# ANALYSIS OF MICRONUTRIENTS AND HEAVY METALS OF INDIGENOUS REED SALTS AND SOILS FROM SELECTED AREAS IN WESTERN KENYA

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## DECLARATION AND RECOMMENDATION

### DECLARATION

This is my original work and has not been submitted in part or whole for an award in any other institution.

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### RECOMMENDATION

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

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## DEDICATION

This work is dedicated to my husband Peter, my children Brian, Teddy and Marvelynne; my parents Patrick and Joyce Fedha and Susan Nyongesa and my sisters and brothers.

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I give thanks to God for giving me grace to accomplish this task. Accordingly, the culmination of a journey that started with a single step and gradually developed into one mighty task is complete with the support of people so dear to me. My joy and sense of fulfillment would not be complete without making mention of everyone who offered help and support, in one way or another, during the entire period of this PhD study. The brevity of this acknowledgement does not in any way downplay the support I have received from anyone mentioned, or not mentioned, herein.

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## ABSTRACT

Most communities in Western Kenya use plant indigenous salts for cooking, medicinal and numerous uses. *Typha latifolia* and *Cyperus rotundus* reeds are widely used in Busia and Lugari regions of Western Kenya to prepare indigenous salts. The suitability of these salts and validation of micronutrients and heavy metals is unknown. The objective of this study was to assess the suitability of the indigenous reed salt used in selected parts of Western Kenya. Micronutrients and heavy metal concentrations in soil habitats, *C. rotundus* and *T. latifolia* and reed salts were determined; In addition, the effect of the various methods of processing, storage conditions and the stability of iron and iodine nutrients has been investigated. Iodometric titration (Iodine), 1, 10-phenanthroline method (Iron II), flame photometric method (Na and K) and Atomic absorption spectrophotometric method (Pb, Cd, Fe, Cr) were used for analysis. Results showed that 85% of Lugari and Busia inhabitants in Western Kenya use *C. rotundus* and *T. latifolia* reed salts. Heavy metal in the soil was of the order Fe> Cr >Pb > Cd for the dry season and Cr > Fe > Cd > Pb for the wet season both in top and sub-surface soils with higher levels in the dry season. Salt iodine was of the order Kensalt >Top-chef salt > Herbal sea salt > Sea salt > *C. rotundus* salt > *T. latifolia* and Herbal sea salt >*C. rotundus* salt = *T. latifolia* salt > Sea salt > Kensalt = Top-chef salt for $Fe^{2+}$. *C. rotundus* and *T. latifolia* reed salts had higher iodine (1.1 mg/kg) than the WHO limit of 0.015 - 1.1 mg/kg , while the concentration of $Fe^{2+}$ (0.9 mg/kg and 1.0 mg/kg) was below the recommended limit of 8 - 45 mg/kg. The Na: K ratio of *T. latifolia* salt (3.2:1) was within the recommended limit of 2.5:1 - 4:1 while that of *C. rotundus* salt (0.9:1) is lower. Fe, Pb and Cd levels in both *C. rotundus* reed and *T. latifolia* reed salts exceeded the WHO/FAO permissible. Effectively all iodine and $Fe^{2+}$ present in the reed salts was lost within six months of storage under normal conditions of temperature and RH with more losses at elevated temperature and relative humidity. *T. latifolia* salt prepared using complete evaporation method and stored in LDPE container for a period not more than three months is suitable for use as table salt. *C. rotundus* prepared by complete evaporation method is ideal for use as a low-sodium salt.

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| | |
|---|---|
| AFR/EA | WHO Regional Office for Africa/ Sub-Regional Office of Eastern Africa |
| ASDS | Agricultural Sector Development Strategy |
| CDI | Centre for Development and Innovation |
| CBS | Central Bureau of Statistics |
| CSB | Corn Soy Blend |
| DON | Division of Nutrition |
| DPH | Division of Public Health |
| ECSA | East, Central and Southern Africa Health Community |
| ECD | Early child development |
| EPZ | Export Promotion Zone |
| ICN | International Conference on Nutrition |
| IDA | Iron deficiency anemia |
| IDD | Iodine Deficiency Disorders |
| IMF | International Monetary Fund |
| FAO | Food and Agriculture Organization |
| FFI | Food Fortification Initiative |
| FNSP | National Food and Nutrition Security Policy (Kenya) |
| GAIN | Global Alliance for Improved Nutrition |
| GDP | Gross Domestic Product |
| GNI | Gross National Income |
| HACCP | Hazard Analysis Critical Control Points |
| HiNi | High Impact Nutrition Interventions |
| IDD | Iodine deficiency disorders |
| IFAD | International Fund for Agricultural Development |
| KEBS | Kenya Bureau of Standards |
| KIFST | Kenya Institute of Food Science and Technology |
| KFSM | Kenya Food Security Meeting |

| | |
|---|---|
| KFSSG | Kenya Food Security Steering Group |
| LDC | Least Developed Countries |
| MCH | Maternal Health Care |
| MI | Micronutrient Initiative |
| MoA | Kenyan Ministry of Agriculture |
| MT | Metric Tons |
| MUAC | Mid upper arm circumference |
| MUI | Median Urinary Iodine |
| NaFeEDTA | Sodium iron EDTA |
| NGO | Non-Governmental Organization |
| NFSCC | National Food Safety Coordination Committee |
| NNAP | National Nutrition Action Plan |
| NPANs | National Plans of Action for Nutrition |
| OHP | Orange House Partnership (Brussels) |
| ORS | Oral Rehydration Solution |
| PEM | Protein Energy Malnutrition |
| RUTF | Ready-to-use therapeutic foods |
| SME's | Small & medium enterprises |
| SPM | Sanitary and Phytosanitary Measures |
| TGR | Total Goiter Rate |
| UNICEF | United Nations Children's Fund |
| UNESCO | United Nations Educational, Scientific and Cultural Organization |
| UNDP | United Nations Development program |
| USAID | U.S. Agency for International Development |
| VAD | Vitamin A deficiency |
| WHO | World Health Organization |
| WFP | World Food Program |
| WTO | World Trade Organization |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

More than 2 billion people in the world today suffer from micronutrient deficiencies caused largely by a dietary deficiency of vitamins and minerals. In the less developed countries these deficiencies have serious health consequences, especially for women and young children. According to the WHO , in the period 1993 to 2003 a total of 54 countries worldwide suffered from iodine deficiency and 3 billion had insufficient iodine intakes, infants, pregnant and lactating women being among those most affected (Al-Hosani *et al*., 2003). Despite significant progress, deficiencies of iron and iodine remain major public health problems affecting ≥30% of the global population (Zimmermann, 2006). The public health importance of these deficiencies lie upon their magnitude and their health consequences, especially in pregnant women and young children, as they affect fetal and child growth, cognitive development and resistance to infection. Poverty, lack of access to a variety of foods, lack of knowledge of appropriate dietary practices and high incidence of infectious diseases are key factors (Shawel *et al*., 2010). In 2000, the *World Health Report* identified iodine, iron, vitamin A and zinc deficiencies as some of the world's most serious health risk factors (Shawel *et al*., 2010).

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well-being. Minerals are essential components of our diet that serve as cofactors in the thousands of enzyme-controlled reactions, control the action of nerves and muscles, help maintain the body's water balance, and buffer the pH (acidity) of the cell and extracellular fluids (Minerals-Learn, 2010). Salt has high physiological importance in human bodies and other living organisms because it helps to regulate the body's metabolism. Salt is consumed at relatively constant, well defined levels by all people within a society, irrespective of socio-economic status. Although various preservatives are available today, salt is still widely used in preservation of meat, fish and for therapeutic purposes. There is commercially available salt as well as indigenous salts. Commercially available salt consists mainly of sodium chloride, which reduces water activity in food thereby preserving it. Also present in salt in a smaller proportion is sodium carbonate which is used to tenderize food during cooking (Olivier and

Dumitroaia, 2005). Despite the reeds being used for processing of indigenous salt, they also provide a range of ecosystem services. These include water purification, flood control and shoreline stability, much as it is a home to a wide range of plants and animal life (Silliman *et al*., 2009). While playing the role of water purification, uptake of potentially harmful chemicals by the reeds may result. These may find their way into the food chain. Furthermore, some of the plants from which indigenous salts are extracted grow in wetlands that are contaminated by heavy metals such as mercury, Hg (Campbell *et al.,* 2004; Chibunda *et al.,* 2006), cadmium, Cd (Kishe and Machiwa, 2003), chromium, Cr and lead, Pb which may be a health risk to its users.

Iron deficiency anemia and iodine deficiency are two major nutrition-related disorders, affecting more than one third of the world's population. In the less developed countries these deficiencies have serious health consequences, especially for women and young children. The public health importance of these deficiencies lies upon their magnitude and their health consequences, especially in pregnant women and young children, as they affect fetal and child growth, cognitive development and resistance to infection. Poverty, lack of access to a variety of foods, lack of knowledge of appropriate dietary practices and high incidence of infectious diseases are key factors (Shawel *et al*., 2010). The lack of iodine in the soil and water and thus in food, leads to iodine-deficiency disorders, which include anemia, goiter and a wide spectrum of mental and intellectual defects of varying degrees of severity, including cretinism, paralysis, and deaf-mutism. Iodine-deficiency disorders can also lead to stunted growth and development, miscarriages, stillbirths, and infant deaths. Low iron intake or poor absorption leads to anemia, resulting in a major reduction in work capacity and impaired immune response, which leads to a higher incidence of infection, an increased risk of maternal and fetal morbidity, and a reduction in body growth. The combined impact of these deficiencies results in severe retardation of social and economic development of entire populations. Deficiencies in iodine or iron can be inexpensively eliminated, prevented, or reduced by increasing their dietary intake (Shawel *et al*., 2010).

Heavy metal accumulation in soils and plants is of increasing concern because of the potential human health risks associated with them. Excessive accumulation of heavy metals in agricultural soils may not only result in soil contamination, but also lead to elevated heavy metal uptake by

crops, and thus affect food quality and safety (Muchuweti *et al*., 2006). Anthropogenic activities, such as mining, industrial and domestic wastewater and sludge, fertilizer and pesticide application to land, as well as atmospheric deposition are the main sources of metal contamination in plants (Szynkowska *et al*., 2009; Wuana and Okieimen 2011). Even at low concentrations, elements such as Ni, Cd, Cr and Pb are harmful to plants and humans (Kirkillis *et al*., 2012; Parsafar and Marofi 2014). This food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body (Uwah *et al*., 2009).There are two main types of salts found world wide, the unrefined and refined that vary in physical and chemical characteristics depending on their source. Refined salt like table salt contains about 97% to 99% sodium chloride with 40% sodium and about 60% chloride with a maximum recommended level of sodium intake being 2,300 mg/day and a minimum of 1,500 mg/day with one table spoon of salt providing 2,000 mg of sodium (Brody, 1994). Contrary to refined salts, the chemical composition of unrefined salt varies depending on its source, either rock or plant.

Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals that make a significant contribution to daily mineral needs at all stages of the life cycle (Grusak, 2002). Besides sources of minerals in the human diet, edible plants are also sources of heavy metals intoxication through the food chain (King'ang'a, 2005). Indigenous salts were made by Kenyan local communities from either rock or plant materials. Depending on the source, each of these salts has a unique flavor, physical and chemical characteristics (Peker *et al.*, 2007). Western Kenya is one of the regions where plant derived salts are largely used in their unrefined forms. The salts are processed as ashes upon burning some plant materials (King'ang'a, 2005). In Lugari and Busia regions of Western Kenya, the reed plant stems and leaves are used for the preparation of the indigenous salt. This plant salt has been used traditionally as a salt substitute in Western Kenya. After the raw salt is obtained, it is neither purified nor refined.

There are five major processes of salt production commonly used; (1) evaporating the sea water using "salt-making potteries"; (2) evaporating water from the salt springs; (3) soaking the dried grass in saline water collected from salt springs followed by burning the grass in firewood and evaporating the filtrate; (4) burning salt-containing grass or plants, lixiviating the ash and

evaporating the solution; and (5) mining the salt rocks from caves and ground to get the "geological salt" (Janarthanan , 2015). Among them, vegetal salts deserve special mention due to their ease of preparation and wide spread utility. Burning grasses, ferns, herbs, shrubs, palms, leaves, barks, shoots, stems or even the whole plant and tree are the major adapted protocols. Throughout the world more or less the same methodology has been practiced, but with little variations in the plants, tools and equipment utilized. In general, plant materials will be burnt in selected firewood, the ashes will be lixiviated through the filters and the resulting brine will be slowly dehydrated to obtain the dry salt (Janarthanan, 2015). In order to take advantage of the health benefits of vegetal salts, an in-depth chemical analysis of the inorganic constituents has to be done. In most developing countries, once the salt has been processed, it is sold in packages or in bulk. Packaging materials include paper, high and low density polyethylene and woven bags made of jute, straw or high density polyethylene. Because iodine is unstable under the storage conditions found during the manufacturing, distribution, and sale of salt in most developing countries, the effects of packaging materials and environmental conditions on the stability of salt with iron and iodine are of concern. Equally, there are predictable chemical interactions when iodine and iron are combined.

In the presence of ferrous ions and oxygen, the iodine moiety of the double-fortified salt is likely to be unstable due to evaporation and catalytic oxidation of $I^-$ to $I_2$. Iron is also readily oxidized to the ferric form, which has a lowered bioavailability, an unpleasant taste and an unsightly, yellowish brown or rust color. The various storage methods and the length of storage may have implications on the quality and composition of the reed salt. The actual availability of micronutrients in the salt can vary over a wide range of methods used as a result of losses due to salt impurities, packaging and environmental conditions during storage and distribution. Other factors include losses due to food processing, washing, and even cooking in the households (Kabaija, 1989). Sodium is the main elemental component in most types of edible salts. High level dietary sodium consumption over extended period is associated with hypertension (Hollenberg, 2006). The chemical composition of the indigenous salts and the variability of the chemical elements that the consumers' metabolic systems are exposed to may not be uniform. Studies have shown that some of the micronutrients in salt including iodine and iron are lost over time if suitable ambient conditions are not established and maintained (Diosady *et al.*, 1998).

Consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Kucera, 2004; Tu¨rkdogan *et al*., 2002). In order to assess the health risks associated with contaminated food consumption, it is necessary to identify the potential sources that introduce risk agents into the environment, estimate the amount of risk agents that come into contact with the human-environment boundaries, and quantify the health consequence of the exposure (Ma *et al*., 2006). The health risk of the toxicity associated with the indigenous reeds salt is done by estimating the level of exposure of the heavy metals through the food chain. Health risk assessment is calculated in terms of estimated daily intake of metal (EDIM) and estimated health risk index (EHRI) by considering the intake of metal through the salts by the consumers and comparing the provisional tolerable weekly intakes (PTWIs) (Pourgheysari, 2012).

Salt is the oldest and commonly used food additive. It contains sodium and chloride ions majorly. These two ions are dominant chemical constituents of the human body that affect a wide range of important metabolic functions (WHO, 2007a). Salt has been used for long in cooking and other culinary applications. Today most people become accustomed to the taste of salt so that its consumption has increased despite numerous challenges it contributes to raise cases of hypertension, cardiovascular and kidney diseases. Besides Na and chloride ions, salt serves as a means of delivery of micronutrients including iodine, iron and potassium to the human body. The suitability of a salt for human consumption depends on the provision of micronutrients while eliminating possible toxicity from non-essential elements like Cd and Pb besides other heavy metals. The Na:K ratio is also key in ensuring that salt is suitable for human consumption. Estimation of heavy metal effect on the consumers is obtained from the EDIM and EHRI values. This study was carried out in selected areas in Busia and Lugari districts of Western Kenya where *T. latifolia* and *C. rotundus* reed species are found respectively. The aim of this study was to investigate and compare the micronutrient and heavy metal content of the indigenous reed salts across the selected areas. Results of the micronutrient and heavy metal composition were further used to estimate the suitability of the salt for human consumption by use of their Na:K ratios, EDIM and EHRI values. In addition, the effect of the reed species, methods of

preparation, modes of packaging, storage conditions of temperature and relative humidity and storage period on the stability of iodine and $Fe^{2+}$ in the salts prior to their consumption was also investigated.

### 1.2 Study Area

The study covered selected sampling areas from Lugari and Busia districts of the Western region of Kenya. Two sites were selected from each region, Matete and Lugari villages of Lugari district, in Kakamega region and Ululo and Bidimbidi villages in Matayos district of Busia region of Western Kenya (figure 1.1). A Global Positioning System (GPS) was used to obtain the grid references of the location of the study sites. The regions were chosen based on their significance in terms of processing of unrefined indigenous crystalline salts and farming agricultural patterns that are similar. *T. latifolia* reeds are used in Lugari district while *C. rotundus* reed species are used in Busia district (figure 1.2). Lugari district comprises of three administrative divisions Likuyani, Matete and Lugari, with a total area of 670.2 $km^2$. Two administrative units, Lugari and Matete divisions were selected in Lugari district for this study. Lugari district lies at an average altitude of between 1600-1999 m above sea level. It lies between longitudes 34028´ and 350 East and between latitude 00 25´ and 10 north of the equator. Lugari Division has temperatures varying from 8.8-28 $^oC$ within the year. Maximum temperature varies over the year between 22-28 $^oC$ while minimum temperature ranges between 12 - 16 $^oC$.

Rainfall pattern is bimodal; the mean being just over 1100 mm annually (900-2200 mm) with the long rains falling between the months of April and June, short rains fall between August and November while December to February are dry months. Well drained deep red to dark, sandy loams to sandy clays that are not very fertile but deep and well drained soils are the characteristic soil types found in Lugari district. Along River Nzoia, there is a complex of imperfectly drained and poorly drained very deep-dark grey to dark grayish brown sandy to clay in higher areas, while some perches of regosil and lithosols are found around Mwamba characterized by being shallow and rocky soils (Otsieno *et al.*, 2014; Republic of Kenya, 2009). Lugari district can be roughly divided into three different land use zones. Small-scale subsistence maize, sorghum, vegetable and sunflower characterize most parts of Lugari division which forms the central portion of the study area.

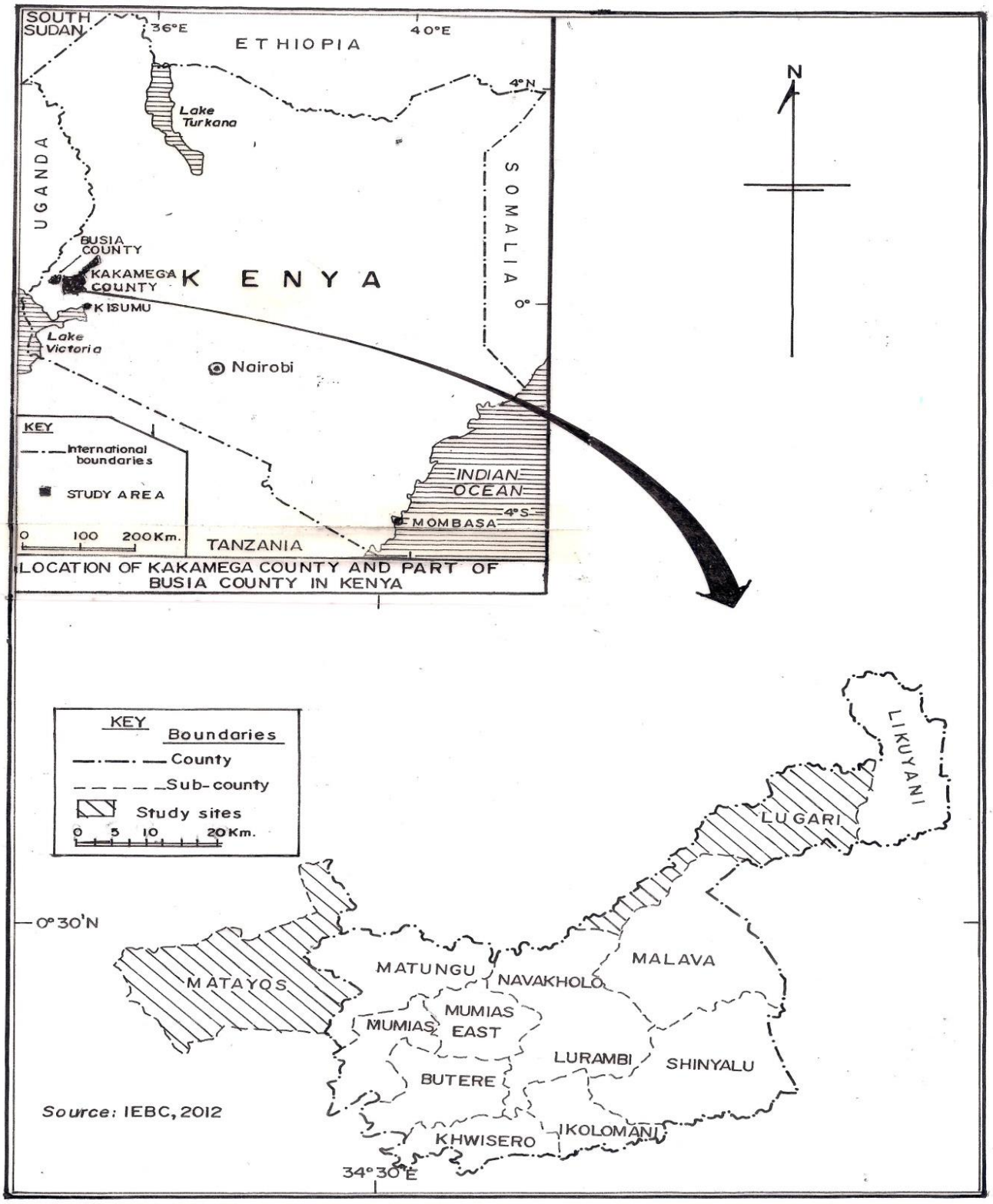



**Figure 1.1:** Map of Kenya Showing the sampling areas in Western Province (IEBC, 2012)

Both large-scale and small-scale sugar plantations and smaller maize schemes are located in Matete division plus livestock keeping.Thirdly, both large scale and small-scale maize farming and livestock keeping characterize the larger Likuyani division. Small-scale tea farmers are also found in the periphery of this division especially in Nzoia location. A part from agriculture, other forms of land use that are common in Lugari district include areas of land under gazzetted which accounts for about 83.9 $Km^2$ while the rest of the land is under urban development, transport and telecommunication network, public institutions, industries, quarries and mining, wetlands, water catchments, hills and rocks.


**Figure 1.2:** *C. rotundus* (left) and *T. latifolia* reeds (right) from Lugari and Busia regions of Western Kenya respectively.

Busia is one of the four Counties that comprise western Kenya and it is situated at the extreme Western border of Kenya. It borders Bungoma County to the north, Kakamega to the east, Siaya to the south west, Lake Victoria to the south east and the Republic of Uganda to the west. It lies between latitude 0º and 0º 45 north and longitude 34º 25 east and covers an area of 1694.5 $km^2$ (CRA, 2011). The larger part of Busia County falls within the Lake Victoria Basin and has an undulating altitude that rises from about 1,130 m above sea level at the shores of Lake Victoria

to a maximum of about 1,500 m in the Samia and North Teso Hills. It receives an annual rainfall of 1300-1800 mm. It has two rain seasons, the long rain season which is at its peak between late March and late May and the short rains occurring between August and October. The dry season with scattered rains falls from December to February. The temperatures for the whole County are more or less homogeneous. The annual mean maximum temperatures range between 26 °C and 30 °C while the mean minimum temperature range between 14 °C and 22 °C. Most parts of Busia have sandy loam soils although dark clay soils cover the northern and central parts of the County whereas. Other soil types are sandy clays and clays. These soils are good for the production of a large range of food and cash crops such as sorghum, finger millet, maize, cassava, cotton, tobacco, robusta coffee, sunflower and sugarcane.

The County is divided into six administrative divisions, namely Nambale, Butula, Funyula, Budalangi, Township and Matayos. Matayos division was randomly selected for this study in Busia district (Republic of Kenya, 2002-2008a). Matayos falls in the southern most part of Busia County and is covered by the Yala Swamp which is a down warped area associated with the formation of Lake Victoria. It is well served by streams and bisected by the River Sio as it flows from Mount Elgon toward Lake Victoria. Matayos is hilly to the south-east, with the land generally slanting to its lowest points in the Sio swamp, and rising again as it sprawls towards the international border with Uganda. The area forms a colony of papyrus growth and is broken by irregular water channels and occasional small dams with grassy islands. This area is covered with locustrine and alluvial deposits of recent and Pleistocene times. Other rivers that form the drainage system in the County includes Malakisi river to the extreme north, Malaba in the northern entry of the central region and River Sio in Samia and Nambale Sub-Counties and river Nzoia that drains into Lake Victoria through Bunyala Sub-County. The inhabitants of Matayos are subsistence farmers who mainly grow cassava, white and yellow (Nyayo) maize, sweet potatoes, ground nuts, soya beans, sorghum, finger millet, yams, bananas and beans on small pieces of land, usually an acre or less. They raise livestock on a similarly small scale, mainly cattle which acts as drought animals for ploughing purposes, some goats, pig farming, fishpond farming, sheep and usually some free-range chickens the area also grow cash crop such as sugarcane farming. The soils in the district are moderately deep, generally rocky and stony consisting of well-drained red clays which have a low natural fertility. In the swamps, there are

heavy clay types, which are very difficult to cultivate, both when it is dry and wet. The County has approximately 925,200 hectares (924 $km^2$) of agricultural land. In the downstream areas the wetlands mainly occur in the floodplain of major rivers mainly Nzoia and Sio. Vegetation is sparse consisting mainly of *Sesbania* spp, *Cyperus* spp, *Sedge* grass and *Typha*.

### 1.3 Statement of the Problem

In the recent past, there has been an increase in occurrences of toxicity associated with food and food additives due to lack or overconsumption of micronutrients laced with heavy metals. The consumption of unrefined indigenous reed salts from wetland plants in Western Kenya is a great concern. Besides the micronutrients such as iodine and iron, this salt can also deliver heavy metals like cadmium, lead among others that could pose a health risk to its consumers. Consequently, the increasing demand for traditional remedies and cheaper commodities with limited scientific knowledge on their chemical composition poses a serious health concern. There have been rising cases of health disorders associated with consumption of sodium, iodine and iron micronutrients (hypertension, goitre and anaemia) in Busia and Lugari regions of Western Kenya (WHO, 2013). In 2004, 47.4% cases of children under 5 years old and 41.8% pregnant women in Kenya suffered from anaemia (WHO, 2004). In 2008, 53.1% cases of iodine deficiency disorders (IDD) and 15.5% of iron deficiency anaemia (IDA) were reported in Kenya. In the same year, Busia region for instance reported 31.6% and Kakamega-Lugari region reported 26.7% of the toatal 53.1% IDD cases (WHO, 2008). The use of these salts as traditional remedies in treatment of various illnesses in this region has also been reported by some researchers. The population is likely to be consuming indigenous reed salt product that has no medicinal or nutritive value or may be contaminated with heavy metals. There is need to scientifically ascertain the chemical composition of these reed salts.

### 1.4 Objectives

#### 1.4.1 General Objective

To assess the suitability of the indigenous reed salt used in selected parts of Western Kenya with regard to micronutrients and heavy metal contamination and their health risk indices.

### 1.4.2 Specific Objectives

1. To determine the concentration of selected micronutrients and heavy metals in soil habitats of papyrus reed plants, *C. rotundus* and *T. latifolia* from selected areas of Lugari and Busia regions of Western Kenya respectively
2. To measure the concentration of selected micronutrients and heavy metals of indigenous reed salts processed from *C. rotundus* and *T. latifolia* and estimate the health risk associated with the consumption of these indigenous reed salts.
3. To determine the effect of the various methods of salt processing, various storage conditions of the unrefined indigenous papyrus salts on the chemical composition, availability and stability of iron and iodine nutrients.

## 1.5 Hypotheses

1. There is no statistically significant difference in the concentration of selected micronutrients and heavy metals in soil habitats of papyrus reed plants, *C. rotundus* and *T. latifolia* of selected areas of Lugari and Busia regions of Western Kenya even on application of the principal component analysis method.
2. There is no statistically significant difference in the concentration of selected micronutrients and heavy metals in indigenous reed salts processed from *C. rotundus* and *T. latifolia* and they pose no health risk.
3. There is no statistically significant difference in the levels and stability of iron and iodine in the reed indigenous salts when processed using the two types of methods of preparation and when packaged and stored under varying conditions.

## 1.6 Justification

Lugari and Busia regions of Western Kenya harvest salt from two different reed species. This indigenous salt is used as a food additive, as a table salt, for cooking and as medicine. With the increasing demand for cheaper commodities and treatment of varied sicknesses, traditional remedies are finding a lot of application irrespective of the chemical composition of such traditional food additives as well as medicine. These findings will enhance the scientific knowledge on iodine and iron II, Na and K levels as well as heavy metals Cd, Pb, Cr and Fe. Equally, knowledge on the effects of methods of preparation, storage conditions and plant

species on the chemical composition of the salt will be enhanced. The amount of data generated in this study can be useful in addressing health, food security and environmental related issues by major decision makers. Knowledge on the effects of method of preparation, plant species, storage methods as well as storage time on the chemical composition of the indigenous reed salt in comparison to commercially available salts and the WHO permissible limits will help address conditions necessary for indigenous reed salt production. It will equally provide a basis for undertaking necessary interventions for ensuring potential cases of goitre; anaemia and hypertension associated with these micronutrients are eliminated. This will also inform on the safety of consumption of this salt and the suitability of indigenous reed salt as a low sodium salt as well as table salt for human consumption. Besides domestic consumption of these salts in the Western Region of Kenya, these indigenous salts are also traded to a smaller scale as a commodity across the EA boarders. Validation of the chemical composition of the reed salt may contribute towards increased trade and food security consequently improving the living standards of people in Western Kenya.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 General Introduction

In the last few decades, growing interests in environmental issues in connection with human and animal health have prompted a renewed focus on micronutrient and trace elements (Korkmaz *et al*., 2010). Air, soil and water pollution leads to presence of harmful elements such as lead, cadmium, mercury and arsenic in foodstuffs (Zukowska and Biziuk, 2008) which are non-essential and toxic even at low concentrations. For non-occupationally exposed humans, water and food consumption is the major route of exposure to toxic elements accounting for more than 90% of exposure to these substances, compared to that from inhalation and dermal contacts (Chen *et al*., 2011). Heavy metals are defined as those groups of elements that have specific weights higher than 5 $g/cm^3$. A number of them (Co, Fe, Mn, Mo, Ni, Zn and Cu) are essential micronutrients and are required for normal growth and take part in redox reactions, electron transfers and other important metabolic processes in plants. Metals which are considered non-essential include Pb, Cd, Cr and are potentially toxic to plants (Sebastiani and Scebbaf, 2004). Other nutrients such as sodium, iodine, iron are essential for humans; they play important roles in biological systems. When their intake exceeds permissible levels, they become harmful and cause toxic effects (Zarei *et al*., 2011).

Food stuffs and food additives including salt may contain both essential and non-essential elements (Zukowska and Biziuk, 2008). Although this salt as a food additive is an ideal vehicle for the delivery of physiological amounts of micronutrients, it may also deliver traces of heavy metals. Research has shown that food additives either lack or have higher micronutrients or are contaminated with heavy metal which causes toxicity to its consumers. Table salt is consumed daily, so any contamination, even at low levels, could be hazardous to the consumer's health. Today most people become accustomed to the taste of salt so that its consumption has increased despite numerous challenges it contributes to raise cases of hypertension, cardiovascular and kidney diseases. Part of its overwhelming influence stems from its role as a source of sodium and chloride, two dominant chemical constituents of the human body with important metabolic functions (WHO, 2007a). In addition to its use as a flavor enhancer, it is also used as a food

preservative to inhibit the growth of spoilage and pathologic bacteria with a smaller proportion of sodium carbonate which is used to tenderize food during cooking (Zarei *et al*., 2011). Salt is a food additive that is considered to be an excellent carrier of micronutrients (Amorin and Ferrerira, 2005).

Elements like iron, sodium and iodine are essential for human life since they play an important role in biological systems; however, in excess these elements can also produce toxic effects. Others like cadmium, chromium, lead and mercury are non-essential and contribute to several disorders (Zukowska and Biziuk, 2008) including cardiovascular, neural, hematopoietic, immunological and gastrointestinal systems (Inbaraj and Chen, 2012; Sabolić, 2006). They can also cause kidney dysfunction, anemia, liver toxicity, cancer and Alzheimer disease (Inbaraj and Chen, 2012). Since most of the salt consumed in the globe and in particular, the Western region of Kenya comes from plant products and mines, it is expected that heavy metal contamination is a serious concern (Zarei *et al*., 2011). Because of chemical similarities, these heavy metals compete with essential elements and interact with several divalent transporters leading to disruption in different physiologic functions (Inbaraj and Chen, 2012).

Heavy metals may enter the food chain as a result of their uptake by edible plants. The high level of heavy metals in the soil could indicate similar concentration in plant by accumulation at concentration causing serious risk to human health when consumed (Singh *et al*., 2010). Plant accumulates a number of mineral elements essential to human nutrition, though it equally accumulates other mineral elements, the heavy metals, such as Cd, Pb and Fe, which are in no direct use to humans but injurious to health (Farell *et al*., 2010). Indigenous table salt has been considered in the present study, with the aim of establishing the presence of essential and non-essential elements in the table salt. Despite the limited knowledge on indigenous reed salt used in Western Kenya, other factors that may affect the composition of these essential and non-essential elements have also not been well understood.

Factors which include method of reed salt production, storage and packaging and their conditions, reed species among others need to be considered. This study is designed to assess the chemical composition of the reed salt as well as effects of its processing, packaging and storage over time with an attempt to use it as edible and Lo-Na salts.

## 2.2 Soil Properties

The interactions between metals and solid phases of soil, soil water, and air within and above soil depend on a variety of chemical factors and determine the heavy metal transport and fate. Absorption of metals from soil water to soil particles is the most important chemical determinant that limits mobility in soils (Curtis *et al*., 2002). As a result of increasing anthropogenic activities, heavy metal pollution of soil, water, and atmosphere represents a growing environmental problem affecting food quality and human health. The chemistry of metal interaction with soil matrix is central to the phytoremediation concept and sorption to soil particles reduces the activity of metals in the system.

### 2.2.1 Soil Texture, Loss on Ignition (LOI) and Bulk Density (BD)

Soil texture is the relative proportions of sand, silt, or clay in a soil. The soil texture has an important role in nutrient management because it influences nutrient retention where finer textured soils tend to have greater ability to store soil nutrients. Soils with the finest texture are called clay soils, while soils with the coarsest texture are called sands. However, a soil that has a relatively even mixture of sand, silt, and clay and exhibits the properties from each separate and is called a loam soil. Soil organic matter is frequently said to consist of humic substances and nonhumic substances (figure 2.1, Dube *et al.*, 2001).

Organic-mineral particles expressed as LOI can vary widely in their adsorption properties because of diverse specific surfaces, charge densities and widely different SOM content. The term SOM is generally used to represent the organic constituents in soils including undecayed plant and animal tissues, their partial decomposition products, and soil biomass. Organic Matter may range in soils from 0.1% in desert soils to 90% in organic soils (Dube *et al*., 2001).

The bulk density of an organic soil is the weight of a given volume of soil usually expressed on a dry weight basis in grams per cubic centimeter. Values range from 0.05 g/cm$^3$ in very fibric, undecomposed materials to less than 0.5 g/cm$^3$ in well decomposed materials (Lucas, 1982). Bulk densities of a mean of 0.12 and 0.09 g/cm$^3$ have been reported (Zamora-Cristales *et al.*, 2014) with values greater than 0.2 g/cm$^3$for well decomposed sapric peats. Bulk density depends on the amount of compaction, the botanical composition of the materials, their degree of

decomposition, and the mineral and moisture contents at the time of sampling with bulk density greater than 1.6 g/cm$^3$ tending to restrict root growth (McKenzie *et al.*, 2004).

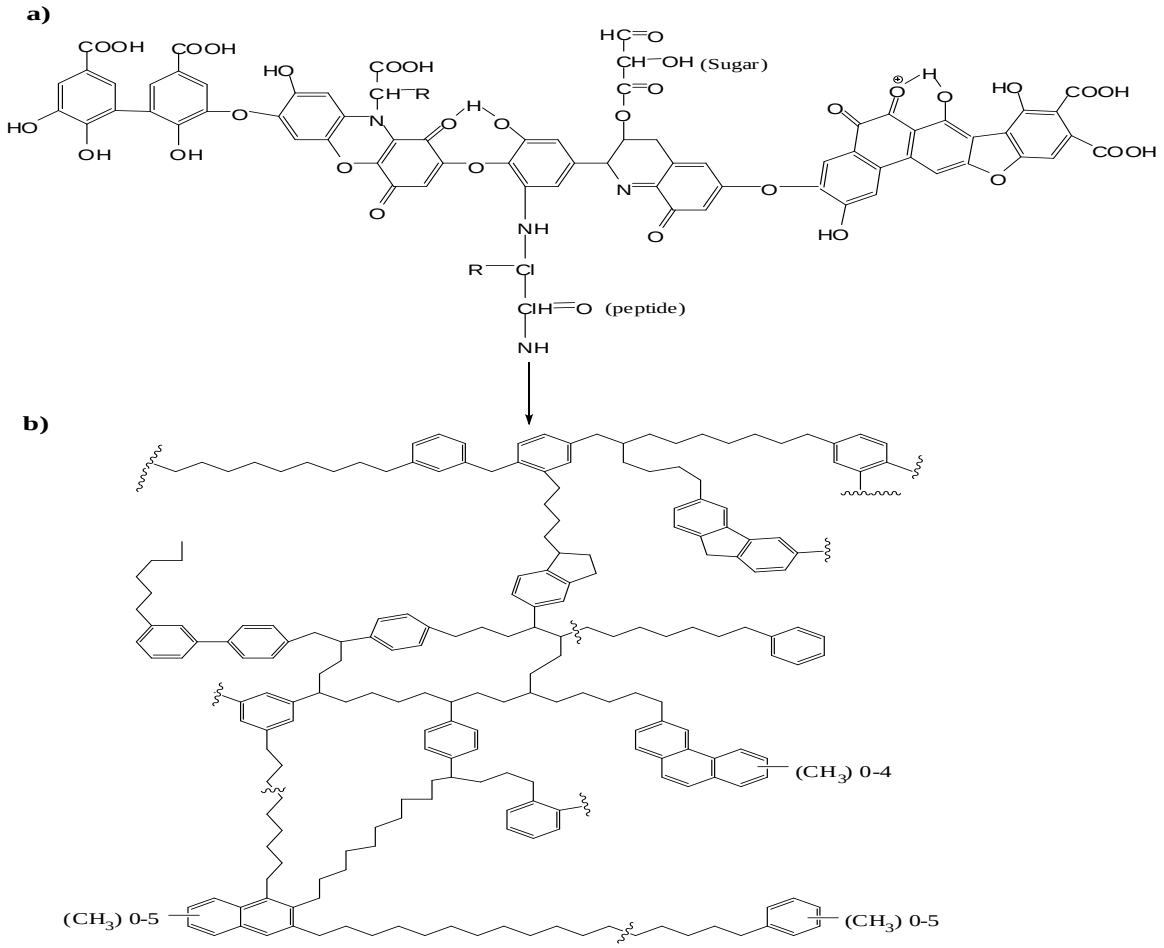



**Figure 2.1:** Model structures of humic acids according to Stevenson (1992) (a) and Schulten, Plaige and Schnitzen (1991) (b) according to Dube *et al.*, (2001)

### 2.2.2 Soil CEC, pH and Nutrient availability

Cation exchange capacity (CEC) is the maximum amount of cations that 100 g of dry soil can absorb. It is a measure of the soil's ability to hold positively charged ions. It influences soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton and Murphy, 2007). Soils with a higher organic matter and clay fraction tend to have a higher CEC. The CEC of soils varies according to the clay percentage, the type of clay, soil pH and amount of organic matter. In general, an increase in CEC decreases uptake of

metals by plantsSandy soils rely heavily on the high CEC of organic matter for the retention of nutrients in the topsoil. The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force. This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. magnesium, potassium and calcium).

The main ions called base cations, associated with CEC in soils are the exchangeable cations $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$ and $K^{+}$ (Schmidt, 2003). In most cases, summing the analysed base cations gives an adequate measure of CEC (CEC by bases). However, as soils become more acidic these cations are replaced by $H^{+}$, $Al^{3+}$ and $Mn^{2+}$, and common methods will produce CEC values much higher than what occurs in the field (McKenzie *et al.*, 2004). Thus, the higher the cation exchange capacity (CEC) of the soil, the greater the sorption and immobilization of the metals. Because a higher CEC usually indicates more clay and organic matter is present in the soil, high CEC soils generally have greater water holding capacity than low CEC soils. The lower the CEC of a soil, the faster the soil pH will decrease with time. Soils with a low CEC are more likely to develop deficiencies in $K^{+}$, $Mg^{2+}$ and other cations while high CEC soils are less susceptible to leaching of these cations (CUCE, 2007). In acidic soils, metal desorption from soil binding sites into solution is stimulated due to $H^{+}$ competition for binding sites. The sum of the base cations provides an estimate of the CEC of each soil layer. The surface soils have higher organic matter content while the sub-soils have lower CEC. Organic matter plays an important role in soil structure, water retention, cation exchange and in the formation of complexes (Alloway and Ayres, 1997).

Soil pH affects not only metal bioavailabilty, but also the very process of metal uptake into roots, depending on the metal in question. Soil pH directly affects the concentration of major nutrients and the forms of microelements available for plant uptake and can result in deficiencies or toxicities. At low pH metals are more soluble in the soil solution, hence more bioavailable to plants. Hence, toxicity problems are more severe in acidic soils than in alkaline soils. Because hydroxide ion activity is directly related to pH, the solubility of metal hydroxide minerals increases with decreasing pH, and more dissolved metals become potentially available for incorporation in biological processes as pH decreases. The adsorption edge and the pH range

over which the rapid change in sorption capacity occurs, varies among metals, which results in precipitation of different metals over a large range of pH units. Consequently, mixing metal-rich, acidic water with higher pH, metal-poor water may result in dispersion and separation of metals as different metals are adsorbed onto various media over a range of pH values (McKenzie *et al.*, 2004).

### 2.2.3 Soil Profiles, Soil Iodine and Plant Iodine

The average iodine concentration in the earth's crust is approximately 0.25 mg $kg^{-1}$ (Fuge and Johnson, 1986). From the atmosphere iodine is transferred to soils by both wet and dry deposition (Amachi *et al*., 2005). Mixture of species including iodate, iodide and organic iodine species have all been reported; the proportion of each species depends on sampling location (Gilfedder *et al*., 2007c; Yoshida *et al*., 2007). Iodine in soils has been studied extensively and a worldwide average concentration in surface soils reported to be ~5 mg $kg^{-1}$ (Fleming, 1980) however, UK soils concentrations range from 0.5 to 98.2 mg $kg^{-1}$ (Whitehead, 1979). Yuita and Kihou (2005) studied iodine depth profiles in three Japanese soils and observed higher concentrations in topsoil compared to subsoil especially where the subsoil was more reducing. Strong correlations were found between soil iodine and sesquioxide content which was attributed to the scavenging characteristics of soil metal oxyhydroxides for inorganic iodine forms (Dai *et al*., 2004; Um *et al*., 2004). Sorption of iodide and iodate on Fe and Al oxides is favourable under oxidative conditions. Under reducing conditions, dissolution of metal oxyhydroxides releases sorbed iodine (Ashworth and Shaw, 2006; Yamaguchi *et al*., 2006). Close association of iodine with soil organic matter has also been widely reported leading to the conclusion that soil iodine is mainly organically-bound (Steinberg *et al*., 2008 a, b).

The inverse relationship between the sorption of iodide and/or iodate and soil pH (Dai *et al*., 2004; Um *et al*., 2004) resembles that of other anions known to be nonspecifically sorbed on soils such as $Cl^-$, $NO_3^-$, and $SO_4^{2-}$ and those specifically adsorbed with low pKa values, such as $F^-$ and $PO_4^{3-}$. Phosphate ($PO_4^{3-}$) is strongly adsorbed by aluminum and iron oxides and, therefore, can be used as an extractant for other ions of equal or lower adsorption affinity such as sulphate and iodine. Transfer of iodine from soil to plants is generally low and locally grown plants often cannot supply a population with the recommended daily intake of iodine (Johnson, 2003). Iodine

enters a plant either by root uptake from soil solution or through leaves from rain drops, atmospheric particulates and gaseous forms of iodine (Fuge, 2005). Studies of root uptake have suggested that most of the iodine remains in the roots and is not translocated to the rest of the plant (Voogt *et al*., 2010). This would imply that atmospheric iodine may be the most important source of the total iodine inventory in the aerial parts of plants. In a recent study, however, Landini *et al*., (2011) found that $125^{I-}$ supplied to tomatoes grown in a hydroponic system was widely distributed throughout the whole plant. Re-distribution of iodide or iodate from nutrient or soil solutions via plants root to the edible parts of the plant has also been reported by Zhu *et al*., (2004) and Weng *et al*., (2008). Zhu *et al*., (2003) and Voogt *et al*., (2010) found that ryegrass, rice and lettuce, grown in hydroponic systems absorbed up to 20 times more iodide than iodate. When rye grass was grown in soil, greater uptake of iodate than iodide was observed which was attributed to the longer residence time of iodate in soils compared to iodide which can be readily fixed into humus and rendered unavailable (Whitehead, 1975). Iodate sorption on soil metal oxides and incorporation into soil organic matter (via reduction to $I_2$ or HOI) both happen to a greater extent under acidic conditions (Um *et al*., 2004).

