ASSESSMENT OF SELECTED ANTIBIOTICS AND HEAVY METALS IN UNTREATED WASTEWATER, VEGETABLES AND SOILS IN EASTERN NAIROBI, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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We confirm that the work reported in this thesis was carried out by the student under our supervision.

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DEDICATION

I dedicate this work to my family and to my dad and mum.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AIDS	Acquired Immune Defficiency Syndrome
ATSDR	Agency for Toxic Substances and Disease Registry
CCREM	Canadian Council of Resource and Environmental Ministers
CD4	Cluster of Differentiation 4
CLEA	Contaminated Land Exposure Assessment
DEFRA	Department of Environment, Food and Rural Affairs
DETR	Department of Environment, Transport and the Regions
DM	Dry Mass
DNA	Deoxy-ribonucleic Acid
DTPA	Diethylene Triamine Penta Acetate
DWB	Dry Weight Basis
EDCs	Endocrine Disrupting Compounds
EDTA	Ethylene Diamine Tetracetic Acid
EPA	Environmental Protection Act
FAO	Food and Agriculture Organization
FDA	Food and Drug Agency
HIV	Human Immunodefficiency Virus
HPLC	High Performance Liquid Chromatography
ICRCL	Inter-departmental Committee on Redevelopment of Contaminated Land

INQWS	Interim Water Quality Standard
MAL	Maximum Allowable Limits
NAS	National Academy of Sciences
NH&MRC	National Health and Medical Research Council
NMED	New Mexico Environmental Department
NRCC	National Research Council of Canada
PAC	Polyaluminum-Chloride
PFA	Prevention of Food Adulteration
PhACs	Pharmaceutically Active Compounds
RNA	Ribonucleic Acid
SGV	Soil Guidance Values
SLD	Scientific Laboratory Division
SMX	Sulfamethoxazole
SPE	Solid Phase Extraction
TMP	Trimethoprim
UK	United Kingdom
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

Untreated wastewater contains pharmaceutical compounds and heavy metals harmful to human health and the environment. People get access to untreated water through broken sewer pipes, blocked manholes and direct disposal into rivers. One such river is the Ngong River which passes through Nairobi County including Njiru and Ruai. People use untreated wastewater for growing of vegetables and washing cars. Soils and vegetables grown or irrigated with untreated wastewater may contain high levels of antibiotics and heavy metals that are detrimental to health. Sulfamethoxazole (SMX) and trimethoprim (TMP) antibiotics, administered in synergy for the management of *pneumocystis carinii* pneumonia, *pneumocystis jiroveci* pneumonia, toxaplasmosis and genitourinary tract infections in HIV-AIDS patients or in cases of oral thrush infections. The two drugs are also cheap and readily available in synergy over the counter even through self-prescription for management of upper respiratory tract and genitourinary tract infections under the brand name septrin or co-trimoxazole. In synergy, the two drugs are also administered to poultry and livestock as growth promoters, prophylaxis and to control microbial infections. Their presence in vegetables could induce microbial resistance and minimize drug sensitivity. The heavy metals may emanate from the waste water discharged by the small scale industries that are based in the study site. However, reports on levels of antibiotic residues and heavy metals in untreated waste water, vegetables and soils in Kenya are very scarce. Therefore this study aimed to determine the concentration of sulfamethoxazole (SMX) and trimethoprim (TMP), Cd, Pb, Zn, Mn, Fe and Cu in the soil, untreated waste water and vegetables irrigated with the untreated waste water in Ruai and Njiru. Untreated wastewater, soil and vegetables were collected during the dry season from various sites in Ruai and Njiru from small scale farms along Ngong River. The samples were also obtained from a control plot at the Kenyatta University. The samples for heavy metal analysis underwent wet digestion pre-analysis procedures while for antibiotics underwent solvent extraction pre-analysis. The heavy metals were determined by atomic absorption spectroscopy while antibiotic residues were determined by high performance liquid chromatography. The heavy metal concentrations in the vegetables ranged from 81.88 to 633.05 mg/kg. The mean concentration for Fe in the vegetables ranged between 481.24 to 1215.49 mg/kg for Mn, 1.94 to 4.58 mg/kg for Cd, 2.19 to 4.46 mg/kg for Pb, 3.19 to 8.70 mg/kg for Zn, 21.17 to 29.70 mg/kg for Cu, 24.71 to 27.52 ng/L for TMP, and 4.93 to 22.64 ng/L SMX. Some of these values were above the local and international safety limits. The concentrations (mg/L) of the heavy metals in untreated wastewater ranged from 3.09 to 3.54 for Mn, 0.01 to 0.03 for Zn, 0.21 to 0.28 for Pb, 4.79 to 8.07 for Fe, 0.17 to 0.22 for Cu and 0.42 to 0.47 for Cd. In the untreated wastewater SMX ranged from 62.09 to 88.66 ng/L. The vegetables TMP concentration ranged from 2.16 to 15.45 ng/L. The levels of some heavy metals and antibiotics were significantly higher than those from control samples. In all cases the results in the farms upstream were higher than in those downstream. The results from this work will be availed to relevant authorities for policy formulation and they can be used to sensitize the public, especially on the antibiotic residues.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Generally, of the sewage generated globally, more than 80% is discharged untreated into the environment (Dreschel *et al.,* 2010). Despite farmers' good reasoning and the advantages accruing from wastewater use, this practice can severely harm human health and the environment (Qadir *et al.,* 2007). This is mainly attributed to the associated pathogens, but also heavy metals, pharmaceutically active compounds (PhACs) and other undesirable constituents depending on the source. Urban 'rivers' and other water bodies are known to be more or less open sewers or cesspits (Cornish and Lawrence, 2001).

There has been an increase of immunosuppressive conditions which are confounded by opportunistic infections managed by a wide array of pharmaceutical compounds that are eventually discharged through the body as metabolites or un- metabolized active forms. Hence there is cause for alarm on just what quantities of pharmaceutically active compounds (PhACs) may be in the wastewater effluent and subsequently transferred into the food consumed. Examples include antibiotics, analgesics and antidepressants (Gulkowska *et al*., 2008). Of concern related to PhACs is the initiation of microbial antibiotic resistance following discharge of antibiotics in the environment (Guardabassi *et al*., 1998).

The majority of antibiotics detected in environmental waters and wastewaters are drugs used for a variety of therapeutic purpose for both humans and animals (Alistair *et al.,* 2011). Wrong disposal of pharmaceutical compounds is an important source of environmental contamination that needs to be noted with concern (Giger *et al.*, 2003). The dominant pathway for antibiotic release in the terrestrial environment is via the application of animal manure and biosolids containing excreted antibiotics to agricultural land as fertilizer (Kemper, 2008). Antibiotics can also be introduced to agricultural land through irrigation with reclaimed wastewater, since they have been frequently detected in the raw and treated sewage wastewaters (Gulkowska *et al.*, 2008).

The soil fertility in such areas is greatly improved because the sewage effluents used for irrigation contains significant proportions of major essential plant nutrients (Anjula and Sangeeta., 2011). The soil ability to function within an ecosystem while promoting both plant health and environmental quality are important markers for the soil health, which is an increasingly popular concept (Langat *et al.,* 2008). Studies have shown that heavy metal concentrations from wastes applied to the farms can accumulate in the soils at toxic levels hence the risk of food crops grown in these areas getting contaminated with heavy metals and consequently endangering human health (Karanja *et al.,* 2010).

One of the major factors of human exposure to metals through the food chain is the soil-to-plant transfer (Lăcătuşu *et al.,* 1996). According to Hough *et al.* (2004) land is contaminated only if the current or intended use of a site has the potential to cause an unacceptable health risk to human occupants or to the environment. It is very difficult to remove heavy metals from the soil once they accumulate and therefore it is not desirable at all to contaminate soils liberally with heavy metals. Even in portions as negligible as parts per billion (ppb), many heavy metals act as biological poisons (Okoronkwo *et al.*, 2005). Indiscriminate release of commercially important but toxic heavy metals into the environment is a hazardous practice (Qadir *et al.*, 2007).

Many poor farmers have been using wastewater sources for years without formal water rights and it has been reported that banning the use of polluted water was estimated to affect about 12,700 households or 90,000 people living around the city of Kumasi by late 90s, depending on dry-season irrigation (Cornish and Lawrence, 2001). Such predicaments faced by farmers in Kumasi and Accra are very similar to those of their counterparts in Nairobi, particularly in Ruai and Maili Saba (Cornish and Kielen, 2004; Karanja *et al.,* 2010).

Njiru District is in the eastern part of Nairobi County (Kenya Gazette Notice, 2005). It is a cosmopolitan environment with both the less privileged communities as well as the well to do co-existing side by side. For most of the vulnerable populations living in the cities, farming is considered an important means of livelihood despite there being no access to agricultural land (Nabulo *et al.,* 2012). The farming carried out in urban and peri-urban sites in Nairobi is irrigated with untreated wastewater (Cornish and Lawrence, 2001). Untreated municipal wastewater comes from residential properties like houses and apartments as well as commercial buildings, hospitals, health centers, industrial and agricultural processes (Gweyi and Osei, 2011). It can contain a wide variety of contaminants and presents a health hazard where humans or animals come into contact with it. It may overflow from a sanitary sewer after a period of very heavy rainfall passing through slums loaded with raw human wastes.

Vegetables are fast growing and even when grown on no-man's land, in this case which happens to be the riparian region along which the City sewer pipes run parallel to Ngong river in Njiru-Ruai Districts the farmers can harvest frequently prior to the rainy season when their farms act as the flood zones and all farming activities stop (Plate 1).

Plate 1.1: Farming activities in Ruai along Ngong River

Although vegetables are a necessary part of the human diet, they have been found to expose man to consumption of heavy metals, nitrates and other anions (Türkdogan *et al.*, 2003; Akan, 2009). In a case-control study on vegetables irrigated with wastewater (Cheraghi *et al.*, 2009), the vegetables irrigated with wastewater had many fold higher concentration of heavy metals in relation to those grown in a controlled area. Even when supported by identical solutions containing minor and major macro elements, each vegetable species has unique nutritive requirements (Anjula, 2011). A report on randomly sampled vegetables from markets in the major cities of Accra and Kumasi in Ghana revealed that the food crops grown on untreated wastewater have excessively high concentration of heavy metals (Chealteau, 2007), with carrots showing as high as 100-fold of the WHO/FAO permissible levels (Chealteau, 2007).

In this study, the antibiotics quantified were sulfamethoxazole (SMX) and trimethoprim (TMP), while the heavy metals determined were cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe).

Sulfamethoxazole falls under the class of sulfonamide antibacterial group (Altenburger, 2004). The medicinal use of sulfamethoxazole is for treatment of tuberculosis, malaria and urinary tract infections (Richards *et al*., 2004). Trimethoprim is a synthetic broad spectrum antibiotic that is used to treat urinary tract infections, respiratory infections and middle ear infections (Lauren *et al.*, 2010).

Heavy metal ions like Fe, Cu, Zn, and Mn at appropriate concentrations are required for structural and catalytic components of proteins and enzymes as cofactors, essential for normal growth and development of human and plants (Gweyi and Osei, 2011). However, supra- optimal concentrations of these micronutrients and other heavy metals in plants operate as stress factors (Singh *et al.*, 2004).

Lead is purely toxic to the human body, and has no positive role even in small proportions in the body (Cherop, 2009). Lead has a cumulative effect and can be stored in the body parts, especially teeth and fatty tissues (Gulson *et al.,* 1997). Toxicity of iron occurs when ingested Fe beyond 3.0 g and symptoms include gastro-intestinal bleeding and restlessness (Williams and Caliendo, 1988). Chronic Fe poisoning leads to siderosis (Krayenbuehl *et al.*, 2005).

Zinc toxicity occurs at levels exceeding 1000 mg and effects include vomiting, nausea and impaired immune function (Mindell and Mundis, 2004). Acute Zn intoxication leads to renal failure characterized by nausea, vomiting, fever and severe anemia. On the other hand, manganese toxicity occurs at levels exceeding 20 mg and symptoms include anorexia, impulsiveness, spastic gait and parkinson's disease (WHO, 1996).

Excess intake of copper can cause liver damage, abdominal pains and postpartum depression (Cherop, 2009). It has been well documented that excessive supply of certain heavy metals such as Cu, Zn or Mn interferes with Fe metabolism and may induce physiological changes that resemble Fe deficiency syndrome (Gweyi and Osei, 2011).

Cadmium is a heavy metal posing severe risks to human health (Nordberg, 2004) and has not been shown to have any physiological function within the human body (Johannes *et al.*, 2006).

1.2 Problem statement

Most urban farmers in Kenya use untreated wastewater to irrigate crops. The vegetables irrigated with this water may accumulate unsafe levels of heavy metals and pharmaceutically active compounds. This poses a health concern to the consumers because of the known health problems that may arise from accumulation of heavy metals and antibiotics in the body. In addition, the presence of antibiotic residues in the vegetables may lead to initiation of bacterial resistance to the drugs. Unlike pesticides used on agricultural land, antibiotics have not aroused attention as potential pollutants until fairly recently (Feng *et al.*, 2009). Bacterial resistance has been a big issue in terms of human and animal health; however, antibiotic ecotoxicological relevance is scarcely known because the potential effects of antibiotics in the environment are very limited (Rooklidge, 2004). In the Kenyan sewerage system, hospital, industrial and domestic wastes are discharged together. No report in Kenya is available about the presence of antibiotic residues in the sewage and the vegetables. Therefore there is need to carry out assessment of the heavy metal and antibiotic residues in different vegetables grown in the same locality and irrigated with the untreated waste water in order to determine whether there is variation.

1.3 Justification of the study

There are many urban farms along the sewer lines in Kenya and studies have shown that food crops grown on contaminated urban soils contain higher concentration of trace metals than those grown in uncontaminated soils. Dietary intake of certain contaminants in foods consumed by urban communities may exceed limits. There is need therefore to determine the levels of heavy metals and pharmaceutical compounds in wastewater and vegetables irrigated with wastewater. Therefore the study will provide a report that will enable the health care policy makers to draw a baseline on the safety of consuming such vegetables; therefore becoming eye opener on the concerns that could arise following the application of untreated wastewater to the vegetable farms.

1.4 Research questions

i) What quantities of hazardous heavy metals and antibiotics are in the untreated wastewater used for irrigation?

ii) What quantities of hazardous heavy metals are in the soil irrigated with the untreated wastewater?

iii) What quantities of hazardous heavy metals and antibiotics do the kales, spinach, cow peas and pumpkin leaves irrigated with the untreated wastewater contain?

1.5 Null hypothesis

There is no significant difference in the levels of heavy metals and antibiotics in soils, untreated wastewater and vegetables irrigated with the wastewater in Eastern Nairobi in those irrigated with tap water.

1.6 Objectives

1.6.1 General objective

To determine the levels of selected heavy metals and antibiotics in some vegetables, soil on which the vegetables are grown and wastewater used to irrigate the farms in Eastern Nairobi Region.

1.6.2 Specific objectives

i) To determine the levels Pb, Cd, Mn, Fe, Zn and Cu in untreated wastewater from Ngong River used to irrigate the farms in Njiru and Ruai.

ii) To determine the concentration of Pb, Cd, Mn, Fe, Zn and Cu in the soil irrigated with the untreated wastewater.

iii) To determine the levels of Pb, Cd, Mn, Fe, Zn and Cu in kales, spinach, cow peas and pumpkin leaves irrigated with untreated wastewater in Njiru and Ruai.

iv) To determine the levels of sulfamethoxazole (SMX) and trimethoprim (TMP) in untreated waste water and vegetables irrigated with the untreated wastewater.

1.7 Significance of the study

The results of this study have generated baseline information about the presence of antibiotics which are among the pharmaceutically active compounds and heavy metals in untreated wastewater and the consequent health risks from consumption of vegetables irrigated with untreated wastewater. This information will be communicated to the policy makers in order to sensitize the public on the accrued health risks of consumption of vegetables irrigated with untreated wastewater.

1.8 Scope and limitations

The study was conducted in the farms where farmers co-operated and availed their farm produce for sampling and analysis. It was purposive to areas where there were irrigation practices undertaken with untreated wastewater. Most of these farms were not readily accessible as there were no clearly marked routes through the slum to the river banks along where the farming takes place. There was also high insecurity in the study area as it is located in the slums, with many un-employed youth or where the farming takes place in disserted areas. Accessibility required accompaniment by a security officer.

Only the parent compounds of SMX and TMP were investigated, and this was only done in wastewater and the common vegetables grown in the region. The study determined the levels of only cadmium lead, manganese, zinc, iron and copper in soils, wastewater and in pumpkin leaves, cowpeas, spinach and kales grown on the soils and irrigated with the waste water. A control was set in Kenyatta University.

It was not possible to state clearly the age of the vegetables but the sample taken was from the vegetables ready for market in the harvest season. Seasonal variations were not considered because irrigation is only carried out during the dry season since during the rainy season, the farms become flooded and no farming is carried out till the river levels go down.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global use of untreated wastewater in food crop farming

Globally, irrigation with untreated wastewater and in some cases raw sewage is carried out largely in China, Italy, Australia, Americas (Goldstein, 2011) and in the Africa continent in countries such as Ghana, Kenya (Cornish and Lawrence, 2001), Rwanda, Uganda (Nabulo *et al.,* 2012), Tanzania and Nigeria (Okoronkwo *et al.,* 2005). Most people, however, tend to become less favorable towards reused water as it physically comes closer to them. In other words, most people are very supportive of the irrigation of public open spaces in some ill defined regions, but baulk at the use of reused water in the household or when the chance of personal physical contact increases (Po *et al.,* 2003; Hartley, 2003).

The amount of public unease about water reuse also depends on the type of reused water and treatment levels. For example, people have much less concern about using untreated captured storm water than they have about highly treated sewage effluent (Po *et al.,* 2003). While the actual physical risk from the sewage effluent can be similar or less than that of untreated storm water, the public perception can lead to a belief within the community of a greater risk from the effluent (Marks, 2003; Nancarrow *et al.,* 2002). Socially it could be considered that public opinion for water reuse would be more favourable when it involves distant concepts such as crop irrigation as compared to recycling wastewater for drinking and kitchen use. Consumers and exporters, however, are still concerned about the potential for negative health and environmental impacts with crops and recycled water and biosolids (Crute *et al.*, 2003).

While many countries (for example Australia and the USA) have guidelines for use of reused water (NH&MRC 1999; USEPA, 1992), these guidelines tend to focus on the health or environmental risk from microbial pathogens and nutrients. Very little is mentioned regarding the potential presence of trace contaminants apart from heavy metals and some brief mention on disinfection-byproducts and pharmaceutically-active compounds (USEPA, 1992). There is concern about the potential health and environmental impacts by the compounds if they accumulate in the environment and enter the food chain.

In wastewater irrigated soil of Harare, Zimbabwe the heavy metal concentrations (mg/kg) reported were 3.4 for Cd, 145 for Cu, 228 for Zn, 21 for Ni, 59 for Pb and 225 for Cr (Mapanda *et al*, 2005). Anita *et al.* (2010) observed that continuous application of treated and untreated sewage water to the soil led to higher concentrations of heavy metals in the soil at waste water irrigated sites as compared to clean water irrigated sites. Singh *et al.* (2004) reported that the concentrations of heavy metals were higher by 109 % for Cd, 152 % for Cu, 25 % for Pb, 32 % and for Zn, 161 % in soil of Dinapur site irrigated by treated and untreated wastewater as compared to those in soil at a clean water irrigated site. Excessive accumulation of trace elements in agricultural soils through wastewater irrigation may not only result in soil contamination but also affect food quality and safety (Muchuweti *et al.*, 2006; Sharma *et al.*, 2007). Risk of cadmium and lead ingestion to the population of the Uganda Capital located close to Lake Victoria has been exposed (Jolocam *et al.,* 2010).

Lone *et al*. (2003), found the levels of the heavy metals in untreated wastewater used for irrigation in Pakistan to be Cu, Zn, Fe, Mn, Cd and Pb to be 0.7 mg/l, 0.42 mg/l, 2.67 mg/l, 0.53 mg/l, 0.31 mg/l and 0.42 mg/l respectively wheres the potable water had 0.005 mg/l Cd, 0.16 mg/l Pb, 8.3 mg/l Cu, 7.71 mg/l Zn, 24.57 mg/l Fe and 15.30 mg/l Mn. This shows that the untreated wastewater had a higher concentration of heavy metals as compared to potable water.

2.2 Use of untreated wastewater for irrigation in Kenya

Despite use of wastewater being illegal in Kenya, it is used to irrigate over 720 ha in Nairobi (Kaluli *et al.,* 2011). A study undertaken in 2006 and 2007, for example, revealed that only 50% of the wastewater generated in Nairobi ends up in the treatment facilities while the rest is used for cultivation of over 720 hactares using raw sewage (Githuku, 2009). With decreasing per capita in freshwater availability, there is increasing dominance of wastewater in the water balance and this makes wastewater a very important source of irrigation water for urban agriculture (Githuku, 2009).

Although wastewater has great fertilizing value when used in crop production, it may contain high levels of toxic heavy metals, organic contaminants and soluble salts (Gweyi and Osei, 2011). Among the inorganic contaminants of wastewater, heavy metals are gaining importance due to the tendency of absorption by soil colloids and thereafter get released to soil solutions (Karanja *et al.,* 2010b).

2.3 Economic importance of Ngong River

The main sources of pollution of the Ngong River basin are uncontrolled disposal of human waste, disposal of solid waste, blockages and breakages of sewage line within the industrial area and untreated wastewater discharged from large scale and cottage (Jua Kali) industries (Karanja *et al.,* 2010b). Lack of adequate income is compounded by the situation of urban food markets where families contend with fluctuating prices and inaccessibility of cheaper foods (Karanja *et al.*, 2010).

Confronted with such situations, millions of families in developing world cities and towns improve their access to food and raise income through agricultural activities in urban and peri-urban areas (Kaluli *et al.,* 2011). Poucher *et al.* (2007) noted that although land application of sewage sludge can improve soil physical properties and increase soil organic matter content, there are also disadvantages such as the possible transfer of pathogenic micro-organisms to the soil which may include *Escherichia coli, faecal coliforms and enterococci.*

As many as 800 million people in cities and towns worldwide are already raising livestock and cultivating crops in vacant plots (Hussain and Al-Saati, 1999). This is now widely accepted as an urban livelihood strategy (Dreschel *et al.,* 2010). Urban agriculture provides benefits to the economy in terms of employment for women and other disadvantaged groups (Karanja *et al.,* 2010) (Plate 2.1).

Plate 2.1: Harvesting of vegetables grown with untreated wastewater from Ngong River in Ruai

As a source of food and self employment, many farmers from informal settlements have developed local knowledge on use of waste water for crops and fodder production (Karanja *et al.*, 2010). Within a 20 km radius of Nairobi center, over 3,700 farmers practice irrigation agriculture and 36% of them use raw sewage water (Ministry of agriculture, 2002); Githuku (2009) reported Cd and Pb levels in excess of the EU safe limits, in all food substances irrigated with wastewater in Nairobi and heavy metal concentration have been reported to be lower during the wet season perhaps because of dilution (Kaluli *et al.*, 2011).

Over 100,000 households in Kahawa, Soweto, Kibera, Mailisaba, Maringo and Kariobangi South use raw sewage for cultivation (Kaluli *et al.*, 2011). Though vegetables may be cooked and the microbes destroyed, they may still retain harmful chemicals that may pose serious health hazards to consumers (Van der Hoek, 2004).

Comparative studies between wastewater and non-wastewater farmers have shown that the former make more income not only from savings in fertilizer but additionally the reliable wastewater supply allows them to grow short-cycle cash crops (Van der Hoek, 2004;Karanja *et al.,* 2010). Wastewater may be used directly or after mixing with sewage channeled into natural drainage systems, from where the polluted water is used for farming (Qadir *et al.,* 2007). Since sewage is rich in other organic nutrients the food crops do so well that the harvest is better than what is grown in conventional farms (Cornish and Lawrence, 2001). There is need therefore to analyze wastewater pollutants (WHO, 2011).

Kenya has a mixed sewer system which receives both domestic and industrial effluent (Ministry of Agriculture, 2002). Mixing industrial effluent and domestic sewage in the sewer system in urban areas is responsible for poor performance of pond treatment system for example the Dandora Waste Stabilization ponds due to toxicity of micro-organisms from industrial effluent which is not pre-treated (Ministry of water and irrigation, 2007). In general, there is poor sewerage service delivery in Kenya and where facilities exist, their standards of operation and maintenance have problems resulting in poor service delivery (Cornish and Lawrence, 2001). Investment over the years has tended to favour the water development and operation, leaving little attention to sewerage. This notwithstanding, in all areas where there are slums, and incidentally they all mushroom in the riparian regions of the

Nairobi rivers, the same areas through which the main sewer line runs, urban farming is practiced and irrigation is carried out by utilizing leaking manholes and other wastewater sources during dry weather (Cornish and Lawrence, 2001).

2.4 Heavy metals

The term heavy metal refers to metals with a density greater than 5g/cm^3 (Sebastian *et al.*, 2012). Heavy metals are generally defined as environmentally stable elements of high specific gravity and atomic weight (Adama, 2012). They have such characteristics as luster, ductility, malleability, and high electric and thermal conductivity (Ochieng *et al.*, 2007). Whether based on their physical or chemical properties, the distinction between heavy metals and non-metals is not sharp (Sebastian *et al.*, 2012). Some heavy metals are essential elements for health. Essential elements are chemical nutrients needed by the body in large or tiny amounts and are vital for growth and development, for example Zn, Mn, Cu and Fe (Skudi, 2011). Most of the essential trace elements have unfilled d-orbital and readily form coordination complexes with electron rich elements like nitrogen, oxygen and sulphur thus competing with each other for ligands (O'Dell, 1989).

