CHAPTER ONE

1.0 INTRODUCTION

Pesticides can be defined as chemical or biological substances used to kill or control specifically targeted pests. The target pests include insects, fungi, molluscs, microbes and nematodes. These pests are vectors of diseases of plants that result in reduced productivity of vegetables and fruits. Pesticides may be classified based on target organisms, chemical structure and physical state of the pesticide. The classification based on the target organisms includes insecticides, rodenticides, herbicides, and weedicides (Gilden et al., 2010). Based on the chemical nature of the pesticide, may have such compounds as organophosphates (OPPs), organochlorides and carbamates.

Vegetables are important ingredient of the human diet for the maintenance of the health and prevention of disease in humans (Hoffman and Gerber, 2015). Among the functions of vegetables include supplying vitamins, minerals and dietary fiber (Yokoyama et al., 2014). Vegetables like other crops are invaded by pests and diseases during production and storage leading to reduction of quality and yield. In the pursuit to avoid loss and maintenance of the quality of fruits and vegetables harvested, pesticides are applied in combination with other pest management techniques to destroy pest and prevent diseases. These vegetables are usually infected with various pests such as aphids, diamond moths and caterpillars. There are several fates of pesticides after they are applied on fruits and vegetables.

When applied to vegetables or any other part of the plant, small amount of pesticide may remain in the crops or animal feed or environment leading to contamination (Tsoutsi et al., 2006). The effect of organophosphates on human health is by virtue of its action on the enzyme acetyl cholinesterase. OPPs exert their effect on pests as well as humans by inhibiting acetyl cholinesterase at nerve endings and nerve junctions (Jin et al., 2013). The function of acetyl cholinesterase is to inactivate the neural transmitter, acetylcholine on the nerve junctions and nerve endings. The inactivation is effected by hydrolyzing it to choline and acetic acid or acetyl CoA. Acetylcholine transmits impulses across the nerve junction to effect various biological functions. This enzyme inactivation, leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission (Colovic et al., 2013).

Zambia is a third world developing country in which agricultural activity in the urban area contributes highly to food security (UNDP, 1996). In this country small scale industries highly grow vegetables for sale as wide population of Zambia consume vegetables as part of a staple diet (Nkhungulu and Msikita, 1985).These vegetables are known to be prone to pest infestation which may destroy vegetables threatening a good harvest for profit, therefore farmers apply pesticides to vegetables to prevents their destruction (Sibanda et al., 2000; Kuntashula et al., 2006). Pesticides applied to vegetables penetrate it by diffusion and some may remain on the vegetable. Most of it degrade and consequently diffuse out of the vegetable. However, small amount of pesticide residues may remain in the vegetable after killing pests which may be a health hazard (Metcalf, R.L., 1980; Ware and Whitacre, 2004).

The Zambian Governments being aware of this risk that pesticides residues may pose on human health has put in place measures to minimizing pesticides residues in food substances (Odhiambo et al., 2004), these include putting in place laws, regulations and regulatory institution such as Food and Drugs Act (Zambian Food and Drugs Act No. 13 of 1994) and Food and Drug Control Laboratory that has set maximum residual levels (MRLs) of all pesticides in foods. The Food and Drug Control Laboratory is supposed to regulate and monitor levels of pesticides residues in foods in Zambia before they are sold to consumers.

1.1 Statement of the problem

The Zambian laws on foods prescribe the maximum allowable organophosphates residues in vegetables. The Maximum residue limits (MRLs) of organophosphate in vegetables according to the Zambian laws (Zambian Food and Drugs Act No. 13 of 1994) on food for Dichlorvos is given as 1.0 ppm (mg/kg) for cabbage, rape and lettuce. As far as we aware there is no one monitoring and regulating the residues of organophosphate in vegetables sold in various areas in Lusaka. Evaluating and documenting the levels of Dichlorvos in vegetables at the point of sale provides necessary protection for the consumers and provides feedback to the farmers about the safety of their vegetables. Consequently, there was need to carry out a study to determine whether people eating these vegetables are being exposed to higher than allowable levels of organophosphates residues in vegetables.

1.2. Rationale of the study

As far as we are aware, there is no field data on Dichlorvos residues in vegetables sold in Lusaka. It was therefore, the purpose of this study to evaluate the Dichlorvos residues levels in vegetables (cabbages, rape and lettuce) at the point of sale if any corrective measures are to be put in place. This study sought to generate information as to whether Dichlorvos residue in vegetable is above the maximum allowable residue limit (MRLs). This data will help to maximize the monitoring and regulation of organophosphate residues in vegetables. It will also add to the body of knowledge on the possible Dichlorvos poisoning in Lusaka.

1.3. Research question

Do vegetables (lettuce, cabbage and rape) sold in some areas in Lusaka meet the required standard as prescribed in the Zambian Food and Drug Act no. 13 of 1994 with respect to maximum residue limit (MRL) of Dichlorvos?

1.4. Objectives

1.4.1 General objective

To determine the Dichlorvos residues in cabbages, rape and lettuce sold in some areas in Lusaka district, Zambia.

1.4.2 Specific objectives:

1.4.2.1 To determine the presence and levels of Dichlorvos residues in cabbages, rape and lettuce sold in areas in Lusaka district.

1.4.2.2 To ascertain whether the Dichlorvos residues found in cabbages, rape and lettuce meet the permissible maximum residue limits as set by the Zambian Food and Drugs Act and FAO/WHO.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The historical aspect and use of pesticides

Dichlorodiphenyltrichloroethane (DDT) was the first synthetic organochlorides. It is a broad spectrum agent used to eradicate human disease vectors and agricultural pests. The other type of pesticide, called organophosphate has it origin from the nerve gas. Organophosphates that have been developed include Diazinon, Parathion, Malathion, Tetraethyl pyrophosphate, Dimethoate, Trichlorophon, Mevinphos and Disulfoton

Research has revealed that since 1950, the use of pesticides worldwide has increased and it has been estimated that worldwide about 22 million kg of pesticides are used annually (Ye et al., 2013) . This is to be expected as food security particularly in developing countries is very high on the international agenda.

Pesticides are extensively used in agricultural production to prevent or control pests, diseases, weeds and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality. Pesticides diffuse into the vegetables and fruits and small amounts may persist in or on food. The pesticides that remain on the vegetable or on fruit may easily be washed away with water or may undergo photolytic degradation. The rate of breakdown is influenced by the intensity and spectrum of sunlight, length of exposure, and the properties of the pesticide (Waldron, 1992; Hill and Comardese, 1986; Wang and Liu, 2007). Even after washing and undergoing degradation small amounts of pesticides may remain in the vegetable or fruit (Akoto et al., 2013, Guler et al., 2010, Latif et al., 2011, Lozowicka, 2015). How much of the pesticide remains in the vegetables depends on factors such as how frequently is the pesticide applied, admixing of pesticide, duration of exposure to sunlight, environmental conditions such aeration, watering humidity and temperature among others. Therefore, the possibility of very rapid pesticide breakdown is increased by using pesticides only when necessary, by avoiding repeated applications of the same chemical and avoiding admixing of different types of organophosphate (Waldron, 1992).

By their very nature, most pesticides show a high degree of toxicity because they are intended to kill certain organisms and this creates some risk of harm to humans. It is because

of this that pesticide use has evoked grave concerns not only of potential effects on human health but also about impacts on wildlife and sensitive ecosystems (Chagnon et al., 2015, Shiff and Garnett, 1961). In developing countries such as Ghana, farmers face immense risks of exposure owing to the use of toxic chemicals that are banned or restricted in other countries (Nasr et al, 2007; Al-Eed et al, 2006; Adhikari, 2010). Wrong application techniques, badly maintained or totally unsuitable spraying equipment and inadequate storage practices exacerbate these risks (Al-Wabel et al, 2011). Often, the reuse (recycle) of old pesticide containers for food and water storage also contributes to the risks of exposure (Ecobichon, 2001; Damalas and Eleftherohorinos, 2011). Pesticide residues in or on plants may be unavoidable even when they are used in accordance with good agriculture practices but it's the bio accumulative effect in human body that is of concern (Castorina et al., 2003). Research conducted for the past decade in a number of countries point to the presence of pesticide residues in a number of food items including strawberries, onions, cucumber, lettuce, cabbage, okra, pepper, tomatoes, beans, rape, oranges and lemons (Esturk et al., 2014, Aysal et al., 2007). Research reveals that if farmers follow religiously the instructions on the labels on the packaging of organophosphate, follow agricultural practices such following appropriate dosage, withdrawal periods and frequency of applying organophosphates, use a single pesticides, the pesticides residue will be below maximum residual limit. However, if these measures are not followed the residue levels in vegetables exceed the maximum residual levels (MRLs) and could lead to diseases to consumers (Ware and Whitacre, 2004).

