UPTAKE OF CADMIUM, COPPER AND ZINC IN SELECTED PLANTS COMMONLY GROWN IN KENYA FROM POST-METHANATION DISTILLERY EFFLUENT

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and / or University of Eldoret.

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Declaration by Supervisors

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

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DEDICATION

This thesis is dedicated to my loving parents, brothers and sisters, for their endless support and encouragement, towards the accomplishment of this study.

ABSTRACT

Pot experiments were carried out on a field plot in University of Eldoret (UoE) to study uptake of selected metal contaminants from distillery effluent in four plants commonly grown in Kenya: *Ipomoea batatas*, *Eucalyptus grandis*, *Saccharum officinarum* and *Zea mays*. The irrigation water included treated distillery effluent (TDE) from the Agrochemicals and Food Company (ACFC), in Kisumu County and tap water (TW) from Eldoret Water and Sanitation Company Limited (ELDOWAS), a public water supply in Uasin Gishu County. The experiment was a 2 (irrigation water qualities) by 4 (test crops) factorial, with 4 replications. The control treatment involved irrigation of pots that comprised soil only. Variation in plant growth characteristics between the irrigation treatments was studied by physical measurements (leaf count, leaf and stem lengths), over a 120-day period. Samples of the irrigation water, leachates, soil and various plant parts were analyzed through selected tests to evaluate the effect of application of TDE on the soil-plant systems. Analysis of the levels of cadmium (Cd), copper (Cu) and zinc (Zn), and total nitrogen (N) and phosphorous (P), in the soil-plant systems, were determined by flame atomic absorption spectroscopy (FAAS) and ultraviolet-visible (UV-Vis) spectroscopy, respectively. Toxicity stress in soil-plant systems was measured in leachate samples by use of Toxi-Screening Kit*Microbiotest* ®. Data was stored in Microsoft Excel Spreadsheets and analyzed by use of Statistical Programme for Social Scientists (SPSS version 20.0.0).The alpha level for all statistical tests (Student's t-test, One-way ANOVA and Spearman's correlation test) was set at 0.05. The metal levels in TDE irrigation water were below the National Environmental Management Authority (NEMA) standards, for heavy metals in irrigation water and heavy metals in effluent for discharge to the environment. Plants irrigated with TDE gave higher leaf count, leaf and stem length measurements as well as higher concentrations of N, P, Cu and Zn than those irrigated with TW. Deposits of Cd, a toxic metal, were higher in TDE irrigation treatment. Uptake of Cd, Cu and Zn varied between test crops. Spearman's correlation determined significant positive correlations between Cu and Zn (r_s = 0.237, n=72, p= 0.045) and Cd and Zn (r_s =0.236, n=72, p = 0.046), N and P (r_s =0.605, n=72, p < 0.0001).Cadmium and Cu had a negative correlation (r_s = -0.59, n=72, p= 0.623). The inhibition of bioluminescence in *Vibrio fischeri* by leachates , an indication of toxicity stress in the plants irrigated with TDE , was determined as follows: maize (31.21%) > sweet potato (28.34%) > eucalyptus (16.43%) > sugarcane (15.20). The detection of Cd, a toxic metal, in sweet potato tuber (0.020 mg L^{-1}), sugarcane stem (0.014 mg L^{-1}) and maize grain (0.002 mg L^{-1}) indicates potential risk of Cd being introduced to the human food chain. For that reason, TDE is not recommended for cultivation of food crops. The production of effluent-irrigated eucalyptus plantations can secure additional treatment of distillery effluent and minimize the adverse environmental effects associated with its disposal.

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LIST OF ACRONYMS

AAS	Atomic Absorption Spectrophotometer
ACFC	Agro-Chemicals and Food Company
AR	Analytical Reagent
ARWR	Annual Renewable Water Resources
ATP	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
ELDOWAS	Eldoret Water and Sanitation Company
EPA	Environmental Protection Agency
DE	Distillery Effluent
FAAS	Flame Atomic Absorption Spectrophotometry
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
IWMI	International Water Management Institute
KEPHIS	Kenya Plant Health Inspectorate Services
MSC	Mumias Sugar Company
MW	Molasses Wastewater
NEMA	National Environment Management Authority
ANOVA	Analysis of Variance
PE	Polyethylene
ROS	Reactive Oxygen Species
RGR	Relative Growth Rate

RLU	Relative Light Units
TDE	Treated Distillery Effluent
TRE	Toxicity Reduction Evaluation
TW	Tap Water
UN	United Nations
UV	Ultra-Violet
UNDP	United Nations Development Program

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

1.1.1 Water resources situation in Kenya

The average annual renewable water resources (ARWR) per capita in Kenya decreased from 1,853 cubic metres in 1969, to 704 cubic metres in 2000, to the current estimate of 647 cubic metres(Institute of Econmonic Affairs, 2007). Because of this, Kenya is chronically water-scarce. Unless effective measures to address the water scarcity challenge are implemented, Kenya's per capita availability of ARWR is projected to reach 235 cubic metres by 2025. This poses a serious threat to socio-economic development and the integrity of national ecosystems(2007. Government of the Republic of Kenya, 2007; Institute of Econmonic Affairs, 2007).

Water availability is also a function of rainfall. The average annual rainfall in Kenya is 630 mm, with a variation from less than 250 mm in the arid and semi-arid land (ASAL) to over l800 mm in the Lake Victoria Basin. Of Kenya's total land area, eighty per cent is ASAL and hence majority of areas in the country receive less than adequate rainfall required for crop cultivation (FAO, 2009).

The agricultural sector accounts for about 64 % of the country's total water consumption and has by far the greatest influence in the annual water balance (FAO AQUASTAT, 2005; 2007. Government of the Republic of Kenya, 2007; Onjala, 2002).

1.1.2 The wastewater challenge

The term ‘wastewater’ collectively refers to water that has been adversely affected in quality by anthropogenic activities. Depending on source of generation and level of treatment, wastewater may contain different types and levels of undesirable constituents. In general , industrial wastewater contains higher levels of contaminants than other sources and needs proper treatment before disposal or use (Qadir & Scott, 2009).In this report, the term ‘effluent’ has been widely used to denote wastewater from industrial operations.

Properly treated wastewater can be used in sectors which not require freshwater for their operations. The World Health Organization (WHO) international guidelines on wastewater use (WHO 2006), were first formulated in 1989, in an effort aimed at developing mechanisms for controlling negative impacts of wastewater use. To date, the WHO recommendations of wastewater treatment and crop restrictions are considered by many governments as the legal framework, though they are not intended for absolute and direct application in every country (Amerasinghe, Bhardway, Scott, Jella, & Marshall, 2013).

A study by (Delgado & Casanova, 2001), recommends use of properly treated effluent for agriculture. The main benefits of effluent irrigation include the provision of alternative water source in regions lacking freshwater (Qadir et al., 2010b), fertilizer substitution by the effluent’s nutrient load (Al-Nakshabandi, Saqqar, Shatanawi, Fayyad, & Al-Horani, 1997) and securing supplemental treatment for wastewater (Pollice, Lopez, Laera, Rubino, & Lonigro, 2004). However, effluent must undergo aerobic or anaerobic

treatment before application. If properly managed, the practice of effluent irrigation reduces the amount of water abstracted from the environment and releases fresh water to be used only by sectors requiring high quality water in their operations (Jimenez & Asano, 2008).

With declining per capita fresh water availability in Kenya, there is increasing dominance annual water balance by wastewater. Rapid population growth, industrial and technological expansions have outstripped improvements the wastewater infrastructure(Onjala, 2002). Even though the Kenyan vision 2030 has attempted to address the water scarcity challenge by advocating for conservation measures such as rain water harvesting and enhanced utilization of groundwater resources , the existing water policy does not recognize wastewater recycling (Kaluli , Githuku, Home, & Mwangi, 2011).

There is growing concern about the quality of water available for use by various sectors due to the implication of wastewater use for the hydrology of natural water resources. Schedules three, five and nine of the water quality regulations set by Kenya's National Environmental Management Authority (NEMA), define the standards for quality of effluent for discharge to the environment and public sewers water , as well as quality of water for use in irrigation (2006. Government of the Republic of Kenya, 2006).

Wastewater recycling is a critical element for conservation of fresh water resources. Despite the informal nature of wastewater reuse in Kenya, research has shown that farmers in urban centres irrigate their crops with poor quality water(Kaluli , et al., 2011).Much of this use is not intentional and is the consequence of water sources being polluted by poor sanitation and waste disposal practices. This increases the exposure of

the public to health and environmental risks. Therefore, an assessment of possible policy and technical options that can reduce or eliminate negative health effects and economic burden resulting from wastewater use, particularly in agriculture, becomes an urgent necessity in Kenya.

Heavy metal contamination is a widespread problem associated with global industrialization. The magnitude of absorption is metals in plants largely depends on their concentration in soil, physicochemical condition and the ability of plant roots to absorb (Ganeshamurthy, Varalakshmi , & Sumangala, 2008).

1.1.3 Use of plants to clean up effluents

Phytoremediation is an emerging technology that involves the use of plants to use of plants to extract, degrade, contain or immobilize pollutants in environmental media such as soil, groundwater and surface water. The development of phytoremediation is driven primarily by the high cost of other soil remediation techniques as well as the desire to use a ‘green’, sustainable process (Pulford, Watson, & McGregor, 2001).

Generally, five techniques are employed to treat a variety of environmental contaminants: i) Phytoextraction , the process where plants remove pollutants from soil and concentrate them in their harvestable plant parts(Kumar, Dushenkov , Motto, & Rasakin, 1995),ii) phytodegradation ,the process where plants and associated microbes that degrade organic pollutants(Burken & Schnoor, 1997), iii) phytostabilisation, the process where plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization of by preventing migration(Vangronsveld, Assche, & Clijsters, 1995) ,iv)phytovolatilisation, the volatilization of pollutants into the atmosphere via

plants(Burken & Schnoor, 1999), and v) rhizofiltration , the absorption of metals from plant roots by plants(Dushenkov, Kumar , Motto, & Raskin, 1995).

Ideal plant species to remediate a heavy metal contaminated soil would be a high biomass producing crop capable of tolerating and accumulating the contaminant of interest. Plants that absorb unusually large amounts of metals in comparison to other plants are known as hyper-accumulators. Metals such as nickel (Ni), Zinc (Zn) and copper (Zn) are best removed by phytoextraction because the majority of the approximately 400 known plants that absorb unusually large amounts of heavy metals and have a high affinity for these metals(Pulford, et al., 2001).

1.2 Statement of the problem

Globally, the cultivation and processing of sugar involves intensive use of water for irrigation, heavy use of agro-chemicals, and discharge of polluted effluent, as well as air pollution.This leads to degradation of soil, air and water quality in areas sugar production areas. The most significant impact from sugar processing is water pollution due to discharge of polluted effluent (WWF, 2004).

Kenya's sugar industry is the second largest contributor to agricultural growth, after tea (Kenya Sugar Board 2011). A study on the impact of agro-industrial activities on the water quality of River Nyando, Lake Victoria basin, Kenya, determined changes in water quality in the river above and below Muhoroni Sugar Company (MSC) and Agrochemicals and Food Company Limited (ACFC). The elevated levels of conductivity, TDS and turbidity below ACFC / MSC can be closely linked to the industrial effluent

from the factories, particularly ACFC whose effluent has a characteristic persistent brown colour(Raburu & Okeyo-Owuor, 2006).

Data on turbidity levels and increase in nutrient and sediment loads in River Nyando in Kenya indicates the river is carrying pollutant loads such that aquatic and animal life is severely impacted. Sugar processing and agro-chemical factories in the "sugar belt" have been shown to contribute significant amount of phosphorous in the river (Okung'u 2004).

The ACFC is a downstream value addition on molasses, an otherwise putative waste from sugar milling, to produce ethanol and spirit beverages. However, the process of ethanol manufacture is highly water intensive and releases large volumes of high strength effluent. Personal communication from ACFC revealed that the raw distillery effluent generated from ethanol processing is characterized by high biological oxygen demand (BOD_5, 20°C (30,000-60,000 mg L^{-1}), high chemical oxygen demand (COD) (80,000-100,000 mg L^{-1}), intense dark brown colour and objectionable odour(Macharia, 2011).

The dark brown colour of distillery effluent (DE) is a property of water-soluble recalcitrant colouring compounds called melanoidins that are contained in molasses. Melanoidins are natural condensation products of sugars and amino acids produced by non-enzymatic browning reactions called Maillard reactions(Plavsic, Cosovic, & Lee, 2006), and are highly resistant to microbial attack. Conventional biological processes such as activated sludge treatment processes, are insufficient to treat them (Evershed et al., 1997).

Researchers across the world have shown that ratio of wastewater generation per litre of alcohol production in distilleries is approximately 15:1(Baskar, Kayalvizhi, & Subash Chandra Bose, 2003; Delgado & Casanova, 2001). In Kenya, the national production of

approximately 25-31 million litres of alcohol per year results in sequential discharge of approximately 420 million litres of DE(Macharia, 2011).

Generally, alcohol distilleries have been classified among the most polluting industries in the world (Saha, Batra, & Balakrishan, 2005). Besides the high organic load and melanoidins, other potential contaminants may result from processes in cooling systems, sanitary and hygienic cleaning to contribute to variability in composition of the DE (e.g. heavy metals, oil and grease, microorganisms and suspended solids (Delgado & Casanova, 2001; Wilkie, Riedesel , & Owens, 2000).

The increasing number of molasses-based distilleries in India, a major producer of sugar in the world, has led to substantial increase in industrial pollutant loads (Malaviya & Sharma, 2011). River pollution near alcohol distilleries utilizing molasses is also a major problem in Brazil (WWF, 2004) .

Generally, main focus of wastewater treatment plants is to reduce the BOD level before discharging into natural waterways. Primary treatment of effluent from ACFC involves anaerobic digestion in the bulk volume fermentor (biomethanation) for 28 days to produce biogas. Secondary treatment involves subjection to activated sludge and eventually it is held in lagoons for stabilization (44 days), before final discharge. These treatment processes result in reduction of the BOD and COD values by 90 and 70 % , respectively (Macharia, 2011).

A number of studies indicate that DE is beneficial for plant growth due to the high nutrient loading, including potassium, sulphur, nitrogen and phosphorous, as well as easily bio-degradable organic matter (Chidankumar, Chandraju , & Nagendraswamy, 2009; Zalawadia, Raman, & Patil, 1997).

The present investigation was a part of systematic work undertaken to study the application of DE on plants in an effort to utilize the nutrient content of the effluent, while providing supplemental treatment to the effluent. This research aimed at providing knowledge on environmentally sound techniques for management of DE so as to minimize pollutant loads and protect the intergrity of the river system downstream of ACFC.Plants commonly grown in the Western Kenya sugar belt were cultivated in pots and their growth characteristics and their abilities for uptake of selected metal contaminants in effluent were studied.The study findings contribute knowledge on the benefits of DE on crops production vis-à-vis social, economic, health and environmental parameters, as well as crop selection criteria for effluent irrigation.

1.3 Objectives of the study

1.3.1 General objective

To evaluate the potential for *Ipomoea batatas*, *Saccharum officinarum*, *Zea mays* and *Eucalyptus grandis* to uptake selected metals contaminants from distillery effluent

1.3.2 Specific objectives

- To determine the levels of Cd, Cu and Zn in soil and tissues of plants irrigated with treated distillery effluentand tap water.
- To find out whether treated distillery effluent contributes additional levels of Cd, Cu and Zn in soils and plants, so as to render them dangerous to human and environmental health.

- To determine the levels of nutrient (N and P) in plants irrigated with treated distillery effluent
- To study the detoxification effects of *Ipomoea batatas*, *Saccharumofficinarum*, *Zea mays* and *Eucalyptus grandis* on treated distillery effluent on marine bacterium *Vibrio fischeri.*

1.4 Research hypothesis

- $H0_1$: Deposits of heavy metals in plant tissues and soil irrigated with tap water do not exceed those deposited by treated distillery effluent
- $H0_2$:Levels of Cd, Cu and Zn in soils and plants irrigated with treated distillery effluent do not exceed NEMA standards
- $H0_3$: There is no difference in plant growth characteristics between plants irrigated with treated distillery effluent and those irrigated with tap water.
- $H0_4$: Leachate that filters from pots irrigated with treated distillery effluent does not reduce the intensity of bioluminescence in *Vibrio fischeri.*

1.5 Justification

Good environmental quality is prerequisite for the health and well-being of human beings, plants and animals. The physical, chemical and biological nature of river systems is greatly altered by wastewater discharges from urban and industrial water uses. Through water conservation and recycling, Kenya can achieve environmental sustainability and still have a viable economy.This study highlightsRiver Nyando as one of the most degraded of all the rivers in Lake Victoria basin, with significant pollution emanating

from the sugarcane industry. The quality of water downstream of the Sugar industries is negatively impacted by effluent discharges, both treated and untreated, particularly from molasses-based alcohol distillation which have intense dark brown colour. Though degradation and decolourisation of distillery effluent has been accomplished in countries that are leading producers of sugar, the methods are not economically feasible in Kenya due to cost limitations. Therefore, distilleries in Kenya employ biomethanation to treat their effluent. The development of phytoremediation is driven primarily by the rising cost of available treatment technologies. Soil-plants systems are capable of utilizing nutrient rich effluents as well as remove, transfer, stabilize or degrade contaminants present in the effluent may be a less destructive and cost-effective approach to safeguard water quality. The production of effluent-irrigated energy crops and forest plantations in close proximity to distillery industries certainly represents an ecologically sound method for disposal of effluent through the avoidance of disposal into natural waterways. This minimizes the pollutant loading of the natural water ways and leads to protection of ecosystems downstream of the distillery industry.

CHAPTER TWO

LITERATURE REVIEW

2.1 Water

Access to water is not just a fundamental right, but also a condition for attaining wider human development goals. With increasing world population, there is intensifying competition for fresh water resources by domestic, agricultural, industrial and commercial sectors (Veenhuizen, 2006). According to the 'Falkenmark Indicator' (Falkenmark, 1989) , a widely applied water scarcity index, a country is categorized as 'water stressed' if its annual renewable freshwater resources (ARWR) are between 1,000 and 1,700 cubic metres per capita, and 'water scarce' if its ARWR are less than 1,000 cubic metres per capita(2006 UN, 2006; UNDP Human Development Report, 2006).

About 8.3 % of the countries around the world are classified as 'water scarce' , while 9.8% are considered as 'water stressed'(Brown & Matlock, 2011). With the rapidly declining ARWR per capita availability, Kenya is in the category of water scarce countries (Transparency International Kenya, 2011).The average annual rainfall over the country is 630 mm with a variation from less than 250 mm in the arid and semi-arid land to over l800 mm in the Lake Victoria Basin (FAO, 2009). So far, the main consumers of water in Kenya are industries, agriculture (horticulture and livestock), energy production and domestic consumption, in reducing order (2007. Government of the Republic of Kenya, 2007).

Agriculture, urbanization and industries contribute to organic, inorganic and aesthetic pollution of water through wastewater discharges. The levels of pollutants in wastewater vary according to source of generation and level of treatment applied (Atwell, Kriedemann, & Turnbull, 1999). In Kenya, industrial wastes of environmental concern include polluted effluents, sludge and solid waste from sugar, coffee pulping and textile factories, leather tanneries, paper mills and slaughter-houses (1999 UN, 1999).

2.2 Wastewater from molasses processing

Molasses is the dark brown, thick, viscous substance produced by sugar mills characterized by an acidic nature, rich salts and also contains sugar which did not crystallize during the process of sugar manufacture. Specific strains of yeast are used in the distillation and fermentation of molasses to produce ethanol for potable and industrial uses(Mohana, Acharya, & Madamwar, 2009).

The terms ‘distillery effluent’ (DE) refers the aqueous by-product resulting from the production of ethanol. The production and composition of DE is highly variable and depends on the quality of molasses and various aspects of the ethanol production process. The concentration of sugars in molasses, through crystallization and evaporation of cane juice increases the content of non-fermentable organics which remain in the DE after fermentation, augmenting COD and increasing the COD/BOD ratio(Atwell, et al., 1999). Researchers have shown that DE is highly acidic and contain large quantities of soluble organic matter and plant nutrients (Baskar, et al., 2003).Unpleasant characteristics include intense dark brown colour and objectionable odour(Ramadurai & Gearard, 1994). The dark brown colour of DE is due to presence of water-soluble recalcitrant colouring

compounds called melanoidins. Melanoidins are natural condensation products of sugars and amino acids produced by non-enzymatic browning reactions called Maillard reactions (Plavsic, et al., 2006) and are a source of soil and aquatic pollution (Chandra, Bharagava, & Rai, 2008).Several studies reveal that these compounds are mutagenic, carcinogenic and cytotoxic (Borrelli et al., 2003; Silvan, Lagemaat, Olano, & Castillo, 2006).

Wash water used for cleaning the fermentation jars, cooling water blow down, and boiler water blow down may contribute to variability in composition of DE. Potential pollutants in DE include heavy metals, oil, grease and cleaning agents (Wilkie, et al., 2000).Other compounds present in DE include low weight molecular components such as lactic acid, glycerol, ethanol and acetic acid.Phenol compounds and heavy metals have also been found in effluent from molasses-based distilleries (Pant & Adholeya, 2007).

2.2.1 Treatment of distillery effluent

The discharge of raw DE to open land or nearby water bodies poses serious environmental risks. In Gorakhpur District of Nepal, the discharge of improperly treated effluent into a nearby stream by two sugar factories and a distillery rendered the stream's water unfit for drinking, bathing or irrigation (WWF, 2004).

Though the degradation and decolourisation of distillery effluents by chemical methods (Chandra & Singh, 1999), flocculation treatment and physico-chemical treatment such as ozonation and activated carbon adsorption have been accomplished, these methods are not economically feasible on large-scale because of cost limitations.

A mix population of microorganisms have been used to degrade organic matter in effluent to result in biogas production, which is a readily usable fuel for alcohol distilleries (Wilkie, et al., 2000). This treatment, otherwise known as biomethanation, reduces the oxygen demand of the effluent and increases in nitrogen (N), potassium (K) and phosphorous (P) and lowers the contents of calcium (Ca), magnesium (Mg), sodium (Na), chlorine (Cl) and sulphates (SO_4^{2-}) (Chidankumar, et al., 2009).

So far, biological decolourisation of DE by use of fungi such as *Coriolus*, *Aspergillus*, *Phanerochaete* and certain bacterial species such as *Bacillus*, *Alkaligenes* and *Lactobacillus* , has been successfully achieved as a bioremediation technique for distillery effluents (Chandra, et al., 2008; Kumar & Chandra, 2006) .

