- 3- Those which are slightly toxic to some life processes.(e.g. tin (Sn) and bismuth (Bi)).*
- 4- Those which are toxic to life processes.(e.g. lead (Pb) and mercury (Hg)).*

Whether an element is essential for life or not depends on its participation in one or several biochemical reactions. An essential element is useful to the organism and to the maintenance of health when a measurable deficit in the diet reduces the growth and vitality of humans,

^{*} Since the term "trace element" has brevity, historical associations and preferable by analytic workers than the term "heavy metal"([Underwood, 1962](#)), it is retained here.

animals or plants to a reproducible degree. Possibly even well known "toxic" elements are needed in minute quantities for the normal function of cell metabolism ([Seiler et al, 1994](#))

1.2 Toxicity of Trace Elements

Trace element poisoning has become an increasingly major health problem when it goes over its natural quantity. The toxicity can cause our mental functions, energy, nervous system, kidneys, lungs and other organ functions to decline. Knowing where these metals can be found and decreasing one's exposure is vital staying healthy ([Hauser et al, 2005](#)) (see Table 1.1).

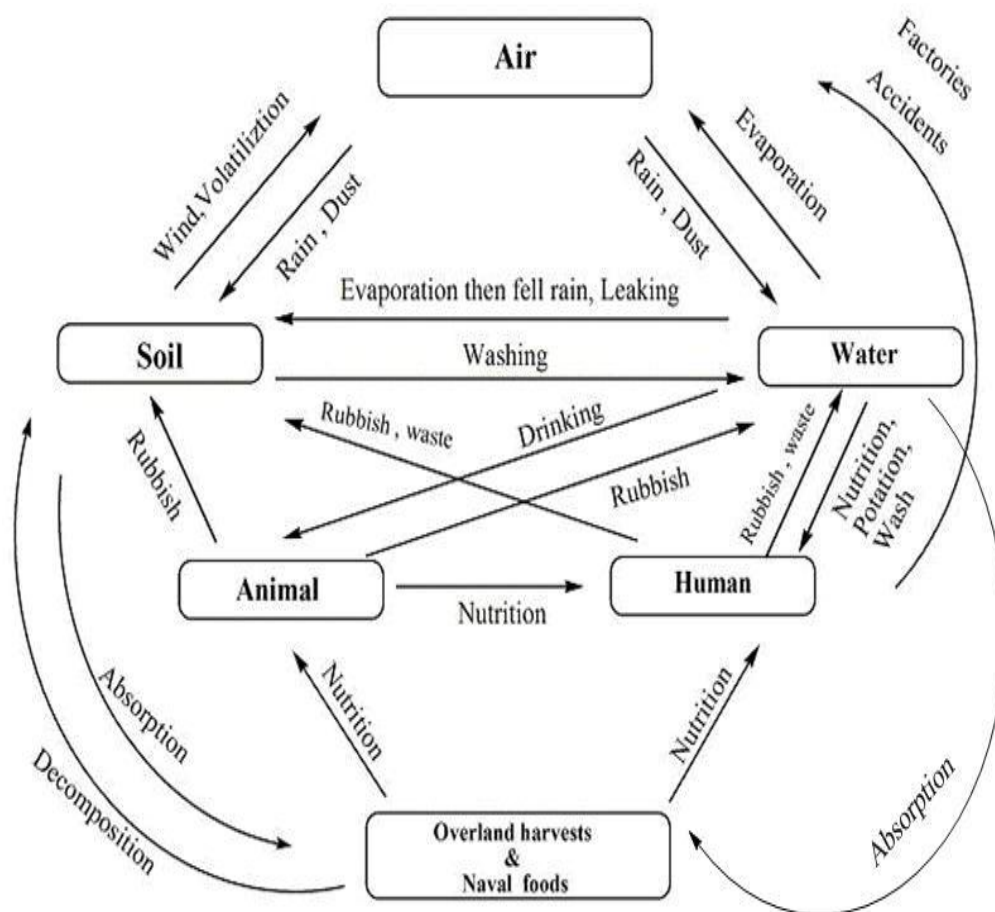
1.3 The Pollutants Cycle in The Environment

In recent decades, many scientists have taken care of the changes on Earth according to the environmental impacts of several pollutants and the exposure to them which destroy our life slowly or quickly depending upon the kind of a pollutant. These pollutants find there way to our bodies and cause serious diseases.

The most important inorganic/ organometallic pollutants are the trace metals such as arsenic, cadmium, cobalt, copper, lead, mercury... etc.

The scheme below describes the relation between the pollutants, especially inorganic and organometallic pollutants, in the environment in relation with human activities.

Figure 1.1: Inorganic and Organometallic Pollutants Cycle in the Nature*



The essentiality /non essentiality of some trace elements together with their biological importance are given in Table 1.1.

* Adapted with modification from [Manahan, 2000](#) and [Technology and services for Mercury Detection and Control site, 2006](#).

Table 1.1: Some Trace Elements and their Biological Functions

Element (symbol)	Essentiality	Health effects , role & deficiency aspects
Aluminum (Al)	Not essential ⁽³⁾	Pathological changes of human lung, central nervous system, and bone causally related to a chronic exposure to aluminum ⁽¹⁾
Arsenic (As)	Essentiality uncertain ^{(3)&(2)}	Striking systemic effects: gastrointestinal, cardiovascular, neurologic, and hematologic symptoms. Deficiency may slow the growth ⁽²⁾
Cadmium (Cd)	Essentiality uncertain ⁽³⁾	Energy disorder, Nervous system hyperactivity and nerve cells damage. Bones and Joints diseases (arthritis, osteoporosis, and neuromuscular diseases) Interference with zinc in the arteries, which contributes to arteries being brittle and inflexible. Accumulation in the kidneys, resulting in high blood pressure and kidney disease. Its toxicity can alter calcium and vitamin D activity, resulting in cavities and tooth deformities. Eyes structure. Cadmium-induced alterations in ocular trace elements. Influence of dietary selenium and copper. ^{(4) & (5)}
Cobalt (Co)	Discovered as an essential elements in 1935 ⁽³⁾	Component of vit. B ₁₂ . Some symptoms of cobalt deficiency: Slow or retarded growth rate, Fatigue, Nerve damage, Poor circulation, Pernicious anemia, and digestive disorders ^{(3)) & (7)}
Copper (Cu)	Discovered as an essential elements in 1928 ⁽³⁾	Linked to oxidative enzymes; interacts with iron. Deficiency results in anemia ⁽³⁾

Table 1.1 continued :

Element (symbol)	Essentiality	Health effects , role & deficiency aspects
Lead (Pb)	Not essential ⁽³⁾	<p>Bones: Instead of calcium, lead is incorporated into bone.</p> <p>Brain: Lead can inhibit copper-dependent enzymes needed for neurotransmitters, causing hyperactivity.</p> <p>Energy: Fatigue is triggered by increasing rate of destruction of red blood cells. Also, lead inhibits copper and iron-dependent enzymes in the Krebs cycle.</p> <p>Kidneys: Gout can occur from lead toxicity raising uric acid levels and impairing kidney functions. Minerals- Lead inhibits calcium, zinc, manganese, copper, and iron causing deficiencies.</p> <p>Thyroid gland: Lead can inactivate the thyroid hormone thyroxin because it interferes with the iodine uptake to the thyroid gland⁽⁴⁾</p>
Nickel (Ni)	Essentiality uncertain ⁽³⁾ .	Interference with iron absorption. Excess exposure causes eczema and cancer. ⁽³⁾
Zinc (Zn)	Discovered as an essential elements in 1896 ⁽³⁾	<p>Constituent of a large number of enzymes. Nails, hair, and teeth have some zinc, and this mineral is important to those tissues⁽⁶⁾</p> <p>Deficiency leads to growth retardation, sexual immaturity, skin lesions, anorexia nervosa, hyperactivity, atherosclerosis, vegetarianism, short stature in childhood, poverty, and insulin dependent diabetes , etc^{(3) & (5)}</p>

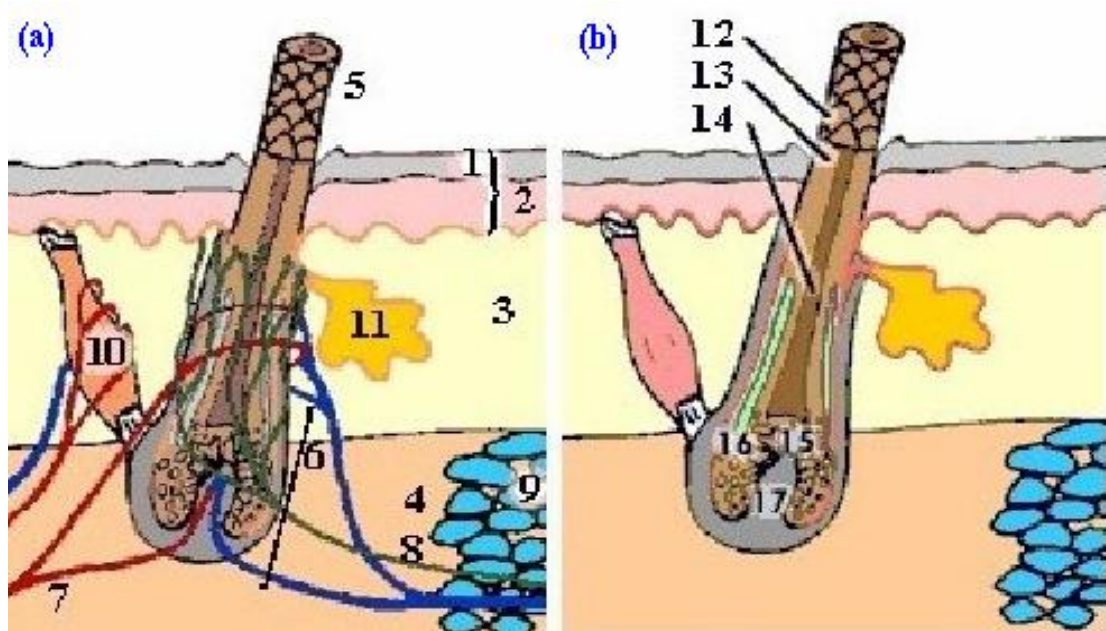
(1) Seiler et al ,1994 . (2) Boyd et al, 1997. (3) Dean, 1997. (4) Hauser et al .2005 ,(5) John ,2005 . (6) Haas ,1992 . (7) Hinkson, 2003.

1.4 Morphology and Chemistry of Human Hair Structure

1.4.1 Morphology

Human hair does not grow continuously. Each hair follicle in the scalp is in a stage of development independent of that of neighboring follicles. One follicle is still in the germinating stage, while another nearby is drying off (Seiler et al, 1994).

Figure 1.2: (a) The Micro-Anatomy of Human Scalp Skin and (b) Human Hair*



1-corneous epithelium 2- epidermis layer 3- dermis 4-hypodermis 5- hair (keratinous secretion) 6- hair follicle 7- blood vessels 8-nerves 9- fat cell 10- erector muscle 11- sebaceous gland

12-scales 13-cortex 14-medulla 15-melanocytes 16-keratinocytes 17-dermal papilla

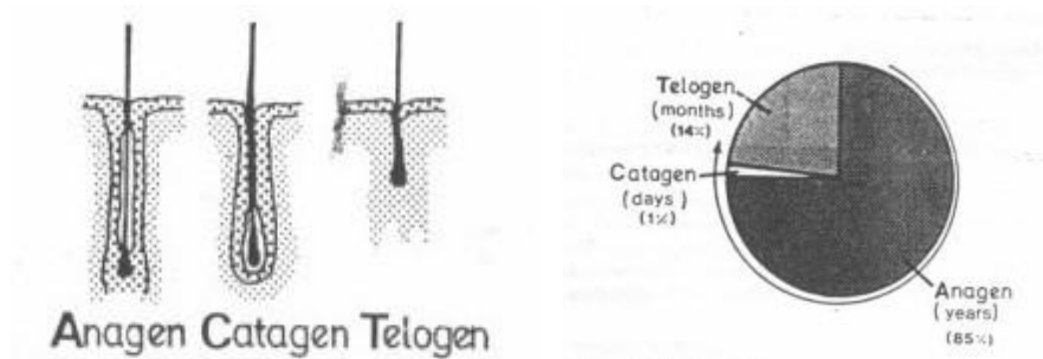
The anagen or growth phase is characterized by the high metabolic selectivity of the hair matrix and lasts 2-7 years. The maximum length reached by the hair depends on the duration of the cycle. There are large

* Taken from [Radboud University site, 2004](#).

individual differences in this. The metabolism of the matrix is wound down over period of 102 weeks in the catagen or transition phase. The dead hair root then remains in the scalp for another 1-6 months in the telogen phase and then falls out (Seiler et al, 1994 and Gray, 2006).

Figure 1.3 shows that normally 85% of the hair is in the anagen, 1% in catagen, and ~14% is dead and will be shed in the following months. These details refer to healthy hair (Seiler et al, 1994).

Figure1.3: Shapes of Different Hair Roots and their Cyclical Development *



The papilla is closely connected to the body by means of the blood circulation, the lymph system, and the extracellular fluid during the anagen growth phase. Not only the components of the hair but also the trace elements that are present are fed into the hair at the concentrations they have at that time and incorporated into the body of the hair (L'OREAL, 2005 and Gray, 2006).

In the region of the root, Seiler (1994) mention that each hair follicle is surrounded by a system of capillary blood vessels that maintains constant information exchange between the root and the rest of the body. The narrowing of a capillary can be a threat to the "life" of the hair-follicle;

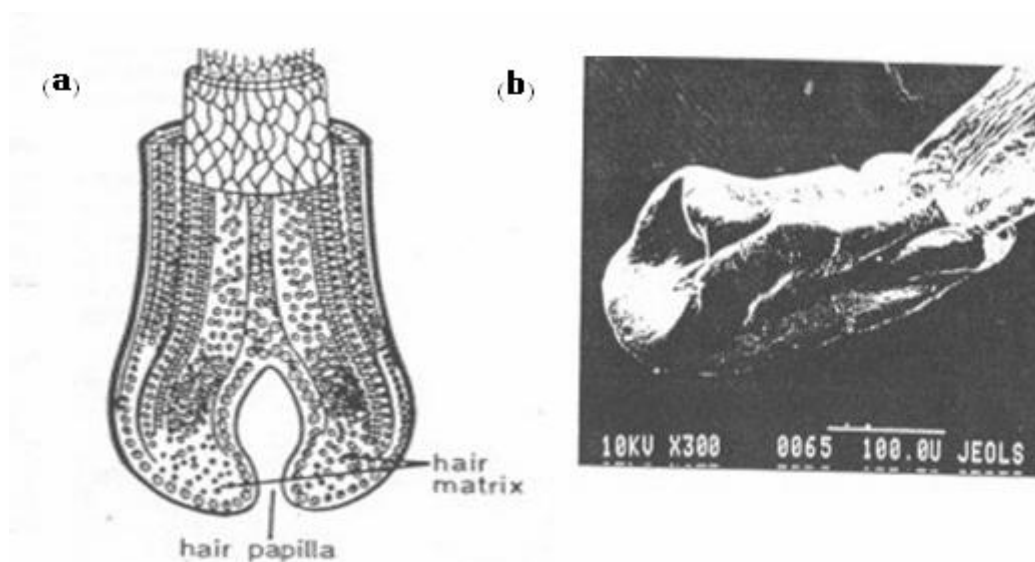
* Taken from Seiler et al, 1994.

when the arterial supply to the vascular loop of the papilla breaks down the hair follicle begins to die off, but remains anchored in the scalp for some months. After certain intoxications the dead hair root exhibits substance-specific alterations (Figures 1.3 and 1.4).

The cortex may be regarded as the major component of human hair. It is made up of cylindrically arranged cells ~100 long.

The center of the human hair follicle, the medulla, is made up of fibrous structures with vacuoles and can be readily differentiated from cortex because of a citrulline-rich protein (Seiler et al, 1994).

Figure 1.4* : (a) Healthy Human Hair Root in the Scalp (Histological Section). (b) Hair Root which has died off because of Exposure to Cd.



* Taken from Seiler et al, 1994.

1.4.2 Chemical structure

The main constituent in the hair is keratin. It is a protein formed by the combination of eighteen amino acids, among which cysteine deserves special mention, being rich in sulfur and playing an important role in the cohesion of the hair by making sulfur bond (L'OREAL, 2005).

Gerhard and Hermes (1987) found eight keratin phenotypes suitable for the identification of persons by considering the number and positions of the individual keratin bands. Ten hair keratins have been identified since 1988 (Heid et al, 1988).

Within the body of the hair, the cortex, the keratin is organized into protofibrils, composed of four chains of keratin. This assembly is held together by bonds or bridges between the atoms of the different chains. These bonds may be of variable strength: weak bonds such as hydrogen bonds can be distinguished from the stronger ionic bonds and sulfur bridges. It is by acting on these bonds that the shape of the hair can be modified (Gray, 2006 and Folk et al, 1977).

The salt bond is also an ionic (electrolytically controlled) bond formed by the electron transfer from the side chain of a basic amino group (an amino acid with an OOC^- group) to the side chain of an acidic amino acid, i.e. NH^{3+} . (This is two positive and negative charges attracting one another.) This occurs in a position paralleled to the axis line of the rotation of the helix of the hair. The salt bond is responsible for approximately 35% of the strength of the hair and 50% of the hair's elasticity.

The sugar bond is formed between the side chain of an amino acid having an OH group and an acidic amino group. This bond is also formed perpendicular to the axis of the hair. Because of its position, it gives the

hair toughness but little strength (5%). Some moisture is contributed to the hair as a by-product of this bonding ([All about Hair site, 2006](#)).

Histological staining reactions confirm a high heavy metal activity in matrix proteins layer: the microfibrils of the cortex ([Seiler et al, 1994](#)).

Oxidative degradation ([Binns et al, 1957](#)) and ([Nicolaus, 1962](#)) have elucidated the structure of melanin.

1.5 Hair Mineral Analysis

Trace element analysis in biological samples has improved significantly over the last 40 years ([Bass et al, 2001](#)).

Hair is an important biological tissue, widely accepted for assessing toxic element exposures and measured by most clinical laboratories capable of making trace element measurements.

[Barrett \(2005\)](#) reported, "Hair analysis is a test in which a sample of a person's hair - typically from the back of the neck - is sent to a laboratory for measurement of its mineral content".

1.5.1 History of Hair Analysis

The detection of toxic trace elements in human hair has a longer tradition than their detection in human blood and urine. Over two hundred years ago, hair testing was used to measure arsenic levels in the body. More recently, the modern introduction of hair as a useful biopsy specimen occurred over fifty years ago, when Flesch proposed using hair as an indicator of body stores of trace elements based on its function as a "minor excretory organ" ([Chatt et al, 1988](#)).

Over the ensuing decades, along with rapid advances in laboratory technology and modern analytical techniques, hair has emerged as a valuable specimen type for providing important clinical information about long-term nutritional status, toxic element exposure, drug and substance

abuse, and other important bio-physiological parameters ([Goulle et al, 1996](#)).