Iodate oxidises iodide to form iodine;

$$IO^-_{3(aq)} + 5I^-_{(aq)} + 6H^+_{(aq)} \rightarrow 3I_{2(g)} + 3H_2O_{(l)} \qquad 2.1$$

Reduction of iodine to iodide;

$$2I_{2(g)} + IO^-_{3(aq)} + 2H_2O_{(l)} + H^+_{(aq)} \rightarrow 5HOI_{(aq)} \qquad 2.2$$

### 2.3 Trace and Heavy metals in Soils and Plants

#### 2.3.1 Trace and Heavy metals in Soils

Soil is the major sink for heavy metals released in the environment through poor agricultural practices, disposal of industrial and urban wastes and most do not undergo microbial or chemical degradation with their total concentrations persisting in the soils for a long time (Tran and Popova, 2013). In soil, metals are associated with several fractions: (1) in soil solution as free metal ions and soluble metal complexes; (2) adsorbed to inorganic soil constituents at ion exchange sites; (3) bound to soil organic matter; (4) precipitated such as oxides, hydroxides, and

carbonates; and (5) embedded in structure of the silicate minerals. For phytoextraction to occur, contaminants must be bioavailable (ready to be absorbed by roots). Bioavailability depends on metal solubility in soil solution. Only metals associated with fractions 1 and 2 (above) are readily available for plant uptake (Khan *et al.*, 2008). Moreover, heavy metals are toxic because they tend to bioaccumulate in plants and animals, bioconcentrate in the food chain and attack specific organs in the body (Balkhair and Ashraf, 2016). Table 2.1, gives the soil concentration ranges and regulatory guidelines for some toxic metal, (Riley *et al*., 1992). Plants grown on polluted land can absorb heavy metals in form of mobile ions present in the soil solution through their roots or through foliar absorption and get bio accumulated in the roots, stems, fruits, grains and leaves of plants (Fatoki, 2000).

**Table 2.1:** Soil concentration ranges and regulatory guidelines for some toxic metal

| **Heavy metal** | **Threshold in mg/kg** | | | |
|---|---|---|---|---|
| | **Soil concentration range[a], mg/kg** | **Regulatory limits[b], mg/kg** | **US-EPA[c], mg/kg** | **WHO values[c], mg/kg** |
| Cadmium | 0.10-345 | 100 | 70 | 3 |
| Chromium | 0.05-3,950 | 100 | 230 | ng |
| Lead | 1.00-69,000 | 600 | 400 | 50 |

[a]Riley *et al*.,1992, [b]NJDEP, 1996; [c]Kananu *et al*., 2014; ng=not given

Some heavy metals like iron serve as plant nutrients depending on their concentrations while lead, cadmium, chromium and many others that are indirectly distributed as a result of human activities could be very toxic even at low concentrations and are non-biodegradable (Korkmaz *et al*., 2010). Fe availability is dictated by the soil redox potential and pH. In soils that are aerobic or of higher pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides. At lower pH, the ferric Fe is freed from the oxide, and becomes more available for uptake by roots. Cadmium occurs primarily in exchangeable, readily bioavailable form while Pb occurs as soil precipitate, a significantly less bioavailable form.The fate and transport of a heavy metal in soil depends significantly on the chemical form and speciation of the metal. Once in the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity. This distribution is believed to be controlled

by reactions of heavy metals in soils such as (i) mineral precipitation and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake (Farrell *et al*., 2010).

The adsorption capacity (both exchange and specific adsorption) of a soil is determined by the number and kind of sites available. Adsorption of metal cations has been correlated with such soil properties as pH, redox potential, clay, soil organic matter, Fe and Mn oxides, and calcium carbonate content with Pb>Cu>Cd>Zn order in organic soils and Pb>Cu>Zn>Cd in mineral soils (Elliott *et al*., 1986). Anion adsorption has been correlated with Fe and Mn oxide content, pH, and redox potential. Adsorption processes are affected by these various soil factors, by the form of the metal added to the soil, and by the solvent introduced along with the metal. The results of these interactions may increase or decrease the movement of metals in the soil water. Korte *et al*., (1976) qualitatively ranked the relative mobilities of 11 metals added to 10 soils to simulate movement of metals under an anaerobic landfill situation. Of the cationic metals studied lead and copper were the least. Griffin and Shimp (1978) found the relative mobility of nine metals through montmorillonite and kaolinite to be: Cr (VI) > Se > As (III) > As (V) > Cd > Zn >Pb > Cu > Cr (III).

### 2.3.2 Trace and Heavy metals in Plants

Plants take up trace elements from the soil solution, where ions are in equilibrium with those located in the solid phase through various reactions, including adsorption, exchange, complexation with organic and inorganic ligands, redox reactions, and precipitation-dissolution (Morel, 1997). The extent of the reactions, and hence the solubility of trace elements, is a function of soil mineral content (e.g. silicate layers, carbonates, oxides and hydroxides), soil organic matter (e.g. humic and fulvic acids, polysaccharides and organic acids), soil pH, redox potential and soil temperature and humidity. The risks of heavy metal transfer into the food chain are dependent on the mobility of the heavy metal species and their availability in the soil (Richards *et al.*, 2000). Among inorganic contaminants, heavy metals are important due to their non-degradable nature leading to bioaccumulation through tropic levels, which may have adverse biological effects (Wagesho, 2015). Even at low concentrations, elements such as Ni, Cd, Cr and Pb are harmful to plants and humans (Parsafar and Marofi, 2014).

The plant species differ widely in their ability to accumulate heavy metals. While plants serve as sources of minerals in the diet they are also sources of heavy metal intoxication to consumers (Islam *et al*., 2007). Uptake of metals into root cells, the point of entry into living tissues, is a step of major importance for the process of phytoextraction (Nouri *et al*., 2009). However, for phytoextraction to occur, metals must also be transported from the root to the shoot. Movement of metal-containing sap from the root to the shoot, termed translocation, is primarily controlled by two processes: root pressure and leaf transpiration. Following translocation to leaves, metals can be reabsorbed from the sap into leaf cells (Kirkillis *et al*., 2012).

Heavy metals are generally present in agricultural soils at low levels (Uwah *et al*., 2009). Due to their cumulative behaviour and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health (Idzi *et al.,* 2013). The source of heavy metals in plant is the environment in which they grow and their growth medium (soil) from which heavy metals are taken up by roots or foliage of plants (Okonkwo *et al.,* 2005). Heavy metal uptake from the soil and accumulation in plants depends upon bioavailability of the metal in the water phase, soil pH, plant species, fertilizers, and soil type and the efficiency of different plants in absorbing metals (Wuana and Okieimen, 2011), as shown in figure 2.2, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water.

Heavy metal uptake by plants may also depend on plant species, properties of medium like chelators and fertilizers, root zone and vegetative cover (Idzi *et al.,* 2013). Furthermore, when metals have been bound to the soil, the pH, redox potential, and organic matter content will all affect the tendency of the metal to exist in ionic and plant-available form. It is evaluated by either plant uptake or soil-to-plant transfer factors of the metals (Rattan *et al*., 2005). Typically, the soil-to-plant transfer factor is one of the key components of human exposure to metals through the food chain (Cui *et al*., 2004). The high transfer values for metals from soil to plants indicate a strong accumulation of the respective metals by food crops, particularly by leafy vegetables. Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals (Sebastiani *et al*., 2004), increasing the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients (Yoneyama *et al*., 2015).

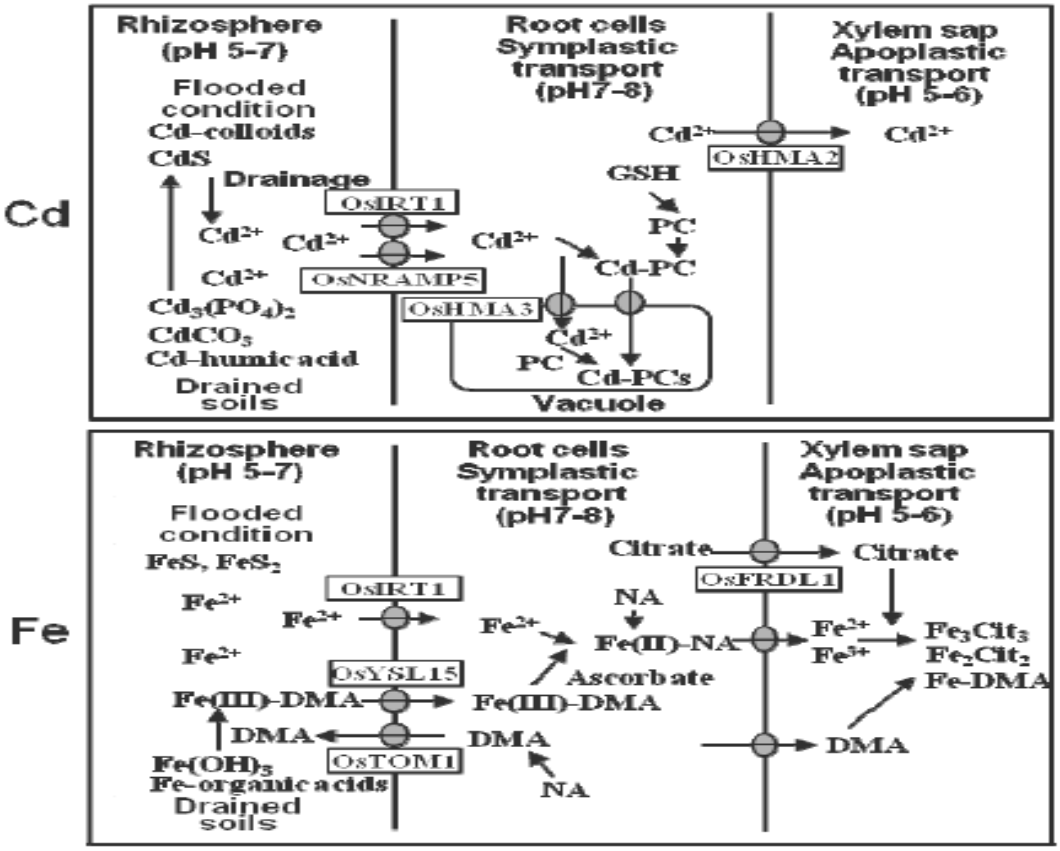



**Figure 2.2:** Models of uptake and transport of Cd and Fe in plant roots. DMA, 2′-deoxymugineic acid; GSH, glutathione; NA, nicotianamine; PC, phytochelatin. (Yoneyama *et al*., 2015), Arrows indicate flows of substances and grey ellipses indicates transporters.

Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals (Sebastiani *et al*., 2004), increasing the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients (Yoneyama *et al*., 2015). Heavy metals transfer in plants may take one of several forms: phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization. Plants tend to remove harmful chemicals in the ground when their roots take in water and nutrients from polluted soil, streams and groundwater and stored in stems, leaves and roots. Consumption of contaminated plant biomass is a cause of concern since contaminants may still enter the food chain through plant materials or products of the same, containing contaminants (Angima, 2010). According to Merkl *et al*., (2005), the plants act both as "accumulators" and "excluders". Water, evaporating from plant leaves, serves as a pump to absorb nutrients and

other soil substances into plant roots (Merkl *et al.*, 2005). This process, termed evapotranspiration, is responsible for moving contamination into the plant shoots as well. Since contamination is translocated from roots to the shoots, which are harvested, contamination is removed while leaving the original soil undisturbed (Mwegoha, 2008). Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times those taken up by nonaccumulator (excluder) plants (Hammer *et al.*, 2003).

Because of their charge, metal ions cannot move freely across the cellular membranes, which are lipophilic structures. Therefore, ion transport into cells must be mediated by membrane proteins with transport functions, generically known as transporters. Trans-membrane transporters possess an extracellular binding domain to which the ions attach just before the transport, and a trans-membrane structure which connects extracellular and intracellular media. The binding domain is receptive only to specific ions and is responsible for transporter specificity. The trans-membrane structure facilitates the transfer of bound ions from extracellular space through the hydrophobic environment of the membrane into the cell. These transporters are characterized by certain kinetic parameters, such as transport capacity (Vmax) and affinity for ion (Km). Vmax measures the maximum rate of ion transport across the cellular membranes. Km measures transporter affinity for a specific ion and represents the ion concentration in the external solution at which the transport rate equals Vmax/2. A low Km value, high affinity, indicates that high levels of ions are transported into the cells, even at low external ion concentration. By studying kinetic parameters, Km and Vmax, plant biologists gain insights to specificity and selectivity of the transport system.

It is important to note that of the total amount of ions associated with the root only a part is absorbed into cells. A significant ion fraction is physically adsorbed at the extracellular negatively charged sites ($COO^-$) of the root cell walls. The cell wall-bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass (phytoextraction). Thus, it is possible for a plant exhibiting significant metal accumulation into the root to express a limited capacity for phytoextraction. For example, many plants accumulate Pb in roots, but Pb translocation to shoot is very low. In support of this, Blaylock and Huang 1999) concluded that the limiting step for Pb phytoextraction is the long-distance translocation

from roots to shoots. Binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoot. Metals can also be complexed and sequestered in cellular structures like vacuole, becoming unavailable for translocation to the shoot (Lasat *et al*., 1998). In addition, some plants, coined excluders, possess specialized mechanisms to restrict metal uptake into roots. However, the concept of metal exclusion is not well understood (Peterson, 1983).

**Lead**

Lead (Pb) exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals (Gzar, *et al*., 2014). Lead (II) is the most common and reactive form of Pb, forming mononuclear and polynuclear oxides and hydroxides (Wang *et al.*, 2009). Ionic lead, Pb (II), lead oxides and hydroxides, and lead-metal oxyanion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters. The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead (hydr) oxides (Raskin and Ensley, 2000). Lead sulfide (PbS) is the most stable solid form within the soil matrix and forms under reducing conditions, when increased concentrations of sulfide are present. Under anaerobic conditions a volatile organolead (tetramethyl lead) can be formed due to microbial alkylation (Wang *et al.*, 2009).

Major sources of lead are exhaust fumes from vehicles, industrial gases and liquid effluents, some phosphate fertilizers and pesticides. Lead accumulates in the upper 8 inches of the soil and is highly immobile, and its contamination is long-term (Lasat, 2000) and its effects are generally limited to especially contaminated areas. Lead is the leading cause of heavy metal poisoning with a major source coming from soils and its uptake by plants is reduced when the soil pH is above 6.5 (Angima, 2010). Typical mean Pb concentration for surface soils worldwide averages 32 $mg\,kg^{-1}$ and ranges from 10 to 67 $mg\,kg^{-1}$ (Wuana and Okieimen, 2011). Since plants do not take up large quantities of soil lead, the lead levels in soil considered safe for plants will be much higher than soil lead levels where eating of soil is a concern (pica) (Gzar *et al*., 2014). Generally, it has been considered safe to use garden produce grown in soils with total lead levels less than 300 ppm. The risk of lead poisoning through the food chain increases as the soil lead level rises above this concentration. Even at soil levels above 300 ppm, most of the risk is from lead

contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant (Rosen, 2002).

Pb accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. Inhalation and ingestion are the two routes of exposure, and the effects from both are the same (Gzar *et al*., 2014). It has been reported by Naithani *et al.*, (2010) that lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk.

**Chromium**

It is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite, $FeCr_2O_4$ (Ghani *et al*., 2012). Chromium (VI) is the form of Cr commonly found at contaminated sites. Chromium can also occur in the +III oxidation state, depending on pH and redox conditions. In acid solution, $Cr^{3+}$ is always an octahedral hexaquo ion, $Cr(H_2O)_6{}^{3+}$. It tends to hydrolyze with increasing pH, resulting in the formation of polynuclear complexes containing OH- bridges (equation 2.3).

$$Cr(H_2O)_6^{3+}{}_{(aq)} \rightarrow [Cr(H_2O)_5OH]^{2+}{}_{(aq)} \rightarrow [Cr(H_2O)_n(OH)_m]^{3+}{}_{(aq)} \rightarrow mCr(OH)_{3(aq)} \qquad 2.3$$

Above pH 8, only $CrO_4^{2-}$ is stable, and as the pH decreases into the pH region 2-6, the equilibria shifts to dichromate according to the overall equilibrium (equation 2.4), figure 2.3:

$$2CrO_4^{2-}{}_{(aq)} + 2H^+{}_{(aq)} \rightarrow Cr_2O_7^{2-}{}_{(aq)} + H_2O_{(l)} \qquad 2.4$$

Chromium (VI) is the dominant form of Cr in shallow aquifers where aerobic conditions exist.Chromium (VI) can be reduced to Cr (III) by soil organic matter, $S^{2-}$ and $Fe^{2+}$ ions under anaerobic conditions often encountered in deeper groundwater. Major Cr (VI) species include chromate ($CrO_4{}^{2-}$) and dichromate ($Cr_2O_7{}^{2-}$) which precipitate readily in the presence of metal cations (especially $Ba^{2+}$, $Pb^{2+}$, and $Ag^+$). Chromate and dichromate also adsorb on soil surfaces,

especially iron and aluminum oxides. Chromium (III) is the dominant form of Cr at low pH (<4). $Cr^{3+}$ forms solution complexes with $NH_3$, $OH^-$, $Cl^-$, $F^-$, $CN^-$, $SO_4^{2-}$, and soluble organic ligands.

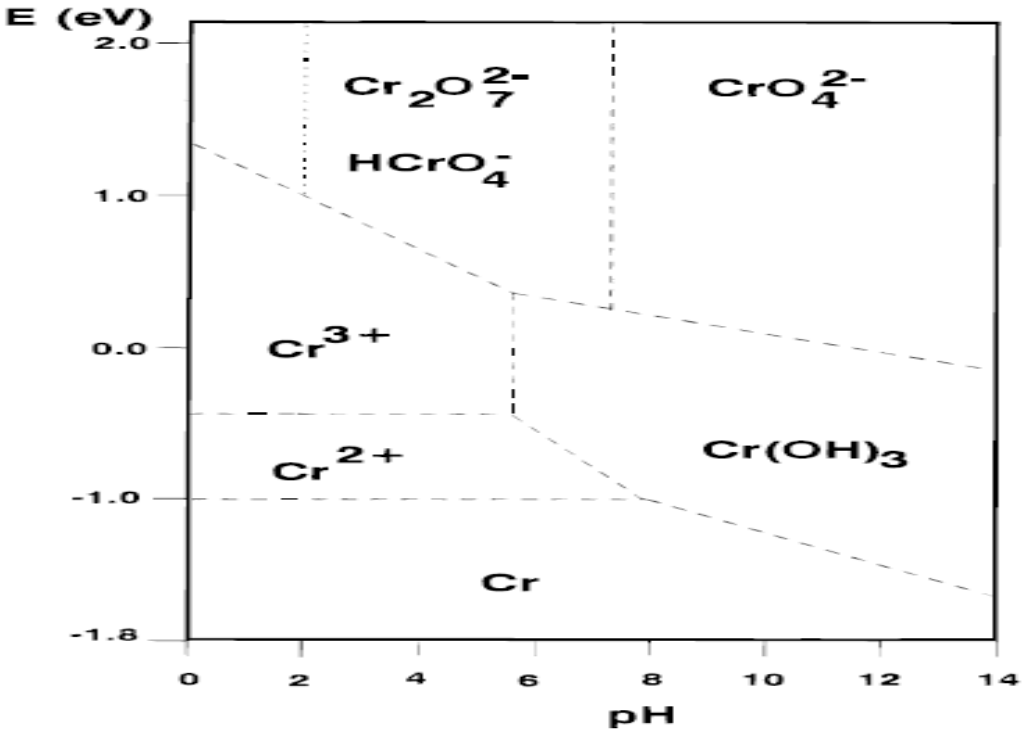



**Figure 2.3:** The Pourbaix diagram of chromium, expressing the Cr speciation as a function of pH and potential (T= 25 °C), (Weckhuysen et al., 1996)

Chromium (VI) is the more toxic form of chromium and is also more mobile. Chromium(III) mobility is decreased by adsorption to clays and oxide minerals below pH 5 and low solubility above pH 5 due to the formation of $Cr(OH)_3(s)$ (Chrostowski *et al*., 1991), (figure 2.3). Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content, and the amount of organic matter present. Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form. Soluble and un-adsorbed chromium complexes can leach from soil into groundwater. The leachability of Cr (VI) increases as soil pH increases. Most of Cr released into natural waters is particle associated, however, and is ultimately deposited into the sediment (figure 2.4), (Maturi and Reddy, 2008). Chromium is associated with allergic dermatitis in humans (Scragg, 2006). Major sources of Cr-contamination include releases from electroplating processes and the disposal of Cr containing wastes (USEPA, 2007). Hexavalent Chromium ($Cr^{6+}$) may originate from industrial applications such as

steelmaking, tanning, plating, and textiles. Hexavalent chromium is considered by the USEPA to be a carcinogen and can be easily absorbed by the body leading to ulceration of the liver and nasal septum. The process that take place in the stomach during digestion tend to change $Cr^{6+}$ to $Cr^{3+}$, but $Cr^{6+}$ is a strong oxidizer agent and can destroy cell walls easily. Chromium is known to cause nephrotoxicity, nasal and lung ulcers, skin ulcers, hypersensitivity reactions and "chrome holes" of the skin (Dzomba *et al.*, 2012).

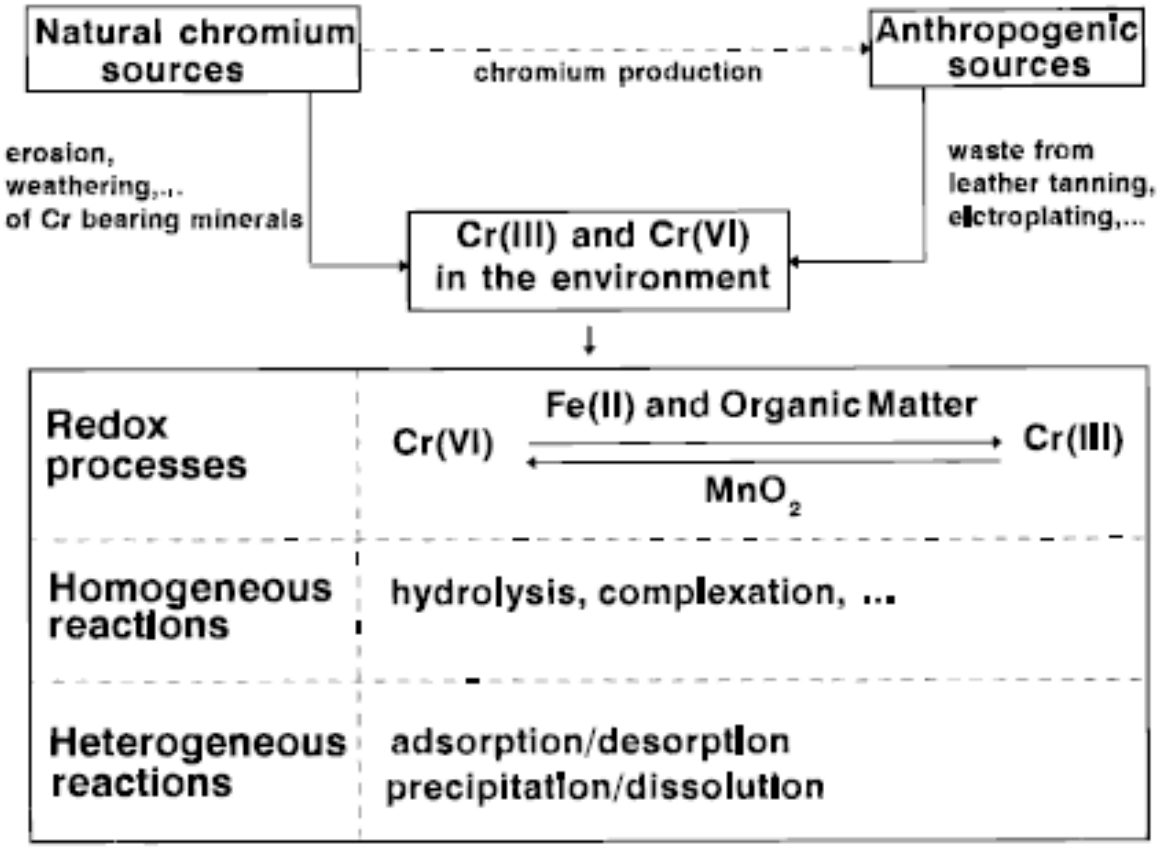



**Figure 2.4:** Behavior of Cr in the environment (Weckhuysen *et al.*, 1996).

**Cadmium**

Cd is a relatively rare element and is not found in a pure state in nature and is rapidly oxidised into cadmium oxide in air. In its compounds, Cd occurs as the divalent Cd (II) ion and can undergo weak bonding to carbon and other more electronegative atoms. Cadmium is directly below Zn in the periodic table and has a chemical similarity to that of Zn, an essential micronutrient for plants and animals partly accounting for Cd's toxicity since Zn being an

essential trace element, its substitution by Cd may cause the malfunctioning of metabolic processes (Campbell, 2006). It easily reacts with carbon dioxide, water vapour, sulphur dioxide, sulphur trioxide, or hydrogen chloride and produces cadmium carbonate, hydroxide, sulphide, or chloride and other halogens (Tran and Popova, 2013).

Cadmium (Cd) may increase in the biosphere due to emissions from batteries, coatings, electroplating steel and cast iron, pigments, plastic stabilizers constituent of low melting or easily fusible alloys, electronic and optics and solder for aluminum, reactor control rods, hardener for copper and catalysts. Moreover, it is presented as a contaminant in phosphatic fertilizer and sewage sludge and is dispersed by mining activities (Reza *et al.*, 2007). Cadmium coatings provide good corrosion resistance coating to vessels and other vehicles, particularly in high-stress environments such as marine and aerospace. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products (Campbell, 2006). In addition, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of Cd, and as a result its surface-water concentrations tend to increase as lake water pH decreases (Zhuang *et al.*, 2010). The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree (Weggler *et al.*, 2004). Cadmium is very biopersistent but has few toxicological properties and, once absorbed by an organism, remains resident for many years. The uptake of Cd in plants from the soil seems to occur mainly via $Ca^{2+}$, $Fe^{2+}$, $Mn^{2+}$, and $Zn^{2+}$ transporters (Rascio & Navari-Izzo, 2011). In general, Cd has been shown to interfere with the uptake, transport, and use of several elements like Ca, Mg, P, and K, (Tran and Popova, 2013).

Cadmium in the body is known to affect several enzymes. It is believed that the renal damage that results in proteinuria is the result of Cd adversely affecting enzymes responsible for reabsorption of proteins in kidney tubules (Tran and Popova, 2013). Cadmium also reduces the activity of delta-aminolevulinic acid synthetase, arylsulfatase, alcohol dehydrogenase, and lipoamide dehydrogenase, whereas it enhances the activity of delta-aminolevulinic acid dehydratase, pyruvate dehydrogenase, and pyruvate decarboxylase (Manahan, 2003). The most

spectacular and publicized occurrence of cadmium poisoning resulted from dietary intake of cadmium by people in the Jintsu River Valley, near Fuchu, Japan (Aoshima, 2012). It was attributed to irrigated rice contaminated from an upstream mine producing Pb, Zn, and Cd and victims were afflicted by itai itai disease with symptoms of bone disease combined with kidney malfunction (Aoshima, 2012).

The major threat to human health is chronic accumulation in the kidneys leading to kidney dysfunction. Food intake and tobacco smoking are the main routes by which Cd enters the body (Manahan, 2003). Consumption of cadmium contaminated food causes enzyme poisoning. Cadmium displaces zinc in many vital enzymatic reactions resulting in disruption or cessation of activity. This leads to acute gastro-enteritis. Epideomologic studies indicate that workers engaged in cad-mium related work are more likely to suffer from prostrate and nasopharynx cancers than their counter parts engaged in other activities (Fergusson, 1990).

**Iron**

Iron is chemically active and forms two major series of chemical compounds, the bivalent iron (II), or ferrous, compounds and the trivalent iron (III), or ferric, compounds. However, these properties of iron which prove so useful for catalysis also make it a threat to life via generation of reactive oxygen species. While living systems must have iron to survive, iron-catalyzed generation of superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (·OH) presents a potential for oxidative stress. Such reactivity mandates that iron acquisition and distribution be tightly regulated.

Iron (III)-O-arsenite, pentahydrate may be hazardous to the environment; special attention should be given to plants, air and water. It is strongly advised not to let the chemical enter into the environment because it persists in the environment.Impurities in food, such as magnesium chloride and magnesium sulfate in common salt, can promote the conversion of iron to the ferric form. High humidity also increases the rate of oxidation. In addition to lowered bioavailability, food fortified with ferrous compounds that undergo oxidation may have poor taste and discoloration due to the formation of ferric compounds. Through oxidation, ferrous iron is converted to the ferric form ($Fe^{3+}$), which is less bioavailable and has a darker color (equation 2.5):

$$Fe^{2+}\,(green) \rightarrow\ Fe^{3+}\ \left(\frac{orange}{red}\right) + e \qquad 2.5$$

This reaction is more likely to occur under alkaline conditions or in the presence of an oxidizing agent, but it may also occur, less rapidly, by air oxidation. Ferrous compounds can react with iodate, resulting in the formation and eventual loss of iodine, and the oxidation of the ferrous iron to the ferric form (equation 2.6):

$$2\,I^{5+} + 10\,Fe^{2+} \rightarrow I_2 + 10\,Fe^{3+} \qquad 2.6$$

Iron may cause conjunctivitis, choroiditis, and retinitis if it contacts and remains in the tissues. Iron is an essential micronutrient required for virtually every aspect of normal cell function. The ability of this metal to interact with $O_2$, reflecting a favorable oxidation–reduction potential, and its abundance in nature have led to its evolutionary selection for a wide range of biological functions.

### 2.3.3 Soil-Plant Transfer Coefficient or Bio-Accumulation Factor (BAF) for Heavy metals

The ability of plant to accumulate a particular metal with respect to its concentration in the soil substrate is called the transfer coefficient or the index of bio-accumulation factor (BAF) (Kachenko and Singh, 2006; Cui *et al.*, 2005). This is determined using the equation below (equation 2.7):

$$TF = Cplant/Csoil \qquad 2.7$$

where, $C_{plant}$ = metal concentration in plant tissue, mg $Kg^{-1}$ fresh weight and

$C_{soil}$ = metal concentration in soil, mg $Kg^{-1}$ dry weight.

Soil-to-plant transfer is one of the key components of human exposure to metals through food chain. Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe the transfer of trace elements from soil to plant body.

## 2.4 Edible Salt

Majority of the world's population in one way or the other depend on herbal medicines for various health ailments due to the immense benefits they have (Annan *et al.*, 2013). Poisonings

from traditional medicine products containing trace or heavy metals is well documented (Street, 2012). There are two forms of packed edible salt also known as table salt for use in food and cooking in Kenyan market, refined and unrefined salts. Refined salt is a commercialised form of table salt that contains about 97% to 99% sodium chloride and other components. It is 40% sodium and about 60% chloride with the maximum recommended level of sodium intake being 2,300 mg per day and a minimum of about 1,500 mg of sodium per day. One table spoon of salt provides 2,000 mg of sodium (Kerkar and Fernandes, 2013). The other kind of packed salt is the unrefined salt obtained from plants.

The production of common salt is one of the most ancient and widely distributed industries in the world. The world Health Organisation (WHO) estimates that in sub-Saharan Africa, 80% of the population relies on plant based salts for culinary and medicinal use as their primary intervention. Plants have been used as source of food, fodder, shelter and medicines. Many plants which are used in different traditional foods and medicine systems of the world are termed as medicinal plants (Ishtiaq *et al*., 2007). These therapeutic plants have always been valued as a mode food additive and or of treatment of variety of ailments in folk cultures and have played a very important role in the discovering of the modern day medicines with novel chemical constituents (Ishtiaq and Khan, 2008). Some studies have been carried out on levels of essential and non-essential metals in some spices and medicinal plants cultivated in Ethiopia (Mekebo and Chandravanshi, 2014).

The efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents like essential oils, vitamins, glycosides and other bio constituents. Among other plants used by man are reed plants that have found wide usage that includes its use for salt production in various regions including some regions of Western Kenya. Reeds accumulate the major, minor and toxic elements such as Cd and Pb, which are toxic to humans (Srek *et al*., 2012). Indeed, the mineral distribution may vary within the reeds and geographical location (LeRiche *et al*., 2009; White *et al*., 2009; Subramanian *et al*., 2011). Kenyan local communities use crystalline and non-crystalline indigenous salts from soils or rock and various plant materials, for cooking and food preservation among other uses. Depending on the source, each salt vary in physical and chemical composition (Peker *et al*., 2007). The chemical composition,

stability of the salts micronutrients, the level of contaminants in the salt are important factors to be considered for production and use of any salt by man and other animals.

### 2.4.1 Edible Salts of Western Kenya- Indigenous Reed Salts

Indigenous salts, a form of unrefined salts, have been processed from different plant materials ranging from wetland plants, papyrus reeds, beans, maize, and banana vegetation among others. The suitability of the indigenous reed salt as a cooking and table salt depends on its chemical composition, both in terms of micronutrients and also presence of impurities. The concentration of sodium in the salt is an important factor in determining its use for cooking purposes since it is associated with hypertension when consumed in high amounts. Although the prices of common salt are generally very low, some rural communities still prefer to use unrefined crystallised indigenous salts which are more tedious to prepare and higher in cost.

The physical and chemical composition of salt produced from the various sources varies widely depending upon the climatic conditions, source, techniques, and processes adopted. In Lugari and Busia regions of Western Kenya, papyrus reeds are used for the preparation of crystalline indigenous salt. Papyrus reed salt has been used traditionally in its unrefined form as a salt substitute for commercial table salt in the Western part of Kenya (Keter *et al*., 2011). Results of this study show that out of the total population in Western Kenya, 71.6 % use indigenous reed salt, with 50.6 % of these from Busia and 49.4 % from Lugari region. There are two main species of papyrus reed plant used in these regions; *T. latifolia* that grows in swampy places and *C. rotundus* that grows in and/or along the river banks within Busia and Lugari regions respectively. *C. rotundus* species is found along and in River Nzoia which stems from the upper ecological zones of Lugari County taking up the fertilizer and other pesticide residues that contain traces of heavy metals as earlier mentioned. This species grows to a height of about two meters and is ready for harvesting when its flowers wilt and the upper leaves are almost dried out. *T. latifolia* has been harvested and cultivated since ancient times for its wide array of uses ranging from the production of the first paper and boats in ancient Egypt's first dynasty to the manufacturing of ropes, baskets, house building material and furniture today (Terer *et al.*, 2012; Keter *et al.*, 2013). *T. latifolia* reeds are characteristic of many wetlands of tropical Africa including Kenya's fresh water lakes. However, like most wetland habitats worldwide, they are vulnerable to human

pressure due to harvesting and reclamation for agriculture. Cattails are herbaceous, rhizomatous perennial plants with long, slender green stalks topped with brown, fluffy, sausage-shaped flowering heads.

*C. rotundus* plants are 15-30 cm tall. The spike-like, terminal, cylindric inflorescence has staminate flowers above and pistillate flowers below with a naked axis between the staminate and pistillate flowers. The spike is green when fresh, becoming brown as it matures. The basal leaves are thin with parallel veins running the long, narrow length of the leaf. These plants are rhizomatous and colonial. *C. rotundus* is found in Busia region and it grows in swampy places only. It is a broad leaf cattail plant species of the Typhaceae family that is widely used for salt preparation. The plant reaches 5 feet when mature and grows in soils of pH 5.5-8.7. *C. rotundus* is widely used for phytoremedation and is known to take up lead from contaminated soils and water and by itself it has no toxicity (Lyubenova *et al*., 2012). *T. latifolia* on the other hand is a perennial aquatic macrophyte. Both these two species have been used in wetlands for wastewater and fecal sludge treatment (Kadlec, 1995).

### 2.4.2 Method of Preparation of Indigenous Reed Salts

The general method used for the preparation of reed salts from Western Kenya involves harvesting and ashing, filtration and evaporation for crystallisation. Five major sources have been found to be useful for producing salt: (1) evaporating the sea water using "salt-making potteries"; (2) evaporating water from the salt springs; (3) soaking the dried grass in saline water collected from salt springs followed by burning the grass in firewood and evaporating the filtrate; (4) burning salt-containing grass or plants, lixiviating the ash and evaporating the solution; and (5) mining the salt rocks from caves and ground to get the "geological salt".

Among them, vegetal salts deserve special mention due to their ease of preparation and wide spread utility. Burning grasses, ferns, herbs, shrubs, palms, leaves, barks, shoots, stems or even the whole plant and tree are the major adapted protocols. Throughout the world more or less the same methodology has been practiced, but with little variations in the plants, tools and equipment utilized. In general, plant materials will be burnt in selected firewood, the ashes will be lixiviated through the filters and the resulting brine will be slowly dehydrated to obtain the

dry salt (Gopalakrishnan, 2015). Three stages are followed in processing of reed salts in Western Kenya; Harvesting and ashing, filtration and evaporation and crystallisation.

### 2.4.3 Preparation of Indigenous Salt in Western Kenya

Reed plant materials are harvested by farmers from the river or swamps using a machete. Once harvested, the reed plant material is dried in the sun for one (1) to seven (7) days depending on the origin, burnt, lixiviated and crystallised.

#### Preparation of *T. latifolia* Salt in Busia Region

Reed plant materials are harvested by farmers from Sio swamps using a machete. The reeds are then dried for two (2) to four (4) days. After drying, the reeds are burnt in the open to completion and the hot ash is left overnight to cool. However, in Busia, the reeds are ashed from the green or dried material unlike in Lugari where the reeds must first be dried before burning. Once fully burnt and cooled, the ash is mixed with water and with modest filtering through perforated plastic containers or clay pots lined with banana leaves to remove the dirt and obtain the salt solution. Filtration is carried out in plastic containers and the solution collected in open metallic containers. The filtrate is used either in liquid form or in crystallised form. For the liquid form, the filtrate is stored either in large pots or plastic bottles or prepared whenever it is required for use. The filtrate can further be heated on fire in open metallic pans and allowed to evaporate to dryness to obtain crystallised salt, a method referred in this study as complete evaporation method. The crystallised salt is then stiored in dried banana stems (figure 2.5), plastic bags or plastic tins for future use.

#### Preparation of *C. rotundus* Salt in Lugari Region

Reed plant materials are harvested by farmers from River Nzoia using a sickle. The reeds are then dried for two (2) to seven (7) days. After drying, the reeds are burnt in the open to completion and the hot ash is left overnight to cool. Once fully burnt and cooled, the ash is mixed with water and with modest filtering through perforated clay pots lined with banana leaves to remove the dirt and obtain the salt solution. The filtrate is used either in liquid form or in crystallised form. For the liquid form, the filtrate is stored either in large pots or plastic bottles or prepared whenever it is required for use. The filtrate can further be heated on fire in open metallic pans and allowed to evaporate to saturation point. The saturated solution is further

scooped into green banana leaves tied and put in hot ash for further drying and crystallisation. The salt is crystallised under hot ash over a period of 8 hours-2 days depending on the person preparing the salt. The crystallised salt is then stored ground and repacked in dried banana stems, plastic bags or plastic tins for future use and sale.

**Figure 2.5:** Salt wrapped in dried banana stems

### 2.4.4 Effect of Methods of Preparation and Storage Media used in Western Kenya on the Composition of Salt Indigenous Reed Salt

Indigenous reed salts of Western Kenya are crystalline unrefined and impure as compared to the commercial salt. No purification or refining processes are carried out hence consumed in their raw forms. They are prepared from reeds with slight variations in preparation, handling and storage methods over time from one community to the other. The effect of the method of preparation, mode of storage and the shelf life are factors that may affect the micronutrients as well as heavy metal composition in the salt. The actual availability of micronutrients in salt like iodine, iron, at the consumer level can vary over a wide range as a result of variability in amount

of micronutrients like iodine, losses due to salt impurities, packaging, environmental conditions during storage and distribution and losses due to food processing, washing, and even cooking in the households (Kabaija, 1989).

Drying agricultural produce by sun drying is widely used in most of the developing countries of the tropical region (Hassan *et al.,* 2007). Drying is considered as a critical factor for the postharvest management and the merchantability of herbs. The drying of herbs inhibits microbial growth and forestalls biochemical changes but, at the same time, it can give rise to other changes that affect the herb quality. The chemistry of the plants is affected by the drying temperature and by the drying amount (temperature x time). The two most significant reactions are the decomposition of water-soluble carbohydrate and the Maillard reaction. But additionally, the fibre concentrations and solubility of certain materials are also affected. The effects can also be registered at low temperatures, and as a result the determination of the drying temperature is a fine balance on how to minimize both the drying effect and the decomposition processes. An aerobe enzymatic decomposition of carbohydrates and protein starts as soon as the green plants have been harvested.

According to Darfour *et al*., (2014), who studied the effect of varying dring methods on the herb product, concluded that each plant sample needs a special drying method to show the best radical scavenging activity and highest phytochemical content, because of differences in the secondary metabolites as well as inorganic composition. Although reports are limited, studies indicate that drying conditions can impact the chemical and biological activities of herbs. Some studies have reported that thermal treatments can alter the mineral and heavy metal content (or their chemical forms), leading to a more toxic produce than the original fresh state (Sartal *et al*., 2012). These two communities employ different methods of drying the plants and this could possibly affect the salt composition.

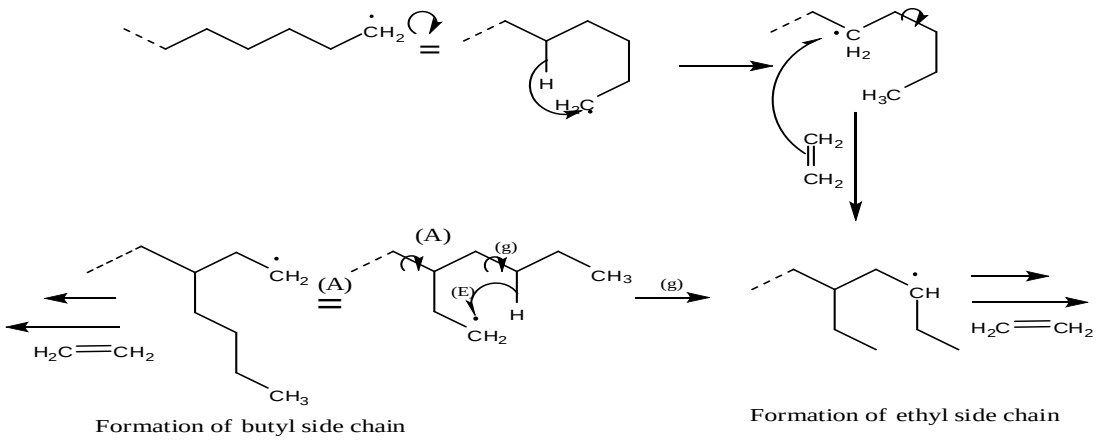
Crystallisation is a separation technique that is used to separate a solid that has dissolved in a liquid and made a solution. It is the most important method of product isolation and purification, the presence of impurities in the crystallisation mediam or in the crystal product is clearly a significant issue. Interfacial properties are of key importance for where and how the crystals form. In most developing countries salt is sold packaged or in bulk. Some of the packaging

materials used include polyethylene bags; plastic tins and dries banana leaves. The storage methods and length of storage of both ash and crystallised salts vary and may have implications on the quality and composition of the salt. Ash is stored in clay pots, sacks or plastic containers. Salt is stored in clay pots, plastic bags and plastic containers with lids and others without and in plastic sacks or in banana stems and leaves. The lixiviated ash is stored in plastic bottles for further use, and this liquid can stay for as long as it is still available. Ash samples can be stored or as long as a full year especially for Lugari region where harvesting of reeds from the river is done periodically. Salt can be stored for a longer period irrigardless of change in texture, color or taste. Whereas this is important on human health little is documented over the same. Because iodine is unstable under the storage conditions found during the manufacturing, distribution, and sale of salt in most developing countries, the effects of packaging materials and environmental conditions on the stability of salt with iron and iodine have been investigated. Several factors including moisture, humidity or excessively aerated environment, exposure to light, presence of impurities and the packaging materials have been found to affect the stability of minerals in refined table salts (Diosady *et al.,* 1997; Diosady *et al.,* 1998). Eqully, the effect of storage materials on the chemical composition of the salt can not be ruled out.

Plastic polythene bags range in quality from LDPE to HDPE polythenes.HDPE is a thermoplastic polymer that is known for its large strength-to-density ratio. HDPE has the chemical Structure:-$CH_2CH_2$-. The density of HDPE can range from 0.93 to 0.97 g/cm$^3$ or 970 kg/m$^3$. Although the density of HDPE is only marginally higher than that of low-density polyethylene, HDPE has little branching, giving it stronger intermolecular forces and tensile strength than LDPE. The difference in strength exceeds the difference in density, giving HDPE a higher specific strength. It is also harder and more opaque and can withstand somewhat higher temperatures (120 °C for short periods, 110 °C continuously). LDPE is a thermoplastic polymer made from the monomer ethylene. LDPE has the chemical Structure: -$CH_2CH_2$-. It is defined by a density range of 0.910–0.940 g/cm$^3$. It is not reactive at room temperatures, except by strong oxidizing agents, and some solvents cause swelling. It can withstand temperatures of 80 °C continuously and 95 °C for a short time. Made in translucent or opaque variations, it is quite flexible, and tough but breakable.

