

PESTICIDE APPLICATION, THEIR RESIDUE LEVELS IN THE ENVIRONMENT, KALES AND TOMATOES IN EWASO NAROK WETLAND, LAIKIPIA COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for the award of any degree in any university or any other institution of higher learning

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DEDICATION

This work is dedicated to my wife Susan, our children Jevins, Layla and Jack for their moral support, patience and inspiration.

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LIST OF ABBREVIATIONS AND ACRONYMS

AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemistry (USA)
APCI	Atmospheric Pressure Chemical Ionization
ARfD	Acute Reference Dose
BDL	Below Detection Limit
CEN	European Commission for Standardization
ChE	Cholinesterase
CNS	Central Nervous System
DCM	Dichloromethane
D-SPE	Dispersive Solid Phase Extraction
EFSA	European Food Safety Authority
ESI	Electrospray Ionization
EU	European Union
FAO	Agricultural Food Organization
GCB	Graphitized Carbon Black
GC-MS	Gas Chromatography- Mass Spectrophotometer
GDP	Gross Domestic Product
HBGV	Human Health-based Guideline Value
ICIPE	International Centre for Insect Pest Ecology
IPM	Integrated Pest Management
LCMS/MS	Liquid Chromatography- Tandem Mass Spectrometer
LOD	Limits of Detection
LWF	Laikipia Wildlife Forum
MDL	Minimum Detection Limit
LEWRUA	Lower Ewaso Ngi'ro Water Resource Users Association
MEMR	Ministry of Environment and Natural Resources
MEWNR	Ministry of Environment, Water and Natural Resources
MRLs	Maximum Residue Limits
PCPB	Pest Control Product Board
PHI	Pre-Harvest Interval.
PSA	Primary Secondary Amine
REI	Re-Entry Interval
SDGs	Sustainable Development Goals
SPSS	Statistical Package for Social Sciences
UNEP	United Nations Environmental Programme
US-EPA	United States Environment Protection Agency
UV	Ultra-Violet
QuEChERS	Quick, Efficient, Cheap, Effective, Reliable and Safe.
QTOF	Quadrupole Time of Flight
QqQ	Triple Quadrupole
WRMA	Water Resource Management Authority
WHO	World Health Organization

ABSTRACT

Pesticide use in agriculture is beneficial in controlling losses from pest and diseases. However, unsafe pesticide use can harm environment and human health. The aim of this study was to assess pesticide use among Ewaso Narok wetland farmers and to determine their residue levels in environment, kales and tomatoes. A total of 86 farmers purposively selected participated in this study. A pre-tested structured questionnaire was administered on the farmers with the help of trained enumerators in which data on pesticide use was collected. For laboratory analysis, a total of 276 samples of soil, water, tomatoes and kales were collected and analyzed during wet/ rainy and dry/ sunny seasons. Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) protocol was followed for sample preparation. Extracts were analyzed using LC/MS/MS and data analyzed using SPSS version 22. The study revealed that farmers use pesticides in violation of safe recommendation; 99% of farmers used unsafe storage facilities, 60.5% did not use appropriate personal protective equipment (PPE), 38.4% mixed pesticides during application and 100% use unsafe disposal methods for the empty pesticide containers. Majority (97%) of the farmers had no training on pesticide use. On laboratory analysis, the results revealed the presence of residual organophosphorus, carbamates, pyrethroids, triazoles, morpholine, phenylamine, carbazate, anilinopyrimidine and beta-methoxyacrylate in soil, water, kales and tomatoes. Residues namely aldicarb, triadimefon, azoxystrobin, chlorpyrifos, buprofezin, cyproconazole I and II, fenpropathrin, paclobutrazole, pyrimethanil, metalaxyl, diazinon, bifentazate, azinphos methyl and spiroxamine were detected and quantified. The levels of triadimefon (132-150) $\mu\text{g/L}$, cyproconazole I(59-66.50) $\mu\text{g/L}$ and II(71-81) $\mu\text{g/L}$, fenpropathrin (27-30) $\mu\text{g/L}$, bifentazate (16-18) $\mu\text{g/L}$, pyrimethanil (62-89) $\mu\text{g/L}$, and spiroxamine (55-150) $\mu\text{g/L}$ in kales and cyproconazole I (54-82) $\mu\text{g/L}$ and II (80-92) $\mu\text{g/L}$, fenpropathrin (16-19) $\mu\text{g/L}$ and spiroxamine (51-105) $\mu\text{g/L}$ in tomatoes were above the European Food Safety authority Limits (EFSA-MRLs) which ranged between 10-100 $\mu\text{g/L}$. All residue levels in tomatoes and kales were within the Maximum Residue Limits as set by the World Health Organization and Agricultural Food Organization. Pesticide residues levels in soil ranged between 2.17-1000.21 $\mu\text{g/L}$ which was significantly higher than the levels in kales and tomatoes. In water, six pesticide residues were detected and quantified in the range of 0.09-5.85 $\mu\text{g/L}$ which were above the European Union-Maximum Contamination Limits (EU-MCL) for the drinking water of 0.10 $\mu\text{g/L}$ for individual residues and 0.50 $\mu\text{g/L}$ for the sum concentrations of all the residues in water. Farmers need training to comply with the safety requirements which include but not limited to the use of appropriate and complete PPEs, reading of pesticide labels before use, adhere to safety intervals (re-entry and pre-harvest intervals), safe storage and disposal of empty pesticide containers. Farmers need to embrace Good Agricultural Practice and Integrated Pests Management to reduce overreliance on synthetic pesticide use. Further study on pesticides residues levels in cabbages, french beans, chilies and potatoes should be done. The findings of this study can be used by policy makers (Government, Non-Governmental Organizations), researchers/ scholars and farmers in making informed decisions on safe pesticide use that ensures safety of environment and human health.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Horticultural farming is an agricultural sub-sector that is experiencing the fastest growth in many global economies (Karungi *et al.*, 2011). In Kenya, horticultural farming is the second largest foreign earner, employing over 500,000 people directly and over 2 million people indirectly in the year 2015 (Tsimbiri *et al.*, 2015). Some of the horticultural crops considered to be of high cost value are tomatoes, flowers, amaranths, French beans, green beans, cowpeas, onion, pepper among others (Tsimbiri *et al.*, 2015). The existing favorable prices and available market both local and international for the fruits and vegetables further encourages the farmers to venture into fruit and vegetable farming. Traditionally undisturbed riparian wetlands like Ewaso Narok in Laikipia County, Kenya are quickly being turned into production areas for horticultural fresh produce (Kamiri *et al.*, 2014).

Horticultural crops are vulnerable to pests and disease attacks making them highly dependent on pesticide usage. This ensures reduced losses, good quality harvest and better returns on investment (Damalas and Eleftherohorinos, 2011). However, pesticides are distributed from target to untargeted areas following various routes such as chemical drift, vaporization, leaching through the soil, accidental spillage and surface runoffs (Otieno *et al.*, 2010). The distribution depends on the prevailing environmental conditions, physical and chemical properties of the individual pesticide (Gavrilescu, 2005). As a result, the beneficial and non-target organisms such as insects, wildlife, aquatic organisms, birds and

human may get exposed to the pesticides (Chindah *et al.*, 2004; Gunasekara *et al.*, 2007; Macharia *et al.*, 2009; Fairchild, 2011).

The bioaccumulation of pesticide residues in larger mammals including man has been linked to increased health related complications such as high cancer, endocrine disruption, neurological cases and sometimes extinction of important organisms from an ecosystem (Fenik *et al.*, 2011; Fennessy and Craft, 2011). Environmental persistence of pesticides is dependent on their chemical and physical properties such as partition coefficient, deposition and degradation rates in addition to environment and climate conditions (Olaniran and Igbinsosa, 2015). Furthermore, some of these residues formed after the degradation process may be more harmful than the parent compound (Muneer and Boxall, 2008). An intact ecosystem provides very important services that ensure human survival. However, the loss of biodiversity and the destruction of natural ecosystems present a lot of perilous consequences threatening the existence of the local communities (Isenring, 2010). Some of the pesticides have been linked to chronic neurotoxicity, mutagenicity, endocrine disruption, ecotoxicity and cancer for instance, chlorpyrifos (organophosphate) has been reported to cause neurological, attention deficit hyperactivity and fetus development disorders, carbofuran (carbamate) is linked to reproductive abnormalities, while carbaryl causes nausea, vomiting, blurred vision, coma and death (Chowdhury *et al.*, 2012). Azinphos methyl and chlorpyrifos exposure was linked to the decrease of earthworms in South African orchards and predatory birds poisoning in USA, UK and Canada (Quinn *et al.*, 2011).

1.2 Problem statement

Ewaso Narok wetland is found in semi-arid parts of Laikipia County, Kenya, making it an important water source for agriculture, domestic animals and wildlife. The area is characterized by bushy grassland receiving low rainfall of less than 750mm annually. The wetland is known for its rich biodiversity ranging from over 170 birds (both resident and migratory), to over 100 plant species (Thenya, 2001; Amler, 2014). It also provides both natural and socio-economic benefits such as availability of papyrus plant known by locals as *Marura* used for building. The dominant land use in the 1970s and early 1980s was mainly large scale ranching and nomadic pastoralism with no farming in the wetland. This trend has since been transformed into high density small scale farming in the wetland. Human settlements along the riverine and wetland areas due to availability of fertile land and water for cultivation has been increasing since 1990s.

Horticultural farming is a fast growing agricultural venture in Ewaso Narok wetland. Many small scale farmers are drawn to horticultural farming due to available market, short harvesting period and high returns on investment. However, horticultural farming is pesticide dependent venture, thus farmers rely on the use of pesticide on horticultural production to control pests and diseases. In the absence of alternative pest control programs such as Integrated Pest Management (IPM), the use of pesticides on vegetables in the wetland has become an obligatory practice among farmers. Tomato and kale are the most grown vegetables in Ewaso Narok wetland and consumed by almost every household. However, majority of local consumers have inadequate abilities to determine the presence of residual pesticides in tomato and kales (Inonda *et al.*, 2015). Furthermore, Kenya have

not established pesticides standards meant to ensure food safety for the local consumers (PCPB, 2012). The situation is made worse if the farmer has little knowledge on good pesticide use and possible consequences of improper pesticide application. Pesticides are toxic substances by nature designed to kill pests and to control crop diseases (El-Kheir, 2004). Various human health problems linked to pesticide exposure include breakdown of the central nervous system, respiratory system, eye and skin irritation, endocrine disruption, interference of fetus development in pregnant women while some pesticides are classified as carcinogens (Oyugi, 2012; Inonda *et al.*, 2015). In addition, the disappearance of important ecological species and the emergence of new crop pests and diseases has equally been associated to pesticide use especially where good agricultural practice (GAP) is not embraced (Mironga, 2005; Karingu and Karanja, 2013).

Absence of local regulatory measures on Maximum Residue limits (MRLs) in vegetables and weak enforcement of adopted international residue levels in Ewaso Narok wetland initiated the interest to carry out this study. Kales and tomatoes grown in the wetland are an important component of human nutrition due to their rich nutrients but are highly prone to pest and diseases attack making their production entirely pesticide dependent. Ewaso Narok River that traverse the wetland is a major water source for both human, domestic animals and wildlife. Pesticide use without following safety rules is likely to contribute to water contamination. The water resource in the area is under Water Resource Management Authority (WRMA) (KENWEB, 2013). The area around Ewaso Narok wetland is categorized as water scarce by WRMA due to several threats including illegal over abstractions, over irrigation, poor irrigation methods and Water pollution resulting from

poor use of agrichemicals, direct discharge of farm effluents into the river, mixing pesticides and spraying of cattle near the river (Mwita *et al.*, 2012).

High pesticide residue levels in the soil may also reduce agricultural production. This is because soil contain important microorganisms which assist in important processes such as nitrogen fixation. When these soil microorganisms are killed by pesticides, then these important processes are halted compromising soil fertility. Failure by farmers to follow pesticide safety precautions and lack of awareness of the pesticide effects on human health and environment raises concerns on human health and environment safety.

Application of pesticide on crops without considering the right dosages is likely to increase phytotoxicity on the vegetables, while some pests may develop resistance against the applied pesticide (s). Some pesticides also binds strongly to the soil and are easily taken up by plants further leading to increased phytotoxicity in vegetable. Empty pesticide containers thrown in the fields may collect water during rainy season posing serious dangers to important insects such as pollinators, resulting into the decline in agricultural productivity. This trend needs to be monitored to ensure safety of both vegetables and environment since the impact of the potential destruction of Ewaso Narok wetland due to pesticide use will be difficult to reverse in future. The inadequate research information on pesticides use, safety and residue levels in vegetables and environment in Ewaso Narok wetland presents a research gap. The purpose of this study therefore was to assess pesticide application and residues levels in soil, water, tomatoes and kales in Ewaso Narok wetland during wet/ rainy and dry/ sunny seasons (Appendix 6).

1.3 Hypotheses

- i. Ewaso Narok wetland vegetable farmers follows unsafe pesticide use from storage, mixing, spraying, use of personal protective clothing and equipment (PPEs) to disposal of empty pesticide containers.
- ii. Pesticide residue levels in soil, water, tomatoes and kales in Ewaso Narok wetland during wet and dry seasons are not within the recommended WHO/FAO and EFSA (EU) maximum residues limits (MRLs).

1.4 Objectives

1.4.1 General objective

To assess pesticide use and residue levels of the selected commonly used pesticides in soil, water, tomatoes and kales in Ewaso Narok wetland, Laikipia County, Kenya.

1.4.2 Specific objectives

- i. To evaluate farmers' knowledge on safe pesticide use in Ewaso Narok wetland in Laikipia County.
- ii. To determine residues levels of some of the selected commonly used pesticides in soils, water, tomatoes and kales in Ewaso Narok wetland in Laikipia County during wet and dry seasons.

1.5 Justification and significance

Ewaso Narok wetland is known for its rich biodiversity ranging from over 170 different birds and 100 plants species (Inonda *et al.*, 2015). Its location in semi-arid part of Laikipia

County makes it an important water source in the region. However, in the past few decades especially from early 1990s, there has been a steady increase of agricultural activities within the wetland (Mwita *et al.*, 2012). Currently, the wetland is a major source of horticultural vegetables namely tomatoes, kales, french beans, chilies, peas, cabbages among others. Though pesticide use is beneficial to farmers leading to reduced losses, they are poisonous in nature and their effect on ecological system and human health are well documented and cannot be ignored.

Kenya is a signatory to a number of environmental conventions and treaties such as Stockholm conventions and the Strategic Approach to International Chemical Management (SAICM) that regulates pesticide use and levels on agricultural produce (Weinberg, 2008). However, lack of implementation programs of these conventions and treaties has led to pesticide use with little or no regard to both human and environment safety. Pesticides application on crops above the recommended limits can lead to high residues levels in food and environment resulting into decline of important environment species and human health complications (Oyugi, 2012). Important soil microorganisms, worms, insects, pollinators, birds, and aquatic life has continue to disappear or die due to pesticide poisoning (Mulati *et al.*, 2018). The effect of the high levels of pesticide residues on the rich biodiversity of over 170 birds species (resident and migrants), over 100 plant species and wild animals earlier documented to be part of Ewaso Narok wetland have remained largely unconfirmed and currently, their numbers cannot be guaranteed (Republic of Kenya, 2015). Tomatoes and kales are an important recipe in every household nutrition, high residues levels in tomatoes and kales raises a major human health concern. Failure by farmers to adhere to

standard safety procedures, poor enforcement of legislations related to pesticide use, illiteracy, poor handling, storage, and disposal of wastes, inadequate protective clothing, and lack of awareness on pesticide use are likely to complicate efforts toward environmental conservation, ensuring food security and having a healthy population.

Over 90% of horticultural produce in Kenya are exported to European market while the use of pesticides are controlled by United States Environmental Protection Agency (USEPA) (Inonda *et al.*, 2015). All agricultural produce for export must have their pesticide residue levels complying with World Health Organization (WHO), Agricultural Food Organization (FAO) and European Food Safety Authority (EFSA) standards (Chowdhury *et al.*, 2012; EFSA, 2014). MRLs are usually set as low as reasonable achievable (ALARA principle) and any food produce with residue levels within the set MRL is assumed to be safe for consumption. MRL can also be used as a tool to determine if pesticides had been applied on crops following Good Agricultural Practice (GAP). Local consumers in Kenya get their foods directly from the farms or local markets. They have no capacity to determine the levels of pesticide residues in the food.

Farmers in Ewaso Narok wetland have no alternative pest control mechanism, thus synthetic pesticides are widely used to control pests and diseases. Failure to comply with Good Agricultural practices put local consumers at risk of consuming vegetables with high levels of pesticide residues. In addition, environment safety is not guaranteed. The area is located in arid and semi-arid part of Laikipia County and is categorized as water scarce by WRMA making the wetland a major water source in the region. Water pollution within the

wetland means that anybody that depend on the river water for domestic use is not guaranteed of water safety including the communities at the downstream. This study assessed pesticide use and residues levels in soil, water tomatoes and kales and the data obtained will help policy makers (government and NGOs), researcher/ scholars and farmers to make evidenced-based policies and decisions on safety of pesticide use that lead to sustained food security but at the same time ensures safe environment and improved human health.

1.6 Scope and limitations of the study

This study was conducted in Ewaso Narok wetland in Kenya during wet/rainy and dry/sunny season). Assessment of farmers' pesticide use was conducted through a structured questionnaire in which the farmers' responses were limited to their correctness, genuinity and accuracy. Fifteen (15) pesticide residues; triadimefon, pyrimethanil, metalaxyl, chlorpyrifos, azinphos methyl, azoxystrobin, spiromaxime, paclobutrazole, aldicarb, buprofezin, bifenazate, cyproconazole I and II, diazinon and fenpropathrin were determined in soil, water, tomatoes and kales. The relationship between the duration and time of pesticide application was investigated. Vegetables varieties, maturity/age were not considered in this study. Pesticide residue levels in other horticultural produce grown in Ewaso Narok wetland such as french beans, cabbages, potatoes, chilies were not done.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wetlands

Wetland is defined in article 1.1 under the Ramsar text of convention as a natural or temporary swampy, fen and turf land area characterized by stagnant or flowing water salty or fresh with a depth not exceeding six meters at low tide. Riparian, coastline areas near wetlands, islands or marine water bodies deeper than six meters at low tide are listed in article 2.1 of the text of convention as wetlands (Ramsar Convention Secretariat, 2006).

Globally, most wetlands are faced with threats attributed to human activities amongst them agricultural intensification characterized by the use of agrichemicals. Wetlands have become important zones for agricultural food production due to availability of nutrients and water for irrigation. However, pests and diseases attack on crops contribute significantly to food losses, hence, use of pesticides on crops has become very essential to the attainment of food security (Kanda *et al.*, 2012). The loss or degradation of most wetland biodiversity has been linked to high levels of pesticides in wetlands (Hardstone and Scott, 2010; Isenring, 2010). In Kenya 2 to 3% of the total surface area is under wetlands (Amler *et al.*, 2015). These wetlands provides a range of benefits which include ecological (habitat to diverse flora and fauna), socio-cultural (religious and cultural sites) and economical (agriculture, eco-tourism benefits (USEPA, 2006). Similarly, wetlands helps in the maintenance of biodiversity through water storage, carbon and nutrients, mitigation of floods, protection of coastal areas from storms, water quality improvement, recharging groundwater aquifers, serving as pollutants sinks, and erosion control (Mironga,

2005; Ramsar Convention Secretariat, 2006). Wetlands are therefore considered to be important conservation and restoration targets.

2.1.1 Ewaso Narok wetland

Ewaso Narok wetland is an important vegetable production area in Kenya where tomatoes, peas, french beans, chilies, kales, spinach and onions are produced for both local markets and export (Thenya *et al.*, 2011; Mwita, 2013). The wetland is fed by seasonal Muruku stream and the Pesi River from the southern side, Kandutura and the seasonal Aiyam Rivers. The wetland stretches for 17km up to the Veterinary Outspan at Ol Maisor (KENWEB, 2013). It is 29.28 Km² in area and it's located in the semi-arid part of Laikipia County making it a major source of fresh water for domestic and wildlife use. In addition, the wetland act as a natural habitat to diverse flora and fauna including over 170 birds and 100 plants species (Thenya 2001). Despite the widespread horticultural farming in Ewaso Narok Wetland, there is inadequate published research that has been conducted to assess pesticides use and their residue levels in environment and food produce. This information is important in assessing both the ecological safety of the wetland in carrying out its functions and evaluating the level of food safety within the wetland.

Horticultural farming is a pesticide dependent thus various types of pesticides ranging from organophosphorus, carbamates, pyrethroids, triazole, are used within the Ewaso Narok wetland. These classes of pesticides are considered to exhibit low environmental persistence compared to organochlorines and high efficacy making them to be widely used for crop protection, food preservation, soil treatment, and disease control (Oyugi, 2012;

Chebai, 2014). However, the toxic effect of these classes of pesticides to beneficial insects such as invertebrates and worker bees are well documented, making them environmental contaminants (Johnson *et al.*, 2010; Fenik *et al.*, 2011).

Ewaso Narok wetland conservation is faced with numerous challenges emanating from the increasing demand for horticultural food produce from the ever growing population. As demand for food and human livelihood increases, more people continue to move into the wetland riparian areas for horticultural farming (Kamiri *et al.*, 2014). The lower Ewaso Narok River flows from the bridge along Rumuruti-Nanyuki road for less than 1km before entering Ewaso Narok wetland. The total length of Ewaso Narok Main River is 95km having two gauging stations at Rumuruti-Nanyuki Bridge (5AC15) and 5AC8 near Loisaba ranch. The water resource in the area is under Water Resource Management Authority (WRMA) while its use is controlled by the Lower Ewaso Ngi'ro Water Resource Users Association (LENWRUA) (KENWEB, 2013). The area is categorized as water scarce by WRMA due to several threats including illegal over abstractions, over irrigation, poor irrigation methods and Water pollution resulting from poor use of agrichemicals, direct discharge of farm effluents into the river, mixing pesticides and spraying of cattle near the river (Mwita *et al.*, 2012).

Poor administration of land and agricultural activities resulting from absent land owners especially national government and lack of agriculture extension support are key areas of concern. The lands are allocated each season to small scale farmers by the local administrators based on the farmers' capability and interest in farming. This has made it

nearly impossible to define the size and the number of farmers within the wetland at any given farming period. As a result, the intense human activities within the wetland have led to the wetland losing most of its vital functions occasioned by the disappearance of some of its important ecological biodiversity (Thenya *et al.*, 2011). The widespread use of pesticides to promote horticultural production within the wetland is likely to lead to unreversed destruction on the biodiversity such as decline in the number of insects and birds thus destabilising the food chain within the ecosystem (Macharia *et al.*, 2009). There is need to build an evidence based conservation ethic around Ewaso Narok Wetland for users by educating them to adopt sustainable utilization of the wetland resources by embracing Good Agricultural Practices (GAP) and Integrated Pest Management (IPM).

