DETERMINATION OF SELECTED HEAVY METALS IN SUGARCANE (*SACCHARUM SP*) GROWN ALONG NGONG TRIBUTARY OF NAIROBI RIVER

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Determination of Selected Heavy Metals in Sugarcane (*Saccharum Sp*) Grown along Ngong Tributary of Nairobi River

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DECLARATION

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

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DEDICATION

This study is especially dedicated to my dear parents whom endured many challenges to make sure I became a resourceful person.

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LIST OF ABBREVIATIONS.

AAS	Atomic absorption spectrophotometer
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BAC	Bioaccumulation coefficient
BAF	Bioaccumulation Factor
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
EDI	Estimated daily intake.
FAO	Food and Agriculture Organization
ICP	Inductively coupled plasma mass spectrophotometer
INECAR	Institute for Environmental Conservation and Research
KIRDI	Kenya industrial development Institute
L.C.T.1	Low Affinity Cation Transporter
L.M.W. T	Low Molecular Weight Thiols
PVC	Polymerizing Vinyl Chloride
SPSS	Statistical Packages for Social Sciences
T.V	Tolerable Values
THQ	Total Hazard Quotient
TTHQ	Sum of Total Hazard Quotient

USA	United States of America
WHO	World Health Organization

ABSTRACT

The transfer of heavy metals from soil or water to plants can pose a health hazard to humans if such plants and their products are consumed. Heavy metals from industrial waste are often deposited in land and water bodies neighbouring the urban and industrial areas. Nairobi river is one such area. The study aimed at establishing heavy metal contamination and safety of consumption of sugarcanes grown along Ngong tributary of Nairobi river. Sugarcane, soil, and water were randomly sampled from the upstream, middle stream, downstream and control points during the wet months of October and November 2016 and dry month of January 2017. Kisii region was the control point. Levels of copper, lead, cadmium, chromium, iron, manganese and zinc in juice soil and water were determined by Atomic absorption spectrophotometer. Transfer factor was determined by comparing levels in juice and soil. Heavy metals analysis, sensory evaluation and health risk assessment of sugarcane juice vendored from various selling joints of fresh sugarcane juice within Kibera, Kayole, Njiru, Kariobangi, and Mukuru areas located along this tributary as well as Kisii region were determined. Sensory evaluation was done through just about scale and preference test while heavy metal concentration of vendored juice was done by atomic absorption spectrophotometer. The results showed that levels of iron, chromium, and lead for sugarcane juice collected along Ngong tributary were higher than safe limits recommended by WHO. In vendored juice chromium, lead, iron, and manganese levels werehigher than WHO recommended limits. In water samples, levels of chromium, iron, lead and manganese were more elevated than WHO recommended levels. The concentration of copper, chromium, iron, manganese, lead, zinc, and cadmium for juice samples ranged from undetected levels to 3.61mg/l. Water samples' heavy metal concentration ranged from undetectable levels to 3.5mg/l while soil samples ranged from undetected levels to 295.2mg/kg. Vendored juice heavy metal levels ranged from undetected levels to 4.74mg/l. There were significant differences ($p<0.05$) of the levels of the heavy metals in upstream, middle stream, downstream as well as control region for sugarcane juice, soil and water. There were also significant differences in heavy metal levels in slums along Ngong tributary and control region for vendored sugarcane juices $P<0.05$. The most preferred vendored juice had significantly lower concentrations of all heavy metals ($P<0.05$) apart from zinc. The least preferred juice had significantly higher levels of manganese, iron, chromium and copper. Most preferred sugarcane juice in sensory evaluation scored highly in aroma and level of sweetness compared to the least preferred, which was most salty. This result indicated that higher levels of heavy metals in juice affected their preference. The transfer factor ranged from 0 to 0.106 while the total estimated daily intakes (EDI) of juice for adults ranged between 0.01mg/kg/d and 0.03mg/kg/d while for children were 0.02mg/kg/d and 0.07mg/kg/d. Total hazard quotients (TTHQ) for adults were 0.14 and 0.3 while those of children ranged between 0.34 and 0.7. In general, most heavy metals concentration were higher than WHO recommended limits in river water, soil and sugarcane juice. However, the risk of consumption of heavy metals from vendored juices in the sampled regions was low compared to TTHQ level of 1 that is considered a high risk.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Sugarcane is one of the crops cultivated along banks of all tributaries of Nairobi river. It is watered either by wastewater draining into the river or the river water itself (Kaluli *et al.*, 2011). Nairobi river basin has heavy metals polluting units ranging from agricultural chemicals, unregulated informal settlements, automotive wastes as well as heavy industrial waste (Hide, Kimani, &Thuo, 2001). Lead, chromium, manganese, iron and isolated elevated levels of mercury and aluminium in levels higher than WHO recommended limits, have previously been detected in Ngong river, a tributary of Nairobi river (Budambula & Mwachiro, 2006). Some studies have shown variations in specific heavy metals bioaccumulation among sugarcane varieties (Xueli *et al.*, 2012). Sugarcanes irrigated with wastewater show significant bioaccumulation of heavy metals (Alghobar & Suresha, 2015). Furthermore, sugarcane has potential to bioconcentrate heavy metals from soils with acceptable heavy metals limits indicating risk of accumulation even in low levels of heavy metals in soil (Salam-Abdus *et al.*, 2008).

Sugarcane cultivated along Nairobi basin has a market share in Nairobi city and its environs (Foeken & Mwangi, 1998). In Kenya's major towns, including Nairobi, Sugarcane hawking is an economic generator for youth and urban poor (Kaluli *et al.*,2011). In all these towns including Nairobi, Sugarcane, and its products are sold to residents, on street markets, schools, hospitals, bus stops, around slums and suburbs (Hide *et al.*, 2001). Sugarcane is either consumed raw, juiced or used to process jaggery and local brews (Ruth *et al.*, 2013). Sugarcane juice contains Sucrose, flavonoids, polyphenols, amino acids, and minerals (Valli *et al*., 2012; Kadam *et al.*, 2008). Polyphenols in this juice are beneficial to humans due to their antioxidant activities (Kadam *et al*., 2008). Consumption of sugarcane juice has become widespread in Nairobi city based on the increasing number of sugarcane and sugarcane juice selling joints (Kaluli *et al.*, 2011; Ruth *et al.*, 2013). Heavy metals are not degraded in the body but accumulate and may damage tissues such as the central nervous system, liver, kidneys, lungs and bones. In excess, they are associated with several health problems such as acute renal failure, autism, lungs cancer, hyperactivity, hepatoxicity, and genotoxicity (Zahir *et al.*, 2005). Sugarcane grown along Ngong tributary of Nairobi river has the potential to bioaccumulate some of the reported heavy metals and cause the outlined health risk. This study, therefore, aimed at determining levels of heavy metals in sugarcane planted along the Ngong tributary of Nairobi river and its products.

1.2 Statement of the Problem

Heavy metals such as Lead, Mercury, Chromium, Cadmium, and Arsenic have been detected in Ngong tributary of Nairobi river and its environs (Mutune *et al.*, 2014). Some of these metals have been detected in crops such as kales (lead (29.06 mg/kg), chromium (17.42 mg/kg), cadmium (5.78 mg/kg)), arrowroots (lead (16.07 mg/kg), chromium (7.03 mg/kg), cadmium (3.33 mg/kg)) and maize (lead (4.55 mg/kg), cadmium (0.63 mg/kg), grown along the river (Kakoi *et al.*, 2015; Mutune *et al.*, 2014). Although sugarcane is grown along the Nairobi river, information on heavy metals levels of sugarcane and its products is scanty and inconclusive. The risk of usage and consumption of sugarcane grown along the river, therefore, needs to be established as consumption of heavy metals above certain limits exposes users to health problems such as central nervous damage, cancers and genotoxicity.

1.3 Justification

Sugarcane is one of the important world crops, especially due to its sucrose production to sugar. At the household level, sugarcane can be consumed when raw or juiced and for the production of local brews. Locally made sugarcane juice is widely consumed in the informal settlements. Sugarcane, when contaminated with heavy metals are bound to have health effects on human beings. Information on translocation factor and concentration of heavy metals in sugarcane from this river will increase awareness to stakeholders along the sugarcane value chain and consumers. In the NEMA integrated guidelines for land (2011), there is continuous monitoring of human activities around rivers and their impact as well as the prohibition of untreated wastewater for agriculture.The government and its agencies can use information from this study to develop and strengthen existing guidelines on the usage of wastewaters and peri-urban farming. This study will also lay the foundation for future studies on different health problems resulting from heavy metal toxicity.

1.4 Objectives

1.4.1 General objective

To determine heavy metal contamination in sugarcane, health risk and consumer preference of the sugarcane juice consumed along the Nairobi River.

1.4.2 Specific objectives

1. To determine levels of manganese, copper, iron, lead, zinc, cadmium, and chromium in Sugarcane (saccharine species) grown along Nairobi river.
2. To determine levels of manganese, copper, iron, lead, zinc, cadmium, and chromium in soils and water sampled where the sugarcane is grown along Nairobi river.
3. To assess the sensory properties, daily Intakes and total hazard quotients in the vendored sugarcane juices whose sugarcane is sourced from the farms along Ngong tributary

1.5 Research hypothesis

1. The levels of manganese, copper, iron, lead, zinc, cadmium, and chromium in sugarcane grown along Nairobi River are not significantly different from the WHO recommended levels.

2. The levels of manganese, copper, iron, lead, zinc, cadmium, and chromium in soil and water along Nairobi River are not significantly different from levels in sugarcane grown in the same area.
3. There is no significant difference in levels of heavy metals concentration and the preference levels in vendored juices sampled in different slums along Ngong tributary.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sugarcane farming

Sugarcane is an important cash crop cultivated for its stalk sucrose that contributes to the world's 75% raw sugar share, while the remaining 25% comes from beetroots (Sentíes-herrera *et al*., 2014). It also contributes to bioethanol production in countries like Brazil (Sentíes-herrera *et al*., 2014). The world's largest sugarcane producers are Brazil and India, and over 90 countries around the globe are sugarcane producers (Grivet & Arruda, 2007). In Kenya, over 6 million people benefit directly or indirectly from sugarcane. Of this, over 260000 are sugarcane farmers, while 11000 are employees (Jamoza, 2013).

In Kenya, intensive sugarcane farming is in Western, Nyanza, and the greater Lake Victoria basin bordering Kenya and Uganda (Netondo *et al*., 2010). However, farming also occurs in other regions, including Nairobi, which receives between 1000mm and 1200mm annual rainfall (Krhoda & Kwambuka, 2016). This rainfall amount is not adequate for crop farming. Consequently, irrigation with both clean and wastewater supports crop farming in this city (Kaluli *et al.*, 2011). Sugarcane farming practices in Nairobi are on plots and along tributaries of the Nairobi river.

Nairobi river system consists of two tributaries; Mathare and Ngong, each transversing through various industries, waste dumping sites, slums, motor garages, and agricultural lands, all of which contain heavy metal pollutants (Tabainjuki,2007). The water from this tributary is therefore contaminated by industrial waste which contains heavy metals such as zinc cadmium, chromium, manganese, iron, and lead which could end up in sugarcane grown along its banks (Karanja *et al.*, 2010; Kakoi *et al*., 2015).

Sugarcane grown along Nairobi river is sold on estate streets as cuttings that are chewed directly or crushed, forming juice that can be used directly as raw juice for consumption, brewed, or converted into jaggery (Ruth, Jane, & Charles, 2013). Despite the widespread use of sugarcane juice and sugarcane farming employing farmers and vendors in Nairobi, there is the potential for heavy metal contamination from the soils and water in the production environment.

2.2 Factors influencing heavy metal uptake

Heavy metals such as copper, cadmium, zinc, manganese, iron, chromium and lead have different absorption patterns. Some of the heavy metals being essential elements in trace amounts have defined absorption and transportation mechanisms. Others either depend on existing mechanisms used by trace elements or form complexes with other elements for easier transport. The most defining factor for absorption is the chemical form (Wuana & Okieimen, 2011). If the chemical form of the heavy metals is in the solution state, then absorption is far much possible than when the form is insoluble. Copper is absorbed by root surface through active transport and passive transport. It can also form complexes with different compounds, such as carboxylate (Rehman *et al.*, 2019). Soluble chemical forms are available to plants. Another important factor is the electrochemical potential of the elements in the soil and the plant. Iron toxicity causes reduction of insoluble iron (III) and iron (II), causing excessive absorption resulting in radical damage to plants (Sreekanth, 2010). If the elements get immobilised in the roots, then the electrochemical potential weakens and his results to minimal absorption. Lead uptake by rice reduces with an increase in redox potential and pH (Weis & Weis, 2004). If there is a defined mechanism, then the electrochemical potential will work for the absorption increasing absorption. Soil Ph is another factor that affects absorption affects redox potential either improving absorption or reducing.

