UNIVERSITY FOR DEVELOPMENT STUDIES

YIELD, NUTRITIONAL QUALITY AND HEAVY METALS TOXICITY IN ROSELLE (*Hibiscus sabdarrifa* L.) AND JUTE MALLOW (*Corchorus olitorius* L.) CROPS AS AFFECTED BY TWO COMMERCIAL COMPOSTS AND CHICKEN MANURE.

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BY

ABUBAKARI MUTARI

STUDENT NUMBER: UDS/DPHP/0001/13

THESIS SUBMITTED TO THE DEPARTMENT OF HORTICULTURE, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (POSTHARVEST TECHNOLOGY) DEGREE

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Student

I hereby declare that this thesis entitled "Yield, nutritional quality and heavy metals toxicity in roselle (*Hibiscus sabdarrifa* L.) and jute mallow (*Corchorus olitorius* L.) crops as affected by two commercial composts and chicken manure" is the outcome of my own original effort and has not been published wholly or partly anywhere as a research output or presented in any institution for academic award. However, published works carried out by other authors have been consulted, cited and duly acknowledged in this study.

Candidate's signature………………………… Date...

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Supervisors

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of dissertation/thesis laid down by the University for Development Studies.

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Name: ………………………………………..…………………………………………..

Head of Department Signature …………………………………Date ……………………………

Name ……………………………………………………………………………………….

ABSTRACT

Field and laboratory experiments were conducted to determine the effect of different compost formulations on yield, nutrient status, and possible heavy metal toxicity in roselle (*Hibiscus sabdarrifa* L.) and jute mallow (*Corchorus olitorius* L.). The vegetable crops were cultivated on an upland field (Latitude 09^{o} 25" N, Longitude 00 ^{o} 58 " W) with an Altitude of 183 m above sea level consisting of the Kpalsawgu soil series, located at Changnaayili in the Northern Region of Ghana. The different compost formulations were DeCo (decentralized compost), ACARP (Accra Compost and Recycling Plant), CDLCM (composted deep litter chicken manure) and the control to which each of the two test-vegetable crops was assigned to as treatments. These treatments were then randomly distributed in each of four replications for the cultivation. Randomized complete block design (RCBD) was used for the experimental field where data was collected on growth parameters, chlorophyll content (using the SPAD meter), and leaf yield. Leaves of sample crops were harvested and shade-dried, sent to the laboratory for the nutrient status analysis. They were subsequently analyzed for the presence and concentration of heavy metals using the Atomic Absorption Spectrophotometer (Buck Scientific Model 210 VGP). Prior to this, the same test was done earlier on the resident soil series and on the different compost formulations. Residual levels of heavy metals (Cd, Pb, Zn, and Cu) found in the sample leaves were then compared with maximum residue levels. Completely randomized design was used for analysis of data obtained from the laboratory experiments. All data were analyzed using the Analysis of Variance (ANOVA) technique and the GENSTAT statistical program (9th edition). Least Significant Difference (LSD) at 5 % probability level was used to determine treatment differences. Heavy metals and some chemical constituents were naturally inherent in the soil of the Kpalsawgu soil series, but at safe levels; and as well contained in ACARP, DeCo and

CDLCM composts but in varying concentrations/rates. Application of compost resulted in leaf-yield increase as compared to the control where CDLCM gave the highest leaf-yield. Dried leaves of roselle and jute mallow contained protein and minerals; they also contained Cu, Zn, Cd, and Pb but concentration levels of Pb and Cd were above recommended maximum residue levels reported by FAO and the European Commission and so considered a risk to the consumer (adults and children). It is recommended that soils be analyzed for the presence and concentration of heavy metals prior to cultivation of leafy vegetables (hyperaccumulators of heavy metals) so that the soil does not become a potential recipe for the bio-transfer of heavy metals to edible parts of the plant. Compost, and for that matter any soil amendment should be analyzed for the presence and concentration of heavy metals prior to application i.e. in situations where this requirement is not displayed on the compost package. ACARP, DeCo and CDLCM composts should be subjected to corrective reformulation to become soil amendments that are either free of heavy metals or at least to contain heavy metals in the range below or at recommended maximum residue levels.

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DEDICATION

I dedicate the work to my family and friends for the encouragement and backing during my research work.

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LIST OF ACRONYMS

ACARP – Accra Compost and Recycling Plant

ATSDR – Agency for Toxic Substances and Disease Registry

CDLCM – Composted Deep Litter Chicken Manure

DeCo – Decentralised Compost

FAO – Food and Agriculture Organisation

GNA – Ghana News Agency

MoH – Ministry of Health

USCC – United States Composting Council

USDA – United States Department of Agriculture

USEPA – United States Environmental Protection Agency

WHO – World Health Organisation

CSIR – Council for Scientific and Industrial Research

SARI – Savanna Agricultural Research Institute

AAS - Atomic Absorption Spectrophotometer

IWMI – International Water Management Institute

UDS – University for Development Studies

UNEP – United Nations Environment Programme

CHAPTER ONE

INTRODUCTION

1.1. Background

For normal functioning of the body, the human as a right requires food that is safe and of a high quality. The term food safety according to the FAO/WHO (1999), can be defined as "the assurance that food will not cause harm to the consumer when prepared and/or consumed according to its intended use''. A food item becomes unsafe for consumption when there is the presence of food hazard in it. The hazards may be physical, chemical or biological. Physical hazards are tangible materials such as broken bottles, twigs etc. found in food with the potential to cause harm to the consumer. Biological and chemical hazards cannot be observed easily by the consumer unless through laboratory examination or when a consumer shows signs and symptoms of illness resulting from the consumption of such an unsafe food. Biological hazards occur due to the presence of pathogenic microorganisms such as bacteria, viruses, and fungi in food (Johnny, 2006). Sources of chemical hazards, which include the incidence of pesticides and heavy metals in food, have lingering or chronic effects on the consumer. Because the residues are deposited into vital organs of the body, their effects are manifested after a prolonged period in the living organism. These hazards come about as a result of poor or improper production and produce handling practices. (FAO/WHO, 1999).

There has been an upswing consumption of vegetables among the growing urban and peri-urban populations as a result of the rise in diet-related illnesses making vegetable consumption a better alternative to diet management and healthy living. With the globalization of the horticulture industry involving trans-border movement of food and other horticultural products, food safety concerns are on a high alert especially on minimally processed foods such as fresh-cut fruits and vegetables. This has stimulated the development of food safety measures and standards (both public and private) to regulate the production and supply of food items from one country to another (Passam *et al*., 2007; Gereffi and Lee, 2009). Recently, in Ghana, a food safety policy was drafted to safeguard the production of food and food products for domestic and international markets (MoH, 2013). Therefore, vegetable production has to conform to these hygiene and safety guidelines in order to ensure that the produce are safe for human consumption.

Swift growth in the urban areas does not only result in challenges with regards to settlement planning and handling of food security concerns but also creates challenges related to waste management e.g. management of municipal solid waste (MSW). In a study by Dreschel and Kunze (2001), it was reported that in order to address waste management challenges in the urban areas, MSW can be recycled into compost for use by urban and peri-urban vegetable gardeners. As regards MSW generation in the urban areas, Annepu and Themelis (2013) reported that a human population of 3.9 million generated about 2,000 tons of municipal solid waste (MSW) per day in 2008. To manage such volumes of waste, the Accra Metropolitan Assembly (AMA) spent 82% of the Assembly's budget to manage that much MSW. To make good of the MSW generated in Ghana, the Zoomlion Company commissioned the Accra Compost and Recycling Plant (ACARP), in 2011 and started the commercial production of compost (ACARP compost) for use especially by vegetable farmers. Similarly, a Company based at the outskirts of Tamale (Decentralised Composting company) recycles MSW generated in and around the Metropolis into commercial compost (DeCo compost) for used by farmers within the Tamale Metropolis and surrounding communities for vegetable crop production especially during the dry season (DeCo, 2010; Kranjac-Bersavljevic and Gandaa, 2013).

According to Beecher (2009), recycling of organic waste for use as a soil amendment minimizes its subsequent handling and disposal problems. It is indicated that if such soil amendments are meet quality and safety standards, they will serve as a better substitute to inorganic fertilizer since it is demonstrated that organic fertilizer does not have detrimental effect on the environment. In Ghana, organic waste are normally applied to the soil as a fertilizer either in a raw form or in some cases after being composted. Examples of such organic waste include sewage sludge, poultry droppings, municipal solid waste, and cow dung etc. A report by the USCC (2008), indicated that the application of organic waste to the soil as an amendment helps to improve the soil texture which in turn impacts the structure, creates a good atmosphere for root growth and development, improves water holding capacity of the soil, supplies nutrients, stabilizes soil pH, and binds or degrades some potentially toxic elements such as lead and cadmium.

According to Grubinger (1999) soil conditioners such as wood ash, lime, and compost contain a number of elements in them which are likely to improve the soil physical properties. Apart from conditioning the soil, compost also serves as source of feed for some organisms in the soil.

Some organizations including the Water, Sanitation and Hygiene (WASH) Alliance have encouraged the use of organic fertilizers including compost prepared from sewage sludge for vegetable production (AVRDC, 1998; Kranjac-Berisavljevic and Gandaa, 2013). Organically-produced vegetables are considered safe and their consumption is reported to improve mental alertness in children, and serves as precursors of natural antibiotics. However, organic soil amendments should be free of pathogens to avoid infection of otherwise healthy produce. (Worthington, 2001; Norwegian Scientific Committee for Food Safety, 2014; Simonne *et al*., 2016).

Amlinger *et al*. (2004) in a report stated that, the application of organic waste as soil amendment may not simply signify a major source of nutrients but could likewise be a probable source of heavy metals. Crops, particularly leafy vegetables may accumulate comparatively higher amounts of these heavy metals. With the consumption of vegetables containing these heavy metals, the heavy metals are often deposited in vital organs such as the liver and kidney of the human body causing chronic health problems. The attendant hazard related with this, is the quantity of the heavy metals in the plant and the recurrence and length of utilization of such vegetables. To assess the risk associated with vegetable consumption, Sarpong *et al*. (2014) evaluated thirteen (13) commonly consumed vegetables (lettuce, carrot, garden eggs, tomato, pepper, onion, green pepper, cabbage, French beans, spring onion, sweet potato, cucumber and okra) in seven (7) different markets (Kumasi, Asante-Mampong, Obuasi, Nkenkesu, Akomadan, Daboase and Nobekwaw) in the Ashanti region of Ghana. They detected that Pb, Fe, Zn, and Cu were present as residues in the vegetables with Pb and Fe having its concentrations above the FAO/WHO Codex Alimentarius Commission Maximum Residue Levels.

1.2. Problem statement

Vegetable production in Ghana, vegetables is usually carried out in backyard gardens, market gardens and truck farms delivering fresh produce to urban, semi-urban and peri-urban communities, and even for export. According to Kamran *et al*. (2013) however, many of the soils in which these vegetable crops are cultivated have been found to contain potentially toxic elements. This is because, these vegetable production sites are usually sited close to motorable roads and busy streets, where the vegetable crops are exposed to vehicular emissions (Drechsel and Keraita, 2014). Moreover, vegetable crops produced from these sites are most frequently watered with sewage, which typically contains high levels of heavy metals and infectious microorganisms. Such vegetable crops are also commonly fertilized with cow dung, sheep droppings, goat droppings, pig droppings, sewage sludge, and poultry droppings. This production system is made worse with the abusive use of pesticides, herbicides, and in some cases fungicides; all of which may result in the bioaccumulation of potentially toxic elements in the production environment (IWMI, 2008).

1.3. Justification

Some studies have been conducted by Obuobie *et al*. (2006), Sarpong *et al*. (2014) and Drechsel and Keraita (2014) on the microbial contamination and heavy metal residues in vegetables including roselle and jute mallow produced locally in some areas and under different contexts in Ghana . For the research conducted by these scholars, samples of some vegetable produce were taken from different market centers without the assessment of the conditions under which they were produced. The investigators did not also conduct tests to determine the presence and concentration of potentially toxic elements (PTE) inherent in the soils, the inorganic and/ organic fertilizer that could be used as soil amendments, or even in eatable portions of the vegetables considered. In order to accomplish these unanswered concerns, the present study sought to scrutinize the concentration of heavy metals in the soil at the experimental site. This was done also for the organic soil amendments (Deco compost, Composted Deep Litter Chicken Manure and ACARP compost), and in the leaves of jute mallow and roselle. Based on the outcome of this process, the study determined the safety of consuming vegetables (roselle and jute mallow) produced with the different organic soil amendments by conducting risk analysis in the two vegetable crops.

Therefore, the fundamental goal of this investigation was:

To determine the safety of jute mallow and roselle cultivated under different organic soil amendments.

The specific objectives were:

1. Determine the presence and concentration of selected heavy metals (Cd, Pb, Zn, and Cu) and other chemical elements (pH, N, P, K, and C) inherent in soils of the Kpalsawgu soil series.
2. Determine the presence and concentration of selected heavy metals (Cd, Pb, Zn, and Cu) and other chemical elements (pH, N, P, K, and C) inherent in composted deep litter chicken manure (CDLCM). Decentralized compost (DeCo), and Accra Compost and Recycling Plant (ACARP) compost.
3. Determine the effect of DeCo compost, ACARP compost and CDLCM and the inherent soil chemical elements (N, P, K, C, and pH,) on the growth, yield and nutrient content of jute mallow and roselle.
4. Determine the presence and selected heavy metals (Cd, Pb, Zn, and Cu) concentration and other chemical elements in leaves of jute mallow and roselle.
5. Compare residual levels of Cd and Pb found in jute mallow and roselle leaves with Maximum Residue Levels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Definition of heavy metals

Heavy metals are generally described as a group of metals and semi-metals that are linked to the contamination of the environment by causing toxicity to the soil, water and air (SEPA, 2001; Khan *et al*., 2008). In nature, they occur in small quantities but at when their concentrations increase, usually due to human activities, they are considered major contaminants in the environment as they pose risk to plants and animals (Sao *et al*., 2014). However, there is no single definition of heavy metals considered as universal. They have been defined differently by different scholars. Accordingly, some scholars classify heavy metals based on their elemental densities thus; elements with densities above 7g/cm^3, 6g/cm^3, 4.5g/cm^3 or 4g/cm^3. Others also classify them based on their atomic numbers thus; elements with atomic numbers greater than 20 while others classify them as elements that are toxic to animals and other living organisms (Duffus, 2002).

The underlining perception about heavy metals is that they are toxic to plants, animals and to the environment. However, their ordinary occurrence in the environment does not certainly result in their being toxic except in high concentrations. The occurrence of essential heavy metals in minute amounts in the growing medium could result in deficiency symptoms in plants. The symptoms could be thinner or shorter internodes and chlorotic flecks on older leaves (McCauley, 2011). When they are present in sufficient quantities, they ensure optimum plant growth and development. In view of this, Yruela (2005) indicated that copper activates enzymes in plants and helps in improving the flavour in vegetables and enhancing the colour of flowers. However, when heavy metal concentration in the soil is high, it results in growth reduction and changes in physiological processes leading to exhibition of necrotic symptoms in plants (Girisha and Ragavendra, 2009; Appenroth, 2010). Further to this, Pandey and Tripathi (2010) indicated that the concentration of cadmium, arsenic and lead at concentrations between 1- 10 ppm significantly reduced chlorophyll content, crude protein, amino acids and soluble sugars in plant leaves. Also, Sharma and Dubey (2005) indicated that, excess lead causes stunting, chlorosis and blackening of root in plants.

2.3. Essential and non-essential elements (heavy metals)

Depending on their functions in biological processes, heavy metals are regarded as diverse elements that can either be described as essential or non-essential. Essential heavy metals are not toxic to living organisms but serve as co-factors of enzymes in metabolic processes. They are therefore required for normal metabolic processes and well-being of living organisms (Arinola *et al*., 2010). When these essential elements are displaced from their protein carriers in the living cell, the living organism exhibit deficiency disorders (Theron *et al*., 2012). Examples of essential elements include iron (Fe), manganese (Mn), cobalt (Co), copper (Cu), molybdenum (Mo), zinc (Zn), and nickel (Ni). However, when the concentration of these essential elements reach above normal levels, they cause toxicity in the organisms.

Non-essential heavy metals on the other hand are toxic to living organisms even at low concentrations as they are not metabolized these organisms (Lenntech, 2016). When these elements are ingested into the body, they are deposited into vital organs in the body such as the liver or kidney and persist for a longer time and damaging these organs. Among these elements are Cd, Pb, As, and Hg (Rascio and Navari-Izzo, 2011). Lead and Cd are rated among six of the most toxic chemicals in the world with about 26 million people at risk of exposure to Pb and 5 million people at risk of exposure of Cd (Pure Earth and Green Cross, 2015).

2.3.1. Cadmium (Cd)

Cadmium in the pure and unadulterated form, is a soft and silver-white element. It is found primarily in the soil together with zinc, copper and lead. In the manufacturing industry, Ii is utilized in the making of batteries, stabilizers for plastics, coating and plating as well as in photovoltaic devices. Cadmium can be released into the soil, air and water bodies through industrial activities such as non-ferrous metal mining, refining, manufacturing of phosphate fertilizer, and combustion of fossil fuel and incineration of solid waste (ATSDR, 2012).

2.3.1.1. Cadmium sources

Natural sinks and human activities are the two major sources of cadmium into the environment. The occurrence of cadmium from natural processes into the environment comes from volcanic activity and weathering of rocks while its contribution from human activities emanate from manufacturing of phosphate fertilizers, exploration of fossil fuel, treatment and recycling of other heavy metals (e.g. zinc and copper), incineration and application of municipal soil waste or sewage sludge into the soil and water bodies (UNEP, 2010).

According to the Nordic Council of Ministers (2003), most of the cadmium deposited into the environment remains in the top 0-20 cm of the soil thereby increasing its availability for plant uptake. A similar report by UNEP (2011) indicated that, the presence of cadmium in fertilizers and their atmospheric deposition in certain European countries was causing the concentration of cadmium in the topsoil to surge. The accumulation of Cd in the soil is contingent on the properties of the soil, with heavy (clay) soils generally holding more cadmium than light (sandy) soils. According to the WHO (2007), the main route of exposure to cadmium is either through food or smoking. However, for non-smokers the main route of exposure to cadmium (more than 90%) is through food consumption. The following food items contain nearly 0.05–0.12 mg cadmium/kg: lettuce, spinach, potatoes, rice, peanuts, soybeans, sunflower seeds, meat and fish (ATSDR, 2012). For meat products, the Food Safety Authority of Ireland (2009) indicated that cadmium can be in high levels in the offals (kidney and liver) of mammals and in mussels, oysters and scallops.

2.3.1.2. Fate of cadmium in soils

The average concentration of cadmium in surface soils in various parts of the world ranges from 0.1 -0.5 ppm (ATSDR, 2012). The availability of the element in the soil however relies on several factors. These includes the soil pH, soil organic matter content, content of hydrous metal oxide, and antagonism from other metals (International Cadmium Association, n.d). Soils that are derived from metamorphic and igneous rocks tend to have lower concentration of cadmium and ranges between 0.02 ppm and 0.2 ppm. Soils derived sedimentary rocks on the other hand, have much higher concentrations of Cd ranging between 0.1 ppm and 25 ppm (Tran and Popova, 2013). When cadmium concentration goes beyond 0.5 mg/kg, it is attribute to anthropogenic activities resulting in the surge. A study by Biernacka and Maluszynski (2006) found that the cadmium content in soils as a result of human activities ranged between 8.0 – 64 mg/kg. For soils that are derived from anomalous parent materials, the concentration of cadmium can reach 30 mg/ kg (WHO, 2007).

Cadmium and its compounds can be itinerant in the soil medium, but their mobility is depends on several factors such as the soil pH and organic matter content. These factors however vary from one environment to the other. Generally, cadmium has a strong affinity to organic matter and binds strongly to it, where it will be immobile in the soil. However, as the soil conditions change in favour of its solubility, it can be translocated into the plant and eventually enters the food chain. Some authors have linked the mobility of cadmium in the soil to cation exchange (de Matos *et al*., 2001; Kabala and Singh, 2001; Vanek *et al*., 2005).

2.3.1.3. Cadmium and plant health

Reports have revealed that that plants that have been grown in cadmium-contaminated medium expressed oxidative stress at the early stage mainly during germination and plant development (Perez-Chaca *et al*., 2008). Residues of cadmium in leaves of active plants could result in accumulation of reactive oxygen species (ROS) which is coupled with alterations in biomolecule contents. Transportation of Cd from the stems to ears of cereal crops through phloem and xylem plays a significant part in Cd translocation. Also, the microclimate around the developing spikes could influence the transport of Cd to the developing grains (Farid *et al*., 2013). According to Skrebsky (208), Cd stress can affect the uptake of other minerals such as manganese. The ability of a plant to detoxify Cd in the soil can vary between and within the plant species. Some plants have the ability to alter the pH around the rhizosphere depending on how high or low the concentration of the heavy metal is and this affects the mobility of the heavy metal (Dong *et al*., 2007). Cadmium is ranked seventh among the 20 toxic elements that affect the human health through the food chain. A report by Chaney (2012) indicated that, cadmium is the most potentially toxic heavy metal found in the food chain. Though Cd can cause phytotoxicity, plants can accumulate high concentration of it enough to pose risk to humans yet will not show signs of phytotoxicity. The report added that, Cd could accumulate in healthy plants for more than 50 years exposing humans to chronic dietary risk. It recommended therefore that, strategies should be adopted to reduce its concentration in soils and soil amendments as much as possible. Plants growing on soil contaminated with Cd easily uptake the Cd by their roots and transport it to above plant parts. The increased concentration of Cd in the plant may result in retardation in growth, reduced stem and root dry weight (Chaturvedi, 2004).

2.3.1.4. Cadmium and human health by route of exposure

The consumption of food containing high concentration of cadmium can cause disruption in calcium metabolism in the body; cause dysfunction of the kidney and can lead to the formation of kidney stones. Populations that are living and /or working in cadmium-contaminated areas are predisposed to the softening of the bones referred to as “osteomalacia” and bone fragility known as “osteoporosis”. Inhaling high amount of cadmium oxide fume is considered fatal and can result in acute pneumonitis with pulmonary oedema (a condition characterized by an excess of watery fluid collecting in body tissues or cavities). Long-term and high exposure to cadmium is associated with changes in lung functions, primarily characterized by chronic obstructive airway disease (ATSDR, 2011; ATSDR, 2012).

Available evidence seeks to suggest that exposure to cadmium over a long period (e.g. through inhalation of cadmium fume) contributes to the development of lung cancer. However, there is little evidence to show that cadmium may also cause cancers of the kidney and the prostate. According to the International Agency for Research on Cancer (IARC), cadmium together with cadmium complexes are classified among Group 1 toxic compounds (carcinogenic to humans) and that implies sufficient evidence for their carcinogenicity (WHO, 2010).

According to Kaji (2012), during the 1930s, chronic exposure to cadmium led to the discovery of the itai-itai (ouch-ouch) disease in Japan which was characterized by weakening and deformation of bones, anaemia, kidney failure and death. Though the element itself and some of its compounds including cadmium carbonate, cadmium selenide and zinc cadmium sulfide are insoluble in vivo, others such as cadmium oxide, cadmium chloride, cadmium sulfide and cadmium sulfate are soluble in vivo. This makes them more toxic to living organisms as the free metal ions interact with body tissues (National Academy of Sciences, 1997; Pure Earth, 2015).

2.3.2. Lead (Pb)

Lead can be described as "a heavy, low-melting, bluish-grey metal that occurs naturally in the earth's crust". Within the periodic table, lead can be found in group IV and period 6 of the table. The atomic number for Pb is 82, atomic mass of 207.2, density of 11.4 g cm^{-3}, melting point of 327.4°C, and boiling point of 1725°C (Duffus, 2002). Naturally, lead is rarely found in isolation as a metal but forms compounds with other elements (ATSDR, 2007). Some of the elements it combines with to form compounds include Sulphur to form lead sulfide (PbS); sulphate to form lead sulphate ($PbSO_4$); oxygen to form lead oxide (Pb_3O_4) or nitrate to form lead nitrate ($Pb(NO_2)_2$) (Kenneth, 1995) . The lead compounds are of two categories – soluble and insoluble lead compounds. The soluble compounds include lead acetate, lead chloride and lead nitrate while the insoluble ones include lead arsenate, lead bromide, lead fluoride and lead sulphate (HSDB, 2016). Lead can also be categorized into inorganic (as in paints, soil, and exhaust from gasoline) and organic (leaded gasoline) with the former more prevalent in the environment (ATSDR, 2012).