2.2 Pesticides

Pesticides are defined as any toxic and poisonous substance or a mixture of substances, chemical or biological in nature that is used to repel, control or destroy any pest such as rodents, insects, fungi and plant weeds that are harmful to health and environment (Zacharia, 2007; Fenik *et al.*, 2011; Noorizadeh and Farmany, 2014). Pesticides can be classified in a number of ways namely; according to their target organisms (insecticides, herbicides, fungicides, acaricides, rodenticides) (Lesueur *et al.*, 2007), and chemical classification (organochlorines, organophosphates, carbamates, pyrethroids, triazole) (Macharia *et al.*, 2009). World Health Organization (WHO) further classifies pesticides according to their hazards such as extremely hazardous (class Ia), highly hazardous (class Ib), moderately hazardous (class II), slightly hazardous (class III), and unlikely to present a acute hazard (class IV) (Dilshad and Imran, 2011).

2.2.1 Organophosphorus pesticides

Organophosphorus pesticides are of thirteen (13) different types containing phosphate group which constitute their basic structure as shown by the Schrader's formula in figure 2.1 (Eleršek and Filipi, 2006).

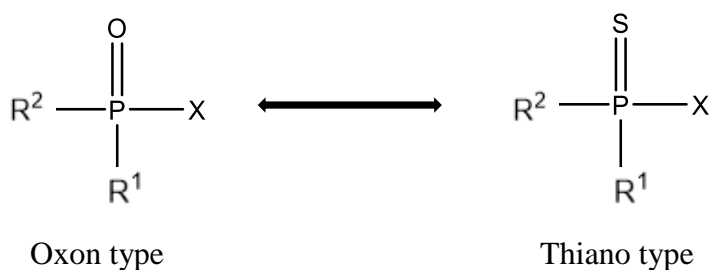


Figure 2.1: General structures of oxon and thiono types of organophosphorus

Where R^1 and R^2 are methoxy and ethoxy groups, X is the leaving group after hydrolysis or reaction with Cholinesterase (ChE). Two categories of organophosphorus exist based on their structural composition namely; Oxon type ($\text{P}=\text{O}$) and thiono type ($\text{P}=\text{S}$). Organophosphates are highly toxic and they act by targeting the acetylcholinesterase (AChE) for inhibition, an enzyme found in nervous tissues, muscles and red blood cells that is responsible for the breakdown of acetylcholine into choline and acetic acid, thereby controlling the nerve impulses transmission (Mwila *et al.*, 2012). Despite being stable and having high penetration power of the insect cuticle, thiono type of organophosphates have poor inhibition potential of AChE. On the other hand, the higher electronegativity of 'O' compared to 'S' in the Oxon type of organophosphate makes it very unstable (Van *et al.*, 2005). Organophosphorus pesticides are a class of synthetic pesticides that have been used for over 60 years (Eleršek and Filipi, 2006). They were widely used in 21 century, until over 10 years ago when some of the organophosphorus (parathion and chlorpyrifos) use

were put under restrictions and even banned following concerns over their toxicity and the general effects on health and environment (Wandiga, 2001).

Organophosphorus pesticides are mainly applied to control crop pests and diseases thus reducing losses. In environment, pesticides are transformed into different metabolites based on the prevailing environmental conditions such as pH, temperature, light intensity etc., (Eleršek and Filipi, 2006). the metabolites may exhibit different toxicity levels and sometimes have pesticide effects as well (Olaniran and Igbinsosa, 2015). Diazinon, chlorpyrifos and azinphos methyl are some examples of organophosphorus pesticides. Organophosphorus are highly toxic to both animals and human (Ingelse *et al.*, 2001). They act by inhibiting enzyme acetylcholinesterase (AChE) as its primary target thus reducing its activity (Gupta and Milatovic, 2012). Toxicity of organophosphorus pesticide is dependent on the compound chemical structure, mode on target organism, the pesticide dosage used, method of application, route of pesticide entry into the body system and degree of decomposition (Eleršek and Filipi, 2006).

2.2.2 Carbamates

Carbamates are a group of synthetic pesticides derived mainly from the methyl esters of carbamic acids (NH₂COOH). Figure 2.2 shows the general structure of carbamates.

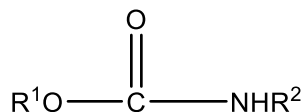


Figure 2.2: General structure of carbamates

Where R^1 and R^2 are alkyl or aryl. Carbamates act by inhibiting Acetylcholinesterase (AChE) enzyme thus damaging the central nervous system (Wijngaarden *et al.*, 2005). Unlike in the case of phosphorylated enzyme, the instability of carbamylation of enzyme results into fast restoration of AChE enzyme in addition to being selective (Oyugi, 2012). Methomyl, aldicarb, bendicarb and propoxur are some carbamates.

2.2.3 Pyrethroids

Figure 2.3 shows the general structure of pyrethroids Where R^1 and R^2 are alkyl or aryl groups.

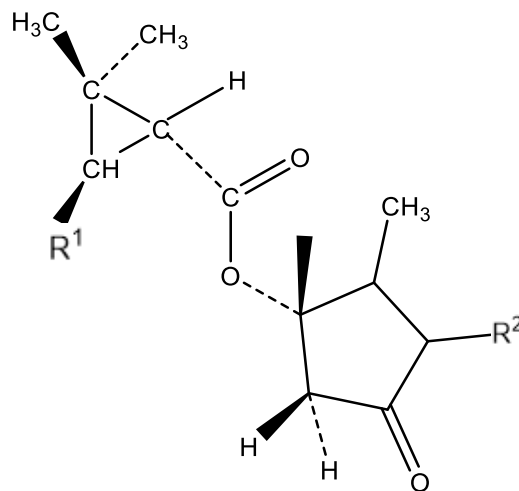


Figure 2.3: General structure of pyrethroid

Pyrethroids are a group of semisynthetic derivatives of the chrysanthemumic acids that have been developed as insecticides. Chrysanthemumic acid is derived from pyrethrins (natural pesticides) found in pyrethrum plants (*Chrysanthemum cinerariaefolium*) flowers (Macharia *et al.*, 2009; Hénault-Ethier and Soumis, 2015). They are used widely in agriculture, domestic, industries, veterinary and medical sector in controlling crop pests (aphids and weevils), crawling or flying pests, animal and human parasites (Hénault-Ethier

and Soumis, 2015). Their use has increased tremendously since the first pyrethroids (Allethrin) was used in 1949 with Canada having over 614 registered pyrethroids and 3500 products used in United States (Hénault-Ethier and Soumis, 2015). When sprayed on the crops or environment, pyrethroids easily degrade to non-toxic products, however, rates of degradation are dependent on the type of soil and physiochemical properties with a half-life ranging from 1-16 weeks (Tchorpe *et al.*, 2000; Sande *et al.*, 2011). In addition, they exhibit high hydrophobic nature making them to be able to bind strongly to soil and sediments. Pyrethroids are fat soluble though can easily be washed off the surfaces by rain water. They act by disrupting the signal processing in neurons (ion channel neurotoxins) causing interference with the functions of the nervous system (Beasley *et al.*, 2013). Interference with the signal processing neutrons on the voltage-sensitive sodium channels found along the cell membrane of the neutron tail (axon) may block the open gates resulting into a repetitive firing and depolarization, as a result incoordination, convulsion, paralysis and death of the insect may occur (Daniel *et al.*, 2003; Beasley *et al.*, 2013; Hénault-Ethier and Soumis, 2015).

2.2.4 Triazole fungicides

Figure 2.4 shows the general structure of 1,2,3-triazole.

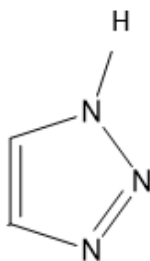


Figure 2.4: General structure of 1,2,3 triazole

Triazole are heterocyclic five-membered ring compounds with a molecular formula $C_2H_3N_3$. They contain two carbon atoms and three nitrogen atoms in their structure having two sets of isomers that differ in the relative positions of the three nitrogen atoms (Elgarhy, 2015). They belong to a class of fungicides of triazole and are used as fungicides with broad spectrum activity, they are site specific and are classified into two main groups namely; imidazole and triazole (Elgarhy, 2015). The members of each group are structurally related and changing the side chain structure determines the level of toxicity and the antifungal activity of the compound (Goetz *et al.*, 2007). Triazole are fat-soluble and easily absorbed in the gastro-intestinal tract (Kjærstad *et al.*, 2007). They are very specific fungicides that act by inhibiting the biosynthesis of the sterols (fungal cytochrome P-450-(CYP)-enzyme ergo sterol), a vital part of the fungal cell membrane (Goetz *et al.*, 2007; Shahinasi *et al.*, 2017). Examples of triazole fungicides include; cyproconazole, triadimefon, paclobutrazole among others.

2.3 Pesticides used for horticultural production

Many developing countries especially in Africa, continue to experience a number of challenges in meeting food safety requirements for fresh horticultural produce for export (Kanda *et al.*, 2012). To ensure compliance, farmers and stakeholders in developing countries are now engaged in developing strategies to improve pesticide use for sound environmental conservation, safety of consumers, compliance and to maintain exports (Mushobozi, 2010). However, local consumers are at risk since they consumes directly from the farms without the pesticide levels being checked. Pesticide use in Kenya has been on the rise showing the continued rate of pesticide consumption. In the year 2012, Kenya

imported approximately 7708 metric tons of pesticides worth Kshs 4.5 billion, of this import, 37% were insecticides, 31% fungicides, 17% herbicides and 15% other chemicals including fumigants, rodenticides, growth regulators, wetting agents etc (PCPB, 2012). Kenya have no standards for domestic pesticide application and residue monitoring in agricultural produce, farmers are not adequately trained in pesticide use. In Ewaso Narok wetland, the information on pesticide application and their levels in various horticultural produce including vegetables are very scanty. The absence of good agricultural practices, integrated pest management program and failure by the relevant regulatory authorities to enforce laws and regulations on pesticide use is a major concern.

2.4 Fate of agricultural pesticide in environment

Only 1% of the applied pesticide reach the target pest while 99% are distributed in the four environmental components namely; water, air, soil and biota (flora and fauna) depending on the prevailing environmental conditions (Otieno *et al.*, 2010; Bansal, 2011). The distribution of the pesticide is dependent on various physico-chemical properties (pH, temperature, salinity, solubility, bioaccumulation factor (BCF)) of individual chemicals (Tan and Chai, 2011). The distribution of pesticides in environment components takes place by several physical processes namely; adsorption, volatilization and sedimentation (Gunasekara *et al.*, 2007; Olaniran and Igbiosa, 2015). After the distribution, the pesticides then may get degraded by several chemical reactions such as oxidation, photolysis and hydrolysis (Daniel *et al.*, 2003; Verster, 2005; Wightwick *et al.*, 2010). Figure 2.5 shows different routes of pesticides distribution into the four main

environmental compartments of air, water, soil and biota (plants and human) (Fenik *et al.*, 2011).

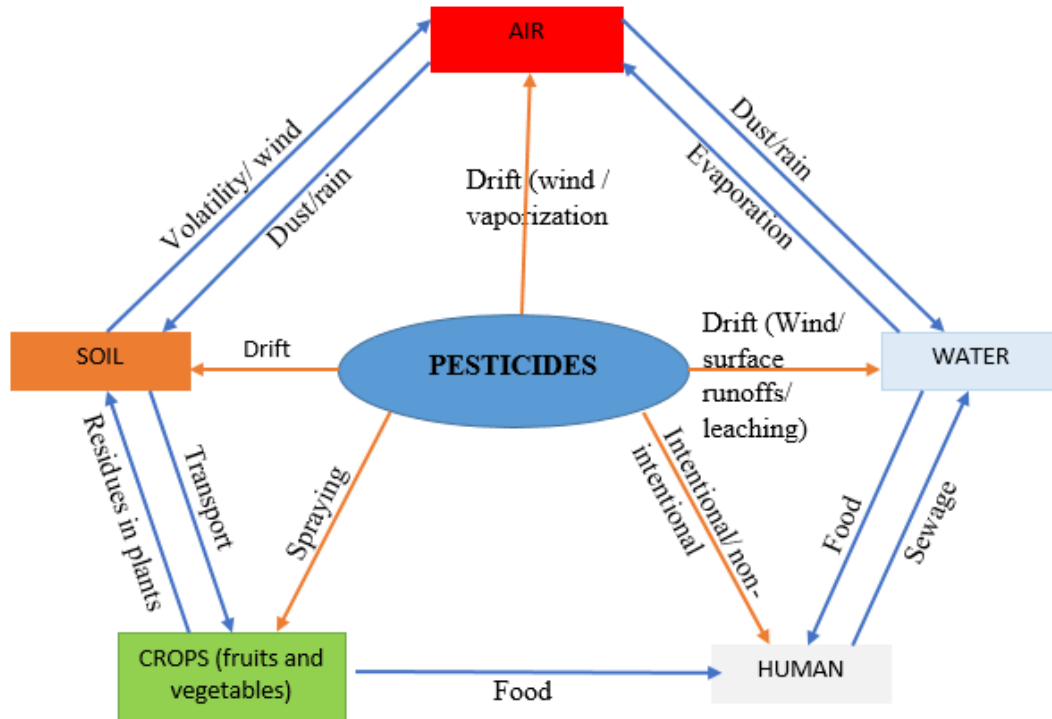


Figure 2.5: The distribution routes of pesticide chemicals in the environment

The process is aided by living organisms in which pesticide are degraded biologically through hydrolysis, oxidation, reduction and conjugation processes (Naqvi *et al.*, 2013; Mekonen *et al.*, 2014). The degradation of pesticides in the environment depends on the environmental matrices (soil, water, plants) in which the pesticides is distributed, chemical nature of the pesticide and the physico-chemical properties (pH, temperature, conductivity).

Pesticides have different toxic effects on the environment which impact negatively on the ecosystem (El-Wakeil *et al.*, 2013). There are approximately 500 registered pesticides

products globally with new agrochemicals being registered daily (Raina, 2011). Unregulated pesticides use is on the rise which is not only a potential health risks on human, but also on wildlife and sensitive ecosystems (Ogada and Keesing, 2010). Improper application techniques and wrong spraying equipment, inadequate storage facilities and reuse of pesticide containers for water and food storage exacerbate these risks (Akan *et al.*, 2013). Furthermore, some pesticides takes long to degrade leading to accumulation in the soil hence reducing the general functionality of the soil by killing the beneficial soil micro bacteria (Aktar *et al.*, 2009).

2.4.1 Pesticides in water

Pesticide residues have been reported in water systems including wells, rainwater and ground water (Kumar *et al.*, 2015). Pesticides are distributed from the targeted sites into the water system through different routes namely; drifts especially during spraying, surface runoffs due to soil erosion, accidental or intentional spillages, percolation or leaching through the soil (Bollmohr *et al.*, 2011). However, these processes are dependent on the physical and chemical properties of the individual pesticides. Farmers also clean pesticide mixing tanks and sprayer equipments along the water ways such as rivers further increasing the levels of pesticide residues in the water (Pazou *et al.*, 2006).

2.4.2 Pesticides in soil

Most pesticides in use today are regarded as soil contaminant and it is believed that the chemical effects may persist in the environment hence endangering the existence of important micro-organisms in the soil (Yasmin and D'Souza, 2010). This may lead to a

serious reduction of the general soil biodiversity and fertility. Important processes such as nitrogen fixation are stopped as a result of the decline in soil microorganism (Hussain and Siddique, 2010). Relatively high pesticide residues concentrations in the soil could be attributed to a number of factors; such as the continued application of pesticides within the study area, past land use or surface runoffs from neighboring fields. The persistence of pesticide in the soil is dependent on the physico-chemical properties of individual residues such as water solubility, soil pH, octanol/water partition coefficient ($\log K_{ow}$) and half-life (DT50) (Moncada, 2001; Kaushik *et al.*, 2009). Pesticide residues with high values for $\log K_{ow}$ shows that the chemicals have high affinity to the organic (non-polar) environment than to the aqueous environment (Olaniran and Igbinsosa, 2015). This gives an indication of the pattern of distribution of pesticides in the environment. Highly soluble pesticides are easily distributed in the environment during rainy season as a result of surface runoffs while some leach into the groundwater. Soil is sink to most environmental pollutants, agrochemicals included. It is also a pathway through which most pollutants gets into and contaminate water systems, plant foods thereby affecting human and other living organisms.

2.4.3 Pesticide in fruits and vegetables

Pesticides are introduced into the environment in different forms namely; powder, concentrates, aqueous solutions, emulsions and others (Fenik *et al.*, 2011). Vegetables considered in this study are tomatoes (*Lycopersicum esculentum* Mill.) and kales (*Brassica oleracea*). Vegetables such as french beans, cabbages, peas, spinach are grown in the region but not in the same scale as tomatoes and kales. Tomatoes and kales are widely

grown crops and consumed not only within Ewaso Narok wetland but also all over Kenya while tomato is a major Mediterranean diet and largely consumed in most European countries (Gambacorta *et al.*, 2005). The use of pesticides therefore helps in preventing losses and improving the quality and the yield of both tomatoes and kales in the region. However, pesticide also is a major contaminant of these crops and may present potential risk to both human and animals that consumes them (Adeola, 2012). The frequency and amount of pesticide application, its nature and the type of fruits and vegetables plays a major role on the levels of contamination. Application of pesticides in high doses than the recommended is a major cause of high residues levels in vegetables (Chowdhury *et al.*, 2012). Aldicarb may bioaccumulate in primary, secondary and tertiary consumers of contaminated vegetables, hence, their presence in the vegetables though in low quantities may have irreparable chronic effects on the health of the consumer (Hussain and Siddique, 2010). Aldicarb degrades through oxidation in the environment to form its aldicarb oxides (sulfoxide and sulfone) (Sagrati *et al.*, 2007). It is highly persistent in underground water with a half-life ranging from a few weeks to several years, its oxide (sulfoxide) is more potent acetylcholinesterase inhibitor than the parent aldicarb, while sulfone is considerably less AChE inhibitor (WHO, 2003).

2.5 Effects of pesticides in the ecological ecosystem

Soil microorganism (fungi and bacteria) which uses pesticides as food has the potential of biodegrading the pesticides to benign substances that are compatible with the environment (Chowdhury *et al.*, 2008). However, at high concentration pesticides impact negatively on the soil microorganisms causing microbial imbalance and this may lead to reduced soil

fertility (Behn *et al.*, 2013). Bioaccumulation of pesticide residues in fish and fish eating birds has been reported in previous studies (Otieno *et al.*, 2010). Carbamates are toxic to fish, invertebrates, worms, insects, and other living organisms in the soil (Jabeen *et al.*, 2016). Previous studies have reported carbamates toxicity effects on honey bees which are the main pollinators (Hardstone and Scott, 2010). Pyrethroids are toxic to fish, honey bee, lobster, aquatic insects shrimp, mayfly, nymphs, zooplanktons and oyster according to Jabeen *et al.* (2016). Decline in the number of arthropods and wetland biodiversity have been linked to over reliance in the use of pesticides (Carter and Thompson, 2015).

2.6 Pesticides and human health

The global annual report by the United Nation on deaths linked to acute pesticide poisoning stands at approximately 200,000 people (Batt, 2017). Approximately 99% of the reported pesticide related deaths occur in the third world countries accounting only for the 25% of the total global pesticides production (Oesterlund *et al.*, 2014; Mahmood *et al.*, 2016). In Kenya, 62 pesticide products containing 36 active ingredients are registered for used in horticultural production, 35% of the registered pesticides are organophosphorus, 25% are carbamates, 22% are pyrethroids while inorganics and others constitute 14% (Macharia *et al.*, 2009). Eight (8) percent of the 62 pesticides products used for vegetable production in Kenya are classified as highly hazardous by World Health Organization (WHO), 25% as carcinogenic, 43% are possible carcinogens, 64% are ground water contaminants while 47% are harmful to beneficial insects (Macharia *et al.*, 2009). Long term pesticide exposure have been linked to various human diseases such as cancer, Parkinson's and Alzheimer's diseases, hormonal disruption, neurological health effects, loss of coordination and

memory, reduced motor skills and visual abilities, development disorder and sterility (Bassey, 2011). Pre-natal pesticide exposure has also been linked to pre-term deliveries, miscarriages, and birth defects (Van *et al.*, 2012). A pesticide residue mixtures have been reported in umbilical cords and first feases of newborns a clear evidence of pre-natal exposure (Rahbar *et al.*, 2016).

Pesticide exposure by pregnant mothers may lead to higher risk of leukemia, autism, cancer and respiratory illness in children (Urayama *et al.*, 2007). Aubertot *et al.* (2005) in their study explained that pesticide effect in human is dependent on the pesticide toxicity level, magnitude and period of exposure. Farmers are in direct contact to pesticides, they are likely to bear the greatest impacts resulting from exposure. Furthermore, failure or laxity in using proper and complete protective clothing and equipments during pesticide handling has only led to increased farmers-pesticide exposures resulting to serious human health concerns (Mengistie *et al.*, 2017). Asogwa and Dongo (2009) linked unregistered pesticide products in the markets to poor enforcement of pesticides regulations. This is an avenue in which farmers knowing or unknowingly access wrong pesticide products, banned or restricted for use in agricultural food production (Dasika *et al.*, 2012).

Pesticide exposure are common through occupational contact by those access the treated fields though cases of suicidal exposure are also common (Printes and Callaghan, 2004; Beasley and Temple, 2013). Pesticide exposure may occur through various routes such as ingestion of contaminated food or water, soil or dust particles, absorption through the skin during application or touching treated surfaces, breathing in fine droplets or dust particles

airborne (Beasley and Temple, 2013). Symptoms of the acute pesticide exposure include dysfunction of parasympathetic system, paresthesia, headache, fatigue, nausea, vomiting, dizziness, moderate toxicity (CNS depression, fever, diaphoresis, blurred vision and increased salivation) and severe toxicity (pulmonary oedema, seizure, respiratory failure, coma and death) (Daniel *et al.*, 2003; Blain, 2011; Hénault-Ethier and Soumis, 2015).

High toxicity of organophosphorus (azinphos methyl) and carbamates (aldicarb) has been linked endocrine disruption, poor masculinization and poor testicular development which affect the general adult sexual behavior, immunotoxic, genotoxic and potential carcinogenic effects in human (Chindah *et al.*, 2004; Kjærstad *et al.*, 2007; Goetz *et al.*, 2007; Liu *et al.*, 2012). Hénault-Ethier and Soumis (2015) reported that synthetic pyrethroids causes the disruption of an endocrine system in human resulting into possible adverse effects on the sexual development and lower immunity. According to a study by Thatheyus and Selvam (2013), pyrethroids contain xenoestrogens which increases estrogen in the body resulting into development of breast cancer cells. In Kenya, pesticides sprayed on vegetables have been found to be possible carcinogen, endocrine disruptors, skin and eye irritants and neurotoxicants among other human health issues (Lalah *et al.*, 2003; Tsimbiri *et al.*, 2015). Bioaccumulation of these pesticides in human due exposure through consumption of contaminated vegetable and drinking contaminated water with residue levels above the recommended limits can be catastrophic to the entire population. There is need to regulate pesticide access and to carry out regular surveillance on the level of the pesticide residues in environment and agricultural food produce.

