ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Determination of the Levels of Pb, Cr, Zn, Cu and Al in Soil from Selected Garage site in Addis Ababa, Ethiopia

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A Thesis submitted to the Department of Chemistry in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry

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This is to certify that the thesis prepared by Kassahun Tesfaye entitled "Determination of the levels of Pb, Cr, Zn, Al and Cu in soil from selected garage site" submitted in partial fulfillment of the requirements for the degree of master of science in chemistry compiles with the regulation of the university and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
AD	Alzheimer Disease
AES	Atomic Emission Spectroscopy
ANOVA	Analysis of Variance
CL	Confidence Level
CSA	Central Statistics Authority
DNA	Deoxyribonucleic Acid
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
MCL	Maximum Contamination Level
MDL	Method Detection Limit
ppm	Parts Per Million
Q_A	Quality Assurance
Q_C	Quality Control
%R	Percentage of Recovery
%RSD	Percentage Relative Standard Deviation
R	Correlation coefficient
ROS	Reactive Oxygen Species
RL	Recommended Limit
SD	Standard Deviation
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Abstract

Both natural and human activities adds heavy metals to soil, natural activities have a little effect on soil pollution, however soil pollution mainly causes from anthropogenic activities of humans that lead to heavy metals toxicity into the soils. Garages are one of the major sources of increase in heavy metal concentration in soils of Addis Ababa, Ethiopia. Three garage sites were selected for the study, which include; Zenebework (Z.W), Amanuel (A) and Kotebe (K). Heavy metals in the soil samples at 0-10 cm depth were collected. After proper sample pretreatment, the type and volume of reagents to be used, digestion temperature and digestion time were optimized. Then using the optimized conditions sample preparation was made and levels of metals such as Lead (Pb), Zinc (Zn), Chromium (Cr), Copper (Cu) and Aluminium (Al) in each soil were determined by microwave plasma atomic emission spectrometry (MP-AES). The accuracy of the optimized procedure was evaluated by analyzing the digestion of the spiked samples with standard solution and the percentage recoveries varied from 86% to 112%.

The mean concentration of metals obtained in each site at 0-10 cm depth by MP-AES (mg/kg) were; (Z.W; Zn: 422 ± 31.8, Cr: 58 ± 6.8, Cu: 71 ± 6.1, Al: 47601 ± 2350), (A; Zn: 657 ± 42, Cr: 146 ± 12.8, Cu: 307.5 ± 14.4, Al: 16132 ± 664), (K; Zn: 447 ± 20, Cr: 57 ± 4.5, Cu: 188 ± 6.1, Al: 17486 ± 953.6). The levels of metal concentration in the soils from these experimental sites were higher than the recommended limits given by World Health Organization (WHO). In these study; Lead (Pb) is below the detection limit.

Keywords: Anthropogenic activities; Addis Ababa; Garage soil; Heavy Metals; concentration of metals; microwave plasma atomic emission spectrometry; recommended limits

1. Introduction

1.1. Background of study

Metals occur naturally in the earth's crust, and their contents in the environment can vary between 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]. Of the 92 naturally occurring elements, approximately 30 metals and metalloids are potentially toxic to humans, Be, B, Li, Al, Ti, V, Cr, Mn, Co, Fe, Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, Pt, Au, Hg, Pb, and Bi are among these.

Pollution is a worldwide problem and its potential in influencing health of the human population is great [2]. Pollution in recent years has increased considerably as a result of increasing human activities such as burning of fossil fuels, industrial and automobile exhaust emissions which were identified as primary sources of atmospheric metallic burden [3]. The most common environmental pollutants in the world are heavy metals [4]. Heavy metal is a general term used to describe a group of metals and metalloids with an atomic density greater than 5.0 g/cm^3 [5]. Among the 35 natural existing metals, 23 possess high specific density above 5 $g/cm^3$with atomic weight greater than 40.04. Thus include: antimony, tellurium, bismuth, tin, thallium, gold, arsenic, cerium, gallium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, nickel, platinum, silver, uranium, vanadium, and zinc [6]. Some of these heavy metals such as cobalt, chromium, copper, magnesium, iron, molybdenum, manganese, selenium, nickel and zinc are essential nutrients that are required for various physiological and biochemical functions in the body and may result to deficiency diseases or syndromes if not in adequate amounts [7] but in large doses they may cause acute or chronic toxicities. These elements occur naturally in soils and rocks at various ranges of concentrations; they are also found in ground and surface water bodies and sediments [8].

Unchecked industrial and human activities have contributed significantly to elevated (pollution) levels of these metals, in surface and subsurface soils when compared to those contributed from geogenic or natural processes [9]. The extent of soil pollution by heavy metals is very alarming because of their toxicity which lead to adverse effects on human and ecosystem health [10].

Chronic exposure to heavy metals leads to serious kidney malfunction, anaemia, hematological and brain damage [11]. According to WHO, 20 million children worldwide suffer from pollution which has become critical.

In industrialized countries, a garage is an enclosed area of land set aside for repair of automobiles [12]. The impact of pollution in the vicinity of overcrowded cities and from industrial effluents and automobiles has reached a disturbing magnitude and is arousing public awareness [3]. Addis Ababa city, the African city, having a growth rate of 2.1% constitutes 32.27% of the total urban population [13]. Thus, the increase in population size coupled with the economic growth and increase in the size of the city demands a transport service supply in line with the increase in the mobility need of the people, which corresponds to an increase of vehicles in the city resulting in an increase in garages, one of sources of soil pollution. The contamination chain of heavy metals almost always follows a cyclic order: industry, atmosphere, soil, water, foods and human. During the rainy season, the waste from garage soils is swept to pollute water ways. If water from these water ways is used for irrigation, the irrigated crops bioaccumulate the contaminants and more especially heavy metals [14]

It is important to analyze some of the heavy metal and pollutants in soil; As such this research is aimed at evaluating the level of heavy metal concentrations in soil. The paper reports a spectroscopic investigation of heavy metals in soil samples from garages in Addis Ababa city where automobile repairs are prominent. The study examines the potential environmental risk of these activities in the area.

1.2. Statement of the problem

Addis Ababa city is one of the African developing cities. The population growth rate of the city is high related to industrialization and mobility of population; hence pollution is the major problem of the city. Soil pollution by the accumulation of heavy metals is directly or indirectly related to industrialization and population mobility. Lead, zinc, chromium, copper, aluminum can cause poisoning when taken at levels exceeding the defined maximum contamination levels (MCL) set by world health organization (WHO). All over the world, due to phenomenal increase in the number of autos, the number of garages has also multiplied. Many of these garages do not have any elementary schemes for disposing off materials generated while servicing vehicles. Almost all waste is thrown directly to the environment to contaminate water either by percolation

or surface runoff [15]. Hence it is important to determine the levels of toxic metals in garages soil for proper management.

1.3. Objectives

1.3.1 General objective

- To determine the levels of heavy metals (Pb, Cr, Zn, Cu) and Al in selected garages soil in Addis Ababa city.

1.3.2 Specific objectives

- ✓ To determine the levels of thus metals in selected garage soils by microwave plasma atomic emission spectrometry.
- ✓ To compare the variation of metals in each garages soil
- ✓ Gives information about the effects of toxic metals for humans and living organisms.
- ✓ To test the significance of analytical method by statistical analysis.