2.3.2.1. Sources of Pb

Lead can be found in different habitats of the environment including the air, soil, water, and even in homes. For the most part, the introduction of animals to lead originates from human exercises including the utilization of non-renewable energy sources, for example, leaded gas, a few kinds of modern activities, and the use of toxic paint in homes. (USEPA, 2015).

a. Industry

In industrial processes, lead can be found in solders/pipes, lead-containing vessels used as storage containers, lead-acid batteries, cosmetics and electronic waste, mining, smelting, and leaded gasoline (WHO, 2010; ATSDR, 2012).

Lead ranks fifth in industrial production of metals after Fe, Cu, Al, and Zn. Generally, Pb usage is more prevalent in the manufacture of storage batteries. However, there are other uses such as in the making of solders, ammunition, bearings, plumbing materials, cable covers, pigments, and caulking (sealant for waterproofing of joints). In industry, Pb is usually combined with one or more different elements to form alloys and used for various purposes. Some of the Metals commonly alloyed with Pb are antimony (Sb), silver (Ag), tin (Sn), calcium (Ca), and strontium (Sr). These alloys are commonly used in storage batteries; for sleeve bearings, printing, in maintenance-free storage batteries; for solder and anodes in electro winning processes; and high-detail castings (Bureau of Indian Standard, 1992; O'Donnell and Finin, n.d; Torres *et al*, 2012).

Ionic lead, lead oxides, lead hydroxides and lead metal oxyanion compounds are the types of Pb that are discharged into or sully the soil, groundwater, and surface water. The most well-known types of lead are Pb (II) and lead-hydroxy groups. Lead (II) is the most receptive and normal mononuclear and polynuclear oxides and hydroxides that can be formed. It is injurious to human even at trace amounts (Edokpayi *et al*., 2015). The principal insoluble Pb compounds that form scale in distribution systems are lead carbonates, lead phosphates, and lead hydroxides, (Maynard, 2008). The most steady type of Pb in the soil grid is lead sulfide (PbS) and this forms under reducing conditions, when the level of sulfide is high (Hem, 1976).

Compounds of Pb (II) are mostly ionic (e.g., Pb^{2+} $SO_4{}^{2-}$), while that of Pb (IV) are mostly covalent (e.g., tetraethyl lead, Pb $(C_2H_5)_4$ (NIOSH, 1994). Some compounds of Pb (IV) are strong oxidants e.g. PbO_2. Lead forms several basic salts, such as Pb $(OH)_2$. $2PbCO_3$, which was considered as a source of protracted poisoning in children because it was commonly used in the manufacture of stain in white paint and children, ate the peeling of the white paint. Several compounds of Pb (II) and a small number of Pb (IV) compounds on the other hand, are beneficial. The most widely recognized of these compounds are lead dioxide and lead sulphate, which are utilized in the reversible retort that occurs during the charging and discharging of lead storage battery (Water Research Foundation, 2012).

Apart from the inorganic lead compounds, there are a quantity of organic compounds of lead such as tetraethyl lead tetramethyl lead (WHO, 2001). They are lead compounds formed by direct binding of lead to a carbon atom and used to be the major source of environmental lead as they were used as fuel additives for anti-knock purposes.

a. Food

There are different ways by which lead enters the food chain. The commonest is from a contaminated soil where crops are grown and the lead in the soil is transported from the roots into edible parts of the plant. Cereals crops can contain high levels of lead while spice crops can be contaminated with lead (EFSA, 2010). The amount of lead taken up by plants is reliant on its level in the soil and this is highest around mines and smelter sites. The use of food and beverage cans soldered with lead may considerably increase the lead content of the food or the beverage, especially in the case of acidic foods and/or drinks. Additionally, the use of any product that contains lead in the manufacture, distribution or storage of alcoholic drinks tends to increase their lead contents, since they are acidic. Migration of lead into food contained in lead-glazed pottery ware or ceramic ware is also a source of exposure to lead. Tobacco contains high amounts of lead and its smoking increases the intake of lead into the body (WHO, 2010). Lead contamination in food is a serious concern in some developing countries as there are no proper food safety standards, monitoring and recall procedures to protect the consumer from consumption of lead-contaminated foods. In the developed countries where these standards and procedures are in place, several product recalls have been made on imported foods containing unsafe levels of lead in them (Giblin, 2010).

2.3.2.2. Fate of Pb in soil

Naturally, the typical concentration of lead in the soil ranges from below 10 mg/kg soil up to 30 mg/kg soil. The concentration of lead in the top layer of soils varies considerably and decreases with soil depth. The concentration results from the accumulation of lead particulates deposited as a result of anthropogenic activities (WHO, 2007). The most important cause of exposure to soil lead especially for children is from through consumption (eating) of soil or dust contaminated with lead. Generally, the uptake and translocation of lead from the soil is low in vegetables compared to cereals and grains. Some reports have discovered that lead does not accumulate freely fruit vegetable such as tomatoes, beans, squash, strawberries, and fruit crops such as apples. However, leafy vegetables tend to have higher concentrations, particularly due to air-borne deposition and on the root surface of crops such as carrots which are in direct contact with the soil. Since plants do not take up huge amounts of lead from the soil, the lead concentration in the soil are regarded as nontoxic for crop production. Nevertheless, higher levels of lead will be of concern in areas where the consumption of soil is common. Usually, it has been considered harmless to consume vegetables grown in gardens with total lead concentration in the soil of between 300 - 600 ppm (Wander, n.d.; Cornell University, 2012).

2.3.2.3. Pb and plant health

The concentration of lead at elevated level in the soil has detrimental consequences on the growth and development of the plant. Hussain *et al*. (2013), considered the effects of lead nitrate $Pb(NO_3)_2$ on maize growth and development and concluded that, increased amounts of the compound (lead nitrate) resulted in reduced percentage germination and seedling growth;

reduced root-shoot length; reduced fresh and dry weight of root, shoot and reduced total protein content. In a related study conducted by Kabir *et al*. (2010), it also established that, increased amounts of Pb resulted in reduced percentage germination and seedling growth; reduced root-shoot length; reduced fresh weight and dry weight of root and shoot and a reduction in the leaf area as well as the number of leaves of *Thespesia populnea* L. With respect to phytotoxicity, Sharma and Dubey (2005) indicated that, excess Pb could result in stunted growth, chlorosis and blackening of roots. They also indicted that photosynthesis as well as nutrient and water balance may be inhibited.

2.3.2.4. Pb and human health by route of exposure

The two main routes of exposure to human are through inhalation from the atmosphere and by ingestion of food but the effects from both sources are the same. Exposure of a population to lead can cause a widespread of biological effects depending on the amount and length of exposure. Lead accumulates in vital organs (heart, kidney and brain) of the body, and at elevated levels can cause poisoning or even death. The gastrointestinal tract, the kidneys, and the central nervous system can also be damaged with exposure to lead. Several effects ensue over a wide range of doses, with the developing babies and infants being more sensitive than their adult counterparts. Studies have shown that children are more at risk of lead exposure, with those below the age of six being at a more substantial threat. As a result of this, they suffer retarded development, lower Intelligent Quotient (IQ), reduced attention time, hyperactivity, and intellectual decline. For adults, the consequences of lead exposure include decreased in response time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints (EFSA, 2010; Subothini, 2010). It is however rare to experience severe illness due to lead poisoning. Lead does not perform any known metabolic function in the body. It is particularly a hazardous chemical, as it can accumulate in individual organisms, but also in the entire food chain.

2.3.3. Zinc (Zn)

Zinc is one of the most well-known elements found in the soil and occurs at a normal concentration of 70 mg/kg (USEPA, 2005). It is a bluish-white, glossy metal in its unadulterated basic (or metallic) form. It is for all intents and purposes, it is insoluble in water with a melting point of 419.5 °C and a boiling point of 908 °C. Zinc is unstable in the powdered form and may blast into blazes whenever kept under soggy conditions (ATSDR, 2005).

2.3.3.1. Sources

In the environment, zinc is found in the soil, water, and air, as well in some foods. Human contribution of zinc into the environment emanate from metal smelters, mining activities and other industrial waste (USEPA, 2005). Zinc input into the soil can also be from the application of certain inputs in crop production such as livestock manures, inorganic fertilizers, sewage sludge and fungicides (Alloway, 2008).

2.3.3.2. Fate of Zn in soil

All soils contain some amount of essential and non-essential elements (heavy metals) including zinc, the concentration of which vary under different soil conditions but on an overall average of 55mg/kg (Ayari *et al.,* 2010). In the soil, zinc is found in different fractions i.e. water-soluble fraction, exchangeable fraction, organically bound fraction, sorbed non-exchangeable fraction and primary mineral pool of weathered materials (Alloway, 2008). Zinc ions that are usually held onto clay and organic matter particles by chelation making them immobile in the soil. Their mobility in the soil and availability to plants therefore depends on the soil texture, the soil pH, soil phosphorous and weather conditions (Schulte, 2004).

2.3.3.3. Zn and plant health

Because Zn is essential in plant growth, its limitation results in Zn deficiency symptoms manifested as thinner and shorter internodes, twisting of leaf borders upwards, and uneven distribution of chlorotic flecks on older leaves (McCauley, 2011). In a study conducted by Kaya and Higgs (2001), the deficiency of zinc in tomato was corrected by the application of foliar Zn at 23 mg L^{-1}. On the other hand, the toxicity of zinc in tomato may be ameliorated by spraying with foliar fertilizers containing P and Fe (Kaya and Higgs 2002).

2.3.3.4. Zn and human health

Zinc is one of the essential elements required for human well-being and its absence in the body leads to deficiency characterized by retardation in growth, loss of appetite, impaired immunity, hair loss, eye and skin lesions, delayed sexual maturation and impotence (Yanagisawa, 2004) which are more of a risk to human than its intoxication (Plum *et al*., 2010).

2.3.4. Copper (Cu)

Copper is a reddish metal that occurs naturally in the earth's crust at an average concentration of 50 parts copper per million parts soil (ppm). It is found in rocks, soil, water and at low levels in the air. It also occurs naturally in all plants and animals. It is an essential element for all known living organisms including humans and other animals. At much higher concentration, copper becomes toxic to living organisms (ATSDR, 2004).

2.3.4.1. Sources of copper (Cu)

Copper is found naturally in both plants and animals, and at high concentrations in oysters and mussels, which are described as filter feeders. It can also be found at various concentrations in many foods and beverages (ATSDR, 2004). Its input to the soil can be attributed to Cu –

containing fertilizers such as copper sulphate, cupric oxide and fungicides such as Bordeaux mixture (Schulte and Kelling, 2004).

2.3.4.2.* Fate *of Cu in soil

Similar to zinc, copper in the soil can be found in different fractions as exchangeable fraction, weakly bound fraction in organic matter, carbonate-bound fraction, manganese oxide-bound fraction, specifically bound-fraction in organic matter and mineral bound fraction (Xiarong *et al*., 2007). The available copper is held as a cation to clay mineral and organic matter surfaces. However, its availability to plants largely depend on the pH of the soil and organic matter content (Schulte and Kelling, 2004).

2.3.4.3. Cu and plant health

. Copper as a co-factor of metallo-proteins, activates some enzymes in plants that are part of the synthesis of lignin. During the process of photosynthesis in plants, it is required in respiration and assists in metabolism of carbohydrates and proteins. Copper also serves to increase the intensity of flavour and color in vegetables as well as in flowers. Copper in excess can have negative effects on the plant such as growth retardation and impairment of cellular processes. When copper toxicity is high, it can lead to reduction in branching and eventual decline in plant growth if not corrected (Yruela, 2005; PROMIX, 2016). Martins and Mourato (2006) studied the effect of excess copper on the growth of tomato and found that, higher concentration of copper in the growing medium restrict root growth, decrease leaf area and dry matter content. The study also found that, high levels of copper compete with plant uptake of iron, calcium and zinc. In a related experiment, Sheldon and Menzies (2005) established that excess copper concentration damages the roots of Rhodes grass by disrupting the root cuticle, reducing the growth of root hair and causing deformation of the root structure.

2.3.4.4. Cu and human health

In the adult, the concentration of copper ranges from 1.4 – 2.1 mg/kg body weight. Though the value is small, it is enough to maintain an essential well-being of the body (Copper Development Association Inc. 2016). Both the lack and excess of copper in the body are considered detrimental for normal functioning. Though Cu deficiency or toxicity is rare in humans, its inadequacy or otherwise in the body is associated with the onset of abnormalities in neurological processes, connective tissue, skeletal and vascular systems (Ward *et al.,* 2003; Desai and Kaler, 2008). Watts (1989) indicated that due to its role in enzymatic activity, Cu deficiency and excess could be associated with type I and type II osteoporosis respectively. Its deficiency is also linked to cardiovascular defects and orthopaedic disturbances. Generally, the toxicity of copper is dependent on the absence of antagonistic elements such Cd, Zn, Mo, Pb, Fe, as well as vitamins B and A.

2.2. Sources of Heavy Metals

The major sources of heavy metals include the soil, fertilizer (organic and inorganic), pesticides and vehicular emissions.

2.2.1. Soil

Heavy metals are naturally occurring elements in the soil environment due to processes of soil formation from weathering of parent materials. Their occurrence are in minute quantities that are considered as *trace* (<1000mg kg^{-1}) and are rarely toxic (Kamran *et al*., 2013). Due to human activities which results in the the disturbance of the soil and the acceleration of nature's slowly occurring geochemical cycle of metals, most soils of rural and urban environments may accumulate one or more of the heavy metals especially the non-essential ones. Their accumulation may increase above some threshold values high enough to cause risks to humans, animals, plants, or the ecosystem (USDA, 2000). The rate of generation of the heavy metals becomes so rapid because of human activities relative to the natural inputs. Eventually, they become contaminants in the soil environment (Singh *et al.*, 2011). They are transported from one location to the other where higher the potential for direct exposure exist. For instance, mercury, lead and cadmium travel long distances before being deposited (Dinis and Fiuza, 2011). However, their bioavailability and toxicity depends on the form (organic, extractable or residual) in which they are deposited. Aikpokpodion *et al.*, (2012) indicated that Cu and Zn in their organic form are more bioavailable for plant uptake while Pb in the extractable form is more bioavailable and Cd is more bioavailable in the residual form.

Soils that originate from igneous rocks have Cd concentrations of between 0.1 – 0.3 mg/kg while those originating from metamorphic rocks contain between 0.1 – 1.0 mg/kg and those from sedimentary rocks contain between 0.3 – 11mg/kg. Around the world, Nickel is found in soils at an average concentration of 20 mg/kg. Clay-rich soils contain above median concentrations of Cr and Ni (Alloway, 1995).

2.2.2. Fertilizers

Agriculture is considered the first major historical human activity affecting the soil (Wuana and Okieimen, 2011). Plants require both macronutrients (N, P, K, S, Ca, and Mg) and micronutrients (such as Co, Cu, Fe, Mn, Mo, Ni, and Zn) for healthy growth and development. However, some soils are deficient in some these nutrients especially, the micronutrients (Nagajyoti *et al.*, 2010). These crops can be supplied with these essential nutrients as an addition to the soil or as a foliar spray. Under intensive systems of farming, substantial amounts of fertilizer are applied regularly to soils to provide adequate N, P, and K for crop growth. The raw materials used in the formulation of the fertilizers contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities. They are thus deposited inadvertently into the soil through the fertilizer application, significantly increasing their concentration in the soil. For instance, the application of certain phosphate fertilizers inadvertently adds Cd as well as Hg, As, and Pb to the soil (Stroebel, 2008). The main organic fertilizers that are known to contain substantial amount of heavy metals are animal manure and sewage sludge (biosolids). Heavy metals found in sewage sludge may be in the inorganic form or may be in an organic complex and this could affect their chemical reactions in soil. With the continuous application of fertizers known to be sources of some heavy metals, their concentration in the soil could increase. Heavy metals that are of great concern due to their carcinogenicity include cadmium, chromium, lead, mercury, nickel, and vanadium (Wuana and Okieimen, 2011; Chaney, 2012).

2.2.3. Pesticides

Pesticides have been used widely in developed and developing countries agriculture system to control insect pests from crop infestation. Some of the pesticides that are used extensively in agriculture and horticulture in the past however, contained substantial amounts of heavy metals which when applied, are released into the soil. Examples of these compounds include Bordeaux mixture (copper sulphate) and copper oxychloride (EFSA, 2008) which are both fungicidal sprays containing copper; lead arsenate (Schooley *et al*., 2008) which was used in orchards to control insects; maneb or mancozeb (ATSDR, 2011), manganese – containing fungicide and organic arsenic containing compounds (ATSDR, 2011) used to control pests in cotton.

2.2.4. Vehicular emissions

Emissions from vehicles does not only release carbon dioxide into the atmosphere but heavy metals as well contributing to high amounts of the heavy metals in the atmosphere. A study by Abechi *et al*. (2010) indicated that there is a strong positive correlation between traffic volumes and heavy metals concentration in the nearby environment. Popescu (2011) supports this in a similar study where it indicated that, there is positive correlation between heavy metals (Pb, Cd) and vehicular emission. In addition, Pal *et al*. (2010) found high levels of Cd, Cu and Zn within a meter stretch of the roadside attributed to vehicular emission.

2.4. Bioavailability of heavy metals

Bioavailability or 'environmentally available fraction' of heavy metals represents the fraction of total heavy metal found in soil, sediment, water, or air that is available for physical, chemical, and biological activities (USEPA, 2007). It is the total pool of metal at a given time within a system that is potentially able to contact or enter an organism. Out of that total pool, the amount that actually interacts with the organism at its contact surface and is potentially available for absorption or adsorption by the organism is referred to as the bioaccessible fraction (BF). Bioavailability is the extent to which bioaccessible metals can be utilized by the living organism. It is expressed as a fraction of the total amount of metal that the organism is proximately exposed to (at the sorption surface) at a given time and under certain conditions.

2.4.1. Factors that influence the bioavailability of heavy metals in plants

The bioavailability of heavy metals to plants can be affected by several factors including the soil pH, oxidation and reduction potential, soil organic matter content, soil texture, and content of macro and micronutrients in the medium.

2.4.1.1. pH

In soils that are highly acidic, the mobility of heavy metals tends to be higher as compared to neutral or alkaline soils. Therefore, low pH values in soils tend to make heavy metals bioavailable while higher values cause them to bind and become unavailable. In a report by Jung

(2008), it was found that the concentrations of Pb, Zn, Cd, and Cu increased with depth as the pH reduces below 4.0. Wang *et al*., (2006) also demonstrated that soluble forms of Zn and Cd occurred at lower soil pH. Hence, decreasing the soil pH resulted in increased percentage extraction of Cd and Zn while increasing it reduces the extraction percentage of the heavy metals.

2.4.1.2. Oxidation – reduction potential

The oxidation and reduction potential of soil significantly influences the mobility of an element that can enter into the biological cycle in relation to the total element content. The mobility of heavy metals such as Cr, Cu, Pb, and Ni is enhanced under anaerobic conditions depending on the particular metal species and the microenvironment (Violante *et al*., 2010).

2.4.1.3. Organic matter content

When organic matter content in the soil is high, it helps to minimize the absorption of heavy metals by plants by actively binding to them (Prasad *et al*. 2001; Bulut and Tez, 2007).. However, the forms in which heavy metals occur in the soil significantly affect their mobility. Some of the heavy metals have a high affinity for specific particulate matter in the soil. For instance, Zn binds well to clay particles in the presence of soil organic matter while Pb binds to mineral surfaces in the presence of soil organic matter (Quenea *et al*., 2009).

2.4.1.4. Soil texture

The particle size distribution of the soil can affect the availability of heavy metals in the soil. For instance, heavy soils have a higher ability to retain metallic elements due to its large amounts of suspended fraction (Fijalkowski *et al.* 2012). Light soils on the other hand, are not able to retain these metallic elements as they lack suspended particles. Soils with high sorption capacity for cations, i.e. soils containing a large amount of clay minerals, have the ability to accumulate more heavy metals. At a comparable state of heavy metal pollution, the soil may contain heavy metals in dissolved form that make them easily available to plants.

2.4.1.5. Content of macro and micronutrients

Plants need appropriate amounts of nutrients, i.e. macronutrients and micronutrients for growth and development. The plants take up heavy metals from the soil in a similar way as they take up the nutrients through the root system. The rate of uptake of these heavy metals by the roots is dependent on the chemical form in which they appear in the soil. When micronutrients are insufficient in the soil, they often result in excessive accumulation of several heavy metals in plants. However, well balanced amounts and level of nutrients in the soil, with a low concentration of heavy metals ensures higher yields (Fijalkowski *et al*., 2012).

2.4.2. Bio-Concentration Factor (BCF)

The BCF is a ratio of plant: soil content of heavy metals. It indicates the rate of transfer of heavy metals from the soil through the roots to the reference plant part (Zhao and McGrath, n.d.). The higher the BCF, the higher the transfer rate of the particular heavy metal.

2.4.3. Risk analysis of heavy metals

Risk is a function of the probability of the occurrence of an adverse health effect. In a study by Khan *et al.* (2008), it is mentioned that, the accumulation heavy metal in soils and its translocation into plants is of great concern because of the potential health risks they pose to humans. The study indicated that heavy metal contamination in the food chain is one of the important pathways through which the toxic pollutants enter into the human body. According to the study, vegetables grown in soils contaminated with heavy metals, may take up these heavy metals in quantities large enough to cause potential health risks to the consumer. According to the FAO/WHO (2009) report on dietary assessment of chemicals in food, dietary exposure combines information on food consumption patterns with available data on the concentration of chemicals in food. Dietary exposure assessments can be computed for acute or chronic scenarios. An acute exposure is transient and covers a period of up to 24 hours while chronic exposure covers average daily exposure over the entire lifetime of a living organism.

The Hazard Quotient (HQ) is defined as a ratio of the determined Average Daily Dose (ADD) of a pollutant to a Reference Dose (RfD). If the ratio is less than one, the exposed population is unlikely to experience adverse health effects. It is a method used in characterizing the health risk of vegetable consumption by people within a local setting (Hough *et al.,* 2004). Moreover, it is used frequently to assess non-cancer health risk related to heavy metals ingested through food consumption (USEPA, 2005). The hazard quotient (HQ) is used to assess individual heavy metals while the hazard index (HI) as a cumulative risk factor (Liu et al, 2013). The values of RfD is 0.001 mg/kg/day for Cd, 0.3 mg/kg/day for Zn, 0.004 mg/kg/day for Pb and for Cu, it is 0.04 mg/kg/day (USEPA, 2007; FAO/WHO, 2014).

The hazard index (HI) is a summation of all the hazard quotients for each heavy metal. When the value obtained is less than one, the population under study is not exposed to any carcinogenic threat but if it is more than one, then there is a potential threat to the exposed population.

2.5. Strategies for reducing heavy metal concentration in the soil

2.5.1. Bioremediation

Bioremediation involves the use of living organisms (microorganisms and/or plants) in the removal of heavy metals from polluted soils (Sarma, 2011). It is a widely accepted method of soil remediation because it is a natural process of cleaning the soil. Phytoremediation involved the use of plants as part of the bioremediation process for the correction of heavy metal-polluted soil, sediment, or water. Bioremediation has four different plant-based technologies, with each having a different mechanism of action. These are rhizofiltration, phytostabilization, phytovolatilization, and phytoextraction.

Rhizofiltration is the process by which aquatic plants are used to clean various aquatic environments (Yadav *et al*., 2011; Krishna *et al*., 2012). Phytostabilization involves process by in which plants are used to stabilize or immobilize heavy metals in the soil (Soudek *et al*., 2012). Phytovolatilization uses plants to extract certain metals from soil and then release them into the atmosphere through volatilization. Phytoextraction also uses plants to absorb metals from the soil and then translocate them to the harvestable shoots where they accumulate (Sakakibara *et al*., 2007; Souza *et al*., 2013).

Agricultural waste materials such as biochar is also being exploited for its potential in the management of heavy metal polluted soils (Chibuike and Obiora, 2014).