2.2.2 Use of distillery effluent

Where industrial effluents are rich sources of plant nutrients, soil provides a logical sink for their disposal (Antil, Arora, & Kuhad, 2011). A number of studies indicate that DE is beneficial for plant growth due to the high nutrient loading, including potassium, sulphur, nitrogen and phosphorous, as well as easily bio-degradable organic matter (Chidankumar, et al., 2009; Zalawadia, et al., 1997).

A study on soil properties and crop yields on a vertisol due to application of DE resulted in increased yield of soybean and wheat crops. It is envisaged that the DE enhanced fertility in soil and improved soil physical environment, which might have led to better germination, root proliferation, nutrient and water uptake by the crops and greater biomass production (Chidankumar, et al., 2009).

Preliminary studies on cotton plants to compare effect of application of DE and fresh water indicated that DE-fed plants fared better than those fed with fresh water, and gave greater plant height, tap root length, and seed cotton yield as well as increased the value of most soil parameters (Rajvanshi & Nimbkar, 2004).

A study on pea plants revealed that DE led to increases in growth of shoot length, leaf number per plant, leaf area and chlorophyll content(Thakare, Chaudhary, & Pokale, 2013). The application of DE to soil also increased yield of sugarcane, wheat and rice (Chidankumar, et al., 2009; Zalawadia, et al., 1997).

Vvarious distilleries in India allow direct application of DE to land for pre-sowing irrigation (Atwell, et al., 1999) and to crops , after proper dilution with irrigation water (1:10 to 1:50) .The effluent is also applied on soil, 40-60 days before planting (pre-sowing application), to allow for the natural oxidation of organic matter (Baskar, et al., 2003). Other applications of DE include their use for composting other sugar industrial by-products, such as bagasse and pressmud, which in turn to enables the degradation of coloured organics and rapid evaporation of water, hence reducing the BOD level. The technology of using DE for composting has been successfully tested in so many places (Baskar, et al., 2003).

Globally, the health considerations of use of wastewater in agriculture are centered on inherent pathogenic organisms, build-up of toxic materials within the soil, and subsequently within plant and animal tissues, eventually reaching the human food chain (Mutengu, Hoko, & Makoni, 2007). There is concern on the ability of DE to pollute soils and groundwater, if used in large amounts. The effect of DE is crop-specific and due care should be taken before use for irrigation purpose (Malaviya & Sharma, 2011).

Irrigation with DE for example, was found to suppress germination of peas in Balrampur, India (WWF, 2004) .

2.2.3 Health risks of using distillery effluents in agriculture

Excess nutrients

Maintenance of adequate levels of nutrients in effluents remains a challenging task due to the possible negative impacts of their excessive addition to soils. These nutrients may cause environmental degradation if over-applied to crops, or if wastewater is discharged into surface or marine waters (Wilkie, et al., 2000).

Nitrogen is a necessary macro element for plants and can be found in effluent in the form of nitrate (NO_3), ammonia (NH_3), organic nitrogen and nitrite (NO_2). Together, these forms constitute total N (WHO 2006).

Phosphorous is an essential element for plants but is usually scarce in soils in a form that is bioavailable to plants. In effluent, P exists in many forms but is normally expressed as total P. Orthophosphates H_2PO_4, $HPO42^-$ and PO_4^{3-} are available immediately for soil-plant biophysical reactions. The polyphosphates are broken down more slowly to orthophosphates while organic phosphates are broken down biologically to polyphosphates and then to orthophosphates (USA EPA, 2004). However, P is relatively stable and may accumulate especially at or near the soil surface (J. H. Lee & Doolittle, 2002).

Excess levels of N and P may lead to excessive vegetative growth, delayed maturity and low quality agricultural yields as well as eutrophication in receiving water bodies. Leaching of N can lead to groundwater contamination and methaemoglobinemia

(decreased ability of blood to carry vital oxygen around the body, generally in infants) in case of drinking N-rich groundwater -particularly high levels of nitrates (A. Scott, Zarazua, & Levine, 2000).

Salinity

There is growing concern onuse of DE due to its significant quantity of salt. According to Chidankumar*et a.*, 2009 , continual application of DE is projected to affect the physical and physicochemical properties of soil and crop growth by increasing soil salinity (Hati, Biswas, Bandyopadhyay, & Misra, 2007).

Excessive salinity stunts crop growth by reducing the availability of soil-water, slowing crop growth and restricting root development. With higher salinity water, sodium and chloride toxicity are also likely to be evident.

Toxicants

Numerous toxicants such as heavy metals and organic compounds are increasingly being released to the environment due to increasing industrialization. Unlike organic contaminants, most heavy metals are not biotransormed and persist in the environment (Wang et al., 2009). Potential pollutants in DE include heavy metals, oil, grease and cleaning agents. While some heavy metals may be introduced from the feed stock (e.g. molasses) and chemicals used, the corrosion of piping , tanks and heat exchangers is expected and they may contribute to heavy metals in the effluent(Wilkie, et al., 2000).

A study to characterize chemical, physico-chemical and physical properties of molasses wastewater (MW) from the Sudanese fermentation industry, determined it contained

relatively high levels of micro-elements: 0.525 mg L^{-1} (cobalt), 0.141 mg L^{-1} (cadmium), 1.17 mg L^{-1} (copper) , 26.82 mg L^{-1} (manganese),0.657 mg L^{-1} (lead) and 243.9 mg L^{-1} (iron), and macro-elements 443 mg L^{-1} (sodium), 7000 mg L^{-1} (potassium), and 3810 mg L^{-1} (calcium) (Sulieman, Yousif, & Mustafa, 2010).

Depending on their concentration levels, heavy metals may exert beneficial or harmful effects on plant, animal and human life. Some heavy metals are toxic to living organisms, even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. When ingested in excess, they combine with biomolecules in the body, such as proteins and enzymes to yield stable biotoxic compounds, mutilating their structure and interfering with normal functioning (Muzyed, 2011).

Although the concentrations of heavy metals in DE are usually relatively low, long-term application of these effluents on land can eventually result in heavy metal accumulation in soil. A study to characterize chemical, physico-chemical and physical properties of molasses wastewater (MW) from the Sudanese fermentation industry, determined it contained relatively high levels of macro-elements and micro-elements: 0.525 mg L^{-1} (cobalt), 0.141 mg L^{-1} (cadmium), 1.17 mg L^{-1} (copper) , 26.82 mg L^{-1} (manganese),0.657 mg L^{-1} (lead) and 243.9 mg L^{-1} (iron), and macro-elements 443 mg L^{-1} (sodium), 7000 mg L^{-1} (potassium), and 3810 mg L^{-1} (calcium). The study also showed that MW had high BOD (640 mg L^{-1}) and COD (4500 mg L^{-1}). The discharge of the organic-rich effluent accelerates bacterial growth and decomposition of organic matter consumes the oxygen levels in natural waterways (Sulieman, et al., 2010).

2.2.4Selected heavy metals and their toxicity

Cadmium

Cadmium is the second element in Group IIB of the periodic table. It has an atomic number of 48, an atomic weight of 112.41 and a valence of 2 .The specific gravity of Cd is 8.65 and ionic forms of the metal (Cd^{2+}), combine with oxygen (cadmium oxide, CdO), chlorine (cadmium chloride, $CdCl_2$), or sulfur (cadmium sulfate, $CdSO_4$) (Muzyed, 2011).

The average abundance of Cd is as follows: 0.16 parts per million (ppm) in the earth's crust 0.1 to 0.5 ppm in soils ,1 mg L^{-1} in streams and 1 to 10 mg L^{-1} in ground waters(APHA, 1999).The source of Cd in the environment is through industrial waste processes such as electroplating, plastic manufacture, mining, paint pigments, alloy preparation and batteries that contain Cd. Household appliances, automobiles and trucks, agricultural implements, airplane parts, industrial tools and fasteners of all kinds (e.g., nuts, bolts, screws and nails) are commonly coated with Cd. Cadmium is widely used for luminescent dials, in photography, rubber curing, and as fungicides (Adriano, 2001).

Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products. Enrichment of agricultural soils with phosphate fertilizers is the most documented source of Cd-contamination (Nziguheba & Smolders, 2008) . In Peninsular Malaysia, a study revealed that elevation of Cd in soil and excessive concentrations of Cd in cocoa (*Theobroma cacao*) was the result of application of phosphate fertilizers (Zarcinas, Ishak, McLaughlin, & Cozens, 2004).

Plants accumulate Cd to the levels may not be toxic to the plants themselves but are toxic to their consumers. Animals accumulate Cd in the kidney, liver and reproductive organs. Cadmium toxicity affects humans more than animals, because of their longevity and the accumulation of Cd in their organs by eating Cd-contaminated food (Tudoreanu & Phillips, 2004).

Elevated levels of Cd in humans can cause kidney damage, while low levels of Cd in the diet are linked to renal dysfunction. Other diseases associated with Cd exposure are pulmonary emphysema and the notorious Itai-Itai ('ouch-ouch') disease that results in painful demineralization (osteoporosis) because Cd replaces calcium in the bones. Because of this reason, Cd is listed as one of the metals under scrutiny by the U.S. Environmental Protection agency (Yeung & Hsu, 2005).

Copper

Copper (Cu) , the first element in Group IB in the periodic table, has an atomic number of 29, an atomic weight of 63.55 and valences of 1 and 2.The specific gravity of Cu is 8.96 (Muzyed, 2011).

The average abundance of Cu is 68 ppm in the earth's crust,9 to 33 ppm in soils,4 to 12 mg L^{-1} in streams, and less than 0.1 mg L^{-1} in ground water. Copper occurs in its native state, but may also found in many minerals. The important ores of Cu include Chalcocite ($CuFeS_2$), Cuprite (Cu_2O) and Malachite [$Cu_2CO3.OH_2$]. It is widely used in electrical wiring, roofing, various alloys, pigments, cooking utensils, piping, and in the chemical industry. The main alloys of Cu are brass (with zinc) and bronze (with tin). Other applications are kitchenware, water delivery systems, and copper fertilizers. Copper salts

are also used in water supply systems to control biological growths in reservoirs and distribution pipes and to catalyze the oxidation of manganese (APHA, 1999).

Copper forms a number of complexes with inorganic and organic ligands in natural waters. Corrosion of copper-containing alloys in pipe fittings may introduce measurable amounts of the metal into the water in a pipe system. Though Cu is considered an essential trace element for plants and animals, some of its compounds are toxic by ingestion or inhalation (APHA, 1999).

Copper is essential as a constituent of metalloenzymes of living organisms and is required for synthesis of haemoglobin and catalysis of metabolic reactions. The metal has distinct oxidation states and when bound to protein, it is a cofactor in many redox reactions because of its ability to cycle between the oxidized Cu^{2+} and Cu^{+} states (Bradi, 2005).

The redox properties of Cu make it particularly useful and yet harmful, in biological systems(Mehta, Templeton D, & O'Brien, 2006). Excess Cu leads to the generation of free radicals by redox cycling in Fenton-like reactions to produce reactive oxygen species(ROS),which cause oxidative damage (Tudoreanu & Phillips, 2004; Xie et al., 2006).

Zinc

Zinc is the first element in Group IIB in the periodic table, has an atomic number of 30, an atomic weight of 65.38, a density of 7.13 and a valence of 2 (Bradi, 2005). The average abundance of Zn is 76 ppm in the earth's crust is; 25 to 68 ppm in ,20 mg L^{-1} in streams and less than 0.1 mg L^{-1} in ground waters. The most common ores of Zn are zinc

sulphide (ZnS), zincite (ZnO), and smithsonite ($ZnCO_3$). Zinc is fourth most used metal after iron (Fe), aluminum (Al), and Cu(APHA, 1999; Momtaz, 2002).

The sources of Zn in the environment include industrial activities such as mining, coal and waste combustion and steel processing. The metal is used in a number of alloys such as brass and bronze, and in batteries, fungicides, and pigments (Bradi, 2005).

Zinc is an essential growth element for plants and animals, but at elevated levels it is toxic to some species of aquatic life. It is present in many enzymes involved in important physiological functions like protein synthesis. In plants and animals therefore , excess amount of Zn can cause system dysfunctions, cause impairment of growth (Duruibe, Ogwuegbu, & Egwurugwu, 2007).

Zinc enters the domestic water supply from deterioration of galvanized iron and dezincification of brass. In such cases, lead (Pb) and Cd also may be present because they are impurities of Zn used in galvanizing. Zinc in water also may result from industrial waste pollution. It may increase the acidity of waters. Some fish can accumulate Zn in their bodies, when they live in Zn-contaminated waterways. When the metal enters the bodies of fish, it is biomagnified up the food chain (Muzyed, 2011).

Leaching of water-soluble Zn from soils can contaminate groundwater. Plants often have a maximum Zn level that their systems cannot handle. Soil activity can also be affected by elevated levels of Zn as it negatively influences the activity of microorganisms and earthworms thus retarding the breakdown of organic matter (APHA, 1999).

2.3 Pot experiment

2.3.1 Test crops

Sweet potato

Sweet potato (*Ipomoea batatas*) belongs to the convolvulaceae family. It is a perennial root crop grown from vine cuttings or transplants produced by bedding mother roots, or from rooted cuttings. The crop has adventitious roots normally found within the top 25 cm of soil. Tubers vary in shape , colour and structure. The vines may runup to 4 m, usually prostrate and slender. Depending on variety, the leaves are green or purplish, chordate, palmately-veined, borne on long petioles (Ecocrop, 2010).

The crop is highly valued for its fibre and starch content and is also rich in vitamins A,C and B6, complex carbohydrates, proteins, iron and calcium. Besides, sweet potato leaves and shoots can be used by humans as greens. It is also a valuable animal feed. Dry vines have feed value which compares favorably with hay as forage. For example, tubers are relished by pigs and cattle and tuber processing by-products may be fed to livestock. The crop is also used industrially in the manufacture of starch and alcohol (Woolfe, 1992).

It is considered a major staple food only in a few countries but is much appreciated as alternative food in most countries. In Africa, the crop is cultivated as a security crop or famine prevention crop (G. J. Scott & Wiersema, 1993).

In Kenya, sweet potato is the third most widely used food after maize and potatoes. It is grown by small-scale farmers mostly in Western, Nyanza and parts of Central and Coastal provinces, as a dual –purpose crop; the vines are fed to livestock whereas tubers are used as human food. Two cultivars of sweet potato, namely *Musinyamu* and *Toilo* are the most commonly grown (Orodho, 1990).

Eucalyptus

Globally, eucalyptus trees are the most widely cultivated forest trees and comprise more than 900 species and unknown hybrids and varieties. Most eucalyptus species (eucalypts) occur naturally in Australia. A few species are naturally found in Philippines, Papua New Guinea, Indonesia and Timor. The major eucalypts growing countries are China, India and Brazil. In Africa, South Africa has the largest area under eucalypts(Oballa, Konuche, Muchiri, & Kigomo, 2010).

Eucalypts grow in diverse ecological conditions with some hardy species growing in semi-arid areas, while others are able to grow on marshy and swampy sites. Eucalypts also grow under a variety of soils including fertile loamy soils, infertile sands and heavy clays. They are used for fuel wood, timber, plywood, transmission poles, pulp, building materials, fencing posts, wind breaks and ornamentals(Stape, 2002; Teketay, 2003).

In Kenya, the major eucalypts growing areas are Western Region, Central Rift valley, Central Kenya and parts of Eastern and Coastal Regions. The area under eucalypts is expected to increase with increasing demand for transmission poles to cater for the ongoing expansion of the rural electrification, and for construction, fuel wood, carbon sequestration and mitigation of climate change effect. Propagation of eucalyptus is from seed and through vegetative means. *Eucalypts grandis* is one of the tree species commonly grown in Kenya. This is a fast growing tree that can grow to a height of up to 50 m and diameter at breast height (dbh) of 2 m (Oballa, et al., 2010).

Sugarcane

The sugarcane plant is a tall bamboo-like grass that grows to a height of up to 6 m and is largely grown in tropical countries. The crop is propagated from stem cuttings or sections

of the stalks called ‘sets’ or seed pieces. The main product of sugarcane is sugar, constituting about 10 percent of the crop. Eighty percent of the world’s sugar is derived from sugarcane, while the remaining 20 percent is produced from sugar beet (WWF, 2004).

Besides sugar production, other by-products of the sugar industry are bagasse, molasses, filter mud cakes and cane wax. Bagasse is a residue used as fuel, livestock feed and for the manufacture of fiber board, paper pulp and cellulose. Molasses are fed to livestock, used for industrial purposes, in confectionary, and also in alcohol distillation. Filter mud cakes are used as fertilizer ,while cane wax is used in the production of furniture, shoe, leather polish, electrical insulating material and waxed paper (Ecocrop, 2010).

Two largest producers of sugarcane in the world are Brazil and India. In Africa, Sudan is the leading producer of sugarcane(Sulieman, et al., 2010).

The Kenyan sugarcane industry is second largest contributor to agricultural growth (about 15 per cent of the agricultural Gross Domestic Product (GDP), after tea. Farm households and businesses depend largely on the injection of cash derived from the sugar industry (Kenya Sugar Board 2011).

Western and coastal climates of Kenya offer ideal climatic and weather conditions for growth and cultivation of sugarcane. With above average rainfall and good temperature supported by deep fertile soils with good water holding capacity, these regions produce the bulk of sugarcane in the country (Kenya Sugar Board, 2008).

Maize

Maize is the second most important cereal grain after wheat in the world. It is an annual grass, whose sizes vary greatly depending on race and growth conditions .The commercial types mostly grow up to a height of 2 m.

Maize is of great economic significance worldwide because it represents a major staple food, particularly in Africa, Latin America and Asia, and a major feedstuff in developed countries. In Africa, the largest producers of maize are Nigeria and South Africa (FAO, 2009). In Kenya, is it is estimated that one out of every two acres of land pt to crop production is under maize (FAO AQUASTAT, 2005).

Maize can also be fed whole to livestock (grazed or chopped and ensiled). Animals can feed on the leaves and stalks left in the field after the harvest (maize Stover). By-products of maize plant (hominy feed, bran, germs, oil meal), starch (corn gluten feed, corn gluten meal) and alcohol / bio-fuel industries (distillers' dried grains and soluble) , can be fed to animals (Ecocrop, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 FIELD EXPERIMENT

Pot experiments were carried out on a field plot in UoE between the months of September 2012 and February 2013. Polyethylene (PE) plastic film was fitted onto the wooden posts by use of nails, to provide cover for the roof and walls of an open greenhouse structure available at the field plot, in which the pot plants were housed to shield them from rain.

The soil used in the pot experiments was obtained from selected spots on the field plot. First the field plot was divided into four and 'V' shapes made on each section with the help of sticks tied to a string. Three sub-sample spots were randomly marked on each arm of the 'V' shapes and soils were collected by use of a garden trowel from upper horizon (0-20 cm) and filled into 20-Kg plastic buckets. A composite sample was made by mixing all the soils collected thoroughly and carefully, by use of a shovel, to ensure exclusion of vegetation, stone or other solid materials in the soil.

A weighing balance (SC-30 KAM series, United Kingdom), was used to weigh 18 kg of soil into34-incomplete conical-shaped plastic buckets (mouth and base diameter measurements of 39 cm and 24 cm)of capacity of 20 L. The buckets were perforated with 7 uniformly spaced holes (1 cm diameter each) at the base. The buckets were loaded onto 5-L basins, of uniform diameter (28 cm), in order to contain leachate that filtered from the buckets.

The planting materials included : certified commercial variety maize seeds 'H626' from the Kenyan market; pre-germinated stem cuttings of sugarcane 'NCO376', obtained

from a farmer in Muhoroni , Kisumu County; vines cuttings of sweet potato ‘Spk004’ obtained from a farmer in Lugari, Kakamega County; and, Kenya Plant Health Inspectorate Services (KEPHIS)-improved eucalyptus seedlings from Pan African Paper Mills nursery in Kaptagat, Uasin Gishu County. The irrigation water included treated distillery effluent (TDE) from the Agrochemicals and Food Company (ACFC), in Kisumu County, and tap water (TW) from Eldoret Water and Sewerage (ELDOWAS), a public supply in Uasin Gishu County.

Four replicates of each test crop were established for the TDE and TW irrigation water treatments. A maximum of two plantlets per test crop were grown in the buckets to yield a total of thirty two pot plants. Two buckets were set up as controls and they comprised of soils only (see Plate 1).

The pot plants were labeled as TDE 1, TDE 2, TDE 3, TDE 4, TW 1, TW 2, TW 3 and TW 4 according to the type of water quality treatment that was applied. Similarly, the control pots were labeled as TDE - C and TW - C (Table 1). The control pots were irrigated TDE and TW irrigation, in the similar manner as pot plants.

Table 1: Layout of the field experiment

	TDE				TDE			
Sweet potato	1	2	3	4	1	2	3	4
Eucalyptus	1	2	3	4	1	2	3	4
Sugarcane	1	2	3	4	1	2	3	4
Maize	1	2	3	4	1	2	3	4
Controls	TDE - C				TW- C			

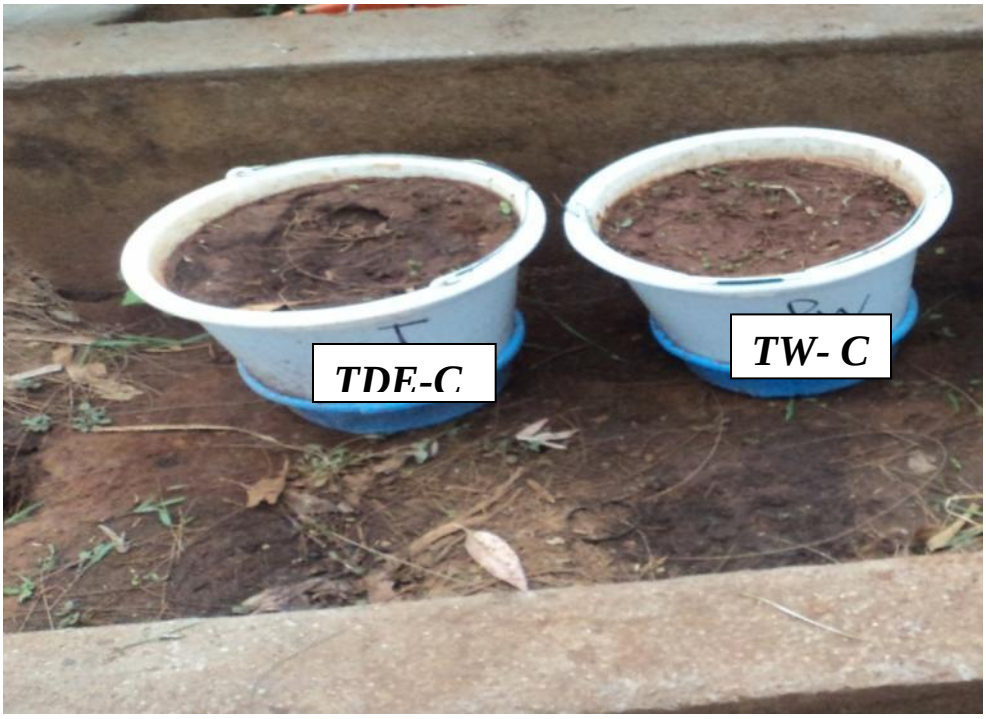

Plate 1: Control pots (Source: Author, 2012)

The plantlets were irrigated with 3 L of TW three times weekly, for the first two months to allow for their germination of and adjustment to the inherent soil environment, and leachate that collected in the basins was regularly returned back to the pots (in the evenings) , to avoid drying up. After two months of irrigation with TW, the irrigation regime was changed to include TDE irrigation as treatment.