As the pH increases (becomes alkaline), the reduction of heavy metals occurs, and they become more unavailable (Sreekanth, 2010). Plant species determines the extent to which heavy metals will be absorbed. There are plants which absorb more (phytoremediators) while others resist. Some plant species of family *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae,* and *Scrophulariaceae* are hyperaccumulators of copper, zinc and cadmium (Wuana & Okieimen, 2011). Plants species also compete for these elements, and some exhibit tolerance to some of heavy metals toxicity (Reichman, 2014). Soil humidity also affects absorption. Absorption of elements occur in solution form, and so if the soil humidity is low, there will be minimal absorption (Weis & Weis, 2004).

Presence of other elements in the soil also influences absorption. Some elements will bind and form insoluble complexes, thereby preventing heavy metal uptakes. Fe forms insoluble complexes of phosphates and oxides containing its absorption (Reichman, 2014). Other elements will react with others forming soluble complexes, thereby increasing absorption. Some heavy metals may severely damage cell structures, thereby disabling plants from absorption of other elements. Some will affect gaseous exchange process, carbon dioxide fixation, respiration and nutrient absorption (Sreekanth, 2010). Excess zinc in plants oxidative damage and retarded growth. This damage inhibits the absorption of copper and manganese by the plants (Sreekanth, 2010). Excess cadmium inhibits iron (II) deficiency, uptake of calcium, magnesium, phosphorous and potassium) and affect the transport of nitrates in the plant. copper and lead induce reactive oxygen species bringing about oxidative stress. Manganese toxicity cause chlorosis in plants and severely affects Fe absorption. In an environment uptake of heavy metal is determined by determining the absorption coefficient. Bioconcentration factor (BCF) is the ratio of heavy metals in plants to the concentration in the soil where the exposure results only from the soil. Bioaccumulation factor (BAC) is the ratio of heavy metals in plants to the concentration in soil. Still, it differs with BCF because heavy metal exposure is from all possible routes leaf, roots and shoot. Transfer factor (TF) is the ratio of heavy metals in plant part or tissue to the concentration in soil (Deforest *et al*., 2007).

2.3 Zinc uptake by plants and effects on humans

Zinc is an essential element to both plants and animals (Prasad, 1998). It's naturally emitted by rocks such as olivine, augite, and hornblende as well as volcanoes (Sreekanth, 2010). Agricultural activities also emit a considerable amount of zinc to the soil.

Fungicides, inorganic fertilisers, use of lime, sewage sludge, smelting, detergents and use of manure will enrich the ground with zinc (Bhatti *et al.,* 2016; Omwoma *et al*., 2010).

The mechanism of uptake is still not very clear. However, studies done on absorption show high zinc root intake at pH 7 with large amounts retained in the root cells compared to other plant parts (Peralta *et al*., 2009). The study also showed zinc uptake across plasmalemma occurs in a biphasic manner (Pae *et al,* 2012). In sugarcane, zinc concentration in sugarcane roots, bagasse leaves, and juice decreases with maturity and is lowest at harvest (Sampanpanish & Tantitheerasak, 2015). An excess amount of zinc in the soil will enhance zinc uptake and will increase competitive advantage over iron and manganese in the sugarcane root storage site.

In human zinc is an essential micronutrient that regulates adaptive immune responses (Hojyo *et al.,* 2014). It plays a role in the signalling of the B cell receptor in humoral immune signalling. Lack of zinc in humans lead to lymphopenia and attenuations of both cellular and humoral immunity, increasing body vulnerability to diseases. 15mg/day for adults and 20-25mg/day in pregnancy and lactation is the recommended daily allowance for zinc (Shankar & Prasad, 1998; Mohamed, 2014). Excessive zinc in the body is toxic and is associated with respiratory, gastrointestinal disorders and renal failure (Prasad, 2014). It may also result in pancreatic damage and anaemia as well as zinc fume fever in high dose inhalation. Zinc poisoning mimics lead poisoning and can cause multiple organ failures (Duruibe, 2007). Excess of zinc intake results in oxidative damage leading to enzyme dysfunction (Cakmak, 1993; Romero, 2004).

2.4 Cadmium uptake by plants and effects on humans

Fertilisers produced from phosphate constitute a significant source of contamination, besides industrial waste and smoking (Holmgren *et al*, 1993; Kumar *et al*. 2016). In non-contaminated soils, levels of cadmium vary from 0.01 to 5mg/kg (Peralta *et al*., 2009). Uptake by roots is influenced by the electrochemical gradient, which pulls cadmium and other cations into the roots (Smolders, 2001). In many plants, cadmium is transported from roots to other parts by low-affinity cation transporter (LCT 1). The concentration of iron of 0-10µM reduces cadmium uptake by plants like *Hordeum Vulgare* (Barley). Iron (II) deficiency has also been found to increase uptake in *Arabidopsis halleri* and *Zea mays* (Peralta *et al*., 2009). This trigger the increased uptake of cadmium and iron (II). In *lactuva Sativa* soil rich with manganese promotes cadmium uptake with over 63% retention in the cell wall and subsequent passage to humans and animals (Peralta *et al*., 2009).

Phytochelatins stores cadmium in the root cell vacuoles and is thought to influence symplastic radial cadmium uptake (Peralta *et al.,* 2009). Low-affinity cation transporter (LCT 1) which transport calcium and cadmium in wheat and yeast *Pichia pastoris*respectively is also a likely carrier of cadmium in many other plants (Peralta *et al*., 2009). In *Zea mays* movement of cadmium from soil to root symplast is unregulated while its movement to shoot is restricted. In rice uptake of cadmium from roots to shoots and shoot to grains will determine grain cadmium concentration. (Smolders, 2001). In a study done in China, high grain cadmium retention in rice was 100 times higher than EU recommendation cadmium concentration in rice (Peralta *et al*., 2009).

Cadmium has a far-reaching toxic effect on human. Once in the alimentary canal, it passes through the placenta to the fetus and damage brain membranes. It is also genotoxic (Peralta *et al*., 2009; Järup *et al*, 2016). It is the only toxic heavy metal that causes toxicity in human and animals even at levels that cannot be phytotoxic to plant (Peralta *et al.*, 2009). WHO puts safe limits to a concentration less than 10nmol/mmol creatinine (200mg/kg kidney cortex) (Järup,2003). The resultant tubular damage markers are urine production of β- microglobulin, α-microglobulin, and enzymes (Järup, 2003). Prolonged exposure potentially leads to prostate cancer, lung cancer and kidney cancer (Järup, 2003). Cadmium toxicity may also affect female reproductive health by damaging ovary proteins, lipids and endocrine system (Järup *et al*., 2016; Peralta *et al*., 2009; Kumar *et al.*, 2016).

2.5 Chromium uptake by plants and effects on humans

Chromium's widespread industrial application makes it a very serious environmental pollutant. Its natural form is chromite (FeCr2O4) found in rocks. It also forms complexes with lead rich crocoite (PbCrO4) and bentorite, among others (Shanker *et al.*, 2005). Electroplating, tanning products, pigment and paint products, and wood preservatives all contain and emits chromium metal to the environment. Stable forms of chromium are the lesser toxic chromium (III) (trivalent) and the most toxic chromium (VI) hexavalent (Peralta *et al.*, 2016). Hexavalent chromium toxicity is enhanced by its high oxidising capacity, high solubility, and mobility through cell membranes (Peralta *et al.*, 2009). Trivalent chromium solubility is relatively low, and at the normal ground, pH forms an OH precipitate with iron. Trivalent chromium is considered essential to humans and animals in trace amounts as they control cholesterol and in plants promote growth (Peralta *et al*., 2009).

The concentration of 1-5ppm will alter biological function in both plants and animals. Uptake of chromium by plants will depend on species (Shankar *et al.,* 2005; Biology *et al.,* 2009). Brassicaceae family (kales and cabbages) are higher accumulators than other *Brassicaceae species*(Narasimha *et al.,* 2003). The primary mechanism of uptake by plants is through the formation of complexes with root exudates such as organic acids with resultant increased solubility and movement through the xylem tissue (Peralta *et al.*, 2009; Shanker *et al.*, 2005). Both trivalent and hexavalent chromium enters the roots through the symplast pathway, but upon entry, the hexavalent chromium reduces to trivalent chromium (Shanker *et al*., 2005). The trivalent chromium accumulates into the root cortex tissue. The translocation to roots is very weak (Shanker *et al*., 2005). The hexavalent chromium reduces uptake of essential elements such as iron, potassium, magnesium, manganese, phosphorous and calcium as well as oxidising roots tissue due to the production of reactive oxygen species (Peralta *et al*., 2009). Chromium (iii) uptake is not inhibited by metabolic inhibitors, unlike chromium (iv) in barley. However, uptake of chromium (iii) by active transport is higher than uptake of chromium (iii) in barley (Peralta *et al*., 2009). After uptake through xylem vessels, the distribution to plants parts does not depend on soil properties, and concentration but rather mode of distribution in plants system (Shanker *et al*., 2005). There are no chromium (iv) reducing enzymes in higher vascular plants; therefore, reduction of chromium (iv) to chromium (iii) of is made by bacteria and fungi (Shanker *et al*., 2005). Their mode of uptake best explains other toxicities of chromium (iv) and chromium (iii), chromium (iv) compete with essential elements and follow the metabolic pathway. At the same time, chromium (iii) uses a passive system and is retained by low ion carriers in the system (Shankar *et al*., 2005; Peralta *et al*., 2009).In human chromium is a potential carcinogen that produces reactive oxygen species that is genotoxic with irreparable damage to various organs, tissues, and cells (Budambula & Mwachiro, 2006). Lesser but potentially fatal effects of chromium include respiratory disorders such as asthma, bronchitis, and pneumonia caused by direct chromium inhalation (Peralta *et al*., 2009). Chromium skin contact leads to dermatitis, various skin allergies, and necrosis (Von *et al.*,1993). When inhaled hexavalent chromium reduces to trivalent chromium after solubilisation in lysosomes (Peralta *et al*., 2009). The free trivalent chromium is bound to DNA and complexes with ligands at the hydrophobic end intoxicating the DNA. Such ligands include 1, 10- phenanthroline and 2, 2- bipyridine as well as the picolinic acid (Peralta *et al*., 2009).

2.6 Lead uptake by plants and effects on humans

Lead is also a widely used environmental toxicant with no biological function (Peralta *et al*., 2009). Smelting, agricultural activities, industrial as well as urban wastes are primary sources of lead pollution (Miah & Buruleanu, 2011). Lead forms insoluble precipitates of phosphates and sulfates with very low plant uptake levels (Gale *et al.*, 2004; Peralta *et al*., 2009). However, these precipitates accumulate in the rhizosphere of plants. Lead can immobilise due to the formation of complexes with organic compounds in the soil (Mganga, 2014; Wauna & Okieimen, 2011). As lead is non-essential, plants do not have established channels for translocation hence lead binds to the carboxylic end of root mucilage uronic acids (Peralta *et al*., 2009). The mechanism of entry to root tissues is unknown.*Zea mays*, *H. ovulgare*, and *Alliumcepa* resist lead toxicity through the formation of complexes and inactivation while the vulnerable plants such *Phaseolus* *vulgaris* and*Brassica napus* will be affected through metabolism inhibition (Peralta *et al.*, 2009). Most lead remains bound as phosphates and carbonates in the roots cell wall and extracellular ion exchange sites while Free lead ions will move through calcium channels and settle near root endodermis (Peralta *et al*., 2009; Vara & Oliveira, 2003). Studies show how casparian strip hampers low levels of lead movement to central plant tissue. In wheat, roots cell wall will retain lead whose removal using citric acid to form a complex is done (Marmoli *et al*., 2005; Peralta *et al*., 2009). In European walnut (*Juglans regia*) lead will be stored in the lignocellulosic root structure. Some studies have indicated lead movement to the leaves through xylem tissue and return to plant body through phloem in the form of structures similar to lead acetate, lead nitrate and lead sulfide like in *Prosopis sp.* Lead complexing with phytochelatins has been reported in other plants (Peralta *et al*., 2009). Lead and aluminium accumulate in the body with minimal turnover and slow half-life. They have no immediate exposure to body tissues but rather, long-term implications such as bone loss gestation and kidney failure (Baldwin & Marshall, 1999). In humans and food chain, lead exposure resulting in the liver concentration of 25µg/g liver (dry weight) and kidney concentration of 10µg/g (dry weight) is considered acute poisoning. (Peralta *et al*., 2009). Studies have recorded 100% translocation of lead to blood in rat fed with 300mg/l (Peralta *et al*., 2009). In brain cytosolic protein binds to lead while polypeptides bind lead in kidneys (Smith *et al*., 1998; Peralta *et al*., 2009). Leaded gasoline contains organic lead a more toxic form than inorganic lead (ATSDR,2007; Biology *et al*., 2009). Binding sites in the body include ergothioneine, glutathione, cysteine and homocysteine. All toxic metals not absorbed in the intestinal mucosal may be eliminated from the body altogether (Baldwin & Marshall, 1999).