The utility and reliability of using hair element analysis has been borne out by a plethora of studies conducted by independent agencies over the years. In 1975, a report for the Global Environmental Monitoring System (GEMS) recommended that human hair be utilized “as one of the important materials” for biological monitoring of trace metal levels in human beings ([EPA-600/4-79-049, 1979](#)). A monitoring program by the International Atomic Energy Agency (IAEA) showed that hair reliably indicates exposure to the major trace element pollutants. And in their classic, comprehensive review of over 250 reports, the U.S. Environmental Agency (EPA) concluded that hair is “a meaningful and representative tissue” for measuring high priority toxic metals, including lead, cadmium, antimony, selenium, copper, and mercury, as well as selected nutrients assuming that the proper washing and analysis procedures were followed ([EPA,1979, Jenkins, and EPA-600/3-80-089 ,1980](#)).

1.5.2 Hair Elements: Previous Studies Comparison

At the earlier of last decade, the researchers and the analytic workers were paying attention to hair elements study.

Those studies that have dealt with hair element together with their correlations and associations with other body tissues and fluids are given in Table 1.2. While the other class of studies correlating hair elements with exposure to toxicity is given in Table 1.3

Table 1.2: The Verified Hair Elements Levels: Correlations and Associations With Other Body Tissues and Fluids

Element	Body Tissue or Fluid	Remarks	Study
Aluminum (Al)	Bone, plasma	Dialysis patients receiving an aluminum-containing phosphate binder (Aludrox) have significantly higher Al levels in hair, bone, and plasma.	Winterberg et al (1987)
	Bone, serum	Hair aluminum is unrelated to serum or bone aluminum	Wilhelm et al (1989)
Arsenic (As)	Blood	Hair arsenic levels “depend” on blood arsenic levels.	Paulsen et al (1996)
	Nails	Hair and fingernail arsenic levels were “significantly correlated.”	Hewitt et al (1995)
Cadmium (Cd)	Blood, urine	Cadmium concentrations in hair, blood, and urine significantly higher in East German children than West German children.	Hoffman et al (2000)
	Urine	Positive correlation between urine and hair Cd , indicating “hair Cd levels [are] influenced by the body burden of this metal.”	Liu (1999)
	Toenail	[Toenail Cd & Pb levels were much higher than those in hair]and [there was a minor relationship between metal levels in hair and these in toenails.	Wilhelm et al (1991)
Cobalt (Co)	Heart	Hair and heart levels of Co higher in two occupationally exposed young men.	Jarvis et al (1992)
Copper (Cu)	Plasma	Plasma and hair levels of Cu, Zn, and Se decreased after three months in subjects on a lacto vegetarian diet	Srikumar et al (1992)
	Serum	Copper in hair and serum was significantly higher in allergic groups compared to controls.	El-Kholy et al (1990)

Table 1.2 continued:

Element	Body Tissue or Fluid	Remarks	Study
Lead (Pb)	Blood	The mean values of [blood lead levels] & [hair lead levels] follows similar patterns in the boys & girls	Sanna et al (2003)
	Blood	Lead levels in the blood & hair of children from [the] kindergarten [located near the battery factory] were higher than in children from the other schools	Esteban et al (1999)
	Bone	"A positive correlation between the lead content in bone and hair was found in this study ($r=0.308$)."	Hac et al (1998)
	Blood	"Substantial decline" in hair and blood Pb levels after leaded gasoline usage was reduced.	Schuhmacher et al (1996)
	Blood, teeth	"Hair lead was significantly related to both blood and teeth values."	Bergomi et al (1989)
Nickel (Ni)	Serum, urine	"The monitoring of nickel exposure levels can be based on blood serum and urine analysis, but also on nickel determination in hair which have proved promising even in groups of non-occupationally exposed individuals"	Bencko (1983)
Zinc (Zn)	Plasma	Low intake of bioavailable zinc correlated with low zinc in plasma and in hair.	Gibson et al (1998)

Table 1.3: The Verified Hair Elements as a Reflection of Toxic Exposure

Element	Exposure Type	Remarks	Study
Aluminum (Al)	Radiography	Concentration of Al, K , and V in hair were significantly higher in radiographers," consistent with changes in trace element concentration in irradiation tissues"	Man et al (1998)
	Hemodialysis	Researchers report a “highly significant difference” between Al hair levels in hemodialyzed patients and those in healthy Controls.	Chappuis et al (1988)
	Aluminum-containing drugs	Aluminum levels “significantly higher” in the plasma, bone, and hair of dialysis patients taking aluminum-containing phosphate binder Aludrox compared to those taking Antiphosphate (which releases only few Al ions in low pH environments).	Winterberg et al (1987)
Arsenic (As)	Soil samples	[The] results showed [positive] significant correlated with arsenic levels [in hair]	Pereira et al (2004)
	Smoking ; Fish & animal protein		Saad et al (2001)
	Groundwater contamination	77% of hair, nail , and urine samples of affected individuals contained toxic As levels ⁷	Chowdhury et al (2000)
	Tap water and diet	“...tap water consumption and dietary habits were significant contributors to arsenic in hair.”	De Peyster et al (1995)
	Burning of chemically treated wood	“Hair and fingernails of all family members [exposed to burning of chemically treated wood] demonstrated pathological levels of arsenic.”	Peters et al (1984)

Table 1.3 continued:

Element	Exposure Type	Remarks	Study
Cadmium (Cd)	Wastewater spreading field	Average hair concentrations of Cd (and lead) higher in children exposed to wastewater spreading field.	Lekouch et al (1999)
	Ceramic workers	Ceramists, especially those with poorly ventilated studios, showed significantly higher Cd and Pb levels in hair than controls.	Bache et al (1991)
Cobalt (Co)	Mineral assay workers	Two young men with confirmed occupational exposure to cobalt had higher Co levels in hair and heart. ⁵	Jarvis et al (1992)
	Production workers	“...hair analysis is a suitable method for the biological monitoring of exposure to these two metals [cobalt and nickel]”	Bencko et al (1986)
Copper (Cu)	Radiography	Increases in hair Cu, Zn, and Fe reported in radiographers compared to controls.	Chatterjee et al (1993)
Lead (Pb)	Men smokers	Positive association was found between Pb in hair of men smokers.	Strumylaite et al (2004)
	Ceramics Plant workers	Workers exposed to lead had significantly higher lead content in hair than [controls]	Strumylaitė et al (2000)
	Chronic lead exposure	“...among the media investigated, Pb concentration in hair is an environmental marker of exposure to this metal.”	Nowak et al. (2000)
	Motor traffic	Outdoor vendors working at sites with higher traffic densities had significantly higher lead levels in hair.	Furman et al (2000)
	Smoking	Smokers show “significantly higher levels of lead” and “slightly increased” levels of cadmium, cobalt and nickel in hair.	Vienna et al (1995)

Table 1.3 continued:

Element	Exposure Type	Remarks	Study
Nickel (Ni)	Soil, plants	Hair levels of Ni, Pb, Cd, Al and other toxic elements in inhabitants correspond with local soil composition of these metals.	Tommaseo et al (1998)
Zinc (Zn)	Radiography	Increases in hair Cu, Zn, and Fe reported in radiographers compared to controls.	Chatterjee et al (1993)

1.5.3 Blood and Hair Analysis

Blood and hair analysis offer different views of the body from different perspectives. Both are important, but in different situations. Blood is ever changing, reacting almost instantly sometimes, to things ingested or to physical stresses. It is our most valuable indicator of sudden onset or acute types of health problems, and can be analyzed in a relatively short period as well. That is why a physician often takes a blood sample in an emergency situation.

Hair analysis, on the other hand, does not show rapid onset problems; It makes an excellent biopsy material. The hair tissue is dead and the minerals are locked inside, leaving a clear record of the hair metabolism. Mineral imbalances show up much sooner in the hair than in the blood. This is because the blood is maintained at the expense of the tissues. That is, minerals are pulled out of the tissues to maintain the blood levels. For this reason, serum mineral levels often appear normal even if one is ill

Hair analysis in people and animals can show problems in malabsorption and nutritional deficiency, as well as possible causes of chronic and long term problems such as: arthritis, anemia, allergies, asthma, diabetes, hypoglycemia, acne, heavy metal poisoning (lead, cadmium, arsenic, nickel, mercury), various sleep disturbances, some learning disabilities, many fertility problems, weight problems, depression, hyperactivity, headaches, chronic infections, abnormal graying of hair, nervousness, irregular heart beat, cravings for abnormal food stuffs, poor healing of wounds, and sometimes aggression ([Vita Royal, 2006](#)).

Table 1.4 summarizes some of the advantages of using hair analysis

Table 1.4: The Advantages of Hair Sample Analysis

- Hair (and nails) samples are good indicators (and biomarkers) for toxic elements to which subjects have been exposed during the previous 2–18 months. The World Health Organization (WHO), Environmental Protection Agency (EPA), and International Atomic Energy Agency (IAEA) have recommended the use of hair as *an important biological material for worldwide environmental monitoring*.⁽¹⁾
- Hair analysis is a very scientific method of detecting tissue disturbances.⁽²⁾
- Hair analysis tells whether the levels of the nutrient minerals are low, normal or high, but more importantly, it tells whether they are *in balance or not*.^{(2) & (8)}
- The Analysis has been used successfully to test for forensic and drug abuse.^{(3)&(4)}
- Comparing to other types of clinical specimens (analysis), hair analysis has even advantages over blood or urine such as:
 - (i) the concentrations of most of the trace elements are higher in hair than in other human materials;
 - (ii) specimens can be collected more quickly and easily than specimens of blood, urine or any other tissue and also special storage conditions are not needed;
 - (iii) unlike blood, hair is an inert and chemically homogeneous sample;
 - (iv) and serum and urine concentrations provide both an acute index and also over a relatively short time period whereas the concentrations in hair provide a retrospective index of trace element supplies.^(2&5)
- For some elements, concentrations can be more than two hundred times higher in hair than in blood or urine. This increased concentration offers greater sensitivity and precision for the detection and quantification of many trace elements in the sample.⁽⁶⁾
- Hair analysis makes it possible to explain environmental pollution by inorganic substances.⁽⁷⁾
- Human hair used as a biologic measure of exposure to persistent organic pollutants (POPs).⁽⁹⁾
- Trace element analysis on hair samples has been widely used to assess wildlife.^(10&11)

(1) Samanta et al ,2004 (2) Tabrizian ,2006 (3) محمد حسن ,2001 (4) Musshoff et al , 2004 (5) Rao et al , 2002 (6) Ho et al ,2001 (7) Jung et al ,2001 (8) Bass et al ,2001 (9) Altshul et al ,2004 (10) Madsen et al ,1987 (11) Stevens et al , 1997 .

1.5.4 Methodology in Hair Analysis

The success in hair analysis requires consideration the conditions below:

1.5.4.1 Factors Influencing the Interpretation of Hair Analysis Results

There are some factors influence the interpretation of hair analysis data; any analyst should treat them if he wants to analyze hair samples ([Agency for Toxic Substances and Disease Registry \(ATSDR\) - Appendix C, 2001](#)):

- Hair sample scalp location and homogenization.
- Laboratory sample preparation and washing methods.
- Laboratory calibration standards and proficiency testing programs (QA/QC procedures).
- "Normal" reference ranges.
- "Abnormal" concentration ranges.
- Exposure of hair sample to the external environment (e.g., shampoos, bleaches, dyes, permanent waving, relaxers, styling products, hair sprays, hot dryers and curlers, tobacco smoke).
- Hair color and hair diameter.
- Gender, race, diet, age and geographical location.

1.5.4.2 Selection Analytical Methods

Choosing the right analytical method is a critical stage because it gives confidence in getting results.

We have to ask ourselves those questions ([ATSDR-Appendix B, 2004](#)):

- What analytical methods currently exist?
- For what substances do reliable analytical methods exist?
- For what purposes are these methods typically used (e.g., diagnostics, forensics, industrial hygiene)?
- What amount of hair is needed? Is it dependent on the substance being tested? If so, specify substance-specific requirements.
- To what extent are multi-element analytical approaches used? Concern: Accuracy and/or sensitivity for a specific element may be sacrificed.
- Interlaboratory variability: How variable are reference ranges, results, and interpretations?

Therefore, before starting hair analysis we should consider the above notes and at this point, the hidden difficulties in making this analysis.

chapter Two

EXPERIMENTAL

Chapter (2)

EXPERIMENTAL

2.1 Equipments and Instruments

- JENCONS analytical balance (± 0.0001 g) model (BP110S), UK.
- JENCONS Temperature adjustable hotplate capable of maintaining a temperature of 105°C, UK.
- JENCONS Conductivity meter model (4010), UK and InoLab Conductivity meter, WTW Company, Germany.
- GRIFFIN Four arms flasks shaker, UK.
- AUTOSTILL Distiller model (GP3), UK.
- LABCONOCO Laboratory fume hood, US.
- Vacuum aspirator filtration system.
- Lec Laboratory refrigerator model (L-LR500s), UK.
- HACH spectrophotometer model (DR/ RD/2010) ,US .
- Vario 6 AAS with WinAAS v3.30 eng software package, Germany.
- UNICAM VP90 AAS with SOLAAR32 AA v5.66 software package,UK.
- Varian spectrAA 220 AAS with Specteraa v2.10 software package, US.
- Perkin Elmer AAnalyst 100 AAS plus WinLab Analyst software package, US.

2.2 Reagents and Standards

- Triply distilled water and measured its conductance.
- Analytical grade nitric acid HNO_3 (Assay 69-72%, sp.gr.1.41-1.42), BDH Limited Poole. England.
- Acetone extra pure, MERCK Ltd., Germany.
- Johnson's baby shampoo pH balanced. Greece.
- The standard solutions used for AAS calibration were prepared from the stocks below :
- As = $1007 \pm 5 \mu\text{g/l}$ (H_3AsO_4 in HNO_3) determined by ICP-OES 0.5 mol/l, MERCK Ltd. Germany.
- Al = $1000 \pm 5 \mu\text{g/l}$ ($\text{Al}(\text{NO}_3)_3$ in HNO_3) determined by complexometric titration 0.5 mol/l, SIGMA Ltd, US.
- Cd = $1003 \pm 2 \mu\text{g/l}$ ($\text{Cd}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l, traceable to SMR from NIST, SIGMA Ltd. , US.
- Co = $1003 \pm 2 \mu\text{g/l}$ ($\text{Co}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l, traceable to SMR from NIST, SIGMA Ltd. ,US.
- Cu = $1000 \pm 2 \mu\text{g/l}$ ($\text{Cu}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l determined by complexometric titration 0.5 mol/l, SIGMA Ltd. , US.
- Ni = $1003 \pm 2 \mu\text{g/l}$ ($\text{Ni}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l, traceable to SMR from NIST, SIGMA Ltd. , US.
- Pb = $997 \pm 2 \mu\text{g/l}$ ($\text{Pb}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l, MERCK Ltd.,Germany.
- Zn = $1001 \pm 2 \mu\text{g/l}$ ($\text{Zn}(\text{NO}_3)_2$ in HNO_3) 0.5 mol/l, MERCK Ltd,Germany.
- Copper Reagent Cu Ver1; Bicichoiate powder pillows (Used for HACH spectrophotometer), US.

2.3 Laboratories

- Chemistry department laboratory, faculty of Education, University of Aden.
- The Central Research laboratory , University of Aden
- The laboratory of Fish Wealth, Al-Tawahi- Aden.
- Al-Areesh laboratory, Khormaksar-Aden.
- Micro Analytical Center ,Cairo University.
- The Central Unit of Analysis and Scientific Services -Atomic Absorption Unit, National Research Center- Cairo.

The reason for using two techniques and several AAS instruments inside and outside our country is to insure our results by comparing among the gotten results and also ,in some times, according to difficulties in testing some elements inside our country especially arsenic and aluminum .

2.4 Target Sites and Peoples

The hair samples were taken from students of two primary governmental schools existing under different environments in Aden governorate (Fig. 2.1).

The first school is Al-Areesh primary school and the second is 30 November primary school.

2.5 Geography

Aden governorate is part of the Republic of Yemen, at the south-west corner of the Arabian Peninsula, which has traded by land and sea since ancient times. Mid-way between Europe and the Far East, Aden lies on a major world trading route through the Suez Canal. It is one of the largest natural harbors in the world with an area of about 70 km² of sheltered water surrounded by Jebel Shamsan, Khormaksar, and the shore which extends to the hills of Little Aden. The Port of Aden consists of the Inner harbor, the

Oil harbor serving Aden Refinery and the anchorage and approach channels of the Outer harbor (Solar Navigator, 2005).

Al-Areesh school is located in the town of Khormaksar .It is far away from traffic and crowdedness, near the sea and south of police center. It has been reported that rubbish dump on the main street adjacent to the school often on fire. Another factor is there is no good drainage (Fig.2.2).

On the other hand, Al- Shaikh Othmaan school, which is located in the town of Al-Shaikh Othmaan, is surrounded by traffic and metal workshops crowdedness .Also, the school is near two petrol stations: Al-Hashmi and Al- Shaikh Othmaan petrol stations, near the main taxi station (Al-Hashmi station) and surrounded by many workshops (See Fig.2.3).

Figure 2.1: Map Section for Aden Governorate*



**Taken with modification from Google earth software package, 2006*

*Figure 2.2: Al-Areesh School Site****Figure 2.3: 30 November (Al-Shaikh) School Site***

***Taken with modification from Google earth software package, 2006*

2.6 Sample Assortment

2.6.1 Strategy

The hair samples were collected over the period of four weeks starting from 12/9/2004 to 18/9/2004 in the first semester and continued in the second semester from 15/3/2005 to 21/3/2005.

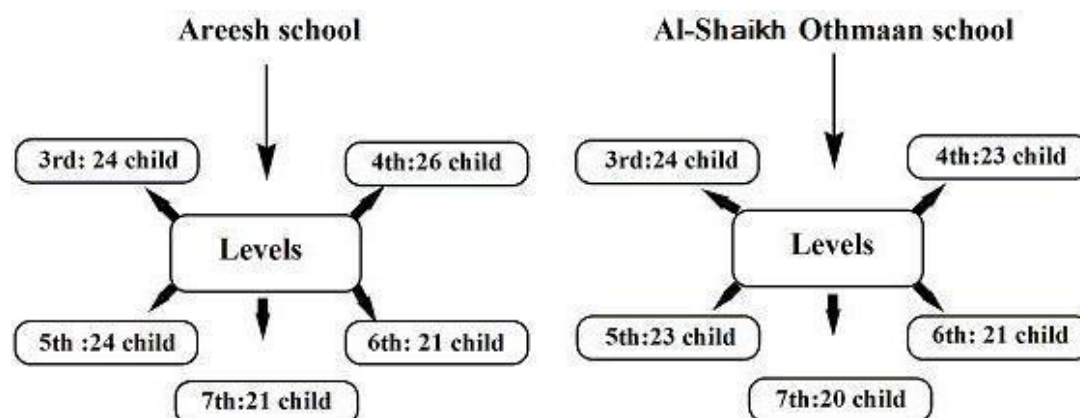
It was decided to divide these samples into subgroups, and assigned according to their main groups.

Al-Areesh's hair samples were collected from 99 students in the first semester and 119 students in the second semester.