2.5.2. Agricultural by-products as biosorbents of heavy metals

A wide range of agricultural and forestry by-products as well as wild and cultivated species have been exploited for use as biosorbents of toxic metals. For instance, rice husk, chaff, sesame, sunflower and tea waste have been demonstrated to adsorb Pb, Fe and Ni from wastewater. Stalks of African spinach and pawpaw seeds have been used to remove Pb and Mn from wastewater (Hegazi, 2013; Egila *et al.*, 2011; Surchi, 2011) while the use of sawdust has also been demonstrated for capability to adsorb cadmium, lead and nickel (Bulut and Tez, 2007).

The bark of *Acacia nilotica* also has the capability to adsorb heavy metals. It has been demonstrated that when 1g of the powdered bark of *A. nilotica* was added to 100 ml of aqueous solution containing 10 mg/ml of metal solution, it exhibited different metal adsorption rates for different metals. From the experiment metal adsorption was in the order of Cr > Ni > Cu > Cd> As > Pb. To confirm the metal removal property of the bark of *A. nilotica*, toxicity bioassay was conducted with *Salix viminalis* stem cuttings in hydroponic system where Cd, Cr and Pb together with *A. nilotica* bark powder was added to the medium. The results confirmed that these metals were adsorbed by the powdered bark (Prasad *et al*., 2003).

2.5.3. Microbial action on heavy metal remediation

Some microorganisms are capable of producing enzymes that degrade hazardous chemicals such as lead chromium, cadmium manganese etc. Some of these microorganisms have been used in the removal of heavy metals in contaminated media. Examples of these microbes include *Bacillus subtilis, Pseudomonas putida*, and *Enterobacter cloacae, Saccharomyces cerevisiae* (yeast); *Fuscus* vesiculosus, *Penicillium chrysogenum* (fungi), and *Ascophyllum nodosum* (marine algae) (Prasad and Freitas, 2003).

In a study conducted by Gayathramma *et al*. (2013), it indicated that *B. subtilis* bioaccumulated lead, manganese and cadmium at different concentrations in a cultured medium. In related experiments, *B. subtilis* was shown to be capable of removing cadmium, lead, chromium and zinc from heavy metal contaminated media (Daboor, 2014; Syed and Chinthala, 2015).

Pseudomonas putida has also been exploited to reduce chromium (Cr VI) in contaminated soil samples (Balamurugan *et al*., 2014), lead, cadmium and nickel from soils (Banerjee *et al*., 2015).

2.6. Compost and its suitability for crop production

Composting is a widely accepted practice used in all systems associated with sustainable/conservation agriculture. It offers enormous benefits for all agro-ecological systems as it combines environmental protection with a sustainable agricultural production. Composting involves recycling of organic waste from forestry, farming and animal production systems by transforming them into inputs to replenish the nutrients in the soil (Ramàn *et al*., 2015). Composting and its application to the soil, provide many benefits such as, increasing organic matter content of the soil, the uptake of carbon, control of soil temperature, increasing soil porosity, providing nutrients and beneficial microorganisms, improving water retention and cation exchange as well as increasing crop productivity (US Composting Council, 2008).

The European Commission (2004) has legislations and standards governing the use of composts for agriculture purposes. Based on their potential toxic elemental (PTE) load i.e. heavy metal or organic contaminants, composts have been classified into three categories: class A+ (top quality compost, suitable for organic farming); class A (high quality, suitable for use in agriculture) and class B (minimum quality, suitable for non-agriculture use). The acceptable mean values (mg/kg d.m.) for the three classes are shown below:

Table 1.European Commission standard on compost heavy metal threshold (mg/kg d.m) for land application

Compost class	Cd	Pb	Zn	Cu
A+ - agriculture	0.7	45	200	70
A – agriculture	1	120	500	150
B – land reclamation	3	200	1200	400

Source: European Commission (2004)

2.6.1. Effect of compost on crop performance

A well-matured compost that is free from contaminants and harmful microorganisms helps in maintaining physico-chemical properties of the soil when applied as amendment for crop cultivation. However, the effect of composts on plants requires some considerations i.e. the general response of different plant species to any compost, the response of any particular plant species to a specific type of compost and the combination of compost and soil for growing plants.

2.6.1. 2. SPAD index

Chlorophyll content in leaves of plant can be measured using a SPAD meter. The meter is a simple, hand-held device for estimation of foliar chloropyll content. It is non-destructive, inexpensive and rapid method in its estimation. However, values provided by the SPAD meter are nominal or unitless and will require calibrations between SPAD values and extracted chlorophyll values. According to Konica Minolta Incorporated (2015), the higher the measured SPAD index, the healthier the plant, as the values are indicative of the amount of nitrogen present in the leaves of the plant.

Measured values from the SPAD meter give an indication of the amount of chlorophyll present in the leaf. When the SPAD meter is used, the values obtained have to be converted into absolute units of chlorophyll concentration by derivation of calibration curves (Ling *et al*., 2011). In similar studies, Gaborcik, 2003; Coste *et al*., 2010; Mielke *et al*., 2010 indicated that there is a positive correlation between SPAD index values, chlorophyll (*chl* a or *chl* b) content and total leaf chlorophyll (*chl* a + *chl* b) content. Apart from the chlorophyll content, Percival *et al*. (2008) also reported that SPAD values are positively correlated with leaf nitrogen content, total carotenoids and chlorophyll fluorescence.

2.7. Hyper accumulators of heavy metals

Depending on its phytotoxicity with respect to a particular heavy metal, hyperaccumulators are plants when grown on native soils can absorb heavy metals in their shoot system, without suffering phytotoxic damage (Rascio and Navari – Izzo, 2011).

Leafy vegetables including roselle and jute mallow are considered hyper accumulators as than translocate high amounts of heavy metals from the soil into their edible parts (Puschenreiter *et al.,* 2005).

2.7.1. Roselle (Hibiscus sabdariffa L.)

The roselle plant is an annual bushy plant that can grow up to 2.4 meters high. It is comparatively easy to grow although it is difficult to produce a high quality crop consistently. In order to obtain high quality crop, it is important to select good seed, choose proper site with good drainage, adopt good agronomic and postharvest handling practices (especially the drying process). Roselle can grow in a range of soils but well-drained soils with good organic matter content are most suitable. In most cases, it is mostly grown as a secondary crop rather than a primary crop. It requires nighttime temperatures above 21^{o}C and 13 hours of daylight during the first 4-5 months of growth in order to prevent premature flowering.

Roselle requires a monthly rainfall of between 130-250 mm in the first 3-4 months of growth. It can withstand dry spells but this is desirable towards the last months of growth. High humidity during the harvest period can result in poor quality of the calyces and can cause yield reduction (FAO, 2004; Da-Costa-Rocha *et al.,* 2014).

Roselle is an underutilized and multipurpose crop (Williams and Haq, 2000) that provide farmer households with food and additional income when other vegetables have become scarce. In many West African countries, the leaves of roselle and its processed products such as the juice or syrup from the red calyx are generally carried out by women providing additional income to the family (McClintock, 2004). Through research on crop improvement, good husbandry and post – harvest practices, a number of cultivars of roselle are available for use as vegetables or for making of beverages. In order to boost the competitiveness of the roselle on the international market, appropriate quality and safety standards (grading, sorting, processing and packaging) are adhered to. The demand for roselle fibre, which are biodegradable, is likely to increase because of the rising interest in natural fibres,

According to the World Crops Database (2012), roselle is a member of the family Malvaceae. It is known differently in different languages as, Indian or Jamaican sorrel (English). In the French language, roselle is known as, oseille de Guinée, bissap, thé rose d'Abyssinie, or groseille pays. In Spanish, it is known as Flor de Jamaica. It is also called Vinagreira, azeda de Guiné, azedinha, caruru azedo, quiabeiro azido in Polish. In Swahilli, it is called Ufuta, ufuta dume and in North Africa, it is commonly referred to as 'karkade' in Arabic (Morton, 1974).

2.7.1.1. Origin and geographic distribution

Roselle (*Hibiscus sabdariffa*) is believed to have originated from Africa, where it was domesticated in western Sudan about 4000 BC (Mohamed *et al*., 2012). It was first cultivated for the seed and subsequently for the leaf and calyx. Later in the 17th century, vegetable types of the crop were introduced into India and the Americas. At the beginning of the 20th century, selection of some cultivars for fibre production took place in parts of Asia (India, Sri Lanka, Thailand, Malaysia and Java). The crop is now commonly grown throughout the tropics and can be found in tropical Africa especially in the savanna zones of West and Central Africa where it serves as an escape crop from main cultivation. Truly wild plants of *Hibiscus sabdariffa* have been collected and preserved in Ghana, Niger, Nigeria and Angola.

2.7.1.2. Description

Roselle is a large annual herb that can grow up to 2.4 m tall and has a deep taproot. The stem is smooth, green or reddish, cylindrical, glabrous to sparsely pubescent and sometimes sparsely prickly.

The leaves are simple, alternate, and between 7.5 – 12.5 cm long, stipules are narrowly lanceolate to linear and the leaf blade shallowly to deeply palmate and 3–5 lobed with toothed margins. The flowers are solitary in leaf axils up to 12.5 cm wide, pale yellowish beige with a deep purplish eye. The flower is bisexual, regular with petals consisting of 5 large sepals. The pedicel is up to 2 cm long and articulate. The epicalyx is segmented into 8–12 and united at the base, subulate to triangular in shape. The calyx is up to 5.5 cm long, campanulate, becoming fleshy in fruit. The lobes are nearly glabrous to hispid hairy with a nectary outside. Petals are free, obovate and up to 5 cm × 3.5 cm, pale yellow or pale pink, often with dark red-purple centre. The stamens are numerous and united into a column of up to 2 cm long, pink, superior ovary, 5-celled style with 5 branches.

The fruit (capsule) is ovoid and up to 2.5 cm long, almost glabrous to appressed-pubescent, enclosed by the calyx and it's many-seeded. The capsule browns when dry and is dehiscent. Seeds are reniform, up to 7 mm long, dark brown, and covered with minute hairs (Morton, 1987; Mahadevan *et al*., 2009; McClintock and El Tahir, 2011).

2.7.1.3. Species

The genus *Hibiscus* comprises of several species (>300), many of which are grown as ornamentals especially in the tropics and subtropics, though some are also grown as vegetables. There are varying opinions among taxonomists concerning the inclusion of other related species into the genus. For this, the estimated number of species varies. *Hibiscus sabdariffa* belongs to the section *Furcaria*, a group that has about 100 species. The common feature of this group is a pergamentaceous calyx (rarely fleshy) with 10 strongly prominent veins, 5 running to the apices of the segments and bearing a nectary, and the other 5 running to the sinuses. Other species that belong to this section include *Hibiscus asper, Hibiscus acetosella* Hook. f. *Hibiscus diversifolius Hibiscus cannabinus,* Jacq. *Hibiscus mechowii* Garcke, *Hibiscus noldeae* Baker f., *Hibiscus* *rostellatus* Guill. and Perr., and *Hibiscus surattensis.* L. these species are used for vegetable production (Qi *et al*., 2005; The Plant List, 2010; Ansari *et al*., 2013).

2.7.1.4. Cultivars

The two main cultivated types of *Hibiscus sabdariffa* are distinguished originally as botanical varieties. These are: *Hibiscus sabdariffa* var. *sabdariffa*, which has a profuse, strongly branching habit and glabrescent calyx, accrescent and becoming fleshy in fruit; and *Hibiscus sabdariffa* var. *altissima*, Wester that is taller, usually unbranched and often hispid hairy calyx, hardly accrescent and not fleshy in fruit. The *Hibiscus sabdariffa* var. *altissima* is grown for its fibre but its not common in Africa. These types are best described as cultivar-groups. Several of the known cultivars are grown as vegetable or for their calyces. In some of these cultivars, there is the presence of anthocyanins, which is responsible for the formation of reddish stems, leaves, calyces, and pinkish petals. In other cultivars where anthocyanins are absent, the plants have green stems, green leaves, pale green calyces and pale yellow petals (Morton, 1987; McClintock and El Tahir, 2011).

2.7.1.5. Uses

In Africa, the leaves and young shoots of roselle (green type) are finely chopped and cooked as soup with grated groundnut or palmnut sauce, salt, chopped onion, dried fish and pepper added. They are boiled in hot water and strained. Spices such as ginger or garlic are usually added and used as a beverage. They are also used fresh in making wine, jam, juice, jelly, gelatin syrup, pudding, cakes, ice cream and flavours (Agriculture Research and Extension Center, 2008).

Plate 1. Roselle tea

Plate 2. Dry roselle calyces

According to Mohamed *et al*., (2012), a tea made from roselle known as Sudanese roselle tea is well known as an organic product that is highly valued for its beneficial health effects. Tea made from roselle flowers is also commonly sold on the domestic market and is widely exported for use in making a sweet herbal tea. In Senegal, the calyces of the green type are stewed and used as a condiment ('bëkëj') in rice dishes. In Côte d'Ivoire, the calyces are dried, ground into powder and used in sauces during the dry season. The red calyces are dried and commonly used to prepare tea with added sugar and drunk either hot or, more commonly, cold. The drink taste sour but refreshing and very popular in Senegal, Sudan, Egypt and other North African countries. The consumption of the juice is more prominent during the evening where Muslims break their fast in the month of Ramadan. It is variously referred to as 'bissap' (Senegal), soborodo (Nigeria) or 'da bilenni' (Côte d'Ivoire, Mali, and Burkina Faso) (Bolade *et al*., 2009; Mady, 2010; Obouayeba *et al*., 2014).

To prepare roselle syrup, the calyces are dried and boiled at a ratio of 1 part dried calyx to 4–5 parts of water. In order to reduce its tart taste, large quantities of sugar is added and the mixture is boiled for several hours. Before the syrup is used, it is diluted with water to make 'da bilenni'. It is estimated that about 1kg of roselle could produce 80litres of 'da bilenni' (McClintock, 2004).

2.7.1.6. Chemical properties

It is estimated that 100 g of edible portion of roselle contain 85.6 g water, 180 kJ (43 kcal), of energy, 3.3 g of protein, 0.3 g of fat, 9.2 g of carbohydrate, 1.6 g of dietary fibre, 213 mg of Ca, 93 mg of P, 4.8 mg of Fe, 4135 μg of β-carotene, 0.2 mg of thiamin, 0.45 mg of riboflavin, 1.2 mg of niacin, and 54 mg of ascorbic acid (Ismail *et al*., 2008).

2.7.1.7. Pharmacological / medicinal benefits

The roselle plant has several pharmacological properties. It has anti-spasmodic, anti-helminthic and bactericidal properties. The tea made from roselle calyces has anti-hypertensive and cardio-protective effects and this has been demonstrated in some clinical studies for various animals. The phenolic compound (protocatechuic acid) extracted from the flowers of roselle showed that is has antioxidant, hepatoprotective and antitumor properties. Antioxidants such as flavonoids, gossypetine, hibiscetine and sabdaretine are also contained in the dry red calyces. Extracts from roselle also showed antipyretic and anodyne properties when tested with mice. The oil from the seed exhibits antibacterial and antifungal properties (Orwa *et al*., 2009; McClintock and El Tahir, 2011; Puro *et al*., 2014; Obouayeba *et al*., 2014; Da-Costa-Rocha, 2014).

2.7.1.8. Planting, Growth and development

Roselle can be direct-seeded on the field or nursed in beds and transplanted. For direct seeding, a seed rate of 6-8kg/ha is required. Because roselle is deep-rooted, ploughing should be deep enough to ensure a good seedbed is prepared. Either sowing can be done by hand or with a seed drill and the planting holes spaced 45-60 cm within rows and 60 cm-1m between rows. Roselle is photoperiod sensitive and flowering is induced when the days are shorter with reduced light intensity. Flowering usually begins in September or thereafter though with spatial variations, and continues through to October or thereafter. Towards the end of October, the flowers begin to drop, as they are diurnal and last only a day. The seedpods begin ripening from the bottom and proceed to the top (FAO, 2004).

2.7.1.9. Diseases

A number of fungal diseases affect the roselle plant. These include root and stem rot caused by several fungi such as *Phytophtera spp*., leaf spot caused by *Cercospors hibisci*, root rot caused by *Fusarium spp*., Collar rot caused by *Phytophtera nicotiana var parasitica*, powdery mildew caused by *Oidium abelmoschi* (Adenji, 1970; Hassan *et al*., 2014).

2.7.1.10. Pests

Flea beetle (*Podogrica spp*.). These are common pests in many vegetables that make tiny holes in the leaves of the crops. Their damage is generally minimal and can be ignored as usually recover soon after attack. Seedlings, because of their small leaf area mostly suffer badly from the pest attack and seedlings occasionally die. The larvae feed on the roots while the adult damages leave and growing points. However, large numbers of the insects can cause considerable damage. A recommended control measure is the use of sticky cards to trap the insects along the row of plants as the beetles dart on the field. Foliar sprays with insecticide can also be used to control them.

Cotton stainer (*Dysdercus superstitiosus*) is another pest that attack roselle by sucking the calyces causing brown spots. In addition, Spiral borers (*Agrilus acutus*) cause galls on the stems leading to reduction in nutrient uptake. Other pests of interest include cutworms, mealy bugs, leafhoppers and snails (Burkness and Hahn, 2007).

2.7.1.11. Harvesting

Three consecutive cuttings from the leaf branches can give a yield of up to 20 t/ha. For the fresh calyx, yields may range from 4–6.5 t/ha but for dried calyces (12% moisture content) yield of 0.8–1.2 t/ha can be obtained. Fresh calyx yields of up to 15 t/ha have been reported in Asia. In

Africa however, average yields are much lower and variable because of unfavourable environmental conditions and poor management. In Sudan for instance, an average yield of dry calyces of 0.093 t/ha has been recorded while in Senegal, maximum production of calyx on a dry weight basis is 0.5 t/ha (McClintock and El Tahir, 2011).

2.7.1.12. Postharvest handling

As a leafy vegetable, the shoots of roselle bearing the leaves are tied in bunches for sale. These are usually kept in basins/vessels containing water to reduce/prevent dehydration. As a result of high daily temperatures and because the vegetable is commonly sold in the open, they are continuously being sprinkled with water (FAO, 2004).

In most African countries, roselle calyces are air-dried prior to marketing. Shade-drying is preferred as sun-drying can lead to reduced quality because it involves simultaneous heat and mass transfer. Therefore, drying which can take various forms is an important post-harvest handling process carried out to dehydrate the calyces and in order to increase their shelf life. Drying with adequate ventilation prevents sun baking, which can reduce quality of the produce, and this can be achieved by using woven nylon mats. A clean plastic sheet placed on the floor can also be used and the hibiscus spread thinly on top of it (Bobadilla-Carrillo *et al*; 2016; Fajar *et al*, 2012). Adequate ventilation is important during the drying process.. Drying by using artificial heat source is practiced though capital-intensive and rare in sub-Saharan Africa. Under this method, the temperature is regulated and must remain below 43°C. Throughout West Africa, dried calyces are bundled and sold in bulk or in individual packs. In Senegal, dried calyces of roselle are compressed into bales of about 80kg for export. The major export destinations of the calyces are the United States of America and Germany.

2.7.2 Jute mallow (*Corchorus olitorius* L.)

Cochorus olitorius is a member of the family Tiliaceae. It is known in other languages as Jute Mallow, Jew's Mallow, bush okra, krinkrin, tossa Jute, and West African sorrel (English). It is called Ayoyo in Hausa. In French, it is known as mauve des Juifs, Corète potagère, Jute potager, craincrain, and krinkrin. In Polish, it is known as Coreté, caruru da Bahia and Mlenda in Kiswahili.

Corchorus olitorius L. is a high quality leafy vegetable with a wide diversity of plant types found in the African continent (Denton and Nwangruka, 2012). It is cultivated widely for its leaves, which becomes slimy when cooked and used in local dishes. Over 100 species constitute the genus *Corchorus*, of which *Corchorus olitorius and Corchorus capsularis* are the most widely and frequently cultivated (Ghosh *et al*., 2013). *Corchorus olitorius* is a vegetable that is popular in both arid and semi-arid regions as well as in the humid areas of Africa (Musa and Ogbadoyi, 2012).

2.7.2.1. Origin and geographic distribution

Tough the geographical origin of *Corchorus olitorius* is often disputed, Africa is considered the primary center of origin due to the diversity of plant types in the continent. In both Asia and Africa, it has been cultivated for the past centuries and occurs in the wild in both continents. Though some have attributed the origin of *Corchorus olitorius* and several other *Corchorus* species to India or the Indo-Burmese area, the presence of more wild *Corchorus* species in Africa and the larger genetic diversity within *Corchorus olitorius* point to Africa as the primary center of origin of the genus. Presently, *Corchorus olitorius* is found all over the tropics and it is reported as either wild or cultivated vegetable in tropical Africa. *Corchorus olitorius* is the most common leafy vegetable in many African countries including Côte d'Ivoire, Benin, Nigeria, Cameroon, Sudan, Kenya, Uganda and Zimbabwe. In Asia (India, Bangladesh and China), it is cultivated as a vegetable as well as for jute in the case of Corchorus *capsularis* L. In Africa however, the fibre is less important although, it may be used domestically.

2.7.2.2. Uses

When the leaves of jute mallow are cooked, they form a slimy sticky sauce, comparable to okra. In Nigeria, the slimy sauce is used as a condiment and served with moulded starchy paste made from cassava, yam or millet. The leaves are dried and made into powder for use during the lean season. The immature fruits of jute mallow referred to as bush okra, are also dried and ground into powder for the preparation of the slimy sauce. In East Africa, several recipes can be made from it. For example, it can be cooked with cocoyam leaves, cowpeas, pumpkin, sweet potato, milk and butter, meat flavoured with lemon.

For more than 100 years, jute mallow has been used widely as fibre for packaging due to its high tensile strength and durability, low cost of production, ease of manufacturing and availability in large and uniform quantities. The *Corchorus olitorius* that are used as leafy vegetable are quite different from those used for jute production. However, jute production is less important in Africa.

Different parts of the jute mallow plant can be used for different purposes. In Kenya, the root scrapings of the plant are used to treat toothache; in Congo, the leafy twigs are used as remedy for heart troubles; in Tanzania, an infusion from the leaves is taken to relieve constipation and in Nigeria, the seeds are used as purgative and for treatment of fever (Fondio and Grubben, 2004).

Fibre from the jute is obtained from the stem described as 'bast fibre'. The bast fibre is used for coarse fabrics, because the length: diameter ratio of the jute filaments is only 100–120mm, which is much below the minimum of 1000mm required for fine spinning quality. The fibre cells are between 2–2.5 mm long and 15–20 µm in diameter. These cells are cemented together into filaments of up to 250 mm long. The tensile strength of jute fibre is 393–773 MPa while the elongation at break is only 1.5–1.8% (Gon *et al*., 2012). The jute fibre has an ignition temperature of 193°C, posing a considerable hazard in warehouses.

2.7.2.3. Chemical properties

On fresh weight, a 100 g portion of *Corchorus olitorius* contains 80.4 g (74.2–91.1%) of water, 243 kJ (58 kcal) of energy, 4.5 g of protein, 0.3 g of fat, 12.4 g of carbohydrate, 2.0 g of fibre, 360 mg of Ca, 122 mg of P, 7.2 mg of Fe, 6410 μg of β-carotene, 0.15 mg of thiamin, 0.53 mg of riboflavin, 1.2 mg of niacin, and 80 mg of ascorbic acid. On average, the dry matter content of fresh jute mallow leaves is higher than other dark green leafy vegetables, their composition is similar. The nutrient composition is strongly affected by external factors such as soil fertility. Application of nitrogen fertilizer to the crop greatly improves its micronutrient content, e.g. Fe, P, Ca, carotene and vitamin C.

The polysaccharide in the leaves that is responsible for its sliminess is rich in uronic acid (65%) consisting of rhamnose, glucose, galactose, galacturonic acid and glucuronic acid in addition to 3.7% acetyl group (Leung *et al*., 1968). The leaves of *Corchorus olitorius* contain also antioxidative phenolic compounds, of which 5-caffeoylquinic acid is the most important one. Some ionone glucosides have also been isolated from the leaves. These inhibit the release of histamine from rat peritoneal exudate cells, which is induced by antigen-antibody reaction. The seeds contain cardiac glycosides and are poisonous to mammals and insects.

