DETERMINATION OF COPPER, ZINC AND LEAD IN CABBAGE (*Brassica oleracea sp.)* HEADS AND LETTUCE *(Lactuca sativa sp.)* LEAVES GROWN ON SOIL AMENDED WITH SEWAGE SLUDGE

By

JEFFERY AMO-ASARE

Bsc. (Hons.) Environmental Science

A Thesis submitted to the Department of Theoretical and Applied Biology,

Kwame Nkrumah University of Science and Technology

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (Environmental Science)

Faculty of Biosciences

College of Science

June, 2012

DECLARATION

I hereby declare that this submission is my own work towards the MSc Environmental Science and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

Jeffery Amo-Asare PG 39967-09 ……………………… …………………………..
(Student) (ID) (Signature) (Date)

Certified by
Dr. Bernard Fei-Baffoe ……………………… ………………………….....
(Supervisor) (Signature) (Date)

Certified by
Dr. P.K. Baidoo ………………………….. … ……………………………
(Head of Department) (Signature) (Date)

ABSTRACT

Sewage sludge from KNUST treatment plant is currently being used by many farmers for cultivation of vegetables on large scales for human consumption. This study focused on some selected heavy metals (lead, zinc and copper) levels in the KNUST treatment sludge, the effect of sewage sludge on soil properties that affect heavy metal uptake and determined levels of metals in lettuce and cabbage which are grown on the sewage sludge amended soil. In this study, three treatments were used base on recommended application rates for both cabbage and lettuce. 100, 150 and 200 kg/ha application rates were used as treatments for lettuce while 160, 210 and 260 kg/ha application rates were used for cabbage. The experiment was pot experiment arranged in a completely randomized design and under climatic conditions of Kumasi. Heavy metals (53.10±0.22, 40.77±0.36 and 24.10±0.13 mg/kg for copper, zinc and lead respectively) were found to be present in the KNUST sewage sludge but were below EU acceptable limits. Effect of sewage sludge on soil properties such as pH, organic matter content and soil conductivity were determined. Soil organic matter content and soil conductivity increased significantly ($P < 0.05$) with increasing application rates. The soil pH was not affected significantly ($P > 0.05$) with the application of sewage sludge. Levels of metals in the soil increased significantly with increasing application rates. The controls for both plants recorded the lowest heavy metal uptake (0.48±0.13, 1.36±0.23 and 2.60±0.29 mg\kg for lead, zinc and copper respectively for cabbage and 0.34±0.19, 1.35±0.31 and 2.30±0.14 mg\kg for lead, zinc and copper respectively for lettuce) while highest metal uptakes were recorded at the highest application rates for both plants (0.66±0.17, 2.66±0.09 and 4.33±0.14 for lead, zinc and copper respectively for cabbage and 0.54±0.01, 2.24±0.17 and 3.88±0.19 mg/kg for lead, zinc and copper respectively for lettuce). The uptake of zinc and copper were statistically significant ($P < 0.05$) while lead uptake was insignificant ($P > 0.05$) for both plants. The levels of metals in both plants for all treatments used were below WHO safe limit. Yields increased significantly with increasing application rates for both plants. Heavy metals residual levels in the soil were found to be higher in the control (13.21±0.15 for cabbage and 14.32±0.12 for lettuce) than other treatments for both plants. The results of this study showed that applying the KNUST treatment sludge based on recommended rates can enhance yields and improve soil fertility. The study also revealed that the sewage sludge can also increase levels of metals especially copper and zinc in soils and consequently leading to uptake by lettuce and cabbage plants.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACKNOWLEDGEMENT

I express my sincere gratitude to Almighty God for how far he has brought me. My appreciation also goes to my academic supervisor Dr. Fei - Baffoe for his professional, academic and moral support throughout the study. My heartfelt gratitude goes to Senior Laboratory Technicians at the FRNR – KNUST for their guidance and contribution to the success of this project. I am also grateful to Mr. Osei and his farm workers for permitting the work to be carried out on his farm premises and for their guidance and advice on cultivation of lettuce and cabbage.

Finally, I sincerely appreciate my family and friends who have been my source of inspiration and support. I say thanks and express my love to you all.

DEDICATION

This work is dedicated to the Lord God Almighty and to those who have put their trust in Him.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Agriculture, traditionally, has been known to perform roles such as provision of food security, supply of raw materials for industry, creation of employment and generation of foreign exchange earnings. Beyond these, agriculture is also recognized to have a greater impact on poverty reduction as well as social stabilization, buffer during economic shocks, support to environmental sustainability, and cultural values associated with farming (FASDEP II, 2007). In Ghana, agriculture is an important economic sector which employs more than half the population on a formal and informal basis and contributes enormously to the country's Gross Domestic Product (GDP) and export earnings (Clark, 1994). Agriculture in Sub-Saharan Africa generates at least 30 per cent of GDP, 40 per cent of exports and over 70 per cent of employment (Båge, 2006). Agriculture has been known to account for about half of all European economic growth during the nineteenth century (Cunningham, 2006) and in 2007, employed one third of the world's workers (ILO, 2009). Agriculture therefore forms an inevitable resource of the entire global economic, environmental and social importance and the world's history provides overwhelming global evidence that general economic growth of any nation must be preceded or at least accompanied by solid agricultural growth.

One of the major challenges to Agriculture especially in developing countries is excessive nutrients loss. Large areas of Sub-Saharan African are affected by nutrient depletion (Smaling and Stoorvogel, 1990). For Sub-Sahara African as a whole, nutrient depletion accounts for about 7 percent of the agricultural gross domestic product (GDP) of both crop and livestock production and amounts to an annual cost of approximately US$32 per farm household, or about US$20 for each hectare of arable land (Choi and Yun, 2003). The Millennium Ecosystem Assessment estimation indicates much of Sub-Saharan Africa and large parts of Asia almost have no highly productive land left (Steiner, 2006). The continual nutrient removal via crop harvests with insufficient nutrient replacement contributes to nutrient depletion which leads to low crop yields and increased erosion (Sanchez, 2002). Burning and ploughing have been known to be contributing factors to loss of nutrients through wind and water erosion and through leaching (Sesay, 2007). Intensive land use has been identified as serious problem in tropical regions which contribute to nutrient loss and eventually decreasing crop productivity (Araya and Takeshita, 2000).

As a result of this, many regions of the world, particularly Africa, are in urgent need of greater nutrient inputs to support food production. This has prompted farmers to amend the soil with different materials in order to enhance plant growth and increase crop yields. In Ghana, Mali and Benin for example, farmers are known to bribe septic truck drivers to dump the fecal matter on their fields (Asare *et al.*, 2003; Cofie *et al.*, 2005). Farmers are currently relying on both synthetic and organic fertilizer to improve the fertility of soil.

Synthetic or inorganic fertilizer is used as plant nutrients amendment and has caused tremendous increased yields from cultivation (US EPA, 2006). India and China, in particular, have shown dramatic improvements in their agricultural practices with attendant increases in the use of commercial fertilizer (Hayami and Otsuka, 1994). Increase in commercial fertilizer has also caused widespread ecological damage and negative human health effects (US EPA, 2006). Eutrophication, which leads to fish kills, loss of biodiversity, and renders water unfit for drinking and other industrial uses, has been enhanced by excessive fertilization nutrient runoff and leaching from agricultural land (Carpenter *et al.*, 1998). Methane emissions from crop fields are increased by the application of ammonium-based fertilizers. These emissions contribute greatly to global climate change as methane is a potent greenhouse gas (Bodelier *et al*., 1999). Fertilizer burn can occur when too much fertilizer is applied, resulting in a drying out of the roots and damage or even death of the plant (Felicity, 2004). Natural gas is overwhelmingly used for the production of ammonia and increases in price of natural gases over the past decade, along with other factors such as increasing demand, have contributed to an increase in fertilizer price (Sawyer, 2001). Africa uses an average of only 19kg of fertilizer per hectare or about one fourth of the world average and in order to increase its crop production, there is the need to raise fertilizer use to world average (Cunningham, 2006) and this involves huge amount of money. Many farmers, especially in the Sub- Saharan Africa cannot afford these synthetic fertilizers and therefore rely on government to subsidize fertilizers. In Malawi's agriculture budget, fertilizer subsidies now consume 60 per cent of Malawi's agriculture budget, which is a huge amount and leave out provisions for other essential factors such as, extension services, irrigation, research, which are not being done as a result (Africa Renewal, 2008). These make the use of synthetic fertilizer as a solution to nutrient loss very expensive especially in Africa.

Organic fertilizer has been identified as possible solution to nutrients restoration in soil and addresses the problems of nutrients loss especially in Africa at a cheaper cost as compared to synthetic fertilizers. Yields of crops grown in organic and inorganic fertilizer can be the same (Stamatoados *et al*., 1999). The use of organic fertilizers contribute to restoring and maintaining a high soil organic matter content which is the principal strategy for attaining economic progress and improving environmental quality. This makes organic fertilizer more environmental friendly than inorganic fertilizers. Increases in soil biomass, biological abundance, and diversity are directly related to increased levels of organic matter and good management practices, which, in turn, positively influence soil structure, nutrient cycling and availability, buffering capacity, and pest and disease control in cultivation systems (Drinkwater *et al.*, 1995). Other properties such as greater plant water-holding and cation exchange capacity, lower bulk density of soils, inducer of beneficial microorganisms and cheaper production cost (Chao *et al*., 1996) have increased the use of organic fertilizers. Despite these benefits from organic fertilizer, one particular type, sewage sludge, has been of public concern because of reported adverse environmental and health effects associated with its usage.

1.2 PROBLEM STATEMENT

The spreading of sewage sludge on agricultural land has been known to result in increased concentration levels of heavy metals in soil. These heavy metals are transferable and are not biodegradable, and at some levels, they become toxic and tend to accumulate along the food chain (Amir *et al*., 2005; Dudka *et al*., 1999). Neurological disorders, CNS destruction, and cancers of various body organs are some of the reported effects of heavy metal poisoning (ATSDR, 2000).

The KNUST Waste Treatment Plant produces huge tons of sewage sludge during waste treatment processes. Farmers, especially those around the treatment plant used the treated sludge for cultivation of various food crops including vegetables. These vegetables are grown on large scales which are sold for human consumption. Vegetables, especially those of leafy vegetables have been known to accumulate high amounts of metals even in the presence of high levels of plant nutrients (Jassir *et al*., 2005). It was found that farmers hardly carried out soil testing before applying sludge as soil amendment and application rates are based on maximization of yields. Many people could therefore be at risk of adverse health effects from consuming vegetables cultivated in soil amended with sludge and this can have both direct and indirect effects on the economy.

It is therefore important to conduct studies on sewage sludge due to the health and environmental implications of these materials to living organisms as well as agricultural soils.

1.3 OBJECTIVES

The general objective of this research is to determine the levels of some selected heavy metals (copper, zinc, lead) concentration in both the cabbage (*Brassica oleracea*) heads and lettuce (*Lactuca sativa*) leaves on soil amended with sewage sludge.

Specific objectives are to:

1. Assess the levels of some selected plant nutrients (nitrogen and phosphorus) and some selected soil properties (pH, soil conductivity and organic matter) in the sewage sludge and soil.
2. Assess the levels of the selected heavy metals in the sewage sludge and the top soil.
3. Determine levels of the selected heavy metals in the soil after applying various treatments before planting.
4. Assess the effect of sewage sludge on some selected soil properties (soil pH, organic matter content and soil conductivity) after treatments application and prior to planting.
5. Determine levels of selected heavy metals in both matured lettuce leaves and matured cabbage heads on various treatments.
6. Determine effect of sewage sludge on cabbage and lettuce yields.
7. Determine residual heavy metal levels after cabbage and lettuce cultivation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. SEWAGE SLUDGE

Sewage sludge is defined as the insoluble residue from wastewater treatment after either aerobic or anaerobic digestion processes (Hussein *et al.*, 2010). Some countries have coiled treated sewage sludge as biosolid because of its agriculture importance (Marmo, 2000). Most sewage sludge are treated before final disposal, therefore the use of sludge and treated sludge in literatures are usually used interchangeably (Przewrocki *et al.*, 2003). It is estimated that an amount of 10,000 tons per day of treated sewage sludge is produced in the world (Dharmappa *et al.*, 1997) and the quantity of treated sludge may be continually increasing with the industrial development and with the improvement of human life (Wang, 1997). Safe disposal of treated sludge has reached environmental concerns throughout the world. Disposal methods such as soil application, ocean dumping, land filling and incineration have been tried (Sánchez - Monedero *et al.*, 2004). The land application is suggested to be one of the most economical disposal methods and has attracted the interest of environmental engineers and scientists (Kim *et al.*, 2002). Incineration of sludge always needs supplementary fuel, which makes this method less economical and generates emissions of greenhouse gases, particles, acid gases, heavy metals, volatile organic compounds into the air, as well as damaging water resources (Dolgen *et al.*, 2006). Landfill of sludge though inexpensive method, requires huge land space and it is a kind of waste because there are lots of organic materials which produce gas that contribute to greenhouse effect. Soil and water in the neighborhood of landfill sites could be contaminated by ions, heavy metals, organic compounds and micro-organisms present in leachate. Other environmental impacts generated by the landfills are noise, dust, odors, land use, disturbance of vegetation and landscape (Wei and Liu, 2005). At present the disposal of sludge on landfills is 40% in the European Union (EU) while 37% of the sewage sludge produced within the EU is used for agricultural purposes (Angelidaki and Ahring, 2000).

Treated sewage sludge can be categorized as either Class A or Class B. Class A sludge does not require special handling or other restrictions because the concentration of pathogens has been reduced to a very low level. In Class B sludge, the pathogens have been reduced, but not totally eliminated from the original wastewater. Class B sludge has been responsible for most of the illnesses reported across the areas where people have been exposed to it either when it is spread or when it is stockpiled (Rockefeller, 2002).

2.1.1 ELEMENTS FOUND IN TREATED SEWAGE SLUDGE

Waste water from industrialized urban centers, industry, institutions, businesses, landfills, and households are discharge into treatment plants. Most waste water treatment plants are designed to remove pathogens, metals and chemical compounds from waste water. The removed substances concentrate in the resulting sludge leading to diverse materials and elements in the resulting sludge. Sludge composition varies from one waste water treatment plant to the other depending on the sludge treatment and stabilization processes employed and the nature of the waste water received (Krebs *et al*., 1998; Przewrocki *et al.*, 2003; Wong *et al*., 2001).

Sludge has been found to contain essential plants nutrients such as; organic matter, nitrogen, phosphorus, other macronutrients, organic pollutants, microorganisms and eggs of parasitic organisms (Hussein *et al.,* 2010**).** Certain endocrine disrupting compounds have also been identified in treated sludge. Five endocrine disrupting compounds namely 4-n-nonylphenol (4-n-NP), nonylphenol monoethoxylate (NP_1EO), nonylphenol diethoxylate (NP_2EO), triclosan (TCS) and bisphenol A (BPA), were identified in sludge produced from eight sewage treatment plants in Greece. Sources of these substances were attributed to an important group of non-ionic surfactants that are widely used in many commercial and household functions, including detergents, cosmetic products and textiles (Birkett and Lester, 2003). Chemicals from medicines and consumer products such as antidepressants, steroids, flame retardants, detergents, fragrances, disinfectants have resulted in more than five hundred (500) synthetic chemical compounds in sewage sludge (Ellen *et al*., 2006; Kinney *et al.,* 2006). According to Smith (1995), not much research have been into organic compounds in sewage sludge to the extent as heavy metals because organic impurities have not been proved to cause permanent damage to microbe activity and no negative impact on growth has been observed as long as the sludge amounts used have corresponded to the nutrient needs of plant. Again, increased awareness of dangers of some organic pollutants which has led to regulations eliminating persistent ones in favor of others that are more easily degraded and volatile nature of some organic pollutants have contributed to their low levels in biosolids (O'Connor, 1993).

Sewage treatment has been known not to eliminate disease-causing microbes in sludge. Human excreted viruses such as Hepatitis A and Polio, parasites including Cryptosporidium and Giardia, bacteria such as Salmonella and Escherichia coli O 157: H7 as well as more virulent, antibiotic resistant strains have been found in sewage sludge (Dumont *et al.,* 2004; Reinthaler *et al.,* 2003)

Heavy metals such as arsenic, cadmium, lead, copper, zinc, mercury and other metals have been detected in sludge (McBride, 1998). Industrial wastes, atmospheric deposition from crowded cities and other domestic wastes are among major sources of heavy metals in the sewage sludge (Hussein *et al.,* 2010**).** The main hazard connected with the application of sludge stems from the unstable amounts of metals and the formation of combinations with various degrees of their release to the environment. The degree and rapid expansion of urbanization and industrialization have been associated in large extent to the presence of heavy metal which often exceeds the admissible concentration, thus limiting the agricultural use of sludge (Jakubus and Czeka, 2001). Some researchers have also attributed the presence of heavy metal in sludge to rain waters. A study carried out on a catchment area of the city of Marseilles concluded that 63% of the annual load of lead discharged to the sewer came from run-off rain water. Another study carried out in Paris showed that the proportion of zinc and lead coming from run-off rain water represents nearly 90% of the pollution sources. This pollution is mainly caused by vehicle traffic, air deposition and roof corrosion (Magoarou, 2000).

2.1.2 THE BENEFICIAL IMPACTS OF TREATED SEWAGE SLUDGE

Sewage sludge predominantly contains sediment and humic substances suitable for reuse as a soil substitute (Elliott and Dempsey, 1991; Elliott and Singer, 1988). Reuse of sewage sludge for agricultural purposes has many beneficial effects, such as; supplying nutrients of nitrogen, phosphorus, and micronutrients to the crops, improving soil physical properties and increasing soil organic matter content (Basta *et al*., 2000). Sewage sludge has been used as an amendment to agricultural soils (Kidd *et al.,* 2007) and the application of sludge also not only increase soil organic matter content that contribute to the structural stability of the soil but also to its resistance to erosion (Hernández, *et al*., 1991; Ortiz and Alcañiz, 2006). Soil aeration, water holding capacity and aggregate stability have been improved in soil amended with sewage sludge (Logan and Harrison, 1995; Aggelides and Londra, 2000).

Sewage sludge has contributed to increasing beneficial soil organisms, reducing the need for fertilizers and pesticides, improving soil physical and biological properties, and helping keep organic wastes out of landfills (Pinamonti and Zorzi, 1996). Application of sludge to substitute costly inorganic nitrogen fertilizer has been shown to be not only profitable but also solves its disposal problems (Bozkurt and Yarilgac, 2003). The agricultural sector needs a secure, long term supply of nutrients and organic matter (humus) to compensate for losses through harvest, grazing and leakage into surface water, groundwater and the atmosphere. Sewage sludge serves both purposes, primarily as a supplier of micro-nutrients and organic matter and also as a supplier of nutrients such as nitrogen, potassium and phosphorous. Even though heavy metals have been reported in sewage sludge, sludge application has been known to produce soluble organic complexes (less harmful effects as compared to free metal ion) with the heavy metals and these complexes are more mobile, less readily adsorbed and possibly more readily taken up by plants than free metal ions (Nouri *et al*., 2006). Organic matter usually constitutes 50–60% of the dry matter of mechanically dried sludge, resulting in an increase in the amount of organic substances in cultivated land. It also effectively binds various harmful substances, such as heavy metals, preventing their action on the soil.

Available heavy metals concentration decreased as related to the complexing actions of the humic compounds to these metallic micro-pollutants (Echab *et al.*, 1998; Paré *et al*., 1999).

The institution of waste treatment plants has greatly reduced transmission and incidence of human diseases. In many cities before the nineteenth century, polluted water as a result of human waste was often contaminated and cholera, typhoid fever and other enteric diseases were common. Systematic collection and treatment has removed pathogens from waste water before releasing into streams (Basta, 1995).

2.1.3 THE ADVERSE EFFECTS OF TREATED SEWAGE SLUDGE

Sewage sludge has been found to potentially carry pollutants, since many wastewater treatment plants receive discharges not only from residential area but also from industry (Dai *et al.*, 2007; Bright and Healey, 2003; Berti and Jacobs, 1998). More than three hundred and thirty (330) of the synthetic chemical contaminants detected in sludge to date have been found to contribute to chronic diseases (Iranpour *et al.*, 2004). Many chemical contaminants (including dioxins, PCBs, and some flame retardants) and heavy metals found in sludge tend to bio-accumulate in fat tissue and milk fat (Rhind, 2002).

