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ANALYSIS OF HEAVY METAL CONTENT IN WATER HYACINTH (*EICHHORNIA CRASSIPES)* FROM LAKE VICTORIA AND ASSESSMENT OF ITS POTENTIAL AS A FEEDSTOCK FOR BIOGAS PRODUCTION

BY

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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

I dedicate this work to my mum; thank you for believing in me.

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ABBREVIATIONS

AIS - Aquatic Invasive Species

APC - Aquatic Plant Central

EAR - Eastern Applied Research

EEA - European Environmental Agency

EUR - Euro

FAO - Food and Agriculture Organization

IUCN - *International Union for Conservation of Nature*

KEBS - *Kenya Bureau of Standards*

Ksh - *Kenyan Shilling*

KWSTCo. - *Kisumu Water and Sewerage Treatment Company*

LVEMP -*Lake Victoria Environmental Management Program*

NCPB - *National Cereals and Produce Board*

ND -*Not Detected*

TC - *Translocation Coefficient*

TXRF - *Total Reflection X-Ray Fluorescence*

UNEP - *United Nations Environmental Program*

US$ - *United States Dollar*

USDA -*United States Department of Agriculture*

WHO - *World Health Organization*

XRF - *X-Ray Fluorescence*

ZEO - *Zambia Environmental Outlook*

ABSTRACT

Water hyacinth (*Eichhornia crassipes*) is an aquatic weed that exhibits prolific growth on the surface of water bodies and it is also a hyper accumulator of heavy metals. Over the past two decades Lake Victoria has been adversely affected by the plant which negatively impacted income generating activities as well as lake's the ecology. The objective of this study was to evaluate the potential of water hyacinth as biomass for biogas production as a strategy to eliminate its threat on the lake and assess the heavy metal content in the weed. One hundred and eighty samples of the plant samples and 90 water samples were collected from 10 sites along Winam Gulf in Lake Victoria. For each sample, triplicate sub-samples for roots, stems and leaves were weighed, dried in room temperature followed by oven drying at 80 ^{0}C, then ashed at 600 ^{0}C and finally acid digested before analysis using TXRF. Water samples were analyzed directly using the same method. Bio-gasification was conducted in 6 m^3 flexi tubular digester. Cow dung to water hyacinth ratios used were 1:1, 1:3 and 1:5 each having water ratio of 1:1. Twenty seven digestate samples were collected for analysis. These samples were prepared and analyzed in the same method as water hyacinth samples. Results showed the presence of manganese, iron, nickel, copper and zinc in water and which had as well been accumulated in the plant tissue. Highest recorded concentrations were Fe and Mn at 27812 ± 3.4 and 16691 ± 2.1 mg kg^{-1} respectively from roots. For water samples, the highest heavy metal concentration was Fe at 6931 ± 57 µg l^{-1}. Order of occurrence for the metals in both water and water hyacinth samples and was found to be: Fe > Mn > Zn > Cu

> Ni. It was observed that the heavy metals were highly accumulated and retained in the roots in comparison to aerial parts and the order of accumulation was: roots > stems > leaves. Biogas volumes were recorded highest at cow dung: water hyacinth ratio of 1: 5 and least at cow dung: water hyacinth ratio of 1:1. The trend was similar in heavy metal concentrations for respective digestate analysis where highest concentrations ranged between 1427 to 1.7 mg kg^{-1} for 1:5 mixture and 1:1 mixture having the least range of concentrations between 505 and < 20 mg kg^{-1}. When compared against heavy metal limits as specified by KEBS in 2011 for organic fertilizers, the quantities of heavy metals in the digestate does not exceed these limits and therefore can be applied as an organic fertilizer.

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CHAPTER ONE

INTRODUCTION

1.0 Background

Water hyacinth, *E. crassipes*, is among the worst aquatic weeds affecting many parts of the world (Gichuki *et al.,* 2012). This is because it exhibits prolific growth and exhausts nutrients and oxygen in high volumes from water bodies thus harmfully affecting the surrounding environment. Its growth also causes complete blockage of channels making commercial and recreational activities very difficult (Bhattacharya and Kumar, 2010). The plant is known to be robust and extremely hard to eliminate, since it is able to withstand severe conditions. For instance, during unfavorable conditions such as drought, the plant sinks into the bottom of the water and remains dormant until the conditions become conducive for growth (Ndimele, 2012).

Great concerns have been raised lately due to the rate at which the weed is spreading, especially in the African Continent (Calvert, 2002). In Nigeria, it was first noticed in 1984 along Badagry creek in Lagos. The weed was reported to have formed a 'mat', a term generally used to describe the physical distribution of water hyacinth invasion on a water body over the surface. By 1990, the weed had spread throughout the entire Nigerian Coastlines (Bolorunduro, 2000). In Kenya, the weed was first spotted in Lake Victoria, a fresh water lake, in 1989 (Abong'o, 2009). The lake stretches over 68,800 km^2, and since the first spotting, the weed has eventually spread to cover nearly 199 square kilometers of the entire lake. (Gichuki *et al.,* 2012; Mehrhoff *et al.,* 2010).

The various techniques that are being applied to eradicate the weed are very expensive and therefore short term. Mechanical methods which employ the use of machinery and manual removal have cost Mali around US$ 80,000 - 100,000 per year (Dagno *et al.,* 2007). In Lake Victoria, manual and mechanized management of the weed at Port Bell, costs Uganda approximately 5 million dollars annually (Mailu, 2001).

Figure 1: Water hyacinth infestation along Winam Gulf in Lake Victoria (Ministry of Environment and Mineral Resources, 2010)

The use of the weevil *Neochetinaei chhorniae* from South America as a biological control mechanism is considered the most effective on huge infestations of water hyacinth. In Benin it was a successful endeavor but the cost rounded up to a value of US$ 2,090,000 (Groote *et al.,* 2003). This application has however been found to be ineffective on Lake Victoria because it takes many years to achieve satisfactory results. The weevil's rate of activity could not keep up with the weed's fast growth, in addition to heavy rainfall experienced in area that limits their performance (Osumo, 2001). Chemical methods are largely cheaper to use. However, chemical methods are least favored for long term applications on account of the potential harm posed by the herbicides on the lake's fauna. Eventually, socio-economic activities could be negatively impacted (Dagno *et al.*, 2007).

Survival of water hyacinth is based on the nutrients provided by various habitats. These can range from clean waters which can be lacking in key nutrients to extremely contaminated waters with high amounts of nutrients. Although the growth of water hyacinth is more vibrant in neutral water bodies, the weed can grow well in waters severely polluted by organic matter and heavy metals, like in sewage lagoons waters, due to phytoaextractive properties (So *et al.*, 2003, Jafari, 2010). Phytoextraction is the uptake of pollutants by roots with successive accumulation in the aerial parts of a plant (Pivetz, 2001). A study by Zhu *et al*. (1999) on phytoaccumulation of various elements by water hyacinth revealed that the weed builds up trace elements like silver, lead, cadmium, among others. In addition, the plant proved to be effective in phytoremediation of wastewater contaminated with cadmium, chromium, copper and selenium. Similar research conducted by Shao and Chang (2004), indicated that water hyacinth is capable of absorbing, as well as translocating heavy metals like Pb, Cd, Ni, Zn, and Cu. Mahamadi (2011) in a related study attributed these properties to numerous poly-functional metal-binding sites in the plant, for both anionic and cationic metal complexes, thus the ability to absorb heavy metals along with other contaminants. In Pakistan, an investigation by Hussain *et al.* (2010) on phytoremediation of nickel ions by water hyacinth, reported an accumulation of heavy metals in the roots.

Water hyacinth has a high content of fermentable matter, as well as nitrogen and essential nutrients. These attributes make it a suitable biomass to produce biogas (Patil *et al.*, 2011). Biogas is a fuel composed of a mixture of carbon dioxide and methane, generated by bacterial degradation of organic matter (Science Dictionary, 2013). This is a beneficial initiative because biomass fuel is sustainable and plays a role in substituting fossil fuels as an energy source (European Commission, 2013).

1.1 Problem Statement

Lake Victoria has been gravely affected by the growth of water hyacinth over the past few decades. Economic activities in Lake Victoria such as commercial fishing, transport, recreational activities have declined due to the invasion of the weed. Water supply to Kisumu and the surrounding towns has suffered regular interruption because the plants block inlet pipes at Kisumu Water and Sewage Treatment Company (KWSTCo.). The growth of water hyacinth has posed a health risk to the people in the Lake region because the mats are habitats for mosquitoes and snails which spread diseases. Additionally, water hyacinth generates large amounts of organic matter. As the organic matter decomposes, biological oxygen demand increases and water quality deteriorates. The oxygen sometimes reduces to such low levels that it leads to massive fish deaths. The weed has limited positive uses. Research shows that it cannot be used directly as a fertilizer due to its high C: N ratio that necessitates supplementation of nitrogenous fertilizer. In addition, it cannot be utilized as fodder for livestock due to excessive silica and potassium content, as well as calcium oxalate. Furthermore, it does not contain sufficient protein (Makhanu, 1997). There is therefore the urgent need to eliminate the weed from the Lake.

Water hyacinth contains vital nutrients as well as high degree of fermentable materials, which makes it suitable biomass to produce biogas. However, it is also known to accumulate high levels of heavy metals from polluted waters. Therefore, before using water hyacinth for this purpose, it is imperative to analyze the heavy metal content. If the metal content is too high in the plant material, it will be challenging to utilize water hyacinth as biomass. This is because the metals remain in the slurry and it could pose a serious environmental hazard during the disposal of the digestate as an organic fertilizer.

1.2 Justification

Converting water hyacinth into a biomass feedstock is important for solving ecological and economic problems facing the Lake Victoria ecosystem. This approach provides a solution to energy conservation and largely solves environmental problems. Biogas is a better fuel than fossil fuels because it does not contain sulphur. As a result, its utilization reduces energy costs and the reliance on firewood as a source of energy. The resultant slurry produced after using water hyacinth for production of biogas can also be applied as a fertilizer because it was found to contain large amounts of nitrogen, phosphorus and potassium. This plan can be applied up to when the weed has been completely eradicated on the lake.

Since water hyacinth derives its nutrients from water, it will also be essential to analyze metal content in water from Lake Victoria. Analysis of heavy metals in water and water hyacinth will not only provide data on the metal concentration levels in the plants but it will also give a general view of the pollution levels along Lake Victoria.

In Kenya, minimal studies have been carried out towards the prospective of water hyacinth as a biomass feedstock. Given that biogas produced from plant material is dependent on its properties, investigation of the composition of water hyacinth growing in Lake Victoria is vital before it is utilized. Additionally, it is crucial to monitor the levels of heavy metals in the plant due to disposal of the slurry to prevent eventual pollution.

In this study, heavy metal content in water hyacinth will be investigated as well as its utilization as biomass for biogas production.

1.3 Objectives

1.3.1 General objective

The main objective is to investigate the heavy metal content in water hyacinth and assess its potential as feedstock for biogas generation.

1.3.2 Specific objectives

1. To identify and determine the concentration of heavy metals in water hyacinth from Lake Victoria using TXRF.
2. To determine the heavy metal content in the water column of Lake Victoria water using TXRF.
3. To evaluate the viability of utilizing water hyacinth as feedstock for bio gasification.
4. To establish the heavy metal levels from the bio digester slurry before applying it as an organic fertilizer.

1.4 Hypothesis

Water hyacinth from Lake Victoria along Winam Gulf does not contain heavy metals and it cannot be used as biomass for biogas production.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Water hyacinth has been described by the International Union for Conservation of Nature (IUCN) as one of the most destructive and aggressive plant species on water bodies (Téllez *et al.*, 2008). UNEP (2013), reported the weed to be the most extensive and destructive water plant in Africa having an economic impact of as much as US$ 100,000,000 annually. In this chapter, we show the weeds occurrence and development on various habitats. We further go on to present a survey that confirms its ecological and economic effects including the huge amount of resources invested towards its eradication. In anticipation to use water hyacinth as biomass for biogas production, we present the various modes in which it has been used for the same. Based on the various deductions made, we show that its potential as a biomass is indeed a tangible strategy towards its utilization. We then look into one of the areas where water hyacinth has caused numerous scientific investigations; its ability to extract and accumulate heavy metals. This is demonstrated by the supporting results described in several research activities. Finally, a brief overview of the analytical technique used in determination of heavy metals will be given

2.1 Description of water hyacinth

There are generally four species of water hyacinth: *Eichhornia crassipes*, *Eichhornia azurea*, *Eichhornia diversifolia* and finally *Eichhornia paniculata*. *Eichhornia crassipes* (common water hyacinth) is found all over the world due to its fast reproductive characteristics and its ability to float. *Eichhornia azurea* (anchored water hyacinth), occurs in the Americas including the Caribbean. Its physical appearance is similar to *Eichhornia crassipes* only that it is rooted and its reproductive period is longer (Aquatic Invasive Species, 2005). *Eichhornia diversifolia* (Variable leaf water hyacinth) is commonly found in Central and South America (Aquatic Plant Centrals, 2007). *Eichhornia paniculata*, a short-lived developing perennial, is abundantly found in some parts of South and Central America and a few regions of the Caribbean (USDA, 2014). The species growing in Lake Victoria is *Eichhornia crassipes* (Osumo, 2001)

Eichhornia crassipes is a floating waterweed of the family Pontederiaceae and draws all its nutrients from water. The plant comprises 95% water and 5% dry matter. It has dark green rounded leaves with a diameter of up to 5 cm, and stems that are engorged into bulbous and spongy structures (Biosecurity Queensland, 2007; Osumo, 2001). It has intense light purple flowers that grow in clusters of 8 - 15 on stalks of up to 30 centimeters (Robinson, 2003). Its roots are fibrous and featherlike. If the plant is growing in deep water, the roots can trail underneath the plant, with lengths of up to 1 meter. In shallow water, the roots usually take hold in the base of sediment or mud (Burton *et al.,* 2010).

2.2 Reproduction and ecological distribution of water hyacinth

Water hyacinth grows in a broad array of fresh water habitats for example marshes, shallow ponds, small streams, rivers and lakes, which do not become saline during drought (Ecocrop, 2011). Wind and currents play a role in their dispersal and distribution (Osumo, 2001). For instance, it may double in size within a week, with a mat of medium sized plants containing approximately 2 million plants per hectare that weighs between 280 to 400 tons (Malik, 2007). The plant reproduces either asexually through stolons or sexually via seeds, with long viability period of close to 20 years, thus hard to control (Center *et al.,* 1999). The infestations spread swiftly through production of new daughter plants. During floods and high water flows, the infestations can easily split up and relocated to other areas. However, anthropogenic activities including deliberate deposition of water hyacinth in dams and ponds are largely to blame for the widespread (Burton *et al.,* 2010). Presently, water hyacinth occurs throughout the subtropical and tropical regions, including; Africa, North and South America, Asia, New Zealand and Australia (FAO, 2002).

Figures 2.1 and 2.2 show the different forms of water hyacinth that develop depending on growth conditions. According to Wilson *et al.,* (2007), nutrient content and temperature are some the key influences for its development and reproduction. Where the water body has high nutritional content, these plants generally have shorter roots which are spread out horizontally, in addition to extended shoots and comparatively larger leaves (Fig. 2.1b). Where there is poor nutrition, the plants tend to have longer roots that are set deeper in search for food, with relatively smaller leaves and shorter shoots (Fig. 2.1a). The most favorable conditions are moderate temperatures (14 - 29 °C), low salinity, neutral pH, N, P, K-rich water, plenty of sunlight and minimal physical disturbances and pests infestation (Hasan and Chakrabarti, 2009).

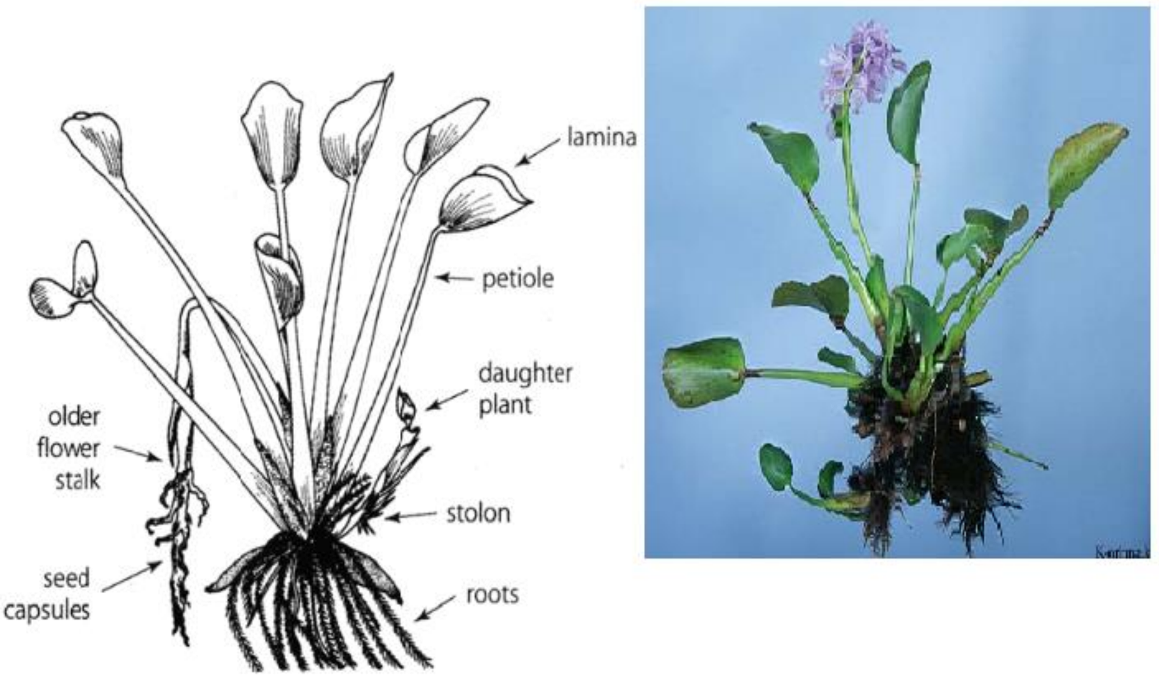

Fig 2.1: First form of water hyacinth; grows in poor nutritional environment (Korhnak, 2015; Wright and Purcell, 1995).

