HEAVY METAL PHYTOEXTRACTION IN SEWAGE SLUDGE USING SUNFLOWER

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Heavy Metal Phytoextraction in Sewage Sludge using Sunflower

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DECLARATION

I certify that this thesis has not been presented for an academic award in any University or any other Institution of Higher Learning.

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

This project is dedicated to my mother Winfred Katila and my sister Linda Mwongeli for their moral, spiritual and financial support.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
GLS	Glucosinolates
U.S. EPA.	United States Environmental Protection Agency
ppm	Parts per million
mg/l	Milligram per litre
mg/kg	Milligram per kilogram
µg/g	Microgram per gram
°C	Degree Centigrade
g	Gram
cm^3	Cubic centimetre

ABSTRACT

Agricultural application of sewage sludge is an effective disposal method as it is beneficial to agricultural productivity. However, there is need to regularly monitor levels of heavy metals in sludge. Such monitoring is currently lacking in our sewage treatment plants-thereby, leading to informal use of sewage sludge in agriculture and lack of quality control. Furthermore, there is an absence of locally available technologies for heavy metal removal. Conventional processes for heavy metal removal such as chemical precipitation and membrane filtration are too expensive, require technologically advanced systems, are difficult to maintain, require a lot of expertise and are therefore not locally accessible. Heavy metals have adverse effects on human life when consumed. There is therefore need to come up with affordable, innovative technologies that can be locally used to remove heavy metals from sewage sludge used in agriculture. This study used phytoextraction, a phytoremediation process in which certain plants have the ability to absorb toxic contaminants from a soil matrix, to remove heavy metals from sewage sludge. The objective was to investigate the potential of heavy metal phytoextraction in sewage sludge using sunflower. The sewage sludge was obtained from Dandora and Kariobangi Wastewater Treatment Plants. The experimental set up was in three sets. The first containing soil and Kariobangi sewage sludge mix in the ratio 1:1. The second containing soil and Dandora sewage sludge mix in the ratio 1:1. The third set contained 100% soil which served as the control experiment. Sunflowers were grown in each of the sets and heavy metal levels were monitored in the plant roots, shoots and soil sludge mixes for a period of four months using atomic absorption spectroscopy. After the four months, cadmium levels in the sewage sludge were reduced by 84%, manganese by 91%, copper by 85%, lead by 89% and zinc by 84%. The stated heavy metals were all brought down to levels acceptable for garden soil. This proved that sunflower phytoextraction is a technology that can be assimilated in wastewater treatment plants to ensure safe agricultural use of sewage sludge. However, this should be done with keen analysis so as to avoid depleting the sludge of useful nutrients as the heavy metals are absorbed. Hence, further study should be done to determine the appropriate density of sunflower plants that should be planted on a given quantity of sewage sludge during the phytoextraction process.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Use of sewage sludge as fertilizer can be traced as far back as 1550 in Bunzlau, Germany (Epstein, 2003). In the 1980's American sewage sludge used to be dumped into the Atlantic and Pacific Oceans. Later, scientists discovered that the practice was killing marine life. This led Congress to ban ocean dumping, forcing the country to find an alternative method for disposing sewage sludge. The result was sewage sludge application to land as fertilizer ("The Commercialization of Sewage Sludge," n.d.). This mode of sewage sludge disposal has proven to be the most effective as it maximizes bulk reduction of disposable sewage sludge and also offers a significant benefit from the valuable nutrient and organic matter contained in sludge for crop production (Smith, 2011).

Currently, sewage sludge is used as fertilizer in western countries in the form of bio solids, which is sludge that has been processed for agricultural reuse. Locally, unprocessed sewage sludge is used by farmers to supplement the use of chemical fertilizers or for total replacement. This unregulated use of sewage sludge in agriculture may result in heavy metal soil contamination. Heavy metals in sewage are mainly as a result of industrial effluents. The heavy metals accumulate in the soil, get into the food chain and eventually in human and animal bodies leading to several health effects and even death (Muhammad, 2015; U.S. EPA., 1998b).

Several recent studies have been carried out to quantify heavy metal levels in sewage sludge and effluents from Kenyan wastewater treatment plants. The results indicate staggering high levels of heavy metals. Sewe (2008), revealed that final effluents from Dandora Wastewater Treatment Plant contained cadmium, manganese and lead levels above the Kenya guideline standards of 0.01, 0.2 and 0.01 mg/L respectively. In his study, cadmium ranged from 0.025 to 0.033 mg/L, manganese concentrations were from 0.085 to 0.748 mg/L and lead concentrations were between 0.083 and 0.332 mg/L. A study by

(Maina, 1984) from the University of Nairobi, Kenya, showed that chromium, titanium, nickel, zinc, lead, gallium and rubidium were present in sewage sludge samples from Kariobangi Wastewater Treatment Plant in excess of safe levels. Mulamu (2014), discovered high levels of lead, mercury and cadmium contamination in soils obtained from the environs of Dandora Wastewater Treatment Plant. Nduta (1992), determined that extremely high levels of zinc, copper, lead, cadmium and mercury were present in sewage sludge from sewage treatment sites in Kariobangi, Dandora, Kiambu, Limuru, Kiserian and Ngong. These high heavy metal levels justify the urgent need to seek methods of heavy metal removal in sewage sludge before application in agriculture.

Conventional techniques used to remove heavy metals are generally expensive, require complex systems and advanced technical know-how. It has been estimated that the overall cost to remediate soils contaminated with heavy metals in European cities costs between €59 and €109 billion. This is in an estimated 52 million hectares of land (Pueke & Rennenberg, 2005). There is a need for alternative, cheaper, easily adoptable and effective methods to clean up heavy metals. This can be achieved by a relatively new green technology called phytoextraction, which has been demonstrated by several studies to have the potential of removing heavy metals from soils using plants such as sunflower (Dushenkov & Kapulnik, 2000). Phytoextraction and other phytoremediation methods are estimated to be 50-80% cheaper than other methods of remediation (Salt et al., 1995).

During phytoextraction, the plants absorb contaminants through their roots and store them within their roots or transport them into their shoots. The plant continues to absorb contaminants from the soil until it is harvested and disposed of in a hazardous waste landfill. After harvest the soil contaminant levels are brought down. The process may be repeated as necessary to bring the contaminant levels to acceptable levels.

New approaches have been developed to enable phytoextraction to be a self-sustaining economic venture. This is through processes such as phytomining, whereby the by-products of phytoextraction are used to recover heavy metals (Elekes, 2014).

1.2 PROBLEM STATEMENT

While the use of sewage sludge in agriculture may be the best method of disposal, there is a need to regularly monitor the levels of heavy metals in sludge. Such monitoring is currently lacking in our sewage treatment plants leading to informal agricultural use of sewage sludge and a lack of proper quality control (Sushanta et al.,2017).

Furthermore, there is an absence of locally available technologies for heavy metal removal from sewage sludge. Conventional processes for heavy metal removal such as chemical precipitation and membrane filtration are too expensive, require technologically advanced systems, are difficult to maintain, require a lot of expertise and are therefore not suited for local use (Epstein, 2003; Jama, 2012).

Exposure to heavy metals leads to high health risks including reduced infant growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death (Mahurpawar et al.,2015). There is therefore a need to develop affordable innovative technologies that can be locally used to remove heavy metals from sewage sludge used in agriculture. This study sought to explore the potential of using sunflower phytoextraction technology to reduce levels of heavy metals in sewage sludge before application in agriculture as a manure.

1.3 STUDY OBJECTIVES

1.3.1 General objective

To investigate the potential of heavy metal phytoextraction in sewage sludge using sunflower.

1.3.2 Specific objectives

- To identify and quantify heavy metals present in sewage sludge samples before and after phytoextraction.
- To identify and quantify heavy metals in the sunflower plants roots and shoots before and after phytoextraction.

- To assess the efficiency of sunflower phytoextraction in removing heavy metals from sewage sludge.
- To indicate the availability of plant nutrients in the sewage sludge after phytoextraction.

1.4 JUSTIFICATION OF THE STUDY

The study addressed the absence of affordable technology that can be used locally to remove heavy metals from sewage sludge used in agriculture. Exposure to heavy metals poses high health risks (Mahurpawar et al.,2015). By quantifying the extent to which sunflower can be used to extract heavy metals from sewage sludge, a way can be found for safe use of sewage sludge in agriculture. Additionally, by addressing this absence of technology, the study adds to the body of knowledge of heavy metal extraction in Kenya. Major beneficiaries of the results of this study include local wastewater treatment plants who can employ these results to develop systems of on-site remediation of sewage sludge at a low cost, with minimal technological requirements, expertise and maintenance.

Researchers will also benefit from findings of this study as it will form a background for future related studies. Policy makers can also use the findings and recommendations of this study to formulate policies on sewage sludge heavy metal management in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Phytoextraction also known as phytoaccumulation is a phytoremediation process that uses plants to remove heavy metal contaminants from the soil. All plants absorb water and heavy metals from the soil that are essential for their growth and development. Such heavy metals include iron, zinc, manganese and molybdenum. However, excessive accumulation of heavy metals is toxic to most plants. The plants used for phytoextraction are called hyperaccumulators. These are plants that absorb unusually large amounts of heavy metal contaminants in comparison to other plants. This could possibly be for defense purposes, as excess metal accumulation can protect plants from certain natural enemies such as pathogens and herbivores to whom the accumulated metals would have a toxic effect (Muhammad, 2015).

During phytoextraction, the plants absorb heavy metal contaminants through their roots and store them within their roots or shoots. The plant continues to absorb these contaminants from the soil until harvest. After harvest, the soil contaminant levels are brought down. The harvested plants are usually incinerated with the ash disposed of in a hazardous waste landfill. The phytoextraction process may be repeated as necessary to bring the contaminant levels down to allowable limits (Abdu et al., 2011; Dushenkov & Kapulnik, 2000).

With the increased advocacy for green technology, recent studies have shown that the harvested sunflower residue from phytoextraction can be used in phytomining, biofumigation, energy production and paper production (Cornelis et al., 2011; Delplanque et al., 2013; Gomes, 2012). Pairing sunflower phytoextraction with such ventures increases the economic viability of this technology.

2.2 PHYTOEXTRACTION MECHANISM

For a plant to extract heavy metals from soil the following processes take place:

- The metal is dissolved and chelated into a substance that the plant can absorb;
- The plant roots absorb the heavy metal;
- The plant moves the chelated metal to storage;
- The plant adapts to damages caused by the metal's transportation and storage (Rascioa & Navari, 2011).

These processes are explained in detail in section 2.2.1, 2.2.2 and 2.2.3.

2.2.1 Dissolution and root absorption

Metals cannot be taken into an organism while in their normal state. The metals need to be dissolved into an ionic solution so as to enable mobility in the organism. Once the metal is mobile, it is transported over the root cell wall. Plant roots facilitate this process by secreting substances such as organic acids that capture, chelate the metal and transport the metal into the root. Chelation is a process whereby a metal is surrounded and chemically bonded to an organic compound. The chelator serves as a case to conceal the hazardous nature of the metal from the rest of the plant. This is a way that a hyperaccumulator protects itself from the toxic effects of poisonous metals (Tangahu et al., 2011).

2.2.2 Root-to-shoot transport

The root-to-shoot transportion of heavy metals in hyperaccumulators is strongly regulated by advanced gene expression. This advanced gene expression speeds up the root-to-shoot transport process thereby limiting the amount of time the metal is exposed to the plant systems before storage (Laghlimi et al., 2015).

2.2.3 Storage

The systems that transport and store heavy metals are the most critical systems in a hyperaccumulator because the heavy metals will damage the plant before they are stored.

Often in hyperaccumulators the heavy metals are stored in the roots and shoots (Dillalogue, 2014).

2.3 PLANT SELECTION CONSIDERATIONS FOR PHYTOEXTRACTION

For a plant to be used in heavy metal phytoextraction, it has to be a heavy metal hyperaccumulator. The properties that qualify a plant as a heavy metal hyperaccumulator include an enhanced rate of heavy metal uptake, a fast root-to-shoot translocation and a great ability to detoxify heavy metals through chelation (Rascioa & Navari, 2011). In addition, plants suited for phytoextraction should possess a high tolerance to metals, a high metal storage capacity and should be easily harvestable (Muhammad, 2015).

Plants that have been used in previous studies for heavy metal phytoremediation include:

- A tea herb, *Orthosiphon stamineus* B. which was used for phytoextraction of heavy metals in soils amended with sewage sludge. A decrease in the concentrations of cadmium, chromium, zinc, copper and lead was observed in the amended soils after phytoextraction. The reduced levels were 0.13 ppm for cadmium, 24.30 ppm for chromium, 252.04 ppm for zinc, 5.96 ppm for lead and 18.56 ppm for copper (Abdu et al., 2011).

- A water cabbage, *Pistia stratiotes* used by (Abubakar et al., 2014) to study remediation of waters that had been polluted with heavy metals. The plant was grown in high concentration solutions of lead, nickel and chromium for 21 days before being harvested. After heavy metal analysis using atomic absorption spectroscopy, it was observed that uptake of the heavy metals had exceeded 300 times over what is obtainable in normal plants.

- An anchored hydrophyte, *Hydrocotyle umbellata* L. that was used by (Khilji & Bareen, 2008) for the removal of toxic metals from tannery sludge effluent. *Hydrocotyle umbellata* L. showed a good tolerance for prepared concentrations of wet tannery sludge. The plants were harvested after 30, 60 and 90 days. Accumulation of toxic metals in the plants was observed to have significantly increased, with a higher amount observed in the roots than in the shoots.

- The willow, *Salix viminalis* L. was used by (Jama & Nowak, 2012) in purifying sewage sludge treated soils. In the study, it was realized that willow could accumulate ten times more cadmium than was the concentration in sewage sludge or soil.
- Water hyacinth was used by (Kulkarni et al., 2007) in reducing heavy metal content in textile processing effluent. He reported 25% - 45% reduction of heavy metal content.

Plant species used in phytoextraction are also selected based on their root depth, nature of the soil and contaminants as well as the regional climate (Laghlimi et al., 2015). The root depth directly impacts the depth of remediation. Root depth varies depending on the type of plant, soil structure and soil depth.

Sunflower is the name for a genus comprising of about 70 flowering plant species. The name is commonly used in reference to the most popular species of sunflower, *Helianthus annuus*. It is a perennial plant, characterized by a long stem and a large flowering head. Sunflowers commonly grow to heights of 2.5 and 3.5 meters, with a rough, hairy stem. Sunflowers have large, broad, toothed leaves and deep tap roots. They are commonly grown as a food crop, for oil production and for ornamental purposes (Prasad, 2007; Wolsey, 2004). The decision to select sunflower for the study was based on the following:

- In 1986 a nuclear explosion occurred at Chernobyl Nuclear Plant in Ukraine. Extensive radioactive contamination was discovered in areas up to 100km from the site. In February 1996, a company called Phytotech Inc., embarked on a project to use sunflower to clean up the contaminants in the soil and water. Mature sunflower plants were found to remove as much as 95% of toxic contaminants originally in the soil in as little as 12 days. Subsequently, sunflowers were planted in the affected areas (Dillalogue, 2014).
- After a devastating earthquake and tsunami struck Japan in March 2011, radiation leakage in Fukushima nuclear plant led to high quantities of radioactive materials and

other toxins contaminating the soil. Following the nuclear cleanup of Chernobyl, numerous projects have come up in Japan to recruit people to plant sunflower in the affected zones. Analysis has shown that the sunflowers have reduced 95% of the radiation in the soil in just 20 days (Gellerman, 2011).

