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M. Sc Thesis

On:

Determination of Concentrations of selected metals in Tobacco Leaves and its farmland Soils of AssosaWoreda, Benshangul Gumuz Regional state, Ethiopia

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Jimma, Ethiopia

Determination of Concentrations of Selected Metals in Tobacco Leaves and Its Farmland Soils of Assosa woreda, Benshangul Gumuz Regional state, Ethiopia

Thesis Submitted to School of Graduate Studies, college of Natural sciences Jimma University, in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Chemistry (M.Sc).

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Declaration

I declare that this is my original work, except where reference is made, and has never been submitted anywhere for award of any degree or diploma in any university.

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A thesis submitted to School of graduate studies, college of Natural sciences Jimma University in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry

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TABLE OF CONTENTS

Content page

LIST OF TABLES

LISTS OF FIGURE

ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
DNA	Deoxyribonucleic Acid
DPTA	Diethylene –triaminePentacetic Acid
FAO	Food and Agricultural organization
ICP-OEP	Inductive coupling plasma-Optical Emission spectroscopy
LOD	Limit of Detection
LOQ	Limits of quantification
UNODC	United Nation of Drug Corporation
WHO	World health organization

ABSTRACT

Tobaccos are a commercial plant that naturally accumulates and concentrate relatively high levels of toxic heavy metals such as; cadmium (Cd) and lead (Pb) and essential elements like Zinc (Zn),Copper (Cu) and Manganese (Mn). Hence, the level of these metals were determined in tobacco and its farmland of AssosaWoreda, BenshangulGumuz Region, Ethiopia. The sample was collected from five different localities of tobacco farmlands of AssosaWoreda. Soil samples were collected from the top to 30 cm depth and leaves of tobacco were collected from the lower to upper part of each tobacco plant. The tobacco and soil samples were digested following the protocol of wet digestion method using analytical grade chemicals. Chemicals were used HCl, HNO_3 and H_2O_2. One way of ANOVA and F-test were used to analyze the obtained data. The analysis of heavy metal in tobacco leaves and soil of its farm land was as certain levels of concentration of total metal content of cadmium, copper, manganese, lead and zinc of digestion was carried out by using wet digestion technique. The efficient of the digestion procedure was checked by recovery test of known concentration of standards solution of Cd, Cu, Mn, Pb and Zn were spiked in tobacco leaves and soil samples then triplicate digestion and triplicate reading was applied. Results show that the concentration values tobacco obtained were as follows: Mn range from (0,75 mg/L to 1.18 mg/L), Cu range from (0.11 mg/L to 0.21 mg/L), Zn range from (0.58 mg/L to 1.16 mg/L) Cd range from (0.008 mg/L to 0.009 mg/L) and the content of heavy metals in soil sample were Mn range from (1.59mg/L to 8.04mg/L),Cu range from (0.32mg/L to 0.38mg/L), Zn range from (0.29mg/L to 1.26mg/L) Cd was below the detection and quantification limits of the instrument and Pb ranged from (0.17mg/L to 0.31mg/L). The content of heavy metal in tobacco leaves and in soil sample is below the permissible value of WHO/FAO except the concentration Mn in the soil.

Keywords: Tobacco leaves, Heavy metals, soil, ICP- OES

1. INTRODUCTION

1.1 Background

Metals occur naturally in the earth's crust, and their contents in the environment can vary in different regions resulting in spatial variations of background concentrations. The distribution of metals in the environment is governed by the properties of the metal and influences of environmental factors [1]. Heavy metals is the generic term for metallic elements having an atomic weight higher than 40.04 (the atomic mass of Ca. These metals enter in to the environment by natural and/or anthropogenic means, such as natural weathering of the earth’s crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants and air pollution fallout [2].

Lead (Pb), cadmium (Cd), Zinc (Zn), Manganese (Mn) and copper (Cu) are some of the heavy metals which are widely dispersed in the environment. Cd and Pb have no beneficial effects in humans, and there is no known homeostasis mechanism for them [3]. Cd and Pb are generally considered as the most toxic substances to humans and animals. Because exposure to these metals even at low concentration could bring an adverse health effect to human being but Mn, Cu and Zn are an essential element for plants and human [4]. In Ethiopia, tobacco is grown for commercial purposes by state owned farms and individual farmers. The National Tobacco Enterprise has been given the mandate to organize tobacco production and processing in the country. The three main types of commercial tobaccos are produced in Ethiopia: Virginia, Oriental and Burley [6]. Virginia accounts for a little more than 74 % of the total production, followed by Oriental, 22 %, and Burley, 4 %. Major cultivation areas are Sidamo, Northern Showa, and Hararghe. Commercial production is concentrated in ShewaRobit (North Showa), Billate, Awassa, Wolaita (Sidamo) and in Nura-Era (Hararghe). State farms in these areas produce about 500–900 MT of cured leaves annually from about 2000 hectares of land [5]. It has been reported that in Ethiopia peoples commonly use Chat, tobacco, and alcohol, which have a share of 48.2, 29.9 and 18.9 %, respectively, of all type of drugs [6]. This clearly shows that tobacco made significant contribution as a drug in Ethiopia.

The heavy metals are widely dispersed in the environment, and at excessive levels, thus making it very toxic to humans [7]. Although, these metals occur naturally, exposure to them may be increased by human activities that release them into the air, soil, water, food, and by-products that contain heavy metals. Certain plants also have the ability to accumulate heavy metals that have no known biological function [8].

Tobacco (NicotianatabacumL.) plants are amenable to absorb and accumulates heavy metal species from the soil into leaves [10]. Tobacco plants transport metal ions from the soil through their roots into the leaves [11]. Trace amounts of heavy metals accumulate in the leaves, and they are known to transfer in trace quantities from the cured and processed tobacco to main stream cigarette smoke. These metals include Zn, Cd and Pb [12]. The most abundant redox inactive metals in cigarette smoke generally are Cd and Pb. The concentration of heavy metals in the soil affects the amount their accumulation in plant grown on it. The degree of accumulation of the metals from the soil depends on the type of soil, the pH value, the quality of water used for irrigation, the chemical composition of the metal, geographical origin and the type of tobacco plant [13]. The farmers use large amounts of fertilizers and pesticides during the production of tobacco plant. The fertilizers and pesticides usually contain high concentrations of metals and contribute a major degree in the pollution of agricultural soil, as well as plants [14]. Tobacco is a commercial plant used for cigarette production and chewing. [15].

