INVESTIGATION OF ESSENTIAL AND POTENTIALLY TOXIC HEAVY METALS IN SOILS AND KHAT LEAVES BY USING ATOMIC ABSORBATION SPECTROSCOPY (AAS) TECHNIQUE.

The Case of Assano Area in Siltie zone

By

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A Thesis Submitted to the Department of Physics, Dilla University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Physics.

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Statement of the Approval

I hereby certify that we have read, evaluate and recommend to the faculty of post graduate studies for the acceptance of a thesis entitled " investigation of essential and potentially toxic heavy metals in soils and khat leaves by using atomic absorbation spectroscopy(AAS) technique "around Silti Woreda in siltie zone by Ayenew Meshesha and submitted in partial fulfillment of the requirements for the degree of master of science in physics compliance with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Advisor: Abebe Hailu (Dr.)	Signature	Date

As a member of the board of examiners of the MSc thesis open defense examination, I certify that I have read and evaluate the thesis prepared by Ayenew Meshesha Anteneh and examined the candidate. I recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of Master of Science in physics.

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Declaration

I declare that, this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this thesis. This thesis has been submitted in partial fulfillment of the requirements for the MSc degree in physics with specialization of Laser spectroscopy at Dilla University. The experimental work is my own work and the collaborative contributions have been indicated clearly and acknowledged.

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This MSc thesis has been submitted for examination with my approval as university advisor.

Advisor

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Date of Submission: August. 25, 2021

Dedication

To the memory of my late Grandfather Enyew beshaw, to my beloved uncle Tebeje Anteneh and aunt Teyeku Enyew.

Table of Contents

List of Table

List Of Figures

List of Abbreviations

AAS	Atomic Absorption Spectroscopy
CRM	Certified Reference Material
FAO	Food and Agricultural Organization
ppm	Parts per million (10-9 g/g or mL/mL)
MDL	Method Detection Limit
SD	Standard Deviation
ATSDR	Agency for Toxic Substances and Disease Registry
EPA	Environmental Protection Agency
H_0	null hypothesis
NPK	Nitrogen Phosphorus Potassium
PTWI	Provisional Tolerable Weekly Intake
PTDI	Provisional Tolerable Daily Intake
RDA	Recommended Daily Allowance
WHO	World Health Organization
ANOVA	Analysis of Variance
APHA	American Public Health Association
H_a	Alternative hypothesis
μ_k	mean concentration of khat leaves
IDL	Instrumental Detection Limit
RSD	Relative Standard Deviation
USEPA	United State Environmental Protection Agency
USPHS	United States Public Health Service
%RSD	Percentage relative standard deviation
μ_s	mean concentration of soil
CDC	Centers for Disease Control and Prevention
μ_c	mean concentration of control soil and khat leaves .
μ_{WHO}	mean concentration of WHO maximum allowable limit.
AOAC	Association of Official Analytical Chemists
TF	Transfer factor

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Abstract

This study was conducted to assess the concentration of heavy metals (Cd, Zn, Pb, Cu, Fe, Cr)in soil and khat leaves by using Atomic Absorbation Spectroscopy(AAS). A1-g of powdered soil sample was accurately weighed Using Zeta citizon. A 10-mL of 1:3mixture of concentrated HNO_3 and HCl were added to the flask and digested on Kjeldahil digestion block for an optimized period of 2:30 hour at the optimized temperature of 150°C.A 0.5-g of powdered khat leaves sample was accurately weighed by using zeta citizon.A4-mL of 1:1 mixture of concentrated HNO_3 and HCl were added to the reaction vessel. Digested by using Kjeldahil digestion block for an optimized period of 2:00 hours at the optimized temperature of 150°C.The percentage recovery for the soil and khat leaves samples are found in the range of 90.9 to 97.17%.The pH value of the soils ranged from 5.6$\pm$0.03 to 6.08$\pm$0.13 then,soil samples collected from khat growing area were moderately acidic. The transfer factor (TF) value of essential heavy metals were found to be for: Fe (0.54), Cu (0.33), for Zn (0.29) and the potentially toxic heavy (Cd, Pb and Cr) were excluded in the khat leaves tissue. The average concentration for the analyte heavy metal in the research sample is higher than the average concentration for the control sample for both the soil and khat leaves, this shows that the area is contaminated. However, it have not reached the pollution stage at 95% confidence level. The average concentration of heavy metals in the research and control sample for both soil and khat leaves are lower than the maximum allowable limit (MAL) set by WHO/FAO standard at 95% confidence level.

***Key Words**: concentration of Heavy metals, soil & khat leaves sample Analyses, AAS, Kjeldahil digestion.*

CHAPTERONE:INTRODUCTION

1.1 Background of the study

Heavy metals are any metallic chemical element that have a relatively high density and is toxic or poisonous at a given concentration when the usage is above the limits of WHO. Thus metals can end up in the soil and khat leaves from different sources which include agricultural practices such as application of phosphatic fertilizers, pesticides, fungicides and refuse derived composts (Alloway 1995). Heavy metals are extremely persistent in the environment and they are non-biodegradable therefore readily accumulate to toxic levels (Akguc2008**).** The most common sources of heavy metals in the environment are the anthropogenic activities such as: Bio Solids ,Manures, fungicides pesticides, herbicides, rodenticides, phosphate minerals, chemical industry, agriculture and domestic activities (L.Jantschi et al 2008,C,Stihi et al 2006).Iron, Zinc and Copper are essential for normal growth and enhancement of proper metabolic processes in human body only if the concentration levels are within allowable safe limits (Islam 2014) while Cr, Cd, Pb have no known health benefit to both animals and plants, on the contrary, they can be hazardous even at trace level (Goyer, 2004).

Level of H. metals	Proven Essential	Non Essential
Trace (mg/L)	Fe, Zn, Cu	
Ultra-trace (μg/L)	Cr	Cd, Pb

Table 1.1:dietary point of view Essential (Islam, 2014) and nonessential (Goyer, 2004) elements

Soil is a main component of rural and urban environment of earth surface that serve as a natural medium for plant growth and other developmental activities (Haliru, H.A 2014). Soil acts as a thin layer of earth's crust and unconsolidated mineral matter influenced by genetic and environmental factors (yousefi,N. N 2009). Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, disposal of high metal wastes, land application of fertilizers, sewage sludge pesticides, wastewater irrigation, coal combustion residues and atmospheric deposition (Khan *et al*., 2008). Heavy metal contamination in agricultural soils may lead to the disorder of soil functionality and retardation of plant growth, and influence human health through a contaminated food chain (Khan 2008).

Toxicity of heavy metal pollution in the soil and khat leaves refers to cases where the quantities of the element in soil and khat leaves are higher than maximum allowable limit set by WHO/FAO and this is potentially harmful to biological life (Adele ken and Abegunde 2011).Heavy Metal refers to the potential of chemicals to cause injury (death) , abnormal functioning of the tissues , ill-health , death to the organism, disorder of soil functionality, retardation of plant growth and influence human health through a contaminated food chain (Khan 2008).Toxicity of Fe, Cu, Zn, Pb, Cr, and Cd in the environment increases the risk of entering in to the living systems directly or indirectly which is causing serious health issues (Guan 2014, Chen 2016).

Heavy Metal	Toxicology (potential risky) of heavy metal
Chromium(Cr)	Skin rashes, Lung cancer, Kidney and liver decreased fertility and sperm count.
Copper(Cu)	Headaches, Vomiting, Diarrhea, and Inclusive osteoporosis in children. Liver damage
Cadmium(Cd)	Bone fracture ,Reproductive failure , infertility, Psychological disorders, DNA damage or cancer development
Lead(Pb)	blood pressure, Kidney damage, Brain damage ,Declined fertility of men through sperm damage.
Iron(Fe)	estrogen-induced cancers, bacterial infections; liver disease; cancer and estrogen therapy
Zinc(Zi)	Stomach cramps, Skin irritations Vomiting

Table 1.2:Heavy metals and the organs systems they target (www.lentech.com, 2010)

Khat (*Catha edulis Forsk*) is an evergreen shrub of the Celastraceous family and the most favored part of the plant is it's leaves, particularly the young shoots near the top of the plant and it is widely cultivated in the equatorial climates mainly in the Arabian Peninsula and the regions around the horn of Africa (Al-Motarreb 2002b).khat leaves cultivation has extending from Southern Africa to the Arabian Peninsula more specifically in Yemen, Ethiopia, Kenya, Madagascar, Somalia, Tanzania and others as well (Abdulsalam 2002, Lemessa 2001).khat plant is known by different vernacular names such as, qat in yemen,khat (chat) in ethiopia,qaad (jade) in somalia and miraa (vive) in Kenya (Tilahun.E 2009, Elmi A.s 1983, Iraqi S.M 2014).The leaf of khat plant were chewed on a daily basis by more than 20million people on the south-western

Arabians peninsula&estern part of Africa (Baker, Broadly K.J 2002). On average, almost 70 % of households in Yemen and 50 % in Djibouti use khat (Milanovic 2008), and more than 30 % of Ethiopians have been reported to use khat (Belew 2000).

Siltie zone is a place where it is located in the south of Addis Ababa and found in SNNPR. It has a total of eleven Woreda, from those, one of the research area is Silti Woreda which is located at 55 ^{0} N and 38 ^{0} 12"E and found in the North East part of Siltie Zone. Kibet is the administrative center of Silti Woreda which is found 27 km from the Zone center, Werabe and 147 km from the Federal center, Addis Ababa

1.2 Statement Of The Problem

So far, very few research works have been conducted to study the concentration level and distribution of heavy metals in soil and in khat Leaves around the selected research area located in Southern Region, Ethiopia.(Ephraim Tilahun, June 2009) assessed the level of contamination of khat leaves and soils by eight heavy metals, Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb using flame atomic absorption spectrometry (FAAS) in Guraghe zone near to the current research study area. (Fenta AD and KidanemariamAA2015**)** conducted a research around the current study area with the objective of determining the quantity of selected essential and nonessential metals of; Co, Mg, Ca, Cu, Mn, Cr, Cd, Fe, Zn and K in the Khat leaf and soil by flame atomic emission spectroscopy (FAES) and flame atomic absorption spectrometry (FAAS) in Konso, Gidole, Koyra and Hadeia.(Mesfin Redi January 2017) has analyzed ten elements (Ca, Mg, Fe, Mn, Cu, Zn, Co, Ni, Cd and Pb) content in soil and in edible portion of khat leaves growing in different parts of sebeta, aletawondo and bonga using flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP, East Norwalk, USA).

However, to the best of my knowledge, there were no research studies conducted, using AAS and Kjeldahil bloke sample digestion techniques, to investigate the level and distribution of six heavy metals (Cd, Cr, Cu, Fe, Pb, and Zn) in agricultural soils and khat leaves. Therefore, this study is carried out to investigate the level of selected essential and potential toxic heavy metals (Cd, Cr, Pb, Zn, Fe and Cu) in the khat (*khat edulis*) leaves and soil samples in the research site using Atomic Absorption spectroscopy (AAS).

1.3 Objectives Of The Study

1.3.1 General Objective

The general objective of this study is to assess the levels of heavy metals (Cd, Cr, Cu, Fe, Pb, and Zn) in soil and khat leaf in Silti Woreda, Southern Ethiopia.

1.3.2 Specific Objectives

The specific objectives of this study are:

(i) To determine the levels of heavy metals in the soil using AAS.

(ii) To determine the levels of heavy metals in the khat leaf using AAS.

(iii) To compare the level of metals in soil and khat leaf to the MAL set by WHO/FAO.

1.4 Research Questions and Hypothesis

1.4.1 Research Questions

(I) Is the soil in the research area was contaminated by the analyte heavy metals?

(II) What are the levels of the heavy metals in the soil of the study area?

(III) Is the khat leaf in the research area was contaminated by the heavy metals?

(IV) What are the levels of the analyte heavy metals in the khat leaves of the Study area?

(V) Is there a significant difference b/n the contaminated levels of khat leaves and soil?

(VI) What are the sources of contamination, if any, for samples by heavy metals?

1.4.2 Research Hypothesis

1. The mean concentration of heavy metals in the soil of the research area is not higher than the mean concentration of the analyte heavy metals in the control soil sample.

$$H_0: \mu_s \leq \mu_c \qquad H_a: \mu_s > \mu_c$$

2.The mean concentration of heavy metals in the soil of the research area is not higher than the maximum allowable limit of WHO/FAO standard.

$$H_0: \mu_s \leq \mu_{WHO} \qquad H_a: \mu_s > \mu_{WHO}$$

3.The mean concentration of heavy metals in the khat leaves of the research area is not higher than the mean concentration of the analyte heavy metals in the control khat leaves

$$H_0: \mu_k \leq \mu_c \qquad H_a: \mu_k > \mu_c$$

4.The mean concentration of heavy metal in Khat leaf sample is not higher than the mean concentration of heavy metals stipulated by WHO/FAO maximum allowable limit.

$$H_0: \mu_k \leq \mu_{st} \qquad H_a: \mu_k > \mu_{st}$$

1.5 Scope and Limitation

There are many heavy metals that needs the attention of curious researcher in the area; however, due to financial limit and time constraints, firstly, only six heavy metal have been consider in the soil and khat leaf. Secondly, the area selected for study covers only the region (place) which is predominantly known for its expansive area under Khat leaves cultivation. Thirdly, the levels of selected heavy metal becomes investigate only on the horizontal profile over 10 cm depth of top soil and the edible part of the khat leaf .Fourthly, The roots, the stems of the khat leaves and the whole region of siltie zone were not considered in this study.

1.6 Significance of the Study

The major significance of this research is to provide information about the level of some selected essential and no essential heavy metals in khat leaves and soil sample. The study will also inform the final investigation related to the concentration levels of the heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in khat leaf and soil samples. Also it will give information for the concerned institutions and organizations to take some necessary actions for the wellbeing of the society. The results from the study will also be used to determine the remedial action to be taken including treatment of the khat leaves and soil to remove the heavy metals where the levels are too high. The findings of this study will create an awareness to the public for the health effects of chewing khat leaf, if the level of heavy metals in khat leaf is high as compared to WHO/FAO standard. This study is believed to give a clue for further studies, which is used as a reference for future large-scale studies in khat leaves cultivated in different parts of the region.

1.7 Organization of the Thesis

This thesis is organized into five chapters: It begins with a general introduction in chapter one (This chapter), which begins with the environmental definition of heavy metals, agricultural soil ,khat leaves ,toxicity of heavy metals and their sources. The remaining topics in this chapter include topics like; Statement of the Problem, General and Specific Objectives, Research Questions and Hypothesis, scope and limitation of the Research, Significance of the Research, and Organization of the Thesis.

The next chapter comprises review of literature based on relevant books and articles written on the environmental issues around themes: heavy metal, Agricultural soil, khat plant, toxicity of heavy metal, source of heavy metal and their healthy effect.

Chapter three deal with materials and methods employed in the implementation of the objectives of this research work. In this chapter we look up description of the study area, Apparatus and Equipment, chemicals, reagents and standard solutions, optimization condition, apparatus and equipments, soil sample collection, pre-treatment and digestion; khat leaves sample collection, pre-treatment and digestion, AAS and Kjeldahil-digestion including descriptive statistical analysis techniques.

In Chapter four, results of instrument calibration, method validation and method detection limit (MDL) for each analyte heavy metal are reported. Then, the descriptive statistical analysis results of soil and khat leaves data including mean, median, range, standard deviation, TF etc were recorded. For the purpose of clear presentation, different methods of data reporting styles including tables and bar graph were employed. For better understanding of the data obtained from AAS analysis, the results of data analysis techniques, including the concentration of heavy metals in the soil and khat leaves sample analyses for results were displayed in tabular form and bar graph.

Chapter five includes short summary of the whole work, conclusions and recommendations based on the analysis results obtained from the research work as described in Chapter four. There is also Appendices, which includes agricultural soils and khat leaves data

CHAPTER TWO:LITERATURE REVIEW

Introduction

Heavy metals such as lead, copper, zinc, chromium, Cadmium and iron may be present in an environment through natural and anthropogenic causes and their presence in trace concentrations is important both to the animal and plant development on the contrary, if the concentration of these heavy metals surpasses the maximum allowable levels as stipulated by various organizations such as World Health Organization (WHO), American Public health Association (APHA, 1989),then plant and animal developments may be retarded because of malfunctions caused by concentration of heavy metals. The chewing of khat leaves (Catha edulis Forsk) is widely practiced in East Africa and parts of the Middle East, where it forms a deep-rooted social and cultural function (Drake1988). In this chapter we will see the meaning of heavy metals, the classification of heavy metals, the nature of soil ,contaminated soil by heavy metals, khat leaves, the toxicological potential of heavy metal in soil & khat leaves, sources of heavy metal in soils and khat leaves, sources and health effects of heavy metals in khat leaf and soil were explained.

2.1 Heavy metal

The term heavy metal refers to any metallic element whose density is relatively high and toxic or poisonous even at low concentration (Kaduna2014).Heavy metals are not degradable and once they enter into an environment, they will stay there for a longtime (Vote al., 2008). The word heavy metal has been used in the literatures environmental pollution for group name of metals and metalloids that have been associated with environmental contamination and potential eco-toxicity (Islam, 2014).Increased heavy metal concentrations in the soil (mostly from anthropogenic activities such as sewage sludge application) are considered to pose possibly serious hazards in the soil-plant-animal system (Jin Qian et al.,1996). The most common heavy metals found at contaminated sites, in order of abundance are Pb, Cr, As, Zn, Cd, Cu, and Hg (USEPA 1996). Plants have ability to absorb heavy metals in soil, know Potential health risks to humans and animals from consumption of khat leaves can be due to heavy metal uptake from contaminated soils via plant roots as well as direct deposition of contaminants from

the atmosphere onto plant surfaces (Ping Zhuanga 2009). The accumulation of heavy metal contents on the khat leaf and soil can be increased or decreased depending on the agricultural practices, such as application of phosphatic fertilizers, insecticides, pesticides, DAP, URIA and refuse derived composts.(Alloway, 1995).heavy metals are metals and metalloids having density greater than 5gm/cm3 (Akwa Ibom State. 2013 Maced. J. Med.Sci. 2015).thus metals are: mercury, chromium, magnesium, zinc and that are widely distributed in nature such as water, soil, air and various forms of organisms (Many in Zhang et al., 2009). Simultaneously, some micronutrient elements (Cu, Cr, Cd, Fe, Pb and Zn) may be toxic to both plants at high concentration (K. Chojnacka 2004).

