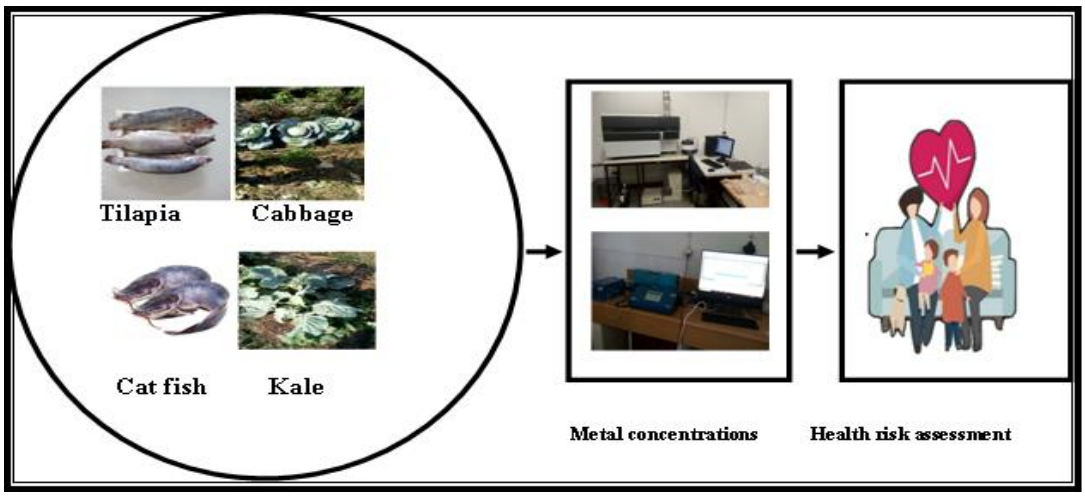
UNIVERSIDADE EDUARDO MONDLANE
FACULTY OF SCIENCE
DEPARTMENT OF CHEMISTRY

Master's in Chemistry and Processing of Local Resources

Dissertation

Potential health risks of trace metals in fish tissue muscle (tilapia and catfish) and vegetables (cabbage and kale) from some agricultural fields and markets in Mozambique



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DECLARATION

I, Vedaste Munyeshuri, do hereby declare to the Master's Commission of Faculty of Science, Chemistry Department of the Eduardo Mondlane University, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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The above declaration is confirmed by:

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ABSTRACT

Concentrations of metals Al, As, Cd, Cu, Fe, Pb and Zn in the muscles of two fish species tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)* and two vegetable cultivars namely cabbage (*Brassica oleracea var. capitata)* and kale *(Brassica oleracea var. acephala)* were determined using inductively coupled plasma atomic emission spectroscopy (ICP-OES) and Hg by Lumex Mercury Analyzer. The metal concentrations in fish samples range are as follows: 38.0-644; 5.65-12.7; 1.05-12.9; 5.25-18.9; 21.0-168; 0.0012-0.033; 1.9-6.5 and 30.8-52.2 mg kg^{-1} for Al, As, Cd, Cu, Fe, Hg, Pb and Zn, respectively. In vegetable samples, the concentration ranges are: 0.25-724.5; 1.6-19.7; 0.8-5.2; 0.5-24.8; 1.0-95.7; 0.001-0.018; 0.4-6.2 and 1.4-76.5 mg kg^{-1} for Al, As, Cd, Cu, Fe, Hg, Pb and Zn, respectively. One-way ANOVA shows that for analysed metals, the concentration is significantly different between sampling sites ($p < 0.05$) for both fish and vegetable samples. The calculated estimated daily intakes (EDI) shows that for all determined analytes, the EDI values (mg $kg^{-1}day^{-1}$) are lower than the established provisional tolerable daily intake (PTDI) for both fish and vegetable samples.

The average EDI values of metals through fish consumption are in a decreasing order as follows: Al >Fe >Zn >Cu >As >Cd >Pb>Hg. In vegetables, the average EDI of metals is in a decreasing order: Al >Fe >Zn >Cu >As >Pb>Cd >Hg for cabbage and Al >Fe >Zn >Cu >As >Pb>Cd >Hg for kale. The target hazard quotient (THQ) value was > 1 for As in all the fish samples whereas for Cd the target hazard quotient (THQ) value is >1 in fish samples from sites of MaRIV, TeEST and NaLAL. Therefore, As and Cd are the major contributors to the hazard index (HI) in the studied fish samples which ranges between 40.6-89.5%. For vegetables, the THQ values of As and Cd are >1 for both vegetable cultivars. Likely to the fish samples, As and Cd are the major contributors to the HI in proportions ranging between 70.4-85.5 and 12.1-20.4 % respectively, for As and Cd. The results of calculated maximum allowable fish consumption **(**CR_{lim}) show that As is associated with the smallest CR_{lim} values while Hg is with highest CR_{lim} values and the decreasing order of CR_{lim} in Kg day^{-1} is Hg >Fe >Al >Zn >Pb>Cu >Cd >As. On the side of vegetables, the decreasing order of CR_{lim} is as follows Al >Fe >Zn > Hg >Cu >Pb >Cd >As and Zn >Al >Fe >Hg >Cu >Pb>Cd >As for cabbage and kale, respectively. Generally, based on the concentration and calculated health risk assessment parameters, we notice that healthcare should be taken on the continuous exposure to the fish and vegetables of the areas involved in this study because all evaluated metals were found in levels which are not quite safe for health with a particular emphasis on Al, As, Cd and Pb.

Keywords: Fish, Vegetable, Heavy metal, Health risk, ICP-OES, Mozambique

RESUMO

As concentrações de metais Al, As, Cd, Cu, Fe, Pb e Zn foram determinadas por espectrometria de emissão atómica com plasma indutivamente acoplado (ICP-OES), e o Hg através de um analisador de mercúrio (Lumex) nos músculos de duas espécies de peixes tilápia (*Oreochromis mossambicus*) e bagre (*Chrysichthys nigrodidatatus*) e duas cultivares que incluem o repolho (*Brassica oleracea var. capitata*) e a couve (*Brassica oleracea var. acephala*). Os resultados revelam que as concentrações de metais nas amostras de peixe variam da seguinte forma: 38,0-644; 5,65-12,7; 1,0-12,9; 5,25-18,9; 21,0-168; 0,0012-0,033; 1,9-6,5 e 30,8-52,2 $mgkg^{-1}$ para Al, As, Cd, Cu, Fe, Hg, Pb e Zn, respectivamente. Em amostras de vegetais, os intervalos de concentração são: 0,25-724,5; 1,6-19,7; 0,8-5,2; 0,5-24,8; 1,0-95,7; 0,001-0,018; 0,4-6,2 e 1,4-76,5 $mgkg^{-1}$ para Al, As, Cd, Cu, Fe, Hg, Pb e Zn, respectivamente. A ANOVA unimodal mostra que para os metais Al, As, Cd, Cu, Fe, Hg, Pb e Zn, a concentração é significativamente diferente entre os locais de amostragem ($p < 0,05$) de peixes e vegetais. As ingestões diárias estimadas calculadas (EDI) mostram que, para todos os metais determinados, os valores de EDI ($mg\ Kg^{-1}dia^{-1}$) são menores do que a ingestão diária tolerável provisória estabelecida (PTDI) para ambos, peixes e vegetais.

O EDI médio dos metais por meio do consumo de peixes apresenta-se por ordem decrescente da seguinte forma: Al >Fe >Zn >Cu >As >Cd >Pb >Hg. Em vegetais, o EDI médio dos metais está em ordem decrescente: Al >Fe >Zn >Cu >As >Pb >Cd >Hg para o repolho e Al >Fe >Zn >Cu >As >Pb >Cd >Hg para a couve. O valor do quociente de risco alvo (THQ) é >1 para As em todas as amostras de peixe, enquanto que para o Cd o valor de THQ é > 1 apenas para as amostras de peixe dos locais MaRIV, TeEST e NaLAL. As e Cd são os maiores contribuintes para o índice de perigo (HI) nas amostras de peixes estudadas que variam entre 40,6-89,5%. Para vegetais, os valores de THQ de As e Cd foram menores que 1 para ambos os vegetais estudados. Para as amostras de peixe, os teores de As e Cd foram os maiores contribuintes para o HI em proporções que variam entre 70,4-85,5 e 12,1-20,4%, respectivamente, para As e Cd. Os resultados do consumo máximo permitido de peixe calculado (CR_{lim}) mostram que o As é associado aos menores valores de CR_{lim}, enquanto que o Hg revelou o maior valor de CR_{lim} (i.e., CR_{lim} em $Kg\ dia^{-1}$ segue a ordem Hg >Fe >Al >Zn >Pb >Cu >Cd >As). Para os vegetais, a ordem de CR_{lim} é na ordem decrescente Al >Fe >Zn >Hg >Cu >Pb >Cd >As e Zn >Al >Fe >Hg >Cu >Pb >Cd >As, respectivamente, para repolho e couve. Com base na concentração e nos parâmetros de avaliação de risco à saúde calculados, recomenda-se a tomada de cuidados de saúde na exposição contínua aos peixes e vegetais das áreas incluídas neste estudo porque todos os metais avaliados apresentam níveis que não são muito seguros para saúde com particular ênfase para o Al, As, Cd e Pb.

Palavras Chaves: Peixe, Vegetal, Metal Pesado, Risco de saúde, ICP-OES, Moçambique

DEDICATION

I wish to dedicate this work to my beloved wife, my son and daughters, my father, mother, brothers as well as sisters

LIST OF ABBREVIATIONSAND SYMBOLS

2M: Dois M

AG: Angónia

ANHMRC: Australian National Health Medical Research Council

ANOVA: Analysis of Variance

ANZFA: Australian New Zealand Food Authority

ATSDR: Agency for Toxic Substances and Disease Registry

BOA: Boane

BoMAF: Boane-Mafuiane

BPA: Bisphenol A

CEC: Cation Exchange Capacity

CR_{lim}: Allowable daily consumption limit

CRM: Certified Reference Material

DALYs: Disability-Adjusted Life Years

DNA: Deoxyribonucleic acid

EDC: Endocrine Disrupting Chemical

EDI: Estimated Daily Intake

EFSA: European Food Safety Authority

EU: European Union

FSAI: Food Safety Authority of Ireland

HI: Hazard Index

HMs: Heavy Metals

ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy

IHME: Institute for Health Metrics and Evaluation

JECFA: Joint Expert Committee on Food Additives

LARD: Larde

MACH: Machava

MaRIV: Matola-River

MOA: Moamba

MoKUR: Moamba-Corumana

MPL: Maximum Permissible Limit

MTZ: Moatize

NaINT: Nampula-Inthaka

NaLAL: Nampula-Lalane

NaMAG: Nampula-Maganha

PAHs: Polycyclic aromatic hydrocarbons

PATR: Patrice

PCBs: Polychlorinated biphenyls

POM: Percentage Organic Matter

PTDI: Provisional Tolerable Daily Intake.

PTWI: Provisional Tolerable Weekly Intake

RfD: Oral Reference Dose
SESA: Strategic Environmental and Social Assessment

TBCFs: Toxicity and Bio-concentration Factors

TeEST: Tete-Estima

TEs: Toxic Elements

THQ: Target Hazard Quotient

USEPA: United States Environment Protection Agency
ZV: Zona Verde

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CHAPTER ONE

1. INTRODUCTION

1.1. Background

Contamination of food products by toxic elements (TEs) has become a worldwide problem for environment and human beings (Cui *et al*., 2005;Chen *et al.*, 2011). Heavy metals (HMs) are a common occurrence of TEs in the environment and have resulted from various natural and anthropogenic activities for the entire history of mankind (Kamunda *et al.*, 2016). Depending on the context, the criteria used to define heavy metals (HMs) can vary, but the term generally refers to metallic/metalloid chemical elements with relatively high densities and toxic properties (Jiwan and Kalamdhad, 2011). According to the World Health Organization (WHO), there are 13 HMs of significance to human and environmental health including: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), iron (Fe), zinc (Zn), nickel (Ni), lead (Pb), tin (Sn), and titanium (Ti)(WHO, 2011). Some of these metals like Cu, Fe and Zn are essential for sustaining life, but the vast majority has no safe exposure level (Akintujoye *et al.*, 2013). Others like As, Cd, Pb and Hg exhibit extreme toxicity even at trace levels (Boyd and Rajakaruna, 2013).

HMs enter the environment from both natural and anthropogenic sources (Ali *et al.*, 2019). Various sources of HMs include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, pesticides and fertilizers applied to crops, among others (Morais *et al.*, 2012). Thus, the contamination of environment with HMs is reported to be of great concern, particularly in agricultural production systems (Jafarian and Alehashem, 2013). This is because plants uptake HMs from the soil, leading to successive accumulation in tissues and biomagnification through the food chain and ultimately causing both human health and environment concerns (Jiwan and Kalamdhad, 2011). Also, unlike organic substances, HMs are non-biodegradable and have long biological half-lives (Muchuweti *et al.*, 2006; Ali *et al.*, 2013; Ametepey *et al.*, 2018; Das and Das, 2018). The deleterious effects of HMs do not normally manifest themselves immediately after the toxin enters the organism; but, usually become apparent only after a few years (WHO, 2007; Singh and Giri, 2015). Once absorbed by the human body,

HMs continue to accumulate in vital organs like the brain, liver, bones, and kidneys for years or decades causing serious health consequences (Kabata-pendias and Pendias, 2001).

Fish is considered as an important food ingredient in many countries worldwide, including Mozambique (Doherty *et al.*, 2012). According to FAO, the consumption of fish accounts for 16% of animal protein consumed and 6% of total protein intake by people of the world (Elnabris *et al.*, 2013). It is estimated that about 3.5% of the animal protein intake of the Mozambican population comes from fish and fish products (UNFAO, 2007). Likewise, vegetables constitute an important part of the human diet since they are rich in nutrients required for human health, and are an important source of carbohydrates, vitamins, minerals, and fibers (Farooq *et al.*, 2008; Ogunkunle *et al*., 2016). Although highly nutritionally recommended, both fish and vegetables may accumulate HMs in quantities large enough to cause potential health risks to the consumers (Singh *et al*., 2010). For instance, several studies done on fish and vegetables have shown the potential to accumulate high concentrations of HMs (Ensley *et al.*, 1997; Rahman *et al*., 2012; Taweel *et al.*, 2013; Gebeyehu and Bayissa, 2020).

As, Cd, Pb and Hg are listed among the ten chemicals of major public concern by the WHO for their potential to be carcinogenic and inflict acute organ damage (Tchounwou *et al.*, 2014). They are also reported to be part of the so called Endocrine Disrupting Chemicals (EDCs) (Georgescu *et al.*, 2011). In their behavior as EDCs, HMs were reported to have the potential to significantly alter endocrine balance (Mocarelli *et al.*, 2008; Balabanič *et al*., 2011; Georgescu *et al.*, 2011). They act to mimic the biologic activity of steroid hormones, including androgens, estrogens, and glucocorticoids (Georgescu *et al.*, 2011). For instance, one of the most important mechanisms of Pb toxicity is the mimicking of Ca or disruption of Ca homeostasis (Pohl *et al.*, 1997; Rigby and Warren, 2003). Studies have suspected EDCs to be associated with increased incidence of breast cancer, abnormal growth patterns, and neurodevelopmental delays in children, as well as with changes in immune function (Monneret, 2017). According to the Institute for Health Metrics and Evaluation (IHME), in 2015 Pb exposure only accounted for nearly 0.5 million deaths and 9.3 million life years lost in terms of disability-adjusted life years (DALYs) among adults of 15 years and older, with the highest occurrence in developing countries (Landrigan *et al.*, 2017).

Despite the very few above mentioned of numerous existing issues, the national and international HMs monitoring is not consistent and the reality emerges with particular force among developing

countries where incidents of HM exposure often go unnoticed or unreported, and the public health laws are not properly enforced (Mamtani *et al.*, 2015). Taking into account the potential toxicity, persistent nature, and cumulative behavior of HMs, it is necessary to analyze HMs in foodstuffs to ensure food safety (Chen *et al.*, 2011). Monitoring and assessment of HMs in foodstuffs from the market sites have been carried out in some developed and developing countries (Al-Busaidi *et al.*, 2011; Doherty *et al.*, 2012; Benti, 2014; Saha and Paul, 2016).

1.2. Problem statement and Justification

HMs contamination and accumulation is a serious problem around the world due to the potential threat to food safety and their detrimental effects on human and animal health (Hu*et al.*, 2017). Although some individuals are primarily exposed to these contaminants in the workplace, for most people the main route of exposure to these toxic elements is through the diet (i.e. food and water) (Chen *et al.*, 2011; Morais *et al.*, 2012). However, the excessive and uncontrolled mineral resources exploitation has caused irreversible environmental damages as contaminations of toxic metals in soil and water (Alves and Denise de La Corte, 2017). Some decades ago agricultural practices have been adopted through the uncontrolled use of inorganic fertilizers, biosolids from slugged and manures in order to obtain maximum yields (Alves *et al.*, 2016). Such practices, may accumulate high levels of potential HMs in soils, which may have significant consequences for the quality of plant health, soil biological processes and thus through bio magnifications enter the human body as well (Alves *et al.*, 2016). Many researchers have reported that HMs are easily accumulated in various edible vegetables and fruits through contaminated soil (Odai *et al.*, 2008; Sun*et al.*, 2012; Sharma *et al.*, 2016; Baghaie and Fereydoni, 2019). Therefore, the consumption of vegetables grown in HM-contaminated soils can lead to harmful metabolic and physiological effects on human body (Hu *et al.*, 2017).

Fish is considered as one of the important foods for humans and is used in a variety of diets as it is a good source of digestible proteins, vitamins, minerals, and polyunsaturated fatty acids which support healthy living (Carvalho *et al.*, 2005; Ikem and Egilla, 2008). Being major components of aquatic habitants, fishes are highly vulnerable to HMs contamination because aquatic environments become the sink of huge number of pollutants including HMs (Rohan *et al.*, 2014).

For this reason, fishes act as bio-indicator or bi-monitor or bio-marker of HM levels in aquatic environments, and can be used to evaluate the health risks of such aquatic ecosystems (Farkas *et al*., 2002). The long persistence, bioaccumulation, bio-magnification and toxicity of HMs in the food chain may cause serious health hazards to the humans on consumption (Baki *et al.,* 2018).

However, in Nampula province, Moma district there is occurrence and extraction of heavy mineral sands/titanium where reserves estimated at 842 million tons in minerals like zircon, rutile and ilmenite are produced from Moma mine (MIREME, 2017). On the other hand, the Tete Province, district of Moatize possesses one of the biggest coal reserves of the Permian age, deriving the Moatize Formation actually considered as the biggest coal field province of Mozambique (Alves and Denise de La Corte, 2017). According to José and Sampaio (2012) the mine reserves is estimated over 2.5 billion of tons. The 2017 final mining Strategic Environmental and Social Assessment (SESA) prospection also reported some reserves in iron, gold and rare earths in the same area (MIREME, 2017). In their studies, Alves and Denise de La Corte (2017) have reported that metals such as Al, As, Fe, Mg, Mn and Zn were present in significant concentrations in Zambezi river basin. The exploitation of coal is done in the field of Moatize where are located the Revúbuè, Moatize and Zambezi rivers, all important in the hydric supply to various activities, mainly to subsistence agriculture and artisanal fishery by the surroundings communities (Alves and Denise de La Corte (2017). The proximity of those mining activities to the riverbeds and consequently to communities that use that water as mean of survival and subsistence are subdue to a potential risk of exposure to toxic metals (Alves and Denise de La Corte, 2017).

Although the above sources are susceptible to generate toxic HMs found in these areas, only few data are available about their status in foodstuffs such as fishes and vegetables sold at Mozambican open markets. Several studies on the determination of HMs' concentrations in both fish and vegetables as well as potential health risk of dietary intake of HMs have been reported in several places of the world (Santos *et al*., 2004; Storelli, 2008; Türkmen *et al.,* 2009; Rahman *et al.,* 2012; Copat *et al.*, 2013; Taweel *etal.*, 2013; Alipour *et al.*, 2014; Zhu *et al.*, 2015; Hu *et al.,* 2017; Ametepey *et al.,* 2018; Vongdala *et al.*, 2018; Gebeyehu and Bayissa, 2020). To our knowledge, there have been few reports on the assessment of potential risks of HMs in edible fish and vegetables locally sold at some markets in Mozambique. In this study, the concentrations of As,

Cd, Cu, Fe, Hg, Pb, and Zn were determined in edible tissue muscle of Mozambique tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)* and vegetables including cabbage (*Brassica oleracea var. capitata)* and kale *(Brassica oleracea var. acephala)* from some local open markets across three chosen provinces of Mozambique, namely Maputo, Nampula and Tete. Therefore, this study aims to evaluate the level of HMs concentration for health risk assessment through the estimation of the daily intakes (EDI), the target hazard quotient (THQ) as well as the maximum allowable limit (CR_{lim}). The measured HMs concentration and calculated EDI are compared with maximum permissible limits (MPLs) and the provisional tolerable daily intakes (PTDI), respectively, set by various standard bodies.

1.3. Hypotheses

- The fish tilapia and catfish collected from Moamba, Boane (Umbeluzi river), Matola (Influene basin) are contaminated by the HMs (Al, As, Cd, Cu, Fe, Hg, Pb and Zn), due to the runoff of agrochemicals used in farming;
- The fish tilapia collected from the Zambezi river (Moatize-Xitima) is possibly contaminated by the same HMs as a result from the coal mining activities occurring in Tete province;
- The vegetables (cabbage and kale) cultivated in Moamba, Boane, Matola, Moatize and Larde agricultural fields are eventually contaminated by the HMs (Al, As, Cd, Cu, Fe, Hg, Pb and Zn), due to the use of contaminated water by pesticides and fertilizers;
- Vegetables locally grown and fish collected from Nampula (Maganha, Lalane and Inthaka) are contaminated by the same HMs as a result of the mining activities during mineral extraction and processing.

1.4. Aims and Objectives of the study

1.4.1. Aims of the study

The aim of this study is to determine the occurrence and concentrations of HMs such as Al, As, Cd, Cu, Fe, Hg, Pb and Zn in fish samples (Tilapia and catfish) of the Zambezi and Umbeluzi rivers and vegetable samples (cabbage and kale) from Moamba, Boane, Matola and Nampula (Maganha, Lalane and Inthaka) for health risk assessment.

1.4.2. Specific objectives of the study

The study aims specifically to:

- determine the concentration of Al, As, Cd, Cu, Fe, Hg, Pb and Zn in fish samples, namely Tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)* and vegetables cabbage (*Brassica oleracea var. capitata)* and kale *(Brassica oleracea var. acephala);*
- compare the metal concentrations in tilapia and catfish, cabbage and kale samples to both national and international safety standards;
- investigate the relationship between HMs concentration and anthropogenic sources;
- evaluate the health risk associated with fishes from local rivers and vegetables locally grown.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Overview on Heavy Metals and their occurrence in environment

HMs are a common occurrence in the environment and have resulted from various natural and anthropogenic activities for the entire history of mankind (Kamunda *et al.,* 2016). The term '*heavy metal* 'has been variously defined based on density, atomic mass, atomic number, or other properties including toxicity (Duffus, 2002). For instance, HMs constitute an ill-defined group of inorganic chemicals which exhibit metallic properties, including transition metals, metalloids, lanthanides, actinides (Sharma *et al.,* 2014; Alves *et al.*, 2016). Heavy metals" is a collective term for metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms, such as lead, mercury, and cadmium (Duffus, 2002). According to Duffus (2002), definitions based on the chemical properties of elements would be more useful.

However, the contamination and pollution by heavy metals is a threat to the environment and is of serious concern (Ali *et al.*, 2019). This is because some of them are systemic toxicants known to induce adverse health effects in humans, including cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic and immunologic disorders, and various types of cancer (Tchounwou *et al.,* 2014). HM enrichment in the environment occurs through a number of sources including acid mine drainage, industrial emissions, traffic, domestic sewage, storm water, atmospheric deposition and building materials (Dhanakumar *et al.,* 2015). In developing countries, rapid increase in domestic, agricultural and industrial activities contribute to high levels of HMs in the air, water and soil (Kumar *et al*., 2007).

2.2. Sources of Heavy Metals in environment

HMs enter the environment from both natural and anthropogenic sources (Ali *et al.*, 2019). The most significant natural sources are weathering of rocks, erosion and volcanic activities (Wuana and Okieimen, 2011; Chehregani and Malayeri, 2014). In addition to natural sources, the amount of HMs entering the environment through anthropogenic activities has also increased tremendously (Jan *et al*., 2015). This comes from the fact that, in developing countries, the economic growth which generally relies on industrial and agricultural development has bypassed

environmental protection guidelines to a greater extent (Ikhuoria and Okieimen, 2000; Sahu and Arora, 2008). Some of the anthropogenic sources include mining, smelting, electroplating, use of pesticides and phosphate fertilizers as well as biosolids in agriculture, sludge dumping, industrial discharge, atmospheric deposition (Fulekar *et al.*, 2009; Ali *et al.*, 2019). Human activities have been found to contribute more to environmental pollution due to the everyday manufacturing of goods to meet the demands of the large population (He *et al.*, 2015). The contamination chain of HMs mostly follows a cyclic order such as: industry, atmosphere, soil, water, air, food and human (Morais *et al.*, 2012). It can be noted in the Figure 1, that the content of metals in tissues builds up from left to right, indicating the vulnerability of toxic metals to humans (Masindi and Khathutshelo, 2018).