Generally, different metals have different health effects. For instance, Cd is a non-essential to both plant and human. It is highly toxic and gets accumulated in different plants including tobacco. Cd is transferred to human being through cigarette smoking [16]. Cd is associated with bone and kidney diseases. Pb is associated with neurological disorders [17]. Excess of Zn causes metabolic disorders potentially resulting in death [18]. Thus, cigarette smoking could be a major source of intake of these toxic elements not only to the smoker but also through passive smoking, to nonsmokers. It is observed that contents of Pb [19] and arsenic (As) [20] in biological samples of human population are much higher in smokers than those in non-smokers. Pb and As are classified as carcinogenic to humans [21], but also various other negative effects of lead and arsenic on human health have been recognized [22].

In general, tobacco is one of the potential sources of heavy metals. Determination of the level heavy metals in tobacco and its farmland soil is important. Tobacco is widely grown in Assosa Woreda and utilize traditionally as cigarette by many people of this area. Therefore, determination of the level of Zn, Cd, Pb, Mn and Cu in tobacco and its farmland soil is important, as no study has been conducted on the determination of these metals in the tobacco and soils of this area. Thus, in this study, the content of selective heavy metals such as Zn, Cd, Pb, Mn and Cu in tobacco leave and its farmland soil were investigated using Inductive coupled plasma-optical emission spectrometry(ICP-OES). The correlation between the content of the target metals in tobacco leave and its farmland soil were also studied using Pearson correlation.

1.2. Statement of the Problem

Several studies were conducted aimed on determinations of the levels of heavy metals in the raw and processed tobacco leaves in the different parts of the world using different techniques [23]. Some studies were conducted out in Ethiopia on the levels of essential and non-essential metals in a psychoactive Chat leaves as well as in cannabis leaves [24].But a few studies were carried out on the levels of nicotine in Ethiopian tobacco leaves [25]. However, to the best of our knowledge, there is no report on the levels of heavy metals in tobacco leaves and its farmland Soil of Assosa Woreda, Benshangul Gumuz Region, Ethiopia. Hence this study is intended to determine the levels of toxic heavy metals, Cd and Pb and other essential elements such as Zn, Mn and Cu in tobacco leaves and its farmland soil of AssosaWoreda Benishangul-Gumuz region of Ethiopia

1.3. Objectives of the study

1.3.1. General objective

The main objective of this work is to know the content of selective heavy metal in tobacco leaves and its farmland soils of AssosaWoreda, BenshangulGumuz Region, Ethiopia.

1.3.2. Specific objective

The specific objective of the present study was:-

- ✓ To analyze the level of Zn, Cd, Pb, Mn and Cu in tobacco leave and its farmland soil of Assosaworeda.
- ✓ To compare the level of the metals among the different tobacco leaves collected from different farm lands of the Assosa Woreda.
- ✓ To investigate the relationship between the content of the target metals in the tobacco leaves and its farmland soils.
- ✓ To compare the level of the heavy metals in tobacco and in soil with WHO/FAO standard.

1.4. Significance of the study

This study was investigating the concentration of selective heavy metals in tobacco leave and its farmland soil of AssosaWoreda. Thus the study will be important,

- ✓ The background information about the concentration of the metals in tobacco product of the Assosa Woreda.
- ✓ Itwill alsouse as formal and valuable document for further research work.
- ✓ It will be used to create awareness to the people, use tobacco as insecticides or as medicinal plant rather than production of cigarettes.

2. REVIEW LITERATURE

2.1. Tobacco (Nicotianatabacum L.)

Tobacco (Nicotianatabacum L) is **an** industrial plant which has the ability to accumulate metals. Tobacco leaves are used for cigarette production and chewing. The accumulation of heavy metals in the tobacco plant is a consequence of a complex interaction between the soil, plant and animal environment. Tobacco smoking is a worldwide problem with 1.3 billion people currently smoking cigarettes and one person loosing life every 6 second due to tobacco related illnesses [17]. Consumption of tobacco products by both smoking and non-smoking ways affect the health of smokers directly as well as non-smokers via passive smoking and also add metal contents to the environment[26].More than 4,000 chemicals have been isolated from tobacco (hydrocarbons, aldehydes, ketones, aromatic hydrocarbons, heavy metals including cadmium, lead, Zinc and arsenic). Lead and arsenic have been identified and measured both in tobacco and tobacco smoke [27]. Exposure to metals through tobacco depends on the amount of metal present in tobacco, a percentage that is transferred to the tobacco smoke and the percentage that is absorbed.

2.2. Heavy Metals

A heavy metal is a member of a loosely-defined subset of elements that exhibits metallic properties. It mainly includes the transition metals, some metalloids, lanthanides, and actinides. Many different definitions have been proposed-some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity [28]. Alloway (1990) defined the term heavy metal to any metallic chemical element that has a relatively higher density and is toxic or poisonous at low concentration[29] also stated the term heavy metal as usually applied to elements such as cadmium, copper, nickel, mercury and lead which are commonly associated with pollution and toxicity problems. An alternative name for this group of elements is trace elements but it is not widely used. Heavy metals are among the major contaminants of food supply and may be considered the most important problem of pollution [30].

Heavy metals may be present either as a deposit on the surface of leaves or may be taken up by the crop roots and incorporated into the edible part of plant tissues. Heavy metals deposited on the surface can often be eliminated simply by washing prior to consumption, whereas bio-accumulated metals are difficult to remove and are of major concern .Tobacco plant take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the tobacco plant that exposed to the air from polluted Environments[31].Different species accumulate different metals depending on environmental conditions, metal species, plant available and forms of heavy metals. Heavy metals may enter the human body through inhalation of dust, consumption of contaminated drinking water, direct ingestion of soil, smoking tobacco and consumption of food plants grown in metal contaminated soil [32].

Heavy metals are not easily bio-degradable and consequently can be accumulated in human vital organs. This situation cause varying degrees of illnesses based on acute and chronic exposures potentially toxic metals are also present in commercially produced food stuffs. The excessive content of heavy metals in food was reported to be associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous, as well as bone diseases.

2.2.1. Lead

Lead as a toxicologically relevant element has been brought into the environment by man in extreme amounts, despite its low geochemical mobility and has been distributed worldwide [33] Lead amounts in deep ocean waters is about 0.01-0.02 μg/L, but in surface ocean waters is 0.3 μg/L [34]. Lead still has a number of important uses in the present day; from sheets for roofing to screens for X-rays and radioactive emissions. Like many other contaminants, lead is ubiquitous and can be found occurring as metallic lead, inorganic ions and salts [35]. Lead has no essential function in man.