Heavy metals may enter the food chine as a result of their uptake by edible plants (Am. J. Anal. Chem. 2013) and the yield of plant depends on fertility, presence of micronutrients and heavy metal in the soil. the soil condition is of great importance ,because it is a universal medium for plant growth ,which supplies essential nutrients to the plants(IJIRR. 2015).Absorption and accumulation of heavy metals in khat leaves are influenced by many factors, including: sources generated by agricultural technologies like irrigation with wastewater, ,mineral fertilizers with the load of heavy metals, application of pesticides, which contain in their structure (Prabu 2009,Hart 2005,

2.2 The classification of heavy metals

Most of the people that chews khat leaves do not have information about the contamination level and the health problem of essential (Fe, Cu, Zn) and non-essential (Pb, Cr, Cd) elements which is caused by chewing khat leaves, again there is still insufficient information on their toxicological implication due to this case some of the signs of heavy metal contamination among the consumer like mental retardation and cancer are evident among the population (Lesamana 2009).

2.2.1 The trace elements (trace essential metals)

Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as ‘rang of safe and adequate intakes’, results in a consistent and reproducible impairment of a physiological function (Xiu, Y.M 1996). Thus, establishment of the optimum daily requirements and determinations of actual daily intake of elements are the problem of trace element in nutrition (Windisch, W 2002).

A. Copper

Copper is an essential trace element to plants, animals and even humans, and although the concentration of copper is usually low in nature, it happens in adequate quantities for growth in all aquatic environment, for bone formation, maintenance of myelin within the nervous system, synthesis of hemoglobin, involved in the redox reactions in the cells of animals (Nursery, 1998). Deficiency of copper causes low white blood cell count and poor growth where as Excess intake of copper can cause vomiting, nervous system disorder and other diseases (Windiest, W.;2002, Manahan, Stanley E).Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health (J. Bjuhr 2007).

B. Iron

Iron is an essential micronutrient for almost all living organisms because it plays critical role in metabolic processes and a significant role in basic biological processes (Benefit HF and Van Deer Mark1983). Iron is being the most abundant element in the lithosphere, is generally present at high quantities in soils; however, its bioavailability in aerobic and neutral pH environments is limited (Benefit HF and Van Deer Mark1983).Iron availability is assumed to affect the natural distribution of species, and it may limit the growth of fast-growing and important plants (Van Deer Mark 1983, Brita Gringo, 1985).

C. Zinc

Zinc is an essential element found in the tissue of animals and plants even at normal ambient concentration; However, if plants and animals are exposed to large concentration of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects (Manahan, Stanley E,2003). Zinc occurs naturally in soil about 70 mg /kg in crustal rocks (B. E. Davies and L. H. P. Jones) but Zn concentrations are rising unnaturally, due to anthropogenic additions; most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing (K. M. Granny 2005).

2.2.2 Toxic metals (non-essential metals)

Toxic metals are among the major causes of health problems on earth today, Their presence in the atmosphere, soil and water can cause serious problems to all organisms (Das et al., 1997). Heavy metal toxicity can result in damaged or reduced mental and

central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (WHO, 1984).

A. Lead

Lead is one of the hazardous heavy metal pollutants of the environment that originates from various sources like burning of coal fertilizers, pesticides and from additives in pigments and gasoline (Eick et al., 1999).lead exposure can occur through multiple pathways, through inhalation of air, water, soil or dust, as it is emitted in the environment from vehicles and automobiles and can also enter the food chain via plants (Wierzbicka and Antosiewiez, 1993). In plants, its accumulation has been reported in stem, leaves, roots and seeds that increase with increase in exogenous Pb levels (Singh et al., 1998). It is well known to be toxic and its can cause serious injury to the brain, nervous system, red blood cells, and kidneys (D. R. Baldwin and W. J. Marshall 1999).

B. Chromium

Chromium is associated with allergic dermatitis in humans and it is a blood sugar mineral is also called an energy mineral b/c it is essential for insulin metabolism (A. Scraggy 2nd edition, 2006). Diabetes and coronary heart disease are associated with low chromium concentration in human tissue (Biomed Environ. Sci. 1996, Manahan, Stanley E.2003.As a heavy metal Cr, is one of the serious pollutants of air, soil and water (Froes et al, 2011; Wilson, 2012).

C. Cadmium

Cadmium is an essential micronutrient for plants and animals but may cause malfunctioning of metabolic processes (Wuana and Okieimen, 2011). The human body can tolerate low levels of Cd but long-term exposure can lead to serious health problems in humans, affects several organ systems, and can cause high blood pressure, heart disease, cancer, and other problems (ICDA, 2001). it is also present as an impurity in several products, including phosphate fertilizers, detergents , refined petroleum products and added to agricultural soils through the use of phosphate fertilizers (WHO, 1989). The application of agricultural inputs such as fertilizers, pesticides, and bio solids (sewage sludge), the disposal of industrial wastes or deposition of atmospheric contaminants increases total concentration of Cd in soils (K. Wiggler, M. J. McLaughlin, and R. D. Graham 2004).

2.3 Soil

Soil is the upper layer of the earth surface, which could be comprised of natural humus or a mixture of roots, leaves and compost in man-made soil material (Yli-Halla & Mokma 2002).Soil is an essential natural resource for support of human life but with time, its degradation has been constantly increasing due to the deposition of pollutants and the background concentration of metals in virgin soil depends primarily on the bedrock type from which the soil parent material was derived (Maldonado et al., 2008).

Heavy metals are introduced into soils from two major sources: Pedogenic (natural) and anthropogenic (human) sources (Ross, 2018). Igneous rocks (which crystallize from molten magma) and metamorphic (which form from other rocks by essentially solid state changes in chemical and physical environment, under very high pressure and temperature) are the commonest natural sources of heavy metals in soils. However, large quantity of heavy metals in the terrestrial and aquatic environment is a result of human intervention (Lindsay, 1979). Anthropogenic activities including manufacturing industries, agricultural activities, transport, municipal wastewater, and power generation are commonly considered as the major causes of elevated heavy metal levels in soils.

2.4 Contaminated soil by heavy metals

Heavy metal pollution in soils refers to cases where the quantities of the elements are higher than maximum allowable concentrations and this is potentially harmful to biological life (Gesso, 2001).Soil is often contaminated by human activities and this is reflected in the high horizontal and vertical variability brought about by the anthropogenic influence on soil formation and development (Fong 2008).The source of contamination in soil are multifarious and include agricultural and industrial pollution (Moor, Dietrich, Mikrochim. 2001).Heavy metals are released in to the environment through man's industrial, domestic and commercial activities, industrial effluents, pesticides, fungicides and manure from poultry farms (Akwa Ibom State The IJES 2013).Soil pollution by heavy metals has led to severe environmental degradation and this has negative impact on plants, animals, and human health directly or indirectly and Polluted agricultural soil is the starting point of contamination throughout food chain, potentially damaging plant life and affecting human health (Itana, 1998). In fact, metals are common natural components of all soils in earth's crust (Kabata-Pendias A 2007).

Environmental contamination by heavy metals has become a worldwide problem due to the fact that heavy metals unlike other pollutant are not biodegradable (Bazrafshan, E. Mohammadi, L.Ansari-Moghaddam2015).

Heavy metal occur naturally in soil which are formed by geological processes such as alteration & erosion of the geological underground material (Moor, C.; Kabir, E2012). Heavy metals occur at typical background in all ecosystems, however, anthropogenic releases can result in higher concentrations of these metals relative to their normal background values of the pollution (Gesso, 2001).The presence of toxic metals in soil can severely inhibit the biodegradation of organic contaminants (P. Maslin and M. Maier 2000) and hazards to humans and the ecosystem through direct ingestion or contact with contaminated soil, food chain (soil-plant-human or soil-plant- animal-human), reduction in land usability for agricultural production causing food insecurity, and land tenure problems (R. E. Harmon 2000 and W. Ling, Q. Sheen 2007).The continuous application of fertilizers to the soil and khat leaves may increase the heavy metal contents making it exceed the natural abundances in soils, Excessive content of these metals in food is associated with a number of diseases (WHO,1992&1995).

2.5 The Khat Plant (Catha Edulis)

Khat (Catha edulis Forsk) is an evergreen shrub of the Celastraceous family then the most favored part of the plant is it's leaves, particularly the young shoots near the top of the plant and it is widely cultivated in the equatorial climate mainly in the Arabian Peninsula and the regions around the horn of Africa (Al-Motarreb 2002). A large number of people are habitually chew fresh leaves owing to its amphetamine-like properties(euphoric properties) that have a verity of pleasurable stimulating effect(who 2006).The optimal altitude and annual rainfall for its growth ranges from 1,500 to 2,100 m and 1,000 to 1,500 mm, respectively (Al-Motarreb 2002 , Lemessa 2001).

Fig 2.1:Khat leaves wrapped up in banana leaves to preserve freshness

2.5.1 The Origin And History Of Khat Leaves

Khat leaves is exporting from Ethiopia to the neighboring and the Middle East countries and in recent years the market for khat leaves has grown to Europe & America (Carlson 2006, Lemessa 2001). (Al-Radii Yemen 1992) claim that khat originated from Yemen, however the literature indicates that khat originated from Ethiopia, specifically in Hararghe with a gradual expansion to different parts of Ethiopia, Yemen and other parts of the world. khat leaves cultivation has extending from Southern Africa to the Arabian Peninsula more specifically in Yemen, Ethiopia, Kenya, Madagascar, Somalia, Tanzania and others as well (Al-Motarreb 2002, Abdulsalam 2002, Elmi 1987, Lemessa 2001). khat plant is known by different vernacular names such as, qat in yemen,khat (chat) in Ethiopia, quad (jade) in somalia and miraa (vive) in Kenya (Tilahun.E 2009,Elmi,A.s 1983,Ireri,S.M 2014).

2.5.2 Social and Environmental Effects Of Khat Leaf (Catha Edulis)

Fresh khat leaves from khat trees are chewed daily by over 20 million people on the Arabian Peninsula and East Africa (Al-Motarreb A, Baker K, Broadly KJ 2002 and Sasha S, Dollery C. 2006). Recent reports suggest that 80–90% of male adult and 10–60% of female adult people in East Africa consume khat on a daily basis (Odenwald M, Neuner F, Schauer M, Elbert Lingenfelder B 2005). Khat is a high value cash crop in Ethiopia and it has become one of the most important sources of hard currency for the country (Dechassa, 2001). Khat cultivation examine the social, economical and ecological impacts of the society in Silti Woreda (Kibrom Alem 2016). Khat use is widely found to be socially accepted habit in most of the countries geographically situated where the herbal drug is cultivated and chewed as a recreational and socializing drug (Ali2010, Manghi2009, Al-Habori, 2005). Opponents of khat consumption claim that it damages health of the individual user and affects many aspects of life with its adverse social, economic and medical consequences, conversely, supporters of the habit of chewing khat maintain contrary to this points of view arguing that khat is useful in diabetic patients because it lowers blood glucose, it acts as a remedy for asthma, it eases symptoms of intestinal tract disorders and upholds social contact as a socializing herb (Hassan, Gun aid & Murray-Lyon, 2007)

Fig 2.2:khat leaves which is ready for chewing

In addition to, the use of chemical pesticides on khat leaves to speed-up adverse effects and imposes health risks (Al Habori 2005). A study by (Datival 2004) shows that people who chew khat which is sprayed with pesticides have the highest health risks due to the combination of the pesticides. The Cathinone and a lesser extent of cathine are held responsible for the effects of khat on the nervous system, and many other constituents of the khat are frequently overlooked (ECDD, 2006). The active ingredient in khat is the alkaloid Cathinone, sometimes called "natural amphetamine"(Chevallier A 1996).

2.5.3 The Chemical Composition Of Khat plant (Catha Edulis)

Khat contains a lot of chemical components that may have different effect on the body system Such as, alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino-acids, vitamins and minerals (Kalix and Brandon 1985,Nencini and Ahmed1989, Cox and Ramps,2003). Trace quantities of vitamins including ascorbic acid, riboflavin niacin and carotene (Szendrei, K.1980)and Elements including copper, Zinc, and toxic metals like lead and cadmium (Matloob MH.2003).The environment and climate conditions determine the chemical profile of khat leaves(WHO,2006). Cathinone is unstable and undergoes decomposition reactions after harvesting and during drying due to this case Cathinone is presumably the main psychoactive component of khat, this explains why fresh leaves are preferred and why khat is wrapped up in banana leaves to preserve freshness (ECDD2006).Additionally, Cathinone is the most abundant of the alkaloids in the fresh leaves of khat and is reported to be responsible for the pharmacological effects observed and Some of the effects desired by khat leaf chewers such as euphoria, alertness and for undesirable effects including drug dependency, hypertension and tachycardia are

considered to be responsible by Cathinone (Hassan2007, Al Habori 2005).However, agricultural practices like application of phosphatic fertilizers, pesticides and refuse derived composts can be the source of heavy metals in the soil and khat leaf (Steen land and Buffett 2000).

2.6 The toxicological potential of heavy metal in soil & khat leaves

Toxicity of iron, copper, zinc, lead, chromium and cadmium in the environment increases the risk of entering in to the living systems directly or indirectly causing serious health issues (Guan 2014, Chen 2016).Chewing its fresh leaves is a widespread habit in the local populations, with several million people consuming khat regularly in social sessions (Al-Motarreb A, Baker K, Broadly KJ 2002).The heavy metals Cd, Cr and Pb have not been shown to be essential for either plants or animals (Misra and Misra and Mani, 2009). Heavy metals are considered serious pollutants because of their toxicity, persistence and non biodegrable conditions in the environment, thereby constituting a threat to human beings and other forms of biological life (Adeleken and Abegunde, 2011).

Heavy metals occur in the atmosphere basically in particulate form and toxicity can result in damaged mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (WHO, 1984).The toxicity and mobility of heavy metals in soils depend not only on the total concentration but also on their specific chemical form, metal properties, environmental factors, soil properties and organic matter content (Osu and Okoro, 2011).A previous study has identified traces of heavy metal (Zn and Cu) samples in teeth dentine humans, that could have toxic effect (Asaduzzaman 2017).All heavy metals are toxic for living organisms at excessive concentrations, but some are essential for normal healthy growth and reproduction by plants at low but critical concentrations (Hemantaranjan, 2005). Users of khat report increased levels of energy, alertness and self-esteem, a sensation of elation, enhanced imaginative ability and a higher capacity to associate ideas, These effects have been attributed to the khat content in Cathinone (Kalix, P 1992).Although, other less potent stimulant substances may also be present, nor pseudo ephedrine (cathine) and nor ephedrine (Baker K, Broadly KJ 2002).Daily intake can also cause chronic constipation, hyperactivity, insomnia and hypertension are other side effects of khat, as well as dental and oral problems (Elmi As 1983). Khat is said to have reproductive toxicity in human

beings and several studies have shown that khat use can lead to a decrease in sperm quality, low birth weight and inhibition of lactation in mothers (Gebissa E 204).The most obvious effect of khat-use can be seen on the digestive system where gastritis is fairly common among regular chewers due to the effects of the acid tannin in it, khat should not be taken during pregnancy (Chevallier A.)

2.7 Sources of heavy metal in soils and khat leaves

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from rapidly expanding industrial areas, land application fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, and atmospheric deposition (S. Khan, 2008 and H. Wang 2010).

2.7.1 Fertilizers

Historically, agriculture was the first major human influence on the soil (A. Scraggy 2nd edition, 2006).Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (Cd and Pb) as impurities, which, after continued fertilizer, application may significantly increase their content in the soil (L. H. P. Jones and S. C. Jarvis 1981). Metals, such as Cd and Pb no known physiological effect. Application of certain phosphatic fertilizers (DAP, UREA) inadvertently adds Cd and other potentially toxic elements to the soil, including F, Hg, and Pb (P. H. Raven, L. R. Berg, and G. B. Johnson 2nd edition, 1998).

2.7.2 Pesticides`

Several common pesticides (DDT, malatine, insecticide e t c) used fairly and extensively in agriculture and horticulture in the past contained substantial concentrations of metals about 10% of the chemicals have approved for use as insecticides and fungicides (L. H. P. Jones and S. C. Jarvis 1981). Pb and Zn have resulted in contamination of soil that poses risk to human and ecological health, like Assimilation pathways include the ingestion of plant material grown in (food chain), or the direct ingestion (oral bioavailability) of contaminated soil (N. T. Baste and R. Gradwohl 1998). In addition, many are potentially hazardous because of their contents of heavy metals (Cr, Pb, and Zn) or toxic organic compounds are applied to land and others are very low in plant

nutrients or have no soil conditioning properties (M. E. Sumner, 2000).Such contamination has potential to cause problems, particularly if sites are redeveloped for other agricultural or non agricultural purposes and the use of such materials has been more localized, being restricted to particular sites or crops (M. J. McLaughlin 2000).

2.7.3 Bio Solids And Manures

Bio solids (sewage sludge) are primarily organic solid products, produced by wastewater treatment processes that can be beneficially recycled (USEPA 93/003).The application of numerous bio solids (livestock manures, composts, and municipal sewage sludge) to land inadvertently leads to the accumulation of heavy metals such as, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and so forth, in the soil (N. T. Baste, J. A. Ryan, and R. L. Chaney 2005). The manures produced from animals on such diets contain high concentrations of As, Cu, and Zn and, if repeatedly applied to restricted areas of land, can cause considerable buildup of these metals in the soil in the long run (R. D. Graham 2004). The potential of bio solids for contaminating soils with heavy metals has caused great concern about their application in agricultural practices (R. Canet, F. Pomares, F. Tarazona, and M. Estela 1998). Heavy metals most commonly found in bio solids are Pb, Ni, Cd, Cr, Cu, and Zn, and the metal concentrations are governed by the nature and the intensity of the industrial activity, as well as the type of process employed during the bio solids treatment (S. V. Matt 1983).

2.8 Sources and health effects of heavy metals in khat leaf and soil

2.8.1 Chromium

Chromium is a naturally occurring element in rocks, animals, plants, soil and volcanic dust (ATSDR, 1998). The sources of chromium in the environment include, cement, leather, textiles, paints, printing ink, detergents, wood preservatives (Hilgenkamp, 2006). Chromium is one of the heavy metals in the environment whose concentration is steadily increasing due to industrial growth, especially the development of metals, chemicals and tanning industries (Adele ken and Abegunde, 2011).The wide use of Cr compounds by modern industries has resulted in the discharge of large quantities into the environment via emission, waste water or solid waste disposal (Towns head, 1995).The general population is exposed to Cr by eating food, drinking water and inhaling air that contains the chemical (ATSDR, 1998).