Samples of TDE irrigation water were obtained from ACFC, at the point of discharge of the effluent treatment plant, by use of clean 20-L plastic jerricans, plastic filter funnels and 1.5-L calibrated measuring jugs. The jerricans were tightly sealed and transported to the site of the field experiment, in order to carry out irrigation on the same day. It is important to note that the frequency of irrigation of the pot plants and volume of water used for irrigation per pot (3L), were maintained constant. This irrigation regime was continued for a period of four months.

Field management of the pots included regular hand weeding and thinning to remove unwanted plant growth and maintain only two plants per pot. Physical measurements of stem and leaf lengths, and leaf counts per plant, were carried out on the 60^{th},90^{th} and 120^{th} day, and recorded in a field note book. Observable changes in plant growth were also noted. The data was used to analyze for growth variations in individual test crop species in the two irrigation treatments.

3.2 SAMPLE COLLECTION

3.2.1 Irrigation water and leachate samples

Samples of the two irrigation water treatments and leachate from the pot plants, including controls, were collected in the 3^{rd}, 5^{th} and 6^{th} months, for the analysis of three heavy metals (Cd,Cu and Zn). All leachates for this purpose were collected a day before carrying out irrigation.

The irrigation water samples and leachates for toxicity screening tests were collected on the 6^{th} month of the experiment and did not require any pretreatment because they were analyzed on the same day of collection.

Clean 500-mL plastic PE bottles were used for sample collection and were labeled appropriately before transporting to the laboratory for analysis.

3.2.2 Soil and plant tissue samples

Plant tissue and soil samples for heavy metal and nutrients (N and P) analysis were collected at the end of the experiment (6^{th} month). Equivalent weights of 100 g of soil were collected from the upper 10-15 cm of each pot, by use of a soil auger, into clear

polythene paper bags. To avoid cross-contamination, the soil auger was cleaned appropriately between samples.

Aerial plant parts(stems and leaves) were sampled using a stainless steel pair of scissors, while roots and tubers were scooped using a stainless shovel. Maize grains were hand-picked together with their cobs. The different plant parts were put into separate clean plastic bags. All samples were assigned appropriate identification codes and transported to the laboratory for analysis.

3.3 LABORATORY ANALYSIS

3.3.1 Apparatus, reagents and chemicals

For heavy metal and nutrient analysis, high purity salts for the preparation of stock solutions and analytical grade reagents (AR) which included concentrated nitric (HNO_3), hydrochloric(HCl),sulphuric(H_2SO_4)acids, and 30 % v/v hydrogen peroxide (H_2O_2) (Merck, Germany) , were used.

High purity (99.99%) metal strips of Cu, Cd and Zn metals were purchased from Sigma Aldrich agents in Nairobi. The Toxi-Screening Kit*MicroBiotest* ® (Kleimoer, Belgium) was used to study detoxification effects of the various pot plants. Distilled water was prepared using ManestyL4 Machine (United Kingdom), temperature set at 400-500°C.

Plastic bottles and glassware were rinsed and soaked in 10% (v/v) HNO_3 overnight and rinsed with double distilled water before use for sample analyses. All the analytical procedures were carried out in clean, dust-free laboratory area to avoid contamination of samples.

3.3.2Sample preparation and digestion

Soil and plant tissue samples

The analysis of environmental samples often requires measurement of total (suspended and dissolved) metals as well as soluble (dissolved) elements. Therefore, digestion of samples was done to reduce interference with organic matter and convert elements associated with particulates to forms that can be determined by instrumental analysis.

Soil samples were air–dried at room temperature and crushed using pestles and agate mortars. Individual soil samples were passed through 2-mm and 0.02-mm sieves, respectively, and exactly 0.3 ± 0.001 g of each sample were weighed with the help of spatula and an analytical balance (CA-124, India) , into clearly labeled paper envelopes.

Plant tissue samples were carefully washed with distilled water for at least 3 minutes to remove any soil particles adhered to them. The plant parts were chopped into small pieces, put in glass petri-dishes and oven-dried for 48 hours at 150°C. The dried plant samples were crushed by use of pestles and agate mortars and 0.3 ± 0.001 g of each sample were weighed into clearly labeled paper envelopes.

The acid digestion mixture was prepared according to the procedure described in Parkinson and Allen wet oxidation digestion method (H_2SO_4-H_2O_2-Li_2SO_4-Se) (Parkinson & Allen, 1975).An equivalent volume of 350mL of 30 % v/v H_2O_2 was measured into a 500-mL volumetric flask followed by the addition of 0.42 ± 0.001 g of selenium (Se) powder and 14 g of lithium sulphate (Li_2SO_4), to the solution. The resultant solution was mixed well and 420 mL of concentrated H_2SO_4 was added slowly, while cooling in an ice bath.

Individual soil samples were digested by transferring the weighed samples (0.3 ±0.001g) to dry and clean digestion tubes and adding 4.4 mL of the digestion mixture. The resultant soil sample solutions were digested in a block digestor (Digestion system 20 model 1015, Sweden) at 450°C for 1 hour. All samples were digested in duplicates. The digests were filtered by use of whatman no. 42 (0.45µm pore size) filter papers, into 50-mL volumetric flasks and topped to the mark with distilled water.

The digests were transferred into 100-mL PE sample bottles and pre-treated by acidification to pH 2 by adding 2 mL of concentrated HNO_3,in order to prevent precipitation of metals, reduce adsorption of analytes onto the walls of the containers and to avoid microbial activity. The samples were stored at 4°C until the day of analysis.

Acid blanks of the digestion reagents were prepared in the same way as sample digests. During analysis, the values of blanks were used as background correction to ensure that the equipments read only the exact values of the parameters being analyzed. Therefore, each set of sample digests had an acid blank.

Plant tissue samples were digested and pretreated in the same manner as soil samples, but their digestion was done for a longer time (at 450°C for 2 hours), including the acid blanks.

Irrigation water and leachate samples

The irrigation water and leachate samples were digested according to the HNO_3 - H_2SO_4 APHA 3030 G method as per the National Environmental methods index (NEMI) (APHA, 1999).First of all, the samples contained in 500 mL PE bottles were mixed by thorough shaking by use of a vortex mixer (VM 300, USA). Equivalent volumes of 25

mL for each individual sample were measured into 25-mL volumetric flasks. The resultant solutions were acidified by use of concentrated H_2SO_4 to the methyl orange end-point. The solutions were mixed well and 3 mL concentrated HNO_3 added to each. The solutions were placed on hot plates, boiled slowly and allowed to evaporate to about 10-15 mL, with sequentially addition of 3 mL of $HNO_3$and 6 mL of H_2SO_4 and continual stirring.

The solutions were cooled and diluted to the mark with distilled water. Further heating was applied to dissolve all salts, after which they were cooled and filtered by use of whatmanno.42 filter papers into 50-mL conical flasks, topped up to the mark with distilled water and mixed thoroughly on vortex machine. At this stage, all the samples were digested and ready for analysis. This procedure was carried out for all samples as well as acid blanks.

Digestion was done in duplicates. The sample digests were transferred into clean, clearly labeled 100-mL PE sample bottles.All digests were pre-treated by acidification to pH 2 by adding 2 mL of concentrated nitric acid (HNO_3)and stored at 4°C until the day of analysis.

3.3.3 Determination of total nitrogen and phosphorous

Total N and P in soils and plant tissues was determined according to the standard operating procedure described by in Laboratory Methods of Soil and Plant Analysis –a working manual prepared by Okalebo*et al.,* 2002 .

Total Nitrogen

Reagent N1 was prepared by weighing 34 g of sodium salicylate, 25 g of sodium citrate and 25g of sodium tartarate and dissolving the salts in 750 mL of distilled water. An equivalent weight of 0.12± 0.001 g of sodium nitroprusside was added and the resultant solution made up to 1 L with distilled water.

A second reagent N2 was prepared by dissolving 30 g of sodium hydroxide in 750 mL of distilled water. The solution was allowed to cool and 10 mL of sodium hypochlorite was measured and added to it. The solution was mixed thoroughly on vortex machine and topped up to the 1L mark.

A stock solution of 2500 mg N $L^{-1}$was prepared by weighing an equivalent weight of 1.793 ± 0.001 g of ammonium sulphate into a 1-L volumetric flask and topping up to the mark with distilled water.

Standards were prepared by measuring 2.5mL of the digestion mixture (from wet oxidation digestion method, H_2SO_4-H_2O_2-Li_2SO_4-Se), into 20mL of distilled water contained in7, 100-mL volumetric flasks. Sequentially, 0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL of the stock solution (2500 mg N L^{-1}), were added to the volumetric flasks. Generally, the standard series contained 0, 25, 50, 75, 100, 125 and 150 mg N L^{-1}, respectively. These series were diluted in the ratio of 1:9 (v/v) with distilled water, to yield final concentrations of 0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg N $L^{-1,}$ respectively.

The digests and blanks were also diluted in the ratio 1:9 (v/v) with distilled water to match the standards. Using a micropipette, 0.1 mL of sample digests and blanks were added into clearly labeled test tubes and 5 mL of ReagentN1 was added while shaking on a vortex. The solutions were allowed to stand for 15 minutes, after which 5 mL of

reagent N2 was added while mixing thoroughly on vortex. This was left to stand for 1 hour for colour development.

The absorbance readings of standards and samples were measured using UV-Vis spectrophotometer (SPECTRO™ 30) at 665 nm and recorded. A graph of absorbance against concentrations was plotted for standards.Nitrogen concentration in a sample was expressed in percentage form. Therefore, to get the percent concentration of N, the formula below was used:

$\% N = (a-b) \times v \times 50 \div 1000 \times w \times al \times 1000$

Where,

a = concentration of N in the solution

b = concentration of N in the blank

v = total volume at the end of analysis procedure

w = weight of the dried sample

al = aliquot of the solution taken

Total phosphorus

A 5N H_2SO_4 solution was prepared by measuring 500 mL of distilled water into a clean 1-L beaker and placing it in cold water in a sink. An equivalent volume of 148 mL of concentratedH_2SO_4 was added to the beaker slowly with continual stirring. The resultant solution was allowed to cool and later diluted to the mark with distilled water.

To prepare Ammonium molybdate / antimony potassium tartrate solution, 12 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$) was weighed and dissolved in 250 mL of warm distilled water (about 50°C). Separately, an equivalent weight of 0.291±0.001g of

antimony potassium tartrate ($KSb.C_4H_4O_6$) was dissolved into 100 mL of distilled water and the two solutions added to 1000 mL of $5NH_2SO_4$. The final solution was mixed thoroughly and diluted with distilled water to make a volume of 2 L. The solution was transferred to a reagent bottle and stored in a cool, dark place.

Ascorbic acid reducing agent was prepared by dissolving 2.108± 0.001 g of ascorbic acid ($C_6H_8O_6$) in 400 mL of ammonium molybdate / antimony potassium tartrate solution. The resultant solution was mixed well on vortex. It is important to note that this particular solution expires within 24 hours and so it was used on the same day of preparation.

A standard stock solution of 1000 ppm P was prepared by weighing 1.0967± 0.001 g of oven-dried KH_2PO_4 into a 250-mL volumetric flask and topping up to the mark with distilled water (1 mL = 1 mg P). A 10-ppm P working solution was prepared by diluting 10 mL of 1000 ppm P standard stock solution to 1 L with distilled water.

Standards were prepared by measuring 0, 1, 2, 3, 4, 5 and 6 mL of the 10 ppm P working solution using a pipette into 7, 50-mL volumetric flasks. These standards contained 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm P, respectively. To each volumetric flask, 10 mL of ascorbic acid reducing solution was added and allowed to stand for 1 hour.

For each sample, 5 mL of the supernatant clear digest solution was measured into to a 50-mL volumetric flask containing 20-mL distilled water using a pipette, and 10 mL of the ascorbic acid reducing agent was added. The solutions were topped up to the mark with distilled water while shaking on a vortex and left to stand for 1 hour.

The absorbance readings of standards and samples were measured at 880nm wavelength using UV-Vis spectrophotometer (SPECTRO™ 30). A graph of absorbance against concentrations was plotted for the standards. The concentrations of P were determined by

subtracting the mean values for the blanks from the sample digests to give a value for corrected concentration (c). For example, if a blank = 0.2 ppm and the sample = 4.05 ppm, the corrected P concentration (c) would be = 4.05 - 0.20 = 3.85 ppm.

Therefore,

$$\% \text{ P in sample} = c \times v \times f \div w$$

Where:

c = the corrected concentration of P in the sample

v = volume of the digest

f = dilution factor

w = weight of the sample

In this study, where 5 mL aliquots were diluted with50 mL of distilled water, the formula for calculating the percent concentration of P was as follows:

$$\% \text{ P in sample} = c \times 0.05 \div w$$

3.3.4 Analysis of cadmium, copper and zinc

Sample digests of plant tissues, soil, water and leachates were analyzed for heavy metals by use of FAAS (AAS, Varian Spectra A200).

Preliminary studies involved the determination of presence of various heavy metals – cadmium (Cd), chromium (Cr); nickel (Ni), copper (Cu) and zinc (Zn) in TDE and TW samples, before application as irrigation water. The concentrations of Cr and Ni in both water samples were below detection limits. This formed the basis for selection of Cd, Cu and Zn, as the metals of concern in this study.

Preparation of heavy metal standards

Varian ® analytical Methods manual for Flame Perkin Elmer Atomic Absorption Spectroscopy (AAS) was used to prepare calibration standards for the heavy metals as follows: a)Copper metal strip (1.000 g) was dissolved in a minimum volume of 1:1 HNO_3 and diluted to 1 L to give 1000 µg mL^{-1} Cu, b) Cadmium metal strip (1.000 g) was dissolved in a minimum volume of 1:1 HNO_3 and diluted to 1 L to give 1000 µg mL^{-1} Cd , and c) Zinc metal granules (1.000 g) were dissolved in 40 mL 1:1 hydrochloric acid (HCl) and diluted to 1 L to give 1000 µg mL^{-1} Zn. These standards bracketed the expected sample concentration and were within the method's working range.

The principle behind AAS technique involved aspiration of samples into a flame which resulted in their atomization. For each sample, a light beam was directed through the flame and the amount of light absorbed by the atomized element in the flame was measured by a detector(APHA, 1999).

Each metal has a characteristic absorption wavelength, and for this reason, source lamps of Cd, Cu and Zn were used. The amount of energy at the characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample. To verify baseline stability, the standard solutions were analyzed after every ten samples. The resulting readings for the three metals were generated as AAS output. The data readings were stored in Microsoft office excel sheets.

3.3.5 Bacterial toxicity screening

The Toxi-Screening Kit (see appendix VIII), a 'field test kit', contained materials and reagents that were required to perform low-cost toxicity analyses on leachate samples.

The test was based on the measurement of bioluminescence which is produced by marine bacteria *Vibrio fischeri*, as a by-product of their cellular respiration. The amount of light produced by the bacteria was proportional to the intensity of cellular respiration and was measured in a portable luminometer in Relative Light Units (RLU).

The luminometer and other materials and reagents as well as easy to follow instructions with detailed illustrations, for the conduction of the toxicity tests on samples were enclosed in a small lightweight 'Luminescence Measurement Case'.

The Toxi-Screening Kit comprised of one box containing 10 vials of freeze-dried *Vibrio fischeri* and 10 boxes containing tubes with Adenosine triphosphate (ATP) reagents. The upper compartments of the tubes comprised a liquid ATP extractant whereas the bottom compartments had solid ATP reagent. All tests were performed at temperature range of 15°C and 25°C, with direct measurement of the number of Relative Light Units (RLU), using a portable luminometer.

A specific strain of *Vibrio fischeri* (NRRLB-1117) was supplied with the kit as freeze-dried reagent and was rehydrated by transferring the total contents of the osmotic adjustment medium tube into the vial containing bacteria, by use of a 1-mL finntip fitted on a 1-mL finnpipette. The vial containing the bacteria was closed and its contents mixed by shaking thoroughly.

To prepare samples for toxicity screening, one of the 2 finntips was fixed on the 200-µlFinnpipette and used to take 200 µL of the leachate sample. The tip of the finntip was pushed through the cover of the upper chamber of one of the 2 tubes with ATP reagents and the leachate sample transferred into the compartment with the liquid ATP extractant. The water sample and ATP extractant were mixed by repeated sucking up and pushing

back the solution in the top chamber, with the same finnpipette. After about 30 seconds, 200 μL of the mixture was sucked up again, and the tip of the finntip slowly pushed further down in the tube, through the partition separating the two compartments. The contents of the finntip were emptied into the bottom chamber which contains the solid ATP reagent. Using the same finnpipette, the entire solution of the upper chamber was transferred into the lower chamber.

One of the two transparent holders was adjusted to the tube and then it's holder swirled gently to mix the water + extractant, with the solid ATP reagent, till the latter is dissolved completely .The luminometer was switched on by pressing the "power" button and allowed to calibrate itself by a 10 seconds countdown, followed by a beep signal. Thereafter, the lid of the luminometer was opened and the tube inserted, with its holder. The lid of the luminometer was closed and the "ENTER" button pressed. After the countdown of 10 seconds, a luminescence score was displayed.

It is important to note that ATP degrades rapidly and so the luminescence scores had to be recorded within few minutes of introduction of the water samples in the tubes with ATP reagents.Therefore, the timer was set at 30 minutes and sample measurements at $\mathbf{t_0}$ and $\mathbf{t_{30}}$ (minutes) were recorded. All tubes were shaken properly before taking the measurements. The scores were recorded in a laboratory notebook and the tube together with its holder was removed from the luminometer before switching it off.

Generally, the inhibition of bacterial respiration under toxic stress automatically led to decrease of the bioluminescence and this decrease of luminescence in the leachate samples was measured after a short exposure time (30 minutes) and compared to the decrease in luminescence in the control water samples. The luminescence scores of

leachate samples were compared to luminescence change in the control water samples, for calculation of the percentage toxicity of the leachatesamples.

Therefore, the degree of toxicity was calculated as the ratio of the magnitude of the luminescence decrease in the water sample, versus that in the control water. The following formula was used to calculate percentage toxicity of the water samples:

% toxicity = (RLU at $\mathbf{t_0}$– RLU at $\mathbf{t_{30}}$) sample / (RLU at $\mathbf{t_0}$ – RLU at $\mathbf{t_{30}}$) control X 100

Where,

RLU at t_0 = relative light units at time 0

RLU at t_{30} = relative light units at the 30^{th} minute

3.4 DATA COLLECTION AND STATISTICAL ANALYSIS

3.4.1 Data collection

The experiment was designed as a 2 (irrigation treatments) by 4 (test crops) factorial with 4 replications. Leachate samples were collected from all the pots and their analysis was done in duplicates, whereas soil and plant tissues were collected from two pots (randomly selected), for each test crop, and their analysis in the laboratory was also done in duplicates. The irrigation water samples were sampled and analyzed in duplicates. Physical measurements of growth were carried on all the test crops and their means were used to determine for variation.

3.4.2 Data analysis

All data was stored in Microsoft office Excel spreadsheets and statistical tests were done by use of Statistical Package for Social Scientists (IBM® SPSS® version 20.0.0). The

alpha level for all the tests was set at 0.05. Student's t-Test for Independent Samples was used to compare the means for total metal concentrations in TDE and TW water quality treatments and One-way analysis of variance (ANOVA), *F*- test, was used to determine variations in means in plant growth, metal and macro element concentrations in soils, plant tissues and leachates, between TDE and TW. Spearman's correlation analysis was used to determine the relationship between the heavy metal and macro elements pairs of 'Cd-Cu', 'Cd-Zn' and 'Cu-Zn' and 'P-N'.

Descriptive statistics including mean (M), standard deviation (SD), and standard errors (SE) were used to present the results in form of graphs. All error bars represented standard errors of mean.

CHAPTER FOUR

RESULTS

4.1 Soil characteristics

Data on characteristics of soils used for this experiment was obtained from records available at the Soil Science laboratory of UoE. (Soil Science 2012).The soil had pH 4.82, organic carbon 0.984 % , and comprised of silt 62 % , clay 29 % and sand 9 % (Figure 1).

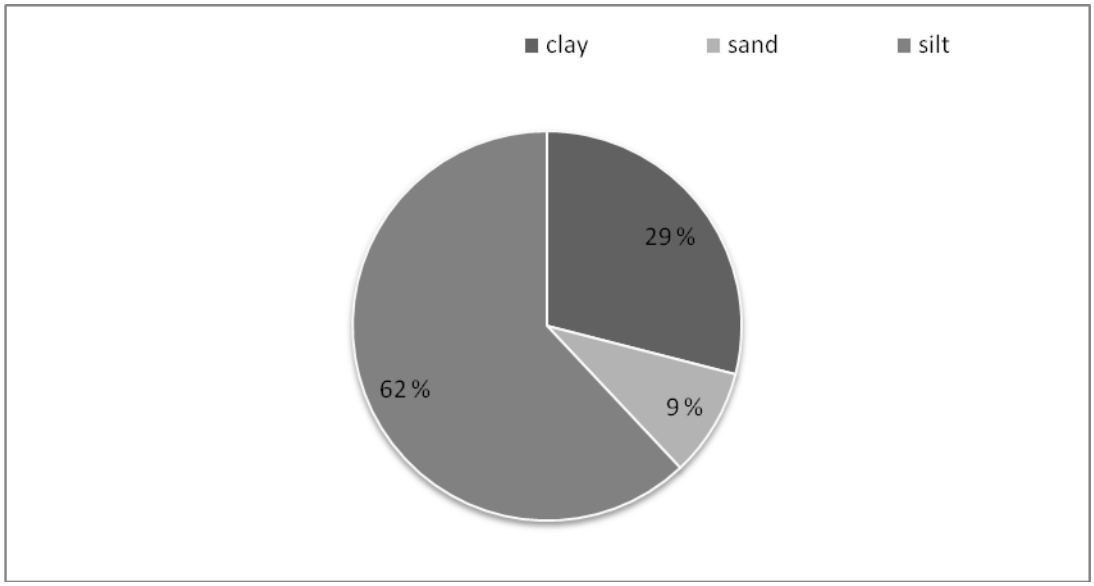

Figure 1: Characteristics of soil sample selected field plot in UoE

4.2 Plant growth measurements

Sweet potato

Plants irrigated with TDE had intense dark green colour compared to those irrigated with TW (Plate 2). The average leaf count was higher in plants irrigated with TDE (*M*=174 *SD*=40.658), than in plants irrigated with TW (*M*=97.080 *SD*=40.827), $F_{(1, 22)} = 21.384$, *p*

< 0.05 (Figure 2).The average stem length measurement for plants irrigated with TDE (*M*=85.420 *SD*=18.759), was higher that of irrigated with TW (*M*=62.170 *SD*=26.274), $F_{(1, 22)} = 6.224$, *p*=0.021 (Figure10).

