ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTIONAL SCIENCES

DEPARTMENTOF CHEMISTRY

Determination of mercury in the soils from Adolla (Shakiso) of gold mining area, Ethiopia

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February, 2020

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A thesis submitted to Department of Chemistry, Addis Ababa University in partial fulfillment of the requirements for Master of Science in Chemistry.

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February, 2020

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DECLARATION

As thesis advisor, I hereby certify that I have read this thesis prepared under my guidance and recommended that it can be accepted as fulfilling the thesis requirement.

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ACKNOWLEDGMENTS

First of all, I would like to thank God who kept and protected me to live according to his plan and will for successful accomplishment of my Master of Science in Chemistry. Next, I would like to thank my advisor Dr.Negash Getachew for giving his constructive ideas, unreserved checking and commenting throughout my activities. And also, I would like to thank Addis Ababa University, College of Computational and Natural Sciences especially the Department of Chemistry for giving me this opportunity. Lastly, special thank goes to Minister of Education which sponsored me to get this chance to learn my Master of Science in Chemistry by financially supporting.

Table of contents

Pages **Titles**

Acknowledgments…….....iii

Table of contents……iv

List of tables……..……..……..vii

List of figures…….……..……viii

Acronyms and abbreviations……..ix

Abstract……x

1. INTRODUCTION.....1

1.1. Background of the study……..…….....1

1.2. Objective of the study……...2

1.2.1. General objective…….……..……2

1.2.2. Specific objectives…….…….....2

1.3. Significances of the study……2

2. REVIEW OF RELATED LITERATURE..……3

2.1. Physical properties of mercury…….....3

2.2. Chemical properties of mercury……..….....4

2.3. Toxic nature of mercury in the environment…….....5

2.4. Health effects of mercury in the environment …….…….....6

2.5. Importance of mercury in the environment…….....7

2.6. Occurrence and extraction of mercury…….....7

2.6.1. Occurrence of mercury in the soil…….…….....7

2.6.2. Extraction of mercury…….8

2.7. How mercury contaminates the environment in gold mining areas……..9

2.8. Sources of mercury in the environment……..10

2.8.1. Natural sources……..11

2.8.2. Anthropogenic sources……..11

2.9. Microwave plasma- atomic emission spectroscopy (MP-AES)…….12

2.9.1. The benefits of microwave plasma-atomic emission spectroscopy (MP-AES)…….13

3. EXPERIMENTAL……..14

3.1. Description of the study area……14

3.2. Sample collection…….14

3.3. Working procedure…….14

3.3.1. Cleaning the apparatus…….14

3.3.2. Moisture content determination of soil samples……15

3.3.3. Sample preparation…….15

3.4. Chemicals, reagents and equipment…….16

3.4.1. Chemicals and reagents……..16

3.4.2. Equipment and apparatus…….16

3.5. Wet acid digestion……..16

3.5.1. Optimization of the soil samples……..16

3.5.2. Digestion of the soil samples……18

3.6. Method validation and measuring performance…….18

3.6.1. Accuracy and precision…….18

3.6.2. Instrumental detection limit……19

3.6.3. Limit of detection (LOD)…….19

3.6.4. Limit of quantification (LOQ)……..…….....19

3.6.5. Method validation…….……..…….....20

3.6.6. Calibration of instrumental …….…….…….....20

3.6.7. Determination of concentration of mercury…….…….....21

4. RESULTS AND DISCUSSION….……....…….....22

4.1. Regression analysis and limit of detection…….……..……22

4.2. Accuracy and precision……..…….....22

4.3. Concentration of mercury in the soil samples……..……...23

4.4.Comparison of concentration of mercury in this study with the related in the literature….……...23

4.5. Conclusions ……..……..……..…….23

5. REFERENCES……..25

LIST OF TABLES

Table 1: Moisture content determination of soil samples……...…...15

Table 2: Optimization of volume …….....17

Table 3: Optimization of temperature …….....17

Table 4: Optimization of time …….....17

Table 5: Concentration of working standard solutions…….....20

Table 6: Linear regression equation, instrumental detection limit, coefficient of determination, limit of detection and limit of quantification…….....22

Table 7: Recovery and spiking test result of mercury in the soil samples…….....22

Table 8: The mean and standard deviation of mercury concentration in the soil samples…….....23

LIST OF FIGURES

Fig.1: Elemental mercury……..…….....4

Fig.2: Chemical properties of mercury……5

Fig.3: Health effects of mercury in the environment……...6

Fig.4: Occurrence of mercury in the soil ……....8

Fig.5: How mercury contaminates the environment in gold mining areas……...……10

Fig.6: Schematic diagram of a microwave plasma atomic emission spectrometer…….....13

Fig.7: The prepared soil samples…….....15

Fig.8: Calibration curve for mercury…….21

Acronyms and Abbreviations

AAS	Atomic Absorption Spectroscopy
Hg (0)	Elemental mercury
Hg(I)	Mercurous
Hg(II)	Mercuric
MeHg	Methyl mercury
mg/kg	Milligram per kilogram
MP-AES	Microwave plasma- atomic emission spectroscopy
µg/kg	Microgram per kilogram
WHO	World Health Organization
FAO	Food Agricultural Organization
SD	Standard deviation
ASM	Artisanal small mining
LOD	Limit of detection
LOQ	Limit of quantification
IDL	Instrumental detection limit

ABSTRACT

Mercury and its compounds are cumulative toxins and hazardous to human and animal health in the ecosystem. The toxicity of mercury in the soil depends on its oxidation states. So, the major effects of mercury poisoning manifest as neurological and renal disturbances as it can easily pass to the blood and damage the functions of brain, liver and kidney. Some of the common sources of mercury found in the environment are natural and anthropogenic sources. The concentration of mercury in soil samples used in the Adolla gold mining of Shakiso in Guji zone, Ethiopia was determined using Microwave plasma - atomic emission spectroscopy instrument. The result obtained revealed that the concentration of mercury is 0.111 mg/kg in the soil. So, the level of contamination of soils by mercury is high at present. As a result, the soil is polluted and toxic due to mercury.