2.8.2 Cadmium

cadmium is a heavy metal characterized by high mobility in biological systems and it is emitted to the atmosphere in combustion processes (Wieczorek et al., 2004). Among the sources of Cd in the environment include; mining and smelting of Zn, Pb and Cu, fossil fuel combustion and also phosphate fertilizers (Challa and Kumar, 2009). Soil receives Cd from two sources. Firstly, phosphate fertilizers invariably contain Cd as a natural contaminant. When land is sprayed with phosphates, Cd becomes incorporated in the soil. Secondly, polluted irrigation water increases the Cd load of soil (Sodhi,2009). Cd taken up by plants from the soil accumulates first of all in the roots, and then transported in smaller quantities to stems and seeds (Wieczorek et al., 2004). (Hanč 2008),reported that addition of manure and fossil fuel burning especially coal is an ongoing source which increase Cd uptake by plants. The exposure of cadmium and especially chronic exposure can cause renal dysfunction, calcium metabolism disorders and also increased incidence of some forms of cancer (Selinus and Alloway, 2005).Cd causes high blood pressures and kidney damage (Mehbrahtu and Zerabruk, 2011).

2.8.3 Copper

The major sources of Cu in land are mining operations, agriculture, solid waste and sludge from treatment works (Atlabachew at al., 2011). (Xiong and Wang2005) found that Cu concentration in the shoots was significantly influenced by Cu concentration in soil and increased markedly with an increase in the soil Cu concentration. Copper is widely distributed in foods and having the highest concentrations and dairy products having relatively low levels (IPCS, 1998).Copper is an essential element and adverse health effects are related to its deficiency as well as excess then the places where copper accumulates are the liver first, then the brain and the reproductive organs (Wilson, 2010). Long term exposure to Cu can cause irritation of the nose, mouth and eyes and causes headaches, stomachaches, dizziness, vomiting and diarrhea (Lenntech, 2009).

2.8.4 Lead

Lead is a toxic metal whose widespread caused extensive environmental contamination and health problems in many parts of the world as well as Lead emitting sources include mining and smelting, coal-burning and incinerators (Hill, 2007) while the addition of

artificial fertilizer and pesticides cause an increase of Pb levels in agricultural soil (Onder etal.,2007).Lead effectively absorbed by roots and leaves (Tyagi and Mehra,1990). Although Pb is not an essential nutrient for plants, majority of Pb is easily taken up by plants from the soil and accumulated in roots while only a small fraction was translocated upward to the shoots (Patriot2004). Chronic exposure to Pb can affect physical growth and can cause anemia, kidney damage, headache, hearing problems, speaking problems, fatigue or irritable mood (Simonov 2010). Exposure to low levels for long period results in loss of weight, body weakness and anemia in children, while severe cases may result in constipation, loss of appetite, nausea and vomiting, insomnia, headache, diarrhea, and even death (Ferguson, 1998).Pregnant women exposed to Pb have higher rates of infertility, miscarriage and still births (Edina 2000).

2.8.5 Zinc

Zinc is an essential trace element for plants, animals and humans found in virtually all food or organic complexes (Swaminathan et al., 2011). The main pollutant sources of Zn in the soils are mining activities, agricultural use of sewage sludge and composed materials and the use of agrochemicals such as fertilizers and pesticides (Alloway, 1995). The application of compost manure not only results in Zn accumulation in soil but also causes an increase in zinc mobility and enhances Zn leaching (Asada 2010). Excess Zn causes toxicity in plant whose symptoms included chloros in young leaves, browning of coralloid roots, and serious inhibition on plant growth (Long et al., 2003). Consumption of such food leads to high Zn levels in blood and Zn accumulation in body organs (Tilahun.E, 2009). Zinc toxicity can occur in both acute and chronic forms, then the acute adverse effects of high Zn intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea and headache (IMFNB, 2001).

2.8.6 Iron

Iron is a most crucial element for growth and survival of almost all living organisms (Valdo 2005). In humans, increased body stores of iron have been shown to increase the risk of several estrogen-induced cancers (Liar and Jones, 2001). Iron deficiency includes symptoms such as reduced resistance to infection, reduced work productivity, reduced physical fitness, weakness, fatigue, impaired cognitive function, and reduced learning ability, increased distractibility, impaired reactivity and coordination, itching, inability to

regulate body temperature and eating pica (Beard, 2001). People with pregnancy, menstruation, acute/chronic blood loss; vegetarian women; infancy, especially pre-maturity; bacterial infections; liver disease; cancer and estrogen therapy are exceptionally in need of high level of iron.

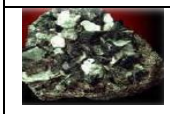
Heavy Metal	**Toxicology**	Reference
 chromium(Cr)	-Skin rashes , Upset stomachs and ulcers, Respiratory problems, Weakened immune systems, Kidney and liver damage, Alteration of genetic material, Lung cancer and death	Anderson, 1988; Truman1977; Offenbach 1986
 copper (Cu)	-Headaches, Stomachaches, Dizziness ,Vomiting ,Diarrhea , liver and kidney damage	Mason,1979; King 1978; Hadley 1988
 cadmium(Cd)	-Diarrhea, stomach pains , vomiting ,Bone fracture, Reproductive failure , infertility and Damage the central nervous system, the immune system, Psychological disorder, DNA and cancer development	Steinhardt, &Puns1993
 lead (Pb)	-A rise in blood pressure , Kidney damage , abortions, Disruption of nervous systems, Brain damage, Declined fertility of men through sperm damage, Diminished learning abilities of children,	National research Council(NRC) 1989
 iron(Fe)	-estrogen-induced cancers bacterial infections; liver disease; cancer and estrogen therapy	F.Oteiza,2002; Kohrle, 2002
 zinc (Zn)	-Stomach cramps, Skin irritations, Vomiting, Nausea, Anemia, Damage the pancreas Disturb the protein metabolism, Cause arteriosclerosis	Baer et al., 1984; Hess et al., 1977

Table 2.1:summery of toxicology heavy metal (www.lentech.com, 2010)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the Study Area

The present research was conducted on an agricultural land of Silti Woreda which is found in the North East part of Siltie Zone and located at a grid reference of 55^0N latitude and 38^0E longitude. Silti Woreda is one of the eleven Woreda as in Siltie zone. Kibet, the area where this research has been done, is found 27 km from the Zone center-Werabe, and 147 km South of the capital city of the country, Addis Ababa.

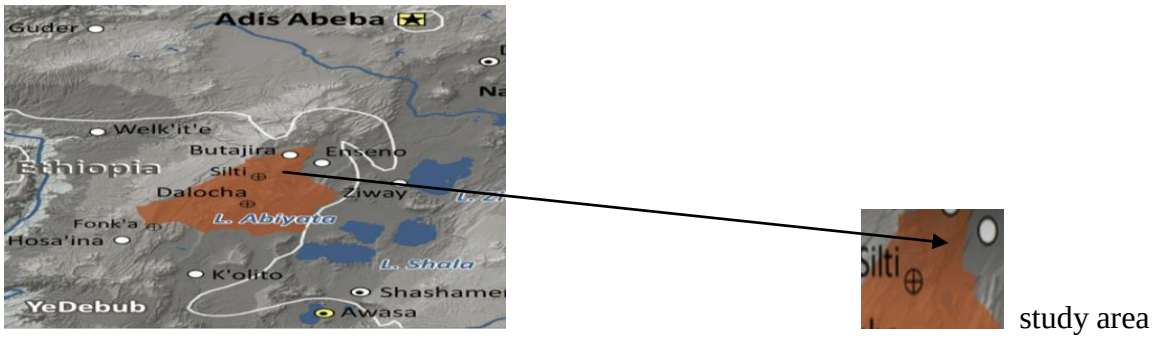


Fig 3.1:A real view of the research area and boundaries of the site (map data @ 2021)

This area is characterized by a semi-arid to semi-humid climate having an altitude of about 1200 m, and the maximum and minimum average daily temperatures of this area are 19 ^{0}C and 14.5^{0}C, respectively (WARDO, 2011). The mean annual rainfall of the study area is about 1500 mm. The climate in the research area is so suitable for cultivation of khat that it has become one of the major khat growing areas in the country. In addition to Khat, there is a considerable product of wheat and maize. However, Khat is the only agro-product for export that procures hard currency.

3.2 Apparatus and Equipment

The instruments used for this study was AAS (Model ZEE Nit 700 P Analytikjena Manufacturing Co. Ltd,Germany) for the investigation of essential and potentially toxic heavy metal in soil and khat leaves sample. Philip Harris standard magnetic stirrer Jenway (3345 ion meter) model was used for the determination of soil pH value. The common laboratory apparatus which were used during the study include; different sized beakers, volumetric flasks, funnels, round bottom flasks, block digester, scrapper (shovel), sieve, digestion flask (reflux condenser), pestle and mortar, spatula, filter paper, plastic knife, gloves, magnetic bar, polyethylene bags, Zeta citizon and oven.

3.3 Reagents and Standard Solutions

Concentrated HCl (Hopkins and Williams, UK) and HNO_3 (Spectrosol, BDH, UK) mixed with Distilled de-ionized water was used throughout the analysis for digestion of soil and khat leaves sample. Certified Stock standard solutions of 1000 mg/kg were prepared for the selected heavy metals (Cr, Cd, Zn, Fe, Pb and Cu). Three point calibration with pH of (4,7 and 10) buffers solutions were used to calibrate the digital pH meter before taking measurements.

3.4 Optimization of Digestion Procedure

To select an optimum procedure for digestion, parameters like digestion time, reagent volume, volume ratio of reagents, and digestion temperature were optimized by varying one parameter at a time and keeping the others constant. Parameters giving color less solution at lower temperature, requiring minimum reagent volume and digestion time were selected as an optimum procedure for digestion of Khat and soil samples.

no	S.m (g)	V.(mL)	HNO_3:HCL(mL)	T(C^0)	t(h)	Observation
1	1	5	2:2	50	1:30	Dark and turbid solution
2	1	6	3:3	50	1:30	Yellow solution with
3	1	7	3:4	50	1:30	Dark colored with liquid
4	1	8	5:5	50	1:30	Light yellow solution
5	1	10	1:1	50	1:30	Light yellow solution
6	1	9	5:4	50	1:30	Clear yellow solution
7	1	12	5:6	50	1:30	Clear but brown solution
8	1	**10**	**1:3**	50	1:30	**Color less solution**
9	1	11	5:6	50	1:30	A clear light yellow
11	1	4	1:3	50	1:30	Light yellow solution

Table 3.1A:Optimization of digestion volume ratio for soil sample

The bold font indicates optimized volume ratio; v=total volume ratio, s.m=sample mass, T=temperature, t- time

no	S.m(g)	V.(mL)	HNO_3:HCL(mL)	T (C^0)	t (h)	Observation
1	1	10	1:3	50	1:30	Clear light yellow
2	1	10	1:3	80	1:30	Clear yellow solution
3	1	10	1:3	120	1:30	Dark colored with liquid
4	1	10	1:3	**150**	1:30	**Clear, Color less**
5	1	10	1:3	200	1:30	Some yellowish matter

Table 3.1B:Optimization of digestion temperature for soil sample

The bold font indicates optimum digestion temperature; v=total volume, s.m=sample mass, T=temperature, t- time.

no	S.m(g)	V.(mL)	HNO_3:HCL(mL)	T (C^0)	t (h)	Observation
1	1	10	1:3	150	1:00	Clear light yellow solution
2	1	10	1:3	150	1:15	Clear yellow solution
3	1	10	1:3	150	1:30	Dark colored with liquid
4	1	10	1:3	150	1:45	Yellow solution \residue
5	1	10	1:3	150	2:00	yellowish turbid matter
6	1	10	1:3	150	2:15	Clear light yellow solution
7	1	10	1:3	150	**2:30**	**clear Color less solution**

Table 3.1C:Optimization of digestion time for soil sample

The bold font is optimum digestion time; v=total volume ratio, s.m=sample mass, T=temp.,t-time

Table 3.1A-3.1C:Optimization of volume ratio , mass, time and temperature for soil sample

no	S.m(g)	V.(mL)	HNO_3:HCL (mL)	T (C^0)	t (h)	Observation
1	0.5	5	2:3	60	1:15	Clear and Pale Yellow
2	0.5	4	3:1	60	1:15	Yellow solution color
3	0.5	3	2:1	60	1:15	Clear and Yellowish
4	0.5	**4**	**1:1**	60	1:15	**Clear and Colorless**
5	0.5	6	1:4	60	1:15	Clear and colorless
6	0.5	5	2:2	60	1:15	Pale Yellow color

Table 3.1D:Optimization of digestion volume ratio for khat leaves sample

The bold font shows optimized volume ratio; v=total volume, s.m=sample mass, T=temp., t- time.

no	S.m(g)	V.(mL)	HNO_3:HCL(mL)	T (C^0)	t (h)	Observation
1	0.5	4	1:1	60	1:15	Clear and Yellow color
2	0.5	4	1:1	90	1:15	Yellow solution color
3	0.5	4	1:1	120	1:15	Clear and pale Yellow
4	0.5	4	1:1	**150**	1:15	**Clear and Colorless**
5	0.5	4	1:1	180	1:15	Clear and Yellowish color

Table 3.1E:optimization of digestion temperature for khat leaves sample

The bold font is optimum digestion temp.;v=total volume, s.m=sample mass, T=temp., t- .time.

no	S.m (g)	V.(mL)	HNO_3:HCL (mL)	T (C^0)	t (h)	Observation
1	0.5	4	1:1	150	1:15	Pale Yellow color
2	0.5	4	1:1	150	1:30	Yellow solution color
3	0.5	4	1:1	150	1:45	Clear and pale Yellow
4	0.5	4	1:1	150	**2:00**	**Clear and Colorless**
5	0.5	4	1:1	150	2:15	Yellowish color

Table 3.1F:optimization of digestion time for khat leaves sample

The bold font indicates optimum digestion for time;

Table 3.1D-3.1F: Optimization volume ratio, mass, time, temperature for khat leaves sample.

3.5 Sample Collection, Preparation and Digestion

3.5.1 Soil Sample

3.5.1.1 Soil Sample Collection

Prior to the soil sampling, Shovel **(**scrape**)** was used to sweep and remove typical organic matter like leaves, roots, and other foreign organic materials on the top surface of each sampling area (3.3A)**.**Composite soil sampling technique was applied to collect soil samples from four sampling points, SA, SB, SC, and SD) over the whole 4 ha of the study area (Fig3.2A).The distance of separation between the corner of each sampling area was 100m apart from each other. The soil sample from each sampling area, say, SA, is in fact, a mixture of five sub-samples collected from five sub-sampling points (within ten meter square area). For instance, taking sampling point SA as a center, a 10m by 10 m square area is marked on the sampling ground. Then, from each corner point of the square and the center, 50 g of soil is excavated and the whole 250 g of the sample is put into a clean polyethylene bag as a representative sample for that point (SA) and labeled (fig 3.2A). Similar procedure is followed to collect the representative samples for the remaining four sampling points, namely, SB, SC and SD (fig3.2A) and the control sample (SE),which is about 500m from the research area (3.2B).The control soil sample was collected from unplowed barren nearby land. All the research samples as well as the control samples were collected on the date May 26/2013 EC at 4:00-6:40 AM, local time.

3.5.1.2 Pretreatment Of Soil Samples

Upon arrive at AAU, the soil sample were brought to the department of chemistry laboratory room to take the first physical treatments prior to Kjeldahil apparatus digestion. The soil samples were air-dried in a clean and dust free environment in the laboratory at a constant temperature of 20 C^0 for 4 days. Then, using an oven (Fig3.7A) the air-dried research samples were put in side it for 24 hours at 105 ^{0}C to reduce the moisture content of research soil (Fig3.7B).Using mortar and pestle, the dried research soil samples were grounded to convert it to powder, and to pass 1mm sieve. The dried, grounded and sieved of the research soil samples were mixed thoroughly and poured separately into clean polyethylene bottles. Similar procedure was followed for the pretreatment of the representative samples of the remaining control soil.

3.5.1.3 Kjeldahil Apparatus Digestion Of Soil

A digestion method reported by (Allen SE, Grim Shaw HM, Rowland AP 1986) was used for the digestion of the soil samples. 1g of powdered research soil sample was accurately weighed Using Zeta citizon (figure3.4A**).** A clean wooden spatula was used to transfer soil sample from each sampling point into 100 mL round bottom flask and moistened with 1 mL of distilled de-ionized water **(**fig 3.4B). Applying the optimization conditions (Table3.1A,3.1B and 3.1C),10 mL of 1:3 mixture of conc. HNO_3 and HCl was added to the flask and kept for 30min until it got stabilized. The round flask were then tightly closed and placed on the fire place (fire proof) of the Kjeldahil digestion apparatus (fig 3.5A).Then**,** digested by Kjeldahil block under reflux condenser for the optimized period of 2:30 hour at the optimized temperature 150°C on June 5/ 2013 EC (fig 3.5B). After two and half hours of the digestion time, the digested mixture was allowed to cool down to room temperature for about 30 min without spread apart on the Kjeldahil digestion block. Then about 100 mL of distilled deionized water was added to the flask, and filtered through What man No. 1 filter paper of 100 mL volumetric flask and top up to the mark with deionized water. Similar procedure was applied to digest the remaining control soil.

Kjeldahil apparatus is one of the wet acid digestion apparatuses by which organic components are decomposed in the form of different gaseous forms and metallic elements are left in the solution.

mass of soil	Reagents(mL)	Digestion Temp.	Digestion time	Results of Heating
1.0 g	1:3(HNO3:HCl)	$150c^0$	2:30 hr	Colorless solution

Table 3.1G:General summery and optimized condition for digestion of soil samples.

