DETERMINATION OF SELECTED ACARICIDE RESIDUES IN SOILS AND WATER RESOURCES AROUND CATTLE DIP TANKS IN MBARARA DISTRICT, WESTERN UGANDA

BY

SSENOGA ERIC

16/U/13368/GMCH/PE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN CHEMISTRY DEGREE OF KYAMBOGO UNIVERSITY

OCTOBER, 2019

Declaration

I, SSENOGA ERIC hereby declare to the best of my knowledge and belief, except for literature cited, that this is my original work and has never been submitted to this or any other University or institute of higher learning for any degree a ward.

SIGNATURE…….

DATE:…….

Approval

This is to certify that this dissertation work has been done under the guidance and supervision of the following

Name: **DR. WILLIAM WANASOLO.**

Signature…….

Date:…….....

DR. JUSTUS MASA

Signature: 

Date: 23.10.2019

Acknowledgements

I wish to express my sincere gratitude to my supervisors Dr. William Wanasolo and Dr. Justus Masa who played a tremendous role and provided guidance, advice, support and constant enthusiasm throughout this work. I would also like to thank them for proofreading this work and making the necessary corrections during the study right from the proposal preparation to the dissertation write up. I would also like to acknowledge Dr. Nicolus Natukunda of Norbrook Pharmaceutical Laboratories Mbarara branch for his assistance during sample collection from the study area. I wish to appreciate the contribution of Dr. Emmanuel Tebandeke from Makerere University for his tireless efforts during data analysis and interpretation.

Special gratitude to my employer Norbrook Pharmaceutical Laboratories for the financial support during my study.

Sincere thanks go to all lecturers of the MSc. Chemistry program at Department of Chemistry Kyambogo University.

Dedication

This study is dedicated to my daughter Nalubwama Lillian and my wife Nabukenya Winfred.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ND	Not Detected
GC/FID	Gas Chromatography, Flame Ionization Detector
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MRL	Maximum Residue Level
OCPs	Organochlorine Pesticides
OPPs	Organophosphate Pesticides
TBD	Tick-borne disease

ABSTRACT

This study determined the levels of selected acaricide residues in soils and water samples around cattle dips in Mbarara District Western Uganda. Samples were collected, prepared and analysed for amitraz, chlorpyrifos and cypermethrin residues by gas chromatography equipped with a Flame Ionization Detector (GC/FID). The obtained data revealed evidence of amitraz, chlorpyrifos and cypermetherine contributing to the presence of acaricide residues in the area. The observed quantities were higher than United States permissible maximum residue limits (MRL) for agricultural soils. The amitraz, chlorpyrifos and cypermethrin residue concentrations at the sampled sites are high near the dip and reduce with all investigated distance of 25 m, 50 m and 75 m away from the cattle dip tank. This could be as a result of the cattle carrying some acaricide as they move away from the cattle dip tank and not all the acaricides flow back into the cattle dip tank.

The data obtained from obtained from analysis of samples indicated presence of amitraz, chlorpyrifos and cypermetherin present in the area for all the studied farms.

There was a significant difference in amitraz residue concentration in water samples from farms A compared to farms B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as acaricide in these particular farms

At farms D and E, only chlorpyrifos and cypermethrin residues were detected and no amitraz residues was detected at any of the studied distances away from the cattle dip tanks.

The data obtained from water resources showed that only amitraz residues were detected from water samples collected from farms A, B, and C at all studied distances away from the cattle dip tanks with no Chlorpyrifos and cypermethrin being detected at the three farms A, B and C

At farms D and E, only Chlorpyrifos and Cypermethrin residues were reported from water samples collected at farms E and D and no amitraz residues was detected at any of the studied distances away from the cattle dip tanks.

The study from cattle dip samples showed that amitraz was found in the three dip tanks from farms A, B, and C while Chlorpyrifos and cypermethrine were detected from farms D and E.

Farm A had the highest amitraz concentration, followed by farm B and then farm C. All the concentrations exceeded the allowable maximum standard values / Normal range of 250 ± 5 ppm of amitraz inside the cattle dip tank an indication of overdosing of the cattle dip tanks by farmers.

At farms D and E, both Chlorpyrifos and cypermethrin were reported with farm E having the highest concentration of Chlorpyrifos and cypermethrine followed by farm D with concentration of Chlorpyrifos and cypermethrine. All the concentrations exceeded the allowable maximum standard values / Normal range of 500 ± 5 ppm Chlorpyrifos and 50 ± 5 ppm for cypermethrin inside the cattle dip tank an indication of overdosing of the cattle dip tanks.

CHAPTER ONE

INTRODUCTION

1.1 Background

Agriculture has for several years formed the backbone of Uganda's economy contributing to approximately 43% of the Gross Domestic Product (GDP) and over 80% of the total population derive its livelihood from this sector (Kyomuhendo, 2002). Agricultural products contribute nearly to all of Uganda's foreign exchange earnings, with coffee (of which Uganda is Africa's second leading producer) contributing the largest percentage of 19% of the country's exports. Exports of products such as hides, skins, vanilla, vegetables, fruits, cut flowers, and fish are growing, while exports of cotton, tea, and tobacco continue to contribute to Uganda's foreign exchange earnings (Bouët & Odjo, 2019). Uganda faces challenges like major tick-borne diseases for example Anaplasmosis, Babesiosis, Cowdriosis and East Coast fever (ECF). Drugs for treatment of these diseases are expensive, and in most cases, the available drugs are not highly specific. In view of this, the control of these diseases has depended on the use of pesticides (Turyahikayo, 2013).

Furthermore, the principal method employed to control tick-borne diseases (TBD) in Uganda is intensive dipping or spraying of cattle with acaricides to free them of tick vectors. The acaricides used include: Amitraz, Chlorpyrifos, Cypermethrin and Alpha Cypermethrin under the brand names of Norotraz, Duodip, Sypertix and others. The use of acaricides to control ectoparasites started with the application of arsenicals from the early years of the last century and it is still the main method used for the control of the parasites (Natala & Ochoje, 2009). In Uganda, the use of acarcides especially in ensuring the sustainability of large quantities of high quality livestock produce has been steadily increasing over the past half century(Kasozi *et al.*, 2006). Chemical tick control was started in Uganda in the early 1930s and gained momentum after the second World War (Okello-Onen *et al.*, 1992).

The introduction of exotic cattle in the 1960s led to the construction and use of several dips as a method of applying the chemicals to the animals. The government encouraged farmers to practice tick control by providing a 50% subsidy on the costs for construction of dips and purchase of spray pumps and acaricides. However, this subsidy was removed in 1984. So far, Uganda has gone through a succession of groups of chemicals used to control ticks. In the past, protection against ticks was dominated by organochlorines namely Toxaphene and Lindane but as in other cases, these have largely given way to organophosphates such as Delnav, Supona and synthetic pyrethroids deltermethrin (Decatix) and cypermethrin (Fendona) (Khopkar, 2007). In order to improve agricultural productivity, farmers in Uganda have resorted to use of synthetic fertilizers and pesticides. However, the farmers use incorrect application techniques, inappropriate spraying equipment and poor storage practices. The pesticides can later find their way into the environment and water bodies via run offs (Marshall, 2018). The pollutants can then bio accumulated and bio magnified by living organisms and may result in into adverse toxic effects. Some of the toxic effects of pesticides range from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption (Srivastava & Kesavachandran, 2019).

In addition, inappropriate use of pesticides has been linked with adverse effects on non-target organisms, water contamination from volatile pesticides or from pesticide drift, air pollution from volatile pesticides, injury on non-target plants from herbicide drift, injury to rotational crops from herbicide residues in the field and crop injury due to high application rates, wrong application timing or unfavorable environmental conditions before and after pesticide application (Winter & Davis, 2006). Despite continuing disagreements over the degree of risk posed by pesticides, it appears that people have become increasingly concerned about

pesticide use and particularly about their impacts on human health and environmental quality (Damalas & Eleftherohorinos, 2011). However, research has indicated that there is an overuse, misuse and abuse of pesticides in farming mainly due to illiteracy and ignorance of the health effects of these chemicals (Winter & Davis, 2006). A number of unapproved pesticides are in use on farms in Uganda, most of which have been banned in other countries due to their environmental toxicity and persistence. Pesticides such as Lindane, Dichlorodiphenyltrichloroethane (DDT), Aldrin, Dieldrin, Heptachlor, Benzene Hexachloride (BHC) and Endosulfan were found to be environmentally persistent (Okoffo, *et al.*, 2016). However, due to a combination of factors including weak enforcement of laws on pesticide importation policies, these chemicals are still being used on animal farms in Uganda(Wambua & Muhigirwa, 2019).

The application of several acaricides on animals has been associated with effective control of ticks, pests and diseases in order to increase animal health and hence productivity. Due to the acaricides potential risk of toxicity to human health, persistence, and tendency to bioaccumulate, much effort has been made for the determination of pesticide residues in environmental samples. However, less monitoring has been done on acaricides residues, as a result; different international bodies such as the European Union (EU), United States Environmental Protection Agency (US EPA) and World Health Organization (WHO) established maximum allowed concentrations for pesticides in water. In 2011, the Brazilian Ministry of Health Enacted Ordinance 2014, which sets the procedures for control and surveillance of the water portability Parameter (Matsumura, 2012). Among other parameters, this legislation set the maximum limits of pesticide residues permitted in water for human consumption. Considering the importance of monitoring the presence of pesticide residues at

low levels in drinking water, low-cost reliable methods with high sensitivity and low detection limits, high selectivity, and speed are still required.

This research investigated the occurrence and concentration of acaricide residues in soils and water resources near cattle dip tanks, as well as inside selected cattle dip tanks in Mbarara District western Uganda.

Effect of exposure to acaricide

Human exposure to agricultural pesticides and the subsequent contamination or poisoning may be occupational, non-occupational, intentional or unintentional. Also, exposure may be through ingestion (oral), through the skin (dermal) or through inhalation (respiratory). Occupational contamination or poisoning has been identified as the most serious problem associated with the use of agricultural pesticides, especially in developing countries (Shachi *et al,* 2018). There is widespread use of pesticides in Uganda handled by persons who are not adequately trained, supervised, informed and guided in the proper procedures for pesticide use and handling. Consequently, populations have been exposed to undesirable levels of acaricides and caused unquantified deleterious effects (Lekei *et al*, 2014) Pesticides and acaricides are the most easily accessible toxic chemicals and most widely stocked, however, the core problem is their poor handling. Handling includes transportation, storage, application, and disposal of pesticides.

The victims of pesticide poisoning are not only those who apply the pesticides. Others working with pesticides, such as storekeepers and farm workers may also be exposed (Lahr *et al,*2016). Even those who do not work with pesticides may be exposed to them unknowingly, for example, by being sprayed accidentally or eating contaminated food. Children are frequent victims of pesticide poisoning when they eat or drink pesticides that are stored in their reach. Pesticides may enter the body orally (through the mouth), they may be inhaled as vapors or

they may enter through the skin. Oral ingestion may occur by accidentally drinking a pesticide, by splashing spray materials or pesticide dust into the mouth or by eating or drinking contaminated foods or beverages. The ability of a pesticide to be absorbed through the skin depends on the chemical characteristics of the pesticide and its formulation (Sarwar & Salman, 2015). Respiratory exposure occurs when dust or vapours enter the lungs, or when aerosols are formed as pesticides are sprayed. Pesticides that are more soluble in oil or petroleum solvents penetrate skin more easily than those that are more soluble in water

Acaricide residues

When an acaricide product is applied in the cattle dips, the chemical is gradually lost as a result of breakdown, leaching and evaporation and the residue is the amount that remains after application (Cox *et al*, 1995). Some pesticides have long residual activity and therefore persist in the environment; others have short residual activity and therefore do disappear from the environment or produce low residue concentration. It is therefore possible to find or detect residues of pesticides in the environment and food crops after usage. Pesticide residues may enter the food chain causing serious hazards to human and animal lives(Agrawal *et al*, 2010). The major sources of acaricide residues in soils, water sources, crops, food, animal products, include the following among others; animal carry-over from dip tank to soil or to growing crops and other water sources, leaching of acaricide into ground water, disposal of acaricide waste in streams, rivers, lakes, effluents of pesticide industry in rivers and streams, and into soil which may be translocated in crops (Vaarst et al., 2006)

1.2 Problem statement

Despite the intense use of acaricides by farmers to control crop pests and ticks against animals particularly cattle, little is known as to whether residues of acaricides find their way into surrounding environments. There are serious health risk concerns arising from spray spillages, misuse and poor disposal techniques of acaricide wastes around the cattle dips in western Uganda. Upon disposal into soak pits, they overflow and end up into nearby soils and water resources. The acaracide degradation products can also contaminate surface water through runoff or leach into the soil and contaminate underground water. In addition, during use, the acaracide in dips dissipate during rainy seasons where flooding of soak pits is likely to take place increasing the runoff and leaching rates, hence, causing potential hazards to human health and the environment. Apparently, little is known on the levels of acaricide residue contamination of the soil and water surrounding areas of cattle-dip tanks in in Uganda.

1.3 Objectives

1.3.1 General objective

To determine acaricide residues in soils and water resources around selected cattle dip tanks in Mbarara District, Western Uganda.

1.3.2 Specific objectives

The specific objectives were:

i. To determine the residue levels of amitraz, chlorpyrifos and cypermethrine in soil samples around cattle dip tanks in Mbarara Distric Western Uganda.
ii. To determine the residue levels of amitraz, chlorpyrifos and cypermethrin in water samples around cattle dip tanks in Mbarara District Western Uganda.
iii. To determine the residue levels of amitraz, chlorpyrifos and cypermethrine in cattle dip tanks in Mbarara District Western Uganda.

1.4 Hypotheses

$H_1$1: The levels of acaricide residues in soils and water resources within the areas around cattle dip tanks exceed the permissible maximum residue levels (MRL) set by European Union (EU), United States Environmental Protection Agency (US EPA) and World Health Organization (WHO) .

$H_0$1: The levels of acaricide residues in soils and water resources within the areas around cattle dip tanks do not exceed the permissible maximum residue levels (MRL).

$H_1$2: The acaricide concetration in cattle dip tanks exceed the permissible ranges.

$H_0$2: The acaricide concetration in cattle dip tanks do not exceed the permissible ranges .

1.5 Scope of the study

The water and soil samples were collected from five farms named and coded as follows:

Farm A = GBK farm, Farm B = Byagagayire farm, Farm C = Ndyabagye farm,

Farm D = Amara farm and Farm E = Rubyerwa farm

Sampling was done for a period of six months from September 2017 to February 2018.

The parameters analysed were residue level of amitraz, chlorpyrifos and cypermethrine in both soil and water samples. The concentration of amitraz, chlorpyrifos and cypermethrine inside the cattle dip tanks was also determined and sampling was done when the last animal had passed through the cattle dip tank in order to obtain homogeneous sampe

1.6 Justification

This study was intended for analysis of selected acaricide residue levels in water resources and soils around cattle dips in the district of Mbarara in Western Uganda. The study was conceived due to the fact that there is extensive use of acaricides for controlling ticks on various cattle farms. Moreover, acaricide residues as runoff end up in drinking water sources and soils, resulting in adverse effects on water and the environment. Secondly, the human population in western Uganda has been growing very rapidly which is a driving force for the farmers to use more acaricides on animal farms that supply milk, beef, hides and skins, among others, to the population. The incentive to increase animal productivity is also driven by the increasing demand for raw materials for dairy product processing industries hence forcing farmers in this area to increasingly use acaricides to enhance animal production in order to meet the demand for raw materials for the existing dairy products processing industries in the area.

1.7 Significance of the study

The results are expected to inform policy making by Government Departments and Agencies involved in monitoring programmes on pesticide residues in livestock products, as well as those involved in monitoring the composition of both locally manufactured and imported pesticides and their proper application. Non-Governmental Organizations (NGOs) and Civil Society Organizations (CSO) among others can use the generated data to raise awareness among consumers and farmers about levels of acaricide residues in soils and water sources, and their potential health risks. It is also hoped that data and information generated can contribute to policy dialogue and inform policy change regarding pesticide residues, public health and trade issues in Uganda. The data could guide cattle farm owners to apply proper levels of acaricides in order to avoid excessive application and consequently reduce on acaricide levels in the environment

CHAPTER TWO

LITERATURE REVIEW

2.1 Acaricide in the study

The category of an acaricide depends on the type of pest they control and their nature e.g chemical acaricides (Arias-Estévez *et al*., 2008) ,These includes amidines (for example amitraz), Organophosphate pesticides (for example chloropyrifos), Pyrethroid pesticides (for example cypermethrin) others include Organochlorine pesticides (for example DDT, lindane)

2.1.1 Amitraz

Amitraz is a triazapentadiene compound, a member of the amidine chemical family and is widely used as an insecticide and acaricide to control red spider mites, leaf miners, scale insects, and aphids (Madbuni & Amini, 2013). On cotton, it is used to control boll-worms, white fly, and leaf worms. On animals it is used to control ticks, mites, lice and other animal pests. The United states environmental protection agency (EPA) classifies Amitraz as class III - slightly toxic.