2.6.1 Regulating pesticide residues in food products

Maximum Residue Limit (MRL) can be used to verify that pesticide have been used correctly following Good Agricultural Practice (GAP) (Neff *et al.*, 2012). MRL is also an important safety standard in many international markets use for trade. All agricultural food or commodity should comply with the MRLs that varies with different countries or regulatory bodies such as World Health Organization (WHO), Agricultural Food Organization (FAO) and European Food Safety Authority (EFSA) to be legally allowed into the markets (Inonda *et al.*, 2015). Maximum residue limits are set as low as reasonably achievable (the ALARA principle), hence, food or commodity that comply with the set MRL is considered not only safe for human consumption but also legal for trade (Neff *et al.*, 2012). However, non-compliance with the MRL does not imply that the food or commodity is not safe for human consumption, though not legal for trade. Further considerations such as compliance to acceptable daily intake (ADI) and/ or acute reference dose (ARfD) (Aktar *et al.*, 2009). If ADI and /or ARfD are exceeded, then that particular residues may be a human health concern given the safety margins incorporated into the ADI and ARfD (Delaplane, 2000).

Fruits and vegetables are prone to pests and disease invasion making pesticide use a major input in production (Lesueur *et al.*, 2008). However, food safety from pesticide contamination have elicited a lot of concern worldwide considering the direct impact of residue contamination on human health (Kaushik *et al.*, 2009). Presence residues in food therefore present risk to the consumers, thus, leading to the rejection of vegetable/ fruits entry into the international markets (Karungi *et al.*, 2011). EFSA, WHO, FAO have set

MRLs for different pesticide residues for tomatoes and kales (Lozowicka *et al.*, 2015; EFSA, 2017). However, the MRL for the same residue in the same food or commodity may differ in different countries or regulatory bodies, thus they cannot be used to disqualify any food and commodity as unfit for human consumption based on residue levels exceeding the set MRL (FAO and WHO, 2011; EFSA, 2017). The ADIs and ARfDs for individual residue based on an individual's weight need to be considered against the residue level for the food or commodity to be declared as safe or unsafe for human consumption (Al-Dabbas *et al.*, 2014). Table 2.1 shows the WHO/FAO and EFSA (EC) MRLs, ADIs and ARfD values.

Table 2.1: WHO/FAO and EU residue limits for kales and tomatoes

Pesticides	Kales (MRL)		Tomatoes (MRL)		ADI		ARfD	
	WHO/FAO	EFSA (EC)	WHO/FAO	EFSA (EC)	WHO/FAO	EC	WHO/FAO	EC
Aldicarb	-	20	-	20	3	-	3	-
Azinphos methyl	500	50*	1000	50*	30	5	100	75
Azoxystrobin	5000	6000	3000	3000	200	100	-	-
Bifenazate	-	10*	500	500	10	10	-	-
Buprofezin	-	50*	1000	1000	10	10	-	100
Chlorpyrifos	-	50*	1000	10*	10	10	100	100
Cyproconazole	-	50*	-	50*	-	10	-	20
Diazinon	50	10*	500	50*	5	5	30	30
Fenpropathrin	-	10*	1000	10*	30	30	-	-
Metalaxyl	-	200	500	200	-	-	-	40
Paclobutrazole	-	-	-	-	100	20	-	100
Pyrimethanil	-	10*	700	1000	200	170	-	-
Spiroxamine	-	10*	-	10*	-	25	-	100
Triadimefon	-	100	-	300	30	-	80	-

SI Units ($\mu\text{g}/\text{kg}$), *(lower limits of analytical determination), ADI (Acceptable Daily Intake), ARfD (Acute Reference Dose), EC (European Commission), WHO (World Health Organization), MRL (Maximum Residue Limit). FAO (Agricultural Food Organization).

2.7 Instrumentation

2.7.1 Principles of liquid chromatography

Liquid chromatography (LC) also known as High Performance Liquid Chromatography (HPLC) is an analytical chromatographic technique used to separate mixtures of compounds in analytical and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture (Sargent, 2013). Figure 2.6 shows a schematic diagram of High Liquid Chromatography or HPLC system (Unger *et al.*, 2013).

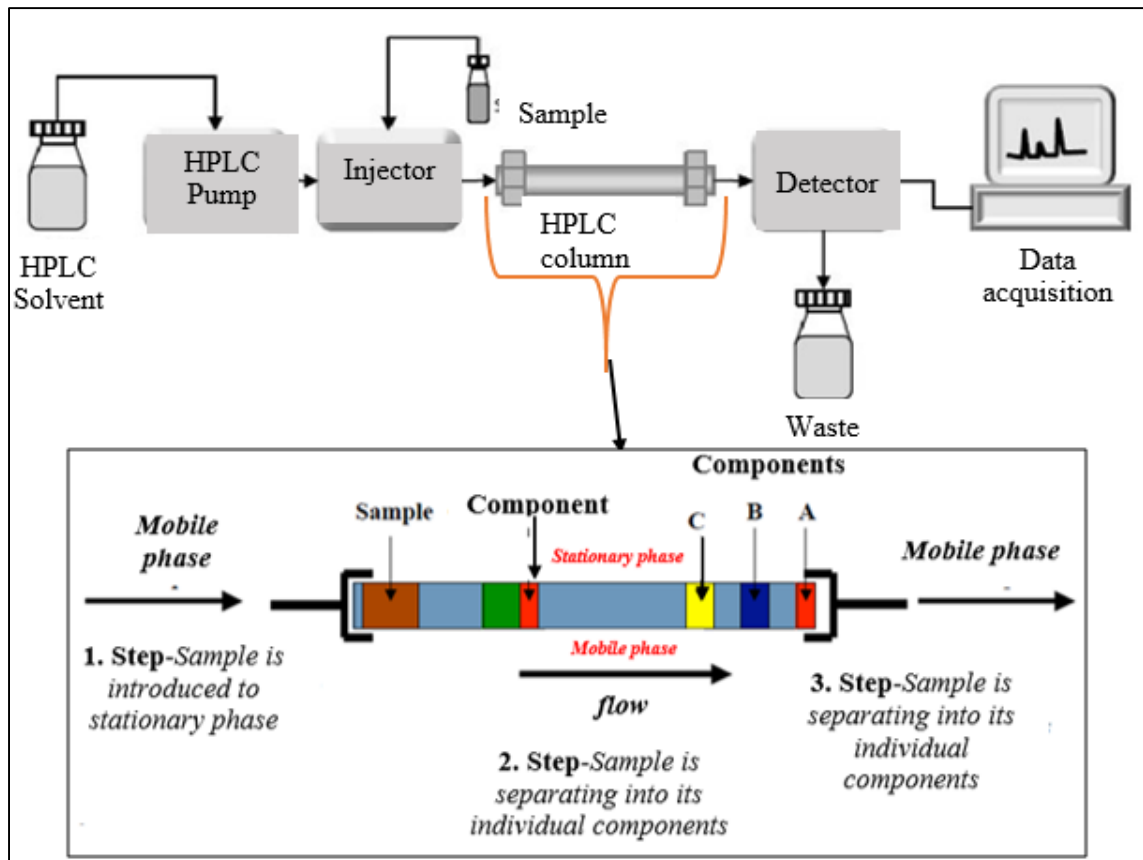


Figure 2.6: Schematic diagram of liquid chromatography (HPLC) system

Separation is accomplished by the injection of a small quantity of a liquid sample into a moving stream of a liquid solvent (mobile phase) that passes through a column packed with

particles (resin) of stationary phase. Separation of a mixture to its components depends on different degrees of retention of each component in the column (Picó *et al.*, 2004). The strength of interaction between the sample components and stationary phase (packing) varies for different compounds determined by the sample component partitioning between liquid mobile phase and stationary phase (Picó *et al.*, 2004). In LC, the partitioning is affected by the relative solute/stationary phase and solute/ mobile phase. As a result, the retention time, t_R (time between injection and detection) at the stationary phase varies for different components thus achieving separation. The analytes of interests in the mobile phase are then separated based on their physicochemical interaction with the mobile phase. The interaction is based on different factors including molecular size (size exclusion chromatography), charge (ion exchange chromatography), hydrophobicity (hydrophobic interaction chromatography), specific binding interaction (affinity chromatography) or a combination of all factors (multimodal or mixed-mode chromatography) (François *et al.*, 2009).

The composition of the mobile phase can be changed during separation run so as to alter the strength of interaction of the compounds of interest (Ferrer *et al.*, 2005). Each compound then elutes from the column in a particular order depending on the relative strength of its interaction with the solid stationary phase and the mobile phase. There are numerous detectors used in LC and their modes of detection depends on the analyte to be detected. Most commonly, a UV-detector or spectrophotometer is attached to the chromatography system (Jeannot *et al.*, 2000). The resulting chromatogram is then analyzed qualitatively (matching t_R of a known compound with the t_{R_s} of components in

unknown mixture) or quantitatively. Each distinct peak in a chromatogram represent a unique sample component resolved by the column and the area under the peak corresponds to the amount of that compound eluted from the column (Carneiro *et al.*, 2013)

2.7.2 Principles of tandem mass spectrometry

Tandem mass spectrometry (MS/MS) is a method in which at least two mass spectrometers are coupled in series for the analysis of specific organic compounds in complex mixtures.

Figure 2.7 shows a schematic diagram of tandem mass spectrometer.

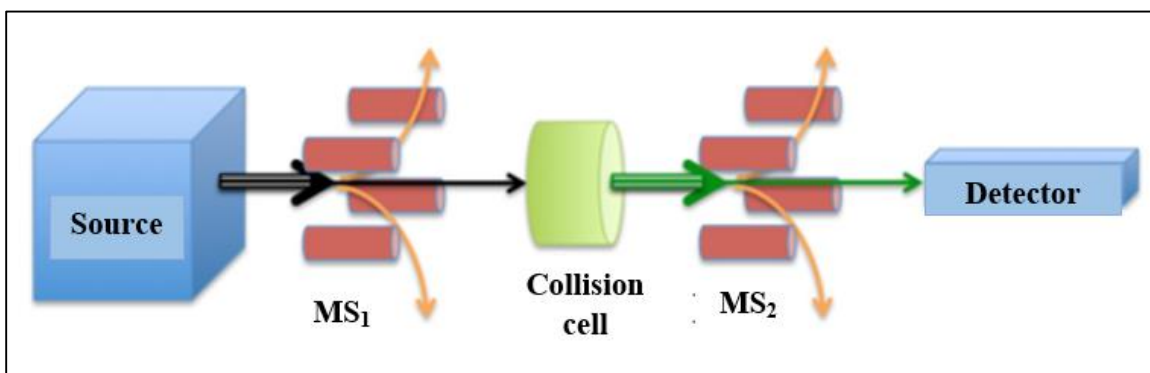


Figure 2.7: A Triple quadrupole (QqQ) Tandem Mass spectrometry (MS/MS)

Tandem Mass Spectrometry involves the activation of a precursor ion formed in the source and the mass analysis of its fragments. There are several ion activation methods used in MS/MS including collision induced dissociation (CID), Infrared Multiphoton Dissociation (IRMPD), Electron Capture Dissociation (ECD), Surface Induced Dissociation (SID), Triple quadrupole (QqQ), Time of Flight (TOF) among others (Sleno and Volmer, 2004). Major components of MS/MS include the source where the sample in liquid phase is injected, ionized and introduced into the first quadrupole MS_1 . The first selection of ions based on their m/z ratio is performed in the first quadrupole (MS_1). From the first

quadrupole, only one mass of the ion (precursor ion) is selected from a mixture of ions and allowed to enter the second quadrupole (collision cell). Collision cell is filled with N₂ or argon gas at a pressure of 0.1 Pa. in the collision cell, the precursor ion selected by the first quadrupole collide with a beam of N₂ or Argon molecules with sufficient energy and breaks to form fragments known as product ions (Douglas and Frank, 2005). The third quadrupole (MS₂) receives product ions and filters the product ion of interest which passes through the detector to identify the presence of molecules of interest. With internal standards to support quantification, an ion detector counts each ion that passes through the MS₂ at each specific m/z ratio and transfers the information to the computer system for analysis (Madeira and Florencio, 2012).

2.8 Methods used for pesticide residues determination

2.8.1 Sample extraction and cleanup methods

Several methods for obtaining pesticide residues in complex matrices like soil, water and vegetables have been developed. These methods include; supercritical fluid extraction (SFE), liquid-liquid extraction (LLE), solid-phase extraction (SPE) , solid-phase micro-extraction (SPME), microwave assisted extraction (MAE) and accelerated solvent extraction (ASE) (Durovic and Dordevic, 2011). Despite the fact that these methods have specific advantages, techniques such as supercritical fluid extraction (SFE) and liquid-liquid extraction (LLE) present a range of practical disadvantages to the users. The limitations include large solvents throughput required, several extraction steps including sequential, large sample throughput, higher costs of maintaining semi-automated

extraction techniques, and the need for extract reconstitution and pre-concentration before analysis (Đurovic and Đordevic, 2011).

The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method was introduced in an attempt to increase the efficiency of old methods for sample preparation (Anastassiades *et al.*, 2003). QuEChERS has several advantages over to traditional methods used for sample preparation namely; good recoveries, cheap and few cleanup steps (Mekonen *et al.*, 2014). In this method in which acetonitrile (C_2H_3N) is used for sample extraction, only a small sample throughput (10-15g) and fewer extraction steps are involved thus reducing sources of errors (Lehotay *et al.*, 2007). The sample is mixed thoroughly with 10-15mL of C_2H_3N (1mL C_2H_3N / 1g sample) in a 50mL tube (Lehotay *et al.*, 2005). Large amounts of salts are added to the extracts to aid in the extraction of both pesticide residues. Pesticides are extracted simultaneously at this stage with other sample matrix components such as fatty acids, sugars and plant pigments (like chlorophyll) (Lehotay *et al.*, 2008). Dispersive solid phase extraction (dSPE) technique is then used for sample extract clean-up.

In the dispersive solid phase clean-up extraction technique, salts and a number of sorbent materials. Primary Secondary Amine (PSA) and Graphitized Carbon Black (GCB) are used to eliminate residual water, sample pigments alongside other sample matrix interferences (Lehotay *et al.*, 2005). PSA is used to remove fatty acids, sugars, and some ionic pigments while GCB is employed to eliminate non-polar pigments (chlorophyll) especially from vegetable samples, anhydrous $MgSO_4$ / NaCl salts is used to remove residual water. The

C₂H₃N extract can be analyzed by both GC/MS and LC/MS/MS with dilution (Lesueur *et al.*, 2008; Sahoo *et al.*, 2011). A number of studies have been undertaken to improve and optimize the performance of QuEChERS extraction method (Chen *et al.*, 2015). This involved the inclusion of buffers at the point of extraction to enhance the stability of analytes and quality of extracts.

Figure 2.8 is an illustration of pesticide residues extraction process using QuEChERS EN protocol for pesticide extraction (Michelangelo and Lehotay, 2003).

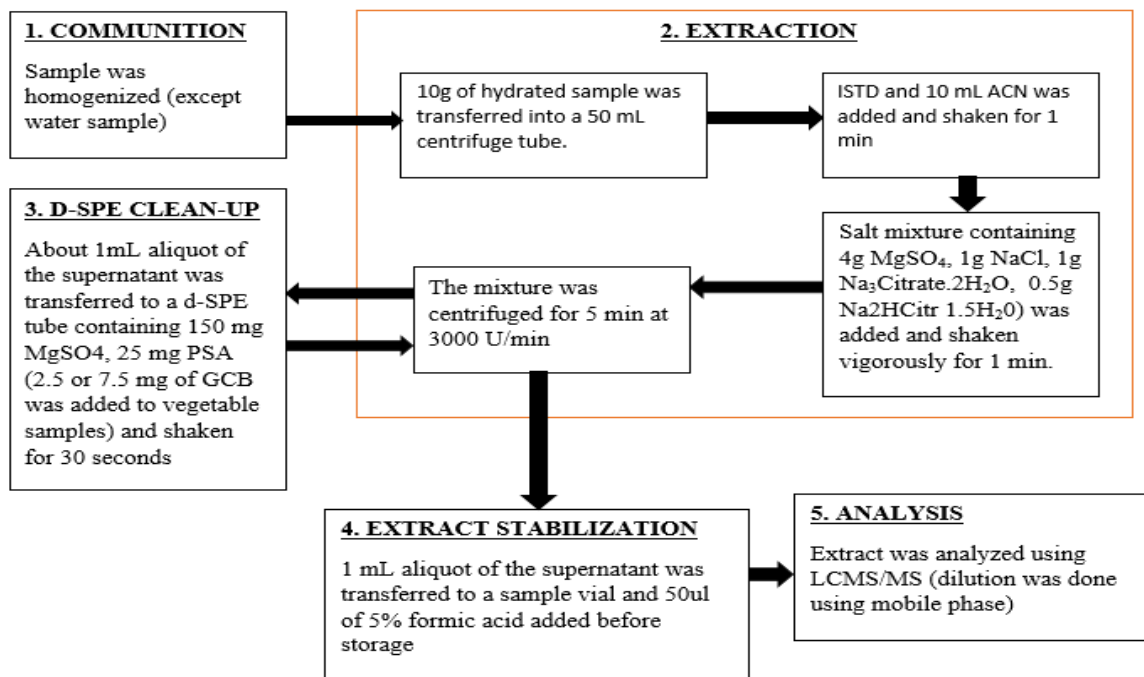


Figure 2.8: QuEChERS EN protocol for pesticide extraction

As a result two buffered methods were developed namely; the European committee for standardization (CEN) method and American version AOAC method. In this study EU-CEN method was used for sample extraction.

2.8.2 Pesticide analysis

Multiresidue analysis by gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (GC-MS, LC-MS) is documented (Davis and Lewis, 2008; Kumar *et al.*, 2015). GC-MS provides confirmation of a range of pesticides in a single analytical run due to its superior selectivity allowing for interference free quantification even with peak co-elution (Chiu *et al.*, 2015). Mass Spectrometry (MS) serves as a quantitative and qualitative detector providing detailed structural information of a compound present in an analyte (Hetherington *et al.*, 2004). Each analyte has a unique mass spectrum that can be compared with the mass spectral databases or standard spectra and thus identified. Recently, strict regulations have been set on the maximum residue limits (MRLs) of pesticide residues in food and commodity (Thurman *et al.*, 2006). The MRLs for all pesticides in Fruits and vegetables for baby food and all compounds without regulations is set at lowest 10 µg/kg (Thurman *et al.*, 2006). To meet these standards in complex samples, development of more sensitive analytical methods was crucial, hence the use of Liquid Chromatography-tandem mass spectrometry (LCMS/MS) with triple quadrupole in multiple reaction monitoring mode (MRM) method for monitoring and quantitation of pesticide residues in food (Ferrer *et al.*, 2005; Thurman *et al.*, 2006; Kiu, 2011; Dasika *et al.*, 2012). This method is suitable for routine pesticide residues surveillance due to the ease of using triple quadrupole as a detection technique and MS/MS capacity to achievable lower limits of detections (Alder *et al.*, 2006; Dasika *et al.*, 2012).

Interfacing LC with MS in LC-MS, together with Electrospray Ionization (ESI) and atmospheric pressure chemical ionization (APCI), makes LC-MS be preferred for the

analysis of polar compounds while GC-MS is used for the analysis of mid-polar to non-polar compounds (Alder *et al.*, 2006; Kiu, 2011). However, with the recent development in LCMS/MS instrumentation, it has become a preferred method in the determination of a wide range of pesticide residues in food and environment in the past 10 years (Zrostlikova *et al.*, 2003; François *et al.*, 2009). LCMS/MS has advantages of improved selectivity, efficiency and sensitivity (Ferrer *et al.*, 2005). Thus, researchers prefer the use of LCMS/MS method for pesticide analysis (Kiu, 2011). Hundreds of potential contaminants including those difficult to detect using GC and LC-MS can easily be monitored by LCMS/MS (Wittrig and Schreiber, 2014).

Tandem mass spectrometer (MS/MS) technology is a technique in which two mass spectrometers are connected in series to help in the identification of pesticides via the selection of specific MRM transitions for each compound (Wittrig and Schreiber, 2014). The function of the first MS is to select the precursor ions as produced at the ion source (ion trap, time of flight (TOF) or quadrupole mass spectrometer) (Alder *et al.*, 2006). The selected precursor ion is then made to fragment into fragment (product) ions through collisional activation or collision induced dissociation (CID). The second MS perform the task of filtering the product ions generated by the fragmentation based on their mass (Bossi *et al.*, 2002). This process takes place in either a triple quadrupole MS (QqQ) or a quadrupole time of flight (QTOF). The resulting MS/MS spectrum consist only of a product ion from the selected precursor ion. LCMS/MS has the advantage of increased sensitivity especially in the QqQ due to noise reduction while QTOF provides more information on the analyte based on the fragmentation pattern (Kiu, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study area was Ewaso Narok wetland, an alluvial floodplain wetland found in Laikipia County, Kenya (shown in figure 3.1).

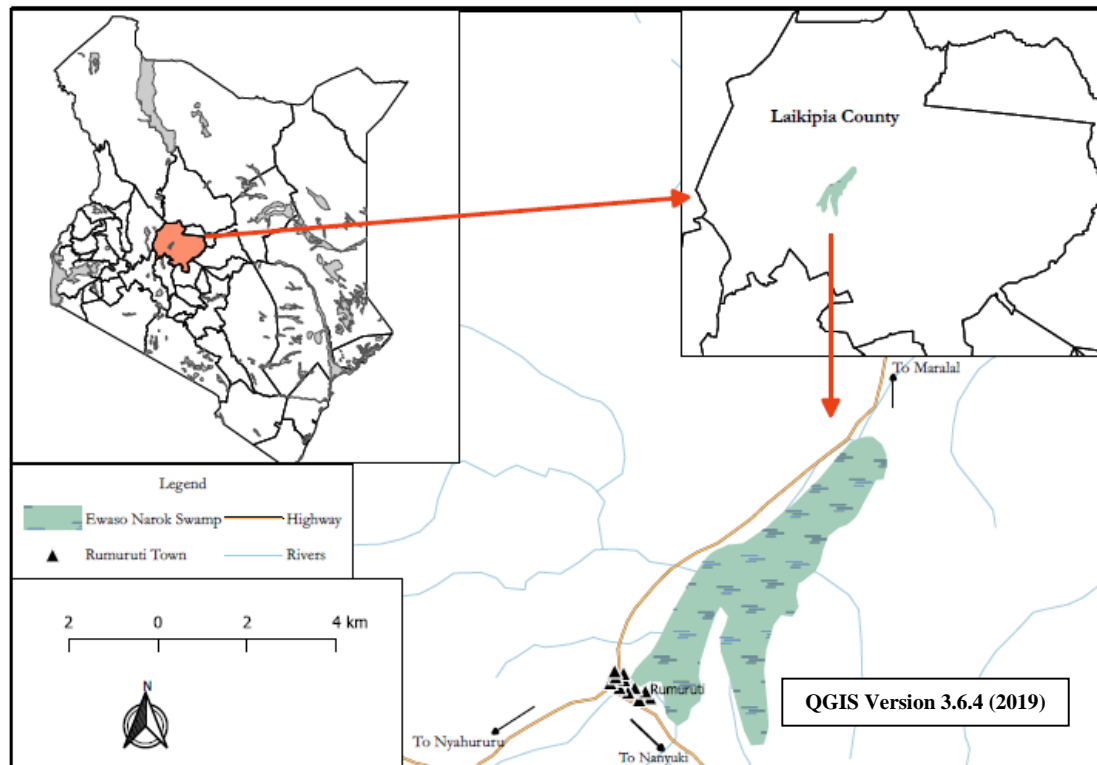


Figure 3.1: Geographical map of Ewaso Narok wetland ecosystem

Ewaso Narok wetland lies between Longitude $36^{\circ}12'17''\text{E}$ to $36^{\circ}45'16''\text{E}$ and Latitude $0^{\circ}28'51''\text{N}$ and $0^{\circ}7'28''\text{S}$ with altitude ranging from 1600-2400 m above the sea level with a total area of 80.5Km^2 . It is located in the semi-arid part of Laikipia County receiving an annual rainfall of 700mm with a total catchment area of 2610Km^2 (Leemhuis *et al.*, 2016). The farmland has a total area fluctuating between 14 and 20Km^2 in the dry and wet seasons,

respectively. Wetland terrain is such that farm runoffs freely flows into the river. On Ewaso Narok River upstream are large-scale farms including Williams Limited, Kandorovo Farm and AAA Farm and others.