Also, from Al-Shaikh school hair samples were collected from 101 students and 111 students in the first and the second semesters respectively.

The samples were separated according to the students' level from the third to the seventh level (9-13 ages). Figure 2.4 below explain the above description:

Figure 2.4: Distribution Students on Their Study Levels in the Second Semester



2.6.2 Preparation

In order to avoid contamination of the samples, the laboratory was cleaned using tap water, distilled water and acetone, respectively. Moreover, all labwares were acid-washed by soaking in diluted nitric acid over night, rinsed thoroughly with distilled water followed by triply distilled water. In case of the chemicals, which were not included in the analysis, were excluded away from the laboratory.

2.6.3 Collection of Hair Samples

All samples were taken from boys and they were orally questioned about their hair treatment by dyes or herbs (e.g. using henna) and the distance between home and school. Then, the representative samples were taken from the least exposed to external contamination.

Samples were directly cut from the scalp in the nape of the students' neck, mixed together, homogenized and kept in sterile polyethylene envelopes. The length of the hair samples were about 1cm long.

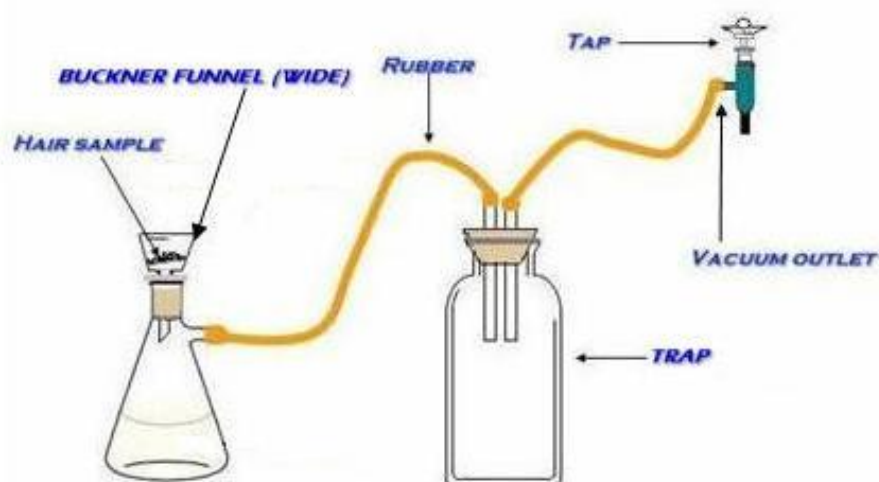
2.7 Treatment of Samples for the Analyzing Trace Metals*

About 90 liters triply distilled water was prepared in the chemistry laboratory. The water was stored in new cleaned 20 liters capacity plastic containers.

Sample pretreatment: The hair samples were transferred to a 500mL conical Stoppard flask containing 10 mL of the shampoo with 350mL distilled water. The flasks were shaken for ca. 15 minutes using a Griffin shaker. An additional washing was made (3-4 times) by putting the samples to vacuum aspirator filtration system as shown below:

* Adapted with modification from [Dombova'ri et al, 1998](#) and [Rao et al, 2002](#).

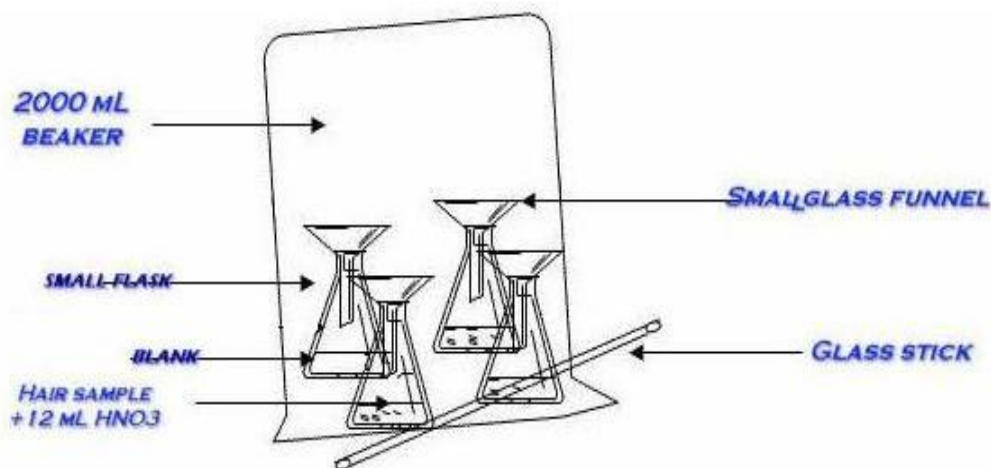
Figure 2.5: Vacuum Aspirator Filtration System



The last wash was done with two times triply distilled water and finally rinsed with extra pure acetone.

After that, the samples were allowed to drain. All samples were left about half a day in the desiccator before weighing. Using cleaned 50mL flasks, glass sticks and stainless steel scissors, the samples, in every level, were parted nearly to three equal parts and weighed carefully around 0.3g on calibrated digital balance far away from air source.

Sample digestion: 12 mL of concentrated HNO_3 were added to each sample and allowed to react 12 hours at room temperature in an empty and cleaned fume hood. To insure no an accidental contamination from the laboratory, the 50mL samples flasks were covered with 2000 mL beaker (Fig.2.6).

Figure 2.6: Samples Protection

The cold digestion was followed by heating the samples in 50 mL flasks covered with cleaned small glass funnel using hotplate heater at 80 °C until dense white fumes were evolved.

All samples were transferred to a 50 mL volumetric flask and diluted to the mark with triply distilled water. Likewise, the same treatments were made for the blanks.

The samples as well as the blanks were kept in sealed polyethylene containers, labeled, and stored in the refrigerator ($\sim 4^{\circ}\text{C}$), ready for analysis.

Some properties of the triply distilled water are given in the table below:

Table 2.1: Characteristic Triply Distilled Water

Test	Result	Mark
Conductivity	0.66 μS -2.5 μS	Taken several times at 25-29.5 °C
TDS	2 mg/lit	At 0.60
NO_3^-	8 mg/lit	--
SO_4^{2-}	1 mg/lit	--

The tables below show the preparation of the hair samples and some of their characters:

Table 2.2: Preparing Hair Samples for Analyzing (First Semester)

Sample No.	Sample Place , Level & Label	Sample Weight	Sample Volume	Dilution Factor (ml/g)	Notes
	Al-Areesh Primary School (Al-Areesh)				
1	3/1	0.3057 g	50 ml	163.56	
2	3/2	0.3047 g	50 ml	164.10	
3	3/3	0.3002 g	50 ml	166.56	
4	4/1	0.3000 g	50 ml	166.67	
5	4/2	0.3002 g	50 ml	166.56	
6	4/3	0.3002 g	25 ml	83.278	
7	5/1	0.3002 g	50 ml	166.56	
8	5/2	0.3009 g	50 ml	166.17	
9	5/3	0.0799 g	25 ml	312.89	Remaining sample
10	6/1	0.3009 g	50 ml	166.17	
11	6/2	0.3006 g	50 ml	166.33	
12	6/3	0.1963 g	25 ml	127.36	Remaining sample
13	7/1	0.3024 g	50 ml	165.34	
14	7/2	0.3000 g	50 ml	166.67	
15	7/3	0.2277 g	25 ml	109.79	Remaining sample

Table 2.2 Continued:

	30-Nov. Primary School (Al-Shaikh Othma'n)				
16	3/1	0.3009 g	50 ml	166.17	
17	3/2	0.1832 g	50 ml	272.93	Remaining sample
	--	--	--	--	--
18	4/1	0.3008 g	50 ml	166.22	
19	4/2	0.3002 g	50 ml	166.56	
20	4/3	0.3005 g	25 ml	83.195	
21	5/1	0.3008 g	50 ml	166.22	
22	5/2	0.3005 g	50 ml	166.39	
23	5/3	0.1417 g	25 ml	176.43	Remaining sample
24	6/1	0.3009 g	50 ml	166.17	
25	6/2	0.3012 g	50 ml	166.00	
26	6/3	0.1372 g	25 ml	182.22	Remaining sample
27	7/1	0.3006 g	50 ml	166.33	
28	7/2	0.3012 g	50 ml	166.00	
29	7/3	0.0401 g	25 ml	623.44	Remaining sample

Table 2. 3: Preparing Hair Samples for Analyzing (Second Semester)

Sample No.	Sample Place , Level & Label	Sample Weight	Sample Volume	Dilution Factor (ml/g)	Notes
	Al-Areesh Primary School (Al-Areesh)				
30	3/1	0.3000 g	50 ml	166.67	Not used
31	3/2	0.3011 g	50 ml	166.06	
32	3/3	0.3007 g	50 ml	166.28	
33	4/1	0.3001 g	50 ml	166.61	Not used
34	4/2	0.3012 g	50 ml	166.00	
35	4/3	0.3002 g	50 ml	166.56	
36	5/1	0.3006 g	50 ml	166.33	Not used
37	5/2	0.3004 g	50 ml	166.44	
38	5/3	0.3002 g	50 ml	166.56	
39	6/1	0.3012 g	50 ml	166.00	Not used
40	6/2	0.3001 g	50 ml	166.61	
41	6/3	0.3005 g	50 ml	166.39	
42	7/1	0.3015 g	50 ml	165.84	Not used
43	7/2	0.3013 g	50 ml	165.95	
44	7/3	0.3003 g	50 ml	166.50	

Table 2.3Continued:

	30-Nov. Primary School (Al-Shaikh Othma'n)				
45	3/1	0.3002 g	50 ml	166.56	Not used
46	3/2	0.3005 g	50 ml	166.39	
47	3/3	0.3005 g	50 ml	166.39	
48	4/1	0.3008 g	50 ml	166.22	Not used
49	4/2	0.3004 g	50 ml	166.44	
50	4/3	0.3011 g	50 ml	166.06	
51	5/1	0.3012 g	50 ml	166.00	Not used
52	5/2	0.3003 g	50 ml	166.50	
53	5/3	0.3005 g	50 ml	166.39	
54	6/1	0.3012 g	50 ml	166.00	Not used
55	6/2	0.3006 g	50 ml	166.33	
56	6/3	0.3010 g	50 ml	166.00	
57	7/1	0.3009 g	50 ml	166.17	Not used
58	7/2	0.3010 g	50 ml	166.11	
59	7/3	0.3012 g	50 ml	166.00	

2.8 Analysis of The Pretreated Hair Samples

2.8.1 Instrumental Techniques

Using the right measurement technique depends on [the sample type, the element type] and the concentrations of tested elements (metals and metalloids) from percentage levels through to trace (parts per million (ppm)) and ultra trace (parts per billion (ppb) and below) levels (Dean , 1997).

The techniques used in this study are a professional Ultraviolet/Visible (UV/Vis) Spectrophotometer (Molecular Absorption Spectrophotometer) and Atomic Absorption Spectrophotometer (AAS).

Those techniques depend on Beer-Lambert law. Beer and Lambert found the relationship between the light absorbed (A) and the concentration (c) of a tested element that is $A = \epsilon.b.c$ * (equation 2.1).

* ϵ = molar extinction coefficient or molar absorptivity ; b = cell length in cm

The wavelength (λ) was fixed to determine specific element.

2.8.1.1 Molecular Spectrophotometer

Instruments using monochromators for wavelength selection are called spectrometers. In absorbance spectroscopy, where the transmittance is a ratio of two radiant powers, the instrument called a spectrophotometer and it measures the amount of light absorbed by a sample ([Harvey ,2000](#) and [Skoog et al ,2000](#)).

An example if a molecular absorption spectrophotometer instrument is a considered HACH spectrophotometer device.

2.8.1.2 Atomic Absorption Spectrometer

This is a method of elemental analysis and astronomers, physicists and chemists have known the principles of it for over 150 years. Only since 1955, however, has the technique of atomic absorption spectroscopy been utilized as a precise analytical tool for elemental analysis ([Gerhard Schlemmer Analytik_Jena \(no date\)](#)).

2.8.1.3 The Quantitative Analysis Steps

The digested samples with their blanks were taken to the mentioned laboratories for analyzing the investigated elements using HACH spectrophotometer and the AAS instruments (single-beam atomic absorption spectrometer).

HACH spectrophotometer was calibrated to determine copper concentration in hair samples at 560 nm:

Bicichoiate powder pillow (reagent) was added to hair sample in the first 25 ml cell and allowed two minutes to react.

The second sample cell (the blank) was filled with 25 ml of sample and placed into the cell holder to zero the spectrophotometer.

Then, within thirty minutes, the prepared sample was placed into cell holder to measure hair's copper concentration.

Using AAS, all elements had were determined by FAAS technique using acetylene- air or acetylene -nitrous oxide as a gas flame. Arsenic, however, was determined by a graphite furnace AAS (Varian SpectrAA 220) equipped with a graphite furnace Auto-sampler (GTA-110).

The elements were determined using a single hollow cathode lamp (HCL) specified for each element.

For the AAS technique, it is necessary to evaluate the response of the unknown sample against a set of standards (known quantities). This method called a standard calibration curve (الزامل، 1998).

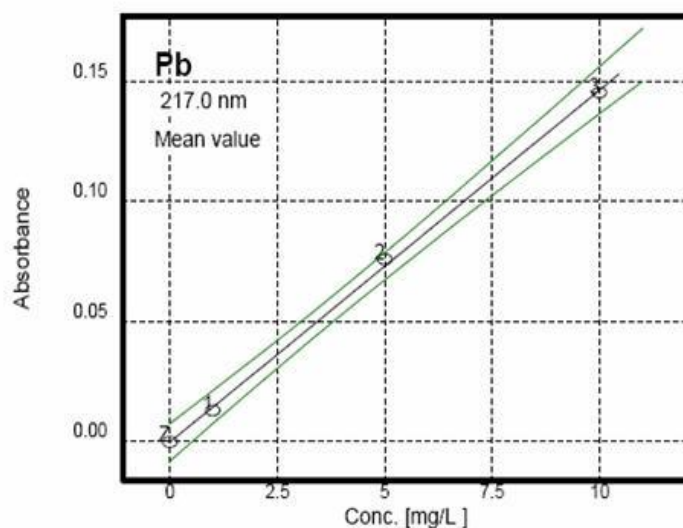
In this method, the solutions with a known concentration (e.g. 3 or 4 solutions) of the investigated element were prepared. Then, the absorbance for each solution was measured at a particular wavelength.

Auto zero blank was used to correct the absorbance of the sample according to the equation below:

Corrected absorbance (A_{net}) = absorbance of sample (A_{sample}) – absorbance of blank (A_{blank}) (equation 2.2)

Subsequently, it was fit at a straight line. Today, most of the instruments do that automatically.

An example of calibration curve is shown in Figure 2.7.

*Figure 2.7: Lead Calibration Curve**

It must be taken into account that; all instrumental methods have a degree of noise associated with the measurement. This limits the amount of analyte that can be detected and increases the error.

The lowest concentration level that can be determined to be statistically different from the analyte blank called "Detection limit".

Generally, the sample signal must be 3x the standard deviation of the background signal (Billing, 2004).

The dilution factor (Dilf) was applied to convert the units used in the laboratories in mg/lit (=µg/ml) to the unit µg/g hair (wt/wt) as it is recorded in the following equation:

$$\mu\text{g/g sample (wt/wt)} = \mu\text{g/ml sample (wt/v)} \times \text{Dilf ml/g (equation 2.3)}$$

Other equation was used to convert relative standard deviation (RSD) as a result from the AAS reports to standard deviation (SD):

$$\text{SD} = \text{RSD} \times \text{Mean} / 100 \text{ (equation 2.4)}$$

*Taken from Vario 6 AAS Report

Table 2.5 below gives AAS parameters used for the analysis of hair samples.

Table 2.5: Some Experimental Parameters Used in AAS Instruments

(1) Flame Atomic Absorption Spectrometer (FAAS):								
M	λ (nm)	Flame Fuel-Oxidant	Gases rate		lamp	Lamp current	Calibration standard solution ppm	Detection Limit (DL) mg/L
			Fuel	Oxidant				
Al	309.3	C ₂ H ₂ -N ₂ O	220NL/h	385NL/h	HCL	6.0mA	1.0/ 5.0/ 10.0	0.3635
Cd	228.8	C ₂ H ₂ -air	1.5L/min	3.5L/min	HCL	4.0mA	0.1/ 0.50/ 1.0	0.0069
Co	240.7	C ₂ H ₂ -air	1.4L/min	4L/min	HCL	14.0mA	1.25 / 5.0 /10.0	0.0252
Cu	324.7	C ₂ H ₂ -air	45NL/h	500NL/h	HCL	5.0mA	1.00/2.00 /4.00	0.0131
Ni	232.0	C ₂ H ₂ -air	55NL/h	501NL/h	HCL	4.0mA	1.00 / 2.00 / 4.00	0.0810
Pb	217.0	C ₂ H ₂ -air	55NL/h	503NL/h	HCL	3.0mA	1.0/5.0/10.0	0.0264
Zn	213.9	C ₂ H ₂ -air	50NL/h	500NL/h	HCL	9.0mA	1.00/2.50/ 5.00/ 10.00	0.0052
(2) Graphite Furnace Atomic Absorption Spectrometer (GFAAS):								
M	λ (nm)	Temperature program	Modifier volume	Ar Gas flow	lamp	Lamp current	Calibration standard solution ppb	Detection Limit (DL) mg/L
As	193.7	Max. Drying Temp. 120 °C Ashing Temp. 1400 °C Atomization Temperature 2600°C	Pd(NO ₃) ₂ 10 µL	3L/min	HCL	10.0mA	20.00 / 40.00/ 60.00 /80.00	0.00026

2.9 Statistical Analysis

The results were calculated using the SPSS software package ver.13 (September-2004) and Origin Professional software package ver.7.5 SR5 (2004), especially using mean analysis, t-test ($p < 0.02$ and $p < 0.05$) and ANOVA (F-test) ($p < 0.05$).

chapter Three

RESULTS

&

DISCUSSIONS

Chapter (3)

RESULTS & DISCUSSIONS

3.1 Introduction

Two groups of students were taken into account in this study. One group was considered as a control group, that is the students from Al-Areesh school, Khormaksar, and the other is the students in Al-Shaikh Othman school.

Using the HACH spectrophotometer and atomic absorption spectrometer for analyzing trace elements in the hair samples had given the results shown in the Table 3.1 below.

Descriptive statistic was undergone for the trace elements in the hair samples studied (Table 3.2).

The Figures 3.1-3.5 show the net concentrations for all elements in the two schools according to the study level while the total trace elements concentrations is figured in Pie chart 3.6 for the target schools.