Lead circulates in the bloodstream and accumulates in tissues and bones, or is eliminated from the body, primarily in urine. The blood brain barrier of children and infants is relatively impermeable to lead but they are at high risk of accumulating lead in the brain and central nervous system which may cause neurodegeneration [36].Tobacco is an important source of lead in secondhand tobacco smokers (children and adolescents) in the United States. Blood levels of lead were 14% and 24% higher in children who lived with 1 or with 2 or more smokers than in children living with non-smokers [37].

Lead plays a significant role in tobacco toxicity, especially radioactive ^{210}Pb. ^{210}Pb is a product of ^{238}U disintegration and its existence in tobacco depends on the tobacco origin and natural level of uranium in the soil where tobacco grows. In Italy, ^{210}Pb dose from inhalation of cigarette smoke is much higher than the dose from ambient air [38].

2.2.2. Cadmium

Cadmium is a non-essential to both plant and human. It is highly toxic and gets accumulated by tobacco plants. Cadmium is transferred to human being through cigarette smoking [17]. Cadmium is naturally present in the environment: in air, soils, sediments and even in unpolluted seawater. Cadmium is emitted to air by mines, metal smelters and industries using cadmium compounds for alloys, batteries, pigments and in plastics, although many countries have stringent controls in place on such emissions .Tobacco smoke is one of the largest single sources of cadmium exposure in humans. Tobacco in all of its forms contains appreciable amounts of the metal. Because the absorption of cadmium from the lungs is much greater than from the gastrointestinal tract, smoking contributes significantly to the total body burden .In general, for non-smokers and non-occupationally exposed workers, food products account for most of the human exposure burden to cadmium. Elevated cadmium levels in lung, liver, and kidney tissue [39], body fat [40], blood [41] and urine [42], have been correlated with smoking history or exposure to second hand smoke. Elevated lead levels in the blood and amniotic fluid [43] and in the cord blood of newborn babies [44] have also been associated with smoking.

2.2.3. Zinc

Zn is anessential element, necessary for the growth, development and the normal functioning of the body. Nevertheless, increased concentrations of zinc in the body can have a detrimental effect on human health. Studies have shown that the increased intake of zinc into the body can lead to a deficiency of copper in the liver, the serum and the heart, and the decrease of the activity of copper metalloenzymes [45]. In addition, the increased intake of Zn into the body can have a detrimental effect on the storage of iron and can lead to the occurrence of anemia [46]. Toxic effects of heavy metals (Zn, Cd and Pb) which can be introduced into the body through tobacco smoke, both in the case of active and passive Smokers.

2.2.4. Copper

Copper is one of the essential elements for human metabolism. It enters the soil through waste deposit dumps, domestic waste water and the use of phosphate fertilizer for farming. Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain [47].Cu is also added to soils from different human activities including mining and smelting of Cu-containing ores. Mining activities generate a large amount of waste rocks and tailings, which get deposited at the surface. Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis [48]. Exposure of plants to excess Cu generates oxidative stress and ROS [49]. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al. 2001).The WHO and FAO recommended values for daily and provisional tolerable weekly intake is 100mg/kg and 500mg/kg respectively.

2.2.5. Manganese

Manganese is the eleventh most common element in the earth's crust, with an average concentration of 0.09%, or 900 mg/kg. Manganese is present primarily as oxides and sulfides; it often occurs in association with iron. Soils have manganese concentrations that are usually in the range of 20 to 3000 mg/kg, with an average of 600 mg/kg. Soil manganese exists in three oxidation states Mn^{2+}, Mn^{3+}, and Mn^{4+}. Manganese absorbed by plant roots is primarily as Mn^{2+}. Oxidation-reduction reactions in the soil influence the amount of each oxidation state.

Total soil manganese may be divided into mineral manganese, organically completed manganese, exchangeable manganese, and solution manganese. Manganese in solution may be either Mn^{2+} or manganese combined with soluble organic compounds. The equilibrium of manganese between these forms is influenced greatly by soil pH and radox conditions [49]. Manganese is an essential element in respiration and nitrogen metabolism; in both processes it functions as an enzyme activator, in chlorophyll development and in the enzyme systems of plants. Manganese is also in some way involved in the oxidation-reduction processes in the photosynthetic electron transport system [50]

Manganese deficiency also appears to have a marked effect on the chloroplast. The chloroplasts lose chlorophyll and starch grains, become yellow green in color, vacuolated, and granular, and finally disintegrate [51]

When Mn is consumed in excess amount in plants it disturbs photosynthetic process. In animals Mn is an important nutrient in bone formation, fat and carbohydrate metabolism, blood sugar regulation, Ca absorption and as cofactor of several enzymes [52, 53]. It activates numerous enzymes and a constituent of some enzymes [53, 54], needed for energy and protein metabolism, regulation of cell metabolism as well as it act as antioxidants and prevent oxidative stress by neutralizing oxidants produced under different stresses like environmental or production stress or stress related to infections or diseases [53, 55, 56]. Mn also plays an essential role in connective tissue growth and blood clotting [54]. Mn deficiency lead to skeletal abnormalities, postural defects, impaired growth and reproductive function, disturbances in lipid and carbohydrate metabolism [53, 54, 50], poor bone formation, reduced fertility and birth defects [57], bone malformation, weakness [52] and impaired insulin synthesis and action [53].

2.3. Comparison of heavy metals in the raw tobacco leaves and processed tobacco samples

The variation in composition of metals in the raw and processed tobacco leaves was observed for all the detected metals).This change in concentration of metals could be due to treatments and handling of tobacco leaves starting from harvesting to the cigarette manufacturing in the factory. During harvesting, transportation from the farm to the site of curing, transportation from site of curing to the factory and system of the storage could make leaves of tobacco be contaminated with dusts and soil, which contain these metals. The processes of packing and packing materials, curing system, treatments in the factory and chemical additives (casing activities) in the manufacturing, could have contribution for the contamination the tobacco leaves with the metals. The other factor that made the large difference in concentration of metals could be: during the collection of tobacco leaves for analysis the collected samples were washed with tape water and rinsed with distilled-deionized water. Since there is no such treatment in processed tobacco or in manufacturing of cigarette, the extraneous substances including soil and dust particles, and foliar spray residues could introduce extra metal contamination. Particularly tobacco leaves from the lower part were highly contaminated with soil. Therefore, metals from the soil that were deposited on the leaves could contribute to the high level of metals in the processed tobacco.