- Scientists at the Centre for Pesticides and Environmental Research, Yugoslavia, studied growth and uranium uptake in sunflower, soybean and maize crops. Sunflowers were found to have the fastest growth rate and highest concentration of uranium, making them an excellent fast-cleaning crop (Jovanovic, 2008).
- Sunflowers adapt to a wide variety of soils and climatic conditions.
- Sunflowers are one of the best hyperaccumulators. Hyperaccumulators are plants with the capability to absorb and retain contaminants within their biomass with no harm caused to the plant (Dillalogue, 2014).
- Sunflowers have a high phytoextraction coefficient compared to other species (Prasad, 2007).
- Sunflowers require minimal agricultural maintenance.
- Sunflowers have deep tap roots thus resulting in a great depth of heavy metal clean up.
- Sunflowers add aesthetic value as they clean up the soil.
- Sunflowers are easy to harvest.
- After the first cycle of phytoextraction, the harvested sunflower seeds can be replanted for future phytoextraction.
- The harvested seeds can be used for energy production in the form of biodiesel and can be used for heavy metal extraction (Diels et al., 2007) thus making it an economical venture.
- The sunflower stalks can be used in energy production in the form of biogas or combustion within specialized facilities (Cornelis et al., 2011; Gomes, 2012).
- Sunflower stalks can be used in paper manufacture (Mcmullen & Mccormack, 1916; Rudi et al., 2016; Wolsey, 2004; Zavertnik, 1914). Thus making sunflower phytoextraction a self-sustaining economic venture.

2.4 PHYTOEXTRACTION AND PLANT NUTRIENT CONTENT

Mineral nutrients that are essential for plant growth are divided into two groups: macronutrients and micronutrients.

Macronutrients are divided into two more groups: primary and secondary nutrients. Primary nutrients are potassium, nitrogen and phosphorous. Plants use these nutrients in large amounts and are essential for plant growth and survival. Primary nutrients are usually lacking from the soil and therefore fertilization is always needed. Secondary nutrients are calcium, magnesium and sulfur. They are usually sufficient in the soil therefore fertilization is not always needed ("Plant Nutrients," n.d.).

Micronutrients, also known as trace elements, are nutrients that plants require in very small (micro) quantities for plant growth. Micronutrients are zinc, copper, iron, chloride, boron, manganese and molybdenum.

The extent to which nutrients are available to plants depends on soil pH and soil texture.

Soil texture refers to the amount of sand, silt, clay and organic matter in the soil. Soil texture affects the retention of water and nutrients in the soil. Clay and organic soils hold nutrients and water better than sandy soils. When water drains from sandy soils, it often carries nutrients along with it, a condition called leaching. When nutrients are leached from the soil, they are not available for plant use. An ideal soil contains equal portions of sand, silt, clay and organic matter ("Plant Nutrients," n.d.).

Soil pH is used as an indicator of the acidity and alkalinity of a soil. It is one of the most important soil properties that affects the availability of plant nutrients. Macronutrients tend to be more available in soils with high pH, while micronutrients tend to be more available in soils with low pH (Mohammad, 2014; Weissert & Kehr, 2017).

Weissert & Kehr (2017) indicate that the favourable soil pH range for plant growth is 6.0 to 6.5. In this pH range, nutrients are readily available to plants at optimum levels. IPNI Plant Nutrition Today (2010) indicates that ideal soils for plant growth have a pH that is close to neutral. Neutral soils are considered to fall within the range of pH 6.5 to 7.5. At this pH range, availability of plant nutrients is high. Northeast Region Certified Crop Adviser Study Resources (2010) indicates that a soil pH of 6.5 is considered the optimum pH for plant nutrient availability. In this regard, therefore, soil pH can be a useful indicator of the presence of plant nutrients after phytoextraction. This is an important determination as during phytoextraction, plants absorb nutrients for their growth as well as the toxins that need to be extracted. It is necessary to give assurance that the plant nutrients shall not be depleted by the phytoextraction process.

Samake et al. (2003) found that with appropriate plant combination, nutrient content in sewage sludge can be maintained at favourable levels after phytoextraction. Thereby, allowing for good potential in agriculture. Abdu et al. (2010) discovered that nutrient levels were maintained at favorable levels for plant growth even after phytoextraction on sewage amended soil. They described suitable pH range for plant growth as between 6.0 to 6.5. Xu et al. (2014) also found that after phytoextraction of sewage sludge and its use in crop production, plant nutrients in the sewage sludge were found to be more than sufficient for plant growth. It is therefore possible to maintain adequate plant nutrients in sewage sludge after phytoextraction and use pH as an indicator of their availability.

2.5 HEAVY METALS

The principal characteristic of heavy metals is that they have an atomic density greater than 5g/cm^3 and an atomic number greater than 20 (Fijalkowski et al., 2012). The most common heavy metal contaminants include zinc, lead, cadmium, chromium and mercury. The occurrence of heavy metals is as a result of two main sources:

- Natural source: Heavy metals occur naturally in soil from pedogenesis, the process in which soil is formed from the weathering of parent rock.
- Anthropogenic sources: Anthropogenic sources of heavy metals include human activities such as smelting, electroplating, mining, sludge dumping, energy and fuel production. These activities are the major sources of heavy metal contamination (Laghlimi et al., 2015).

Heavy metals are divided into two groups. Essential heavy metals and non-essential heavy metals. Essential heavy metals are also known as micronutrients. These heavy metals are necessary for plant growth and include iron, zinc, manganese and nickel. Non-essential heavy metals are not needed for plant growth and include chromium, mercury, lead, cadmium and arsenic. Essential and non-essential heavy metals are toxic to plants and animals above specific concentrations for each element. The general effects of heavy metals on plants are shown in Table 2.1.

Table 2.1: Functions and effects of heavy metals on plant growth

Heavy metals		**Functions in plants**	**Effects on plants**
Essential heavy metals	Nickel	Constituent of plant enzymes	Reduction of protein production, seed germination, enzyme and chlorophyll production
	Copper	Enables strong disease resistance, is an enzyme constituent, is involved in photosynthesis and reproductive processes	Reduced plant growth, photosynthesis and reproductive processes
	Zinc	Determines yield and quality of crops, is a component of various enzymes and of the cell membrane, involved in the reproductive phase	Reduces seed germination
Non-essential heavy metals	Cadmium	No use	Decreases plant resistance to fungal infection, reduced plant growth, distorted enzyme activity, decreased plant activity, decreased plant growth and seed germination
	Chromium	No use	Causes membrane damage and root damage,

Heavy metals	Functions in plants	Effects on plants
		decreases plant growth and enzyme activity
Lead	No use	Affects seed germination, causes reduced biomass production, inhibits root and shoot growth and reduced chlorophyll production

Source: Laghlimi et al., 2015

The toxicity of heavy metals to animals is as a result of the chemical reactivity of heavy metal ions with cellular structural enzymes, proteins and the membrane system. The primary sources of heavy metals to animals include industrial dust and fumes, aerosols, polluted water and food (Mahurpawar, 2015).

The health effects associated with common heavy metals are given in Table 2.2.

Table 2.2: Health effects associated with common heavy metals

Heavy metal	Health effects
Cadmium	In humans, long-term exposure to cadmium is associated with renal dysfunction, obstructive lung disease, bone defects, lung cancer and increased blood pressure.
Copper	Extreme doses of copper leads to fatigue and exhaustion, dry skin, paranoia, constipation, eating disorders, muscle cramps, anaemia, nerve damage, varicose veins and haemorrhoids.
Manganese	High doses of manganese leads to nausea and vomiting, muscle weakness, low blood pressure, respiratory distress, cardiac arrest, urine retention and irregular heartbeat.
Lead	In humans exposure to lead can result in problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints

Heavy metal	Health effects
	and reproductive system, and acute or chronic damage to the nervous system.
Mercury	Mercury poisoning is associated with tremors, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation. Monomethyl mercury causes damage to the brain and the central nervous system, while foetal and postnatal exposure have given rise to abortion, congenital malformation and development changes in young children.
Zinc	High doses of zinc leads to nausea, vomiting, muscle cramps and diarrhoea

Source: Jaishankar et al. ,2014

2.6 FACTORS AFFECTING HEAVY METAL PHYTOEXTRACTION

Phytoextraction rates are dependent on the phytoavailability of metals in contaminated soils. Phytoavailability refers to the degree to which contaminants are available for uptake by the living organisms exposed to them. Plants absorb heavy metals depending on the fraction that is phytoavailable to them (Chang et al., 2014). Factors that affect phytoextraction include soil properties, plant species and properties of heavy metals.

2.6.1 Soil properties

Soil pH

Soil pH influences the phytoavailability of heavy metals as soil acidity influences metal solubility and its mobility in the soil solution (Semple et al., 2003). In soils, concentrations of metal contaminants tend to increase with decreasing pH because of their displacement from exchangeable sites on solid surfaces due to the increased activity of hydrogen ions. Therefore, at low pH, metal phytoavailability increases as more metals are released in the soil solution due to competition with hydrogen ions (Laghlimi et al., 2015; Bhargava et al, 2012). At neutral or alkaline pH, most of the metals in the soil are unavailable to plants.

This is especially with lead and chromium, as they are rendered immobile (Semple et al., 2003).

Soil texture

Soil texture reflects on the particle size distribution of the soil and thus the percentage of fine particles such as clay and oxides (Laghlimi et al., 2015). Particle size distribution influences the level of metal contamination in a soil. Fine particles (<100µm) are more reactive and have a larger surface area than coarser material. Due to this, finer particles of a soil contain higher contamination rates. Studies have reported that fine textured soils contain higher amounts of lead (3889mg·kg^{-1}) than coarse textured soils (530mg·kg^{-1}). Coarser soils are also easily leached by running water than fine soils, thus enabling fine soils to hold more heavy metals than coarse soils (Sherene, 2010).

Soil organic matter

Soil organic matter has a dominant role in controlling the behaviour of metals in soils. This is because organic matter can delay both the accumulation and movement of metals in the soil (Elekes, 2014). An increase in the amount of organic matter in soils, minimizes the absorption of heavy metals by plants. Soils with high levels of organic matter actively retains a high concentration of metallic elements (Fijalkowski et al., 2012). Soils with low organic matter levels are more susceptible to contamination by trace elements (Olaniran et al., 2013).

Redox potential

Redox potential in soil results from microbial activity which cause oxidation and reduction reactions (Bolan et al., 2010). These redox reactions convert metal contaminants into less toxic or non-hazardous compounds which are more stable, less mobile and/or inert. In this way therefore, microbes play a part on the availability and mobility of metals (Muhammad, 2015).

Root zone

Plant organic acids exuded from the roots play a key role in the chelation of heavy metals in soils, which facilitate the rate of uptake by plants (Shenker et al., 2001). Plant root absorption rates are also indirectly affected by the weather. This is because weather affects the amount of water in the environment, and in this case the root zone (Kabata, 2010). Water is required in the roots zone of plants to dissolve metals into ionic form that can be absorbed by plants.

2.6.2 Plants species

Plants exhibit a large variation in their metal uptake capabilities (Muhammad, 2015). Many authors have concluded that mechanisms which may be responsible for plant species differences in metal uptake include differences in rhizosphere chemistry, xylem loading of metals and translocation within the plant (Hamon & McLaughlin, 2003). Rhizosphere chemistry refers to the chelation process. Phytoextraction rates are higher in plants that exude large quantities of organic acids for chelation. Xylem loading of metals and their translocation within the plant affects their root to shoot transportation and the translocation factor. Translocation factor is the ratio of metal concentration in the shoots to the metal concentration in the roots during phytoextraction. For a plant to be a good hyperaccumulator, the translocation factor is equal to or greater than one. Implying that the root to shoot transportation of metals within a good hyperaccumulator plant is faster and a larger concentration of heavy metals is absorbed and stored than when compared to a non-hyperaccumulator. Also, the age and growth stage of a plant can affect the uptake of metals. Plants in their exponential stage of growth absorb the highest quantity of water and nutrients and are therefore expected to contain more metals (Nouri et al., 2009; Elekes, 2014).

2.6.3 Heavy metal properties

The forms of occurrence of heavy metals in soil significantly influence their mobility. That is, whether in ionic form or chemically bonded to the mineral elements of the sample. The most mobile elements in either forms include zinc, cadmium and molybdenum while the least mobile are nickel, lead and chromium. Heavy metals that are in ionic form are more available for absorption by a plant resulting in higher rates of phytoextraction. (Fijalkowski et al., 2012).

The chemical speciation of heavy metals determines their bioavailability. Chemical speciation is majorly related to a metal’s bonding strength (Chaney & Oliver, 1996).

2.7 ECONOMIC VENTURES ASSOCIATED WITH PHYTOEXTRACTION

Plants that have been used in phytoextraction contain high levels of contaminants in their tissues. As a result, the plant residues from the phytoextraction process need to be appropriately disposed of. For a long time, the only mode of doing this was incineration. Nowadays, this biomass is increasingly being used in energy production, phytomining, paper production and biofumigation (Cornelis et al., 2011; Fukuta et al., 2004)

2.7.1 Phytomining

Phytomining is an upcoming environmentally friendly technology of growing heavy metal hyperaccumulator plants and using their harvested biomass for heavy metal extraction (Ha et al., 2010). Different methods have been adopted for heavy metal extraction from the plant biomass. These processes include:

- Thermal treatment: During thermal treatment, the organic matter from biomass is incinerated and the metals remain in the ash as oxides which can be recovered by flotation, ion exchange or magnetic field procedures (Elekes, 2014).

- Electrochemical processes: Electrochemical processes uses electrolysis for the extraction of heavy metals. In this way, this method allows for the selective recovery of metals, as each metal has a specific ion discharge potential (Fukuta et al., 2004).
- Hydrometallurgical processes: This is a complex chemical process which entails the use of chemicals for ash leaching. The solutions obtained after leaching are purified, concentrated and can be processed to extract metal ions of interest (Elekes, 2014).

Phytomining can be paired with phytoextraction so as to make use of the harvested biomass. The extracted metals can be used in various industries for tanning, production of alloys, manufacture of defense explosives, batteries and paints (Fukuta et al., 2004).

2.7.2 Energy production

A study conducted by Diels et al. (2010) looked into the pairing up of energy crop production with phytoremediation. Findings from the study indicated that the seeds from oil crops such as rape seed, maize and sunflower can be used for biodiesel production in specialized facilities after phytoremediation. Additionally, the plant biomass can be incinerated, gasified and anaerobically digested to produce energy.

Delplanque et al. (2013) conducted a study on the behaviour of metals after the combustion of leaves and shoots of *Salix,* which had been grown and used in phytoremediation. The authors concluded from the study that the combustion of biomass after phytoremediation reduced the waste volume, therefore a useful mode of waste management after phytoremediation. The residue from this process is deposited in a hazardous land fill.

2.7.3 Paper production

Several authors have investigated the option of using phytoextraction plant residue in paper production. The results indicate successful applications especially if the dried plant stalks are woody and containing substantial amounts of cellulose (Rudi, 2016; Wolsey, 2004).

2.7.4 Biofumigation

Published research results show that the accumulation of heavy metals in plants can stimulate the synthesis of glucosinolates (GLS). GLS is an organic compound containing sulphur. The product of their enzymatic degradation called isothiocyanates exhibit biocidal properties which are used in biofumigation. This can be used to combat bacteria, parasites and fungi attaching crops (Piekarska et al., 2011; Rios, 2017).