Furthermore, the existence of the investigated elements follows the order:

$$\text{Zn} > \text{Al} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Co} > \text{Cd} \approx \text{As}$$

Table 3.1: The Mean Concentrations ($\mu\text{g/g} \pm \text{SD}$) for the Investigated Elements

No.	Element Sample (level)	Al $\mu\text{g/g} \pm \text{SD}$	As $\mu\text{g/g} \pm \text{SD}$	Cd $\mu\text{g/g} \pm \text{SD}$	Co $\mu\text{g/g} \pm \text{SD}$	Cu $\mu\text{g/g} \pm \text{SD}$	Ni $\mu\text{g/g} \pm \text{SD}$	Pb $\mu\text{g/g} \pm \text{SD}$	Zn $\mu\text{g/g} \pm \text{SD}$
1	Areesh (3)	27.18 \pm 4.379 ^d	1.078 \pm 0.033 ^c	0.963 \pm 0.118 ^d	1.285 \pm 0.068 ^d	9.760 \pm 1.39 ^d	3.689 \pm 0.03 ^d	25.92 \pm 2.337 ^a 27.59 \pm 2.235 ^b 25.74 \pm 1.923 ^d	111.3 \pm 0.8588 ^a 104.1 \pm 0.7569 ^a 98.88 \pm 1.523 ^c
2	Areesh (4)	17.18 \pm 1.926 ^d	0.626 \pm 0.033 ^c	0.548 \pm 0.362 ^d	1.370 \pm 0.057 ^d	9.661 \pm 0.782 ^d	3.729 \pm 0.1316 ^d	16.27 \pm 2.489 ^b 12.60 \pm 1.900 ^d	96.91 \pm 5.33 ^c
3	Areesh (5)	7.373 \pm 0.354 ^d	0.404 \pm 0.026 ^c	0.578 \pm 0.205 ^d	1.360 \pm 0.017 ^d	11.87 \pm 1.068 ^b 9.787 \pm 1.0 ^d	2.256 \pm 0.0413 ^d	8.586 \pm 4.117 ^a 13.71 \pm 1.495 ^d	117.5 \pm 0.8994 ^a 114.7 \pm 1.347 ^a 104.2 \pm 2.50 ^c
4	Areesh (6)	27.36 \pm 2.569 ^d	0.751 \pm 0.029 ^c	0.584 \pm 0.133 ^d	1.706 \pm 0.123 ^d	9.198 \pm 0.138 ^b 9.713 \pm 0.058 ^d	2.660 \pm 0.595 ^d	22.90 \pm 3.539 ^a 18.81 \pm 2.157 ^a	125.0 \pm 1.969 ^a 113.2 \pm 1.661 ^c
5	Areesh (7)	27.39 \pm 3.794 ^d	3.377 \pm 0.209 ^c	0.332 \pm 0.279 ^d	2.170 \pm 0.072 ^d	7.920 \pm 0.499 ^b 10.1 ^c 9.625 \pm 0.25 ^d	1.305 \pm 0.202 ^d	15.42 \pm 1.203 ^a 18.34 \pm 2.190 ^d	105.4 \pm 1.227 ^a 97.02 \pm 1.94 ^c

Table 3.1 Continued:

No.	Element Sample (level)	Al μg/g ± SD	As μg/g ±SD	Cd μg/g ± SD	Co μg/g ±SD	Cu μg/g ± SD	Ni μg/g ± SD	Pb μg/g ± SD	Zn μg/g ± SD
6	Shaikh (3)	57.24±19.70 ^d	0.454±0.037 ^c	0.998±0.151 ^d	3.286±0.325 ^d	9.650±0.609 ^d	6.103±1.018 ^d	32.71±5.651 ^d	87.09±21.59 ^a
7	Shaikh (4)	67.24±17.597 ^d	2.110±0.103 ^c	0.745±0.469 ^d	2.859±0.160 ^d	9.819±0.799 ^d	5.551±0.0398 ^d	29.37±0.8884 ^a 25.09±4.553 ^d	98.75±1.269 ^a 103.8±1.074 ^a
8	Shaikh (5)	67.27±13.34 ^d	0.826±0.068 ^c	1.111±0.361 ^d	2.258±0.147 ^d	9.990±0.932 ^d	2.608±0.0659 ^d	20.76±2.164 ^d	103.8±2.687 ^a
9	Shaikh (6)	67.36±14.27 ^d	0.775±0.049 ^c	1.315±0.679 ^d	2.719±0.494 ^d	11.44±0.5834 ^b 9.55 ^c 10.15±0.7755 ^d	5.469±1.263 ^d	31.73±1.634 ^d	100.9±11.5 ^c
10	Shaikh (7)	57.47±5.517 ^d	0.980±0.046 ^c	1.970±0.422 ^d	2.503±0.508 ^d	10.3 ^c 9.967±0.8920 ^d	5.526±0.0293 ^d	27.80±2.699 ^d	101.9±0.6915 ^a 101.0±0.707 ^c

Keys: a: Central research laboratory (Aden university), b: Laboratory of fish wealth- Aden, c: Al-Areesh Laboratory, d: Micro Analytical Center-Cairo, e: National Research Center- Cairo.

Notes: (1)The analysis of the hair samples in a, b and c Laboratories was done in the first semester .On the other hand, the d and e Laboratories were selected to analyze hair samples in the second semester.(2) no SD details for c laboratory using HACH spectrophotometer.

Table 3.2: Descriptive Statistic for Trace Elements in the Hair Samples of the two Investigated Groups

Element	N _{total}	Group	Mean	5% T M	Min-Max	Median	SE (±)	SD (±)	Var	CoefVar	CIL	CIU	P25-P95
Al	116	Areesh	21.29	21.73	7.37 -27.39	27.18	3.99	8.93	79.83	0.42	10.20	32.39	17.18-27.39
	111	Shaikh	63.32	63.42	57.24 -67.36	67.24	2.43	5.44	29.62	0.09	56.56	70.07	57.47-67.36
As	116	Areesh	0.715	0.712	0.40-1.08	0.69	0.14	0.28	0.08	0.39	0.27	1.16	0.40-1.08
	111	Shaikh	0.860	--	0.78-0.98	0.83	0.06	0.11	0.01	0.12	0.59	1.13	0.78-0.98
Cd	116	Areesh	0.57	--	0.55-0.58	0.58	0.01	0.02	0.00	0.03	0.52	0.62	0.55-0.58
	111	Shaikh	1.042	1.044	0.75-1.32	1.05	0.12	0.24	0.06	0.23	0.66	1.42	0.75-1.32
Co	116	Areesh	1.578	1.561	1.29 -2.17	1.37	0.16	0.37	0.14	0.23	1.12	2.04	1.36-2.17
	111	Shaikh	2.725	2.719	2.26-3.29	2.72	0.17	0.39	0.15	0.14	2.24	3.21	2.50-3.29
Cu	99-116	Areesh	9.737	9.72	7.92-11.87	9.71	0.34	1.02	1.04	0.10	8.95	10.52	9.63-11.87
	101-111	Shaikh	10.02	10.06	9.65-10.38	9.98	0.11	0.28	0.08	0.03	9.72	10.31	9.82-10.38
Ni	116	Areesh	2.728	2.751	1.31-3.73	2.66	0.46	1.02	1.04	0.37	1.46	3.99	2.26-3.73
	111	Shaikh	5.52	--	5.47-5.55	5.53	0.02	0.04	0.00	0.01	5.41	5.62	5.47-5.55
Pb	99-116	Areesh	18.72	18.79	8.59-27.59	18.34	1.86	6.16	37.96	0.33	14.58	22.86	13.71-27.59
	101-111	Shaikh	27.91	28.04	20.76-32.71	28.59	1.82	4.45	19.77	0.16	23.24	32.58	25.09-32.71
Zn	99-116	Areesh	108.02	107.69	96.91-125	105.4	2.74	9.08	82.52	0.08	101.92	114.12	98.88-125
	101-111	Shaikh	101.69	101.74	98.75-103.8	101.5	0.79	1.93	3.74	0.02	99.66	103.72	100.9-103.8

Keys:

N_{total} :The total number of students, 5%TM: 5% Trimmed Mean , Min-Max: Minimum-Maximum , SE(±):Standard Error, SD(±) : Standard Deviation, Var :Variance, CoefVar: Coefficient of Variance ,CIL: Lower 95%Confidence Interval of the Mean, CIU: Upper 95%Confidence Interval of the Mean, P25- P95: 25th-95th Percentile

* No outlier values included.

Figure 3.1: Trace Elements Concentrations ($\mu\text{g/g}$) In the Analyzed Hair of Third Level Samples

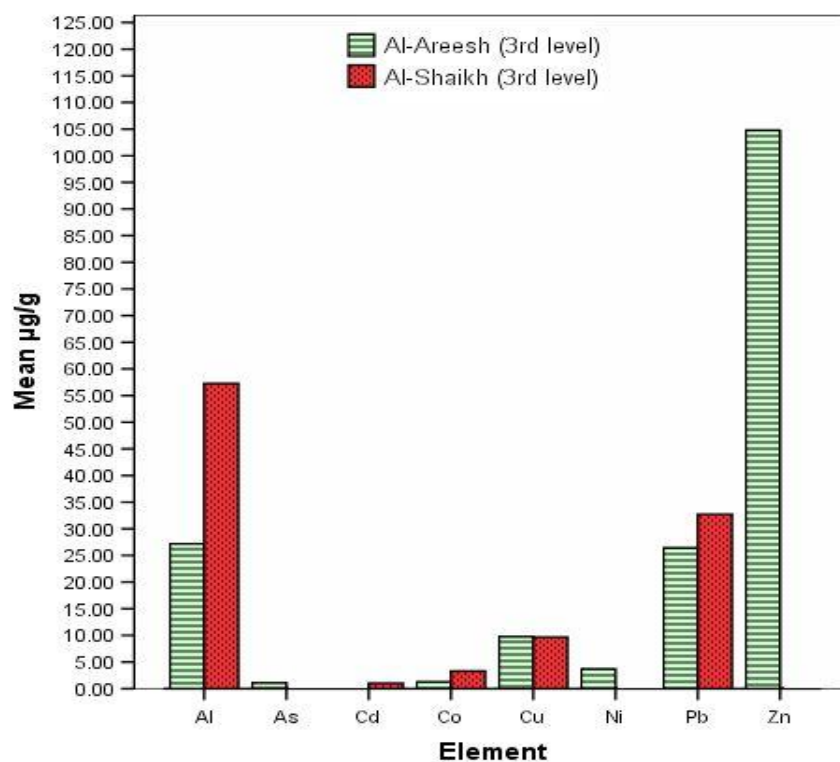


Figure 3.2: Trace Elements Concentrations ($\mu\text{g/g}$) In the Analyzed Hair of Fourth Level Samples

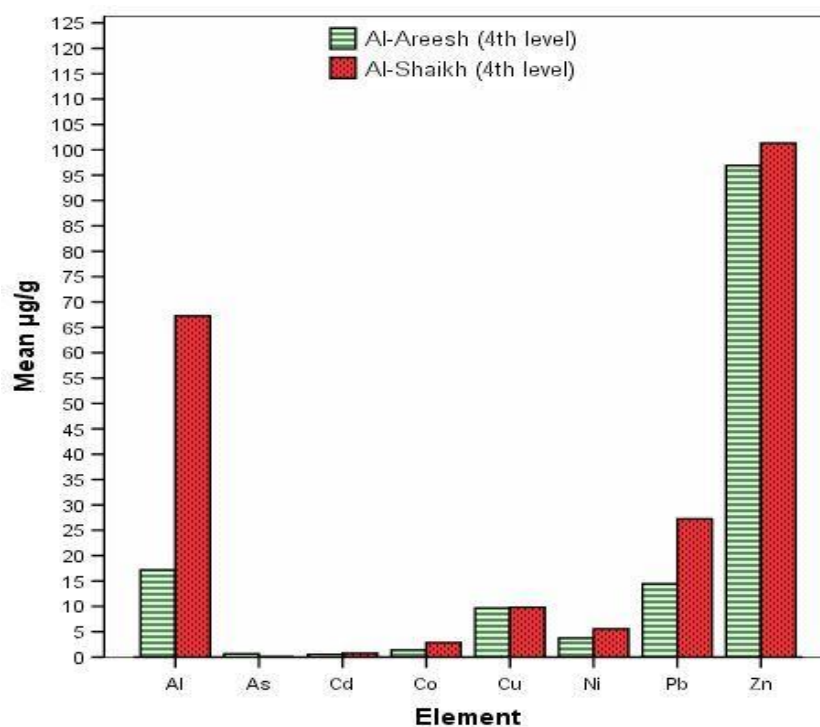


Figure 3.3: Trace Elements Concentrations ($\mu\text{g/g}$) In the Analyzed Hair of Fifth Level Samples

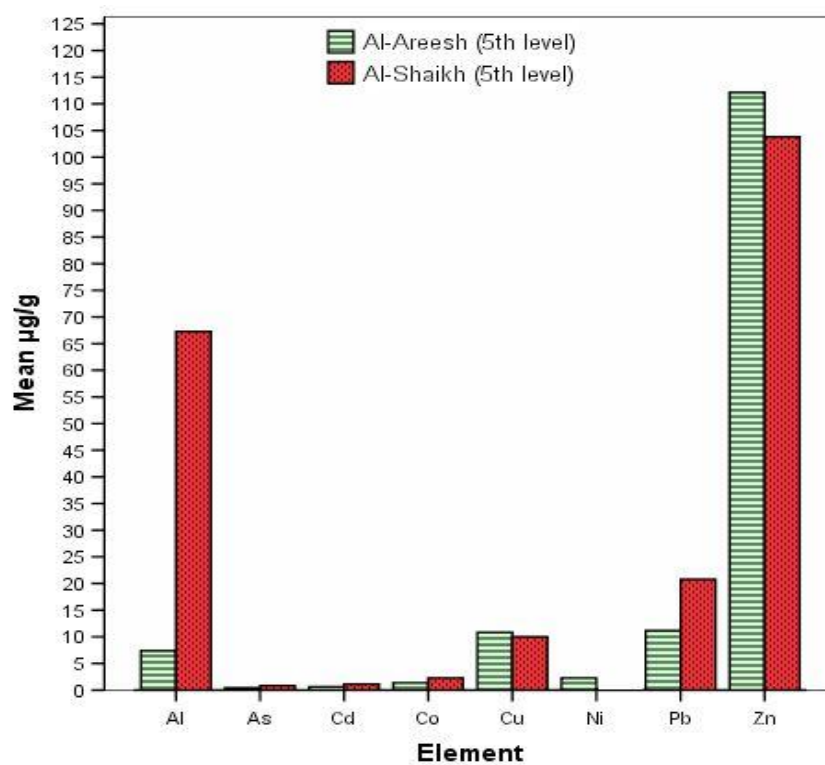


Figure 3.4: Trace Elements Concentrations ($\mu\text{g/g}$) In the Analyzed Hair of Sixth Level Samples

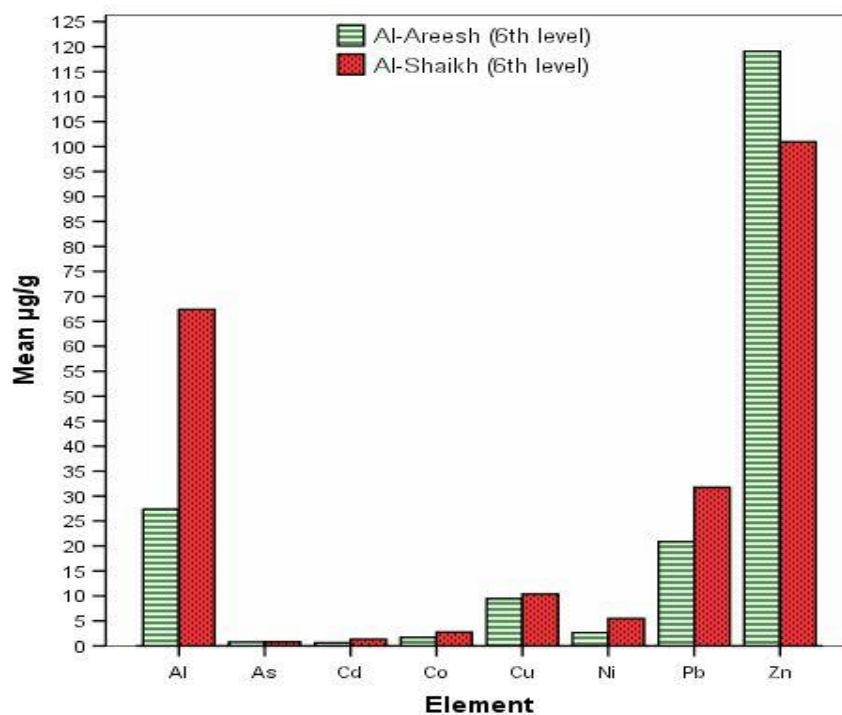


Figure 3.5: Trace Elements Concentrations ($\mu\text{g/g}$) In the Analyzed Hair of Seventh Level Samples

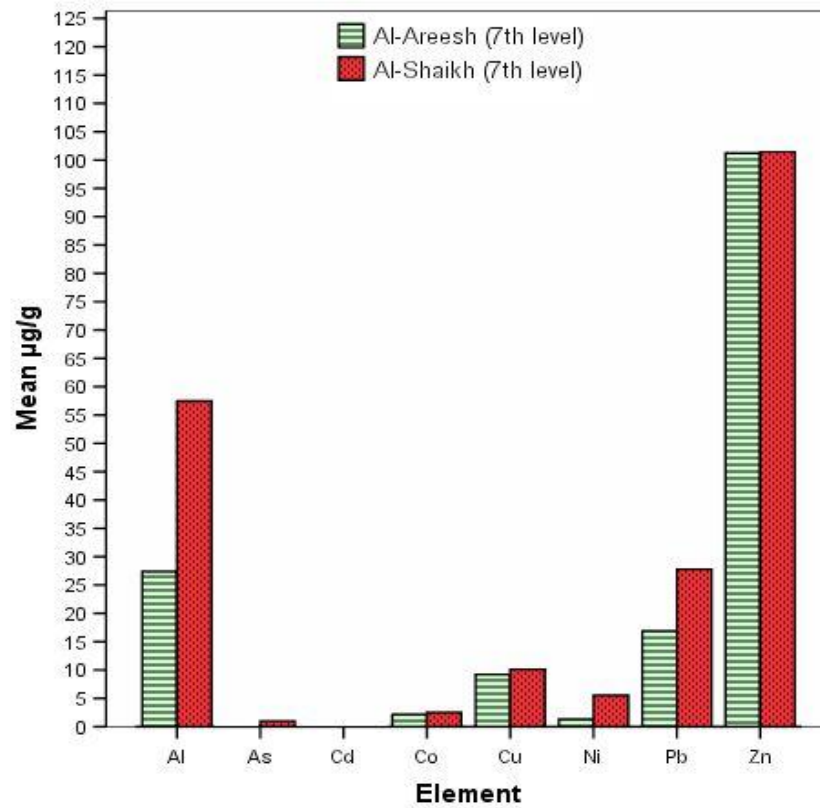
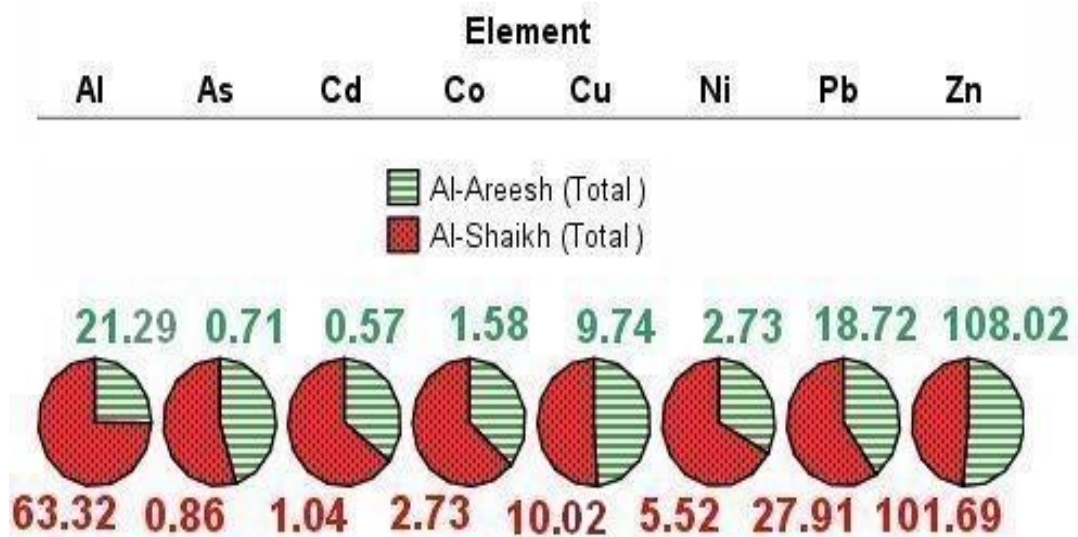


Figure 3.6: Total Trace Elements Concentrations ($\mu\text{g/g}$) for All Analyzed Level Samples



3.2 Statistical Review

3.2.1 Dealing With Outliers

The first step in analyzing data is to investigate the true values resulted from the analysis laboratory.