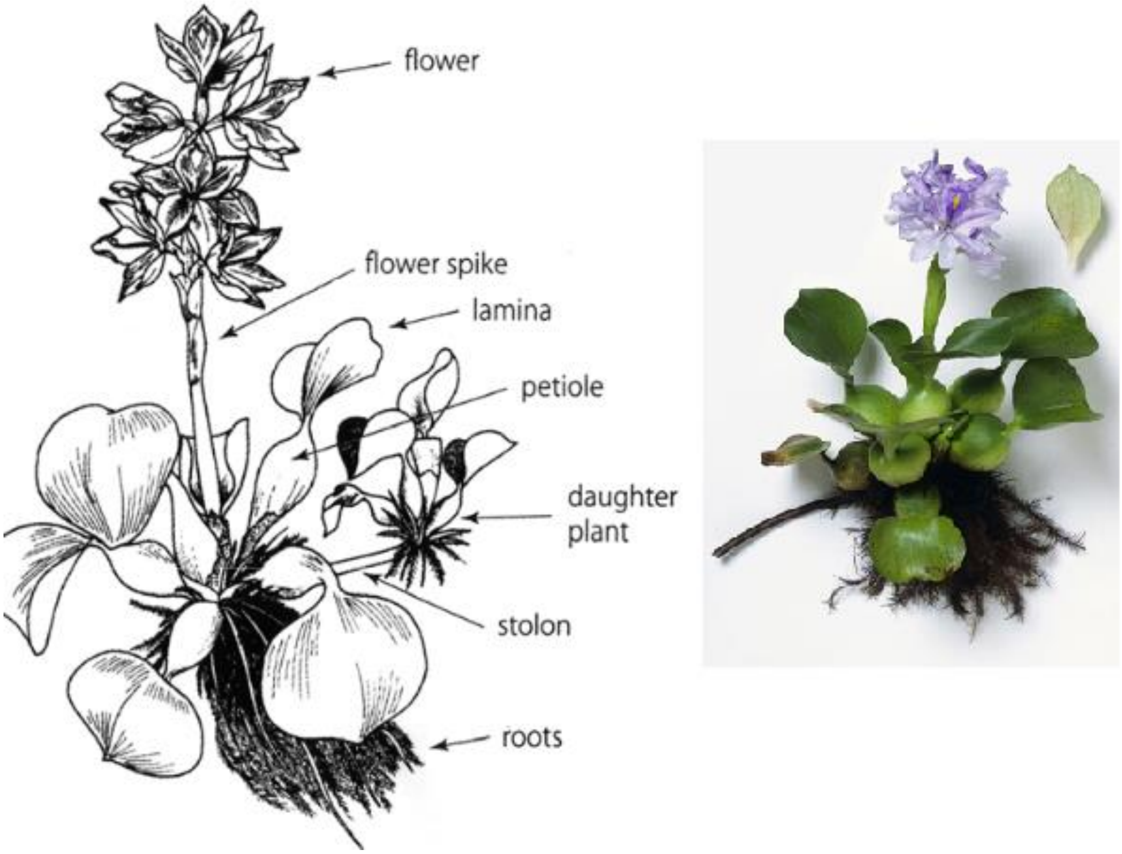

Figure 2.2: Second form of water hyacinth; grows in high nutritional environment (Diszhal, 2007; Wright and Purcell, 1995).

2.3 Environmental and economic effects of water hyacinth

2.3.1 Ecological degradation

Water hyacinth challenges the ecologic balance of the affected water body (Khanna *et al.,* 2011). It inhibits the development of other plant life and thus negatively impacting microbes. In addition, the weed also suppresses the emergence of phytoplankton under its bulky mats, eventually affecting fisheries (Gichuki *et al.,* 2012). In Lake Victoria, fish haul rates along the Kenyan segment decreased by over 40 %, since water hyacinth renders some fishing grounds inaccessible, hinders entrance to food markets, and increases cost (material and effort) of fishing (Kateregga and Sterner, 2009). Additionally, vast thick water hyacinth mats inhibit the exchange of oxygen between the atmosphere and the water surface, therefore diminishing oxygen yield by algae and other plants (Hill *et al.,* 2011). Once the plant dies out and drops under, the rotting biomass exhausts oxygen levels in the water body. Its thick infestation increases water loss through transpiration, approximately three times the amount lost during regular evaporation (European Environmental Agency, 2012).

2.3.2 Impacts on public health

Over the past few years, water hyacinth has been connected in harboring different agents of several diseases. For instance, from 1994 to 2008, the surrounding region to Lake Victoria in Kenya, reported higher cholera incidences than anticipated, relative to its population size (38 % of reported cholera cases as opposed to 15 % nationwide). The annual water hyacinth occupation in Lake Victoria, especially on the Kenyan side, was positively linked to the high incidences of cholera in the area (Feikin *et al.,* 2010). In addition, the ability of the plant's mass of stringy, loosely floating roots and semi-sunken stalks and leaves to reduce water flow intensifies rearing grounds for anopheles mosquito that causes malaria as demonstrated in the region (Minakawa *et al.,* 2008). According to Varshney *et al.* (2008), Mansonioides mosquitoes which are the vectors of the human lymphatic filariasis causing nematode Brugia, breed on water hyacinth plants. Schistosomiasis (Bilharzia) was also on the rise in the region because snails which are transmitters for the parasite occupied the entangled weed mattings (Borokini and Babalola 2012). There had been also increased incidences of crocodile assaults that were ascribed to the substantial invasion of the weed which provided concealment to the reptiles (Ndimele and Jimoh2011).

2.3.3 Blockage of waterways

Water hyacinth infestation literally makes all water based activities impossible by blocking water courses owing to its fast replication and proliferation rate (Seyoum and Fetali, 2012). For example, in the Wouri River Basin of Cameroon the lives of close to 900,000 residents was disrupted as a result of the blockage and flooding of the entire Abo and Moundja Moussadi creeks. In addition to being made inaccessible, the two creeks flooded during the wet season due to obstruction by the enormous weed mattings. Ultimately, all the socioeconomic activities came to a complete standstill resulting in subsequent rural mass departure (Mujingni, 2012). In Nigeria, water hyacinth has turned fishing and navigation nearly an unachievable undertaking (Ndimele and Jimoh 2011). Patel (2012) reported that in India along River Brahmaputra the weed had also clogged irrigation canals as well as impeded the water flow to the paddy fields. This caused a yearly loss of rice paddy by instantly overwhelming the crop, constraining rice growth and deterring harvesting

2.3.4 Effects to electricity supply

Many large hydropower schemes have also been reported to be negatively sustaining the consequences of water hyacinth colonization of water bodies (Shanab *et al.*, 2010). Choked up turbines on Kafue Gorge in Zambia, rendered the loss of water for power generation and ultimately into loss of income estimated at US$ 15,000,000 every year for the power generating company (Zambia Environmental Outlook, 2008). Cleanup of the weed on the uptake screens at the Owen Falls hydroelectric power plant, in Uganda, were totaled to a million dollars each year (Mailu, 2001). In Malawi, water hyacinths aggregated as islands, float along the river clogging the hydroelectric turbines generators causing frequent blackouts. Losses of About 140 MW power are experienced daily (Mellhorn, 2014).

2.4 Control and management

Due to the numerous problems that water hyacinth has caused, the need to eliminate it has become a priority. The three most common control and eradication methods used are: biological, chemical and mechanical methods. They are presented in the following sections.

2.4.1 Biological Control

Biological control was explored as early as the 1970’s by the USDA and Centre for Agricultural Bioscience International (CABI) (Center *et al.*, 2002). They used the weevils Neochetinabruchi, Neochetinaeichhorniae, and most recently the pyralid moth *Niphograpta albiguttalis*. These insects feed on leaves of the weed, creating tiny scars. Their eggs are usually laid inside the bulbous stems, plus the larvae burrow through the weed tissue, making it venerable to bacteria and fungi attack. The plant becomes waterlogged and eventually dies especially when under severe attack (Center *et al.,* 2002). One major drawback when using biological means is that it may take quite a while to kick-off since it takes a number of years for the pest population to attain required level to effectively take on the weed (Calvert, 2002). For example, in Rwanda, Agaba *et al.,* (2009) studied a program that was introduced in 2000 on biological control of water hyacinth in the Kagera River. The report showed that there was need to expand the application method and integrate other methods for effective results. Jones (2009) also made a similar observation in a project implemented on Lake Nsezi - Nseleni River in South Africa. He highlighted the importance of integrating other control methods to support the biological application. Biological control in Kenya on Lake Victoria was initiated in January in 1997 by Kenya Agricultural Research Institute (Mailu *et al.,* 2000). The project is still underway, but the weevils cannot keep up with the multiplication rate of water hyacinth.

Fig. 2.3: *Neochetina eichhorniae* and *Neochetina bruchi* weevils (Julien *et al.*, 1999).

2.4.2 Chemical Control

Chemical control has proved to be an effective control method for water hyacinth in developed countries, but it is generally considered inappropriate in developing nations. The reason being the high cost and it calls for highly trained personnel, since herbicides are usually considered to be toxins (Hill *et al.*, 2010). The three main herbicides commonly used in control of water hyacinth are; Glyphosate, Diquat, and 2, 4-D (Calvert, 2002). Unfortunately, these chemical substances strongly bind to soil particles and this presents a risk of contaminating groundwater (WHO, 2004). In Kenya application of the herbicides on the lake was found only to be effective for a short while (Osumo, 2001).

Public opinion all over the world is against the application of herbicides in water bodies for the control of the weed mainly due environmental concerns (Charudattan *et al.*, 1996).

2.4.3 Mechanical and manual extraction

The application of machineries such as weed cutters is very costly, nearly US$ 700 - 1,300 per hectare (Villamagna and Murphy, 2010). Additionally, this technique has been demonstrated as unfeasible for parts bigger than a hectare given the rapid propagation of the weed (Malik, 2007). In Europe, it was reported that control expenditure to eradicate 250,000 tons of the weed in a 75 kilometer stretch on Guadiana River, along the Portuguese-Spanish border at a cost of over fourteen million Euros in three years (EEA, 2012). Dagno *et al.* (2007) estimated that mechanized management of water hyacinth would cost about US$ 75,000 to 110,000 per annum in Mali. In Kenya, it was projected that the venture would cost KSh.500, 000,000 for a harvester to shred water hyacinth material from 1500 hectares of water hyacinth on Lake Victoria. This method is not fully effective because the vegetative material could regenerate and seed dropping into the sediment can be a source of future re-infestation (Mailu *et al.*, 2001).

Figure 2.4: Mechanical clearing of water hyacinth from Lake Victoria (Aquarius Systems, 1999)

Since all the possible eradication methods are not offering effective long term results, then we can consider the utilization of the weed for biogas production as a sustainable solution towards elimination of the weed.

2.5 Biogas production using water hyacinth

The prospect of converting water hyacinth to biogas continues to be a field of great interest all over the world. Research experiments conducted in China showed that a combination of biomass of pulped water hyacinth with pig compost resulted in better biogas yield as compared to use of pig dung alone (Lu et al., 2010). In a related study, Dilhani (2004) illustrated that cow dung and water hyacinth mixtures produced biogas even though the carbon: nitrogen ratios were not within the optimum range of 20-30. In Niger, West Africa, Almoustapha et al. (2009)

successfully installed a biogas digester for a maternity facility from water hyacinth and rumen slurry.

Some local communities took up this idea as an alternative eradication method. For instance, in Kampala, water hyacinth from Port Bell was used in generating biogas for the prisoners at Luzira Maximum Prison (Keith and Hirt, 2000). The gas was stored and used for cooking meals during the frequent power outages. In this way, the prison was able to save six million Uganda shillings per year (about US$ 5,000) as well as about 300m^3 firewood (equivalent to 8 hectares of woodland).

Considering all other technologies for disposal and management of water hyacinth, using the weed for biogas purposes is environment friendly and economically viable. However, hardly ever does utilization alone offer a lasting remedy to the rapid spread as well as effects of water hyacinth (EEA, 2012). It could actually offer a bad incentive to retain the aggressive weed that destroys the environment as well as production schemes at great socio-economic costs.

2.6 Theory of anaerobic digestion

Anaerobic digestion also referred to as bio-gasification, is a biochemical process whereby decomposition of complex organic matter takes place in limited oxygen supply by use of a variety of anaerobic microorganisms to generate biogas (Teodorita *et al.,* 2008). Biogas is made up of CO_2 and CH_4 with smaller amounts of water vapor, H_2S and in some cases NH_3 (Dana, 2010). It can be burnt to generate heat for domestic uses or for generation of electricity (Friends of the Earth, 2007).

Anaerobic digestion is dependent on a number of diverse factors for optimal production. Some of these are: feedstock type, carbon nitrogen ratio (C/N ratio), mixing, pH and temperature, (Rajendran *et al.*, 2012). Of particular importance is C/N ratio, as the bacteria liable for the anaerobic process needs both carbon and nitrogen. However, its consumption of carbon is thirty times higher than of nitrogen. Fowler (2003) reported that if all the other parameters are ideal, then a carbon nitrogen ratio of 30:1 is perfect for the raw material fed into a digester.

There are four stages involved in the process of anaerobic digestion. Van Haandel and Van der Lubbe (2007), reported that these stages occur simultaneously. The first stage is hydrolysis and it involves the breaking of polymer chains in the feedstock and dissolving the smaller molecules. The composite organic substances are broken down into basic sugars, fatty acids and amino acids. The second stage is acidogenesis whereby advanced digestion of the remaining components by acidogenic bacteria takes place. In this stage NH_4, CO_2, H_2S alongside other by-products are created. In the third stage, known as acetogenesis, the simple molecules produced in the second stage are further broken down by acetogens to generate CO_2, hydrogen and acetic acid. Methanogenesis is the final phase in which the intermediate products from all the other stages are converted into CO_2, methane and water. These three components form the bulk of the biogas released from the digester.

2.7 Phytoremediation process by water hyacinth

Phytoremediation can be described as the use of plants to moderately or even eradicate certain pollutants in adulterated soil, ground water, surface water, sediment, sludge and waste water (Pivetz, 2001). Water hyacinth is among the most frequently utilized plant in marshlands for heavy metal elimination from a range of sources due to its prolific growth rate as well as high absorption capacity of contaminants and nutrients (Rai, 2009). Over the years, comprehensive studies on water sources decontamination have revealed that water hyacinth that grows in wastewater, tend to effectively accumulate heavy metals (Jafari, 2010). According to Shao and Chang (2004), water hyacinth has the ability to absorb and translocate Cd, Ni, Cu, Zn and Pb in the plant's tissue. In a similar study by Greenfield *et al.*, (2007), water hyacinth foliage tissues were found to accumulate Hg levels similar to the residue below, signifying that reaping the weed could help regulate Hg contamination. Wang *et al.*, (2002), in a pot experiment to determine the effectiveness of water hyacinth for its probable usage in refining contaminated waters observed that water hyacinth highly accumulates cadmium, with levels of up to 14,200 mg kg^{-1} being recorded. Ingole and Bhole (2003), in a related study on uptake of Cr, As, Hg, Pb, Ni and Zn by water hyacinth from aqueous solution of different concentration levels, at a range of 5 to 50 mg l^{-1} dry matter, observed that for the aqueous solution that contains 5 mg l^{-1} of Hg, Cr and As, the maximum uptake was determined at 328, 109 and 26 mg kg^{-1} respectively. In conclusion, water hyacinth's ability to take up nutrients and contaminants makes it a potential biological means in treatment of contaminated waters. However, the key challenge is on ways to appropriately get rid of the large quantities of the plant residues that are considered as harmful waste (Zhang *et al.,* 2012).

2.8 Analysis of heavy metals

Heavy metals are abundantly distributed in the environment. Despite the fact that a number of these metals possess significant toxicity, some are generally not believed to have considerable harmful properties. Several of these metals such as Fe, Cr, Co, Cu, and Zn are essential for the metabolic function in many organisms (Buta *et al.*, 2011)

When choosing a technique to determine heavy metals one must consider cost, physical nature of the matrix, limits of detection i.e. sensitivity and most importantly availability of the instrument (de Gennaro *et al.,* 1999). One of the techniques that is widely used for the analysis of heavy metals is X-Ray fluorescence (XRF). This technique involves the interaction of X-rays with a sample in order to determine its elemental composition. It is well suited for both solid and liquid samples (Horiba Scientific, 2013). The method is fast, accurate, is multi-elemental and covers a wide range of concentrations (ppm - ppb) (Brouwer, 2003). It involves three specific stages taking place at the atomic level (Figure 2.4). First, an incoming energetic X-ray radiation knocks out an electron from one of the inner orbital within an atom of the material. The vacancy created causes instability of the atom. To regain stability, an electron from a higher-energy exterior orbital moves in to fill the vacancy. This transition results in the emission of the characteristic X-rays which are absorbed by the detector. The pulses from the detector are then amplified and digitized according to their magnitude (Oxford Labs, 2009). The equipment available for the purpose of this research was a total reflective X-ray fluorescence (TXRF) spectrometer. In TXRF, the incident beam is reflected by the substrate and only the particles on the surface are excited resulting to characteristic X-ray fluorescence emissions. In this way the background normally associated with XRF measurements is greatly reduced, leading to higher sensitivity and lower detection limits (de Gennaro *et al.*, 1999).

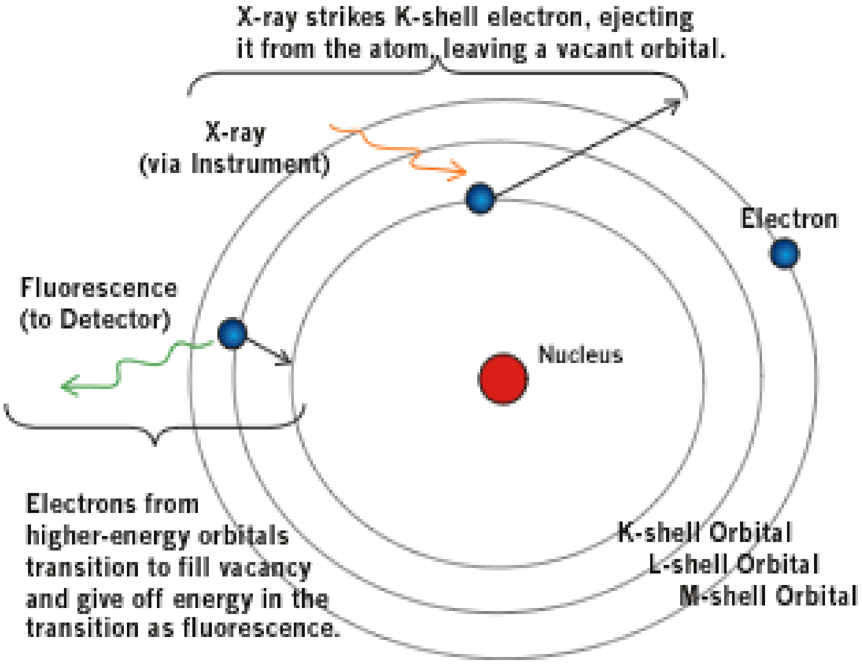

Figure 2.5: Emission of X-ray fluorescence (Eastern Applied Research, 2015)

2.9 Summary of literature review

Water hyacinth is a global menace which literally makes all water related activities impossible. This has prompted the undertaking of various measures to control the weed. Unfortunately, each of the techniques being applied worldwide has major drawbacks to the environment and in the recent past have proven to be short term. Therefore, utilization of the weed i.e. making it into feedstock for bio digestion, is increasingly being opted as a sustainable solution towards eradication of the weed. Even though the set-up of such a venture demands monetary input, the resultant benefits to the environment and socio-economic welfare of the community outweigh its costs.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Introduction

A brief description and illustration of the study area along with the sampling sites will be given. This will be followed by a presentation of the chemicals and reagents used during the study. After this, a step by step account of all the activities carried out from the sampling leading to analysis will be presented.