The uptake of heavy metals from the soil is determined by many factors, including their amounts in the soil, genetic makeup of the plant species, physical conditions of the soil and pH of soil (Cherop, 2009). Certain plant species have the ability to accumulate uniquely high concentrations of a particular essential element. Many interactions between elements in the soil have been shown to be of profound nutritional importance. Interactions can result in competitive inhibition or in stimulation of uptake, for example;

high concentration of Cd and P in the soil can prevent uptake of Zn by plants (Skudi, 2011). Even when trace element intake can be estimated, the amount available to the body may be significantly less because the absorption and metabolism of individual trace element is strongly influenced by mineral interactions (Kiende *et al.*, 2012).

Only a relatively small number of heavy metals such as cadmium , copper , iron, cobalt, zinc, mercury , vanadium, lead , nickel , chromium, manganese, molybdenum, silver, and tin as well as the metalloids arsenic and selenium are associated with environmental, plant, animal, or human health problems (Singh and Angrawal, 2010) and this is common in areas with high anthropogenic pressure (Islam *et al*., 2007). Their presence in the atmosphere, soil and water, even in traces can cause serious problems to all organisms, and heavy metal bioaccumulation in the food chain especially can be highly dangerous to human health (Lone *et al.*, 2008). Heavy metals enter the human body mainly through two routes namely: inhalation and ingestion, ingestion being the main route of exposure to these elements in human population (Singh and Taneja, 2010).

Heavy metals intake by human populations through food chain has been reported in many countries (Sharma *et al.,* 2009). Soil threshold for heavy metal toxicity is an important factor affecting soil environmental capacity of heavy metal and determines heavy metal cumulative loading limits (Singh and Angrawal, 2010). For soil-plant system, heavy metal toxicity threshold is the highest permissible content in the soil that does not pose any phytotoxic effects or heavy metals in the edible parts of the crops (Oleszczuk, 2008).

Factors affecting the thresholds of dietary toxicity of heavy metal in soil-crop system include: soil type which includes soil pH, organic matter content, clay mineral and other soil chemical and biochemical properties; and crop species or cultivars regulated by genetic basis for heavy metal transport and accumulation in plants (Yadav, 2010). In addition, the interactions of soil-plant root-microbes play important roles in regulating heavy metal movement from soil to the edible parts of crops (Tsekova *et al*., 2010).

Agronomic practices such as fertilizer and water managements as well as crop rotation system can affect bioavailability and crop accumulation of heavy metals. This influences the thresholds for assessing dietary toxicity of heavy metals in the food chain (Singh and Taneja, 2010). Heavy metals are not easily biodegradable and it leads to their accumulation in human vital organs causing varying degree of illness on acute and chronic exposure (Ward *et al.,* 1995).

The U.S. Environmental Protection Agency's Maximum Contaminant Level (MCL) of all heavy metals in drinking water is 1.3 milligrams per liter (USEPA, 1992).

2.4.1 Cadmium

Cadmium is a chemical element with the symbol Cd and atomic number 48 (Morrow, 2010). It is soft; bluish-white metal is chemically similar to the two other stable metals in group 12, zinc and mercury (Michael, 2010). Like zinc, it prefers oxidation state +2 in most of its compounds and like mercury it shows a low melting point compared to transition metals (Lane *et al.*, 2005). Cadmium and its congeners are not always considered transition metals, in that they do not have partly filled d or f electron shells in the elemental or common oxidation states (Lane and Morel, 2000). The average concentration of cadmium in the Earth's crust is between 0.1 and 0.5 parts per million (ppm) (Nogawa *et al.*, 2004).

Cadmium occurs as a minor component in most zinc ores and therefore is a byproduct of zinc production (Swandulla and Armstrong, 1989). It was used for a long time as a pigment and for corrosion resistant plating on steel while cadmium compounds were used to stabilize plastic (Morrow, 2010).

Cadmium is also an environmental hazard (Hayes, 2007). Human exposures to environmental cadmium are primarily the result of fossil fuel combustion, phosphate fertilizers, natural sources, iron and steel production, cement production and related activities, nonferrous metals production, and municipal solid waste incineration (Michael, 2010). Root crops and vegetables also contribute to the cadmium in modern populations (Jarup, 1998). There have been a few instances of general population toxicity as the result of long-term exposure to cadmium in contaminated food and water, and research is ongoing regarding the estrogen mimicry that may induce breast cancer (Nogawa *et al.*, 2004). Interest has therefore risen in its biohazardous potential. The highest concentration of cadmium has been found to be absorbed in the kidneys of humans, and up to about 30 mg of cadmium is commonly inhaled throughout childhood and adolescence (Michael, 2010).

The respiratory system is affected severely by the inhalation of cadmium-contaminated air which leads to shortness of breath; lung edema and destruction of mucous membranes as part of cadmium-induced pneumonitis (Seidal *et al.,* 1993). Kidney damage has long been described to be the main problem for patients chronically exposed to cadmium (Barbier, 2005). Low dosages of cadmium are reported to stimulate ovarian progesterone biosynthesis, while high dosages inhibit it (Henson and Chedrese, 2004). Maternal exposure to cadmium is associated with low birth weight and an increase of spontaneous abortion (Frery *et al.,* 1993; Shiverick, 1999). Some evidence exists also that cadmium is a potent nonsteroidal estrogen in vivo and in vitro. Studies in rats showed that cadmium precipitates enhanced mammary development and increased uterine weight (Johnson *et al.,* 2003). Kazantzis (1979) showed a connection between cadmium intoxication and bone damage, in workers exposed to cadmium-polluted fume and dust. A subcutaneous injection of cadmium chloride can induce prostate cancer in Wistar rats (Waalkes *et al.,* 1988), which is proof of the carcinogenic role of cadmium.

2.4.2 Lead

Lead is a soft and malleable metal, which is regarded as a heavy metal (Bergeson, 2008). Lead is a highly poisonous metal (regardless if inhaled or swallowed), affecting almost every organ and system in the body (Jagadish, 2010). The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system (Schoeters *et al.,* 2008). Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy, and colic-like abdominal pains (Needleman *et al*., 1990).

In the human body, lead inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into proporphrin IX, the final step in heme synthesis (Levin *et al*., 2008). This causes ineffective heme synthesis and subsequent microcytic anemia (Marino *et al*., 1990). At lower levels, it acts as a calcium analog, interfering with ion channels during nerve conduction. This is one of the mechanisms by which it interferes with cognition (Levin *et al*., 2008).

Whenever lead finds its way into the human body, it can cause severe brain damage and malfunctioning of bone marrow which leads to deficiency in the production of blood cells which may cause leukemia (Counter *et al.,* 1998). Of all the lead that enters the human body, a child's body is only able to excrete a third in about two weeks while an adult may excrete up to 99% within the same two weeks (ATSDR, 1999). This makes children more susceptible to lead poisoning. When lead in its mobilized form is deposited in the human soft tissues, it may seriously affect the human health (Todd *et al.*, 1996; ATSDR, 1999).

2.4.3 Copper

Copper, is in group 11 of the periodic table, and elements in this group have one s-orbital electron on top of a filled d-electron shell and are characterized by high ductility and electrical conductivity (Brewer, 2007).

Copper in the blood exist in two forms: bound to ceruloplasmin (85–95%) and the rest is loosely bound to albumin and small molecules (Faller, 2009). Loosely bound copper causes toxicity, as it generates reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical (Hureau and Faller, 2009). These damage proteins, lipids and DNA (Genderen *et al.*, 2005).

Copper has a tendency to accumulate in the blood and deplete the brain zinc supplies. High dietary levels of Zn induce signs of copper defficieny, anaemia and decreased activity of the enzyme cytochrome oxidase, whereas the presence of Fe reduces the absorption of Mn (Lehninger, 2007). There is unequivocal evidence that physiological levels of Cu interact positively with Fe to facilitate absorption and utilization by extra-hepatic tissues (O'Dell, 1989).

Acute symptoms of copper poisoning by ingestion include vomiting, hematemesis, hypotension, melena, coma, jaundice and gastrointestinal distress (Brewer, 2007). Individuals with glucose-6-phosphate deficiency may be at increased risk of hematologic effects of copper (Faller, 2009). Chronic (long-term) effects of copper exposure can damage the liver and kidneys (Flemming and Trevors, 1989). Mammals have efficient mechanisms to regulate copper stores such that they are generally protected from excess dietary copper levels (Prociv, 2004).

2.4.4 Zinc

Zinc is a bluish-white, lustrous, diamagnetic metal (Blake, 2007), though most common commercial grades of the metal have a dull finish (Fosmire, 1990). It is less dense than iron and has a hexagonal crystal structure (Connie and Christine, 2009).

Zinc is a versatile trace element required as a co-factor by more than 200 enzymes (Grodner *et al.,* 2000). Zinc acts as a traffic director, overseeing the efficient flow of body processes, maintenance of enzyme systems and cells (Cherop, 2009). The free zinc ion is a powerful Lewis acid up to the point of being corrosive (Maret and Sandstead, 2006). Stomach acid contains hydrochloric acid, in which metallic zinc dissolves readily to give corrosive zinc chloride (Rosado, 2003). Zinc toxicity, which is commonly fatal causes a severe hemolytic anemia, and also liver or kidney damage; vomiting and diarrhea (Bothwell, 2003).

2.4.5 Iron

It is a metal in the first transition series (Rouault, 2003). It is the most common element (by mass) forming much of Earth's outer and inner core (Milman, 1996). Like other group 8 elements, iron exists in a wide range of oxidation states, −2 to +6, although +2 and +3 are the most common (Brar, 2009). Iron plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates (Nanami *et al.,* 2005). Iron is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals (Ramzi *et al.*, 1999). Iron boosts the immune system of the body because it is responsible for the formation of white blood cells as well as haemoglobin formation and functions in the blood (Cherop, 2009). The recommended daily allowance of Fe is 10 to 15 mg for adults and 30 mg for pregnant women (Krayenbuehl *et al.,* 2005).

Large amounts of ingested iron can cause excessive levels of iron in the blood (Durupt *et al.*, 2000). High blood levels of free ferrous iron react with peroxides to produce free radicals, which are highly reactive and can damage DNA, proteins, lipids, and other cellular components (Milman, 1996). Iron typically damages cells in the heart, liver and elsewhere, which can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, and even death (Boukhalfa and Crumbliss, 2002). Humans experience iron toxicity above 20 milligrams of iron for every kilogram of mass, and 60 milligrams per kilogram is considered a lethal dose (Brar, 2009).

Excess of Zn or Fe in the body can interfere with absorption of other minerals (Solomon, 1986). Essential mineral interaction must be based on the physic-chemical properties of their ions. Ions whose valence shell structure are similar would be antagonistic to each other, therefore minerals such as Mn, Fe, Zn and Cu compete with each other because they have similar molecular weights, sizes and charges (Cherop, 2009).

2.4.6 Manganese

Manganese is a silvery-gray metal that resembles iron and is hard and very brittle, difficult to fuse, but easy to oxidize (Zhang and Cheng, 2007). Manganese metal and its common ions are paramagnetic and it tarnishes slowly in air and "rusts" like iron, in water containing dissolved oxygen (Verhoeven, 2007).

Manganese plays the role of activating enzymes necessary for the body's proper use of biotin, vitamin B_1 and vitamin C (Skudi, 2011). It is also responsible for synthesis of glycoprotein in body cells that act as a protein coating against viruses (Bakhru, 2006).

2.5 Toxicity of heavy metals in food crops

Plants take up metals by absorbing them from contaminated soils, as well as from deposits on parts of the vegetables exposed to the air from polluted environments (Anita *et al.*, 2010). Vegetables constitute essential diet components by contributing protein, vitamins, iron, calcium and other nutrients, which are usually in short supply (Thompson and Kelly, 2003). They also act as buffering agents for acidic substances produced during the digestion process (Kiende *et al*., 2012). However, they contain both essential and toxic aspects over a wide range of concentrations. Metal accumulation in vegetables may pose a direct threat to human health (Türkdogan *et al.*, 2003; Damek-Poprawa and Sawicka-Kapusta, 2003). Vegetables, especially that are leafy grown in heavy metals contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their leaves (Al Jassir *et al*., 2005).

It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals) (Islam *et al.*, 2007). These metals enter the human body mainly through two routes namely: inhalation and ingestion, and with ingestion being the main route (Kiende *et al.*, 2012). Prolonged consumption of unsafe concentrations of heavy metals through food stuffs may lead to the chronic accumulation of heavy metals in the kidney and bone diseases (Jarup, 2003). The European Union does not accept food substances with more than 0.2 ppm of Cadmium and 0.3 ppm of lead (Kaluli *et al.,* 2011).

Singh *et al.* (2001) analyzed six and Itanna (2002) three green leafy vegetables respectively and both reported higher concentration of Zn in spinach. 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. They also investigated the concentrations of heavy metals such as Cd, lead (Pb), Zinc (Zn), Cu and Ni in different vegetables, grown in various parts of Turkey.

In other studies, the levels of heavy metals (lead, cadmium, copper and zinc) were examined in selected fruits and vegetables sold in the local markets of Egypt with levels ranging from 1.47 to 2.51 mg/kg (Radwan and Salama, 2006). Fytianos *et al.* (2001) studied heavy metals in vegetables grown in an industrial area of North Greece and reported high levels ranging from 3.69 to 79.54 mg kg^{-1} which exceeded the health safety limits. Manganese forms soluble salts and complexes which are readily taken up by the plants and this leads to the elevated levels of manganese in the vegetables (Fytianos *et al.,* 2001). Lead is a toxic element that can be harmful to plants although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield (Mohsen and Mohsen, 2008).

Demirezen and Ahmet (2006) analyzed different samples of vegetables and reported a significantly high concentration (3.0-10.7 mg kg^{-1}) of Pb which poses health risks to human life. In another study, Sharma *et al.* (2006) found the concentration of Pb to range from 17.54-25.00 mg kg^{-1} in vegetables grown in wastewater industrial areas in India which were above the safe limit of 0.3 mg kg^{-1}. Muchuweti *et al.*, (2006) reported the level of Pb (6.77 mg kg^{-1}) in vegetables irrigated with mixtures of wastewater and sewage from Zimbabwe to be higher than EU (2006) safe limit of 0.3 mg kg^{-1}. Fytianos *et al.* (2001) reported a high concentration of Pb in spinach grown in industrial and rural areas of Greece. Al Jassir *et al.* (2005) reported Pb levels in coriander (0.171 mg kg^{-1}) and purslane (0.226 mg/kg). Demirezen and Ahmet (2006) reported Cd levels in vegetables ranging from 0.24–0.97 mg kg^{-1} while Muhammad *et al.* (2008) levels ranged from 0.4-0.7 mg kg^{-1}, which were beyond the safe limits. Similar findings beyond the safe limits were also reported by Iqrar *et al.* (2006).

Globally, there are differing threshold limits that have been set as safety standards for heavy metals in the soil, water for irrigation and the vegetables irrigated with the water. Table 2.1 shows a comparative set of the safety limits as set by various standardizing bodies.

Table 2.1: Guidelines for safe limits of heavy metals

Sample	**Standards**	**Cd**	**Cu**	**Pb**	**Zn**	**Mn**
Soil (mg/l)	Indian Standard (Awashthi, 2000)	4.33	135-270	250-500	300-600	-
	WHO/FAO (2007)	-	-	-	-	-
	European Union Standards (EU 2002)	3.0	140.0	300.0	300.0	-
	FAO (2006)	3.0	135.0	300.0	300.0	
Water (mg/l)	Indian Standard (Awashthi, 2000)	0.01	0.05	0.1	5.0	0.1
	FAO (1985)	0.1	0.2	5.0	2.0	0.2
	European Union Standards (EU 2002)	-	-	-	-	-
	NEMA (2006)	0.5	0.05	5.0	2.0	
Vegetables (mg/l)	Indian Standard (Awashthi 2000)	1.5	30.0	2.5	50.0	-
	WHO/FAO (2007)	0.2	40.0	5.0	60.0	-
	European Union Commission regulation (EU 2006)	0.2	-	0.3	-	-
	FAO/WHO (Codex Alimentarius, 2001)	0.1	73.3	0.3	100.0	500.0

Source: Anita *et al.* (2010)

Plant species have a variety of capacities in removing and accumulating heavy metals, so there are reports indicating that some species may accumulate specific heavy metals, causing a serious health risk to human health when plants based food stuff are consumed (Wenzel and Jackwer, 1999). For bioaccumulation of heavy metals in different vegetables, various mechanisms like binding metal with organic acids, proteins or other ligands (Godbold *et al.*, 1984) has been speculated. Similarly, adsorption of metals in different plant parts like cell wall, middle lamella or cell organelles (Chettri *et al.*, 2000) has been suggested for their bioaccumulation. Tolerance of heavy metals in plants is either by detoxification mechanisms or accumulation in different plant parts or cell organelles or in vacuoles or in cell wall. These heavy metals are some of the contaminants that can be found in the tissues of fresh vegetables and leads to food chain contamination as crops and vegetables absorb them from the soil. Certain trace elements are essential in plant nutrition, but plants growing in a polluted environment can accumulate trace elements at high concentrations causing a serious risk to human health when consumed (Voutsa *et al.*, 1996). Prolonged exposure to heavy metals such as cadmium, copper, lead and zinc can cause deleterious health effects in humans (Reilly, 1991). The heavy metals not only affect the nutritive values of vegetables but also affect the health of human beings and therefore, the safe limits of these heavy metals are lowered regularly in these vegetables.

2.6 Anthropogenic activities leading to discharge of heavy metals

Major urban inputs of sewage water include household effluents, drainage water, business effluents for example car washes, atmospheric deposition, and traffic related emissions such as vehicle exhaust, brake linings, tires, asphalt wear, gasoline/oil leakage among others transported with storm water into the sewage system (USEPA, 1992; Sorme and Lagerkvist, 2002).

Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure (Islam *et al.,* 2007). Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms. The mobilization of heavy metals into the biosphere by human activity has become an important process in the geochemical cycling of these metals (Islam *et al.,* 2007). This is acutely evident in urban areas where various stationary and mobile sources release large quantities of heavy metals into the atmosphere and soil, exceeding the natural emission rates (Bilos *et al.,* 2001). Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries (Qadir *et al.,* 2007).

2.7 Antibiotics

Vast quantities of pharmacologically active substances (PhACs) are used annually in human and livestock disease management (Sarmah *et al.,* 2006). The majority of PhACs detected in environmental waters and waste waters are drugs used for a variety of therapeutic uses for both humans and animals (Jjemba, 2006). Pharmaceutically active compounds (PhACs) such as antibiotics can enter municipal and natural water systems via residential or commercial discharges, including hospital effluent (Lauren *et al*., 2010). Another major route by which pharmaceuticals can enter the environment is by the disposal of out-of-date or unwanted medicines, which may occur via the sink/toilet or in household waste that is then taken to landfill sites (Jjemba, 2006).

Antibiotics are specifically designed to control bacteria in human or animals and help to protect their health (Jjemba *et al.*, 2010). After treatment, most antibiotics are excreted from the treated body, either unaltered or as metabolites, some of which are still bioactive (Sarmah *et al.,* 2006). This makes them potentially hazardous to bacteria and other organisms in the environment (Baguer *et al.,* 2000). Of all emerging contaminants, antibiotics are of greatest concern, since their emission in the environment can increase the occurrence of resistant bacteria (Soto-Chinchilla *et al.,* 2007; Barnes *et al.,* 2008) and enhance the transfer of genes that resist antibiotics between different bacteria taxonomic affiliations (Davidson, 1999). Chee-Sanford *et al.* (2001) demonstrated that antibiotics occur in groundwater as a direct result of effluent infiltration into aquifers and suggested that groundwater may be a potential source of antibiotic resistance bacteria (Jjemba, 2006). Detection of these negative effects in the environment is difficult; although *in vivo* and *in vitro* laboratory tests generally show that the toxic effects of these compounds are not seen at the low levels currently detected in the environment, the possibility of variations in sensitivity, chronic exposure, and mixture effects such as concentration addition and synergism (Altenbureger *et al.,* 2004; Cleuvers 2004; Richards *et al.* 2004). This implies that other negative effects cannot be ruled out and hence the need to analyze them continuously in wastewater, soils and vegetables irrigated with untreated wastewater.

Different types of drugs have different anticipated exposure routes to the environment (Halling-Sørensen *et al.,* 1998). The dominant pathway for antibiotic release in the terrestrial environment is through the application of animal manure and biosolids containing excreted antibiotics to agricultural land as fertilizer (Kemper, 2008). Antibiotics can also be introduced to agricultural land through irrigation with reclaimed wastewater, since they have been frequently detected in the raw and treated sewage wastewaters (Gulkowska *et al.,* 2008).

When antibiotics get into the arable land, they could negatively impact vegetation growth and development as well as soil microbial activity (Jjemba, 2002). The effects of antibiotics on plants in soils are found to be different between compounds and between plant species (Farkas *et al.,* 2007). Tetracyclines increase radish yields, but decrease pinto bean yields (Batchelder, 1982). When grown in chlortetracycline-treated soil, a significant increase in the activities of the plant stress proteins glutathione S-transferases and peroxidases was observed in maize plants, but not in pinto beans (Farkas *et al.,* 2007).

The presence of antibiotic residues in groundwater was intensively investigated mainly below effluent recharge ponds, feedlots, landfills and riverbanks (Batt *et al.,* 2006). In many cases, where geohydrological information is available, it can be seen that antibiotic residues were mainly detected in shallow phreatic aquifers 3–6 m below ground surface levels (Batt *et al.,* 2006). Organisms in the surface waters are adversely affected by PhACs and showed increased resistance to drugs (Jobling *et al.,* 1998).

Although PhACs are intended to be utilized by the human body, in some instances as much as 50 to 90 percent of an administered drug may be excreted by the body in a biologically active form (Raloff, 1998, Guardabassi *et al.,* 1998). In the year 2000, the

New Mexico Environment Department (NMED) and the Scientific Laboratory Division (SLD) of the New Mexico Department of Health initiated a study of PhACs in New Mexico waters. NMED detected a variety of drug residues in 11 of 15 sewage effluent samples and in 4 of 23 surface water samples (Mc Quillan *et al.*, 2001). The presence of antibiotics in streams is a global concern (Heberer and Adam, 2004). This is due to their possible connection to the development of antibiotic-resistant organisms, the potential for disruption of microbial ecology, complications surrounding development of water reuse technologies, and even increased human health risks (Daughton and Ternes, 1999; Guardabassi *et al.,* 1998).

Some of these chemicals are easily removed during sewage treatment and environmental processes (Andreozzi *et al.,* 2002), while others are more persistent (Ternes *et al.,* 2002; Drewes, 2004). In the third world populations that use untreated wastewater, the levels of exposure are high (Guardabassi *et al.*, 1998). With the increased use of antibiotics due to the rise in immunosuppressive conditions that expose people to opportunistic infections, there is cause for alarm on just what quantities of PhACs may be in the wastewater effluent and subsequently transferred into the food consumed (Jjemba, 2002).

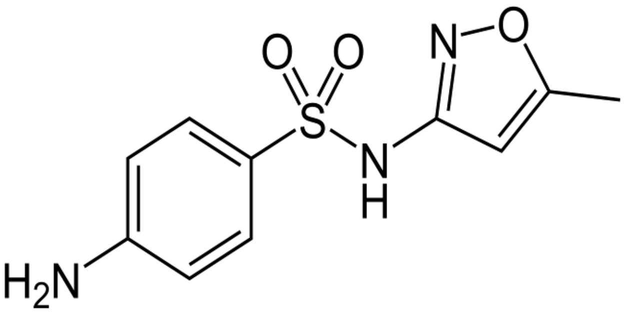
A report on the determination of the concentration of sulfamethoxazole in untreated hospital wastewater discharged into sewer systems states that sulfamethoxazole has high persistence and was detected at concentrations of 300 ng/l (Kathryn, 2004). In addition to predictions regarding fate and persistence, Huang *et al.* (2001) also reported the sulfamethoxazole concentrations in untreated hospital wastewater to range from 3.9 ng/l to approximately 27,000 ng/l. Hartig *et al.* (1999) reported a sulfamethoxazole level in the primary effluent of a German wastewater treatment programme (WWTP) of 2.4 μg/L, whereas analysis of the secondary treated sewage gave residual concentrations in a range from 0.3 to 1.5 μg/L. Another study monitoring a series of pharmaceuticals in sewage effluents found median concentrations of 0.40 μg/L for sulfamethoxazole and 0.32 μg/L for trimethoprim (Pérez *et al.,* 2005). Unlike sulfamethoxazole, trimethoprim has been reported to persist in sewage sludges mush longer (Pérez *et al.,* 2005).

2.7.1 Sulfonamides

The sulfonamide antibacterial group is considered to be one of the first antimicrobial drugs used and paved the way for the antibiotic revolution in medicine (Lindsey *et al.*, 2001). It was intensively manufactured since the mid 1940s. Presently, only few sulfonamide derivatives are still in clinical use because of developed bacterial resistance (Tilles, 2001). Sulfamethoxazole (4-amino-N-(5-methyl-3-isoxazolyl) benzene-sulfonamide), (SMX, $C_{10}H_{11}N_3O_3S$), a representative antibacterial compound of the sulfonamides group, is considered as an emerging micro contaminant of concern due to its potential adverse effects on ecosystems and human health (Lindsey *et al.,* 2001). The SMX was first synthesized in 1957 (Kano and Ogata, 1957) and approved by the FDA in 1961. It is a synthetic antibiotic of the sulfonamide group. Its mode of action is prevention of folic acid synthesis in the bacteria (Richard *et al.*, 2008).