A recent published study used white cabbage to reduce the level of heavy metals in soil. At the same time plant residues from the cabbage were used for biofumigation. Interesting to note from this study, is that the heavy metals taken up during phytoextraction intensified the synthesis of bioactive compounds including GLS and facilitated the needed resistance to bacteria, parasites and fungi (Piekarska et al., 2011). This shows that the use of plant residues after phytoextracation for biofumigation can be justified.

2.8 SEWAGE TREATMENT PLANTS IN NAIROBI

2.8.1 Dandora Wastewater Treatment Plant

Dandora Wastewater Treatment Plant is located 30 km to the East of Nairobi City. The plant has stabilization ponds in eight series, all totalling to 38 ponds (Sewe, 2008). The plant treats about 80,000 m^3/day, which is about 80% of the waste water generated within Nairobi County. The major source of wastewater treated in this plant is from industries (Mbugua, 2015; Sewe, 2008). Wastewater in this plant is treated through two processes; physical treatment which consist of screen chambers where large solids are removed either mechanically or manually from the waste water and biological treatment using waste stabilization ponds where naturally occurring bacteria remove organic and inorganic loads (Mara et al., 1998). From the screen chambers, the wastewater is pumped to a splitting chamber and distributed over two anaerobic ponds and facultative ponds. Final treatment is achieved in the maturation ponds (Sewe, 2008).

Anaerobic ponds have anaerobic bacteria which decompose the organic matter in raw wastewater into carbon dioxide and methane. Solids in the raw waste water settle down in the sludge layer (Dominica et al., 2009). In the facultative ponds, aerobic oxidation of the upper layers of the wastewater and anaerobic decomposition at the bottom layers of the waste water occur simultaneously. Maturation ponds provide the final level of treatment. They are shallow ponds, allowing sunlight to penetrate to the full depth for photosynthesis to occur. Photosynthetic algae release oxygen into the water and at the same time consume carbon dioxide produced by bacterial respiration. At this shallow depth, the sun's UV rays are also able to kill pathogens within the wastewater ((Mara, 2004; Mbugua, 2015).

Sewe (2008) collected samples from the final treatment ponds in Dandora Wastewater Treatment Plant. He analysed the samples for heavy metal content and found that cadmium, manganese and lead levels had exceeded safe levels for discharge into surface water bodies. Cadmium levels were 0.01 mg/l, manganese levels 0.2 mg/l and lead levels 0.01 mg/l. He recommended that measures be put in place to improve the quality of the final effluent.

2.8.2 Kariobangi Wastewater Treatment Plant

Kariobangi Wastewater Treatment Plant handles a daily treatment capacity of 32,000m3 from domestic effluent from areas such as Westlands, Kariobangi and Nairobi CBD (Mbugua, 2015). Gross solids are removed by use of grit chambers and bar screens. After this stage of separation, the raw wastewater moves to the second stage of treatment in the sedimentation tanks or primary clarifier. At the primary and secondary sedimentation tanks, sludge is generated. This sludge is taken to the sludge pump house and further pumped to primary digesters which are enclosed tanks that offer anaerobic conditions. The anaerobic microorganisms in these tanks digest the sludge and in the process pathogens are destroyed (Mara, 2004).

2.9 CRITERIA USED TO SELECT HEAVY METALS TO BE INVESTIGATED

The criteria used to select the heavy metals to be investigated in the study was based on previous studies done at Kariobangi and Dandora Wastewater Treatment Plants. The studies used are shown below.

- Andere (2016) investigated the efficiency of Kariobangi Wastewater Treatment Plant. He conducted his study for a period of four months on samples collected from the inlet, the clarifiers and the outlet. He discovered that the highest levels of cadmium levels during the four months was 1.4465 mg/l, 0.39 mg/l for lead, 10.16 mg/l for chromium and 4.2335 mg/l for zinc.
- Mbugua (2015) investigated the suitability of wastewater sludge for agricultural use. He used (Ruai) Dandora Wastewater Treatment Plant as the case study. He found the highest levels of zinc in sludge obtained from the anaerobic ponds to be 5937.61 mg/kg, copper was at 1750 mg/kg, cadmium was at 40 mg/kg, chromium was at 7714 mg/kg, lead was at 3464.33 mg/kg and nickel at 400 mg/kg.
- Nduta (1992) conducted a study to determine the levels of heavy metals in sewage sludge, sewage effluent, garden soils and food crops grown in ordinary and sewage sludge amended soils. She found that vegetables grown in soil amended with sludge from Kariobangi Wastewater Treatment Plant were highly contaminated with heavy metals. The zinc content of the samples ranged between 40.0 - 840.0 µg/g. The cadmium content from 0.1 – 25.0 µg/g. The copper content from 40.0 – 220.0 µg/g. While the mercury content ranged from 0.1 – 12.0 µg/g.
- Maina (1984) in his study on heavy metal analysis of sewage sludge by x-ray fluorescence discovered the following heavy metals in sludge samples collected from Kariobangi Wastewater Treatment Plant: Titanium (2220 - 8550 ppm); Chromium (90 - 530 ppm); Iron (28000 - 61270 ppm); Nickel (26 - 97 ppm); Copper (296 - 490 ppm); Zinc (1350 - 2400 ppm); Gallium (11 - 24 ppm); Lead (248 - 580 ppm);

Rubidium (16 - 50 ppm); Strontium (79 - 170 ppm); yttrium (29 - 67 ppm); Zirconium (210 - 460 ppm) ; and Niobium (53 - 116 ppm).

- Sewe (2010), conducted a study to establish the efficiency of Dandora Wastewater Treatment Plant. She collected samples of the treated wastewater, analysed them for heavy metals and found cadmium levels ranged between 0.025 – 0.033 mg/l, manganese levels were from 0.085 – 0.748 mg/l and lead levels were between 0.083 and 0.332 mg/l.

Based on the above studies it was established that the most likely heavy metals to be found in the sewage sludge samples from Dandora and Kariobangi Wastewater Treatment Plants were cadmium, zinc, lead, copper and manganese. This allowed narrowing down of the wide possibility of heavy metals and focusing on the phytoextraction of these metals. Thereby, saving on time and finances for the study.

2.10 METHOD OF TESTING

Atomic absorption spectroscopy(AAS) was used in the study to determine heavy metal levels. It is an analytical technique used for the determination of elements using the absorption of optical radiation by free gaseous atoms. Each of the elements present in an atomic vapour phase absorbs radiation at a limited number of wavelengths over an exceedingly narrow spectral region. The wavelength bands which each element can absorb are different from another element, thereby allowing analysis of different elements with little spectral interference. This implies that traces of one element can be determined in presence of a high concentration of other elements. An atomic absorption spectrometer can either have a flame or graphite tube atomizer. In this study flame atomic absorption spectroscopy was used.

2.11 FLAME ATOMIC ABSORPTION SPECTROSCOPY

Flame atomic absorption spectroscopy uses a flame atomizer. In this technique a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator and onto a detector that measures the amount of light frequency absorbed by the atomized element in the flame. Each element absorbs a specific light frequency when in vaporized form (Kasima, 2014).

The schematic diagram of flame AAS is in Figure 2.1.

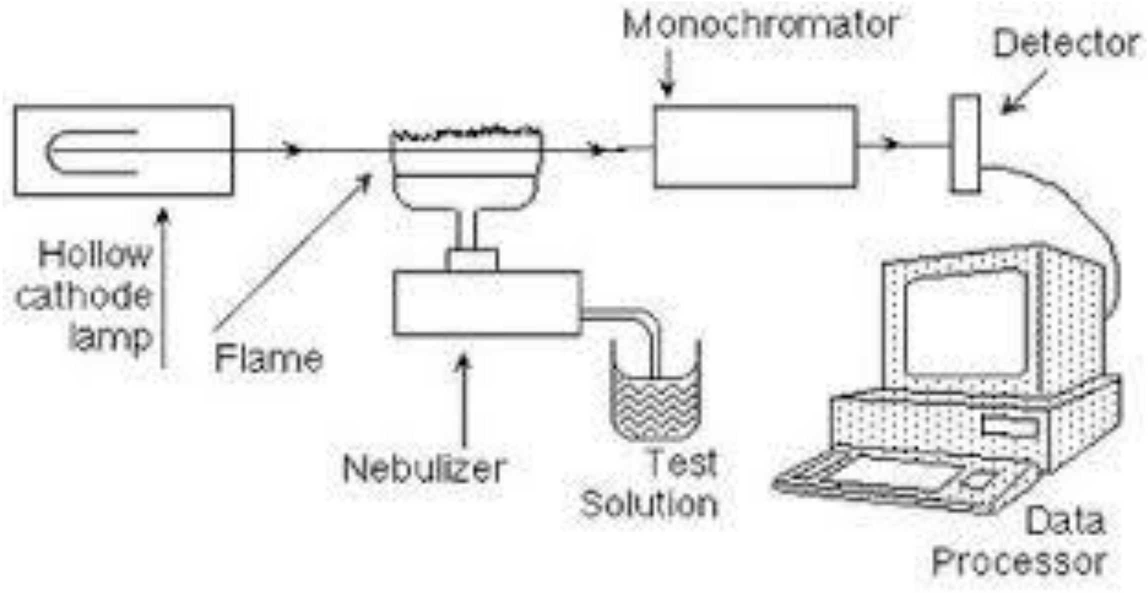


Figure 2.1: Schematic diagram of a flame AAS (Sharma, 2012)

A flame atomic absorption spectrometer consists of the following;

- A light source: Hollow cathode lamps are the most common radiation source in AAS. It comprises of a tungsten anode and a hollow cylindrical cathode made of the element to be determined. This is sealed in a glass tube filled with an inert gas such as neon or argon. Each element has its own unique lamp used for its analysis.
- A nebulizer: A nebulizer sucks up liquid samples at a controlled rate and creates a fine aerosol spray for introduction into the flame.
- An atomizer: Atomization is the separation of particles into individual molecules and breaking molecules into atoms. This is done by exposing the analyte to high temperatures in a flame or granite furnace. Atomizers can either be flame atomizers or granite tube atomizers. In flame atomizers, a flame is created by mixing an oxidant

gas and a fuel gas. In most cases, air-acetylene flame or nitrous oxide-acetylene flame is used. Liquid or dissolved samples are typically used in flame atomizers. Graphite tube atomizers uses a graphite coated furnace to vaporize the sample. The graphite tubes are heated using a high current power supply.

- A monochromator: A monochromator is used to select the specific wavelength of light absorbed by the sample and to exclude other wavelengths. The selection of the specific light allows the determination of the specific element in the presence of others.
- A detector: The light selected by the monochromator is directed into a detector which is typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity. The processing of electrical signals is done by a signal amplifier.

The following steps occur within a flame atomic absorption spectrometer to determine the concentration of a certain metal ion in a sample:

- A hollow cathode lamp emits light of a particular frequency.
- The light emitted by the lamp passes through the test sample vaporized in a flame.
- The degree of light absorption is directly proportional to the concentration of the metal in the sample.
- The intensity of the light passing through the flame is measured by a photomultiplier tube.
- By comparing the intensity with that produced from a control sample containing none of the metal ions being tested for, the degree of absorption can be determined.
- The absorbance is then compared to that of a series of diluted standard solutions in order to determine the concentration.
- This involves the use of a calibration graph.

- A linear calibration curve is produced when the absorbance is plotted against the concentration of the standard solutions. The equation of this line can be displayed in the form: $y = mx + b$

  Where y = absorbance, m = gradient of the line, x = concentration (mg/l), b = y intercept

- The absorbance recorded for the test sample can be matched with a concentration using the graph.

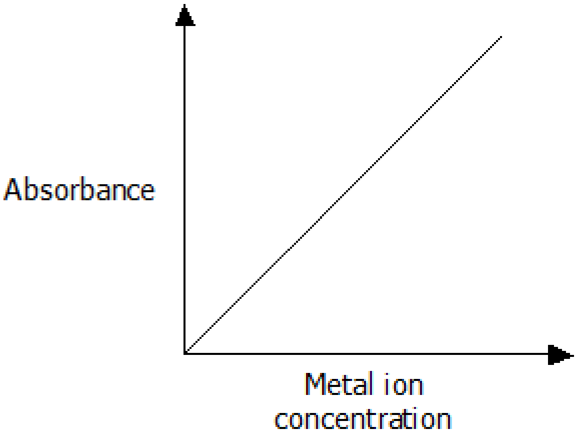


Figure 2.2: General layout of a calibration graph (Sharma, 2012)

2.12 RESEARCH GAPS IN PREVIOUS USES OF SUNFLOWER PHYTOEXTRACTION IN SEWAGE SLUDGE

Kilongi (2017) compared the phytoremediation potential of mustard plant, sunflower plant and turnip in the phytoremediation of heavy metals from sewage sludge collected at Kariobangi Wastewater Treatment Plant. She discovered that copper, zinc, iron and manganese were accumulated up to 91% in the plants. However, she did not assess the availability of plant nutrients in the sewage sludge after phytoremediation. She also did not relate the rates of phytoremediation to the plants' age. In her study, she used citric acid as a chelating agent to accelerate the phytoremediation process. In this study, no chelating agent was used. Evaluation of the phytoextraction process solely depended on the sunflower's performance with no aid.

CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

Sewage sludge samples were collected from Dandora and Kariobangi Wastewater Treatment Plants, which are the two major sewage treatment plants in Nairobi, Kenya. Dandora treatment plant is lagoon based while Kariobangi uses conventional sewage treatment.

For the study, sunflower seedlings planted in a nursery were transplanted after 3 weeks to three experimental sets. One set contained 1:1 ratio of soil and sludge from Dandora Sewage Treatment Plant. The second set contained 1:1 ratio of soil and sludge from Kariobangi Sewage Treatment Plant. The third set contained 100% soil and served as the control experiment. A mix ratio of 1:1 was used in line with a study previously done by Nduta (1992). In her study she managed to successfully grow spinach, cabbage, carrots, lettuce and potatoes, using a mix of 1 part of sewage sludge to 1 part of soil. The experimental code used was K1, K2 and K3 for buckets containing soil and Kariobangi sludge mix. D1, D2 and D3 for buckets containing soil and Dandora sludge mix. C for the control experiment. The experimental set up is shown in Figure 3.1.

Heavy metals analysis for the sunflower plants roots, shoots, soil and soil sludge mixes was done when transplanting. Thereafter monthly analysis measurements were conducted on the soil sludge mixes for a period of 3 months to obtain conclusive results. A second heavy metal analysis for the sunflower plants roots and shoots was done during harvesting.

Atomic absorption spectroscopy was used to analyze heavy metals in this study. This is because atomic absorption spectroscopy can determine over 70 different elements in a sample, has high precision and the equipment required was available within the university campus.

pH tests were done on the soil sludge mix samples after phytoextraction to indicate the availability of mineral nutrients for plant growth.

3.2 STUDY AREA

The area for sewage sludge collection was located within two wastewater treatment plants. The first, Dandora Wastewater Treatment Plant and the second, Kariobangi Wastewater Treatment Plant. These two are the main wastewater treatment plants in Nairobi. Combined, they serve approximately 48 percent of Nairobi's population (Andere, 2016; Kenya National Bureau of Statistics, 2002).

