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Sequential Extraction/Fractionation of Selected Heavy Metals and Their Bioavailability in the Wastewater, Sewage Sludge and Soil Samples of Saygin Dima Textile and Nefas Silk Paint Factories

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By

Shisho Haile Geleta

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Sequential Extraction/Fractionation of Selected Heavy Metals and Their Bioavailability in the Wastewater, Sewage Sludge and Soil Samples of Saygin Dima Textile and Nefas Silk Paint Factories

Shisho Haile Geleta

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This is to certify that the thesis prepared by Shisho Haile, Sequential Extraction/Fractionation of Selected Heavy Metals and Their Bioavailability in the Wastewater, Sewage Sludge and Soil Samples of Saygin Dima Textile and Nefas Silk Paint Factories and submitted in partial fulfillment of the requirements for Degree of Masters of Science in Chemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Dr. Negussie Megersa ______________ ______________________
Advisor Signature Date

Dr. Merid Tessema ______________ ______________________
Examiner Signature Date

Prof. B. S. Chandravanshi ______________ ______________________
Examiner Signature Date

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Chair of Department or Graduate Program coordinator

Abstract

This study focuses on the sequential extraction/fractionation of some heavy metals from industrial wastes of Saygin Dima Textile and Nefas Silk Paint factories in Ethiopia. The investigated heavy metals were Cd, Cr, Cu, Ni, Pb and Zn. Both total concentration and fractional concentration level of the selected heavy metals were determined for comparison of their concentration level and their mobility in the soil. Digestion was used for sample preparation to determine the total concentration and the six step fractionation procedure developed by Tessier was followed for fractional concentration determination. Finally, the concentration level of selected heavy metals was determined by Flame Atomic Absorption Spectrometer (FAAS) and compared with permissible international standard concentration limit.

The experimental results indicated that, Cd was not detected in both total concentration level determination and fractionation. Ni was detected only in total concentration determination. Pb may be mobile in NPSP sample while Zn may be mobile in NPSD, NPSP and SPS samples. Both of these heavy metals are available for animals and plants from the soil polluted by the waste of Nefas Silk paint factory. In addition to this, Zn may be available for animal and plants from the soil polluted by the waste of Saygin Dima textile while the rest heavy metals are not bioavailable from the waste of both factories. The concentration of Cr in NPSD and NPSP, Ni and Cr in SSS, Pb in NSS, and both Ni and Pb in the soil samples of both factories is above permissible international standard concentration limit. Therefore, an appropriate treatment plant must be implemented to minimize the concentration of Ni in the Saygin Dima textile sewage sludge, Pb in Nefas Silk paint factories sewage sludge and both elements in the polluted soil samples of both factories.

Key words: sequential extraction/fractionation, mobility, bioavailability, sewage sludge, wastewater, heavy metals, soil samples, factory

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List of Abbreviations

CBR	Community Bureau of Reference
CEC	Cation Exchange Capacity
DDW	Double Distilled Water
DIW	Deionized Water
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrometer
GSC	Geological Society of Canada
HCL	Hollow Cathode Lamp
ICP-AES	Inductively Coupled Plasma- Atomic Emission Spectrometer
MF	Mobility Factor
PMT	Photoelectron Multiplier Tube
NPSD	Nefas Silk paint factory Polluted Soil from Distance
NPSP	Nefas Silk paint factory Polluted Soil from Point
NSS	Nefas Silk paint factory Sewage Sludge
NTWD	Nefas Silk paint factory Treated Water from Distance
NTWP	Nefas Silk paint factory
NUW	Nefas Silk paint factory Untreated Water Treated Water from Point
RSS	Reference Soil Sample
SEP	Sequential Extraction Procedure
SPS	Saygin Dima textile factory Polluted Soil
SSS	Saygin Dima textile factory Sewage Sludge
STW	Saygin Dima textile factory Treated Water
SUW	Saygin Dima textile factory Untreated Water

1. INTRODUCTION

1.1. Industrial Wastewater

Wastewater from manufacturing or chemical processing factories contributes to water pollution. Industrial waste water usually contains specific and readily identifiable chemical compounds (Lokhande et al., 2011). One of the main causes of industrial pollution is the discharge of effluents containing heavy metals. Since most of the heavy metals are nonrenewable and non-degradable into non-toxic end products, effective recovery of heavy metals is as important as their removal from waste streams. Disposal of industrial wastewater has always been considered as the major environmental issue. Pollutants in industrial wastewater are almost invariably so toxic that wastewater has to be treated before its reuse or disposal in water bodies.

Industrial processes generate wastewater containing heavy metal contaminants that is their concentrations must be reduced to the acceptable levels before discharging them into environment. Otherwise these could pose threats to public health and/or affect the aesthetic quality of waters (Badmus et al., 2007). Major contributors to heavy metal water pollution are tanneries, brewery, textile, pottery, electroplating, metal finishing, mining, dyeing and printing factories, ceramic, photographic and pharmaceutical factories (Rauf et al., 2009).

1.2. Sewage Sludge of the Industrial Waste

Sewage sludge is defined by the U.S. Environmental Protection Agency (EPA) as the “solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works” (Mathney, 2011). Hence, it is a by-product of wastewater treatment process, (Jia-yin et al., 2006) during which liquids and solids are being separated (Fytili and Zabaniotou, 2008).

Industrial waste sewage sludges are the sink for trace metals, as a result of adsorption, precipitation, diffusion processes, chemical reactions, biological activity and a combination of these phenomena. When some physical disturbance occurs, changes in pH or redox potential, they can become a source of metals, releasing them in the overlying water column. This phenomenon can occur even long after the end of the direct discharge and its extent depends on the metal association with different mineralogical fractions of the sludges (Turki, 2007).

Very common heavy metals found in sludge include Cd, Zn, Cu, Cr, Co, Pb, Mn, Ni, Hg, etc. (Adyel et al., 2012). The source of heavy metals in wastewater, whether domestic or industrial, also has profound effects on the total content as well as chemical fractions of heavy metals in sludge (Wang et al., 2005). Several methods for determining the different forms of these metals in sludges are described in scientific literatures. The most widely used methods are based on sequential extraction procedures, where several reagents are used consecutively to extract operationally-defined phases from the sludge in a set sequence (Turki, 2007).

1.3. Polluted Soils by Industrial Wastes

Heavy metals are rather loosely defined group consisting of mainly transition metals (Bates, 2000). They constitute a group of about 40 elements and are considered to be one of the main sources of the environmental pollution, since they have a significant effect on its ecological quality (Al-Oud et al., 2011). A feature of heavy metal physiology is that even though many of them are essential for growth, they are also reported to have comprehensively toxic effects on cells, mainly as a result of their ability to denature protein molecules (Bates, 2000). They are elements that have more than 4.5 g/cm^3 density (Gawdzik and Gawdzik, 2012). Very common heavy metals found in include Cd, Zn, Cu, Cr, Co, Pb, Mn, Ni, Hg, etc. (Filgueiras et al., 2002).

Although the natural presence of heavy metals is limited to a few soil habitats, they are locally present as a consequence of human activities including mining, processing of the extensive use of metals. These activities typically result in soils with gradients of metal distribution where heavy metals are often present as a mixture. In contrast to organic contaminants, heavy metals are nondegradable, and although they exhibit some mobility in the environment, the contamination is usually relatively stable over time (Sherameti and Varma, 2009). Hence, they cannot be destroyed but can only be transformed from one oxidation stage or organic complex to another (Marques et al., 2009).

Waste disposal whether domestic, commercial or industrial in the world, is a problem that continues to grow with human civilization and none of the waste disposed so far is completely safe.

Experience has shown that all forms of waste disposal have negative effects on the environment, public health and local economics, pointed out that pollution in soil systems is strictly related to human activities such as factory, agriculture, burning of fossil fuels, mining and metallurgical processes and their waste disposal (Okeyode and Rufai, 2011).

Toxic elements, such as heavy metals can be retained by soils and/or mobilised to soil solution by biological and chemical mechanisms with a potential impact on human health (contamination of drinking water supplies, uptake by vegetation and input into the food chain) (Okeyode and Rufai, 2011). Sorption is an important chemical process that regulates partitioning of trace elements between solution and solid phases in soil (Berbecea et al, 2010). Even though heavy metals occur naturally in the environment (Wuana and Okieimen, 2011**)**; they are of great concern when they were continuously added (Okeyode and Rufai, 2011).

Heavy metal pollution refers to cases where the quantities of these elements are higher than the maximum allowable concentrations and this is potentially harmful to biological life at such locations (Adelekan and Abegunde, 2011). Due to rapid industrialization, the soils in the industrial areas are polluted by various toxic substances such as heavy metals (Shivakumar et al., 2012).

Soil contamination with heavy metals is an issue of concern worldwide. Consequently, several studies were carried out intensively to investigate contaminated soils for evaluating the level of contamination in relation to human health and/or environmental conservation. It is also very important to investigate the process of soil contamination itself with special attention to available fractions because it is directly related to the management and prevention of soil contamination (Yanai et al., 2010). Pollution of the environment with toxic heavy metals has increased dramatically since the onset of the industrial revolution (Onder et al., 2007).

The concentration of heavy metals in soil is associated with biological and geochemical cycles and is influenced by anthropogenic activities such as agricultural practices, industrial activities, and waste disposal (Zauyah et al., 2004).

The toxicity and the mobility of heavy metals in soils depend not only on the total concentration, but also on their specific chemical form, their binding state, the metal properties, environmental factors and soil properties such as pH, organic matter content and type, redox conditions, root exudates acting as chelates (Ghrefat et al., 2012) and cation exchange capacity (CEC) (Aydinalp and Marinova, 2003).

Soils are the reservoir for many harmful constituents, elemental and biological, including heavy metals and trace metals, hence forth referred to as just metals (Zimmerman and Weindorf, 2010). Metals in soil can be divided into two fractions: (i) Inert fraction assumed as the non-toxic fraction and (ii) The labile fraction assumed to be potentially toxic. To assess the availability of heavy metals, only the soil of labile fraction is taken into account because this fraction is often called by extension, the bioavailable fraction (Aikpokpodion et al., 2012).

The behavior and fate of heavy metals are governed by a range of different physicochemical processes, which dictate their availability and mobility in the soil system. In the water phase, the chemical form of a metal determines the biological availability and chemical reactivity (sorption/desorption, precipitation/dissolution) towards other components of the system. The binding form in the solid phase is related to the kinetics and equilibriums of the metal released to the liquid phase and hence the likelihood of mobility and bioavailability (Tack and Verloo, 1995).

The determination of the total concentration of an element is often only an initial step. If the pathways, mobility, bioavailability and potential toxicity of the elements to humans and animals are to be elucidated, it is necessary to obtain information on the different chemical forms of the elements in the samples (Maina et al., 2012). Metal cations in soils may be present in several different physicochemical forms (i) as simple or complex ions in soil solutions (ii) as easily exchangeable ions (iii) carbonates (iv) occluded or co-precipitated with metal oxides, or phosphates and other secondary minerals, (v) organically bound or (vi) as ions in a crystal lattice of primary minerals (Jopony, 1985). Heavy metals present in each category have different mobility behaviors in environment (Adamma et al., 2013). Metal cations present in soil in the first three forms are considered to be most available to plants while the other forms are less available. Each form, except for the most unavailable ones may presumably be estimated by an extraction procedure (Jopony, 1985).

It is generally recognized that information about the physicochemical forms of the elements is required for understanding their environmental behaviors (mobility, pathways and bioavailability) (Bates, 2000). Chemical speciation can broadly be defined as the identification and quantification of the different species, forms or phases in which an element occurs (Fuenteset al., 2003).

The term "fractionation" is frequently used interchangeably with speciation but emphasizes the concept of subdividing a "total content". Furthermore, the analytical preparations for separating metal species are referred to as "fractionation" (Tack and Verloo, 1995). Operationally, the definition of speciation includes simple and sequential extraction to relate the species associated with particular phases of the samples.

Different species, forms or phases present in a sample of material can be defined as:

(1) Functionally, those species that can be assimilated by plants,

(2) Operationally, according to the procedures or reagents used in their extraction and

(3) Specifically, as particular components or oxidation states of an element (Fuenteset al., 2003).

Therefore, the determination of heavy metals and their impact in the environment were broadly studied in the last years, the success of which usually requires two procedures namely, digestion and extraction (Marın et al., 2001). Acid digestion usually renders all metal species into the same form and therefore cannot be used for speciation analysis (Zeiner et al., 2007). It poses organic matter destruction to form more soluble metal compounds. On the other hand, extraction permits speciation or at least the measurement of specific metal fractions (Marın et al., 2001).

Metals are distributed throughout soil components and are associated with them in various ways including ion exchange, adsorption, precipitation and complexation (Uwumarongie-Ilori and Okieimen, 2011). The extraction methods used for determination of metals in soil can be accomplished via single reagent leaching, ion-exchange resins and sequential extraction procedures (Mohammed et al., 2012). Single and sequential extraction schemes have also been designed for the determination of binding forms of trace metals in solid samples (Dalmacija et al., 2010).

These methods are based on the rational use of a series of more or less selective reagents chosen to solubilise successively the different mineralogical fractions of soils (Quevauviller, 2002).

Many of the sequential extraction methods are, in fact, variants of the Tessier procedure, in which the exchangeable metals and those nominally associated with carbonate, Fe-Mn oxides, organic material, and silicate residues are extracted with different reagents (Dalmacija et al., 2010).

Information given by sequential extraction is noteworthy and despite the drawbacks aforementioned for the application of extraction schemes, an increasing number of publications have appeared in the literature. General applications of sequential extraction schemes can be summarized as follows:

(i) Characterization of pollution sources

(ii) Evaluation of metal mobility and bioavailability

(iii) Identification of binding sites of metals for assessing metal accumulation, pollution and transport mechanisms (Filgueiras et al., 2002).