2.7 Heavy metal contamination in food crops in Kenya

In Kenya, there are various studies on heavy metals absorption in plants. In a study near Moi Teaching and Referral Hospital (MTRH), heavy metals in the water had copper (0.18 ppm); lead (0.46 ppm) and zinc (0.70 ppm). Sediments had copper (1.62 ppm); lead (1.27 ppm) and zinc (6.73 ppm). Bioconcentration coefficient for zinc, copper and lead recorded were 15.1,5.2 and 2.8 respectively. This BAC indicated a high plant preference for zinc

(Jepkoech *et al.*, 2013). Another study in Peri-urban sites in Nairobi Kenya investigating the use of wastewater for irrigation recorded elevated levels of chromium, zinc, nickel-cobalt boron and lead in leafy vegetables and agricultural soils. The heavy metals in Kale recorded were arsenic (0.01-0.4mg/kg), lead (0.39-3.06mg/kg),copper (3.6-6.7mg/kg), nickel (0.76-4.54mg/kg),chromium (trace levels),boron (22-28.67mg/kg)and cadmium (0.01-0.02mg/kg) (Karanja *et al.*, 2012). Lead, and cadmium levels of 48.4 and 26.5 ppm in edible crops were recorded in a study investigating the use of untreated wastewater at Kibera and Maili saba in Nairobi, Kenya against safe limits of 0.3 and 0.2ppm respectively. Plots with Nightshade and kales recorded the highest Enrichment factor(EF) of the lead of about 2200, indicating the environmental risk associated with the industrial waste (Kaluli *et al.,*2014).A study done along Nairobi river investigated ten commonest vegetables grown along the river as well as the soil in the 25 sites analysed for copper, zinc, cadmium and chromium. Soil recorded lead (20mk/kg), copper (75.37mg/kg), zinc (198,3mg/kg), chromium(1.4mg/kg) as well as cadmium (2.6mg/kg). In vegetables the values were lead between 0 - 2.4 mg/kg while copper recorded 0.52 - 21.34 mg/kg. Zinc levels were 20.13 - 89.85 mg/kg, cadmium levels were 0 - 3.02 mg/kg and chromium had 0 - 1.24 mg/kg. (Mutune, 2014). A phytoremediation study involving *Amaranthus hybridus (A. hybridus*) along with Nairobi river concentrations of; cadmium, copper and zinc to be 4.19mg/kg,8.73mg/kg and 17.42 mg/kg respectively (Orwa, 2000). Other studies have dwelt on heavy metal accumulation in soil and water.

2.8 Uptake of heavy metals by sugarcane

Heavy metal contamination has significant effects on the growth and development of sugarcane (Azevedo & Carvalho, 2011). Cases of some varieties with low, as well as high levels of bioaccumulation, have been recorded with some, having significant phytoremediation characteristics (Azevedo & Carvalho, 2011). According to Sereno *et al.* (2007) sugarcane has proved to be cadmium phytoremediator with 500mµ accumulation with no signal intoxication and notable 451mm/kg dry weight shoot content (Azevedo& Carvalho, 2011). The accumulation of heavy metals in sugarcane varies between species as well as soil content and specific heavy metals with no standard threshold across the world (Azevedo & Carvalho, 2011). Another study has shown a high growth in sugarcane irrigated with treated sugar industrial waste. Compared to groundwater irrigation, the overall high levels of totals solids, BOD, COD, phosphates, and sulphates levels, as well as heavy metals, are high (Damodharan & Reddy, 2012). Enrichment factor is significant in sugarcane irrigated with wastewater for 12 years (Alghobar & Suresha, 2015). Soil pH organic matter, carbonates, phosphates soil types influence mobility and heavy metal uptake (Alghobar & Suresha, 2015).

Bioaccumulation and biomagnification of iron, manganese, zinc, lead and cadmium in sugarcane irrigated with feed water with almost undetectable concentration are significant (Adekola, 2008). Studies done in Ngong River have detected too high lead and cadmium content of 48.4 and 26.5ppm against WHO recommended a concentration of wastewater of 0.3 and 0.2 ppm, respectively (Kaluli *et al.*,2014).

2.9 Sensory evaluation, heavy metals and food safety.

Sugarcane juice has become a popular juice sold in various vendor points within Nairobi city, mainly due to its nutritional strength. Environmental conditions for the growth of sugarcane plants may affect sensory and chemical properties of the juice which may influence preference (Schramm, 2014). The unfavourable parameters of sugarcane juice may influence either blending or additions of sugarcane juice additives to hide the original taste (Singh & Gaikwad, 2014). Sugarcane grown in polluted areas such as farms along Ngong tributary may pick heavy metals among other pollutants and pass it to the communities which consume these juices. Studies, however, have pointed out the existence of several health risks of consumption of sugarcane juice (Sreekanth, 2010). These risks are microbiological as well as chemical in nature. Heavy metals are one such risk which depends on the levels of pollution in the area where sugarcane is grown. Studies have indicated bioaccumulation of heavy metals in different parts of sugarcane plant among this the juice which is most likely to cause heavy metal contamination. (Pandey *et al.,* 2016). Data on heavy metals risk assessments of vendored sugarcane juice and their influence on consumer behaviour is not yet known. The level of preferences is suspected to vary as a result of varying pollutants being absorbed by the sugarcane (Singh & Gaikwad, 2014). Blending of sugarcane juice from various sources to factor in cost and taste preferences is common among vendors. Health risk associated with heavy metals ranges from being known carcinogens, mutagens and also causes harm to internal organs such as the liver and lungs. (Griswold & Ph, 2009). Some heavy metals such as iron and zinc are essential microelements to both plants and animals. However, in amounts exceeding recommended levels, they become toxic. Among the influencers of sensory parameters of sugarcane juice, include soil pH, presence of ions such as Sodium, potassium as well as other pollutants (Schramm, 2014). These factors are connected to environmental pollution and degradation. Food quality and safety are significant issues of current world with substantial consequences on economic, social and ecological systems. Food consumed should be free of harmful agents or below toxic limits (Banu *et al*., 2005). There is a possibility of food products to become potentially dangerous to people through contamination with microorganisms, other organisms, toxins, pollution with chemicals and heavy metals (Banu *et al*., 2005). This concept of food security dates back to the 1948 human rights declaration of adequate and standard healthy living, including food. Right to secure food has been considered as a fundamental right (World food summit,1996). At the household level concept of food security concern adequate, nutritionally sound, safe and culturally acceptable food and in variety ((WHO & Consultation, 2003). While urbanisation is a necessity, there has been a sharp increase in Sub-Saharan Africa countries compared to growth of service delivery and employment (World food summit,1996). Dependent on market purchases to food supplies has led to 60-70% spending of household incomes on food in an urban setup. Subsequently, this has led to the rise of urban agriculture in a couple of decades to supplement income (World food summit,1996). The emergence of various types of markets coupled with dynamic consumer needs has led markets such as vending of sugarcane juice. People have become health conscious and consequently adopted diets based on Nutritional benefits they provide to the body. However, other aspects of nutrition, such as safety have been neglected, or adequate information, has not been disseminated. Knowledge of risk of heavy metal consumption in vendored juice will bridge such gaps. This food insecurity risk needs to be adequately addressed by the determination of heavy metals levels and other factors that needs to be addressed.

CHAPTER THREE

METHODOLOGY

3.1 Study design

This research was a cross sectional descriptive study where heavy metals (manganese, lead, copper, iron ,Chromium,cadmium and zinc) levels were determined in sugarcane, soil, water and vendored sugarcane juice along Ngong tributary and a control region. Comparison were made between levels in samples from different region and maximum safe WHO limits.

3.2 Study site

The Ngong tributary of the Nairobi river was the study area with three sampling points depending on industrial pollution. The sampling points were Montoine dam (upstream), which is the source of this tributary, industrial area region (middle stream) and confluence (downstream) to Nairobi river. The stream is a 37.5km from Montoine swamp and Dagoreti forest down, streaming down Kibera slums, Mukuru slums, industrial area, Kayole, Chokaa, Njiru and joins main Nairobi river at Njiru confluence. Control samples were collected at Gesere river in Kisii. Nairobi basin lies at longitudes 10'S36049'E. The annual rainfall is 1000-1200mm, with the long rainy season between March and June and short rainy season of October to December. Mean annual temperature is 17C (Foeken & Mwangi, 1995). September to mid- October is the driest period, January to mid-march is hot and dry while June to mid-October is cold, cloudy and dry. Sampling was done in October 2016 and February 2017. Figure 3.1 shows the Ngong river tributary and particularly the area of study.

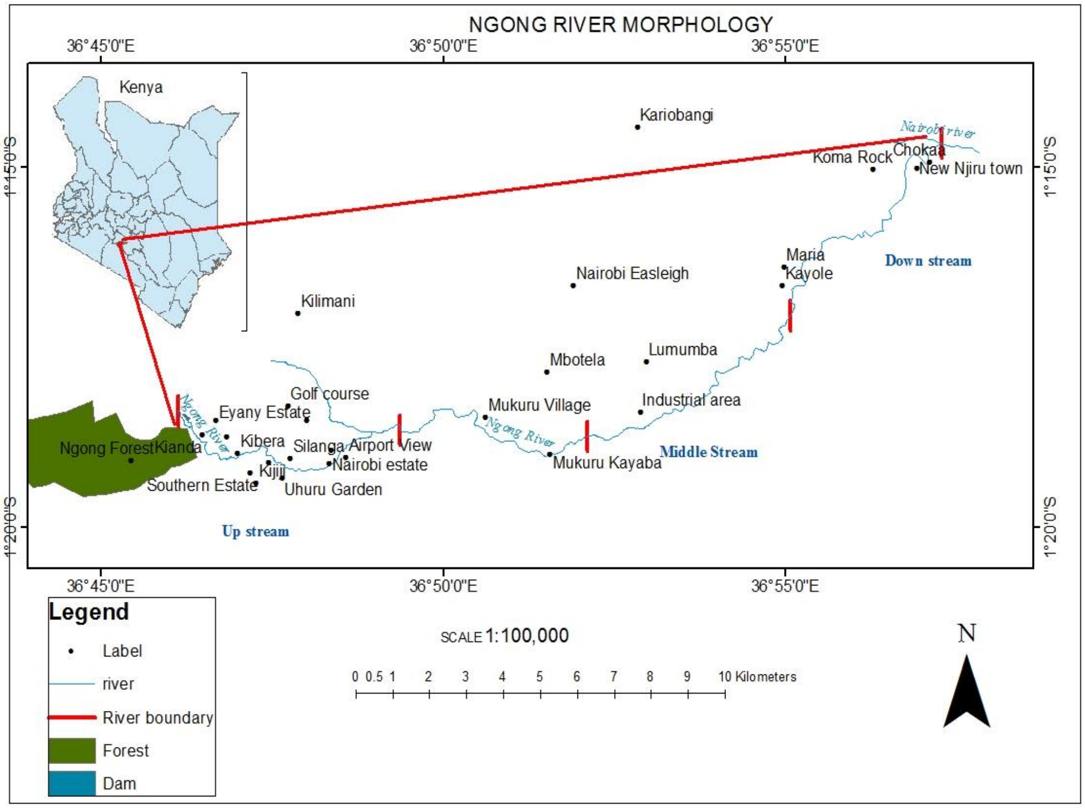

Figure 3.1: Ngong tributary basin

3.3 Sample collection

3.3.1 Sugarcane sample collection

Sugarcane was collected randomly in the upstream region of Ngong tributary region covering a distance of 5km long and 7m in either side of the river. This procedure was replicated in the middle stream, downstream and finalised on the control region. A total of 15 samples were collected on every region making a total of 45 samples. Control samples were collected from Kisii county as the area has had different geographical features and no industrial activities. The uprooted samples were washed with de-ionised water, dried, cut, and kept in a labelled polyethene paper and transported in a cool box for analysis (Xueli *et al*., 2012).

