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Evaluation of the Essential Trace Metals in Soils and African Spider Plants: Case Study of Molo Ward-Nakuru County

by:

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DECLARATION

This thesis is my original idea and has not been presented for research at any other University.

Sign………………………Date……………………….

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Supervisors' approval

This thesis has been submitted with our knowledge as University supervisors

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DEDICATION

I dedicate this study work to my guardian Francis Siele, Mum Annah Kebenei, Lucy Maina, schoolmates and Molo Ward residents for their continued support during the entire research process. They are the pillars of the success of my studies and continuous efforts on my academics in general. Above all, I thank my Almighty God for the knowledge, health and skills that He granted me in the due process.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AFSIS	Africa Soil Information Service
AXIL	Analysis of X-ray spectra by Iterative Least-squares fitting
CCME	Canadian Council of Ministers of the Environment
CRM	Certified Reference Material
Cu	Copper
EDXRF	Energy Dispersive X-ray Fluorescence
FAO	Food and Agriculture Organization
Fe	Iron
FNB-IM	Food and Nutrition Board, Institute of Medicine
GOK	Government of Kenya
GPS	Global Positioning System
IAEA	International Atomic Energy Agency
KNBS	Kenya National Bureau of Statistics
Mn	Manganese
MoH	Ministry of Health
R	Correlation value
RDI	Recommended Nutrient Intake
SRM	Standard Reference Material
pH	Potential Hydrogen ions
WHO	World Health Organization
Zn	Zinc

ABSTRACT

In Kenya, cases of malnutrition and nutritionally related ailments have been on the rise. This calls for the search of food crops with vital trace elements constituting their diets. The aim of the current research was to assess the concentrations of the Zn, Fe, Cu, and Mn in stems and leaves of the African Spider plant (C*leome gynandra*). The trace metals were also determined in the soils from highlands and lowland regions in Molo Ward, Kenya, in which the Spider plant was grown. In addition, the pH levels in soils were determined. Both the plant and soil samples were analyzed using an EDXRF spectrometer. Fe was found to be the most abundant trace element in the soils with a concentration trend of Cu < Zn < Mn < Fe being observed. The mean concentration for the elements in the soil samples ranged from 63000 to 77000 mg kg^{-1}for Fe, 3100 to 3600 mg kg^{-1} for Mn, 19 to 21 mg kg^{-1} for Cu, and 180 to 260 mg kg^{-1} for Zn. There were no notable distinction in concentrations of these elements in the two study regions ($P > 0.05$) given in Appendix 16. The soils in the area of the study were found to be slightly acidic with a mean range of pH 5.2 to 6.3. The Spider plant stems grown in the Highland were found to contain total available Zn at 160 ± 50 mg kg^{-1}, Mn at 400 ± 140 mg kg^{-1}, Cu at 16 ± 5 mg kg^{-1} and Fe at 4100 ± 1600 mg kg^{-1} while the leaves contained Fe at concentration mean of 3100 ±180 mg kg^{-1}, Mn at 380 ± 120 mg kg^{-1}, Cu at 14 ± 4 mg kg^{-1}, and Zn of 225 ± 60 mg kg^{-1} respectively. For the Spider plants grown in the Lowland, the stem was found to contain Fe of 5600 ± 2100 mg kg^{-1}, Zn at 140 ± 5 mg kg^{-1}, Mn at 500 ± 20 mg kg^{-1}, and Cu at 15 ± 6 mg kg^{-1} at while the leaves were found to contain Fe at 2200 ± 800 mg kg^{-1}, Zn at 230 ± 60 mg kg^{-1}, Mn at 350 ± 70 mg kg^{-1}, and Cu at 13 ± 3 mg kg^{-1}. There was no correlation between the pH and the essential trace elements in the soils in the present study ($P < 0.05$) given in Appendix 10. In comparison to the recommended daily dietary requirement, the Spider plants was found to provide enough amounts of Fe, Zn, Cu, and Mn thus making it a nutritious traditional vegetable (WHO,2005). Therefore, farmers should be encouraged and empowered to grow more of the vegetable.

CHAPTER ONE

INTRODUCTION

1.1 Background

Essential trace metals or micronutrients are nutrients required by the human beings, animals and plants in trace amounts, that is, < 100 mg daily (Goldhaber, 2003). The availability of these crucial trace metals in the body system assists in numerous metabolic activities and physiological processes. Some of the known essential trace metals are Cu, Fe, Zn, and Mn. Trace elements are necessary for the prevention of various disorders in human beings including anemia, acute diarrhea, anosmia (loss of smell sense, either total or partial), poor wound healing, reduced immunity, among others (Prasad, 2004). However, excessive intake of these essential trace metals can have adverse effects on health. For instance, excess Cu intake results in oxidative damage to biological systems, stomach upsets, nausea and diarrhea (Biljana et al., 2015).

The main source of trace metals for animals, human beings and plants is the soil. Soil's trace element content is dependent on the parent material from which it was formed. However, subsequent nutrient cycling and leaching creates both enrichment and depletion, usually in certain soil horizons. Other ways in which soil can be enriched with trace elements include, pollution resulting from human activities, deposition of dust especially in areas prone to dust storms and adsorption from water draining into the soil (Johnston, 2004).

The trace element content of plants is dependent on the soils on which they are growing, and plant species/ variety (Hajar et al., 2014). Plants adsorb trace elements dissolved in the soil. A positive correlation has been demonstrated by different studies between trace metals concentration in the soils and plants (Hajar et al., 2014; Khan et al., 2013; Nayak et al., 2015). This means that, plants growing in contaminated areas tend to accumulate these elements to higher concentrations, relative to those growing in mineral deficient soils. Additionally, the uptake of these trace elements varies with plant species and is distinct for each element.

Plants are a key diet for both human beings and animals, and a major source of the essential trace elements/ minerals. Deficiencies and excesses of these elements both have an adverse effect on human and plant health. For instance, research has been done to assess the nutritional significance of each of these elements, requirements for health, and safe range of dietary intakes (Bhattacharya et al., 2016, Mehri and Marjan, 2013). Below is a brief discussion of the trace elements investigated in this study.

Fe is a major element in the in the soils and in earth's crust which is found at a concentration range of 600 to 50000 mg kg^{-1}, with a global mean of 38000 mg kg^{-1}. It has two oxidation states; Fe^{3+} and Fe^{2+}, which exists in the soils mainly as hydroxides and oxides. For Zn, its concentrations range is 60 to 100 mg kg^{-1}. It has an oxidation state of Zn^{2+}. Its higher concentrations are associated with calcareous and organic soils whereas light sandy soils record the lowest concentrations. However, a concentration range of 20 to 9200 mg kg^{-1} has been reported for Mn in uncontaminated soils. Its common oxidation states are Mn^{2+}, Mn^{4+}, Mn^{6+}, and Mn^{7+}. Calcareous and loamy soils record higher levels of Mn. Nonetheless, in uncontaminated soils, Cu is determined at a range of 5 to 140 mg kg^{-1}, depending on the soil’s parent material and its prominent oxidation states are Cu^{1+} and Cu^{2+}. It accumulates in the top soils, with a tendency to adsorb to oxyhydroxides of Fe and Mn, organic matter, clay minerals, and carbonates (Kabata-Pendias and Pendias, 2011).

1.2 Cleome gynandra (African Spider plant)

Spider plant (*Cleome gynandra*), also known as the cat's whiskers is an indigenous vegetable in Kenya belonging to the family *Cleomaceae*, which has approximately 150 - 200 species globally, and about fifty of them being in different parts of Africa (Chweya and Eyzaguirre, 2009). In Kenya, it is known as Saget in Kalenjin and Chinsaga in Kisii. Generally, it has got hairy and oily stems with leaves growing on lengthen stalks, divided into either 3, 5 or 7 leaflets. The leaves are bitter, and they are locally prepared for consumption with other vegetables such as *Amaranth*, in order to reduce the level of bitterness.

Plate 2.1: Photograph of *Cleome gynandra* (African Spider plant) at Molo Ward. November 12, 2017

The African Spider plant grows up to approximately 1.3 meters, with compound leaves having close to 5 leaflets hence the name African Spider plant. Generally, it has hermaphrodite kind of flowers, possessing both female (gynandroceum) and male (androecium) organs thus acquiring the name *Androphore* (male) and *Gynandropsis gynophore* (female). For that reason, it has also been referred to as *Gynandropsis gynandra L* with regular flowers, whereby sometimes are hypogynous or perigynamous, zygomorphic and bisexual (Dutta, 2001).

1.3 Research hypothesis

There is a notable distinction in the concentration amounts of Fe, Cu, Mn, and Zn between the soils and African Spider plants sampled from the highland region and lowland region of Molo Ward, Nakuru county due to distinct weather patterns and geographical setups.

1.4 Problem statement

In the developing countries, malnutrition is a major challenge which has led to increased cases of nutrition-related diseases that demand on soils and plants. Soil is known to be the primary source of essential trace metals for animals, plants and human beings. Substantial concentrations of trace elements in the soil improve the health of the crops, which attributes to quality food crops for the betterment of nutritional status for human beings and animals. In Kenya, a high mortality rate has been reported in children under the age of five, which can be related to lack of enough nutritious food due to poverty in many Kenyan households (GOK/MoH, 2006). To address the problem of food undernutrition and insecurity, Kenyan government sees the need of sensitizing farmers/ communities on the importance of growing crops of high nutritional value, mostly traditional vegetables such as African Spider plant. C*leome gynandra* (African Spider plant***)*** is one of the crops which is currently grown in Kenya, but not popular in most Kenyan communities because of the absence of awareness on its nutritional benefits. The crop is habitually utilized as a vegetable in the Rift valley region in Kenya, Nyanza region and some parts of the Western region which is believed to be of high nutritional value whereby the edible stems and leaves are consumed. However, minimal studies have been done in Kenya to assess the actual nutritional status of this crop. Hence, it is crucial to find the levels of crucial trace in the stems and leaves of Spider plants in different parts of Kenya.

1.5 Study objectives

1.5.1 Broad objective

To assess the micronutrient status of the Spider Plants and the elemental content of the soil in which they are grown in Molo Ward, Nakuru County in the perspective of healthy food security.

1.5.2 Specific objectives

1. To assess the pH of agricultural soils in Molo Ward.
2. To assess total available Fe, Mn, Cu, and Zn in stems and the leaves of the Spider plants in Molo Ward.
3. To assess the variation of trace elements in soils and in Spider plant for both highland and lowland regions in Molo Ward.

1.6 Justification of the study

Most Kenyans today are faced with the challenges of food security and nutrition-related diseases which are associated with the deficiency in micronutrients. This observation has been associated with high poverty levels resulting in peoples' inability to acquire quality foods of high nutritional value and food supplements. In addition, Kenya's food shortage has been greatly related to intense land-use which leads to the severe degradation of the soil as a result of low soil fertility and erosion. Additionally, most of the farmers have got scanty information on the soil status and most of them depend on the information that is passed down generations. It is crucial therefore to study essential trace metals in food crops and in the soils in which they are grown.

Approximately 36 % of Kenya's population has been identified as food insecure, with close to 46 % living in poverty (Gordon et al., 2012). For that reason, there is an immediate need to increase farmers' capacity and building resilience in rural communities to meet challenges associated with food production. One way of achieving this objective is by providing data that can enable them to make informed decisions. This study aims at establishing the levels of essential trace elements in the African Spider plant, and variations with geographical locations. The study focused on Zn, Fe, Mn and Cu since they are the most vital micronutrients that form the basis of the normal supplements in a human diet. The information obtained from this study will be crucial in sensitizing the public and popularizing the use of Spider plant as vegetable and source of essential trace elements.

1.7 Scope and limitation of the study

This research entails analysis of four essential trace elements; Zn, Fe, Cu, and Mn in African Spider plants and the soils in which they grow. Samples were collected from Molo Ward in Nakuru County from the following locations; Sachangwan, Mukinyai, Kabianga, Tayari, Molo and Tumaini. The plant's parts investigated in this study were the edible parts of the stems and leaves. The roots of the plants were not considered for analyses of the trace elements content since they are not edible. In addition, for every plant sample, a corresponding soil sample was collected at the same point where it was growing, representative of the top soils and subsoil. Seasonal variations and age were not considered for this study. Additionally, the Soil types were not considered in this study. Finally, the impact of fertilizers and compost manure was not considered due to the scarcity of the crop.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Generally, a balanced diet is very important in the control of diseases and in the recovery from illness or physical injuries. Well-nourished people have a strong immune system which helps them to resist many diseases and fight infections. The immune system relies on a balanced diet rich in essential trace elements among other micronutrients (FAO, 2002), which plays a very vital role in improving the health status of human beings.

2.2 Trace metals in soils

Studies on trace element content in the soils, ground and surface waters have gained interest with a raising number of documentations indicating bio-accumulation of these micronutrients in both human beings and animals. The concentration levels and distribution of trace elements in soil depend mainly on the biogeochemical cycles and vary extensively depending on the parent rock and mineral composition (Foti et al., 2017; Kabata-Pendias and Pendias, 2011).

In uncontaminated soils, the total elemental content is reliant on the parent rock/ material, texture of the soil and depth, and the contents of the organic matter (Alloway et al., 2005). However, anthropogenic activities like fertilizer application, use of pesticides, mining and smelting wastes, in addition to land disposal of sewerage sludge, coal, fly ash, and the wastes from industries affects the content of the essential trace elements in the earth's crust (Ngure et al., 2014; Park et al., 2011; Li et al., 2007). These activities result in the release of significant amounts of trace elements, hence their accumulation in soils. For instance, in a study by Florido et al. (2011), a statistical correlation was reported between the elemental content of Pb with correlation of 0.6), Cu with R = 0.7 and Zn with R = 0.9 in soils and the respective total concentration upon amendment of the soil with bio-solid from wastewater treatment plant. Similarly, a study by Yun-Guo et al. (2006), disclosed that soils and crops near mine were of higher elemental content relative to a control area far from the mine.

The bio-availability and solubility of the trace metals to plants and their leaching to the groundwater are controlled by determinants such as micro-organisms, cation exchange capacity, pH, organic matter content, among others (Alloway et al., 2013; Kabata-Pendias and Pendias, 2011; Zeng et al., 2011). The pH affects the desorption of these trace metals in soil. For instance, Fe, Zn, and Mn tend to be more bioavailable in low pH while Molybdenum is more available at high pH (Tohomiro et al., 2016). The pH of the soil has a positive correlation with the bio-availability of trace metals since it influences their capacity to form chelates and solubility in the soil (Kabata-Pendias and Pendias, 2011). Different studies have demonstrated a significant correlation between pH and bio-availability of trace elements in plants (Xu et al., 2013; Bronick and Lal, 2005). The soil pH influences the mobility and solubility of essential trace elements, which are key factors that determine the uptake of these metals in plants. The influence of pH on bio-absorption is however specific to plant species and particular trace element. A study by Wang et al. (2000), aimed at determining the influence of pH on the trace element uptake by tomato and soya bean regarding the acidic rain. The uptake of Ru, Sr, Rh, Zn, Tc, Y, and Co by soya bean were found to increase with acidification of soil, while absorption of Se and uptake of the trace elements by tomato were found to decrease with acidification of soil. Comparative finding was observed by Zhao et al. (2012), where lower pH levels resulted in increased bio-availability of most elements in soil hence the risk of increased uptake and retention of harmful elements. However, contrary findings were reported by Segura-Mun˜oz et al. (2006), whereby, the insignificant correlation between soil pH and metal concentration in stems, roots, and leaves of sugarcane was observed.

The organic matter of the soil plays a key role in cycling and solubilization of trace metals in the soil. Basically, the soil microorganisms support in the degradation of organic matter, nutrient cycling, altering soil structure, in addition to immobilization and solubilization of trace elements (Kabata-Pendias and Pendias, 2011).

Soil management is important to avail and improves the distribution of essential trace metals in areas with deficiencies. This deficiency problem in soils can be rectified by applying organic fertilizers containing the deficient trace metals, organic manure application, prevention of soil erosion and excess leaching in agricultural soils (Alloway et al., 2005). The farming practices influence the trace element distribution in soils. For example, the application of inorganic fertilizers complexes with trace metals. Unguided applications of these fertilizers could lead to excessive concentrations of these trace metals in soil.

Trace element mobility in a soil environment is directly related to the solubility and bioavailability to plant uptake. The main factors impacting element mobility are soil redox, pH and the presence of other minerals and nutrient groups. Additional factors include temperature, biologic activity, and water flux through the soil column. The soil chemistry at this level is not fully dependent on one factor but is instead a function of the interaction between several factors.

2.3 African Spider Plants

The African Spider plant has been used to prepare herbal medicines which possess antifungal properties, but little research has been conducted to ascertain the validity of these findings (Stangeland et al., 2009). These products possess chemical compounds such as flavonoids, antibiotics, alkaloids and natural phenols with medicinal capabilities (Kokate et al., 2010). In Northern and Southwestern Uganda, (Stangeland et al., 2009), founded that the shoots and leaves of the Spider plant are crashed and used for the treatment of Tinea capitis. Tinea capitis is one of the most prevalent fungal infections amongst the children under 12 years and adults whose immunity is weak or suppressed. In a comparable study by Namukobe et al. (2011) on traditional medicinal uses of Spider plant, it was noted that the plant is used in management of ailments such as stomach ache, epilepsy, migraine headache, sepsis and ear pain, diphtheria, vomiting, promotion of labor at the end of pregnancy and management of the bites from the snakes. According to Biel et al. (2017), the leaves contain substantial amounts of vitamin C and A, and moderate levels of Mn, Ca, and Fe which forms a very strong basis in the diet of the human beings.

Asiimwe et al. (2013), conducted an ethnobotanical study on medicinal plants used by traditional healers and local communities in Sub-Saharan Africa in the management of HIV/AIDS opportunistic infections. These include fungal/bacterial infections, cough, diarrhea, fever, herpes simplex, boosting of immunity, skin infections, cryptococcal meningitis, wounds, tuberculosis and oral candidiasis. Spider plant was identified as one of 74 plants identified to possess anti-HIV active compounds. However, there is an urgent need for scientific documentation of plants being used as herbal treatments by traditional healers, that is, their efficacy and safety.

