CYANIDE LEVELS IN RAW SWEET CASSAVA VARIETIES AND PEOPLE'S PERCEPTION ON CYANIDE POISONING IN KAGERA AND MOROGORO REGIONS OF TANZANIA

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ABSTRACT

Cassava (*Manihot esculenta* Crantz), an edible crop that is renowned out of its growth advantages over other crops, carries cyanide which is potentially poisonous to humans. The threat is intensified by human habit of consuming raw cassava tubers whose toxicological status is not established. Cases and indicators of cyanide poisoning due to cassava consumption are evident in Tanzania, particularly in Kagera and Morogoro regions. This study was launched to quantify cyanide in tubers of sweet cassava varieties grown in Kagera and Morogoro regions and thereafter to assess if it is safe for humans to consume raw tubers of sweet cassava varieties grown in the study area. Another objective was to assess whether the habit of consuming raw cassava tubers was out of people's lack of awareness about cyanide and its poisoning effects or negligence. The study employed cross-sectional research design to collect participants' responses on cyanide and its associated poisoning effects and to determine cyanide levels in sweet cassava tubers by using alkaline titration method. Sixty six tubers of 12 sweet cassava varieties from the study area were analyzed and 386 participants were involved in the study. The study findings showed that cyanide levels in the raw sweet cassava tubers were above the internationally accepted level in human consumables (10 mg/kg) and thus unfit for human consumption in their raw state. Some sweet varieties were found to be wrongly classified as sweet because their cyanide content was above 50 mg/kg. The inconsistency of cyanide levels in tubers of similar variety showed that a variety can exist in both sweet and bitter forms depending on environmental factors, making the categorization of varieties into sweet and bitter varieties misleading. Furthermore, the habit of consuming raw cassava tubers was found to be mostly cultivated by people's lack of awareness with regard to the presence of cyanide in cassava tubers (86%) and on cyanide poisoning effects (81%) respectively. It was also found out that the slippery tissue and the inner tissue of the cassava parenchyma differ significantly in cyanide content so that the habit of scratching off the slippery tissue contributes to reduction of cyanide in cassava tubers. This research work recommends that the public should be sensitized on the issue of cyanide in cassava, the poisoning effects it has to human health as well as ways of identifying and dealing with the poison contained in cassava tubers prior to human consumption. Apart from inventing simple, inexpensive and efficient cyanide quantifying devices, genetically modified cassava varieties need be produced and disseminated in which the gene for cyanide expression is either masked or removed as an attempt to protect people against cyanide poisoning.

DECLARATION

I, CORNELIUS BENEDICTO MUSHUMBUSI, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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Life and its protection are a gift from God and without this gift human beings are nothing and inexistent. May the Almighty God be praised for his unfailing love to me which made this academic adventure and work a reality!

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To my Parents, Benedicto Mutakyamirwa and Blandina Mukarunyaga, who have never been selfish in bringing up, supporting and loving their twelve children (May Josephine and Willibald rest in peace) and who keep on blessing and wishing us well in this life and one to come.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
MASL	Metres above sea level
CIAT	Center for International Tropical Agriculture
CL	Confidence Level
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FAOSTAT	The Food and Agriculture Organization Corporate Statistical Database
FSANZ	Food Standard Australia New Zealand
HCN	Hydrocyanic acid/Hydrogen cyanide/ Cyanide
IM	Institute of Medicine
IPCS	International Programme on Chemical Safety
KB	KIBAHA sample
KI-H	KIKANIKI from highland
KI-L	KIKANIKI from lowland
MK-H	MZURIKWAO from highland
MK-L	MZURIKWAO from lowland
MWECAU	Mwenge Catholic University
MW-H	MWANGA from highland
MW-L	MWANGA from lowland
NY	NYACHILO sample
NBS	National Bureau of Statistics
OCGS	Office of Chief Government Statistician
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TAN	Tropical ataxic neuropathy

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Cassava (*Manihot esculenta* Crantz) is a perennial crop native to tropical America (Allen, 1994) and an important and cheap source of carbohydrate in tropical regions, particularly in Sub-Sahara Africa. The crop has more growth advantages than other crops (Nhassico *et al*., 2008; Tivana *et al*., 2014; Ubwa *et al.*, 2015). Its ability to withstand hard climatic conditions has made the diverse edible varieties of cassava to be an outstanding crop that saves at least 500 million of human lives from hunger in the world. About 56% of the world's cassava production has its source in Africa (FAO, 2013). The same organization ranked Tanzania as the 8th best consumer and the 14th producer of cassava worldwide. It is also noted that in Tanzania, cassava consumption is higher than its production (Chilongola, 2017a).

Cassava has many advantages to human health: highest-calorific energy; gluten-free (hence useful for celiac disease patients); low glycemic index- GI (good for diabetics and for reduction of risk to coronary heart diseases); a buffer to lipid levels and a source of vitamins and important minerals including Fe, Zn, Ca, Mg, K and Mn (Mburu, 2013; Mercola, 2016). However, cassava carries the potential danger of cyanide poisoning (Babalola, 2014), because it contains cyanogenic glycosides, linamarin: 2-β-D-glucopyranosyloxy-2-methylpropane nitrile, and lotaustralin: 2-β-D-glucopyranosyloxy-2-methylbutyronitrile (Idible, 2008). On hydrolysis in the human gut, these cyanogens produce hydrocyanic acid (HCN) which is toxic (Tivana *et al.*, 2014). This poisonous biochemical is responsible for several health complications such as Konzo disease, thyroid goiter and tropical ataxic neuropathy (TAN) (Kobawila *et al.*, 2005), incidences of cretinism, stunted growth in children and deaths (Nhassico *et al.*, 2008). Symptomatic effects of acute poisoning may include nausea, dizziness, vomiting, stomachache and headache (Mercola, 2016). Cyanide poisoning is also associated with epilepsy (Ngugi *et al*., 2013) as well as behavioral and emotional abnormalities in children (Kariuki *et al*., 2017).

Traditional methods including boiling, roasting, drying, grating, soaking and fermentation are renowned for their effectiveness to significantly reduce cyanide level in cassava tubers (Ernesto *et al.*, 2002; Nzwalo and Cliff, 2011). Nevertheless, given the vast pool of cassava varieties (bitter and sweet) which differ in cyanide levels and the inconsistency of cyanide level in tubers of the same variety due to different factors (Cardoso *et al.*, 2004; Famurewa and Emuekele, 2014; Ubwa *et al.*, 2015), the toxin remains a point of great concern due to the danger it poses to human health. Whereas some researchers believe that acyanogenic cassava tubers have never been confirmed (Bradbury and Holloway, 1988), some go further by asserting that all edible cultivars contain cyanide above the internationally acceptable level (10 mg/kg) in human consumables (White *et al.*, 1998; FAO, 2007). Another group of researchers hint to the existence of non-toxic varieties (Tivana, 2012; Famurewa and Emuekele, 2014).

Although people are cautioned not to consume raw cassava tubers even if sweet (Cardoso, 2004; FSANZ, 2005; Ubwa *et al.,* 2015), sweet cassava tubers are habitually consumed raw in a good number of localities in Tanzania, for instance in Kagera and Morogoro regions. Raw sweet cassava is taken as part of breakfast, hunger suppressor and for other unproved reasons such as increasing reproductive fertility and fighting against reproductive impotency among men. The goal of this study was to establish if cassava tubers of varieties from two selected regions in Tanzania have acceptable levels of cyanide for human consumption. The main objective was to quantify cyanide in tubers of sweet cassava varieties grown in Kagera and Morogoro regions as well as assessing people's awareness on the danger of cyanide to human health.

1.2 Statement of the Problem and Justification of the Study

Knowledge of cyanide levels in raw cassava tubers is very important. Cyanide is proved to cause complications to human health. Sweet cassava tubers are consumed raw without knowledge of their poison status. People take for granted that sweet cassava cultivars are free of cyanide and thus fit for consumption. The only criteria employed to identify what should not be consumed raw include bitterness of cassava and type of variety. It is worth noting that even those cassava cultivars considered sweet have been a source of disaster to humans in East Africa (Mburu *et al.*, 2011). In 2016, five people were poisoned and three of them died at Kauzeni in Mvomero District, Morogoro (IMG, 2016). Furthermore, eleven family members in Ilala Municipality (Dar-es-Salaam) were poisoned costing life of a child (Chilongola, 2017b). There is also an increasing awareness to maximize production of alternative food crops, cassava being on the first line, in areas where outstanding crops like banana are challenged by diseases. Intensifying cassava production implies also an increasing possibility of long term cyanide poisoning in places where cassava is not effectively processed. Health complications such as thyroid goiter, epilepsy and persisting leg weakness and pain as well as partial leg paralysis, evident in areas where cassava production is in full swing, make cassava consumption to be suspected as the source of the problems.

Cyanide level in various cultivars is affected by various factors and thus inconsistent (Cardoso *et al*., 2004; Gitebo *et al*., 2009; Famurewa and Emuekele, 2014; Ubwa *et al*., 2015). There are also contradictory views with regard to cyanide levels in edible cassava tubers (Tivana, 2012; Famurewa and Emuekele, 2014). This entire scenario calls for a need to quantify cyanide in cassava tubers consumed raw in different geographical locations (Tivana, 2012; Ubwa *et al.*, 2015). Also understanding people's knowledge and perception on cyanide and its poisoning effects will help to change people's habit of consuming raw cassava tubers and provide protection education to people.

1.3 Research Objectives

1.3.1 Overall objective

To assess cyanide levels in sweet cassava varieties and people's awareness about cyanide poisoning in Kagera and Morogoro regions.

1.3.2 Specific objectives

a) To identify varieties of sweet cassava commonly grown in Kagera and Morogoro regions.
b) To quantify cyanide in the sweet cassava tubers eaten raw in Kagera and Morogoro regions using alkaline titration method.
c) To compare the measured levels of cyanide in sweet cassava tubers with the internationally acceptable cyanide level in human consumables.
d) To compare cyanide levels in cassava tubers by considering types of varieties and locations.
e) To compare cyanide levels between the outer slippery tissue and the inner tissue of the cassava tuber parenchyma.
f) To assess people's awareness about cyanide poisoning using a structured questionnaire.

1.4 Research Hypotheses

a) H_0**1** There are no many sweet cassava varieties whose tubers are commonly consumed raw in Kagera and Morogoro regions

   H_A**1** There are many sweet cassava varieties whose tubers are commonly consumed raw in Kagera and Morogoro regions

b) **$H_0$2** There is no cyanide in the cassava tubers eaten raw in Kagera and Morogoro regions

   H_A2 There is cyanide in the cassava tubers eaten raw in Kagera and Morogoro regions

c) **$H_0$3** Cyanide levels from different varieties of sweet cassava do not differ from the internationally acceptable cyanide levels in human consumables

   H_A3 Cyanide levels from different varieties of sweet cassava differ from the internationally acceptable cyanide levels in human consumables

d) **$H_0$4** Cyanide level in cassava tuber does not vary with type of variety or location

   H_A4 Cyanide level in cassava tuber varies with type of variety and location

e) **$H_0$5** There is no significant difference in cyanide levels between the outer slippery tissue and the inner tissue of the cassava tuber parenchyma

   H_A5 There is significant difference in cyanide levels between the outer slippery tissue and the inner tissue of the cassava tuber parenchyma

f) **$H_0$6** The people of Kagera and Morogoro regions are not aware about cyanide poisoning

   H_A6 The people of Kagera and Morogoro regions are aware about cyanide poisoning

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Biochemistry of Cyanide

2.1.1 Cyanogenic glycosides and their hydrolysis

Cassava cyanogenic glycosides mainly linamarin (2-β-D-glucopyranosyloxy-2-methylpropane nitrile) and lotaustralin (2-β-D-glucopyranosyloxy-2-methylbutyronitrile) are a natural defense against destructive herbivores (Jones, 1962; Newton, 2017), have regulatory function (Eck and Hageman, 1974) and play part in protein synthesis (Ellis *et al.*, 1977). A cyanogenic glycoside is made up of a reactive component, α-hydroxynitrile, stabilized by conjugating with either D-glucose or gentiobiose. Whereas the cyanogenic glycosides are stored in cell vacuoles, their hydrolyzing enzymes, β-glycosidases and hydroxynitrile lyases reside in the cell wall. Destruction of the cell wall by maceration or fermentation allows the hydrolyzing enzyme to come in contact with cyanogenic glycosides which causes enzymatic cleavage of the carbohydrate moiety. The free α-hydroxynitrile can then either be enzymatically cleaved or spontaneously dissociate to a ketone or aldehyde and HCN (Nhassico *et al*., 2008; Tivana, 2012; Abraham *et al*., 2016). Hydrogen cyanide, which is a weak acid, boils at 25.6^{o}C and is extremely poisonous.