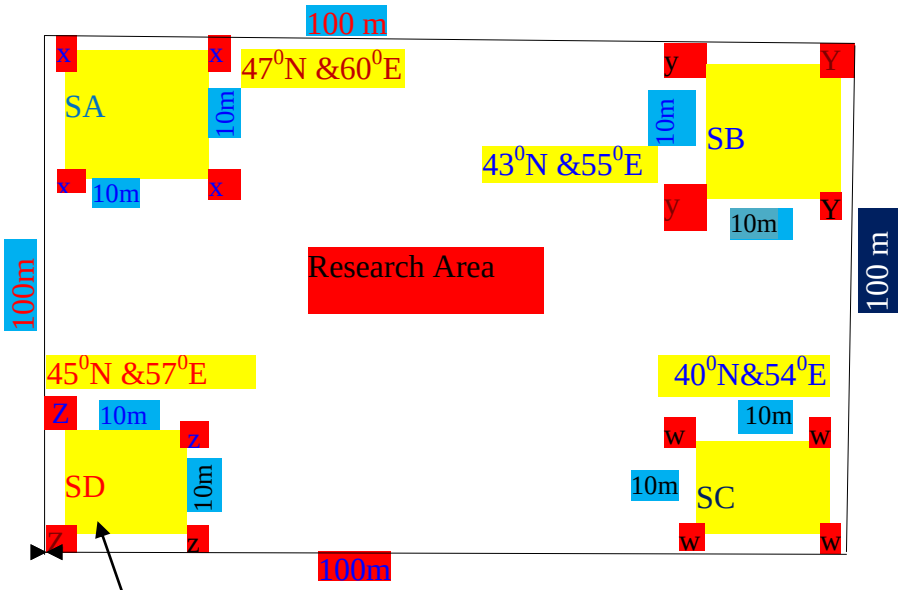


fig 3.2A:Research samples collecting area (SA, SB, SC and SD).

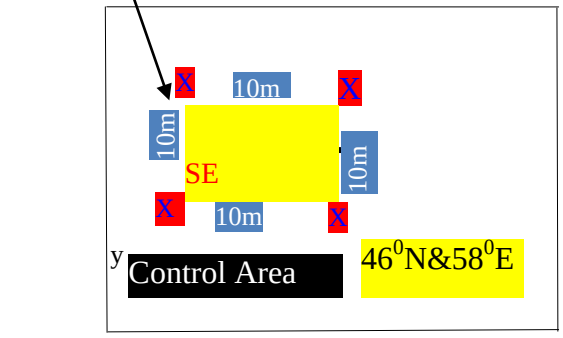


fig 3.2B:Control samples collecting area (SE)

fig 3.2A and 3.2B:the research and control sample collecting area for soil & khat leaves

The letters (x, y, z, w) and the center, from a 10m by 10 m square area marked, are used to represent five sub-sampling point for each research and the control samples for the whole four areas (SA,SB, SC, SD) and the control sample (SE) to that of both soil and khat leaves sample.

fig 3.3A:Cleaning the top surface

fig 3.3B:Placing the soil sample into poly- bag.

fig 3.4A:(AA 200DS,Instrument Company, Germany)

fig 3.4B:samples put in to 100 ml flask

fig 3.5A:Kjeldahil digestion Apparatus

fig 3.5B:samples digest on Kjeldahil block

fig 3.3A-3.5B:various methods used from collecting to digesting of soil sample

3.5.2 Khat Leaves sample

3.5.2.1 Khat Leaf Sample Collection

Before khat leaves sampling, Glove was used to protect the sample from contact in hand for each khat leaves sample collection. Simultaneously, with the same procedure as soil sampling method, The edible part of the khat leaves which is ready for chewing were collected from four sampling points (SA, SB, SC, and SD) over the whole 4 ha of the study area (Fig 3.6A). The distance of separation between each sampling point of the research area was 100m apart from each other (Fig 3.2A). The khat leaves sample from each sampling area, say, SD, is in fact, a mixture of five sub-samples collected from five sub-sampling area (within ten meter square area). For instance, taking sampling point SD as a center, a 10m by 10 m square area is marked on the sampling ground. Then, from each corner point of the square and the center, 50 g of khat leaves was collected, make it dray and mix thoroughly (Fig 3.2A).Then, the whole 250 g of the sample is put into a clean polyethylene bag as a representative sample for that point (SD) labeled it (Fig 3.6B). Similar procedure is followed to collect the representative samples for the remaining three sampling points, namely SA,SB and SC. The whole 250 g were collected for each sampling point, then transferred on a big clean polyethylene bag separately and firmly closed with its screw cap as a representative sample for each sampling area SA,SB,SC (fig 3.2A). Similar procedure was followed to collect 250g of representative sample for the control sampling area of SE (fig 3.2B), which was 500m distance from the research sample (SA, SB, SC and SD), were put carefully in a high density and clean polyethylene bag (HDPB).All the research samples as well as the control samples were collected on the date May 26/2013 E.C at 4:00-6:40 AM, local time. The research sample SA, SB, SC, &SD which has1000g and control sample SE which has 250g of the whole sample-holding HDPB were packed in a clean cardboard box and the package was then transported to AAU, the Department of chemistry for further treatment.

fig 3.6A:Khat leaves ready for harvest

fig 3.6B: samples was stored on poly- bag.

fig 3.7A:p-Selecta oven (model J.P. Selecta A,)

fig 3.7B:samples dried at 105 ^{0}C

fig 3.6A-3.7B: method of khat leaves collecting and drying using an oven at105 ^{0}C

3.5.2.2 Pretreatment of Khat Leaf Sample

In the laboratory, the collected khat leaves samples were washed with tap water and then rinsed with de-ionized water for the purpose to remove absorbed soil particles and other particulates on the surfaces of the sample during Khat leaf collection processes. The khat leaves samples were cut into small pieces using plastic knife in order to facilitate drying. The washed khat leaf samples were air-dried in a clean and dust free environment at a constant temperature of 20^{0}c for 6 days by using dish . The air-dried khat leaf samples

were dried in an oven (Fig3.7A) for 24 hours until each khat leaf sample attained constant weight, to remove moisture content and maintain constant mass at 105 ^{0}C (Fig3.7B). Using mortar and pestle, the dried research khat leaves samples were grounded to convert it into powder and to pass in 0.5mm sieve. After grinding, the powder research samples were mixed thoroughly and poured separately into clean, labeled and decontaminated polyethylene bag and kept in desiccators until the time of Kjeldahil apparatus digestion. The control khat leaves sample was Pretreated by following similar procedure as the research khat leaves.

3.5.2.3 Kjeldahil Digestion Of Khat Leaves Samples

The samples were digested following the procedure recommended by the (AOAC 1990), 0.5 g of powdered and homogenized research khat leaves sample was accurately weighed by using citizon (Fig 3.4A).A clean spatula was used to transfer the measured research khat leaves sample in to 250 ml round bottom flask and moistened with 1 mL of distilled de-ionized water (fig 3.4B).4 mL of 1:1 mixture of conc. HNO_3 and HCl was added to the reaction vessel and kept for 20min until it got stabilized. The round reaction vessel were tightly closed and placed on the fire place (fire proof) of the Kjeldahil digestion apparatus(fig 3.5A).Then**,** digested on Kjeldahil digestion block under reflux condenser for 2:00 (8:40-11:10 AM) hour at the temperature of 150°C on June 5/ 2013EC(fig 3.5B). After two hours of the digestion time, the digested mixture was changed from orange/yellow color to color less under reflux condition, and the tubes were removed from Kjeldahil block and cooled to room temperature. To this, about 100 ml of distilled-deionized water was added and mixed thoroughly, and then, the clear and colorless solution was filter out into 100 mL volumetric flask through What man No.1 filter paper and top up to the mark with distilled-deionized water.

mass of khat leaves	Reagents(mL)	Digestion Temp.	Digestion time	Heating Result
0.5 g	1:1 (HNO3:HCl)	150c^0	2:00 hr	Colorless solution

Table 3.1H:General summery and optimized condition for digestion of khat leaf samples



Soil sample collection collect khat leaves sample samples put in poly.bag



Grounded samples in to Powder make drying by using an oven put samples in side an oven



Digested on Kjeldahil bl. App. Filtered on volumetric flask Stock solution 1000mg/kg




Put on AAS for analysis samples are in to pipe intr. solution for each metal cupper

Kjeldahil bl. App.=Kjeldahil block apparatus, Poly.bug=polyethylene bag, inter. solution =intermediate solution

The diagram shown above represents the general explanation for soil sample and khat leaves sample; starting from sample collection to the end of the metals analysis that we obtain the result by using Atomic Absorbation Spectroscopy.

3.6 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy is one of the most common analytical techniques used to determine the concentration of desired essential and potentially toxic elements in the sample accurately. The technique uses spectrometry to assess the concentration of heavy metals in a sample. It requires a standard intermediate solution for known metal content to establish the relation between the measured and the analyte concentrations and relies on Beer Lambert's law (Skoog *et al*., 2005).This Law describes the relationship between light absorbation and concentration of the element. According to the BEER'S Law:

$$\mathbf{A= B*X \times C}$$

Where: (A) is the absorbance (Abs) measured by AAS, **(B)** is the path length(atom width=the slop of calibration curve), **(C)** is the concentration of the element For flame AAS.

3.6.1 Principle of AAS

AAS is a powerful technique for the analysis of elements present in complex samples by measuring the radiation absorbed by the target element. The instrument used for this research was an AAS; model ZEE nit 700P AAS (analytikjena manufacturing co. ltd,Germany 150Z7P1025 Tech: Flame SW-Version: AS pect LS 1.3.1.0RC1 Created: 14/06/2021 16:16).

fig 3.8:AAS (Model ZEE Nit 700 P Analytikjena Manufacturing Co. Ltd,Germany)

The atomic absorbation spectroscopy technique consists of four standards component:

(i) the light (radiation source)

(ii) the sample introduction area

(iii) the monochromatic(polychromatic)

(iv) the detector.

Modern AAS techniques was equipped and comprised with, energy source(light lamp), monochromatic, detector (phototube),chopper and a computer.

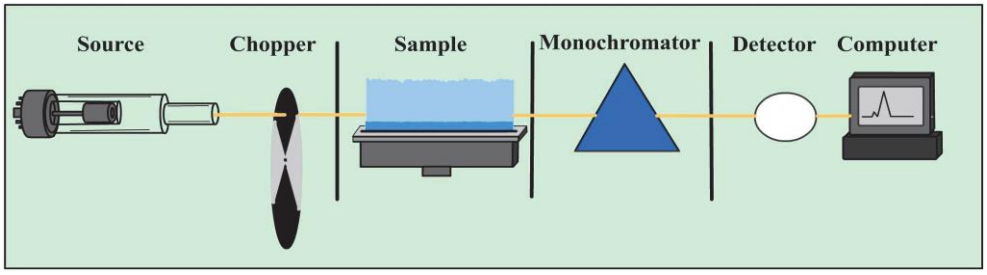


Fig 3.9:Schematic Diagram for the Components Of an AAS

light **s**ources is a hollow cathode lamp and a deuterium lamp which produces broad band (continuous) radiation source. **A C**hopper or modulated power supply is used to modulate the source radiation that passes through the atomizer (flame). The function of the chopper is to chop the light leaving the source when the incident beam hits the chopper at the solid surface, the beam will be blocked and detector will only read the emitted signal from the flame. **S**ample is a determined phenomena which is collected from the sampling area to obtain the desired analysis. **M**onochromator is a device that selects and transmits a specified wavelength and light appropriate to the element from the cathode lamp direct it into a detector to set a desired wavelength to readout. **D**etector (phototube) applies to convert the energy received from Monochromator during experiment into measurable signal. **S**ignal is a measurable process, produces when atoms will absorb light radiation at specific wavelength during AAS process**. p**hototube and photomultipliers include a photosensitive surface that absorbs radiation in the UV-visible light. **C**omputer is necessary to display the quantitative result.

3.6.2 Instrumentation of AAS

Atomic Absorption Spectroscopy is analytical technique used for the quantitative and qualitative determination of elements in a sample. To understand the workings of the atomic absorption spectroscopy, it must have components which fulfill the three basic requirements, a light source, a sample cell, specific light measurement. Know, to calibrate AAS solution containing none of the element of interest is measured by using AAS. This solution is called the ‘blank’. Immediately after calibration, the sample

solutions were placed into the instrument and direct triplicate readings of the metal concentrations are record. After the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument then the parameters are set according to the specifications given in the manufactures manual.

Ana	W. length (m)	S. w(nm)	L. cur. (mA)	Energy(mJ)
Cr	357	0.7	2.0	3.623
Cu	324	0.7	1.5	3.775
Fe	248	0.2	5.00	4.015
Pd	283	0.7	2.0	3.507
Zn	213	0.7	2.0	3.001
Cd	228	0.7	2.0	3.065

Table 3.2:Instrumental operating conditions for determination of metals using AAS Working

Where: Analysis (Ana),wale length (w. length),slit width (s. w),lamp current(L.cur.)

Atomic Absorbation Spectroscopy have the following main component with their basic principle of the working condition.

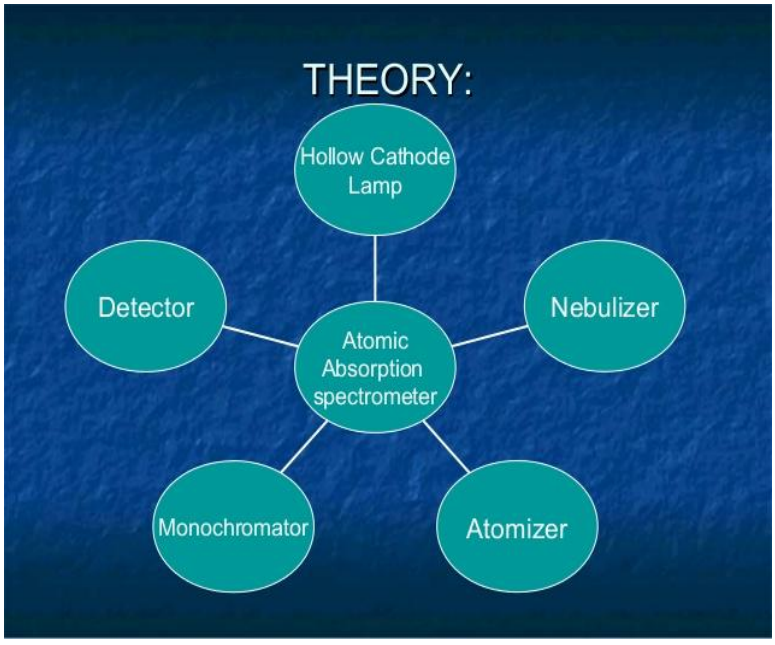


Fig 3.10:The important Principle for the instrumentation of AAS

A. Light source: A hollow cathode lamp are the most common radiation source in AAS. Light source contains hollow cylindrical cathode made of the element being analyzed, and an anode electrode. A hollow cathode lamp consists of a hollow tube filled with an

inert gas (noble gas) such as Ne or Ar, an anode made from tungsten, and a cathode made of the metallic form of the element to be detected. Usually, for AAS, each element has its own unique lamp which must be used for that analysis.

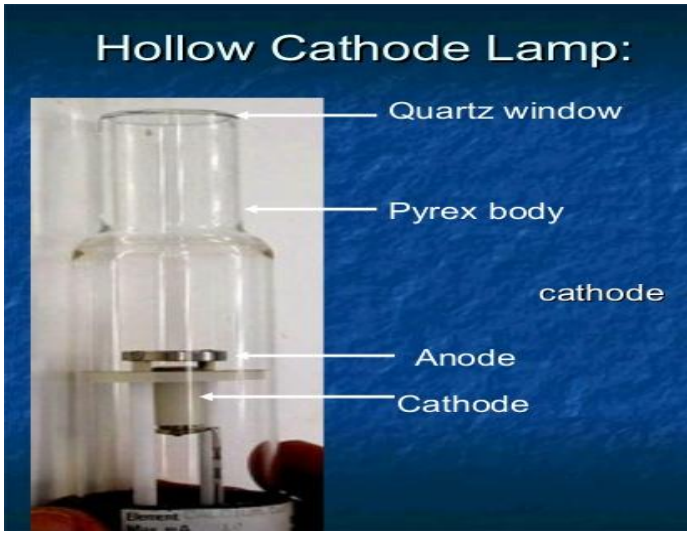


Fig 3.11:The schematic depiction of a hollow cathode lamp

B. Nebulizer: Nebulizer is used to convert a solution in to AA spray and to suck up ("uptake") liquid sample up the tube and into the nebulizer chamber at controlled rate, a process called aspiration. The nebulizer chamber mixes acetylene (fuel) and oxidant (air or nitrous oxide)thoroughly to create an aerosol spray for introduction in to the flame.

C. Atomizer: Atomization is the separation of particles in to individual molecules and breaking molecules in to atoms, then the sample is converted into atomic vapors by a process known as atomization. To create flame, we need to mix an oxidant gas and fuel gas and in most of the cases air acetylene flame or nitrous oxide acetylene flame is used.

D. Monochromatic: The monochromatic is part of an AA spectroscopy which is used to separate the thousands of lines generated by all of the elements in a sample. A monochromatic is used to select the specific wavelength of light that is absorbed by the sample and to exclude other wavelengths (fig 3.12)**.**

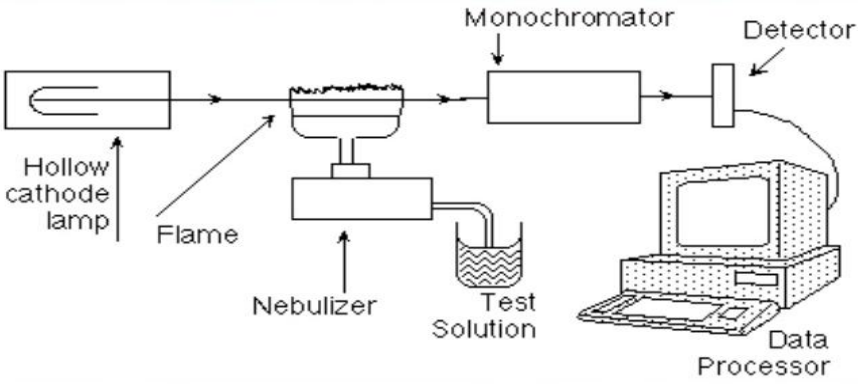


fig 3.12:schematic diagram for the Working component of AAS

E. Detector: The detector is placed in front of the exit slit and receives the determined photons by the monochromatic(fig 3.12).Some commonly used detectors are photocells and photo multiplier tubes (Navies *al*., 2000).The light selected by monochromatic is directed on to a detector that is typically a photomultiplier tube. The function of a photomultiplier tube is to convert the light signal in to an electrical signal proportional to the light intensity. The signal could by displayed for readout and print out by the requested format for further fed in to a data.

F. Calibration curve: The sample solution is feed in to the instrument, and the absorbance of the element in this solution is measured, then the un known concentration of the heavy metal was calculated from the calibration curve. The calibration curve determines the relationship between the absorbance of the light and the concentration of the element in the solution.