Dandora Sewage Treatment Plant treats 80% of Nairobi's wastewater with the exception of wastewater from Nairobi's Central Business District (CBD), Kariobangi South, Kariobangi North, Mathare, Uhuru, Eastleigh, Jericho and Buru Buru whose wastewater is treated at Kariobangi Sewage Treatment Plant (Andere, 2016)

Locations of these plants are as shown in Figure 3.2.

The site for growing the sunflower plants was at Syokimau, Machakos County.

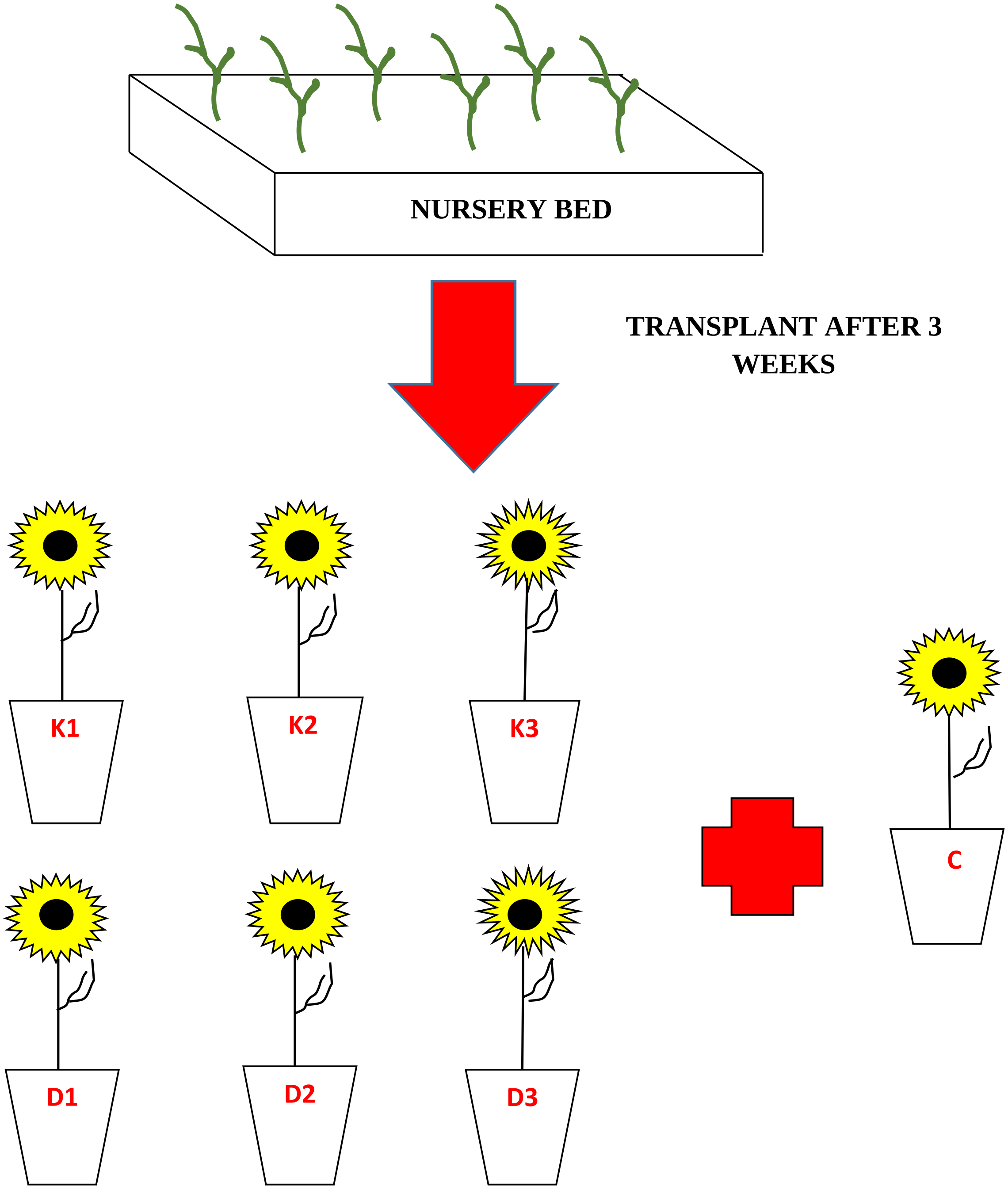
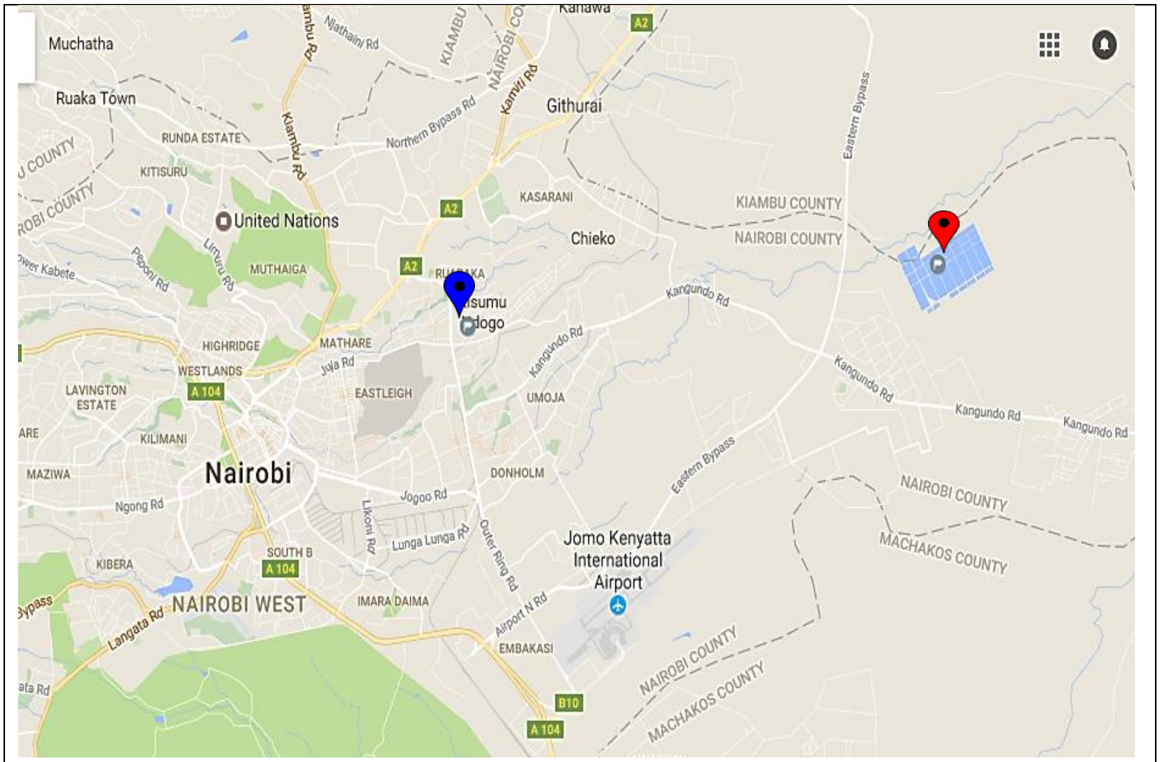


Figure 3.1: Experimental set up for the sunflower plants



KEY:

🔴 Dandora Sewage Treatment Plant

🔵 Kariobangi Sewage Treatment Plant

Figure 3.2: Location map for sewage sludge collection sites

3.3 EXPERIMENTAL PROCEDURES

The experimental protocals followed Nduta (1992), Haswell (1991), Reynolds et al. (1970), Prakash (2014), Schrenk (1975) and Varma (1985). The details are in Section 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 and 3.5.6.

3.5.1 Reagents

All the reagents used were of analytical grade unless otherwise stated. The specific reagents are given in each section.

3.5.2 Cleaning of glassware

All glassware which included beakers, pipettes, measuring cylinders, volumetric flasks etc. were thoroughly cleaned with tap water and detergent. They were rinsed with water and twice with 1:1 nitric acid solution. They were then rinsed with water and finally with distilled–deionized water. The glassware was dried in the oven at 105 ^{0}C.

3.5.3 Preparation of standard solutions

Zinc stock solution was prepared by dissolving 1.2450 g of zinc nitrate in 100 ml 0.5 M nitric acid. This was then diluted to 1 litre in a volumetric flask with 0.5M nitric acid.

Lead stock solution was made by dissolving 1.5980 g of lead nitrate in 100 ml of 0.5 M nitric acid. This was then diluted to 1 litre in a volumetric flask with 0.5 M nitric acid.

Cadmium stock solution was made by dissolving 2.1032 g of cadmium nitrate in 250 ml 0.5 M nitric acid. Thereafter, it was diluted to1 litre in a volumetric flask using 0.5 M nitric acid.

Copper stock solution was prepared by dissolving 3.7980 g of copper nitrate in 250 ml of 0.5 M nitric acid. This was then diluted to 1 litre in a volumetric flask with 0.5M nitric acid.

Manganese stock solution was made by dissolving 3.6077 g of manganesse chloride in 50 ml of 0.5 M nitric acid. Thereafter, it was diluted to 1 litre in a volumetric flask with 0.5 M nitric acid

Each of the above 1000 ppm stock solutions was stored in a plastic bottle. Standards for AAS analysis were then obtained by serial dilution of the 1000 ppm stock solutions. These standard solutions were used for calibration of the AAS instrument and calibration curves prepared.

3.5.4 Sampling

At the Dandora and Kariobangi Wastewater Treatment Plants, sludge is put in sludge drying beds to dry. For both treatment plants, one sample of sludge was taken for every 3 sludge drying beds. The samples were weighed and stored in labelled plastic buckets. In most cases immediate sample analysis was not performed after collection. For this reason, appropriate storage conditions, at or below 4 ℃, was employed for a period not exceeding 3 days.

3.5.5 Sample preparation

Sludge and soil samples:

The samples were dried in the open for 2-3 days, ground using a pestle and mortar to a very fine powder then dried in the oven overnight at 105 ^{o}C.

Sunflower samples:

Sunflower samples were thoroughly washed using tap water and then washed twice with distilled-deionized water. The samples were then dried by sandwiching in filter paper before being weighed ready for digestion.

3.5.6 Procedure for AAS

Sample digestion

The soil, sewage sludge, sunflower root and shoot samples were openly digested using aqua regia $HCl:HNO_3$ digestion as follows:

Sewage sludge and soil samples:

- 0.1 g of oven-dry sludge/soil was weighed and placed in a digestion flask.
- 25 cm^3 of aqua regia $HCl:HNO_3$ was added. This was digested at boiling point for 2 hours. Complete digestion was indicated by a light-coloured, clear solution.
- Heating was then stopped and the solution allowed to cool.
- The solution was filtered using filter paper no. 42 and made up to 50 cm^3 with distilled - deionized water to make it ready for analysis.

Sunflower samples:

- 1 g of dried sunflower sample, root and shoot separately, were weighed and placed in an oven 550 °C for ashing for 5-6 hours.
- 50 ml of 0.5 M nitric acid was added to the ashed samples and filtered ready for AAS analysis.

AAS instrument calibration:

The operating conditions for AAS analysis is based on a cathode lamp for a single element. The instrument type used was Shimadzu AA-6200. A hollow cathode lamp was installed in the instrument for the metal under investigation. That is lead, zinc, manganese, copper and cadmium. The wavelength dial was roughly set according to the table below:

Table 3.1: Wavelength instrument detection level sensitivity and optimum concentration range for elements

Element	Wavelength (nm)	Flame gases	Instrument Detection Level (mg/l)	Sensitivity	Optimum Concentration Range (mg/l)
Cd	228.8	Air-Acetylene	0.002	0.025	0.05-2
Cu	324.7	Air-Acetylene	0.01	0.1	0.01-2
Mn	279.5	Air-Acetylene	0.01	0.05	0.001-2
Pb	283.3	Air-Acetylene	0.05	0.5	0.001-2
Zn	213.9	Air-Acetylene	0.005	0.02	0.0025-2

Source: Prakash, 2014

The slit width was set according to the manufacturer's setting for the element being measured.

3.5.7 Procedure for pH Test

The experimental procedure for pH was adopted in accordance to Prakash (2014).

- 8 g of soil-sewage sludge mix was put in a plastic cylinder.

- 500ml of distilled water was added to the mix and vigorously mixed for one minute.
- The mixture was let to stand for 30 minutes.
- Filter paper was used to filter the solution into a plastic cylinder.
- A pH test strip was inserted into the solution.
- The pH test strip colour was compared to the colour chart provided with the strips to determine the pH.

3.6 METHOD OF ANALYSIS

The data collected was subjected to statistical analyses using Microsoft Excel package. Means and plotting of graphs were used to establish the correlation between heavy metal levels and to establish whether any significant difference would exist at different stages of the phytoextraction process.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results of the heavy metals identified and quantified in the soil sludge mixes, sunflower plants roots and shoots. Thereby, identifying the efficiency of sunflowers in phytoextraction. This chapter also presents the pH test results for the soil sludge mixes and soil, which will serve as an indicator of the availability of plant nutrients.

4.1 HEAVY METALS PRESENT IN SEWAGE SLUDGE SAMPLES

The heavy metals identified in the soil sludge mixes and their mean concentrations for the phytoextraction period are as in Table 4.1.

Table 4.1: Heavy metal levels in the soil sludge mixes and pure soil during the phytoextraction process

Heavy metal	Experimental samples	Concentration in µg/g			
		1st Month	2nd Month	3rd Month	4th Month
Cadmium	**D1**	66.72	43.44	22.16	8.31
	D2	59.48	37.67	29.88	11.41
	D3	57.02	32.76	20.97	11.53
	Mean D	**61.07**	**37.96**	**24.34**	**10.42**
	K1	47.81	24.73	10.77	9.64
	K2	51.20	21.58	11.52	9.72
	K3	45.83	22.68	12.73	8.01
	Mean K	**48.28**	**23.00**	**11.67**	**9.12**
	C	**24.51**	**11.22**	**6.15**	**1.96**
Manganese	**D1**	3906.09	2001.59	694.55	473.11
	D2	3930.95	2020.76	657.43	441.97
	D3	3758.83	2953.11	600.19	392.25
	Mean D	**3865.29**	**2325.15**	**650.72**	**435.78**
	K1	2952.78	1868.59	511.14	379.99
	K2	2927.14	1799.10	502.46	350.48
	K3	2976.30	1600.39	587.96	445.73

	Mean K	**2952.07**	**1756.03**	**533.85**	**392.07**
	C	**2443.92**	**567.39**	**233.34**	**38.95**
Copper	**D1**	3479.97	2480.46	1463.20	1184.37
	D2	4726.38	2590.63	1009.16	687.59
	D3	4586.43	2788.44	916.46	487.11
	Mean D	**4264.26**	**2619.84**	**1129.61**	**786.36**
	K1	2388.54	999.73	352.84	201.72
	K2	1998.51	897.60	253.55	189.31
	K3	1764.32	689.59	97.11	54.41
	Mean K	**2050.46**	**862.31**	**234.50**	**148.48**
	C	**36.9**	**17.49**	**5.33**	**1.07**
Lead	**D1**	600.93	298.46	102.46	84.43
	D2	588.74	203.14	92.43	77.49
	D3	550.47	193.72	76.60	70.03
	Mean D	**580.05**	**231.77**	**90.50**	**77.32**
	K1	463.82	322.10	95.07	57.22
	K2	401.44	159.42	88.92	43.12
	K3	496.68	358.66	99.89	60.11
	Mean K	**453.98**	**283.39**	**94.63**	**53.48**
	C	**157.18**	**47.74**	**10.98**	**4.48**
Zinc	**D1**	4603.52	2555.37	1001.73	888.40
	D2	4596.48	2434.32	994.53	870.61
	D3	4566.41	2305.47	1084.11	860.16
	Mean D	**4588.80**	**2431.72**	**1026.79**	**873.06**
	K1	2947.45	1955.91	979.40	504.76
	K2	2920.11	1677.39	926.53	478.46
	K3	3050.55	1980.72	987.85	363.17
	Mean K	**2972.70**	**1871.34**	**964.59**	**448.80**
	C	**1192.11**	**881.10**	**421.94**	**113.13**

Cadmium, zinc, lead, manganese and copper were identified in the sludge soil mixes and soil samples. The possible industries discharging heavy metals into the Wastewater Treatment Plants include tanneries, pharmaceutical industries, metal plating factories, automotive battery industries, paint manufacturing industries amongst others (Sewe, 2010; Nduta, 1992).