The Dixon's Q-test is the simplest test of this type and it is usually the only one described in textbooks of analytical chemistry in the chapters of data treatment. This test allows us to examine if one (and only one) observation from a small set of replicate observations (typically 3 to 10) can be "legitimately" rejected or not.

The equation of this test is given next:

$$Q_{\text{exp}} = \frac{x_2 - x_1}{x_N - x_1} \quad Q_{\text{exp}} = \frac{x_N - x_{N-1}}{x_N - x_1} \quad (\text{equation 3.1})$$

Q experimental value (Q_{exp}) then, compared to table of Q critical values (Q_C) at 90%, 95% and 99% confidence.

If Q_{exp} is larger than Q_C the outlier can be discarded with the required confidence. ([University of Athens, 2006](#))

Dixon's Q-test was applied firstly to detect the single outlier value within the same taken sample.

In addition, Q-test was applied among the samples in the same school environment. Then, the outlier values were removed (Figure 3.1 :As, Cd Ni and Zn; Fig.3.2 :As; Fig.3.3: Ni and Fig.3.5: As and Cd).

According to the differences in the samples, samples time taken, the laboratories and the studied levels, it is not preferable to depend on Q-test only to remove the outlier values. The better way is to use special measures of central tendency such as 5% Trimmed Mean and M-Estimators rather than arithmetic mean to reduce or omit the effects of the

outlier and the extreme values showed in the results (الزّعبى وآخرون, 2004).

Pox plot diagram is also a visual way used to determine the outlier and the extreme values (see the point 3.2.5 below).

3.2.2 Tests Normality

The normality tests tell us which suitable statistical analysis is to be followed either the parametric or the nonparametric ones.

There are many normality tests such as Shapiro-Wilk test and Kolmogorov-Smirnov test.

3.2.2.1 Shapiro-Wilk Normality Test

The Shapiro-Wilk Normality test (W) is used to determine whether or not a random sample of values follows a normal distribution (Origin reference v7.5 SR5, Descriptive Statistics, 2004). The Normality test is useful because other statistical tests (such as the t-Test) assume that data is sampled from a normally distributed population. The Shapiro-Wilk statistic and a probability value (p -value)* for testing null hypothesis are computed, from which a statistical decision can be made by comparison with a level of significance.

Kolmogorov-Smirnov test is the same as Shapiro-Wilk test ,but the main difference between those two tests is the sample's volume size where Shapiro-Wilk test is used if sample size is between 3 and 2000 while Kolmogorov-Smirnov test is used if sample size is greater than 2000 (NIST/SEMATECH. (no date)).

* Rumsey (2003) defined the p -value as "a number between 0 and 1 that reflects the strength of the data that are being used to evaluate the null hypothesis".

3.2.2.2 Applying Shapiro-Wilk test

1-Investigated Elements in Al-Areesh School's Samples:

Applying Shapiro-Wilk test to examine the investigated elements for Al-Areesh samples distribution gives the Table below:

Table 3.3: Shapiro-Wilk test for Al- Areesh School

Element	N _v *	W**	P-Value
Al	5	0.77789	0.05366
As	5	0.73706	0.02442
Cd	5	0.88180	0.31490
Co	5	0.82725	0.13017
Cu	9	0.84671	0.06799
Ni	5	0.91650	0.50927
Pb	11	0.95683	0.71033
Zn	11	0.94567	0.56693

At 0.05 level, all the investigated elements, exception arsenic, showed a normal distribution.

2- Investigated Elements in Al-Shaikh School's Samples:

Table 3.4: Shapiro-Wilk test for Al-Shaikh School

Element	N _v	W	P-Value
Al	5	0.69749	0.01051
As	5	0.82307	0.12107
Cd	5	0.92467	0.56422
Co	5	0.98592	0.95093
Cu	8	0.80830	0.03645
Ni	5	0.71055	0.01405
Pb	6	0.94901	0.75092
Zn	7	0.72720	0.00709

Aluminum, copper nickel and zinc did not show a normal distribution at 0.05 level and hence both the analysis of variance and the

* N_v=the number of values mentioned in Table 3.1 for the tested samples. ** W= Shapiro-Wilk value.

Post Hoc tests were applied.

3.2.3 Analysis of Variance

3.2.3.1 One-Way ANOVA (F-test) and Post Hoc Tests

The One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal. This technique is an extension of the two-sample t test.

There exist two types of tests for the sake of comparing means: a priori contrasts and the post hoc tests. Prior contrasts are tests set up before running the experiment and post hoc tests are applied to check after the experiment has been conducted. It can be also used for the trends across the categories.

Dunnett, Scheffé, and LSD (least significant difference) can be used if equal variance is assumed (بشير، 2003).

When the variances are unequal, Dunnett's C (pairwise comparison test based on the Studentized range) is used in analysis (الزّعبى وآخرون، 2004 and SPSS v13 base system topics, GLM Multivariate Analysis, 2004).

3.2.4 Independent Two-Sample t-Test

The Independent-Sample t test procedure is a parametric test. It compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups (SPSS v13 base system topics, T tests, 2004).

The two-sample t-Test can either be a one- or two-tailed test depending on the nature of the variable under study and the objectives of the experimenter. The experimenter develops a null hypothesis (H_0) which is the logical counterpart, mutually exclusive and exhaustive, to an alternative hypothesis (H_1) that the experimenter is attempting to evaluate. Depending on the outcome of the test, the null hypothesis is either rejected or retained. Rejection of the null hypothesis logically leads to acceptance of the alternative hypothesis and retention of the null hypothesis leads to an inability to accept the alternative hypothesis (Origin reference v7.5 SR5, Descriptive Statistics, 2004) and (منتديات الإحصائيون العرب، 2005).

Independent t-Test on Al-Areesh school and Al-Shaikh school for the studied elements where based on the following hypotheses:

Null Hypothesis (H_0): $\text{Mean}_1 - \text{Mean}_2 = 0$

Alternative Hypothesis (H_1): $\text{Mean}_1 - \text{Mean}_2 \neq 0$

3.2.5 Independent Two-Sample U-Test

The nonparametric test equivalent of the t-test is due to Mann and Whitney, called the U test or Mann-Whitney test.

So it can be used for comparing two groups without assumptions about normality or any other distribution (NIST/SEMATECH. (n.d.)).

3.2.6 Applying t-Test and/or U-Test

3.2.6.1 Aluminum (Al) Analysis

School	N_v
Al-Areesh	5
Al-Shaikh	5

Mann-Whitney U= .000

P-value (Asymp . Sig. (2-tailed)) = 0.009

Using U-test analysis at the 0.05 level and 0.02 level explains that the difference of the population means is significantly different than the test difference (that means the null hypothesis (H_0): $\text{Mean}_1 - \text{Mean}_2 = 0$ is not true); and the mean concentration of aluminum was higher 300% (or three times) in Al-Shaikh school than in Al-Areesh school.

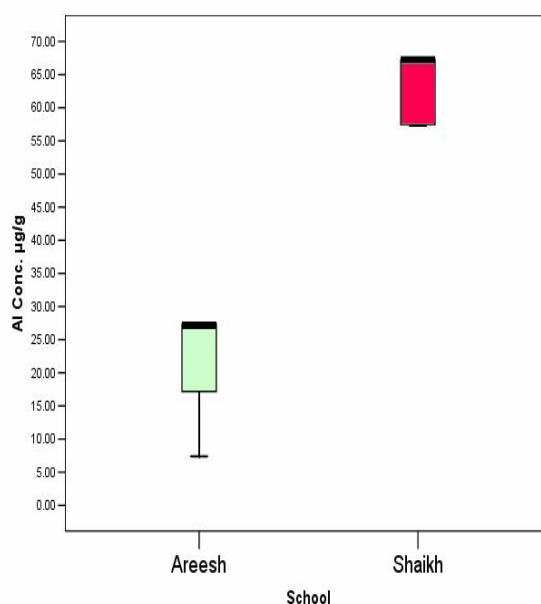
Aluminum is elevated in hair only in extreme exposures ([Wilhelm et al, 1989](#)). That is, the resulted concentrations may be according to the high pollution by aluminum occupations and workshops .Moreover, the high concentrations can be resulted from eating habits using food cooked in aluminum cookware, beverages from aluminum cans when the contents are acidic ([Wilson, 2002](#)).

Other common sources include antiperspirants and antacids.

However, aluminum is also added to table salt, drinking water, and baking powders. Peppermint and spearmint tea, and to some degree regular tea, are high in aluminum ([Wilson, 2002](#)).

Box plot (Figure 3.7) explains the difference in aluminum concentrations of the studied samples.

Figure 3.7: Box Plot Diagram for Al Concentrations in the Target Schools



The Figure above shows that the elevation in the concentration of aluminum in Al-Shaikh site has no clear min-max concentrations (low and high T-bars) that is because of the convergence in the levels of concentration.

Al-Areesh samples concentrations revealed low T-bar (minimum concentration) and this is a smallest observed value that is not outlier.

The bold horizontal line showed the median of the concentrations.

The down marginal box line represented the first quarter (Q1) (or 25% of cases).

The upper marginal box line represented the third quarter (Q3) (75% of cases).

The whole box gives the 50% of cases that have values within it.

It can be used the equation below to get the values within the box area:

$$\text{inter quartile range (IQR)} = Q3 - Q1 \quad (\text{equation 3.2})$$

The box plot was used for all the investigated elements because it is "an excellent tool for conveying location and variation information in data sets, particularly for detecting and illustrating location and variation changes between different groups of data" ([NIST/SEMATECH. \(n.d.\)](#)).

3.2.6.2 Arsenic (As) Analysis

<u>School</u>	<u>N_v</u>
Al-Areesh	5
Al-Shaikh	5

Mann-Whitney U= 11.00

P-value (Asymp. Sig. (2-tailed)) = 0.754

For arsenic mean concentration, the U-test results at the 0.02 and 0.05 levels gave no significant differences between the two schools and

the arsenic concentration in Al-Shaikh is higher approximately 0.2 $\mu\text{g/g}$ than Al-Areesh.

The box plot drawing for arsenic concentrations with and without the outlier and the extreme values (that are 3.377, 0.454 and 2.110 $\mu\text{g/g}$ respectively) can be shown below (see the Figures 3.1, 3.2 3.5 and 3.6 too):

Figure 3.8a: Box Plot Diagram for As Concentrations in the Target Schools

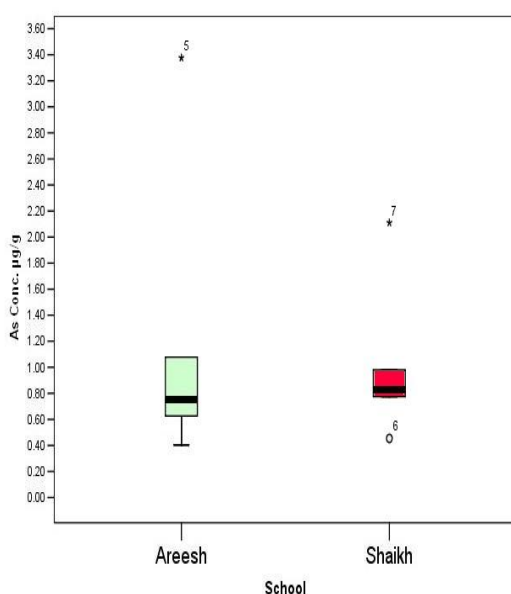
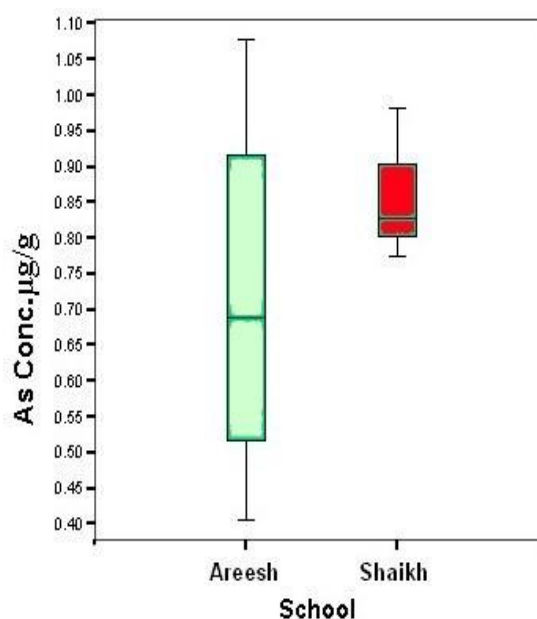


Figure 3.8b: Box Plot Diagram for Modified As Concentrations in the Target Schools



This concentration mostly comes from water pollution and studies have noted a correlation between levels of arsenic in hair and water (Valentine et al, 1979). Adsorption of arsenic to hair may also be substantial following contact with arsenic containing dust (Atalla et al, 1965).

The modified mean (no outlier values included) and other statistics for arsenic are tabled below:

Table 3.5: Descriptive Statistics for Modified As Concentrations in the Target Schools

School				Statistic	Std. Error
Element As	Areesh	Mean		.7148	.14074
		95% Confidence Interval for Mean	Lower Bound	.2668	
			Upper Bound	1.1627	
		5% Trimmed Mean		.7118	
		Median		.6885	
		Variance		.079	
		Std. Deviation		.28149	
		Minimum		.40	
		Maximum		1.08	
		Dataset	N	W	P Value
		Areesh	4	0.98543	0.90959
		Decision			
		Normal at 0.05 level			
	Shaikh	Mean		.8603	.08162
		95% Confidence Interval for Mean	Lower Bound	.5952	
			Upper Bound	1.1255	
		5% Trimmed Mean		.	
		Median		.8260	
		Variance		.011	
		Std. Deviation		.10673	
		Minimum		.78	
		Maximum		.98	
		Dataset	N	W	P Value
		Shaikh	3	0.92308	0.46326
		Decision			
		Normal at 0.05 level			

The t-test for modified arsenic concentrations obtained below:

t	df*	P Value

-0.83511	5	0.44172

Again, at the 0.02 and 0.05 levels, the difference of the population means is not significantly different.

*df : degree of freedom.

3.2.6.3 Cadmium (Cd) Analysis

School	Mean	N _v
Al-Areesh	0.6010	5
Al-Shaikh	1.2278	5

$t = 2.716$ $p = 0.026$ $df = 8$

At the 0.02 level, the difference of the mean is not significantly different from the test difference (0).

The mean concentration of the cadmium element at the 0.05 level is statistically different between the two schools with high value (nearly 1.8:1) for Al-Shaikh school and this increased values returns to the paint workshops circulation.

The box plot diagram for cadmium concentrations with outlier values is indicated in the Figure (3.9a).

Figure 3.9a: Box Plot Diagram for Cd Concentrations in the Target Schools

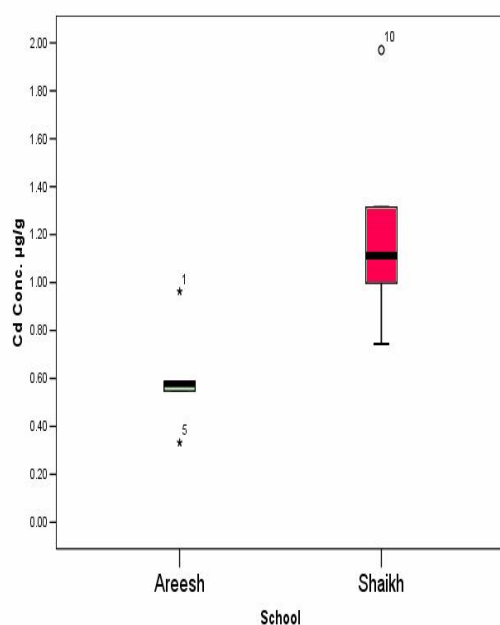
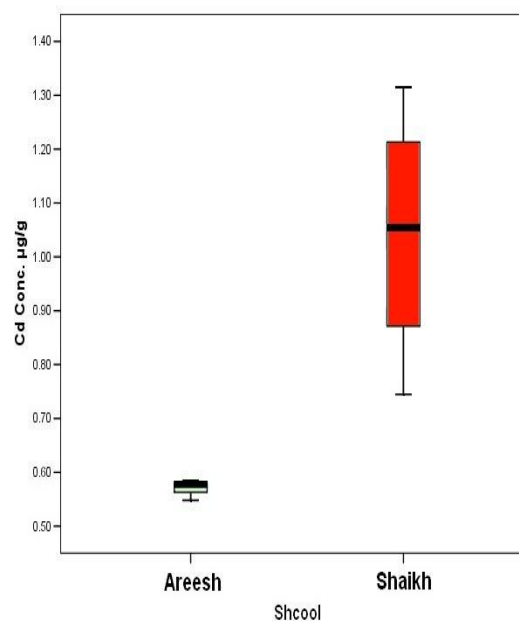


Figure 3.9b: Box Plot Diagram for modified Cd Concentrations in the Target Schools



Removing the outlier and extreme values (0.963, 0.332 and 1.970 $\mu\text{g/g}$) yield the Figure 3.9b.

The pie chart (Figure 3.6) contains the net concentrations for cadmium while the t-test for those Cd concentrations is obtained below:

t	df	P Value

-3.37781	5	0.01972

For a second time, at 0.05 level and least at the 0.02 level, the difference of the population means is significantly different.

The most significant non-occupational exposure to cadmium occurs through tobacco smoke (Hoffmann et al, 2000) and smoking 20 cigarettes/day inserts 40 μg Cd to our bodies (محمد حسن, 2001).

Junk food and tap water contain cadmium too (Wilson, 2002).