3.7 Basic Statistical Analysis

The statistical methods applied in analytical technique are the standard deviation, variance, relative standard deviation and range of series measurements (Skoog 1996).The agreement between the results can be determined by mean value ($\bar{x}$), standard deviation (SD),percentage relative standard deviation (%RSD) and variety of series measurements and the results of the measurements are expressed as the mean of the measurements with the standard deviation of the triplicate measurements of each sample$(\bar{x} \pm SD)$ (Miller and Miller, 2005; Meseret, 2013).

3.8 Soil pH

A soil sample of (30 g) was weighted and placed into 100ml glass beaker and 30 ml of distilled deionized water was added. The magnetic bar was inserted on a glass beaker and the beaker becomes placed on Harris standard magnetic stirrer to Agitate the soil vigorously by stirring or swirling the mixture .The mixture was allowed to stand for one hour and after stirring the mixture decant for 15 minutes then after the solution becomes filtered by using filter paper and put on round volumetric flask. The Buffer solutions were recommended to have a three point calibration with pH of (4,7 and10) solutions were used to calibrate the digital pH meter before taking measurements.

fig 3.13A:Harris standard magnetic stirrer (Philip Harris)

fig 3.13B:Jenway (3345 ion meter)

fig 3.13A-3.13B: Philip Harris standard magnetic stirrer Jenway (3345 ion meter)

CHAPTER FOUR:RESULTS AND DISCUSSION

Introduction

In this chapter the levels of heavy metal analysis in soil and khat leaves samples from Assano region are reported. The results of the analysis that will be presented and discussed in the section are instrument calibration, method detection limit (MDL),method validation (percentage recovery, precision and accuracy),analysis of heavy metals in soil sample, P_H value of soil, analysis of heavy metals in khat leaves sample, comparison of heavy metals between research soil and khat leaves sample and heavy metal transfer factor from soil to the edible part of khat leaves will be explained briefly. The mean values were determined from triplicate analysis of each sample and the results were reported in terms of mean values ($\bar{x} \pm$ SD) for the selected heavy metals in this study.

4.1Instrument Calibration

In AAS, Secondary intermediate standard solutions containing 10 mg/kg were prepared From certified standard stock solution that contains 1000 mg/kg. These secondary standards solutions were diluted with the deionized water and obtain four working standards for each metal of interest and calibrate the instrument. The corresponding volume of the standard solution need to prepare each calibration solution was calculated:

$$C_1V_1 = C_2V_2 \text{.....(4.1)}$$

C_1 = is conc. of analyte in the stock or intermediate standard solution to dilute

V_1= is the volume of the stock or intermediate standard solution to be used

C_2=is the final concentration of standard solution to be prepared

V_2=is the total volume of the standard solution to be prepared

For instance, if 10 ml of a 2 mg/l standard will be prepare from a 1000 mg /L stock standard solution ,the volume of the stock or intermediate standard to be withdrawn can be calculated by using the above equation

1000 mg /l (c_1) x v_1 =2mg/l (c_2) x 10ml (v_2)

V_1=2mg/l x 10ml /1000 mg /l, V_1=0.02ml

To prepare a 2 mg/l standard solution, a 0.02 ml of 1000 mg /l standard will be added in to a 10-ml volumetric flask which contains multi-element calibration standard a sample

Ana	St.So.mg/kg	In.so.mg/kg	W. Sta. mg/kg	Co. Cal.R^2	Eq. Cal. Curve
Cr	1000	10	0, 1, 2, 3, 4	0.99968	Y=0.05518x+0.0028
Cu	1000	10	0, 0.25, 0.5, 1, 2	0.99995	Y=0.10225x+0.0005
Fe	1000	10	0, 2, 4, 6, 8	0.99997	Y=0.2606x+0.0008
Pd	1000	10	0, 0.5, 1, 2, 4	0.99953	Y=0.01692x-0.0008
Zn	1000	10	0,0.25,0.5,0.75,1	0.99951	Y=0.45659x+0.0063
Cd	1000	10	0,0.25,0.5,0.75,1	0.99937	Y=0.22241x+0.0035

Table 4.1:stock standard solution (st.sol), intermediate standard solution (in.sol), working standard (w. sta.),correlation coefficients (R^2) equations of calibration (eq. cal.) and analysis (Ana.).

The Pearson's correlation coefficient values that are closer to the absolute value 1 indicate that there is a strong relationship between the concentration levels of the analyte and the absorbation ,whereas values closer to 0 indicate that there is no linear relationship (Gezahegn, 2013).

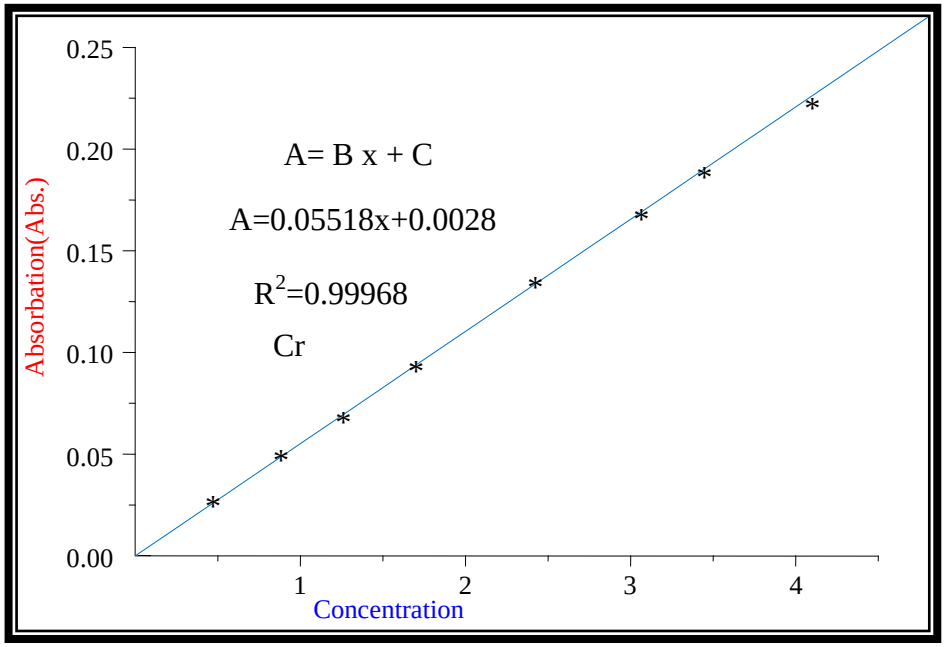


fig 4.1A:Calibration graph of Cr Standard solution

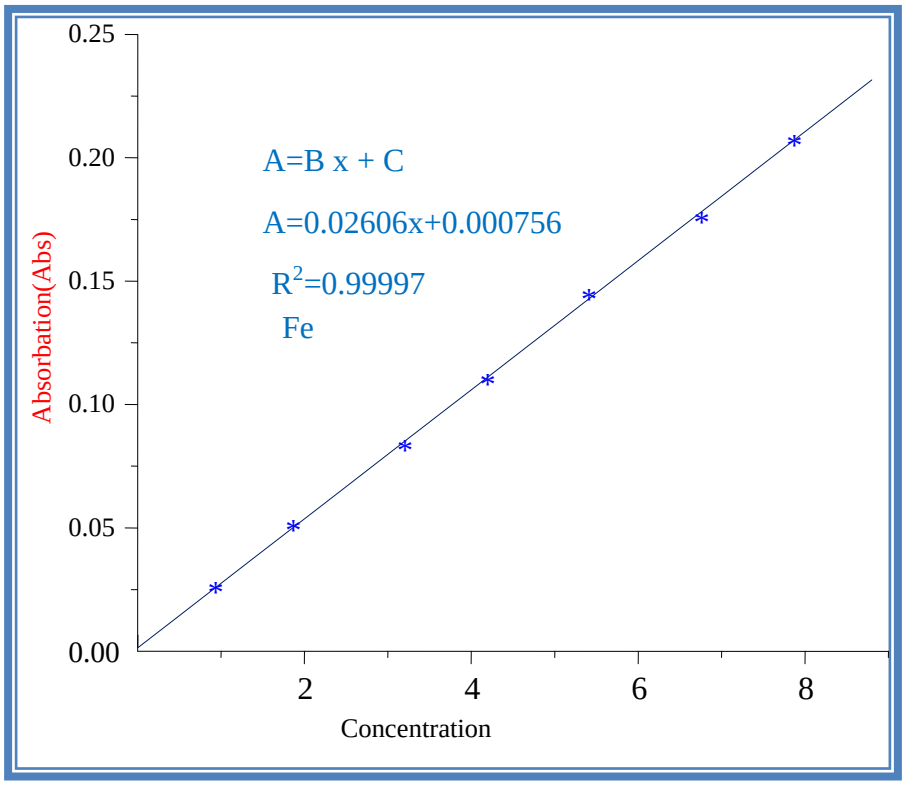


fig 4.1B:Calibration graph of Fe Standard solution

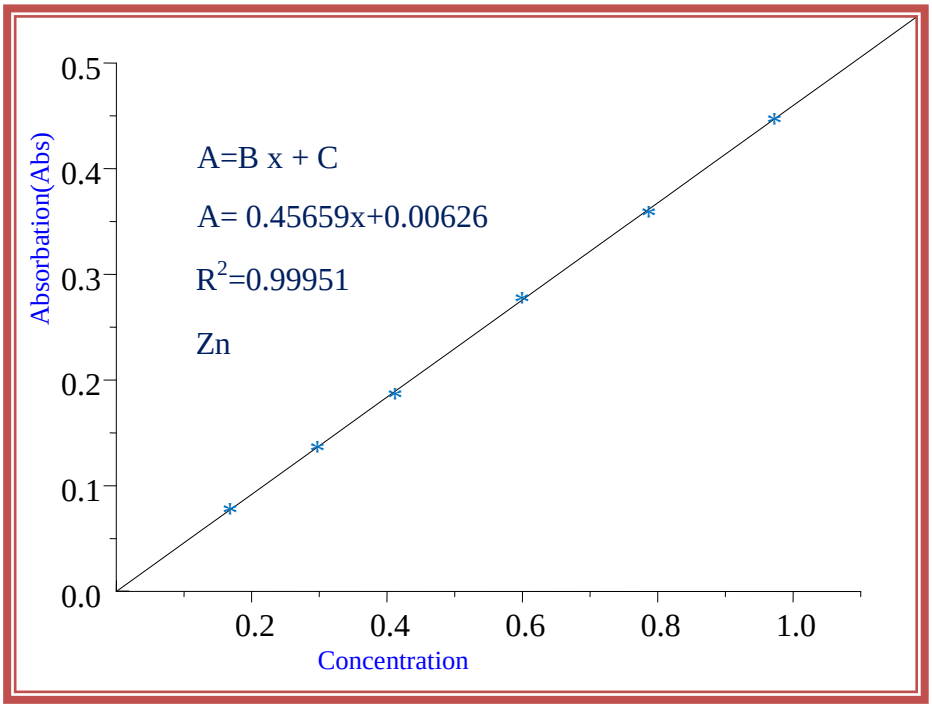


fig 4.1C:Calibration graph of Zn Standard solution

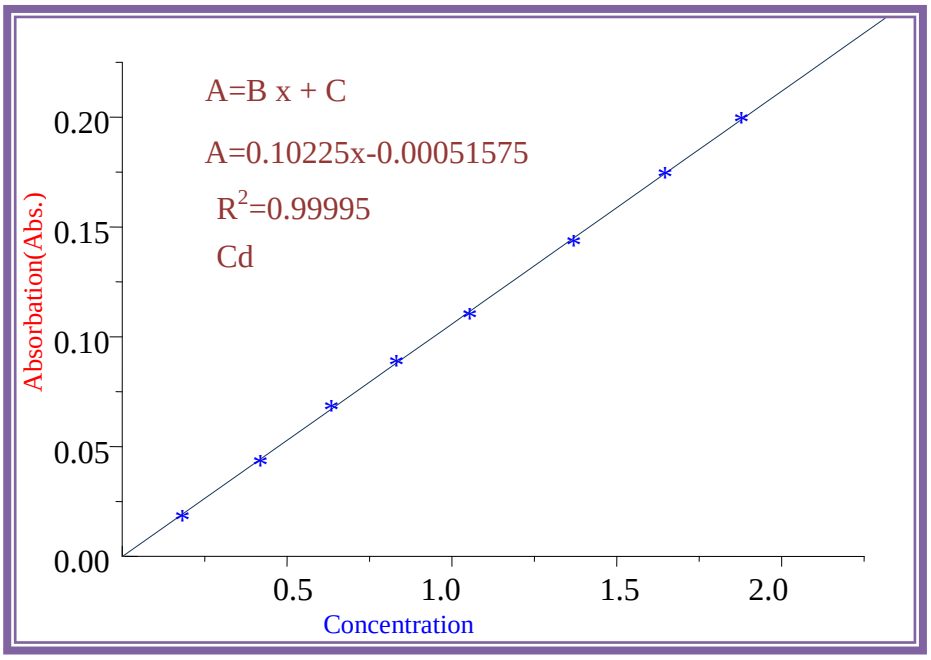


fig 4.1D:Calibration graph of Cd Standard solution

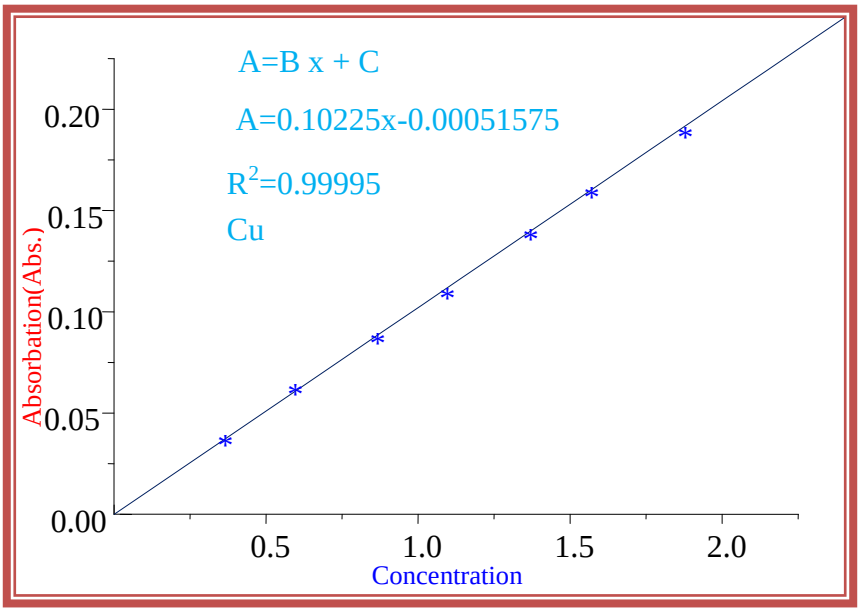


fig 4.1E:Calibration graph of Cu Standard solution

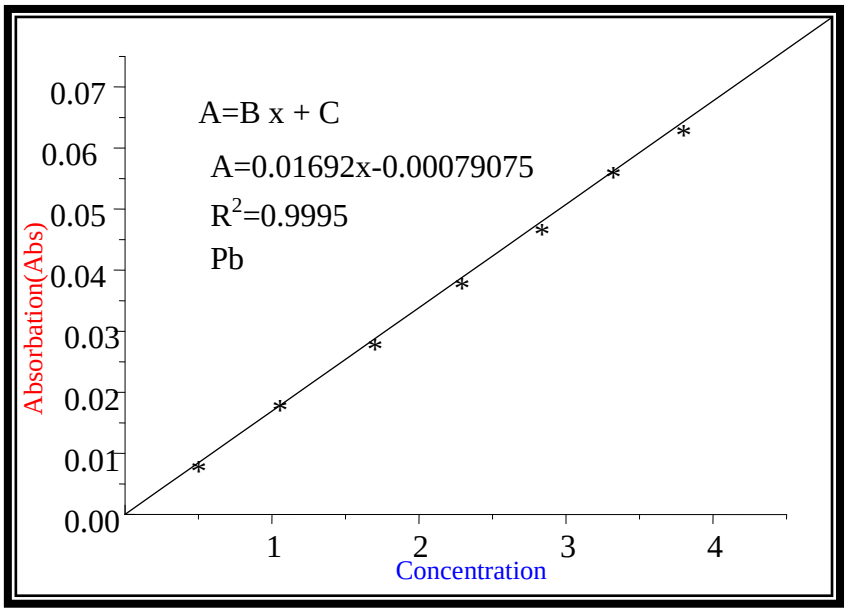


fig 4.1F:Calibration graph of Pb Standard solution

fig 4.1A-4.1f: Calibration graph of Pb, Cr, Cd, Fe, Zn and Cu Standard solution

From Table 4.1: the correlation coefficients of metals were found to be from 0.99937-0.99997 and their corresponding calibration curves for each metal from (Fig. 4.1A-4.1 F) indicates that concentration and absorbation (Abs) are linearly fit each other. Then, it is possible to say that, there is a strong relationship and good positive correlation.

4.2Method Detection Limit (MDL)

Method Detection Limit (MDL) the minimum concentration that can be detected by the analytical method with a given confidence limit (America Environmental Protection Agency).Detection limit is the lowest analyte concentration which is distinguished from statistical fluctuations in a blank (Gezahegn, 2013). The general accepted and common definition of method detection limit is three times the standard deviation of the blank (background signal) divided by the slope (Regassa, 2007).The detection limits were obtained by multiplying the standard deviation of the reagent blank by three divided by the slope of calibration curve (Thompson, Wood 1993 and L. Winefordner, J. D. 1983)

$$\mathbf{M\ D\ L} = \frac{\mathbf{3 \times standard\ deviation\ of\ blank\ readings}}{\mathbf{Slope\ of\ calliberation\ curve}} \qquad \textbf{(4.2)}$$

From the table 4.2 the method detection limit of each element is greater than the instrument detection limit. The method detection limits for all the selected metals are less than 0.1 mg/kg. then AAS is good, reliable and applicable for the analysis of metals.