Land application of sewage sludge is likely to increase the concentrations of bio-available metals especially copper and zinc in soil (Wei and Liu, 2005). Crops raised on the soil amended with sludge can accumulate metals in quantities excessive enough to cause clinical problems both to animals and human beings consuming these metal rich plants (Tiller, 1986). The pollutants such as heavy metals are transferable and are not biodegradable, and at some levels, they become toxic and tend to accumulate along the food chain, where man is the last link (Dudka and Miller, 1999; Amir *et al.*, 2005). Long-term irrigation of plants by waste water and sludge increased heavy metal contents in soil (Behbahaninia *et al*., 2009). Among the heavy metals in sewage sludge, the most hazardous ones to humans are cadmium, mercury, and lead, while copper, zinc, chromium, and nickel in high concentrations are particularly poisonous to plants (Levinen, 1991). The presence in sludge of human antibiotics and heavy metals may also increase the ecological pressures in selecting for bacteria that are antibiotic-resistant. Resistant bacteria can be transferred from sludge-contaminated soil and plants to grazing animals and then to humans if meat is not thoroughly cooked or handled properly (Summers, 2006).

Farmers or workers working in direct contact with sludge could acquire serious health effects. High concentrations of *Legionella pneumophila* was found in sludge along with positive antibody titers in five workers who were repairing a decanter used for concentrating sludge at a sewage treatment plant. They experienced fever and flu like symptoms indicating they have developed Pontiac fever from contact with sludge (Gregersen *et al.,* 1999).

2.1.4 EFFECTS OF SEWAGE SLUDGE ON PLANTS GROWTH

Extensive researches have documented the fertilizer benefits of sewage sludge. Research results have shown sewage sludge benefits are similar to commercial fertilizers because of high crop yields and high quality crops (Barriquelo *et al.,* 2003; Coker, 1983; Ippolito *et al.,* 1992; Knuteson *et al.,* 1988; Azam and Lodhi, 2001; Chatha *et al.,* 2002; Mohammad and Athamneh, 2004; Dursan *et al.,* 2005; Casado-Vela *et al.,* 2006 and 2007; Jamil *et al.,* 2006).

Again sewage sludge looks preferable because of inclusion of micronutrients which play vital role in plants growth. McCaslin *et al.,* (1987) reported that land application of sewage sludge corrected the deficiencies of iron in sorghum plants. Plants nutrients such as protein, straw nitrogen, grain zinc, copper, nickel, cadmium increased as a result of sewage sludge application (Ippolito *et al.*,1992, Kirleis *et al.*, 1984). Wang *et al.*, (2008) indicated that growth rates of Chinese cabbage were enhanced by application of sewage sludge. Dolgen *et al*., (2004), Kim *et al.*, (2002), and Taek-Keun *et al*., (2009) found that maximum plant growth of lettuce was obtained by application of sludge. Taek-Keun *et al*., (2009) concluded in his work that sludge application on agriculture land would not present a problem of any hazardous and adverse effects but rather a suitable material for improvement of the physical and chemical properties of soil. Increasing sewage sludge rates have resulted in increasing plant yields. Dorn *et al*., (1985) and Muhammad *et al*., (2007) reported highest sewage sludge application rate supplied more nutrients to plant, consequently yield is increased.

2.1.5 EFFECTS OF SEWAGE SLUDGE ON HEAVY METAL LEVELS IN BOTH PLANT AND SOIL

Content of several trace elements have been reported to be larger in sewage sludge than in their natural abundance in soil. These include cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), mercury (Hg), molybdenum (Mo), lead (Pb), and zinc (Zn). Heavy metals occur in sewage sludge as insoluble precipitates (carbonates, phosphates, sulfides), surface-adsorbed mineral complexes, and insoluble organic matter chelates (Corey *et al.,* 1987). After sewage sludge has been applied on the land, metals in the sludge enter soil chemical pathways common to natural-occurring heavy metals in the soil. Metals are strongly adsorbed by soil minerals and soil organic matter. Other chemical reactions further limit their solubility (McLean and Bledsoe, 1992). Because of their limited solubility, heavy metals are not easily leached or moved through the soil by water and pose a low risk to water quality (Dowdy and

Volk, 1983; Logan and Chaney, 1983). Conversely, heavy metals accumulate in soil with residence times of several hundreds to thousands of years (Alloway, 1992; Bowen, 1979).

The levels of heavy metals in sewage sludge have raised several questions on its usage. Wei and Liu, (2005) reported abundant nutrition in sewage sludge can increase the output of vegetables but the bio-available concentration of metals was also increased in soils amended with sewage sludge. Higher amounts of Fe, Cu and Zn were absorbed by the plants grown in sludge-amended soil than those grown in the control soil (Hernández *et al*., 1991). Most of the content of metals increased in Chinese cabbage with the increase in sludge amendments ratio, and the content of heavy metal As, Cd, Cr and Zn exceeded the limits of metals in the regulation (Wang *et al.*, 2008). The increased in heavy metals such as cadmium and zinc have been noted to occur in plants but some researchers have reported that controlling application rates of sewage sludge will control such situations (Zwarich and Mills, 1979). Some have reported levels of heavy metals in plants after applying sewage sludge do not exceed phytotoxic limits. MacLean *et al.,* (1987) reported uptake of heavy metals by grass and legume plants was variable after application of sewage sludge but levels of the metals were all within the levels normally found in grass and legume plants except copper that was significantly higher. Hinesly *et al.,* (1979) noted that within 3 to 4 years after sludge application grain tissue from control and treated plots could not be distinguished by differences in zinc and cadmium concentrations. Studies conducted by Dai *et al.,* (2007) found that heavy metal concentrations in wheat hay after continuous application of sewage sludge at recommended plant-available nitrogen rates were similar to the heavy metal concentrations in wheat hay fertilizers with inorganic nitrogen. Some researchers have also indicated otherwise. Metal content in Chinese cabbage increased in sludge amendments ratio and the content of heavy metal such as arsenic, cadmium, chromium and zinc exceeded the limits of metals (Wang *et al.,* 2008).

Zinc contents of soil were increased from marginally low to an adequate level by the sewage sludge application. Lowest Zn contents resulted in control as a result of no sewage sludge applied in the control (Ngole, 2007 and Rappaport *et al*., 1988). Analysis of soil samples for copper contents showed that the Cu concentration of soil was significantly increased by the application of sewage sludge. These findings are in good agreement with the results of Adao *et al.,* (2003) who found that Cu-concentration increased with the increasing sewage sludge rate. This result is in line with Reddy *et al*., (1996) who pointed out that soils having pH 7 to 9 inhibited the copper availability in soils and consequently their uptake by plants. Land disposal of sewage sludge is unlikely to cause copper toxicity in plants except in cases where very heavy repeated applications are made to acidic soil (Sloan *et al*., 1997).

Lead contents of sludge-amended soil were increased non-significantly with the increasing rate of sewage sludge (Bride *et al*., 2000, and Teresa *et al*., 1991). This might be due to the fact that sewage sludge addition improves organic matter fraction of the soil and Pb has a strong capability, in the presence of organic matter, to combine with other ions to form a stable compound (Haar, 1991).

Numerous studies have shown that soil pH has dramatic effects on heavy metal mobility and plant uptake (Logan and Chaney, 1983; Sommers, 1977; Dowdy and Volk, 1983). Zn concentrations in plants were greater in the acidic soil than in the alkaline ones. These results confirm that Zn absorption depends on soil pH (Lübben *et al*., 1991; Smith, 1994; Planquart *et al.*, 1999).

2.1.6 EFFECTS OF SEWAGE SLUDGE ON SOIL PHYSICAL AND CHEMICAL PROPERTIES

Sewage sludge is predominantly organic matter and decomposition decrease with time after application. Decomposition rates depend on sewage sludge treatment process and the soil conditions. Decomposition of sludge releases plant nutrients including nitrogen, phosphorus and sulfur (U.S.EPA, 1993).

Kazi *et al*., (2005) reported that metal mobility and bioavailability increase with decreasing pH below pH 6.5 and decrease with increasing pH above pH 6.5. The pH was not affected significant (pH7.61- pH7.72) in soil amended with sewage sludge. An increase in soil pH has been reported in soils applied with municipal sewage sludge (Tasdilas, 1997). Lowering of soil pH is also reported (Epstein *et al.*, 1976). The changes in soil pH have been correlated with the calcium carbonate content of sludge and acid production during sludge decomposition.

The amended soils particularly those to which high doses of sludge had been applied showed a higher nitrogen and phosphorus content than the control (Wang *et al.*, 2008). Soil conductivity also varied when the sludge amendment was carried out and it increased compared to the control due to the formation of metallic salts (complexes of organic matter and heavy metals). Soil conductivity of the maximum sludge amended soil was 4.48 times higher than the control. In the soil amended with sewage sludge the CEC content significantly increased (Corey *et al.*, 1987).

Land application of the sewage sludge is becoming more popular due to the possibility of recycling valuable components such as organic matter, N, P and other plant nutrients (Martinez, 1999). Sewage sludge application to soil enables the recycling of nutrients and may eliminate the need for commercial fertilizers in cropland (Sommers, 1977). The fertility of soil increased over a long period of time, as sludge are organic fertilizers (Archie and Smith, 1981). Unwise sludge amendment may, however, disturb the soil properties especially when it bears high concentrations of metals and toxic constituents.

Organic matter added to the soil as sewage sludge composts improved the soil properties, such as bulk density, porosity and water holding capacity (Ramulu, 2002). The chemical properties of sludge-soil mixtures not only depend on the properties of the soil and sludge and the application rates of the mixtures, but also on their interaction and soil pH (Jacobs, 1981). Relatively high rates of sludge application increased the cation exchange capacity, which helped to retain essential plant nutrients within the rooting zone due to additional cation binding sites (Soon, 1981). The higher organic matter proportion in sludge decreased bulk density and increased the aggregate stability (Ojeda *et al.*, 2003). These improvements in soil physical properties increased water-holding capacity by promoting higher water retention in sludge-amended soils (Ojeda *et al.*, 2003)

2.2. HEAVY METALS

Heavy metal is defined as any metallic chemical element that has a relatively high density (density higher than that of water) and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), zinc (Zn), copper (Cu) and lead (Pb) (Lenntech, 2009). Naturally, heavy metals are found everywhere; in soils, water, sediments, plants and even the arctic (Szefer, 1998; Glasby, 1998; Pacyna; 1994). Heavy metals move from one environmental components to the other once introduced to the environment and may cause interactions within the natural systems and change their forms of existence in the environment. The presence of lead deposited in soft tissues of the body for example, can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive, and developmental effects (ATSDR, 1999). High levels of zinc in both plants and animals inhibit copper absorption which produces Cu deficiency and associated anemia (Broun *et al.* 1990; Willis *et al.* 2005).

One characteristic feature of Heavy metals is that they are not easily biodegradable and consequently can be accumulated in human vital organs. This situation causes varying degrees of illness based on acute and chronic exposures (Demirezen and Ahmet, 2006). Chronic low-level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen *et al*., 1990).

Neurological disorders, Central Nervous System (CNS) destruction, cancers of various body organs, low birth weight and severe mental retardation of newborn children have been reported in some cases where the pregnant mother ingested toxic amounts of a heavy metal through direct or indirect consumption of vegetables (ATSDR, 2000). The presence of heavy metals in sewage sludge has limited the use of sludge for land application (Wang *et al.*, 2008).

2.2.1. INTERACTION OF HEAVY METALS IN SOIL

Soil acts as filters of toxic chemicals and may adsorb and retain heavy metals from wastewater and other sources. However, the capacity of soils can be reduced due to continuous loading of pollutants or changes in pH to retain toxic metals, heavy metals can be released into groundwater or soil solution and are available for plant uptake (Ortiz and Alcañiz, 2006). Many soils contain a wide range of heavy metals with varying concentration ranges depending on the surrounding geological environment and anthropogenic and natural activities occurring or once occurred. Heavy metals can enter into soil from mineral and organic fertilizers, air pollutants and irrigation water (Lombi *et al.*, 2001). The metals in the soil can occur in water-soluble fraction, exchangeable, bound to carbonates, bound on oxides and hydroxides of iron (Fe) and manganese (Mn), bound to soil organic matter and bound to structural matrix of minerals (residual). The other forms except residual ones are potentially available and changing soil properties (Makovníková, 2000). Work on heavy metal by Jakubus and Czeka, (2001), in sewage sludge indicated that the hazard connected with heavy metals does not result from their total contents, even those exceeding admissible standards were rare in the analyzed material, but rather from the quantitative distribution in the fractions easily transferred to the soil solution. The binding and sorption of heavy metals to substances in soil may increase and decrease mobility of metals through soil profile (Kanugo, 2000; Amir *et al*., 2005).

The uptake of metals from the soil depends on different factors such as their soluble content in it, soil pH, plant growth stages, types of species, soil organic matter content, clay fraction content, mineralogical composition, fertilizers and soil type (Sharma *et al*., 2006; Hamon *et al.*, 1995; Otte *et al.*, 1995; Su *et al.*, 2004). The pH of the soil is known to influence the solubility and availability of metals for plants most significantly (Tlustoš *et al.*,1995, Hooda and Alloway 1996, Krebs *et al.,* 1998, Podlešáková *et al.,* 1998). The majority of ion fractions start getting mobile with decreasing pH (Wenzel *et al.,* 1999). Farooq *et al.,* (2008) reported that the amount of Pb in the leaves of different vegetables was higher as compared to those in the other investigated parts because Pb uptake was promoted by the pH of soil and the levels of organic matter. Chu and Wong (1987), reported that though high heavy metal contents was found in refuse compost, crops grown on the compost accumulated lower levels of metal than those with less heavy metal content due to the high pH and organic matter content of the composted refuse.

The inorganic colloidal fraction of soil is also responsible for sorption of metals by its mineral particles. It is comprised of clay minerals, oxides, sesquioxides and hydrous oxides of minerals. The clay minerals have two types and the major difference between expandable, type 2:1, and nonexpanding clays is in the surface area. The 2:1 type of clays have much higher total surface area than the 1:1 type has because of the existence of the internal surface area (Weber, 1991; Stevenson, 1992). The expandable clay minerals have also a much greater cation exchange capacity (C.E.C.) than the nonexpanding types and thus have a much greater propensity for immobilizing chemicals such as metal ions. Soil conductivity measurement correlates with soil properties that affect crop productivity including cation exchange capacity. Clays have high conductivity and CEC increase as clay content increases. Research bears out the correlation between conductivity and CEC through its relationship to clay (Grisso *et al*., 2006).

The existence of humic material in soils strongly influences sorption of chemicals. Humic and fulvic acids can exist in a dissociated form and thus are negatively charged. The main sources of these charges are carboxylic and phenolic groups in which hydrogen can be replaced by metal ions. This source of negative charges in soil colloids is strongly pH-dependent so the sorption of heavy metals in organic soils or in soils with relatively high organic content is mostly pH dependent. The Cation Exchange Capacity (C.E.C.) is also very high for soil organic matter, especially for fulvic acids according to clay minerals (Dube *et al.,* 2000).

Reports have shown that the amount of heavy metals especially Zn accumulated by any vegetable grown on sludge-amended soil is influenced by several factors including the bioavailability of Zn in the sludge-amended soil, the type of soil on which the sludge is applied, the makeup of the sludge, as well as the rate at which it is applied to the soils (Jung, 2008; Merrington *et al.,* 2003). For example soils such as luvisols, which is slightly acidic with low organic matter content, enhance the mobility of heavy metals through the soil profile into groundwater and soil solution, increasing availability of heavy metals to plants while vertisols have high organic matter and clay content, high carbon exchange capacity and presence of swelling clays which tend to bind heavy metals to the soil matrix reducing its availability to plants and its mobility through soil profile (Brady and Weil, 1999).

2.2.2 HEAVY METALS IN HUMAN EXCRETA

Human excreta consist of faeces and urine. The two are waste products of the bodily metabolism. The appearance, physical and chemical characteristics of urine or faeces depend largely on the health of the person excreting the material, as well as on the amount and type of food and liquid consumed. It has been reported that older people excreted larger amounts of total wet matter than younger, which was linked to a larger water intake intended to reduce the risk of constipation (Schouw *et al*., 2002). It is estimated that the annual amount of human excreta of one person corresponds to the amount of fertilizer needed to produce 250 kg of cereal which is also the amount of cereal that one person needs to consume per year (Wolgast, 1993).

Urine is the excreta fraction that is filtered from the blood and combined with excess water by the kidneys. At excretion, urine is usually yellow and does not have a foul smell but when stored it acquires a pungent odor as the urea breaks down to ammonia and carbon dioxide due to bacterial action (Drangert, 1998). Urine largely consists of water, approximately 93-96% (Polprasert, 1995; Vinnerås *et al*., 2006), and large amounts of nutrients that are mainly in water-soluble form (Jönsson and Vinnerås 2004). The nutrient content of faeces also originates from the food consumed (Fraústo da Silva and Williams, 1997).

Human faeces and to a small extent urine contain trace metals, which if present in excess concentrations may be harmful to man and to the environment. The amounts of harmful heavy metals in the urine are miniscule (WHO, 2006). Uptake of heavy metals occurs by two main pathways, ingestion and inhalation. The percentage uptake is higher for inhaled metals than for ingested metals (Vahter *et al*., 1991; Kim and Fergusson, 1993). The metal content and mass flows in faeces are usually reported to be far higher than in urine. The amounts of Zinc, Copper, Nickel, Cadmium, lead and mercury were reported larger in faeces than in urine, while amounts of some non-metal elements were larger in urine than in faeces (Schouw *et al.*, 2002). All the metals are excreted in larger amounts in faeces than in urine (Jönsson and Vinnerås 2005). Essentially all the heavy metals in the excreta come from the food ingested (Guyton, 1992).

2.2.3 SOME SELECTED HEAVY METALS

2.2.3.1 COPPER

Copper is an essential trace element that is vital to the health of all living things. The human body normally contains copper at a level of about 1.4 to 2.1 mg for each kg of body mass. Copper is distributed widely in the body and occurs in liver, muscle and bone. Copper is transported in the bloodstream on a plasma protein called ceruloplasmin. When copper is first absorbed in the gut it is transported to the liver bound to albumin. Copper metabolism and excretion is controlled delivery of copper to the liver by ceruloplasmin, where it is excreted in bile.

Copper has been associated with low mobility. This is as a result of its firm binding with the mineral fraction of sludge, high stability of the complexes with slightly soluble humic acids and other components of organic matter having high molecules (Carlson and Morrison 1992; Marschner *et al.*, 1995). The stability of the complexes of copper with other molecules is affected by pH. Decreasing percentage shares of copper in organic bonds with an increasing percentage of the less stable ones were observed under influence of high acidity (pH 0.5) of sewage sludge (Rudd *et al.*, 1988). The acceptable limit for human consumption of Copper (Cu) is 10 ppm and when Cu exceeds its safe level concentration, it causes hypertension, sporadic fever, uremias, coma etc (Nair *et al.*, 1997).

2.2.3.2 ZINC

Among all metals, Zinc (Zn) is the least toxic and an essential element in the human diet as it is required to maintain the proper functions of the immune system. It is also important for normal brain activity and is fundamental in the growth and development of the foetus. Zinc deficiency in the diet may be more detrimental to human health than too much Zinc in the diet. Although the average daily intake of Zinc is 7-16.3 mg Zn/day, the recommended dietary allowance for it is 15 mg Zn/day for men and 12 mg Zn/day for women (ATSDR 1999). On the contrary, the high concentration of Zinc in vegetables may cause vomiting, renal damage, cramps etc. Zinc is necessary for enzymes and enzymatic function and is therefore a constituent of about 100 enzymes in both plants and animals. Each of these enzymes has diverse processes and functions such as cell reproduction, immunity, protein synthesis, wound repair, vision, free radical protection and immunity. It is also required for protein synthesis, carbohydrate metabolism and is a constituent of insulin and semen. In plants, Zn is essential for growth because it controls the synthesis of indoleacetic acid, which regulates plant growth.