Amidines (also called formamidines) are a special group of active ingredients with activity against ticks, mites and lice. Their parasiticidal properties were discovered in the 1960's (Del Pino *et al*., 2013). The most relevant active ingredient of this chemical class is amitraz which is a tertiary amino compound; 1,3,5-triazapenta-1,4-diene substituted by a methyl group at position 3 and 2,4-dimethylphenyl groups at positions 1 and 5, with a Molecular Weight of 293.414 g/mol. It has a role as an acaricide thus a potential environmental contaminant. It is a tertiary amino compound and a member of formamidines and is still extensively used in livestock, especially in cattle, but also in dogs, mainly against ticks and mites (Harrison *et al*., 1973). Amitraz has a detachment effect: if they are not directly killed, the ticks leave the host before completing or even initiating their blood

meal. Amitraz also has a repellent effect that keeps many ticks away from treated animals. This repellent effect can however have a drawback. Amitraz treated livestock may be clean of ticks because larvae in the pastures are repelled but the pastures can remain highly infested. As soon as the repellent effect declines or another not repelling product is used, livestock on such pastures may become highly infected(Mbaria *et al*,2008).

Amitraz is also effective against scab and mange mites (*Psoroptes* spp, *Sarcoptes* spp, etc.) and against certain lice species but it does not control biting flies and other blood-sucking insects. It is unstable in cattle dips that have to be stabilized with lime or fully replenished, toxic for horses and cats (Agin *et al*, 2004)

Shortly after the introduction of amitraz in the 1970's, synthetic pyrethroids came to the market that where not only good against ticks, but excellent insecticides safe for livestock, not toxic for horses and stable in cattle dips. Synthetic pyrethroids vastly replaced amitraz in the 1980's and 1990's. However, cattle ticks (*Boophilus* spp) quickly developed resistance against synthetic pyrethroids. Nowdays, this resistance is so strong and widespread that amitraz has experienced a strong comeback in all tropical and subtropical regions where *Boophilus* ticks are a problem and cattle dips are still popular. In the 1990's there were only a few commercial brands of amitraz available for cattle (basically the original ones: TAKTIC, TRIATIX, etc.). Today there are dozens of brands on the market, also from multinational companies. Usage of amitraz has sky rocketed, probably because it is the only reliable tickicide left for dipping and spraying cattle after the failure of synthetic pyrethroids and the general rejection of organophosphates. Alternative tickicides such as macrocyclic lactones and fluazuron are not available for cattle dipping or spraying. Amitraz has also experienced a certain revival for use on dogs. The reason is that a number of insecticides (for example imidacloprid, spinetoram) used in spot-ons are highly

effective against fleas, but only poorly or not at all effective against ticks. They are sometimes mixed with amitraz to improve their efficacy against ticks (W. Chen & Plewig, 2014)

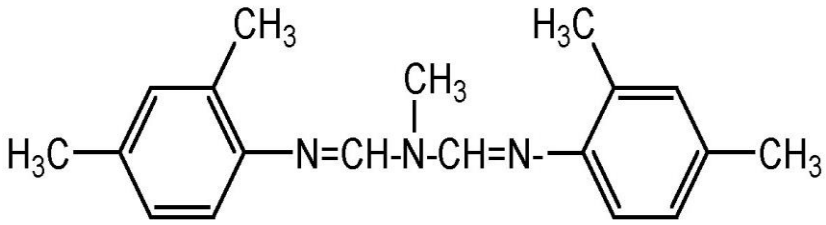


Figure 2.1: The chemical structure of amitraz (1,3,5-triazapenta-1,4-diene)

Efficacy of amitraz

The efficacy of amitraz on cattle ticks was assessed by susceptibility tests, spraying and dipping trials. Tests on the susceptibility of three tick species, Rhipicephalus appendiculatus, Amblyomma variegatum and Boophilus decoloratus to amitraz (technical grade-purity 98,6% w/w) showed all 15tick strains tested to be highly susceptible, Spraying trials were carried out on calves infested with R. appendiculatus, A. variegatum and B. deco/oratus. Amitraz wettable powder was tested against B. decoloratus, and amitraz emulsifiable concentrate against the other two species. Both formulations showed instant action, with ticks detaching from the calves between 30 min and 8 h after spraying. More than 50% of the detached engorged females failed to lay eggs. The remainder laid few eggs, and these had a low hatching rate of 0-2%, compared with 90-98% in the controls. The detached nymphs failed to moult, and the males and non-engorged females also detached, were immobilized and finally died. In the dipping trials, cattle heavily infested with ticks (mean tick counts of about 800) were dipped once weekly in amitraz . Weekly tick counts showed that the reinfestation rate was reduced to zero after the ninth dipping. The results of the three trials complement each

other, showing that amitraz is at present effective in the control of African tick species on cattle (Mathivathani *et al;* 2011)

Pharmacokinetics of amitraz

Dermal absorption of amitraz applied on the surface of animal's skin is quite low, less than 10% in dogs and pigs. However, treated animals may ingest amitraz through licking and grooming. Amitraz is vastly broken down to metabolites in the liver. This occurs rather fast in ruminants, pigs and dogs, but much slower in horses, which may explain why they do not tolerate amitraz. Excretion is achieved through the kidneys. More than 60% of the administered dose is excreted in 24 hours after treatment (Pass & Mogg, 1995)

Mode of action of amitraz

The acaricidal activity of amitraz is due to its antagonistic effect on octopamine receptors of the nerve cells in the brain and inhibition of monoamine oxidases and prostaglandin synthesis. Parasites become hyper excited, paralyzed and eventually die. This mode of action is different from those of synthetic pyrethroids, organophosphates and other ectoparasiticides (Fishel, 2008)

2.1.2 Chlorpyrifos

Chlorpyrifos belongs to organophosphorus pesticides (OPPs). It is widely used in agriculture to protect plants and animals from insects and hence provide numerous benefits in terms of production and quality. These groups of compounds (OPPs) are highly liposoluble but are also soluble in water. Due to their instabilities, the residue levels of organophosphorus pesticides in foods are affected by a number of physical factors applied in food processing, including fermentation, heat treatment and drying. In addition, the chemical nature of organophosphorus pesticides and some environmental factors such as pH, light, metal ions and ozone, also have impacts on the degradation of pesticide residues (Bogialli *et al*., 2006). Organophosphate insecticides include chlorpyrifos, diazinon, dimethoate, disulfoton,

malathion, methyl parathion, and ethyl parathion among others. The most toxic of all pesticides for vertebrates are the organophosphates (Ware *et al;* 2000). Exposure to organophosphates occurs via inhalation, absorption into the skin, and ingestion (Wei *et al;* 2012). Organophosphate insecticides share a common mechanism of toxicity, through inhibitory effects on cholinesterase enzymes in the nervous system. This results in elevated levels of acetylcholine (ACh), which acts on the muscarinic receptors situated at cholinergic junctions in skeletal nerve-muscular junctions, at nicotinic receptors in autonomic ganglia, and receptors in the central nervous system (CNS).

They poison the nervous system by inhibition of cholinesterase enzyme. Health effects of chlorpyrifos are headache, nausea, dizziness, salivation, excess sweating, blurred vision, chest tightness, muscle weakness, abdominal cramps and diarrhea (Nganchamung *et al*, 2017). Chlorpyrifos is moderately toxic following acute oral, dermal and inhalation exposures. Chlorpyrifos affects the nervous system by reversibly inhibiting the activity of cholinesterase (ChE), an enzyme necessary for the proper functioning of the nervous system (Smegal, 2000)

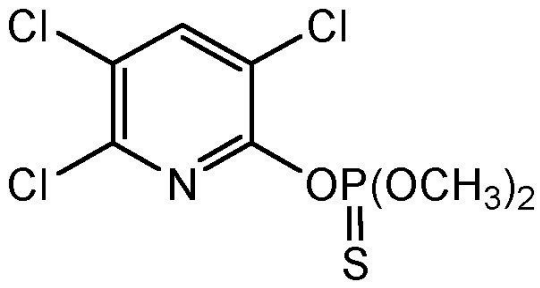


Figure 2.2: Chemical structure of chlorpyrifos

Efficacy of Chlorpyrifos

Chlorpyrifos is abroad-spectrum insecticide, acaricide and larvicide. Chlorpyrifos has average efficacy against most external parasites, but not outstanding against any particular

one. However, resistance of important veterinary parasites to all organophosphates, including chlorpyrifos is widespread, especially in cattle ticks (*Boophilus* spp), horn flies (*Haematobia irritans*), sheep lice (*Damalinia ovis*), poultry mites (*Dermanyssus gallinae*), mosquitoes, dog and cat fleas (*Ctenocephalides* spp) and houseflies (*Musca domestica*)**.** Consequently, products with this active ingredient may not achieve the expected efficacy in many places. The same applies to all other organophosphates. This is also a reason for their progressive replacement with newer active ingredients having different modes of action

(Mack *et al;* 1989).

Pharmacokinetics of chlorpyrifos

Percutaneous absorption (that is to say through the skin) of topically administered chlorpyrifos depends on the animal species, the administered dose, and the extension of the treated body surface. Animals treated topically can ingest chlorpyrifos and through licking and grooming. Orally administered chlorpyrifos is partly excreted unchanged through the feces. Once absorbed into blood it is quickly metabolized in the liver to less toxic compounds and rapidly excreted through urine. Excretion half-life is about 2 to 3 days. (Timchalk *et al.*, 2002)

Mode of Action of chlorpyrifos on pest

All Chlorpyrifos act on the nervous system of the parasites as inhibitor of acetylcholinesterase (actylchlolinesterase enzyme), the enzyme that hydrolyzes acetylcholine (Ach). Ach is a molecule involved in the transmission of nervous signals from nerves to muscles (so-called neuromuscular junctions) and between neurons in the brain (so-called cholinergic brain synapses). AchE's role is to terminate the transmission of nervous signals where Ach is the neurotransmitter (there are several other neurotrasmitters). By inhibiting the activity of AchE, carbamates prevent the termination of those nervous signals, that is to say the neurons remain in constant activity and excitation,

massively disturbing the normal movements of the parasites. Generally, the parasites get paralyzed and die more or less quickly. Organophosphates bind irreversibly to AchE, in contrast to carbamates, another chemical class of parasiticides, which bind reversibly to AchE (Jett *et al;* 1999).

2.1.3 Cypermethrine

Cypermethrine belongs to Pyrethroid pesticides and were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. The US Environmental Protection Agency has classified cypermethrin as a possible human carcinogen though available information on its carcinogenic properties is inconclusive (Marino & Ronco, 2005). This class of insecticides or acaricides includes permethrin, cypermethrine, resmethrin and allethrin. Often the formulations contain a synergist (something that enhances the effectiveness of the active ingredient) called piperonyl butoxide (PBO) which itself is relatively non-toxic. The synthetic pyrethroids show properties of low mammalian toxicity but good activity against insects, ticks and mites. They do not appear to be readily absorbed through the skin (Turyahikayo, 2013)

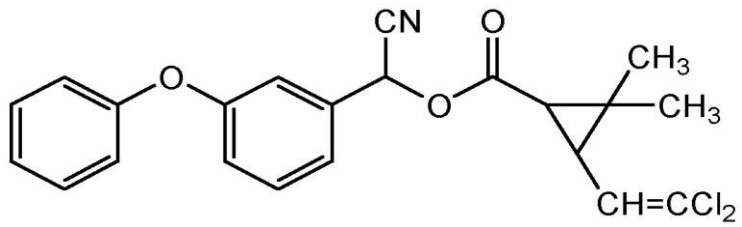


Figure 2.3: Chemical structure of cypermethrin

Pharmacokinetics of cypermethrin

Percutaneous absorption (that is to say through the skin) of topically administered cypermethrine depends on the animal species, the administered dose, and the extension of the treated body surface. Animals treated topically can ingest cypermethrine and through licking

and grooming. Orally administered cypermethrine is partly excreted unchanged through the feces. Once absorbed into blood it is quickly metabolized in the liver to less toxic compounds and rapidly excreted through urine. Excretion half-life is about 2 to 3 days. (Timchalk *et al*., 2002)

Mode of action of cypermethrin

Synthetic pyrethroids, including cypermethrin, have a similar mode of action as organochlorines. They act on the membrane of nerve cells blocking the closure of the ion gates of the sodium channel during re-polarization. This strongly disrupts the transmission of nervous impulses, causing spontaneous depolarization of the membranes or repetitive discharges. At low concentrations, insects and other arthropods suffer from hyperactivity. At high concentrations, they are paralyzed and die. Sensory and nervous cells are particularly sensitive

Organochlorine pesticides

Organochlorine pesticides (OCPs) are insecticides composed primarily of carbon, hydrogen, and chlorine. They break down slowly and can remain in the environment long after application and in organisms long after exposure. The OCPs are a class of non-polar toxic chemical compounds classified as dichlorodiphenylethane cyclodienes and chlorinated benzenes (Ademoroti, 1996). They may be grouped into three general classes; the dichlorodiphenylethanes such as DDT, DDD and dicofol. The chlorinated cyclodienes such as aldrin, dieldrin, heptachlor, and hexachlorocyclohexanes (lindane). These compounds differ substantially between and within groups with respect to toxic doses, skin absorption, fat storage, metabolism, and elimination. The signs and symptoms of toxicity in humans, however, are remarkably similar except for DDT (Hayes & Laws, 1991). These lipophilic

compounds accumulate and even magnify their concentration along the food chain, especially in fatty food (Manirakiza *et al.*, 2002)

Organochlorine pesticides are widely used by farmers because of their effectiveness and broad-spectrum activity (Darko & Acquaah, 2007) They are liposoluble compounds and are easily absorbed in lipids of the insect cuticles. In vertebrate animals, variable amounts of chlorinated insecticides are retained in the adispose tissues, from where they are gradually released in the circulatory system. The OCPs were commonly used in the past, but many have been removed from the market due to their health and environment effects and their persistence. In Uganda, the use of most OCPs has been banned or restricted under the Rotterdam and Stockholm Convention due to high levels of persistence in the environment and toxicity to non-target organisms.

Carbamate pesticides

Carbamates are similar to organophosphates in activity in that they inhibit cholinesterase. These insecticides can cause cholinesterase inhibition poisoning by reversibly inactivating the enzyme acetyl cholinesterase and cause a cholinergic crisis that is to say muscles stop responding leading to paralysis and respiratory failure (Lorke & Petroianu, 2019)

2.2 Studies on acaricides

In Uganda, an assessment on the levels of pesticide residues in livestock products and water around Lake Mburo National Park, South Western Uganda was carried out in the year 2010/2011. This study was necessary in view of the fact that there is inadequate data on the levels of pesticide residues in live stock products and water despite the increasing use of pesticides to control animal disease vectors specially ticks.

A structured questionnaire was used to interview the farmers on the types of pesticides used to control animal disease vectors, and their practices, knowledge and attitudes on the use of pesticides. A total of sixty (60) farmers were interviewed and data analysed using Statistical Package for Social Sciences (SPPSS 19). Samples of milk, muscle and water were also collected and analyzed in a laboratory for organochlorine, organophosphate and pyrethroid residues.

From the study, 100% of the farmers controlled animal disease vectors by use of pesticides. The survey revealed a total of ten (10) different pesticides that were being used by the farmers in the study area with synthetic pyrethroids (33.6%) being widely used followed by organophosphates and formamidine (22.1%). There was no organochlorine pesticide being used by the farmers and no organochlorine pesticide residues were detected. However, chlorfenvinphos residues of 0.13 mg/l, 0.11 mg/l, were detected in water sample 1 and 2 from Kanyarweru subcounty while 0.17 mg/l, 0.12 mg/l, 0.41 mg/l, 0.12 mg/l of chlorfenvinphos residues were detected in water sample 1, 2, 3 and 4 from Nyakashara subcounty. In addition, chlorfenvinphos residues of 0.32 mg/l, were detected in milk sample 5 from Sanga Subcounty while 0.28 mg/l and 0.31 mg/l were detected in milk sample 2 and 5 from Nyakashashara subcounty. Also, unquantifiable levels of chlorpyrifos residues, an organophosphate were detected in meat samples 1, 2 and 4 from Nyakasharara Sub County. No pyrethroid residues

were detected in the animal products and water samples analyzed. The presence of pesticide residues in animal products and water could be attributed to the practices, attitudes and knowledge about the use of pesticides. The study revealed that 78% of the farmers disposed their pesticide containers around the spray area.