1. INTRODUCTION

1.1. Background of the study

Soil is unconsolidated materials found on the earth's surface which is made of organic and inorganic materials typically consists of minerals, organic matter, living organisms, water and air. Also, it is upper layer of the Earth's crust transformed by weathering of physical, chemical and biological processes. It has a vital function to serve as a natural medium for plant growths, control of substances and energy cycles within ecosystems**,** means of storage, agriculture and forestry, documents of natural history, archaeological documents, basis for the stability of buildings and road, supply and purification& other developmental activities [1].

Environmental contamination by heavy metals has become a world-wide problem in the recent years due to the fact that heavy metals unlike some other pollutants are not biodegradable. So, soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, spillage of petrochemical, chloro-alkali production, dentistry, gold mining sites, and agricultural sources such as fertilizers, pesticides and fungicide in paints, disposal of heavy metal wastes, leaded gasoline, and coal combustion residues. Heavy metals have density higher than $5g/cm^3$.Heavy metal constitutes inorganic hazardous chemicals and those most commonly found at contaminated sites are lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel [2].

Mercury is a naturally occurring substance which found in the several form in the environment. It has the lowest melting point ($-39\ ^0C$) of all pure metals and it is the only pure liquid metal at room temperature. Mercury and its compounds are cumulative toxins and hazardous substances to human and animal health in the ecosystem.

The extents of toxicity depend strongly on the type of compound and the oxidation states of mercury. The major effects of mercury poisoning manifest as cause of cancer, attacks of liver and kidney, neurological and renal disturbances as it can easily pass the blood and it has effects on brain or loss of memory. Today, due to its unique physico-chemical properties, it is widely used in industry, mining, medicine, agriculture, dentistry, as catalyst and other everyday life [3].

1.2. Objective of the study

1.2.1. General objective

To assess mercury in the soil of Adolla gold mining area of Shakiso in the southern part of Oromia regional state

1.2.2. Specific objectives

1. To collect the soil samples from Adolla gold mining site.
2. To develop a procedure for digestion of the soil samples.
3. To analyse the presence of mercury in the soils of Adolla gold mining area of Shakiso.
4. To determine the concentration of mercury in the soils of Adolla gold mining area of Shakiso.

1.3. Significance of the study

The major significance of this study is to provide information about the presence of mercury in soils of Adolla (Shakiso) gold mining area of Ethiopia. It gives a clue for further studies for toxicity and effect of mercury in gold mining areas in different parts of the country. As well as it also will give information for the concerned institutions and organizations to take some necessary actions for the wellbeing of the society.

CHAPTER TWO

2. REVIEW OF RELATED LITERATURE

2.1. Physical properties of mercury

Mercury is an element which has been known for at least 4000 years. It is a heavy, shiny, toxic, silvery-white metal with an atomic number 80, an atomic weight of 200.6 g/mol, and the only liquid metal at room temperature. It is a silvery white metal with a mirror like surface when it is liquid. It is a volatile metal at room temperature and has a vapor pressure of 0.002 mm Hg at 25 ^{0}C. As a vapor, it is odorless, colorless metal. It is insoluble in water but soluble in lipid, nitric acid, and sulfuric acid upon boiling. It is a naturally occurring transitional element in which chemically symbolized as Hg. This abbreviation comes from the Latin word hydrargyrum means water or synonyms with quick silver [4].

As compared to other metals, it has low melting points (-39 ^{0}C) and boiling points (357 ^{0}C) and also it is poor conductor of heat but a fair conductor of electricity. It is a metallic element present in small quantities in the Earth's crust. It is the most toxic, hazardous substance to the environment. It occurs as elemental, inorganic or organic forms in various oxidation states. When it has a zero oxidation, Hg (0) means exists as vapor or as liquid state whereas Hg (I)and Hg(II) may occur either as organic or inorganic compound forms such as methyl mercury (CH_3Hg) and cinnabar (HgS) or calomel($HgCl_2$) respectively [5].

Mercury and its compounds are dispersed throughout rocks, soils, airs, water, and living organisms by a complex system of physical, chemical, and biological process. So, mercury in the soils can be converted from inorganic compound to organic compound such as methyl mercury (CH_3Hg) or (MeHg) by methylation of aerobic and anaerobic bacteria. Methyl mercury is the most frequently encountered compound of the organic form found in the environment, and formed as a result of the methylation of inorganic forms of mercury by microorganisms found in soils. The toxicity of mercury in the soil depends on its oxidation states and types of compounds it form [6]. It is released into the environment in both natural and anthropogenic process. It can be obtained as a by-product from the mining and refining of heavy metals such as copper, gold, lead, and zinc extractions. It can also be recovered by recycling operations and sometimes removed from natural gases or fossil fuels.

(a) (b)

Fig.1: Elemental mercury (a).Liquid mercury (b).Silvery white mercury

It readily forms amalgam or an alloy with most metal and a process called amalgamation. This tendency of mercury to form amalgam makes it especially dangerous to be handled by people wearing gold jewelry as it can diffuse into the gold [7].

2.2. Chemical properties of mercury

Mercury reacts with different inorganic and organic compounds or elements to form simple and complex molecules like chlorides, sulfide, carbonates and phosphates. It also combines with carbon to form organic compounds such as methyl mercury or dimethyl mercury. Mercury may occur in three different oxidation states in nature as Hg(0), Hg(I), and Hg(II), of which Hg(0) and Hg(II) are the states normally encountered in soils. Among, the oxidation states of mercury, Hg (I) ion is not stable under normal conditions since it converts into Hg(0) and Hg(II) state. Hg(II) also forms strong complexes with humus matter where bonding to reduced organic sulphur groups plays a major role. Mercury (II) occurs in the soils as major fraction being either bound in soil minerals or adsorbed to inorganic or organic solid surfaces [8].