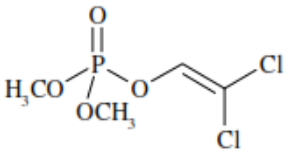
Various researchers have analyzed pesticide residues in fruit and vegetables worldwide and found a lot of pesticides residues in vegetables and fruits which are well above the maximum residue limits (Ferrer at al., 2005; Yamagami et al., 2009; Karanth, 2000; Dasika et al., 2011; Hrouzkova and Matisova, 2011). However there are limited documented pesticides residual analyses conducted using HPLC for vegetable at point of sale in Lusaka. In one study it was shown that many farmers in Zambia applied pesticides that are considered hazardous, indiscriminately used organophosphate and used over-dosage of organophosphate (Nyirenda et al.; 2011). All these factors lead to increased pesticide residue in vegetables. However the amount of pesticide residues on vegetable crops sold in Lusaka have not been studied extensively. A few isolated studies that have been conducted, observed higher levels of

Chlorpyrifos and monocrotophos on tomato and cabbage (Inambo T.M.P et al., 1997). Therefore, Zambia is faced with the challenge of the lack of enough information on the organophosphate residues in vegetables being sold in various areas.

2.2. Physical and chemical properties of Dichlorvos

Dichlorvos pesticide is common name for 2, 2-Dichlorovinyl dimethyl phosphate (DDVP). Its empirical formula is $C_4H_7C_{l2}0_4P$. Dichlorvos is an organophosphate pesticide the may be classified as a systemic insecticide and ascaricide which is frequently used for crop protection. Some of the trade names as used in Lusaka include Boam and Doom. Dichlorvos is a liquid with high vapor pressure at room temperature, highly volatile and is used for fumigation. Its target pests include house flies, mosquitoes, ticks, cockroaches, armyworms, chinch bugs, clover mites, crickets, cutworms, grasshoppers, aphids and sod webworms.

Table2.2.1.Dichlorvos nomenclature

Chemical Structure	
Empirical Formula	$C_4H_7Cl_2O_4P$
Common name	Dichlorvos (ISO) or DDVP
Company experimental name	
IUPAC name	2,2-dichlorovinyl dimethyl phosphate
CAS name	2,2-dichloroethenyl dimethyl phosphate
CAS Registry Number	62-73-7
End-use product/EP	Alco, Amvos
Chemical Class	organophosphate
Known Impurities of Concern	none
PC Code No.	084001

(Source: United State Environmental Control Agency 2010)

Table 2.2.2 Physiochemical properties of Dichlorvos

Parameter	**Value**
Molecular Weight	221.0
Physical State	Liquid
Boiling point/range	117˚ C at 10 mmHg
pH	~ 4 as 1 % aqueous solution
Specific gravity	1.424 at 25˚ C
Water solubility (20 C)	1.5 g/100 g
Solvent solubility (temperature not specified)	~0.5 % in glycerine; Miscible with aromatic hydrocarbons, ketones, and esters.
Vapor pressure	0.032 mmHg at 32˚ C
Dissociation constant	N/A
Octanol/water partition coefficient, log KOW (25˚ C)	38.4 log KOW = 1.58

Source: (Feiler, W., 1988)

2.3. Mode of action of Dichlorvos

Dichlorvos like any other organophosphate inhibits acetyl cholinesterase in both targeted pests and non-targeted organisms including humans which may lead to short and/or longer term neurotoxic effects. Dichlorvos is absorbed through all routes of exposure and may damage DNA of insects. Acute poisoning with an acetyl cholinesterase inhibitor in humans may cause weakness, headache, tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, and abdominal cramps (Maryse et al., 2010, Espeland et al., 2010). The mode of action is used for its insecticidal activity. In addition, organophosphates have also been shown to act by inhibiting cAMP-adenylyl cyclase signaling pathway (Meyer et al., 2004a, Meyer et al., 2004b, Meyer et al., 2003, Zhang et al., 2002). This mode of action may account for disruption of metabolic, cardiovascular and hormonal responses which can possibly lead to metabolic syndrome.

2.4. General characteristics of cabbage, rape and lettuce

Cabbage is botanically called *Brassica oleracea var capitata*. (Figure1.) It is globally cultivated and highly consumed by most people. It is a member of the family cruciferae which may be grown twice a year. The Brassicaceae family comprises a variety of vegetables such as rape (Brassica napus L.), Cauliflower (Brassica oleracea L. var. botrytis) among other. The edible part of the cabbage plant is made up of series of overlapping expanded leave which cover a small terminal bud.

Rape is botanically called *Brassica napus.* It is globally cultivated and highly consumed by most people. It is grown as a leafy vegetable used as relish. Lettuce is a plant belonging to the family Asteraceae. Its botanical name is *Lactuca sativa* which is biennial crop.

Figure 1. Cabbages at one of the study sites.

CHAPTER THREE

3.0. METHODOLOGY

3.1. Study Area and Sampling sites

The study was conducted in Lusaka, the capital city of the Republic of Zambia. The selected areas for sampling were Chisamba, Ngwerere, Mwembeshi, Kenneth Kaunda Airport area, Kasisi, Chilanga and Mimosa based on widespread cultivation and readily availability of the vegetables under investigation.

3.2. Sample Collection

Fresh vegetables were collected in polypropylene sealable bags and labeled with a unique sample identity and placed in an iced chest box. A simple random sampling method was used to select the vegetables. This was done by numbering the various selling points at a particular sampling site and every 2^{nd} selling points number were sampled. The sampling was repeated three times in different month to increase the probability of sampling different cultivation batches. The repeat was one month apart (30 days). 15 rolls cabbages, 14 bunches of lettuce and 9 bunches of rape were sampled from the study sites. These vegetables were collected between January to June, 2015. During the sample collection disposable gloves were used and changed every time before collecting the next sample. Dust from the samples was removed with light brushing, without washing prior to placing them into, the collection bags. Samples were double packed, and transported from the field to the Zambia Bureau of Standards (ZABS) Laboratory, Lusaka, Zambia. In the laboratory, samples were placed in the refrigerator at 4˚ C and analysed within 14 days of sampling. A vegetable collection sheet was used, which provided information on the date, place, and conditions of purchase of the vegetables. (Appendix 1)

3.3. Materials and reagents

All the chemicals and reagents that were used were of analytical grade. Pesticides standards were provided by ZABS. Ethyl acetate (HPLC grade) and analytical grade acetone were supplied by HIMEDIA, sodium sulphate (purities greater than 98 %) was purchased from GLASSWORLD, while sodium chloride was supplied by UNIV. Activated charcoal was purchased from Wako Pure Chemical Industries (Japan). Methanol was supplied by

RANKEN. The methanol had a boiling point of 65˚ C, viscosity of 0.54, miscible with water and dielectric constant of 32.7. Acetonitrile was supplied by Avon Chem. It had a boiling point of 82˚ C, viscosity of 0.34 cp at 25˚ C, miscible with water at 20˚ C and dielectric constant of 37.5. Dichlorvos reference standards (98.0 % purity) were obtained from AccuStandards.

3.4. Principle of chromatography

Chromatography was coined in from the Greek term meaning color which was first reported by Tswett in 1906 (Christian G.D, 1994). It is a separation technique which allows the separation of closely related compounds of complex mixtures which differ in polarities, masses, affinities, size and many more properties. The technique is made of two phases, the mobile phase and stationary phase through which the mixture's components distribute themselves differently (Christian G.D, 1994). High performance liquid chromatography (HPLC) is one of the common techniques commonly used for pesticide residue analysis. In this method separation involves the injection of a small volume of liquid sample into a tube packed with tiny particles called the stationary phase where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump. These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount. High performance liquid chromatography HPLC, has found its applications for pesticide analysis with ultraviolet and fluorescent properties. The HPLC was selected for analysis because it is used for a broad spectrum analysis, it can determine all ionic, polar, non-polar, acidic, basic, neutral and thermally unstable pesticides (Siddique et al., 2003). And a reversed phase mode high performance liquid chromatography was used for separation and identification because it is effective in separation and identification of organophosphate pesticides (Tariq et al., 2007; Hernandez-Borges et al., 2009).