Identifying cadmium, zinc, lead, manganese and copper in the control experiment, which consisted of 100% soil is an indication of heavy metal contamination.

A reduction in heavy metal levels in the sludge soil mixes and control experiment was observed during the phytoextraction process. This is as a result of the heavy metal uptake by the sunflowers as they are hyperaccumulators and are thus able to store toxic matter within their biomass without getting damaged (Kilongi, 2017).

A higher concentration of heavy metals was observed from Dandora sewage sludge soil mix as compared to Kariobangi sewage sludge soil mix. This is because Dandora Sewage Treatment Plant treats 80% of Nairobi's wastewater with the exception of wastewater from Nairobi's Central Business District (CBD), Kariobangi South, Kariobangi North, Mathare, Uhuru, Eastleigh, Jericho and Buru Buru whose wastewater is treated at Kariobangi Sewage Treatment Plant (Andere, 2016). These mentioned areas served by Kariobangi Sewage Treatment Plant are mainly residential areas. This leaves treatment of wastewater from the vast industrial and commercial environs of Nairobi city to Dandora Sewage Treatment Plant. Therefore, a larger heavy metal load would be expected in Dandora sewage sludge soil mix when compared to Kariobangi sewage sludge soil mix. Industries that discharge wastewater containing a high load of heavy metals to Dandora Sewage Treatment Plant include East African Leather, Regal Pharmaceuticals, Sunflag Textiles and Knitwear Mills Ltd and Modern Lithographic Kenya Ltd (Sewe, 2008). The control experiment presented the least heavy metal concentration as no sewage sludge was added to it.

Figure 4.1, 4.2, 4.3, 4.4 and 4.5 are graphs that represent the heavy metal concentration reductions observed.

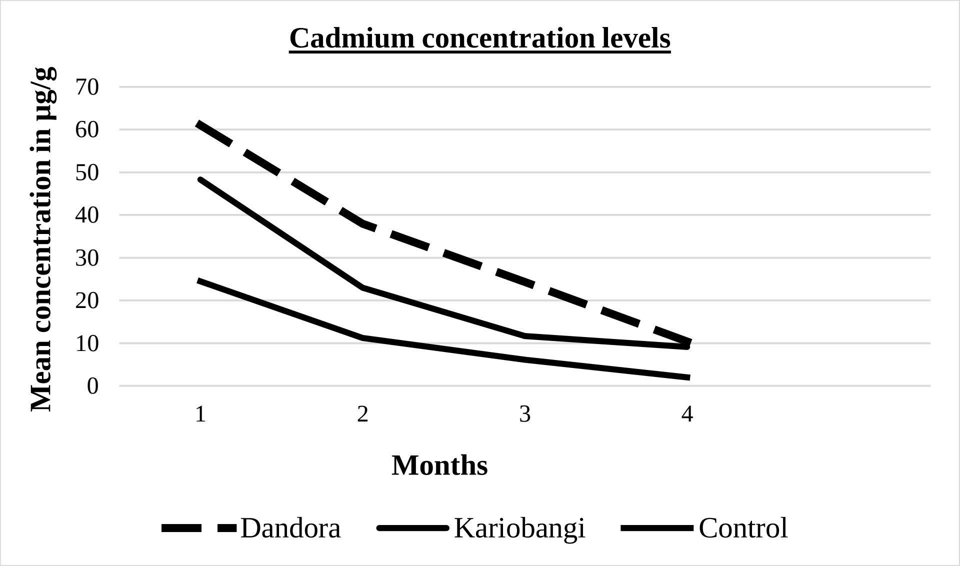


Figure 4.1: Cadmium concentration levels in the soil sludge mixes and soil

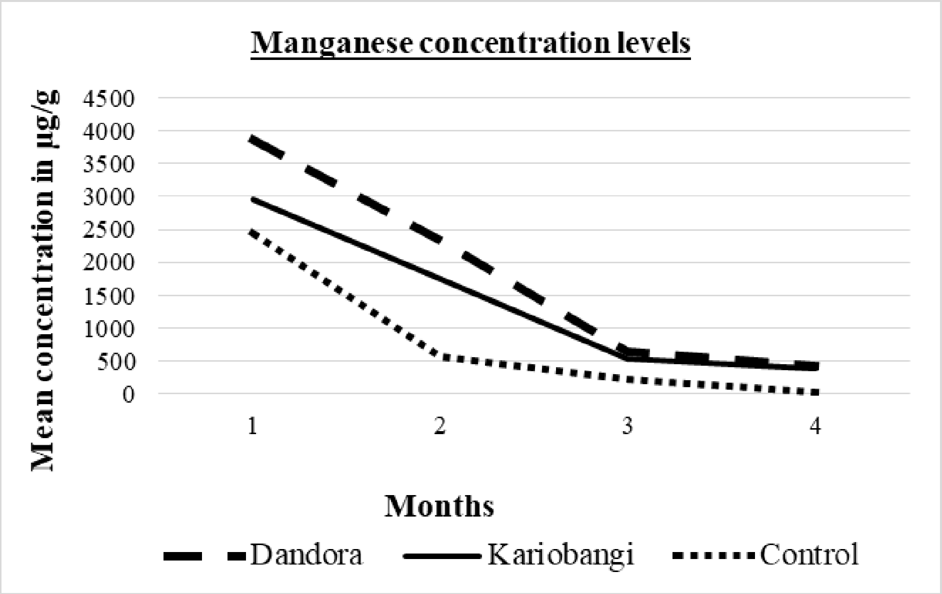


Figure 4.2: Manganese concentration levels in the soil sludge mixes and soil

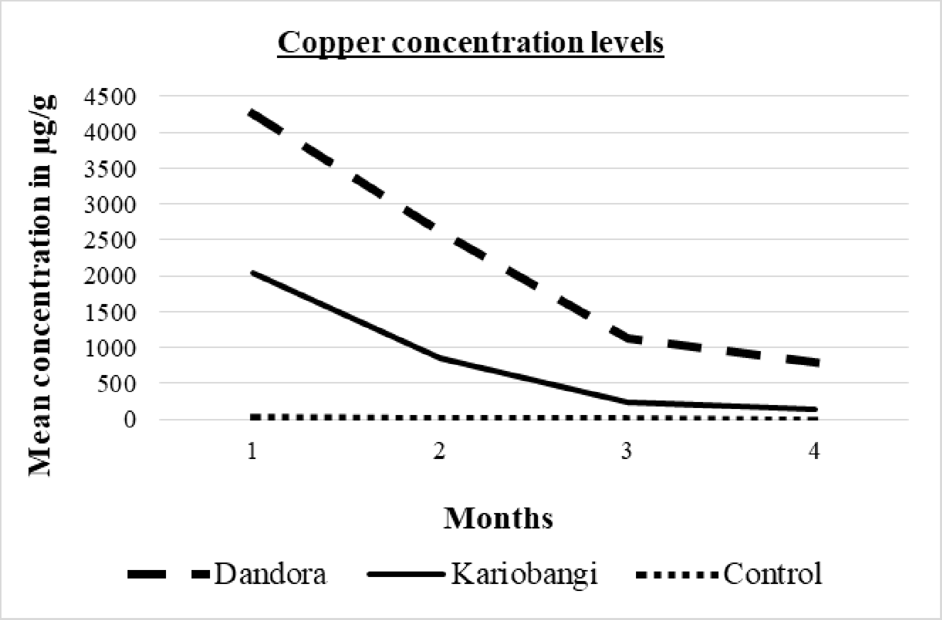


Figure 4.3: Copper concentration levels in the soil sludge mixes and soil

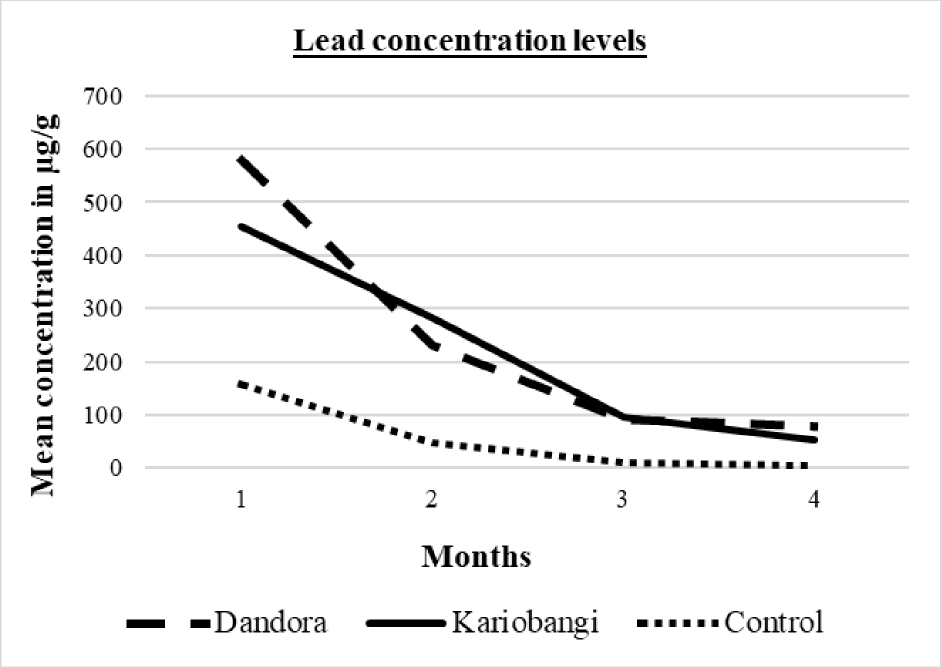


Figure 4.4: Lead concentration levels in the soil sludge mixes and soil

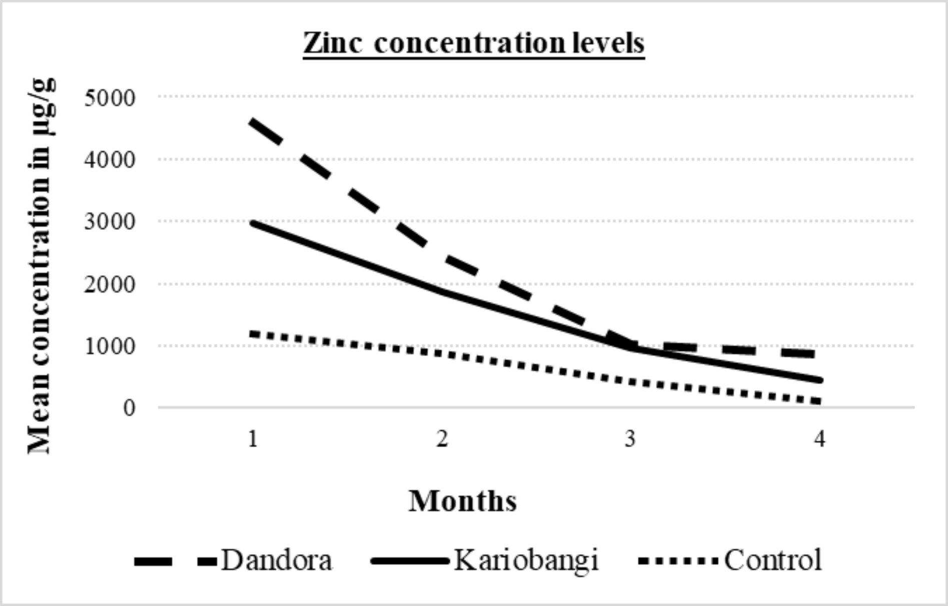


Figure 4.5: Zinc concentration levels in the soil sludge mixes and soil

It can be observed from the graphs that there's a rapid decline in heavy metal levels in the sludge soil mixes and soil during the first, second and third months. After which, the reduction of heavy metals slows down. Indicating that the heavy metal reductions increased with the age of the plants until the last month where they dropped. This is observed because a plant's rate of absorption increases with increase in age/size. Especially during the periods of exponential growth, as the uptake of water and nutrients must be increased to maintain maximum plant growth (Tolman et al., 1990). During the last month the sunflowers absorbed reducing values of heavy metals until their death. This is because a plant progressively absorbs less nutrients and water after the reproductive phase till its death (Tolman et al., 1990).

4.2 HEAVY METALS PRESENT IN THE SUNFLOWER PLANTS ROOTS AND SHOOTS

The heavy metal's concentration in the sunflower roots and shoots during transplanting and harvest are presented in Figure 4.6, 4.7, 4.8, 4.9 and 4.10.