3.2 Research design

The research design involved descriptive survey using a pre-tested questionnaire (Appendix 1b) to gather information on pesticide application. The data obtained was analyzed using non-parametric tests (Kruskal-Wallis and Mann-Whitney). Samples of soil, water, tomatoes and kales were collected from the farmland. Sample preparation and analysis of pesticide residue were achieved using QuEChERS and LCMS/MS, respectively. Data obtained was analyzed using parametric tests (ANOVA and Student Newman-Keuls) to determine significance difference.

3.3 Chemical and reagents

Pesticide standards of the highest analytical purity (aldicarb, azinphos methyl, cyproconazole I and II, buprofezin, bifenazate, pyrimethanil, diazinon, paclobutrazole, fenpropathrin, chlorpyrifos, metalaxyl, spiromamine, azoxystrobin and triadimefon) of purity ranging between 97.5-99.8% were obtained from Dr. Ehrenstorfer GmbH, 6A-86199 Augsburg, Germany. Solvents such as acetone of purity 99.9% (BDH, England), HPLC grade n-hexane (99.9%) and de-ionized water of 150M Ω cm (Merck, Germany), Analytical grade acetonitrile (Sigma-Aldrich Taufkirchen, Germany). Analytical grade acetonitrile (C₂H₃N) was used as the most appropriate solvent for pesticide residues extraction using QuEChERS method (Anastassiades *et al.*, 2003; AOAC, 2007; Payá *et al.*, 2007). 50mL

polypropylene centrifuge tubes, d-SPE packed tubes containing Primary Secondary Amine (PSA) for organic acids and polar pigments removal, Graphitized Carbon Black (GCB) powder for removing chlorophyll and polar pigments from vegetable extract, octadecyl (C_{18}) for removing lipids and sterols from the sample matrix and QuEChERS EN Pouch part number 5982-0650 containing $MgSO_4$, NaCl, NaAc, and citric acid disodium salt were obtained from Agilent Technologies (Santa Clara, USA).

3.4 Cleaning and storage of apparatus

All the glasswares used in the study were cleaned with detergent, rinsed with distilled water and pure acetone of purity 99.9%. All glassware except measuring cylinders were oven dried and stored in the oven overnight at $100^\circ C$. Measuring cylinders were placed on the rack and left to dry. Pre-cleaned dark amber glass bottles used during sampling were rinsed three times with the same water from the river before collecting the water.

3.5 Instrumentation

Analytical balance model UX-420H from Shimadzu (Kyoto, Japan), Vortex mixer model QL 901 Microtecnica (Brazil), Centrifuge model NT 825 Novatecnica (Brazil), SL 703 Centrifuge Solab (Germany), Juicer/ blender/grinder-model RM 298 (Ramtons, China). The chromatographic analysis was achieved using Waters Acquity Ultra-High Performance Liquid Chromatography (UHPLC) system (Waters, Milford, MA, USA) with tandem mass spectrometry (LCMS/MS). BEH C18, 2.1×50 mm, $1.7 \mu m$ column, which was equipped with a guard pre-column. The flow rate was 0.45 mL/min, the injection volume was $5 \mu l$ and the column was operated at $30^\circ C$.

A binary mobile phase was composed of 10 mM ammonium formate (A) in water and 10 mM ammonium formate in methanol (B). A mobile phase gradient starting at 2% B (0.0-0.25 min) to 99% B at 12.25 minutes was held until 13 minutes and column equilibration at initial condition for 4 min for a total run time of 17 min. The UHPLC system was interfaced to a Xevo TQ triple quadrupole mass spectrometer (MS) (Waters, Milford, MA, USA). The MS determination was performed in a positive electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitioning using scheduled multiple-reaction monitoring (MRM) program for 60s for each analyte with an electrospray ionization operated both in positive ion mode. Source conditions were set as follows: ion source temperature 150°C, capillary voltage 1.5kV, desolvation temperature 500°C. Nitrogen was used as desolvation and cone gas with a flow rate of 800 and 150 l/h, respectively. Argon was used as a collision gas and the flow rate was set at 0.12 ml/min.

3.6 Sampling

3.6.1 Administration of questionnaire

Field study was done using pre-tested questionnaire to gather information from farmers on pesticide application. A structured questionnaire was developed, pre-tested on 30 vegetable farmers who were not part of the study in Kikuyu, Kiambu County and reviewed. Ewaso Narok farmers were taken through the study requirements, expectations and requested to sign a consent form (Appendix 1a) before embarking on the study. The questionnaire was administered on tomatoes and kales farmers of Ewaso Narok wetland. This was achieved by distributing questionnaires among farmers who were able to read and answer the

questions while those who were not able to read were assisted by trained enumerators who read, translated and asked questions using farmers native language mainly Kikuyu. Out of 91 questionnaires distributed, 86 (94.5%) fully filled questionnaires were received back which was a suitable sample size based on Krejcie and Morgan table (Chua, 2006). The survey was conducted in the first week of June 2016.

3.6.2 Sampling of grids and vegetables

Soil, tomatoes and kales were collected at Ewaso Narok wetland using rectangular grids measuring 50m by 100m which were overlaid on the study area prior to sampling as described by Wollenhaupt and Wolkowski (1994). Figure 3.2 shows sampling protocol used to collect soil cores from the Ewaso Narok wetland crop field.

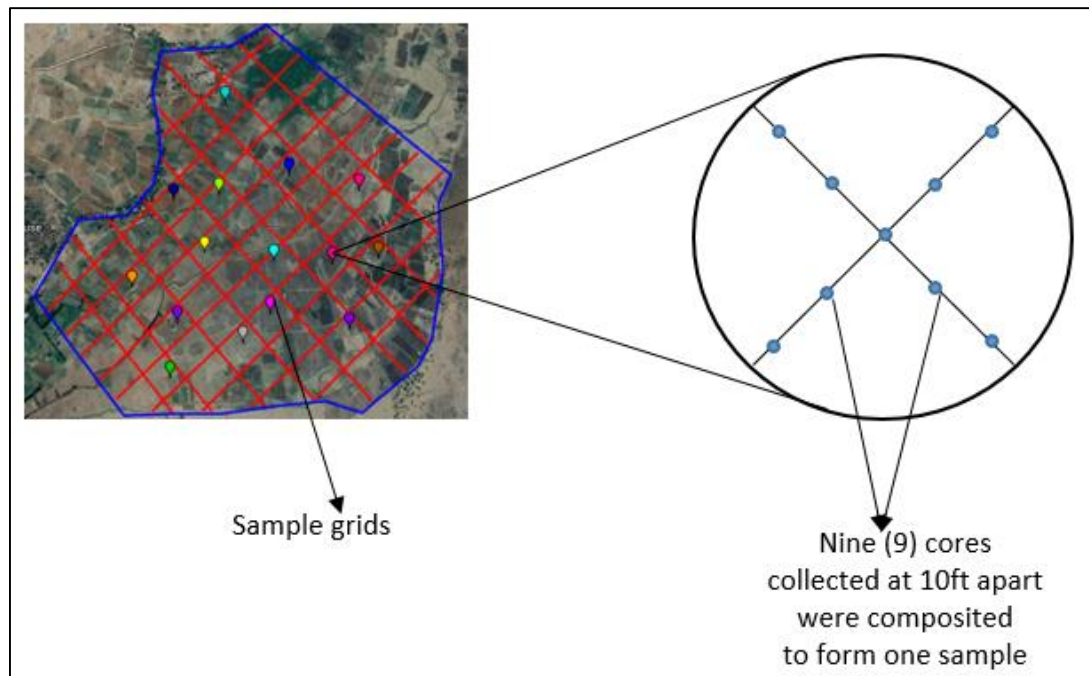


Figure 3.2: Layout of rectangular grids and locations where soil were taken

Sampling period covered wet season (June, 2016) which was the period of short rains. The dry season sampling period was during the month of August, 2016. Rainfall data was retrieved from the GlobE Wetland EA Project weather station stationed at Rumuruti (WRMA compound). (Appendix2). Fifteen (15) grids that fell on the farmland that had tomatoes and kales were randomly selected for sampling. For kales sampling, nine kale plants were randomly selected from each grid following an 'X' pattern. Four most recently matured fresh, undamaged leaves were collected from various areas of each plant, thirty six (36) fresh leaves were composited to form one kale sample. For tomatoes sampling, five ripe, undamaged tomatoes were collected from various tomato vines of each of the nine tomato plants randomly selected in a sampling grid following 'X' pattern, composited to form one sample.

Both kales and tomatoes samples were placed in separate clean polyethene bags, properly labelled and kept in an ice packed cool box before being transported to the project house in Rumuruti town for blending. The same procedure was replicated in all the 15 grids in wet/ rainy and dry/ sunny seasons. After blending, about 20g each of the blended kales and tomatoes were transferred into clean pre-labelled eppendorf plastic bottles and stored in a freezer at 4°C before being transferred to ICIPE chemistry laboratory for extraction and analysis. To stop the occurrence of microbial and chemical activity processes before extraction all samples were kept at -86°C. Samples were collected in triplicates in the three consecutive weeks in the months of wet season and dry season, 2016. A total of 90 samples each were collected for kales and tomatoes. All the sampling coordinates were recorded and reported (Appendix 3).

3.6.3 Soil sampling

Hand-held stainless steel soil auger having a diameter of 2.5cm was used to core 50g of soil each from the nine (9) different points in the grid following an X pattern at a depth of 1 to 30 cm into a clean bucket. The soil cores were composited in a clean bucket to form one sample. About 50g of the composite sample was bagged in a well labeled Ziploc bags and placed in an ice-packed cooler box. The same procedure was replicated for all the 15 grids in wet and dry seasons. Sampling was done in triplicate (three consecutive weeks) in both June and August, 2016. A total of 90 soil samples were collected for wet and dry seasons.

3.5.3 Water sampling

Water was collected along Ewaso Narok River in Ewaso Narok wetland. The river was classified into three sections and water collected using grab sampling method (Danielson, 2014; Madrid *et al.*, 2014). Figure 3.3 shows a sketch map of the wetland ecosystem showing water sampling points.

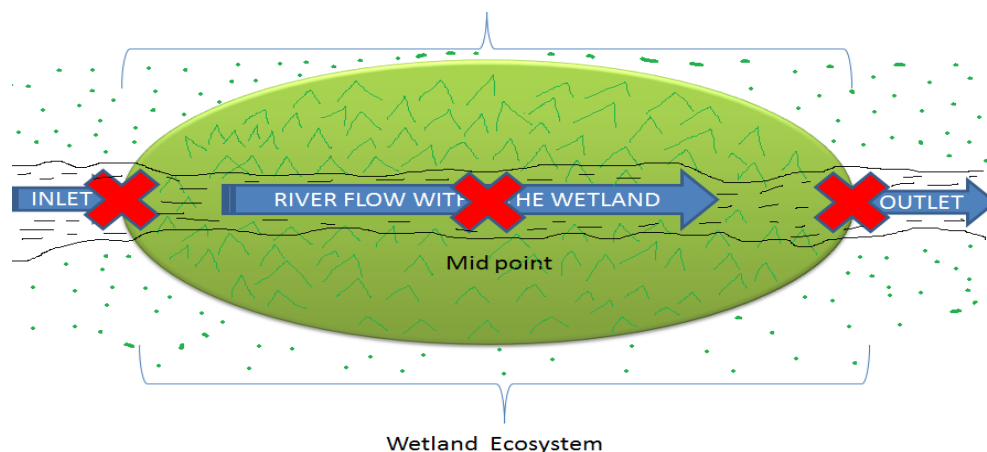


Figure 3.3: A sketch map of the wetland showing water sampling points

The water sampling sections were; up-stream (inlet) near Rumuruti–Nanyuki bridge where gauging station 5AC15 is located at latitude N00.256634° and longitude E036.539835°, Mid-stream (mid-point) before Ewaso Narok swamp characterized by increased agricultural activities lying at Latitude N00.258765° and longitude E036.543129°, Down-stream (outlet) just after Ewaso Narok swamp before Sosian ranch where water exits the Ewaso Narok wetland located at latitude N00.259352° and longitude E036.546620°. From each region, 10 water samples of 50mL were collected after every 5 minutes using a 50mL stainless steel mug at a depth of 1-30cm into a pre-cleaned 1000mL dark amber glass bottle. The collected water samples were composited to form one sample of volume 500 ml. The bottle containing water sample was properly sealed, labelled correctly before it was placed into an ice packed cool box. The sampling procedure was repeated for the rest of the water sampling points. Surface water was avoided during sampling so as not to collect oil and other contaminants. The pre-cleaned sample bottles were washed three (3) times with water from the exact sampling sites before taking the samples. Samples were stored at 4°C in ice-packed cool box. The samples were then transported to the ICIPE chemistry laboratory for samples pre-treatment and analysis. A total of 6 water samples were collected and analyzed for the dry and wet seasons.

3.6 Sample pre-treatment

3.6.1 Extraction and clean-up of pesticide residues in soil

Ten (10) g of soil sample was separately weighed into a clean 50ml falcon tube into which 10mL of water and 10mL of acetonitrile were added. The mixture was vigorously shaken for one minute and mixed using a vortex for 1 minute. QuEChERS salt pouch (Agilent

Technologies, USA) containing a mixture of 4g magnesium sulphate, 1g sodium chloride, 1g sodium citrate and 0.5g sodium hydrogencitrate sesquihydrate was added to the tubes to promote phase separation. The tube was shaken and mixed using a vortex for 1 minute and centrifuged for 5 min at 4, 000 round per minute (rpm). The upper organic layer (acetonitrile extract) containing pesticide residues was then transferred into a clean tube for sample clean-up procedure. The same extraction procedure was repeated for all the 90 soil samples collected during wet and dry seasons respectively. For the clean-up of the soil extracts, 3mL of the acetonitrile extract was transferred to a tube containing 900 mg anhydrous MgSO₄/150 mg PSA. The mixture was shaken for 30 seconds and centrifuged for 3 minutes at 3000 rpm. 1mL of the supernatant layer was transferred into a sample vial for LCMS/MS analysis. The same procedure for sample clean-up was replicated for all the 90 soil samples collected during the dry and wet seasons respectively.

3.6.2 Extraction and clean-up of pesticide residues in water

Ten (10) mL of acetonitrile was added into 10mL of each water sample in a 50 mL falcon centrifuge tube. The mixture was vigorously shaken and mixed using a vortex for 1 minute. QuEChERS salt containing 4g magnesium sulphate, 1g sodium chloride, 1g sodium citrate and 0.5g sodium hydrogencitrate sesquihydrate was added to the tubes to promote phase separation. The tubes were shaken and mixed using a vortex for 1 minute and centrifuged for 5 minutes at 4,000 rpm. The same procedure for pesticide extraction in water was replicated for all the 18 water sample collected during wet and dry seasons. For the clean-up of water extract, 3ml of the acetonitrile extract was transferred to a tube containing 900 mg anhydrous MgSO₄/ 150mg PSA. The mixture was shaken for 30 seconds and

centrifuged for 3 minutes at 3000 rpm. One (1) mL of the supernatant layer was then transferred into a sample vial for LCMS/MS analysis. The same procedure for sample clean-up was replicated for all the six water samples collected during dry season and wet season.

3.6.3 Extraction and clean-up of pesticide residues in vegetables

Ten (10) g of blended vegetable sample was placed in a 50mL falcon tube, 10ml of distilled water and 10mL acetonitrile containing 0.01% formic were then added. The mixture was stirred and vortexed for 1 minute. QuEChERS salt (Agilent Technologies, USA) containing 4g magnesium sulphate, 1g sodium chloride, 1g sodium citrate and 0.5g sodium hydrogencitrate sesquihydrate was added to the tubes to promote phase separation. The tube was shaken, vortexed for 1 minute and centrifuged for 5 minutes at 4,000 rpm. In sample clean-up, 1mL of the acetonitrile extract (supernatant layer) was transferred to a tube containing a mixture of 150mg anhydrous MgSO₄ /50mg PSA/50mg Graphitized Carbon Black (GCB) and the mixture shaken for 30 seconds. The mixture was then centrifuged for 3 minutes at 3000 rpm. The supernatant layer was finally transferred to a pre-cleaned sample vial for LCMS/MS analysis. The same sample clean-up procedure was replicated for both kales and tomatoes samples.

3.7 Preparation of stock standard solutions

Stock solution was prepared by weighing 1mg of pesticide standards (aldicarb, triadimefon, azoxystrobin, buprofezin, cyproconazole, bifenazate, chlorpyrifos, diazinon, fenprothrin, paclobutrazole, azinphos methyl, spiromamine, pyrimethanil and metalaxyl)

into a 1000 mL volumetric flasks and dissolving to the mark with acetonitrile to make a standard stock solutions of 1ppm (1000 µg/L). Five different concentrations for each pesticide that is (50, 100, 150, 200 and 250) µg/L were prepared from the stock solutions by serial dilution based on the dilution formula $C_1V_1 = C_2V_2$, where C is concentration and V is volume. The working standards were then kept in a refrigerator at 4°C.

3.8 Method validation

3.8.1 Accuracy (recovery) tests

Procedure and method validation involved the use of matrix spikes and the reference samples (soil, water, tomatoes and kales not spiked). The 10g of hydrated homogenized sample was spiked with 50 µg/L of the pesticide standards (analyte) and allowed to go through QuEChERS EN sample extraction and d-SPE clean-up procedures. Another 10g of the same sample (unspiked) was also weighed and allowed to go through QuEChERS EN sample extraction and d-SPE clean up procedure as the spiked sample. Extracts were analyzed using LCMS/MS under the same instrumental conditions. All the analysis were done in triplicates and the average recoveries calculated. The percentage recoveries were calculated using the equation 3.1. The same procedure was replicated for all the pesticide standards in all sample matrices of soil, water, tomatoes and kales.

$$\% \text{ Recovery} = \frac{\text{SSR} - \text{USR}}{\text{SSC}} \times 100 \quad \dots\dots\dots \text{Eq 3.1}$$

Where; SSR (spiked sample results), USR (unspiked sample results) and SSC (spiked standard concentration)

3.8.2 Linearity test and calibration curves

Linearity range was investigated by checking the concentration of the standard solutions in the range of 0.1-500 μ g/L. Calibration study was tested by spiking the working standard solutions of concentrations (50, 100, 150, 200 and 250) μ g/L into C₂H₃N containing NaCl solution. Calibration curves were obtained by plotting the peak areas versus concentration of each pesticide.

3.8.3 Precision (repeatability)

To test for precision, repeatability was performed at level of 50 μ g/L of the pesticides for all the sample matrices (soil, water, tomatoes and kales). The test was done in triplicates (n=3) and the mean percentage relative standard deviation calculated (%RSD). The relative standard deviation was less than 20% ($\leq 20\%$ RSD) as described by AlMulla (2016).

3.8.4 Selectivity and limits of detection (LOD)

The ability of LCMS/MS instrument to detect the concentration of the pesticide residue of interest from other responses of the interfering matrices in the sample was tested. The pesticide standards in acetonitrile at 50 μ g/L and the sample extract containing the pesticide (analyte) plus all the other potential interfering components were analyzed using LCMS/MS at the same conditions. The resolutions of the analyte peak from the nearest eluting peak was evaluated and the retention times of the eluting analytes were recorded. LOD is the minimum quantity of an analyte that can be distinguished from the absence of that substance (blank) at a stated confidence level. It is the concentration of an analyte in a sample that gives rise to a peak with a signal-noise (S/N) ratio of 3.3. Limits of detections

of the analytes were determined by replicate chromatographic runs (3 times) of the least concentration of the analytes standards and then multiplying standard deviation obtained by three (3). Equation 3.2 was used to calculate the LOD.

$$\text{LOD} = 3.3 \times \frac{\alpha}{S} \dots\dots\dots \text{Eq 3.2}$$

Where α (standard deviation of a replicate measurement) and S (slope of the calibration curve)

3.9 Data analysis

3.9.1 Questionnaire data analysis

The data obtained in the questionnaire were analyzed using Statistical Package Software for Social Sciences (SPSS) version 22. Non-parametric tests (Kruskal-Wallis and Man-Whitney) were performed at 95% confidence.

3.9.2 Laboratory data analysis

Data was analyzed using software for the Statistical Package for Social Sciences (SPSS) version 22. One way analysis of variance (ANOVA) and Student–Newman Keuls (SNK) multiple comparison method was used. The statistical significance tests were carried at 95% confidence level ($p < 0.05$). .

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The results of farmers' pesticide application and analysis of pesticide residue in soil, water, kales and tomatoes in Ewaso Narok wetland are presented and discussed in this chapter. The samples were collected during wet season (June, 2016) and dry season (August, 2016). Each of the samples (soil, water, tomatoes and kales) were collected in triplicate in each season from the study area and analyzed.

4.2 Farmers' awareness and knowledge on pesticide use

4.2.1 Farmers' socio-demographics

Table 4.1 shows Ewaso Narok wetland farmers' socio-demographics background based on education, age and gender.

Table 4.1: Percentage frequency on age and education levels of farmers

ITEM	Frequency (F)	Percentage (%)	
Education (N= 86)			
No formal education	4	4.7	
Primary (class 1-8)	25	29.1	
Secondary level (form 1-4 or A- level)	40	46.5	
Tertiary (colleges or university)	17	19.8	
Age (Years) (N= 86)		Gender	
≤30	Male	17	19.8
	Female	2	2.3
31-50	Male	48	55.8
	Female	6	7
>50	Male	10	11.6
	Female	3	3.5

Within all the three categories of age brackets (≤ 30 , 31-50 and >50) years, there were more males (87.2%) than females (12.8%). 62.8% farmers were between 31 to 50 years of age, 22.1% were 30 years and below while 15.1% were above 50 years old. Majority (66.3%) had gone through secondary to tertiary education, 29.1% had primary education (class 1-8), while 4.7% had no formal education. These results are comparable to 89.4 and 80% literacy levels reported in Lake Naivasha region by Tsimbiri *et al.* (2015) and in Tehran, Iran by Shafiee *et al.* (2012), respectively. The number of farmers with no formal education (4.7%) were however much lower than the 55% reported for a study done in Ethiopia by Mengistie *et al.* (2017). Adeola (2012) in a similar study done in Nigeria, categorized farmers age groups as 25-55 years and >55 years which constituted 92.2 and 7.8%, respectively. According to Adeola (2012), 93% of the farmers were male and 7% female with 63.3% having at least primary education while 12.5% had no formal education. The result of the current study revealed that 95.4% had at least primary education while 4.7% had no formal education.