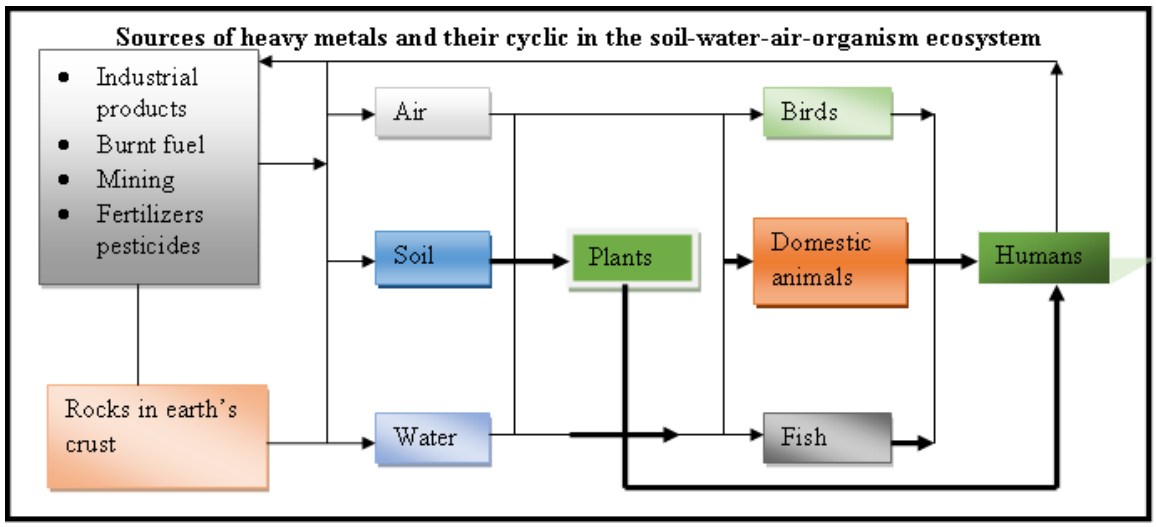

Figure 1: Source of HMs and their cycle in environment, adapted from Masindi and Khathutshelo (2018)

2.3. Bioaccumulation of Heavy Metals in food chains

HM contamination in agricultural lands leads to bioaccumulation of these elements in agricultural crops while such contamination in freshwater bodies like rivers, lakes, and streams leads to bioaccumulation of these elements in fish from freshwater (Ali *et al.*, 2019). The accumulation of HMs in soils and waters poses a risk to the environment and humans (Salem *et al.*, 2000). These elements accumulate in the body tissues of living organisms (bio-accumulation) and their concentrations increase as they pass from lower trophic level to higher trophic level (bio-magnification) (Khan *et al.*, 2010; Ali *et al.*, 2019). Food contamination by HMs is of serious

global concern and particularly in cities of both developing and developed countries where the anthropogenic pressure is very high (Das and Das, 2018).

HMs are persistent environmental pollutants, non-biodegradable, have long biological half-lives, and therefore, pose a serious threat to food chain contamination and humans health (Muchuweti *et al.*, 2006; Sahu and Arora, 2008; Ametepey *et al.*, 2018). The consumption of HM contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intrauterine growth retardation, impaired psychosocial faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyengar and Nair, 2000; Singh *et al.*, 2010). The bioaccumulation of HMs in food chains is due to the buildup of such environmental toxins in soils and also from atmospheric deposition (Wong *et al.*, 2003).

2.4. Bioaccumulation of Heavy Metals in fish

Fish have high levels of unsaturated fatty acids and low levels of cholesterol and constitute an important source of proteins (Malakootian *et al.*, 2016). However, fish are source of 3-omega fatty acids content that are known to contribute to good health (Copat *et al.*, 2012). The use of an edible fish in human diet is beneficial and therefore recommended in balanced diet (Ali *et al.*, 2019). On the other hand, HMs are potentially accumulated in marine and aquatic environments including water, sediments, and fish, and subsequently transferred to human beings through the food (Youssef and Tayel, 2004).

Fishes are exposed to different toxic HMs released to freshwater bodies from different natural and anthropogenic sources (Ali *et al.*, 2019). Thus, the contamination of fish by HMs has become an important global issue because it presents a threat to fish and poses health risks to fish consumers (Rahman *et al.*, 2012). Contamination of fish by toxic HMs is considered as a risk for human health and has raised concerns about their consumption especially in more sensitive groups of human population such as women, children, and people at risk of diseases from other causes (Ali *et al.*, 2019). Different researches have been conducted on HMs bioaccumulation in tilapia fish (Youssef and Tayel, 2004; Abdel-Baki *et al.*, 2011; Ismail and Saleh, 2012; Kaile and Nyirenda, 2016; Saha *et al.*, 2016). The Figure 2 illustrates how humans are contaminated by heavy metals as a result of

the bioaccumulation and biomagnification in aquatic food chain.

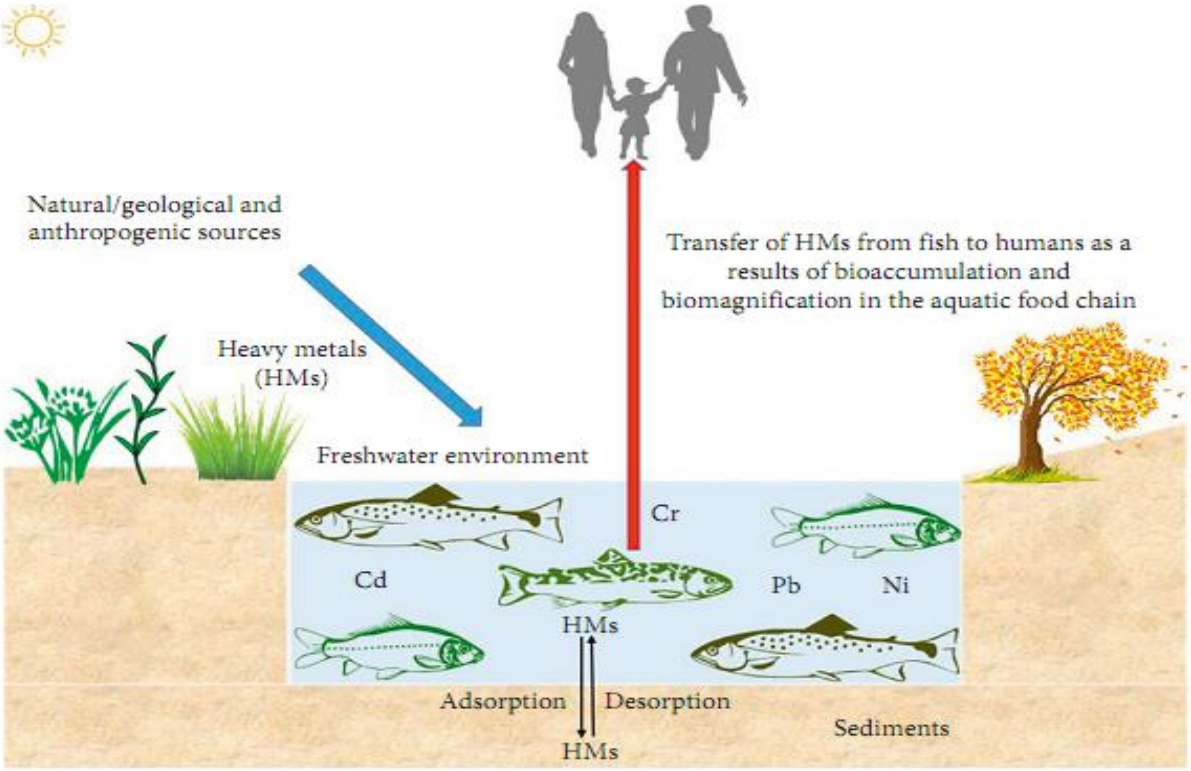

Figure 2: Trophic transfer of HMs from freshwater fish to humans in the food chain (Ali *et al.*, 2019).

2.5. Bioaccumulation of Heavy Metals in vegetables (Case of cabbage and kale)

Vegetables constitute an important part of the human diet since they are rich in nutrients required for human health, and are an important source of carbohydrates, vitamins, minerals, and fibers (Khan *et al.*, 2009; Ogunkunle *et al.*, 2016). Vegetables act as buffering agents for acid generation during digestion (Maleki and Zarasvand, 2016). Most research has focused on beneficial phytochemicals in cabbage, particularly its indole-3-carbinol (I_3C), sulforaphane and indoles (Brooks *et al.*, 2001). These compounds help activate and stabilize the body's antioxidant and detoxification mechanisms that dismantle and eliminate cancer-producing substances(Brooks *et al.*, 2001). Nevertheless, leafy vegetables such as cabbages and amaranth have been reported to be good absorbers of HMs from soil (Cui *et al.*, 2005). However, different studies conducted to evaluate the level of accumulation of heavy metals in cabbages have proven their ability to uptake heavy metals (Singh *et al.*, 2010). For instance, Boamponsem *et al.* (2012) carried out a study to

quantify HMs accumulation in the stems, leaves and roots of different vegetables including cabbage, lettuce and carrot in Nagodi mining Site in Ghana. Mumba *et al.*(2008) carried out a study in Malawi to determine the levels of HMs in cabbages grown in gardens irrigated with reservoir and tap water. The results have shown that HMs like Cd, Pb and Cr were potentially uptaken by both the reservoir and tap water irrigated cabbages. In their study on HMs content in cabbage cultivated in the Bezi bar farm area of Katima Mulilo, Namibia, Abah *et al.* (2015) reported that concentration levels of Cr, Cd, Co, Pb, Ni and As were accumulated in different cabbage species.

2.6. Factors influencing the bioaccumulation of Heavy Metals in abiotic ecosystems

HMs are transferred from the abiotic environment (sediments, water, soils) to living organisms and are accumulated in biota resulting in contamination of food chains with these elements (Ali *et al.*, 2019). For organic chemicals, toxicity and bio-concentration factors (TBCFs) for structurally-related chemicals can be predicted using quantitative structure activity relationships (Larsen *et al.*, 1992; Angelique*et al.*, 1993). For metals however, the situation is somewhat more complicated. The TBCFs, for a given element, are altered by soil factors that influence availability such as pH, cation exchange capacity (CEC), clay content, chemical processes, percentage organic matter (POM) among other factors (Spurgeon and Hopkin, 1996).

2.6.1. pH

Generally the pH is acknowledged to be the most important influencing factor on metal bioavailability in soils (Rieutwerts *et al.*, 1998). The solubility and pH usually show an inverse relationship, that is, metal solubility tends to increase at lower pH and decrease at higher pH values (Rieutwerts *et al.*, 1998).

2.6.2. Cation exchange capacity (CEC)

The CEC of metals is dependent on the density of negative charges on the surfaces of soil colloids, the relative charges of metal species in solution and on the soil surface (Evans, 1989). In order to maintain electroneutrality, the positively charged cations in solution are attracted by electrostatic or coulombic forces to the negatively charged edges and surfaces of soil particles (Evans, 1989).

The surface negative charge is balanced by an equal quantity of cations from the soil solution, and this cation exchange, between the balance and solution cations, is reversible (McLean and Bledsoe, 1992).

2.6.3. Clay content

Clays are thought to adsorb metal ions through both ion exchange and specific adsorption (Farrah and Pickering, 1979). Specific adsorption involves the exchange of metal cations with surface ligands to form partly covalent bonds with charged mineral surfaces and it is strongly pH dependent (Rieutwerts *et al.,* 1998; Evans, 1989;Jones, 1987).

2.6.4. Chemical processes

The metal bioavailability can be altered by chemical processes such as redox, precipitation, complexation reactions, among others (Avenant-Oldewage and Marx, 2000). Their influences are:

- High redox potentials (E_h) are typically recorded in dry, well aerated soils whilst soils prone to water logging and rich in organic matter tend to have low E_h values (Evans, 1989). Several authors reported that metals and other inorganic constituents are more readily dissolved in waterlogged soils (Swaine and Michell, 1960). In general, oxidizing conditions favor retention of metals in soils, while reducing conditions contribute to accelerated migration (McLean and Bledsoe, 1992).

- The concentrations of many HMs in industrial and municipal wastes applied to soils generally is several orders of magnitude higher than their concentrations in nature (Evans, 1989). Thus, the precipitation of the metals as secondary minerals often may occur when such wastes are added to soil (Evans, 1989). Among the most important of these precipitates are the oxides, oxyhydroxides, hydroxides, and carbonates; phosphates and silicates probably are of lesser importance (Lindsay, 1980).

- In general, the decrease in positive charge on the complexed metal reduces adsorption to a negatively charged surface as in the example:Cd^{2+},$CdCl^{+}$,$CdCl_2{}^{0}$,$CdCl_3^{-}$(McLean and Bledsoe, 1992). But the actual effect of complex formation on sorption depends on the

properties of the metal of interest, the type and amount of ligands present, soil surface properties, soil solution composition, pH and redox conditions (McLean and Bledsoe, 1992).

2.6.5. Percentage Organic Matter (POM)

Organic matter accumulates at the soil surface, mainly as a result of decomposing plant material. Whilst the organic matter content of soils is often small compared to that of clay, the organic fraction has a significant influence on metal binding (Zimdahl and Skogerboe, 1977). Organic matter has a particular potential for the retention of atmospherically derived metal inputs in the surface humic layer of soils: this has important implications for metal mobility down the soil profile and for the bioavailability of metals to surface dwelling organism (McLean and Bledsoe, 1992).

However, in aquatic systems HMs concentration is usually monitored by measuring concentration in water, sediment and biota (Camuso *et al.,* 1995). Metal concentration in organs or in tissues of aquatic organisms depend not only on concentrations in the environment but also on several geochemical and biological factors that affect metal bioavailability, such as temperature, pH, organic ligands and size of organisms (Luoma, 1983;Campbell and Stokes, 1985;John *et al*., 1987). Also, water hardness associated with factors such as alkalinity and dissolved organic matter can change the speciation of metals and thus their toxicity and bioaccumulation (Franklin*et al*., 2005).

2.7. Human Health hazards of Heavy Metals

Although soil, water and air are the major environmental compartments which are affected by HMs pollution, human beings are the most vulnerable due to the ecological continuous build up in food chain (Herawati *et al*., 2000). As , Cd, Hg and Pb are HMs known to have serious health implications since they have no beneficial effects in humans, and there is no known homeostasis mechanism (Draghici *et al.,* 2011; Morais *et al.,* 2012; Sharma *et al*., 2014). However, another group of essential metals like Cu, Fe, and Zn among others, are strongly regulated by metabolic processes, as they are important constituents of enzymes and other living compounds (Jakimska *et al.,* 2011). Although suggested as essential cofactors in biochemical and many other physiological processes in organisms, any increased concentration of these metals may result in deleterious

effects in the organism such as impairment of growth and reproduction (Rattan *et al*., 2005).

Metals like Al have no proven essential functions in humans and are likely to have adverse physiological effects (Santos *et al*., 2004). The deleterious effects of HMs do not normally manifest themselves immediately after the toxin enters the organism; but, usually become apparent only after a few years (Sharma and Singh, 2015). Their effects are diverse and include, but not limited to neurotoxic and carcinogenic actions (Jakimska *et al.*, 2011). They affect deoxyribonucleic acid (DNA) and enzymatic processes (Jakimska *et al.*, 2011). The summary on the sources, targeted organs and health hazards of selected metals is provided in Table 1. The Figure 3 shows the arsenic keratosis and arsenicosis (skin lesions) side effects of arsenic.

Figure 3: Arsenic keratosis (raindrops) (left) and arsenicosis (middle-right) (Skin lesions) adapted from Jaishankar *et al*.(2014)

Table 1: Summary of sources, targeted organs and health effects of selected metals

Metal	Source	Targeted organ	Health effects	Reference
Al	Air, water and soil, Earth's crust as part of silicates, (mica or feldspar)	Brain, bones, kidney and liver	Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, dialysis syndrome, inhibition of enzymes	(Klaassen*et al.*, 1986; Trond, 2001; Jaishankar *et al.*, 2014; Abubakar *et al.*, 2015; Hardisson *et al.*, 2017)
As	Atmospheric deposition through burning of charcoal, activities of metal foundry, excessive use of pesticides and fertilizers and mining, paints, dyes, soaps, semi-conductors and drugs	Skin, lung, kidney, bladder,	Cancer (lung, kidney, bladder, and skin), skin lesions (arsenicosis), internal cancers, neurological problems, pulmonary disease, peripheral vascular disease, hypertension, cardiovascular disease and diabetes mellitus	(Smith *et al.*, 2000; Adriano, 2001; Alloway, 2012; Jaishankar *et al.*, 2014)
Cd	Metal-ore refining, pigments, alloys and electronic compounds, phosphate fertilizers, detergents and refined petroleum products	Liver, kidney, center of nervous system(CNS)	Cancer, Renal abnormalities (proteinuria and glucosuria), osteoporosis,anemia, eosinophilia,anosmia,chronicrhinitis	(WHO/IARC, 1993; Lafuente *et al.*, 1999; Castro-González and Méndez-Armenta, 2008; Sharma *et al.*, 2014; Gautam *et al.*, 2014)
Cu	Mining, metallurgy, pesticides, fertilizers, industry and sewage sludge	Liver ,brain, eyes	Component of many metal-loproteins (antioxidant)*,mense disease, wilson disease, hepatic cirrhosis, neurological degeneration	(Yan-Hong e*t al*., 2002; Siraj and Kitte, 2013; Gautam *et al.*, 2014)
Fe	Mining, manufacture of chemicals (ex: H_2SO_4production)		Components of O_2-transporting proteins (hemoglobin and myoglobin)*,cancer (oxidation of DNA molecules),asbestosis	(Nelson, 1992; Vuori, 1995;Bhasin *et al.*, 2002; Andrade *et al*., 2017)

			(lung cancer),Fe-deficiency causes Cd and Pb absorption	
Hg	Municipal wastewater discharges, mining, incineration, pharmaceuticals, paper and pulp preservatives, chlorine and caustic soda production industry, fish and marine mammals consumption	Brain, kidney, nervous system	Cancer, neuroendocrine, renal damage, damage of tertiary and quaternary protein structure, causes disappearance of ribosomes and eradication of endoplasmic reticulum in cells	(Chen *et al.*, 2011; Chiu-Wen *et al.*, 2012;Morais *et al.*, 2012;Hsu-Kim *et al.*, 2013; Jaishankar *et al.*, 2014; Azaman *et al.*, 2015)
Pb	Mining, manufacturing and fossil fuel burning, metal plating and finishing operations, Fertilizers and pesticides, Wastes from battery industries, additives in gasoline and pigment, exhaust from automobiles, smelting of ores	Bones, brain, blood, kidneys, and thyroid gland	Allergies, dyslexia, weight loss, hyperactivity, paralysis, muscular weakness, brain damage, kidney damage and death, mimicking of Ca-action and/or disruption of Ca-homeostasis	(Gerhardsson *et al.*,2002; Rigby and Warren, 2003; Griswold and Martin, 2009; Jaishankar *et al.*, 2014; Baby *et al.*, 2010)
Zn	Mining activities, Zn purification process, Pb and Cd ores, steel production, coal burning, and burning of wastes		Cofactor to enzymes*, stabilization of proteins*, diarrhea, bloody urine, icterus, Parkinson's disease, liver failure, kidney failure and anemia	(Gorell *et al.*, 1997; Duruibe *et al.*, 2007; Song *et al.*, 2009; Song *et al.*, 2010)

(*) Health benefi

2.8. Heavy Metals as Endocrine Disrupting Chemicals (EDCs)

The pollution effects of Endocrine Disrupting Chemicals (EDC) gain more and more attention worldwide (Daughton and Ternes, 1999). EDCs refer to exogenous agents, which interfere with the synthesis, secretion, transport, binding action, or elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development, or behavior (Juvancza *et al.*, 2008). Many environmental pollutants, including organic compounds like polychlorinated biphenyls (PCBs), dioxins, polycyclic aromatic hydrocarbons (PAHs), phthalates, bisphenol A (BPA), pesticides, alkylphenols and heavy metals (As, Cd, Hg and Pb), have been shown to significantly alter endocrine balance (Schantz and Widholm, 2001; Birkett and Lester, 2003; Järup, 2003; Mocarelli *et al.*, 2008). Most EDCs are mutagenic and highly carcinogenic (Balabanic *et al.*, 2011). EDCs can be found in many products including plastic bottles, metal food cans, detergents, flame retardants, foodstuffs, toys, cosmetics, and pesticides (Yang*et al.*, 2015). These chemicals have also been referred to as endocrine modulators, environmental hormones, and endocrine active compounds (NIEHS, 2010). The Table 2 contains some elements with their endocrine disrupting effects.

Human exposure to EDCs may result from the ingestion of contaminated food and water, inhalation of air and absorption of the EDCs through the skin. However, in most cases, human exposure to EDCs is through the ingestion of contaminated food (Balabaničet *al.*, 2011). Evidences from many countries show that exposure to EDCs can result to reduced fertility and increased progression of some diseases, including obesity, diabetes, endometriosis, and some cancers (Toppari *et al.*, 1996).

Table 2: Some Metals as Endocrine Disrupting Chemicals

Metal	**Impact on human health**	**Reference**
Al	Alzheimer's disease, Parkinson's disease	(Hardisson *et al.*, 2017)
As	Decreased birth weight and miscarriage	(Balabanič *et al.*, 2011; Bornman *et al.*, 2017)
Cd	Infertility and prostate cancer	(Georgescu *et al*, 2011; Bornman *et al.*, 2017)
Hg	Infertility, miscarriage, low birth weigh	(Bornman *et al.*, 2017)
Pb	Infertility, miscarriage, disturbances in the menstrual cycle	(Bornman *et al.*, 2017)

CHAPTER THREE

3. MATERIAL AND METHODS

3.1. Laboratory equipment, materials and reagents

3.1.1. Equipments

Analytical balance (Model AD-1672, Tabletop Breeze Break), Electric heating plate, drying oven (Biobase Biodustry, Model BOV-T30C, Temp-Range 50-200^{o}C), Refrigerant (model BD-300), Inductively Coupled Plasma-Atomic Emission Spectrometer (model ICPE-9820, Shimadzu), Lumex mercury analyser (PYRO-915^{+}).

3.1.2. Materials

Teflon mortar, petri dishes, stainless steel spatula, stainless steel knife, washing bottle, Glass volumetric flasks (25 mL, 50 mL), polyethylene vials (ICP), whatman filter paper, funnel, measuring cylinder, micro-pipette, teflon crucibles (for digestion) and spatula.

3.1.3. Reagents

Perchloric acid, $HClO_4$, 70% (Glassworld, Johannesburg, South Africa), sulphuric acid, H_2SO_4, 98% (Rochelle chemicals, Johannesburg, South Africa), nitric acid, HNO_3, 70% (Glassworld, Johannesburg, South Africa), hydrochloric acid, HCl 37% (Glassworld, Johannesburg, South Africa), Certified Aqueous Reference Material (Multi-element Standard)-CRM004-Sanas ULTRASPEC®, Fish Certified Reference Material Dolt-3, double deionized water (Milli-Q water) and argon gas.

3.2. Samples and Sampling area

The process of sample collection took place within the period from September-2019 to December-2020. Two kind of samples were collected namely fish (tilapia and catfish) and vegetables (cabbage and kale) and the details about the samples and sampling area are provided in the next

pages. Samples were collected from sellers in agriculture fields and others from the sellers in markets or places located in the targeted area.

3.2.1. Fish samples

Fish samples were collected from seven (7) sites all in five (5) districts across three Mozambican provinces. The districts are Moamba, Boane, Matola (Maputo province), Moma (Nampula province) and Moatize (Tete province). The sampling sites were Moamba-Corumana (MoKUR), Boane-Mafuiane (BoMAF), Matola river (MaRIV), Tete-Xitima (TeEST), Nampula-Maganha (NaMAG), Nampula-Lalane (NaLAL) and Nampula-Inthaka (NaINT). The fishes were purchased either at open markets or from the local sellers near the above areas. Mainly two kinds of fish species were collected namely tilapia fish (*Oreochromis mossambicus)* and cat fish (*Chrysichthys nigrodidatatus)*. All sites provided the tilapia except Nampula-Inthaka (NaINT) where catfish samples were taken.

3.2.2. Vegetable samples

Vegetable samples were collected from the same areas as for the fish samples described in the sections 3.2.1. For vegetables, two cultivars of the brassica species were collected namely cabbage (*Brassica oleracea var. capitata)* and kale (*Brassica oleracea var. acephala)*. The samples were purchased some from farmers in local agricultural fields and sellers at open markets in each one of the below mentioned sites. Both cultivars were successfully collected from the sites MOA1 (Moamba 1), MOA2 (Moamba 2), MOA3 (Moamba 3), BOA1 (Boane 1), BOA2 (Boane 2), MACH (Machava), AG1 (Angónia 1), AG2 (Angónia 2) and LARD (Larde) whereas from other sites only one kind of cultivar was purchased as fellows: Boane 3_BOA3 (cabbage), Patrice_PATR (kale), T-Tres_T3 (cabbage), Dois-M_2M (kale), Zona Verde_ZV (kale) and Moatize_MTZ (kale). The Figure 4 shows the geographical location of the three provinces on Mozambique's map and locations of sampling sites.

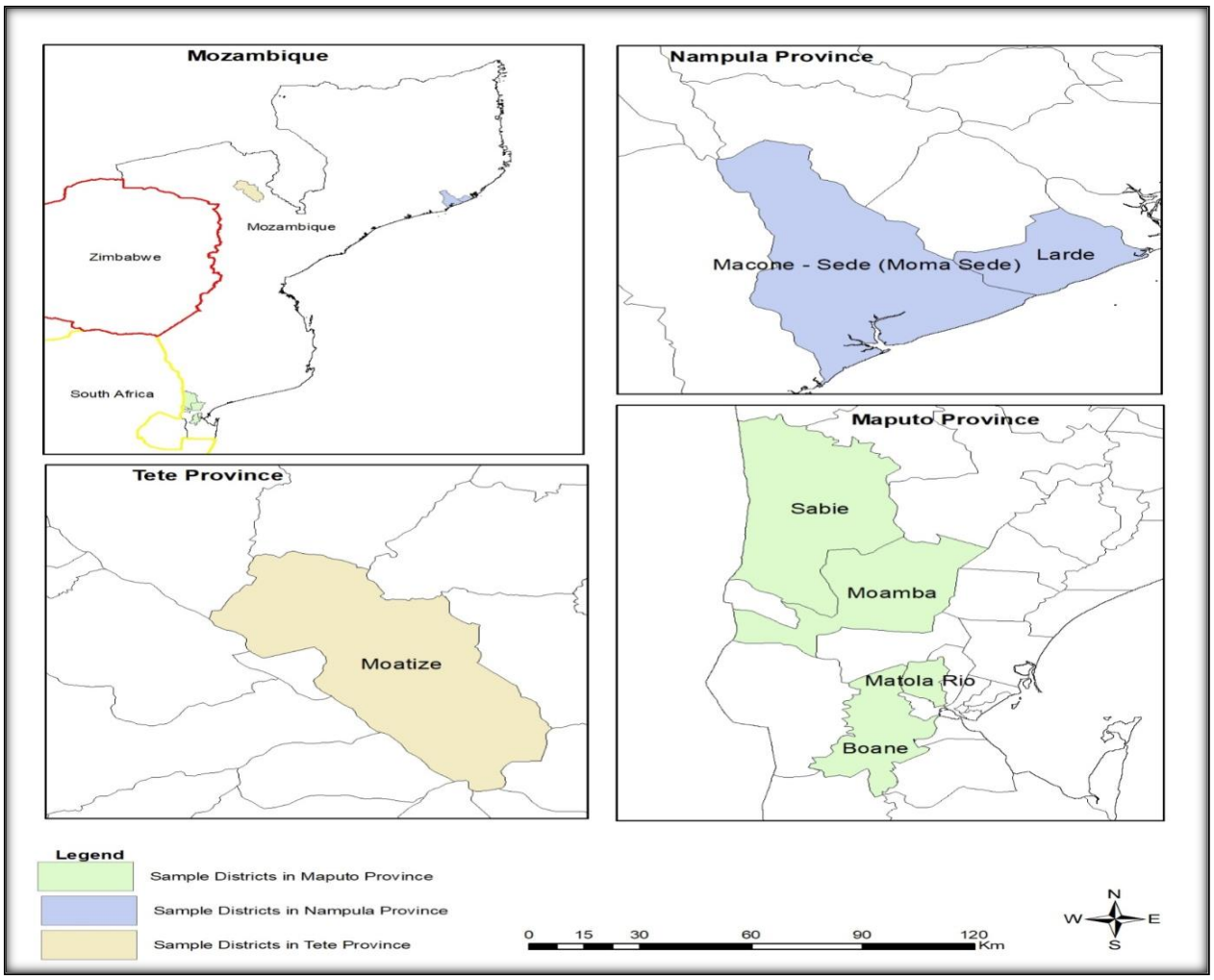

Figure 4: Map showing sampling location sites in three provinces, Mozambique

3.3. Samples collection and preservation

3.3.1. Fish samples

A total of 156 fish species were collected from each of the above mentioned sites within the period of September-2019 and December-2020. After collection, fishes were packaged in clean polyethylene bags and then in clean cooler boxes containing ice. The fish samples were transported to the chemistry laboratory, Department of Chemistry of Universidade Eduardo Mondlane. At laboratory, the fish samples were first washed with double deionized water in order to remove any eventual contaminant and then stored in the fridge (Model: BD-300) at -20 °C until further processing. The Figure 5 illustrates some of the collected fish samples.