1.4. Objective of the Study

1.4.1. General Objective

- To quantify the chemical form that determines heavy metal behavior in the environment mobility its bioavailability.

1.4.2. Specific Objectives

The specific objectives of this study are to:

- Determine the level of chemical form of the heavy metals in soil samples affected by wastewaters, waste water effluent and sewage sludge from the different operation units of Nifas Silk Paint factory and Saygin Dima Textile factory.
- Compare and correlate the physicochemical parameters and heavy metals quantity in surface soils.
- Identify the bioavailability of selected heavy metals found from the supernatant solution and residue solid samples.

2. LITERATURE REVIEW

2.1. Sources of Metals in the Soils

The background concentration of metals in natural soil depends primarily on the bedrock type from which the soil parent material was derived. In addition, anthropogenic inputs may increase metal concentrations, especially in highly industrialized parts of the world producing heavy metals (Maldonado et al., 2008).

Weathering of rock and anthropogenic sources are the two main pathways of metal input to soils. Anthropogenic sources of metal contamination can be divided into five major groups:

1. Metalliferous mining and smelting (arsenic, cadmium, lead, and mercury).
2. Factory (arsenic, cadmium, chromium, cobalt, copper, mercury, nickel, and zinc).
3. Atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury and uranium).
4. Agriculture (arsenic, cadmium, copper, lead, selenium, uranium and zinc).
5. Waste disposal (arsenic, cadmium, chromium, copper, lead, mercury and zinc) (Heidary-Monfared, 2011).

2.2. 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. Those metals are important since they are capable of decreasing crop production due to the risk of bioaccumulation and biomagnifications in the food chain. There is also the risk of superficial and groundwater contamination. Knowledge of the basic chemistry, environmental and associated health effects of these metals is necessary in understanding their speciation, bioavailability and remedial options. The fate and transport of a heavy metal in soil depends significantly on the chemical form and speciation of the metal. Once in the soil, heavy metals are absorbed 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. This distribution is believed to be controlled by reactions of heavy metals in the 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 (Wuana and Okieimen, 2011).

Metals of concern in this study are cadmium (Cd), Chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) and these are briefly described in the following section (Wuana and Okieimen, 2011).

2.2.1. Cadmium (Cd)

Cadmium is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g/cm^3, melting point 320.9^{O}C, and boiling point 765^{O}C. Together with Hg and Pb, Cd is one of the big three heavy metal poisons and is not known for any essential biological function (Wuana and Okieimen, 2011). Cadmium (Cd) occurs naturally in the form of CdS or $CdCO_3$. Cadmium is recovered as a by-product from the mining of sulfide ores of lead, zinc and copper. Sources of cadmium contamination include plating operations and the disposal of cadmium-containing wastes (Evanko and Dzombak, 1997). The major threat to human health is chronic accumulation in the kidneys leading to kidney dysfunction. Food intake and tobacco smoking are the main routes by which Cd enters the body (Wuana and Okieimen, 2011).

Cadmium is relatively mobile in surface water and ground-water systems and exists primarily as hydrated ions or as complexes with humic acids and other organic ligands. Under acidic conditions, cadmium may also form complexes with chloride and sulfate. Cadmium is removed from natural waters by precipitation and sorption to mineral surfaces, especially oxide minerals, at higher pH values (> pH 6). Removal by these mechanisms increases as pH increases. Sorption is also influenced by the cation exchange capacity (CEC) of clays, carbonate minerals, and organic matter present in soils and sediments. Under reducing conditions, precipitation as CdS controls the mobility of cadmium (Evanko and Dzombak, 1997).

2.2.2. Chromium (Cr)

Chromium can occur in each of the oxidation states from -2 to +6; however, only the +2 (divalent), +3 (trivalent), and +6 (hexavalent) states occur naturally. Chromium compounds in the divalent state are relatively unstable and are easily oxidized to trivalent compounds (Aplin, 2009).

Chromium in contaminated soil is mainly present in two stable forms: trivalent, Cr(III) which is low toxic and hexavalent, Cr(VI) (Palma et al., 2012). Cr(VI) is the more toxic form of chromium and is also more mobile. Cr(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 $Cr(OH)_3$ (Evanko and Dzombak, 1997). Cr(VI) is the product of oxidation of Cr(III) with atmospheric oxygen. The Cr(III) reactivity increase when the inert crystals and amorphous mineral are transformed in organic and hydroxide forms, smaller and more mobile. The equilibrium between the two chromium forms in soil depends upon soil physical and chemical characteristics. The oxidation process is only controlled by the reaction kinetics, due to Cr(III) species immobility and insolubility. Cr(III) tends to be strongly bound by soil humic acid polymers, and this affinity restricts the availability of Cr(III) to be oxidized and reduce the organic matter decomposition (Palma et al., 2012).

Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content and the amount of organic matter present. Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form. Soluble and unadsorbed chromium complexes can leach from soil into groundwater. The leachability of Cr(VI) increases as soil pH increases. Most of chromium released into natural waters is particle associated, however, and is ultimately deposited into the sediment (Evanko and Dzombak, 1997).

2.2.3. Copper (Cu)

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^{O}C and boiling point 2595^{O}C. The metal's average density and concentrations in crustal rocks are 8.1 × 103 kg/m^3 and 55 mg/kg, respectively (Wuana and Okieimen, 2011). Copper occurs in four oxidation states of Cu, Cu^{1+}, Cu^{2+}, and Cu^{3+}. However the Cu^{2+} is the most common form (Heidary-Monfared, 2011).

Copper is an essential micronutrient required in the growth of both plants and animals. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain (Wuana and Okieimen, 2011).

The cupric ion (Cu^{2+}) is the most toxic species of copper. Copper toxicity has also been demonstrated for $CuOH^+$ and $Cu_2 (OH)_2^{2+}$ (Evanko and Dzombak, 1997).

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). The solubility of Cu is drastically increased at pH 5.5 (Wuana and Okieimen, 2011). The cupric ion, Cu^{2+}, and hydroxide complexes, $CuOH^+$ and $Cu(OH)_2$, are also commonly present. Copper forms strong solution complexes with humic acids. The affinity of Cu for humates increases as pH increases and ionic strength decreases. In anaerobic environments, when sulfur is present CuS will form (Evanko and Dzombak, 1997).

Solution and soil chemistry strongly influence the speciation of copper in groundwater systems. In aerobic, sufficiently alkaline systems, $CuCO_3$ is the dominant soluble copper species. Copper mobility is decreased by sorption to mineral surfaces. Cu^{2+} sorbs strongly to mineral surfaces over a wide range of pH values (Evanko and Dzombak, 1997).

2.2.4. Nickel (Ni)

Nickel is a transition element with atomic number 28 and atomic weight 58.69. In low pH regions, the metal exists in the form of the nickelous ion, Ni (II). In neutral to slightly alkaline solutions, it precipitates as nickelous hydroxide, $Ni(OH)_2$, which is a stable compound. This precipitate readily dissolves in acid solutions forming Ni(III) and in very alkaline conditions; it forms nickelite ion, $HNiO_2$, which is soluble in water. In very oxidizing and alkaline conditions, nickel exists in form of the stable nickelo-nickelic oxide, Ni_3O_4, which is soluble in acid solutions. Other nickel oxides such as nickelic oxide, Ni_2O_3, and nickel peroxide, NiO_2, are unstable in alkaline solutions and decompose by giving off oxygen. In acidic regions, however, these solids dissolve producing Ni^{2+} (Wuana and Okieimen, 2011).

Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of Ni is an ingredient of steel and other metal products. The major sources of nickel contamination in the soil are metal plating factories, combustion of fossil fuels, and nickel mining and electroplating.

It is released into the air by power plants and trash incinerators and settles to the ground after undergoing precipitation reactions. It usually takes a long time for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all Ni compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic soils, however, Ni becomes more mobile and often leaches down to the adjacent groundwater. Microorganisms can also suffer from growth decline due to the presence of Ni, but they usually develop resistance to Ni after a while. Nickel is not known to accumulate in plants or animals and as a result Ni has not been found to biomagnify up the food chain. For animals Ni is an essential foodstuff in small amounts (Wuana and Okieimen, 2011).

2.2.5. Lead (Pb)

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^{O}C, and boiling point 1725^{O}C (Wuana and Okieimen, 2011). Lead predominantly exists in the nature in its stable plumbous ion (Pb^{2+}) (Heidary-Monfared, 2011). It is a naturally occurring, bluish gray metal usually found as a mineral combined with other elements, such as sulphur (i.e. PbS, $PbSO_4$), or oxygen ($PbCO_3$). 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. Lead phosphates, lead carbonates and lead hydroxides insoluble compounds. Lead sulfide (PbS) is the most stable solid form within the soil matrix and forms under reducing conditions (Wuana and Okieimen, 2011).

The primary industrial sources of lead contamination include metal smelting and processing, secondary metals production, lead battery manufacturing, pigment and chemical manufacturing, and lead-contaminated wastes. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and lead-metal oxyanion complexes (Evanko and Dzombak, 1997).

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. Lead is not an essential element. It is well known to be toxic (Wuana and Okieimen, 2011).

Most lead that is released to the environment is retained in the soil. The primary processes influencing the fate of lead in soil include adsorption, ion exchange, precipitation, and complexation with sorbed organic matter. These processes limit the amount of lead that can be transported into the surface water or groundwater. The relatively volatile organolead compound tetramethyl lead may form in anaerobic sediments as a result of alkyllation by microorganisms. The amount of dissolved lead in surface water and groundwater depends on pH and the concentration of dissolved salts and the types of mineral surfaces present. In surface water and ground-water systems, a significant fraction of lead is undissolved and occurs as precipitates ($PbCO_3$, Pb_2O, $Pb(OH)_2$, $PbSO_4$), sorbed ions or surface coatings on minerals or as suspended organic matter (Evanko and Dzombak, 1997).

2.2.6. Zinc (Zn)

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^{O}C, and boiling point 906^{O}C. Its most common oxidation form is (Zn^{+2}) (Heidary-Monfared, 2011).

Zinc occurs naturally in soil, 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. Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems (Wuana and Okieimen, 2011).

Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values. At higher pH values, zinc can form carbonate and hydroxide complexes which control zinc solubility. It readily precipitates under reducing conditions and in highly polluted systems when it is present at very high concentrations, and may coprecipitate with hydrous oxides of iron or manganese (Evanko and Dzombak, 1997).

2.3. Analysis of Heavy Metals in the Soil Samples

Analysis of heavy metals in the soil takes a few forms and they depend on the purpose of the analysis. Firstly, it is the determination of the ‘total’ element contents in the soil. Secondly, analysis of strong acid digests of soil is carried out to understand the extent of heavy metal pollution accumulated in the topsoil and provide information on the long-term potential toxicity effects of pollution as well as of long-term potential deficiency situations where pollutant is not the issue. To further understand, for understanding the chemistry of heavy metals and their interaction with other soil components such as clay minerals, organic matter and soil solution, or to investigate their mobility and retention as well as their availability to plants, the usual approach is to use selective chemical extraction (Sabienë et al., 2004).

2.4. Sequential Extraction

Selective sequential extraction procedures were commonly used for studying metal mobility and their availability in the soils. The extraction scheme in this technique is based on operationally defined fractions: exchangeable, carbonate, Fe-Mn oxides, organic, and residual fractions (Aikpokpodion et al., 2012).

2.4.1. Different Sequential Extraction Procedures

2.4.1.1. Tessier Procedure

In the extraction procedure given by Tessier et al., 1 g of the sample is placed in a 50 mL tube. The sample is exposed to the appropriate reagents and shaken. Each fraction is then separated from the supernatant by centrifugation at 10,000 rpm, (≈12,000 gravity) for 30 min, and then the supernatant is collected for lab analysis. The sediment is rinsed with 8 mL of deionized water (DIW) and centrifuged again.

For the fourth fraction, a 1 g (dry weight) sample is exposed to 12 mL of 5:1 HF-$HClO_4$ acid mixture and evaporated to near dryness. A 10:1 HF-$HClO_4$ acid mixture is added to the sample and again evaporated to near dryness followed by 1mL of $HClO_4$, evaporated until white fumes are visible. The final digestion is performed with 12 N HCl and diluted to 25 mL (Zimmerman and Weindorf, 2010).

In the modified Tessier procedure, two soil samples are analyzed: one with moderate metal contamination and the other with heavy contamination. Fraction one is run as normal. The reagent used for fraction two is increased from 8 mL to 50 mL with continuous agitation for 5 hr. The reagent used in fraction three for the heavily contaminated soil is also increased to 50 mL with continuous agitation for 6 hr. Fractions four and five remain unchanged Rauret et al., determined that an increase in the amount of reagent used increased the concentration of metals extracted for fractions two and three. They determined that the level/type of contamination of the tested sediment had a direct effect on the results obtained and by increasing the amount of used solution from 8 mL and 20 mL respectively to 50 mL and were able to extract the maximum amount of metal without saturation (Zimmerman and Weindorf, 2010).

2.4.1.2. Community Bureau of Reference (CBR) Procedure

Based on the Tessier procedure, the Community Bureau of Reference (CBR) of the Commission of the European Union produced the definition of an extraction protocol and a purely operational definition of sequential fractionation and partitions the heavy metals into four fractions: exchangeable and carbonate bound, iron and manganese oxides bound, organic matter bound, and residual metal. However, extraction schemes were widely criticized and the great variety of protocols that were developed reflects the complexity of the techniques; lack of uniformity in the procedures, lack of selectivity of the reagents used, lack of quality control, results highly dependent on the procedure used, etc. (Dalmacija et al., 2010).