3.1 Study site

Lake Victoria is the second largest fresh water lake in the world and supports the livelihood of about 35 million people. The lake has existed for over 35,000 years and it provides drinking water, water transport, hydroelectric power, and sustains various industries such as trade, agriculture, fisheries, tourism and wildlife (Gichuki *et al.,* 2012). However, these resources have been adversely affected due to the water hyacinth menace. The most affected region since the hyacinth outbreak in 1998 was the Winam Gulf (LVEMP, 2003). The gulf is a large inlet in the northeast corner of Lake Victoria that extends into the Kenyan region. It has a shoreline measuring about 550 km. To date, 17,231 ha of the plant have grown on its surface. Its climate is generally characterized by both wet and dry seasons. The wet season runs through the long rains in March to May and also during short rains in the month of October to December. The dry season ensues thereafter from January to February and then later from June to September. Average annual rainfall in the basin ranges from 1000 mm to 1800 mm. Over the past two decades the Committee for Inland Fisheries of Africa has noted Winam Gulf to be the most polluted catchment area on Kenya's side of the Lake (Abong'o, 2009).

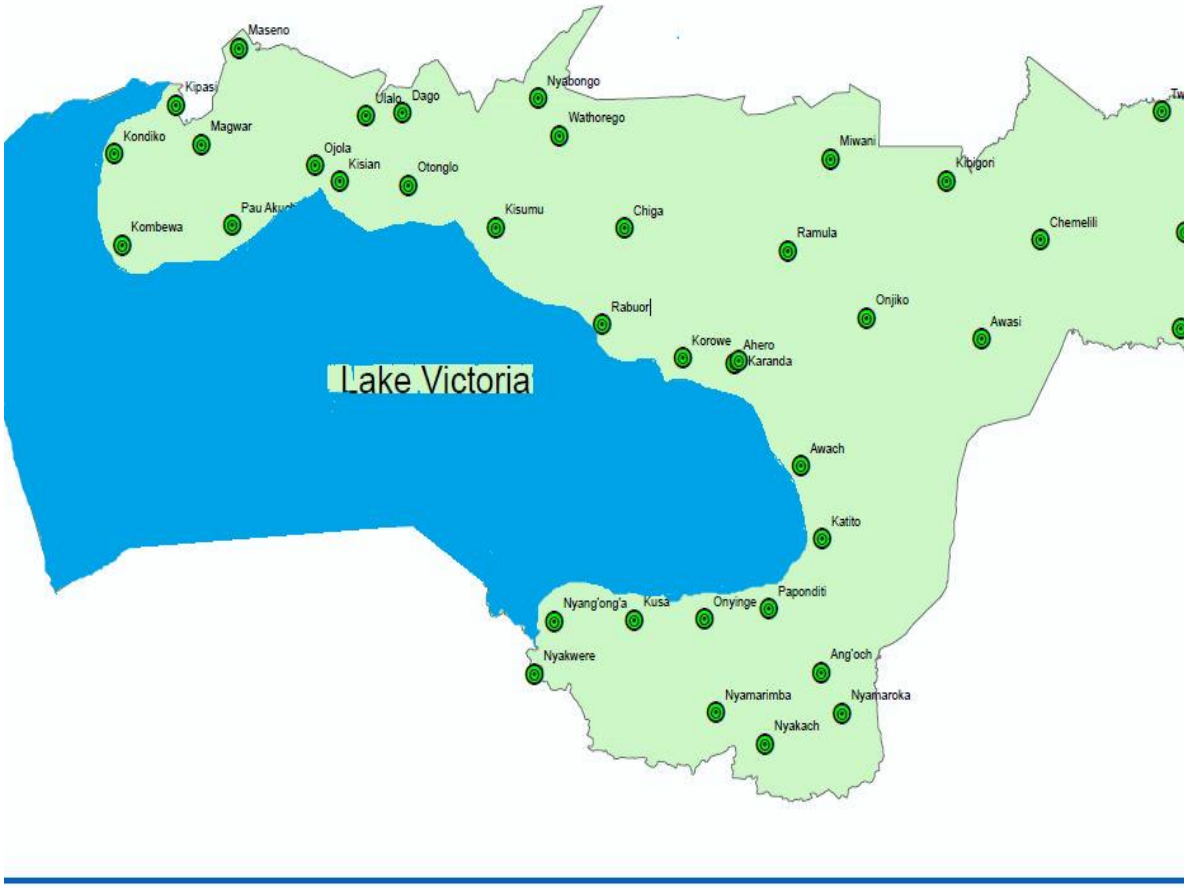

Figure 3.1: Kenyan portion of Lake Victoria and surrounding towns in the region

The highest sources of pollution along the Winam gulf are mainly municipal and industrial discharge. Rain runoff from surrounding commercial large-scale farms that grow tea, coffee, maize and sugarcane is also a contributor (Abong'o, 2009). The pollution is sometimes so high that it is visible and due to its high retention time, the pollutants drained into the Lake Victoria stay in it for a long time. Reports from analysis along the shallow shorelines of the Winam Gulf show that the main composition of pollutants in the water are pesticides and various nutrients including heavy metals (Abong'o, 2009). Ten sampling sites were selected along the Winam Gulf; from Kichinjio Bay to Kiboko Bay.

3.2 Sampling sites

Figure 3.2 shows a map out of the sampling sites along Winam Gulf. Sites were selected based on the activities occurring there.

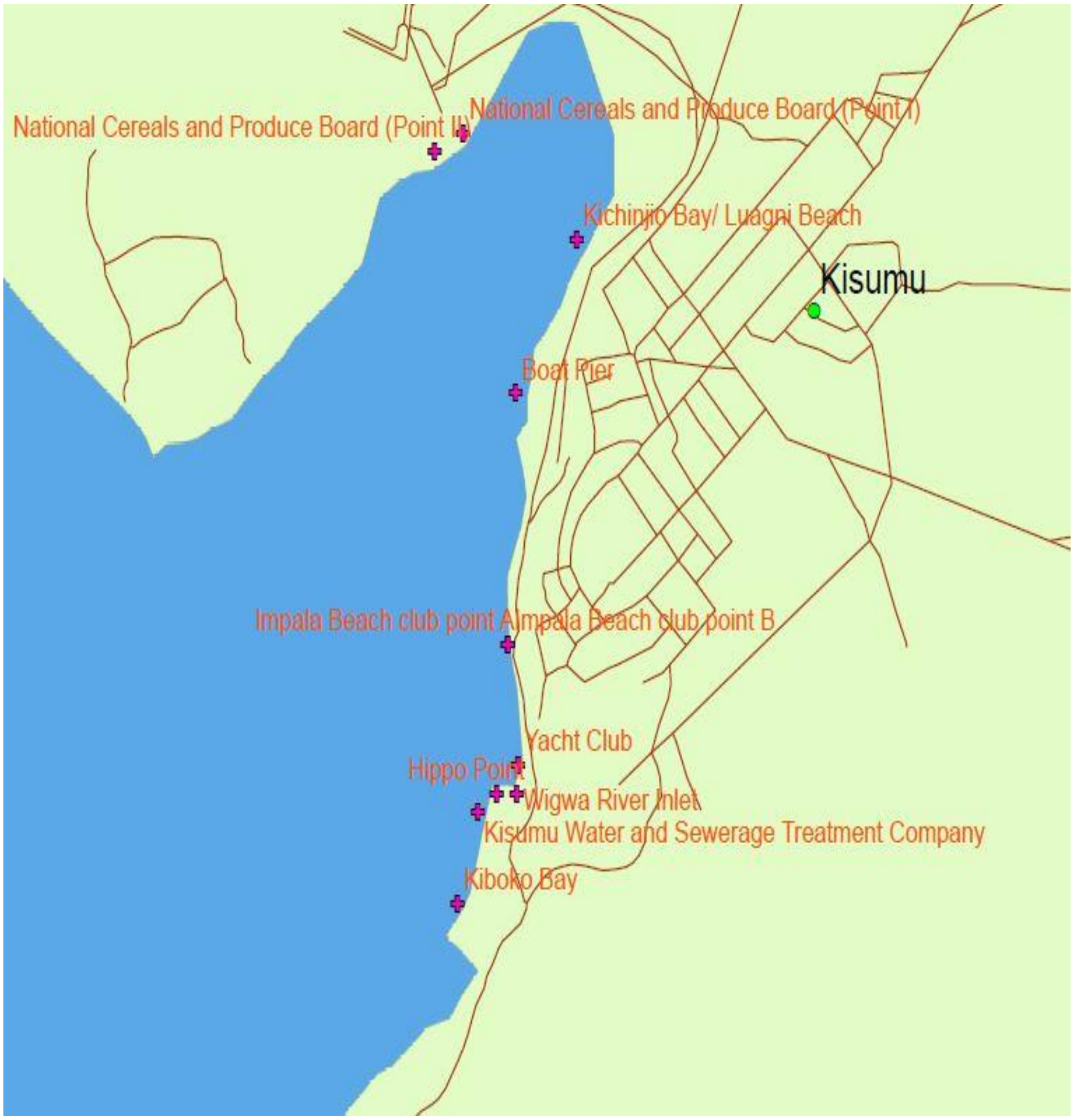

Figure 3.2: Sampling sites along Winam Gulf.

Table 3.1: Description of the sampling sites

Sampling point	**Name**	**Description**
1	Kichinjio Bay	Kichinjio Bay is considered to be the entry point for all local water related activities at the Winam Gulf from Kisumu. These include; fishing boat dock, the local car-wash and laundry area. It is one of the few areas that the local community takes initiative to manually clear when the plant is at its peak.
2	National Cereals and Produce Board	NCPB in Kisumu is the regional Lake/ Western branch of the National Cereals and Produce Board. It is located on the shores of Lake Victoria, in Kisumu town, on Nkrumah Road, off Kisumu Uganda highway.
3	(NCPB)I (NCPB)II	The shoreline by the NCPB covered a large distance and therefore sampling was conducted at two points.
4	Boat Pier	The Boat Pier is the main dock for ships and marine vessels from the countries in the lake region

5	Impala Beach Club	These are high end eateries. They both also offer accommodation for tourists. Some of their activities their activities are boat tours for guests.
6	Yacht Club	
7	Wigwa River Inlet	There are many activities carried out at Wigwa River Inlet area. There is a slum set up on the up side of the bridge, charcoal vending and locally setup eating kiosks.
8	Hippo Point	It is known as so because it is a favorite spot for hippos. Local and international tourists frequent the area to see them. When water hyacinth was at its peak and had carpeted the entire area, the hippos relocated to other points of the lake. This affected the tourism industry along Winam Gulf.
9	Kisumu Water and Sewerage Treatment Company (KWSTCo.)	This is the municipal council's water supply and sewage treatment management authority. The company has been struggling with water distribution into the city because water hyacinth causes blockage to pipes. A fence was built around the inlet area to try and prevent water hyacinth from clogging the pipe

		but this did not work because water hyacinth seeds are dispersed by wind.
10	Kiboko Bay	*Kiboko* is Kiswahili for hippopotamus. It is a spot which was formerly frequented by hippos but they relocated due to human activity.

3.3 Solvents and reagents

Chemicals used were of analytical standard; acetone, EDTA, ethanol and HNO_3. Acetone was used for cleaning the high speed rotor miller and the stainless steel cutters. EDTA and ethanol were used for cleaning TXRF carriers while $HNO_3$was used in the digestion procedure of the samples.

The procedure for cleaning the glassware and crucibles included; soaking in 1:3 nitric acid: water mixture for four hours, washing twice in tap water using locally obtained liquid detergent and rinsing thoroughly in distilled water. The glassware was left to dry under a hood while the crucibles were dried out in the furnace oven at 600 ^{0}C for 4 hours and then left to cool overnight in silica gel dessicators.

TXRF carriers were soaked for four hours in detergent: water mixture (1:3) washed and rinsed in distilled water and then re-soaked again for 4 hours in a freshly prepared detergent: water mixture. The carriers were then rinsed with distilled water, set in a holder and soaked in EDTA (preheated for an hour at 100 ^{0}C) for 4 hours then rinsed thoroughly in distilled water. They were then heated in 1:3 nitric acid/ water mixture for an hour, rinsed twice in distilled water and left to drip off water on a holder in a covered container. They were then lightly dabbed in an outward direction using Velvex ® 3 ply tissues soaked in absolute ethanol before testing for impurities using the S2 PicoFox Bruker ® Bench Top TXRF Spectrometer.

3.4 Sampling plan

Sampling of both water and water hyacinth samples was conducted three times; January, May and in September 2013. This coincided with the growth patterns of the water hyacinth. In January 2013, the water hyacinth had just freshly invaded the gulf (Figure 3.3). In May 2013, the water hyacinth carpet had receded only to the shoreline and the lake was partially accessible. In September 2013, the water hyacinth had cleared therefore only water sampling was conducted.

Figure 3.3: Invasion of water hyacinth in Winam Gulf in January, 2013

3.4.1 Sampling of water hyacinth

Water hyacinth plants were harvested whole (one plant consisting of several stalks) approximately 5 meters from the shore of each sampling site. This was done from aboard a boat that helped navigate the water patches. The plants were stored in individual labelled bin packing bags and transported to INST laboratory for analyses. At the laboratory, the samples were cleaned thoroughly and then rinsed using distilled water. The roots, stems and leaves were separated, weighed and dried in the open under average room temperature of 24.6 ^{0}C for 5 days in a drying room.

This was done because water hyacinth contains a lot of water and it would have required leaving them in an oven for longer period which could have possibly caused degradation. They were then dried for 24 hours in an oven at 80 ^{0}C to stabilize and boost homogenization. Afterwards, they were cooled and reweighed. The roots, stems and leaves were ground using a stainless steel Col-Int Tech Lab Crusher and Grinder FW-100 and mixed to give a composite sample. Three replicate samples each weighing 10 g were packaged in polyethene bags and stored at average room temperature of 23 ^{0}C to maintain the powder form awaiting digestion.

3.4.2 Sampling of water

Water samples were collected in triplicates from the growth point of the corresponding water hyacinth by grab method into 300 ml plastic bottles and stored in a 6 ^{0}C cooler box for transportation. At the laboratory, the water was filtered through Whatman 185 mm diameter filter papers to remove precipitates. A 100 ml aliquot was measured and acidified with 1ml of HNO_3 then preserved in plastic bottles under refrigeration at 4 ^{0}C.

3.4.3 Sampling and preparation of digester feed for anaerobic digestion

Water hyacinth samples were pretreated by removal of the sediments attached to the roots. These were later pulped using an electric driven pulping machine into a fine slurry. Cow dung was obtained from the cowshed at the Department of Bio-mechanical and Agricultural Engineering at Jomo Kenyatta University of Agriculture and Technology. It was collected using 20 liter buckets and homogenized in a trough. The water hyacinth slurry was then introduced and mixed with cow dung at ratios of cow dung to water hyacinth ratios of, 1:1, 1:3 and 1:5. Each of these portions was mixed with an equal portion of water (1:1). The resultant mixture was introduced into the 6 m^3 tubular digester using a hopper (6 inches internal diameter).

3.4.4 Sampling of digestate

One hundred g of triplicate samples were taken from every (1:1, 1:3 and 1:5) pulp mix digestate and mixed thoroughly to make a composite sample. For each, the samples were oven dried at 80 ^{0}C for 24 hours. The samples were then mixed into a composite and ground into a powder. 3 replicates weighing 10 g were packaged in

A6 size khaki envelopes and stored under average room temperature of 23 ^{0}C to prevent caking and maintain powder form to await digestion.

3.4.5 Biogas production and recording of volumes

The production of biogas was done from a 6 m^3 flexi tubular digester (Figure 3.4) with a UV resistant enclosure supplied by Biogas International Kenya ltd. The volume of biogas produced was recorded from the 10th day. Gas volume was measured using a standardized gas meter in 2 to 3 day intervals for 10 to 12 days for the different ratios of cow dung and water hyacinth with constant monitoring and stirring.

Figure 3.4: Tubular digester set up for the study

3.5 Sample preparation and analysis

3.5.1 Reference samples

Bowen kale from IAEA was used as the certified reference sample for the solids. A 2 g portion of powdered sample was weighed in three replicates into crucibles and made to ash at 600 ^{0}C in a Carbolite ® furnace oven for 6 hours and then left to cool in a silica gel dessicator. The ash was digested in 3 ml conc. HNO_3 and made up to 100 ml with distilled water. 20 ml of this was drawn by use of a pipette and spiked with 1 ppm Ga internal standard. An aliquot of 10 µl of this was then drawn using a hand held micropipette and set upon a pre-scanned TXRF glass carrier, dried in a covered heating plate at 30 ^{0}C and packed in a 10 cm diameter plastic Petri dish. Heavy metal analysis was conducted using S2 PicoFox Bruker ® Bench Top TXRF Spectrometer for a run time of 1000 seconds.

Bernd Kraft® Multi-element Standard (10 mg l^{-1}) was used as the certified reference sample for the water samples (See Appendix 2 for Bernd Kraft ® Multi-element Standard (10 mg l^{-1}) internal standards spectra). The matrix blanks were prepared in triplicates and were treated in the same way as actual samples for analysis. The blanks comprised of 20 ml of twice distilled deionized water spiked with 1 ppm Ga in triplicates.

3.5.2 Preparation and analysis of water hyacinth samples.

A 2 g portion of powdered sample was weighed in three replicates into crucibles and made to ash at 600 ^{0}C in a Carbolite ® furnace oven for 6 hours and then left to cool in a silica gel dessicator. The ash was digested in 3 ml conc. HNO_3 and made up to 100 ml with distilled water. A 20 ml aliquot of this was drawn by use of a pipette and spiked with 1 ppm Ga internal standard. An aliquot of 10 µl of this was then drawn using a hand held micropipette and set upon a pre-scanned TXRF glass carrier, dried in a covered heating plate at 30 ^{0}C and packed in a 10 cm diameter plastic Petri dish. Analysis was conducted using S2 PicoFox Bruker ® Bench Top TXRF Spectrometer for a run time of 1000 seconds.