4.2.2 Impact of farmers' socio-demographics on good pesticide use

The good pesticide use investigated included; knowledge of pesticide entry routes into the body system, use of personal protective clothing and equipments (PPEs), mixing of different pesticide chemicals before use, disposal of empty pesticide containers, reading of pesticide labels before use and the use of alternative pest control methods. Table 4.2 shows the impact of the farmers' socio-demographic on pesticide use. Farmers' Socio-demographics (age, education and gender) had significant influence on a number of pesticide use ($p < 0.05$).

Table 4.2: Impacts of farmers' socio-demographics on good pesticide use

Knowledge of good pesticide use	p-value		
	Kruskal-Wallis test		Mann-Whitney test
	Age	Education	Gender
Mixing of different pesticide products	0.211	0.490	0.519
Use of PPEs	0.007	0.005	0.132
Pesticide storage before and after use	0.757	0.074	0.007
Disposal methods for pesticide containers	0.622	0.022	0.140
Use of pesticide containers for other purposes	0.333	0.597	0.003
Risk of exposure during pesticide use	0.004	0.031	0.248
Routes of pesticide entry into the body	<0.001	0.007	0.029
Practices of alternative pests control mechanisms	1.000	1.000	1.000
Reading of pesticide labels before use	<0.001	0.003	0.482
Observing pesticide safety intervals	0.273	0.009	0.208

$\alpha=0.05$

This included the ability to rate risks of pesticide exposure which was significantly dependent on farmers' age ($p=0.04$) and education ($p=0.031$), knowledge of the routes of pesticide entry into the body was significantly dependent on age ($p<0.001$), education ($p=0.007$) and gender ($p=0.029$). Similarly, decision to use personal protective clothing during pesticide application was significantly dependent on farmers' age ($p=0.007$) and education ($p=0.005$), pesticide storage before and after use and use of pesticide empty containers for other farm use were significantly dependent of the gender ($p=0.007$) and ($p=0.003$), respectively. Education was significantly important in influencing the disposal methods for pesticide containers ($p=0.022$), observing pesticide safety intervals ($p=0.009$), while reading of pesticide labels before pesticide use was significantly dependent on age ($p<0.001$) and education ($p=0.003$). A study conducted by Macharia (2015) in Kenya, concluded that farmers with higher education are more knowledgeable and have a better understanding of the dangers posed by pesticides. However, a study done in Zimbabwe by

Maumbe and Swinton (2003) found that farmers with higher knowledge were more likely to report more health symptoms related to poor use of pesticide.

However, the study revealed that decision to mix different pesticide products for use was not significantly dependent on farmers' age ($p=0.211$), education ($p=0.490$) and gender ($p=0.519$). Pesticide storage before and after use and use of pesticide containers for other purposes was not significantly dependent on farmers age and gender ($p>0.05$). The choice of disposal methods for pesticide containers, observation of safety intervals and practicing alternative pest control mechanisms were significantly independent on farmers' age and education ($p>0.05$).

Gender did not significantly influence knowledge on rating risk of pesticide exposure ($p=0.248$), use of personal protective clothing ($p=0.132$), use of alternative pests control methods ($p=1.000$), disposal for pesticide container ($p=0.140$), observing pesticide safety intervals ($p=0.208$) and reading of pesticide labels before use ($p=0.482$). This was contrary to a study done by Atreya (2007) that found that gender had significant influence on household decision on pesticide ($p=0.016$), reading and understanding pesticide labels ($p<0.001$), prior knowledge on safety measures ($p=0.016$) and awareness of pesticide labels and use of personal protective clothing ($p<0.001$). In a study done in India by Kumari and Reddy (2013), age and gender had no significant influence on knowledge and practice on safety use of pesticide.

4.2.3 Knowledge of pesticide exposure routes

Table 4.3 below shows farmers' knowledge on pesticide exposure routes in the body based on age, Education and gender.

Table 4.3: Knowledge on pesticide entry routes into the body

Variables	Yes (%)	No (%)
Age		
Less than 30 years	78.9	21.1
31-50 years	90.7	9.3
Over 50 years	38.3	61.5
Education		
No formal education	30	70
Primary education	46.7	53.3
Secondary education	84	16
Tertiary education	100	0
Gender		
Male	61.6	38.4
Female	18.6	81.4
Pesticide entry routes		
Inhalation	17	83
Skin/ eye contact	15	85
Ingestion	34	66

The majority (90.7%) of the farmers of age 31-50 years, 38.3% aged over 50 years and 78.9% aged less than 30 years correctly identified different routes. The routes mentioned were inhalation of vapours, dusts or mists (17%), skin/ eye contact with residues (15%), or ingestion (34%). Most (80.2%) of farmers with better understanding of pesticide entry routes had primary to tertiary education levels. Farmers with better understanding on the pesticide entry routes into the body, 46.7% had primary, 84% secondary and 100% tertiary level of education. Ninety three (93%) of farmers with no formal education had their information on pesticide entry routes limited to ingestion of pesticide at high concentrations (100%). Male farmers (61.6%) knew pesticide entry routes than female

farmers (18.6%). Female farmers did not take part in pesticide mixing and spraying in the farms. Jallow *et al.* (2017) in a study done in Kuwait, dermal (54%), inhalation (86%) and ingestion (42%) were the most common routes of pesticide exposures listed while 14% had no knowledge of how pesticides enters the human body. This finding concurs with finding of a study done by Abong'o *et al.* (2014) in River Nyando catchment in Kenya and Mengistie *et al.* (2015) in Ethiopian Central Rift Valley in which out of the farmers who did not know of the pesticide routes of exposure into human body, more than 70% had no formal education.

4.2.4 Use of personal protective clothing and equipments (PPEs)

Table 4.4 shows personal protective equipment (PPEs) that were used by farmers and the reasons for their incomplete use.

Table 4.4: Use of PPEs and factors influencing their use

Variables		Frequency (n)	Percentage (%)
PPEs	Overall	21	24.4
	Gloves	1	1.2
	Face masks	0	0
	Eye glasses	2	2.3
	Boots/shoes	18	20.9
	Long pants	3	3.5
	Long sleeved shirts	5	5.8
	Respirators	0	0
Age	<30 years	9	47.4
	31-50	25	46.3
	>50 years	0	0
Education	Non- formal	2	50
	Primary	8	32
	Secondary	20	50
	Tertiary	4	23.5
Gender	Male	25	33.3
	Female	9	81.8

The types of PPEs that were used by farmers during pesticide application included; overall (24.4%), hand gloves (1.2%), eye glasses (2.3%), boot and shoes (20.9%), long pants (3.5%), and long sleeved shirts (5.8%). Face mask and respirators were not used by any of the farmers. Though none of the farmers reported to be using complete PPEs, 47.4% of farmers aged less than 30 years and 46.3% aged 31-50 years reported the use of at least PPEs during pesticide application. All farmers above 50 years of age (100%) did not use any PPE, instead they preferred using their home cloths while 50% and 23.5% of farmers with secondary and tertiary education, respectively, reported the use of at least one type of PPE. Fifty (50%) of the farmers with no formal education reported to be using overalls. However, 68% of the farmers with primary education did not use any PPEs during pesticide use. 33.3% male farmers used at least one PPE while 81.8% of female farmers reported that they wore at least one PPEs when entering freshly sprayed farms. However, Farmers including those who did not used PPEs reported discomfort (90.7%), inaccessibility (79.1%) and high cost (88.4%) as their reasons for not using appropriate or complete PPEs. In a study done by Mengistie *et al.* (2017) in Ethiopia, 19% of farmers used incomplete PPEs which did not adequately cover their body, and 81% wore their normal home clothes during spraying.

Failure by farmers to use proper PPEs such as goggles and gloves during pesticide application presented a risk of exposure to the farmers of Ewaso Narok wetland since the nose and eyes serve as routes of exposure as documented by Mekonnen and Agonafir (2002). Majority of farmers with formal education ignored basic pesticide safety rules like wearing appropriate PPEs and observing basic hygiene. However, there was a general poor

pesticide use among farmers with no formal education and this was linked to their lack of knowledge on safe pesticide use. Most of the farmers who were using at least one personal protective clothing had at least secondary education and above. Education literacy is important as it contribute to good pesticide use among farmers (Wandiga, 2001; Yassin *et al.*, 2002; Bond *et al.*, 2007).

4.2.5 Mixing different pesticide products before spraying

Table 4.5 shows some of the reasons why farmers mixed and applied pesticides as ‘cocktail’ on vegetables based on gender, education and age.

Table 4.5: Reasons for mixing pesticides before use

Farmers’ socio-demographics	Reasons for mixing different pesticide products before application				
	unsure about the quality of the individual pesticide	uncertain about the effectiveness of one particular pesticide	advice from agricultural officers	advice from retailers/suppliers	advice from fellow farmers
Gender					
Male	32%	34.7%	12%	8%	13.3%
Female	63.6%	36.4%	0	0	0
Education					
Non- formal	75%	100%	50%	75%	75%
Primary	96%	44%	20%	4%	8%
Secondary	0	15%	5%	2.5%	2.5%
Tertiary	11.8%	17.6%	0	0	0
Age					
<30 years					
31-50 years	42.1%	31.6%	10.5%	15.8%	0
>50 years	42.6%	33.3%	11.1%	1.9%	11.1%
	38.5%	46.2%	7.7%	7.7%	0

Thirty three (38.4%) of farmers had mixed different pesticides and sprayed them as a cocktail. Reasons for mixing pesticide products included; uncertainty about quality of pesticide product (34.9%), doubts about pesticide effectiveness towards a particular pest (41.9%), advice from some retailers and suppliers (5.8%) and farmers (7.0%). Mengistie *et al.* (2017) in a study done in Ethiopia, 87% of farmers mixed two pesticides before application while 13% used both cocktail and single spray. Farmers preferred to mix different pesticides and apply the chemicals as a ‘cocktail’ believing that the mixture was more effective than applying single pesticide product. Those who embraced this practice reported cheap labour and the need to save on time as the main reasons for mixing pesticides and spraying them as a cocktail.

Majority (96%) of the farmers had only one tank in their farms where all pesticides were mixed before being applied, this was done without cleaning off the previous pesticides. It was observed that mixing of different pesticides and using them as cocktail was common among farmers with over 10 years farming experience a practice linked to lack of training on pesticide use and failure to read pesticide labels before use. Pesticide labels do not contain information about mixing of pesticides. Thus, mixing different pesticide products and applying the pesticide as a cocktail can present adverse effect on human health and environment (Halimatunsadiah *et al.*, 2016). The efficacy of the individual pesticides could also be compromised significantly as a results of the chemical ingredients incompatibility and possible chemical reaction (Hamby *et al.*, 2015).

4.2.6 Disposal of empty pesticide containers

Levels of education was the only key variable that was found to significantly influence the choice of disposal methods for empty pesticide containers ($p=0.022$). About 58% and 62% of farmers with secondary and tertiary education levels, respectively, had their pesticide empty containers kept in dug pits before being burned in the open fields or buried within the farm. Majority (98%) farmers having primary education and below had their wastes thrown all over the farms. 97% had not received training on pesticide use, hence they had no specific method for waste disposal. As a result, empty pesticide containers were either thrown away in the fields (28.6%) or burnt (38.1%) while 20% were re-using pesticide containers to perform other farm activities such as watering seedlings. Education literacy is important in enabling good pesticide use among farmers (Wandiga, 2001; Yassin *et al.*, 2002; Bond *et al.*, 2007). However, this was not reflected practically among the farmers who had more knowledge on pesticide safety use.

Burning and burying of containers was done without taking any safety precautions. Pesticide containers were buried without protecting the wastes from possible leaching into the underground water. Burning was done in the open further exposing the nearby workers to toxic fumes. Jallow *et al.* (2017) in a study done in Kuwait explained that unsafe pesticide waste disposal methods was a recipe to the increased pesticide levels in agricultural produce, water and soil contamination. Unsafe pesticide containers disposal methods by farmers has continued to raise lots of safety concerns (Shafiee *et al.*, 2012). Re-use of pesticide containers for other domestic purposes as observed during this study could aggravate the situation of human pesticide exposures within Ewaso Narok wetland.

4.2.7 Reading of the pesticide labels and observation of safety intervals

Table 4.6 shows farmers socio-demographic characteristics that influenced farmers' knowledge and ability to read the instruction on pesticide labels before use

Table 4.6: The impact of age, education and gender on reading of pesticide labels

Variables		Yes	No
Age (years)	<30	68.4	31.6
	31-50	81.4	18.6
	Over 50	61.5	38.5
Education	Non-formal	25	75.0
	Primary	40	60.0
	Secondary	92.0	8.0
	Tertiary	100	0.0
Gender	Male	73.3	26.7
	Female	87.5	12.5

Farmers (68.4%) aged 30 years and below, 31-50 years (81.4%) and above 50 years (61.5%) read pesticide labels before use. 92 and 100% of farmers with secondary and tertiary education levels, respectively, read pesticide labels before use. However, 60% of farmers with primary and 75% of farmers with no formal education did not read the pesticide labels before use. Some of the reasons given for not reading pesticides labels before use included; low level of education (60%) making it difficult to read and understand the meaning of the information on the labels, small and unfriendly fonts used (30%), while 10% did not care to read instructions on the pesticide labels before use. Majority of farmers with secondary (92%) and tertiary (100%) education levels had knowledge of two pesticide safety intervals such as re-entry interval (REI) and pre-harvest interval (PHI). However, it was observed that the intervals were not observed strictly as the vegetables especially tomatoes were sold whenever the buyer was available. Most

farmers (85%) having primary education and below did not observe the safety intervals. Similarly, Jallow *et al.* (2017) in their study reported 70% of farmers not reading or following instructions on pesticide labels. Halimatunsadiyah *et al.* (2016) states that failure to comply with the pesticide safety intervals such as PHI is a recipe for the possible consumption of vegetables having pesticide residues above the recommended maximum residues limits (MRLs). Furthermore, Inonda *et al.* (2015) reported that adherence to the recommended pesticide safety intervals such as PHI resulted in 99% reduction of pesticide residues concentrations in vegetables. To minimize the effects of pests and diseases on farm crops, farmers embraced the alternative pest control methods such as rotational farming (57%) and intercropping (43%). (Appendix 4).

4.2.8 Farmers' knowledge on pesticide products used on vegetables

Most farmers (89%) were able to mention names of pesticide products they were using at the time of the study. The table of the common pesticide metabolites and WHO classification is presented. (Appendix 5). This was similar to the 92% of farmers who knew the names of pesticides they were using in a research conducted by Mengistie *et al.* (2017) in Ethiopia. The possible uncontrolled pesticides accessibility by farmers in the study area is a risk to both human health and the entire wetland ecosystem. Some of the pesticide classified as extremely or highly toxic to both human and environment (aldicarb and azinphos methyl) were found to be in use (WHO, 2004). Chlorpyrifos, fenprothrin and diazinon are classified as highly hazardous pesticides (HHPs) as a result, their use is restricted and not allowed on vegetables (Grube *et al.*, 2011 and PCPB, 2017).

4.2.9 Farmers' pesticide storage practices

Figure 4.1 shows farmers' storage facilities for the pesticide still in use and new pesticides.

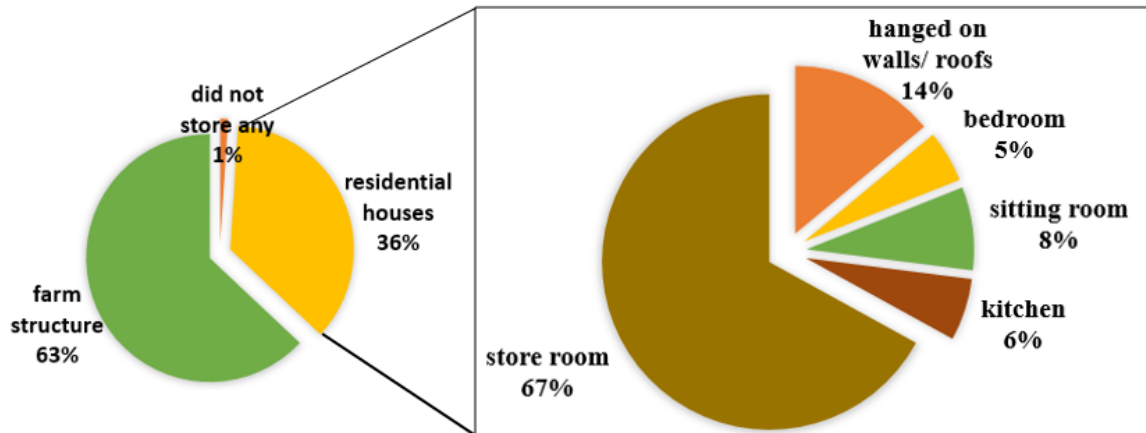


Figure 4.1: Farmers' pesticide storage practices

Main pesticide storage areas were farm structures (63%) and residential houses (36%), only 1% of the farmers reported not to store any pesticide. Farm tools such as knapsack sprayers and water pumps were stored in the farm structures. Similarly, some farmers lived, slept and even cooked in the farm structures sometimes with their families increasing the risk of exposure. In the residential houses, 67% stored pesticides in store-rooms, 14% hanged them on the roof and walls, 5% had pesticides in their bedrooms or under their beds, 8% had pesticides kept in their living rooms while 6% had pesticides kept in the kitchen. Store rooms, wall or roof storage are accessible areas to the family members including children presenting the risks of accidental or suicidal attempts through pesticide poisoning. In a study done in Kuwait by Jallow *et al.* (2017), farmers stored their pesticides in open shade (34%), open field (30%), animal house (15%), inside refrigerator with other food items (8%) and living areas (20%). Mengistie *et al.* (2017) in a study done in Ethiopia, main pesticide storage areas included own house (32%) and farm structures (57%). Tsimbiri *et*

al. (2015) in a study done in Lake Naivasha asserts that no part of the population is completely safe from the effects of pesticides whether from intended nor un-intended exposure due to poor pesticide storage. The most vulnerable part of this population are farmers and their family members due to poor storage at home and in farm structures.

4.2.10 Pesticide related acute poisoning symptoms on farmers

Table 4.7 shows the examples of the common acute pesticide poisoning symptoms reported by farmers 24 hours after pesticide application.

Table 4.7: Acute pesticide poisoning symptoms reported by farmworkers

Symptoms	Frequency (f)	Percentage (%)
Excessive sweating	2	2
Hand tremor	3	4
Convulsion staggering	1	1
Nausea / vomiting	1	1
Narrow pupils/ miosis	6	7
Blurred vision	3	4
Headache	40	47
Dizziness	17	20
Irregular heartbeat	2	2
Skin rushes	9	11
Sleeplessness/ insomnia	2	2

The study revealed that headache (47%), dizziness (20%) and skin rushes (11%) were the main problems farmers associated with pesticide use. This could be related to the inadequate use of PPEs such as gloves, face masks, overall and failure to used respiratory equipment. As a result, pesticide inhalation and direct skin contact were the main routes of exposure among farmers in the study area. Shafiee *et al.* (2012) in a study done in Tehran, reported dizziness (57.1%) and cough (44.3%) as the main pesticide poisoning symptoms

linked to failure by farmers to use proper PPEs. This was emphasized by Jallow *et al.* (2017) in a study done in Kuwait, where headache (82%), dizziness (41%), nausea (49%) and skin problem (58%) were the main symptoms of acute pesticide poisoning among farmers after pesticide use.

4.2.11 Knowledge of common vegetables pests and diseases

Figure 4.2 shows some of the common pests that were listed by farmers to be affecting vegetable production in Ewaso Narok wetland during the study.

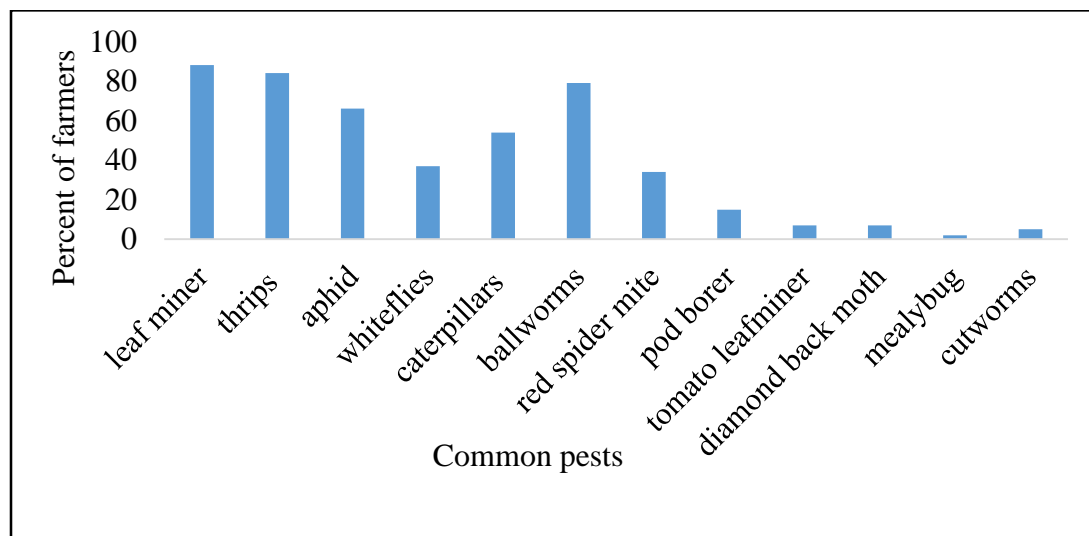


Figure 4.2: Common pests listed as a threat to tomatoes and kales production

Leaf miner (88%) and thrips (84%) were the most common vegetable pests listed by farmers. However the mealybug (2%) and cutworms (5%) were least reported. Omolo, (2011) list the common horticultural pests mentioned by farmers during his study in the Rift Valley and Central Kenya as thrips (19%), aphid (23%) and mealy bugs (23%) among others. Halimatunsadiyah *et al.* (2016) and Moncada (2001) reported several insects and pests namely cutworms, thrips, aphids, caterpillars, leafminer and diamond back moth as

having been mentioned by farmers during the studies. Figure 4.3 shows a list of the common fungal disease that affected vegetable production in Ewaso Narok wetland.

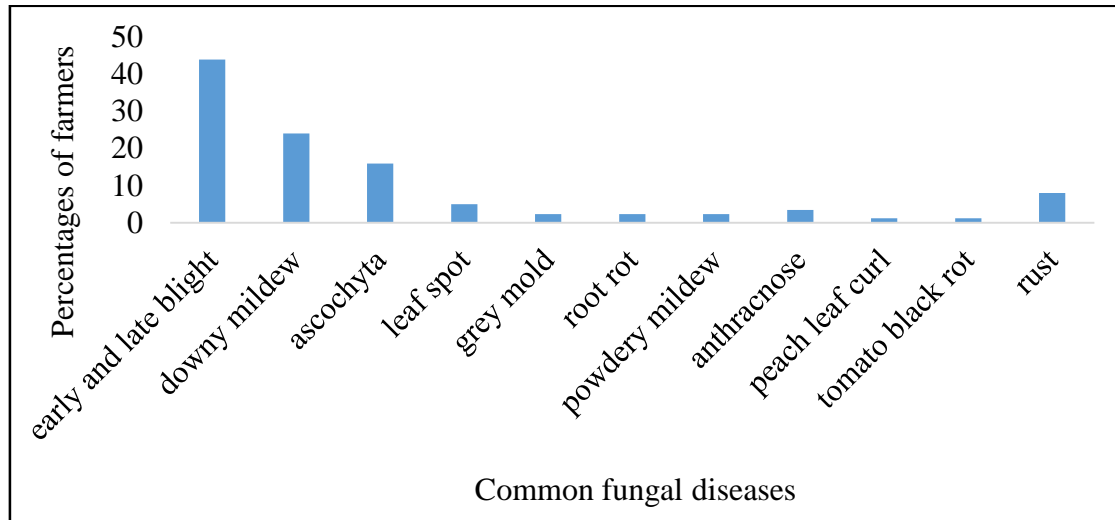


Figure 4.3: Common fungal diseases listed as a threat to tomato and kales

All farmers (100%) listed some of the diseases that normally affect their tomato farms. Early blight and late blight were mentioned by most farmers (43.9%) while powdery mildew, root rot and grey mold were mentioned by 2.3% of farmers. Insecticides (51%) and fungicides (42%) were the most widely used pesticides in the tomato and kales farms. However, few farmers reported to have used acaricides (4%), miticide (1%) on their tomatoes. Herbicides were not used since the weeds were hand-plucked by farmworkers. The list of common pesticide metabolites found in pesticide products reported by farmers to be in use at the time of the survey is provided. (Appendix 5).