2.4 Trace Metals in Plants

Plants need essential trace metals for healthy growth. The ability to absorb these essential trace metals from soil differ considerably with plant species (Alloway et al., 2005). For instance, some plant species such as *Pinus massonian*, *Castanea henri,* and *Phytolacca acinosa*, have played a useful role in phytoremediation of contaminated soils, especially in the mining areas (Zhao et al., 2012; Abreu et al., 2012). In addition, different parts of the plant e.g. leaves, roots, and the shoots tend to accumulate the trace metals at varying amounts. For example, Mn is taken into the plants in divalent form Mn^{2+} and tends to assemble in the shoots of the plant as compared to the roots (Biljana et al., 2015).

The trace metals are transferred from soils to plants through absorption in different chemical forms and speciation (Yeasmin, 2013). This absorption depends on factors like pH of the soil, the stages of plant growth, a variety of the plant species, and the chemical speciation in the soil of each trace metals. For instance, Kabata-Pendias and Pendias, (2011) stated that, the mobility of Fe in the soil is greatly affected by pH and soil aeration, while its solubility is mainly affected by complexation and hydrolysis. (Rahman et al., 2014), attributed Fe deficiencies in plants to unavailability of Fe, rather than its content in the soil. A similar observation was reported by Kabata-Pendias and Pendias, (2011), where extractable Fe accounted for 10 to 100 mg kg^{-1} of the total Fe content, with higher extractable amounts being observed in plants growing in slightly acidic and sandy soils as compared to loamy and calcareous soils.

In plants, Mn exists in trace amounts which act as an activator of the enzymes in the photosynthesis process. In addition, it plays a central role in the electron transport systems, oxidation-reduction reactions, and in metalloproteins. Although Mn can occur in minerals and soils in different forms such as $Mn^{2+,}$ Mn^{3+}, and Mn^{4+}, only Mn^{2+} can be adsorbed by the plants (Rahman et al., 2014).

Zn is another vital element to plants and by extension, human beings and animals. Its main source is the weathering of minerals releasing Zn^{2+} ions that can be adsorbed by plants (Shackleton et al., 2009). Relative to the solubility of other trace elements, Zn^{2+} are more soluble, with even higher solubility being associated with slightly acidic soils (Ngure et al., 2014). Elements with similar chemical and physical properties like Zn for instance Cu and Fe results in interactions that are very competitive and when they are in large amounts, they tend to reduce the absorption rate of Zn.

Zn deficiencies in plants can be associated with strongly alkaline or acid, low organic matter, high N and P content and free $CaCO_3$ in the soil. Cu is a very essential element in the plant. Its availability for plants is greatly affected by excess N, excess P, organic matter which reduces its levels and excess Zn which reduces its levels for uptake by the plants. Among the major sources of Cu in food includes beef, avocado, vegetables, nuts, grains, legumes, fruits and shellfish (Mutune et al., 2014).

Transfer of these trace metals (Fe, Zn, Cu and Mn) from soil to vegetables and other food crops in excessive quantities can be toxic. For instance, excess amounts of Cu inhibit root growth (Fargasova, 2004). The availability of these trace elements in soil determines if a plant receives enough amounts of the elements to meet their nutritional demand or absorbs the elements in toxic amounts (Nabulo et al., 2010; Ryan, 2013). Through the process of absorption, the trace elements are transported into food materials in the crops (Morgan and Connolly, 2013). For the plants to accumulate a certain element, it must be able to resist its associated negative effects. The degree of tolerance of a certain species is associated with the balance between the rate of metal uptake and their detoxification effectiveness within that specific plant (Hajar et al., 2014).

An increased amount of trace elements in plants has been observed in areas with a high content of these elements. A study by Nayak et al. (2015), observed a significant correlation between trace elements Fe, Zn, Mn, and Cu in rice grain, straw and in the soils. Relatively higher concentration levels were observed in straw than in the rice grain for Fe, Zn, and Mn. However, higher Cu concentrations were reported in the grains. This observation was associated with differences in cellular bioaccumulation mechanisms that influence the translocation of minerals in the plant system. Corresponding research by Hu et al. (2014), in a rice paddy field, revealed that a rise in metal concentrations in soil corresponds to an increase in metal content in the plants. Minkina et al. (2012) reported higher bioaccumulation of trace element (Zn and Cu), in plants with an increase in soil's content. An addition of 100 mg kg^{-1} of Zn in soil resulted in higher Zn concentrations in rice grains to amounts surpassing the maximum allowable limits for Zn.

The pH of the soil is one of the most significant determinants that influence the trace element content in food crops. pH influences the solubility and mobility of these elements by changing their ionic forms in soil. For example, a change in pH could result in chemical changes in the soil that could, in turn, make the contained elements bioavailable by altering their chemical speciation and influencing chemisorption (Omwoma et al., 2010; Zhao et al., 2012). A research by Zeng et al. (2011), noted a negative correlation between the soil pH with concentrations of Zn with $R = -0.6$ at $P < 0.01$, , Mn with $R = -.7$ at $P < 0.01$, Cu with $R = -0.6$ at $P < 0.001$, Fe with $R = -0.5$ at $P < 0.01$, and in the straw, implying that concentrations of Mn, Zn, Cu , and Fe were lower at higher pH, and vice versa.

Distinct parts of crops such as roots, leaves, stems, and shoot tend to accumulate trace elements at varying degree. An investigation on the accumulation of trace metals in different parts of the Stevia rebaudiana plant was conducted by Hajar et al. (2014). Different elements were concentrated differently in leaves, flowers, and stems. For instance, while highest Cu levels were recorded in flowers, followed by leaves then stems while Fe varied in the order of leaves > flowers > stems. Li et al. (2007), recorded an elevated amount of Pb, Cd, and Cu in roots than in leaves in domestic crops grown on reclaimed tidal flat soil in the Pearl River Estuary. In a related study by Minkina et al. (2012), the Zn, Cu, and Pb levels varied in different parts of barley. For example, Cu concentrations were higher in grains, Zn in the roots, and Pb in the straw.

2.5 Trace elements in the Human Diet

In human health, trace elements play a crucial role in biological functions, with significant contributions to good health and growth. While intake of essential trace elements greatly contributes to good health and immunity against infections, toxicity effects are manifested when in excess. Dietary consumption is a key source of these essential minerals. Some of the effects associated with inadequate trace elements in the body include reduced immunity, decreased reproductive performance, impaired mental and physical growth, and the impact on work productivity (Maina et al., 2012). For example, Fe is one of the essential trace metals whose deficiency in the body is associated with decreased resistance to infections, poor concentration and decreased work performance. The FNB-IM, (2001), recommended that the daily allowance of Fe is set to approximately 10 mg per day for men of age 25 to 50 years and for women of age 20 to 50 years, 15 mg daily is recommended. However, studies have shown that dietary Fe consumption is consistently at low levels, hence the body Fe reserves are depleted resulting in Fe deficiency. Therefore, to improve Fe levels in human beings, infant cereals, grain products and formulas are recommended to be fortified with this mineral.

Another vital trace element is Zn which is vital in various metabolic and physiological activities in human beings. The recommended daily allowance of this element for adults is 15 mg per day and 10 mg per day for children of 1-year-old and above. It is a very essential element during periods of rapid growth and developments in both human beings and animals, more so in processes such as recovery from illness and during pregnancy (Kilavi et al., 2014). Moreover, it is crucial for the efficient perfomance of the body immune system. Generally, the best dietary sources of this element are pork and lean red meat amongst others. Its deficiency is shown by loss of taste, orifical, alopecia, growth retardation, and delayed skeletal and sexual maturation.

Mn on the other hand is crucial when it comes to lowering total cholesterol, for hypoglycemic individuals and stabilization of blood sugar in the body system. It is essential in the nutritional treatment of osteoporosis, post-partum depression and menopausal symptoms. Its supplementation is necessary in cases of infertility, deafness, carpal tunnel syndrome, lack of libido in both sexes, and epilepsy. However, its requirement for children and adults are 2 and 5 mg per day respectively. Its deficiency is accompanied by depression, fatigue, gastrointestinal disorders, low blood sugar, joint dislocation, asthma, infrequent menstrual cycles and high cholesterol levels. Underwood, (2017) founded that, whole-grain products, tea, dried fruits and nuts have got the highest amounts of Mn in the range of 20 - 23 mg kg^{-1}. However, precaution has to be taken since high amounts of Mn in body system results in dizziness, fibroid tumors, frequent menstrual cycles, insomnia, nausea, colitis and liver disease.

Nonetheless, Cu is essential in the synthesis of haemoglobin as well as playing a crucial role in important processes such as protection of the body from heart diseases, generation of hormones and blood vessels. Lack of enough Cu in humans is associated with neutropenia, bone abnormalities and anemia (De Romaña et al., 2011). Nonetheless, its high amounts in the human body can result in complications such as Cardiovascular disease and increased deaths from cancer (Klevay, 2000).

Deficiency in vitamin A, Zn and Fe is among the leading causes of fatalities in the developing nations. Unfortunately, the affected population is unaware of the deficiency since no clinical symptoms are being exhibited, hence the phrase hidden hunger. Maziya-Dixon et al. (2010), associated the deficiencies of Zn, Fe and vitamin A in sub-Saharan Africa with high intake of plant-based products as compared to animal-based products.

The most common food crop in Kenya and sub-Saharan Africa is maize which provides essential trace elements. Other common food crops include beans, finger millet, sorghum, yams, and cassava. According to Kilavi et al. (2014), these crops provide a large proportion of daily intake of energy. In addition, the cereals were recognized as a source of Fe, Cu, and Mn, with the approximate contribution of 20 to 30 mg kg^{-1}for Mn, and 30 to 50 mg kg^{-}

^{1} for Zn. Hemalatha et al. (2007), analyzed different cereals for Zn and Fe content. For sorghum, the Zn content was found to be 11 ± 1 mg kg^{-1} and Fe at 65 ± 2 mg kg^{-1}.

The concentrations of Fe and Zn were reported at 21 ± 1 mg kg^{-1} and 17 ± 0.3 mg kg^{-1} respectively in finger millet and 32 ± 1 mg kg^{-1}and 15 ± 1 mg kg^{-1} respectively in maize. In Nigeria, Edeogu et al. (2007), observed values in maize for Fe, Zn, and Cu at 44 ± 2 mg kg^{-1}, 25 ± 3 mg kg^{-1}, 3 ± 0 mg kg^{-1} respectively, while in beans, values were reported at 48 ± 3 mg kg^{-1} for Fe, 26 ± 2 mg kg^{-1} for Zn and 5 ± 0.1 mg kg^{-1} for Cu.

Maina et al. (2012), carried out an assessment of micronutrients in traditional diets common in Eastern Kenya. The samples were prepared and cooked as per the traditional procedure. In the decorticated maize, Cu levels were reported below 20 mg kg^{-1}, Zn was reported at a range of 40 to 70 mg kg^{-1}, Mn at 35 to 80 mg kg^{-1}, and Fe at significantly higher concentrations 160 to 290 mg kg^{-1}. For beans, Cu levels ranged from 10 to 40 mg kg^{-1}, Mn at 30 to 100 mg kg^{-1}, Zn at 20 to 40 mg kg^{-1}and Fe at a concentration range of between 250 mg kg^{-1} to 700 mg kg^{-1}. Regional variations in trace element concentrations in cooked 'muthokoi' were reported with samples from Machakos district recording relatively higher levels. Extremely elevated amounts of Fe of up to 1600 mg kg^{-1} were however observed in some samples from Mwingi district. For finger millet, the Fe, Cu, Zn, and Mn levels were determined at a mean of 130 to 300 mg/kg, 10 to 20 mg/kg, 15 to 20 mg/kg and 100 to 300 mg/kg respectively for the three-sampling region. In conclusion, finger millet was observed to be a good supplement of Mn and Fe, whereas beans were found to be a vital source of Fe. All the food crops were however found to be low sources of Cu.

Kilavi et al. (2014), reported high variability in Fe, Mn, Cu, and Zn content in indigenous complementary infant flours from rural Kenya. For example, Mn ranged from 2 ± 0.2

mg/kg to 300 ± 10 mg/kg, Cu from 1 ± 0 mg/kg to 6 ± 1 mg/kg, Fe ranged from 30 ± 4 mg/kg to 300 ± 60 mg/kg, and Zn varied from 14 ± 0.3 mg/kg to 100 ± 4 mg/kg. The high variability was attributed to the difference in ingredients and portions in the complimentary flour, the soil and climatic condition on which the food crops are grown. Some of the ingredients composing the flour included finger millet, maize, soya, sorghum, cassava, groundnuts, fine maize, fish powder, milk powder and green grams in different rations.

Ngeno, (2012), did an assessment on the presence of essential immune boosting trace elements in leaves, stems and grains of amaranth grown in different parts of Kenya, in relation to the content in soils they are grown in two species selected for the study; *Amaranthus cruentus* and *Amaranthus hypochondriacus*. The two-grain amaranth species at different maturity stage, in different geographical locations, had varying levels of trace elements. The levels of Zn and Cr in the leaves of both species increased with the plants' maturity from 25 to 50 days and then declined from 50 to 75 days while those of Cu, Mn, and Se decreased with the plants' ages.

2.6 Instrumentation

Figure 2.2: Amptek- EDXRF Spectrometer

2.6.1 EDXRF Spectrometer Hardware

The spectrometer is made of a control circuit, a personal computer, a light path subsystem and a power supply. Basically, the light path subsystem consists of the detector which picks the fluorescence signal from the sample, filter which removes the low energy photons from the monochromatic light, x-ray tube which act as source of the X-rays and the collimator which change the diverging radiation from the source into a parallel beam (Yao et al., 2015). In general, the light path system is responsible for receiving, emitting and counting the X-ray Fluorescence photons. The high-voltage power supply generates high-voltage energy to the X-ray tube stimulating it to generate the primary X-rays (Yao et al., 2015).

These primary X-rays is transmitted through the beryllium window, filter, and the collimator which irradiates the sample finally.

2.6.2 EDXRF Principle

This is an analytical technique that utilize the X-rays to energize the atoms in the sample which they emit X-rays as they de-excite at energies characteristic of each element. Sample preparation is easy and its results are fast due to its improved detection limits and sensitivity. for this technique. The characterization abilities of this technique are based on the uniqueness of atomic structure of each metal which allows a unique set of signals inform of peaks to be identified from the electromagnetic spectrum (Lyman et al., 2012). When a specimen interacts with the X-ray beams of high energy, its atoms are energized in the inner shell making them be excited to the higher energy levels leaving a hole behind where the electron was. In the outer shell, the excited atom is unstable and due to that, they de-excite to the ground state with the release of the characteristic energy of the specific elements present (Lyman et al., 2012). The silicon drift detector with high counts and good resolution then measures the intensity of the signal due to the incoming photon giving the output inform of peaks with specific energy which is a characteristic of each element present. Basically, the preamplifier transforms the received characteristic X-rays are into a low-voltage pulse (Yao et al., 2015). The amplitude of the pulse is directly proportional to the energy of the acquired characteristic X-rays which then undergoes further amplification by the main amplifier. The amplified voltage is then transformed into the digital signal by the analog to digital converter (Yao et al., 2015). The digital signal goes through further shaping which is then sorted and transformed into a pulse counter together with the information of the amplitude. The multichannel analyzer stores this information in accordance with its amplitude which is finally formatted to an X-ray fluorescence spectral line. The spectral information is then sent to the personal computer by the detector for qualitative and quantitative analysis.

2.6.3 Weaknesses of EDXRF

The accuracy of the quantitative analysis relies on the specimen type under the study since the X-rays are emitted by any particle that is successfully excited by the incoming photons. These characteristic X-rays are emanated in all directions whereby not all of them will escape from the specimen. The probability of an X-ray escaping from the sample and its availability for detection relies on the energy of the incoming photon, density, amount and composition of the specimen (Lyman et al., 2012). Therefore, matrix correction procedures are required. The instrument may utilize a filter in reducing the continuum energies manifested at the elemental lines which will aid in increasing the number of X-rays above the element absorption edge.

2.7 Summary of the literature review

A balanced diet is very important in the control of diseases and in the recovery from illness or physical injuries. Basically, the immune system relies on a balanced diet rich in essential trace elements among other micronutrients. The main source of these essential trace metals is the soils from which the plants, human beings and animals acquire them through distinct mechanisms. The concentration levels and distribution of trace elements in soil depend mainly on the cycles of the biogeochemical and vary extensively depending on the parent rock and mineral composition. The bio-availability and solubility of the trace metals to plants and their leaching to the groundwater are controlled by determinants which are inclusive of micro-organisms, cation exchange capacity, organic matter content, and pH among others. The ability to absorb these essential trace metals from the soil differ considerably with plant species and it also varies with different parts of the plants. Presence of these trace elements is analyzed by the use of the EDXRF technique whose characterization capabilities relies on the unique atomic structure of different elements.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This study aimed at determining the levels of Fe, Cu Mn, and Zn in the selected parts of the Spider plants and in the soils in which they were grown. Molo Ward, Nakuru County was identified as the area of the study due to the popularity of the crop as a vegetable and its geographical set up. The highland and lowland regions of Molo Ward have different weather patterns. In this chapter, the study area is described, and the sampling procedure is outlined. In addition, sample preparation, quality control procedures and analysis are given. The chapter is concluded with the method used for data analyses.

3.2: Description of the study area

Nakuru County is located at about 125 km to the north west of Nairobi with an area of approximately 7,500 km^2 with a general GPS coordinate of 0° 18' 11.1564" S and 36° 4' 48.0900" E. It is home to approximately 2 million people as per Kenya's national census conducted in 2009. The County is cosmopolitan, with Kalenjin and Kikuyu communities dominating the region, accounting for approximately 70 % of the total population of the County (KNBS, 2010).

The weather patterns of the County are predictable with an approximate mean temperature of 25 °C during the dry season and 10 °C in cold seasons. Generally, the region does receive an approximate annual rainfall of 700 to 1200 mm, with an approximate average of 800 mm. Two rainy seasons are experienced in the area; short rains between October and December and long rains between mid-March and May.

Agriculture is the extensive activity in the County due to favorable weather conditions allowing dairy farming, horticulture, and large-scale farming. Most of the crops being grown in the County for consumption include carrots, kales, beans, maize, peas, cabbages, and wheat, with some of the product being sold within and outside the County for income generation.

This study was localized in Molo Ward which is approximately 36 km from Nakuru town with an area of about 100 km ^{2}. The samples of the plant and soil were collected in farms located in Molo Sub-County, Molo Ward with six locations being selected to represent the highlands and lowland regions: Molo, Tayari, Tumaini, and Kabianga locations are in the Highland region while Mukinyai and Sachangwan locations are in the Lowland regions of Molo Ward. Figure 3.2 is a map of the area of the study with the sampling points.