It was also revealed that most of the spray areas were located in the radius of not more than 1km from the water source. This could be the probable cause of water contamination with pesticide residues as a result of runoff. In addition, 88% of the farmers interviewed had no knowledge on the withholding periods of the pesticides. Despite the fact that some of the pesticide residues detected in the livestock products and water are below the Maximum Residue Levels(MRLs) with increasing pesticide use for tick control in Uganda, there is a possibility of increased pesticide residue levels in livestock products and water above the MRLs. In addition, given that pesticides are toxic compounds their presence in food, even in trace amounts, should be avoided(Turyahikayo, 2013)

In Kenya, the study was undertaken to determine the concentrations of carbofuran residues in water, soil and plant samples from selected sites in the farmlands in the country. To demonstrate the impact of Furadan use on the local environment. Soil, water and plant samples obtained from agricultural farmlands where the technical formulation Furadan has been used extensively showed high environmental contamination with concentrations of carbofuran and its two toxic metabolites 3-hydroxycarbofuran and 3-ketocarbofuran, separately, ranging from 0.010–1.009 mg/kg of dry surface soil, 0.005–0.495 mg/L in water samples from two rivers flowing through the farms and bdl-2.301 mg/L in water samples from ponds and dams located close to the farms. Maize plant samples contained these residues in concentrations ranging from 0.04–1.328 mg/kg of dry plant tissue.

The significantly high concentration levels of carbofuran and its metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran, found in various matrices demonstrate that Furadan was used extensively in the two areas and that there was environmental distribution and exposure of residues in water which posed risks when used for domestic purposes or as drinking water for animals in two wildlife conservancies where the dams and ponds are located. Surface soil contamination was also high and posed risks through run-off into the dams and rivers as well as through secondary exposure to small birds and mammals.(Otieno *et al*, 2010)

The increasing number of incidences of alleged wildlife poisoning with Furadan in Kenya has sparked off a strong lobby fronted by wildlife conservationists against Furadan use in the country and prompted this study. The worst-case scenario was in 2004 in Athi River, where a massive number of 187 African white-backed vultures (*Gyps africanus*) and hyenas were found dead at a spot where poisoning was suspected to have occurred through a Furadan-laced camel carcass bait. This study was initiated by the Peregrine Fund—Africa Project, and the objective was to provide evidence for Furadan exposure, its misuse and involvement in vulture poisoning and potential impact on areas near two wildlife conservancies in two most affected districts.

The study found evidence for ready availability of Furadan 5G in local veterinary retail shops and its illegal misuse by pastoralists and farmers against wildlife to protect their animals and crops. Analysis of soil, water and plants taken from the farms and water sources by high-perfomance liquid chromatography (HPLC) and gas liquid chromatography–mass spectrometry (GC–MS) found residues of carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran, indicating that Furadan was used extensively in farming causing residual environmental distribution and contamination and posing risks to

small birds and mammals. Forensic analysis of residues in beaks, feet and crop content of the dead vultures as well as in a laced camel carcass bait and soil samples from one site of poisoning also showed carbofuran and its two metabolites supporting allegations of Furadan involvement in wildlife poisoning and high-mortality cases of African white-backed vultures (*Gyps africanus*) in Kenya.(Otieno et al., 2010)

In Tanzania, this study was conducted to assess farming practices, agrochemical usage and environmental pollution in Manyara basin, Tanzania. Field surveys, interviews, questionnaires and Lake Manyara nutrient analysis were used in data collection. The highest number (95%) of households grew crops, namely, maize, rice, banana and vegetables with median farm size of 3 ha. Irrigated farming was common (75%) which enhanced cultivation on same piece of land up to 6 times a year. Farmers indiscriminately used pesticides, namely, insecticides (50%), fungicides (37.5%) and herbicides (12.5%). Uses of endosulfan in vegetable farms poses public health threats to consumers. Most respondents (85%) applied insecticides in vegetables up to 4 times per cropping season. Excessive use of pesticides and haphazard disposal of pesticide remnants and containers caused environmental pollution.

The average amount of acaricides used was 1109±915 ml (mean ± SD) per livestock keeper per month per. Most farmers (78%) used inorganic fertilizers and animal manure (43.4%). Low levels of ammonium (3.6±3.1 µg/L), nitrate (1±0.8µg/L) and phosphate (36.1±42 µg/L) were recorded in the lake. Easy access to agrochemicals, limited knowledge of pesticide on environmental health and limited extension services were factors for indiscriminate uses of agrochemicals. Increasing farmers awareness and training aimed at sustainable agriculture, agrochemical uses and integrated pest management is suggested(Nonga ***et al,***, 2011)

The objective of this review was to analyze the existing information on the use of agrochemicals (Fertilizers and Pesticides) in the Tanzanian horticulture industry especially the Northern regions and their potential to impacting water resources. Agrochemicals play an important role in horticulture, and have been widely used in Tanzania for crop protection and increasing productivity. Apart from these benefits, agrochemicals have the potential to impair the quality of water resources for different end uses. Majority of communities in Tanzania depend on surface water from rivers and lakes for potable uses such as washing, drinking and domestic animals also drink from these sources. Reports from studies done in Northern Tanzania have indicated the presence of significant levels of pesticides, phosphates and nitrates in surface and groundwater. It is apparent that most of the horticultural farms in Northern Tanzania are located on gently sloping land adjacent to water bodies. Thus discharges of wastewaters from horticulture farms may affect the quality of water resources through run-off and groundwater through infiltration if proper management of the agrochemicals is not well adhered to.

The agrochemicals that have been widely used and identified as potential environmental pollutants from their use as horticultural chemicals are reviewed. The potentially adverse impacts of these agrochemicals to water resources are discussed. The review concludes with a discussion of the directions for further investigation (Lema *et al*, 2014)

2.2.2 Studies of acaricides in the rest of the world

In European countries, over 2000 surface, ground and raw drinking water samples have been analyzed in the frame of different monitoring projects in Hungary and watercourses in neighboring countries between 1990 and 2015. Effects of pesticide contamination on ecological farming and drinking water supply have been assessed. Main water pollutant ingredients of agricultural origin in Hungary are herbicides related to maize production. After

EU pesticide re-registration, diazinon, atrazine, and trifluralin gradually disappeared as contaminants. High levels of water soluble pollutants (e.g., acetochlor) in surface water result in temporarily enhanced levels in raw drinking water as well. Extreme levels observed for herbicide residues were of agrochemical industrial origin(Székács *et al,* 2015)

The efficacy of formic acid, sulfur, fluvalinate and amitraz in controlling *T. clareae* infecting the European honey bee, *A. mellifera,* was determined. Mite mortality under hive conditions varied between 80.51 and 85.14% after two weeks of treatment. All the treated colonies became mite-free within 22-25 days, and the tested chemicals had no adverse effects on the brood and bees or queens.(Sharma *et al,*, 2003)

Further more, in Asian countries the mountain cold-trapping of soil in the Tibetan Plateau may be an important global sink of organochlorine pesticides (OCPs). However, there are limited data on OCPs in the soils of the Tibetan Plateau. In addition, the atmospheric transport and deposition mechanisms of OCPs also need to be further studied.

In this study, the sampling area covered most regions of the Tibetan Plateau. The detection frequencies of ΣChlordane (sum of *trans*-chlordane, *cis*-chlordane and oxychlordane), HCB, ΣNonachlor (sum of *trans*- and *cis*-nonachlor), DDTs, ΣEndo (sum of endosulfan-I, endosulfan-II and endosulfate), aldrin, HCHs, ΣHeptachlor (sum of heptachlor and heptachlor epoxide), mirex and dieldrin were 100%, 98.3%, 96.6%, 94.8%, 89.7%, 87.9%, 62.1%, 55.2%, 32.8% and 6.9%, respectively. DDTs (with arithmetic mean values of 1050 ng kg^{-1} dw) and HCHs (393 ng kg^{-1}) were the principal OCPs in cultivated soils, whereas ΣEndo (192 ng kg^{-1}) and ΣChlordane (152 ng kg^{-1}) were the principal OCPs in non-cultivated soils. Local use of DDTs, dicofol and HCHs may be an important source of OCP accumulation in the soil of the Tibetan Plateau.

Aldrin and endosulfan are considered to be good indicators for studying atmospheric transport and deposition of OCPs from South Asia and Southeast Asia. Two zones with high OCP levels were found in the southeast and northwest of the Tibetan Plateau. The zones have dissimilar pollution sources of OCPs and are influenced by different factors that affect their precipitation scavenging efficiency. The amount of precipitation was the dominant factor in the southeast, whereas large differences in temperature and wind speed were the dominant factors in the northwest.(L. Chen et al., 2017)**.**

Hipicephalus (Boophilus) microplus, a one host tick, has been reported to have developed resistance to all major classes of acaricides, including synthetic pyrethroids and formamidines. Fully engorged female R. (B.) microplus ticks were collected from various Gaushalas (cow shelters) located in district Hisar, Haryana. The ticks were subjected to adult immersion test with a discriminating dose against deltamethrin (1.25%) and amitraz (12.5% EC). Prevalence of resistance was determined based on the number of ticks that laid eggs or which died before laying eggs. Prevalence of resistance against deltamethrin ranged from 46.6% to 76.6%, and against amitraz 10% to 23.3% depending on the location. It seemed that a long time exposure to synthetic pyrethroids and comparatively less exposure to amitraz resulted into this type of prevalence pattern.(Suman *et al;* 2018)

In the USA, cyflumetofen is a novel benzoyl acetonitrile acaricide without cross-resistance to existing acaricides. In the present study, for the first time, the environmental behaviors of cyflumetofen and the formation of its main metabolites, 2-(trifluoromethyl) benzoic acid (B-1) and 2-(trifluoromethyl) benzamide (B-3), in the four types of soil (black soil, sierozem, krasnozem, and fluvo-aquic soil) and three types of water/sediment systems (Northeast Lake, Hunan paddy field, and Beijng Shangzhuang reservoir) under aerobic and anaerobic conditions were investigated.

The degradation dynamics of cyflumetofen followed first-order kinetics. Under aerobic environment, the half-lives of cyflumetofen in black soil, sierozem, krasnozem and fluvo-aquic soil were 11.2, 10.3, 12.4, and 11.4 days. Under water anaerobic conditions, the half-lives were 13.1, 10.8, 13.9, and 12.8 days. The effects of different conditions and soil types on the half-lives of cyflumetofen were studied using a one-way ANOVA test with post hoc comparison (Tukey's test).

It was shown that the differences in black soil, krasnozem, and fluvo-aquic soil were extremely significant difference ($p < 0.05$) under aerobic and water anaerobic conditions. And there is a strong correlation between half-life and pH. Under aerobic environment, the half-lives of cyflumetofen in Northeast Lake, Hunan paddy field, and Beijng Shangzhuang reservoir were 15.4, 16.9, and 15.1 days. Under anaerobic conditions, they were 16.5, 17.3, and 16.1 days. Analyzing the differences of the half-lives under aerobic and anaerobic conditions, the difference only in Shangzhuang reservoir was extremely significant difference ($p < 0.05$). In soils, cyflumetofen degraded metabolites B-1 and B-3, from the first day 0.24 % B-1 was generated, while, only very low levels of B-3 generated at the same time. As time increased, B-3 gradually increased, cyflumetofen reduced gradually. Until 100 days, there were about 3.5 % B-1 and B-3 in the soils. In the water/sediment systems, from the first day, it degraded into B-1 in the sediment, and in the water mainly degraded into B-3 (Wang et al., 2016)

Although several of the studies have reported the prescence of acaricides in the environmental matrix in various countries, little is known as to whether acaricides are present in area surrounding cattle dip areas in mbarara district

2.3 Impact of acaricides on human health

Acaricides applied to livestock most frequently include those used to control disease vectors, such as ticks and tsetse flies. Chemical use may therefore have positive impacts on animal health and thus productivity thereby curbing disease transmission to humans. At the same time, chemicals applied to livestock or infiltrating livestock products from the broader environment can impact negatively on human health for example through residues or tainting of food products, pollution of drinking water sources and bioaccumulation in the food chain (Karabelas *et al.*, 2009)

Acute effects

Injuries may be caused either by a single massive dose being absorbed in a single pesticide exposure, or from smaller doses absorbed during repeated exposures over an extended period of time. Illness or damage is referred to as acute when it has a sudden onset and lasts for a short period of time. The type and severity of the symptoms depend on the chemical mode of action and toxicity and the amount of chemical the victim has been exposed to. WHO estimates of 2000 showed that each year three million Farmers in the developing world were experiencing severe poisoning from pesticides, about 18,000 of whom would die. Fifty percent (50%) of modern pesticides are mutagens, that is to say, cause heritable changes in the genetic material, DNA. This is of concern since it poses a threat to the gene pool of Uganda's biodiversity, which is quite extensive (Damalas & Koutroubas, 2016).

Chronic effects

Chronic toxicity refers to the effects that occur after exposure over a long period of time, or to symptoms that occur long after exposure and/or persist for a long time. In general, these effects can occur with doses as low as a few micrograms of pesticide per kilogram body weight of the person or animal exposed. Examples of the chronic effects of pesticides on humans are described below.

Carcinogenic effect

Acaricides can exert a carcinogenic effect through a variety of mechanisms, including:

Genotoxicity: Here, is suspected that reaction with DNA to cause mutations or cancer takes place for example Chlorpyrifos and cypermethrine Before DNA replication, they may lead to gene mutations and initiate carcinogenesis **(**Cui *et al.,* 2006)

Hormonal action: In this effect, lengthening of the oestrous cycle occurs, prolonging exposure to endogenous oestrogen, and can cause mammary and uterine tumours, these are mainly induced by amitraz and cypermethrine.

mmunotoxicity: This effect alters immune function in a number of ways that can cause cancer. Cypermethine is considered a potential immunotoxin. Cypermethrin can cross the blood-brain barrier and exert its effect on nigrostriatal, It has been classified by the US Environmental Protection Agency [56] as a possible carcinogen (Hussien *et al*., 2013)

Neurological effects

There is a growing concern of developmental neurotoxicity by recent epidemiological observations where children exposed prenatally or during early postnatal life suffer from various neurological effects. Examples of neurological effects are numbness or weakness of arms, legs, feet or hands, lethargy, memory loss, loss of concentration, and anxiety (Costa, *et al.,* 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out on selected farms in Mbarara District, western region of Uganda, the farms were specifically in the counties of Kashari and Rwampara. Mbarara district covers a land area of 1,778.4 Square Kilometres (686.6sq mi), with an average elevation of about 1,800 metres (5,900 ft) above sea level. The district receives average rainfall of 1,200 millimetres (47 in), the temperature ranges between 17°**C** (63°**F**) and 30°**C** (86°**F**). The district had an estimated population of 472,629 people by the end of 2014 and population was projected to 524,400 people by 1st July 2019. Mbarara district is part of the cattle corridor that extends from the southwestern region of the country, through central to the north eastern region. There is extensive use of acaricides for controlling ticks on various cattle farms. Moreover, acaricide residues as runoff end up in drinking water resources and soils, resulting in adverse effects on water and the environment. The incentive to increase animal productivity is also driven by the increasing demand for raw materials for dairy product processing industries hence forcing farmers in this area to increasingly use acaricides to enhance animal production in order to meet the demand for raw materials for the existing dairy products processing industries in the area. An area with no spraying at all with acaricides was used as a control.

3.2 Sampling

For soils, 150 samples were collected, picking 75 samples during each of two visits that were made. Sample where picked at farms A, B , C, D and E picking 15 soil samples per farm

For water, 60 samples were collected, picking 30 samples during each of two visits that were made. Sample where picked at farms A, B , C, D and E, picking 6 water samples per farm

For cattle dip samples, 30 samples were collected, picking 3 dip samples per farm making 15 sample per visit and two visits were made.

A total of 240 samples were collected in two visits with the second visit coming one month after the first one. Water and soil samples were collected from five farms named and coded as follows: Farm A = GBK farm, Farm B = Byagagayire farm, Farm C = Ndyabagye farm, Farm D = Amara farm and Farm E = Rubyerwa farm

Mbarara district has a total of 30 cattle dips in general and five were selected for this research. The selection was based on the number of cows on the farm and the number of years the farm has been in existence. Selection was further done basing on only cattle dip tanks that were still in use and those with poor engineering status.

Soil samples

From each farm, 0.5 kg soil samples were collected in triplicate at intervals of 25 m, 50 m, 75 m away from the cattle dip tank, soak pits and control samples using a soil auger, hoe and speed, soil samples were packed in both white and black polythene bags and put in a cool box and transported to Norbrook Pharmaceutical laboratories.

From each farm, at intervals of 25 m, 50 m, 75 m away from the cattle dip tank, 9 homogenous samples were picked, picking three homogenous samples per interval, 3 samples were picked at soak pits and 3 samples were picked as control samples from soils to far from the cattle dip tank, this resulted in a total of 15 soil samples per farm. Making a total of 75

samples (15 soil samples from each farm X 5 farms) for the first visit and 150 soil samples for second visit. The samples were frozen until the time of analysis.