Knowing the type of pests/ disease is important to the farmer as it helps select the type of insecticide to be acquired and used. In Ewaso Narok wetland, some farmers (34%) kept

referring to the pests or diseases in Swahili language as *dudu* or *magonjwa*. This was evident when some farmers reported *tuta absoluta* as a new fungal disease instead of a pest pointing to inadequate knowledge and education on pesticide use. A study done by Moncada (2001) in Lake Naivasha, Kenya, powdery mildew, rust and stem borer as the fungal diseases that affected vegetable production. Most pesticides are very specific, thus may not be useful when applied on the crops for the purpose of controlling pest/ disease that it is not meant for. The choice of pesticide used in the crop field should be largely influenced by the type of pests and diseases in the farm. Ability to identify the type of pests or fungal diseases in the farms is important in reducing possible misuse of pesticides. During the survey, farmers with at least secondary school education were reported to apply pesticides in their farms only when there is pest (s) and disease (s) attack in their farms or neighboring farms.

4.2.12 Training and awareness of farmers on safe pesticide use

Majority (97%) of farmers had not received any formal training on safe pesticide use (Appendix 4). World Health Organization (WHO) and Agricultural Food Organization (FAO) recommends that any person handling pesticides must be trained on safe pesticide use (FAO/WHO, 2014). Millard *et al.* (2004) in a study done among Michigan Migrant farmworkers, emphasized on the importance of both formal and informal training in the enhancement of farmers' knowledge on pesticide safety. This lack of training contributed to the poor use of pesticide in Ewaso Narok wetland. Inadequate use of PPEs, poor pesticide disposal mechanism, wrong spraying equipments, mixing of different pesticides, failure to observe pesticide safety intervals are some of the poor pesticide use that were

linked to lack of proper training. Lack of awareness on the importance of complying to safe pesticide use, led to farmers reluctant to put the right knowledge into practical use (Mekonnen and Agonafir, 2002; Jallow *et al.*, 2017). Some farmers relied on pesticide vendors and agricultural extension officers where they got information on safe pesticides use. This could have contributed to possible poor pesticide use as some of the vendors were not experts on pesticide handling.

Farmers' levels of knowledge on pesticide use is a key determinant on the level of safety of pesticide use. Mengistie *et al.* (2015) and Yassin *et al.* (2002) in studies done in Ethiopia and Gaza Strip, respectively, linked adequate knowledge on the impact of pesticide on human health and environment to improved pesticide use. However, Ouédraogo *et al.* (2011) linked inadequate knowledge on the potential risks of pesticides on human health and environment among Burkina Faso pesticide sprayers to the high level of illiteracy which stood at 80%. It is evident that lower levels of education and lack of awareness and training of the farmers on safe pesticide use was a major contributor to the unsafe use. The study revealed that farmers were using uncalibrated containers for measuring pesticide, as a result, it was difficult to ascertain if the right quantity of pesticide and water are used during pesticide mixing.

Application of wrong pesticide quantity was therefore possible leading to either overdose or under dose (Eshun *et al.*, 2011; Lozowicka *et al.*, 2015). Risk of pests and diseases developing resistance to the pesticide due to under-dose is a major threat to horticultural production while overdose could lead to high residue levels in food and environment. The

use of manual knapsack sprayers are not considered safe since they are prone to leakages. This may expose the sprayers to pesticide poisoning through skin contact and even inhalation. As observed during the study, most of the knapsack sprayers available for use in the farms were worn out mostly as a result of wear and tear. This was discovered through the observed leakages, poor atomization through the nozzles and nozzle blockage.

4.3 Pesticide residue levels in soil, water, tomatoes and kales

4.3.1 Accuracy (recovery) tests

Table 4.8 shows the percentage recoveries and standard deviations error for the pesticides. Standard of 50µg/L was spiked in soil, water, kales and tomatoes samples (n=3).

Table 4.8: Mean percentage recoveries (\pm SE) of pesticide standards

Pesticide	Soil	Water	Kales	Tomatoes
Aldicarb	90.1 \pm 5.2	83.1 \pm 3.5	70.7 \pm 3.6	84.8 \pm 3.8
Triadimefon	92.1 \pm 7.2	78.1 \pm 2.6	79.4 \pm 7.8	79.3 \pm 7.8
Azoxystrobin	97.5 \pm 2.7	90.5 \pm 7.2	82.0 \pm 3.5	80.0 \pm 4.3
Chlorpyrifos	96.2 \pm 3.7	93.3 \pm 4.3	89.1 \pm 2.2	85.1 \pm 3.7
Buprofezin	93.4 \pm 6.2	85.1 \pm 9.2	70.2 \pm 0.6	77.1 \pm 1.2
Cyproconazole I	95.3 \pm 4.2	78.0 \pm 5.7	86.1 \pm 2.6	90.1 \pm 7.2
Cyproconazole II	97.1 \pm 2.0	81.2 \pm 3.2	78.9 \pm 1.8	80.2 \pm 4.2
Fenpropathrin	96.3 \pm 3.8	90.9 \pm 9.0	75.4 \pm 2.9	75.0 \pm 4.0
Paclobutrazole	93.1 \pm 6.9	94.6 \pm 5.1	88.0 \pm 2.5	87.5 \pm 1.6
Pyrimethanil	83.5 \pm 2.6	73.6 \pm 3.2	72.0 \pm 0.5	92.0 \pm 0.8
Metalaxyl	94.7 \pm 5.3	77.8 \pm 7.5	70.1 \pm 1.7	76.3 \pm 1.4
Diazinon	94.1 \pm 5.7	91.8 \pm 4.2	78.3 \pm 4.0	78.5 \pm 5.2
Bifenazate	94.4 \pm 5.0	82.7 \pm 1.9	76.3 \pm 2.2	76.0 \pm 0.9
Azinphos methyl	92.4 \pm 4.4	96.0 \pm 3.8	70.5 \pm 2.6	82.1 \pm 1.7
Spiroxamine	92.8 \pm 4.7	79.3 \pm 5.3	79.0 \pm 2.9	89.0 \pm 2.3

^a values are means \pm SE of three replicates measurements (n=3).

These values were comparable to the recoveries reported for DI-SPME (70-120%) (Sapahin *et al.*, 2014), modified QuEChERS (70-120%) Carneiro *et al.* (2013) and solvent extraction (75-125%) Njoroge (2014). The percent recoveries showed that QuEChERS method and UHPLC coupled by tandem mass spectrometry (MS/MS) technique are reliable, accurate and suitable methods for simultaneous determination of the 15 pesticide residues in soil, water, kales and tomatoes respectively.

4.3.2 Linearity and calibration tests

Table 4.9 shows the linear equations and regression coefficients (r^2) obtained for this study.

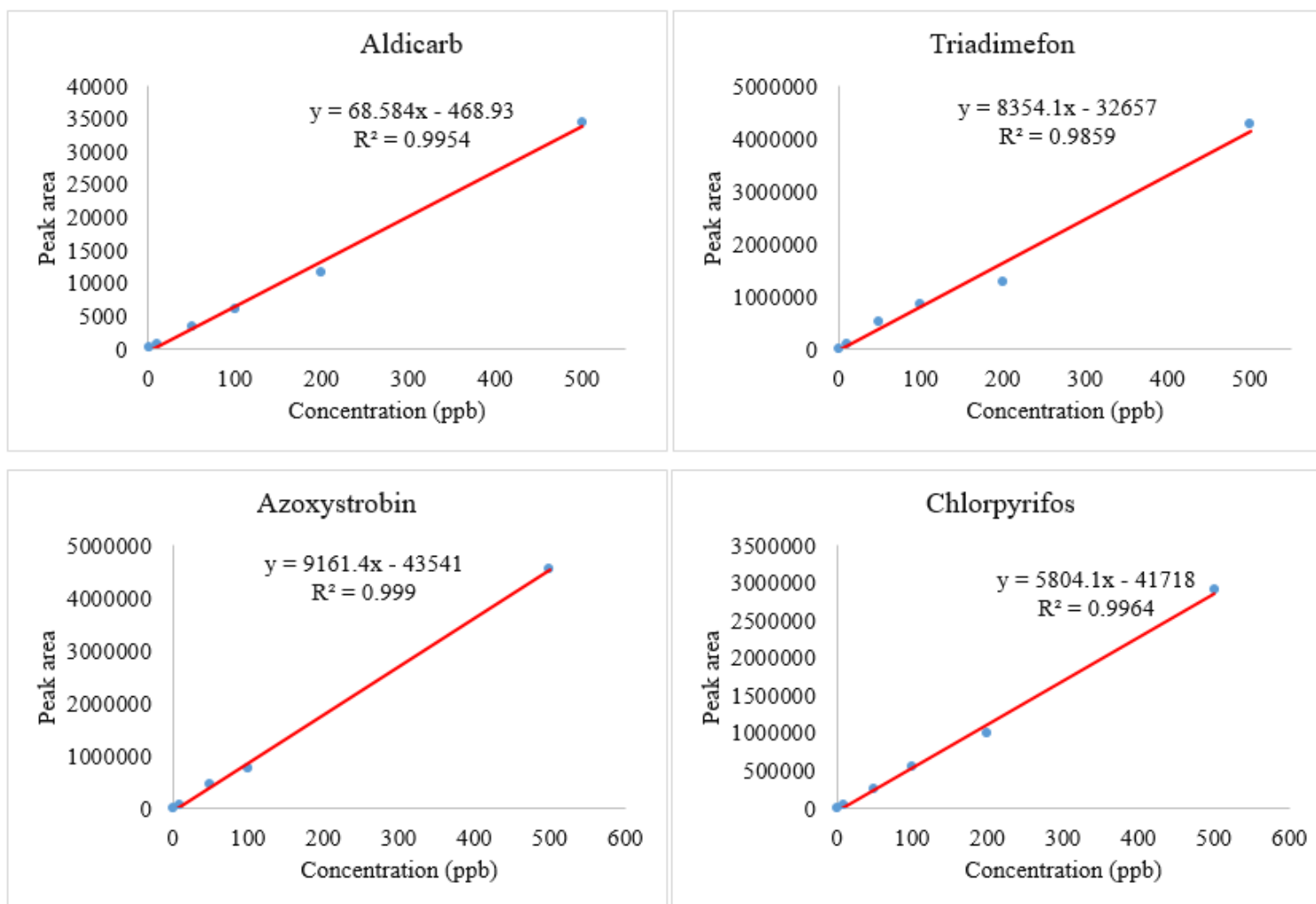
Table 4.9: Linear equations, regression coefficients (r^2) and LOD

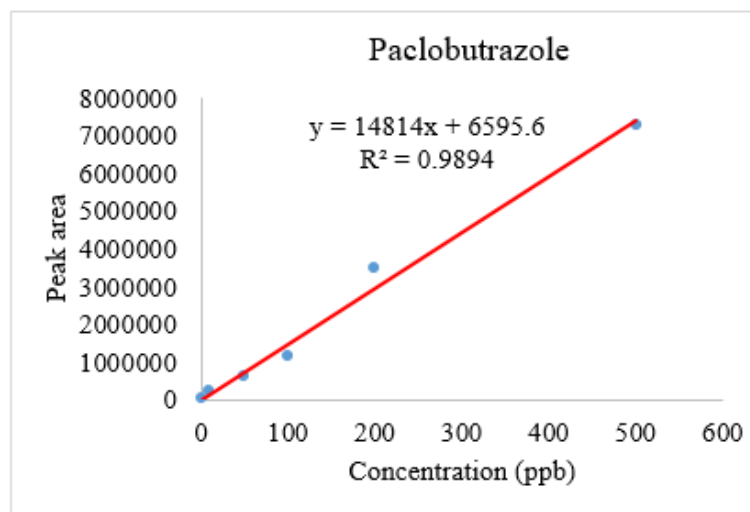
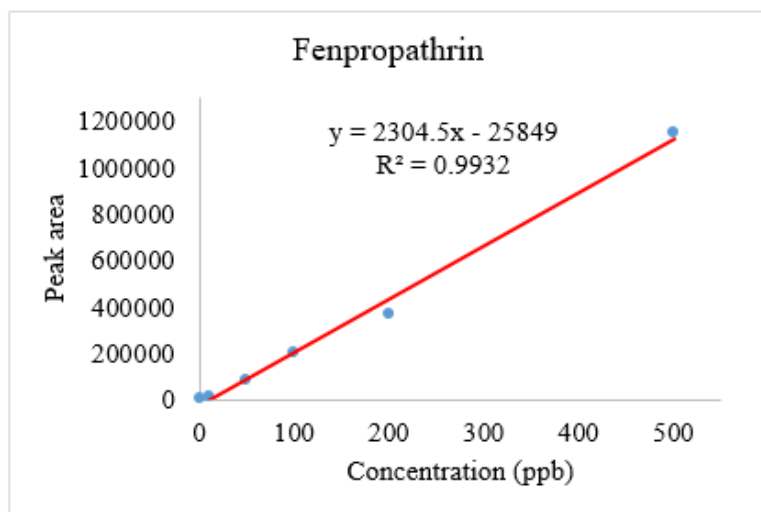
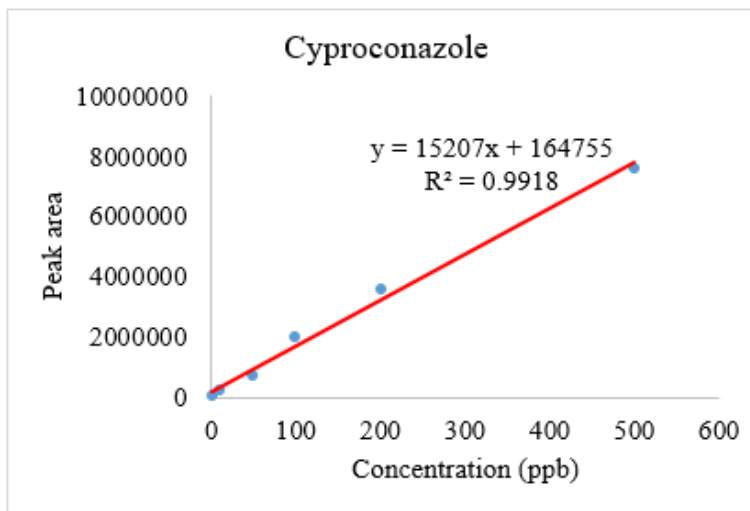
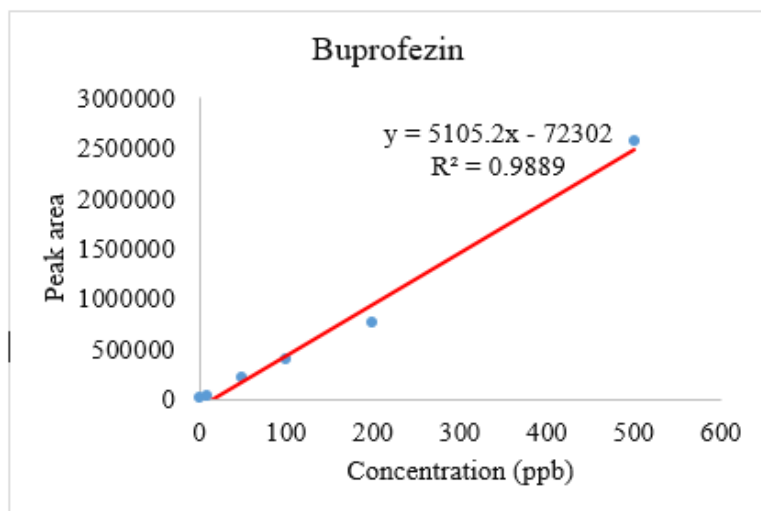
Pesticides	Regression equation (y)	Regression coefficient (r^2)	LOD $\mu\text{g/L}$
Aldicarb	$68.584x - 468.93$	0.9954	0.214
Triadimefon	$8354.1x - 32657$	0.9859	0.158
Azoxystrobin	$9161.4x - 43541$	0.9990	<0.1
Chlorpyrifos	$5804.1x - 41718$	0.9964	<0.1
Buprofezin	$5105.2x - 72302$	0.9889	<0.1
Cyproconazole I	$15207x - 164755$	0.9918	<0.1
Cyproconazole II	$7357x + 142402$	0.9985	<0.1
Fenpropathrin	$2304.5x - 25849$	0.9932	0.237
Paclobutrazole	$14814x + 6595.6$	0.9894	<0.1
Pyrimethanil	$5196.7x + 93820$	0.9906	0.24
Metalaxyl	$11528x + 21508$	0.9610	<0.1
Diazinon	$1554x - 1328.5$	0.9975	<0.1
Bifenazate	$2048.4x + 12246$	0.9753	<0.1
Azinphos methyl	$2300.6x - 24369$	0.9930	<0.1
Spiroxamine	$2304.5x - 25849$	0.9932	0.241

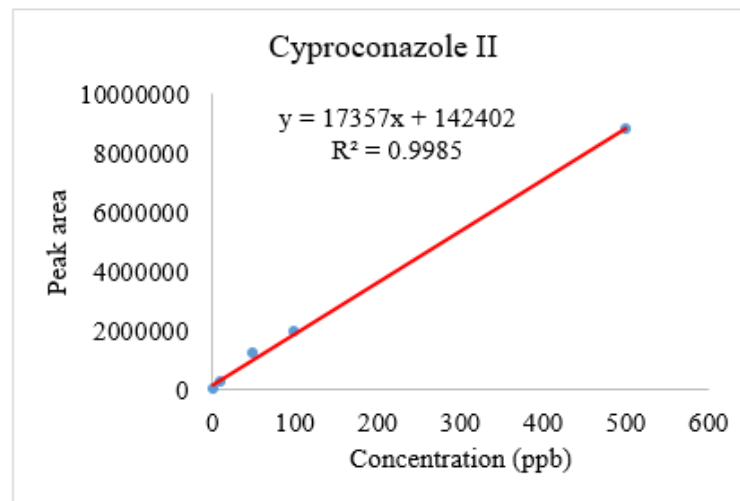
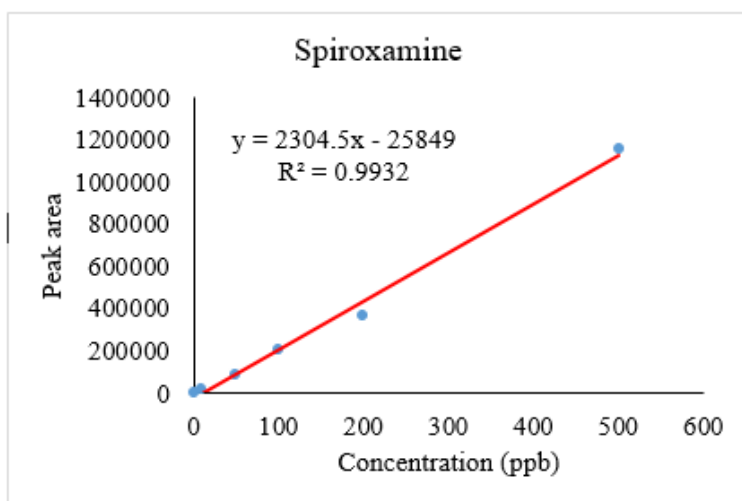
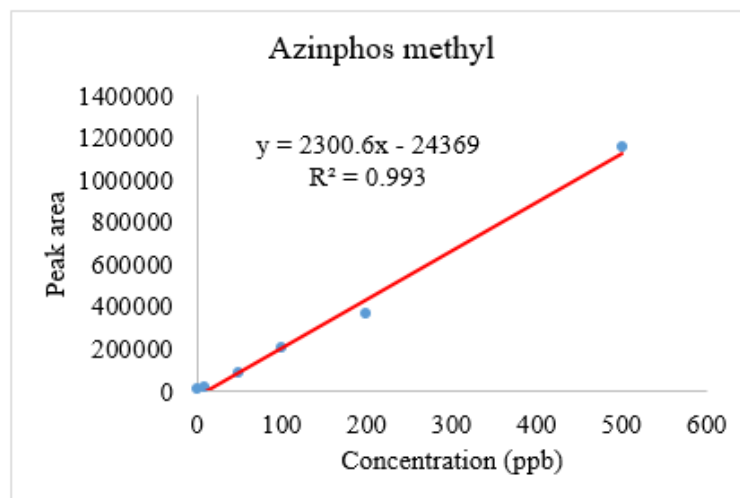
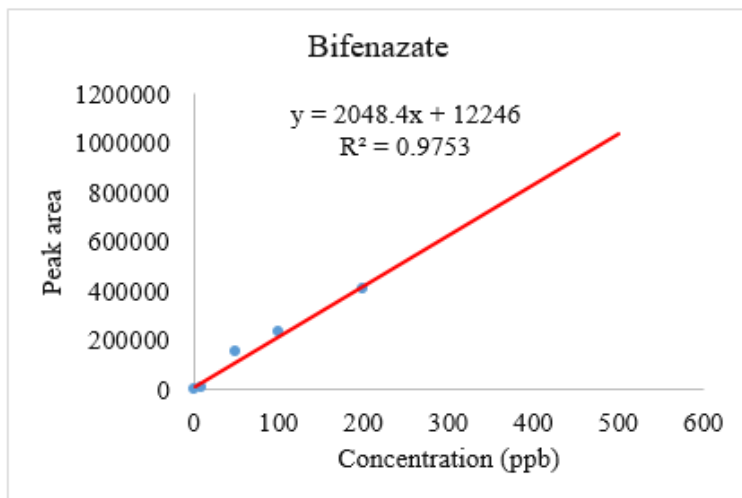
Regression coefficient (r^2) ranged between 0.961 and 0.999 with most analyte giving a regression coefficient of determination ($r^2 \geq 0.99$) which were adequate for residues analysis as reported by Rizzetti *et al.* (2016).

