Determination of elemental concentrations in edible seaweeds, sea sediments and seawater samples from the Kenyan Coast using X-ray Fluorescence techniques

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DECLARATION

This thesis is my original work and has not been presented to any other examination body/ University for the award of a degree.

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DEDICATION

This thesis research is dedicated to my family, friends, coastal communities and all those who gave financial and moral support.

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ABBREVIATIONS

ANZFA-Australia-New Zealand Food Agency

AXIL – Analysis of X-ray spectra by Iterative Least-square

USEA – United Sates Environmental Agency

EBO - Encyclopedia Britannica Online

EDXRF- Energy Dispersive X- ray Fluorescence

EPA- Environmental Protection Agency (US)

EAME- East Africa Marine Ecoregion

EUFIC-European Food Information Council

FAO - Food and Agriculture Organization (UN)

FNB – US Food and Nutrition Board (2004), Institute of Medicine, National Academy of Sciences

IAEA – International Atomic Energy Agency

IOSHIC- International Occupational Safety and Health Information Centre

KMFRI - Kenya Marine and Fisheries Research Institute

MDGs- Millennium Development Goals (UN)

NAAS -National Academy of Agricultural Sciences India

NDP – National Development Plan (Kenya)

NEMA- National Environment Management Authority (Kenya)

TXRF – Total Reflection X-ray Fluorescence technique

UN - United Nations

UNEP - United Nations Environmental Programme

WHO- World Health Organization

XRF- X-ray Fluorescence

ABSTRACT

Since ancient times, seaweeds have been used as human food, animal feed, fertilizers, salt extractors and pharmaceuticals in orient countries but not to any great extent in Kenya. Currently, world seaweed industry is estimated at over US$ 6 billion annual income and there is steady growing demand for consumption and direct uses of seaweeds as food across the globe. This makes Kenya a potential producer of edible seaweeds in the region especially the *Eucheumoids* and *Gracilaroids* used for *Agar* and *Carrageenan* hydrocolloids production in food industries.

Despite the growing consumption, marine pollution remains a major threat at the Kenyan coast due to increasing populations and industrial developments at the coastal city of Mombasa. Previous studies at the coast on heavy metal pollution remain implicit especially with regard to the use of biological indicators such as edible seaweeds to monitor trace element contamination and pollution of the marine ecosystem. On the other hand, presence of trace elements in edible seaweeds is an aspect that can compromise its use as human food.

Therefore, this study sought to establish concentration levels for total and bio-available trace metals in the edible seaweed samples using X-ray Fluorescence (XRF) technique to specifically determine the trace element content and to address the safety of seaweeds as food product as well as for their use as biological indicators of marine pollution.

In this study, a total of 40 seaweeds samples, 20 sea sediment samples and 20 sea water samples were collected by random method from the sampling sites at the Kenyan coast that included: Kibuyuni, KWS (Shimoni), Gazi Bay, Makupa and Tudor Creek. Seaweed samples were prepared by digestion method for TXRF analysis. This analytical method was also applied for seawater samples. The sea sediments were oven dried crushed, grounded and pelletized then

analyzed using ED200EDXRF machine comprised of cadmium radioactive source and Canberra Si-Li detector with a resolution of 190 eV at 5.9 KeV line.

For seaweed samples, the concentration levels (in $\mu g\ g^{-1}$) obtained varied as follows: **Ca** [217-1253; 36.1 - 398], **Mn** [1.0 – 10.1; 0.1 - 3.9], **Fe** [7.0 - 123; 1.8 – 16.6], **Cu** [0.3 – 4.6; 0.0 – 0.6] and Zn [0.3 - 7.6; 0.0 – 1.6], for total and bioavailability contents in seaweeds respectively. In sediment and seawater, concentrations obtained were: **Ca** [<962 - 2,555; 54.8 - 175], **Mn [**<99; 4.7 - 43.8], **Fe** [<62 - 225; 7.1 - 61.2], **Cu** [14 – 41.2; 2.9 - 19.8] and **Zn** [19.0 – 78.4; 3.7 - 52] in $\mu g\ g^{-1}$ and $\mu g\ ml^{-1}$ respectively.

From the findings, concentrations of trace elements in seaweeds were relatively high while the toxic elements such as As and Pb were below detection limits hence within EPA/WHO regulations. Therefore, the direct consumption of edible seaweeds as food should be encouraged. On the other hand, measurement of trace elements in sediments and seawater presents marked inconsistencies that studies have proved to be associated with changes in organic composition of the sediments and seasonal changes affecting dissolved matter in the sea. This makes seaweeds the preferred option as pollution indicators in the marine environment.

CHAPTER 1

INTRODUCTION

1.0 Background

Seaweed has long been part of the traditional coastal diet. It is widely consumed in East Asia, particularly Japan, China and Korea (Almela *et al*., 2006, Miguel *et al*., 2008) but not to any great extent in African countries (Wakibia *et al.,* 2007). In Western countries, especially the Caribbean, its use is concentrated mainly in the extraction of compounds for use in the pharmaceutical, cosmetics and food industries (Caliceti *et al.,* 2002; Mabeau *et al*., 1993). In recent years, there has been an increase in direct consumption of seaweed particularly because of the nutritional (Darcy-Vrillon, 1993; Fleurence, 1999) and therapeutic benefits that these products provide.

From a nutritional point of view, seaweeds constitutes high dietary fibre content (Almela *et al*., 2006, EUFIC, 2008) rich in soluble fractions with hypocholesterolemic and hypoglycemic effects (Mabeau *et al.,* 1993; Jime´- nez-Escrig, *et al*, 2000). In addition, they are a source of proteins, with an amino acid composition of nutritional interest (Fleurence, 1999). The mineral uptake in seaweeds reaches considerable levels (8–40%), making it a potential food supplement for macro-minerals and trace elements (Ru´ perez, 2002, Rajfur *et al*., 2010), although it constitutes a negligible energy source because of their low lipid content; $1-2\%$ (Darcy-Vrillon, 1993).

However, seaweed has a high metal pollutants accumulation capacity and has been used as a bio-indicator in several of marine environment contamination studies (Amado − Filho *et al.,* 2008; Bryan, 1973; Ferreira *et al*, 1998; Vasquezl *et al*., 1996). Metal contamination is an aspect that can affect the safety of edible seaweeds as a food product (Almela *et al.,* 2006).

Developed countries such as France, the USA, Australia and New Zealand have developed specific regulations for limiting toxic elements intake in edible seaweed (ANZFA, 1997, Mabeau *et al.,* 1993). In Kenya, such regulations do not exist.

1.1 The ocean chemistry: an overview

Oceans constitute about 70% of the earth's surface that is under water cover (De Mora, 2007). Environmental chemistry in regard to oceans takes a geochemistry approach which is the traditional way of conceptualizing the study of an ocean system as a huge reservoir. For a given component, the reservoir has sources (inputs) and sinks (outputs). Weigner (2000), postulates that, the system is said to be at equilibrium and operating under steady-state conditions when a mass balance between inputs and outputs is achieved. An imbalance could signify that an important source or sink has been ignored. He further asserts that, alternatively the system may be perturbed, possibly through anthropogenic activities, and therefore be changing towards a new equilibrium state.

According to De Mora (2007) processes within the reservoir that affect the temporal and spatial distribution of a given component are transportation and transformations. The former is dominated by the hydrodynamic regime, while the latter involves both chemical and geological processes. The chemical processes involves dissolution, redox reactions and speciation changes whereas geological is principally a sedimentation process. Generally, biological activity controls nutrient and trace metal distributions. Material supplied to the ocean originates (Weigner 2000) from the atmosphere, rivers, glaciers and hydrothermal waters (Ahmad *et al*., 2012). Although volatilization and subsequent evasion to the atmosphere can be important for removal of some elements, Sedimentation acts as the major removal mechanism.

Research shows that, transported material may be either dissolved or particulate, but discharges into surface water are confined to coastal regions (De Mora, 2007). Hydrothermal water is formed when seawater circulates into the fissured rock matrix, and under conditions of elevated temperature and pressure, compositional changes in the aqueous phase occur due to seawater – rock interactions resulting in release of this water from the vents on the sea floor. Weigner (2000) identified this as an important source of some elements, such as Li, Rb and Mn among other metal contaminants.

Weigner (2000) asserts that aerial deposition of particulate material occurs globally on the ocean surface. In recent years, this has been the most prominent pathway of lead (Pb), identified by its isotopic signature as originating from petrol additives, especially the Tetra-Ethyl Lead (TEL). Wind-borne transport is greatest in low latitudes and the Sahara Desert is known to act as an important source of dust (Weigner, 2000). Also, the airborne flux of nutrients, notably nitrogenous compounds, has become increasingly recognized as important both in some coastal waters, such as Chesapeake Bay (US), and large seas, including the Mediterranean Sea (De Mora, 2007). On the other hand, glacial activity makes little impact on the World Ocean. Glacier-derived material tends to be comprised of physically weathered rock residue, which is relatively insoluble. In addition, (De Mora, 2007) the input is largely confined to Polar Regions, with Antarctica responsible for approximately 90% of the material.

1.2 The project conceptual frame work

In the preceding discussion, the geochemistry approach of the study of marine system has been greatly adopted by environmental chemists (De Mora, 2007). The ocean trough can be exemplified as a huge reservoir in an equilibrium state. An imbalance implies a shift occasioned

by fluctuations in inputs, mainly from the atmosphere, river, runoffs and glaciation and outputs predominated by Sedimentation and hydrothermal processes (Figure 1.1).

1.3 The project conceptual frame work (illustration)

Based on the primary objectives, this study can be summarized as shown in the flow chart of figure (a) and (b)

(a)

(b)
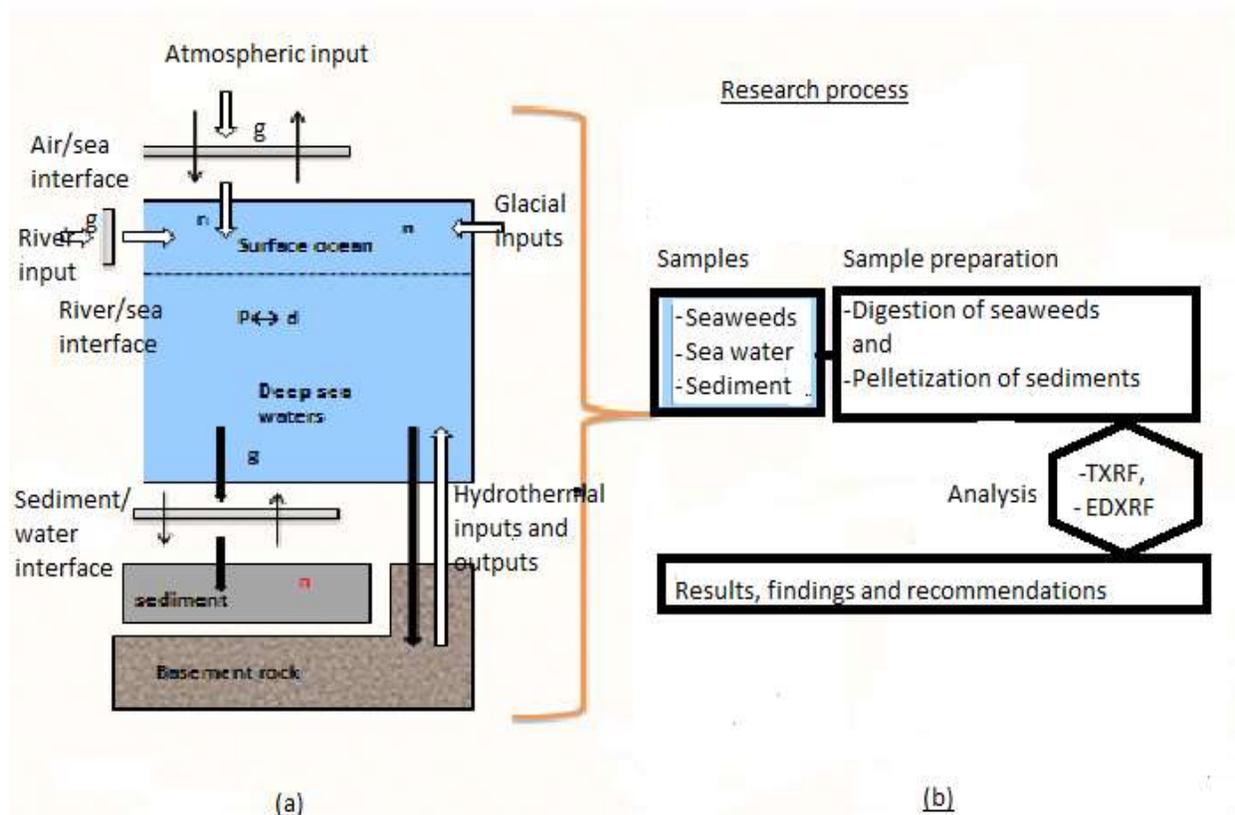
Fig.1.1: (a). A schematic representation of the ocean reservoir. The source and sink fluxes are designated as g and n, referring to gross and net fluxes respectively, thereby indicating that interactions within the boundary regions can modify the mass transfer. Within seawater, the p↔d term signifies that substances can undergo particulate–dissolved interactions. However, it must be appreciated that several transportation and transformation processes might be operative (Weigner, 2000; De Mora, 2007). (b). A schematic representation of research process.

1.4 Statement of the Problem

Anthropogenic activities have increased lately in coastal urban centers due to increase in number of people visiting these centers for human tourism and for employment. Increase in discharges from untreated domestic and industrial effluents into the sea results in the pollution of the marine life. These principally, form the pathways of heavy metal contamination in seafood that includes edible seaweed, an aspect that can affect their consumption as human food. With increasing population, food demand and economic challenges are inevitably growing calling for diversification of food production and livelihood improvement activities at the coast.

1.5 Research hypothesis

i. The edible seaweeds are safe for human consumption and form a good dietary supplement.

ii. Seaweeds are not safe for human consumption.

1.6 Objectives
1.6.1 General objective

The overall goal of this research is to obtain information on trace element levels in seaweeds, the bioavailability of trace elements and also assess the potential of seaweeds as marine pollution bio-monitors.

1.6.2 Specific Objectives

The objectives of the study included the following;

1)   To determine the total content and bioavailability levels of trace elements in the edible seaweed from Kenyan coast using X-ray fluorescence technique;

2)   To evaluate trace elements concentration in sea sediment and sea water from the Kenyan Coast using X-ray fluorescence technique;

3)   To assess marine pollution, using seaweed as a bio-indicator of metal contamination and compare with findings using water and sediments.

1.7 Justification and Significance of the study

Seaweeds have been widely consumed as part of diet in other regions of the world but not to any great extent in Kenya. In other countries, there is growing demand of *Carrageenan*-hydrocolloid produced mainly from red seaweeds (*Euchemoids* and *Gracilaria*) species that thrives well in this region.

The consumption and use of seaweed as food is growing steadily across the globe and the industry is estimated to be around US$ 6 billion (FAO, 2002; FAO, 2012). Currently, there is a pilot project for seaweed cultivation at the Kenyan south coast initiated by non-state actors with technical support of the Kenya Marine and Fisheries Research Institute. Consequently, this has presented the opportunity for Kenya coast to become one of the leading seaweed producers and as economic potential.

However, the increased human activities have led to high degree of marine pollution in particular leading to trace metal contamination in sea foods at the Kenyan coast (Onyari, J.M., 1985). Bioaccumulation of heavy metals and trace elements is enhanced in seaweed (Bryan, 1973) due to their inability to regulate mineral uptake as compared to other marine vegetation. This makes it a good bio-indicator of marine pollution (Bryan, 1973; NAAS, 2003; Vasquez *et al*., 1996). In addition, most seaweed research at the Kenyan coast has been taxonomic and implicit in nature (Bolton *et al*., 2007), and no information on total content and bioavailability of trace elements in edible seaweeds has been comprehensively documented. This study evaluated concentrations in terms of iron, manganese and other trace element content of endemic species of edible seaweeds available from Kenyan coast using X-Ray Fluorescence. The findings can be used to address food safety, document the state of marine pollution and provide information for future legislative enforcements by the government.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

This chapter presents an overview of marine pollution with specific reference to the Kenyan coast, trace elements concentrations in marine ecosystem, review related studies done elsewhere.

2.1 Overview of marine contamination and pollution

Both contamination and pollution is the perturbation of the natural state of the environment by anthropogenic activities (UNEP, 2006b; Brown *et al.,* 2006). The two terms are interchangeably used, but distinguishable in terms of the severity of the effect, for example, pollution induces the loss of potential resources. There is a clear cause–effect relationship, for a substance to be classified as a pollutant towards a particular organism or system.

The human-induced disturbances take many forms, but the greatest effects tend to be in coastal environments due to the source, strengths and pathways (Brown *et al.,* 2006).

Water and sediments in coastal regions bear the greatest brunt of industrial and sewage discharges, and are also subject to dredging and dumping. Agricultural run-off may contain pesticide residues and elevated nutrients, the latter of which may over-stimulate biological activity producing *eutrophication* and anoxic conditions (Brown *et al*., 2006; UNEP, 2006b). The manifestations of deep sea contamination comprise crude oil, petroleum products and solid (e.g. plastics) pollutants. Additionally, the Aeolian transport of heavy metals has enhanced natural fluxes of some elements; particularly lead (De Mora, 2007).

2.2 Marine contamination and pollution at the Kenyan coast.

A study by Muohi *et al.,* (2002) indicates that pollution is on the increase at the Kenyan coastal city of Mombasa. With the increasing human population and the rapid industrial growth of the coastal urban centers and the development of the coastal tourism sector in Kenya, high degree of pollution is envisioned from untreated domestic and industrial effluents, posing a threat to the marine ecosystem.

Pollution from marine transportation and oil spills reportedly on increase around Mombasa resulting in damaging nearby marine ecosystem; mangrove forests and beaches (EAME, 2004) in addition to the monsoon winds from the east, though to a small extent (De Mora, 2007). For instance, domestic oil spills at Mombasa can be felt at Maputo (Mozambique) and vice versa. This serves both as enrichment and dispersion mechanism of contaminants. Apart from point source contamination, the hydrological circle plays a big role in entry of pollutants into water bodies (De Mora, 2007).

2.3 Sources of heavy metal contamination and their effects on human health

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water at 4°C (IOSHIC, 2009). There are 35 elements in this category and their toxicity depends on the degree of concentration in the environment and food materials. Some examples of well-known toxic metallic elements include; arsenic, cadmium, iron, lead and mercury (WHO, 1996). In small quantities, certain heavy metals are nutritionally essential e.g., iron, copper, manganese, and zinc (IOSHIC, 2009). The contamination of marine ecosystems by heavy metals is a worldwide problem and is exacerbated by the ability of ecosystems to concentrate and accumulate some in the food chains (Rajfur *et al.,* 2010; Wattling, 1983).

Marine organisms, particularly sea plants, can take up these metals through their mineral uptake mechanisms, thus increasing the potential for their entry into food chain (EUFIC, 2008). Therefore, there is need to continuously monitor the levels of heavy metals in aquatic environments.

Bioaccumulation of these metals in seaweed is made possible because of their ability to be in a biologically available form in water and sediments (EUFIC, 2008). The bioavailability of sediment-bound trace metals is dependent not only on the total metal concentration, but also on the strength of the association between the metal and sediment compounds (Mwashote, 2003, Nyatebe, 1990) and also the water column. These metals do not occur freely in nature, but are incorporated in compounds that have a higher degree of solubility (Harrisons, 2007).

In Kenya, studies by Onyari (1985), Oteko (1987), Muohi *et al., (*2002), Mwashote (2003) have reported high levels of toxic heavy metals such as lead and cadmium in seafood and other living organisms.

In the following sections, some of the commonly encountered heavy metals contaminants causing concern in water and food consumption are briefly discussed.

2.3.1 Iron

Iron is naturally released into waters by weathering of pyritic ores containing iron sulfide (FeS) (Weigner, 2000) and other iron-bearing minerals in igneous, sedimentary, and metamorphic rocks. Other sources includes; mineral processing, coke and coal burning, acid-mine drainage, iron and steel industry wastes, and corrosion of iron and steel contribute to presence of iron in the environment(Weigner, 2000). Iron is present in organic matter, in soil, sewage and land leachate (De Mora, 2007) hence available as mineral to plants. Major sources of iron in food stuffs includes: meat, vegetables and cereals and also in low concentrations in milk and fruits.

Iron is an essential nutrient for animal and plant metabolism. It plays an important role in the body's immune function, cognitive development, temperature regulation, energy metabolism and work performance (WHO, 1996). It is a component of various proteins involved in the transportation and metabolism of oxygen in hemoglobin (EBO, 2009). Its deficiency leads to anaemia in human beings.

Seafood products including seaweed have reportedly high iron content which varies between 30% to 40% of their mineral content (Almela *et al*., 2006; EUFIC, 2008). Generally, iron is considered a non-toxic substance though excessive ingestion (excess of 27µg/day according to US Food and Nutrition Board, 2004) may result in haemochromatosis; a condition of tissue damage from iron accumulation (EBO, 2009). This condition rarely occurs from dietary intake alone but may result from prolonged consumption of acidic foods cooked in iron utensils and from the ingestion of large quantities of iron tablets (Weigner, 2000).

2.3.2 Manganese

Manganese is an abundant, widely distributed metal in the environment. It does not occur in nature as the elemental metal (Weigner, 2000) but is found in various salts and minerals frequently along with iron compounds. Soils, sediments, and rocks are significant natural sources of manganese though weathering also contributes small amounts deposited into natural waters. Other anthropogenic sources of manganese includes; wastes from steel industry where it is used to manufacturing metal alloys and dry cell batteries, and in the chemical industry for making paints, varnishes, inks, dyes, glass, ceramics, matches, fireworks, and fertilizers(Weigner, 2000). The iron & steel industry, acid mine drainage release a large portion of the manganese found in the environment (Weigner, 2000). Manganese is absorbed by plant tissues and ingested by human being (EUFIC, 2008).

Manganese is essential for normal physiologic functioning in all animal species and other organisms though problems may arise from its deficiency and excessive intakes. Grains and cereals are the richest dietary sources of manganese followed by fruits and vegetables; while meat, fish, and poultry contain little manganese (WHO, 1996).

2.3.3. Zinc

Zinc does not occur freely in nature but is incorporated in minerals mainly sulfides, oxides, carbonates, and silicates which are associated with other metals, particularly lead, copper, cadmium, mercury, and silver (Weigner, 2000). In natural waters, it occurs in both suspended and dissolved forms. The dissolved form is the divalent cation, Zn^{2+}, that is readily sorbed to or occluded in mineral clays and humic colloids hence it gets into the food chain through mineral uptake by aquatic plants.

Zinc is a common contaminant in surface and groundwater, storm water run-off, and industrial waste streams (UNEP, 2006b). Industries with waste streams containing significant levels of zinc include steel works with galvanizing operations, zinc and brass metal works, zinc and brass plating, and production of viscose rayon yarn, ground wood pulp, and newsprint paper. Reported highest concentrations of zinc in industrial waste streams reach 48,000 mg/L (Weigner, 2000). According to US Food and Nutrition Board (FNB, 2004) dietary recommendations (Appendices Table 4.26), zinc is an essential nutrient and is toxic to humans at levels exceeding regulatory standards (11mg/d for male adults). Toxicity from chronic ingestion of zinc usually presents with the features of copper deficiency as prolonged intake of zinc ranging from $100 - 150$ μgg^{-1} interferes with copper metabolism and causes low copper status, reduced iron function, red blood cell microcytosis, neutropenia, reduced immune function and reduced levels of high density lipoproteins. Ingesting 20 to 80 µg/day of zinc can cause abdominal pain, nausea,

vomiting and diarrhea. (EUFIC, 2008, EBO, 2009, EPA, 1996, FNB, 2004). US EPA guidelines in seawater for Zn is 81.0 μgl^{-1} and in sediments is 271 μgg^{-1} dry weight while the US Food and Nutrition Board dietary recommended level is 11μg/day (EPA, 1996, FNB, 2004).

2.3.4 Lead

Lead minerals are found mostly in igneous, metamorphic and sedimentary rocks. The most abundant lead mineral is galena (PbS). Oxide, carbonate, and sulfate minerals are lanarkite (PbO), cerrusite ($PbCO_3$), and anglesite ($PbSO_4$), respectively (Weigner, 2000). Metallic lead and the common lead minerals have very low solubility. Most environmental lead (perhaps 85%) is associated with sediments; the rest is in dissolved form. Although some lead enters the environment from natural sources by weathering of minerals, particularly galena, anthropogenic sources are predominant pathways (De Mora, 2007). Mining, milling and smelting of lead and metals associated with lead, such as zinc, copper, silver, arsenic and antimony, are major sources, as well as combustion of fossil fuels and municipal sewage.