2.4. Comparison of heavy metals concentration in Ethiopian tobacco with literature values

Many researchers have reported the concentration of metals in cigarette tobacco as well as tobacco leaves. (Moulin et al. 2006) analyzed 755 tobacco's leaves samples during 2001–2003 and found that cadmium concentrations in the samples ranged from 0 to 6.78 μg/g dry mass.

As compared the report of the concentration of Cd in Ethiopia tobacco leaves(1.20-1.30 μg/g dry mass) was higher than flue cured tobacco of India(0.21-0.49 μg/g dry mass)and New Zealand tobacco leaves (0.23-0.56 μg/g dry mass) but the concentration of Cd in Ethiopian tobacco leaves (1.20-1.30μg/g dry mass)was less than America tobacco leaves(1.7-2.9 μg/g dry mass), Canada flue cured tobacco concentration(1.25-7.02 μg/g dry mass) and also the concentration of lead in Ethiopian tobacco leaves was not detected, but the concentration of lead in other country such as India,America,GermanyNewzland and Canada were(0.311–0.416 μg/g dry mass,0–200 μg/g dry mass,2.4–4.3μg/g dry mass,0.48–0.5530μg/g dry mass and 0.8–9.15μg/g dry mass)respectively.

Generally the level of Cd in Ethiopian tobacco leaves was within the range of the literature values which can range from 0 to 6.78 (Moulin et al. 2006). Whereas the concentration of lead was not detected, this was below the range of the literature values.

3. MATERIALS AND METHODS

3.1. Instrument and apparatus

Ceramic pestle and mortar was used for grinding and homogenizing of the raw a tobacco leaves and the soil samples. Digital analytical balance (KERN ABJ-NM/ABS-N, Germany) and oven (N50C England) was used for weighing and drying the samples, respectively. Glass beakers (100 -400 mL) and hot plate for wet digestion of tobacco and soil sample. Inductive coupling plasma-Optical Emission spectrometry (PerkinElmer® Optima™ 8000, USA) were used for total determination of heavy metals.

3.2. Chemicals, reagents and standard solutions

Reagent that were used in the analysis are analytical grade, such as HNO_3 (69%, LOBA, chem. India), HCl (37% LOBA,chem,India) and H_2O_2 (30% RdHLaborchemikalien,GmbH & CO.KG) were used for the digestion of tobacco leaves sample and soil sample. 10 % of HNO_3 solution was used for socking and cleaning laboratory apparatus. From stock standards solutions containing (1000mg/L) of Mn, Cu, Zn, Cd and Pb for spiking recovery test and calibration curve preparation for each metal. Double distilled water was used for dilution, cleaning and for preparation of all solution throughout the laboratory work

3.3. Study Area and study period

The study was conducted in Assosa Woreda Benshangul Gumuz Region, Ethiopia, which is located 659 km from Addis Ababa this has a latitude and longitude of 10°04′N and 34°31′E, with an elevation of 1570 m. The study was conducted from January to October, 2017 G. C.

3.4. Study Design. .

3.4.1. Sample collection

Depending on the availability of tobacco plant, representative tobacco leaves were collected from five different tobacco farmlands of the AssosaWoreda having highly cultivated tobacco plant by systematic method. The collected samples were washed with tap water and rinsed three times with distilled water to make them free of extraneous substances, including soil and dust particles and foliar spray residues that may influence analytical results. Similarly, soils samples are also collected from the sites that tobacco sample were collected. Both types of samples were sealed in polyethylene bag and transported to the laboratory where further sample pre-treatments were made.

Tobacco leaves sample was collected from five different farmland of tobacco plantation, the distance between each sample on the same farmland was on averages of 50 cm, and the lower part leaves of the sample and the upper part of the leaves sample were combine together. The soil sample was also collected where the tobacco leaves collected by systematic method at five position of the same tobacco farmland and the collected sample was combined and powdered to homogenize them.

3.4.2. Digestion of tobacco sample

Tobacco leaves were collected from five different sample stations in Assosa woreda. The leaf samples were washed with distilled water carefully and allowed to dry in oven at 105°C. The dried leaves were pound using mortar and pestle and sieve with the mesh sieve. About 0.5g of ground tobacco leaves was weighed into a clean 125mL Erlenmeyer flask. A mixture of 5 ml of Conc. HNO_3 (69%) and 4mL Conc.H_2O_2 (30%) was added to the flask. The mixture was heated gently on a hot plate under a fume–hood for 30 minutes. The flask was allowed to cool and 5mL of Conc. HNO3 was further added. The mixture was finally heated strongly to a medium heat and (1:1) 5 mL of HCl: HNO_3 was added heat for 15 minutes and allowed to cool. The solution was completely filtered (using Whatman No 42 filter) into 50mLvolumetric flask and make up to the mark with double-distilled water. The filtrate solution was stored in the refrigerator waiting for heavy metal analysis using ICP-OES.

3.4.3. Wet Digestion Methods of Soil sample

Air-dried samples of soils were ground ceramic pestle and mortar and sieve through a 300 inch mesh sieve.0.5g of well powdered soil sample weight and added to 125 mL Erlenmeyer flask. The aqua regia method was used for digestion soil involving concentrated HNO_3 and HCl (1:3 proportion) with addition of 2mL of (H_2O_2) for removing of the remaining organic matter using 125 mL Erlenmeyer flask socked overnight, then the mixture was heated using hot plate for 45 min inwell ventilated hood for the total analysis of Cd, Pb, Zn, Mn and Cu.. Then the digested mixture solution cooled. After cooling the mixture was filtered with (What man no 41) filters paper and diluted to50mL with double distilled water.

The blank solutions was prepared by digesting the mixture of reagents following the same digestion procedure and diluted to 50 mL with double distilled water.

3.4.4. Procedure of spiking

To confirm the efficiency of the utilized procedures recovery study was conducted, spiking experiments in which known volume and concentration of standard solutions, were employed. (2.5 mg/L) of Mn, Cu, Zn and (0.25mg/L) of Cd and Pb were prepared a mixture of standard solution was prepared in 10mLvolumetric flask from the stock solution containing (1000 mg/L) and from the mixed standard solution (2 mg/L)Mn, Cu and Zn and (0.2mg/L) Cd and Powered spiked to(0.5 g) tobacco leaves and soil sample collected from five different Keble of AssosaWoreda.