For other statistics and the customized cadmium mean, see ANNEX1-table 2.

3.2.6.4 Cobalt (Co) Analysis

School	Mean	N _v

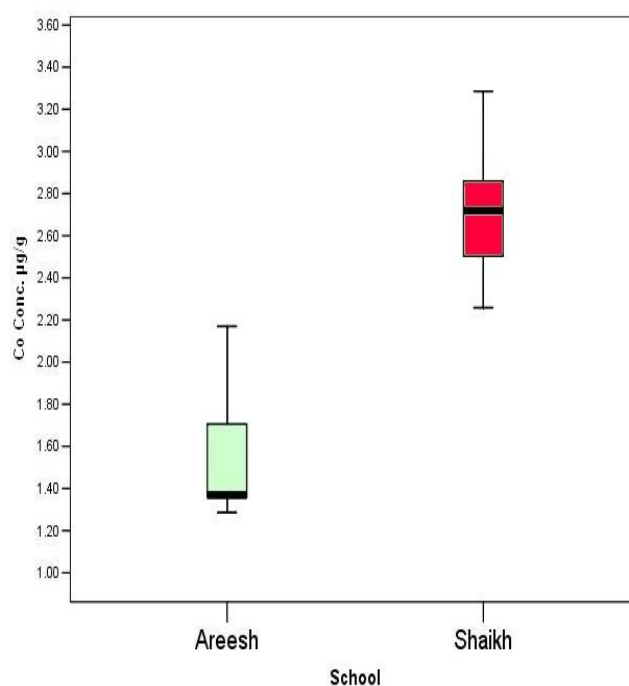
Al-Areesh	1.5782	5
Al-Shaikh	2.7250	5

t = 4.796	p = 0.001	df = 8

At the 0.02 level and 0.05 level, the mean concentration of cobalt element is statistically different with ratio ~1.7:1 higher for Al-Shaikh samples than Al-Areesh samples (see Figure 3.10).

The high concentration in Al-Shaikh resulted from surrounding paint workshops and the burning of coal or oil.

Figure 3.10: Box Plot Diagram for Co Concentrations in the Target Schools



3.2.6.5 Nickel (Ni) Analysis

School	N _v
Al-Areesh	5
Al-Shaikh	5

Mann-Whitney U= 3.00

P-value (Asymp . Sig. (2-tailed)) = 0.047

At $\alpha=0.05$, the mean concentration of nickel element is significantly different between the two schools with ratio 2:1 higher for Al-Shaikh school than Al-Areesh.

This increasing in nickel concentration in Al-Shaikh environment may returns to weld workshops and dust.

Cocoa, soybeans, some dried legumes, various nuts, and oatmeal contain high concentrations of nickel.

Wilson (2002) mentioned other sources include hydrogenated oils found in margarine, commercial peanut butters and vegetable shortening. In adults, dental materials and occupational exposure to metals may also raise the nickel level.

Removing the outlier and the extreme values (6.103 and 2.608 $\mu\text{g/g}$) results in the diagram below:

Figure 3.11a: Box Plot Diagram for Ni Concentrations in the Target Schools

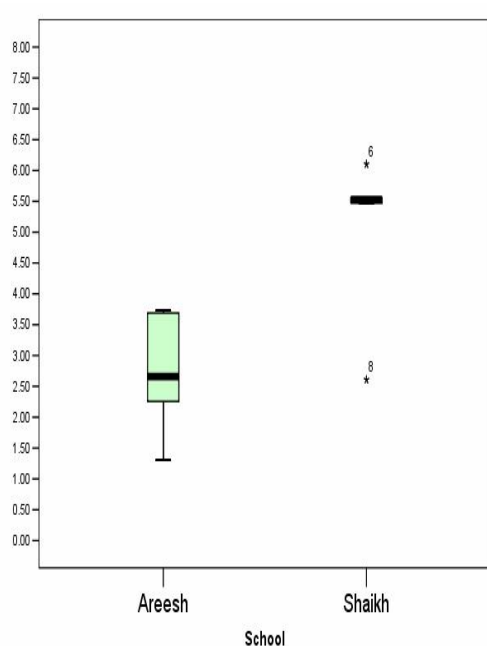
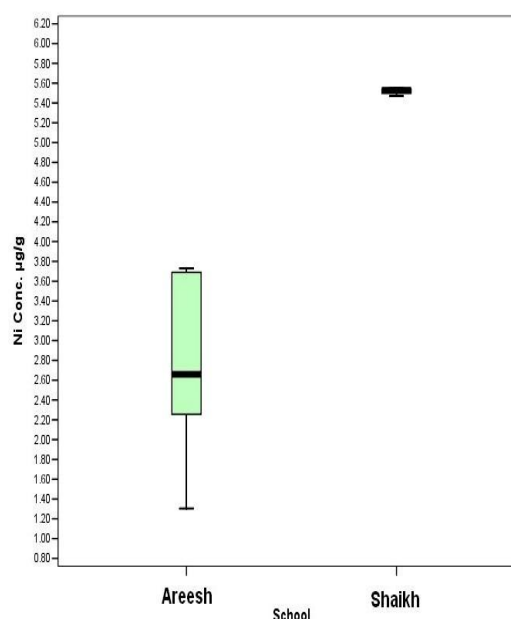


Figure 3.11b: Box Plot Diagram for modified Ni Concentrations in the Target Schools



The adapted mean and other statistics for nickel are scheduled in ANNEX 1-Table 3.

Therefore, it is obvious that, except the arsenic element at $\alpha = 0.02$, $\alpha = 0.05$ and Cd at $\alpha = 0.02$ (Cd concentration no modified), there are significant differences in the concentrations of Al, Cd, Co and Ni between the two studied schools*.

* Note: The analysis for the elements Cu , Pb and Zn are separated in the point 3.2.7 below according to their extra analysis.

3.2.7 Copper, Lead and Zinc Analysis

3.2.7.1 Copper (Cu) Analysis

Previous study was done for determination copper (and zinc) in the Yemeni students with schistosomiasis disease ([Al-Attaas et al, 2002](#)).

However, the analyses here focus on the students seem healthy in two different environments:

School	N _v
Al-Areesh	9
Al-Shaikh	8

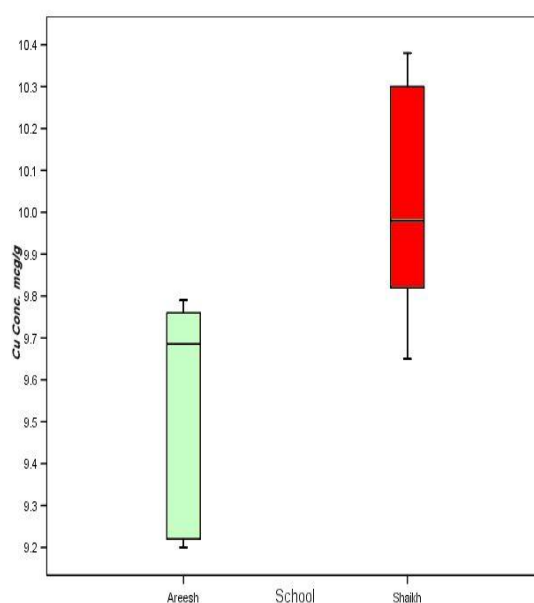
Mann-Whitney U= 22.00

P-value (Asymp . Sig. (2-tailed)) = 0.178

Using Mann-Whitney test at the 0.05 level, the difference between the population means is not significantly different from the null hypothesis.

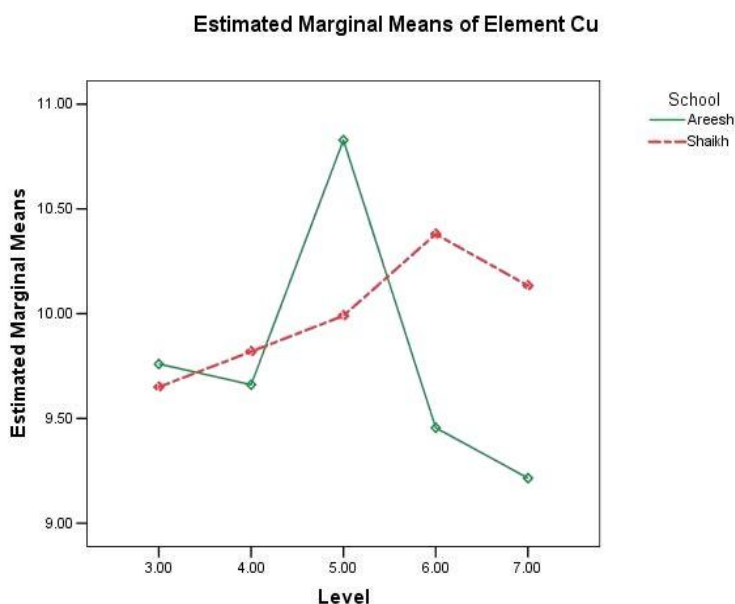
It is clear if we looked at the box plot diagram (Figure 3.12), the values rang of copper concentration in Al-Shaikh samples is slightly higher than Al-Areesh samples.

Figure 3.12: Box Plot diagram for Cu Concentrations in the Target Schools



The estimated marginal copper means showed high concentrations for the fifth level in Al-Areesh and the sixth level in Al-Shaikh school (Fig.3.13).

Figure 3.13: Profile Plots for Copper Element



3.2.7.1.1 Analysis of Variance (ANOVA) and Post Hoc Test for Cu Element

The effects of independent variables (school, lab and level) * on dependent variable (element concentration) were tested by analyzing the factorial variance (ANNIX1-Table 4 and 5) and the results revealed as the points below:

I- There are no differences between the means of two schools where Al-Shaikh school mean is equal 10.02 ± 0.28 while Al-Areesh school is equal 9.737 ± 1.02 and that was assured by comparing F value ($=0.535$) at 0.05 level.

* Semester variable was fixed to avoid the interference with laboratory variable.

2- According to $F = 0.392$ (not statistic significant value), there are no differences within the study levels for each school.

3- At the 0.05 level, the effect of laboratory variable revealed no statistical significant value ($F = 0.082$) by using L.S.D for the lowest significant value and this signs to the following:

a- The data from Al-Areesh and Microanalysis laboratories were lower than Fish laboratory in which the mean difference of Al-Areesh and Fish laboratories was not significant value ($0.1237 \mu\text{g/g}$) at $\alpha = 0.05$. The difference between the means of Microanalysis and Fish laboratories was not statically significant value ($0.2948 \mu\text{g/g}$) $\alpha = 0.05$.

b- There is no significant difference between Al-Areesh and Microanalysis laboratories.

c- Using Microanalysis laboratory as a control laboratory* (Dunnett t-tests), the means of Fish and Microanalysis laboratories was not significant value; and still no statistic difference was noticed between Al-Areesh and Microanalysis laboratories.

4- The tests of between-subjects effects (school vice level) give a certain impression that is no significant difference between the two schools' levels.

3.2.7.2 Lead (Pb) Analysis

School	Mean	N _v
Areesh	18.72	11
Shaikh	27.91	6

t = -3.207	p = 0.0059	df = 15

*According to AAS features and high experience technicians...

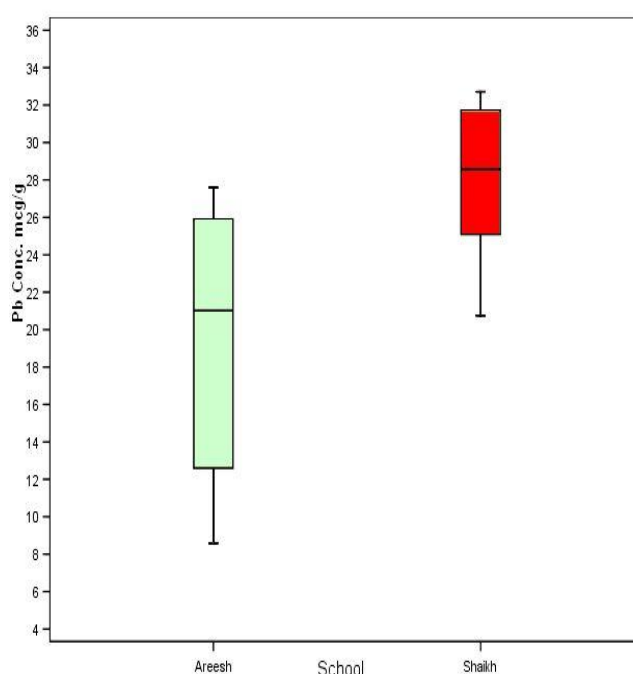
At the 0.02 level and at the 0.05 level, the difference of the population means is significantly different than the test difference (0). The main accused is the crowd traffic in Al-Shaikh site.

Doctor's data (2006) have mentioned other sources of exposure to Pb including welding, old leaded paint (dust/chips), drinking water and newsprint.

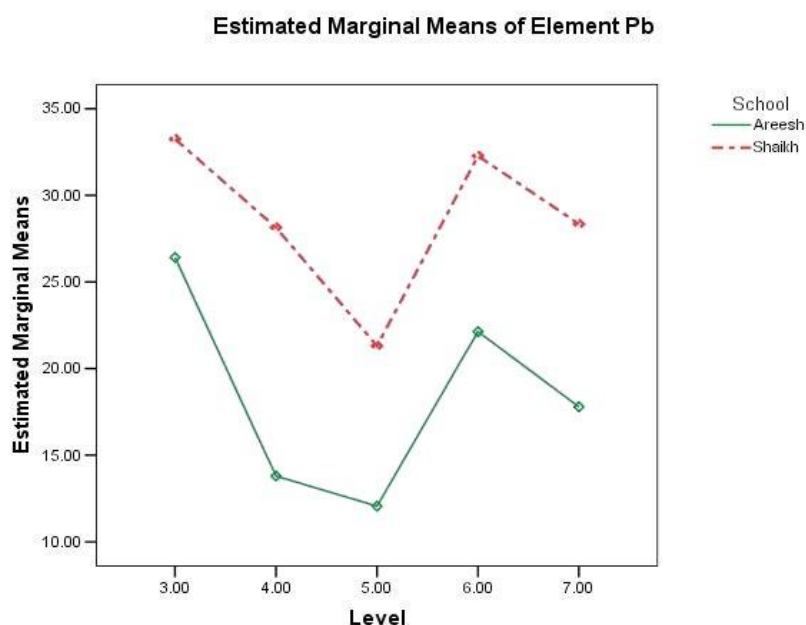
Analysis of variance for lead (ANNEX1-Table 6) shows that the significant difference is due to the schools' environment and the levels while the interaction school-level and the laboratories give no significant difference.

Lead concentrations are plotted in the Figures 3.6 and 3.14.

Figure 3.14: Box Plot Diagram for Pb Concentrations in the Target Schools



The Figures 3.1-3.5 and 3.15 give an idea about the estimated lead means for the five levels in the two schools, and they point that the third level in Al-Shaikh school showed the highest lead concentration.

Figure 3.15: Profile Plots for Lead Element

3.2.7.2.1 Post Hoc Tests for Lead

According to the LSD, Dunnett C and Dunnett t values (ANNEX1-Table 7), the concentrations of lead recorded by all laboratories had statistically no difference at $\alpha = 0.05$.

3.2.7.3 Zinc(Zn) Analysis

School	Mean	N _v

Areesh	108.02	11
Shaikh	101.69	6

t = 1.662	p = 0.117	df = 15

At the 0.02 level and at 0.05 level, the difference of the population means is not significantly different than the test difference.

This agreed with the box plot diagram (see Fig. 3.16).

The high concentration of the zinc element comes from the fact of its appearance in the earth.

3.2.7.3.1 Post Hoc Tests for Zinc

In accordance with the use of only two laboratories to analyze zinc hair contents, it cannot be available to use those tests for a comparison.

The box plot for zinc concentrations with and without the extreme value 87.09 $\mu\text{g/g}$ (number 1 in the box plot) can be shown below:

Figure 3.16a: Box Plot Diagram for Zn Concentrations in the Target School

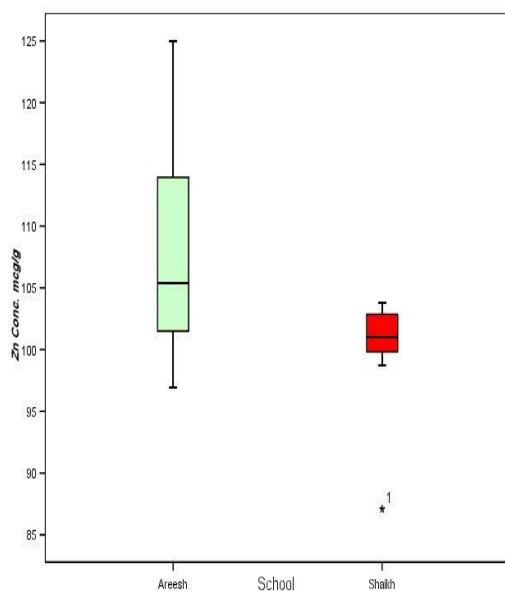
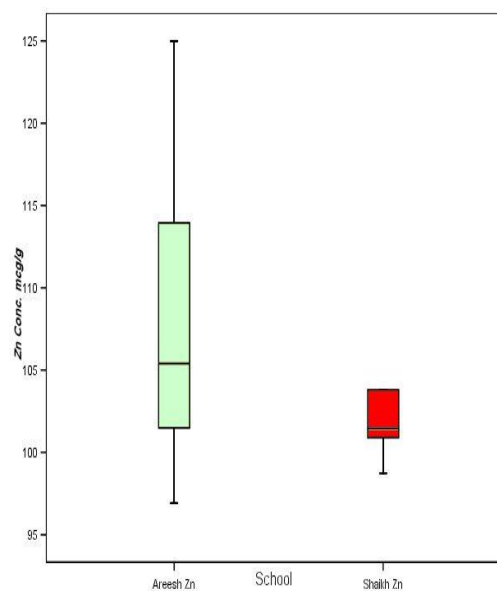
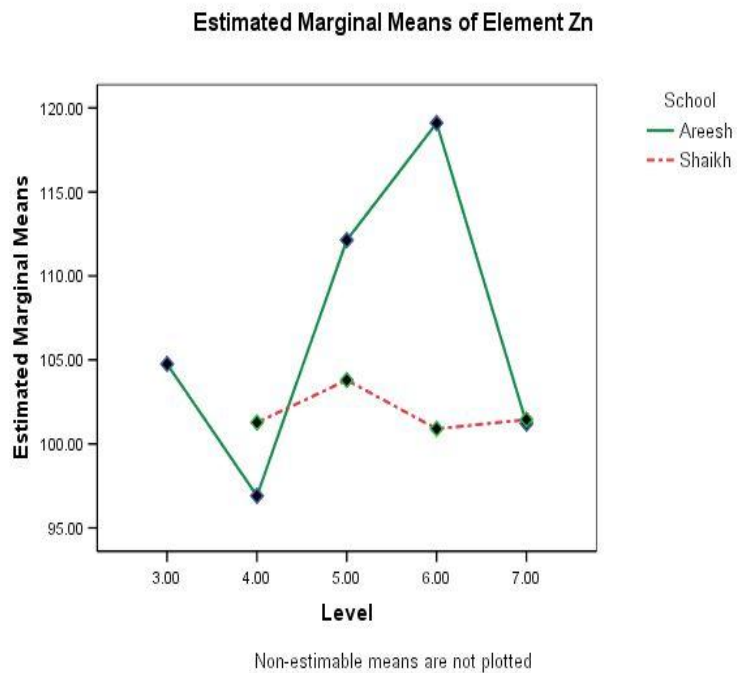


Figure 3.16b: Box Plot Diagram for the net Zn Concentrations in the Target School



The estimated marginal zinc means (Figure 3.17) in comparison with Figures 3.1-3.5 showed high concentrations for the sixth level in Al-Areesh school.