This procedure is largely similar to that produced by Tessier with the little difference in the first fraction of the procedure. Instead of evaluating the exchangeable and carbonate bound separately, the CBR procedure combines both in the first fraction (Zimmerman and Weindorf, 2010).

2.4.1.3. Short Extraction Procedure by Maiz

This method compared the Short and Tessier procedures and found that the Short procedure produced strong correlation data for metals tested. Three grams of residue are placed in a 50 mL tube, exposed to reagents and shaken. After the first extraction, the solution is centrifuged at 3000 rpm (≈ 1000 gravity) for 10 min, the supernatant removed, and analyzed. The sample is then washed in 10 mL of distilled water and centrifuged. For the residual fraction, the residue is placed in teflon tubes with aqua regia-HF acid for an undetermined time (Zimmerman and Weindorf, 2010).

2.4.1.4. Galan Procedure

This procedure is also similar in structure to the Tessier and CBR procedures. However, this procedure was used in extracting metals from soils severely affected by acid drainage in. Amorphous iron oxy-hydroxides can coat soils resulting is unobtainable data from regularly used techniques such as x-ray diffraction. Initial use of the Galan et al., method showed increased accuracy of metals extracted in these soils than the Tessier and CBR methods.

One-half a gram of soil the sample is placed into tubes and exposed to reagents. Centrifugation of the sample, collection of the supernatant, washing and fraction 4 are analyzed in the same manner as in the aforementioned Tessier extraction (Zimmerman and Weindorf, 2010).

2.4.1.5. Geological Society of Canada (GSC) Procedure

One gram of the soil sample is placed in a 50 mL tube and exposed to reagents and shaken. In between each fraction, samples are centrifuged for 10 min at ≈ 1000 g (2800 rpm). The supernatant is collected and the samples are washed in 5 mL of water, centrifuged, adding the wash water to the previous supernatant. Repeat the washing procedure. The amount of time needed to complete the fraction is proportional to the time for the reduction of sample to an appropriate volume. The modified GSC is the most modified of the sequential extraction procedures. The run time is drastically shortened and the reagents changed.

Benitez and Dubois modified the GSC procedure by testing various reagents at various time in varying order. They determined that no one sequence of events were fully satisfactory for a sequential extraction procedure but recommended one particular method above the others. That experiment was later adapted by Doelsch into the modified GSC (Zimmerman and Weindorf, 2010).

2.5. Target phases in environmental solid samples

2.5.1. Water Soluble Fraction

Trace elements extracted by H_2O are relatively labile and thus may be potentially bioavailable. The water-soluble fraction of a soil or sewage sludge is the first to be brought. This fraction is usually negligible, except in areas where evaporates are present. This phase contains the water-soluble species made up of free ions and ions complexed with soluble organic matter and other constituents. It constitutes the most mobile and potentially the most available metal and metalloid species.

The use of water as an extractant is connected with analytical and methodological problems: (a) the organic matter content of the sample significantly influences the interaction between the water and the solid phase (e.g. by suspension forming); (b) the water as an extractant medium has no buffering capacity, therefore the easily soluble major components of the soil or sewage sludge strongly influence the results of a long-term leaching of trace elements. When this procedure is used the pH may be indeterminate because of the low buffering capacity of the extractant and problems with readsorption occur; (c) the efficiency of the aqueous extraction for trace elements is too low and therefore the dissolved concentration may be below detection limits of available routine analytical methods (FAAS, ICP-AES). The water-soluble metals can be determined from the saturation paste extract of a soil or by extracting soil with deionised water at a certain soil/water ratio.

The water-soluble fraction may be obtained by two ways, by sampling soil or sewage sludge pore solution using in situ filtration, dialysis tubes or bags, or by a laboratory procedure such as centrifugation, filtration or displacement. Considerable amounts of specifically sorbed trace metals may be solubilized at pH 7 and the sufficient extraction time is 2 hr (Filgueiras et al., 2002).

2.5.2. Exchangeable Fraction

Heavy metals in the exchangeable fraction held by electrostatic adsorption represent the most mobile and readily available for biological uptake in the environment thus this fraction can be regarded as a pollution indicator. The concentration of metals in this phase indicates the environmental impact (Kumar et al., 2011). This fraction reflects the amount of heavy metals that would be released into the environment under normal conditions (Nemati et al., 2010). It is likely to be affected by changes in water ionic composition as well as sorption-desorption processes (Shivakumar et al., 2012). In this fraction the metals are adsorbed on the sludge or on their essential components, denominated clays, Fe and Mn hydroxides and humic acids. Metal adsorption is related with changes in the ionic composition of the water, which may affect the processes of adsorption–desorption (Fuentes et al., 2003). Sewage sludge or their major constituents have demonstrated the adsorption of trace metals; changes in water ionic composition are likely to affect sorption-desorption processes (Tessier and Campbell, 1979).

Salts solutions of replaceable cations such as $MgCl_2$, $CaCl_2$, $NaNO_3$, $Mg(NO_3)_2$, $BaCl_2$, KNO_3, $Ca(NO_3)_2$, CH_3COONH_4, $Sr(NO_3)_2$, NH_4NO_3, LiCl/ CsCl in methanol etc. are usually employed for leaching the metal fraction bound via electrostatic forces to the negative sites on the solid surface. Ammonium salts of strong acids, such as NH_4Cl or NH_4NO_3, however, can lower the pH and encourage the hydrolysis of clays. Nitrate salts are advantageous over chloride and acetate salts, since no metal complexing takes place due to the nitrate anion, and as a result, cation exchange is the only operating mechanism. Despite this drawback, $MgCl_2$ and CH_3COONH_4 at concentration are the most widely employed reagents for leaching the exchangeable fraction. Considerable amounts of specifically sorbed trace metals may be solubilized at pH 7 and the sufficient extraction time is 1 hr (Filgueiras et al., 2002).

2.5.3. Bound to Carbonates Fraction

Significant trace metal concentrations can be associated with carbonates. The carbonate fraction is susceptible to changes in pH (Shivakumar et al., 2012). It was reported that trace metals extracted from soil and sewage sludge with sodium acetate (1M) adjusted to pH 5 may have also been specifically sorbed to low energy sites on the surfaces of clay minerals, organic matter and oxide minerals.

Therefore, heavy metals recovered within this fraction are not strongly bound and can be released in acidic conditions (pH < 5). Heavy metals extractable with 1M sodium acetate adjusted to pH 5 (with acetic acid) are therefore likely to be bioavailable (Kumar et al., 2011).

The reagent most widely employed in sequential extraction sequence for releasing the metal fraction associated to carbonates is the sodium acetate - acetic acid buffer and pH 5, but the effect of the reagent is not limited to carbonate dissolution. Considerable amounts of specifically sorbed trace metals may be solubilized at pH 5 and also metal complexation must be assumed. The time required with this reagent for complete solubilization of carbonates depend on several factors such as particle size of the solid, type and amount of carbonate in the sample, etc. A 5 hr time is sufficient for extraction of more than 99.9% of the metal content associated with carbonate in soil and sewage sludge (Filgueiras et al., 2002).

2.5.4. Oxides Bound Fraction

In comparison with carbonate minerals, Fe-Mn oxide minerals have relatively large area and surface site density. The Fe-Mn oxide, the reducible phase of the soil under oxidizing conditions is a significant sink for the heavy metals. The association of higher concentration of metals with this fraction is caused by adsorption of these metals by the Fe-Mn mineral surface. The extent and intensity of this process will vary depending on several factors associated with oxygen dynamics in the soil (Kumar et al., 2011).

This fraction represents the concentration of metal oxides that would be released if the substrate was subjected to more reductive condition (Nemati et al., 2010). The heavy metals are strongly bound to these oxides but are thermodynamically unstable in anoxic conditions (Fuentes et al., 2003). Iron and manganese oxides exist as nodules, concretions, cement between particles, or simply as a coating on particles; these oxides are excellent scavengers for trace metals and are thermodynamically unstable under anoxic conditions (Tessier and Campbell, 1979).

Hydroxylamine hydrochloride in nitric acid medium is the most widely used reagent for leaching the easily reducible fraction. One of the problems which appear is that it releases substantial amounts of trace elements bound to organic matter, consequently, the recovery of trace elements in the oxide fraction may be overestimated at the expense of the oxidizable fraction. A selective leaching of the fraction associated with amorphous Fe oxides can be attained with hydroxylamine hydrochloride in acetic acid medium, which is capable of breaking the bonds between metals and amorphous and poorly crystallised Fe oxides without attacking either the silicates or the organic matter fraction, being included in the past in some extraction schemes to leach the moderately reducible fraction. Considerable amounts of specifically sorbed trace metals may be solubilized at pH 3 and the sufficient extraction time is 6 hr (Filgueiras et al., 2002).

2.5.5. Organic Bound Fraction

Organic matter plays an important role in the distribution and dispersion of metals by mechanisms of chelating and cation exchange. In this phase a reaction between a metal ion and an organic ligand leading to a species which can either precipitate directly or be adsorbed on soil materials. Carboxyl, phenolic, hydroxyl and carbonyl functional groups are assumed to be primarily responsible for metal binding. Metal humic complexes are reversible, and metals can be desorbed by salting out or by hydrogen ion competition (Kumar et al., 2011).

The oxidizable fraction represents the amount of metal bound to the organic matter and sulfides that will be released into the environment if conditions become oxidative (Nemati et al., 2010). The metals may be complexed or peptized by the natural organic substances is well known. Soluble metallic forms are liberated when organic matter is attacked in oxidant conditions (Fuentes et al., 2003). Trace metals may be bound to various forms of organic matter. The complexation and precipitation properties of natural organic matter are well recognized, as is the phenomenon of bioaccumulation in certain living organisms. Under oxidizing conditions in natural waters, organic matter can be degraded, leading to a release of soluble trace metals (Tessier and Campbell, 1979).

In general, the hydrogen peroxide applied to a heated medium (e.g. 85^{O}C) for several hours is the reagent preferred for dissolving organic matter as a compromise between a complete attack of organic matter and minimum alteration of silicates with ammonium acetate at pH 2. A considerable amount of specifically sorbed trace metals may be extracted time is 5 hr. The combination H_2O_2/CH_3COONH_4, where the addition of CH_3COONH_4 prevents readsorption of extracted metals onto the oxidized substrate, was adopted in most schemes for leaching metals associated with organic matter and sulfides (Filgueiras et al., 2002).

2.5.6. Residual (Silicate Bound Fraction)

The residual fraction is concerned with the most stable and least bioavailable of all the chemical fractions of the soil and sewage sludge, since it is believed that metals are occluded within the crystal lattice of silicates and well crystallized oxide minerals. The residual fraction is a major carrier of metals in most environmental systems. The percent of this fraction can be taken as a guide to the degree of non-availability of metals to biota or diagenetic processes except over long time scales. The metals of the residual fraction are usually considered to be fragments of the primary mineral phase. All other fractions can be of secondary mineral phases as they involve materials formed through physical and chemical processes of weathering of primary minerals. The smaller the percentages of the metal present in this fraction, the greater the pollution of the area (Kumar et al., 2011).

The metals in the residual fraction are strongly bound to the crystalline structures of the minerals. These metals are not easily extracted or removed (Nemati et al., 2010). These metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature (Shivakumar et al., 2012). The residual solids mainly contain primary and secondary solids that occlude the metals in their crystalline structures (Fuentes et al., 2003).

2.6. Major Parameters for a Sequential Extraction Schemes

A wide range of extraction procedures is readily available for different metals and variations of the extraction conditions are utilized due to varying soil composition. The following experimental parameters are considered when designing an adequate extraction procedure:

- ***Extractants (chemicals to be extracted)***: Chemical and physical interferences both in the extraction and analysis steps, respectively.

- ***Extraction steps***: Selectivity, readsorption processes, and redistribution processes. If the single extractants for the different steps are chosen with respect to their ion-exchange capacity or reduction/ oxidation capacity, each step has to be designed individually following special considerations.

- ***Concentration of the chemicals***: The efficiency of an extractant to dissolves or desorbs trace metals from sediment and soils will usually be increased with increasing concentration or ionic strength. A thermodynamic law predicts the efficiency of an extractant to dissolve or desorbs trace metals from solid samples.

- ***Extraction pH***: Extractants with a large buffering capacity or extractants without buffer capacity can be used.

- ***Solution/solid ratio and extraction capacity***: The relative amount of extractant added to the sewage sludge and soil has various implications on the results. Essentially, Wenzel distinguished four cases, e.g. (1) pure dissolution of metal compounds according to the solubility product, (2) pure ion exchange by 0.1–1 mol/L neutral salt solutions, or (3) by water or highly diluted neutral salt solutions (<<0.1 mol/L), and (4) combinations of (1) with either (2) or (3). If, over a sufficiently wide solution/solid ratio, the capacity of the extractant to dissolve a metal fraction exceeds its total amount present in the solid sample, then the metal concentration in the extract (mg/L extract) will decrease with an increase in solution/solid ratio. However, the total amount (mg/kg) extracted will be constant with increasing solution/solid ratio. Nevertheless, as sediment and soils are multiphase/multicomponent systems, dissolution of other compounds due to the nonselectivity of the extractant may confuse this behavior. Wenzel et al. concluded that the efficiency of mild reagents for extraction of abundant metal cations (e.g. Ca, Al, Mg, K) usually increased by increasing the solution/soil ratio, although often the concentrations in the extract concurrently decreased. With stronger reagents, this should also be valid for the more abundant metal cations as long the capacity of the extractant to dissolve a particular compound exceeded the amount present in the soil.