3.3.2 Vendored sugarcane juice sample collection

For purposes of evaluating the risk of consumption of sugarcane juice within slums along Ngong tributary of Nairobi river, samples were collected in five vendors points who sources sugarcane from farms along Ngong tributary and a control region. The heavy metal analysis was carried out on the samples, and the results of the heavy metal levels were used to establish whether there was a risk of consumption or not. Vendored juice was collected in Kibera slums, Mukuru kayiaba, Kariobangi, Sinai, Kisii (keumbu) and Mukuru rube. Three samples were collected from 3 vendors per slum, making it a total of 54 samples.

3.3.3 Soil sample collection

Soil samples were collected randomly in the upstream region of Ngong tributary region at the point of sugarcane sampling covering the 5km distance and 7m in either side of the river. This procedure was replicated in the industrial area of Kayole up to Mukuru Kwa Ruben and finalised on the confluence area of Chokaa to Njiru confluence. Control samples were collected from Kisii. The soil was scooped within a depth of 10cm to 20cm on uprooted sugarcane root. A total of 15 samples each from the four sampling points were taken. Labelling was done accordingly (Xueli *et al*., 2012).

3.3.4 Water sample collection

The river water was collected randomly in the upstream region of Ngong tributary region within a radius of 7 meters from the point of sugarcane sampling and middle of the river. It was collected at a depth of 20cm in replicates covering a distance of 5km (Greaney, 2005). This procedure was replicated in the industrial area of Kayole up to Mukuru Kwa Ruben and finalised on the confluence area of Chokaa to Njiru confluence. The water samples were put in a clean PTFE plastic bottle acidified to pH of less than two by addition of concentrated analytical grade Nitric acid and transported and stored for analysis (Adekola, 2008).

3.4 Heavy metals determination

3.4.1 Glassware cleaning

All glassware and plastic used during sampling, sample storage, and analysis were immersed overnight in concentrated Nitric acid. They were rinsed with lots of de-ionised water and left to dry in the rack as described in the AOAC official method 999.11. Pre-cleaning was done again for every analysis.

3.5 Sample digestion

3.5.1 Soil sample digestion

The soil sample was selected through coning and quartering method (Gerlach *et al*., 2002), ground to a fine powder using pestle and mortar. About 0.01g was digested by the open conventional digestion method using Aqua regia solution (1:2 Nitric acid &hydrochloric acid respectively). The digestion was done for 4 hours on a hot plate at a temperature of 80° C and filtered using a Whatman filter and topped to 50ml.

3.5.2 Digestion of sugarcane juice and water samples

Sugarcane samples were thoroughly cleaned with distilled water and allowed to dry. Juice extraction was done using a stainless-steel juicer in JKUAT fruit workshop. A modified EPA 3050b method of digestion was adopted, 20ml of the sample was placed in a 250ml beaker (Peña-icart *et al*., 2011).50ml 8.5M nitric acid was added and boiled to half the volume.10ml of hydrogen peroxide was added, and further boiling with the addition of a small amount of concentrated nitric acid until brown fumes disappeared and the liquid became clear indicating a complete oxidation process. About half the volume remained which was filtered on 45μm Whatman filter paper into 100ml volumetric flask with constant washing with distilled water, topped to the mark, then analysed using Atomic absorption spectrophotometer. Vendored sugarcane juice was digested and analysed using |the same method. Blanks were done using the same procedure without the sample.

3.6 Standard solution preparation

The stock solution, calibration standards, and working standards were prepared from analytical grade multi-element standards with high purity of approximately 99.9%, The multi-element standard which was in concentration of 100ppb was made into 10ppb, 20ppb 50ppb and 200ppb to draw the calibration curve. This was by topping up 1ml, 2ml 5ml and 20mls of standard solution to 10mls each respectively. To eliminate background interferences blanks consisting the digesting solution for every analysis without the sample were used and digested together with other samples and finally topped to 50ml for analysis.

3.7 Quality control for heavy metal analysis

Appropriate quality assurance procedures and precautions were carried out to ensure the reliability of the results. Glasswares were adequately cleaned, and reagents were of analytical grade. Double distilled water was used for AAS determined samples. Blanks determinations were used together with samples used to correct the reagents interferences. Standards were prepared for each metal from their multi-elements stock solution for calibration of equipment. Precision and accuracy were checked through repeat analysis. A 20.0 µg /g, 30.0 µg /g and 50.0 µg /g blank spike sample in triplicates was prepared and analysed to determine if contamination or sample loss was occurring during the digestion process. Percentage recoveries recorded for chromium, copper, manganese, iron, and lead were 98%, 96.4%, 99.63%, 90.1% and 100% on average. Samples were run in Atomic absorption spectrophotometer and analysed.

3.8 Heavy metal Transfer Factor (TF) in sugarcane

TF was calculated as follows:

TF= Heavy metal concentration in sugarcane ÷ Heavy metal concentration in soil (dry weight basis)

3.9 Sensory evaluation

3.9.1 Consumption pattern

A questionnaire was issued to assess the consumption pattern of sugarcane juice along Ngong tributary. Both farmers and sugarcane vendors were targeted.

Questions dealt with the source of sugarcane, primary customers, their consumption patterns and the ratio of blending.

3.9.2 Sensory evaluation method

Twenty-one untrained panellists did the sensory evaluation. Two scales were used. The first scale was just about the right scale that evaluated sweetness, saltiness and aroma based on questionnaire findings on individual perception on major differences between sugarcane grown along Ngong tributary and other areas (Epler *et al.*, 1998). The description for sweetness was; too sweet, moderately sweet, slightly sweet and flat with a score of 4,3,2 and 1 respectively. Description for saltiness was; too salty, moderately salty, slightly salty and flat with a score of 4,3,2 and 1 respectively. The description for the odour was too pleasant, moderately pleasant, pleasant and unpleasant with a score of 4,3,2 and 1 respectively.

The second scale was the 9-point hedonic scale and description for the preference was; like extremely, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely with a score of 9,8,7,6,5,4,3,2 and 1 respectively (Singh & Gaikwad, 2014; María *et al*., 2017).

3.10 Health risk assessment

The consumption and health risk assessment was calculated by a method by EPA (2010) as used by (Pandey, Suthar, & Singh, 2016).

$EDI=C\times DI/BW$ (Pandey et al., 2016).

Where

EDI = Estimated daily intake in mg/kg/d,

C= concentration of specific heavy metal in the sugarcane juice in mg/l,

DI= The daily intake of the juice as per survey; Adults on average took 0.5litres per day, while children took 0.25litres per day

BW =Body weight .70kg was considered BW for adults and 15kg for children

$THQ=EDI \times EF \times ED/RFD \times AT$

Where

THQ =Total hazard quotient

EDI = Estimated daily intake in mg/kg/d,

EF = Exposure frequency per year considered to be 350 days

ED = Lifetime exposure duration considered to be 67.5yrs on average for both male and females as per Kenya's life expectancy

RfD =WHO specific heavy metals' recommended levels in juice in mg/l

AT=Average lifetime exposure in days considered to be 67.5 ×365days.

$TEDI=\Sigma 7\ EDI$

$TTHQ= \Sigma 7 THQ$

Where,

TEDI = Sums of specific metals EDIs.

TTHQ =The sums of specific metals THQs

3.11 Statistical Analysis

Statistical analysis of the results of heavy metal concentration in soil samples, water samples, sugarcane juice and vendored sugarcane juice as well as sensory evaluation results was done using Graphpad (version 6). The descriptive statistic that included the mean, standard deviation, range and standard errors were used using a one-way ANOVA test procedure at a 95% level of confidence at turkey's test range. The results obtained were compared with the WHO maximum recommended limits.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Heavy metal concentration in sugarcane juice

The lowest amount recorded for zinc in sugarcane juice was at the upstream region (1.88mg/l) while the highest values were at the downstream (2.5mg/l) and control region (2.5mg/l) (Table 4.1). There was no significant difference in the zinc levels in sugarcane juice for the middle stream (2.22mg/l) and upstream (1.88mg/l). However, levels in sugarcane juice at downstream and control region were significantly different compared with levels in sugarcane juice in both middle stream and the upstream region at $p<0.05$. WHO limits in juices and drinking water are 5mg/kg, a value way above the values in this study. Zinc absorption depends on various environmental and physiological factors which would have contributed to the absorption pattern. Absorption of zinc is high at pH 7 (Preet & Sidhu, 2016) and excess zinc levels in soil enhance its absorption (Tsonev & Lidon, 2012). Zinc levels decrease with plant maturity (Sampanpanish & Tantitheerasak, 2015). These factors could have explained the differences in levels in various regions. Zinc is an essential element to plants, but above certain limits (125ppm), it becomes toxic to both plants and animals (Badoni *et al*., 2016). Zinc amounts in sugarcane juice irrigated with wastewater were found to be in the range of 4.55-48.9mg/kg in a study in India (Pandey *et al*., 2016). The levels in the current study (1.88-2.5mg/l). were above these levels. However, levels of contamination and geographical location vary. The levels in the present study were also lower compared to levels in another study in China which reported levels of 10.64mg/l in sugarcane juice (Liao *et al*., 2016). Zinc is dispensed by fertilisers, pesticides, industrial waste and also naturally occurs in soil. These sources could have explained the non-significance difference in control and downstream region, considering the level of industrial pollution is different in the two regions (Sreekanth, 2010).

Table 4.1: Heavy metal concentration of sugarcane juice in different points of the Ngong tributary and control region.

Heavy metal	Upstream	Middle stream	Down stream	Control (Kisii)	WHO (mg/l)
Zn	1.88 ± 0.34^{a}	2.22 ± 0.23^{a}	2.50 ± 0.23^{b}	2.50 ± 0.03^{b}	5
Fe	0.76 ± 0.06^{a}	3.06 ± 0.07^{b}	3.61 ± 0.08^{c}	3.61 ± 0.08^{c}	0.01
Cu	0.13 ± 0.01^{a}	0.2 ± 0.01^{b}	0.27 ± 0.02^{c}	0.02 ± 0.00^{d}	2
Cr	0.02 ± 0.01^{a}	1.28 ± 0.89^{b}	2.81 ± 2.33^{b}	0.01 ± 0.00^{a}	0.1
Cd	ND	ND	ND	ND	0.1
Pb	0.31 ± 0.01^{a}	0.43 ± 0.02^{b}	0.46 ± 0.02^{b}	ND	0.01
Mn	2.58 ± 0.04^{b}	2.24 ± 0.04^{a}	2.41 ± 0.03^{a}	2.58 ± 0.03^{b}	0.5

Different letters in the column indicates a significance difference ($P \leq 0.05$) while similar letter indicates values are not significantly different. ND refers to not detected. All concentrations are in mg/l.

The lowest levels of iron in sugarcane juice were in the upstream region of the river (0.76mg/l) and were significantly lower than all other regions ($P<0.05$) (Table 4.1). The levels of iron in sugarcane juice from the middle stream (3.06mg/l), downstream (3.61mg/l) and control (3.61mg/l) were not significantly different. All levels had higher than the WHO recommended limits of 0.01mg/l. Fe absorption in plants depends on the electrochemical gradient. Iron also competes with cadmium, and this may have explained the non-detection status of cadmium in juices and thus, higher iron levels (Peralta et al., 2009). Iron concentration in soil is also generally affected by concentration on the ground and natural rocks of different soils. pH and chemical forms which affect the absorption of iron in plants (Pandey et al., 2016). These factors may have explained the differences in levels in the juice especially in the downstream and control regions which had significantly higher amounts. In a previous study, iron in sugarcane juice was found to be in a range of 1.6- 12.5mg/l (Jaffe, 2015). The current study levels (0.76-3.61mg/l) were within the same range.