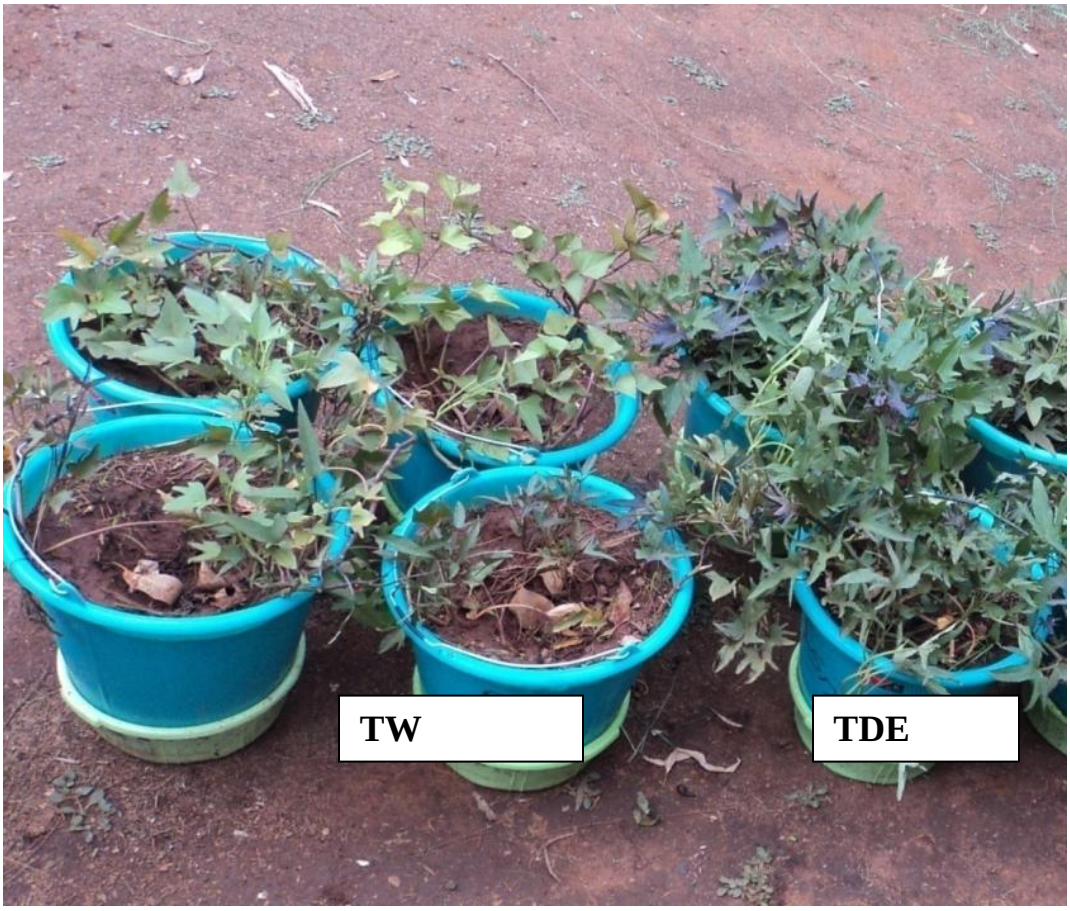

Plate 2: Sweet potato plants irrigated with TDE and TW (Source: Author, 2012)

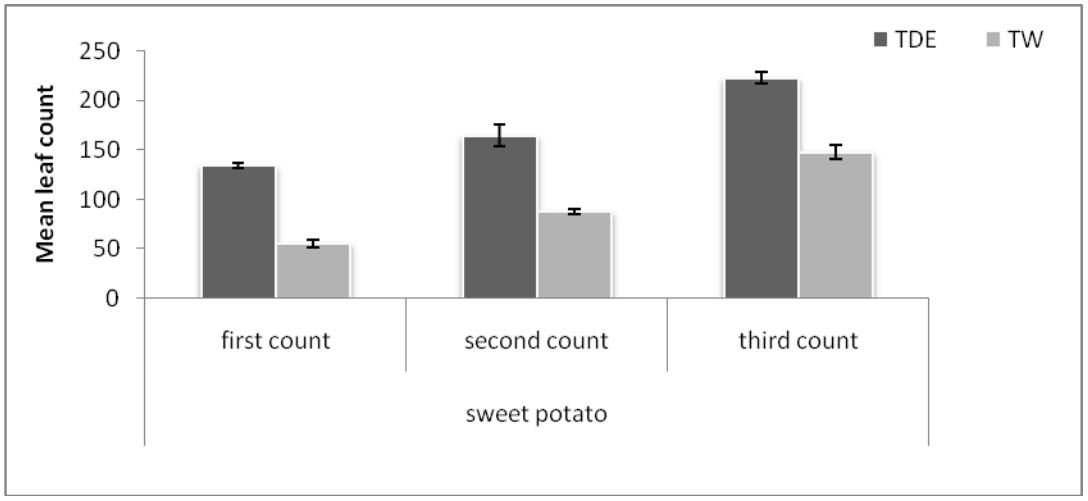

Figure 2: Mean leaf count in sweet potato plant

The average rate of increase in leaf count per day was higher in TW - irrigated plants(*M*=0.012 *SD*=0.003), than TDE - irrigated plants (*M*=0.006 *SD*= 0.003), $F_{(1, 14)}$ = 14.555, *p*=0.002 (Figure 3).Similarly, the average rate of increase in stem lengths was higher in TW (*M*=0.0136 *SD*=0.0055) , than TDE (*M*=0.0063 *SD*= 0.0043), $F_{(1,14)}$=8.62, *p*=0.011.

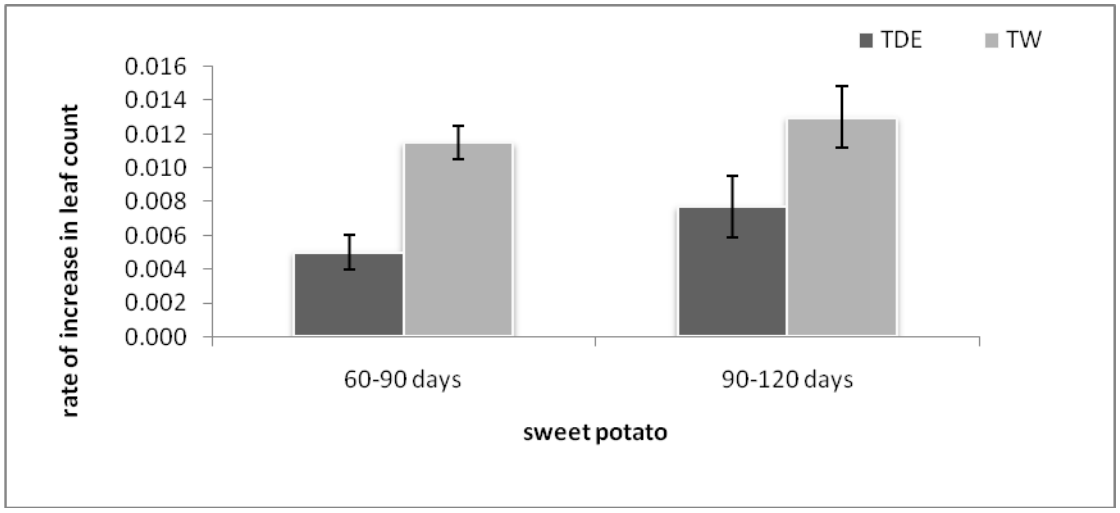

Figure 3: Relative rate of increase in leaf count in sweet potato plant

However, sweet potato plants under both treatments recorded decrease in stem elongation rates during the second time interval (90-120 days) (Figure 11).

Eucalyptus

Plants irrigated with TDE had intense dark green colour compared to those irrigated with TW (Plate 3). The average leaf count was higher in plants under TDE irrigation (M=299 SD=108.339), than those under TW irrigation (M=125 SD= 59.560) $F_{(1, 22)}$ =23.611, p < 0.05(Figure4).The average stem length measurement was also higher in TDE (M =151.670 SD=32.740) than TW (M =110.330 SD=26.085), ($F_{(1, 22)}$ = 11.7, p=0.002) (Figure10).

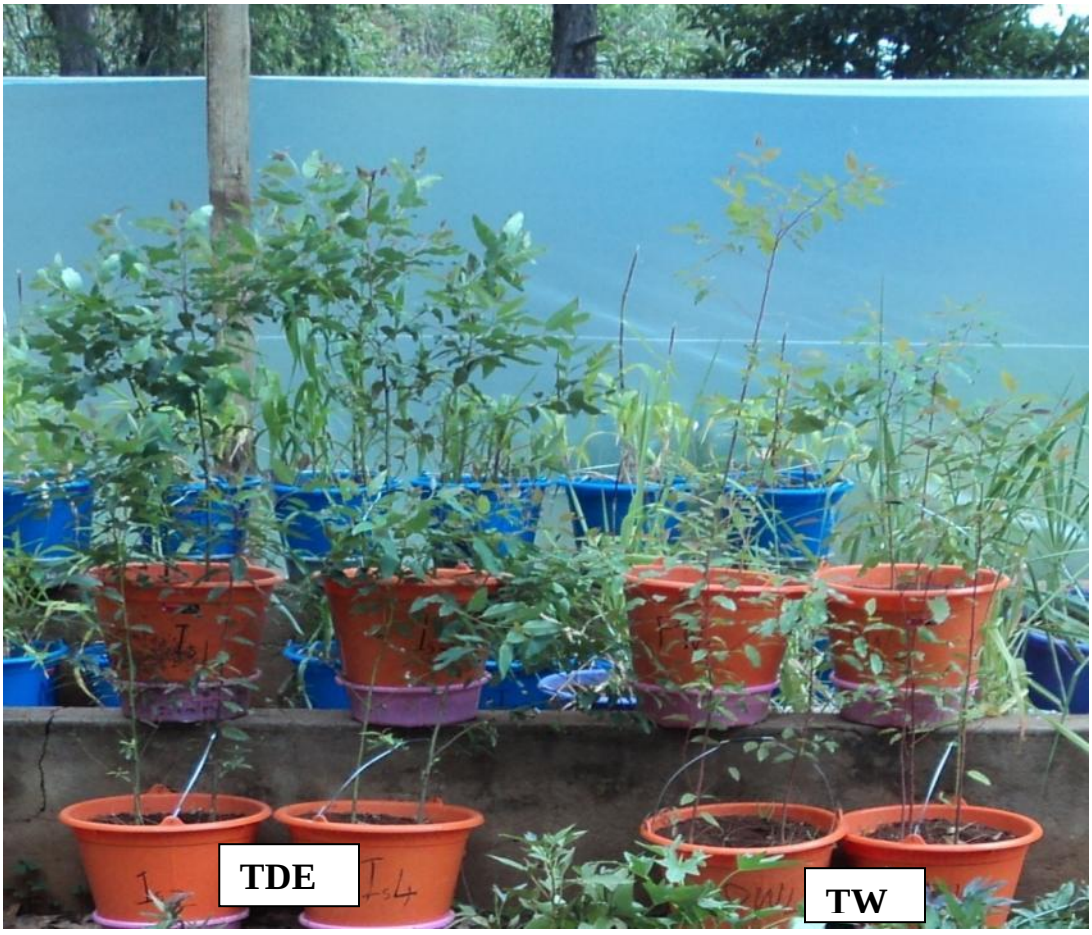

Plate 3: Eucalyptus plants irrigated with TDE and TW (Source: Author, 2012)

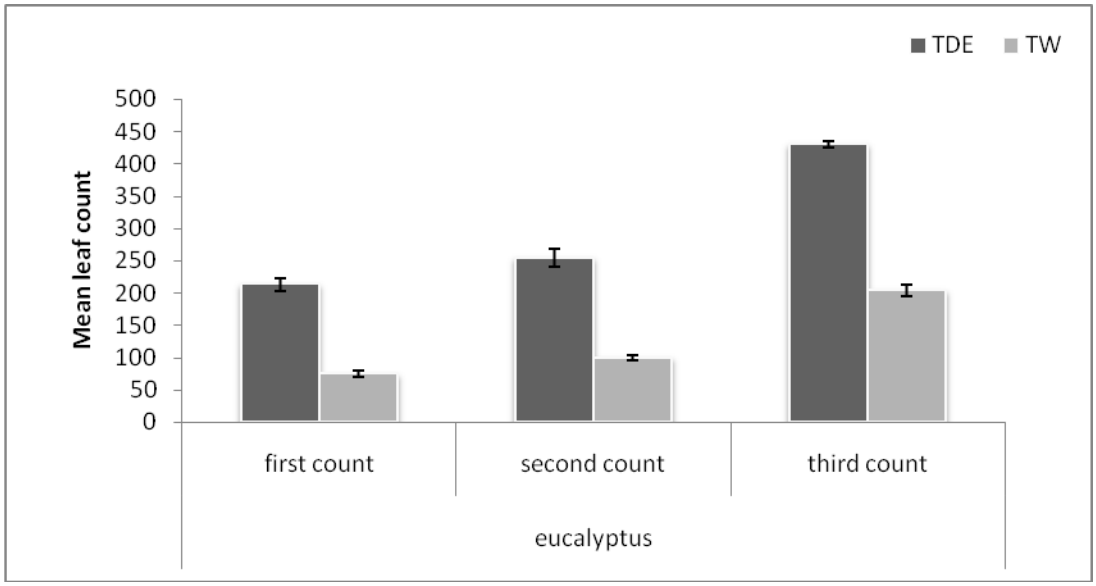

Figure 4: Mean leaf count in eucalyptus plant

There was no significant difference determined between the average rates of increase in leaf count in TDE (*M*= 0.009, *SD*=0. 0.005) and TW (*M*= 0.013, *SD*= 0.006) irrigation treatments, ($F_{(1, 14)}$ = 2.001, *p*=0.179) (Figure5).There was also no difference between the average rates of increase in stem lengths for plants irrigated with TDE (*M*= 0.006, *SD*= 0.001) and those irrigated with TW (*M*= 0.007, *SD*=0. 0.001), ($F_{(1, 14)}$ = 2.001, *p*=0.29) (Figure 11).

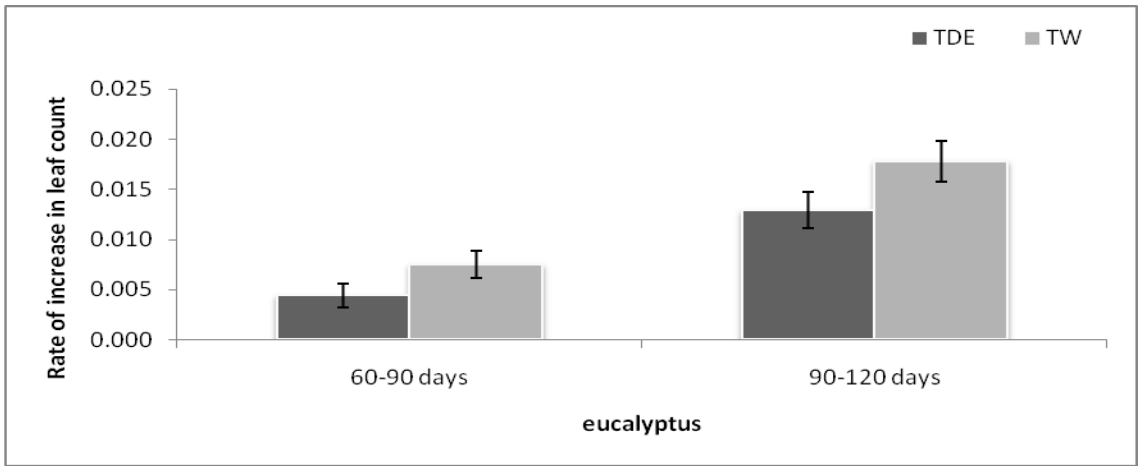

Figure 5: Relative rate of increase in leaf count in eucalyptus

Sugarcane

Plants irrigated with TDE had intense dark green colour compared to those irrigated with TW (Plate 4). The average leaf length measurement was higher in plants under TDE irrigation (M=1184.750 SD = 99.062) than those under TW irrigation (M=987 SD = 64.522), $F_{(1, 22)} = 33.575$, $p<0.05$ (Figure6), and the average stem length measurement was also higher in TDE (M=130.580 SD=21.869), than TW (M=93.580 SD=17.096), $F_{(1,22)}=21.31$, $p<0.05$(Figure10).

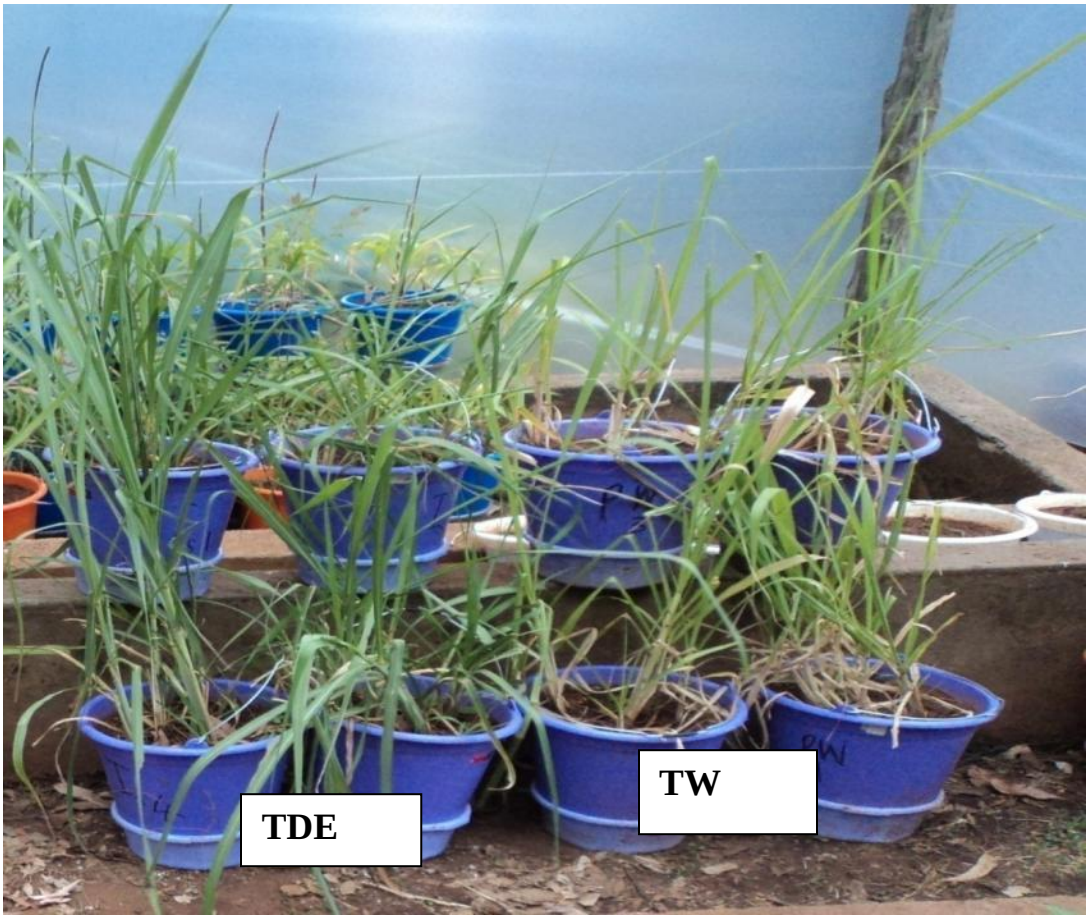

Plate 4: Sugarcane plants irrigated with TDE and TW (Source: Author, 2012)

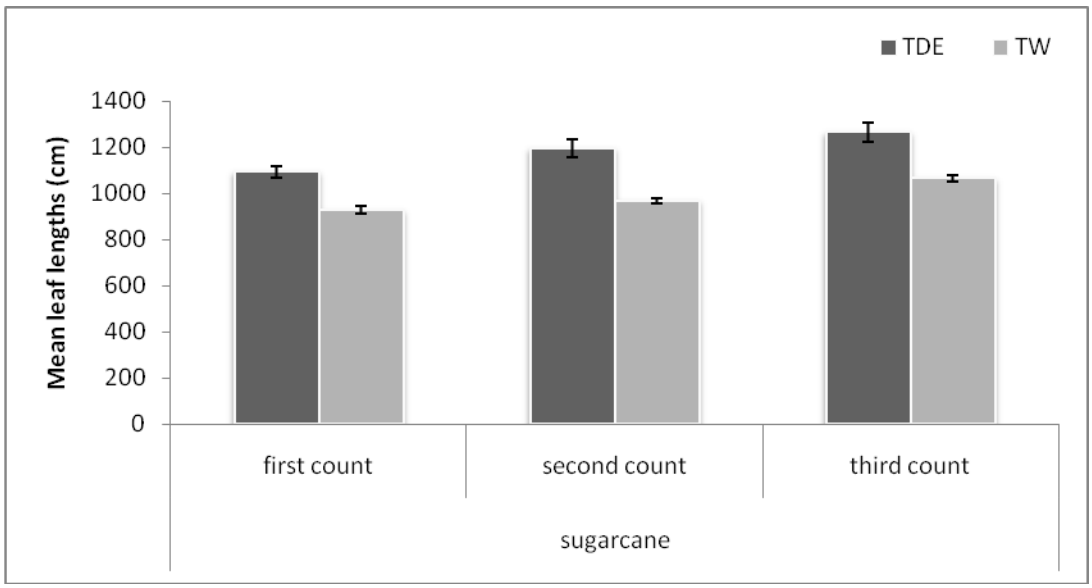

Figure 6: Mean leaf length measurements in sugarcane plant

There was no significant difference determined between average rate of increase in leaf length in TDE - irrigated plants (M=0.002 SD=0.001) and TW-irrigated plants (*M*=0.002 *SD*= 0.001), $F_{(1, 14)} = 0.051$, p=0.825, (Plate 5, Figure7).Similarly, the average rate of increase in stem lengths in plants under TDE (*M*=0.004 *SD*=0.002) and TW (*M*=0.005 *SD*= 0.003) treatments, showed no significant difference ($F_{(1,14)}$=0.061, p=0.809) (Figure 11).