2. Literature Review

2.1. Introduction

Human activities such as industrial production, mining, agriculture and transportation, release high amounts of heavy metals into surface and ground water, soils and ultimately to the biosphere. Accumulation of heavy metals in crop plants is of great concern due to the probability of food contamination through the soil root interface. Though the heavy metal like, Cd, Pb and Ni are not essential for plant growth, they are readily taken up and accumulated by plants in toxic forms. Ingestion of vegetables irrigated with waste water and grown in soils contaminated with heavy metals possess a possible risk to human health and wildlife. Heavy metal concentration in the soil solution plays an important role in controlling metal bioavailability to plants. Most of the studies show that the use of waste water contaminated with heavy metals for irrigation over long period of time increases the heavy metal contents of soils above the permissible limit. Ultimately, increasing the heavy metal content in soil also increases the uptake of heavy metals by plants depending upon the soil type, plant growth stages and plant species [16].

The most important sources of heavy metals in the environment are the anthropogenic activities such as mining, smelting procedures, steel and iron industry, chemical industry, traffic, agriculture as well as domestic activities [17]. These heavy metals may adversely affect soil ecology, agricultural production or product quality, and ground water quality, and will ultimately harm the health of living organism by food chain. These effects are closely related to the biological availability of heavy metals, which in turn are controlled by the metal ion speciation in the soil. Therefore, the determination of free metal ion concentrations in soil solution becomes important. The free metal ion concentration not only depends on the total metal content in soils, but also on the metal species that exist in the soil [18].

2.2. Soil pollution

“Soil pollution” refers to the presence of a chemical or substance out of place and/ or present at a higher than normal concentration that has adverse effects on any non-targeted organism [19]. Although the majority of pollutants have anthropogenic origins, some contaminants can occur naturally in soils as components of minerals and can be toxic at high concentrations. Soil pollution often cannot be directly assessed or visually perceived, making it a hidden danger.

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition [20]. Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) [21]. Soils are the major sink for heavy metals released into the environment by the aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation [22], and their total concentration in soils persists for a long time after their introduction [23]. Changes in their chemical forms (speciation) and bioavailability are, however, possible. The presence of toxic metals in soils can severely inhibit the biodegradation of organic contaminants [24]. Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems [25].

2.3. Sources of heavy metals for garage soil contamination

Due to phenomenal increase in the number of autos, garage waste is also on the increase. This waste includes waste oil, lubricants, discarded oil filters, tyres, acid batteries, paints, gas filters, brake pads, bearings and nuts. Heavy metals from the waste contaminate water through percolation or surface run off [15]. Motor vehicles are complex “contaminant sources” due to the number of emission sources and the range of metals used in motor vehicles. There is a wealth of information on potential sources of copper, lead and zinc in motor vehicles. Two recent studies have examined the distribution and presence of metals in vehicles in relation to life cycle analysis [26].

The automobile industry uses large proportion of lead in car battery and in solder and the principal use of lead is in lead accumulators. Major sources of chromium are air conditioning

coolants, engine parts, brake lining, catalytic converters, wear and tear of chrome plated vehicular parts, yellow paints on the roads used for marking and metal industries [27].

2.4. Basic soil chemistry and potential risks of heavy metals

The most common heavy metals found at contaminated sites, in order of abundance are Pb, Cr, As, Zn, Cd, Cu, and Hg [28]. These metals are important since they are capable of decreasing crop production due to the risk of bioaccumulation and biomagnification in the food chain. There is also the risk of surface and groundwater contamination. Knowledge of the basic chemistry, environmental and associated health effects of these heavy metals is necessary in understanding their speciation, bioavailability, and remedial options. The fate and transport of a heavy metal in the soil depends significantly on the chemical form and speciation of the metal. Once in the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity [30]. This distribution is believed to be controlled by reactions of heavy metals in soils such as (i) mineral precipitation and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake [30].

2.4.1. Lead

Lead is a metal belonging to group IV and period 6 of the periodic table with atomic number 82, atomic mass 207.2, density 11.4 g cm^{-3}, melting point 327.4°C, and boiling point 1725°C. It is a naturally occurring, bluish gray metal usually found as a mineral combined with other elements, such as sulfur (i.e., PbS, $PbSO_4$), or oxygen ($PbCO_3$), and ranges from 10 to 30 $mgkg^{-1}$ in the earth's crust [31].

Ionic lead, Pb(II), lead oxides and hydroxides, and lead metal oxy-anion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters. The most stable forms of lead are Pb(II) and lead-hydroxy complexes. Lead(II) is the most common and reactive form of Pb, forming mononuclear and polynuclear oxides and hydroxides [21]. The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead hydroxides [32]. Under anaerobic conditions a volatile organolead (tetramethyl lead) can be formed due to microbial alkylation [21].

Lead(II) compounds are predominantly ionic (e.g., Pb^{2+}, SO_4^{2-}), whereas Pb (IV) compounds tend to be covalent (e.g., tetraethyl lead, Pb $(C_2H_5)_4$). Some Pb (IV) compounds, such as PbO_2, are strong oxidants. Lead forms several basic salts, such as $Pb(OH)_2 \cdot 2PbCO_3$, which was once the most widely used white paint pigment and the source of considerable chronic lead poisoning to children who ate peeling white paint.

Inhalation and ingestion are the two routes of exposure, and the effects from both are the same. Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead [33]. Lead is not an essential element. It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals. Lead can cause serious injury to the brain, nervous system, red blood cells, and kidneys [34]. Exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure.

The most serious source of exposure to soil lead is through direct ingestion (eating) of contaminated soil or dust. In general, plants do not absorb or accumulate lead. However, in soils testing high in lead, it is possible for some lead to be taken up. Higher concentrations are more likely to be found in leafy vegetables (e.g., lettuce) and on the surface of root crops (e.g., carrots).

2.4.2. Chromium

It is the 21st most abundant mineral in the crust of the earth. It is a first-row *d*-block transition metal of group VIB in the periodic table with the following properties: atomic number 24, atomic mass 52, density 7.19 g cm^{-3}, melting point 1875°C, and boiling point 2665°C. It is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite, $FeCr_2O_4$. Major sources of Cr contamination include releases from electroplating processes and the disposal of Cr containing wastes [35]. Chromium (VI) is the more toxic form of chromium and is also more mobile. Chromium(III) mobility is decreased by adsorption to clays and oxide

minerals below pH 5 and low solubility above pH 5 due to the formation of $Cr(OH)_3(s)$ [36]. The leachability of Cr (VI) increases as soil pH increases. Hexavalent chromium (Cr^{6+}) is the second most stable form and a strong oxidizing agent, especially in acidic media. Hexavalent chromium is bound to oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) with a strong oxidative capacity. This form of Cr crosses biological membranes easily, reacting with protein components and nucleic acids inside the cell while being deoxygenated to Cr^{3+}. The reaction with genetic matter provides for the carcinogenic properties of Cr^{6+}.Trivalent chromium (Cr^{3+}) is the most stable oxidation state in which Cr is found in living organisms. It does not have the capacity to cross cell membranes easily [37] and has a low reactivity, which is the most significant biological feature distinguishing it from Cr^{6+}. Trivalent Cr forms a number of coordination complexes, hexadentate ligands being the basic form. Some forms of Cr^{3+} (e.g. Cr_2O_3) are, thanks to their low reactivity and absorption from the gastrointestinal system, used as markers in the study of digestion processes [38].

Trivalent chromium is essential to normal carbohydrate, lipid and protein metabolism. Chromium is biologically active as part of an oligopeptide – chromodulin – potentiating the effect of insulin by facilitating insulin binding to receptors at the cell surface. With chromium acting as a cofactor of insulin, Cr activity in the organism is parallel to insulin functions.