3.5. Instrumentation- Shimadzu High Performance Liquid Chromatography-UV-VIS spectroscopy overview

The HPLC has 5 components namely the pump, injector, column, detector and computer. The pump forces a liquid, mobile phase through the stationary phase. The injector introduce the liquid sample into the flow stream of the mobile phase. While the column separates the components of the sample and the detector sense and measures the separated components: The detector provides an output to a recorder or computer that results in the chromatogram (i.e., the graph of the detector response) which is measured as peak area and peak height. The peak of a particular component appears at a particular time called retention time. When determining the concentration of a sample there are two main ways to interpret a chromatogram, determination of the peak height of a chromatographic peak as measured from the baseline and determination of the peak area. In order to make a quantitative assessment of the compound, a sample with a known amount of the compound of interest is injected and its peak height or peak area is measured. The accurate determination of the concentration analyte depends on a linear relationship between the height or area and the amount of sample.

The Shimadzu High Performance Liquid Chromatography (HPLC) was used in the quantification of Dichlorvos (Figure 2). It is made of Reservoir bottle, on-line degasser DGU-20A3, Solvent delivery system LC-20AB (Pump), Auto sampler (injector), Column oven, CTO-1OASVP, SPD-20AV Detector, System controller, CMB-2OA and waste collector. It uses only HPLC grade mobile phase filter with a filter of 0.45µM mesh or finer before use to remove particulate and foreign matter. The HPLC was operated on isocratic system. In this system the mobile phase is drawn out of the reservoir bottle and pumped through the tubing by pumps. The degasser removes dissolved air from the mobile phase, preventing air bubbles and consequent rise, drift or other baseline irregularity caused by dissolved air. The pump sends the mobile phase through the manual injector column and detector in that order and finally into the waste container. The samples are injected into the system by the manual injector with a syringe. In the column, the components are separated by means of mutual interactions of the mobile phase and the column packing or the stationary phase. Finally the detector detects the components eluted from the column and sends the signal data to a monitor which has LabSolutions software.

Figure 2. The HPLC and lab Solution monitor used in the analysis of the sample and generation of chromatograms.

3.6. PROCEDURE

3.6.1. Sample preparation, extraction and cleanup

Samples for cabbages, rape and lettuce were purchased from the selected sites of Lusaka city. One kg of each vegetable was collected for the laboratory analysis. The extraction of the analyte was conducted according to Sadia Ata et al. (Sadia Ata et al., 2013). Each sample was chopped in an electrical chopper and 50 g of chopped sample was taken in an Erlenmeyer flask together with 2.5g sodium chloride, 10g anhydrous sodium sulfate and 60ml ethyl acetate and mixed with a horizontal shaker for an hour followed by filtration with an ordinary filter paper. Filtered extract was cleaned using 6 g of activated charcoal. The cleaned extract was concentrated on a rotary evaporator and was then dried by bubbling nitrogen gas through it. The dried extract was then re-dissolved in an acetonitrile solvent and final extract of 10ul was injected in the HPLC.

3.6.2. HPLC Instrumentation-Extract analysis

HPLC separations were carried out on an HPLC apparatus (Shimadzu 10 Ac VP; Kyoto, Japan) consisting of a Shim-Pack VP-ODS column (150mm length, 4.6mm i.d., 5µm particle size), a UV–visible detector and a degasser system (a DGU-20A3 degasser, two LC-20AB pumps, a SIL-20AC auto sampler with volume injection capacity ranging from 0.1ul to 2,000ul, a CT0-10ASVP column oven, a SPD-20AV UV-VIS Detector and a system controller). Acetonitrile–water was used mobile phase in the ratios 60:40, v/v respectively. The HPLC analyses were carried out at column temperature 40˚C under isocratic condition at a flow rate of 1.0 $mLmin^{-1}$ using a UV wavelength of 254nm. Separation time was 15min and an additional 10min post-run time was added for reconditioning of the column to initial conditions. An injection volume of 10µL was used in all experiments. The chromatographic apparatus was controlled by Liquid Chromatography Shimadzu Lab Solutions software.

3.7. Quantification and identification of Dichlorvos residues

3.7. 1. Preparation of calibration curve

The preparation of samples was by the official methods Association of Official Analytical Chemist (AOAC, 1990). The calibration curves were constructed for target analyte by injecting four calibration standards directly into the HPLC, at the concentrations of 0.1, 0.5, 10, and 20 ug/l for Dichlorvos standards for samples with a limit of detection of 0.03 mg/kg. The calibration curve was constructed each time a new sample was analysed in order to accurately compensate for the day to day variation of the control standard. The calibration curve was constructed from the known concentration and the peak area values of the standard samples.

3.7. 2. Quantification and identification of Dichlorvos residues

To determine the quantities of Dichlorvos residues in the sample extracts an external standard method was used. The Standard Dichlorvos solutions were run and the response of the detector for each standard was determined. The area of the corresponding peak in the sample was compared with that of the standard (whose concentration is known). This comparison was automatically done using the LabSolutions software. The software computer

generated retention time of the Dichlorvos in the vegetables corresponds to the concentration of Dichlorvos in the vegetables detected by the HPLC.

3.8. Ethical consideration

Although the study will not involve human subjects or mice, clearance was sought from the University of Zambia Biomedical Research ethics committee (UNZA BREC). The clearance was granted (Assurance No. FWA 00000338, IRB00001131 of IORG0000774, dated 23rd April 2015, appendix 8). Vegetables were bought from the study sites and no ethical issues arose. The results will be disseminated to the local authority- Lusaka City Council and Food and drug Department of Ministry of Health.

CHAPTER FOUR

4.0. RESULTS

4.1. Calibration of standard Dichlorvos solution

The standard solutions of Dichlorvos of different concentrations were eluted. For preparation of a calibration curve with the standard solutions areas of peaks for every concentration were plotted against concentration (Table 4.1.1)

Table 4.1.1 Scheme used for creation of a five standard calibration solutions

Calibration level	Concentration of Dichlorvos in mg/kg	Peak area
1	0.097	25610
2	0.504	4896
3	9.756	5684
4	19.835	5654

Using the LabSolutions software linear regression was done to obtain the equation y= ax + b. to prove linearity.

The following data was obtained from the equation (Table 4.1.2)

Table 4.1. 2. Data for the linear regression obtained from the LabSolutions software

Parameter	Value
A	24.7635
B	12945.7
R	0.9070478
R2	0.8227357
RSS	2.512570e +004
Mean RF	9.343491e+003
RF SD	1.447073e+004
RF% RSD	154.875020

4.2. Levels of Dichlorvos Residues found in Lettuce from the study site

This study revealed that out of the 14 lettuce samples four of them did have detectable Dichlorvos residues, two samples had residue levels below the MRL as prescribed by the law (Zambian Food and Drug Act) and 8 samples had Dichlorvos residue above the MRL. This represents 28.57 %, 14.29 % and 57.14 %, of lettuce samples that had no Dichlorvos residues, below the MRL and above the MRL respectively (Figure 3.). The mean Dichlorvos residues in lettuce were found to be 5.23 mg/kg. The average Dichlorvos residues in lettuces were significantly higher than the MRL ($p < 0.05$) (Table 4.2.1 and appendix 2.). Pesticides residues levels (Dichlorvos residues) are defined as the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986). Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible. The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994).

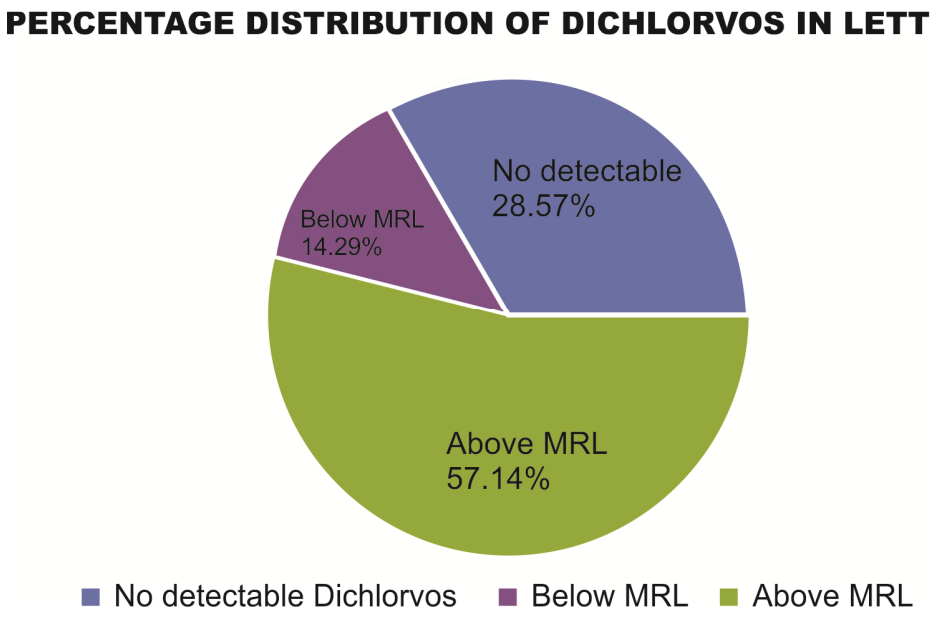


Figure 3. Proportion of lettuce vegetable samples with Dichlorvos residues above or below MRL.