The SMX is water soluble, has a half life of 19 days under sunlight and is highly resistant to further biodegradation in the subsurface (Lam *et al.*, 2004). It has a low K_{ow} (− 0.1 to

1.7) and is considerably hydrophilic and polar. Such properties enable SMX to be transported over long distances without being adsorbed to sediments (Pérez *et al.,* 2005). Furthermore, under typical environmental pH conditions (pH ~ 7–8) SMX is negatively charged approximately 95–100% a property that can increase its transport velocity in porous media due to anion exclusion. The molecular structure of a sulfamethoxazole molecule is shown below.



Sulfamethoxazole (4-amino-N-(5-methyl-3- isoxazolyl) benzene-sulfonamide)

The medicinal use of sulfamethoxazole is for example treatment of tuberculosis, malaria and urinary tract infections (Richards *et al.,* 2004). Due to development of resistant bacteria against sulfamethoxazole when it was used as a single substance, sulfamethoxazole is nowadays mainly used in combination with trimethoprim. In combination with trimethoprim, it is used to treat *Pneumocystis carinii* pneumonia, *Pneumocystis jirovecii* pneumonia and toxoplasmosis among the HIV-AIDS patients (De Ruiter *et al.*, 2008). Like other sulfonamides, SMX disrupts the folate biosynthetic pathway in bacteria, which was recently established as identical to that of plants, raising concerns over nontarget toxicity (Richard *et al.,* 2008).

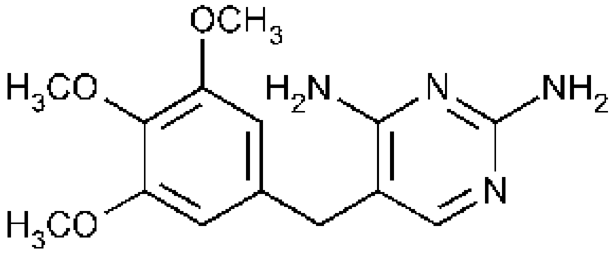
In a study carried out to determine the presence and concentration of sulfonamide antibioitic sulfamethoxazole in untreated hospital wastewater, the drug displayed high persistence and was detected at concentrations of 300 ng/l in the Rio Grande (Kathryn, 2004). The finding of sulfamethoxazole in the Rio Grande is consistent with results obtained by the USGS in their surveillance of US streams in 1999 and 2000 where sulfamethoxazole and trimethoprim were both detected in 12.5 percent of 104 streams with a median concentration of 150 ng/l (Kolpin *et al.*, 2002). In an earlier study in Rio Grande on the presence of trimethoprim and sulfamethoxazole in untreated wastewater, trimethoprim was however not detected although these two drugs frequently appeared to be detected together and in similar concentrations by the USGS (Kolpin *et al.,* 2002).

In addition to predictions regarding fate and persistence, Huang *et al.* (2001) also estimated sulfamethoxazole concentrations in untreated hospital wastewater to range from 3.9 ng/l to approximately 27,000 ng/l. The free forms of sulfamethoxazole and trimethoprim are considered to be the therapeutically active forms (Kolpin, 2002). The average percentage of the dose recovered in urine from 0 to 72 hours after a single oral dose of sulfamethoxazole/trimethoprim is 84.5% for total sulfonamide and 66.8% for free trimethoprim (Kathryn, 2004). Thirty percent of the total sulfonamide is excreted as free sulfamethoxazole, with the remaining as N4-acetylated metabolite (Huang, 2001). Perhaps this is due to the differences of removal from wastewater where trimethoprim is reduced by approximately 69 percent and sulfamethoxazole by only 20 percent (Kathryn, 2004).

In a study carried out to determine the concentration of sulfamethoxazole in sewage sludge, the concentration of SMX detected was in the range of 28 and 68 µg/kg (Gobel, 2005).

2.7.2 Trimethoprim

Trimethoprim (5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine) is a synthetic broad spectrum antibiotic that inhibits the dihydrofolic acid synthesis in bacteria and in the absence of Dihydrofolate, the bacteria die out (Ryan *et al.,* 2011). The molecular structure of trimethoprim molecule is shown below.



Trimethoprim [5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine]

This active substance is used to treat many different bacterial infections such as urinary tract infections, respiratory infections and middle ear infections. The combination of sulfamethoxazole and trimethoprim obstruct two consecutive steps in the folic acid metabolism, thus interrupts the micro-organisms synthesis of RNA and DNA (Pérez *et al.,* 2005). In a study carried out by Ryan *et al.* (2011), the rate of loss of sulfamethoxazole was found to be enhanced in wastewater effluent due to indirect photolysis reactions, specifically reactions with hydroxyl radicals and triplet excited state effluent organic matter. Photolysis in the presence of natural organic matter, however, did not lead to enhanced degradation of sulfamethoxazole. Trimethoprim was also found to be susceptible to indirect photolysis in wastewater effluents, with hydroxyl radical and triplet excited effluent organic matter being the responsible species (Ryan *et al.*, 2011). Research findings from a study by Kathryn (2004) showed a concentration range of 2900-5000 ng/l of trimethoprim in untreated hospital wastewater. Similar studies done earlier by Kolpin (2002) reported a lower concentration range of 180-590 ng/l of trimethoprin in untreated wastewater. This is in line with current increase in the use of trimethoprim/sulfamethoxazole in combination in the management of opportunistic infections among the immunocompromised HIV/AIDS patients.

2.8 Methods for analysis of metals

For screening heavy metals, the principal methods used are energy dispersive X-Ray fluorescence (Albaiges, 2000), neutron activation analysis (NAA), (Jamieson and Hancock, 2004), mass spectrometry (MS), (Swart *et al*., 1998) flame atomic absorption spectroscopy (AAS) (Taylor *et al.*, 2006) and flame atomic emission spectrometry (AES) (Taylor *et al.*, 2006) chemical polarography (Joseph, 1983), voltametric methods (Melluci and Locatelli, 2007) and anodic stripping voltammetry (Salisbury *et al.*, 1991). This study used the atomic absorption spectroscopy (AAS) because of its availability.

2.9 Principles of atomic absorption spectroscopy (AAS)

The AAS is a method of elemental analysis that works on the principle of absorption of radiation energy by free atoms. The concentration of an element is measured by the absorption of radiation with a characteristic frequency by free atoms of an element. Light of certain wavelength produced by monochromatic or hollow cathode lamp emits spectral lines corresponding to energy required for excitation of an element of interest (Skoog, 2007).

The analytical signal is obtained from the signal between the intensity of the source in the absence of the element of interest and the decreased intensity obtained when the element of interest is present in the optical path. Absorption of light is associated with the transition process from one steady state to another. For instance the case of a steady state O and J, where $E_o<E_j$, the O-J transition results in the absorption of light with frequency given in Equation 2.1 (Skudi, 2011)..

$V_{oj} = (E_j-E_o) / h$ ……………………………………………………..……………….. (2.1)

Where:

h = plank's constant
V = frequency
Eo = energy at ground state
Ej = energy at excited state
O-J = transtitions stimulated by absorption of external radiation

The number of atoms in the excited state relative to the number in the ground state is given by the Maxwell-Boltzman law (Skoog *et al.*, 1998), given in Equation (2.2).

$N_1/N_0 = g_1/g_0 \exp [E_g-E_1/KT]$ ………………………..………………..………… (2.2)

Where;

N_1 = Number of atoms in excited state
N_0 = number of atoms in ground state
g_1 and g_0 = Statistical weight of excited and ground state respectively

K = Boltzman’s constant
T= Absolute temperature
E_g = Energy at ground state
E_1 = Energy at excited state

Relative fraction of atoms in excited state is dependent on temperature whereas intensity is independent on temperature. Sample solution is aspirated through nebulizer into the air/acetylene flame or nitrous oxide/acetylene flame (Taylor *et al.,* 2006). An electrically heated graphite furnace is used when very high sensitivity is required. The sample solution gets dispersed into mist of droplets and then gets evaporated into dry salt.

The dry salt goes into vapour then dissociates into atoms that absorb resonance radiation from external source. The unabsorbed radiation is allowed to pass through the monochromator which isolates the existing spectral lines. The isolated analyte line falls on the detector and the output of which is amplified and recorded. The parameter measured is absorbance (A) and related to concentration by Equation (2.3).

$A = \log I_0/I = \varepsilon cl$……………………………………………………….……………… (2.3)

Where;

A = Absorbance
I_0 = Incident radiation
I = Attenuated radiation
ε = molar absorptivity (L $mol^{-1}cm^{-1}$)
c = Concentration (mol dm^{-3})
l = Path length (cm)

Since the relationship between absorbance (A) and concentration (C) is linear over a wide range of concentration (Beer’s law), standards are used to obtain calibration curve from which concentration of analyte is established through interpolation method (Van loon, 1980). The most important components of atomic absorption spectroscopy are:

a) Radiation source

There are two types of radiation sources, namely;

i) Continuous source

The continuous source gives a wide range of radiation and includes deuterium lamp and mercury vapour lamp. It is less sensitive because only a small band of radiation passed by monochromator is absorbed while a large portion falls on the detector.

ii) Hollow cathode source

It is commonly used in atomic absorption spectroscopy (AAS) instrument made up of metallic or alloy of element of interest. Hollow cathode lamp consists of tungsten anode and cylindrical cathode sealed in a glass tube that is filled with neon or argon gas at a pressure of 1-5 torr.

b) Atomizer

The two types of atomizers are flame and electro-thermal atomizers. In flame atomizer, temperature is determined by flowrate and the ratio of oxidant and fuel. In flame atomizer, solvent is evaporated to produce solid molecular aerosol during dissolvazion process.

Dissociation leads to atomic gas whereas some of the atoms ionize to give cations and electrons. In electro-thermal atomizer, few molecules of the sample are first evaporated at low temperature and ashed at higher temperature in electrically heated graphite. After ashing, the temperature is increased to 2000-3000 ^{0}C to atomize the sample.

c) Monochromators

They are analyzers that present monochromatic radiation to the detector. They are filters, prisms or gratings that disperse or separate radiation so that selected wavelength corresponding to a particular energy of the sample is transmitted. Diffraction gratings are preferred to prisms as they offer accuracy over a wide range of wavelengths.

d) Detectors

Detectors convert radiation energy into electrical signal and include phototube, photomultiplier tube and photodiode array detectors.

e) Read out system

These are digital and interfaced with microprocessors that allow the programming of various aspects bringing simplicity in operations. However, AAS is a single elemental method, in which one element is determined in a series of samples and instrumental parameters optimized for the next element.

2.10 Methods for analysis of antibiotics

For screening antibiotics, the principal methods used are microbiological assays (Viola and DeVincent, 2006), enzyme linked immune sorbent assay (ELISA) (Bogialli, 2009), biosensors (Turnidge, 2004) and High performance liquid chromatography (HPLC) (Stolker et al., 2007). This study used the HPLC method because of its availability.

2.11 The principles of high performance liquid chromatography (HPLC)

This is a type of chromatography where the stationary phase and the mobile phases are either both liquid, liquid stationary phase and gaseous mobile phase, or solid and liquid or gaseous mobile phase (Boyer, 2000). The samples to be separated are partitioned between the two phases on account of their unique affinities for one of the phases. The main influencing factor of separation is polarity, non-polarity, hydrophobic and hydrophilic interactions between the solutes and the two phases. The HPLC columns are packed with superfine beads with a diameter of less than 20 µm and a large surface area to volume ratio. In HPLC the flow rates are increased by applying a pressure differential across the column. A combination of high pressure and adsorbents of small particle size leads to the high resolving power and short analysis times characteristic of HPLC.

The retention time of a solute in HPLC (t_R) is defined as the time necessary for maximum elution of a particular solute. Retention volume (V_R) of a solute is the solvent volume required to elute the solute, and is defined by Equation (2.4).

$V_R = Ft_R$ …………………………………………………………………… (2.4)

Where F is the flow rate of the solvent:

tR is retention time of solute

V_R is the retention volume of the solute

The measure of column efficiency is resolution, (R). Resolution indicates how well solutes are separated; it is defined by Equation (2.5).

$R = 2 (t_{R} - t^1{}_R)/ (w + w^1)$ ……………………………………………………….(2.5)

Where: t_R and t^1_R are retention times of two solutes
w and w^1 are the base peak widths of the same two solutes.

2.11.1 The basic components of HPLC equipment

The basic components are a solvent reservoir, high pressure pump, packed column, detector and recorder. A computer is used to control the process and to control and analyze data.

a) Solvent reservoir

The solvent chamber should have a capacity of at least 500 ml for analytical applications, but larger reservoirs are required for preparative work. In order to avoid bubbles in the column and detector, the solvent must be degassed. Several methods may be used to remove unwanted gases, including refluxing, filtration through a vacuum filter, ultrasonic vibration, and purging with an inert gas. The solvent should be filtered to remove particulate matter that would be drawn into the pump and column.

b) Pumping systems

The purpose of the pump is to provide a constant, reproducible flow of solvent through the column. The two types of pumps available are the constant pressure and the constant volume pump. The pump must be capable of pressure outputs of at least 500 psi and preferably up to 5000 psi. It should have a controlled, reproducible flow delivery of about 1mL/min for analytical applications and up to 100 mL/min for preparative applications. It should also yield pulse-free solvent flow and should have a small hold-up volume. Although neither type of pump meets all these criteria, constant –volume pumps maintain a more accurate flow rate and a more precise analysis is obtained.

c) Injection port

A sample must be introduced onto the column in an efficient and reproducible manner. One of the most popular injectors is the syringe injector. The sample in a microliter syringe is injected through a neoprene/Teflon septum. This type of injection can be used at pressures up to 3000 psi.

d) Columns

HPLC columns are prepared from stainless steel or glass/Teflon tubing. Typical column diameters are 2.1, 3.2 or 4.5 mm for analytical separations and up to 30 mm for preparative applications. The length of the column can range from 5 to 100 cm but 10 to 20 cm columns are common.

e) Detector

Liquid chromatographs are equipped with a means to continuously monitor the column effluent and to recognize the presence of solute. Only small sample sizes are used with most HPLC columns, so a detector must have high sensitivity. The type of detector that has the most universal application is the differential refractometer. This device continuously monitors the refractive index difference between the mobile phase (pure solvent) and the mobile phase containing sample (column effluent). The most widely used HPLC detectors are the photometric detectors. These detectors measure the extent of absorption of ultraviolet or visible radiation by the sample. A third type of detector that has only limited use is the fluorescence detector. This type of detector is extremely sensitive; its use is limited to samples containing trace quantities of biological materials.

Its response is not linear over a wide range of concentrations, but it may be up to 100 times more sensitive than the UV detector.

2.11.2 Analysis of HPLC data

Most HPLC instruments are on line with an integrator and a computer for data handling. For quantitative analysis of HPLC data, operating parameters such as rate of solvent flow must be controlled. In modern instruments, the whole system including the pump, injector, detector and data system is under control of a computer.

2.11.3 Stationary phases in HPLC

The adsorbents in HPLC are typically small diameter porous materials. In the early days of HPLC, solid supports were coated with a liquid stationary phase. Columns with these packings had short lifetimes as a gradual decrease in resolution because there was continuous loss of the liquid stationary phase with use of the column. This problem was remedied by the discovery of methods for chemically bonded stationary phases. The major advantage of a bonded stationary phase is stability. Since it is chemically bonded, there is very little loss of stationary phase with column use. The use of nonpolar chemically bonded stationary phases with a polar mobile phase is referred to as reverse phase HPLC. This technique separates sample components according to hydrophobicity. It is widely used for the separation of all types of biomolecules. Typical solvent systems are water, methanol, water acetonitrile, and water-tetrahydrofuran mixtures

The mobile Phase selection of a column packing that is appropriate for a given analysis does not ensure a successful HPLC separation. A suitable solvent system must also be chosen. Several critical solvent properties to be considered are;

i) Purity: Very high purity solvents with no particulate matter are required.

ii) Reactivity: The mobile phase must not react with the analytical sample or column packing. This does not present a major limitation since many relatively unreactive hydrocarbons, alkyl halides and alcohols are suitable.

iii) Detector compatibility: A solvent must be carefully chosen to avoid interference with the detector. Since refractometer detectors monitor the difference in refractive index between solvent and column effluent, a greater difference leads to greater ability to detect the solute.

2.11.4 Solvents for HPLC operation

The eluting power of a solvent is related to its polarity. Chromatographic solvents have been organized into a list according to their ability to displace adsorbed solutes (eluting power, ε°). The ε° increases with an increase in polarity. Using the eluotropic series makes solvent choice less a matter of trial and error. Occasionally, a single solvent does not provide suitable resolution of solutes. Solvent binary mixtures can be prepared with eluent strengths intermediate between the ε° values for the individual solvents.

2.11.5 Gradient elution in HPLC

The common difficulty encountered in the analysis of multicomponent samples is referred to as the general elution problem. It is due to the fact that the components have a wide range of K_D values and no single solvent system is equally effective in displacing all components from the column. The general elution problem is solved by the use of gradient elution. This is achieved by varying the composition of the mobile phase during elution. In practice, gradient elution is performed by beginning with a weakly eluting solvent (low ε°) and gradually increasing the concentration of a more strongly eluting solvent (higher ε°). The weaker solvent is able to improve resolution of components having low K_D valued. In addition, the gradual increase in ε° of the solvent mixture decreases the line broadening and provides a more effective separation. The choice of solvents for gradient elution is still somewhat empirical, but data from eluotropic series may give some guidelines. Modern HPLC equipments are equipped with solvent programming units that control gradient elution in a stepwise or continuous manner.

2.11.6 Sample preparation and selection of HPLC operating conditions

The following procedure is needed in order to convert a sample into a clean one: de salting, removal of anions and cations, removal of metal ions, concentration of desired macromolecules, removal of detergent, and particulate removal. Sample preparation techniques used to achieve these results are gel exclusion chromatography, ion exchange chromatography, microfiltration, and metal affinity chromatography. These procedures may be completed by commercial available prefilters or precolumns.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study site was in Njiru/Ruai locations in Nairobi County located in the riparian region of the Ngong River that flows through Nairobi County. The sample collection points were upper Njiru, which is the area just past the Mwiki Police Station towards Njiru shopping centre in the Gituamba quarry site vicinity, Lower Njiru, which is the area that is within the vicinity of the Eastern by-pass road running at a point where two untreated water streams that make the Ngong Nairobi River converge.

The two streams flow down, ridden with sewage into the Ruai sewage treatment site. Upper Ruai is approximately two kilometers behind a shopping center referred to as Sewage shopping centre, a site occupied by internally displaced people, which is a flood zone, and lower Ruai which is below the Sewage Shopping centre vicinity. The study site is arid, characterized by thorny shrubs and short thorny trees, in the middle of which there are the lash green vegetable farms. The geographical terrain of the study site is that Upper Njiru is upstream, with the river flowing through Lower Njiru, then Upper Ruai and finally Lower Ruai downstream at the lower end. The GPS bearing points for some of the sampling sites are: S114.654 E36 57.255, S114.663 E36 57.260, S1 14.666 E36 57.269, S114.669 E 36 57.273, S114.667 E36 57.198, S114.672 E36 57.206.

Plate 3.1 (a) shows the farms irrigated with the wastewater in close proximity to the river, while the background is dry vegetation due to lack of water. Between the farms is a river (Plate 3.1 b) in which the sewage is disposed. In this case, both flood irrigation and manual over head irrigation is practiced widely. This is done using the wastewater disposed into the river.

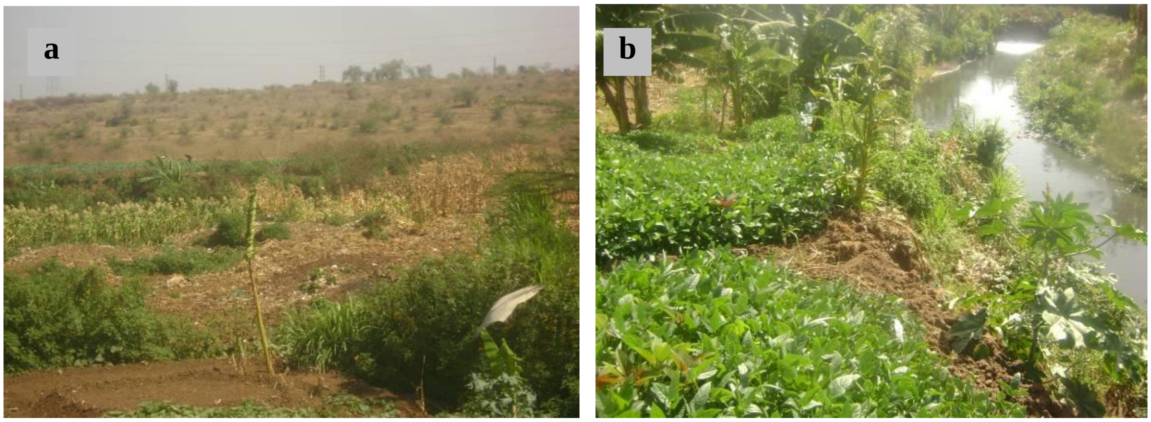


Plate 3.1: The sampling site where the vegetables are grown (a) The study site and background of the dry farms at Njiru where the vegetables are grown by irrigation. (b) Cow pea vegetables grown along the Ngong River polluted with wastewater used for irrigation.

The vegetable farms directly get the untreated waste water from the exhausters in areas where exhausters discharge. The sewage exhausters dispose the raw sewage into the river which pollutes the water as Plate 3.2 shows.

Plate 3.2: The sewage exhausters disposing off the raw sewage into the river

This wastewater is then used for irrigation of agricultural crops and vegetables by the farmers. The study site also receives waste water from the Kariobangi small scale industries and part of the industries that are in the Njiru Township. Most of these industries deal with reconditioning of used cars, spray painting cars, smelting ores and there is also a large steel industry in the neighbourhood. Plate 3.3 shows the contamination of the river with sewage.

Plate 3.3: Foamy and dark coloured water of Ngong River used to irrigate the farms with vegetables. (a&b) Sewage and dumped materials disposed into a flowing river. (c) The flowing river carrying the sewage and dumped materials. (d) Stagnant wastewater along the river used for irrigating the vegetables.

Seasonal variations were not considered because the farming takes place along the River banks of the Ngong River parallel to the Nairobi sewer lines and these acts as a flood zone during the rainy season. No farming is carried out during the rainy season until the rivers dry down and so the farmers use the banks exclusively in the dry season.

3.2 Research design

Quasi comparative study design among four areas was used, and there was a control plot at the Kenyatta University. The control plot comprised of potted plants that were grown on the soil collected from the study site but away from the farm irrigated with the untreated wastewater. The potable water used was from Ruiru dam which is the same water used by the population in the study area for domestic purposes. The samples were collected on Monday, Wednesday and Friday during the dry season and for each of the three samples from the same site at the same season, they were homogenized. This was taken to be one sample and the procedure was repeated two more times during two other consecutive dry seasons in the month of January. Therefore the sampling was carried out in triplicate for three alternate days in the months of January. The respective vegetables grown in the sample farms were also grown in the control plot. A total of 240 vegetable samples, 240 soil samples and 240 wastewater samples were collected.

3.3 Materials

3.3.1 Chemicals and solvents

All chemicals and solvents used for AAS were of analytical grade and those used for HPLC were HPLC grade. Acetronitrile HPLC grade, methanol HPLC grade, hexane HPLC grade, ethyl acetate HPLC grade, analytical grade potassium dihydrogen phosphate, ultra pure water, standards for SMX,TMP, standards for Cd, Mn, Fe, Zn, Pb and Cu in solution for AAS, distilled-deionized water was used.

3.3.2 Cleaning of glass ware and sample containers

All glassware and plastic containers were thoroughly washed with 10% HCl and rinsed thoroughly with de-ionized water and then oven dried. Prior to use, the glassware was rinsed out finally with 10% HCl acid solution. Storage self sealing bags used were clean and used as they were bought for packaging of vegetables.

3.4 Sampling and sample pretreatment

The samples of vegetables, soil and water were taken from different plots irrigated with untreated wastewater during the dry season in areas namely; Upper Njiru, Lower Njiru, Upper Ruai and Lower Ruai. In each area, five farms that grew a specific vegetable of interest were identified and from each such farm, five samples of vegetables , water and soil were collected as described in the following subsections. The sampling was carried out in the morning before 8.00 am to limit the concentration effects of evaporation due to the hot sun during the dry season. The samples were collected on Monday, Wednesday and Friday during the dry season and for each of the three samples from the same site, they were homogenized. This was taken to be one sample and the procedure was repeated two more times during two other consecutive dry seasons in the month of January. Therefore the sampling was carried out in triplicate for three alternate days in the months of January for three consecutive years. The respective vegetables grown in the sample farms were also grown in the control plot. A total of 240 vegetable samples, 240 soil samples and 240 wastewater samples were collected. Samples from the control plot were also collected on the same days as the test samples in triplicate and homogenized prior to analysis just as the test samples.

Plate 3.4 shows the vegetables grown in the wastewater contaminated with raw sewage. Surface irrigation was done in which the wastewater is pumped by a generator to flood the surface of the cultivated farm.

Plate 3.4: Vegetables grown with untreated waste water. (a) Spinach, (b) Cow peas grown adjacent to river. (c&d) Cow peas irrigated with wastewater being harvested for sale.

3.4.1 Water samples for metal analysis

Water samples were collected using 500 ml plastic bottles. The sampling bottles for heavy metal determination were pre-soaked overnight with 10% HCl and rinsed with distilled water and rinsed using river water four times before sample collection. The wastewater is pumped using a generator to flood the farm land and sampling was carried out in triplicate from each source point for irrigation of a particular vegetable.

3.4.2 Water samples for antibiotic analysis

Water samples were collected using 500 ml plastic bottles that were washed with de-ionized distilled water and at the collection point, the bottles were rinsed with the untreated waste water twice before filling them with the sample water. The untreated waste water is pumped using a generator to flood the farm land and sampling was carried out in triplicate from each source point of irrigation of a particular vegetable. No additives to preserve the water were added to this water and upon arrival to the laboratory; it was subjected to solid phase extraction (Santos *et al.,* 2005).