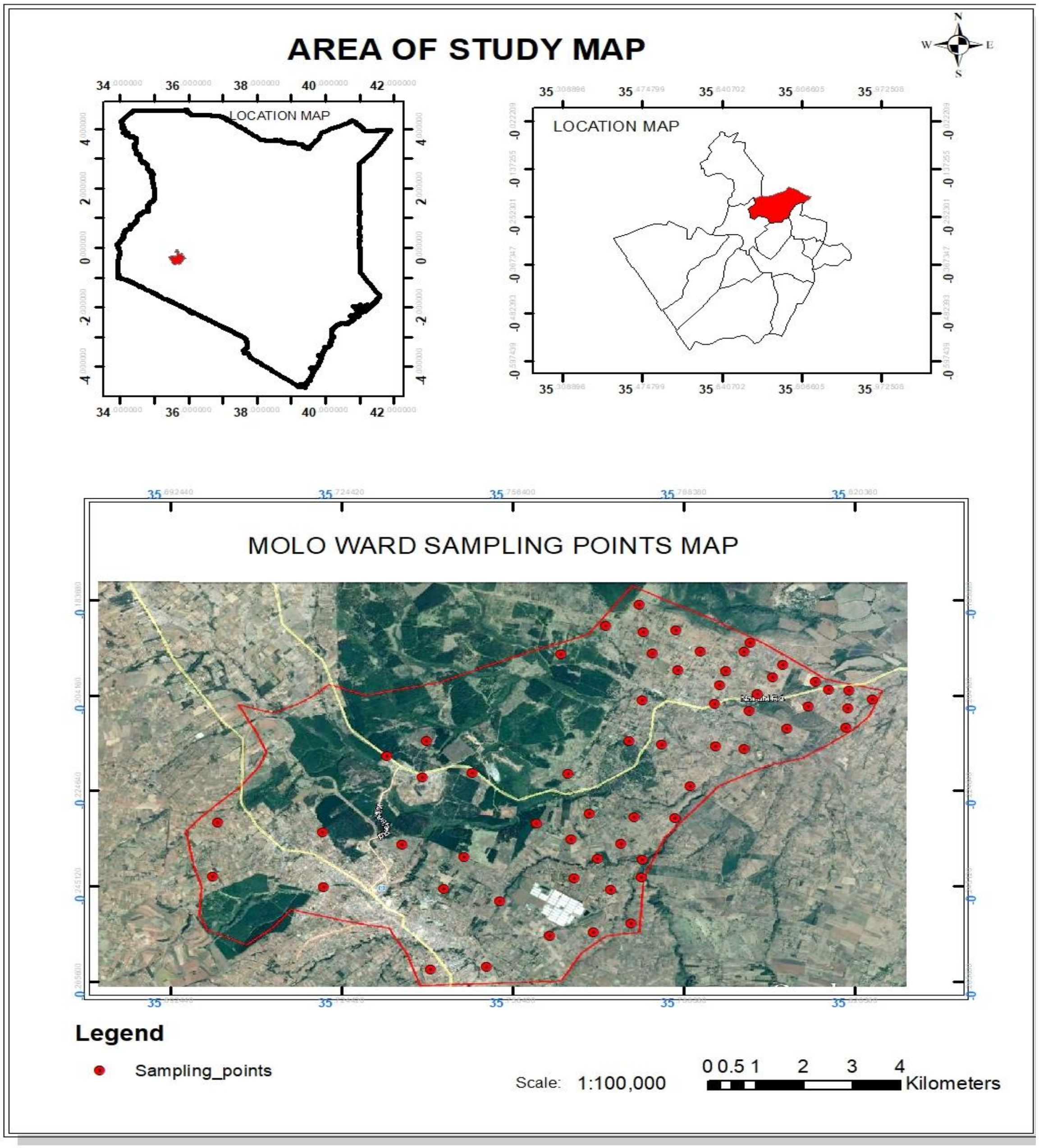

Figure 3.1: Geographical positioning of Mukinyai, Sachang'wan, Kabianga, Tumaini, Molo, and Tayari locations with 60 sampling points obtained using the Garmin GPS device.

3.3 Sampling procedures

3.3.1 Soil sampling

60 farms of varying sizes were selected for the study (30 farms from the highland region and 30 from the lowland region). An Auger was used in soil sampling. Y sampling technique adopted from AfSIS was applied in the collection of the samples. Four sampling points were generated from the central point located in the middle of the farm as shown in Figure 3.3 (Vågen et al., 2010). The radius (r) and the distance (d) were customized depending on the farm size with the Spider plants. The samples were obtained from depths of 0 to 20 cm for the top soils and 20 to 50 cm for the subsoils. The soil samples from the four points were homogeneously mixed using a trowel in a bucket for varying time depending on the properties of the soils into two composite samples; one for the top and another for the subsoil (Vågen et al., 2010). Light soils were mixed for 10 minutes while the thick soils were mixed for 20 minutes. Approximately 0.5 kg of the top soils and 0.5 kg of the sub soils were placed using a trowel into a clean labelled sampling bag. A total of 120 soil samples were collected i.e. 60 for top soils and 60 for subsoils, with 30 from the Highland and 30 from the Lowland and sent to INST laboratories in Nairobi for analysis.

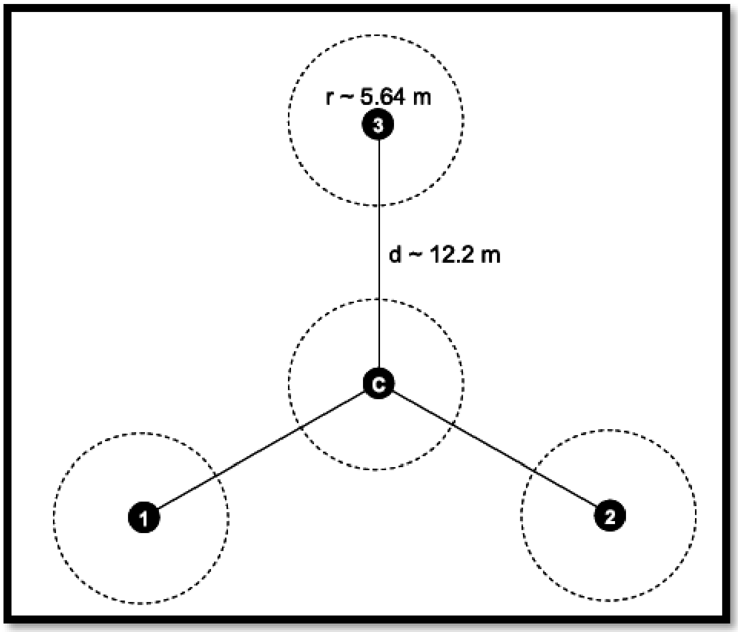

Figure 3.2: A plot showing the layout for the four-soil sampling sub-points (Vågen et al., 2010).

3.3.2 Plant sampling

Selected food crop samples were sampled from every site marked out for soil sampling. The healthy matured leaves of the African spider plants were collected by pruning. A knife was used for stem sampling where the tender part of the shoot was cut for nutritional analysis. Stems and leaves of the African spider plants were handled using gloves then cleaned using running tap water to ensure that the contaminants from the soils and other sources were excluded from the samples. A total of 120 plant samples were sampled i.e. 60 stems and 60 leaves with 60 both stems and leaves from Highland and 60 both stems and leaves from Lowland. The plants were then packed in a clean sampling bag for laboratory analysis.

3.4 Method Validation

3.4.1 Analysis of Standard Reference Material (SRM)

For every analytical technique, it is very important to assess the degree at which the obtained values conform to the true or standard value. In this study, EDXRF spectrometer was utilized in the assessment of the trace metal concentrations in plants and soil samples. To evaluate the accuracy and suitability of the EDXRF spectrometry technique, standard reference materials (SRMs) which should be of similar matrix characteristics as the samples were used. In this case, PTXRF-IAEA-09 river clay SRM from the International Atomic Energy Agency (IAEA) laboratories, Seibersdorf, for soil samples and Bowen Kale SRM from the University of Reading for plant samples were used. The reference samples were prepared in a similar manner as the analyzed samples, and the obtained values compared to the certified values. The experimental concentrations of Fe, Cu, Mn, and Zn were determined at levels within the recommended range of the certified concentrations. Additionally, a significant test of these mean values for each element was performed in accordance with the method described by Miller and Miller, (2010).

3.5 Sample preparation

3.5.1 Determination of soil pH

5 g of each soils sample was transferred into a 250 ml beaker. Thereafter, addition of the 10 ml of double distilled water was done, stirred thoroughly and left to stand for approximately 15 minutes. A standardized pH meter was used to measure and record the pH levels after stabilization (Agrawal et al., 2015).

3.5.2 Preparation of soil samples for analysis with EDXRF

Soil samples placed on a spread sheet representative of each sample. Thereafter, they were air-dried for 3 weeks days at room temperature of approximately 25°C after which they were oven dried at a temperature of 40 °C for 6 - 7 h to further remove the moisture content. The sample was then pulverized and sieved using a 2 mm diameter sieve. Further sieving was done using a 75 µm sieve. For each sample, 3 pellets of approximately 0.4 g with an approximate diameter of 2.5 cm were then prepared using a hydraulic pellet press under pressure of 5 to 8 tons without cellulose/starch because they were binding (IAEA, 1997).

3.5.3 Preparation of plant samples for analysis with EDXRF

Air-drying was done on the plant samples 3 weeks days at room temperature of approximately 25°C for 3 weeks on spread sheet representative of each sample. Thereafter oven dried at a temperature of 40 °C for 6 - 7 hours to further reduce the moisture content was done. The sample was then crashed and sieved through 2 mm diameter sieve. Three (3) pellets of each of the plant sample with an approximate weight of 0.7 g were prepared from the sieved sample using hydraulic pellet press under a pressure of 5 to 8 tons in absence of cellulose/starch (IAEA, 1997).

3.6 Analysis of soils and plants samples using EDXRF

Amptek-EDXRF spectrometer at the INST, University of Nairobi, was used to analyze the pellets where the samples were placed on a sample holder and irradiated for 200 S. The instrument was set at a current of 80 µA and a maximum energy of 30 KeV from an X-ray tube with a silver target and silicon drift detector. The output of the machine was peaks of varying intensities depending on the quantity of the essential trace elements in the sample under the study. Spectra de-convolution and quantification of the obtained spectral data were done using AXIL software. These involved spectrum format conversions from CSV files to SPE format files, spectrum fitting and finally performing quantitative analysis of various pellets representing a specific sample (Van Espen et al., 1994).

To confirm the reliability of the EDXRF technique, Certified Reference Materials (CRM) were used, and detection limits determined. For this study, river clay CRM from IAEA was prepared and analyzed in a similar way as the other samples. The values that were obtained were compared with the certified values. Additionally, the reproducibility of the values was monitored by analyzing three replicates per sample.

Figure 3.3: Photos summarizing the research procedure (A-Sampling of the Soil and Spider plants, B-Pulverization of the samples, C-Pellet preparation by the use of the hydraulic pellet press and D- Pellet ready for EDXRF analysis)

3.7 Statistical methods

The mean essential trace metal concentrations values were calculated and reported with their standard deviations. The data was further subjected to Pearson's correlation analyses to test if there was correlation of trace element content in plant samples to their respective soil samples. R programming software was used in displaying the distribution of the concentrations of various elements in different sampling sites. The test of the notable distinction at 95% confidence interval was done by utilization of the t-test:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$ …………………. **equation 3.1**,

Where $\bar{\mathbf{X}}$ is the mean of the sample

N is the sample space

S is the variance of the sample

When t-stat < t-critical ($P > 0.05$) then there is no notable distinction between the two means and the vice versa is true.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, the concentration results of Cu, Fe, Zn and Mn, of both soils and Spider plant samples are presented. Furthermore, the validation of the EDXRF method using certified control samples is also presented. The presentation was done using the tables, graphs, box plot, and whisker.

4.2 Validation of the EDXRF method using SRM materials

4.2.1 Bowen kale SRM

The T-test (Appendix 12) revealed that the certified values and experimental values in Table 4.1 exhibited no statistical differences ($P > 0.05$) where the concentration average of the three pellets was considered. The experimental value obtained was within the certified range of values for Cu, Zn, Mn, and Fe under the study. Based on the results obtained in the validation process by use of Bowen Kale SRM, it shows that the technique which was utilized in analyzing the plant samples was reliable and accurate. This means that the obtained concentration values for all the plant samples under the study were valid and dependable.

Table 4.1: Experimental and certified values of the Bowen kale Standard Reference Material (mg/kg)

Element	Experimental values	Certified Value
Fe	120 ± 19	122 ± 20
Mn	15 ± 3	16 ± 4
Zn	30 ± 4	34 ± 6
Cu	5 ± 1	5 ± 0.6

4.2.2 PTXRF-IAEA-09 River clay SRM

The PTXRF-IAEA-09 River clay SRM concentration values are presented in Table 4.2 where concentration average of the three pellets was considered. From the results, it was noted that Fe, Mn, Cu, and Zn were determined at levels within the certified range. The experimental and certified values were analyzed using t-test as shown by Miller and Miller, (2010), where the two concentrations were noted to be similar statistically since $P > 0.05$ (Appendix 13). This means that the EDXRF technique applied in the analysis of the soil samples was reliable and well grounded.

Table 4.2: Experimental and certified values for the PTXRF-IAEA-09 River clay Standard Reference Material (mg/kg)

Element	Experimental values	Certified values
Fe	31000 ± 1600	30000 ± 1000
Cu	25 ± 4	20 ± 3
Zn	90 ± 7	100 ± 8
Mn	1100 ± 100	1000 ± 100

4.3 Soil pH of highland and lowland sites

The pH levels in the soil samples was determined at the Public Health Engineering, University of Nairobi Laboratory using a calibrated pH electrode. The results of the analyses are presented in Table 4.3 and summary given in appendix 2 to 5. The soils were found to be slightly acidic. A mean pH of between 5.6 ± 1.0 and 6.3 ± 1.0 was recorded for the soils in the lowland regions for both soil profiles under the study. In contrast, a mean pH value of between 5.9 ± 1.0 to 6.0 ± 0.6 was observed for both soil profiles under research in highland sampling sites. A pH range of 4.3 to 7.6 was recorded in the top soils for both highland and lowland regions. A pH range of 4.4 to 7.4 was reported in overall for the subsoils in highland and lowland regions. These results exhibited no significant statistical difference between the two sampling areas; highland and lowland ($P > 0.05$) given in Appendix 14. Higher pH values were reported in the top profiles in comparison to the bottom profiles. For instance, in the highlands, the mean pH for the top soils was reported at 6.3± 1.0, as compared to 5.6 ± 1.0 in the subsoils. That means that the bottom soil profile was slightly acidic as compared to the top soils profile. A similar trend was reported for lowland soil samples. This observation could be attributed to the decomposition of organic matter leading to the release of the acids mainly in the top soils layer which finally settles at the subsoil profile. Organic matter is vital in the modification of the soil pH which alters water retention ability of the soils (Xu et al., 2013). Soil pH is a crucial parameter that impacts the bio-availability of trace elements in plants. It strongly influences the mobility and solubility of trace elements by modifying their chemical speciation (Zhao et al., 2012). In turn, the chemical speciation of an element in soil controls its bio-availability and bio-accumulation in plants. Soils with pH values above 7.5 are categorized as alkaline, while those with pH value less than 6.5 are classified as acidic, with pH < 5.5 as strongly acidic. The soils with pH levels between 6.5 and 7.5 are classified as neutral soils.

Table 4.3: pH levels in Highland and Lowland soils

	Lowlands		Highlands	
	Top soils	Subsoils	Top soils	Subsoils
Average	**6.3 ± 1.0**	**5.6 ± 1.0**	**6.0 ± 0.6**	**5.9 ± 1.0**
Minimum	4.8 ± 0.0	4.4 ± 0.1	4.3 ± 0.0	4.5 ± 0.2
Maximum	7.6 ± 0.2	7.3 ± 0.1	7.4 ± 0.2	6.9 ± 0.2

The soil pH generally meets the Canadian and Chinese recommendations for the agricultural soils. The Canadian standards for agricultural soils recommend a pH range of 6 - 8 (CCME, 2001), while the Chinese environmental quality standards for soils recommends a pH < 6.5. This means the pH values reported in this study mostly fall within this range, hence suitable for agricultural activities. However, some areas were noted to be too acidic hence the need for pH modification to make them more suitable for crop production. Apparently, there are no documented pH values in Nakuru County.

4.4 Essential trace element concentrations

Micronutrients plays vital functions in animals, human and plant nutritional status. Soil is a key source of these nutrients to the plants and by extension, human beings. As part of the objectives of this study, the trace element content in Spider plants (*Cleome gynandra*), which is a vegetable that is popular in some parts of the Rift Valley region, in addition to their respective soils on which they were growing was determined. Fe, Cu, Mn, and Zn were analyzed, and the obtained results are outlined and discussed in this section.

4.4.1 Iron

Figure 4.2 shows the variation of the Fe concentration in the top soils and subsoils samples collected from the highland and lowland regions respectively. The results obtained from the analyses of the soils samples for Fe are presented for both top soils and subsoils in highland and lowland regions (Appendix 2 to 5).

A mean concentration of 70000 ± 10000 mg/kg for lowland top soils and 77000 ± 10000 mg/kg for lowland subsoils was recorded respectively. A mean concentration of 65000 ± 20000 mg/kg and 63000 ± 25000 mg/kg was reported for highland top soils and subsoils respectively. Fe was found to be the most readily available metal in the soil samples with a mean concentration level of between 63000 ± 25000 mg/kg and 77000 ± 10000 mg/kg for the overall sampling regions and depth profiles. The high Fe concentrations could be attributed to high earth crust concentrations, giving the soils a reddish-brown color. The values obtained in this study are slightly higher than the global mean reported by Kabata-Pendias and Pendias, (2011), where Fe was identified amongst the major elemental constituents in the earth crust at a mean of 38000 mg/kg in uncontaminated soils. That could be attributed to the elevated background levels of Fe in the area under the study. High levels of the Fe in the soils does not translate to high fertility of the soil since fertility of the soil is a function of different parameters.

Comparatively higher concentrations of Fe were recorded in the lowlands (71000 to 77000 mg/kg), as compared to the highlands. This observation could be attributed to intensive agricultural activities leading soil nutrient mining (Drechsel, 2001) in the highlands (Molo, Tayari, Tumaini and Kabianga locations) that receive high rainfall as compared to the lowlands that are mostly dry due to geographical location.