Commercial products that are major sources of lead pollution include lead-acid storage batteries, electroplating, construction materials, ceramics and dyes, radiation shielding, ammunition, paints, glassware, solder, piping, cable sheathing, roofing, and gasoline additives such as tetra-methyl lead and tetra-ethyl lead (Weigner, 2000). Lead is bio-accumulated by aquatic organisms, including benthic bacteria, freshwater plants, invertebrates, benthic flora and fish hence finds its way to human being through food chain mechanism.

Lead accounts for most of the cases of pediatric heavy metal poisoning (IOSHIC, 2009). Its exposure at relatively low concentrations can cause interference with red blood cell chemistry, delays in normal physical and mental development in babies and young children, slight deficits in the attention span, hearing, and learning abilities of children, and slight increases in the blood pressure of some adults (IOSHIC, 2009). It appears that some of these effects, particularly

changes in the levels of certain blood enzymes and in aspects of children's neurobehavioral development may occur at blood lead levels so low as to be essentially without a threshold (EBO, 2009). Other studies indicate that long term exposure to lead has been linked to cerebrovascular and kidney disease in humans. It also has the potential to cause cancer from a lifetime exposure at levels above the action level. Target organs are the bones, brain, blood, kidneys, and thyroid gland (IOSHIC, 2009).

2.3.5. Copper

Copper occurs naturally as mineral ores that contain 2% (EPA, 1996), or less of the metal. The most common copper-bearing ores are sulfides, arsenites, chlorides, and carbonates. Chalcopyrite ($CuFeS_2$) is the most abundant of the copper ores (Weigner, 2000), accounting for about 50% of the world's copper deposits. The weathering of copper deposits is the main natural source of copper in the aquatic environment, but dissolved copper rarely occurs in unpolluted source water above 10 mg/L (EPA, 1996), limited by the solubility of copper hydroxide ($Cu(OH)_2$), co-precipitation with less soluble metal hydroxides, and adsorption.

Anthropogenic sources include; addition of copper salts to reservoirs (for the control of algae), smelting operations and municipal incineration which enrich its levels in surface water (UNEP, 2006b). Corrosivity of water toward copper metal increases with decreasing pH, especially below pH 6.5 and it is the main source of copper contamination in domestic supplies. This eventually gets into seawaters through domestic wastes discharges (EBO, 2009).

Copper is an essential nutrient, but at high doses it has been shown to cause stomach and intestinal distress, liver and kidney damage, and anemia (IOSHIC, 2009). Persons with Wilson's disease, may be at a higher risk than the general public (WHO, 1996). There is inadequate evidence to state whether or not copper has the potential to cause cancer from a lifetime exposure from dietary intake (Weigner, 2000).

2.3.6 Chromium

Chromium occurs in minerals mostly as chrome iron ore or chromite ($FeCr_2O_2$), in which it is present as Cr^{3+}. Chromium in soils occurs mostly as insoluble chromium oxide (CrO_3), where it is present as Cr^{6+} (EPA, 1996). In natural waters, dissolved chromium exists as either Cr^{3+} cations and in anions such as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$), where it is hexavalent with oxidation number +6. Though widely distributed in soils and plants, it is generally present in low concentrations in natural waters. Trivalent chromium ($Cr^{3+)}$ readily sorbs to negatively charged soils and minerals compared to Cr (VI) which is stable under aerobic conditions. Under anaerobic conditions, Cr (VI) is quickly reduced to low mobility Cr^{3+}. Thus, most of the chromium in surface waters will be present in particulate form as suspended and bed sediments (EPA, 1996).

The main natural environmental source is chromium weathering of rocks and soil while major anthropogenic sources includes metal alloy production, metal plating, cement manufacturing, paint pigments and incineration of municipal refuse and sewage sludge (De Mora, 2007).

Trivalent chromium is an essential trace nutrient and plays a role in prevention of diabetes and atherosclerosis (WHO, 1996). Trivalent chromium is essentially nontoxic. The harmful effects of chromium to human health are caused by hexavalent chromium (EBO, 2009). Since oxidants such as chlorine and ozone readily oxidize trivalent chromium to the toxic hexavalent form, water quality limits are usually set for total chromium concentrations. Acute exposures of Chromium at levels above the minimum contamination level (WHO, 1996) have the potential to cause skin irritation or ulceration. It also has the potential to cause damage to the liver, kidney circulatory, and nerve tissues, and dermatitis due to long-term exposures at levels above the minimum contamination level (IOSHIC, 2009).

2.4. Seaweeds

Seaweeds are macroscopic marine algae that constitute one of the important resources of the ocean. They are ubiquitous primary producers in these habitats, attached and stock-still and easy to collect and preserve. In addition, they have relatively similar species numbers in any one large region from temperate to tropical regions (Bolton, 2002), and comprise representatives of three major phyla, which, though widely divergent phylogenetically, have a series of convergent functional forms (Bolton *et al.,* 2007; FAO, 2002; FAO, 2006).

2.4.1 Classification of seaweeds

According to FAO (2002), seaweeds can be categorized into three broad groups based on colour: brown, red and green representing *Phaeophyceae*, *Rhodophyceae* and *Chlorophyceae* respectively. FAO further indicate that brown seaweeds are usually large and range from the giant kelp that is often 20 metres tall, thick, leather-like seaweeds from 2-4m tall, to smaller species from 30 - 60cm long. Red seaweeds are usually smaller, generally ranging from a few centimeters to about a meter in length; however red seaweeds are sometimes purple, even brownish red, but classified by botanists as *Rhodophyceae* because of other characteristics (FAO, 2006). Green seaweeds are also small, similar in size to the red seaweeds. Because of their relative large sizes, seaweeds are also called macro-algae which distinguishes them from microalgae that are microscopic in size (Almela *et al.,* 2006; UNEP, 2006a), often unicellular and are best known by the blue-green algae that sometimes blossom and contaminate rivers and streams.

Naturally growing seaweeds are sometimes referred to as wild seaweeds, in contrast to seaweeds that are cultivated or farmed (FAO, 2002: UNEP, 2006a).

2.4.2 General uses of seaweeds

From the classification of seaweeds; *Agar*, *Carrageenan* and *Alginate* are the most popular seaweeds. They have been used as food for human beings, feed for animals, fertilizers for plants and source of various chemicals (Almela *et al*., 2006). In the recent past, seaweeds have also been centre of interest by researchers as new experimental systems for biological research and integrated aquaculture systems (Miguel *et al.,* 2008). Seaweed products are used for example, in the manufacture of toothpastes, soaps, shampoos, cosmetics, milk products, ice creams, meat products, processed food, air fresheners and a host of other items (Miguel, 2008). In several oriental countries like Japan, China, Korea, etc., seaweeds are a staple food diet (Almela *et al*., 2006; NAAS, 2003).

Other typical examples of seaweed applications are presented in the following section.

Studies by Almela *et al*., (2006); FAO, (2002); NAAS, (2003**)** gives vivid descriptions of the uses of seaweeds. *Agar* is widely used in paper manufacturing, culture media, packaging material, photography, leather industry, plywood manufacturing, and preservation of foodstuffs, dairy industry, cosmetics industry and pharmaceutical industry (Almela *et al*., 2006).

Carrageenan is commonly used in food industry to manufacture sausages, corned beef, meat balls, ham, preparations of poultry and fish, chocolates, dessert gels, ice creams, juice concentrates, marmalade, sardine sauces. It is also used in the manufacture of non-food items like beer, air fresheners, textiles, toothpastes, hair shampoos, sanitary napkins, tissues, culture media, fungicides, etc. The applications of *Alginate* find place in frozen foods, pastry fillings, syrups, bakery icings, dry mixes, meringues, frozen desserts, instant puddings, cooked puddings, chiffons, pie and pastry fillings, dessert gels, fabricated foods, salad dressings, meat and flavour sauce **(**Almela *et al*., 2006; FAO, 2002; NAAS, 2003**).**

2.4.3 Nutritional content of edible seaweeds

Recent scientific studies have demonstrated that edible seaweeds have high contents of carbohydrates, proteins, vitamins and especially mineral salts (FAO, 2002; Almela *et al*., 2006; EUFIC, 2008). Compared with milk products, algae provides up to 10 percent more calcium and iron, in addition to other important minerals (Almela *et al*., 2006; EUFIC, 2008). On therapeutic aspects, seaweeds have antibacterial, antiviral, anticancer effects, and can reduce high cholesterol levels, prevent hypertension and atherosclerosis development and improve fat metabolism.

Seaweeds are rich in minerals and trace elements because they absorb minerals from the sea (Almela *et al.,* 2006). Calcium and iron tend to accumulate at much higher levels in seaweed than in terrestrial plants (Amado-Filho *et al*., 2008). For instance, an 8g portion of dried *Kombu* (seaweed) provides much more calcium than a cup of milk, and a portion of *dulse* contains more iron than a 100g sirloin steak although maximum absorption is not guaranteed (EUFIC, 2008). Seaweed also provides large quantities of iodine vital for thyroid function though excess iodine content has potential health risks. Additionally, seaweed can accumulate heavy metals like lead, cadmium and arsenic which are toxic at levels exceeding the daily recommended intakes (Almela *et al.*, 2006).

Besides minerals, seaweeds are rich in soluble fibres (*Alginates*, *Carrageenan* and *Agar*) which are not digested in the gut and can help increase feelings of satiety (Almela *et al.,* 2006; Noda, 1993). Seaweed *Alginates* and *Carrageenan*s are also used in processed foods (e.g., sausages, croissants) to give favourable texture and stability. Although seaweed fibre extracts may have some potential as slimming aids, seaweed itself is likely to have an effect on satiety (and weight control) similar to ordinary fruits and vegetables. An 8 g portion of dried seaweed provides (EUFIC, 2008) about an eighth of an adult's daily fibre needs, similar to the amount found in a bunch of banana.

Besides essential elements and fibre, seaweeds also contains little amounts of fat (Almela *et al.,* 2006), and some varieties are rich in protein. Many contain levels of essential amino acids similar to *dulses* and eggs. Vitamins A, C and E are found in seaweed in useful amounts, and it is also one of the few vegetable sources of Vitamin B12, making it a useful adjunct to a vegetarian diet (EUFIC, 2008).

2.4.4 Potential health benefits of edible seaweeds

Seaweed has been used extensively in Chinese medicine, but still largely unexplored as a therapeutic agent in the West. Preliminary research suggests that certain polysaccharides called fucoidans, typically found in brown seaweeds such as *Kombu* and *Wakame* may exert anti-cancer activity (EUFIC, 2008). Seaweed fibres have beneficial effects on the digestive system and lipid metabolism. They are also reported to exhibit anti-inflammatory and antioxidant activities, although no information is available on humans (Almela *et al*., 2006).

2.4.5 Biodiversity of seaweed distribution

Taxonomists have identified approximately 36,000 known seaweed species which represent 17 percent only of those currently in existence worldwide (Oza *et al.*, 2001). This indicates that the total number of species should exceed 200,000.

The distribution of these species across sub-Sahara Africa has been presented by renowned botanists (Bolton *et al*., 2003; Bolton *et al.,* 2007; John, 1997). These studies indicate that, knowledge of seaweeds diversity patterns is an imperative component in planning the conservation and sustainable use of inshore marine resources. East Africa has over 400 species (Silva *et al*., 1996); West Africa has over 200 species (John, 1997) while Southern Africa region has over 850 species (Bolton *&* Anderson, 1997). The latter is favoured because of its geographical dispensation and the climate.

Table 2.1 shows seaweed distribution in East Africa marine ecoregion that comprises the coasts of

Somalia, Kenya, Tanzania and Mozambique, and the extreme north of South Africa, as having a

predominantly tropical flora (Fig. 2.1).

Fig. 2.1: Map showing the coast lines of East Africa marine ecoregion (Shaded area).

2.4.6 Biodiversity of seaweeds in Kenya compared to other regions of Sub-Saharan Africa

According to the study by Bolton *et al*.,(2007), it is reported that southern coast of Kenya has, a total of 386 species which includes; 214 red algae, 116 green algae and 56 brown algae and an additional 19 infra-specific taxa giving a total of 405 species.

Table 2.1: Seaweed distribution in tropical East Africa, West Africa and South Africa

Ecoregion	Countries	Number of species	Information source
Tropical East Africa {Eastern Africa marine ecoregion}	Somalia Kenya Tanzania Mozambique	211 405 428 243	Silva *et al*., (1996) and Bolton *et al.,* (2007).
Tropical west Africa	From; Angola-Gambia Angola- Mauritania	 382 583	John, (1997)
Temperate South African	Namibia South Africa	196 850	(Bolton & Anderson 1997; Bolton *et al*.,2007)

Seaweed species in Somalia and Mozambique coasts are under estimated as they share the same ecosystem with the other countries in the region. In the tropical Indian Ocean, there is homogeneous sea surface temperature coupled with a continuous coastline, which ensure wide dispersal of marine macro-algae, resulting in a rather homogenous Indian Ocean marine benthic flora (Oza *et al*., 2001).

2.5 The Seaweed Industry - An Overview

Baseline surveys by FAO (2002) indicate that the use of seaweed as food can be traced back to the fourth and sixth century in Japan and China respectively. Today, the two countries and the Republic of Korea are the largest consumers of seaweed food and their requirements provide the basis for an industry that, worldwide harvests 6 million tonnes of wet seaweed per annum valued over US$ 6 billion (FAO, 2006). Due to an increasing demand over the last fifty years worldwide, scientific research into the life cycles of these algae has led to the development of cultivation industries that now produce more than 90 percent of the market's demand.

China is the leading producer of edible seaweeds in the world with about 5 million tonnes annually (mostly *Kombu*), is produced from hundreds of hectares of *Laminaria japonica* that is grown on suspended ropes in the ocean (FAO, 2006). The Republic of Korea grows about 800 000 tonnes annually of three different species and about 50 percent of this is for *Wakame*, produced from *Undaria pinnatifida* grown in a similarly to *Laminaria* in China (Krishnaiah *et al.,* 2008).

Japan produces nori from *Porphyra* species which make 75% of its annual seaweed production (600,000 tonnes). It is a high value product, about US$ 16 000 per tonne, compared to *Kombu* at US$ 2 800 per tonne and *Wakame* at US$ 6 900 per tonne (FAO, 2002). Norway has also pioneered the production of seaweed meal, made from dried and powdered brown seaweed, used as an additive to animal feed. Norwegian seaweed industry is approximated at US$ 5 million per year (FAO, 2002; FAO, 2006).

According to FAO (2002) survey, *Alginate* (introduced in 1930s), *Agar* (discovered in Japan, 1958) and *Carrageenan* (from red seaweeds) are thickening and gelling agents (hydrocolloids) extracted from seaweeds and the three form the main basis of the industrial uses of seaweeds.

Currently, approximately 1 million tonnes of wet seaweed are harvested and extracted yearly to produce the three hydrocolloids (extracts from brown, red and green seaweed species); 55 000 tonnes of hydrocolloids are produced with a total value of US$ 585 million per year (FAO, 2002; FAO, 2006). *Alginate* production (US$ 213 million/year) is by extraction from brown seaweeds, all of which are harvested from the wild since cultivation of brown seaweeds is too expensive to provide raw material for industrial uses (NAAS, 2003). *Agar* and *Carrageenan* production, principally from red seaweed is valued at US$ 132 million and US$ 240 million per year respectively. While the total value of the industrial products from seaweeds is estimated at US$ 590 million annually, the total value of all products from the seaweed industry is estimated at US$ 6 billion per year (FAO, 2002; FAO, 2006; FAO, 2012).

2.6 Kenya's seaweed cultivation

Kenya experiences severe economic challenges and food shortages. Perhaps, the surest way to overcome this labyrinth is to exploit the full potential of natural resource utilization.

Currently, the pilot project of *Euchemoid* seaweed cultivation at Kenya's South Coast is as a result of studies indicating the potential in seaweed for food security (Bolton *et al*., 2006; Mshigeni, 1998; Wakibia., 2007,). These studies have also shown that in East Africa region, communities around Zanzibar have benefited from the seaweed farming for over two decades now and Kenya's potential is immense.

Despite the diminutive biomass of seaweeds to sustain such an industry, cultivation and production of *Alginates* and *Carrageenan* precursors; *Eucheumoids* and *Gracilarioids* introduced following the high demand for *Carrageenan,* a few international bio-polymers companies are promoting the geographical spread of *Eucheumoids* to non-endemic regions such

as Indonesia, Tanzania, Madagascar, Fiji and Kiribati (Yarish *et al.,* 1990; Wakibia *et al*., 2007). This should encourage more investors to venture into seaweed farming in the country. Kenya is well endowed for supply of raw material for either the hydrocolloid industry or edible seaweeds (FAO, 2002) and cultivation can be done either by vegetative or sexual reproduction methods which are easily implemented by most coastal communities across the globe.

2.7 Heavy metal contamination in seaweeds

Despite of the implicit nature of studies done on heavy metal pollution in seaweeds at the Kenya coast, elsewhere, especially in Europe and Asia, there are reported concentrations of trace elements in seaweeds. For instance, a study in Spain by Ru´perez (2002) reported concentrations (μg g^{-1}) of Ca [41.5 − 101], Fe [0.3 − 1.1] and Zn [0.18 − 0.72]. Krishnaiah *et al*., (2008) from Malaysia reported Ca [41.1 − 100], Fe [0.7 − 1.1] and Zn [0.2 − 0.7] (μg g^{-1}). Two studies done in Southeast Coast of India by Karthikai *et al.,* (2009) reported Ca, Mn, Cu, and Zn as follows; Ca [534 - 3055], Fe [3.94 − 50.82], Mn [0.31 − 4.44], Cu [0.38 - 1.54] and Zn [0.65 − 4.28] (μg g^{-1}) and Anantharaman *et al.,*(2010) reported Mn [0.72 − 4.08],

Fe [17.53 - 62.47], Cu [0.87 - 1.70] and Zn [0.32 − 0.74] (μg g^{-1}). These studies indicate relatively low concentrations in seaweeds an aspect that is attributed to the varying degree of pollution experienced in the particular regions and to some extent the varying metal accumulation capacity of the seaweeds.

2.8 Heavy metals in sea sediments and seawater

A few studies have been done at the Kenyan Coast to evaluate heavy metal concentration in sediments. Nyatebe (1990) reported concentrations (in mg kg^{-1}) of Cu, Zn and Pb in Port Kilindini as Cu [12 - 52], Zn [34 - 186] and Pb [8 - 102]. Muohi *et al*., (2002) did a study at Makupa and

Port Reitz and reported the following corresponding concentrations (in mg kg^{-1}) Cu   [50.9 - 229], Zn [276 - 3193] and Pb [54.6 - 165] for Makupa and Cu [11.3 - 45.1], Zn [30.7 – 92.9] and Pb [15.7 - 71.2] for Port Reitz.

Elsewhere, Wendy (2005) reported heavy metals in sediments of Gulf of Paria, Trinidad and Tobago. The heavy metals reported included Cu [0.8 - 3.7] mg kg^{-1}, Zn [13.6 - 170.3] mg kg^{-1} and Pb [3.1-73.3] mg kg^{-1}

As discussed in the previous sections of this study, the sediments act as the primary reserves for the heavy metals in the ocean ecosystems. The degree of contamination varies with extent of anthropogenic activities in the surrounding environments such as mining and the industrial effluents. Concentrations of calcium tend to be associated with the bed rocks and most research have failed to give precise contamination of Ca (Muohi *et al,* 2002) as its concentrations exhibit a lot of inconsistencies, due to the parent bed rock.

CHAPTER 3

MATERIALS AND METHODS

3.0 Introduction

This chapter presents the materials and methods of study used in this research work. It also includes description of sample collection, preparation and analysis of samples for elemental content. It also discusses the instrumentation used in the study which included mainly the Total Reflection X- Ray Fluorescence (TXRF) and the Energy Dispersive X- ray Fluorescence (EDXRF).

3.1 Description of the study area

According to the Kenya Ministry of Fisheries Development website description of the Kenyan coast, coastline is some 640 km, lies, within the tropical zone extending from Kiunga at 1.5° South (S) of the equator to Vanga at 4.5°S *(http://www.fisheries.go.ke)*. Its most distinctive feature is the continuous fringing coral reef that runs parallel to the coast. The coral reefs occupy the shallow inshore zone extending offshore to about 45 meters (m) depth, and at a distance of 500 m to 2 km from the shore, except where river systems enter the sea creating conditions of low salinity and high turbidity which limit coral growth.

Tana River, the country's longest river discharges its waters into Ungwana Bay, Sabaki River that incorporates Athi and Galana Rivers discharges into the sea just north of Malindi.