Excessive uptake of Zn by plants causes stunting of shoot, curling and rolling of young leaves, death of leaf tips and chlorosis (Rout and Das, 2003). High Zn intake in humans could cause nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, headaches (Panel on Micronutrients, 2001) and inhibition of Cu absorption which sometimes produce Cu deficiency and associated anemia (Broun *et al.,* 1990; Willis *et al.*, 2005). Foods such as beans, whole grains, dairy products, cereals, nuts, red meat, sea foods and poultry are excellent zinc sources. Zinc has been found in sewage sludge and application of sewage sludge has been documented to be a major input of zinc to agricultural soils (Alloway, 1995; Berti and Jacobs, 1998; Sloan *et al.,* 1997; Tlustoš *et al.,* 2001; Wong *et al.,* 2001).

2.2.3.3 LEAD

The permissible limit of lead in vegetables for human consumption is 2.0 - 2.5 ug/g dry weight (Samara *et al.,* 1992). Sources of lead include; leaded paints, cans, plumbing fixtures, leaded gasoline, deterioration of leaded paint used in the past, soldering, vehicle exhaust, lead crystal; ingesting leafy vegetables grown in lead contaminated soil, storing acidic foods in improperly-glazed ceramics, battery manufacturing, demolition, painting and paint removal, smelting operations, and many more (Rubin, 1998). Behbahaninia *et al.*, (2009) reported that lead, as compared to other heavy metals, has the highest concentrations in sludge and drainages in southern part of Tehran, because the presence of electroplating industries, car battery manufactures, dry cell manufactures and leather tanneries are located in the study region.

Accumulated lead in the human lead is mostly sequestered in the bones and teeth and this lead to brittle bones and weakness in the wrists and fingers and finally enters blood stream during the periods of increased bone mineral recycling thus pregnancy, lactation, menopause, advancing age, etc (Todd, 1996). Mobilized lead can be re-deposited in the soft tissues of the body and can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive, and developmental effects (ATSDR 1999). Lead may accumulate in bone and lie dormant for years and then pose a threat later.

Low levels of lead in blood have been associated with increase blood pressure, decreased creatinine clearance, subtle decrements in cognitive performance, iron and deficiency (ATSDR, 2000). Selective neurological deficits in the following areas have been observed in children with lead poison; mental retardation, language, cognitive function, balance, behavior and school behavior. Kidney diseases including; interstitial nephritis, tubular damage, hyperuricemia, decline in glomular filtration rate, chronic renal failure have been observed in adults as a result of lead poison (Rubin, 1998). It has been suggested that the formation of Lead phosphates in soils contaminated with both Lead and Phosphate is responsible for immobilizing Lead, thereby reducing the bioavailability of Lead (Ruby *et al.*, 1994). Lead phosphates, and in particular pyromorphites, are some of the most stable forms of Lead in soils under a wide range of environmental conditions and experimental evidence supports the hypothesis that lead phosphates can form rapidly in the presence of adequate lead and phosphate in aqueous systems (Ma *et al.*, 1994; Zhang *et al.*, 2000) and in Lead-contaminated soils (Zhang *et al.*, 2007).

2.2.4 HEAVY METAL LIMITS IN SOIL AND SEWAGE SLUDGE

According to the EU Directive 86/278/EEC, 1,000-1,750 mg/kg of dry matter represent the maximum range of copper in sewage sludge allowable for land application. 750-1,200 mg/kg dry matter and 2,500-4,000 mg/kg dry matter are maximum ranges for lead and zinc respectively (Marmo, 2000; Przewrocki *et al.*, 2003). Limits in some countries include: 1500, 3000 and 1000mg/kg dry matter of sludge for copper, zinc and lead respectively in china (Wang *et al.*, 2008). Limits in china are within the range of EU directive even though China is not a member of the EU. In Poland the maximum value cannot be higher than 800 mg/kg of dry matter for copper which is lower than that of EU directives, limit of 500 mg/kg dry matter for lead in Poland which is also lower than that of EU directives and Zinc limit in Poland is equal to the lower limit of EU regulation, which is 2,500-4,000 mg/kg dry matter (Przewrocki *et al.*, 2003). The EU Directive 86/278/EEC described maximum limit of heavy metal in soil as follows: copper 50-140mg/kg dry matter, lead 50-300mg/kg dry matter and zinc as 150-300mg/kg dry matter for soils (Marmo, 2000). Limits of heavy metals in soil for china include; 350mg/kg for lead, 100 for copper and 300 for zinc (Wang *et al.*, 2008),

2.3 VEGETABLES

Vegetables are an important part of human's diet. In addition to a potential source of important nutrients, vegetables constitute important functional food components by contributing protein, vitamins, iron and calcium which have marked health effects (Arai, 2002). Vegetables provide a rich source of antioxidant vitamins, and other phyto-chemicals with antioxidant characteristics. The antioxidant content of vegetables may contribute to the protection against diseases.

2.3.1 HEAVY METALS IN VEGETABLES

Many researchers have shown that vegetables are capable of accumulating high levels of metals from the soil (Xiong 1998, Cobb *et al.,* 2000). Vegetables grown in heavy metals contaminated soils accumulate higher amounts of metals than those in uncontaminated soils. This is because heavy metals absorb these metals through their leaves (Al Jassir *et al*., 2005). The heavy metals are absorbed by vegetables along with other essential plant nutrients and contamination of soils and crops with these metals may have adverse effects on soil, plants, animals and human beings. (Varalakshmi and Ganeshamurthy, 2010). Certain species of *Brassica* (Cabbage) have been classified as hyper-accumulators of heavy metals into the edible tissues of plant (Xiong, 1998). High level of heavy metals was detected in Chinese cabbage grown in soil amended with sewage sludge (Wang *et al*., 2008)

Heavy metals levels in vegetables increase as soil background concentration increases as a result of irrigation of vegetables with wastewater, sewage sludge application as well as increased in fertilizer and pesticides application. This has limited especially the use of sewage sludge land application although there is abundant nutrition for plants. Demirezen and Ahmet (2006) and Sharma *et al.*, (2006) reported high concentration of Lead above the safe limit in vegetables grown in soil irrigated with wastewater from industrial areas. Muchuweti *et al.*, (2006) reported the level of Lead in vegetables irrigated with mixtures of wastewater and sewage from Zimbabwe to be higher than WHO safe limit (2 mg kg^{-1}). High concentration of lead was identified in vegetables grown in industrial areas of Greece (Fytianos *et al.*, 2001). Al Jassir *et al.*, (2005) reported the levels of Zn to be higher in the vegetable species for both washed and unwashed samples. Tandi *et al.*, (2005) also reported a higher uptake of Cu in lettuce and mustard rape and pointed out that leafy vegetables produced around industrial sites pose a health risk to poor communities, especially to children.

Levels of metals in background concentration may not exceed the safe limits but vegetables uptake of metals in such areas can exceed their acceptable limits. Awasthi (2000) reported levels of heavy metals in soil under study were within safe limits as per the standards but the levels in vegetables exceeded official Indian standards by many folds. This indicates that though levels in background soil may not exceed official acceptable limits, it does not guarantee lower levels in vegetables grown in those areas.

Research has shown that vegetables bio-accumulate heavy metals differently and this has been attributed to plant differences in tolerance to these metals and within the plant, there is accumulative partitioning of the metals in the leaves, fruit or roots (Itanna, 2002; Fitzgerald, *et al.*, 2003; Delibacak, *et al.*, 2002; MacFarlane, *et al.*, 2003). Demirezen and Ahmet (2006) analyzed various vegetables (cucumber, tomato, green pepper, lettuce, parsley, onion, bean, eggplant, pepper mint, pumpkin and okra) and reported that the Zn concentration (3.56–4.592 mg kg^{-1}) was within the recommended international standards as compared to Lead levels.

Analysis of the vegetables from an industrial area in some studies demonstrated the concentration of Zn to be within the set limits of international standards (5.00 mg kg-1, WHO, 2006) (Fytianos *et al.*, 2001). Sharma *et al.*, (2006) reported the concentration of Cu (2.25-5.42 mg kg-1) in vegetables grown in wastewater areas of Varanasi, India to be within the safe limit.

It was found that heavy metals accumulated more in roots and leaves than those in other parts because both are the entry points of heavy metals from soils and air, respectively. Demirezen and Ahmet (2006) reported that levels of Cu (22.19-76.50 mg kg^{-1}) were higher in the leafy species than the non-leafy vegetable species from Turkey. Some metals levels in soil have been correlated with their uptake by plant. The edible portions of five varieties of green vegetables, viz. Amaranth, Chinese Cabbage, Cowpea leaves, Leafy Cabbage and Pumpkin leaves collected from several areas in Dar Es Salaam, Africa, were analyzed for Lead, Cd, Cr, Zn, Ni and Cu. There was a direct positive correlation between Zn and Lead levels in soils with the levels in vegetables. The relation was absent for other heavy metals (Othman, 2001).

The ability of some vegetables to remove and accumulate heavy metals though may cause a serious health risk to human health when plants based food stuff are consumed (Wenzel *et al.*,1999), but may also have the potential in the remediation of heavy metals contaminated soils. Several reports (Wei and Liu, 2005; Zhao *et al.,* 2006; Zheng *et al.,* 2007; Gr_man *et al.,* 2003) had shown that cabbage can uptake the As, Zn, U, Hg, Lead, Cd Cr and Cu and accumulate them in a higher level and therefore be used in remediation of heavy metals in contaminated soils.

2.3.2 CABBAGE CULTIVATION

Cabbage belongs to the *Cruciferae* (mustard) family which includes: Brussels sprouts, broccoli, cauliflower, and kale. Cabbage is of the genus *Brassica*, species *oleracea*, and variety *capitata*. This shallow-rooted, cool-season crop (grows best when temperatures are 50-75 degrees F.) is cultivated for its large leafy head and is thought to have originated in Western Europe. Before being thought of as a food, cabbage was valued for medicinal purposes in treating headaches, gout, and diarrhea. Cabbage juice was reportedly used as an antitoxin for poisonous mushrooms. Cabbage is a good source of vitamin C, contains some vitamin A, and has a fair amount of thiamin, riboflavin, potassium, and soluble and insoluble fiber. Fresh market cabbage is low in calories and sodium, and free of fat and cholesterol.

Cabbage is a cool-season crop generally requiring 60 to 100 days from sowing to reach market maturity, depending on the variety. Although it can be direct seeded, most cabbage production relies on the use of transplants. The ideal monthly temperatures for optimal growth and development ranges from 60°F to 65°F and temperatures over 75°F can induce “bolting” in cabbage, but varieties differ in their susceptibility to this disorder. Bolting is the process in which the plant switches from vegetative growth (heading) to reproductive growth (formation of flowers and seeds). The optimal range for germination is between 45°F and 95°F. Cabbage seeds can be directly sowed and then thinned to the desired spacing or can be sowed in nursery beds and then transplanted in field beds. Transplants are dug within 3-7 weeks depending on the variety. One ounce of cabbage seed will produce about 5,000 transplants. A good watering immediately after transplanting is essential to ensure that the young plants become well established.

Soil analysis prior to applying fertilizers is strongly advisable. Cabbages require large amounts of fertilizer but are not as demanding as cauliflowers. Cabbages benefit from high levels of organic matter, therefore animal manure normally forms the basis of the fertilizer program. Cabbages grown in beds will require more irrigation than those grown on the flat. Soil type and weather will also influence the frequency of irrigation. Space plants 12 to 15 inches apart in 36- to 42-inch rows. This will produce heads that range from 2 to 3 pounds

.

Cabbage can be grown on a wide range of soil types. A well-drained sandy loam soil with good organic content is preferred. Required soil pH for cabbage is between 6.0 and 6.5. Cabbages are planted in soils that have not been used for production of cabbage. This helps to avoid potential soil borne disease and nematode problems. Soil testing is essential for determining the amount of fertilizer and lime required to successfully produce a crop of cabbage. The best strategy is to have a soil test performed yearly. Timely and appropriate applications of fertilizer can make a significant difference in the quality and quantity of cabbage harvested. Fertilizer applied before planting or transplanting should be well mixed into the soil to prevent any chance of plant injury.

Many experiments have shown that cabbage gives a good response to manure and most of earlier experiments indicate a superiority of manure over commercial fertilizers. Muhammad *et al*., (2007) reported that cabbage production increased by 54 percent in rotation with combination of fertilizers, manure and green manure. Excessive nitrogen, on the other hand, may cause loose head formation and internal decay.

Irrigation is an essential element of a successful vegetable production operation and is critical to the consistent production of quality produce. Cabbage is a fast-growing, shallow-rooted crop whose roots penetrate only 12 to 15 inches into the soil. Although cabbage is relatively drought tolerant, adequate soil moisture levels should be maintained to maximize yields. In cabbage, the most critical period for irrigation is following direct seeding or transplanting and during head development. Any stress related to a lack of water during these periods can lead to small head size (reduced yields), growth cracks, or tip burn. Any of these problems will result in the production of poor quality heads, reducing their marketability and value.

Yields will vary with the season of production, variety, and production system used. With proper management, cabbage can produce 10 to 12 tons per acre (400 to 500 50- pound crates or bags). Harvesting involves cutting stems close to the ground near the base of the head and this done when heads are well formed and firm. Typically, outer wrapper leaves are removed. Harvested cabbages are removed from direct sunlight because cabbage wilts quickly when exposed to sunlight. Wilting results from a loss of water from the head (Primefacts, 2006).

2.3.3 LETTUCE CULTIVATION

Lettuce is a cool-season crop that grows best on well-drained muck soils and the crop does best on fertile, high organic matter soils that have good water-holding capacity. Lettuce is adapted to cool growing conditions with the optimum temperatures for growth of 60 to 65^{0}F. Lettuce has a relatively high water requirement therefore shortage of rainfall will seriously stunt growth and head quality. Irrigation greatly reduces risk of crop failure. Adequate nutrients and a continuous moisture supply are essential to vigorous growth. A soil test is the only way of knowing the amount of lime and fertilizer required. The pH should be 6.0 to 6.7. Plants should be spaced 9 to 12 inches in row, depending on plant size (Primefacts, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The project comprised:

1. Cultivation of cabbage with sewage sludge as an amendment at recommended rates (160, 200 and 260Kg/ha).
2. Cultivation of lettuce with sewage sludge as an amendment at recommended rates (100, 150 and 200Kg/ha).

The experiments were conducted simultaneously under local climatic condition of Kumasi in the Ashanti Region.

3.1 SITE AND SAMPLING

3.1.1 EXPERIMENTAL SITE

The study was conducted at a selected cabbage farm located at Chirapatre in Kumasi, Ashanti Region with climatic conditions as follows; temperature ranges between 21.5°C – 30.7°C, average humidity about 84.16 per cent at 0900 GMT and 60 per cent at 1500 GMT and maximum rainfall as 214.3mm in June and minimum rainfall as 165.2mm in September. The choice of site was based on its nearness to source of tap water which is the main water source for the experiment, good farm management practices by the farmers and its location, which is sited away from vehicular traffics and industrial activities.

3.1.2 SOIL SAMPLING

Undisturbed sandy – loam top-soils were identified at the site of experiment. The soils were collected at a depth of 5.0 – 8.0 cm and thoroughly homogenized. Samples of the soil were obtained for analyses of the selected heavy metals (zinc, lead and copper) and some selected soil properties (soil pH, organic matter and soil conductivity). 20kg of the soil was weighed into each experimental pot. Sample of the soil was used to nurse both the cabbage and lettuce seeds.

3.1.3 SLUDGE SAMPLING

Sludge samples were obtained from the KNUST Treatment Plant. The sludge at the time of sampling was three months old from treatment processes. The sampled sludge was air dried within a period of two weeks at room temperature and analyzed for heavy metal levels and some selected soil properties as mentioned above.

3.2 EXPERIMENTAL DESIGN

The experiments were conducted in a completely randomized design with three replications. Jerry cans were used as experimental pots. Jerry cans are widely used for storing drinking water and vegetable oil and this project seeks to extend its use in agriculture for growing shallow rooted vegetables. This can enable cultivation of shallow rooted vegetables to be grown in containers especially in soil degraded areas. The cans were placed at distance of 30cm from each other. A similar set up was designed for the cultivation of lettuce.

3.3 DETERMINATION AND APPLICATION OF TREATMENTS

Three treatments were used in this experiment. Treatments were based on recommended application rates based on nitrogen needs for cabbage and lettuce. Calculations were based on formula for determining amount of sludge to be applied designed by Cooperation Extension

Service, University of Purdue, Indiana. The formula involves the following parameters; the application rates of crop, the area of plot or pot where sludge is applied and the nitrogen levels of sludge. Below shows the relationship between the parameters in order to determine amount of sludge to apply:

$$\text{Amount of sludge to be applied} = \frac{\left(\text{Application rates of crops } \frac{\text{kg N}}{\text{ha}} \times \text{Area of pot (ha)}\right)}{(\text{ Nit`rogen levels of sludge \%})}$$

The nitrogen levels of sludge = 3.43%

The area of the experimental pot = 42cm by 28cm = 1176cm^2 = 0.00001176ha

3.3.1 TREATMENTS FOR CABBAGE CULTIVATION

Treatments for cabbage cultivation were based on recommended nitrogen application rates for cabbage provided by the Fertilizer Society of South Africa (2000). The recommended nitrogen application rates ranged from 160 Kg N / ha – 260 Kg N/ha. Three rates were selected based on the upper limits (260 Kg N/ha), median (210 Kg N/ha) and lower limit (160 Kg N/ha) of the required application rates. The table below indicates the calculated amount of sludge applied as treatment at various application rates:

Table 3.1 Amount of sludge used as treatment for cabbage cultivation

Application Rates (kg N / ha)	Calculated Amount Of Sludge As Treatment (mg)
160	5.49 mg
210	7.2 mg
260	8.9 mg

The treatments for cabbage were labeled as follows:

Tc0 – control – no amendment

Tc1 – Treatment 1 – 5.49mg (160 kg N/ ha)

Tc2 – Treatment 2 – 7.2mg (210 kg N/ ha)

Tc3 – Treatment 3 – 8.9mg (260 kg N/ha)

3.3.2 TREATMENTS FOR LETTUCE CULTIVATION

Treatments for lettuce cultivation were also based on recommended nitrogen application rates for lettuce provided by the Fertilizer Society of South Africa (2000). The recommended nitrogen application rates ranged from 100 kg N / ha – 200 kg N/ha. Three rates were selected based on the upper limits (200 kg N/ha), median (150 kg N/ha) and lower limit (100 kg N/ha) of the required application rate. The table below indicates the calculated amount of sludge applied as treatment at various application rates:

Table 3.2 Amount of sludge used as treatment for lettuce cultivation

Application Rates (kg N / ha)	Calculated Amount Of Sludge As Treatment (mg)
100	3.4mg
150	5.14mg
200	6.86mg

The treatments for lettuce were labeled as follows:

Tl0 – control – no amendment

Tl1 – Treatment 1 – 3.4mg (100 kg N/ ha)

Tl2 – Treatment 2 – 5.14mg (150 kg N/ ha)

Tl3 – Treatment 3 – 6.86mg (200 kg N/ha)

Treatments were applied, stirred and watered to equilibrate with soil for four weeks before seedlings were sown into various experimental pots. Samples of amended soil were analyzed for zinc, copper, lead and soil chemical properties such as organic matter, pH and conductivity which influence heavy metal uptake by plants.

3.4 CULTIVATION OF CABBAGE

Seeds of cabbage were purchased from Dapsy Agro-chemicals located at Kejetia, Kumasi. The cabbage was of variety Oxylius, which is a variety grown in the country.

Plate 3.1 Treatments applied on soil

3.4.1 NURSING OF SEEDS

The seeds were nursed in jerry cans. The seeds were broadcasted on soil surface and watered with tap water. Palm fronts were used as a cover to obtain the appropriate temperature for germination. The seeds were watered twice daily, early morning and late evening.

3.4.2 TRANSPLANTING SEEDLINGS

Seedlings were ready for transplant after three weeks from nursing. Unhealthy seedlings were thinned out with hands. The healthy seedlings were transplanted a week later into the various experimental pots randomly by hands. The seedlings were watered immediately after transplant. Transplanting was done after four weeks of nursing. One transplant was sown on each pot.

Plate 3.2 Seedlings of cabbage

3.4.3 CULTURAL PRACTICES

Soils were stirred once every week to enhance aeration and control weeds. No chemical was applied for controlling insects and weeds. This was done to reduce heavy metal inputs from such chemicals. Insects were controlled by hand picking. Watering was done twice daily. Tap water was used to irrigate the plants since heavy metal levels of the tap water used were negligible.