2.4.3. Zinc

Zinc is a transition metal with the following characteristics: period 4, group IIB, atomic number 30, atomic mass 65.4, density 7.14 g cm^{-3}, melting point 419.5◦C, and boiling point 906°C. Zinc occurs naturally in soil (about 70 $mgkg^{-1}$ in crustal rocks) [39], but Zn concentrations are rising unnaturally, due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Many foodstuffs contain certain concentrations of Zn. Drinking water also contains certain amounts of Zn, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc shortages can cause birth defects. The world's Zn production is still on the rise which means that more and more Zn ends up in the environment. Water is polluted with Zn, due to the presence of large quantities present in the wastewater of industrial plants. A consequence is that Zn polluted sludge is continually being

deposited by rivers on their banks. Zinc may also increase the acidity of waters. Some fish can accumulate Zn in their bodies, when they live in Zn-contaminated waterways. When Zn enters the bodies of these fish, it is able to biomagnify up the food chain. Water-soluble zinc that is located in soils can contaminate groundwater. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter [40].

2.4.4. Copper

Copper is a transition metal which belongs to period 4 and group IB of the periodic table with atomic number 29, atomic weight 63.5, density 8.96 g cm^{-3}, melting point 1083°C and boiling point 2595°C [39].

Copper is the third most used metal in the world [41]. Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain. In the soil, Cu strongly complexes to the organic implying that only a small fraction of copper will be found in solution as ionic copper, Cu(II).

Copper and Zn are two important essential elements for plants, microorganisms, animals, and humans. The connection between soil and water contamination and metal uptake by plants is determined by many chemical and physical soil factors as well as the physiological properties of the crops. Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income. Some food

importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops [42].

Table 1. Countries and FAO/WHO limits of heavy metals in soil (mg/kg)

Element	Germany	Poland	Australia	Taiwan	Canada	Tanzania	South Africa	FAO/ WHO
Pb	70.0	100	300	300	200	200	20	100
Cr	60.0	100	50	250	250	100	6.5	100
Cu	40.0	100	100	200	150	200	16	100
Zn	150	300	200	600	500	150	240	300
Reference	[43]	[44]	[45]	[43]	[46]	[47]	[48]	[49]

2.5. Mechanism of heavy metal toxicity

2.5.1. Heavy metal-induced oxidative stress and oxidation of biological molecules

Certain heavy metals are known to generate free radicals which may lead to oxidative stress and cause other cellular damages [50]. The mechanism of free radical generation is specific to the type of heavy metal.

I. Copper: Copper ions have been identified to participate in the formation of reactive oxygen species (ROS) as cupric (Cu^{2+}) and cuprous (Cu^{+}) which can participate in oxidation and reduction reactions. The Cu^{2+} in the presence of biological reductants such as glutathione (GSH) or ascorbic acid can be reduced to Cu^{+} which is capable of catalyzing the decomposition of H_2O_2 to form $OH^{\bullet}$ *via* the Fenton reaction [51] as shown below.

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{-}$$

The OH• radical formed is capable of reacting with several biomolecules. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases *via* oxygen free radicals [52].

2.5.2. Heavy metal-induced carcinogenesis and neurotoxicity

Some heavy metals are known to have carcinogenic effect. Several signaling proteins or cellular regulatory proteins that participate in apoptosis, cell cycle regulation, DNA repair, DNA methylation, cell growth and differentiation are targets of heavy metals [53]. Thus, heavy metals

may induce carcinogenic effect by targeting a number of these proteins. Some heavy metals such as lead and manganese may affect the brain and cause neurological toxicity [54].

I. Lead: The mechanism of lead-induced carcinogenic process is postulated to induce DNA damage, disrupt DNA repair system and cellular tumor regulatory genes through the generation of ROS [55]. Studies have supported with evidence that ROS generation by lead is key in altering chromosomal structure and sequence [55]. Lead can disrupt transcription processes by replacing zinc in certain regulatory proteins [55].

Lead toxicity is also targeted towards the memory and learning processes of the brain and can be mediated through three processes. Lead can impair learning and memory in the brain by inhibiting the N-methyl-d-aspartate receptor (NMDAR) and can block neurotransmission by inhibit neurotransmitter release, block the neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs) and reduce the expression of brain-derived neurotrophic factor (BDNF).

2.5.3. Biochemical mechanism of heavy metal toxicity

When heavy metals are ingested through food or water into the body, they are acidified by the acid medium of the stomach. In this acidic medium, they are oxidized to their various oxidative states (Zn^{2+}, Cd^{2+}, Pb^{2+}, As^{3+}, Ag^{+}, Hg^{2+}, etc.) which can readily bind to biological molecules such as proteins and enzymes to form stable and strong bonds. The most common functional group that heavy metals bind is the thio groups (SH group of cysteine(CSH) and SCH_3 group of methionine(MSH)) [56]. The equations of these reactions are shown below (Figure 2).

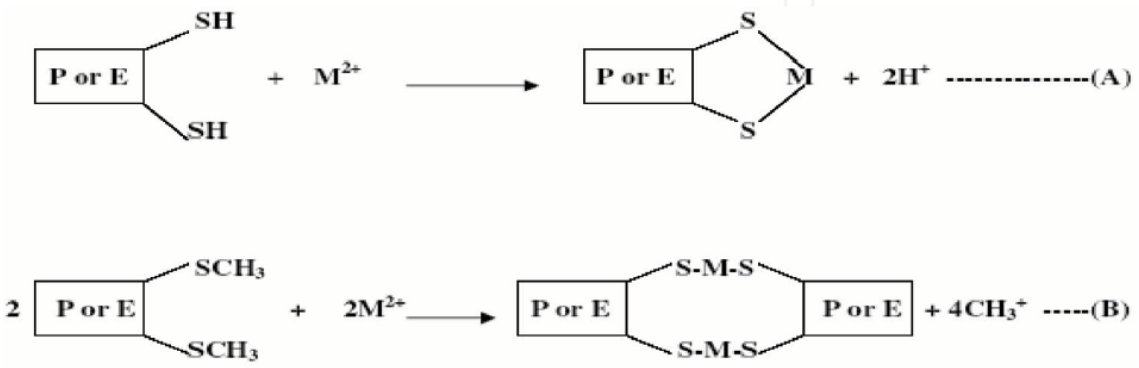


Figure 1. Reactions of heavy metals (M) with sulphydryl groups of proteins (P) or enzymes (E)

A = Intramolecular bonding, B = Intermolecular bonding

In the above reaction, the oxidized heavy metal replaces the hydrogen of the SH group and the methyl of the SCH_3 group thereby inhibiting the function of the protein or activity of the enzyme.

Heavy metal-bound proteins may be a substrate for certain enzymes. In such situations, the heavy metal-bound protein fits into an enzyme in a highly specific pattern to form an enzyme-substrate complex and thus cannot accommodate any other substrate until it is freed. As such, the product of the substrate is not formed as the enzyme is blocked and therefore, the heavy metal remains embedded in the tissue leading to dysfunctions, abnormalities and damages in the body. Inhibition of thiol transferases lead to increased oxidative stress and cell damage.

2.6. Remediation of heavy metal-contaminated soils

The overall objective of any soil remediation approach is to create a final solution that is protective of human health and the environment [57]. The regulatory authorities will normally accept remediation strategies that centre on reducing metal bioavailability only if reduced bioavailability is equated with reduced risk, and if the bioavailability reductions are demonstrated to be long term [57]. Information about the physical characteristics of the site and the type and level of contamination at the site must be obtained to enable accurate assessment of site contamination and remedial alternatives. The contamination in the soil should be characterized to establish the type, amount, and distribution of heavy metals in the soil. Once the

site has been characterized, the desired level of each metal in soil must be determined. This is done by comparison of observed heavy metal concentrations with soil quality standards for a particular regulatory domain, or by performance of a site-specific risk assessment. Remediation goals for heavy metals may be set as total metal concentration or as bleachable metal in soil, or as some combination of these.