Coastal features include Lamu Archipelago with its extensive mangrove forests. Mombasa Island at the entrance to a very extensive bay including Mombasa harbour, Kilindini harbour, Port Tudor and Port Reitz, and the southern complex of Gazi Bay, Chale Island, Funzi Bay, Funzi Island, Wasini Island, and some smaller coral islands.
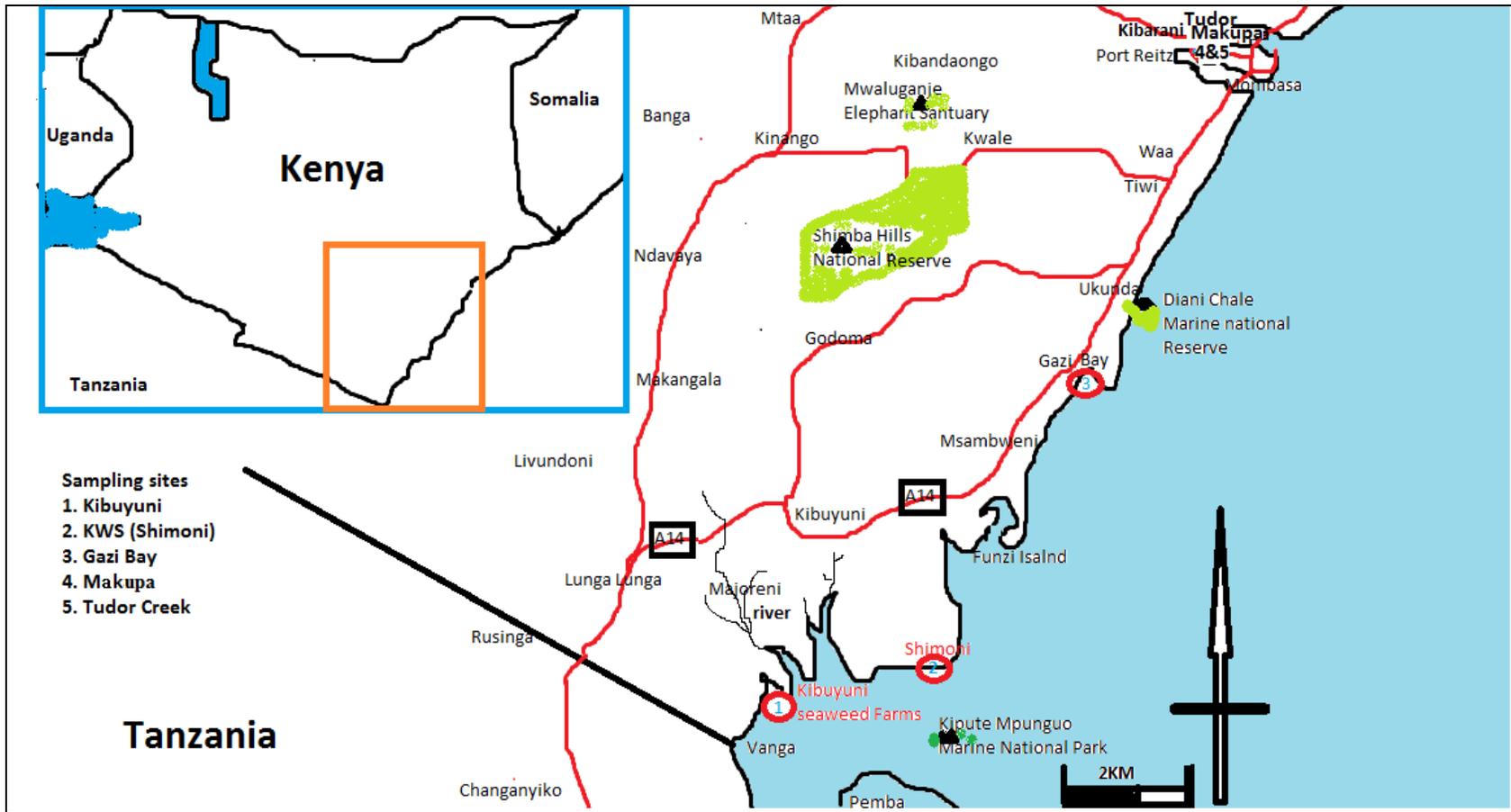
Fig.3.1: (a) Map showing outline of Kenya, Kenyan coast (cropped area) and the sampling sites (Courtesy of Google map).
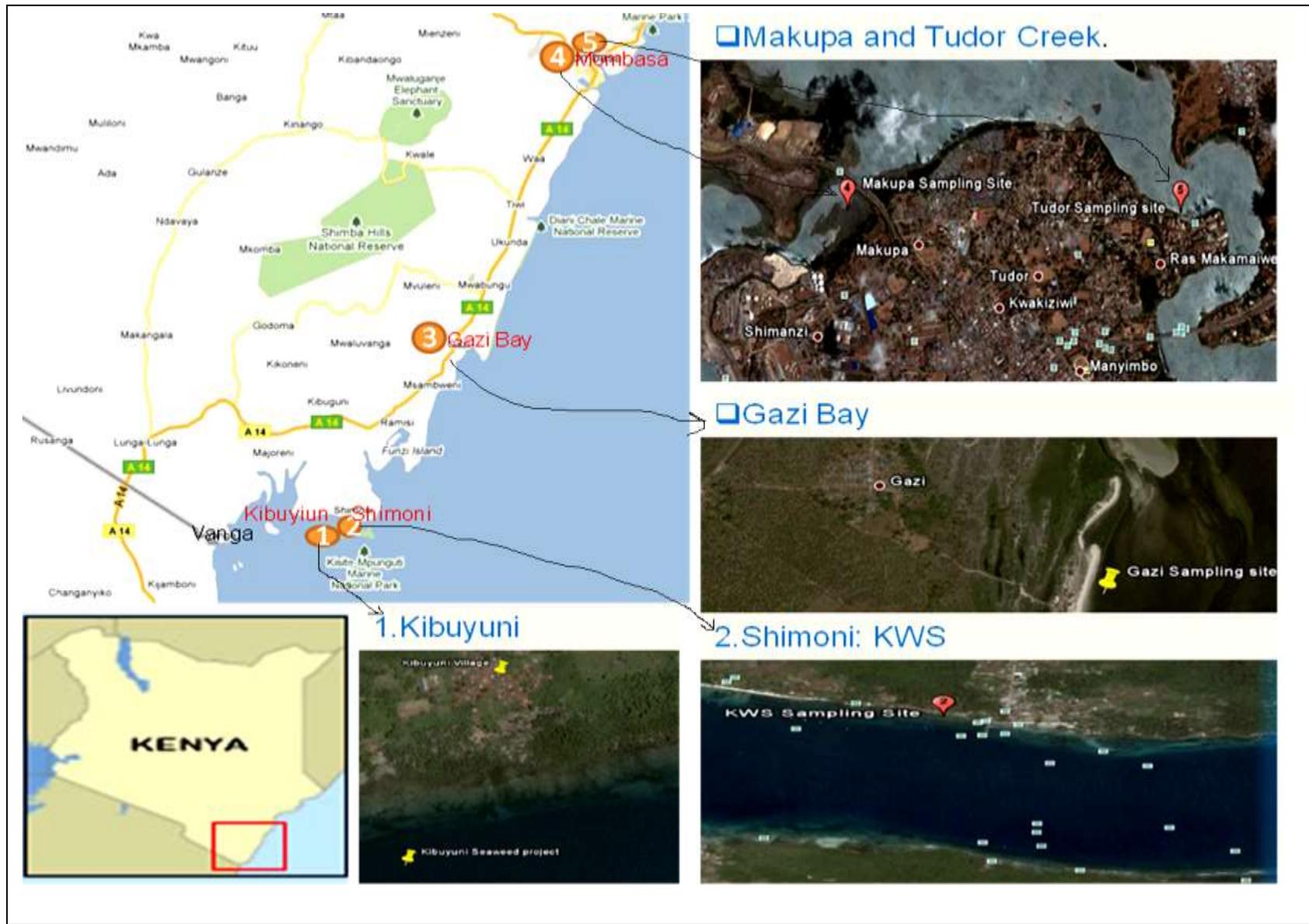
Fig. 3.1 (b) Map showing the sampling sites (Google maps)

3.2 Location of the sampling sites

 In this study, the samples were collected from the following sites; Kibuyuni, KWS, Gazi Bay, Tudor Creek and Makupa (Figure 3.1). These sites were categorized as shown in the following;

a)   Mombasa sites (Makupa and Tudor Creeks)

Mombasa district has a coastline of about 32 km and covers approximately an area of 275 km^2, consists of; the island (13 km^2), mainland (197 km^2) and the Tudor, Kilindini/Port Reitz and Makupa creeks (65 km^2) (Figure 3.1).

The study areas around Mombasa City were Makupa and Tudor creeks which lies on 4° 2'7.75"S, 39°39'3.89"E) and 4° 2'8.30"S, 39°40'21.01"E respectively.

Makupa creek forms part of a larger creek system in which the port of Mombasa (Kilindini) is located. Tudor creek is on the eastern side of Mombasa Island and is separated from Makupa creek by a narrow strip of landfill, the Makupa causeway, which prevents mixing of the waters (Muohi *et al.,* 2002; Mwashote, 2003).

The Port- Reitz- Kilindini system supports a wide spectrum of associated manufacturing industries including oil refineries, manufacturing and shipping activities.

The shores of Makupa creek, harbors Kibarani that is a dumpsite of domestic wastes which contribute significantly to marine pollution loading (Muohi *et al.,* 2002).

b)   Kenya south coast (Gazi Bay, Shimoni and Kibuyuni)

Gazi bay lies on 4°25'43.54"S, 39°30'44.12"E, located between Ukunda and Shimoni and has limited human activities though fed by a small tributary from the mainland. Shimoni site specifically KWS lies on 4°38'50.02"S, 39°22'40.43"E at the south coast overlooking Wasini Island. Shimoni town is a business hub with a lot of human activities including tourism at the Shimoni slave caves and the wild life sanctuary at KWS. Kibuyuni which lies on 4°38'33.92"S,

39°20'22.71"E is an isolated place located South coast, a few miles from Pemba. A few kilometers from Kibuyuni are some rivers at Mojerini which discharges into the Indian Ocean.

In general, these sites have coral reefs and carbonaceous base rock rich in calcium deposits that release calcium under hydrothermal regime.

3.3 Sampling procedure

Samples were randomly sampled twice for the seaweed samples; from 22nd to 25th of October, 2009, mainly for the various seaweed species (red/brown in colour) and from 26th to 29th of June, 2010, for the commercial *euchemoid* seaweed species *(*originally from the Philippines*)* comprising of green and brown *Kappaphycus alvarezii* from Kibuyuni as they were being introduced in pilot sea farms in Kibuyuni village. Samples from Kibuyuni and Kenya Wildlife Service station collected in 2009 included various seaweed species comprised of red/brown and the green seaweeds. Botanical identification and naming of the seaweeds was done at the Kenya Marine and Fisheries Research Institute in Mombasa (Table 3.1).

Sediments and water samples were also sampled in June, 2010 from near shores of each of the five sites selected for this study (Makupa, Tudor, Gazi Bay, KWS and Kibuyuni) by scooping sediments while water samples were contained in plastic bottles that were thoroughly cleaned and rinsed with double distilled water.

A total of 40 seaweed samples (20 samples collected in October, 2009 and 20 samples collected in June, 2010 were put in polythene bags and kept in ice-cold box, 20 water samples put in plastic bottles and 20 sediment samples put in polythene bags before transporting them to the laboratory for analysis in this study. All samples were labeled for identification and ease of handling (Table 3.1).

Table 3.1: Sample codes and their corresponding species names/identity.

Seaweeds collected in October, 2009		Seaweeds collected in June, 2010 (B = Brown, G = Green		Totals
Site, Sample Code	**Species name**	**Site** *Euchemoids* **Seaweeds**	**Site Sediments water**	
KWS KS1A	*Padina pavonica*	Kibuyuni: Farm 1 1B	Gazi Bay GS1 GW1	
" KS1B	*Padina pavonica*	" 1G	GS2 GW2	
" KS2	*Chondrophycus papillosus*	" Farm 2 2B	GS3 GW3	
" KS3	*Acanthophora dendroides*	" 2G	GS4 GW4	
" KS4	*Cystoseira trinodis*	" Farm 3 3B	Makupa MS1 MW1	
" KS5	*Gracilaria corticata*	" 3G	MS2 MW2	
" KS6A	*Gracilaria salicornia*	" Farm 4 4B	MS3 MW3	
" KS6B	*Gracilaria salicornia*	" 4G	MS4 MW4	
Kibuyuni " K1A	*Hypnea cornuta*	" Farm 5 5B	Tudor TS1 TW1	
"K1B	*Hypnea cornuta*	" 5G	TS2 TW2	
" K2A	*Chondrophycus papillosus*	" Farm 6 6B	TS3 TW3	
" K2B	*Chondrophycus papillosus*	" 6G	TS4 TW4	
" K3	*Gracilaria corticata*	" Farm 7 7B	KWS KWS1 KW1	
" K4A	*Gracilaria canaliculata*	" 7G	KWS2 KW2	
" K4B	*Gracilaria canaliculata*	" Farm 8 8B	KWS3 KW3	
" K5	*Solieria robusta*	" 8G	KWS4 KW4	
" K6A	*Acanthophora spicifera*	" Farm 9 9B	Kibuyuni KIBS1 KIBw1	
" K6B	*Acanthophora spicifera*	" 9G	KIBS2 KIBw2	
" K7A	*Kappaphycus alvarezii*	" Farm 10 10B	KIBS3 KIBw3	
" K7B	*Euchema dendiculatum*	" 10G	KIBS4 KIBw4	
Totals	**20**	**20**	**20** **20**	**80**

3.4 Sample Preparation

Cleaning: All glass ware and plastic ware used in this study were soaked in a soap solution overnight in a plastic bucket. The apparatus were then first rinsed with tap water followed by de-ionized water. They were put and left to stand for 24hrs in 10% (v/v) concentrated HNO_3 solution at room temperature then rinsed thoroughly with double distilled (deionized) water and allowed to dry in open air. The dried apparatus were put in closed plastic polythene bags and stored.

Reagents (for digestion): All chemicals used in sample preparation were of analar grade. Concentrated HCl, H_2O_2, and HNO_3; NaCl, KCl, $CaCl_2.2H_2O$, $MgCl_2$; and Food enzymes (pepsin).

3.4.1 Preparation of seaweed samples for TXRF and EDXRF Analysis

a) Sample preparation for total elemental content in seaweeds

Approximately 1g of each of sample powder was weighed, placed in digestion tubes and 20ml conc.HNO_3 then added and heated for 30-40 minutes at 100^oC followed by cooling the digestion mixture to room temperature. For EDXRF analysis, 10 ml of perchloric acid ($HOClO_3$) was carefully added to the cooled mixture and heated further to a colourless solution then left to cool to room temperature. Further pre-concentration was done and then filters prepared by using a suction pump. The filters were air dried at room temperature and kept in labeled sample holders prior to Tube Excited EDXRF analysis.

For TXRF analysis, the addition of $HOClO_3$ (cooled mixture) was avoided as it makes drying of sample spots on carriers difficulty. In every 1ml of the sample's supernant (cooled mixture), an aliquot of $1\mu gg^{-1}$ of each of the internal standards (yttrium and gallium) was added and stirred to ensure homogeneity. 10 µl of the resulting mixture was then pipetted using Eppendorf pipette

and spiked on a clean sample carrier (reflector). It was then dried in an oven at 25°C and reflectors kept in specimen holders prior to analysis.

Sample preparation for bio-available content in seaweeds

Samples for trace elements bioavailability in edible seaweeds were prepared according to the procedure described by Svanberg *et al*., (1993), and as modified by Matuscheck *et al.,* (2001).

A sample aliquot was prepared by suspending 0.5g of sample powder in 5 ml of distilled water and 5 ml of 0.3% pepsin solution (in 0.1m of HCl). To the mixture a 1ml of physiological amounts of Na (49 mMol/l as NaCl), K (12 mMol/l as KCl), Ca (10 mMol/l as $CaCl_2.2H_2O$), Mg (2.4 mMol/l as $MgCl_2$) and Phosphate (3.5 mMol/l as KH_2PO_4) was added. The mixture was then homogenized by stirring before digestion in an incubator for 90 minutes at 37°C.

The pH was adjusted to 2 by adding between 3 - 5 drops of 1.0 M NaOH followed by 1 ml of a combination of pancreatic acid (0.012 g) and bile (0.075 g) in 0.1M $NaHCO_3$ and then the pH to adjusted 5. The mixture was then incubated at 37°C for another 30 minutes before adjusting pH to 6 followed by centrifugation at 66,800 rev/ min for 20 minutes. The supernant solution obtained after centrifuging was filtered through 0.45 µm filters.

In 1ml of the sample's supernant, an aliquot of 1µl g^{-1} of each of the internal standards (yttrium and gallium) was added and stirred to ensure homogeneity. 10 µl of the mixture was then pipetted using Eppendorf pipette and spiked on a clean sample carrier (reflector). It was then dried in an oven at 25°C prior to TXRF analysis.

Powered seaweeds samples for EXDRF analysis were prepared using the procedure as described for the sea sediment samples in the subsequent section (3.4.3)

3.4.3 Sea sediment samples

Samples were air dried and further oven dried at 105°C overnight (24hrs) to a constant weight, then crushed, ground and sieved through a <63 µm sieve. Pellets were prepared by weighing 1g

of each sample powder then pressing the material in 2.5 cm of diameter discs for analysis, using EDXRF (Kinyua, 1982).

3.4.4 Seawater samples

In 1ml of the sea water sample, an aliquot of 1µl g^{-1} of each of the internal standards (yttrium and gallium) was added and stirred to ensure homogeneity. 10 µl of the mixture was then pipetted using Eppendorf pipette and spiked on a clean sample carrier (reflector). It was then dried in an oven at 25°C prior to TXRF analysis.

3.5 Sample analysis

(a) TXRF analysis

The TXRF (Figure 3.2) method used for elemental determination was validated for use by running sample equivalence amount of multi-element standard. Sample carrier (Reflector) was then placed on sample holder in TXRF machine and run for 500s at optimum operating conditions as described in section 3.6 in this report. The resulting spectral were measured and calculated to obtain concentrations of the respective elements (Equation 1).

(b) EDXRF analysis

Calibration of EDXRF (Figure 3.3) instrument was done with a standard sample (IAEA soil 7 for Sediment samples, and Bowens Kale for the seaweeds) prepared using procedure used in sample preparation. Pellets of samples were mounted on sample holder in the EDXRF instrument then irradiated for 1000s and the resulting spectral characteristic of energy intensities of each element were observed and recorded. The spectrum was measured simultaneously and the area of a peak profile calculated to determine the concentration of the element using AXIL software. Optimum operating conditions and instrumentation for EDXRF machine are described in section 3.7 of this report.

3.6 Total Reflection X- ray Fluorescence spectroscopy

The X-ray beam, generated by the Molybdenum tube, is reflected on a multilayer monochromatic resulting in a monochromatic X-ray beam. This small beam impinges on the sample holder at a very small angle ($< 0.1°$) causing total reflection of the beam. The characteristic fluorescence radiation emitted by the sample is detected by an energy-dispersive detector and the intensity is measured by means of an amplifier coupled to a multichannel analyzer.

The difference with respect to common XRF spectrometers is the use of monochromatic radiation and the total reflection optics. Illuminating the sample with a totally reflected beam reduces the absorption as well as the scattering of the beam in the sample matrix resulting in reduced background noise, and consequently much higher sensitivities and a significant reduction of matrix effects.

Another major advantage of TXRF, compared to atomic spectroscopy methods like Atomic Absorption Spectrophotometry (AAS) or Iinduced Coupled Plasma-Optical Emission Spectrometry (ICP –OES), is the avoidance of memory effects. The technical parameters of the S2 PICOFOX spectrometer used are summarized in Table 3.2.

Table 3.2: Typical features of TXRF S2 PICOFOX

X-ray tube	Air-cooled metal ceramic Mo or W anode, max. power 50 W
Optics	Multilayer monochromator (17.5 keV)
Detector	Si drift detector XFlashR Area: 10 mm^2, FWHM: < 160 eV (Mn Ka))
Size (D x W x H)	450 x 590 x 300 mm
Weight	37 kg

The complete process of analysis and quantification is described by the following steps:

- Measurements of the complete spectrum - All detectable elements are measured simultaneously.

Evaluation of the measurement spectra - All identified elements are marked for quantification, which can be done manually or automatically by the software.

- Spectra deconvolution - On the basis of the chosen elements, the software performs the deconvolution of the spectra. The net intensities of the element peaks are calculated with regard to corrections for line overlaps, background factors, escape peak correction etc.

- Calculation of concentrations - The element concentration is calculated by the simple formula:

$$C_x = \frac{N_x/S_x}{N_{is}/S} \, x \, C_{is} \text{ ---1}$$

Where N is the net intensity, S is the relative sensitivity and C_x is the concentration of the analyte x and C_{is} is the concentration internal standard (is).
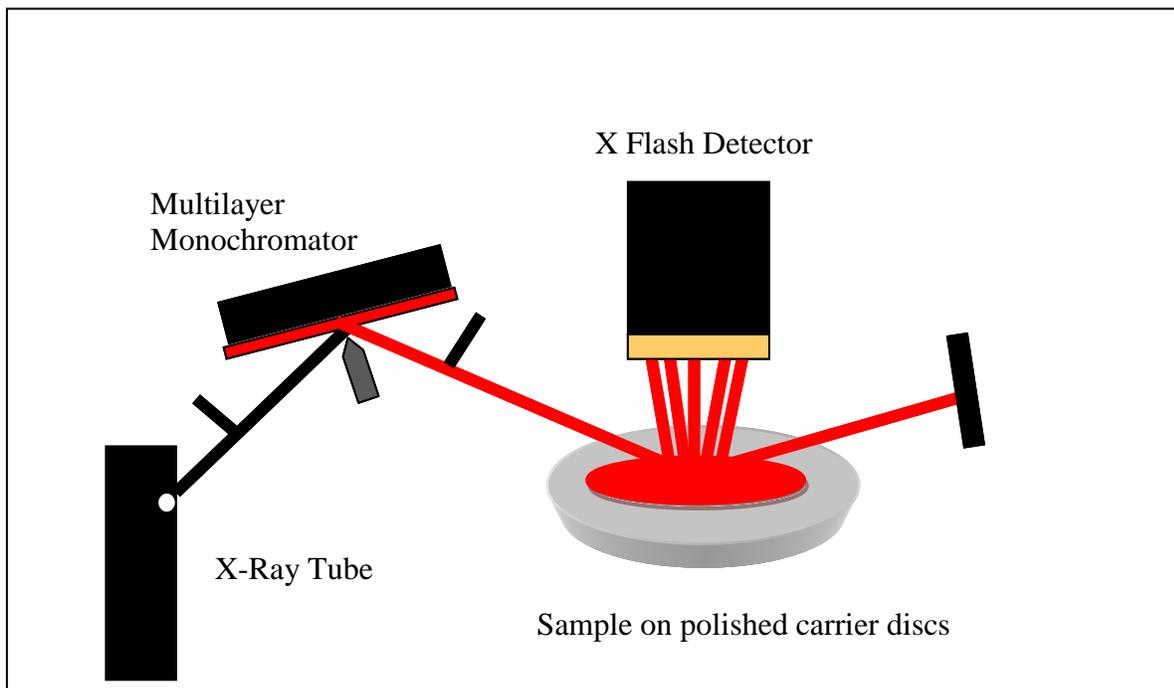
Figure 3.2: Schematic working principle of S2 PICOFOX TXRF spectrometer

3.7 Energy Dispersive X- ray Fluorescence spectrometry

In this study, the EDXRF instrumentation used for elemental analyses consisted of Oxford ED200EDXRF machine. It comprises of isotopic ^{109}Cd x-ray source, sample holder, Si (Li) detector and output system (computer). The prepared pellets were positioned in front of the Si (Li) detector and irradiated with X-rays for 1000s. The resulting photons were detected by a Canberra Si (Li) detector with a resolution of 190 eV at 5.9 KeV was used for X-ray detection, connected to Canberra NIM electronics, and 8keV multi-channel analyzer. The system was also coupled to IBM-PSI computer for data storage and analysis. Spectra data obtained were evaluated by the Analysis of X-ray spectra by Iterative Least-square (AXIL) software program.
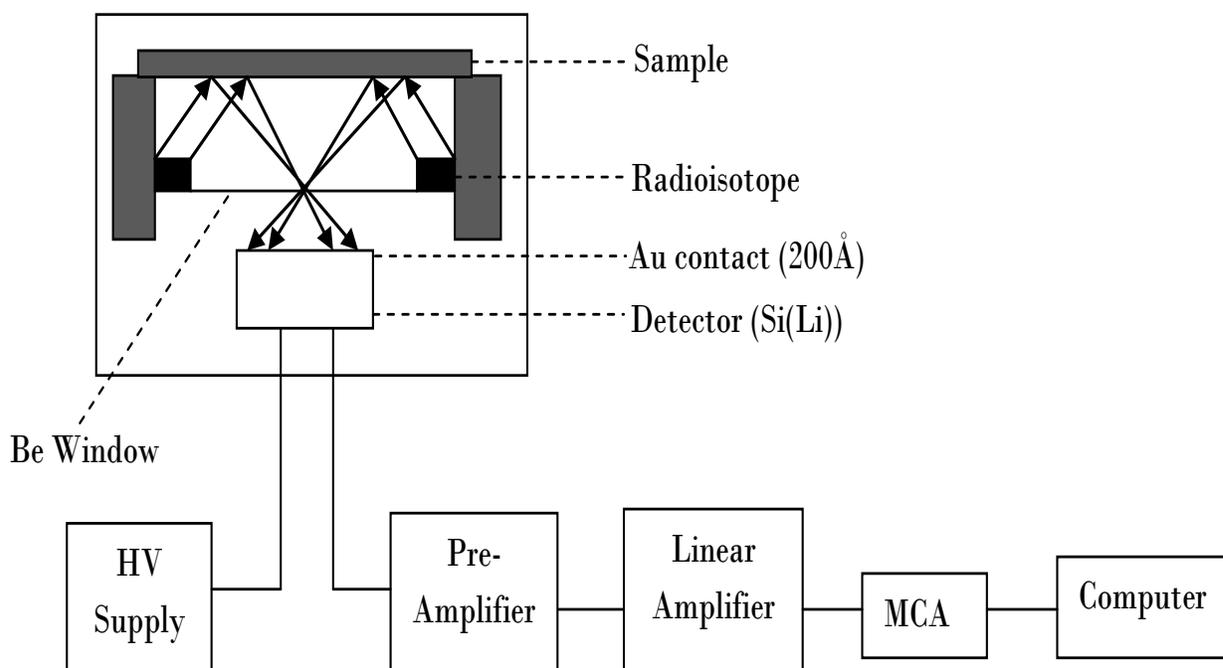
Fig.3.3 Arrangement in Energy- Dispersive X – ray Fluorescence spectrometer

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction.

Trace elements are required for optimum health of human beings and are obtained from our diets. Edible seaweeds are part of our diets and in particular in the Coastal region of Kenya. Unfortunately, they may be contaminated by heavy metals which may be present in sea sediments and seawater. In this study, edible seaweeds were analysed for their total and bioavailable trace element content. In addition, sediments and seawater were analysed for their heavy metal content to determine whether they could be sources of contamination. The results and their significance are reported in this chapter.

4.2 Detection limits

Detection limits of elements of interest in this study were determined for EXDRF analyses using equation 1 in this chapter.

where;

$$Detection\ limit = 3C\ x \frac{\sqrt{N_B}}{N_P} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 1$$

C = concentration of the element of interest in appropriate measurement units.

N_B = Background area under the element

N_p = Peak area of the element.

Table 4.1 shows the results of detection limits for elements of interest in this study analyzed using the TXRF and EDXRF methods.