2.7.2.4. Description

The plant is an upright annual herb that grows up to 2 m tall, usually strongly branched with reddish stems, which are fibrous and tough.

The leaves are simple, alternate and stipules are narrowly triangular with long point; petiole is 1–7 cm long; the blade is narrowly ovate or elliptical, 4–15 cm × 2–5 cm, cuneate or obtuse and with setaceous appendages of up to 2.5 cm long at the base, acuminate to acute at apex, margin is serrate or crenate, almost glabrous, usually shiny and dark green, 3–7-veined from the base.

The inflorescence is a 1–4 flowered axillary fascicle, bracteate. Flowers are bisexual, regular, usually 5-merous, shortly stalked; sepals are free, narrowly obovate, 5–7 mm long; petals are free, obovate, 5–7 mm long, yellow, caducous; stamens are numerous; ovary is superior, usually 5-celled, style is short.

The fruit is many-seeded, a cylindrical capsule up to 7 cm long, ribbed, with a short beak, usually dehiscent.

The seeds are angular, 1–3 mm long, and dark grey. Seedling germinate epigeally; hypocotyl is 1–2 cm long; cotyledons are foliaceous, broadly elliptical to circular, 3–8 mm long.

The genus *Corchorus* consist of number of species ranging from 40–100 (Ghosh *et al*., 2013). There are two important cultivar-groups of *Corchorus olitorius*. These are the types used in fibre production and are classified the Textilis Group. Plant in this group grow up to 4–5 m tall and are slightly branched at the top. The other group are the vegetable types classified as the Olitorius Group, characterized by a plant height lower than 2 m, and a more or less heavily branched. There are numerous local cultivars within the Olitorius Group. There are early and late flowering types, and other have differences in growth habit and leaf shape. In Nigeria, the popular 'Amugbadu' has finely serrate, elliptical-ovate leaves and considered suitable for transplanting and harvesting by repeated cuttings; whereas 'Oniyaya' has smaller and coarsely serrate leaves, strongly branched and more suitable for direct sowing and once harvest. In Cameroon, 'Géant de Bertoua' has very large, broadly ovate leaf blades while cultivars with deeply and irregularly serrate leaves ('Incisifolius') can be found in Benin and Cameroon as well ((Fondio and Grubben, 2011; Belay, n.d.).

2.7.2.5. Pharmacological / medicinal benefits

The leaves of *Corchorus olitorius* are rich in vitamins, minerals, antioxidants, fatty acids, and mucilaginous polysaccharides. In a study by Yokoyama *et al*., (2014), it was indicated that extract from the leaves of *Corchorus olitorius* applied on the skin of mice reduced transepidermal water loss, and increased skin hydration in atopic dermatitis (AD)-like lesions in NC/Nga mice. Leaf extracts of jute mallow have varying compositions in terms of the physical and chemical properties hence, can be used for different purposes. The leaves are rich in beta – carotene that is essential for good eyesight, contains calcium for strong bones and teeth, iron for healthy red blood cells, and vitamin C for smooth, clear skin, strong immune cells, and fast wound-healing (Islam, 2013).

2.7.2.6. Planting, growth and development

Planting of *C. olitorius* can be by direct seeding or can be nursed and transplanted onto prepared beds. The seed coat is hard and therefore requires scarification to break seed dormancy. For direct seeding, seed rate required ranges from 5-10 kg/ha depending on size of the seed and its viability (Palada and Chang, 2003). Seedlings of *Corchorus olitorius* are fast-growing and in short-day conditions, flowering starts about a month after emergence and continues for between 1–2 months depending on the environmental conditions. The flowers are usually self-pollinated, but a little cross-pollination of up to 10% occurs. The fruits ripen between 3–4 months as the leaves begins to drop and the plant dies.

2.7.2.7. Management

Jew's Mallow is usually grown under rain-fed conditions with little care. During the dry season production in peri-urban areas, growers practice manual irrigation, at least 6 mm of irrigation water daily is ideal. Organic fertilizer may be applied at a rate of up to 20 t/ha. A basal application with NPK compound fertilizer (e.g. 15–15–15 at a rate of 400 kg/ha) and a side dressing with nitrogen are recommended for an optimal yield. Weeds particularly grasses are problematic in jute fields and can be managed by hand weeding or with post-emergence herbicides (Sakar, 2006).

2.7.2.8. Diseases and pests

Jew's Mallow is rather tolerant to diseases and pests. However, stem and root rot caused by *M. phaseolina* (Tassi) are the important diseases that affect both *C. olitorius* and *C. capsularis.* Other diseases including leaf blight, seedling blight, anthracnose and leaf mosaic also cause failure in jute performance (Meena *et al.*, 2014). Foot rot caused by *Sclerotium rolfsii* results in wilting and it is sometimes a problem in crop fields. Fungal diseases such as black leaf spot caused by *Curvularia species* and circular leaf spot caused by *Cercospora species* are kept under control by ensuring well-drained beds and a wider spacing.

Rahman and Khan (2012) found seventeen different species of pest belong to insects, mites and nematodes that attacked and caused economic damage to jute mallow

The most damaging pests are grasshoppers (*Zonocerus variegatus*), caterpillars (*Acrea* spp.), armyworm (*Spodoptera littoralis*) and flea beetles (*Podagrica* spp.). During the dry season, red spider mites (*Tetranychus cinnabarinus*) often attack the leaves. Chemical control is rarely applied.

Jew's Mallow is also highly susceptible to root-knot nematodes (*Meloidogyne* spp.). these can be controlled by crop rotation, avoiding other crops susceptible to root-knot nematodes for at least one year, and ensuring a high organic matter content in the soil.

2.7.2.9. Harvesting

Harvesting of jute mallow is done either by plucking the young leaves and shoots or by pulling off the entire plant from the soil. The first harvest may take place at 4–6 weeks after transplanting when plants attain a height of 10–20 cm above the ground by cutting shoots 20–30 cm long. This cutting stimulates more leaf yield through the development of side shoots (Asiegbu, 1983). Subsequently, harvesting can be done every 2–3 weeks giving a total of about 2–8 cuttings. For a once-over harvest the crop was direct seeded, the plants are uprooted or cut at ground level when they are up to 30–40 cm tall, at 3–5 weeks after emergence and before they develop fruits. The uprooted plants are then bundled for marketing. In intercropping systems, the crop is harvested at irregular intervals. For wild Jew's Mallow, it is whenever necessary and usually for home consumption (Department of Agriculture, Forestry and Fisheries, 2012).

2.7.2.10. Yield

The leaf yield in jute mallow can vary depending on factors such as plant spacing and frequency of harvest, soil fertility management and water management. The closer the spacing, the higher the plant population and hence, higher yield. Madakazde *et al*., (2007) in an experiment found a significant difference in leaf yield between a closer spacing of 50 cm x 10 cm at a week harvest interval (24.97 t/ha) and a wider spacing 50 cm x 50 cm (5.7 t/ha). Adediran *et al*. (2015) recorded fresh yields of 1.40 t/ha for control, 2.02 t/ha for organo-mineral, 2.58 t/ha for NPK and 3.05 t/ha for poultry manure treated plots. In assessing the effect of water management on the biomass yield of jute mallow, Fasinmirin and Olufayo (2009) recorded 5.2 t/ha at full irrigation, 4.41 t/ha at medium level irrigation and 1.96 t/ha at low level irrigation.

2.7.2.11. Postharvest handling

Like other leafy vegetables, Jew's Mallow leaves cannot be kept for long hence, the leaves are mostly sold on the day of harvest. They are constantly kept wet by sprinkling water on them to maintain freshness within a short period (Mutuli and Mbuge, 2015).

To improve on the quality, dried leaves can blanched (scalding them in boiling water, steam or a micro-wave oven) for a short period to stop enzymatic action (Andress and Harrison, 2006). Oulai *et al.*, (2015) in a study on nutrient content and antioxidant properties of some blanched leafy vegetables recommended blanching time of less than 15 minutes to ensure retention and bioavailability of essential nutrients. Blanching kills microorganisms that serves as pathogens and neutralizes enzymes that can affect the flavour of dried leaves during storage. It is important to ensure that that leaves well dried and crispy before storing. It is essential to dry the leaves to a moisture content of 12 – 14% n the first eight hours of drying to prevent the growth of mould that can produce aflatoxins.

It is important to completely and effectively dry the leaves in one day to avoid reabsorption of moisture in subsequent drying. The leaves can be spread thinly on the drying platform to accomplish a single-day drying process. During drying, the leaves need to be protected from rain, insects, birds and dust. After drying, the dried leaves should be kept in an airtight container, away from sunlight and in a dry cool place. When leaves are dried in direct sunlight the dark green colour quickly fades to a paler grayish green. This is caused mainly by ultraviolet rays in the sunlight that breaks apart molecules of the chlorophyll and carotenoid pigments giving the leaves their characteristic colour. Not only is the colour of the faded leaves less appealing but most of their vitamins (A and C) are destroyed. This is why it is advisable not to dry leaves in direct sunlight (Musa and Ogbadoyi, 2012; Mutuli and Mbuge, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study area

The study was conducted at the upland field of the Council for Scientific and Industrial Research – Savanna Agricultural Research Institute (CSIR-SARI), Nyankpala, in the Tolon District of the Northern Region of Ghana during the main growing season in 2014 and 2015. The upland field (Latitude 09^{o} 25" N, Longitude 00^{o} 58" W) with an altitude of 183 m above sea level is about 200 m west of the Changnaayili village.

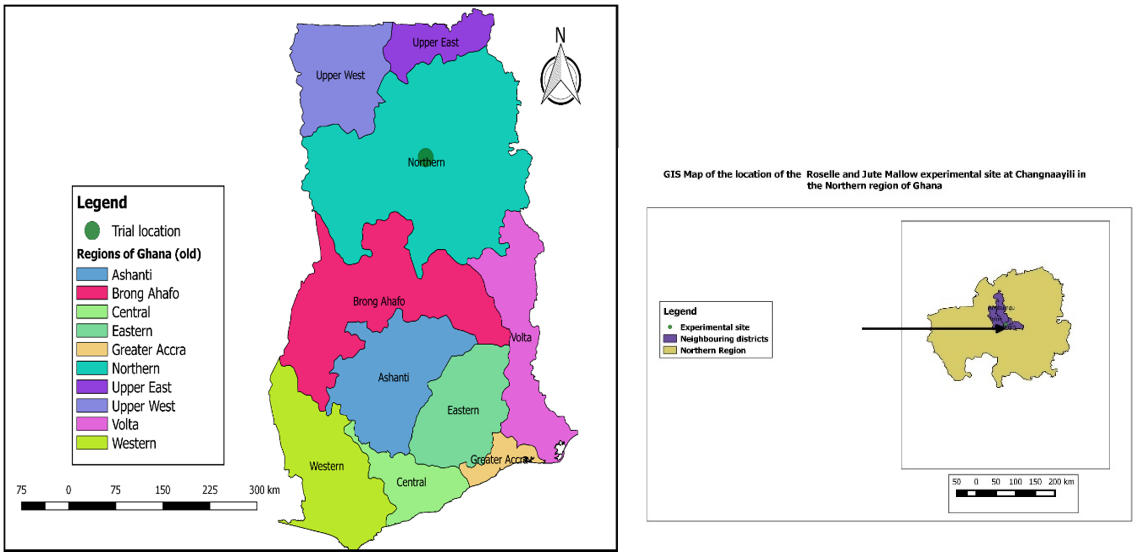

Plate 3. Map of Ghana and the experimental site

The soils found in the experimental site have been described as *Ferric luvisols* (FAO-UNESCO, 2002). They reported to have been formed from ground water laterite soil with concretions and are classified into: (a) Kpalsawgu series which are imperfectly drained, and occurs within the east on the low-lying uplands and (b) Changnayili series which are poorly drained, and occupies the lower slopes and the valley bottoms. They are both sandy loam soils that are slightly acidic and with a pH of about 5.8 (Obeng, 2000).

The Guinea Savannah zone of the northern part of Ghana has two distinct seasons (rainy season and dry season). The rainy season begins from May and ends around October. According to the MoFA (2013), the annual rainfall in the area varies between 750 mm and 1050 mm and has an estimated cropping period of between 180-200 days. The dry season begins from November and ends somewhere in March/April. High temperatures are recorded around March and April while low temperatures recorded around December and early January. The harmattan (northeast trade winds) which occurs between December to early February affects the temperature in the region considerably. The temperatures during the harmattan period varies between 14 °C in the night and 40 °C during the daytime. Because relative humidity is very low during harmattan, it reduces the intensity of the high temperature during daytime.

The ecology of the area consists of vast grassland, which is interspersed with short trees without distinct storeys like in the forest ecology. The trees as described as drought-resistant trees and include dawadawa, shea, baobab, neem, acacia and mango among others.

3.2. Determining the presence and concentration of selected heavy metals (Cd, Pb, Zn and Cu) and other chemical constituents (, N, P, K, C, and pH) inherent in the Kpalsawgu soil series

3.2.1. Soil sampling and analysis

The analysis of the soil samples was done by following standard procedures referenced in the handbook of methods on tropical soil biology and fertility by Anderson and Ingram (1993). At the experimental site, soil samples were taken in the morning at 9:00 am GMT at a depth of 15 cm using a soil augur. Ten (10) samples were collected randomly into polyethylene bags at different locations in the field. The samples were oven-dried at 104 ^{o}C for 24 hours, pulverized into finer particles. They were sieved and composited to run an analysis. The samples were divided into three replicated analysed for the presence and concentration of N, P, K, C, Cu, Cd, Pb, Zn, and pH. This was carried out at the analytical laboratory of the Soil Research Institute in Kwadaso, Kumasi, Ghana.

The Ethylene diamine tetracetic acid (EDTA) with ammonium acetate as a universal extractant was used for the isolation of the selected heavy metals (Cd, Pb, Cu, and Zn). The isolation procedure was as outlined by Lindsay and Norvell (1978) as follows:

- 10 g of the soil sample was weighed into a 100 millilitre (ml) bottle.
- 30 ml of 0.05 of the EDTA was added to the sample and kept on a reciprocal shaker for 2 hrs.
- This was filtered into a 50 ml flask.

- An aliquot (1ml) of the filtrate was taken each time for the determination of the various heavy metals using the Atomic Absorption Spectrophotometer (AAS) (Thermo Scientific I C E 3000 series). This enables a calibration curve of absorbance from the AAS reading against concentration to be plotted and the heavy metal concentrations determined by extrapolation.

The other soil chemical constituents (pH, percent organic carbon, percent nitrogen, available phosphorous and available potassium) were determined in various ways as follows:

Thus:

3.2.1.1. Soil pH

Normally, a soil: water ratio in the range of 1:1 – 1:4 in addition to a standardized pH electrode are required for the determination of soil pH depending on the soil type and a particular laboratory. In this study, the determination of the pH was done in water in a ratio of 1:2.5 using a pH meter of model 3330 (Jenway Ltd., Essex, UK). Ten (10) grams of the experimental soil sample was added to 25 ml of water, stirred and left to stand for about an hour and the pH electrode after standardizing, was dipped into the set up and the reading taken (Karla, 1995; Seer, 2002).

3.2.1.2. Organic carbon (%) content

The Wakley and Black method adopted from Schumacher (2002) was used to determine the organic carbon content. With this method, a weight of 2 g of the experimental soil sample was transferred into a conical flask in which 10 ml of 1 N of potassium dichromate ($K_2Cr_2O_7$) was added as an oxidizing agent. Twenty (20) ml of sulphuric acid (H_2SO_4) was then added to ensure thorough solubility and mixing of the soil with the reagent. This was left in the fume hood to cool. After cooling, 100 ml of water was added and left to cool. Thereafter, 2-3 drops of Diphenylamine were added as an indicator and titrated against 0.5 N Iron (II) sulphate (Fe_2SO_4). At the endpoint, there was a change in colour from violet to green and the titre value (T) was recorded. The organic carbon (%) content was calculated as follows:

Organic carbon (%) = T x 0.2 x 0.3/W

Where T = titre value

W = sample weight

3.2.1.3. Nitrogen (%) content

The nitrogen content was determined by the wet oxidation / Kjedahl method (Persson *et al.*, 2008). The Kjeldahl method enables a precise determination of available nitrogen (N) in the soil. This method involves three successive phases, viz:

1. Digestion of the organic material to convert N into nitric acid (HNO_3).

2. Distillation of the released ammonia into an absorbing surface or medium.
3. Volumetric analysis of the ammonia formed during the digestion process.

A Kjeldahl digestion mixture was made by adding 0.42 g of selenium powder and 14 g of lithium sulphate in 350 ml of 30 % hydrogen peroxide. Four hundred and twenty (420) ml of concentrated sulphuric acid (H_2SO_4) was added slowly and cooled in a water bath. The soil sample (0.2 g) was weighed and transferred into a digestion tube (100 ml) and digested with 4.4 ml of the Kjedahl digestion mixture. It was then heated at 360 ^oC for 2 hours at which temperature the mixture turned from dark brown to a colourless solution. The content was allowedto cool and distilled water was added to it while swirling until it reached the 100 ml mark. An aliquot (50 ml) of the sample was then taken and distilled through the vapodest, into a conical flask containing pink boric acid. As the boric acid reacted with the nitrogen, it turned green. It was then titrated with 0.1 M HCl from the green colour back to pink, giving the sample titre value. A blank titre was also carried out by steaming ammonia-free distilled water and the distillate was collected and titrated with the HCl. The value obtained was subtracted from the sample titre value to give the corrected titre value.

The nitrogen content (%) was then calculated as:

$$N (\%) = (T \times S \times 0.01) / (A \times W)$$

Where N = nitrogen (%)
T = corrected titre value (ml)
S = final digest solution volume (ml)
A = aliquot volume (ml)
W= sample weight (g)

3.2.1.4. Phosphorus content

A sample of the soil (5 g) was weighed into a shaken bottle. Thirty five (35) ml of Bray 1 extraction solution was added and shaken on a mechanical shaker for 8 minutes. The solution was then filtered through a Whatman filter paper number 42. A blue colour developed and the intensity of the colour (absorbance) was measured on the Ultra Violet Visible (UV-Vis) Spectrophotometer (model 7305, Bibby Scientific, Staffordshire, UK) at 690 nm (DSNR, 1995; vlab.amrita.edu, 2013). The measured value in the UV – Vis represented the amount (ppm) of phosphorous (P) in the solution.

The P concentration was therefore calculated as follows:

$$P = Ps \times St/W$$

Where P = phosphorous in soil (ppm)

Ps = phosphorous in solution (ppm)

St = total extracting solution (ml) used

W = weight (g) of soil sample

3.2.1.5. Potassium

Five (5) grams of the soil sample was weighed and transferred into a shaken bottle and 50 mls of 1 N ammonium acetate ($NH_4CH_3CO_2$) extraction solution was added and shaken on a mechanical shaker for 2 hours. The solution was filtered through a Whatman filter paper number 42. Then, the solution was dispensed in a Flame Photometer of model PFP7 (Bibby Scientific, Stafford-shire, UK) where the potassium concentration was read (IRD, 2009). The reading (intensity) was then plotted against concentration and the concentration of the potassium in the sample extrapolated from the graph.

3.3. Determining the presence and concentration of selected heavy metals and chemical constituents identifiable in Composted Deep Litter Chicken Manure, ACARP compost, and DeCo

3.3.1. Soil amendment sampling and analysis

Samples of DeCo compost, ACARP compost and Composted Deep Litter Chicken Manure were taken to determine the presence and concentration of Pb, Cd, Cu, Zn, pH, N, P, K, and organic carbon content at the Chemistry laboratory, UDS, Navrongo Campus. Each of the three different organic soil amendments were contained in three different 50 kg polypropylene bags. Each bag was opened and ten (10) grams of the soil amendment taken from the top, middle and bottom and then mixed well for the determination process in each case of Cd, Pb, Zn, Cu, N, P, K, C, and pH as treatments. The determination was done using the same reagents and procedures as in 3.2.1.

3.4. Determining the effect of DeCo compost, ACARP compost, Composted Deep Litter Chicken Manure and the inherent soil chemical constituents (pH, N, P, K, and C) on the growth, yield and nutrient content of roselle and jute mallow

3.4.1. The experimental site

The experimental site was as described in **3.1.**

3.4.2. Seed acquisition

Seeds of common cultivars of jute mallow were acquired from a seed grower in Gbulahgu, a farming community in the Tolon district while that of roselle were obtained from farmers at Builpela in the Tamale metropolitan area.

3.4.3. Nursery management

The seeds of both crops were sown and nursed in nursery boxes. For the jute mallow, the seeds were thermally primed in luke warm water of about 60 ^{o}C for 30 minutes to soften the seed coat and facilitate germination/uniform germination (Ghassemi-Golezani *et al*., 2010; Bölek *et al*., 2013). Before sowing, seeds were shade – dried and thinly laid in drilled lines in the sowing/ nursery boxes, buried lightly with soil, mulched lightly with grass straw and watered. The seeds of roselle were however not primed as its seed coat is not as hard as jute mallow and so will normally germinate and emerge. Seedling emergence started from the third day after sowing and continued up to a week; at which time, about ninety percent (90%) of the seedlings had emerged. The seedlings were watered as and when necessary.

Plate 4. Seedlings of jute mallow and roselle raised in nursery boxes

3.4.4. Land preparation

A one-acre land was prepared by ploughing and harrowing to obtain a fine tilth. An area of 20 m x 15 m was lined and pegged to demarcate the experimental plots from the prepared area.

3.4.5. Experimental design and layout

The experiment was laid out in a randomized complete block design (RCBD) replicated four times in each case of roselle and jute mallow. A block contained 4 plots representing 4 treatments {ACARP compost applied soil amendment, DeCo compost applied soil amendment, Composted Deep Litter Chicken Manure applied soil amendment and no soil amendment application (control)}. Each experimental plot measured 3 m x 3 m. the plots were spaced 1 m apart. Thus, 16 plots each of roselle and jute mallow were used for the field experiments.

Plant height, number of leaves, leaf yield and SPAD index were parameters considered for determining the performance of jute mallow and roselle in relation to the application of DeCo compost, ACARP compost and Composted Deep Litter Chicken Manure as organic soil amendments.

3.4.6. Application of the organic soil amendments

The organic soil amendments were applied at a rate of 10 t/ha. This was done spreading them gently on the soil and incorporating them into the plots using a hand hoe. The amendment of the soil was carried out two weeks prior to transplanting.

3.4.7. Transplanting

The seedlings of both crops were transplanted onto prepared plots on the flat. Transplanting distances were 40 cm x 60 cm for roselle (McClintock and El Tahir, 2011) and 50 cm x 50 cm (Fondio and Grubben, 2011) for jute mallow. Plant population of each experimental plot was thirty eight (38) for roselle and thirty six (36) for jute mallow.

3.4.8. Weed management

The weed population increased from the first week after transplanting. Therefore, weeding was carried out the second weeks after transplanting and two weeks after the first weeding. This was done manually using a hand hoe.

3.4.9. Pest control

A broad-spectrum insecticide (Bastion extra) with Imidacloprid as active ingredient was applied to control insect pests. It applied once by burying the granules 15-20 in the middle of the rows at a rate of 10 kg/ha.

3.4.10. Data collection

Data was collected on agronomic and yield parameters specifically, plant height, number of leaves, SPAD index and leaf yield.

Plate 5.Women harvesting Roselle and Jute mallow in the field

3.4.10.1 Plant height

Data was collected on the three inner rows constituting sixteen (16) plants in each experimental plot leaving out border rows.

Plant height measurements were done at four weeks after transplanting (4WAT), six weeks after transplanting (6WAT), and eight weeks after transplanting (8WAT). Plant height (cm) was measured from soil level to the terminal growing point of the main stem using a measuring tape.

3.4.10.2. Number of leaves

Number of leaves per plant was conducted on the four inner rows constituting sixteen plants in each experimental plot leaving out border rows. The number of leaves was estimated by counting the total number of sound (green, fully open leaves, and without infestations or infections) leaves on the sampled plants.