KEY

- Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).
- Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the Dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible
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Table 4.2.1 Dichlorvos residue levels in lettuce

Sample	Measured level	Maximum Residue Limit (MRL)	Category
L1	**0.000**	**1.000**	**No detectable residues**
L2	**1.837**	**1.000**	**Above MRL**
L3	**0.000**	**1.000**	**No detectable residues**
L4	**7.475**	**1.000**	**Above MRL**
L5	**1.115**	**1.000**	**Above MRL**
L6	**0.000**	**1.000**	**No detectable residues**
L7	**16.740**	**1.000**	**Above MRL**
L8	**11.110**	**1.000**	**Above MRL**
L9	**0.000**	**1.000**	**No detectable residues**
L10	**0.504**	**1.000**	**Below MRL**
L11	**3.643**	**1.000**	**Above MRL**
L12	**0.049**	**1.000**	**Below MRL**
L13	**8.284**	**1.000**	**Above MRL**
L14	**22.448**	**1.000**	**Above MRL**

Table comparing the maximum Dichlorvos residue and measured Dichlorvos residues in lettuce samples

Key:

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4.3. Levels of Dichlorvos Residues found in cabbage from the study site

This study revealed that out of the 15 cabbage samples nine of them had Dichlorvos residues below the maximum residue limit as prescribed by the law (Zambian Food and Drug Act) and 6 samples had Dichlorvos residue above the MRLs. This represents 60.0 %, and 40.0 % of cabbage samples with Dichlorvos residues below and above the MRLs respectively (Figure 4.). The mean residue levels in cabbage were found to be 6.35 mg/kg. The average Dichlorvos residue of 6.35 mg/kg was on the borderline between significant and non-significant (p=0.05) (Table 4.2.2 and appendix 3)

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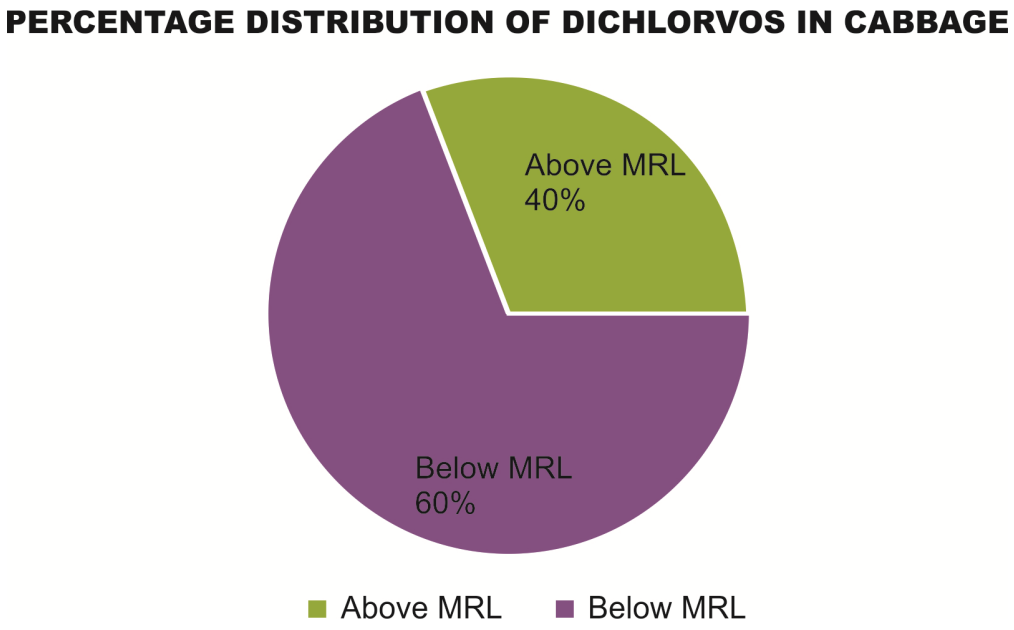


Figure 4. Proportion of cabbage samples with Dichlorvos residues above or below MRL.

KEY:

- Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).
- Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the Dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible
- The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994).

Table 4.2.2 Shows the Dichlorvos' residue levels for Cabbage

Sample	Measured level	Maximum Residue Limits (MRL)	Category
C1	0.6555	1.000	Below the MRL
C2	0.919	1.000	Below the MRL
C3	0.184	1.000	Below the MRL
C4	1.165	1.000	Above the MRL
C5	0.231	1.000	Below the MRL
C6	0.298	1.000	Below the MRL
C7	3.783	1.000	Above the MRL
C8	0.538	1.000	Below the MRL
C9	28.463	1.000	Above the MRL
C10	0.860	1.000	Below the MRL
C11	0.281	1.000	Below the MRL
C12	11.801	1.000	Above the MRL
C13	20.66	1.000	Above the MRL
C14	24.51	1.000	Above the MRL
C15	0.91	1.000	Below the MRL

Table comparing the maximum dichlorvos residue and measured Dichlorvos residues in cabbage samples.

Key:

Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).

Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect.

The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994).

4.4. Levels of Dichlorvos Residues found in rape from the study site

This study revealed that out of the 9 rape samples all of them had Dichlorvos residues level above the maximum residue limit as prescribed by the law (Zambian Food and Drug Act). This represents 100.0 % of rape samples had Dichlorvos residues above the MRLs. The mean Dichlorvos residue in rape was found to be 398.28 mg/kg. The average Dichlorvos levels in rape vegetables were significantly higher than the MRL (Table 4.2.3 and appendix 4)

KEY:

Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).

Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible

The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994

Table 4.2.3 Shows the Dichlorvos' residue levels for rape

Samples	Measured level	Maximum Residue Level (MRL)	Category
R1	412.00	1.000	Above the MRL
R2	208.230	1.000	Above the MRL
R3	23.942	1.000	Above the MRL
R4	616.480	1.000	Above the MRL
R5	430.360	1.000	Above the MRL
R6	336.179	1.000	Above the MRL
R7	659.244	1.000	Above the MRL
R8	876.839	1.000	Above the MRL
R9	21.267	1.000	Above the MRL

Table comparing the maximum Dichlorvos residue and measured Dichlorvos residues in rape samples

Key:

Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).

Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the Dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible

The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994

4.5. **The mean levels of Dichlorvos Residues found in the vegetables from the study site**

This study revealed that the mean values of Dichlorvos residues in the vegetables were 5.228, 6.350 and 398.28 mg/kg for lettuce, cabbage and rape respectively (Figure 5.). It is evident that the mean levels of Dichlorvos in the three vegetables are significantly higher than the accepted maximum residue limit. This study also revealed that out of the 38 vegetables samples 10 of them had Dichlorvos residues level below the maximum residue limit as prescribed by the law (Zambian Food and Drug Act) and 24 samples had Dichlorvos residue above the MRLs. This represents 63.15 %, and 26.32 % of samples had Dichlorvos residues above and below the MRLs respectively. There are 4 samples that did not have Dichlorvos residues representing 10.53 % of the samples.

Key:

Pesticides residues levels (Dichlorvos residues) are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).

Detectable pesticides (Dichlorvos) residues refer to the pesticides residue level that instrument of detection (HPLC) is able to detect. The Shimadzu High Performance Liquid Chromatography (HPLC) that was used for detecting the Dichlorvos had a limit of detection of 0.003mg/kg. This means that any Dichlorvos level less than 0.003mg/kg was undetectable and is very negligible