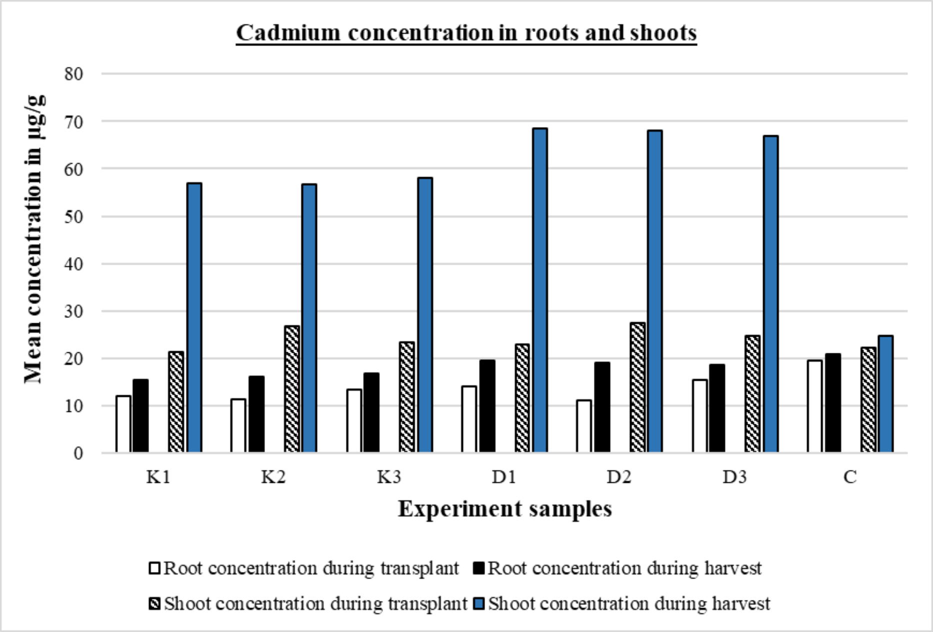


Figure 4.6: Cadmium concentration in sunflower roots and shoots

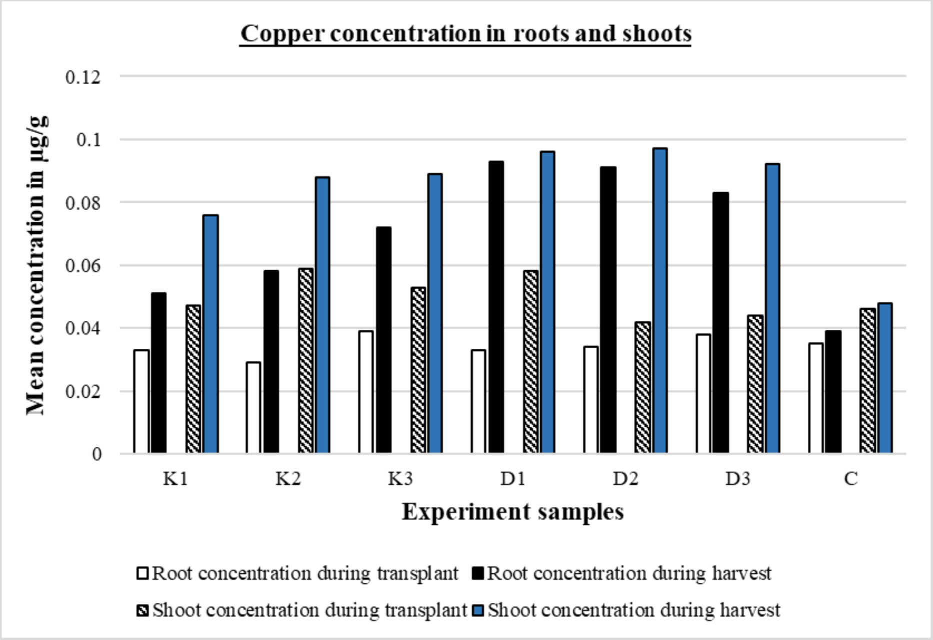


Figure 4.7: Copper concentration in sunflower roots and shoots

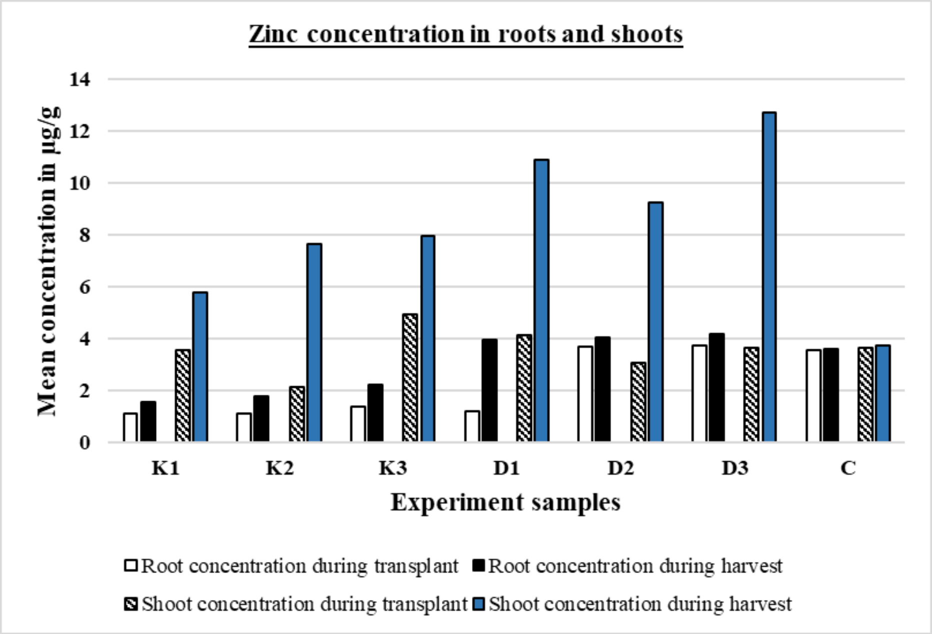


Figure 4.8: Zinc concentration in sunflower roots and shoots

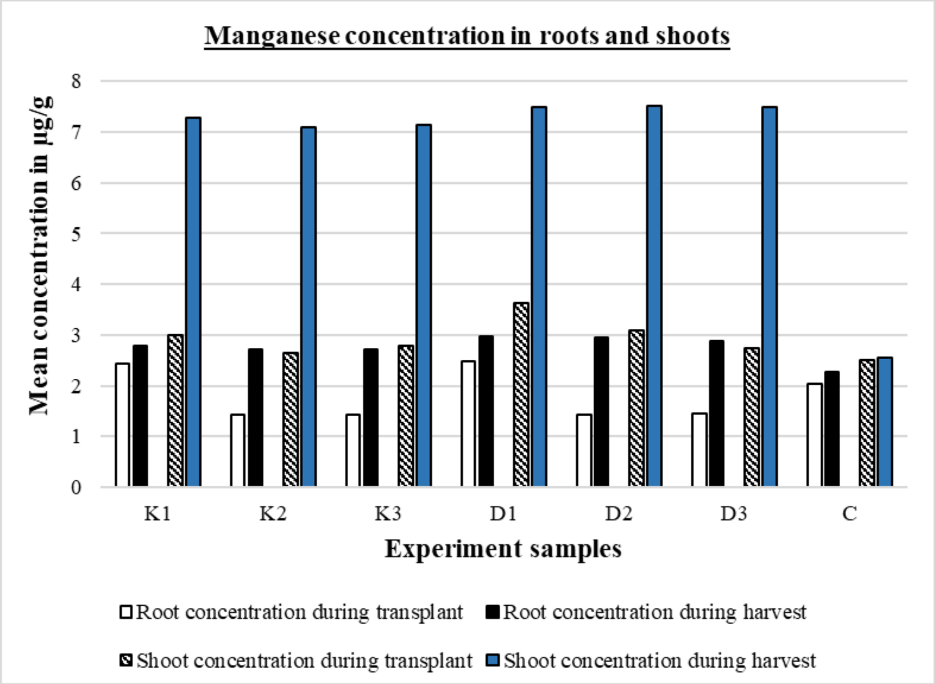


Figure 4.9: Manganese concentration in sunflower roots and shoots

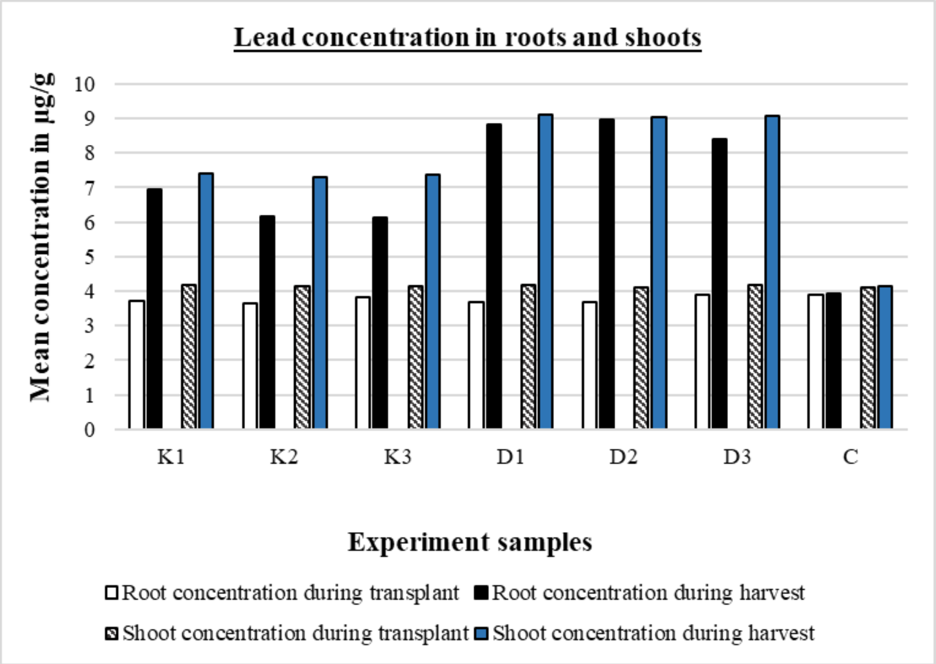


Figure 4.10: Lead concentration in sunflower roots and shoots

It can be seen from the graphs that a general increase of the identified heavy metal levels was noted in the sunflower roots and shoots after phytoextraction. Cadmium levels in the shoots and roots increased by an average of 83.5%; manganese by 90.3%; copper by 85.0%; lead by 85.6% and zinc by 83.6%. This results more or less tally with the average percentages absorbed from the sewage sludge soil mixes as has been tabulated in Table 4.4.

A higher percentage of the heavy metals was observed in the sunflower shoots than the roots after the phytoextraction process. This is because good hyperaccumulators, sunflower being one of them, absorb and quickly move the toxins to the shoot for storage (Rascioa & Navari, 2011; Muhammad, 2015).

A higher concentration of heavy metals was observed in sunflowers planted in Dandora sewage sludge soil mix than in Kariobangi sewage sludge soil mix. This is attributed to the higher heavy metal load in Dandora sludge as compared to Kariobangi which was observed in the heavy metal concentration analysis to the sludge soil mixes (Refer Table

4.1). The sunflower planted in 100% soil, which served as the control experiment, presented the lowest concentration in all the heavy metals. The control also presented the least translocation of heavy metals from the roots to the shoot during phytoextraction. Studies reveal that uptake, translocation and storage of heavy metals in hyperaccumulator plants increases with increasing metal concentration in the growth medium (Rizwan et al., 2016). The control sunflower plant was grown in a medium with the least heavy metal concentration. Thereby, had the least translocation and storage of heavy metals during phytoextraction. The sunflower plants planted in Dandora sewage sludge soil mix had the highest root to shoot translocation as they were planted in the highest heavy metal concentrated mix.

4.3 THE EFFICIENCY OF SUNFLOWER PHYTOEXTRACTION

Studies indicate that the efficiency of phytoextraction is majorly dependent on the ability of the plant to uptake toxins by the roots and translocate them to its shoot. This is measured by the translocation factor, which is the ratio of metal concentration in the shoots to the metal concentration in the roots after phytoextraction. Rascioa & Navari (2011) report that for phytoextraction to be efficient the translocation factor has to be equal to or greater than one. The translocation factors for all the experimental samples was greater than one, as shown in Table 4.3. This indicates that sunflower has a high efficiency for phytoextraction. Other indicators for efficient phytoextraction is the ability of the plant to tolerate large quantities of toxins without affecting its growth, health and production (Rizwan et al., 2016; Turgut et al., 2004). All through the study, the sunflowers were observed to be healthy, grew steadily and productivity was not affected (See appendix VIII). Common indicators of a healthy plant include brightly coloured flowers and dark green leaves (Holder, 2015). All the sunflower plants were observed to bear dark green leaves and bright yellow flowers. However, wilting was observed a month after transplanting. This is a common occurrence when transplanting as some roots are usually damaged during the move (Pavlis, 2014). With regular watering, the wilting disappeared within the second month after transplanting. (See appendix VII and VIII)

Table 4.2: Experiment samples translocation factors

Heavy metal	Experiment samples	Heavy metal concentration in sunflower shoots after phytoextraction (µg/g)	Heavy metal concentration in sunflower roots after phytoextraction (µg/g)	Translocation factor (concentration in shoots: concentration in roots)
Lead	**D1**	9.10	8.81	1.03
	D2	9.04	8.96	1.01
	D3	9.06	8.39	1.08
	Mean D	**9.07**	**8.72**	**1.04**
	K1	7.41	6.95	1.07
	K2	7.31	6.15	1.19
	K3	7.37	6.13	1.20
	Mean K	**7.36**	**6.41**	**1.15**
	C	**4.16**	**3.92**	**1.06**
Zinc	**D1**	10.87	3.93	2.77
	D2	9.21	4.02	2.29
	D3	12.71	4.15	3.06
	Mean D	**10.93**	**4.03**	**2.71**
	K1	5.78	1.55	3.73
	K2	7.63	1.76	4.34
	K3	7.95	2.22	3.58
	Mean K	**7.12**	**1.84**	**3.88**
	C	**3.71**	**3.60**	**1.03**
Manganese	**D1**	7.49	2.98	2.51
	D2	7.52	2.96	2.54
	D3	7.50	2.89	2.60
	Mean D	**7.50**	**2.94**	**2.55**
	K1	7.28	2.78	2.62
	K2	7.09	2.71	2.62
	K3	7.13	2.71	2.63
	Mean K	**7.17**	**2.73**	**2.62**
	C	**4.36**	**2.26**	**1.93**
Copper	**D1**	0.096	0.093	1.03
	D2	0.097	0.091	1.07

	D3	0.092	0.083	1.11
	Mean D	**0.095**	**0.089**	**1.07**
	K1	0.076	0.051	1.49
	K2	0.088	0.058	1.52
	K3	0.089	0.072	1.24
	Mean K	**0.084**	**0.060**	**1.41**
	C	**0.048**	**0.039**	**1.23**
Cadmium	**D1**	68.57	19.59	3.50
	D2	67.95	19.01	3.57
	D3	66.98	18.64	3.59
	Mean D	**67.83**	**19.08**	**3.55**
	K1	56.97	15.38	3.70
	K2	56.77	16.05	3.54
	K3	57.98	16.85	3.44
	Mean K	**57.24**	**16.09**	**3.56**
	C	**24.69**	**20.88**	**1.18**

To further investigate the efficiency of sunflower phytoextraction, the heavy metal levels after phytoextraction were compared to allowable safe heavy metal limits for garden soil. The United States Environmental Protection Agency (U.S. EPA.) published in 1998 the maximum recommended limit of heavy metals for home vegetable gardens. The safe maximum concentration for the heavy metals in this study is as given in Table 4.4 for comparison. On this basis, cadmium mean concentration had exceeded by 40.2%, manganese by 13.6%; copper by 110%; zinc by 72.3% and lead by 35% of the allowable limit. This implies that the sewage sludge sampled from Dandora and Kariobangi Sewage Treatment Plants was not safe for use in agriculture without removal of these heavy metals.

Table 4.3: Safe heavy metal concentration ranges

Heavy metal	Recommended limit (µg/g)	Mean concentration before phytoextraction (µg/g)	Mean concentration after phytoextraction (µg/g)	Percentage heavy metal reduction (%)
Cadmium	39	44.62	7.17	83.93
Manganese	3000	3087.09	288.93	90.64
Copper	1500	2117.21	311.97	85.27
Lead	300	397.07	45.09	88.64
Zinc	2800	2917.87	478.33	83.61

Source: U.S. EPA. (1998a)

As shown in Table 4.3, all the heavy metals were reduced by phytoextraction to the allowable limits for garden soil.