Figure 5: Fishes, tilapia (left) and catfish (right)

3.3.2. Vegetable samples

From each sampling site, about 500 g of each cultivar were taken. After collection, vegetables were packaged in clean polyethylene bags and then in a clean cooler boxes containing ice. The vegetable samples were transported to the chemistry laboratory of Universidade Eduardo Mondlane. At laboratory the vegetable samples were first washed with double deionized water in order to remove any eventual contaminant and then stored in the fridge (Model: BD-300) at -20 °C until further processing. The Figure 6 below illustrates the samples of vegetables collected.

Figure 6: Vegetables, cabbage (left) and kale (right)

3.4. Sample preparation

Prior to any further handling, either fish or vegetable samples were let thawed at room temperature for about two hours and the specific processes are specifically described below.

3.4.1. Fish samples

The fish samples were dissected by use of cleaned stainless steel knife, to separate the muscle, gills and liver as recommended by UNEP/IOC/IAEA/FAO (1990). Then, the edible portion (muscles) were kept and cut into small pieces (2–3mm) over a clean polyethylene sheet (Rahman *et al.,* 2012). About four grams (4g) of the homogenized muscles were taken from each species and placed on labelled acid washed petri dishes. The homogenized fish muscles were allowed to dry to constant weight at 80°C for a period of 48hours (Taweel *et al.,* 2013).

3.4.2. Vegetable samples

Vegetables were cut into small pieces (2–3mm) by use of cleaned stainless steel knife on a polyethylene work-sheet. Then, the edible portion was cut over a clean polyethylene sheet. About four grams (4 g) of the homogenized edible potion were taken from each vegetable cultivar and placed on labelled acid washed petri dishes. The homogenized and chopped portions were allowed to dry to constant weight at 70°C for a period of 48hours (Tiwari *et al.*, 2011).

For fish or vegetable sample, the constant temperature drying oven Biobase Biodustry, Model BOV-T30C, Temp-Range 50-200^{o}C was used for drying. By use of Teflon mortar, the dried tissues were pulverized, homogenized and stored in clean polyethylene containers before digestion.

3.5. Sample digestion

3.5.1. Fish samples

The dried fish samples were digested according to the method described in the open literature (Sadeghi *et al*., 2020). Briefly, 0.1 g dry weight of fish powder were accurately weighed on the analytical balance (Model AD-1672), transferred into 200 mL Teflon digestion crucible and then moistened with 2 mL of deionized water. Then, 10 mL 70 % of HNO_3 and 5mL 70% of $HClO_4$were

added. The system was allowed to digest at 100-120 ºC, on an electric heating plate, repeated until the solution was clear, up to a volume of 1 mL. The sample was left to cool and then filtered using an acid-resistant 0.45µm-filter and the filtrate was diluted to a final volume of 10 mL, using ultra-pure water (Milli-Q water).

3.5.2. Vegetable samples

The dried vegetable samples were digested according to the method described by Gupta *et al.*(2012). Briefly, 0.1 g of powdered vegetable sample was digested on the electric heating plate with a mixture in proportion of 5:1:1 respectively in 70% HNO_3, 98% H_2SO_4 and 70% $HClO_4$solution at 100-120°C until the solution became transparent (Gupta *et al.*,2012).The sample was left to cool and then filtered with the acid-resistant 0.45µm-filter paper and the filtrate was diluted to a final volume of 10 mL volumetric flask using the ultra-pure water (Milli-Q water).
For either fish or vegetable sample, the final labelled solutions were stored at about 4ºC until the measurement of concentrations of Al, As, Cd, Cu, Fe, Pb and Zn were determined with ICP-OES (model ICPE–9820 Plasma Atomic Emission Spectrometer, Shimadzu Corporation, Japan), using argon plasma with digital read out.

Hg-analysis was conducted through the direct Lumex analyserfor solid sample analysis. About 200 mg of dried, pulverized and ground fish sample was put in the sample boat. After switching on the integration of the analytical cell, the sample boat was inserted into PYRO-915$^+$ attachment. Within 60-120 sec the integration is finished and the analytical signal goes back to the baseline.

3.6. Instrumentation

The concentration of metals Al, As, Cd, Cu, Fe, Pb and Zn was determined with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Model ICPE–9820, Shimadzu Corporation, Japan) using argon plasma with digital read out system while for Hg the concentration was determined with Lumex Mercury Analyzer PYRO-915$^+$. For ICPE–9820, the instrument calibration standards were made by diluting the standard solution of a multielemental aqueous reference material (CRM004) of 100 µg/mL supplied by ULTRASPEC® from South Africa, to prepare calibration solutions of 0, 25, 50, 100, 250, 500, 1000 and 1500 µg/L. For Lumex Mercury

Analyzer PYRO-915^{+}, the instrument calibration was made with CARBON NWC Activated coconut shell charcoal for mercury analysis.

3.7. Quality Assurance and Control

The accuracy and precision of the analytical procedure was checked using a fish Certified Reference Material (CRM-DOLT-3, dogfish liver, from National Research Council Canada). The CRM-DOLT-3 was repetitively analysed in triplicates following the same procedure for fish samples and the results are shown in Table 3. Any other apparatus that comes in contact with the sample was subjected to the cleaning process as adapted from Rothery (2012). All glassware and plastics were soaked over-night in soapy water, rinsed with distilled and deionized water. They were respectively immersed in 20% (v/v) HCl and 20% (v/v) HNO_3 each for 48 hours, rinsed in deionized water and oven-dried before being used.

3.8. Health risk assessment

Health risk is defined as the likelihood of harmful effects to human health as a result of environmental pollution (Liang *et al.*, 2017). Health risks caused by different contaminants that enter the body through diverse exposure pathways exist as, either carcinogenic risk or non-carcinogenic risk (Xiao *et al.*, 2017). The *carcinogenic risk* refers to the incremental probability of an individual developing any kind of cancer in a lifetime as a result of exposure to pollutants (Chen *et al.*, 2015). Carcinogenic risk can be evaluated by the following linear equation:

$$\text{Cancer risk} = \text{EDI} \times \text{SF} \quad \textbf{(1)}$$

Where cancer risk is a unit less probability of an individual developing cancer, **EDI** is the estimated daily intake dose of carcinogens (mg $kg^{-1}day^{-1}$) and **SF** is the carcinogenicity slope factor (mg $kg^{-1}day^{-1}$). The *Non-carcinogenic risk* (which is used in this study) is evaluated by comparing an exposure level over a specified time period (e.g., lifetime), with a reference dose derived for a similar exposure period (Liang *et al.*, 2017). The non-carcinogenic risk can be characterized as a hazard quotient (HQ)(U.S.EPA, 1989).

3.8.1. Estimated daily intake (EDI)

The estimated daily intake (EDI) refers to the presumed daily exposure to or consumption of a nutrient or chemical residue (Liang *et al.*, 2017). The EDI ($mgkg^{-1}day^{-1}$) of each HM was calculated in accordance to the equation **(2)** below:

$$EDI = \frac{E_F \, x \, E_D \, x \, F_{IR} \, x \, C}{W_{AB} \, x \, T_A} \, x \, 10^{-3} \qquad \textbf{(2)}$$

Where $\boldsymbol{E_F}$ is exposure frequency (156 days/year, assuming that consumers have three meals of fish or vegetable a week), $\boldsymbol{E_D}$ is exposure duration (60 years) equivalent to the estimated average Mozambican lifetime (António *et al.*, 2013). $\boldsymbol{F_{IR}}$ is fish ingestion rate (23.3 g $day^{-1}person^{-1}$) according to published national consumption values (FAO, 2013). $\boldsymbol{F_{IR}}$ for vegetable ingestion rate (145 g $day^{-1}person^{-1}$) (Ruel *et al.*, 2005). **C** is metal concentration (edible part of fish and vegetable) (mg kg^{-1}); $\boldsymbol{W_{AB}}$ is average body weight of consumer (70 Kg the average standard body weight of adult (U.S.EPA, 2000). $\boldsymbol{T_A}$ is average exposure time for non-carcinogens (365 days/year x $\boldsymbol{E_D}$)(Saha and Zaman, 2013).

3.8.2. Target Hazard Quotient (THQ)

The target hazard quotient (THQ) is defined as the ratio of exposure to the toxic element and the reference dose which is the highest level at which no adverse health effects are expected (Chen *et al.*, 2011). The hazard quotient is explained as the ratio of estimated daily intake (EDI) and oral reference dose (RfD)(U.S.EPA, 2000). The target hazard quotient for selected metals through food consumption is evaluated as a way to determine the non-carcinogenic risk (U.S.EPA, 1989).

$$THQ = \frac{EDI}{RfD} \qquad \textbf{(3)}$$

The RfD represents the oral reference dose that is an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects (Akoto *et al.*, 2014;Nuapia *et al.*, 2018). The RfD values in $mgkg^{-1}day^{-1}$are as follows: Al(1.0),(0.0003),Cd(0.0005),Cu(0.04),Fe(0.7),Hg(0.0001), Pb(0.0035) and Zn(0.3)(U.S.EPA, 2000). If the THQ is <1, the contaminant is unlikely to cause adverse non-carcinogenic effects to the exposed consumers. If the value of THQ is >1, the contaminant is not in the acceptable threshold, and the greater the value, the greater the probability of the occurrence

of adverse non-carcinogenic effects (Liang *et al.,* 2017). It is further assumed that cooking has no effect on the toxicity of HMs in food (Cooper *et al.,* 1991; Cooper *et al.,* 1991). In order to assess the overall potential risk of non-carcinogenic effects posed by more than one element, the hazard index (HI) approach has been developed (U.S.EPA, 1989).

$$HI_{Indivivual\ food} = THQ_{tox1} + THQ_{tox2} + THQ_{tox3} + \ldots\ldots\ldots THQ_{toxn} \quad \textbf{(4)}$$

In the present study the toxicants are Al, As, Cd, Cu, Hg, Fe, Pb and Zn and the foodstuffs are fish and vegetables from the respective sites.

$$HI_{Individualfood} = THQ_{Al} + THQ_{As} + THQ_{Cd} + \ldots\ldots\ldots THQ_{Zn} \quad \textbf{(5)}$$

The HI value expresses the combined non-carcinogenic effects of multiple toxicants in studied foodstuffs (Chen *et al.,* 2011). When the HI value exceeds 1, there is a chance of non-carcinogenic effects, with an increasing probability as the value increases (Akoto *et al.,* 2014).

The THQ and HI proposed by U.S.EPA (2000) are parameters for risk assessment which compare the ingestion amount of a pollutant with a standard reference dose and have been widely used in the risk assessment of metals in contaminated foods (Alipour*et al.,* 2014).

3.8.3. Allowable daily consumption limit (CRlim)

In order to calculate the allowable daily consumption limit (CR_{lim}) of fish, we assume that no other sources of Al, As, Cd, Cu, Fe, Hg, Pb and Zn exist in the diet of consumers. The equation (6) was used, and the results were expressed in Kg day^{-1} of fish (Taweel *et al.,* 2013).

$$CR_{lim} = \text{RfD} \times \frac{BW}{C_m} \quad \textbf{(6)}$$

Where CR_{lim} = maximum safe daily consumption limit of fish (Kg day^{-1}); RfD = reference dose of metal (mg kg^{-1}day^{-1}); B_W = average consumer body weight (Kg) (In this study 70Kg for adults); C_m = measured concentration of the chemical in fish (mg kg^{-1}).

3.9. Statistical analysis

The results from analytical instruments were first stored and processed in Microsoft excel 2010. The means, standard deviations, minimum and maximum concentrations of the HMs for the fish and vegetable samples were calculated by use of IBM-SPSS statistics version 20 software. One-way analysis of variance (ANOVA) was performed to test the significance difference of concentrations between sampling sites. The significance level was ($p < 0.05$).

CHAPTER FOUR

4. RESULTS AND DISCUSSION IN FISH SAMPLES

4.1. Metals concentration in fish Certified Reference Material

In other to check for precision and accuracy of the applied analytical protocols, a series of analyses of the certified reference materials (i.e., CRM-Dog fish) were performed and the results are presented in Table 3.

Table 3: Metal concentrations in fish CRM (dog fish), determined by ICP-OES and Lumex Hg-Analyzer (mg kg^{-1}, mean ± SD, n=3).

Metals	LOQ	Certificate value (CRM-dog fish)	Measured value	Recovery (%)
As	0.34	10.2 ± 0.5	10.7 ± 1.21	105
Cd	1.42	19.4 ± 0.6	17.6 ± 0.28	91
Cu	17.11	31.2 ± 1.0	31.6 ± 2.68	101
Fe	18.45	1484 ± 57	1321 ± 28	89
Pb	0.187	0.32 ± 0.05	0.37± 0.034	115
Zn	24.73	86.6 ± 2.42	85.3 ± 2.62	98
Hg*	NA	3.37 ± 0.14	3.04 ± 0.236	90

CRM = Certified Reference Material, LOQ = Limit of quantification, NA= Not Available

(*) = Analyzed by Lumex mercury analyzer (Direct mercury analysis)

4.2. Metal concentrations in fish samples

The concentrations of eight metal elements (Al, As, Cd, Cu, Fe, Hg, Pb and Zn) in muscle tissue of two fish species (tilapia and catfish) from seven locations across three provinces of Mozambique were listed in the Table 4. The average concentration (mg kg^{-1}) of total HMs in fish samples was in the following decreasing order of Al (236) > Fe (59.4) > Zn (38.8) > Cu (10.1) >As (7.9) > Cd (5.1) > Pb (3.5) > Hg (0.013). The highest mean concentrations of metals were 644 $mgkg^{-1}$ for Al from NaINT; 168 $mgkg^{-1}$ for Fe from NaINT; 52.2 $mgkg^{-1}$for Zn from NaMAG; 19.3 $mgkg^{-1}$ for Cu from NaINT; 14.5 $mgkg^{-1}$ for Cd from TeEST; 12.7 $mgkg^{-1}$ for As from NaINT; 7.9 $mgkg^{-1}$for Pb from MaRIV; and 0.033 $mgkg^{-1}$ for Hg from BoMAF (Table 3). Al showed the highest concentration values followed by Fe, amongst the HMs reported in this study. Moreover, the marine sediments are said to contain about 50.000 mg $kg^{-1}$of Fe, which can be the source of contamination on aquatic organisms including fishes. (Panayotidis and Florou, 2008). The study

of heavy metals accumulation in fish muscles have been carried out and reported by a good number of researchers across the world. The Table 5 exhibits some of the literatures with their reported values for the metals investigated in this work.

Table 4: Metal concentrations in fish muscles (tilapia and catfish) (means ± SD, in mg/kg as dry wt) by ICP-OES

Site	Species	Al	As	Cd	Cu	Fe	Hg	Pb	Zn
MoKUR[x]	Tilapia	38.0 ± 2.0	5.8± 0.08	1.05±0.074	5.25± 0.21	25.5 ± 0.7	0.032 ± 4E-03	2.3 ± 0.28	33.1 ± 1.27
BoMAF[x]	Tilapia	129 ± 4.2	10.1 ± 0.92	2.13 ± 0.15	9.97± 0.18	28.6 ± 0.77	0.033 ± 1E-04	2.6 ± 0.81	44.2 ± 1.06
MaRIV[x]	Tilapia	369 ± 28.9	7.11 ± 1.11	5.46 ± 0.24	12.9± 0.14	35.0 ± 0.5	0.0014±2.8E-04	6.5 ± 0.21	28.2 ± 0.50
TeEST[y]	Tilapia	30.8 ±0.91	5.65 ± 1.46	12.9±1.06	6.60± 0.41	21.6± 0.52	0.0019±1.9E-04	3.7 ± 0.33	30.8± 5.32
NaMAG[z]	Tilapia	94.0 ± 3.5	7.14 ± 0.45	1.28 ± 0.10	5.6 ± 0.28	44.6 ±6.12	BLQ	1.9 ± 0.11	52.2 ± 3.18
NaLAL[z]	Tilapia	290.7 ± 11.3	6.60 ± 0.51	6.51 ± 0.15	7.1 ± 0.41	92.2±1.08	BLQ	2.6 ± 0.23	38.7 ± 3.77
NaINT[z]	Cat fish	644 ±12.8	12.7 ± 1.44	1.15 ± 0.04	18.9± 0.28	168 ± 0.70	0.0013±2.8E-04	3.6 ± 0.07	44.2 ±2.97
Range		30.8-644	5.65-12.7	1.05-12.9	5.25-18.9	21.0-168	BLQ -0.033	1.9-6.45	28.2-52.2
MPLs (mg kg^{-1})		70[adf]	0.1[a]	0.05[b]	30[e]	56[a]	0.5[abcd]	0.3[ab]	1000[e]
			2.0[c]	2.0[e]	NP	NP	NP	0.5 [c]	NP
sig			0.000	0.002	0.000	0.000	0.000	0.000	0.000

[x] Sites from Maputo province [y] Sites from Tete province [z] Sites from Nampula province sig: coeffiency of significance

MPLs: Maximum permissible limits mg kg^{-1} **NP**: Not Provided; **BLQ**: Below the limit of quantification

[a](FAO/WHO,2011) [b](FSAI, 2009;EU, 2006;Eritrea, 2003) [c](ANZFA, 2011) [d](FAO/WHO, 2006)

[e](Bebbington *al.*, 1977) [f](EFSA,2011)

4.2.1. Aluminium (Al) concentration in fish

The highest concentration of Al was 644±12.8 mg kg^{-1} dry wt. in catfish from NaINT while the lowest one was found at 30.8±0.9 mg kg^{-1}in tilapia from TeEST (Table 4). The European Food Safety Authority (EFSA) and FAO/WHO Expert Committee on Food Additives set the Tolerable Weekly Intake (TWI) of Al equal to 1.0 mg kg^{-1}bw equivalent to 70 mg $kg^{-1}$for a 70 Kg adult-body weight (EFSA, 2011;FAO/WHO, 2011). Among all the fish samples, only 2 out of 7 samples (28.5 %) have concentrations lower than the MPL of 70 mg kg^{-1}while 5 out of 7 (71.5%) show concentrations above. Al levels are significantly different between sites ($p < 0.05$). In comparison with other studies, Addo-Bediako *et al*. (2014) reported the concentration of Al ranging between 32-59.8 mg kg^{-1} in tilapia fish muscles from Limpopo, South Africa. According to WHO reports, humans get inevitably exposed to Al through food, cooking utensils, deodorants, and antacids (Kaur *et al.,* 2006). Al has no proven essential functions in humans and is likely to have adverse physiological effects (Santos *et al.*,2004). The variations of Al concentrations in this study are indicated in Figure 7.

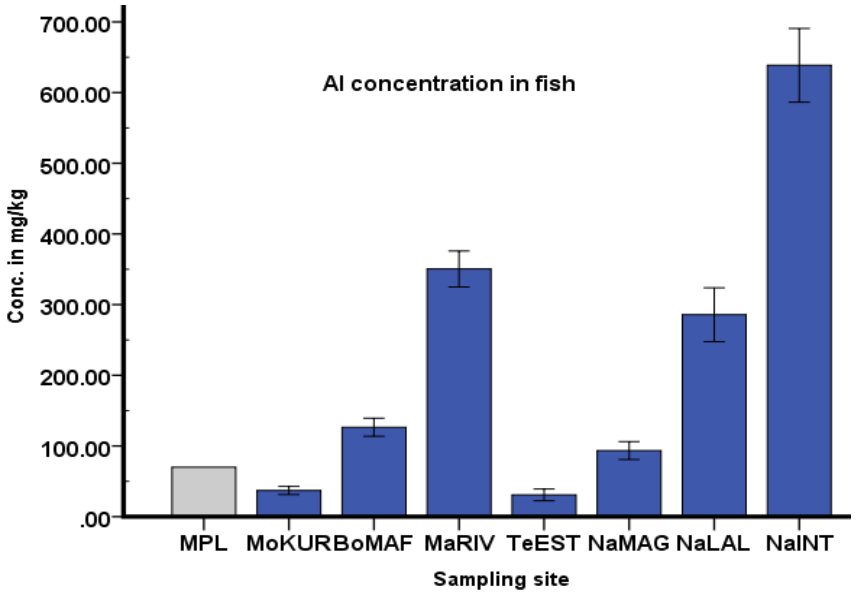

Figure 7: Variations of Al concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.2. Arsenic (As) concentration in fish

Arsenic (As) is a ubiquitous, but potentially toxic heavy metal (Rahman *et al.*,2012). For the present study the lowest concentration was 5.65±1.46 mg kg^{-1}in tilapia fish from TeEST and the highest 12.7±1.44 mg kg^{-1}in tilapia fish from NaINT as presented in Table 4. The concentration of As was significantly different between sampling sites ($p<0.05$). All fish measured concentrations are higher than the MPLs of 0.1 and 2.0 mg kg^{-1}recommended by FAO/WHO, 2011 and ANZFA, 2011 respectively. Exposure to As can lead to skin and lung cancers, kidney and heart diseases, neurological and respiratory malfunctions, among others (Zhu *et al.*, 2015). In comparison with other researchers, Nuapia *et al.*(2018) reported concentrations of fish ranging between 9.81-14.21 and 2.45-3.89 mg kg^{-1}, respectively from Kinshasa (DRC) and Johannesburg (RSA). The concentration from Kinshasa (DRC) was higher while that from Johannesburg (RSA) was lower than the concentration obtained in this study. The variations of As concentrations in this study are indicated in Figure 8.

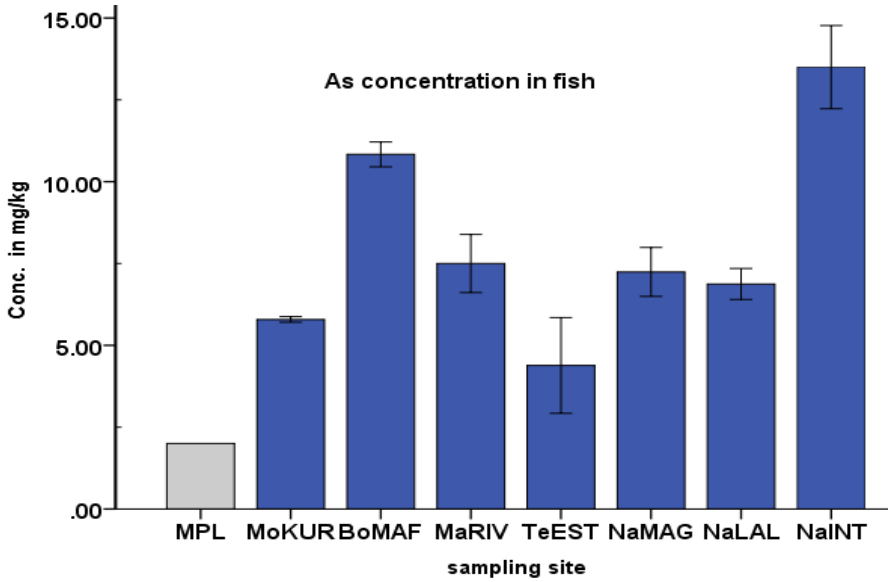

Figure 8: Variations of As concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.3. Cadmium (Cd) concentration in fish

Cadmium (Cd) causes negative effects on kidney, lungs, liver, reproduction organs, skeleton, blood, and nervous systems, among others (Raknuzzaman *et al.*, 2016). From the Table 4, the measured concentration ranged between 1.05±0.074 mg kg^{-1} in tilapia fish from MoKUR and 12.8±1.06 mg kg^{-1} in tilapia fish from TeEST. Cd concentration was significantly different between location sites ($p<0.05$). The measured concentrations were outside the acceptable limit of 0.05 mg kg^{-1} prescribed in the open literature (Eritrea, 2003; EU, 2006;FSAI, 2009). The Australian National Health Medical Research Council (ANHMRC) recommended the maximum tolerable standard for Cd in seafood at 2.0 mg kg^{-1}(Bebbington*et al.*, 1977). It was noted that the concentrations of some samples from MaRIV, BoMAF, TeEST and NaLAL were outside the MPL indicated by ANHMRC while others from MoKUR, NaMAG and NaINT were found within acceptable limits. In comparison with other studies Mbewe *et al.* (2016) reported the Cd concentration relatively higher than that of this study, in range of 0.3-20 mg kg^{-1} in fish from Kafue River of Zambia. The variations of Cd concentrations in this study are indicated in Figure 9.