[Cyanogenic glycoside (+β-glycosidase) →cyanohydrin-unstable (+lyase/optimum condition)→ HCN + Acetone + Glucose]

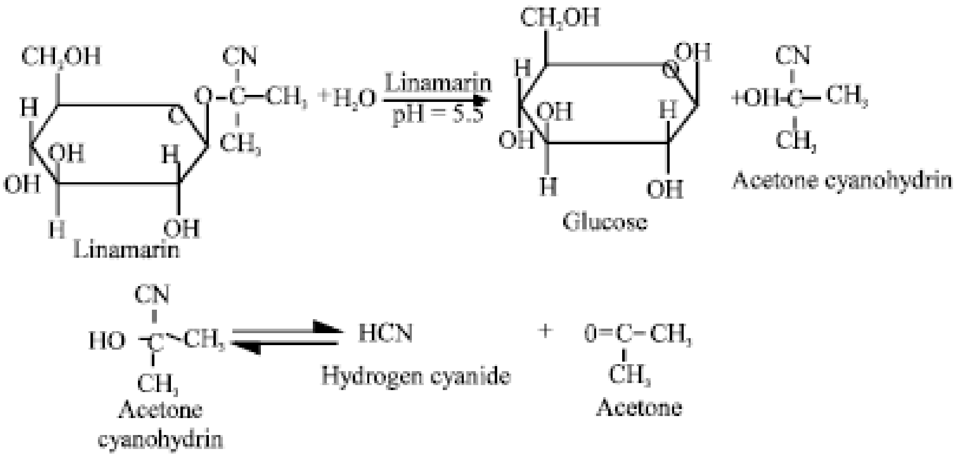

Figure 1: Hydrolysis of cyanogenic glycoside (linamarin) into HCN

2.1.2 The biochemistry of cyanide poisoning

Subsequent to its intake, cyanide gets readily absorbed and rapidly distributed in the body through blood (Eisler, 1991), with highest levels in the liver, lungs, blood (prior to chronic or repeated exposure) and brain (IPCS, 2004). According to EPA (1990), during fatal cases, in human tissues, HCN concentration (mg/100g) was found to be: 0.03 (gastric content), 0.5 (blood), 0.03 (liver), 0.11 (kidney), 0.07 (brain) and 0.2 (urine). The primary target of cyanide toxicity in humans and animals are the cardiovascular, respiratory and the central nervous system. The endocrine system is a potential target for long term toxicity (IPCS, 2004). While in blood, cyanide binds to both reduced and oxidized forms of iron in hemoglobin and methemoglobin respectively (Barrett *et al.*, 1977; Romano *et al.*, 2007). This reaction poses potential cyanide poisoning by reducing the ability of the blood to circulate oxygen by changing the structural identity of oxygen-carrier molecules. The major body defense against cyanide poisoning is cyanide conversion to thiocyanate, a reaction catalyzed by the enzyme thiosulfate-cyanide sulfur transferase (also called rhodanese) in the presence of sulfur donors, for example cysteine (Beasley *et al.*, 1998; Way *et al.*, 2008). The enzyme has an active sulphide group which reacts with the thiosulphate and cyanide to produce thiocyanate.

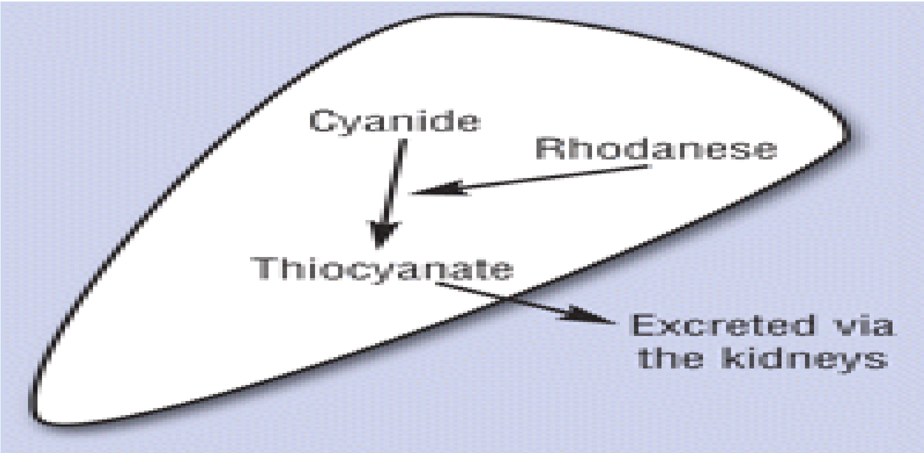

Figure 2: Simple diagram depicting detoxification of HCN in normal situation

Cyanide poisoning begins when hydrogen cyanide level in the body, exceeds the detoxifying capacity of the body natural system (FSANZ, 2005). Cyanide exerts its toxic effects by combining with cytochrome oxidase enzyme through its ferric ion to which it has high affinity. In so doing, tissue utilization of oxygen is inhibited (IM, 1995; Baskin *et al.*, 2004; Keren, 2004; Way *et al.*, 2008), a situation termed histotoxic anoxia. The function of cytochrome oxidase is to catalyze the oxidation of cytochrome c whereby oxygen is reduced to water and four hydrogen ions are moved to the mitochondrial intermembrane thus creating a proton gradient used to produce ATP.

[4 Cytoc (reduced) +8H^+ (matrix) + O_2 (+complex IV) → 4 Cytoc (oxidized) + 2H_2O + 4H^+ (intermembrane)].

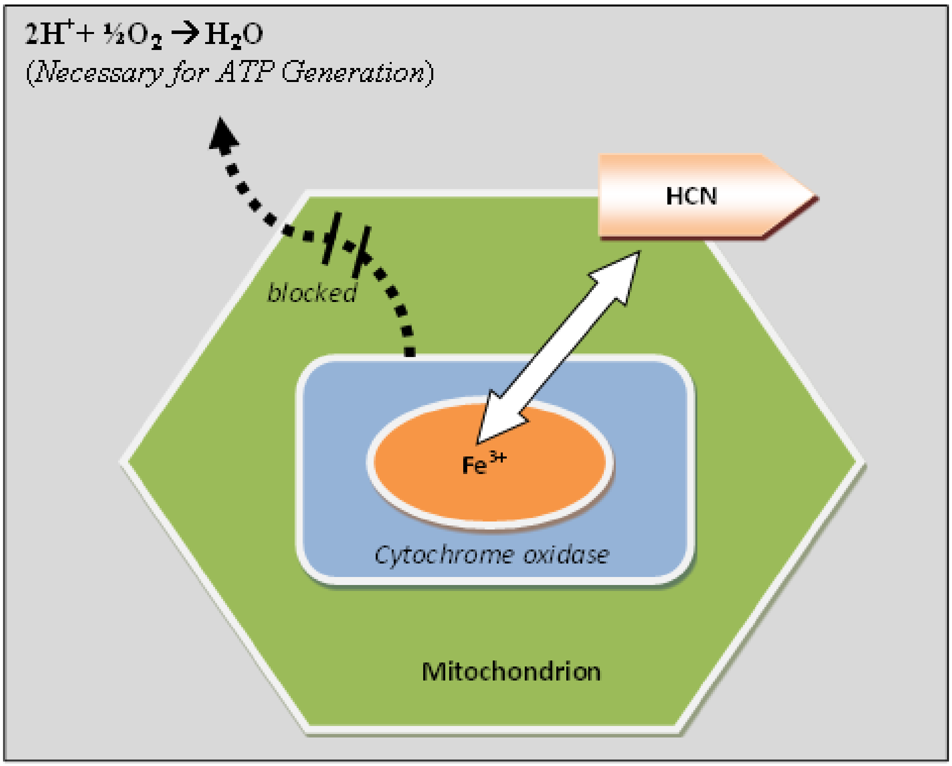

Figure 3: Simple diagram showing cyanide interrupting cytochrome oxidase

The reaction of cyanide with the multimeric iron enzyme complex is made easy by the penetration of hydrogen cyanide into the protein followed by binding of the poison to the heme ion. The resulting cyanide-heme cytochrome oxidase complex renders the enzyme incapable of oxygen utilization. This inhibition interrupts the electron transport chain and the mitochondrial oxidative phosphorylation (Keren, 2004; Romano *et al*., 2007). Oxidative phosphorylation is essential to the synthesis of adenosine triphosphate (ATP) and the continuation of cellular respiration (Hamel, 2011). The resultant anaerobic metabolism with drastic decrease of ATP generation and the implied increase in lactic acid formation at last leads to tissue hypoxia and metabolic acidosis (Beasley and Glass, 1998; Keren, 2004). More rapid effects of HCN appear to take place on neural transmission (Alexander and Baskin, 1987).

In acute cyanide-poisoning, abrupt death may occur; otherwise, long term cyanide poisoning with sub-lethal doses results in increased blood cyanide levels which can result in several health complications (Kobawila *et al.*, 2005; Nhassico *et al.*, 2008). However, the inhibitory properties of cyanide are ascribed to its ability to complex with metallo-enzymes (containing cytochrome oxidase) like iron, molybdenum, zinc and copper which are equally sensitive to cyanide. Thus, cyanide toxicity should rather be viewed as a complex phenomenon (Keren, 2004).

2.1.3 Three cyanide poisoning effects: Konzo, tropical ataxia neuropathy (TAN) and thyroid goiter

Long term consumption of cassava that contains sub-lethal doses of cyanide (i.e. high levels of cyanogenic glycosides) is believed to result into Konzo and TAN as well as into the development of hypothyroidism, thyroid goiter and cretinism in areas with low iodine intake (IPCS, 2004). Whereas neurotoxicity is the interference of the poison with the central nervous system, endocrine system poisoning is associated with the interference with iodine uptake by the thyroid gland. The exact mechanism by which cyanide causes neurological damage is unclear (Newton, 2017). Probably, routine reduction of oxygen to the central nervous system (due to habitual consumption of unprocessed cassava) deprives nerve cells of oxygen. This leads to nerve cell deformation and destruction (neuropathy) rendering them incapable of playing their role, with eventual development of neurological diseases like Konzo and TAN.

(1) Konzo: a distinctive neurological disease with selective upper motor neuron damage, characterized by an acute or sub-acute abrupt onset of irreversible, non-progressive and symmetrical spastic paraparesis or tetraparesis, due to consumption of insufficiently processed high-cyanide containing cassava (Adamolekun, 2010a; Nzwalo and Cliff, 2011; Newton, 2017). The initial symptoms of Konzo are

trembling or cramping in the legs, heaviness or weakness of the legs, and a tendency to fall or inability to stand (Eeg-Olofssona and Tshala-Katumbay, 2004). The disease also causes cognitive impairment in children, and an exacerbation of thiamine deficiency results in its clinical manifestations (Ravindra, 2007; Boivin *et al.,* 2013; Tshala-Katumbay *et al.,* 2013). A toxico-nutritional etiology is strongly supported by both clinical and epidemiological studies. The postulated mechanism is that in Konzo patients, a severe exacerbation of thiamine deficiency results from the inactivation of thiamine. This happens in the absence of dietary sulfur-containing amino acids in which case the sulfur in thiamine is utilized for the detoxification of cyanide consumed in improperly processed bitter cassava. Thiamine is rendered inactive when the sulfur in its thiazole moiety is combined with hydrogen cyanide (Adamolekun, 2010b).

(2) Tropical ataxic neuropathy (TAN): This neurodegenerative disease is attributed to prolonged cyanide intoxication from monotonous diet of cassava: thiamine deficiency (Ravindra, 2007; Madhusudanan *et al.,* 2008; Selvan, 2013). It is multi-fold in expression as sensory polyneuropathy, ataxic gait, optic atrophy, and sensory deafness (Ravindra, 2007; Madhusudanan *et al.,* 2008). The metabolism of cyanogens leads to the release of cyanide and thiocyanate; and the resulting development of deficiency of sulphur containing amino acids leads to the neurotoxicity which presents as predominant sensory neuropathy with ataxia (Selvan, 2013). Vitamin deficiencies are implicated (chronic thiamine deficiency) in the etiology of TAN. For instance, abnormal pyruvate metabolism is shown to be reversed by thiamine in patients with TAN. It is causally associated with deficiency of sulfur donor- thiamine. A long-term thiamine supplementation program for

susceptible individuals in endemic areas may be effective in the control and eventual eradication of the disease (Adamolekun, 2010a).