3.5.3 Preparation and analysis of water samples

Twenty ml of undiluted water sample was drawn by use of a pipette and spiked with 1 ppm Ga internal standard. An aliquot of 10 µl of this was then drawn using a hand held micropipette and set upon a pre-scanned TXRF glass carrier, dried in a covered heating plate at 30 ^{0}C and packed in a 10 cm diameter plastic Petri dish. Analysis was conducted using S2 Pico Fox Bruker ® Bench Top TXRF Spectrometer for a run time of 1000 seconds.

3.5.4 Preparation and analysis of digestate samples

A 2 g portion of powdered sample was weighed in triplicates into crucibles and made to ash at 600 ^{0}C in a Carbolite ® furnace oven for 6 hours and then left to cool in a silica gel dessicator. The ash was digested in 3 ml concentrated HNO_3 and made up to 100 ml with purified water. 20 ml of this was drawn by use of a pipette and spiked with 1 ppm Ga internal standard. An aliquot of 10 µl of this was then drawn using a hand held micropipette and set upon a pre-scanned TXRF glass carrier, dried in a covered heating plate at 30 ^{0}C and packed in a 10 cm diameter plastic Petri dish. Analysis was conducted using S2 Pico Fox Bruker ® Bench Top TXRF Spectrometer for a run time of 1000 seconds.

3.6 Statistical Analysis

Statistical analysis was conducted using S2Picofox 6.2.0.0. ® analysis software and Microsoft Excel 2013 ® data analysis software to achieve quantification values.

3.6.1 Quantification of elements

Since the samples were prepared in liquid form, and internal standard method was preferable for quantification of the individual elements (Bruker, 2008).

Elemental concentration was calated as:

$$C_i = \frac{C_{is}.\ N_{is}.\ S_{is}}{N_{is}.\ S_i} \text{............ eqn. 1 (Bruker, 2008).}$$

Where

C_i: element i concentration

C_{is}: internal standard concentration

N_i: element net count rate

N_{is}: internal standard net count rate

S_i: element i sensitivity factor

S_{is}: internal standard sensitivity factor

For the lower limits of detection, it was assumed that an element is considered to be detected if the peak area is three times larger as the counting statistics of the background. This procedure is known as the 3-sigma criterion (Bruker, 2008).

This was achieved by:

$$LLD_i = \frac{3.C_i.\sqrt{N_{BG}}}{N_i} \text{ ……………eqn. 2}$$

Where;

LLD_i: lowest limit of detection of the element i

C_i: concentration of the element i

N_i: area of the fluorescence peak in counts

N_{BG}: background area subjacent the fluorescence peak

3.6.2 Quantification of actual concentrations

Plant and digestate samples analyzed were a fraction of the whole. Therefore, computation of concentration values achieved from 10 µl sample to the total concentration in one kilogram of water hyacinth and one kilogram of digestate was required. It was calculated as:

$$C_{i\,tot} = \frac{(C_{i\,avg} - C_B)\,V_D}{W_{RP}} \text{ …………… eqn. 3}$$

Where;

$C_{i\,tot}$: total concentration of the element i in one kilogram of the plant

$C_{i\,avg}$: average concentration of element i from the triplicates

C_B: concentration of the blank sample

V_D: volume of dilution

W_{RP}: weight of representative sample

Standard error for water hyacinth and digestate samples was calculated as:

$$\bar{\sigma} = \frac{(\sqrt{\sum_{i=0}^{n} \sigma^2}\,)\,V_D}{W_{RP}} \text{…………… eqn.4}$$

Where;

$\bar{\sigma}$: standard error

n: number of replicates

σ^2: square of the system error

V_D: volume of dilution

W_{RP}: weight of representative sample

Water samples did not require any dilution. Calculation was carried out for elemental concentration in one liter.

$$C_{wi} = (C_{i\,avg} - C_B)\,1000 \text{ ....eqn. 5}$$

Where;

C_{wi}: total concentration of the element *i* in a litre of water sample

$C_{i\,avg}$: average concentration of element i from the triplicates

C_B: concentration of the blank sample

Standard error for water samples was calculated as:

$$\bar{\sigma} = (\sqrt{\textstyle\sum_{i=0}^{n} \sigma^2}\ )\,1000 \text{.............. eqn.6}$$

Where;

$\bar{\sigma}$: standard error

n: number of replicates

σ^2: square of the system error

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

The Winam Gulf has been the worst affected by the water hyacinth invasion. Water in this area is also the most polluted in the region with heavy metals being part of the pollution. In this chapter the results of the heavy metal concentrations in the water column and the water hyacinth are reported and discussed. This is followed by the discussion of the results obtained from anaerobic digestion.

4.1 Quality assurance

A paired two tailed t- test was used to determine the level of significance for the reference material for the water hyacinth samples at $P < 0.05$. It was assumed that H_0; the certified reference sample and the experimental reference sample are the same (Table 4.1). It was found that for $t = 4$, $p = 0.97$ and $p > 0.05$ we do not reject the null hypothesis. Therefore the analytical method was considered as adequate for the study Limits of detection for heavy metals in water hyacinth samples were calculated as shown in Table 4.2.

Table 4.1: Comparison of the reference and experimental values for Bowen kale

Metal	**Experimental value (mg kg^{-1})**	**Reference value (mg kg^{-1})**	**Percentage difference**
Mn	13.7 ± 2.0	14.8 ± 1.7	11.76%
Fe	123 ± 20	119.3 ± 14.8	-3.25%
Ni	10.4 ± 0.2	10.0	4%
Cu	3.9 ± 1.0	4.9 ± 0.6	20.13%
Zn	26.3 ± 3.4	32.3 ± 2.75	18.62%

Table 4.2: Limits of detection for water hyacinth samples and digestate samples

Metal	Limit of detection (mg kg^{-1})
Mn	0.120
Fe	0.095
Ni	0.030
Cu	0.065
Zn	0.055

Significance for the analysis water samples was tested at 95 % confidence intervals. The reference concentration for Bernd Kraft® Multi-element Standard was 10 mg l^{-1} (Table 4.3).

Average concentration for the experimental value was calculated to be 9.8 mg l^{-1}. Confidence interval for the test at 95 % was calculated to be 9.3, 10.3. We therefore do not reject the null hypothesis because the confidence intervals showed that 9.8 mg l^{-1}is a satisfactory value for the water sample analysis. Limits of detection for water samples in reference to the Bernd Kraft® Multi-element Standard were computed and tabulated in Table 4.4.

Table 4.3: Comparison of the reference and experimental values for Bernd Kraft ® multi-element standard

Metal	Experimental value (mg l^{-1})	Reference value (mg l^{-1})	Percentage difference
Ca	10.7 ± 0.2	10	7%
Ti	9.1 ± 0.4	10	-9%
V	9.5 ± 0.2	10	-5%
Cr	8.8 ± 0.2	10	-12%
Mn	10.8 ± 0.2	10	8%
Fe	9.0 ± 0.2	10	-10%
Co	10.4 ± 0.2	10	4%
Ni	10.4 ± 0.2	10	4%
Cu	10.0 ± 0.2	10	0%
Zn	10.3 ± 0.1	10	3%
As	8.3 ± 0.1	10	-17%
Se	9.1 ± 0.2	10	-9%

Table 4.4: Limits of detection for water samples

Metal	Limits of detection (mg l^{-1})
Ca	0.160
Ti	0.095
V	0.080
Cr	0.065
Mn	0.055
Fe	0.045
Co	0.040
Ni	0.030
Cu	0.025
Zn	0.020
As	0.015
Se	0.015

4.2 Concentrations of heavy metals in water and water hyacinth samples

4.2.1. Concentration of manganese in water hyacinth samples

It was observed that Mn is largely accumulated in the roots during January and May sampling periods (Table 4.5). It was also observed that in May the concentration of Mn was much higher than in January, especially in the roots. There is a general clustering of sampling points based on this observation; sampling points 6 - 10 showed very high amounts in comparison to the other sampling points for both sampling periods and sampling points 2 - 5 which showed considerably low concentrations in comparison to the other sites also for both periods. The concentration of Mn in the roots at sampling points 6 - 10 ranged from 2194 to 3361mg kg^{-1}in January and 2257 to 18267 mg kg^{-1}in May. The concentration of in the roots at sampling points 2 - 5 ranged from 484 to 971 mg kg^{-1}in January and 890 to 1248 mg kg^{-1}in May. However, in May point 4 had a higher amount than its cluster counterparts. The concentrations at points 2 - 5 were approximately half the quantity than in the points 6 - 10.

The reason for this huge amount of Mn at these sampling points (6 – 10) is possibly due to human activities such as informal settlement, business activities etc. and the influence of rainfall. The same argument can be applied for points 2 - 5. In this case, pollution levels are lesser because of fewer human activities in comparison to the other sites.

In January, the concentrations are generally low because it is a dry season with little occurrence of rainfall. The consequent increase in May could be attributed to the heavy rainfall season that occurs during this period. Since Mn is abundantly found in soil and biological material (Van der Ent *et al.*, 2012), runoff coming in from the surrounding farms as well as from the municipal could cause a spike in the amount off Mn in the lake water at Winam Gulf.

Table 4.5: Concentration of Mn (mg kg^{-1}) in water hyacinth samples in January and May, 2013

January, 2013 sampling				**May, 2013 sampling**		
Sampling point	Roots	Stem	Leaves	Roots	Stem	Leaves
1	1822 ± 80	687 ± 54	1224 ± 103	7302 ± 186	722 ± 30	218 ± 14
2	620 ± 15	1094 ± 25	707 ± 12	1248 ± 44	4438 ± 91	591 ± 21
3	484 ± 11	852 ± 25	936 ± 30	947 ± 47	322 ± 16	819 ± 37
4	721 ± 19	683 ± 31	1004 ± 43	7544 ± 286	650 ± 29	501 ± 19
5	971 ± 33	595 ± 29	408 ± 36	890 ± 25	864 ± 31	518 ± 21
6	2406 ± 75	655 ± 18	439 ± 8.4	4008 ± 179	745 ± 32	308 ± 14
7	3361 ± 80	1197 ± 44	1961 ± 82	18267 ± 438	3135 ± 133	389 ± 14
8	2933 ± 59	2136 ± 58	1750 ± 38	2257 ± 47	460 ± 5	145 ± 12
9	2194 ± 37	1359 ± 31	1835 ± 44	4438 ± 126	693 ± 30	629 ± 26
10	2579 ± 128	929 ± 34	1039 ± 66	16691 ± 494	1138 ± 39	536 ± 26
Range	Roots: 484 - 3361 Stem: 595 - 2136 Leaves: 408 - 1835			Roots: 890 - 18267 Stem: 322 - 4438 Leaves: 145 - 819		

The concentration of Mn in the roots had the highest ranges in comparison to the aerial parts. Overall Mn concentrations ranges in the roots (maximum value) was approximately twice as much as in the leaves and the stems (maximum value) in January. In May, the overall maximum concentration of Mn in roots was over 22 times more than the overall maximum concentration in the leaves and four times as much as the maximum concentration observed in the stem. Generally, the stems had higher concentrations ranges than the leaves. However, looking at individual points this applies only during May sampling period. In January, points 1, 4, 7, 9 and 10 had significantly higher concentrations in the leaves in comparison to the stem. The stems having higher concentrations than the leaves could be because the stem is a conduit for the transportation of minerals.

This fluctuation in the amounts Mn in the plant parts is attributed to translocation. This is the movement of metal-containing sap from the root to the shoot. It is primarily controlled by two processes: root pressure and leaf transpiration and measured in terms of a translocation coefficient. The Translocation Coefficient (TC) is computed as a ratio of a metal's concentration in the shoot (in this study, the aerial parts) to its concentration in roots. (eqn. 4).

$$TC_{i=}\frac{C_{mi}}{C_{mr}} \text{………………… eqn. 7}$$

where; TC_i is the translocation coefficient of the aerial plant tissue, C_{mi} is the metal concentration in the selected aerial plant tissue and C_{mr} is the metal concentration in the root.

In January, there was more translocation of Mn to the leaves than to the stem (Fig. 4.1). There were 6 out of the 10 sites showing this trend; 1, 3, 4, 7, 9 and 10. Average TC in the leaves at these points in January was 1.16 in comparison an average of 0.73 TC in the stems at these during the same period. In May, the trend changed in all sampling points, with the exception of sampling point 3, having more translocation of Mn to the stems than to the leaves. Average TC to the stem for all sampling points was 0.6 and that of the leaves for all sampling points was 0.2.

In January, it is observed that as one moved further along the sampling points that the translocation of Mn to the leaves and stems decreased. Only four points (2, 3, 4 & 7) had TC > 1 for the leaves and two points (2 and 3) had TC > 1 for the stems. Although the general translocation to the aerial plant parts is quite low in May, it was during this period that there was the highest TC i.e. TC for stem at sampling point 2 was calculated to be 3.56. Deng *et al.*, (2004) suggested that for a plant to have effective translocation abilities, it must be of TC > 1. Therefore, larger ratios imply good translocation capability like for points 2 in January and 3, 4, and 7 in May. Having good TC improves the potential of a plant to be used for phytoremediation. After a laboratory study on the growth of water hyacinth in various metal concentrations, Soltan and Rashed (2001), observed that TC is affected by the weakness and wilting of plant tissues that are responsible for transportation of chemical components from the roots to aerial parts of the plant this could be a cause for low TC values observed.

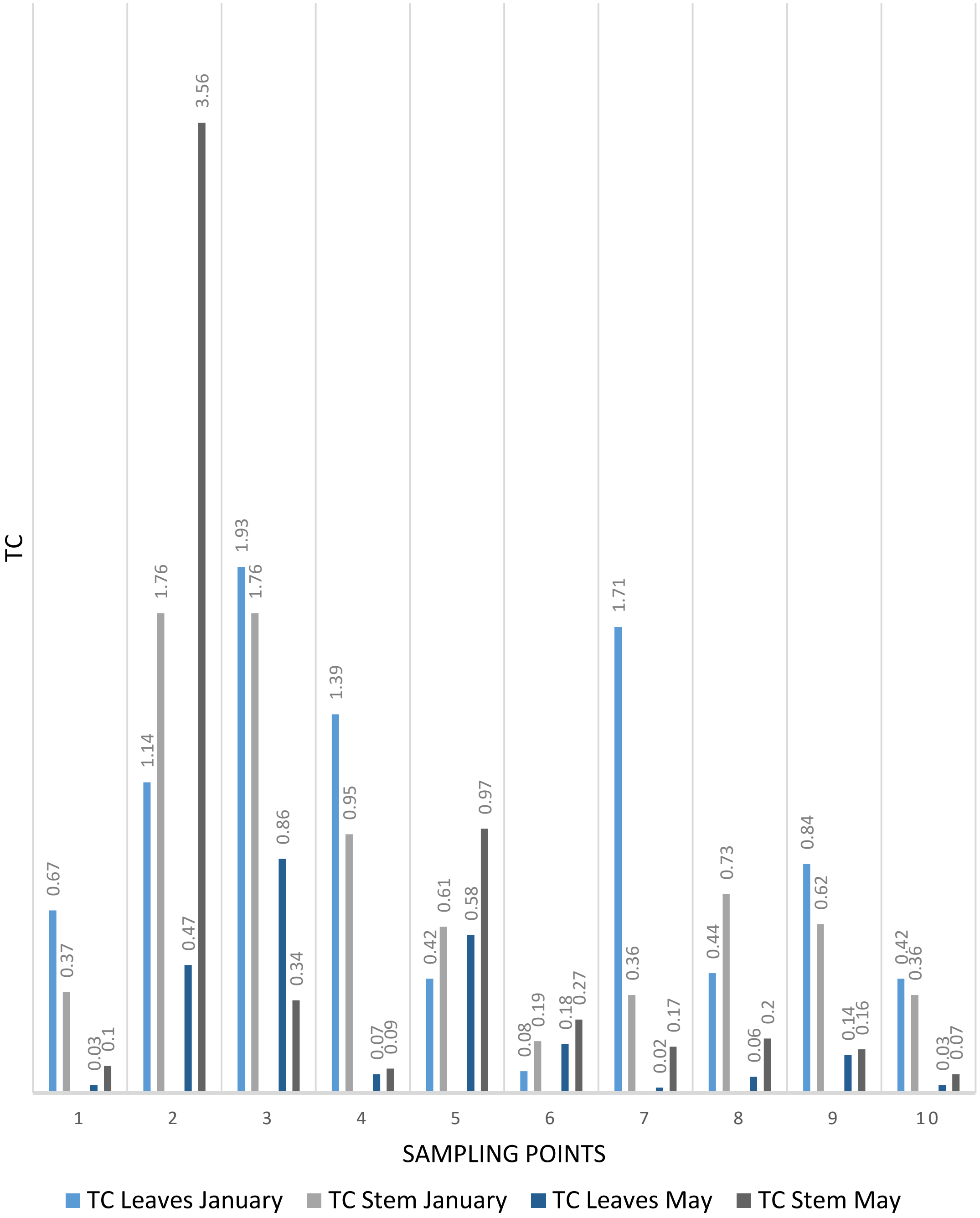

Figure 4.1: TC for Mn in water hyacinth samples from Winam Gulf

4.2.2. Concentration of iron in water hyacinth samples

In this study, Fe was observed to be the highest occurring metal in water hyacinth along Winam Gulf. Iron was highly accumulated in the roots of the plants in comparison to other plant parts. This pattern is observed for all points during both sampling periods (Table 4.6). In January iron concentrations in the plant parts ranged as follows; 1857 to 5103 mg kg^{-1}, 316 to 2761 mg $kg^{-1}$and 282 to 3715 mg kg^{-1}in the roots, stems and leaves respectively. In May, the concentrations ranged between 2464 to 27812mg kg^{-1}in the roots, 667 to 4322mg kg^{-1}in the stem and 506 to 2925 mg kg^{-1}in the leaves. However, in May, at sampling point 5 the concentration of Fe in the stem (4322 ± 111mg kg^{-1}) is higher than in the roots (2464 ± 62 mg kg^{-1}) and the leaves (1450 ± 43 mg kg^{-1}). These high amounts recorded for Fe in all the sampling points could be an indication of its high abundance in the Lake water.

Ratan and Verma (2014), also found that Fe was the highest and easily accumulated heavy metal by water hyacinth in a river polluted by industrial and municipal waste. Comparing with results from their average Fe concentrations from the plants were found to be 311.17, 4.89 and

2.49 mg kg^{-1} in the roots, stem and leaves. Although their values are much lower than those observed in this study, the trend is similar to the observation at Winam Gulf as the sequence of accumulation for Fe was roots > stems > leaves.

It was also observed that sampling points 5 and 6 had low concentrations of Fe in the roots when compared to the other points especially during May sampling period when significantly high concentrations of Fe were recorded for all the points in all plant parts. Iron concentration in the roots at sampling point 5 was 2464 ± 62mg kg^{-1} in May and 5501 ± 232 mg kg^{-1} at sampling point 6. This in comparison to a range of 11265 – 27812 mg kg^{-1} for the other points during the same period can be considered to be significantly low is significantly low. It can be considered that there is minimal pollution at these two points.