Table 4.1: Detection limits for elements of interest

| Element | TXRF method of analysis | | EDXRF method of analysis |
	Seaweeds (µg g^{-1})	water (µg ml^{-1})	Sea sediments (µg g^{-1})
Ca	9.00	9.00	962
Cr	0.01	0.01	200
Mn	0.02	0.02	99
Fe	0.10	0.10	62
Co	0.01	0.01	42
Cu	0.01	0.01	13
Zn	0.04	0.04	20
As	0.01	0.01	12
Pb	0.06	0.06	20

4.3 Quality Assurance - Certified Reference Materials (CRMs)

To evaluate the accuracy of the analytical techniques, CRMs from International Atomic Energy Agency (IAEA), Soil-7 and Bowens Kale, were analyzed using the same procedure used for sample analysis and the results obtained are presented in table 4.2 and 4.3 respectively.

Table 4.2: Results for Bowens Kale analyses by EDXRF ($\mu g\ g^{-1}$)

Element	Experimental Value (± 1SD)	Certified Value	Confidence Interval
Ca	41932 ± 90	41060	38840 – 43280
Cr	<200	0.4	0.27 – 0.47
Mn	<99	14.8	13.1 – 16.5
Fe	120 ± 1	119	105 – 134
Co	<42	0.06	0.05 – 0.07
Cu	<13	4.9	4.3 – 5.5
Zn	31.7 ± 0.4	32.3	29.5 – 35.0
As	< 12	0.1	0.1 – 0.2
Pb	< 20	2.5	1.9 – 3.1

Table 4.3: Results for IAEA Soil -7 analyses by EDXRF ($\mu g\ g^{-1}$)**.**

Element	Experimental Value ± 1SD)	Certified Value	Confidence Interval
Ca	16300 ± 1100	16300	15700 – 7400
Cr	< 200	60	49 – 74
Mn	608 ± 104	631	604 – 650
Fe	2600 ± 200	2570	2520 – 2630
Co	< 42	8.9	8.4 – 10.1
Cu	< 13	11	9 – 13
Zn	120 ± 17	104	101 – 113
As	< 12	13.4	12.5 – 14.2
Pb	< 20	60	55 – 71

Tables 4.2 and 4.3 shows the results of analysis of certified reference materials used in this study. The experimental values were not significantly different from certified values since they were relatively within the confidence intervals. Therefore, the analytical technique was validated.

4.4 Results of elemental analyses of seaweeds, sea sediments and seawater.

The result of concentrations values for calcium, manganese, iron, copper and zinc in seaweeds, sea sediment and sea water are presented and discussed in the subsequent sections with results tabulated in tables 4.4 to 4.20. Assorted pictures relating to this study are presented in figure 5.1 in the appendices.

4.4.1 Trace elements in seaweeds

In this section, the results for total and bio-available concentrations levels of calcium, manganese, iron, copper and zinc in various seaweed samples from KWS & Kibuyuni and the *Euchemoid* seaweeds are presented in the tables 4.4 to 4.18 respectively. The mean concentration levels of these elements are presented in figures 4.1 to 4.5 respectively.

a)   Calcium in seaweeds

Results of calcium concentration levels in various seaweeds samples collected in October, 2009 during the dry season from KWS ranged from 285 ± 14 µg g^{-1} to 632 ± 32 µg g^{-1} for total content and the bioavailability values varied from 123 ± 6 µg g^{-1} to 213 ± 11 µg g^{-1} (Table 4.4). Calcium concentrations for the various seaweeds from Kibuyuni; also sampled in the dry season varied from 228 ± 11µg g^{-1} to 955 ± 48 µg g^{-1}for total content and for the bioavailability fraction, it varied from 38.1 ± 2µg g^{-1} to 253 ± 13µg g^{1} (Table 4.5). Higher values in some samples can be attributed to presence of calcium in the carbonaceous rocks at the sites.

Total calcium concentrations in *Euchemoids* seaweeds sampled in June, 2010 during the wet season varied from 475 ± 23 µg g^{-1}to 1193 ± 60 µgg^{-1}as the bio-available content varied from a minimum of 153 ± 8 µg g^{-1}to a maximum of 379 ± 19 µg g^{-1}(Table 4.6). The results showed that calcium concentrations were highest in brown *Euchemoids* species and lowest in the green

ones with bio-available fraction decreasing with an increase in total content as illustrated in correlation plots in the subsequent section. This can be attributed to the cell structure and difference in the color pigment and the mineral balance by the seaweeds (Almela *et al.,* 2006).

Inferring from the mean concentrations of calcium in seaweeds from KWS and Kibuyuni as shown in figure 4.1, the results of measurements obtained for samples collected during dry season were relatively lower indicating that there is elemental enrichment in rainy seasons due to surface run off coupled with hydrodynamic ocean regime and other anthropogenic activities (Weigner 2000). With reference to other published data; Ru´perez, (2002) and Krishnaiah *et al.,* (2008) reported calcium values in the range of 41.5 μg g^{-1} to

101 μg g^{-1} and 41.1μg g^{-1} to 100 μg g^{-1} in Spain and Malaysia respectively (Appendices table 5.4). The reported calcium levels in the studies were below the nutritional threshold levels, while a study in Gulf of Mannar in Southeast Coast of India by Karthikai *et al.,* (2009) reported values in the range of 534 μg g^{-1} to 3055 μg g^{-1} which were higher than the nutritional threshold. The recommended calcium intake is in the maximum range of 1300 μg day^{-1} for adults and 270 μg day^{-1} for infants according to US Food and Nutrition Board (FNB, 2004) dietary references (Appendices table 5.7). The results obtained in the present study (range 217 μg g^{-1} to 253 μg g^{-1}) were within the recommended levels hence the consumption of edible seaweeds is an important source of calcium intake.

The increased level of calcium in the seaweeds is associated with the presence of carbonaceous base-rocks and also enrichments from surface run-offs as reported in the related studies elsewhere. Findings from this study show the same trend as observed elsewhere for calcium accumulation in seaweeds hence seaweeds can form a good biological indicator of marine pollution.

Table 4.4: Total and bio-available calcium concentrations in various seaweeds from KWS.

Sample	Species name	Total concentration	Bio-available concentration	
		($\mu g\ g^{-1} \pm 1SD$)	($\mu g\ g^{-1} \pm 1SD$)	% of total content
KS1A	*Padina pavonica*	312 ± 16	192 ± 10	61.6
KS1B	*Padina pavonica*	378 ± 19	184 ± 9	48.7
KS2	*Chonrophycus papillosus*	297 ± 15	123 ± 6	41.4
KS3	*Acanthophora dendroides*	632 ± 32	191 ± 10	30.2
KS4	*Cystoseira trinodis*	550 ± 28	213 ± 11	38.7
KS5	*Gracilaria corticata*	285 ± 14	164 ± 8	57.5
KS6A	*Gracilaria salicornia*	540 ± 27	201 ± 10	37.3
KS6B	*Gracilaria salicornia*	617 ± 31	125 ± 6	20.3

Table 4.5: Total and bio-available of calcium in various seaweed species from Kibuyuni.

Sample	Species name	Total concentration	Bio-available concentration	
		($\mu g\ g^{-1} \pm 1SD$)	($\mu g\ g^{-1} \pm 1SD$)	% of total content
K1A	*Hypnea cornuta*	869 ± 44	176 ± 9	20.2
K1B	*Hypnea cornuta*	955 ± 48	161 ± 8	16.9
K2	*Chonrophycus papillosus*	228 ± 11	116 ± 6	50.8
K2B	*Chonrophycus papillosus*	246 ± 12	38.1 ± 2	15.5
K3A	*Gracilaria corticata*	415 ± 21	158 ± 8	38.1
K4A	*Gracilaria canaliculata*	326 ± 16	111 ± 5	34.0
K4B	*Gracilaria canaliculata*	652 ± 33	169 ± 8	25.9
K5	*Solieria robusta*	842 ± 42	108 ± 5	12.8
K6A	*Acanthophora spicifera*	540 ± 27	176 ± 9	32.7
K6B	*Acanthophora spicifera*	955 ± 48	152 ± 7	16.0
K7A	*Kappaphycus alvarezii*	575 ± 29	253 ± 13	43.9
K7B	*Euchema dendiculatum*	869 ± 44	240 ± 12	27.6

Table 4.6: Total content and bio-available calcium in *Euchemoids* seaweeds from Kibuyuni (Samples collected in 2010).

Sample	Total concentration	Bio-available concentration	
	$(\mu g\ g^{-1} \pm 1SD)$	$(\mu g\ g^{-1} \pm 1SD)$	% of total content
1B	636 ± 32	270 ± 14	42.5
2B	708 ± 35	177 ± 9	25.0
3B	593 ± 30	234 ± 12	39.5
4B	521 ± 26	295 ± 15	56.6
5B	551 ± 27	276 ± 14	50.0
6B	602 ± 30	228 ± 11	37.8
7B	1131 ± 57	235 ± 12	20.8
8B	1172 ± 59	379 ± 19	32.4
9B	1193 ± 60	345 ± 17	29.0
10B	1013 ± 50	232 ± 11	22.9
1G	634 ± 32	284 ± 14	44.8
2G	634 ± 32	153 ± 8	24.1
3G	564 ± 28	224 ± 11	39.7
4G	491 ± 24	274 ± 14	55.8
5G	475 ± 23	245 ± 12	51.7
6G	503 ± 25	200 ± 10	39.8
7G	953 ± 48	181 ± 9	19.0
8G	853 ± 43	341 ± 17	40.0
9G	1009 ± 50	239 ± 12	23.7
10G	976 ± 49	237 ± 12	24.3

Correlations for calcium total concentration versus bioavailable fraction in various seaweeds from KWS, Kibuyuni and *Euchemoids* from Kibuyuni

Plotting the values for total content versus bioavailable fraction for calcium concentration in seaweed, (Tables 4.4, 4.5 and 4.6) the correlation coefficients obtained were nearer zero implying a weaker correlation of bioavailable concentration with the increase in calcium total content in samples from both KWS and Kibuyuni.
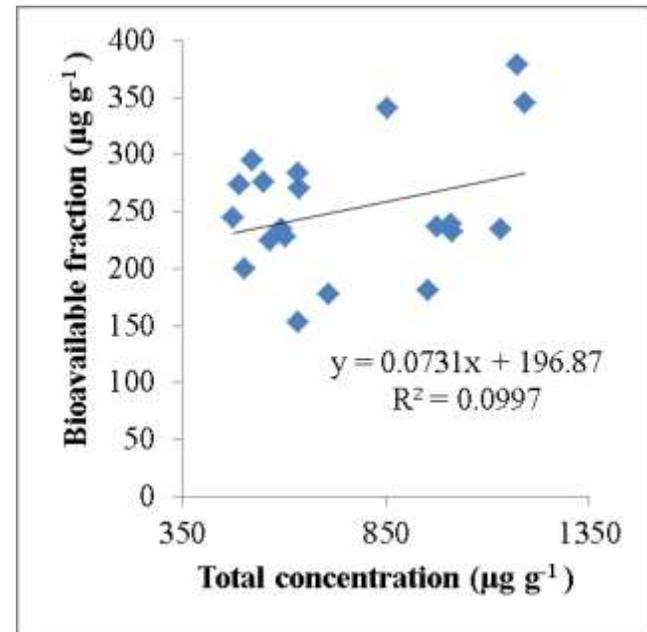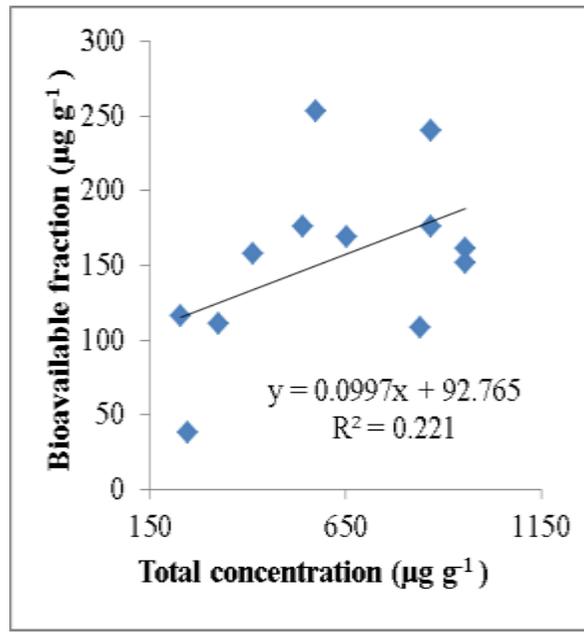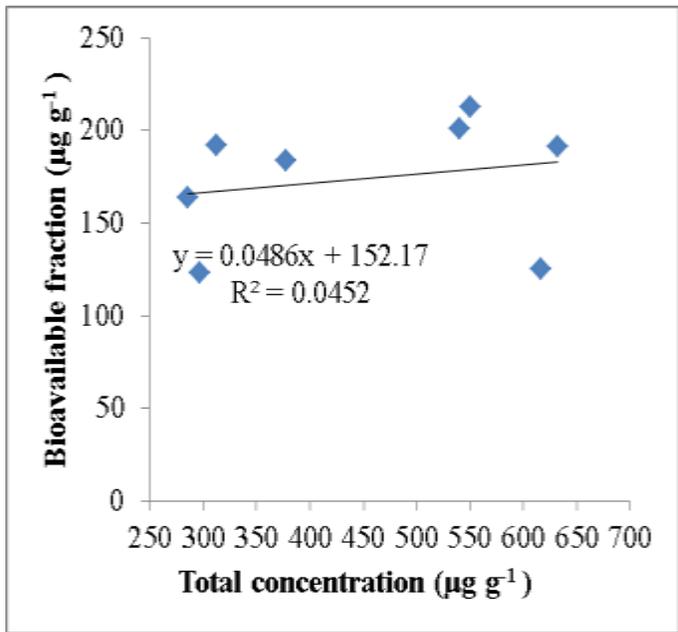
(i) Ca content correlations in KWS samples, (ii) Ca content correlations in Kibuyuni samples, (iii) Ca content correlations in *Euchemoids*
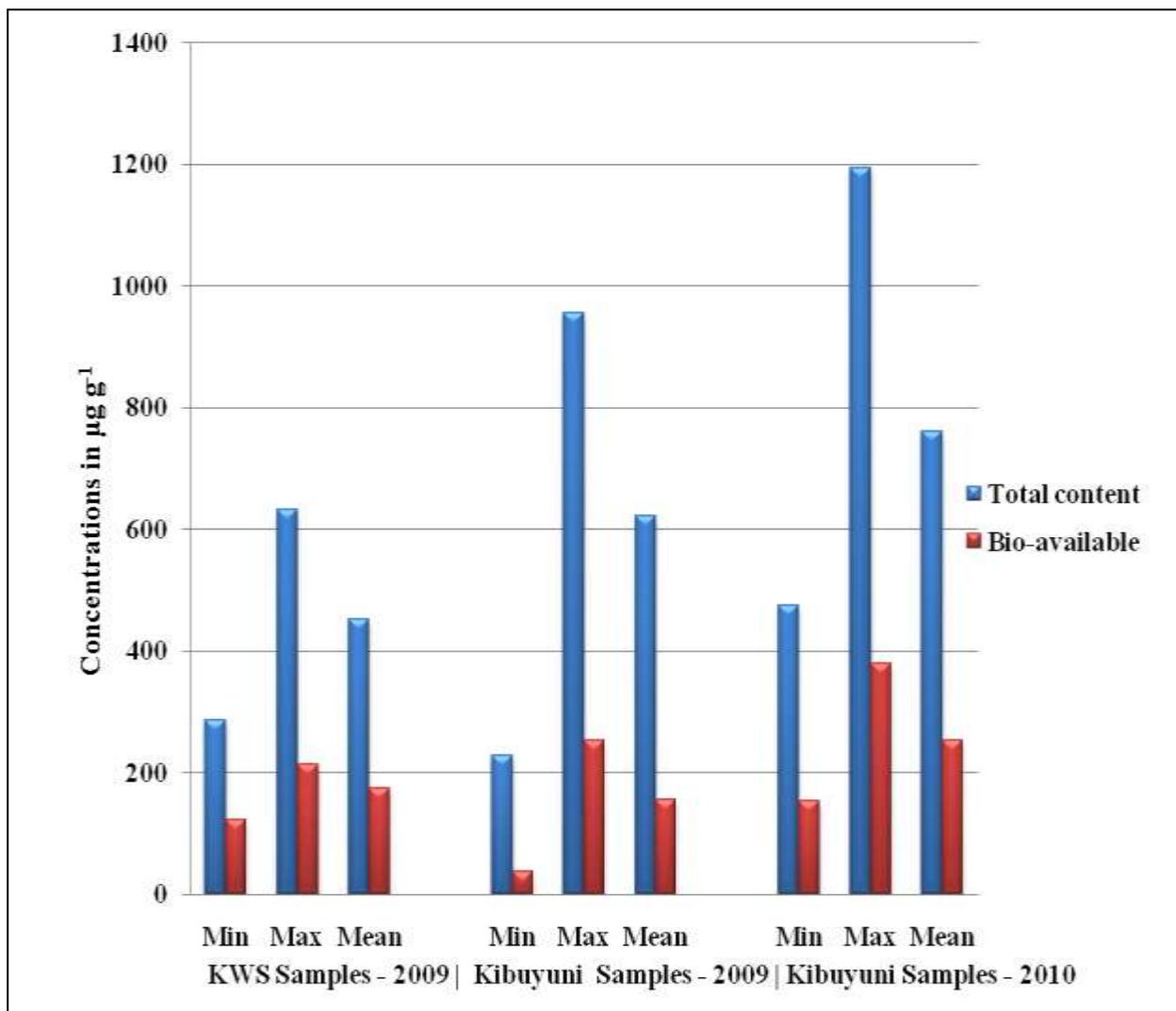
Fig. 4.1: Comparison for calcium concentrations (µg g^{-1}) in seaweeds from KWS & Kibuyuni.

b) **Manganese in seaweeds.**

Total manganese concentration levels obtained in various seaweed species from KWS varied from 1.7 ± 0.1µg g^{-1} to 8.7 ± 0.4 µg g^{-1} (Table 4.7). The corresponding bioavailability content ranged from 0.4 ± 0.01 µg g^{-1} to 0.9 ± 0.01 µg g^{-1} with higher bio-available content reported in lower values of total content. Various seaweed species from Kibuyuni had manganese

concentration levels that varied from $1.1 \pm 0.1 \mu g\ g^{-1}$ to $7.4 \pm 0.4\ \mu g\ g^{-1}$ whereas the corresponding bioavailability fraction ranged from $0.1 \mu g\ g^{-1}$ to $1.7 \pm 0.1 \mu g\ g^{-1}$.

Total content manganese concentrations values in *Euchemoids* seaweeds samples from Kibuyuni varied from $3.6 \pm 0.2\ \mu g\ g^{-1}$ to $9.7 \pm 0.4 \mu g\ g^{-1}$ while the bio-available fraction ranged from $0.8 \pm 0.01 \mu g g^{-1}$ to $3.7 \pm 0.2\ \mu g\ g^{-1}$ in the samples collected in June, 2010. The overall range of manganese concentration varied from $1.0\ \mu g\ g^{-1}$ to $10.1 \mu g\ g^{-1}$ in this study. Elsewhere, studies by Anantharaman *et al.,*(2010) and Karthikai *et al.,* (2009) reported manganese concentration that varied from $0.72\ \mu g\ g^{-1}$ to $4.08\ \mu g\ g^{-1}$ and $0.31\ \mu g\ g^{-1}$ to $4.44\ \mu g\ g^{-1}$ in Mandapan Coastal regions and Gulf of Mannar in Southeast India respectively. According to the US Food and Nutrition Board (FNB, 2004) , the recommended manganese dietary allowances (RDAs) among the children range from $1.2\ \mu g$ to$1.5\ \mu g$ per day and for the adults, it ranges from as low as $1.6\ \mu g\ day^{-1}$ among females to a maximum of $2.6\ \mu g\ day^{-1}$ for the lactating mothers (Appendices table 5.7).

The manganese concentrations level in the samples collected in wet season were relatively high in comparison to those sampled during in dry season. This is can be attributed to surface run-offs and seabed enrichment. Bioavailability fraction was highest with lower values of total content an aspect that can be attributed to relative abundance of the trace elements and the mineral balance in the seaweed.

Generally, the highest manganese values were recorded in samples from Kibuyuni and the lowest in samples from KWS (Tables 4.6, 4.8, 4.9). The high manganese concentrations in the seaweeds are attributed to enrichment during rainy seasons and hence its measurement can be a basis for bio- monitoring of marine metal contamination.

Table 4.7: Total content and bio-available manganese in various seaweeds from KWS.

Sample	Species name	Total concentration	Bio-available concentration	
		(µg g^{-1} ± 1SD) 1x10^{-1}	(µg g^{-1} ± 1SD) 1x10^{-1}	% of total content
KS1A	*Padina pavonica*	16.6 ± 0.8	3.6 ± 0.2	21.5
KS1B	*Padina pavonica*	18.6 ± 0.9	6.0 ± 0.3	32.3
KS2	*Chonrophycus papillosus*	35.0 ± 1.8	9.0 ± 0.5	25.7
KS3	*Acanthophora dendroides*	27.6 ± 1.4	3.6 ± 0.2	13.1
KS4	*Cystoseira trinodis*	51.6 ± 2.6	5.3 ± 0.3	10.2
KS5	*Gracilaria corticata*	47.6 ± 2.4	8.1 ± 0.4	16.9
KS6A	*Gracilaria salicornia*	62.2 ± 3.1	5.7 ± 0.3	9.2
KS6B	*Gracilaria salicornia*	87.2 ± 4.4	5.5 ± 0.3	6.3

Table 4.8: Total content and bio-available manganese in various seaweeds from Kibuyuni.