Several technologies exist for the remediation of metal contaminated soil. Gupta et al [58] have classified remediation technologies of contaminated soils into three categories of hazard-alleviating measures: (i) gentle *in situ* remediation, (ii) *in situ* harsh soil restrictive measures, and (iii) *in situ* or *ex situ* harsh soil destructive measures. USEPA [59] has broadly classified remediation technologies for contaminated soils into (i) source control and (ii) containment remedies. Source control involves *in situ* and *ex situ* treatment technologies for sources of contamination. *In situ* or in place means that the contaminated soil is treated in its original place; unmoved, unexcavated; remaining at the site or in the subsurface. *In situ* treatment technologies treat or remove the contaminant from soil without excavation or removal of the soil. *Ex situ* means that the contaminated soil is moved, excavated, or removed from the site or subsurface. Implementation of *ex situ* remedies requires excavation or removal of the contaminated soil. Containment remedies involve the construction of vertical engineered barriers (VEB), caps, and liners used to prevent the migration of contaminants.

Another classification places remediation technologies for heavy metal-contaminated soils under five categories of general approaches to remediation: isolation, immobilization, toxicity reduction, physical separation, and extraction [20]. In practice, it may be more convenient to employ a hybrid of two or more of these approaches for more cost effectiveness. The key factors that may influence the applicability and selection of any of the available remediation technologies are: (i) cost, (ii) long-term effectiveness/ permanence, (iii) commercial availability, (iv) general acceptance, (v) applicability to high metal concentrations, (vi) applicability to mixed wastes (heavy metals and organics), (vii) toxicity reduction, (viii) mobility reduction, and (ix) volume reduction. Soil washing, phytoremediation, and immobilization techniques are among the best demonstrated available technologies for heavy metal-contaminated sites.

2.7. Aluminium

2.7.1. Occurrence and major uses

Aluminium is the most abundant metallic element and constitutes about 8% of the Earth's crust. It occurs naturally in the environment as silicates, oxides, and hydroxides, combined with other elements, such as sodium and fluorine, and as complexes with organic matter.

Aluminium metal is used as a structural material in the construction, automotive, and aircraft industries, in the production of metal alloys, in the electric industry, in cooking utensils, and in food packaging. Aluminium compounds are used as antacids, antiperspirants, and food additives [60]. Aluminium salts are also widely used in water treatment as coagulants to reduce organic matter, colour, turbidity, and microorganism levels [61]. Use of aluminium salts as coagulants in water treatment may lead to increased concentrations of aluminium in finished water.

2.7.2. Environmental fate, levels and human exposure

Aluminium is released to the environment mainly by natural processes. Several factors influence aluminium mobility and subsequent transport within the environment. These include chemical speciation, hydrological flow paths, soil–water interactions, and the composition of the underlying geological materials. Acid environments caused by acid mine drainage or acid rain can cause an increase in the dissolved aluminium content of the surrounding waters [62].

I. Air: Aluminium enters the atmosphere as a major constituent of atmospheric particulates originating from natural soil erosion, mining or agricultural activities, volcanic eruptions, or coal combustion. Atmospheric aluminium concentrations show widespread temporal and spatial variations. Airborne aluminium levels range from 0.0005 $\mu g/m^3$ over Antarctica to more than 1 $\mu g/m^3$ in industrialized areas [63].

II. Water: The concentration of aluminium in natural waters can vary significantly depending on various physicochemical and mineralogical factors. Dissolved aluminium concentrations in waters with near-neutral pH values usually range from 0.001 to 0.05 mg/l but rise to 0.5–1 mg/l in more acidic waters or water rich in organic matter. At the extreme acidity of waters affected by acid mine drainage, dissolved aluminium concentrations of up to 90 mg/l have been measured [62].

III. Food: Aluminium is present in foods naturally or from the use of aluminium-containing food additives. The use of aluminium cookware, utensils, and wrappings can increase the amount of aluminium in food; however, the magnitude of this increase is generally not of practical importance. Foods naturally high in aluminium include potatoes, spinach, and tea. Processed dairy products, flour, and infant formula may be high in aluminium if they contain aluminium-based food additives [63, 64]

Adult dietary intakes of aluminium (mg/day) have been reported in several countries: Australia (1.9–2.4), Finland (6.7), Germany (8–11), Japan (4.5), Netherlands (3.1), Sweden (13), Switzerland (4.4), United Kingdom (3.9), and USA (7.1–8.2). Intake of children 5–8 years old was 0.8 mg/day in Germany and 6.5 mg/day in the USA. Infant intakes of aluminium in Canada, the United Kingdom, and the USA ranged from 0.03 to 0.7 mg/day [62].

2.7.4. Effects on humans

There is little indication that aluminium is acutely toxic by oral exposure despite its widespread occurrence in foods, drinking-water, and many antacid preparations [62].

In 1988, a population of about 20,000 individuals in Camelford, England, was exposed for at least 5 days to unknown but increased levels of aluminium accidentally distributed to the population from a water supply facility using aluminium sulfate for treatment. Symptoms including nausea, vomiting, diarrhea, mouth ulcers, skin ulcers, skin rashes, and arthritic pain were noted. It was concluded that the symptoms were mostly mild and short-lived. No lasting effects on health could be attributed to the known exposures from aluminium in the drinking-water [65].

It has been hypothesized that aluminium exposure is a risk factor for the development or acceleration of onset of Alzheimer disease (AD) in humans. WHO [62] has evaluated some 20 epidemiological studies that have been carried out to test the hypothesis that aluminium in drinking-water is a risk factor for AD. However, each of the studies had some deficiencies in the study design (e.g. ecological exposure assessment; failure to consider aluminium exposure from all sources and to control for important confounders, such as education, socioeconomic status, and family history; the use of surrogate outcome measures for AD; and selection bias). In general, the relative risks determined were less than 2, with large confidence intervals, when the

total aluminium concentration in drinking-water was 0.1 mg/l or higher. Based on current knowledge of the pathogenesis of AD and the totality of evidence from these epidemiological studies, it was concluded that the present epidemiological evidence does not support a causal association between AD and aluminium in drinking-water [62].

In addition to the epidemiological studies, two studies examined cognitive dysfunction in elderly populations in relation to the levels of aluminium in drinking-water. The results were again conflicting. Such data are insufficient to show that aluminium is a cause of cognitive impairment in the elderly.

2.8. Quantitative analytical methods

2.8.1. Atomic spectroscopy

In general, atomic spectroscopy is comprised of atomic absorption, emission and fluorescence. Basically, each atom is composed of a nucleus bordered by electrons. Every element has a specific number of electrons connected to its nucleus. The most stable orbital configuration of an atom is known as the “ground state”. When energy is applied to an atom, a known quantity of energy with a given wavelength will be absorbed and an outer electron then promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will spontaneously return to the "ground state," releasing light energy. The process of excitation and decay to the ground state is involved in all three fields of atomic spectroscopy [66].

Atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES) are the most widely used techniques for heavy metals quantitative analysis in environmental samples.

For the study the most efficient, sensitive and recent technology micro plasma atomic emission spectroscopy was used, and explained as follows.

2.8.2. Microwave plasma atomic emission spectroscopy /MP-AES/

Microwave Induced Plasma Atomic Emission Spectrometry (MP-AES) is a plasma technique. The first commercially available microwave plasma atomic emission spectrometer (MP-AES) was released in 2011, which applies a robust excitation source for stable and continuous measurements. The MP-AES 4200 using microwave plasma and an atomic emission spectroscopy detector provide a new and improved instrument to the analytical field. The

magnetron generates electromagnetic wavelengths at 2.5 GHz and the magnetic field is focused axially around the torch. Plasma is supplied with nitrogen produced by a generator from air which makes the technique the most cost-effective one in the field of atomic spectrometry. It was recently used for different elemental determination in various matrices.