3.4.3 Soil samples for metal analysis

Soil surface samples were collected using an augur to the depth of 9 1nches in triplicate from each of the four study locations named and described above. The sampling took place at the location where the vegetable of interest was growing. The sampling was repeated thrice. The soils collected from the irrigated plots where the vegetables were grown were then analyzed for heavy metal accumulation.

3.4.4 Vegetable samples for metal analysis

The vegetables were collected in triplicate from each of the four study sites. The vegetables collected were kales *(Brassica oleraceae),* spinach *(Spinacia oleraceae),* cow peas *(Vigna unguiculata),* pumpkin *(Cucubita pepo)* leaves. The fresh vegetables were collected in the morning between 8 and 9 am and packed in well labeled plastic paper bags. They were transported to the laboratory within 1 hour after collection.

Once in the lab, the vegetable and soil samples were spread on labeled aluminum foil and dried in the hot air oven at 70 °C for 72 hours. They were then labeled and stored awaiting digestion.

3.4.5 Vegetable samples for antibiotic analysis

The vegetables were collected in triplicate from each of the four study sites. The vegetables collected were kales *(Brassica oleraceae),* spinach *(Spinacia oleraceae),* cow peas *(Vigna unguiculata),* pumpkin *(Cucubita pepo)* leaves. The fresh vegetables were collected in the morning between 8 and 9 am and packed in well labeled plastic paper bags. They were transported to the laboratory within 1 hour after collection. They were then put through solvent extraction.

3.5 Sample preparation for AAS analysis

3.5.1 Wet digestion of vegetables for AAS analysis

This was digested using the procedure recommended by Gupta *et al.,* (2008) as described. An acid mixture of two parts concentrated nitric acid and one part perchloric acid (2:1) was prepared. One gram (1.0 g) dried vegetable material was weighed and put in a 100 ml digestion tube. 5 ml of the acid mixture was added and the sample was placed on a hot plate. The sample was heated at 60 °C for 15 minutes. The heat was then increased to 120 °C and digested for 75 minutes or until the sample cleared. The tube was removed from the hot plate when the sample was clear.

The sample was then cooled and sufficient distilled water added to bring the solution to 100 ml and preserved in the cold room at 4 °C awaiting analysis by AAS -6200 Atomic Absorption Flame Emission Spectrophotometer (Shimadzu). A blank comprising of the reagents in the proportions as for the samples but containing no sample material was prepared likewise.

3.5.2 Wet digestion of soil sample for AAS analysis

The soil sample was dried in the oven at 70 °C for 72 hours and ground into fine powder. One gram 1.0 g of each sample was weighed into a 100 ml beaker. This was digested using the procedure recommended by Gupta and colleagues (2008) which involves addition of 5 ml of an acid mixture of concentrated nitric acid, sulphuric acid and perchloric acid, ratio 6:3:1 was then added. This was shaken well and allowed to stand for 5 minutes. The sample was then digested on a hot plate starting at 70 °C through to 120 °C until the volume reduced to approximately 1 ml and production of floating suspended white fumes of SO_3 was clearly observed. This was allowed to cool to room temperature and 20 ml of 5% HCl acid solution added. This was then heated on a hot plate at about 75°C for 15 minutes and then allowed to cool. The sample was then filtered through whatman number 42 filter paper into a 100 ml volumetric flask and topped up to volume with 5% HCl and preserved in the cold room at 4 °C awaiting analysis by AAS -6200 Atomic Absorption Flame Emission Spectrophotometer (Shimadzu). A blank comprising of the reagents in the proportions as for the samples but containing no sample material was prepared likewise.

3.5.3 **Digestion of wastewater samples for AAS analysis**

This was digested using the procedure recommended by Gupta and colleagues (2008) which involves transferring 100 ml of water into a beaker and 5 ml concentrated HNO_3 was added. The beaker with the content was placed on a hot plate and evaporated down to about 20 ml. The beaker was cooled and another 5ml concentrated HNO_3 was also added. The beaker was covered with watch glass and returned to the hot plate. The heating was continued, and then small portion of HNO_3 was added until the solution appeared light coloured and clear. The beaker wall and watch glass were washed with distilled water and the sample was filtered to remove any insoluble materials that could clog the atomizer. The volume was adjusted to 100 ml with distilled water (Ademoroti, 1996) and preserved in the cold room at 4 °C awaiting analysis by AAS. Determination of heavy metals in the wastewater samples was done using AAS-6200 Atomic Absorption Flame Emission Spectrophotometer (Shimadzu).

3.6 Analytical conditions for AAS sample analysis

Table 3.1 shows the analytical conditions as specified in the equipment model. These were the optimal conditions under which the AAS analysis using the Atomic Absorption Flame Emission Spectrophotometer (AAS-6200, Shimadzu) was carried out to determine the concentration of selected heavy metals in the soil, untreated waste water and vegetables from farms irrigated with untreated wastewater as well as from a control plot irrigated with potable water.

Table 3.1: The AAS instrumental parameters

	The operating conditions used for the AAS instrument (Air/acetylene flame)					
	Mn	**Zn**	**Pb**	**Fe**	**Cu**	**Cd**
Wavelength (nm)	279.4	213.9	283.3	248.3	324.2	228.8
Slit width (nm)	0.4	1.0	0.4	0.2	0.2	0.4
Fuel flow	2.0	2.0	1.8	2.2	1.8	2.2
Oxidant flow	8	8	8	8	8	8

Mn – manganese, Zn – zinc, Pb – lead, Fe – iron, Cu – copper, Cd – cadmium.
Fuel and oxidant flow are in (L/min)

3.7 Method validation

Stock standard solutions from Sigma Aldrich at a concentration of 1000 µg/ml were used. Working solutions were made by appropriate dilution of the standard stock solutions. The method of validation was done using recovery analysis of the elements as well as the analysis of standard solutions before and after spiking.

3.7.1 Recoveries

The recovery was determined by spiking a sample whose concentration of a heavy metal of interest was known with an analyte material of known concentration and putting the mixture through the AAS analysis to determine the concentration. This evaluates the presence of background hindrances in detection relative to the solvent medium and other experimental conditions which may lower the sensitivity of the analytical procedure chosen.

3.7.2 Determination of the minimum detection limits

This was tested by gradually lowering the concentration of the standard material by diluting with double distilled de-ionized water and determining the concentration of the heavy metals in the standard up to a point where the levels could not be further detected. The lowest concentration that the equipment was able to read was the minimum detection limit.

3.8 Analysis of antibiotic residues

Vegetables and untreated waste water were collected from the farms irrigated with untreated waste water for three days during the dry season in January and it was repeated two more times during the dry season in January in two consecutive years. They were immediately delivered to the laboratory for processing as described in the following subsections.

3.8.1 Solvent extraction of vegetable material

For solvent extraction, initial methanol extraction was used (Alanis *et al.,* 2005). Five grams of powdered plant samples was covered with 100 ml methanol in a flask and allowed to stand for 48-72 hours. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator at 60 °C until methanol free solid powder was obtained. The resulting extracts were then subsequently labeled as methanol extracts and preserved at 5 °C in airtight bottles until further use.

3.8.2 Untreated wastewater sample preparation

Prior to extraction, 500 ml of wastewater was filtered through Whatman filter paper No. 1 to remove suspended matter (Santos *et al.,* 2005). The solid phase extraction (SPE) cartridges were conditioned using 3 ml ethyl acetate, 3 ml methanol and 3 ml de-ionized water at a flow rate of about 3 ml/min. Water samples were transferred to the SPE cartridges through a Teflon tube at a flow rate of 15 ml/min using a vacuum pump. The loaded cartridges were rinsed with 3 ml of methanol/water (5:95) and 3 ml n-hexane at a flow rate of about 1 ml/min. The combined aliquots were evaporated to dryness in a gentle stream of nitrogen. The residues were then dissolved in 0.5 ml of methanol and injected into C18 HPLC system. The elution gradient for SMX and TMP in a reverse phase C18 HPLC column was as follows and the wavelengths of detection used in the UV range are SMX 260 nm and TMP 230 nm. Mobile phase of water and acetronile was adjusted to pH 3.3 by addition of formic acid at a flow rate of 0.75 ml/min.

Table 3.2: The gradient of mobile phase

Time	% Water	% Acetonitrile
0	65	35
2	15	85
5	15	85
7	65	35
17	65	35

3.8.3 Method validation

The method of validation was also done using recovery analysis of the sample before spiking to know the concentration of the analyte of interest, then spiking the sample with a standard containing known concentration, and determining the concentration of the spiked material that was detected relative to that which was introduced in the sample.

3.8.4 Recoveries

The recovery was determined by spiking a sample whose concentration of antibiotic of interest was known with an analyte standard material putting the mixture through the HPLC analysis to determine the concentration. This evaluates the presence of background hindrances in detection relative to the solvent medium and other experimental conditions which may lower the sensitivity of the analytical procedure chosen.

3.9 Data management and analysis

Data was stored in both hard copy and electronically. MS excel was used as the data base. Table statistics was used to present data on mineral composition. Differences in mean concentrations of heavy metals were determined using ANOVA for analysis of more than 2 means. Within sample species and between sample species heavy metal differences were determined using ANOVA. Genstat was used as the analysis software. The means were deemed significantly different when $P \leq 0.05$ and insignificant when $P > 0.05$ at 95% confidence interval. The Pearson's correlation test was used to test the strength of association between the parameters analyzed.

CHAPTER FOUR

4.0 RESULTS

4.1 Validation analysis

4.1.1 Atomic absorption spectroscopy

The percentage recoveries of various analytes are shown in Table 4.1. Copper reported the highest recovery as compared to the other elements whereas the percentage recovery for Cd was the lowest among them.

Table 4.1: Percentage recoveries

Parameter	USR (mg/kg)	SSR (mg/kg)	% Recovery
Manganese	63.01	120.58	91.4
Zinc	3.75	6.98	86.1
Lead	0.18	0.33	83.4
Iron	83.24	157.04	88.7
Copper	9.29	18.87	103.2
Cadmium	0.61	1.11	81.6

USR – unspiked sample result, SSR – spiked sample result

As can be seen from Table 4.1, percentage recoveries ranged from 81.6-103.2 %. Atomic absorption spectroscopy gave results that were reliable since the recoveries fell between the acceptable recovery ranges of between 80 – 105% (Singh and Taneja, 2010).

4.1.2 Results for standard soil sample

The results of the analysis of the standard sample of soil are presented in Table 4.2.

Table 4.2: Concentration of trace elements in standard sample

Parameter	Standard's value (mg/kg)	concentration obtained (mg/kg)	t-calculated	P-value
Manganese	316.15	282.09±15.12	1.56	0.26
Zinc	101.21	97.06±7.21	1.31	0.32
Lead	3.52	3.33±0.50	1.86	0.59
Iron	136.11	134.54±6.33	0.20	0.86
Copper	30.80	29.12±1.21	0.14	0.90
Cadmium	0.78	0.71±0.01	2.30	0.15

Values are given as means of replicates ± SD n = 3 t-critical = 1.8595

From the results, there was no significant difference between the standard soil material and the values obtained using the present method. This clearly implies that the procedure and the AAS instrument used in this study were reliable thus the results also are reliable.

4.2 The detection limits for the heavy metals analyzed

The limits of detection were established in order to know the concentration below which the equipment could not detect. Table 4.3 shows the limit of detection (practical) which was calculated as three times the standard deviation (3σ) of the blank signal (Jianrong and Khay, 2001).

Table 4.3: The limit of detection for the heavy metals analyzed

	Concentration (mg/kg)	
Metal	**Practical**	**Theoretical**
Cadmium	0.002	0.002
Copper	0.003	0.003
Iron	0.008	0.006
Manganese	0.01	0.02
Lead	0.02	0.01
Zinc	0.005	0.001

Ref from Thermoelemental (2001)

4.3 Percentage recoveries of the antibiotics

The detection of the percentage recoveries of the antibiotic reference samples are shown in Table 4.4. The percentage recoveries ranged from 83.33–100.00 %. The method of analysis chosen for determination of the concentration of antibiotics was acceptably effective because the percentage proportions of antibiotics recovered were within the statistically acceptable limits (Table 4.4).

Table 4.4: Percentage recoveries of standard material from biological reference preparations (BRP) under British Pharmacopoeia

Parameter	USR (ppb)	SSR (ppb)	% Recovery
Trimethoprim	0.06	0.11	83.33
Sulfamethoxazole	0.06	0.12	100.00

USR – unspiked sample result, SSR – spiked sample result

The method for analysis was reliable since the percentage recovery results fall between the acceptable recovery ranges of between 80–105% reported by Singh and Taneja (2010).

4.4 Limits of detection

Table 4.5 shows the limit of detection (practical) which was calculated as three times the standard deviation (3σ) of the blank signal (Jianrong and Khay, 2001). The reference drugs were from Biological Reference Preparations (BRP) under British Pharmacopoeia.

Table 4.5: Limits of detection

Drug	**Concentration (ng) (practical)**
Sulfamethoxale[x]	0.02
Trimethoprim[xx]	0.02

[x] Drug code BP 314; [xx] Drug code BP 344

4.5 The concentration of heavy metals in untreated waste water

Concentrations of heavy metals found in wastewater used for irrigation are summarized in Table 4. 6.

Table 4.6: Heavy metal concentrations in water used for irrigation (mg/l) and allowed levels

	The concentration of heavy metal in water used for irrigation (mg/l) n=15 , (ẍ ±SD)					
	Manganese	**Zinc**	**Lead**	**Iron**	**Copper**	**Cadmium**
Upper Njiru	3.54 ± 0.40^{n}	0.03 ± 0.01^{g}	0.28 ± 0.08^{i}	8.07 ± 1.14^{j}	0.22 ± 0.10^{l}	0.47 ± 0.06^{m}
Lower Njiru	3.31 ± 0.39^{p}	0.02 ± 0.01^{g}	0.27 ± 0.06^{i}	6.92 ± 1.67^{j}	0.19 ± 0.02^{l}	0.42 ± 0.09^{m}
Upper Ruai	3.16 ± 0.27^{p}	0.02 ± 0.01^{g}	0.21 ± 0.05^{i}	4.79 ± 1.39^{k}	0.17 ± 0.03^{l}	0.43 ± 0.04^{m}
Lower Ruai	3.09 ± 0.25^{p}	0.01 ± 0.00^{g}	0.21 ± 0.07^{i}	5.62 ± 1.54^{k}	0.18 ± 0.02^{l}	0.43 ± 0.05^{m}
Control	0.45 ± 0.02^{f}	ND^{h}	ND^{o}	2.12 ± 0.06^{n}	ND^{p}	ND^{q}
[a]FAO/[b]NAS	0.2	2	5	5	0.2	0.1
[c]NEMA	-	2	5	1	0.05	0.5
[d]Awashthi	0.1	5	0.1	-	0.05	0.1
[e]INWQS	0.20	2.0	5.0	-	0.20	0.01

Values are given as means of triplicates ± SD. SD – Standard deviation, ND – Not detected.

The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval, whereas having different letters in the same row indicates significant differences.

Source: [a]FAO guidelines for trace metals in irrigation water (2006). [b]NAS guidelines (1973). [c]NEMA guidelines for irrigation water, (NEMA, 2006). [d]Awashthi (2000). [e]Class IV Interim water quality standard for Malysia (INWQS, 1998).

From Table 4.6, it is evident that the wastewater is contaminated with Mn, Fe, and Cd and the concentration of these heavy metals vary. The concentrations (mg/L) of the heavy metals in untreated wastewater ranged from 3.09 to 3.54 mg/L for Mn, 0.01 to 0.03 mg/L for Zn, 0.21 to 0.28 mg/L for Pb, 4.79 to 8.07 mg/L for Fe, 0.17 to 0.22 mg/L for Cu and 0.42 to 0.47 mg/L for Cd. Of all the heavy metals examined, the concentration of Fe in wastewater used for irrigation in the study area was significantly higher than the control and ($P < 0.05$). According to Adhikari *et al.* (1998), iron accumulates more than any other metal ion in plants. In comparison with the standard guideline of irrigation water it was found that mean Mn, Fe and Cd concentrations of the wastewater exceeded the FAO/WHO recommended level (FAO, 2006).

From the results, it was evident that wastewater carries appreciable amounts of trace toxic metals and this concur with the reports from other researchers (Yadav *et al.,* 2002). The results obtained are also in line with Lone *et al.* (2003) who studied the effect of wastewater on vegetables.

Figure 4.1 shows the distribution of heavy metals in the various study sites arranged from upstream to downstream for each of the first four sites per cluster. The control plot was at an independent site and therefore is exempted from the effects of composition of heavy metals as the river flows downstream.

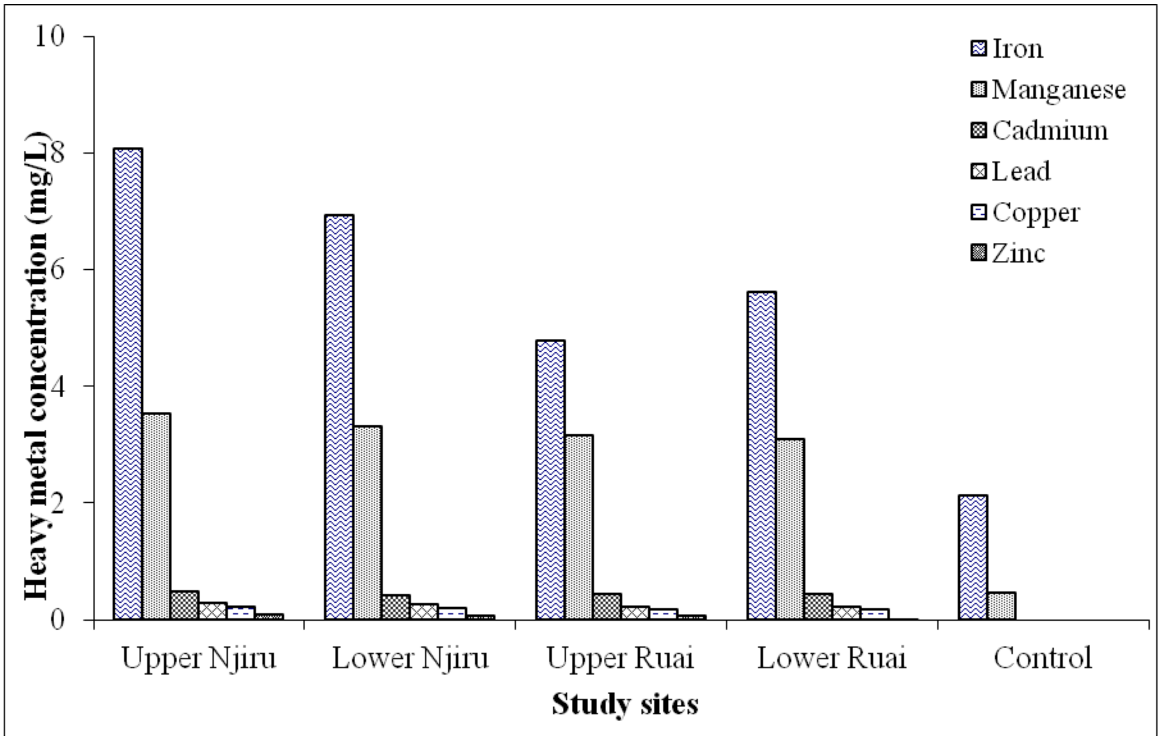


Figure 4.1: Concentration of heavy metals in untreated waste water

From the results, the heavy metal concentration decreased downstream and Upper Njiru showed a higher concentration of all the heavy metals as compared to the other plots downstream. However the results also revealed that the concentration of all the metals was significantly higher in the wastewater compared to control. The concentrations of some of the heavy metals viz; zinc, lead, copper and cadmium in control water used in this study were below the detectable limits. The control plot was at an independent location away from the River gradient terrain.

4.6 The concentration of heavy metals in the soil

The subsections that follow give the concentrations of various heavy metals in the soils picked in each farm where a specified vegetable was grown.

4.6.1 The concentration of manganese in the soil

The concentration of manganese in soil as picked from farms from where each specified vegetable was grown is shown in Table 4.7.

Table 4.7: The concentration of manganese in the soil

	Manganese contents of the soil (mg/kg, DWB) **n=15, (ẍ ±SD)**			
	Spinach farm	Pumpkin farm	Kales farm	Cow peas farm
Upper Njiru	3663.50±51.66^{a}	3710.69±46.67^{b}	3819.38±48.39^{d}	3957.00±14.84^{f}
Lower Njiru	3326.81±58.33^{a}	3415.94±65.78^{b}	3239.74±27.75^{e}	3444.02±95.15^{g}
Upper Ruai	3222.09±96.37^{a}	3304.25±47.20^{b}	3183.44±23.15^{e}	3262.76±26.47^{g}
Lower Ruai	3083.96±72.61^{a}	2940.30±71.68^{c}	2992.08±67.02^{e}	3104.82±51.09^{h}

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

Safe limit. 2000 mg/kg. Source: Ewers, 1991

There was no significant difference between the Mn contents in all the plots where the vegetables were grown. Manganese accumulation in soil ranged from 3083.96-3663.50 mg/kg for spinach plots, 2940.30-3710.69 mg/kg for pumpkin plots, 2992.08-3819.38 mg/kg for kales plots and 3104.82-3957.00 mg/kg for cow peas plots.The trends for the Mn accumulation in the different plots reduced downstream. The plots at upper Njiru reported higher accumulation of Mn and the accumulation in lower Njiru, upper Ruai, lower Ruai decreased in that order.

4.6.2 The concentration of zinc in the soil

The concentration of zinc in the soil as picked from farms where each specified vegetable was grown is shown in Table 4.8.

Table 4.8: Concentration of zinc in the soil

	Zinc contents of the soil (mg/kg, DWB) n=15 , (ẍ ±SD)			
	Spinach farm	Pumpkin farm	Kales farm	Cow peas farm
Upper Njiru	10.75±4.69^{a}	13.94±4.74^{d}	15.40±2.63^{f}	17.32±3.68^{h}
Lower Njiru	13.81±3.40^{b}	16.22±2.57^{e}	15.32±5.35^{f}	14.99±4.31^{j}
Upper Ruai	11.52±3.32^{b}	13.69±2.92^{d}	12.31±5.17^{g}	16.71±4.13^{h}
Lower Ruai	11.10±4.87^{c}	16.68±2.12^{e}	11.70±3.98^{g}	15.18±4.65^{i}

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

Safe limit.300mg/kg. Source: Ewers, 1991, 300 mg/kg. Source: European Union (2002)

In the plots where spinach was grown, the zinc concentration was lowest as compared to the zinc concentrations in the other plots. Except in Lower Njiru, for all the other plots with spinach, there was no significant difference in zinc concentration. The plots with the highest zinc concentration are those where the cow peas were grown and those with the lowest zinc concentration are where the spinach was grown. In all cases however, the zinc levels were significantly lower than the stipulated threshold values.

4.6.3 The concentration of lead in the soil

In all the sites, there was no significant difference in the lead concentration from most of the farms from which each specified vegetable was grown as shown in Table 4.9.

Table 4.9: The concentration of lead in the soil

	Lead contents of the soil (mg/kg, DWB) n=15, (ẍ ±SD)			
	Spinach farm	**Pumpkin farm**	**Kales farm**	**Cow pea farm**
Upper Njiru	70.05±5.47^{a}	73.15±4.94^{b}	72.74±3.99^{d}	75.79±9.08^{f}
Lower Njiru	72.68±4.45^{a}	67.76±9.89^{c}	70.78±8.03^{d}	76.94±4.33^{f}
Upper Ruai	73.66±5.23^{a}	70.85±7.33^{b}	74.72±4.98^{e}	73.95±8.01^{f}
Lower Ruai	70.90±9.57^{a}	68.79±6.94^{c}	73.86±3.81^{e}	68.06±7.36^{g}

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

Safe limit.100mg/kg. Source: Ewers, 1991, 300 mg/kg. Source: European Union (2002), 250-500 mg/kg. Source: Indian Standard; Awashthi (2000).

The concentration ranged from 68.06-76.94 mg/kg. The plots with cow peas had the highest lead concentration levels in the soil and the cow peas though the numerically visible difference is statistically not significant at 95% confidence.

4.6.4 The concentration of iron in the soil

Table 4.10 shows the concentration of iron in the soil as picked from farms from which each specified vegetable was grown.

Table 4.10: The concentration of iron in the soil

	Iron contents of the soil (mg/kg, DWB) n=15, (ẍ ±SD)			
	Spinach farm	**Pumpkin farm**	**Kales farm**	**Cow pea farm**
Upper Njiru	7439.40±86.72^{a}	7608.95±96.24^{b}	7284.12±83.10^{c}	7560.55±72.59^{d}
Lower Njiru	7533.35±82.57^{a}	7562.64±93.81^{b}	7496.66±98.47^{c}	7546.42±70.65^{d}
Upper Ruai	7512.05±35.49^{a}	7563.38±11.05^{b}	7434.77±18.67^{c}	7491.81±86.50^{d}
Lower Ruai	7533.40±66.23^{a}	7515.27±77.47^{b}	7487.48±15.05^{c}	7585.98±99.99^{d}

Values are given as means of triplicates ± SD. SD = Standard deviation. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

There was no significant difference in the iron concentrations in the soil irrespective of what vegetable was growing.

4.6.5 The concentration of copper in the soil

Table 4.11 shows the concentration of copper in the soil as picked from farms from which each specified vegetable was grown.