The highland region considered for this study lay at approximately 2300 m above the sea level. With enhanced crop production, the soil mineral content in the highlands may get depleted with time (Drechsel, 2001). The lowlands regions considered for this study lay at about 1700 m above sea level and it includes Mukinyai and Sachangwan locations. The two locations generally have fertile soils for crop production. Minimal agricultural activities take place due to the geographical setup with semi-arid weather conditions covering major parts of the region. Furthermore, most farmers have very little income to practice irrigation farming; hence they rely on traditional and drought-resistant crops. The higher Fe content in lowlands may also be attributed to the difference in the geological structure of the area and the silting process taking place thus enhancing the fertility of soils.

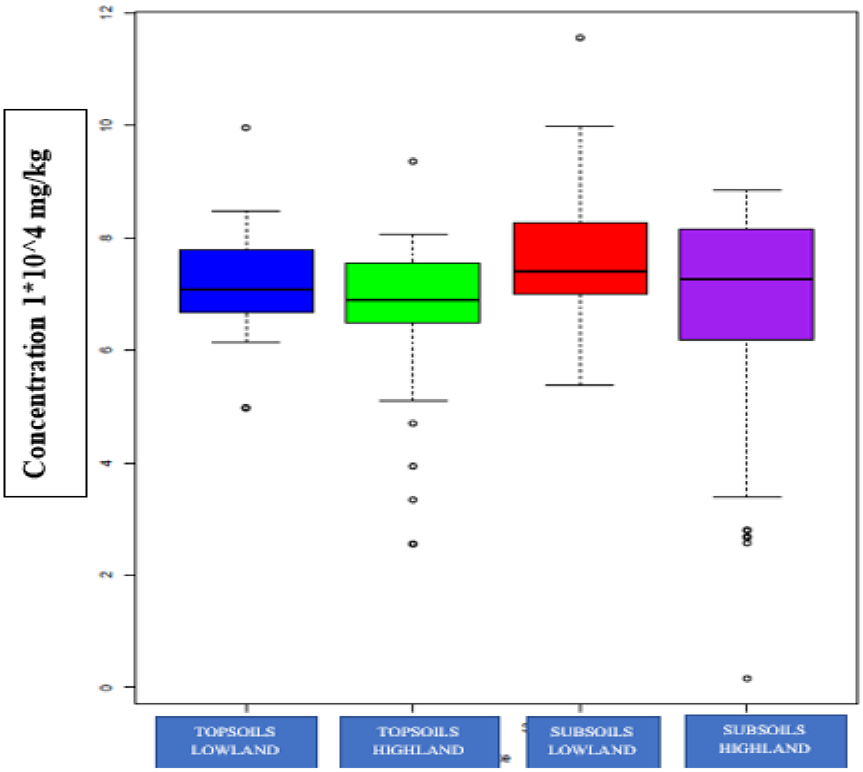

Figure 4.1: Concentration distribution of Fe in highland and lowland soil samples (mg/kg)

In regards to the variation of Fe in the two soil profiles no distinct distribution pattern was identified. For instance, in the highlands, slightly higher mean values were reported in the top soils, while in the lowland, the top soils recorded a slightly lower mean. However, there was no distinct statistical contrast between the two soil sampling profiles in both the lowland and highland regions ($P > 0.05$) shown in Appendix 15. Moreover, there was no statistical distinction between the concentration of the Fe in highland and lowland regions respectively. Further analysis showed a strong correlation between the top soils and subsoils for both lowland samples and highlands samples as shown in Figure 4.3 below ($R = 0.8$).

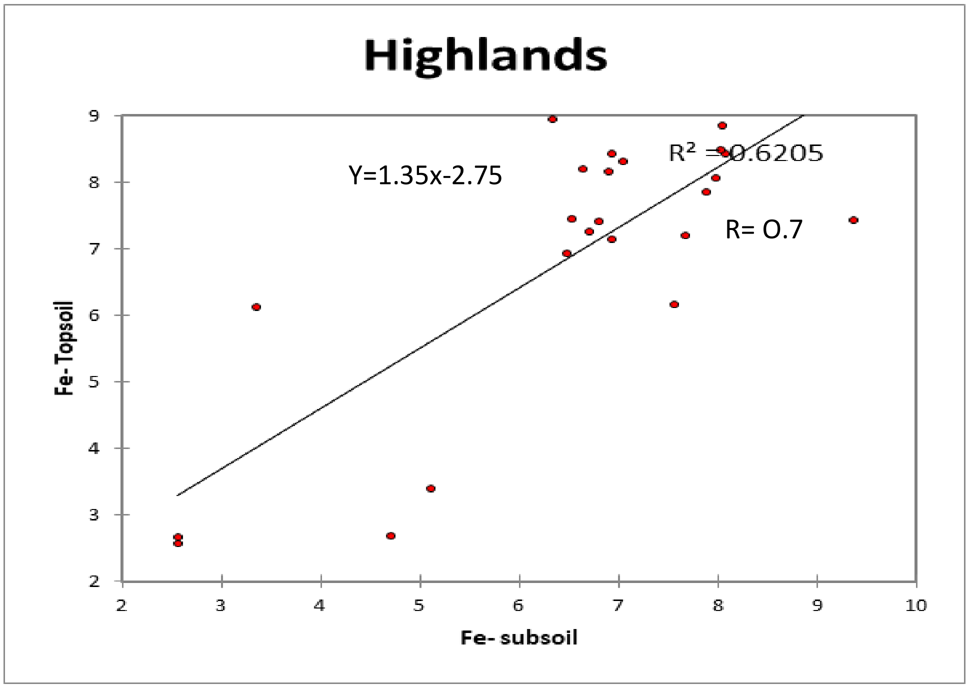

Figure 4.2: Correlation between Fe levels in top soils and subsoils in the highlands

Correlation between Fe and other elements considered in this study was also determined as presented in Appendix 10. A strong correlation (positive) was exhibited between Fe and Mn (R = 0.9), and moderately with other trace elements. This means that the two elements could possibly share similar formation and depletion factors (Ding et al., 2018). However, there was a moderate correlation between the Fe, Cu, and Zn with a poor correlation between the pH and Fe being observed (R = 0.1). Therefore, the pH variation does not affect the total available Fe in the soils and by extension the Spider plants from the sampling site (Table 4.5). Also, there was a notable distinction in the levels of Fe in soils and in the African spider plants ($P < 0.05$) shown in Appendix 11.

Nutritional analysis was also done on the Spider plant sampled from the study area. Figure 4.4 shows the distribution of the Fe concentration in the Spider plant stems and leaves sampled from lowland and highland regions. For the edible parts of the Spider plants (stems and leaves), Fe concentration range of between 1300 to 9000 mg kg^{-1} dry matter, was recorded with the stems having a higher concentration. A mean concentration of 4100 ± 1600 mg kg^{-1} for the stems sampled from the highland region and 3100 ± 1900 mg kg^{-1} for the leaves sampled from highland regions was reported. A mean concentration of 5600 ± 2000 mg kg^{-1} for lowland sampled stems of the Spider plants and 2200 ± 800 mg kg^{-1} for the leaves sampled from lowland was observed (Appendix 6 to 9).

The Fe content in Spider plants in this study area is higher than those reported in different vegetables and cereals. The high Fe content in Spider plants could be associated with high Fe concentrations in soils, high bio-accumulation and tolerance levels in the Spider plants. In general, Spider plant stems were found to contain higher Fe levels which means that the stems accumulate more Fe in comparison to the leaves (Appendix 6 to 9). Also, the leaves from the highland region were found to have a slightly higher mean concentration than the leaves from the lowland regions. This could be linked to the distinction in the soil type and content plus the ability of the leaves in highland regions to accumulate more Fe than stems. However, no notable statistical contrast was noted between the stems and leaves ($P > 0.05$) shown in Appendix 17. The observation can be linked to the sampling procedure adopted for this study where only the tender part of the stems, close to the leaves was considered.

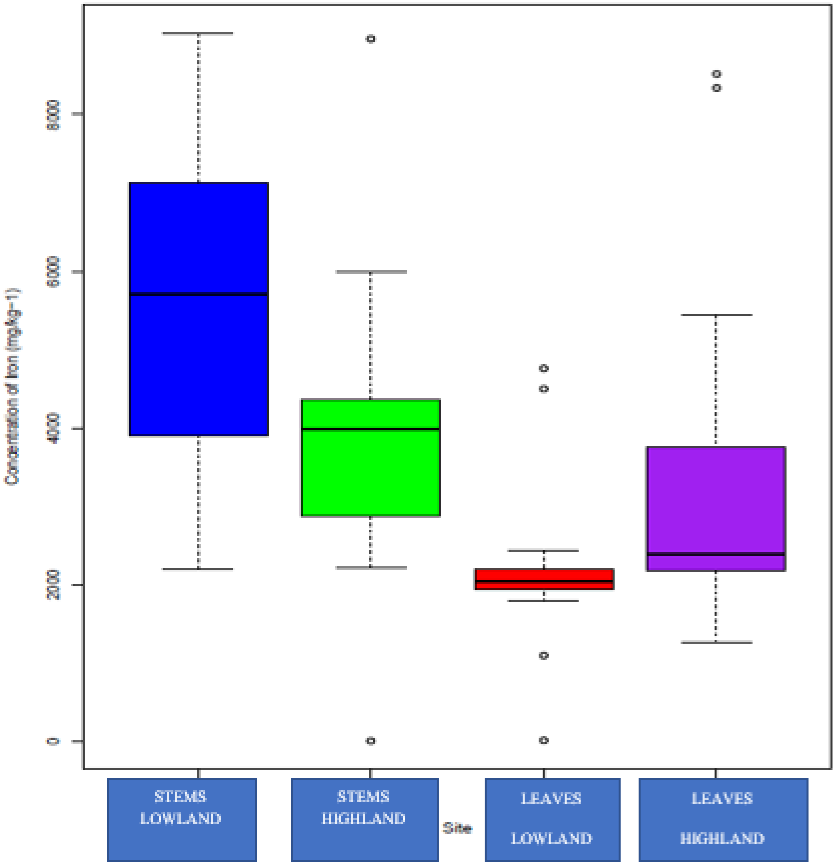

Figure 4.2: Concentration distribution of Fe in highland and lowland stems and leaves of Spider plant samples (mg kg^{-1}).

Fe is an essential nutrient in the human body that plays an important role in various cellular activities. It acts as a co-factor in enzymatic roles in hormones, collagen, neurotransmitters, and biosynthesis of proteins (Anderson et al., 2012). The dietary requirement of Fe varies with age, sex and WHO standards. For instance, higher amounts of Fe are recommended for pregnant women and those in the childbearing age as compared to men. A minimum dietary intake of 8 mg per day is however recommended for human beings (FAO, 2002). The results from this study, where a concentration mean of the total available Fe of between 2200 to 9000 mg kg^{-1} dry matter was reported in the selected parts of the Spider plants. This indicates that Spider plants can act as a good source of Fe for the dietary requirements.

4.4.2 Manganese

Mn is among the most vital micronutrients in soil and plays an essential function in plants and human health. The concentration values of all the soil samples together with their respective means are presented in Appendix 2 to 5 for the area under the study. Figure 4.5 shows the Mn concentration distribution in the top soils and subsoils samples from highland and lowland regions. An overall concentration range of 200 mg kg^{-1} to 8300 mg kg^{-1} was reported. For the lowland region, a concentration of 3600 ± 1200 mg kg^{-1} for top soils and 3500 ± 1300 mg kg^{-1} for subsoils was recorded. For the highland region, an average concentration of 3600 ± 1300 mg/kg for top soils and 3100 ± 1900 mg/kg for subsoils was observed. These reported levels are higher than the threshold level set for agricultural soils which are at > 10 mg kg^{-1}, hence the soils in the study area are not Mn deficient (Kabata-Pendias and Pendias, 2011). In addition, the values are within the regulatory limits for agricultural soils set at 1000 mg kg^{-1}, hence does not pose a significant risk of toxicity in plants, human beings and animals.

Generally, the mean global concentration of Mn is 450 mg/kg, with a range of 2 to 9200 mg/kg being recommended (Kabata-Pendias and Pendias, 2011). Although the Mn concentrations for this study fall within this range, the overall mean is higher than the global mean. The higher values could be attributed to the soil contamination from the environmental pollutants.

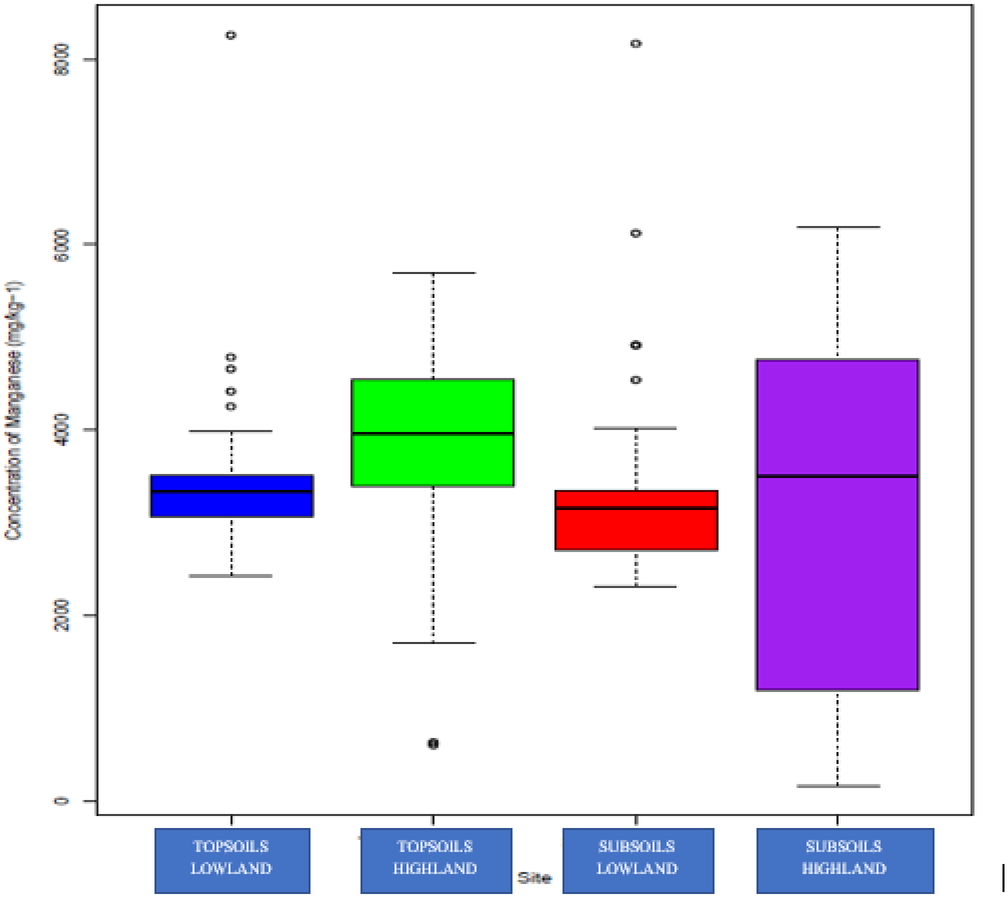

Figure 4.4: Concentration distribution of Mn in highland and lowland soil samples (mg kg^{-1})

Generally, the lowest Mn concentrations in soils were recorded in the highland regions, while the highest values were reported in the lowland regions (Appendix 2 to 5). For example, in the highlands, a concentration range of between 200 to 6200 mg kg^{-1} was reported as compared to a range of 2300 to 8300 mg kg^{-1} in the lowland regions.

A comparative trend was reported for the Fe content which could be attributed to similarities in formation and depletion factors (Ding et al., 2018). The slight difference in concentration between the two regions could be associated with depletion of nutrients due to enhanced cultivation in the highlands where favorable climatic conditions for crop production is experienced. Although a high overall mean was recorded in the top soils, there was no notable distinction between the two sampling depths ($P > 0.05$) given in Appendix 15. Mn tend to accumulate in top soils due to its fixation with organic matter (Kabata-Pendias and Pendias, 2011). The levels of Mn in the two depth profiles were noted to moderately correlate ($R = 0.7$) as exhibited in Figure 4.6. Moreover, a positive correlation between the Mn and Fe ($R = 0.9$) was noted, meaning the two elements have the same formation and depletion factors (Ding et al., 2018). A moderate correlation between Mn, Cu, and Zn was observed (Appendix 10). There was a poor correlation between total available Mn and the soil pH ($R = 0.2$) for the soil in the sampling site (Appendix 10). This means that pH does not affect the total available Mn in both the Spider plants and the soils. Additionally, there was a notable contrast in the levels of Mn in soils and in the African spider plants i.e. ($P < 0.05$) as shown in Appendix 11.