Wuana & Okieimen (2011) related the efficiency of phytoextraction to the amount of heavy metal removed using Equation 4.1 below. Where t_p is the phytoextraction time needed to achieve a target value of heavy metal reduction. It is assumed that the plant is cropped n times during the phytoextraction period and that heavy metal pollution occurs at 0-20 cm of the active root zone area (Wuana & Okieimen, 2011).

$$t_p = \frac{Metal\ concentration\ in\ soil\ needed\ to\ decrease \times Soil\ mass}{Metal\ concentration\ in\ plant\ shoot \times Plant\ shoot\ biomass \times n} \qquad \text{(Equation 4.1)}$$

Using the safe levels for garden soils in Table 4.4 to get the metal concentration needed to decrease and the mean metal concentration in plant shoots derived from Appendix XIII. Phytoextraction time in months to bring the heavy metal concentration of cadmium, zinc, manganese, lead and copper to safe levels using sunflower can be achieved using the following equations:

Cadmium: $$t_p = \frac{5.62 \times Msoil}{57.13 \times Mshoot \times n} \qquad t_p = \frac{0.098\ Msoil}{n\ Mshoot} \qquad \text{(Equation 4.2)}$$

Zinc: $t_p = \frac{117.87 \times Msoil}{8.27 \times Mshoot \times n}$ $t_p = \frac{14.25\ Msoil}{n\ Mshoot}$ (Equation 4.3)

Lead: $t_p = \frac{97.07 \times Msoil}{7.64 \times Mshoot \times n}$ $t_p = \frac{12.71\ Msoil}{n\ Mshoot}$ (Equation 4.4)

Copper: $t_p = \frac{617.21 \times Msoil}{0.084 \times Mshoot \times n}$ $t_p = \frac{7347.74\ Msoil}{n\ Mshoot}$ (Equation 4.5)

Manganese: $t_p = \frac{87.09 \times Msoil}{6.65 \times Mshoot \times n}$ $t_p = \frac{13.10\ Msoil}{n\ Mshoot}$ (Equation 4.6)

Msoil denotes the soil mass and Mshoot denotes the plant shoot mass.

4.4 PLANT NUTRIENTS IN THE SEWAGE SLUDGE AFTER PHYTOEXTRACTION

After phytoextraction the soil sludge mixes and soil were tested for pH. This was done to determine whether there were sufficient nutrients for plant growth after the process. Results of the pH test are presented in Table 4.4 below.

Table 4.4: pH results

Sample	**K1**	**K2**	**K3**	**C**	**D1**	**D2**	**D3**
pH	5.9	5.8	6.0	3.7	6.3	6.6	6.1

The pH of Dandora sewage sludge soil mixes were the highest, therefore indicating they had the highest availability of plant macronutrients. Which are potassium, nitrogen and phosphorous. These nutrients are needed by plants in large quantities and are the ones added to a soil during manuring or fertilization (Mohammad, 2014; Weissert & Kehr, 2017). Sources of potassium, nitrogen and phosphorous in wastewater include effluents from slaughter houses, tanneries and dairy processing factories (Arienzo et al., 2009). Dandora Sewage Treatment Plant treats wastewater from factories such as Nyongara Slaughter house in Dagoretti, Hurlingham Slaughter House, Kenya Meat Commission, Kapiti Dairies, Eldoville Dairy, Kinangop Dairy, Aziz Tanneries Ltd and Babar Tannery in Industrial Area (Andere, 2016). These factories are the likely contributors of these nutrients.

On the contrary, Kariobangi sewage sludge soil mixes had lower pH values indicating lower availability of macronutrients. This could be attributed by the nature of the wastewater treated at Kariobangi Sewage Treatment Plant which is dominantly from residential areas (Andere, 2016).

The control experiment had the lowest pH, implying low availability of plant macronutrients. This is the case as the control had 100% soil and no sewage sludge. The same scenario would be expected in unmanured or unfertilized soils (Mohammad, 2014; Weissert & Kehr, 2017).

pH results for Kariobangi and Dandora sewage soil sludge mixes range from 5.8 to 6.6. These values are within the pH ranges that favour high nutrient content for plant growth (Mohammad, 2014; IPNI Plant Nutrition Today, 2010; Northeast Region Certified Crop Adviser Study Resources, 2010; Weissert & Kehr, 2017).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study investigated the potential of heavy metal phytoextraction in sewage sludge using sunflower plant. The study identified and quantified heavy metals present in the sewage sludge soil mixes, soil, sunflower roots and shoots before and after phytoextraction. Thereby, assessing the efficiency of sunflower plant in phytoextraction and establishing the availability of plant nutrients in the sludge soil mixes after phytoextraction. The following were the conclusions from the study.

1. The mean concentrations of cadmium, manganese, copper, lead and zinc from the sludge soil mixes had exceeded safe standard concentrations for garden soil by 40.2%, 13.6%, 110%, 35% and 72.3% respectively.
2. After phytoextraction cadmium, manganese, copper, lead and zinc levels in the sludge soil mixes were reduced by 83.9%, 90.6%, 85.3%, 88.6% and 83.6% respectively.
3. Phytoextraction rates increased with the age of the sunflower plants till their reproductive phase. After which, the phytoextraction rates progressively slowed down till the plants' death.
4. A higher concentration of heavy metals was found in the shoots than in the roots of the sunflower plants. The average translocation factors ranged between 1.03 to 3.88.
5. The pH results for Kariobangi and Dandora soil sludge mixes ranged from 5.8 to 6.6 and were within the suitable pH range for plant growth.
6. Dandora soil sludge mixes were found to be richer in plant nutrients than Kariobangi soil sludge mixes.

5.2 RECOMMENDATIONS

The study recommends further work to:

1. Establish heavy metal removal in a hectare of land fertilized with a known sludge dosage and sunflower as the phytoextractor.
2. Establish the optimal sunflower phytoextraction period without complete depletion of plant nutrients from sewage sludge.
3. Investigate sunflower phytoextraction on other toxins and heavy metals.

REFERENCES

Abdu, A., Aderis, N., Abdul, H. H., Majid, N. M., Jusop, S., Karam, D. S. & Ahmad, K. (2011). Using Orthosiphon stamineus B. for Phytoremediation of Heavy Metals in Soils Amended with Sewage Sludge. *American Journal of Applied Sciences, 8*(4), 323-331. doi:10.3844/ajassp.2011.323.331.

Abubakar, M. M., Ahmad, M. M. & Getso, B. U. (2014). Rhizofiltration of Heavy Metals from Eutrophic Water using Pistia Stratiotes in a Controlled Environment. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *8*(6), 27-34. Retrieved from http://www.researchgate.net/publication/271254281.

Andere, C. M. (2016). *The Efficiency of Nairobi's Kariobangi Wastewater Treatment Plant* (MSc thesis). University of Nairobi, Nairobi, Kenya.

Arienzo, M., Christen, E. W., Quayle, W. & Kumar, A. (2009). A Review of the Fate of Potassium in the Soil-Plant System after Land Application of Wastewaters. *Journal of Hazardous Materials, 164*(2-3), 415-422. doi: 10.1016/j.jhazmat.2008.08.095.

Bhargava, A., Fuentes, F.C., Bhargava, M. & Srivastava, S. (2012). Approaches for Enhanced Phytoextraction of Heavy Metals. *Journal of Environmental Management, 105*, 103-120. doi: 10.1016/j.jenvman.2012.04.002.

Bolan, N., Naidu, R., Choppala, G., Park, J., Mora, M. L., Budianta, D. & Panneerselvam, P. (2010). *Solute Interactions in Soils in Relation to the Bioavailability and Environmental Remediation of Heavy Metals and Metalloids.* Food and Agriculture Organization of the United Nations. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=JP2010005544.

Chaney, R. L. & Oliver, D. P. (1996). Sources, Potential Adverse Effects and Remediation of Agricultural Soil Contaminants. In R. Naidu (Ed.), *Proceedings of the First Australasia-Pacific Conference on Contaminants and the Soil*

Environment in the Australasia-Pacific Region (323-359). Adelaide, Australia: Springer Link.

Chang, Y. T., Hseu, Z.Y. & Zehetner, F. (2014). Evaluation of Phytoavailability of Heavy Metals to Chinese Cabbage (Brassica chinensis L.) in Rural Soils. *Scientific World Journal, 2014.* doi: 10.1155/2014/309396.

Cornelis, E., Guisson, R., Elst, K., Van, S.S., Peene, A. & Dejonghe, W. (2011). Combining Bio-energy Production and Phytoextraction: Examination of the Fate of Heavy Metals of Various Conversion Routes: Recycling of Conversion Residues. *European Biomass Conference and Exhibition Proceedings,* 1828-1835. doi: 10.5071/19thEUBCE2011-VP2.8.1.

Delplanque, M., Collet, S., Del, G. F., Schnuriger, B., Gaucher, R., Robinson, B. & Bert, V. (2013). Combustion of Salix Used for Phytoextraction. The Fate of Metals and Viability of the Processes. Biomass and Bioenergy. *Science Direct, 49,* 160-170. doi: 10.1016/j.biombioe.2012.12.026.

Diels, L., Dejonghe, W., Geurds, M., Guisson, R., Ginneken, L., Van, D. L., Ugent, E. M., Ruttens, A., Witters, N., Thewys, T., Vangronsveld, J., Kegels, B., Defoort, E., Beeckman, J., Smis, S., Schepper, D., & Fastenaekels, H. (2007). *Energy Crop Production Combined with Phytoremediation for Heavy Metal Contaminated Soils*. Retrieved from http://www.mipvlaanderen.be/file.aspx?id=109.

Dillalogue, E. (2014). *Phytoremediation: The Power of Plants to Clean Up the Environment.* Vision Learning. Retrieved from https://www.visionlearning.com/blog/2014/07/12/phytoremediation-power-plants-clean-environment/.

Dominica, D., Sandhya, B. & Parkpian, P. (2009) Potential for land application of contaminated sewage sludge treated with fermented liquid from pineapple wastes, Thailand. *Journal of Hazardous Materials*. 1(7) 866-877. doi: 10.1016/j.jhazmat.2009.01.064.

Dushenkov, S. & Kapulnik, M. (2000). Phytoremediation: A Novel approach to an Old Problem. Global Environmental Biotechnology. *Science Direct, 66*, 563-572. doi: 10.1016/S0166-1116(97)80071-4.

Elekes, C.C. (2014). Eco-Technological Solutions for the Remediation of Polluted Soil and Heavy Metal Recovery. *Intech Open.* doi: 10.5772/57314.

Epstein, E. (Ed.). (2003). *Land Application of Sewage Sludge and Biosolids*. United States of America: CRC Press.

Fijalkowski, K., Kacprzak, M., Grobelak, A. & Placek, A. (2012). The Influence of Selected Soil Parameters on the Mobility of Heavy Metals in Soils. *Research Gate*, 15(1), 81-92. Retrieved from https://www.researchgate.net/publication/282734701_The_influence_of_selected_soil_parameters_on_the_mobility_of_heavy_metals_in_soil.

Fukuta, T., Ito, T., Sawada, K., Kojima, Y., Matsuda, H. & Seto, F. (2004). Separation of Cu, Zn and Ni from Plating Solution by Precipitation of Metal Sulfides. *Research Gate, 30*(2), 227-232. doi: 10.1252/kakoronbunshu.30.227.

Gellerman, B. (2011). *Sunflowers Used to Clean Up Radiation*. Japan Today. Retrieved from https://japantoday.com/category/national/sunflowers-used-to-clean-up-radiation.

Gomes, H.I. (2012). Phytoremediation for bioenergy: Challenges and Opportunities. *Environmental Technology Reviews*, 1(1), 59-66. doi: 10.1080/09593330.2012.696715.

Hamon, R. & McLaughlin, M. (2003). *Food Crop Edibility on the Ok Tedi/Fly River Flood Plain.* Research Publications Repository. Retrieved from procite:52e2a3a6-0d87-4d84-b7fe-b47c4af7a8da.

Haswell, S.J. (Ed.). (1991). *Atomic Absorption Spectroscopy: Theory, Design and Applications*. Elsevier, Amsterdam: Elsevier Science Publishers B.V.

Holder, C. (2015). *Healthy and Unhealthy Plants*. LinkedIn Learning. Retrieved from https://www.slideshare.net/CarlosHolder/healthy-and-unhealthy-plants.

IPNI Plant Nutrition Today. (2010). Retrieved May 8, 2019, from http://www.ipni.net/ipniweb/pnt.nsf/5a4b8be72a35cd46852568d9%20001a18da/97c1b6659f3405a28525777b0046bcb9.

Jaishankar, M., Tseten, T., Anbalagan, N., Blessy, B. M. & Beeregowda, K. N. (2014). Toxicity, Mechanism and Health Effects of Some Heavy Metals. *Interdisciplinary Toxicology*. doi: 10.2478/intox-2014-0009.

Jama, A. & Nowak, W. (2012). Willow (Salix viminalis L.) in Purifying Sewage Sludge Treated Soils. *Polish Journal of Agronomy, 9,* 3-6. Retrieved from https://pdfs.semanticscholar.org/399e/996fe58512bb858ff03a02ad4187fe3a7ec6.pdf.

Jovanovic, L. (2008). Sunflowers that Love Heavy Metal. *Biology Online*. Retrieved from https://www.biology-online.org/articles/sunflowers-love-heavy-metal.html.

Kabata, P. A. (Ed.). (2010). *Trace Elements in Soils and Plants*. Boca Raton, Florida: CRC Press.

Kasima, E. (2014). *Efficiency of Municipal Wastewater Treatment Plants in Kenya: A Case Study of Mombasa Kipevu Treatment Works*. (MSc thesis). University of Nairobi, Nairobi, Kenya.

Kenya National Bureau of Statistics. (2002). *Analytical Report on Population Projections*. (Volume VII). Retrieved from https://www.knbs.or.ke/download/analytical-report-on-population-projections-volume-vii/.

Khilji, S. & Bareen, F. (2008). Rhizofiltration of Heavy Metals from the Tannery Sludge by the Anchored Hydrophyte, Hydrocotyle umbellata L. *Africa Journal of Biotechnology, 7*(20), 3711-3717. doi: 10.5897/AJB08.608.

Kilongi, E.M. (2017). *Phytoremediation of Heavy Metals in Sewage Sludge by Brassicae Family Plants; Turnip, Sunflower and Mustard.* (MSc thesis). University of Nairobi, Nairobi, Kenya.

Kulkarni, B. V, Ranade, S. V. & Wasif, A. I. (2007). Phytoremediation of Textile Process Effluent by Using Water Hyacinth – A Polishing Treatment. *Jr. of Industrial Pollution Control,* 23(1), 97-101. Retrieved from http://www.icontrolpollution.com/articles/phytoremediation-of-textile-process-effluent-by-using-water-hyacinth--a-polishing-treatment-.pdf.

Laghlimi, M., Baghdad, B., Hadi, H. E. & Bouabdli, A. (2015). Phytoremediation Mechanisms of Heavy Metal Contaminated Soils: A Review. *Scientific Research Publishing Inc, 5*(8), 375-388. doi: 10.4236/oje.2015.58031.

Mahurpawar, M. (2015). Effects of Heavy Metals on Human Health. *Social Issues and Environmental Problems*. Retrieved from http://granthaalayah.com/Articles/Vol3Iss9SE/152_IJRG15_S09_152.pdf.

Maina, D.M. (1984). *Heavy Metal Analysis of Sewage Sludge by X-ray Fluorescence Technique and the Environmental Implications.* (MSc thesis). University of Nairobi, Nairobi, Kenya.

Mara, D.D. (Ed.). (2004). *Domestic Wastewater Treatment in Developing Countries*. London: Earthscan Publications.

Mara, D.D. and Pearson, H.W. (1998). *Waste Stabilization Ponds: Design Manual for Mediterranean Europe*. Retrieved from https://www.ircwash.org/resources/design-manual-waste-stabilization-ponds-mediterranean-countries.