The concentration of copper in sugarcane juice was highest at the downstream region (0.27mg/l) and lowest at the control region (0.02mg/l). Upstream region had 0.13mg/l and middle stream had 0.2mg/l. Copper levels in sugarcane juice from downstream and middle stream regions were not significantly different but were significantly higher than sugarcane juice heavy metal levels from upstream and control region ($p<0.05$). The levels were also below the WHO limits of 2mg/l. Copper absorption increases with a concentration in the soil (Krhoda & Kwambuka, 2016; Roberto & Camilotti, 2014.). However, when introduced in the environment, copper rapidly stabilises in the soil and form insoluble complexes. Only a small percentage forms a solution of ions and thus absorbed (Wuana & Okieimen, 2011). The high amounts of copper in sugarcane juice from both the downstream and middle stream regions could have emanated from industrial and slums waste (Krhoda & Kwambuka, 2016). However, the low absorption by the sugarcane plant was possibly due to factors such as insolubility complexes or unfavourable environmental conditions. The current study levels (0.13-0.27mg/l) were lower compared to a previous study done in sugarcane juice which was found to be between 3.56-22.38mg/kg (Pandey et al., 2016).In another study, levels of 1.82mg/l for sugarcane juice planted in industrial waste zones were recorded in China (Liao et al., 2016). The current study values were lower compared to levels in this study.

Chromium levels in the juice were highest at the downstream region of the river (2.81mg/l) while the lowest level was in juice from the control region(0.01mg/l) (Table 4.1). Levels in the middle region (1.28mg/l) of the river were not significantly different from the downstream region. Still, they were significantly higher than levels at the river upstream region (0.02mg/l) and the control region (0.01mg/l) ($p<0.05$). Sugarcane harvested along Ngong tributary regions had higher than the WHO Chromium recommended levels of 0.1mg/l of chromium in drinking juices. Uptake of chromium by sugarcane juice depends on plant species (Shanker et al., 2005). The primary mechanism of absorption of chromium in plants is through the formation of complexes with root exudates such as organic acids with resultant increased solubility and movement through xylem tissue (Peralta et al., 2009). Upon entry to roots through the symplast pathway, the hexavalent form is reduced to trivalent and stored in the root cortex. Translocation is weak, and also, they inhibit the uptake of iron and manganese (Shanker et al., 2005). These factors explain why levels in juices are generally low compared to other heavy metals, although higher than WHO recommended levels of chromium of 0.1mg/l in drinking juices. Since higher levels of chromium inhibit the uptake of manganese, their levels in these juices were subsequently lower. Sugarcane harvested along Ngong tributary regions had higher than the WHO chromium recommended limits. The high levels of chromium in sugarcane juice from the middle stream and downstream region of Ngong tributary indicated possible contaminations from industrial and anthropogenic activities. Previously recorded levels of chromium in sugarcane juice were in the range of 10.54-60.22mg/kg in India (Pandey et al., 2016). All the regions in the current study recorded lower values than the levels in this Indian study. Chromium contaminations mostly emanate from fertilisers and organic manure as well as sewage sludge (Sreekanth, 2010). Anthropogenic activities, sewage sludge and industrial sludge, could have contributed to the high levels in both the middle stream and downstream areas. Iron carriers facilitate chromium absorption. Thus, the absorption of chromium is inhibited by high Iron levels. Environmental factors such as temperature and pH may also have influenced chromium absorption (Shanker et al., 2005).

Cadmium in the sugarcane juice was not detected in all samples. WHO limits for cadmium in juice and water is 0.1mg/l. Uptake of cadmium is influenced by electrochemical gradient which pulls cadmium and other cations into the roots. The high concentration of iron reduces cadmium intake (Peralta et al., 2009). The undetected cadmium levels in all regions could have been due to poor absorption in sugarcane crops (Onyedika & Okon, 2014) and unfavourable electrochemical gradient. Mobility of cadmium from soil to plants is also severely restricted at its roots (Onyedika & Okon, 2014). These may have contributed to non-detection of cadmium. Previous studies in different regions in the world recorded cadmium levels of 0.0001-0.1mg/l in India (Pandey et al., 2016), 0.18mg/l in China (Liao et al., 2016), and 0.152mg/l in China. (Wang et al., 2018). These figures were slightly above the WHO recommended levels of 0.1mg/l.

Lead levels in the sugarcane juice were undetected in the control region. The highest levels of lead in sugarcane juice were recorded in the middle stream region (0.43mg/l) and downstream region (0.46mg/l) while upstream recorded 0.31mg/l. There was no significant difference in lead concentration in juice from sugarcane harvested in middle stream areas and downstream areas. However, the levels of lead in sugarcane juice in both middle stream and downstream region were significantly higher than upstream (0.31mg/l) and control region ($p<0.05$) (Table 4.1). All levels of lead in sugarcane juice were above the WHO recommended limits of 0.01mg/l apart from the sugarcane juice from the control region where no detectable amounts were recorded. Previous studies recorded 0.01-1.11mg/kg lead levels in sugarcane juice from India (Pandey et al., 2016). In China lead levels in sugarcane grown in similar conditions were 0.2mg/l (Liao et al., 2016). The levels in the current study were within the same range of levels in these studies. As lead is non-essential, plants do not have established channels for translocation hence binds to the carboxylic end of root mucilage uromic acid. The insoluble phosphates and sulfates formed by lead immobilise to the root while free lead ions will move through calcium channel and settle through root endodermis (Pourrut et al., 2011). Some studies show levels of lead movement to central plant tissue being hampered by casparian strip. Some other studies have shown lead movement to the leaves through xylem tissues and return to plant body through phloem in the form of lead acetate, lead nitrate and lead sulfide (Peralta et al., 2009). These factors could have accounted to high levels of lead in the sugarcane juice in the middle stream and downstream and non-detected levels in the control region. Lead is released to the environment through corrosion of commercial waste products, chemical combustions and petroleum products (Sreekanth, 2010). Higher levels of lead in sugarcane juice the middle stream and downstream region of Ngong river indicated higher pollution rates and absorption in the region as opposed to non-detected levels in the control region.

Manganese concentration in sugarcane juice in the upstream region (2.58mg/l) and control region (2.58mg/l) was significantly higher than the rest of the regions ($p<0.05$). The levels of manganese in sugarcane juice in the middle stream (2.24mg/l) and downstream regions (2.41mg/l) were significantly lower ($p<0.05$). However, they were not significantly different. Manganese absorption is enhanced in acidic soils and the transpiration stream. The immobilisation in leaves lowers its movement to other plants parts (Millaleo et al., 2010). The highest concentration of the soluble manganese ions is high after hot, dry summer in waterlogged conditions due to containment of manganese oxidising organism hence chemical reduction of manganese occurs (Millaleo et al., 2010; Peralta-videa et al., 2009). Sugarcane juice manganese levels from all regions had values higher than the WHO recommended limits of 0.5mg/l. Upstream and control regions main activities are agriculture-based, which could have contributed to higher absorption in sugarcane compared to sugarcane in other regions under study. A previous study in India found levels of manganese in sugarcane irrigated with eight different treatments of iron, manganese and sulphur to be 0.46-0.86mg/kg and this included the levels in bagasse and juice (Mishra et al., 2014). The current study had levels higher than the levels in the above research. In non-centrifuged sugarcane juice (jaggery) manganese was found to be 8mg/kg (Jaffe, 2015). The current study had lower levels than the above study. Excess zinc in soils could have led to reduced manganese availability and absorption in plants (Jamal et al., 2013).

4.2 Heavy metal concentration in soil

The highest level for zinc was in soil in the upstream region (109.8mg/kg) while the lowest level was in soil from the control region (18.99mg/kg). Levels of zinc in soil from all the regions were significantly different ($p< 0.05$) (Table 4.2).

Table 4.2: Heavy metal concentration in soil collected along Ngong river tributary

Soil sampling points heavy metal concentration in mg/kg					
Heavy metals	Upstream	Middle stream	Downstream	Control	WHO
Zn	109.8 ± 0.09^{d}	20.9 ± 1.27^{b}	47.49 ± 0.26^{c}	18.99 ± 0.07^{a}	300
Fe	295.2 ± 6.82^{b}	176.0 ± 4.3^{a}	250.2 ± 22.16^{b}	295.2 ± 6.82^{b}	50000
Cu	4.08 ± 0.59^{a}	122.2 ± 0.66^{d}	59.94 ± 2.45^{c}	22.35 ± 0.26^{b}	100
Cr	12.63 ± 1.06^{a}	40.96 ± 0.87^{b}	121.3 ± 4.20^{c}	59.74 ± 0.23^{d}	200
Cd	ND	0.43 ± 0.57^{b}	0.01 ± 0.02^{a}	0.29 ± 0.25^{b}	100
Pb	99.00 ± 0.326^{a}	98.33 ± 0.78^{a}	98.31 ± 0.31^{a}	99.42 ± 0.25^{a}	100
Mn	109.8 ± 0.09^{a}	102.7 ± 0.55^{a}	111.1 ± 0.17^{b}	110.6 ± 0.94^{b}	2000

Different letters in the column indicates a significance difference ($P<0.05$) while similar letter indicates values are not significantly different. ND refers to not detected. All concentrations are in mg/kg.

However, levels of zinc in soil from the middle stream and upstream were not significantly different. Zinc content in soil can be influenced by soil type and the presence of other metals. Generally, iron, manganese, and copper share the same soil matrix and are usually higher in red soil (Liao *et al*., 2016). Previous studies along Ngong tributary indicated zinc levels in the soil of between 40.34-190.05mg/kg (Mutune *et al.,* 2014). The levels in soil in the current study were within the same range as the previous study. However, a previous study found zinc levels in the soil to be within a range of 4.55-48.9mg/kg in China (Pandey *et al*., 2016). The current study values were higher than this study. Levels of zinc in soil within a range of 0.432-0.807mg/kg were also found in a study in Nigeria and the levels in the current study were far much higher than levels in soils in this study (Funtua *et al.,* 2014). Levels in the present study were within the same range as levels in a study in India with an average level of 49.10-57.09mg/kg found in a soil contaminated by industries (Pandey *et al*., 2016). High levels in the upstream and downstream region of Ngong river could have been explained by the presence of zinc rich soils and anthropogenic activities in these two regions respectively. Subsequent leaching experienced in rainy season transporting the zinc rich soils along the river and subsequent deposition on the river banks may also explain the high levels in downstream.

The highest concentration of iron in soil was from the upstream region of Ngong river (295.2mg/kg) and control region (295.2mg/kg) followed by downstream (250.2mg/kg) while the middle stream had the lowest value (176.0mg/kg). The levels in upstream, control and downstream regions were not significantly different ($p>0.05$) but significantly higher than the middle stream($P<0.05$) (Table 4.2). The soil matrix at the control region and the upstream regions are mainly red soil. High levels of Fe found in the upstream region and control region still indicated the influence of natural rock type. The values in the current study were within the same range as previous studies in India under similar conditions (220mg/kg) (Kumar *et al.*, 2016). A mean level of 566mg/kg in both topsoil and subsoil levels in contaminated soil was found in a study in India (Pandey *et al*., 2016). Another study found iron levels of 565.7-566.94mg/kg in an industrial waste contaminated soil in India (Pandey *et al.*, 2016). The levels in the current study were slightly lower compared to levels in the above studies and variation could be due to the level of contamination or the natural soil type variations.

The highest level of copper in soil was in the middle stream (122.2 mg/kg), followed by the soil in the downstream region (59.94mg/kg) (Table 4.2). The lowest level was in soil from the upstream region (4.08mg/kg) while levels of copper in soil from the control region was 22.35mg/kg. There was a significant difference in levels of copper in soil from all regions ($p<0.05$). Only soil from the downstream region had levels higher than the WHO limits of 100mg/kg. The highest amounts of copper in soil were detected at the middle stream, which is the area with most industries and human settlement and this could have produced higher copper contamination in the soil. High levels in the middle stream indicated likely industrial waste contamination along this region as well as contaminants arising from other anthropogenic activities. Increased levels in middle stream region and downstream indicated the probability of industrial waste or waste arising from dense human settlement finding its way to the soil and possible surface runoffs, flooding and silting on the lower edges of the river. In a study along the Nairobi river, Ruiru river and Juja rivers, soil recorded copper levels of 6.43-45.61mg/kg (Mutune *et al*., 2014). A previous study involving heavy metals in African leafy vegetables along the Nairobi river and its tributaries, soil recorded copper levels ranging from 3.59-75.37mg/kg (Mutune *et al*., 2014). In an urban setup study in Uganda, Copper in soil was found to be 36.23mg/kg while a review for copper content in waste contaminated soils in England found copper levels to be 38-57mg/kg (Gleadthorpe, 2008). The current study concentrations were with the same range as the above studies.