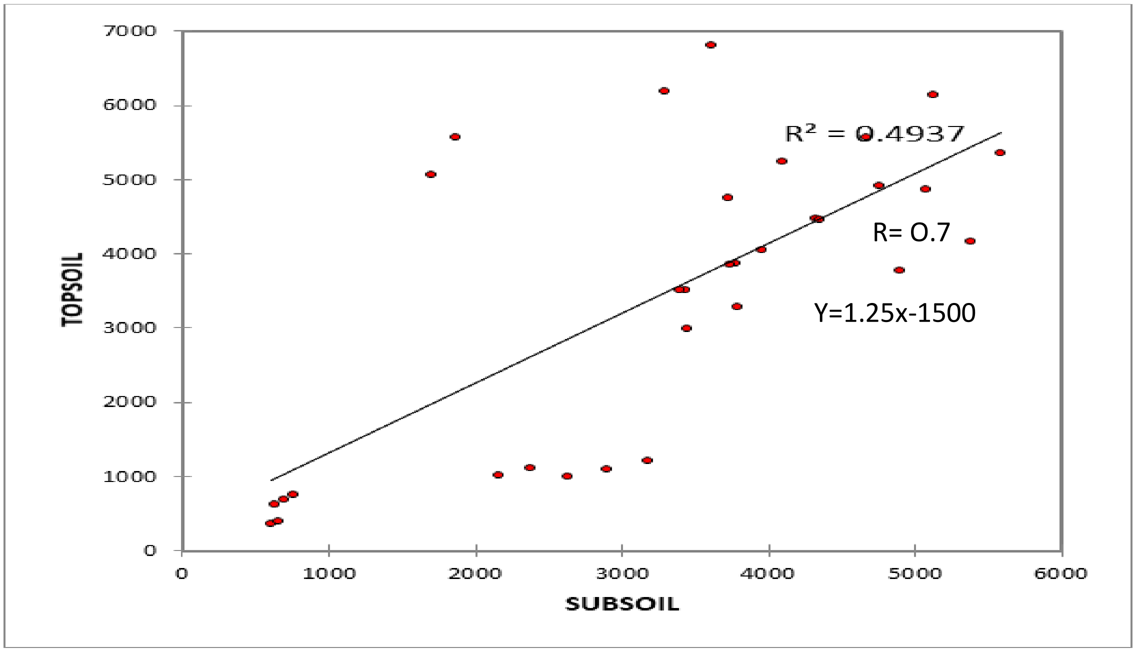

Figure 4.5: Correlation between Mn concentrations in top soils and subsoils

Mn is one of the essential micronutrients in the human body. Some of its key functions in the body include regulation of blood sugar levels, assisting in the proper functioning of thyroid glands, the formation of connective tissues, bone formation, and absorption of calcium (Kazi et al., 2008). Figure 4.7 shows the distribution of Mn in stems and leaves of the Spider plants sampled from the two regions of study. In this study, a mean concentration of 400 ± 140 mg kg^{-1} and 380 ± 120 mg kg^{-1} was reported in stems and leaves respectively for the Spider plants samples collected from the highland regions. However, a concentration means of 500 ± 20 mg kg^{-1} and 350 ± 70 mg kg^{-1} was obtained for the stems and leaves in the lowland region respectively (Appendix 6 to 9).

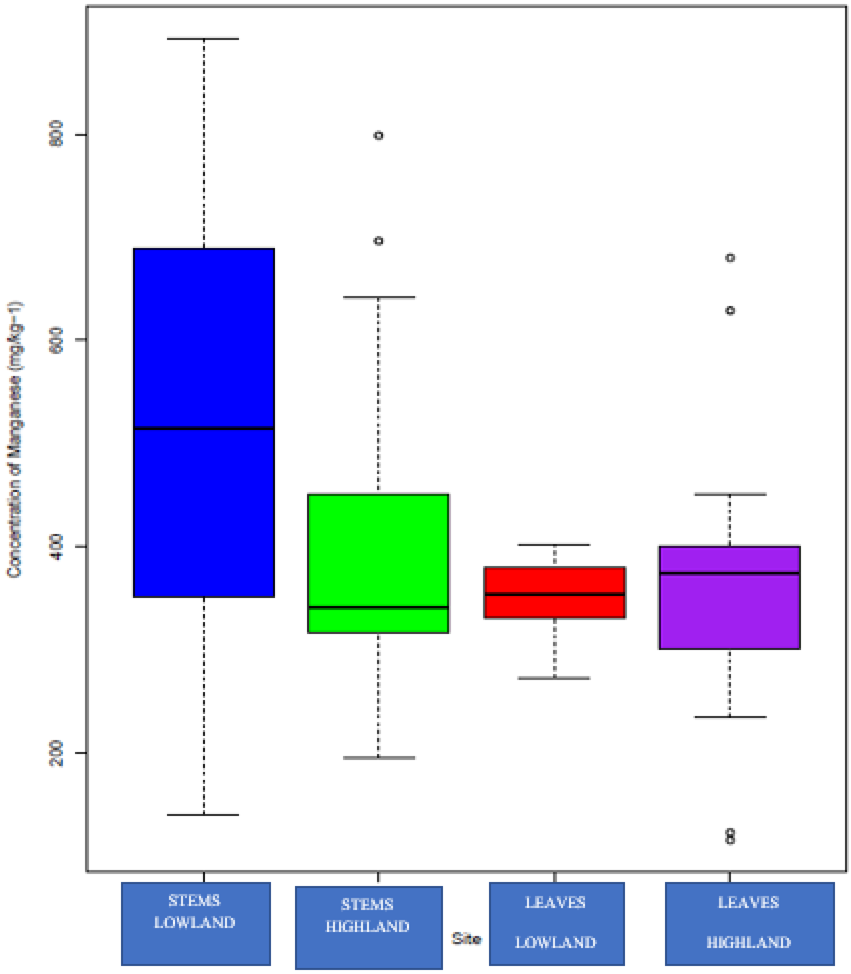

Figure 4.6: Concentration distribution of Mn in highland and lowland Spider plant leaves and stems (mg kg^{-1})

The Mn content in stems was higher in comparison with the leaves for both highland and lowland (Appendix 6 to 9). For example, in the highlands, the mean Mn content for the stems was 400 ± 140 mg kg^{-1} compared to 380 ± 120 mg kg^{-1}, with a similar trend being observed for the lowland Spider plants samples. However, there was no notable distinction between the concentrations in stems and leaves for the two study regions ($P > 0.05$) given in Appendix 17, with a translocation factor of 0.02 (ratio of Mn content in the plant to soil) being reported. This means the Mn accumulates more in the stems than the leaves (Idris et al.,2011).

Basically, Mn plays a vital function in different physiological processes and it is a key constituent of enzymes and a catalyst. However, both deficiencies and excesses, could result in adverse effects on human health. While Mn toxicity has been associated with bronchitis, nerve damage and lung embolism and its deficiency being associated with skin problems, glucose intolerance, skeleton disorder, and neurological symptoms (Crossgrove and Zheng, 2004). With reference to the recommended dietary daily intake of 2 to 5 mg per day for adults and 2 to 3 mg per day for children, the Mn content in Spider plants (50 to 360 mg kg^{-1}) can be concluded to be enough, hence the plant can serve as a good Mn supplement (WHO, 2005).

4.4.3 Copper

In line with Kabata-Pendias and Pendias, (2011), the reported range for Cu concentrations in uncontaminated soils is between 14 and 109 mg kg^{-1}. Figure 4.8 shows the concentration distribution of Cu in highland and lowland soils. From Appendix 2 to 5, a mean concentration of Cu in highland top soils and subsoils were noted to be 19 ± 3 and 16 ± 2 mg/kg respectively. For the lowlands, a mean concentration for the top soils and subsoils were observed to be 18 ± 4 and 21 ± 1 mg/kg respectively as displayed in Figure 4.8. The values observed in this study falls within the range of < 10 to 92 mg kg^{-1}(Kabata-Pendias and Pendias, 2011).

The amounts of Cu detailed in this study are within the permissible levels for agricultural lands set at 90 mg kg^{-1}, hence suitable for cultivation. Cu is a vital element in plant growth that is required in micro amounts. Some of the key functions of Cu in plants include activation of enzymatic functions like lignin synthesis, essential in photosynthesis and respiration processes, assists in the metabolism of proteins and carbohydrates. Moreover, it assists in intensifying the color and flavor in flowers and vegetables. However, both excesses and deficiencies of Cu have unfavorable impact on plant growth and quality. Excess Cu in the soil can cause restricted root growth by burning the root tips and it affects the uptake of Fe, Zn, and Mo. Cu deficiencies have been attributed to Cu immobility. Some of the symptoms associated with Cu deficiency is slight chlorosis in leaves and cupping.

There was no striking difference between the concentration levels in the highlands and those for the lowland's region ($P > 0.05$) given in Appendix 16, although a slightly higher overall mean was recorded for the lowland regions. This could mean that, the area shares a similar geological structure among other environmental factors. Similar observations were made where high concentrations values were reported in the lowlands for other elements considered in this study. This observation could be attributed to intensive agricultural activities in the highlands that experiences a favorable climate leading to depletion of elements (Drechsel, 2001).

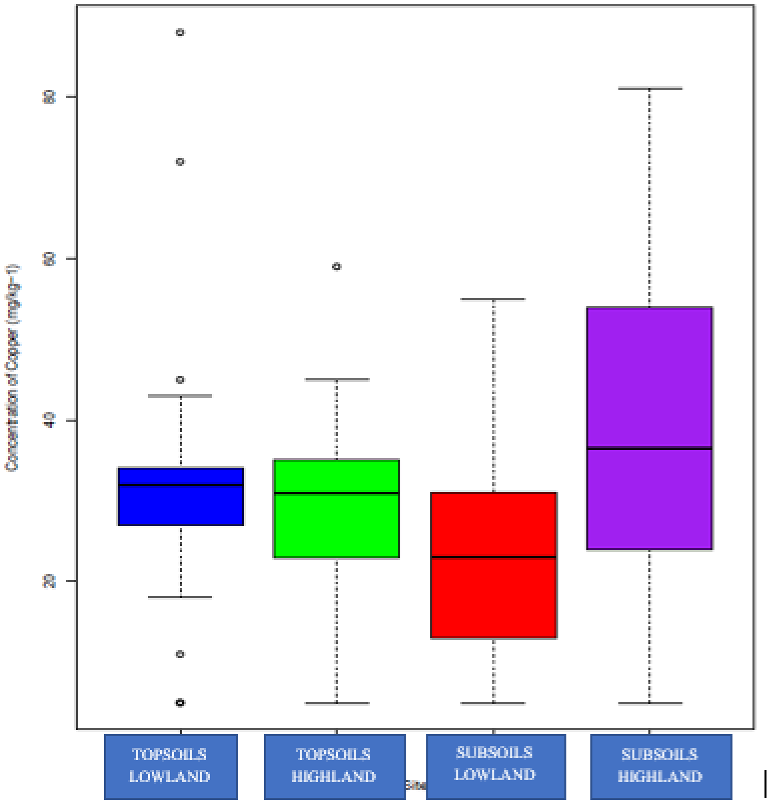

Figure 4.7: Concentrations distribution of Cu in highland and lowland soil samples (mg kg^{-1})

There was no notable distinction in concentrations in the top soils and subsoils in the study region (P > 0.05) given in Appendix 15. These findings support a report by Kabata-Pendias and Pendias, (2011), where Cu was noted to be rather immobile in soils, exhibiting little variability within the soil profiles. In addition, a weak positive correlation was observed between these two sampling depth profiles (R = 0.5) as shown in Figure 4.9. There was a weak correlation between the total Cu, Zn, Mn, and Fe and absence of correlation noted between the pH of the soil and Cu (R = 0) being reported. Therefore, an increase or decrease in pH has no effect on the total availability in both the Spider plants and the soil (Appendix

10). Moreover, there was a notable distinction in the levels of Cu in soils and in the African spider plants i.e. ($P < 0.05$) depicted in Appendix 11.

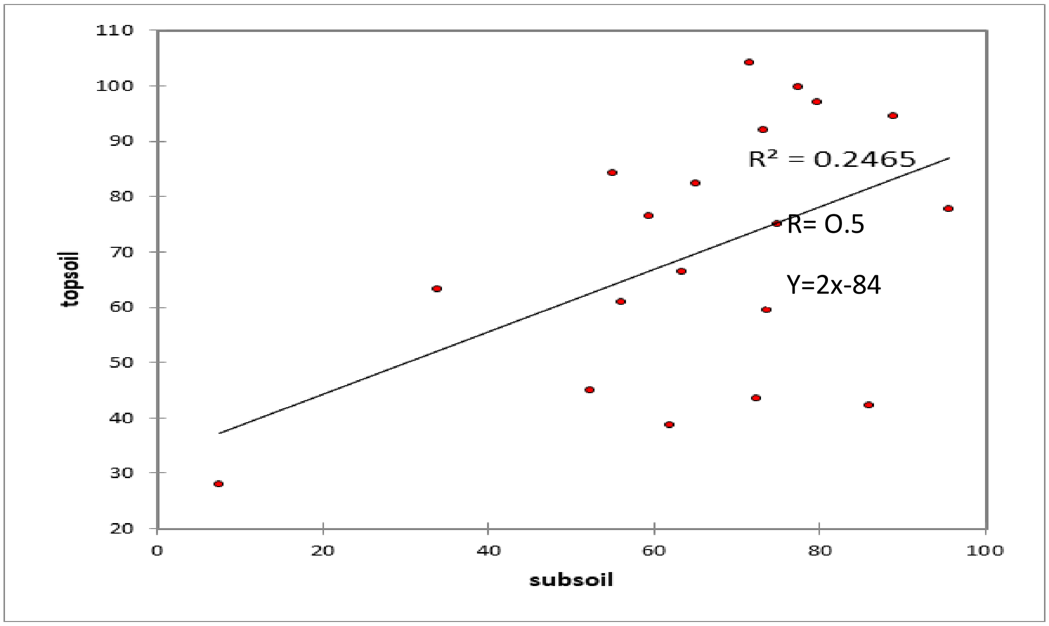

Figure 4.8: Correlation between Cu concentrations in top soils and subsoils

Figure 4.10 presents the distribution of the obtained concentration results for Cu content in leaves and stems of Spider plants from highland and lowland regions. From the study, the levels of Cu were found to be high in stems from the highland region as compared to stems in the lowland region. Also, the leaves from highland had a high concentration of Cu as compared to lowland leaves. A mean concentration of 15 ± 6 mg kg^{-1} was obtained in stems and 13 ± 3 mg kg^{-1} in leaves from the lowland region while 16.2 ± 5.4 mg kg^{-1} was obtained for the stems and 14 ± 4 mg kg^{-1} in leaves of Spider plants in the highland region. A general concentration range of 5 to 30 mg/kg was observed in the Spider plants as depicted by Figure 4.10 and Appendix 6 to 9.

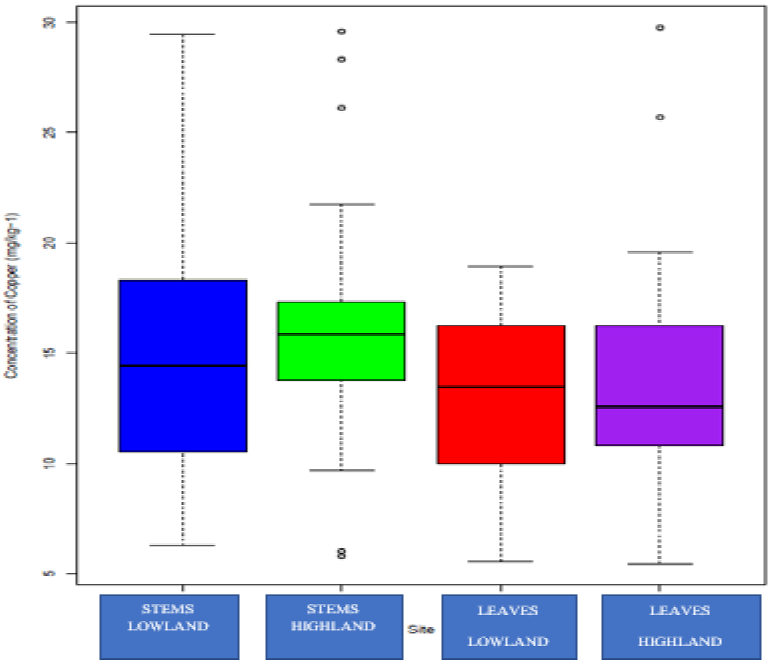

Figure 4.9: Concentration distribution of Cu in Spider plant stems and leaves (mg kg^{-1})

From the results, there was no notable distinction between the Cu content in Spider plants growing in the lowlands and highlands regions ($P > 0.05$) given in Appendix 16. However, a slightly higher mean was recorded in the highlands, an observation that could be attributed to higher total Cu concentration in the area. In both regions, higher Cu content was realized in the leaves as compared to stems which means leaves accumulate Cu more than the stems (Idris et al., 2011; Satter et al.,2016).

Cu is known to be a very crucial element in the human body aiding various physiological process and the production of collagen which are major structural components in the human body. It assists in the management of Osteoporosis and a vital element in the prevention of the same (Zheng et al., 2006). In addition, it is a good immune booster which helps the body to stand the foreign antigens from attacking the body cells which will lead to diseases and complications in the human body. However, high and low amounts of Cu in the human body have been known to cause some adverse effects, with deficiency being linked to bone fractures, thyroid problems, loss of skin pigmentation and low recorded body temperatures. High levels of Cu are associated with the adverse effects in the central nervous system whereby it is known to damage the brain tissue hence altering the functioning of the brain (Zheng et al., 2006). The recommended dietary daily intake is known to be a maximum 0.1 mg/day, hence with < 5 to 9 mg kg^{-1} of Cu reported in Spider plants is enough to maintain the required amounts in the body (WHO, 2005).

4.4.4 Zinc

Figure 4.11 presents the distribution of the obtained results for Zn concentration in the study area. From Appendix 2 to 5, a concentration means of between 180 ± 40 mg kg^{-1} for subsoils in the lowlands and 210 ± 60 mg kg^{-1} for the lowland top soils was reported from the study. A mean concentration of 260 ± 10 mg kg^{-1} for top soils in highland and 180 ± 40 mg kg^{-1} for the subsoils with an overall range of 100 to 500 mg kg^{-1}, was observed as displayed by Figure 4.11. These values are within the global range for uncontaminated soils for Zn reported at 150 - 300 mg kg^{-1} (Kabata-Pendias and Pendias, 2011). There was no notable distinction realized between the top soils and subsoils in the two study regions ($P > 0.05$) shown in Appendix 15. However, higher concentration values of Zn were recorded in the top soils as compared to the subsoils. For example, in the lowlands, a mean value of 260 ± 90 mg kg^{-1} was reported in the top soils as compared to a mean of 180 ± 70 mg kg^{-1} for the subsoils. A similar trend was noted in the highlands where a mean of 210 ± 60 mg kg^{-1} was recorded in the top soils in comparison to 180 ± 40 mg kg^{-1} for the subsoils. This observation could be attributed to adsorption of Zn to mineral clay and organic matter, hence its tendency to accumulate in the surface soils (Kabata-Pendias and Pendias, 2011).