LDPE has more branching (on about 2% of the carbon atoms) than HDPE, so its intermolecular forces (instantaneous-dipole induced-dipole attraction) are weaker, its tensile strength is lower, and its resilience is higher. Also, since its molecules are less tightly packed and less crystalline because of the side branches, its density is lower. LDPE is used for both rigid containers and plastic film applications such as plastic bags and film wrap. Normally the use of LDPE will be limited to less than 100 $^0$C as especially LDPE starts to soften around 80$^0$ C. LDPE is not resistant to UV radiation. LDPE is very tight against water, but open for diffusion of gasses e.g. oxygen and vapor. Low-density polyethylene (LDPE) bottles are permeable to oxygen and the migrated oxygen is responsible for the considerable loss of Thiomersal through oxygen-induced degradation (Kumar and Singh, 2011).

The properties of polyethylene are highly dependent on type and number of chain branches. The chain branches in turn depend on the process used: either the high-pressure process (only PE-LD) or the low-pressure process (all other PE grades). Low-density polyethylene is produced by the high-pressure process by radical polymerization; thereby numerous short chain branches as well as long chain branches are formed. Short chain branches are formed by intramolecular chain transfer reactions, they are always butyl or ethyl chain branches because the reaction proceeds after the following mechanism (Scheme 2.1):




**Scheme 2.1:** Mechanism for the emergence of side chains during synthesis of polyethylene (PE-LD)

Polyethylene is not hazardous to health, and pure ethylene the raw material is not physiologically toxic but simple asphyxiants. The danger arises from the additives and degradation products.

Polyethylene may contain vinyl chloride and acrylic acid as co-monomers; acrolein and formaldehyde are released in heat induced hazardous degradation products from polyethylenes. Common blowing agent is a yellow fine powder of azodicarbonamide (AC) which may cause asthma. Some dyes, benzidine based and reactive dyestuffs may also have sensitizing effects. Some filler have been found to contain hazardous asbestos fibres, and anti-block powder may contain free crystalline silica. These hazards are avoided by substituting, e.g. AC powder may be substituted with paste to prevent dust formation, and careful planning of enclosure and/or ventilation in production line. Polyethylene is mong the resins which contain antioxidants. So-called hindered phenols and phosphite are used to prevent colour changes and deterioration in polyethylenes. Silica is commonly used as an antiblocking agent for LDPE film-grade resins. The silica is usually diatomaceous earth that creates little mounds (asperites) on the surface of the LDPE film and reduces sticking or blocking between adjacent film surfaces. Erucamide, chemically known as cis -13-docosenamide, is a fatty acid amide $CH_3$-$(CH_2)_7$-CH=CH-$(CH_2)_{11}$-CO-$NH_2$ commonly used in LDPE film to provide "slip." Erucamide, which is incompatible with PE, migrates to the surface of the LDPE film over ~24 h. It is essentially a surface-active molecular lubricant used to lower the film's coefficient of friction (COF), allowing adjacent surfaces to slide smoothly over one another and bags to slide smoothly in automatic operations such as form, fill, and seal. Erucamide is an unsaturated compound highly susceptible to oxidation forming nitrile compounds. The oxidation of erucamide can occur before it is incorporated into the polymer during storage at ambient temperatures. This degradation leads to yellowing of the erucamide because nitrile compounds form. Moreover, erucamide can degrade significantly during compounding at processing temperatures that exceed 200 °C.

The properties of polymers deteriorate under the combined effects of high and ambient temperatures, atmospheric radiation, oxygen, ozone, water, microorganisms, and other atmospheric agents. The deterioration is attributed to the degradation or chain scission and crosslinking and to the formation of chromophoric and polar groups in the polymer. The overall effects on the polymer are loss of strength, hardening and color formation and/or reduction of optical clarity, changes in chemical activity, and a decrease in electrical insulation properties. The gas and water vapor permeability (only polar gases) is lower than for most plastics; oxygen, carbon dioxide and flavorings on the other hand can pass it easily (Kumar and Singh, 2011).

Thus, for any meaningful use, the polymer needs to be protected from such detrimental influences. Polymers with photosensitive functional groups such as carbonyls are likely to degrade during use when exposed to light. The deterioration is attributed to the degradation or chain scission and crosslinking and to the formation of chromophoric and polar groups in the polymer. The primary route for degradation being autoxidation and the prkocess is automatic once polymers are exposed to oxygen. In addition, impurities in the polymer also tend to accelerate the process.

### 2.5 Essential and Non-essential Elements in Edible Salt

In areas of subsistence farming in rural Africa, salt is one of the few regularly purchased food items that could be a good vehicle for delivery of micronutrients such as iodine and iron. The human body requires both the metallic and the non-metallic elements within certain permissible limits for growth and good health. Therefore, the determination of element compositions in food and related products is essential for understanding their nutritive importance.

#### 2.5.1 Iodine and the Prevalence of Iodine Deficiency Disorders

Elemental iodine ($I_2$) is toxic and its toxicity derives from its oxidizing properties, which make it able to denaturate proteins (including enzymes). Human iodine intake is closely related to iodine concentration of water, soil and salt. Iodine concentration in water and soil reflects the environmental iodine distribution, and is also an important index of human's natural iodine intake and an indirect index of environmental pollution. Table 2.2 gives the estimated average requirements; recommended dietary allowances; adequate intake and tolerable upper intake levels for various elements. EARs, RDA/AIs and ULs for an average healthy 44-year old male are shown below. EARs shown as "NE" have not yet been established or not yet evaluated.

Elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion. Moisture is naturally present in the salt, or is abstracted from the air by hygroscopic impurities such as magnesium chloride. The pH of the condensed moisture on the salt is very much influenced by the type and quantity of impurities present, and this affects the stability of the iodine compounds. Elevated temperatures increase the rates of iodine loss. Based on the chemical properties of salt aimed at human consumption, there have been numerous published and unpublished studies on iodine stability in salt during the past 75 years. A review of this

literature showed that iodate is superior to iodide in terms of stability as a fortificant in salt. Published evidence of the stability of iodine, added in the form of iodate without stabilizers, is relatively meagre, but indicates iodine losses ranging from around 5% to 66% after 12 months.

**Table 2.2:** EAR, RDA, AI and UL for various elements.

| **Nutrient** | **EAR** | **RDA/AI** | **UL[5]** |
|---|---|---|---|
| Calcium | 800 mg | 1000 mg | 2500 mg |
| Chromium | ne | 35 | nd |
| Iodine | 95 µg | 150 µg | 1100 µg |
| Iron | 6 mg | 8 mg | 45 mg |
| Phosphorus | 580 mg | 700 mg | 4000 mg |
| Potassium | ne | 4700 | nd |
| Sodium | ne | 1500 mg | 2300 mg |

**EAR**: Estimated Average Requirements; **RDA**: Recommended Dietary Allowances; **AI**: Adequate Intake; **UL**: Tolerable upper intake levels, nd=not detected, ne=not estimated.

Variations between rates of iodine loss reflect impurities, moisture content, and processing methods. Conditions of packaging and storage, such as humidity and temperature also affect the final iodine content of the salt, yet these factors were not always clearly defined in earlier studies. Sample sizes and reproducibility of results were not always reported, making it more difficult to assess the statistical significance of results. A comprehensive review of the literature by Kelly (1953) concluded that the stability of iodine in salt is determined by (i) the moisture content of the salt and the humidity of the atmosphere (ii) light, (iii) heat (iv) impurities in the salt (v) alkalinity or acidity (vi) the form in which the iodine is present. He concluded that the iodine content will remain relatively constant if the salt is packed dry with an impervious lining, and kept dry, cool, and away from light. Chauhan *et al*., (1992) compared iodine stability over 300 days in common salt iodized with iodate, packed in 5 kg solid high density polyethylene (HDPE) bags or left in open heaps. The relative humidity and temperature varied from 41 – 83% and 30-39 °C respectively. Both the salt packed in HDPE bags and in the open lost 9-10% of the added iodine within the first month, after which values remained practically constant.

Iodine is an essential trace element that plays a role in the synthesis of thyroid hormones, which are fundamental for brain development, growth, and modulation of metabolic rate, thermogenesis

and energy (Delshad *et al.,* 2010). Low dietary intake of iodine is the main cause of iodine deficiency (Xie *et al.*, 2014). Iodine deficiency occurs in region where the soil is poor in iodine so that food and animal products lack iodine as well as the population living in iodine deficient area. Iodine deficiency may lead to a range of clinical abnormalities including mental retardation, deafness, stunted growth, neurological problems and goiter. These health issues are known collectively as iodine deficiency disorders (IDD) (Fuge, 2007), however, the main consequence of iodine deficiency is impaired development of fetal brain (Delange *et al*., 2001), an IDD, which data shows is the most common cause of preventable brain damage in children and which inhibits the social and economic development of countries (Padilla and Fagela-Domingo, 2008). IDD are reported by the World Health Organization to affect around 35% of the world's population (Xie *et al.*, 2014). WHO recommends that goiter prevalence above 10% in a population should be taken to indicate a public health problem that requires preventative measures. Although the number of iodine deficiency disorders affecting people is declining, on a global scale the number of affected people is still over 740 million which is 13% of the world's population with 30% of the remainder being at risk (Asobayire *et al*., 2001).

According to World Health Organization (WHO, 2004) between 1993 to 2003 a total of 54 countries worldwide suffered from iodine deficiency and 3 billion had insufficient iodine intakes, infants, pregnant and lactating women being among those most affected (Al-Hosani *et al*., 2003; Andersson *et al*., 2005). Mirmiran *et al*., 2012, findings showed that the losses of iodine are considerable and only the concentrations at the production site were sufficient to ensure an adequate intake. The loss in iodine concentration at the household level is more than that estimated by the WHO a reduction of 20% of iodine concentration from the production to the household level (World Health Organization, 2008). Table 2.3 gives the prevalence of iodine deficiency based on two indicators, the proportion of the population with median urinary iodine (MUI) below the threshold and total goiter prevalence (TGP). MUI is an indicator of dietary iodine intake and TGP, an indicator of iodine deficiency disease.

The control and prevention policy for IDD are currently based on the prevalence of MUI. The data show that although MUI is below 100 μg/l in 36.5% of the population, MUI for the general population is at 115 μg/l which means that the iodine nutrition in Kenya is adequate. However a

program of salt iodization is required for the 36.5% of the population which iodine intake is insufficient. Compared to Eastern Africa or even Africa, the proportion of the population with insufficient iodine intake is higher. Moreover, 15.5% of school-aged population suffers from goiter which indicates that IDD is a public health problem of moderate significance. TGP in Kenya is lower than in the Eastern region of Africa.

**Table 2.3:** Prevalence of Median Urinary Iodine (MUI) and Total Goiter Rate (TGR) in Kenya and in Africa in school-aged children

| **UN Regions** | **Population (%) with median urinary iodine (MUI) ≤ 100 µg/L** | **Total goiter prevalence (TGP,) in the population (%)** |
|---|---|---|
| Kenya | 53.1 | 15.5 |
| Global | 36.5 | 15.8 |
| Africa | 42.7 | 26.8 |
| Eastern Africa | 45.1 | 29.5 |
| Middle Africa | 32.4 | 23.3 |
| Northern Africa | 50.7 | 25.3 |
| Southern Africa | 31.6 | 29.1 |
| Western Africa | 41.4 | 25.9 |

Source: Gitau, 1994; WHO, 2004.

### 2.5.2 Iron and the Prevalence of Iron Deficiency Anaemia

Iron is an absolute requirement for most forms of life, including humans and most bacterial species. Iron is essential to life, because of its unique ability to serve as both an electron donor and acceptor (Mirmiran *et al*., 2012). Iron can also be potentially toxic and its ability to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and ultimately kill the cell. To prevent that kind of damage, all life forms that use iron bind the iron atoms to proteins. That allows the cells to use the benefits of iron, but also limit its ability to do harm (Mirmiran *et al*., 2012).

The daily requirement of iron for an adult male is 8-10 mg, for women, 10-18 mg, and for pregnant and lactating women up to 20 mg (Ghani *et al*., 2012). Iron deficiency can make the content of hemoglobin and the activity of physiological decrease and cause oxygen significantly

to reduce, thus affecting the supply of the nutrient and oxygen in the brain. Anemia is a public health problem in the most countries, which affects 2 billion (over 30%) worldwide (WHO, 2008). According to WHO, 47% of children under 5 years old, 30% of non-pregnant women and 42% of pregnant women suffer from anemia (WHO, 2004). Iron deficiency, the most common nutritional disorder worldwide especially among children, pregnant and non-pregnant women, is one of the risk factors for the global burden of disease in 2000, which caused 841,000 deaths and 35,057,000 disability-adjusted life years (DALYs) lost (Stoltzfus, 2003). The main causes of iron deficiency are low intake of heme iron, high intake of non-heme iron, high consumption of phytate and phenolic compounds that inhibit iron absorption, increased requirement and heavy blood losses (Bagchi, 2004). In Kenya, anemia is a problem of severe public health significance in the three groups of population where anemia is documented: preschool children, pregnant and non-pregnant women table (2.4).

**Table 2.4:** Prevalence of anemia in school aged children, pregnant women and non-pregnant women in Africa (1993-2005)

| UN Regions | Proportion (%) of preschool-aged children (6-59 months) with a blood Hb concentration below 110 g/L | Proportion (%) of pregnant women with a blood Hb concentration below 110 g/L | Proportion (%) of women of reproductive age (non- pregnant and > 15 yrs) with a blood Hb concentration below 120 g/L |
|---|---|---|---|
| Kenya | 47.4 | 41.8 | 30.2 |
| Africa | 64.6 | 55.8 | 44.4 |
| Global | 69 | 55.1 | 46.4 |

Source: WHO, 2004

Nearly half preschool children and pregnant women are anemic (table 2.4). As it is usual in most countries anemia prevalence in preschool children and in pregnant women are comparable (47% and 42% respectively). These figures are slightly lower than the average prevalence observed in Africa of 44.4% (WHO, 2004). Iron deficiency and iron deficiency anemia (IDA) are major public health problems globally (Tegegne, 2015).

According to the WHO, the highest number of pre-school children, pregnant and non-pregnant women suffering from IDA live in the Eastern Mediterranean countries (WHO, 2008). The prevalence of IDA among preschool children was 20–67%, among school children was 12.6–

50% and among pregnant women was 22.7–54% (Musaiger, 2002). In Iran, the prevalence of iron deficiency and IDA was 31.7% and 19.7–26.2% in 12–15 month old infants, respectively, statistics which are too high (Monajemzadeh and Zarkesh, 2009). Among children 6–60 months, 43.9% were anemic and 29.1% had IDA. The prevalence of IDA was severe in 12–24 month old children (Mirmiran et al., 2012). 23.7% of female adolescents and 40.9% of young female adults were iron deficient and 12.2% and 3.8% have IDA, respectively (Shams *et al*., 2010). In Turkey, 17.2% of population have iron deficiency; 48% of infants, 21–42% of children and 14.7% of adults (Manios *et al*., 2007). The prevalence of IDA was 3.1% and 13.5% among children 6–16 years old and pregnant women, respectively (Karaoglu *et al*., 2010).

Surveys carried out in Saudi Arabia have reported that iron deficiency was not found among newborns and 3–4 month old infants, whereas the prevalence of iron deficiency had increased significantly and reached to 14.5% among 12–15 month old infants; IDA is, also, prevalent among school students, affecting 16.1% of 7–14 year old children and 40.5% of female adolescents (16–18 years old) (Abalkhail and Shawky, 2002). The prevalence of iron deficiency and IDA were not assessed among pregnant and non-pregnant women. Among Jordanian infants IDA is a severe public health problem (Kilbride *et al*., 1999), with one survey showing 72% of infants had IDA and 57% were iron deficient. Percentage of anemic infants born to anemic mothers was higher than to healthy mothers (81% vs. 65%) (Kilbride *et al*., 2000). Anemia in pregnancy is a mild- moderate public health, but percentage of pregnant women with IDA has not been assessed (Albsoul-Younes *et al*., 2004). However in one study over 50% of young and pregnant women had symptoms of IDA includes dizziness, fatigue, depression, headache and loss of memory and concentration (Khatib and Elmadfa, 2009). 28.4% of Jordanian children (5.5–10 years old) had iron deficiency (Jarrah *et al.,* 2007). In the United Arab Emirates, Lebanon and Egypt, iron deficiency and IDA are more prevalent among pregnant women and children (Muwakkit *et al*., 2008; Alper *et al*., 2004). The prevalence of IDA in the Middle Eastern countries is the same as the prevalence in other developing countries (25–35%), much higher than that of industrialized countries (5–8%). However, in the Middle East data on the prevalence of IDA is limited. Most surveys have assessed anemia rather than IDA and the few surveys that have assessed IDA were provincial, not national (Shams *et al*., 2010). Apparently, IDA is an important health problem also in Kenya. According to the survey conducted by the

government and UNICEF in Kenya in 1999, 89% of children under 6 years were anemic. Prevalence was as high as 91% in the Lake Basin region (GOK & UNICEF, 2002), table 2.5.

**Table 2.5:** Prevalence of anemia in preschool children, women of child bearing age and adult men.

| **Prevalence of anemia in preschool children** | **Background characteristics** | | | |
|---|---|---|---|---|
| | **Age (years)** | **Sex** | **Sample size** | **% children with any anemia (Hb<11.0 g/dL)** |
| Total/ Region | < 5.99 | M/F | 2738 | 89 |
| Lake basin | < 5.99 | M/F | 450 | 91.3 |
| Western highlands | < 5.99 | M/F | 686 | 70.1 |
| Central highlands | < 5.99 | M/F | 442 | 31.5 |
| Dry, humid and semi-arid areas | < 5.99 | M/F | 510 | 58.3 |
| Coastal region | < 5.99 | M/F | 659 | 85.1 |
| **Prevalence of anemia in women of child bearing age** | | | | |
| Total /Region | 24-34 | M/F | 388 | 55.5 |
| Lake basin | 24-34 | M/F | 77 | 72.9 |
| Western highlands | 24-34 | M/F | 113 | 47 |
| Central highlands | 24-34 | M/F | 41 | 17.1 |
| Dry, humid and semi-arid areas | 24-34 | M/F | 58 | 41.4 |
| Coastal region | 24-34 | M/F | 99 | 68.2 |
| **Prevalence of anemia in adult men** | | | | |
| Total /Region | 24-34 | M/F | 1173 | 46.1 |
| Lake basin | 24-34 | M/F | 188 | 42.7 |
| Western highlands | 24-34 | M/F | 343 | 25.1 |
| Central highlands | 24-34 | M/F | 151 | 24.6 |
| Dry, humid and semi-arid areas | 24-34 | M/F | 221 | 49.6 |
| Coastal region | 24-34 | M/F | 270 | 31.4 |

Source: Kenya nutrition profile- FAO Food Nitrition Division, 2005; GoK and UNICEF, 2002; Anemia and the status of iron in Kenya, 1999 (GoK and UNICEF, 2002). Hb: Hemoglobin.

Among women of childbearing age, 56% were anemic. Again prevalence was particularly high in the Lake Basin and Coastal regions (73% and 68% respectively), areas where malaria is highly endemic. Men are also affected by IDA. In 1999, 46% of men aged 25 to 34 years were anemic,

table 2.5 (GOK & UNICEF, 2002). Presently, no data on severe anemia of preschool children, women of childbearing age or adult men are available. However, according to WHO (2004), 47% of children under 5 years old, 30% of non-pregnant women and 42% of pregnant women suffer from anemia. Between 4 and 5 billion people suffer from iron deficiency and an estimated 2 billion are anaemic. Women and young children are most vulnerable: 50 per cent of pregnant women and 40 to 50 per cent of children under five in developing countries are iron deficient. Overall, despite of FAO efforts and WHO guidelines in order to combat iron deficiency, it is a public health problem in Kenya. Therefore further investigations are needed to reduce the prevalence of iron deficiency and IDA in populations especially at high risk groups.

### 2.5.3 Other Essential Elements in Edible Salt

#### Sodium

Sodium is an indispensable nutrient and an appropriate amount of it in the body is critical for proper cellular functions of living animals (Pohl *et al*., 2013). Sodium is essential for maintenance of osmotic pressure, distribution of body fluids, normal pH and most metabolic processes. The influx of sodium ions across plasma membrane is required for action potential that is fundamental for nerve impulses and muscle contraction. Too little sodium is insufficient to maintain these important functions. On the contrary, sodium is readily available and consumed in large amounts from diets in modern societies (Powles *et al*., 2013), posing severe threats to public health (O'Donnell *et al.*, 2014). However, how excessive amounts of sodium cause health problems remains poorly understood. High level dietary sodium, which is the main elemental component in most types of salts, is associated with hypertension (Hollenberg, 2006; Diosady *et al.*, 1997; Diosady *et al.*, 1998). Some of the indigenous salts may also contain lower sodium content than the commercial salts thus being a potential of being used as a preferred product for healthy consumption to avoid hypertension (Hetzel, 1989).

#### Magnesium

Magnesium plays an important role in human body since it is the activator of many enzymes. Magnesium has a sedative effect and strengthens the elderly neural, inhibition function of the nervous system. Magnesium deficiency can lead to metabolic disorder of striated muscle. The ecological importance of heavy metals has attracted a great deal of attention from governmental

and regulatory bodies who are concerned in reducing the human health risk associated to the environmental pollution (Sabolić, 2006).

### 2.5.4 Heavy Metal in Edible Salt

Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc. Excessive levels of non-essential metals, however, can be detrimental to the organism. Naturally, heavy metals are accumulated in the environment through the processes of weathering and dissolution while artificially; they may be introduced to the environment (soil and water) and humans during mining, agricultural and industrial activities (Navarro *et al*., 2008), and food processing packaging (Liu *et al*., 2005 and Kachenko and Singh, 2006). The high level of heavy metals in the soil could indicate similar concentration in plant by accumulation at concentration causing serious risk to human health when consumed (Singh *et al*., 2010). Absorption of heavy metals in low doses by humans over a long period of time through food has been shown to have resulted in serious health consequences, declining economic development in terms of low productivity as well as direct costs of treating illnesses. Some common health implications of heavy metals in humans include kidney disease, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and death (Jarup, 2003).

Table salt is one of the mostly used food additive with unique place in food consumption (Soylak *et al.,* 2008). Although purified table salt is expected to have lower level of contamination, some people still prefer to use unrefined plant salt. Several researchers have reported the presence of trace elements in the unrefined salts consumed by humans, which include: Al, As, Ba, Br, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, Fe, Hf, La, Mn, Na, Ni, Pb, Rb, Sb, Sc, Sm, Sr, Ta,Tb, Th and Zn (Cheraghali *et al.,* 2010). Lead, cadmium, mercury and arsenic are the most important heavy metals which may cause health risks to human health following consumption of contaminated foods (Peker *et al.,* 2007). Indigenous salts are extracted from plants growing in wetlands that are contaminated with heavy metals such as Hg, Cd, Cr and Pb and may find their way into the food chain over time and also pose a threat to human health (Kishe and Machiwa, 2003; Chibunda *et al.,* 2006). Heavy metal contamination of food is a great concern for human health because of their toxicity and even at relatively low concentrations can cause harmful effects (Anim-Gyampo

*et al*., 2012). Edible salt is biologically necessary because it provides two important macro elements of sodium and chlorine for human body. The toxicity of various heavy metal elements has been well-known and documented for many years. Lead is a heavy metal that accumulates in the body and affects different systems and organs such as central and peripheral neural system, gastrointestinal tract, muscles, kidneys and hematopoietic system (Ciobanu *et al*., 2012). The maximum permitted level of lead in food grade salt is 2.0 mg/kg according to the Codex legislation, (2006) and 1.0 mg/kg according to the Iranian food standards (ISIRI) is given in table 2.6.

**Table 2.6:** Permitted Levels of heavy metals in refined table salt, mg/kg.

| **Element** | **Iranian food Standards, mg/kg** | **Codex Standards, mg/kg** | **KEBS Standards, mg/kg** |
|---|---|---|---|
| Lead | 1.0 | 2.0 | 4.0 |
| Cadmium | 0.2 | 0.5 | 1.0 |
| Chromium | - | - | 10.0 |
| Iron | 10.0 | - | 30-50 |

Source: Am-Euras. J. Toxicol. Sci., 3 (2): 59-62, 2011; KEBS, (2007).

Non-essential heavy metals of particular concern are cadmium, chromium, mercury, lead, arsenic, and antimony. Ingestion of metals such as Pb, Cd, Hg, As, Ba, and Cr, may pose great risks to human health. Trace metals such as lead and cadmium will interfere with essential nutrients of similar appearance, such as $Ca^{2+}$ and $Zn^{2+}$. Studies carried out by Zarei, *et al.,* 2011, reported the presence of heavy metals in table salt, with 0.01-5.8 mg/kg for lead, 0.01-0.4 mg/kg range for cadmium and 1.5-37 mg/kg for iron. The maximum permissible level of iron in food grade salt is 10 mg/kg according to Iranian food standards (ISIRI, 2006), provisional tolerable intake of 400-500 mg Cd per week for man and maximum permissible level of 0.5 mg/kg according to codex legislation (Codex Alimentarius Commission, 2006) and 0.02 mg/kg according to to Iranian food standards . According to Soylak *et al.*, 2008, the concentration of cadmium in 53% of the refined and unrefined table salt samples from Turkey, Egypt and Greece was below 0.14 mg/kg and the highest was 0.30 mg/kg, while table salt from Brazil had 0.01-0.03 mg/kg of cadmium (Amorin and Ferreira, 2005).

### 2.5.5 Risk Assessment

Risk assessment, as a part of risk analysis, a process of evaluating the possibility of adverse health effects that may occur as a consequence of exposure to a hazard, was evaluated. The health risk of the toxicity associated with the indigenous reeds salt was done by estimating the level of exposure of the heavy metals through the food chain. The degree of toxicity of heavy metal to human depends on the daily intake. *C. rotundus* and *T. latifolia* reed salts were selected and their health risk assessment calculated in terms of estimated daily intake of metal (EDIM) and estimated health risk index (EHRI) by considering the intake of metal through the salts by the consumers. The dietary intake of the studied heavy metals was estimated and their associated risks were studied by comparing to the provisional tolerable weekly intakes (PTWIs). Considering the average daily salt intake to be 5 g/day, PTWI was calculated using the formula provided by Pourgheysari, (2012). The Estimated daily oral intake of metals (EDIM) from soil through the consumption of reed salt in mg was calculated as in equations 2.8- 2.11 below given by Pourgheysari, (2012).

$$Daily\ intake\ of\ HM = (conc.of\ HM\ in\ the\ salts) * (mean\ salt\ intake\ )\ (g/\ person)/day) \quad 2.8$$

and

$$Weekly\ intake\ of\ heavy\ metals, (\text{PTWIs}) = daily\ intake\ x\ seven\ days\ /\ week \quad 2.9$$

$$Weekly\ intake\ per\ bodyweight\ kg, = (PTWIs\ weekly\ intake)/(reference\ bodyweight\ 60kg) \quad 2.10$$

$$EDI = CHM\ x\ \frac{WCASS}{BW} \quad 2.11$$

where; CHM (mg/kg, on fresh weight basis) is the concentration of heavy metals in salt; WCASS represents the daily average consumption of salt by consumers in the study areas; and BW is the adult's body weight. The WCASS for this study was taken as 5 g/day. An adult's average body weight of 60 kg was used for the EDI evaluation.

## 2.6 Micronutrient Status of Kenyan Population.

More than 2 billion people in the world today suffer from micronutrient deficiencies caused largely by a dietary deficiency of vitamins and minerals. Mineral deficiencies exist even among population groups with sufficient food in terms of meeting energy requirements. Although people in all population groups in all regions of the world may be affected, the most widespread and severe problems are usually found amongst resource poor, food insecure and vulnerable households in developing countries. Poverty, lack of access to a variety of foods, lack of knowledge of appropriate dietary practices and high incidence of infectious diseases are key factors. In 2000, the *World Health Report* 1 identified iodine, iron, vitamin A, and zinc deficiencies as being among the world's most serious health risk factors (Shawel *et al*., 2010).

Interest in micronutrient malnutrition has increased greatly over the last few years. One of the main reasons for the increased interest is the realization that micronutrient malnutrition contributes substantially to the global burden of disease. In addition to the more obvious clinical manifestations, micronutrient malnutrition is responsible for a wide range of non-specific physiological impairments, leading to reduced resistance to infections, metabolic disorders, and delayed or impaired physical and psychomotor development. Another reason for the increased attention to the problem of micronutrient malnutrition is that, contrary to previous thinking, it is not uniquely the concern of poor countries. While micronutrient deficiencies are certainly more frequent and severe among disadvantaged populations, they do represent a public health problem in some industrialized countries. This is particularly true of iodine deficiency in Europe, where it was generally assumed to have been eradicated, and of iron deficiency, which is currently the most prevalent micronutrient deficiency in the world (World Health Report, 2000).

Micronutrient deficiencies are highly prevalent in Kenya. The main forms of micronutrient malnutrition of public health significance include vitamin A and iron and iodine deficiencies. They affect in priority the most vulnerable groups: preschool children (births to 59 months) and pregnant women (WHO, 2004). Despite significant progress, deficiencies of iron and iodine remain major public health problems affecting ≥30% of the global population. These deficiencies often coexist in children. Recent studies have demonstrated that a high prevalence of iron deficiency among children in areas of endemic goiter may reduce the effectiveness of iodized

salt programs. These findings argue strongly for improving iron status in areas of overlapping deficiency, not only to combat anemia but also to increase the efficacy of iodine prophylaxis (Zimmermann, 2006). The public health importance of these deficiencies lies upon their magnitude and their health consequences, especially in pregnant women and young children, as they affect fetal and child growth, cognitive development and resistance to infection. Overcoming micronutrient malnutrition is therefore a precondition for ensuring rapid and appropriate national development. The best way of preventing micronutrient malnutrition is to ensure consumption of a balanced diet that is adequate in every nutrient. Unfortunately, this is far from being achievable everywhere since it requires universal access to adequate food and appropriate dietary habits. However, this is not the case in all regions since in western Kenya where indigenous reed salt is adversely used, there is no fortification done, implying that the population may be using table salt deficient of some micronutrients including iron and iodine. Alike all forms of malnutrition; micronutrient malnutrition is the result of the joined intervention of multiple factors such as food security, education, sanitary environment, adequate health services and proper care and feeding practices to ensure a healthy life for all household members (UNSCN 6$^{th}$ Report on the World Nutrition Situation). Table 2.7 gives the prevalence of the micronutrient deficiencies of public health significance present in Kenya.

**Table 2.7:** Prevalence of the main micronutrient deficiencies in Kenya and in the world (%)

| **Regions/Countries** | **Iodine deficiency (2004)** | **Anemia (2008)** |
|---|---|---|
| Kenya | 36.7 | 69 |
| Africa | 42 | 67.6 |
| America | 11 | 29.3 |
| South East Asia | 30 | 65.5 |
| Europe | 52 | 21.7 |
| Eastern Mediterranean | 47.2 | 44.2 |
| Western Pacific | 21.2 | 30.7 |
| Global | 36.5 | 24.8 |

UNSCN, 2004; WHO, 2009; Mwaniki *et al*., 2002; WHO, 2008;

Over one third of the population (36.7%) is deficient in iodine and consequently at risk of IDD. This figure is similar to that observed at global level (36.5%) and slightly lower than that observed in the African Region (41.0%). The prevalence of anemia is almost the same in Kenya

(69.0%) and in the African region (67.6%) and higher than in other Regions of the world apart from South East Asia (65.0%). Over the past two decades, the magnitude and consequences of global micronutrients deficiencies have been increasingly recognized. Iodine deficiency disorders and iron deficiency anemia affect > 30% of the global population (Asobayire *et al., 2001*). In the less developed countries these deficiencies have serious health consequences, especially for women and young children. In regions of West Africa, 20-38% of school children may suffer from both iron and iodine deficiencies (Zimmermann *et al*., 2003).

According to the results almost half (48%) the population is iodine deficient and 15.5% has a goiter, anemia is present in more than half of pregnant women (55.1%) and preschool children (69.0%) have anemia and almost half women of reproductive age (15-49 years) and 84.4% of preschool children are at risk of VAD and 17.3% of pregnant women (Mwaniki *et al*., 2002; WHO, 2004).

**2.7 Total Iodine Determination**

Iodine can be a difficult element to assay because of its volatility, especially in acidic conditions (Gilfedder *et al*., 2007a). Neutron Activation Analysis (NAA) is one of the most accurate methods for determination of iodine in environmental samples and is characterized by both high sensitivity and selectivity (Kucera *et al*., 2004). NAA has been widely used for iodine determination in a range of environmental samples, including foodstuffs, rock and soil samples, algae, seaweed, plant samples, and rain and river waters (Osterc *et al*., 2007; Suzuki *et al*., 2007). The main limitation of NAA is that it requires access to a nuclear reactor and is generally expensive hence not used. Another method as described by the Association of Official Agricultural Chemists (AOAC) utilises the volatility of halogens to effect separation from the solid phase with subsequent trapping in a suitable solution (Schnetger and Muramatsu, 1996) including 0.05 M NaOH, trap solution with 1% TMAH (tetramethyl ammonium hydroxide) and 0.1% $Na_2SO_3$ solution and then detected by automated colorimetery with a detection limit of 0.05 μg g-1and ICP-MS with a detection limit of 0.1 μg $L^{-1}$ respectively (Sahoo *et al*., 2009).

Bing *et al*., (2004) examined the efficiency of extracting total iodine from soil and biological samples with dilute ammonia under pressure. Samples were extracted in screw-top PTFE-lined stainless steel bombs using 10% v/v ammonia solution at 185 °C for 18 hours; iodine in the

extract was measured using ICP-MS. The method was applied to geological certified reference materials and gave good agreement with certified values (1.8 – 4.32% RSD; n = 10). In other studies, slightly varied approaches have been applied to extract iodine from food samples (Mesko *et al*., 2010), plants samples (Tagami *et al*., 2006) and seaweed (Romaris-Hortas *et al*., 2009). There are many analytical methods available for detecting, and/or measuring iodine and its various species in complex matrices. Unfortunately, there is no perfect method which would be accurate, sensitive, cheap, fast, simple, and free of interferences at the same time.

**2.8 Spectrophotometric Techniques**

Flame photometric method (for analysis of Na and K), UV-VIS Spectrophotometric method (for $Fe^{2+}$) and Atomic Absorption spectrophotometry (AAS for heavy metals analysis) are analytical techniques for quantitative determination of the analyte concentrations as described by the Beer-Lamberts law (equations 2.12 and 2.13) and calculation using a calibration curve by direct-reading methods ( Balkhair and Ashraf 2016). The law states that absorbance A of the medium is linearly proportional to the concentration of the absorbing atoms.

$$I = I_o e^{-kcx} \qquad 2.12$$

$$A = \log\left(\frac{Io}{I}\right) = kcx \qquad 2.13$$

where;

I = Intensity of outgoing radiation

$I_o$ =Intensity of incoming radiation

A= Absorbance

K = Absorptivity constant

C = Concentration of the absorbing species and x is the path length.

By using the direct-reading flame photometric method, calculation of the sodium and potassium concentration in the sample solutions (equations 2.14 and 2.15) is done;

$$mg\frac{Na}{L} = \left(mg\frac{Na}{L}\,in\ portion\right) x\ D \qquad 2.14$$

and

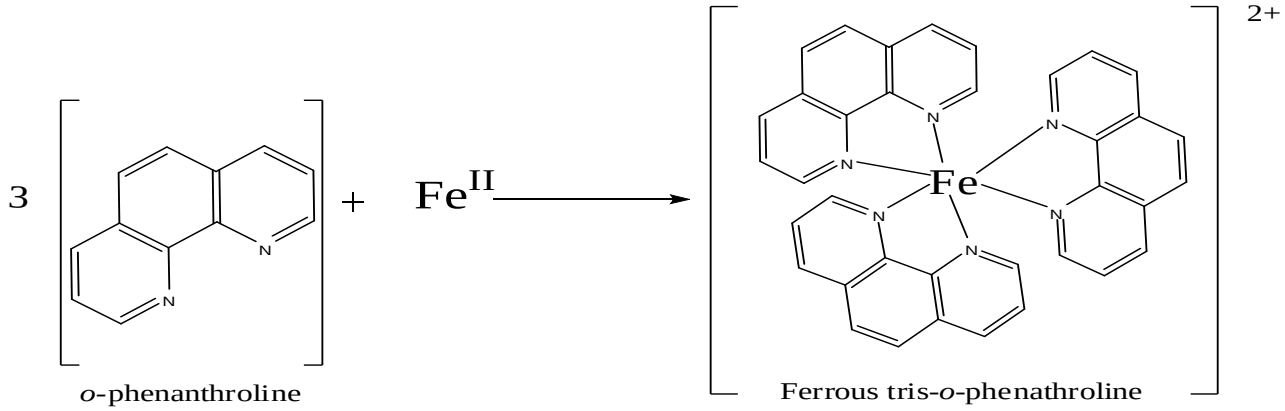
$$mg\frac{K}{L} = \left(mg\frac{K}{L} in\ portion\right) x\ D \qquad 2.15$$

where D= dilution ratio

Ultra Violet-Visible absorption spectrophotometry provides a convenient method of determination of concentration of any substance which can be treated to form a coloured solution in which the colour intensity is proportional to the concentration of the substance. This experiment is based on the determination involving the formation of a complex species that absorbs in the visible region.

**Principle:** Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1, 10-phenanthroline at pH 3.2 to 3.3. Three molecules of phenanthroline chelate each atom of ferrous iron to form an orange-red complex. In the determination of iron (II) in aqueous solutions, a tricyclic nitrogen heterocyclic compound. 1, l0-phenanthroline ($C_{12}H_8N_2$, *ortho-phenantlnuline* or o-Phen) is used as the ligand that reacts with a metal such as iron to form a strongly coloured complex. With ferrous ions ($Fe^{2+}$), it reacts in a ratio of 1:3 to form an orange red coloured complex $[(C_{12}H_8N_2)_3Fe]^{2+}$ in aqueous medium as per equation 2.16 and represented in scheme 2.6.

$$Fe^{2+}(aq) + 3\ Phen(aq) \rightarrow Fe\ (Phen)_3^{2+}(aq) \qquad 2.16$$




*o*-phenanthroline Ferrous tris-*o*-phenathroline

**Scheme 2.2:** Formation of Ferrous tris-o-phenanthroline

The ligand is a weak base that reacts to form phenanthrolinium ion, $PhenH^+$, in acidic medium. Accordingly, the complex formation may be represented as follows (equation 2.17).

$$Fe^{2+}(aq) + 3PhenH^+(aq) \rightarrow Fe(Phen)_3^{2+}(aq) + 3H^+(aq) \qquad 2.17$$

To determine the concentration of iron (II) ions in the analyte sample, it must be free from any iron (ill) ions which may be present due to the partial oxidation of the ferrous ions. This is achieved by adding a reducing agent before the coloured complex is formed. Ferrous ion ($Fe^{3+}$) is reduced to ferric state ($Fe^{2+}$) by hydroxylamine before complexation as in equation 2.18.

$$4Fe^{3+}(aq) + 2NH_2OH(aq) \rightarrow 4Fe^{2+}(aq) + N_2O(g) + 4H^+(aq) + H_20(l) \qquad 2.18$$

(Hydroxylamine)

Under these conditions the complex obeys Beer-Lambert's law in the range of the concentrations being determined (~0.5-2.0 ppm). Atomic absorption spectroscopy is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lamberts law. Absorption of electromagnetic radiation in the visible and ultraviolet regions of the spectrum by atoms results in changes in electronic structure. This is observed by passing radiation characteristic of a particular element through an atomic vapour of the sample. The Beer–Lambert law relates the attenuation of light to the properties of the material through which the light is traveling.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Area

The study area comprised of two sites: Lugari and Busia regions of Western Kenya. Two sites were selected from each region, Matete and Lugari villages of Lugari division, in Kakamega region and Ululo and Bidimbidi villages in Matayos division of Busia region of Western Kenya (figure 1.1). These regions were chosen based on the method of processing of unrefined indigenous crystalline salts and the different reed species used in processing of the indigenous salts. Two types of papyrus reeds were used in this study; *T. latifolia* reeds (Matayos) and *C. rotundus* reeds (Lugari). Lugari's *C. rotundus* reeds grow mainly along river Nzoia banks while the Busia ones grow in swampy areas within the region. Details of the study sites are given in section 1.2.

## 3.2 Chemicals, Reagents and Equipment

Double distilled water was used for dilution of reagents, preparation of both the samples and the working standards. All glassware and apparatus were cleaned by soaking in detergents for 24 hours and rinsed with double distilled water. Then they were soaked in 10% $HNO_3$ for 24 h, rinsed with double distilled water and oven-dried at 110 °C. The standard solutions of the analytes used for calibration were produced by diluting the respective stock solutions of 1000 mg/L of the given element.

## 3.3 Sample Collection

The reeds and corresponding soil and ash samples were collected from the selected sites in Lugari and Busia regions of Western Kenya, (figure 1.1). The samples were collected for both the wet (March-Aug) and dry (Jan-March) periods between January 2012 and December 2013. Sampling was done using randomized method.

### 3.3.1 Soil Samples

Top soils (0-15cm depth) and subsoil (15-30 cm depth) samples were collected from randomLy selected sampling points by the 5 points mixture method from a 10 m square plot of land (Provisional manual for soil survey related to dioxins", Soil and Pesticide Division, Water

Quality Reservation Bureau, Japan Environment Agency, Jan. 199), using a clean stainless steel auger and a corer for bulk density determination. They were mixed to form one pooled soil. Approximately 0.5 kg (wet weight) of soil samples were collected into separate polyethylene plastic bags. The same was done to subsoil (15-30 cm depth) to give a composite sub soil sample for each of the sampling areas selected, which were packed in polythene bags. A mud (Bucket) auger was used to obtain the wet clay boggy soil for Busia soil samples since the place is swampy (Bukata *et al*., 2015). Using a steel core, an undisturbed flat horizontal surface in the soil was prepared with a spade at the depth of 15 cm and 30 cm. The steel ring was gently hammered into the soil to collect soil for bulk density. Excavation was done around the ring without disturbing or loosening the soil it contained and carefully removed with the soil intact.

### 3.3.2 Plant Samples

At each of the above sampling sites used for soils, about 220 kg full growth stage (8 months) fresh reed samples were collected and mixed as well to obtain a 1kg pooled sample per site. *T. latifolia* reeds measuring 150 cm long and *C. rotundus* reeds measuring 30 cm long were harvested at full growth respectively.The reeds were harvested from the base of the reed stem, together with leaves using a stainless steel panga.

### 3.3.3 Ash Samples

From the same sites, ash from reeds was collected from randomLy selected homes. The soil, plant and ash samples were stored in a cool box (at 4 $^{o}$C) and transported to the laboratory at Egerton University for analysis.

### 3.3.4 Salt Samples

Salt samples from the two sampling regions vary in color and particle size, with Busia *T. latifolia* salt being grey-white while Lugari *C. rotundus* salt being slightly dark grey in color. Equaly the particle size of Busia salt is larger compared to Lugari's salt particle. Using this variation therefore the salts were distinguished during collection. There were two batches of salt samples that were collected. The first batch consisted of salts prepared by members from the selected sampled homesteads from each of the four study sites with the consent of local people. A total of 18 salt samples were collected from the Busia sites and 15 samples from Lugari making a total of thirty three salt samples. The salt samples were collected using standard sampling methods

(Gupta and Sinha, 2007) from randomly selected homesteads on burning of the above collected and well prepared reeds. Prepared salt samples were thoroughly mixed to ensure a uniform mixture. Salt samples were collected from the top, middle and bottom of the storage containers. These were mixed to form a 500 g composite sample for each respective homestead. The samples were packed in polythene bags and transported to Egerton University laboratory in a cooler where they were preserved for further analysis. The second batch was salts prepared from on-site with a variation in drying conditions as discussed under smple preparation section.

### 3.4 Sample Preparation and Preservation

#### 3.4.1 Soil Samples

In the laboratory, portions of the soil samples collected were cored using stainless steel corers/moisture cans for moisture determination. For other measurements, the soil samples were dried in the open in aluminium trays at ambient temperature for 6 days gently disaggregated using a pestle and mortar where necessary. Samples were homogenized using the 'cone method'. Sieved soil was poured into a cone-shaped pile and strips from the cone base to the top used to build a new cone and the entire process repeated four times (Schumacher *et al.,* 2010). The homogenised soil samples were crushed and finely ground using a Moulinex coffee and spice mill, model 980. Solid ingredients were mixed using a 5 L ribbon blender from the Department of Soil Science, Egerton University. Finely ground soil samples were sieved through a 2 mm mesh, and then sealed in Kraft paper envelopes prior to analysis.

#### 3.4.2 Plant Samples

The plants were divided into two unequal batches of 20 kg (for plant sample analysis) for plant analysis and 200 kg for salt preparation. The 20 Kg batch plant samples were kept in separate Ziploc polythene bags and properly labeled. In the laboratory, the reed plants were prepared according to the procedure described by Khan *et al.,* (2006). The plant parts were washed in fresh running water to eliminate dust, dirt, possible parasites or their eggs followed with deionized water and cut into pieces of about 10 $cm^3$ and air dried in the open for for 6 days. The respective dried plant samples were homogenized by grinding using a Retsch, Model PM400 agate ball miller. Each oportion of the finely ground samples were sieved with 2 mm mesh before being stored in respective labeled polythene bags at ambient temperature prior to analysis.

### 3.4.3 Ash Samples

Ash samples were prepared by scouping burned plant material using a stainless spoon, and packing in polythene backs for further analysis.