(3) Thyroid goiter: This is the enlargement of the thyroid gland that creates a visible mass in the neck (Norman, 2017). It is either intrinsic: stimulation of goiter development stems from intrinsic alteration of the gland; or extrinsic: cases due to excess of thyroid gland stimulators extrinsic to the gland. In the latter case some examples are iodide deficiency with elevated thyroid stimulating hormone (TSH) and goitrogens that interfere with iodine intake by the gland (Pisarev and Kleiman de Pisarev, 1980). One of the external goitrogens is associated with long-term consumption of insufficiently processed or unprocessed cassava. In the course of cyanide detoxification, thiocyanate is produced. Continued exposure of the body to thiocyanate production following detoxification of cyanide, prevents iodine uptake in the thyroid gland hence acting as a goiter-causing agent (IPCS, 2004). That is, through thiocyanate, cyanide limits the iodine storing and processing ability of thyroid glands (Dhas *et al.,* 2011) thus stimulating its growth for compensating iodine absorption. Here the problem is not iodine availability but rather the interference of thiocyanate.

2.2 Cyanide Quantification in Cassava Tubers

A research by Ubwa *et al.* (2015) determined cyanide level in tubers of three sweet cassava cultivars grown in Nigeria. The research findings showed that the tubers contained cyanide levels above the one acceptable in human consumables. The author advises that such tubers should not be eaten raw. However, it is not established if those cassava cultivars are the same varieties found in Kagera and Morogoro region. Another researcher (Mburu, 2013) determined cyanide levels in sweet cassava tubers collected from Kakamega, Kisii, Kitui, Nairobi and Thika in Kenya. According to this study, the studied cultivars were wrongly placed under the category of sweet varieties with the exception of the cultivar collected from Kisii. This is a caution that it is categorically misleading to base on mere names to classify cassava varieties.

Diallo *et al.* (2014) insists on the necessity of quantifying and evaluating cyanide in cassava fresh roots and its derivative products to develop strategies against cyanide poisoning and its entailed diseases. Their study on some Senegalese cassava varieties, showed that some fresh-consumed Senegalese cassava varieties are highly toxic (>100 mg/kg); thus a risk to consume fresh cassava roots whose cyanide level-status is not established. According to Mlingi *et al.* (1992), acute intoxications occurred in drought stricken areas in southern Tanzania in 1988. The intoxication was due to high amount of cyanide ingested from insufficiently processed cassava roots. From this information, eating cassava products whose poison status is not established poses a danger of cyanide poisoning

2.3 Factors that Affect Cyanide Levels in Cassava

2.3.1 Water stress

Water stress is shown by several researchers to affect cyanide levels in cassava. According to Tan (1995), drought increases cyanide levels in edible cassava varieties. This was proven by imposing water stress on Medan and Perintis varieties in which case cyanide levels in cassava shoots increased. This stand is also held by Bokanga *et al*. (1994) that in cassava, cyanide level tends to increase during periods of droughts or prolonged dry weather due to water stress on plant. Expressing similar idea but in a different language, Simwambana *et al*. (1992) revealed that cyanide levels are inversely related to amount of rainfall so that the levels are lower during rainy months but higher in drier months. According to Splittstoesser and Tunya (2010), cassava grown in wet areas have relatively lower amount of cyanide than those grown in drier areas. On animals' tolerability in digesting small amounts of cyanide, Barley (1999) cautions during drought as the amount of the chemical in plants increases. In Mozambique, 55% or so of fresh sweet cassava tubers were found to be extremely toxic and the remaining ones moderately toxic during drought like conditions. Similar observations were noted in the Democratic Republic of Congo (Gitebo *et al*., 2009). Other studies that share the same idea include CIAT (1989), Ekanayake (1994) and Cardoso *et al*. (2005).

2.3.2 Cassava variety/cultivar

Although all cassava varieties are said to contain cyanogenic glycosides (Bradbury and Holloway, 1988), a wide variation in the level of cyanogens exists among different varieties which can range from 1 to 2000 mg/kg (Cardoso *et al*., 2005; CIAT, 2007). That is, cyanide level is influenced by the variety of cassava. According to the findings by Cuvaca *et al*. (2015), cyanide level in cassava tuber appears to be a function of the physiology of the crop or possibly cassava variety. These views hint to the relationship between a variety and cyanide levels.

2.3.3 Use of fertilizers

De Bruijn (1973) in studying the factors influencing cyanogenesis, found that cassava tuber cyanide levels increased with nitrogen (N), decreased with potassium (K), and did not change with phosphorus (P) fertilization. Endris (1977) suggested that potassium application significantly reduced cyanogenic content (hence cyanide levels) in cassava tubers, thus supporting partly De Bruijn's findings. Studying the influence of nitrogen fertilizer on cyanide levels in forage sorghum, Wheeler *et al*. (1990) showed that the fertilizer increased cyanide levels. But researches in Philippines showed that there is no significant effect to cyanide levels with the application of fertilizers (Rolinda *et al*., 2008). This was also observed by Cuvaca *et al*. (2015). So, the relationship between fertilizer application and cyanide level in cassava is not conclusive. However, Cuvaca *et al*. (2015) did not talk of the interaction effects among the three nutrients N, K and P which were applied together. This, in one way or another, might have affected nutrient's individual effect on cyanide levels.

2.3.4 Soil type

Sandy soil, due to poor water retention, contributes to water stress especially during drought. This, indirectly and positively, affects cyanide levels in cassava (CIAT, 1989; Ekanayake, 1994). Conclusively, soils with high water retention would indirectly and negatively affect cyanide level in cassava. Furthermore, soils rich in cyanide as a result of natural processes (e.g. volcanoes, wildfire and microbiological activities), human activities (e.g. electroplating, gold mining, textiles and plastics production) or both, are a reservoir of cyanide taken up by cyanogenic plants (CHU, 2015) including cassava. Thus, cyanide levels in cassava are likely to be influenced by soil cyanide levels where cassava is grown.

2.3.5 Age of cassava harvesting

A study by Hidayat *et al*. (2002) on cassava varieties showed that cyanide potential of roots and leaves correlate significantly. Since cyanide level was higher in younger roots and leaves compared to older ones, this suggests that cyanide potential of roots drops as the plant ages. This observation was supported by later findings of Chotineeranat *et al*. (2006). So, age of cassava has a bearing on cyanide levels. A study by Wheeler *et al*. (1990) on factors that affects cyanide levels in forage sorghum showed similar trends of cyanide levels decreasing with age.

2.3.6 Cassava post-harvest practices

There are numerous and diverse processing methods to decrease cassava's cyanogenic potential and these include peeling, chopping, grating, soaking, drying, frying, boiling and fermenting. Cassava cooking, for example, was found to reduce cyanide levels in cassava tubers by 25-75% (Nambisan and Sundarsan, 1985; Aalbersberg and Limalevu, 1991). Methods which use grating and crushing are very effective; and when in combination with wetting, fermentation and drying, they can reduce cyanide level by up to 99%. This is due to the fact that maceration enables the intimate contact in the grated or crushed wet parenchyma between linamarin and the hydrolyzing enzyme linamarase, thus promoting rapid breakdown of linamarin to hydrogen cyanide gas which is volatile (Cardoso *et al*., 2005).

2.4 Gap of Knowledge

Prior to this study, no research had been launched in Kagera region to quantify cyanide in sweet cassava tubers. Comparison of cyanide levels between Kagera and Morogoro regions' cassava varieties is original. Thirdly, no research so far had addressed the issue of people's knowledge on cyanide and its potential danger, particularly in Kagera and Morogoro region. Further, there are conflicting views with regard to cyanide levels in sweet cassava varieties. Therefore, there was a reason of undertaking this research work in order to bridge the identified gaps of knowledge for basic and application purposes.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study area included Bukoba Rural and Missenyi districts for Kagera Region and Morogoro municipal and Mvomero district for Morogoro Region. These were chosen because they are among the regions where cassava is widely grown and consumed as staple food. Kagera region, which is located in the northwestern corner of Tanzania on the western shore of Lake Victoria, lies between 1^{o} 00’2.45”S, 30^{o}25’32.41”E and it is 1199 MASL. It consists of eight administrative districts. Although experiencing two rainy seasons, the region has undergone soil overuse in some parts causing soil exhaustion and need for fertilizer use. Morogoro region is located in central eastern part of Tanzania. It lies between 6^{o}41’15.67”S, 37^{o}39’40.39”E and its elevation is 523 MASL. Made up of six districts, it is characterized by lowland with protruding mountains including Uluguru Mountains. A good number of the natives live and undertake their agricultural activities, including cassava and banana growing, in the highlands. With regard to population, Kagera has 2 458 023 people whereas Morogoro region has 2 218 492 people; and the representative districts for each region together contribute 20% and 41% of the total population respectively (NBS and OCGS, 2013).

Figure 4: Location of Kagera and Morogoro Regions

3.2 Study Design and Sampling

This study used cross-sectional design for the collection of participants' responses and for determining cyanide levels in raw cassava tubers. The number of participants was estimated using the formula by Smith (2013): $n = Z^2 \times (1 - SD) / S_x^2$; where n = sample size; Z = Z-score (1.96); SD = standard of deviation (0.5); S_x = standard error (0.05). This was done at 95% confidence level. A total of 386 participants were selected and the inclusion criteria were cassava growing, consuming or both. Having identified common sweet varieties grown in Kagera and Morogoro regions by using agricultural officers and cassava growers, simple random sampling was employed to obtain sample varieties that were used for the study. Names (each on a separate piece) of all identified varieties for each region were mixed together in a box. The researcher randomly picked up the pieces according to the number of varieties to be used in the study.

3.3 Data Collection

3.3.1 Participants' responses

Through ward officers in selected wards, the researcher and research assistants met the participants. Having built a rapport, participants' responses were collected from them through answering structured questionnaires. Those unable to read and write were interviewed using the same questionnaire guide. The responses were then organized and kept for statistical analysis.

3.3.2 Cyanide level determination

In total, 66 cassava tubers for cyanide quantification were obtained from cassava growers in the study area. Each variety's tubers were collected from not less than five sampling points. The tubers had to be intact and were well packed in containers to avoid acceleration of their destruction. Transportation to Food Science Research Laboratory at Sokoine University of Agriculture was done in less than two days to meet the limited shelf life of cassava tubers. At the research laboratory, the tubers were preserved in a refrigerator (at 4^{o}C) until analysis.

Quantitative analysis of cyanide involved the following steps. Having washed the sample tuber, it was peeled to obtain the parenchyma. The parenchyma was then longitudinally cut into several portions enough of which were ground using an electric blender to obtain ground parenchyma tissue that produced two portions each weighing 20 g. Each of the portions was placed into 800 mL Kjeldahl flask and then mixed with 200 mL distilled water. The mixture was left to stand for at least two hours for complete enzymatic process. Then followed steam-distillation of the mixture to collect 150 mL distillate in NaOH solution (0.5 g in 20 mL H2O) contained in a flat-bottomed flask. To 100 mL of the distillate, 8 mL 6 N NH4OH and 2 mL 5% KI solution were added and then titrated with 0.02 N AgNO3 using micro-burette. The average titre volume for the results obtained from the two 20 g -portions of the sample tuber was obtained. This was repeated for each sample cassava tuber. Eventually, HCN level for each average titre volume was calculated by using the formula for alkaline titration method (1 mL 0.02 N $AgNO3$ = 1. 08 mg HCN; AOAC, 1995). The calculated values were in turn used to work out mg/kg of HCN in fresh cassava. The results were kept for statistical analysis.

Another case involved three samples each representing a different variety notably MZURIKWAO, MWANGA and KIKANIKI. From each sample, 20 g were scratched off from the outer slippery tissue and 20 g from the following inner tissue of the tuber parenchyma. Each of these portions was placed into 800 mL Kjeldahl flask and then mixed with 200 mL distilled water and underwent the remaining steps of the procedure as already described above to obtain the titre volume with eventual determination of cyanide level for each titre volume. Again, cyanide level (mg/kg) of fresh cassava was worked out in each case and noted down for later statistical analysis.