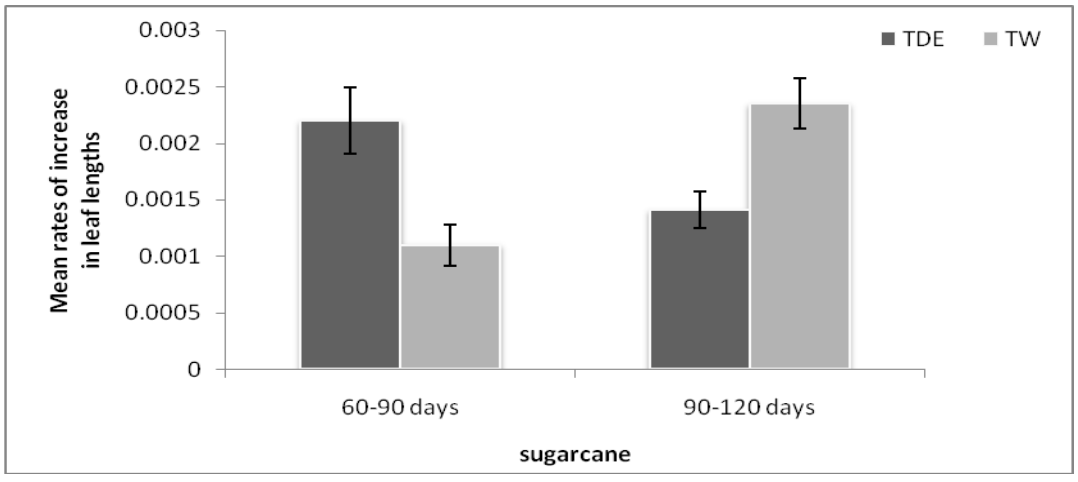

Figure 7: Relative rate of increase in sugarcane leaf lengths

Maize

Plants irrigated with TDE had intense dark green colour compared to those irrigated with TW (Plate 5). There was no significant difference determined between average leaf length measurementsof plants irrigated withTDE(M=479.420 SD=103.864) and those irrigated withTW (M=551.750 SD=68.617), $F_{(1,22)}$=0.01, p=0.922 (Figure8).Also, stem length measurements in TDE-irrigated plants (M=67.330 SD=13. 210) and that of TW-irrigated plants (M=62 SD= 4.459), did not vary significantly ($F_{(1,22)}$ = 0.524, p=0.229) (Figure10).

Plate 5: Maize plants irrigated with TDE and TW (Source: Author, 2012)

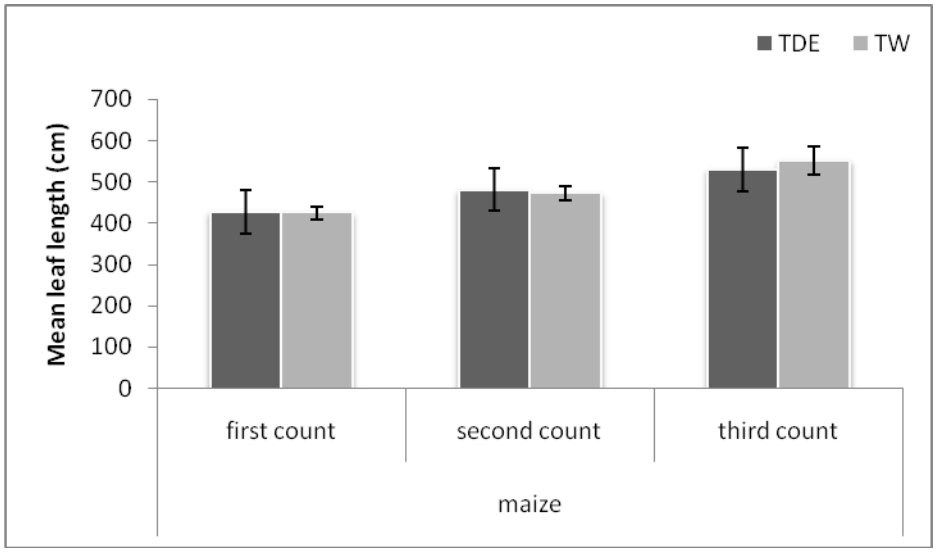

Figure 8: Mean leaf lengths measurements in maize plant

There was no significant difference determined between the average rates of increase in leaf lengths of TDE-irrigated plants (M=0.003 SD= 0.001)and TW-irrigated plants (M=0.003 SD=0.002) treatments, $F_{(1,14)}$=0.524, p=0.481 (Figure9).The average rates of increase in stem lengths in plants under TDE (M=0.0044 SD= 0.0020) and those under TW (M=0.0047 SD= 0.0028), did not show significant difference ($F_{(1,14)}$=0.708, p=0.414) , (Figure 11).

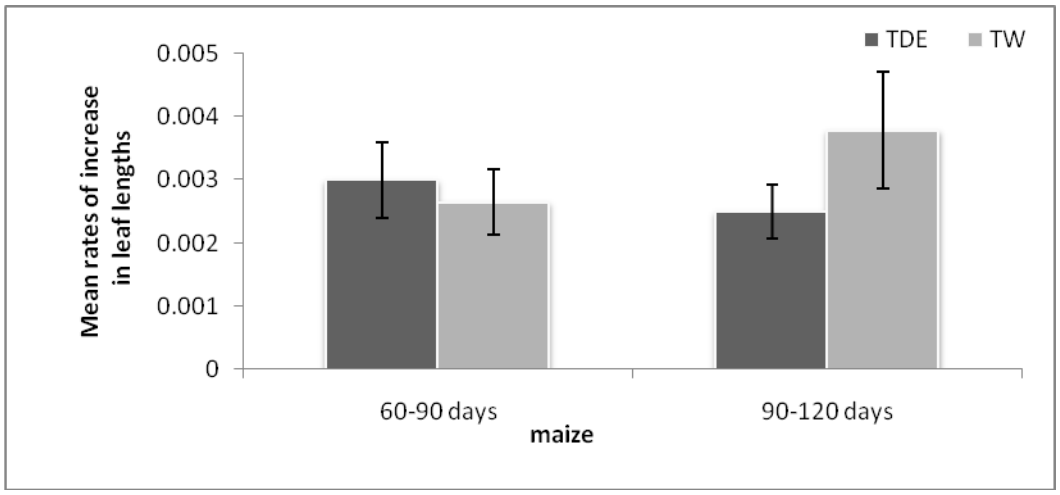

Figure 9: Relative rate of increase of leaf lengths in maize plant

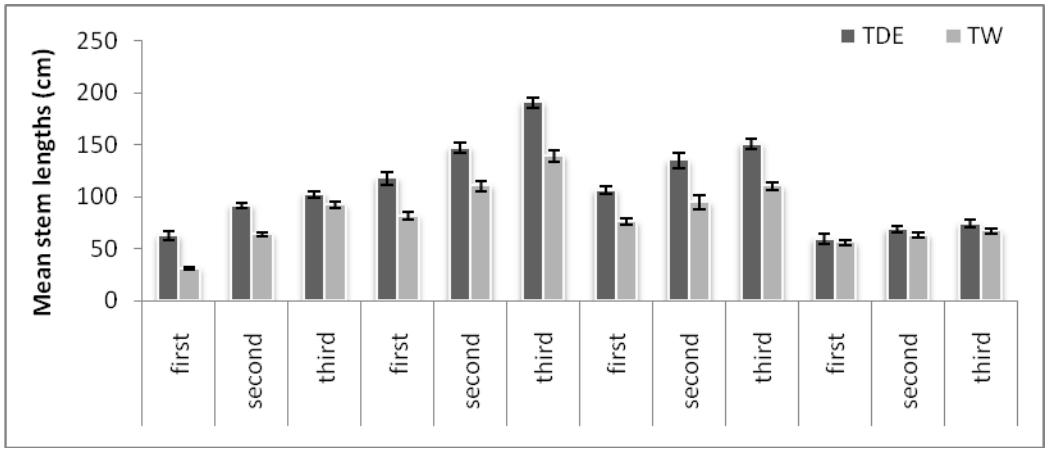

Figure 10: Mean stem lengths of sweet potato, eucalyptus, sugarcane and maize

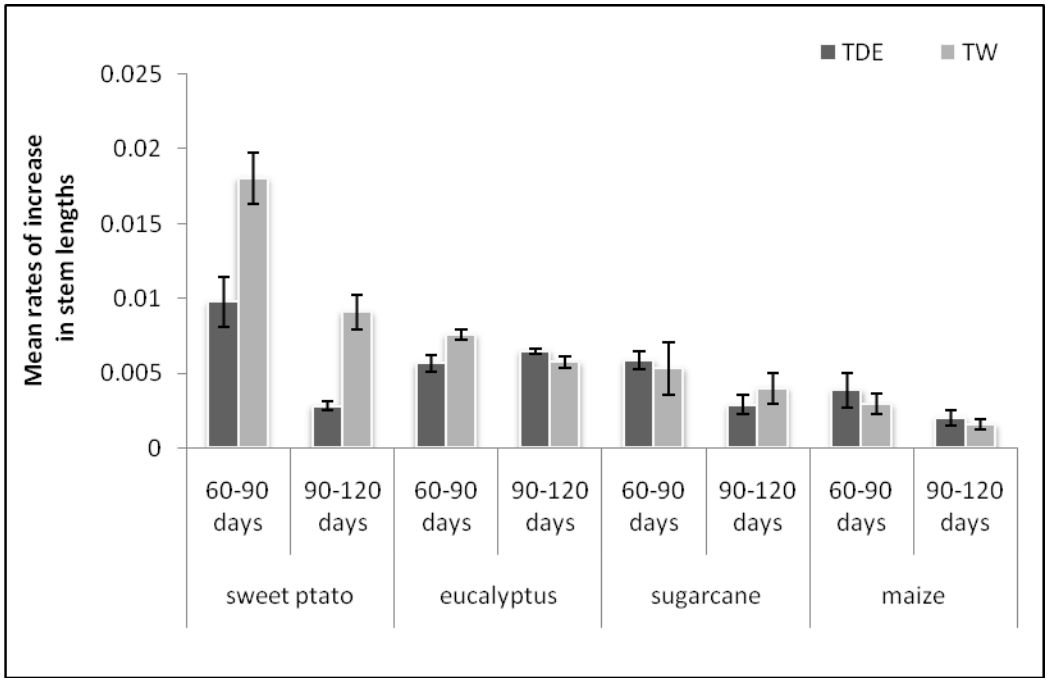

Figure 11: Mean rates of increase in stem lengths of sweet potato, eucalyptus, sugarcane and maize

4.3Heavy metals

4.3.1 Heavy metals in irrigation water samples

Students t-test determined that the level of Cd was higher in TDE water samples (*M*=0.104 *SD*=0.004), than that in TW water samples (*M*=0.046 *SD*= 0.002) $t_{(6)}$

=24.885, $p<0.0001$), and the level of Cu was higher in TDE water samples (M=0.105 SD=0.003), than that in TW water samples (M=0.004 SD= 0.003), t ($_6$) =44.835, $p<0.0001$).On the other hand, no significant difference was determined between the level of Zn in TDE water samples (M=0.370 SD=0.011) and that in TW water samples (M=0.535 SD=0.005), t ($_6$) = -0.61, p=0.563 (Table 2).

Table 2: Levels of cadmium, copper and zinc in irrigation water samples (mg L-1)

Heavy metal	TDE	TW
Cd	0.104 ± 0.002	0.046 ± 0.001
Cu	0.105 ± 0.002	0.004 ± 0.002
Zn	0.370 ± 0.005	0.535 ± 0.027

*All values are mean values of triplicate determinations ± SE

4.3.2 Heavy metals in soils and leachates from control pots

The levels of heavy metals measured in control pots (Table 3, see also Appendix I),were recorded in order to estimate degree to which pot plants absorbed and accumulated the metals and how they were partitioned in various plant tissues and soil (see Appendices II,II,IV and V).

Table 3: Levels of heavy metals in control pots (mg L^{-1})

	TDE		TW	
	Soil	Leachate	Soil	Leachate
Zn	0.145 ± 0.001	0.273 ± 0.030	0.539 ± 0.012	0.306 ± 0.038
Cd	0.045 ± 0.021	0.006 ± 0.000	0.065 ± 0.013	0.049 ± 0.001
Cu	0.024 ± 0.001	0.013 ± 0.003	0.017 ± 0.010	0.019 ± 0.002

4.3.3 Heavy metals in pot plants

Cadmium in sweet potato

Pots irrigated with TDE recorded higher levels of Cd, as compared to those under TW irrigation, $F_{(1, 30)} = 10.382$, p=0.003). In TDE treatment, Cd was deposited in the order of leachate (M=0.036 SD=0.006) > tuber (M=0.020 SD = 0.003) > root (M=0.015 SD=0.002) > soil (M=0.004 SD = 0.003) > stem (M=0.003 SD=0.001) and lacked in leaf samples. In TW treatment, Cd was only detected in leaf (M=0.006, SD=0.003) and leachate (M=0.005 SD= 0.005) (Figure 12).

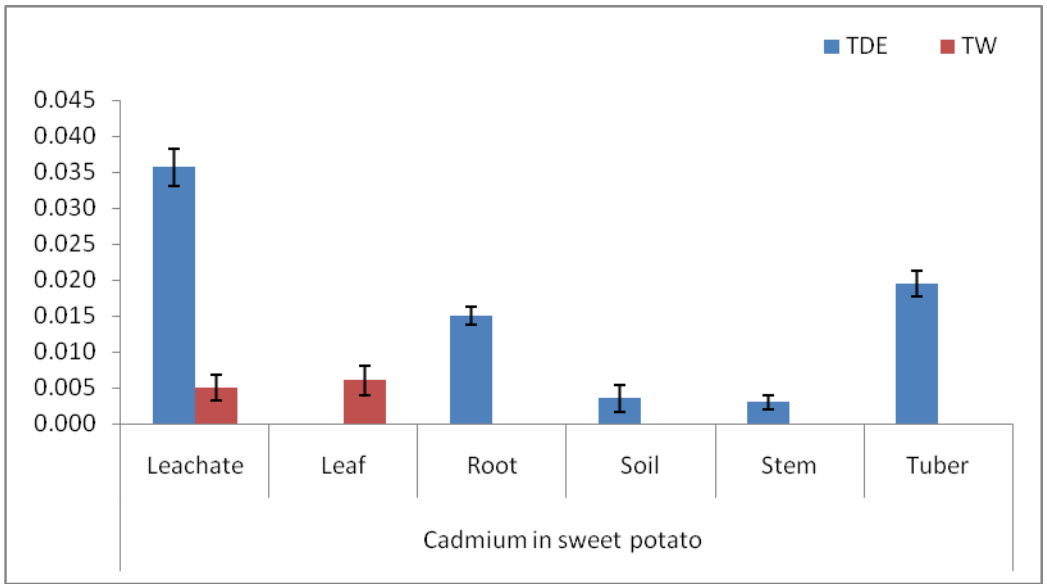

Figure 12: Cadmium in plant tissues, soils and leachates of sweet potato

Cadmium in Eucalyptus

There was no significant difference determined in levels of Cd between pots treated with TDE and those under TW irrigation treatment ($F_{(1, 26)} = 0.218$, p=0.645). Cadmium in TDE was deposited in the order of root (M=0.019 SD=0.006) > soil (M=0.016 SD=0.004)

> stem (*M*=0.013 *SD*=0.004) > leaf (*M*=0.009 SD=0.003), and lacked in leachate samples.

On the other hand, in plants irrigated with TW Cd in was deposited in the order of root (*M*=0.064 *SD* = 0.002) > leaf (*M*=0.012, *SD*=0.003) > stem (*M*=0.012 *SD*= 0.001).The metal was not detected in leachate and soil samples (Figure 13).

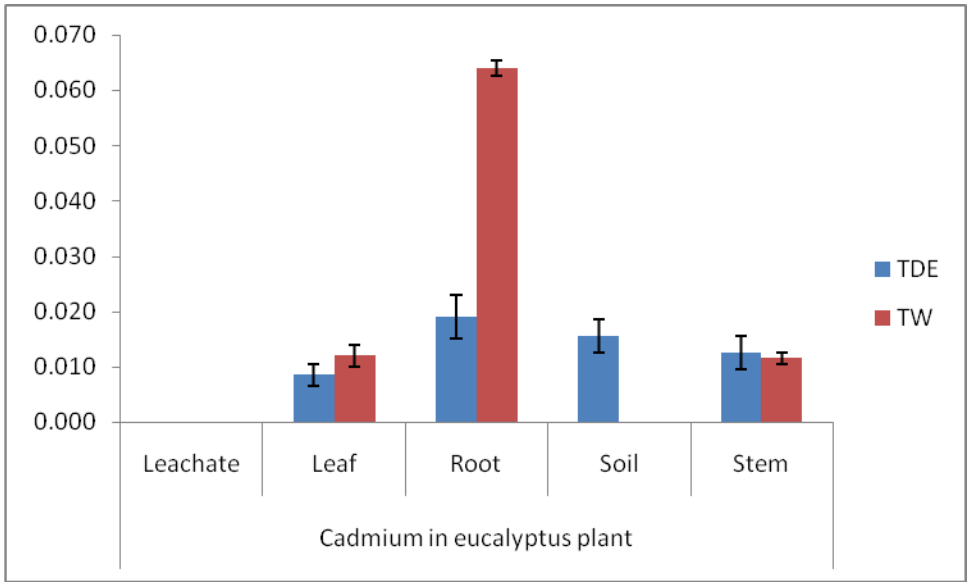

Figure 13: Cadmium in plant tissues, soils and leachates of eucalyptus

Cadmium in Sugarcane

Levels of cadmium were higher in pots under TDE treatment than those under TW, ($F_{(1, 26)}$ = 0.5.863, *p*=0.023), with TDE depositing Cd in the order of leachate (*M*= 0.023 *SD*=0.003) > soil (*M*=0.015 *SD*=0.002) > stem (*M*=0.014 *SD*=0.003), root (*M*=0.014 *SD*=0.003) >leaf *(M*=0.007 *SD*= 0.002). Irrigation with TW deposited Cd in leachates only (*M*=0.002 *SD*= 0.003) (Figure 14).

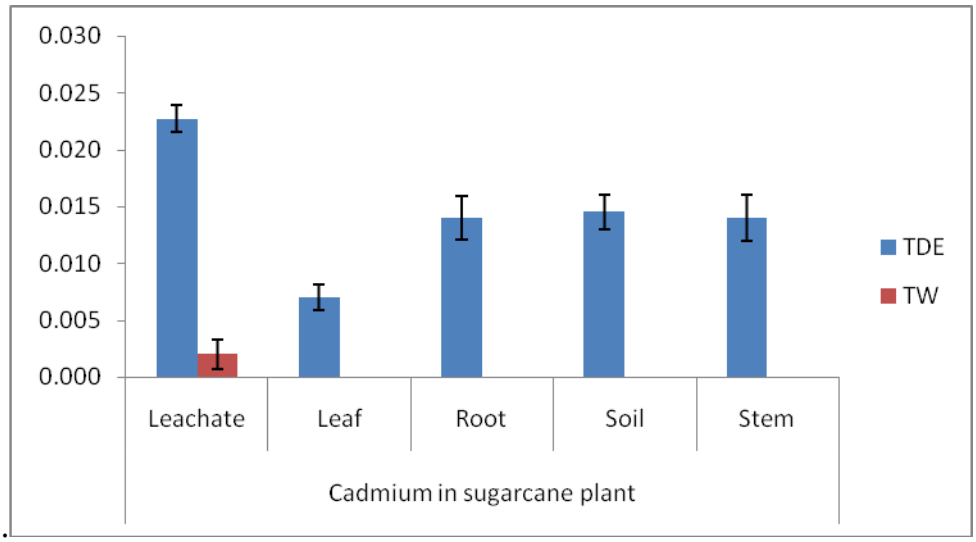

Figure 14: Cadmium in plant tissues, soils and leachates of sugarcane

Cadmium in Maize

Deposits of Cd were higher in TDE- treated pots as compared to TW, $F_{(1, 30)} = 6.359$, $p = 0.017$. In TDE treatment, the metal was deposited in the order of leachate (M=0.049 SD=0.011) > soil (M=0.012 SD=0.003) > grain (M=0.002 SD=0.000), and was lacking in leaf, root and stem samples, whereas in TW treatment, the metal was not detected (Figure 15).

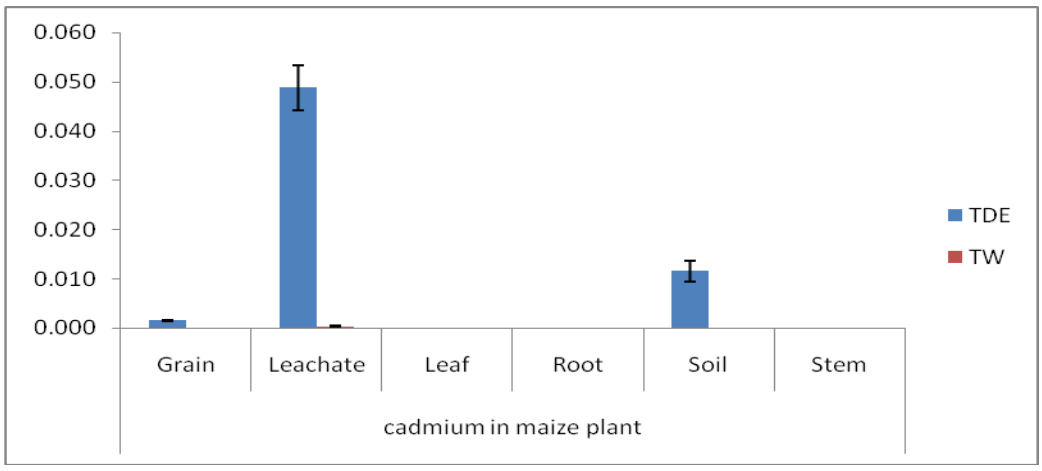

Figure 15: Cadmium in plant tissues, soils and leachates of maize

Copper

Sweet potato

The level of Cu in TDE-irrigated plants was higher than that of TW-irrigated plants, $F_{(1, 30)} = 7.109$, p=0.012. The concentrations of Cu in TDE was in the order of leaf (M=0.037 SD=0.001) > leachate (M=0.023 SD=0.001) > root (M=0.019 SD=0.000) >soil (M=0.011 SD=0.001) > tuber (M=0.009 SD=0.000) > stem (M=0.001 SD=0.001).