Element	MDL (mg/kg)	IDL (mg/kg)
Cr	0.0215	0.0152
Cu	0.0314	0.0213
Fe	0.0031	0.0004
Pb	0.0149	0.0124
Zn	0.0224	0.005
Cd	0.0071	0.005

Table 4.2:comparison of MDL and IDL for all metals based on AAST

Source: AAS result analysis at AAU 2013

4.3 Method validation

Method validation is the process of providing that a given analytical method is acceptable for its desired purpose (Duane 2003). A mean recovery of the matrix was evaluated at 95% confidence level (Borosova 2002).In Spiking, a fixed amount of each metal from standard solutions is spiked into a sample and digested in the same manner as the original sample (Zealand, K.A and Chandravanshi, B.S, 2014). To check the efficiency of the procedure, appropriate amount of stock solution of Zn, Cu,Cr,Cd,Fe and Pb are spiked at once in to 0.5 g of khat sample and appropriate amount of the stock solution of metals in to another digestion flask containing unspiked solution. A recovery test is also performed for the 1g spiked soil samples and unspiked samples appropriate amount of stock solution, which is digested using the same procedure and analyzed by AAS. Each recovery test for both samples is performed in triplicate and the amount that was recovered after digestion of the spiked samples was used to calculate % recovery (Al-weher, 2008).Then the percentage recoveries of the analyte are calculated by using the equ 4.3 (Deribachew 2015, Kediri 2015).

$$\% \text{ Recovery} = \frac{\text{CM in spiked sample} - \text{CM in un spiked sample}}{\text{added Amount}} \text{x100\% .....(4.3)}$$

Where, CM = concentration of metal

4.3.1 Recovery test

Because of the absence of certified reference material (CRM) in the laboratory to check the efficiency of the analytical procedure percentage recovery was calculated. The small and known amount of the heavy metals from the stock solution were added or spiked in to the soil and khat leaves sample to digest. Then, the concentration of both spiked and non-spiked samples were read. Results are obtained through calculation using (equ.4.3).

Ele.	Unspiked C_m mg/kg	Amount added mg/kg	Spiked C_m mg/kg	$R_{percentage}$(%)
Fe	5.203	0.50	5.678	95
Cd	0.0574	0.02	0.0764	95
Zn	1.749	0.08	1.8235	93.1
Cr	0.2917	0.05	0.3371	90.9
Cu	0.4989	0.03	0.5281	97.2
Pb	0.0375	0.02	0.0557	91

Table 4.3:percentage Recovery test for the optimized procedure of the samples

Where: C_m=concentration of metals, $R_{percentage}$=percentage recovery

As shown in table 4.3, the percentage recovery for the soil and khat leaves samples lie in the range 90.9 to 97.17% which are within the acceptable range between 70%-125% according to (Duane 2003),This reveals that the digestion method and the AAS analysis in the present study were reliable.

4.3.2 Precision and accuracy

Precision is a statistical method that measures the closeness or agreement between a set of data obtained from multiple sampling under the prescribed condition (Kikuchi et al.,2002). Accuracy is how close a measurement in to its desired or theoretical value, usually reported as a percent error (% error).

$$\%\mathbf{RSD} = \frac{\mathbf{SD}}{\mathbf{MEAN}}\mathbf{X100}\%\text{.....}(4.4)$$

The analysis of the results are report with (%RSD) at 95% confidence limit for the triplicate readings of metals in each khat leaf and soil sample. The values of percentage relative standard deviation (%RSD) are less than 10% for all mean value of samples collected in the research area (Regassa, 2007).The % RSD result of elements in each soil sample (Table 4.4) and khat leaves sample (Table 4.11) are below 10% ,this indicates there exists good precision in measurement.

Results And Discussion

4.4 Analysis of heavy metals in agricultural soil

4.4.1 Level of heavy metals in each soil sample

Table 4.4 shows ($\bar{x}$± SD) (mg/kg),of metals in each of five soil sample (Appendix Table 3).

Sample	**Fe** (mg/kg)	**Cd** (mg/kg)	**Zn** (mg/kg)
S1	5.08±0.29 (5.7) *	0.06± 0.006 (10) *	1.73± 0.02 (1.2)*
S2	6.11±0.22 (3.6) *	0.13±0.01 (7.7) *	1.84± 0.01 (0.5)*
S3	4.71±0.1 (2.1) *	0.09±0.008 (8.9) *	1.8± 0.01 (0.6)*
S4	5.17±0.14 (2.7) *	0.14± 0.01 (7.1) *	1.83±0.01 (0.6)*
S5(cont.)	4.97 ±0.07(1.4) *	0.099 ± 0.0067 (6.8) *	1.79±0.01 (0.6)*
Sample	**Cr** (mg/kg)	**Pb** (mg/kg)	**Cu** (mg/kg)
S1	0.3±0.02 (6.7) *	BDL	0.5± 0.01 (2.0)*
S2	0.3±0.02 (6.7) *	BDL	0.55±0.01 (1.8) *
S3	0.31±0.01 (3.2) *	BDL	0.63±0.01 (1.9) *
S4	0.54±0.02 (3.7) *	BDL	0.62±0.01 (1.6) *
S5(cont.)	0.34± 0.02 (5.8) *	BDL	0.49±0.01 (2.01)*

Table 4.4: Heavy metal concentration ($\bar{x}$± SD)mg/kg, Standard deviation (S.D), below detection limit (BDL) and percentage relative standard deviation (*=%RSD)(n=25)for soil samples.

Sample	Cr (mg/kg)	Pb	Cu (mg/kg)	Fe (mg/kg)	Cd (mg/kg)	Zn (mg/kg)
S1	0.283-0.308	-	0.489-0.499	4.755-5.318	0.052-0.063	1.719-1.75
S2	0.279-0.308	-	0.544-0.547	5.86-6.266	0.116-0.137	1.829-1.849
S3	0.306-0.320	-	0.618-0.635	4.604-4.801	0.082-0.098	1.795-1.813
S4	0.526-0.557	-	0.605-0.628	5.024-5.286	0.134-0.148	1.829-1.84
S5(cont.)	0.426 -0.462	-	0.584-0.595	4.916-5.043	0.002-0.015	1.776-1.8

Table 4.5:The range value of heavy metals in each soil sample in mg/kg

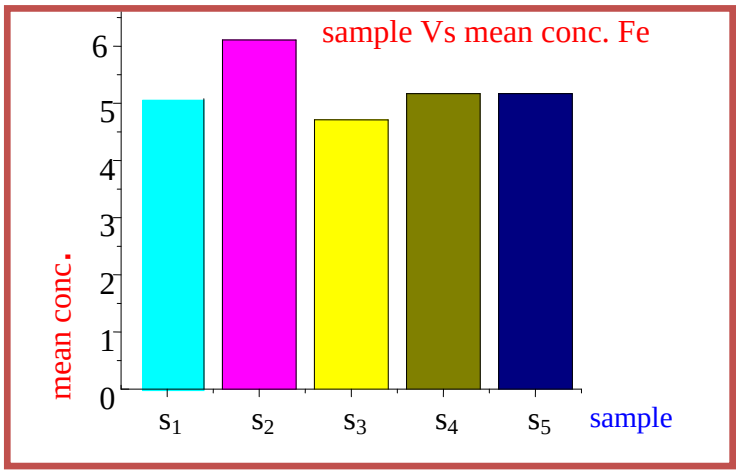


fig 4.2 A:The overall comparative result of iron between five soil sample mg/kg

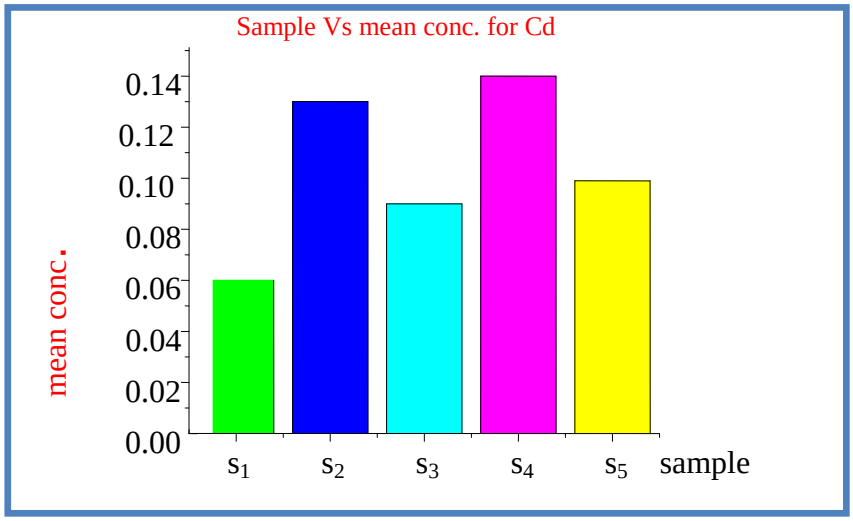


fig 4.2B:The overall comparative result of cadmium between five soil samplein mg/kg

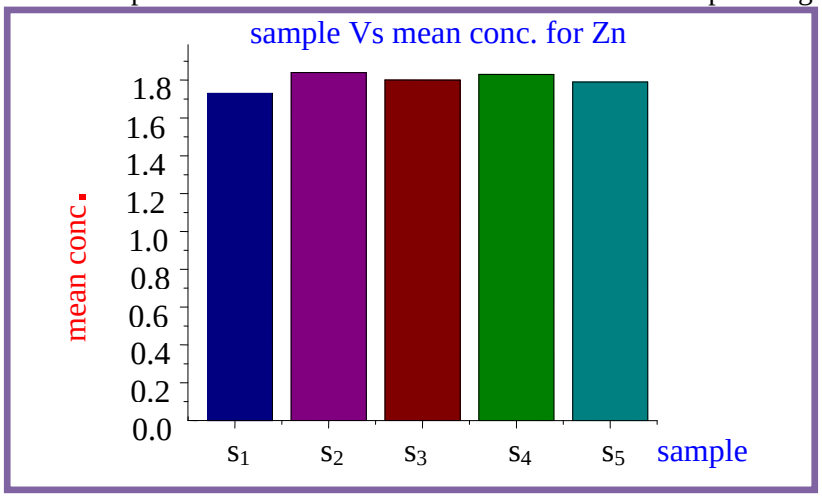


fig 4.2C:The overall comparative result of zinc between five soil sample in mg/kg

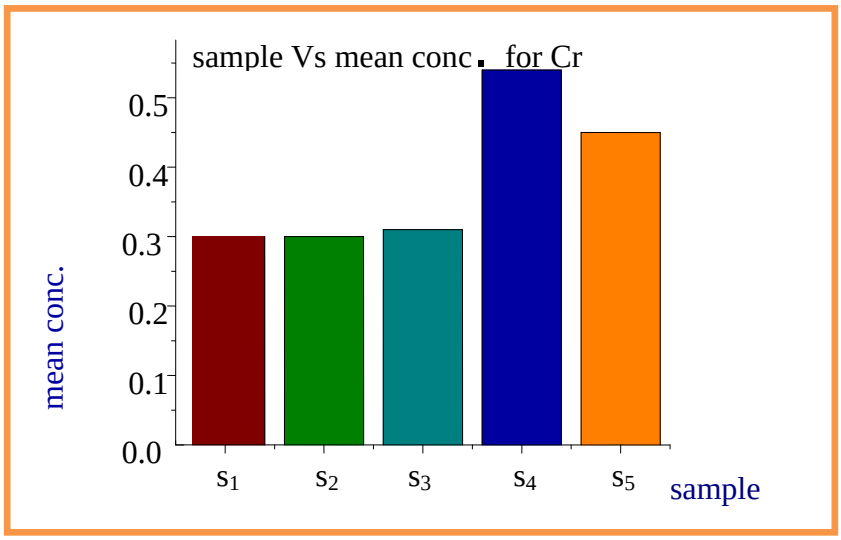


fig 4.2D:The overall comparative result of chromium between five soil sample in mg/kg

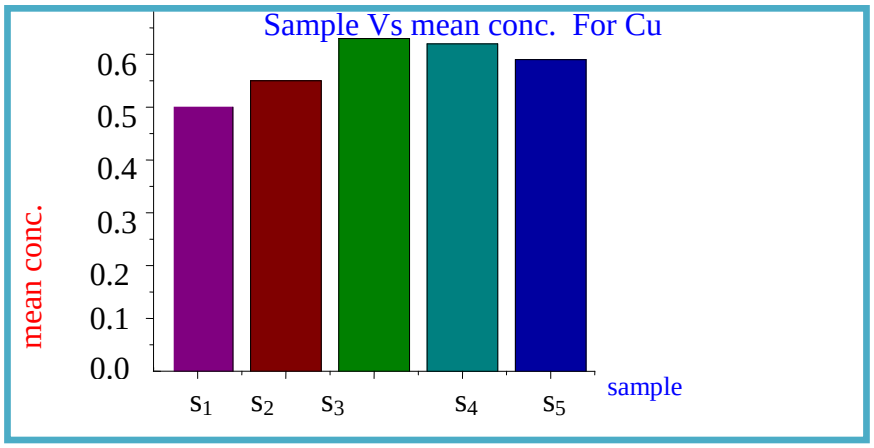


fig 4.2E:The overall comparative result of copper between five soil sample in mg/kg.

fig 4.2A-4.2E: The comparative result of Fe, Cd, Zn, Cr, Cu, between five soil sample

Table 4.4 shows that, sample1 and sample 3 have higher cadmium concentration with significant difference at 95% confidence level. (fig 4.2A – 4.2E) represents comparison of the concentration level of heavy metal in each soil sample. The concentration level of essential and potentially toxic metals assessed in each soil sample are Fe: S_2> S_4> S_5> S_1>S_3, for Zn: S_2 > S_4> S_3> S_5>S_1, for Cu: S_3> S_4> S_5> S_2>S_1,for Cr: S_4> S_5> S_3> S_1=S_2,and Cd: S_4> S_2> S_5> S_3>S_1.This indicates the average concentration of heavy metals in each sample is significantly different at 95% confidence

4.4.2 Average Concentration of heavy metals in soil sample

However, the research soil sample have relatively higher average concentration as compared to the mean concentration of all heavy metal in the control soil sample with significant difference at 95% confidence interval, as shown in Table4.6 bellow.

Element	$\bar{x}$ ± SD for R_S (mg/kg)	$\bar{x}$ ± SD for C_S (mg/kg)	FAO/WHO (mg/kg)
Cr	0.3625±0.1118	0.3427 ± 0.0179	50
Cd	0.1051±0.0348	0.0099 ± 0.0067	3
Zn	1.8021±0.0443	1.7847 ± 0.0133	300
Fe	5.2675± 0.5632	4.9703 ± 0.0655	500
Pb	BDL	BDL	100
Cu	0.575±0.0573	0.4882 ± 0.0056	100

Table 4.6:The average concentration comparison of heavy metals b/n research and control soil sample to that of maximum allowable limit (MAL) set by WHO/FAO (mg/kg).

Source: Mamtaz and Chowdhury(2006);Atieno 2011) and element (ele), mean value ($\bar{x}$) standard deviation (SD),research soil sample(R_S),control soil sample(C_S) for (n=25).

The mean concentration of heavy metals in the research soil sample is bellow the maximum allowable limit set by WHO/FAO standard. Based on this result we can say that we fail to nullify null Hypothesis; this means we don't accept alternative Hypothesis and then we continue to accept null Hypothesis**.** The average concentration value of heavy metals for both the research and control soil samples are bellow the maximum allowable limits set by WHO/FAO. The level of Pb in the soil samples is found to be bellow the detection limit of the spectrometer. For instance the mean concentration of Cr in the research soil is (0.3625±0.1118) mg/kg ,the mean concentration of Cr in the control soil sample is (0.3427 ± 0.0179) mg/kg and MAL set by WHO/FAO for Cr is 50mg/kg. This indicates that, the value is significantly different ($p<0.05$) limit. The same is true for the remaining heavy metals (Table 4.6).

Ele	Range of Research soil sample	Range Control soil sample	FAO/WHO mg/kg
Cr	0.2791 - 0.5565	0.4263 - 0.4619	4.123-6.744
Cd	0.0519 - 0.1475	0.0024 - 0.0151	0.029-0.328
Zn	1.719 - 1.849	1.776 - 1.8	50-100
Fe	4.604 - 6.266	4.916 - 5.043	143.1-313
Pb	BDL	BDL	0.061-0.461
Cu	0.4896 -0.6354	0.5839 - 0.5945	50-100

Table 4.7: Range of metal concentration comparison b/n soil sample to that of WHO/FAO

The range of heavy metals for the research and control soil samples are bellow the maximum allowable limits set by WHO/FAO. The mean range value of Cd in the control soil sample is (0.0024 - 0.0151) mg/kg; the rang value of Cd in the research soil (0.0519 - 0.1475) mg/kg and maximum allowable limits set by WHO/FAO for Cd is (0.029-0.328) mg/kg, This comparison indicates that, the range value of soil samples are bellow the maximum allowable limits set by WHO/FAO at 95% confidence limit. The same is true for the remaining analyte element (Table 4.7).

4.4.3 Comparison level of heavy metal between research and control soil

As a result of long-term irrigation domestic sewage, NPK fertilizer& compost residue, etc; the average concentration of heavy metal in soil were significantly higher than the background values (Bourennane 2006, Dere 2007, Rodriguez 2008).

Element	$\bar{x}$ ± SD& R_S (mg/kg)	$\bar{x}$ ± SD,&C_S (mg/kg)	p-value
Cr	0.3625±0.1118	0.3427 ± 0.0179	0.0015
Cd	0.1051±0.0348	0.0099 ± 0.0067	0.0012
Zn	1.8021±0.0443	1.7847 ± 0.0133	0.0010
Fe	5.2675± 0.5632	4.9703 ± 0.0655	0.0010
Pb	BDL	BDL	-
Cu	0.575±0.0573	0.4882 ± 0.0056	0.0025

Table 4.8:Thep-valuecomparison of metals for the research and control soil.

Where: Research soil sample(R_S; n=20) and control soil sample(C_s ;n=5)

All the analyte metals in the research soil have higher average concentration than the average concentration of the control soil. Then, the result indicates that we reject null Hypothesis and we accept alternative hypothesis . let's take the average concentration of Fe in the research soil have (5.2675± 0.5632) mg/kg and the average concentration of Fe in the control soil is (4.9703 ± 0.0655) mg/kg. This in fact shows that, the research soil are contaminated as compared to that of the control soil sample with significantly difference limit ($p<0.05$).The contamination of the research sample may be due to the continuous application of fertilizers, pesticides, herbicides etc. As can be observed from table 4.8the contamination level of each metal is below the maximum allowable limit set by WHO/FAO. The concentration level of heavy metals in the research and the control soil sample are shown below based on bare graph clearly.