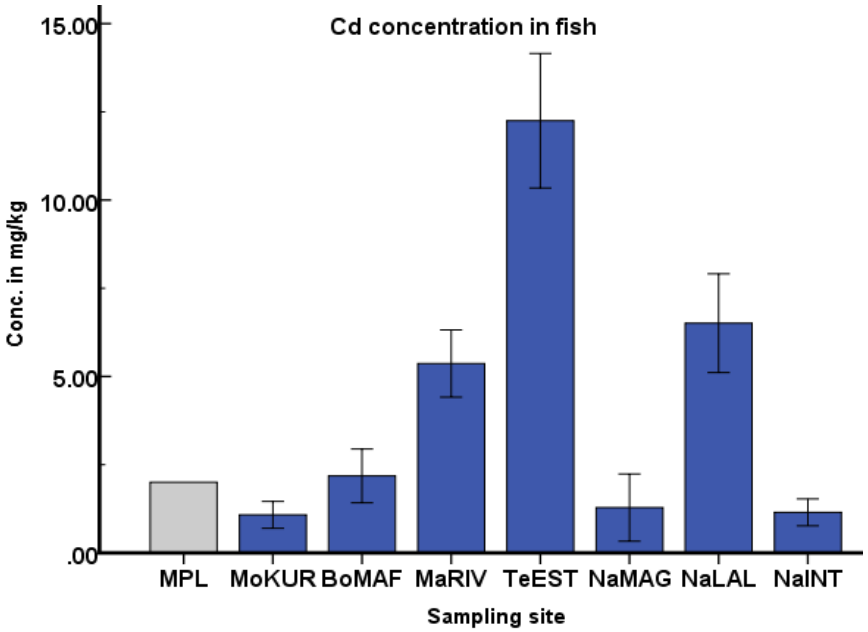

Figure 9: Variations of Cd concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.4. Copper (Cu) concentration in fish

Although Cu is essential for the formation of hemoglobin and some enzymes in humans, high intakes can result to damage the livers and kidneys (Alipour *et al.,* 2014; Gautam *et al.,* 2014). In the present study, the Cu concentration ranges between 5.25±0.21 mg $kg^{-1}$for tilapia from MoKUR and 18.9±0.28 mg kg^{-1} in catfish from NaINT. None of the fish samples exceeds the recommended MPL value of 30 mg kg^{-1} set by the ANHMRC (Bebbington *et al.,* 1977; Meltem *et al.,* 2007; Rahman *et al.,* 2012). Report by UK Food Standards and the Spanish legislation, estimated that the concentration of Cu in food should not exceed 20 mg kg^{-1} (Cronin *et al.,* 1998; Demirak *et al.,* 2006). All the measured concentration values were lower than this standard limit. Cu concentrations varied significantly between sites ($p<0.05$). Nuapia *et al.*(2018) reported the mean concentration of Cu in fish samples collected from the open markets in Johannesburg (South Africa) and Kinshasa (DRC) and this was close the one presented in this study as it ranged between 6.53-11.8mg kg^{-1}. The variations of Cu concentration in this study are shown in Figure 10.

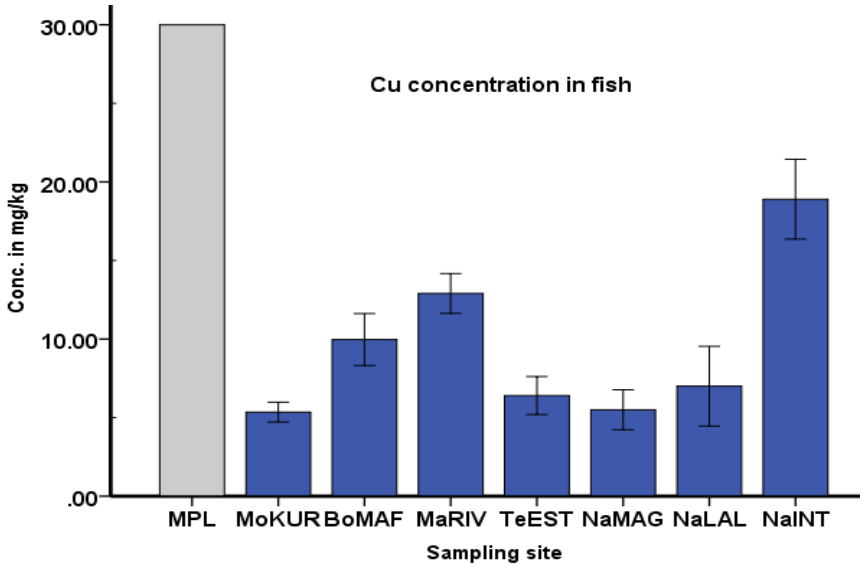

Figure 10: Variations of Cu concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.5. Iron (Fe) concentration in fish

Iron (Fe) is another essential metal for plant and animal growth (Khan *et al.,* 2007; FAO/WHO, 2011). The lowest concentration was 21.6±0.52 mg kg^{-1}in tilapia fish from TeEST and the highest

was 168.5±0.7 mg kg^{-1} in catfish from NaINT as presented in Table 4. FAO/WHO(2011 and 1983) have set a provisional tolerable maximum daily intake (PTMDI) for iron to 0.8 mg kg^{-1}bw, equivalent to 56 mg kg^{-1} for a 70 Kg body weight, as a precaution against storage in the body of excessive Fe. It was noted that two samples from NaLAL and NaINT of the analyzed fish samples were above the PTMDI set by FAO/WHO (2011 and 1983) while the five remaining were below the PTMDI of 56 mg kg^{-1}. Fe levels are significantly different between sites ($p<0.05$). Ejike and Liman (2017) and Abubakar *et al.*(2015) did studies on tilapia fish from Sokoto (Nigeria) and Zaria Metropolis (Nigeria) and reported concentration values ranging between 14.7-544 and 11.45-375.93 mg kg^{-1} respectively, which is far higher than that presented in this study. The variation of Fe concentrations in this study is indicated in Figure 11.

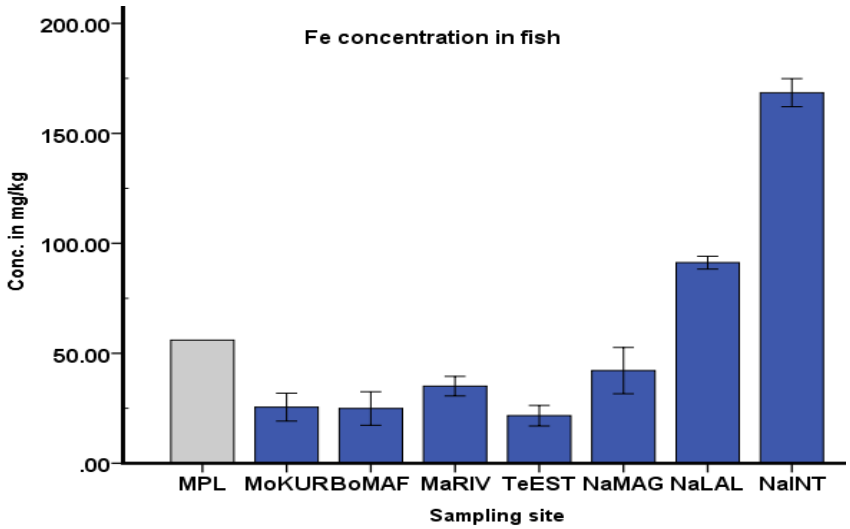

Figure 11: Variation of Fe concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.6. Mercury (Hg) concentration in fish

Mercury (Hg) is considered as one of the most toxic HMs in our environment (Castro-González and Méndez-Armenta, 2008; Jaishankar *et al.*, 2014). In the present study, the highest detected concentration of Hg was 0.035 ± 10^{-4}(0.0001) mg kg^{-1} in tilapia fish from BoMAF followed by MoKUR with $0.0326\pm2 \times 10^{-04}$(0.0002) mg kg^{-1} while the lowest detected is $0.0013\pm2.8 \times 10^{-4}$(0.0001) mg kg^{-1} in catfish from NaINT as presented in Table 4. Such measured concentration was lower than the acceptable limits of 1.0 mg kg^{-1} as recommended by FAO/WHO(1983), 0.5

mg kg^{-1} recommended by FSAI and ANHMRC. Hg concentrations are significantly different between sampling sites ($p < 0.05$). Hg is a neurotoxic agent that affects the development of the nervous system, resulting in psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control and general debilitation (Monteiro *et al.*, 2010;Monteiro *et al.*, 2010; Perugini *et al.*, 2016). In their study, Nuapia *et al.*(2018) reported the concentration of Hg, which is higher than that presented in this study and was in range of 1.0-2.0 and 2.7-3.2 mg kg^{-1} in fish from Johannesburg (South Africa) and Kinshasa (RDC), respectively. The variations of Hg concentration in this study are indicated in Figure 12.

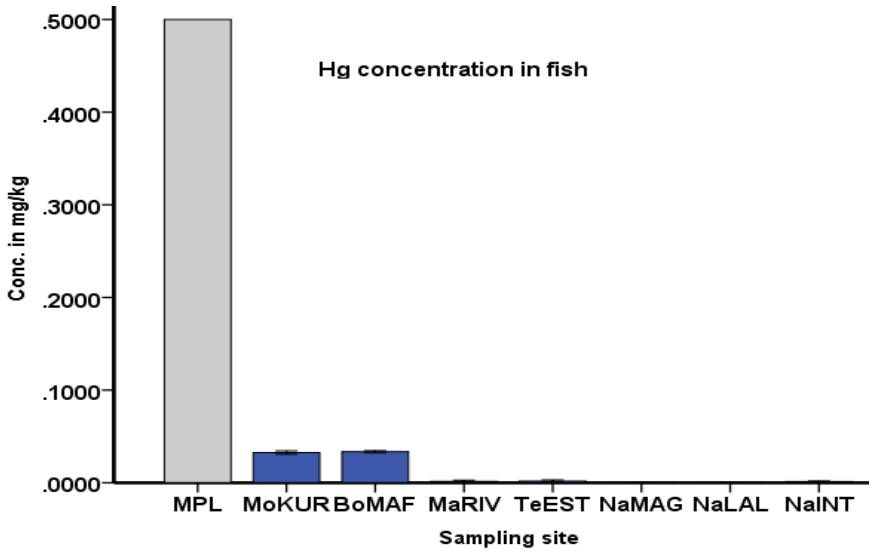

Figure 12: Variation of Hg concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.7. Lead (Pb) concentration in Fish

Lead (Pb) is categorized among the six most toxic pollutants threatening human health (Csavina *et al.*, 2012; Andrade *et al.*, 2017). In the present study, the highest mean concentration of Pb was 6.45±0.21 mg kg^{-1}in tilapia fish from MaRIV while the lowest is 1.9±0.11 mg kg^{-1} in tilapia from NaMAG. All the concentrations exceeded the maximum recommended values of 0.3 and 0.5 mg kg^{-1}proposed in the open literature (Eritrea, 2003; EU, 2006; FSAI, 2009; FAO/WHO, 2011; ANZFA, 2011). The ANHMRC recommended the maximum tolerable standard of Pb in seafood as 2.0 mg kg^{-1}(Bebbington*et al.*, 1977). However, Pb concentrations are significantly different between sampling sites ($p<0.05$). In comparison with other researches (Table 5), Mbewe *et al.*

(2016) assessed the concentration of Pb in fish muscles of tilapia from Kafue River (Zambia) and reported higher values than those of this study ranged between 11.6-110 mg kg^{-1}. The variations of Pb concentration in this study are indicated in Figure 13.

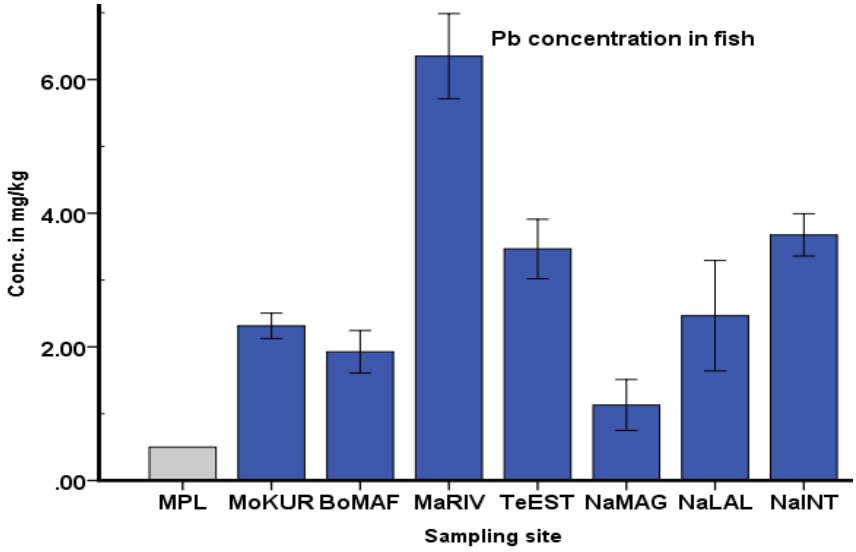

Figure 13: Variation of Pb concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.2.8. Zinc (Zn) concentration in Fish

Zinc (Zn) being a HM, has a tendency to get bio-accumulated in the fatty tissues of aquatic organisms, including fish and is known to affect reproductive physiology in fishes (Rahman *et al.*, 2012). Excessive intake of Zn by human is associated with acrodermatitis enteropathica, diabetes mellitus, high myopia, schizophrenia, among others (Vu *et al.*, 2017). The measured concentrations in fish samples are presented in Table 4 and they ranged between 28.25±0.50 mg kg^{-1} in tilapia fish from MaRIV and 52.25±3.18 mg kg^{-1} in Tilapia fish from NaMAG. The ANHMRC and WHO indicated that the health risks associated to zinc are possible at a level of 1000 mg kg^{-1} (Bebbington *et al.*, 1977;WHO, 2001). All the Zn determined concentrations in fish samples were lower than this standard. Zn concentrations varied significantly between sampling sites ($p<0.05$). In their study, Akpanyung *et al*. (2014) assessed the level of Zn in fish muscles from AkwaIbom (Nigeria) and reported the values which were higher than that presented in this

study and ranged between 145.5-250.6 mg kg^{-1}. The data from the existing literature (Table 5) shows that the HM concentrations in the muscles of fish vary widely. The variations of Zn concentration in this study are indicated in Figure 14.

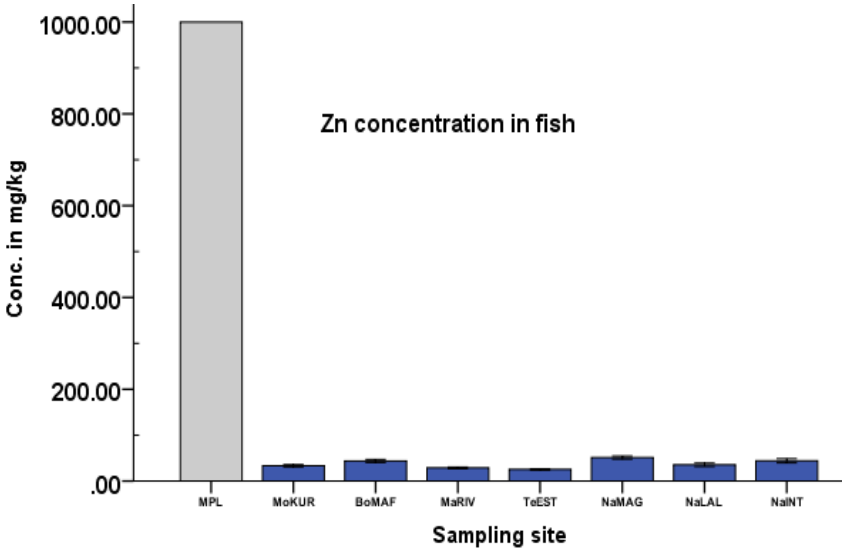

Figure 14: Variation of Zn concentration in fish

MPL: Maximum Permissible Limit **MoKUR**: Moamba-Corumana **BoMAF**: Boane-Mafuiane, **MaRIV**: Matola river, **TeEST**: Tete-Xitima, **NaMAG**: Nampula-Maganha, **NaLAL**: Nampula-Lalane **NaINT**: Nampula-Inthaka

4.3. Variation of metal concentration in fish versus the anthropogenic sources (mg kg^{-1})

Amongst the metals reported in this study, Al shows the highest average concentration in all studied sampling areas (districts) (Figure 15). Food is unquestionably the main source of Al intake either from the geologic surrounding or food additives as well as veterinary drugs, fertilizers and the air (Stahl *et al.*, 2011). The highest average concentration of both Fe and Zn is found in fish samples from MOMA respectively at 102 mg kg^{-1} and 45mg kg^{-1}. For other metals As, Cd, Cu, Hg and Pb the highest concentration is 10.1; 12.8; 12.9; 0.033 and 6.45mg kg^{-1} respectively from Boane (BOA); Moatize (MTZ); Matola (MAT); Boane (BOA) and Matola (MAT). Generally, the fish samples from MOMA and MAT show the relatively high concentration level compared to other places as we can see in the following decreasing order: MOMA >MAT >BOA >MOA >MTZ. The MOMA district is more characterized by anthropogenic activities which include the

heavy mineral sands extraction where the soil erosion is susceptible to spread the metal contamination. Mining development can impact water quality through direct and indirect contamination with inorganic and organic compounds, sediments, and biological wastes (Kirshner and Power, 2015).

However, an issue closely linked to the health hazards of metal contaminated land is soil erosion and land degradation (Singh *et al.,* 2018). The MAT (Matola) as sampling area where is located the Influene basin, is a semi urban area where different sources of urban runoff, sewage discharge and industrial effluents can be released. The fish samples from MTZ (Moatize), although bought at the markets located in Moatize districts, the sellers transported them from Angónia located in Tete province. The area of Angónia is characterized by fishing and agriculture as the main activities for human surviving and the area is less prone to the metal-contaminating sources. The fish samples from BOA and MOA sampling areas were taken from the rivers of Umbeluzi and Incomati, the places mostly characterized by agricultural activities which might be less metal-contaminated areas in comparison to MAT and MTZ. The comparative study of concentration of fish samples is presented in the Figure 15.

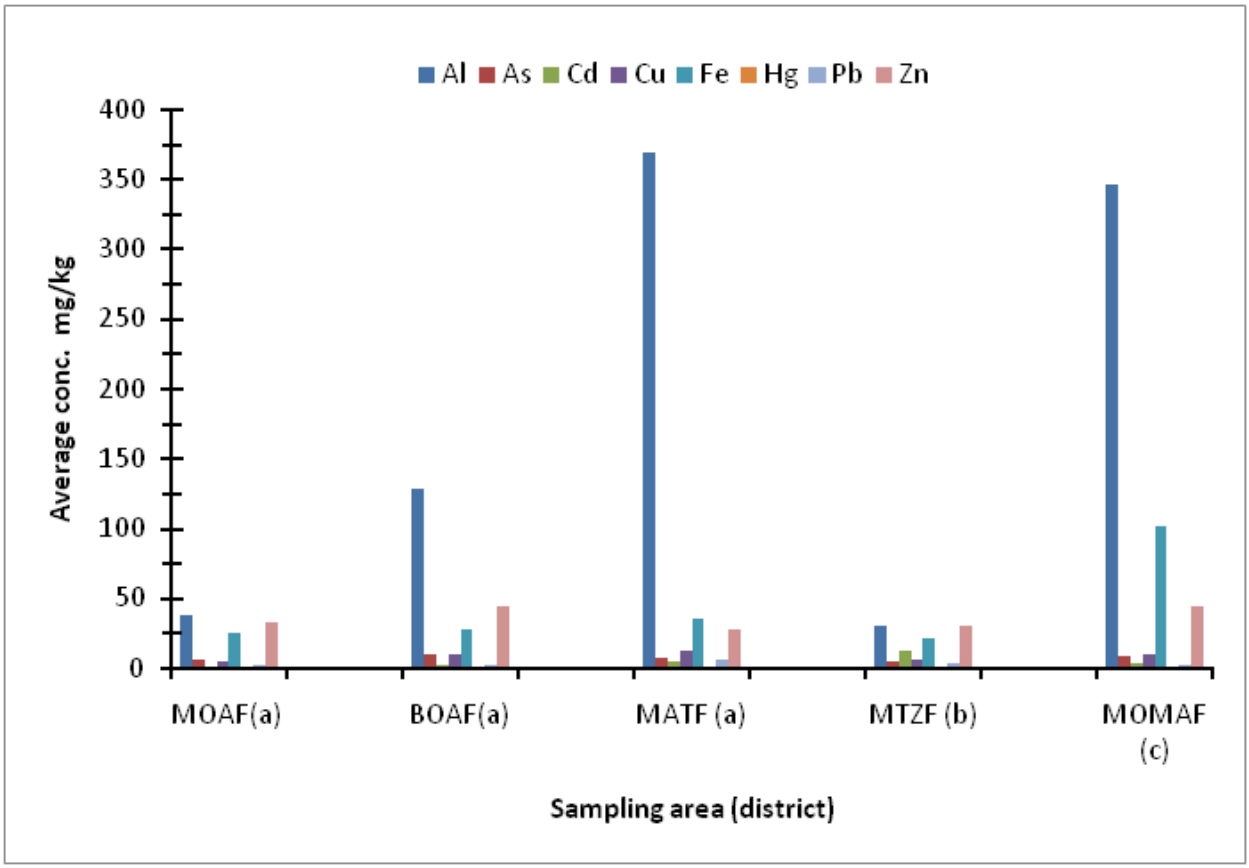

Figure 15: Metal concentration in fish/comparison between sampling site

MOAF: Moamba; **BOAF**: Boane; **MATF**: Matola river; **MTZF:** Moatize; **MOMA:** Moma (Larde,Maganha, Inthaka):

(a) Maputo **(b)** Tete **(c)** Nampula province

Table 5: Comparison of heavy metal concentrations in fish muscle with the reported values in the literatures

Sampling area	Al	As	Cd	Cu	Fe	Hg	Pb	Zn	Reference
Markets from Maputo, Tete & Nampula, Mozambique	30.8-644	5.65-12.7	1.05-12.8	5.25-18.9	21.0-168	ND-0.0033	1.88-6.45	28.2- 52.2	This study
Sokoto (Nigeria)	NA	NA	NA	10.8-31.9	14.7-5440	NA	10.8-25.4	44.2-85.1	(Ejike and Liman, 2017)
Johannesburg (RSA)	5.3-12.33	2.45-3.89	0.52-0.75	5.17-7.89	NA	1.06-2.01	0.21-0.45	12.76-15.17	(Nuapia *et al.*,2018)
DurbanSouth Africa	NA	4.2-8.9	NA	0.75-1.18	9.7-22.8	NA	0.09-1.09	12.2-21.4	(Moodley *et al.*, 2021)
Kinshasa (DRC)	3.85-5.41	9.81-14.21	1.72-3.28	2.52-4.60	NA	2.71-3.17	0.58-2.50	43.74-57.64	(Nuapia *et al.*,2018)
Kafue River (Zambia)	NA	NA	0.3-20	3.9-51	271-3300	NA	11.6-110	NA	(Mbewe *et al.*, 2016)
Lake Kariba (Zambia)	NA	NA	0.002-0.02	2-33	NA	NA	0.04-1.36	21-78	(Nakayama *et al.*, 2010)
Bangshi river (Bangladesh)	NA	1.97-6.24	0.09-0.87	8.83-43.18	NA	NA	1.76-10.27	42.83-413.0	(Rahman *et al.*,2012)
Zaria Metropolis (Nigeria)	NA	NA	1.12-19.75	NA	11.5-375.9	66.54-80.35	3.95-17.55	NA	(Abubakar *et al.*, 2015)
AkwaIbom(Nigeria)	NA	0.001-0.09	0.01-0.022	NA	NA	NA	0.0013-0.09	145.5-250.6	(Akpanyung *et al.*, 2014)
Egyptian inland (Egypt)	NA	NA	0.03-0.11	0.25-1.85	1.41-4.74	NA	NA	3.38-8.46	(Youssef and Tayel, 2004)
Mediterranean seas(Turkey)	NA	NA	0.01-0.39	0.51-7.05	9.18-136	NA	0.21-128	3.51-53.5	(Türkmen *et al.*, 2009)
Puchong (Malaysia)	NA	ND	ND	ND-20.8	31.9-743	NA	ND	45.5-86.1	(Ismail and Saleh, 2012)
Langat River(Bangladesh)	NA	NA	0.03-0.05	1.01-1.69	NA	NA	0.26-0.99	20.58-26.13	(Taweel *et al.*,2013)
Rivers (Bangladesh)	NA	NA	0.04-013	1.48-23.30	NA	NA	0.29-10.05	33.01-286.4	(Sharif *et al.*, 1993)
Markets of India	NA	ND-4.14	ND-1.32	0.14-14.7	NA	ND-2.31	ND-0.76	0.66-39.2	(Sivaperumal *et al.*, 2007)

NA: Not analyzed **ND**: Not detected

4.4. Health risk assessment of metals in fishes

4.4.1. Estimated daily intake (EDI)

The EDI values of metals As, Cd, Cu, Fe, Hg Pb and Zn in fish are presented in Table 6. They were evaluated according to the mean concentration of each metal in each species of fish (Islam *et al.,* 2018). The average EDI of metals through fish consumption is in a decreasing order as follows: Al >Fe >Zn >Cu >As >Cd >Pb>Hg. However, the calculated EDIs data range between 1.85 x 10^{-7} and 1.01 x 10^{-1} mg $kg^{-1}day^{-1}$ for all metals and both fish species (*Oreochromis mossambicus* and *Chrysichthys nigrodidatatus*) where they all fall within the safety range of the established provisional tolerable daily intake (PTDI) values, as provided in Table 6 (FAO/WHO, 2005; FAO/WHO, 2003). The PTDI data were established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Alipour *et al.*, 2014). The PTDI is an estimate of the amount of a chemical that can be ingested over a lifetime without appreciable risk. An intake above the PTDI does not automatically mean that health is at risk but it an alert (RCFS/FEHD, 2009).

This shows that there is no health-threatening concern due to the consumption of tilapia and catfish sampled in the locations sites MoKUR, BoMAF, MaRIV, TeEST, NaMAG, NaLAL and NaINT. However, in a comparable study Addo-Bediako *et al*.(2014) reported the EDI values (4.55-4.58; 0.00-0.06; 0.00-0.01; 0.37-0.81; 9.61-49.49; 0.31-0.37 and 1.97-300 µg $kg^{-1}day^{-1}$) for Al, As, Cd, Cu, Fe, Pb and Zn respectively, in one fish species (*Oreochromis mossambicus)* from Flag Boshielo Dam and Phalaborwa barrage (South Africa). For all metals, the EDIs are less than the acceptable levels for safe consumption.