3.4.5. Method of Data analysis

Quantitative determination of the target analytes were done by Inductive coupling plasma-Optical emission spectroscopy (ICP-OES). Statistical data analysis of the sample were analyzed by one way ANOVA and F-test at confidence level of 95 % was employed to assess the presence or absence of significant difference among tobacco leaves and soils collected from different farmlands of the study area and the statistic result was reported using(Mean ± RSD).
(10mg/L) of standard solution of each metals (Zn, Pb, Mn, Cu and Cd) was prepared in100mL of volumetric flask from stock solution containing (1000mg/L)for the preparation of calibration curves for total determination of selective heavy metals in the samples. Double Distilled water was used for preparation of standard solutions and dilution.

3.4.6. Precision and Accuracy

In this study, the precision of the result was evaluated by the standard deviation of the results of triplicate samples (n = 3), analyzed under the same condition. Alternatively, the accuracy of the result was evaluated by recovery studies.

To perform a recovery study, a known concentration of target metals was added into Tobacco and soil samples and its response is measured (recovered).From known (1000mg/L) stock standard solutions of (Pb, Cd, Mn, Zn and Cu) of intermediate solution was prepared. From the intermediate solutions based upon the amount that make the concentration of the final solution2 mg/L of (Mn, Zn and Cu) and 0.2mg/L of (Pb and Cd) were added to 0.5 g of tobacco and soil samples. Then they were digested with the same digestion method and condition for tobacco and soil sample. After digestion the spiked tobacco and soil samples were diluted to the required volume with double -distilled water, and analyzed with the same method used for the analysis of the tobacco and soil samples. Triplicate samples were prepared and triplicate analyses were carried out. As shown in the Table 3

$$\% \text{ Recovery} = \frac{\text{conc. in spiked sample - conc. in unspiked sample}}{\text{Concentration added}} \times 100 \quad \text{----------} \quad (1)$$

3.4.7. Method of detection limit

Method detection limit is the smallest concentration of analyte that can be distinguished from statistical fluctuations in the blank, which usually corresponds to the standard deviation of the blank solution times a constant. In this study, the digested blank solutions containing HNO_3 and HCl and H_2O_2 analyzed by ICP-OES triplicate readings were taken for each blank and the standard deviation of the blank solution was calculated. The method of detection limit of each element was obtained by multiplying the standard deviation of the reagent blank by three and the mean concentration of the blank was added [58]. As shown in Table -1.

$$\textbf{LOD} = 3 \times \delta\text{-blank} + \text{mean of blank} \quad \text{-------------------------------} \quad (2)$$

Where: δ-is standard deviation of the blank readings

3. 4. 8. Limit of quantification (LOQ)

The lowest concentration level at which a measurement is quantitavely meaningful .The LOQ is most often defined as 10 times the signal/noise ratio plus the mean concentration of blank if the noise is approximated as the standard deviation of the blank. In this study, LOQ was obtained from triplicate analysis of five reagents blanks which were digested in the same digestion procedure for tobacco and soil samples .The LOQ was calculated by multiplying mean of standard deviation of the reagent blank and the values for the elements[58] was listed in table 1

$$LOQ = \text{mean of blank} + 10 \times \delta\text{- blank} \quad (3)$$

The table below shows instrumental detection limits, limit of detection and limit of quantification

Table 1: LOD and LOQ for determination of metals in tobacco and soil samples (mg/L)

Metals	Mn	Cu	Zn	Cd	Pb
IDM	0.0014	0.0097	0.0059	0.0027	0.042
LOD	0.07	0,068	0.125	0.005	0.05
LOQ	0.08	0.07	0.132	0.008	0.06

3.4.9. Determination of Metals in the tobacco and soil sample

For the determination of the concentration of metals in tobacco and soil sample series of standard metal solutions were prepared by diluting the stock solutions of the metal with double distilled water. Standard solution was run in ICP- OES and their points of calibration curve were established. Tobacco and soil sample solutions of each sample were aspirated into the ICP-OES instrument and the intensity reading of metals was recorded.

Triplicate samples were prepared and triplicate determinations were carried out on each tobacco and soil sample. The operating conditions of ICP- OES employed for each analyte are given in Table. 2

This table 2 indicates that the instrumental operation condition during the analysis of metals

Table 2: Instrumental Operating conditions for determination of metals using ICP-OES

No	Metal	Wavelength(nm)	Gas Flows(L/min)			RF power (Watt)	Pump flow rate (mL/min)
			Plasma	Auxiliary	Nebulizer		
1	Mn	257.61					
2	Cu	327.39					
3	Zn	206.20	8.00	0.20	0.70	1500	1.00
4	Cd	228.80					
5	Pb	220.35					

3.4.10. Calibration curve and Analytical Performance Characteristics

The quantitative determination of the metals using ICP-OES, external calibration curves were construct by using five working standard solution. Intermediate standard solutions of each metal containing 10 mg/l were prepared in 100 ml volumetric flask from the standard stock solutions that contained 1000 mg/L. The intermediate standards were diluted with double-distilled water to obtain five working standards solution of each metal interest for calibration purpose. The instrument was calibrated using five series of working standards.

Concentrations of working standards and value of correlation coefficient obtained from intensity verses concentration calibration curve for each metal and all selected metals have good linearity with a coefficients of ($R^2 = 0.999$).

4. RESULT AND DISCUSSION

4.1. Validation of the procedure

Since there was no certified reference material for heavy metal in our laboratory, in order to ascertain the accuracy of wet digestion procedure, recovery tests were performed using spiked samples with known concentration. Accordingly, known amounts from stock solution (1000mg/L) of each metal element were spiked on 0.5 g samples. The spiked and non-spiked samples were digested in parallel using the wet digestion method and concentration of each metal was determined in triplicate samples by taking three reading for each. The obtained recoveries were in the range of 82 - 126% with RSD (0.03 - 9.52%) and 78 – 128% with RSD (0.20 – 2.44%) for tobacco and soil samples, respectively. The results are given in Table 3. These values are within the accepted range. But recoveries of same metals in sample were unexepectingly, higher in both tobacco and soil samples, but the precision is good. In general, the proposed method demonstrated its good accuracy and precision [58].