Sample	Species name	Total concentration	Bio-available concentration	
		(µg g^{-1} ± 1SD) 1x10^{-1}	(µg g^{-1} ± 1SD) 1x10^{-1}	% of total content
K1A	*Hypnea cornuta*	41.4 ± 2.1	14.9 ± 0.7	36.0
K1B	*Hypnea cornuta*	48.2 ± 2.4	16.9 ± 0.8	35.0
K2A	*Chonrophycus papillosus*	73.8 ± 3.7	6.33 ± 0.3	8.6
K2B	*Chonrophycus papillosus*	73.8 ± 3.7	12.5 ± 0.6	16.9
K3	*Gracilaria corticata*	15.8 ± 0.8	1.0 ± 0.1	6.2
K4A	*Gracilaria canaliculata*	22.4 ± 1.1	4.9 ± 0.2	22.0
K4B	*Gracilaria canaliculata*	45 ± 2.3	9.5 ± 0.5	21.1
K5	*Solieria robusta*	11.1 ± 0.6	2.8 ± 0.1	25.5
K6A	*Acanthophora spicifera*	62.2 ± 3.1	9.0 ± 0.5	14.5
K6B	*Acanthophora spicifera*	48.2 ± 2.4	5.3 ± 0.3	11.0
K7A	*Kappaphycus alvarezii*	25.8 ± 1.3	2.8 ± 0.1	10.8
K7B	*Euchema dendiculatum*	41.4 ± 2.1	4.0 ± 0.2	9.6

Table 4.9: Total content and bio-available manganese in *Euchemoids* seaweeds from Kibuyuni

Sample	Total concentration (µg g^{-1} ± 1SD) 1x10^{-1}	Bio-available concentration (µg g^{-1} ± 1SD) 1x10^{-1}	% of total content
1B	71.6 ± 3.6	27.0 ± 1.3	37.7
2B	58.0 ± 2.9	30.2 ± 1.5	51.9
3B	59.8 ± 3.0	20.4 ± 1.0	34.1
4B	75.7 ± 3.8	37.2 ± 1.9	49.1
5B	63.1 ± 3.2	12.6 ± 0.6	20.0
6B	51.9 ± 2.6	8.6 ± 0.4	16.6
7B	75.3 ± 3.8	20.6 ± 1.0	27.4
8B	95.7 ± 4.8	17.4 ± 0.9	18.2
9B	35.8 ± 1.8	10.1 ± 0.5	28.2
10B	37.0 ± 1.9	11.0 ± 0.6	29.8
1G	67.5 ± 3.4	26.2 ± 1.3	38.8
2G	57.4 ± 2.9	32.4 ± 1.6	56.4
3G	54.2 ± 2.7	20.4 ± 1.0	37.6
4G	44.5 ± 2.2	21.2 ± 1.1	47.6
5G	43.8 ± 2.2	7.6 ± 0.4	17.4
6G	53.3 ± 2.7	8.6 ± 0.4	16.1
7G	39.4 ± 2.0	10.6 ± 0.5	26.9
8G	96.7 ± 4.8	17.8 ± 0.9	18.4
9G	40.5 ± 2.0	10.6 ± 0.5	26.2
10G	38.1 ± 1.9	10.0 ± 0.5	26.2

Correlations for manganese total concentration versus bioavailable fraction in various seaweeds from KWS, Kibuyuni and *Euchemoids* from Kibuyuni

Plotting the values for total content versus bioavailable fraction for manganese concentration in seaweed, (Tables 4.7, 4.8 and 4.9) the correlation coefficients obtained indicated a weak correlation of bioavailable concentration with the increase in manganese total content in samples from both KWS and Kibuyuni.
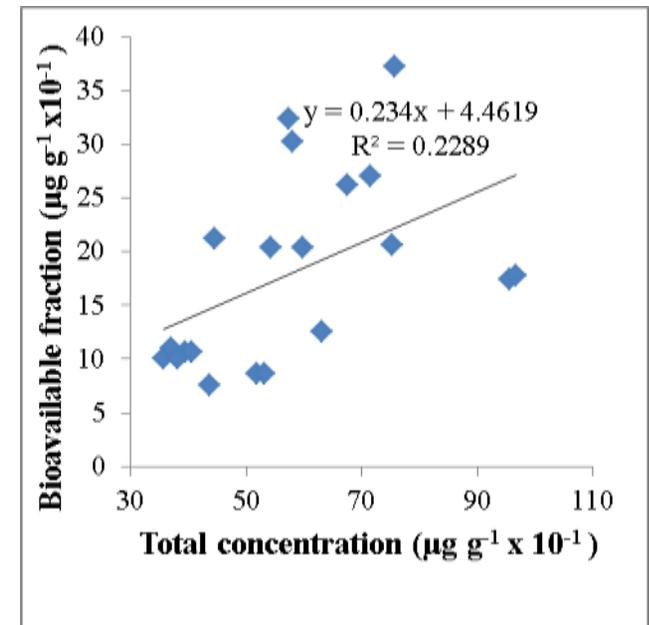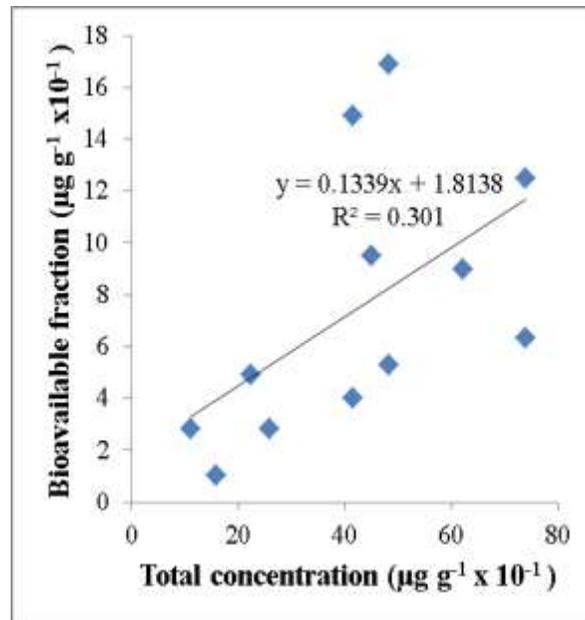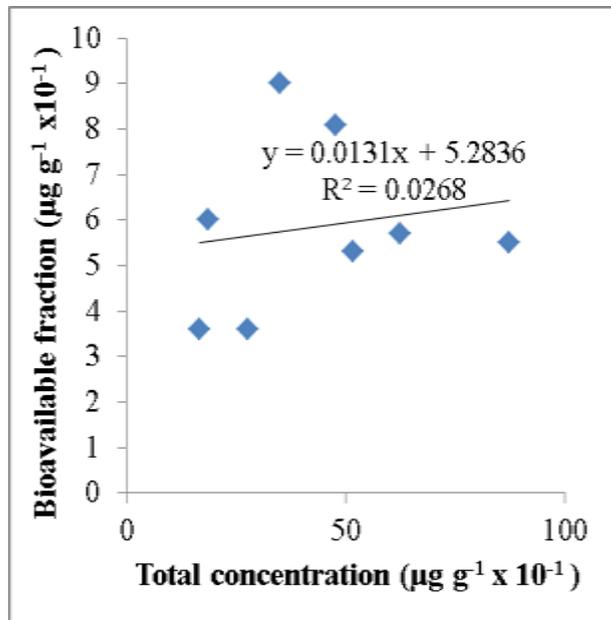
(i) Mn content correlations in KWS samples, (ii) Mn content correlations in Kibuyuni samples, (iii) Mn content correlations in *Euchemoids*
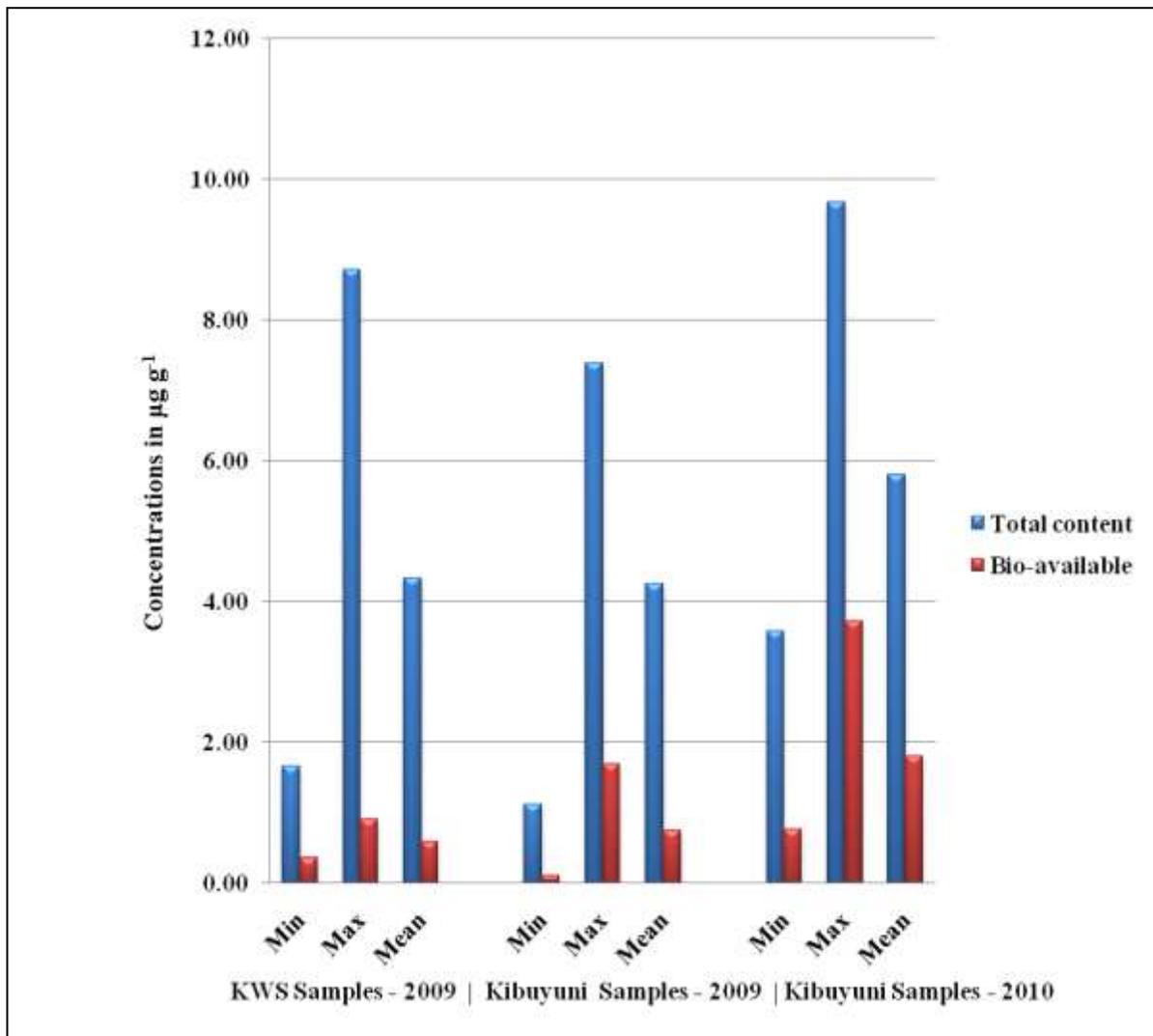
Fig. 4.2: Comparison for manganese concentrations (µg g^{-1}) in seaweeds from KWS & Kibuyuni.

c)   **Iron in seaweeds**

From the analysis, various seaweed samples collected in October, 2009, had iron total concentrations that varied from 18.5 ± 1.0 µg g^{-1} to 58.7 ± 2.9 µg g^{-1} for the samples from KWS and the corresponding bioavailability values ranged from 2.0 ± 0.1µg g^{-1} to 15.8 ± 0.8 µg g^{-1} as shown in table 4.10.

Various seaweed species from Kibuyuni had iron content in the range of 7.4 ± 0.4 µg g^{-1} to 117 ± 5.9 µg g^{-1} for total content and a range of 1.9 ± 0.1µg g^{-1} to 15.7 ± 0.8 µg g^{-1} bioavailability fractions (Table 4.10). Generally, the bioavailability fractions decreased with the increasing total content implying the significance in mineral balance in seaweeds.

Iron content in *Euchemoids* seaweeds ranged from 12.9 ± 0.6 µg g^{-1} to 95.5 ± 4.8 µg g^{-1} while the bioavailability fraction varied from 2.2 ± 0.1µg g^{-1} to 9.2 ± 0.4 µg g^{-1} with same correlation as observed in the various seaweeds (Table 4.11). Generally, higher iron levels were obtained in brown seaweeds samples and hence can be a good source of iron in diet.

It was observed that iron concentration values were higher in samples from Kibuyuni, ranking second after calcium which is associated with the mineral forming rocks in the sampling sites. Samples collected during rainy season (*Euchemoids* seaweeds) had higher iron values than those collected during dry season owing to enrichment from surface run off and from base rock enrichment in Kibuyuni sites. Studies by Anantharaman *et al.,* (2010) and Karthikai *et al*., (2009) reported a range of 17.53 µg g^{-1} to 62.47µg g^{-1} and 3.94 µg g^{-1} to 50.82 µg g^{-1} in Southeast Coastal regions of India respectively (Appendices table 5.4). These values were within the nutritional thresholds of $7 - 10$ µg day^{-1} for children and a range of $8 - 27$µg day^{-1} for adults (FNB, 2004). Ruperez, (2002) reported iron values in the range of $0.3 - 1.1$µg g^{-1} from Spain while Krishnaiah *et al.,* (2008) reported a range of $0.7 - 1.1$ µg g^{-1} in Malaysia, both were below the Nutritional thresholds of toxicity. This study concurs that marked changes in chemical composition of the seaweeds occurs due to changes in seasons, environmental conditions and the varied stages of seaweed growth that affects its mineral accumulation (Anantharaman *et al.,* 2010, Krishnaiah *et al*., 2009).

Table 4.10: Total content and bio-available iron in various seaweeds from KWS.

Sample	Species name	Total concentration	Bio-available concentration	
		$(\mu g\ g^{-1} \pm 1SD)$	$(\mu g\ g^{-1} \pm 1SD)$	% of total content
KS1A	*Padina pavonica*	56.7 ± 2.9	15.8 ± 0.8	27.9
KS1B	*Padina pavonica*	58.7 ± 2.9	12.3 ± 0.6	21.0
KS2	*Chonrophycus papillosus*	36.1 ± 1.8	6.8 ± 0.3	18.7
KS3	*Acanthophora dendroides*	29.6 ± 1.4	4.8 ± 0.2	16.2
KS4	*Cystoseira trinodis*	19.3 ± 1.0	2.0 ± 0.1	10.2
KS5	*Graciaria corticata*	19.2 ± 1.0	3.2 ± 0.1	16.5
KS6A	*Graciaria salicornia*	18.5 ± 1.0	6.2 ± 0.3	33.5
KS6B	*Graciaria salicornia*	19.4 ± 1.0	10.3 ± 0.5	53.2

Table 4.11: Total content and bio-available iron in various seaweeds from Kibuyuni.

Sample	Species name	Total concentration	Bio-available concentration	
		$(\mu g\ g^{-1} \pm 1SD)$	$(\mu g\ g^{-1} \pm 1SD)$	% of total content
K1A	*Hypnea cornuta*	86.1 ± 4.3	2.9 ± 0.1	3.4
K1B	*Hypnea cornuta*	117 ± 5.9	5.1 ± 0.2	4.3
K2A	*Chonrophycus papillosus*	45.7 ± 2.3	10.4 ± 0.5	22.7
K2B	*Chonrophycus papillosus*	66.3 ± 3.3	10.4 ± 0.5	15.6
K3	*Graciaria corticata*	19.2 ± 1.0	2.6 ± 0.1	13.6
K4A	*Graciaria canaliculata*	10.1 ± 0.5	1.9 ± 0.1	18.6
K4B	*Graciaria canaliculata*	17.1 ± 0.9	3.8 ± 0.2	22.0
K5	*Solieria robusta*	50.1 ± 2.6	15.5 ± 0.8	31.0
K6A	*Acanthophora spicifera*	53.0 ± 2.7	6.2 ± 0.3	11.7
K6B	*Acanthophora spicifera*	49.1 ± 2.5	5.1 ± 0.2	10.3
K7A	*Kappaphycus alvarezii*	9.0 ± 0.5	2.0 ± 0.1	22.2
K7B	*Euchema dendiculatum*	7.4 ± 0.4	1.9 ± 0.1	26.0

Table 4.12: Total content and bio-available iron in *Euchemoids* seaweeds from Kibuyuni.

Sample	Total concentration ($\mu g\ g^{-1} \pm 1SD$)	Bio-available concentration ($\mu g\ g^{-1} \pm 1SD$)	% of total content
1B	34.5 ± 1.7	4.7 ± 0.2	13.7
2B	26.8 ± 1.3	5.7 ± 0.3	21.1
3B	31.2 ± 1.6	7.8 ± 0.4	24.9
4B	60.1 ± 3.0	6.7 ± 0.3	11.2
5B	18.2 ± 0.9	3.4 ± 0.2	18.8
6B	33.5 ± 1.7	3.4 ± 0.2	10.2
7B	12.9 ± 0.6	3.3 ± 0.2	2.6
8B	63.8 ± 3.2	4.4 ± 0.2	6.9
9B	26.5 ± 1.3	2.7 ± 0.1	10.0
10B	37.4 ± 1.8	7.6 ± 0.4	20.3
1G	24.0 ± 1.2	4.4 ± 0.2	18.1
2G	17.4 ± 0.9	9.2 ± 0.4	53.1
3G	31.3 ± 1.6	4.3 ± 0.2	13.7
4G	18.3 ± 0.9	3.0 ± 0.2	16.2
5G	95.5 ± 4.8	3.2 ± 0.2	3.3
6G	84.4 ± 4.2	2.2 ± 0.1	2.6
7G	32.2 ± 1.6	3.5 ± 0.2	10.9
8G	42.7 ± 2.1	3.1 ± 0.2	7.3
9G	31.1 ± 1.5	3.0 ± 0.2	9.7
10G	15.4 ± 0.8	3.6 ± 0.2	23.5

Correlations for iron total concentration versus bioavailable fraction in various seaweeds from KWS, Kibuyuni and *Euchemoids* from Kibuyuni

Plotting the values for total content versus bioavailable fraction for manganese concentration in seaweed, (Tables 4.10, 4.11 and 4.12) the correlation coefficients obtained indicated a strong correlation of bioavailable concentration with the increase in iron total content in samples from KWS, moderate correlation in various seaweed samples from Kibuyuni and a weak correlation in *Euchemoids* samples from Kibuyuni. This could be associated with variation in iron absorption.
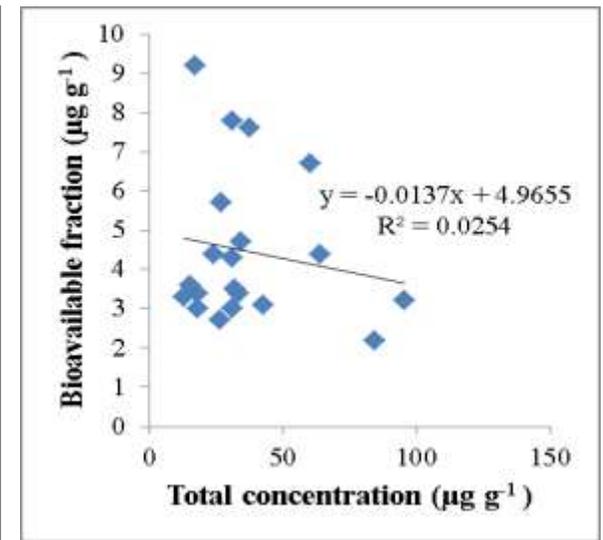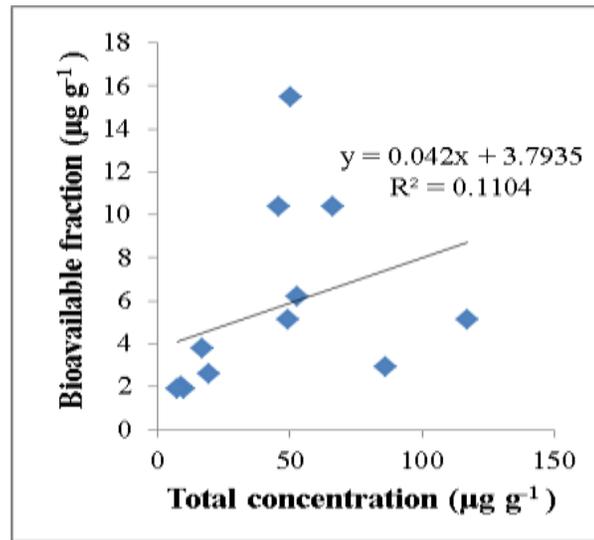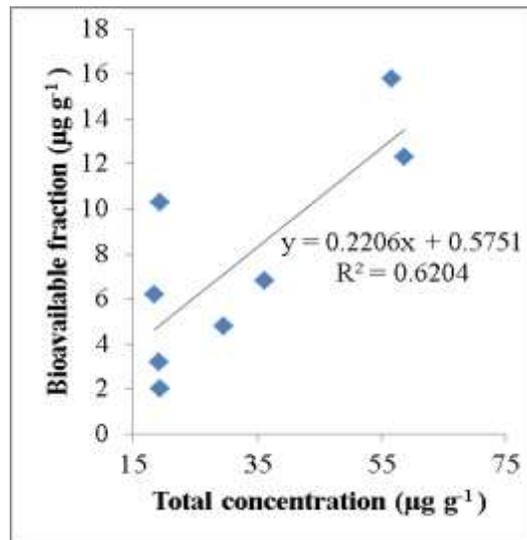
(i) Fe content correlations in KWS samples,   (ii) Fe content correlations in Kibuyuni samples,   (iii) Fe content correlations in *Euchemoids*
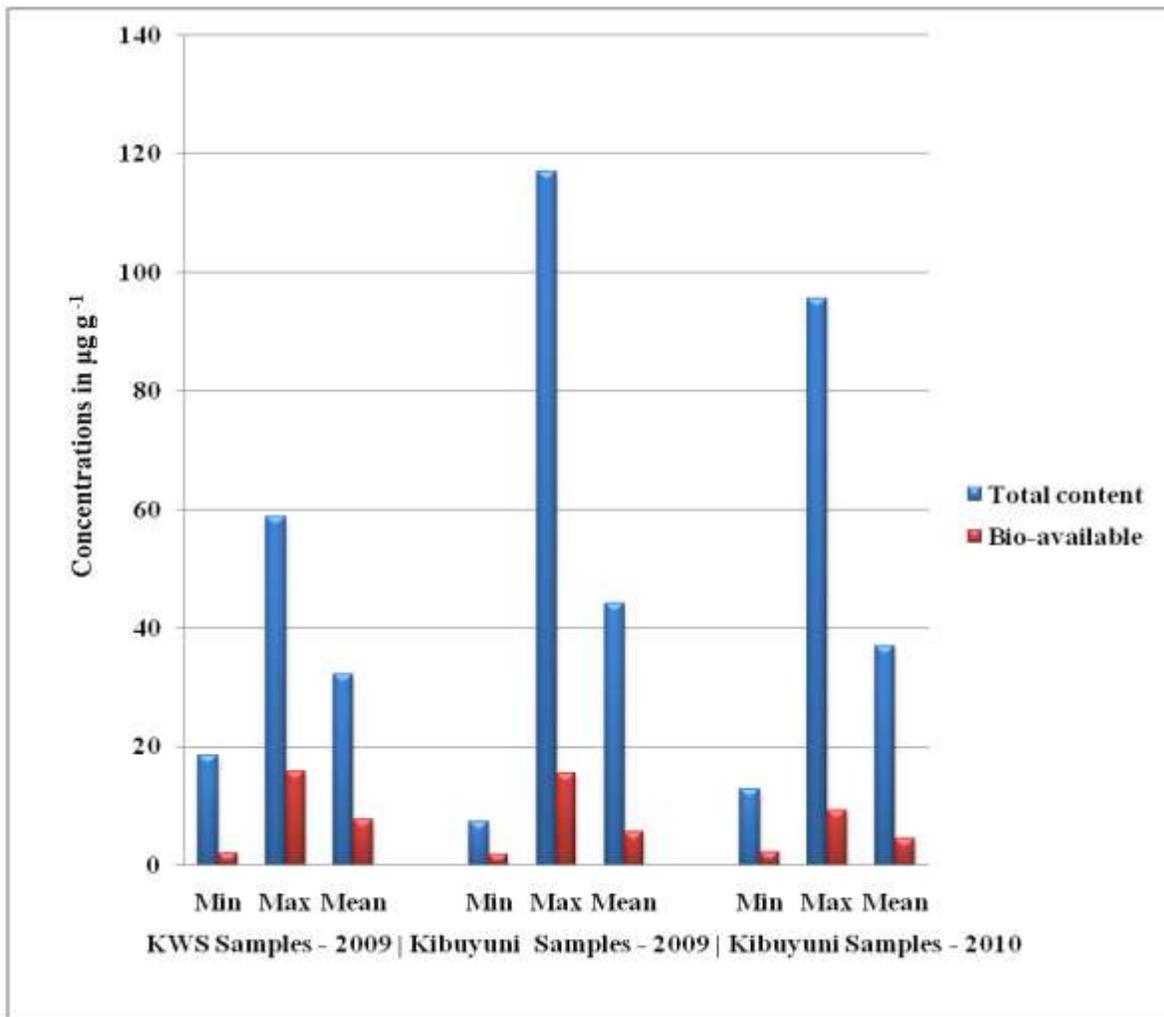
Fig. 4.3: Comparison for iron concentrations (µg g^{-1}) in seaweeds from KWS & Kibuyuni.

d)   **Copper in seaweeds**

Generally, samples from KWS had higher copper concentration levels as compared to those from Kibuyuni. This can be associated to the increased human activities at Shimoni market centre and KWS station.

Tabulated results for total and bio-available copper concentrations in various seaweed samples from KWS and Kibuyuni and *Euchemoids* seaweeds from Kibuyuni are presented

in the tables 4.13, 4.14 and 4.15 respectively. The mean concentrations of the seaweeds are presented in comparison graph as shown in figure 4.4

Total copper concentration levels in various seaweed samples were relatively low compared to other trace elements in the samples. Copper levels in samples from KWS ranged from 0.35 ± 0.02 µg g^{-1} to 1.32 ± 0.07 µg g^{-1} whereas the corresponding bioavailability fraction ranged from 0.03 µg g^{-1} to 0.14 ± 0.01 µg g^{-1} (Table 4.13). Copper in samples from Kibuyuni ranged from 0.26 ± 0.02 µg g^{-1} to 1.26 ± 0.06 µg g^{-1} and the corresponding bioavailability fraction ranged relatively low from 0.01µg g^{-1} to 0.13 ± 0.01µg g^{-1} (Table 4.15).