Moreover, in their study Sadeghi *et al.*(2020) determined the EDIs in three tuna species and they found the values in range of 0.83-2.56, 0.24-0.46 and 5.56-11 µg $kg^{-1}day^{-1}$ for Cu, Zn, and Pb respectively. All EDIs were found to be within the safety limits of tolerable daily intake, suggesting that consumption of the fish species *Euthynnus affinis*, *Katsuwonus pelamis* and *Thunnus albacares* has no risks for people around the Oman Sea, a similar finding as in the present study. The daily intakes estimated in this study also are in agreement with the values reported in other studies such as Alipour *et al.* (2014) and Taweel *et al.*(2013).

Table 6: Estimated daily intake (EDI, mg $kg^{-1}day^{-1}$) of metals due to consumption of fish

		Heavy metal, EDI							
Site	**Species**	**Al**	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**
MoKUR	Tilapia	5.41E-03	8.28E-04	1.49E-04	7.47E-04	3.62E-03	4.55E-06	3.66E-04	6.29E-03
BoMAF	Tilapia	1.82E-02	1.43E-03	3.03E-04	1.41E-03	4.06E-03	4.69E-06	3.02E-04	7.49E-03
MaRIV	Tilapia	4.96E-02	1.00E-03	8.55E-04	1.83E-03	4.98E-03	1.99E-07	1.13E-03	4.01E-03
TeEST	Tilapia	7.14E-03	8.04E-04	2.06E-03	1.15E-03	2.99E-03	2.70E-07	5.21E-04	4.38E-03
NaMAG	Tilapia	1.37E-02	1.01E-03	1.89E-04	1.03E-03	6.34E-03	NA	2.67E-04	7.43E-03
NaLAL	Tilapia	4.06E-02	9.39E-04	1.33E-03	1.15E-03	1.31E-02	NA	3.64E-04	5.51E-03
NaINT	Catfish	1.01E-01	1.80E-03	2.21E-04	2.74E-03	2.39E-02	1.85E-07	5.19E-04	6.29E-03
$EDI_{(aver)}$ mg kg^{-1} day^{-1}		3.36E-02	1.12E-03	7.30E-04	1.44E-03	7.75E-03	1.98E-06	4.96E-04	6.11E-03
PTDI mg kg^{-1} day^{-1}		10[a]	0.15[a]	0.07[a]	35[a]	56[a]	0.016[b]	0.25[a]	70[a]

PTDI: Provisional tolerable daily intake; NA: Not Available (for concentration below the limit of quantification); [a](FAO/WHO, 2005); [b](FAO/WHO, 2003); $EDI_{(aver)}$: Average estimated daily intake.

4.4.2. Target Hazard Quotient (THQ)

The computed THQ from metal concentration is shown in Table 7. As and Cd were the major contributors to the hazard index (HI) in the studied fish samples. The THQ value is < 1 for all studied HMs except As and Cd. For As the THQ > 1 in fish samples from all sampling sites, while for Cd the THQ > 1 only in fish samples from MaRIV (1.70), TeEST (4.12) and NaLAL (2.66). However, exposure to more than one contaminant may produce a synergistic effect on the consumer health (Nuapia *et al.*, 2018). The combined impact of all metals (hazard index, HI) under consideration is higher than the acceptable limit of 1 in both species of fish, in all sampling sites. The As contribution to the HI ranges between 40-90% which is considerably higher than 12.5 % as the minimum possible contribution expected for each one of eight studied metals. The highest Cd contribution to the HI is 32.6, 58.2 and 45.3% in fish samples from MaRIV, TeEST and NaLAL, respectively. The HI value is > 1 for all the studied heavy metals, in all the sampling sites and it is in range of 3.15-7.08. These results indicate the probable potential risk to the local consumers of the fish sold in the open markets of the concerned area in this study. However, THQ and HI do not directly measure risk because they do not define any dose–response relationship (U.S.EPA, 1989).

In comparison with other researches, Nuapia *et al.*(2018) computed the THQ in fish from Johannesburg and Kinshasa cities and they found that the combined THQ values of metals (Al, As, Cd, Cr, Cu, Mn, Pb, Se and Zn) were > 1 for all the fish samples. Such results indicate high

potential risk to the local consumers both in Kinshasa and Johannesburg via consumption of the fish sold at the open markets. Likewise Copat *et al*. (2012) determined the THQs for Cd, Hg and Pb and in fish from Sicily, Mediterranean Sea and the results ranged between 64 x 10^{-6}-0.035; 27 x 10^{-6}-195 x 10^{-6} and 2 x10^{-6}-19 x10^{-6} respectively, indicating that there is no non-carcinogenic risk to the fish consumers. Zhu *et al.* (2015) calculated the THQ values of individual metals As, Cd, Cu, Ni, Zn and Fe in 10 species of edible fishes from Nansi Lake, China, and found the THQs of individual metals < 1 in range between 0.007-0.439 for both general population and fishermen and this information revealed that this population had no non-carcinogenic risks due to the consumption of such fish diet. For the HI, the values were < 1 (between 0.480 and 0.679) for the general population and were > 1 (between 1.165 and 1.742) for the fishermen, indicating that local fishermen may experience some adverse health effects. On the other hand, Krishana *et al*.(2014) did a study on accumulation of HMs through fish consumption, from Machilipatnam Coast, Andhra Pradesh, India. They reported that the calculated average THQ values for individual HMs like Hg, Cu, Zn, Pb and Zn were all >1 and they ranged between 1.8 and 17.9 except for Cd.

Table 7: Target Hazard Quotient (THQ, mg kg^{-1}) of metals due to consumption of fish

	Heavy metal, THQ								**HI**
Site	**Species**	**Al**	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**	
MoKUR	Tilapia	0.005	2.75	0.29	0.018	0.005	0.045	0.023	0.015	3.15
BoMAF	Tilapia	0.018	4.77	0.60	0.030	0.005	0.046	0.026	0.020	5.51
MaRIV	Tilapia	0.050	3.37	1.70	0.045	0.007	0.001	0.080	0.013	5.26
TeEST	Tilapia	0.007	2.88	4.12	0.020	0.004	0.002	0.037	0.014	7.08
NaMAG	Tilapia	0.013	3.38	0.37	0.025	0.009	NA	0.019	0.024	3.84
NaLAL	Tilapia	0.040	3.12	2.66	0.028	0.018	NA	0.020	0.018	5.90
NaINT	Catfish	0.100	6.00	0.44	0.068	0.034	0.001	0.037	0.020	6.70

NA: Not Available (for concentration below the limit of quantification); **HI**: Hazard Index, Sum of THQ values (from one kind of foodstuff)

4.4.3. Allowable daily consumption limit (CRl_{im})

The Table 8 illustrates the results of the calculated maximum allowable fish consumption limit **(**CR_{lim}). According to U.S.EPA(2000), the risk-based consumption limits are estimated as the maximum daily consumption rates of contaminated fish that would not be expected to cause immediate adverse health effects for human consumers. However, in the present study the highest average CR_{lim} of the tilapia fish from TeEST shows that it would be, relatively the most tolerated

for consumption in the present fish diet whereas; on the contrary the tilapia fish from NaLAL, is the least tolerated for consumption. Based on the average CR_{lim} the decreasing order in terms of sampling site is: TeEST>MaRIV>NaINT>MoKUR>BoMAF>NaMAG>NaLAL with the average CR_{lim} values; 1.08 >0.95 >0.83 >0.69 >0.45 >0.44 >0.29 Kg day^{-1}, respectively. In terms of the individual metals (in different foodstuffs), the ranges of CR_{lim} were 0.09-1.84; $2.08x10^{-3}$-$3.71x10^{-2}$; $2.41x10^{-3}$-$3.33x10^{-2}$;0.14-053;0.29-2.32; 0.21-5.38; 0.12-0.52 and 0.40-0.74 kg day^{-1} respectively for Al, As, Cd, Cu, Fe, Hg, Pb and Zn.

In comparison with other studies, Taweel *et al.*(2013) reported the values of CR_{lim} in tilapia fish which were in ranges 1.51-2.53; 0.64-1.06; 0.73-0.93; 0.4-0.93 and 0.13-0.49 for Cu, Cd, Zn and Pb, respectively. Such values are higher than the reported results in the present study for the same metal. As shows, the lowest average CR_{lim}value ($2.99x10^{-3}$ kg day^{-1}) which means that it would be the least allowed for consumption in the present fish samples. On the contrary, the higher average CR_{lim} values of Hg (2.60 Kg day^{-1}) suggest that, in this diet it is the most likely tolerated metal for consumption, based on the measured concentration and its associated RfD value. Based on the average $CR_{lim,}$ the decreasing order of studied metals was Hg >Fe >Al >Zn >Pb> Cu >Cd >As. This order opposes to the one for average THQ from different fish samples (As >Cd >Cu>Al>Pb>Zn >Hg >Fe). The reason is that both THQ and CR_{lim} vary inversely with respect to the RfD as shown in equations above, (2) and (5).

Table 8: Maximum allowable fish consumption limit (CR_{lim}, Kg day^{-1})

		Heavy metal, CR_{lim}								$CR_{lim\ (aver)}$
Site	**Species**	**Al**	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**	
MoKUR	Tilapia	1.84	3.60E-03	3.33E-02	0.53	1.92	0.21	0.41	0.63	0.69
BoMAF	Tilapia	0.54	2.08E-03	1.64E-02	0.28	1.71	0.21	0.38	0.47	0.45
MaRIV	Tilapia	0.20	2.95E-03	5.82E-03	0.21	1.39	5.00	0.12	0.74	0.95
TeEST	Tilapia	1.39	3.71E-03	2.41E-03	0.34	2.32	3.68	0.26	0.68	1.08
NaMAG	Tilapia	0.72	2.94E-03	2.63E-02	0.38	1.09	NA	0.52	0.40	0.44
NaLAL	Tilapia	0.24	3.18E-03	3.73E-03	0.34	0.53	NA	0.38	0.54	0.29
NaINT	Catfish	0.09	2.48E-03	2.25E-02	0.14	0.29	5.38	0.26	0.47	0.83

NA: Not Available (for concentration below the limit of quantification); **$CRlim_{(aver)}$:** Average of maximum allowable fish consumption limit

CHAPTER FIVE

5. RESULTS AND DISCUSSIONS IN VEGETABLE SAMPLES

5.1. Heavy metal concentrations of vegetables

The mean concentrations ($mgkg^{-1}$) in vegetable samples for eight metals Al, As, Cd, Cu, Fe, Hg, Pb and Zn are presented in the Table 9. The bioaccumulation of metal was found in a decreasing order as follows: Al (221.6) > Fe (29.1) > Zn (19.67) > Cu (10.51) >As (8.07) >Pb (7.32) > Cd (2.23) > Hg (0.009) in cabbage and Al (724.5) >Fe (95.7) > Zn (76.5) > Cu (24.8) > As (19.7) >Pb (6.25) > Cd (5.18) > Hg (0.018) in kale. The ranges of concentration for all metals in both cultivars are presented in the Table 9. Comparing the uptake level of metals, it can be seen that the metal uptake of kale cultivar was higher (68%) than the uptake of cabbage (32%). This estimation was made based on the sampling sites where both cultivars, cabbage and kale were simultaneously collected such as Moamba site one (MOA1), Moamba site two (MOA2), Moamba site three (MOA3), Boane site one (BOA1), Boane site two (BOA2), Machava (MACH), Angónia site one (AG1), Angónia site two (AG2) and Larde (LARD).

However, the same finding was reported by Santos *et al.* (2004) who analysed trace metals in food stuffs in Rio de Janeiro city (Brazil). For all studied metals (Al, Cu, Mn, Zn, Cd, Cr, Ni, Pb and U), the metal uptake capacity of kale cultivar was higher (89%) than the uptake capacity of cabbage (11%). The mean concentrations of metals Al, As, Cd, Cu, Fe, Hg, Pb and Zn were compared with the maximum limits in foodstuffs as recommended by different organizations (EU, 2006; FSAI, 2009; FAO/WHO,2006; 2007&2011). The coefficient of significance (sig) was determined by use of one-way ANOVA. However, it was found that all studied metals and for the two vegetable cultivars, cabbage and kale Al, As, Cd, Cu, Fe, Hg, Pb, and Zn, the concentrations of each one was significantly different between sampling sites ($p < 0.05$). The studies of heavy metals accumulation in vegetables have been done and reported by various researchers across the world. The Table 10 contains some of the literatures with their reported values for the presently studied metals.

Table 9: Metals concentration in vegetable samples (Mean ± SD mg kg^{-1} dry wt)

Site	Cultivar	Al	As	Cd	Cu	Fe	Hg	Pb	Zn
MOA1[a]	**Cabbage**	1.8 ± 0.55	1.65± 0.26	0.88±0.05	1.2±0.29	1.7±0.44	0.001±0.00	0.4± 0.01	1.7±0.17
	Kale	6.7±0.07	3.54± 0.27	1.7 ± 0.06	5.9±0.41	8.0±0.22	0.011±0.00	1.4± 0.01	1.3±0.03
MOA2[a]	**Cabbage**	0.65 ±0.03	1.6 ± 0.4	0.82± 0.08	0.51±0.04	2.0±0.05	0.001±0.00	1.3±0.00	1.45±0.08
	Kale	11.9±0.84	3.52 ±0.31	1.7 ±0.08	6.1±0.4	8.4±0.57	0.009±0.0007	3.7±0.00	1.4±0.05
MOA3[a]	**Cabbage**	0.25 ± 0.07	3.6 ± 0.32	1.7±0.07	6.1±0.34	8.1±0.24	0.001±0.00	1.4±0.01	1.4±0.06
	Kale	6.5 ± 0.70	3.5±0.25	1.6±0.06	6±0.43	19.7±0.00	0.009±0.0007	3.6±0.01	1.3±0.03
BOA1[a]	**Cabbage**	66.5±0.70	7.8 ± 2.23	2.0 ±0.07	10.0±0.19	17.7±1.25	0.009±0.00	1.3±0.1	5.9±0.7
	Kale	6.0±0.00	3.5 ± 0.2	1.65 ±0.06	5.9±0.43	19.65±0.07	0.015±0.0007	3.5±0.02	1.3±0.04
BOA2[a]	**Cabbage**	64.6±0.77	8.1 ±2.56	2.1 ±0.09	9.8±0.07	14.4±0.84	0.002±0.00	1.5±0.07	8.8±0.62
	Kale	8.5 ± 0.7	3.5 ± 0.26	1.65 ±0.05	5.9±0.44	7.8±0.02	0.018±0.00	3.5±0.02	1.3±0.05
BOA3[a]	**Cabbage**	59.4 ± 1.9	7.61 ± 2.3	2±0.02	9.7±0.28	11.1±0.63	0.002±0.00	0.8±0.06	12.2±0.86
MACH[a]	**Cabbage**	222±1.41	7.2 ± 2.54	1.8 ±0.07	7.1±0.00	1.0±0.21	0.009±0.00	3.9±0.08	16.1±7.52
	Kale	51.6±2.05	14.1 ± 8.25	4.68 ±0.07	21.7±0.07	>95.7±1.7	0.003±0.00	6.2±0.1	41.7±1.0
PATR[a]	**Kale**	51.6 ±2.05	19.7 ± 3.94	5.36 ±0.14	24.0±0.07	95.7±1.7	0.01±0.00	4.64±0.14	22.9±1.4
T3[a]	**Cabbage**	84.3 ± 6.1	7.8 ±2.2	2.2± 0.15	10.5±0.07	13.1±1.5	0.001±0.00	7.3±0.14	19.7±3.7
2M[a]	**Kale**	230 ± 3.81	17.9 ±3.84	4.9 ± 0.16	20.8 ±0.77	94.0±1.5	0.014±0.00	4.0±0.17	32.6±7.84
ZV[a]	**Kale**	225.1 ± 3.6	17.6 ± 3.84	4.7±0.05	18.7±1.13	60.4±0.14	0.017±0.00	3.8±0.08	32.6±3.6
AG1[b]	**Cabbage**	76.5 ± 2.85	7.45 ± 2.84	2 ±0.00	9.5±0.77	29±2.06	0.0017±0.0003	0.8±0.06	16.2±1.42
	Kale	269.3 ± 0.7	16.9 ± 3.38	4.8 ±0.12	21.1±1.27	>95.7±1.7	0.007±0.00	3.5±0.16	35±2.95
AG2[b]	**Cabbage**	78.2 ± 1.27	7.2± 7.77	1.91 ±0.02	10±0.42	26.4±1.18	0.0019±0.0001	0.8±0.01	17.1±1.6
	Kale	724±4.94	15± 3.38	4.57± 0.09	19.8±1.41	>95.7±1.7	0.0065±0.0007	2.9±0.07	76.5±2.23
MTZ[b]	**Kale**	328±5.09	18.1 ± 3.98	5.2± 0.07	24.8±0.24	>95.7±1.7	0.008±0.001	3.8±0.09	34.8±2.74
LARD[c]	**Cabbage**	41.4±0.28	6.6 ± 2.6	1.6 ±0.01	6.9±0.14	12.3±1.9	0.001±0.00	0.57±0.04	12.6±2.15
	Kale	264±2.12	15.5 ± 3.16	4.4± 0.29	18.2±0.49	>95.7±1.7	0.0085±0.0007	3.3±0.3	19.8±1.55
MPL mg kg^{-1}		70 [d]	0.1 [e]	0.2 [efgh]	40 [g]	450 [g]	0.1 [g]	0.3 [efgh]	100 [g]

[a]Maputo province, [b]Tete province, [c]Nampula province, MPL: Maximum Permissible Limits(FAO/WHO, 2006) ,[e] (FAO/WHO, 2011) , [f](EU, 2006) [g] (FAO/WHO, 2007) , [h](FSAI, 2009)

5.1.1. Aluminium (Al) concentration in vegetables

The highest Al mean concentration is 222±1.41 mgkg^{-1} in cabbage from MACH and 724±4.94 mg kg^{-1}in kale from AG2 (Table 9). The two concentrations reflected the highest ones of other metals in this study. The lowest mean concentrations are 0.25±0.07 mg kg^{-1} and 6.5±0.70 mgkg^{-1} in cabbage and kale, respectively, both from MOA3. Al is widely present in the diet and its levels in vegetable, fruit or seafood groups are higher than in other groups(Hardisson *et al.*, 2017). The provisional tolerable weekly intake (PTWI) of Al is 1.00 mg kg^{-1}bw, equivalent to 70 Kg for an adult of 70 Kg (FAO/WHO,2006). However, 7 out of 11 cabbage samples (64%) were lower than the limit of 70 mg kg^{-1} and 7 out of 13 kale samples (54%) are lower than the same limit. In different sampling sites, Al was more bioaccumulated in kale (66%) than in cabbage (34%). A similar finding was reported by Santos *et al.*(2004) where 1.8 mg kg^{-1} and 8.6 mg kg^{-1} Al were found in cabbage and kale, respectively. Santos *et al.*(2004) determined the concentration in different vegetables and reported Al in the concentration range of 1.4-2.2 and 5.3-17 mg kg^{-1} respectively, in cabbage and kale. The variation of Al concentration in vegetables is shown in Figure 16.

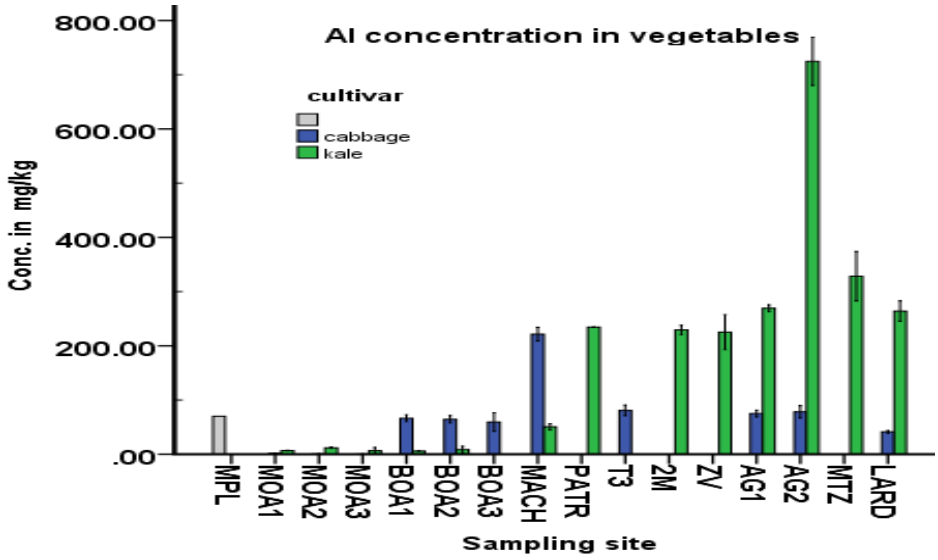

Figure 16: Variation of Al concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.2. Arsenic (As) concentration in vegetables

The highest measured concentrations are 8.1±2.56 mg kg^{-1} in cabbage from BOA2 and 19.7±3.94 mg kg^{-1}in kale from PATR. The lowest measured concentrations for As are 1.6±0.4 mg kg^{-1} in cabbage and 3.5±0.26 mgkg^{-1} in kale from MOA2 and BOA2, respectively. As concentrations in all samples both cabbage and kale are higher than the FAO/WHO stipulated limit of 0.1 mg kg^{-1} (FAO/WHO, 2011). It was reported that exposure to As can lead to skin and lung cancers, kidney and heart diseases, neurological and respiratory malfunctions, among others (Zhu *et al.*, 2015;Baghaie and Fereydoni, 2019). As is released into the environment by the smelting process of copper, zinc and lead, as well as by the manufacturing of chemicals and glasses (Baby *et al.*, 2010). In Bangladesh, both Islam *et al.* (2014) and Islam *et al.*(2018) did a study on HMs in vegetables where they found As in concentration ranging between 0.04-0.5 and 0.01–0.75 mg kg^{-1},respectively (Table 9). The variation of As concentration in vegetables is presented in the Figure 17.

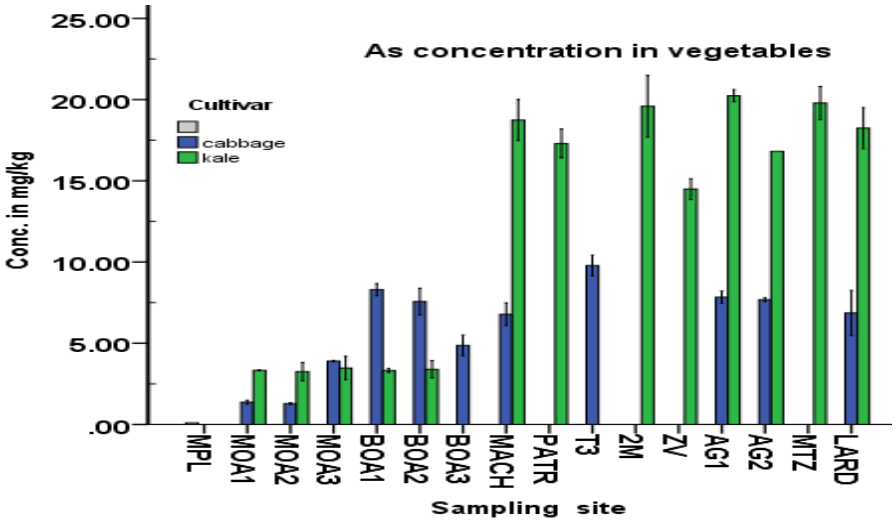

Figure 17: Variation of As concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.3. Cadmium (Cd) concentration in vegetables

The Cd maximum detected concentration is 2.2±0.15 mg kg^{-1} in cabbage from T3 and 5.3±0.06 mg kg^{-1} in kale from PATR. The lowest detected concentration was 0.82± 0.08 mg kg^{-1} in cabbage

from MOA2 and 1.65±0.06 mg kg^{-1} in kale from both of BOA1. Cd concentration measured in both cultivars was all above the recommended maximum limit of 0.2 mg kg^{-1}(EU, 2006; FSAI, 2009; FAO/WHO, 2006,2007,2011). It is found that Cd was more up taken by kale (66%) than cabbage (34%) when we consider the sampling sites with both cultivars. The similar finding was reported by Guerra *et al.*(2012) who found Cd concentration at 0.04 mg $kg^{-1}$and 0.12 mg kg^{-1},respectively, in cabbage and kale (Table 9). This concentration was below the MPL as recommended by (FAO/WHO, 2011). Cd may accumulate in human body and induce kidney dysfunction, skeletal damage, and reproductive deficiencies (Chen *et al.*, 2011; Gautam *et al.*, 2014). The variation of Cd concentration in vegetables is shown in Figure 18.

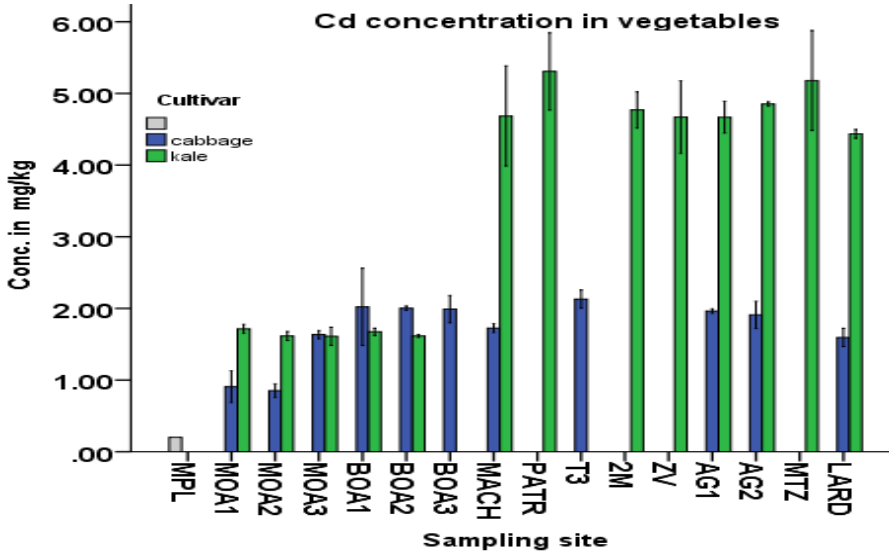

Figure 18: Variation of Cd concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.4. Copper (Cu) concentration in vegetables

The highest Cu concentrations are 10.5±0.07 mg kg^{-1} in cabbage from T3 and 24.8±0.24mgkg^{-1} in kale from MTZ whereas the lowest measured concentrations were 0.51±0.04 mg kg^{-1} in cabbage from MOA2 and 5.9±0.44 mgkg^{-1} in kale both from BOA2. The MPL of 40 mg $kg^{-1}$is within the measured concentration values for both vegetable cultivars. For cabbage samples, 19% of the

studied samples are above the acceptable limit while 81% are within the acceptable limit of 40 mg kg^{-1}. However, on the part of kale samples, 54% of the total analyzed samples are above the acceptable limit whilst 46% are within the limits. In Ghana, the studies conducted by both Lente *et al.*(2012) and Ametepey *et al.* (2018) reported Cu concentration values ranging between 3.3–8.1 mg $kg^{-1}$and 0.04-0.09 mg kg^{-1},respectively, in vegetables (Table 9). Cu is essential for humans as a trace dietary mineral but excessive consumption can lead to adverse effects on human health (Rahman *et al.*, 2012; Gautam *et al.*, 2014). The variation of Cu concentration in vegetables is shown in Figure 19.