3.4.10.3. Leaf weight (g)

In each case of a plot, leaves were harvested on the sixteen plants innermost part of the plot when the leaf cover could be considered economical at each point of the plant's growth. Harvest at each point in time was weighed fresh using a digital scale and the cumulative fresh weight computed at the end of the final harvest. The weight was recorded in grams (g) and converted to kilograms per hectare (kg/ha). At each harvest, the leaves were shade-dried (to retain the nutrient status) after taking the fresh weight. Drying continued until the samples maintained constant weight. They were then weighed to obtain the dry weight. The difference between the fresh weight and the dry weight was taken as the weight of moisture content. Percent moisture content was calculated as follows:

$$\%\ \text{Moisture content} = \qquad \text{fresh weight} - \text{dry weight}/$$

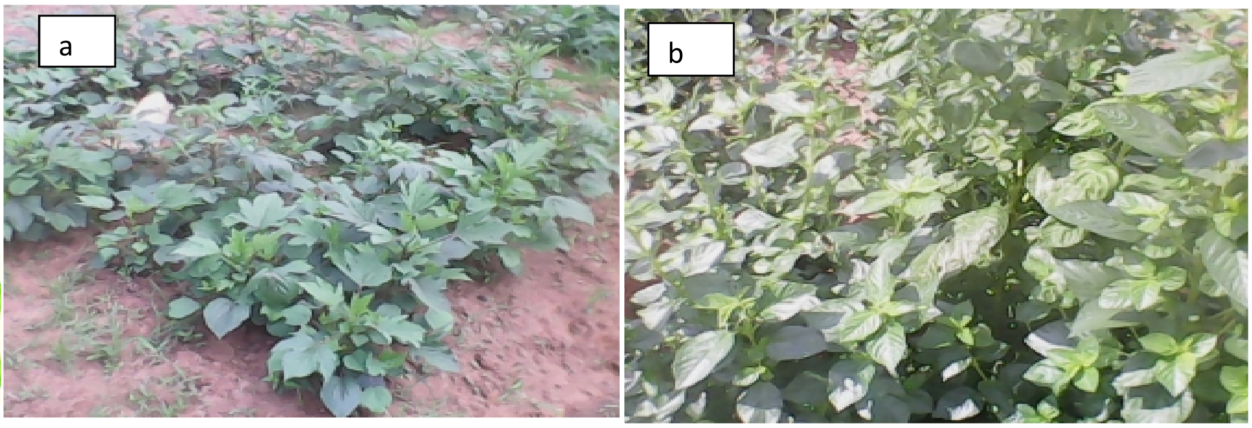

Plate 6. . Roselle (a) and Jute mallow (b) plants in the field

3.4.10.4 SPAD index

Data on SPAD index was measured on the sixteen inner plants in each plot for both crop species. SPAD index was measured using a SPAD meter (SPAD 502, Konica Minolta, Osaka, Japan). SPAD index is a measure of the greenness of the leaf based on optical response of the leaf upon exposure to light (Konica Minolta Inc., 2015). The SPAD meter was clipped to the leaf blade and the resultant optical response values displayed on the screen of the meter. Readings were made and recorded on two sound leaves each in case of a sample plant to represent the reading (SPAD index) for a treatment at a time. This was done for the sixteen sampled plants and then averaged. The higher the value, the greener the leaves and hence its nitrogen content and health status

3.5. Determining the presence and concentration of selected heavy metals (Cd, Pb, Zn, and Cu) and other chemical constituents (pH, N, P, K, and C) in harvested leaves of jute mallow and roselle

Harvested leaves of sample plants of both jute mallow and roselle crop species were dried in an oven at 70 ^{o}C for 48 hours. They were then removed, milled and sieved through a 1mm mesh. The samples were weighed into different crucibles (0.5 g) and positioned in a muffle furnace to char. This was done at a temperature of 450 ^{o}C for 3 hours. They were left to cool after which the samples were removed from the furnace and 10 ml of dilute Nitric acid solution was added to each of the samples. The samples were kept on a hot plate until the first sign of boiling (fine water bubbles) was detected. They were filtered into a 20 ml flask and with diluted distilled water to the 20 ml mark. One milliliter (1 ml) of the solution was injected into an AAS of model 210 VGP (Buck Scientific, East Norwalk, USA) for the determination of the of Cd, Pb, Zn and Cu. The absorbance of these elements at different wavelengths by their corresponding detection lamps in the AAS were plotted against concentration in a calibration curve by extrapolation.

Plate 7. Atomic Absorption Spectrophotometer Buck Scientific, Model 210 VGP

The concentration of the target heavy metals in the leaves of jute mallow and roselle crop species was determined as follows:

Concentration of heavy metal (ppm) = C x df.

Where C = heavy metal concentration from AAS reading

df = dilution factor as outline by Motsara and Roy (2008).

3.6. Determination of Bio-Concentration Factor (BCF) in leaves of jute mallow and roselle

The BCF is a ratio of the concentration of heavy metals in the plant to that in the soil (Ndeda and Manohar, 2014). A higher ratio of plant to soil heavy metal concentration indicates that there is a higher transfer of a heavy metal from the soil to the plant and vice versa. After the determination of each of the selected heavy metals (Pb, Cd, Zn, and Cu) concentration in the soil as in 3.2.1, that determined in the leaves of each of jute mallow and roselle for the different soil amendments were divided by that of the soil and the values obtained represented the BCF for each heavy metal determined at a time. Determination of the BCF was therefore accomplished by reference to Ndeda and Manohar (2014), and Zhao and McGrath (n.d.)

Thus, BCF = PTc / Sc

Where PTc = plant tissue concentration of heavy metal (mg/kg)

Sc = soil concentration of heavy metal (mg/kg)

3.7. Comparing the levels of the selected heavy metals (Pb, Cd, Zn, and Cu) found in leaves of jute mallow and roselle with maximum residue levels

The concentration of Pb, Cd, Zn, and Cu found in the leaves of jute mallow and roselle crops were in each case of the different test crops, compared to standards on contaminants in foodstuff (European Commission, 2006) and maximum residue levels of chemicals in food (FAO, 2011) to ascertain their safety for consumption. The hazard quotient (HQ) was computed for the heavy metals (Pb, Cd, Zn, and Cu) to determine the health risk that could be linked with the consumption of these test crops according to Sharma *et al*. (2016). The HQ is the average daily dose (ADD) of a pollutant determined in a sample relative to a reference dose (RfD). The ADD is defined as "dose rate averaged over a pathway-specific period of exposure expressed as a daily dose on a per-unit body weight basis" while RfD is defined as "the numerical estimate of a daily oral exposure to the human population, including sensitive subgroups such as children, that is not likely to cause harmful effects during a lifetime". If the ratio of the HQ is less than 1, it means the population exposed to the heavy metal is unlikely to experience adverse effects form it. However, if it is greater than 1, then the is likely to suffer adverse health effect from it. The HQ is used to characterize the health risk of consuming vegetables within a locality (Hough *et al*., 2004), and this is frequently used to assess the non-cancer health risk associated with heavy metals consumed through food (USEPA, 2005).

The hazard quotient (HQ) is used to assess individual heavy metal. Thus:

$$HQ = \frac{ADD}{RfD} = \frac{C \ x \ IR \ x \ EF \ xED}{BW \ x \ AT \ x \ RfD}$$

Where *HQ* = Hazard Quotient (unitless)

ADD = Average Daily Dose (mg/kg-day) of a heavy metal

RfD = Reference Dose (mg/kg-day) of a heavy metal

C = heavy metal concentration on individual heavy metal basis in the edible part of the vegetable (mg/kg fresh weight basis).

IR = amount of daily vegetable consumption per head (kg/day per person).

EF = Frequency of exposure to a heavy metal (350 days/year).

ED = exposure duration (6 years for child, 30 years for adult).

BW = body weight of the consumer (24.5 kg for child, 60.3 kg for adult).

AT = average lifetime for non-carcinogens (ED-365 days/year).

NB: The IR in Ghana was 0.137 kg/day per person (Ruel *et al.*, 2005).

The HQ of heavy metals was calculated separately for children (3–12 years old) and for adults (18– 45 years old).

The occurrence of a potential carcinogenic threat to any population under this type of study was also assessed for each of the test crops. This was accomplished by computing the hazard index (HI) of the heavy metals in leaves of jute mallow and roselle as described by Lui *et al*. (2013). The hazard index (HI) is a summation of all the hazard quotients for each heavy metal. When the value obtained is less than one, the population under study is not exposed to any carcinogenic threat and vice versa.

Thus: HI $= \sum_{i=1}^{n} HQi$

Where HI = hazard index

$\sum_{i=1}^{n} HQi$ = the summation of the individual hazard quotients (HQs).

Data obtained from analysis of the concentration of Cd, Pb, Zn, and Cu in jute mallow and roselle leaves, and the reported values for the consumption pattern of vegetables in Ghana together with the estimated body weights of adults and children from literature were used in calculating the HQ for each of Cd, Pb, Zn, and Cu in jute mallow and roselle leaves.

3.4.8. Data analysis

The analysis of variance (ANOVA) on the data collected was carried out using GenStat statistical package version 9.2. Treatment means were separated at the 5% level of significance using the Fisher's least significant difference (LSD) (Gomez and Gomez, 1984).

CHAPTER FOUR

4.0. RESULTS

4.1. Concentration of the selected heavy metals (Cd, Pb, Zn, and Cu) and other chemical constituents (pH, N, P, K, and C) inherent in the experimental soil (Kpalsawgu series)

The Cd, Pb, Zn, and Cu concentrations in the soil (Table 2) were either within or even below the levels reported as being safe for crop cultivation.

4.1.2. pH

The pH (4.4) of the soil (Table 2) indicated an acidic condition as it was below the neutral level of 7.0. The pH of the soil is one of the important factors that influence the bioavailability of heavy metals in plants. Low pH tends to make heavy metals readily bioavailable to plants. Hence, the pH of the soil under this study could not be an exception.

4.1.3. Nitrogen (N)

The N concentration (%) in the soil was found to be above what was reported by the MoFA (2011).

4.1.4. Phosphorous (P)

The P concentration in the soil was above the concentration range reported by FAO (2005) for the Guinea savannah soils.

4.1.5. Potassium (K)

The K concentration in the soil was similar to reported values by the MoFA (2013) for the Guinea savannah soils.

Table 2. Comparison of the heavy metals and other chemical constituents in the experimental soil with some standards

Soil chemical constituent	Concentration	Reference values
pH	4.4	[a]4.5 -6.7
C (%)	2.0	[a]0.6 -2.0
N (%)	0.2	[a]0.02 -0.05
P (mg / kg)	38.7	[a]2.5 -10
K (mg / kg)	261.3	[b]140 -450
Zn (mg kg)	17.6	[c]70
Cd (mg / kg)	0.5	[d]0.1 -0.5
Pb (mg / kg)	5.5	[e]10 -30
Cu (mg / kg)	6.5	[f]50

The values in the table are means of three replicates on dry matter basis

[a]Ministry of Food and Agriculture (MoFA), Ghana (2011, 2013); FAO (2005). [b] FAO (1982). [c]USEPA (2005), [d] ATSDR (2012). [e]WHO (2007). [f]ATSDR (2004)

4.2. Concentrations of the selected heavy metals (Cd, Pb, Zn, and Cu) and other chemical constituents (pH, N, P, K, and C) in DeCo, ACARP compost, and Composted Deep Litter Chicken Manure

4.2.1. Heavy metals

All the three organic soil amendments contained Pb, Zn, Cd, and Cu (Table 3). The concentrations of Zn in Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost were 3.2 mg/kg, 19.5 mg/kg, and 1.8 mg/kg respectively. Cadmium concentrations were 0.1 mg/kg, 3.4 mg/kg and 0.5 mg/kg in Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively. For Pb, the concentrations were 0.7 mg/kg, 4.2 mg/kg and 1.6 mg/kg Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively while that of Cu was 1.1 mg/kg, 6.8 mg/kg and 2.8 mg/kg in Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively.

4.2.2. pH

The pH of the various amendments (Table 3) range from 3.9, 4.3 and 4.8 for the Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively. All the values indicated acidic conditions being below 7.0 (McCauley et al., 2009). The pH was however not significantly different among the various amendments.

4.2.3. Nitrogen (N)

The N concentrations (Table 3) in the amendments were 0.6 %, 0.3 % and 0.3 % for Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost.

4.2.4. Phosphorous (P)

The P concentrations (Table 3) were 72.3 mg/kg, 59.6 mg/kg and 79.1 mg/kg in Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively.

4.2.5. Potassium (K)

The K concentrations in the amendments (Table 3) were 446.9 mg/kg, 368.6 mg/kg and 494.4 mg/kg in Composted Deep Litter Chicken Manure, ACARP compost and DeCo compost respectively.

4.2.6. Organic carbon (C)

As can be seen in Table 3, the C content in the Composted Deep Litter Chicken Manure (7.5 %) was significantly different from DeCo compost (3.6 %) and ACARP compost (1.6 %).

Table 3. Comparison of the heavy metals and other chemical constituents in the soil amendments with some standards

Chemical constituent	CDLCM	ACARP Compost	DeCo Compost	Reference values
pH	3.9	4.3	4.8	[a] **4.5 -6.7**
C (%)	7.5	1.6	3.6	[a] **0.6 -2.0**
N (%)	0.6	0.3	0.3	[a] **0.02 -0.05**
K (mg/kg)	446.9	368.6	494.4	[b] **140 -450**
P (mg/kg)	72.3	59.6	79.1	[a] **2.5 -10**
Cd (mg/kg)	0.1	3.4	0.5	[d] **0.1 -0.5**
Cu (mg/kg)	1.1	6.8	2.8	[f] **50**
Zn (mg/kg)	3.2	19.5	1.8	[c] **70**
Pb (mg/kg)	0.7	4.2	1.6	[e] **10 -30**

[a] MoFA, Ghana (2011, 2013); FAO (2005). [b] FAO (1982). [c] USEPA (2005) [d] ATSDR (2012). [e] WHO (2007). [f] ATSDR (2004)

4.3. Effect of Composted Deep Litter Chicken Manure, ACARP compost, DeCo compost on the growth, yield and nutrient content of roselle and jute mallow

4.3.1. Plant height (cm) of roselle

The effect of the different soil amendments on plant height of roselle was not significantly different at 4WAT (Figure 1). However, at 6WAT, there was a significant difference between the ACARP compost and the control but the Deco compost and Composted Deep Litter Chicken Manure were not significantly different from each other nor with the control. Also at 8WAT, there was a significant difference in plant height between the DeCo compost and the control but the ACARP compost and the Composted Deep Litter Chicken Manure were not significantly different from each other.

For jute mallow, there were no significant differences among the various soil amendments including the control with respect to plant height at 4 and 6WAT (Figure 1). At 8WAT, Composted Deep Litter Chicken Manure with the highest plant height of 53.4cm was significantly different from the ACARP compost soil amendment and the control but not with DeCo compost. The DeCo compost was significantly different from the control, which had the least plant height of 36.7 cm. The ACARP compost and the control were however not significantly different from each other.

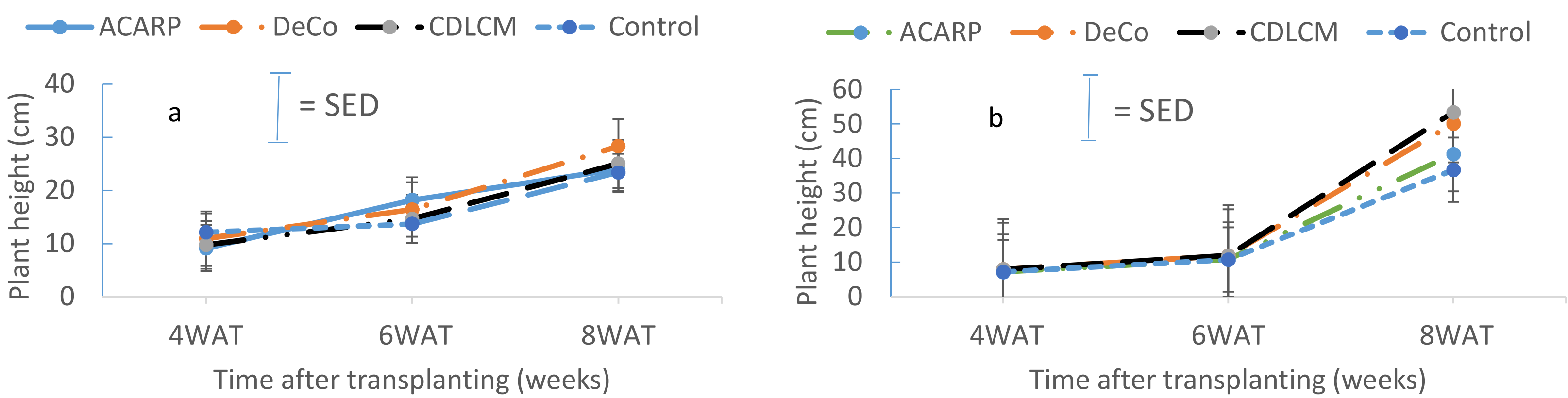

Figure 1. Effect of organic soil amendments on plant height of Roselle (a) and Jute mallow (b)

4.3.3. Number of leaves of jute mallow

Soil amendment had no significant effect on the number of leaves at 4 weeks after transplanting and 6 weeks after transplanting (Figure 2). However at 8 weeks after transplanting, Composted Deep Litter Chicken Manure treated plants had the highest number of leaves per plant (123.6) and was significantly different from the control but not significantly different from DeCo compost (109 leaves) and ACARP compost (88 leaves) composts.

4.3.4. Number of leaves of roselle

At 4 weeks after transplanting and 6 weeks after transplanting (Figure 2), there were no significant differences among all the soil amendments with respect to number of leaves but at 8 weeks after transplanting, all the treatments were significantly different from the control. Number of leaves was highest in Composted Deep Litter Chicken Manure followed by DeCo compost, ACARP compost, and the least in the control treatment.

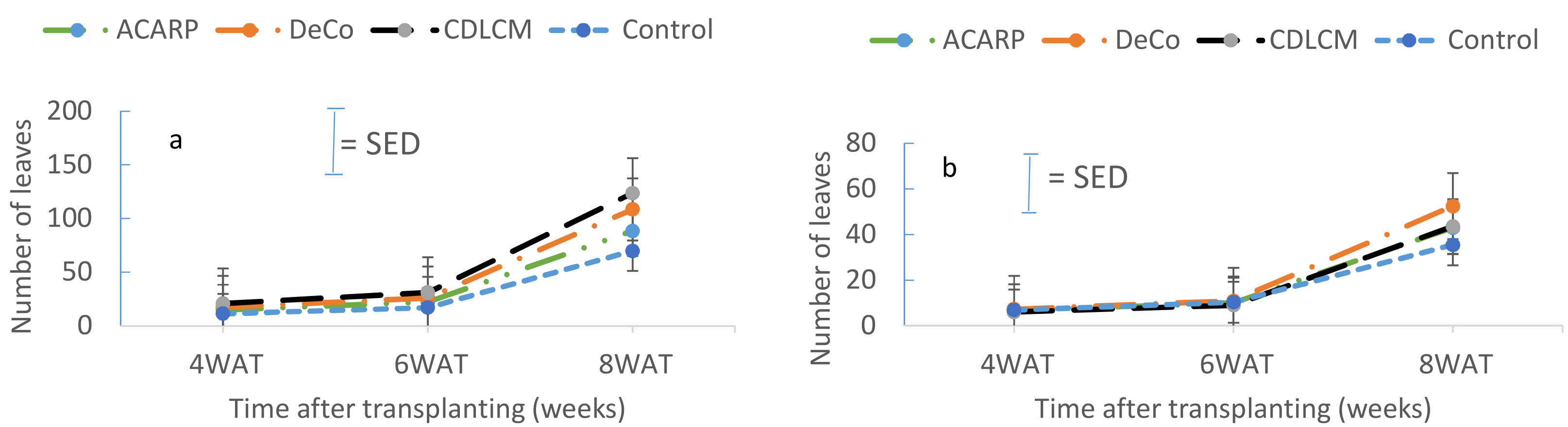

Figure 2. Effect of organic soil amendments on number of leaves in Jute mallow (a) and Roselle (b)

4.3.5. SPAD index of jute mallow

With the exception of ACARP compost, jute mallow plants treated with Deco compost and Composted Deep Litter Chicken Manure resulted in increase in SPAD values (Table 4). The SPAD indices of plants raised from DeCo compost and Composted Deep Litter Chicken Manure treated plots were significantly ($P \leq 0.05$) different from that of ACARP compost but not with the control. The mean SPAD index were 42.97, 47.67, 47.42 and 44.92 for ACARP compost, DeCo compost, Composted Deep Litter Chicken Manure and control respectively.

4.3.6. SPAD index of roselle

The application of soil amendments resulted in increase in SPAD values in roselle leaves (Table 4). The SPAD index of plants raised in DeCo compost treated plot was significantly different from that of the control but the rest of the soil amendments were not significantly different from the control. The SPAD index values were 45.25, 46.7, 44.83 and 43.6 for ACARP compost, DeCo compost, Composted Deep Litter Chicken Manure and control respectively.

4.3.7. Leaf yield of jute mallow

The analysis of variance showed that, cumulative leaf yield (Table 4) from the Composted Deep Litter Chicken Manure treated plot was significantly ($P \leq 0.05$) different from the control and the other amendments. Also, DeCo and ACARP composts were also significantly different from the control but were not significantly different from each other. Leaf yield in the control plot was the lowest (319 kg/ha) while that of Composted Deep Litter Chicken Manure recorded the highest yield of 799 kg/ha.

4.3.8. Leaf yield of roselle

It was observed that leaf yield (Table 4) of Composted Deep Litter Chicken Manure treated plot had significantly highest yield of 1498 kg/ha and this was significantly ($P \leq 0.05$) different from

ACARP compost (1008 kg/ha) and the control (1112 kg/ha) but not significantly different from DeCo compost (1323 kg/ha). Leaf yield from ACARP compost and DeCo compost amended plots were not significantly different from that of the control.

4.3.9. Moisture content of jute mallow leaves

The application of soil amendment resulted in increased moisture content (Table 4) of jute mallow leaves. The leaf moisture content from Composted Deep Litter Chicken Manure treated plot was significantly different from that of the control but not with DeCo compost and ACARP compost. In addition, moisture content in leaves from ACARP compost and DeCo compost plots were not significantly different from each other nor with the control.

4.3.10. Moisture content of roselle leaves

From the results (Table 4), the moisture content (%) of roselle leaves in the Composted Deep Litter Chicken Manure amended plot was significantly different from that of the ACARP compost, DeCo compost and the control. However, there were no significant differences in moisture contents between the ACARP compost and DeCo compost amended plots and that of the control.

Table 4. SPAD index, total leaf yield and leaf moisture content of Roselle and Jute mallow

Soil amendment	SPAD Index		Total leaf yield (kg/ha)		Leaf moisture content (%)	
	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow
Control	43.6	44.92	1,112	319	89.25	79.72
ACARP	45.25	42.97	1,008	483	88.57	82.55
DeCo	46.7	47.67	1,323	558	89.05	83.22
CDLCM	44.83	47.42	1,498	799	90.72	86.90
LSD (5%)	2.89	4.3	323	159.8	3.92	4.55
p-value	0.15	0.16	0.03	<0.001	0.76	0.06

4.3.11. Nutrient content in roselle leaves

It was observed (Table 5) that the protein content of the control was higher (29.2) than the rest of the soil amendments and was significantly different (P≤ 0.05) from the DeCo compost (26.4) compost but not significantly different from ACARP compost (28.6) and Composted Deep Litter Chicken Manure (27.6). The application of the soil amendments therefore decreased the protein content in the leaves of roselle

There were no significant differences among the soil amendment with respect to the moisture content, percent carbon and ash content. The nitrogen content had a similar trend with the protein content.

4.3.12. Nutrient content in jute mallow leaves

In jute mallow leaves (Table 5), there were no significant differences (P≤ 0.05) among the soil amendments for all the nutrients determined. From the proximate analysis carried out, the protein content in leaves of the jute mallow decreased with application of soil amendments and followed a decreasing order of control > Composted Deep Litter Chicken Manure > ACARP compost > DeCo compost. For moisture content, the order of decrease was DeCo compost > Composted Deep Litter Chicken Manure > ACARP compost > control. With respect to the nitrogen content, the control, ACARP compost and Composted Deep Litter Chicken Manure had the same mean value of 4.4 while DeCo compost had a mean of 4.0. The carbon content was also in the decreasing order of ACARP compost > Composted Deep Litter Chicken Manure > control > DeCo compost while the ash content also varied in decreasing order of DeCo compost> control > Composted Deep Litter Chicken Manure > ACARP compost. This meant that contents of the nutrients varied from one compost to another in terms of quantities and therefore none of the composts nor the control maintained a higher or lower ranking for all the nutrients.