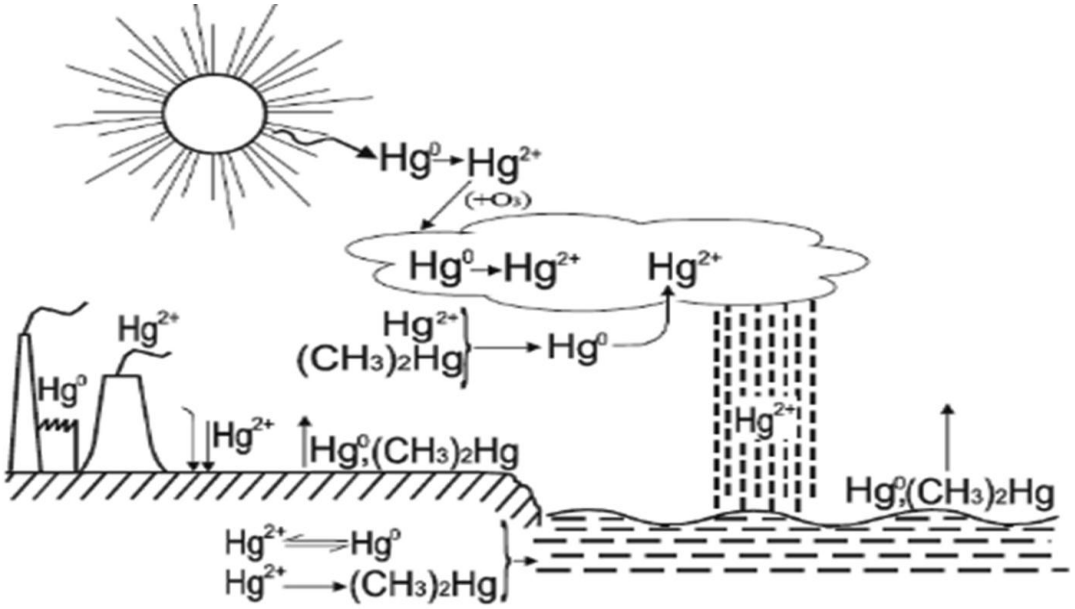

Fig.2: Chemical properties of mercury

2.3. Toxic nature of mercury in the environment

Mercury is the most toxic, heavy metal to human being found naturally in the contaminated environments. It is a widely spread environmental toxic element which induces severe alterations in the body tissues and causes a wide range of adverse health effects to both humans and animals. A variety of mercury species exist in the environment and its various chemical forms can differ in their bioavailability, persistence and toxicity [9]. All chemical forms of mercury species are toxic to human beings especially methyl mercury being the most toxic species. Any form of mercury in the environment may evolve into a more toxic species (methyl mercury) under biogeochemical transformation processes. The impact of mercury depends strongly on its chemical species; understanding mercury transformations and the impact of its various chemical forms are vital to preventing harmful effects on humans and the environment. So, mercury compounds tend to be much more toxic than the elemental mercury itself and they may be responsible in causing in brain and kidney damage [10].

2.4. Health effects of mercury in the environment

One of the first scientifically documented incident effects of mercury occurred in Minamata, Japan in 1950s and related to the accumulation of methyl mercury in fish and the subsequent poisoning of the local inhabitants [11]. It is a persistent environmental toxic with bioaccumulation ability in fish, animals, and humans. So, methyl mercury is the dominant toxic species in the environment which causes the main hazard to humans such as carcinogenic effects and also affects brain development resulting in a lower IQ, and consequently a lower learning potential.

Even though, elemental mercury is used in a variety of household products, such as barometers, thermometers and fluorescent light bulbs. So, using these devices in house hold or laboratory or industry, does not cause any health problems [12]. However, when a thermometer will break a significantly high exposure to mercury through breathing problems will occur for a short period of time while it vaporizes. This can cause harmful effects such as nerve, damage brain and kidney functions, lung or eye irritation, skin rashes or allergic, vomiting and diarrhea. Damaged brain functions can cause degradation of learning abilities, personality changes, tremors, vision changes, lack in muscle coordination and memory loss [13].

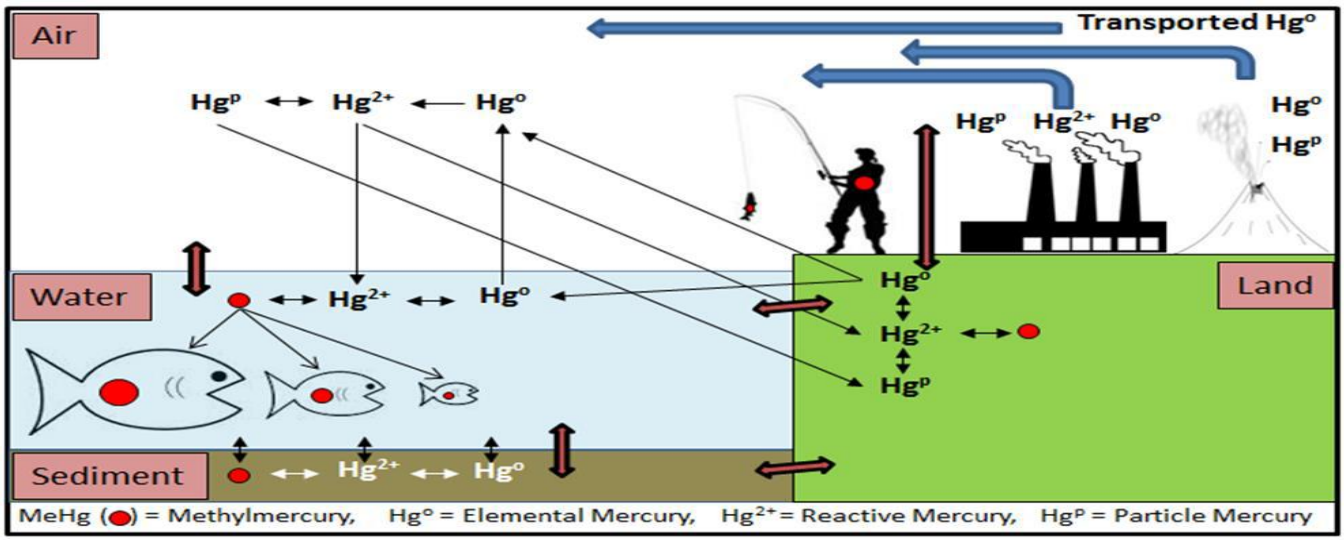

Fig.3: Health effects of mercury in the environment

2.5. Importance of mercury in the environment

Mercury has no well known biological function in any living organism in the environment but it has many applications in the process of gold mining, medicine, agriculture, industrial catalysis, in dental amalgams to fill cavities, in industry such in chloro-alkali production, the manufacturing of paints, soaps, plastics and as catalysts in academic laboratory or hospitals, as well as measuring and medical devices such as thermometers, blood pressure devices and other everyday life. Because of its high density it is mainly used in manufacturing of barometers and manometers devices. It is extensively used in thermometer production because it has high rate of thermal expansion that is fairly constant over a wide temperature range. Industry such as in chloro-alkali production, mercury is used as a liquid electrode in the manufacture of chlorine and sodium hydroxide for electrolysis of brine [14].