Soil samples were picked at several points along the intervals and mixed together to obtain a homogenous samples. Two visits were made to cater for any slight changes that may occur due to environmental changes.

25 m distance covers a draining pen in which the cattle cluster whilst still wet with dip solutions, while both 50 m and 75 m covers a radial areas around the cattle dip tanks.

Water samples

From each farm, 2 L water samples were collected in triplicate using clean plastic containers. The chosen water sources, including ponds and dams, were those closest (< 300 m) to the selected cattle dip tank. The water sources away from the cattle dip tanks at a distance of 300 m and above that is to say an area with no spraying at all with acaricides were used as controls. From each farm, 6 water samples where obtained, picking 3 from water sources and 3 samples as control and this resulted in a total of 30 water samples (6 water samples from each farm X 5 farms). Sample bottles were rinsed three times before collection of the water samples. The collected samples were kept in a cool box and transported to Norbrook Pharmacetical laboratories in Kampala for analysis. Prior to analysis, all the samples were kept frozen at – 24°C on the day of collection.

Samples from cattle dip tanks

From each farm, three 1 L cattle dip samples were collected into clean plastic bottles immediately after dipping the animals making a total of 15 cattle dip samples from all the five farms. Sample bottles were rinsed three times before collection of samples from dip tanks. The collected samples were kept in a cool box and transported to Norbrook Pharmacetical laboratories in Kampala for analysis. The samples were stored under refrigeration at – 24°C until the time of analysis.

3.3 Laboratory reagents and materials

The analytical standards of chlorpyrifos (purity 98.5 %), amitraz (purity 99.8 %), cypermethrine (purity 98.4 %) were procured from Sigma Aldrich, India and switzerland. The standards were obtained from M/s Premier Sales Agency, Srinagar, Jammu & Kashmir, India. Analysis of hexane extracts of each separate standards showed only Chlorpyrifos, amitraz and cypermethrine respectively and none of their metabolic products. Solvents and reagents like, hexane (purity 98 %) ethyl acetate (purity 99.9 %), sodium chloride (purity 99 %)) and analytical grade anhydrous MgSO4 (purity 98 %), were obtained from Merck, Darmstadt, Germany. Sodium sulphate anhydrous AR grade (purity 99 %), was obtained from perkins laboratories William street kampala, Uganda. All solvents were redistilled prior to use and the suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

Preparation of standard solutions

25 mg of standard Amitraz powder was weighed on a calibrated Shimadzu analytical balance (model ATX 224) into a 100 ml volumetric flask. 50 mls of n-hexane was added using a 25 ml pipette and contents shaken for 5 minutes. The solution of then topped up to the 100 ml mark with n-hexane. Further shaking was done for 30 minutes using the orbital flask shaker (SF1) at a speed of 600 Osci/min. The resulting solution was used as amitraz standard solution.

A 50 mg of standard chlorpyrifos and 5 mg of standard cypermethine powder were weighed on a calibrated Shimadzu analytical balance into a 100 ml volumetric flask. 50 mls of n-hexane was added using a 25 ml pipette of class A and pipette filler and the mixture hand shaken for 5 minutes to ensure dissolution. The solution was topped up to the 100 ml mark with n-hexane. The solution was shaken further for 30 minutes using the orbital flask shaker (SF1) at a speed of 600 Osci/min. The resulting solution was used as chropyrifos and cypermethrine standards.

3.4 Analytical procedure

This section is concerned with extraction and clean-up of soils, water samples and cattle dip tank samples

3.4.1 Extraction and clean-up of soil samples

The soil samples were air-dried for 5 days and then oven dried at 105 °C until constant weight was obtained. They were then ground and passed through a 2 mm sieve to remove all the the graval prior to extraction at this stage.

The extraction of the soil samples was carried out by (Benedicta *et al*; 2016). Only ten grams (10 g) of the sub-samples were weighed and transferred into 250 ml separating flasks. A 10 ml of n-hexane and 5 g of sodium hydroxide were added and sonicated for 5 min. An additional 10 ml of n-hexane was added, and the separating flasks closed tightly. The contents of the flasks were placed on a horizontal mechanical shaker (Ika-Werke HS 501 Digital), for 30 min at 600 rpm/min, and allowed to stand for 10 min to sufficiently separate the phases. The supernatants (organic layers) were carefully transferred into 50 ml centrifuge tubes and centrifuged at 3000 rpm for 5 min. A 10 ml aliquot of the supernatants (organic phases/top layers) equivalent to 5.0 g soil weight were pipetted and dried/passed over 5 g anhydrous sodium sulfate into a round bottomed flask.

The contents in the flask were reduced to 2 ml on a rotary evaporator at 35°C and kept for clean-up.

The extracted samples were cleaned by passing through silica cartridges with a 1 g layer of anhydrous magnesium sulfate on top (conditioned using 6 ml n-hexane). 50 ml pear shaped flasks were placed under the columns in a vacuum manifold, and the concentrated extracts loaded onto the cartridges. The extracts were allowed to pass through the cartridges and eluted with 10 ml of acetonitrile. The eluate collected were concentrated to near dryness

using the rotary film evaporator set at 40°C and transferred into 2 ml chromatography (GC) standard vials and kept for analysis.

3.4.2 Extraction and clean-up of water samples

Water samples were extracted as reported by (Aneani & Ofori-Frimpong, 2013), with slight modification. After filtration of the water samples through 0.45 µm fiberglass filters (WHATMAN) to remove debris and suspended material, 1000 ml portion of the filtered water sample were transferred into 2 L capacity glass-separating flasks. 30 ml of a saturated sodium chloride solution (NaCl) was added to produce a salt out effect.

The samples were then thoroughly mixed by inverting the flask 3 to 4 times. 100 ml of n-hexane as extraction solvent was added to each sample and vigorously shaken manually for 2–3 min, while releasing the pressure intermittently. The phases were allowed to separate for 5 min and the n-hexane extracts (organic layers) were separated from the aqueous layers by decanting. The extraction for each water sample was repeated two times with 100 ml of n-Hexane and the organic layers put together and dried over anhydrous magnesium sulfate through filter papers into 50 ml round bottom flasks. The extracts from the water samples were then concentrated using a rotary evaporator (Buchi Ratovapor R-210, USA) to about 2 ml prior to clean-up.

The extracted samples were cleaned by passing through silica cartridge (conditioned using 6 ml n-hexane) with a 1 g layer of anhydrous magnesium sulfate on top. The concentrated extracts were then loaded onto the cartridges and 100 ml round-bottom flasks were placed below the columns to collect the eluates. 20 ml n-hexane was then used to elute the cartridges afterwards. The eluate collected were concentrated to near dryness using the rotary film evaporator (Buchi Ratovapor R-210) set at 40 °C and transferred into 2 ml standard opening vials and kept for instrument analysis.

3.4.3 Extraction and homogenization of cattle dip tank samples

The sample was shaken thoroughly and an aliquot of 40 ml was taken for extraction. 40 ml were then transferred into a 250 ml separating funnel. To the separating funnel, 15 g of sodium chloride was added and shaken for 30 minutes using an orbital shaker at 600 Osci/min. The water phase was drained off by opening the tap. The extract was collected in a 100 ml volumetric flask.

The extract obtained was transferred into a vial and concentrated to 3 ml by evaporation at 40 °C in a warm water bath. The extract was further concentrated to 1 ml and was transferred into a GC Vial for GC-FID analysis.

3.5 Instrumental analysis of analytes

This section is caters for the analysis of amitraz, chloropyrifos and cypermethrine

3.5.1 Analysis of amitraz

The extracts from water, soil and dip water tank samples were analyzed at Norbrook Pharmaceutical Laboratories using a Gas Chromatographic system equipped with a Flame Ionization Detector (GC/FID) model Shimdzu GC- 14A to determine the levels of acaricide residues. The samples were analyzed according to the procedures reported by (Benedicta *et al;* 2016) The GC conditions and detector response were adjusted so as to match the relative retention times and response as spelt out by Japanese analytical methods for agricultural chemicals (Batarseh & Tarawneh, 2013)

For each sample, 1.0 µl aliquots of the sample extract was injected and the separation was performed on a fused silica gel capillary column (RTX035, L 30, ID .53, DF .50). The column initial temperature was 200 °C for 0.5 min, then raised to 280 °C at the rate of 40 °C/min, where it was held for 1 min. The injector and detector temperatures were 280 °C and 280 °C, respectively. The carrier gas was nitrogen at a flow rate of 1 ml/min. The FID

detector response was in form of a chromatogram. A 10 ml µl volume syringe was used for the injections. The percentage relative standard deviation (% RSD) was not more than 5 % for manual injection and 2 % for an automatic injection. 1.0 µl was injected for both samples and standards with 3 replicate injections for the sample and 5 replicate injections for the standards.

3.5.2 Analysis of chlorpyrifos and cypermethrine

The column initial temperature was 180 ^{o}C for 1.0 min, then raised to 280 ^{o}C at a rate of 40 ^{o}C/min where it was held for 3 min. The injector and detector temperatures were still set at 280 °C and 280 °C respectively. Chlorpyrifos is a less polar molecule so it eluted first while cypermethrine is relatively more polar and thus eluted last.

3.6 Quantification of residue levels

The residue levels of all the studied acaricides were quantitatively determined by the general method of using peak area. For the quantitative determinations, the concentrations of the analytes were kept within the linear range of the detector. The peak areas whose retention times coincided with the standards were recorded in an excel sheet to obtain the concentration. The formula used was:

$$Concentration\ of\ sample = \frac{Average\ peak\ area\ of\ sample}{Average\ peak\ area\ of\ \ standard} x Concentration\ of\ the\ standard$$

3.7 Quality assurance/ quality control

This section covers method validation, limit of detection, limit of quantitation and how recoveries determine to ensure accuracy of the methods.

3.7.1 Method validation

Specificity, Linearity and Range, Accuracy and Recoveries, LOD, LOQ, Ruggedness and Precision were performed at different levels by following the SANCO guidelines (SANCO (2013) to examine the efficiency of extraction, reliability of GC method, by analyzing reference compound and the analyses.

Specificity

The specificity of the method was obtained by analyzing standards, sample and due to blank interference technique: The hexane solution was injected to find out any peaks observed at the retention time (RT) corresponding to the peak of Chlorpyrifos, cypermethrin and amitraz. The standard solutions were also injected and their retention time for chlorpyrifos, cypermethrin and amitraz was recorded. There was no interfering peaks at the retention time (RT) corresponding to the peaks of chlorpyrifos, cypermethrin and amitraz. The detected peaks were solely due to the analyte, not another compound. The absence of any interfering peak indicated that the method is specific.

Linearity

Linearity levels in the range 80 % to 120% of the working level was prepared and the linearity regression coefficient and Y-intercept determined. This was done by preparing 1000ppm standard solution for Chlorpyrifos (solution A) and 100ppm of Cypermethrin (solution B). To obtain the 80% to 120% the following dilution scheme was used:

80%: 4ml of solution A and 4ml of solution B were pipetted into 10ml volumetric flask. Toped up with hexane and shaken to mix.

90%: 4.5 ml of solution A and 4.5 ml of solution B were pipetted into 10 ml volumetric flask. Toped it up with hexane and shake to mix.

100%: 5 ml of solution A and 5 ml of solution B were pipetted into 10 ml volumetric flask. Toped up with hexane and shaked to mix.

110%: 5.5 ml of solution A and 4 ml of solution B were pipetted into 10 ml volumetric flask. Toped up with hexane and shaken to mix.

120%: 6 ml of solution A and 4 ml of solution B into 10 ml volumetric flask and shake to mix.

The Linearity Regression Coefficient was 0.999 and the Y-intercept 0.05.

Range

The first linearity and last linearity levels were injected each five replicates and determined their RSD of the replicate injections. The RSD for replicate injections was not more than 5.0%.

Accuracy and Recoveries

Accuracy levels in the range of 80% to 120% of working level were prepared. This was done by spiking in triplicate the blank with the active ingredients at 80% to 120%. The % Recoveries were found to be between 98.0% to 102.0%. The values obtained are within the acceptance criteria for Accuracy and recovery studies. Hence the method is Accurate .

Robustness

Precision study was carried out by altering the detector temperature from 280ºC to 275ºC. The cumulative RSD for content of chlorpyrifos, cypermethrin and amitraz was determined. The cumulative RSD for the content of chlorpyrifos and amitraz cypermethrin was found to be 5.0%.

Limit of detection

Limit of detection is the lowest analyte concentration of a sample which can still be detected by the analysis method but not necessarily quantitated as an appropriate value. LOD was calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels according to the formula: LOD = 3.3(SD/S). where SD is the standard deviation of the response and S is slope of the calibration curve. Hence the data obtained from linearity was used to calculate the SD and S. LOD in all cases was found to be 0.005ppm.

Limit of quantification

Limit of quantification is the lowest sample concentration which can still be quantitatively detected with accuracy and an acceptable precision. LOQ is calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula: LOQ = 10(SD/S). Where SD is the standard deviation of the response and S is slope of the calibration curve. Hence the data obtained from linearity was used to calculate the SD and S. The LOQ in all cases was found to be 0.001 ppm

Precision

Assay determination for eighteen samples from three different batches (6 samples from each batch) of chlorpyrifos, amitraz and cypermethrin. Their RSD of Chlorpyrifos, amitraz and cypermethrin content of the six samples of each batch was determined.

The RSD for the content of chlorpyrifos, amitraz and cypermethrin for the six samples was found to be 5.0% hence the method is precise.

3.8 Statistical data analysis

Data was subjected to IBM SPSS statistical software version 22 and analysed using analysis of variance (ANOVA) to determine the significant difference in the mean values at $P \leq 0.05$. Duncan's test was used to test for significance difference among means.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

This section is concerned with the results and discussion from all the studied farms

4.1.1: Residue levels of amitraz, chlorpyrifos and cypermethrine in soil samples around cattle dip tanks.

The residue levels of amitraz, chlorpyrifos and cypermethrin in soil samples around cattle dip tanks were summarized in Table 4.1 indicating the farm, distance away from the cattle dip tanks and the residue concentrations in ppm.

The data obtained showed evidence of presence of amitraz, chlorpyrifos and cypermetherin present in the area for all the studied farms. Only amitraz residues were detected from farms A, B, and C at all studied distances away from the cattle dip tanks with no amitraz detection from farms D and E. At farms D and E, only chlorpyrifos and cypermethrin residues were detected and no amitraz residues was detected at any of the studied distances away from the cattle dip tanks.

At 25m away from the cattle dip tanks, farm A had had the highest amitraz residue level recorded as 87±1 ppm, followed by farm C with 55 ± 2 ppm and then farm B with 45 ± 1 ppm, farm E had the highest chlorpyrifos residue level recorded as 213±2 ppm, and cypermethrine residues as 15 ± 1 followed by farm D with chlorpyrifos residue level of 194 ± 4 ppm and cypermethrine residues as 18 ± 0 ppm

At 50 m away from the cattle dip tanks, farm A had had the highest amitraz residue level recorded as 58 ± 3 ppm, followed by farm B with 44 ± 3 ppm and then farm C with 41 ± 2 ppm, farm E had a chlorpyrifos residue level recorded as 178 ± 5 ppm, and cypermethrine residues as 14 ± 1 ppm while farm D had a residue level of 159 ± 2 ppm

and cypermethrine residues as 14 ± 1 ppm.both farm D and E had similar values of cypermethrine residues

At 75m away from the cattle dip tanks, farm B had had the highest amitraz residue level recorded as 33 ± 2 ppm, followed by farm A with 32 ± 3 ppm and then farm C with 28 ± 2 ppm, farm E had a Chlorpyrifos residue level recorded as 154 ± 3 ppm, and cypermethrine residues as 10 ± 1 ppm while farm D had a residue level of 133 ± 2 ppm and cypermethrine residues as 13 ± 1 ppm.

Table 4.1**:** Mean concentrations of the analytes in soil samples

LOCATION	DISTANCES (m) FROM DIP TANK	RESIDUES LEVELS (ppm)		
		AMITRAZ	CHLORPYRIFOS	CYPERMETHERIN
FARM A	25 m	87±1	ND	ND
	50 m	58±3	ND	ND
	75 m	32±3	ND	ND
FARM B	25 m	45±1	ND	ND
	50 m	44±3	ND	ND
	75 m	33±2	ND	ND
FARM C	25 m	55±2	ND	ND
	50 m	41±2	ND	ND
	75 m	28±2	ND	ND
FARM D	25 m	ND	194±4	18±0
	50 m	ND	159±2	14±1
	75 m	ND	133±2	13±1
FARM E	25 m	ND	213±2	15±1
	50 m	ND	178±5	14±1
	75 m	ND	154±3	10±1

Mean± SD

SD standard deviation, ND non-detectable, the limits of detection in all cases was 0.005 ppm US MRLs United States maximum residue limits for pesticides in agricultural soils < 0.01 mg/kg or < 0.01ppm.