2.8.2.1. Atomic excitation

Microwave plasma atomic emission spectroscopy is an atomic emission technique. It uses the fact that once an atom of a specific element is excited, it emits light in a characteristic pattern of wavelengths – an emission spectrum, as it returns to the ground state. Sources for atomic emission include the microwave plasma (MP) and the inductively coupled argon plasma (ICP) both of which are high temperature sources, and therefore excellent excitation sources for atomic emission spectroscopy. The nitrogen fuelled microwave plasma reaches temperatures nearing 5,000 K. At these temperatures, atomic emission is strong, producing excellent detection limits and linear dynamic range for most elements. Inside a MP-AES instrument, microwave energy from an industrial magnetron is used to form plasma from nitrogen that has been extracted from compressed air by Agilent's Nitrogen Generator. Effectively, the MP-AES runs on air.

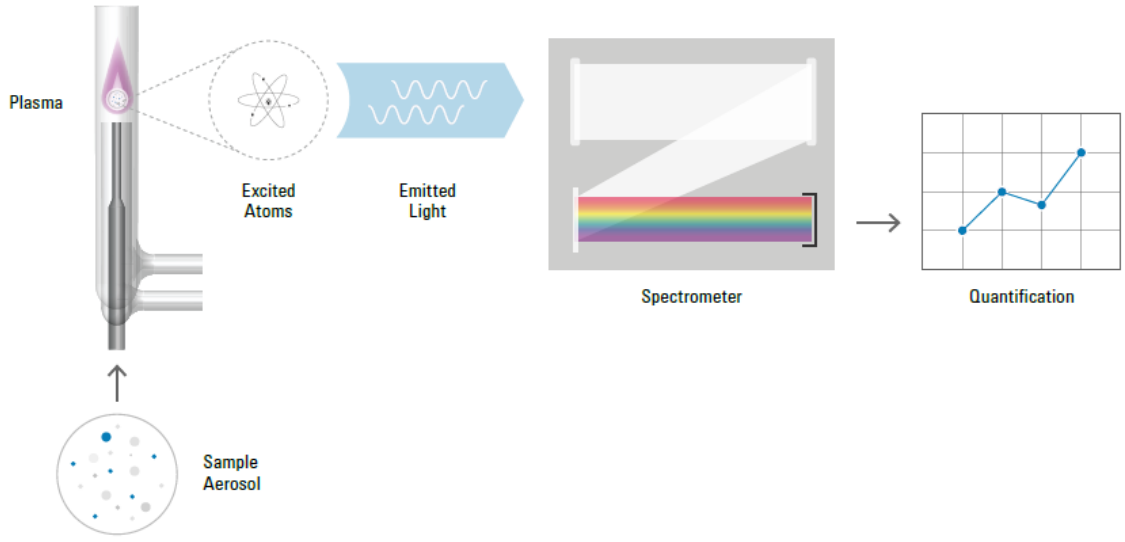


Figure 2. Schematic diagram of a microwave plasma atomic emission spectrometer

2.8.2.2. The benefits of MP-AES

Low cost of ownership

Gas supply is one of the highest costs associated with elemental analysis in most spectrometric techniques such as flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), etc except the Agilent technology spectrometers; Mp-AES, ICP-MS. The MP-AES runs on air, it vastly reduces the cost of ownership and eliminates the need for ongoing supply of flammable or expensive gases. There is no gas cylinders or lamps to buy and no standby operating costs. When a MP-AES is off, no gas or power is used. Simply switch it on again when needed for analysis

Safety

Removing the requirement for flammable gases means no gas leaks and ordering and transporting of cylinders. Removing all of these risks makes the laboratory a safer workplace.

Better performance than AA

An MP-AES has high sensitivity with detection limits down to sub ppb levels and is faster than conventional flame Atomic Absorption (AA) for a typical multi-element analysis.

Handling difficult matrices

The robust, magnetically excited microwave plasma source of a MP-AES handles difficult matrices with ease, including fuels and organic solvents, geochemical samples, fertilizers and foods. A vertically positioned torch gives the best performance with difficult samples, and features end-on axial viewing for excellent detection limits.

Remote operation

Requiring only electricity, an MP-AES can be located at a sampling point, instead of in a lab. This allows measurement turnaround to be much quicker, delivering timely data that could deliver huge benefits, such as preventing environmental spills or incorrectly manufactured products.

Fast and easy to use

Agilent's MP-AES instruments feature easy-to-use, application-specific software applets that automatically load a pre-set method so one can start analysis immediately without method development or alignment, and with minimal training. The instrument´s torch loader automatically aligns the torch and connects gases for fast start up and

2.9. Sample preparation procedure

2.9.1. Solid samples

Compared to liquids, preparation of solid samples is more complex. In general, unless the analytical method involves direct analysis of solid samples, they need to be in solution before analysis. Many types of solid samples are converted into aqueous solution and therefore dissolution of sample matrices prior to determination is a vital stage of analysis aimed at releasing analytes into simple chemical form. The composition of sample matrices varies from purely inorganic (*e.g.*, ash, rocks, metallurgical samples) and purely organic (*e.g.*, fats) to mixed matrices (*e.g.*, soils, sediments, plant and animal tissues). Dissolution of inorganic matrices leads to clear solutions, where analytes are in their ionic forms. Both, purely organic and mixed matrices are more troublesome and dissolution does not guarantee complete matrix decomposition. Of the methods for total decomposition of organic samples and normally used for sample preparation are: (1) dry ashing and (2) wet digestion procedures.

2.9.2. Dry ashing

Dry oxidation or ashing eliminates or minimizes the effect of organic materials in mineral element determination. It consists of ignition of organic compounds by air at atmospheric pressure and at relatively elevated temperatures (450-550°C) in a muffle furnace. Resulting ash residues are dissolved in an appropriate acid. Dry ashing presents several useful features: (1) treatment of large sample amounts and dissolution of the resulting ash in a small acid volume result in element preconcentration; (2) complete destruction of the organic matter, which is a prerequisite for some detection techniques (3) simplification of the sample matrix and the final solution condition (clearness, colourless and odourless); (4) application to a variety of samples. Nevertheless, dry ashing presents either some limitations: (1) high temperature provokes volatilization losses of some elements; to avoid losses of volatile As, Cd, Hg, Pb and Se, and

improve procedure efficiency, ashing aids (high-purity $Mg(NO_3)_2$ and MgO) are used; (2) on the other hand, the addition of ashing aids significantly increases the content of inorganic salts, which may be a problem in subsequent determinations of trace elements and contribute to contamination that necessitates careful blank control; (3) it does not ensure dissolution of silicate compounds and consequently of all elements associated with them (it can be encountered during plant analysis); after a procedure without elimination of Si (by evaporation with HF), poor recoveries for some elements can be observed, particularly traces; (4) open dry ashing exposes samples to airborne contamination [67, 68].

2.9.3. Wet digestion

Wet digestion is used to oxidize the organic part of samples or to extract elements from inorganic matrices by means of concentrated acids or their mixtures. Commonly it is carried out in open vessels (in tubes, in beakers, on a hot plate, in a heating block) or in closed systems at elevated pressure (digestion bombs) using different forms of energy: thermal, ultrasonic and radiant (infrared, ultraviolet and microwave) [67, 68].