The evident sharp difference between January and May periods could be as a result of the heavy rains. January is a dry season while May is during the heavy rains season.

Table 4.6: Concentration of Fe (mg kg $^{-1}$) in water hyacinth samples in January and May, 2013.

	January, 2013 sampling			**May, 2013 sampling**		
Sampling point	Roots	Stem	Leaves	Roots	Stem	Leaves
1	2894 ± 118	471 ± 33	1307 ± 101	20581 ± 454	1536 ± 53	506 ± 23
2	2347 ± 45	316 ± 9.0	1063 ± 17	13235 ± 303	2533 ± 86	1053 ± 32
3	1857 ± 35	950 ± 26	904 ± 27	11265 ± 344	2093 ± 62	2925 ± 102
4	2413 ± 53	549 ± 26	759 ± 33	14979 ± 518	1064 ± 42	678 ± 24
5	2581 ± 78	576 ± 30	282 ± 28	2464 ± 62	4322 ± 111	1450 ± 43
6	3553 ± 105	419 ± 12	351 ± 7.1	5501 ± 232	1746 ± 58	768 ± 25
7	3758 ± 86	1009 ± 34	3715 ± 137	21390 ± 501	1855 ± 84	822 ± 23
8	5103 ± 83	2761 ± 63	2843 ± 71	13692 ± 216	667 ± 20	936 ± 39
9	3256 ± 53	934 ± 22	940 ± 24	15474 ± 343	1153 ± 36	547 ± 27
10	3129 ± 143	919 ± 35	1395 ± 78	27812 ± 787	2746 ± 112	536 ± 75
Range	Roots: 1857 - 5103 Stem: 316 - 2761 Leaves: 282 – 3715			Roots: 2464 - 27812 Stem: 667 - 4322 Leaves: 506 - 2925		

In addition to anthropogenic activities that cause pollution to the lake, runoff draining in the lake from farms may possibly have increased the amounts of Fe in the water. This could be related to the presence of microorganisms found in soil that produce chemicals which increase the availability and uptake of iron (Hamdollah, 2011). This is similar to the trend observed for Mn. Having these two metals in high concentrations during May sampling period could be expected as Fe like Mn is abundantly found in surface soils and is easily reducible (Kabata - Pendias and Pendias, 1984).

Iron is largely retained in the roots because as shown from Figure 4.2 the TC ratios are quite low with the average TC for stems and leaves in January being 0.30 and 0.42 respectively. In May, average TC for the stems and the leaves was 0.29 and 0.18 respectively. These results show that in January, Fe was more translocated to the leaves than to the stems but was more translocated to the stem than to the leaves in May. From Table 4.6 and Figure 4.2, it is observed that other factors and not the concentration of the metal in the roots affects the movement of Fe in the plants. According to Lu *et al.* (2004), one of these factors could be physiological barriers such as the strength of the plant tissue. An example to demonstrate this observation is sampling point 8 which had the highest concentration in the roots at 5103 ± 83 mg kg^{-1} in January and had relatively low amounts in the stems (2761 ± 63mg kg^{-1}) and the leaves (2843 ± 71mg kg^{-1}) during the same sampling period. TC values were 0.54 and 0.56 in stems and leaves respectively. Sampling point 8 had comparatively close TC values with sampling point 3 which had the lowest concentration of Fe in the roots in January at 1857 ± 35 mg kg^{-1} while the concentration of Fe in the stem and the leaves at this point was 950 ± 26 and 904 ± 27 mg kg^{-1}. Resultant TC values at this point were 0.51 and 0.49 in the stems and the leaves respectively.

In May, the trend was similar; sampling point 10 had the highest concentration of Fe in the roots (27812 ± 787 mg kg^{-1}) with 2746 ± 112 mg kg^{-1} and 536 ± 75 mg kg^{-1} in the stem and the leaves respectively. Corresponding TC values were 0.1 and 0.01. Also in during May sampling period, sampling point 5 had the lowest concentration of Fe in the roots, 2464 ± 62 mg kg^{-1}, while the stems and the leaves had 4322 ± 111 mg kg^{-1}, 1450 ± 43 mg kg^{-1} of Fe respectively. Corresponding TC values at this point were 1.68 and 0.56 for the stem and the leaves. Similar observations were made by Win *et al.*, (2002) where absorption of Fe occurred predominantly in the roots of water hyacinth plants growing in 0.001 M and 0.01 M solutions of Fe. In their study, there was preferential transportation of Fe to leaves than to the stems.

In a study by Jayaweera (2007), water hyacinth was exposed to Fe concentration of 9.27 mg kg^{-1} for 15 weeks. Results showed that the roots accumulated the highest amount in comparison to the other parts of the plant, with concentrations of up to 6707 mg kg^{-1}. The report concluded that the high uptake of Fe in plants is due to the low nutrient content in the water over a duration of time. This study brings in a factor of time. For the water hyacinth plants growing along Winam Gulf to have accumulated such large amounts of Fe, there is indication to have been a consistent presence of high Fe for a long period of time. This could be possibly as a result of pollution caused by human activities in the area.

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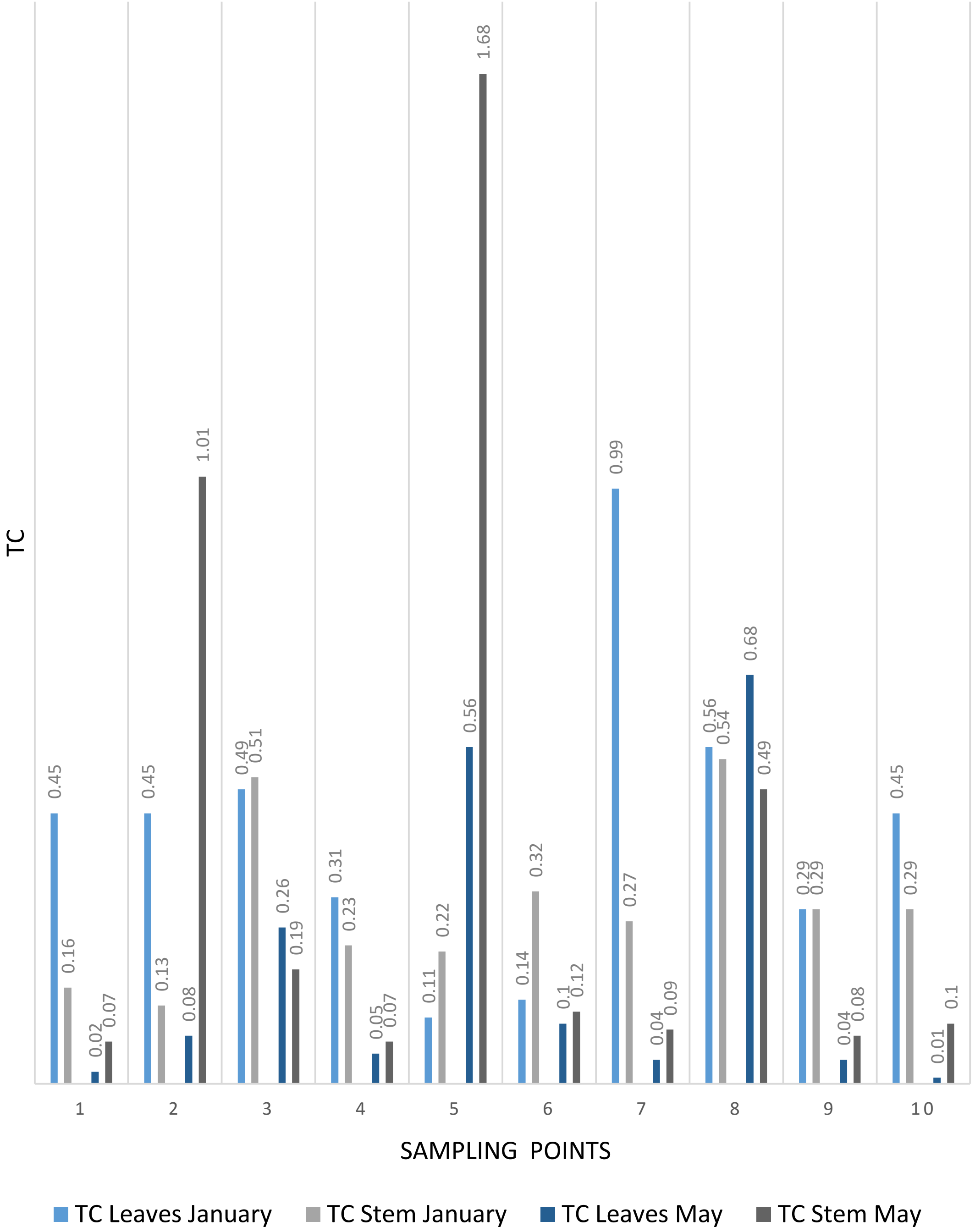

Figure 4.2: TC for Fe in water hyacinth samples from Winam Gulf

4.2.3 Concentration of nickel in water hyacinth samples

There was poor abundance of Ni in the water hyacinth plants from Winam Gulf. The highest amount recorded for Ni in the plant samples from both sampling periods was 7.5 ± 0.6 mg kg^{-1} at point 7 in May. Nickel concentrations at sampling points 1, 2 and 3 were below detection limits and therefore only results from points 4 - 10 were presented in this study as shown in Table 4.7. It is also observed that the high concentrations of Ni are retained in the roots during both sampling periods. From Table 4.7, the range of concentration for Ni in the roots during January was between < 0.03 to 5.5 mg kg^{-1}, while the stem and the leaves ranges between < 0.03 to 2.1 and < 0.03 to 3.4 mg kg^{-1} respectively. In May, the roots ranged between < 0.03 to 7.5 mg kg^{-1}, while the stems and the leaves ranged between < 0.03 to 5.4 and < 0.03 to 4.5 mg kg^{-1} respectively. This indicated that there was also low presence of the metal in the water for the plants to absorb. When comparing absorption of Ni by water hyacinth in the Nile Hammad *et al.* (2011), obtained similar results where Ni recorded the lowest concentrations in comparison to other heavy metals having a range of 0.5 to 10.85 mg kg^{-1}other metals ranged between 2.5 – 660 mg kg^{-1}. The range for Ni in their study was quite high compared to that in this study.

Results showed that water hyacinth plants from some sampling points were able to absorb Ni during one sampling period but not in the subsequent sampling period possibly due to the fluctuating abundance of Ni in the water. Points 4, 8, and 9 showed measurable Ni amounts in their plant parts in January but in May, Ni levels were below detection limits (< 0.03 mg kg^{-1}). Points 5 and 6 showed Ni levels in May but Ni concentrations in January at these points for all plant parts was below detection limits. Sampling points 7 and 10 had quantifiable amounts of Ni during both sampling periods. They were also the points with the highest concentrations of Ni in their plant parts for both sampling periods. This goes on to indicate the substantially low abundance the Ni in the water.

Sampling point 7 showed the highest concentrations in the roots in comparison to the other sites at 5.5 ± 0.6 and 7.5 ± 0.6 mg kg^{-1} in January and May respectively indicating that pollution in the area was quite high. Pollution could have been caused by the numerous human activities around the site. It was observed that Ni was the least accumulated metal by water hyacinth plants at all the sites in comparison to the other metals during both sampling times. It further goes to show the low abundance of Ni in water and consequently its low accumulation rates in water hyacinth plants

Table 4.7: Concentration of Ni (mg kg^{-1}) in water hyacinth samples in January and May, 2013

	January, 2013 sampling			**May, 2013 sampling**		
Sampling point	Roots	Stem	Leaves	Roots	Stem	Leaves
4	0.5 ± 0.2	1.1 ± 0.2	1.9 ± 0.3	< 0.030	< 0.030	< 0.030
5	< 0.030	< 0.030	< 0.030	3.4 ± 0.4	5.4 ± 0.5	4.5 ± 0.4
6	< 0.030	< 0.030	< 0.030	1.3±0.5	2.7±0.6	0.5 ± 0.3
7	5.5 ± 0.6	1.3 ± 0.4	2.3 ± 0.6	7.5 ± 0.6	3.0±0.4	3.0 ± 0.6
8	4.3 ± 0.3	2.1 ± 0.4	3.4 ± 0.5	< 0.030	< 0.030	< 0.030
9	1.8 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	4.2 ± 0.3	2.1 ± 0.3	< 0.030
10	2.5 ± 0.5	0.7 ± 0.2	2.2 ± 0.9	7.3 ± 0.8	4.9 ± 0.3	3.8 ± 0.6
Range	Roots: < 0.030 - 5.5 Stem: < 0.030 - 2.1 Leaves: < 0.030 - 3.4			Roots: < 0.030 - 7.5 Stem: < 0.030 - 5.4 Leaves: < 0.030 - 4.5		

As shown in Figure 4.3, Ni was highly translocated to the aerial parts of the plant. In January average TC to the stems for the points shown in Table 4.7 was 0.8 (sampling points 4, 7, 8, 9 and 10). Average TC to the leaves in January for the same sampling points was calculated to be 1.3. This indicated very good transportation of the metal to the leaves than to the stem in January. In fact, sampling point 4 in January showed the highest TC value (3.8) in comparison to all the other metals for both sampling periods. In May the overall average TC for stems was found to be 1.2 (sampling points 5, 6, 7, and 10). The overall average TC for the leaves in May was computed to be 0.6 (sampling points 5, 6, 7, 9 and 10). From this it was concluded that Ni was more translocated to the stems than to the leaves in May. Although Ni is generally well translocated, from Figure 4.3 it is clear to see that as one moves along the sites, the movement of Ni to aerial plant parts is decreasing.

For plants to have a TC > 1, the aerial plant parts must have a higher concentration than the roots this is the case for point 4 in January and sampling points 5 and 6 in May. However, this is not the case in research conducted by Mahamadi *et al.* (2011). Their study showed that Ni concentrations were up to 17 times higher in roots than in aerial parts in comparison to Zn concentrations in the roots which were up to 6 times higher than that in the other plant parts. Similar trends were also observed by Shao and Chang (2004) who found that water hyacinth roots accumulated Ni up to 15 times more than did the aerial parts. From these studies, water hyacinth showed to be a poor transporter of Ni, whereas in the present study water hyacinth growing along Winam Gulf was shown to be a good transporter of Ni. Yahaya (2011) explained that high accumulation of Ni in the aerial parts could be because the metal is mobile in plants, and accumulates readily in the leaves and seeds therefore important to plant growth. This may possibly be the case in this study.

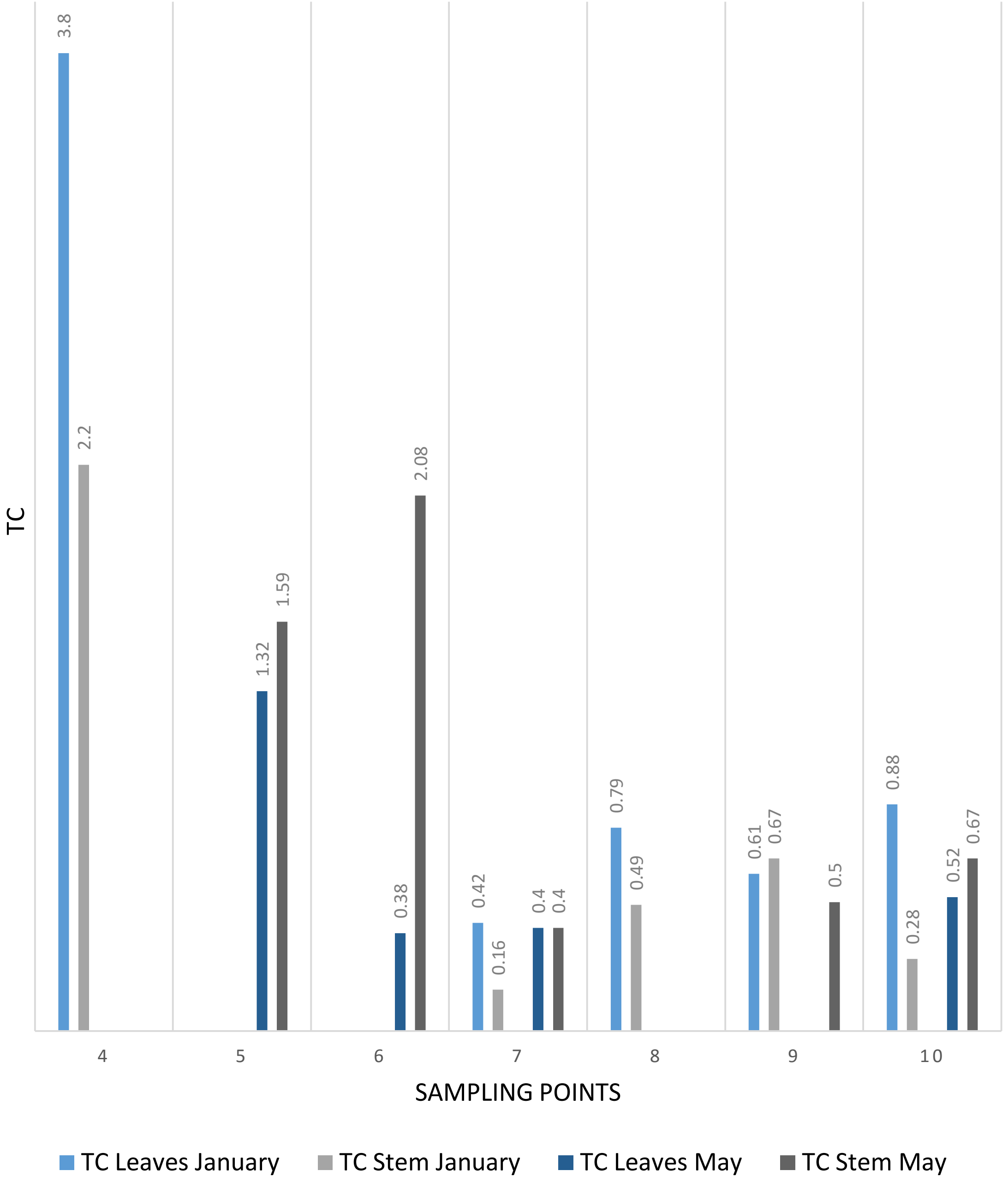

Figure 4.3: TC for Ni in in water hyacinth samples from Winam Gulf

4.2.4 Concentration of copper in water hyacinth samples

As shown in Table 4.8, concentration ranges were: < 0.065 to 9.1 mg kg^{-1}in the roots, < 0.065 to 5.5 mg kg^{-1} in the stem and < 0.065 to 6.4mg kg^{-1}in the leaves during January sampling period. During May, the Cu concentrations ranged between < 0.065 to 31 mg kg^{-1}, < 0.065 to 52 mg kg^{-1} and < 0.065 to 12 mg kg^{-1} for roots stem and leaves respectively. Sampling point 6 was not presented in Table 4.8 because copper concentrations in water hyacinth plants from this point were below detection limits in all plant parts for both sampling periods.