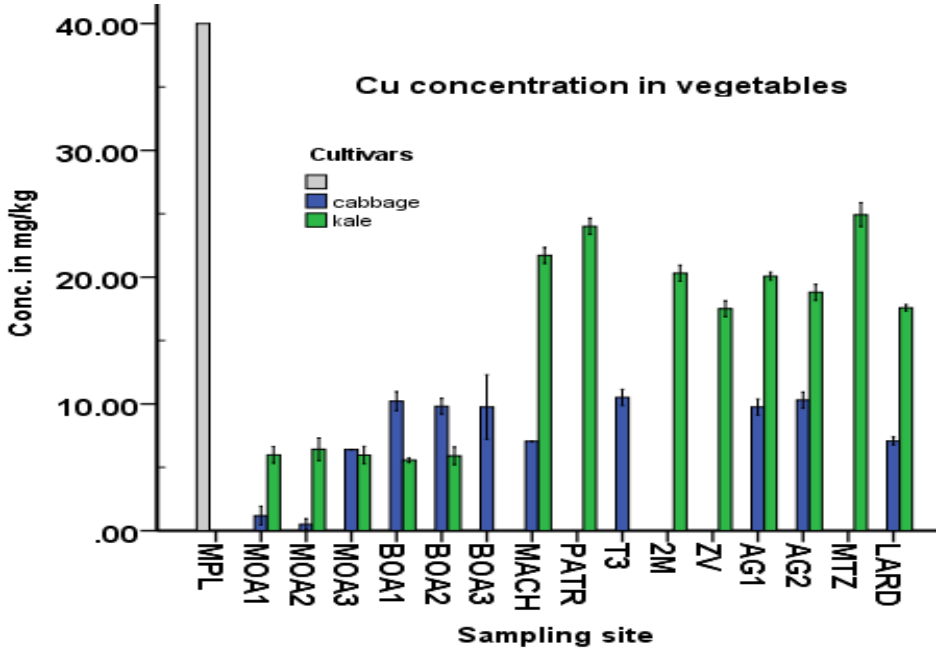

Figure 19: Variation of Cu concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.5. Iron (Fe) concentration in vegetables

The highest Fe concentration in cabbage is 29±2.06 mg $kg^{-1}$from AG1. However, for kale cultivar the highest detected was 95.7 mg kg^{-1} from PATR. The lowest concentration of Fe was 1.0±0.21 $mgkg^{-1}$in cabbage from MACH and 7.8±0.02 $mgkg^{-1}$ in kale from BOA2. Generally, the Fe concentration in both cabbage and kale is below the FAO/WHO recommended limit of 450 mg

kg^{-1}(FAO/WHO, 2007). In a study on HMs bioaccumulation in wastewater-irrigated vegetables, in India (Arora*et al.*, 2008) reported Fe concentration ranging between 111- 412 mg kg^{-1}. For the study carried out in Ghana, Ametepey *et al.* (2018) reported the Fe concentration in different vegetables and it ranged between 3.04 – 4.47 $mgkg^{-1}$ (Table 9). Although Fe is an essential metal, excess ingestion can result in deposition of iron in tissues (siderosis), in adrenals, liver, pancreas, thyroid, pituitary among others (FAO/WHO, 2011). The variation of Fe concentration in vegetables is shown in Figure 20.

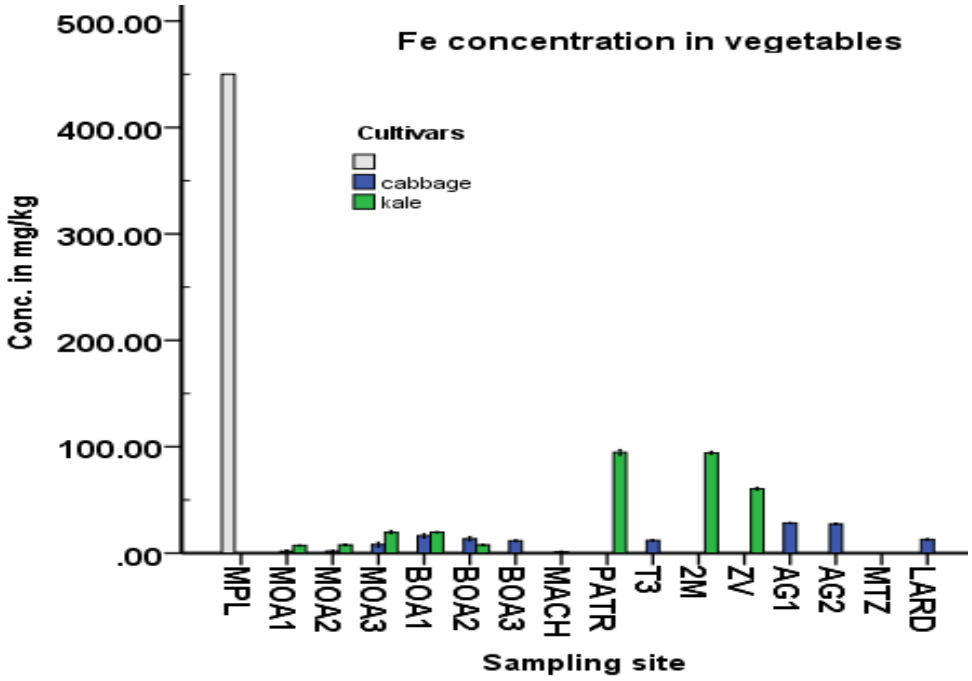

Figure 20: Variation of Fe concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.6. Mercury (Hg) concentration in vegetables

The maximum concentration of Hg is 0.009±0.00 mg kg^{-1} and 0.018±0.00 mg kg^{-1} respectively in cabbage from MACH and kale from BOA2. The lowest values were recorded as 0.001±0.0001mg kg^{-1} in cabbage from MOA1 and 0.003±0.0001 $mgkg^{-1}$ in kale from MACH. However, all vegetable samples have lower levels of Hg than the MPL of 0.1 mg kg^{-1} as set by FSAI(2009) and

FAO/WHO(2007). Chen *et al.*(2011) reported the concentration of HMs ranging between 0.001-0.046 mg kg^{-1} in vegetables and fruits from China, Xiamen, which is lower than the MPL. Hg is a neurotoxic poison that causes neurobehavioral effects, neuroendocrine, and renal damage and gastrointestinal toxicity (Chen *et al.*, 2011). It is also said to be carcinogen (Azaman *et al.*, 2015; Ikem and Egilla, 2008). The variation of Hg concentration in vegetables is shown in Figure 21.

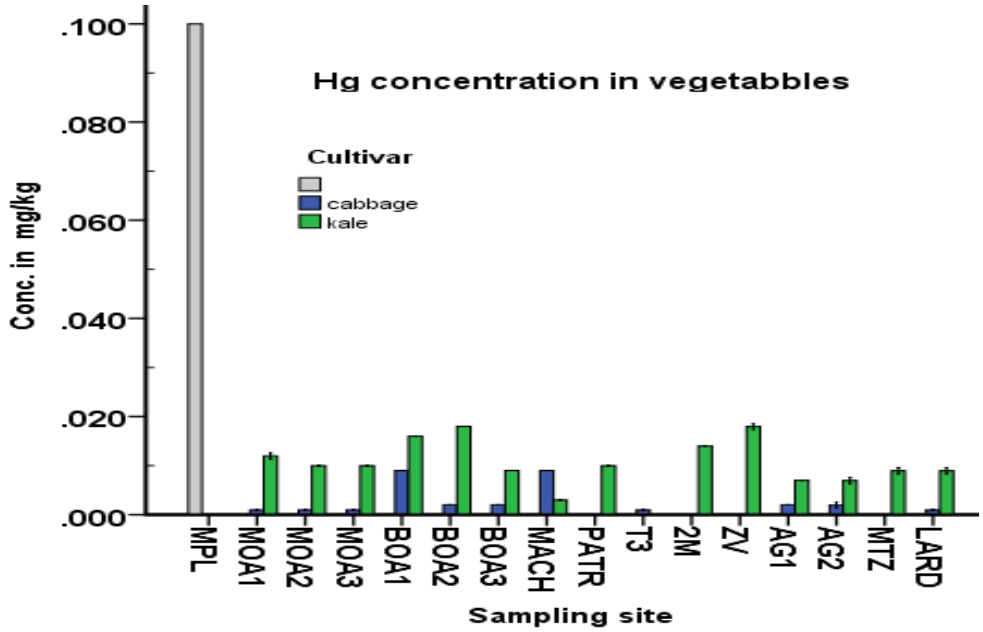

Figure 21: Variation of Hg concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.7. Lead (Pb) concentration in vegetables

The highest Pb measured concentration is 7.3±0.14 mg kg^{-1} in cabbage from T3 and 6.2±0.1 $mgkg^{-1}$ in kale from MACH while the lowest measured concentrations are 0.4± 0.01 mg kg^{-1} in cabbage and 1.4± 0.01 $mgkg^{-1}$ in kale both from MOA1. In the present study, Pb concentration is all higher than the FAO/WHO recommended limit of 0.30 mg kg^{-1} (FAO/WHO, 2011, 2007, 2006)**.** Pb as well as other metals in the present study is highly accumulated by kale (89 %) than cabbage (11 %). Guerra *et al.*(2012) reported a similar finding in their study and they found 0.60 $mgkg^{-1}$ and 1.66 mg kg^{-1} of Pb, respectively, in cabbage and kale. For other studies, in Ghana (Accra), Lente *et al.* (2012) reported Pb concentration that range between 7.61-10.51 mg kg^{-1} (Table 9). However,

lead is one of the metals which are toxic to humans where it targets important body organs such as the bones, brain, blood, kidneys, and thyroid gland (ATSDR, 2007;Baby *et al*., 2010). The variation of Pb concentration in vegetables is shown in Figure 22.

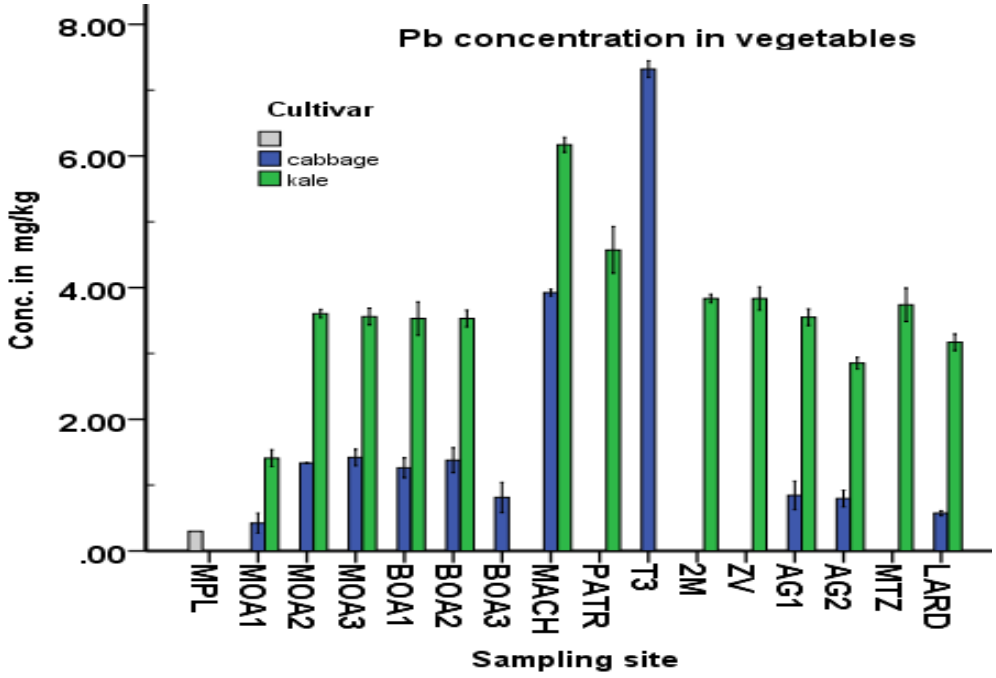

Figure 22: Variation of Pb concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.1.8. Zinc (Zn) concentration in vegetables

The maximum concentration of Zn is 19.7±3.7 mg kg^{-1} in cabbage from T3 and 76.5±2.23 mg kg^{-1} in kale from AG2. The lowest Zn concentration values are recorded as 1.35±0.06 mg kg^{-1} in cabbage from MOA3 and 1.3±0.05mg kg^{-1}in kale from BOA2. However, all vegetable samples are within the safe limits estimated to 100 mg kg^{-1}according to FAO/WHO (2007). In Ghana, Odai *et al.*(2008) reported the mean concentration of HMs in vegetables ranging between 26.77-106.83 mg kg^{-1} (Table 9). Although Zn is an essential element, if taken orally, excess amount can cause system dysfunctions that result in impairment of growth and reproduction (Krishna *et al.*, 2014;

Rahman *et al.*, 2014; Ametepey *et al.*, 2018). The variation of Zn concentration in vegetables is shown in Figure 23.

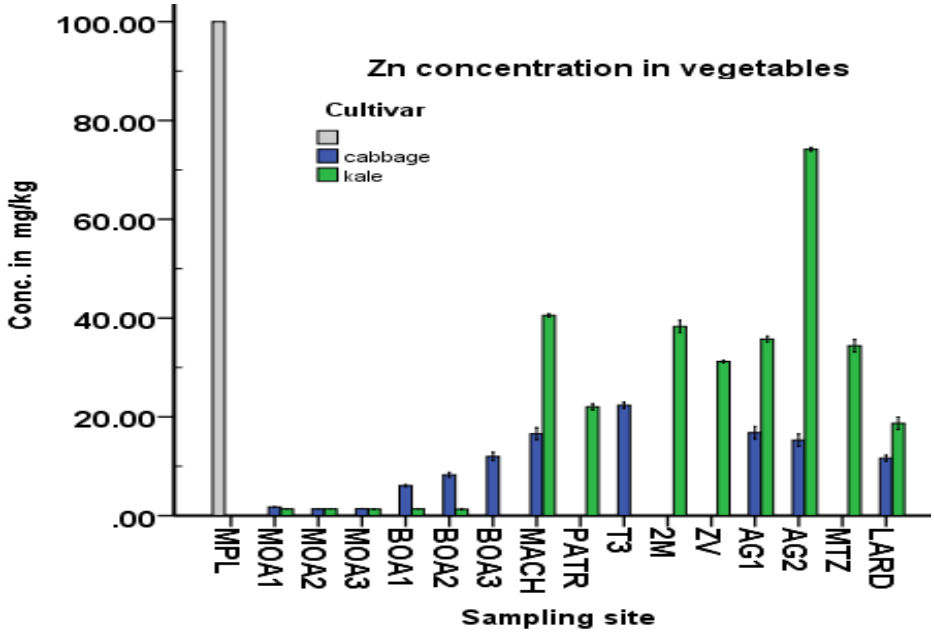

Figure 23: Variation of Zn concentration in vegetables

MPL: Maximum Permissible Limit**, MOA1**: Moamba site one, **MOA2**: Moamba site two, **MOA3**: Moamba site three, **BOA1**: Boane site one, **BOA2**: Boane site two, **BOA2**: Boane site three, **MACH**: Machava, **PATR**: Patrice, **T3**: T-Três, **2M**: Dois M, **ZV**: Zona verde, **AG1**: Angónia site one, **AG2**: Angónia site two, **MTZ**: Moatize and **LARD**: Larde.

5.2. Variation of metal concentration in vegetables versus the anthropogenic sources

In vegetable samples, amongst the metals reported in this study, Al shows the highest concentration in all sampling areas for both cabbage and kale. The exception is in Moamba sampling area where the Al average concentration is relatively lower than other metals. The highest Al average concentration is observed in kale from MTZ (Moatize), followed by kale from Moma (Figure 24). In cabbage, Al highest average concentration is found in MAT (Matola) followed by MTZ. This finding can be ascribed to one of the fact that Al is one of the main constituents of the Earth's crust in rocks commonly ranging from 0.45 to 10% (Kabata-pendias and Pendias, 2001). Specifically in the present study the coal mining in the Zambezi Coal Basin located in the Moatize sub-basin; Tete province with reserves estimated over 2.5 billion of tons can be one of indices for metal contamination (MIREME, 2017). Also the occurrence and extraction of heavy mineral

sands/titanium in the Larde district estimated at grades of 3%, 0.2% and 0.061% respectively for ilmenite, zircon and rutile is an alert of metal contamination (USGS, 2013). However, in Matola sampling sites (a semi-urban area) some farmers are found using river water contaminated by the urban household wastewaters as well as the wastewater discharged from Mozal aluminium smelter (sewage) for watering the crops.

Moreover, some discarded containers of fertilizers and pesticides are witnessed across the field this confirms another anthropogenic source of metal contamination. The highest average concentration of As was encountered in kale from MAT followed by kale of MTZ. In cabbage the highest average concentration of As in MTZ is followed by that from BOA. The highest average concentration of Cd is encountered in kale from MAT followed by kale in MTZ. In cabbage the highest average concentration of Cd is from both MTZ and BOA. For copper the highest average concentration in kale from MTZ followed in kale from MAT. The highest average concentration of Cu in cabbage was found in BOA followed by the one from MTZ. The highest average concentration of Fe is in kale from MTZ and MOMA followed by that from MAT. In cabbage the highest average concentration of Fe was from MAT. For Hg the highest average concentration in kale from BOA while in cabbage it was from MAT.

The highest average Pb concentration in cabbage and kale are both from MAT. For Zn the highest average concentration of Zn in kale from MTZ while in cabbage the highest average concentration in kale from MAT. Generally, the metals are more distributed in MAT and MTZ than in other sampled areas and the decreasing order of sampling areas can be approximately ranked as MAT>MTZ>BOA>MOMA>MOA for cabbages and MTZ>MAT>MOMA>MOA>BOA for kale. However, these soils might pose severe contamination due to the frequent use of municipal contaminated water and wastewater. With regard to agricultural soil, the major inputs of metals are the application of agrochemicals and other soil amendments (Wong *et al.,* 2002). The comparative study of concentration of metals in vegetable samples is presented in Figure 24.

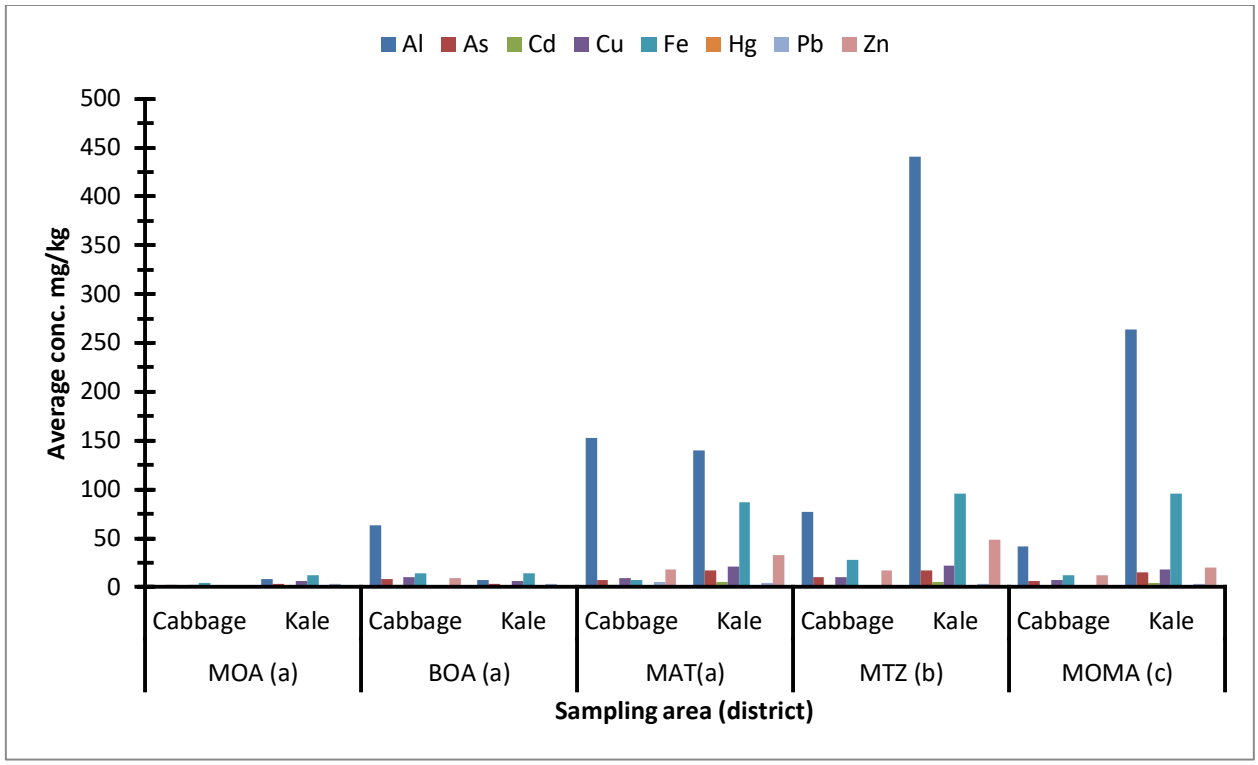

Figure 24: Metal concentration in vegetables/comparison between sampling areas

MOA: Moamba (1, 2&3**): BOA:** Boane (1, 2&3): **MAT:** Matola (MACH, PATR, T3, and 2M&ZV**): MTZ: Moatize** (AG1, AG2&MTZ): **MOMA:** Moma district (LARD)

(a) Maputo **(b)**Tete **(c)**Nampula province

Table 10: Comparison of measured heavy metal levels in cabbage and kale with concentrations $mgkg^{-1}$ from the literatures

Vegetable	Al	As	Cd	Cu	Fe	Hg	Pb	Zn	Sampling site	Reference
Cabbage	61.5	5.6	1.8	20	13.2	0.003	3.6	9.	Maputo, Tete & Nampula, Mozambique	This study
Kale	159	8.8	2.9	25.7	13.5	0.009	4.3	19.2	Maputo, Tete & Nampula, Mozambique	This study
Cabbage	1.8	NA	2.1	0.2	NA	NA	0.4	1.9	Rio de Janeiro city	(Santos *et al.*, 2004)
Kale	8.6	NA	7.0	0.2	NA	NA	0.17	2.6	Rio de Janeiro city	(Santos *et al.*, 2004)
Cabbage	NA	NA	0.04	0.04	3.23		BDL	0.06	Tamale, Ghana	(Ametepey *et al.*, 2018)
Cabbage	NA	5.73	1.56	9.42	490.46	4.23	7.56	23.53	Mojo area ,Ethiopia	(Gebeyehu and Bayissa, 2020)
Cabbage	NA	NA	0.04	NA	NA	NA	0.6	NA	São Paulo State	(Guerra *et al*., 2012)
Kale	NA	NA	0.12	NA	NA	NA	1.16	NA	São Paulo State	(Guerra *et al*., 2012)
Cabbage	NA	NA	0.22	NA	NA	NA	0.31	NA	Adama, Ethiopia	(Benti, 2014)
Cabbage	NA	NA	0.26	1.18	NA	NA	NA	38.1	Eastern Cape	(Bvenura and Afolayan, 2012)
Cabbage	NA	0.013	0.005	NA	NA	0.001	0.055	NA	Xiamen, China	(Chen *et al.*, 2011)
Cabbage	NA	NA	0.68	16.17	NA	NA	7.5	26.77	Kumasi,Ghana	(Odai *et al.*, 2008)

NA = Not Analyzed

5.3. Health risk assessment of metals in vegetables

5.3.1. Estimated daily intake (EDI)

The Table 11 illustrates the EDI values of metals Al, As, Cd, Cu, Fe, Hg, Pb and Zn calculated based on the mean concentration of metals in each vegetable cultivar (Santos *et al.,* 2004; Islam *et al.,* 2018). The computed EDI values were compared with the recommended provisional tolerable daily intake values (PTDI)(FAO/WHO, 2003; FAO/WHO,2005&2006; EFSA, 2011). Thus, the calculated EDIs data of vegetables range between 8.85 x 10^{-7} and 0.660 mg $kg^{-1}day^{-1}$ for both vegetable species *(Brassica oleracea var. capitata) and kale (Brassica oleracea var. acephala*. That is, they are within the safety limits with respect to the established provisional tolerable daily intake (PTDI) values, which re 10, 0.15, 0.07, 35, 56, 0.016, 0.25 and 70 mg $kg^{-1}day^{-1}$respectively, for Al, As, Cd, Cu, Fe, Hg, Pb and Zn, as provided in Table 6(FAO/WHO, 2005; FAO/WHO, 2003). The results reveal that the EDI values of metals in kale cultivar were generally higher than in cabbage cultivar. Generally, the EDI of metals is in a decreasing order as follows: Al >Fe >Zn > Cu >As >Pb>Cd >Hg for cabbage and Al >Fe >Zn >Cu >As >Pb> Cd >Hg for kale, based on the average values. The same order is observed based on the calculated maximum EDI values.

According to Liang *et al.* (2017), EDI depends on the metal concentration, food consumption, and body weight. However, for all metals, the calculated EDI values were within the safe limits of recommended PTDI (Table 11). In general, based on EDI values determined in this study, there would be no health-threatening concern due to the consumption of vegetables of cabbage and kale cultivars from the studied sites. The daily intakes of metals estimated in this study are in agreement with values reported by other researchers for metal-contaminated vegetables like (Santos *et al.,* 2004; Chen *et al.*, 2011; Islam *et al.,* 2018; Gebeyehu and Bayissa, 2020). The dietary exposure approach for HMs via vegetables consumption is a reliable tool for investigating a population's diet in terms of intake levels of nutrients, bioactive compounds and contaminants, providing important information about the potential nutritional deficiencies or exposure to food contaminants (WHO, 1985).