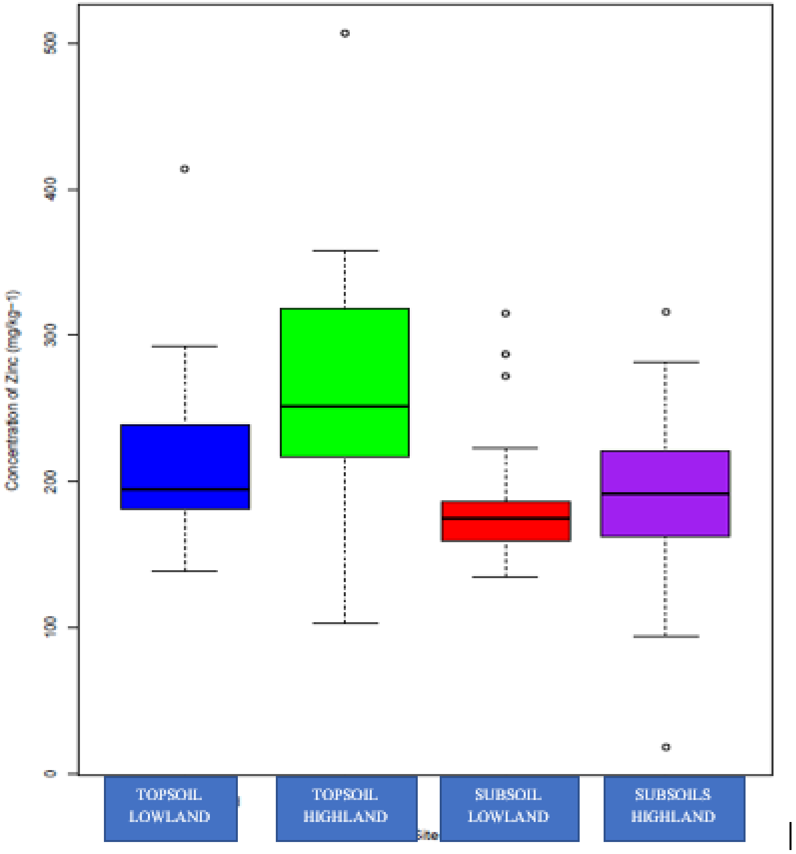

Figure 4.10: Concentrations distribution of Zn in lowland and highland soil samples (mg kg^{-1})

Zn is an essential micronutrient with a threshold concentration values above 5 mg kg^{-1} for crop production. The main source of Zn in the soil is weathering of Zn minerals released mainly as Zn^{2+} ions, a form that is most mobile and bio-available form to plants depending on soil parameters like pH, clay and hydrous oxides content. In this study, Zn levels above worldwide range in contaminated soil (150 - 300 mg kg^{-1}), and regulatory limits at 500 mg kg-1 (Yadav, 2009), were reported. Based on the total Zn content in the soil, it can be concluded that soils in the region have adequate amounts of Zn and does not pose a risk of toxicity. However, Zn deficiency in plants can be experienced even in soils with high total content. Studies have associated Zn deficiencies with soil conditions such as very high or low pH, high N and P content, free $CaCO_3$ and low organic matter (Kabata-Pendias and Pendias, 2011).

From Table 4.5, there was a weak correlation between Zn and the other trace elements (Cu, Mn, and Fe), in both depth profiles. There was a weak correlation between the total available Zn and the pH of the soil samples in both depth profiles ($R = 0.3$) meaning, pH variation does not affect the total available Zn in both soil profiles and the Spider plants (Table 4.5). Figure 4.12 shows that there was a very weak correlation between Zn content in top soils and subsoils ($R = 0.1$). This means that Zn concentration in top soils did not affect the respective concentration in the subsoils. Moreover, there was a notable distinction in the levels of Zn in soils and in the African spider plants i.e. ($P < 0.05$) displayed in Appendix 11.

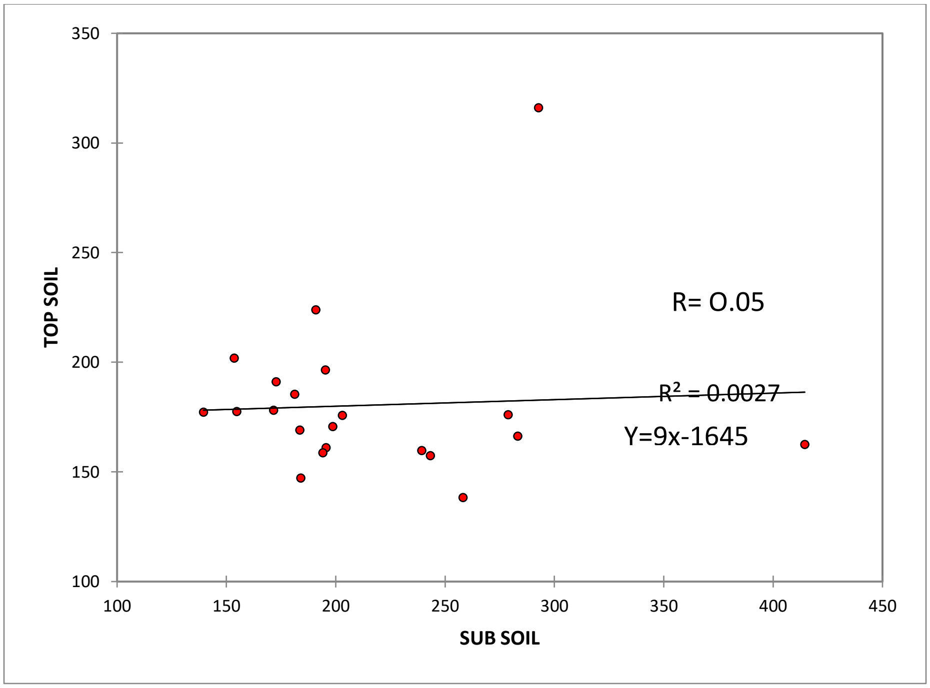

Figure 4.11: Correlation between Zn concentrations in top soils and subsoils

Figure 4.13 presents the distribution of the obtained results for Zn levels in stems and leaves of the Spider plants from lowland and highlands regions. From the box plot distribution of Zn, the levels of the Zn in stems from the highland region are higher than those in the lowland region. However, the levels of Zn in the leaves from the lowland region were high as compared to the highland region leaves. A mean concentration for the highland region stem and leaves were found to be 160 ± 50 mg kg^{-1} and 225 ± 60 mg kg^{-1} respectively while lowland stems and leaves were determined at 140 ± 50 mg kg^{-1} and 230 ± 60 mg kg^{-1} respectively with a concentration range of 57 to 310 mg kg^{-1} being observed in general as shown in Figure 4.13 and in Appendix 6 to 9.

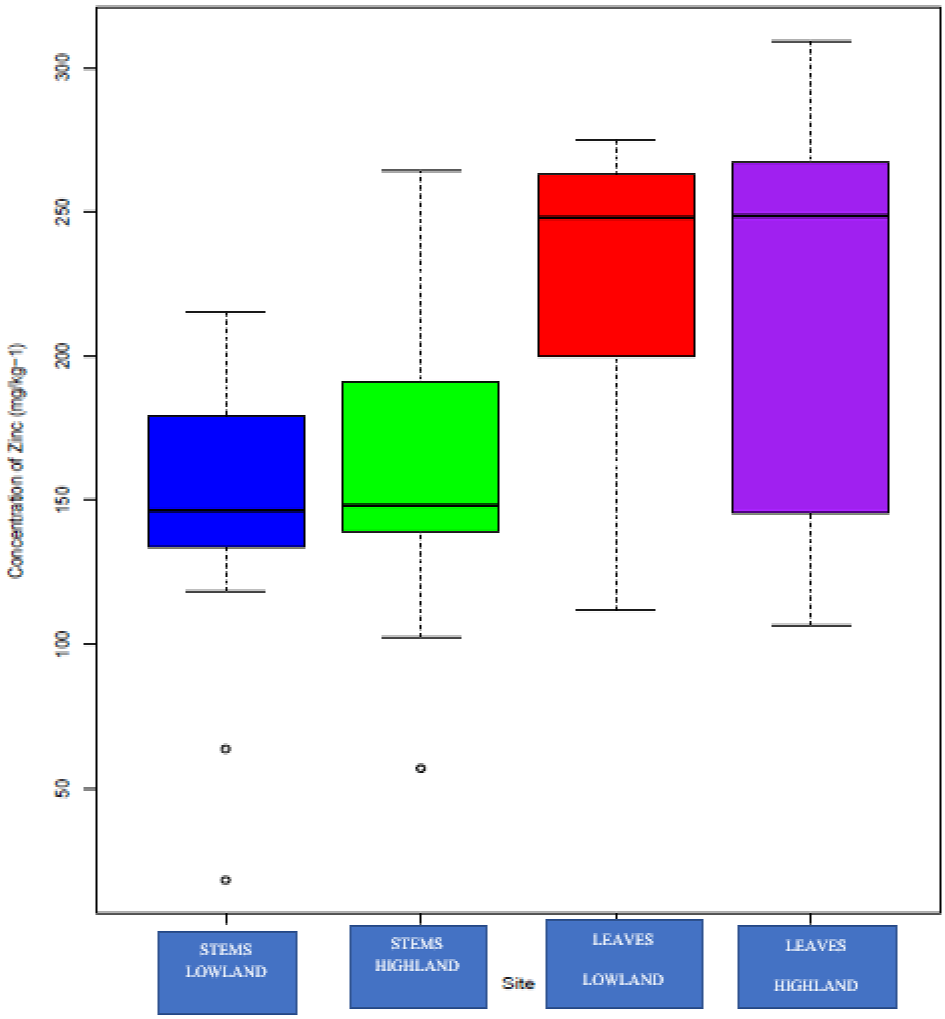

Figure 4.12: Concentration distribution of Zn in Spider plant stems and leaves (mg kg^{-1})

From the study, there was no notable distinction between the Zn content in the Spider plants growing in highland and lowlands regions ($P > 0.05$) given in Appendix 16. Nonetheless, there was a slight increase in the levels of Zn in leaves than in stems for Spider plant growing in highlands, with a similar trend being noted for the lowland Spider plants. From the results, it can be argued that Zn bioaccumulates more in leaves as compared to stems due to differences in structural and adsorption capabilities of stems and leaves. Similar observation after conducting research on different parts of the wild vegetables (Satter et al., 2016)).

Zn is known to be a very vital and necessary element in the human body. It is essential in the promotion of healthy growth in children, synthesis of the deoxyribonucleic acid, regulation of the immune system and healing of the wounds (Maret and Sandstead, 2006). However, high concentrations and low amounts of Zn in the human body have been known to cause some complications in the body system. High levels of Zn promote the development of the kidney stones which leads to stomach pains, headaches, vomiting and loss of appetite. Zn deficiency is associated with slow healing of the wounds, depressed growth, loss of appetite and hair loss (Maret and Sandstead, 2006). With reference to the daily recommended dietary intake of 4.2 - 14.0 mg per day, the Zn content in the Spider plant (80 to 230 mg kg^{-1}) could be concluded to be sufficient, hence Spider plant can be used as Zn supplement (WHO, 2005).

4.5 Summary of the results

The soils in the study region were found to be slightly acidic with an approximate mean of 5.6 in overall. From the elemental analysis of the soils in the area of research, the levels of Fe, Mn, Cu, and Zn were found to be within the recommended global range therefore the farmers in the study area can be encouraged to enhance crop cultivation. There was notable difference between the concentration levels of Cu, Zn, Mn, and Fe in the soils and African Spider plants ($P < 0.05$) shown in Appendix 11. That means the variation of the Cu, Zn, Mn, and Fe in the soils does not affect the total available the Cu, Zn, Mn, and Fe in the African Spider plants. The African Spider plant from the study area was found to be nutritious with no notable distinction being reported in the levels of Fe, Mn, Cu, and Zn between the two regions considered for the study. The information obtained from this study will be crucial in sensitizing the public and popularizing the use of Spider plant as vegetable and source of essential trace elements. Below is the summary of the elemental content of Mn. Fe, Cu, and Zn in soil profiles and African Spider plants.

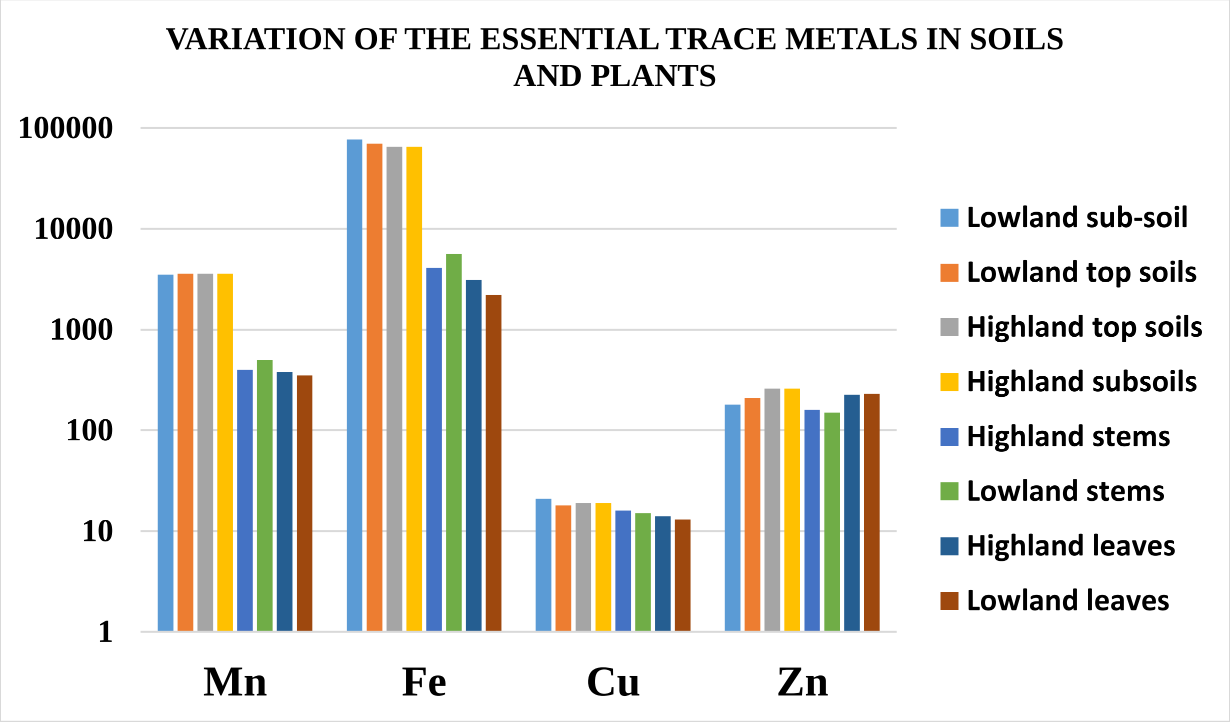

Figure 4.13: Summary results of the concentration levels of Mn, Fe, Cu, and Zn in both soil profiles and the African Spider plant's stems and leaves.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The soils in the study area were found to be slightly acidic i.e. a mean pH range of between 4.3 to 7.3 being recorded for the soils in the lowland regions for both soil profiles under the study in contrast to a mean pH value of between 4.4 to 7.4 being observed for both soil profiles under research in highland sampling sites.

The Spider plant was found to contain high levels of total available Zn, Mn Cu, and Fe i.e. Cu at 15- 16 mg kg^{-1}, Fe at 4100 -5600 mg kg^{-1}, Zn at 140-160 ± 50 mg kg^{-1} , Mn at 400 - 500 mg $kg^{-1}$for the stem .For the leaves , the mean concentration was reported for Fe at 2200-3100 mg kg^{-1}, Mn at 350 -380 mg kg^{-1}, Cu at 13-14 mg kg^{-1}, Zn at 225-230 mg kg^{-1} was recorded; hence the plant was found to be a nutritious vegetable and a suitable source of essential trace elements.

Fe was noted to be the most abundant trace element in soil and a trend in the order of $Cu < Zn < Mn < Fe$ i.e. 63000 to 77000 mg $kg^{-1}$for Fe, 19 to 21 mg kg^{-1} for Cu, 3100 to 3600 mg kg^{-1} for Mn, and 180 to 260 mg kg^{-1} for Zn being observed. Therefore, the farmers from lowland should be encouraged to intensify cultivation of the Spider plants.

There was no notable distinction in the levels of the essential trace metals from the soils collected in highland and lowland regions with the same trend being observed in African Spider plants ($P > 0.05$). There was a notable distinction between the levels of Fe, Mn, Cu, and Zn in soils and in African Spider plants ($P < 0.05$).

5.2 Recommendations

1. The Spider plant was found to contain substantial amounts of essential trace metals. Therefore, the government and community-based organization should sensitize farmers on the importance of the crop and encourage the farmers to cultivate it.
2. This study focused only on total trace elements content. More research should be conducted on the plant to assess its other parameters such as medicinal values and the bio-availability of the essential trace metals.
3. Age of the plant was not considered during sampling since the farmers do not harvest it at a particular age. Therefore, more research should be conducted to ascertain the optimum age of harvest of the crop based on the period at which the crop is of the highest nutritional value.