The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994

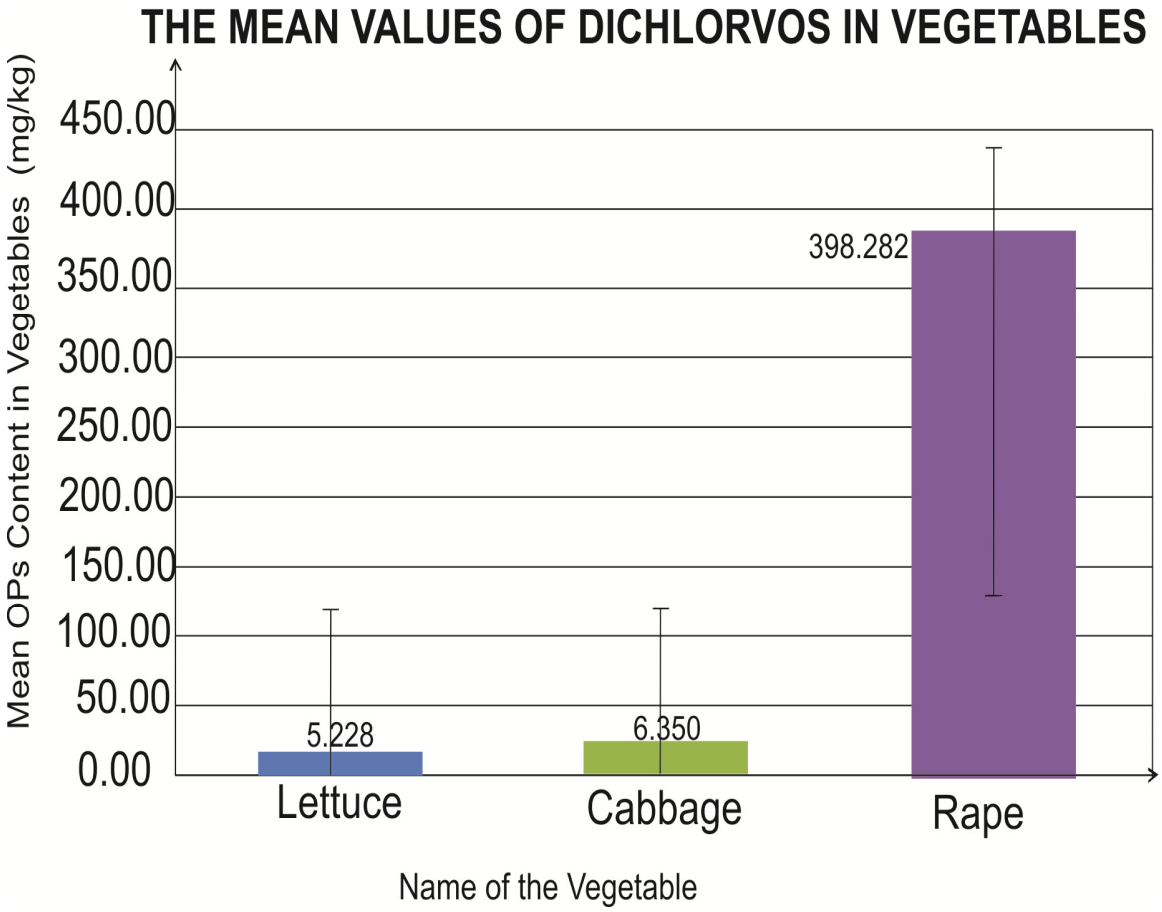


Figure 5. Average Dichlorvos levels in lettuce, cabbage and rape

Table 4.2.4 Shows the mean Dichlorvos' residue levels for vegetable samples

Sample	**Mean Dichlorvos residues (mg/kg)**	**Maximum Residue Level (MRL) (mg/kg)**	**Category**
Lettuce	5.228	1.000	Above the MRL
Cabbage	6.350	1.000	Above the MRL
Rape	398.28	1.000	Above the MRL

Table comparing the maximum Dichlorvos residue Limit (MRL) and average Dichlorvos residues in vegetable samples

Key:

Pesticides residues levels are the small amounts of active pesticides compounds that may remain in or on food and in the environment after being used in killing pests (Hill and Comardese, 1986).

The maximum residual levels (MRLs) are legal guidelines used to determine whether pesticide residue levels in agricultural crops are within acceptable limits in relation to human and environmental health (FAO/WHO (1993); Zambian Food and Drug Act, 1994).

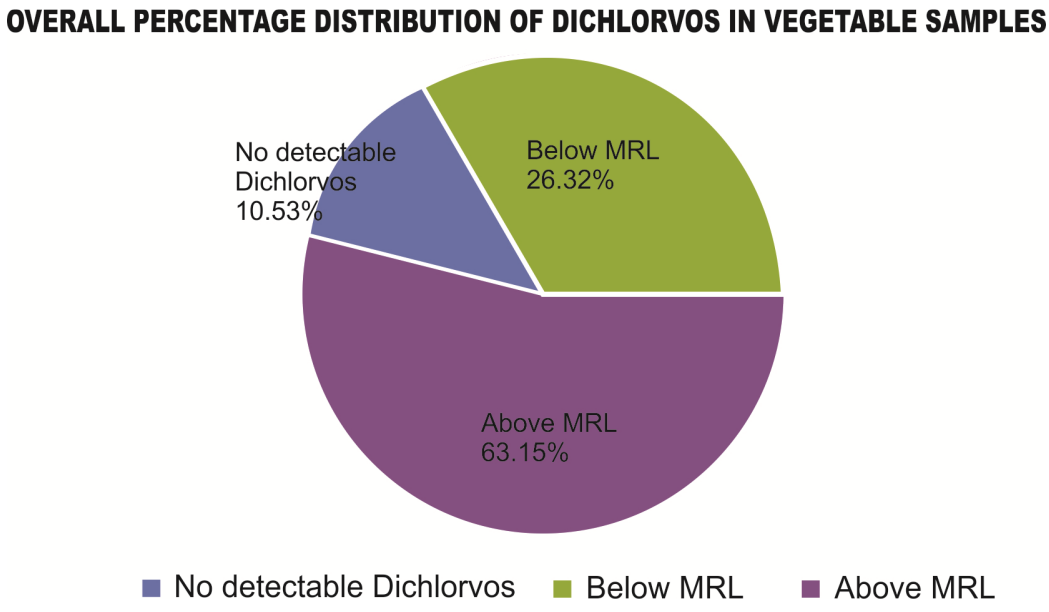


Figure 6. Overall % distribution of Dichlorvos in the three vegetables

CHAPTER FIVE

DISCUSSION

On average the Dichlorvos residues that were recorded in the vegetables were higher than the Food and Drugs Act (Zambian), FAO and WHO recommended standard values. The mean Dichlorvos residue levels were highest in rape followed by cabbage and then lettuce samples. This high level of Dichlorvos could be due to the misapplication such as over applying the pesticides and the indiscriminate use of chemical pesticides to achieve higher vegetable yields. Improper disposal of insecticide containers that bring about the liberation of these insecticide residues may also be a contributing factor. This improper use of pesticides may lead to emergency of resistant pest populations, which would be insensitive to normal pesticide dosage (Bardin et al., 2015, Li, 2009).

It is evident that there are high levels of Dichlorvos in vegetables consumed in Zambia. It is highly likely that this could be due to lack of following proper methods of spraying vegetables, use of fault equipment and not following proper guidelines on when to consume vegetables that have just been sprayed (Wang et al., 2012). It is therefore important that levels of Dichlorvos are ascertained in order to protect human health due to cumulative effect of the pesticides (De Silva et al., 2006, Darko and Akoto, 2008). In addition pesticides like Dichlorvos have also been shown to have a detrimental effect on soil microflora and fauna. According to this study rape is a bigger accumulator of Dichlorvos followed by cabbage and lettuce. Rape may apparently appear so possibly because rape sells faster on the market and in order to increase profit margin farmers may be applying their insecticides on rape more aggressively. There is also a possibility that rape may be supplied on the market much earlier than the minimum time that is allowed between spraying and harvesting.

The higher levels of Dichlorvos in vegetables as revealed by this study imply that the vegetables sold in the study site do not meet the standards as set by Zambian Food and drugs Act. The other implication is that higher levels of Dichlorvos in vegetable may accumulate in the consumers which have a potential to gradually affect the health and wellbeing of consumers. For instance studies have shown that even low level chronic exposure to organophosphates can cause neuropsychiatric conditions (Mackenzie Ross et al., 2010). This though was not the case in one study where no association was observed between the behavioral score in children and exposure to low levels of organophosphates (Oulhote and

Bouchard, 2013). Perhaps even more worrying is the possible link between organophosphates and the development of obesity and type 2 diabetes mellitus as reported in one study (Slotkin, 2011). The suggested mechanism through which organophosphate residues may lead to Diabetes Mellitus type 2 is that they have an effect on glucose metabolism. Studies have revealed that organophosphate such as Dichlorvos may increase the activity of glycogen phosphorylase (GP) and phosphoenolpyruvate carboxykinase (PEPCK) which are the vital liver and brain enzymes that convert non carbohydrate compounds and glycogen to glucose respectively (Martin and Husain, 1987; Martin et al,. 1990; Sarin and Gill 2000). The likely mechanism for obesity is that organophosphate pesticides may initiate increase in rate of growth of immature fat cell to mature fat cells (Chadwick et al,. 1988). Other studies reveal that organophosphates may induces hypothyroidism and euthyroid syndrome which both cause abnormal weight gain (Satar s et al., 2005). Furthermore studies show that Dichlorvos inhibits the plasma enzymes butyrylcholinestrase and lipase leading to increase in triglycerides (Kozlowska et al 1990, Goldberg I.L. et al., 1982). Emphasis has been on diet, life style, genetics as factors associated with obesity and type 2 diabetes mellitus but the possible role of organophosphates in etiology of the two conditions has not received much attention. It is plausible to think that nearly every person shows organophosphate residues in their bodies and that most exposures are below that threshold and thus go unnoticed and undetected (Casida and Quistad, 2004).