The total copper concentrations levels in *Euchemoids* seaweed samples varied from 0.43 ± 0.02 µg g^{-1} to 4.51 ± 0.23 µg g^{-1}. The corresponding bioavailability fraction ranged from 0.1 ± 0.01µg g^{-1} to 0.62 ± 0.03 µg g^{-1} (Table. 4.13) with a general trend of decreasing concentration with the increase in total content. Comparing copper levels in various seaweeds collected in October, 2009 and the *Euchemoids* seaweeds collected in June, 2010 during the wet season from Kibuyuni, the latter had higher values owing to seasonal changes and the water–rock interactions in marine ecosystem that leads to mineral enrichment.

Related studies by Anantharaman *et al*., (2010) and Karthikai *et al*., (2009) in India reported a relatively low range of 0.87-1.70 µg g^{-1} and $0.38 - 1.54$ µg g^{-1} for copper respectively (Appendices table 5.4) as compared to results from this study. With respect to recommended dietary intakes, copper concentration levels in edible seaweeds are within permissible intake limits i.e. 340 mg day^{-1} for children and 1300 mg day^{-1} for lactating mothers(adults) as per RDI guidelines according to the US National Academy of Science (FNB, 2004, Appendices table 5.7).

Table 4.13: Total content and bio-available copper in various seaweeds from KWS.

Sample	Species name	Total concentration	Bio-available concentration	
		(μg g^{-1} ± 1SD) 1x 10^{-1}	(μg g^{-1} ± 1SD) 1x 10^{-1}	% of total content
KS1A	*Padina pavonica*	4.4 ± 0.2	0.40 ± 0.02	9.0
KS1B	*Padina pavonica*	3.5 ± 0.2	0.36 ± 0.02	10.3
KS2	*Chonrophycus papillosus*	7.1 ± 0.4	1.44 ± 0.07	10.8
KS3	*Acanthophora dendroides*	5.8 ± 0.3	0.26 ± 0.01	20.4
KS4	*Cystoseira trinodis*	13.2 ± 0.7	1.15 ± 0.06	4.5
KS5	*Gracilaria corticata*	5.8 ± 0.3	0.62 ± 0.03	8.7
KS6A	*Gracilaria salicornia*	6.4 ± 0.3	1.3 ± 0.06	20.0
KS6B	*Gracilaria salicornia*	6.2 ± 0.3	1.3 ± 0.06	20.7

Table 4.14: Total content and bio-available copper in various seaweeds from Kibuyuni.

Sample	Species name	Total concentration	Bio-available concentration	
		(μg g^{-1} ± 1SD) 1x 10^{-1}	(μg g^{-1} ± 1SD) 1x 10^{-1}	% of total content
K1A	*Hypnea cornuta*	5.0 ± 0.3	0.22 ± 0.01	4.4
K1B	*Hypnea cornuta*	8.8 ± 0.4	0.58 ± 0.03	6.6
K2A	*Chonrophycus papillosus*	5.3 ± 0.3	0.54 ± 0.03	10.3
K2B	*Chonrophycus papillosus*	12.6 ± 0.6	0.54 ± 0.03	4.3
K3	*Gracilaria corticata*	5.8 ± 0.3	0.36 ± 0.02	6.3
K4A	*Gracilaria canaliculata*	2.6 ± 0.2	0.36 ± 0.02	14.0
K4B	*Gracilaria canaliculata*	5.7 ± 0.3	0.72 ± 0.04	12.6
K5	*Solieria robusta*	3.0 ± 0.2	0.08 ± 0.01	2.7
K6A	*Acanthophora spicifera*	6.4 ± 0.3	1.28 ± 0.06	20.1
K6B	*Acanthophora spicifera*	5.9 ± 0.3	0.58 ± 0.03	9.9
K7A	*Kappaphycus alvarezii*	3.8 ± 0.2	0.57 ± 0.03	15.1
K7B	*Euchema dendiculatum*	2.7 ± 0.1	0.22 ± 0.01	8.3

Table 4.15: Total content and bio-available copper in *Euchemoids* seaweeds from Kibuyuni.

Sample	Total concentration (µg g^{-1} ± 1SD) 1x 10^{-1}	Bio-available concentration (µg g^{-1} ± 1SD) 1x 10^{-1}	% of total content
1B	15.2 ± 0.8	3.4 ± 0.2	22.4
2B	13.1 ± 0.7	1.8 ± 0.1	13.7
3B	11.5 ± 0.6	2.6 ± 0.1	22.6
4B	18.9 ± 1.0	4.4 ± 0.2	23.3
5B	45.1 ± 2.3	6.2 ± 0.3	13.7
6B	15.0 ± 0.8	3.1 ± 0.2	20.5
7B	26.0 ± 1.3	6.1 ± 0.3	23.5
8B	24.3 ± 1.2	3.8 ± 0.2	15.6
9B	18.4 ± 0.9	2.0 ± 0.1	10.9
10B	10.6 ± 0.5	3.2 ± 0.2	30.2
1G	14.3 ± 0.7	2.6 ± 0.1	18.2
2G	13.4 ± 0.7	2.1 ± 0.1	15.8
3G	28.0 ± 1.4	5.6 ± 0.3	20.0
4G	11.7 ± 0.6	2.6 ± 0.1	22.2
5G	12.0 ± 0.6	1.6 ± 0.1	13.3
6G	4.7 ± 0.2	1.0 ± 0.1	21.7
7G	10.6 ± 0.5	2.2 ± 0.1	20.8
8G	11.2 ± 0.6	2.1 ± 0.1	18.6
9G	19.9 ± 1.0	2.4 ± 0.1	12.1
10G	4.3 ± 0.2	1.4 ± 0.1	32.6

Correlations for copper total concentration versus bioavailable fraction in various seaweeds from KWS, Kibuyuni and *Euchemoids* from Kibuyuni

Plotting the values for total content versus bioavailable fraction for copper concentration in seaweed, (Tables 4.13, 4.14 and 4.15) the correlation coefficients obtained indicated a moderate and a weak correlation of bioavailable concentration with the increase in copper total content in various seaweed samples from both KWS and Kibuyuni respectively. The correlation in *Euchemoids* samples from Kibuyuni was strong as illustrated diagram (iii).
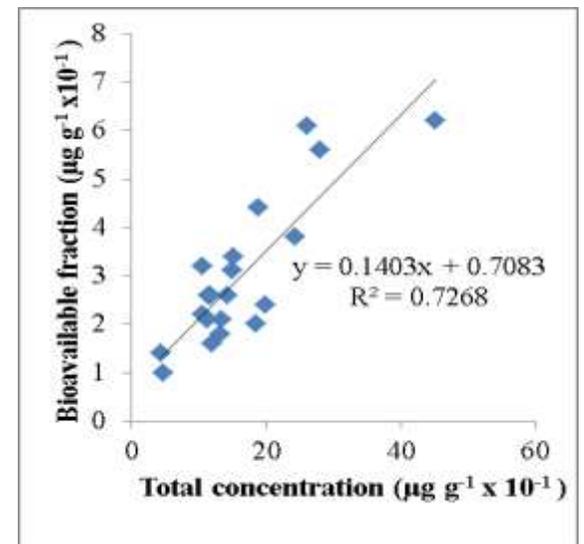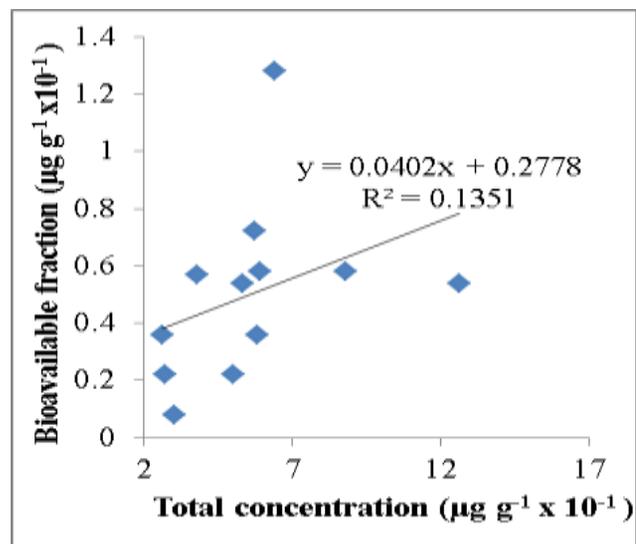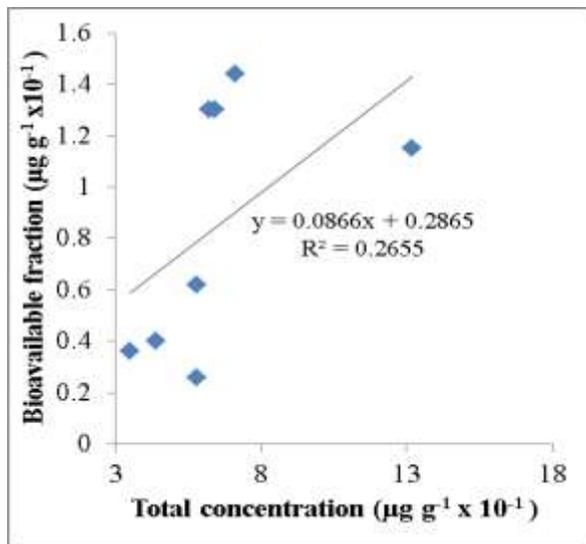
(i) Cu content correlations in KWS samples,   (ii) Cu content correlations in Kibuyuni samples,   (iii) Cu content correlations in *Euchemoids*
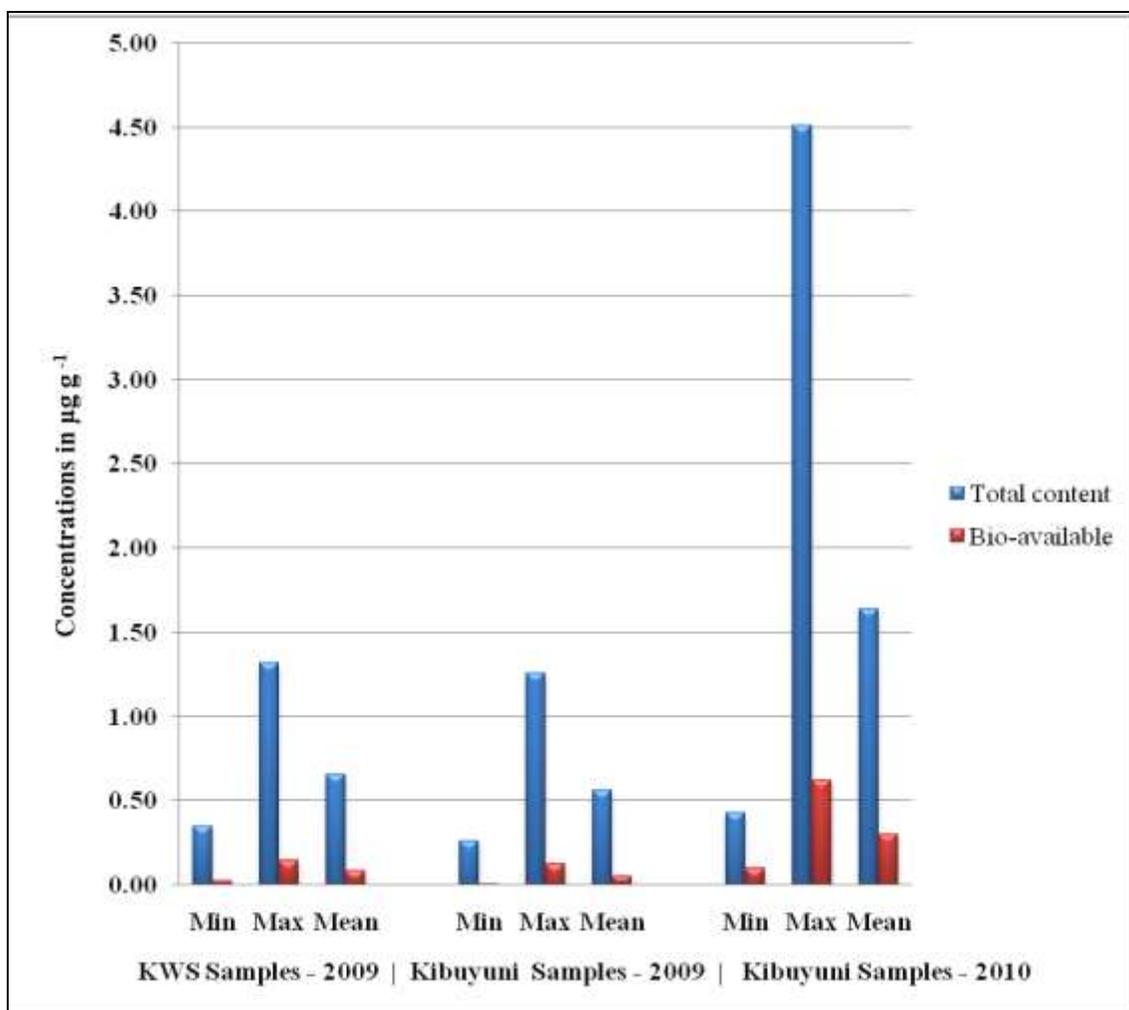
Fig.4.4: Comparison graph for copper concentrations (µg g^{-1}) in seaweed from KWS & Kibuyuni.

e) **Zinc in various seaweed species from KWS and Kibuyuni**

Results for total and bio-available zinc concentration levels in various seaweed samples from KWS and Kibuyuni, *Euchemoids* seaweeds from Kibuyuni are presented in the tables 4.16, 4.17 and 4.18 respectively. Mean concentration levels of zinc in seaweeds are presented in the comparison graph shown in figure 4.5. Zinc concentrations in various seaweed species from KWS varied from 0.89 ± 0.05 µg g^{-1} to 2.94 ± 0.07 µg g^{-1} for the total content and

$0.08 \pm 0.01 \ \mu g \ g^{-1}$ to $0.28 \pm 0.02 \ \mu g \ g^{-1}$ for the bio-available fraction. Samples from Kibuyuni gave concentrations that varied from $0.27 \pm 0.01 \mu g \ g^{-1}$ to $2.18 \pm 1.01 \mu g \ g^{-1}$ for the total content, and from $0.01 \ \mu g \ g^{-1}$ to $0.17 \pm 0.01 \ \mu g \ g^{-1}$ for the bio-available fraction. There was a positive correlation for bioavailable fraction decreasing with an increase in total content as illustrated in correlation plots in subsequent section.

The results for zinc concentration levels in *Euchemoids* seaweeds for total content varied from $1.94 \pm 0.1 \mu g \ g^{-1}$ to $7.21 \pm 0.36 \ \mu g \ g^{-1}$ and for the bioavailability levels varied from $0.38 \ \mu g \ g^{-1}$ to $1.54 \pm 0.08 \ \mu g \ g^{-1}$ (Table 4.18). Lower levels were observed for brown seaweeds which can be attributed to variation in cell composition and relative difference in zinc metal accumulation capacity (Almela *et al.,* 2006). According to US Food and Nutrition Board (FNB, 2004) dietary recommendations (Appendices table 5.7), zinc is an essential nutrient and toxic to humans at levels exceeding regulatory standards of $11 mg \ day^{-1}$ for male adults.

Generally, it was observed that concentration levels for samples collected in dry season were relatively lower than those sampled in wet season. This can be due to seasonal changes and change in environmental conditions that favours zinc enrichments in the seawater.

In comparison with published data, the results of this study were comparable and within range as Karthikai *et al*., 2009 reported zinc concentration levels that varied from $0.65 \mu g \ g^{-1}$ to $4.28 \ \mu g \ g^{-1}$ in India and in another study by Anantharaman *et al.,* (2010) reported a range of $0.32 - 0.74 \ \mu g \ g^{-1}$ also in Southeast Coastal regions of India. Krishnaiah *et al.,* (2008) and Ruperez, (2002) each reported a range of $0.2 - 0.7 \ \mu g \ g^{-1}$ from Malaysia and Spain respectively (Appendices table 5.4). Though some of the reported values in other regions were slightly higher than the recommended dietary levels, this study reported values below the threshold of zinc toxicity. According to the US Food and Nutrition Board (FNB, 2004), recommended Daily Allowances for zinc is $2 \ mg \ day^{-1}$ for infants and a range of $3 - 13 \ mg \ day^{-1}$ for Children.

Table 4.16: Total content and bio-available zinc in various seaweeds from KWS.

Sample	Species name	Total concentration (μg g^{-1} ± 1SD) 1x 10^{-1}	Bio-available concentration (μg g^{-1} ± 1SD) 1x 10^{-1}	% of total content
KS1A	*Padina pavonica*	13.7 ± 0.3	2.7 ± 0.6	19.8
KS1B	*Padina pavonica*	10.2 ± 0.5	0.6 ± 0.1	5.9
KS2	*Chonrophycus papillosus*	29.4 ± 0.7	2.7 ± 0.2	9.3
KS3	*Acanthophora dendroides*	8.9 ± 0.5	0.8 ± 0.1	9.0
KS4	*Cystoseira trinodis*	18.4 ± 0.9	2.7 ± 0.2	14.4
KS5	*Gracilaria corticata*	13.2 ± 1.5	1.0 ± 0.1	7.8
KS6A	*Gracilaria salicornia*	18.3 ± 1.5	2.5 ± 0.3	13.8
KS6B	*Gracilaria salicornia*	20.2 ± 1.0	2.8 ± 0.2	13.8

Table 4.17: Total content and bio-available zinc in various seaweeds from Kibuyuni.

Sample	Species name	Total concentration (μg g^{-1} ± 1SD) 1x 10^{-1}	Bio-available concentration (μg g^{-1} ± 1SD) 1x 10^{-1}	% of total content
K1A	*Hypnea cornuta*	11.9 ± 0.6	0.76 ± 0.04	6.4
K1B	*Hypnea cornuta*	18.2 ± 0.9	0.90 ± 0.05	5.0
K2A	*Chonrophycus papillosus*	20.7 ± 1.0	1.66 ± 0.08	8.0
K2B	*Chonrophycus papillosus*	5.5 ± 0.3	0.17 ± 0.09	3.0
K3	*Gracilaria corticata*	13.2 ± 0.7	0.72 ± 0.04	5.5
K4A	*Gracilaria canaliculata*	2.7 ± 0.1	0.07 ± 0.01	2.5
K4B	*Gracilaria canaliculata*	5.4 ± 0.3	0.14 ± 0.01	2.5
K5	*Solieria robusta*	12.4 ± 0.6	0.25 ± 0.01	2.0
K6A	*Acanthophora spicifera*	19.9 ± 1.0	0.25 ± 0.01	1.3
K6B	*Acanthophora spicifera*	15.1 ± 0.8	0.90 ± 0.5	6.0
K7A	*Kappaphycus alvarezii*	21.8 ± 1.1	1.33 ± 0.07	6.1
K7B	*Euchema dendiculatum*	14.1 ± 0.7	0.76 ± 0.04	5.4

Table 4.18: Total content and bio-available zinc concentration in *Euchemoids* seaweed samples from Kibuyuni.

Sample	Total concentration ($\mu g\ g^{-1} \pm 1SD$) $1x\ 10^{-1}$	Bio-available concentration ($\mu g\ g^{-1} \pm 1SD$) $1x\ 10^{-1}$	% of total content
1B	27.6 ± 1.4	8.0 ± 0.4	29.0
2B	36.7 ± 1.8	13.7 ± 0.8	37.3
3B	24.2 ± 1.2	4.9 ± 0.6	20.3
4B	72.1 ± 3.6	12.0 ± 1.0	16.7
5B	34.3 ± 1.7	4.6 ± 0.2	13.4
6B	62.7 ± 3.1	11.6 ± 0.4	18.5
7B	49.2 ± 2 5	8.4 ± 0.2	17.1
8B	71.7 ± 3.6	9.2 ± 0.2	12.8
9B	19.4 ± 1.0	3.8 ± 0.4	19.6
10B	38.2 ± 1.9	9.3 ± 0.8	24.3
1G	40.2 ± 2.0	14.0 ± 1.2	34.9
2G	51.1±2.6	15.4 ± 0.8	30.1
3G	49.1 ± 2.5	10.2 ± 0.6	20.8
4G	54.7 ± 2.7	8.4 ± 0.6	15.4
5G	45.6 ± 2.3	6.4 ± 0.4	14.1
6G	19.4 ± 1.0	4.0 ± 0.2	20.6
7G	70.2 ± 3.5	9.4 ± 0.2	13.4
8G	57.2 ± 2.9	5.9 ± 0.3	10.3
9G	40.2 ± 2.0	7.0 ± 0.4	17.4
10G	25.3 ± 1.3	5.2 ± 0.4	20.6

Correlations for zinc total concentration versus bioavailable fraction in various seaweeds from KWS, Kibuyuni and *Euchemoids* from Kibuyuni