Table 5. Effect of different organic soil amendments on the nutrient content of Roselle and Jute mallow leaves.

Soil amendment	Protein content (%)		Moisture content (%)		Nitrogen (%)		Carbon (%)		Ash content (%)	
	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow
Control	29.2	27.8	13.6	16.5	4.7	4.4	42.2	41.8	15.7	16.4
ACARP	28.6	27.4	9.9	19.4	4.6	4.4	43.8	42.7	12.4	14.6
DeCo	26.4	25.2	25.5	28.5	4.2	4.0	41.7	40.9	16.7	18.2
CDLCM	27.6	27.6	8.7	26.2	4.4	4.4	42.2	42.0	15.7	16.0
LSD (5%)	2.5	4.4	19.9	23.9	0.4	0.7	3.7	3.9	7.4	7.7
p-value	0.2	0.7	0.4	0.8	0.2	0.7	0.8	0.9	0.8	0.9

Values in the table denote means of three replicates on dry matter basis

4.4. Effect of soil amendments on the concentration of Cd, Pb, Zn, Cu, and other chemical constituents in leaves jute mallow and roselle

4.4.1. Concentration of heavy metals in leaf of roselle

The results showed that (Table 6) all the four metals (Zn, Cu, Cd, and Pb) tested for were found in the leaf samples of roselle. The DeCo compost had the highest concentration of 31.5 mg/kg of Zn compared to the ACARP compost and Composted Deep Litter Chicken Manure amendments. This was significantly ($P \leq 0.05$) different from that of the ACARP compost (18.8 mg/kg) and Composted Deep Litter Chicken Manure (18.6 mg/kg) but not with the control (22.3 mg/kg). There were however, no significant differences among the soil amendment with respect to the concentrations of Cd, Pb and Cu in the leaves of roselle.

4.4.2. Concentration of heavy metals in leaf of jute mallow

All the four heavy metals (Zn, Cu, Cd, and Pb) were found (Table 6) in the leaf samples of jute mallow. Leaves of jute mallow from the ACARP compost-amended plots recorded the highest concentrations (26.5 and 17.6 mg/kg) of Zn and Cu respectively but these were not significantly different from those of DeCo compost and Composted Deep Litter Chicken Manure nor with the control. Among the various soil amendments, there were no significant differences with respect to Pb and Cd concentrations of jute mallow leaves.

Table 6. Concentrations (mg/kg) of Zn, Cu, Cd, and Pb in harvested leaves of jute mallow and roselle

Soil amendment	Zn		Cu		Cd		Pb	
	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow	Roselle	Jute mallow
Control	22.3	16.7	12.5	15.9	0.6	0.9	3.6	5.9
ACARP	18.8	26.5	12.5	17.6	0.6	0.6	4.1	6.7
DeCo	31.5	22.8	11.5	16.3	1.1	1.0	5.9	5.2
CDLCM	18.6	16.6	11.4	15.3	0.9	0.4	4.9	5.4
LSD (5%)	12.1	9.1	5.4	2.8	1.2	0.8	3.4	5.2
p-value	0.2	0.1	0.7	0.3	0.9	0.6	0.6	1.0

NB: the values in the table denote means of four replicates expressed on dry weight basis

4.4.3. Effect of organic soil amendments (ACARP compost, Deco and Composted Deep Litter Chicken Manure on Bio-Concentration Factors (BCF) in leaves of jute mallow and roselle

The values of the BCF obtained in both jute mallow and roselle were in the decreasing order of Zn > Cu > Pb > Cd as shown in Table 7 below. This indicates a higher accumulation of the two essential) heavy metals (Zn and Cu) and lower accumulation for the two carcinogenic heavy metals (Pb and Cd) in the leaves of jute mallow and roselle.

Table 7. Bio-Concentration Factors of Pb, Cd, Cu, and Zn in leaves of jute mallow and roselle

Heavy metal	BCF – roselle	BCF - jute mallow
Zn	1.30	1.17
Cu	1.84	2.50
Cd	1.60	1.45
Pb	0.84	1.05

Values in the table denote means of three replicates expressed on dry matter basis

4.5. Comparing residual levels Cd and Pb determined in the leaves of roselle and jute mallow with standards (maximum residue levels)

Though all the four heavy metals (Zn, Cu, Cd, and Pb) tested for were found in the leaf samples of roselle, the two carcinogenic metals – Cd and Pb which are more important with respect to human health were compared (Table 8) with Maximum Residue Levels (MRLs) in Codex Alimentarius Commission and European Commission standards. Both were found to be at levels above the MRLs of 0.2 mg / kg for Cd and 0.3 mg / kg for Pb as set by the European Commission (2006) and FAO/WHO (2011) for leaf vegetables, brassicas, fresh herbs and cultivated fungi.

Table 8. Comparison between heavy metals (Cd and Pb) concentration (mg/kg) in leaves of jute mallow and roselle with maximum residue levels

Test crop	Type of soil amendment	Heavy metal concentration	
		Cd	Pb
Jute mallow	Control	0.9	5.9
	ACARP	0.6	6.7
	DeCo	1.0	5.2
	CDLCM	0.4	5.4
Roselle	Control	0.6	3.6
	ACARP	0.6	4.1
	DeCo	1.1	5.9
	CDLCM	0.9	4.9
Standard European Commission (2006); FAO/WHO (2011)		**0.2**	**0.3**

4.5.1. Effect of organic soil amendments on adult health risk of heavy metals in leaves of jute mallow and roselle

Generally, HQs for Zn and Cu in each case of the study on the leaves of jute mallow and roselle were below 1.0 (Figure 3 and 4). However, the HQs of Pb and Cd on average were more than 1.0 indicative of a potential risk to children and adult populations. From the analysis, the risk was more distinct in roselle for the adult population than in jute mallow but variable in children.

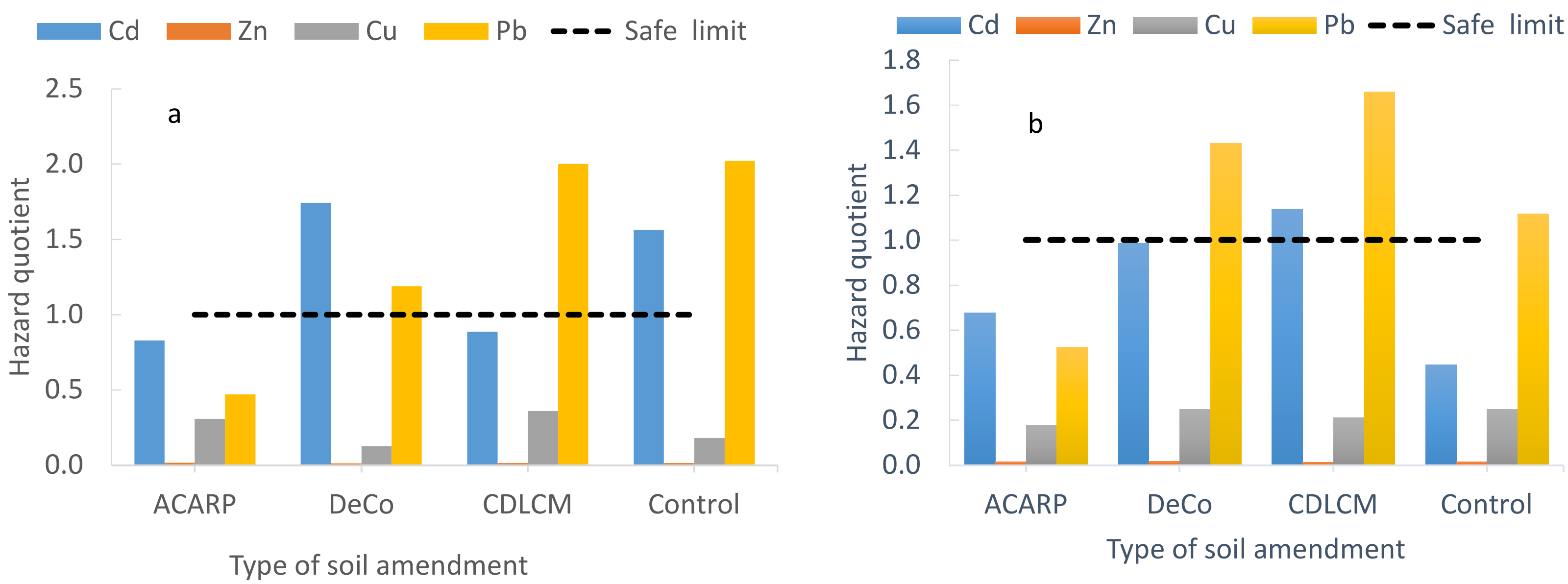

Figure 3. Health risk assessment of heavy metals in Roselle (a) and Jute mallow (b) leaves for adults

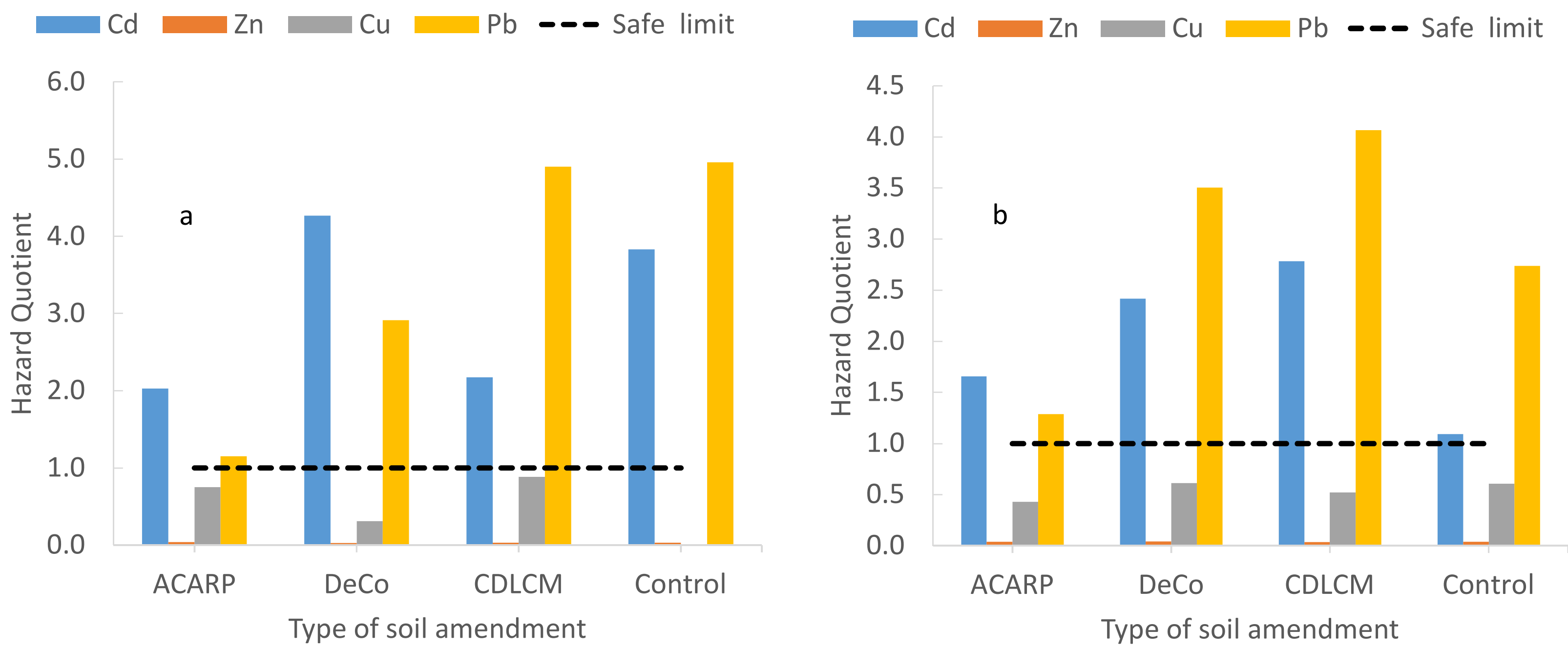

Figure 4. Health risk assessment of heavy metals in Roselle (a) and Jute mallow (b) leaves for children

4.5.2. Hazard indices for Cd, Zn, Cu and Pb in jute mallow and roselle leaves

It was observed that, the HI (Table 9) in order of high risk were Pb > Cd > Cu > Zn for roselle and jute mallow as well as for both adult and children. The results have shown that the levels of Zn in both roselle and jute mallow, could not pose any carcinogenic risk because HIs were less than one (1). However, Pb, Cd and Cu had HIs greater than one (1). The risk levels were the same for both the adult and the child but the average weight of 24.7 kg was used for children in the calculation. This average weight which was lower than that of the adult (60 kg), led to the greater risk of exposure relative to adults.

Table 9. Hazard indices for Cd, Zn, Cu and Pb in Jute mallow and Roselle leaves

Crop	Heavy metal	HI (adult)	HI (child)
Roselle	Cd	2.8	6.9
	Zn	0.3	0.6
	Cu	0.9	2.3
	Pb	3.9	9.5
Jute mallow	Cd	2.4	5.9
	Zn	0.2	0.5
	Cu	1.3	3.2
	Pb	4.7	11.5

HI = Hazard Index

CHAPTER FIVE

5.0. DISCUSSIONS

5.1. Presence and concentration of heavy metals and other chemical constituents in the soil (Kpalsawgu series)

The heavy metals (Cd, Pb, Cu and Zn) were found to be present in the study site. The experimental site is situated in a vicinity of 200 m from the main Tamale – Nyankpala road where vehicular activity on the road is brisk on daily basis. The experimental site had also been under continuous agriculture activities with the application of various agro-inputs (fertilizers, pesticides and weedicides) for several years. Hence, the presence of the heavy metals in the soil at the site could be inherent because of the natural soil forming processes at the site but could also be due the human activities outlined. With respect to heavy metal deposition in the environment due to vehicular emission, Abechi *et al*. (2010) in their findings reported that, there is a positive correlation between the concentration of Pb and Cd in the environment and traffic volumes. Similar to this findings were as reported by Popescu (2011) and Pal *et al*. (2010) where they indicated that there was a positive relationship between vehicle emissions and Pb and Cd concentration in the environment. Adedeji *et al.* (2013) found that the concentration of these heavy metals tend to decrease with distance from the roadside. At high levels, Pb adheres to plant roots and its mobility into plant tissues is expedited by the absence of phosphates. Water and nutrient in rice fields to a high uptake of Cd into the plant roots.

In this study, the heavy metals concentrations in the topsoil were 0.5 mg/kg Cd, 5.5 mg/kg Pb, 6.5 mg/kg Cu and 17.6 mg/kg Zn. These concentrations are similar to that of a study conducted by Ayari *et al*. (2010), in which they found that the top 0-20 cm of soil contained Cu, Zn, Pb and Cd and their concentrations were 48.23 mg/kg Cu, 92.12 mg/kg Zn, 55.22 mg/kg Pb and 1.10 mg/kg Cd. The concentrations of Cd and Pb reported in this study were considered characteristic for most soils as contained in study reports by WHO (2007) and by Biernacka and Maluszynski (2006).

The concentration (0.5 mg/kg) of cadmium in the soil fell in line with results of other studies conducted by Chaney (2012) and Tchounwou *et al*. (2014) who indicated that, cadmium concentration normally range from 0.1 to 2 mg/ kg in most agricultural soils. These studies stipulated that, subsequent increase from this value could be due to human activities. Similarly, Alloway (1995) reported that soils that originate from igneous rocks have Cd concentrations ranging between 0.1 – 0.3 mg/kg while those from metamorphic rocks contain Cd ranging between 0.1 – 1.0 mg/kg and those from sedimentary rocks contain between 0.3 – 11 mg/kg of Cd.

The Pb concentration (5.5 mg/kg) found in the experimental soil was far below the 10 -50 mg/kg threshold by ATSDR (2012). The ATSDR in its report mentioned that Pb concentration in soils could reach as high as 200 mg/kg in urban areas due to industrial deposition. According to the WHO (2007), lead concentration in the top layer of soils varies considerably with soil depth and inversely related to the soil depth. Its deposition and accumulation, depends on the intensity of anthropogenic activities.

The concentration of Zn (17.6 mg/kg) found in the Kpalsawgu soil series was below averaged Zn concentrations in the soil (55 mg/kg) reported by Alloway (2008). According to the USEPA

(2005) Zn is one of the most common elements in the soil and it occurs at an average concentration of 70 mg/kg. Considering the concentration in the soils of the Kpalsawgu series, Zn toxicity could not be expected as its concentration was far below the concentrations that have been reported above. Alloway (2008) has also indicated that Zn availability is dependent on the soil pH, and it decreases as the pH increases. In this study however, the pH was low and so therefore, the risk of Zn toxicity was comparatively low.

The concentration (6.5 mg/kg.) of Cu found in this study was considered low relative to other findings. For instance, a report by the ATSDR (2004), indicated that the mean concentration of Cu in the soil is about 50 mg/kg. However, other studies such as that of Nachtigall *et al*., (2007) found Cu concentrations in some Brazilian soils to be as high as 1,300 – 1,400 mg/kg. Suciu *et al*., (2008) in a similar study, also found Cu levels in Zlatna, Romania to be as high as 1197.6 mg/kg. Therefore, the concentration of Cu in the soil varies from one location to the other.

For the chemical elements of the soil at the experimental site, it was found that the pH was 4.4. This was slightly below the reported range of 4.5 - 6.7 as indicated in reports by FAO (2005); MoFA (2011) and MoFA (2013) on the fertility status for the soils of the Guinea Savannah agro-ecological zone. These reports also indicated nitrogen concentration in the soils to be in the range of 0.02 – 0.05%. However, the average nitrogen content in the soil for this particular study was 0.1%, being higher than the reported values. Similarly, phosphorous which was 12 mg/kg in the experimental soil was slightly higher than the reported range of 2.5 – 10 mg/kg for the Guinea Savannah soils. A joint report submitted by IFPRI, IFDC, ILFSP, MSU and IITA (2015) on the fertility status of the Guinea Savannah soils of Ghana, showed the chemical constituents of the soil as follows: pH, 6.2 - 6.6; organic carbon, 0.51 – 0.99%; total N,0.02 - 0.12%, available P 0.06 -1.80 mg/kg and K and 36.96 - 44.51 mg/kg. Compared with reports provided by Antonio and John (2013), Pariera and Clain (2013), the concentrations of potassium, phosphorous copper and zinc in this study were relatively higher. The higher values obtained for the various parameters from the experimental site could be because the land had been under cultivation and the fact that various agro-chemicals such as fertilizer, weedicides, pesticides and fungicides have been applied to the soil, which could modify the chemical constituents of the soil. According to McCauley *et al*. (2009) macronutrients are more available when the soil pH ranges from 6.5 - 8.0 while most micronutrients are available at a pH range of 5 - 7. When the soil pH increases, metals are tightly bound to soil particles making them unavailable. Carbon: Nitrogen (C: N) ratio of the soil was 10:1 which was far below the ideal level of 24:1 (PerkinElmer Inc., 2010, USDA, 2011). This means that nitrogen mineralization under such condition is accelerated by soil microbes (Zhu, 2007), making the depletion of nitrogen in the soil very fast.

5.2. Presence and concentration of heavy metals and other chemical constituents in DeCo, ACARP compost, and Composted Deep Litter Chicken Manure

From the study, the Composted Deep Litter Chicken Manure was found to contain the four heavy metals (Cd, Pb, Cu and Zn) analysed for. In support of this report, similar studies conducted by Delgado Arroyo *et al.*, (2014); Agriculture and Agri-food Canada (1990) and Chastain *et al*., (n.d.) reported that poultry manure also contains heavy metals such as Pb, Cd, Zn, and Cu apart from the nutrients that it contains for plant growth. A correlated report by McCall (1980) showed that the quantity of heavy metals found in chicken manure varies with respect to certain factors. These include the age of the chicken, the age of the manure and its moisture content, the type and quantity of litter in the pen and storage and handling practices of the manure. The report also mentioned that, due to the variations in the chemical composition of the manure, it is important to conduct compositional analysis on it to be certain of particularly, the heavy metal concentration in it.

In the Composted Deep Litter Chicken Manure, the concentrations of Cd was 0.1 mg/kg, 0.7 mg/kg Pb, 1.1 mg/kg Cu and Zn 3.2 mg/kg. The Composted Deep Litter Chicken Manure was obtained from an intensive management system with sawdust used as the litter material. The manure was further composted and therefore, the heavy metal concentration could reflect what pertains to that particular management system as well as the handling process. The feed material fed to the birds also affect the heavy metal concentration in the manure. For instance, Nicholson *et al*., (1999) reported that, feed for layers generally contain higher levels of heavy metal contents than that for broilers. In that study, they observed that concentration of Zn reached up to 400 mg/kg and that of Cu up to mg/kg on dry matter basis for a classic poultry feed. Similarly, Adesoye *et al*. (2014) also found that manure from layers contained more of Zn, Pb, Ni and Mg than for manures from other domestic animals. As far as litter material is concerned, Delgado Arroyo *et al*. (2014) reported that there are variations in Cd, Zn, Pb, Cu and other heavy metals in straw and sawdust litter material for poultry manure.

DeCo compost, a municipal solid waste compost which is source-separated, contained the four heavy metals (Zn,Cu, Cd, and Pb) in addition to N, P, K and C. The Zn concentration in this study was 1.8 mg/kg and Cu was 2.8 mg/kg while that of Cd was 0.5 mg/kg and Pb was 1.6 mg/kg. Related to this findings, Ghaly and Alkoaik (2010), found that, an average value for Zn and Cu the organic portion of municipal solid waste was 211.0 mg/kg being higher that what was found in this study. According the European Commission (2004), the acceptable level for Cu in waste material for agricultural use is 150 mg/kg making the experimental soil suitable for use in agriculture.

The ACARP compost, which is also a municipal waste compost had the highest concentration of heavy metals (19.5 mg/kg Zn, 6.8 mg/kg Cu, 3.4mg/kg Cd and 4.2mg/kg Pb) compared to the DeCo compost and the Composted Deep Litter Chicken Manure. The higher availability of the heavy metals could be due to the lower pH values observed in the composts materials, which makes the heavy metals more labile. A similar study by Ayari *et al*. (2010) though with higher concentrations compared to this study, mentioned that compost from municipal solid waste contained 1174.5 mg/kg of Zn, 337 mg/kg of Cu, 411.5 mg/kg of Pb and 5.17 mg/kg of Cd.

The concentrations of heavy metal in these composts were compared to the European Commission standards (EC, 2004) Annex 2 on compost suitability for crop production. In the ACARP compost, the Cd content (3.4 mg/kg) exceeded the 1mg/kg limit threshold in this standard and could be accordingly graded as B (low quality: suitable for non-agriculture use). However, the concentrations of Zn, Pb and Cu in it were all within the acceptable limit making the combined concentration suitable for use in agriculture. The DeCo compost and the Composted Deep Litter Chicken Manure per their composition of the heavy metals, could be classified as grade A (suitable for agriculture production).

The concentrations of Cd, Zn, and Pb in the ACARP compost as embossed on the packing material were 1.07 mg/kg, 0.06 mg/kg, and 0.26, mg/kg respectively while the N, P and K were 0.3 mg/kg, 9.0 mg/kg, and 7.5 mg/kg respectively. In this current study however, the analysis done prior to its application showed Cd to be 3.4 mg/kg, Pb to be 4.2 mg/kg, Zn to be 19.5

mg/kg and Cu to be 6.8 mg/kg which are far above what the company estimated. Also, the percent nitrogen was found to be 0.3, while phosphorous was 59 mg/kg and potassium 368.6 mg/kg. Though these concentrations obtained differed from that of the company, it could not necessarily be a deliberate underestimation but could be due variations in each production batch and procedural variations.