Chromium levels in the soil ranged from 12.63mg/kg to 121.3mg/kg (Table 4.2). Highest levels of chromium in soil were from the downstream region (121.3mg/kg) followed by a control region (59.74mg/kg), middle stream region (40.96mg//kg) while the lowest levels of chromium in soil were in the upstream region (12.63mg/kg). All levels were significantly different ($p< 0.05$). All the regions had lower than the WHO recommended limits of 2000mg/kg. Chromium sources are generally industrial and agricultural-based (Bhatti *et al*., 2016). Levels of chromium in the soil in the control region and upstream regions indicated agricultural chemical-based pollution.In contrast, low levels of chromium in the soil in the middle stream area could have been associated with dilutions from large water volumes that moved along the river. Previous studies along Nairobi river found values of 0.03-1.4mg/kg of chromium in soil (Mutune *et al*., 2014) while in naturally occurring soils chromium ranged between 10-1000mg/kg (Shanker *et al*., 2005). The values in the current study were slightly higher than values in above studies. A study in China soil that was heavily polluted recorded levels of chromium of 74.58mg/kg (Pan *et al*., 2017), while another one had 24.1-67.58mg/kg (Pandey *et al*., 2016). The values in the current study were within the range of heavy metals in above studies.

The results showed that cadmium levels in soil were in the range of 0 to 0.43mg/kg (Table 4.2). The highest amount was in soil from the middle stream region (0.43mg/kg) followed by the soil from control region (0.29mg/kg), downstream region (0.01mg/kg) and no levels were detected at the upstream area. Middle stream region had significantly higher levels of cadmium in soil than the rest ($p<0.05$) but lower than recommended WHO limit of 100mg/kg. Previous studies along the Nairobi river had levels of cadmium in the soil of 2.02-2.64 mg/kg (Karanja *et al*., 2012). The levels in the current study were lower compared to the above study. Cadmium levels in soil in India ranged from 0.516-1.58mg/kg in another study involving contaminated soil (Bhatti et al., 2016). Another study in China found levels of cadmium in the soil of 0.41-1.71mg/kg in industrially contaminated soil (Holmgren *et al*., 2010). The levels in the current study were within the range in these studies though slightly lower. High levels in the middle stream indicated the possibility of industrial and dense settlements waste finding its way to the soil along the banks of Ngong river.

Lead levels in the soil were in the range of 98.31mg/kg to 99.42mg/kg (Table 4.2) and with no significant difference ($p>0.05$). The highest levels of lead were in soil from the upstream region (99.00 mg/kg) while the lowest was in soil from the downstream region (98.31mg/kg). Upstream and middle stream (industrial area) had soil with lead levels of 99.00mg/kg and 98.33mg/kg, respectively. All levels were below the WHO recommended limit of 100mg/kg. Previous studies in agricultural soils in the USA recorded values of 47.7-52.6mg/kg (Holmgren *et al*., 2010) and 109.8-240mg/kg in Dandora dumpsite along the Nairobi river (Mulamu, 2014). These levels were below the levels of lead in soil found in the current study apart from those results around Dandora dumpsite which had higher results possibly due to lead waste accumulated in the dumpsite. There was no significant difference in lead levels in all regions at $p<0.05$ indicating lead pollution was in both along Ngong tributary and control region.

Manganese levels in soil were in the range of 102.7mg/kg and 111.1mg/kg (Table 4.2). The highest levels in soil were in the downstream region (111.1mg/kg) followed by control region (110.6mg/kg), upstream region (109.8mg/kg) and the lowest levels were in the middle stream (102.7mg/kg). The levels of manganese in the soil at the middle stream were significantly higher($P<0.05$) while the rest of the regions were not significantly different. All regions had values lower than the WHO recommended limit of 2000mg/kg. High levels in the control region indicated the soils could generally be rich in manganese, and also pollution been predominantly agricultural-based (Sreekanth, 2010). Depending on the season and magnitude of Manganese pollution levels in the soil are expected to vary. In a previous study, soil Manganese levels in contaminated soil in Turkey ranged from 167mg/kg -382mg/kg (Rudmick & Gao,2004). Another study found manganese levels of 182-806mg/kg in an industrial waste contaminated soil in Turkey (Ekmekyapar, 2012). Values of Manganese in the current study were below this level, possibly due to geographical and pollution variations.

4.3 Heavy metal concentration in river water

The lowest amount of zinc recorded in water was 0.03mg/l at the control region followed by the upstream region, which had a concentration of 0.38mg/l in water. The downstream region had 1.13mg/l in the water while the middle region had 0.59mg/l (Table 4.3). All levels were significantly different ($p<0.05$). All the values were lower than the WHO recommended levels of 5mg/l. The levels in water along the middle stream and the downstream had the highest values possibly due to the constant dispensation of waste at these points. A study done before along Ngong river indicated levels of zinc in river water of between 0.01-0.02mg/l respectively (Kithiia, 2007). The levels of zinc in water in the current study were within the same range of concentration as in the above study. Zinc levels in wastewater in India were found to contain 0.133-0.278mg/l (Alghobar & Suresha, 2015). Studies in Nigeria recorded zinc levels in river water of 0.073-1.67mg/l (Gimba *et al*., 2015) as well as 0.2mg/l in wastewater used in irrigation (Chiroma *et al*., 2014). The levels of zinc in the current study were within the same range as levels of zinc in water in these studies. High levels in both middle stream and downstream of Ngong river indicated likely industrial effluent pollution. However, the effects of surface runoffs were evident as the highest levels were in the downstream compared with other regions along the river.

Table 4 3: Heavy metals concentration in river water collected along Ngong tributary

River water sampling points' heavy metal concentration in mg/l					
Heavy metals	Upstream	Middle stream	Downstream	Control	WHO
Zn	0.38±0.18^b	0.59±0.01^b	1.13± 0.17^c	0.03±0.03^a	5
Fe	0.77±0.02^a	1.41±0.03^c	3.37± 0.06^b	3.50±0.07^b	0.01
Cu	ND	ND	ND	ND	2
Cr	ND	0.75±0.11^c	0.09± 0.01^b	ND	0.1
Cd	ND	ND	ND	ND	0.03
Pb	ND	0.06±0.02	ND	ND	0.03
Mn	1.49±0.08^b	1.58±0.05^b	2.66±0.07^c	0.07±0.01^a	0.5

Different letters in the column indicates a significance difference ($P \leq 0.05$) while similar letter indicates values are not significantly different. ND refers to not detected. All concentrations are in mg/kg.

Iron levels in river water ranged from 0.77mg/l to 3.50mg/l. The Iron levels in river water at the control region were (0.77mg/kg), and the middle region had (1.41mg/kg). Downstream region river water had 3.37mg/kg iron content while the upstream region river water had the lowest value of iron (0.77mg/kg) (Table 4.3). The levels in all region were significantly different ($p<0.05$) except the downstream and control regions that were not significantly different. The middle stream region had the lowest levels. Upstream region and control region have red soil whose iron content is higher than clay soil found in other regions. The previous study along Ngong river indicated iron levels in the water of 1.99-1.44mg/l (Kithiia, 2007). Other studies recorded Iron levels in river water of 0.395-22.90mg/l (Gimba *et al.*, 2015),0.5-13.94mg/l in Nigeria (Chiroma *et al.*, 2014), 2.48-2.93mg/l in India (Alghobar & Suresha, 2015). The levels in the current study were within these ranges. High levels in river water in downstream, middle stream and control indicated that iron pollution was in all regions eliminating the likelihood of only industrial effluent contamination. Rock type, industrial and domestic effluents are likely sources of iron contamination, and this also explained the high levels in the control region (Sreekanth, 2010). The reduced human activities on the upstream region of Ngong river characterised by lowest human activities compared to other regions and characteristic clay soil low in Fe content compared with red soil explained the lower levels of Fe in the upstream region. Levels of iron in river water observed in this study were higher than WHO and European standards limits of 0.5mg/l (Chiroma *et al*., 2014).

The concentration of copper in water was not detected in all samples. The undetected levels were hence below WHO limits of 2mg/l. A study done before along Ngong river indicated levels of copper between 0.04-0.18mg/l (Ndeda & Manohar, 2014). Undetected levels of copper in water in all regions could have been due to increased dilutions in the river water and also the fact that copper is generally low in industrial effluent apart from where smelting is done and even mining. These factors may also have indicated the infrequency of copper contaminations along Ngong tributary. However, low levels and to an extent, undetectable levels may have been recorded in upstream and control regions due to absence of copper contamination sources. Copper mobility from soil to water is severely restricted as to its heavily bound in the soil, and this also may have explained the low levels in water (Fernandes & Henriques, 2018). Previous studies in different regions in the world recorded copper levels in the water of 0.0 3-0.6mg/l in Nigeria (Gimba *et al*., 2015),0.017-0.46mg/l in Nigeria (Chiroma *et al*., 2014), and 0.04-0.29mg/l in Kenya (Jepkoech *et al*., 2013).

The highest level of chromium in the water was 0.75mg/l in the middle stream region, followed by downstream (0.09mg/l) while the water from the rest of the areas had undetected levels of chromium. Levels in all regions were significantly different ($p<0.05$). Middle stream region had the highest levels. The levels were also than the WHO recommended limits of 0.1mg/l. Chromium contamination could be from industries or agriculture. High levels in the middle stream may have been due to industrial and agricultural activities around the middle stream region. Agricultural activities could have contributed to high levels in the control region. Undetected levels at the control region could have been associated with low levels of pollution or insolubility of chromium complexes. A study in India recorded chromium levels in the water of 0.031-0.032mg/l (Alghobar & Suresha, 2015). The levels in the current study were within this range.

In all river water samples collected there was no detection of cadmium. The undetected levels were therefore below the recommended WHO limits of 0.03mg/l and could have been associated with diluted amounts of cadmium-based waste or infrequency of cadmium contaminations. Cadmium pollution arises from industrial effluents and diesel oils (Sreekanth, 2010) and the latter could have explained the levels of cadmium in the upstream due to its proximity to the highway. Previous studies recorded cadmium levels of 0.009-0.446mg/l in Nigeria (Gimba *et al*., 2015) and 0.047mg/l in India (Alghobar & Suresha, 2015).

Pb levels in the water were only detected in middle stream region 0.06mg/l. and the values were above WHO recommended limits of 0.01mg/l. Dilution effects could have been responsible for undetected lead levels in water in other region coupled with very low lead-based waste pollution across the river in the dry season. Lead waste is both fuel-based and manufacturing-based pollution, and because of this entry point could have been any point along the river (Sreekanth, 2010). Detection of lead in water in the middle stream to a level higher than recommended limits indicated the possibility of industrial effluent pollution. Previous studies done indicated levels of 0.053mg/l in river India, (Alghobar & Suresha, 2015) and 0 - 0.07mg/l in Athi and Nairobi river basin (Kithiia, 2007). The current study levels were within the range of these studies levels.

Manganese levels in the water were in the range of 0.07mg/l and 2.64mg/l. The highest levels were in the downstream region (2.66mg/l) followed by middle stream (1.58mg/l), upstream (1.49mg/l), while the lowest levels were at the control region (0.07mg/l) (Table 4.3). All levels were significantly different ($p< 0.05$). All regions apart from the control region had values higher than the WHO recommended limit of manganese in water in (0.5mg/l). Manganese pollution is associated with agricultural fertilisers and industrial activities. These sources may explain the high levels at the middle stream region compared with other regions. Low levels in the control region were probably due to dilution effect by the water, absence of water contaminations or due to the growth of huge plant mass grown along the river. Compared to Ngong river, the plant mass has been severely hampered due to human activities (Sreekanth, 2010). Previous studies recorded levels of 0.157mg/l in India (Alghobar & Suresha, 2015),0.046-1.85mg/l in groundwater in Nigeria (Gimba *et al*., 2015). Levels in the current study were within the range of these previous studies. Higher levels in the downstream indicated the likely hood of industrial effluent contamination movement from middle stream region to downstream region.