3.4.4 HARVESTING AND SAMPLING

Cabbage plants were harvested on the fourth month after transplant when the heads were fully formed. The fresh head from each pot was weighed and samples were taken for heavy metals analysis. Soil samples were taken for analysis of heavy metals residual levels.

3.5 CULTIVATION OF LETTUCE

Seeds of lettuce were purchased from Dapsy Agro-chemicals located at Kejetia, Kumasi. The lettuce was of variety Creat Lakes, which is a common variety grown in the country.

3.5.1 NURSING OF SEEDS

The seeds were nursed in jerry cans. The seeds were broadcasted on soil surface and watered with tap water. Palm fronts were used as a cover to obtain the appropriate temperature for germination. The seeds were watered twice daily, early morning and late evening.

3.5.2 TRANSPLANTING SEEDLINGS

Seedlings were ready for transplant after four weeks from nursing. Unhealthy seedlings were thinned out with hands. The healthy seedlings were transplanted a week later into the various experimental pots randomly by hands. The seedlings were watered immediately after transplant. Two transplants were sown on each pot.

3.5.3 CULTURAL PRACTICES

Soils were stirred once every week to enhance aeration and control weeds. No chemical was used for controlling insects and weeds. This was done to reduce heavy metal inputs from such chemicals. Insects were control by hand picking. Watering was done twice daily. Tap water was used to irrigate the plants and heavy metal levels of the tap water used were negligible.

Plate 3.3 Seedlings of lettuce plant

3.5.4 HARVESTING AND SAMPLING

Lettuce plants were harvested on the third month after transplanting. The fresh leaves from each pot were weighed and samples were taken for heavy metals analysis. Soil samples after cultivation were taken for residual heavy metal levels.

Plate 3.4 Matured lettuce plants

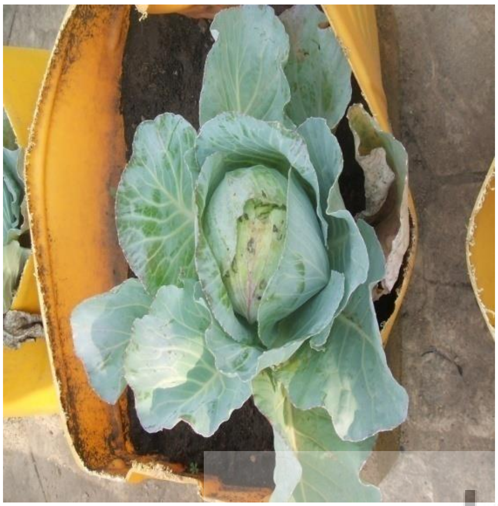

Plate 3.5 Folded cabbage plant

3.6 CHEMICAL ANALYSES

Chemical analyses were done under the following parameters;

3.6.1 pH

10g air- dried soil was weighed into a 100 ml beaker. 25ml distilled water was added and stirred vigorously for 20minutes. Soil – water suspension was allowed to stand for about 30minutes by which most of the suspended soils have settled out from the suspension. The pH meter was calibrated with blank at pH of 4 and 7 respectively. The electrode of the pH meter was inserted into the partly settled suspension. The pH was read off using the pH meter and the results were recorded. This procedure was repeated for the sewage sludge and amended soils.

3.6.2 SOIL CONDUCTIVITY

10g air- dried soil was weighed into a 100 ml beaker. 25ml distilled water was added and stirred vigorously for 20minutes. Soil – water suspension was allowed to stand for about

30minutes by which most of the suspended soils have settled out from the suspension. The conductivity cell is calibrated with the 0.01 KCl. The cell is then inserted into the suspension and conductivity is read in micro-simens / cm using the conductivity meter. This procedure was used for measuring the conductivity of the sludge and all the amended soil.

3.6.3 ORGANIC MATTER

The mass of an empty, clean and dry porcelain dish was determined and recorded and labeled as Mp. Sample of the soil was placed in the known-mass porcelain dish and the mass of the dish and soil specimen were determined and recorded as Mps. The dish was then placed in a muffle furnace. The temperature in the furnace was gradually increased to 440 °C. The specimen was left in the furnace overnight. The porcelain was removed carefully using tongs and allowed to cool to room temperature. The mass of the dish containing the ash (burned soil) was determined and recorded as Mpa.

The mass of the dry soil (Md) was determined as Md = Mps – Mp

The mass of ashed soil (Ma) was determined as Ma = Mpa – Mp

The organic matter (Mo) was determined as Mo = Md – Ma

The organic matter content (OM) was determined as OM = (Mo / Md) × 100

This procedure was repeated for sewage sludge and amended soils.

3.6.4 NITROGEN CONTENT

One gram (1g) of dry soil sample was weighed with a mettlar balance into a kjeldahl flask of 300 ml size. 25 ml concentrated sulphuric acid was added with a selenium catalyst tablet (kjeldahl tablet). The flask was then heated in a fume chamber to digest the mixture until clear solution is obtained. The digested sample was then allowed to cool and diluted to 300 ml with distilled water. 50 ml of sodium hydroxide thiosulphate and 10 ml of sodium hydroxide were added to the diluted digest to provide the alkaline condition necessary for the release of organic nitrogen. 200 ml of the mixture was then distilled into a conical flask containing 50ml of boric acid indicator. The solution in the conical flask was then titrated against standard 0.02 N sulphuric acid until the indicator turns pale lavender with volume used representing V1. A blank was prepared by heating 25 ml of concentrated sulphuric acid and a tablet of selenium catalyst and treated as a digest to get V0.

The nitrogen of the sample was calculated using the relationship:

Nitrogen (mg/kg) = $\frac{V1-V0}{M} \times 280$

Where:

V1 is the volume of the sulfuric acid used in the titration of the sample in milliliters (ml),

V0 is the volume of the sulfuric acid used in the titration of the blank test in milliliters (ml),

m is the mass of test sample in gram (g).

The nitrogen content (mg/kg) was then converted to percentage.

3.6.5 PHOSPHORUS

Composite samples were air dried, crushed lightly, and then passed through a 2-mm sieve. 2g of the sieved sample was weighed into a porcelain crucible. The crucible was placed in a muffle furnace and heated at 500°C for four hours which was then removed and allowed to cool. The ignited residue was moisten with 2ml distilled water and slowly and carefully 5ml of 8N HCl was added. The crucible was covered and placed on steam water bath for 20mins. It was then filtered through Whatman No. 42 filter paper, a 100ml volumetric flask was used to collect the filtrate. The crucible was washed as well as the filter paper several times with distilled water. The solution was then made up to 100ml and was shaken vigorously to assure complete mixing to complete the digestion process. 5ml of the digest was measured into 50 ml volumetric flask. 10ml Vanadomolybdate reagent was added and the volume was made up with distilled water and shaken vigorously and kept for 30mins. A yellow color developed and read at 430nm on a colorimeter. The percentage transmittance (% T) values were obtained and recorded. The absorbance was determined and P content was determined from the standard curve,

Calculation;

Percent (%) T values were converted to 2 – Log T. a graph was plotted using P Standard solutions to obtain actual concentration of P. The concentration of P in the extract is obtained by comparing the results with a standard curve plotted. From the standard curve, this equation is obtained:

Y=AX ………………………….. (1)

Therefore available phosphorous (P) ppm or mg/Kg

X=(Y/A) ÷10

Where

Y= 2- log T of the sample

A =a constant obtained from the graph

3.6.6 HEAVY METAL DETERMINATION IN SOIL, SLUDGE AND SOIL AMENDED SAMPLES

The digestion process used in determining Phosphorus content was used in this procedure. 5ml of the digest solution was made up to 100 ml with distilled water. The test solution was then analyzed for the concentration of copper using Atomic Absorption Spectrophotometry (AAS) with copper lamp attached to it. With AAS, the sample was aspirated and atomized. The light Beam emitted was directed through the flame into a mono-chromator and onto a detector that measured the amount of light absorbed by the atomized element in the flame. The concentration of the copper in the sample was then shown in milligram per liter (mg/l) which was converted to mg/kg. Similar procedure was done for other metals by changing the attached lamp to the specific metal.

3.6.7 HEAVY METAL DETERMINATION IN PLANT SAMPLES

The collected vegetable samples were washed with distilled water to remove dust particles. The samples were then cut to separate the roots, stems and leaves using a knife. The leaves of lettuce and heads of cabbage were air-dried and then placed in a dehydrator at 80 °C for 2-3 days and then dried in an oven at 100 °C. Dried samples of different parts of vegetables were ground into a fine powder. 1.00g of finely grinded plant tissue was weighed into a porcelain crucible. The crucible was placed in a muffle furnace and heated at 500°C for four hours, it was then removed from furnace and allowed to cool. The ignited residue was moisten with 2ml distilled water and slowly and carefully 5ml of 8N HCl was added. The crucible was covered and placed on steam water bath for 20mins. It was then filtered through Whatman No. 42 filter paper, a 100ml volumetric flask was used to collect the filtrate. The crucible was washed as well as the filter paper several times with distilled water. The solution was then made up to 100ml and was shaken vigorously to assure complete mixing to complete the digestion process. The procedure used for determining the various metals in the soils using the Atomic Absorption Spectrophotometry (AAS) was repeated to determine the various metals in the lettuce and cabbage plants.

3.6.8 DETERMINATION OF YIELD

The average fresh weight of the two lettuce plants grown in each pot was determined with a mettler balance. The weight of fresh head of the cabbage in each pot was determined with the mettler balance.

3.7 STATISTICAL ANALYSIS

All analyses were carried out with three replicates per sample. Data were reported as mean ± standard deviation. One way analysis of variance was used to determine significant difference between treatments considering a level of significance of less than 5% by using Microsoft Excel 2010. Graphs and tables were done with Microsoft Excel 2010.

CHAPTER FOUR

4.0 RESULTS

4.1 BACKGROUND CONCENTRATION

The table below shows a comparison of background concentration of selected parameters of the topsoil and sewage sludge used in this experiment.

Table 4.1 Mean values of background and concentration of topsoil and sludge

PARAMETER	SLUDGE	SOIL
pH	4.72±0.27	6.55±0.16
Phosphorus (ppm)	21.81±2.57	5.22±0.43
Nitrogen %	3.48±0.12	0.15±0.03
Organic Matter (%)	65.63±1.63	6.93±0.40
Zinc (mg/Kg)	40.77±0.36	6.05±0.13
Copper (mg/Kg)	53.10±0.22	15.43±0.38
Lead (mg/Kg)	24.10±0.13	4.39±0.17
Conductivity (us/cm)	1365.33±21.57	257±9.54

The pH of soil was within the required recommended soil pH for plant growth while the sludge pH was within the acidic range of the pH scale. The nutrient levels, soil conductivity and organic matter contents in sewage sludge were higher than the levels in topsoil. Lower heavy metal levels were present in the topsoil as compared to the sewage sludge for all the selected heavy metals. Levels of metals in both soil and sewage sludge were all within the EU directives of acceptable limits of heavy metals in sewage sludge and soil.

4.2 EFFECTS OF SEWAGE SLUDGE ON SOIL AFTER APPLYING TREATMENTS

The results below are the effects of sewage sludge on soil at various treatments for both cabbage and lettuce before plant cultivation.

4.2.1 CABBAGE

The table below shows the effect of sewage sludge on the soil properties (pH, organic matter and soil conductivity) after applying various treatments before cabbage cultivation.

Table 4.2 Mean values of selected soil properties after sludge application on soils used for cabbage cultivation

Parameters	Control	Treatment 1 (160kg/ha)	Treatment 2 (210kg/ha)	Treatment 3 (260kg/ha)
pH	6.55±0.16	6.69±0.05	6.71±0.15	6.75±0.06
Organic Matter Content (%)	6.93±0.40	17.435±0.82	20.475±0.59	24.425±0.67
Soil Conductivity (us/cm)	257±9.54	346.75±17.82	365.25±5.68	382.25±13.30

The table above revealed that increasing application rates increased the pH slightly but pH values were within the optimum pH values for plant growth (6.69±0.05 - 6.75±0.06). The pH of the treatments and control were not significantly different ($P > 0.05$). The organic matter contents were within the range (17.434±0.82 – 24.425±0.67) %, after treatment application. Increasing application rates increased the organic matter contents. Increasing application rates increased the soil conductivity of the soil. The increase of soil conductivity and organic matter content were statistically significant ($P < 0.05$).

The figure below shows the effect of heavy metal levels in soil after application of treatments before cabbage cultivation.

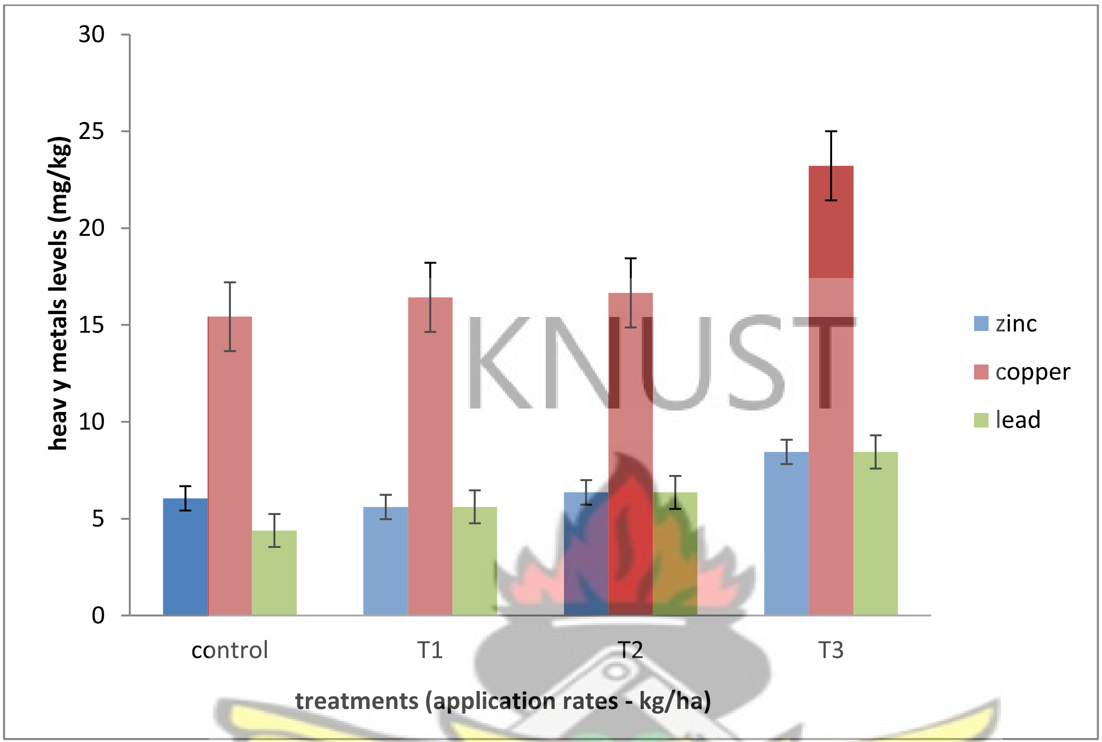

Fig 4.1 Mean values of heavy metal levels in soils used to cultivate cabbage after sewage application

The above figure shows the levels of lead increased as application rates increase. Levels of lead were in the range of (5.61±0.22-8.4475±0.18) mg/kg. The level of lead at the highest application rate (260kg/ha) was almost doubled (92.4%) with respect to the background concentration. The levels of lead were all in EU directives of acceptable limits of heavy metals in soil. A similar trend was found in the levels of copper and zinc. Copper levels ranged from (16.43±1.12-23.22±1.18) mg/kg with a percentage increase within the range of 6.48% for lowest application rates (160kg/ha) to 50.49% (260kg/ha). Highest level of zinc was found in the highest application rate (8.21mg/kg) with a percentage increase ranging within 21.03-35.07%. The increase of heavy metal levels in the soil as a result of sewage sludge application was statistically significant for all the metals.

4.2.2 LETTUCE

The table below shows the effect of sewage sludge on the soil properties (pH, organic matter and soil conductivity) after applying various treatments before lettuce cultivation.

Table 4.3 Mean values of selected soil properties after sludge application on soils used for lettuce cultivation

Parameters	Control	Treatment1 (100kg/ha)	Treatment2 (150kg/ha)	Treatment3 (200kg/ha)
pH	6.55±0.16	6.71±0.10	6.68±0.13	6.74±0.11
Soil conductivity us/cm	6.93±0.40	14.675±1.07	16.5±0.28	18.87±0.79
Organic matter %	257±9.54	331±19.77	343.75±19.21	364.75±10.31

The results above show that soil pH was slightly increased after application of treatments. The pH increased by 1.91- 2.90% and were within the neutral range of the pH scale (6.68 – 6.74). Sewage sludge applications had no significant effect on the soil pH. Organic matter content increased as a result of sludge application. Increasing the application rates increased the soil conductivity. The increase of soil conductivity and organic matter were statistically significant ($P < 0.05$).

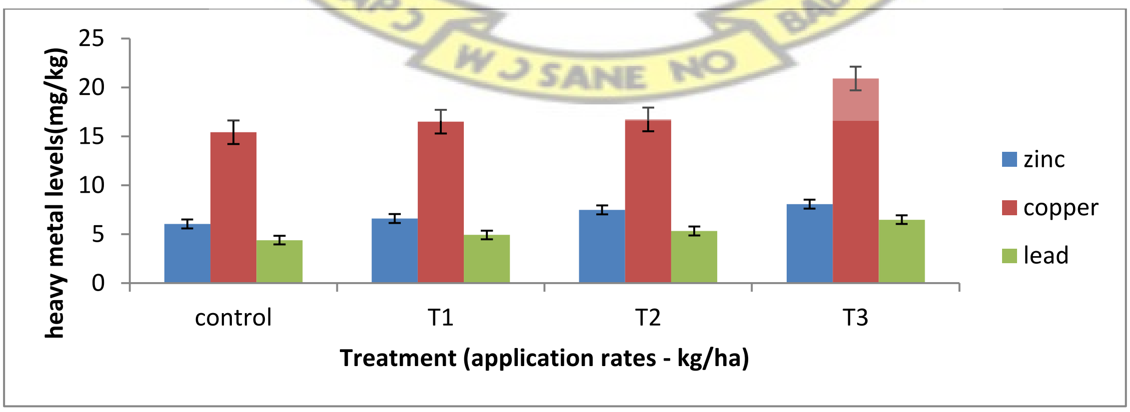

Fig 4.2 Mean values of heavy metal levels in soils used to cultivate lettuce after treatments application

The figure above revealed that heavy metal levels increased as the application rates increased. Heavy metal levels after sewage sludge application were statistically significant ($P < 0.05$). The levels of heavy metals were within the EU directives of acceptable limits of heavy metals in soil.

4.3 HEAVY METAL LEVELS IN PLANTS GROWN ON VARIOUS TREATMENTS

The results below show the heavy metal levels in both lettuce and cabbage for various treatments after harvesting.

4.3.1 CABBAGE

The figure below shows the result of heavy metal levels in cabbage after harvesting.