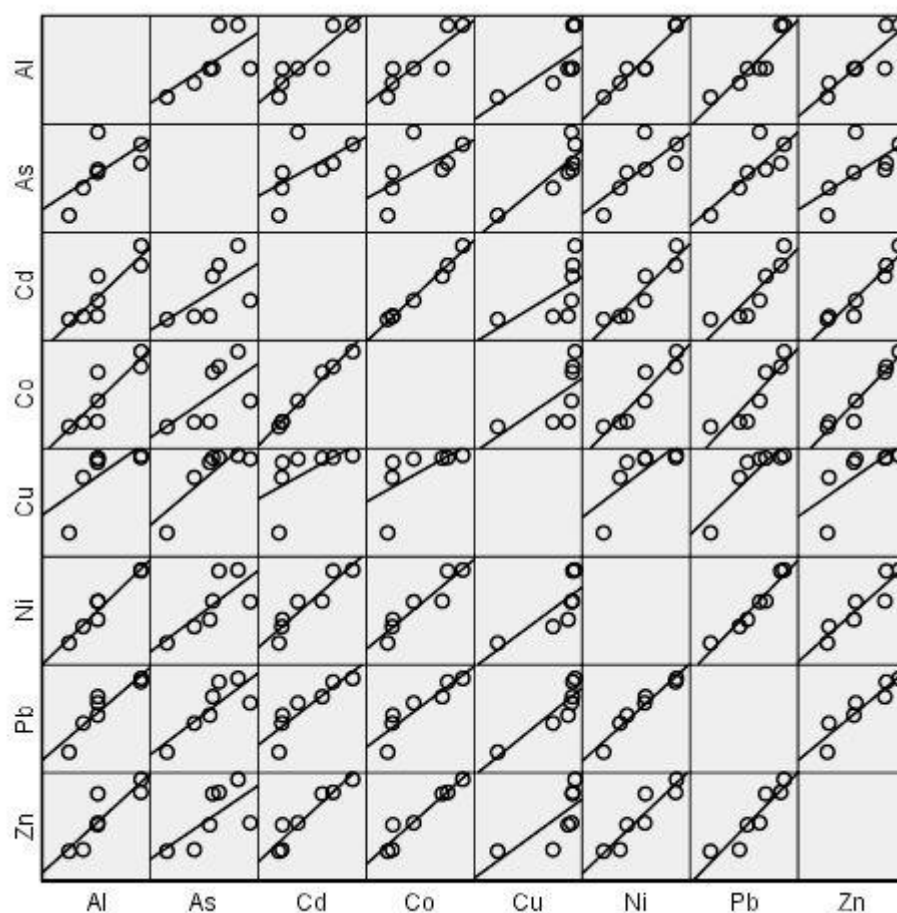
Figure 3.17: Profile Plots for Zinc Element



3.2.8 The Correlations Between the Elements

Although there are not enough values to make a good image about the correlations between the elements within the two studied environments, it seems that the overall correlations between the elements in Aden town can be made as it shown below:

Figure 3.18: Matrix Scatter Correlation Graph for All Investigated Elements



The total significant correlations using Pearson Correlation Coefficient values (r)^{*} for the two environments at the 0.01 level are in the order:

(Cd, Co) $r=0.992 >$ (Co, Zn) $r=0.972 >$ (Ni, Pb) $r=0.970 >$ (Al, Ni),
 (Co, Pb) $r=0.964 >$ (Cd, Zn) $r=0.949 >$ (Al, Zn) $r=0.946 >$ (Al, Pb)
 $r=0.942 >$ (Co, Ni) $r=0.938 >$ (Cd, Ni) $r=0.932 >$ (Al, Co) $r=0.924 >$ (Ni,
 Zn), (Pb, Zn) $r=0.903 >$ (Al, Cd) $r=0.893 >$ (Cu, Zn) $r=0.884 >$ (Cd, Pb) $r=$
 $0.882 >$ (Cu, Pb) $r=0.866$

* Pearson Correlation (r) is used to correlate between two numerical variables and it is always between -1 (downhill linear relationship) and +1 (uphill linear relationship). (الزّعي وآخرون, 2004)

Also, the correlation is significant at 0.05 level for the bivariate in the order:

(As, Cu) $r=0.839$ > (As, Pb) $r=0.812$ > (Cu, Ni) $r=0.762$ > (Al, Cu) $r=0.712$ > (Co, Cu) $r=0.642$

Those positive correlations declare the relationship between the elements but are still need further study.

3.3 Conclusion

Form the above discussions and results, it concluded that there are significant differences between the two schools, the mean concentrations in Al-Shaikh school revealed higher than Al-Areesh school.

First, the differences retune to the environment pollutions.

The food and the water polluted may be the second reason for those differences.

The most significant difference was shown for aluminum element.

There were no significant differences either between the levels of the same school or between the corresponding levels of the two schools .

Moreover, it was considered interest to compare the present values on this thesis with those available in the literatures and the references range concentration as written in Table 3.6. Since this kind of information is regretfully rare, data reported therein are not homogeneous in terms of eligibility criteria, age, sampling procedure and treatment .Nevertheless, a comparison shows rather overlapping ranges (observed in many cases).

Figure 3.18 graphically displays the obtained results for each of the elements studied in this work and how they compare to the established "Population Reference Range" ^{*}.

Values that are above or below the reference range indicate a deviation from "normal". The more significant the deviation, the greater the possibility a deficiency or excess may be present.

Examination of the Figure 3.18 reveals that the levels of the trace elements: lead (Pb), cadmium (Cd), arsenic (As), cobalt (Co), nickel (Ni) and aluminum (Al) ,all, are above the established reference range for these trace elements.

Aluminum was exception high. These findings support the need for reducing exposure to those elements.

On the other hand, the levels of the elements copper (Cu) and zinc (Zn) were within the acceptable range.

^{*} Generally, reference ranges should be considered as guidelines for comparison with the reported test values. These reference ranges have been statistically established from studying an international population of "healthy" individuals.

^{*} Important Note: The reference ranges should not be considered as absolute limits for determining deficiency, toxicity or acceptance.

Table 3.6: Content Range for Elements in the Hair from Different Countries (µg/g)

Element	Yemen (this study): Min-Max Values µg/g*	Bulgaria ¹	Canada ²	England ³	Italy ⁴	Japan ⁵	New Zealand ⁶	USA ⁷	Reference range (µg/g hair) ^{8*}
Al	7.37-67.36	2.7-21.3	1.0-17.0	1.79-9.43	0.1-27.6	0.6-36.0	6.2-10.8	1-14	<18
As	0.40-1.08	0.04-0.62	--	0.10-2.41	0.02-0.93	1.00-2.70	0.28-1.05	1.00	<0.2
Cd	0.55-1.32	0.56-2.71	--	0.11-0.99	0.03-1.72	0.05-0.57	0.36-1.51	0.10-0.43	<0.15
Co	1.29-3.29	0.03-0.17	--	0.01-0.20	0.03-3.83	0.13-0.49	0.04-0.10	0.20-0.23	0.013-0.035
Cu	7.92-11.87	7.2-19.4	4-245	4.6-19.4	0.29-280	6.0-69.1	3.42-8.12	6.5-18.0	9-30
Ni	1.31-5.55	0.55-3.59	--	0.44-7.10	0.03-10.0	0.17-3.00	1.62-4.52	0.40-1.30	0.79-1.71
Pb	8.59-32.71	--	2.00-4.00	--	0.12-36.7	1.4-18.0	--	2.00-4.00	<10
Zn	96.91-125	144-284	120-220	142-260	24-477	72-327	158-293	120-220	110-210

1- Ward et al 1987.

2- Rya et al 1988.

3- Ward, Personale Communication 1988.

4- Senofonte et al 2000.

5- Kamakura, M. 1983.

6- Ward et al 1986.

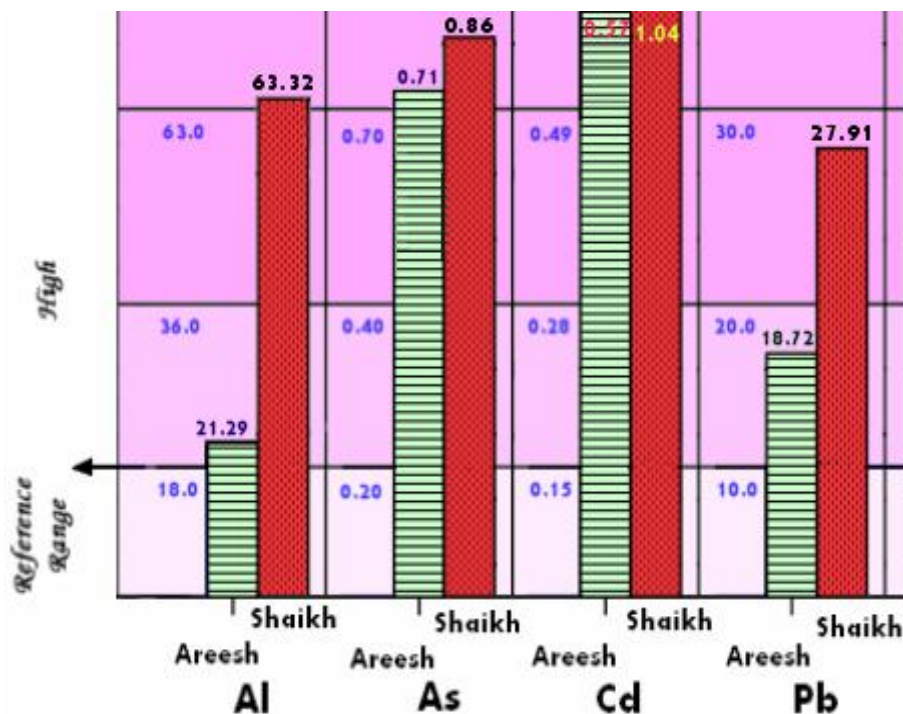
7- Mineral Lab. USA, Data Supplied, 1989

8- Al (Trace elements, 2006), Pb (Haas, 1992), As (Trace elements, 2006), Cd, Co and Cu (Doctor's data, 2006), Ni (Samanta et al, 2004) . Zn (Trace elements reports, 2006, Doctor's data reports, 2006 and Haas, 1992).

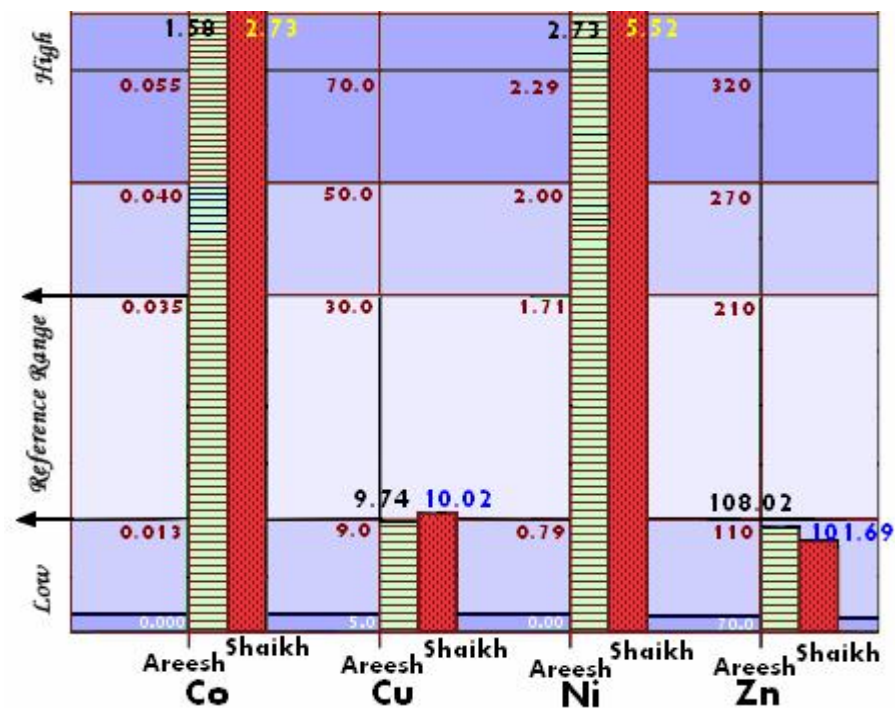
* Note: The reference range for the toxic elements wrote less than (<) one value while the essential and/or other elements wrote as a rang between two values (min-max) .

Figure 3.19: Population Reference Ranges and the Obtained Results for Each of the Investigated Elements*

(a) Toxic Elements



(b) Essential and Other Elements



* Adapted with modification from (trace elements and Doctor's data reports 2006)
<http://www.traceelements.com> and <http://www.doctorsdata.com>

Chapter Four

RECOMMENDATIONS

Chapter (4)

RECOMMENDATIONS

In light of the results obtained in this study, the researcher recommends the following:

4.1 General Recommendations

- Other schoolchildren in Aden governorate and in other governorates of the Republic of Yemen should also be subjected to a similar study as in this study.
- Gardening schools from inside and the outside surrounding is important to minimize pollution from various sources.
- Ensure that children schools are located in places far away from pollution sources such as heavy traffic, fuel and power stations, handcrafts and car maintenance workshops ...etc.
- Carry out similar studies involving other toxic elements such as mercury (Hg) and chromium (Cr).

- Continue monitoring trace elements in children hairs in a periodical manner.
- Yemen Standardization, Metrology and Quality Control Organization (YSMQCO) should be given a more active by putting standard specifications so that it includes information about the maximum allowable exposure to toxic trace elements.
- Organize regular campaigns to assure that schools are not surrounded by random and illegal handcrafts and car maintenance workshops ...etc.
- Increase awareness of the harmful effects of toxic elements by initiating public awareness campaigns to call attention for the danger and risk of these elements.

4.2 Specific Recommendations

First : Al-Shaikh Othman School

The present study has showed that students of the schools suffer from relative high concentration ratios of some trace elements specifically Al, Pb, Ni and Co. Hence, the author recommends the following:

- School principals in corporation with the local authority take the action to evacuate the area around the school from all sources of pollution such as fuel stations, handcrafts and car maintenance workshops ...etc.
- Follow up the healthy situation of the students specifically exposure to toxic elements.
- Foster a love of having clean and healthy environment among the students.
- Educate the students about the risk of toxic elements and the dangers of being affected by them.

Second: Al-Areesh School

- Maintain and keep the school surroundings clean and far away from pollution sources.
- Foster a love of having clean and safe environment.
- Raise awareness about the risk of heavy and trace elements.
- Cultivate the school indoors and outdoors.

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ANNEXES

ANNEX 1

Some Statistical Processes Applied In the Thesis

Dixon's Q-test:*

The test is very simple and it is applied as follows:

(1) The N values comprising the set of observations under examination are arranged in ascending order:

$$x_1 < x_2 < \dots < x_N$$

(2) The statistic experimental Q-value (Q_{exp}) is calculated. This is a ratio defined as the difference of the suspect value from its nearest one divided by the range of the values (Q: rejection quotient). Thus, for testing x_1 or x_N (as possible outliers) we use the following Q_{exp} values:

$$Q_{exp} = \frac{|\text{outlier} - \text{value closest to the outlier}|}{|\text{highest value} - \text{lowest value}|}$$

(3) The obtained Q_{exp} value is compared to a critical Q-value (Q_c) found in tables. This critical value should correspond to the confidence level (CL) we have decided to run the test (usually: CL=95%).

(4) If $Q_{exp} > Q_c$, then the suspect value can be characterized as an outlier and it can be rejected, if not, the suspect value must be retained and used in all subsequent calculations.

A table containing the critical Q values for CL 90%, 95% and 99% and N=3-10 is given below

* University of Athens site <http://www.chem.uoa.gr/applets/> , Retrieved May,2006

Table 1: critical values of Q

N	Q _{crit} (CL:90%)	Q _{crit} (CL:95%)	Q _{crit} (CL:99%)
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

Table 2: Descriptive Statistics for Modified Cd Concentrations in the Studied Schools

School					Statistic	Std. Error
Element Cd	Areesh	Mean			.5700	.01114
		95% Confidence Interval for Mean	Lower Bound	.5221		
			Upper Bound	.6179		
		5% Trimmed Mean				
		Median		.5780		
		Variance		.000		
		Std. Deviation		.01929		
		Minimum		.55		
		Maximum		.58		
		Dataset II W P Value Decision				
		Areesh 3 0.75000 0 Not Normal at 0.05 level				
		Shaikh	Mean			
	95% Confidence Interval for Mean		Lower Bound	.6641		
			Upper Bound	1.4204		
	5% Trimmed Mean		1.0436			
	Median		1.0545			
	Variance		.056			
	Std. Deviation		.23765			
	Minimum		.75			
	Maximum		1.32			
	Dataset II W P Value Decision					
	Shaikh 4 0.99522 0.97164 Normal at 0.05 level					

Table 3: Descriptive Statistics for Modified Ni Concentrations in the Studied Schools

School				Statistic	Std. Error
Element Ni	Areesh	Mean		2.7278	.45705
		95% Confidence Interval for Mean	Lower Bound	1.4588	
			Upper Bound	3.9968	
		5% Trimmed Mean		2.7512	
		Median		2.6600	
		Variance		1.044	
		Std. Deviation		1.02199	
		Minimum		1.31	
		Maximum		3.73	
		Dataset	II	W	P Value
				Decision	

		Areesh	5	0.91650	0.50927
				Normal at 0.05 level	
	Shaikh	Mean		5.5153	.02426
		95% Confidence Interval for Mean	Lower Bound	5.4109	
			Upper Bound	5.6197	
		5% Trimmed Mean		.	
		Median		5.5260	
		Variance		.002	
		Std. Deviation		.04203	
		Minimum		5.47	
		Maximum		5.55	
		Dataset	II	W	P Value
				Decision	

		Shaikh	3	0.92308	0.46326
				Normal at 0.05 level	

Table 4: Tests of Between-Subjects Effects for Copper

Source	Type II Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.695(a)	11	.427	.322	.946
Intercept	1670.132	1	1670.132	1258.588	.000
Lab	.216	2	.108	.082	.923
School	.710	1	.710	.535	.497
Level	2.078	4	.520	.392	.808
School * Level	1.584	4	.396	.298	.868
Error	6.635	5	1.327		
Total	1681.462	17			
Corrected Total	11.330	16			

a R Squared = .735 (Adjusted R Squared = .564)