Therefore the higher levels of Dichlorvos in vegetables may lead to increased prevalence of obesity and type 2 diabetes especially in urban area may in part be due to pesticide residues in fruits and vegetable eaten. There has been a general decline in fertility in many countries as manifested by declining sperm counts. In one study which looked at the effect of food pesticide residues on sperm count it was found that there was a negative correlation between pesticide levels in semen and the sperm count (James, 1985), which suggests that pesticides may be responsible to some extent for the worldwide decline in fertility. Organophosphates have also been linked to malignancy, Parkinson's disease. Findings of a study in France suggested that there was neurologic impairments in elderly persons who were exposed occupationally to pesticides (Baldi et al., 2003). Another study carried out in France showed

that pesticides in vineyards contributed to mortality from brain cancer among farmers (Viel et al., 1998)

It must be underscored that illiteracy, poor or bad attitudes of farmers on dangers of pesticides could contribute to higher levels of Dichlorvos in vegetables. For instance some farmers may be mixing one pesticide with the other in the pursuit of higher concentration of pesticide that is more effective in eradicating the pests without realizing the risk to consumer (Orr and Ritchie; 2004).

The higher levels of Dichlorvos could be due to lack of following Good Agricultural Practices such as not following the recommended withdraw period, which is the minimum time one must wait between applying a pesticide and final harvesting of the crop or vegetable (Al-Wabel et al, 2011)..

In Zambia, laws have been enacted and regulatory agencies have been established that regulate the importation of pesticides and pesticide residues in the vegetables (Zambian Food and Drugs Act). However lack of effective implementation of these laws may contribute to higher levels of Dichlorvos in the vegetables. The enforcement agencies have to endure logistical constraints like poor funding, lack of transport and manpower. Furthermore the cost of conducting pesticides residue monitoring is prohibitive for the local authorities to embark on. To crown it all, these agencies may be faced with lack of clearly defined policy and programs on how and when the monitoring of the pesticide residue may be conducted in vegetables.

The study had a number of limitations which included inadequate funding which restricted study to four areas within Lusaka. Soil samples from the farms were not analyzed for the presence of Dichlorvos due to prohibitive costs that were going to be incurred. Furthermore it is not clear whether the sampled vegetables were harvested from farms where owners followed good agricultural practices such following appropriate withdrawal period. This study only looked at Dichlorvos and only three vegetable. Therefore it is highly recommended that another study be conducted that will include a variety of pesticides and a variety of fruits and vegetables.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMEDATION

6.1. Conclusion

This study has found that out of the 38 vegetables sampled (15 cabbages, 9 rape and 14 lettuce) 63.15 % had Dichlorvos residues well above the safe limit, 26.32 % contained Dichlorvos below the safety limit and 10.53 % did not contain any Dichlorvos residues. The vegetable rape had the highest mean residue levels while lettuce had the lowest. The mean levels for Dichlorvos (DDVP) in cabbages, lettuce, and rape are 6.35, 5.23 and 398.28 mg/kg respectively. Generally this study agrees with studies conducted in the sub region and internationally which found that in general that the organophosphate residues in vegetables and fruits are higher than the MRLs (Elliion, J. et al., 2000; Mukherjee, L., and Gospal, M. , 2003; Taneja, A., 2005). The mean residue levels in all samples were found to be 98.76 mg/kg.

In conclusion, the studies reveals that the three vegetables contained Dichlorvos and the residues was well above the maximum residue limit as set by the Zambian Food and Drugs Act .

6.2. Recommendations

1. As an outcome of this study, it is suggested that a wide range of studies on monitoring of all vegetables should be undertaken for determining the levels of pesticides and pesticide residues.
2. It is suggested that one reason for higher levels of residues is lack of monitoring of pesticides residues in vegetables at the point of sale by the Authorities; it is therefore recommended that inspectorate system of the Local council be funded and strengthened to be able to conduct monitoring of pesticides residues in vegetables at the point of sale.
3. It is recommended that a simpler method of detecting levels of organophosphate in vegetable other than HPLC be identified.

REFERENCES

AL-Wabel, M.I, M.H.El- Saeid, A.M. Al-Turki And G. Abdel-Nasser, 2011. monitoring of pesticide residues in Saudi Arabia agricultural soils.. Res J. environ. sci., 5: (2011) 269-278.

Akoto, O., Andoh, H., Darko, G., Eshun, K. and Osei-Fosu, P., 2013. Health risk assessment of pesticides residue in maize and cowpea from Ejura, Ghana. *Chemosphere*, *92*(1), pp.67-73.

Aysal, P., Ambrus, A., Lehotay, S. J. & Cannavan, A. 2007. Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *J Environ Sci Health B,* 42**,** 481-90.

Baldi, I., Lebailly, P., Mohammed-Brahim, B., Letenneur, L., Dartigues, J. F. & Brochard, P. 2003. Neurodegenerative diseases and exposure to pesticides in the elderly. *Am J Epidemiol,* 157**,** 409-14.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M. & Nicot, P. C. 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Front Plant Sci,* 6**,** 566.

Casida, J. E. & Quistad, G. B. 2004. Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. *Chem Res Toxicol,* 17**,** 983-98.

Castorina, R., Bradman, A., Mckone, T. E., Barr, D. B., Harnly, M. E. & Eskenazi, B. 2003. Cumulative organophosphate pesticide exposure and risk assessment among pregnant women living in an agricultural community: a case study from the CHAMACOS cohort. *Environ Health Perspect,* 111**,** 1640-8.

Chagnon, M., Kreutzweiser, D., Mitchell, E. A., Morrissey, C. A., Noome, D. A. & Van Der Sluijs, J. P. 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res Int,* 22**,** 119-34.

Colovic, M. B., Krstic, D. Z., Lazarevic-Pasti, T. D., Bondzic, A. M. & Vasic, V. M. 2013. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr Neuropharmacol,* 11**,** 315-35.

Darko, G. & Akoto, O. 2008. Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food Chem Toxicol,* 46**,** 3703-6.

De Silva, H. J., Samarawickrema, N. A. & Wickremasinghe, A. R. 2006. Toxicity due to organophosphorus compounds: what about chronic exposure? *Trans R Soc Trop Med Hyg,* 100**,** 803-6.

Esturk, O., Yakar, Y. & Ayhan, Z. 2014. Pesticide residue analysis in parsley, lettuce and spinach by LC-MS/MS. *J Food Sci Technol,* 51**,** 458-66.

Gilden, R. C., Huffling, K. & Sattler, B. 2010. Pesticides and health risks. *J Obstet Gynecol Neonatal Nurs,* 39**,** 103-10.

Guler, G. O., Cakmak, Y. S., Dagli, Z., Aktumsek, A. & Ozparlak, H. 2010. Organochlorine pesticide residues in wheat from Konya region, Turkey. *Food Chem Toxicol,* 48**,** 1218-21.

Helrich, K.C., 1990. Official methods of Analysis of the AOAC. Volume 2 (No. Ed. 15). Association of Official Analytical Chemists Inc.

Hoffman, R. & Gerber, M. 2015. Food Processing and the Mediterranean Diet. *Nutrients,* 7**,** 7925-7964.

James, W. H. 1985. Pesticide residues in food: possible effect on sperm counts. *J R Soc Med,* 78**,** 416.

Jin, S., Sarkar, K. S., Jin, Y. N., Liu, Y., Kokel, D., Van Ham, T. J., Roberts, L. D., Gerszten, R. E., Macrae, C. A. & Peterson, R. T. 2013. An in vivo zebrafish screen identifies organophosphate antidotes with diverse mechanisms of action. *J Biomol Screen,* 18**,** 108-15.

Latif, Y., Sherazi, S. T. & Bhanger, M. I. 2011. Assessment of pesticide residues in commonly used vegetables in Hyderabad, Pakistan. *Ecotoxicol Environ Saf,* 74**,** 2299-303.

LI, S. G. 2009. [Drug resistance evolution of dichlorvos-resistant and cypermethrin-resistant strains of Culex pipiens pallens]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi,* 27**,** 311-2.

Lozowicka, B. 2015. Health risk for children and adults consuming apples with pesticide residue. *Sci Total Environ,* 502**,** 184-98.