3.4 Statistical Data Analysis

Raw data obtained in section 3.3 above were fed into computer programme SPSS and Microsoft Excel for further processing. With SPSS, mean cyanide levels in sweet cassava tubers were obtained and compared using analysis of variance (ANOVA) at the significance level of $\alpha = 0.05$. This helped to get p-values (used to judge the study hypotheses), descriptive statistics with means, standard deviations and standard error (highlighting on the degree of data consistency) and post hoc multiple comparisons (for sensing out what differed significantly). The programme also assisted in tallying participants' responses with eventual production of frequencies and percentages of data. The Excel was employed to produce diverse graphs that were a great help in describing trends of sets of data.

CHAPTER FOUR

4.0 RESULTS

4.1 Sweet Cassava Varieties Grown in Kagera and Morogoro Regions

Fifty two common sweet cassava varieties were identified; 22 from Morogoro and 30 from Kagera regions, as presented in Tables 1 and 2. From this pool, a sample of 12 varieties was selected for cyanide quantification comprising of PIKIPIKI, SHELEKERA, KATAAKYA, SUNGUSUNGU, AKIBA, KAEMPU and RUSHULA (from Kagera Region), KIKANIKI, MZURIKWAO, MWANGA, NYACHILO and KIBAHA (from Morogoro Region). From these results, there are numerous cassava varieties from each region but more in Kagera than Morogoro.

Table 1: Cassava varieties grown in Morogoro Region including frequency and percent of variety's turns of mention

S/N	Variety	Frequency	Percent
1	KIBAHA	65	32.18
2	KIGOMA-TANGA	85	40.08
3	MOSHI-WA-TAA	72	35.64
4	BETAUJE	107	52.97
5	NYACHILO	95	47.03
6	MZUNGU	50	24.75
7	KIKANIKI	75	37.13
8	KIBANGA-MENO	54	26.73
9	KISWAHILI	27	13.37
10	MZURI-KWAO	56	27.72
11	MTWIKE	35	17.33
12	SIGARA-BARIDI	68	33.66
13	SIGARA-MWEUPE	62	30.96
14	KIBIBI	22	10.89
15	MNDUNGA	36	17.82
16	CHELEMA	25	12.38
17	MWANGA	45	22.28
18	SUPER-TALL	58	28.71
19	KISAMBAA	20	9.90
20	MPERA	15	7.42
21	MWALI-MWEUPE	61	30.20
22	MWARUSHA	33	16.34

Table 2: Cassava varieties grown in Kagera Region including frequency and percent of variety's turns of mention

S/N	Variety	Frequency	Percent
1	RUSHULA	113	61.41
2	BUKARAZA	125	67.93
3	KAEMPU	86	46.74
4	AKIBA	67	36.41
5	SHELEKERA	82	44.57
6	KATAAKYA	102	55.43
7	PIKIPIKI	87	47.28
8	KASHANJE	57	30.98
9	KAGINGO	45	24.46
10	KOMUBEGI	40	21.74
11	MAREKANI	68	36.96
12	KINOGOFU	35	19.02
13	SUNGUSUNGU	85	46.20
14	KACHALI	35	19.02
15	NJUBU	81	44.02
16	MATOKE	30	16.30
17	GONYA	31	16.85
18	JOJIA	36	19.57
19	RUTUKU	77	41.85
20	KONYU	55	29.89
21	SILA	22	11.96
22	KYA-MWANZA	33	17.93
23	MANIMAJEGE	40	21.74
24	MAMA-ASHA	46	25.00
25	MPOROGOMA	37	20.11
26	KARANDILIRA	55	29.89
27	NAZARETI	40	21.74
28	MBARA	45	24.46
29	SEZARIO	50	27.17
30	KWATAMPALE	33	17.93

4.2 Cyanide Levels in Cassava Tubers and Varieties

Tables 3 and 4 present cyanide levels for the 66 cassava tubers collected from Kagera (38 tubers) and Morogoro (28 tubers) regions. Each tuber was collected from not less than 5 sampling points. From the results, all the tested tubers contained cyanide with levels ranging from 19.84 - 226.80 mg/kg for Kagera varieties and from 22.68 - 117.05 mg/kg for Morogoro varieties. Overall, the cyanide levels were all above the acceptable level in human consumables (i.e. 10 mg/kg).

Taking strictly cyanide level of 50 mg/kg as the cutoff point between sweet and bitter tubers (FAO, 1977; Mercola, 2016), 34 sample tubers (52%) were bitter and the rest were sweet (49%). With the exception of the varieties NYACHILO, SUNGUSUNGU and PIKIPIKI, the remaining varieties contained samples in both categories, suggesting that a variety can exist in both sweet and bitter forms.

Table 5 provides descriptive statistics for the sample varieties. The mean cyanide level is highest for variety PIKIPIKI (114.58 mg/kg) and lowest for variety NYACHILO (33.13 mg/kg). The three cassava varieties leading in mean cyanide levels were SUNGUSUNGU, PIKIPIKI and MWANGA; whereas RUSHULA, KAEMPU and NYACHILO form a group of three varieties with comparatively low cyanide levels. The fact of PIKIPIKI and SUNGUSUNGU peaking high in cyanide level was also observed generally in all sample wards of Kagera region, but much highest in Kaagya and Kassambya respectively. With the exception of AKIBA and SHELEKERA, Kagera varieties had the lowest cyanide levels in Kishogo ward (Figure 5).

Mean cyanide levels revealed that 58% of the sample varieties fell into the category of bitter varieties and 42% into the category of sweet varieties. The former category consisted of PIKIPIKI, SHELEKERA, KATAAKYA, SUNGUSUNGU, KIKANIKI, MWANGA and MZURIKWAO. The latter group consisted of AKIBA, KAEMPU, RUSHULA, NYACHILO and KIBAHA. It should be noted that cyanide levels in individual tubers and mean cyanide levels for varieties provide a hint to the degree of poisoning that can be incurred by people who have a habit of consuming raw cassava tubers. For example, variety PIKIPIKI suggests conferring highest degree of poisoning whereas NYACHILO gives the lowest degree of cyanide poisoning.

Table 3: Cyanide levels in tubers from Kagera Region

Variety	**Sample**	**HCN conc. (mg/kg)**
PIKIPIKI	Kishogo	83.03
	Minziro	92.34
	Kaagya-1	66.42
	Kaagya-2	104.30
	Kassambya	226.80
SHELEKERA	Kassambya	55.89
	Minziro	61.56
	Kaagya-1	20.66
	Kaagya-2	66.83
	Kishogo	50.05
KATAAKYA	Kishogo	30.38
	Kaagya-1	77.76
	Kaagya-2	35.24
	Minziro	68.04
	Kassambya	63.59
SUNGUSUNGU	Minziro	80.91
	Kaagya-1	103.28
	Kaagya-2	190.35
	Kishogo	56.70
	Kassambya	113.40
AKIBA	Kishogo-1	60.35
	Kishogo-2	28.35
	Minziro	19.85
	Kassambya	55.06
	Kaagya	85.05
KAEMPU	Kassambya	56.70
	Minziro	58.73
	Kaagya-1	42.12
	Kaagya-2	49.41
	Kishogo-1	24.30

Table 4: Cyanide levels in tubers from Morogoro Region

Variety	Sample	HCN conc. (mg/kg)
KIKANIKI	KI-H-1	51.44
	KI-H-2	41.72
	KI-H-3	40.50
	KI-L-1	117.05
	KI-L-2	85.86
	KI-L-3	52.25
MZURIKWAO	MZ-H-1	36.45
	MZ-H-2	39.29
	MZ-H-3	42.53
	MZ-L-1	100.85
	MZ-L-2	89.91
	MZ-L-3	45.36
MWANGA	MW-H-1	35.64
	MW-H-2	74.93
	MW-H-3	76.14
	MW-L-1	65.21
	MW-L-2	70.47
	MW-L-3	116.64
NYACHILO	NY-1	39.69
	NY-2	22.68
	NY-3	47.39
	NY-4	27.95
	NY-5	27.95
KIBAHA	KB-1	40.91
	KB-2	51.84
	KB-3	64.80
	KB-4	31.19
	KB-5	44.96
MZURIKWAO	Upper- tissue	65.61
	Inner-tissue	40.50
KIKANIKI	Upper-tissue	72.90
	Inner-tissue	48.60
MWANGA	Upper-tissue	68.85
	Inner-tissue	44.55

Table 5: Mean cyanide levels (mg/kg) of cassava varieties from Kagera and Morogoro regions

Variety	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
PIKIPIKI	5	114.58	64.24	28.73	34.81	194.35	66.42	226.80
SHELEKERA	5	51.00	18.08	8.09	28.55	73.45	20.66	66.83
KATAAKYA	5	55.00	20.97	9.38	28.96	81.04	30.38	77.76
SUNGUSUNGU	5	108.93	50.48	22.58	46.24	171.61	56.70	190.35
AKIBA	5	49.73	26.17	11.70	17.24	82.22	19.85	85.05
KAEMPU	6	42.59	15.33	6.26	26.50	58.68	24.30	58.73
RUSHULA	7	44.84	21.13	7.99	25.30	64.38	27.14	75.33
KIKANIKI	6	64.80	30.44	12.43	32.86	96.74	40.50	117.05
MZURIKWAO	6	59.06	28.50	11.63	29.15	88.97	36.45	100.85
MWANGA	6	73.17	26.00	10.66	45.88	100.46	35.64	116.64
NYACHILO	5	33.13	10.11	4.52	20.57	45.69	22.68	47.39
KIBAHA	5	46.74	12.56	5.62	31.14	62.34	31.19	64.80
Total	**66**	**61.32**	**36.46**	**4.49**	**52.36**	**70.28**	**19.85**	**226.80**

The standard deviations (SDs) in Table 5 provide the degree of inconsistency in cyanide levels for the varieties. Whereas varieties PIKIPIKI and SUNGUSUNGU have comparatively high inconsistency (SD = 64.24 and 50.48, respectively), varieties NYACHILO, KAEMPU and SHELEKERA have comparatively low inconsistency (SD =10.11, 15.33 and 18.08 respectively). This is also deduced when one studies Figure 5 for Kagera varieties. The remaining varieties were averagely inconsistent in their cyanide level. From the same figure, Kishogo Ward generally had tubers with lowest cyanide levels when compared with similar varieties' tubers in their respective wards in Kagera region. The SD for Kishogo mean cyanide level (SD = 20.20) is the lowest for Kagera's sample wards.

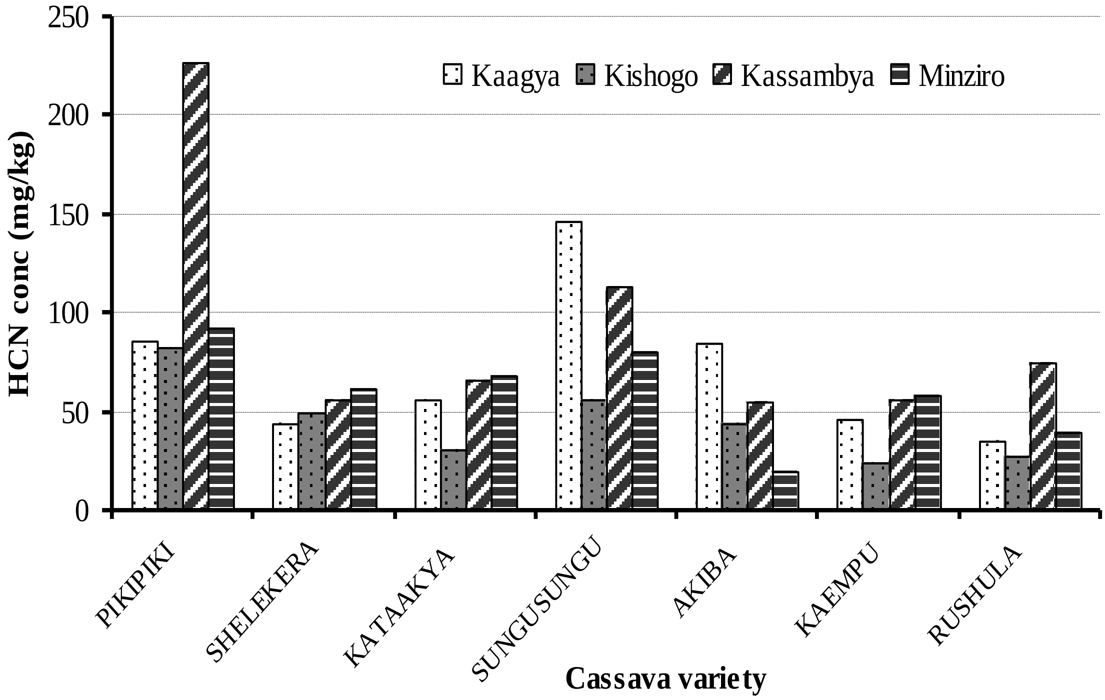

Figure 5: Kagera varieties' cyanide levels in different locations

4.2.1 Effect of variety type on cyanide levels

With respect to cyanide levels, the varieties were found to differ significantly from each other (p=.001). With this finding, the null hypothesis that cyanide levels in cassava tubers are independent of types of varieties is rejected. Table 6 displays the varieties that differed significantly in cyanide level. PIKIPIKI differed significantly from all varieties except SUNGUSUNGU which in turn differed from all varieties except PIKIPIKI and MWANGA; and MWANGA differed only significantly from NYACHILO and PIKIPIKI. The remaining varieties differed significantly only from PIKIPIKI and SUNGUSUNGU (Appendix 4).