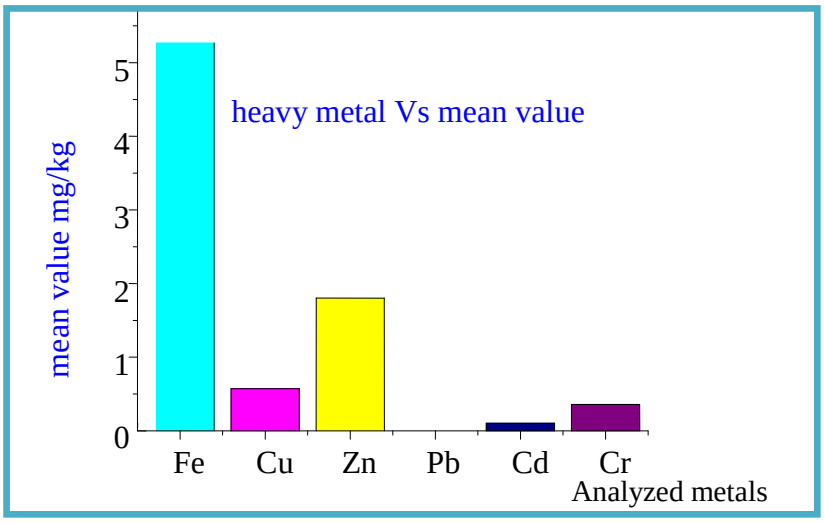


fig 4.3A:Mean concentration of heavy metal in research soil based on bar graph

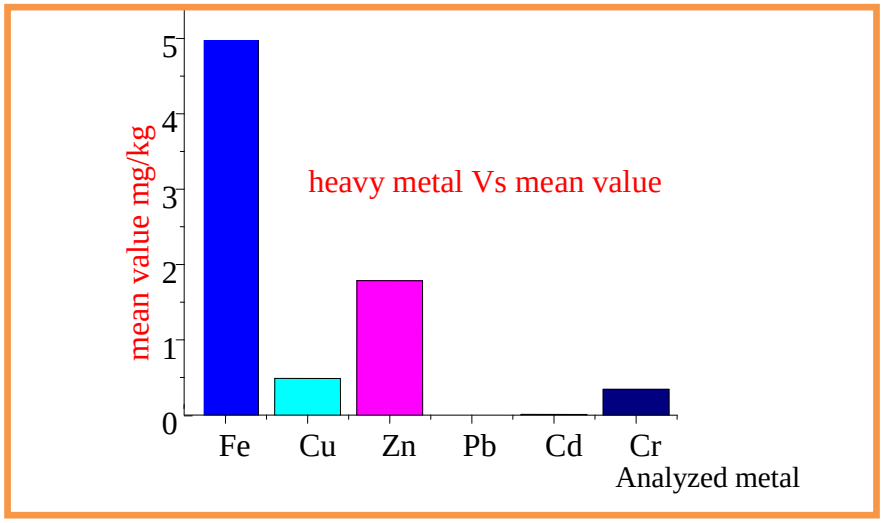


fig 4.3B:Mean concentration of heavy metal in the controle soil sample based on bar graph

Fig 4.3A and 4.3B: concentration of heavy metal in research and control soil sample

However, based on the comparison of results on this study, the concentration of heavy metal in the research soil are determine in the order of: Fe>Zn > Cu > Cr > Cd where as for the control soil: Fe>Zn > Cu > Cr, The Comparison of heavy metals in the research soil sample with that of the control soil sample indicates that; the higher level found in research soil sample. The content of Fe in the sample site was the highest and the content of Pb in the sample site is BD L as compared to the other heavy metals. The soil sample is contaminated but not polluted by essential and potentially toxic heavy metals at different amount. However, continuous application of fertilizer (DAP and UREA),insecticide, sewage sludge and pesticide may pollute the soil yet to come.

4.4.4 Comparison of heavy metal content in soil sample with other literature study

Element	Australia	Canada	Poland	Uk	Germany	USA	$\bar{x}$ ±SD,this soil study
Cr	100	75	100	50	200	1000	0.3625±0.1118
Cd	5	8	3	3	NR	0.7	0.1051±0.0348
Zn	300	400	300	300	300	300	1.8021±0.0443
Fe	NR	NR	NR	NR	NR	NR	5.2675± 0.5632
Pb	100	200	100	100	500	200	BDL
Cu	NR	NR	NR	NR	NR	NR	0.575±0.0573

Table4.9:Comparison of heavy metals (mg/kg) in soil with MAL values of other country.
Source: Mamtaz and Chowdhury (2006).

The mean values of all heavy metals in the research study is below the MAL as compared for all the countries literature except lead and iron. The level of Pb in this study becomes BDL and the mean value of iron does not recommended the MAL from all country. Then this study have good agreement with studies done in other countries.

4.4.5 Soil pH analysis

pH refers to the potential hydrogen concentration of hydrogen ions in a solution and the degree of acidity or alkalinity is measured using pH ion electrode (Stewart, 1989).

Calibration buffer point	Soil sample	pH value
4 7 10	Sample 1	5.6±0.03
	Sample 2	6.08±0.13
	Sample 3	5.99±0.15
	Sample 4	6.00±0.03
	Control sample 5	5.62±0.15

Table 4.10:The mean pH values of soil sample from khat growing regions.

The pH value of the soils ranged from 5.6±0.03 to 6.08±0.13 (Table 4.10). According to (Hizkeal 2012) soils with pH range of 5.6- 6.0 moderately acidic, 6.1-6.5 slightly acidic, 6.6-7.4 neutral or nearly neutral, 7.4-7.8 slightly alkaline and 7.8-8.4 are moderately basic and soil with pH above 8.5 are strongly alkaline. The acidic condition in soil may be due to application of NPK fertilizers. The increasing use of nitrogenous fertilizers generally increases soil acidity (Ishibashi et al., 2004). The fertilizer amended soil have generally lower pH than natural soils Nartey et al., (2012). Based on this, soil samples collected from khat growing areas are acidic with significant different ($p < 0.05$).

4.5 Analysis of Heavy metals in khat leaves

4.5.1 Level of heavy metals in each khat leaves sample

The concentration of six heavy metals (Zn, Pb, Cd, Cu, Fe and Cr) in the digested solutions for each Khat leaves are identified by AAS. As we see in table 4.11, the average concentration of potentially toxic heavy metals in each khat leaves sample from the study area are bellow detection limit (BDL).Then, no need of explanation for Cd, Cr, Pb based on tabular and bare graph. for more refer (Appendix Table 4).

Sample Code	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
S1	3.16± 0.123 (3.9) *	0.204±0.05 (9.8)*	0.48±0.02 (4.2) *
S2(control)	1.84± 0.12 (6.5) *	0.151±0.0012 (0.79) *	0.46±0.006 (1.3) *
S3	2.61±0.06 (2.3) *	0.16±0.01 (6.3) *	0.44±0.002 (0.1) *
S4	3.89±0.12 (3.1) *	0.21±0.01 (4.8) *	0.63±0.004 (0.6) *
S5	1.78±0.12 (6.7) *	0.18± 0.006 (3.3) *	0.51±0.007 (1.4) *

Table 4.11:Average metal concentrations ($\bar{x}$± SD) mg/kg, Standard deviation (S.D) and percentage relative slandered deviation (*) of khat leaves sample for (n=25).

Sam	Cr (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Cd (mg/kg)	Zn (mg/kg)
S1	BDL	BDL	0.174-0.261	2.396-4.572	BDL	0.462-0.496
S2	BDL	BDL	0.1498-0.152	1.727-1.965	BDL	0.457-0.468
S3	BDL	BDL	0.1553-0.1695	2.551-2.675	BDL	0.438-0.442
S4	BDL	BDL	0.2009-0.2167	3.75-3.983	BDL	0.623-0.631
S5	BDL	BDL	0.1734-0.184	1.629-1.858	BDL	0.499-0.5121

Table 4.12:The range value of heavy metals in each khat leaves sample in (mg/kg)

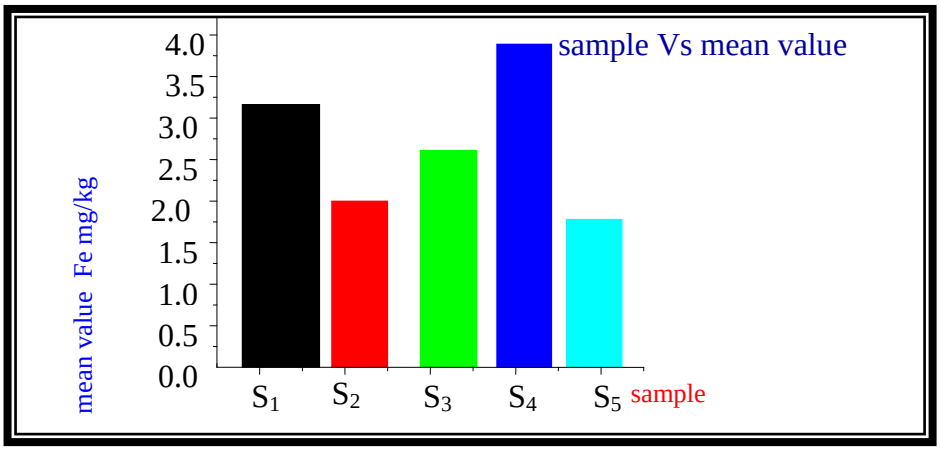


fig 4.4A:The overall comparative result of iron between five khat leaves sample

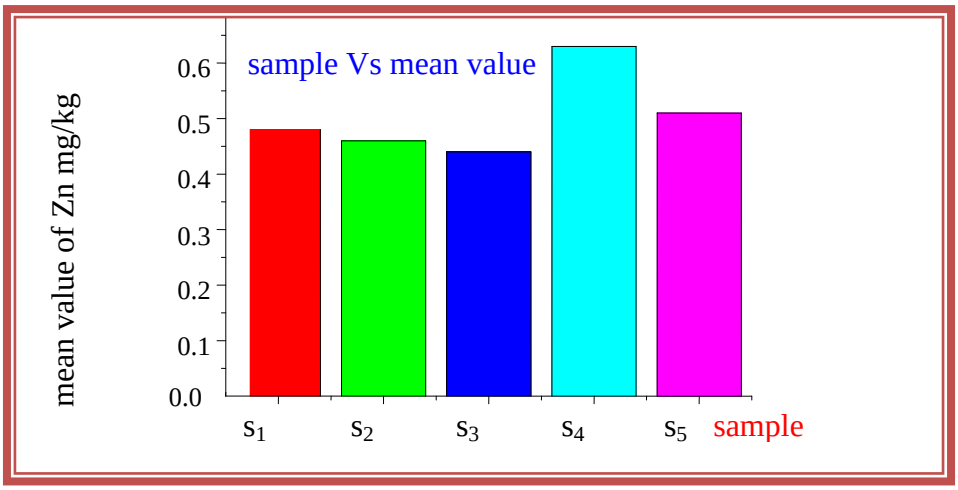


fig 4.4B:The overall comparative result of zinc between five khat leaves sample

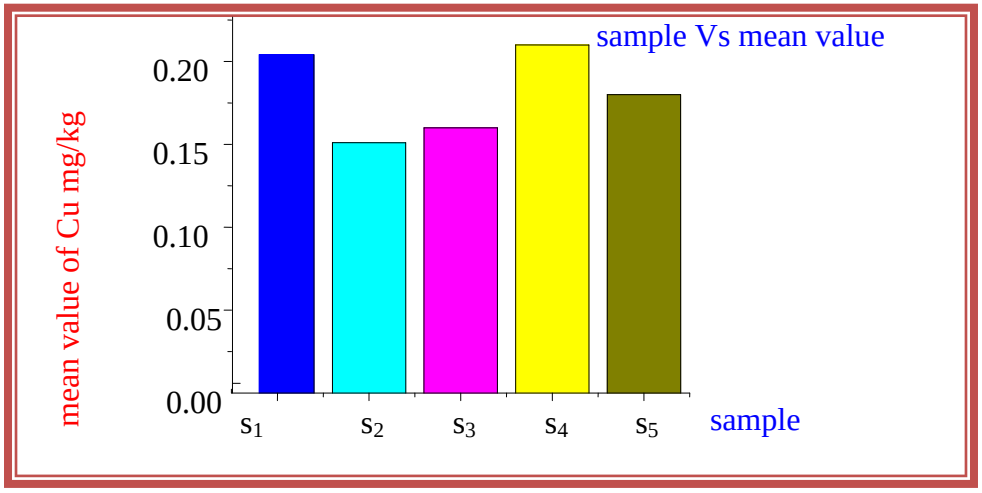


fig 4.4C:The overall comparative result of copper between five khat leaves sample

fig 4.4A-4.4C:the comparative result of Fe, Cu, Zn for five khat leaves sample.

In table 4.11, from the five khat leaves sample, copper have high concentration in sample 1 while iron have high concentration in sample 2 and 5 respectively with significant difference 95% confidence interval.fig 4.4A-4.4C represents comparison of heavy metal content based on each khat leaves sample. The concentration of essential metals determined in each khat leaves are Fe: S_4> S_1> S_3> S_2>S_5, for Zn: S_4> S_5> S_1> S_2>S_3,and for Cu: S_4> S_1> S_5> S_3>S_2.The sample collected from the area becomes contaminated by Fe, Zn, Cu where as the toxic heavy metals (Pb, Cr, Cd) are below detection limit (BDL) of the spectrometer.

4.5.2 Concentration Level of heavy metals in Khat leaves sample

The level of the essential and potentially toxic heavy metals in the research and control khat leaves sample are discussed in the following table.

Element	$\bar{x}$ ± SD for R_{kh} (mg/kg)	$\bar{x}$ ± SD for C_{kh} (mg/kg)	FAO/WHO (mg/kg)
Cr	BDL	BDL	1.2
Cd	BDL	BDL	0.2
Pb	BDL	BDL	0.5
Zn	0.515±0.0688	0.4632±0.0055	1.5
Fe	2.8624±0.9555	1.8433± 0.1191	150
Cu	0.1885±0.0302	0.1505±0.0012	2.0

Table 4.13:The average concentration comparison of heavy metals b/n research and control khat leaves sample to that of maximum allowable limit (MAL) set by WHO/FAO (mg/kg).

Source: WHO/FAO (1999) element (ele), mean value ($\bar{x}$), standard deviation (SD), research khat leaves sample (R_{kh}), control khat leaves sample (C_{kh}).

The mean concentration of heavy metals in the research khat leaves is less than the maximum allowable limit set by WHO/FAO standard. Then ,this result implies that we fail to reject null Hypothesis, this means we don't accept alternative Hypothesis and then we continue to accept null Hypothesis. The average concentration value of heavy metals for both the research and control khat leaves samples are bellow the maximum allowable limits set by WHO/FAO.As can be observed from table 4.13 the contamination level of each metal is below the maximum allowable limit set by WHO/FAW.There level of Pb, Cr, Cd in the khat leave samples are found to be bellow the detection limit of the spectrometer. For instance the mean concentration of Cu in the research khat leaves is (0.1885±0.0302) mg/kg the mean concentration of Cu in the control khat leaves sample

is (0.1505±0.0012) mg/kg and maximum allowable limits set by WHO/FAO for Cu is (2.0) mg/kg; then, this value are significantly different ($p<0.05$) limit. In similar procedure the average concentration of all heavy metals are below the WHO/FAO limit (Table 4.13).

Ele	Range of Research khat leaves	Range of Control khat leaves	FAO/WHO
Cr	BDL	BDL	3.123-5.744
Cd	BDL	BDL	0.035-0.428
Zn	0.438-0.631	0.457-0.468	50-150
Fe	1.629-4.572	1.727-1.965	145.71-300.5
Pb	BDL	BDL	0.071-0.561
Cu	0.1498 - 0.2608	0.1498-0.1519	50-150

Table 4.14: Range value comparison b/n khat leaves samples to that of WHO/FAO(mg/kg)

The range of heavy metals for the research and control khat leaves samples are bellow the maximum allowable limits set by WHO/FAO. The mean range value of Zn in the control khat leaves sample is (0.457-0.468) mg/kg; the rang value of Zn in the research soil (0.438-0.631) mg/kg and maximum allowable limits set by WHO/FAO for Zn is (50-150) mg/kg, This shows, the range value of the research and control khat leaves samples are bellow the range of maximum allowable limits set by WHO/FAO at 95% confidence limit. In similar way all heavy metals are below the range of WHO/FAO maximum allowable limit (Table 4.14)

4.5.3 Comparison of heavy metal between research and control khat leaves

Element	$\bar{x}$ ± SD& R_{kh} (mg/kg)	$\bar{x}$ ± SD,& C_{kh} (mg/kg)	p-value
Cr	BDL	BDL	-
Cd	BDL	BDL	-
Pb	BDL	BDL	-
Zn	0.515±0.0688	0.4632±0.0055	0.0097
Fe	2.8624±0.9555	1.8433± 0.1191	0.0039
Cu	0.1885±0.0302	0.1505±0.0012	0.0015

Table 4.15:Thecomparison of metals and p-value for the research and control khat leaves

Where: Research khat leaves (R_{kh}; n=20) and control khat leaves (C_{kh}; n=5)

All the analyte metals in the research khat leaves have higher average concentration than the average concentration of the control khat leaves sample. This result shows that we

nullify null hypothesis and so we accept alternative hypothesis. This in fact indicates that, the research khat leaves are contaminated as compared to that of control khat leaves. let's take the average concentration of Fe in the research khat leaves is (2.8624± 0.9555) and the average concentration of Fe in the control khat leaves is (1.8433± 0.1191) this indicates that the research khat leaves is contaminated by heavy metal than the control khat leaves significantly ($p<0.05$) difference limit. The contamination of the research khat leaves sample may be due to the continuous application of fertilizers, pesticides, herbicides, land application fertilizer (DAP,UREA),wastewater irrigation, atmospheric dust, plant protection agent, and atmospheric deposition in the research khat leaves. The concentration level of heavy metals in the research and control khat leaves sample are indicated below on bare graph simply.