### 3.4.4 Salt Samples

This consisted of the second batch of samples from on-site preparation of the remaining 200 kg of the collected reeds. These batch was used to prepared salt sample for analysis of effects of method of preparation, reed species, packaging, storage time and storage period on the salt. The reeds were dried for four days, under shade and burned for four hours to obtain reed ash. To each 5 kg of ash in a plastic drum, 120 litres of water collected from a well was added and mixed thoroughly using a wooden pole followed by filtration using a perforated container prepared from a 20 litre plastic can. The filtrate was divided into two equal portions for further processing using the two methods of salt preparation. The first portion of 60 litres was boiled to complete evaporation in a steel open pan (and not a normal aluminium pan for fear of corrosion) for 6 hours (2 hours per 20 litres solution), to give 2.5 kg of salt. The second portion of 60 liters was exposed to evaporation crystallisation process where the contents were boiled in a steel open pan for about 6 hours to obtain a saturated solution. Using a steel spoon, spoon scoops of the saturated solution was packed into washed and dried green banana leaves, tied well and placed into hot ash for drying to obtain the salt crystals, a drying process that lasted for about 8 hours. These banana sachets contained 50 grams of the dried salt at the end of the crystallisation period.

From the complete evaporation procedure, five salt samples weighing 500 g each were collected and packed into polythene bags for transportation to the Laboratory for further analysis. The salt samples from evaporation-crystallization method were unpacked and repacked into five different polythene bags weighing 50 g each, to obtain 500 g salt. In total, ten salt samples, each weighing 500 g, were obtained for each sampling site, Ululo, Bidimbidi, Lugari and Matete, with five per each sampling site per method of preparation, (evaporation-crystallisation and complete evaporation methods). These salt samples were well labeled, packed and transported to the Laboratory for further analysis.

In the laboratory, the salts were packed in different packing materials weighing 50 g each and subjected to different storage methods for further analysis. To test the effect of storage

conditions, five packaging methods were used. This included (1) woven high density polyethylene (HDPE) bags of 0.15 mm thickness, (2) low-density polyethylene (LDPE) film bags of 0.07 mm thickness, (3) open plastic, (4) closed plastic containers and (5) banana leaves packs. Clear transparent, Continuous film, low-density polyethylene (LDPE) bags of 0.07 mm thickness and high density polyethylene (HDPE) bags of 0.15 mm thickness were made into bags by folding the sheets into appropriate shape, and welding the seams, by heating them to form bags. HDPE has a much higher tensile strength as it is made of longer molecular chains than LDPE.

A set of storage conditions were selected as a representation of the extreme conditions applied for home storage, normal distribution and sale of salt in developing countries. From the 500 g batch from the four sites, five 50 g samples were prepared for each storage method. To test the effect of aging on the concentration of Iodine and Iron, two sets of temperature and humidity were adopted: ambient temperature and humidity; (22 °C, 50% RH) for analysis over 6 months and high temperature and humidity (40 °C, 100% RH) for analysis of samples over a period of 18 days.

For room temperature storage analysis; packages of the salts were sampled for analysis at the start of the experimental and after 1, 2, 3, and 6 months of storage. For accelerated temperature storage analysis; packages of the salts were sampled for analysis at the start of the experimental series (denoted as starting days in the results) and after 3, 6, 9, 12, 15 and 18 days. If the iodine content in the salt dropped below 20 ppm, no further analyses were performed on the sample. To test the effect of temperature and humidity as well as storage, two sets of temperature and humidity were adopted: ambient temperature and humidity; (22 °C, 50% RH) for analysis over 6 months and high temperature and humidity (40 °C, 100% RH) for analysis of samples over a period of 18 days.

**3.5 Sample Analysis**

The chemicals used during the entire study were of analytical grade. Sample containers made of TFE were used for storage of the sample digests. Oven dried Pyrex glass apparatus which were properly washed were used as well. All sample containers were thoroughly cleaned with a metal-free non-ionic detergent solution, rinsed with tap water, soaked in acid (1:1 $HNO_3$) at 70 $^oC$ for

24 hours and then rinsed with metal-free water. A series of standard metal solutions were prepared, as per the standard methods (AOAC, 1996) by appropriate dilution of the respective stock metal solutions. In general, reagents of the highest purity were used. For hydrates, fresh reagents were used.

### 3.5.1 Determination of pH in Soil

Air-dried soil sample (5 g) of the sieved soil (< 2mm), was weighed using Shimadzu type electronic analytical balance model AUY 120 and put into a 100 mL beaker. It was mixed with a 1:1 ratio of water to soil and shaken on a reciprocal shaker for 30 minutes to make the soil into a wet soil solution. The wet soil solution was left to sit for half an hour to equilibrate and the wet soil solution stirred again. The mixture was stirred occasionally with a glass rod and the pH measured by inserting the electrode of the pH meter into the suspension after standing for 30 minutes. There was an allowance of 5 minutes for the reading to stabilize before recording the pH values. The pH of the soil suspension was measured using a JENWAY pH meter model 3505, at a temperature of 20.8 $^{o}$C in triplicate. The pH meter which consisted of a combined glass electrode (Ag/AgCl; PHE 1004), had earlier been calibrated with pH 7 and pH 4 buffers before he analysis of the samples.

### 3.5.2 Determination of Soil Texture

Forty grams of air dried (crushed to <2 mm) soil was shaken for 16 hr with 100 mL of 5% sodium hexametaphosphate (Bereta *et al*., 2014). The suspension was quantitatively transferred to a sedimentation cylinder and brought to a total volume of 1 L with distilled water. After a 2 hour temperature equilibration, the suspension was stirred vigorously for one minute to re-suspend the particles. An ASTM No. 152H hydrometer was carefully placed in the suspension and used to take two readings, one at 40 sec. and another at 6-8 hours (exact time depends on the temperature of the suspension). The percentage of sand, silt and clay in the soil is calculated from the resulting hydrometer readings.

### 3.5.3 Determination of Soil Bulk Density

A known volume of soil is collected using a metal ring pressed into the soil (intact core), and the weight determined after drying (McKenzie *et al.*, 2004). The soil core is then excavated from the ground ensuring that the cylinder is full of soil, while at the same time ensuring that the total

volume of the cylinder is full but not overflowing. Once soil core is collected it is "bagged" so that soil does not dry out and lose any moisture. Laboratory analysis of soil core starts with the soil core being weighed as it was found in the field -air dry. This weight is recorded and the core is placed into an oven at 105 $^{o}$C to obtain oven dry (OD) soil whereby soil moisture has been removed. The OD soil is weighed and the water content can then be calculated by the volume of water ($cm^3$) divided by the total volume of soil ($cm^3$). Bulk density will be calculated by the mass of OD soil (g) divided by the total volume of soil ($cm^3$). The soil core was weighed as it was found in the field and air dried. This weight was recorded and the core was placed into an oven at 105 $^{o}$C to obtain oven dry (OD) soil. The OD soil was weighed and the water content calculated by the volume of water ($cm^3$) divided by the total volume of soil ($cm^3$). Bulk density was calculated by the mass of OD soil (g) divided by the total volume of soil ($cm^3$).

The volume of the ring was determined by measuring the height of the ring with the ruler in cm to the nearest mm, and the diameter of the ring to get the radius. Ring volume ($cm^3$) = 3.14 *x* $r^2$*x* ring height; (Soil volume = ring volume). To calculate the dry weight of the soil, the ovenproof container was weighed in grams ($W_1$). The soil was dried for 2 hours in a conventional oven at 105 ºC. The dry soil sample was weighed to give ($W_2$); (Dry soil weight (g) = ($W_2 - W_1$) and p is the BD. Bulk density is expressed in g/$cm^3$ (1 mg/$m^3$ = 1 g/$cm^3$ = 1 t/$m^3$) using equation 3.2 below (Cresswell and Hamilton, 2002):

$$\text{Bulk density, } \rho, \left(\frac{\text{g}}{\text{cm}^3}\right) = \frac{\text{Dry soil weight, g}}{\text{Soil volume (cm}^3\text{)}} = \frac{\left[\frac{\text{W} - \text{W1}}{\text{V}}\right]\text{g}}{\text{cm}^3} \qquad 3.1$$

**3.5.4 Determination of Soil Moisture Content**

Erlenmeyer flasks (100 mL) and glass funnels were cleaned and dried in a Memmert oven model UNB 500. They were weighed to 0.1 g to give the weight, labeled as $W_1$. About 20 g of soil sample was placed in the container and weighed to 0.1 g ($W_2$). The container with a funnel cork were placed in the oven and dried for 8 hours at 105 °C. After drying, the container was removed from the oven, and allowed to cool in a silica gel desiccator. The container with 0.5 g of fresh soil samples was weighed to 0.1 g ($W_3$) (Ren *et al.,* 2015), (until the difference in weight of the cooled sample after two successive periods did not exceed 0.1% of the original sample weight).

The moisture content of the soil as a percentage of the dry soil weight was calculated using equation 3.2 below, (Ren *et al.*, 2015):

$$MC\% = (W_2 - W_1) - (W_3 - W_1)/(W_2 - W_1)X\ 100 \qquad 3.2$$

where; $W_1$= Weight of tin (g)

$W_2$= Weight of moist soil + tin (g)

$W_3$= Weight of dried soil + tin (g)

The same procedure was repeated for salt samples using 5 g of salt sample.

### 3.5.5 Determination of Loss on Ignition (LoI)

Triplicate samples of approximately 5 g oven-dried soil samples in silica crucibles were ignited in a muffle furnace for 16 h at 550 $^{o}$C Crucibles were re-weighed after cooling and the% LOI was calculated.

### 3.5.6 Determination of Cation Exchange Capacity, CEC, by Direct Method

Air dried soil samples weighing 3 g each (1 g peat) was leached with 60 mL 1 M $NH_4OAc$, pH 7, to saturate exchange sites with ammonium ions. Excess free ammonium ions were rinsed from the soil using isopropyl alcohol. The remaining ammonium ions held on cation exchange sites was replaced by leaching the soil with successive aliquots of a solution of 10% KCl acidified to 0.005 N HCl. Ammonium was determined on the KCl leachate by colorimetry on a Lachat QuikChem 8500 Flow Injection Analyzer using the salicylate/nitroprusside method.

### 3.5.7 Determination of the Concentration of Iodine in Soil, Plant and Salt samples

Iodine was determination according to Ann (2012) and Lena *et al*., (2015). Potassium iodide (KI, AR) was prepared by dissolving 100 g KI in 1 Litre of double-distilled water and stored in a cool, dark place. Soluble chemical starch was prepared by dissolving reagent-grade sodium chloride (NaCl) crystals in 100 mL boiled, double-distilled water. While stirring, NaCl was added until no more dissolved and heated till excess salt dissolved. When it was completely cooled, the supernatant was decanted into a clean bottle. About 1 g of chemical starch was dissolved in 10 mL boiling double-distilled water and boiling continued till it completely dissolved. The saturated NaCl solution was added to make 100 mL starch solution. 0.005M Sodium thiosulphate ($Na_2S_2O_3$) was prepared by dissolving 1.24 g sodium thiosulphate crystals

($Na_2S_2O_3.5H_2O$) in 1 L boiled, double-distilled water. This was stored in a cool, dark place. Using an electronic analytical balance, (Shimadzu model AUY 120) 5 grams of sieved soil, air dried plant, ash and salt samples were weighed into 250 mL beakers. Each of the samples was digested for iodine analysis using the wet digestion method (Yadata, 2014). Ten (10) mL of 1:1$HNO_3$ and 20 mL of 1:4 HCl, for 30 minutes at a temperature of 300 ºC. Each of the digested samples were filtered using a Whatman No. 1 filter paper into respective 100 mL volumetric flasks and the filtrates made to 100 mL mark and kept for analysis.

Duplicate samples of 25 mL of respective soil, plant, ash and salt digests were pipetted into 250 mL Erlenmeyer flasks. 1mL of hydrochloric acid and 5 mL 10% potassium iodide were added to each one of them. The liberated iodine was titrated with 0.005 M Sodium thiosulphate solution using 1mL of 1% starch indicator near the end of the titration. The level of thiosulphate used, was recorded as titre used. From the redox equations, based on the weight of each of the soil, plant, ash and salt samples used to prepare the solutions, the iodate content was calculated in mg of iodate per Kg of the salt (ppm). Determination of iodine by redox method takes place in two stages:

**Reaction 1:** Liberation of free Iodine from salt, where addition of $H_2SO_4$ liberates free iodine from the iodate in the salt sample, and excess KI is added to help solubilize the free iodine, which is quite insoluble in pure water under normal conditions.

Formation of iodine from the iodate in salt solution, equation 3.3:

$$IO_3^- (aq) + 5I^- (aq) + 6H_2O (aq) \rightarrow 3I_2 (aq) + 9H_2O (l) \qquad 3.3$$

**Reaction 2:** Titration of free Iodine with thiosulfate, where free iodine is consumed by sodium thiosulfate in the titration step. The resulting iodine which is highly soluble is titrated with 0.002 mol $L^{-1}$ thiosulfate using 0.5% starch indicator solution. The amount of thiosulfate used is proportional to the amount of free iodine liberated from the salt. Formation of triiodide and blue complex with starch (iodine reacts with iodide to form triiodide, which is slightly soluble and not volatile (equation 3.4);

$$I_2 (aq) + I^- \rightarrow I^-_3 (aq) + starch \rightarrow starch - iodine\ blue\ complex \qquad 3.4$$

and

Reduction of iodide with thiosulfate (the triiodide reacts with thiosulfate to form iodine and tetrathionate), (equation 3.5);

$$I^{3-}\,(aq)\ +\ starch\ +\ 2S_2O_3^{2-}\,(aq)\ \rightarrow\ S_4O_6^{2-}\,(aq)\ +\ 3I^{-}\,(aq) \qquad 3.5$$

(Blue solution) (thiosulfate) (Colourless, tetrathionate)

Starch is added as an external (indirect) indicator of this reaction, and reacts with free iodine to produce a blue colour. When added towards the end of the titration (that is, when only a trace amount of free iodine is left) the loss of blue colour, or endpoint, which occurs with further filtration, indicates that all remaining free iodine has been consumed by thiosulfate. The end point is visually determined by the disappearance of the blue color from solution when no more iodine is present. The overall reaction is presesnted in equation 3.6 below:

$$IO^{-3}\,(aq)\ +\ 5I^{-}\,(aq)\ +\ 6H_3O^{+}\,(aq) +\ 6S_2O_3^{2-}\,(aq)\ \rightarrow\ 6\,S_4O_6^{2-}\,(aq) +\ 6\,I^{-}\ \,(aq)\ \ 3.6$$

The overall reaction implies that one equivalent of iodate ($IO^{3-}$) reacts with 6 equivalents of thiosulfate. Therefore, in terms of iodate/iodine weight, one equivalent of thiosulfate means 35.667 grams of potassium iodate (FW $KIO_3$/6 =214/6 = 35.667), or 21.222 grams of iodine, knowing that potassium iodate contains 59.5% iodine (35.667 x 0.595 =21.222).

### 3.5.8 Determination of Na and K Metals in Soil, Plant, Ash and Salt samples

Sodium stock solution, 1000 mg/L, was prepared by dissolving 2.5435 g sodium chloride, NaCl, dried at 140 $^{o}$C, in water and 10 mL concentrated nitric acid ($HNO_3$) added and diluted to 1000 mL with de-ionised water. Potassium stock solution, 1000 mg/L, was prepared by dissolving 1.907 g of potassium chloride, KCl, in deionized water and diluted to 1 liter with deionized water. Sodium standard solutions of 5, 10, 25, 75 and 100 mg/mL concentrations were prepared from the stock solution by volumetric dilution using distilled water. Potassium stock solution was prepared by dissolving 1.9070 g potassium chloride analytical reagent salt dried at 110 $^{o}$C and diluted using distilled water to 1000 mL. Potassium standard solutions of 0, 2, 4, 6, 8 and 10 mg/mL concentrations were prepared from the stock solution by volumetric dilution using distilled water. The instrument was calibrated by setting the readout to zero using distilled water as a blank. The peak reading was set using the most concentrated sodium solution (100 mg/mL).

The concentrations of Na and K in each of the respective soil, plant, ash and salt samples were determined spectrophotometrically upon digestion as described in section 3.4.6. A corning flamephotometer, Corning model 410 was used for determination of Na and K in the samples. The analytical wavelength (nm), detection limits (mg/kg), regression equation and correlation coefficient were done and the results indicate that the linearity for Na and K was good, with Regression equations and correlation coefficients (r) as Na; Y= 1.0006X+1.1694, r = 0.9992 while Y= 0.9925X+0.2594, r = 0.9982 was obtained for K at wavelengths of 589.0 nm and 766.5 nm respectively. Precision and revovery of the method was also determined and results show the method with good recovery and precise (table 3.1).Samples were diluted where necessary to obtain emission values in the range 0 to 100 mg/L for Sodium and 0-25 mg/L for Potassium. Using prepared blank potassium and sodium calibration standards in the range 0 to 100 mg/L, calibration curves for sodium and potassium were constructed respectively. The concentration of sodium and potassium in the unknown samples were determined from the curves.

### 3.5.9 Determination of Iron (II) Content in Salt Samples

Standard ferrous solution 10 ppm (10 mg/ $dm^3$) was prepared by weighing 0.0702 g of analytical grade ferrous ammonium sulphate hexahydrate, ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) into a 1 $dm^3$ volumetric flask and sufficient distilled water added to dissolve it. 2.5 $cm^3$ of concentrated sulphuric acid was added and the solution made up to the mark using distilled water. Hydroxylamine hydrochloride was prepared by dissolving 10 g of hydroxylamine hydrochloride in 100 $cm^3$ of distilled water. 1, 10 phenanthroline solution: 200 mg 1, 10 phenanthroline monohydrate (ACS grade) was weighed into a 100 mL volumetric flask in which 40 mL distilled water and 2 drops of concentrated hydrochloric acid were added, stirred and diluted to volume with distilled water. Sodium acetate (0.l M) was prepared by dissolving 109 of sodium acetate in 100 $cm^3$ of water. Acetic acid (0.1M) was prepared by diluting about 6 $cm^3$ of glacial acetic acid to 100 $cm^3$. Acetic acid-sodium acetate buffer (pH = 4.5) was prepared by mixing 65 $cm^3$ of 0.1 M acetic acid and 35 $cm^3$ of 0.1 M sodium acetate in a 100 $cm^3$ flask. The reaction under study involves the formation of a metal ion complex. Metal ions, especially transition metal ions, possess the ability to form complexes with both organic and inorganic molecules called ligands. These complexes are produced when lone pair electrons from the ligand are donated into empty orbitals of the metal ion (resulting in a coordinate covalent bond). Here, iron (II) cations will be mixed with the

ligand 1, 10-phenanthroline (Scheme 3.1) to produce an iron (II)-phenanthroline complex (equation 3.7):

$$Fe^{2+} + y\,phen \rightarrow Fe_x(phen)_y; \qquad 3.7$$

where phen = 1, 10-phenanthroline

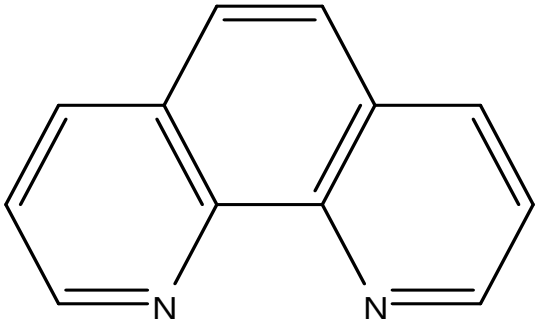



**Figure 3.1:** Structure of 1, 10-phenanthroline

Phenanthroline method was used for determination of iron (II) in the salt samples (Lipson *et al*., 2010). A UV-VIS spectrophotometer, single beam, Nova spec II model was used, at a wavelength of maximum absorbance, $\lambda_{max}$ =508 nm. A blank solution was used for zeroing the machine after every two readings. A series of standard ferrous ion solution containing 1, 2, 3, 5, 10, 15 and 20 $cm^3$ of 10 ppm were pipetted into 100 $cm^3$ standard flasks and labeled from 1 to 7. In another flask, labeled 'Sample', 10 $cm^3$ of the unknown sample was placed. To another 100 $cm^3$ standard flask, labeled 'Blank', about 20 $cm^3$ of distilled water was added to prepare the blank solution. To each of the above flasks (standards, sample and blank) 1 $cm^3$ of hydroxylamine hydrochloride and 5 $cm^3$ of 1, 10-phenantbroline were added. Each solution was buffered by adding 8 $cm^3$ of acetic acid-sodium acetate buffer and left to stand for 15 minutes after the addition of the reagents for full colour development (The colour once developed is stable for hours). Each solution was diluted to 100 $cm^3$ mark with distilled water and mixed well. The standard solutions so obtained correspond to 0.1, 0.2, 03, 0.5, 1.0, 1.5 and 2.0 ppm respectively. The absorption spectrum for the 2.0 ppm standard solution was recorded against the reagent blank in the range of 400 - 700 nm and used to obtain the spectrum: by plotting the absorbance as a function of the wavelength. The wavelength which gave maximum absorbance (λ max) was 508 nm; this was used to calculate the molar absorption coefficient *(E)* of the complex from the molar concentration and path length of the cuvette, using the relationship A =

*e c b.* the absorbance of all the standard solutions was measured at the wavelength of maximum absorption and recorded. The absorbance for the 'Sample' was measured and recorded also in the same way. A plot of absorbance at Y-axis versus concentration of the standard solutions at X-axis was made to obtain the calibration curve. (The linear region of the curve obeys Beer-Lambert's law and is used for the estimation of unknown samples). The concentration of the samples was determined with the help of the calibration curve. The ferrous ion content was calculated by accounting for the dilution factor.

**Table 3.1:** Precision Test and Recovery of Developed Method for Na and K and Iodine analysis

| **Element** | **Precision Test** | | | | | | **Recovery of Developed Method** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Quantity in (mg/kg )** | | | | | **% precision** | **Quantity in (mg/kg )** | | | **Recovery,%** |
| | 1 | 2 | 3 | 4 | Mean | | Base value | Quantity added | Quantity found | |
| Mg | 128 | 127.8 | 128.7 | 126.4 | 127.7 | 0.22 | 128 | 100 | 229 | 101% |
| Ca | 1212.4 | 1531.9 | 2120 | 1413.9 | 1569.6 | 22.75 | 1212.4 | 500 | 1701.6 | 98% |
| Na | 1397.6 | 1417.6 | 1497.7 | 1354.2 | 1416.8 | 1.35 | 1397.6 | 500 | 1854.4 | 91% |
| K | 2386.7 | 2585.2 | 2386.7 | 2849.8 | 2552.1 | 6.48 | 2386.7 | 1000 | 3306.3 | 92% |
| Iodine | 66 | 78.7 | 79.9 | 69.7 | 73.6 | 10.3 | 66 | 10 | 76.1 | 101% |

### 3.5.10 Determination of the Concentration of Pb, Cr, Cd and Fe Heavy metals in Soil, Plant, Ash and salt samples

**Preparation of the Blank Solution**

50 ml of distilled water was transferred into 100 ml beaker and 5 ml of nitric acid was added. It was then digested to almost dryness on a hot plate and filtered using Whatman filter paper 42and washed thoroughly using warm distilled water. The filtrate was allowed to cool and then ransferred into 50 ml volumetric flask and topped up to the mark using distilled water. The blank was ready to be aspirated by atomic absorption spectrophotometer.

formula $C_1V_1 = C_2V_2$ where C and V are concentration and volume, respectively. From this 100 ppm, a series of dilution was done to give working standard; 0.00 ppm, 0.20 ppm, 0.50 ppm, 1.00 ppm and 2.00 ppm lead using dilution formula $C_1V_1 = C_2V_2$. Similarly, solutions with different metal concentrations were prepared. The absorbance for the standards was recorded and a calibration curve of the absorbance against concentration was plotted. Pb equivalent to 1 gm= (molecular weight of Pb $(NO_3)_2$ x 100)/ (Atomic weight of Pb x purity).

**Preparation of Chromium Stock Solution**

Chromium stock metal solution (1000 mg/L) was prepared by dissolving 0.1923 grams of chromium oxide ($CrO_3$) in water. When solution is complete, acidification with 10 mL concentrated $HNO_3$ was done and diluted to 1000 mL with water. From 1000 ppm, dilution was made to give 100 ppm using dilution formula $C_1V_1 = C_2V_2$ where C and V are concentration and volume respectively. From this 100 ppm, a series of dilution was done to give working standard; 0.00 ppm, 0.20 ppm, 0.50 ppm, 1.00 ppm, 2.00 ppm, and 4.00 ppm chromium using dilution formula $C_1V_1 = C_2V_2$. The absorbance for the standards was recorded and a calibration curve of the absorbance against concentration was plotted.

**Preparation of Cadmium Stock Solution**

1.00 g of cadmium metal strip (99.9%) was accurately weighed and dissolved in 4 mL volume of 1:1 $HNO_3$ acid and was diluted using distilled water to a volume of 1000 ml to make 1000 ppm cadmium. From 1000 ppm, dilution was made to give 100 ppm using dilution formula $C_1V_1 = C_2V_2$ where C and V are concentration and volume respectively. From this 100 ppm, a series of dilution was done to give working standard; 0.00 ppm, 0.50 ppm, 1.00 ppm, 1.50 ppm and 2.00 ppm cadmium using dilution formula $C_1V_1 = C_2V_2$. The absorbance for the standards was recorded as shown and a calibration curve of the absorbance against concentration was plotted.

**Preparation of Iron Stock Solution**

Stock iron solution (1000 mg/L) was prepared by dissolving 1.000 g of iron wire in a mixture of 1:1 HCl and 3 mL concentrated $HNO_3$ acid, followed by addition of 5 mL concentrated $HNO_3$ and diluted to 1000 mL with water. From 1000 ppm, dilution was made to give 100 ppm using dilution formula $C_1V_1 = C_2V_2$ where C and V are 28 concentration and volume respectively. From this 100 ppm a series of dilution was done to give working standard; 0.00 ppm, 0.50 ppm,

1.00 ppm, 2.00 ppm, 4.00 ppm and 8.00 ppm iron using dilution formula $C_1V_1 = C_2V_2$. The absorbance for the standards was recorded and a calibration curve of the absorbance against concentration was plotted.

### 3.5.11 Analysis of Pb, Cr, Cd and Fe using Atomic absorption spectrometric (AAS)

Heavy metals were determined using the Atomic absorption spectrometric (AAS) method as described by Afrasiab *et al*., 2014. An Atomic Absorption Spectrophotometer, Thermo Jarell AshS11 AAS model, equipped with an air acetylene burner was used for analysis. The hollow Cathode Lamps for Pb, Cd, Fe and Cr were used at a specific wavelength hand band pass. Cadmium was analysed at a wavelength of 228.8 nm of wavelength, with a band pass of 1.0 nm. The sensitivity of the AAS was 0.01 µg/mL. Chromium was determined at a wavelength of 357.9 nm, band pass of 0.5 nm and 0.04 µg/mL sensitivity. Lead at 217.0 nm, band pass of 1.0 nm, sensitivity of 0.1 µg/mL and for total iron, at 248.3 nm, band pass of 0.3 nm and sensitivity of 0.4 µg/mL. Analysis of each sample was carried out in triplicate to obtain representative results and the data reported in mg/kg (on a dry matter basis), (Muneer *et al.*, 2010). About 0.5 grams salt sample was weighed using an electronic analytical balance, Shimadzu type model AUY 120 into a beaker. The conventional wet digestion method as described in section 3.4.6 was used to prepare salt extracts for analysis. The salt filtrate was transferred into a 100 mL plastic container for the analysis of pH, essential and toxic heavy metals in salt materials using the AAS machine.

The analytical wavelength (nm), detection limits (mg/kg), regression equation and correlation coefficient were done and the results indicate that linearity for all trace elements was good (Cr as $Y=100.08X - 0.4209$, $r = 0.9564$; Fe as $Y=198.80X+0.3283$, $r = 0.9854$; Pb as $Y=182.13X-0.6364$, $r = 0.9908$ and Cd as $Y= 1.4132X+0.4612$, $r = 0.7804$). Detection limits ranged from 0.0005-0.0100 mg/kg. Equally calibaration and quality control were checked. A standard was aspirated for every 10 samples to check for instrument drift. The method of standard addition was used occasionally to check for interferences. The method of digestion and AAS analysis were validated by preparation of a multistandard solution which was prepared from commercially available standards. A sample from a given sampling site of *T. latifolia* reed salt was first digested, run through an AAS and metal contents in the unspiked sample were

determined from the calibration curve. An aliquot of the multi-element standard solution was obtained using graduated pipette and used to spike a sample from the same sampling site. This was followed by the digestion of the spiked sample, aspiration and finally determination of metal contents from the calibration curve. The amount of spiked metal content recovered after the digestion of the spiked samples was used to calculate the percentage recovery results were considered satisfactory because recovery ranged from 91 to 102.0% (table 3.2). Spiking, digestion and analysis were done in triplicate. The mean ranges and standard deviations of the data collected were determined using SAS.The accuracy and precision was carried out by use of randomly chosen reed samples. The results indicate good precision under the analytical conditions used since the relative standard deviations were < 5% and the method was considered precise.

**Table 3.2:** Precision test and recovery of developed method for AAS determination of heavy metals

| **Element** | **Precision Test** | | | | | | **Recovery of Developed Method** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Quantity in (mg/kg )** | | | | | **Precision, %** | **Quantity in (mg/kg )** | | | **Recovery, %** |
| | 1 | 2 | 3 | 4 | Mean | | Base value | Qty added | Qty found | |
| Cr | 4703.8 | 4603.8 | 4503.7 | 4203.4 | 4503.7 | 4.4 | 4703.8 | 1000.0 | 5610.2 | 91 |
| Fe | 540.0 | 659.3 | 659.3 | 540.0 | 599.7 | 9.9 | 540.0 | 200.0 | 743.7 | 102 |
| Pb | 6.9 | 4.8 | 4.3 | 4.1 | 5.0 | 37.3 | 6.9 | 5.0 | 11.4 | 90 |
| Cd | 4.6 | 4.6 | 4.7 | 4.6 | 4.6 | 0.5 | 4.6 | 5.0 | 9.4 | 96 |

### 3.6 Calculation of the accelerated aging factor ($Q_{10}$) of the salt

To calculate the accelerated aging factor ($Q_{10}$) of the salt, equation 35 will be used, (Labuza *et al.*, 2007), equation 3.8.

$$Q_{10}^{\Delta/10} = e^{E_a/RT_1T_2} \qquad 3.8$$

where; $E_a$ = activation energy $(20 - 30\text{kcal/mol})$

$R$ = gas law constant $(8.314\text{J/mol k})$

$T_1 =$ room temperature (298 K)

$T_2 =$ accelerated temperature (313 k)

The testing frequency at accelerated temperature then determined using equation 3.9, (Labuza *et al.,* 2007);

$$F_2 = F_1 \, Q_{10}{}^{\Delta/10} \qquad 3.9$$

where $F_2 =$ the time between tests at room temperature

$F_1 =$ the time between tests at higher temperature

$\Delta =$ the difference in $\circ C$ between the two temperatures .

### 3.7 Descriptive Cross Section Methods

In this study a pre-tested questionnaire and a face-to-face interview were used to capture data on the respondent's knowledge, education, wealth and perceptions on whether the salt pose any health risk to them. Simple random sampling was done from a list of the households obtained in 2013. The study population included all residents who live in the two study areas of Busia and Lugari regions that have lived I this area for more than six months and with consent. Sample size was determined using the Fischer *et al*., (1998) formula.

### 3.8 Ethical Considerations

Permission was sought from the National Commission for Science, Technology and Innovation. Informed consent was sought from individual participants after explaining to them the objective of the study. Confidentiality was ensured throughout the study.

### 3.9 Statistical Analysis

Statistical analysis was done using SAS (SPSS Inc., Chicago, IL, USA) based on three replicates. Means were calculated and compared using t-test , standard errors of each individual nutrient of the samples were computed, and variations among the species were evaluated by least significance difference (LSD) at 5% level of probability ($p<0.05$). A Randomized Completely Block Design with Analysis of covariance (ANOVA) was done for nutrients iodine, Fe, K and Na with the mean separation done by Fischers protected LSD for significance. A multivariate comparison of means as well as Regression was done to establish each independent variable's contribution to the change in elemental contents of the salts. Correlation was done to establish the relationship between soil-plant-salt elemental concentrations.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Chemical and Physical Characteristics of Soils, Reed Plants, Ash and Reed Salt

### 4.1.1 pH of Soils, Ash and Salt

The pH values of soils were in the range of 6.2 - 6.4 with a mean of 6.3 for Busia soils and 4.9-6.3 with a mean of 5.6 for Lugari soils (table 4.1). This means that Busia soils are weakly acidic while Lugari soils acidic. The variation in pH between Busia sites and Lugari sites is probably due to the presence of swamps and mulching effect in Busia, characteristicws which are absent in Lugari. It was also observed that the pH of the soils during the wet season were higher than the dry season (table 4.2) and slightly acidic. It was aslo noted that the pH of the top soil (0-15 cm) was higher than the pH in the subsoil (15-30 cm) except for Matete where the pH was the same (table 4.2). Under normal circumstances top soil is excpected to have low pH due to the presence of humus, however in this situation the reverse was observed. The acidic conditions of the soils of Lugari and Busia might be due to ferrolysis, which happens due to the poor drainage and flooding during the rainy season.

**Table 4.1:** Selected soil Properties of Busia and Lugari Soils.

| **Sampling area** | **Ululo** | **Bidimbidi** | **Lugari** | **Matete** |
|---|---|---|---|---|
| pH | 6.4±0.01 [a] | 6.2±0.03 [b] | 6.3±0.01 [c] | 4.9±0.02 [d] |
| % moisture | 57.5±0.03 [a] | 66.5±0.07 [b] | 42.2±0.20 [c] | 22.2±0.02 [d] |
| % N | 0.3±0.01[a] | 0.3±0.002[b] | 0.2±0.01[a] | 0.2±0.01[b] |
| CEC meq/100g | 165.0±15.0 [a] | 91.0±1.00 [b] | 92.0±2.00 [c] | 86.8±0.11 [d] |
| SBD, g/cm3 | 0.3±0.01 [a] | 0.3±0.02 [b] | 0.3±0.01 [c] | 0.3±0.01 [d] |
| % clay | 23.8±3.80 [a] | 18.9±0.93 [b] | 10.5±0.50 [c] | 11.0±1.00 [d] |
| % sand | 70.3±9.30 [a] | 60.9±0.05 [b] | 88.8±0.15 [c] | 88.9±0.28 [d] |
| % silt | 5.9±5.50 [a] | 19.6±0.20 [b] | 1.5±0.09 [c] | 1.4±0.02 [d] |
| % LOI | 59.3±4.30 [a] | 61.0±1.00 [b] | 41.5±0.50 [c] | 42.5±0.50 [d] |
| P, mg/kg | 16.7±0.79 [a] | 12.3±0.01 [b] | 18.3±5.79 [c] | 6.7±5.79 [d] |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations. Within rows, means with different alphabets are statistically different at $p<0.05$ by ANOVA and lsd test. n=12, lsd=0.0836; s.e.d=0.0396;

**Table 4.2:** The concentrations of K, Na and iodine soil samples collected during the dry and wet seasons from Busia and Lugari regions at varying depths.

| Sampling area | Depth, cm | Dry Season | | | | Wet Season | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | pH | K, mg/kg | Iodine, mg/kg | Na, mg/kg | pH | K , mg/kg | Iodine, mg/kg | Na , mg/kg |
| Ululo | 0-15 cm | 6.5± 0.34 | 640.3±19.85 | 208.8±0.95 | 1671.1±24.05 | 6.9±0.02 | 269.9±8.24 | 197.8±0.95 | 2025.3±6.25 |
| | 15-30 cm | 6.4±0.41 | 560.9±19.85 | 124.0±1.16 | 897.3±0.001 | 6.8±0.03 | 262.6±0.69 | 129.3±0.16 | 1562.8±2.25 |
| Bidimbidi | 0-15 cm | 6.2±0.73 | 680.0±13.23 | 170.3±5.72 | 850.6±13.34 | 6.9±0.01 | 274.0±2.83 | 155.3±1.72 | 1011.0±0.50 |
| | 15-30 cm | 6.1±0.67 | 349.2±19.84 | 112.7±7.34 | 470.3±6.67 | 6.7±0.01 | 272.7±4.22 | 120.1±6.23 | 1123.8±13.75 |
| Matete | 0-15 cm | 6.3±0.51 | 964.4±20.92 | 139.6±9.15 | 603.7±42.19 | 7.1±0.02 | 266.8±6.46 | 123.6±1.23 | 1060.5±7.00 |
| | 15-30 cm | 6.3±0.50 | 640.3±159.76 | 107.1±4.22 | 640.4±61.67 | 7.1±0.03 | 267.7±7.31 | 107.1±2.25 | 1064.8±11.25 |
| Lugari | 0-15 cm | 6.1±0.01 | 964.4±33.08 | 86.4±0.56 | 510.3±6.68 | 6.9±0.02 | 260.4±0.02 | 92.4±0.29 | 1248.5±3.80 |
| | 15-30 | 4.3±0.02 | 865.2±33.08 | 91.1±1.11 | 470.3±6.67 | 4.8±1.02 | 0.25±0.10 | 101.1±1.12 | 24.25±3.08 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12

This process assumes that repeated redox and leaching cycles lead to strong acidic conditions and exces-sive weathering of clays and consequently to a very small cation ex-change capacity (CEC) of soil minerals in the top-soils (Kannan *et al*., 2014). The binding forces of metal ions to soils decrease with increasing pH of the environment. ANOVA indicated that pH in soils varied significantly with region ($p<0.05$, df =3, F=33.07, appendix 3) and depth ($p<0.05$, df = 285.1, df =8, appendix 3). However, there was no significant difference pH between the wet and dry season ($p>0.05$, df=2, F=0.12) and in between locations ($p>0.05$, df = 1,5, F =156.12; appendix 3). This is supported with t-test which showed significant differences in the pH of soil samples from all locations (table 4.1). The pH ash samples from the two plants both in Busia and Lugari were the same during the dry season (table 4.3), however, during the wet season the pH of samples from Lugari were higher than the ash samples from Busia probably due to increased Na content that may be due to chemicals application in farming during the wet period.

The pH of *T. latifolia* salt samples in Busia had pH values ranging from 10.23- to 10.55 (table 4.4) with an average of 10.53. However, salt samples from *C. rotundus* in *Lugari* ranged from 9.56 to 9.76 (table 4.4) with an average of 9.66. The lower pH reported in Lugari was expected due to the high K and Na concentrations given in soils (table 4.2).

**Table 4.3:** Chemical composition of ash samples obtained from Lugari and Busia.

| **Season** | **Sample source** | pH | **Concentrations of Elements , mg/kg** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **Na** | **Fe** | **Cr** | **Pb** | **Cd** | **Iodine** | **K** |
| Dry season | Busia | 11.1 | 7461.1±0.34 | 441.6±0.02 | 6.0±3.67 | 12.76±3.62 | 4.9±2.01 | 62.9±21.20 | 2095.6±9.21 |
| | Lugari | 11.1 | 2476.6±1.23 | 669.3±3.52 | 3.1±4.92 | 10.37±0.07 | 4.5±0.06 | 57.1±4.90 | 1896.3±23.7 |
| Wet season | Lugari | 12.2 | 16712.1±14.25 | 3757.5±3.91 | 0.5±0.01 | nd | 3.6±0.01 | - | 2929.0±8.23 |
| | Busia | 11.6 | 11548.6±34.20 | 480.5±11.9 | 3.0±1.20 | nd | 3.6±0.03 | - | 15039.1±3.29 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=24; nd= not detected

### 4.1.2 Soil Texture

Busia soils and Lugari soils are both sandy loam soils though the Lugari soils have higher sand. These soils can be classified as Typic Xerofluvent (American Soil Taxonomy) (table 4.1). Clay, silt and sand contents varied significantly with location ($p<0.05$, df = 3; appendix 3). This was also shown in the t-test in table 4.1.

### 4.1.3 Soil Bulk Density

The soil bulk density for the two regions was similar with very insignificant variation. Soil bulk density varied significantly with location of sampling as indicated with t-test results (table 4.1). This is supported with significant difference due to location from the ANOVA table ($p<0.05$, df = 3, F= 2.83; appendix 3).

### 4.1.4 Moisture

The percentage moisture of the soils used ranged from 22.2% in Matete to 66.5% in Bidimbidi (table 4.1). This was done during the dry season. The moisture content varied from 0.5 % in Lugari to 15.6% in Bidimbidi (table 4.4) with significant difrences with regions ($p<0.05$, df =1, F= 67.90; appendix 3), location of sampling ($p<0.05$, df =1, F= 53.85) where $R^2 = 0.9223$, and while there was insignificant difference with season ($p>0.05$, df =1, F= 0.86; appendix 3). Salts obtained from *T. latifolia* reed plants that thrive in swampy areas have higher moisture contents than those from *C. rotundus*. Using the ISO 2483, the moisture content of food grade salt shall not be greater than 0.5% by mass (East African Community, 2013). Lugari samples meet this specification but not the Busia salt.

**Table 4.4:** Chemical composition of salt samples obtained from Lugari and Busia.

| Season | Sampling area | pH | % Moisture | iodine | $Fe^{2+}$ | Na | K |
|---|---|---|---|---|---|---|---|
| **Dry** | Lugari | 9.7 | 0.5±0.21 | 1.2±0.50 | 1.1±0.001 | 3398.4±43.28 | - |
| | Matete | 9.8 | 1±0.05 | 1.4±0.60 | 1.0±0.002 | 5957.2±24.00 | - |
| | Ululo | 10.4 | 8.2±1.80 | 0.2±0.10 | 1.0±0.00 | 12650.1±47.09 | - |
| | Bidimbidi | 10.0 | 15.6±0.001 | 1.6±0.90 | 1.0±0.002 | 15752±46.60 | - |
| **Wet** | Lugari | 9.7 | 0.5±0.25 | 1.3±0.65 | 1.1±0.01 | 3016.8±73.39 | 4305.2±8.75 |
| | Matete | 9.8 | 1.0±0.05 | 0.6±0.28 | 1.0±0.01 | 3403.3±9.12 | 4966.7±59.36 |
| | Ululo | 10.2 | 8.7±1.52 | 1.6±0.79 | 1.0±0.01 | 9081.9±26.41 | 3114.4±36.38 |
| | Bidimbidi | 10.5 | 15.6±4.27 | 0.1±0.01 | 1.0±0.01 | 10419.2±29.35 | 3809.0±87.51 |
| | Ululo | 10.6 | 11.2±0.22 | 2.1±1.06 | 1.0±0.03 | 10125.6±6.13 | 3974.4±6.45 |
| | Ululo | 10.5 | 4.9±0.34 | 2.1±0.65 | 1.0±0.01 | 9929.9±10.93 | 3809.0±7.56 |
| | Bidimbidi | 9.6 | 15.9±2.87 | 0.4±0.24 | 1.1±0.01 | 10517.0±159.79 | 3974.4±86.45 |
| | Lugari | 9.7 | 0.5±0.25 | 1.3±0.70 | 1.1±0.01 | 3016.8±73.40 | 4305.2±75.10 |
| | Matete | 9.8 | 1.0±0.21 | 0.6±0.30 | 1.0±0.01 | 3403.3±9.10 | 4966.7±9.40 |
| | Ululo | 10.4 | 8.2±1.80 | 1.9±0.40 | 1.0±0.01 | 9636.5±40.34 | 3632.6±26.20 |
| | Bidimbidi | 10.0 | 15.6±0.01 | 0.2±0.10 | 1.0±0.03 | 10468.3±15.58 | 3891.7±13.50 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12

### 4.1.5 Loss on Ignition (LOI)

The organic matter content indicated by loss on ignition (LOI) showed that Busia had higher LOI (59.3-61 %) compared to Lugari samples which had a lower LOI (41.5-42.5%), table 4.1. This may be due to the increased humus as a result of high plant material decomposition Busia region. From ANOVA table, the location of soil sampling significantly determines the LOI ($P<0.05$, df =3 and F= 5.67; appendix 3). The results indicate a positive correlation that LOI or organic matter content is a function of % moisture ($p_{value}=0.001$, r = 0.455), % silt ($p_{value}=0.002$, r= 1.00) and % clay ($p_{value}<0.05$, coeff 1.40) which have a $p_{value}$ <0.05 (appendix 3). Generally, the higher % clay contents of the soil the higher the organic matter content. The results obtained do agree with studies by Skaven-Haug (1972) who found that clay content is associated with organic matter content. Finding of this study reveal that LOI and SD vary from those of Tie and Kueh (1979) who reported very high LOI (95%) and low BD bulk densities of 0.15 and 0.13 $g/cm^3$ at depths of 0-15 and 15-30 cm respectively. It is possible that the present peats are less decomposed and hence have a high BD of and a low LOI (table 4.1). The high LOI may be due to increased organic matter accumulation, decomposition rates and evapotranspiration (Tsheboeng *et al.,* 2014) factors which wre observed in Busia soils.

### 4.1.6 Cation Exchange Capacity (CEC)

The CEC of soil samples ranged from 86.8 meq/100g in Matete to 165.0 meq/100g in Ululo (table 4.1). The higher the clay content the higher the CEC. Ululo has high clay content hence a high CEC. The CEC is a function of the clay content and SBD ($p_{values}$ of 0.021 and 0.049 < 0.05; appendix 3) showing statistical signicance difference in their compositions. The CEC is also significantly influenced by the location of sampling ($p<0.05$, df = 3, F = 4.25; appendix 3).Waterlogged soils are submitted to temporal variations of aeration and aerobic–anaerobic conditions which influence redox conditions and thus element mobility.

## 4.2 Concentration of Micronutrients Iodine, $Fe^{2+}$, K and Na in Soils, Plants, ash and salt Samples

### 4.2.1 Concentration of Micronutrients Iodine, K and Na in Soils,

The concentrations of iodine, K and Na in soils in Busia and Lugari regions are presented in table 4.2.