The concentration of amitraz residues was highest close to the dip tank and decreased with distance from the cattle dip tank. This could be due to the fact that as the cows move away from the cattle dip tank they carry some acaricides along and not all the acaricides flow back into the cattle dip tanks. As cows move back to the grazing areas when they are not completely dry, the acaricide residue continue dripping off the cattle skin, and the rate of dripping off decreases as the cattle move further away from the dip tanks.

.

Both Chlorpyrifos and cypermethrin residues were not detected in the soils samples from the three farms A, B and C. This is due to the fact that the 3 farms mentioned have been using only amitraz acaricides for a number of good years without changing. Secondary could be due to the short half-life of the compounds.

4.1.2: Residue levels of amitraz, chlorpyrifos and cypermethrine in water samples around cattle dip tanks.

The residue levels of amitraz, chlorpyrifos and cypermethrine in water samples around cattle dip tanks are summarized in Table 2 indicating the farm and the residue concentrations in ppm

The data showed (Refer to Table 2) that only amitraz residues were detected from water samples collected from farms A, B, and C at all studied distances away from the cattle dip tanks with no Chlorpyrifos and cypermethrin being detected at the three farms A, B and C

At farms D and E, only Chlorpyrifos and Cypermethrin residues were reported from water samples collected at farms E and D and no amitraz residues was detected at any of the studied distances away from the cattle dip tanks.

At 20 m away from the cattle dip tanks, farm A had had the highest amitraz residue level recorded as 73±3 ppm, followed by farm C with 39±2 ppm and then farm B with 38±2 ppm.

At 14 m away from the cattle dip tanks, farm D had both Chlorpyrifos and cypermethrin residue level recorded as 179±3 ppm and 13±1 ppm respectively.

At 13 m away from the cattle dip tanks, farm E had both Chlorpyrifos and cypermethrin residue level recorded as 197±1 ppm and 15±1 ppm respectively.

Table 4.2: Residue level of amitraz, chlorpyrifos and cypermethrine in water samples

LOCATION	**DISTANCE (m) FROM CATTLE DIP TANK**	**Concentrations (ppm)** AMITRAZ	CHLORPYRIFOS	CYPERMETHERIN
FARM A	20	73±3	ND	ND
FARM B	20	38±2	ND	ND
FARM C	20	39±2	ND	ND
FARM D	14	ND	179±3	13±1
FARM E	13	ND	197±1	15±1

Mean± SD

SD standard deviation, **ND** not-detected, **LOD** limit of detection = 0.005 mg/kg or 0.005 ppm United States maximum residue limits (US MRLs) for pesticides in agricultural soils. < 0.01 mg/kg or <0.01 ppm and the maximum residue limits (MRL) for OPPs in water is 0.05 µg/l or 0.00005 ppm.

Total number of water samples was 15 that is to say picking in triplet samples per every farm for all the five farms.

Water sources from farm A and C were open and close to the cattle dip tank surrounded more dry grass, water source from farm B was also open and close to the cattle dip tank and surrounded by a little more less dry grass as compared to water sources from farms A and C. Water sources from farm E and D were both open and nearer to the cattle dip tank surrounded by more dry grass.

Water sources nearer the cattle dip tank had high residue level compared to those far away from the dip. All the three residues were not detected in the soil control samples. Amitraz residues were not detected at Amara and Rubyerwa farms. This is due to the fact that Amara and Rubyerwa have been using both chlorpyrifos and cypermethrin for a long period of time. The concentration of amitraz, chlorpyrifos and cypermethrine residues of water samples were highest for those farms close to the dip tank and decreased with distance from the cattle dip tank. This could be due to the fact that as the cows move away from the cattle dip tank they carry some acaricides along. As cows move back to the grazing areas when they are not completely dry, some cows move into these water sources to drink and some put part of their skin into water source, in this process, the acaricide residue continue to wash off from the cattle skin into these water sources. Both Chlorpyrifos and cypermethrin residues were not detected in the water samples from the three farms A, B and C. This is due to the fact that the 3 farms mentioned have been using only amitraz acaricides for a number of good years without changing.

4.1.3: Concentration of amitraz, chlorpyrifos and cypermethrin in cattle dip tanks.

The study showed that amitraz was found in the three dip tanks from farms A, B, and C while Chlorpyrifos and cypermethrine were detected from farms D and E.

Farm A had the highest amitraz concentration of 935 ± 3 ppm, followed by farm B with 816 ± 2 ppm and then farm C with 285 ± 2 ppm. All the concentrations exceeded the allowable maximum standard values / Normal range of 250 ± 5 ppm of amitraz inside the cattle dip tank an indication of overdosing of the cattle dip tanks by farmers.

At farms D and E, both Chlorpyrifos and cypermethrine were reported with farm E having the highest concentration of 990 ± 5 ppm as Chlorpyrifos and 99 ± 4 pmm as cypermethrine followed by farm D with concentration of 970 ± 5 ppm as Chlorpyrifos and 90 ± 4 pmm as cypermethrine. All the concentrations exceeded the allowable maximum standard values / Normal range of 500 ± 5 ppm Chlorpyrifos and 50 ± 5 ppm for cypermethrine inside the cattle dip tank an indication of overdosing of the cattle dip tanks.

The amitraz, Chlorpyrifos and Cypermethrine concentration inside cattle dip tanks were summarized in table 4.3 indicating the farm and the concentrations in ppm.

Table 4.3**:** Concentration of amitraz, chlorpyrifos and cypermethrine in farms A, B, C, D and E inside the cattle dip tanks**.**

LOCATION	**CONCETRATION (ppm)**		
	AMITRAZ	CHLORPYRIFOS	CYPERMETHERIN
FARM A	935±3	ND	ND
FARM B	816±2	ND	ND
FARM C	285±2	ND	ND
FARM D	ND	970±5	90±4
FARM E	ND	990±5	99±4

Mean ± SD

Total number of water samples analyzed was 15 that is to say picking in triplet samples per every farm for all the five farms.

Variation of Amitraz concentration inside the cattle dip tank for farms A, B, and C plus their standard values were summarized in figure 4.1

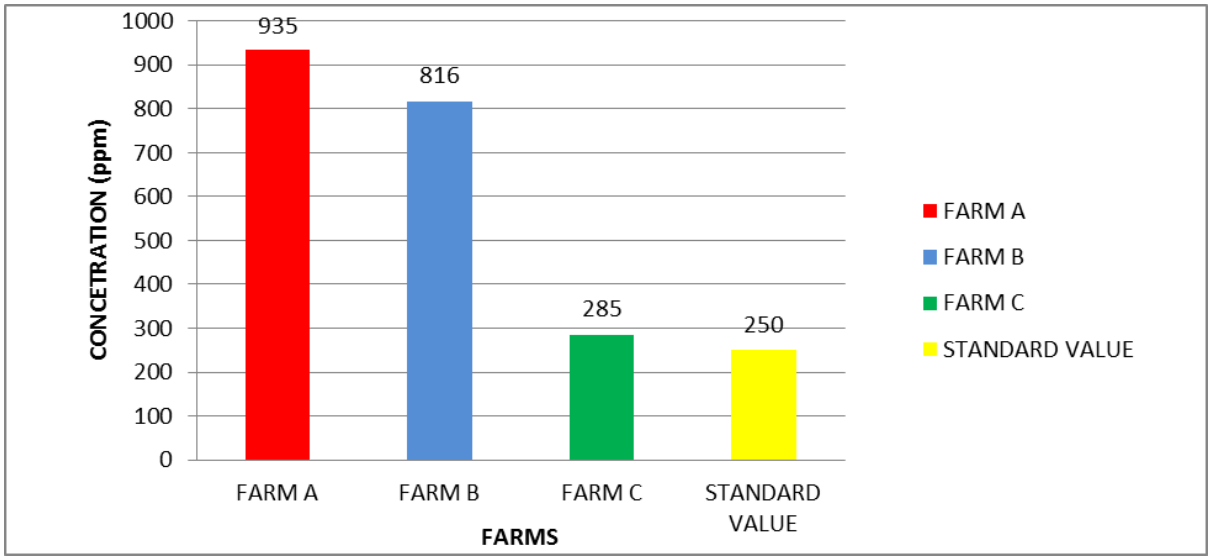


Figure 4.1: Amitraz concentration (ppm) inside the cattle dip tank for farms A, B and C

Variation of Chlorpyrifos and Cypermethrine concentration inside the cattle dip tank for farms E and E plus their standard values were summarized in figure 4.2.

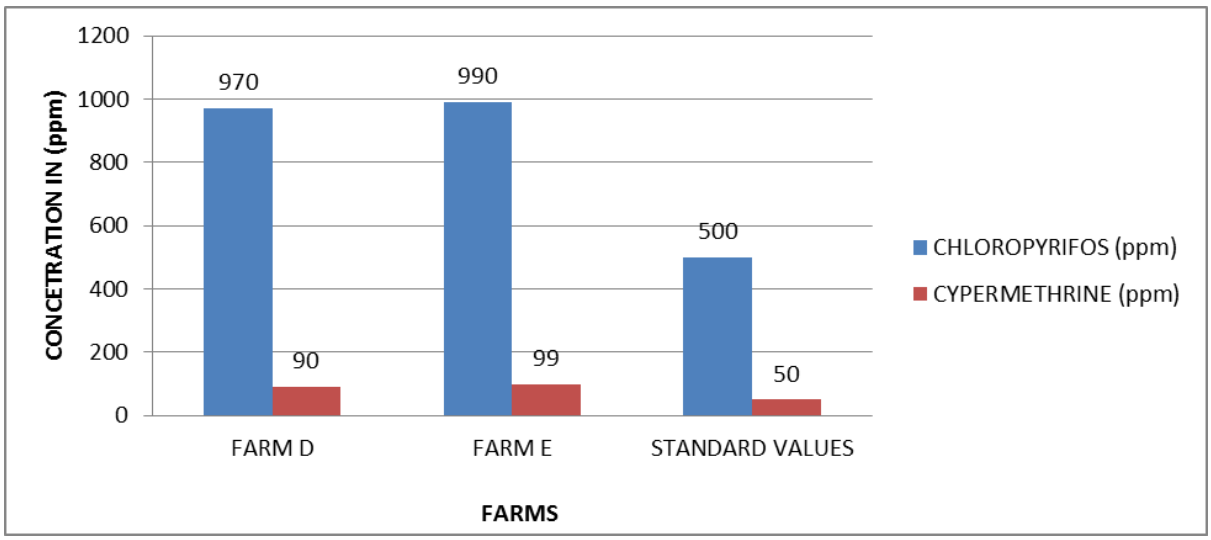


Figure 4.2: Chlorpyrifos and cypermethrine concentration inside the cattle dip tanks for farms D and E.

4.1.4: Residue levels of amitraz, chlorpyrifos and cypermethrine in soak pits

The residue levels of amitraz, chlorpyrifos and cypermethrine in soak pit samples around cattle dip tanks were summarized in Table 4.4 indicating the farm, and the residue concentrations in ppm. The study results indicate that amitraz residues were found in soak pits only from farm A, B, and C. Farm A had the highest residue amitraz levels of (109 ± 4 ppm) followed by farm farm C with (69 ± 2 ppm) and farm B had (57 ± 1 ppm). Chlorpyrifos and cypermetherin were detected from soaks pits of farms D and E ,with farm D having the highest chlorpyrifos residue level of (258 ± 2 ppm) and cypermethrin residue level of (21 ± 2 ppm). Farm E had (254 ± 3 ppm) chlorpyrifos and (20 ± 1 ppm) of cypermethrin.

Amitraz residues where not detected from farms D and E while both Chlorpyrifos and cypermethrin were not detected from farm A, B, C, D and E.

Variation of Chlorpyrifos and cypermethrine concentration of soak pit soil samples for farms A, B, C, D and E were summarized in Table 4.4

Table 4.4: Residue level of amitraz, chlorpyrifos and cypermethrine in soakpits.

LOCATION	**SOAKPITS**	**RESIDUES LEVEL CONCETRATION (ppm)**		
		AMITRAZ	CHLORPYRIFOS	CYPERMETHERINE
FARM A	SOAK PIT	109±4	ND	ND
FARM B	SOAK PIT	57±1	ND	ND
FARM C	SOAK PIT	69±2	ND	ND
FARM D	SOAKPIT	ND	258 ± 2	21 ± 2
FARM E	SOAK PIT	ND	254±3	20 ± 1

Mean ± SD

Total number of soak pit samples was 15 that is to say picking in triplet samples per every farm from the soak pits for all the five farms.

Soak pits have the highest level of residue concentration as compared to the residue concentrations in the soils as you move away from the cattle dip tank, which is attributed to accumulation of acaricide residues over time, during dipping process, the dip tanks dissipate/drain into the soak pit and when it rains, the dip tanks over flow leading to spreading allover into the surrounding environment. Being found at the lower gradient, acaricide residues tend to accumulate inside the soak pits at the lower gradients since soak pits are not constructed on high level areas but lower level area are more preferred.

There were no acaricides detected in all the control soil samples collected at 150 m away from the cattle dip tank indicating that acaricide residues are only present in areas close to catte dip tanks (Table 4.6 and Table 4.7)

The table below gives a summary of the standard values for Amitraz, Chlorpyrifos and cypermethrine concentration inside the cattle dip tanks

Table 4.5: Normal ranges in the cattle dip tanks

ACARICDE	**STANDARD RANGES IN CATTLE DIP TANK (ppm)**
AMITRAZ	250 ± 5
CHLORPYRIFOS	500 ± 5
CYPERMETHERINE	50 ± 5

Table 4.6: Residue level of amitraz, chlorprifos and cypermethrin in soil contol sample at 150M distance from cattle dip tank.

LOCATION	**RESIDUES LEVEL CONCETRTION (ppm)**		
	AMITRAZ	CHLOROPYRIFOS	CYPERMETHERIN
FARM A	ND	ND	ND
FARM B	ND	ND	ND
FARM C	ND	ND	ND
FARM D	ND	ND	ND
FARM E	ND	ND	ND

There was no detection of acaricide in all the soil control samples indicating that residues are only detected in only areas nearer the catte dip tank.

Table 4.7: Residue level of amitraz, chloropyrifos and cypermethrine in water contol samples at 300 M distance from cattle dip tanks.

LOCATION	**RESIDUES LEVEL (ppm)**		
	AMITRAZ	CHLOROPYRIFOS	CYPERMETHERIN
FARM A	ND	ND	ND
FARM B	ND	ND	ND
FARM C	ND	ND	ND
FARM D	ND	ND	ND
FARM E	ND	ND	ND

There was no detection of acaricide in all the water control samples indicating that residues are only detected in areas nearer the catte dip tank.

4.2 Discussion

The concentrations of amitraz, chlorpyrifos and cypermethrin residues were high near dip tanks but decreased with increasing distance away from the tanks (Table 4.1). This is certainly due to the fact that as the cattle exit the dip tanks, not all the acaricides flow back into the cattle dip tank and the animal bodies are not entirely dry, thus the acaricides keep dripping off. The rate of acaricide residues' drip off evidently decreases as the animals move further away from the dip tank. It is also expected that the soils upon which the animals freshly emerging from the dip tank lie is likely to get contaminated. Both chlorpyrifos and cypermethrine residues were not detected in the samples taken from farms A, B and C. It was established that on these particular farms, only amitraz acaricides are used.