5.3. Effect of Composted Deep Litter Chicken Manure, ACARP and DeCo composts on the performance of roselle and jute mallow

5.3.1. Plant height and number of leaves

This study showed that compost application has an effect of plant growth parameters. To support this claim, Khatab (2016) reported that, compost application has a significant effect on plant height, number of leaves and number of branches of roselle. In a similar study, Akinfasoye *et al.*, (2008) examined the effect of compost fertilizer from maize stover on growth parameters of spinach and stated that the compost application led to significant differences in plant height, stem girth and number of leaves. However, the significant differences in this study on plant height of jute mallow was not realised at the early stages of the plant growth i.e. 4 weeks after transplanting and 6 weeks after transplanting. At this early stage, the plants were still not fully recovered from transplanting shock and the roots not well developed to absorb adequate nutrients for growth. However, as the plants advanced in growth, differences in the plant height began to show. For instance at 8WAT, the Composted Deep Litter Chicken Manure – treated plots recorded the highest plant height and this was significantly different from the ACARP compost and the control plots but not with the DeCo compost. Related to this, a study by Aluko *et al.* (2014) evaluated the effect of fertilizer application on plant height and indicated that, differences in plant height was not expressed at the early stage until at eight weeks after planting.

Masarirambi *et al*. (2010) also mentioned that plants treated to chicken manure recorded higher values of plant height, number of leaves and leaf yield compared to the other composts. This trend was similarly observed for the number of leaves in both jute mallow and roselle. This conclusion is corroborated by Mahmoud *et al*., (2014) who indicated that increasing the level of nitrogen could significantly influence the growth characteristics in jute mallow.

5.3.3. SPAD index

SPAD values range between 0.0 – 50.0 (Konica Minolta Incorporated, 2015) and the higher the value, the higher the relative nitrogen concentration in the leaf blade. In this study, the SPAD values varied from one soil amendment to the other and between roselle and jute mallow in no specific order. For instance, mean SPAD value recorded for DeCo compost – treated plants in jute mallow was 47.67 which meant that their nitrogen concentration was higher compared to those of the other soil amendments. Subsequent to this was the Composted Deep Litter Chicken Manure, followed by the control and ACARP compost in decreasing order. Thus, the SPAD indices of DeCo compost and Composted Deep Litter Chicken Manure were significantly ($P \leq 0.05$) different from that of ACARP compost but not with the control. Also, mean SPAD value of DeCo compost – treated plants of roselle was highest, followed by ACARP compost, Composted Deep Litter Chicken Manure and the control. The leaf nitrogen content however, was not examined unlike in the study by Percival *et al*., (2008) and Coste *et al*., (2010) where they mentioned that, SPAD values correspond directly to nitrogen concentration in plants and thus, signifies health status of the plants. Mielke *et al*., (2010), supported this explanation where they mentioned that there is a positive relationship between SPAD values and the nitrogen concentration in plants. In this study, it was observed that there were positive correlations between SPAD values and growth parameters particularly, plant height and number of leaves in both roselle and jute mallow.

5.3.4. Leaf moisture content (%)

Though leaf moisture content were not significantly different for all the organic soil amendments, the recorded values did not vary from the reported mean of 82.7 % for jute mallow according to Fondio and Grubben (2004) and 85.6 % for roselle according to McClintock and El Tahir (2011) per 100 g edible portion of the plant. This means that the moisture contents measured in this study for jute mallow and roselle were as anticipated as far as these reference values are concerned.

5.3.5. Leaf yield

Leaf yield cumulated over the period indicated that the yield from the Composted Deep Litter Chicken Manure-treated plots were significantly different ($P \leq 0.05$) from that of the ACARP compost, DeCo compost and the control treatments in jute mallow. Also, DeCo and ACARP composts were significantly different from the control but were not different from each other. The control had the lowest leaf yield of 319 kg/ha compared to the compost-amended plots. The Composted Deep Litter Chicken Manure had the highest yield of 799 kg/ha followed by DeCo compost of 558 kg/ha and ACARP compost of 483 kg/ha. In a related study by Eifediyi *et al.*, (2013), they reported on the effect of organic fertilizer on the performance of jute mallow where the control plots had the lowest yields compared to different levels of the organic fertilizer applied. Ginindza *et al*., (2015) also observed a similar trend in different rates of fertilizer applied to jute mallow.

From this study, it was observed that, the yield of roselle in the Composted Deep Litter Chicken Manure was highest (1498 kg/ha) and this was significantly different ($P \leq 0.05$) from that of the ACARP compost of 1008 kg/ha and the control of 1112 kg/ha. The higher yield in the Composted Deep Litter Chicken Manure could be the result of its relatively higher percentage of nitrogen (0.3%) which was higher than ACARP compost of 0.2% and DeCo compost of 0.2%. Similar studies conducted by Mera *et al.,* (2009); Oyewole and Mera (2010); Atta *et al.*, (2010) and Haruna *et al*., (2011) stated that, the use of poultry manure, farmyard manure or increasing N application rates of nitrogenous fertilzers matched with the increase in growth and yield of roselle.

5.3.6. Nutrient content

With the exception of the protein content, the ash, carbon and moisture contents were not significantly different among the various soil amendments. The moisture content of the roselle was in the range of 8.7 % - 25.5 % which according to a study by Asaolu *et al.*, (2012) is suggestive of the ability of the plant tissue to support enzymatic activity. They found percent moisture content in the dry leaves of roselle to be 12.5%, protein content to be 46.6% and the ash content to be 7.5 %.

In the jute mallow leaves, this study noted higher values of protein, moisture, and ash contents which were comparable to a related study on leafy vegetables by Idoko *et al*., (2014) where they determined protein, moisture, and ash contents to be 3.5%, 14.2% and 13.7% respectively for jute mallow. There were however no significant differences amongst the soil amendments for the moisture, carbon, protein and ash contents. In a related report by Adediran *et al*., (2015), it was found that fertilizer application did not affect nutrient content significantly in harvested plant leaves.

5.4. Concentration of Cd, Pb, Zn and Cu in leaves of jute mallow and roselle

In this study, Cd concentration was found to be above the 0.2 mg/kg Maximum Residue Level while Pb concentration was also above the 0.3 mg/kg Maximum Residue Levels as set by the European Commission (2006) standards for leaf vegetables such as brassicas, fresh herbs and cultivated fungi. It has been reported that though the soil is considered the main translocation route of heavy metals into plants, wastewater use in vegetable production (Girisha and Ragavendra, 2009) also increases the concentration and uptake of heavy metals by plants. For most of the heavy metals, their uptake into roots of plant takes place in the aqueous phase. A strong binding of heavy metal to soil particles and/or precipitation renders a greater portion of heavy metal in the soil fraction insoluble, and largely unavailable for plant uptake. Low bioavailability of heavy metals such as lead in the soil is a major factor limiting the potential for phytoextraction of significant heavy metal contaminants (Lasat, 2002). However, each heavy metal need to be assessed separately as each has its unique chemistry.

Some heavy metals have little potential for redox change with changes in the redox status of soils. Reduced soils can form sulfide, and sulfide forms low-solubility compounds with most of the heavy metals of concern in soils, including Pb, Zn, Cd, Cu, and Ni. For essential heavy metals (e.g., Zn, Cu, Ni), low-solubility species can result in deficiency symptoms. When there is oxidation in the soil, sulfide is quickly oxidized, and the heavy metals are returned to more normal equilibrium reactions of aerobic soils (USEPA, 2007).

5.4.1. Cadmium

Cadmium concentration in the harvested leaves of roselle was found to be 0.6 mg/kg in the control, 0.6 mg/kg in the ACARP compost, 1.1mg/kg in DeCo and 0.9 mg/kg in Composted Deep Litter Chicken Manure. In the jute mallow leaves, the concentrations were 0.9 mg/kg in the control, 0.6 mg/kg in ACARP compost, 1.0 mg/kg in DeCo and 0.4 mg/kg in Composted Deep Litter Chicken Manure. In this study, though the values obtained in both jute mallow and roselle were not significantly different for the different organic soil amendments, they were higher than the Maximum Residue Levels set by Codex Alimentarius Commission (FAO/WHO, 2011) and European Commission (EC, 2006) standards of 0.2 mg/kg for leafy vegetables.

The slight variations in the Cd levels for jute mallow and roselle could be because of the different Cd uptake capacities of the different plant species. Different studies mentioned that different factors could affect the uptake and bioaccumulation of Cd in crops. These include the species and cultivar (Stritsis and Claasen, 2013), soil pH (Wang *et al*., 2006), soil and feriliser Zn, P fertiliser rate and Cd concentration (Grant *et al*., 2010), chloride content in the soil and Zn deficiency (Smolders, 2001), rate and form of N fertilizer (Alpha *et al*., 2009) and additional factors such as soil drainage, cropping systems and tillage practices (Pavlikova *et al*., 2007).

The pH of the soil is usually found to be a highly significant factor in influencing the uptake and accumulation of Cd in plants. Because of this, any changes in soil pH (especially in the rhizosphere) due to the form and rate of N-fertilizer applied may significantly affect Cd levels in plants (Chaney 2012). According to McCauley *et al.*, (2009), the lower the soil pH, the higher the mobility of Cd and its subsequent uptake in some plants that can tolerate the level of acidity. Similarly, Smolders (2001) indicated that there is a negative relationship between Cd uptake in field grown plant and soil pH. The pH of the soil in this study was 4.4, which could be the additional reason apart from other factors that accounted for the higher concentration of Cd in the leaves of jute mallow and roselle. Hattori *et al*., (2006), studied the effect of chloride application and low pH on Cd uptake in kenaf and sorghum and found that low pH treatment resulted in increased Cd uptake by kenaf plants.

5.4.2. Lead

The concentration of Pb on dry matter basis in roselle was 3.6 mg/kg in the control, 4.1 mg/kg in ACARP compost, 5.9 mg/kg in DeCo and 4.9 mg/kg in the Composted Deep Litter Chicken Manure. In jute mallow, Pb concentration was 5.9 mg/kg in control, 6.7 mg/kg in ACARP, 5.2mg/kg in DeCo and 5.4 mg/kg Composted Deep Litter Chicken Manure. These concentrations were all above the Codex Alimentarius Commission and European Commission maximum residue levels (MRLs) of 0.3 mg/kg for vegetables. In a similar study conducted by Wamalwa *et al*., (2015), leafy vegetables were analysed for heavy metal and the results showed that Pb levels in the leaves were above the MRLs. Tangahu *et al*., (2011), found that, even though Pb in the soil is immobile as compared to other heavy metals, there is a genotypic variation in its uptake even in the same species. In relation to this, Cheng *et al*., (2006) studied the genotypic and environmental variation in Pb among five heavy metals in nine rice genotypes and found that there was both genotypic and environmental variation in the concentration of Pb in the rice grains. According to a study conducted by Sharma and Dubey (2005), it was indicated that the uptake of Pb in plants is influenced by the soil pH, cation exchange capacity, particle size of the soil, and other physico- chemical properties. Wierzbika (1995) in a study found out that, plants are capable of accumulating high amount of Pb without showing any visible signs of stress because Pb accumulates within the cell wall without penetrating the protoplast and that the accumulated Pb is therefore transferred into the body of the consumer.

5.4.3. Zinc

As an essential plant element, Chao *et al*., (2007) indicated that Zn concentration in plants below 50 mg/kg dry matter satisfies the physiological requirements of the plants. However, it becomes toxic to the plant when the concentration goes above this threshold. Therefore, the concentrations determined in the study implies an ideal phenomenon for the different amendments in both jute mallow and roselle.

In roselle leaves, zinc (Zn) was found to be 22.3 mg/kg in the control, 18.8 mg/kg in ACARP, 31.5 mg/kg in DeCo and 18.6 mg/kg in Composted Deep Litter Chicken Manure amended plots.

In jute mallow leaves, the concentration of Zn was 16.7 mg/kg in the control, 26.5 mg/kg in ACARP, 22.8 mg/kg in DeCo and 16.6 mg/kg in Composted Deep Litter Chicken Manure amended plots.

5.4.4. Copper

The concentration of Cu in the leaves of roselle on dry matter basis was 12.5 mg/kg in control, 12.5 mg/kg in ACARP, 11.5 mg/kg in DeCo and 11.4 mg/kg in Composted Deep Litter Chicken Manure amended plots. Considering the concentrations determined in this study, Chao *et al*., (2007) indicated that Cu concentration in plants below 20 mg/kg dry matter satisfies the physiological requirements of the plants. However, above this threshold, toxicity effects will be manifested.

In jute mallow, the Cu concentration was 15.9, 17.6, 16.3 and 15.3 mg/kg for control, ACARP, DeCo compost and Composted Deep Litter Chicken Manure respectively. With the exception of the ACARP compost, there were no significant differences among the various amendments with respect to Cu concentration. Related to this, Aydinalp and Marinova (2012) reported that the concentration of Cu in fruits varied from 2.4 – 25 mg/kg and 1.2 – 2.2 mg/kg in vegetables.

5.4.5. Bio-concentration factor (BCF)

From the study, it was found that the uptake (transfer of heavy metals from the soil to leaves) capability of both roselle and jute mallow of the heavy metals being investigated was in a decreasing order of Zn>Cu>Pb>Cd. A similar trend was reported exactly by Yan *et al*., (2012), where they studied the relationship between heavy metals in soil and grass and found the BCF in the order as presented above. It is worth noting however that, the uptake of a heavy metal is dependent on several factors. These include the heavy metal concentration in the soil, the soil pH and the plant species/characteristics among others (USEPA, 2007). Strongly acidic soils increase Zn, Cu and Cd uptake and increases their phytotoxicity. In this study, there were variations between roselle and jute mallow as far as the heavy metal concentration is concerned. For instance, the application of Deco compost tendered to increase Zn bio-concentration in roselle and jute mallow. Additionally, ACARP compost also increased Zn bio-concentration in jute mallow. For Cu, ACARP compost and Deco compost increased its bio-concentration in jute mallow. For Cd, ACARP compost and Composted Deep Litter Chicken Manure decreased its bio-concentration in jute mallow. Bio-concentration of Pb decreased with the application of Deco Compost and Composted Deep Litter Chicken Manure. Laboratory studies conducted by the Environmental Protection and Heritage Council (2003) also affirmed that there are variations in the ability of plants to bioaccumulate heavy metals. The studies observed that differences in bioaccumulation exist for plant types as well as the nature of the heavy metal.

5.5. Comparing residual levels of heavy metals in leaves of roselle and jute mallow with standards to ascertain the safety of the leaves for consumption

The concentrations Pb, Cd, Zn and Cu assessed in the harvested leaves were compared with the FAO/WHO Codex Alimentarius Commission (2011) and European Commission (2006) standards. Cd concentration in the leaves was found to be above the MRL of 0.2 mg/kg while Pb concentration was also above MRL of 0.3 mg/kg. Similar studies by Ramteke *et al*., (2016), Islam and Hoque (2014) and Yang *et al*., (2017) reported that Pb and Cd levels in vegetables were above the MRLs. Zn and Cu concentrations were however within acceptable safety levels. A study by Aydinalp and Marinova (2012) also reported, that the concentrations of Zn and Cu were within safety levels in the fruits and vegetables they assessed.

5.5.1. Health risk (HQ and HI) of Zn, Cd, Pb, and Cu in leaves of jute mallow and roselle

5.5.1.1. HQ

In this study, the HQ of Cd in roselle for adults was 6.3 and 15.4 for children and in jute mallow; it was 4.0 for adults and 9.8 for children. From the study, the risk of exposure was higher in children as compared to their adult counterparts. This finding is corroborated by SCOOP (2004), where it was indicated that, as children consumed less food compared to adults, they are predisposed to higher health risk due to their lower body weight. A higher body weight results in a dilution effect as the heavy metal concentration diminishes with increased body weight. A health risk assessment of heavy metal toxicity conducted by Singh *et al*., (2010) in thirteen (13) vegetables, found that the HQ of Cd exceeded one (1) in nine (9) of the vegetables while three (3) vegetables exceeded one (1) for Pb with one (1) and two (2) vegetables exceeding 1 in Cu and Zn respectively. According to the WHO (2011), heavy metals may be harmful to humans through the consumption of edible plants containing heavy metals through normal uptake, or the consumption of plants splashed with contaminated soil or by accidental direct ingestion of soil (especially in the case of children).

This study reported the HQ of Pb in roselle in adults as 7.3 and 18.0 in children and in jute mallow, it was 5.7 for adults, 18.0, and 13.9 for children. A similar risk assessment by Jena *et al*., (2012) in leafy vegetables, found HQ for Pb to be 7.22, which per the value exposes the consumer to health risk linked to the consumption of the vegetables. Based on the risk analysis conducted in this study, children were more at risk than their adult counterparts were. The WHO (2010) indicated in a study that, the susceptibility of children to the risk of lead exposure is greater than that of adults. This is because the developing brain of human undergoes rapid growth, development and differentiation, and Pb can interfere with these extraordinarily complex and delicate transformation processes. The study also mentioned that absorption of Pb into the gastrointestinal tract is enhanced in childhood i.e. up to 50% of lead ingested by children is absorbed into the body, as compared with 10% absorption in adults. In a related study conducted by Zhou *et al*., (2016), the HQ in vegetables for Pb was found to be 1.15 for adults and 1.51 for children and that of Cd was 2.49 for adults and 3.27 for children, indicating a higher risk of children to exposure to these heavy metals.

In this study, the HQ of Cu in roselle was 1.2 for adults and 1.3 for children. In jute mallow, it was 2.9 for adults and 3.2 for children. The assumed adult body weight 60 kg and that of the child was 24.5 kg. The HQ of Zn in roselle and jute mallow in adults (60 kg body weight) was 0.1 for both vegetables and 0.2 for children (24.5 kg body weight). As seen from the hazard analysis, Cd, Pb and Cu for both roselle and jute mallow had HQ values above 1.0 implying their potential toxicity to both the adult and child consumer. This is similar to a study conducted by Sharma *et al*., (2016) where the health risk of 12 vegetables were assessed and 11 were found to be most hazardous to children in terms of the HQ for Cu, Cd and Pb. In this study however, the HQ for Zn was below 1 for both crops making the consumption of these vegetables less risky as regards Zn toxicity. In another study conducted by Winiarska-Mieczan (2009), it was reported that, infants and little children are the highest risk group as far as their exposure to heavy metals is concerned. The report indicated that newly born babies do not have effective functioning regulatory mechanisms and the absorption of mineral elements, including toxic ones, is higher than in older children and adults whose regulatory mechanisms are more advanced. A health risk analysis by Yang *et al.*, (2017) also put children at a higher risk of exposure to heavy metals than adolescents and adults.

5.5.1.2. HI

The Hazard Index for both jute mallow and roselle were both higher than 1.0 indicating the collective effect of all the heavy metals in the two vegetables. This implies that summing all the heavy metals in each crop still pose a risk to the consumer though their individual risk may be lower. In a study on the effect of gold mining on heavy metal pollution around Obuasi and its surrounding communities in Ghana and the risk on consumption of vegetables irrigated with the polluted water, Bempa and Ewusi (2016) found that the HI in vegetables cultivated in the area was higher than 1, indicative of a hazard risk from the consumption of the vegetables. A similar study by Lente *et al.*, (2012) found that jute mallow and cabbage irrigated with wastewater in Accra, Ghana, posed a risk from their consumption as their HIs were above 1. Zeng *et al.*, (2015), found that in the Tianjin area in China, the HI index in wheat was more than 1 for 36.4% of adults and 63.6 % children, attesting to the vulnerability of children to the potential risk of heavy metals in dietary intake. Other studies have found much higher HI for children and adults, such as that carried out by Zhuang *et al.*, (2014) where the computed HI were for adults was

10.3 and 11.1 for children. Ghosh *et al*., (2013) also found HI of Pb and Cd to be higher than 1 in a number of vegetables evaluated.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

Since the study was centered on certain objectives, its outcome in terms of results and observations led to the following conclusions based on the specific objectives of the study:

The experiment confirmed presence of heavy metals (Zn, Cu, Cd, and Pb) and other chemical constituents (N, P, K, and C) naturally inherent in the soil of the Kpalsawgu soil series on which the experiment was conducted. Compared to some reference standards, they were considered to be at safe levels. Because the heavy metals are inherent in the soil, the continuous and indiscriminate application of heavy metal – containing substrates (both organic and inorganic) could increase the soil load and transfer of heavy metals to cultivated crops.

The soil amendments - ACARP compost, Composted Deep Litter Chicken Manure, and DeCo compost also contained heavy metals (Zn, Cd, Cu, and Pb) and other chemical constituents (N, P, K, and C) and when these are amended to the soil could increase the soil load and transfer of heavy metals to cultivated crops.

It was found that the application of these composts resulted in yield increase as compared to the control. Among the three organic soil amendments, the Composted Deep Litter Chicken Manure generally gave the highest leaf yield. From the nutritional point of view, roselle and jute mallow were found to contain protein and minerals, which are essential for the well-being of the human body.

It was also observed that there was bio-transfer of the heavy metals from the soil to edible portions of roselle and jute mallow i.e. the leaves. This implies that, the continuous consumption of the leaves of the crops make bioavailable the heavy metals to the human body. There were however, variations in the bio-transfer of the heavy metals in jute mallow and roselle. Overall Zn was more bio-accumulated followed by Cu, then Pb and Cd in both roselle and jute jute mallow. The bio-accumulation also varied with the different soil amendments. For Zn, high accumulation was observed in roselle under the Deco compost – treated plots while in jute mallow, it was observed in ACARP and Deco-compost plots. For Cu, high bioaccumulation was observed in jute mallow and this occurred in ACARP compost and Deco compost plots. With Cd, high bioaccumulation was observed in Deco compost and Composted Deep Litter Chicken Manure plots in roselle but in jute mallow, this occurred only in Deco compost plot. High Pb bioaccumulation was in ACARP compost plots for both roselle and jute mallow but Deco compost and Composted Deep Litter Chicken Manure plots had high accumulation of Pb in roselle. With the exception of Cd and Pb in roselle, the Composted Deep Litter Chicken Manure decreased the bio-concentration of all the heavy metals studied.

In comparing the residue levels of the non-essential heavy metals, it was found that Cd and Pb concentrations in the leaves of jute mallow and roselle exceeded Maximum Residue Levels as set by the the FAO/WHO (Codex Alimentarius Commission) and European Commission standards. From the health risk analysis conducted, it was realized that the heavy metal Pb followed by Cd posed the greatest risk to consumers (both adults and children). Zinc and Cu being essential heavy metals did not pose a health threat to the consumers.

6.1 Recommendations

As leafy vegetables are regarded as hyperaccumulators of heavy metals, it is recommended that the soils on which these are cultivated be assessed for their heavy metal content to enable the gardener determine the appropriate fertilizer to apply to reduce the potential biotransfer of the metals into edible parts. This is because, most leafy vegetables are minimally processed and even if it has to be processed, the process will not be able to eliminate the heavy metal in the value chain.

It is recommended that the concentration of heavy metals in compost and/or any soil amendment including inorganic fertilizer intended to be applied to vegetable gardens be known so that a reasonable rate can be applied to avoid heavy metal build up in the soil. For this, manufacturers should be display the heavy metal concentrations on the compost package.

As lower pH tends to make the heavy metals more labile than at higher pH, the pH of soils and soil amendments should be managed to limit the easy transfer of the heavy metals into the edible parts of plants

Given the rate of application under this study, which led the concentration of heavy metals as residues in the roselle and jute mallow, it is recommended that consumers be circumspect in the consumption of such vegetables produced without recourse to safety measures in the production and handling process.