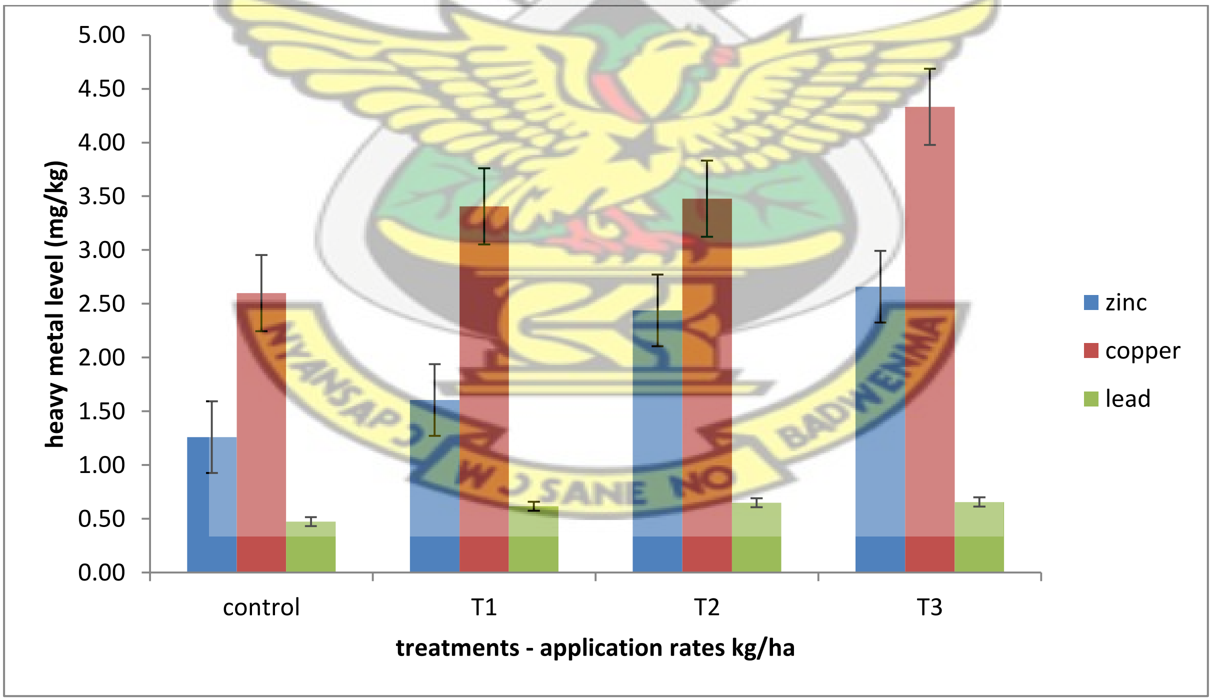

Fig 4.3 Mean values of heavy metal uptake in cabbage after cultivation on sewage amended soil

The result above shows that heavy metal levels in the cabbage increased with increasing application rates. The control has lower levels of heavy uptake (0.48±0.13, 1.36±0.23 and 2.60±0.29 mg\kg for lead, zinc and copper respectively). The highest levels were recorded in the highest application rates (0.66±0.17, 2.66±0.09 and 4.33±0.14 for lead, zinc and copper respectively). Heavy metals uptake was statistically significant ($P < 0.05$) for all the metals except lead uptake which was not significant. The heavy metals uptakes in the cabbage at various treatments were all within the WHO safe limit of the selected heavy metals in plants for human consumption.

4.3.2 LETTUCE

The figure below shows the results of heavy metal levels in lettuce after harvesting.

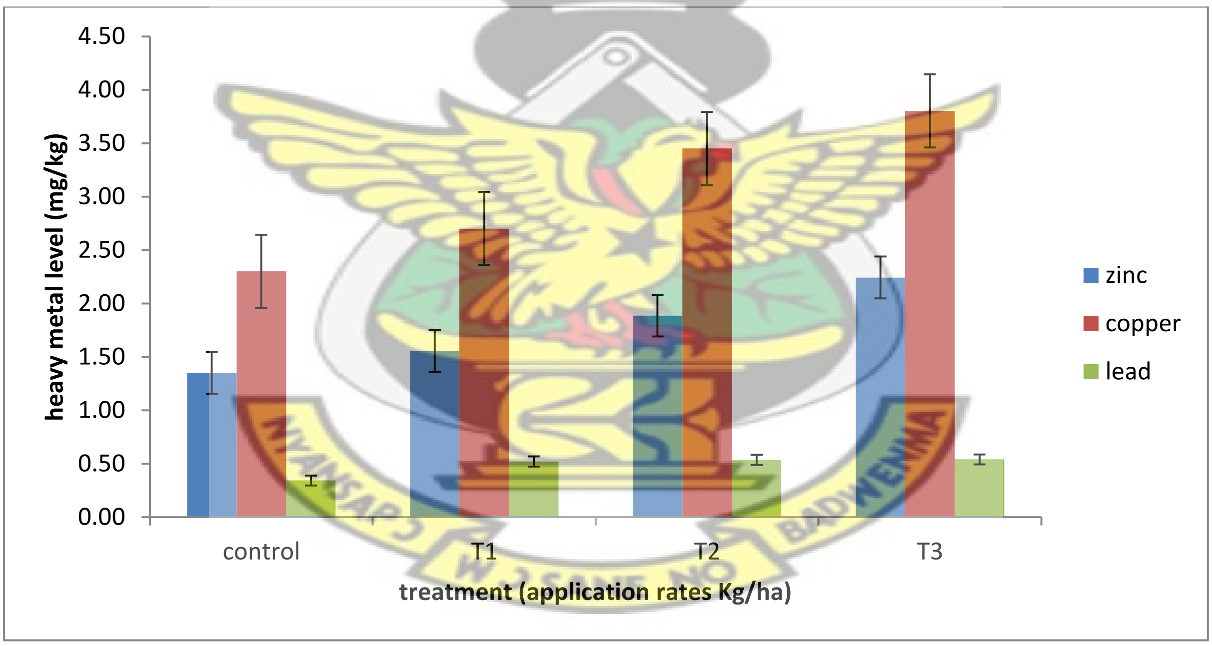

Fig 4.4 Mean values of heavy metal uptake in lettuce after cultivation on sewage amended soil

The figure above shows that heavy metal levels increased as the application rates increased. The control has low heavy metal uptake (0.34±0.19, 1.35±0.31 and 2.30±0.14 mg\kg for lead, zinc and copper respectively). The highest application rate recorded the maximum heavy metal uptake (0.54±0.01, 2.24±0.17 and 3.88±0.19 mg/kg for lead, zinc and copper respectively). The uptake of metal was statistically significant ($P < 0.05$) for all the metals except lead. The heavy metal uptakes in the lettuce at various treatments were all within the WHO safe limit of the selected heavy metals in plants for human consumption.

4.4 RESIDUAL HEAVY METAL LEVELS IN SOIL AFTER CULTIVATION

The results below indicate the residual heavy metal levels in soil after lettuce and cabbage cultivation.

4.4.1 CABBAGE

The figure below is the results of the residual heavy metal levels after cabbage cultivation for various treatments.

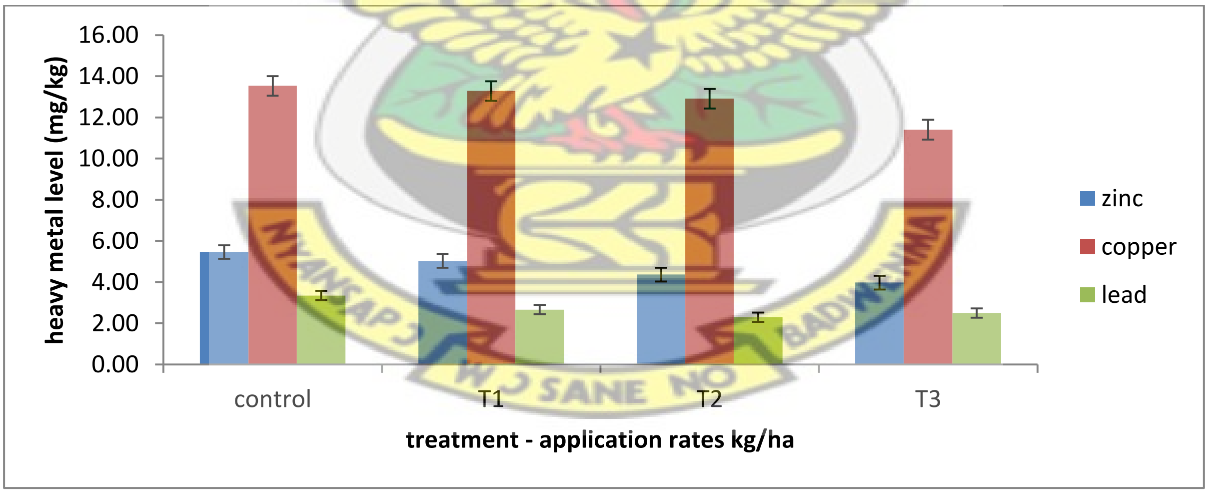

Fig 4.5 Mean values of residual heavy metal levels in sewage amended soil after cabbage cultivation

The results in Fig 4.5 reveal that residual heavy metal levels in the control were higher for all the metals compared to other treatments. Residual levels of zinc and copper decreased with increasing application rates. Levels of lead among treatments did not decrease with increasing application rates but were within the range of 2.66 – 2.29 mg/kg.

4.4.2 LETTUCE

The result for residual heavy metal levels in the soil after cultivation is shown below.

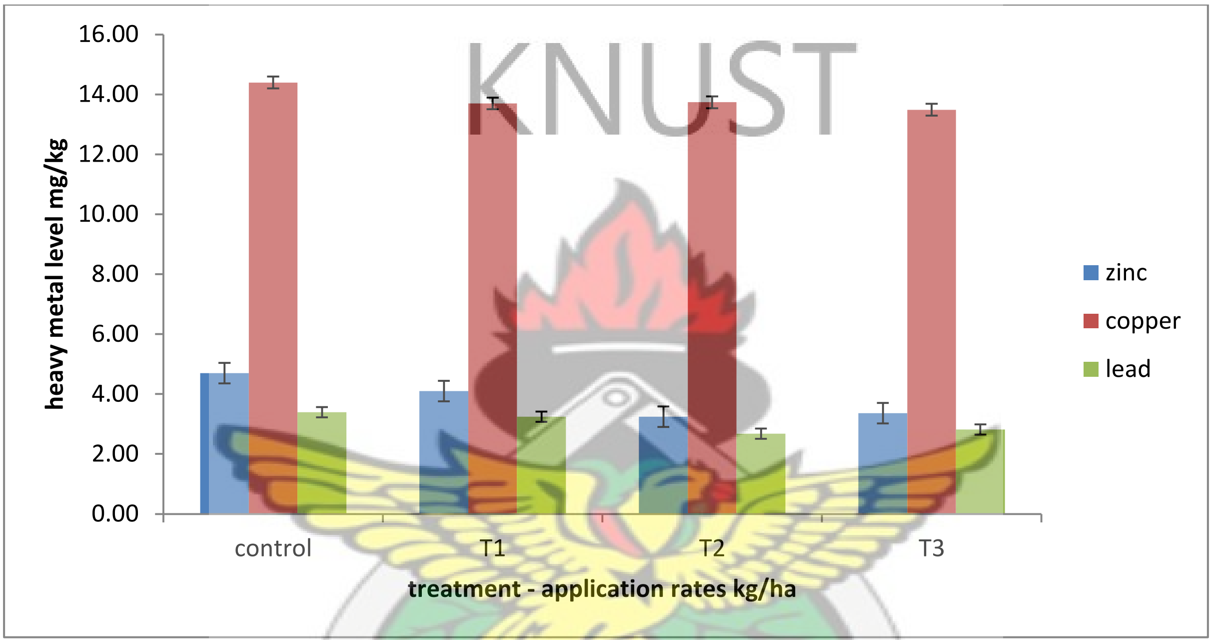

Fig 4.6 Mean values of residual heavy metal levels in sewage amended soil after lettuce cultivation

Residual heavy metal levels in the soil of the control were higher than in the other treatments (Fig. 4.6). Heavy metal levels among treatments were within the range of 4.10-3.24, 13.70-13.49 and 3.25 – 2.82 mg/kg for zinc, copper and lead respectively.

4.5 PLANT YIELD

The results below are mean values of fresh weight of lettuce and cabbage.

4.5.1 CABBAGE

The figure shows the result of yields of cabbage after cultivation.

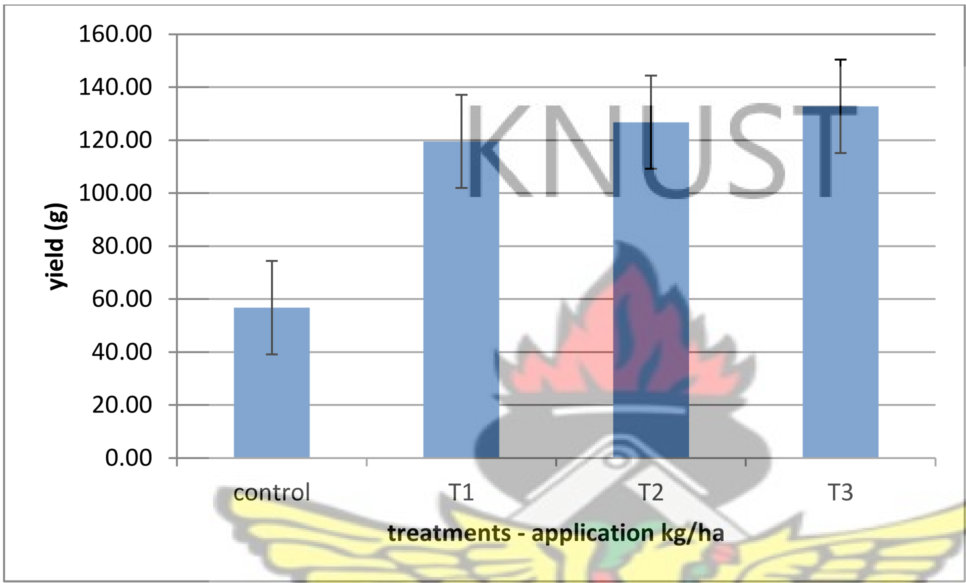

Fig 4.7 Mean values of cabbage yields

The results above revealed that yields of cabbage increased as application rates increased. Lower yields were recorded in the control (324.50±17.92 g). Yields of other treatments (536.75 - 610.50 g) were found to be within market yield (above 450g). The increase in yields was statistically significant ($p < 0.05$) with respect to the control.

4.5.2 LETTUCE

The figure shows the result of yields of lettuce after cultivation.

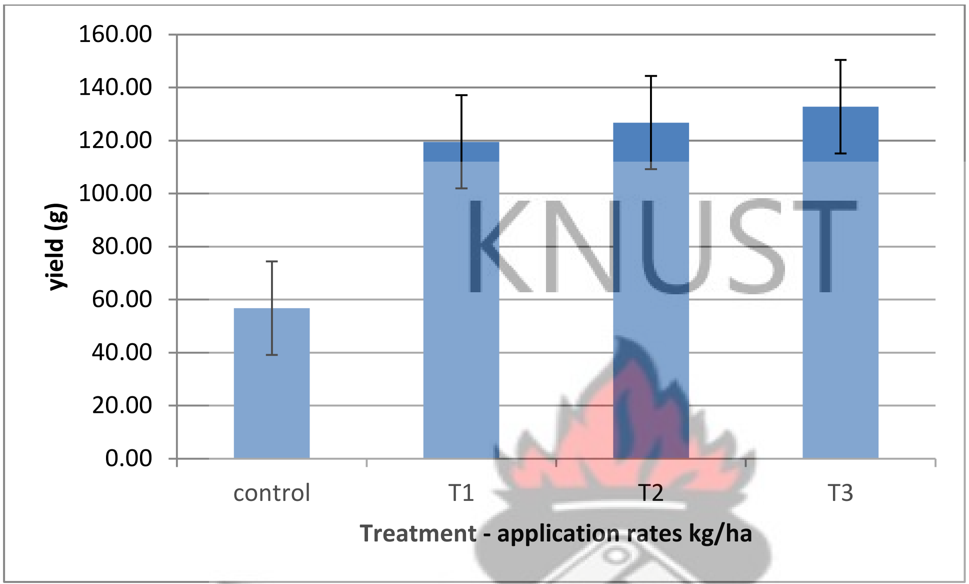

Fig 4.8 Mean values of lettuce yields

Yields of lettuce increase as application rates increased. Yield of lettuce were within the range of 56.75 - 132.75g, which fall within the market yield (above 30g). The increase in yields was statistically significant ($p < 0.05$) with respect to the control.

CHAPTER FIVE

5.0 DISCUSSION

5.1 BACKGROUND CONCENTRATION OF SEWAGE SLUDGE AND TOP SOIL

The use of sewage sludge in agriculture as amendment has been encouraged and practiced for many years. It has been known to be economically attractive as compared to other waste management options such as landfill and incineration. Sewage sludge applications enable waste materials to be recycled therefore saving the natural resources and energy effectively (Dolgen *et al.,* 2006; Kim *et al*., 2002). In this study, the content of nitrogen, phosphorus and organic matter were high in the sewage sludge. This revealed the potential of sewage sludge to support agriculture and enhance soil fertility. The source of these nutrients and organic matter may be attributed to human excreta, which mainly originates from consumed food (Jönsson and Vinnerås, 2004; Fraústo da Silva and Williams, 1997). Detergents, use for cleaning lavatories, have been reported to have phosphates compounds (Knud-Hansen, 1994; Carpenter *et al.*, 1998) and may also contribute to the high phosphorus content in the sewage sludge. Many researchers have reported high nutrient levels in sewage sludge (Martinez *et al*., 1999; Sommers, 1977; Bozkurt and Yarilgac, 2003). The high nutrient values in the KNUST waste treatment sludge might contribute to the high demand of the sludge by farmers for cultivation of many crops including vegetables. The high organic matter content contributes to the structural stability of the soil and resistance to erosion (Hernández, *et al*., 1991; Ortiz and Alcañiz, 2006) as well as improving soil aeration, water holding capacity and aggregate stability of the soil (Logan and Harrison, 1995; Aggelides and Londra, 2000). This makes the sludge preferable to synthetic fertilizers which have no or little organic matter content (Chao *et al*., 1996).

The background concentration of the topsoil used has low nitrogen, phosphorus and organic matter content. The soils used were undisturbed soil which has not been engaged in agriculture activities within three to four years. The soil may represent conditions of undisturbed soils which are exposed to environmental conditions without good soil management practices. Factors such as high rainfall, low oxygen levels in soil, warm temperatures, erosion, excessive burning, contribute to low levels and leaching of soil nutrients (Araya and Takeshita, 2000; Johnson, 1995; Sesay, 2007). Crops grown on soil with low nutrients result in lower yields and market values which consequently threaten food security and decline in economic growth especially countries whose economic growth depends on agriculture (Steiner, 2006; Sanchez, 2002; Smaling and Stoorvogel, 1990). This might be the reason why farmers are using different soil amendment materials to enhance fertility of the soil.

The heavy metals content in the sludge were higher than the soil used (table 4.1). This agrees with Corey *et al.*, (1987) who reported that trace elements are higher in sewage sludge than in their natural abundance in soil. The presence of metals in sludge accord with several research works which reported presence of heavy metals in sewage sludge which is a major setback to its application in agriculture (McBride, 1998; Hussein *et al.*, 2010; Jakubus and Czeka, 2001). Copper and Zinc are essential minerals in the human diet. Zinc enhances the functions of the immune system and as well as important for normal brain activity and is fundamental in the growth and development of the foetus (ATSDR, 1999). Copper is essential for the formation of healthy nerves, bones, blood, hair, skin, connective tissue, and so it is vitally important to maintain healthy levels of copper (Carlson *et al.*, 1992). Higher levels of copper and zinc have been found to be detrimental to the body and causes serious health problems and plant growth disorders (Rout and Das, 2003; Panel on Micronutrients, 2001). Food sources of copper include; beans, leafy vegetables, cereals, broccoli, oranges, coconut, wheat, oats, garlic, meats, and many more. Foods that provide the body with zinc include; chicken, eggs, fish, cashew, liver, oyster and many more.

Levels of copper and zinc in sewage sludge can be attributed to the human excreta. Guyton, 1992 reported that heavy metals in human excreta come from ingested food. The WHO reported presence of heavy metal in human excreta (WHO, 2006). Some researchers have reported heavy metals in human consumed foods (Xiong 1998, Cobb *et al.*, 2000; Wang *et al.*, 2008).

The levels of lead in sludge were lower than the other metals. The presence of lead in sewage sludge may indicate its presence in consumed food. Pesticides, fertilizers, waste water, sewage sludge, atmospheric deposition, have been reported to contribute to lead uptake in plants (Demirezen and Ahmet 2006; Sharma *et al.*, 2006). Colored plastic bags, wrapping papers, cardboard containers that contain lead or are colored with lead-containing dyes, lead foil capsules on wine bottles, and lead-glazed ceramic, lead crystal, or lead-containing metal vessels used for packaging or storing foods have been identified as leading lead sources in food (Carpenter *et al.*, 1998). Lead has been known to have no nutritional benefits to the human body but causes health problems even at a very low concentration (ATSDR 1999). The levels of lead, copper and zinc in sewage sludge used for this experiment were found to be far below the EU directives of acceptable limits of heavy metals in sewage sludge for land application. A comparison of the levels of heavy metals in the KNUST treated sludge to other treatment plants which incorporate the treatment of industrial, hospital, domestic waste water and landfill leachate revealed that levels in other treatment plants sludge were higher than those in KNUST treatment plants. This is in agreement with recommendation made by

Shiming, (2002) thus, treatment of human excreta should be separated from other waste water sources because of low levels of heavy metals in human excreta. WHO (2006) also reported miniscule heavy metals levels in human excreta.