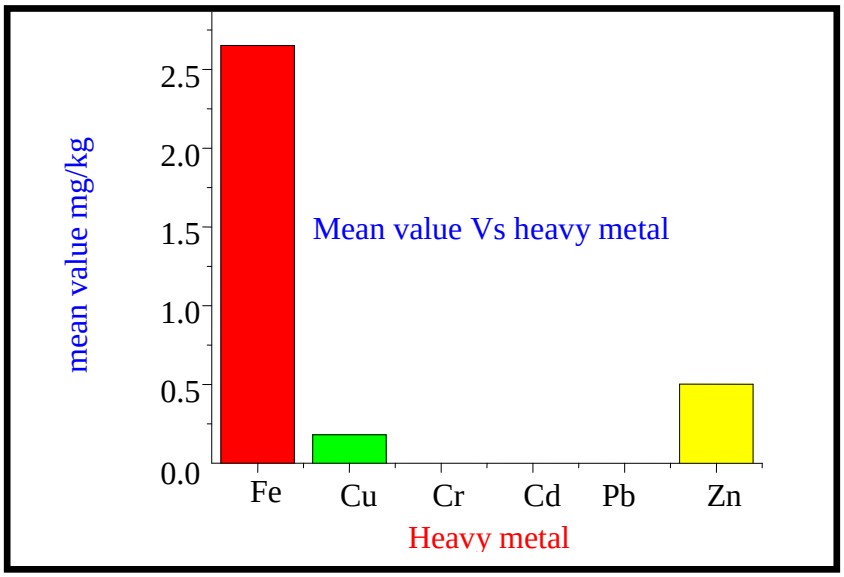


fig 4.5A:mean value of heavy metals in research and control khat leaves based on bar graph

4.5.4 Comparison of heavy metal content in this study with other country literature

The quantitative analysis found that the chemical profile of khat leaves was largely determined by the environment in which it grows rather than by the cultivators (Raman R.1983).The heavy metal concentration of Khat leaves varies from place to place depending on the climate, the soil mineral content, soil pH and age of the plant being harvested. As shown in Table 4.16, the level of Cu, Zn, Cd and Pb was higher in Djibouti

and Yamani khat compared to the current study except Cr and Fe which are not recommended.

Heavy Metal	Yemeni(mg/kg)	Djibouti(mg/kg)	Range(current research) (mg/kg)
Cr	NR	NR	0 - 0.0123
Cd	0.01 -0.031	0.325 - 0.7	0 - 0.043
Zn	4.8 - 8.44	6.03 - 11.73	0.438-0.6305
Fe	NR	NR	1.629-4.572
Pb	0.921 - 2.1	1.2 - 2.28	0 - 0.080100
Cu	3.32 - 5.19	4.8 - 6.1	0.1498 - 0.2608

Table 4.16:the comparison of the result obtained in the study to (MAL) in different countries of khat leaves sample

4.6 Comparison of metals in research Soil and khat leaves Samples

The concentration of all essential and potentially toxic heavy metals were found to be higher in the soil sample than in the khat leaves. This is an indicative that a small allotment (percentage) of metals in the soil is transferred to the khat leaves because the roots and stems acts as a barrier to the translocation of heavy metals within khat leaves (Davies and White, 1981). This shows that the main source of metal content of khat leaves is from their corresponding soil content which might be highly affected by the Zn, Fe, Cu, Cd, Cr, Pb based application, the environmental interference like pesticides, insecticides, NPK fertilizers (DAP,URIA) and other additives that farmers use.

element	Soil sample	Range	Khat leaves sample	range
Cr	0.3625±0.112	0.279- 0.557	-	
Cd	0.1051±0.035	0.052 - 0.148	-	
Pb	-	-	-	
Zn	1.802±0.044	1.719 - 1.849	0.515±0.069	0.438-0.631
Fe	5.2675± 0.563	4.604 - 6.266	2.8652±0.956	1.629-4.572
Cu	0.575±0.057	1.719 -1.849	0.1885±0.031	0.149 -0.261

Table 4.17: Heavy metals concentration comparison in the soil and khat leaves samples in mg/Kg

4.7 Heavy metal transfer factor from soil to khat

Transfer Factor is the relative measure of the transfer of an element (essential or potentially toxic) from the soil to the edible part of the khat leaves (Hood, 2010).

The transfer factor is calculated by equ (4.5) (Tsarina *et al*., 2015).

$$TF = \frac{\text{Conc.of Heavy Metal in the edible part of khat leaves}}{\text{Conc.of metal in soil}} \ldots\ldots\ldots\ldots (4.5)$$

Higher transfer factor represents relatively poor retention in soils or greater efficiency of khat leaves to absorb metals while Low transfer factor shows the strong sorption of metals to the soil (Coutate, 1992, Wierzbicka, 1995).

element	Khat leaves
Fe	0.54± 0.03
Cd	-
Zn	0.29±0.01
Cr	-
Pb	-
Cu	0.33± 0.02

Table 4.18:the transfer factor of heavy metals from soil to khat leaves

According to Table 4.18the TF or PCF values ranges were: Fe(0.54), Zn(0.29), Cu(0.33) while Cr, Cd, Pb does not have transfer value. The transfer factor for heavy metals in khat leaves sample are in the order of: Fe>Cu>Zn. Transfer quotient less than 0.1 indicates that the khat leaves is excluding the element from its tissues and the greater the transfer factor than 0.50 the greater the chances for metal contamination by anthropogenic (human made) activities (Sagged *et al.,* 2009). Reference to this idea, Cd, Pb and Cr were excluded in the khat leaves tissue, while Fe (0.54) could be contaminated by anthropogenic activities. High TF of Fe indicates that Fe is more available to khat leaves than other metals and Fe can be transferred from the soil to khat leaves more easily. The medium TF values were found to be 0.29 and 0.33 for Zn and Cu respectively. These might be due to medium mobility of these heavy metals with a natural occurrence in soil than other toxic element (Alloway and Ayres, 1997; Tsarina *et al*., 2015).

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The main objective of this research was to assess the status of essential and potentially toxic heavy metals in the khat leaves and agricultural soil sample collected from an agricultural land located southern Ethiopia of siltie zone, Silti Woreda of assano region. To achieve this goal, a total of 5 soil samples and 5 khat leaf samples were collected from the research site. Composite soil sampling technique was applied to collect the soil samples. The khat leaves and soil sample analysis using the AAS instrument, the instrument was calibrated for accuracy and precession using standard calibration solutions and validated using standard reference materials. The blank and the actual sample were all subjected to similar Kjeldahil digestion processes. In this study the concentration of Pb, Cr, Cd, Fe, Zn, and Cu were analyzed in khat leave and soil sample collected from the research areas by AAS (ZEE nit 700P,ATOMIC FLAME MODE,Analaytik Jena) technology giving due emphasis on their relative concentration.

The average concentration level of the analyte metals in the control soil samples are, Fe>Zn>Cu>Cr>Cd for the value 4.970> 1.785> 0.488>0.3427>0.0099 mg/kg respectively where as the mean value of heavy metal for the research soil samples are: Fe>Zn>Cu>Cr>Cd at a value of 5.2675> 1.802 > 0.575> 0.3625> 0.1053 mg/kg. The concentration level of all the analyte metals in the research soil samples were found to be higher than the corresponding control soil samples (background values). However, in both the control and research soil sample, the levels of the analyte heavy metals were below the maximum allowable limit (MAL) set by FAO/WHO.

The mean concentrations of the essential heavy metal in the research khat leave sample in increased order of values are: Cu (0.1885)< Zn (0.515) < Fe (2.86) whereas the mean concentrations of the potentially toxic heavy metals were below detection limit. For the Control khat leaves sample the average concentration of heavy metals are: [Fe (1.8433± 0.11909),Zn (0.4632±0.00547), Cu (0.1505±0.00118), Cd (BDL), Cr (BDL), Pb (BDL)].The average concentration for the analyte heavy metal in the research khat leaves sample is higher than the average concentration for the control khat leaves sample.

The percentage recovery for the soil and khat leaves samples are found in the range of 90.9 to 97.17%.The percentage relative standard deviation (% RSD) of all the analyzed elements in each soil and khat leaves sample were below 10%. The pH value of the soil ranges from 5.6 ± 0.03 to 6.08 ± 0.13 then, soil samples collected from khat growing area were moderately acidic. The transfer factor (TF) value of essential heavy metals were found to be for: Fe (0.54), Cu (0.33),Zn (0.29) and the potentially toxic heavy (Cd, Pb and Cr) were excluded in the khat leaves tissue.

A statistical analysis at 95% confidence level indicated that there is significant difference in the level of all metals among the khat leave and soil sample. The ANOVA results indicates that there is significant different ($p < 0.05$) in the level of heavy metals between soil and khat leaves from the research region. The average concentration of heavy metals in the research and control sample for both soil and khat leaves are lower than the maximum allowable limit (MAL) set by WHO/FAO standard at significantly different of ($p<0.05$).

5.2.Recommendation

5.2.1 Recommendations from this study

Based on the levels of heavy metals investigated in soil and khat leaves sample collected from Assano, the following are recommended:

$. The levels of Pb, Cr and Cd in the soil and khat leaves should be continuously regulate to check on their levels b/c thus heavy metals are very poisonous even in their smallest quantities.

$.The use of agrochemicals should be minimized as this are the source of contamination by heavy metals but For this study, the concentration of heavy metals are bellow the maximum permissible limit set by WHO/FAO standard.

$.The soil sample is contaminated by Fe, Zn, Cu, Cr, and Cd except Pb and khat leaves sample is contaminated by Fe, Zn, Cr but not Cd, Pb, Cr but this is not polluted by thus heavy metals. This shows, it needs a great concern for monitoring the levels of heavy metals in soil and khat leaves to ensure that they do not exceed the maximum allowable limit, is the matter of the society and the consumer.

$.Sources of heavy metals in soils like inorganic fertilizers, pesticides and insecticides ,NKP fertilizer need to be controlled. The anthropogenic sources or ingredients of agricultural inputs are; known to be the sources of some of the heavy metals like zinc, chromium, copper, iron and Cd which have been detected both in soil and khat leaves at low levels.

$.Small holder farmers, agricultural professionals, environmentalists, agrochemical suppliers and others should be aware on the ingredients of the fertilizers, herbicides, insecticides and others.

$.Under these study, the soil is contaminated by to Fe, Cu, Zn , Cr , Cd and khat leaves is also contaminated by Fe, Cu, Zn then, community should aware on the danger of thus heavy metals and the government should come up with policies that will control the continues distribution, sale and use of khat leaves.

$. The higher level of Fe, Cu and Zn in soil sample is may be related to human's activities, the use of fertilizers with different chemical compositions. then farmers should use other alternative to decrease the contamination level of this heavy metal.

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APPENDICES

Appendix Table 1 : Experimental determination for the Mean concentration of Cr, Cd, Zn Fe, Pb and Cu of in khat leaves sample based on AAS.

	Cd	Cr	Cu	Fe	Pb	Zn
Mean	-0.008847	-0.165640	0.180700	2.652400	-0.732533	0.501840
Median	-0.013000	-0.183300	0.174200	2.501000	-0.794000	0.467600
Maximum	0.043000	0.012300	0.260800	4.572000	0.080100	0.630500
Minimum	-0.034300	-0.250100	0.149800	1.629000	-1.132000	0.438000
Std. Dev.	0.019800	0.065747	0.030214	0.955500	0.0329352	0.068830
Skewness	1.124565	1.601917	1.263433	0.764487	0.962142	1.113028
Kurtosis	4.144235	5.029337	4.269273	2.227543	3.444734	2.728992
Jarque-Bera	3.979909	8.989229	4.997564	1.834031	2.437911	3.142980
Probability	0.136702	0.011169	0.082185	0.399710	0.295539	0.207735
Sum	-0.132700	-2.484600	2.710500	39.78600	-10.98800	7.527600
SumSq. Dev.	0.005489	0.060518	0.012781	12.78172	1.518615	0.066325
Observations	15	15	15	15	15	15

Appendix Table 2: Experimental determination for the Mean concentration (mg/kg) of Cr, Cd, Zn Fe, Pb and Cu in soil sample based on AAS.

	Cd	Cr	Cu	Fe	Pb	Zn
Mean	0.084487	0.375907	0.574880	5.207933	-0.242280	1.798533
Median	0.089400	0.308100	0.586200	5.043000	-0.152300	1.800000
Maximum	0.147500	0.556500	0.635400	6.266000	0.121200	1.849000
Minimum	0.002400	0.279100	0.489600	4.604000	-0.718700	1.719000
Std. Dev.	0.019461	0.0105139	0.57287	0.574479	0.0295184	0.3035240
Skewness	-0.345604	0.674010	-0.460542	1.019106	-0.429607	-0.628902
Kurtosis	1.782663	1.813588	1.831184	2.882150	1.845069	2.275376
Jarque-Bera	1.224798	2.015456	1.384079	2.605122	1.295073	1.316970
P-value	0.542049	0.365047	0.500554	0.271835	0.523333	0.517635
Sum	1.267300	5.638600	8.623200	78.11900	-3.634200	26.97800
Sum Sq. Dev.	0.034249	0.154759	0.036825	3.705643	1.219874	0.022670
Observations	15	15	15	15	15	15

Appendix Table 3: Heavy metal concentration (mg/kg) range, mean, maximum, minimum, SD for each soil sample (n = 10).

	Mean	S.D	Min.	Max.	Range	Sum
sample for Fe						
Sample1	5.077	0.29011	4.755	5.318	0.563	15.231
Sample2	6.106	0.21623	5.86	6.266	0.406	18.318
Sample3	4.713	0.10016	4.604	4.801	0.197	14.139
Sample4	5.17333	0.13479	5.024	5.286	0.262	15.52
Sample5	4.97033	0.06545	4.916	5.043	0.127	14.911
Sample for Zn						
Sample1	1.73433	0.0155	1.719	1.75	0.031	5.203
Sample2	1.83867	0.01002	1.829	1.849	0.02	5.516
Sample3	1.802	0.00964	1.795	1.813	0.018	5.406
Sample4	1.833	0.00608	1.829	1.84	0.011	5.499
Sample5	1.78467	0.01332	1.776	1.8	0.024	5.354
Sample for Cu						
Sample1	0.49527	0.00503	0.4896	0.4992	0.0096	1.4858
Sample2	0.5451	0.00191	0.544	0.5473	0.0033	1.6353
Sample3	0.62873	0.00946	0.6179	0.6354	0.0175	1.8862
Sample4	0.6171	0.01163	0.6046	0.6276	0.023	1.8513
Sample5	0.4882	0.00558	0.5839	0.5945	0.0106	1.7646
Sample for Pb						
Sample1	-0.4121	0.02876	-0.4403	-0.3828	0.0575	-1.2362
Sample2	-0.0347	0.02012	-0.0566	-0.017	0.0396	-0.1042
Sample3	-0.167	0.03504	-0.207	-0.1417	0.0653	-0.501
Sample4	-0.1013	0.02994	0.0669	0.1212	0.0543	0.304
Sample5	-0.6989	0.01793	-0.7187	-0.6837	0.035	-2.0968
Sample for Cr						
Sample1	0.29127	0.01458	0.2825	0.3081	0.0256	0.8738
Sample2	0.29163	0.01489	0.2791	0.3081	0.029	0.8749
Sample3	0.31093	0.00803	0.3062	0.3202	0.014	0.9328
Sample4	0.54303	0.01563	0.5259	0.5565	0.0306	1.6291
Sample5	0.34267	0.01797	0.4263	0.4619	0.0356	1.328
Sample for Cd						
Sample1	0.0562	0.00611	0.0519	0.0632	0.0113	0.1686
Sample2	0.127	0.01019	0.1163	0.1366	0.0203	0.381
Sample3	0.08993	0.00801	0.0822	0.0982	0.016	0.2698
Sample4	0.1394	0.00714	0.134	0.1475	0.0135	0.4182
Sample5	0.099	0.00666	0.0024	0.0151	0.0127	0.0297

Appendix Table 4: Heavy metal level (mg/kg) range, mean, maximum, minimum and SDof each khat leaves sample (n = 10).

	Mean	S.D	Min.	Max.	Range	Sum
sample for Fe						
Sample1	3.156	1.227	2.396	4.572	2.176	9.469
Sample2	1.8411	0.1191	1.727	1.965	0.238	5.53
Sample3	2.606	0.063	1.727	2.675	0.124	7.816
Sample4	3.889	0.123	2.551	3.983	0.233	11.668
Sample5	1.767	0.121	3.75	1.858	0.229	5.301
Sample for Zn						
Sample1	0.745	0.0183	0.462	0.496	0.10335	1.4246
Sample2	0.46323	0.00547	0.4571	0.4676	0.0105	1.3897
Sample3	0.4632	0.0022	0.438	0.4676	0.004	1.3186
Sample4	0.4395	0.0038	0.623	0.442	0.0075	1.8816
Sample5	0.627	0.0069	0.4989	0.6305	0.0132	1.5131
Sample for Cu						
Sample1	0.204	0.049	0.1742	0.2608	0.0866	0.6127
Sample2	0.15053	0.00118	0.1498	0.1519	0.0021	0.4516
Sample3	0.1613	0.0074	0.1553	0.1695	0.0142	0.4839
Sample4	0.2074	00083	0.2009	0.2167	0.0158	0.6222
Sample5	0.1800	0.00578	0.1734	0.184	0.0106	0.5401
Sample for Pb						
Sample1	-0.2274	0.2702	-0.4267	0.0801	0.5068	-0.6823
Sample2	-0.6057	0.07598	-0.6828	-0.5309	0.1519	-1.8171
Sample3	-0.7875	0.0601	-0.8441	-0.7244	0.1197	-2.365
Sample4	-0.9574	0.0425	-1.002	-0.9173	0.0847	-2.8721
Sample5	-1.0847	0.044	-1.132	-1.045	0.087	-3254
Sample for Cr						
Sample1	-0.0975	0.0956	-0.1622	0.0123	0.1745	-0.2924
Sample2	-0.1951	0.01076	-0.2062	-0.1847	0.0215	-0.5854
Sample3	-0.1381	0.0775	-0.2042	-0.0529	0.1513	-0.4144
Sample4	-0.1751	0.0074	-0.1833	-0.1686	0.0144	-0.5252
Sample5	-0.2224	0.0241	-0.2501	-0.2067	0.0434	-0.6672
Sample for Cd						
Sample1	0.0194	0.0215	0.0011	0.043	0.0419	0.0581
Sample2	-0.0045	0.0067	-0.0118	0.0012	0.013	-0.0131
Sample3	-0.0205	0.0062	-0.0268	-0.0144	0.0124	-0.0616
Sample4	-0.0096	0.0112	-0.0188	0.0029	0.0217	-0.0289
Sample5	-0.0291	0.0056	-0.0343	-0.0232	0.011	-0.0872