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APPENDIX

1. ANALYSIS OF VARIANCE TABLES

```
"Data taken from unsaved spreadsheet: New Data;1"
DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
READ [PRINT=*; SETNVALUES=yes] _stitle_
PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 24-Feb-2019 17:47:23

```
DELETE [REDEFINE=yes] Crop,Treatment,Rep,Jute_Plant_ht_4WAT,\
Jute_Plant_ht_6WAT,Jute_Plant_Ht_8WAT,Jute_No_of_leaves_4WAT,\
Jute_No_of_leaves_6WAT,Jute_No_of_leaves_8WAT,Jute_Chlorophyll_content,\
Fresh_wt_g,Fresh_wt_2_g,Leaf_yield_Total_g,Jute_Fresh_wt_kg_ha,leaf_dry_wt_g\
,leaf_dry_wt_kg_ha,leaf_%_moisture_content,leaf_dry_matter_%,Root_fresh_wt_g\
,Root_fresh_wt_kg_ha,Root_dry_wt_g,Root_dry_wt_kg_ha,root_%_moisture_content\
,root_dry_matter_%
UNITS [NVALUES=*]
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('cochorus')\
; REFERENCE=1] Crop
READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Crop	16	0	1

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
'Control','Deco'); REFERENCE=1] Treatment
READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
VARIATE [NVALUES=16] Jute_Plant_ht_4WAT
READ Jute_Plant_ht_4WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_Plant_ht_4WAT	5.600	7.519	11.20	16	0

```
VARIATE [NVALUES=16] Jute_Plant_ht_6WAT
READ Jute_Plant_ht_6WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_Plant_ht_6WAT	10.20	11.28	12.30	16	0

```
VARIATE [NVALUES=16] Jute_Plant_Ht_8WAT
READ Jute_Plant_Ht_8WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing

Jute_Plant_Ht_8WAT	32.00	45.36	63.80	16	0

```
213  VARIATE [NVALUES=16] Jute_No_of_leaves_4WAT
214  READ Jute_No_of_leaves_4WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_No_of_leaves_4WAT	7.100	15.99	32.60	16	0

```
216  VARIATE [NVALUES=16] Jute_No_of_leaves_6WAT
217  READ Jute_No_of_leaves_6WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_No_of_leaves_6WAT	14.60	23.94	43.60	16	0

```
219  VARIATE [NVALUES=16] Jute_No_of_leaves_8WAT
220  READ Jute_No_of_leaves_8WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_No_of_leaves_8WAT	37.60	97.57	151.8	16	0

```
223  VARIATE [NVALUES=16] Jute_Chlorophyll_content
224  READ Jute_Chlorophyll_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_Chlorophyll_content	38.70	45.75	52.50	16	0

```
226  VARIATE [NVALUES=16] Fresh_wt_g
227  READ Fresh_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Fresh_wt_g	84.00	139.2	248.0	16	0

```
229  VARIATE [NVALUES=16] Fresh_wt_2_g
230  READ Fresh_wt_2_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Fresh_wt_2_g	63.00	132.2	211.0	16	0

```
232  VARIATE [NVALUES=16] Leaf_yield_Total_g
233  READ Leaf_yield_Total_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Leaf_yield_Total_g	147.0	271.4	431.0	16	0

```
235  VARIATE [NVALUES=16] Jute_Fresh_wt_kg_ha
236  READ Jute_Fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_Fresh_wt_kg_ha	245.0	452.3	718.3	16	0

```
239  VARIATE [NVALUES=16] leaf_dry_wt_g
240  READ leaf_dry_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_g	34.00	43.81	55.00	16	0

```
242  VARIATE [NVALUES=16] leaf_dry_wt_kg_ha
243  READ leaf_dry_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_kg_ha	56.70	73.01	91.70	16	0

```
245  VARIATE [NVALUES=16] leaf_%_moisture_content
246  READ leaf_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_%_moisture_content	76.50	83.08	88.30	16	0

```
248  VARIATE [NVALUES=16] leaf_dry_matter_%
249  READ leaf_dry_matter_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_matter_%	11.70	16.93	23.50	16	0

```
251  VARIATE [NVALUES=16] Root_fresh_wt_g
252  READ Root_fresh_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_fresh_wt_g	116.0	285.6	572.0	16	0

```
254  VARIATE [NVALUES=16] Root_fresh_wt_kg_ha
255  READ Root_fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_fresh_wt_kg_ha	193.3	475.9	953.3	16	0

```
258  VARIATE [NVALUES=16] Root_dry_wt_g
259  READ Root_dry_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_dry_wt_g	53.00	85.56	152.0	16	0

```
261  VARIATE [NVALUES=16] Root_dry_wt_kg_ha
262  READ Root_dry_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_dry_wt_kg_ha	88.30	142.6	253.3	16	0

```
265  VARIATE [NVALUES=16] root_%_moisture_content
266  READ root_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
root_%_moisture_content	39.70	66.57	81.30	16	0

```
269  VARIATE [NVALUES=16] root_dry_matter_%
270  READ root_dry_matter_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
root_dry_matter_%	18.70	33.43	60.30	16	0

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_No_of_leaves_4WAT
```

Analysis of variance

Variate: Jute_No_of_leaves_4WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	34.90	11.63	0.23	
Rep.*Units* stratum					
Treatment	3	192.65	64.22	1.29	0.336
Residual	9	448.06	49.78		
Total	15	675.62			

Tables of means

Variate: Jute_No_of_leaves_4WAT

Grand mean 16.0

Treatment	ACARP	CDLCM	Control	Deco
	14.7	20.7	11.3	17.3

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	4.99

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	11.29

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_No_of_leaves_6WAT
```

Analysis of variance

Variate: Jute_No_of_leaves_6WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	55.08	18.36	0.58	
Rep.*Units* stratum					
Treatment	3	412.73	137.58	4.33	0.038
Residual	9	285.65	31.74		
Total	15	753.46			

Message: the following units have large residuals.

Rep 2 *units* 3 9.9

Tables of means

Variate: Jute_No_of_leaves_6WAT

Grand mean 23.9

Treatment	ACARP	CDLCM	Control	Deco
	22.1	30.8	17.0	25.9

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	3.98

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	9.01

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_No_of_leaves_8WAT
```

Analysis of variance

Variate: Jute_No_of_leaves_8WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1500.9	500.3	0.76	
Rep.*Units* stratum					
Treatment	3	6629.2	2209.7	3.34	0.070
Residual	9	5951.5	661.3		
Total	15	14081.5			

Tables of means

Variate: Jute_No_of_leaves_8WAT

Grand mean 97.6

Treatment	ACARP	CDLCM	Control	Deco
	88.4	123.6	69.7	108.5

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	18.18

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	41.13

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_Chlorophyll_content
```

Analysis of variance

Variate: Jute_Chlorophyll_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	37.485	12.495	2.20	
Rep.*Units* stratum					
Treatment	3	59.570	19.857	3.50	0.063
Residual	9	51.005	5.667		
Total	15	148.060			

Message: the following units have large residuals.

Rep 1 *units* 4 -3.82

Tables of means

Variate: Jute_Chlorophyll_content

Grand mean 45.75

Treatment	ACARP	CDLCM	Control	Deco
	42.97	47.42	44.92	47.67

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.683

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	3.808

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_Plant_ht_4WAT
```

Analysis of variance

Variate: Jute_Plant_ht_4WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.442	1.481	0.43	
Rep.*Units* stratum					
Treatment	3	2.327	0.776	0.22	0.878
Residual	9	31.216	3.468		
Total	15	37.984			

Tables of means

Variate: Jute_Plant_ht_4WAT

Grand mean 7.52

Treatment	ACARP	CDLCM	Control	Deco
	7.15	7.90	7.12	7.90

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.317

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	2.979

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_Plant_ht_6WAT
```

Analysis of variance

Variate: Jute_Plant_ht_6WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0769	0.0256	0.11	
Rep.*Units* stratum					
Treatment	3	5.1769	1.7256	7.09	0.010
Residual	9	2.1906	0.2434		
Total	15	7.4444			

Tables of means

Variate: Jute_Plant_ht_6WAT

Grand mean 11.28

Treatment	ACARP	CDLCM	Control	Deco
	10.72	11.85	10.70	11.85

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	0.349

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	0.789

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_Plant_Ht_8WAT
```

Analysis of variance

Variate: Jute_Plant_Ht_8WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	183.71	61.24	2.02	
Rep.*Units* stratum					
Treatment	3	717.72	239.24	7.89	0.007
Residual	9	272.93	30.33		
Total	15	1174.36			

Message: the following units have large residuals.

Rep 3 *units* 4 9.1

Tables of means

Variate: Jute_Plant_Ht_8WAT

Grand mean 45.4

Treatment	ACARP	CDLCM	Control	Deco
	41.2	53.4	36.7	50.1

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	3.89

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	8.81

```
316  "Data taken from unsaved spreadsheet: New Data;1"
 317  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 318  READ [PRINT=*; SETNVALUES=yes] _stitle_
 321  PRINT [IPRINT=*] _stitle_; JUSTIFY=left
```

Data imported from Clipboard
on: 24-Feb-2019 17:53:35

GenStat Release 9.2 (PC/Windows) 24 February 2019 21:33:19

GenStat Ninth Edition
GenStat Procedure Library Release PL17.1

```
  1  %CD 'C:/Users/user/Documents'
  2  "Data taken from unsaved spreadsheet: New Data;1"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  7  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 24-Feb-2019 21:33:41

```
  8  DELETE [REDEFINE=yes] Crop,Treatment,Rep,Leaf_yield_Total_g,\
  9  Jute_Fresh_wt_kg_ha
 10  UNITS [NVALUES=*]
 11  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('cochorus')\
 12  ; REFERENCE=1] Crop
 13  READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Crop	16	0	1

```
 15  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
 16  'Control','Deco'); REFERENCE=1] Treatment
 17  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
 19  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
 20  READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
 22  VARIATE [NVALUES=16] Leaf_yield_Total_g
 23  READ Leaf_yield_Total_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Leaf_yield_Total_g	147.0	271.4	431.0	16	0

```
 25  VARIATE [NVALUES=16] Jute_Fresh_wt_kg_ha
 26  READ Jute_Fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_Fresh_wt_kg_ha	245.0	452.3	718.3	16	0

```
UNITS [NVALUES=*]

"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_Fresh_wt_kg_ha
```

Analysis of variance

Variate: Jute_Fresh_wt_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	24104.	8035.	0.58	
Rep.*Units* stratum					
Treatment	3	101408.	33803.	2.45	0.130
Residual	9	124067.	13785.		
Total	15	249579.			

Tables of means

Variate: Jute_Fresh_wt_kg_ha

Grand mean 452.

Treatment	ACARP	CDLCM	Control	Deco
	392.	558.	361.	499.

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	83.0

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	187.8


```
DELETE [REDEFINE=yes] Roselle_Plant_ht_4WAT,Roselle_Plant_ht_6WAT,\
Roselle_Plant_Ht_8WAT,Roselle_No_of_leaves_4WAT,Roselle_No_of_leaves_6WAT,\
Roselle_No_of_leaves_8WAT,Roselle_Chlorophyll_content,Roselle_Fresh_wt_kg_ha
UNITS [NVALUES=*]
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('Roselle')\
; REFERENCE=1] Crop
READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels

Crop	16	0	1

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
'Control','Deco'); REFERENCE=1] Treatment
READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
VARIATE [NVALUES=16] Roselle_Plant_ht_4WAT
READ Roselle_Plant_ht_4WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Plant_ht_4WAT	7.500	10.48	13.70	16	0

```
VARIATE [NVALUES=16] Roselle_Plant_ht_6WAT
READ Roselle_Plant_ht_6WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Plant_ht_6WAT	11.80	15.71	18.30	16	0

```
VARIATE [NVALUES=16] Roselle_Plant_Ht_8WAT
READ Roselle_Plant_Ht_8WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Plant_Ht_8WAT	17.60	25.17	33.20	16	0

```
VARIATE [NVALUES=16] Roselle_No_of_leaves_4WAT
READ Roselle_No_of_leaves_4WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_No_of_leaves_4WAT	4.800	6.688	9.300	16	0

```
VARIATE [NVALUES=16] Roselle_No_of_leaves_6WAT
READ Roselle_No_of_leaves_6WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_No_of_leaves_6WAT	7.000	10.05	13.10	16	0

```
VARIATE [NVALUES=16] Roselle_No_of_leaves_8WAT
READ Roselle_No_of_leaves_8WAT
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_No_of_leaves_8WAT	29.10	43.59	55.80	16	0

```
VARIATE [NVALUES=16] Roselle_Chlorophyll_content
READ Roselle_Chlorophyll_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Chlorophyll_content	42.20	45.09	47.90	16	0

```
360  VARIATE [NVALUES=16] Fresh_wt_g
361  READ Fresh_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Fresh_wt_g	135.0	316.8	492.0	16	0

```
363  VARIATE [NVALUES=16] Fresh_wt_2_g
364  READ Fresh_wt_2_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Fresh_wt_2_g	197.0	345.6	790.0	16	0

```
366  VARIATE [NVALUES=16] Leaf_yield_Total_g
367  READ Leaf_yield_Total_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Leaf_yield_Total_g	332.0	662.3	1089	16	0

```
369  VARIATE [NVALUES=16] Roselle_Fresh_wt_kg_ha
370  READ Roselle_Fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Fresh_wt_kg_ha	553.3	1104	1815	16	0

```
373  VARIATE [NVALUES=16] leaf_dry_wt_g
374  READ leaf_dry_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_g	39.00	61.63	80.00	16	0

```
376  VARIATE [NVALUES=16] leaf_dry_wt_kg_ha
377  READ leaf_dry_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_kg_ha	65.00	102.7	133.3	16	0

```
380  VARIATE [NVALUES=16] leaf_%_moisture_content
381  READ leaf_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_%_moisture_content	86.80	90.24	94.70	16	0

```
384  VARIATE [NVALUES=16] leaf_dry_matter_%
385  READ leaf_dry_matter_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_matter_%	5.300	9.756	13.20	16	0

```
387  VARIATE [NVALUES=16] Root_fresh_wt_g
388  READ Root_fresh_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_fresh_wt_g	131.0	222.1	480.0	16	0

```
390  VARIATE [NVALUES=16] Root_fresh_wt_kg_ha
391  READ Root_fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_fresh_wt_kg_ha	218.3	370.1	800.0	16	0

```
394  VARIATE [NVALUES=16] Root_dry_wt_g
395  READ Root_dry_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_dry_wt_g	50.00	74.62	123.0	16	0

```
397  VARIATE [NVALUES=16] Root_dry_wt_kg_ha
398  READ Root_dry_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Root_dry_wt_kg_ha	83.30	124.4	205.0	16	0

```
401  VARIATE [NVALUES=16] root_%_moisture_content
402  READ root_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
root_%_moisture_content	29.00	63.54	79.50	16	0

```
404  VARIATE [NVALUES=16] root_dry_matter_%
405  READ root_dry_matter_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
root_dry_matter_%	20.50	36.46	71.00	16	0

```
407
408  "General Analysis of Variance."
409  BLOCK Rep
410  TREATMENTS Treatment
411  COVARIATE "No Covariate"
412  ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
413  lsd; LSDLEVEL=5] Roselle_No_of_leaves_4WAT
```

GenStat Release 9.2 (PC/Windows) 24 February 2019 22:10:11
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GenStat Procedure Library Release PL17.1

```
1  %CD 'C:/Users/user/Documents'
2  "Data taken from unsaved spreadsheet: New Data;1"
3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
4  READ [PRINT=*; SETNVALUES=yes] _stitle_
7  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 24-Feb-2019 22:10:38

```
DELETE [REDEFINE=yes] Crop,Treatment,Rep,Jute_leaf_%_moisture_content
UNITS [NVALUES=*]
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('cochorus')\
; REFERENCE=1] Crop
READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Crop	16	0	1

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
'Control','Deco'); REFERENCE=1] Treatment
READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
VARIATE [NVALUES=16] Jute_leaf_%_moisture_content
READ Jute_leaf_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Jute_leaf_%_moisture_content	76.50	83.08	88.30	16	0

```

"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Jute_leaf_%_moisture_content
```

Analysis of variance

Variate: Jute_leaf_%_moisture_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	51.04	17.01	1.37	
Rep.*Units* stratum					
Treatment	3	21.22	7.07	0.57	0.649
Residual	9	111.69	12.41		
Total	15	183.95			

Tables of means

Variate: Jute_leaf_%_moisture_content

Grand mean 83.07

Treatment	ACARP	CDLCM	Control	Deco
	82.52	84.50	81.50	83.77

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	2.491

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	5.635

Analysis of variance

Variate: Roselle_No_of_leaves_4WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	8.793	2.931	2.02	
Rep.*Units* stratum					
Treatment	3	3.353	1.118	0.77	0.540
Residual	9	13.073	1.453		
Total	15	25.218			

Tables of means

Variate: Roselle_No_of_leaves_4WAT

Grand mean 6.69

Treatment	ACARP	CDLCM	Control	Deco
	6.65	6.00	6.82	7.27

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	0.852

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	1.928

```
 414  "General Analysis of Variance."
 415  BLOCK Rep
 416  TREATMENTS Treatment
 417  COVARIATE "No Covariate"
 418  ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
 419  lsd; LSDLEVEL=5] Roselle_No_of_leaves_6WAT
```

Analysis of variance

Variate: Roselle_No_of_leaves_6WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	10.335	3.445	1.63	
Rep.*Units* stratum					
Treatment	3	7.015	2.338	1.11	0.396
Residual	9	19.030	2.114		
Total	15	36.380			

Tables of means

Variate: Roselle_No_of_leaves_6WAT

Grand mean 10.05

Treatment	ACARP	CDLCM	Control	Deco
	10.03	9.03	10.30	10.85

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.028

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	2.326

```
 420  "General Analysis of Variance."
 421  BLOCK Rep
 422  TREATMENTS Treatment
 423  COVARIATE "No Covariate"
 424  ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
 425  lsd; LSDLEVEL=5] Roselle_No_of_leaves_8WAT
```

Analysis of variance

Variate: Roselle_No_of_leaves_8WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	238.42	79.47	1.23	
Rep.*Units* stratum					
Treatment	3	581.75	193.92	3.00	0.088
Residual	9	581.90	64.66		
Total	15	1402.08			

Tables of means

Variate: Roselle_No_of_leaves_8WAT

Grand mean 43.6

Treatment	ACARP	CDLCM	Control	Deco
	43.0	43.5	35.4	52.4

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	5.69

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	12.86

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Roselle_Chlorophyll_content
```

Analysis of variance

Variate: Roselle_Chlorophyll_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.557	1.519	0.77	
Rep.*Units* stratum					
Treatment	3	19.632	6.544	3.32	0.070
Residual	9	17.721	1.969		
Total	15	41.909			

Message: the following units have large residuals.

Rep 4 *units* 4 2.42

Tables of means

Variate: Roselle_Chlorophyll_content

Grand mean 45.09

Treatment	ACARP	CDLCM	Control	Deco
	45.25	44.83	43.60	46.70

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	0.992

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	2.245

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Roselle_Plant_ht_4WAT
```

Analysis of variance

Variate: Roselle_Plant_ht_4WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	9.437	3.146	1.15	
Rep.*Units* stratum					
Treatment	3	21.092	7.031	2.56	0.120
Residual	9	24.716	2.746		
Total	15	55.244			

Message: the following units have large residuals.

Rep 2 *units* 4 -2.49

Tables of means

Variate: Roselle_Plant_ht_4WAT

Grand mean 10.48

Treatment	ACARP	CDLCM	Control	Deco
	9.12	9.75	12.12	10.92

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.172

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	2.651

```
"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Roselle_Plant_ht_6WAT
```

Analysis of variance

Variate: Roselle_Plant_ht_6WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.1425	0.7142	1.21	
Rep.*Units* stratum					
Treatment	3	46.9925	15.6642	26.59	<.001
Residual	9	5.3025	0.5892		
Total	15	54.4375			

Message: the following units have large residuals.

Rep 2 *units* 4	-1.46

Tables of means

Variate: Roselle_Plant_ht_6WAT

Grand mean 15.71

Treatment	ACARP	CDLCM	Control	Deco
	18.15	14.63	13.68	16.40

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	0.543

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	1.228

```
 444  "General Analysis of Variance."
 445  BLOCK Rep
 446  TREATMENTS Treatment
 447  COVARIATE "No Covariate"
 448  ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
 449  lsd; LSDLEVEL=5] Roselle_Plant_Ht_8WAT
```

Analysis of variance

Variate: Roselle_Plant_Ht_8WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	104.540	34.847	5.22	
Rep.*Units* stratum					
Treatment	3	57.635	19.212	2.88	0.095
Residual	9	60.035	6.671		
Total	15	222.210			

Message: the following units have large residuals.

Rep 4 *units* 3 -4.28

Tables of means

Variate: Roselle_Plant_Ht_8WAT

Grand mean 25.18

Treatment	ACARP	CDLCM	Control	Deco
	24.00	25.02	23.38	28.30

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.826

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	4.131

```
67  "Data taken from unsaved spreadsheet: New Data;1"
68  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
69  READ [PRINT=*; SETNVALUES=yes] _stitle_
72  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 24-Feb-2019 21:42:15

```
  73  DELETE [REDEFINE=yes]
Roselle_Fresh_wt_kg_ha,leaf_dry_wt_g,leaf_dry_wt_kg_ha
  74  UNITS [NVALUES=*]
  75  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('Roselle')\
  76  ; REFERENCE=1] Crop
  77  READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Crop	16	0	1

```
  79  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
  80  'Control','Deco'); REFERENCE=1] Treatment
  81  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
  83  FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
  84  READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
  86  VARIATE [NVALUES=16] Leaf_yield_Total_g
  87  READ Leaf_yield_Total_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Leaf_yield_Total_g	332.0	662.3	1089	16	0

```
  89  VARIATE [NVALUES=16] Roselle_Fresh_wt_kg_ha
  90  READ Roselle_Fresh_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Roselle_Fresh_wt_kg_ha	553.3	1104	1815	16	0

```
  93  VARIATE [NVALUES=16] leaf_dry_wt_g
  94  READ leaf_dry_wt_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_g	39.00	61.63	80.00	16	0

```
  96  VARIATE [NVALUES=16] leaf_dry_wt_kg_ha
  97  READ leaf_dry_wt_kg_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
leaf_dry_wt_kg_ha	65.00	102.7	133.3	16	0

```
UNITS [NVALUES=*]

"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7; FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Roselle_Fresh_wt_kg_ha
```

Analysis of variance

Variate: Roselle_Fresh_wt_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	871680.	290560.	3.52	
Rep.*Units* stratum					
Treatment	3	100106.	33369.	0.40	0.754
Residual	9	743262.	82585.		
Total	15	1715048.			

Message: the following units have large residuals.

Rep 3 *units* 2	-460.	s.e.	216.
Rep 3 *units* 4	466.	s.e.	216.

Tables of means

Variate: Roselle_Fresh_wt_kg_ha

Grand mean 1104.

Treatment	ACARP	CDLCM	Control	Deco
	1048.	1008.	1158.	1202.

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	203.2

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	459.7

```
"Data taken from unsaved spreadsheet: New Data;1"
DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
READ [PRINT=*; SETNVALUES=yes] _stitle_
PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 24-Feb-2019 22:15:28

```
DELETE [REDEFINE=yes] Rosele_leaf_%_moisture_content
UNITS [NVALUES=*]
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=1; LABELS=!t('Roselle')\
; REFERENCE=1] Crop
READ Crop; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Crop	16	0	1

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; LABELS=!t('ACARP','CDLCM',\
'Control','Deco'); REFERENCE=1] Treatment
READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	16	0	4

```
FACTOR [MODIFY=yes; NVALUES=16; LEVELS=4; REFERENCE=1] Rep
READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	16	0	4

```
VARIATE [NVALUES=16] Rosele_leaf_%_moisture_content
READ Rosele_leaf_%_moisture_content
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Rosele_leaf_%_moisture_content	86.80	90.24	94.70	16	0

```

"General Analysis of Variance."
BLOCK Rep
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7;
FPROB=yes; PSE=diff,\
lsd; LSDLEVEL=5] Rosele_leaf_%_moisture_content
```

Analysis of variance

Variate: Rosele_leaf_%_moisture_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	14.562	4.854	0.94	
Rep.*Units* stratum					
Treatment	3	3.832	1.277	0.25	0.861
Residual	9	46.506	5.167		
Total	15	64.899			

Message: the following units have large residuals.

Rep 3 *units* 2	-3.96	s.e.	1.70
Rep 3 *units* 4	3.64	s.e.	1.70

Tables of means

Variate: Rosele_leaf_%_moisture_content

Grand mean 90.24

Treatment	ACARP	CDLCM	Control	Deco
	90.47	89.40	90.60	90.50

Standard errors of differences of means

Table	Treatment
rep.	4
d.f.	9
s.e.d.	1.607

Least significant differences of means (5% level)

Table	Treatment
rep.	4
d.f.	9
l.s.d.	3.636