Heavy metals were found in the top soil used for this work (table 4.1). This supports works by some researchers who indicated that heavy metals are found naturally in the soil. (Szefer, 1998; Glasby, 1998; Pacyna, 1994). Lombi *et al.*, (2001) reported that anthropogenic activities such as irrigation with waste water, application of mineral and inorganic fertilizers and air pollution can increase the levels of heavy metals in the soil. The lower levels of metals in the soil might be as a result of its inactiveness in agricultural practices as well as the location of the soil which was found to be away from industrial settings and vehicular traffics, which are major sources of air pollutants. The levels of lead, copper and zinc in the soil were found to be far below the EU directives of acceptable limits of heavy metals in soil for agriculture purposes.

5.2 THE EFFECTS OF SEWAGE SLUDGE ON SOIL

5.2.1 EFFECTS ON SOME SELECTED SOIL PROPERTIES

Three soil properties have been noted to affect the heavy metals uptake by plants. These are the pH, organic matter and the cation exchange capacity (CEC) (Dube *et al.,* 2000, Grisso *et al*., 2006, Sharma *et al*., 2006; Otte *et al.,* 1995; Su *et al.,* 2004). Soil conductivity is at times substituted with CEC because research bears out the correlation between conductivity and CEC through its relationship to clay (Grisso *et al*., 2006). Applying sewage sludge to the soil had no significant effect on the soil pH for both cabbage treatments (P>0.05) and lettuce treatments (P>0.05). The pH of the soil was still within the required pH for optimum plant growth range even after sewage sludge application. The pH of sludge was within the acidic range (4.72) and this could explain why most farmers who use the KNUST sludge report plant burn after applying the sludge at the base of plants. Acidic conditions have been reported to cause plant burns (Cunningham *et al.*, 2005). Micro-organisms have been known to alter pH of sludge as a result of acid production during decomposition and therefore altering their environmental conditions can alter the pH (Bina *et al.*, 2004). This could explain why farmers who use the sludge identify composting and thorough mixing of sludge and soil before plant cultivation as a solution to the problem of burns. This work also confirmed thoroughly mixing of sludge with soil can affect the pH of sewage sludge. Kazi *et al*., (2005) reported that pH of soil was insignificantly affected after amending with sewage sludge. An increase in soil pH has been reported in soils applied with sewage sludge (Tasdilas, 1997) and lowering of soil pH is also reported (Epstein *et al.*, 1976). The changes in soil pH have been correlated with the calcium carbonate content of sludge and acid production during sludge decomposition (Tasdilas, 1997).

The organic matter contents of the soil significantly increased as application rates (treatments) increase in both the cabbage and lettuce. This accord with most research works. Archie and Smith, (1981) reported of an increase in organic matter which increased soil fertility as a result of sewage sludge application. Ramulu, (2002) also reported an increase in organic matter as a result of sewage sludge application which improved soil properties, such as bulk density, porosity and water holding capacity. Ojeda *et al.*, (2003), Hussein *et al.*, (2010), Basta *et al*., (2000), Hernández, *et al*., (1991), Ortiz and Alcañiz, (2006) have reported increase in organic matter as a result of sewage sludge application. Increasing application rates have been correlated with increasing organic matter (Hue and Ranjith, 1994). A similar trend was found in this work. Though sludge increase organic matter content, excessive application of sludge can also lead to increasing heavy metal levels (Behbahaninia *et al*., 2009) and excessive leaching of plant nutrients into ground water and surface waters (Kanugo, 2000). Therefore soil testing is normally recommended to enhance the correct application rates of sludge to be applied for optimum plant growth and minimize negative environmental effects. Most farmers who engaged sludge application hardly carry out soil testing before sludge application and the presence of algae growth around surface waters used for irrigation in some farms is an indication of excessive sludge application.

Soil conductivity increased significantly after sludge application for both lettuce and cabbage soils. Increasing application rates increased soil conductivity. Some works have associated increased in soil conductivity with organic matter increase. Serna and Pomares (1992) reported that organic matter contains metallic salts which increase the conductivity of soils. Dube *et al*., (2000) also associated the increase of conductivity with an increase in organic matter. Zucconi and Bertoldi,(1987) indicated that excessive application of sludge can increase conductivity to levels that can decrease yield. Levels of soil conductivity for all treatments were within the desire level for plant growth.

5.2.2 EFFECT OF SEWAGE SLUDGE APPLICATION ON HEAVY METAL LEVELS IN SOIL BEFORE CULTIVATION

Heavy metal levels significantly increased after sewage sludge application for all the metals. This study confirms most research works concerning increase in soil heavy metal levels as a result of sewage sludge application. Wei and Liu, (2005), Dudka and Miller, (1999); Amir *et al.*, (2005), Adao *et al.*, (2003) have reported increase in heavy metal levels as a result of sewage sludge application. This work also is in agreement with the work by Zwarich and Mills, (1979), which reported an increase in heavy metals as application rates increase and also reported controlling the application rates of sewage sludge controlled heavy metals loading. The heavy metal levels after sewage sludge application were below the EU acceptable limits. The increase of heavy metal in soils as a result of sewage sludge application has become a major setback to its use.

5.3 HEAVY METAL UPTAKE BY LETTUCE AND CABBAGE

Levels of metal in cabbage and lettuce plants increased as a result of increasing application rates of sludge. The control recorded the lowest heavy metal uptake for all the metals. This indicates that sewage sludge enhance heavy metal uptake by lettuce and cabbage. This corresponds to some literatures which recorded lower uptake of heavy metal in control (without amendment) than other treatments. Ngole, (2007) and Rappaport *et al*., (1988) recorded lowest zinc uptake in control as a result of no sewage sludge application. Adao *et al.*, (2003) also found that copper concentration in plants increased with increasing sewage sludge rate. The uptake of copper and zinc were statistically significant for both plants but lead uptake was statistically insignificant compared with the controls though levels increased as rates of sludge increased. Again, among the metals, uptake of lead was the lowest for both lettuce and cabbage. Though heavy metals uptake by lettuce and cabbage were enhanced by sewage sludge application especially for copper and zinc, levels in lettuce and cabbage were all below the WHO safe limit of the selected heavy metals in plants for human consumption. This may be attributed to lower levels of heavy metal in background concentration of both sewage sludge and the top soil used, the type of water used for irrigation, soil pH, organic matter and soil conductivity.

Fytianos *et al*., (2001) reported levels of lead in vegetables exceeded WHO safe limit because of high levels of lead in the background soil as a result of industrial activities. Tandi *et al*., (2005) also reported a higher uptake of Copper in lettuce grown on industrial sites. Alloway, (1995), Berti and Jacobs, (1998), Sloan *et al*., (1997), Tlustoš *et al*., (2001), Wong *et al*., (2001) have reported higher uptake of zinc in plants as a result of higher levels of zinc in sewage sludge used. Lower levels of metals in background soil and sewage sludge were found in this work and could have contributed to lower metal uptake by both lettuce and cabbage.

In this work, applying sewage sludge increased the organic matter content and soil conductivity which have been associated with immobilization of heavy metals for plant uptake and could possibly have contributed to low uptake of metal by cabbage and lettuce. Bride *et al*., (2000), Haar, (1991) and Teresa *et al*., (1991) recorded lower or no translocation of lead into plants. They attributed the cause of low or no translocation of lead to the presence of organic matter which combined with ions to form a stable compound. Ruby *et al*., (1994) suggested that the formation of lead phosphates in soils is responsible for immobilizing lead, thereby reducing the bioavailability of lead. Sloan *et al*., (1997) reported that organic matter tied up excess amount of copper making it unavailable to plant and land application of sewage sludge is unlikely to cause copper toxicity in plants except in cases of heavy repeated application to acidic soil. McBride, (1998) found that organic matter is tightly bound to heavy metal reducing their bioavailability. Conductivity increase indicate an increase in cation exchange capacity which provide binding sites for heavy metals making them unavailable for plant uptake (Soon, 1981).

The pH of the soil is also a contributing factor that could have contributed to the low heavy metal uptake by the plants (Logan and Chaney, 1983; Sommers *et al*., 1987; Dowdy and Volk, 1983). pH (<6) has been reported to enhance leaching of heavy metal and making the metals available for plants uptake (Kazi *et al*., 2005). Lübben *et al.*, (1991), Smith, (1994) and Planquart *et al.*, (1999) reported that Zn concentrations in plants were greater in the acidic soil than in the alkaline ones confirming Zn absorption dependency on soil pH. Hooda and Alloway (1996) stated that the use of urea to raise the soil pH affected the availability of heavy metals to wheat grain. Reddy *et al*., (1996) pointed out that soils having pH 7 to 9 inhibited the copper availability in soils and consequently their uptake by plants. The sewage sludge used in this work did not affect the soil pH significantly and the pH of the soil was within the neutral range of the pH scale (6.55 – 6.75). This could also have resulted to the lower uptake of heavy metals by both plants.

Wastewater used for irrigation has also been noted to increase heavy metal uptake by plants (Behbahaninia *et al*., 2009). Demirezen and Ahmet (2006) and Sharma *et al*., (2006) reported high concentration of Lead above the safe limit in vegetables grown in soil irrigated with wastewater from industrial areas. Muchuweti *et al*., (2006) reported the level of Lead in vegetables irrigated with mixtures of wastewater and sewage to be higher than WHO safe limit. Most farmers who use the KNUST sludge rely on streams and rivers loaded with waste materials to irrigate their plants and this could enhance heavy metal uptake by plants. In this study, tap water was used to irrigate the plants and levels of heavy metals in the tap water were insignificant contributing to low heavy metals uptake. The percentage uptake of metals by lettuce and cabbage with respect to background soil heavy metal levels confirmed the ability of leafy vegetables to uptake metals (Demirezen and Ahmet (2006). Comparing the uptake of metals by both plants, cabbage leaves have a higher uptake levels than lettuce and this agrees with works by Wang *et al.,* (2008), Wei and Liu, (2005), Zhao *et al.,* (2006), Huang *et al.,* (1998), Zheng *et al.,* (2007) and Gr_man *et al.,* (2003) that cabbage absorbed more metals and may be a candidate plant to remediation of soil contaminated with heavy metal.

5.4 EFFECT OF SEWAGE SLUDGE ON CABBAGE AND LETTUCE YIELD

Yields of cabbage and lettuce responded positively to application of sewage sludge. The controls recorded the least yields for both plants and highest yields were recorded at highest application rates for both plants. The percentage increase of yield for lettuce ranged between 110.38 - 133.71% with respect to the control while that of cabbage ranged between 65.41 - 88.14% with respect to the control. The increased in yield were found to be statistically significant. With the exception of yields of controls of both lettuce and cabbage, the yields of the other treatments were found to be within market values. The low yield in control can be attributed to low nutrient contents and organic matter content. This really shows that Ghanaian farmers cannot rely solely on our natural soil for production without amendment with fertilizers to meet market values both for local production and exportation. The increase in yield as result of sewage sludge application conformed to works by researchers that sewage sludge has the potential of increasing the yield of crops. Barriquelo *et al.,* (2003), Coker, (1983), Ippolito *et al.,* (1992), Knuteson *et al.,* (1988), Azam and Lodhi, (2001), Chatha *et al.,* (2002), Mohammad and Athamneh, (2004), Dursan *et al.,* (2005), Casado-Vela *et al.,* (2006) and (2007) Jamil *et al.,* (2006) have shown sewage sludge benefits are similar to commercial fertilizers because of high crop yields and high quality crops. Dorn *et al.,* (1985) and Muhammad *et al.*, (2007) reported highest sewage sludge application rate supplied more nutrients to plant resulting in an increased yield. Boswell, (1975) reported an approximately 30% higher yield under application of sewage sludge. Nielson *et al.,* (1998) reported an increase in yield by sewage sludge amendment and the yield was higher than those grown on soil amended with recommended NPK fertilizer. The increase in yield as a result of sewage sludge is attributed to its high nutrient content, and high organic matter content which affect positively the soil structure, improve soil aeration and enhance activities of living organisms within the soil.

5.5 RESIDUAL HEAVY METAL LEVELS

The control recorded the highest heavy metal residual levels for both plants and decrease as application rates decreased. Though the levels decreased as rates of application increased, the difference in residual levels were insignificant among the treatments and statistically significant with respect to the control for both plants. This is attributed to the presence of organic matter content and other metal binding substances in soil amended with sewage sludge. The binding of heavy metal with organic matter in soil makes them unavailable for plant uptake and residual form. This might explain why lower residual levels were recorded in the amended soils than the control. Work done by Eriksson (2006) shows that a continuous application of 0.7 ton (700.112 kg/ha) sewage sludge dewatered to a single field would theoretically lead to a doubling of concentrations in the topsoil for copper in 170 years and 200 years for lead and zinc. This shows that increasing sewage sludge increase organic matter content and other heavy metal substances therefore increasing the capacity of the soil to bind metals to decrease their bioavailability for plant uptake.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study showed that heavy metals (copper, lead and zinc) were present in the sewage sludge from the KNUST treatment plant. The sewage sludge according to the EU directives can be used in land application because levels of metals were below acceptable limits. Application of sewage sludge to soil improved the soil organic matter content, increased soil conductivity and has no influence on soil pH. Thou levels of heavy metals increased significantly as a result of sewage sludge application, they were still below acceptable limits. The heavy metal levels and organic matter content as well as soil conductivity increased significantly as the application rates of sewage sludge increased. The KNUST treatment sludge can therefore be used as soil amendment material to enhance and improve soil fertility and will not pose any ill effects on the soil even at the highest recommended rates.

This study revealed uptake of metals by lettuce and cabbage increased as the application rates of sewage sludge increased. The levels of metals in the lettuce and cabbage were all below WHO safe limits of metals in plants for human consumption. Cabbage and lettuce yield increased with increasing application rates. The yield increased at the highest application rates while the lowest yields were recorded at the control which has no amendment. This shows that the use of KNUST treatment sludge for cultivation of cabbage and lettuce can improve the yields and result in low heavy metal uptake even at highest application rates. The heavy metal residue in the soil after cultivation were minimal among treatment for both plants, indicating the sewage sludge application has the capacity to bind heavy metals or change their form into complexes which incorporate metals into the soil matrices. The initial background concentration of soil to be amended with sewage sludge, water used for irrigation and soil pH should be assessed before applying sewage sludge because of their potential to increase the levels of metals in the soil.

6.2 RECOMMENDATIONS

In order to improve and enhance the use of sewage sludge in agriculture, it is recommended that;

1. Heavy metals such as nickel, cadmium and arsenic levels in the sewage sludge and their uptake by plants should be assessed.
2. Though organic pollutants have been reported to be low in sewage sludge, it will be advisable to assess the levels of these organic pollutants in sewage sludge.
3. Non leafy vegetables and crops should also be grown on sewage sludge amended soil to analyze the heavy metal levels in them.
4. Wastewater used for irrigation should be assessed in order to measure the levels of heavy metals input in both soil and plant as a result of wastewater used for irrigation.
5. Lettuce and cabbage should be used in bioremediation program because of high uptake of heavy metals by these plants.

REFERENCES

Adao L., M., Ordinocleante B., and Otavio A. (2003).Copper, nickel and zinc phytoavailability in an oxisole amended with sewage sludge and liming. *Sci, Agric.*, 60(4): 1-13.

Africa Renewal. (2008). A harvest of hope for African farmers. *Vol.22#3 pg. 3.*

Agency for Toxic Substances and Disease Registry (ATSDR). (2000). Toxicological Profile for Arsenic, Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services. *Public Health Service*, 205-1999-00024.

Agency for Toxic Substances and Disease Registry (ATSDR). (1999). Toxicological Profile for Lead; Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services. *Public Health Service*, 205-93-0606.

Aggelides, S. M., and Londra P. A. (2000). Effects of compost produced from town wastes and sewage sludge on the physical properties of a loamy and clay soil. *Bioresource Technol*., 71(3): 253–259.

Al Jassir, M.S., Shaker A., and Khaliq, M.A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabea. *B. Environ. Contam. Tox,* 75:1020-1027.

Alloway, B.J. (1992). Soil processes and behaviour of metals. p. 7-28. In B.J. Alloway (ed.) Heavy metals in soils. John Wiley, New York.

Alloway, B.J. (1995). Soil Processes and Behavior of Heavy Metals. *Blackie Academic and Professional*, *Glasgow*. pp. 11-37.

Amir, S., Hafidi, M., Merlina, G., and Revel, J.C. (2005). Sequential extraction of heavy metals during composting of sewage sludge. *Chemosphere.59: 801-810.*

Angelidaki, L., and Ahring, B. K. (2000). Anaerobic digestion of sludge: focusing on degradation of the contained organic contaminants; Workshop on problems around sludge, 18-19 November 1999 - Stresa (NO) Italy, Proceedings; European Commission Joint Research Centre. *EUR 19657 EN.*

Arai, S. (2002). Global view on functional foods: Asian perspectives. *Brit. J. Nutr*., 88: S139 S143.

Araya, T., and Takeshita, M. (2000). Soil nutrient loss caused by intensive land use and the retention of nutrients inside termite mounds in Niger, Africa; Graduate School of Agriculture, Hokkaido University, Sapporo, Japan. *Japanese Journal of Ecology*.

Archie, S.G., and Smith, M. (1981). Survival and growth of plantations in sewage sludge treated soil and older forest growth study. University of Washington, College of Forest Resources, Washington, DC, pp. 105–113.

Asare, I., Kranjac-Berisavljevic, G., and Cofie, O. (2003). Faecal sludge application for agriculture in Tamale. *Urban Agriculture Magazine,* vol 10, pp31-3.

Awasthi, S.K. (2000). Prevention of Food Adulteration Act No 37 of 1954. Central and state rules as amended for 1999', 3rd edition. (Ashoka Law House: New Delhi).

Azam, F., and Lodhi, A. (2001). Response of wheat (*Triticum aestivum* L.) to application of nitrogen fertilizer and sewage sludge. *Pakistan J. Biol. Sci.,* 4: 1083–1086.

Båge, L. (2006). Seeds of Hope; Agriculture and Economic Development. The magazine of the United Nations Environment Programme, *Special Edition (Our Planet).*

Barriquelo, M., Marines, J., Silva, M., and Lenzi, E. (2003). Lead behavior in soil treated with contaminated sewage sludge and cultivated with Maize, Brazilian. *Archives of Biology and Technology Journal* 46: 499-505.

Basta, N. T., Zupancic, R. J., and Dayton, E. A. (2000). Evaluating Soil Tests to Predict Bermudagrass Growth in Drinking Water Treatment Residuals with Phosphorus Fertilizer. *J. environmental quality,* 29: 2007–2012.

Basta, N. T. (1995). Historical Perspective; Land Application of Biosolids; A Review of Research Concerning Benefits, Environmental Impacts, and Regulations of Applying Treated Sewage Sludge B-808 Oklahoma Agricultural Experiment Station and Center for Agriculture and the Environment Division of Agricultural Sciences and Natural Resources Oklahoma State University.

Behbahaninia, A., Mirbagheri, S. A., Khorasani, N., Nouri, J., and Javid A.H. (2009). Heavy metal contamination of municipal effluent in soil and plants. *Journal of Food, Agriculture & Environment Vol.7* (3&4) : 851-856. 2009.

Berti W.R., and Jacobs L.W. (1998). Distribution of Trace Elements in Soil from Repeated Sewage Sludge Applications. *J. Environ. Qual 27*: 1280-1286.

Bina, B., Movahedian, H., and Kord, I. (2004). The Effect of Lime Stabilization on the Microbiological Quality of Sewage Sludge. *Iranian J Env Health Sci Eng, Vol.1, No. 1, pp.*34-38, 2004.

Birkett, J.W., Lester, J.N., (2003). Endocrine Disrupters in Wastewater and Sludge Treatment Processes. *CRC Press LLC, Florida.*

Bodelier, L.E., Roslev, P., Henckel, T., Frenzel, P. (1999). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature 403 (6768): 421–424.*

Bowen, H.J.M. (1979). Environmental chemistry of the elements. *Academic Press, Inc.,* New York.