Table 11: Estimated daily intake (EDI, $mgkg^{-1}day^{-1}$) of metals in vegetables

Site	Cultivar	Heavy metal, EDI							
		Al	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**
MOA1	**Cabbage**	0.0020	0.0014	0.0007	0.0010	0.0016	8.85E-07	0.0003	0.0014
	Kale	0.0060	0.0031	0.0014	0.0052	0.0075	9.75E-06	0.0012	0.0011
MOA2	**Cabbage**	0.0005	0.0014	0.0007	0.0004	0.0019	8.85E-07	0.0011	0.0012
	Kale	0.0100	0.0031	0.0014	0.0053	0.0079	7.96E-06	0.0032	0.0012
MO3	**Cabbage**	0.0002	0.0032	0.0014	0.0054	0.0076	8.85E-07	0.0012	0.0012
	Kale	0.005	0.0031	0.0014	0.0052	0.0187	7.96E-06	0.0031	0.0011
BOA1	**Cabbage**	0.059	0.0069	0.0017	0.0088	0.0168	7.96E-06	0.0011	0.0052
	Kale	0.006	0.0030	0.0014	0.0052	0.0187	1.32E-05	0.0031	0.0011
BOA2	**Cabbage**	0.058	0.0071	0.0018	0.0086	0.0137	1.77E-06	0.0012	0.0078
	Kale	0.007	0.0030	0.0014	0.0052	0.0074	1.59E-05	0.0031	0.0011
BOA3	**Cabbage**	0.052	0.0067	0.0017	0.0086	0.0106	1.77E-06	0.0007	0.0108
MACH	**Cabbage**	0.196	0.0063	0.0015	0.0062	0.001	7.96E-06	0.0034	0.0142
	Kale	0.045	0.0125	0.0041	0.0192	>0.0912*	2.65E-06	0.0055	0.0368
PATR	**Kale**	0.045	0.0174	0.0047	0.0212	0.0912	8.85E-06	0.0041	0.0202
T3	**Cabbage**	0.074	0.0068	0.0019	0.0093	0.0124	8.85E-06	0.0064	0.0174
2M	**Kale**	0.203	0.0158	0.0043	0.0184	0.0898	1.23E-05	0.0035	0.0288
ZV	**Kale**	0.199	0.0155	0.0041	0.0165	0.0575	1.50E-05	0.0033	0.0288
AG1	**Cabbage**	0.067	0.0065	0.0017	0.0084	0.0277	1.50E-06	0.0007	0.0143
	Kale	0.238	0.0149	0.0042	0.0187	>0.0912*	6.19E-06	0.0031	0.0309
AG2	**Cabbage**	0.069	0.0063	0.0016	0.0088	0.0251	1.68E-06	0.0006	0.0151
	Kale	0.660	0.0132	0.0040	0.0175	>0.0912*	5.75E-06	0.0025	0.0677
MTZ	**Kale**	0.295	0.016	0.0045	0.0219	>0.0912*	7.08E-06	0.0033	0.0308
LARD	**Cabbage**	0.036	0.0058	0.0014	0.0061	0.011	8.85E-07	0.0005	0.0111
	Kale	0.242	0.0137	0.0039	0.0161	>0.0912*	7.52E-06	0.0029	0.0175
Average EDI ($mg\ kg^{-1}day^{-1}$)	**Cabbage**	0.055	0.0053	0.0014	0.0065	0.0117	3.18E-06	0.0015	0.0090
	Kale	0.150	0.0103	0.0031	0.0135	0.0580	9.23E-06	0.0032	0.0205
(PTDI, $mg\ kg^{-1}day^{-1}$)		**10[a]**	**0.15[b]**	**0.07[b]**	**35[b]**	**56[b]**	**0.016[c]**	**0.25[b]**	**70[b]**

(*) = Minimum quantified EDI (for the concentration above the limit of detection)

PTDI: Provisional tolerable daily intake. **NA** = Not Available (for concentration below the limit of quantification)

[a](FAO/WHO, 2006;EFSA, 2011) [b](FAO/WHO, 2005) [c](FAO/WHO, 2003)

5.3.2. Target hazard quotient (THQ

The THQ values of metals through vegetables consumption are shown in Table 12. However, it is found that all metals showed the THQ <1 values except for As, Cd and Pb (in some samples) whose THQs are >1. The THQ values range between 4.86-58.13 and 1.55-9.49 respectively for As and Cd in both species *Brassica oleracea var. capitata* and kale *Brassica oleracea var. acephala*. On the side of Pb, the higher THQs >1 are found only in some samples namely 1.58, 1.17, 1.85 and 1.01 from MACH (kale), PATR (kale), T3 (cabbage) and 2M (kale) respectively. According to Ametepey *et al.* (2018) the THQ>1 means an unacceptable risk of non-carcinogenic effects on health, whilst the THQ<1 means an acceptable level of risk.

Exposure to more than one contaminant may produce a synergistic effect on the consumer health (Nuapia *et al.*, 2018). The hazard index (HI) values of the studied metals are all>1 and they range between6.55-30.1 in cabbage and 14.2-69.6 in kale which indicate the probability of occurrence of non-carcinogenic adverse effects to the consumers. As and Cd are the major contributors to the HI with proportions ranging between 70.4-85.5 and 12.1-20.4% for As and Cd, respectively. Although the THQ-based risk assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of the risk level due to exposure to pollutants (Guerra *et al.*, 2012). Therefore, such observations indicate that continuous consumption of vegetables from these locations might pose potential non-carcinogenic risk especially in regard of As and Cd.

Compared to other studies done, Gebeyehu and Bayissa (2020) on levels of HMs in vegetables (tomato and cabbage) and associated health risks in Mojo area, Ethiopia, they reported values of THQ which are >1 for As and Hg, i.e.,5.99 and 4.42respectively,in cabbage. In the same report, Gebeyehu and Bayissa (2020) presented the hazard index (HI) values of 8.014, 1.003 and8.014 for As, Pb and Hg respectively as seen, which are >1. Likewise, in their study Islam *et al.*(2018) investigated the potential health risks in vegetables where the calculated THQ values of As are >1 in some vegetable samples 3.689, 1.517, 1.222, 1.011 and 1.250 L. siceraria, S. lycopersicum, C. maxima, D. carota and L. culinaris, respectively.

Table 12: Target hazard quotient (THQ, mg kg^{-1}) and hazard index (HI)of metals in vegetables

Site	Cultivar	Heavy metal, THQ								HI
		Al	As	Cd	Cu	Fe	Hg	Pb	Zn	
MOA1	**Cabbage**	0.0020	4.860	1.55	0.026	0.002	0.008	0.100	0.004	6.55
	Kale	0.0060	10.44	2.93	0.132	0.010	0.097	0.356	0.003	13.9
MOA2	**Cabbage**	0.0005	4.750	1.45	0.011	0.002	0.008	0.336	0.004	6.56
	Kale	0.0109	10.38	2.97	0.134	0.010	0.079	0.925	0.004	14.5
MO3	**Cabbage**	0.0002	10.68	2.97	0.135	1.010	0.008	0.359	0.004	15.1
	Kale	0.005	10.35	2.93	0.131	0.024	0.079	0.900	0.003	14.4
BOA1	**Cabbage**	0.059	23.07	3.55	0.221	0.022	0.079	0.326	0.017	27.3
	Kale	0.006	10.15	2.92	0.131	0.024	0.132	0.892	0.003	14.2
BOA2	**Cabbage**	0.058	25.81	3.66	0.217	0.018	0.017	0.364	0.026	30.1
	Kale	0.007	10.15	2.92	0.130	0.009	0.159	0.890	0.003	14.2
BOA3	**Cabbage**	0.052	22.45	3.52	0.216	0.014	0.017	0.209	0.036	26.5
MACH	**Cabbage**	0.196	21.27	3.13	0.156	0.001	0.079	0.976	0.047	25.8
	Kale	0.045	41.72	8.28	0.480	>0.120*	0.026	1.580	0.122	52.3
PATR	**Kale**	0.045	58.13	9.49	0.530	0.120	0.088	1.170	0.067	69.6
T3	**Cabbage**	0.074	22.90	3.94	0.232	0.016	0.008	1.850	0.058	29.0
2M	**Kale**	0.203	52.79	8.62	0.460	0.118	0.123	1.014	0.096	63.4
ZV	**Kale**	0.199	51.96	8.26	0.413	0.070	0.15	0.956	0.096	62.1
AG1	**Cabbage**	0.067	21.98	3.47	0.210	0.036	0.015	0.220	0.047	26.0
	Kale	0.238	49.84	8.44	0.468	>0.120*	0.061	0.887	0.103	60.1
AG2	**Cabbage**	0.069	21.30	3.38	0.220	0.033	0.016	0.199	0.050	25.2
	Kale	0.660	44.14	8.09	0.439	>0.120*	0.057	0.733	0.225	54.4
MTZ	**Kale**	0.295	53.53	9.17	0.549	>0.120*	0.07	0.953	0.102	64.7
LARD	**Cabbage**	0.036	19.53	2.91	0.153	0.015	0.008	0.144	0.037	22.8
	Kale	0.242	45.77	7.84	0.403	>0.120*	0.075	0.839	0.058	55.3

(*) = Minimum **THQ** (for the concentration above the limit of quantification)

HI = Sum of THQ values (from one kind of foodstuff)

5.3.3. Maximum daily consumption limits (CR_{lim})

The CR_{lim} values of metals in vegetables are determined and presented in Table 13. The CR_{lim} changes proportionally to RfD and body weight but inversely proportional to the metal concentration in foodstuff (U.S.EPA, 2000). The results show that the CR_{lim} values of metal-contaminated cabbage are generally higher than the CR_{lim} values of metal-contaminated kale. This is because the kale cultivar exhibits generally a higher metal uptake capacity compared to the cabbage. The higher average CR_{lim} of the cabbage from MOA3 (38.6 Kg day^{-1}) and of kale from MOA1 (4.15 kg day^{-1}) shows that it would be the most relatively allowed for consumption in the present vegetable diet whereas the cabbage from AG1 (1.04 Kg day^{-1}) and kale from 2M (0.27 Kg day^{-1}) is the least tolerated for consumption.

Based on the average CR_{lim} the approximate decreasing order of allowable vegetable diet is: MOA3 >BOA1 > MOA2 >MOA1 >MACH > LARD >T3>BOA3 >BOA2 >AG2 >AG1 for cabbage and MOA1 >BOA2 >MOA3 >BOA1 >MOA2 >MACH >PATR >AG1 >AG2 >MTZ>LARD > ZV>2M for kale. Generally, it is to be noted that vegetable samples taken from MOA and BOA (Maputo) are more likely to be tolerated for consumption than those from AG, MTZ (Tete) and LARD (Nampula). The 2M place is located around the McMahon brewery, famously known in Maputo. This place receives domestic sewage and water which goes to the river nearby. However, in addition to the use of agrochemicals, farmers around use the sewage released from the brewery for irrigation of vegetables. This may explain why the vegetables sampled in that place show a relatively high concentration and consequently the calculated CR_{lim} classifies it as the least allowable for consumption when compared to other vegetables from other places.

In terms of the individual metals Al, Fe and Zn were the three more tolerated for consumption in the diet while As, Cd and Pb are the least tolerated for consumption in the present vegetable diet. The decreasing order of CR_{lim} due to vegetable consumption can be shown as follows: Al> Fe > Zn > Hg > Cu >Pb>Cd >As and Zn>Al> Fe > Hg > Cu >Pb> Cd >As for cabbage and kale, respectively. Such an order explains the sequence in which the metals are relatively allowed for consumption in the studied vegetables. Therefore, the more is the metal uptake concentration in vegetable; less will be allowed the metal in the vegetable. The CR_{lim} represents the maximum lifetime daily consumption rate (in kilograms of food) that would not be expected to cause adverse

non-carcinogenic health effects (Alipour *et al*., 2014). The maximum allowable consumption rate data are shown in the Table 13.

Table 13: Maximum allowable consumption rate (CR_{lim}, Kg day^{-1}) for studied metals

Site	**Cultivar**	**Heavy metals, CR_{lim}**								**Aver CR_{lim}**
		Al	**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**	
MOA1	**Cabbage**	29.16	1.27E-02	3.97E-02	2.35	24.4	7.00	0.583	12.5	9.52
	Kale	10.21	5.93E-03	2.10E-02	0.46	6.14	0.63	0.173	15.5	4.15
MOA2	**Cabbage**	104.4	1.30E-02	4.26E-02	5.49	24.1	7.00	0.184	14.4	19.5
	Kale	5.64	5.96E-03	2.08E-02	0.46	5.84	0.77	0.066	15.2	3.50
MOA3	**Cabbage**	280	5.80E-03	2.08E-02	0.45	6.07	7.00	0.172	15.4	38.6
	Kale	10.7	5.98E-03	2.10E-02	0.47	2.48	0.77	0.068	15.9	3.81
BOA1	**Cabbage**	1.03	2.68E-03	1.74E-02	0.27	2.77	0.77	0.189	3.55	24.7
	Kale	10.0	6.10E-03	2.12E-02	0.47	2.49	0.46	0.060	16.0	3.69
BOA2	**Cabbage**	1.06	2.60E-03	1.69E-02	0.28	3.40	3.50	0.170	2.37	1.35
	Kale	8.23	6.10E-03	2.12E-02	0.47	6.30	0.38	0.069	16.1	3.95
BOA3	**Cabbage**	1.17	2.76E-03	1.75E-02	0.28	4.39	3.50	0.295	1.71	1.42
MACH	**Cabbage**	0.31	2.91E-03	1.97E-02	0.39	46.6	0.77	0.063	1.30	6.19
	Kale	1.35	1.48E-03	7.47E-03	0.12	<0.51*	2.33	0.039	0.50	0.62
PATR	**Kale**	1.35	1.06E-03	6.53E-03	0.11	0.51	0.70	0.052	0.91	0.45
T3	**Cabbage**	0.83	2.70E-03	1.56E-02	0.26	3.75	7.00	0.033	1.06	1.62
2M	**Kale**	0.30	1.17E-03	7.18E-03	0.13	0.52	0.50	0.061	0.64	0.27
ZV	**Kale**	0.31	1.19E-03	7.49E-03	0.15	0.81	0.41	0.064	0.64	0.30
AG1	**Cabbage**	0.91	2.81E-03	1.78E-02	0.29	1.68	0.41	0.281	1.29	0.61
	Kale	0.25	1.24E-03	7.33E-03	0.13	<0.51*	1.00	0.069	0.60	0.39
AG2	**Cabbage**	0.89	2.90E-03	1.83E-02	0.28	1.85	3.68	0.310	1.23	1.04
	Kale	0.09	1.40E-03	7.65E-03	0.14	<0.51*	1.07	0.084	0.27	0.38
MTZ	**Kale**	0.20	1.15E-03	6.75E-03	0.11	<0.51*	0.87	0.064	0.60	0.36
LARD	**Cabbage**	1.69	3.17E-03	2.20E-02	0.40	3.98	7.00	0.429	1.67	1.90
	Kale	0.25	1.35E-03	7.90E-03	0.15	<0.51*	0.82	0.073	1.06	0.33

(*) = Maximum CR_{lim} (for the concentration above the limit of quantification)

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Consuming the foods contaminated with toxic metals involves different detrimental effects on human health. In reference to the specific objectives set, the concentration of Al, As, Cd, Cu, Fe, Hg, Pb and Zn in fish samples, namely Tilapia (*Oreochromis mossambicus)* and catfish (*Chrysichthys nigrodidatatus)* and vegetables cabbage (*Brassica oleracea var. capitata)* and kale *(Brassica oleracea var. acephala);* was determined. The results of this study reveal the presence of various concentrations of the metals in the fish and vegetables collected in seven agricultural fields across five districts namely Moamba, Boane, Matola (Maputo province), Moma (Nampula province) and Moatize (Tete province).

The metal concentrations in tilapia and catfish, cabbage and kale samples were compared to both National and International Safety Standards. Generally, the concentration of metals As, Cd, Pb and Al in both fish and vegetable samples are outside the safety limits set by various bodies such as ANHMRC, ANZFA, FSAI, EU, and FAO/WHO, which is an indication of possible health risks. On contrary, the concentration of Cu, Fe, Hg and Zn in both fish and vegetable samples generally fall within acceptable limit of safety in reference to the recommended maximum permissible limits of metals.

However, the calculated average concentration (mg kg^{-1}) per district as sampling area reveals the extent at which the existing anthropogenic activity may influence the metal contamination of the place. For instance, the decreasing order of metal concentration in fish samples is deduced as MOMA >MAT >BOA >MOA >MTZ.

The relationship between HMs concentration and anthropogenic sources was investigated. The Moma district with the sampling sites of Maganha, Lalane and Inthaka is more characterized by anthropogenic activities which include the heavy mineral sands extraction combined with the use of agrochemicals around the water bodies hosting the fishes. The soil erosion, percolation and runoff are processes susceptible to spread the metal contamination. The MAT (Matola-Influene basin) is located in a semi-urban area which is relatively more prone to contamination due to the sewage from various household wastewater as well as the industrial wastewaters from Mozal

aluminium smelter. On the other side, MTZ (Tete-Estima) is a place which is located in rural area where polluting anthropogenic activities are relatively low to water bodies since water is mobile.

Thus, for the vegetable samples, the order of decreasing metal concentration (mg kg^{-1}) is as follows: MAT >MTZ >BOA>MOMA >MOA for cabbages and MTZ >MAT >MOMA >MOA>BOA for kale. The use of wastewater released from factories and households to water vegetables in Matola's agricultural fields (MAT) and the extraction of coal in Moatize (MTZ) are among the eventual anthropogenic activities to influence the metal concentration in vegetables from these areas. Moamba (MOA) and Boane (BOA) are located in rural areas with relatively less polluting anthropogenic activities which is why they rank the last. It is particularly noted that kale cultivar seems to have higher metal uptake capacity compared to cabbage cultivar in the same sampling site.

The health risk associated with fishes from local rivers and vegetables locally grown was evaluated. For health risk assessment, the calculated EDI values are all within acceptable limits in regard to the daily dietary allowance recommended by various standard authorities for both fish and vegetable samples. The THQ determined is >1 for both As and Cd in both fish and vegetable samples indicating probable health risks to the consumers. For other metals, the THQs are less than one indicating acceptable limits for human health. The hazard index (HI) is >1 in all fish and vegetable samples, which is an unacceptable threshold for human health and safety. Based on the average maximum allowable fish and vegetable rate (CR_{lim}), the three metals namely As, Cd and Pb are the least allowed for consumption compared to others in both fish and vegetable samples (Hg >Fe >Al >Zn >Pb> Cu >Cd >As; Al> Fe > Zn > Hg > Cu >Pb>Cd >As and Zn>Al> Fe > Hg > Cu >Pb> Cd >As for fish, cabbage and kale, respectively).

In overall, the consumption of the investigated fish and vegetable are relatively unsafe for public health particularly for As, Cd, Pb and Al. Due to the health hazard presented by these metals, the continuous exposure to the fish and vegetable from the studied areas may induce health risks to consumers including for instance neurological problems such as Alzheimer's disease, Parkinson's disease, cancers, skin lesions (arsenicosis), hypertension, cardiovascular disease, diabetes, allergies, weight loss, paralysis, muscular weakness, brain damage, kidney damage and ultimately death.

6.2. Recommendations

- It is suggested that the evaluation of HMs level and associated health risks on the fishes and vegetables even other foodstuffs, daily consumed in the same areas as the present study and others of Mozambique, should be performed continuously and consistently in order to confirm with more findings.

- More chemistry studies are recommended in order to compare the metal uptake capacity between cabbage and kale of the same place, which can serve as important information for vegetable consumers, especially for the areas which have been used in sampling.

- Many metal-related diseases are known to go unnoticed that is why is recommended a public health campaign of awareness about these contaminants (toxic metals) and their health effects especially in areas of high risk.

- There should be a close collaboration between the UEM-Chemistry Department, community health-based institution as well as non-communicable disease department of ministry of health in terms of investigation of metal-contaminated foods and their related diseases.

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APPENDECES

AppendixI: Instrumental conditions for measurement of heavy metals using ICP-OES

Parameter	Conditions
RF Power (W)	1200
Plasma gasflow (/L min^{-1})	10.0
Auxiliary gas flow (L min^{-1})	0.6
Nebulizer gas flow (L min^{-1})	0.7
Spray chamber	Cyclonic
Nebulizer	Cross flow
Wavelength (nm)	189.042 (As), 214.438 (Cd), 213.598 (Cu), 238.204 (Fe), 216.999 (Pb), 202.548 (Zn)
LOQ (mg kg^{-1})	3.4 (As), 15.71 (Cd), 170.82 (Cu), 181.15 (Fe), 18.64 (Pb), 247 (Zn)

For ICP OES determinations, argon 99.996% (Afrox Moçambique LDA, Mozambique) was used for plasma generation, nebulization and auxiliary gas, LOQ = Limit of Quantification (mg kg^{-1}).

Appendix II: Metal concentrations (mg kg^{-1}, dry wt.) in fish muscles of tilapia and catfish

[x]

Site	Species	Al	As	Cd	Cu	Fe	Hg	Pb	Zn
MoKUR[x]	Tilapia	39.5	5.75	1.01	5.40	25.00	0.0325	2.53	31.50
		36.6	5.81	1.11	5.10	26.00	0.0354	2.03	33.00
			5.94					2.20	33.40
			5.81					2.63	34.60
BoMAF[x]	Tilapia	125.5	10.72	2.24	9.84	28.00	0.0336	1.95	43.50
		131.5	11.03	2.02	10.10	29.10	0.0334	1.80	45.00
			9.43						
			9.11						
MaRIV[x]	Tilapia	389.5	7.57	5.64	13.00	34.70	0.0016	6.30	27.90
		348.5	7.03	5.29	12.80	35.40	0.0014	6.60	28.60
TeEST[y]	Tilapia	30.10	4.50	12.12	6.31	22.00	0.0020	3.43	25.30
		31.40	4.27	13.60	6.90	13.56	0.0018	3.90	27.30
			6.92			28.40			34.30
			6.93			20.26			36.30
NaMAG[z]	Tilapia	91.50	6.95	1.21	5.44	40.3	BLQ	1.26	50.00
		96.50	6.64	1.36	5.80	42.96		1.10	54.50
			7.70						
			7.28						
NaLAL[z]	Tilapia	282.70	6.69	6.40	7.40	93.00	BLQ	2.40	41.54
		298.70	7.07	6.62	6.82	91.46		2.73	42.30
			6.06						35.10
									35.70
NaINT[z]	Cat fish	634.51	13.40	1.18	18.70	169.0 0	0.0014	3.70	41.10
		652.70	11.00	1.12	19.15	168.0 0	0.0013	3.60	42.40
			13.62						45.90
									47.50

Maputo province ; [y] Tete province; [z] Nampula province; **BLQ** = Below the limit of quantification

Appendix III: Metals concentration (mg kg^{-1} dry wt.) in vegetable samples

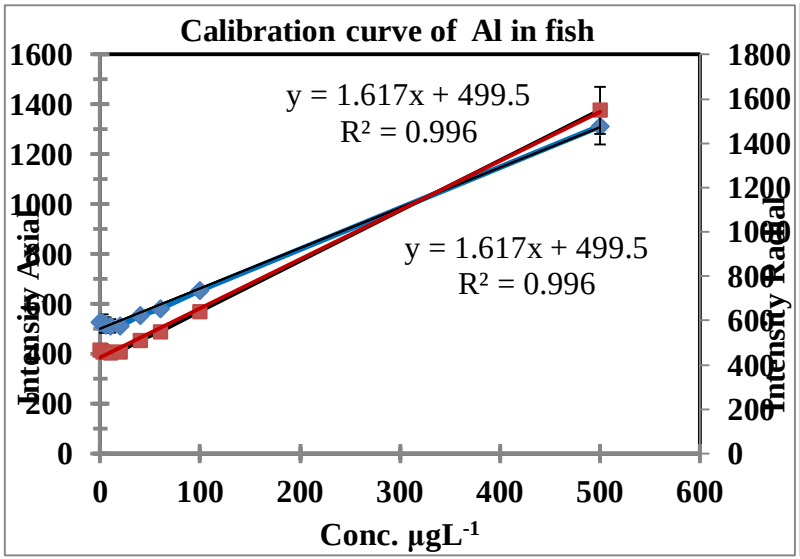
Site	Cultivar	Al		As				Cd				Cu			
MOA1[a]	Cabbage	1.8	1.88	1.66	1.91	1.38		0.93	0.85			1.16	1.5	0.91	
	Kale	6.7	6.8	3.64	3.32	3.89	3.34	1.59	1.71	1.62	1.72	5.64	6.31	5.61	6.37
MOA2[a]	Cabbage	0.66	0.65	1.93	1.28	1.98	1.28	0.77	0.92	0.73	0.88	0.55	0.48		
	Kale	11.8	12	3.7	3.3	3.87	3.21	1.62	1.71	1.61	1.79	5.65	6.35	5.8	6.49
MOA3[a]	Cabbage	0.2	0.3	3.89	3.89	3.29	3.92	1.62	1.76	1.62	1.74	5.84	6.4	5.76	6.4
	Kale	6	7	3.81	3.31	3.65	3.3	1.62	1.72	1.6	1.71	5.64	6.37	5.53	6.29
BOA1[a]	Cabbage	67	66	8.93	5.21	10.27	6.87	2.07	1.97			10.16	9.88		
	Kale	6	6	3.69	3.28	3.58	3.3	1.6	1.71	1.59	1.7	5.55	6.34	5.58	6.31
BOA2[a]	Cabbage	64	65.1	8.63	4.98	11.17	7.5	2.01	2.15			9.76	9.86		
	Kale	8	9	3.64	3.3	3.69	3.15	1.6	1.69	1.6	1.71	5.53	6.26	5.52	6.32
BOA3[a]	Cabbage	60.8	58.1	8.33	4.92	10.37	6.82	2.01	1.98			9.56	9.96		
MACH[a]	Cabbage	222.6	220.6	7.84	4.13	10.24	6.63	1.72	1.83			7.06	7.06		
	Kale	50.1	51	18.84	13.14	21.64		4.63	4.74			21.76	21.66		
PATR[a]	Kale	234.4	234.4	21.53	15.23	24.17	17.87	5.27	5.47			23.96	24.06		
T3 [a]	Cabbage	81.6	80.04	10.14	6.04	9.14	5.74	2.34	2.12			10.56	10.46		
2M [a]	Kale	230	228.6	19.24	13.44	22.44	16.44	4.75	4.99			21.36	20.26		
ZV[a]	Kale	222.6	227.7	19.44	13.24	21.94	15.84	4.63	4.71			17.86	19.46		
AG1[b]	Cabbage	75.5	74.5	7.87	4.14	11.01	6.81	1.97	1.97			8.95	10.05		
	Kale	269.8	268.8	19.27	13.17	20.21	14.91	4.86	4.69			22.05	20.25		
AG2[b]	Cabbage	79.1	77.3	7.67	4.14	10.41	6.69	1.93	1.9			10.25	9.65		
	Kale	721	728	16.17	10.97	18.91	13.81	4.86	4.69			18.85	20.85		
MTZ [c]	Kale	324.8	332	19.87	13.57	22.71	16.41	5.13	5.24			25	24.65		
LARD[c]	Cabbage	41.2	41.6	6.97	3.35	9.61	5.75	1.61	1.59			7.05	6.85		
	Kale	265.6	262.6	18.14	12.34	18.34	13.24	4.43	4.43			18.56	17.86		