The soil samples from farm A had the highest amitraz residue content of (87±1 ppm) followed by farm C with (55±2 ppm) and then farm B with (45±1) ppm (Table 4.1). Both chlorpyrifos and cypermethrine residues were detected for farms D and E as shown in Table 4.1

At 25 m away from the cattle dip tanks, there was a significant difference in amitraz residue concentration in soil samples from farms A, B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as analyte. At 50 m away from the cattle dip tank, there was no significant difference in amitraz residue concentration in soils at farm B and C this is due to degradation process and similar half-lives of the analyte used in these farms. However, amitraz residue concentration in soils at farm A was significantly different to that of B and C. while farms D and E were at zero because amitraz was not used as analyte. At 75 m away from the cattle dip tank, there was no significant difference in amitraz residue concentration in soil samples at farm B and A this is due to degradation process and similar halflives of the analyte. However, amitraz

residue concentration at farm C was significantly different to that of A and B while farm D and E were at zero because amitraz was not used as acaricide in these particular farms

At 25 m away from the cattle dip tank, there was a significant difference in chlorpyrifos residue concentration in soil samples at farm E and D this is due to different dosing in farm E compared to D. However, farms A, B and C were at zero because chlorpyrifos was not used as analyte. This trend was similar to all other investigated distances of 50 m and 75 m away from the cattle dip tanks. At 25 m away from the cattle dip tank, there was a significant difference in cypermethrine residue concentration in soil samples at farm E and D this is due to different dosing in farm E compared to D. However, farms A, B and C were at zero because cypermethrin was not used as analyte while at 50 m there was no significant difference in cypermetherine residues detected in soils at farms D and E but farms A, B and C where still at zero. Finally at 75 m , there was a significant difference in cypermethrine residues detected at both farms E and D(Table 4.1)

There was a significant difference in amitraz residue concentration in water samples from farms A compared to farms B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as analyte. Similarly there was no significancy difference in residue concentration in water samples obtained from farm B and C . There was a significant difference in both chlorpyrifos and cypermethrine residue concentration in water samples at farm E and D this is due to different dosing in farm E compared to D. However, farms A, B and C were at zero because both chlorpyrifos and cypermethrin were not used as analytes in these particular farms A, B and C (Table 4.2)

There was a significant difference in amitraz concentration in cattle dip tank samples from farms A, B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as analyte. There was a significant difference in chlorpyrifos concentration in cattle dip tank samples from farms D and E this is due to different dosing in farm D compared to E. However, farms A, B and C were at zero because chlorpyrifos was not used as analyte. There was a significant difference in cypermethrin concentration in cattle dip tank samples from farms D and E this is due to different dosing in farm D compared to E. However, farms A, B and C were at zero because cypermethrin was not used as analyte (Table 4.3)

There was a significant difference in amitraz concentration in soak pit soil samples from farms A, B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as analyte. There was a significant difference in chlorpyrifos concentration in soak pit soil samples from farms D and E this is due to different dosing in farm D compared to E. However, farms A, B and C were at zero because chlorpyrifos was not used as acaricide in these particular farms. There was no significant difference in cypermethrin concentration in soak pit soil samples from farms D and E this is due to similar dosing and same halflives of the analyte in farms D and E. However, farms A, B and C were at zero because cypermethrin was not used as acaricides in these particular farms(Table 4.4)

The concentrations of both chlorpyrifos and cypermethrine in the soil samples from farms D and E were significantly higher than the maximum residue limits this is in agreement with earlier research by (Benedicta *et al*; 2016), who assessed organochlorine pesticide residues in soils and drinking water sources from agricultural farms.

In all the farms where acaricide residues were detected in the soil samples, water samples and cattle dip tank samples the obtained values exceeded the United States maximum residue limits (US MRLs) for pesticides in agricultural soils < 0.01 mg/kg or <0.01 ppm.

From table 2, it can be observed that amitraz residues were detected in the water samples from farms A, B and C but neither chlorpyrifos nor cypermethrine was detected, meanwhile, the water samples from farms E and D both contained residues of chlorpyrifos and cypermethrin but amitraz was not detected (Table 4.2). This occurrence is due to the continuous selective of specific acaricides by the farmers for a long period of time.

One-way Analysis of variance (ANOVA) at 95 % confidence interval revealed that there was a significant difference in amitraz residue concentration in water samples from farms A compared to farms B and C this is due to different dosing in farm A compared to B and C. However, farms D and E were at zero because amitraz was not used as analyte. Similarly there was no significancy difference in residue concentration in water samples obtained from farm B and C . There was a significant difference in both chlorpyrifos and cypermethrine residue concentration in water samples at farm E and D this is due to different dosing in farm E compared to D. However, farms A, B and C were at zero because both chlorpyrifos and cypermethrin were not used as analytes in these particular farms A, B and C (Table 4.2).

Amitraz, chlorpyrifos,and cypermethrine residues in water resources around cattle dip tanks on animal farm exceeded the United States maximum residue limits (US MRLs) for pesticides in agricultural soils < 0.01 mg/kg or <0.01 ppm. This confirms earlier research by (Del Prado-Lu, 2015) who determined insecticide residues in soil, water, and eggplant fruits on agricultural farms.

Analysis of variance at 95 % confidence interval revealed that there was a significant difference in both chlorpyrifos and cypermethrine residue concentration in water samples at farm E and D this is due to different dosing in farm E compared to D. However, farms A, B and C were at zero because both chlorpyrifos and cypermethrin were not used as acaricide in these particular farms A, B and C (Table 4.2)

From table 3, the concentration of all the studied acaricides inside the cattle dip tanks, amitraz (250 ± 5 ppm), Chlorpyrifos (500 ± 5 ppm) and Cypermethrine (50 ± 5 ppm), exceed the recommended standard values this indicating that farmers tend to overdose the dip tanks.

From table 4, soak pits have the highest level of residue concentration with respect to amitraz detected on farms A, B and C while chlorpyrifos and cypermethrin were detected on farms D and E. This high concentration is due to frequent change of dips, draining of dips into soak pits, being found at the lower gradient and the acaricide residues tend to accumulate at the lower gradients.

No acaricide residues were detected in all the soil and water control samples indicating low mobility of the residues that remain localized in areas near the catte dip tanks.

The precence of these acaricide residues in soils confirms with earlier reserch by (Edwards, 1966) who determined Analysis of insecticides and acaricide residues in soils. Also earlier research by (Nonga, *et al.*, 2011) who Assessed farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania also confirm to the presence of acaricide residues in soils..

However, results for water sources from this study revealed a significant difference (Table 2) than the maximum residue limits and therefore donot agree with earlier research by (Turyahikayo, 2013) who assessed the levels of pesticide residues in livestock products and water around Lake Mburo National Park, South Western Uganda . This could be attributed to seasonal and climate changes for all the years

Presence of acaricides residues in the water samples could be due to animal carry-over, drift during acaricides application, direct overspray, direct spillage, pesticides misuse, improper disposal of left-over spray solutions, sprayer wash water and pesticide containers, run-off from treated areas or leaching, among others.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results from this study show that acaricide residues are present in soil and water resources within and around animal cattle dip tanks on selected farm in the two subcounties of Mbarara district. Three acaricide residues (amitraz , Chlorpyrifos, cypermethrine) were detected. Amitraz was only detected on farms A, B and C, while Chlorpyrifos and cypermethrin were only detected on farms D and E

The levels of the studied acaricide residues (amitraz, Chlorpyrifos and cypermethrin) in the soils and water resources in the areas around cattle dip tanks exceeded the allowable maximum residue levels (MRL) hence hypothesis $H_1 1$ is upheld.

The acaricide concetration in cattle dip tanks exceeded the allowable standard ranges hence hypothesis $H_1 2$ is upheld.

5.2 Recommendations

Basing on the conclusions from the study, it was recommended since the acaricide residues were present in soil and water resources around animal farms in Mbarara District, the water should not be used for direct human consumption because it poses health risks.

Also, the soils should not used for agriculture since the residues could enter the food chain in the crops grown on them.

There is also need to enhance the laboratory capacities and facilities in acaricide residue analysis in order to cope with the newly emerging challenges of acaricide residues.

Proper engineering of the dip tanks with slanting surfaces to make sure that all the acaricide remains flow back into the cattle dip tank. Increased holding time to enable longer dripping off of acaricides remains from the animal skin.

There should be a coordinating team of public private partnership for close monitoring of appropriate use of acaricides at the field level. In addition, there is need for a massive campaign to all farmers, retailers, distributors of pesticides about the use and safe handling of acaricides.

The need to sensitize farmers on safe pesticide use is thus crucial to reduce the levels of acaricides residues in soils and water in the study area.

There is need for comprehensive assessment of pesticide residues in livestock products for a longer period to generate data for more research and guiding policy makers.

Routine monitoring of pesticide residues in the study area is necessary for the prevention, control and reduction of environmental pollution, so as to minimize health risks to human.

Further study should be done on other acaricides that are not covered in this research.

REFERENCES

Ademoroti, C. (1996). Environmental chemistry and toxicology. *Ibadan: Foludex Press Ltd Journal of Environmental science*.

Agin, H., Calkavur, S., Uzun, H., & Bak, M. (2004). Amitraz poisoning: clinical and laboratory findings. *Indian pediatrics, 41*(5), 482-485.

Agrawal, A., Pandey, R. S., & Sharma, B. (2010). Water pollution with special reference to pesticide contamination in India. *Journal of Water Resource and Protection, 2*(05), 432.

Aneani, F., & Ofori-Frimpong, K. (2013). An analysis of yield gap and some factors of cocoa (Theobroma cacao) yields in Ghana. *Sustainable Agriculture Research, 2*(4), 117.

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C., & García-Río, L. (2008). The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, Ecosystems & Environment, 123*(4), 247-260.

Batarseh, M., & Tarawneh, R. (2013). Multiresidue analysis of pesticides in agriculture soil from Jordan Valley. *Jordan journal of chemistry, 146*(897), 1-17.

Bogialli, S., Bruno, M., Curini, R., Di Corcia, A., Fanali, C., & Laganà, A. (2006). Monitoring algal toxins in lake water by liquid chromatography tandem mass spectrometry. *Environmental science & technology, 40*(9), 2917-2923.

Bouët, A., & Odjo, S. P. (2019). *Africa agriculture trade monitor 2019*: Intl Food Policy Res Inst.

Chen, W., & Plewig, G. (2014). Human demodicosis: revisit and a proposed classification. *British Journal of Dermatology, 170*(6), 1219-1225.

Costa, L. G., Giordano, G., Guizzetti, M., & Vitalone, A. (2008). Neurotoxicity of pesticides: a brief review. *Front Biosci, 13*(4), 1240-1249.

Cox, J. D., Stetz, J., & Pajak, T. F. (1995). Toxicity criteria of the radiation therapy oncology group (RTOG) and the European organization for research and treatment of cancer (EORTC). *International Journal of Radiation Oncology* Biology* Physics, 31*(5), 1341-1346.

Cui, Y., Guo, J., Xu, B., & Chen, Z. (2006). Potential of chlorpyrifos and cypermethrin forming DNA adducts. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 604*(1), 36-41.

Damalas, & Eleftherohorinos. (2011). Pesticide exposure, safety issues, and risk assessment indicators. *Int J Environ Res Public Health, 8*(5), 1402-1419. doi: 10.3390/ijerph8051402

Damalas, & Koutroubas, S. (2016). Farmers' exposure to pesticides: toxicity types and ways of prevention: Multidisciplinary Digital Publishing Institute.

Darko, G., & Acquaah, S. O. (2007). Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science & Technology, 4*(4), 521-524.

Del Pino, J., Martinez, M., Castellano, V., Ramos, E., Martinez-Larranaga, M., & Anadon, A. (2013). Effects of exposure to amitraz on noradrenaline, serotonin and dopamine levels in brain regions of 30 and 60 days old male rats. *Toxicology, 308*, 88-95.

Del Prado-Lu, J. L. (2015). Insecticide residues in soil, water, and eggplant fruits and farmers' health effects due to exposure to pesticides. *Environmental health and preventive medicine, 20*(1), 53.

Edwards, C. (1966). Insecticide residues in soils *Residue Reviews* (pp. 83-132): Springer.

Fishel, F. M. (2008). IRAC's Insecticide mode of Action Classification. *Publication# PI-83 Available from:* http://edis. *ifas. ufl. edu/pi121, 18*(10).

Fosu-Mensah, B. Y., Okoffo, E. D., Darko, G., & Gordon, C. (2016). Assessment of organochlorine pesticide residues in soils and drinking water sources from cocoa farms in Ghana. *SpringerPlus, 5*(1), 869.

Benedicta Yayra Fosu-Mensah, B. Y., Okoffo, E. D., Darko, G., & Gordon, C. (2016). Organophosphorus pesticide residues in soils and drinking water sources from cocoa producing areas in Ghana. *Environmental Systems Research, 5*(1), 10.

Harrison, I. R., Kozlik, A., McCarthy, J. F., Palmer, B. H., Wakerley, S. B., Watkins, T. I., & Weighton, D. M. (1973). 1, 3, 5-Triazapenta-1, 4-dienes: Chemical aspects of a new group of pesticides. *Pesticide Science, 4*(6), 901-910.

Hayes, W. J., & Laws, E. R. (1991). Handbook of pesticide toxicology *Handbook of pesticide toxicology*: Academic Press.

Hsu, W. H., & Lu, Z. X. (1984). Amitraz-induced delay of gastrointestinal transit in mice: Mediated by α2-adrenergic receptors. *Drug development research, 4*(6), 655-660.

Hussien, H. M., Abdou, H. M., & Yousef, M. I. (2013). Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: the protective effect of sesame oil. *Brain research bulletin, 92*, 76-83.

Jett, D. A., Navoa, R. V., & Lyons, M. A. (1999). Additive inhibitory action of chlorpyrifos and polycyclic aromatic hydrocarbons on acetylcholinesterase activity in vitro. *Toxicology letters, 105*(3), 223-229.

Karabelas, A., Plakas, K., Solomou, E., Drossou, V., & Sarigiannis, D. (2009). Impact of European legislation on marketed pesticides—a view from the standpoint of health impact assessment studies. *Environment International, 35*(7), 1096-1107.

Kasozi, G., Kiremire, B., Bugenyi, F., Kirsch, N., & Nkedi-Kizza, P. (2006). Organochlorine residues in fish and water samples from Lake Victoria, Uganda. *Journal of environmental quality, 35*(2), 584-589.

Khopkar, S. (2007). *Environmental pollution monitoring and control*: New Age International.

Kulkarni, A., Soppimath, K., Dave, A., Mehta, M., & Aminabhavi, T. (2000). Solubility study of hazardous pesticide (chlorpyrifos) by gas chromatography. *Journal of hazardous materials, 80*(1-3), 9-13.

Kyomuhendo, P. (2002). *A bioeconomic model for Uganda's Lake Victoria Nile Perch fishery*. Universitetet i Tromsø.

Lahr, J., Buij, R., Katagira, F., & van der Valk, H. (2016). Pesticides in the Southern Agricultural Growth Corridor of Tanzania (SAGCOT): a scoping study of current and future use, associated risks and identification of actions for risk mitigation: Wageningen Environmental Research.

Lekei, E. E., Ngowi, A. V., & London, L. (2014). Farmers' knowledge, practices and injuries associated with pesticide exposure in rural farming villages in Tanzania. *BMC public health, 14*(1), 389.

Lorke, D. E., & Petroianu, G. A. (2019). Reversible cholinesterase inhibitors as pretreatment for exposure to organophosphates. A review. *Journal of Applied Toxicology, 39*(1), 101-116.

Mack, T., Funderburk, J., Lynch, R., Braxton, E., & Backman, C. (1989). Efficacy of Chlorpyrifos in Soil in 'Florunner'Peanut Fields to Lesser Cornstalk Borer (Lepidoptera: Pyralidae). *Journal of economic entomology, 82*(4), 1224-1229.

Madbuni, M. Z., & Amini, M. (2013). Risk Assessment Process of Amitraz on Environment and Human Health.

Manirakiza, P., Covaci, A., Nizigiymana, L., Ntakimazi, G., & Schepens, P. (2002). Persistent chlorinated pesticides and polychlorinated biphenyls in selected fish species from Lake Tanganyika, Burundi, Africa. *Environmental Pollution, 117*(3), 447-455.

Marino, D., & Ronco, A. (2005). Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. *Bulletin of environmental contamination and toxicology, 75*(4), 820-826.

Marshall, e. a. (2018). Investigating sediment sources and quality of sediment in Kananook Creek and associated waterways.

Matsumura, F. (2012). *Toxicology of insecticides*: Springer Science & Business Media.

Mbaria, J. M., Ogara, W. O., & Midiwo, J. O. (2008). Topic Document and assess the acaricidal potential of extracts of Psidia punctulata (Labaai) and Acokanthera schimperi (Lmorijoi) against prevalent tick species in Samburu district using the recommended in vitro bioassay techniques at University of Nairobi.

Natala, A., & Ochoje, O. (2009). Survey of pesticides used in the control of ectoparasites on farm animals in Kaduna State, Northern Nigeria. *Journal of Animal & Plant Sciences, 4*(1), 276-280.

Nganchamung, T., Robson, M. G., & Siriwong, W. (2017). Association between blood cholinesterase activity, organophosphate pesticide residues on hands, and health effects among chili farmers in Ubon Ratchathani Province, northeastern Thailand. *Roczniki Państwowego Zakładu Higieny, 68*(2).

Nonga, H., Mdegela, R., Lie, E., Sandvik, M., & Skaare, J. (2011). Assessment of farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania.

Okello-Onen, J., Ssekitto, C., Ssentongo, Y., & Kudamba, C. (1992). Tick situation and control strategies in Uganda. *International Journal of Tropical Insect Science, 13*(4), 657-660.

Okoffo, E. D., Fosu-Mensah, B. Y., & Gordon, C. (2016). Persistent organochlorine pesticide residues in cocoa beans from Ghana, a concern for public health. *International Journal of Food Contamination, 3*(1), 5.