- ***Extraction time and batch processes***: The effect of extraction time is related to the kinetics of the reactions between solid sample and extractant. Extractions may be predominantly based either on desorption or dissolution reactions. For desorption of metal cations from heterogeneous soil systems, Sparks identified four rate-determining steps, e.g. (i) diffusion of the cations in the (free) bulk solution, (ii) film diffusion, (iii) particle diffusion, and (iv) the desorption reaction. Accordingly, the rates of most ion-exchange reactions are film- and/or particle diffusion-controlled (Hlavay et al., 2004).

Vigorous mixing, stirring, or shaking significantly influences these processes. Film diffusion usually predominates with small particles, while particle diffusion is usually rate-limiting for large particles. Dilute solutions usually favor film-diffusion-controlled processes. The time to reach equilibrium for ion exchange on soils varies between a few seconds and days and is affected by soil properties. For mineral dissolution, essentially three rate-controlling steps were identified, e.g. (i) transport of solute away from the dissolved crystal (transport-controlled kinetics) (ii) surface reaction-controlled kinetics where ions are detached from the surface of crystals and (iii) a combination of both. Batch processes (e.g. stirring or shaking) increase the rate of transport-controlled reactions, while they do not affect surface-controlled reactions. Shaking and other batch processes may enhance the dissolution of readily soluble salts effectively, but are unlikely to affect the dissolution rate of less soluble minerals. Experiments reported by several authors generally revealed an increase of the extractable amounts of metals with time of extraction as expected from the theory of reaction kinetics.

- ***Extraction temperature****:* Within the normal range of extraction temperatures (20–25°C or room temperature), the effect of temperature on metal extractability is usually small, but has to be considered for interpretation of small differences. Finally, the whole procedure has to be optimized with regard to selectivity, simplicity and reproducibility (Hlavay et al., 2004).

2.7. Determination of Physicochemical Parameters of the Samples

There are different physicochemical parameters of the waste water, sewage sludge and soil samples. From these parameters electrical conductivity and pH were determined for all of the samples under study because they can affect the mobility of toxic heavy metals in the soil while organic matter content was determined for the soil and sewage sludge samples by using the following method.

1) Determine the mass of the dry soil.

$$M_D = M_{PDS} - M_P \qquad (1)$$

2) Determine the mass of the ashed (burned) soil.

$$M_A = M_{PA} - M_P \qquad (2)$$

3) Determine the mass of organic matter

$$M_O = M_D - M_A \qquad (3)$$

4) Determine the organic matter (content).

$$\%\ \mathrm{OM} = \frac{M_O}{M_D} \mathrm{X} 100 \qquad (4)$$

Where,

MP = Mass of empty, clean porcelain dish (grams); MPDS = Mass of dish and dry soil (grams); MPA = Mass of the dish and ash (Burned soil) (grams); MD= Mass of the dry soil (grams); MA= Mass of the ash (Burned soil) (grams); MO= Mass of organic matter (grams); OM = Organic matter, %

2.8. Mobility of Heavy Metals in the Soil

The mobility of metals in soil samples may be assessed on the basis of absolute and relative contents of fraction weakly bound to soil components. The relative index of metal mobility was calculated as a mobility factor (MF) on the basis of the following equation (Aikpokpodion et al., 2012).

$$\mathrm{MF} = \frac{(\mathrm{F1+F2+F3})}{(\mathrm{F1+F2+F3+F4+F5+F6})} \mathrm{X}\ 100 \qquad (5)$$

Where: MF-mobility Factor

F1-Water Soluble Fraction

F2-Exchangeable Fraction

F3-Carbonate Bound Fraction

F4-Oxides Bound Fraction

F5-Organic Bound Fraction

F6-Residual (Silicate Bound Fraction)

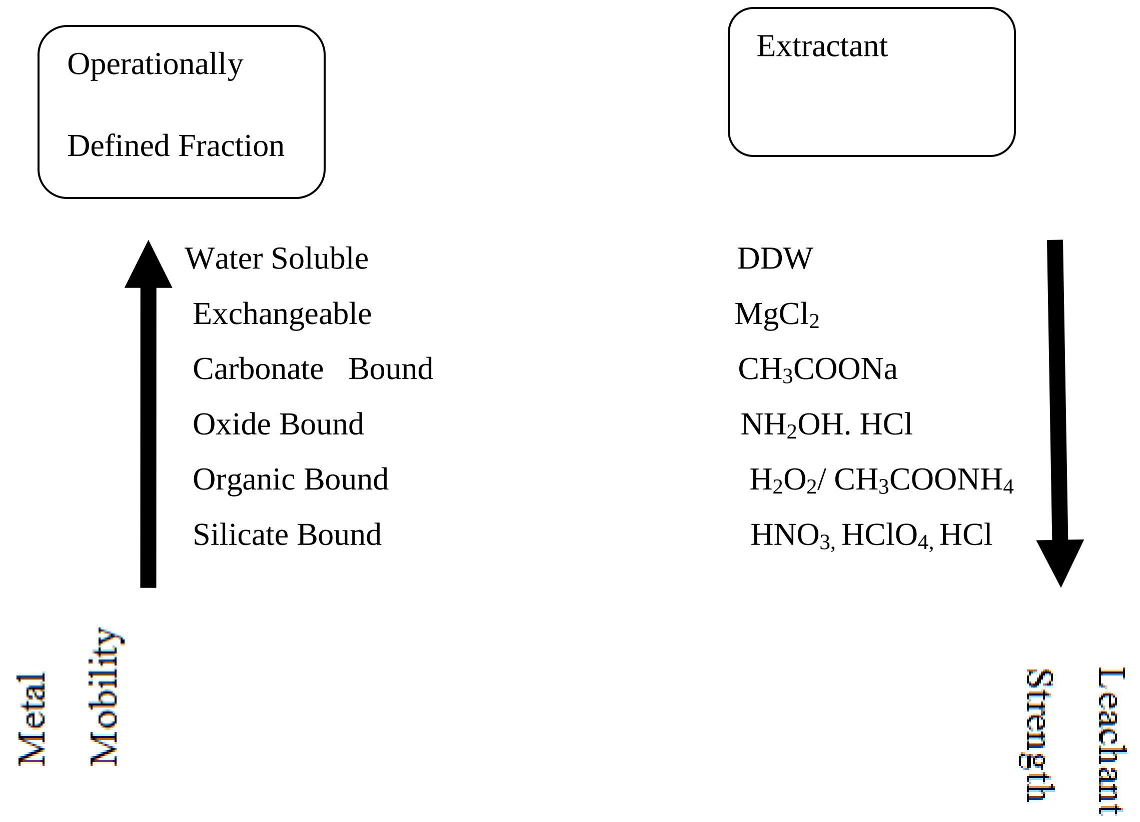

Figure 1 Relationship between Metal Mobility and Leachant Strength

3. MATERIALS AND METHODS

3. 1. Description of the Study Area

The samples in the present study were collected from two factories located in different localities. Saygin Dima Textile Factory is located in the central part of Ethiopia, the Oromia Special Zone surrounding Finfine in Sebeta town. It is about 30 kilometers southwest of the capital, Addis Ababa and its elevation is 2,356 meters above sea level. On the other hand, Nefas Silk Paint Factory is located at the southern part of Addis Ababa and has geographical location of 2,326 meters above sea level.

3. 2. Chemicals and Reagents

All of the chemicals used throughout the experiments were in high-purity grade. In all dilutions and standard preparation, double distilled water was used. Standards of Cd, Cr, Cu, Ni, Pb and Zn were used for instrumental calibration. 69%-71% HNO_3; Sigma Aldrich (Steinheim, Germany), 37% HCl; Sigma Aldrich (Steinheim, Germany) and 70% $HClO_4$; Research laboratory fire chemical factory (Mombai, India) were used for sample digestion. DDW, 97% $MgCl_2$; BDH chemicals ltd (Poole, England), 99% CH_3COONa; BDH chemicals ltd (Poole, England), 30% H_2O_2; BDH chemicals ltd (Poole, England), 99% CH_3COONH_4; Sigma Aldrich (Steinheim, Germany), 99.7% CH_3COOH; May & Baker ltd (Dagenham, England), 98% $NH_2OH.HCl$; Sigma Aldrich (Steinheim, China) were used for sequential extraction. High purity C_2H_2 gas was used for FAAS.

3. 3. Instrumentation

Polyethylene bags, polyethylene bottles and spade were used for sample collection. Mortar and pestle and Stainless steel sieve were used for sample preparation. Electronic balance; Adam Equipment (PW250,) was used for measuring of the mass of samples. Oven; (PLT 125) was used for drying purpose. Kjeldahl digester was used for the digestion of the samples. Orbital Shaker (QSZ-100A), Water Bath Shaker; Gemmyco industrial corporation (YCW-012S, Taiwan) and Centrifuge; centurion scientific ltd (K240, UK) were used for sample preparation in both digestion and sequential extraction technique. All glass wares were cleaned with 10% HCl and rinsed with double distilled water. Muffle Furnace (L51/S, Germany) was used for determination of organic matter content of the soil and sewage sludge samples. Conductivity meter; Thermo Orion ($145A^{+}$, USA), and pH meter (AD1020, Romania) were used to measure the electrical conductivity and pH of the samples respectively. Refrigerator was used for sample storage. Flame Atomic Absorption Spectrometry (FAAS); Zeenit (700P, Germany) was used for determination of the concentration level of selected toxic heavy metals in the samples under study.

3. 4. Sample Collection, Preparation and Analysis

3. 4.1. Sample Collection

For both factories the day temperature was $24^{O}C$. Instruments used for sample collection were cleaned with 10 % HCl. Solid samples and liquid samples were collected into polyethylene bags and polyethylene bottles respectively. Freshwater samples were collected at the entrance to the processing unit of both factories. Untreated wastewater samples were collected at the discharge point. The treated wastewater samples were collected at the middle point of the water flow at different distances from the wastewater discharge point and composited. Sewage sludge samples were collected by spade randomly from different point of stable point and composited. The polluted soil samples were collected at 20 cm deeper from the surface by spade at the same distances with the treated wastewater from the wastewater discharge point.

The treated wastewater and polluted soil samples were collected at the distance about (0 m, 40 m, 80 m, 120 m and 160 m) for Sayigin Dima Textile factory and (0 m, 120 m, 160 m, 200 m and 240 m) for Nefas Silk Paint Factory from the wastewater discharge point and composited. The distance of sample collection for Nefas Silk Paint Factory was because of the wastewater line was covered by cement nearby the point of discharge. The reference soil samples were collected from the distance of unpolluted area by spade for both factories.