Mackenzie Ross, S. J., Brewin, C. R., Curran, H. V., Furlong, C. E., Abraham-Smith, K. M. & Harrison, V. 2010. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. *Neurotoxicol Teratol,* 32**,** 452-9.

Meyer, A., Seidler, F. J., Aldridge, J. E., Tate, C. A., Cousins, M. M. & Slotkin, T. A. 2004a. Critical periods for chlorpyrifos-induced developmental neurotoxicity: alterations in adenylyl cyclase signaling in adult rat brain regions after gestational or neonatal exposure. *Environ Health Perspect,* 112**,** 295-301.

Meyer, A., Seidler, F. J., Cousins, M. M. & Slotkin, T. A. 2003. Developmental neurotoxicity elicited by gestational exposure to chlorpyrifos: when is adenylyl cyclase a target? *Environ Health Perspect,* 111**,** 1871-6.

Meyer, A., Seidler, F. J. & Slotkin, T. A. 2004b. Developmental effects of chlorpyrifos extend beyond neurotoxicity: critical periods for immediate and delayed-onset effects on cardiac and hepatic cell signaling. *Environ Health Perspect,* 112**,** 170-8.

Nyirenda, S.P., Sileshi, G.W., Belmain, S.R., Kamanula, J.F., Mvumi, B.M., Sola, P., Nyirenda, G.K. and Stevenson, P.C., 2011. Farmers' ethno-ecological knowledge of vegetable pests and pesticidal plant use in Malawi and Zambia. African Journal of Agricultural Research, 6(6), pp.1525-1537.

Oulhote, Y. & Bouchard, M. F. 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. *Environ Health Perspect,* 121**,** 1378-84.

Shiff, C. J. & Garnett, B. 1961. The short-term effects of three molluscicides on the microflora and microfauna of small, biologically stable ponds in Southern Rhodesia. *Bull World Health Organ,* 25**,** 543-7.

Slotkin, T. A. 2011. Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity? *Reprod Toxicol,* 31**,** 297-301.

Tsoutsi, C., Konstantinou, I., Hela, D. & Albanis, T. 2006. Screening method for organophosphorus insecticides and their metabolites in olive oil samples based on headspace solid-phase microextraction coupled with gas chromatography. *Anal Chim Acta,* 573-574**,** 216-22.

Viel, J. F., Challier, B., Pitard, A. & Pobel, D. 1998. Brain cancer mortality among French farmers: the vineyard pesticide hypothesis. *Arch Environ Health,* 53**,** 65-70.

Wang, J. L., Xia, Q., Zhang, A. P., Hu, X. Y. & Lin, C. M. 2012. Determination of organophosphorus pesticide residues in vegetables by an enzyme inhibition method using alpha-naphthyl acetate esterase extracted from wheat flour. *J Zhejiang Univ Sci B,* 13**,** 267-73.

Ye, M., Beach, J., Martin, J. W. & Senthilselvan, A. 2013. Occupational pesticide exposures and respiratory health. *Int J Environ Res Public Health,* 10**,** 6442-71.

Yokoyama, Y., Barnard, N. D., Levin, S. M. & Watanabe, M. 2014. Vegetarian diets and glycemic control in diabetes: a systematic review and meta-analysis. *Cardiovasc Diagn Ther,* 4**,** 373-82.

Zhang, H., Liu, J. & Pope, C. N. 2002. Age-related effects of chlorpyrifos on muscarinic receptor-mediated signaling in rat cortex. *Arch Toxicol,* 75**,** 676-84.

Appendix 1. Vegetable data collection tool.

UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

PHYSIOLOGICAL SCIENCES DEPARTMENT

To be entered by the researcher/research assistants

(i) Name of the site where vegetables are collected: ……

(ii) Type of source of vegetables

Supermarket/traditional market/market adjacent to a farm (Circle the appropriate answer)

(iii) How much is collected: Number of balls (e.g. 1 cabbage)

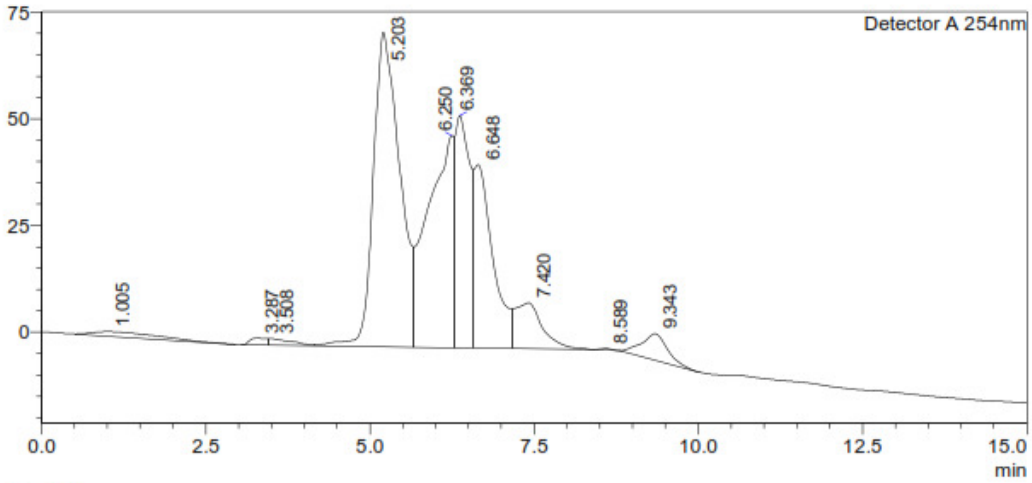
(iv) Date of collection: …….

Name of Veg	Number of samples collected	Physical condition of Vegetables	Type of cabbages

(v) Name of the collector ……..

(vi) Signature of collector ……

Appendix 2. Chromatogram for lettuce generated by the labSolution software.



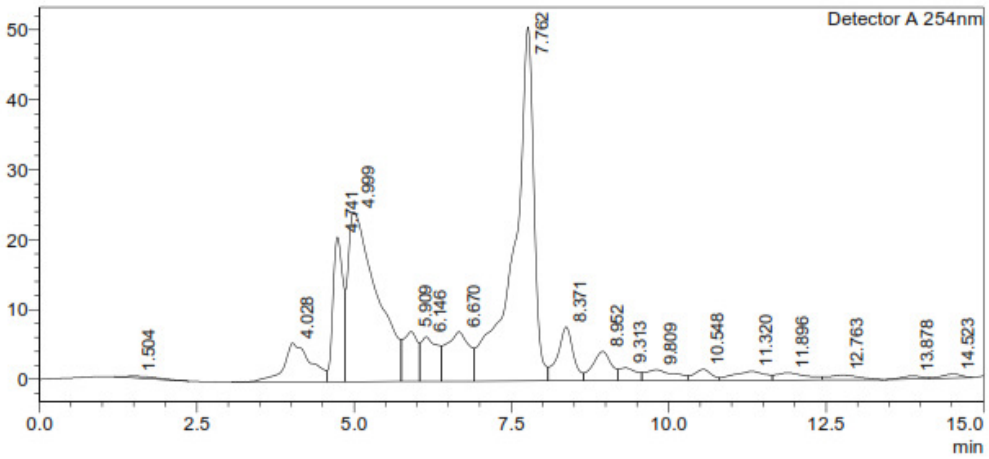
<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.005	80451	1026	0.000			
2	3.287	28190	1594	0.000			
3	3.508	37746	1528	0.000		V	
4	5.203	2070560	73667	0.000		V	
5	6.250	1352368	49656	0.000		V	
6	6.369	851362	54456	0.000		V	
7	6.648	859068	42997	0.000		V	
8	7.420	308150	10735	0.000		V	
9	8.589	4401	370	0.504	mg/kg		DICHLORVOS
10	9.343	172506	6236	0.000			

Chromatogram and peak tables for lettuce generated by the labSolution software.

Appendix 3. Chromatogram for cabbage generated by the labSolution software.