**Soil Iodine**

Iodine concentration in soils ranged from 86.4 mg/kg in Lugari to 208.8 mg/kg in Ululo (table 4.2). Generally, concentration of iodine decreases with soil depth and is thought to be as a result of the mild oxidative conditions as the soil depth increases. Under reducing conditions, dissolution of metal oxyhydroxides releases sorbed iodine (Ashworth and Shaw, 2006; Yamaguchi *et al*., 2006). This is in agreement with the findings of Yuita and Kihou (2005) who observed higher concentrations of iodine in topsoil of three Japanese soils. The increase in iodine concentration may be primarily ascribed to changes in environmental conditions, such as soil moisture, $E_h$ value and groundwater level, which control the dissolution ratio of soil iodine (Yuita *et al.,* 2005). The iodine concentration at 0-15 cm depth was much higher at 15-30 cm depths (table 4.2). The limitation of the high concentration of iodine in soil 15 cm depth was ascribed to the fact that the high pH and high phosphate content in the plow horizon, associated with liming and the application of fertilizer phosphates, promotes the desorption of iodine from soil. Flooding occurs on the soil surface (development of reductive conditions in the surface soil) during heavy rain events because of the formation of a plow pan and drying of the topsoil during the dry season (particularly during the interval between harvest and planting), and these two conditions result in an increase in the the dissolution ratio of soil iodine (Yuita *et al.* 2006).

Lugari samples have generally lower iodine concentrations than Busia samples (table 4.2) with an increase in iodine concentration with depth probably due to the mild reducing conditions and the high moisture and high organic matter. The observed decrease in iodine concentrations with depth may be a result of reduction of weakly organic-bound iodine to iodide at low redox potentials resulting in the release of iodine from soil surfaces into the soil solution (Yamaguchi *et al.,* 2010). The results also show that the lower the moisture content the lower the iodine level with the exception of Lugari samples. In this study, it was found that iodine increased with increase in SBD and % clay ($p > 0.05$ with $r = 5650$ and 17.25 respectively, appendix 8). It is thought that iodine adsorbs strongly on clay matter.

**Soil Potassium**

The concentration of K ranged from 349.2 mg/kg in Bidimbidi to 964.4 mg/kg in Matete and Lugari (table 4.2). Just like iodine concentrations, K concentrations decreased with soil depth. K

varied significantly with depth ($p<0.05$, df = 8, F = 10.75, appendix 3), season ($p<0.05$, df = 1, F = 295.67; appendix 3), as well as region ($p<0.05$, df = 3, F = 43.6, v), of soil sampling but not with region ($p>0.05$, df = 5, F = 0.41) and location ($p>0.05$, df = 2, F = 0.56; appendix 3).

**Soil Sodium**

The concentration of Na ranged from 470.3 to 1671.1 mg/kg in Ululo (table 4.2). There is a general decresase of Na concentration in the top soil and subsoil with an exception of Lugari which is in agreement with Whitehead (1978) probably due to soil water dilution and increased leaching as water infiltrates the soil. However, the concentrations of Na were significantly higher during the wet season (1011.0-2025.3 mg/kg) as compared to the dry season (470.3 mg/kg - 1671.1 mg/kg). Na varied significantly with depth ($p<0.05$, df = 8, F = 2.64; appendix 3), region ($p<0.05$, df = 5, F = 0.64; appendix 3; appendix 3), season ($p<0.05$, df = 1, F = 25.66), location ($p<0.05$, df = 2, F = 24.24; appendix 3), as well as region ($p<0.05$, df = 3, F = 0.26), of soil sampling, appendix 3. During the wet season only K and Na were analysed. The concentration of K in all the soil samples analysed were lower than the concentrations obtained in the dry season (table 4.2). K concentrations during this season ranged from 260.4 mg/kg in Lugari to 272.7 mg/kg in Bidimbidi, probably due to increased leaching as well as wash away and plant uptake.

### 4.2.2 Concentration of Iodine, Na and K in Plants

The levels of micronutrients (Iodine, Na and K) were determined in plant samples collected in Busia and Lugari regions of Western Kenya. Results are given in table 4.5.

**Concentration of Iodine**

There was higher concentration of iodine in *T. latifolia* reeds from Bidimbidi than in Ululo during the dry seasons however they were the same during the wet season. In Lugari and Matete, there are no major differences in the two seasons. As indicated in table 4.6, TF of iodine from the soils to the plants, there is generally more uptake of iodine by *T. latifolia* reeds than by *C. rotundus* 0.164 versus 0.138 which is in agreement with findings by Zhu *et al*., (2004) and Wang *et al*., (2008). Iodine exhibited a significant difference with location of sampling ($p<0.05$, F =8.29. df =2; appendix 11).The low uptake of iodine translocation from the soil to the reed plants is thought to be low due to leaching and sorption to soil surfaces.

**Concentration of Sodium and Potassium**

Busia reeds have more Na and K than Lugari reeds for both the wet (2871.8 mg/kg > 2519 mg/kg) and the dry (367 mg/kg > 223 mg/kg) seasons (table 4.5). K showed significant variation in their concentrations with season ($p<0.05$, $F= 426.23$, $df =1$; appendix 8), location ($p<0.05$, $F= 4.34$, $df =4$), region ($p<0.05$, $F= 12.45$, $df =1$) and depth ($p<0.05$, $F= 40.62$, $df =1$). Equally, Na showed significant variation in their concentrations with season ($p<0.05$, $F= 56.68$, $df =1$), location ($p<0.05$, $F= 52.26$, $df =4$) and depth ($p<0.05$, $F= 53.71$, $df =1$) while there was no statististical significant variation between Na and the region of samplimg ($p>0.05$, $F= 0.12$, $df =1$), appendix 8. Iodide may directly interact with clays by forming ion-pairs with K which may concentrate within the interlayer space as well as the thin areas surrounding the clay particles. Due to dissolution, there is more loss of K and Na from soil into the soil solution. However, it wasIt was found that the concentrations of K and Na are higher in soils (table 4.2) than in plants (table 4.5).

**Table 4.5:** Seasonal K, Na and iodine concentrations in Busia and Lugari reed plants

| Sampling point | Wet season | | | Dry season | | |
|---|---|---|---|---|---|---|
| | **$I_2$, mg/kg** | **K, mg/kg** | **Na, mg/kg** | **$I_2$, mg/kg** | **K, mg/kg** | **Na, mg/kg** |
| Ululo | 21.4±5.19 | 3279.8±9.23 | 286.8±19.85 | 21.2±5.64 | 442.5±4.80 | 8677.7±7.83 |
| Bidimbidi | 22.0±2.00 | 2055.9±66.15 | 783.8±13.34 | 36.8±3.58 | 292.1±5.81 | 4024.5±18.05 |
| Matete | 27.6±3.83 | 2452.9±11.79 | 1235.3±12.15 | 23.8±3.05 | 209.6±4.42 | 1767.3±9.72 |
| Lugari | 7.8±5.63 | 2618.2±60.99 | 1360.9±9.06 | 4.2±0.14 | 263.1±0.002 | 1085.7±7.43 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12

The ranking order for TF for Na, K and iodine in studied reed samples was Na > K > > I for both Busia and Lugari regions (table 4.6). A sequence of decreasing TF values: Na> K> I can be generalized for plants in both regions for the dry season, and K> Na> I for the wet season. K had the highest TF values (10472) in the wet season, while Na was the highest (69) in the dry season (table 4.6).

### 4.2.3 Concentration of Iodine, Na, K and Iodine in Ash

The concentration of elements in ash is observed to be of a similar trend; Na > K > I in both regions (table 4.3). The concentration of iodine was higher in Busia ash (61.4 mg/kg) than in

Lugari ash samples (58.1 mg/kg) (table 4.3). The concentration of Na was higher in the wet season (11548.6-16712.1 mg/kg) than in the dry season (2476.6-7461.1 mg/kg) for both the two regions (table 4.3). During the dry season, the concentration of Na was higher in Busia ash (7461.1 mg/kg) than in Lugari ash samples (2476.6 mg/kg) while during the wet season, the concentration of Na was higher in Lugari ash (16712.1 mg/kg) than in Busia ash sample (11548.6 mg/kg) (table 4.3). The concentration of K was highest during the wet season (1896.3-2095.6 mg/kg) than the dry season (2929.0-15039.1 mg/kg). However, the concentration of K was higher in Busia ash samples than in Lugari ash samples both during the dry (2095.6 mg/kg > 1896.3 mg/kg) and the wet seasons (15039.1 mg/kg >2929.0 mg/kg) (table 4.3). The use of fertilizer during the planting season may have contributed to the increase in Na and K uptake by plants, thereby increasing their levels during the wet season.

**Table 4.6:** Transfer factor of micronutrients, trace and heavy metal from soils into reed samples

| Element/sampling point | Iodine | K | Na |
|---|---|---|---|
| BRPU | 0.128 | 4.294 | 2.912 |
| BRPB | 0.208 | 2.980 | 2.783 |
| LRPM | 0.208 | 2.489 | 1.782 |
| LRPL | 0.068 | 2.757 | 2.172 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12

### 4.2.4 Concentration of Micronutrients (Iodine, Na, K, $Fe^{2+}$) in Salt Samples.

The trend from the highest to the lowest was Na> K >Mg for Busia while for Lugari it was K >Na >Mg, although the difference between Na and K in Lugari salts was very minimal.

### Concentration of Iron (II)

The level of iron (II) is higher than iodine comparatively in all the salts from the two reed species of the two sampled regions respectively. Iodine and $Fe^{2+}$ levels in *T. latifolia* salts from Busia region increased from the dry season to the wet season, from 0.6 to 1.2 mg/kg and 0.9 to 1.0 mg/kg respectively (table 4.4). On the other hand the iodine content for *C. rotundus* salt decreased from 1.3 to 0.9 mg/kg from dry to wet season while that of $Fe^{2+}$ increased from 1.1 to

1.4 mg/kg in the same seasons (table 4.4). However, both Iodine and Iron were higher in *C. rotundus* reeds than *T. latifolia* reeds (table 4.5).

**Concentration of Iodine**

The concentration of iodine was higher in the salt samples collected during the dry season (0.2-1.6 mg/kg) than in the wet season (0.1-2.1 mg/kg) (table 4.4). During the dry season Busia reeds had iodine in the range of 1.2-1.4 mg/kg as compare to Busia salts which were in the range of 0.2-1.6 mg/kg respectively. The mean salt iodine concentrations for Lugari's *C. rotundus* salts was 1.1 ± 0.18 mg/kg and 0.9 ± 0.31 mg/kg for Busia's *T. latifolia* salts respectively, lower than the worldwide average of 5 mg/kg (Fleming, 1980; Ure and Berrow, 1982) and the UK average of 9.2 mg/kg (Whitehead, 1979).

**Table 4.7:** Comparison of *C. rotundus* salt, *T. latifolia* Salt and Commercial Salts

| **Element** | **Type of salt** | | | | | |
|---|---|---|---|---|---|---|
| | ***C. rotundus salt*** | ***T. latifolia salt*** | **HSSA** | **SSSA** | **KNSA** | **TCSA** |
| | **Concentration, mg/kg** | | | | | |
| Iodine | 0.9±0.18 | 1.6±0.31 | 19.5±4.79 | 7.4±0.89 | 95.6±1.20 | 69.4±2.39 |
| $Fe^{2+}$ | 1.0±0.01 | 1.0±0.001 | 2.3±0.03 | 0.2±0.03 | nd | nd |
| Na | 3943.8±7.34 | 9969.2±0.0001 | 20099.6±33.54 | 21285.6±77.20 | 18913.6±59.07 | 23855.1±33.94 |
| K | 4635.8±0.001 | 8813.5±7.33 | 10047.0±13.4 | 3819.0±0.0001 | 4294.0±67.19 | 4769.0±0.001 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12; nd= not detected; HSSA= herbal sea salt; SSSA; KNSA= Kensalt; TCSA=Top-Chef salt.

Comparing this with the wet season, the range of iodine concentration in Lugari samples was 0.6-1.3 mg/kg while Busia salts had a range of was 0.1-2.1 mg/kg respectively. Commercial salts however contained more iodine than reed salts in the order TF<CP<SSSA>HSSA<TCSA<KNSA (table 4.7).

**Concentration of Potassium**

The concentration of K ranged from 3114.4-4966.7 mg/kg during the wet season (table 4.7). Busia reeds salts sampled during the wet season contained lower K (3114.4-3974.4 mg/kg) as compared to Lugari reed salts which had a range of 4305.2-4966.7 mg/kg (table 4.7). However, salts from *C. rotundus* reeds collected from Matete contained higher K levels than *C. rotundus* salts from Lugari, while *T. latifolia* salts from Bidimbidi had higher K levels than similar reeds from Ululo. The concentration of K in reed salts and commercial salts was of the order HSSA> TF>CP >TCSA > SSSA (table 4.7). The concentration of K in commercial salts was lower (3819.0-10047.0 mg/kg) than what was observed in reed salts apart from K in HSSA which was higher (table 4.7).

**Concentration of Sodium**

The concentration of Na was higher reed salts collected during the dry season (3398.4-15752.0 mg/kg) than in the wet season (3016.8-10517.0 mg/kg), (table 4.7). Busia reeds salts sampled during the wet season contained lower Na as compared to Lugari reed salts while in the dry season, Busia salts were higher in Na than Lugari reed salts (table 4.7). There was more Na in Matete samples than in Lugari *T. latifolia* salt samples while Bidimbidi *C. rotundus* salt showed higher Na levels than Ululo reed salts (table 4.7). It is apparent from the results that the sodium levels increased with increase in pH and% moisture of the salt.The concentration of Na in reed salts and commercial salts was of the order TCSA > SSSA >HSSA> KNSA>CP >TF (table 4.7).The concentration of Na in commercial salts was higher (18913.6-23855.1 mg/kg) than what was observed in reed salts (table 4.7).

**Concentration of Iron II**

The concentration of $Fe^{2+}$ was the same for both the two types of salts from Lugari and Busia regions in both the wet and dry seasons with a range of 1.0±0.01–1.1±0.001 mg/kg. The order of $Fe^{2+}$ concentration was of the order HSSA>SSSA>TL and CP>KNSA and TCSA with higher levels in commercial salts than in reed salts except for KNSA and TCSA which had undetectable levels of $Fe^{2+}$ (table 4.7). The recommended Dietary Allowances (RDA) for iodine and iron are 0.015 mg/kg and 8 mg/kg respectively, with an Upper Limit (UL) of 1.1 mg/kg and 45 mg/kg respectively. It therefore shows that *C. rotundus* and *T. latifolia* reed salts have slightly higher

iodine (2.0 mg/kg) than required while that of iron is within the recommended limit. Since the K content of *C. rotundus* was higher than that of *T. latifolia*, it is expected that iodine should vary proportionally as supported by the correlation of K to Iodine (r = 0.5466), due to their chemical reaction to form KI compounds. Salt iodine was observed to positively relate with the plant iodine as well as soil iodine (r = 0.5247 and 0.6449; appendix 10 and 11). $Fe^{2+}$ levels seemed to generally decrease with increase in pH while there was no significant relationship with% moisture. On average, Busia *T. latifolia* salt has higher Na, pH and moisture content but lower K, 11,930 mg/kg, 10.3, 10.1% and 3,736.2 mg/kg compared to Lugari *C. rotundus* salt whose Na, K, pH and moisture are 3,943.8 mg/kg, 4,635.8 mg/kg, 9.7 and 0.8% respectively (figure 4.1 and 4.2).

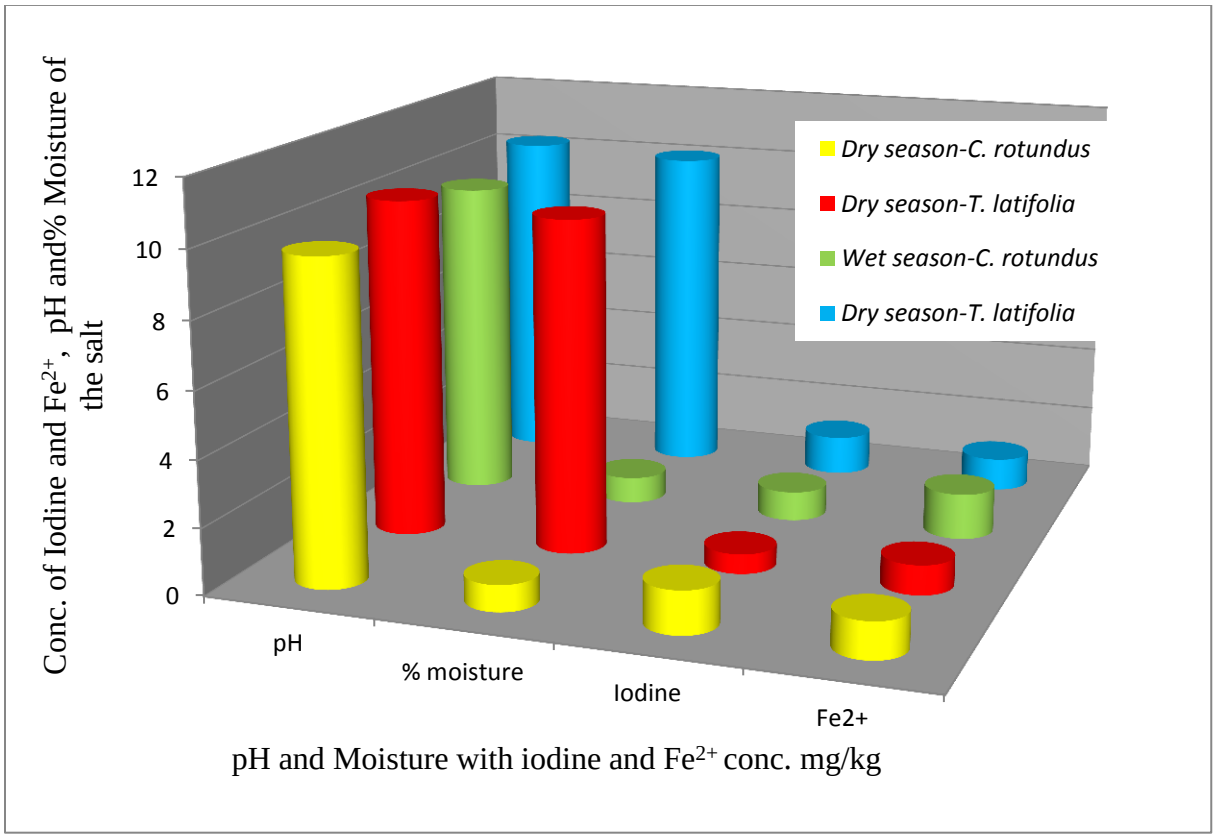
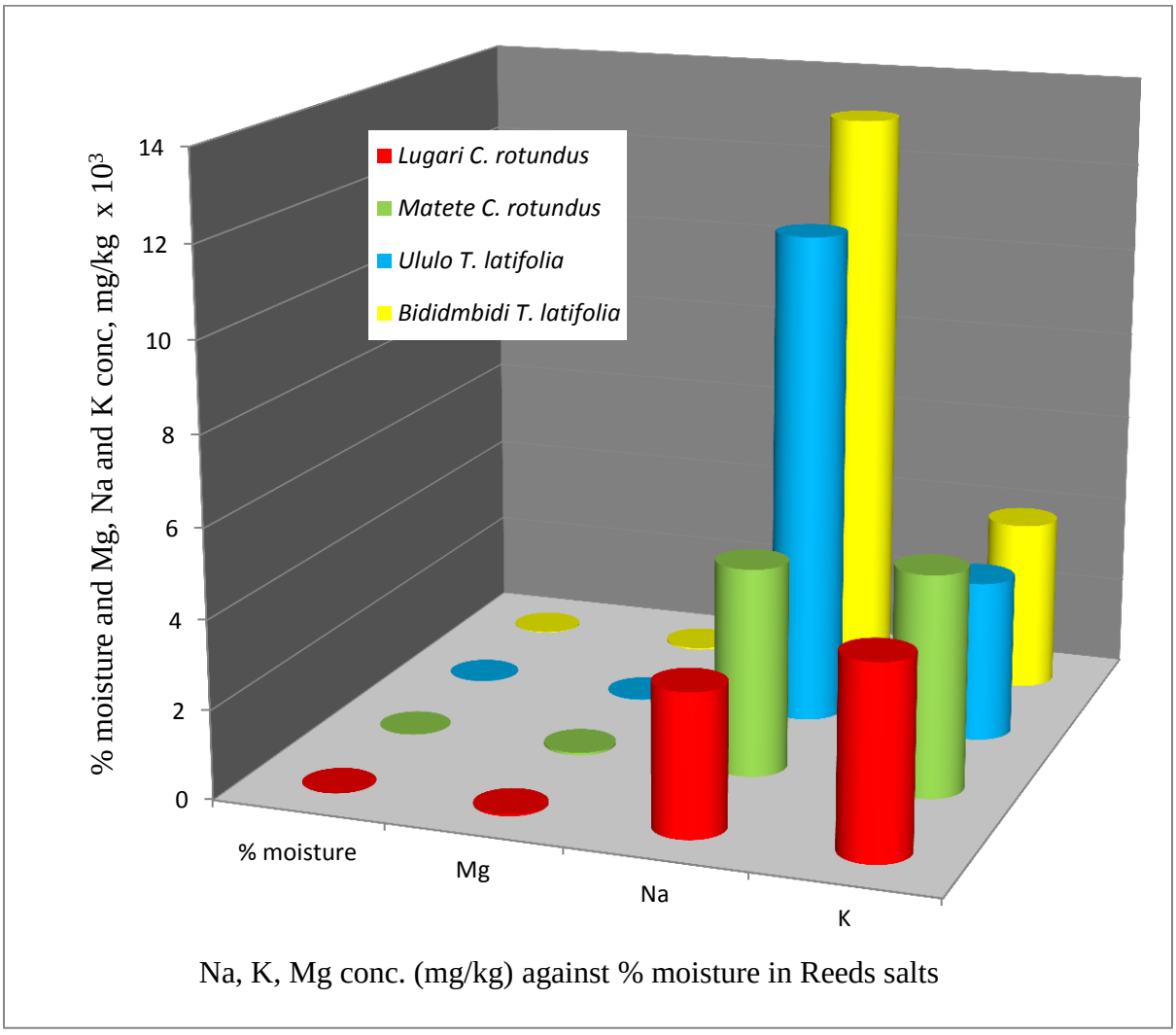



**Figure 4.1:** Concentration of Iodine and $Fe^{2+,}$ mg/kg in salt samples from various sites

Equally, *C. rotundus* reed salts tend to have higher levels of K than Na which the reverse is true for *T. latifolia* that tends to contain higher Na and lower K. The method used for preparing *C. rotundus* salt tends to remove moisture than that used in Busia on *T. latifolia* salt. *T. latifolia* is a

better bioaccumulator for Na than K while *C. rotundus* is better accumulating K than Na. This is expected since Busia plants have higher Na and K due to a higher translocation of the same from the soil to the plants as compared to Lugari, as indicated from their TF value where K and Na TF (table 4.6). From the correlation Table, the amount of Na will decrease when that of K increases (r = -0.7014) while salt K will increase as that of soil K increases (r= 0.7418). The amount of Na increases as the amount of Fe increases (p > 0.05 0.0475, r =180.5; appendix 10 and 11). On the other hand an increase in Cd level in the salt causes a decrease in Na content of the salt (p > 0.05, r = -139.6; appendix 12). *C. rotundus* salts have a lower pH and moisture content compared *T. latifolia* salts.




Na, K, Mg conc. (mg/kg) against % moisture in Reeds salts

**Figure 4.2:** Effect of % moisture on the concentration of Na, K, Mg (mg/kg) in reed salts

Moisture is naturally present in the salt, or is abstracted from the air by hygroscopic impurities such as magnesium chloride. The presence of moisture and some hygroscopic impurities and metal ions impurities such as iron accelerates the loss of iodine (Diosady *et al.*, 1998). As is the case for moisture content, it is clear that the salts from Busia recorded on average the lowest iodate contents, which is true with the moisture contents that were high. It is therefore important to prepare this salt to ensure lower moisture levels so as to have higher iodate contents. Iodine availability in the absence of dietary seafood sources depends largely on its transfer from soil to food and fodder crops (Johnson *et al.*, 2002). Transfer of iodine from soil to plants is generally low and locally grown plants often cannot supply a population with the recommended daily intake of iodine (Johnson, 2003). There is therefore a need to increase understanding of iodine behaviour in soils of Lugari and Busia if the resulting implications for transfer to crops and livestock are to be understood. Although some of the plant salts contain iodate the amount is very low compared to the recommended levels and there is need for iodization of the salt as well.

Comparing the salt samples' Na/K ratio with the recommended ratio of 2.5:1 and 4:1, it can be observed that Lugari samples are well below the 2.5:1 to 4:1 ratio (mean of Na: K of 0.9) recommended by (Xie *et al*., 2014) while for Busia salts processed from *T. latifolia* is slightly below the recommended limit of 2.5-4 for Na: 1 for K, with a mean Na:K ratio of 3.2 (figure 4.3). This suggests that indigenous reed salt from Busia's *T. latifolia* reeds is the more ideal salt for an acceptable Na: K ratio as table or common salt, while Lugari's *C. rotundus* salt could be used as a Lo-Na salt. Currently dietary guidelines in the US recommend limiting salt intake to 1.5-2.4 grams of sodium per day while the American Heart Association suggests 1.5 gram limit. For a frame reference, one tea spoon of regular table salt contains about 2.3 grams of sodium. Data from around the world suggest that the population average sodium consumption is well above the minimal physiological needs, and in many countries is above the value recommended by the 2002 Joint World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) Expert Consultation (WHO, 2013) of 2 g sodium/day (equivalent to 5 g salt/day) (WHO, 2013). The degree of toxicity of heavy metal to human depends on the daily intake. *C. rotundus* and *T. latifolia* reed salts were selected and their health risk assessment calculated in terms of estimated daily intake of metal (EDIM; appendix 7) and estimated health risk index (EHRI; appendix 6) .

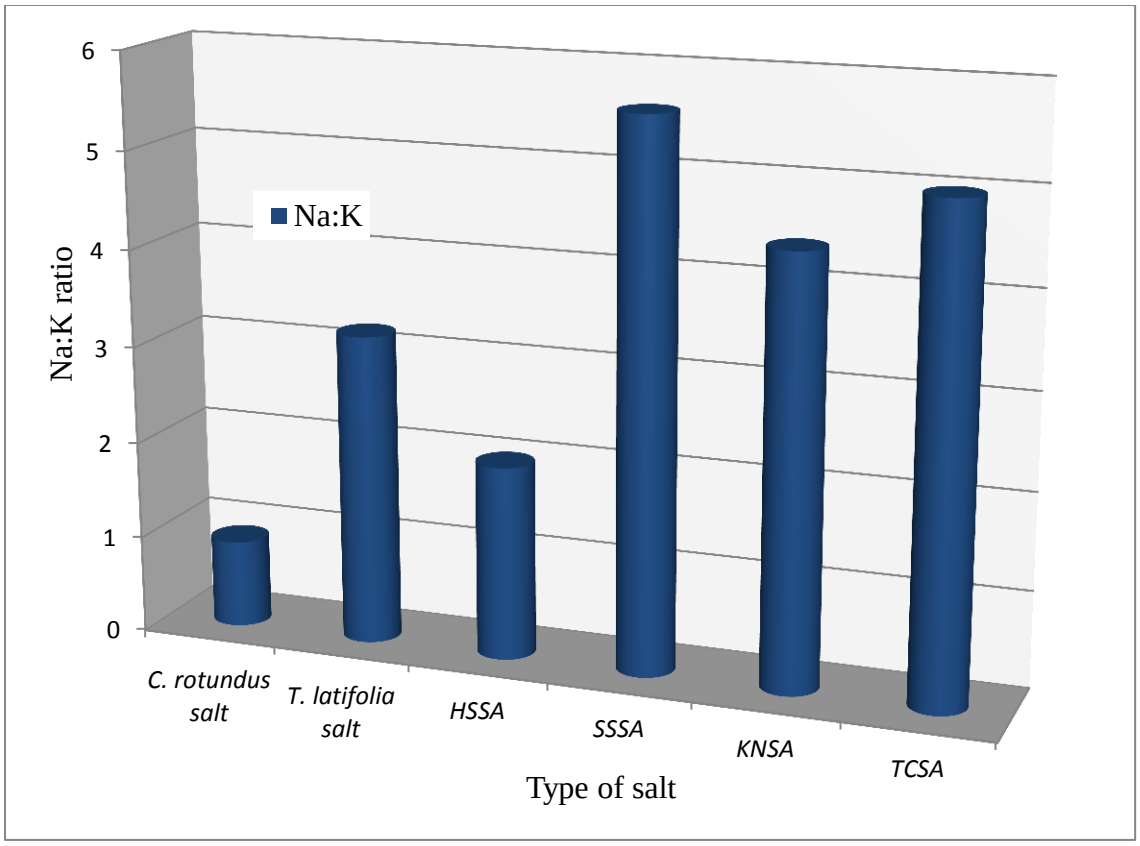



**Figure 4.3:** Na: K ratio for *C. rotundus* and *T. latifolia* and the commercial salts

The dietary intake of the studied heavy metals was estimated and their associated risks were studied by comparing to the provisional tolerable weekly intakes (PTWIs; appendix 6). The commercial salts had K indices ranging between 36-87, with Herbal sea salt having the highest, whereas *C. rotundus* has 311 and 230 for *T. latifolia*, appendix 6. On the other hand Na indices for *C. rotundus* and *T. latifolia* were 311 and 1032 respectively while commercial salts range from 1576-1988, which are higher than the reed salts (appendix 6). There is high K and lower Na levels in the reed salts as compared to the Kenya Bureau of statistics certified commercial salts analysed which are in the market. The Adequate Intake (AI) for sodium is 1,500 mg daily for males and females ages 9-50 (Nguyen *et al*., 2013). This value is less than 1 teaspoon of table salt per day. The maximum recommended level of sodium intake is 2,300 mg daily (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). The daily intake for both Busia and Lugari people is higher than the maximum recommended level of sodium intake of 2,300 mg daily.

### 4.3 Heavy metals Concentration in Soil, Plant, Ash and Salt Samples

#### 4.3.1 Concentration of Fe, Cr, Pb and Cd in Soil Samples

The toxic heavy metals Cd and Pb and the trace elements Cr and Fe, accumulated in the soil of reed plant fields in the dry season. In all the sampling sites, the level of Fe was highest, while Pb was the least, with a ranking order of Fe > Cr > Cd > Pb for both top surface and sub-surface soils (table 4.8).

**Table 4.8:** Concentration of heavy metals Fe, Pb, Cd and Cr concentrations of Busia and Lugari soils, wet and dry season, at 95.0% confidence level.

| **Dry season, at 95.0% confidence level** | | | | | | |
|---|---|---|---|---|---|---|
| **Sampling area** | **Depth cm** | **pH** | **Fe, mg/kg** | **Pb, mg/kg** | **Cd, mg/kg** | **Cr, mg/kg** |
| Ululo | 0-15 cm | 6.5 | 635.5±79.95 | 0.3±1.21 | 4.7±0.01 | 45.8±5.78 |
| | 15-30 cm | 6.4 | 811.7±73.79 | 9.4±4.25 | 4.7±0.01 | 125.8±5.77 |
| Bidimbidi | 0-15 cm | 6.2 | 228.6±13.25 | 1.5±0.61 | 4.7±0.02 | 79.1±3.33 |
| | 15-30 cm | 6.1 | 758.7±39.76 | nd | 4.7±0.03 | 79.1±3.33 |
| Matete | 0-15 cm | 6.3 | 559.9±27.16 | 3.4±1.74 | 4.7±0.02 | 65.8±3.65 |
| | 15-30 cm | 6.3 | 619.6±52.09 | 4.6±1.76 | 4.7±0.01 | 69.1±5.58 |
| Lugari | 0-15 cm | 6.1 | 440.6±39.76 | nd | 4.7±0.02 | 92.5±3.33 |
| | 15-30 | 4.3 | 559.9±45.91 | 7.0±0.61 | 4.6±0.01 | 72.5±3.33 |
| **Wet season, at 95.0% confidence level, (Ns=4, Nr=8).** | | | | | | |
| Ululo | 0-15 | 6.9 | 364.0±3.08 | nd | 2.3±0.02 | 26.9±1.01 |
| | 15-30 | 6.8 | 23.2±3.58 | nd | 2.4±0.01 | 41.7±0.25 |
| Bidimbidi | 0-15 | 6.9 | 102.6±2.14 | 1.1±0.23 | 2.4±0.02 | 17.4±1.00 |
| | 15-30 | 6.7 | 63.9±1.99 | 0.5±0.27 | 2.5±0.01 | 26.9±3.50 |
| Matete | 0-15 | 7.1 | 272.7±6.96 | 0.2±0.32 | 2.5±0.01 | 17.2±1.25 |
| | 15-30 | 7.1 | 46.1±1.11 | 0.5±0.03 | 2.7±0.26 | 15.7±2.25 |
| Lugari | 0-15 | 6.9 | 80.9±0.91 | nd | 1.3±1.12 | 18.9±0.32 |
| | 15-30 | 6.9 | 119.7±0.84 | nd | 6.0±3.54 | 19.9±0.50 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12; nd = not detected.

**Concentration of Iron**

Findings indicated higher iron levels accumulation in the soil samples during the wet season than in the dry season (table 4.8). The Fe concentration in the area of study increased from 440.6-

811.7 mg/kg during the dry season. In addition it was noted that the Fe concentration increased with increase in depth. This is probably due to the preconcentration of Fe in the subsoil in the absence of the rains. However, during the wet season, the concentration ranged from 23.2-364.0 mg/kg and there was a decrease in Fe concentration with increase in depth except for Lugari (table 4.8). The decrease in Fe concentration may be due to dissolution and leaching effect during the rains. Reeds grow to a depth of about 35 cm and therefore have utilization of soil-Fe. The concentration of iron varied significantly with the sampling region ($p<0.05$, $F=101.88$, $df=1$), location ($p<0.05$, $F=12.66$, $df=1$; appendix 3) while it was insignificant with season of sampling ($p>0.05$, $F=0.33$, $df=1$; appendix 3).

**Concentration of Chromium**

The concentrations of Cr during the dry season were higher (45.8-125.8 mg/kg) than the wet season (15.7-41.7 mg/kg) (table 4.8). The concentration of Cr in Busia was in the range of 45.8-125.8 mg/kg and 17.4 - 41.7 mg/kg while the range for Lugari was between 65.8-92.5 mg/kg and 17.2 – 19.9 mg/kg during the wet and dry seasons respectively. The concentration of Cr was generally lower in the top-soils as compared to the sub-soils in both regions. The concentration of Cr varied significantly with the sampling region ($p<0.05$, $F=17.95$, $df=1$), location ($p<0.05$, $F=123.20$, $df=1$) and season of sampling ($p>0.05$, $F=160.23$, $df=1$), appendix 3.

**Concentration of Lead**

The concentrations of Pb ranged from 0.2- 9.4 mg/kg with relatively higher levels during the dry season (0.3-9.4 mg/kg) than the wet season (0.2-1.1 mg/kg), (table 4.8). However, during the wet season, Pb levels in Ululo and Lugari areas were below the detection limit. The amount of Pb was higher in sub-soils than in top-soils. The concentration of Pb varied significantly with the sampling region ($p<0.05$, $F=54.46$, $df=1$), location ($p<0.05$, $F=14.16$, $df=1$) and season of sampling ($p>0.05$, $F=19.77$, $df=1$), appendix 3. The high levels of Pb in soils could be attributed to the various sources of Pb such as vehicle exhaust emissions, sewage sludge, manure, mining and smelting activities.

**Concentration of Cadmium**

The Cd concentrations during the in the dry season (4.6-4.7 mg/kg) were higher than in the wet season (1.3-2.5 mg/kg for the top soil), (table 4.8). The concentration of Cd did not seem to

change with depth in both the two seasons. The Cd concentrations in soils from this study were found to be higher than those obtained by Demi˙rezen and Aksoy (2006), who found the concentrations of Cd to be 1.83 mg/kg, in non-contaminated soils in rural area. pH is the most significant characteristic and is directly related to mobility and bioavailability of Cd and other high risk heavy metals. However, the broad spectra of metals (including Cd) are highly available below neutral pH (table 4.8). The concentration of Cd varied significantly with the sampling region ($p<0.05$, $F=54.46$, $df=1$), location ($p<0.05$, $F=14.16$, $df=1$) and season of sampling ($p>0.05$, $F=18.22$, $df=1$), appendix 3. However, the concentrations of Pb, Cd and Cr were least affected by pH since the soils were almost neutral. Pb interacts positively with Cd in both plants and soil. These findings agree with those reported by Panich *et al.,* 2010 who observed a positive correlation between Cd and Pb. While Cd interacted positively with Cr and Fe in plants it interacted negatively with the same in soil. Pb interacted negatively with Fe in plants. The concentrations of Fe, Pb and Cr in reed soils are comparable to those for worldwide normal soils (Ghani *et al.,* (2012). Ghani *et al.,* (2012) reported maximum concentrations of Fe and Pb metals in the analyzed samples as 207.6 and 0.39 mg/L respectively. However, Cd level in both soils are higher than the regulatory limits of 0.6 mg/kg, and hence indicating Cd contamination. The high levels of Cd in soils could be attributed to heavy use of phosphate fertilizers and farm manure during farming. Further, the fertilizer inputs, batteries, fungicides, incineration of tyres, rubber, iron roofs and motor oil, which contain Cd, could have elevated the heavy metal in the soils.

### 4.3.2 Concentration of Fe, Pb, Cd and Cr in Plant Samples

The level of heavy metals in plant samples was generally of the order Fe> Cr >Pb > Cd for the dry season and Cr > Fe > Cd > Pb for the wet season (table 4.9).

#### Concentration of Iron

The concentration of Fe during the wet season was higher than that during the dry season in both regions. In the wet season, Fe concentration ranged from 440.6-705.7 mg/kg, while during the dry season it ranged from 10.0-19.8 mg/kg (table 4.9). Busia reed plants have more Fe than the reeds collected from Lugari region during the wet season.However, during the dry season the plants in Lugari had a higher concentration of Fe than those in Busia (table 4.9). The concentration of Fe in reeds varied significantly with the sampling location ($p<0.05$, $F=418.47$,

df =2), appendix 3. In Busia, Fe was highest in soils (table 4.2) than in the reed plants, while it was highest in plants than in soils in the Lugari region. Concentrations of Fe in reed plants from this studty area agree with Ghani *et al.,* 2012. The WHO recommended level of iron in plants is 20 mg/kg (Nazir *et al*., 2015). This means that the level of Fe in reed plants during the wet season was higher than the WHO permitted levels while the dry season Fe concentration was slightly lower than recommended. The TF of Fe was 0.956 in Busia and 1.698 in Lugari respectively, appendix 5. There is more uptake of Fe by *C. rotundus* than by *T. latifolia* reeds within the Western Kenya region (table 4.9).

**Table 4.9:** Heavy metals Fe, Pb, Cd and Cr concentrations in plants from Busia and Lugari regions sampled during the wet and dry season (Confidence Level, 95.0%).

| **Season** | **sampling point** | **Concentration in mg/kg** | | | |
|---|---|---|---|---|---|
| | | **Fe** | **Pb** | **Cd** | **Cr** |
| Wet season | Ululo | 705.7 ±93.76 | nd | 4.6 ±0.01 | 47538.8 ±376.62 |
| | bidimbidi | 699.1 ±35.84 | 0.9 ±1.05 | 4.7 ±0.01 | 52042.4 ±77.81 |
| | Matete | 682.5 ±56.40 | nd | 4.7 ±0.03 | 46037.6 ±13.60 |
| | Lugari | 440.6 ±72.59 | nd | 4.7 ±0.02 | 59548.4 ±64.37 |
| Dry season | Ululo | 11.9±7.82 | 3.0±0.46 | 0.1±0.02 | 7.5±3.07 |
| | Bidimbidi | 10.0±2.95 | 1.9±0.19 | 5.2±2.58 | 22.5±6.15 |
| | Matete | - | 3.0±0.66 | 0.1±0.91 | 22.5±6.24 |
| | Lugari | 19.8±0.58 | 1.8±0.03 | 1.0±0.58 | 42.5±3.33 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12; nd= not detected

**Concentration of Lead**

The concentration of Pb in the plant species was higher in the dry season (1.8-3.0 mg/kg) while in the wet season only one sample was detected with 0.9 mg/kg (table 4.10). There was no variation in the two regions and Pb concentration varied significantly with the sampling location ($p<0.05$, $F=13.73$, df =2; appendix 10 and 11). However, Ghani *et al.,* 2012 found lower levels of Pb (0.39 ± 0.01 mg/kg) compared to the levels obtained in this study. European regulations provide maximum levels of Pb as 0.3 mg/kg f.w and the permissible limit in plants recommended by WHO is 2mg/kg (Nazir *et al*., 2015). Given this limit, only *C. rotundus* is recommended for use since its Pb level is below the recommended limit (Commission

Regulation (EC) No 466/2001). Pb uptake in plants was low as shown by the TF values (table 4.10). The low TF values are probably due to a higher Pb sorption affinity for soil surfaces and poorer translocation to plants (Ghani *et al.,* 2012). The mean Pb values in *T. latifolia* and *C. rotundus* reeds are more than the legally estimated value of 0.20 mg/kg specified by Wisnu (2008). *C. rotundus* tends to be less contaminated with Pb since its uptake is less (TF=0.205) compared to *T. latifolia* reeds with TF of 1.40 (table 4.10).

**Concentration of Cadmium**

The level of Cd in Busia reeds was the same with that for Lugari region during the wet season (table 4.10), while in the dry season the Cd levels were higher in Busia reeds (0.1-5.2 mg/kg) than for Lugari reeds (0.1-1.0 mg/kg). The permissible limit of Cadmium in plants, recommended by WHO, is 0.02 mg/kg implying that the reed Cd levels are higher (Nazir *et al*., 2015). The concentration of Cd in plant samples was lower than soil an indication iof restricted translocation from soils to plants. However, comparing the mean Cd levels in Busia and Lugari reed plants having transfer factors in the range of 0.667-1.385 mg/kg and 0.658-0.687 mg/kg respectively (table 4.10), then it is clear that Lugari *C. rotundus* reeds have a lower uptake of Cd than *T. latifolia*. The concentration of Cd varied significantly with the sampling location ($p<0.05$, $F=164.59$, df =2; appendix 11). This could probably be due to other competing metals present in Lugari than in Busia soils. However, there is also a possibility of Pb aerial deposition on reed leaves from use of fertilizers since a lot of fertilizers are used in farming within this region.

**Concentration of Chromium**

The concentration of Cr in reed plants was higher during the wet season (46037-59548 mg/kg) than in the dry season (7.5-42.5 mg/kg) (table 4.10). The permissible limit of Chromium for plants is 1.30 mg/kg recommended by WHO (Nazir *et al*., 2015). In reed plants, concentration of chromium was higher than the WHO recommendations. (Chemistry of cr in water logged soils, normal soils and plants). There is a higher uptake of Cr by *C. rotundus* than by *T. latifolia* reed species. The TF for Cr in Busia samples was 443 while Lugari recorded 569 showing that *C. rotundus* is a better bioaccumulator of Cr than *T. latifolia* reed (table 4.10). WHO limit is 2.3 mg/kg in plants (WHO/FAO, 1999). The mean concentration of Cr in reed plants was higher

than in soils for both *C. rotundus* and *Typha latifolia*. The concentration of Cr varied significantly with the sampling location ($p<0.05$, $F=5.67$, df =2; appendix 11).

**Table 4.10:** Transfer factor of micronutrients, trace and heavy metal from soils into reed samples

| **Element/sampling point** | **Transfer factors for respective elements** | | | |
|---|---|---|---|---|
| | **Fe** | **Pb** | **Cd** | **Cr** |
| BRPU | 0.782 | 0.968 | 0.667 | 395.889 |
| BRPB | 1.229 | 2.947 | 1.385 | 514.221 |
| LRPM | 1.822 | 0.483 | 0.658 | 548.988 |
| LRPL | 0.767 | ND | 0.687 | 584.798 |
| BUSIA | 0.956 | 1.400 | 1.014 | 443.135 |
| LUGARI | 1.698 | 0.205 | 0.641 | 569.352 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and LSD test. n=12

Although the levels of these metals are within normal range for plants, however continual consumption could lead to accumulation and adverse health implication (Sebastiani *et al.,* 2004) particularly for Pb and Cd. Also the variation in values obtained for these heavy metals in the soil and crop plant samples along river Nzoia and Ululo down from Bidimbidi is an indication of their mobility particularly through leaching and runoffs. This is in agreement with the report of Oluyemi *et al.,* 2008.

The presence of heavy metals tends to affect the Na/ K ratios and therefore an indication of toxicity. The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of plant species, and is altered by innumerable environmental and human factors (Zurera *et al.,* 1987). The Tf of iodine and Fe seem to be related for both Busia and Lugari reed plants/ regions. The Tf value for Iodine is lower than that of Fe in both cases and the Tf values for Iodine, Cd and Fe are lower for Busia than for Lugari (table 4.6). While this is true, the Tf values for Cr, Mg and Pb are higher in Lugari than in Busia (table 4.10). It is necessary to take into consideration the fact that the content of metal in plant is influenced by a variety of factors, including: plant species, type of soil and its physico-chemical properties. Also, metals are taken up by plants not only from soil, but also from air deposition and water. Generally, from farming and other human activities, chemicals entering the water system, subsequently, enter the soil. The soil absorbs part of the chemicals which, subsequently, become

part of the photosynthetic processes. It was therefore expected that the concentrations of metals in the reed plants would reflect the concentrations in the soil samples. On the basis of transfer factor values the higher the value of transfer factor, the more element would be accumulated by plants. Additionally, soil pH, soil texture and BD also have influence on the metal content in plants, since this determines the amount of the metals in the soils thereby affecting the amount translocated to the plant either by increase or decrease of the same. This is consistent with conclusions of Sipter *et al.,* (2008) that Tf of Cd and Pb in soils varied substantially with the concentrations found in plants growing on the soils in contaminated areas. Cr and Pb were found to be the most accumulated in both reed species in the dry season with cadmium being the least. This implies that the local inhabitants are at high risk of being exposed to lead and cadmium related health diseases. It was further observed that Cd which was the third most accumulated heavy metal in the soils was the least absorbed in the reed plants while Pb which was the second least accumulated in the soil is amongst the most absorbed metal by the two reed species. According to Liu *et al.,* (2005), there is significant correlation between metal concentrations in rhizosphere soils and grown crops as has been observed in this study.