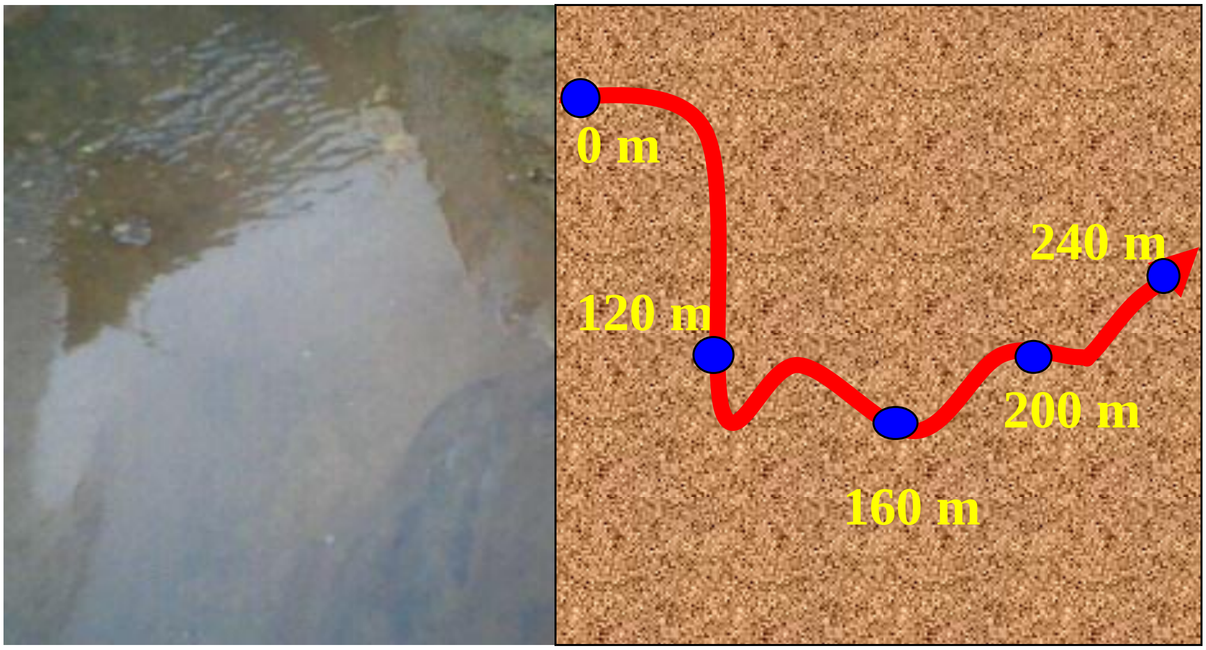

Figure 2 Nefas Silk Paint Factory Sampling Area

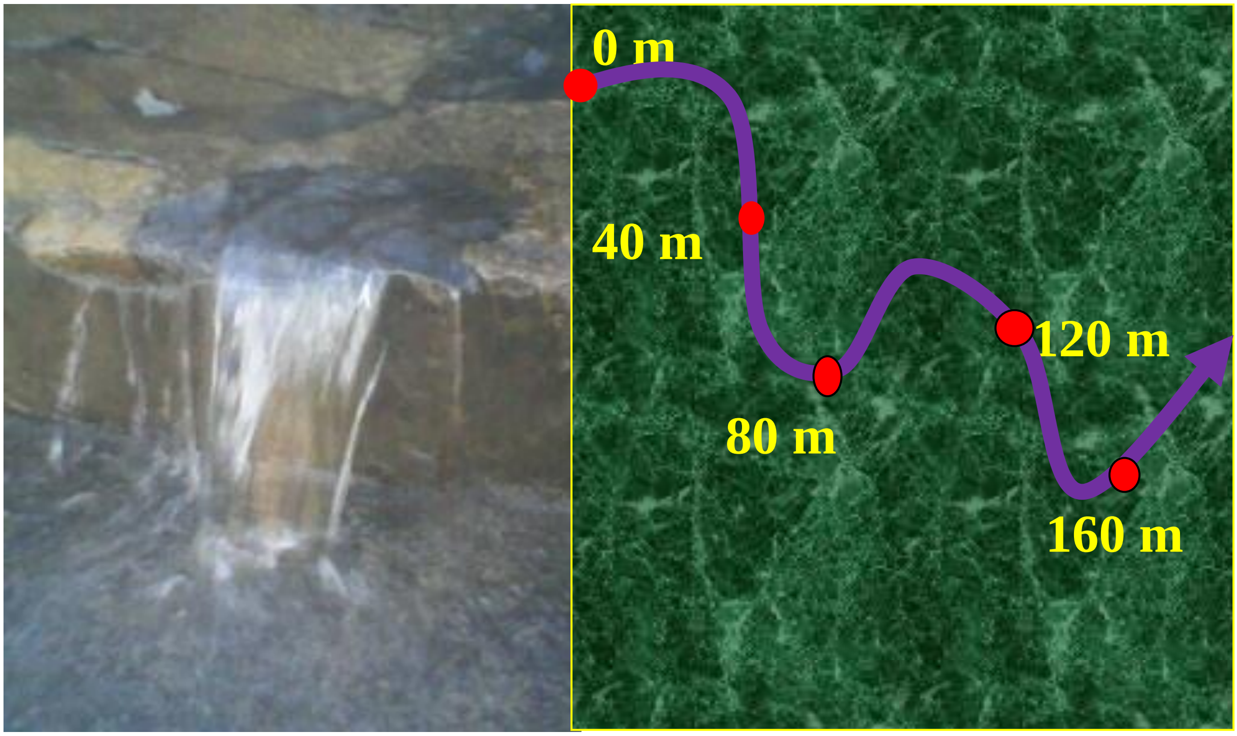

Figure 3 Saygindima Textile Factory Sampling Area

3. 4.2. Sample Preparation

The wastewater and fresh water samples collected from both factories were filtered by filter paper. 1 mL of concentrated HNO_3 was added to 100 mL of the filtrate for preservation and the solution was stored in refrigerator until digestion and analysis. The addition of HNO_3 is used to prevent the precipitation of metals.

The soil and sewage sludge samples were freed from extraneous matter (stones, pebbles etc) and air dried (Poornima et al., 2011). This was done so that the samples can be mixed uniformly for subsampling. The samples were ground with mortar and pestle and mixed. Then the samples were divided into two. The first portion was sieved with stainless steel sieve of 2 mm mesh for sequential extraction and the second portion with 0.425 mm mesh and stored separately for digestion. Samples were mixed thoroughly and stored in a clean, closed container. Representative subsamples of the samples were taken for analysis (Adepetu et al., 1996).

3.4.3. Sample Analysis

3. 4.3.1. Physicochemical Parameters Analysis

Organic matter, electrical conductivity and pH were the selected physicochemical parameters determined.

3.4.3.2. Sample Digestion for Determination of Total Concentration of Selected Heavy Metals

The water samples were shaken well to homogenize before sub-sampling for digestion according to EPA. 50 mL of water samples were taken into round bottom flask. Then, the samples were digested with (HNO_3 and HCl, 1:3) at 90°C for 2 hr (Srikanth et al., 2013). 0.5 g of soil samples were digested with the mixture of HNO_3 and $HClO_4$ (3:1 v/v) at $90^{O}C$ for 2 hr (Rahman et al., 2012**;** Kalembkiewicz and Soco, 2002 and Kumar et al., 2011) and the same amount of sewage sludge samples were digested with aqua regia (HCl and HNO_3, 3:1 v/v) at 120 ºC for 2 hr (Gawdzik and Gawdzik, 2012 and Venkateswaran et al., 2007). Then, the samples were removed from heat source and cooled for at least 30 minutes. The solutions were filtered with filter paper and diluted to 50 mL.

3.4.3.3. Sequential Extraction of Heavy Metals

To determine the geochemical forms of metals, the soil and sewage sludge samples sieved with 2 mm mesh sieve were subjected to the following sequential extraction. 1 g of air dried sample was subjected to sequential extraction technique proposed by Tesser (Ghrefat et al., 2012). The procedure of Tessier, selected for this study was designed to separate heavy metals into six operationally defined fractions: water soluble, exchangeable, carbonate bound, Fe-Mn oxides bound, organic bound and silicate bound(residual) fractions (Aikpokpodion et al., 2012, Chang et al., 2005). One gram of each soil and sewage sludge samples were weighed into 50 mL polyethylene sample bottle and the following fractions obtained.

(F1) Water soluble: Soil and sludge samples were extracted with 15 mL of double distilled water and shaken for 2 hr at room temperature.

(F2) Exchangeable: The residues from water soluble fractions were extracted with 8 mL of 1 M $MgCl_2$ (pH 7.0) and shaken for 1 hr at room temperature.

(F3) Carbonate –Bound: The residues from exchangeable fractions were extracted with 8 mL of 1 M CH_3COONa + CH_3COOH (pH 5.0) and shaken for 5 hr at room temperature.

(F4) Fe-Mn Oxides-Bound: The residues from carbonate fractions were extracted with 20 mL of 0.04 M NH_2OH. HCl in 25% (v/v) CH_3COOH + CH_3COOH (pH 3.0) and shaken in water bath shaker at 96°C for 6 hr.

(F5) Organic- Bound: The residues from Fe-Mn oxides bound fractions were extracted with 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 + HNO_3 (pH 2) and shaken in water bath shaker at 85°C for 2 hr. A second 3 mL aliquot of 30% H_2O_2 + HNO_3 (pH 2) and shaken in water bath shaker at 85°C for 3 hr. After cooling, 5 mL of 3.2 M CH_3COONH_4 in 20% (v/v) HNO_3 was added and diluted to 20 mL with DDW. Finally, the solutions were shaken continuously for 30 min at room temperature.

(F6) Residual (Silicate Bound)

To determine the residual fraction concentration of each heavy metals, the residual fraction of soil samples were digested with the mixture of HNO_3 and $HClO_4$ (3:1 v/v) at 90^OC for 2 hr

(Rahman et al., 2012**;** Kalembkiewicz and Soco, 2002 and Kumar et al., 2011) and sewage sludge samples were digested with aqua regia (HCl and HNO_3, 3:1 v/v) at 120 ºC for 2 hr (Gawdzik and Gawdzik, 2012 and Venkateswaran et al., 2007). The solutions were filtered and diluted to 50 mL.

For all steps shaking was done at 100 rpm at respective temperatures (Lim et al., 2009). After each successive extraction separation was taken place by centrifuging at 3000 rpm for 30 min (Maina et al., 2012). The supernatant solutions were removed with a pipette whereas the residues were washed with 8 mL of double distilled water and these second supernatant solutions were discarded after centrifugation for 30 min.

For the purpose of comparison of metal level with soil background levels, reference soil samples (RSS) were collected from each site not affected by the effluents. For both total concentration and sequential extraction the blank sample was subjected to the same procedure. Finally, the solutions collected from sequential extraction and digestion were analyzed for selected toxic heavy metals by Flame Atomic Absorption Spectrometer (FAAS).

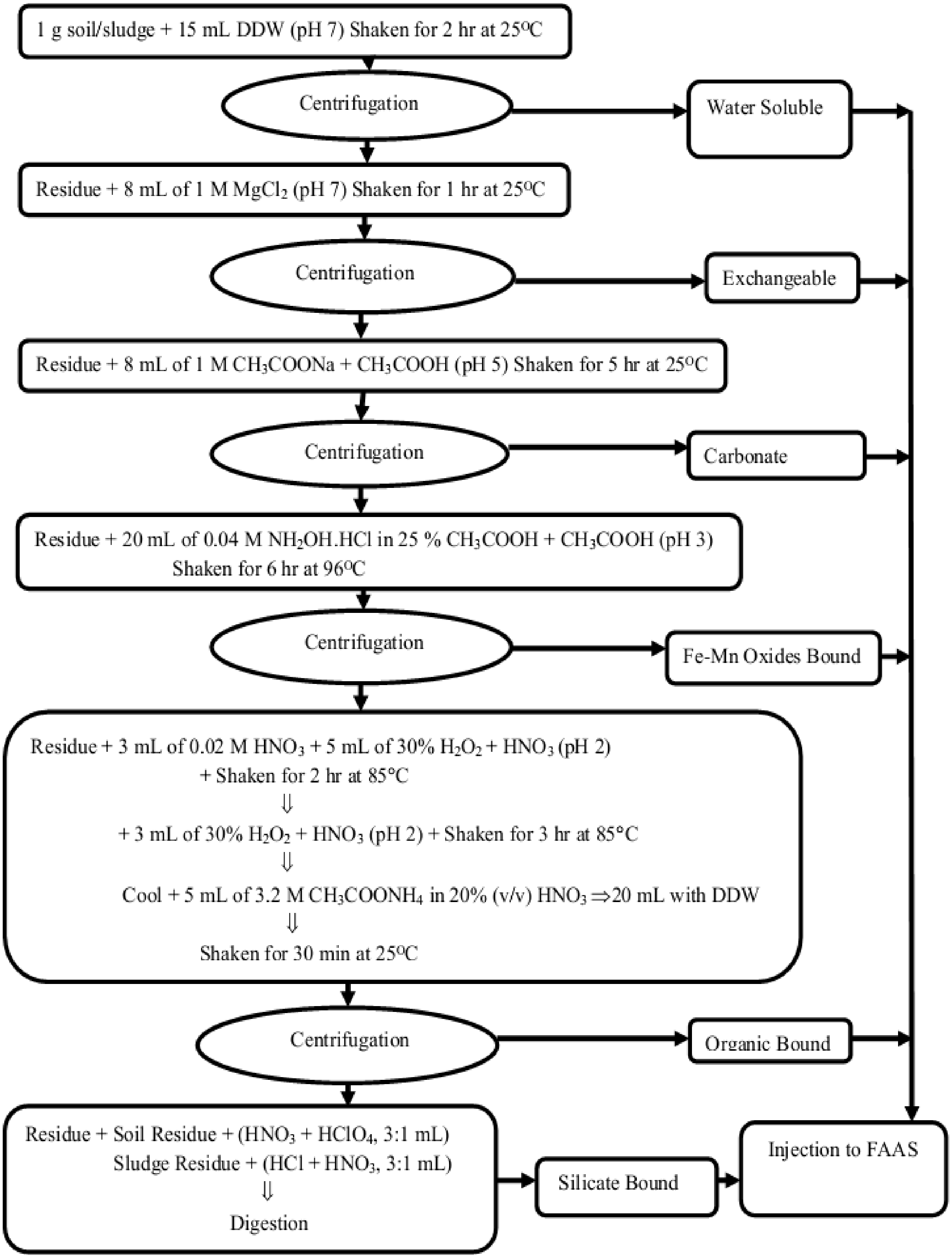

Figure 4 Flow Chart of Sequential Extraction Procedure

4. RESULTS AND DISCUSSION

4.1. Physicochemical Parameters Analysis

The toxicity and the mobility of heavy metals in soils depend not only on the total concentration, but also on their specific chemical form, their binding state, the metal properties, environmental factors and soil properties such as pH, organic matter content and type, redox conditions, root exudates acting as chelates (Ghrefat et al., 2012) and cation exchange capacity (CEC) (Aydinalp and Marinova, 2003). From the physicochemical parameters affecting the toxicity and mobility of heavy metals organic matter, electrical conductivity and pH were determined and showed in the table 1.

Table 1 Physicochemical Parameters of the Samples

Samples	Organic Matter (%)	EC (mS/cm) (25ºC)	pH (25ºc)
NPSD	17.745	0.792	7.747
NPSP	11.714	0.393	8.533
NSS	48.688	0.005	8.727
SPS	12.617	0.306	7.803
SSS	47.304	0.003	8.210
NTWD	-	0.003	8.647
NTWP	-	0.011	8.650
NUW	-	1.826	8.223
STW	-	0.004	9.590
SUW	-	0.008	9.283

Organic Matter: NSS and SSS sewage sludge samples have high organic matter. NPSD> SPS> NPSP from polluted soil samples have medium organic matter. Hence, both sewage sludge samples have high organic matter than all polluted soil samples.

Electrical Conductivity: SSS and NSS sewage sludge samples and NTWD, STW, SUW and NTWP from polluted water samples have low electrical conductivity values. NPSP and SPS polluted from polluted soil samples have medium electrical conductivity values.

NUW from polluted water samples and NPSD from polluted soil samples have high electrical conductivity values.

pH: STW and SUW from polluted water samples are slightly basic while other samples are neutral.

4.2. Optimization Condition of Flame Atomic Absorption Spectrometer

Flame atomic absorption spectrometer was optimized to obtain the maximum energy for absorption of analytes at the respective wave length. These instrumental optimization parameters used throughout this study for the elements of interest can be seen in Table 2.