Table 4.11: The concentration of copper in the soil

	Copper contents of the soil (mg/kg, DWB) n=15 , (ẍ ±SD)			
	Spinach farm	**Pumpkin farm**	**Kales farm**	**Cow pea farm**
Upper Njiru	41.33 ± 2.12^a	40.31 ± 2.59^b	41.43 ± 3.05^c	40.97 ± 2.31^d
Lower Njiru	40.67 ± 2.49^a	40.82 ± 2.09^b	40.68 ± 2.48^c	40.10 ± 3.05^d
Upper Ruai	42.03 ± 2.06^a	39.53 ± 2.73^b	39.51 ± 1.44^c	40.70 ± 3.91^d
Lower Ruai	39.72 ± 2.54^a	41.21 ± 1.95^b	41.32 ± 2.46^c	40.01 ± 2.59^d

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

Safe limit. 100mg/kg. Source: Ewers, 1991 140 mg/kg. Source: European Union (2002), 135-270 mg/kg. Source Indian Standard; Awashthi (2000).

Copper levels were spread from 39.51- 42.03 mg/kg across the plots in the various sites. There was no significant difference in the concentration of the copper within plots irrespective of what vegetable was growing on the plot.

4.6.6 The concentration of cadmium in the soil

Table 4.12 shows the concentration of cadmium in the soil as picked from farms from which each specified vegetable was grown.

Table 4.12: The concentration of cadmium in the soil

	Cadmium contents of the soil (mg/kg, DWB) n=15 , (ẍ ±SD)			
	Spinach farm	Pumpkin farm	Kales farm	Cow pea farm
Upper Njiru	24.43±1.99^{a}	23.30±2.08^{b}	22.75±2.96^{c}	22.88±1.54^{d}
Lower Njiru	23.35±2.34^{a}	22.38±3.09^{b}	21.70±2.21^{c}	23.40±2.51^{d}
Upper Ruai	23.11±0.96^{a}	21.72±1.49^{b}	22.32±2.24^{c}	21.92±2.80^{d}
Lower Ruai	23.50±2.52^{a}	20.85±1.02^{b}	22.45±3.10^{c}	21.98±3.64^{d}

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

Safe limit. 3mg/kg. Source: Ewers, 1991, 3 mg/kg. Source: European Union, (2002), 3-6 mg/kg. Source: Indian Standard; Awashthi (2000).

Cadmium concentration in the soil was spread from 20.85-24.43 mg/kg. There was no marked variation in concentration as one varied the vegetables or the locations. There was no significant difference in concentration across the sites irrespective of what vegetable was growing.

4.6.7 The average concentration of heavy metals in the soil.

Table 4.13 shows the results for the average concentration of heavy metals in the soil from the study sites.

Table 4.13: The average concentration of heavy metals in the soil

	The concentration of heavy metals in the soil mg/kg, DWB **n=15, (ẍ ±SD)**					
	Manganese	Zinc	Lead	Iron	Copper	Cadmium
Upper Njiru	3603.26±830.74	15.05±3.99	73.29±6.41	7534.77±86.81	41.01±2.47	23.34±2.21
Lower Njiru	3481.63±786.39	14.35±4.56	72.93±7.71	7530.53±119.70	40.57±2.46	22.72±2.53
Upper Ruai	3275.70±952.43	13.67±4.54	72.16±7.74	7500.75±134.86	40.44±2.78	22.28±2.00
Lower Ruai	3194.30±848.65	13.56±4.30	70.40±7.58	7473.26±422.76	39.57±2.40	22.20±2.80
Control	964.72±85.36	4.03±0.95	20.55±1.58	1137.93±67.29	11.57±1.66	2.44±0.75
Safe limits (mg/kg)						
FAO, 2006	-	300	300	-	135	3.0
Ewers, 1991	2000	300	100	-	-	3.0
Indian Standard	75 – 150	300 - 600	250 - 500	-	135 - 270	3.0 - 6.0
EU Standards	75	300	300	-	140	3.0

Values are given as means of triplicates ± Standard deviation. DWB – Dry weight basis.

FAO Irrigation and drainage paper 47 (2006); Indian Standard (Awashthi 2000); European Union Standards (EU, 2002)

All the target heavy metals were found to be present in the soil in all the sites. The concentration of Cd and Mn were significantly higher ($P < 0.05$) than the values quoted as the threshold limits. However, the concentration ranged from 3194.30 to 3603.26 mg/kg for Mn, 13.56 to 15.05 mg/kg for Zn, 70.40 to 73.29 mg/kg for Pb, 7500.75 to 7534.77 mg/kg for Fe, 40.44 to 41.01 mg/kg for Cu and 22.20 to 23.34 mg/kg for Cd. The results on table 4.13 revealed relatively high concentrations of Mn and Fe. The iron content was significantly high as compared to the other elements ($P < 0.05$). The contents of lead did not vary significantly ($P > 0.05$) with that of cadmium. The highest mean concentration recorded was for Fe followed by Mn, Pb, Cu, Cd and the minimum concentration was observed for Zn.

Lead and Cu levels on the other hand, was not significantly different ($P > 0.05$) from the threshold levels and the concentration of Pb was below the maximum permissible limit of 0.3 mg Pb/kg. The values of iron detected in all the plots were significantly lower ($P > 0.05$) than the threshold values according to the WHO reference quoted.

The data showed that heavy metal accumulation in soils from vegetable fields irrigated with wastewater was higher than the control field irrigated with tap water. The low accumulation of heavy metals in the control soil was expected if water was contributing to the iron levels in the soil because the tap water had much lower iron concentration as compared to the untreated waste water. However, the heavy metal contents in all vegetables fields irrigated with wastewater decreased downstream.

In addition, the concentration of the control soils was within the safe limits for agricultural practices. The trend of heavy metal concentration in the four plot soils were iron>manganese>lead >copper>cadmium>zinc.

4.7 The concentration of heavy metals in the vegetables

The results that show the concentration of each heavy metal in the selected vegetables are shown in the subsections that follow.

4.7.1 The concentration of manganese in the vegetables

The average concentrations and range of manganese in the selected vegetables grown in the four plots are listed in Table 4.14.

Table 4.14: The concentration of manganese in the vegetables

	Manganese contents of the Vegetables (mg/kg DWB) n=15 , (ẍ ±SD)			
Vegetable	**Spinach**	**Pumpkin**	**Kales**	**Cow peas**
Upper Njiru	633.05 ± 66.06^{a}	159.81 ± 56.50^{c}	153.48 ± 63.97^{c}	286.33 ± 23.34^{b}
Lower Njiru	532.46 ± 91.81^{a}	142.65 ± 64.77^{c}	146.50 ± 43.97^{c}	279.97 ± 29.52^{b}
Upper Ruai	307.35 ± 64.20^{a}	131.61 ± 60.51^{c}	129.65 ± 59.61^{c}	178.37 ± 73.60^{b}
Lower Ruai	240.50 ± 60.89^{a}	110.25 ± 34.61^{c}	81.88 ± 12.02^{c}	175.81 ± 37.62^{b}
Control	51.73 ± 5.14^{a}	28.36 ± 2.54^{b}	21.02 ± 0.99^{b}	48.03 ± 1.22^{a}

Values are given as means of triplicates ± SD. Means with different letters within a row are significantly different ($P < 0.05$). SD= Standard deviation. DWB – Dry weight basis.

Permissible levels set by FAO/WHO (Codex Alimentarius Commission, 2001) (500.00 mg/kg)

From the results in Table 4.14, spinach showed significantly higher Mn concentration ($P < 0.05$) as compared to the other vegetables with values ranging from 240.50 to 633.05

mg/kg between the different plots. Cow peas on the other hand showed a significantly lower Mn levels as compared to spinach but significantly higher levels than those of kales and pumpkin ($P < 0.05$). Kales and pumpkin reported significantly lower levels of manganese than spinach and cow peas. In kales and pumpkin, Mn values ranged from 81.88 - 153.48 mg/kg and 110.25 – 159.81 mg/kg, respectively and their concentrations did not differ significantly ($P > 0.05$).

The concentration of manganese also differed between the different plots and as the farms moved from upstream to downstream (Fig 4.2). Since the sewage is deposited at the upper part of the river, at upper Njiru, the maximum Mn accumulation, 633.05 mg/kg (Spinach), 159.81 mg/kg (Pumpkin), 153.48 mg/kg (Kales) and 286.33 mg/kg (Cow peas). The concentration was followed slightly by lower Njiru, upper Ruai and the minimum accumulation, 240.50 mg/kg (Spinach), 110.25 mg/kg (Pumpkin), 81.88 mg/kg (Kales) and 175.81 mg/kg (Cow peas) was on lower Ruai site.

Figure 4.2 shows the concentration of manganese in the vegetables grown in the various study sites arranged from upstream to downstream for each of the first four sites per cluster. The control plot was at an independent site and therefore is exempted from the effects of composition of heavy metals as the river flows downstream.

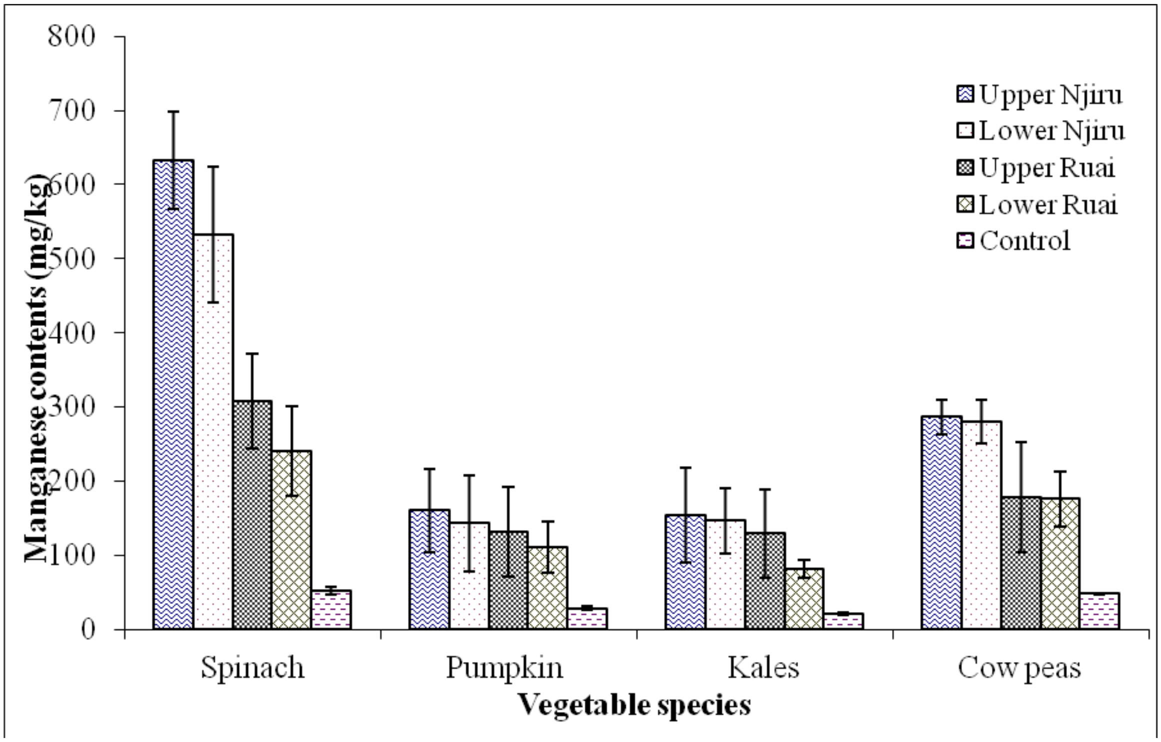


Figure 4.2: Concentration of manganese in vegetables

The Mn concentration of spinach in upper Njiru was above the permissible levels (500.00 mg/kg) set by FAO/WHO (Codex Alimentarius Commission, 2001). On the other hand the results for manganese in the other vegetables in all the plots were within the permissible levels.

The results showed that Mn level in all the vegetable samples from the study sites were significantly higher ($P<0.05$) than the control site. Therefore, the highest Mn accumulation was observed due to irrigation with untreated waste water and minimum Mn accumulation was in the control plot irrigated with tap water.

4.7.2 The concentration of zinc in the vegetables

The concentration of zinc in the vegetables is shown in Table 4.15.

Table 4.15: The concentration of zinc in the vegetables

	Zinc contents of the Vegetables (mg/kg, DWB) n=15 , (ẍ ±SD)			
Vegetables	Spinach	Pumpkin	Kales	Cow peas
Upper Njiru	8.70±3.17^a	4.38±1.80^b	6.72±3.08^a	7.29±2.45^a
Lower Njiru	8.46±1.20^a	3.80±0.85^b	6.09±1.12^a	7.03±3.14^a
Upper Ruai	7.77±3.02^a	3.57±0.63^b	5.79±1.65^a	7.00±2.34^a
Lower Ruai	6.90±2.50^a	3.19±0.27^b	5.70±1.10^a	6.80±2.34^a
Control	2.65±0.12^c	1.25±0.05^c	2.03±0.25^c	2.34±0.42^c

Values are given as means of triplicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD= Standard deviation.

Safe limit = <100 mg/kg {Source: FAO/WHO (Codex Alimentarius Commission, 2001)}, 50 mg/kg. Source: Indian Standard: Awashthi (2000), 60 mg/kg. Source: WHO/FAO (2007).

Of all the vegetables, pumpkin reported a significantly lower ($P > 0.05$) zinc contents between 3.19-3.57 mg/kg with no significant difference ($P > 0.05$) between the plots. Between the four plots, the concentration of Zn in all the vegetables did not differ significantly although the upper Njiru and lower Njiru plots reported higher concentration than plots at Ruai. The control vegetables reported significantly low Zn levels as compared to the vegetables irrigated with untreated wastewater.

From the results, the highest zinc concentration was found in spinach followed by cow peas, kales and pumpkin. Although the reported zinc concentration in spinach was higher, there was no significant difference with that of cow peas and kales ($P > 0.05$).

Figure 4.3 shows the concentration of zinc in the vegetables in the various study sites arranged from upstream to downstream for each of the first four sites per cluster. The control plot was at an independent site and therefore is exempted from the effects of composition of heavy metals as the river flows downstream.

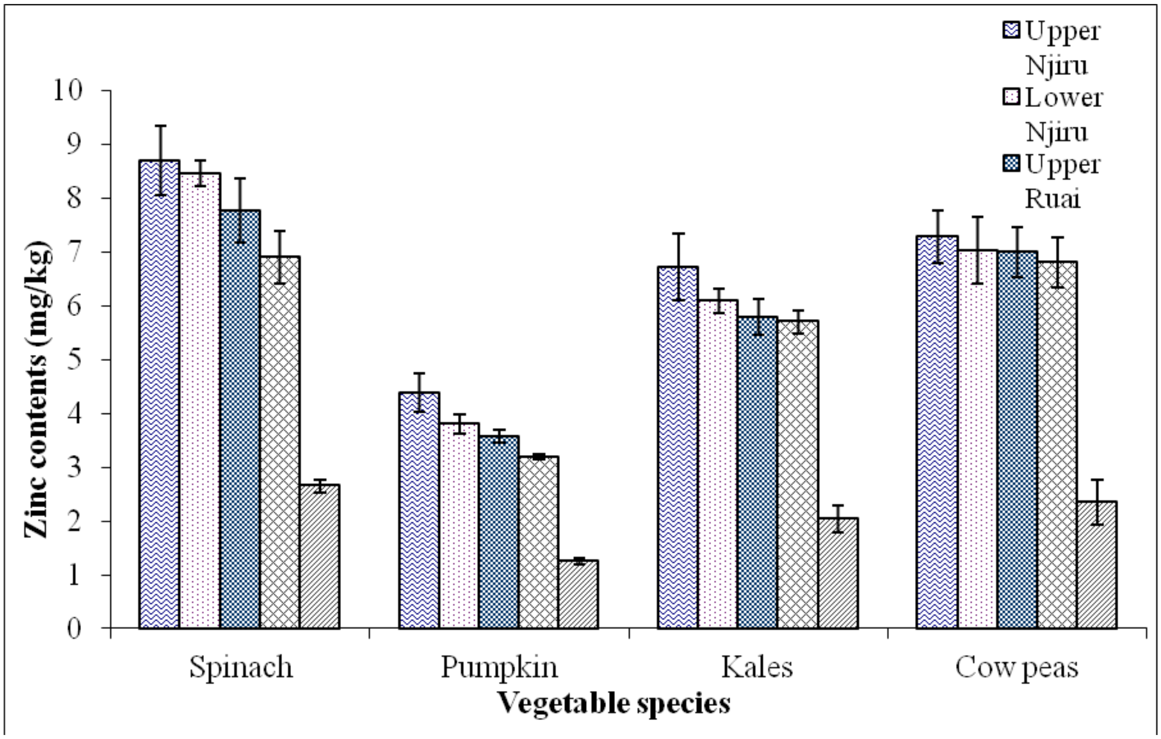


Figure 4.3: The concentration of zinc in the vegetables

The concentration of zinc in the vegetables had a pattern of decreasing downstream as illustrated in figure 4.3. The upper parts of the river with the untreated wastewater used for irrigation had a higher concentration of heavy metals as compared to the lower parts and this was transferred to the vegetables in a similar pattern.

4.7.3 The concentration of lead in the vegetables

Table 4.16 shows the mean levels of lead in the vegetables from different plots.

Table 4.16: The concentration of lead in the vegetables

	Lead contents of the vegetables (mg/kg, DWB) n=15, (ẍ ±SD)			
	Spinach	**Pumpkin**	**Kales**	**Cow peas**
Upper Njiru	2.79±0.38^{b}	4.04±0.46^{a}	2.60±0.41^{b}	4.46±0.68^{a}
Lower Njiru	2.58±0.57^{b}	3.92±0.43^{a}	2.55±0.43^{b}	4.43±0.97^{a}
Upper Ruai	2.42±0.40^{b}	3.86±0.69^{a}	2.49±0.51^{b}	4.20±0.84^{a}
Lower Ruai	2.19±0.48^{b}	3.77±0.69^{a}	2.37±0.52^{b}	4.14±0.64^{a}
Control	0.11±0.01^{b}	0.18±0.02^{a}	0.07±0.00^{b}	0.16±0.04^{a}

Values are given as means of triplicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD= Standard deviation.

Safe limit = <0.3 mg/kg {Source: FAO/WHO (Codex Alimentarius Commission, 2001)}, 2.5 mg/kg: Indian standard; Awashthi (2000), 5.0 mg/kg, Source: WHO/FAO (2007), 0.30 mg/kg. Source: European Union (2006).

From the results in Table 4.16, pumpkin and cow peas had a significantly higher concentration of lead as compared to spinach and kales which had significantly lower values. Lead concentrations in the vegetables did not differ significantly ($P > 0.05$) between the four plots. The highest Pb concentration was found in cow peas (4.46 mg/kg) followed by pumpkin (4.04 mg/kg) from the upper Njiru.

From all the farms, the vegetables had a high concentration of lead as compared to the recommended threshold levels acceptable in the vegetables. Comparison of the mean concentrations of Pb with the safe limits showed that the concentrations for pumpkin and cow peas were above the recommended limits in all the examined farms. Concentration of Pb in all the vegetables grown in untreated waste water irrigated land was significantly higher than Pb concentrations in vegetables grown in the control plot whose concentrations ranged between 0.71 and 1.84 mg/kg (Table 4.16).

Figure 4.4 illustrates the lead concentration in the vegetables in the various study sites arranged from upstream to downstream for each of the first four sites per cluster. The control plot was at an independent site and therefore is exempted from the effects of composition of heavy metals as the river flows downstream.

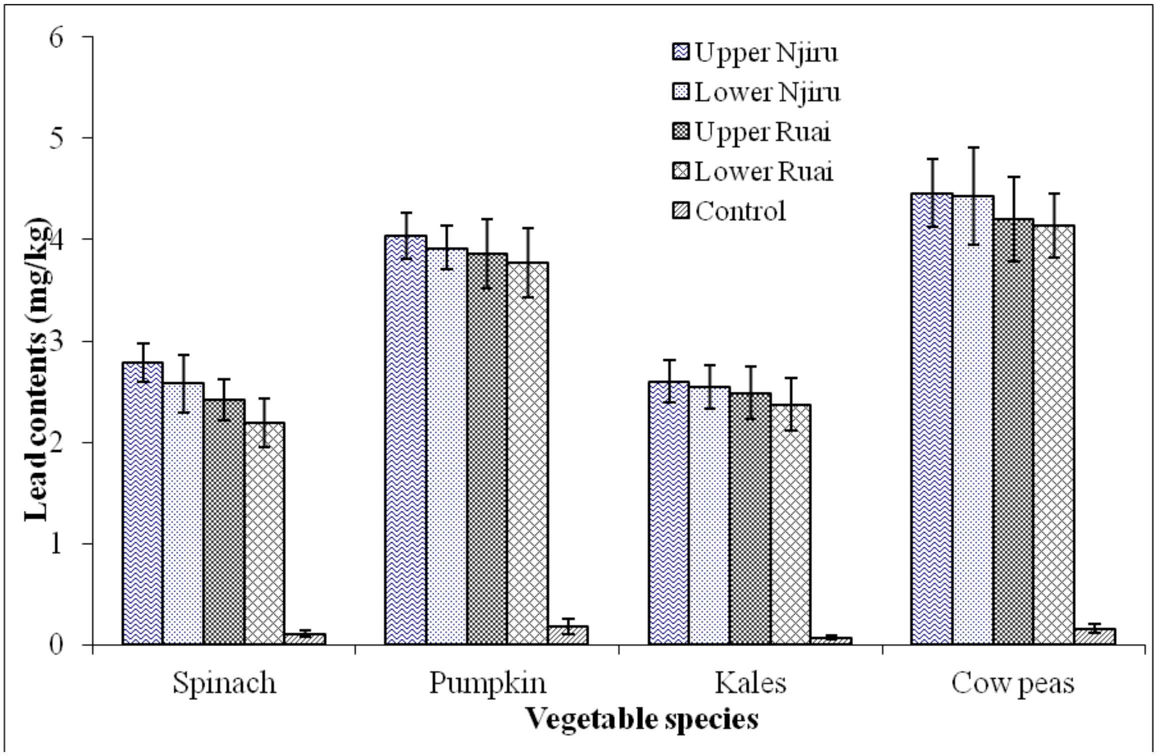


Figure 4.4: Lead concentrations in the vegetables

The concentration of lead was higher in the vegetables grown upstream as compared to the vegetables grown downstream as shown in Figure 4.4. This is accounted for by the adsorption of the heavy metal ions to the soil as the untreated wastewater moves down stream.

The pumpkin leaves and the cow peas leaves had significantly high concentration of lead when compared to kales and spinach ($P<0.05$). There was no significant difference in the concentration of lead in the spinach and kales. In all cases, the control plot had very little lead detected, but in all vegetables, it was detected.

4.7.4 The concentration of iron in the vegetables

Table 4.17 shows the mean concentration of iron in the various vegetables.

Table 4.17: The concentration of iron in the vegetables

	Iron contents of the vegetables (mg/kg, DWB) n= 15, (ẍ ±SD)			
	Spinach	**Pumpkin**	**Kales**	**Cow peas**
Upper Njiru	1187.84 ± 205.18^{a}	1215.49 ± 396.05^{a}	555.55 ± 44.69^{b}	1019.63 ± 71.96^{a}
Lower Njiru	1109.42 ± 130.07^{a}	1126.64 ± 342.67^{a}	509.25 ± 87.57^{b}	1006.33 ± 71.66^{a}
Upper Ruai	1039.22 ± 278.33^{a}	1105.81 ± 185.37^{a}	484.69 ± 24.02^{b}	962.89 ± 92.78^{a}
Lower Ruai	989.36 ± 199.17^{a}	1080.16 ± 323.30^{a}	481.24 ± 55.07^{b}	867.29 ± 104.50^{a}
Control	262.99 ± 14.20^{a}	277.34 ± 33.48^{a}	69.23 ± 15.23^{b}	221.35 ± 11.84^{a}

Values are given as means of replicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD= Standard deviation.

Permissible levels set by FAO/WHO (Codex Alimentarius Commission, 2001) (425.50 mg/kg)

The mean concentrations of Fe in vegetables in the present study areas varied between 1019.36-1187.84 mg/kg, 1080.16-1215.49 mg/kg, 481.24-555.55 mg/kg and 867.29-1019.63 mg/kg in spinach, pumpkin, kales and cow peas, respectively (Table 4.17).

Although the contents in spinach, pumpkin and cow peas were significantly higher, there was no significant difference between them (*P > 0.05*). Kales on the other hand reported significantly lower levels of iron.

Figure 4.5 illustrates the concentration of iron in the various study sites arranged from upstream to downstream for each of the first four sites per cluster. The control plot was at an independent site and therefore is exempted from the effects of composition of heavy metals as the river flows downstream.