Plotting the values for total content versus bioavailable fraction for zinc concentration in seaweed, (Tables 4.16, 4.17 and 4.18) the correlation coefficients obtained indicated a strong correlation of bioavailable concentration with the increase in zinc total content in various seaweed samples from both KWS and Kibuyuni and a moderate correlation in *Euchemoids* samples from Kibuyuni.
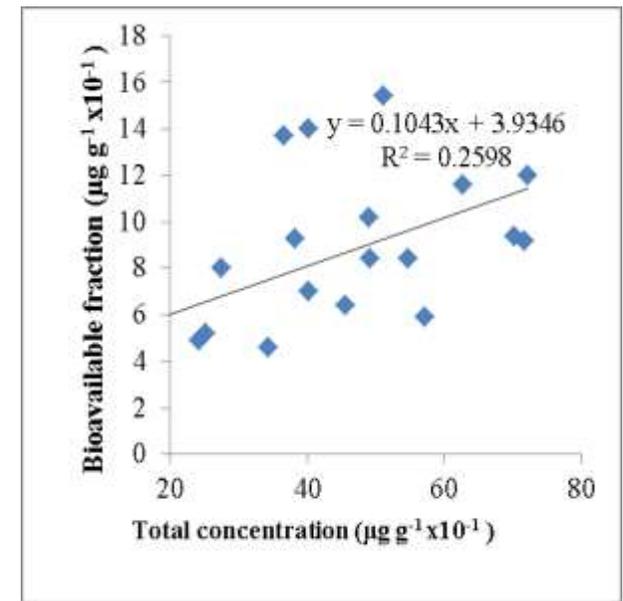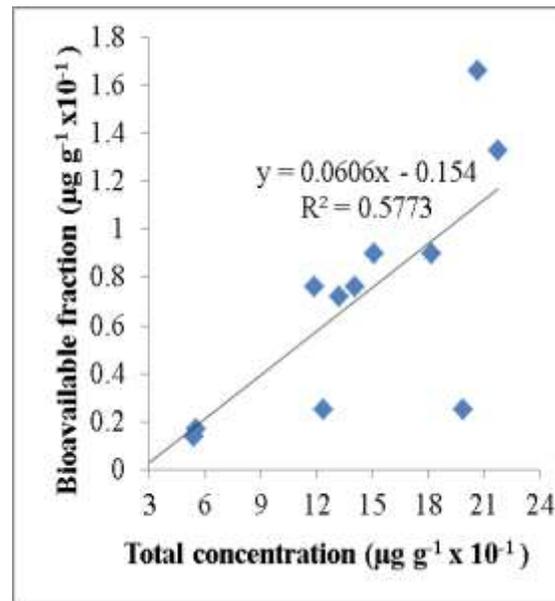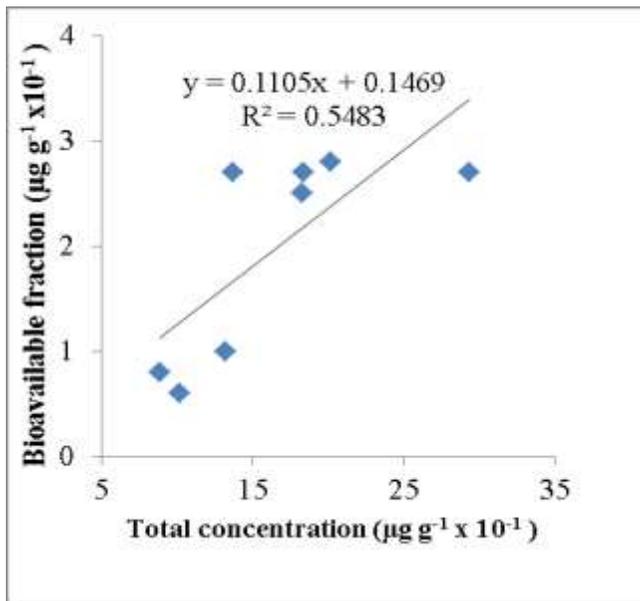
(i) Zn content correlations in KWS samples,   (ii) Zn content correlations in Kibuyuni samples,   (iii) Zn content correlations in *Euchemoids*
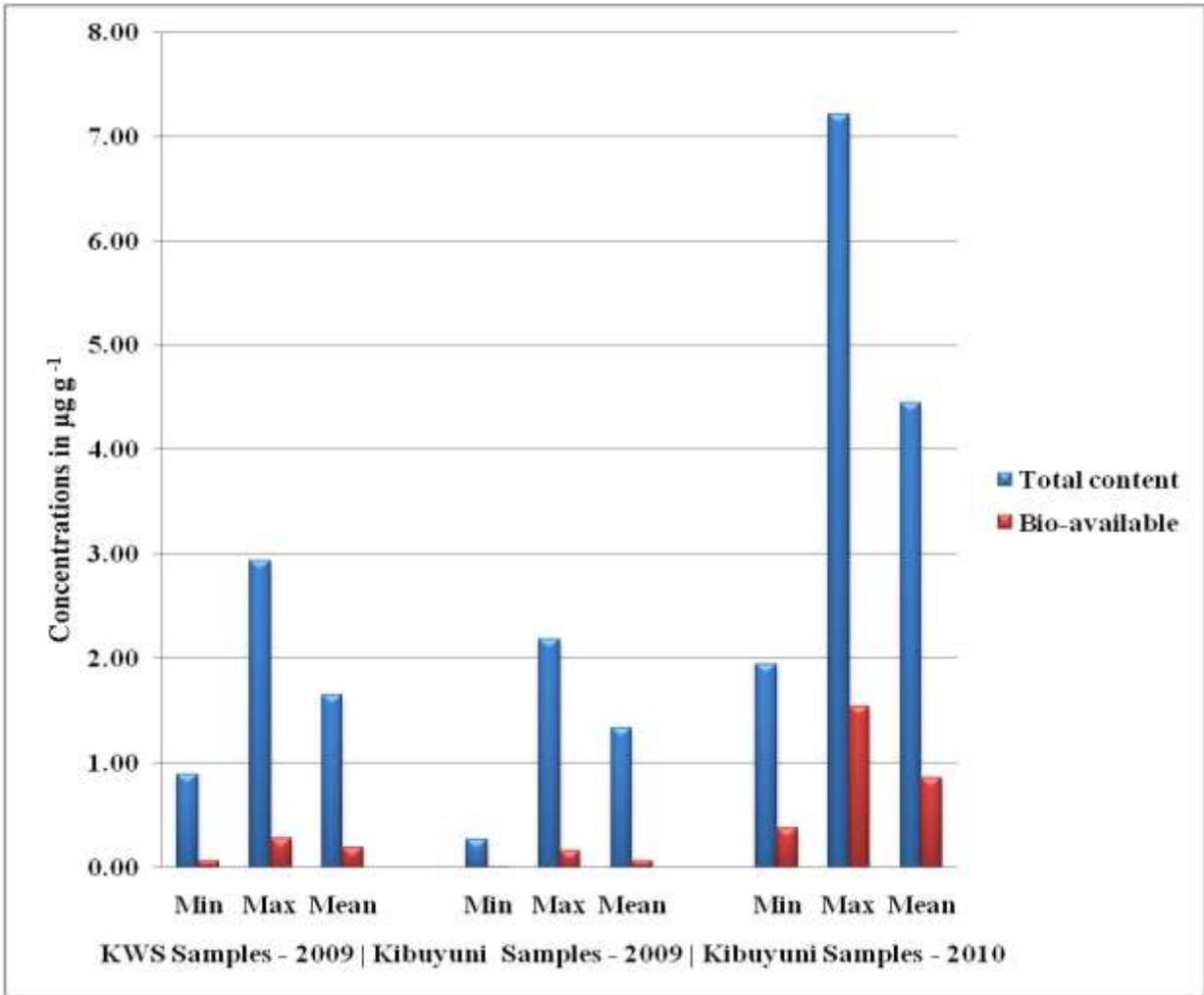
Fig. 4.5: Comparison graph for zinc concentrations (μg g^{-1}) in seaweeds from KWS & Kibuyuni.

4.4.2. Trace elements in sea sediments.

In this section, results for calcium, manganese, iron, copper and zinc concentrations in sea sediments are presented. Tabulated results are shown in table 4.19 and the mean concentrations presented graphically in figure 4.19.

a) **Calcium in sea sediments.**

In general, calcium concentration levels in the sediments samples ranged from minimum of

988 ± 49 µg g^{-1} to a maximum of 2433 ± 122 µg g^{-1}. However, calcium concentrations levels

for samples from Makupa, Tudor and Gazi Bay were below the detection limit (Table 4.19).

This can be attributed to the absence of carbonaceous rocks in these sites and also low

enrichment from other sources. Other related studies at the Kenya coast on calcium

concentrations remain implicit in nature as measurement of calcium in sea sediments presents

difficulties due to matrix effects (Muohi *et al*., 2002). Digestion of samples reduces the matrix

effect.

b) **Manganese in sea sediment.**

Manganese concentrations in the sea sediments samples studied were below the detection limits

(Table 4.19). Manganese enrichment in sea sediments is associated to industrial effluents

(Weigner, 2000) and higher levels of manganese indicate a high degree of industrial pollution in the

marine ecosystem. The observation can be attributed to low levels of manganese in the study area.

Oyugi *et al*., (2000) reported highest manganese levels at 1,100 µg g^{-1} in sediments from Vanga.

Hashim, (2001) reported manganese levels that varied from 97.54 ± 19.33 µg g^{-1} in Gazi to

550.83 ± 52.55 µg g^{-1} in Malindi. In another regional study, Sheikh *et al*., (2007) reported levels of

$537 - 812$ µg g^{-1} in Zanzibar harbor and 277-725 µg g^{-1} in Dares Salaam port. Elsewhere, other

related studies from the coastal regions indicate a wider range of manganese concentrations in sea

sediments for instance a study by Wendy (2005) reported a range of 46.1-1175 mg kg^{-1} in the Gulf

of Paria, Trinidad and Tobago (Table 5.5). These studies indicate that manganese enrichment is

generally associated with increased anthropogenic activities in the study sites (Weigner, 2000).

c) Iron in sea sediments.

Iron concentrations in sediments were varied for: Gazi, below detection limit to a maximum of 86.4 ± 4.3 µg g^{-1}]; Kibuyuni, below detection limit to a maximum of 117 ± 6 µg g^{-1}]; KWS, below detection limit to a maximum of 75.1 ± 3.8 µg g^{-1}]; Makupa [124 ± 6 µg g^{-1} to 152 ± 8 µg g^{-1}]; Tudor [82.4 ± 4.2 µg g^{-1} to 14 ± 11µg g^{-1}]. However, higher average iron concentrations were reported in samples from Tudor creek followed by Makupa, Kibuyuni, KWS and Gazi Bay. This can be explained by the fact that iron accumulation in sediments is associated with natural release into waters by weathering of pyritic ores containing iron sulfide (FeS) (Weigner, 2000, EPA, 1996). Other industrial processes such as mining and industrial effluents can contribute to the high levels of iron in water and sediments.

In a related study, Fatoki *et al.,* (2001) reported maximum iron concentration level of 15,185 µg g^{-1} at Port Elizabeth in South Africa. The study indicated that iron levels and other trace elements enrichment occurs due to pollution from storm water drains, and discharges from streams which carry runoff from industrial, urban and residential sources. Iron is considered essential element and US EPA has not set specific limits for its toxicity in sediments but ingestions exceeding 27µg day^{-1} in adults according to US Food and Nutrition Board, (FNB, 2004).

d) Copper in sea sediment

In general, the concentration levels for copper in both sediments and water samples were relatively low as compared to other trace elements. Table 4.18 shows the results of copper concentrations in sediment sampled in the five sampling sites that varied as follows; Gazi [13.6 ± 0.1 µg g^{-1} to 20.1 ± 1.0 µg g^{-1}]; Kibuyuni [13.4 ± 0.1 µg g^{-1} to 39.3 ± 1.9 µg g^{-1}]; KWS [27.8±1.4 µg g^{-1} to 30.9 ± 1.5 µg g^{-1}]; Makupa [13.4 ± 0.1 µg g^{-1} to 39.3 ± 1.9 µg g^{-1}]; Tudor [25.7 ± 1.3 µg g^{-1} to 31.8 ± 1.6 µg g^{-1}]. In a decreasing order, higher concentrations were

reported in; KWS, Tudor, Makupa, Kibuyuni and Gazi Bay. This observation can be attributed to lots of anthropogenic activities and to the weathering of copper-bearing rocks such as chalcopyrite from the sea bed.

The results of this study are comparable to other related studies; Muohi *et al.,* (2002) reported a higher range of 50.9 - 229 µg g^{-1} copper concentration from the Mombasa region while studies by Hashim (2001) reported relatively lower copper levels of 47.68 ± 4.83 µg g^{-1} for Mombasa area and 13.96 ± 1.47 µg g^{-1} for Gazi area. In other related study, Oyugi *et al.,* (2000) reported copper concentration levels ranging from 18.7 µg g^{-1} at Mombasa Marine Park to 49.97 µg g^{-1} at Diani. Individual differences could be due to difference in sampling points and variations in the sampling seasons. In the East Africa region, Sheikh *et al*., (2007) reported copper levels in the range of 4 – 75 µg g^{-1} in Zanzibar port and 36 µg g^{-1} in Dar es Salaam harbour. Elsewhere, Wong *et al.,*(1980) reported a range of 6.80 - 231 µg g^{-1} for Tolor Habour in Hong Kong China while Wendy, (2005) reported a range of 0.8 - 39.7 µg g^{-1} copper concentrations in Gulf of Paria, Tronidad & Tobago (Table 5.5).

The results of this study shows a similar variations for copper concentration levels in Kibuyuni and Makupa which can imply a relatively comparable degree of copper contamination in the two sites especially associated with weathering of copper – bearing rocks.

e) Zinc in sea sediment.

The concentration levels of zinc in sediment varied as shown in the Table 4.19, for:

Gazi [20.0 ± 1.0 µg g^{-1} to 54.8 ± 2.7 µg g^{-1}]; Kibuyuni [20.1 ± 1.0 µg g^{-1} to 23.6 ± 1.2µg g^{-1}]; KWS [26.4 ± 1.3 µg g^{-1} to 29.3 ± 1.4µg g^{-1}]; Makupa [44.2 ± 2.2 µg g^{-1} to 74.7 ± 3.7 µg g^{-1}]; Tudor [26.4 ± 1.3 µg g^{-1} to 71.3 ± 3.6 µg g^{-1}] with Makupa posting the highest concentration

while the lowest concentration was reported from Kibuyuni. General trend in high zinc concentrations was in order of Makupa followed by Tudor, KWS, Gazi Bay and Kibuyuni.

Zn does not occur freely in nature but is incorporated in its minerals (mainly sulfides, oxides, carbonates, and silicates) which are associated with minerals of other metals, particularly lead, copper, cadmium, mercury, and silver (Weigner, 2000). Makupa which habours the Mombasa dumpsite could be having high zinc contaminations compared to other sites.

Other studies have indicated higher zinc in sediment that varied from 276 - 3193 mg kg^{-1} in Makupa and 30.7 - 2.9 mg kg^{-1} in Port Reitze as reported by Muohi $et\ al.,$ (2002). The relative variation in the results can be attributed to seasonal changes.

In another related study in East London and Port Elizabeth harbours, Fatoki $et\ al.$, (2001) reported zinc levels that varied from 26.1 - 332 µg g^{-1} and 18.8 - 1260 µg g^{-1} in sediments respectively. These results indicated significant contributions of heavy metal pollution from storm water drains and streams which carry run off from industrial, urban and residential sources (Fatoki $et\ al.$, 2001; Weigner, 2000).

Table 4.19: Concentration of trace elements in sea sediment from the Kenyan Coast ($\mu g\ g^{-1}$).

Sample	Ca	Mn	Fe	Cu	Zn
GS1	1,064 ± 53	< 99	86.4 ± 4.3	15.2 ± 0.1	54.8 ± 2.7
GS2	<962	< 99	<62	14.0 ± 0.1	20.5 ± 1.0
GS3	< 962	< 99	<62	13.6 ± 0.1	26.9 ± 1.3
GS4	< 962	< 99	<62	20.1 ± 1.0	20.0 ± 1.0
KIBS1	2,433 ± 122	< 99	74.4 ± 3.7	31.9 ± 1.6	20.4 ± 1.0
KIBS2	2,127 ± 107	< 99	92.8 ± 4.7	13.4 ± 0.1	20.1 ± 1.0
KIBS3	2,073 ± 104	< 99	117± 6	39.3 ± 1.9	22.9 ± 1.1
KIBS4	2,157 ± 108	< 99	<62	30.4 ± 1.5	23.6 ± 1.2
KWS1	1,055 ± 52	< 99	75.1 ± 3.8	27.8 ± 1.4	28.2 ± 1.4
KWS2	1,730 ± 87	< 99	<62	30.9 ± 1.5	29.3 ± 1.4
KWS3	1,080 ± 54	< 99	63.6 ± 3.4	27.4 ± 1.4	26.4 ± 1.3
KWS4	988 ± 49	< 99	76.8 ± 3.9	29.7 ± 1.4	26.8 ± 1.3
MS1	< 962	< 99	152 ± 8	39.3 ± 1.9	74.7 ± 3.7
MS2	< 962	< 99	147 ± 7	13.5 ± 0.1	61.8 ± 3.1
MS3	< 962	< 99	124 ± 6	13.6 ± 0.1	45.1 ± 2.2
MS4	< 962	< 99	143 ± 7	13.4 ± 0.1	44.2 ± 2.2
TS1	< 962	< 99	214 ± 11	26.4 ± 1.3	69.5 ± 3.5
TS2	< 962	< 99	194 ± 20	25.7 ± 1.3	71.3 ± 3.6
TS3	< 962	< 99	82.4 ± 4.2	31.8 ± 1.6	33.3 ± 1.7
TS4	< 962	< 99	90.7 ± 4.5	30.5 ± 1.5	26.4 ± 1.3

4.4.3. Trace elements in sea water.

In this section the results for concentration of calcium, manganese, iron, copper and zinc in sea water are presented. Tabulated results are shown in table 4.20 and mean concentrations presented in the graph (figure 4.7)

a) **Calcium in sea water**

Table 4.20 shows calcium concentration levels in water varied as follows:

Gazi [72.5 ± 3.6 µg ml^{-1} to 114 ± 6 µg ml^{-1}]; Kibuyuni [57.7 ± 2.9 µg ml^{-1} to 167 ± 8 µg ml^{-1}]; KWS [87.8 ± 4.4 µg ml^{-1} to 118 ± 6 µg ml^{-1}]; Makupa [72 ± 3.6 µg ml^{-1} to 95 ± 4.7 µg ml^{-1}]; Tudor [99.1 ± 4.9 µg ml^{-1} to 118 ± 6µg ml^{-1}]. This observation can be attributed to solubility of calcium salts which is limited to a few soluble salts of its compounds. In general, calcium was lowest in the sediments from Makupa and Tudor Creek and was highest in sediments from Kibuyuni, KWS and Gazi Bay respectively. This can be explained by the fact that calcium is associated with coral reefs which are in high abundance at Kibuyuni.

It is imperative to mention that other studies regarding calcium concentration in seawater remains scanty and implicit in nature as its determination presents a lot of inconsistencies due to matrix effect. The use of multi-element standard in TXRF analysis reduces the effects.

b) **Manganese in sea water**

Manganese concentration varied from 4.9 ± 0.2µg ml^{-1} to 41.7 ± 2.1µg ml^{-1} with the highest concentration reported in Tudor Creek and the lowest from KWS. The concentrations in decreasing order varied as; Tudor, Gazi, Makupa, Kibuyuni and KWS. The variation is relatively low and the enrichment of manganese in both sediments and water is associated with surface run-offs and anthropogenic activities. Tudor harbors a lot of human activities and with industrial discharges from the surrounding environs.

c) Iron in seawater.

Table 4.20 shows the results of iron concentration in water that varied for:

Gazi [11.5 ± 0.6 µg ml^{-1} to 24.3 ± 1.2 µg ml^{-1}]; Kibuyuni [12.6 ± 0.6 µg ml^{-1} to

58.8 ± 2.4 µg ml^{-1}]; KWS [7.5 ± 0.4 µg ml^{-1} to 16.2 ± 0.8 µg ml^{-1}]; Makupa [24.8 ± 1.2 µg ml^{-1}

to 52.1 ± 2.6µg ml^{-1}]; Tudor [10.0 ± 0.5 µg ml^{-1} to 46.1 ± 2.3 µg ml^{-1}]. Higher average iron

concentration levels were reported in samples from Kibuyuni followed by Makupa, Tudor,

KWS and Gazi Bay. This observation can be attributed to solubility of iron and water - rock

interactions in marine environment (Weigner, 2000; EPA, 1996).

d) Copper in seawater.

Concentration of copper in water varied for: Gazi [5.3 ± 0.3 µg ml^{-1} to 10.7 ± 0.5 µg ml^{-1}];

Kibuyuni [3.1 ± 0.2 µg ml^{-1} to 15.2 ± 0.8 µg ml^{-1}];

KWS [12.8 ± 0.6 µg ml^{-1} to 15.3 ± 0.8 µg ml^{-1}];

Makupa [8.3 ± 0.4 µg ml^{-1} to 18.9 ± 0.9 µg ml^{-1}];

Tudor [11.7 ± 0.6 µg ml^{-1} to 16.2 ± 0.8 µg ml^{-1}] with highest mean concentration reported from

KWS, Tudor, Makupa, Kibuyuni and Gazi Bay in that order. It can be said that copper

accumulation is relatively low in sediments and water. Dissolved copper rarely occurs in

unpolluted source water above 10 mg l^{-1} (EPA, 1996), limited by the solubility of copper

hydroxide (Cu (OH)$_2$), co-precipitation with less soluble metal hydroxides, and adsorption. A

related study in North West Spain (Pe´rez-Lo´pez *et al*., 2003) reported a range of 0.12 ± 0.04 to

0.88 ± 0.23 µg l^{-1} in seawaters of an estuary using differential pulse anodic stripping

voltammetry (DPASV) method. Studies of copper metal pollution in seawater using XRF

method of analysis remain implicit especially in relation to the Kenyan coast.

e) Zinc in sea water.

The concentration levels of zinc in water samples are presented in Table 4.22 and they varied as follows; Gazi [12.6 ± 0.6 µg ml^{-1} to 17.4 ± 0.9 µg ml^{-1}];

Kibuyuni [4.5 ± 0.2 µg ml^{-1} to 27.0 ± 1.4 µg ml^{-1}];

KWS [3.9 ± 0.2 µg ml^{-1} to 21.0 ± 1.0 µg ml^{-1}];

Makupa [7.9 ± 0.4 µg ml^{-1} to 12.0 ± 0.6 µg ml^{-1}];

Tudor [6.9 ± 0.4 µg ml^{-1} to 49.5 ± 2.5 µg ml^{-1}]. The highest mean concentration levels were reported for Tudor samples while the lowest in Makupa. There is a nearby dumpsite in Makupa and hence likelihood of zinc forming insoluble compounds with the other contaminant/pollutants. Zinc enrichment at Tudor can be explained by the fact that zinc is a common contaminant of surface and groundwater, storm water, run-offs, and industrial waste streams (UNEP, 2006b). Industrial effluents containing significant levels of zinc include steel works with galvanizing operations, zinc and brass metal works, and production of viscose rayon yarn, ground wood pulp, and newsprint paper.

Reported highest concentration levels of zinc in industrial waste streams reach $48,000$ µg l^{-1} (Weigner, 2000) and US EPA (1996) guidelines levels of zinc stands is 81.0 µg l^{-1}. A study by Pe´rez-Lo´pez *et al*., (2003), reported a range of 38.47 ± 5.29 µg l^{-1} to 52.85 ± 6.04 µg l^{-1} in seawater of an Estuary in North West Spain which were below the threshold levels for zinc toxicity in water intakes (EPA, 1996).

In another study in East London and Port Elizabeth harbours, Fatoki *et al.,* (2001) reported Zn levels of 0.5 - 27.6 µg l^{-1} in seawater 0.7 - 16.2 µg l^{-1} in seawater respectively. These results indicated significant contributions of heavy metal pollution from storm water drains and discharges from streams which carry run-offs from industrial, urban and residential sources (Fatoki *et al.* 2001; Weigner, 2000).

Table 4.20: Concentration levels of trace elements in seawater (μg ml^{-1}).

Sample	Ca Conc.	Mn Conc.	Fe Conc.	Cu Conc.	Zn Conc.
Gw1	114 ± 6	21.8 ± 1.0	24.3 ± 1.2	10.7 ± 0.5	17.4 ± 0.9
Gw2	99.2 ± 4.9	17.5 ± 0.9	11.5 ± 0.6	10.0 ± 0.5	14.0 ± 0.7
Gw3	72.9 ± 3.6	15.8 ± 0.8	18.3 ± 0.9	9.9 ± 0.5	12.6 ± 0.6
Gw4	72.5 ± 3.6	19.5 ± 1.0	12.9 ± 0.7	5.3 ± 0.3	15.6 ± 0.8
KIBw1	167 ± 8	25.4 ± 1.2	50.4 ± 2.0	15.2 ± 0.8	23.1 ± 1.2
KIBw2	110 ± 6	25.2 ± 1.2	58.8 ± 2.4	9.8 ± 0.5	27.0 ± 1.4
KIBw3	83.7 ± 4.2	5.6 ± 0.3	12.6 ± 0.6	3.1 ± 0.2	4.5 ± 0.2
KIBw4	57.7 ± 2.9	7.5 ± 0.4	26.2 ± 1.3	4.1 ± 0.2	6.0 ± 0.3
KW1	87.8 ± 4.4	4.9 ± 0.2	7.5 ± 0.4	13.8 ± 0.7	3.9 ± 0.2
KW2	113 ± 6	13.9 ± 0.7	12.8 ± 0.6	15.3 ± 0.8	11.1± 0.6
KW3	118 ± 6	26.3 ± 1.3	16.2 ± 0.8	13.7 ± 0.7	21.0 ± 1.0
KW4	112 ± 6	13.9 ± 0.7	12.6 ± 0.6	12.8 ± 0.6	11.1 ± 0.6
Mw1	94 ± 4.7	10.5 ± 0.5	30.1 ± 1.5	18.9 ± 0.9	8.4 ± 0.4
Mw2	95 ± 4.7	9.9 ± 0.5	52.1 ± 2.6	8.3 ± 0.4	7.9 ± 0.4
Mw3	91 ± 4.5	12.0 ± 0.6	24.8 ± 1.2	9.9 ± 0.5	9.6 ± 0.5
Mw4	72 ± 3.6	15.0 ± 0.8	29.8 ± 1.5	9.8 ± 0.5	12.0 ± 0.6
Tw1	108 ± 5	18.6 ± 0.9	10.0 ± 0.5	12.0 ± 0.6	14.9 ± 0.8
Tw2	118 ± 6	22.9 ± 1.1	26.3 ± 1.3	11.7 ± 0.6	18.3 ± 0.9
Tw3	99.1 ± 4.9	41.7 ± 2.1	46.1 ± 2.3	16.2 ± 0.8	49.5 ± 2.5
Tw4	110 ± 6	8.6 ± 0.1	17.3 ± 0.9	15.2 ± 0.8	6.9 ± 0.4

4.4.4: Graphical presentation of elemental variations in sea sediment samples.