Table 3: Recovery (%R) tests for the Tobacco and soil samples

Sample site	Recovery (%R)									
	Mn		Cu		Zn		Cd		Pb	
	Tobacco	Soil	Tobacco	soil	Tobacco	soil	Tobacco	soil	Tobacco	soil
Tsentseh	112	86	93	105	119.	112	101	128.	90	115
Belmili	102	81	125	118	118	110	116	119.	115	115
Ateto	103	83	118	118	82	98.	86	117.	120	115
Amba	118	87	108	89	91	119	125	106.	90	105
Basha	83	78	108	94	84	109	126	128.	105	100

4.2. Concentration of metals in the tobacco leaves

The table below shows that the mean concentration metals in tobacco leaves

Table 4: Average concentration (mean mg/L ± RSD, n = 3) of metals in Tobacco samples from the five sites (mg/L)

Sample site	Mn	Cu	Zn	Cd	Pb
Tsentsehalo	1.09 ± 1.19	0.11 ± 0.74	0.58 ± 1.14	0.01± 1.23	ND
Belmili	0.96 ± 0.52	0.15 ± 0.03	0.58 ± 0.28	NQ	NQ
Ateto	1.09 ± 0.72	0.20 ± 0.10	0.74 ± 0.74	NQ	NQ
Amba	0.75 ± 1.26	0.21 ± 1.26	1.16 ± 1.56	0.01 ± 0.61	0.07±0.95
Basha	1.18 ± 0.39	0.17 ± 0.03	1.05 ± 1.32	0.01 ± 9.52	NQ

NQ: not quantified, ND:not detected

Tobacco plant is known to easily absorb heavy metals from soil and accumulate in the leaves. In this study;the highest concentration of Mn was recorded in the raw tobacco leaves ranges from (0.75mg/L to 1.18mg/L)as shown in Table 4. The highest content of Mn was obtained in tobacco leaves from Basha area (1.18mg/L) and the lowest concentration of Mn was found in sample Ameba site (0.75mg/L) of Assosa woreda. Average daily requirement for Mn to be 2 – 3 milligrams per day for ages 1- 8 and 6 – 11 mg/day for ages 9 and above. The concentration of Mn was below WHO permissible daily intake.

The mean of the Cu contents in tobacco leave range from (0.11mg/L) to (0.21mg/L) with the maximum value of (0.21mg/L) from the sample location Ameba and the minimum value was below WHO/FAO recommended daily and provisional tolerable weekly intake limits in food and plant100 mg/kg and 500 mg/kg respectively. As shown in Table 4

The mean concentration of the Zn contents in tobacco leaves sample range from (0.58 mg/L) to (1.16mg/L).The maximum value of Zn was (1.16mg/L) obtained from sample site Amba and the minimum content of Zn is found in Tsentsehalo site (0.58mg/L) as shown in Table 4.The concentration of Zn was below permissible limit of WHO/FAO recommended value for daily and provisiononal tolerable weekly intake of 5mg/L and 25mg/L respectively.

The concentration of Pb was below the detection limit of the instrument in sample area Tsentsehalo and in sample site Ateto, Belmili and Basha, the content of Pb was below the quantification limit. The concentration of Cd in sample area Tsentsehalo, Ameba and Basha were very small in amount and in Belmili and Ateto was below the quantification limit. As shown in Table 4

In all tobacco samples, Mn was found in higher concentration than Cu, Zn and Cd intobacco sample but Pb was not detected in Tsentsehalo tobacco sample and not quantifiable in Belmili, Ateto and Basha. As shown in Table 4

In general the concentration of metals in all tobacco sample was: Mn > Zn> Cu> Cd in sample area Tsentsehalo Belmili, Ateto and Basha. But in sample area Ameba Zn > Mn > Cu > Cd > Pb. The. Result in this study indicated that the metal contents of tobacco varied with the geographical origin and chemical composition of the soil in which the tobacco plant grows. This variation may be due to the mineral content of the soil on which the tobacco plant grows. The metal content of tobacco plant depends on geographical origin, chemical composition of the soil and types of soil. [13]

4.3. Concentration of heavy Metals in the Soil sample

The table below Shows that the concentration metals obtained from the soil……………………………………….

Table 5: Average concentration (mean mg/L ± RSD, n = 3) of metals in soil samples from the five sites. (mg/L)

Sample site	Mn	Cu	Zn	Cd	Pb
Tsentseha	8.04± 0.28	0.35 ± 0.45	0.69± 1.14	ND	0.17±0.87
Belmili	1.59± 0.20	0.38± 1.76	0.45± 0.29	ND	0.18± 0.38
Ateto	7.28± 0.55	0.37± 0.41	0.29± 2.44	NQ	0.24± 1.03
Amba	6.38± 0.72	0.35± 0.30	0.43± 2.33	NQ	0.22± 1.72
Basha	5.98± 0.54	0.32± 1.05	1.26± 1.19	ND	0.31± 0.59

NQ-not quantified, ND- not detected

The mean concentration of Mn contents in soil range from (1.59mg/L) to (8.04mg/L) with the maximum value of (8.04mg/L) obtained from Tsentsehao and the minimum concentration was recorded(1.59mg/L)from sample location Belmili, (see Table.5).

The mean concentration of Cu contents in soils sample range from (0.32mg/L)to (0.38mg/L).There was no much difference on the values among of Cu concentration in each sample site is close to each other.as noted in(table 5)

The mean concentration of Zn in the digested soil sample range from (0.29 mg/L to 1.26 mg/L).The samples from Basha were resulted the highest concentration (1.26 mg/L) while the samples from Ateto were found to be the least (0.29 mg/L) among the soil samples.(see Table.5)

The Pb content of soil were range from (0.17mg/L to 0.31mg/L).The highest concentration of Pb were obtained in Basha sample area (0.31mg/L) while, the lowest concentration of Pb was found in Tsentsehalo soil sample (0.17 mg/L) (Table 5).

The concentration of Cd in the soil samples ofTsentsehalo, Belmili and Basha were below the detection limit of the employed method and the concentration of Cd in the sample areas of Ateto and Ameba were below limit of quantification.