Observations show that Cu concentrations at some points were quantifiable during one sampling period but were below detection limits during the subsequent sampling period. This was observed for sampling points 1 and 4 in January and May. On the other hand, sampling points 2 and 5 had measureable concentrations in May but the concentrations in January were below detection limits. Looking at Table 4.8, sampling points 7 - 10 form a group where during both sampling periods, quantifiable amounts of Cu were detected in all parts. Fluctuating amounts in water hyacinth plants could reflect fluctuating abundance of Cu in the water column.

Copper concentrations follow the same trend as other heavy metals found in the water hyacinth; a large retention of the metal in the roots and there is significant increase of the amount of Cu in the plants in May as compared to January. It is expected that the concentration of a metal is largely retained in the roots as this is the point of contact with the matrix. This was also the case for Vesk *et al*. (1999 where in their study, they found that Cu was retained in the roots of water hyacinth. Their investigations went further to show that Cu was not accumulated at the root surface of the plants but rather centrically across the root. High concentrations of Cu in the root system could also be caused by co-precipitation of heavy metals in plates of Fe and Mn that form on the roots (Vesk and Allaway, 1997). As for the increase in May, it may be attributed to the heavy rains which occur during this period that drain runoff from the farms and the municipal hence bringing in biologically available forms of Cu into the Lake.

Table 4.8: Concentration of Cu (mg kg^{-1}) in water hyacinth samples in January and May, 2013.

January, 2013 sampling				**May, 2013 sampling**		
Sampling point	Roots	Stem	Leaves	Roots	Stem	Leaves
1	< 0.065	< 0.065	< 0.065	31 ± 2.8	33 ± 3.7	6.6 ± 0.7
2	5.9 ± 0.4	2.9 ± 0.3	3.6 ± 0.3	< 0.065	< 0.065	< 0.065
3	34 ± 4.1	3.4 ± 0.4	6.1 ± 0.5	28 ± 3.8	52 ± 5.5	18 ± 2.8
4	< 0.065	< 0.065	< 0.065	34 ± 0.8	36 ± 0.8	6.7 ± 0.3
5	5.3 ± 0.6	< 0.065	6.4 ± 1.9	< 0.065	< 0.065	< 0.065
7	4.7 ± 0.9	4.4 ± 1.1	5.0 ± 1.1	14 ± 2.8	5.6 ± 1.2	7.0 ± 1.0
8	9.1 ± 0.3	5.5 ± 0.4	5.8 ± 0.5	7.9 ± 0.3	6.9 ± 0.3	9.9 ± 0.5
9	4.7 ± 0.3	3.2 ± 0.4	3.7 ± 0.4	14 ± 0.4	26 ± 0.5	12 ± 0.5
10	5.8 ± 0.9	3.5 ± 0.6	3.2 ± 0.6	25 ± 4.0	25 ± 3.3	8.7 ± 1.0
Range	Roots: < 0.065 - 34 Stem: < 0.065 - 5.5 Leaves: < 0.065 - 6.4			Roots: < 0.065 - 31 Stem: < 0.065 - 52 Leaves < 0.065 – 12		

There are several sampling points where the aerial parts have a higher concentration of Cu than the roots. In January, the leaves at point 5 and 7 had 6.4 ± 1.9 and 5.0 ± 1.1mg kg^{-1} respectively while the roots had 5.3 ± 0.6 and 4.7 ± 0.9 mg kg^{-1} respectively. This translated to TC values of 1.2 and 1.06 for sampling points 5 and 7 as shown in Figure 4.4. This showed that there was good translocation of Cu to the leaves than to the stem at these points. In May, the translocation trend changed too with more points having high concentration of Cu in the stems than in the roots and the leaves i.e. sampling points 1, 3, 9 and 10. The TC ratios to the stems at these point were all > 1 (1.07, 1.86, 1.32 and 1 respectively). Sampling point 3 showed the highest concentration of Cu in the water hyacinth plants. From Figure 4.4 it is observed that this point had extremes in translocation of Cu during both sampling periods. In January, the roots from this point had 34 ± 4.1 mg kg^{-1}while the stems and the leaves had concentrations of 3.4 ± 0.4 and 6.1 ± 0.5mg kg^{-1} respectively during the same period. This calculated to the lowest TC ratios of 0.1 and 0.18 respectively. On the other hand, in May point 3 had the highest concentration recorded in the stem at 52 ± 5.5 mg kg^{-1} while the roots and the leaves had 28 ± 3.818 ± 2.8 mg kg^{-1} respectively. TC values translated to 0.64 for the leaves and 1.68 for the stems (the highest TC value for Cu).

Research by Ratan and Verma (2014) show similar trends for Cu accumulation and translocation in water hyacinth with results showing an average TC≅1. This observation was also supported by Gomati *et al.* (2014), whose average TC for Cu in water hyacinth plants in various controlled environments was ≅1. From these studies, it can be concluded that water hyacinth transports Cu to the aerial parts well.

Copper is an essential micronutrient for plants, but it can be toxic at high concentrations (Kabata-Pendias and Pendias, 1984). The metal contributes to several physiological processes in plants including photosynthesis and respiration (Kabata- Pendias and Pendias, 1984). Even though Cu concentrations are generally higher in all plant parts than in Ni, its abundance is not as high as Mn and Fe. In the study by Hammad, (2011), similar observations were made in that Cu was medially accumulated as much as Zn and Ni with order of absorption being Zn > Cu > Ni. It therefore would be better suited to cluster Cu with Ni than with Fe and Mn as a low occurring metal in Winam Gulf for this study.

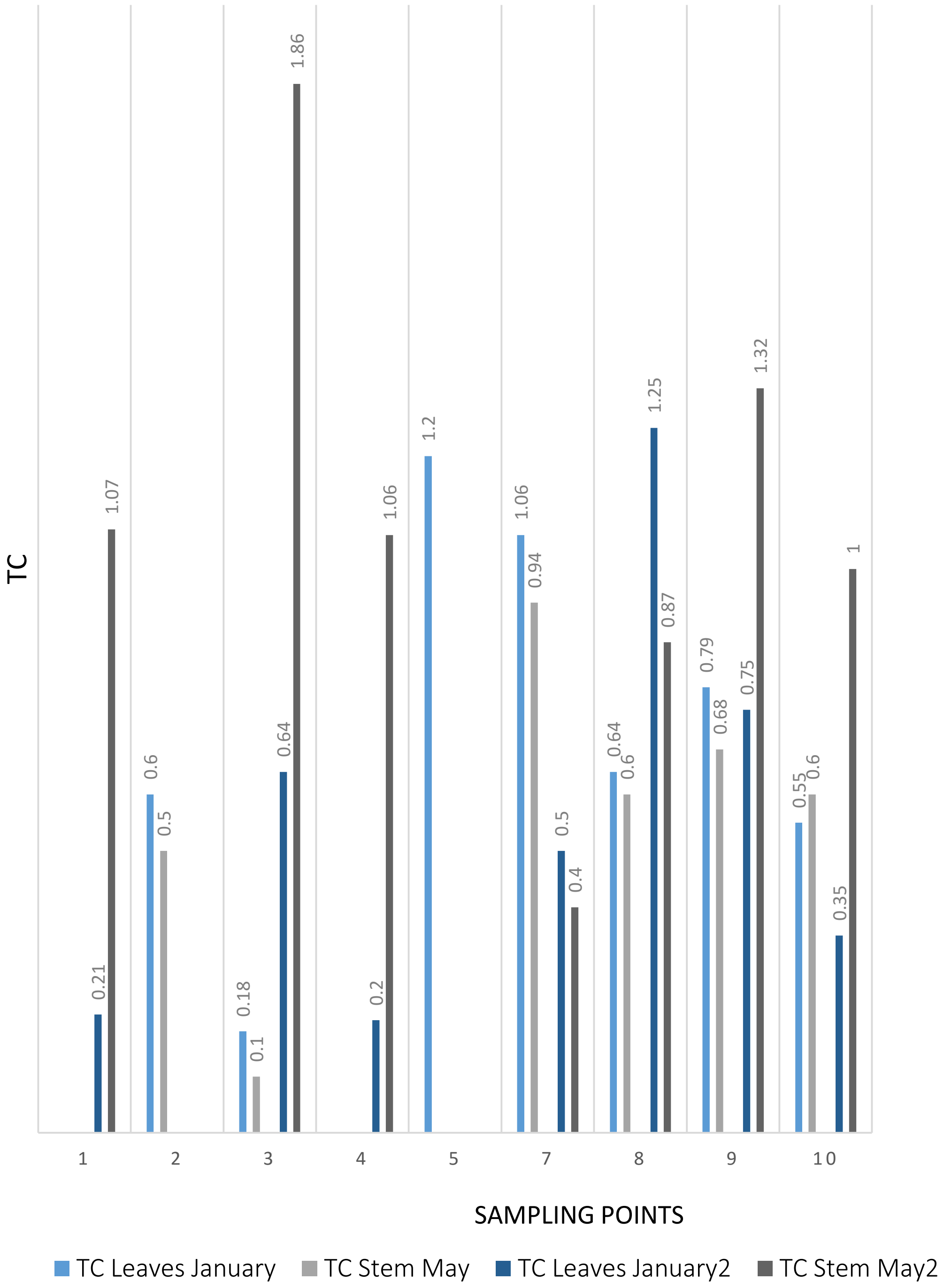

Figure 4.4: TC for Cu in water hyacinth samples from Winam Gulf

4.2.5 Concentration of zinc in water hyacinth samples

From Table 4.9, higher concentrations of Zn were observed in May than in January. This follows a similar trend observed with all the other metals accumulated by water hyacinth along Winam Gulf. This observation goes further to support the possibility that the high concentration of heavy metals observed in May is greatly influenced by the heavy rains season occurring during this time. In addition to pollution from urbanization and industrialization, runoff swept into Winam Gulf during this period also contains pollutants from farming activities. In January, the concentration ranges for Zn were; < 0.055 to 79 mg kg$^{-1 <}$ 0.055 to 64 mg kg^{-1} < 0.055 to 46 mg kg^{-1} for the roots, stem and leaves respectively. In May concentration ranges for Zn were; < 0.055 to 280 ± 13 mg kg^{-1}, < 0.055 to 774 mg kg^{-1} and < 0.055 to 59 mg kg^{-1} for the roots, stem and leaves respectively.

Another trend that is maintained by the accumulation of Zn in water hyacinth plants from Winam Gulf is the overall high retention of its amounts in the roots system. Although in comparatively high amounts, related observations were made in a study by Lu *et al.* (2004) where average accumulated concentration of Zn was found to be 9652.1 mg kg^{-1}in roots and 1926.7 mg kg^{-1}in aerial parts of water hyacinth plants grown in controlled water concentration of 40 mg l^{-1}. Buta *et al.* (2011) also reported that harvested water hyacinth growing in contaminated water had high levels of Zn in roots at 84 mg kg^{-1} while the shoots of the plants had concentrations of 51 mg kg $^{-1}$. The effect of having high retention of Zn concentrations in the roots could be due to a number of contributing factors one of which is co precipitation in Fe and Mn plaques (Vesk and Allaway, 1997).

Zinc is also comparable to Cu and Ni in that water hyacinth growing in some of the points did not show the presence of Zn in quantifiable amounts. These were points 1 and 10 during January, and points 6 and 8 during May where the concentration of Zn was below detection limits in all plant parts (Table 4.9).

Table 4.9: Concentration of Zn (mg kg^{-1}) in water hyacinth samples in January and May, 2013.

January, 2013 sampling				**May, 2013 sampling**		
Sampling point	Roots	Stem	Leaves	Roots	Stem	Leaves
1	< 0.055	< 0.055	< 0.055	106 ± 9.8	29 ± 6.1	30 ± 6.2
2	14 ± 1.2	13 ± 1.2	15 ± 1.4	280 ± 23	44 ± 5.8	15 ± 1.9
3	7.3 ± 0.8	4.3 ± 0.6	18 ± 1.9	158 ± 12	539 ± 49	59 ± 9.4
4	12 ± 1.4	17 ± 2.0	18 ± 1.6	269 ± 28	774 ± 51	< 0.055
5	14 ± 1.1	15 ± 1.4	2.9 ± 0.9	65 ± 6.8	32 ± 4.8	34 ± 3.9
6	25 ± 1.2	64 ± 7.1	46 ± 3.1	< 0.055	< 0.055	< 0.055
7	73 ± 2.8	24 ± 1.2	28 ± 1.5	84 ± 18	26 ± 6.2	< 0.055
8	79 ± 10	50 ± 9.6	28 ± 2.3	< 0.055	< 0.055	< 0.055
9	25 ± 3.0	37 ± 7.0	21 ± 0.9	44 ± 5.5	9.3 ± 0.8	47 ± 6.0
10	< 0.055	< 0.055	< 0.055	116 ± 11.1	11 ± 1.6	< 0.055
Range	Roots: < 0.055 - 79 Stem: < 0.055 - 64 Leaves: < 0.055 - 46			Roots: < 0.055 - 280 Stem: < 0.055 - 774 Leaves: < 0.055 - 59		

This could be an indication that Zn was in low abundance at these sampling points during the respective sampling periods as was seen with Cu and Ni which have been classified as low occurring metals along Winam Gulf. Gopal and Charma (1981), noted that water hyacinth's elemental content varies greatly due to unique variances of each in study site, the plant part in question and the biochemical conditions of the environment in which the plant is growing. This is true for this study as in January, points 1- 5 were observed to have least accumulation of Zn in all plant parts. The concentration range for Zn at these points was < 0.055 to 15 mg kg^{-1} in the roots, < 0.055 to 17 mg kg^{-1} in the stems and < 0.055 to 18 mg kg^{-1} in the leaves. These ranges were significantly low compared to other parts whose range was 21 to 79 mg kg^{-1} (sampling points 6 – 9).

Observations in May from Table 4.9 show substantially high amounts of Zn concentration even in points where during the preceding sampling period, had very low Zn levels or somewhat high levels of Zn. Examples include sampling point 1 where in January, Zn concentrations were below detection limits (0.055 mg kg^{-1}) but when the season changes in May (consequently changing the chemical conditions of water environment) the Zn levels increase to 106 ± 9.8 mg kg^{-1}, 29 ± 6.1 mg kg^{-1} and 30 ± 6.2 mg kg^{-1} in the roots, stems and leaves respectively. The opposite occurs for sampling point 8 where in January, water hyacinth plants had relatively high amounts of Zn 79 ± 10 mg kg^{-1}, 50 ± 9.6 mg kg^{-1}and 28 ± 2.3 mg kg^{-1} in the roots, stems and leaves respectively but then in May the levels were below detection limits.

In the cluster of low occurring heavy metals (Ni, Cu and Zn) along the Winam Gulf, it was observed that Zn had the highest abundance in comparison to Cu and Ni. A study conducted to investigate the concentration of Zn in whole water hyacinth plants growing in Ologe Lagoon, Nigeria showed that Zn was in low abundance. Concentration of the metal in the plants ranged between 1.73 and 4.63 mg kg^{-1} (Ndimele and Jimoh, 2011). The ranges in their study were quite low in comparison to our study.

There is higher transportation of Zn to the stem than to the leaves during both sampling periods as shown in Figure 4.5. Average TC ratio for stems in January was calculated to be 1.11 and average TC ratio for the leaves was found to be 1.07. In May, average TC values for the stems and the leaves were compute as 0.98 and 0.43 respectively. Highest translocation factors are recorded at sampling points 3 and 4 in January as well as in May, and sampling point 6 in January (Figure 4.5). Considering the individual plant part concentrations at these sampling points from Table 4. 9, the cause of these large TC values are not necessarily high values of metal concentration. Zinc showed to be the most translocated metal from the roots to the aerial parts of the plants in comparison other metals. From Figure 4.5, Zn recorded the highest number (10) of TC values > 1. Supporting observations were also made by Hammad *et al.* (2011) where their results showed that Zn was translocated more to the aerial parts of water hyacinth plants than Cu and Ni. Efficient transportation of Zn could be because it is an essential micronutrient required for plant metabolism (Lu *et al.*, 2004).

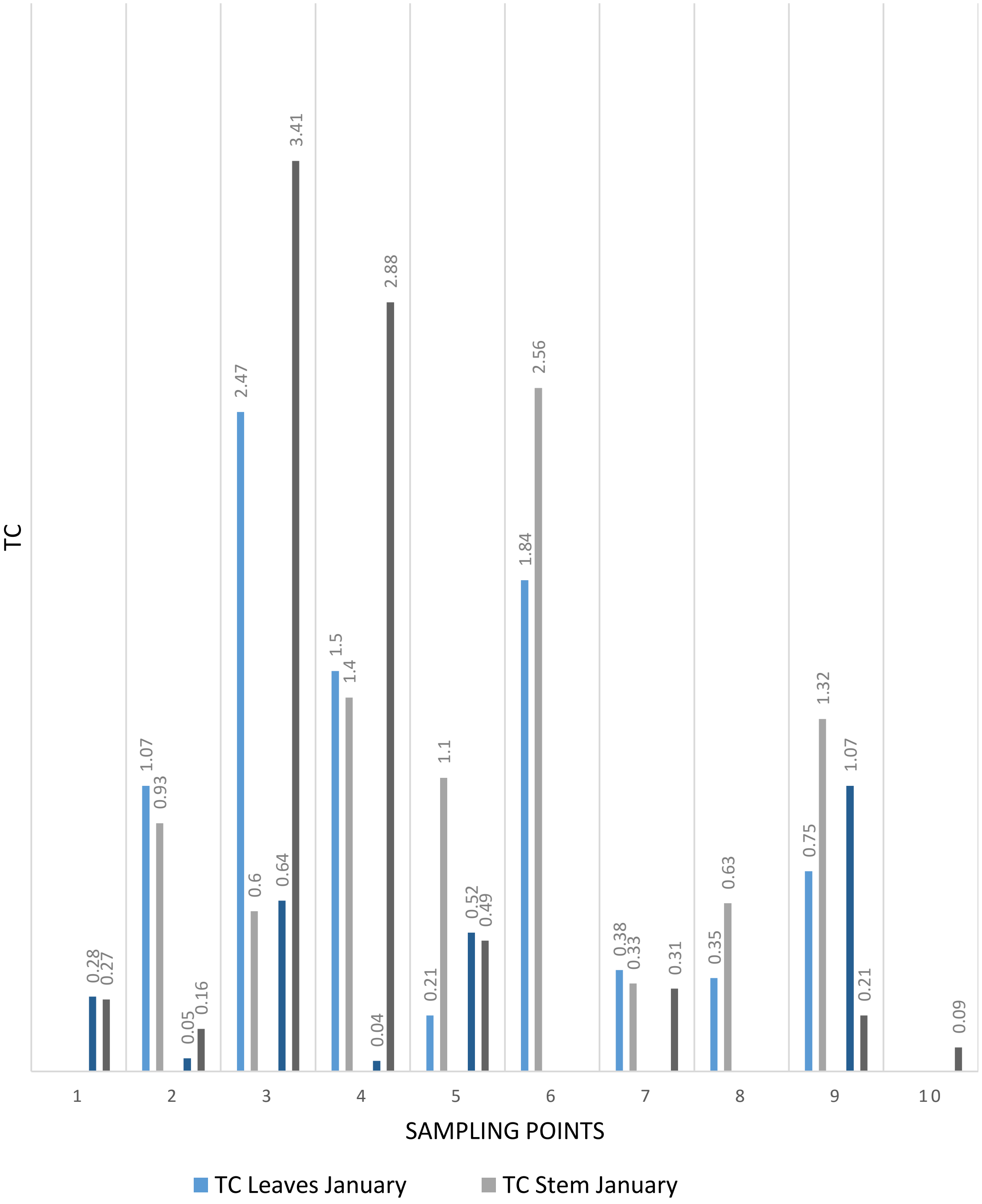

Figure 4.5: TC for Zn in water hyacinth samples from Winam Gulf

4.2.6 Heavy metal concentration in water samples

In September, water hyacinth did not grow on the lake and therefore only water sampling was conducted. Results from our study showed that Ni was below detection levels ($< 30\mu g\ l^{-1}$) in the water and therefore Ni was not included the results (Table 4.10). Its low levels correlate with the poor abundance observed in water hyacinth plants.