On the other hand, Cu deposits in TW treatment were in the order of soil (M=0.033 SD= 0.002) > leaf (M=0.011 SD=0.000) > root (M=0.004 SD= 0.000) > tuber (M=0.002 SD= 0.000) and lacked in leachate and stem samples (Figure 16).

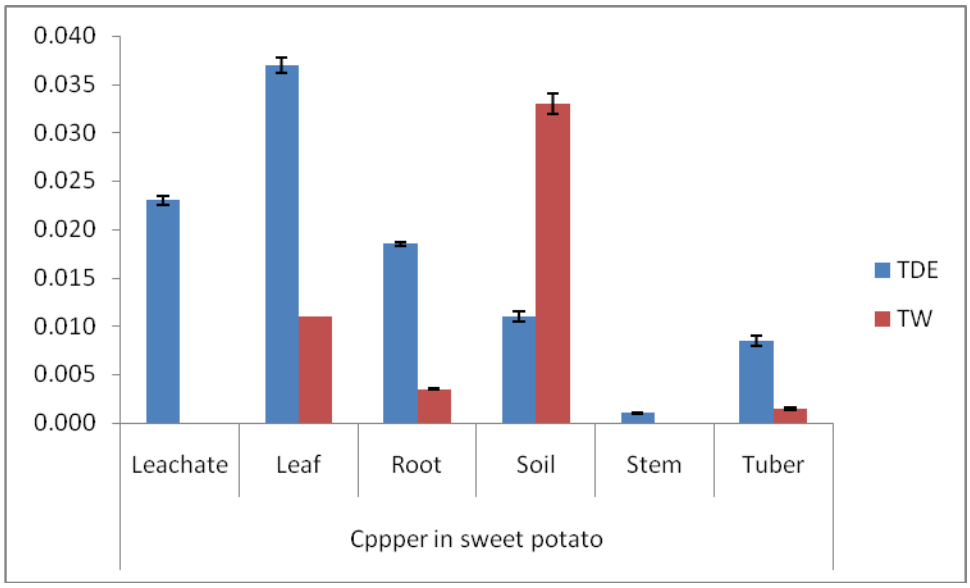

Figure 16: Copper in plant tissues, soils and leachates of sweet potato

Eucalyptus

The levels of Cu were higher in TDE than TW treatments, $F_{(1, 26)} = 4.657$, p = 0.04. In TDE, the metal was deposited in the order of root (M=0.030 SD=0.003) > soil (M= 0.027

SD=0.002) > stem (*M*= 0.018 *SD*=0.005) > leaf (*M*=0.017 *SD*= 0.004) > leachate (*M*=0.011 *SD*=0.005).

On the other hand, in plants irrigated with TW, Cu deposits were found in soil (*M*=0.033 *SD*= 0.001) and root (*M*=0.020 S*D*= 0.002), but lacked in leachate, leaf and stem samples (Figure 17).

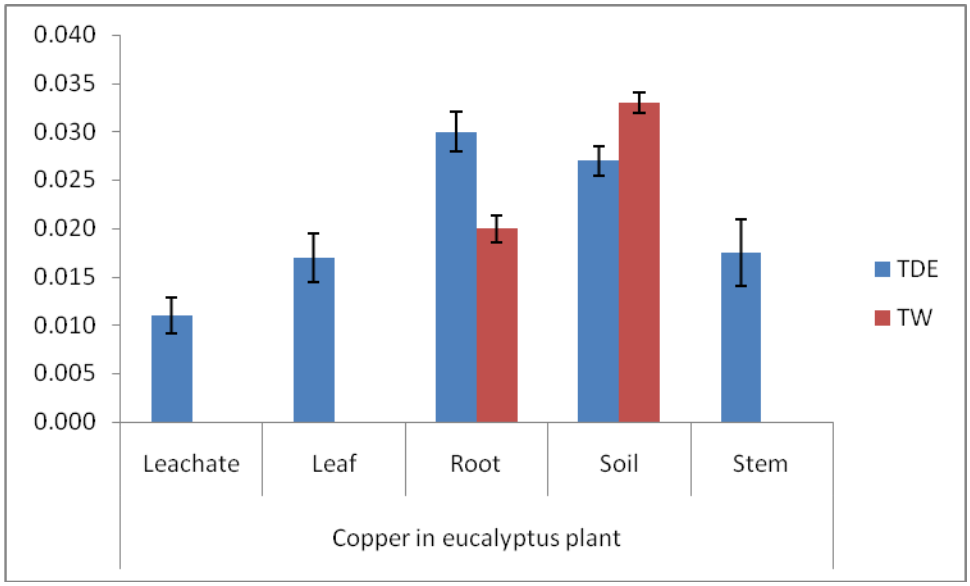

Figure 17: Copper in plant tissues, soils and leachates of eucalyptus

Sugarcane

On average, there was no difference in Cu deposited by TDE and TW treatments, $F_{(1, 26)} = 0.451$, p=0.508. In TDE, the metal was deposited in the order of root (*M*=0.041 *SD*=0.005) > leachate (*M*=0.020 *SD*=0.023) > soil (*M*=0.011 *SD*=0.002) and lacked in leaf and stem samples. In TW, Cu was deposited in the order of root (*M*=0.067 *SD*=0.001) >stem (M=0.047 SD=0.022) >soil (*M*=0.018 *SD*=0.003)>leaf (*M*=0.006 *SD*=0.001)> leachate (*M*=0.004 *SD*=0.005) (Figure 18).

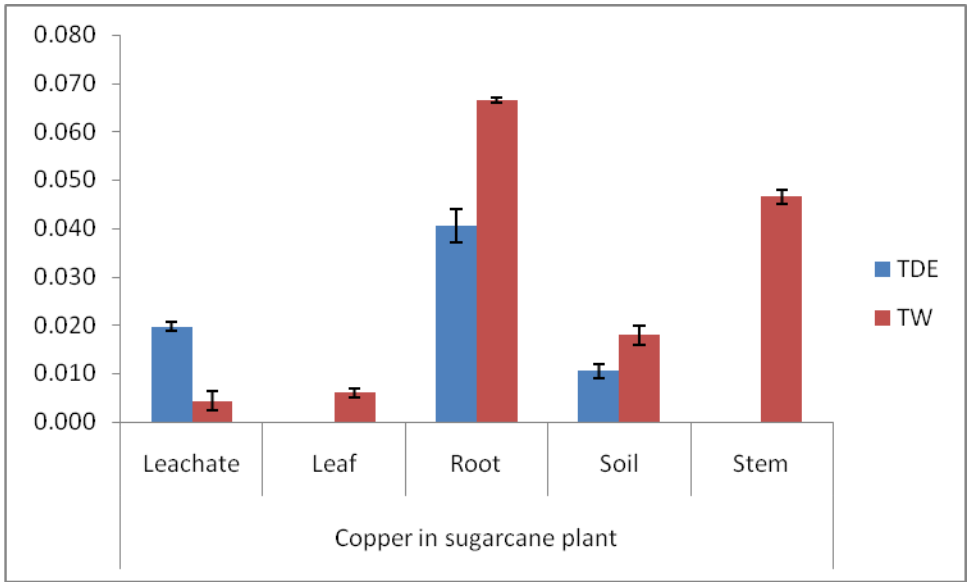

Figure 18: Copper in plant tissues, soils and leachates of sugarcane

Maize

There was no difference in levels between TDE and TW irrigation treatments, $F_{(1, 30)}$ = 0.059, p=0.809. In TDE, Cu was deposited in the order of leaf (M=0.032 SD=0.004) > grain (M=0.030 SD=0.006) > stem (M=0.012 SD=0.003) > leachate (M=0.003 SD=0.003) > soil (M=0.001 SD=0.001), and lacked in root samples.

On the other hand, TW treatment had Cu deposits in the order of grain (M=0.034 SD= 0.006) > leaf (M=0.024, SD=0.001) > stem (M=0.017 SD= 0.001) > root (M=0.007 SD= 0.004) > leachate (M=0.004 SD=0.004), and lacked in soil samples (Figure 19).

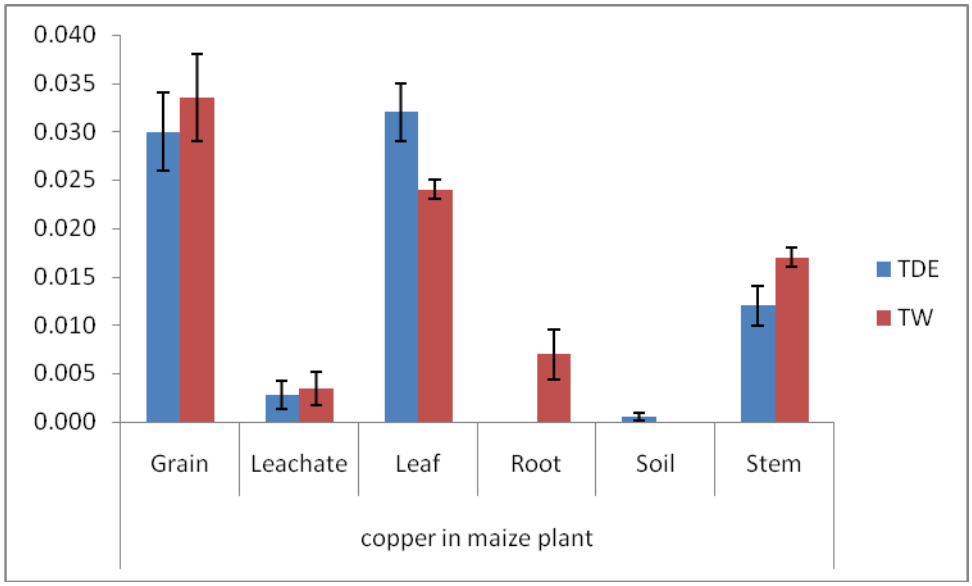

Figure 19: Copper in plant tissues, soils and leachates of maize

Zinc

Sweet potato

The level of Zn was higher in TDE-irrigated plants than that of TW plants($F_{(1, 30)}$ = 5.455, p=0.026), with TDE treatment depositing the metal in the order of leaf (M=0.428 SD=0.028) > root (M=0.239 SD=0.005) > stem (M=0.092 SD=0.014) > leachate (M=0.081 SD=0.052) > soil (M=0.013 SD=0.000) > tuber (M=0.007 SD=0.001). In TW treatment, Zn was only detected in root (M=0.161 SD=0.033), followed by leachate (M=0.049 SD= 0.060), and lacked in leaf, soil, stem and tuber samples (Figure 20).

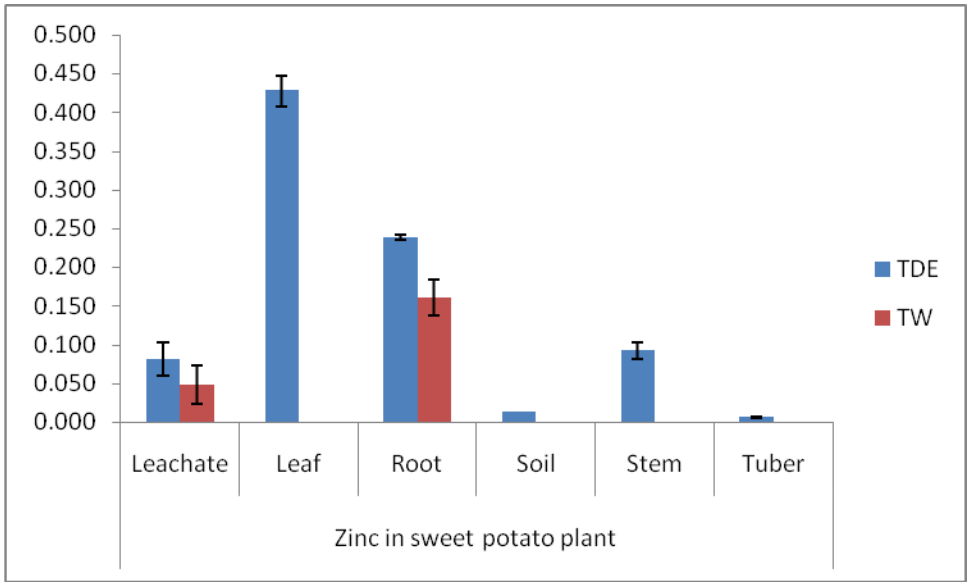

Figure 20: Zinc in plant tissues, soils and leachates of sweet potato plant

Eucalyptus

The level of Zn in TDE treatment was higher than that of TW treatment, ($F_{(1, 26)}$ = 6.214, p=0.019). In TDE, Zn was deposited in the order of root (M=0.520 SD=0.016) > soil (M=0.432 SD=0.035) > stem (M=0.199 SD=0.021) >leaf (M=0.191 SD=0.014) > leachate (M=0.008 SD= 0.013). On the other hand, Zn deposits in TW treatment were found in root (M=0.211 SD=0.025) and soil (M=0.130 SD=0.004), in reducing order, and lacked in leachate, leaf and stem samples (Figure 21).

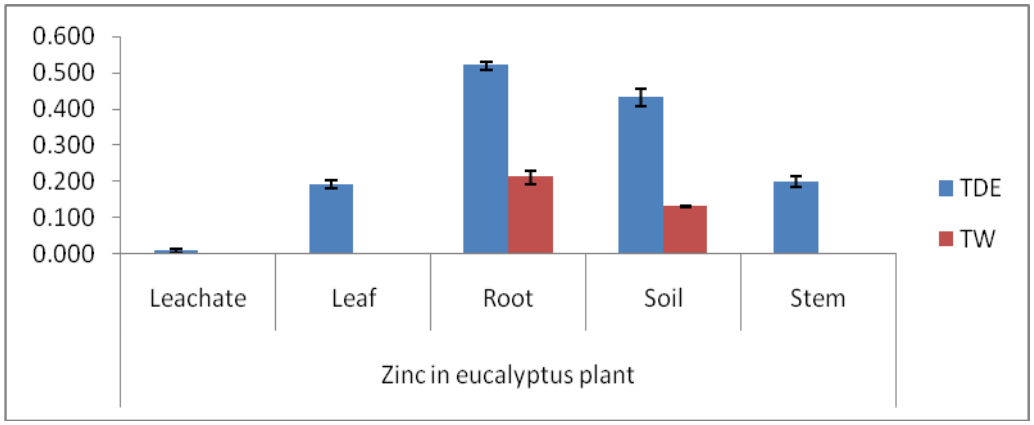

Figure 21: Zinc in plant tissues, soils and leachates of eucalyptus

Sugarcane

There was a significant difference in levels of Zn ($F_{(1, 26)}$ = 11.229, p=0.002). The metal concentrations were higher in TDE than TW treatment, with TDE deposits following the order of soil (M=0.504 SD=0.013) > stem (M=0.184 SD=0.006) > root (M=0.179 SD=0.007) > leaf (M=0.132 SD=0.016) > leachate (M=0.020 SD=0.033). On the other hand, the deposits of Zn TW treatment were detected in leachate only (M=0.004 SD=0.001) (Figure 22).

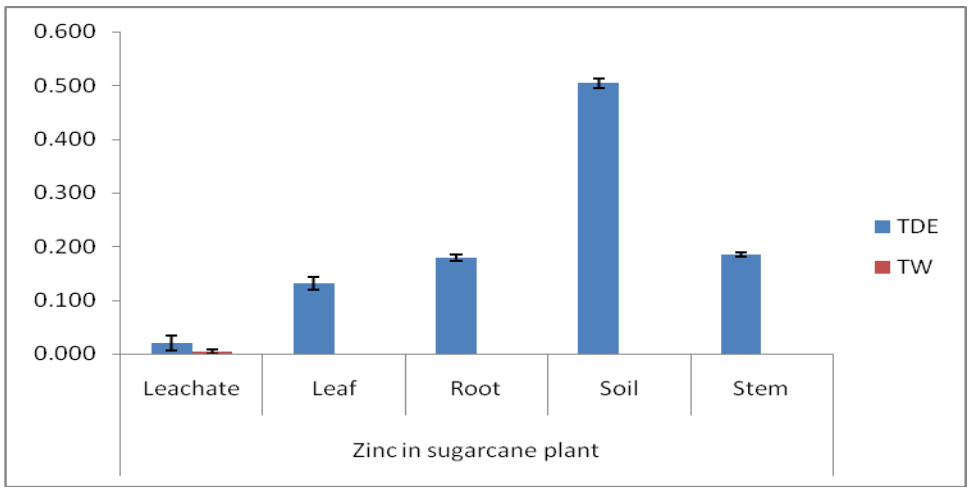

Figure 22: Zinc in plant tissues, soils and leachates of sugarcane

Maize

Irrigation with TDE deposited higher levels of Zn than TW, $F_{(1, 30)} = 19.381$, $p<0.05$. In TDE treatment, the metal was deposited as follows: leaf (*M*=0.239 SD=0.032) > grain (*M*=0.214 SD=0.004) > stem (*M*=0.190 *SD*=0.040) > root (*M*=0.136 *SD*=0.011) > leachate (*M*=0.042 *SD*=0.006) > soil (*M*=0.026 *SD*=0.004).

Deposits of Zn due to TW treatment were only present in soil (*M*=0.063 *SD*=0.007), (Figure 23).

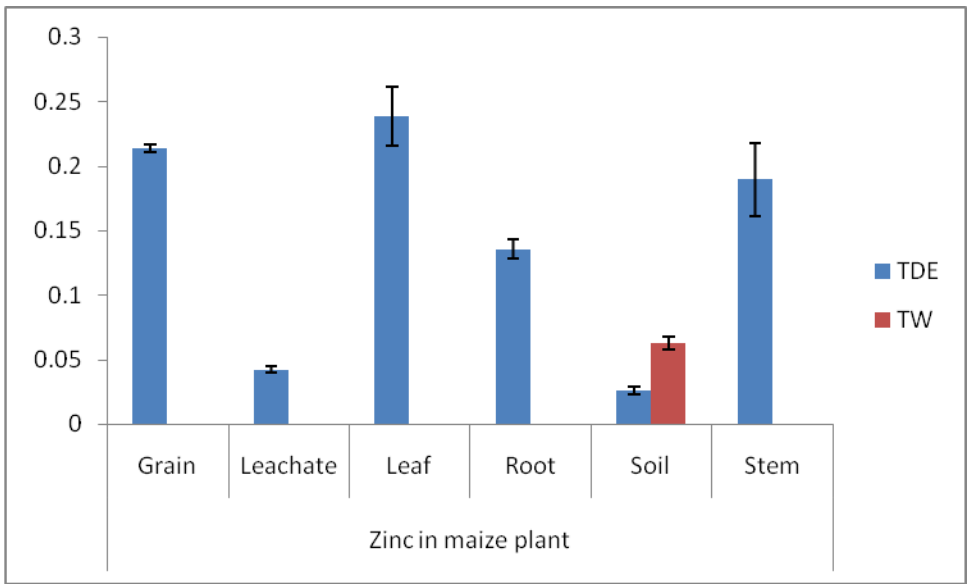

Figure 23: Zinc in plant tissues, soils and leachates of maize

4.4 Macro elements

4.4.1 Macro elements in control pots

The levels of N and P in control pots were recorded for purposes of comparison with pots containing plants to determine the degree of nutrient uptake by individual test crops from soil. It is evident from the control pots (Table 4), that the levels of N were higher in TDE (*M* = 0.192 *SD* = 0.014) than those TW-irrigated soils (*M*=0.107 *SD*=0.016). Also, the

levels of Pin TDE were higher (*M*= 0.075 *SD*= 0.009), than the levels of P in TW (*M*=0.073 *SD*= 0.012).

Table 4: Levels of nitrogen and phosphorous in control pots (soil only) (mg L^{-1})

	TDE	TW
N	0.192 ± 0.011	0.107 ± 0.040
P	0.075 ± 0.007	0.073 ± 0.009

*All values are mean values of triplicate determinations ± SE

4.4.2. Macro elements in pot plants

Total phosphorous

Sweet potato

There was no significant difference determined levels of P, between plant tissue and soil samples under TDE and TW irrigation treatments, ($F_{(1, 18)}$ = 3.429, p=0.081). In TDE treatment, P was deposited in order of root (*M*=0.204 *SD*=0.009) > leaf (*M*=0.175 *SD*=0.008) > stem (*M*=0.105 *SD*=0.018) > tuber (*M*=0.063 *SD*=0.004), and in TW , P was deposited in the order of stem (*M*=0.130 *SD*=0.051) > root (*M*=0.057 *SD*=0.029) > leaf (*M*=0.043 *SD*=0.048) > tuber (*M*=0.040 *SD*=0.039. The element was lacking in soil samples of both TDE and TW treatments (Figure 24).

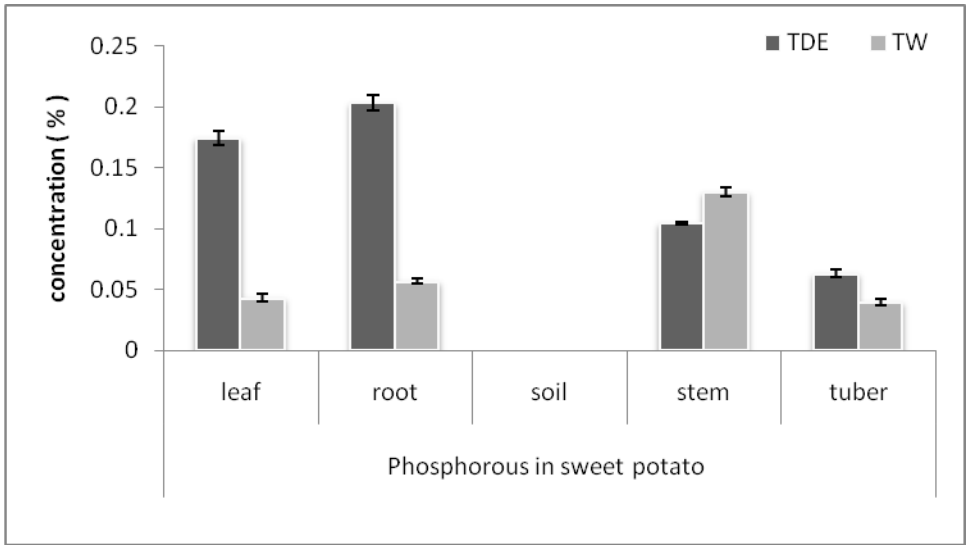

Figure 24: Percent concentration of P in plant tissues and soil samples of sweet potato

Eucalyptus

There was no difference determined in levels of P between TDE and TW treatments, $F_{(1, 14)} = 0.466$, $p=0.506$). In TDE, P was deposited in the order of root ($M=0.113$ $SD=0.010$) > stem ($M=0.054$ $SD=0.012$) > leaf ($M=0.015$ $SD=0.002$) > soil ($M=0.005$ $SD=0.007$). In TW treatment, P was deposited in stem ($M=0.122$ $SD=0.006$) > leaf ($M=0.094$ $SD=0.002$) > root ($M=0.038$ $SD=0.019$) and was lacking in soil samples (Figure 25).