In soil sample the concentration of metals was: Mn > Zn> Cu> Pb in sample area Tsentsehalo, Belmili Ameba and Bashabut in sample area Ateto Mn > Cu > Zn > Pb. The obtained result shows that there was metal content variation in soil sample it may be due to the difference in geographical location, the mineral composition of the soil, the type of soil and pH of the soil. [13]

4.4. Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) is the widely used statistical method to compeer group mean. The one- way ANOVA can compare the mean of more than two groups of sample. ANOVA uses F-statistical test to compare whether the difference between samples means are significant or not [60]. During the process of sample preparation and analysis of tobacco leaves and soil sample, random error may be introduce in each sample solution and in each replicate measurement. The variation in sample mean of the analyte was tasted using ANOVA. ANOVA showed that there was the statistical significant difference at 95 % ($P< 0.05$) confidence level in mean concentration of all metals of tobacco and soil sample. The variation it may be the mineral content of the soil or the experimental procedures.as show in Table 6

The table 6 and 7 below shows the Analysis of variance (ANOVA) of metals in tobacco and soil sample

Table 6: ANOVA between and within tobacco leave sample at 95% confidence level

Metals	source of variation	DF	F calculated	P-Value	F-critical
Mn	Between sample	4			
	Within sample	10	1293	0.00	3.48
	Total	14			
Cu	Between sample	4			
	Within sample	10	18,364	0.00	3.48
	Total	14			
Zn	Between sample	4			
	Within sample	10	1893.7	0.00	3.48
	Total	14			
Cd	Between sample	4			
	Within sample	10	11.638	0.00	3.48
	Total	14			
Pb	Between sample	4			
	Within sample	10	518.7	0.00	3.48
	Total	14			

Table 7: ANOVA between and within soil sample at 95% confidence level

Metal	Source of vibration	Degree of freedom	F-calculated	P-Value	F-critical
Mn	Between sample	4			
	Within sample	10	24858	0.00	3.48
	Total	14			
Cu	Between sample	4			
	Within sample	10	650	0.00	3.48
	Total	14			
Zn	Between sample	4			
	Within sample	10	367.50	0.00	3.48
	Total	14			
Cd	Between sample	4			
	Within sample	10	88.42	0.00	3.48
	Total	14			
Pb	Between sample	4			
	Within sample	10	2214.70	0.00	3.48
	Total	14			

4.5. Pearson correlation of metals in tobacco leaves and soil sample

The table below shows that the correlation between metals in soil and tobacco sample.

Table 8: The Pearson correlation coefficient metal in tobacco leaves and soil sample

Metals	Mn in soil	Cu in soil	Zn in soil	Pb in soil
Mn in leave	0.23	-0.33	0.57	0.40
Cu in leave	0.04	0.01	-0.30	0.49
Zn in leave	0.22	-0.64	0.32	0.67

To correlate the effect of metal concentration on tobacco leave with the concentration of metal in the soil sample, Pearson correlation matrices was used to correlate for the sample [60].The correlation coefficient is given in Table 8. The content of Mn in the soil has week positive correlation with Mn in tobacco leave this verifies that the dependence of Mn concentration in the tobacco leaves on the amount of metals under supporting soil of the plant was low. Cu in soil with Cu in tobacco leave have negligible relationship, Zn in soil with Zn in tobacco leaves have moderately positive relation. This shows that the concentration Zn in tobacco leaves is dependent on concentration of Zn metals in the soil.

4.6. Comparison of concentration of metals of Assosa woreda tobacco leaves with literature value

The table below shows that comparison of metals concentration in Assosa tobacco leaves with other Ethiopia tobacco leave sample**…………………………………………….**

Table 9. Comparison of metal concentration (mean ± SD) in Assosa raw tobacco leaves with other Ethiopian sample location (μg/g)

Metal	Assosa	Shewa robit	Billate
Cu	0.21±0.01	7.30 ± 0.19	4.38 ± 0.11
Zn	1.56±0.01	33.2 ± 1.90	53.7 ± 0.96
Cd	0.009±0.01	1.30 ± 0.04	1.20 ± 0.05
Pb	0.07±0.01	ND	ND

ND – not detected

The mean concentration of some metals in tobacco leaves of Assosa woreda compare with the mean concentration of tobacco leaves of other tobacco farmland of shewa Robite and Bilate which produce high amount of tobacco in Ethiopia. The concentration of all metals obtained from Assosa woreda less than the metals concentration of shewa Robite and Billate but Pb was not detected in both tobacco farmland of shewa Robite and Billate.

5. CONCLUSION AND RECOMANDATION

5.1. Conclusion

In this study the concentration of toxic heavy metals Cd and Pb essential heavy metals Cu,Mn, and Zn were determined in leaves of tobacco and its farmland soil of five kebeles of Assosa Woreda of Benishangul-Gumuz region of Ethiopia.. In contrast to compare the concentration of those heavy metals in tobacco and soil sample Mn have higher concentration in all sample location and in both tobacco and soil sample, However the concentration of Cd and Pb were very small in amount. In some sample location the contents of Cd in the soil sample and Pb concentration in tobacco leave on sample site Tsentsehalo was below the detection limits of the instrument and the concentration of Cd on sample area Belmili and Ateto was not quantified in tobacco leaves and on sample area Belmili, Ateto and Basha the concentration of Pb in tobacco samples were detected by the instrument but not quantified. The results showed that the concentration of heavy metal in the tobacco and soil samples were below the permissible limits of WHO/FAO .The significant difference between the soil and tobacco sample of each element are significantly different and the mean concentration of each metal in tobacco leave and soil sample was significantly different at the level of 95%

5.2. Recommendation

In light of the findings and the conclusion drawn above, the following recommendations are forwarded.

- It might be repeated with GFAAS or ICP-MS to draw strong conclusion about the metals content of tobacco in Assosa woreda of Benishangul-Gumuz region of Ethiopia.
- Where tobacco is grown for use as insecticide, or medicinal plant, restriction should be placed on the type of fertilizer used to avoid environmental pollution with heavy metals.
- The community should support government by stop tobacco consumption and Non-smokers are advised to keep the good habit and avoid passive smoking by staying away from environment polluted with cigarette smokers.