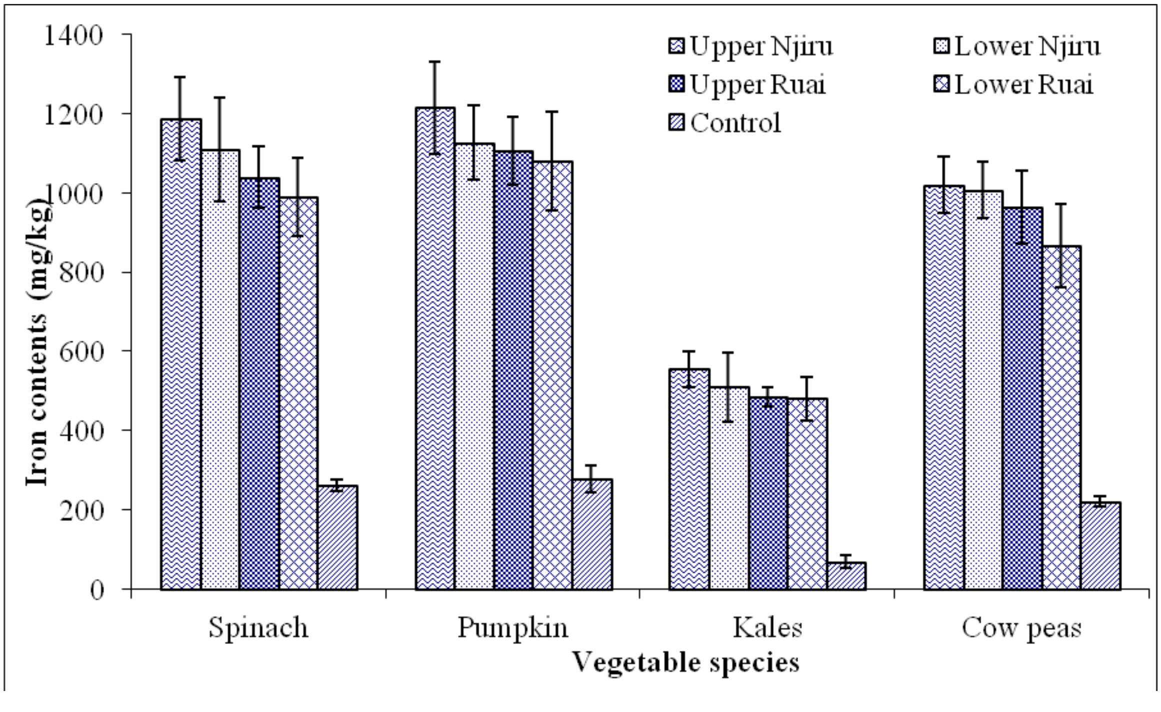


Figure 4.5: Iron concentrations in the vegetables

Following the criteria proposed by FAO/WHO (Codex Alimentarius Commission, 2001) the mean values of Fe exceeded the set permissible levels (425.50 mg/kg). Besides, as expected, the concentration of iron for the control vegetables grown at Kenyatta University was significantly lower (P < 0.05) compared to the wastewater irrigated vegetables.

4.7.5 The concentration of copper in the vegetables

The results for copper concentration in the vegetables are shown in Table 4.18.

Table 4.18: The concentration of copper in the vegetables

	Copper contents of the vegetables (mg/kg, DWB) n=15, (ẍ ±SD)			
	Spinach	**Pumpkin**	**Kales**	**Cow peas**
Upper Njiru	24.79±3.27	25.37±4.40	28.63±5.09	29.70±3.44
Lower Njiru	24.36±3.66	24.47±3.27	27.16±3.69	27.10±3.33
Upper Ruai	23.42±1.21	23.60±2.62	25.39±3.97	27.20±2.33
Lower Ruai	22.54±2.19	21.17±6.70	26.38±4.03	26.36±2.24
Control	8.51±1.28	11.25±0.92	4.20±0.51	5.59±0.87

Values are given as means of replicates ± SD.

Safe limit <73.3 mg/kg. {Source: FAO/WHO (Codex Alimentarius Commission, 2001)}, 30.0 mg/l Source: Indian Standard; Awashthi, 40.0 mg/kg Source: WHO/FAO (2006).

Although the concentration of Cu in cow peas was high as compared to the three vegetables, the difference was not significant (*P > 0.05*). As well, there was a slight change in the copper concentrations from the upper Njiru to lower Ruai. This change did not show any significant difference between the levels of Cu in all the plots. In addition, the Cu levels for all the vegetables from the different plots fall within the safe limit as the contents were lower than the FAO/WHO guidelines (73.3 mg/kg) for maximum limits of Cu in vegetables (Codex Alimentarius Commission, 2001). The control vegetables also reported significantly lower Cu contents. From the results, it might be concluded that all the vegetables can accumulate almost the same amount of copper.

4.7.6 The concentration of cadmium in the vegetables

Table 4.19 shows the concentration of cadmium in the vegetables (mg/kg, dry weight basis).

Table 4.19: The concentration of cadmium in the vegetables

	Cadmium contents of the vegetables (mg/kg, DWB) n=15, (ẍ ±SD)			
	Spinach	**Pumpkin**	**Kales**	**Cow peas**
Upper Njiru	5.58 ± 0.19^{a}	3.68 ± 0.45^{b}	3.55 ± 0.31^{b}	3.60 ± 0.32^{b}
Lower Njiru	4.52 ± 0.25^{a}	2.65 ± 0.22^{b}	2.31 ± 0.29^{b}	2.55 ± 0.28^{b}
Upper Ruai	4.47 ± 0.16^{a}	2.49 ± 0.18^{b}	2.27 ± 0.72^{b}	2.53 ± 0.28^{b}
Lower Ruai	4.25 ± 0.87^{a}	2.47 ± 0.25^{b}	1.94 ± 0.46^{b}	2.41 ± 0.38^{b}
Control	0.01±0.00	ND	ND	ND

Values are given as means of replicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD = Standard deviation.

Safe limit = <0.1 mg/kg {Source: FAO/WHO (Codex Alimentarius Commission, 2001)}, 1.5 mg/kg Source: Indian Standards; Awashthi (2000), 0.2 mg/kg Source: WHO/FAO (2007), 0.2 Source: European Union (2006).

From the results, cadmium was significantly higher in spinach ($P < 0.05$) as compared to the other three vegetables; pumpkin, kales and cow peas. In addition, there was no significant difference between levels of cadmium in pumpkin, kales and cow peas ($P >$

0.05). For all the vegetables, the concentration of Cd at upper Njiru was significantly higher than the other plots, but the concentration decreased downstream.

The concentration of Cd in the present study was high in all the vegetables (1.94-4.58 mg/kg) for the waste water irrigated vegetables and this exceeded the safe limits. The Cd values also exceeded the <0.1 mg/kg permissible limits as set by FAO/WHO (Codex Alimentarius Commission, 2001). This therefore may pose serious health hazards to the consumers of the vegetables. The control vegetables on the other hand reported significantly lower Cd levels and these were within the safe limits of the standards from various international bodies. Since spinach reported a significantly higher concentration of cadmium, it is an indication that spinach has high affinity of accumulation of cadmium in their leaves. Like the other elements the level of Cd gradually decreased downstream.

4.8 Correlation analysis of heavy metals

A Pearson's correlation analysis was done on the effect of the concentration of heavy metals in untreated waste water had on the soil and the vegetables. Likewise, the correlation test was carried out to determine if the various concentrations of heavy metals in the soil had an impact on the vegetables. In all cases, they had a positive correlation, the results of which are shown in Table 4.20.

Table 4.20: Pearson's correlation of heavy metals in untreated wastewater, soil and vegetables

	The r values of heavy metals in untreated wastewater, soil and vegetables								
	Water					**Soil**			
	Soil	Spinach	Pumpkin	Kales	Cow peas	Spinach	Pumpkin	Kales	Cow peas
Manganese	0.39	0.51	0.47	0.21	0.58	0.42	0.48	0.12	0.31
Zinc	0.35	0.61	0.35	0.19	0.47	0.58	0.27	0.09	0.29
Lead	0.37	0.79	0.67	0.27	0.38	0.63	0.49	0.19	0.29
Iron	0.34	0.42	0.69	0.28	0.58	0.38	0.49	0.09	0.49
Copper	0.38	0.59	0.28	0.19	0.69	0.49	0.37	0.19	0.34
Cadmium	0.29	0.58	0.76	0.18	0.56	0.48	0.35	0.08	0.25

The concentration of heavy metals in the untreated wastewater had a positive correlation on the concentrations of heavy metals that were found in the soil and the vegetables. As the heavy metals increased in the water, so did the levels rise in the vegetables. The soil too had a positive correlation with the vegetables, as it went up, so did the levels of the heavy metals go up (Table 4.20).

4.9 Concentration of antibiotics in untreated wastewater

The results for the concentration of sulfamethoxazole and trimethoprim in water samples are shown in Table 4.20.

Table 4.21: The concentration of sulfamethoxazole and trimethoprim in water

	Concentration of the antibiotics in untreated wastewater (ng/litre)	
	Sulfamethoxazole	**Trimethoprim**
Upper Njiru	88.66±1.23^{a}	27.52±1.56^{a}
Lower Njiru	87.21±4.32^{a}	25.66±0.89^{a}
Upper Ruai	79.15±2.81^{b}	24.99±1.11^{b}
Lower Ruai	62.09±1.77^{c}	24.71±0.62^{b}
Control	ND^{d}	ND^{c}

Values are given as means of triplicates ± SD. Means with different small letters within a column are significantly different ($P < 0.05$). SD = Standard Deviation. ND = Not detected, n=15**,** ($\ddot{x}$ ±SD)

From the results, upper Njiru was found to contain significantly higher concentrations of Sulfamethoxazole and Trimethoprim as compared to the other sites ($P < 0.05$). The concentration decreased down the stream. As shown in Table 4. 20, the concentrations of the two antibiotics in different sites in general followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai.

Figure 4.6 shows the concentration of SMX and TMP in the untreated wastewater in the various study sites arranged from upstream to downstream.

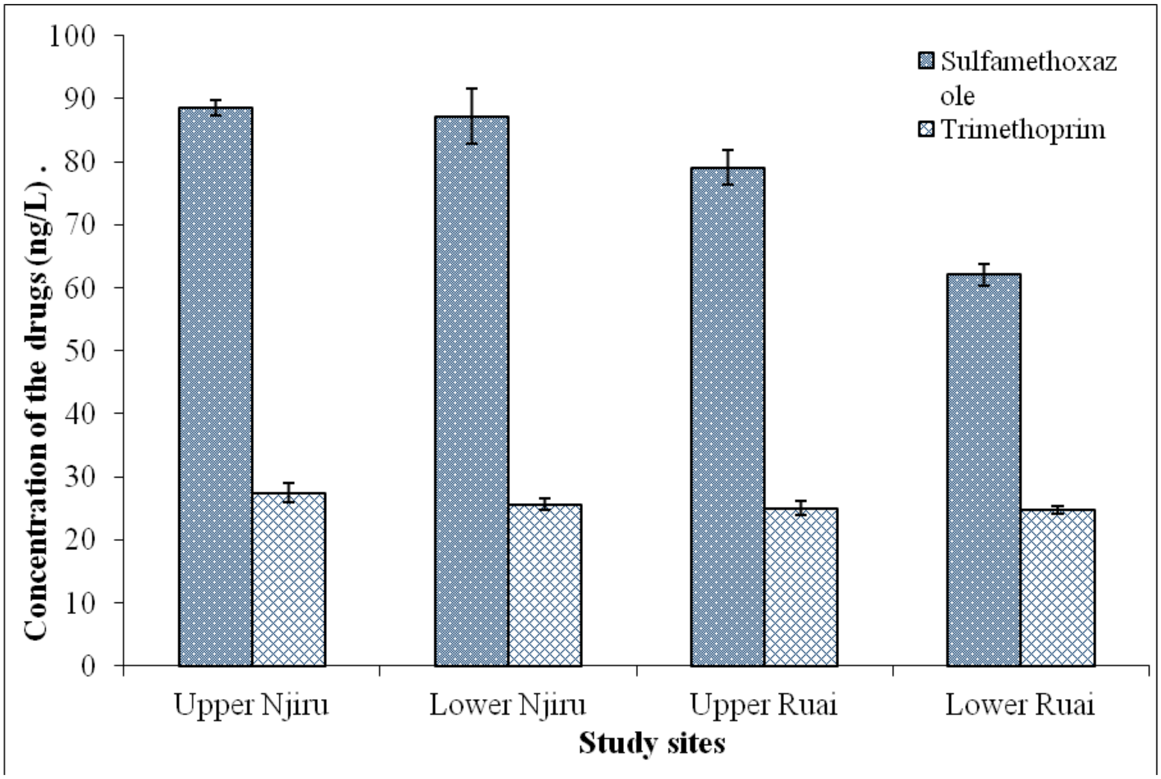


Figure 4.6: Concentration of antibiotics in the wastewater

The concentration of TMP was fairly constant across the various study sites but the concentration of SMX decreased considerably as the untreated wastewater moved downstream.

4.10 Concentration of antibiotics in vegetables

The subsections that follow show the concentrations of various antibiotics in the vegetables.

4.10.1 Concentration of trimethoprim in the vegetables

The concentration of TMP in the vegetables is reported in Table 4.22.

Table 4.22: The concentration of trimethoprim in vegetables

	Concentration of trimethoprim in vegetables (ng/kg)			
	Cow pea	**Kales**	**Pumpkin**	**Spinach**
Upper Njiru	12.12 ± 0.93^{b}	4.15 ± 0.05^{c}	15.45 ± 0.10^{a}	14.97 ± 0.58^{a}
Lower Njiru	11.03 ± 0.85^{a}	3.27 ± 0.01^{b}	12.62 ± 0.86^{a}	10.21 ± 1.20^{a}
Upper Ruai	8.99 ± 1.01^{a}	2.96 ± 0.02^{b}	10.73 ± 0.21^{a}	10.64 ± 1.44^{a}
Lower Ruai	4.78 ± 0.03^{b}	2.16 ± 0.05^{c}	7.17 ± 0.32^{a}	7.54 ± 0.84^{a}
Control	ND	ND	ND	ND

Values are given as means of triplicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD = Standard Deviation, n=15 **, ($\ddot{x}$ ±SD)**

The levels were found to be in the range of 4.78 - 12.12 ng/kg, 2.16 - 4.15 ng/kg, 7.17 - 15.45 ng/kg and 7.54 - 14.97 ng/kg for Cow pea, Kales, Pumpkin and Spinach, respectively. As it was for the water samples, the levels of trimethoprim were significantly higher in the upper Njiru as compared to the other sites. The direct discharge of untreated wastewater was responsible for the highest levels of the trimethoprim in upper Njiru. The level of trimethoprim was also significantly higher in pumpkin and spinach and significantly lower in kales ($P < 0.05$).

Figure 4.7 shows the concentration of TMP in the vegetables in the various study sites arranged from upstream to downstream for each of the four sites per cluster.

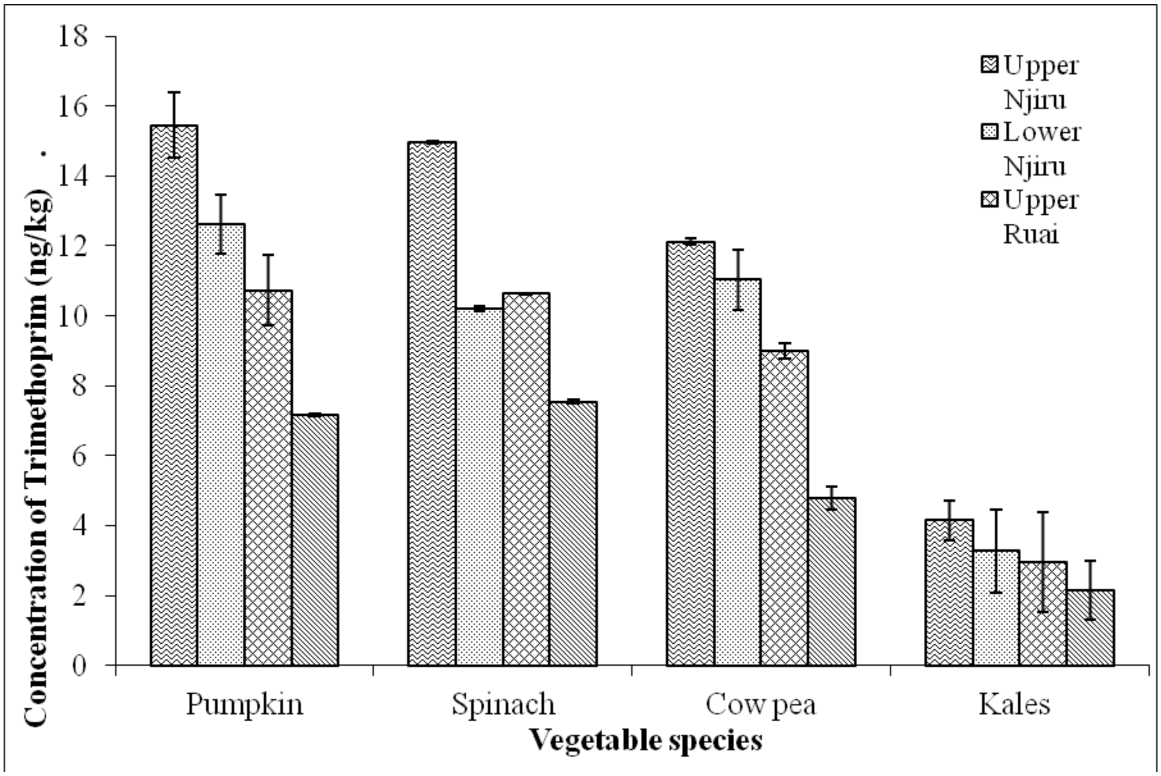


Figure 4.7: The concentration of TMP in the vegetables

4.10.2 The concentration of sulfamethoxazole in the vegetables

The mean concentration of SMX in the vegetables is reported in Table 4.23.

Table 4.23: The concentration of sulfamethoxazole in vegetables

	Concentration of Sulfamethoxazole in vegetables (ng/kg)			
	Cow pea	**Kales**	**Pumpkin**	**Spinach**
Upper Njiru	21.04±0.21^{a}	7.26±0.88^{c}	15.57±0.95^{b}	22.64±2.01^{a}
Lower Njiru	16.95±0.54^{a}	6.64±0.25^{c}	11.73±0.86^{b}	18.86±2.25^{a}
Upper Ruai	15.93±1.92^{a}	5.16±0.41^{b}	13.91±0.12^{a}	14.35±1.33^{a}
Lower Ruai	15.39±0.27^{a}	4.93±0.98^{c}	8.27±0.05^{b}	12.56±1.00^{a}
Control	ND	ND	ND	ND

Values are given as means of triplicates ± SD. Means with different small letters within a row are significantly different ($P < 0.05$). SD = Standard Deviation, n=15**,** (ẍ ±SD)

For sulfamethoxazole in solvent vegetable extracts, the concentrations as reported in Table 4.22 ranged between 15.39 - 21.04 ng/kg, 4.93 - 7.26 ng/kg, 8.27 - 15.57 ng/kg and 12.56 - 22.64 ng/kg for cow pea, kales, pumpkin and spinach, respectively. The levels were significantly higher in the upper Njiru as compared to the other sites. However, this is attributed to the direct discharge of untreated wastewater in upper Njiru. The level of sulfamethoxazole was significantly higher ($P < 0.05$) in spinach and cow pea and significantly lower in kales. As compared to trimethoprim, the level of sulfamethoxazole was significantly higher in all the sites.

Figure 4.8 shows the concentration of SMX in the vegetables in the various study sites arranged from upstream to downstream for each of the four sites per cluster.

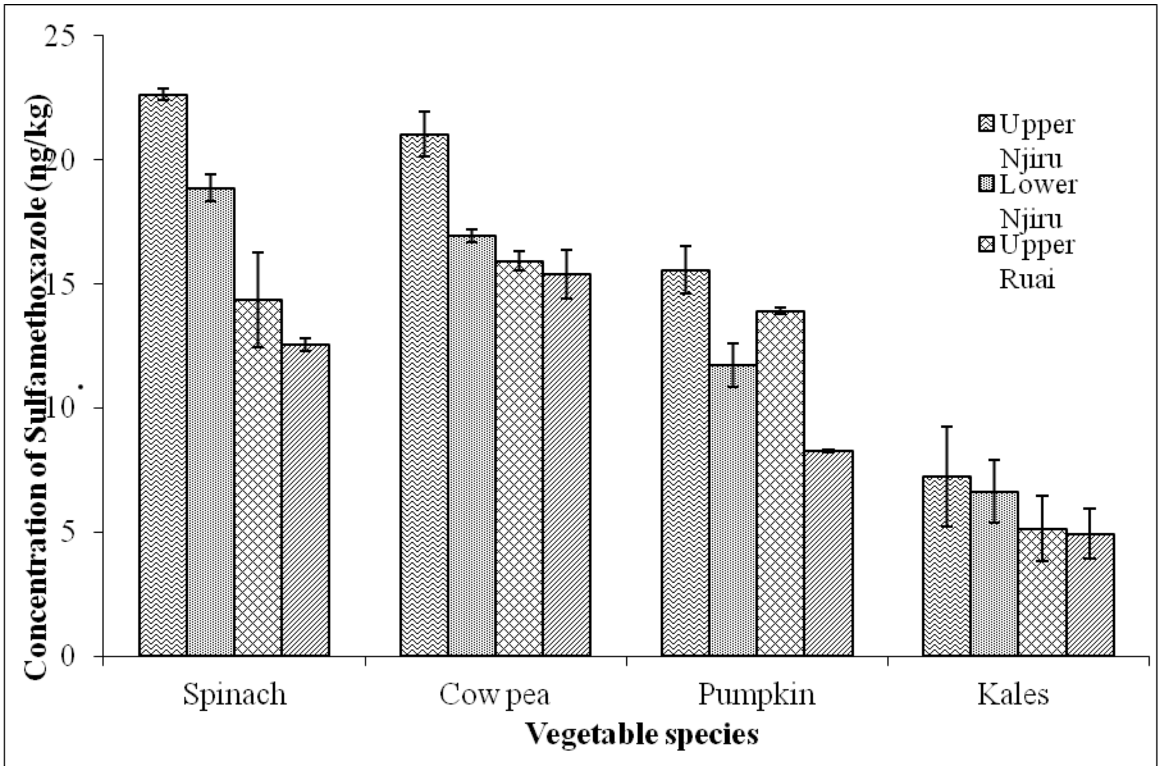


Figure 4.8: The concentration of sulfamethoxazole in vegetables

There was a general decrease in the concentration of SMX as one moved from upstream to downstream in the study region.

4.11 Correlation analysis of antibiotics in untreated wastewater

A Pearson's correlation was done on the association between the concentration of antibiotics in untreated waste water and the vegetables irrigated with the untreated wastewater. It was found to be positive. Table 4.24 shows the correlation results.

Table 4.24: Pearson's correlation of antibiotics in untreated wastewater and vegetables

	The correlation (r values) of antibiotics in wastewater and vegetables.			
	Cow peas	**Kales**	**Pumpkin**	**Spinach**
Trimethoprim	0.39	0.14	0.41	0.54
Sulfamethoxazole	0.32	0.25	0.43	0.59

The concentration of antibiotics in the waste water influenced the concentration of the antibiotics in the vegetables positively (Table 4.24). The vegetable that tended to accumulate the highest concentration of the two antibiotics was spinach, followed by pumpkin leaves and cowpeas. Kales had the lowest correlation value.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The heavy metals in the untreated wastewater

The results of heavy metal concentrations in the untreated wastewater used for irrigation varied widely, but in all cases, the concentrations upstream where the exhausters discharge the waste into the river were higher than the concentration downstream. In the study sites, the sewage exhausters discharge untreated sewage to the rivers at Njiru, which then flows downstream to Ruai. This is due to silting and sedimentation that arises as the water flows down stream. More so, upstream were the points of direct discharge and the farmers had made mini-dams to trap the nutrient rich wastes and apply them into the farms together with the humus rich dark irrigation water.

The concentration (mg/L of heavy metals in wastewater was highest for Fe (5.62-8.07) followed by Mn (3.09-3.54), Cd (0.42-0.47), Pb (0.21-0.28), Cu (0.17 – 0.22) and Zn (0.01-0.03). Heavy metal concentrations in clean irrigation water were below the detectable limits except for Mn (0.45) and Fe (2.12) (Table 4.6).

With reference to the NEMA standards for irrigation water, the heavy metal content in the water did not exceed the safe limits specified except for the case of iron and copper. Corrugated iron sheets are used widely in roofing. Many houses in the slum dwellings that neighbor the urban farms are constructed of iron sheets, therefore the waste water runoff from the iron sheets could account for this. Copper is used in industries to make electrical wires for electrical appliances. Compared to the concentrations (mg/L) of Cd, Pb, and Zn in Dinapur (Sharma *et al.* (2006), the untreated wastewater analyzed during this study had higher concentrations of Cd (0.03), Pb (0.26) and Zn (0.30). Concentrations of Zn, Pb and Cd in the wastewater were below the permissible limits of heavy metals allowed in the irrigation water, (NEMA, 2006), but Cu and Fe were above the safe limits. The concentrations of Mn, Fe and Cd were above the FAO limits of Mn 0.2, Fe 5.0 and Cd 0.1 mg/kg, whereas, the concentration of Zn, Cu and Pb were within the FAO safe limits (FAO, 1985; Pescod, 1992). In a study by Anita (2010) in Varanasi India, the concentrations of Cd (0.02) and Pb (0.09) mg/ L in waste water were much lower than the levels detected in this study with the exception of Zn (0.13 mg/L).

The levels of heavy metals in the untreated wastewater were significantly higher than those in potable water. Rattan *et al.* (2002) reported higher concentrations of heavy metals in sewage effluents as compared to the ground water. Many small scale industries such as dyeing, electro- plating, metal surface treatment, fabric printing, battery and paints discharge their effluents in sewage water, which may be the cause of elevated heavy metals in untreated wastewater.

Water is a solvent for many chemical complexes (Anita, 2010). It therefore dissolves the ionic compounds of the heavy metals and makes them readily available to the plants in the soluble nutrient form. Without adequate water, plants and other forms of life do not thrive well. Since water is flowing, unlike the farm soil which is immobile, the water cannot accumulate as much of the heavy metals as the soil that collects the silt and acts as a sieve as the water flows past. This is the reason why the untreated waste water has less heavy metal load as compared to the soil.