### 4.3.3 Concentration of Fe, Pb, Cd and Cr in Ash Samples

The concentration of heavy metals in ash samples were of the order Fe>Pb>Cd>Cr for Lugari and Fe>Pb>Cr>Cd for Busia ash samples (table 4.3).

**Concentration of Iron**

The concentration of Fe was higher in Lugari ash samples (669.3-3757.5 mg/kg) as compared to the Busia ash samples (441.6-480.5 mg/kg) in both seasons. During the dry season, the concentration of Fe was 3757.5 mg/kg in Lugari ash and 480.5 mg/kg in Busia ash while in the wet season; Lugari ash contained 3757.5 mg/kg as compared to 480.5 mg/kg for Busia ash samples. The mean concentration of Fe was found to be highest in Lugari (2213.4 mg/kg) as compared to Busia's ash samples (461 mg/kg), table 4.3.

**Concentration of Lead**

There was more Pb in Busia ash samples (12.76 mg/kg) than Lugari ash samples (10.37 mg/kg) during the dry season. However, no Pb was detected in ash samples collected during the wet season in both regions (table 4.3)

**Concentration of Cadmium**

The concentration of Cd was higher in Busia ash (4.9 mg/kg) than in Lugari ash samples (4.5 mg/kg) for samples collected during the dry season. However, samples collected during the wet season contained the same concentration of Cd of 3.6 mg/kg (table 4.3).

**Concentration of Chromium**

The concentration of Cr was higher in Busia ash than in Lugari ash samples both during the wet (6.0 mgKg > 3.1 mg/kg) and the dry seasons (3.0 mg/kg > 0.5 mg/kg), (table 4.3). The concentration of Cr was higher during the dry season as compared to the wet season. There is a negative relationship between the plant-element and the ash-element such that while there is an increase in the element concentration in the plant, there is a decrease in the ash. It can be concluded that ashing process tends to affect the chemical composition of the elements in the ash product.

### 4.3.4 Concentration of Fe, Pb, Cd and Cr in Salt Samples

From the results, it was observed that for both dry and wet seasons, the trend of heavy metals presence in the salt samples was of the order Fe > Pb > Cd> Cr, for all the areas sampled (table 4.11). Comparing the average concentrations of the heavy metals Cd and Pb for the two regions, Lugari salt samples had slightly higher mean concentrations. Of the heavy metals chromium was the least detected and for the detected chromium, the concentration was fairly insignificant.

**Concentration of Iron**

The concentration of Fe was higher during the dry season than in the wet season with a range of 72.5-209.2 mg/kg for the dry season and 15.2-53.6 mg/kg for the wet season (table 4.11). Busia *T. latifolia* salt contained higher Fe than Lugari *C. rotundus* salt in both the two seasons, with Ululo having higher Fe than Bidimbidi in both seasons while Lugari reeds contained higher Fe than Matete in both seasons (table 4.11). These may be may be attributed to increased dilution

and dissolution both within the swamp and downstream. The concentration of Fe in reed salts was higher than that in commercial salts in the order was of the order CP >TL > KNSA> SSSA >HSSA >TCSA salt in the range of 1.2-26.1 mg/kg (table 4.11), (Pourgheysari *et al.,* 2012). The concentration of Pb in the salt was found to increase with a decrease in plant Pb ($p > 0.05$, $r = -0.5666$).

**Table 4.11:** Concentration of heavy metals Fe, Cd, Pb and Cr in salt samples for the wet and dry seasons.

| **Season of Sampling** | **Sampling area** | **pH** | **% Moisture** | **Concentration, mg/kg** | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | **Fe** | **Cd** | **Pb** | **Cr** | **Mg** |
| Dry | Lugari | 9.7 | 0.5±0.02 | 142.2±13.50 | 8.4±0.90 | 58.9±2.40 | nd | 17.6±0.80 |
| | Matete | 9.8 | 1.0±0.01 | 72.5±8.20 | 7.9±0.40 | 54.0±3.10 | nd | 23.1±1.10 |
| | Ululo | 10.4 | 8.2±1.80 | 209.2±15.90 | 7.4±0.60 | 57.7±4.40 | nd | 11.1±1.60 |
| | Bidimbidi | 10 | 15.6±0.01 | 169.1±19.30 | 7.1±0.70 | 55.8±4.50 | 0.1±0.50 | 19.2±1.50 |
| Wet | Lugari | 9.7 | 0.5±0.08 | 33.1±1.20 | 4.7±0.02 | 8.8±0.60 | 2.5±1.02 | 69.3±0.58 |
| | Matete | 9.8 | 1.0±0.20 | 15.2±1.20 | 4.7±.003 | 4.0±2.70 | nd | 100.3±0.22 |
| | Ululo | 10.4 | 8.2±1.80 | 53.6±5.90 | 4.7±0.01 | 4.4±1.10 | 5.8±3.33 | 32.5±7.44 |
| | Bidimbidi | 10 | 15.6±0.12 | 23.5±7.30 | 4.6±0.03 | 4.6±1.60 | 0.8±0.42 | 61.6±0.38 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12; nd=not detected

### Concentration of Lead

The concentration of Pb was higher in the salt samples collected during the dry season (54.0-58.9 mg/kg) than the wet season (4.0 -8.8 mg/kg). Lugari salts contained the highest concentration of Pb as compared with the other sites in wet (8.8 mg/kg) and dry seasons (58.9 mg/kg), table 4.12. The findings in this study are much higher than those reported by other researchers. The contribution that automobiles have on Pd contamination cannot be over emphasized; however industrial wastes from Webuye and Mumias could be a contributing factor to the high levels of Pb in these regions.

The concentrations of Pb in this study were also found to be far much above the WHO permissible limit of 0.20 mg/kg Pb (Eftekhari *et al.,* 2014) and indicate a potential health hazard to users. The maximum permitted level of lead in food-grade salt is 2.0 mg/kg according to the Codex legislation (Codex Alimentarius Commission, Rev 1-1997 Amend 1-1999 Amend 2-2001; Siulapwa and Mwambungu, 2015). The concentration of Pb in both the reed salts and the commercial salts was of the order TL > CP > KNSA =HSSA = SSSA= TCSA with no detectable Cd in aal the selected commercial salts (table 4.12).

**Table 4.12:** Comparison of *C. rotundus* salt, *T. latifolia* salt and commercial Salts

| **Element** | **Type of salt** | | | | | |
|---|---|---|---|---|---|---|
| | *C. rotundus* salt | *T. latifolia* salt | HSSA | SSSA | KNSA | TCSA |
| | Concentration, mg/kg | | | | | |
| Fe | 65.8±4.16 | 117.4±75.79 | 5.7±9.59 | 8.0±6.39 | 26.1±19.20 | 1.2±3.20 |
| Cd | 6.4±1.7 | 6.0±1.29 | 4.2±0.25 | 4.2±0.25 | 4.4±0.001 | 4.0±0.001 |
| Pb | 31.4±2.50 | 30.7±26.25 | nd | nd | Nd | nd |
| Cr | nd | 1.8±1.96 | 1.3±0.0001 | 1.3±0.0001 | 1.3±0.0001 | 1.3±0.001 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; statistically different at $p<0.05$ by ANOVA and lsd test. n=12; nd= not detected; HSSA= herbal sea salt; SSSA; KNSA= Kensalt; TCSA=Top-Chef salt.

**Concentration of Cadmium**

The concentration of Cd was higher during the dry season in the range of 7.4-8.4 mg/kg than the wet season that had a range of 4.6-4.7 mg/kg respectively. Although Lugari salt samples had higher Cd levels for both the two seaons, there was however very insignificant variation in the Pb concentration in Lugari and Busia during the wet season (table 4.12). Heshmati *et al.,* (2014) reported the lower levels of Cd (mean ± SD, mg/kg ) in table refined salt samples as 0.229 ± 0.012 mg/kg as compared to the cd levels in reed salts while results for the same metal in bakery refined salt samples was 0.240 ± 0.018 mg/kg . Reed salts therefore have relatively high Cd compared to the WHO permissible limit of 0.20 mg/kg Pb and 0.21 mg/kg for Cd respectively (Eftekhari *et al.,* 2014). It is thought that the use of fertilizers for farming and the possibility of industrial wastes contribute the high Cd levels. The concentration of Cd metal in reed salts was also compared with Pb levels in selected commercial salts. The Cd concentration in the reed and commercial salts was of the order CP > KNSA =HSSA = SSSA=TCSA >TL salt (table 4.12),

with reed salts having higher levels (6.0-6.4 mg/kg) than the commercial salts (4.0-4.2 mg/kg). The levels of Cd in both the two reed salts under study were higher than the WHO permissible limit of 0.21 mg/kg as well as the permitted consumption level defined by Codex for Cd is 0.5 mg/kg (Eftekhari *et al.,* 2014).

**Concentration of Chromium**

The concentration of Cr in the reed salts was highest during the wet season (0.8-2.5 mg/kg) than in the dry season which had Cr in Bidimbidi salts only (0.1 mg/kg), while the rest had undetectable levels (table 4.12). It was also noted that salts collected during the wet season from Busia reeds contained higher Cr than those of Lugari reeds. The concentration of Cr in reed salts as compared to commercial salts was of the order CP > TL while it was undetected in KNSA, HSSA, SSSA and TCSA (table 4.12).

The relationship observed between salt-Cr and plant and soil-Cr was showed that an increase in plant Cr increased the salt Cr content (pvalue= 0.0439, coeff= 0.0161). This was supported by the positive correlation observed for salt Cr with plant and soil Cr (r= 0.6622). On the other hand, an increase in soil Cr caused a decrease in salt Cr (pvalue=0.0382, Coeff= -0.0161; appendix 9 and 12) which was observed with the correlation analysis (r= -0.6969; appendix 12).

At low concentration, chromium is essential for human health; however, high levels of this element is toxic (Liu and Kaffes, 2012). Despite the positive effects of optimal levels of chromium, harmful effects may occur if the threshold level is exceeded. In the present study, the mean Cr concentrations found in the salts were less than the permitted levels of chromium of 20-25 mg/day for women and 30-35 mg/day for men (WHO, 1999). It was however observed that reed salts contained lower Cr levels as compared to the commercially available salts which had similar Cr levels (1.3 mg/kg), table 4.12.

The degree of toxicity of heavy metal to human depending on the daily intake showed that the consumption of reed salt processed from *T. latifolia* is better than for *C. rotundus* as the dietary intake of Cr and Fe in adults is lower than the permissible limits (WHO, 1996), while its Pb and Cd EDIM values are higher than for *T. latifolia* reed salt (table 4.13 and 4.14). The HRI has been recognized as a very useful index to evaluate the health risk associated with the consumption of

heavy metal contaminated food and food additives (Wang *et al.,* 2005). The oral reference dose (RD) for Cd, Pb and Cr are 1.0E-03, 3.5E-03 and 1.5E-00 mg/kg/ day, respectively given by US-EPA (2002), IRIS (2003).

**Table 4.13:** Exposure to Pb, Cd, Cr and Fe metals through salt intake- Daily and Weekly intake of metals in the reed salts.

| Salt Type | Dep. Var | Conc, mg/kg | DI, mg/dy/person | PTWIs, mg/pers/wk | EDI, mg/kg /dy |
|---|---|---|---|---|---|
| *C. rotundus* | Iod | 1.1 | 0.0055 | 0.0385 | 0.0001 |
| | $Fe^{2+}$ | 1 | 0.005 | 0.035 | 0.0001 |
| | Fe | 65.8 | 0.329 | 2.303 | 0.0055 |
| | Cd | 6.4 | 0.032 | 0.224 | 0.0005 |
| | Pb | 31.4 | 0.157 | 1.099 | 0.0026 |
| | Cr | nd | nd | nd | nd |
| | Mg | 52.5 | 0.2625 | 1.8375 | 0.0044 |
| | Na | 3943.8 | 19.719 | 138.033 | 0.3287 |
| | K | 4635.8 | 23.179 | 162.253 | 0.3863 |
| *T. latifolia* | Iod | 0.9 | 0.0045 | 0.0315 | 0.0001 |
| | $Fe^{2+}$ | 1 | 0.005 | 0.035 | 0.0001 |
| | Fe | 117.4 | 0.587 | 4.109 | 0.0098 |
| | Cd | 6 | 0.03 | 0.21 | 0.0005 |
| | Pb | 30.7 | 0.1535 | 1.0745 | 0.0026 |
| | Cr | 1.8 | 0.009 | 0.063 | 0.0002 |
| | Mg | 29.2 | 0.146 | 1.022 | 0.0024 |
| | Na | 9969.2 | 49.846 | 348.922 | 0.8308 |
| | K | 8813.5 | 44.0675 | 308.4725 | 0.7345 |
| KNSA | Iod | 95.6 | 0.478 | 3.346 | 0.008 |
| | $Fe^{2+}$ | nd | nd | nd | nd |
| | Fe | 26.1 | 0.1305 | 0.9135 | 0.0022 |
| | Cd | 4.4 | 0.022 | 0.154 | 0.0004 |
| | Pb | nd | nd | nd | nd |
| | Cr | 1.3 | 0.0065 | 0.0455 | 0.0001 |
| | Mg | 38.9 | 0.1945 | 1.3615 | 0.0032 |
| | Na | 18913.6 | 94.568 | 661.976 | 1.5761 |
| | K | 4294 | 2.147 | 15.029 | 0.0358 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; n=12; nd= not detected; HSSA= Herbal sea salt; SSSA; KNSA= Kensalt; TCSA=Top-Chef salt.

From the estimated Health Risk index (EHRI) (table 4.15) values obtained it is evident that the exposed population is not safe since the Cd and Pb EHRI values for both salt samples are greater than one indicating that there is a potential risk associated with Cd and Pb metals.

**Table 4.14:** Exposure to Pb, Cd, Cr and Fe metals through salt intake- Daily and Weekly intake of metals in the reed salts.

| Salt Type | Dep. Var | Conc, mg/kg | DI, mg/dy/person | PTWIs, mg/pers/wk | EDIM, mg/kg /dy |
|---|---|---|---|---|---|
| HSSA | Iod | 19.5 | 0.0975 | 0.6825 | 0.0016 |
| | $Fe^{2+}$ | 2.3 | 0.0115 | 0.0805 | 0.0002 |
| | Fe | 5.7 | 0.0285 | 0.1995 | 0.0005 |
| | Cd | 4.2 | 0.021 | 0.147 | 0.0004 |
| | Pb | nd | nd | nd | nd |
| | Cr | 1.3 | 0.0065 | 0.0455 | 0.0001 |
| | Mg | 156.5 | 0.7825 | 5.4775 | 0.013 |
| | Na | 20099.6 | 100.498 | 703.486 | 1.675 |
| | K | 10047 | 5.235 | 36.645 | 0.0873 |
| SSSA | Iod | 7.4 | 0.037 | 0.259 | 0.0006 |
| | $Fe^{2+}$ | 0.2 | 0.001 | 0.007 | 0 |
| | Fe | 8 | 0.04 | 0.28 | 0.0007 |
| | Cd | 4.2 | 0.021 | 0.147 | 0.0004 |
| | Pb | nd | nd | nd | nd |
| | Cr | 1.3 | 0.0065 | 0.0455 | 0.0001 |
| | Mg | 48.1 | 0.2405 | 1.6835 | 0.004 |
| | Na | 21285.6 | 106.428 | 744.996 | 1.7738 |
| | K | 3819 | 1.9095 | 13.3665 | 0.0318 |
| TCSA | Iod | 69.4 | 0.347 | 2.429 | 0.0058 |
| | $Fe^{2+}$ | nd | nd | nd | nd |
| | Fe | 1.2 | 0.006 | 0.042 | 0.0001 |
| | Cd | 4 | 0.02 | 0.14 | 0.0003 |
| | Pb | nd | nd | nd | nd |
| | Cr | 1.3 | 0.0065 | 0.0455 | 0.0001 |
| | Mg | 15.4 | 0.077 | 0.539 | 0.0013 |
| | Na | 23855.1 | 119.2755 | 834.9285 | 1.9879 |
| | K | 4769 | 2.3845 | 16.6915 | 0.0397 |

Results are expressed as means ± standard error of the mean (MSE) for 3 determinations; n=12; nd= not detected; HSSA= Herbal sea salt; SSSA; KNSA= Kensalt; TCSA=Top-Chef salt.

The higher the EHRI value, the higher the probability of experiencing long term carcinogenic effects (USEPA, 2002). The order of dominance of HRI index for the metal is Pb > Cd. This clearly shows that the local inhabitants are most likely to be exposed to potential health risk from dietary Pb and Cd.

**Table 4.15:** Estimated Health Risk index (EHRI)

| Element | *C. rotundus* | | | *T. latifolia* | | |
|---|---|---|---|---|---|---|
| | **EDIM, mg/kg /dy** | **RD** | **EHRI** | **EDIM, mg/kg /dy** | **RD** | **EHRI** |
| Cd | 0.0005 | 2.03E+02 | 2.46E-06 | 0.0005 | 2.03E+02 | 2.46E-06 |
| Pb | 0.0026 | 4.92E+02 | 5.28E-06 | 0.0026 | 4.92E+02 | 5.28E-06 |
| Cr | 0.0000 | 1.79E-01 | 0.00E+00 | 0.0002 | 1.79E-01 | 1.12E-03 |

EDMI = Estimated dietary heavy metal intake; RD = Reference dose; EHRI= Estimated health risk index.

## 4.6 Effect of Method of Preparation, Packaging Materials, Storage Conditions, Storage Time and Reed Species on the Stability of Iodine and $Fe^{2+}$ in Reed Salts

Indigenous reed salts of *C. rotundus* and *T. latifolia* were analysed for pH and moisture content and for the presence of iodine, $Fe^{2+}$ and Heavy metals Fe, Pb, Cr and Cd. The results are tabulated in the below.

### 4.6.1 Effect of Method of Preparation and Reed Species on Iodine and Iron in Salt Samples

The effect of method of preparation on the concentration of iodine and $Fe^{2+}$ were compared under normal conditions of temperature and relative humidity (22 $^{o}$C and 50% RH). The results are represented in table 4.16. Iodine concentration varied by region with higher concentration in Busia salt samples than in Lugari salts. Equally, in both Busia and Lugari regions, salts prepared using complete evaporation method contained higher iodine levels than those prepared using evaporation crystallization method (table 4.16). The concentration of iodine in Lugari salt samples ranged from 7.7-12.5 mg/kg when prepared by complete evaporation method and 4.0-4.7 mg/kg when evaporation crystallization method is used (table 4.16). The concentration of iodine varied by region and by the method of preparation. On the other hand, the concentration of $Fe^{2+}$ was higher in Lugari salts than in Busia salts when both methods are used (table 4.16). Salt samples obtained from Lugari and prepared using complete evaporation method contained higher $Fe^{2+}$ levels (2.7-3.1 mg/kg) than those that were prepared using evaporation crystallization

method (2.6-2.7 mg/kg) as shown in table 4.16. However, for Busia, salts prepared by both complete evaporataion and evaporation crystallization methods contained similar $Fe^{2+}$ levels (0.1 mg/kg) with very minimal variations as indicated in table 4.16. This shows that complete evaporation method is better than evaporation crysallisatin method in iodine and $Fe^{2+}$ availability.

**Table 4.16**: A comparison of iodine and $Fe^{2+}$ concentrations for complete evaporation and evaporation-crystallisation methods for both *T. latifolia* and *C. rotundus* salts done at 22 $^{o}$C and 50% RH.

| | **Complete evaporation method** | | | | **Evaporation-crystallisation method** | | | |
|---|---|---|---|---|---|---|---|---|
| Region | % moist. | pH | Iodine, mg/kg | $Fe^{2+,}$ mg/kg | % Moist. | pH | Iodine, mg/kg | $Fe^{2+}$, mg/kg |
| Lugari | 9.7 | 9.7 | 12.5±0.38 | 2.6±0.01 | 9.5 | 9.9 | 4.0±0.24 | 2.7±0.04 |
| | 9.5 | 9.73 | 7.7±0.31 | 2.6±0.04 | 8.9 | 9.9 | 4.2±0.20 | 2.9±0.08 |
| | 9.7 | 9.73 | 7.8±0.30 | 2.6±0.05 | 9.6 | 10.0 | 4.7±0.20 | 2.9±0.06 |
| | 9.8 | 9.68 | 11.2±0.28 | 2.6±0.03 | 9.2 | 10.1 | 4.4±0.22 | 3.1±0.06 |
| | 9.8 | 9.73 | 7.2±0.32 | 2.7±0.04 | 9.8 | 10.1 | 4.3±0.21 | 2.8±0.07 |
| Busia | 10.1 | 9.85 | 299.9±21.15 | 0.1±0.001 | 9.8 | 9.7 | 45.7±1.05 | 0.1±0.002 |
| | 9.7 | 9.86 | 299.2±2.54 | 0.1±0.002 | 9.7 | 9.7 | 38.1±2.35 | 0.1±0.001 |
| | 9.8 | 9.97 | 299.6±2.38 | 0.1±0.002 | 10.1 | 9.7 | 45.0±1.15 | 0.1±0.002 |
| | 15.6 | 10.14 | 299.9±1.69 | 0.1±0.002 | 10.3 | 9.7 | 42.3±2.38 | 0.1±0.002 |
| | 15.1 | 10.14 | 299.6±5.18 | 0.1±0.001 | 9.8 | 9.7 | 43.2±2.12 | 0.1±0.002 |

### 4.6.2 Effect of Packaging Material on Iodine and $Fe^{2+}$ in Reed Salts.

Packaging materials used affected the levels of iron (II) and iodine in the salt samples when stored for over a period of six months (figure 4.4). It was noted that the order of loss of $Fe^{2+}$ was LDPE<HDPE, closed container, banana leaves and open container for Busia and LDPE< banana leaves< closed container< HDPE< open container for Lugari salts samples for the entire storage period. However, it was observed that by the third month of storage the loss in $Fe^{2+}$ was of the order LDPE<HDPE< banana leaves<closed container < open container< closed container for Busia and LDPE< banana leaves<HDPE <closed container < open container for Lugari salts.

During the 1$^{st}$ month, the losses were 55%, 63%, 77%, 82% and 96% of $Fe^{2+}$ for LDPE, banana leaves, closed container, HDPE and open container respectively by Lugari salts while for the same order of containers it was 30%, 33%, 33%, 43% and 56% respectively for Busia samples.

By comparing across the storage period for Busia salts, LDPE lost 0-99% $Fe^{2+}$ while HDPE, closed container, open container and banana leaves lost 0-100%. However, considering a 3rd month storage period, the losses were 43%, 60%, 83%, 100% and 71% for LDPE, HDPE, closed container, open container and banana leaves respectively. Lugari salts on the other hand had 0-81%, 0-94%, 0-95%, 0-92% and 0-99% for LDPE, HDPE, closed container, banana leaves and open container respectively over the entire storage period.

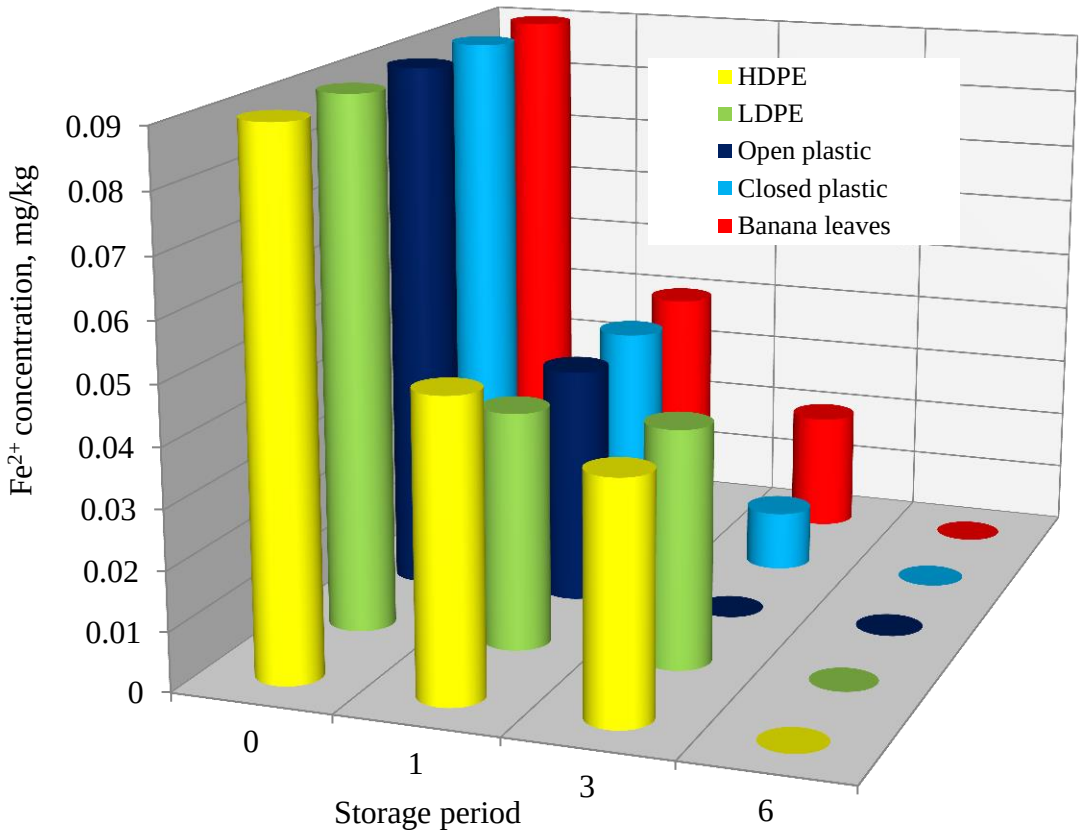



**Figure 4.4:** Effect of packaging material on $Fe^{2+}$ retention over time

However, in 3rd month of storage the losses noted were 79%, 92%, 86%, 83% and 98% for LDPE, HDPE, closed container, banana leaves and open container respectively. This confirms that LDPE container is more ideal to storing salts for the purpose of retaining $Fe^{2+}$ (figure 4.4). It was noted that both Lugari and Busia salts lost all iodine by the end of the storage period. However, it was observed that by the end of the third month of storage the loss in iodine was of the order LDPE<HDPE< banana leaves<closed container < open container for Busia salt

samples while all there was 100% loss of iodine by all the containers in Lugari. During the 1st month, the losses in iodine by Lugari salt samples were 58%, 59%, 64%, 70% and 75% of $Fe^{2+}$ by LDPE, HDPE, banana leaves, closed container, and open container respectively while for the same order of containers it was 61%, 49%, 46%, 43% and 55% respectively for Busia samples. However, considering a 3rd month storage period, the losses were upto 100% in all the containers. Busia salt samples however had 89%, 93%, 96%, 94% and 95% for LDPE, HDPE, closed container, banana leaves and open container respectively of iodine losses by the end of the 3rd month of storage.This confirms that LDPE container is more ideal to storing salts for the purpose of retaining iodine (figure 4.5). These findings suggest that the LDPE is the most preferred method of storing indigenous reed salt which agrees with the findings of Diosady and Venkatesh (2015).

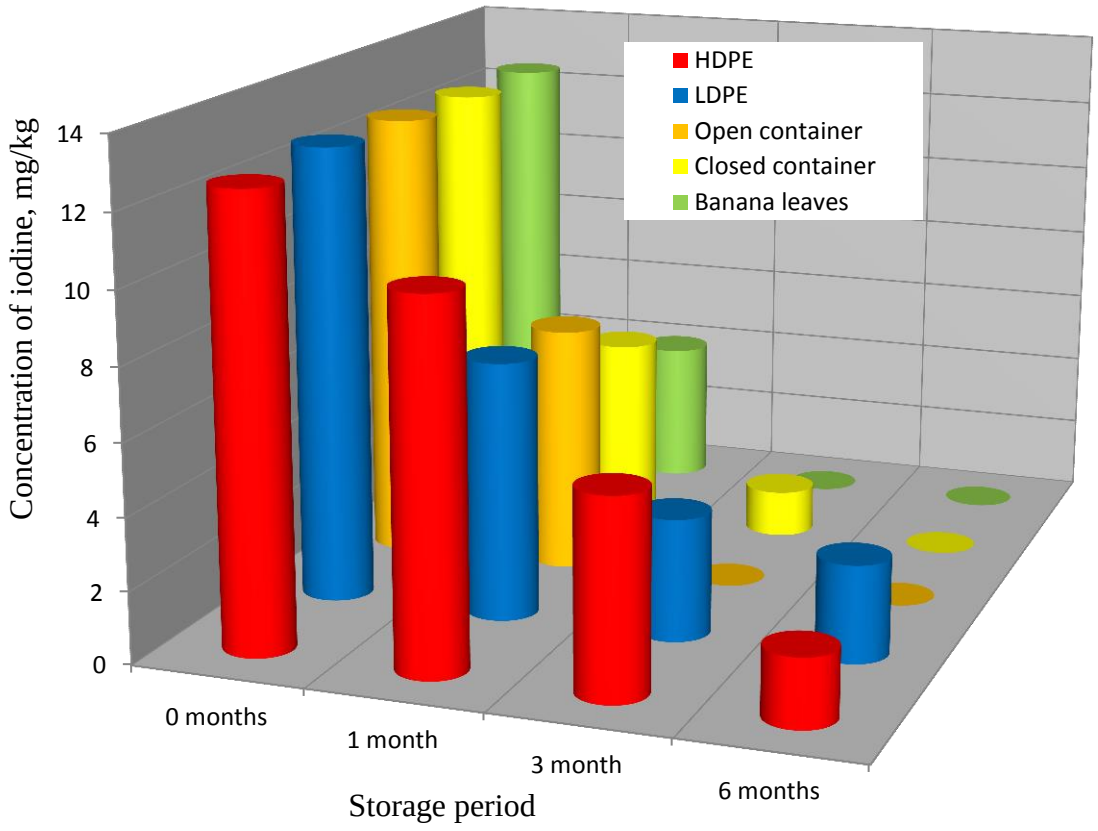



**Figure 4.5:** Effect of packaging material on iodine retention over time

The findings of this study agrees with Diosady and Alberti (1998) who observed that salts with stabilizers lost 33% of their iodine on average after 12 months at 60% relative humidity in LDPE while salts from Canada, India, Philippines and Senegal lost less than 15% of the added iodine over the first six months even at 100% relative humidity when stored in low density polyethylene bags (Abebe *et al*., 2012). This also agrees with Laar and Pelig-Ba (2013) who observed that the length and choice of storage greatly affects the iodine levels in salts for either iodated or non-iodated and found out that iodine losses over the period ranged from 10% to 100%. The above findings however differs with the findings of Abebe *et al*., (2012) that showed, HDPE retaining more iodine from iodized salt better than LDPE. Since LDPE is very tight against water, but open for diffusion of gases e.g. oxygen and vapor, the absorption of these gases could have contributed to the reactions that led to reduced iodine and iron II concentrations in the salt samples. The formation of the hypohalite ion ($IO^-$), equation 4.1, in neutral aqueous solutions of iodine is negligible. Iodine, $I_2$, reacts with water to produce hypoiodite, $OI^-$, equation 4.2, Abebe *et al*., (2012). The position of the equilibrium depends very much upon the pH of the solution.

$$I_{2(l)} + H_2O_{(l)} \rightleftharpoons OI^-_{(aq)} + 2\,H^+_{(aq)} + I^-_{(aq)} \qquad 4.1$$

and

$$I_{2(l)} + H_2O_{(l)} \rightarrow H^+_{(aq)} + I^-_{(aq)} + HIO_{(aq)} \qquad 4.2$$

In basic solutions (such as aqueous sodium hydroxide), iodine converts in a two stage reaction to iodide and iodate, equations 4.3 and 4.4 respectively, Abebe *et al*., (2012):

$$I_2(aq) + 2\,OH^-(aq) \rightarrow I^-(aq) + IO^-(aq) + H_2O(l) \qquad 4.3$$

$$3\,IO^-(aq) \rightarrow 2\,I^-(aq) + IO_3^-(aq) \qquad 4.4$$

The ionic reaction is particularly efficient in stretched LDPE, suggesting that the reaction cavities in the stretched polymer catalyze the formation of extended poly-iodide chain structures **(**Karlsen and Spanget-Larsen, 2009). HDPE had 4% iodine while the rest had lost almost all at the end of the storage period. Though salts stored in HDPE containers behaved similarly to the open containers at 22 $^oC$ and 50% RH (figure 4.5) since the retained moisture initiated ionic reactions where iodine could have undergone a redox reaction, through oxidation of iodide to iodine leading to the slow loss of iodide content in the salt and which was more accelerated since

the salt had no stabilizers. Equally, this phenomenon can be attributed to the solubility of the iodide in the moisture of the salt and its transportation to the surface by capillarity there by escaping by sublimation since it is volatile. Moisture plays a critical role in the stability of iodine (Laar and Pelig-Ba, 2013). Iodine formed can bind to many different substances, for example other halogens as well as react with the heavy metal impurities in the salt, for example reaction with lead ions in the salt, equation 4.5, (Laar and Pelig-Ba, 2013).

$$Pb^{2+}(aq) + 2I^{-}(aq) \rightarrow PbI_2(s) \qquad 4.5$$

Salts in the open and closed containers, as well as banana leaves appeared to have absorbed more moisture and allowed in more air and contributed to the instability of iodine and $Fe^{2+}$. From these findings, LDPE can be considered a more superior packaging material for indigenous reed salts under normal conditions of temperature and relative humidity, for retaining $Fe^{2+}$ and iodine. It provided a better moisture barrier hence lessening the effects of decomposition for both iodine and $Fe^{2+}$.

### 4.6.3 Effect of Storage Conditions of Tempertue and Relative Humidity

In all cases, the samples stored under normal conditions of temperature and humidity lost iodine at a lower rate than those stored at elevated temperature and humidity (40 $^{o}$C temperature and 100% RH), table 4.17. After six months of storage at normal conditions of temperature and relative humidity, 22 $^{o}$C and 50% relative humidity, the losses ranged from 72% to 100% for a storage period of six months and 52-100% at elevated conditions of temperature and humidity for a storage period of 15 days which is higher than expected basing on the ICCIDD/WHO/UNICEF tables (Diosady *et al.,* 1997). Under normal conditions of temperature and RH, *C. rotundus* salt retained 18% iodine when stored in LDPE as compared to the rest of the containers while *T. latifolia* salt stored in LDPE material retained 13% iodine retention as compared to the rest (table 4.17). There was between 26-72% loss in iodine after one month of storage under normal conditions of temperature and relative humidity and 22-24% at 15$^{th}$ day of storage at elevated conditions of temperature and relative humidity. This implies that increase in temperature and relative humidity affects the rate of iodine loss as confirmed by regression results (appendix 14 and 15). These results agree with Diosady and Venkatesh (2015) who also observed that salt samples stored at 40 $^{o}$C and 100% RH lost iodine at a higher rate than those

stored at 60% RH. Diosady *et al.*, (1998) reported 30% to 98% loss of iodine from iodized salt of the original iodine when the salt was exposed to high humidity. Also, these findings are in agreement with the results of Abebe and Negussie (2012) who found high amount of iodine loss when iodized salt was stored at accelerated temperature (40 °C) and at high RH (70-100%).

The effect of temperature on the iodine levels suggest that heat affects the rate of iodine losses as rapid decreases were registered at higher temperatures. This result can be attributed to the exposed nature of the reaction vessel and the presence of heat at such high temperatures causing elemental iodine to readily sublime which is then lost to the atmosphere due to the high volatility of the element. The effect of temperature on the iodine levels suggest that heat affects the rate of iodine losses as rapid decreases were registered at higher temperatures and humidity. Equally, increase in humidity increases the amount of water absorbed by the salt as which apparently also increases with increased temperature. This in turn increases the redox reactions in the salt as well as decomposition reactions thereby losses in iodine content. There was more loss in $Fe^{2+}$ at elevated conditions of temperature and humidity, ranging between 81-100% under normal conditions and 50-100% at elevated conditions.

The rate of loss in $Fe^{2+}$ at elevated conditions of temperature and humidity was of the order LDPE< HDPE < Banana leaves, Closed container and open container, with over 50% loss. Loss in $Fe^{2+}$ was more pronounced with *T. latifolia* salt than with *C. rotundus* salt at 40 $^{o}$C temperature and 100% RH (table 4.18). There was upto 100% (*T. latifolia* salt) and 98% (*C. rotundus* salt) losses in $Fe^{2+}$ by the $15^{th}$ day of storage period at 40 $^{o}$C temperature and 100% RH. Although this was observed, however, at 1 month of storage at 22$^{o}$C and 50% relative humidity, there was more loss in *C. rotundus* salt (55%) as compared to *T. latifolia* salt (33%) while at elevated conditions *C. rotundus* lost 69% and *T. latifolia* lost 26% of $Fe^{2+}$. By exposing the packaging containers to sunlight or in storage containers heated by the sun, high humidity would be retained once moisture is absorbed into the packaged contents, since the containers are permeable. The presence of moisture and permeability to oxygen by the containers provided conditions for oxidation of $Fe^{2+}$ to $Fe^{3+}$ hence the decrease in the concentration of $Fe^{2+}$ Equation 4.6;

$$Fe^{2+}\ (green) \rightarrow Fe^{3+}\left(\frac{orange}{red}\right) + e^{-} \qquad 4.6$$

$Fe^{2+}$ will undergo oxidation resulting in enhanced losses as increase in temperatures increases the rates of reactions. Polyethylene containers are known to be open to diffusion of gases e.g. oxygen and vapor, the absorption of these gases could have contributed to the reactions that led to reduced iodine and iron II concentrations in the salt samples. These ionic reactions are further catalyzed by reaction cavities (Karlsen and Spanget-Larsen, 2009) and at elevated temperature and humidity this reactions are more enhanced. The presence of impurities like heavy metals could also have contributed to increase in the loss by proving surface area foe the reactions leading to these losses. The losses of up to 100% clearly indicate that effectively all of iodine and $Fe^{2+}$ present in the salt had been lost within six months of storage at normal temperature and RH as well as within 15 days of storage at elevated conditions of normal temperature and RH. This demonstrates the large effect of high temperature and ambient humidity on the stability of iodine and $Fe^{2+}$ and especially since the indigenous reed salt has no stabilizers, the loss was more drastic. The results of this study give an indication of the expected iodine and $Fe^{2+}$ loss in the field. It is likely that the relative humidity and temperature may not necessarily remain at these extremes for six months.

### 4.6.4 Effect of Storage Time on the Concentration of Iodine and $Fe^{2+}$ in the Salt

Both Iron (II) and Iodine declined in concentration with time (table 4.17 and table 4.18). *T. latifolia* salt lost more of the iodine present in the salt than *C. rotundus* salt; its loss of iodine was more pronounced than for the later during the first two months of storage. However, after the two months *C. rotundus* salt lost almost all iodine apart from two samples stored in HDPE (94%) and LDPE (72%) as compared to *T. latifolia* that had between 85-98% losses. It is thought that the absence of stabilizers coupled with the presence of moisture as well as high alkaline conditions contributed to the high losses in iodine. The loss of iron II is generally followed the same trend as Iodine from the $0^{th}$ month to the $6^{th}$ month with upto 100% loss for all samples apart from the one stored in LDPE container. However, there was more loss in *C. rotundus* salt between 0-$3^{rd}$ months as compared to *T. latifolia* salt. While *C. rotundus* salt lost between 68-100% of $Fe^{2+}$, *T. latifolia* salt lost all $Fe^{2+}$ that was present in the initial salt sample. It is likely that the presence of moisture and impurities accelerated the loss of iodine from the salt.

### 4.6.5 Effect of Reed Plant Species on Iodine and $Fe^{2+}$ in Reed Salts

The effect of plant species on the retention of iodine (table 4.16) and iron II (table 4.18) in the indigenous reed salts of *C. rotundus* and *T. latifolia* salts was also investigated and findings reveal that *T. latifolia* salt proved to be more superior to *C. rotundus.* It was observed that the concentration of iodine was higher in *T. latifolia* reed salt as compared to the *C. rotundus* reeds salt (table 4.17) when the salt was prepared using the complete evaporation method. It was also observed that while using the same method of preparation for the reed salt and at almost the same pH for the salt obtained, there was 44-88% more loss of iodine in *T. latifolia* reed salt as compared to 85-87% iodine and in *C. rotundus* reed salt samples. On the other hand, considering $Fe^{2+}$ in the reed salt, there was no significant difference in method of preparing the salt since they displayed varying behavior the amount of $Fe^{2+}$ loss (table 4.17). It was observed that with the *C. rotundus* reeds, and at a pH of 10 for the salt obtained, there was 3-16% more $Fe^{2+}$ in the salt samples obtained using evaporation-crystallisation method as compared to complete evaporation method. However, with *T. latifolia* reeds salt samples at pH 10, there was 0-29% more $Fe^{2+}$ when using complete evaporation method for preparation.

### 4.6.6 Effect of Heavy metals Fe, Cd, Pb and Cr on Iodine and $Fe^{2+}$ in Reed Salts

*C. rotundus* and *T. latifolia* salts contained Fe, Cd and Pb contaminants although Cr was undetected in salt samples from both the two reed species. The level of heavy metals in both *T. latifolia* and *C. rotundus* was of the order Fe > Pb > Cd > Cr (table 4.18). There was more Fe in Busia salts ranging from 143.5-235.8 mg/kg as compared to 72.5-142.2 mg/kg in Lugari salts. The presence of more Fe impurity in *T. latifolia* salts could have contributed to the 95% loss in iodine as compared to 72% loss in *C. rotundus* salt which contained less Fe when packed in LDPE packing material. The mean concentration of Cd was 8.1±0.47 mg/kg (with a range of 7.9-8.4 mg/kg) in *C. rotundus* salt and 7.3±0.46 mg/kg (with a range of 7.1-7.4 mg/kg) in *T. latifolia* salt. The results of the present work were higher than the values reported in other studies. In Turkey, Cd content found in refined and unrefined was 0.14 - 0.3 mg/kg and 0.14 – 0.21 mg/kg, respectively (table 4.19). All values for toxic metals were significantly lower than the permitted maximum for human consumption as prescribed by Codex and ISIRI (Heshmati, *et al*., 2014).