Mercury is applied as fungicides, pesticides, disinfectants, antimicrobials, insecticide and in rat poison in agriculture, due to its toxin especially calomel while ethyl mercury is used as part of some fungicides and bactericides. Mercury compounds are also sometimes used as catalysts or feed stocks in chemical manufacturing other industrial processes. Mercury is utilized in the electrical industry as switches, thermostats, batteries, and numerous industrial processes including the production of caustic soda, in nuclear reactors, as antifungal agent for wood processing, as a solvent for reactive and precious metal, and as a preservative of pharmaceutical or in measuring equipment such as thermometers, medical equipments. Though, mercury is used in many of the above items being produced now is restricted or banned to use it, due to its toxic [15].

2.6. Occurrence and Extraction of mercury

2.6.1. Occurrence of mercury in the soil

Mercury is a rare element in the earth's crust & it has an average crustal abundance on earth crust of approximately 0.05-0.10 mg/kg**,** the majority of which occurred as the mineral cinnabar. Mercury is occurred naturally in the soil as the pure elemental form (e.g. liquid mercury), as inorganic mercury in ores such as cinnabar (HgS, mercuric sulfide) and calomel (HgCl, mercurous chloride), and mercuric chloride ($HgCl_2$). Additionally, it is also occurred as organic form such as the organo-metallic cations such as methyl mercury, $[CH_3Hg]^+$ and ethyl mercury, $[C_2H_5Hg]^+$. Mercury enters to the environment as a result of normal breakdown of minerals in rocks and soils through exposure to wind and water [16].

(a) (b)

Fig.4: Occurrence of mercury in the soil (a). Red mercury sulfide (b).Liquid mercury

2.6.2. Extraction of mercury

Soils may contain various species of mercury such as organic, in organic and elemental mercury, as result of deposition and/or microbial and chemical activity. More than 98% of mercury in soil is inorganic, originating from geological sources and from atmospheric deposition. Mercury in soil is subjected to a variety of chemical and biological reactions, determining concentrations and composition of species and their complexes [17]. These reactions depend on soil conditions such as redox potential, dissolved and solid phase organic substances, soil pH, mineral content and composition, temperature and moisture content. So, mercury is mainly extracted from inorganic compounds especially from the ore of cinnabar with the chemical formula of HgS. Traditionally, the ore was heated in a wood fire and the mercury was collected from the ash. However, modern methods, the cinnabar is crushed and separated, and then it is heated with a stream of air. The reaction is:

$$HgS\,(s) + O_2\,(g) \xrightarrow{600^0C} Hg\,(l) + SO_2\,(g)$$

If the ore is especially rich, scrap iron or quicklime can be added to promote the extraction process:

$$HgS\,(s) + Fe(s) \longrightarrow Hg\,(l) + FeS\,(s) \text{ or}$$

$$4HgS(s) + CaO(s) \longrightarrow 4Hg\ (l) + 3CaS(s) + CaSO_4(s)$$

Crude mercury can also be washed in nitric acid to remove oxides and other impurities. More purification can be achieved if the process is performed at lowered pressures [18].

2.7. How mercury contaminates the environment in gold mining areas

Gold miners use mercury to extract gold from ore adding liquid mercury to the milled gold containing ore. This results in a mercury gold compound, called amalgams. Miners smelt this amalgam to obtain gold, vaporizing it and finally inhaling the toxic mercury fumes. When gold miners use mercury to amalgamate gold, they should be aware that they can easily contaminate themselves, their families, and their neighbors, as well as people living far away from their worksites [19]. The ways of the contamination in the environment takes place depends on how the miners do their work. For example, if the whole ore is amalgamated and discharged without adequate settling ponds, or if mercury contaminated materials are dumped directly from a raft into the river, mercury will be carried downstream and dispersed over a very wide area where it can transform into methyl mercury in the sediments at the bottom of the rivers, lakes or streams [20].

Methyl mercury is easily absorbed by worms, snails and insects and becomes highly concentrated in fish, especially the piscivorous species (fish that eat other fish). Eating fish contaminated by mercury from ASM activities can pose a great health risk to people living downstream of mining areas. Likewise, mercury vapor emitted from open pan amalgam burning is dispersed in the air. Most of the vapor settles onto the ground and can contaminate soils up to two kilometers downwind from burning. Some vapor however travels long distances and comes down with rain. If the miners amalgamate concentrates in pools, mercury tends to be concentrated within a relatively small area, forming a local “hotspot” i.e. a site with high concentration of mercury contaminated materials [21].