From Table 4.10 it is observed that Mn and Fe have the highest abundances in all sampling periods with concentrations ranging between < 55 to 618 $\mu g\ l^{-1}$ and 966 to 6931 $\mu g\ l^{-1}$ respectively. Copper and Zn have the lowest abundances with concentrations ranging between < 25 to 98 $\mu g\ l^{-1}$ and < 20 to 173 $\mu g\ l^{-1}$ respectively. These concentration patterns agree with observations made in accumulation of the heavy metals in water hyacinth plant samples where Mn and Fe were classified as highly occurring metals along Winam Gulf and Ni, Cu and Zn were clustered as low occurring metals along the Winam Gulf.

May had the highest range of metal concentrations (< 20 – 6931 $\mu g\ l^{-1}$). This was expected because May is a long rains period. Overflow from the farms and the municipal sweeps in elements that cause increase in the chemical composition of the water which includes pollutants such as heavy metals that occur as a result of human activities. January and September have closely comparable ranges with minor differences taking place in the upper limit due to Fe concentrations. Consecutive overall ranges were: < 20 to 6324$\mu g\ l^{-1}$ and < 20 to 6195 $\mu g\ l^{-1}$.

September is a short rains season. Although the average volume of rainfall is not as high as in the long rains season, the effect is somewhat reflected on the individual heavy metal concentration ranges with Mn, Cu and Zn having greater amounts than in January as shown in Table 4.10. Sequence of abundance of heavy metals in the sampling periods was concluded to be May > September > January.

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Table 4.10: Concentration of heavy metals (µg l^{-1}) in water samples from Winam Gulf in January, May and September, 2013.

point metal	January, 2013				May, 2013				September, 2013			
	Mn	Fe	Cu	Zn	Mn	Fe	Cu	Zn	Mn	Fe	Cu	Zn
1	179 ± 16	1319±39	< 25	< 20	618±40	4452±116	51±8	137±15	190±40	1452±61	< 25	< 20
2	286±26	6063±119	< 25	< 20	192±14	4055±61	< 25	27±9	297±25	6195±116	< 25	77±18
3	128±20	3764±90	30±6	< 20	167±11	4544±55	< 25	< 20	128±20	3765±82	26±6	< 20
4	< 55	1199±52	< 25	< 20	141±24	2850±96	< 25	< 20	<55	1200±96	< 25	< 20
5	85±21	2058±80	< 25	< 20	< 55	1434±55	< 25	106±13	96±21	2190±64	< 25	173±15
6	127±21	3043±88	< 25	< 20	123±28	2755±100	45±11	104±10	134±15	2895±80	< 25	45±8
7	182±14	6324±80	< 25	< 20	58±10	1671±34	< 25	110±37	199±14	6325±79	< 25	42±6
8	72±16	2132±59	< 25	43±6	114±20	2081±50	< 25	< 20	83±20	2264±67	< 25	44±9
9	107±17	2213±64	90±8	129±12	72±7	6931±150	< 25	< 20	118±20	2213±86	98±8	132±18
10	< 55	835±29	< 25	< 20	107±22	2385±87	< 25	< 20	< 55	966±55	< 25	< 20
Ranges	Overall January range: < 20 - 6324				Overall May range: < 20 - 6931				Overall September range: < 20 - 6195			
	Mn: < 55 - 286 Fe: 835 - 6324 Cu: < 25 - 90 Zn: < 20 - 129				Mn: < 55 - 618 Fe: 1434 - 6931 Cu: < 25 - 51 Zn: <20 - 137				Mn: < 55 - 297 Fe: 966 - 6195 Cu: < 25 - 98 Zn: < 20 - 173			
	Overall metal ranges: Mn: < 55 - 618 Fe: 835 - 6931 Cu: < 25 - 98 Zn: < 20 - 173											

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Owing to its phytoaextractive properties, it would be expected that the presence of water hyacinth would significantly minimize the concentration of heavy metals in the water as the weed can exhaustively deplete heavy metals from the water column (Lu *et al.,* 2004). However, this is not the case since concentrations of heavy metals in January are about the same range in September. This could be an indication that pollution is continuously high in the Winam Gulf regardless of the presence of water hyacinth. General order of occurrence for the metals in the water from the three periods is: Fe > Mn > Zn > Cu > Ni.

In natural aquatic systems, metals occur normally at ng - µg l^{-1}. Unfortunately, due to population growth, urbanization, industrialization, exploitation of natural resources and lack of environmental regulations, heavy metal contaminants have polluted water bodies (Ndimele *et al.*, 2011). A study conducted by Lalah *et al.* (2008), on the analysis of heavy metal source pollutants along Winam Gulf revealed the presence of various metals in the water. Concentrations ranged from 5 - 157.5 µg l^{-1} (Cu), 50 - 3276 µg l^{-1} (Mn), nd - 54.1µg l^{-1} (Ni), and 25 - 219.5 (Zn) µg l^{-1}. These levels are significantly low in comparison to the range in this study.

Based on WHO standards (2008), water from Lake Victoria is not safe for domestic use such as cooking and drinking. This is because heavy metals such as Fe and Zn concentrations are above stipulated limits which are 300 µg l^{-1} and negligible levels respectively. Fe has the highest concentration recorded at 6931 ± 57 µg l^{-1} from sampling point 9 in May while Zn recorded well above detectable levels of up to 173 ± 15 μg l^{-1} in September at sampling point 5.

4.2.7 Results from anaerobic digestion of water hyacinth

4.2.7.1 Volume of gas produced

Figure 4.6 shows the volume of gas produced from each cow dung: water hyacinth ratio. Gas production was recorded at 2 - 3 day intervals over a maximum period of 12 days. The higher the amount of water hyacinth, the higher the amount of gas recorded at each recording interval. The volume increases steadily over the period of time. During the last interval at 1:3 ratio it is noted that the volume of gas decreases by 2.0 % unlike in the other ratios where the volume of gas increases. The highest increase was recorded during the third interval at 1:1 ratio where the volume of gas increases by 4.9 %. 1: 1 ratio recorded the highest average percentage increase of 3.0 %. Average percentage increase at 1:3 was 2.1 % and at 1:5 the average percentage increase was recorded to be 2.7 %. Overall average percentage increase in gas production was 2.6 ± 0.45 %. Total average volume of gas produced was 17.0, 19.6, and 23.4 m^3 for 1:1, 1:3 and 1:5 ratios respectively.

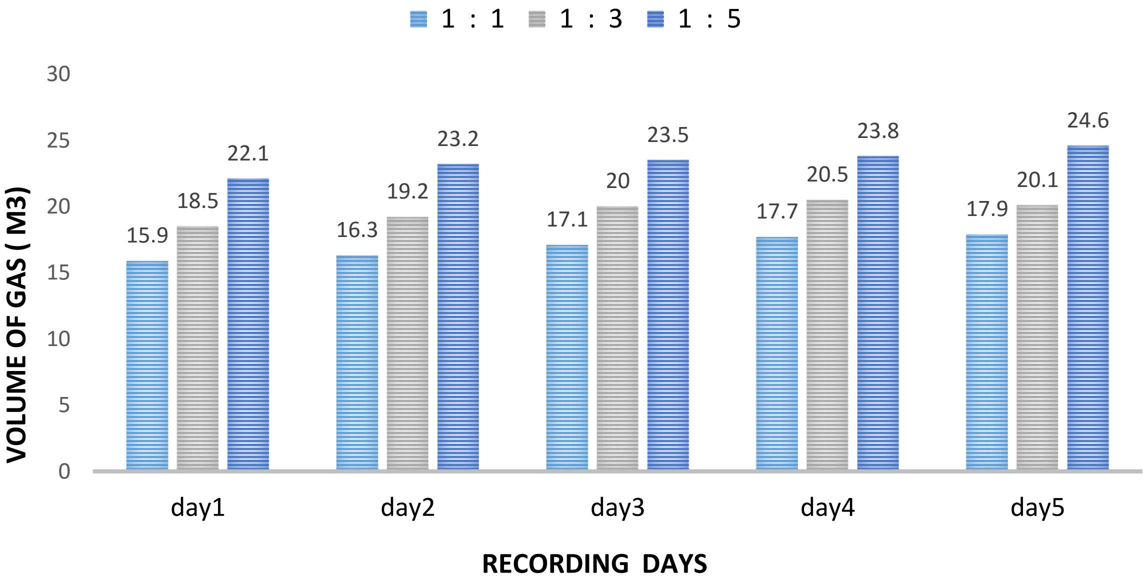

Figure 4.6: Volume of gas produced from various cow dung: water hyacinth cffeed ratios

Using water hyacinth on its own to produce biogas gives very low volumes in comparison to other types of biomass like cow dung and chicken litter because it does not contain microbes to enhance anaerobic digestion. However, the use of enriched water hyacinth with inoculum greatly improves biogas yield. This showed that water hyacinth is a viable substrate for biogas production. Verma *et al.*, (2006) reported that the effect of heavy metals accumulated by the water hyacinth plants on biogas production (using 1:1 and 1:3 inoculum: substrate ratios) was based on the concentration of the metals. The study showed maximum production in biogas was produced from plants with lowest metal concentrations while increased heavy metal levels in plants showed conspicuous reduction in biogas production. In the current study, the reverse is true. It is argued that the heavy metal content in water hyacinth samples used is diverse. The samples used in the previous study were grown in industrial effluent and hence the intensity of concentration could also be a contributing factor. It is observed that the highest volumes of biogas were recorded at 1:5 ratio. From these results, using water hyacinth as for biogas generation could be a good solution to clearing it as well as obtaining an alternative energy source.

4.2.7.2 Heavy metal concentrations in digestate sample

It was observed that increase in the amount of water hyacinth in each ratio increased the concentration of the heavy metals (Table 4.11). This was observed for all the detectable metals except for Ni's and Cu's 1:5 ratio feed concentrations that are much lower than the digestate concentrations. Fe recorded the highest concentration for all ratios used whereas Zn was only recorded at 1:5 ratio. Water hyacinth can be used in farming either as a green manure or as compost. When used as a green manure it can be either ploughed into the ground or used as mulch.

In Sri Lanka water hyacinth is mixed with organic municipal sludge, soil and ash and sold to local farmers and gardeners as compost (Jafari, 2010). Additional reports by Singal and Rai (2003), conclude that digestate obtained after anaerobic digestion of water hyacinth, the primary sludge contains almost all nutrients and can therefore be used as a good fertilizer with no negative effects on the environment.

Table 4.11: Heavy metal concentrations (mg kg^{-1}**)** in digestate samples from various pulp mixtures

METAL	**Cow dung: water hyacinth**		
	1:1 **(mg kg^{-1})**	**1:3** **(mg kg^{-1})**	**1:5** **(mg kg^{-1})**
Mn	170 ± 9.0	350 ± 22	513 ± 51
Fe	505 ± 41	947 ± 55	1427 ± 92
Ni	0.4 ± 0.2	0.4 ± 0.1	2.5 ± 0.8
Cu	1 ± 0.4	1.9 ± 0.3	5.3 ± 1.1
Zn	< 0.055	< 0.055	1.7 ± 0.2

In all the ratios the digestate concentrations did not exceed specifications set by the Kenya Bureau of Standards (Tables 4.12 and 4.13). The levels for secondary elements and levels of toxic metals were all within acceptable range. Using it as manure is therefore a feasible initiative.

Table 4.12: Organic fertilizer- specification (KENYA STANDARD KS 2290: 2011): Secondary Plant Nutrients

Element	**Percent Minimum**
Calcium	1.0000
Magnesium	0.5000
Sulphur	1.0000
Boron	0.0200
Cobalt	0.0005
Copper	0.0500
Iron	0.1000
Manganese	0.0500
Molybdenum	0.0005
Zinc	0.0500

Table 4.13: Organic fertilizer- specification (KENYA STANDARD KS 2290: 2011): Heavy Metal contaminants

Parameter	**Limit (mg kg^{-1})**
Arsenic, max	10
Cadmium, max	5
Chromium, max	50
Copper, max	300
Lead, max	30

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

This chapter presents the conclusions and recommendations of this study.

5.1 Conclusions

The analysis of heavy metal content in water hyacinth and water samples from Lake Victoria along Winam Gulf indicates that water hyacinth is a hyper accumulator of various metals. Pollution increases during the rainy season due to municipal and farm runoff. Should the water be polluted, phytoremediation will occur and this can be a good indicator for pollution control. Results showed that a high concentration of the metals accumulated was retained in the roots in comparison to the aerial parts of the plant.

The study also supports the view that water hyacinth is a good biomass for biogas production. In addition to its utilization, digestate recovered as waste from anaerobic digestion can be used as an organic fertilizer based on its content and commendations made in other studies.

5.2 Recommendations

1)It is important to consider regular monitoring and control of heavy metals in the lake as high levels can be toxic to human beings and animals through bioaccumulation.

2) Research should be carried the on the effects of heavy metals in water hyacinth to anaerobic digestion and the relation to quality and quantity of biogas produced. There after, introducing the bio gasification of water hyacinth as an economic venture for the community. It should be noted however that this venture should only be undertaken as an eradication measure for the weed, because the benefits outweigh the cost of its setup, and not as a permanent commercial project.

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APPENDICES

APPENDIX 1: Coordinates for the sampling sites

	Sampling point	Longitude	Latitude
1	Kichinjio Bay	34° 44' 50.6322" E	0° 05' 34.3000" S
2	National Cereals and Produce Board (Point I)	34° 44' 23.1108" E	0° 05' 14.1562" S
3	National Cereals and Produce Board (Point II)	34° 44' 16.1810" E	0° 05' 17.4181" S
4	Boat Pier	34° 44' 35.7394" E	0° 06' 3.3006" S
5	Impala Beach club point	34° 44' 33.9322" E	0° 06' 50.8716" S
6	Yacht Club	34° 44' 33.9286" E	0° 06' 50.8428" S
7	Wigwa River Inlet	34° 44' 36.3297" E	0° 07' 13.8128" S
8	Hippo Point	34° 44' 35.9985" E	0° 07' 19.1341" S
9	KWSTCo.	34° 44' 31.1710" E	0° 07' 19.0585" S
10	Kiboko Bay	34° 44' 26.6783" E	0° 07' 22.6840" S

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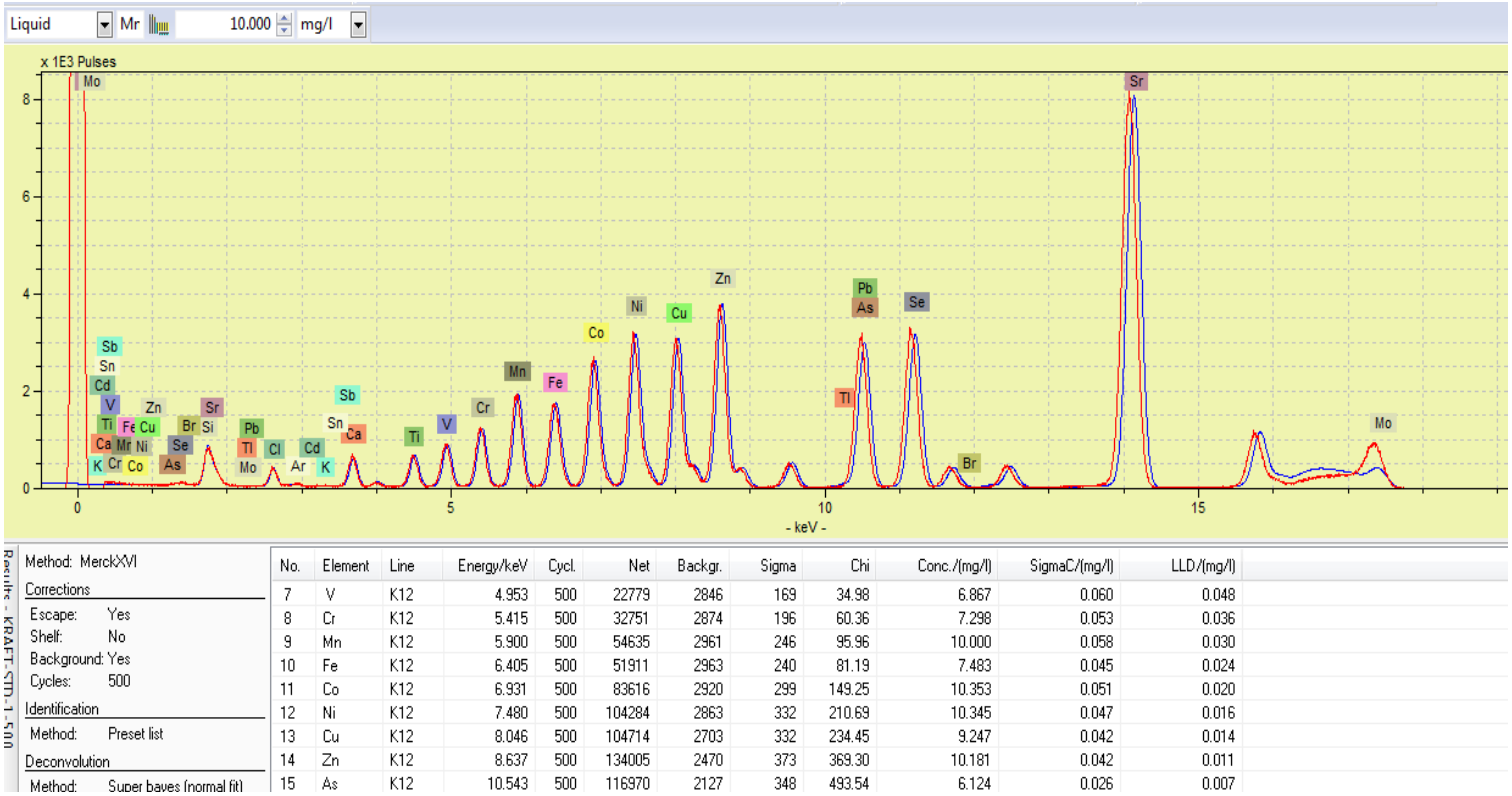
APPENDIX 2: Bernd Kraft® multi-element reference standard (10 mg l^{-1})