**Table 4.17:** Iodine concentrations in Lugari's *C. rotundus* reed salts at 22 $^{o}$C and 50% RH and at 40 $^{o}$C and 100% RH

| **Concentration of iodine (mean±S.D, mg/kg ), 22 $^{o}$C and 50% RH** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type of salt** | | **Complete evaporation method** | | | | | **Evaporation Crystallisation method** | | | | |
| *C. rotundus* | **Pack** | **0 months** | **1 month** | **3 month** | **4 months** | **6 months** | **0 months** | **1 month** | **3 month** | **4 months** | **6 months** |
| | HDPE | 12.5±0.22 | 10.2±0.20 | 4.5±0.42 | 2.1±0.02 | 1.9±0.21 | 4.0±0.24 | 3.0±0.85 | nd | nd | nd |
| | LDPE | 12.7±0.20 | 7.2±0.14 | 3.4±0.42 | 3.0±0.21 | 2.7±0.21 | 4.2±0.20 | 2.3±0.21 | nd | nd | nd |
| | O.C | 12.6±0.43 | 6.9±0.42 | nd | nd | nd | 4.7±0.20 | 1.7±0.42 | nd | nd | nd |
| | C.C | 12.5±0.12 | 5.3±0.20 | 1.3±0.85 | 0.2±0.01 | nd | 4.4±0.22 | 2.3±0.21 | nd | nd | nd |
| | B.L | 12.5±0.32 | 4.0±0.11 | ND | ND | nd | 4.3±0.21 | 0.8±0.01 | nd | nd | nd |
| T. latifolia | HDPE | 299.9±21.15 | 90.9±0.12 | 40.2±2.10 | 18.0±1.06 | 14.8±2.12 | 45.7±1.05 | 23.3±2.51 | 14.8±0.12 | 8.0±0.42 | 3.3±1.12 |
| | LDPE | 299.2±2.54 | 80.4±0.00 | 36.0±1.79 | 15.9±1.20 | 12.7±0.02 | 45.1±2.35 | 24.8±2.11 | 12.3±2.32 | 5.3±1.06 | 3.2±1.06 |
| | O.C | 299.6±2.38 | 95.2±2.12 | 44.4±1.92 | 7.4±1.01 | 6.3±2.00 | 45.0±1.15 | 20.1±1.06 | 10.6±3.14 | 6.3±1.13 | 1.3±1.06 |
| | C.C | 299.9±1.69 | 44.4±2.12 | 23.3±2.12 | 11.6±1.16 | 6.5±2.14 | 42.3±2.38 | 24.3±1.06 | 10.6±1.11 | 8.5±0.00 | 1.9±3.14 |
| | B.L | 299.6±5.18 | 59.2±4.23 | 31.7±2.14 | 14.8±2.12 | 14.8±2.10 | 43.2±2.12 | 23.3±2.17 | 10.6±2.32 | 7.4±1.06 | 2.3±2.18 |
| **Concentration of iodine (mean±S.D, mg/kg ), 40 $^{o}$C and 100% RH** | | | | | | | | | | | |
| *C. rotundus* | Pack | 0 days | 3 days | 6 days | 9 days | 15 days | 0 days | 3 days | 6 days | 9 days | 15 days |
| | HDPE | 12.5±0.38 | 8.34±1.69 | 4.13±0.21 | 4.60±0.24 | 1.48±0.231 | 4.0±0.24 | 3.81±0.00 | 2.75±0.21 | 1.90±0.21 | 1.27±0.42 |
| | LDPE | 7.7±0.31 | 7.44±0.21 | 4.61±0.42 | 4.29±0.21 | 2.75±0.21 | 4.2±0.20 | 3.96±0.42 | 2.75±0.63 | 2.54±0.42 | 1.90±0.63 |
| | O.C | 7.8±0.30 | 3.60±0.21 | 1.48±0.21 | 0.85±0.00 | nd | 4.7±0.20 | 2.12±0.42 | 1.06±0.21 | nd | nd |
| | C.C | 11.2±0.28 | 9.94±1.90 | 3.61±0.42 | 2.60±0.21 | 1.06±0.97 | 4.4±0.22 | 2.33±0.21 | 1.90±0.21 | 0.42±0.02 | nd |
| | B.L | 7.2±0.32 | 4.86±0.21 | 3.38±0.42 | 2.75±0.21 | 0.21±0.25 | 4.3±0.21 | 1.90±0.26 | 1.27±0.42 | 0.21±0.19 | nd |
| C. Papyrus salt | HDPE | 299.9±21.15 | 38.71±0.30 | 25.38±2.99 | 9.52±1.20 | 2.33±0.30 | nd | 16.92±0.60 | 11.63±1.50 | 4.65±1.50 | 1.90±0.30 |
| | LDPE | 299.2±2.54 | 22.21±0.30 | 13.75±1.50 | 7.40±0.90 | 1.48±0.30 | nd | 8.46±2.99 | 7.40±1.50 | 4.02±0.60 | 1.06±0.30 |
| | O.C | 299.6±2.38 | 44.42±1.80 | 28.55±4.49 | 2.98±1.50 | 0.67±0.90 | nd | 9.52±1.50 | 14.81±2.99 | 8.04±0.60 | 3.38±0.60 |
| | C.C | 299.9±1.69 | 14.81±0.00 | 10.58±2.99 | 3.17±0.30 | 1.48±0.30 | nd | 7.40±0.30 | 5.29±1.55 | 2.75±1.50 | 1.27±0.60 |
| | B.L | 299.6±5.18 | 30.67±1.50 | 15.86±1.50 | 11.63±1.50 | 5.92±0.60 | nd | 6.35±0.60 | 7.40±1.80 | 0.64±0.30 | 1.90±0.30 |

nd= not detected; oc=open container, cc= closed container, bl= banan leaves, HDPE=high density polyethylene, LDPE= low density polyethylene

**Table 4.18:** $Fe^{2+}$ concentrations in *T. latifolia* reed salts at 22 $^{o}$C and 50% RH and at 40 $^{o}$C and 100% RH

| **Concentration of $Fe^{2+}$ (mean±S.D, mg/kg ), 22 $^{o}$C and 50% RH** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Complete evaporation method** | | | | **Evaporation Crystallisation method** | | | | |
| *T .latifolia salt* | Pack | 0 months | 1 month | 3 month | 6 months | 0 months | 1 month | 3 month | 6 months | |
| | HDPE | 12.5±0.38 | 2.6±0.01 | 1.2±0.01 | 0.9±0.01 | 9.82±0.29 | 2.7±0.04 | 1.1±0.01 | 0.5±0.02 | |
| | LDPE | 7.7±0.31 | 3.6±0.04 | 1.2±0.01 | 1.8±0.01 | 9.780±0.35 | 2.9±0.08 | 1.1±0.01 | 0.7±0.00 | |
| | O.C | 7.8±0.30 | 0.3±0.05 | 0.2±0.01 | nd | 8.97±0.38 | 1.7±0.06 | 0.2±0.01 | 0.0±0.00 | |
| | C.C | 11.2±0.28 | 2.6±0.03 | 1.5±0.14 | nd | 9.83±0.32 | 3.1±0.06 | 1.1±0.03 | 0.1±0.01 | |
| | B.L | 7.2±0.32 | 2.7±0.04 | 1.2±0.01 | nd | 9.90±0.34 | 1.9±0.07 | 0.2±0.01 | 0.1±0.01 | |
| C. papyrus salt | HDPE | 0.51±0.003 | 0.05±0.002 | 0.04±0.001 | nd | 0.070±0.001 | 0.04±0.020 | 0.02±0.003 | nd | |
| | LDPE | 0.5±0.001 | 0.04±0.001 | 0.04±0.002 | nd | 0.07±0.002 | 0.06±0.001 | 0.04±0.001 | nd | |
| | O.C | 0.5±0.001 | 0.04±0.001 | nd | nd | 0.11±0.002 | 0.04±0.021 | nd | nd | |
| | C.C | 0.54±0.003 | 0.04±0.003 | 0.01±0.001 | nd | 0.07±0.002 | 0.02±0.001 | nd | nd | |
| | B.L | 0.5±0.001 | 0.04±0.001 | 0.02±0.001 | nd | 0.07±0.000 | 0.04±0.002 | 0.03±0.009 | nd | |
| **Concentration of $Fe^{2+}$ (mean±S.D, mg/kg ), 40 $^{o}$C and 100% RH** | | | | | | | | | | |
| *C. rotundus* | Pack | 0 days | 3 days | 6 days | 9 days | 15 days | 0 days | 3 days | 6 days | 9 days | 15 days |
| | HDPE | 12.5±0.38 | 3.00±0.01 | 1.20±0.02 | 0.78±0.00 | 0.60±0.02 | 9.82±0.29 | 3.00±0.01 | 1.40±0.00 | 0.90±0.02 | 0.50±0.02 |
| | LDPE | 7.7±0.31 | 3.80±0.01 | 1.10±0.02 | 0.90±0.02 | 0.80±0.01 | 9.780±0.35 | 3.10±0.30 | 1.10±0.01 | 0.79±0.02 | 0.70±0.00 |
| | O.C | 7.8±0.30 | 3.10±0.00 | 1.20±0.00 | 0.83±0.02 | 0.50±0.00 | 8.97±0.38 | 3.10±0.30 | 1.20±0.00 | 0.94±0.02 | 0.50±0.02 |
| | C.C | 11.2±0.28 | 3.74±0.00 | 1.10±0.01 | 0.80±0.02 | 0.50±0.02 | 9.83±0.32 | 3.05±0.31 | 0.90±0.00 | 0.80±0.00 | 0.80±0.02 |
| | B.L | 7.2±0.32 | 2.99±0.00 | 1.10±0.02 | 0.86±0.01 | 0.50±0.00 | 9.90±0.34 | 2.90±0.36 | 0.93±0.00 | 0.92±0.01 | 0.80±0.02 |
| C. papyrus | HDPE | 0.51±0.003 | 0.4±0.012 | 0.41±0.002 | 0.34±0.01 | 0.28±0.006 | 0.070±0.001 | 0.05±0.001 | 0.03±0.001 | 0.07±0.001 | 0.03±0.001 |
| | LDPE | 0.50±0.001 | 0.37±0.007 | 0.41±0.002 | 0.3±0.006 | 0.45±0.006 | 0.07±0.002 | 0.02±0.001 | 0.05±0.001 | 0.03±0.001 | 0.03±0.001 |
| | O.C | 0.51±0.001 | 0.42±0.015 | 0.39±0.005 | 0.33±0.001 | 0.23±0.01 | 0.11±0.002 | 0.04±0.001 | 0.03±0.004 | 0.04±0.003 | 0.02±0.001 |
| | C.C | 0.54±0.003 | 0.44±0.02 | 0.39±0.006 | 0.28±0.006 | 0.18±0.006 | 0.07±0.002 | 0.04±0.001 | 0.04±0.033 | 0.05±0.003 | 0.02±0.001 |
| | B.L | 0.53±0.001 | 0.34±0.154 | 0.4±0.004 | 0.3±0.006 | 0.21±0.01 | 0.07±0.000 | 0.03±0.003 | 0.03±0.001 | 0.06±0.001 | 0.02±0.002 |

nd= not detected; oc=open container, cc= closed container, bl= banan leaves, HDPE=high density polyethylene, LDPE= low density polyethylene; nd=not detected.

The maximum permitted level of Cd in food grade salt is 0.5 mg/kg according to the Codex legislation (Codex Alimentarius Commission. 2006) and 0.2 mg/kg according to the Iranian food standards (Institute of Standards and Industrial Research of Iran (ISIRI), 2006). The results of this study indicate higher levels of Cd than the maximum permitted level of Cd in food grade salt. The mean concentration of Pb was 56.4±2.03 mg/kg (with a range of 54.0-58.9mg/kg) in *C. rotundus* salt and 56.9±3.11mg/kg (with a range of 55.8-57.7 mg/kg) in *T. latifolia* salt (table 4.19). The results of the present work were higher compared with values reported by Cheraghali et *al*., 2010. Pb Lead is one of the most toxic heavy metal that accumulates in the body and data published in literature indicates that its excessive intake harm different systems and organs such as central and peripheral neural system, gastrointestinal tract, muscles, kidneys and hematopoietic system (Ciobanu *et al*., 2012).

**Table 4.19:** Heavy metal concentration of the *C. rotundus* and *T. latifolia* reed salt samples

| **Concentration of Heavy Metal (mean ±S.D, mg/kg ,)** | | | | | | |
|---|---|---|---|---|---|---|
| Source of the reed salt | pH | % Moisture | Fe | Cd | Pb | Cr |
| *C. rotundus* (Lugari) | 9.7±0.03 | 0.5±0.06 | 142.2±13.49 | 8.4±0.89 | 58.9±2.37 | nd |
| *C. rotundus* (Matete) | 9.8±0.01 | 1.0±0.01 | 72.5±8.19 | 7.9±0.44 | 54.0±3.11 | nd |
| *T. latifolia* (Ululo) | 10.2±0.04 | 8.7±0.004 | 176.1±6.63 | 5.2±1.03 | 56.0±8.24 | nd |
| *T. latifolia* (Ululo) | 10.6±0.002 | 11.2±0.001 | 219.9±39.38 | 9.0±1.19 | 57.7±10.09 | nd |
| *T. latifolia* (Ululo) | 10.5±0.02 | 4.9±0.05 | 235.8±21.04 | 7.1±0.64 | 63.9±5.48 | 1.0±0.04 |
| *C. papyrus* (Bidimbidi) | 10.5±0.03 | 15.6±0.01 | 194.8±13.10 | 6.1±0.56 | 47.8±2.02 | nd |
| *T. latifolia* (Bidimbidi) | 9.6±0.01 | 15.3±0.01 | 143.5±5.01 | 8.1±1.06 | 63.9±5.21 | 1.0±0.01 |

The maximum permitted level of lead in food grade salt is 2.0 mg/kg according to the Codex legislation (Codex Alimentarius Commission. 2006) and 1.0 mg/kg according to the Iranian food standards (Institute of Standards and Industrial Research of Iran (ISIRI), 2006). The results of this study indicate higher levels of Pb than the maximum permitted level of Cd in food grade salt. The presence of these impurities contributed to the loss in iodine which eventually affected the amount of $Fe^{2+}$ in the salt. The presence of moisture and metal ion impurities, such as iron accelerated the loss of iodine. This is in agreement with findings by Diosady *et al.*, 1997 and 1998 who showed that impure iodated salt can lose most of the added iodine during extended storage at high temperature and humidity. Laar and Pelig-Ba (2013) findings also agree with this

study where they showed the effect of impurities on iodine loss which in turn affected iron loss. They found a drop in iodine levels in most iodated salt that ranged from less than 10% to 30% of the added iodine but dropped rapidly from less than 10% to 100% in the raw non iodated salts. According to WHO/FAO (2007) and WHO/FAO in Chaitali, (2015), Fe, Pb and Cd in the study area exceeded the permissible limits table 4.20). It therefore suggests that people in Western Kenya use indigenous reed salt that is highly contaminated with Fe, Pb and Cd while the Cr level is below the permissible limit.

**Table 4.20:** Permissible limits for selected elements

| **Sample code** | **Mean concentration, mg/kg** | | | | | |
|---|---|---|---|---|---|---|
| | **Na** | **Fe** | **Cd** | **Pb** | **Cr** | **K** |
| Permissible limit | 2300 mg/dy | 20[b] | 0.21 | 0.20[a] | 2.30[a] | 3500 |
| PTDI | - | - | - | 3.57[c] | 3.33[a] | - |
| Codex standards | - | - | 0.5 | 2 | - | - |
| Iranian standards | - | 10 | 0.2 | 1 | - | - |
| KEBS | | | 1.0[c] | 4.0[c] | 10.0[c] | 50 – 84[c] |

[a]Kihampa *et al.,* (2011); [b]Tegegne (2015; [c]KEBS, 2007.

### 4.7 Accelerated aging Factor ($Q_{10}$) of the Salt

To calculate the accelerated aging factor ($Q_{10}$) of the salt, using equation 33 gives;

$$e\text{^}\left(\frac{20}{8.315}\ \text{x}\ 298\ \text{x}\ 313\right) = 1.00$$

The testing frequency at accelerated temperature determined using equation 34 gives;

(3 days x 1.00) = 3 days.

The aging factor at accelerated temperature and humidity should therefore be less than 3 days.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusion

Communities living in Lugari and Busia regions of Western Kenya use unrefined plant derived indigenous reed salts .Out of the total population sampled, 71.6%, 50.6% use *T. latifolia* reed salt while 49.4% use *C. rotundus reed salt*. *C. rotundus* reed salts are consumed in Lugari while *T. latifolia* reed salts are used in Busia region. Busia soils are sandy loam soils, classified as Typic Xerofluvent (American Soil Taxonomy), rich in silt (5.9 ±5.5 -19.6± 0.2%), higher in organic matter content (59.3-61%) with 0.316-0.322 g/cm$^3$ BD. Lugari soils are sandy loam and higher sand content (88.8 ±0.15-88.9 ±0.28%) with low organic matter content (41.5-42.5%) and a BD of 0.315-0.322 g/cm$^3$. Soil nutrients and heavy metal content largely depend on soil depth as well as seasonal variation. There was a decrease in micronutrients concentration iodine, iron, Na and K with increase in depth. The concentrations of Cd, Pb, Cr and Fe were higher in the dry season than the wet season in both regions with a ranking order of Fe > Mg > Cr > Cd > Pb for both top surface and sub-surface soils with the sub-soil samples recording higher metal concentration than the top soils.

*T. latifolia* reeds contained higher iodine, Na and K than *C. rotundus* reeds. The level of heavy metals in reeds was generally of the order Fe> Cr >Pb > Cd for the dry season and Cr > Fe > Cd > Pb for the wet season with a relatively high mean concentrations than the maximum permitted level. The mean Pb present in *T. latifolia* and *C. rotundus* reeds was higher than the legally estimated value of 0.20 mg/kg specified by WHO/FAO (1999). The concentrations of K and Na were higher in soils than in plants with a Tf ranking order of Na > K > Mg > I for both Busia and Lugari regions. The concentrations of K and Na were higher in soils than in plants with a Tf ranking order of Na > K > Mg > I for both Busia and Lugari regions. The presence of heavy metals in ash was of the order Fe>Pb>Cr>Cd in both Busia and Lugari regions. The concentrations of Iodine, K, Na, Fe and Pb increased with ashing process while a decrease in Cr was noted. However, Cd concentration remained the same even after ashing of the reed plants. *C. rotundus* reed ash accumulated less K, Pb and Cd as compared to *T. latifolia* ash. The ashing process is an important step in regard to indigenous reed salt production. The Plant-Ash TF

values for the metals for the dry season were higher than those obtained for the wet season which implies that more metals are present in the environment during the dry season than the wet season. Plant-Ash TF varied from region to region with Fe having the highest followed by Iodine.

*T. latifolia* salts had higher moisture content (10.08%), higher % ratio of salt to ash (10%) and to the *raw* material 0.125%), 10% for ash and 0.125% for raw material, as compared to 0.73% moisture content, lower % ratio of salt to ash (4%) and to the raw material (0.003%) in *C. rotundus* salts. The concentration of micronutrients in the salt was of the order Na> K >Mg for Busia and K >Na >Mg for Lugari. *C. rotundus* salt Na/K ratio (0.9:1) was found to be below the WHO recommended range of 2.5:1 - 4:1 while that of *T. latifolia* salt (3.2:1) was within the recommended ratio. Reed salts obtained during the dry season had higher iodine than those harvested in the wet season. A comparison of iodine concentration in reed salts and commercial salts gave the order Kensalt >Top-chef salt > Herbal sea salt > Sea salt > *C. rotundus salt* > *T. latifolia* salt. *C. rotundus* salts contained more iodine than *T. latifolia* during the dry season than in the wet season. $Fe^{2+}$ was of the order Herbal sea salt >*C. rotundus salt* = *T. latifolia* salt > Sea salt > Kensalt = Top-chef salt. *C. rotundus* salts contained more $Fe^{2+}$ than *T. latifolia* during the dry season than in the wet season. The concentration of Iodine: $Fe^{2+}$ was 1:1 in both wet and dry seasons in both regions and for both species of reeds. The recommended Dietary Allowances (RDA) for iodine and iron are 0.015 mg/kg and 8 mg/kg respectively, with an Upper Limit (UL) of 1.1 mg/kg and 45 mg/kg respectively. *C. rotundus* and *T. latifolia* reed salts have slightly higher iodine (2.0 mg/kg) than required while that of $Fe^{2+}$ is within the recommended limit.

The heavy metals presence in the salt samples was of the order Fe > Pb > Cd > Cr, for all the areas sampled both in the dry and wet seasons. The concentrations of Fe, Pb and Cd in the reed salts exceeded the WHO/FAO permissible limits. Pb and Cd WHO permissible limit in food-grade salt are 0.20 mg/kg and 0.21 mg/kg respectively. The Pb concentration in the reed and commercial salts follows the order *C. rotundus salt* > *T. latifolia* salt > KNSA > HSSA = SSSA> TCSA while for Cd is *T. latifolia* salt > KNSA =HSSA = SSSA=TCSA > *C. rotundus* salt. Chromium metal was highest in samples collected during the wet season with no detectable Cr in samples collected during the dry season. In the present study, the mean Cr concentrations found

in the salts was less than 20-25 mg/day for women and 30-35 mg/day for men recommended by WHO/FAO. Results of EDIM indicate that the consumption of reed salt processed from *T. latifolia* is better than for *C. rotundus* as the dietary intake of Cr and Fe in adults is lower than the permissible limits, while it's Pb and Cd EDIM values are higher than for *T. latifolia* reed salt. There's more toxicity in *C. rotundus* reed salt than in *T. latifolia* reed salt in the levels of Fe, Cd, Cr and Pb.

Lugari reed salts are processed using evaporation crystallisation method while Busia reed salts are processed by complete evaporation method. Although *C. rotundus* and *T. latifolia* salts were found to be both alkaline with a pH over 9.5, *T. latifolia* salts contained higher moisture content. The concentration of iodine was higher in *T. latifolia* reeds salt than in *C. rotundus* reeds salt when prepared by complete evaporation method as compared to evaporation crystallisation method, at room temperature and relative humidity. Evaporation crystallisation method contributed more to the loss of iodine from indigenous reed salt. The concentration of $Fe^{2+}$ on the other hand was found to reduce more with complete evaporation method than with evaporation crystallisation method as observed for the case of iodine. The type of packaging materials used affected the amount of iodine and iron (II) in the indigenous reed salt when stored over time. The losses in iodine and iron II were of the order LDPE<HDPE< banana leaves < open container and closed container for both complete evaporation and evaporation crystallisation methods. This was also observed at elevated conditions of temperature and RH, where iodine loss was of the order LDPE < HDPE < Banana leaves < Closed container < open container. The LDPE film provided a better moisture barrier, and thus maintained water content in each bag approximately constant retaining upto 8% and 1% of iodine and $Fe^{2+}$ respectively at six months of storage.

Effectively all iodine and $Fe^{2+}$ present in the reed salts was lost within six months of storage at normal conditions of temperature and RH as well as within 18 days of storage at elevated conditions of temperature and RH. However, there were more losses at elevated temperature (40 $^{o}$C) and relative humidity (100%). After six months of storage at normal conditions of temperature and relative humidity, 25 $^{o}$C and 50% relative humidity, the loss was higher than the ICCIDD/WHO/UNICEF levels. The effect of plant species on the retention of iodine and iron II in the indigenous reed salts showed that *C. rotundus* reed salt retains less iodine than *T. latifolia* when the same method of preparation and same conditions are observed. The concentration of

iodine was found to be higher in *T. latifolia* reeds salt than in *C. rotundus* reeds salt when prepared using the complete evaporation method. Results of EDIM indicate that the consumption of reed salt processed from *T. latifolia* is better than for *C. rotundus* as the dietary intake of Cr and Fe in adults is lower than the permissible limits. However, from the estimated Health Risk index (EHRI) values obtained it is evident that the exposed population is not safe since the Cd and Pb EHRI values for both salt samples are greater than one indicating that there is a potential risk associated with Cd and Pb metals.

It therefore suggests that people in Western Kenya use indigenous reed salt that is contaminated with Fe, Pb and Cd while the Cr level is below the permissible limit. The rate of iodine and $Fe^{2+}$ losses is influenced by the plant species, packaging material used for storage, method of preparation of the indigenous salt, storage period and temperature and relative humidity during storage. The results indicate that with careful control of these conditions, indigenous reed salt could be stabilized for a three-month period for consumption. In summary *T. latifolia* salt prepared using the complete evaporation method is ideal for use as table salt while *C. rotundus* prepared by complete evaporation method is ideal for low sodium salt. The salts should be obtained from reed ash upon burning of the reed plants that have been dried for a period of four days, followed by filtration of the ash to obtain a solution which is should be evaporated to dryness.

### 5.2 Recommendations

It is recommended that indigenous reed salt be sourced from Busia's *T. latifolia* reeds and obtained from reeds during the wet season. In order for the salt to retain its iodine and $Fe^{2+}$, the reed plants should be dried for a period of four days, ashed, filtred and evaporated to dryness and not crystallised under hot ash. The salt should be stored in a dry place avoiding excess exposure to sunlight and heat and a clean airtight plastic LDPE container for a period not exceeding three months under normal conditions of temperature and relative humidity.

EDIM results show that the consumption of this reed salt is better than for *C. rotundus* as the dietary intake of Cr and Fe in adults is lower than the permissible limits while the toxicity associated with Pb and Cd are lower. Additionally, reed plants or plant parts used for processing of reed salts should be collected from unpolluted environment and should be checked for heavy

metal contamination before use in order to make safe for human consumption since even *T. latifolia* salt has been shown to contain some considerable levels of heavy metals.

To ensure reduction in iodine and $Fe^{2+}$ loss there is need to sensitize the communities in Western Kenya to use *T. latifolia* reeds for production of indigenous reed salt. Since the rate of iodine and $Fe^{2+}$ losses was influenced by the plant species, packaging material used for storage, method of preparation of the indigenous salt, storage period and temperature and relative humidity during storage, it follows that with careful control of these conditions, indigenous reed salt could be stabilized for a three-month period for consumption. As losses of iodine and $Fe^{2+}$ in all salt samples was significant after 6 months, efforts must be made to minimize the time required for distribution, retail and consumption to ensure efficient and effective use of the available iodine as well as $Fe^{2+}$.

Indigenous reed salt from Busia's *T. latifolia* reeds is more ideal salt for use as table or common salt, since its Na: K ratio is within the recommended 2.5:1 and 4:1 bracket. On the other hand, Lugari's *C. rotundus* salt could be used as a Lo-Na salt.

### 5.3 Further Work

There is need to investigate the possible sources of heavy metal contaminants present in reed salts as well as their presence in other types of indigenous salts needs as well as other areas of Western Kenya. Further work needs to be done on reeds in other regions to establish any similarity or variation in the micronutrients and heavy metals contamination, with the present findings. There is need to create awareness about proper storage and handling of indigenous reed salt is necessary to limit losses of important nutrients. It is recommended that fortification of the indigenous reed salt *C. rotundus* could be carried out and further commercializes the product as a Lo-Na salt. It is recommended that more investigations be done to establish the possible sources of these heavy metal contaminants so that they can be eliminated or reduced in the food chain. Meanwhile, people living in this area and other areas with similar activities and plant species are encouraged not to consume large quantities of these salts, so as to minimize or avoid excessive accumulation of heavy metals in their bodies. There is need to routinely check for heavy metal contamination of the reed salts in order to make safe for human consumption since the results of this study on *T. latifolia* salt has been shown to contain some considerable levels of heavy

metals. There is need for a regular and stringent quality control mechanism so that its use in humans and animals is safe. It is therefore recommended that urgent attention is needed to devise and implement appropriate means of monitoring heavy metal concentrations in reed plants used for production of indigenous food additives grown in swampy areas and along river banks, to prevent their excessive build-up in the food chain. These findings call for the establishment of an effective monitoring and evaluation system for locally processed indigenous reed salt for iodine and $Fe^{2+}$ levels to ensure consumption of standardized salt composition.

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## APPENDICES

**Appendix 1:** Variable Comparisons of effect of depth, season and location on pH and Fe, Pb, Cd, Cr, Mg Concentrations

| **Variable** | **Variable** | **Source** | **Difference between Means** | **Simultaneous 95% confidence Limits** |
|---|---|---|---|---|
| Season | pH | wet - dry | 0.8875 | 1.00693*** |
| | Fe | wet - dry | -442.68 | -406.15*** |
| | Pb | wet - dry | -2.9875 | -2.3252*** |
| | Cd | wet - dry | -1.925 | -1.6186*** |
| | Cr | wet - dry | -55.725 | -49.762*** |
| Depth | pH | 15-30cm - 0-15cm | -0.2875 | -0.16807*** |
| | Fe | 15-30cm - 0-15cm | 39.75 | 76.28*** |
| | Pb | 15-30cm - 0-15cm | 1.9375 | 2.5998*** |
| | Cd | 15-30cm - 0-15cm | 0.625 | 0.9314*** |
| | Cr | 15-30cm - 0-15cm | 10.8 | 16.763*** |
| Location | pH | Matete - Bidimbidi | 0.225 | 0.42819*** |
| | | Ululo - Bidimbidi | 0.175 | 0.37819 |
| | | Lugari - Bidimbidi | -0.425 | -0.22181*** |
| | Fe | Ululo - Bidimbidi | 170.15 | 232.30*** |
| | | Matete - Bidimbidi | 86.13 | 148.27*** |
| | | Lugari - Bidimbidi | 11.83 | 73.97 |
| | Pb | Ululo - Bidimbidi | 1.65 | 2.7768*** |
| | | Matete - Bidimbidi | 1.4 | 2.5268*** |
| | | Lugari - Bidimbidi | 0.975 | 2.1018 |
| | Cd | Ululo - Bidimbidi | -0.05 | 0.4714 |
| | | Matete - Bidimbidi | 0.075 | 0.5964 |
| | | Lugari - Bidimbidi | 0.575 | 1.0964*** |
| | Cr | Ululo - Bidimbidi | 9.25 | 19.395 |
| | | Matete - Bidimbidi | -8.675 | 1.47 |
| | | Lugari - Bidimbidi | 0.325 | 10.47 |

Dunnett's t Tests; Comparisons significant at the 0.05 level are indicated by ***

**Appendix 2:** ANOVA for the effect of Season, Location, Depth on pH, Fe, Pb, Cd, Cr and Mg concentrations in the soil samples

| **Dep. Variable** | **Source** | **df** | **F Value** | **Pr > F** | **Dep. Var.** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|---|---|
| pH | Rep | 3 | 1.23 | 0.3031 | Cr | 0 | 1 |
| | season | 1 | 218.45 | <.0001 | | 345.48 | <.0001 |
| | Loc | 3 | 24.2 | <.0001 | | 5.96 | 0.001 |
| | Depth | 1 | 22.92 | <.0001 | | 12.98 | 0.0005 |
| | season*loc | 3 | 22.23 | <.0001 | | 0.86 | 0.463 |
| | season*depth | 1 | 12.52 | 0.0007 | | 2.81 | 0.0975 |
| Fe | Rep | 3 | 0 | 1 | Mg | 0.03 | 0.9914 |
| | season | 1 | 581.02 | <.0001 | | 100965 | <.0001 |
| | Loc | 3 | 18.32 | <.0001 | | 11.46 | <.0001 |
| | Depth | 1 | 4.68 | 0.0333 | | 6.98 | 0.0099 |
| | season*loc | 3 | 2.63 | 0.0555 | | 16.28 | <.0001 |
| | season*depth | 1 | 97.75 | <.0001 | | 0 | 0.9447 |
| Pb | Rep | 3 | 0.04 | 0.9892 | K | 0.04 | 0.9879 |
| | season | 1 | 80.49 | <.0001 | | 426.23 | <.0001 |
| | Loc | 3 | 4.76 | 0.0041 | | 4.35 | 0.0028 |
| | Depth | 1 | 33.86 | <.0001 | | 40.62 | <.0001 |
| | season*loc | 3 | 10.07 | <.0001 | | 12.45 | 0.0006 |
| | season*depth | 1 | 36.53 | <.0001 | | | |
| Cd | Rep | 3 | 0.19 | 0.9048 | Na | 0.01 | 0.9976 |
| | season | 1 | 156.11 | <.0001 | | 56.68 | <.0001 |
| | Loc | 3 | 3.49 | 0.0192 | | 52.26 | <.0001 |
| | Depth | 1 | 16.46 | 0.0001 | | 53.71 | <.0001 |
| | season*loc | 3 | 4.12 | 0.009 | | 0.12 | 0.7254 |
| | season*depth | 1 | 17.8 | <.0001 | | | |

**Appendix 3:** ANOVA Table for the Salt Sample-Effect of Region, Location and season on the salts's pH, Moisture and the of Fe, Pb, Cd, Cr concentration

| Dep. Var. | Source | DF | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | rep | 3 | 0.0494 | 2.21 | 0.1024 |
| | region | 1 | 2.2818 | 101.88 | <.0001 |
| | loc | 1 | 0.2836 | 12.66 | 0.001 |
| | season | 1 | 0.0074 | 0.33 | 0.5681 |
| | loc*season | 1 | 0.0074 | 0.33 | 0.5681 |
| Moist | rep | 3 | 0.1118 | 0.03 | 0.9923 |
| | region | 1 | 1465.2818 | 416.68 | <.0001 |
| | loc | 1 | 189.3771 | 53.85 | <.0001 |
| | season | 1 | 3.0092 | 0.86 | 0.3605 |
| | loc*season | 1 | 3.0092 | 0.86 | 0.3605 |
| Fe | rep | 3 | 69.089 | 0.15 | 0.9286 |
| | region | 1 | 35255.351 | 76.91 | <.0001 |
| | loc | 1 | 12179.926 | 26.57 | <.0001 |
| | season | 1 | 152693.24 | 333.12 | <.0001 |
| | loc*season | 1 | 2412.5642 | 5.26 | 0.0271 |
| Cd | rep | 3 | 0.0358 | 0.65 | 0.59 |
| | region | 1 | 3.0181 | 54.46 | <.0001 |
| | loc | 1 | 0.7849 | 14.16 | 0.0005 |
| | season | 1 | 101.0006 | 1822.38 | <.0001 |
| | loc*season | 1 | 0.2046 | 3.69 | 0.0619 |
| Pb | rep | 3 | 0.7552 | 0.5 | 0.681 |
| | region | 1 | 0.6848 | 0.46 | 0.5025 |
| | loc | 1 | 60.9687 | 40.77 | <.0001 |
| | season | 1 | 29877.58 | 19977.2 | <.0001 |
| | loc*season | 1 | 6.0088 | 4.02 | 0.0518 |
| Cr | rep | 3 | 0.0031 | 0.01 | 0.9991 |
| | region | 1 | 7.4427 | 17.95 | 0.0001 |
| | loc | 1 | 51.0796 | 123.2 | <.0001 |
| | season | 1 | 66.4346 | 160.23 | <.0001 |
| | loc*season | 1 | 48.61 | 117.24 | <.0001 |

**Appendix 4:** Tf values for soil-plant for respective samples and sampling sites

| **0-15 cm depth dry season** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **sampling area** | **sample type** | **iodine** | **Na** | **K** | **Fe** | **Cd** | **Pb** | **Cr** | **Mg** |
| Lugari | plant/soil | 0.005 | 11.918 | 0.559 | 0.035 | 0.217 | 0.257 | 0.586 | 0 |
| | ash/plant | 13.598 | 2.281 | 7.208 | 33.804 | 4.51 | 5.761 | 0.073 | - |
| | salt/ash | 0.021 | 1.372 | 0 | 0.212 | 1.863 | 5.68 | - | 1.161 |
| | salt/plant | 0.286 | 3.13 | 0 | 7.182 | 8.4 | 32.722 | - | - |
| Matete | plant/soil | 0.03 | 14.91 | 0.348 | 0 | 0.021 | 0.652 | 0.326 | 0 |
| | ash/plant | 3.022 | 1.551 | 8.504 | - | 45.1 | 3.457 | 0.137 | - |
| | salt/ash | 0.025 | 2.405 | 0 | 0.108 | 1.752 | 5.207 | - | 1.524 |
| | salt/plant | 0.074 | 3.73 | 0 | - | 79 | 18 | - | - |
| Ululo | plant/soil | 0.038 | 69.981 | 0.493 | 0.015 | 0.021 | 0.319 | 0.06 | -0.165 |
| | ash/plant | 2.966 | 0.86 | 4.736 | 37.111 | 48.6 | 4.253 | 0.801 | 0.529 |
| | salt/ash | 0.003 | 1.695 | 0 | 0.474 | 1.523 | 4.522 | - | 1.023 |
| | salt/plant | 0.009 | 1.458 | 0 | 17.58 | 74 | 19.233 | - | 0.541 |
| Bidimbidi | plant/soil | 0.105 | 35.71 | 0.621 | 0.013 | 0.014 | 1.106 | 0.284 | 0.084 |
| | ash/plant | 1.854 | 1.708 | 7.174 | 44.162 | 0.935 | 6.716 | 0.267 | 1.033 |
| | salt/ash | 0.025 | 2.111 | 0 | 0.383 | 1.461 | 4.373 | 0.017 | 1.77 |
| | salt/plant | 0.043 | 3.914 | 0 | 16.91 | 1.365 | 29.368 | 0.004 | 1.829 |
| Lugari | plant/soil | 1.86 | 2.102 | 0 | 0 | 0.064 | 0.711 | 0.379 | 0 |
| | ash/plant | 0.036 | 11.106 | - | - | 15.033 | 3.841 | 0.112 | - |
| | salt/ash | 0.023 | 1.889 | 0 | 0.16 | 1.796 | 5.439 | - | 1.339 |
| | salt/plant | 0.001 | 20.977 | - | - | 27 | 20.889 | - | - |
| Busia | plant/soil | 0.052 | 41.241 | 0.378 | 0.018 | 0.553 | 1 | 0.182 | 0.147 |
| | ash/plant | 2.168 | 1.175 | 5.705 | 40.516 | 1.869 | 5.104 | 0.401 | 0.603 |
| | salt/ash | 0.01 | 1.862 | 0 | 0.437 | 1.502 | 4.459 | - | 1.318 |
| | salt/plant | 0.021 | 2.187 | 0 | 17.725 | 2.808 | 22.76 | - | 0.794 |
| **at 0-15 cm depth wet season** | | | | | | | | | |
| sampling area | sample type | iodine | Na | K | Fe | Cd | Pb | Cr | Mg |
| Lugari | plant/soil | - | 56.12 | 10472.8 | 3.681 | 0.783 | 33 | 2992.382 | 18.687 |
| | ash/plant | 0 | 12.28 | 1.119 | 8.528 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.181 | 3.576 | 0.001 | 0.219 | - | 5984.764 | - |
| | salt/plant | 0.167 | 2.217 | 0 | 2.317 | 0.972 | - | 0 | 0 |
| Matete | plant/soil | - | 1.16 | 9.163 | 14.805 | 1.741 | - | 2932.331 | 11.697 |
| | ash/plant | 0 | 13.529 | 1.194 | 5.506 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.204 | 1.696 | 0.004 | 1.313 | - | -8.4 | - |
| | salt/plant | 0.022 | 2.755 | 2.025 | 0.022 | 1 | - | 0 | 0.787 |
| Ululo | plant/soil | - | 0.184 | 12.49 | 30.418 | 1.917 | - | 1140.019 | 21.678 |
| | ash/plant | 0 | 40.267 | 4.585 | 0.681 | 0.778 | - | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | salt/ash | - | 0.834 | 0.242 | 0.112 | 1.313 | - | 1.933 | - |
| | salt/plant | 0.089 | 33.6 | 1.108 | 0.076 | 1.022 | - | 0 | 0.254 |
| Bidimbidi | plant/soil | - | 0.697 | 7.539 | 10.941 | 1.88 | 1.8 | 1934.662 | 18.687 |
| | ash/plant | 0 | 14.734 | 7.315 | 0.687 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.906 | 0.259 | 0.049 | 1.285 | - | 0.267 | - |
| | salt/plant | 0.009 | 13.356 | 1.893 | 0.034 | 0.979 | 5.11 | 0 | 0.492 |
| Lugari | plant/soil | - | 1.513 | 12.671 | 4.51 | 1.516 | - | 2873.849 | 13.052 |
| | ash/plant | 0 | 13.001 | 1.163 | 6.414 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.192 | 1.583 | 0.006 | 1.313 | - | -1.8 | - |
| | salt/plant | 0.046 | 2.497 | 1.84 | 0.041 | 1 | - | 0 | 0.67 |
| Busia | plant/soil | - | 0.316 | 10.644 | 5.083 | 1.917 | -0.6 | 1739.007 | 19.844 |
| | ash/plant | 0 | 25.522 | 5.237 | 0.683 | 0.778 | - | 0 | 0 |
| | salt/ash | - | 0.863 | 0.248 | 0.087 | 1.313 | - | 1.267 | - |
| | salt/plant | 0.056 | 22.031 | 1.301 | 0.059 | 1.022 | 14.667 | 0 | 0.347 |
| **at 15-30 cm depth dry season** | | | | | | | | | |
| sampling area | sample type | iodine | Na | k | Fe | Cd | Pb | Cr | Mg |
| Lugari | plant/soil | 0.004 | 12.566 | 0.516 | 0.045 | 0.213 | - | 0.459 | 0 |
| | ash/plant | 13.598 | 2.281 | 7.208 | 33.804 | 4.51 | 5.761 | 0.073 | - |
| | salt/ash | 0 | 0.005 | 0 | 6.71E-05 | 4.72E-02 | - | 1.49E-01 | 0 |
| | salt/plant | 3122.272 | 0.182 | 13.979 | 7.52E+02 | 2.12E+01 | - | 1.58E-01 | - |
| Matete | plant/soil | 0.02 | 11.439 | 0.369 | 0.00E+00 | 2.13E-02 | 8.82E-01 | 3.42E-01 | 0 |
| | ash/plant | 3.022 | 1.551 | 8.504 | - | 45.1 | 3.457 | 0.137 | - |
| | salt/ash | 0.025 | 2.405 | 0 | 0.108 | 1.752 | 5.207 | - | 1.524 |
| | salt/plant | 0.074 | 3.73 | 0 | - | 79 | 18 | - | - |
| Ululo | plant/soil | 0.033 | 41.56 | 0.265 | 0.019 | 0.021 | 10 | 0.164 | 0.171 |
| | ash/plant | 2.966 | 0.86 | 4.736 | 37.111 | 48.6 | 4.253 | 0.801 | 0.529 |
| | salt/ash | 0.003 | 1.695 | 0 | 0.474 | 1.523 | 4.522 | - | 1.023 |
| | salt/plant | 0.009 | 1.458 | 0 | 17.58 | 74 | 19.233 | - | 0.541 |
| Bidimbidi | plant/soil | 0.054 | 23.63 | 0.343 | 0.044 | 1.106 | 1.267 | 0.284 | 0.087 |
| | ash/plant | 1.708 | 1.854 | 7.174 | 44.162 | 0.935 | 6.716 | 0.267 | 1.033 |
| | salt/ash | 0.025 | 2.111 | 0 | 0.383 | 1.461 | 4.373 | 0.017 | 1.77 |
| | salt/plant | 0.043 | 3.914 | 0 | 16.91 | 1.365 | 29.368 | 0.004 | 1.829 |
| Lugari | plant/soil | 1.86 | 2.102 | 0 | 0 | 0.064 | 0.711 | 0.379 | 0 |
| | ash/plant | 0.036 | 11.11 | - | - | 15.033 | 3.841 | 0.112 | - |
| | salt/ash | 0.023 | 1.889 | 0 | 0.16 | 1.796 | 5.439 | -0.032 | 1.339 |
| | salt/plant | 0.001 | 20.98 | - | - | 27 | 20.889 | -0.004 | - |
| Busia | plant/soil | 0.052 | 41.24 | 0.378 | 0.018 | 0.553 | 1 | 0.182 | 0.147 |
| | ash/plant | 2.168 | 1.175 | 5.705 | 40.516 | 1.869 | 5.104 | 0.401 | 0.603 |
| | salt/ash | 0.01 | 1.862 | 0 | 0.437 | 1.502 | 4.459 | -0.017 | 1.318 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | salt/plant | 0.021 | 2.187 | 0 | 17.725 | 2.808 | 22.76 | -0.007 | 0.794 |
| **at 15-30 cm depth wet season** | | | | | | | | | |
| sampling area | sample type | iodine | Na | K | Fe | Cd | Pb | Cr | Mg |
| Lugari | plant/soil | - | 1.09 | 10.055 | 5.446 | 3.615 | 11 | 3150.709 | 12.038 |
| | ash/plant | 0 | 12.28 | 1.119 | 8.528 | 0.762 | - | 8.40E-06 | 0 |
| | salt/ash | - | 0 | 0.003 | 0.001 | 1.01 | - | 6301.418 | - |
| | salt/plant | - | 11.266 | 0.111 | 1.566 | 0.211 | - | 2.66E-09 | 0 |
| Matete | plant/soil | - | 1.165 | 9.194 | 2.503 | 1.88 | - | 2676.605 | 11.697 |
| | ash/plant | 0 | 13.529 | 1.194 | 5.506 | 0.762 | - | 1.09E-05 | 0 |
| | salt/ash | - | 0.204 | 1.696 | 0.004 | 1.313 | - | -8.4 | - |
| | salt/plant | 0.0217 | 2.755 | 2.025 | 0.022 | 1 | - | -9.10E-05 | 0.787 |
| Ululo | plant/soil | - | 0.142 | 12.152 | 1.939 | 2 | - | 1767.242 | 20.629 |
| | ash/plant | 0 | 40.27 | 4.585 | 0.681 | 0.778 | - | 6.31E-05 | 0 |
| | salt/ash | - | 0.834 | 0.242 | 0.112 | 1.313 | - | 1.933 | - |
| | salt/plant | 0.089 | 33.6 | 1.108 | 0.076 | 1.022 | - | 0 | 0.254 |
| Bidimbidi | plant/soil | - | 0.775 | 7.503 | 6.814 | 1.958 | 0.818 | 2990.943 | 18.97 |
| | ash/plant | 0 | 14.73 | 7.315 | 0.687 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.906 | 0.259 | 0.049 | 1.285 | - | 0.267 | - |
| | salt/plant | 0.009 | 13.356 | 1.893 | 0.034 | 0.979 | 5.111 | 0 | 0.492 |
| Lugari | plant/soil | - | 1.513 | 12.671 | 4.51 | 1.516 | - | 2873.849 | 13.052 |
| | ash/plant | 0 | 13.00 | 1.163 | 6.414 | 0.762 | - | 0 | 0 |
| | salt/ash | - | 0.192 | 1.583 | 0.006 | 1.313 | - | -1.8 | - |
| | salt/plant | 0.046 | 2.497 | 1.84 | 0.041 | 1 | - | 0 | 0.67 |
| Busia | plant/soil | - | 0.316 | 10.644 | 5.083 | 1.917 | -0.6 | 1739.007 | 19.844 |
| | ash/plant | 0 | 25.52 | 5.237 | 0.683 | 0.778 | - | 0 | 0 |
| | salt/ash | - | 0.863 | 0.248 | 0.087 | 1.313 | - | 1.267 | - |
| | salt/plant | 0.056 | 22.03 | 1.301 | 0.059 | 1.022 | 14.667 | 0 | 0.347 |