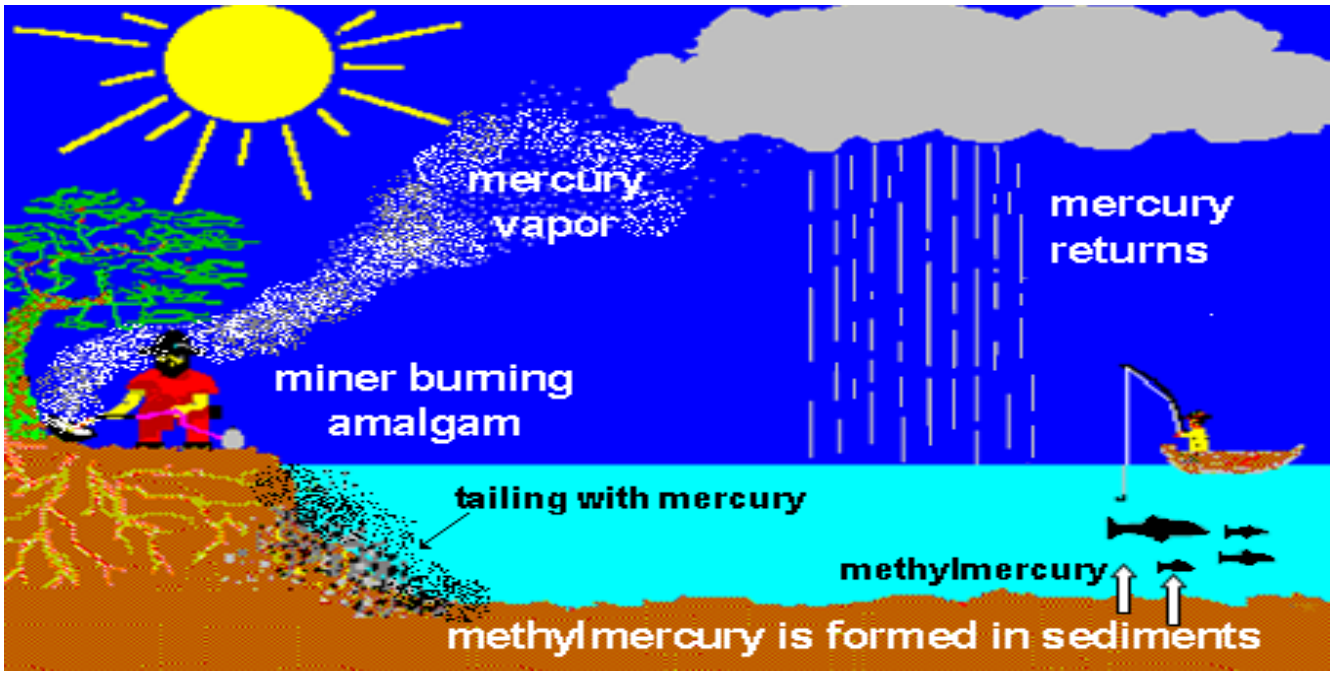

Fig.5: Show how mercury contaminates the environment in gold mining areas

2.8. Sources of mercury in the environment

Mercury occurred naturally & widely distributed in the environment through natural process as well as anthropogenic process. The large amounts of inorganic mercury have accumulated in the environment, especially in soils as a result of these past emissions & releases from human activities. Cement production, mining and smelting, artisanal and small-scale gold mining, volcanic eruptions, geothermal evaporations, burning coal and oil refining are some of the activities emitting mercury which can build up in soils [22]. Consumer products such as electronic devices, switches, batteries, energy-efficient light bulbs and certain cosmetics, dentistry, plastic production, and the chloro-alkali industry are also contributors to mercury emissions. After it is deposited in soils and sediments, bacteria and microbes are mainly responsible for changing mercury to methyl mercury. The most common sources of mercury to all soils are the minerals constituting the rocks forming the soil parent materials. Both natural and anthropogenic sources are the main contributor to mercury in the environment [23].

2.8.1. Natural sources

The natural sources of mercury are estimated to be about one third of the total releases where as two thirds are anthropogenic sources. The major natural sources of mercury in the environment are degassing from the Earth's crust, emissions from soil and rocks through weathering, geothermal evaporations from lakes and oceans or during outburst from volcanic eruptions, forest fires, or degrading of minerals and by degasification from land and water surfaces [24]. As previously stated, some mercury occur naturally in the soils and also in minor amounts in the oceans and in water or land but some of the mercury released by the processes stated above is actually a remobilization of historically deposited anthropogenic mercury. Therefore, it is difficult to estimate natural versus anthropogenic release in the environment correctly.

2.8.2. Anthropogenic sources

Apart from natural contribution of mercury in soil there is a significant anthropogenic contribution, mainly from industrial and agricultural activities. The major drivers of anthropogenic sources of mercury are mining activities, metallurgical and chemical industries, waste disposal, and electronics, shooting and military operations. Most of the mercury currently in the global environment entered as a result of human activity such as combustion of fossil fuel, roasting and smelting of ores (mainly cinnabar (HgS)) or refining of mercury-containing ores, industrial production of caustic soda and chlorine, production of cement and incinerating waste, and deforestation leading to soil erosion and lixiviation (the process of soluble substances in the soil being dissolved in water), can also emit mercury into the environment [25].

Examples of industrial processes include chemical manufacturing that use mercury compounds as catalysts, especially in vinyl chloride monomer production and chloro-alkali production that use pools of elemental mercury as a cathode in electrolysis. Anthropogenic sources of mercury arise when an intentional decision is made to create a product that contains mercury or to operate a process that uses mercury. Examples of products that contain mercury or a mercury compound include fluorescent lamps, thermometers, batteries, switches, and other similar products. Mercury is also present as a result of mining for mercury, gold (where mercury is used to form an amalgam before being burnt off), and other

metals such as copper, zinc and silver from refining [26]. Therefore, artisanal mining is an environmental problem because mercury amalgamation is still largely preferred for gold extraction. A non-industrial process that uses mercury is small-scale gold mining, in which elemental mercury is used to capture gold from mixtures of crushed rocks, sediments soils, or other particles. Also, agricultural activities may contribute to the overall heavy metal loading to the environment through the spreading of pesticides, fungicides, insecticide and wood preservatives, lime and manures. Some compounds of mercury are also used in agriculture as fungicides and seed disinfectants [27].

2.9. Microwave plasma-atomic emission spectroscopy (MP-AES)

Microwave plasma atomic emission spectroscopy consists of microwave induced plasma interfaced to an atomic emission spectrophotometer (AES). It is used for simultaneous multi analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gases in remote locations or foreign countries. The atomized sample passes through the plasma and electrons are promoted to the excited state.

The light emitted electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent (wt. %). MP-AES is a technique comparable to traditional AAS and AES but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gases [28].

Fig.6: Schematic diagram of a microwave plasma atomic emission spectrometer

2.9.1. The benefits of microwave plasma-atomic emission spectroscopy

Some of the following are the main advantages of microwave plasma-atomic emission spectroscopy. First and for most, it removes the requiring for flammable gases, it runs on air and reduces the cost need of flammable gases. Also, it has high sensitivity with detection limit and better performance than AAS and it is fast, easy to handle and use [28].