Compared to dry ashing, wet digestion presents a wide range of varieties, concerning the choice of reagents as well as devices used. However, the sample nature and its composition as well as the composition and concentration of the reactive mixture should be considered before analysis. It includes: strength of the acid, its oxidizing power and boiling point, solubility of resulting salts, safety and purity of the reagent. In general, HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$, HF and H_2O_2 are used for organic samples, alloys, minerals, soils, rocks and silicates. At present, the mixture of HNO_3, H_2SO_4 and H_2O_2 is a very efficient medium for different wet digestion procedures. Main disadvantages associated with the use of H_2SO_4 are its tendency to form insoluble compounds and its high boiling point. The high boiling point makes difficult to remove its excess after completion of oxidation. While $HClO_4$ is a strong oxidizing agent, it is extremely hazardous. Aqua regia (HCl with HNO_3 (3:1)) is widely used to dissolve soils, sediments and sludges. Main problems associated with wet digestion methods are: (1) much lower temperatures as compared to dry ashing procedures, however minimizing volatilization, they may lead to incomplete solubilisation of sample constituents and (2) co-precipitation of analytes. They both represent a real danger concerning reliability of analysis and hence, a good choice of a procedure and adequate reagents is critical for QA/QC of results.

3. Experimental

3.1. Collection and preparation of garage soil samples

3.1.1. Collection of samples

The samples of garage soil were collected in a clean polyethylene plastic from Addis Ababa city from three different garages located at Zenebework, Amanuel (near "autobus tera") and Kotebe. These sampling sites were chosen because of geographical location. Kotebe is located at the highland of the city than zenebework and amanuel. Zenebework is relatively located at lowland. The soil contents might depend on geographical factors, also relative activities of these garages and duration; hence different results were expected. At each sampling site, approximately 0.5 kg of soil was collected at 0-10 cm depth using a stainless steel sampler. The sample size was reduced to 100 g for the next step.

3.1.2. Preparation of samples

100 g of the prepared samples were taken and kept in a clean plastic and then dried in an open air for several days (more than one week) in the laboratory in the direction of sunlight instead of oven drying because each soil sample were much oily from the source they were obtained. The organic components are expected to undergo ignition and damage if oven dried. Samples were thus dried naturally by sunlight energy. Then after each dried sample was powdered using mortar and pestle and sieved. 5 g from each of the three samples was mixed together homogenously and from which 0.5 g was weighed 18 times using digital balance for optimization process.

3.2. Materials and reagents

Mortar and pestle was used to grind and powder the dried soil samples. Clean plastics were used to keep the sample. A digital analytical balance with ± 0.0001 g precision was used to weigh soil samples. 250 ml round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus to digest the dried and powdered soil samples. Reagents that were used in the analysis were all analytical grade. HNO_3 (69%) and HCl (37%);1:3 v/v were used for digestion of soil samples. Stock standard solutions containing 1000 mg/L of the metals Zn, Cr, Cu were used for the preparation of calibration standards and spiking experiments. Throughout the experiment, dilution and rinsing apparatus prior to analysis and during analysis, de-ionized water was used.

3.3. Instrumentation

An Agilent 4200 Microwave Induced Plasma Atomic Emission Spectrometer (MP-AES) (Agilent Technologies, Melbourne, Australia) equipped with an Inert One Neb nebulizer and a double-pass glass cyclonic spray chamber (Agilent Technologies, Melbourne, Australia) were used for the analysis of the analyte metals (Pb, Cr, Zn, Cu, Al) throughout this study.

Figure 3. MP-AES Instrument

3.4. Digestion procedures

3.4.1. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinders, digestion flasks and all the necessary glass ware used for the experiment were washed with detergents and tap water, rinsed with de-ionized water, soaked in 10% nitric acid for 24hrs, rinsed with de-ionized water many times and kept in dust free place until analysis.

3.4.2. Optimization and digestion of soil samples

3.4.2.1. Optimization

Optimization is the way or reaction condition with some specified set of parameters to obtain maximum possible result. For elemental analysis of the soil samples optimization was done using parameters; volume, temperature, and time change on a Kjeldahl digestion apparatus.

0.5 g each of the prepared dried garage soils were placed in the six cleaned 250 ml round bottom flask, and then 1:3 v/v nitric acid (69%) and 37% hydrochloric acid (aqua ragia) added in a varying volume from 3 ml to 8 ml. Each mixture was digested in a Kjeldahl apparatus with a constant temperature and time of 300^0C and 3 hr. The digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 5 min after removing the condenser and then filtered through acid washed Whattman filter paper into a 50 ml volumetric flask and diluted to the mark. The same processes were conducted at by varying temperature and time respectively. In each sequential process, the optimal volume, temperature and time were determined respectively based on the clearness of the digested sample solution. During the process, 5 ml aqua rage, 300^0C and 3 hr were determined as the optimal condition and aqua ragia is already reported as widely used reagent for soils, sludges and sediments [67, 68]

3.4.2.2. Digestion of soil samples

Based on the pre-determined optimal condition (volume, temperature, time); 5 ml aqua ragia, 300^0C and 3hr; 0.5 g of soil from each sample were transferred into a 250 ml round bottomed flask, then aqua ragia (1:3 v/v HNO_3 and HCl) was added and the mixture was digested on the Kjeldahl digestion apparatus. The same digestion procedure was followed as optimization. The sample solution was then aspirated into the Atomic Emission Spectroscopic machine (MP-AES) at intervals.

Figure 4 Soil samples, Kjeldahl digestion apparatus and digested solution

3.5. Determination of metals

For the preparation of calibration curve a series of four working standards were prepared for each metal from the intermediate standards. These working standards were prepared fresh for each metal from the intermediate standards by appropriately diluting with de-ionized water for calibration purpose Then; Zn, Pb, Cr , Cu, Al were analyzed with MP-AES, three replicate determinations were carried out for each sample. The metals were determined by emission intensity and then, the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of metals in the six digested blank solutions.

3.6. Recovery test

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. The efficiency of the optimized procedure was checked by adding known concentration of each metal into 0.5 g sample. Then the same digestion process was followed as for the samples. Each recovery test for the sample was performed in triplicates. The percentage recovery of the spiking experiment was calculated using the equation below.

$$\%R = \frac{C_M \text{ in the spiked sample} - C_M \text{ in the non spiked sample}}{C_A} x\ 100\% \quad \text{----- (1)}$$

Where: %R - percentage of recovered amount.

C_M - Concentration of the metal and

C_A- the amount spiked

4. Results and discussion

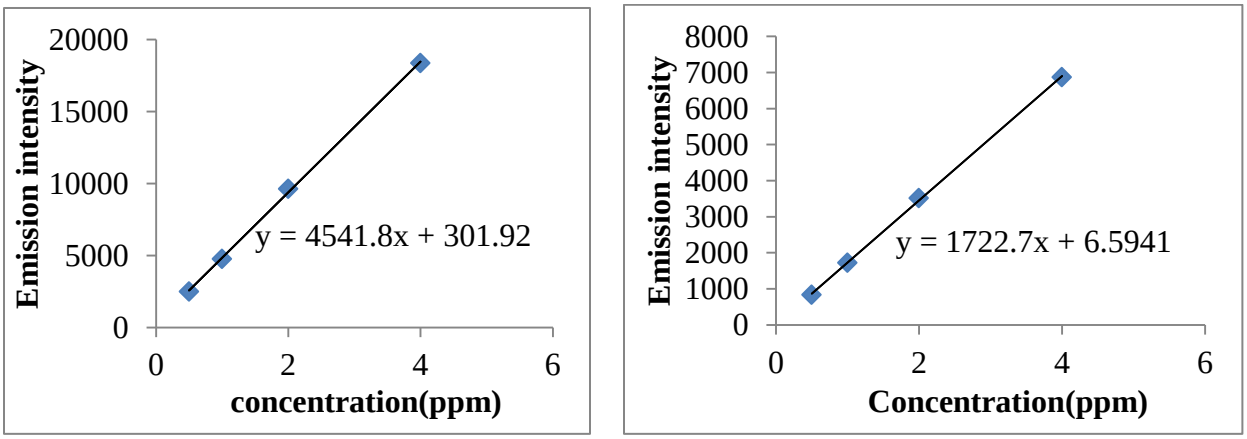
4.1. Instrument calibration

The qualities of results obtained for metals analysis using MP-AES are seriously affected by the calibration and standard solution preparation procedures. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared fresh by diluting the intermediate standard solutions. Concentrations of the working standards and value of correlation coefficient of the calibration graph for each metal is shown in Table 1.