Pass, M., & Mogg, T. (1995). Pharmacokinetics and metabolism of amitraz in ponies and sheep. *Journal of veterinary pharmacology and therapeutics, 18*(3), 210-215.

Sarwar, M., & Salman, M. (2015). Overall notable health challenges about the toxicity of pesticides concerning to end users. *International Journal of Bioinformatics and Biomedical Engineering, 1*(3), 323-330.

Shachi, K., Kumar, S., Dubey, U., Dubey, N., & Sinha, R. (2018). Indian Journal of Agriculture and Allied Sciences.

Smegal, D. C. (2000). Human health risk assessment chlorpyrifos. *US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Health Effects Division, US Government Printing Office: Washington, DC*, 1-131.

Srivastava, A. K., & Kesavachandran, C. (2019). *Health effects of pesticides*: CRC Press.

Timchalk, C., Nolan, R., Mendrala, A., Dittenber, D., Brzak, K., & Mattsson, J. (2002). A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicological Sciences, 66*(1), 34-53.

Turyahikayo, E. (2013). *Assessing the levels of pesticide residues in livestock products and water around Lake Mburo National Park, South Western Uganda.*

UBOS, I. (2011). International Inc. *Uganda demographic and health survey*, 315.

Vaarst, M., Roderick, S., Byarugaba, D. K., Kobayashi, S., Rubaire-Aiikii, C., & Karreman, H. J. (2006). Sustainable veterinary medical practices in organic farming: A global perspective *Global Development of organic Agriculture: Challenges and Prospects* (pp. 241-276): CAB International.

Wambua, B. N., & Muhigirwa, E. (2019). An Assessment of the Causes of Tick Resistance to Acaricides use on Cattle in kazo County Kiruhura District, Uganda. *International Journal of Applied, 9*(2).

Ware, J. E., Kosinski, M., Dewey, J. E., & Gandek, B. (2000). *SF-36 health survey: manual and interpretation guide*: Quality Metric Inc.

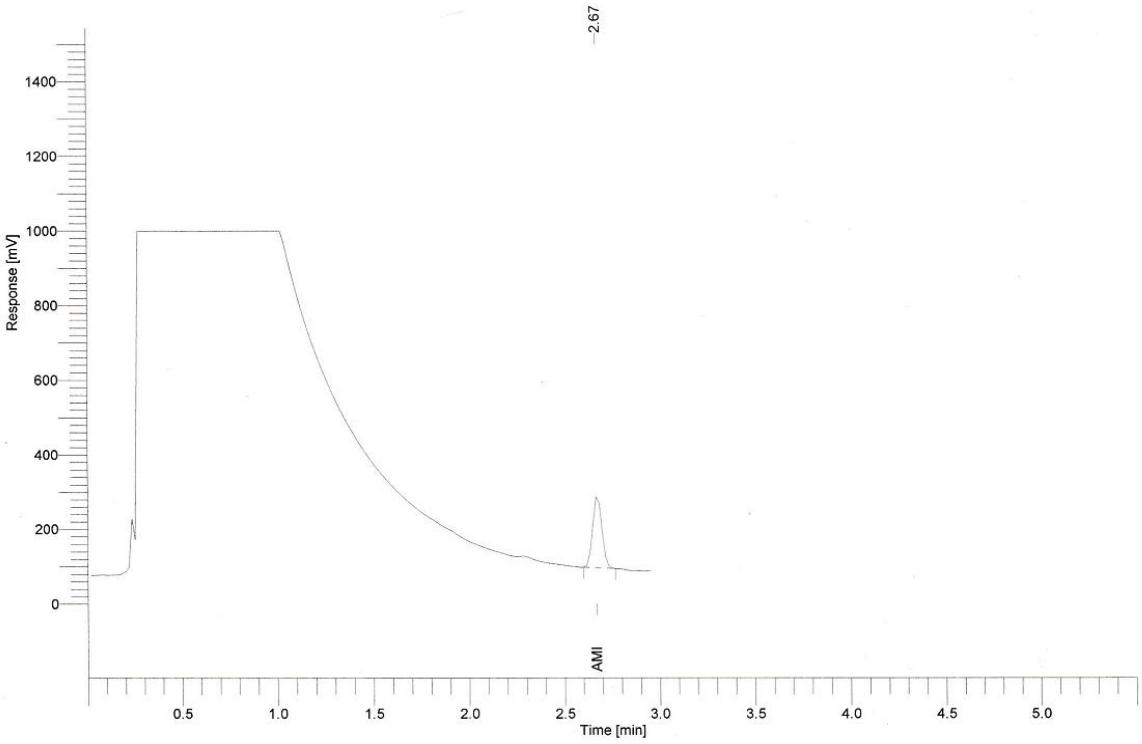
Wei, B., Mohan, K. R., & Weisel, C. P. (2012). Exposure of flight attendants to pyrethroid insecticides on commercial flights: urinary metabolite levels and implications. *International journal of hygiene and environmental health, 215*(4), 465-473.

Winter, C. K., & Davis, S. F. (2006). Organic foods. *Journal of food science, 71*(9).

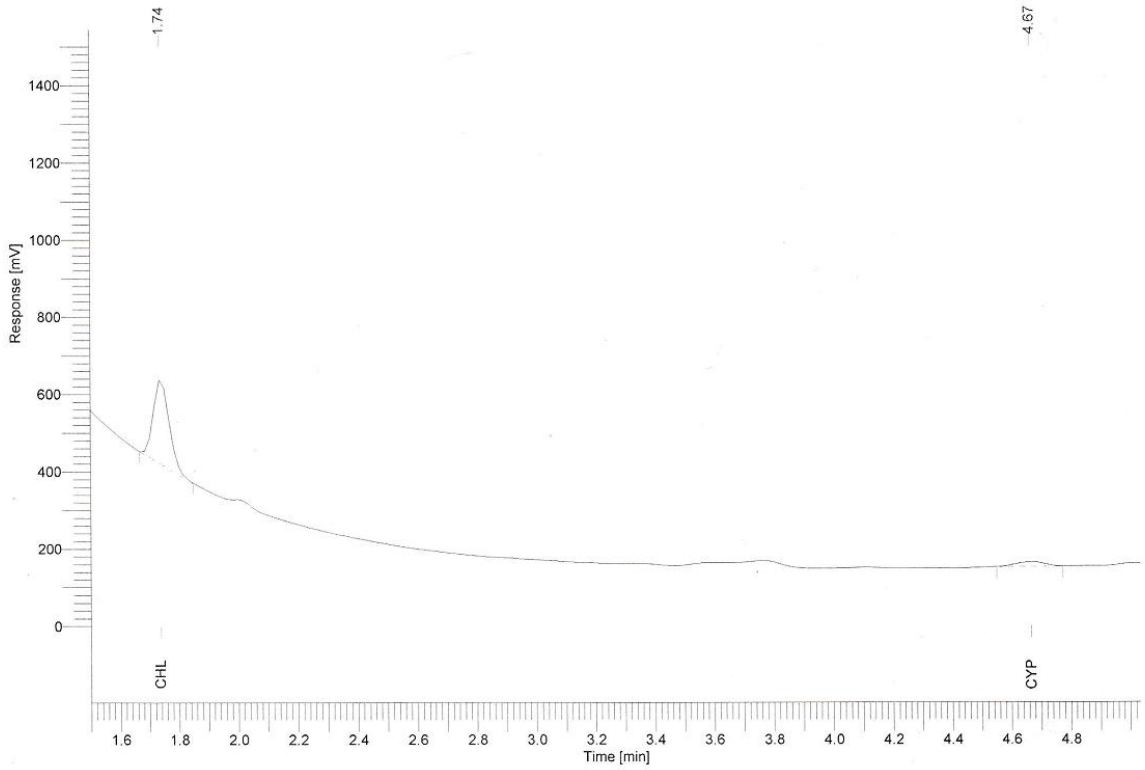
Appendix 1: Acaricide waste disposal

Appendix 2: Disposal of acaricide empty containers around the Farm.

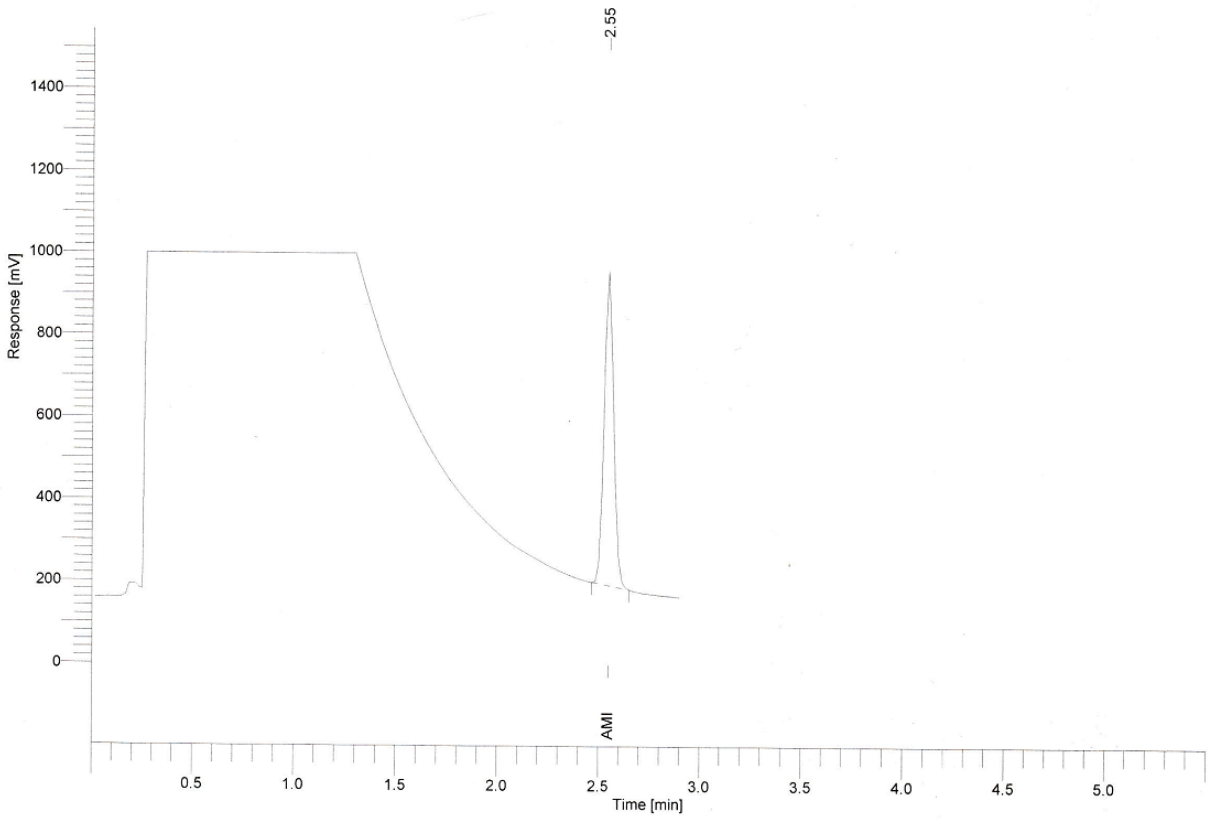
Appendix 3: Amitraz chromatogram for soil samples



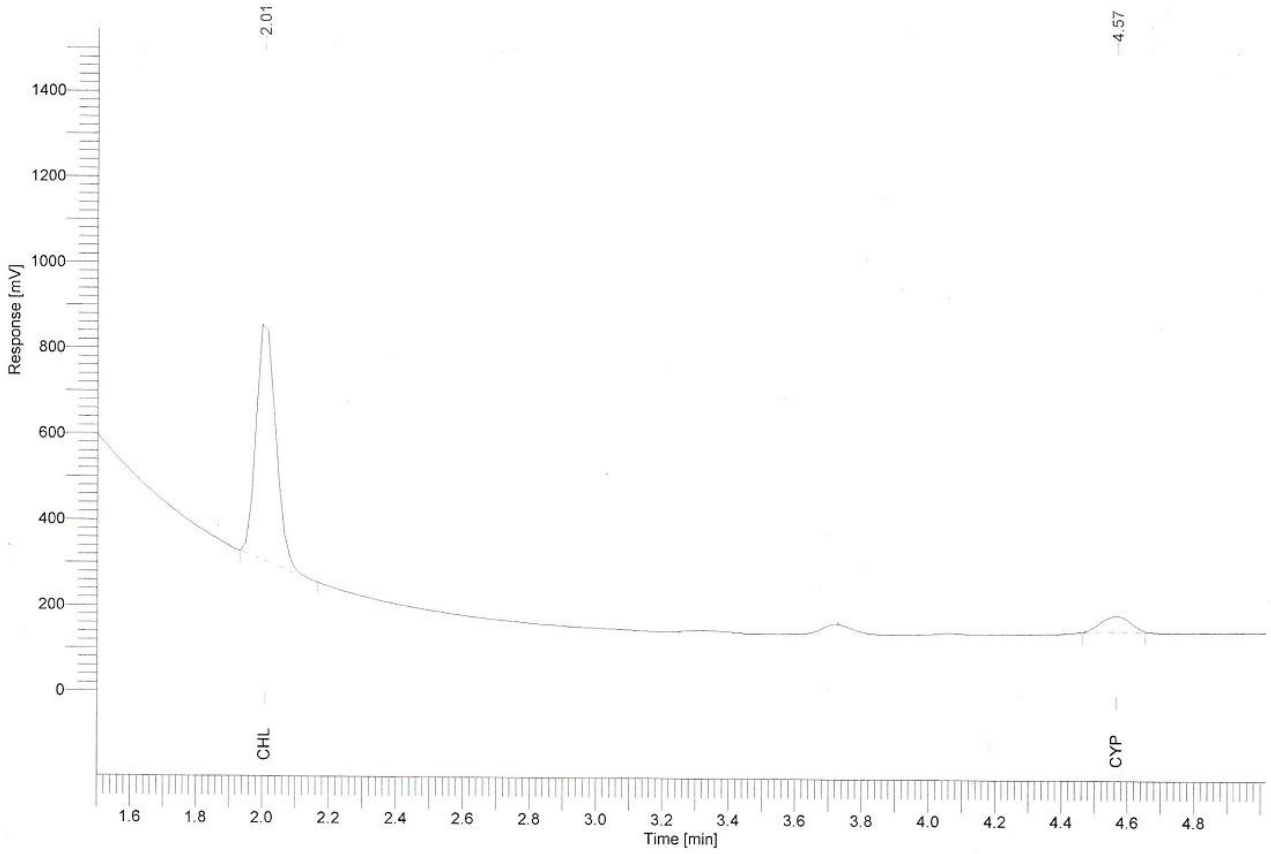
Appendix 4: Chlorpyrifos and cypermethrine chromatograms for soil samples



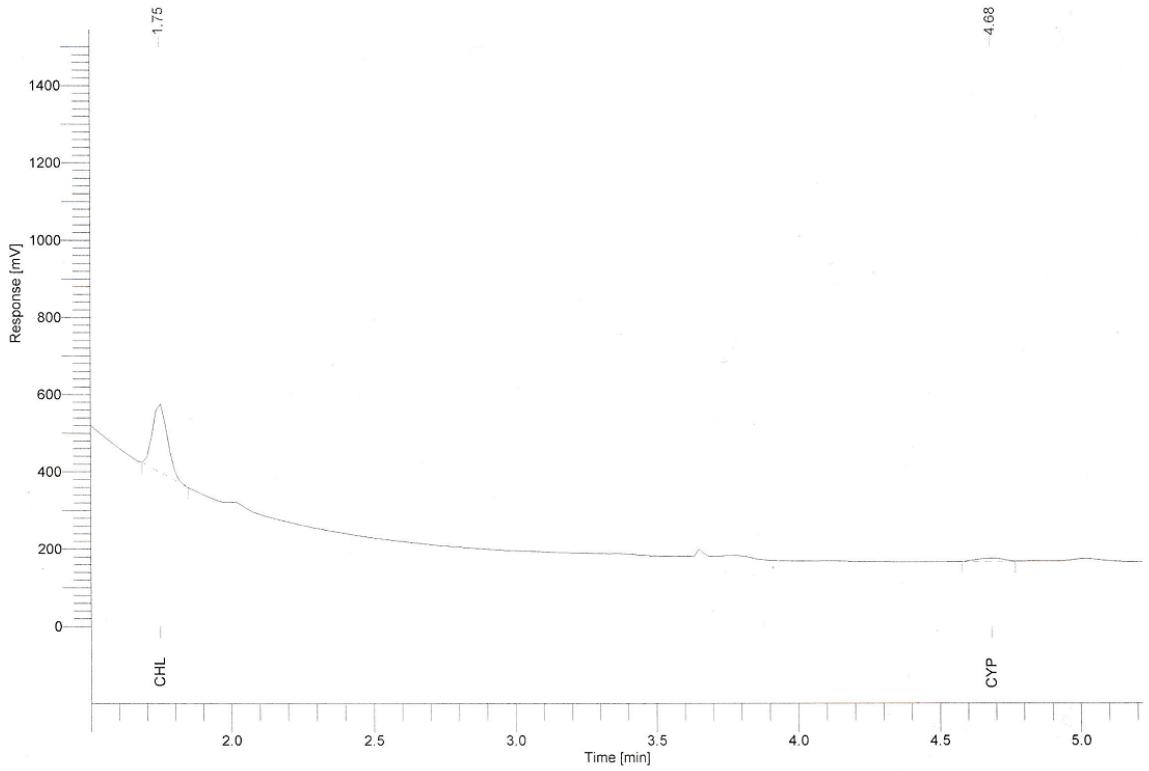
Appendix 5: Amitraz chromatogram for cattle dip tank samples