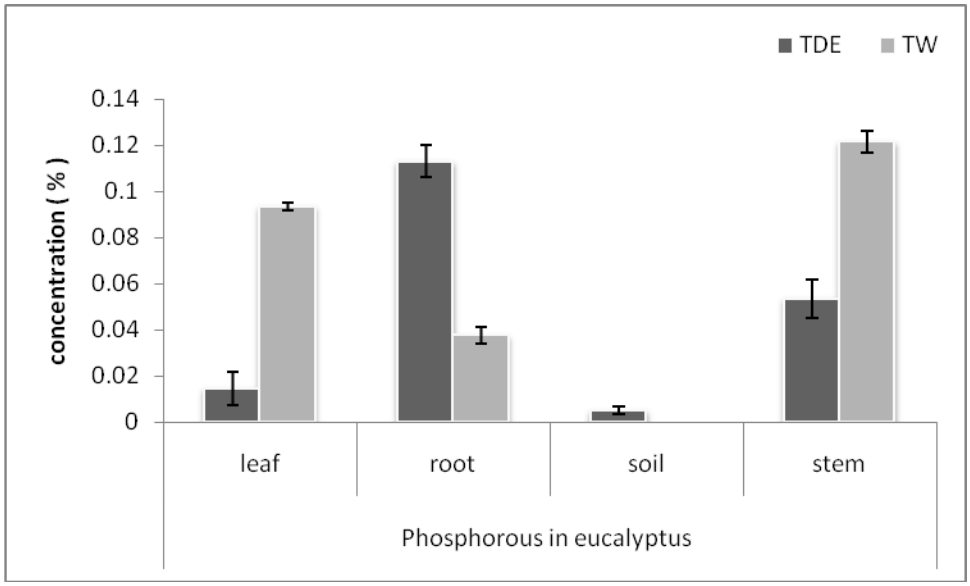

Figure 25: Percent concentration of P in plant tissues and soil samples of eucalyptus

Sugarcane

There was no significant difference in levels P between TDE and TW treatments, $F_{(1, 14)} = 0.193$, $p=0.667$). In TDE treatment, P was deposited in the order of leaf ($M=0.047$ $SD=0.002$) > stem ($M=0.006$ $SD=0.008$) > soil ($M=0.003$ $SD=0.004$) > root ($M=0.001$ $SD=0.004$).In TW treatment, P was detected in leaf ($M=0.025$ $SD=0.035$) and stem ($M=0.009$ $SD=0.012$) only, and was lacking in root and soil samples (Figure 26).

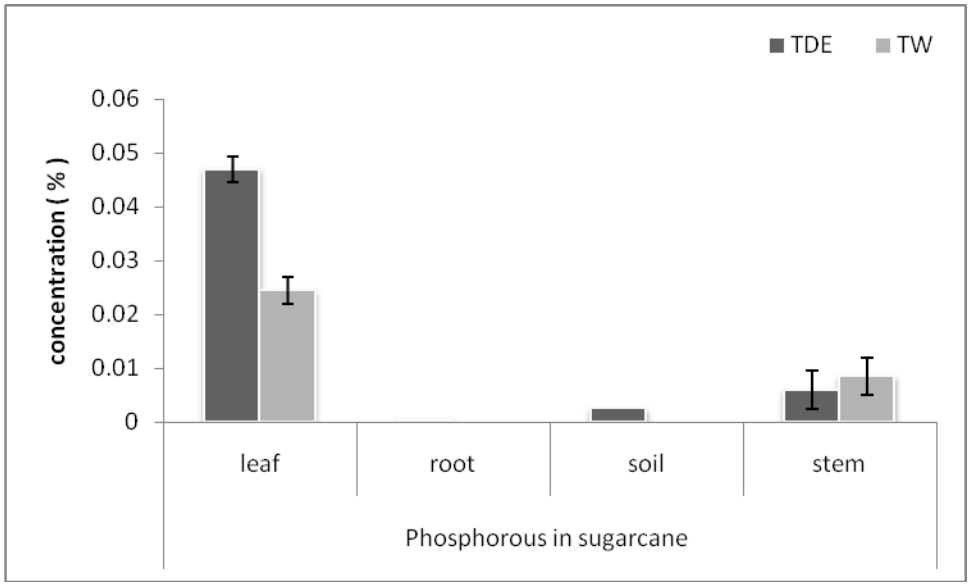

Figure 26: Percent concentration of P in plant tissues and soil samples of sugarcane

Maize

There was no significant difference in levels of P, between TDE and TW treatments, $F_{(1, 18)} = 0.197$, p=0.662). In TDE, the element was deposited in the order of grain (M=0.269 SD=0.014) > leaf (M=0.100 SD=0.019) > stem (M=0.016 SD=0.011) and lacked in root and soil samples.

In TW treatment, P was detected in the order of grain (M=0.261 SD=0.003) > stem (M=0.130 SD=0.024) > leaf (M=0.071 SD=0.016) > root (M=0.025 SD=0.006) > soil (M=0.002 SD=0.002) (Figure 27).

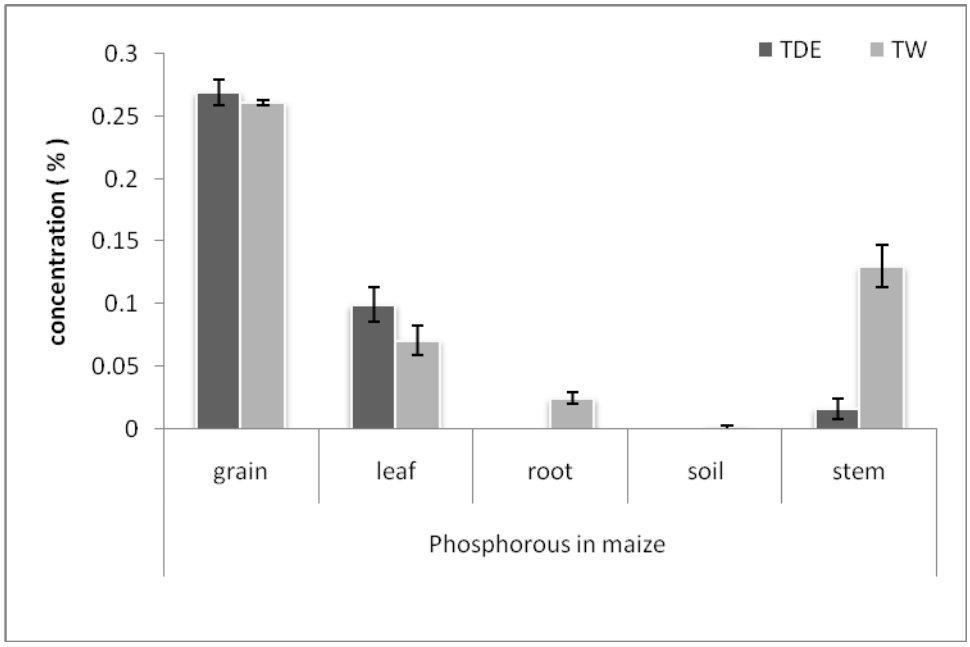

Figure 27: Percent concentration of P in plant tissues and soil samples of maize

Total Nitrogen

Sweet potato

There was no significant difference in levels N, between TDE and TW treatments, $F_{(1, 18)} = 2.02$, p=0.172). In TDE, N was deposited in the order of leaf (M=1.670 SD=0.158) > root (M=0.906 SD = 0.046) > stem (M=0.238 SD=0.134) > tuber (M=0.225 SD=0.078) and was lacking in soil samples.

In TW treatment, N was deposited in the order of leaf (M=0.635 SD=0.100) > root (M=0.622 SD=0.176) > stem (M=0.111 SD=0.093) > tuber (M=0.039 SD=0.055) > soil (M=0.019 SD=0.026) (Figure 28).

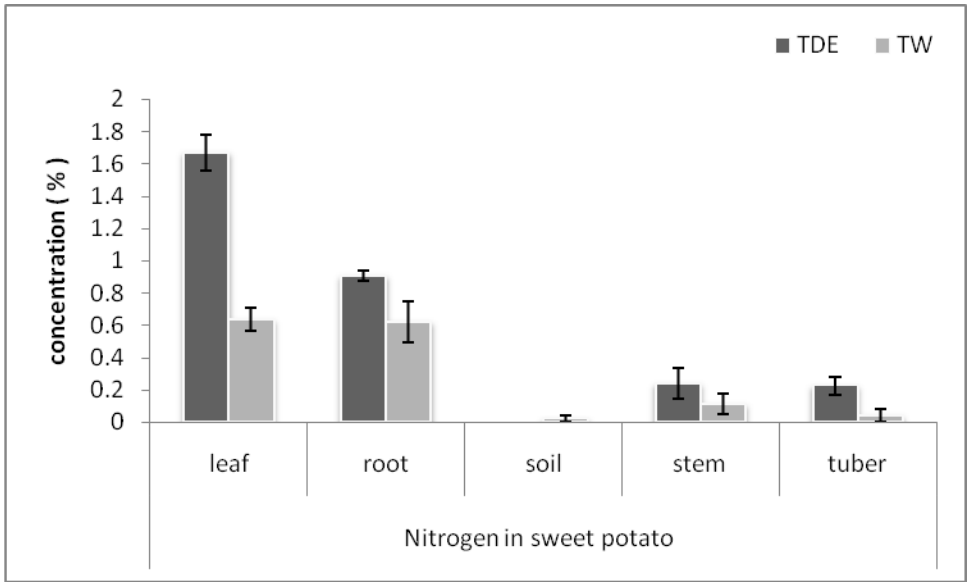

Figure 28: Percent concentration of N in plant tissues and soil samples of sweet potato

Eucalyptus

There was no significant difference in means for concentrations of N, between TDE and TW treatments, $F_{(1, 14)} = 2.094$, $p = 0.17$). In TDE treatment, N was deposited in the order of leaf (M=0.998 SD=0.110) > root (M=0.607 SD=0.037) > stem (M=0.159 SD=0.068) and lacked in soil samples.

In TW treatment, N was deposited in the order of leaf (M=0.378 SD=0.032) > root (M=0.315 SD=0.008) > stem (M=0.141 SD=0.080) >soil (M=0.011 SD=0.016) (Figure 29).

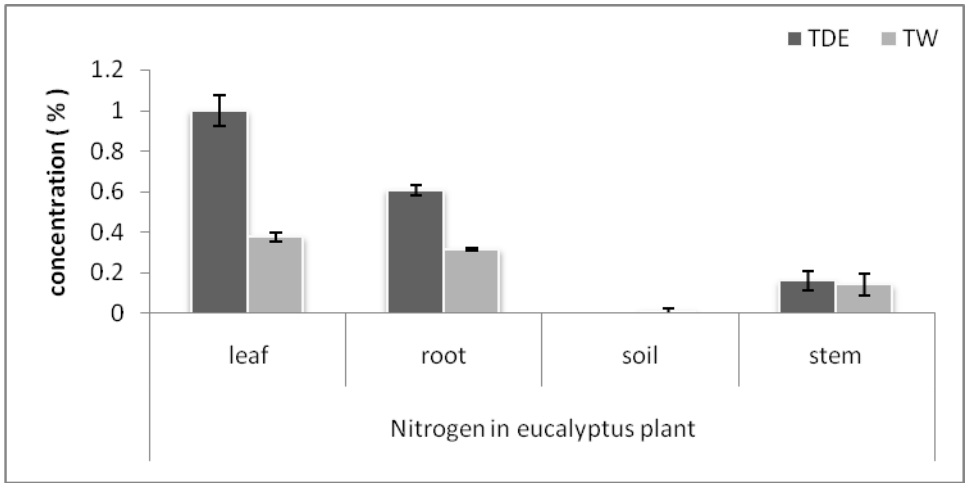

Figure 29: Percent concentration of N in plant tissues and soil samples of eucalyptus

Sugarcane

There was no significant difference determined in levels of N between TDE and TW treatments, $F_{(1, 14)} = 1.846$, $p = 0.196$). In TDE treatment, N was deposited in the order of stem (M=0.358 SD=0.027) > leaf (M=0.352 SD=0.020) > root (M=0.269 SD=0.006) and lacked in soil samples.

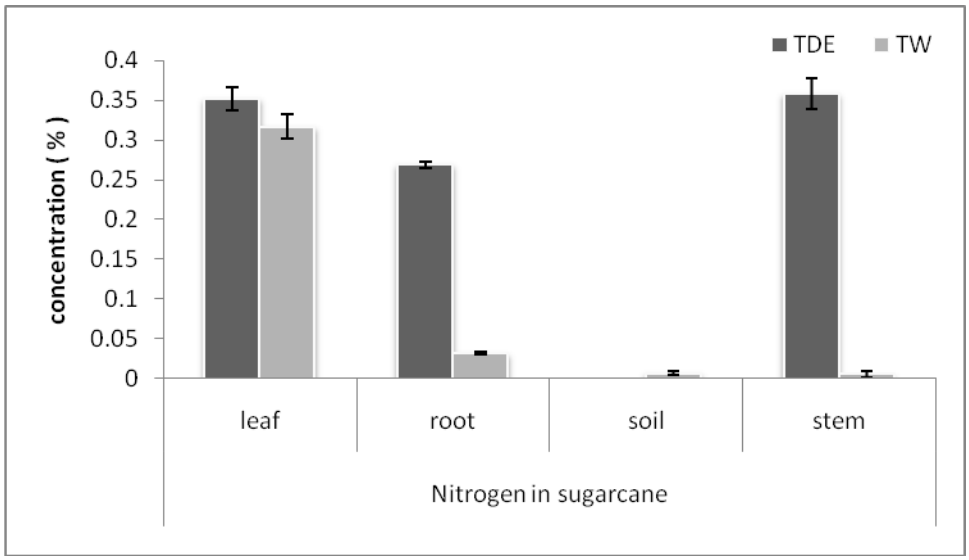

Figure 30: Percent concentration of N in plant tissues and soil samples of sugarcane

In TW treatment, N was deposited in the order of leaf (*M*=0.317 *SD*=0.021) > root (*M*=0.032 *SD*=0.003) > soil (*M*=0.007 *SD*=0.004) > stem (*M*=0.005 *SD*= 0.005) (Figure 30).

Maize

There was no significant difference in levels of N between TDE and TW treatments, $F_{(1, 18)} = 1.722$, $p=0.206$). In TDE, N was deposited in the order of leaf (*M*=0.904 *SD*=0.112) > grain (*M*=0.840 *SD*=0.058) > root (*M*=0.195 *SD*=0.021) > stem (*M*=0.098 *SD*=0.083) and lacked in soil samples.In TW treatment, N deposits were in the order of leaf (*M*=0.462 *SD*=0.050) > grain (*M*=0.336 *SD*=0.146)>stem (*M*=0.226 *SD*=0.051) > root (*M*=0.068 *SD*=0.045) >soil (*M*=0.012 *SD*=0.017) (Figure 31).

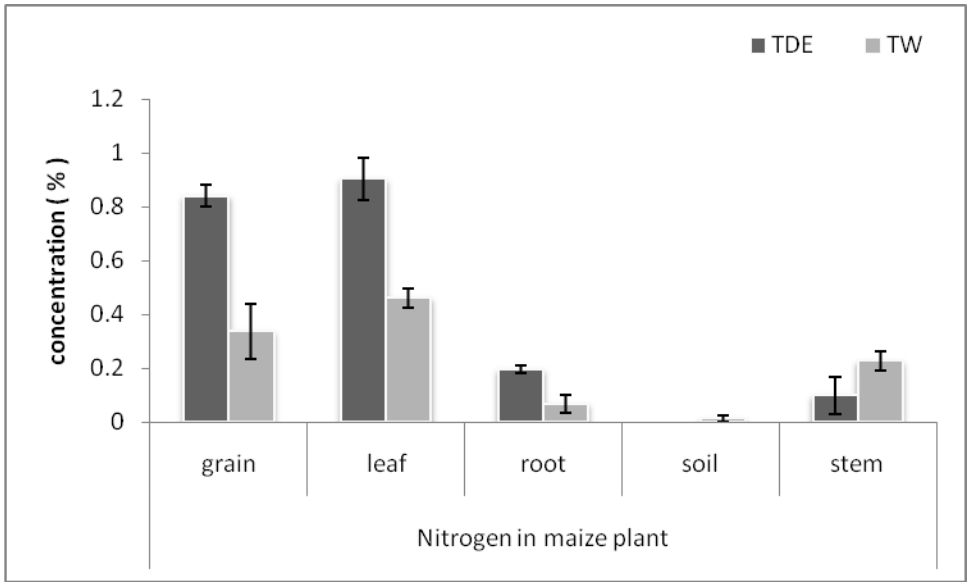

Figure 31: Percent concentration of N in plant tissues and soil samples of maize

4.5. Correlation tests

A spearman's correlation determined positive correlations between Cu and Zn (r_s =0.237, n=72), and Cd and Zn (r_s =0.236, n=72). However, there was a negative correlation between Cd and Cu (r_s = - 0.59, n=72), (Table 5).

Table 5: Spearman's correlation between cadmium, copper and zinc concentrations between the two water quality treatments

	Cadmium	Copper	Zinc
Cadmium	1.000	**-0.59**	**0.236**
Copper	**-0.59**	1.000	**0.237**
Zinc	**0.236**	**0.237**	1.000

* Correlation is significant at the 0.05 level

A strong positive monotonic correlation was determined between the levels of N and P in samples of plant tissues and soils from TDE and TW treatments (r_s =0.605, n=72, $p < 0.001$), (Table 6).

Table 6: Spearman's correlation between phosphorous and nitrogen in soil and plant tissues

	P	N
P	1.000	**0.605**
N	**0.605**	1.000

*Correlation is significant at the 0.01 level

4.6 Toxicity screening test

Generally, leachates collected from test crops irrigated with TDE were more toxic to *Vibrio fischeri*, as compared to leachates for test crops under TW irrigation. Toxicity stress in the plants were the order of maize (31.21) > sweet potato (28.34)>eucalyptus (16.43) > sugarcane (15.20), in TDE and maize (13.96) > sweet potato (10.47) > sugarcane (9.45) > eucalyptus (6.98), in TW. Control pots toxicity levels were TDE (40.86) and TW (15.20), (Table 7).

Table 7: Percentage toxicity levels of leachates from pots under TDE treatment

	TDE	TW
Sweet potato	28.34	10.47
Eucalyptus	16.43	6.98
Sugarcane	15.20	9.45
Maize	31.21	13.96
Control	40.86	15.20

CHAPTER FIVE

DISCUSSION

5.1 Distillery effluent and plant growth

The present study was an effort aimed at determining the abilities of sweet potato, eucalyptus, sugarcane and maize plants to uptake selected metal contaminants in TDE while utilizing nutrients present in the effluent, for their growth. Physical forms and external structures as well as patterns of development in the four test crops, varied between the two irrigation treatments. Generally, the application of TDE as irrigation water showed that the effluent supported plant growth.

One-way ANOVA determined significant differences ($p < 0.05$) in plant growth between the two water quality treatments. For all the test crops, the absolute leaf count, leaf and stem length measurements in TDE irrigation treatment were higher than those measured in TW irrigation treatment. Data obtained from control pots showed that TDE- irrigated soils had higher levels of N (0.192 %) and P (0.75%) than soils irrigated with TW (N 0.107% and P 0.073%), an indication that TDE irrigation treatment provided plants with more nutrients and favourable soil environment than TW.

In plant physiology, relative growth rate (RGR) is the most widely used measure of growth efficiency in plants. It is measured as the mass increase per aboveground biomass per day or rate of production of new dry mass per unit of existing dry mass , per day (RGR, d^{-1})(Hoffman & Poorter, 2002).

Generally, RGR is calculated using the following equation:

$$RGR = (\ln w_2 - \ln w_1) / (t_2 - t_1)$$

Where:

ln = natural logarithm

t_1= time one (in days)

t_2 =time two (in days)

w_1= dry weight of a plant at time one (in grams)

w_2 = dry weight of a plant at time two (in grams)

This study attempted to calculate the RGR from the physical measurements of the above ground plant parts and applied the equation defined in Hoffman and Pooter 2002, to show the variation in production of biomass between TDE and TW irrigation treatments, as a function of nutrient availability. Leaf count and stem length measurements in sweet potato and eucalyptus, as well as leaf and stem lengths measurements in sugarcane and maize plants, were used in place of dry weight, as defined in the equation.

However, it is important to note that this study was not designed to compare the rates of growth between the different test crops. The plants selected for this study were of different species and therefore have different growth characteristics .These plants had been selected from the basis that they were the most commonly cultivated species in the western Kenya region.

The physical measurements were made from the 60^{th} day because the test crops were uniformly irrigated with TW from the time of planting (day 0) to 60^{th} day, to allow the plantlets to germinate and adjust to the soil environment in the pots, after which the TDE irrigation treatment was included from the 60^{th} day, to the end of the experiment. Therefore, the 0^{th} - 60^{th} day, 60- 90^{th} day and 90-120^{th} day time intervals in this experiment denote t_0,t_1, and t_2,respectively.

Plants irrigated with TW treatment had higher rate of increase / production of biomass per day, during the measurement period (60^{th} to 120^{th} day), than plants irrigated with TDE. This is despite the fact that TDE irrigation water had higher nutrient loading than TW. A possible explanation for this difference can be linked to the changes in irrigation regime, whereby, plants were first irrigated with TW from the time of planting to the 60^{th} day(two months), and inclusion of TDE irrigation in the third month. This means that by the time the first measurements were being recorded, the plants had adjusted to TW treatment and the inclusion of TDE irrigation as treatment could have led to a change in soil environmental conditions. Therefore, the plants that were irrigated with TDE treatment had lower growth rate as they were responding to the changed soil environment.