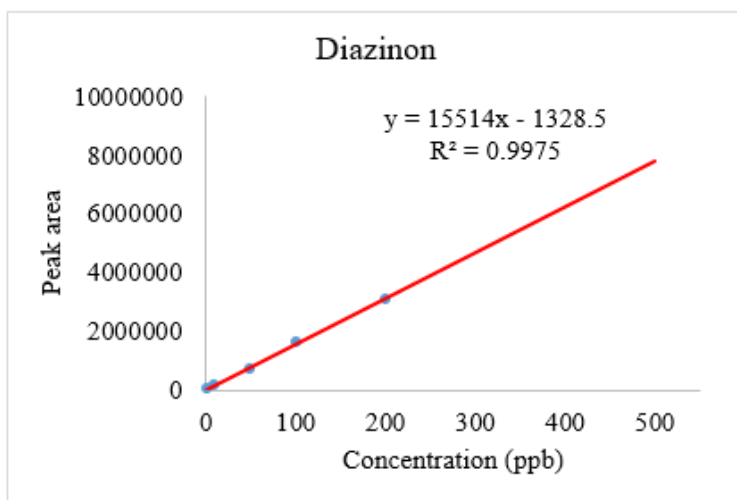
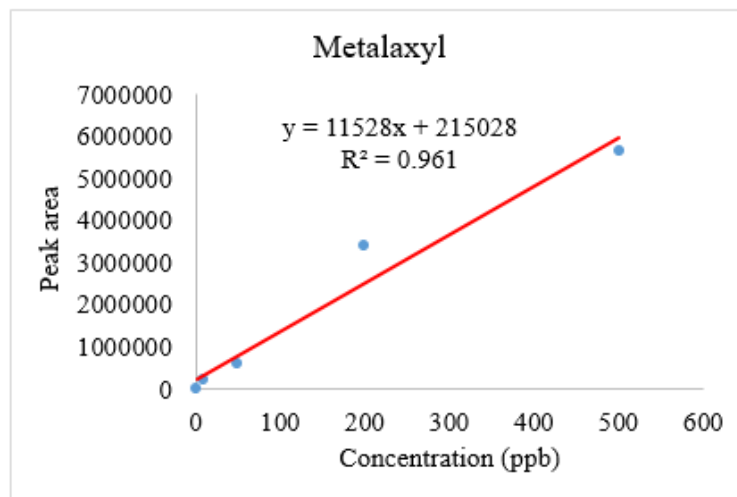
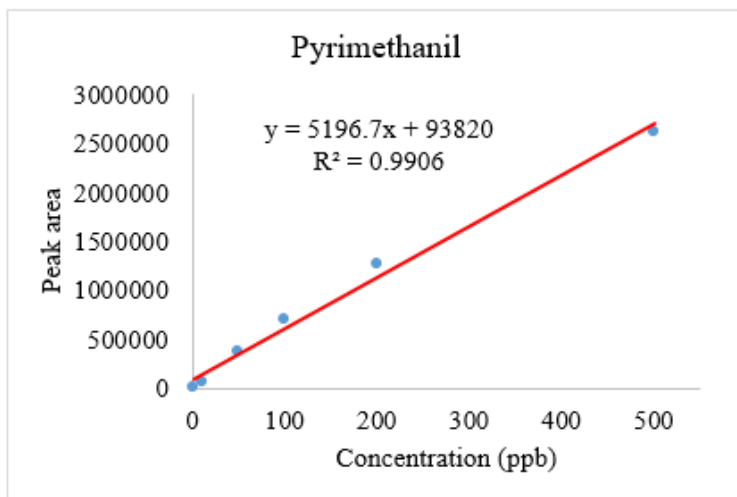
Figure 4.4 show the calibration curves for all the analytes (aldicarb, triadimefon, azoxystrobin, chlorpyrifos, buprofezin, cyproconazole I, fenpropathrin, paclobutrazole, bifenazate, azinphos methyl, spiroxamine, cyproconazole II, pyrimethanil, metalaxyl and diazinon). The analytes that had regression coefficient of determinations ($r^2 < 0.99$) with the linear model, had suitable calibration curve. Over the calibration range (0.1-500) ug/L all the calibration curves were linear with a correlation coefficient range of (0.961-0.999). The degree to which the target analyte concentrations range had linear link with the analyte response of the instrument making the instrument conditions suitable for the residues analysis.

The baseline chromatographic resolution of ≥ 1.5 from the other sample components indicated a suitable chromatographic conditions in terms of column type, flow rate and detection mode chosen for selectivity (Ferrer *et al.*, 2005; Njoroge, 2014). All LOD values obtained ranged from < 0.1 to 0.241 which were below the maximum residue limits (MRLs) of the respective pesticides. These LOD values are comparable to the values earlier reported in the range of 0.01-0.14 (Sapahin *et al.*, 2014).

Figure 4.4: Calibration curves for pesticide standards in C₂H₃N







4.3.3 Precision

Table 4.10 shows the percent relative standard deviations for the triplicate measurements for soil, water, kales and tomato samples.

Table 4.10: Percent relative standard deviations (%RSD)

Pesticides	% RSD (n=10)			
	Soil	Water	Kales	Tomatoes
Aldicarb	5.77	4.21	5.09	4.48
Triadimefon	7.82	3.33	9.82	9.84
Azoxystrobin	6.87	7.96	4.27	5.38
Chlorpyrifos	3.85	4.61	2.47	4.35
Buprofezin	6.64	10.81	0.85	1.56
Cyproconazole I	6.51	7.31	3.02	7.99
Cyproconazole II	2.06	3.94	2.28	5.24
Fenpropathrin	6.02	9.90	3.85	5.33
Paclobutrazole	7.41	9.62	2.84	1.83
Pyrimethanil	3.11	4.35	0.69	0.87
Metalaxyl	6.23	9.64	2.43	1.83
Diazinon	6.06	4.58	5.11	6.62
Bifenazate	5.30	2.30	2.88	1.18
Azinphos methyl	4.76	3.96	3.69	2.07
Spiroxamine	5.06	6.68	3.67	2.58

The percent relative standard deviations (%RSD) were calculated and found to be in the range of 0.69%-10.81%. All the determined percent relative standard deviations were less than 20% this shows that the method used was precise and suitable (AlMulla, 2016). The obtained %RDS were comparable to earlier reported values which were in the range of 2.44-17.95% (Sapahin *et al.*, 2014), 6-11% (Kruve *et al.*, 2008) and 3.9-14.3% (Martins *et al.*, 2013). The chromatographs of the standards showing the retention times of the standards are shown. (Appendix 6).

4.4. Levels of pesticide residues in soil, water, kales and tomatoes

Table 4.11 shows the mean concentrations of all the fifteen pesticide residues detected in soil, tomatoes and kales from Ewaso Narok wetland during the wet and dry seasons.

Table 4.11: Mean concentration (\pm SE) of residues in soil, kales and tomatoes (n=3)

Pesticides	Season	Soil ($\mu\text{g/L}$)	Kales ($\mu\text{g/L}$)	Tomatoes ($\mu\text{g/L}$)	P-value
Triadimefon	Wet	608.10 \pm 32.06 ^b	132.98 \pm 5.56 ^a	117.76 \pm 4.79 ^a	0.003
	Dry	1000.21 \pm 50.12 ^b	149.86 \pm 3.07 ^a	131.42 \pm 1.37 ^a	<0.001
Metalaxyl	Wet	640.18 \pm 49.33 ^b	96.29 \pm 7.11 ^a	91.96 \pm 7.28 ^a	<0.001
	Dry	800.23 \pm 15.25 ^c	108.52 \pm 4.49 ^a	135.75 \pm 6.64 ^a	<0.001
Azoxystrobin	Wet	167.75 \pm 47.87 ^b	49.83 \pm 13.47 ^a	40.13 \pm 11.26 ^a	0.039
	Dry	450.49 \pm 56.89 ^c	56.16 \pm 8.29 ^a	86.15 \pm 9.27 ^b	<0.001
Azinphos methyl	Wet	29.46 \pm 6.20 ^b	14.44 \pm 2.53 ^a	12.54 \pm 2.21 ^a	0.049
	Dry	100.09 \pm 4.66 ^c	16.16 \pm 1.31 ^a	34.42 \pm 2.02 ^b	<0.001
Buprofezin	Wet	5.19 \pm 1.30 ^b	2.76 \pm 0.69 ^{ab}	1.02 \pm 0.32 ^a	0.040
	Dry	6.36 \pm 2.19 ^b	3.11 \pm 0.63 ^{ab}	1.17 \pm 0.21 ^a	0.005
Cyproconazole I	Wet	193.87 \pm 50.70 ^b	59.00 \pm 18.06 ^a	54.04 \pm 19.68 ^a	0.040
	Dry	437.49 \pm 26.26 ^c	66.50 \pm 14.48 ^a	82.15 \pm 13.96 ^b	<0.001
Cyproconazole II	Wet	261.75 \pm 9.18 ^b	71.75 \pm 10.89 ^a	80.08 \pm 2.87 ^a	<0.001
	Dry	420.64 \pm 5.38 ^b	80.86 \pm 7.94 ^a	92.09 \pm 2.42 ^a	<0.001
Diazinon	Wet	2.17 \pm 0.66	1.68 \pm 0.69	1.13 \pm 0.46	0.523
	Dry	5.66 \pm 0.60 ^b	1.90 \pm 0.13 ^b	0.30 \pm 0.12 ^a	<0.001
Fenpropathrin	Wet	207.91 \pm 74.18 ^b	27.03 \pm 8.49 ^a	16.82 \pm 4.71 ^a	0.035
	Dry	480.69 \pm 47.69 ^c	30.46 \pm 5.75 ^b	19.34 \pm 2.30 ^a	<0.001
Chlorpyrifos	Wet	3.52 \pm 0.38 ^b	1.22 \pm 0.19 ^a	0.70 \pm 0.06 ^a	<0.001
	Dry	4.31 \pm 0.25 ^b	1.37 \pm 0.27 ^b	0.81 \pm 0.02 ^a	<0.001
Bifenazate	Wet	37.47 \pm 0.41 ^b	16.13 \pm 0.01 ^a	12.30 \pm 0.12 ^a	<0.001
	Dry	45.90 \pm 0.37 ^c	18.18 \pm 0.05 ^a	24.15 \pm 0.08 ^b	<0.001
Paclobutrazole	Wet	9.34 \pm 1.01	<LOD	<LOD	-
	Dry	21.45 \pm 0.72	<LOD	<LOD	-
Pyrimethanil	Wet	342.24 \pm 27.32	88.83 \pm 36.51	46.28 \pm 17.31	0.096
	Dry	419.13 \pm 22.17 ^b	62.87 \pm 15.41 ^a	42.31 \pm 20.11 ^a	0.001
Spiroxamine	Wet	128.37 \pm 56.06	55.79 \pm 24.56	51.57 \pm 23.13	0.338
	Dry	357.25 \pm 21.15 ^c	149.86 \pm 3.07 ^b	105.42 \pm 1.37 ^a	<0.001
Aldicarb	Wet	10.39 \pm 0.76 ^b	6.89 \pm 0.90 ^a	5.23 \pm 0.75 ^a	0.011
	Dry	32.73 \pm 0.49 ^b	7.76 \pm 0.82 ^a	8.01 \pm 0.58 ^a	<0.001

Mean values followed by the same superscript within the same row do not differ significantly (one-way ANOVA, SNK-test =0.05). LOD (limit of detection).

Relatively high concentrations for all the residues were observed in the soil in the range of 4.31-1000.21 µg/L during the dry season than in the wet season (2.17-640.18) µg/L. In kales, pyrimethanil concentration was 62.87 and 88.83 µg/L in dry and wet seasons, respectively. All the remaining residues concentration in kales were relatively higher in the dry season (1.37-149.86) µg/L than in the wet season (1.22-132.96) µg/L. In tomatoes, there were relatively high residues concentrations in the dry season (0.30-135.75) µg/L than in the wet season (0.70-117.76) µg/L except for pyrimethanil (42.31 µg/L) and diazinon (0.30 µg/L) in dry season (pyrimethanil 46.28 µg/L and diazinon 1.13 µg/L). This could be attributed to lower temperatures during the wet/ rainy season which favored the partitioning of the pesticide compounds from the vapour phase into the suspended particles in the atmosphere (Ritter *et al.*, 1995). The increased surface runoffs, leaching and degradation of pesticides through hydrolysis in the wet season also could be the reason for the lower residue concentrations in the wet season (Moncada, 2001; Fenik *et al.*, 2011). This increases the chances of the pesticides distributed by floods into other untargeted areas (Antić *et al.*, 2015). Hence, there were significant difference in the concentrations of the detected pesticide residues in soil, kales and tomatoes ($p \leq 0.05$) except for the level of diazinon during the dry season ($p \leq 0.05$, $t = 0.523$) and spiroxamine during the wet season ($p \leq 0.05$, $t = 0.338$) that showed no significant differences.

The results showed presence of pesticide residues in the sample matrices in the following range of concentrations; triadimefon (117.76-1000.21) µg/L, metalaxyl (91.96-800.23) µg/L, azoxystrobin (40.13-450.49) µg/L, cyproconazole I (54.04-437.49) µg/L, cyproconazole II (80.08-420.64) µg/L, fenpropathrin (16.82-480.69) µg/L, pyrimethanil

(42.31-448.13) $\mu\text{g/L}$ and spiroxamine (5.23-357.21) $\mu\text{g/L}$. The residue levels could be due to frequent use of the pesticide, short time elapsed between the pesticide applications and sampling. Some pesticides also take long to degrade in the environment. Soil samples recorded relatively higher pesticide concentrations compared to the levels recorded by kales and tomatoes in the two seasons. Paclobutrazole was only detected in soil pointing to a possible persistence from historical use. In a study done by Elgueta *et al.* (2017) in Chile, the mean levels of azoxystrobin, chlorpyrifos and metalaxyl in vegetables were 2.25, 0.54 and 0.13 mg/kg, respectively. Golge and Kabak (2015) in a study done in Turkey, the levels of chlorpyrifos and azoxystrobin in oranges were in the range of 10-90 $\mu\text{g/kg}$ and 20-30 $\mu\text{g/L}$, respectively. while Arjmandi *et al.* (2010) in a study done in Mazandaran Province reported the levels of diazinon in rice paddies in the range of 480-923.7 $\mu\text{g/L}$.

4.4.1 Pesticide residue levels in the soil of Ewaso Narok wetland

A total of 90 soil samples were collected and analyzed from the 15 grids during the wet and dry seasons. Table 4.12 shows the number of soil samples that were contaminated by the various pesticide residues. Out of the 15 pesticide residues analyzed in soil, 46.7% were insecticides (fenpropathrin and bifentazate), 53.3% fungicide (PCPB, 2017). Metalaxyl, fenpropathrin, azoxystrobin were the most widely detected pesticides in soil at 82.4%, 70.6% and 58.8%, respectively. Bifentazate was detected in 5.8% of the soil samples. Aldicarb, azinphos methyl, diazinon, chlorpyrifos, buprofezin, pyrimethanil, cyproconazole, triadimefon, paclobutrazole, azoxystrobin and spiroxamine were detected in soils number ranging from 11.8-23.5%. In a study done by Silva *et al.* (2019) in Europe,

83% of the tested agricultural soil contained pesticide residues with 58% of the soil containing multiple residues.

Table 4.12: Pesticide residues and number of contaminates soil samples

Use	Pesticide residues	No of contaminated soil samples (n)	No of contaminated soil samples (%)
Insecticide (46.7%)	Aldicarb	21	23.3
	Azinphos methyl	16	17.6
	Diazinon	11	11.8
	Chlorpyrifos	11	11.8
	Buprofezin	21	23.5
	Fenpropathrin	61	70.6
	Bifenazate	5	5.9
	Fungicide (53.3%)	Pyrimethanil	11
Cyproconazole I		16	17.6
Cyproconazole II		11	11.8
Triadimefon		21	23.5
Paclobutrazole		16	17.6
Azoxystrobin		53	58.8
Metalaxyl		74	82.4
Spiroxamine		16	17.6

Lower residue levels during wet season compared to dry season was attributed to the increased surface runoffs through flooding and leaching. In the dry season, the residues binds with the soil particles and other soil organic matter increasing residue levels in the soil. However, In the wet season, precipitation and leaching of the pesticide into the underground water is enhanced coupled with both chemical and physical degradation processes such as hydrolysis and dilution, thus residues levels are much reduced. The highest concentration levels were observed for metalaxyl (640 µg/L) and triadimefon (618.79 µg/L) and metalaxyl (800.23 µg/L), triadimefon (1000 µg/L) during wet and dry seasons, respectively. The two pesticides are broad based phenylamine (metalaxyl) and triazole (triadimefon) fungicides used for fungal disease control in tomatoes and kales

production. The least concentration levels were observed for chlorpyrifos (3.52 µg/L) and buprofezin (5.19 µg/L) and chlorpyrifos (4.31 µg/L) and buprofezin (6.36 µg/L) during wet and dry seasons, respectively. While the use of chlorpyrifos is limited to ornamental plants, Ewaso Narok wetland farmers may not be aware of the local or international restrictions put on chlorpyrifos use, the uncontrolled market or poor pesticide use by farmers. All the detected pesticide residues in the soil for the two seasons did not differ significantly in their concentrations ($p < 0.05$) except pyrimethanil that showed a significant difference of ($p = 0.696$) at 95% confidence level.

4.4.2 Pesticide residue levels in tomatoes and kales

In tomatoes during wet and dry seasons, the highest values were for triadimefon in the range of (117.76-135.42) µg/L. The least values were for chlorpyrifos (0.70-0.81) µg/L during wet and dry season. Aldicarb and azinphos methyl were detected in the range of (5.23-8.01) µg/L and (12.45-34.42) µg/L during wet and dry seasons, respectively. In kales the highest values were for triadimefon in the range of (132.98-249.86) µg/L during wet and dry season. While the least values were for chlorpyrifos in the range of (1.22- 4.37) µg/L during wet and dry season. Kales were also contaminated with aldicarb (6.89-9.76) µg/L and azinphos methyl (14.44-36.16) µg/L during wet and dry seasons. World Food Organization (WHO) and Agricultural Food Organization (FAO) have classified aldicarb and azinphos methyl as extremely (class 1a) and very (1b) hazardous chemicals and European Union (EU) among other countries (Watts *et al.*, 2016). This is due to their extremely and very hazardous nature. In addition, chlorpyrifos, paclobutrazole, fenpropathrin and diazinon use is restricted to ornamental plants and not vegetables due to

their highly hazardous nature (HHPs) (PAN, 2015). A similar study of pesticide residues in locally consumed vegetables in Kenya reported the range of chlorpyrifos to be (50-127) $\mu\text{g}/\text{kg}$ (Inonda *et al.*, 2015). Their presence in kales and tomatoes present both human and environmental health risk resulting from their possible bioaccumulation in higher organisms. There was a significant difference in the pesticide residues concentrations in tomatoes and kales during wet and dry seasons ($p < 0.05$). This was because tomato farmers within Ewaso Narok wetland were dependent on irrigation farming as opposed to rain fed agriculture. Therefore, the seasons did not influence significantly the volumes or amount of pesticides applied on the crops at any time of the year.

Pesticide residue concentrations in tomatoes and kales during wet and dry seasons were within the acceptable World Health Organization (WHO) maximum residues limits (WHO-MRLs). Cyproconazole I and II, spiroxamine and aldicarb had no set WHO maximum residue limits (MRLs) values for both tomatoes and kales. The study revealed that the residues levels of cyproconazole I and II, spiroxamine, fenpropathrin in tomatoes and triadimefon, cyproconazole I and II, fenpropathrin, bifenazate and pyrimethanil in kales exceeded the European Union maximum residues (MRLs) (EFSA, 2017). El-zaher *et al.* (2011) in a study done in Egypt reported 60 $\mu\text{g}/\text{L}$ of bifenazate in tomatoes after 15 days of treatment with 4060 $\mu\text{g}/\text{L}$ of bifenazate pesticide, Yalçin and Turgut (2016) in a study done in Aydin province of Turkey, the concentrations of chlorpyrifos in tomatoes collected ranged between (200-400) $\mu\text{g}/\text{L}$ while Lozowicka *et al.* (2015) in a study done in Kazakhstan reported the levels of metalaxyl, pyrimethanil, triadimefon, azoxystrobin and buprofezin in tomatoes as having a range of 50-150, 70-100, 10-40, 0-20 and 0-170 $\mu\text{g}/\text{L}$

respectively. High pesticide residues in food above the recommended maximum residues limits could be attributed to the failure by farmers to adhere to Good Agricultural Practices (GAP) (Aboagye, 2002). Petersen (2000) in a study done in Dublin, Ireland stressed that when pesticides are applied on crops following Good Agricultural Practices (GAP), then the residues in crop after the pre-harvest interval (PHI) are expected to be within the recommended maximum residues limits (MRLs). Daanu (2011) in a study done in Ghana, linked high pesticide residues levels above the recommended maximum residues limits (MRLs) to a number of human health conditions such as cancer, endocrine disruptors, neurological problems and disruption of growth development in children. However, according to Lozowicka *et al.* (2015), agricultural food crops can only be declared unfit for human consumption after considering the nature, type, acceptable daily intake (ADIs), acute reference dose (ARfDs) for the individual pesticide and individual weight. For the current study, the safety of the pesticide residues in tomatoes and kales which exceeded the MRLs were further determined based on the theoretical ADIs and ARfDs set by the World Health organization (WHO), European Union (EU) and Australia for an adult having a body weight of 60kgs. The concentrations of triadimefon, spiroxamine, cyproconazole I and II in kales for both seasons exceeded their respective ADI values as set by the WHO or EU. The levels of fenpropathrin and pyrimethanil in kales exceeded the EU recommended ADIs of 30 and 20 μ g/kg, respectively. Fenpropathrin had no acute reference dose (ARfD) while triadimefon and cyproconazole had their concentrations above ARfD values of 80 and 20 μ g/kg, respectively. Elgarhy (2015) in a reviewed article on an overview of the triazole of interest, reported various health effects associated with triazole exposure as ranging from cardiopulmonary complications to reproductive disorders.

4.4.3 Levels of pesticide residues in water

Table 4.13 gives the mean concentrations of pesticide residues in Ewaso Narok river water during wet and dry seasons.

Table 4.13: Mean concentrations (\pm SE) of pesticide residues in water (n=3)

Pesticide residues	Seasons	Upstream $\mu\text{g/L}$	Mid-stream $\mu\text{g/L}$	Down-stream $\mu\text{g/L}$	P-value
Triadimefon	Wet	2.28 \pm 0.05 ^c	2.15 \pm 0.03 ^b	0.91 \pm 0.03 ^a	<0.01
	Dry	1.38 \pm 0.01 ^c	1.01 \pm 0.01 ^b	0.39 \pm 0.03 ^a	<0.01
Metalaxyl	Wet	0.25 \pm 0.03 ^b	0.15 \pm 0.01 ^a	0.37 \pm 0.02 ^c	<0.01
	Dry	0.15 \pm 0.01 ^a	0.20 \pm 0.02 ^b	0.29 \pm 0.01 ^c	0.01
Azoxystrobin	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Azinphos methyl	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Buprofezin	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Cyproconazole I	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Cyproconazole II	Wet	3.51 \pm 0.15 ^c	3.30 \pm 0.03 ^b	2.75 \pm 0.02 ^a	<0.01
	Dry	2.12 \pm 0.01 ^c	1.55 \pm 0.01 ^b	1.45 \pm 0.01 ^a	<0.01
Diazinon	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Fenpropathrin	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
chlorpyrifos	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Bifenazate	Wet	0.23 \pm 0.03	0.25 \pm 0.01	<LOD	-
	Dry	0.14 \pm 0.01	0.12 \pm 0.00	<LOD	-
Paclobutrazole	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Pyrimethanil	Wet	0.18 \pm 0.02 ^b	0.18 \pm 0.01 ^b	0.13 \pm 0.02 ^a	0.044
	Dry	0.09 \pm 0.01 ^a	0.30 \pm 0.01 ^c	0.14 \pm 0.01 ^b	<0.01
Spiroxamine	Wet	<LOD	<LOD	<LOD	-
	Dry	<LOD	<LOD	<LOD	-
Aldicarb	Wet	4.33 \pm 0.04 ^b	5.85 \pm 0.03 ^c	2.56 \pm 0.02 ^a	<0.01
	Dry	2.93 \pm 0.02 ^b	2.20 \pm 0.01 ^b	1.08 \pm 0.01 ^a	<0.01

Mean values followed by the same small letter within the same row do not differ significantly from one another (one-way ANOVA, SNK-test =0.05), LOD-limit of detection.