Table 2 Optimization Condition of Flame Atomic Absorption Spectrometer

	Cd	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Wavelength (nm)	228	357	324	232	283	213
Current (A)	2	4	2	3	2	2
Slit width (nm)	1.2	0.2	1.2	0.2	1.2	0.5
Energy (KJ)	89.9	71.3	71.4	72.3	79.3	71.6
Burn Height (mm)	7	9	4	6	6	6
Oxidant	Air	Air	Air	Air	Air	Air
Fuel/Oxidant	0.085	0.245	0.085	0.117	0.138	0.085
Acetylene Flow (mL/min)	40	115	40	55	65	40
Read time (sec)	3	3	3	3	3	3
Lamp	HCL	HCL	HCL	HCL	HCL	HCL
PMT (V)	300	300	300	300	300	300

4.3. Linearity

Stock solutions of Cd, Cr, Cu, Ni, Pb and Zn were prepared from the series of different concentrations of respective metals standards. Working solutions were prepared freshly by diluting 1000 mg/L to 100 mg/L. 1ppm, 2ppm, 3ppm and 4ppm standards concentration were prepared for Cr, Ni and Pb. 0.25 ppm, 0.5 ppm, 0.75 ppm and 1 ppm standards concentration were prepared for Cd and Zn. 0.25 ppm, 0.5 ppm, 1 ppm and 2 ppm standards concentration were prepared for Cu. Finally, the calibration curves were plotted by using these metal standards. The linearity of the curves shows that its (R^2) ranges from 0.991 to 0.999.

Table 3 Calibration Data of Flame Atomic Absorption Spectrometer

Elements	**Concentration**	**Absorbance**	**R^2**	**Equation**
Cd	0.25	0.10434	0.991	Y= 0.407x + 0.011
	0.5	0.22248		
	0.75	0.33165		
	1	0.40768		
Cr	1	0.12769	0.997	Y= 0.076x + 0.047
	2	0.19476		
	3	0.27348		
	4	0.35592		
Cu	0.25	0.062	0.999	Y= 0.200x + 0.014
	0.5	0.11626		
	1	0.21567		
	2	0.41468		
Ni	1	0.17076	0.999	Y= 0.130x + 0.040
	2	0.29839		
	3	0.43619		
	4	0.55858		
Pb	1	0.03296	0.996	Y= 0.033x - 0.0009
	2	0.06227		
	3	0.10147		
	4	0.12997		
Zn	0.25	0.17162	0.994	Y= 0.570x + 0.039
	0.5	0.33276		
	0.75	0.48262		
	1	0.59745		

4.4. Total Concentration of Selected Heavy Metals

4.4.1. Total Concentration of Cr

Cr(VI) is the more toxic form of chromium and is also more mobile. The equilibrium between the two chromium forms in soil depends upon soil physical and chemical characteristics.

Table 4 Total Concentration of Chromium

Samples	Unit	Mean ± SD	RSD%	LOD	LOQ
SSS	mg/kg	5248 ± 0.11	8.62	0.26	0.34
NPSD	mg/kg	4424 ± 0.03	2.31	0.32	0.53
NPSP	mg/kg	12960 ± 0.13	4.02	0.27	0.36

Key: SD- standard deviation

RSD-relative standard deviation

LOD-limit of detection

LOQ-limit of quantification

Cr was detected in SSS, NPSD and NPSP samples. The samples are in the order is: NPSP > SSS > NPSD in their Cr concentration level. It was not detected in SPS, NSS, SUW, STW, NUW, NTWD and NTWP samples

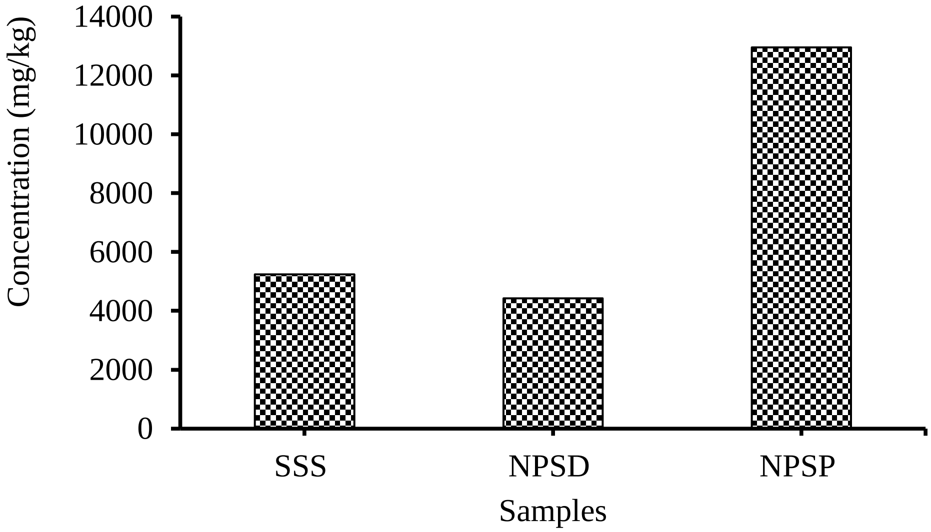

Figure 5 Total Concentration of Chromium

4.4.2. Total Concentration of Cu

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). The solubility of Cu is drastically increased at pH 5.5 (Wuana and Okieimen, 2011). The cupric ion, Cu^{2+}, and hydroxide complexes, $CuOH^+$ and $Cu(OH)_2$, are also commonly present. Copper forms strong solution complexes with humic acids. The affinity of Cu for humates increases as pH increases and ionic strength decreases (Evanko and Dzombak, 1997).

Table 5 Total Concentration of Copper

Samples	Unit	Mean ± SD	RSD%	LOD	LOQ
SSS	mg/kg	2.81 ± 0.00	0.69	0.08	0.10
SUW	mg/L	18 ± 0.13	9.53	0.20	0.21
SPS	mg/kg	772 ± 0.04	4.80	0.15	0.18
NPSD	mg/kg	320 ± 0.06	6.57	0.06	0.09
NPSP	mg/kg	555 ± 0.02	1.45	0.05	0.07
NSS	mg/kg	94 ± 0.02	1.92	0.05	0.07

Cu was detected in SSS, SUW, SPS, NPSD, NPSP and NSS samples. The samples are in the order: SPS > NPSP > NPSD > NSS > SUW > SSS in their Cu concentration level. But, it was not detected in STW, NUW, NTWD and NTWP samples. It was detected in the total concentration determination for NPSD but it was not detected in its fractional forms and hence, it may not be mobile in this sample.

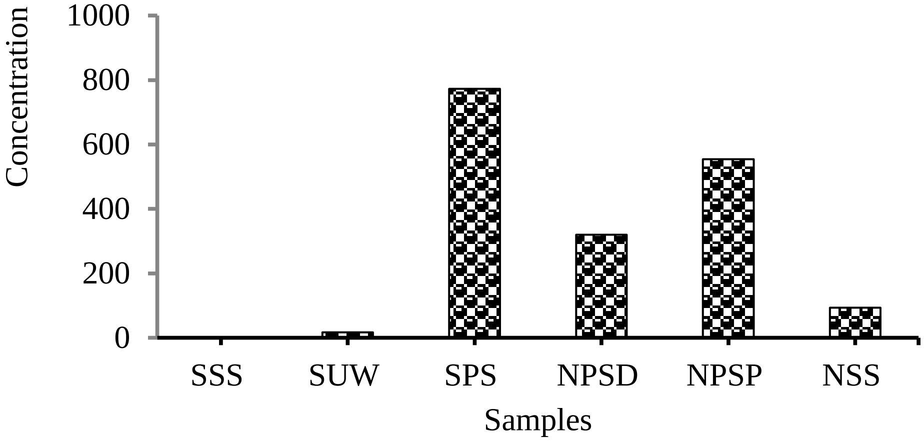

Figure 6 Total Concentration of Copper

4.4.3. Total Concentration of Ni

Nickel can end up in surface water when it is a part of wastewater streams. The larger part of all Ni compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic soils, however, Ni becomes more mobile and often leaches down to the adjacent groundwater (Wuana and Okieimen, 2011).

Table 6 Total Concentration of Nickel

Samples	Unit	Mean ± SD	RSD%	LOD	LOQ
SSS	mg/kg	1745 ± 0.09	4.87	0.27	0.89
SPS	mg/kg	3463 ± 0.13	3.72	0.69	0.89
NPSD	mg/kg	3409 ± 0.47	4.87	0.70	0.98
NPSP	mg/kg	2647 ± 0.16	6.21	0.16	0.29

Ni was detected in SSS, SPS, NPSD and NPSP samples. The samples are in the order: SPS > NPSD > NPSP > SSS in their Ni concentration level. Ni was not detected in SUW, STW, NUW, NTWD, NTWP and NSS samples. But, it was detected only in the total concentration determination rather than any fractional form for all samples and hence, it may not be mobile in all of the samples.

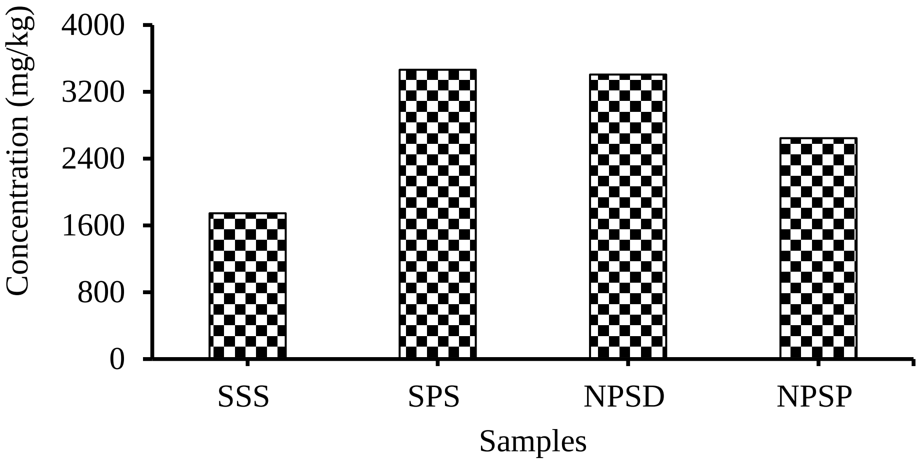

Figure 7 Total Concentration of Nickel

4.4.4. Total Concentration of Pb

Most lead that is released to the environment is retained in the soil. The primary processes influencing the fate of lead in soil include adsorption, ion exchange, precipitation, and complexation with sorbed organic matter. These processes limit the amount of lead that can be transported into the surface water or groundwater. The relatively volatile organolead compound tetramethyl lead may form in anaerobic sediments as a result of alkyllation by microorganisms. The amount of dissolved lead in surface water and groundwater depends on pH and the concentration of dissolved salts and the types of mineral surfaces present. In surface water and ground-water systems, a significant fraction of lead is undissolved and occurs as precipitates ($PbCO_3$, Pb_2O, $Pb(OH)_2$, $PbSO_4$), sorbed ions or surface coatings on minerals or as suspended organic matter (Evanko and Dzombak, 1997).

Table 7 Total Concentration of Lead

Samples	Unit	Mean ± SD	RSD%	LOD	LOQ
SSS	mg/kg	15288 ± 0.10	3.35	0.61	0.62
NPSD	mg/kg	731 ± 0.09	6.47	0.46	0.51
NPSP	mg/kg	806 ± 0.09	6.02	0.62	0.67
NSS	mg/kg	10650 ± 0.01	1.38	0.88	0.89

Pb was detected in SSS, NPSD, NPSP and NSS samples. The samples are in the order: SSS > NSS > NPSP > NPSD in their Pb concentration level. But, it was not detected in SPS, SUW, STW, NUW, NTWD and NTWP samples.

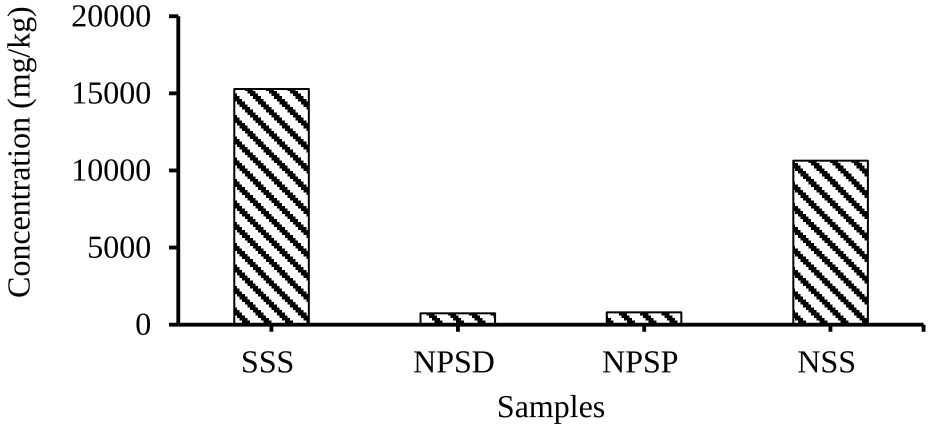

Figure 8 Total Concentration of Lead

4.4.5. Total Concentration of Zn

Industrial sources or toxic waste sites may cause the concentrations of Zn in drinking water to reach levels that can cause health problems (Wuana and Okieimen, 2011). Zinc readily precipitates under reducing conditions and in highly polluted systems when it is present at very high concentrations, and may coprecipitate with hydrous oxides of iron or manganese (Evanko and Dzombak, 1997).