4.2.2 Cyanide levels with respect to location-wards, districts and regions

In order to compare the effect of location on cyanide level, mean cyanide levels in tubers (irrespective of variety type) at ward, district and at region level were determined (Tables 7, 8 and 9) producing respective p-values of 0.014, 0.089 and 0.357 (Appendices 5 and 6). At the ward level, the p-value was less than the level of significance ($\alpha = 0.05$) indicating that at least two wards differed significantly to cause significant difference in mean cyanide levels. On applying LSD, the wards that differed significantly with respect to mean cyanide levels in cassava tubers were: Kaagya vs. Kishogo; Kassambya vs. Kishogo, Homboza, Mlali and Magadu; Luhungo vs. Kishogo, Homboza, Mlali and Magadu (refer Appendix 5). Mean cyanide levels for Kaagya (70.08 mg/kg), Kassambya (90.21 mg/kg) and Luhungo (82.62 mg/kg) were considerably high when compared with Kishogo (41.21 mg/kg); Homboza (39.61 mg/kg); and Mlali (40.26 mg/kg) respectively (Table 7).

At district and region levels, it was noted that there was no significant difference as the p-values were less than the significance level. Nevertheless, with the application of LSD, Mvomero (39.93 mg/kg) and Missenyi (76.16 mg/kg) districts were found to differ significantly ($p = 0.015$) in their cyanide levels (Appendix 6). The SD for Missenyi district's mean cyanide level (46.96) is almost four times that of Mvomero (12.93) district, hinting to significant difference between the two localities in terms of the factors that influence cyanide levels.

Table 6: Cassava varieties (first-three-letter abbreviation; X= do not differ; V= do differ)

Variety	PIK	SUN	SHE	KAT	AKI	KAE	RUS	KIK	MZU	MWA	NYA	KIB
PIK	X	X	V	V	V	V	V	V	V	V	V	V
SUN	X	X	V	V	V	V	V	V	V	X	V	V
SHE	V	V	X	X	X	X	X	X	X	X	X	X
KAT	V	V	X	X	X	X	X	X	X	X	X	X
AKI	V	V	X	X	X	X	X	X	X	X	X	X
KAE	V	V	X	X	X	X	X	X	X	X	X	X
RUS	V	V	X	X	X	X	X	X	X	X	X	X
KIK	V	V	X	X	X	X	X	X	X	X	X	X
MZU	V	V	X	X	X	X	X	X	X	X	X	X
MWA	V	X	X	X	X	X	X	X	X	X	V	X
NYA	V	V	X	X	X	X	X	X	X	V	X	X
KIB	V	V	X	X	X	X	X	X	X	X	X	X

Table 7: Mean cyanide levels (mg/kg) of cassava tubers for sample wards of Kagera and Morogoro

					95% Confidence Interval for Mean			
Ward	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**Lower Bound**	**Upper Bound**	**Minimum**	**Maximum**
Kaagya	13	70.08	45.09	12.51	42.83	97.33	20.66	190.35
Kishogo	10	41.21	20.20	6.39	26.76	55.66	24.30	83.03
Kassambya	8	90.21	58.43	20.66	41.37	139.06	55.06	226.80
Minziro	7	60.10	24.47	9.25	37.47	82.73	19.85	92.34
Homboza	5	39.61	10.21	4.57	26.93	52.29	27.95	51.84
Mlali	5	40.26	16.47	7.37	19.80	60.71	22.68	64.80
Magadu	9	48.74	15.86	5.29	36.55	60.92	35.64	76.14
Luhungo	9	82.62	26.22	8.74	62.47	102.77	45.36	117.05
Total	**66**	**61.32**	**36.46**	**4.49**	**52.36**	**70.28**	**19.85**	**226.80**

Table 8: Mean cyanide levels (mg/kg) of cassava tubers for sample districts of Kagera and Morogoro

District	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Bukoba Rural	23	57.53	38.60	8.05	40.84	74.22	20.66	190.35
Missenyi	15	76.16	46.96	12.12	50.15	102.16	19.85	226.80
Morogoro	18	65.68	27.31	6.44	52.10	79.26	35.64	117.05
Mvomero	10	39.93	12.93	4.09	30.69	49.18	22.68	64.80
Total	**66**	**61.32**	**36.46**	**4.49**	**52.36**	**70.28**	**19.85**	**226.80**

Table 9: Mean cyanide levels (mg/kg) of cassava tubers for Kagera and Morogoro regions

Region	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Kagera	38	64.88	42.49	6.89	50.92	78.85	19.85	226.80
Morogoro	28	56.48	26.14	4.94	46.35	66.62	22.68	117.05
Total	**66**	**61.32**	**36.46**	**4.49**	**52.36**	**70.28**	**19.85**	**226.80**

4.2.3 Cyanide levels with respect to location-effect of altitude/topography

Mean cyanide levels of three varieties, KIKANIKI, MZURIKWAO and MWANGA, were compared between their highland and lowland samples collected from Morogoro Municipality. The p-value obtained ($p = 0.004$) was less than the level of significance ($\alpha = 0.05$). This means that there was significant difference in cyanide level between highland and lowland samples, the latter being richer in cyanide (82.62 mg/kg) than the former (38.74 mg/kg). Thus altitude/topography was found to have significant influence on cyanide levels in cassava varieties grown in Morogoro region. It was also noted that the SD for lowland mean cyanide level (26.22) is comparatively high (Table 10) signifying implied inconsistency in cyanide levels' impacting factors in the lowland zone.

Figure 6 graphically highlights on the difference between highland and lowland samples of the three varieties with respect to cyanide level.

Table 10: Mean cyanide levels (mg/kg) of cassava tubers from highland and lowland zones of Morogoro Municipality

Topography	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Highland	9	48.74	15.86	5.29	36.55	60.92	35.64	76.14
Lowland	9	82.62	26.22	8.74	62.47	102.77	45.36	117.04
Total	**18**	**65.68**	**27.31**	**6.44**	**52.10**	**79.26**	**35.64**	**117.04**

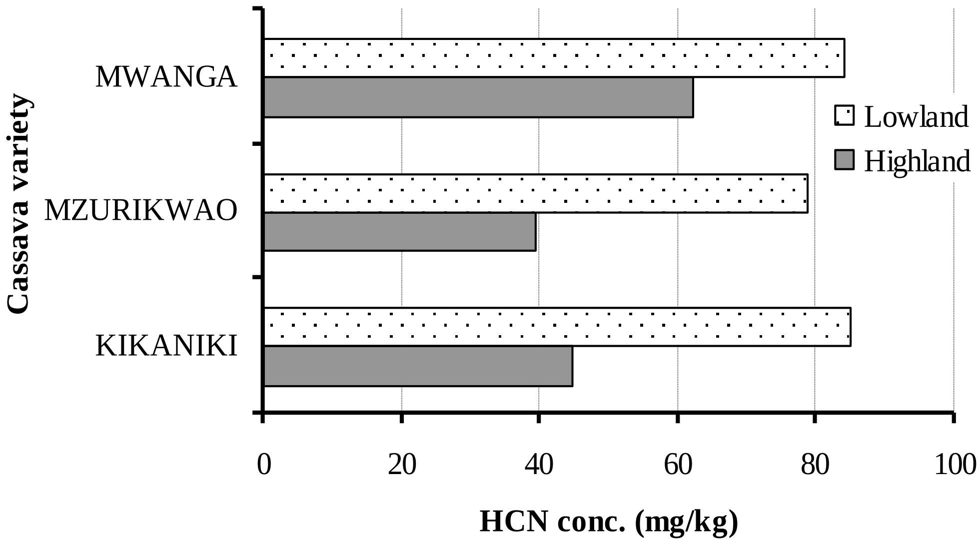

Figure 6: Effect of altitude on cyanide level in selected three cassava varieties

4.2.4 Distribution of cyanide between the slippery and inner tissue of cassava parenchyma

The distribution of cyanide in the slippery outer tissue and inner tissue of the cassava parenchyma is summarized in Table 11 and Figure 7. The information therein shows that there is higher cyanide level in the outer slippery issue than in the following inner tissue of the cassava parenchyma. Figure 7 shows the range in difference of cyanide level between the slippery and inner tissues of each variety. The comparison of mean cyanide levels between the two tissues gave a p-value of 0.001 which is less than the significance level ($\alpha = 0.05$). This indicates the significant difference in cyanide level between the two compared tissues. It can thus be deduced that cyanide is not evenly distributed within the cassava parenchyma. With these results, the null hypothesis that cyanide is uniformly distributed in the cassava tuber parenchyma is rejected.

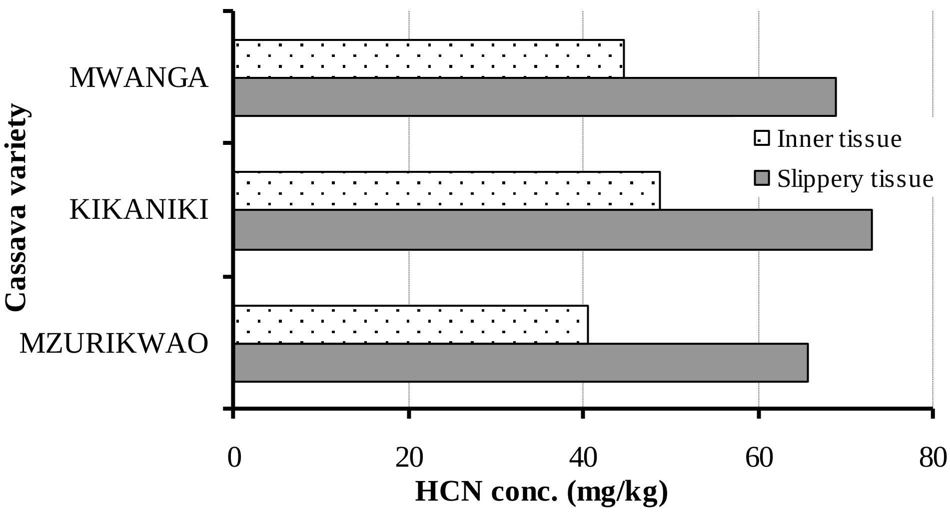

Figure 7: Distribution of cyanide between slippery and inner tissues of cassava parenchyma for three varieties.

Table 11: Mean cyanide levels (mg/kg) of the slippery and inner parenchyma's tissues

Tissue	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
SLIPPERY	3	69.12	3.65	2.11	60.05	78.19	65.61	72.90
INNER	3	44.55	4.05	2.34	34.49	54.61	40.50	48.60
Total	**6**	**56.84**	**13.89**	**5.67**	**42.26**	**71.41**	**40.50**	**72.90**

4.3 Assessment of People's Knowledge on Cyanide and Its Poisoning Effects

4.3.1 Demographic characteristics and distribution of participants

Tables 12 and 13 show socio-demographic distribution of the 386 respondents with regard to sexes, age, regions, districts and wards in terms of frequency and percentage. As it can be deduced from the tables, the representation of the study population in terms of sex, regions, districts and wards was generally balanced. It also shows that both sexes are equally engaged in growing and consuming raw cassava tubers and thus equally at risk of incurring cyanide poisoning.