Six pesticide residues were detected in water at different concentration levels during wet (June, 2016) and dry (August, 2016) season. During wet season, the pesticide residues concentration had a range of; triadimefon (0.91-2.28)µg/L, metalaxyl (0.15-0.37) µg/L, cyproconazole II (2.75-3.51) µg/L, bifenazate (0.23-0.25) µg/L, pyrimethanil (0.13-0.18) µg/L and aldicarb (2.56-5.85) µg/L. During the dry season, pesticide residues concentrations were in the range of; triadimefon (0.39-1.38) µg/L, metalaxyl (0.15-0.29) µg/L, cyproconazole II (1.45-2.12) µg/L, bifenazate (0.12-0.14) µg/L, pyrimethanil (0.09-0.30) µg/L and aldicarb (1.08-2.93) µg/L. Azoxystrobin, azinphos methyl, buprofezin, cyproconazole I, paclobutrazole, diazinon, fenpropathrin, chlorpyrifos and spiroxamine were not detected in water and were assumed to have been below the respective limits of detections (LOD)

In the wet season, concentrations of the residues along the three regions of the river were in the range of; Upstream (0.18-4.33) µg/L, mid-stream (0.15-5.85) µg/L, down-stream (0.13-2.75) µg/L. During dry season, the residues concentration range were; upstream (0.09-2.93) µg/L, mid-stream (0.12-2.20) µg/L and down- stream (0.14-1.45) µg/L. Significantly high concentration ranges were detected for aldicarb (2.93-4.33) µg/L, triadimefon (1.38-2.28) µg/L and cyproconazole II (2.12-3.51) µg/L. Pesticide application in farms in the catchment area upstream contributed to the pesticide pollutants found in Ewaso Narok River. The pollutants get into the water mainly through floods during wet season when farm effluents flow into the river. In the mid-stream, all the residue levels were significantly lower than the upstream level for both the two season except for aldicarb

(5.85) $\mu\text{g/L}$ and bifenazate (0.25) $\mu\text{g/L}$ for the wet season, metalaxyl (0.20) $\mu\text{g/L}$ and pyrimethanil (0.30) $\mu\text{g/L}$ for the dry season that showed slight increase.

Lower levels of pesticide residues were detected down-stream of the river where water flows out of the wetland. This shows that part of the detected pesticide residues were dissipated in the wetland to other pesticide metabolites through different chemical, physical and biological mechanisms. This also depended on the solubilities of the individual pesticides in water with highly soluble pesticides exiting the wetland at high concentrations. Pesticide residues concentrations were significantly high in the wet season compared to the dry season. This could be attributed to the increased surface runoff as a result of rainfall described to be above normal at the time of the study. Antić *et al.* (2015) also reported high pesticide residues in Danube River during the wet season compared to the dry season. Musa *et al.* (2011) in a study done in Kenya, pesticide residues levels in the range of 0.05-59.01 $\mu\text{g/kg}$ and ND-24.54 $\mu\text{g/kg}$ for the wet and dry seasons respectively for River Yala and River Nzoia in Lake Victoria basin were reported.

Flood and leaching are the main routes through which pesticides used in farms can get into the main water system especially during wet/ rainy seasons. In the dry/ hot season, there are no floods and leaching processes are much reduced. As a result, there are reduced amount of farms effluents both from the wetland or river catchment areas that get back into the main water system. Pesticides are also taken by wind, volatilization or evaporation during application, however, these routes may not lead to the distribution of a significant amount of pesticides. General poor pesticide use by farmers which include washing of farm

tools near the river or poor disposal of pesticide waste containers. However, in both wet and dry seasons, no significant differences ($p < 0.05$) in residues concentrations was observed for the upstream, midstream and downstream respectively. The findings of this study therefore offers a clear proof that Ewaso Narok River serves as a drainage route especially during wet/ rainy season for agricultural pesticides from their points of applications.

According to the European union (EU), the maximum admissible concentration of individual pesticides in drinking water is set at $0.10 \mu\text{g/L}$ and the total concentrations of all pesticides in drinking water should not exceed $0.50 \mu\text{g/L}$ (Rezaee *et al.*, 2012; EPA, 2014). In the current study the levels of aldicarb, triadimefon and cyproconazole in water was above the EU recommended maximum admissible concentration ($0.10 \mu\text{g/L}$). The sum concentrations pesticide residues in water also above the recommended EU maximum admissible limit of $0.50 \mu\text{g/L}$ for both wet and dry seasons. This shows over-reliance and widespread pesticide use within the wetland and its catchment areas. A similar study carried out by WHO, (2003) found 111 samples contaminated with aldicarb at a maximum concentration of $28 \mu\text{g/L}$ in Canada and a concentration range of $(10-500) \mu\text{g/L}$ for USA (WHO, 2003).

Dahshan *et al.* (2016) in a study carried in Egyptian river Nile reported pesticide concentration in the range of $0.403-3.33 \mu\text{g/L}$. The presence of pesticide residues in water present not only a risk to the wetland biodiversity but also raises numerous human health concerns. Triadimefon is a possible human carcinogen under group C, known to produce

thyroid tumor and inhibits anti-thyroid activity in rats (USEPA, 2006). Muller *et al.* (2012) reported low concentrations of pyrimethanil found to be responsible for the genetic variability in the multigenerational study of *Chironimos riparius* population.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the result of this study, it can be concluded that farmers of Ewaso Narok wetland did not follow appropriate safe pesticide use. The study revealed unsafe pesticide use from storage, mixing of different pesticide products, disposal of empty pesticide containers, lack of /or incomplete use of personal protective clothing and equipment (PPEs). Majority (95.3%) of the farmers had received formal education from primary to tertiary levels and had better knowledge on safe pesticide use. However, famers' education and knowledge on various safe pesticide use was not reflected on their practical use of pesticide. As a result, this led to significant pesticide exposures among farmers considering the reported poisoning health symptoms reported by farmers where headache and dizziness were the most prevalent symptoms.

Soil, water, tomatoes and kales collected and analyzed in this study were contaminated with various pesticide residues at different concentrations. Apart from paclobutrazole which was only found in soil, triadimefon, metalaxyl, azoxystrobin, azinphos methyl, buprofezin, bifenazate, cyproconazole I and II, spiroxamine, aldicarb, fenpropathrin, diazinon, chlorpyrifos and pyrimethanil were found in soil, tomatoes and kales. The study revealed the presence of aldicarb, triadimefon, metalaxyl, cyproconazole II, bifenazate and pyrimethanil. There was significantly high pesticide residues in the soil, tomatoes and kales during dry season than during wet season. For water, high pesticide residues levels were found during wet season than during dry season. All the pesticide residues levels in soil,

tomatoes and kales during wet and dry seasons were within the recommended WHO-MRLs. However, the levels of cyproconazole I and II, fenpropathrin and spiroxamine in tomatoes and triadimefon, cyproconazole I and II, fenpropathrin, bifenazate, fenpropathrin, and spiroxamine in kales collected during wet and dry seasons were above EFSA MRLs. Six pesticide residues (triadimefon, metalaxyl, cyproconazole II, bifenazate, pyrimethanil and aldicarb) were found to be present in water during wet and dry seasons at concentrations higher than the allowed Maximum Contamination Limits (MCLs) of 0.10µg/L for individual pesticide residues levels and 0.50µg/L for sum total residues concentrations in drinking water. The presence of residue levels in water at concentrations higher than the recommended levels pose both ecological and human health concerns. This is because some of the residues have the ability to remain in the environment for long while some may bioaccumulate in food chain. It can be concluded that vegetable farmers in Ewaso Narok wetland did not embrace Good Agricultural Practices (GAP) and Integrated Pest Management (IPM). The presence of aldicarb and azinphos methyl pointed to lack of knowledge of internationally banned or restricted pesticides by local farmers considering their extremely and very hazardous nature, respectively.

5.2 Recommendations

5.2.1 Recommendation from the study

- i. If pesticides have to be used on vegetables, then farmers need to undergo training to comply with the safety requirements which include but not limited to the use of appropriate and complete personal protective clothing and equipment (PPEs), reading of instructions on pesticide labels before use, adhering to safety intervals

(re-entry and pre-harvest intervals) after pesticide application, safe storage of pesticides and safe disposal methods of empty pesticide containers.

- ii. Farmers need to ensure that right pesticide quantities are applied on vegetables (tomatoes and kales) as this will ensure that the pesticide residue levels in the vegetables are within the recommended maximum residue limits (at the time of harvest and consumption. Misuse and over-reliance on synthetic pesticide use to control vegetable pests and diseases can be controlled by embracing Good Agricultural Practices (GAP) and Integrated Pests Management (IPM) together with periodic surveillance of pesticide use and their levels in environment and food produce.

5.2.2 Recommendations for further research

- i. A study to evaluate pesticide residue levels in other horticultural produce grown in Ewaso Narok wetland such as french beans, cabbages, potatoes, chilies to determine their possible impact on environment and human health.
- ii. Tomatoes and Kales should be planted in different farms and analysed to determine the level of pesticide residue in the two vegetables, farmers should be informed of internationally banned and restricted pesticides to curb their use locally.

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APPENDICES

Appendix 1a: Farmers consent form

RUMURUTI WETLAND AGRICULTURAL PESTICIDES SURVEY.

Name of the Respondent _____

Village _____ County _____ Mobile number: _____

(In case of another person's mobile, please give name of person _____)

I Peter B.M. Otieno Ngolo (I56/CE/27737/2013) a student at Kenyatta University, undertaking Masters of Science project at Rumuruti wetland with an aim of evaluating the level of farmers' exposure on the sound pesticides management in terms of potential risks and safety. Determining the types/ range of pesticides used by farmers within the wetland and carrying out the screening of the levels of these pesticides residues within the wetland ecosystem. The results of this survey are solely meant for educational purposes and not for profit making and as such any **participation** on this study shall be purely on **voluntary basis** with **no financial benefits** attached. This study has been authorized by express permission of Kenyatta University Board of Postgraduate studies. I am inviting you to be part of this study. Your participation is voluntary and has no immediate financial benefits. The outcomes of this study will be shared with the farmers.

If you agree to participate in the study, you will be:

1. Asked questions about the types of exposure you have on sound pesticides management which include safety precautions, first aid mechanisms and waste disposal by means of filling or being assisted to fill in a questionnaire.
2. Requested to provide a list of pesticides that you use in your farm on different crops and the pest / disease they help control.
3. Requested to allow us pick Kales and soil samples from your farm for the laboratory analysis of the pesticides levels.

By signing this form you are consenting to be part of the study/survey. Should you need more information you can contact **Peter B. M. Otieno Ngolo, Tel. number: +254720627109**. If you change your mind about taking part in the study, you are free to do so but we encourage you to participate. If you wish, all your information will be kept confidential. Please let us know your preferred choice (Y) (N).

I declare that the study/survey team has given me all the information I need about the study in a language that I understand and that I have been given a chance to ask all the questions I may have had and that these have been answered to my satisfaction. I voluntarily consent to participate in the study/survey.

Name of the person giving consent

Signature

Date

Appendix 1b: Farmers Questionnaire

<u>SECTION A: PERSONAL DETAILS</u>			
KENYATTA UNIVERSITY	Ewaso Narok Wetland Agricultural Pesticide Study		
	Farmers questionnaire	Farm code: <input style="width: 150px; height: 20px;" type="text"/>	Date: <input style="width: 40px; height: 20px;" type="text"/> Enumerator <input style="width: 150px; height: 20px;" type="text"/>
Farmers' level of education: <input style="width: 150px; height: 25px;" type="text"/>	Sex: <input style="width: 40px; height: 25px;" type="text"/>	Age: <input style="width: 250px; height: 25px;" type="text"/>	

SECTION B: RESOURCE UTILIZATION AND PRODUCTION

1. What is the approximate size of your farm in acres? 0 – 1ha 1 – 5 ha
 over 5 ha
2. For how long have you been farming? Less than 1 year 1year 2- 5 years
 5 – 10 years Over 10 years
3. What types of crops do you plant in your farm? (Maize, kales (Sukuma wiki), spinach, tomatoes, cabbages etc.) Maize Kales (Sukuma wiki)
Spinach Cabbages Tomatoes f) French beans Others specify
4. How long have you been using pesticides on your farm? 0 – 2 years 2 – 5 years
5 – 10 years Over ten years
5. At what stage of crop life do you apply the pesticides? During planting,
weeding storage)
6. Have you ever received formal training on good pesticide practices? Yes No
 if you have not received any training, do you have access to someone who provides such training? Yes No If YES, who?
7. Do you buy the pesticides from one Agroveter retailer or many different retailers? One many
8. When you buy pesticides, does it happen sometimes that the container(s) has no label? Never happen It does happen sometimes Often I don't know

9. Who is the main person with the responsibility of deciding when to apply the pesticides? The respondent Farm owner Farm worker. Farm manager

10. What influences your decision while choosing pesticide to use on your crops/ farm Supplier (vendors and Agrovot) Commercial sources of information (advertisements, labels on the container) Fellow farmers Income media

11. Do you mix different brands of pesticides before application? Yes No

12. What is the main reason why you mix the pesticides this way? Unsure about the quality of pesticides Uncertain about the effectiveness of pesticides for a particular pest Advice by retailers/ suppliers Following the suggestion of others Other reason (please specify)

13. What kind of chemical means of plant protection (pesticides) have you been using, for which crops, pests /diseases, and how much?

Product/trade name	Frequency daily/Weekly/ monthly	Which crop being treated	Target/pest weed/ disease	Results

14. Who is the main person with the responsibility of applying the pesticide in the farm?

Respondent Farm owner other family members Hired applicator

15. On a scale of 1-5, how much risk do you think you are exposed to while using pesticides on this farm? No risk at all Some small risks A medium amount of risk A large and significant amount of risk Dangerous and very toxic risks I don't know

16. Do you know how pesticide can get into your body system (routes) Yes No If yes please give examples (inhalation, skin contact, oral, etc.?)

17. Do you wear protective clothing when applying pesticides? Yes No

If no why? Please pick one: too expensive not available uncomfortable If yes, check one or more of the following;

PPE	YES	NO	I DON'T KNOW
Gloves			
Face masks			
Overalls			

Eye glasses			
Boots/shoes			
Long pants			
Long sleeve shirt			
Respirator			

18. How do you apply the pesticides on your crops? With hand pump with tractor with brush with leaves
19. Do you currently practice any pest control techniques to reduce the need of using pesticides? Yes No If YES, which methods do you use: Organic production Biological control Mechanical-physical techniques Rotation of crop
20. In your opinion, can you rate how harmful the chemical (synthetic) pesticides are for the environment and health? If yes, please specify; not harmful moderately harmful Very harmful
21. When using pesticides or being exposed to them have you experienced (check one or more of the following):

Symptoms	Yes	No	I don't know
Excessive sweating			
Hand tremor			
Convulsion Staggering			
Excessive salivation			
Narrow pupils/miosis			
Blurred vision			
Headache			
Dizziness			
Irregular heartbeat			
Skin rashes			
Diarrhea			
Difficulty breathing			

Sleeplessness/insomnia			
Nausea/vomiting			

22. How do you store pesticides before and after use? in their original containers
 In my own containers in my storage room in the house farm house
 others
23. Are the pesticide containers used for other purposes afterwards? Yes No
 If yes, are you aware that you should not do this? Yes No
24. How are the containers or packages disposed of? Thrown in open field Buried
 Burnt Put in rubbish/trash
25. From whom do you receive consultations about the right use and storage of pesticides? From retailer from consultancy services from fellow farmers
 others (please specify)
26. Are there agricultural extension services in Rumuruti? Yes No
 If yes, are the service or advices by these extension officers available to you?
 Yes No
27. How many times do you apply pesticides in your farm crops before harvesting?
 Once twice thrice more than thrice
28. Do you observe pesticide safety intervals? Yes No. If yes, list the pesticide safety intervals
29. Do you read the label of pesticide product container before use? Yes No
30. Rate the effectiveness of pesticide use in your farm Excellent Good
 Fair Poor

Appendix 2: Average rainfall amounts and temperature readings for 2015-2016

Month, year	Average monthly Rainfall (mm)	Minimum temperature (°C)	Maximum temperature (°C)	Average Monthly average temperature (°C)
May, 2015	11.6	11.53	29.88	20.1
June, 2015	57.2	11.73	29.57	19.56
July, 2015	28.6	8.5	29.88	18.27
August, 2015	3.2	8.6	31.14	19.3
September, 2015	3.1	10.12	31.41	20.64
October, 2015	22.9	13.26	30.57	21.14
November, 2015	102	13.66	27.1	18.08
December, 2015	79.1	12.01	27.38	19.04
January, 2016	116.80	12.51	27.40	19.34
February, 2016	0	9.60	28.68	18.72
March, 2016	0	12.90	30.8	22.17
April, 2016	12.8	13.67	31.00	21.09
May, 2016	84.70	12.26	28.61	19.44
June, 2016	68.6	9.51	27.7	17.71
July, 2016	3.5	11.06	28.31	18.54
August, 2016	0	11.54	30.13	22.26
September, 2016	4.2	7.98	30.21	18.68
October, 2016	24.1	9.54	30.73	19.8

Source: Globe Wetland EA Project Weather Station located at Rumuruti (WRMA compound)

Appendix 3: GPS sampling coordinates and some sampling pictorials

No.	SOIL		TOMATOES/ KALES	
	Latitude	Longitude	Latitude	Longitude
1	N00.257449 ⁰	E036.544173 ⁰	N00.257720 ⁰	E036.545312 ⁰
2	N00.258343 ⁰	E036.545312 ⁰	N00.258670 ⁰	E036.545474 ⁰
3	N00.258992 ⁰	E036.545180 ⁰	N00.258861 ⁰	E036.546453 ⁰
4	N00.259594 ⁰	E036.545850 ⁰	N00.259878 ⁰	E036.546796 ⁰
5	N00.259524 ⁰	E036.547105 ⁰	N00.260609 ⁰	E036.547516 ⁰
6	N00.260609 ⁰	E036.547151 ⁰	N00.260861 ⁰	E036.548465 ⁰
7	N00.261570 ⁰	E035.547813 ⁰	N00.262141 ⁰	E036.548511 ⁰
8	N00.261432 ⁰	E036.549140 ⁰	N00.262039 ⁰	E036.549711 ⁰
9	N00.262359 ⁰	E036.549380 ⁰	N00.262679 ⁰	E036.549117 ⁰
10	N00.263319 ⁰	E036.549723 ⁰	N00.263972 ⁰	E036.550478 ⁰
11	N00.263298 ⁰	E036.550983 ⁰	N00.264213 ⁰	E036.551303 ⁰
12	N00.265210 ⁰	E036.551591 ⁰	N00.264431 ⁰	E036.552265 ⁰
13	N00.264913 ⁰	E036.552026 ⁰	N00.262164 ⁰	E036.549466 ⁰
14	N00.262827 ⁰	E036.549037 ⁰	N00.260895 ⁰	E036.548557 ⁰
15	N00.263128 ⁰	E036.549666 ⁰	N00.260895 ⁰	E036.551682 ⁰

Appendix 4: Knowledge of farmers towards safe use of pesticides

Pesticide safety practices	Response	Frequency	%
Do you know pesticides products by name?	Yes	76	89.0
	No	10	11.0
Do you know effects of pesticides on environment?	Yes	33	38.0
	No	53	62.0
Have you received any training on pesticide use?	Yes	3	3.5
	No	83	96.5
Have you experienced discomfort after pesticide use?	Yes	71	82.6
	No	15	17.4
How long have you been farming? (years)	0-1	30	34.9
	1-5	34	39.5
	5-10	22	25.6
For how long have you used pesticides in your farm? (years)	0-2	69	80.2
	2-5	16	18.6
	5-10	1	1.2
At what time do you apply pesticide?	Planting	11	12.8
	Weeding	14	18.6
	Pest/disease attack	61	70.9
Who decide when to apply pesticide?	Farmer	64	74.4
	Farmworker	13	15.1
Who apply pesticide?	Farmer	9	10.5
	Hired applicator	70	81.4
	Family member	7	8.1
Rate the level of risks you are exposed to when using pesticide	No risk	25	29.1
	Small risk	55	64.0
	Medium risk	1	1.2
	I don't know	5	5.8
Do you use personal protective clothing?	Yes	34	39.5
	No	52	60.5
List alternative pest control method you use	Rotational farming	49	57.0
	Intercropping	37	43.0
Where do you get advice on pesticide use	Farmers	20	23.3
	Retailers	10	11.6
	Income	7	8.1
	Advertisements	6	7.0
	Pest/disease symptoms	38	44.2
	Social media	5	5.8

Do you observe pesticide safety intervals?	Yes	81	94.2
	No	5	5.8
List pesticide safety intervals	Re-entry Interval (REI)	28	32.6
	Pre-harvest Interval (PHI)	72	
Are extension services available for farmers?	Yes	9	10.5
	No	77	89.5
How effective is pesticide use?	Excellent	15	17.4
	Good	30	34.9
	Fair	41	47.7
	poor	0	0.0
Do you mix pesticide products for use on vegetables	Yes	33	38.4
	No	53	61.6
If yes, what are some of the reasons for mixing pesticide products before use?	Uncertain about the quality of pesticide	30	34.9
	Unsure about pesticide effectiveness against a particular pest	36	41.9
	Advice by retailers/suppliers	9	5.8
	Advice from farmers	6	7.0

Appendix 5: Commonly used pesticides in Ewaso Narok wetland

Use	Active ingredient	Chemical classification	WHO/ FAO classification
Insecticide	Aldicarb*	Carbamate	Ia
	Azinphos methyl*	Organophosphates	1b
	Buprofezin	Unclassified	III
	Diazinon	Organophosphate	II
	Fenpropathrin (A)	Pyrethroid	II
	Chlorpyrifos	Organophosphate	II
	Bifenazate (M)	Carbazate	U
Fungicide	Pyrimethanil	Anilinopyrimidine	III
	Cyproconazole I	Azole	II
	Cyproconazole II	Azole	II
	Azoxystrobin	Beta-methoxyacylate	U
	Triadimefon	Triazole	II
	Metalaxyl	Phenylamine	II
	Spiroxamine	Morpholine	II
Paclobutrazole*	Triazole	II	

*pesticide active ingredients not approved to be used on crops in Kenya. 1a extremely hazardous; 1b highly hazardous; II moderately hazardous, III slightly hazardous; U unlikely to present acute hazard; F fungicide; I insecticide; A: acaricides; M: miticide (PCPB, 2006; WHO, 2010).

Appendix 6: Chromatograms showing retention times of the pesticides