Table 8 Total Concentration of Zinc

Samples	Unit	Mean ± SD	RSD%	LOD	LOQ
NTWD	mg/L	0.26 ± 0.02	6.42	0.12	0.14
SSS	mg/kg	23.35 ± 0.78	0.03	0.17	0.23
SUW	mg/L	32.55 ± 0.01	0.62	0.19	0.20
SPS	mg/kg	8.26 ± 0.02	4.02	0.22	0.24
STW	mg/L	2.47 ± 0.01	0.69	0.07	0.08
NUW	mg/L	0.85 ± 0.02	2.51	0.19	0.21
NTWP	mg/L	0.36 ± 0.00	0.04	0.14	0.15
NPSD	mg/kg	8.16 ± 0.00	0.25	0.23	0.24
NPSP	mg/kg	8.74 ± 0.01	3.23	0.18	0.20
NSS	mg/kg	9.96 ± 0.01	1.63	0.21	0.22

Zn was detected in all samples. The samples are in the order: SUW > SSS > NSS > NPSP > SPS > NPSD > STW > NUW > NTWP > NTWD in their Zn concentration level.

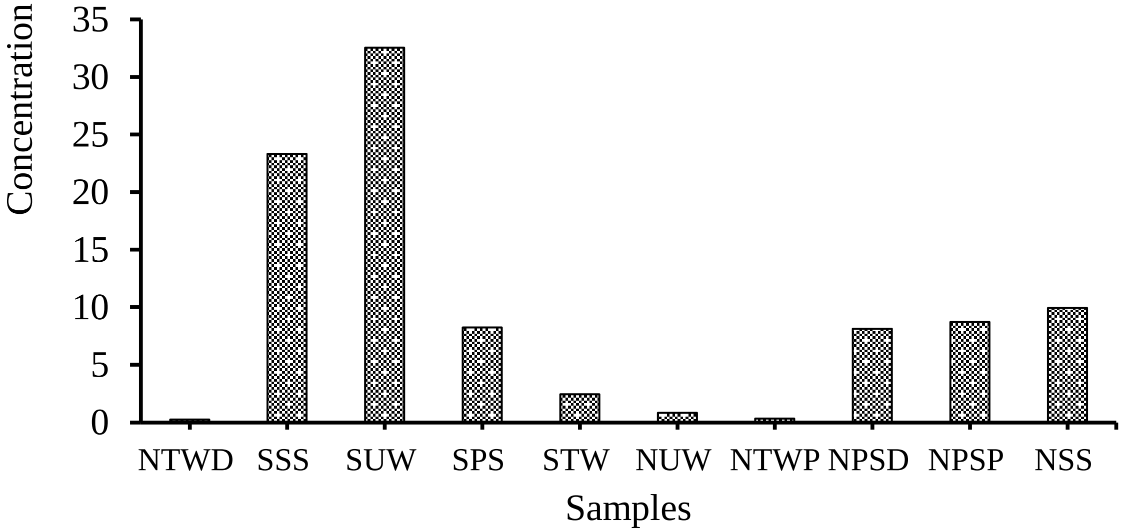

Figure 9 Total Concentration of Zinc

4.5. Fractionation of Selected Heavy Metals

4.5.1. Fractionation of Cr

Cr(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 $Cr(OH)_3$ (Evanko and Dzombak, 1997). The oxidation process is only controlled by the reaction kinetics, due to Cr(III) species immobility and insolubility. Cr(III) tends to be strongly bound by soil humic acid polymers, and this affinity restricts the availability of Cr(III) to be oxidized and reduce the organic matter decomposition (Palma et al., 2012).

Table 9 Fractional Concentration of Chromium

a)

Samples	Unit	F4	SD	RSD%
NPSD	mg/kg	781 ± 0.06	0.06	3.67
NPSP	mg/kg	1215 ± 0.23	0.23	9.66
NSS	mg/kg	639 ± 0.01	0.01	0.44
SSS	mg/kg	1210 ± 0.15	0.15	6.08
LOD	mg/kg	0.09		
LOQ	mg/kg	0.18		

b)

Samples	Unit	F6	SD	RSD%
NPSD	mg/kg	1121 ± 0.19	0.19	8.58
NPSP	mg/kg	ND		
NSS	mg/kg	ND		
SSS	mg/kg	ND		
LOD	mg/kg	0.30		
LOQ	mg/kg	0.39		

Key: - ND— Not Detected

F4— Oxide bound fraction

F6— Silicate bound fraction

Cr was detected in oxide fractions of NPSP, NPSD, NSS and SSS samples and in the residual fraction of NPSD sample. For NPSD sample, Silicate bound > Oxide fraction in Cr concentration. For NPSP, NSS and SSS samples, Cr was detected only in their oxide fractions. It was not detected in the water soluble, exchangeable and carbonate fraction of these samples. Hence, it may not be bioavailable. Even though it was not detected in the total concentration determination; Cr was detected in the fractional form of NSS sample. This is because of Cr may be mobile in this sample.

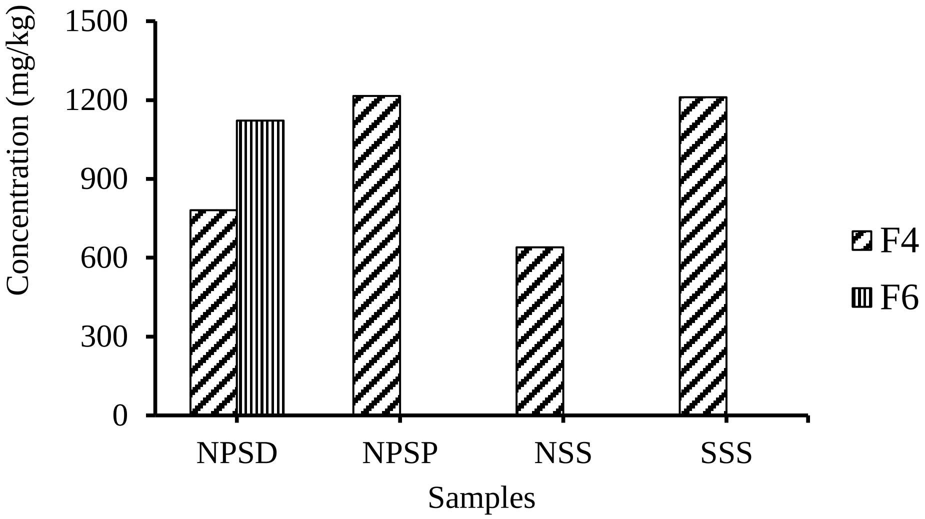

Figure 10 Fractional Concentration of Chromium

4.5.2. Fractionation of Cu

Solution and soil chemistry strongly influence the speciation of copper in ground-water systems. Copper mobility is decreased by sorption to mineral surfaces. Cu^{2+} sorbs strongly to mineral surfaces over a wide range of pH values (Evanko and Dzombak, 1997).

Table 10 Fractional Concentration of Copper

a)

Samples	**Unit**	**F5**	**SD**	**RSD%**
NPSD	mg/kg	370 ± 0.07	0.07	8.79
NSS	mg/kg	ND		
SPS	mg/kg	ND		
SSS	mg/kg	15 ± 0.01	0.01	0.88
LOD	mg/kg	0.19		
LOQ	mg/kg	0.47		

b)

Samples	**Unit**	**F6**	**SD**	**RSD%**
NPSD	mg/kg	ND		
NSS	mg/kg	2125	0	0
SPS	mg/kg	54.15 ± 0.00	0.00	0.54
SSS	mg/kg	23.99 ± 0.03	0.03	3.07
LOD	mg/kg	0.09*, 0.13**		
LOQ	mg/kg	0.75*, 0.96**		

*For soil samples ** For sewage sludge samples

Key: - F5— Organic bound fraction

F6— Silicate bound fraction

Cu was detected in organic fractions of NPSD, SSS and in the residual fractions of NSS, SPS and SSS samples. For SSS, silicate bound fraction > organic fraction. For NPSD sample it was only detected in organic fraction while for NSS and SPS it was detected only in their silicate bound fraction. It was not detected in any water soluble, exchangeable and carbonate bound fraction of any sample. Hence, it may not be bioavailable.

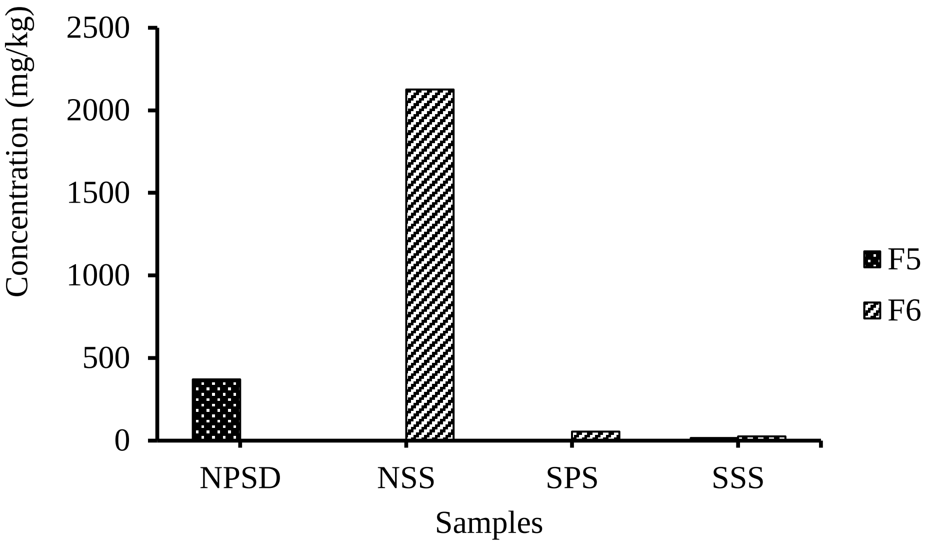

Figure 11 Fractional Concentration of Copper

4.5.3. Fractionation of Pb

The amount of dissolved lead in surface water and groundwater depends on pH and the concentration of dissolved salts and the types of mineral surfaces present. In surface water and ground-water systems, a significant fraction of lead is undissolved and occurs as precipitate, sorbed ions or surface coatings on minerals or as suspended organic matter (Evanko and Dzombak, 1997).

Table 11 Fractional Concentration of Lead

a)

Samples	**Unit**	**F2**	**SD**	**RSD%**
NPSD	mg/kg	ND		
NPSP	mg/kg	ND		
NSS	mg/kg	ND		
SPS	mg/kg	1598 ± 0.08	0.08	1.94
SSS	mg/kg	325 ± 0.02	0.02	1.74
LOD	mg/kg	0.56		
LOQ	mg/kg	0.97		

b)

Samples	**Unit**	**F3**	**SD**	**RSD%**
NPSD	mg/kg	ND		
NPSP	mg/kg	583 ± 0.10	0.1	6.27
NSS	mg/kg	472 ± 0.04	0.04	1.83
SPS	mg/kg	ND		
SSS	mg/kg	ND		
LOD	mg/kg	0.35		
LOQ	mg/kg	0.36		

c)

Samples	**Unit**	**F4**	**SD**	**RSD%**
NPSD	mg/kg	956 ± 0.07	0.07	3.81
NPSP	mg/kg	215 ± 0.10	0.10	6.27
NSS	mg/kg	246 ± 0.04	0.04	1.83
SPS	mg/kg	ND		
SSS	mg/kg	ND		
LOD	mg/kg	0.38		
LOQ	mg/kg	0.39		

d)

Samples	**Unit**	**F5**	**SD**	**RSD%**
NPSD	mg/kg	683 ± 0.07	0.07	3.81
NPSP	mg/kg	830 ± 0.10	0.01	6.27
NSS	mg/kg	ND		
SPS	mg/kg	ND		
SSS	mg/kg	ND		
LOD	mg/kg	0.36		
LOQ	mg/kg	0.37		

Key: - F2— Exchangeable fraction F4— Oxide bound fraction

F3— Carbonate bound fraction F5—Organic bound fraction

Pb was detected in exchangeable fractions of SPS, SSS, in the carbonate fractions of NPSP, NSS, in the oxide fractions of NPSD, NPSP and NSS and as well as in the organic fractions of NPSD and NPSP samples. For NPSD sample, oxide bound fraction > organic bound fraction. For NPSP sample, organic bound > carbonate bound > oxide bound fraction. For NSS sample, carbonate bound fraction > oxide bound fraction. For SPS and SSS samples, it was detected only in their exchangeable fraction. It was not detected in water soluble and residual fraction of these samples. It was detected in the fractional forms of NSS but it was not detected in its total concentration determination because it may be mobile in these samples.

It was detected in exchangeable fractions of SPS and SSS samples and carbonate bound fractions of NPSP and NSS samples. Hence it may be bioavailable in SPS, SSS, NPSP and NSS samples. But, it was detected only in oxide fractions of NPSD sample and hence, it may not be bioavailable in this sample.