Table 12: Locational distribution of respondents

Region		District		Ward	
Kagera	184	Bukoba Rural	98 (25.4%)	Kaagya	49 (12.7%)
				Kishogo	49 (12.7%)
		Missenyi	86 (22.3%)	Kassambya	40 (10.4%)
				Minziro	46 (11.9%)
Morogoro	202	Morogoro Munic.	102 (26.4%)	Magadu	50 (13.0%)
				Luhungo	52 (13.5%)
		Mvomero	100 (25.9%)	Mlali	55 (14.2%)
				Homboza	45 (11.7%)

Table 13: Sex and age distribution of research participants

		Frequency	Percent
Sex	Males	196	(50.8)
	Females	190	(49.2)
Age	Below 18	13	(3.4)
	18 and above	373	(96.6)

4.3.2 People's awareness about cyanide and its poisoning effects

Table 14 summarizes tested areas, questions and participants' responses in terms of percentages on peoples' awareness about cyanide and its entailed effects to human health. From the analysis, 37% of the respondents did not know if tubers of bitter cassava varieties contain cyanide. Furthermore, of all the respondents in the study area, only 14% had the knowledge of the presence of cyanide in sweet cassava tubers indicating that 86% were not aware. Respondents who were found unaware about effects of cyanide to human health were 28% (on early symptoms following acute poisoning), 99% (on Konzo), 100% (on TAN and thyroid goiter), 63% (on death) and 95% (mere mention of word disease). It means on average, about 81% of respondents were ignorant about the effects of cyanide poisoning (Table 15 and Figure 8).

Regarding knowledge of the methods for depleting cyanide from cassava tubers, 81% were not aware about them. About 15% of the study population proposed boiling and scratching off the slippery tissue which is more or less a real reflection of the percentage of the respondents who agreed that sweet cassava tubers were poisonous (14%). This indicates that 86% of the people in Kagera and Morogoro regions did not know that sweet cassava tubers need to undergo processing before being consumed by humans. With regard to ways of identifying poisonous cassava varieties, the results were: tasting (56%); by variety (40%); both tasting and variety (4%); poison quantification (0%) as reflected in Table 14. Overall average percent of people not aware about cyanide poisoning and related aspects was 79 (Table 15). Furthermore, the difference between those who were aware and those unaware was highly significant ($p < 0.001$).

Table 14: **People's extent of awareness about cyanide poisoning**

Knowledge tested	Question aspect	Respondents (%) Yes*	No	No**
A. Presence of cyanide in cassava tubers	1.Bitter cassava poisonous	63.2	34.5	2.3
	2.Sweet cassava poisonous	14.2	85.8	0.0
B. Effects of cyanide in the human body	1. Symptoms	72.0	--	28.0
	2. Konzo	1.3	--	98.7
	3. TAN	0.0	--	100.0
	4. Goiter	0.0	--	100.0
	5. Death	37.6	--	62.4
	6. Disease	5.2	--	94.8
C. Methods of depleting cyanide in cassava tubers	1. Boiling	8.3	--	91.7
	2. Soaking/Fermentation	51.3	--	48.7
	3. Sun-drying	10.1	--	89.9
	4.Scratching off slippery layer	6.5	--	93.5
D. Ways of identifying poisonous cassava varieties	1. Variety	39.6	--	--
	2. Tasting	56.0	--	--
	3. Variety and Tasting	4.4	--	--
	4. Quantification method	0.0	--	100.0

(For B, C and D (4) in Table 12, (*) means respondents mentioned the point; and (**) means respondents did not mention the point. For other cases, it means respondents made choice from the given alternatives.)

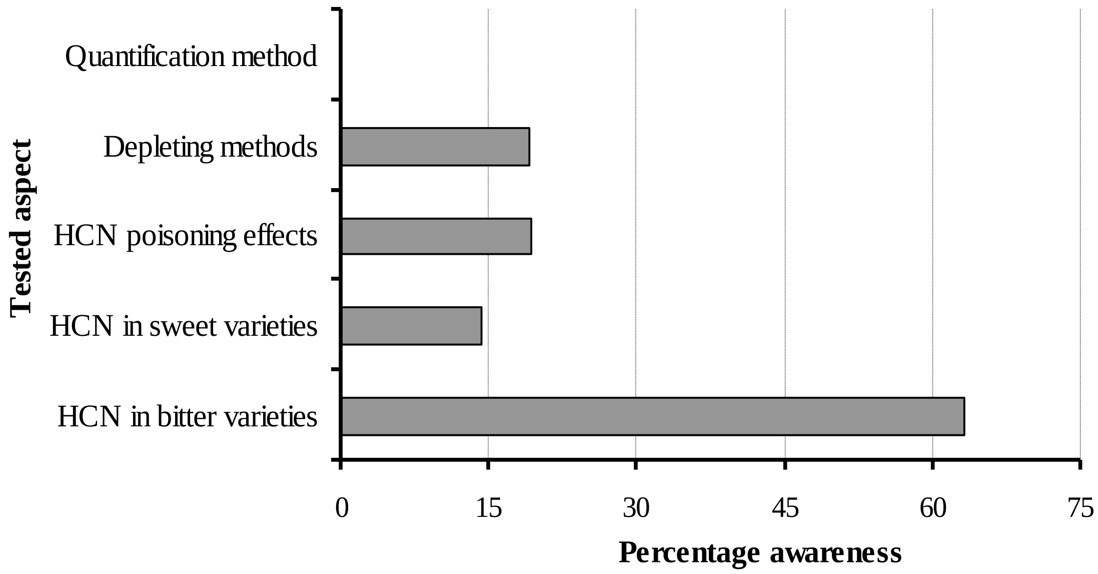

Figure 8: People's awareness on cyanide poisoning

Table 15: Percent of people on awareness about cassava poisoning aspects

People					95% Confidence Interval for Mean			
	N	**Mean**	**Std. Deviation**	**Std. Error**	**Lower Bound**	**Upper Bound**	**Minimum**	**Maximum**
Aware	13	20.74	25.93	7.19	5.07	36.42	.00	72.00
Unaware	13	79.25	25.93	7.19	63.58	94.93	28.00	100.00
Total	26	50.00	39.19	7.69	34.17	65.83	.00	100.00

CHAPTER FIVE

5.0 DISCUSSION

5.1 Cassava Varieties and Cyanide Levels

A good number of sweet cassava varieties are grown in both Kagera and Morogoro regions and thus growers are not limited to a few varieties. According to respondents' views, different varieties are grown on the basis of one or a combination of qualities including palatability, maturity attainment duration, productivity, resistance to diseases and tuber shelf life. Growth to maturity may differ from one variety to another, some maturing within six months like KATAAKYA while others maturing in a year or longer such as RUSHULA. This is in line with the observations made by Allem (2002) and Montagnac *et al*. (2009) that cassava cultivars differ in their ability to tolerate pest and diseases, yield, nutritional and cooking qualities of food products as well as maturity attainment. In fact, the meanings of some local names of cassava varieties hint to the qualities of such varieties. For instance, SHELEKERA ("hide for") signifies productivity; SUNGUSUNGU ("fierce local guard") hints to bitterness; KATAAKYA ("flowers not") signifies early maturity; MATOKE ("like banana fruit") gives the idea of ease to cook; and KONYU ("salty") refers to palatability. These qualities are essential to cassava growers. One respondent commented that the same variety may exhibit inconsistent maturity age due to environmental factors such as climate and soil type. KIBAHA variety was cited as an example which matures within 8 months in Kibaha (Coastal region), but maturity attainment is delayed when grown in the highlands of Morogoro region. On SUNGUSUNGU, a note was made that its harvest should be delayed otherwise one should be ready to bear with its bitterness in its early maturity. This supports the observation that cyanide levels decrease as cassava plants age (Hidayat *et al*., 2002; Chotineeranat *et al*., 2006).

With regard to cyanide levels in cassava tubers, the findings of this research are consistent with authors who hold that acyanogenic cassava varieties are non-existent and that cyanide levels in all cassava tubers are above the accepted level in human consumables (Bradbury and Holloway, 1988; White *et al*., 1998; FAO, 2007). Human consumables containing cyanide levels above 10 mg/kg are not eligible for direct consumption unless appropriate methods for depleting cyanide are employed (FAO, 2007). Moreover, the noted inconsistency of cyanide levels within samples of the same variety means that cyanide level in one sample tuber can in no way be used as a standard for other samples of the same variety collected from different sampling points. In other words, the same variety can exist in both sweet and bitter forms due to factors that affect cyanide levels in cassava including water stress (Bokanga *et al*., 1994; Tan, 1995; Gitebo *et al*., 2009), soil type (CIAT, 1989; Ekanayake, 1994; CHU, 2015) and application of fertilizers (De Bruijn, 1973; Endris, 1977; Wheeler *et al*., 1990).

The observation that some sweet cassava varieties grown in Kagera and Morogoro regions differ significantly concurs with observations made by previous researchers that although all cassava cultivars/varieties contain cyanogenic glycosides, a wide variation in the concentration of cyanogens exists among different cultivars (Cardoso *et al.*, 2005; CIAT, 2007). The categorization of sample tubers and varieties into sweet and bitter lines calls for conscious consideration of methods appropriate for reducing cyanide in consumables to acceptable levels. Tubers in sweet cassava form need be boiled prior to human consumption (Cardoso, 2004; FSANZ, 2005; Ubwa *et al*., 2015). For bitter ones, a more effective process for depleting cyanide than mere boiling is required before being cooked for human use (Ernesto *et al.,* 2002; Cardoso *et al*., 2005; Nzwalo and Cliff, 2011). Whereas bitterness in taste is not always a guarantee of cyanide level (Dufour, 1994) as this could stem from chemicals other than cyanide (Bokanga, 1994), there are cases in which varieties known to be sweet have tubers with high cyanide level disqualifying them to be called sweet (CIAT, 1989; Ekanayake, 1994; Tan, 1994).

In other words, neither taste nor name of variety is a sure method for identifying poisonous and no-poisonous cassava varieties.

It was also noted that mean cyanide levels for Kaagya, Kassambya and Luhungo were considerably high in comparison to the other wards. This knowledge is essential for identifying localities that either intensify or lower cyanide level in cassava tubers and studying the characteristics of such localities in order to find out the influencing factors. This would further assist in making the right choice of where to grow cassava whose tubers would contain comparatively low cyanide levels. For example, Kassambya which was found to differ significantly from Kishogo, Homboza, Mlali and Magadu, consists of sandy soils whose water-retaining capacity is very low thus creating water stress during drought which intensifies cyanide level in cassava tubers (CIAT, 1989; Ekanayake, 1994). This same reason can be used to explain the significant difference in mean cyanide levels that was found to exist between Missenyi and Mvomero districts. The former's soils are generally sandy characterized by low water retention, intensifying water stress during dry season which may account for the high cyanide level in comparison to other sample districts.

At region level, it was found that mean cyanide levels between the two regions did not differ significantly. Probably similar environmental factors in certain locations such as soil type and fertilizer application may account for this observation. Although Kagera region gave a tuber with overall highest cyanide level of 226.80 mg/kg (PIKIPIKI-Kassambya) as well as one with overall lowest cyanide level 19.85 mg/kg (AKIBA-Minziro), in each region there were tubers found with cyanide levels below 50 mg/kg as well as tubers with cyanide levels above 100 mg/kg. Nevertheless, Morogoro and Kagera regions generally have no similar geographical conditions, particularly with respect to topography and climate. Lack of significant difference between the two regions would partly be explained on the basis of sample size. Probably at regional level, there was considerably small sample size which was not well distributed across the region to capture wide variations that exist within each region.

Altitude has been shown by this research to have a significant bearing on cyanide level in cassava tubers collected from Morogoro highland and lowland zones. The fluctuation in cyanide level with regard to same variety tubers collected from different points as it has been seen with all sample tubers may partly be explained by topographical aspects. In Morogoro region, particularly Morogoro municipality and Mvomero district, most cassava tubers consumed raw are grown in highland parts of the region. One respondent from Magadu said that there is a big difference in climate between Uluguru highlands and other lowlands in the area and added that delicious tubers are from highland zone. He identified lowlands as a dry zone. In fact, common experience in Morogoro municipality shows that lowland zone has more stresses due to high temperatures and drought than highland zone. This may account for high cyanide levels in lowland cassava samples. On the contrary, Mt. Kilimanjaro and neighboring parts consist of volcanic soils which are said to intensify cyanide levels in cassava to such an extent that it is held a taboo to consume raw cassava tubers of cultivars grown there. If that is true, deductively Uluguru Mountains are unlikely to be of volcanic soils otherwise one would expect to get high cyanide levels from tubers of cassava varieties grown in the highlands.