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APPENDICES

Appendix 1: Global positions of the sampling points of Molo Ward

	Location	**Elevation**	**X**	**Y**
1	Mukinyai	1814	35° 48' 28.735" E	0° 12' 40.589" S
2	Mukinyai	1732	35° 48' 43.187" E	0° 12' 22.922" S
3	Mukinyai	1988	35° 49' 9.490" E	0° 12' 24.317" S
4	Mukinyai	1984	35° 48' 47.667" E	0° 12' 4.269" S
5	Mukinyai	1985	35° 48' 56.753" E	0° 12' 10.252" S
6	Mukinyai	1986	35° 49' 8.290" E	0° 12' 39.429" S
7	Mukinyai	1765	35° 49' 10.007" E	0° 12' 10.813" S
8	Mukinyai	1945	35° 49' 25.847" E	0° 12' 17.538" S
9	Mukinyai	1764	35° 47' 59.383" E	0° 12' 55.942" S
10	Mukinyai	1802	35° 48' 3.117" E	0° 12' 26.564" S
11	Mukinyai	1865	35° 47' 40.116" E	0° 12' 20.840" S
12	Mukinyai	1988	35° 47' 40.652" E	0° 12' 54.174" S
13	Mukinyai	1784	35° 47' 4.177" E	0° 12' 52.799" S
14	Mukinyai	1765	35° 46' 42.173" E	0° 12' 50.190" S
15	Mukinyai	1877	35° 46' 50.811" E	0° 12' 18.360" S
16	Sachangwan	1982	35° 45' 56.512" E	0° 11' 42.665" S
17	Sachangwan	1995	35° 47' 14.937" E	0° 11' 55.254" S
18	Sachangwan	1820	35° 47' 43.312" E	0° 12' 6.654" S

19	Sachangwan	1998	35° 48' 8.778" E	0° 12' 13.417" S
20	Sachangwan	1996	35° 47' 47.302" E	0° 11' 55.397" S
21	Sachangwan	2001	35° 48' 18.843" E	0° 12' 0.359" S
22	Sachangwan	1980	35° 47' 59.471" E	0° 11' 40.904" S
23	Sachangwan	2014	35° 48' 25.956" E	0° 11' 50.971" S
24	Sachangwan	2011	35° 48' 4.046" E	0° 11' 34.085" S
25	Sachangwan	1787	35° 46' 58.021" E	0° 11' 41.936" S
26	Sachangwan	1715	35° 47' 30.165" E	0° 11' 40.547" S
27	Sachangwan	2010	35° 46' 26.145" E	0° 11' 20.849" S
28	Sachangwan	1991	35° 46' 51.909" E	0° 11' 25.361" S
29	Sachangwan	2001	35° 47' 13.979" E	0° 11' 23.936" S
30	Sachangwan	2000	35° 46' 49.144" E	0° 11' 4.364" S
31	Kabianga	2213	35° 47' 23.213" E	0° 13' 25.098" S
32	Kabianga	2211	35° 47' 13.266" E	0° 13' 49.444" S
33	Kabianga	2237	35° 46' 45.793" E	0° 13' 48.497" S
34	Kabianga	2270	35° 46' 1.251" E	0° 13' 15.385" S
35	Kabianga	2310	35° 46' 15.780" E	0° 13' 46.128" S
36	Kabianga	2273	35° 46' 36.531" E	0° 14' 9.490" S
37	Kabianga	2332	35° 46' 3.414" E	0° 14' 5.899" S
38	Kabianga	2290	35° 46' 20.677" E	0° 14' 21.035" S
39	Tumaini	2291	35° 46' 51.028" E	0° 14' 21.723" S
40	Tumaini	2298	35° 46' 50.177" E	0° 14' 35.263" S
41	Tumaini	2285	35° 46' 29.595" E	0° 14' 44.985" S

42	Tumaini	2292	35° 46' 4.943" E	0° 14' 35.965" S
43	Tumaini	2299	35° 45' 48.470" E	0° 15' 20.740" S
44	Tumaini	2329	35° 46' 18.462" E	0° 15' 17.947" S
45	Tumaini	2338	35° 46' 43.883" E	0° 15' 10.743" S
46	Molo	2423	35° 45' 15.339" E	0° 14' 53.966" S
47	Molo	2439	35° 44' 37.286" E	0° 14' 44.523" S
48	Molo	2448	35° 45' 6.398" E	0° 15' 44.732" S
49	Molo	2413	35° 44' 28.832" E	0° 15' 46.888" S
50	Molo	2413	35° 44' 51.500" E	0° 14' 19.952" S
51	Molo	2434	35° 44' 9.574" E	0° 14' 9.937" S
52	Molo	2449	35° 45' 40.141" E	0° 13' 53.891" S
53	Tayari	2473	35° 42' 5.708" E	0° 13' 52.990" S
54	Tayari	2489	35° 43' 16.581" E	0° 14' 42.828" S
55	Tayari	2460	35° 42' 2.024" E	0° 14' 34.499" S
56	Tayari	2482	35° 43' 15.903" E	0° 14' 0.525" S
57	Tayari	2416	35° 43' 59.386" E	0° 13' 1.497" S
58	Tayari	2436	35° 44' 56.912" E	0° 13' 14.704" S
59	Tayari	2476	35° 44' 25.915" E	0° 12' 49.866" S
60	Tayari	2419	35° 44' 22.929" E	0° 13' 18.023" S

Appendix 2: Concentration of Mn, Fe, Cu, and Zn in the Lowland's subsoils

Sample code	**Mn (mg kg^{-1})**	**Fe (W%)**	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	**pH**
S1MMB	3166 ± 124	11.7 ± 0.3	34 ± 2	272 ± 21	6.1 ± 0.2
S1SHB	2817 ± 123	9.9 ± 0.2	59 ± 5	178 ± 17	4.6 ± 0.1
S2MMB	2830 ± 125	8.4 ± 0.2	35 ± 2	315 ± 19	6.1 ± 1.0
S2SHB	2478 ± 101	7.9 ± 0.1	31 ± 2	177 ± 13	4.4 ± 0.1
S3SHB	2622 ± 134	7.8 ± 0.3	27 ± 3	162 ± 15	4.4 ± 0.1
S3MMB	3126 ± 102	8.1 ± 0.1	45 ± 3	178 ± 31	5.8 ± 0.7
S4SHB	2659 ± 128	6.3 ± 0.2	30 ± 1	166 ± 15	6.1 ± 0.5
S4MMB	3340 ± 98	9.1 ± 0.2	43 ± 8	221 ± 23	6.0 ± 1.2
S5MMB	2824 ± 128	6.2 ± 0.1	33 ±2	138 ± 13	6.1 ± 0.8
S5SHB	3279 ± 126	8.3 ± 0.2	31 ± 2	186 ± 16	6.1 ± 0.4
S5SHB	2666 ± 117	6.3 ± 0.1	37 ± 2	157 ± 13	6.0 ± 0.2
S8MBB	4918 ± 153	7.8 ± 0.3	11 ± 1	287 ± 16	5.6 ± 0.3
S6MDB	4912 ± 196	7.9 ± 0.2	39 ± 2	161 ± 17	5.5 ± 0.9
S6SHB	2628 ± 104	6.8 ± 0.1	32 ± 2	170 ± 11	5.8 ± 0.1
S7MMB	4540 ± 151	7.9 ± 0.2	< 10	135 ± 18	5.3 ± 0.3
S7SHB	2683 ± 102	7.5 ± 0.2	22 ± 1	158 ± 14	4.9 ± 0.5
S8SHB	2697 ± 115	7.3 ± 0.2	14 ± 2	201 ± 16	4.6 ± 0.7
S9MBB	3352 ± 146	8.3 ± 0.2	16 ± 2	185 ± 14	5.0 ± 0.2
S9SHB	2305 ± 131	8.8 ± 0.3	30 ± 2	157 ± 16	5.8 ± 0.1
S10SHB	2889 ± 120	7.6 ± 0.2	< 10	190 ± 16	7.3 ± 0.9
S11SHB	3162 ± 134	5.4 ± 0.1	23 ± 2	177 ± 15	5.8 ± 0.6
S12MBB	3365 ± 134	6.5 ± 0.2	24 ± 1	155 ± 15	6.6 ± 0.7
S12SHB	2914 ± 143	7.2 ± 0.2	31 ± 2	196 ± 15	6.6 ± 0.4
S11MDB	8169 ± 307	9.2 ± 0.2	34 ± 2	176 ± 17	6.3 ± 0.5
S13MMB	4020 ± 164	7.4 ± 0.2	37 ± 2	147 ± 15	5.2 ± 0.1
S10MMB	3199 ± 146	7.1 ± 0.1	19 ± 1	161 ± 17	4.9 ± 0.2
S14MDB	6123 ± 165	9.3 ± 0.3	32 ± 2	175 ± 15	5.3 ± 0.5
S14SHB	3323 ± 149	7.3 ± 0.2	35 ± 4	169 ± 13	5.4 ± 0.3
S15MAB	3219 ± 114	7.1 ± 0.2	28 ± 1	223 ± 13	5.4 ± 0.2
S15SHB	3205 ± 147	7.0 ± 0.3	33 ± 3	159 ± 17	5.5 ± 0.3
AVERAGE	**3500 ± 1300**	**7.7 ± 1.0**	**21 ± 1**	**180 ± 40**	**5.6 ± 0.7**
MIN	**2305 ± 131**	**5.3 ± 0.1**	**< 10**	**135 ± 18**	**4.4 ± 0.1**
MAX	**8169 ± 307**	**11.6 ± 0.3**	**59 ± 4**	**315 ± 19**	**7.3 ± 0.9**

Appendix 3: Concentration of Mn, Fe, Cu, and Zn in Lowland's top soils

Sample code	Mn (mg kg^{-1})	Fe (W%)	Cu (mg kg^{-1})	Zn (mg kg^{-1})	pH
S1MMT	3479 ± 139	6.9 ± 0.2	72 ± 7	172 ± 16	6.0 ± 0.3
S1SHT	3160 ± 124	7.5 ± 0.2	43 ± 5	171 ± 13	5.6 ± 0.1
S2MMT	2574 ± 116	7.7 ± 0.2	34 ± 2	292 ± 19	6.9 ± 0.2
S2SHT	2422 ± 145	6.3 ± 0.1	88 ± 7	154 ± 14	5.4 ± 0.5
S3MMT	3401 ± 163	7.8 ± 0.3	33 ± 6	186 ± 16	7.4 ± 0.7
S3SHT	3290 ± 147	8.5 ± 0.2	34 ± 6	414 ± 26	6.1 ± 0.2
S4MMT	3418 ± 143	7.3 ± 0.2	37 ± 3	258 ± 15	5.4 ± 0.3
S4SHT	2696 ± 108	4.9 ± 0.1	37 ± 2	283 ± 16	6.7 ± 0.8
S5SHT	2898 ± 104	6.8 ± 0.1	< 10	198 ± 24	6.3 ± 0.6
S5MMT	3342 ± 134	6.7 ± 0.1	45 ± 8	198 ± 13	6.3 ± 1
S6MMT	4254 ± 182	7.4 ± 0.3	< 10	195 ± 19	7.6 ± 0.9
S6SHT	3434 ± 147	4.9 ± 0.2	18 ± 2	198 ± 14	5.3 ± 0.3
S7SHT	2866 ± 117	6.1 ± 0.1	26 ± 1	194 ± 19	7.2 ± 1
S7MMT	3342 ± 112	7.8 ± 0.1	33 ± 5	201 ± 12	7.1 ± 0.7
S8MMT	4659 ± 157	6.7 ± 0.2	29 ± 1	153 ± 14	6.0 ± 0.4
S8SHT	3320 ± 139	7.1 ± 0.1	38 ± 2	239 ± 16	6.1 ± 0.6
S9MDT	3521 ± 151	7.9 ± 0.2	11 ± 2	181 ± 13	5.6 ± 0.1
S9SHT	3198 ± 154	6.9 ± 0.1	32 ± 3	243 ± 22	7.2 ± 0.5
S10MDT	3049 ± 151	7.1 ± 0.3	32 ± 2	172 ± 14	6.2 ± 0.4

S10SHT	3456 ± 143	7.0 ± 0.1	< 10	245 ± 21	5.5 ± 0.1
S11SHT	3252 ± 144	7.5 ± 0.2	34 ± 2	196 ± 17	6.6 ± 0.2
S11MDT	3063 ± 129	6.2 ± 0.2	31 ± 8	139 ± 11	4.8 ± 0.1
S12SHT	2890 ± 135	7.1 ± 0.2	31 ± 2	195 ± 14	6.9 ± 0.2
S13MDT	8261 ± 259	9.9 ± 0.3	32 ± 2	278 ± 21	5.7 ± 0.5
S13SHT	3153 ± 153	6.2 ± 0.2	18 ± 2	184 ± 14	6.2 ± 0.7
S14MDT	4417 ± 167	7.9 ± 0.2	30 ± 2	203 ± 18	5.6 ± 0.8
S14SHT	3504 ± 122	6.3 ± 0.1	28 ± 1	183 ± 14	6.0 ± 0.9
S15MDT	4784 ± 182	7.8 ± 0.2	27 ± 2	190 ± 16	5.9 ± 0.1
S15SHT	3985 ± 123	8.1 ± 0.3	34 ± 9	178 ± 11	6.2 ± 0.3
AVERAGE	**3600 ± 1200**	**7.0 ± 1.0**	**18 ± 4**	**210 ± 60**	**6.1±0.6**
MIN	**2422 ± 145**	**4.9 ± 0.1**	**< 10**	**139 ± 11**	**4.8 ± 0.1**
MAX	**8261 ± 259**	**9.9 ± 0.3**	**88 ± 7**	**414 ± 26**	**7.5 ± 0.9**

Appendix 4: Concentration of Mn, Fe, Cu, and Zn in Highland top soils

Sample code	**Mn (mg kg^{-1})**	**Fe (1*10^4) mg kg^{-1}**	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	**pH**
S1KAT	4237 ± 154	7.0 ± 0.2	32 ± 1	224 ± 16	6.1 ± 0.2
S1MLAT	3877 ± 126	7.2 ± 0.2	19 ± 2	247 ± 21	5.1 ± 0.4
S1TABT	630 ± 50	2.6 ± 0.1	22 ± 1	103 ± 8	5.2 ± 0.3
S3TAT	2628 ± 97	5.1 ± 0.1	16 ± 2	343 ± 19	6.0 ± 0.7
S1TMAT	4338 ± 187	6.8 ± 0.2	12 ± 3	318 ± 20	5.7 ± 0.1
S2KAT	4893 ± 211	8.1 ± 0.2	20 ± 4	219 ± 15	4.9 ± 0.1
S2MLAT	3433 ± 125	6.9 ± 0.1	11 ± 3	221 ± 17	5.1 ± 0.7
S2TAT	2155±108	4.7±0.1	32±2	251±16	5.5 ± 0.1
S2TBT	600 ± 67	2.6 ± 0.1	29 ± 1	131 ± 10	5.9 ± 0.2
S2TMAT	4327 ± 179	6.9 ± 0.2	23 ± 2	291 ± 19	4.8 ± 0.1
S3KBBT	3724 ± 155	8.0 ± 0.3	11 ± 2	214 ± 19	6.4 ± 0.4
S3MLAT	3448 ± 178	6.5 ± 0.2	< 10	217 ± 18	6.3 ± 0.2
S5TBT	3287 ± 118	6.7 ± 0.1	32 ± 4	507 ± 23	6.1 ± 0.7
S3TMAT	5073 ± 231	8.0 ± 0.3	55 ± 8	341 ± 27	5.9 ± 0.2
S4KAT	3954 ± 1239	6.6 ± 1.7	17 ± 4	161 ± 25	5.8 ± 0.5
S4MLAT	3397 ± 164	6.5 ± 0.2	31 ± 2	323 ± 17	6.1 ± 0.3
S4TAT	1696 ± 103	3.9 ± 0.1	23 ± 7	336 ± 17	5.8 ± 0.5
S4TMAT	5050 ± 157	7.9 ± 0.2	12 ± 3	358 ± 18	6.9 ± 0.6
S5KBT	4850 ± 148	7.6 ± 0.1	30 ± 3	239 ± 18	5.5 ± 0.1
S5TMAT	4702 ± 149	7.7 ± 0.2	< 10	139 ± 14	5.9 ± 0.9
S5MLT	3838 ± 141	6.8 ± 0.1	13 ± 2	286 ± 17	5.8 ± 0.3
S6KBT	5696 ± 177	9.4 ± 0.2	37 ± 4	268 ± 21	5.8 ± 0.2
S6MLBT	4232 ± 174	7.9 ± 0.2	25 ± 5	260 ± 18	5.4 ± 0.6
S6TMBT	4170 ± 170	6.9 ± 0.2	24 ± 2	333 ± 17	6.0 ± 0.3
S6TAT	3989 ± 134	6.8± 0.1	33 ± 13	298 ± 19	5.6 ± 0.5
S7KBT	3873 ± 149	6.3 ± 0.1	14 ± 1	170 ± 13	5.4 ± 0.1
S7TAT	1752 ± 86	3.3 ± 0.1	26 ± 1	196 ± 11	6.4 ± 0.8
S7MLAT	5121 ± 78	6.9 ± 0.1	34 ± 3	235 ± 13	6.6 ± 0.6
S7TMAT	4546 ± 112	7.8 ± 0.1	< 10	312 ± 23	5.9 ± 0.4
S8KAT	4321 ± 115	7.2 ± 0.2	28 ± 2	276 ± 21	6.4 ± 0.3
S8MLAT	4333 ± 135	6.8 ± 0.1	45 ± 5	196 ± 11	5.9 ± 0.2
AVERAGE	**3600 ± 1300**	**6.5 ± 2.0**	**19 ± 3**	**260 ± 10**	**5.8 ± 0.4**
MIN	**600 ± 67**	**2.6 ± 0.1**	**< 10**	**103 ± 8**	**4.8 ± 0.3**
MAX	**5700 ± 177**	**9.4 ± 0.2**	**55 ± 8**	**507 ± 23**	**6.9 ± 0.6**

Appendix 5: Concentration of Mn, Fe, Cu, and Zn in the Highland's subsoils

Sample code	Mn (mg kg^{-1})	Fe (1*10^4) mg kg^{-1}	Cu (mg kg^{-1})	Zn (mg kg^{-1})	pH
S1KAB	5069 ± 233	8.3 ± 0.2	27 ± 25	238 ± 20	6.1 ± 0.1
S1TAB	994 ± 70	3.4 ± 0.1	< 10	184 ± 12	5.1 ± 0.2
S6TAB	628 ± 53	2.7 ± 0.1	81 ± 1	103 ± 12	4.5 ± 0.1
S2KAB	3780 ± 141	8.4 ± 0.2	19 ± 1	184 ± 15	6.0 ± 0.7
S2MLAB	3517 ± 154	8.2 ± 0.3	51 ± 2	204 ± 17	5.7 ± 0.4
S2TAB	1010 ± 68	2.7 ± 0.1	29 ± 1	123 ± 9	4.9 ± 0.3
S4TBB	364 ± 40	2.5 ± 0.1	26 ± 1	94 ± 12	5.8 ± 0.5
S1MLAB	4470 ± 189	7.2 ± 0.2	31 ± 2	261 ± 21	5.5 ± 0.1
S3KAB	4759 ± 162	8.8 ± 0.2	15 ± 2	180 ± 20	5.8 ± 0.2
S3MLAB	2983 ± 150	6.9 ± 0.3	24 ± 4	162 ± 14	4.8 ± 0.1
S3TAB	613 ± 52	2.8 ± 0.1	27 ± 1	138 ± 15	6.4 ± 0.4
S5TBB	6184 ± 201	7.3 ± 0.2	15 ± 1	193 ± 19	4.8 ± 0.3
S3TMAB	4874 ± 197	8.5 ± 0.3	35 ± 5	282 ± 27	6.8 ± 0.6
S4KAB	4058 ± 154	8.2 ± 0.2	15 ± 1	160 ± 16	5.9 ± 0.3
S4MLAB	3509 ± 132	7.5 ± 0.2	14 ± 2	204 ± 15	5.8 ± 0.6
S4TMBB	5056 ± 195	7.8 ± 0.2	30 ± 2	316 ± 19	6.1 ± 0.2
S5KBB	164 ± 130	1.6 ± 0.3	22± 18	18 ± 22	5.8 ± 0.7
S7TAB	620 ± 43	2.8 ± 0.1	45 ± 3	134 ± 23	6.5 ± 0.1