CHAPTER THREE

3. EXPERIMENTAL

3.1. Description of the study area

This study was conducted in southern part of gold mining area of Adolla in Shakiso, Ethiopia. Shakiso is a town in the southern part of Oromia regional state found in the Guji zone located 500 Km South of Addis Ababa. This town has a latitude and longitude of 5°45′N, 38°55′E and an elevation of 1758 meters above sea level respectively. Based on the Central Statistical Agency in 2005, Shakiso has an estimated total population of 28,260, of whom 15,463 are men and 12,797 are women.

3.2. Sample collection

The samples were collected in the month of September, 2019 from the sample location using clean stainless steel materials. The soil samples were collected at depth of 50 cm from the sample location in Adolla gold mining in Shakiso. The samples were transferred into clean and labeled plastic containers for sample preparation, digestion, and spiking analysis in the laboratory. The samples were taken from the sampling sites labeled as site 1, site2, and site 3 and triplicate samples were prepared from each sample.

3.3. Working procedure

3.3.1. Cleaning the apparatus

To avoid contamination in the analysis of mercury in the soils, the apparatus such as glass wares, volumetric flasks(50 ml), micro pipette, measuring cylinders,250 ml round bottom flasks (24/29), beakers, funnels, plastic containers and all other material needed were first washed with detergent and rinsed with distilled water followed by soaking in diluted nitric acid for 24 h and cleaned in de-ionized water after soaking in acid, and allowed to dry at room temperature and stored in clean dry place to free from contamination.

3.3.2. Moisture content determination of soil samples

The soil samples were dried at a temperature of 120 ^{0}C for 6hand dried to constant weight. The samples were removed and cooled in desiccators and weighed again. The weights lost were obtained by subtracting the weight of dry sample from original weight of the sample using the following equation:

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial weight of sample}} \times 100$$

Table 1: Moisture content determination of soil samples

Sources	Moisture content (g)	Dried soil (g)	Loss in weight (g)	Moisture content (%)
Adolla Shakiso	800	480	320	40

3.3.3. Sample preparation

The soil samples were first dried in air to evaporate moisture and placed in an electric oven at a temperature of 120 ^{0}C for 6 h. Then, the soil samples were ground or crushed in a mortar and pestle until homogenized and fine powdered were sieved through mesh sieve to obtain the fine soil powder fraction. The resulting fine powder was kept at room temperature until required for the purposes of digestion, optimization and spiking of soil samples.

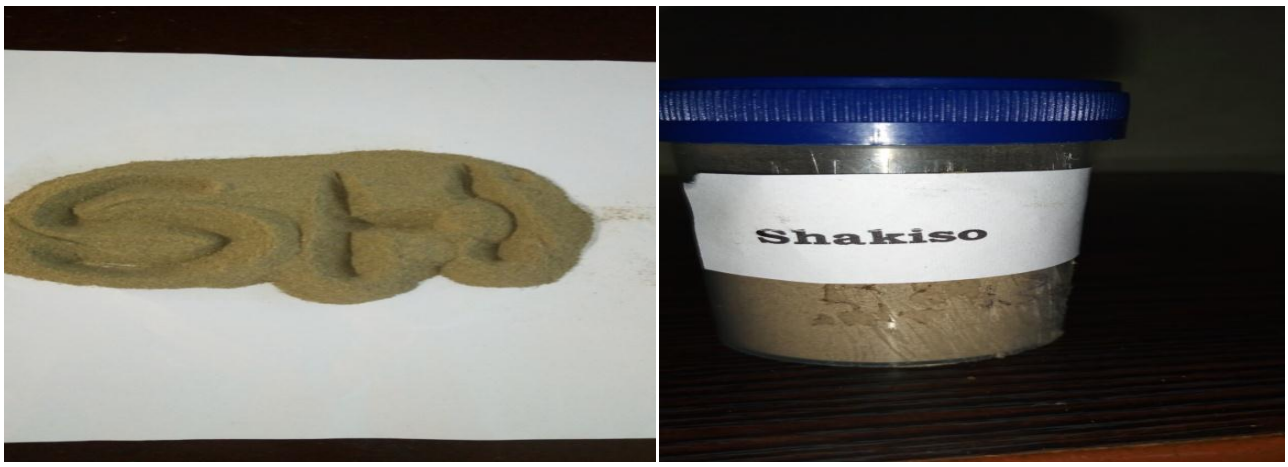

Fig.7: The prepared soil samples

3.4. Chemicals, reagents and equipment

3.4.1. Chemicals and reagents

Reagents that were used for analysis of mercury were all analytical grade. Nitric acid [HNO_3 (69-72%)] and hydrochloric acid [HCl (37%)] mixtures were used to prepare aqua-regia for digestion, optimization and spiking of soil samples. The standard stock solutions in aqua-regia were used to prepare working standard solutions for the determination of mercury in the spiked and non spiked soil samples.

3.4.2. Equipment and apparatus

The instruments used for this study was MP-AES (Agilent, model 4200) for determination of mercury in the soil samples. An electrical oven was used to dry the soil samples at a temperature of 120 ^{0}C for 6 h. Analytical balance was used for weighing the homogenized soil samples. 250 ml round bottom flasks fitted with reflux condenser were used on Kjeldahl apparatus to digest the homogenized soil samples.

3.5. Wet acid digestion

In order to analyze metal present in the soil samples efficiently, different procedures for soil sample digestions were carried out. Wet acid digestion is one of the methods that are involved to determine free metal ions dissolved from complex organic matrix based on changing different digestion parameters like digestion temperature, volume and time. One of the wet acid digestion methods can be carried out on kjeldahl apparatus which is used to digest the prepared soil samples. Hence, the optimization procedures for the soil samples were made in the soil for determination of mercury. Finally, the one that consumed smaller reagent volume with smaller digestion time and produced clear solutions with no residue and suspended materials was selected for the routine digestion of the soil samples.

3.5.1. Optimization of the soil samples

It is important to develop an optimum working procedure to obtain a clear and color less digested soil sample solutions. The digestion was carried out using mixtures of aqua-regia (69-72% $HNO_3$and 37% HCl in 1:3 ratio respectively) by varying the digestion of volume, temperature and time turn by turn as shown in table 2, 3 and 4 until clear, colorless and no suspension solution were obtained. After optimizations, the sample solutions were allowed to cool at room temperature and diluted with distilled water. Then, the optimum working conditions were selected based on clarity of the digested solution and applied for complete digestion of soil samples.