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APENDEX: -Calibration curve of the five metals

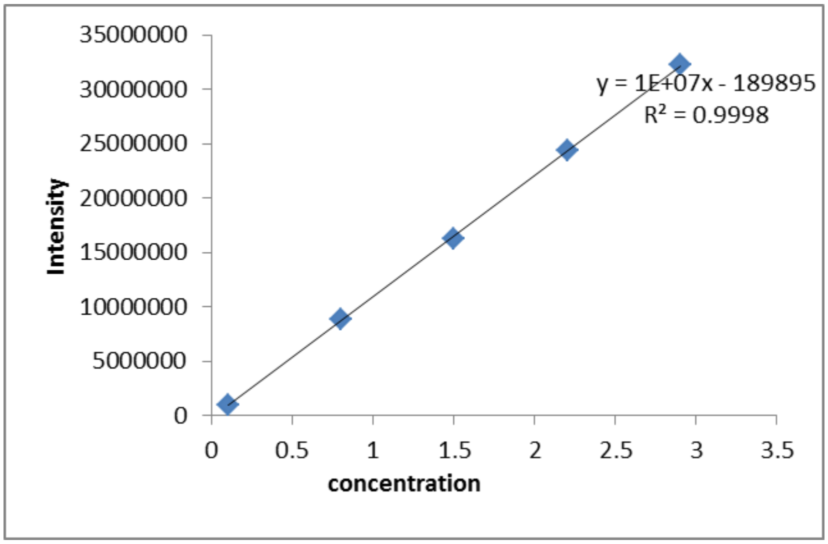

Figure 1: Calibration curve of Mn

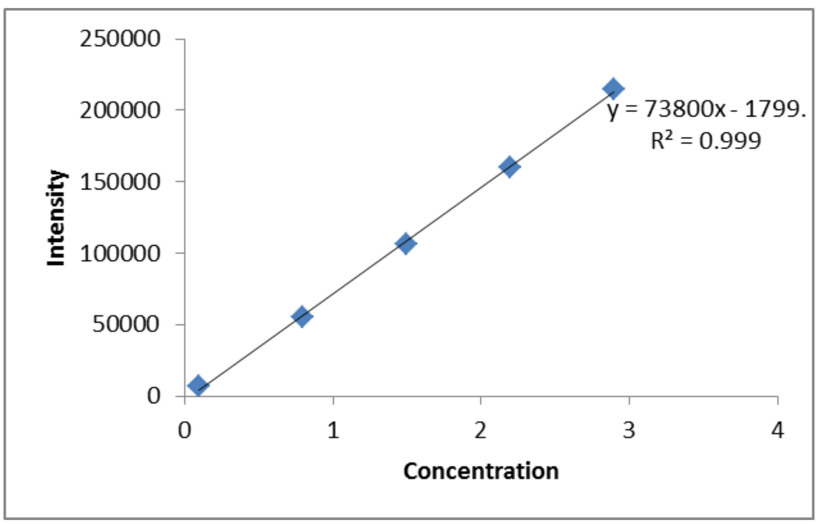

Figure 2: Calibration curve of Pb

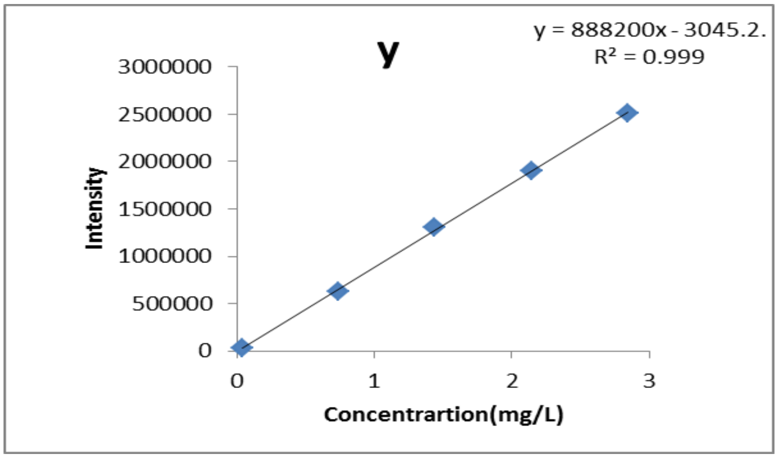

Figure 3: Calibration curve of Cd

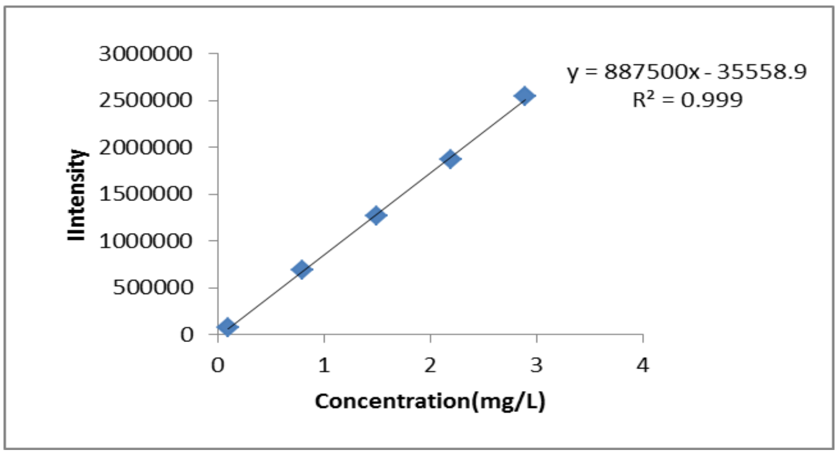

Figure 4: Calibration curve of Cu

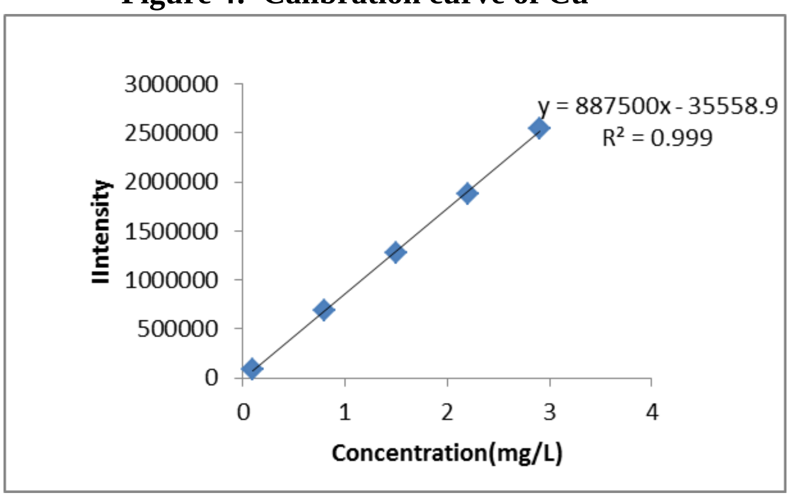

Figure 5: Calibration curve of Zn