Table 2.Concentration of working standards and correlation coefficients of metals

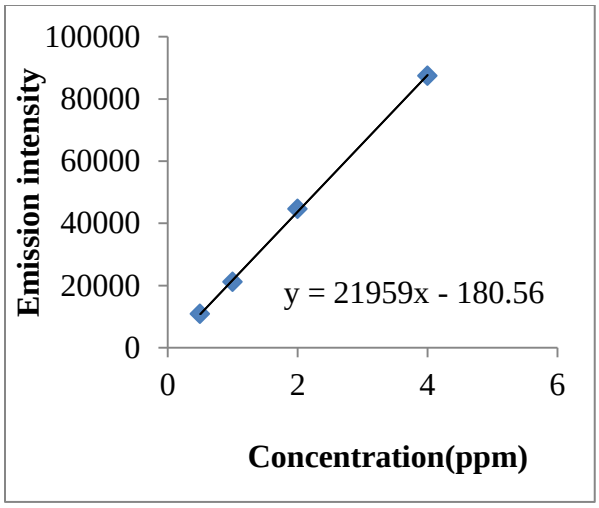
Metals	Concentration of Working standards(ppm)	Correlation coefficient (R^2)
Zn	0.5, 1, 2, 4	0.999
Pb	0.5, 1, 2, 4	0.999
Cr	0.5, 1, 2, 4	0.999
Cu	0.5, 1, 2, 4	0.999
Al	0.5, 1, 2, 4	0.999

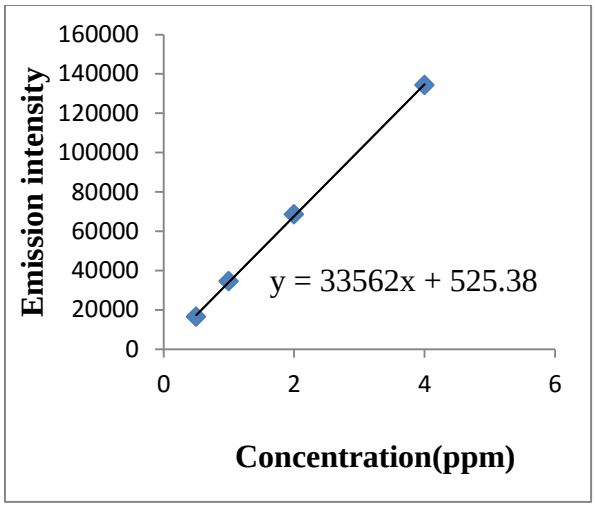
The calibration graph of each of metal standard solutions is shown in Figure 4-6



(A) Calibration curve for zinc (Zn) (B) Calibration curve for lead (Pb)

Figure 5. Calibration curve of zinc (Zn) and lead (Pb)





(C) Calibration curve for chromium (Cr) (D) Calibration curve for copper (Cu)

Figure 6. Calibration curve of chromium and copper (Cu)

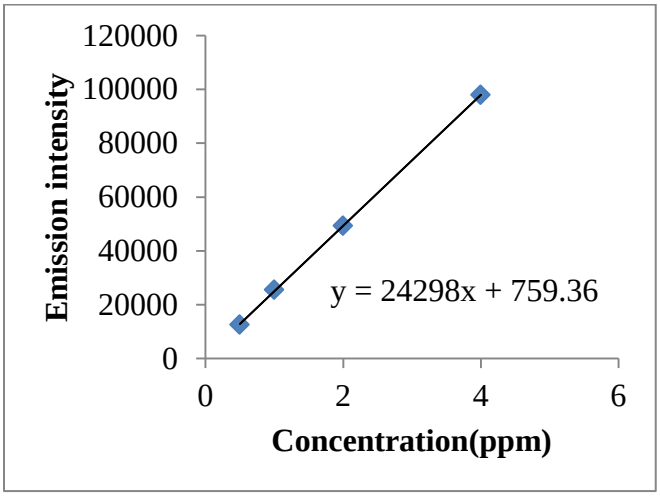


Figure 7. Calibration curve for aluminium (Al)

4.2. Method validation

The validation parameters included in this study were linearity, accuracy, precision and detection limit (DL). The validation method followed the protocol guidelines on validation of analytical methods (69).

4.2.1. Linearity

Linearity is the ability of a method to elicit test results that are directly proportional to analyte concentration within a given range. The equation of the calibration curve that relates the dependent (y) and independent (x) variables is $y = ax+b$. The mathematical method known as

linear regression was used to estimate the coefficients of the calibration curve, from one set of experimental measurements. The value of 0.99 was used for the coefficient of correlation (R^2) as the limit for setting the coefficients of the curve.

As shown in table 2 the linearity of the calibration curves in each standard were acceptable since the correlation factors (R^2) is greater than 0.99

4.2.2. Precision

Precision is an analytical parameter called variability. It expresses the variations within the same laboratory, when the same sample is analyzed by the same procedure, by instrument and/or different analysts in different time interval. The variation or error in analytical results must be evaluated by applying different statistical methods. Most of the common statistical methods applied in analytical chemistry are the standard deviation, variance, coefficient of variance, relative standard deviation and range of series of measurements. In this particular study the results of the measurements are expressed as the mean of the measurements together with the standard deviation of the triplicate samples with triplicate measurements of each sample [70]. The mean, standard deviation and relative standard deviation were calculated mathematically as follows (equations 2, 3 and 4).

$$\text{Mean} = \frac{\sum_{i=1}^{n} Xi}{n} \quad \text{.....(2)}$$

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{n} (Xi - mean)}{n-1}} \quad \text{.....(3)}$$

$$\text{RSD} = \frac{SD}{mean} \quad \text{.....(4)}$$

4.2.3. Method detection limit (MDL)

The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results [69]. Limit of detection was estimated from the calibration function for a signal equal to the net signal of blank and three times its standard deviation [71]. In this study the detection limit of each element; Zn, Cr, Cu and Al was calculated as three times the standard deviation of the blank as expressed in equation 5. The MDL for each metal is expressed below in Table 3 except lead which is below the detection limit.

$MDL = 3SD_{blank}$……..(5)

Table 3. Method detection limit for all metals determined in soil samples

Element	Zn	Pb	Cr	Cu	Al
MDL(ppm)	0.192	BDL	0.001671	0.024	0.164

BDL- Below detection limit

4.2.4. Accuracy

Accuracy of an analytical method, also called recovery, is defined as the closeness between the true value of the analyte in the sample and the value obtained by the analytical procedure and is expressed as percentage recovery (%R). It is a parameter that ensures that no loss or contamination occurred during the test procedure, contributing to quantify the sample. The efficiency of the method was assessed by spiking the soil samples with known amounts of metals. Percentage recovery was calculated using equation (1), given on page 34

Table 4. Analytical result for recovery test (mg/kg) of each sample mean.