Method: MerckXVI

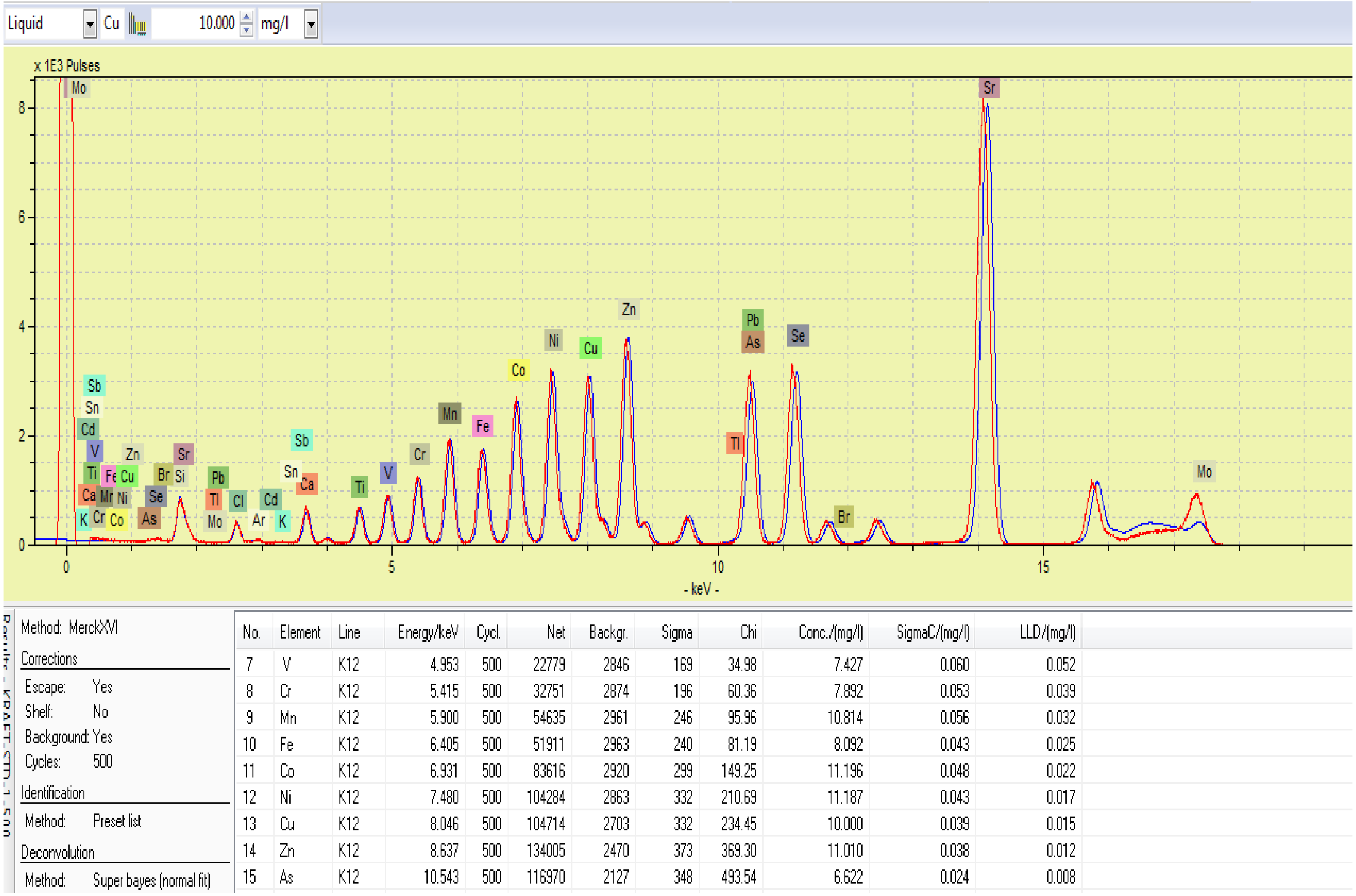
Corrections
Escape: Yes
Shelf: No
Background: Yes
Cycles: 500

Identification
Method: Preset list

Deconvolution
Method: Super bayes (normal fit)

No.	Element	Line	Energy/keV	Cycl.	Net	Backgr.	Sigma	Chi	Conc./(mg/l)	SigmaC/(mg/l)	LLD/(mg/l)
7	V	K12	4.953	500	22779	2846	169	34.98	6.867	0.060	0.048
8	Cr	K12	5.415	500	32751	2874	196	60.36	7.298	0.053	0.036
9	Mn	K12	5.900	500	54635	2961	246	95.96	10.000	0.058	0.030
10	Fe	K12	6.405	500	51911	2963	240	81.19	7.483	0.045	0.024
11	Co	K12	6.931	500	83616	2920	299	149.25	10.353	0.051	0.020
12	Ni	K12	7.480	500	104284	2863	332	210.69	10.345	0.047	0.016
13	Cu	K12	8.046	500	104714	2703	332	234.45	9.247	0.042	0.014
14	Zn	K12	8.637	500	134005	2470	373	369.30	10.181	0.042	0.011
15	As	K12	10.543	500	116970	2127	348	493.54	6.124	0.026	0.007

Manganese



Method: MerckXVI

Corrections

Escape: Yes
Shelf: No
Background: Yes
Cycles: 500

Identification

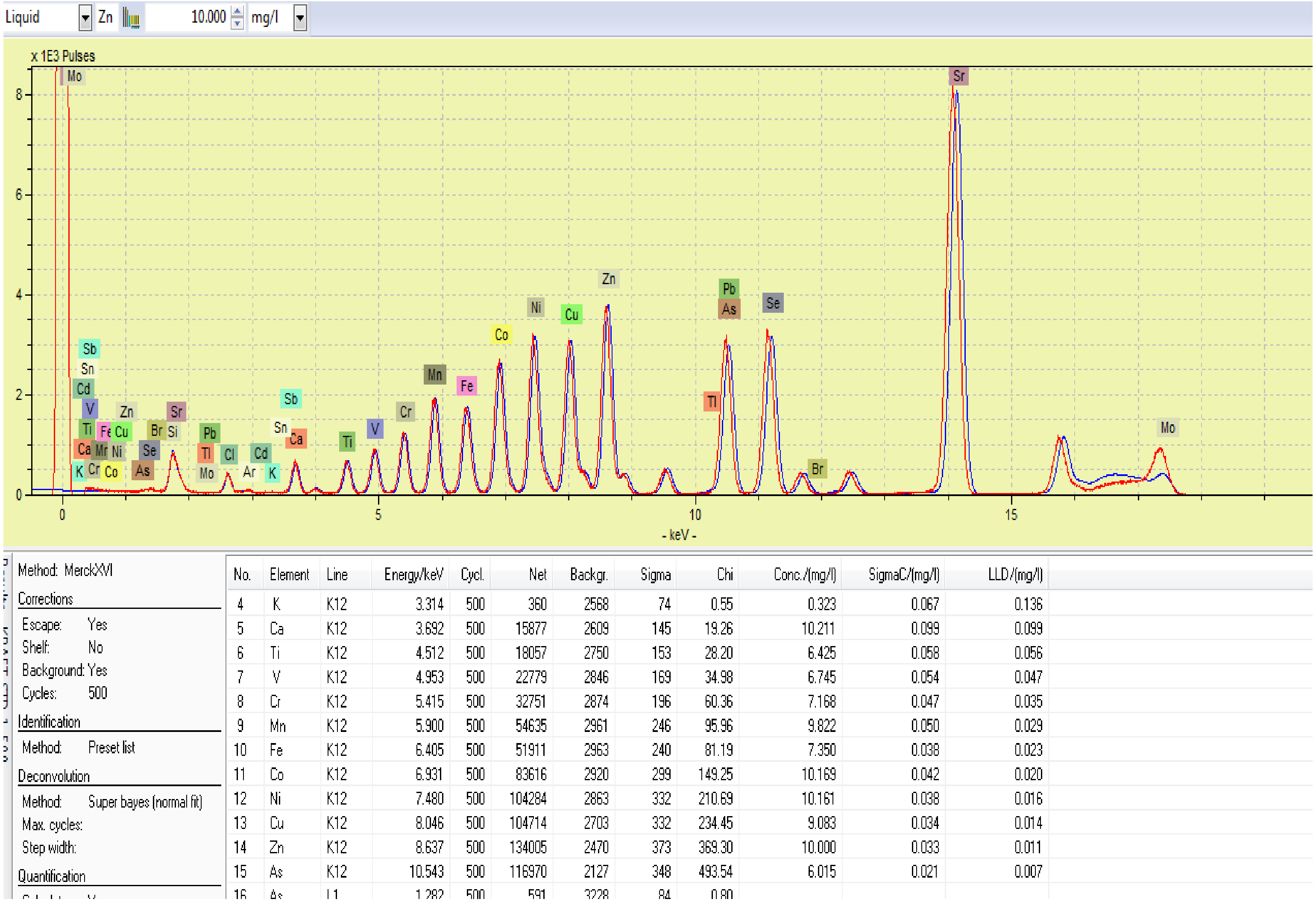
Method: Preset list

Deconvolution

Method: Super bayes (normal fit)

No.	Element	Line	Energy/keV	Cycl.	Net	Backgr.	Sigma	Chi	Conc./(mg/l)	SigmaC/(mg/l)	LLD/(mg/l)
7	V	K12	4.953	500	22779	2846	169	34.98	7.427	0.060	0.052
8	Cr	K12	5.415	500	32751	2874	196	60.36	7.892	0.053	0.039
9	Mn	K12	5.900	500	54635	2961	246	95.96	10.814	0.056	0.032
10	Fe	K12	6.405	500	51911	2963	240	81.19	8.092	0.043	0.025
11	Co	K12	6.931	500	83616	2920	299	149.25	11.196	0.048	0.022
12	Ni	K12	7.480	500	104284	2863	332	210.69	11.187	0.043	0.017
13	Cu	K12	8.046	500	104714	2703	332	234.45	10.000	0.039	0.015
14	Zn	K12	8.637	500	134005	2470	373	369.30	11.010	0.038	0.012
15	As	K12	10.543	500	116970	2127	348	493.54	6.622	0.024	0.008

Copper



Liquid Zn 10.000 mg/l

Method: MerckXVI

Corrections

Escape: Yes
Shelf: No
Background: Yes
Cycles: 500

Identification

Method: Preset list

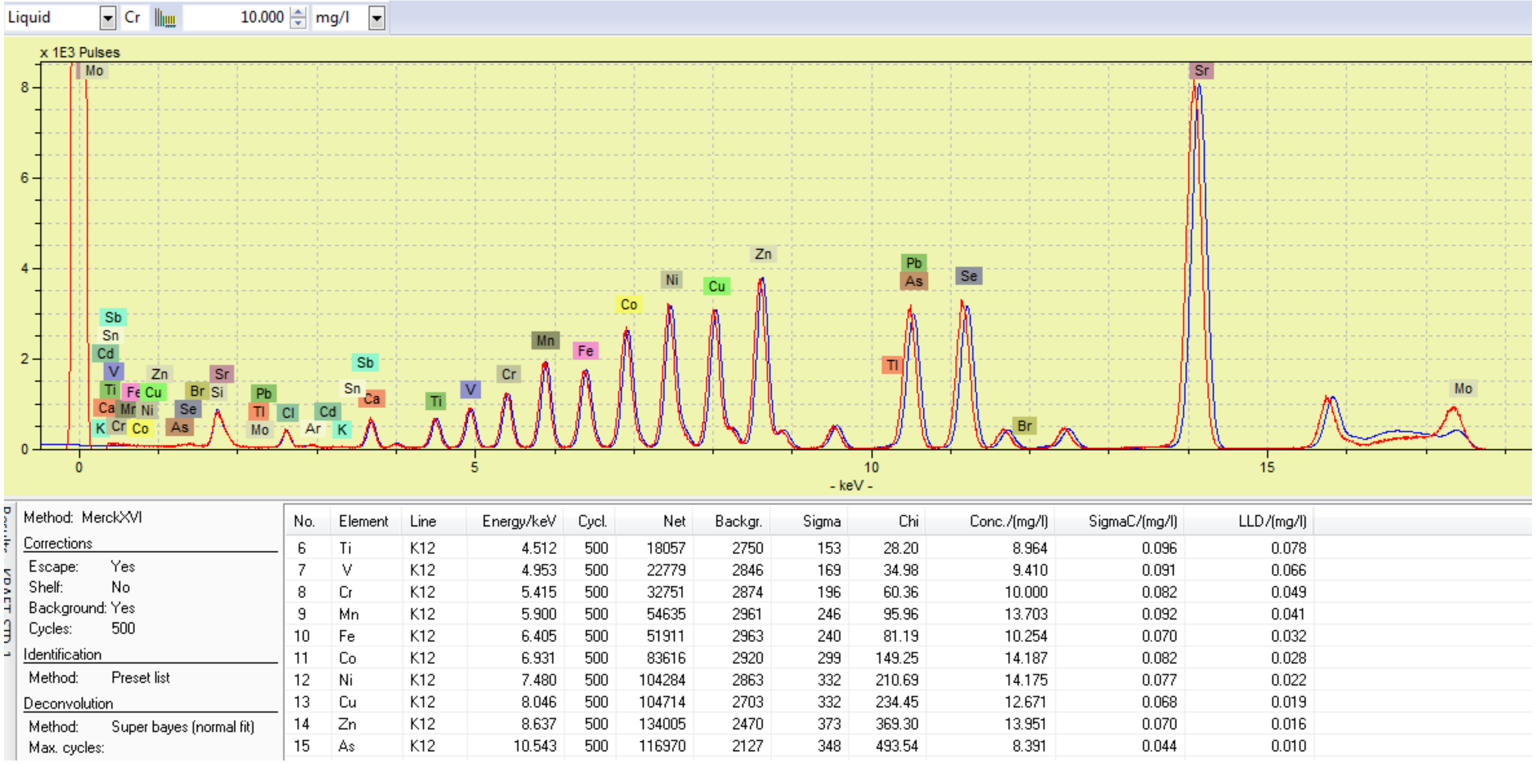
Deconvolution

Method: Super bayes (normal fit)
Max. cycles:
Step width:

Quantification

No.	Element	Line	Energy/keV	Cycl.	Net	Backgr.	Sigma	Chi	Conc./(mg/l)	SigmaC/(mg/l)	LLD/(mg/l)
4	K	K12	3.314	500	360	2568	74	0.55	0.323	0.067	0.136
5	Ca	K12	3.692	500	15877	2609	145	19.26	10.211	0.099	0.099
6	Ti	K12	4.512	500	18057	2750	153	28.20	6.425	0.058	0.056
7	V	K12	4.953	500	22779	2846	169	34.98	6.745	0.054	0.047
8	Cr	K12	5.415	500	32751	2874	196	60.36	7.168	0.047	0.035
9	Mn	K12	5.900	500	54635	2961	246	95.96	9.822	0.050	0.029
10	Fe	K12	6.405	500	51911	2963	240	81.19	7.350	0.038	0.023
11	Co	K12	6.931	500	83616	2920	299	149.25	10.169	0.042	0.020
12	Ni	K12	7.480	500	104284	2863	332	210.69	10.161	0.038	0.016
13	Cu	K12	8.046	500	104714	2703	332	234.45	9.083	0.034	0.014
14	Zn	K12	8.637	500	134005	2470	373	369.30	10.000	0.033	0.011
15	As	K12	10.543	500	116970	2127	348	493.54	6.015	0.021	0.007
16	As	L1	1.282	500	591	3228	84	0.80			

Zinc



Method: MerckXVI

Corrections

Escape: Yes
Shelf: No
Background: Yes
Cycles: 500

Identification

Method: Preset list

Deconvolution

Method: Super bayes (normal fit)
Max. cycles:

No.	Element	Line	Energy/keV	Cycl.	Net	Backgr.	Sigma	Chi	Conc./(mg/l)	SigmaC/(mg/l)	LLD/(mg/l)
6	Ti	K12	4.512	500	18057	2750	153	28.20	8.964	0.096	0.078
7	V	K12	4.953	500	22779	2846	169	34.98	9.410	0.091	0.066
8	Cr	K12	5.415	500	32751	2874	196	60.36	10.000	0.082	0.049
9	Mn	K12	5.900	500	54635	2961	246	95.96	13.703	0.092	0.041
10	Fe	K12	6.405	500	51911	2963	240	81.19	10.254	0.070	0.032
11	Co	K12	6.931	500	83616	2920	299	149.25	14.187	0.082	0.028
12	Ni	K12	7.480	500	104284	2863	332	210.69	14.175	0.077	0.022
13	Cu	K12	8.046	500	104714	2703	332	234.45	12.671	0.068	0.019
14	Zn	K12	8.637	500	134005	2470	373	369.30	13.951	0.070	0.016
15	As	K12	10.543	500	116970	2127	348	493.54	8.391	0.044	0.010

Chromium

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APPENDIX 3: Data from biogas volume collection

Ratio of cow dung water hyacinth	day	date of recording	volume of gas(m^3)
1:1	1	20/01/2014	15.9
	2	22/01/2014	16.3
	3	24/01/2014	17.1
	4	27/01/2014	17.7
	5	29/01/2014	17.9
1:3	1	5/2/2014	18.5
	2	7/2/2014	19.2
	3	11/02/2014	20.0
	4	13/2/2015	20.5
	5	17/2/2014	20.1
1:5	1	19/03/2014	22.1
	2	21/03/2014	23.2
	3	24/03/2014	23.5
	4	26/03/2014	23.8
	5	29/4/2014	24.6

APPENDIX 4: WHO 2008 Guidelines for heavy metal concentration limits in drinking water (WHO, 2008).

Heavy Metal	**Limit ($\mu g\ l^{-1}$)**
As	10
Cd	3
Co	-
Cr	-
Cu	2000
Fe	300
Mn	400
Ni	70
Pb	10
Zn	-