Bozkurt, M.A., and Yarilgac, T., (2003). The effects of sewage sludge applications on the yield, growth, nutrition and heavy metal accumulation in apple trees growing in dry condition. *Turk. J. Agric. For.* 27, 285–292.

Brady,N., and Weil, R. (2002). The nature and property of soils. Prentice Hall, Upper Saddle River, NJ.

Bride, M.B., Martinez, E., and Evans, L. (2000). Trace metal solubility and speciation in a calcareous soil 18 years after No-till sludge application. *Soil Sci.*, 165(8): 646-656.

Bright, D.A., and Healey, N. (2003). Contaminant risk from biosolids land application: contemporary organic contaminant levels in digested sewage sludge from five treatment plants in greater Vancouver, British Columbia. *Environ. Pollut*. 126: 39-49.

Broun, E.R., Greist, A., Tricot, G., and Hoffman, R. (1990). Excessive zinc ingestion. A reversible cause of sideroblastic anemia and bone marrow depression. *Jama*, 264: 1441-1443.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W.,. Sharpley, A.N., et al., (1998). Non-point pollution of surface waters with P and nitrogen. *Issues in Ecology*, 3, pp. 1–12.

Carlson, C., Morrison, G. (1992). Fractionation and toxicity of metals in sewage sludge. *Environmental Technology* 13: 1, 1992.

Casado-Vela, J., Sellés, J., Navarro-Pedreno, S., Bustamante, M.A., Mataix-Beneyto J., and Gomez, I. (2006). Evaluation of composted sewage sludge as nutritional source for horticultural soils. *Waste Manag.*, 26: 946–952.

Casado-Vela, J., Sellés, S., Diaz-Crespo, C., Navarro-Pedreno, J., Mataix- Beneyto, J. and Gomez, I. (2007). Effcet of composted sewage sludge application to soil on sweet pepper crop (*Capsicum annuum var. annuum)* grown under two exploitation regimes. *Waste Manag.*, 27:1509–1518.

Chao, W. L., Tu, H. J., and Chao, C. C. (1996). Nitrogen transformations in tropical soils under conventional and sustainable farming systems. *Biol. Fertil. Soils*, 21: 252-256.

Chatha, T.H., Haya, R., and Latif, I. (2002). Influence of sewage sludge and organic manures application on wheat yield and heavy metal availability. *Asian J. Plant Sci.*, 1: 79–81

Choi, E., and Yun, Z. (2003). Proceedings of the Strong N and Agro 2003, Two volumes, IWA Specialty Symposium on Strong Nitrogenous and Agro-Wastewater, Seoul, Korea, 1,118 pp.

Chu, L.M., and Wong, M.H. (1987). Heavy metal contents of vegetable crops treated with refuse compost and sewage sludge. *Plant and Soil* 103: 191-197.

Clark, N. L. (1994). Agriculture; A Country Study; Ghana. Library of Congress Federal Research Division, La Verle Berry, Editor.

Cobb, G.P., Sands, K., Waters, M., Wixson B.G., and Dorward-King, E. (2000). Accumulation of Heavy Metals by garden vegetables. *Journal of Environmental Quality*. 29: 934-939.

Cofie, O., Kranjac-Berisavljevic, G., and Drechsel, P. (2005). The use of human waste for peri-agriculture in northern Ghana. *Renewable Agriculture and Food Systems*, vol 20, no 2, pp73–80.

Coker, E. G. (1983). Biological aspects of the disposal-utilization of sewage sludge on land. Pages 257–322 in T. H. Coaker, ed. Advances in applied biology. *Academic Press, Inc. London, UK.*

Corey, R.B., King, L.D., Fanning, D.S., Street, J.J., and Walker, J.M. (1987). Effects of sludge properties on accumulation of trace elements by crops. *Lewis Publishers, Inc, Chelsea, MI.*

Cunningham, A. (2006). Tainted by Cleanser; *Science News*, Vol. 169, No. 18, pp. 275.

Cunningham, and Cunningham, S., (2005). Environmental Science; a global concern 8th Edition, ISBN- 0072951729.

Dai, J.Y., Xu, M.Q., Chen, J.P., Yang, X.P., and Ke, Z.S. (2007). PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China. *Chemosphere*. 66: 353-361.

Delibacak, S., Elmaci, O. L., Secer, M., and Bodur, A., (2002). Trace element and heavy metal concentrations in fruits and vegetables of the Gediz River region. Int. *J. water*, 2, (2/3), 196-211.

Demirezen, D., and Ahmet, A. (2006). Heavy metal levels in vegetables in turkey are within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb. *J. Food Qual.*, 29: 252-265.

Dharmappa, H. B., Hasia, A., and Hagare., P. (1997). Water treatment plant residuals management. *Water Sci. Technol*., 35(8):45–56.

Dolgen, D., Alpaslana, M. N., and Delen, N. (2006). Agricultural recycling treatment-plant sludge; A case study for a vegetable-processing factory. *J. Environ Manage.* doi:10.1016.

Dorn, C.R., Reddy, C.S., Lamphere, D.N., Gaeuman, J.V., and Lanese, R. (1985). Municipal sewage sludge application on Ohio farms: Health effects. *Environ, Res.*, 38: 332-359.

Dorronsoro, D., Martin, F., Oritz, I., Garcia, I., Simon, M., Fernandez, E., Aguilar, J., and Fernandez, J. (2002). Migration of trace elements from pyrite tailings in carbonate soils; *J. Environ. Qual.* 31:829-835.

Dowdy, R.H., Latterell, J.J., Hinesly, T.D., Grossman, R.B., and Sullivan, D.L. (1991). Trace metal movement in an aericochraqualf following 14 years of annual sludge applications; *J. Environ. Qual*.20:119–123.

Dowdy, R.H., and Volk. V.V., (1983). Movement of heavy metals in soils. *Spec. Pub.*, No.11. Soil Sci. Soc. Am., Madison, WI.

Drangert, J. O. (1998). Fighting the urine blindness to provide more sanitation options. *Water South Africa*, 24 (2), 157-164.

Drinkwater, L. E., Letourneau, D. K., Workneh, F., H.van Bruggen, A. C., and Shennan, C. (1995). Fundamental differences between conventional and organic tomato agro-ecosystems in California. *Ecol. Appl*., 5: 1098-1112.

Dube A., Kowalkowski T., Zbytniewski R., Kosobucki P., Cukrowska E., Buszewski B. (2000). Proceedings of the XVth International Symposium on Physico-chemical Methods of the Mixtures Separation. *Borowno n. By dgoszcz*, pp.21.

Dudka, S., and Miller, W.P. (1999). Accumulation of potentially toxic elements in plants and their transfer to human food chain. *J. Environ. Sci. Health* B. 34: 681-708.

Dumont et al., (2004). Frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge. *Canadian Journal of Microbiology* 50 653-656.

Dursan, A., Turkmen, O., Turan, M., Sensoy, S., and Cirka, M. (2005). Effect of sewage sludge on seed emergence, development and mineral contents of pepper (*Capsicum annuum*) seedling. *Asian J. Plant Sci*., 4: 299–304.

Echab, A., Nejmeddine., Hafidi, M., Kaemmerer M., Guiresse M., and Revel J.C. (1998). Evolution de la fraction échangeable des éléments métalliques traces (Pb, Cu, Zn et Cd) lors du compostage des boues de lagunage, *Agrochimica XLII- 6.*

Ellen, K., Silbergeld, Amy, R., Sapkota, Lance, B., Price and Kellogg Schwab (2006). Arsenic Resistance in Campylobacter spp. Isolated from Retail Poultry Products." *Applied Environmental Microbiology* 72 3069-3071.

Ellen, G., Loon, J.W., and Tolsma, K. (1990). Heavy metals in vegetables grown in the Netherlands and in domestic and imported fruits. *Z Lebensm Unters Forsch*. 190(1):3439. doi: 10.1007/BF01188261.

Elliott, H. A., and Dempsey, B. A. (1991). Agronomic effects of land application of water treatment sludge. *J. Am. Water Works Assoc.*, 83(4): 126–131.

Elliott, H. A., and Singer, L. M. (1988). Effect of water treatment sludge on growth and elemental composition of tomato shoots. *Commun. Soil Sci. Plant Anal*., 19(3): 345–354.

Epstein, E., Taylor, J.M., and Chaney, R.L., (1976). Effects of sewage sludge and sludge compost applied to soil on some soil physical and chemical properties. *J. Environ. Qual.* 5, 422–426.

Eriksson, J., (2006). Concentrations of 61 trace elements in sewage sludge, farmyard manure, mineral fertilizer, precipitation and in soil and crops. *Swedish Environmental Protection Agency, report* 5159.

Farooq Muhammad, Farooq Anwar, and Umer Rashid (2008). Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrial area. *Pak. J. Bot*., 40(5): 2099-2106.

Felicity, L.(2004). Penguin. pp. 213. *ISBN* 0-14-101566-7

Fitzgerald, E. J., Caffrey, J. M., Nesaratnam, S. T., and McLoughlin, P., (2003). Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. *Environ. Pollut.*, 123 (1), 67-74.

Food and Agriculture Sector Development Policy (FASDEP II) Ghana (2007).

Fraústo da Silva, J.J.R., and Williams, R.J.P. (1997). The biological chemistry of the elements – The inorganic chemistry of life. Oxford UK.

Fytianos, K., Katsianis, G., Triantafyllou, P. and Zachariadis, G. (2001). Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. *B. Environ. Contam. Tox*., 67: 423-430.

Glasby, G.P. (1998). The Sci. of Tot. *Environ.* 212, 49.

Gr_man, H., Vodnik, D., Velikonja-Bolta, S., and Leštan, D., (2003). Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *J. Environ. Qual*. 32: 500–506.

Gregersen, P.T., Grunnet, K., Uldum, S.A., Anderson, B.H., and Madson, H. (1999). Pontiac fever at a sewage treatment plant in the food industry. *Scand J Work Environ. Health*, 25(3): 291-295.

Grisso Robert, Mark Alley David Holshouser, and Wade Thomason (2006). Precision farming tools: Soil electrical conductivity. Virginia Cooperation Extension. *Publication* 442-508

Guyton, A.C., (1992). Human physiology and mechanisms of disease. W. B. Saunders Co, Philadelphia, USA.

Haar, G.J. (1991). Composition of airborne lead particles. *Nature,* London., 232: 553-554.

Hamon, R.E., Lorenz, S.E., Holm, P.E., Christensen. T.H., and McGrath, S.P. (1995). Changes in trace metal species and other components of the rhizosphere during growth of radish. *Plant Cell Environ.* 18: 749-756.

Hayami, Y., and Otsuka, K. (1994). Beyond the green revolution; Agricultural development strategy into the new century. In: Agricultural Technology: Policy Issues for the International Community, J.R. Anderson. (ed.), *CAB International, Wallingford, UK, pp.* 15–42.

Hernández, T., Moreno, J.L., and Costa, F. (1991). Influence of sewage sludge application on crop yields and heavy metal availability; *Soil Sci. Nutr*. 37: 201-210.

Hinesly, T.D., Ziegler, E.L., and Barret, G.L. (1979). Residual effects of irrigating corn with digested sewage sludge. *J. Environ. Qual.* 8:35-41.

Hooda, P.S., and Alloway B.J. (1996). The effect of liming on heavy metals concentration in wheat, carrots and spinach grown on previously sludge applied soils. *J. Agric, Sci*., 127: 289-294.

Hue, N.V., and Ranjith, S.A., (1994). Sewage sludges in Hawaii: chemical composition and reactions with soils and plants. *Water Air Soil Pollut.* 72, 265–283.

Hussein K A., Hassan A. F., and Abdel-Hady E.S., (2010). Study of sewage sludge use in agriculture and its effect on plant and soil. *Agriculture and Biology Journal of North America;* doi:10.5251/abjna.2010.1.5.1044.1049 ISBN Print: 2151-7517.

International Labor Organization (ILO) (2009). Key Indicators of the Labor Market Programme. (*http://www.ilo.org/public/english/employment/strat/kilm/index.htm).* Accessed on 20th November, 2010.

Ippolito, J.A., Barbarick, K.A., Westfall, D.G., Follett, R.H., and Jepson, R. (1992). Application of anaerobically digested sewage sludge to dryland winter wheat. *Colorado Agric. Exp. Stn. TR*92-5.

Iranpour, R., et al., (2004). Regulations for Biosolids Land Application in U.S. and European Union. *Journal of Residuals Science & Technology* 1 209-222.

Itanna, F., (2002). Metals in leafy vegetables grown in Addis Ababa and toxicological implications. Ethiopian. *J. Health Dev*., 16 (3), 295-302.

Jacobs, L. W., (1981). Agricultural application of sewage sludge. *Ann Arbor Science Publishers, Michigan*, pp.109-126.

Jakubus, M., and Czeka, J. (2001). Heavy Metal Speciation in Sewage Sludge. *Polish Journal of Environmental Studies Vol. 10, No. 4 245-250.*

Jamil, M., Qacim M., and Umar, M. (2006). Utilization of sewage sludge as organic fertilizer in sustainable agriculture. *J. Appl. Sci.*, 6: 531–535

Jassir, M.S., Shaker, A., and Khaliq, M.A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh city, Saudi Arabia. Bull. *Environ. Contam. Toxicol*. 75, 1020–1027.

Johnson, J.W. (1995). Nitrogen losses from soil. Tri-state Fertilizer Recommendations for Corn, Soybeans, Wheat and Alfalfa, *Extension Bulletin E*-2567 (New).

Jönsson, H., and Vinnerås, B. (2004). Adapting the nutrient content of urine and faeces in different countries using FAO and Swedish Data. Peer reviewed paper in the proceedings of the 2nd International Symposium on ecological sanitation, incorporating the 1st IWA specialist group conference on sustainable sanitation, Division 44, Environment and Infrastructure sector project ecosan; 7th–11th April, 2003, Lübeck, Germany. Published by GTZ, Postfach 5180, 65726 Eschborn, Germany. http://www.gtz.de.

Jung, M.C. (2008). Heavy metal concentrations in soil and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W mine. *Sensors*, 8: 2413-2423

Kanugo, S.B. (2000). *J. Environ. Qual.* 29, 188,

Kazi, T.G., Jamali, M.K., Kazi, G.H., Arain, M.B., Afridi, H.I., and Siddiqui, A. (2005). Evaluating the mobility of toxic metals in untreated industrial wastewater sludge using a. BCR sequential extraction procedure and a leaching test. *Anal. Bioanal. Chem.* 383: 297-304.

Kidd P.S., Domínguez-Rodríguez M.J., Díez, J., and Monterroso, C. (2007). Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere*. 66: 1458-1467.

Kim, N., and Fergusson, J. (1993). Concentrations and sources of Cadmium, Copper, Lead and Zinc in Dust in Christchurch, New Zealand. *Science of the Total Environment* 138, 1-21.

Kim, J. G., Lee, S. S., Moon, H. S., and Kang, I. M. (2002). Land application of alum sludge from water purification plant to acid mineral soil treated with acidic water. *Japanese Society of Soil Sci. and Plant Nutri*., 48(1): 15–22

Kinney, C. A. *et al.*,(2006). Survey of Organic Wastewater Contaminants in Biosolids Destined for Land Application. *Environmental Science and Technology* 40 7207-7215.

Kirleis, A.W., Sommers, L.E., and Nelson, D.W. (1984). Yield, heavy metal content, and milling and baking properties of soft red winter wheat grown on soils amended with sewage sludge. *Cereal Chem.* 61(6)518-522.

Knud-Hansen, C (1994). Historical Perspective of the Phosphate Detergent Conflict. Conflict Research Consortium. *Working Paper* 94-54.

Knuteson, J., Clapp, C.E., Dowdy, R.H., and Larson, W.E. (1988). Sewage sludge management: Land application of municipal sewage sludge to a terraced watershed in Minnesota. Minn. *Agric. Exp. Stn. Misc. Publ*. 56-1988*, St. Paul, MN.*

Krebs, R., Gupta, S. K., Furrer, G., and Schulin, R., (1998). Solubility and Plant Uptake of Metals With and Without Liming of Sludge-amended Soils*. J. Environ. Qual*. 27: 18-23.

Lenntech. (2009). Water treatment and purification Holding B.V

Levinen, R. (1991). Puhdistamoliete ja sen hyödyntäminen. Kemi-Kemi 18. 2: 116–119.

Logan, T. J. and Harrison, B. J. (1995). Physical characteristics of alkaline stabilized sewage sludge (N–Viro soil) and their effects on soil physical properties. *J. Environ. Qual*. 24: 153–164.

Logan, T. J., and Chaney. R.L., (1983). Utilization of municipal wastewater and sludge on land — metals. p. 235-295.

Lombi, E., Wenzel, W.W., Gobran, G.R., and Adriano, D.C. (2001). Dependency of phytoavailability of metals on indigenous and induced rhizosphere processes: *CRC Press, LLC:* 3–24.

Lübben, S., Rietz, E., and Sauerbeck, D. (1991). Metal uptake and crop growth on sewage sludge field trials with heavy metal contents near the recommended limit values. Pages 535–543. Elsevier, Amsterdam, The Netherlands

Ma, Q.Y., Traina, S.J., and Logan, T.J. (1994). Effect of aqueous Al, Cd, Fe(II), Ni and Zn on Pb immobilization by hydroxyapatite. *Environ Sci Technol.*, 28(7):1219-228

MacLean, K.S., Robinson, A.R., and MacConnell, H.M. (1987). The effect of sewage sludge on the heavy metal content of soils and plant tissue. *Commun. Soil Sci. Plant Anal.* 18:1303-1316.

MacFarlane, G. R., Pulkownik, A., and Burchett, M. D. (2003). Accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina(Forsk.*) Vierh: Biological indication potential. *Environ. Pollut.*, 123, (1), 139-151.

Magoarou, P., (2000). Proceedings of Workshop on Problems around Sludge; *EUR* 19657 *EN*, p.8

Makovníková, J. (2000). Relations between some soil parameters and available contents of cadmium, lead, copper and zinc. *Rostl. Výr.*, 46: 289–296.

Marmo, L., (2000). Sewage Sludge and the Community Waste Strategy. www.europa.eu.int/com/environment/sludge. Accessed on 20th November, 2010.

Martinez, C.E., Sauve, S., Jacobson, A., and McBride. M.B. (1999). Thermally induced release of adsorbed Pb upon aging ferrihydrite soil oxides. *Environ. Sci. Technol.* 33:2016–2020.

Marschner, B., Henke, U., and Wessolek, G. (1995). Effects of meliorative additive on the adsorption and binding forms of heavy metals in contaminated topsoil from a former sewage farm. Z. *Pflanzenernahr. Boden.* 158: 9.

McBride Murray, B. (1998). Growing Food Crops on Sludge-Amended Soils: Problems with the U.S. Environmental Protection Agency Method of Estimating Toxic Metal Transfer. *Environmental Toxicology and Chemistry* 17-2274-2281.

McCaslin, B. D., Davis, J.G., Cihacek, L., and Schluter, L.A. (1987). Sorghum yield and soil analysis from sludge-amended calcareous iron-deficient soil. *Agron. J. 79:204-209.*

McLean, J. E., and Bledsoe, B. E. (1992). Behavior of metals in soils. *EPA/540/S-9*2/018.

Merrington, G., Oliver, I., Smernik, R. J., and McLaughlin, M. J. (2003). The Influence of Sewage Sludge Properties on Sludge-Borne Metal Availability. Adv. *Environ. Res*. 8(1): 2136.

Mohammad, M. J., and Athamneh, B. M. (2004). Changes in soil fertility and plant uptake of nutrients and heavy metals in response to sewage sludge application to calcareous soils. *J. Agron.* 3: 229–236

Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., and Lester, J.N. (2006). Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe. Implications for human health. *Agric. Ecosys. Environ.*, 112: 41-48.

Muhammad Din, Muhammad Qasim, And Maraj Alam *(2007).* Effect of Different Levels of N, P and K on the Growth and Yield of Cabbage. *J. Agric. Res.*, 2007, 45(2).

Nair, M., Balachandran, K. K., Sankarnarayan, V.N., and Joseph, T. (1997). Heavy metals in fishes from coastal waters of Cochin, South West Coast of India. *Indian Journal of Marine Science.* 26: 98-100.

Ngole, V. M. (2007). Response of Copper, Lead and Zinc Mobility and Bioavailability to Sludge Application on Different Soils. *Pol. J. Soil. Sci.* 40(2): 125-138.