Mbugua, P.K. (2015). *Suitability of Wastewater Sludge for Agricultural Use: A Case Study of Ruai Sewage Treatment Plant, Nairobi, Kenya*. (MSc thesis). Kenyatta University.

Mcmullen, E.W. & Mccormack, H. (1916). *Process for the Manufacture of Paper-Pulp and the Like.* The Paper Trade Journal. Retrieved from https://archive.org/stream/readinglistonpa00westgoog/readinglistonpa00westgoog_djvu.txt.

Mohammad, M. (2014). *Soil Nutrients*. Nova Science Publishers Inc. Retrieved from https://www.bookdepository.com/Soil-Nutrients-Mohammad-Miransari/9781629489094.

Muhammad, K.A. (2015). Improving the Phytoextraction Capacity of Plants to Scavenge Metal(loid) – Contaminated Sites. *Canadian Science Publishing, 23*(1), 44-65. doi: 10.1139/er-2014-0043.

Mulamu, L.O. (2014). Heavy Metal Contamination of Land and Water around Nairobi City Dandora Dumpsite, Kenya. *Global Journal of Environmental Science and Technology*, 2(9), 360-367. Retrieved from http://www.springjournals.net/full-articles/springjournals.netgjestarticlesindex=4mulamu.pdf?view=inline.

Nduta, J. (1992). *Determination of Heavy Metals in Sewage Sludge, Sewage Effluent, Garden Soils and Food Crops Grown in Ordinary and Sewage-Sludge Amended Soils*. (MSc thesis). Kenyatta University, Nairobi, Kenya.

Northeast Region Certified Crop Adviser. (2010). Retrieved May 8, 2017, from https://nrcca.cals.cornell.edu/nutrient/CA5/CA0539.php

Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A.H. & Yousefi, N. (2009). Accumulation of Heavy Metals in Soil and Uptake by Plant Species with Phytoremediation Potential. *Environmental Earth Sciences*, *59*(2), 315-323. doi: 10.1007/s12665-009-0028-2.

Olaniran, A.O., Balgobind, A. & Pillay, B. (2013) Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. *International Journal of Molecular Sciences, 14*(5), 197-228. doi: 10.3390/ijms140510197.

Pavlis, R. (2014). *Water When Plants Wilt*. Garden Myths – Learn The Truth About Gardening. Retrieved from https://www.gardenmyths.com/water-plant-leaves-wilt/.

Peuke, A. D. & Rennenberg, H. (2005). Phytoremediation. Molecular Biology, Requirements for Application, Environmental Protection, Public Attention and Feasibility. *EMBO Reports*, 6(6), 497-501. doi: 10.1038%2Fsj.embor.7400445.

Piekarska, A., Konieczka, P., Namiesnik, J. & Gloska, M.S. (2011). Use of Brassica Plants in the Phytoremediation and Biofumigation Processes. *International Journal of Molecular Sciences,* 12(11), 7760-7771. doi: 10.3390/ijms12117760.

Plant Nutrients. (n.d.). Retrieved February 11, 2017, from https://www.ncagr.gov/CYBER/kidswrld/plant/nutrient.htm.

Prakash, S. (Ed.). (2014). *Laboratory Protocols in Applied Life Sciences*. Gwalior, India: CRC Press.

Prasad, M.N.V. (2007). Sunflower (Helinathus annuus L.) - A Potential Crop for Environmental Industry. *Helia, 30*(46), 167-174. doi: 10.2298/HEL0746167P.

Rascioa, N. & Navari, I.F. (2011). Heavy Metal Hyperaccumulating Plants: How and Why Do They Do It? And What Makes Them So Interesting? *Plant Science: An*

International Journal of Experimental Plant Technology, 180(2), 169-181. doi: 10.1016/j.plantsci.2010.08.016.

Reynolds, R.J., Aldous, K. & Thompson, K.C. (1970). *Atomic Absorption Spectroscopy*. Unites States of America, New York: Barnes & Noble Inc.

Rios, P., Gonzalez, M., Obregon, S., Carbonero, M. D., Leal, J. R., Fernandez, P., Antonio, D. H. & Sanchez, M. E. (2017). Brassica-based Seedmeal Biofumigation to Control Phytophthora Cinnamomi in the Spanish "dehesa" Oak Trees. Phytopathologia Mediterranea, 56(6), 392-399. doi: 10.14601/Phytopathol_Mediterr-20771.

Rizwan, M., Ali, S., Rizvi, H., Rinklebe, J., Tsang, D.C.W., Meers, E., Ok, Y.S. & Ishaque, W. (2016). Phytomanagement of Heavy Metals in Contaminated Soils Using Sunflower: A Review. *Critical Reviews in Environmental Science and Technology, 46*(18), 1498-1528. doi: 10.1080/10643389.2016.1248199.

Rudi, H., Kermanian, H., Resalati, H. & Eshkiki, R. B. (2016). Sunflower Stalk Neutral Sulfite Semi-Chemical Pulp: An Alternative Fiber Source for Production of Fluting Paper. *Journal of Cleaner Production*, 127, 562-566. doi: 10.1016%2Fj.jclepro.2016.04.049.

Salt, D. E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I. & Raskin, I. (1995). Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment using Plants. *Bio-Technology, 13*(5), 468-474. doi: 10.1038/nbt0595-468.

Samake, M., Wu, Q.T., Mo, C.H. & Morel, J.L. (2003). Plants Grown on Sewage Sludge in South China and its Relevance to Sludge Stabilization and Metal Removal. *Journal of Environmental Sciences (China), 15*(5), 622-627. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14562922.

Semple, K. T., Morriss, A.W.J. & Paton, G.I. (2003). Bioavailability of Hydrophobic Organic Contaminants in Soils: Fundamental Concepts and Techniques for Analysis. *European Journal of Soil Science*, 54, 809-818. doi: http://dx.doi.org/10.1046/j.1351-0754.2003.0564.x.

Sewe, H.A. (2008). *A study on the Efficiency of Dandora Domestic and Industrial Wastewater Treatment Plant in Nairobi.* (MSc thesis). Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya.

Schrenk, W.G. (1975). *Analytic Atomic Spectroscopy*. United States of America, New York: Plenum Press.

Sharma, S. (2012). *Atomic Absorption Spectroscopy*. LinkedIn Learing. Retrieved from https://www.slideshare.net/sharmasuriti/atomic-absorption-spectroscopy-15185397.

Shenker, M., Fan, T. W. M. & Crowley, D. E. (2001). Phytosiderophores Influence on Cadmium Mobilization and Uptake by Wheat and Barley Plants. *Journal of Environmental Quality, 30*(6), 2091-2098. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11790018.

Sherene, T. (2010). Mobility and Transport of Heavy Metals in Polluted Soil Environment. *An International Journal, 2*(2), 112-121. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=680D7E3812544F8B891CB142EE290DE1?doi=10.1.1.672.3178&rep=rep1&type=pdf.

Smith, S.R. (2011). *Management, Use and Disposal of Sewage Sludge*. Waste Management and Minimization. Retrieved from https://www.eolss.net/sample-chapters/C09/E4-13-01-11.pdf.

Sushanta, S., Bholanath, S., Sajal, P., Biplab, P. & Gora, C. H. (2017). Agricultural Use of Sewage Sludge in India: Benefits and Potential Risk of Heavy Metals Contamination and Possible Remediation Options – A Review. *International*

Journal of Environmental Technology and Management. doi: 10.1504/IJETM.2017.10010695.

Tangahu, B. V., Abdullah, S. R. S., Bari, H., Idris, M., Anuar, N. & Mukhlisin, M. (2011). A Review on Heavy Metals (As, Pb and Hg) Uptake by Plants through Phytoremediation. *International Journal of Chemical Engineering*. doi: 10.1155/2011/939161.

The Commercialization of Sewage Sludge. (n.d.). Retrieved March 29, 2016, from http://online.fliphtml5.com/hmds/czks/#p=1.

Tolman, D.A., Niemiera, A.X. & Wright, R.D. (1990). Influence of Plant Age on Nutrient Absorpton for Marigold Seedlings. *Hortscience 25*(12), 1612-1613. Retrieved from http://hortsci.ashspublications.org/content/25/12/1612.full.pdf.

Turgut, C., Pepe, M.K. & Cutright, T.J. (2004). The Effect of EDTA and Citric Acid on Phytoremediation of Cd, Cr and Ni from Soil using Helianthus annuus. *Environmental Pollution, 131*(1), 147-154. doi: 10.1016/j.envpol.2004.01.017.

United States Environmental Protection Agency (1998a). *Heavy Metals and Gardens.* Retrieved from http://www.epa.gov/empact.

United States Environmental Protection Agency (1998b). *Supplemental Environmental Projects Policy Report*. Retrieved from https://www.epa.gov/enforcement/supplemental-environmental-projects-seps.

Varma, A. (Vol. 1). (1985). *Handbook of Atomic Absorption Analysis*. Boca Raton: CRC Press.

Wauna, R. A. & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Notices.* Retrieved from http://dx.doi.org/10.5402/2011/402647.

Weissert, C. & Kehr, J. (2017). Macronutrient Sensing and Signaling in Plants. In M.A. Hossain, T. Kamiya & T. Fujiwara (Eds.), *Plant Micronutrient Use Efficiency* (pp. 45-64). Hamburg, Germany: Academic Press.

Wolsey, L. (2004). Sunflower – Nature's Perfect Plant. *Herbal Legacy Articles*. Retrieved from http://articles.herballegacy.com/sunflower-natures-perfect-plant/.

Xu, T., Xie, F., Wei, Z., Zeng, S. & Wu, Q.T. (2014). Phytoremediation of Sewage Sludge and Use of its Leachate for Crop Production. *Environmental Technology, 36*(23), 3000-3007. doi: 10.1080/09593330.2014.955061.

Zavertnik, J. (1914). *Utilization of Sunflower Stalks in Paper Manufacture*. (Bsc. Thesis). Armour Institute of Technology. Chicago, United States of America. Retrieved from http://share.iit.edu/bitstream/handle/10560/506/utilizationofsun00zave.pdf?sequence=1&isAllowed=y.

APPENDICES

Appendix I: Dandora sludge drying bed

Appendix II: Kariobangi sludge drying bed

Appendix III: Sludge samples

Appendix IV: Sunflower nursery bed

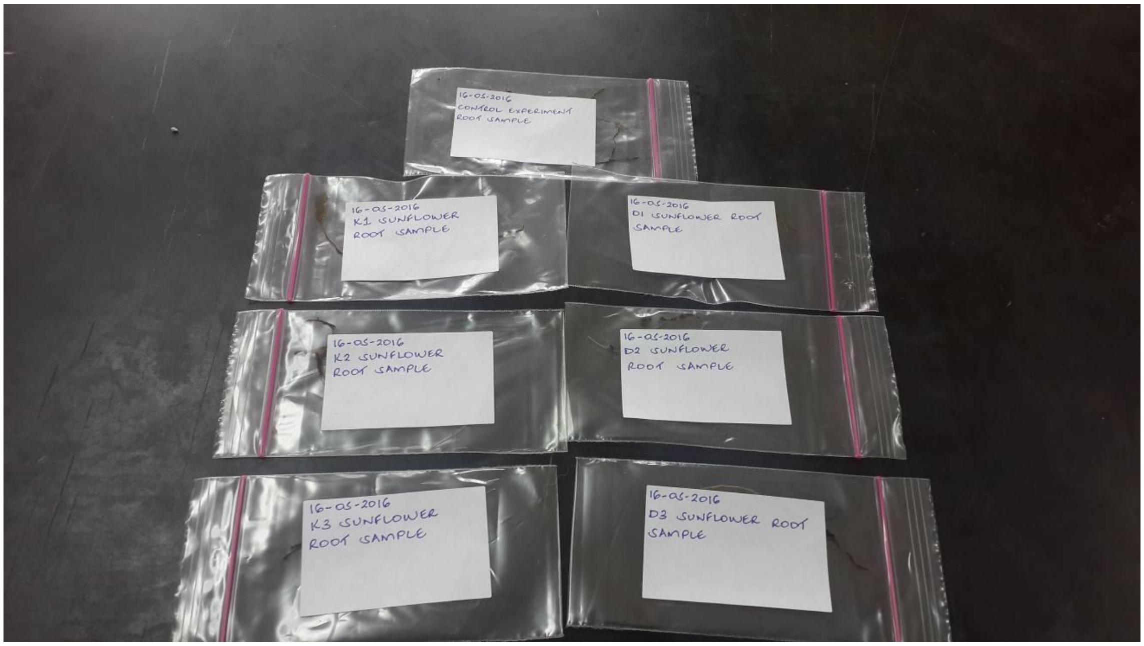
Appendix V: Sunflower seedlings at one week

Appendix VI: Sunflower seedlings at three weeks, ready for transplant

Appendix VII: Sunflower plants, one month after transplant

Appendix VIII: Sunflower plants, two months after transplant

Appendix IX: Sunflower plants, three months after transplant



Appendix X: Sunflower root samples

Appendix XI: Sunflower shoot samples

Appendix XII: Soil sludge mix samples

Appendix XIII: Heavy metal concentration in sunflower plants roots and shoots

Heavy metal	Root samples	Concentration in µg/g		Shoot samples	Concentration in µg/g	
		During transplant	**During harvest**		**During transplant**	**During harvest**
Cadmium	**K1**	12.130	15.380	**K1**	21.330	56.970
	K2	11.450	16.050	**K2**	26.760	56.770
	K3	13.270	16.850	**K3**	23.410	57.980
	D1	14.110	19.590	**D1**	22.960	68.570
	D2	11.180	19.010	**D2**	27.510	67.950
	D3	15.530	18.640	**D3**	24.680	66.980
	C	19.470	20.880	**C**	22.190	24.690
Copper	**K1**	0.033	0.051	**K1**	0.047	0.076
	K2	0.029	0.058	**K2**	0.059	0.088
	K3	0.039	0.072	**K3**	0.053	0.089
	D1	0.033	0.093	**D1**	0.058	0.096
	D2	0.034	0.091	**D2**	0.042	0.097
	D3	0.038	0.083	**D3**	0.044	0.092
	C	0.035	0.039	**C**	0.046	0.048
Zinc	**K1**	1.100	1.550	**K1**	3.560	5.780
	K2	1.090	1.760	**K2**	2.110	7.630
	K3	1.350	2.220	**K3**	4.920	7.950
	D1	1.180	3.930	**D1**	4.120	10.870
	D2	3.660	4.020	**D2**	3.040	9.210
	D3	3.740	4.150	**D3**	3.610	12.710
	C	3.550	3.600	**C**	3.620	3.710
Manganese	**K1**	2.440	2.780	**K1**	2.990	7.280
	K2	1.430	2.710	**K2**	2.650	7.090
	K3	1.420	2.710	**K3**	2.780	7.130
	D1	2.470	2.980	**D1**	3.630	7.490
	D2	1.430	2.960	**D2**	3.090	7.520
	D3	1.450	2.890	**D3**	2.730	7.500
	C	2.040	2.260	**C**	2.500	2.540
Lead	**K1**	3.730	6.950	**K1**	4.180	7.410
	K2	3.630	6.150	**K2**	4.160	7.310
	K3	3.810	6.130	**K3**	4.150	7.370
	D1	3.680	8.810	**D1**	4.170	9.100
	D2	3.670	8.960	**D2**	4.120	9.040
	D3	3.910	8.390	**D3**	4.180	9.060
	C	3.890	3.920	**C**	4.110	4.160