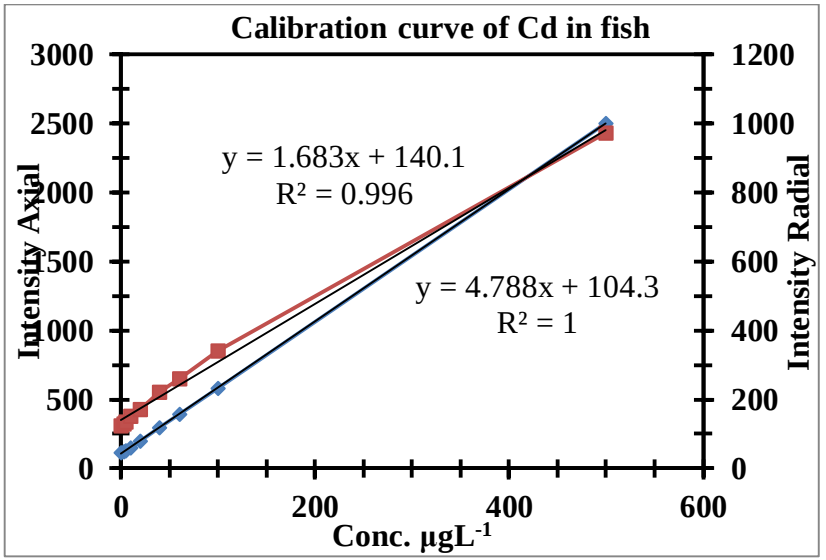
Appendix IV: Metals concentration (mg kg^{-1} dry wt.) measured in vegetable samples

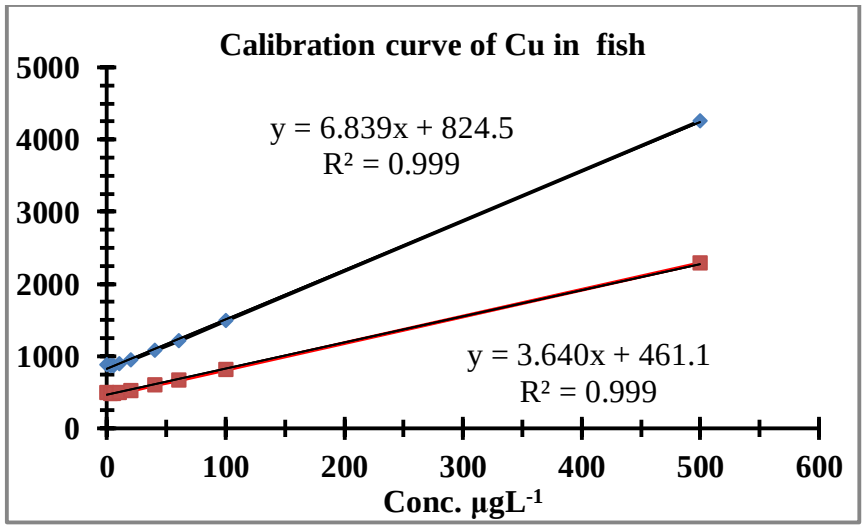
Site	Cultivar	Fe				Hg		Pb		Zn			
MOA1[a]	Cabbage	1.37	1.58	2.22		0.001	0.001	0.43	0.41	1.74	1.86	1.45	1.64
	Kale	7.81	8.13			0.012	0.011	1.42	1.4	1.31	1.38	1.34	1.38
MOA2[a]	Cabbage	2.07	1.99			0.001	0.001	1.34	1.34	1.39	1.55	1.37	1.49
	Kale	7.98	8.79			0.01	0.009	3.6	3.61	1.31	1.39	1.37	1.45
MOA3[a]	Cabbage	8.25	7.9			0.001	0.001	1.43	1.41	1.3	1.42	1.32	1.41
	Kale	19.7	19.7			0.01	0.009	3.57	3.55	1.29	1.36	1.3	1.36
BOA1[a]	Cabbage	19	17.5	16.5		0.009	0.009	1.37	1.22	6.7	5.9	6	5
	Kale	19.6	19.7			0.016	0.015	3.55	3.51	1.28	1.35	1.28	1.36
BOA2[a]	Cabbage	15	13.8			0.002	0.002	1.39	1.49	9.1	8.2	9.6	8.5
	Kale	7.75	7.79			0.018	0.018	3.5	3.54	1.26	1.36	1.26	1.35
BOA3[a]	Cabbage	11.6	10.7			0.002	0.002	0.88	0.79	13.3	12.3	12.1	11.2
MACH[a]	Cabbage	1.2	1.1			0.009	0.009	3.81	3.93	15.7	14.8	17.3	16.5
	Kale	ADL	ADL			0.009	0.009	6.18	6.33	42.9	41.4	41.9	40.5
PATR[a]	Kale	94.5	96.9			0.003	0.003	4.54	4.74	24.4	22	23.7	21.4
T3 [a]	Cabbage	14.1	12			0.01	0.01	7.43	7.22	23.4	22.3	16.8	16.2
2M [a]	Kale	94.1	94.3			0.001	0.001	3.89	4.14	27.2	24.6	40.2	38.4
ZV[a]	Kale	60.3	60.5			0.014	0.014	3.73	3.85	31.1	29.9	31.5	30
AG1[b]	Cabbage	31.3	28.8	27.2		0.017	0.018	0.83	0.92	17.9	15.9	16.7	14.5
	Kale	ADL	ADL	ADL		0.002	0.002	3.64	3.4	35	31	38.1	35.8
AG2[b]	Cabbage	27.4	25.4	27.5	25.4	0.007	0.007	0.79	0.81	19.1	16.8	17.2	15.2
	Kale	ADL	ADL	ADL		0.002	0.002	2.85	2.96	76	74	79.4	76.6
MTZ [c]	Kale	ADL	ADL	ADL		0.009	0.008	3.71	3.85	35	31.6	38.3	34.5
LARD[c]	Cabbage	10.2	13.9	12.8		0.001	0.001	0.57	0.58	15.1	13.4	11.7	10.1
	Kale	ADL	ADL	ADL		0.009	0.008	3.54	3.11	21.7	18.8	20.4	18.3

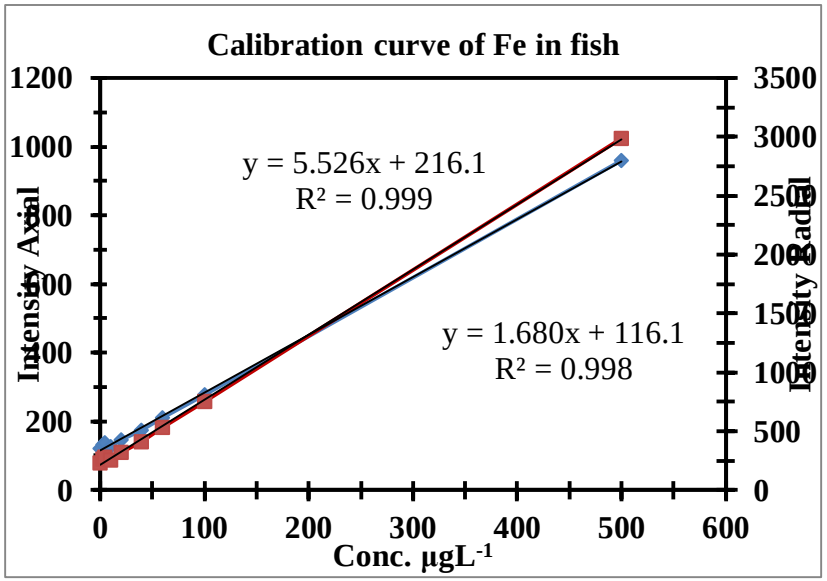
[a] Maputo province [b]Tete province [c]Nampula province ADL= Above Detection Limit

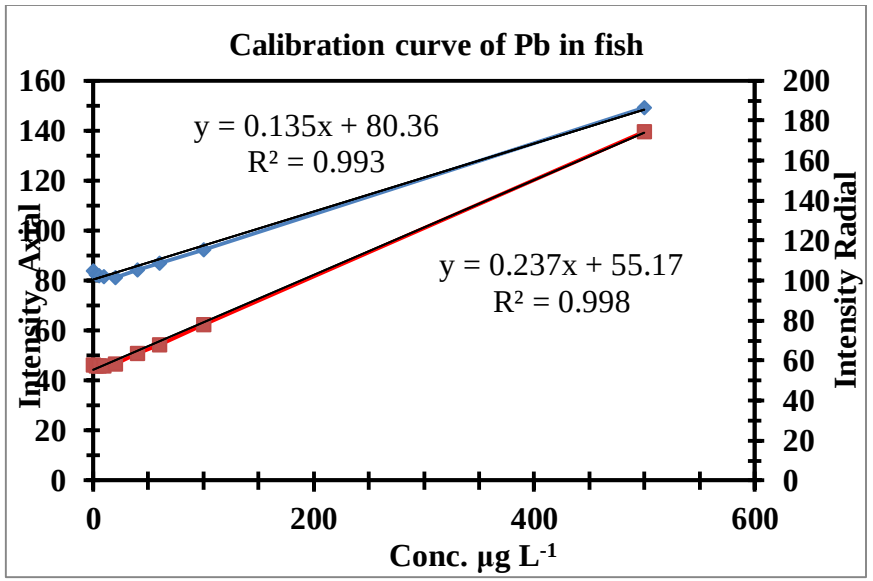
AppendixV: Calibration curves of metals in fish samples

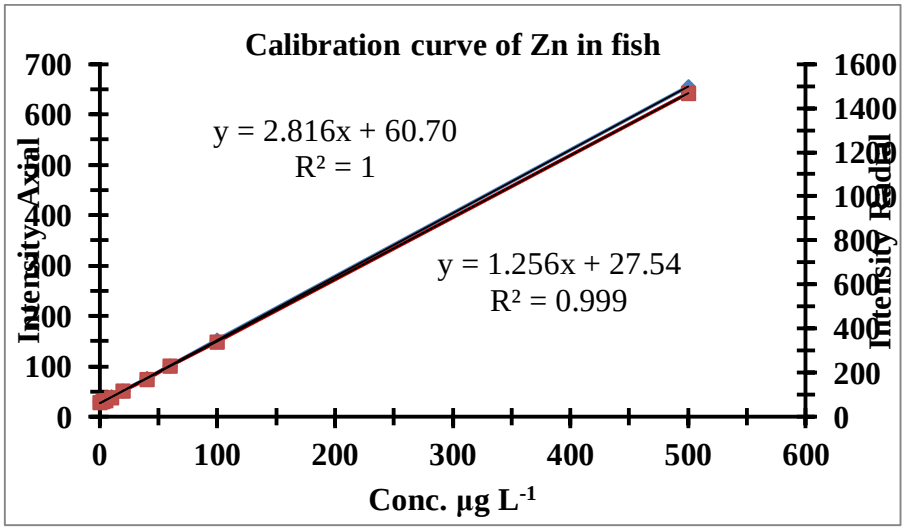




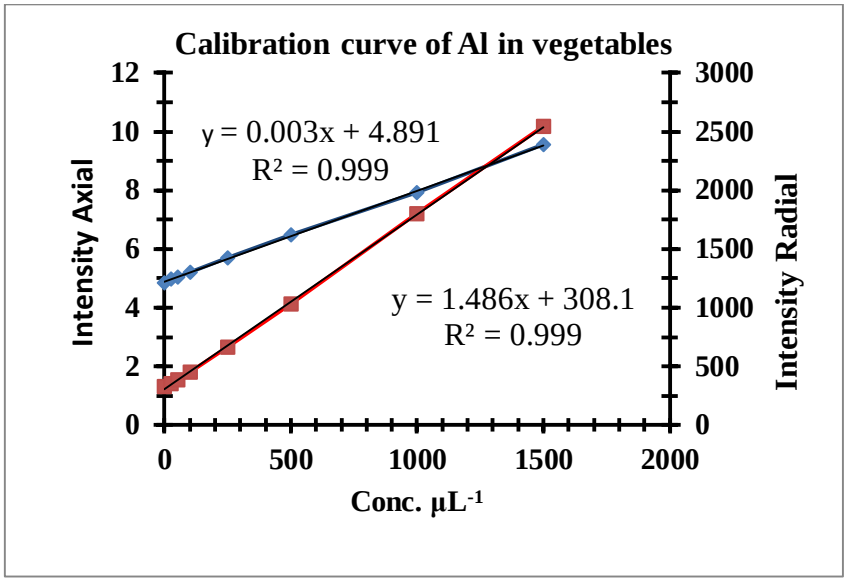


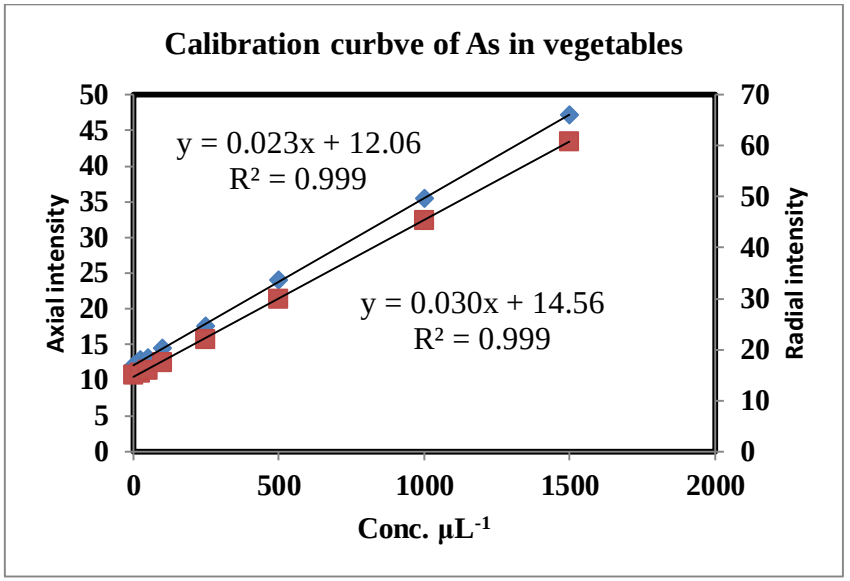

Calibration curve of Fe in fish
y = 5.526x + 216.1
R² = 0.999
y = 1.680x + 116.1
R² = 0.998
Intensity Axial
Intensity Radial
Conc. µgL-1

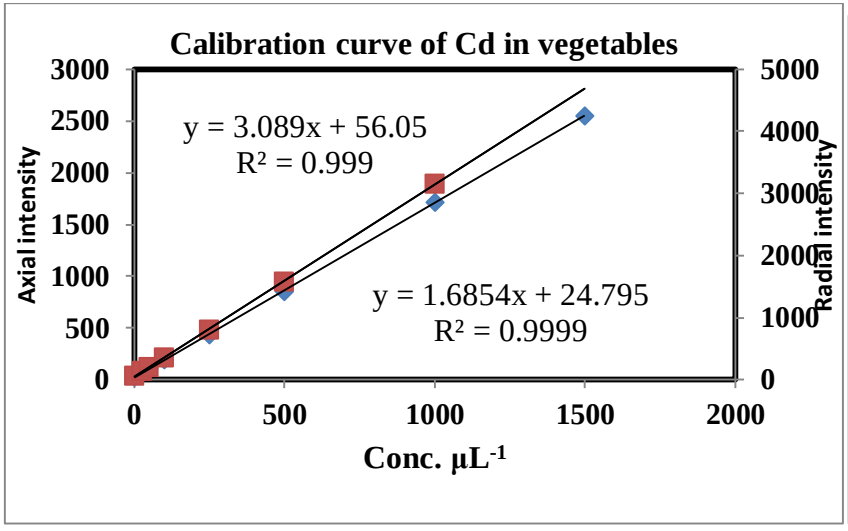

Calibration curve of Pb in fish
y = 0.135x + 80.36
R² = 0.993
y = 0.237x + 55.17
R² = 0.998
Intensity Axial
Intensity Radial
Conc. µg L-1

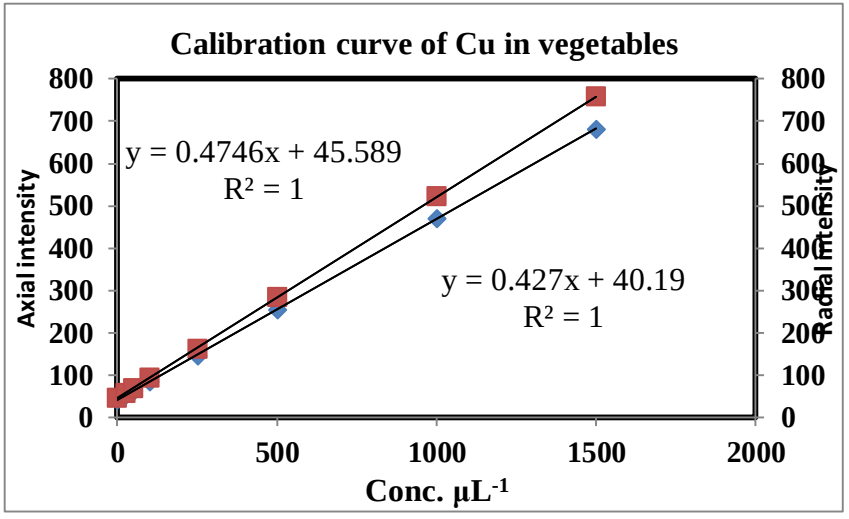

Calibration curve of Zn in fish
y = 2.816x + 60.70
R² = 1
y = 1.256x + 27.54
R² = 0.999
Intensity Axial
Intensity Radial
Conc. µg L-1

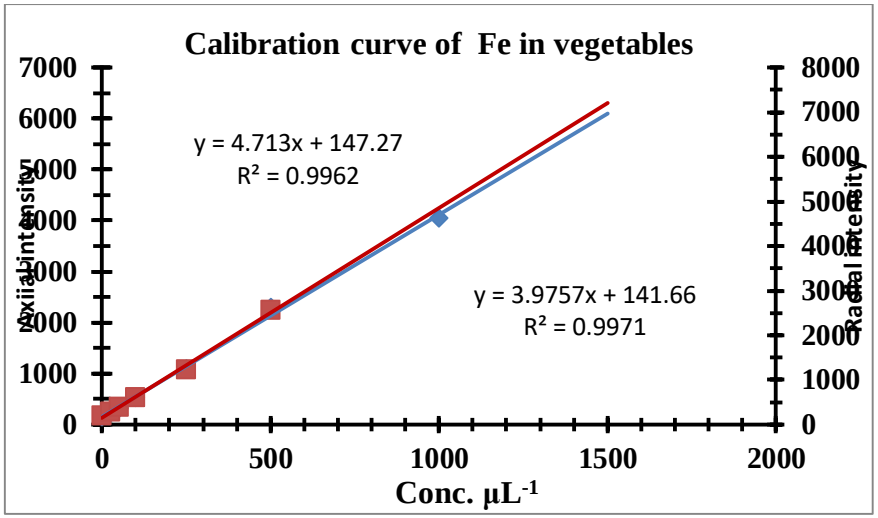
AppendixVI: Calibration curves of metals in vegetable samples

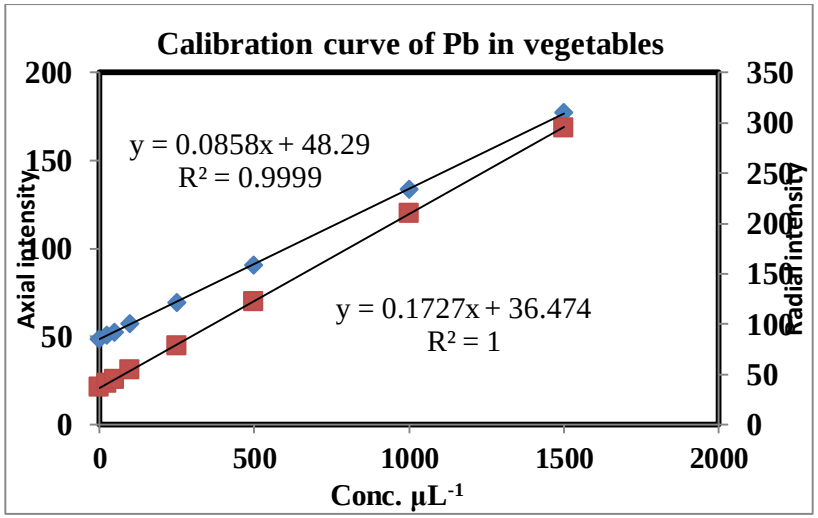


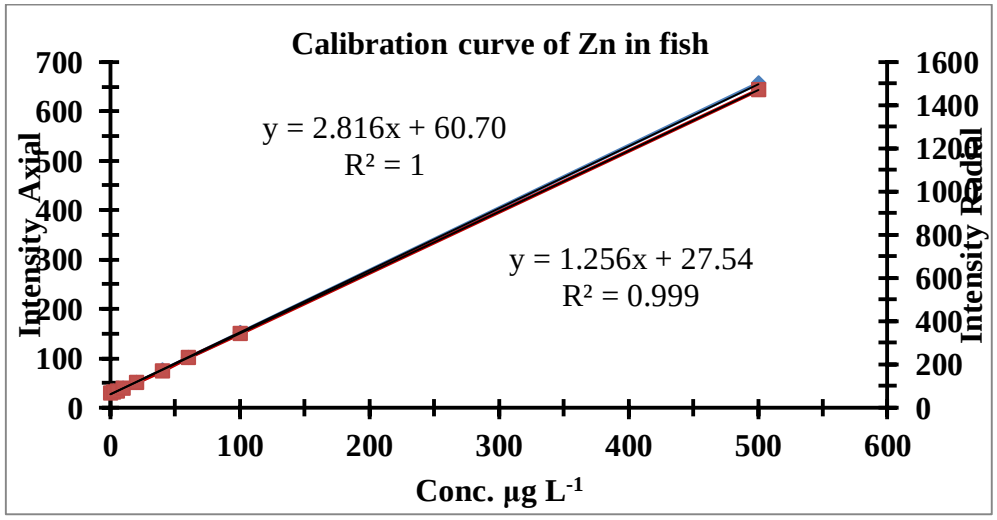







Calibration curve of Fe in vegetables
y = 4.713x + 147.27
R² = 0.9962
y = 3.9757x + 141.66
R² = 0.9971
Axial intensity
Radial intensity
Conc. µL-1
0
1000
2000
3000
4000
5000
6000
7000
8000
500
1500


Calibration curve of Pb in vegetables
y = 0.0858x + 48.29
R² = 0.9999
y = 0.1727x + 36.474
R² = 1
Axial intensity
Radial intensity
Conc. µL-1
0
50
100
150
200
250
300
350
500
1000
1500
2000


Calibration curve of Zn in fish
y = 2.816x + 60.70
R² = 1
y = 1.256x + 27.54
R² = 0.999
Intensity Axial
Intensity Radial
Conc. µg L-1
0
100
200
300
400
500
600
700
800
1000
1200
1400
1600

Appendix VII: Conversion of concentration from mgL^{-1} to $mgkg^{-1}$

$$C\ (mg\ kg^{-1}) = \frac{ICPreading\left(\frac{mg}{L}\right)*FinalVolume(afterdigestion)(L)}{Sampleweight(kg)}$$

For example:

- ICP reading = 0.522 mg L^{-1}
- Final volume (after digestion) = 25 mL
- Sample weight = 0.5 g

Therefore, C (mg kg^{-1}) = $\frac{0.522\frac{mg}{L}*\ 25*10^{-3}L}{0.5*10^{-3}\ (kg)}$ = 26.1 mg kg^{-1}

Note: In case the ICP reading is expressed in μL^{-1} a factor of 10^{-3} will be needed on the numerator.

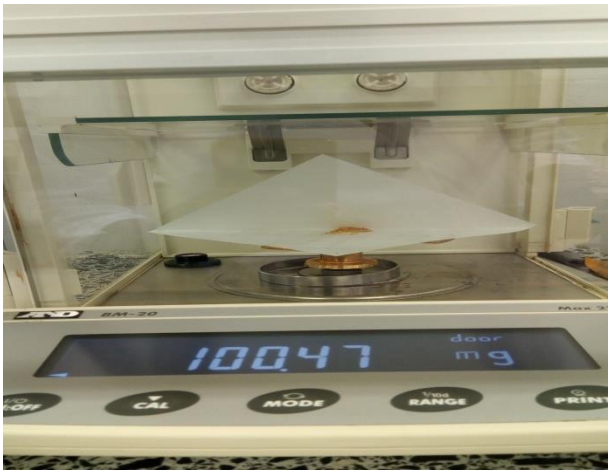
AppendixVIII: Field of sampling and samples processing at chemistry Laboratory-UEM





NIHON FREEZER
Refrigerant
この製品は、オゾン層を破壊する
フロンガスは使用しておりません。
(HFC対応)
CAUTION
取扱説明書はよくお読み下さい。
安全に関する重要な内容を記載しています。
メンテナンス連絡先
日本フリーザー株式会社
JICA
Japan International Cooperation Agency
水かけ
厳禁
-22°




AND
100.47
door
mg
CAL
MODE
RANGE