S5MLAB	3430 ± 120	6.9 ± 0.1	46 ± 10	198 ± 13	6.5 ± 0.9
S1TMAB	4578 ± 123	7.9 ± 0.2	38 ± 7	234 ± 23	6.6 ± 0.8
S6KBB	3356 ± 123	8.3 ± 0.1	54 ± 7	206 ± 12	6.2 ± 0.5
S8MLAB	2997 ± 112	7.1 ± 0.2	54 ± 9	201 ± 7	6.9 ± 0.7
S6MLAB	3517 ± 110	7.3 ± 0.2	62 ± 11	172 ± 15	5.6 ± 0.4
S2TMAB	5034 ± 156	8.1 ± 0.1	46 ± 17	221 ± 14	6.7 ± 0.9
S7KBB	3452 ± 145	7.9 ± 0.1	67 ± 0.11	223 ± 15	5.2 ± 0.1
S5TMBB	4789 ± 134	7.6 ± 0.2	39 ± 6	178 ± 18	6.6 ± 0.2
S7MLAB	3323 ± 115	7.0 ± 0.1	78 ± 9	192 ± 13	5.6 ± 0.1
S6TMBB	4997 ± 120	8.3 ± 0.2	56 ± 16	256 ± 23	6.4 ± 0.7
S8KBB	1198 ± 121	6.9 ± 0.1	57 ± 9	234 ± 14	5.8 ± 0.3
S7TMBB	5120 ± 135	8.1 ± 0.2	67 ± 11	197 ± 20	6.8 ± 0.1
AVERAGE	**3100 ± 1900**	**6.3 ± 2.5**	**16 ± 2**	**180 ± 70**	**5.9±0.7**
MIN	**364 ± 40**	**2.6 ± 0.1**	**< 10**	**94 ± 12**	**4.5 ± 0.1**
MAX	**6184 ± 201**	**8.8 ± 0.2**	**81 ± 1**	**316 ± 19**	**6.9 ± 0.7**

Appendix 6: Concentration of Mn, Fe, Cu, and Zn in Highland's Spider plant stems (mg kg^{-1})

Sample code	**Mn**	**Fe**	**Cu**	**Zn**
S1KBS	310 ± 33	2263 ± 93	16 ± 1	170 ± 8
S2MLS	342 ± 34	4240 ± 164	17 ± 1	210 ± 12
S1TAS	327 ± 30	3157 ± 68	6 ± 2	128 ± 7

S1TMS	493 ± 31	4655 ± 153	16 ± 1	168 ± 13
S2KBS	330 ± 23	2224 ± 70	15 ± 2	148 ± 7
S2TAS	311 ± 35	3074 ± 93	13 ± 3	135 ± 8
S2TMS	306 ± 30	3014 ± 127	17 ± 1	144 ± 8
S3KBS	575 ± 43	7699 ± 191	14 ± 1	151 ± 8
S3MLS	316 ± 36	4308 ± 135	26 ± 1	214 ± 11
S3TAS	256 ± 30	2882 ± 88	17 ± 1	126 ± 6
S3TMS	393 ±33	4006 ± 141	28 ± 1	152 ± 8
S4MLS	696 ± 47	2983 ± 104	13 ± 2	164 ± 10
S4TAS	195 ± 25	2789 ± 86	20 ± 0	139 ± 7
S4TMS	372 ± 40	3976 ± 160	19 ± 0	140 ± 7
S5MLS	798 ± 44	3651 ± 152	29 ± 2	237 ± 13
S5TAS	589 ± 46	3984 ± 110	17 ± 1	191 ± 10
S5TMS	321 ± 33	4058 ± 105	13 ± 1	138 ± 9
S6KBS	412 ± 30	5231 ± 125	21 ± 2	147 ± 8
S6MLS	641 ± 43	8964 ± 228	15 ± 1	216 ± 10
S6TAS	334 ± 31	5990 ± 180	10 ± 0	102 ± 6
S6TMS	322 ± 38	4204 ± 146	15 ± 1	139 ± 7
S7KBS	432 ± 31	4558 ± 138	6 ± 1	140 ± 8
S7TAS	451 ± 32	2511 ± 80	20 ± 2	264 ± 10
S8KBS	339 ± 30	4364 ± 122	16 ± 1	142 ± 9
S8TMS	331 ± 37	3808 ± 112	16 ± 2	147 ± 8

S4KBS	490 ± 32	2668 ± 92	15 ± 1	261 ± 10
S1MLS	285 ± 33	3654 ± 111	17 ± 1	145 ± 8
S7MLS	304 ± 45	5990 ± 37	15 ± 2	56 ± 8
S5KBS	798 ± 35	3651 ± 32	11 ± 1	237 ± 13
S7TMS	412 ± 46	5231 ± 33	13 ± 1	147 ± 21
AVERAGE	**400 ± 140**	**4100 ± 1600**	**16 ± 5**	**160 ± 50**
MIN	**195 ± 25**	**2224 ± 70**	**6± 1**	**56 ± 8**
MAX	**798 ± 44**	**8964 ± 228**	**29 ± 2**	**264 ± 10**

Appendix 7: Concentration of Mn, Fe, Cu, and Zn in Lowland's Spider plant stems (mg kg^{-1})

Sample code	**Mn**	**Fe**	**Cu**	**Zn**
S1MKS	437 ± 46	3902 ± 146	29 ± 1	166 ± 10
S1SHS	591 ± 44	5672 ± 153	19 ± 1	140 ± 8
S2SHS	699 ± 44	7077 ± 186	9 ± 2	141 ± 8
S3SHS	681 ± 48	7126 ± 167	20 ± 3	152 ± 11
S4MKS	519 ± 45	8058 ± 229	10 ± 1	128 ± 8
S4SHS	401 ± 36	5974 ± 206	12 ± 1	181 ± 9
S5MKS	549 ± 46	8260 ± 146	22 ± 2	139 ± 9
S5SHS	509 ± 47	6718 ± 185	15 ± 1	184 ± 8
S6MKS	763 ± 54	3268 ± 79	18 ± 1	138 ± 7

S6SHS	559 ± 42	4338 ± 149	7 ± 0	140 ± 8
S7MKS	450 ±36	6396 ± 230	17 ± 1	118 ± 7
S7SHS	335 ± 30	2475 ± 89	6 ± 1	160 ± 8
S8MKS	602 ± 40	6516 ± 78	13 ± 1	141 ± 8
S8SHS	398 ± 33	2771 ± 104	29 ± 2	179 ± 9
S12MKS	156 ± 44	5691 ± 217	13 ± 2	15 ± 5
S9MKS	225 ± 45	5744 ± 213	15 ± 2	215 ± 5
S9SHS	841 ± 44	6793 ± 69	11 ± 1	140 ± 8
S10MKS	316 ± 27	2351 ± 72	16 ± 1	150 ± 9
S10SHS	664 ± 41	5134 ± 189	10 ± 1	121 ± 8
S3MKS	856 ± 47	7167 ± 80	20 ± 2	171 ± 10
S11MKS	140 ± 40	5236 ± 264	18 ± 2	18 ± 6
S11SHS	370 ± 37	5523 ± 123	18 ± 1	122 ± 8
S12SHS	161 ± 30	5592 ± 83	15 ± 2	177 ± 5
S13MKS	722 ± 48	8486 ± 165	10 ± 2	185 ± 12
S13SHS	124 ± 43	5196 ± 223	9 ± 2	21 ± 6
S2MKS	351 ± 35	5095 ± 198	7 ± 1	125 ± 8
S14MKS	689 ± 44	8403 ± 227	14 ± 2	153 ± 8
S14SHS	318 ± 42	3454 ± 160	13 ± 1	63 ± 8
S15MKS	379 ± 29	2202 ± 69	13 ± 0	211 ± 8
S15SHS	892 ± 56	9035±237	11 ± 1	181±10
AVERAGE	**500 ± 20**	**5600 ± 2000**	**15 ± 6**	**150 ± 40**

MIN	**140 ± 40**	**2202 ± 69**	**6 ± 1**	**18 ± 6**
MAX	**892 ± 56**	**9035 ± 237**	**29 ± 3**	**215 ± 5**

Appendix 8: Concentration of Mn, Fe, Cu, and Zn in Highland's Spider plant leaves (mg kg^{-1})

Sample code	**Mn**	**Fe**	**Cu**	**Zn**
S1KBV	392 ± 31	4968 ± 160	12 ± 1	179 ± 9
SIMLV	368 ± 27	1977 ± 74	19 ± 2	248 ± 10
SITAV	451 ± 32	2522 ± 106	16 ± 4	288 ± 13
SITMV	123 ± 17	1259 ± 41	10 ± 1	106 ± 7
S2MLV	628 ± 42	8338 ± 253	25 ± 2	268 ± 11
S2TAV	398 ± 28	2399 ± 93	15 ± 3	275 ± 12
S2TMV	328 ± 27	3719 ± 107	13 ± 1	129 ± 6
S3KBV	373 ± 28	2075 ± 74	10 ± 5	247 ± 11
S3MLV	680 ± 50	8516 ± 107	29 ± 1	309 ± 16
S3TAV	360 ± 34	2127 ± 82	12 ± 2	260 ± 13
S3TMV	341 ± 37	3765 ± 97	9 ± 1	136 ± 7
S4KBV	390 ± 35	2180 ± 70	8 ± 1	244 ± 11
S4MLV	399 ± 29	2277 ± 94	5 ± 1	261 ± 11
S4TAV	400 ± 25	2352 ± 70	8 ± 3	271 ± 15
S4TMV	115 ± 17	1477 ± 60	17 ± 2	113 ± 7

S5KBV	373 ± 29	2125 ± 95	10 ± 3	248 ± 12
S5MLV	402 ± 31	2281 ± 88	12 ± 1	263 ± 15
S5TAV	431 ± 32	2318 ± 74	17 ± 1	267 ± 12
S5TMV	376 ± 27	2266 ± 88	15 ± 2	249 ± 11
S6KBV	389 ± 30	2185 ± 97	16 ± 3	258 ± 11
S6TAV	416 ± 36	2435 ± 83	12 ± 2	268 ± 12
S6TMV	300 ± 30	3831 ± 130	18 ± 3	135 ± 8
S7KBV	272 ± 34	4930 ± 158	12 ± 2	123 ± 11
S7MLV	383 ± 34	2375 ± 117	16 ± 1	249 ± 12
S7TAV	406 ± 33	2400 ± 87	12 ± 1	278 ± 12
S7TMV	341 ± 37	3765 ± 97	14 ± 4	136 ± 7
S2KBV	389 ± 30	2185 ± 97	5 ± 1	258 ± 11
S8KBV	390 ± 35	2180 ± 70	29 ± 1	244 ± 11
S6MLV	680 ± 50	8516 ± 107	11 ± 2	309 ± 16
S8TMV	376 ± 27	2266 ± 88	16 ± 1	249 ± 11
AVERAGE	**380 ± 120**	**3100 ± 1900**	**14 ± 4**	**225 ± 63**
MIN	**115 ± 17**	**1259 ± 41**	**5 ± 1**	**106 ± 7**
MAX	**680 ± 50**	**8516 ± 107**	**29 ± 1**	**309 ± 16**

Appendix 9: Concentration of Mn, Fe, Cu, and Zn in Lowland's Spider plant leaves ($mg\ kg^{-1}$)

Sample code	**Mn**	**Fe**	**Cu**	**Zn**
S1MKV	347 ± 58	1986 ± 108	18 ± 1	262 ± 13
S2MKV	400 ± 30	2021 ± 53	11 ± 6	254 ± 14
S2SHV	273 ± 21	1725 ± 58	7 ± 1	199 ± 8
S3MKV	392 ± 34	1915 ± 110	10 ± 4	244 ± 13
S3SHV	335 ± 24	1950 ± 65	16 ± 2	232 ± 10
S4MKV	280 ± 26	4499 ± 130	13 ± 1	112 ± 8
S4SHV	363 ± 28	2003 ± 81	13 ± 2	246 ± 12
S5MKV	307 ± 30	4760 ± 187	10 ± 1	127 ± 7
S6MKV	372 ± 27	2202 ± 82	16 ± 2	255 ± 11
S6SHV	336 ± 27	2001 ± 70	18 ± 1	233 ± 12
S7SHV	356 ± 31	2247 ± 92	18 ± 1	265 ± 12
S8MKV	351 ± 34	1992 ± 87	15 ± 4	251 ± 11
S8SHV	366 ± 26	2129 ± 71	17 ± 3	253 ± 10
S9SHV	310 ± 27	1098 ± 354	10 ± 2	112 ± 2
S10MKV	380 ± 29	2075 ± 97	16 ± 2	266 ± 12
S10SHV	389 ± 30	2140 ± 87	17 ± 1	263 ± 13
S11MKV	334 ± 26	1787 ± 74	15 ± 1	243 ± 10
S11SHV	400 ± 28	2205 ± 67	15 ± 3	261 ± 9
S12MKV	331 ± 29	1951 ± 85	5 ± 1	249 ± 11

S12SHV	337 ± 30	2024 ± 89	14 ± 2	268 ± 12
S13SHV	379 ± 35	2174 ± 102	13 ± 1	275 ± 13
S14MKV	329 ± 29	1834 ± 87	11 ± 2	243 ± 12
S14SHV	360 ± 29	2139 ± 103	14 ± 3	273 ± 13
S15MKV	367 ± 30	1855 ± 80	15 ± 2	273 ± 12
S15SHV	388 ± 29	2162 ± 80	8 ± 1	258 ± 11
S13MKV	380 ± 29	2075 ± 97	13 ± 3	266 ± 12
S9MKV	402 ± 30	2121 ± 53	7 ± 2	275 ± 14
S7MKV	307 ± 30	4760 ± 187	11 ± 1	127 ± 7
S1SHV	356 ± 31	2247 ± 92	6 ± 1	265 ± 12
AVERAGE	**350 ± 70**	**2200 ± 800**	**13 ± 3**	**230 ± 60**
MIN	**273 ± 21**	**1098 ± 354**	**5 ± 1**	**112 ± 2**
MAX	**402 ± 30**	**4760 ± 187**	**18 ± 1**	**275 ± 14**

Appendix 10: Correlation matrix between the Mn, Fe, Cu, and Zn in soil samples

Variables	**Mn**	**Fe**	**Cu**	**Zn**	**pH**
Mn	**1.0**	**0.9**	**0.5**	**0.4**	**0.2**
Fe		**1.0**	**0.5**	**0.4**	**0.1**
Cu			**1.0**	**0.5**	**0.0**
Zn				**1.0**	**0.3**

pH					1.0

Appendix 11: T-test for two sample means assuming unequal variances for Mn, Fe, Cu, and Zn in soils and African Spider plants

Mn		**Fe**		**Cu**		**Zn**	
t Stat	77	t Stat	22.3	t Stat	5.3	t Stat	4.3
P(T<=t) one-tail	0	P(T<=t) one-tail	0	P(T<=t) one-tail	0	P(T<=t) one-tail	0.1
t Critical one-tail	1.9	t Critical one-tail	2.4	t Critical one-tail	1.9	t Critical one-tail	1.9
P(T<=t) two-tail	0	P(T<=t) two-tail	0	P(T<=t) two-tail	0	P(T<=t) two-tail	0.3
t Critical two-tail	2.4	t Critical two-tail	3.1	t Critical two-tail	2.4	t Critical two-tail	2.4

Appendix 12: T-test for the notable distinctions between the experimental and certified means of the Bowen kales

t Stat	-2.0
P(T<=t) one-tail	0.1
t Critical one-tail	2.3

P(T<=t) two-tail	0.1
t Critical two-tail	3.1

Appendix 13: T-test for the notable distinctions between the experimental and certified means of the PTXRF-009 River Clay

t Stat	1.1
P(T<=t) one-tail	0.1
t Critical one-tail	2.3
P(T<=t) two-tail	0.3
t Critical two-tail	3.1

Appendix 14: T-test for two samples means assuming equal variances for pH in soils

t Stat	0
P(T<=t) one-tail	0.5
t Critical one-tail	2.919986
P(T<=t) two-tail	1
t Critical two-tail	4.302653

Appendix 15: T-test for two samples with equal variances for the subsoils and top soils for Highland and Lowland regions

Mn		Fe		Cu		Zn	
t Stat	-1.5	t Stat	0.336336	t Stat	0	t Stat	-2.2
P(T<=t) one-tail	0.136197	P(T<=t) one-tail	0.384314	P(T<=t) one-tail	0.5	P(T<=t) one-tail	0.079404
t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986
P(T<=t) two-tail	0.272393	P(T<=t) two-tail	0.768628	P(T<=t) two-tail	1	P(T<=t) two-tail	0.158809
t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653

Appendix 16: T-test for two samples with equal variances for Highland and Lowland regions in the overall study for both the soils and African Spider plants

Mn		Fe		Cu		Zn	
t Stat	-0.78446	t Stat	-2.60985	t Stat	-0.94281	t Stat	0.585206
P(T<=t) one-tail	0.257464	P(T<=t) one-tail	0.060392	P(T<=t) one-tail	0.22265	P(T<=t) one-tail	0.30882
t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986

P(T<=t) two-tail	0.514929	P(T<=t) two-tail	0.120785	P(T<=t) two-tail	0.4453	P(T<=t) two-tail	0.61764
t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653

Appendix 17: T-test for two samples with equal variances between the stems and leaves in Highland and Lowland regions

Mn		Fe		Cu		Zn	
t Stat	1.628305	t Stat	2.515313	t Stat	2.828427	t Stat	-12.9692
P(T<=t) one-tail	0.122502	P(T<=t) one-tail	0.064164	P(T<=t) one-tail	0.052786	P(T<=t) one-tail	0.002946
t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986	t Critical one-tail	2.919986
P(T<=t) two-tail	0.245003	P(T<=t) two-tail	0.128328	P(T<=t) two-tail	0.105573	P(T<=t) two-tail	0.005893
t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653	t Critical two-tail	4.302653

Appendix 21: List of the definition of the terminologies

Micronutrients- This are essential trace metals which are required in trace amounts by plants, animals and human beings.

African Spider plant- This is an indigenous traditional vegetable popularly known as Chinsaga in Kisii community and Sagek in Kalenjin community among other distinct names from various communities.

Trace metal- This are elements which are available in soils, plants, animals and human beings in minute amounts.