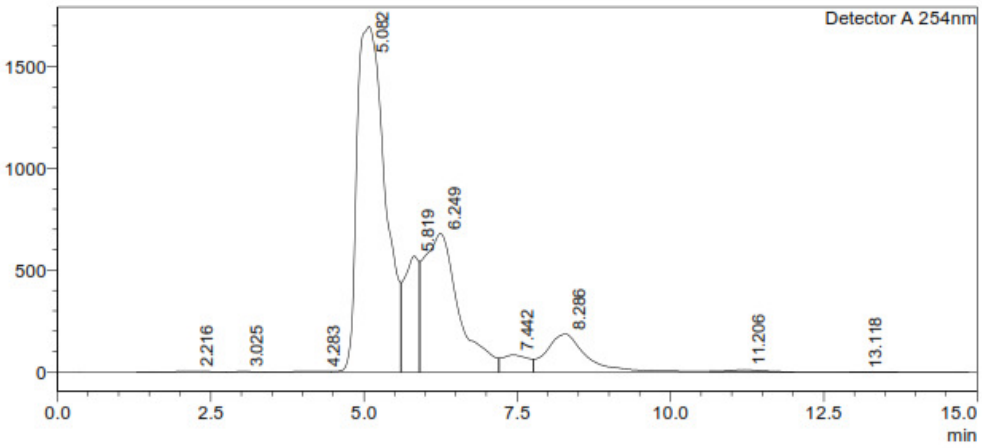
<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.504	9888	242	0.000			
2	4.028	165995	5563	0.000			
3	4.741	231952	20698	0.000		V	
4	4.999	761099	24237	0.000		V	
5	5.909	111925	7150	0.000		V	
6	6.146	118217	6363	0.000		V	
7	6.670	180150	7069	0.000		V	
8	7.762	1101198	50620	0.000		V	
9	8.371	132821	7685	0.000		V	
10	8.952	86845	4184	0.000		V	
11	9.313	34339	1820	0.298	mg/L	V	DICHLORVOS

Chromatogram and peak tables for cabbage generated by the labSolution software.

Appendix 4. Chromatogram for rape generated by the labSolution software.



<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.216	207108	4121	0.000			
2	3.025	98078	3754	0.000		V	
3	4.283	204583	4524	0.000		V	
4	5.082	53483619	1698333	0.000		V	
5	5.819	9593246	571158	0.000		V	
6	6.249	27163821	680836	0.000		V	
7	7.442	2524885	85022	0.000		V	
8	8.286	9047178	187178	-430.360	mg/kg	SV	DICHLORVOS
9	11.206	283275	8748	0.000		T	
10	13.118	4232	145	0.000		T	
Total		102610025	3243818				

Chromatogram and peak tables for rape generated by the labSolution software

Appendix 5. Statistical analysis for lettuce.

SAMPLE	x	ẍ =∑x/n	(x- ẍ)	$(x- ẍ)^2$	$\sum (x- ẍ)^2$ /N-1	S=√E$(x- ẍ)^2$ /N-1
L1	**0**	5,228928571	-5,228929	27,34169401	51,3411253	7,165272172
L2	1,837	5,228928571	-3,39193	11,50517943	51,3411253	7,165272172
L3	**0**	5,228928571	-5,22893	27,34169401	51,3411253	7,165272172
L4	7,475	5,228928571	2,246071	5,044836862	51,3411253	7,165272172
L5	1,115	5,228928571	-4,11393	16,92440829	51,3411253	7,165272172
L6	**0**	5,228928571	-5,22893	27,34169401	51,3411253	7,165272172
L7	16,74	5,228928571	11,51107	132,5047654	51,3411253	7,165272172
L8	11,11	5,228928571	5,881071	34,58700115	51,3411253	7,165272172
L9	**0**	5,228928571	-5,22893	27,34169401	51,3411253	7,165272172
L10	0,504	5,228928571	-4,72493	22,32495001	51,3411253	7,165272172
L11	3,643	5,228928571	-1,58593	2,515169434	51,3411253	7,165272172
L12	0,049	5,228928571	-5,17993	26,83166001	51,3411253	7,165272172
L13	8,284	5,228928571	3,055071	9,333461434	51,3411253	7,165272172
L14	22,448	5,228928571	17,21907	296,4964209	51,3411253	7,165272172
∑x/n				$\sum(x- ẍ)^2$ =667,4346289		

From the calculation the following computed mean residues concentration (mg/kg) was 5.23mg/kg and Standard deviation was 7.165. The t -test for a population mean in which the variance is not known was used to analyze the result. The objective was to investigate the significance of the difference between an assumed population mean μ0 and a sample mean ¯x. The assumption made was that the population was normally distributed. The T test is given by

$$t = \frac{\mathrm{x} - \mathrm{u}}{\mathrm{s}\sqrt{\mathrm{n}}}$$

Where x is sample mean

μ is population assumed mean

s is the standard deviation

n is the sample size

Calculations:

t =5.23-1/1.9152 = 4.23/1.9152= **2.20 (α=0.05)**

Our computed t value is +2.20 and acceptance region -2.160 < t < 2.160. We reject the null hypothesis and accept the alternative hypothesis of a difference between the sample and population means. Therefore the levels of dichlorvos are significantly higher in lettuce samples than the MRL

Appendix 6. Statistical analysis for cabbage.

SAMPLE	x	ẍ	(x- ẍ)	$(x- ẍ)^2$	$\sum (x- ẍ)^2$ /N-1	$S=\sqrt{E(x- ẍ)^2}$ /N-1
C1	0,6555	6,350566667	-5,69507	32,43378434	99,36363546	9,968130991
C2	0,919	6,350566667	-5,43157	29,50191645	99,36363546	9,968130991
C3	0,184	6,350566667	-6,16657	38,02654445	99,36363546	9,968130991
C4	1,165	6,350566667	-5,18557	26,89010165	99,36363546	9,968130991
C5	0,231	6,350566667	-6,11957	37,44909619	99,36363546	9,968130991
C6	0,298	6,350566667	-6,05257	36,63356325	99,36363546	9,968130991
C7	3,783	6,350566667	-2,56757	6,592398588	99,36363546	9,968130991
C8	0,538	6,350566667	-5,81257	33,78593125	99,36363546	9,968130991
C9	28,463	6,350566667	22,11243	488,9597079	99,36363546	9,968130991
C10	0,86	6,350566667	-5,49057	30,14632232	99,36363546	9,968130991
C11	0,281	6,350566667	-6,06957	36,83963952	99,36363546	9,968130991
C12	11,801	6,350566667	5,450433	29,70722352	99,36363546	9,968130991
C13	20,66	6,350566667	14,30943	204,7598823	99,36363546	9,968130991
C14	24,51	6,350566667	18,15943	329,765019	99,36363546	9,968130991
C15	0,91	6,350566667	-5,44057	29,59976565	99,36363546	9,968130991
∑x/n				$\sum(x- ẍ)^2$ =1391.0909		

From the calculation the following were computed mean residues concentration (mg/kg) was 6.350mg/kg and Standard deviation was 9.968. The T test is given by

$$t = \frac{\mathrm{x} - \mathrm{u}}{\mathrm{s}\sqrt{\mathrm{n}}}$$

Where x is sample mean

μ is population assumed mean

s is the standard deviation

n is the sample size

Calculations:

t =6.350-1/2.574= 5.350/2.574= 2.08 (α=0.05)

Our computed t value is +2.08 and acceptance region -2.160 < t < 2.160. We accept the null hypothesis no difference between the sample and population means. There is no significant difference in the organophosphate residues in cabbage and the assumed mean.

Appendix 7. Statistical analysis for rape.

SAMPLE	x	ẍ	(x- ẍ)	$(x- ẍ)^2$	$\sum (x- ẍ)^2 /N-1$	$S=\sqrt{E(x- ẍ)^2 /N-1}$
R1	412	398,2823333	13,71767	188,1743788	83524,10096	289,0053649
R2	208,23	398,2823333	-190,052	36119,88941	83524,10096	289,0053649
R3	23,942	398,2823333	-374,34	140130,6852	83524,10096	289,0053649
R4	616,48	398,2823333	218,1977	47610,22174	83524,10096	289,0053649
R5	430,36	398,2823333	32,07767	1028,976699	83524,10096	289,0053649
R6	336,179	398,2823333	-62,1033	3856,824011	83524,10096	289,0053649
R7	659,244	398,2823333	260,9617	68100,99147	83524,10096	289,0053649
R8	876,839	398,2823333	478,5567	229016,4832	83524,10096	289,0053649
R9	21,267	398,2823333	-377,015	142140,5616	83524,10096	289,0053649
∑x			$\sum (x- ẍ)^2=$	668192.8		

From the calculation the following were computed mean residues concentration (mg/kg) was 398.28mg/kg and Standard deviation was 289.00. The T test is given by

$$t = \frac{\mathrm{x} - \mathrm{u}}{\mathrm{s}\sqrt{\mathrm{n}}}$$

Where x is sample mean

μ is population assumed mean

s is the standard deviation

n is the sample size

Calculations:

t =398.28-1/96.33 = 397.28/96.33= 4.12 **(α=0.05)**

Our computed t value is +4.12 and acceptance region -2.160 < t < 2.160. We reject the null hypothesis and accept the alternative hypothesis of a difference between the sample and

population means. There is a significant difference in the organophosphate residues in rape and the assumed mean.

Appendix 8. Biomedical Sciences Research Ethics approval.