**Appendix 5**: Regression table for chemical characteristics of the reed Plants

| Dry Season | Variable | Coefficients | Standard Error | t Stat | P-value | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|---|
| iodine | Na mg/kg | 0.00682 | 0.001788 | 3.813843 | 0.163249 | -0.0159 | 0.029542 |
| | K mg/kg | -0.06924 | 0.031522 | -2.19651 | 0.27198 | -0.46977 | 0.331288 |
| | Fe mg/kg | 0.401483 | 0.344739 | 1.164599 | 0.451683 | -3.97884 | 4.781809 |
| | Pb mg/kg | 8.58482 | 3.960821 | 2.167435 | 0.275194 | -41.7422 | 58.91182 |
| | Cd mg/kg | 8.595648 | 3.562054 | 2.413116 | 0.250102 | -36.6645 | 53.85584 |
| | Cr mg/kg | 0.845262 | 0.286471 | 2.950604 | 0.208025 | -2.79469 | 4.485218 |
| Na | K mg/kg | 10.24321 | 3.391211 | 3.020516 | 0.203535 | -32.8462 | 53.33262 |
| | Fe mg/kg | -53.0664 | 53.07599 | -0.99982 | 0.500057 | -727.461 | 621.328 |
| | Pb mg/kg | -1239.52 | 511.5471 | -2.42309 | 0.249175 | -7739.34 | 5260.3 |
| | Cd mg/kg | -1256.81 | 402.3899 | -3.12337 | 0.19726 | -6369.66 | 3856.037 |
| | Cr mg/kg | -118.506 | 44.48977 | -2.66366 | 0.228637 | -683.802 | 446.7905 |
| | Iod mg/kg | 137.1926 | 35.97227 | 3.813843 | 0.163249 | -319.878 | 594.2635 |
| K | Fe mg/kg | 4.435823 | 5.357989 | 0.82789 | 0.559767 | -63.6439 | 72.51553 |
| | Pb mg/kg | 118.1787 | 38.44782 | 3.073742 | 0.20024 | -370.347 | 606.7045 |
| | Cd mg/kg | 110.782 | 51.82291 | 2.137703 | 0.278553 | -547.691 | 769.2544 |
| | Cr mg/kg | 10.15986 | 5.865423 | 1.732162 | 0.333316 | -64.3674 | 84.68712 |
| | Iod mg/kg | -11.9632 | 5.446435 | -2.19651 | 0.27198 | -81.1667 | 57.24035 |
| | Na mg/kg | 0.087982 | 0.029128 | 3.020516 | 0.203535 | -0.28213 | 0.458091 |
| Fe | Pb mg/kg | -13.6311 | 11.54964 | -1.18022 | 0.447495 | -160.383 | 133.121 |
| | Cd mg/kg | -13.8226 | 10.86689 | -1.27199 | 0.424148 | -151.899 | 124.2543 |
| | Cr mg/kg | -1.38978 | 0.955471 | -1.45455 | 0.383427 | -13.5302 | 10.75062 |
| | Iod mg/kg | 1.433695 | 1.231063 | 1.164599 | 0.451683 | -14.2084 | 17.07583 |
| | Na mg/kg | -0.00942 | 0.009422 | -0.99982 | 0.500057 | -0.12914 | 0.110299 |
| | K mg/kg | 0.091678 | 0.110738 | 0.82789 | 0.559767 | -1.31538 | 1.498732 |
| Pb | Cd mg/kg | -0.92015 | 0.349068 | -2.63602 | 0.230831 | -5.35547 | 3.515178 |
| | Cr mg/kg | -0.08588 | 0.039192 | -2.19121 | 0.272561 | -0.58386 | 0.412105 |
| | Iod mg/kg | 0.096041 | 0.044311 | 2.167435 | 0.275194 | -0.46698 | 0.659063 |
| | Na mg/kg | -0.00069 | 0.000284 | -2.42309 | 0.249175 | -0.0043 | 0.002925 |
| | K mg/kg | 0.007652 | 0.002489 | 3.073742 | 0.20024 | -0.02398 | 0.039283 |
| | Fe mg/kg | -0.0427 | 0.036183 | -1.18022 | 0.447495 | -0.50245 | 0.417044 |
| Cd | Cr mg/kg | -0.09059 | 0.031543 | -2.87179 | 0.213321 | -0.49138 | 0.310208 |
| | Iod mg/kg | 0.099287 | 0.041145 | 2.413116 | 0.250102 | -0.42351 | 0.622083 |
| | Na mg/kg | -0.00072 | 0.000231 | -3.12337 | 0.19726 | -0.00366 | 0.002214 |
| | K mg/kg | 0.007406 | 0.003465 | 2.137703 | 0.278553 | -0.03661 | 0.051427 |
| | Fe mg/kg | -0.04471 | 0.03515 | -1.27199 | 0.424148 | -0.49134 | 0.401918 |
| | Pb mg/kg | -0.95006 | 0.360413 | -2.63602 | 0.230831 | -5.52954 | 3.62943 |
| Cr | Iod mg/kg | 1.061176 | 0.359647 | 2.950604 | 0.208025 | -3.50857 | 5.630925 |
| | Na mg/kg | -0.0074 | 0.002777 | -2.66366 | 0.228637 | -0.04268 | 0.027884 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | K mg/kg | 0.073822 | 0.042619 | 1.732162 | 0.333316 | -0.4677 | 0.615343 |
| | Fe mg/kg | -0.4886 | 0.335911 | -1.45455 | 0.383427 | -4.75675 | 3.77955 |
| | Pb mg/kg | -9.63725 | 4.39815 | -2.19121 | 0.272561 | -65.5211 | 46.24655 |
| | Cd mg/kg | -9.84551 | 3.428354 | -2.87179 | 0.213321 | -53.4069 | 33.71585 |
| **Wet season** | | | | | | | |
| Iodine | Mg mg/kg | 1.191945 | 7.222694 | 0.165028 | 0.884094 | -29.8848 | 32.26869 |
| | Fe mg/kg | 0.003563 | 0.062206 | 0.057285 | 0.959526 | -0.26409 | 0.271215 |
| | Pb mg/kg | 7.712487 | 3.462998 | 2.227113 | 0.155817 | -7.18759 | 22.61256 |
| | Cd mg/kg | -66.4666 | 169.5387 | -0.39204 | 0.732858 | -795.933 | 662.9997 |
| | Cr mg/kg | -0.00361 | 0.00064 | -5.63581 | 0.002439 | -0.00525 | -0.00196 |
| | K mg/kg | -0.01474 | 0.022226 | -0.66309 | 0.543532 | -0.07645 | 0.046971 |
| | Na mg/kg | -0.02025 | 0.028184 | -0.71844 | 0.512215 | -0.0985 | 0.058003 |
| Fe | Pb mg/kg | -61.6834 | 109.2216 | -0.56475 | 0.672713 | -1449.48 | 1326.109 |
| | Cd mg/kg | -554.35 | 2711.188 | -0.20447 | 0.871602 | -35003.3 | 33894.56 |
| | Cr mg/kg | 0.052932 | 0.061847 | 0.85586 | 0.549346 | -0.73291 | 0.838775 |
| | Iod mg/kg | 20.02637 | 22.80618 | 0.878111 | 0.541259 | -269.754 | 309.8064 |
| | K mg/kg | 0.178 | 0.274244 | 0.649058 | 0.63349 | -3.3066 | 3.662602 |
| | Na mg/kg | -0.14232 | 0.163184 | -0.87217 | 0.43234 | -0.5954 | 0.310747 |
| | Mg mg/kg | 43.74534 | 42.08884 | 1.039357 | 0.357335 | -73.112 | 160.6027 |
| | Cr mg/kg | 0.000172 | 0.000583 | 0.295421 | 0.817131 | -0.00723 | 0.007576 |
| | Iod mg/kg | 0.149403 | 0.142396 | 1.049211 | 0.484715 | -1.65991 | 1.958714 |
| | K mg/kg | 0.000961 | 0.002363 | 0.406836 | 0.754019 | -0.02906 | 0.030987 |
| | Na mg/kg | 0.000787 | 0.004446 | 0.176991 | 0.888479 | -0.0557 | 0.057275 |
| | Mg mg/kg | 1.25551 | 0.925254 | 1.356935 | 0.267874 | -1.68906 | 4.200082 |
| | Fe mg/kg | -0.00798 | 0.009753 | -0.8178 | 0.473381 | -0.03902 | 0.023063 |
| Cd | Cr mg/kg | -4.00E-06 | 1.24E-05 | -0.32398 | 0.776697 | -5.70E-05 | 4.92E-05 |
| | Iod mg/kg | -0.00051 | 0.003076 | -0.16681 | 0.882863 | -0.01375 | 0.012722 |
| | K mg/kg | 9.72E-06 | 5.09E-05 | 0.190817 | 0.866284 | -0.00021 | 0.000229 |
| | Na mg/kg | 9.49E-05 | 6.96E-05 | 1.362747 | 0.306117 | -0.0002 | 0.000394 |
| | Mg mg/kg | 0.008693 | 0.024079 | 0.361022 | 0.742009 | -0.06794 | 0.085322 |
| | Fe mg/kg | -0.0001 | 0.000203 | -0.51689 | 0.640923 | -0.00075 | 0.000541 |
| | Pb mg/kg | -0.00056 | 0.011789 | -0.0471 | 0.965391 | -0.03807 | 0.036961 |
| Cr | Iod mg/kg | -320.465 | 61.94962 | -5.173 | 0.035397 | -587.013 | -53.9177 |
| | K mg/kg | -5.60101 | 3.049947 | -1.83643 | 0.207705 | -18.7239 | 7.521857 |
| | Na mg/kg | 2.144637 | 2.251694 | 0.952455 | 0.441389 | -7.54362 | 11.83289 |
| | Mg mg/kg | 1900.441 | 1090.989 | 1.741944 | 0.223643 | -2793.7 | 6594.586 |
| | Fe mg/kg | 3.186567 | 13.59316 | 0.234424 | 0.829744 | -40.0729 | 46.44606 |
| | Pb mg/kg | -1823.64 | 802.0015 | -2.27386 | 0.107542 | -4375.97 | 728.6868 |
| | Cd mg/kg | 27141.91 | 48587.46 | 0.55862 | 0.615406 | -127485 | 181768.9 |

**Appendix 6:** Regression table for Salt variables in dry seasons

| **Dry season** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Salts** | **Variable** | **Coefficients** | **Standard Error** | **t-Stat** | **P-value** | **Lower 95%** | **Upper 95%** | **$R^2$** |
| pH | % Moist. | 0.0399 | 0.0180 | 2.2135 | 0.0913 | -0.0102 | 0.0900 | <0.500 |
| | iodine | -0.2399 | 0.2990 | -0.8024 | 0.5695 | -4.0386 | 3.5588 | <0.500 |
| | $Fe^{2+}$ | -11.5330 | 11.3006 | -1.0206 | 0.4935 | -155.1210 | 132.0551 | <0.500 |
| | Fe | 0.0058 | 0.0028 | 2.0474 | 0.2892 | -0.0299 | 0.0414 | <0.500 |
| | Cd | 0.0846 | 0.1462 | 0.5784 | 0.6661 | -1.7731 | 1.9422 | <0.500 |
| | Pb | -0.0340 | 0.0080 | -4.2675 | 0.1465 | -0.1352 | 0.0672 | <0.500 |
| | Cr | -0.1343 | 0.0720 | -1.8666 | 0.3131 | -1.0488 | 0.7801 | <0.500 |
| | Mg | -0.0316 | 0.0061 | -5.2021 | 0.1209 | -0.1089 | 0.0456 | <0.500 |
| | Na | 0.0000 | 0.0000 | 5.3097 | 0.1185 | 0.0000 | 0.0001 | <0.500 |
| % Moist | iodine | 0.5264 | 1.7304 | 0.3042 | 0.8120 | -21.4609 | 22.5136 | <0.500 |
| | $Fe^{2+}$ | -232.0830 | 65.4096 | -3.5481 | 0.1749 | -1063.1900 | 599.0251 | <0.5 |
| | Fe | 0.1134 | 0.0163 | 6.9719 | 0.0907 | -0.0933 | 0.3200 | 0.9869 |
| | Cd | 0.6446 | 0.8462 | 0.7618 | 0.5856 | -10.1077 | 11.3969 | <0.500 |
| | Pb | -0.0798 | 0.5460 | -0.1461 | 0.9077 | -7.0175 | 6.8580 | <0.500 |
| | Cr | 1.4310 | 4.9352 | 0.2900 | 0.8203 | -61.2763 | 64.1383 | <0.500 |
| | Mg | -0.0055 | 0.4169 | -0.0131 | 0.9916 | -5.3022 | 5.2912 | <0.500 |
| | Na | 0.0013 | 0.0003 | 3.9700 | 0.1571 | -0.0028 | 0.0053 | <0.500 |
| | pH | 13.7930 | 6.2312 | 2.2135 | 0.0913 | -3.5076 | 31.0936 | <0.500 |
| Iodine | $Fe^{2+}$ | -16.5569 | 33.2658 | -0.4977 | 0.7060 | -439.2390 | 406.1251 | <0.500 |
| | Fe | -0.0029 | 0.0085 | -0.3458 | 0.7881 | -0.1110 | 0.1051 | <0.500 |
| | Cd | 0.2916 | 0.3717 | 0.7844 | 0.5766 | -4.4318 | 5.0149 | <0.500 |
| | Pb | -0.0702 | 0.1618 | -0.4341 | 0.7393 | -2.1260 | 1.9855 | <0.500 |
| | Cr | -1.6125 | 0.4638 | -3.4769 | 0.1783 | -7.5051 | 4.2802 | <0.500 |
| | Mg | 0.0917 | 0.0217 | 4.2298 | 0.1478 | -0.1838 | 0.3671 | <0.500 |
| | Na | 0.0001 | 0.0000 | 3.2221 | 0.1916 | -0.0004 | 0.0007 | <0.500 |
| | pH | -2.8904 | 1.1214 | -2.5775 | 0.2356 | -17.1388 | 11.3581 | <0.500 |
| | % Moist. | 0.0113 | 0.0487 | 0.2327 | 0.8274 | -0.1240 | 0.1467 | <0.500 |
| $Fe^{2+}$ | Fe | -0.0002 | 0.0003 | -0.4685 | 0.7211 | -0.0041 | 0.0038 | <0.500 |
| | Cd | -0.0016 | 0.0103 | -0.1506 | 0.9048 | -0.1323 | 0.1292 | <0.500 |
| | Pb | 0.0050 | 0.0034 | 1.4546 | 0.3834 | -0.0386 | 0.0486 | <0.500 |
| | Cr | -0.0331 | 0.0373 | -0.8855 | 0.5386 | -0.5074 | 0.4413 | <0.500 |
| | Mg | -0.0031 | 0.0021 | -1.4789 | 0.3785 | -0.0295 | 0.0234 | <0.500 |
| | Na | 0.0000 | 0.0000 | -0.5556 | 0.6772 | -0.0001 | 0.0001 | <0.500 |
| | pH | -0.0161 | 0.0629 | -0.2561 | 0.8404 | -0.8158 | 0.7836 | <0.500 |
| | % Moist. | 0.0023 | 0.0069 | 0.3376 | 0.7927 | -0.0850 | 0.0896 | <0.500 |
| | pH | -0.0089 | 0.0286 | -0.3114 | 0.7710 | -0.0883 | 0.0705 | <0.500 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fe | Cd | 6.8478 | 70.2333 | 0.0975 | 0.9381 | -885.5510 | 899.2464 | <0.500 |
| | Pb | -7.9708 | 34.4065 | -0.2317 | 0.8551 | -445.1470 | 429.2057 | <0.500 |
| | Cr | 39.1210 | 343.0134 | 0.1141 | 0.9277 | -4319.2800 | 4397.5200 | <0.500 |
| | Mg | -9.3706 | 23.2052 | -0.4038 | 0.7557 | -304.2210 | 285.4797 | <0.500 |
| | Na | -0.0189 | 0.0191 | -0.9917 | 0.4259 | -0.1009 | 0.0631 | <0.500 |
| | pH | 144.0005 | 81.3130 | 1.7709 | 0.2186 | -205.8610 | 493.8622 | <0.500 |
| | % Moist. | 14.3699 | 13.0566 | 1.1006 | 0.3858 | -41.8079 | 70.5477 | <0.500 |
| | iodine | -30.2632 | 47.7246 | -0.6341 | 0.5711 | -182.1440 | 121.6176 | <0.500 |
| | $Fe^{2+}$ | -166.7500 | 1504.5870 | -0.1108 | 0.9188 | -4955.0200 | 4621.5180 | <0.500 |
| Pb | pH | 1.4013 | 4.9560 | 0.2827 | 0.8246 | -61.5705 | 64.3730 | <0.500 |
| | % Moist. | -0.0592 | 0.2817 | -0.2101 | 0.8682 | -3.6387 | 3.5203 | <0.500 |
| | iodine | 1.5100 | 1.8896 | 0.7991 | 0.5708 | -22.4991 | 25.5190 | <0.500 |
| | $Fe^{2+}$ | 57.0411 | 60.3909 | 0.9445 | 0.5182 | -710.2970 | 824.3797 | <0.500 |
| | Fe | -0.0038 | 0.0138 | -0.2721 | 0.7990 | -0.0422 | 0.0347 | <0.500 |
| | Pb | 0.5662 | 0.1115 | 5.0787 | 0.1238 | -0.8504 | 1.9829 | <0.500 |
| | Cr | -5.0462 | 1.0077 | -5.0075 | 0.1255 | -17.8507 | 7.7583 | <0.500 |
| | Mg | 0.3743 | 0.0851 | 4.3972 | 0.1424 | -0.7073 | 1.4559 | <0.500 |
| | Na | 0.0001 | 0.0001 | 1.3081 | 0.4155 | -0.0008 | 0.0009 | <0.500 |
| Cr | pH | -1.9945 | 0.0430 | -46.372 | 0.0137 | -2.5410 | -1.4480 | 0.9997 |
| | % Moist. | 0.0451 | 0.0024 | 18.4510 | 0.0345 | 0.0140 | 0.0762 | 0.9997 |
| | iodine | -0.3515 | 0.0164 | -21.434 | 0.0297 | -0.5599 | -0.1431 | 0.9997 |
| | $Fe^{2+}$ | -9.0745 | 0.5241 | -17.314 | 0.0367 | -15.7339 | -2.4151 | 0.9997 |
| | Fe | -0.0068 | 0.0034 | -1.9826 | 0.1859 | -0.0217 | 0.0080 | <0.500 |
| | Cd | -0.1848 | 0.1447 | -1.2776 | 0.3297 | -0.8073 | 0.4377 | <0.500 |
| | Pb | 0.0548 | 0.0522 | 1.0503 | 0.4038 | -0.1697 | 0.2793 | <0.500 |
| | Mg | 0.0463 | 0.0295 | 1.5704 | 0.2144 | -0.0475 | 0.1400 | <0.500 |
| | Na | 0.0000 | 0.0000 | -0.4063 | 0.7118 | -0.0001 | 0.0001 | <0.500 |
| Na | pH | 3063.1640 | 4178.5670 | 0.7331 | 0.5973 | -50030.600 | 56156.8900 | <0.500 |
| | % Moist. | 613.4117 | 237.5208 | 2.5826 | 0.2352 | -2404.5800 | 3631.4000 | <0.500 |
| | iodine | -189.4830 | 1593.1500 | -0.1189 | 0.9246 | -20432.400 | 20053.4100 | <0.500 |
| | $Fe^{2+}$ | -20725.700 | 50917.680 | -0.4070 | 0.7539 | -667696.00 | 626244.800 | <0.500 |
| | Fe | 114.3659 | 84.6034 | 1.3518 | 0.4055 | -960.6220 | 1189.3530 | <0.500 |
| | Cd | 1010.7920 | 2778.0640 | 0.3638 | 0.7778 | -34287.900 | 36309.4400 | <0.500 |
| | Pb | -860.7260 | 926.1906 | -0.9293 | 0.5233 | -12629.100 | 10907.6400 | <0.500 |
| | Cr | 6017.5930 | 10075.290 | 0.5973 | 0.6572 | -122001.00 | 134036.300 | <0.500 |
| | Mg | -289.5510 | 367.2887 | -0.7884 | 0.4746 | -1309.310 | 730.2062 | <0.500 |

**Appendix 7:** Regression table for salt-plant-soil elemental concentrations

| **Wet season** | | **Coefficients** | **Standard Error** | **t-Stat** | **P-value** | **Lower 95%** | **Upper 95%** | **$R^2$** |
|---|---|---|---|---|---|---|---|---|
| iodine | plant | -0.00321 | 0.018245 | -0.17588 | 0.876583 | -0.08171 | 0.075291 | 0.091664 |
| | soil | 0.005337 | 0.015259 | 0.349781 | 0.759902 | -0.06032 | 0.070993 | 0.091664 |
| K | plant | 0.259556 | 0.494584 | 0.524796 | 0.652095 | -1.86847 | 2.38758 | 0.445251 |
| | soil | 78.36655 | 62.09895 | 1.261963 | 0.334198 | -188.824 | 345.5568 | 0.445251 |
| Na | plant | -9.7033 | 4.010241 | -2.41963 | 0.13665 | -26.958 | 7.551371 | 0.805746 |
| | soil | -5.18269 | 4.924235 | -1.05249 | 0.402972 | -26.37 | 16.00458 | 0.805746 |
| Fe | plant | 0.021999 | 0.044139 | 0.49839 | 0.667621 | -0.16792 | 0.211915 | 0.135342 |
| | soil | 0.033003 | 0.074159 | 0.445036 | 0.699824 | -0.28608 | 0.352083 | 0.135342 |
| Pb | plant | -0.56661 | 0.047402 | -11.9532 | 0.006926 | -0.77056 | -0.36265 | 0.990329 |
| | soil | 0.066757 | 0.143895 | 0.463931 | 0.688295 | -0.55237 | 0.685887 | 0.990329 |
| Cd | plant | 0.518857 | 0.139367 | 3.722965 | 0.065174 | -0.08079 | 1.118504 | 0.898358 |
| | soil | -0.73548 | 0.17625 | -4.17295 | 0.05291 | -1.49382 | 0.02286 | 0.898358 |
| Cr | plant | -0.0001 | 0.000314 | -0.33076 | 0.772263 | -0.00146 | 0.001248 | 0.266906 |
| | soil | 0.196064 | 0.232929 | 0.84173 | 0.488545 | -0.80615 | 1.198278 | 0.266906 |
| **Dry season** | | | | | | | | |
| iodine | plant | -0.00177 | 0.026999 | -0.06545 | 0.95193 | -0.08769 | 0.084156 | 0.416709 |
| | soil | 0.02096 | 0.024578 | 0.852806 | 0.456442 | -0.05726 | 0.099178 | 0.416709 |
| K | plant | 0.026044 | 0.935007 | 0.027855 | 0.979527 | -2.94956 | 3.001652 | 0.550337 |
| | soil | 1.532324 | 0.836049 | 1.832817 | 0.164208 | -1.12836 | 4.193005 | 0.550337 |
| Na | plant | 0.338841 | 1.112825 | 0.304488 | 0.780657 | -3.20266 | 3.880346 | 0.033031 |
| | soil | -1.15541 | 9.249791 | -0.12491 | 0.908493 | -30.5924 | 28.28155 | 0.033031 |
| Fe | plant | -0.23856 | 1.567374 | -0.15221 | 0.888684 | -5.22665 | 4.749519 | 0.150415 |
| | soil | 0.087543 | 0.132085 | 0.662779 | 0.554846 | -0.33281 | 0.507896 | 0.150415 |
| Pb | plant | 0.13341 | 1.716747 | 0.077711 | 0.942951 | -5.33005 | 5.596865 | 0.121748 |
| | soil | 0.45271 | 0.761967 | 0.594134 | 0.594246 | -1.97221 | 2.877629 | 0.121748 |
| Cd | plant | -0.28441 | 0.369681 | -0.76934 | 0.497713 | -1.4609 | 0.892078 | 0.168583 |
| | soil | -0.41403 | 30.23364 | -0.01369 | 0.989934 | -96.631 | 95.8029 | 0.168583 |
| Cr | plant | 0.016128 | 0.004808 | 3.354424 | 0.043914 | 0.000827 | 0.03143 | 0.891726 |
| | soil | -0.01613 | 0.004553 | -3.54356 | 0.038266 | -0.03062 | -0.00164 | 0.891726 |

**Appendix 8:** Correlation coefficients for respective elements in plant samples

| **Sample** | **Region** | **Location** | **Iodine/K** | **Iodine/Na** | **Iodine/Fe** | **Iodine/Pb** | **Iodine/Cd** | **Iodine/Cr** | **K/Na** | **K/Mg** |
|---|---|---|---|---|---|---|---|---|---|---|
| Soils | Busia | BRPU | -0.6174 | 0.6645 | 0.652 | -0.4144 | -0.9543 | 0.6357 | 0.2322 | 1.3336 |
| | | BRPB | -0.7859 | 0.3489 | 0.3454 | -0.592 | -0.9526 | 0.3731 | 0.432 | 1.7528 |
| | Lugari | LRPM | -0.9294 | -0.868 | 0.271 | -0.0616 | 0.683 | -0.8817 | 1.9771 | 1.9936 |
| | | LRPL | -0.9085 | -0.8424 | 0.0657 | -0.1876 | 0.0622 | -0.9028 | 1.9762 | 1.9284 |
| Plants | Busia | BRPU | -0.1773 | 0.374 | 0.4227 | 0.9188 | 0.4415 | -0.7756 | 1.5401 | -0.5474 |
| | | BRPB | -0.3056 | 0.5892 | 0.4331 | 0.9836 | 0.4746 | -0.8246 | 0.7966 | -0.0169 |
| | Lugari | LRPM | 0.7305 | 0.778 | 0.8544 | 0.4905 | 0.6751 | 0.5662 | 1.5835 | 1.6497 |
| | | LRPL | 0.8256 | 0.9252 | 0.8517 | -0.1738 | 0.8207 | 0.7062 | 1.7039 | 1.7873 |
| **Sample** | **Region** | **Location** | **K/Fe** | **K/Pb** | **K/Cd** | **K/Cr** | **Na/K** | **Na/Mg** | **Na/Fe** | **Na/Pb** |
| Soils | Busia | BRPU | -0.1031 | 0.6418 | 0.515 | -0.4904 | 0.1161 | -0.6572 | 0.6629 | -0.0844 |
| | | BRPB | -0.1937 | 0.6433 | 0.5915 | -0.698 | 0.216 | -0.2666 | -0.16 | -0.3375 |
| | Lugari | LRPM | 0.0823 | -0.0682 | -0.9006 | 0.9873 | 0.9885 | 0.9763 | 0.1905 | -0.1712 |
| | | LRPL | 0.2112 | 0.0056 | -0.4334 | 0.8975 | 0.9881 | 0.9538 | 0.3223 | -0.1211 |
| Plants | Busia | BRPU | -0.2399 | 0.1762 | -0.5795 | -0.2658 | 0.7739 | -0.8227 | -0.3287 | 0.6965 |
| | | BRPB | -0.2331 | -0.139 | -0.3602 | 0.0149 | 0.3983 | -0.8892 | -0.2816 | 0.7083 |
| | Lugari | LRPM | 0.673 | -0.1794 | 0.9609 | 0.9649 | 0.7918 | 0.3266 | 0.4155 | 0.3231 |
| | | LRPL | 0.8292 | -0.602 | 0.9489 | 0.9779 | 0.8519 | 0.5328 | 0.6588 | -0.2085 |
| **Sample** | **Region** | **Location** | **Na/Cd** | **Na/Cr** | **Mg/Fe** | **Mg/Pb** | **Mg/Cd** | **Mg/Cr** | **Mg/Iod** | **Mg/K** |
| Soils | Busia | BRPU | -0.6195 | 0.5783 | -0.587 | 0.5211 | 0.8894 | -0.7525 | -0.9791 | 0.6668 |
| | | BRPB | -0.4812 | -0.0932 | -0.1553 | 0.7543 | 0.8661 | -0.5569 | -0.9673 | 0.8764 |
| | Lugari | LRPM | -0.9486 | 0.9942 | 0.0152 | -0.0609 | -0.8732 | 0.9818 | -0.9523 | 0.9968 |
| | | LRPL | -0.503 | 0.8839 | 0.1275 | 0.1027 | -0.6191 | 0.7489 | -0.8222 | 0.9642 |
| Plants | Busia | BRPU | 0.0736 | -0.4887 | 0.3477 | -0.699 | -0.5767 | 0.2858 | -0.5548 | -0.3266 |
| | | BRPB | 0.5953 | -0.3697 | 0.4463 | -0.6646 | -0.8968 | 0.1605 | -0.6026 | -0.0085 |
| | Lugari | LRPM | 0.8955 | 0.6155 | 0.789 | -0.4407 | 0.6442 | 0.9076 | 0.5384 | 0.8248 |
| | | LRPL | 0.9104 | 0.7255 | 0.838 | -0.7705 | 0.7502 | 0.9597 | 0.5917 | 0.8937 |
| | | | | | | | | | | |

| Sample | Region | Location | Cr/Fe | Cr/Pb | Cr/Cd | Pb/Mg | Pb/Fe | Pb/Cd | Fe/Cd | - |
|---|---|---|---|---|---|---|---|---|---|---|
| Soils | Busia | BRPU | 0.462 | -0.4853 | -0.3869 | 0.5211 | 0.3638 | 0.3475 | -0.5748 | |
| | | BRPB | -0.2487 | -0.7694 | -0.0894 | 0.7543 | 0.4983 | 0.4447 | -0.3708 | |
| | Lugari | LRPM | 0.1533 | -0.192 | -0.9429 | -0.0609 | -0.0418 | 0.2429 | -0.4686 | |
| | | LRPL | 0.3344 | -0.1324 | -0.0585 | 0.1027 | -0.4928 | 0.0511 | -0.299 | |
| Plants | Busia | BRPU | -0.6343 | -0.8533 | 0.2009 | -0.7132 | 0.2561 | 0.2822 | -0.3258 | |
| | | BRPB | -0.7668 | -0.8321 | 0.0894 | -0.6646 | 0.3608 | 0.4705 | -0.5284 | |
| | Lugari | LRPM | 0.6273 | -0.4201 | 0.8893 | -0.4407 | 0.1706 | -0.1057 | 0.4848 | |
| | | LRPL | 0.8086 | -0.7141 | 0.8933 | -0.7705 | -0.4393 | -0.3732 | 0.7015 | |

**Appendix 9:** Correlation table for element concentration in salt versus its presence in plant and soil

| | **Salt-Pb** | **plant-Pb** | **soil-Pb** | **salt-I** | **plant-I** | **soil-I** | **salt-K** | **plant-K** | **soil-K** | **salt-Cd** | **plant-Cd** | **soil-Cd** | **soil-Cr** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salt-Pb | 1 | | | | | | | | | | | | |
| plant-Pb | 0.1357 | 1 | | | | | | | | | | | |
| soil-Pb | 0.3464 | 0.275 | 1 | | | | | | | | | | |
| salt-I | -0.4705 | 0.1157 | -0.6394 | 1 | | | | | | | | | |
| plant-I | -0.7378 | -0.1615 | -0.5627 | 0.5247 | 1 | | | | | | | | |
| soil-I | -0.6347 | -0.3047 | -0.3708 | 0.6449 | 0.838 | 1 | | | | | | | |
| salt-K | -0.0478 | 0.5466 | -0.5213 | 0.5052 | -0.0396 | -0.2681 | 1 | | | | | | |
| plant-K | 0.1567 | 0.8423 | 0.6886 | -0.2011 | -0.457 | -0.4566 | 0.2164 | 1 | | | | | |
| soil-K | 0.1357 | 0.2537 | -0.168 | 0.0707 | -0.5555 | -0.6521 | 0.7418 | 0.2778 | 1 | | | | |
| salt-Cd | 0.5236 | 0.585 | 0.4153 | 0.1289 | -0.6792 | -0.3939 | 0.3348 | 0.6596 | 0.4375 | 1 | | | |
| plant-Cd | -0.9034 | -0.3962 | -0.2161 | 0.4225 | 0.5366 | 0.6447 | -0.1691 | -0.2491 | -0.1259 | -0.4105 | 1 | | |
| soil-Cd | 0.3684 | -0.1387 | 0.8887 | -0.8087 | -0.4577 | -0.283 | -0.8039 | 0.2977 | -0.376 | 0.0674 | -0.1815 | 1 | |
| salt-Na | -0.4415 | -0.124 | 0.3282 | 0.0318 | 0.5528 | 0.7079 | -0.7014 | -0.0082 | -0.8742 | -0.3293 | 0.4593 | 0.3997 | |
| plant-Na | 0.6555 | 0.3315 | 0.5503 | -0.6355 | -0.2595 | -0.3533 | -0.3472 | 0.3201 | -0.4198 | 0.1189 | -0.769 | 0.587 | |
| soil-Na | 0.4399 | -0.3834 | 0.2695 | -0.8486 | -0.1727 | -0.3432 | -0.5815 | -0.2633 | -0.3596 | -0.4594 | -0.4348 | 0.6111 | |
| salt-Fe | -0.0462 | -0.4678 | 0.6058 | -0.3579 | -0.0301 | 0.3161 | -0.9456 | -0.0644 | -0.6349 | -0.1348 | 0.3254 | 0.7856 | |
| plant-Fe | 0.1927 | -0.3896 | -0.1797 | -0.6384 | -0.068 | -0.4439 | -0.1101 | -0.3994 | 0.0757 | -0.6131 | -0.2866 | 0.1354 | |
| soil-Fe | -0.3698 | -0.2295 | 0.3277 | -0.0747 | -0.3119 | -0.0951 | -0.1478 | 0.2198 | 0.3747 | 0.1334 | 0.6258 | 0.2532 | |
| salt-Cr | 0.198 | 0.0093 | -0.7697 | 0.3285 | 0.3324 | 0.0709 | 0.4836 | -0.5012 | 0.0307 | -0.2213 | -0.4186 | -0.6664 | |
| plant-Cr | 0.2684 | 0.3714 | -0.5259 | 0.6787 | 0.0007 | 0.0474 | 0.7217 | -0.0393 | 0.3348 | 0.4969 | -0.3548 | -0.7218 | |
| soil-Cr | 0.2215 | 0.4828 | 0.7859 | -0.0556 | -0.4839 | -0.1253 | -0.1676 | 0.7693 | 0.0055 | 0.7785 | -0.0663 | 0.4825 | 1 |

**Appendix 10:** Correlation table for various soil properties and heavy metals in the soils samples

| **wet season** | **depth, cm** | **pH** | **%moisture** | **%N** | **CEC meq/100 g soil** | **%sand** | **%silt** | **SBD, g/cm3** | **%LOI** | **%clay** | **Cr mg/kg** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | -0.308 | 1 | | | | | | | | | |
| %moisture | 0.417 | -0.653 | 1 | | | | | | | | |
| %N | 0.344 | -0.621 | 0.717 | 1 | | | | | | | |
| CEC meq/100 g soil | -0.215 | -0.325 | 0.486 | 0.47 | 1 | | | | | | |
| %sand | -0.444 | 0.862 | -0.801 | -0.795 | -0.367 | 1 | | | | | |
| %silt | 0.537 | -0.795 | 0.689 | 0.607 | -0.041 | -0.907 | 1 | | | | |
| SBD, g/cm$^3$ | 0.645 | 0.406 | -0.339 | -0.283 | -0.715 | 0.337 | -0.094 | 1 | | | |
| %LOI | 0.307 | -0.847 | 0.831 | 0.808 | 0.61 | -0.96 | 0.763 | -0.511 | 1 | | |
| %clay | 0.26 | -0.757 | 0.755 | 0.835 | 0.719 | -0.897 | 0.627 | -0.519 | 0.974 | 1 | |
| P, mg/kg | 0.311 | -0.029 | -0.351 | -0.414 | -0.046 | 0.23 | -0.254 | 0.388 | -0.201 | -0.159 | |
| Mg mg/kg | -0.131 | 0.9 | -0.388 | -0.571 | -0.473 | 0.705 | -0.526 | 0.467 | -0.754 | -0.75 | |
| Ca mg/kg | -0.138 | 0.08 | 0.397 | -0.259 | -0.018 | 0.069 | 0.054 | -0.203 | -0.049 | -0.191 | |
| Fe mg/kg | -0.578 | 0.588 | -0.096 | -0.477 | 0.302 | 0.537 | -0.638 | -0.313 | -0.366 | -0.327 | |
| Pb mg/kg | -0.275 | 0.326 | -0.276 | -0.74 | -0.59 | 0.503 | -0.187 | 0.142 | -0.584 | -0.738 | |
| Cd mg/kg | 0.277 | 0.441 | -0.267 | -0.322 | -0.869 | 0.23 | 0.132 | 0.672 | -0.467 | -0.561 | |
| Cr mg/kg | 0.287 | -0.654 | 0.522 | 0.833 | 0.66 | -0.775 | 0.48 | -0.36 | 0.847 | 0.932 | 1 |
| Iod mg/kg | -0.145 | 0.772 | -0.135 | -0.252 | -0.286 | 0.57 | -0.46 | 0.31 | -0.582 | -0.572 | -0.619 |
| K mg/kg | -0.049 | 0.489 | 0.005 | -0.268 | -0.441 | 0.158 | 0.066 | 0.197 | -0.279 | -0.362 | -0.533 |
| Na mg/kg | -0.306 | -0.385 | 0.335 | 0.36 | 0.928 | -0.234 | -0.147 | -0.715 | 0.486 | 0.585 | 0.568 |
| **dry season** | **depth, cm** | **pH** | **%moisture** | **%N** | **CEC meq/100gsoil** | **%sand** | **%silt** | **SBD, g/cm3** | **%LOI** | **%clay** | **K, mg/kg** |
| pH | -0.312 | 1 | | | | | | | | | |
| %moisture | 0 | 0.254 | 1 | | | | | | | | |
| %N | 0.055 | 0.156 | 0.767 | 1 | | | | | | | |
| CEC meq/100 g soil | -0.098 | 0.441 | 0.394 | 0.438 | 1 | | | | | | |
| %sand | -0.15 | -0.141 | -0.828 | -0.762 | -0.295 | 1 | | | | | |
| %silt | 0.15 | 0.002 | 0.75 | 0.601 | -0.058 | -0.931 | 1 | | | | |
| SBD, g/cm$^3$ | 0.557 | -0.2 | -0.239 | -0.237 | -0.622 | 0.273 | -0.088 | 1 | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| %LOI | 0.097 | 0.227 | 0.843 | 0.786 | 0.526 | -0.967 | 0.815 | -0.42 | 1 | | |
| %clay | 0.118 | 0.283 | 0.767 | 0.815 | 0.667 | -0.897 | 0.674 | -0.444 | 0.971 | 1 | |
| P, mg/kg | -0.018 | 0.073 | -0.002 | -0.07 | 0.051 | 0.147 | -0.183 | 0.323 | -0.113 | -0.075 | |
| Fe, mg/kg | 0.554 | -0.022 | 0.09 | 0.176 | 0.079 | -0.169 | 0.114 | 0.056 | 0.161 | 0.194 | |
| Pb, mg/kg | 0.67 | -0.415 | 0.052 | 0.314 | 0.223 | -0.097 | -0.025 | 0.231 | 0.14 | 0.228 | |
| Cd, mg/kg | -0.295 | -0.159 | -0.2 | 0.075 | 0.014 | 0.221 | -0.276 | -0.008 | -0.188 | -0.113 | |
| Cr, mg/kg | 0.25 | 0.284 | -0.069 | 0.325 | 0.064 | -0.373 | 0.282 | 0.093 | 0.326 | 0.416 | |
| Mg, mg/kg | -0.262 | 0.403 | -0.068 | -0.129 | 0.066 | -0.094 | 0.078 | -0.404 | 0.09 | 0.095 | |
| K, mg/kg | -0.218 | 0.256 | -0.534 | -0.473 | -0.192 | 0.498 | -0.46 | -0.04 | -0.506 | -0.453 | 1 |
| Iod mg/kg | 0.271 | 0.36 | 0.561 | 0.67 | 0.433 | -0.587 | 0.413 | 0.147 | 0.617 | 0.695 | -0.418 |
| Na, mg/kg | -0.308 | 0.383 | 0.438 | 0.247 | 0.886 | -0.206 | -0.075 | -0.59 | 0.424 | 0.509 | -0.225 |

**Appendix 11:** Regression showing the the dependence of iodine on species, method of salt preparation, storage period and packaging.

| Analysis of variance; Variate: iod_conc | | | | | |
|---|---|---|---|---|---|
| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
| Spp | 1 | 4.20E+05 | 4.20E+05 | 82287.45 | <.001 |
| Method | 1 | 2.35E+05 | 2.35E+05 | 46061.25 | <.001 |
| packaging | 4 | 1.98E+03 | 4.95E+02 | 97.17 | <.001 |
| period | 3 | 5.54E+05 | 1.85E+05 | 36186.35 | <.001 |
| spp.method | 1 | 1.95E+05 | 1.95E+05 | 38250.23 | <.001 |
| spp.packaging | 4 | 1.26E+03 | 3.16E+02 | 61.93 | <.001 |
| method.packaging | 4 | 1.50E+03 | 3.75E+02 | 73.57 | <.001 |
| spp.period | 3 | 4.58E+05 | 1.53E+05 | 29918.41 | <.001 |
| method.period | 3 | 3.15E+05 | 1.05E+05 | 20593.15 | <.001 |
| packaging.period | 12 | 1.95E+03 | 1.62E+02 | 31.85 | <.001 |
| spp.method.packaging | 4 | 1.10E+03 | 2.75E+02 | 54.03 | <.001 |
| spp.method.period | 3 | 2.83E+05 | 9.43E+04 | 18500.67 | <.001 |
| spp.packaging.period | 12 | 1.41E+03 | 1.18E+02 | 23.06 | <.001 |
| method.packaging.period | 12 | 2.49E+03 | 2.07E+02 | 40.63 | <.001 |

**Appendix 12:** Regression analysis at elevated temperature and humidity

| **Estimates of parameters for *C. rotundus* in accumulated analysis of variance** | | | | | |
|---|---|---|---|---|---|
| Parameter/change | df | estimate | s.e. | t(467) | t pr. |
| spp Typha | 1 | -59.13 | 4.75 | -12.46 | <.001 |
| method Ev_cryst | 1 | -44.24 | 4.75 | -9.32 | <.001 |
| packaging Closed_container | 4 | -1.71 | 7.5 | -0.23 | 0.82 |
| packaging HDPE | 4 | 3.92 | 7.5 | 0.52 | 0.602 |
| packaging LDPE | 4 | 2.49 | 7.5 | 0.33 | 0.74 |
| packaging Open_container | 4 | 2.63 | 7.5 | 0.35 | 0.726 |
| period 1_month | 4 | -63.71 | 6.71 | -9.49 | <.001 |
| period 3_month | 4 | -78.01 | 6.71 | -11.62 | <.001 |
| period 6_month | 4 | -86.61 | 6.71 | -12.9 | <.001 |
| **Estimates of parameters for *T. latifolia* in accumulated analysis of variance** | | | | | |
| spp | 1 | 419527 | 419527 | 155.2 | <.001 |
| method | 1 | 234835 | 234835 | 86.88 | <.001 |
| packaging | 4 | 1982 | 495 | 0.18 | 0.947 |
| period | 3 | 553468 | 184489 | 68.25 | <.001 |

**Appendix 13:** Relationship of perception with sex, level of education, occupation, household wealth category and plant do you get local salt from by County

| Variable under Test | Sex | Count and % within County | County | | Total |
|---|---|---|---|---|---|
| | | | Busia | Kakamega | |
| Sex of respondent by County | Male | Count | 11 | 28 | 39 |
| | | % within County | 27.50% | 50.90% | 41.10% |
| | Female | Count | 29 | 27 | 56 |
| | | % within County | 72.50% | 49.10% | 58.90% |
| | Total | Count | 40 | 55 | 95 |
| | | % within County | 100.00% | 100.00% | 100.00% |
| Sex of the household head | male | Count | 30 | 41 | 71 |
| | | % within County | 75.00% | 74.50% | 74.70% |
| | female | Count | 10 | 14 | 24 |
| | | % within County | 25.00% | 25.50% | 25.30% |
| | Total | Count | 40 | 55 | 95 |
| | | % within County | 100.00% | 100.00% | 100.00% |
| Highest level of education of household head by County | None | Count | 2 | 7 | 9 |
| | | % within County | 5.00% | 12.70% | 9.50% |
| | Primary | Count | 35 | 30 | 65 |
| | | % within County | 87.50% | 54.50% | 68.40% |
| | Secondary | Count | 3 | 17 | 20 |
| | | % within County | 7.50% | 30.90% | 21.10% |
| | Post-secondary | Count | 0 | 1 | 1 |
| | | % within County | 0.00% | 1.80% | 1.10% |
| | Total | Count | 40 | 55 | 95 |
| | | % within County | 100.00% | 100.00% | 100.00% |
| Occupation of the household head by County | Farming and business | Count | 11 | 3 | 14 |
| | | % within County | 27.50% | 5.50% | 14.70% |
| | Boda boda | Count | 2 | 2 | 4 |
| | | % within County | 5.00% | 3.60% | 4.20% |
| | Farming | Count | 26 | 46 | 72 |
| | | % within County | 65.00% | 83.60% | 75.80% |
| | Business | Count | 1 | 1 | 2 |
| | | % within County | 2.50% | 1.80% | 2.10% |
| | Teaching | Count | 0 | 1 | 1 |
| | | % within County | 0.00% | 1.80% | 1.10% |
| | Security | Count | 0 | 2 | 2 |
| | | % within County | 0.00% | 3.60% | 2.10% |
| | Total | Count | 40 | 55 | 95 |
| | | % within County | 100.00% | 100.00% | 100.00% |

| | | | | | |
|---|---|---|---|---|---|
| **Variable under Test** | **Sex** | **Count and % within County** | **County** | | **Total** |
| | | | Busia | Kakamega | |
| Household wealth category by County | medium | Count | 40 | 44 | 84 |
| | | | | | |
| | | % within County | 100.00% | 80.00% | 88.40% |
| | poor | Count | 0 | 11 | 11 |
| | | % within County | 0.00% | 20.00% | 11.60% |
| | Total | Count | 40 | 55 | 95 |
| | | % within County | 100.00% | 100.00% | 100.00% |
| What plant do you get local salt from by County | omuverenyi grass | Count | 40 | 0 | 40 |
| | | % within County | 100.00% | 0.00% | 49.40% |
| | Lukhaye Nzioa | Count | 0 | 41 | 41 |
| | | % within County | 0.00% | 100.00% | 50.60% |
| | Total | Count | 40 | 41 | 81 |
| | | % within County | 100.00% | 100.00% | 100.00% |