Figure 4.6 shows the variation of trace elements in the sea sediment and seawater samples from Gazi, Kibuyuni, KWS, Makupa and Tudor sites respectively. In general, the results obtained shows that calcium concentration levels were relatively high in the sea sediments whereas manganese concentration values were below detection limit. The graph presents mean values for iron, copper and zinc from the five sampling sites.
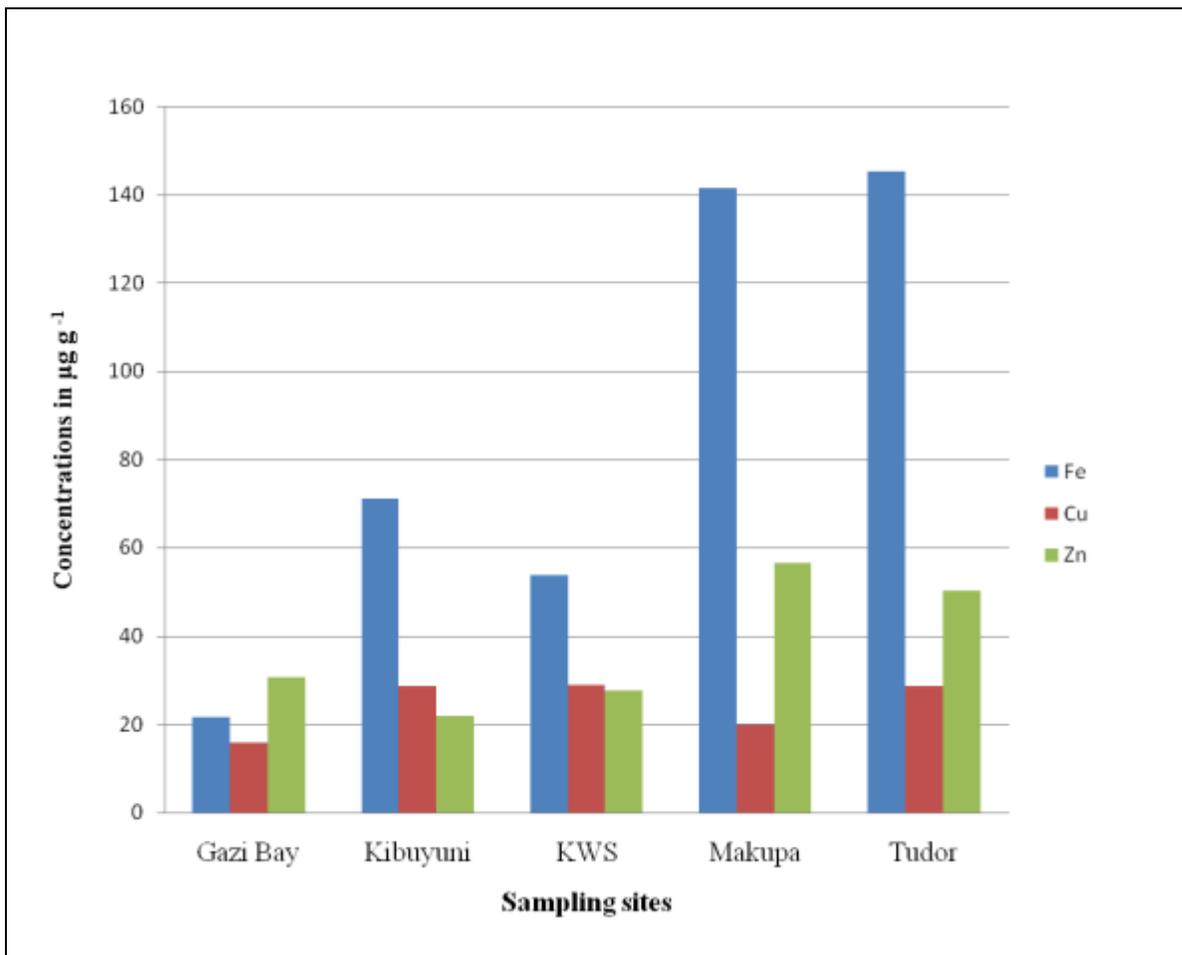
Fig. 4.6: Mean concentrations of trace elements in sea sediments (μg g^{-1}) from Kenyan Coast

4.4.5: Graphical presentation of elemental variations in seawater samples.

Figure 4.7 shows the variation of trace elements in the seawater samples from Gazi, Kibuyuni, KWS, Makupa and Tudor sites respectively. Calcium concentration levels in seawater were generally higher as compared to other trace elements and was not included in the plot. The graph presents mean values for manganese, iron, copper and zinc from the five sampling sites.
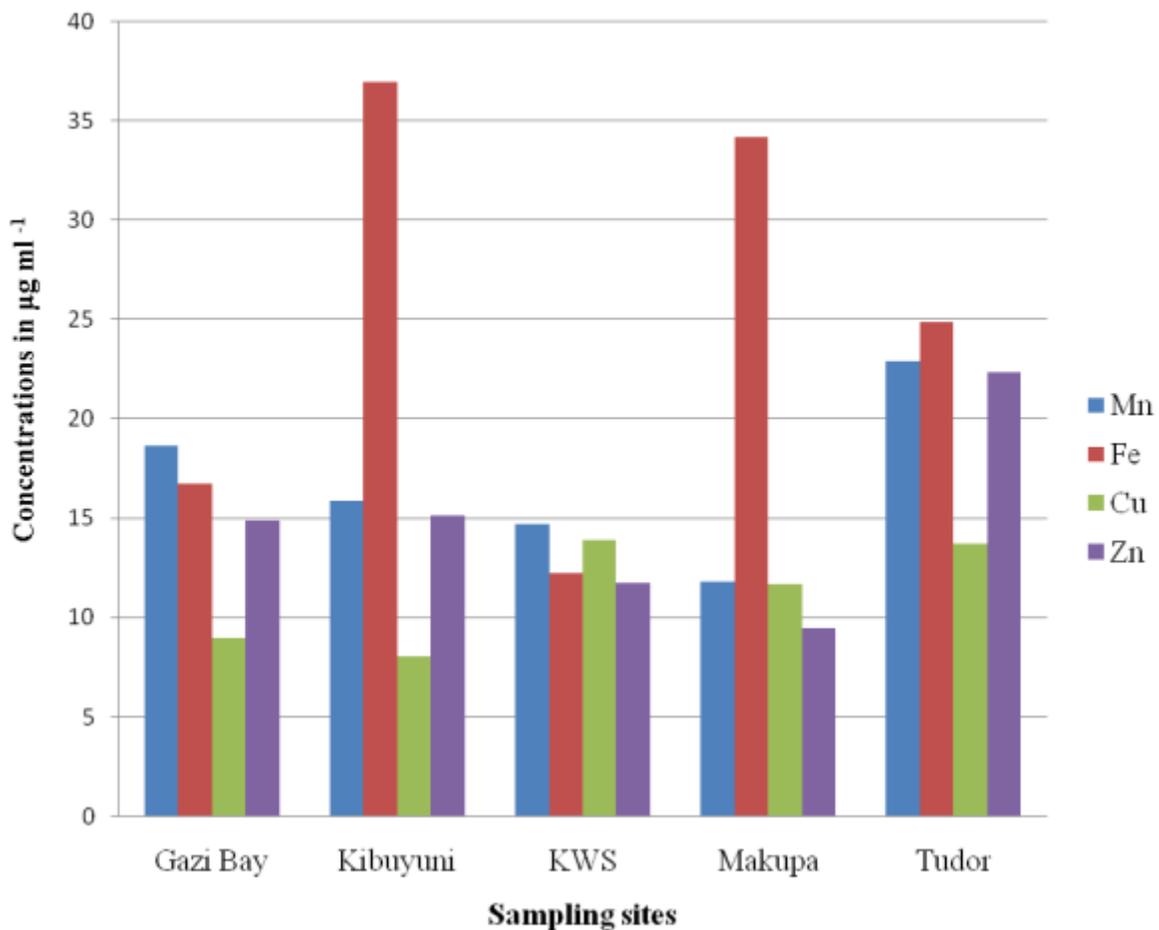
Fig. 4.7: Mean concentrations of trace elements in sea water (µg ml^{-1}) from Kenyan Coast

4.5   General discussion

In this section, the findings of this study is discussed further with reference to the literature review regarding the trace elements in seaweeds, sea water and sea sediments.

4.5.1: Variation of trace elements in seaweeds

Seaweeds samples collected in June, 2010 from Kibuyuni had higher concentration levels of trace elements than those sampled in October, 2009. This can be attributed to variability of the seasons with June being in the mid of rainy season and October shifting towards dry season. Ocean chemistry differs markedly between seasons. The ocean is fed in the wet season by rainwater and surface run-off which carries nutrients and organic matter thus enriching trace element concentrations.

The trace elements concentrations levels in seaweed samples decrease in the following order of calcium, iron, manganese, zinc and copper. Calcium was highest in all samples followed by iron and manganese with zinc recording low values as shown in the results tables in the preceding sections. With reference to other studies, results obtained in this study are comparable and shows same trends and attributes in trace metal enrichments in the seaweeds (Appendices Table 5.4). The bioavailability concentrations varied relatively in a decreasing order with increase in the total content. This can be attributed to the relative mineral balance in the seaweeds and also the seasonal variations due to stages in seaweed of growth.

4.5.2: Variations of trace elements concentration levels in sea sediments and sea water

Sea sediment and sea water samples had high variations in concentration levels for most trace elements as compared to seaweeds. Trace elements concentration levels in sediment in decreasing order were; calcium, iron, zinc, copper and lastly manganese that was below detection limit (<99 μg g^{-1}).

Calcium concentration levels were highest in Kibuyuni samples followed by those sampled from KWS and Gazi respectively. Makupa and Tudor samples had concentration levels below detection limit (<962 µg g^{-1}).

Iron concentration levels were highest in Tudor samples followed by those sampled from Gazi, Kibuyuni, KWS and Makupa respectively.

Zinc concentration levels were highest in Makupa samples, then Tudor, Gazi, KWS and Kibuyuni samples respectively.

For copper, concentration levels were highest in samples from KWS, Kibuyuni, Tudor, Makupa and Gazi Bay in that order. Manganese concentration levels were below detection limit

(<99 µgg^{-1}) in all the sea sediment samples.

For the sea water samples, the trace elements concentration levels, in a decreasing order were; calcium, iron, manganese, zinc and copper respectively. Highest concentration levels were recorded in samples from Makupa and lowest in those sampled at the Kenya Wildlife Service in Shimoni, South Coast (Table 4.20).

The high values of calcium in Kibuyuni samples can be associated to the nearby presence of coral reefs that forms the sea bed on which seaweeds are attached and or float. However, the mean concentration levels were varied as follows in decreasing order: calcium mean concentration - Tudor, KWS, Makupa, Kibuyuni and Gazi; iron mean concentration - Kibuyuni, Makupa, Tudor, Gazi and KWS respectively; manganese mean concentration -Tudor, Gazi, Kibuyuni, KWS and Makupa in that order; zinc mean concentration −Tudor, Gazi, Kibuyuni, KWS and Makupa in that order; copper mean concentration-KWS, Kibuyuni, Tudor, Makupa and Gazi respectively.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Summary of the findings

It was observed that calcium and iron concentrations in seaweeds, water and sediment were relatively higher than other trace elements in all of the five sampling sites. Manganese, copper and zinc were relatively low while chromium, cobalt, mercury, arsenic and lead concentration levels were below detection limits in the samples analyzed.

5.2 Research hypothesis

5.2.1 The edible seaweeds are safe for human consumption and form a good dietary supplement.

From the findings of this study, edible seaweeds are highly rich in calcium and iron and also manganese, though presence in low concentration levels. These are essential elements in dietary supplement. Other trace elements such as copper and zinc were also detected in all seaweed samples and are as well useful in dietary intakes due to their unique metabolic functions in the body. Therefore, the findings of this study justify the hypothesis of the study.

5.2.2 Seaweeds are not safe for human consumption.

Preceding studies done elsewhere in the world indicate that seaweed has a high metal accumulation capacity and hence toxic to human beings. However, this study reported relatively low concentrations of heavy metal in seaweed samples implying that they are safe for human consumption. Relatively high heavy metal concentrations were reported in sea sediments from Makupa and Tudor whereas low mean levels were recorded in the samples from Kibuyuni and Gazi Bay because of (i) no or little anthropogenic activities and (ii) no industrial/domestic discharges at these sites. Therefore, it can be said that Makupa and Tudor Creek experiences

high levels of pollutants as compared to other sampling sites.

Due to wide variations in concentrations of trace elements in sea sediments and sea water, seaweeds are most preferred as marine pollution indicators.

5.3 Conclusion

From the findings of this study, the use of seaweeds as dietary supplement is supported by its high concentration levels of calcium and iron. Manganese, copper and zinc were also found to form part of the mineral content of the seaweeds and they are essential due to their unique metabolic functions in the body. The levels of trace elements were within the recommended dietary allowance and intakes.

Toxic elements such as lead and arsenic were not detected implying that their concentrations are not to any alarming levels in seaweeds from the Kenyan coast hence the edible seaweeds can be harnessed freely as food supplement. With the immense nutritional benefits associated with the edible seaweeds as discussed in this study, its use as food product should be greatly encouraged. Regarding its immense economic potential, seaweed farming will create employment opportunities to Kenyan coastal communities thereby improving their livelihoods and contribution to the national gross domestic product (GDP).

In relation to marine pollution monitoring, the elemental concentration levels in seaweeds collected in October, 2009 during dry season were relatively lower than those sampled in June, 2010 - the wet season. This can be attributed to the increased surface run-off during the rainy season as well as the hydrodynamic regime; water-rock interactions in the ocean.

In general, the measurement results of concentration levels of trace elements in seaweed samples were consistent as compared to results for sea sediments and sea water. This observation is concurrent with studies done in other parts of the world.

Sea sediments and seawater had wide variations and inconsistencies as discussed in this study and also backed by results obtained in other regions. At the same time, as postulated by ocean chemists and researchers, heavy metal levels in the sediment samples can be changed by organic matter content, grain size composition, pH and oxidation-reduction potential among other factors which fundamentally do not affect the concentrations in marine organisms.

Therefore, the variations in trace element concentrations in sea sediment and seawater make seaweeds the most preferred option for marine pollution (biological indicators) due to their high absorption capacity and consistency in the measurements of concentration levels of trace elements.

5.4 Recommendations

1. Seaweeds have great economic potential as witnessed in China, Japan and other Orient countries with the world market estimated at approximately US$ 6 billion per annum. Regionally, communities in Zanzibar have reaped seaweeds benefits for over two decades now. Therefore, the government should encourage its cultivation along the Kenyan coast through an Economic Stimulus Plan. This will greatly improve the livelihoods of the coastal communities as well as boost the gross domestic product (GDP) hence achievement of MDG's and the Kenya's Vision 2030.

2. Government authorities are advised to legislate on recommended dietary intakes of metals in edible seaweeds to promote its direct use as food product in Kenya.

3. The uses of seaweeds as bio re-mediators and/or biological indicators (have high metal accumulation capacity) of marine pollution should be encouraged to ensure sustainable marine pollution monitoring.

4. From the findings of this study, it is evident that anthropogenic pollution is on increase around Mombasa city as indicated by data obtained from Makupa and Tudor samples. Appropriate measures such as legislations on environmental sustainability and physical planning in coastal developments should be undertaken to protect the marine ecosystems.

5.5 Suggestion for further research.

1. Due to limitation of time and resources, this study did not cover the entire Kenyan coastal region hence the need to expand and add more information on current state of the Kenyan marine ecosystem with regard to heavy metal and trace element contamination as well as climate change implications.

2. Research on social economic impacts of seaweeds cultivation in Kenya is highly recommended in order to have fast hand information on product markets and general public opinions on the way forward in regard to seaweed industry.

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Appendices

Picture 1: Farmers at Kibuyuni harvest Seaweeds

Picture 2: Extensive seaweed farms at the coast

Picture 3: Close-up photograph of *Euchemoids*

Picture 4: *Euchemoids* sprouding in the sea

Picture 5: Brown/red and green seaweeds

Picture 6: *Eucheuma* Seaweed salads

Fig. 5.1 Assorted pictures regarding seaweeds

Table 5.1: Summary data for total content and bio-available ($\mu g\ g^{-1}$) trace elements in seaweeds per sampling sites.

Sampling Sites		Ca	Mn	Fe	Cu	Zn
Kibuyuni, 2010	Range	447 -**1253***	3.4 - **10.1***	12.3 - 100	0.4 -**4.6***	1.8 - **7.6***
	Mean	761	5.8	42.4	1.6	4.4
KWS, 2009	Range	271 - 664	1.6 - 9.1	17.5 – 60.6	0.4 -.1.3	0.8 - 3.0
	Mean	451	4.3	32.2	0.7	1.7
Kibuyuni,2009	Range	**217** – 1003	**1.0** - 7.4	**7.0** -**123***	**0.3** -.1.3	**0.3** - 2.3
	Mean	554	4.3	44.2	0.6	1.3
Bio-available ($\mu g\ g^{-1}$)						
Kibuyuni, 2010	Range	145 - **398***	0.8 - **3.9***	2.1 - 9.6	0.1 - **0.6***	0.4 - **1.6***
	Mean	253	1.8	4.5	0.3	0.9
KWS, 2009	Range	115 - 224	0.4 - 0.9	1.9 – **16.6***	**0.01** - 0.1	0.1 - 0.3
	Mean	174	0.6	7.7	0.1	0.2
Kibuyuni,2009	Range	**36.1** - 266	**0.1** - 1.8	**1.8** -16.5	**0.01** - 0.1	**0.01** - 0.2
	Mean	163	0.7	5.6	0.1	0.1

(Bold)* = Highest concentration for the particular element as per the theme of study

(Bold) = Lowest concentration for the particular element as per the theme of study (Total content or bioavailable)

Table 5.2: Summary of elemental concentrations in sea sediment ($\mu g\ g^{-1}$)

Sample		Ca Sediment	Mn Sediment	Fe Sediment	Cu Sediment	Zn Sediment
Gazi	Range	**<962**-1117	**<99**	**<62**-90.7	13.5-21.1	**19.0**-57.5
	Mean	1,064	**<99**	86.4	15.7	30.5
Kibuyuni	Range	1969-**2555***	**<99**	<61.6-123	**<13-41.2***	19.1-24.8
	Mean	2,198	**<99**	94.6	28.8	21.7
KWS	Range	939-1817	**<99**	<62-80.7	26.0-32.4	25.1-30.7
	Mean	1,213	**<99**	71.8	28.9	27.7
Makupa	Range	**<962**	**<99**	118-160	13.4-41.2	42.9-**78.4***
	Mean			141	19.9	56.5
Tudor	Range	**<962**	**<99**	78.2-**225***	24.4-33.4	25.1-74.9
	Mean			145	28.6	50.1

(Bold)* = Highest concentration for the particular element , **(Bold)** = Lowest concentration for the particular element

Table 5.3: Summary of elemental concentrations in sea water (µg ml^{-1})

Sample		Ca Water	Mn Water	Fe Water	Cu Water	Zn Water
Gazi	Range	68.9 - 120	15.0-22.8	10.9-25.5	5.0-11.2	12.0-18.3
	Mean	89.5	18.6	16.7	9.0	14.9
Kibuyuni	Range	**54.8**- **175***	5.3-26.6	12.0-**61.2***	**2.9**-16.0	4.3-28.4
	Mean	86.5	15.9	37.0	8.1	15.2
KWS	Range	83.4 - 124	**4.7**-27.6	**7.1**-17.0	12.2-16.1	**3.7**-22.0
	Mean	108	14.7	12.3	13.9	11.8
Makupa	Range	68.4– 99.7	9.4-15.8	23.6-54.7	8.1-**19.8***	7.5-15.7
	Mean	106	11.8	34.2	11.7	9.5
Tudor	Range	94.2 - 124	8.5-**43.8***	9.5-48.4	11.1-17.0	6.5-**52***
	Mean	109	22.9	24.9	13.8	22.4

(Bold)* = Highest concentration for the particular element , **(Bold)** = Lowest concentration for the particular eleme

Table 5.4: Comparison of trace element content in seaweeds from Kenyan south coast and other regions (µg g^{-1}, DW seaweed)

Origin	Ca	Mn	Fe	Cu	Zn
Spain (Ru´perez , 2002)	41.5-101	-	0.3 -1.1	-	0.18 – 0.72
Malaysia (Krishnaiah *et al*., 2008)	41.1 – 100	-	0.7 – 1.1	<0.55	0.2 – 0.7
Gulf of Mannar, Southeast Coast of India (Karthikai *et al.,* 2009)	534 - 3055	0.31 – 4.44	3.94 – 50.82	0.38 - 1.54	0.65 – 4.28
Mandapan Coastal Regions, Southeast Coast of India (Anantharaman P. *et al.,* 2010)		0.72 – 4.08	17.53 -62.47	0.87 - 1.70	0.32– 0.74
Kenyan coast (This study)	217 – 1253	1.0 – 10.1	7.0 – 123	0.3 – 4.6	0.3 -7.6

DW = Dry weight

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Table 5.5: Comparison of trace elements in sea sediments from Kenyan Coast and other select regions (μg g^{-1}, per dry weight)

Region	Mn	Fe	Cu	Zn
Kenyan coast: This study				
Makupa	<99	118-160	13.4-41.2	42.9-78.4
Tudor	<99	78.2-225	24.4-33.4	25.1-74.9
Gazi Bay	<99	<62-90.7	13.5-21.1	19.0-57.5
KWS	<99	<62-80.7	26.0-32.4	25.1-30.7
Kibuyuni	<99	<62-123	13.3-41.2	19.1-24.8
Kenyan Coast: Other studies				
Makupa (Muhoi *et al,.*2002)	-	-	50.9 -229	276 -3193
Port Reitz (Muhoi *et al,.*2002)	-	-	11.3 -45.1	30.7 - 92.9
Port Kilindini (Nyatebe , 1990)	-	-	32 ± 20	34 -186
Worldwide:				
Gulf of Paria, Trinidad & Tobago (Wendy , 2005)	46.1 - 1175	3200 -18900	0.8 - 39.7	13.6 - 170
Tolor harbour, Hong Kong China (Wong *et al.,*1980)	-	-	6.80 -231	38.9

Table 5.6: Comparison of trace element in seawater from Kenyan Coast (µg/ml) and other selected regions (µg/L)

This study: Kenyan Coast	Ca	Mn	Fe	Cu	Zn
Sample	**Water**	**Water**	**Water**	**Water**	**Water**
Gazi	89.5	18.6	16.7	9.0	14.9
Kibuyuni	86.5	15.9	37.0	8.1	15.2
KWS	108	14.7	12.3	13.9	11.8
Makupa	106	11.8	34.2	11.7	9.5
Tudor	109	22.9	24.9	13.8	22.4
Other Studies					
Fatoki *et al.,* (2001) East London Harbour	-	-	-	-	0.5-27.6
Port Elizabeth harbor, SA					0.7-16.2
North West Spain (Peˊrez-Loˊpez *et al.*, 2003)	-	-	-	0.08 -1.1	33.18 – 58.89

Table 5.7: Dietary Reference Intakes

Dietary Reference Intakes (DRIs): Recommended Intakes for Individuals, Elements

Life Group	Calcium (µg/d)	Chromium (µg/d)	Copper (mg/d)	Iron (µg/d)	Manganese (µg/d)	Zinc (mg/d)
Infants						
0–6	210*	0.2*	200*	0.27*	0.003*	2*
7–12	270*	5.5*	220*	**11**	0.6*	**3**
Children						
1–3	500*	11*	**340**	**7**	1.2*	**3**
4–8	800*	15*	**440**	**10**	1.5*	**5**
Males						
9–13	1,300*	25*	**700**	**8**	1.9*	**8**
14–18	1,300*	35*	**890**	**11**	2.2*	**11**
19–30	1,000*	35*	**900**	**8**	2.3*	**11**
31–50	1,000*	35*	**900**	**8**	2.3*	**11**
51–70	1,200*	30*	**900**	**8**	2.3*	**11**
>70	1,200*	30*	**900**	**8**	2.3*	**11**
Females						
9–13	1,300*	21*	**700**	**8**	1.6*	**8**
14–18	1,300*	24*	**890**	**15**	1.6*	**9**
19–30	1,000*	25*	**900**	**18**	1.8*	**8**
31–50	1,000*	25*	**900**	**18**	1.8*	**8**
51–70	1,200*	20*	**900**	**8**	1.8*	**8**
>70	1,200*	20*	**900**	**8**	1.8*	**8**
Pregnancy						
14–18	1,300*	29*	1,000	**27**	2.0*	**12**
19–30	1,000*	30*	1,000	**27**	2.0*	**11**
31–50	1,000*	30*	1,000	**27**	2.0*	**11**
Lactation						
14–18	1,300*	44*	1,300	**10**	2.6*	**13**
19–30	1,000*	45*	1,300	**9**	2.6*	**12**
31–50	1,000*	45*	1,300	**9**	2.6*	**12**

NOTE: This table presents Recommended Dietary Allowances (RDAs) in bold type and Adequate Intakes (AIs) in ordinary type with an asterisk (*).
RDAs and AIs may both be used as goals for individual daily intakes

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