Metals	Concentration of Non-spiked sample (mg/kg)	Concentration of amount added(mg/kg)	Concentration of spiked sample (mg/kg)	%Recovery
Zn	422	84	496	88
Cr	58	46.4	110	112
Cu	71.4	28.6	96	86

4.3. Determination of metals in soil sample

The accuracy and precision of the results were checked by the aid of different statistical methods after the determination of the levels of metals in the soil samples. The concentration of three heavy metals (Zn, Cr, Cu) and the light metal; Aluminium (Al) in the digested and diluted solutions of soil samples were analyzed with MP-AES. The mean values were determined from triplicate analysis of each sample and triplicate samples were used from each soil sampling. The results are reported in terms of mean values (X) ± SD (standard deviation), for all the metals in this study. Among all the determined metals the concentration of lead was below detection limit of the instrument, hence it was not calculated.

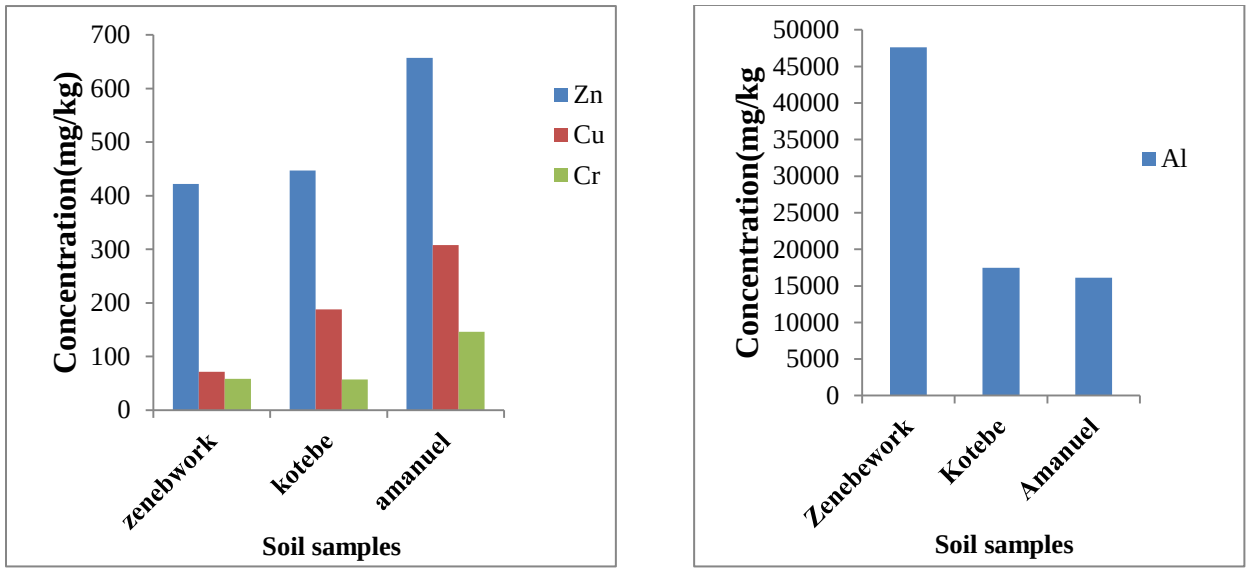
The levels of metals analyzed are shown in Table 5 with their respective percentage RSD. The %RSD was calculated using the equation below.

$$\%RSD = \frac{SD}{X(mean)} \mathbf{x100\%} \quad \text{..... (6)}$$

Table 5.Concentration (mg/ kg) and (%RSD) of metals in soil samples

Metals	Zenebe work soil		Kotebe soil		Amanuel soil	
	Mean ± SD	%RSD	Mean ± SD	%RSD	Mean ± SD	%RSD
Zn	422 ± 31.8	7.5	447 ± 20	4.5	657 ± 42	6.4
Cr	58 ± 6.8	11.7	57.3 ± 4.5	7.8	146 ± 12.8	8.8
Cu	71.4 ± 6.1	8.6	188 ± 6	3.2	307.5 ± 14.4	4.7
Al	47601 ± 2350	5	17486 ± 954	5.4	16132 ± 664	4.1
Pb	BDL	-	BDL	-	BDL	-

Relative concentrations of the metals in the three samples are presented in the figures 8 shown below



(A) Comparison among trace metals (B) Comparison between Aluminium

Figure 8. Comparison among trace metals and Aluminium

4.4. Comparison of Metals within soil Samples

When the concentrations of metals in the soil sample were compared, aluminium is very much higher concentration in all the three soil samples compared to the heavy metals; Zn, Cr, Cu and much higher in zenebework garage soil sample which may be due aluminum is the third most abundant element and the first most abundant light metal in the earth's crust. In addition to that the extent of relative activities in each garage and the geographical location makes difference between each sample. The heavy metals (Zn, Cr, Cu) contents of Amanuel garage soil sample is much higher than Zenebework and Kotebe. The concentration of thus metals is above the limits of FAO/WHO except Cr and Cu in Zenebework sample.

4.5. Statistical Analysis

Statistical analysis is used to check whether there is contribution from random errors for difference in results of the analysis or not. If there are differences, statistical analysis will tell us whether the differences are significant or not at a specified confidence level.

One way Analysis of Variance (ANOVA) is used to perform the statistical analysis with soil as independent and concentration of the metals as dependent variable to test whether there are significant differences between means of each soil types at the stated confidence level. Excel used to compare the statistical parameters and the results of analysis are shown in Table 6.

Table 6.Results of significance test (p-test) for mean comparison between the three samples of garage soil

soil sample compared A/K/Z.W	Metals compared at 95% confidence level and their p-values			
	Zn	Cr	Cu	Al
p-value	7.4×10^{-5}	5.87×10^{-6}	8.65×10^{-7}	7.87×10^{-7}

Z.W = Zenebework, K = Kotebe, A = Amanuel

The p-value in table 6 shows that there is significant difference in the levels of Zn, Cr, Cu, Al among the Amanuel, Kotebe & zenebework garage soils at 95% ($p < 0.05$) confidence level.

5. Conclusion and recommendation

The current fast rate of urbanization and industrialization adds toxic metals in soils which causes adverse effect on plants and animals, humans. Garages are one of the main sources of toxic metals (mostly heavy metals) that grow with urbanization and industrialization. Automobiles release a complex source of heavy metals into our environment. Addis Ababa city, the capital city of Ethiopia is one of the developing cities. Related to urbanization and industrialization, different toxic metal sourced wastes including garage waste of the city were discharged into different water ways (like Akaki River). Thus wastes affect mainly the neibouring cities and agricultural soils around the city. The concentration of the metals under study (Zn, Cr, Cu, Al) in the three garage soil samples obtained above the limits of World Health Organization (WHO) with the exception of Cr and Cu in zenebework sample. FAO/WHO limit (mg/kg); Zn = 300, Cr = 100, Cu = 100, which affects the soil quality and human health. The impact of soil pollution is found to be more hazardous for developing countries like Ethiopia due to lack of proper consideration and management. A lots of study showed, exposure to heavy metal would result in reduced oxygen flow in red blood cells, disruption of ecosystem function, increased risk of chest pain for person with heart disease and death. Therefore, in order to reduce the contamination of soil by heavy metals around garage, appropriate areas should be selected like developing garage and auto-mechanical zone, regulation and management of hazardous waste released from garage should be carried out by environmental authority and trade and industry bureau as well. Furthermore, application of phytoremediation is also required in order to reclaim extremely contaminated soils.

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