Several methods have been employed to reduce cyanide levels in cassava tubers (Ernesto *et al.*, 2002; Nzwalo and Cliff, 2011). In the current study, the interest was focused on finding out if the practice of scratching off the slippery tissue of sweet cassava tuber (evident in Kagera region) and which was among the methods proposed by some respondents for cyanide depletion in cassava tubers was able to significantly reduce cyanide content in the tuber. The findings of this research have shown that the outer slippery parenchyma tissue is significantly richer in cyanide than the inner tissues. Thus, the habit of scratching off the slippery tissue may help to reduce cyanide level in cassava tubers and it warrants further testing. It is a common experience in Kagera region to see people scratching off the slippery layer of sweet cassava tubers prior to consumption or cooking. In fact, one of the respondents cautioned not to give the scratched-off tissues to chicks lest they die.

5.2 People's Awareness about Cyanide and Its Poisoning Effects

Demographic information about the respondents revealed that, in Kagera and Morogoro regions, both sexes are equally engaged in growing and consuming raw sweet cassava varieties. They are thus at the same risk of incurring cyanide poisoning effects. On the question of people's awareness about the presence of cyanide in cassava tubers, the 37% of the respondents who did not know if tubers of bitter cassava varieties contain cyanide were all from Morogoro region. This may be due to the fact that bitter cassava varieties are rarely grown in the area and so only a few people are accustomed to such varieties. It is thus not so much surprising with regard to the case of cyanide poisoning that happened in Kauzeni, Mvomero District that cost lives of three people as hinted before.

It has been shown by this research that 86% of the people living in Kagera and Morogoro regions did not know if sweet cassava varieties contain cyanide (poison). This would suggest that cyanide poisoning in this group of people is out of ignorance since it has been shown by this study that all sweet cassava tubers in the study area contain cyanide beyond the internationally acceptable level in human consumables. Furthermore, average number of people who were not aware of cyanide poisoning effects to human health in

Kagera and Morogoro was 81%. Logically, lack of awareness contributes to the habit of consuming raw sweet cassava tubers evident in Kagera and Morogoro regions. This could not be the case if people had enough knowledge about cyanide poisoning effects. Ravindra (2007) supports this observation when lack of education is mentioned as one of the factors that lead to the production of three food-related neurotoxic disorders, lathyrism, Konzo, and TAN.

With regard to cyanide depleting methods and reliable way of identifying poisonous cassava tubers (cyanide quantification), average percent of unaware people was 81% and 100% respectively. It is of utmost importance for people to be availed with the toxicological identity of cassava tubers and methods for depleting cyanide to acceptable levels. People might be deterred from growing cassava following cyanide poisoning incidents as what happened in Senegal due to lack of information on the toxicological properties of varieties and the processing technology (FAOSTAT, 2013).

The mentioned ways commonly and locally used by people to identify poisonous cassava tubers, including experience of variety and tasting, are misleading. It has been shown by this study that all varieties studied display inconsistency of cyanide level due to different factors (Tan, 1994; Ekanayake, 1994; CIAT, 1989) and the same variety can exist in both sweet and bitter forms. Moreover, the degree of sweetness or bitterness does not necessarily foretell the degree of cyanide level in cassava tubers (Bokanga, 1994; Dufour, 1994). It is thus risky to consume raw cassava tubers whose cyanide level is unknown (Ubwa *et al*., 2015; FSANZ, 2004).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the research findings and discussion as presented in Chapters Four and Five respectively, the following are the conclusions that are deemed main according to the objectives of the study.

1. There are many "sweet" cassava varieties grown in Kagera and Morogoro regions. Selection of varieties to be grown is based on attributes including palatability, maturity-attainment duration, resistance to diseases, shelf life and productivity. Moreover, a variety may exhibit variation in maturity age depending on different environmental factors.

2. Cyanide level in all sweet cassava tubers of varieties grown in Kagera and Morogoro regions is above the internationally accepted level in human consumables and thus not eligible for human consumption in their raw state. By eating such tubers in their raw state, people are highly exposed to the risk of cyanide poisoning.

3. Cassava tubers of the same variety may contain different cyanide levels; thus, the same variety may exist in both sweet and bitter forms making the practice of characterizing a variety as sweet or bitter misleading. With this inconsistency, there is still the possibility of cyanide poisoning if cassava tubers mistakenly taken as sweet are merely boiled.

4. Cyanide level in sweet cassava tubers may be influenced by a number of factors such as type of variety and location. Moreover, cyanide level in the cassava tuber parenchyma is not uniformly distributed. The slippery outer tissue contains more cyanide than the inner tissues. Therefore, scratching off the slippery tissue of the parenchyma before boiling would be beneficial by reducing cyanide level in consumable cassava tubers.

5. Human consumption of raw sweet cassava tubers is on the most part cultivated by people's lack of awareness on the presence of cyanide in cassava tubers and its toxic effects to human health. There is a convincing association between indicators of cyanide poisoning evident in the study area and the habit of consuming raw cassava tubers.

6.2 Recommendations

Since cassava is gaining much attention due to its growth advantages and its nutritive values, we cannot stop people from growing it on the mere basis of containing cyanide. The following recommendations are meant to protect people from cyanide poisoning as well as to maximize production of edible cassava varieties.

1. Provision of education: It is a human weakness to talk good of our products forgetting the negative side of them. Availing people with new cassava varieties should go hand-in-hand with provision of education on the reality of cyanide in cassava cultivars, cyanide poisoning effects, inconsistency of cyanide in cultivars as well as appropriate methods for depleting cyanide to acceptable levels in human consumables. It means, there must be a national programme of attaining this crucial objective.

2. Devices for cyanide quantification: Scientists should invest in developing simple, inexpensive but efficient devices that common people can use to quickly quantify cyanide in cassava tubers and thus enable them to choose appropriate methods for depleting cyanide prior to human consumption.

3. Correlational and cause-effect studies: Since cases of thyroid goiter, epilepsy, mild Konzo and leg weakness are witnessed in areas where cassava is grown, correlational and cause-effect studies are required to build strong grounds for educating the mass with regard to bad habits that are contributory factors to cyanide poisoning and the importance of treating cassava tubers prior to human consumption.

4. Assessment of cyanide depleting methods: Learning from the responses to questionnaires, respondents had different answers as to the number of days for soaking or fermenting cassava tubers in attempt to reduce cyanide level in cassava tubers. This is an area that warrants further study. The findings of such a study would help to secure a common solution to this inconsistency of answers.

5. Acyanogenic cassava varieties: There is a need of intensifying efforts in producing genetically modified cassava varieties in which a gene responsible for cyanide expression in cyanogenic glycosides is either absent or masked.

6. Cyanide level and geographical factors: cyanide level in cassava tubers is inconsistent due to different factors. As some areas differ on geographical basis such as soil type, climate and topography, it is evidenced by this research work that in some cases average cyanide level between two or more locations differs significantly.

While some locations heighten cyanide levels, other locations lower them. Thus, more conscientious choices should be made as to where to grow cassava that would produce minimal cyanide level in cassava tubers.

7. Sulphur-donor diet: Since HCN detoxification requires sulphur donors, essential sulphur-containing amino acids should be part of a diet. That is to say, dietary good quality protein needs to be insisted in areas where cassava is the main staple food.

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APPENDICES

Appendix 1: Questionnaire/structured-interview guide on cassava varieties and cyanide poisoning

A. **Respondent's demographic information** (answer by ticking in a box or filling the blank as required)

1. Your Sex: **Male** ☐ **Female** ☐
2. Year age: **Below 18yrs** ☐ **Above 18yrs** ☐
3. Your District: **Bukoba R.** ☐ **Missenyi** ☐ **Morogoro** ☐ **Mvomero** ☐
4. Your Ward: ……………………………………………
5. Cassava grower/consumer: **Yes** ☐ **No** ☐

B. **Information directly related to the study** (Tick in the box or fill the blank as required)

1. Do you grow bitter cassava varieties in your area? **Yes** ☐ **No** ☐
2. Is bitter cassava poisonous? **Yes** ☐ **No** ☐ **Don't Know** ☐
3. How do you identify poisonous cassava? By: **Variety** ☐ **Taste** ☐ **Variety And Taste** ☐ ;
   Mention any other method you know……………………………………
4. Is sweet cassava poisonous? **Yes** ☐ **No** ☐ **Don't Know** ☐
5. Mention names of sweet cassava varieties grown in your area.
   i) ……………………………… ii)………………………………..
   iii) …………………………… iv) ……………………………
   v) …………………………….. vi) ………………………………
   vii) ……………………………viii) ………………………………
6. What health problems would you incur by eating poisonous cassava?
   i)………………………………………………………………………
   ii)………………………………………………………………………
   …
   iii)……………………………………………………………………

7. Enlist ways used to reduce HCN in cassava tubers before they are allowed for human consumption
   i)---
   ii) ---
   iii) --

Appendix 2: Some activities towards HCN quantification

Plate A: Cassava farm; **Plate B:** Packages of collected cassava samples; **Plate C&D**: Preparation of cassava solution; **Plate E**: Steam distillation of sample solution to obtain HCN solution; **Plate F**: Alkaline titration of HCN solution

Appendix 3: Details on HCN content analysis (based on 20g of cassava)

Region	**Variety**	**Sample**	**$AgNO_3$-TIT. VOL./100mL/150mL**	**mg/kg HCN**
KAGERA	PIKIPIKI	Kishogo	1.025	83.025
		Minziro	1.140	92.340
		Kaagya-1	0.820	66.420
		Kaagya-2	1.287	104.300
		Kassambya	2.800	226.800
	SHELEKERA	Kassambya	0.690	55.890
		Minziro	0.780	61.560
		Kaagya-1	0.255	20.655
		Kaagya-2	0.825	66.825
		Kishogo	0.618	50.050
	KATAAKYA	Kishogo	0.375	30.375
		Kaagya-1	0.960	77.760
		Kaagya-2	0.435	35.235
		Minziro	0.840	68.040
		Kassambya	0.785	63.585
	SUNGUSUNGU	Minziro	0.990	80.910
		Kaagya-1	1.275	103.275
		Kaagya-2	2.350	190.350
		Kishogo	0.700	56.700
		Kassambya	1.400	113.400
	AKIBA	Kishogo-1	0.745	60.345
		Kishogo-2	0.350	28.350
		Minziro	0.245	19.845
		Kassambya	0.655	55.055
		Kaagya	1.050	85.050
	KAEMPU	Kassambya	0.700	56.700
		Minziro	0.725	58.725
		Kaagya-1	0.520	42.120
		Kaagya-2	0.610	49.410
		Kishogo-1	0.300	24.300
		Kishogo-2	0.300	24.300
	RUSHULA	Kaagya-1	0.435	35.235
		Kaagya-2	0.425	34.427
		Kishogo-1	0.340	27.540
		Kishogo-2	0.335	27.135
		Kassambya	0.925	74.925
		Minziro	0.485	39.285
		Kassambya	0.930	75.330
MOROGORO	KIKANIKI	KI-H-1	0.635	51.435
		KI-H-2	0.515	41.715
		KI-H-3	0.500	40.500
		KI-L-1	1.445	117.045
		KI-L-2	1.060	85.860
		KI-L-3	0.645	52.245
	MZURIKWAO	MZ-H-1	0.450	36.450
		MZ-H-2	0.485	39.285

	MZ-H-3	0.525	42.525
	MZ-L-1	1.245	100.845
	MZ-L-2	1.110	89.910
	MZ-L-3	0.560	45.360
MWANGA	MW-H-1	0.440	35.640
	MW-H-2	0.925	74.925
	MW-H-3	0.940	76.140
	MW-L-1	0.805	65.205
	MW-L-2	0.870	70.470
	MW-L-3	1.440	116.64
NYACHILO	NY-1	0.490	39.690
	NY-2	0.280	22.680
	NY-3	0.585	47.385
	NY-4	0.345	27.945
	NY-5	0.345	27.945
KIBAHA	KB-1	0.505	40.905
	KB-2	0.640	51.840
	KB-3	0.800	64.800
	KB-4	0.385	31.185
	KB-5	0.555	44.955
MZURIKWAO	Upper-tissue	0.810	65.610
	Inner-tissue	0.500	40.500
KIKANIKI	Upper-tissue	0.900	72.900
	Inner-tissue	0.600	48.600
MWANGA	Upper-tissue	0.850	68.850
	Inner-tissue	0.550	44.550

Appendix 4: Multiple comparisons (LSD) of cassava varieties from Kagera and Morogoro Regions with respect to HCN levels