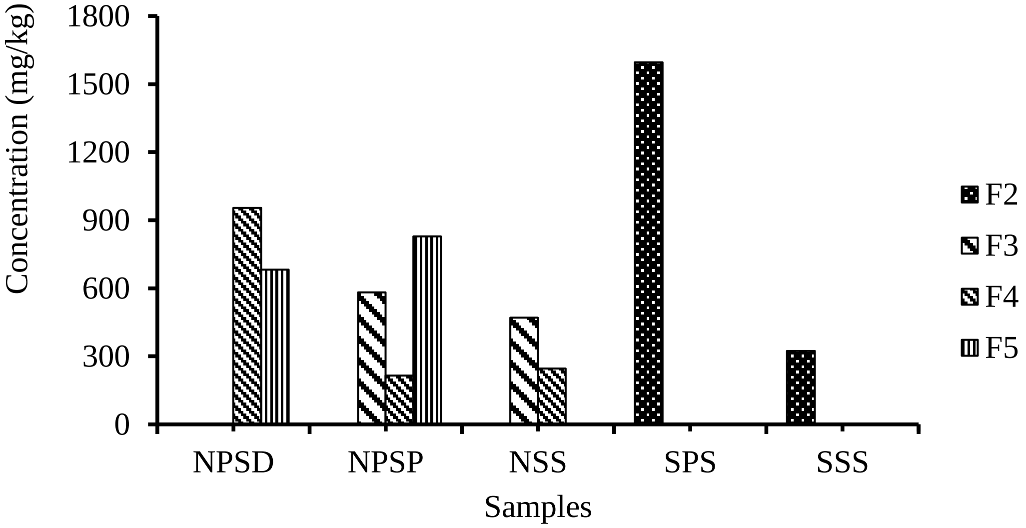

Figure 12 Fractional Concentration of Lead

4.5.4. Fractionation of Zn

Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values. At higher pH values, zinc can form carbonate and hydroxide complexes which control zinc solubility (Evanko and Dzombak, 1997).

Table 12 Fractional Concentration of Zinc

a)

Samples	**Unit**	**F1**	**SD**	**RSD%**
NPSD	mg/kg	ND		
NPSP	mg/kg	ND		
NSS	mg/kg	ND		
SPS	mg/kg	ND		
SSS	mg/kg	73 ± 0.02	0.02	3.01
LOD	mg/kg	0.09		
LOQ	mg/kg	0.22		

b)

Samples	**Unit**	**F2**	**SD**	**RSD%**
NPSD	mg/kg	46 ± 0.04	0.04	9.53
NPSP	mg/kg	12 ± 0.02	0.02	8.52
NSS	mg/kg	41 ± 0.01	0.01	3.26
SPS	mg/kg	70 ± 0.03	0.03	9.74
SSS	mg/kg	22 ± 0.01	0.01	1.61
LOD	mg/kg	0.11		
LOQ	mg/kg	0.26		

c)

Samples	**Unit**	**F3**	**SD**	**RSD%**
NPSD	mg/kg	23 ± 0.01	0.01	0.94
NPSP	mg/kg	21 ± 0.06	0.06	7.41
NSS	mg/kg	64 ± 0.01	0.01	4.41
SPS	mg/kg	24 ± 0.02	0.02	3.29
SSS	mg/kg	15 ± 0.01	0.01	4.24
LOD	mg/kg	0.15		
LOQ	mg/kg	0.36		

d)

Samples	**Unit**	**F4**	**SD**	**RSD%**
NPSD	mg/kg	18 ± 0.004	0.004	1.06
NPSP	mg/kg	17 ± 0.03	0.03	9.87
NSS	mg/kg	16 ± 0.00	0.00	0.38
SPS	mg/kg	65 ± 0.02	0.02	5.32
SSS	mg/kg	28 ± 0.01	0.01	1.00
LOD	mg/kg	0.11		
LOQ	mg/kg	0.16		

e)

Samples	Unit	F5	SD	RSD%
NPSD	mg/kg	13 ± 0.01	0.01	0.24
NPSP	mg/kg	88 ± 0.00	0.00	0.13
NSS	mg/kg	ND		
SPS	mg/kg	88 ± 0.02	0.02	5.32
SSS	mg/kg	27 ± 0.01	0.01	2.04
LOD	mg/kg	0.15		
LOQ	mg/kg	0.22		

f)

Samples	Unit	F6	SD	RSD%
NPSD	mg/kg	14 ± 0.00	0.00	0.03
NPSP	mg/kg	28.2 ± 0.01	0.01	1.52
NSS	mg/kg	13 ± 0.00	0.00	0.27
SPS	mg/kg	27 ± 0.00	0.00	0.16
SSS	mg/kg	18 ± 0.08	0.08	10.82
LOD	mg/kg	0.21*, 0.38**		
LOQ	mg/kg	0.23*, 0.65**		

*For soil samples **For sewage sludge samples

Key: - F1—Water soluble fraction

F2— Exchangeable fraction

F3—Carbonate bound fraction

F4— Oxide bound fraction

F5— Organic bound fraction

F6— Silicate bound fraction

Zn was detected in exchangeable, carbonate bound, oxide bound organic bound and silicate bound fractions of NPSD, NPSP, SPS and SSS samples. It was also detected in exchangeable, carbonate bound and silicate bound fractions of NSS and all fractions of SSS sample. For NPSD sample, exchangeable fraction > carbonate bound > oxide bound fraction > silicate bound fraction > organic bound fraction. For NPSP sample, organic bound fraction > silicate bound fraction > carbonate bound fraction > oxide bound fraction > exchangeable fraction. For NSS sample, carbonate bound fraction > exchangeable fraction > oxide fraction > silicate bound fraction. For SPS sample, organic bound fraction > exchangeable fraction > oxide bound fraction > silicate bound fraction > carbonate bound fraction. For SSS sample, water soluble fraction > oxide bound fraction > organic bound fraction > exchangeable fraction > silicate bound fraction > carbonate bound fraction. It was detected in water soluble, exchangeable and carbonate bound fractions and hence, it may be mobile and bioavailable in all samples.

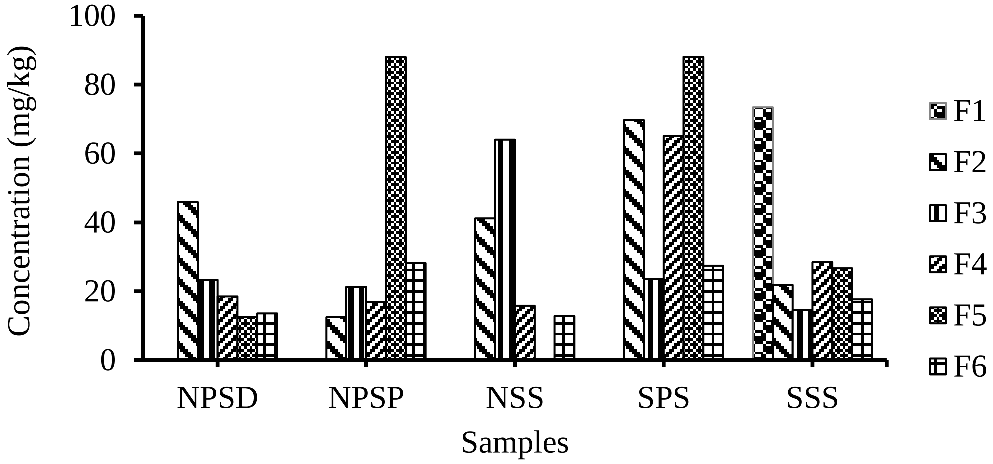

Figure 13 Fractional Concentration of Zinc

4.6. Mobility factors of selected heavy metals in the soil samples

Table 13 Mobility Factors of Selected Metals in the Soil Samples (%)

Samples	Pb	Zn
NPSD	-	61
NPSP	36	20
SPS	1	34

Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values (Evanko and Dzombak, 1997). All of the soil samples under study are neutral and Zn may be mobile in all of the samples. The amount of dissolved lead in surface water and groundwater depends on pH and the concentration of dissolved salts and the types of mineral surfaces present (Evanko and Dzombak, 1997). Hence, Pb is mobile in NPSP which is neutral.

Pb may be mobile and bioavailable in NPSP sample while Zn may be mobile and bioavailable in NPSD, and SPS samples. For SPS sample, the Mobility factor of Pb is unity and hence it may not be mobile in this sample.

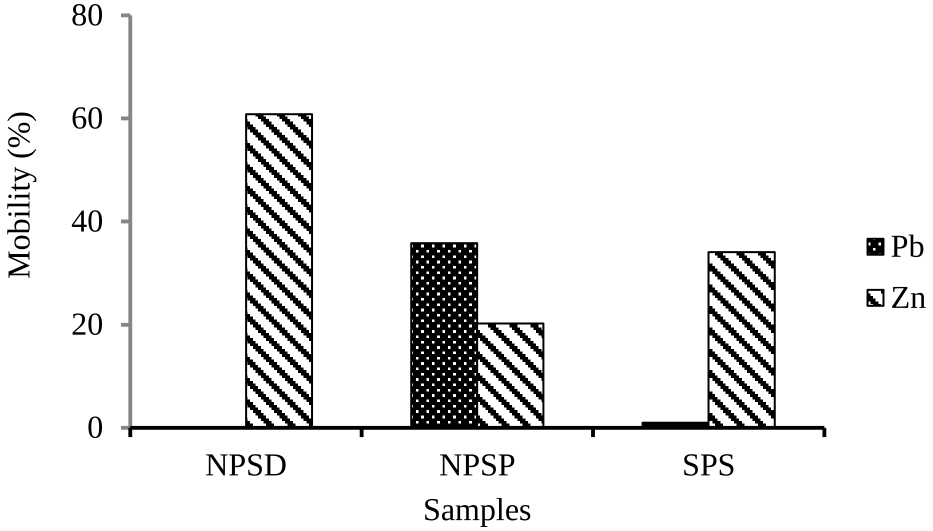

Figure 14 Mobility Factors of Selected Metals in the Soil Samples

5. CONCLUSION AND RECOMMANDATION

In this study, Cd was not detected in both total concentration determination and fractionation in all samples. Ni was only detected in total concentration determination but, it was not detected in sequential extraction. In both determinations the concentration of elements in polluted samples was greater than the reference samples in which they were detected. In total concentration determination, Cr was detected in SSS, NPSD and NPSP samples. The samples are in the order is: NPSP > SSS > NPSD in their Cr concentration level. It was not detected in other samples. Cu was detected in SSS, SUW, SPS, NPSD, NPSP and NSS samples. The samples are in the order: SPS > NPSP > NPSD > NSS > SUW > SSS in their Cu concentration level. It was not detected in other samples. It was detected in the total concentration determination for NPSD but it was not detected in its fractional forms and hence, it may not be mobile in this sample. Ni was detected in SSS, SPS, NPSD and NPSP samples. The samples are in the order: SPS > NPSD > NPSP > SSS in their Ni concentration level. It was not detected in other samples. It was detected only in the total concentration determination rather than any fractional form for all samples and hence, it may not be mobile in all of the samples. Pb was detected in SSS, NPSD, NPSP and NSS samples. The samples are in the order: SSS > NSS > NPSP > NPSD in their Pb concentration level. It was not detected in other samples. Zn was detected in all samples. The samples are in the order: SUW > SSS > NSS > NPSP > SPS > NPSD > STW > NUW > NTWP > NTWD in their Zn concentration level.

In sequential extraction, for Cr determination, NPSD and NRS samples, Silicate bound > Oxide fraction in Cr concentration. For NPSP, NSS and SSS samples, Cr was detected only in their oxide fractions. It was not detected in the water soluble, exchangeable and carbonate fraction of these samples as well as in fraction of SPS sample. Even though it was not detected in the total concentration determination, Cr was detected in the fractional forms of NSS sample. This is because of Cr may not be bioavailable in these samples. For Cu determination, SSS, silicate bound fraction > organic fraction. For NPSD sample it was only detected in organic fraction while for NSS and SPS it was detected only in their silicate bound fraction.

But, it was not detected in any fraction of NPSP and hence, it may not be mobile in this sample. For Pb determination, for NPSD sample, oxide bound fraction > organic bound fraction. For NPSP sample, organic bound > carbonate bound >oxide bound fraction.

For NSS sample, carbonate bound fraction > oxide bound fraction. For SPS and SSS samples, it was detected only in their exchangeable fraction. It was not detected in water soluble and residual fraction of these samples. It was detected in the fractional forms of NSS but it was not detected in its total concentration determination because it may be mobile in this sample. For Zn determination, NPSD sample, exchangeable fraction > carbonate bound > oxide bound fraction > silicate bound fraction > organic bound fraction. For NPSP sample, organic bound fraction > silicate bound fraction > carbonate bound fraction > oxide bound fraction > exchangeable fraction. For NSS sample, carbonate bound fraction > exchangeable fraction > oxide fraction > silicate bound fraction. For SPS sample, organic bound fraction > exchangeable fraction > oxide bound fraction > silicate bound fraction > carbonate bound fraction. For SSS sample, water soluble fraction > oxide bound fraction > organic bound fraction > exchangeable fraction > silicate bound fraction > carbonate bound fraction.

Based on their mobility in the soil samples, Pb may be mobile in NPSP sample while Zn may be mobile heavy metal in the rest samples. Both of these heavy metals are available for animals and plants from the soil polluted by the waste of Nefas Silk paint factory. In addition to this, Zn may be available for animal and plants from the soil polluted by the waste of Saygin Dima textile factory while the rest heavy metals are not bioavailable from the waste of both factories according to this study.

Finally, the concentration of Cr in NPSD and NPSP, Ni and Cr in SSS, Pb in NSS, and both Ni and Pb in the soil samples of both factories is above permissible international standard concentration limit. Therefore, the an appropriate treatment plant should be implemented to minimize the concentration of Ni in the Saygin Dima textile factory sewage sludge, Pb in Nefas Silk paint factory sewage sludge and both elements in the polluted soil samples of both factories. An appropriate treatment plant also should be implemented to minimize the mobility of Pb in the polluted soil of Nefas Silk Paint factory and that of Zn in the polluted soil samples of both factories.

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DECLARATION

“I hereby declare that the project submitted for the Degree of Master of Science (Analytical Chemistry), at Addis Ababa University, Addis Ababa, Ethiopia is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.”

Name: Shisho Haile Geleta

Signature: ________________

Place of submission: Addis Ababa University, June 2014

This project has been submitted for examination with my approval as University advisor

Name: Negussie Megersa (PhD.)

Signature ______________________

Addis Ababa University
June 2014