Table 5: Multiple Comparisons for Copper

	(I) Laboratory	(J) Laboratory	Mean Difference (I-J)	SE(±)	Sig.	95% Confidence Interval	
						CIL	CIU
LSD	b	c	.1237	.87982	.894	-2.1380	2.3853
		d	.2948	.68150	.683	-1.4571	2.0467
	c	b	-.1237	.87982	.894	-2.3853	2.1380
		d	.1711	.75831	.830	-1.7782	2.1204
		b	-.2948	.68150	.683	-2.0467	1.4571
Dunnett C	b	c	-.1711	.75831	.830	-2.1204	1.7782
		c	.1237	.96167		-3.9844	4.2317
		d	.2948	.93675		-3.6152	4.2048
		b	-.1237	.96167		-4.2317	3.9844
		d	.1711	.23075		-1.1483	1.4906
Dunnett t (2-sided)(a)	d	b	-.2948	.93675		-4.2048	3.6152
		c	-.1711	.23075		-1.4906	1.1483
		d	.2948	.68150	.887	-1.8012	2.3908
		c	.1711	.75831	.967	-2.1611	2.5034
		d					

a Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 6: Tests of Between-Subjects Effects for Lead

Source	Type II Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	771.668(a)	11	70.152	10.062	.010
Intercept	8199.249	1	8199.249	1176.075	.000
School * Level	22.114	4	5.528	.793	.577
School	330.603	1	330.603	47.421	.001
Level	393.102	4	98.275	14.096	.006
Lab	8.871	2	4.436	.636	.567
Error	34.859	5	6.972		
Total	9005.776	17			
Corrected Total	806.527	16			

a R Squared = .957 (Adjusted R Squared = .862)

Table 7: Multiple Comparisons for Lead

	(I) Laboratory	(J) Laboratory	Mean Difference (I-J)	SE(±)	Sig.	95% Confidence Interval	
						CIL	CIU
LSD	a	b	-1.7623	2.15588	.451	-7.3042	3.7795
		d	-2.9968	1.39161	.084	-6.5740	.5805
	b	a	1.7623	2.15588	.451	-3.7795	7.3042
		d	-1.2344	2.06410	.576	-6.5404	4.0715
Dunnett C	d	a	2.9968	1.39161	.084	-.5805	6.5740
		b	1.2344	2.06410	.576	-4.0715	6.5404
	a	b	-1.7623	6.44160		-101.4028	97.8782
		d	-2.9968	3.92177		-15.1592	9.1657
	b	a	1.7623	6.44160		-97.8782	101.4028
		d	-1.2344	6.16095		-103.1647	100.6958
	d	a	2.9968	3.92177		-9.1657	15.1592
		b	1.2344	6.16095		-100.6958	103.1647
Dunnett t (2-sided)(a)	a	d	-2.9968	1.39161	.147	-7.2747	1.2811
	b	d	-1.2344	2.06410	.800	-7.5796	5.1107

Based on observed means.

a Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 8: The Correlations between the Elements

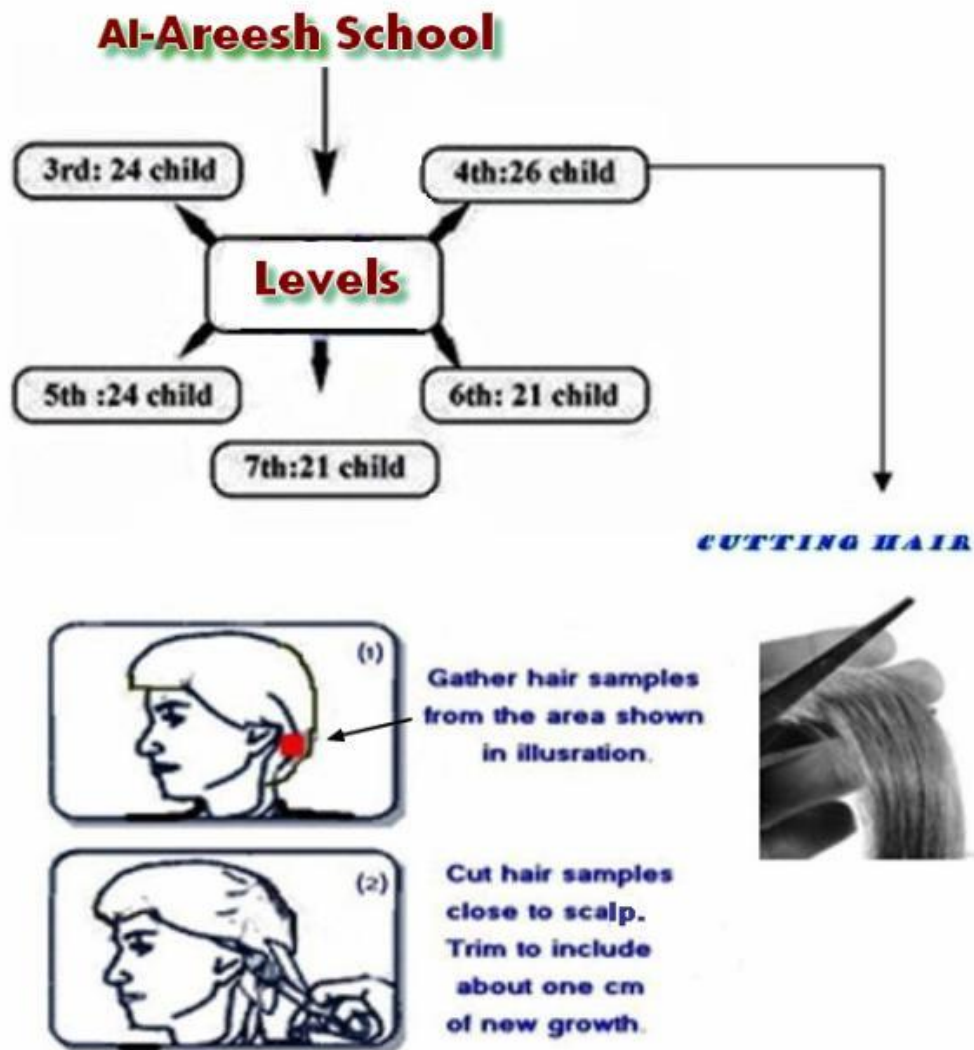
		Al	As	Cd	Co	Cu	Ni	Pb	Zn
Al	Pearson Correlation	1	.652	.893(**)	.924(**)	.712(*)	.964(**)	.942(**)	.946(**)
	Sig. (2-tailed)		.113	.007	.000	.021	.000	.000	.000
	N	10	7	7	10	10	8	10	10
As	Pearson Correlation	.652	1	.583	.610	.839(*)	.751	.812(*)	.654
	Sig. (2-tailed)	.113		.170	.146	.018	.052	.027	.111
	N	7	7	7	7	7	7	7	7
Cd	Pearson Correlation	.893(**)	.583	1	.992(**)	.580	.932(**)	.882(**)	.949(**)
	Sig. (2-tailed)	.007	.170		.000	.172	.002	.009	.001
	N	7	7	7	7	7	7	7	7
Co	Pearson Correlation	.924(**)	.610	.992(**)	1	.642(*)	.938(**)	.964(**)	.972(**)
	Sig. (2-tailed)	.000	.146	.000		.045	.001	.000	.000
	N	10	7	7	10	10	8	10	10
Cu	Pearson Correlation	.712(*)	.839(*)	.580	.642(*)	1	.762(*)	.866(**)	.884(**)
	Sig. (2-tailed)	.021	.018	.172	.045		.028	.000	.000
	N	10	7	7	10	17	8	17	17
Ni	Pearson Correlation	.964(**)	.751	.932(**)	.938(**)	.762(*)	1	.970(**)	.915(**)
	Sig. (2-tailed)	.000	.052	.002	.001	.028		.000	.001
	N	8	7	7	8	8	8	8	8
Pb	Pearson Correlation	.942(**)	.812(*)	.882(**)	.964(**)	.866(**)	.970(**)	1	.903(**)
	Sig. (2-tailed)	.000	.027	.009	.000	.000	.000		.000
	N	10	7	7	10	17	8	17	17
Zn	Pearson Correlation	.946(**)	.654	.949(**)	.972(**)	.884(**)	.915(**)	.903(**)	1
	Sig. (2-tailed)	.000	.111	.001	.000	.000	.001	.000	
	N	10	7	7	10	17	8	17	17

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

ANNEX 2

Cutting Hair Steps



ANNEX 3

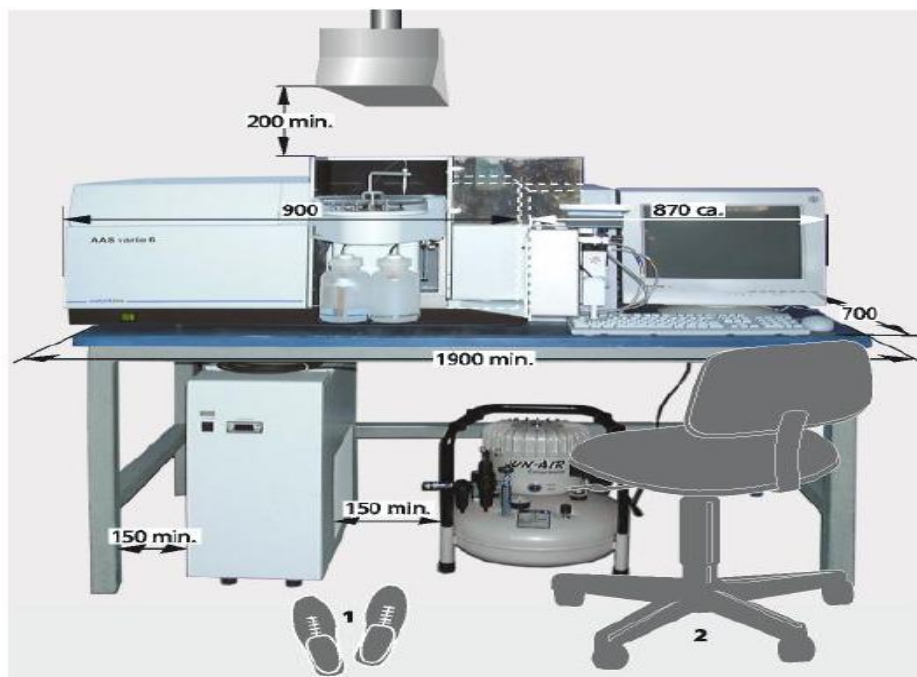
Spectrophotometer Techniques

**Figure : (a) HACH Spectrophotometer Device with its Kit#
(b) :AAS Device Layout and Operating Positions##**

(a)



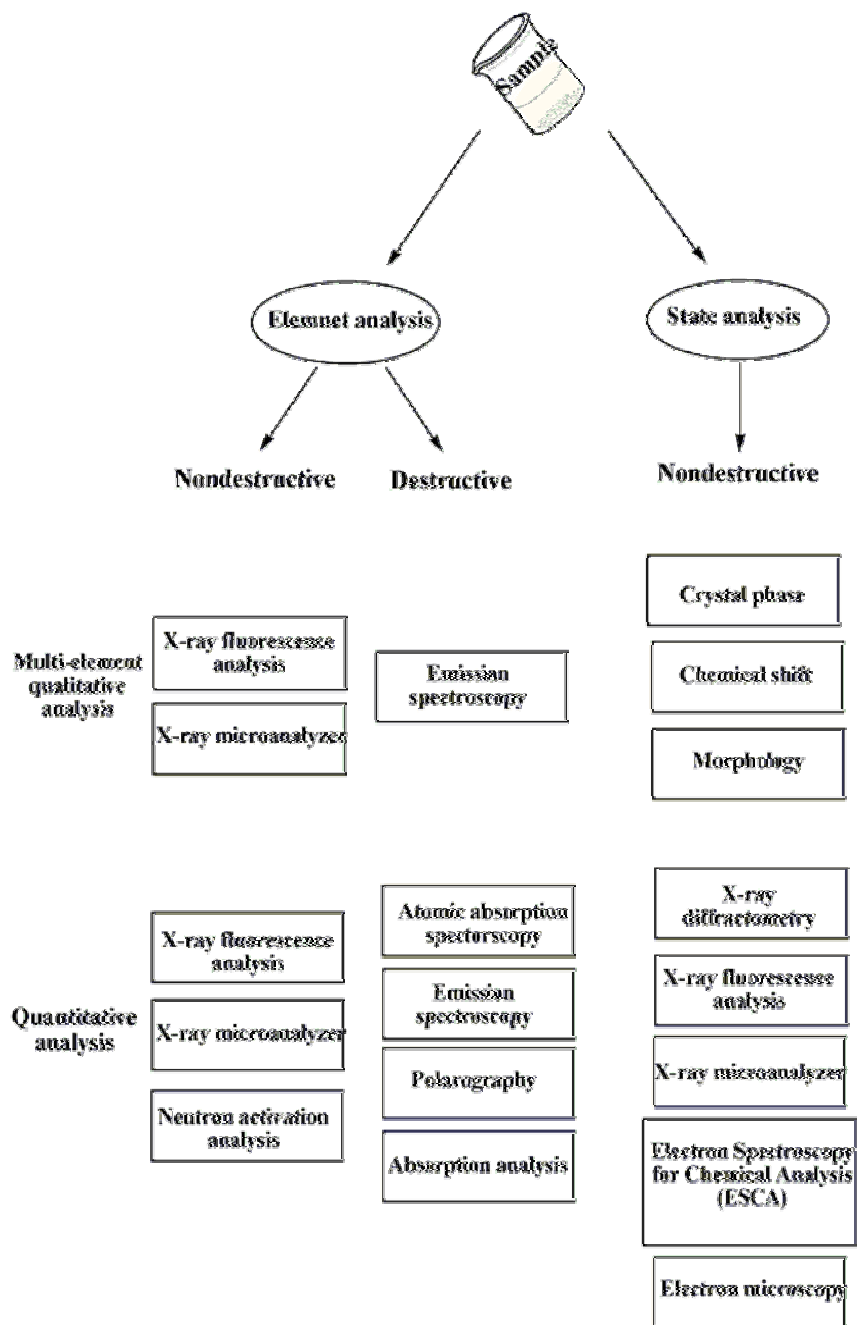
(b)



- 1 Position when installing
- 2 Position when operating the WinAAS

ANNEX4

Proposed Diagram for Metal Element Analysis*



* Adapted from “Kikuo OIKAWA, (1977) Trace Analysis of Atmospheric Samples, Japan, Kodansha Ltd. ”

إنّ سبب تطبيق تقنيّات مختلفة وإستخدام العديد من أجهزة الامتصاص الذري يرجع بدرجة رئيسة لفحص مدى دقة وصحة البيانات المتحصّل عليها للعناصر المدروسة باستخدام هذه التقنيّات والأجهزة .

سبب آخر مهمّ وراء استخدام أجهزة امتصاص ذري خارج الوطن هو عدم توقّر مصباح المهبط المجوّف لقياس بعض العناصر المدروسة باستخدام أجهزة الامتصاص الذري المحليّة. أظهرت النتائج وجود فروق معنويّة بين تراكيز العناصر؛ حيث سجّلت معظم تراكيز العناصر النّزرة المدروسة في عيّنات الشّيخ عثمان ارتفاعاً واضحاً عن تراكيز نفس العناصر في عيّنات العريش ووصل الفرق في تراكيز عنصر الألمنيوم في العيّنات أعلى مايمكن . لم تُظهر العناصر: الزرنيخ، النحاس والخارصين اختلافاً معنوياً في التركيز في عيّنات المحيطين البيئتين المدروسين .

يستنتج من الارتفاع في تراكيز العناصر قيد الدّراسة على تلوث منطقة الشّيخ عثمان . أظهرت النتائج ايضاً ارتفاعاً في تراكيز الكاديوم ،النّيكل والكوبلت للمدرستين أعلى من القيم القياسية العالميّة والمذكورة في تقارير الموقعين : <http://www.traceelements.com> و <http://www.doctorsdata.com/> .

كذلك أظهرت النتائج وجود ارتباط ثنائي لتواجد العناصر المفحوصة في منطقتي محافظة عدن المدروستان .

المُلخَص

يتناول هذا البحث دراسة مستوى تراكيز بعض العناصر النّزرة (أساسية وسامة) في عيّاتٍ شعر أخذت من التلاميذ الذكور للمراحل الأساسية الثالثة- سابعة (عمر 9 - 13 سنة) في مدرستين واقعتين في منطقتين مختلفتين في محافظة عدن هما : مدرسة العريش (منطقة العريش- خورمكسر) ومدرسة 30 نوفمبر (منطقة الشيخ عثمان) والمقارنة بين هذه التراكيز وأثر المحيط البيئي (انظر الخارطة في الفصل الثاني) على تراكم العناصر في أجسام التلاميذ والاستدلال على هذا التراكم من خلال تحليل الشعر كوسيلة استنتاجيه وإثباتية .

تمّ جمع عيّات الشعر (~200 طالب: 100 طالب / مدرسة) خلال الفترة سبتمبر ومارس 2004/2005 من فروة مؤخرة رأس التلاميذ لمدرسة العريش والشيخ عثمان وبمعدل عشرين تلميذ لكل مستوى دراسي. وضعت عيّات شعر تلاميذ كلّ مستوى معاً في ظرف بلاستيكي نظيف ومعقم وأغلقت بإحكام تمهيداً لنقلها للمختبر.

تمّ غسل العيّات وهضمت بحمض النتريك وجُهزت للتحليل كما هو موصوف في الجانب العملي من الدراسة (الفصل الثاني) .

استُخدمت طريقتين لتقدير تراكيز العناصر الأساسية والسامة قيد الدراسة [الخاصين (Zn) ، النحاس (Cu) ، الكوبالت (Co) ، النيكل (Ni) ، الزرنيخ (As)، الكاديوم (Cd) ، الرصاص (Pb) والألمنيوم (Al)] : (أ) طريقة المطيافية الجزيئية باستخدام جهاز مقياس طيف الضوء HACH موديل DR/RD/2010 [Professional Spectrophotometer] صنع أمريكي ؛ (ب) طريقة طيف الامتصاص الذري- اللهبى FAAS باستخدام أربعة أجهزة في أربعة مختبرات مختلفة اثنان منهما مختبرات محلية هما: مختبر عدن المركزي- جامعة عدن باستخدام جهاز Vairo 6 صنع ألماني ، ومختبر الأسماك (التواهي- عدن) باستخدام جهاز UNICAM VP90 صنع بريطاني ؛ ومختبران اثنان خارج الوطن (القاهرة- مصر): مختبر التحاليل الدقيقة باستخدام جهاز Perkin Elmer AAnalyst 100 أمريكي الصنع والمركز القومي للبحوث باستخدام Varian spectra 220 أمريكي .

تمّ استخدام طريقة طيف فرن الجرافيت الذري GFAAS بشكل خاص لفحص عنصر الزرنيخ باستخدام جهاز Varian spectra 220 مرتبطاً بفرن الجرافيت GTA-110 ؛ صنع أمريكي .



الإهداء

في ذكرى أبي الحبيب – رحمه الله تعالى

إلى عائلتي، مدرّسي وأصدقائي

إلى كل من ساعدني

عادل – عدن

2006

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عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿٥٠﴾ الرَّحْمَنِ



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مُقدّم من قبل

عادل أحمد محمد سعيد

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