CASSAVA VARIETY	(J) CASSAVA VARIETY	Mean Difference (I-J)	Std. Error	Sig.
PIKIPIKI	SHELEKERA	63.5810(*)	19.14420	.002
	KATAAKYA	59.5780(*)	19.14420	.003
	SUNGUSUNGU	5.6500	19.14420	.769
	AKIBA	64.8480(*)	19.14420	.001
	KAEMPU	71.9845(*)	18.32917	.000
	RUSHULA	69.7374(*)	17.72408	.000
	KIKANIKI	49.7770(*)	18.32917	.009
	MZURIKWAO	55.5145(*)	18.32917	.004
	MWANGA	41.4070(*)	18.32917	.028
	NYACHILO	81.4480(*)	19.14420	.000
	KIBAHA	67.8400(*)	19.14420	.001
SHELEKERA	PIKIPIKI	-63.5810(*)	19.14420	.002
	KATAAKYA	-4.0030	19.14420	.835
	SUNGUSUNGU	-57.9310(*)	19.14420	.004
	AKIBA	1.2670	19.14420	.947
	KAEMPU	8.4035	18.32917	.648
	RUSHULA	6.1564	17.72408	.730
	KIKANIKI	-13.8040	18.32917	.455
	MZURIKWAO	-8.0665	18.32917	.662
	MWANGA	-22.1740	18.32917	.232
	NYACHILO	17.8670	19.14420	.355
	KIBAHA	4.2590	19.14420	.825
KATAAKYA	PIKIPIKI	-59.5780(*)	19.14420	.003
	SHELEKERA	4.0030	19.14420	.835
	SUNGUSUNGU	-53.9280(*)	19.14420	.007
	AKIBA	5.2700	19.14420	.784
	KAEMPU	12.4065	18.32917	.501
	RUSHULA	10.1594	17.72408	.569
	KIKANIKI	-9.8010	18.32917	.595
	MZURIKWAO	-4.0635	18.32917	.825
	MWANGA	-18.1710	18.32917	.326
	NYACHILO	21.8700	19.14420	.258
	KIBAHA	8.2620	19.14420	.668
SUNGUSUNGU	PIKIPIKI	-5.6500	19.14420	.769
	SHELEKERA	57.9310(*)	19.14420	.004
	KATAAKYA	53.9280(*)	19.14420	.007
	AKIBA	59.1980(*)	19.14420	.003
	KAEMPU	66.3345(*)	18.32917	.001
	RUSHULA	64.0874(*)	17.72408	.001
	KIKANIKI	44.1270(*)	18.32917	.020
	MZURIKWAO	49.8645(*)	18.32917	.009
	MWANGA	35.7570	18.32917	.056
	NYACHILO	75.7980(*)	19.14420	.000
	KIBAHA	62.1900(*)	19.14420	.002

CASSAVA VARIETY	(J) CASSAVA VARIETY	Mean Difference (I-J)	Std. Error	Sig.
AKIBA	PIKIPIKI	-64.8480(*)	19.14420	.001
	SHELEKERA	-1.2670	19.14420	.947
	KATAAKYA	-5.2700	19.14420	.784
	SUNGUSUNGU	-59.1980(*)	19.14420	.003
	KAEMPU	7.1365	18.32917	.699
	RUSHULA	4.8894	17.72408	.784
	KIKANIKI	-15.0710	18.32917	.415
	MZURIKWAO	-9.3335	18.32917	.613
	MWANGA	-23.4410	18.32917	.206
	NYACHILO	16.6000	19.14420	.390
	KIBAHA	2.9920	19.14420	.876
KAEMPU	PIKIPIKI	-71.9845(*)	18.32917	.000
	SHELEKERA	-8.4035	18.32917	.648
	KATAAKYA	-12.4065	18.32917	.501
	SUNGUSUNGU	-66.3345(*)	18.32917	.001
	AKIBA	-7.1365	18.32917	.699
	RUSHULA	-2.2471	16.84047	.894
	KIKANIKI	-22.2075	17.47618	.209
	MZURIKWAO	-16.4700	17.47618	.350
	MWANGA	-30.5775	17.47618	.086
	NYACHILO	9.4635	18.32917	.608
	KIBAHA	-4.1445	18.32917	.822
RUSHULA	PIKIPIKI	-69.7374(*)	17.72408	.000
	SHELEKERA	-6.1564	17.72408	.730
	KATAAKYA	-10.1594	17.72408	.569
	SUNGUSUNGU	-64.0874(*)	17.72408	.001
	AKIBA	-4.8894	17.72408	.784
	KAEMPU	2.2471	16.84047	.894
	KIKANIKI	-19.9604	16.84047	.241
	MZURIKWAO	-14.2229	16.84047	.402
	MWANGA	-28.3304	16.84047	.098
	NYACHILO	11.7106	17.72408	.512
	KIBAHA	-1.8974	17.72408	.915
KIKANIKI	PIKIPIKI	-49.7770(*)	18.32917	.009
	SHELEKERA	13.8040	18.32917	.455
	KATAAKYA	9.8010	18.32917	.595
	SUNGUSUNGU	-44.1270(*)	18.32917	.020
	AKIBA	15.0710	18.32917	.415
	KAEMPU	22.2075	17.47618	.209
	RUSHULA	19.9604	16.84047	.241
	MZURIKWAO	5.7375	17.47618	.744
	MWANGA	-8.3700	17.47618	.634
	NYACHILO	31.6710	18.32917	.090
	KIBAHA	18.0630	18.32917	.329

CASSAVA VARIETY	(J) CASSAVA VARIETY	Mean Difference (I-J)	Std. Error	Sig.
MZURIKWAO	PIKIPIKI	-55.5145(*)	18.32917	.004
	SHELEKERA	8.0665	18.32917	.662
	KATAAKYA	4.0635	18.32917	.825
	SUNGUSUNGU	-49.8645(*)	18.32917	.009
	AKIBA	9.3335	18.32917	.613
	KAEMPU	16.4700	17.47618	.350
	RUSHULA	14.2229	16.84047	.402
	KIKANIKI	-5.7375	17.47618	.744
	MWANGA	-14.1075	17.47618	.423
	NYACHILO	25.9335	18.32917	.163
	KIBAHA	12.3255	18.32917	.504
MWANGA	PIKIPIKI	-41.4070(*)	18.32917	.028
	SHELEKERA	22.1740	18.32917	.232
	KATAAKYA	18.1710	18.32917	.326
	SUNGUSUNGU	-35.7570	18.32917	.056
	AKIBA	23.4410	18.32917	.206
	KAEMPU	30.5775	17.47618	.086
	RUSHULA	28.3304	16.84047	.098
	KIKANIKI	8.3700	17.47618	.634
	MZURIKWAO	14.1075	17.47618	.423
	NYACHILO	40.0410(*)	18.32917	.033
	KIBAHA	26.4330	18.32917	.155
NYACHILO	PIKIPIKI	-81.4480(*)	19.14420	.000
	SHELEKERA	-17.8670	19.14420	.355
	KATAAKYA	-21.8700	19.14420	.258
	SUNGUSUNGU	-75.7980(*)	19.14420	.000
	AKIBA	-16.6000	19.14420	.390
	KAEMPU	-9.4635	18.32917	.608
	RUSHULA	-11.7106	17.72408	.512
	KIKANIKI	-31.6710	18.32917	.090
	MZURIKWAO	-25.9335	18.32917	.163
	MWANGA	-40.0410(*)	18.32917	.033
	KIBAHA	-13.6080	19.14420	.480
KIBAHA	PIKIPIKI	-67.8400(*)	19.14420	.001
	SHELEKERA	-4.2590	19.14420	.825
	KATAAKYA	-8.2620	19.14420	.668
	SUNGUSUNGU	-62.1900(*)	19.14420	.002
	AKIBA	-2.9920	19.14420	.876
	KAEMPU	4.1445	18.32917	.822
	RUSHULA	1.8974	17.72408	.915
	KIKANIKI	-18.0630	18.32917	.329
	MZURIKWAO	-12.3255	18.32917	.504
	MWANGA	-26.4330	18.32917	.155
	NYACHILO	13.6080	19.14420	.480

Appendix 5: Multiple comparisons (LSD) of wards from Kagera and Morogoro Regions with respect to HCN levels (α=.05)

WARD	(J) WARD	Mean Difference (I-J)	Std. Error	Sig.
KAAGYA	KISHOGO	28.8697(*)	14.03452	.044
	KASSAMBYA	-20.1289	14.99334	.185
	MINZIRO	9.9810	15.64227	.526
	HOMBOZA	30.4727	17.55840	.088
	MLALI	29.8247	17.55840	.095
	MAGADU	21.3467	14.46851	.146
	LUHUNGO	-12.5383	14.46851	.390
KISHOGO	KAAGYA	-28.8697(*)	14.03452	.044
	KASSAMBYA	-48.9986(*)	15.82693	.003
	MINZIRO	-18.8887	16.44299	.255
	HOMBOZA	1.6030	18.27536	.930
	MLALI	.9550	18.27536	.959
	MAGADU	-7.5230	15.33066	.625
	LUHUNGO	-41.4080(*)	15.33066	.009
KASSAMBYA	KAAGYA	20.1289	14.99334	.185
	KISHOGO	48.9986(*)	15.82693	.003
	MINZIRO	30.1099	17.26860	.087
	HOMBOZA	50.6016(*)	19.02160	.010
	MLALI	49.9536(*)	19.02160	.011
	MAGADU	41.4756(*)	16.21301	.013
	LUHUNGO	7.5906	16.21301	.641
MINZIRO	KAAGYA	-9.9810	15.64227	.526
	KISHOGO	18.8887	16.44299	.255
	KASSAMBYA	-30.1099	17.26860	.087
	HOMBOZA	20.4917	19.53719	.299
	MLALI	19.8437	19.53719	.314
	MAGADU	11.3657	16.81493	.502
	LUHUNGO	-22.5193	16.81493	.186
HOMBOZA	KAAGYA	-30.4727	17.55840	.088
	KISHOGO	-1.6030	18.27536	.930
	KASSAMBYA	-50.6016(*)	19.02160	.010
	MINZIRO	-20.4917	19.53719	.299
	MLALI	-.6480	21.10257	.976
	MAGADU	-9.1260	18.61072	.626
	LUHUNGO	-43.0110(*)	18.61072	.024
MLALI	KAAGYA	-29.8247	17.55840	.095
	KISHOGO	-.9550	18.27536	.959
	KASSAMBYA	-49.9536(*)	19.02160	.011
	MINZIRO	-19.8437	19.53719	.314
	HOMBOZA	.6480	21.10257	.976
	MAGADU	-8.4780	18.61072	.650
	LUHUNGO	-42.3630(*)	18.61072	.027

WARD	(J) WARD	Mean Difference (I-J)	Std. Error	Sig.
MAGADU	KAAGYA	-21.3467	14.46851	.146
	KISHOGO	7.5230	15.33066	.625
	KASSAMBYA	-41.4756(*)	16.21301	.013
	MINZIRO	-11.3657	16.81493	.502
	HOMBOZA	9.1260	18.61072	.626
	MLALI	8.4780	18.61072	.650
	LUHUNGO	-33.8850(*)	15.72893	.035
LUHUNGO	KAAGYA	12.5383	14.46851	.390
	KISHOGO	41.4080(*)	15.33066	.009
	KASSAMBYA	-7.5906	16.21301	.641
	MINZIRO	22.5193	16.81493	.186
	HOMBOZA	43.0110(*)	18.61072	.024
	MLALI	42.3630(*)	18.61072	.027
	MAGADU	33.8850(*)	15.72893	.035

Appendix 6: Multiple comparisons (LSD) of districts in Kagera and Morogoro regions with respect to HCN levels (α=.05)

DISTRICT	(J) DISTRICT	Mean Difference (I-J)	Std. Error	Sig.
BUKOBA RURAL	MISSENYI	-18.6297	11.75907	.118
	MOROGORO MUNICIPAL	-8.1478	11.15019	.468
	MVOMERO	17.5967	13.42096	.195
MISSENYI	BUKOBA RURAL	18.6297	11.75907	.118
	MOROGORO MUNICIPAL	10.4818	12.38699	.401
	MVOMERO	36.2263(*)	14.46488	.015
MOROGORO MUNICIPAL	BUKOBA RURAL	8.1478	11.15019	.468
	MISSENYI	-10.4818	12.38699	.401
	MVOMERO	25.7445	13.97441	.070
MVOMERO	BUKOBA RURAL	-17.5967	13.42096	.195
	MISSENYI	-36.2263(*)	14.46488	.015
	MOROGORO MUNICIPAL	-25.7445	13.97441	.070