4.4 Heavy metals transfer from soil to sugarcane

4.4.1 Transfer Factor

In general zinc, iron and chromium were highest at the middle stream region, lead was highest at the downstream region while copper, lead and manganese were highest at the upstream region. Transfer factor determined for the juice (Table 4.4), showed values way less than one, indicating the very low uptake of heavy metals in juice.

Table 4 4: Transfer factor of the heavy metals in sugarcane juice

	Sugarcane sampling points			
Heavy metals	Upstream	Middle stream	Downstream	Control (Kisii)
Pb	0.003	0.004	0.005	0
Zn	0.017	0.106	0.053	0.037
Fe	0.003	0.017	0.014	0.012
Mn	0.024	0.022	0.022	0.023
Cr	0.001	0.031	0.023	0
Cd	0	0	0	0.001
Cu	0.032	`0.002	0.004	0.001

According to the results (Table 4.4), zinc transfer factor was highest at the middle stream region followed by downstream, control and upstream at 0.106, 0.053, 0.037 and 0.017 respectively. All values were not significantly different. The transfer factor depends on various factors such as soil physical-chemical properties, plant biochemical reactions, as well as heavy metals chemical forms (Sreekanth, 2010). Zinc levels in the soil were highest at the control region, probably due to favourable environmental characteristic within the area. It follows that zinc levels in soil did not translate directly into an increase in transfer factor. Heavy metals uptake is not linear to the increase in soil concentrations. It is due to a combination of other factors including soil pH, different uptake routes such as leaves, chemical nature of the elements, temperature and aeration among many other factors that influence heavy metals uptake (Sreekanth, 2010). Transfer factor of zinc was less than 1 in all regions, indicating very low levels of heavy metal transfer from soil to the juice.

Iron transfer factor order from highest to lowest was as follows: middle stream, downstream, control and the upstream region at 0.017, 0.014, 0.012 and 0.003 respectively. Again, it follows that high iron levels in soil did not directly translate into high levels in the juice. Iron is a macro element in plants, and its availability to roots is a combination of complexation -chelation processes in the root/medium interface (Pandey *et al*., 2016). The soil characteristics could have influenced more iron intake at the middle stream and downstream region compared to other regions. This fact was because iron may exist in an insoluble state or soluble state as well as the presence of ions such as zinc that improves iron absorption in soil (Sreekanth, 2010).

Copper order of transfer factor was; upstream, downstream, middle stream and control region at 0.032, 0.004, 0.002 and 0.001 respectively. It follows again that high soil copper levels did not translate into higher levels in the juice. The pH below 7 produces several less mobile species of copper, which leads to slow accumulation by plants (Pandey *et al*., 2016). The pH levels, together with other factors, could have contributed to variations in transfer factors in lower regions. Previous studies have recorded pH of below seven around the industrial area along Ngong tributary with subsequent reduction of transfer factor (Karanja *et al*., 2010) compared with other regions. In general, the transfer factor was below 1.

Chromium transfer order from highest to lowest was; middle stream, downstream, upstream, and control region at 0.031, 0.023, 0.001 and 0.0002 respectively, as indicated in table 4.4. Chromium transfer factor was highest at the middle stream probably due to favourable absorption factors around the industrial area. Such factors could have been environmental, biochemical or physical-chemical.

Pb transfer order from highest to lowest was; downstream, middle stream, upstream and control region at 0.005, 0.004, 0.003, and 0 respectively as indicated in table 4.4. Pb is one of the most widely distributed toxic element in soil (Karanja *et al*., 2010). Lead absorption is inhibited in roots by most plants for lack of channels for its absorption. Some lead is bound in exchangeable ion sites in the cell wall and extracellular precipitation as carbonates and nitrates (Peralta *et al*., 2009). Unbound lead is transported by calcium channel to leaves, some inhibited by casparian strip near endodermis, while some studies suggest lead movement through xylem and phloem to leaves and stems (Peralta *et al.*, 2009). These factors could have contributed to low lead transfer factors across all regions.

Manganese transfer order from highest to lowest was upstream, control, middle stream and downstream at 0.024, 0.023, 0.022, and 0.022 respectively, as indicated in table 4.4. Manganese is absorbed by plants and moved to shoot, but its remobilisation to other organs through phloem is not as fast (Sreekanth, 2010). Levels, concentration, presence of different elements, pH and plants genotypes affects its absorption (Soetan *et al.,* 2010). These factors could have contributed to variations in absorption of manganese as is the case with the four regions.

Cadmium in soil was not detected in upstream, middle stream and downstream. The control region had transfer factor of 0.001, as indicated in table 4.4. Transfer factor evaluated possible heavy metal transfer from soil to the edible portion of the sugarcane plant which could lead to potential health risk. Metals with higher transfer risk indicate an easier transfer from soil to the crop (Liao *et al*., 2016). Availability of heavy metals to sugarcane crop depends on soil properties, metal speciation, and crop genetic features (Liao *et al*., 2016). High soil pH and total organic carbon stabilise toxic soil elements resulting in their decreased leaching. Root cell wall, water transport in the xylem as well as ions transport system in the endoderm membranes cytoplasm membrane will also affect metal ions transfer from soil to plants (Liao *et al*., 2016). In the wet season, plants heavy metal contamination is generally reduced though other factors still play a role in absorption compared to the dry season (Kaluli *et al*., 2014). Previous studies have shown that the concentration of heavy metals in sugarcane roots and stem are far much more than the concentration in the juice (Liao *et al.*, 2016). Studies also show that roots uptake is the main route for heavy metals in sugarcane plant and the plant also contain special novel metallothionein (ScMT2-1-3) that gives the sugarcane a characteristic heavy metal tolerance and accumulation (Liao *et al*., 2016). This ability provides sugarcane bioaccumulation abilities.

4.5 Heavy metal concentration in sugarcane juice vendored in settlements along Ngong Tributary

Only juice collected in Sinai had significantly higher in copper levels than others ($p<0.05$) (Table4.5). The levels at Kibera and Mukuru kayiaba juices had no detected levels of copper. Levels at Mukuru Kwa Ruben were significantly higher than levels at Kariobangi and Kisii juices which had the lowest levels detected levels (Table 4.5). All copper levels were below WHO recommended limits of 2mg/l.

Chromium levels in Sinai, and Mukuru Kwa Ruben regions were significantly different than the rest of the juices($p<0.05$) (Table 4.5). Lowest levels were from Mukuru kayiaba followed by Kariobangi. All chromium levels were above WHO recommended limits of 0.1mg/l.

The Zinc levels in vendored juice from Mukuru kayiaba were significantly different from all other samples ($p<0.05$). Kisii and Sinai levels were not significantly different. The lowest detected levels were from Mukuru Kwa Ruben. Zinc was undetected in Kibera and Kariobangi vendored sugarcane juice. All samples had zinc levels below the WHO recommended limits of 5mg/l.

Lead levels in all regions were not significantly different($p>0.05$). All samples collected and analysed had levels above the WHO recommended limits of 0.01mg/l.

Highest levels of iron recorded were in Sinai, Kariobangi and Kibera vendored juices. These levels were significantly higher than the rest of the samples($p<0.05$). Levels at Kisii and Mukuru Kayiaba were not significantly different but were significantly lower than all other samples($p<0.05$). Iron levels in all samples were above the WHO recommended limits of 0.01mg/l.

Table 4.5: Heavy metal concentration in vendored juice along Ngong tributary (mg/l)

Heavy metals	Kibera	Mukuru kayiaba	Kisii	Kariobangi	Sinai	Mukuru kwa Ruben	WHO (RL)
Cu	ND	ND	0.01±0.02^{a}	0.01±0.02^{a}	0.22±0.11^{c}	0.06±0.04^{b}	2
Cr	0.24±0.02^{b}	0.17±0.01^{a}	0.24±0.10^{b}	0.22±0.07^{b}	0.31±0.09^{b}	0.35±0.10^{b}	0.1
Zn	ND	0.97±0.35^{c}	0.37±0.28^{b}	ND	0.49±0.21^{b}	0.25±0.057^{a}	5
Pb	0.40±0.12^{b}	0.32±0.04^{b}	0.35±0.16^{b}	0.38±0.18^{b}	0.26±0.13^{b}	0.33±0.19^{b}	0.01
Fe	3.88±0.65^{c}	0.75±0.26^{a}	0.59±0.07^{a}	3.28±0.70^{c}	4.74±0.49^{c}	2.63±1.30^{b}	0.01
Mn	2.28±1.68^{c}	0.66±0.01^{a}	0.99±0.38^{b}	0.73±0.58^{b}	2.76±1.16^{c}	0.74±0.41^{b}	0.5
Cd	ND	ND	ND	ND	0.01±0.01^{a}	ND	0.03

Different letters in the column indicates a significance difference while similar letter indicate non-significance. ND refers to not detected. RL refers to recommended limits.

Highest manganese levels recorded were in Sinai vendored juice and Kibera juice. They were not significantly different but significantly higher than the rest of the regions ($p<0.05$). Lowest levels recorded were from Mukuru kayiaba juice collected for sensory analysis. All samples had levels above the WHO recommended limits of 0.5mg/l.

Cadmium levels in Kibera, Mukuru kayiaba, Kisii, Mukuru Kwa Ruben and Kariobangi were not detected. Sinai recorded 0.01mg/l. However, the levels were lower than the WHO recommended limits of 0.03mg/l.

In general, Sinai recorded the highest levels of copper, iron, manganese and cadmium. Kibera juice was highest in lead while Mukuru Kayiaba was highest in zinc and Mukuru rube was highest in chromium. Lowest values of chromium, iron and manganese were found in Mukuru Kayiaba. This difference could have arisen due to variation of factors that promote absorptions from the soil. The heavy metals components could have influenced taste, odour and preference (María *et al*., 2017; Lo *et al*., 2007). However, copper, chromium, and cadmium levels in the juice were below WHO recommended limits while the rest of the heavy metals were above WHO recommended limits.

4.6 Sensory properties of sugarcane juice

Sensory evaluation was carried out to determine whether the sugarcane juice levels of sweetness, aroma and saltiness influence levels of acceptability by consumers (Table 4.6).In terms of the aroma score, Kariobangi, Mukuru kwa Ruben and Mukuru Kayiaba vendored sugarcane juice were significantly higher than the rest of the juice ($P<0.05$). The worst-rated was Sinai vendored juice with significantly lower in aroma score than the rest ($p<0.05$). When sugarcane is grown in saline soils juice quality is lowered in terms of aroma, appearance, brix and taste (Vasantha & Gomathi, 2009). This low score of Sinai vendored juice could have attained the unfavourable characteristics due to the farming environment and juice content.

In terms of saltiness, the worst-rated was Sinai vendored juice with significantly higher levels of saltiness score than the rest ($p<0.05$). It was followed by Kibera, Mukuru kayiaba, Mukuru kwa Ruben and Kariobangi vendored juice, all being not significantly different. The best-rated juice was Kisii with significantly lower saltiness score levels than the rest of the vendored juices ($p<0.05$).

Sweetness and brix are indicators of high-quality juice, and the higher the parameters, the higher the juice quality (Vasantha & Gomathi, 2009). In terms of sweetness, the highest rated was Kisii, followed by Kariobangi, Mukuru kwa Ruben and Mukuru kayiaba vendored juice and were significantly higher than the rest ($p<0.05$).

The worst-rated was Sinai vendored juice which had significantly lower sweetness score ($p<0.05$) than the rest of the samples followed by Kibera vendored juice. In the preference test, the most preferred juices were Kisii, Kariobangi vendored juice and Mukuru kayiaba with no significant difference in scores, but significantly higher than the rest of the samples ($p<0.05$). Mukuru kwa Reuben and Kibera vendored juices had no significant difference.