5.2 The heavy metals in the soil

From this study, there was an elevated level of heavy metals in the soil from the study sites, and in some cases, they exceeded the WHO/FAO safe limits. To make a correlation between the concentration of heavy metals in the untreated waste water and the soil, the Pearson's correlation test was carried out, and there was a positive correlation between the concentration of heavy metals in the untreated waste water and in the soil (Table 4.23). Wastewater irrigation has been known to have a significant contribution to the heavy metal content of soils (Mapanda *et al.,* 2005; Nan *et al.*, 2002).

The soil draws heavy metals from the untreated waste water and adsorbs the ions to its surfaces. The soil had a high content of iron, manganese and cadmium exceeding the safe limits. These findings are similar to those by Mapanda *et al.* (2005) that the long-term use of industrial and/or municipal wastewater in irrigation is known to make significant contribution to the trace elements load (Cd, Cu, Zn, Fe, Pb, and Mn) in surface soils. They reported maximum concentration (mg kg^{-1}) of 3.4 for Cd, 145 for Cu, 228 for Zn, 21 for Ni, 59 for Pb and 225 for Cr in the waste water irrigated soil of Harare, Zimbabwe. In the present study, the maximum concentrations for Cu, Pb, Zn and Fe were lower and for Cd and Mn (Table 4.13) were significantly higher than the values reported by Mapanda *et al.* (2005,) and Muchuweti *et al*, (2006). In a similar study by Anita *et al.* (2010), it was observed that continuous application of treated and untreated sewage water to the soil led to higher concentrations of heavy metals in the soil at wastewater irrigated sites as compared to clean water irrigated sites. This is in agreement with the findings from this study because the control plot had much lower concentration of heavy metals as compared to the farms irrigated with wastewater.

From the study the heavy metals in the plots irrigated with untreated wastewater were higher than the control plot by the following percentages: Mn: 373%, Zn: 375%; Pb: 380%; Fe; 662%, Cu; 441%, Cd; 1121%. These results were significantly higher than the findings by Singh *et al.* (2004) where the concentrations of heavy metals were higher by 109 % for Cd, 152 % for Cu,25 % for Pb, 32 % and for Zn, 161 % in soil of Dinapur site irrigated with treated and untreated wastewater as compared to those in soil in a clean water irrigated site. He reported from his studies that among all the heavy metals, Zn was found to be highest and Cd was lowest. This study also had a similar conclusion that zinc was the lowest but in this case, manganese was the highest. This is attributed to the long-term accumulation of zinc ions and adsorption in the soil. Singh *et al.* (2004) and Sharma *et al.* (2007) have also found similar trends of highest and lowest concentrations of Zn and Cd in soil of Dinapur area.

Continuous cultivation and regular absorption by plants, possibly keep the concentrations of heavy metals in soil within safe limits at waste water irrigated sites. These usually accumulate in the upper part of the soil due to strong adsorption and precipitation phenomena (Qadir *et al.,* 2007). Excessive accumulation of trace elements in agricultural soils through wastewater irrigation may not only result in soil contamination but also affect food quality and safety (Muchuweti *et al.*, 2006; Sharma *et al.*, 2007).

The farms in the study site collect a lot of silt during the rainy season because the Ngong River overflows its banks. The untreated wastewater is laden with a lot of garbage, which is applied to the farms because it is rich in humus and nutrients. Garbage utilization in the agricultural land has also been reported to be a likely source of heavy metal pollution in soil of the cultivated land (Gupta *et al.*, 2008). It has been reported that the heavy metals in soils reduce the yield of vegetables because of disturbing the metabolic processes of plants **(**Al-Qurainy and Abdel-Megeed, 2009**)**. From this study however, the vegetables sampled were all fast growing and were harvested thrice a week. The soils irrigated with untreated waste water are rich in humus and nitrates and therefore this favors rapid plant growth. This is in line with observations made by Keraita and Drechsel (2004) and Scott *et al.* (2004) that the farmers even deliberately use undiluted wastewater as it provides nutrients or is more reliable or cheaper than other water sources.

The heavy metal concentration of Zn, Cu and Pb was below the threshold limits for agricultural soil. The soil contributes to the heavy metal content in the vegetables because in the control plot where the soil had less heavy metal, the vegetables grown there had lower or undetectable levels of heavy metals. The Pb accumulation in the soil might be due to the continuous irrigation of soils with metal containing effluents and also due to less mobility of Pb within soils, particularly under the alkaline soil conditions as reported by McBride (1994). Similar results were observed by Lone and colleagues in the analysis of sol and vegetables irrigated with sewage water and tube well water (Lone *et al.,* 2003).

This loading of heavy metals often leads to degradation of soil health and contamination of food chain mainly through the vegetables grown on such soils (Rattan *et al.,* 2002). In addition, the accumulation of heavy metals in the soil greatly affects crops planted in the same farm. The results showed that exposure to wastewater containing heavy metals may have contributed to the level of heavy metals reported in the soil as well as the vegetables grown in the same soil. This is because the amount of heavy metals in the soil samples analyzed was mostly higher than those in the vegetable leaves planted or harvested from the same site. Irrigation is ongoing throughout the dry season and a lot of wastes accumulate in the soil as a result of this activity. The metal ions get solubilized gradually and they are then taken up by the plants. Vegetables take up minerals from the soil, and being grown in soil that was rich in the minerals, there is selective uptake of minerals by the different vegetables.

The results of heavy metals contamination differed from area to area as the concentration of heavy metals in water used for irrigation differed at each site. The possible reason for this may be due to soil factors such as pH, organic matter and nutrients which vary in different areas (Sharma and Chettri, 2005). This indicates that metal concentrations vary from soil to soil. The Upper Njiru, which is in the relatively upper part of Ngong River accumulated a large amount of heavy metals and this increased the soil heavy metal accumulation as well. These heavy metals are absorbed in plants and accumulate in the tissues and may be transferred to food chain through consumption of these plants; therefore accumulation in the soil may lead to toxic effects in the plants grown in the same soil. The organic chemicals in the soil may also chelate the heavy metals and therefore lead to the high concentrations of heavy metals in the soil (Pendias and Pendias, 2002). The vegetables grown in soil that contains relatively high concentration of heavy metals are able to draw the minerals from the soil and thus have a higher concentration of the minerals as compared to controls grown in soil that contains much lower levels of heavy metals.

Cadmium was a notable contaminant and made a concentration that was significantly higher than the FAO (2006) safe limit. The concentration of cadmium in the soil was also significantly higher than the Indian standard (Awashthi, 2000) and the EU (2002) standard (Table 2.1). It is found in paints, and the locality of the sampling has got lots of small scale industries that engage in spray painting of machinery and perhaps discharge the wastes into the adjacent river. Cadmium is known to be highly toxic and therefore it is hazardous to have high concentrations beyond the safe limits recommended for irrigation water.

5.3 The heavy metals in the vegetables

The mean concentrations of Pb, Cu, Zn, Fe, Mn and Cd in vegetables studied are given in Tables 4.14-4.19. The concentrations of heavy metals in these samples are quite variable but the general trend is that the vegetables grown in the Njiru area, which is topographically upstream, had a higher concentration of heavy metals as compared to the downstream sites in Ruai. This is because of silting and sedimentation of the humus and debris as one move further away from the points of discharge into the environment.

The concentration of heavy metals in wastewater irrigated vegetables was significantly higher ($P<0.05$) than that in potable tap water irrigated vegetables. This is due to the higher concentration of these metals in the wastewater irrigated soils as compared to the tap irrigated soils. The results reported a high concentration of iron, manganese, lead and cadmium in all the vegetable samples as compared to the control samples and the levels were above the threshold limits for vegetables. These results concur with the reports by other researchers which explain that, elevated levels of heavy metals in vegetables are reported with long term uses of treated or untreated wastewater (Sharma *et al.*, 2007; Sinha *et al.*, 2005). Plants require some of these heavy metals as trace elements essential for their existence, such as zinc and manganese, but beyond certain limits, they are hazardous to both the plants and the animal that consume the plants for food (Cherop, 2009). This pre-disposition is what raises health concerns on the population that consumes the vegetables regularly. Zinc and copper were within the safe limits.

The concentration of manganese in the vegetables was above the threshold limits of 500 mg/kg (Codex, 2001) (Table 4.14). Manganese forms soluble salts and complexes which are readily taken up by the plants and this leads to the elevated levels of manganese in the vegetables (Fytianos *et al.*, 2001). Spinach showed significantly higher manganese concentrations as compared to the other vegetables. The general trend in concentration of manganese in the vegetables decreased downstream. This shows that the higher the concentration on manganese in the field, the higher the concentration of the element in the vegetable.

The zinc concentration in the vegetables was significantly lower than the threshold limit. The amount of Zn in the samples of leaves of different vegetables that is, spinach, pumpkin, cow peas and kales was found to be 6.90-870, 3.19-4.38, 6.80- 7.29 and 5,70-6.72 mg kg^{-1}, respectively (Table 4.15). This is attributed to overabundance of iron in the untreated wastewater and soils which can interfere with the absorption of other minerals (Solomon, 1986). Singh *et al.* (2001) analyzed six and Itanna (2002) three green leafy vegetables respectively and both reported higher concentration of Zn in spinach. 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. Fytianos *et al.* (2001) also reported a significant uptake of Zn in spinach and endive produced around industrial areas of Greece.

Furthermore, Radwan and Salama (2006) carried out a survey of various fruits and vegetables in Egypt and were able to conclude that the highest mean levels of Pb, Cd, Cu and Zn were present in strawberries, cucumber, dates and spinach, respectively. Al Jassir *et al.* (2005) reported the levels of Zn to be higher in the purslane vegetable species for both washed and unwashed samples. Analysis of the vegetables from the present study demonstrated the concentration of Zn to be within the set limits of international standards (100.00 mg/kg) by FAO/WHO (Codex Alimentarius Commission 2001). The concentration of zinc was significantly low even when compared to the Indian standard (Awashthi, 2000) and the WHO/FAO (2007) safelimits. The results from this study tally with the other results quoted above; where the spinach leaves had the highest concentration of zinc, though it did not get to toxic levels. This implies that spinach is a good source of zinc as a trace element in nutrition. The pumpkin leaves had the lowest concentration of zinc.

All the vegetables sampled had a high lead concentration as compared to the WHO threshold limit of 0.3 mg/kg (Table 4.16). Although the maximum Pb limit for human health has been established for edible parts of vegetables as 0.3 mg/kg (Codex Alimentarius Commission, 2001) and 0.2 mg/kg (Chinese Department of Preventive Medicine, 1994). When evaluated against the WHO/FAO (2006) (Table 2.1) standard, all the vegetables were safe for consumption because the lead concentration was below the threshold limit for toxicity. However, when compared to the Indian standards (Awashthi, 2000) (Table 2.1), none of the vegetables was safe for consumption because the concentration of the heavy metals was beyond the safe limit (Table 4.16). This is comparable to the EU (2006) (Table 2.1) standard, where levels of the heavy metals in the vegetables were beyond safe limits.

Lead is a toxic element that can be harmful to plants although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield (Mohsen and Mohsen, 2008). The amount of Pb in the leaves of different vegetables was varied, with cow peas (4.14-4.46) > pumpkin (3.77 – 4.04) >spinach (2.19-2.79)> kales

(2.37-2.60) leaves. One possible explanation for this situation is that Pb uptake can be promoted by the pH of soil and the levels of organic matter. Demirezen and Ahmet (2006) analyzed different samples of vegetables and reported a significantly high concentration (3.0-10.7 mg kg^{-1}) of Pb which poses health risks to human life. In another study, Sharma *et al.* (2006) reported that concentration of Pb (17.54-25.00 mg kg^{-1}) in vegetables grown in wastewater industrial areas were above the safe limit (0.3 mg kg^{-1}). Muchuweti *et al.* (2006) reported the level of Pb (6.77 mg kg^{-1}) in vegetables irrigated with mixtures of wastewater and sewage from Zimbabwe to be higher than WHO safe limit (0.3 mg kg^{-1}). Fytianos *et al.* (2001) examined a high concentration of Pb in spinach grown in industrial and rural areas of Greece. Al Jassir, *et al.* (2005) studied six washed and unwashed green leafy vegetables from Saudi Arabia and noted the highest concentrations of Pb in the coriander (0.171 mg kg^{-1}) and purslane (0.226 mg/kg). Despite the detection of lead in the vegetables in this case, they were within all the safe limits quotes in Table 2.1. However, the populations feeding habits can also predispose them to excessive quantities of the heavy metal if they are vegetarians and consumption of toxic vegetables comprises a large part of their diet. Muhammad *et al.* (2008) observed a high concentration of lead (1.33-3.65 mg/kg) in vegetables grown with industrial waste water in the vicinity of an industrial area.

In many plants, lead accumulation exceeds the threshold maximum level permissible for human consumption and therefore, it is not safe to consume the vegetables irrigated with such contaminated waste water. The introduction of lead into the food chain may affect human health, thus studies concerning Pb bioaccumulation in vegetables has increased. On the whole, vegetables that were studied in this study were contaminated with lead but it may not be conclusive to say they are totally unsafe for consumption considering that according to some international standards such as WHO/FAO (2006) and EU (2006), they are within the safe limits of consumption. It is therefore important to establish the safe limits depending on the feeding habits of a population for their unique safety.

The pumpkin leaves, spinach and cow peas had a high concentration of iron that was significantly higher than the threshold limits but the kales had a concentration of iron that was within the threshold limit (Table 4.17). Accumulation of Fe is high in all the vegetables as compared to the control vegetable values in the order pumpkin>spinach>cowpeas>kales. Iron accumulation above normal plant concentration may be due to bioaccumulation of it as iron phosphate in the vascular bundles along the veins of the leaf (Sharma and Chettri, 2005).

The amounts of Cu in the samples of spinach, pumpkin, cow pea and kale leaves was 22.54 – 24.79, 21.17 – 25.37, 26.36 – 28.63, 25.39 – 28.63 mg kg^{-1}, respectively (Table 4.18). 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. This may be related to the richness in chlorophyll (Muhammad *et al.*, 2008). 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. Singh *et al.* (2001) analyzed six leafy vegetables from India and reported that copper contents were higher in spinach. 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. Furthermore, Radwan and Salama (2006) carried out a survey of various fruits and vegetables in Egypt and noted that the highest levels of Pb, Cd, Cu and zinc were present in strawberries, cucumber, dates and spinach, respectively. Fytianos *et al.* (2001) analyzed different vegetables from industrial and rural areas of Greece and reported no significant differences in the concentration of metals in most of the vegetables analyzed. Likewise, in this study, no significant difference in the concentration of copper in the vegetables was observed, and in all cases copper was within the safe limits of 73.3 mg/kg by FAO/WHO (Codex Alimentarius Commission, 2001). This is also in line with the Indian safelimits for copper in the vegetables (Awashthi, 2000) and the WHO/FAO (2006) limits (Table 2.1) The concentration was of the order cow peas >kales >pumpkin > spinach.

In a similar study by Lone et al. (2003), he found the levels of the heavy metals in untreated wastewater used for irrigation which included Cu, Zn, Fe, Mn, Cd and Pb to be 0.7 mg/l, 0.42 mg/l, 2.67 mg/l, 0.53 mg/l, 0.31 mg/l and 0.42 mg/l respectively wheres the potable water had 0.005 mg/l Cd, 0.16 mg/l Pb, 8.3 mg/l Cu, 7.71 mg/l Zn, 24.57 mg/l Fe and 15.30 mg/l Mn. This is comparable to the level got in the study, and ascertains that the untreated wastewater has a higher concentration of heavy metals as compared to the potable water. When the vegetables were evaluated for levels of heavy metals, the spinach had the following concentrations Cd, Pb, Cu, Zn, Fe, Mn: 3.2 mg/kg, 10.43 mg/kg, 28.56 mg/kg, 26.169 mg/kg, 500 mg/kg and 104.10 mg/kg respectively. These values were above the safe limits for vegetables set by various international standards organization bodies.

Despite the trace levels of Cd detection in wastewater both in Njiru and Ruai sites, appreciable concentrations of Cd were detected in vegetables. Large amounts of cadmium were also estimated in the different vegetables in the order spinach (4.25-5.58) > pumpkin (2.47-3.68) > cow peas (2.41-3.60) > kales (1.94–3.55) mg/kg (Table 4.19). A significantly ($P<0.05$) higher amount of cadmium was found in the samples of spinach leaves, while lower ($P<0.05$) in the pumpkin leaves. However, there was no significant ($P>0.05$) difference between the leaves of cow peas and kales with regard to Cd content. Demirezen and Ahmet (2006) analyzed various vegetables (cucumber, tomato, green pepper, lettuce, parsley, onion, bean, eggplant, pepper mint, pumpkin and okra) from Turkey and reported that the Cd content (0.24–0.97 mg kg^{-1}) was in higher concentration and not suitable for human consumption. Since the vegetables grown in the study sites from this report are also evidently contaminated with unsafe amounts of cadmium, they are not fit for consumption.

Fytianos *et al.* (2001) reported that spinach and lettuce grown in the soil of industrial area of Greece are enriched with Cd. Al Jassir *et al.* (2005) reported that levels of Cd were higher in the garden rocket vegetable species for both washed and unwashed samples. Muhammad and colleagues (2008) found that the vegetables grown in the vicinity of an industrial area were contaminated with cadmium (0.4-0.7 mg/kg) which is beyond the safe limits. In this study, concentration of Cd was noted to be above the critical level of 0.3 mg kg^{-1} as reported by Codex (2001) and thus might be a threat for the consumers. This may be caused by long-term use of wastewater. According to Nabulo *et al.* (2008), compared to other heavy metals, Cd is more mobile in aquatic environments and is readily available for uptake by grains and vegetables explaining its accumulation in soil and plant parts. Reports have suggested that cadmium uptake by plants is determined by the total available cadmium concentration, soil pH and organic matter content. Although most vegetables showed Cd accumulation, the bioaccumulation of Cd in spinach leaf is greatest, showing tendency for hyper accumulation. These results on cadmium concur with the report by Iqrar *et al.* (2006) which found out that maximum Cd concentration was found highest in spinach followed by eggplant, okra, bitter gourd and pumpkin.

Within the same environment, different vegetables have different accumulation capabilities. This is made evident by the case of manganese whereby the spinach in Upper Njiru and Lower Njiru were the only vegetable that had significantly high manganese content from the mean. This may be due to difference in the ligands at the binding sites of each vegetable (Sharma and Chettri, 2005). Besides this, high pH, lime, organic matter and phosphate reduce heavy metal uptake (Streit and Stumm, 1993). Evidently, the untreated wastewater has a high organic matter and humus content. Higher concentrations of heavy metals in the vegetables were evident in Upper Njiru, than the lower sites. Moreover, this contamination was highest in two of the vegetables spinach and cow peas. Spinach and cow peas are both very fast growing as compared to kales and pumpkin leaves. The fast growing vegetables are able to pick up more organic matter and in due process, uptake of the heavy metals that may have been chelated to the organic chemicals occurs (Qadir *et al.*, 2007).

Of the two vegetables showing a significantly higher concentration of heavy metals, spinach appeared to bioaccumulate the highest concentrations of heavy metals. These results are in line with those obtained by Giordano and Mays which showed that among different vegetables, the highest amounts of heavy metal accumulation is in lettuce, spinach and radish (Giordano and Mays, 1977). The concentrations of heavy metals in the spinach from almost all sites were much higher ($P<0.05$) than the concentrations of the other vegetables and spinach in the control site. This could be an indication of hyper-accumulation in the spinach. This clearly explains that, metal concentration of vegetables not only depends on soil or media on which they grow but also depends on the type and nature of plant.

Levels of heavy metal concentration in the vegetable varied between the four sites and in the different vegetables. No significant differences ($P>0.05$) were observed in the concentrations detected in the upper and lower Njiru although the upper Njiru demonstrated a slightly higher amount than lower Njiru. Generally, heavy metal accumulation was higher in Njiru compared to Ruai. Njiru farms were upstream of the Nairobi River, and sewage exhausters were deposited in the upper Njiru thus the effluents are carried from upper Njiru to lower Njiru, upper Ruai and all the way to lower Ruai.

Upstream of that river, in Njiru farms, the wastewater also comes from household sewage. This suggests that uptake of metals in plants is higher when soils are irrigated with wastewater contaminated by sewage effluents as well as those irrigated with household-derived wastewater. Silting and sedimentation takes place upstream because the farmers create mini barriers to pool the water and tap the high organic matter and debris which is then spread onto the farms. The River is therefore cleaner downstream because most of the filtration is carried out upstream in effort to collect as much of the waste in the river as possible. Indeed, the waste is rich in organic matter but it may also be rich in the much undesired heavy metals.

Studies by Brandy and Weil (2002) showed that plant bioaccumulation of metals depends on the metal and soil conditions such as acidity and organic matter content. Emongor (2007) showed that kale plants irrigated with water of pH 6.5 had significantly higher fresh leaf yield and dry matter content compared to plants irrigated with water having an extreme pH. This holds true for the kales grown on these farms too because there was a socially acceptable high leaf yield, which made the kales look bigger and healthier than those grown in the control plot. The levels of Fe and Cu in Njiru were about twice as high as those observed in Ruai. Compared to heavy metal loads in soil, the different vegetables accumulated less heavy metal loads. Increased organic matter (Bassuk, 1986) and increased clay particles (Hodgson, 1963) can bind metal cations and become unavailable to plants and microorganisms in soil. This indicates that only a small portion of soil heavy metals is transferred to the vegetables and the root acts as a barrier to the translocation of heavy metals within plant.

The concentration of heavy metals from Kenyatta university (control) soil were more than three times less than in soil from Njiru and Ruai. Studies by Nabulo *et al.* (2008) have shown that *Brassica oleracea acephala* kales have high leaf to root ratio efficiency in concentrating the heavy metals from both soil and atmosphere as compared to other vegetables and implies that kale is more predisposing to metal toxicity than other vegetables. In this study, however, the kales did not show this trend.

According to the farmers, the spinach is more water loving and not very resilient to dry weather as compared to the kales and pumpkin leaves. The spinach farms were therefore flooded with the untreated wastewater and silt in the early morning as compared to the kales that got irrigated in alternate days. The evidently more abundant exposure to untreated wastewater contributed to the spinach having a much higher concentration of heavy metals as compared to the other vegetables.

Cow peas are grown and uprooted for the market within 21 days. They are therefore grown next to the river banks in small paddocks and flooded with the wastewater. The results of this study concur with other studies which have shown that leafy vegetables accumulates higher levels of trace and heavy metals (Yusuf *et al.,* 2003; Nabulo *et al.,* 2008). Irrigating the vegetables with water that contains a high concentration of heavy metals leads to uptake of the metals by the vegetables. The heavy metal concentrations vary between the farms and vegetables. The concentrations of heavy metals observed in the vegetable samples from the wastewater irrigated farms are related to the high concentrations of the metals in the soil as reported by Akinola and Ekiyoyo (2006). However, the accumulation of heavy metals may also be attributed to differential absorption capacity of test vegetables for different heavy metals (Anita *et al.,* 2010). This is evident from the comparative study because the vegetable irrigated with potable tap water had a lower concentration of heavy metals. There was also variation in the concentration of heavy metals in the vegetables from one study site to the next. In a similar study by Khan *et al.* (2008), it was observed that long term wastewater irrigation leads to build up of heavy metals in soils and food crops.

Vegetables get their nutrients from soil in a solubilized form and therefore, the well irrigated soils using water with a high concentration of heavy metals offers a more ready source of nutrients for the vegetables. In the process of nutrient uptake by the plants, the heavy metals are taken up as well.

The results of the present analysis showed that the concentration of cadmium and lead in the leaves of the vegetables is significantly high. The Mn and Fe contents in the leaves of the vegetables were significantly higher ($P<0.05$) as compared to the other elements, whereas, the Zn, Pb and Cd contents were found to be significantly ($P<0.05$) lower. In a similar study Iqrar *et al.* (2006), also observed that the concentration of Mn is generally higher in spinach. In addition, they found that samples of several vegetables contained Pb and Mn above the permissible limits.

The concentration of heavy metals in contaminated soils has a positive correlation to the heavy metals found in the vegetables (Table 4.23). As the levels of heavy metals rise in the soil, so do they relate to a proportionate rise in the vegetables. Vegetables, grown in heavy metals contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils and atmosphere because they absorb these metals through their leaves. This explains the heavy metal toxicity of the untreated water and the accumulation of these heavy metals in the soil. Vegetables absorb heavy metals from the soil and polluted water and thus lead to contamination of the food chain. Heavy metals 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). The introduction of these elements into the food chain may affect human health (Coutate, 1992).

The results presented demonstrate that there is a risk associated with consumption of vegetables irrigated with wastewater since there is a positive correlation between the concentration of heavy metals in the untreated wastewater and in the vegetables. The vegetables look apparently healthy and growing well despite accumulating heavy metals to concentrations which substantially exceed maximum values considered safe for human consumption. Long term irrigation can induce changes in the quality of soil as trace element inputs are sustained over long periods. There are various reports (Barman *et al.*, 2000; Singh *et al.*, 2004), that show that when wastewater is used for the irrigation of edible plants for prolonged period, soil health is affected. It is therefore becoming a matter of concern to environmentalists since the presence of pollutants, particularly; toxic metals apparently accumulate in soils. Thus, introducing these pollutants into the plants growing therein through roots, which are translocated to foliage and even to edibles fruit parts (Zayed *et al.*, 1998; Barman *et al.*, 2000; Fytianos *et al.*, 2001) easily take place in such environments.

5.4 The antibiotics in the wastewater

The antibiotics, sulfamethoxazole and trimethoprim detected in the wastewater are commonly used for treatment of human infections. These antibiotics entered the irrigation water through untreated sewage effluent disposed to the river at upper Njiru. This is mainly because the wastewater came from household sewage. These antibiotics were detected in all samples with the exception of control samples.