Nouri, J., Mahvi, A.H., Babaei, A. A., and Ahmadpour, E. (2006). Regional pattern distribution of groundwater fluoride in the Shush aquifer of Khuzestan County. *Fluoride* 39(4):321-325.

O'Connor, G.A. (1993). Sewage sludge: Land utilization and the environment. Toxic organic considerations; August 11-13, 1993, Bloomington, MN.

Ojeda, G., Alcaniz, J. M., and Ortiz, O. (2003). Runoff and losses by erosion in soils amended with sewage sludge. *Land Degrad. Dev.*, 14 (6), 563–573.

Ortiz, O., and Alcañiz, J. M. (2006). Bioaccumulation of heavy metals in *Dactylis glomerata* L. growing in a calcareous soil amended with sewage sludge; *Technol.* 97: 545–552.

Othman, O.C. (2001). Heavy metals in green vegetables and soils from vegetable gardens in Dar Es Salaam, Tanzania. *Tanzania Journal of Science*. 27: 37-48.

Otte, M. L., Kearns C. C., and Doyle, O.M. (1995). Accumulation of arsenic and zinc in the rhizosphere of wetland plants. *Bull. Environ. Contam. Toxicol.* 55: 154-161.

Pacyna, J.M. (1994). Global Perspectives on Lead, Mercury and Cadmium Cycling in the Environment. Edited by T.C. Hutchingson Wiley Eastern Ltd. pp 315-328.

Panel on Micronutrients (2001). Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Zinc. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of medicine, Food and Nutrition Board, Institute of Medicine. Washington DC Report, pp. 442-501.

Paré, T., Dinel, H., and Schnitzer, M. (1999). Extractibility of trace metals during co-composting of biosolids and municipal solid wastes; *Biol Fertil Soils*, 29, 31-37.

Pinamonti, F., and Zorzi, G. (1996). Experiences of compost use in agriculture and in land reclamation projects. The Science of Composting. *Blackie Academic and Professional, London, pp.* 515–527.

Planquart, P., Bonin, G., Prone, A., and Massiani, C. (1999). Distribution, movement and plant availability of trace metals in soils amended with sewage sludge composts: Application to low metal loadings. *Sci. Total Environ. 241: 161–179.*

Podlešáková, E., Nìmeèek, J., and Vácha, R. (1998): Remediation needs of agriculturally used soils. *Rostl. Výr.*, 5: 209–215.

Polprasert, C. 1995. Organic waste recycling. John Wiley & Sons Ltd. London.

Primefacts, (2006). Profitable and Sustainable Primary Industries. Cabbage and Lettuce growing. Primefact 90 (Replaces Agfact H8. 1. 27).

Przewrocki, P., Kulczycka, J., Wzorek, Z., Kowalski, Z., Gorazda, K., and Jodko, M. (2003). Risk Analysis of Sewage Sludge - Poland and EU; Comparative Approach; *Polish Journal of Environmental Studies Vol.* 13*, No.* 2 (2004), 237-244

Ramulu, U., S., Sree.,(2002). Reuse of municipal sewage and sludge in agriculture. *Scientific Publishers, Jodhpur, India.*

Rappaport, B. D., Martens, D. C., Reneau, R. B. and Simpson, T.W. (1988). Metal availability in sludge-amended soils with elevated metal levels. *J. Environ. Qual. 17: 42–47.*

Reddy, M.R., and Perkins, H. (1996). Effect of biosolids applications on trace elements in wheat grain. *Soil Sci. Sos. Am. Proc*., 38: 229.

Reinthaler, F. F. et al., (2003). Antibiotic resistance of E. coli in sewage and sludge. *Water Research* 37 1685-90.

Rhind, S. M. (2002). Endocrine disrupting compounds and farm animals: their properties, actions and routes of exposure (Abstract).” *Domestic Animal Endocrinology* 23 179-187.

Rockefeller, A. (2002). Sewers, sewage treatment, sludge: Damage without end. *New Solutions*, Vol. 12(4) 341-346

Rout, G.R., and Das, P. (2003). Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc. *Agronomie*, 23: 3-11

Rubin, A. (1998). Presentation on sludge and [the] 503 rule. Presentation before the NH Environment and Agriculture Committee, Nov 19

Ruby, M. V., Davis, A., and Nicholson, A. (1994). *In situ* formation of lead phosphates in soils as a method to immobilize lead. *Environ. Sci. Technol.* 28: 646-654.

Rudd T, Lake D, Mehrotra I, Sterritt R, Kirk P, Campbell J A, Lester J. (1988).Charakterisation of metal forms in sewage sludge by chemical extracrion and progressive acidification. T*he Science of the total Environment.* 74: 149.

Samara, C., Misaelides, P., Tsalev, D., Anousis I., and Kouimtzis (1992). Trace elements distribution in vegetables grown in the industrial area of Thessaloniki. Greece. *Fresenius Environ. Bull.* 1: 577-582.

Sánchez-Monedero M. A., Mondini, C., de Nobili, M., Leita, L., and Roig, A. (2004). Land application of biosolids; Soil response to different stabilization degree or treated organic matter. *Waste Management*, 24(4): 325–332

Sanchez, P.A., (2002). Soil fertility and hunger in Africa; *Science*, 295, pp. 2019–20.

Sawyer, J.E. (2001). Natural gas prices affect nitrogen fertilizer costs. *IC*-486 1: 8.

Schouw, N.L., Danteravanich, S., Mosbaek, H., and Tjell, J.C. (2002). Composition of human excreta – a case study from Southern Thailand. *Science of the Total Environment Journal 286* (1-3), 155-166.

Sesay, B. (2007). Agriculture Boosts Economy. AfricaNews (www.react.nl) Accessed on 10th October, 2010.

Sharma, O.P., Bangar., Rajesh, Jain, K.S., and Sharma, P.K. (2004). Heavy metals accumulation in soils irrigated by municipal and industrial effluent. *Journal of Environmental Science and Engineering.* 46(1): 65-73.

Sharma, R. K., Agrawal, M., and Marshall, F. (2006). Heavy metals contamination in vegetable grown in wastewater irrigated areas of Varanasi, India. *B. Environ. Contam. Tox.,* 77: 312-318.

Shiming, L., (2002). The Utilization Of Human Excreta In Chinese Agriculture And The Challenge Faced. South China Agricultural University. *ECOSANRES*

Sloan, J. J., Dowdy, R. H., Dolan, M. S., and Linden, D.R. (1997). Long-term Effects of Biosolid Application on Heavy Metal Bioavailability in Agricultural Soils. *J. Environ. Qual.*, 26: 966-974.

Smaling, E., and Stoorvogel, J. (1990). Assessment of Soil Nutrient Depletion in Sub-Saharan Africa. 1983–2000, *Vol. I, Main report (2nd ed.), Report 28,* The Winand Staring Centre for Integrated Land, Soil, and Water Research, Wageningen, The Netherlands.

Smith, S.R. (1995). The fate and environmental activity of heavy metals in sewage sludge applied to soil; *International Conference "Heavy Metals in the Environment", September - Hamburg,* 2: 459.

Smith, S. R. (1994). Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. I. Nickel, copper and zinc uptake and toxicity to ryegrass. *Environ. Pollut.*, 85: 321–327.

Soon, K. (1981). Solubility and sorption of cadmium in soils amended with sewage sludge. *J. Soil Sci.* 31, 85-95.

Sommers, L. E. (1987). Chemical composition of sewage sludges and analysis of their potential as fertilizers. *J. Environ. Qual.* 6:225-229.

Stamatoados, S., Werner, M., and Buchanam, M. (1999). Field assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California). *Appl. Soil Ecol.,* 12: 217-225.

Steiner, A. (2006). United Nations Under-Secretary-General and Executive Director, UNEP, Editorial Report. *The magazine of the United Nations Environment Programme, Special Edition (Our Planet).*

Stevenson, F. J. (1992). Humus Chemistry. Genesis, Composition and Reactions. Wiley-Intersc. Publ. New York.

Su, D.C., Wong, J. W. C., and Jagadeesan, H. (2004). Implications of rhizospheric heavy metals and nutrients for the growth of alfalfa in sludge amended soil. *Chemosphere.* 56: 957-965.

Summers, A. O. (2006). Genetic linkage and horizontal gene transfer, the roots of the antibiotic multi-resistance problem (Abstract). *Animal Biotechnology* 17 125-35.

Szefer, P. (1998). Applied Geochemistry. 13, 305.

Taek–Keun O. H., Nakaji, K., Chikushi, J., and Seok–Gon P. (2009). Effects of the Application of Water Treatment Sludge on Growth of Lettuce (*Lactuca sativa* L.) and Changes in Soil Properties. *J. Fac. Agr., Kyushu Univ.*, 55 (1), 15–20

Tandi, N.K., Nyamangara, J., and Bangira, C. (2005). Environmental and potential health effects of growing leafy vegetables on soil irrigated using sewage sludge and effluent: A case of Zn and Cu. *J. Environ. Sci. Health*, 39: 461-471.

Tasdilas, C.D., (1997). Impact of waste water reuse on some soil properties. Salinity and Pollution Control Towards Sustainable Irrigation in the Mediterranean Region, pp: 213–226.

Teresa, H., Juan, M., and Fran, A. (1991). Influence of sewage sludge application on crop yields and heavy metal availability. *Soil Sci. Plant Nurition*., 37(2): 201-810.

Tiller, K.G. (1986). Essential and toxic heavy metals in soils and their ecological relevance, Trance. XIII Congr. *Inter.Soc.Soil Sci.Vol .1*, pp 29-44.

Tlustoš, P., Balik, J., Dvoák, P., Száková, J., and Pavlíková, D. (2001). Zinc and Lead Uptake by Three Crops Planted on Different Soils Treated by Sewage Sludge. *Rostlinná Výroba,* 47(3): 129-134.

Todd, G. C. (1996). Vegetables Grown in Mine Wastes. *Environmental Toxicology and Chemistry*. 19 (3): 600-607.

US EPA (2006).Human Health Issues; Pesticides. *(www.epa.gov/opp0001/health/human.htm.*) Accessed on 20th November, 2010.

U.S. Environmental Protection Agency. (1993). 40 CFR Parts 257, 403, and 503. Standards for the use or disposal of sewage sludge. *Fed. Reg.* 58:9248-9415.

Vahter, M., Berglund, M., Lind, B., Jorhem, L., Slorach, S., and Friberg, L. (1991). Personal monitoring of lead and cadmium exposure – a Swedish study with special reference to methodological aspects. *Scandinavian Journal of Work, Environment and Health 17*, 65-74.

Varalakshmi, A. L. R., and Ganeshamurthy, A. N. (2010). Heavy metal contamination of water bodies, soils and vegetables in peri urban areas of Bangalore city of India. 19th World Congress of Soil Science, Soil Solutions for a Changing World.

Vinnerås, B., Palmquist, H., Balmér, P., Weglin, J., Jensen, A., Andersson, Å., and Jönsson, H. (2006). The characteristics of household wastewater and biodegradable waste – a proposal for new Swedish norms. *Urban Water 3*, 3-11.

Wang, C., Zhang, S. H., Wang, P. F., Hou, J., Li, W., and Zhang, W. J. (2008). Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte Vallisneria natans (Lour.) Hara, Aquat. *Toxicol doi:*10.1016/*j.aquatox*.2008.01.009.

Wang, M. J. (1997). Land application of sewage sludge in China. *The science of the total environ*., 197: 149–160

Weber, J.B., (1991). Applied Plant Science 5, 1.

Wei, Y., and Liu, Y. (2005). Effects of Sewage Sludge Compost Application on Crops and Cropland in a 3-Year Field Study. *Chemosphere*. 59: 1257-1265.

Wenzel, W.W., Lombi, E., and Adriano, D. C. (1999). Biogeochemical processes in the rhizosphere: Role in phytoremediation of metal-polluted soils. *Springer- Verlag, Berlin,* 273–303.

WHO, (2006). Guidelines for the safe use of wastewater, excreta and greywater. Volume 4. Excreta and greywater use in agriculture. ISBN 92 4 154685 9.

Willis, M. S., Monaghan, S. A., Miller, M. L., McKenna, R. W., Perkins, W. D., Levinson, B. S., Bhushan, V., and Kroft, S. H. (2005). Zinc-induced copper deficiency: a report of three cases initially recognized on bone marrow examination. *Am. J. Clin. Pathol.* 123: 125-131.

Wolgast, M. (1993). Rena vatten. Om tankar i kretslopp. Uppsala. 186 p.

Wong, J. W. C., Lai, K. M., Su, D. S., and Fang, M. (2001). Availability of Heavy Metals for *Brassica chinensis* Grown in an Acidic Loamy Soil Amended with Domestic and Industrial Sewage Sludge; *Water Air Soil Pollut*. 128: 339-353.

Xiong, Z.T. (1998). Lead Uptake and Effects on Seed Germination and Plant Growth in a Pb Hyperaccumulator *Brassica pekinensis* Rupr. Bulletin of Environmental Contamination and Toxicology. 60: 285-291.

Zhang, M., Heaney, D., Solberg, E., and Heriquez, B. (2000). The effect of MSW compost on metal uptake and yield of wheat, barley and conola in less productive farming soils of Alberta. *Compost Sci. Util.* 8 (3), 224–235.

Zhang, J. L., Liu, J. G., Li, C., Nie, Y. F., and Jin, Y. Y. (2007). Comparison of the fixation of heavy metals in raw material, clinker and mortar using a BCR sequential extraction procedure and NEN7341 test, Cement and Concrete Research. doi:10.1016/j.cemconres.2007.09.004.

Zhao, R., Zhao, M., Wang, H., Taneike, Y., and Zhang, X. (2006). Arsenic speciation in moso bamboo shoot – a terrestrial plant that contains organoarsenic species. *Sci. Total Environ.* 371: 293-303.

Zheng, N., Wang, Q. C., and Zheng, D.M. (2007). Health risk of Hg, Pb, Cd, Zn, and Cu to the inhabitants around Huludao Zinc Plant in China via consumption of vegetables. *Sci. Total Environ.* 383: 81-89.

Zucconi, F., and De Bertoldi, M. (1987). Compost specifications for the production and characterization of compost from municipal solid waste. *Elsveir Applied science*, New York. p.30-51,

Zwarich, M.A., and Mills, J.G. (1979). Effects of sewage sludge application on the heavy metal content of wheat and forage crops. *Can. J. Soil Sci. 59:231-239.*

APPENDICES

APPENDIX 1: ANOVA FOR SOIL PH AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.133369	3	0.044456	3.629699	0.045112	5.952545
Within Groups	0.146975	12	0.012248			
Total	0.280344	15				

APPENDIX 2: ANOVA FOR SOIL PH AFTER APPLYING TREATMENTS ON LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.126475	3	0.042158	2.91585	0.077775	5.952545
Within Groups	0.1735	12	0.014458			
Total	0.299975	15				

APPENDIX 3: ANOVA FOR ORGANIC MATTER CONTENT AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	671.9407	3	223.9802	568.9952	3.38E-13	5.952545
Within Groups	4.7237	12	0.393642			
Total	676.6644	15				

APPENDIX 4: ORGANIC MATTER CONTENT AFTER APPLYING TREATMENTS ON LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	319.510075	3	106.5034	218.3826	9.85E-11	5.952545
Within Groups	5.8523	12	0.487692			
Total	325.362375	15				

APPENDIX 5: ANOVA FOR CONDUCTIVITY AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	39318.69	3	13106.23	84.0929	2.52E-08	5.952545
Within Groups	1870.25	12	155.8542			
Total	41188.94	15				

APPENDIX 6: ANOVA FOR CONDUCTIVITY AFTER APPLYING TREATMENTS ON LETTUCE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27992.25	3	9330.75	38.77714	1.88E-06	5.952545
Within Groups	2887.5	12	240.625			
Total	30879.75	15				

APPENDIX 7: ANOVA FOR LEAD AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	34.80103	3	11.60034	312.7128	1.18E-11	5.952545
Within Groups	0.44515	12	0.037096			
Total	35.24618	15				

APPENDIX 8: ANOVA FOR LEAD AFTER APPLYING TREATMENTS ON LETTUCE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.516519	3	3.172173	78.05624	3.85E-08	5.952545
Within Groups	0.487675	12	0.04064			
Total	10.00419	15				

APPENDIX 9: ANOVA FOR ZINC AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.380369	3	3.12679	51.8521	3.83E-07	5.952545
Within Groups	0.723625	12	0.060302			
Total	10.10399	15				

APPENDIX 10: ANOVA FOR ZINC AFTER APPLYING TREATMENTS ON LETTUCE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.401169	3	3.133723	51.58215	3.95E-07	5.952545
Within Groups	0.729025	12	0.060752			
Total	10.13019	15				

APPENDIX 11: ANOVA FOR COPPER AFTER APPLYING TREATMENTS ON CABBAGE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	152.9662	3	50.98872	46.13503	7.3E-07	5.952545
Within Groups	13.26248	12	1.105206			
Total	166.2286	15				

APPENDIX 12: ANOVA FOR COPPER AFTER APPLYING TREATMENTS ON LETTUCE SET-UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	70.19352	3	23.39784	20.69098	4.92E-05	5.952545
Within Groups	13.56988	12	1.130823			
Total	83.76339	15				

APPENDIX 13: ANOVA FOR LEAD UPTAKE IN CABBAGE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.08695	3	0.028983	1.181988	0.357635	5.952545
Within Groups	0.29425	12	0.024521			

Total	0.3812	15

APPENDIX 14: ANOVA FOR LEAD UPTAKE IN LETTUCE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.109025	3	0.036342	1.20022	0.351439	5.952545
Within Groups	0.36335	12	0.030279			
Total	0.472375	15				

APPENDIX 15: ANOVA FOR COPPER UPTAKE IN CABBAGE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.015169	3	2.005056	43.28822	1.03E-06	5.952545
Within Groups	0.555825	12	0.046319			
Total	6.570994	15				

APPENDIX 16: ANOVA FOR COPPER UPTAKE IN LETTUCE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.642269	3	1.880756	62.53987	1.35E-07	5.952545
Within Groups	0.360875	12	0.030073			
Total	6.003144	15				

APPENDIX 17: ANOVA FOR ZINC UPTAKE IN CABBAGE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.788219	3	1.596073	16.53426	0.000146	5.952545
Within Groups	1.158375	12	0.096531			
Total	5.946594	15				

APPENDIX 18: ANOVA FOR ZINC UPTAKE IN LETTUCE PLANT

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.834169	3	0.61139	14.36662	0.000282	5.952545
Within Groups	0.510675	12	0.042556			
Total	2.344844	15				

APPENDIX 19: ANOVA FOR RESIDUE LEAD IN CABBAGE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.514969	3	0.838323	10.89291	0.000966	3.490295
Within Groups	0.923525	12	0.07696			
Total	3.438494	15				

APPENDIX 20: ANOVA FOR RESIDUE LEAD IN LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.380825	3	0.460275	3.94874	0.035862	3.490295
Within Groups	1.39875	12	0.116563			
Total	2.779575	15				

APPENDIX 21: ANOVA FOR RESIDUE COPPER IN CABBAGE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10.89253	3	3.630842	16.87553	0.000133	3.490295
Within Groups	2.58185	12	0.215154			
Total	13.47438	15				

APPENDIX 22: ANOVA FOR RESIDUE COPPER IN LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.859269	3	0.619756	13.0802	0.000432	3.490295
Within Groups	0.568575	12	0.047381			

Total	2.427844	15

APPENDIX 23: ANOVA FOR RESIDUE ZINC IN CABBAGE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.30515	3	1.768383	17.35907	0.000116	3.490295
Within Groups	1.22245	12	0.101871			
Total	6.5276	15				

APPENDIX 24: ANOVA FOR RESIDUE ZINC IN LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.554875	3	1.851625	9.800847	0.001507	3.490295
Within Groups	2.2671	12	0.188925			
Total	7.821975	15				

APPENDIX 25: ANOVA FOR YIELD IN CABBAGE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	203550.3	3	67850.08	202.7645	1.52E-10	3.490295
Within Groups	4015.5	12	334.625			
Total	207565.8	15				

APPENDIX 26: ANOVA FOR YIELD IN LETTUCE SET UP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14877.69	3	4959.229	234.9882	6.4E-11	3.490295
Within Groups	253.25	12	21.10417			
Total	15130.94	15				