Table 4 6: Sensory properties mean scores of the vendored sugarcane juice for just about right scale and preference

	Aroma	**Saltiness**	**Sweetness**	**Overall acceptance**
Kibera	2.33 ± 0.21^{b}	2.0 ± 0.20^{a}	2.33 ± 0.20^{b}	6.10 ± 0.49^{b}
Mukuru Kaiyaba	3.0 ± 0.18^{c}	2.0 ± 0.18^{a}	3.0 ± 0.18^{c}	7.05 ± 0.36^{c}
Kisii	2.50 ± 0.18^{b}	1.62 ± 0.19^{a}	3.33 ± 0.16^{c}	7.24 ± 0.28^{c}
Kariobangi juice	3.10 ± 0.17^{c}	1.81 ± 0.18^{a}	3.10 ± 0.17^{c}	6.62 ± 0.31^{c}
Sinai juice	1.76 ± 0.18^{a}	3.05 ± 0.19^{c}	1.76 ± 0.18^{a}	3.33 ± 0.54^{a}
Mukuru kwa Ruben	3.0 ± 0.18^{c}	1.81 ± 0.21^{a}	3.0 ± 0.18^{c}	6.10 ± 0.49^{b}

Different letters in the column indicates a significance difference while similar letter indicate non-significance.

The worst preferred was Sinai farm juice which was significantly lower in score than the rest of the sample ($p<0.05$). In general, those juices that scored highly in aroma development and sweetness scores also scored high in the preference test. Thus, the aroma and sweetness contributed positively to being quality parameters that most customers pick out in rating. Sweetness, aroma and general appearance are due to a combination of various components including pH, total soluble sugars, titratable acidity, as well as maturity index (Aleem & Ramteke, 2017). Soil salinity and general soil drainage characteristics, as well as sugarcane species, influence overall sugar development in sugarcane plants (Jaganathan *et al*., 2018).

4.7 Comparison between heavy metal concentration and preference

In terms of Aroma score juices from Mukuru kayiaba, Mukuru kwa Ruben and Kariobangi had a significantly higher score than the rest of the juices ($P<0.05$). In saltiness score, vendored sugarcane juice from Sinai had a significantly higher score than the rest ($p<0.05$). In sweetness and overall preference scores, Mukuru kayiaba, Kisii, Kariobangi, and Mukuru kwa Rube had a significantly higher score than the rest ($p<0.05$). Kisii which had the preference score had significantly lower levels of copper, iron and cadmium ($p<0.05$). Mukuru kayiaba, which had the second highest preference score had significantly lower levels of copper, chromium, iron, manganese and cadmium ($p<0.05$). Vendored juice from Kariobangi had significantly lower levels copper, zinc, manganese, and cadmium ($p<0.05$). Vendored juice which had the lowest preference score had significantly higher levels of copper, zinc, chromium, iron, manganese and cadmium ($p<0.05$). Vendored juice from Kibera, which was the second least preferred juice had significantly higher levels of chromium, iron and manganese ($p<0.05$). It follows that the vendored juice, which had significantly higher levels of heavy metals, scored the lowest in preference score and highest in saltiness score. These results indicated that the presence of heavy metals in sugarcane juice affected its sensory properties and preference.

4.8 Consumption risk assessments

The total THQ (TTHQ) and the total EDIs for vendored cane juice were determined and analysed (Table 4.7). The total EDIs for adults and children for cane juice ranged between 0.01 to 0.03mg/kg/d and 0.02-0.07mg/kg/d respectively. TTHQ values for adults and children ranged from 0.14-0.3 and 0.34-0.7, respectively. The ranks from the highest THQ for adults was Kariobangi>Sinai>Kibera>Mukuru kwa Ruben>Kisii>Mukuru Kayiaba juices. However, all the THQS adults were less than 1, indicating a low risk.

Table 4.7: Health risk assessment for vendored and cane juices

	THQ	**THQ**	**EDI (mg/Kg/day)**	**EDI (mg/Kg/day)**
Sampling points	Adults	Children	Adults	Children
Kisii	0.16	0.37	0.01	0.02
Kibera	0.26	0.6	0.02	0.04
Kariobangi	0.3	0.7	0.02	0.06
Sinai	0.28	0.66	0.03	0.07
Mukuru kayiaba	0.14	0.34	0.01	0.02
Mukuru kwa Ruben	0.22	0.51	0.02	0.04

THQ means Total Hazard Quotient and it has no units. EDI means Estimated daily intake and its units are in mg/kg/d

TTHQ for children were also less than one but very close indicating increased risk for children compared to adults (Table 4.7). The TTHQ ranks for children from the highest to lowest were Kariobangi>Sinai>Kibera>Mukuru kwa Ruben >Kisii>Mukuru kayiaba. The samples from selling points analysed for sensory evaluation had lead, iron, chromium, zinc and manganese posing the most significant risk to consumers, respectively. A study was done in India for chromium, cadmium, copper, lead, zinc, nickel and had TTHQ of 0.0008 for adults and 0.0001 for children. The levels in the current study were far much compared values in the study (Pandey *et al*., 2016). Since level above one is considered as high risk, all the samples collected showed a low risk to heavy metals hazard.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results showed that levels of zinc, iron, copper, chromium, lead and manganese in sugarcane juice were higher than WHO recommended levels in regions where they were most elevated.

The heavy metal concentration in sugarcane juice from sugarcane collected along Ngong river were most elevated either in middle stream and downstream regions of the river apart from manganese which was most elevated in Control. These results indicated that anthropogenic activities and waste levels in these regions provided adequate environment for absorption of more heavy metals by sugarcane plant than the rest of the regions

The heavy metals in soil were lower than WHO recommended levels apart from copper which was higher in region where it was most elevated. With exception of zinc and copper, the rest of the heavy metals in soil were most elevated either in middle stream or downstream region of Ngong tributary. In river water, levels of zinc, iron, chromium, lead and manganese were higher than WHO recommended values in regions where they were most elevated. With exception of copper and cadmium, the rest of heavy metals were most elevated in middle stream and downstream region showing industrial activities contributed to the bulk of heavy metals in water. The results of transfer factor from soil to sugarcane juice showed that, with exception of cadmium and copper, the rest of the heavy metals had the highest transfer factors in either middle stream and downstream regions. This indicated that downstream and middle stream region had the most favourable factors for absorption of heavy metals from soil to sugarcane plant.

In Vendored juices, levels of chromium, lead, iron, and manganese were higher than WHO recommended levels in areas where they were most elevated. The least preferred juices had higher levels of copper, chromium, iron, manganese and cadmium. In contrast, the most preferred had lower amounts of the above heavy metals, indicating that higher levels of heavy metals affect the quality characteristics of the sugarcane juice. All vendored juices had TTHQ lower than 1 in both adults and children, showing no serious risk of heavy metals consumption for vendored sugarcane juice. However, Children TTHQ were higher than adults TTHQ indicating that children are more vulnerable than adults, especially due to weight.

5.2 Recommendations

Recommendations are made for the controlled treatment of waste so that it does not find its way to agricultural soil and rivers. Soil is the main route of transfer of heavy metals to plants while river water transport and deposit heavy metals in soil. A further suggestion is made for the use of clean water to irrigate sugarcane crops as well as other crops planted within the peri-urban zones to avoid contaminations. Close monitoring of levels of heavy metals in irrigation water is needed to avoid excessive uptake by plants. Phytoremediation is recommended in already contaminated soils along Ngong tributary to curtail the movement of heavy metals from soil to crops. A final recommendation is made to closely monitor sources of sugarcane used in vendor points to avoid heavy metal, microbiological and chemical contaminations to both adult and children consumers.

5.3 Areas of further research

A recommendation is made for studies of heavy metal bioaccumulation levels that will entail other sugarcane parts as well as other products made from these parts. This will determine actual levels of bioaccumulation factors (BAF) and bioaccumulation coefficient (BAC).

A recommendation is also made for a controlled study of heavy metal absorption behaviours of sugarcane plant species. A wider range of heavy metals is recommended to evaluate risk even in heavy metals not included in the study.

Risk assessment studies are recommended to include pesticide residues, microbiological and other heavy metals not included in the current study.

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APPENDICES

Appendix I: Vendored sugarcane juice sensory evaluation questionnaire

Introduction

I am researching the quality of sugarcane juice from different cane varieties. I am requesting that you take some time (about 10 minutes) to participate in this sensory evaluation exercise. Thanks for agreeing.

Instructions (Please read the instructions carefully and fill the questionnaire part)

You are provided with different samples of sugarcane juices expressed from different cane varieties grown across the country

1. The tasting consists of two parts/types of evaluation.
2. Keep in mind that you are asked to answer the question as a representative of the consuming population; it is your personal opinion, please do not talk to the other participants during the evaluation.
3. Along with each question, there is space for comments. Use this space to explain the reason for your choice in detail.
4. To help you reset your taste buds in between the samples, use the plain water and bread crumbs provided to rinse your mouth in between tasting. DO NOT SWALLOW THE CONTENT. SPIT IN THE SINK OR CONTAINER PROVIDED

Personal data

Gender:	Male	Female
	☐	☐

Age group: 20-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ 61-70 ☐

Do you in general like………...? ☐ ☐

In the last three months, about how often have you used the type of product of today's tasting?

☐ Not a single time

☐ Less than once a month

☐ More than once a month, but less than once a week

☐ More than once

1. Just About Right Scale

You are provided with nine (9) sugarcane juice samples. Evaluate each attribute one by one separately by ticking in the box. Please try to give the reasons for your opinion under comments.

Sweetness									
Sample #	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Too sweet									
Moderately sweet									
Slightly sweet									
Flat (sweetness not detectable)									

Saltiness									
Sample #	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Too salty									
Moderately sweet									

Slightly salty									
Flat (saltiness not detectable)									

Aroma									
Sample #	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Too pleasant									
pleasant									
Neither pleasant nor unpleasant									
Unpleasant									

2. The 9- point hedonic scale / the degree of liking

Rate how you like or dislike the provided sample by ticking in the appropriate box. Do not forget to rinse your mouth with water in between the samples.

9-point Hedonic Scale									
Sample #	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
☐ Like extremely									
☐ Like very much									
☐ Like moderately									
☐ Like slightly									
☐ Neither likes not to dislike									
☐ Dislike slightly									
☐ Dislike moderately									
☐ Dislike very much									
☐ Dislike extremely									

Description of scores.

1. Too sweet-4, Moderately sweet-3, Slightly sweet-2, Flat-1
2. Too salty-4, Moderately salty- 3, Slightly salty-2, Flat-1.
3. Too pleasant-4, Moderately pleasant-3, Pleasant-2, Unpleasant-1
4. Like extremely-9, Like very much-8, Like moderately-7, Like slightly-6, Neither likes not to dislike-5, Dislike slightly-4, Dislike moderately-3, Dislike very much-2, Dislike extremely-1.

Appendix II: Questionnaire on sugarcane availability and juice consumption

Date ______________________ Age ______________________

Estate/company ______________________ (tick where appropriate)

1. How do you get sugarcane from a) Farmers [ ] b) Market [ ] c) Own farm [ ]d)Any other[ ]
2. Who are your main customers a) Children [ ] b) Women [c) Men [ ] d) All [ ]
3. Other than own juice consumption how else is the juice used here in Nairobi a) Brew [ ] b)Molasses[ ]c)Jaggery[ ]d)Others[ ]
4. Do you know your most frequent customers _a) Yes [ ] b)No[ ]
5. Approximately how many visit daily a) Once [ ] b) Twice [ ]c)Others[ ]
6. Approximately how many visit Weekly a) Once [ ] b) Twice [ ] c)Others[ ]
7. On a single visit approximately how much does the highest consumer take a) 1 big cup (500ml) [ ] b)1 small cup(250ml) [ ] c) More than 1 cup (above 500ml) [ ]
8. Do you blend sugarcane juice from Nairobi and other regions a) Yes [ ] b) No[ ]
9. Is the blend a) Half/Half [ ] b)Quarter/3 Quarter c) Any other [ ]
10. Which sugarcane is most profitable to deal with a) Sugarcane from Nairobi [ ] b)Sugarcane from other sources [ ]
11. On a single visit approximately how much does the lowest consumer take a) 1 big cup (500ml) [ ] b)1 small cup(250ml) [ ] c) Less than 1 cup(below 250ml) [ ]
12. What does consumers prefer most a) Sugarcane from Nairobi [ ] `b) Sugarcane from other sources[ ]
13. Currently there are few sugarcane plantations grown along Nairobi river will it be a good idea to increase this plantation for purposes of manufacturing juice a) Yes [ ] b) No [ ]
14. What is your reason for your answer to question 6 above a) Increase profit [ ] b) Increase supply [ ] c) Both a and b [ ]

d) Any other [ ]