From the results, it was evident that the concentration of the antibiotics was significantly higher in upper Njiru as compared to the other sites (P <0.05). In addition, the concentration of the two antibiotics decreased down the stream and followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai. The high levels of the antibiotics in Upper Njiru resulted from direct discharge of untreated wastewater since the river flows from Upper Njiru and ends in Lower Ruai. The high concentration of these antibiotics is in tandem with the findings of Andreozzi *et al.* (2002) who reported that antibiotics are found more commonly in sewage effluent than in other recycled waters.

The concentration of sulfamethoxazole in the upper Njiru was also significantly higher than in the other sites. Its concentrations ranged from 62-89 ng/L in the four sites. In the wastewater samples, the concentration of the sulfamethoxazole drug was significantly higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials (Pérez *et al.,* 2005).

The present study clearly showed that levels and detection frequencies of trimethoprim in wastewater samples were significantly lower ($P < 0.05$) in all four sites as compared to sulfamethoxazole. However, the concentration was slightly higher at upper Njiru than the other sites and this was consistent as with sulfamethoxazole.

A study carried out by Kathryn (2004) to determine the concentration of sulfamethoxazole in untreated hospital waste water discharged into sewer systems found out that, the drug had high persistence and was detected at concentrations of 300 ng/l. In addition to predictions regarding fate and persistence, Huang *et al.* (2001) also reported the sulfamethoxazole concentrations in untreated hospital wastewater to range from 3.9 ng/l to approximately 27,000 ng/l. These findings are significantly higher than the levels obtained in this study but it is noteworthy that the sampling in this case did not concentrate on hospital untreated waste water but rather a mixture of domestic, industrial hospital and other urban sources of waste water.

5.5 The antibiotics in the vegetables

The concentration of the antibiotics in waste water irrigated vegetables was significantly higher ($p < 0.05$) than in the control vegetables. However, the concentration was higher in upper Njiru as it followed the following order: Upper Njiru > Lower Njiru > Upper Ruai > Lower Ruai. This is because Njiru farms were upstream of the Nairobi River, and sewage exhausters directly discharged the untreated sewage to the river at upper Njiru thus the effluents are carried from upper Njiru to lower Njiru, upper Ruai and all the way to lower Ruai. Therefore, this discharge was responsible for the highest levels of the antibiotics in the irrigation water. During irrigation, these antibiotics accumulated in the soil and thus absorbed by the vegetables. The concentration of sulfamethoxazole drug was significantly higher than that of trimethoprim in all the vegetables.

In addition, the level of trimethoprim was significantly higher in cow pea, pumpkin and spinach ($P < 0.05$) while for sulfamethoxazole the levels were significantly higher in spinach and cow pea. However kales reported significantly lower levels of both sulfamethoxazole and trimethoprim ($P < 0.05$). This may be attributed to the fast growth of spinach and cow peas as compared to kales since the fast growing vegetables are able to pick up more organic matter. Similarly, spinach appeared to bioaccumulate significantly higher ($P<0.05$) concentrations of heavy metals as compared with the other vegetables. This is an indication of hyperaccumulation in the spinach and clearly explains that, the antibiotic concentration of vegetables not only depends on soil or media on which they grow but also depends on the type and nature of plant.

There was no significant differences ($P>0.05$) in the concentrations of the antibiotics in the upper and lower Njiru although the upper Njiru demonstrated a slightly higher amount than lower Njiru. This suggests that uptake of antibiotics in plants is higher when soils are irrigated with wastewater contaminated by sewage effluents as well as those irrigated with household-derived wastewater.

In the primary effluent, the concentration of the sulfamethoxazole was about four times higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials (Ryan *et al.,* 2011). Traces of both compounds were still detectable in the treated sewage. Compared to other studies on the occurrence of sulfonamides in sewage treatment plants, Hartig *et al.* (1999) reported a sulfamethoxazole level in the primary effluent of a German WWTP of 2.4 µg/L, whereas analysis of the secondary treated sewage gave residual concentrations in a range from 0.3 to 1.5 µg/L. Another study monitoring a series of pharmaceuticals in sewage effluents found median concentrations of 0.40 µg/L for sulfamethoxazole and 0.32 µg/L for trimethoprim (Pérez *et al.,* 2005). In the primary effluent, the concentration of the sulfa drug was about four times higher than that of trimethoprim, which is consistent with the typical ratio in medications (5:1) containing the two synergistically acting antimicrobials (United States Department on FDA, 2004). Traces of both compounds were still detectable in the treated sewage. In this study, the mean concentration range was from 62.09-88.66 ng/l for sulfamethoxazole and 24.71-25.66 ng/l for trimethoprim. These values are much lower than those detected in the study above. This could be due to the dilution effect of flowing water and improved disposal of drugs by the users and the health institutions. Unlike sulfamethoxazole, trimethoprim has been reported to persist in sewage sludges mush longer (Pérez *et al.,* 2005).

There is a positive correlation between the concentration of the two antibiotics in untreated waste water and the concentration in the vegetables.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The untreated wastewater contained high levels of manganese, iron and cadmium. The levels of lead, zinc and copper were below the safe limits concentrations when compared to FAO (2006) and NEMA (2006) limits for irrigation water. The copper levels in the untreated wastewater were however on the border limit for unsafe levels.

In the soils from the farms, the manganese, and cadmium levels were above the FAO (2006) and Ewers (1991) safe limits. The concentration of zinc, lead and copper were below the safe limits.

The growing of vegetables in the heavily contaminated soils leads to their uptake of the heavy metals, and this has been confirmed by the strong positive Pearson's correlation between the heavy metals in the soil and in the vegetables.

From the study, the level of heavy metals in the untreated wastewater affects the concentration of heavy metals in both soil and the vegetables. When the untreated wastewater has a high concentration of heavy metals, the soil adsorbs the heavy metals and the vegetables take up solubilized nutrients from the soil and water. This was confirmed by the Pearson's correlation test.

The extent to which vegetables take up heavy metals differs from one vegetable to the next. There are some vegetables that accumulate higher levels of heavy metals than others. The extent of accumulation differs from one heavy metals to the next, where by one vegetable may accumulate the highest concentration of one or more heavy metals but not all of them.

Spinach vegetables had the highest concentration of most of the heavy metals except lead. Cowpeas came next followed by pumpkin leaves and finally kales. The pumpkin leaves accumulated the highest concentration of lead followed by cow peas, spinach and finally kales.

In all cases, the kales had the lowest levels of heavy metals. The levels of heavy metals in the vegetables were beyond the safe limits when compared to the various local and international standards. It is therefore not safe to consume the vegetables irrigated with untreated wastewater.

Based on the results, it was concluded that spinach and cow peas are the relatively high accumulators for most heavy metals whereas kales was the least accumulator of the elements.

From this study a clear association has been shown to exist between heavy metal concentration in soil and vegetables grown in that soil. As well, an association is also evident in the soil and water within the vicinity of that area. This is the water used for irrigation. Untreated wastewater from Njiru and Ruai were found to be unfit for irrigation with respect to the standard safe limits.

The soil in the wastewater irrigated farms is contaminated with heavy metals higher than the threshold values. The practice of irrigation using untreated wastewater is hazardous because the inputs contain hazardous levels of heavy metals.

The wastewater used for irrigation was contaminated with the antibiotics sulfamethoxazole and trimethoprim. These antibiotics detected in the untreated wastewater were also present in the vegetables. Owing to the medical importance of these drugs in the management of opportunistic infections in HIV-AIDS patients, more stringent measures need to be applied in ridding the wastewater of these drugs so that bacteria do not develop resistance due to persistence exposure.

All the vegetables contained residues of the drugs and therefore it can be concluded that it is not safe to consume vegetables contaminated with the antibiotic even though it is in small quantities because it will lead to undue tolerance to the drugs. Irrigation with untreated wastewater leads to production of vegetables that contain hazardous contaminants.

6.2 Recommendations

6.2.1 Recommendations from this study

Since most levels of heavy metals in untreated wastewater reported in this study were above the allowed levels, the untreated wastewater should not be used for irrigation because exposure leads to uptake by the vegetables which are eventually consumed, and this is a health hazard.

The soils irrigated with untreated wastewater end up being contaminated with heavy metals that they adsorb, and this makes them unfit for agricultural use in the farming of vegetables.

The vegetables irrigated with untreated wastewater contain high levels of some heavy metals and are therefore not safe for consumption.

The wastewater used for irrigation contains SMX and TMP antibiotics. These two antibiotics are used to manage human diseases and exposure of microbes to them may lead to development of resistance. The exposure of the human body to low quantities of the drugs gradually leads to tolerance build in the body and this will render the drugs non-effective in disease management.

Antibiotics should be discharged responsibly, especially from hospital

The policy makers should have regislation banning use of untreated wastewater for irrigation in farms growing vegetables and fruits.

6.2.2 Recommendations for further work

i) It is important for further research to be carried out on the levels of antibiotics in hospital wastewaters discharged into the environment

ii) Research should be carried out on the impacts the antibiotics have on the bacterial and fungal pathogens that may be isolated from the soils in the farms and from the untreated wastewater, with intent to establish whether there is any variation to drug sensitivity that may be imparted to these pathogens by the small quantities of antibiotics they are exposed to.

iii) It is suggested that the antibiotic metabolites that may arise from the parent molecule and perhaps have some pharmaceutical activity be considered in a research to determine their quantities.

iv) The concentrations of heavy metals in the blood of consumers of these vegetables could be monitored over a long duration of time to determine whether there is gradual accumulation in the body because of the already discussed health hazards. The correlation between the heavy metals in the vegetable and the blood should be determined.

v) Soil contamination with heavy metals should receive increasing attention from the public as well as governmental agencies in developing countries, particularly in Kenya. Determination of heavy metals concentration in vegetables and food products is important for health risk assessment during food consumption. This kind of study can be used as a tool for the farmers so that they may adopt such strategies which lead them to save the population by minimizing the problems related to metal toxicities. Such assessment for the contaminants is required for the well-being of the population.

vi) Assessment of heavy metals and antibiotics in vegetables sold in markets in Nairobi county should be carried out.

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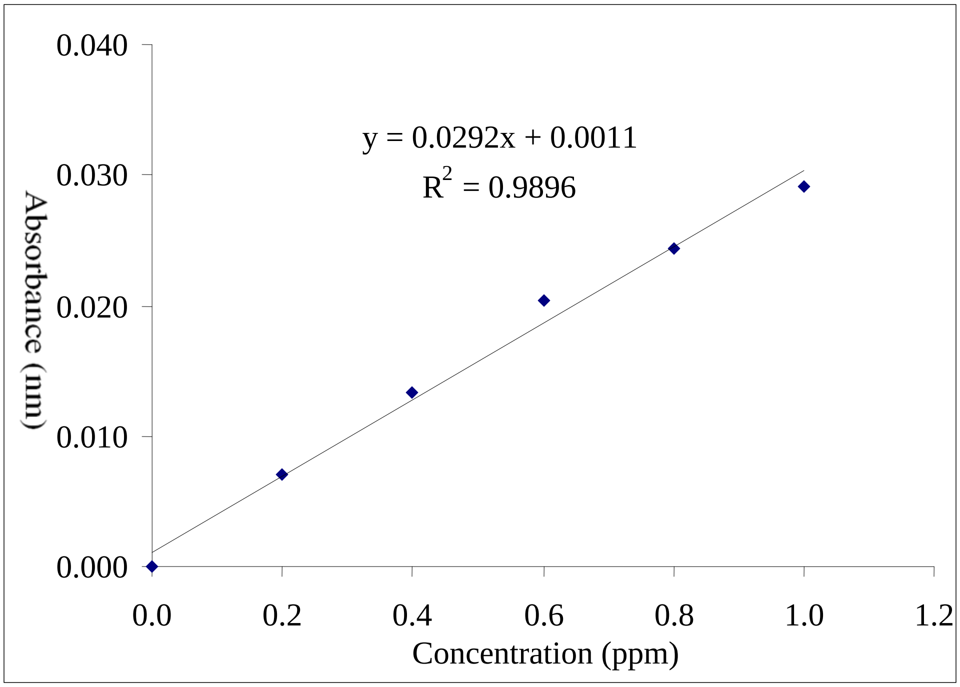
APPENDICES

Appendix I: Njiru farm with irrigated section and dry one

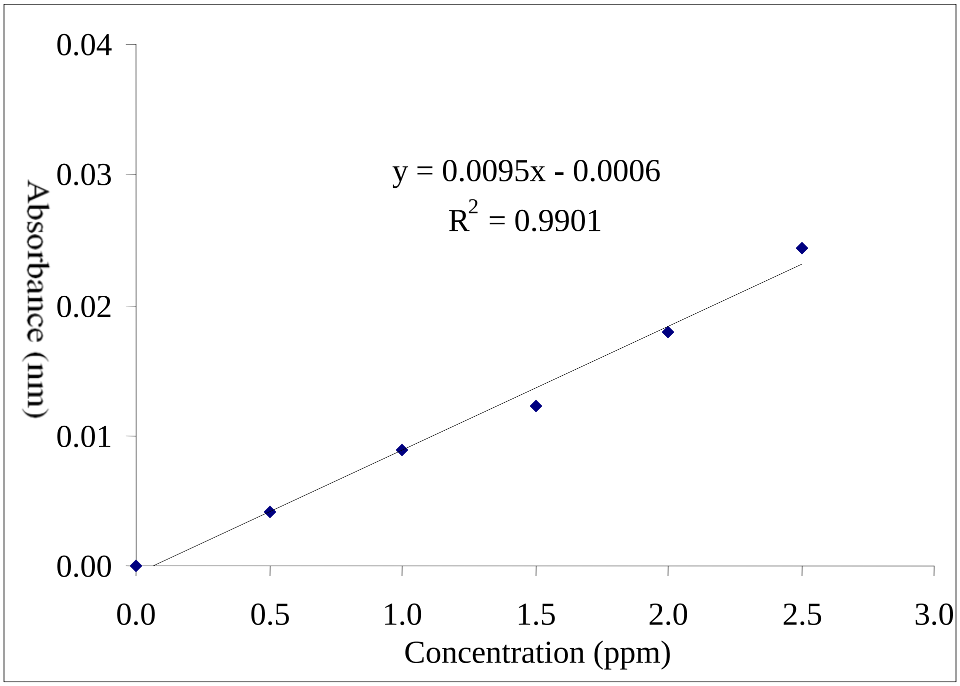
Appendix II: Harvesting of the vegetables irrigated by the waste water

Appendix III: Collection of vegetable samples.

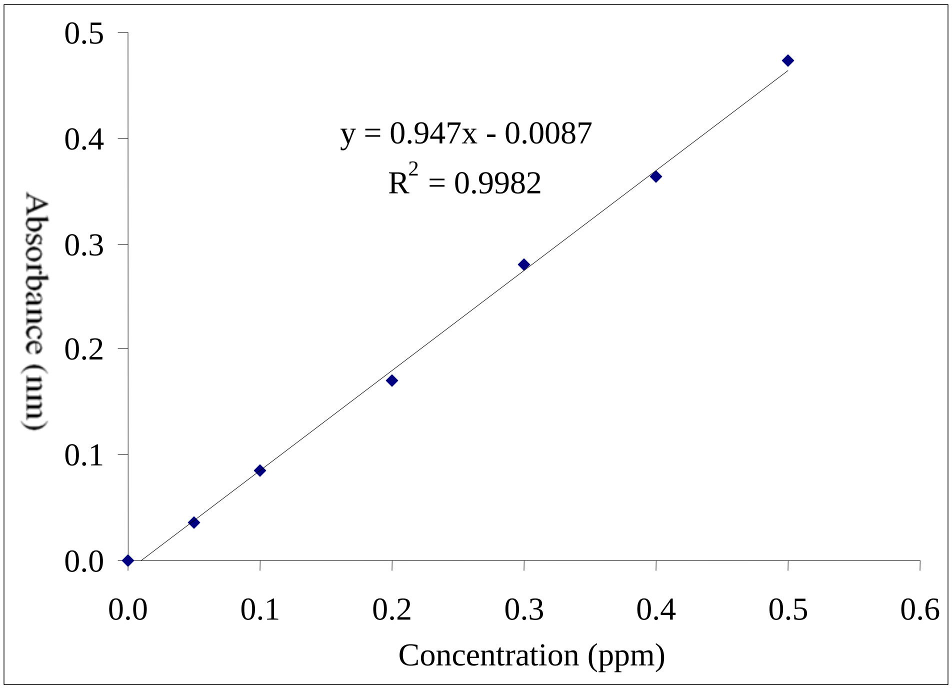
Appendix IV: Standard curve for manganese



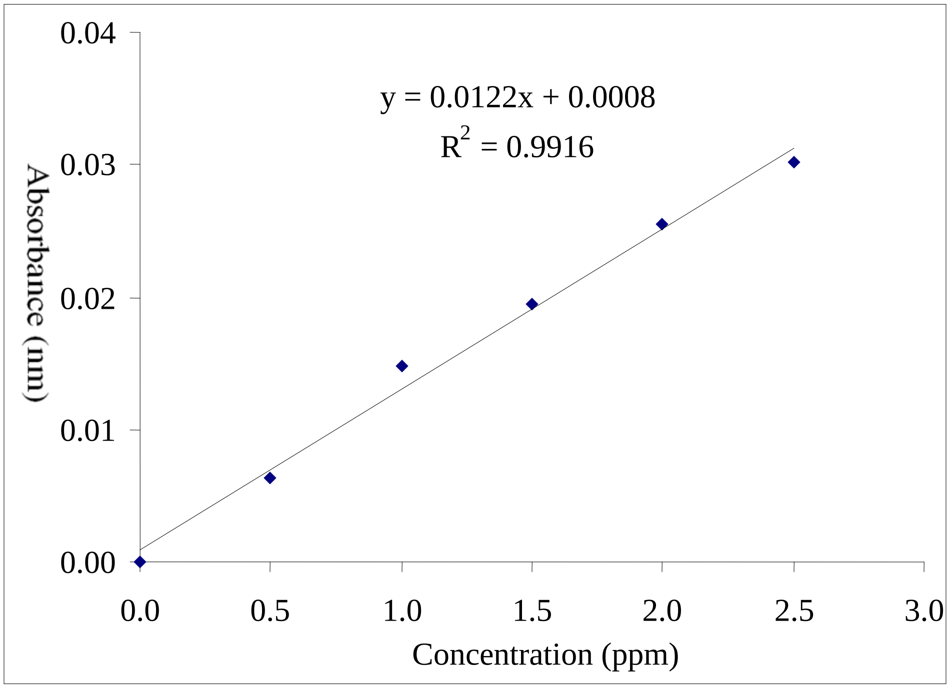
Appendix V: Standard curve for copper



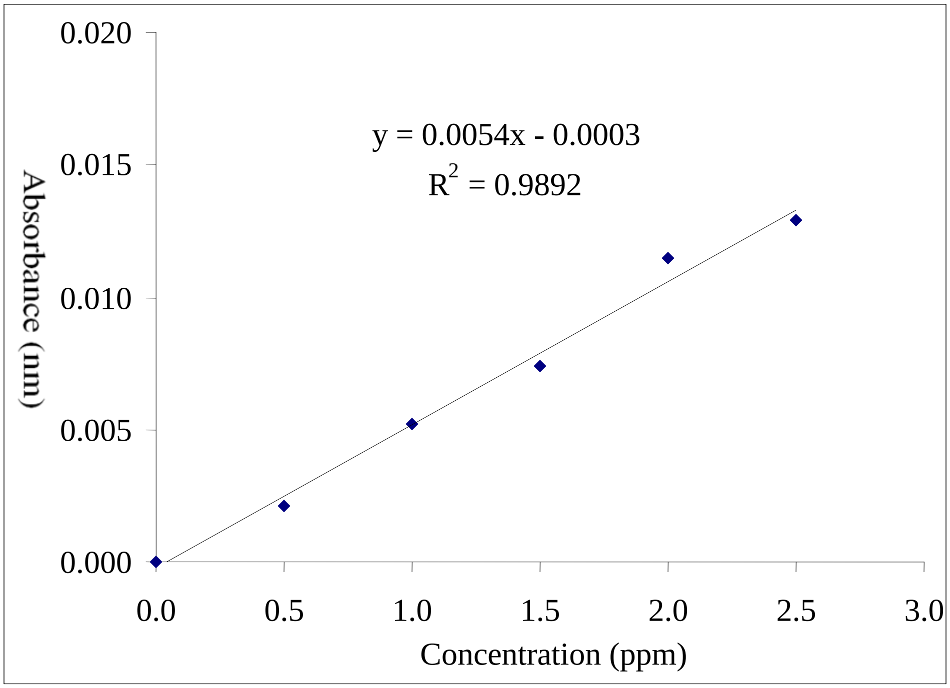
Appendix VI: Standard curve for zinc



Appendix VII: Standard curve for iron

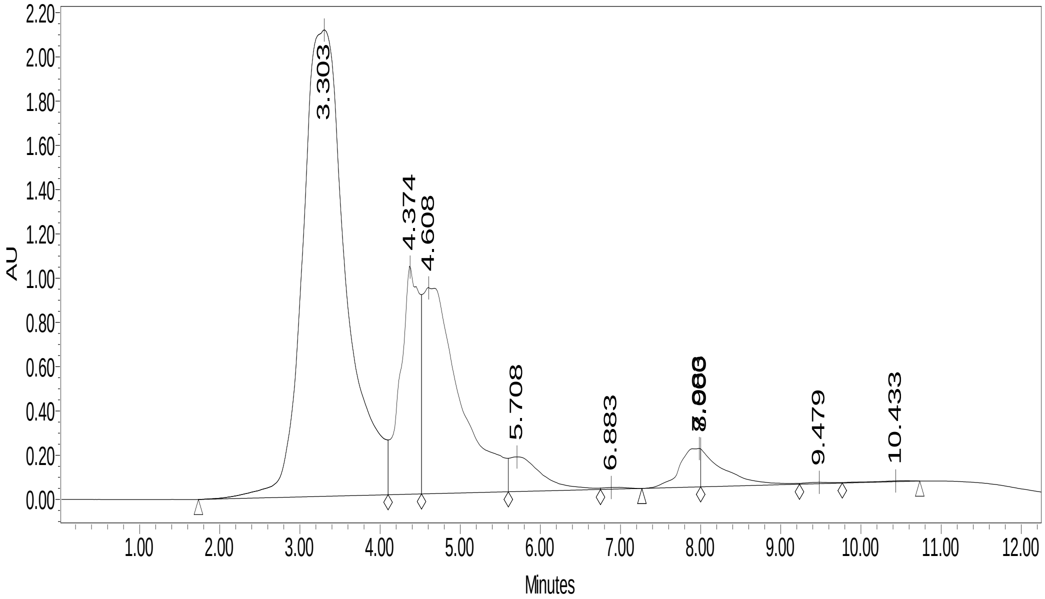


Appendix VIII: Standard curve for cadmium



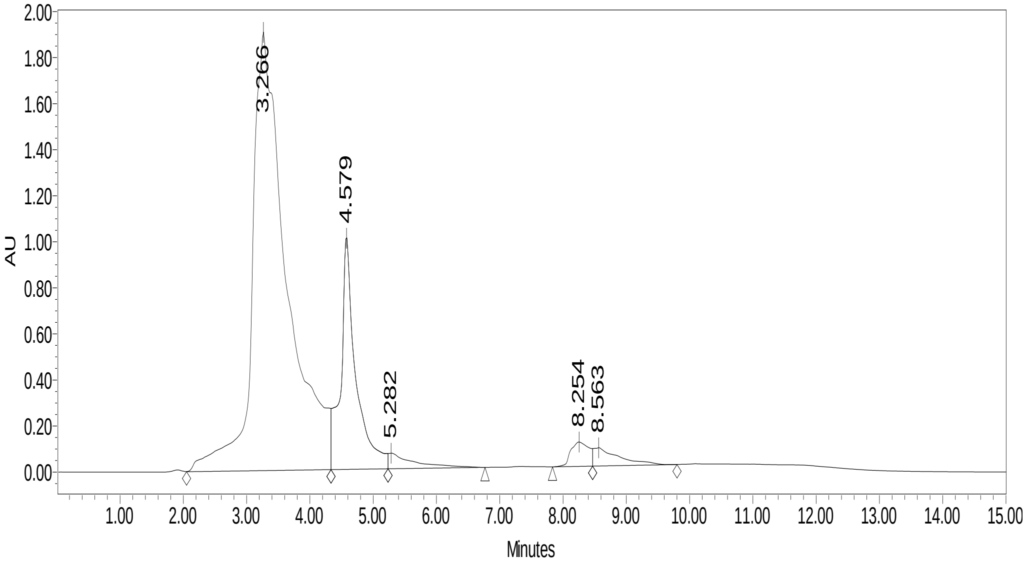
Appendix IX: HPLC chromatogram SMX and TMP for untreated waste water from Upper Njiru

Appendix IX shows the chromatograph for SMX and TMP analyzed in untreated waste water from Upper Njiru.



The peak at 3.303 minutes shows the sulfamethoxazole time of elution. The peak at 4.374 minutes shows trimethoprim time of elution. The small peaks at 5.708, 8.000and 9.479 are the elution peaks for the metabolites of trimethoprim (the 1st peak) and trimethoprim (the 2nd peak). The area under the peaks was used to quantify the concentrations of the antibiotics. There were additional peak at 4.608, 6.883 and 10.433 from other contaminant drugs that were not eluted at the expected retention times.

Appendix X: HPLC chromatogram for TMP and SMX for kales from Upper Njiru



The peak at 3.268 minutes shows the sulfamethoxazole time of elution. The peak at 4.579 minutes shows trimethoprim time of elution. The small peaks at 5.282, 8.254 and 5.563 are the elution peaks for the metabolites of trimethoprim (the 1st peak) and trimethoprim (the 2nd peak). The area under the peaks was used to quantify the concentrations of the antibiotics.