Besides the irrigation regime changes in this experiment, a more logical explanation for the variation would be that plant growth assumes an 'S' shaped curve when plotted against time. The growth curve is also called 'sigmoid curve' and mainly shows four phases of growth: a) Initial slow growth (lag phase), b) The rapid period of growth (log phase / exponential growth phase) where maximum growth is seen in a short period, c) The diminishing phase which is characterized by slow growth, and d) The stationary / steady phase in which plant growth finally stops(Hoffman & Poorter, 2002). Therefore, in this case, the plants under TDE irrigation could have accumulated nutrients in their tissues leading to rapid addition of new biomass (exponential growth), as was seen from the high values of absolute measurement of all parameters studied, and eventually they reached a where growth diminished due to overuse of nutrients available in soil to cause depletion.

On the other hand, the application of TW irrigation water, which had low nutrient loading, resulted in plants with much lower production of new biomass over time compared to TDE-irrigated plants. The TW-irrigated plants took longer time to progress to the exponential phase as compared to TDE-irrigated plants. However, it is possible that the TW plants attained exponential growth at the time plant measurements were being taken. This could be the reason for their higher growth rate compared to TDE, which had reached the stationary phase of growth. Perhaps if this study included recording measurements of the parameters to the end of the experiment (6^{th} month), the plants irrigated with TDE would record highest growth due to availability of nutrients and improved soil conditions. Plants under TW would not show a similar trend with TDE plants because of limited availability of soil nutrients.

Some cases existed where the plants recorded decrease in growth including: average rate of increase in leaf length measurements of sugarcane and maize plants irrigated with TDE, average rate of increase in stem lengths for sweet potato, sugarcane and maize under TDE irrigation, and average rate of increase in stem lengths for all the test crops irrigated with TW treatment.

According to (Atwell, et al., 1999), RGR tends to decrease over as biomass of plant increases is because of factors such as increases in non-photosynthetic biomass (roots and stems), shading of lower leaves by top leaves and limiting soil nutrients. Perhaps this could be the situation in this experiment. A possible explanation for the overall decrease in rates of growth in stem lengths is that soil water and nutrients had become limiting. As plant biomass increased the water and nutrient requirements also increased, and hence their depletion had a bearing on plants' rates of growth.

5.2 Nitrogen and phosphorous in distillery effluent

In the present study, N and P were measured in soils and plant tissue samples to evaluate their contribution to the soil environment and crops. The soil in control pots had relatively higher N and P compared to soil in the pot plants because there were no plants to utilize the nutrients available in the soils.

Generally, the uptake of N and P and their sequential partitioning in the soil-plant systems varied according to test crops and irrigation treatments. The percent concentrations of N and P of dry weight of plant tissues and soil were higher in TDE irrigation treatment, as compared to the levels in plant tissues and soils irrigated under TW treatment. However, there was no much difference in total P between the two irrigation treatments. Soils in the control pots irrigated with TDE irrigation had higher levels of N (0.192 %) and P (0.075 %) than those irrigated with TW (N 0.107 % and P (0.073 %), (Table 4).

The percent concentration of N in soils samples from the pot plants of sweet potato, eucalyptus and sugarcane irrigated with TDE were below detection levels, and P was also not detected in soils under sweet potato and maize plants irrigated with TDE. However, the soil under maize plants irrigated with TDE had detectable N (0.195%), and soils under eucalyptus and sugarcane plants irrigated with TDE had detectable P 0.005% and 0.006 %, respectively. The low contents of N and P in soil samples are an indication that plants had taken absorbed these nutrients to cause their depletion in soils.

Metabolic processes leading to increases in vegetative and reproductive growth and yield are totally dependent upon the adequate supply of N. There is a strong positive correlation between leaf N content and photosynthesis (Cechin & Fumis, 2004).

Generally, all the test crops in the two irrigation water treatments had high content of N deposited in the leaves. However, the leaf nitrogen content in TDE-irrigated plants was higher compared to that in TW-irrigated plants. This contributed to the high increases in production of vegetative matter as seen from the physical measurements.

Sweet potato, eucalyptus and maize plants irrigated with TDE deposited the highest P in roots, that is, (0.204%), (0.113%) and (0. 100%), respectively. Sugarcane plants irrigated with TDE had the lowest deposit of P in the root (0.001%).

Spearman's correlation analysis determined a strong positive correlation between N and P. The concentrations of P increased with increase in N, in both irrigation treatments.

5.3 Cadmium, copper and zinc in distillery effluent

During absorption, plants do not differentiate between nutrient and non-nutrient elements. Because of this, crops grown on polluted soils become major sinks and a gate-way for entry of heavy metals into the food chain. The magnitude of absorption is largely determined by their concentration in soil, physicochemical condition and ability of plant roots to absorb(Ganeshamurthy, et al., 2008).

While some pollutants in DE may be introduced from feed stock (e.g. molasses) and chemicals used , the corrosion of piping , tanks and heat exchangers is expected and this may contribute to heavy metal contents in the effluent (Wilkie, et al., 2000).

Although some metals are essential for plant growth, many are toxic at elevated concentrations and their toxicity may be increased if soil is acidic(USA EPA, 2004).

5.3.1 Compliance of the heavy metal loading in the treated distillery effluent with NEMA standards

One aspect of this research that has been followed is the adherence of the DE to the standards for heavy metals in irrigation water and standards for heavy metals in effluent for discharge to the environment and public sewers, as set by NEMA (Table 8)(2006. Government of the Republic of Kenya, 2006).

Table 8: Recommended standards for heavy metals (mg L^{-1}) in water for use in agriculture as set by NEMA

Heavy metal	Irrigation water	Effluent for discharge
Cd	0.50	0. 1
Cu	0.05	1.0
Zn	2.00	5.0

Although soils normally contain low background levels of heavy metals, our study findings reveal that irrigation with TDE led to additional concentrations of Cd, Cu and Zn in all the test crops and soils, as compared to TW irrigation.

Generally, the distribution and accumulation of heavy metals in soil-plant systems is quite heterogeneous and is controlled by genetic factors, environmental and toxic factors. The degree of heavy metal accumulation in the soil is determined by analysis of accumulation of heavy metals in roots, which in turn offers clues on the soil pollution

degree. On the other hand, analysis of above ground parts may even suggest the atmosphere pollution degree (Ganeshamurthy, et al., 2008).

Regarding heavy metal bioavailability, Cd is the metal of greatest concern in this study due to human health impacts resulting from exposure, particularly through ingestion of plants that accumulated.

Cadmium has great mobility in soil than other metals and is usually taken up in varying degrees by plants. Increasing amounts of Cd in the environment affects various physiological and biochemical processes in plants. Even at low concentration it inhibits plant growth and disturbs photosynthesis, sugar metabolism, sulphate assimilation and several enzyme activities (Kevresan, Kirsek, Kandrac, Petrovic , & Kelemen, 2003).

Findings from this study showed that TDE irrigation resulted in deposition of Cd in leachate of sweet potato (0.036 mg L^{-1}), sugarcane (0.023 mg L^{-1}) and maize (0.049 mg $L^{-1)}$, an indication that these plants were not effective accumulators of this metal. On the other hand, Cd was not detected in leachates of eucalyptus plants irrigated with TDE. Therefore, the eucalyptus plant showed characteristics of an effective accumulator of Cd.

The deposition of Cd in edible tissues of the sugarcane (0.014 mg L^{-1}), maize (0.002 mg L^{-1}) and sweet potato (0.020 mg L^{-1}) indicates potential public health risk. Continual application would impact on the health of consumers (animals and plants) due to introduction of the metal to the food chain.

Metal pairs ‘Zn and Cd’ and ‘Cu and Zn’ showed positive correlation in the soil-plant systems, meaning an increase in one metal led to increase in the other and vice versa. On the other hand, ‘Cu and Cd’ showed negative correlation; an increase in Cu resulted in subsequent decrease in Cd concentration.

Many researchers have studied bivalent transition metal interactions in soil-plant systems because of their chemical similarity, including ‘Cd and Zn’, 'Cu’ and Zn’ and ‘Cu and Cd’. However, most of the research done focused on uptake or adsorption rather than availability of those metals in soils(J. Lee & Doolittle, 2006) .

Owing to the chemical similarities in Cd,Cu and Zn, a high concentration Cu and Cd in soil, relative to Zn , may reduce Zn availability to plant and vice-versa due to competition for the same absorption sites in plant roots (Mousavi, Mohammad, & Maryam, 2012). In our study, the levels of Zn were high compared to Cu and Cd and thus the plant growth did not suffer from Zn deficiency.

A study on the effect of Cd application on Cd and Zn uptake by plants, reported that Cd treatment increased the concentration and total uptake of Zn in plant tops (Turner, 1973) . Other researchers found that Zn application reduced the concentration of Cd in plants. Uptake of Zn by plants was best related to the soil organic fraction. A higher organic matter soil was more effective than other soil factors in reducing Zn uptake. Also, soil type also can strongly influence Cd and Zn uptake, and the Zn levels differ with soil pH. The availability of Cd and Zn in soils can be also affected by phosphorus fertilizer application(J. H. Lee & Doolittle, 2002).

The solubility of Cd and Zn is dependent on soil pH. Lee and Doolittle (2006) determined that the availability of these metals was Cd > Zn in acidic and neutral soils but Zn > Cd in calcareous alkaline soil.

5.4 Toxicity of distillery effluent

There is emerging concern about the release toxic pollutants such as heavy metals and organic compounds into the environment due to increasing industrialization. Unlike organic contaminants, most heavy metals are not bio-transformed and thus persist in the environment.

Two recent applications of toxicity assays for environmental monitoring include sediment toxicity testing and toxicity reduction evaluation (TRE). It is important to carry out TRE at wastewater treatment plants whose effluents fail to meet recommended toxicity standards (Bitton & Koopman, 1992).

Microbial assays using bacteria or enzymes are increasingly applied to measure chemical toxicity in the environment. Bacterial toxicity screening tests are based on bioluminescence, motility, growth viability, ATP, oxygen uptake, nitrification or heat production.

The present study involved toxicity tests based on inhibition of bioluminescence of marine bacterium *Vibrio fischeri*. Bioluminescence ,the property of visible light emission in living organisms that accompanies the oxidation of organic compounds (luciferins),is mediated by an enzyme catalyst (luciferase). Bacterial luminescence reaction is catalyzed by luciferase and involves the oxidation of a long-chain aliphatic aldehyde and reduced flavin mononucleotide ($FMNH_2$) (Bitton & Koopman, 1992), with the liberation of excess free energy in the form of a blue-green light at 490nm:

$$FMNH_2 + RCHO + O_2 \text{ ----> } FMN + RCOOH + H_2O + \text{light (490nm)}$$

Toxicants affect natural bioluminescence of *Vibrio fischeri*. A decrease in luminescence in leachate samples ($\mathbf{t_0\text{-}t_{30}}$) was measured in relative light units (RLU) and compared with

luminescence of the control samples ($\mathbf{t_0}$-$\mathbf{t_{30}}$). Findings from the study revealed that leachate collected from test crops irrigated with TDE were more toxic, compared to those treated with TW. Maize and sweet potato plants were less effective accumulators of toxicants than sugarcane and eucalyptus plants. Therefore, these study findings indicate that soil-plants systems have varying abilities to extract contaminants from wastewater.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The discharge of polluted effluents by sugar and sugar by-product processing plants results in degradation of downstream ecosystems. Effluent from molasses-based distilleries has high organic matter whose decomposition leads to depletion of oxygen levels and negatively affects natural biochemical processes and the species inhabiting in the river systems.

The effluent can also contain concentrations of chemical contaminants (including heavy metals), salts and pathogens that are potentially detrimental to soil health and plant growth.

With the dropping per capita fresh water availability in Kenya, water recycling is becoming an urgent necessity for managing the limited water resources. Properly treated effluents can be recycled for use in sectors that do not require freshwater in their operations such as agriculture and ecosystem services.

The high organic matter and nutrients in distillery effluent can be utilized for agricultural production. Plants can to utilize the nutrient-rich potential of this effluent for their growth and development, while providing an alternative cost-effective and ecologically sound way of disposal.

The major benefit of effluent irrigation is the decrease in wastewater discharges into natural waterways. When discharge of effluent into natural waterways is removed or reduced, the pollutant loadings in these waters are decreased. Substances that would have been discharged into waterways as pollutants are beneficially used for irrigation. For

example; plant nutrients such as nitrogen and phosphorous can lead to algal blooms in waterways but are a valuable fertilizer for crops. Another benefit of effluent irrigation is that water extracted from rivers can be released for use in other sectors that require fresh water for their operation.

Sweet potato, sugarcane and eucalyptus plants showed an improvement in leaf production per day, leaf and stem length measurements, with application of treated distillery effluent, as compared to irrigation with tap water. Maize plants did not show any difference in growth between the two irrigation water treatments. This shows that maize plant was negatively affected by substances present in the effluent to cause its' retardation /poor growth.

The levels of Cd, Cu and Zn in treated distillery effluent were within the region's standards (NEMA) for heavy metals in water for irrigation as well as effluent for discharge into the environment and public sewer. Therefore, this means treatment plant at ACFC was efficient in meeting the environmental regulations as defined by NEMA.

Though crop growth and development was improved, the application of distillery effluent to crops can be projected to lead to adverse effects, on the long-term. The present study revealed that the plants extracted Cd, Cu and Zn from the soils and accumulated the metal deposits in plant tissues, including in the edible tissues. This means that food crops cultivated with distillery effluent pose a risk to the health of consumers of the crops.

Regarding the ability of the test crops studied eucalyptus to uptake metal contaminants in effluent, eucalyptus plants showed the highest potential, followed by sugarcane plants. Leachate from maize plants and sweet potato were shown to have more toxicity stress and thus the plants were not effective in cleaning up the distillery effluent.

Further studies on appropriate crop selection criteria for maximization of use of distillery effluent on best suitable crops would contribute to reduction of soil and water pollution load due to the DE and thus safeguard public health.

6.2 Recommendation

Continuous monitoring of quality of industrial effluents available in the country and intensive studies on their impacts on soil-plant health is required in order to make use of their nutrient- rich potential.

Distillery effluent is not recommended for use as irrigation water for food crops. Plants absorb contaminants from the soil and accumulate them in their tissues, including the edible parts, as was seen in the present study. Farmers around Muhoroni ACFC have been alerted on the projected adverse effects of application of the effluent in for food crop irrigation.

A more sustainable approach would be the cultivation of effluent-irrigated energy crops and forest plantations. In the Kenyan case, the production of eucalyptus trees by use of distillery effluents is highly recommended because there is no risk of contaminants reaching the human food chain. The trees can accumulate the contaminants over the years and contribute significantly to reduced pollutant loads in the environment.

Also, the production of forest plantations in close proximity to ACFC's wastewater treatment plant can secure additional treatment of the effluent, which will in turn reduce pollutant loads in downstream ecosystems and improve water quality.

Further studies on effects of continual application of distillery effluents on soil biological health and crop productivity as well as the development of eco-friendly technology for their use, should be carried out.

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APPENDICES

Appendix I

Mean concentrations for phosphorous and nitrogen in control soils

Treatment	Element	Mean (%)	Minimum	Maximum
TDE	N	0.192 ± 0.011	0.181	0.202
	P	0.075 ± 0.007	0.068	0.081
TW	N	0.107 ± 0.040	0.067	0.147
	P	0.073 ± 0.009	0.064	0.082

Mean concentrations for cadmium, copper and zinc in control soils

Treatment	Metal species	Mean(mg L^{-1})	Minimum	Maximum
TDE	Cd	0.0450 ± 0.021	0.024	0.066
	Cu	0.0240 ± 0.001	0.023	0.025
	Zn	0.1450 ± 0.001	0.144	0.146
TW	Cd	0.06500 ± 0.013	0.052	0.078
	Cu	0.01700 ± 0.010	0.016	0.018
	Zn	0.53850 ± 0.012	0.527	0.550

Mean concentrations for cadmium, copper and zinc in control leachate samples

Treatment	Metal species	Mean(mg L^{-1})	Minimum	Maximum
TDE	Cd	0.006 ± 0.000	0.005	0.007
	Cu	0.013 ± 0.003	0.004	0.021
	Zn	0.273 ± 0.030	0.182	0.365
TW	Cd	0.049 ± 0.001	0.046	0.052
	Cu	0.019 ± 0.002	0.013	0.030
	Zn	0.306 ± 0.038	0.199	0.472

Mean concentrations for cadmium, copper and zinc in irrigation water control samples

Treatment	Metal species	Mean(mg L^{-1})	Minimum	Maximum
TDE	Cd	0.104 ± 0.002	0.099	0.108
	Cu	0.105 ± 0.002	0.102	0.109
	Zn	0.370 ± 0.005	0.361	0.385
TW	Cd	0.046 ± 0.001	0.044	0.049
	Cu	0.004 ± 0.002	0.001	0.008
	Zn	0.535 ± 0.270	0.143	1.327

Appendix II

Concentration of Cd,Cu and Zn in pot plants

Mean levels of cadmium, copper and zinc in sweet potato (concentration in mg L-1 ± SE)

Treatment	Metal species	Leaf n=2	Stem n=2	Root n=2	Tuber n=2	Soil n=2	Leachate n=6
PMDE	Cd	ND	0.003 ± 0.001	0.015 ± 0.001	0.020 ± 0.002	0.004 ± 0.002	0.036 ± 0.003
	Cu	0.037 ± 0.001	0.001 ± 0.000	0.019 ± 0.000	0.009 ± 0.001	0.011 ± 0.001	0.023 ± 0.001
	Zn	0.428 ± 0.020	0.092 ± 0.010	0.239 ± 0.004	0.007 ± 0.001	0.013 ±0.000	0.081 ± 0.021
TW	Cd	0.006 ± 0.002	ND	ND	ND	ND	0.005 ± 0.002
	Cu	0.011 ± 0.000	ND	0.004 ± 0.000	0.002 ±0.000	0.033 ± 0.001	ND
	Zn	ND	ND	0.161 ± 0.024	ND	ND	0.049 ± 0.024

Mean levels of cadmium, copper and zinc in eucalyptus (concentration in mg L^{-1} ± SE)

Treatment	Metal species	Leaf n=2	Stem n=2	Root n=2	Soil n=2	Leachate n=6
PMDE	Cd	**0.009 ± 0.002**	**0.013 ± 0.003**	**0.019 ± 0.004**	**0.016 ± 0.003**	ND
	Cu	**0.017 ± 0.003**	**0.018 ± 0.004**	**0.030 ± 0.002**	**0.027± 0.002**	**0.011± 0.002**
	Zn	**0.191 ± 0.010**	**0.199 ± 0.015**	**0.520 ± 0.011**	**0.432 ±0.025**	**0.008 ±0.005**

	Cd	0.012 ± 0.012	**0.012 ± 0.002**	**0.064 ± 0.001**	ND	ND
TW	Cu	ND	ND	**0.020 ± 0.001**	0.033 ± 0.001	ND
	Zn	ND	ND	**0.211 ± 0.018**	**0.130 ± 0.003**	ND

Mean levels of cadmium, copper and zinc in sugarcane (concentration in mg L^{-1} ± SE)

Treatment	Metal species	Leaf	Stem	Root	Soil	Leachate
	Cd	**0.007 ±0.001**	**0.014 ± 0.002**	**0.014 ± 0.002**	**0.015 ±0.002**	**0.023 ±0.001**
PMDE	Cu	ND	ND	**0.041 ± 0.002**	**0.011 ±0.002**	**0.020 ±0.001**
	Zn	**0.132 ±0.016**	**0.184 ± 0.004**	**0.179 ± 0.005**	**0.504 ±0.009**	**0.020 ±0.014**
	Cd	ND	ND	ND	ND	**0.002 ±0.001**
TW	Cu	**0.006±0.001**	**0.047 ± 0.016**	**0.067 ± 0.001**	**0.018 ±0.002**	**0.004 ±0.002**
	Zn	ND	ND	ND	ND	**0.004 ±0.000**

Mean levels of cadmium, copper and zinc in maize (concentration in mg L^{-1} ± SE)

Treatment	Metal species	Leaf	Stem	Root	Grain	Soil	Leachate
PMDE	Cd	ND	ND	ND	**0.002±0.000**	**0.012 ±0.002**	**0.049 ± 0.005**
	Cu	**0.032±0.003**	**0.012±0.002**	ND	**0.030±0.004**	**0.001 ±0.000**	**0.003 ± 0.001**
	Zn	**0.239±0.023**	**0.190±0.029**	**0.136 ± 0.008**	**0.214±0.003**	**0.026 ±0.003**	**0.042 ± 0.002**
TW	Cd	ND	ND	ND	ND	ND	ND
	Cu	**0.024±0.001**	**0.017±0.001**	**0.007±0.003**	**0.034±0.005**	ND	**0.004± 0.002**
	Zn	ND	ND	ND	ND	**0.063 ±0.005**	ND

- ND=Not detected

Appendix VI

Mean Percentage concentration of N in pot plants

Treatment	Plant tissue	Sweet potato	Eucalyptus	Sugarcane	Maize
TDE	leaf	1.670±0.112	0.998±0.110	0.352±0.485	0.840±0.058
	root	0.906±0.033	0.607±0.037	0.269±0.006	0.904±0.112
	soil	ND	ND	ND	0.195±0.021
	stem	0.238±0.095	0.159±0.068	0.358±0.410	ND
	tuber	0.225±0.055			0.098±0.126
TW	leaf	0.635±0.071	0.378±0.032	0.317±0.121	0.336±0.146
	root	0.622±0.125	0.315±0.008	0.032±0.045	0.462±0.050
	soil	0.019±0.019	0.011±0.016	0.007±0.009	0.068±0.045
	stem	0.111±0.066	0.141±0.080	0.005±0.007	0.012±0.017
	tuber	0.039±0.039			0.226±0.051

Mean Percentage concentration of N in pot plants

Treatment	Plant tissue	Sweet potato	Eucalyptus	Sugarcane	Maize
TDE	leaf	0.175±0.008	0.015 ±0.011	0.047±0.066	0.269±0.014
	root	0.204±0.009	0.113 ±0.010	0.001±0.001	0.100±0.019
	soil	ND	0.005 ±0.007	0.003±0.004	ND
	stem	0.105±0.018	0.054 ±0.012	0.006±0.008	ND
	tuber	0.063±0.004			0.016±0.011
TW	leaf	0.043±0.048	0.094 ±0.002	0.025±0.035	0.261±0.003
	root	0.057±0.029	0.038 ± 0.019	ND	0.071±0.031
	soil	ND	ND	ND	0.025±0.035
	stem	0.130±0.051	0.122 ±0.006	0.009±0.012	0.002±0.002
	tuber	0.040±0.039			0.130±0.024

Appendix VII

Layout of the field pot experiments (Source: Author, 2012)

Appendix VIII

Toxi-Screening Kit*Microbiotest* ® (Source: Kleimoer, Belgium , 2011)

Materials, reagents and bench protocol

Portable luminometer

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