Table 2: Optimization of volume of 0.5 g of soil samples at 300 ^{0}C for 3 h with aqua-regia reagent

Trial No	Volume (ml)	Temperature (^{0}C)	Time (h)	Observation
1.	4	300	2:00	Deep yellowish color
2.	5	300	2:30	Light yellowish color
3.	**6**	**300**	**3:00**	**Clear and colorless solution**
4.	7	300	3:00	Clear and colorless solution

- The bold font shows the optimized digestion volume, V = 6 ml

Table 3: Optimization of temperature of 0.5 g of soil samples for 3 h with aqua-regia reagent

Trial No	Temperature (^{0}C)	Time (h)	Observation
1.	150	3:00	Lightly yellow color
2.	**180**	**3:00**	**Clear and colorless solution**
3.	210	3:00	Clear and colorless solution

- The bold font indicates optimum digestion temperature, T = 180 ^{0}C

Table 4: Optimization of time of 0.5 g of soil samples at 180 ^{0}C with aqua-regia reagent

Trial No	Temperature (^{0}C)	Time (h)	Observation
1.	300	2:00	Deep yellowish color
2.	300	2:30	Light yellowish color
3.	**300**	**3:00**	**Clear and colorless solution**
4.	300	3:30	Clear and colorless solution

- The bold font shows the optimized digestion of time, t = 3 h

Therefore, in the optimization process with a total of 6ml reagents volume, 180 ^{0}C of temperature and 3 h of time were selected for the soil sample digestion.

5.5.2. Digestion of the soil samples

0.5 g of dried and homogenized soil samples were weighed and placed in to 250 ml digestion flask in triplicate. After that, 6 ml of freshly prepared aqua-regia mixtures (1:3 ratio of HNO_3 to HCl) were added. The mixtures were digested on Kjeldahl apparatus at 180 ^{0}C for 3 h and kept to cool at room temperature.

After, digestion was completed; clear and colorless solutions were filtered out into 50 ml volumetric flask by washing the residues with distilled water. Finally, the filtered sample solutions were analyzed for mercury using MP-AES (Agilent, Model 4200). The blank reagent was also digested following the same procedure as the soil samples.

3.6. Method validation and measuring performance

The analysis of mercury in the digested samples were checked by performing the following validation parameters such as instrumental detection limit (IDL), Limit of detection (LOD), limit of quantification (LOQ)**,** precision and accuracy [29].

3.6.1. Accuracy and precision

Accuracy and precision are the most common terms related to analytical quality procedures to express the extent of errors in analytical measurements. Such qualities of data are performed by applying different statistical methods to analytical data such as the standard deviation, relative standard deviation of series of measurements. Also, the precision and accuracy of the results were assessed by determining recovery and repeatability of the analysis of spiking of samples. In this particular study the results of the measurements are expressed as the mean of the measurements together with the relative standard deviation (RSD) of the triplicate samples [30]. The relative standard deviations of the samples were obtained as:

$$\%RSD = \frac{\text{Standard deviation}}{\text{Mean value}} \times 100$$

3.6.2. Instrumental detection limit (IDL)

Instrumental detection limit is the smallest signal above background noise that an instrument can detect reliably. The IDL is calculated to be the concentration equal to three times the standard deviation of the blank signal [31]. In this study, IDL for mercury was determined from analysis of triplicates of calibration blank signal.

3.6.3. Limit of detection (LOD)

Limit of detection is defined as the minimum concentration of analyte that can be detected but not necessarily quantified with an acceptable uncertainty. In other words, it is the lowest concentration that can be determined to be statistically different from a blank solution which detected by the analytical method with a given confidence limit. The SD of the triplicate blanks was calculated to determine the LOD [31]. Limit of detection (LOD) was calculated according to the equation indicated below.

LOD = 3xSD, where, SD is the standard deviation of blank solution.

3.6.4. Limit of quantification (LOQ)

Limit of quantification is the lowest concentration of an analyte in the soil samples which can be quantitatively determined with acceptable limit. It was obtained from triplicate analysis of blanks which were digested in the same digestion procedure as the actual samples [31]. The LOQ was calculated according to the equation indicated below.

LOQ = 10x SD, where SD is the standard deviation of blank solution.

3.6.5. Method validation

The validity of the optimization and digestion soil samples were checked by performing spiking of soil samples with standard solutions. The spiked and non-spiked samples were digested and analyzed in similar way using the optimized procedure for sample analysis. The percentage recoveries of the analytes were calculated to evaluate the accuracy of the analytical procedures [32]. Then, the percentage recoveries of the analytes were calculated by using the following equation:

$$\% R = \frac{C_M \text{ (in the spiked sample)} - C_M \text{ (in the non−spiked sample)}}{C_M \text{ added for spiking}} \times 100\ \%$$

Where: C_M is concentration of mercury metal.

3.6.6. Calibration of the instrument

The calibration curve was drawn to determine the concentration of mercury in the soil sample solutions. The instrument was calibrated using intensity and correlation coefficient (R^2) of the calibration graph for mercury in Table5. The correlation coefficient (R^2) of the calibration curve of mercury was determined by plotting working standard concentration versus their corresponding intensity as shown in Fig.8.

Table 5: Working standard solutions

Standards	Intensity	Results of working standard solutions
S_1	1323.9760	0.9745
S_2	2734.1895	2.0593
S_3	3851.4687	2.9604
S_4	5088.1311	4.0036

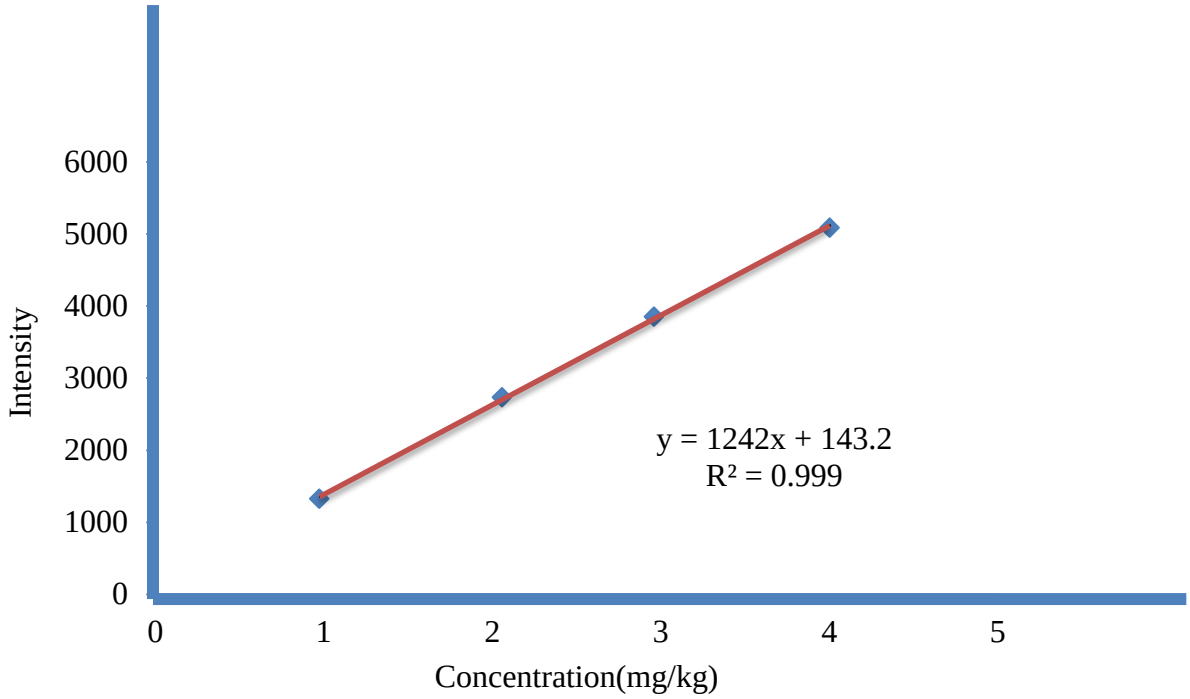

Fig.8: Calibration curve for mercury

3.6.7. Determination of concentration of mercury

The digested samples were determined for the concentrations of mercury using microwave plasma-atomic emission spectroscopy. The concentrations of mercury in the soil samples were determined as the mean of triplicate digested soil samples and it is reported in terms of mean values (mean± SD) standard deviation [32]. Finally, the concentrations of the mercury in the soil samples were calculated using the following formula

$$\text{Concentration of mercury (mg/kg)} = \frac{\text{Concentration (mg/L)} \times \text{V (L)}}{\text{W (kg)}}$$

Where, V = Final volume (50 ml) of solution & W= Initial weight (0.5 g) of soil sample measured.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Regression analysis and Limit of detection

As can be seen from Fig.8, the calibration curve for mercury showed a good linearity with coefficient of determination (r^2) 0.9999 that is greater than the acceptable limit (0.998) for the linearity of the regression line [33]. This showed that there is a good correlation between concentration and intensity, indicating good calibration of the instrument. The instrumental detection limit is below the limit of detection limit, which indicates good sensitivity of the measuring instrument for analysis. The limit of detection and the limit of quantification value are 0.074 and 0.248 mg/kg, respectively as we can see in table 6. The result showed both the LOD and LOQ values are greater than the IDL; hence, the result of the analysis could be reliable.

Table 6: Linear regression equations, Instrumental detection limit, limit of detection, coefficient of determination and limit of quantification.

Metal	IDL (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Coefficient of determination (r^2)	Linear regression equation
Hg	0.002	0.074	0.248	0.999	y= 1242x + 143.2

4.2. Accuracy and precision

As it can be seen from Table 7, the mean percent recovery for the studied metal in the spiked sample was 98.63% which is found within the acceptable range of 80–120% for mercury analysis [33]. The precision of the method was expressed in terms of % RSD of the triplicate readings. So, the %RSD value obtained for spiked soil sample is 22.47% from Table 7.

Table 7: Recovery and spiking test result of mercury in the soil samples

Metal	Average conc. of non-spiked (mg/kg)	Amount added (mg/kg)	Conc. in spiked Sample (mg/kg)	Recovery a (%)	RSD (%)
Hg	0.111	3	3.07	98.6	22.47

4.3. Concentration of mercury in the soil samples

The results of mercury analysis are expressed as mean ± standard deviation of triplicate analyses. The result shows that the concentration of mercury in the soil sample was found to be 0.111 mg/kg. This mean value result shows the presences of high level of mercury in the soil samples as compared to the World Health Organization recorded in the soils 2003 [34].

Table 8: The mean and standard deviation of mercury concentration in the soil samples

Concentration (mg/kg) (mean ± SD) of mercury metal in soil sample							
Metal	S_1	S_2	S_3	Mean	SD	Mean ± SD	%RSD
Hg	0.138	0.103	0.09	0.111	0.025	0.111±0.025	22.47

4.4. Comparison of concentration of mercury this study with those reported in the literature

The concentration of mercury in soil samples of Adolla gold mining site was high as compared to the World Health Organization record in 2003 [34]. The presences of high concentration of mercury in the soil sample may be the result of the widely usage of mercury for reasons of amalgamation during the extraction of gold mining activity. So, the amalgamation and burning of the gold mining waste materials can result in the emission of toxins such as mercury in high concentration. Thus causing of health problem in the workers and the communities of living around the gold mining area

4.5. Conclusions

The concentration of mercury in soil samples collected from Adolla gold mining in southern part of Guji Zone of the Oromia region has been determined using MP-AES instrument. The mean of mercury (0.111 mg kg^{-1}) in the soils of the study area is high as compared to the World Health Organization record in 2003, due to application of mercury for amalgamation purpose during the gold mining extractions in Adolla site. So, the amalgamation and burning of the gold mining waste materials can result in the emission of toxic such as mercury in high concentration. As a result, the soil around the gold mining area is contaminated by toxic nature of mercury. So, it has an effect on health problems in the workers and the communities living around the gold mining area.

In conclusion, the present study will give brief information about the presences of mercury in the soil of Adolla gold mining area. These results may serve as a base line data for determination of mineral content and physico-chemical properties of the mercury in the soils of the study area. Also, the need for periodic monitoring of toxic metal in this area is crucial issue to protect human health.

5. REFERENCES

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