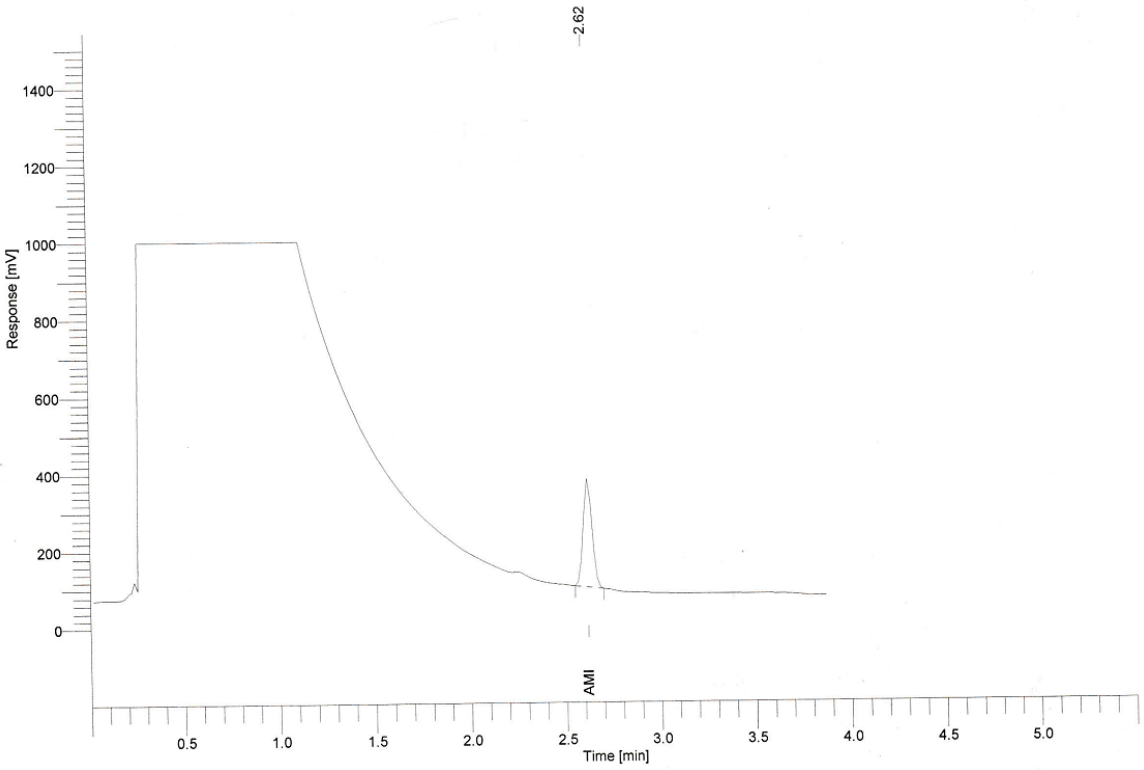
Appendix 6: Chlorpyrifos and cypermethrine chromatograms for dip tank samples



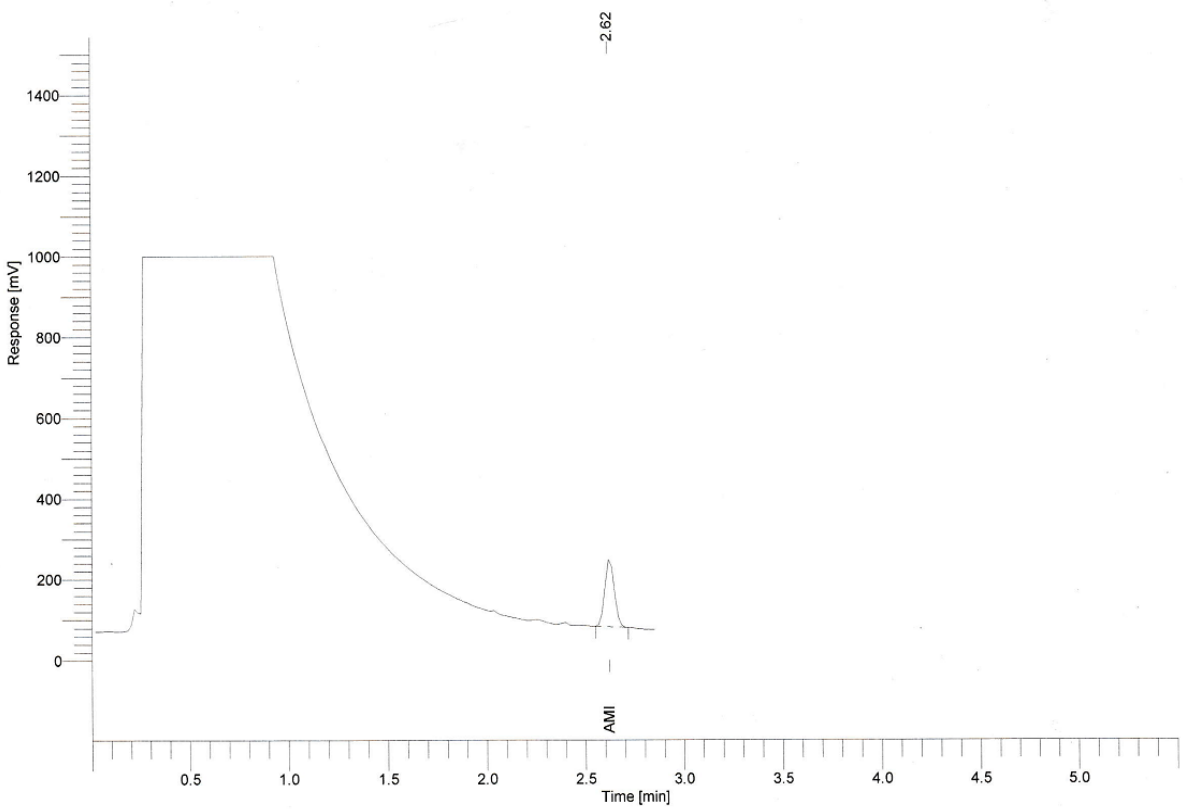
Appendix 7: Chlorpyrifos of and cypermethrine chromatograms for water sources



Appendix 8: Amitraz chromatogram for soak pit samples



Appendix 9: Chromatogram of amitraz for water samples



Appendex 10: Short description

A short description is explained as below for different factors (250 ppm, 500 ppm, and 50 ppm) for the three acaricides (amitraz, chlorpyrifos and cypermethrine)

AMITRAZ LABEL CLAIM IS 12.5% W/V

100 mls of solution contains = 12.5 g

100 mls of solution contains =12500 mg

1ml contains = 125 mg of amitraz

After taking 1ml, it is put into 100 ml volumetric flask and made up to the mark with hexane

100mls contains = 125 mg

1000 mls contains = 1250 mg/L or ppm

From C1V1 = C2V2

1250 ppm X 10 ml = C2 X 50

C2 = 250 ppm

Chlorpyrifos and cypermethrine label claims are 50% W/V and 5% W/V respectively

Chlorpyrifos label claim is 50%

100 mls of solution contains = 50 g of Chlorpyrifos

100 mls of solution contains =50,000 mg of Chlorpyrifos

1ml of solution contains = 500 mg of Chlorpyrifos

After taking 1ml, it is put into 100 ml volumetric flask and made up to the mark with hexane

100 mls contains = 500 mg

1000 mls contains = 5,000 mg/L or ppm

From C1V1 = C2V2

5,000 ppm X 5 ml = C2 X 50

C2 = 500 ppm

Cypermethrine label claim is 5%

100 mls of solution contains = 5 g of Cypermethrine

100 mls of solution contains =5,000 mg of Cypermethrine

1ml of solution contains = 50 mg of cypermethrine

After taking 1 ml, it is put into a 100 ml volumetric flask and made up to the mark with hexane

100 mls contains = 50mg

1000 mls contains = 500 mg/L or ppm

From C1V1 = C2V2

500 ppm X 5 ml = C2 X 50

C2 = 50 ppm

Appendex 11: Amitraz data analysis in soils for farms A, B, C, D and E at investigated distances of 25 m, 50 m and 75 m

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
25 M	FARM A	3	86.6667	1.15470	.66667	83.7982	89.5351	86.00	88.00
	FARM B	3	45.0000	1.00000	.57735	42.5159	47.4841	44.00	46.00
	FARM C	3	55.3333	1.52753	.88192	51.5388	59.1279	54.00	57.00
	FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	37.4000	34.66328	8.95002	18.2041	56.5959	.00	88.00
50 M	FARM A	3	57.6667	2.88675	1.66667	50.4956	64.8378	56.00	61.00
	FARM B	3	43.6667	2.51661	1.45297	37.4151	49.9183	41.00	46.00
	FARM C	3	41.3333	1.52753	.88192	37.5388	45.1279	40.00	43.00
	FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	28.5333	24.84773	6.41566	14.7731	42.2935	.00	61.00
75 M	FARM A	3	31.6667	3.05505	1.76383	24.0775	39.2558	29.00	35.00
	FARM B	3	33.3333	1.52753	.88192	29.5388	37.1279	32.00	35.00
	FARM C	3	28.3333	1.52753	.88192	24.5388	32.1279	27.00	30.00
	FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	18.6667	15.92692	4.11231	9.8466	27.4867	.00	35.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
25 M	Between Groups	16812.267	4	4203.067	4503.286	.000
	Within Groups	9.333	10	.933		
	Total	16821.600	14			
50 M	Between Groups	8609.733	4	2152.433	633.069	.000
	Within Groups	34.000	10	3.400		
	Total	8643.733	14			
75 M	Between Groups	3523.333	4	880.833	314.583	.000
	Within Groups	28.000	10	2.800		
	Total	3551.333	14			

Twenty five meters

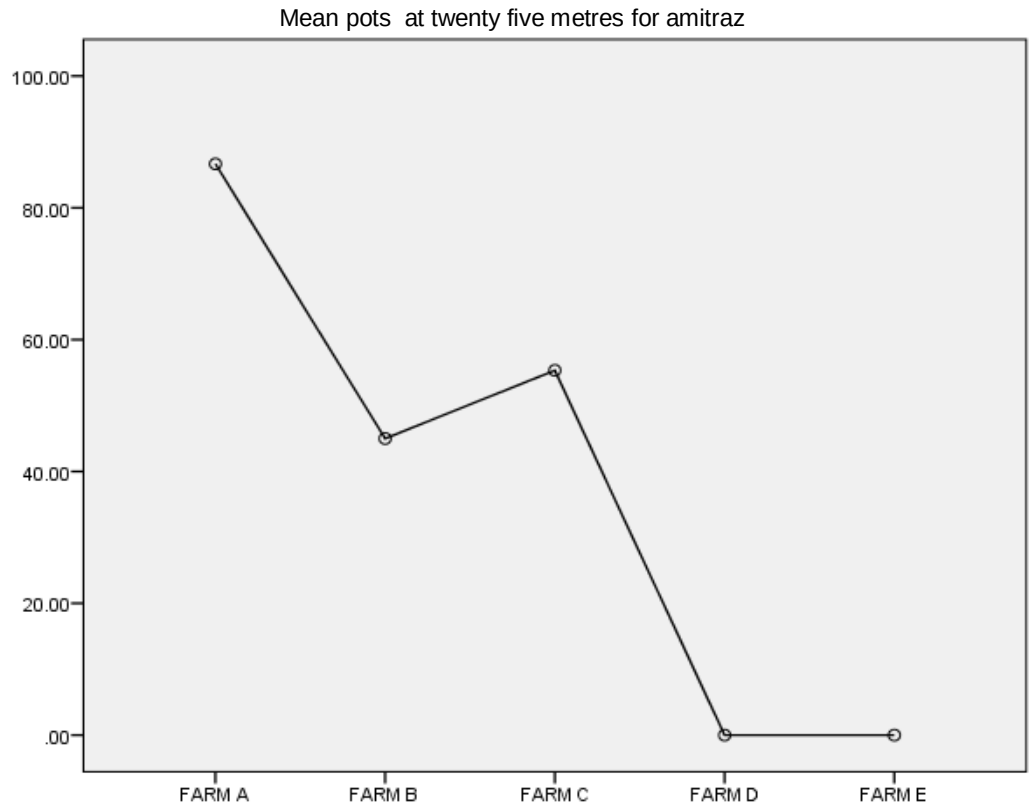
	CONCENTRATION OF AMITRAZ ON FARMS	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM B	3		45.0000		
	FARM C	3			55.3333	
	FARM A	3				86.6667
	Sig.		1.000	1.000	1.000	1.000
Duncan[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM B	3		45.0000		
	FARM C	3			55.3333	
	FARM A	3				86.6667
	Sig.		1.000	1.000	1.000	1.000

Fifty meters

	CONCENTRATION OF AMITRAZ ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM C	3		41.3333	
	FARM B	3		43.6667	
	FARM A	3			57.6667
	Sig.		1.000	.557	1.000
Duncan[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM C	3		41.3333	
	FARM B	3		43.6667	
	FARM A	3			57.6667
	Sig.		1.000	.152	1.000

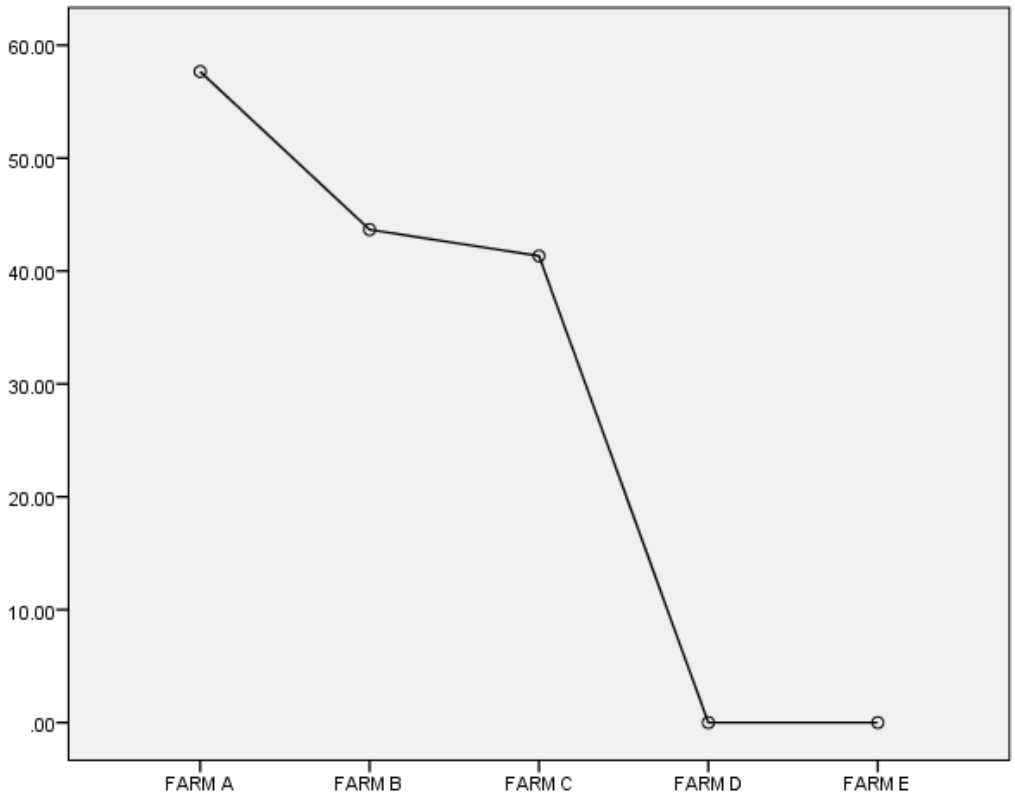
Seventy five meters

	CONCENTRATION OF AMITRAZ ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM C	3		28.3333	
	FARM A	3		31.6667	31.6667
	FARM B	3			33.3333
	Sig.		1.000	.182	.741
Duncan[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM C	3		28.3333	
	FARM A	3			31.6667
	FARM B	3			33.3333
	Sig.		1.000	1.000	.251

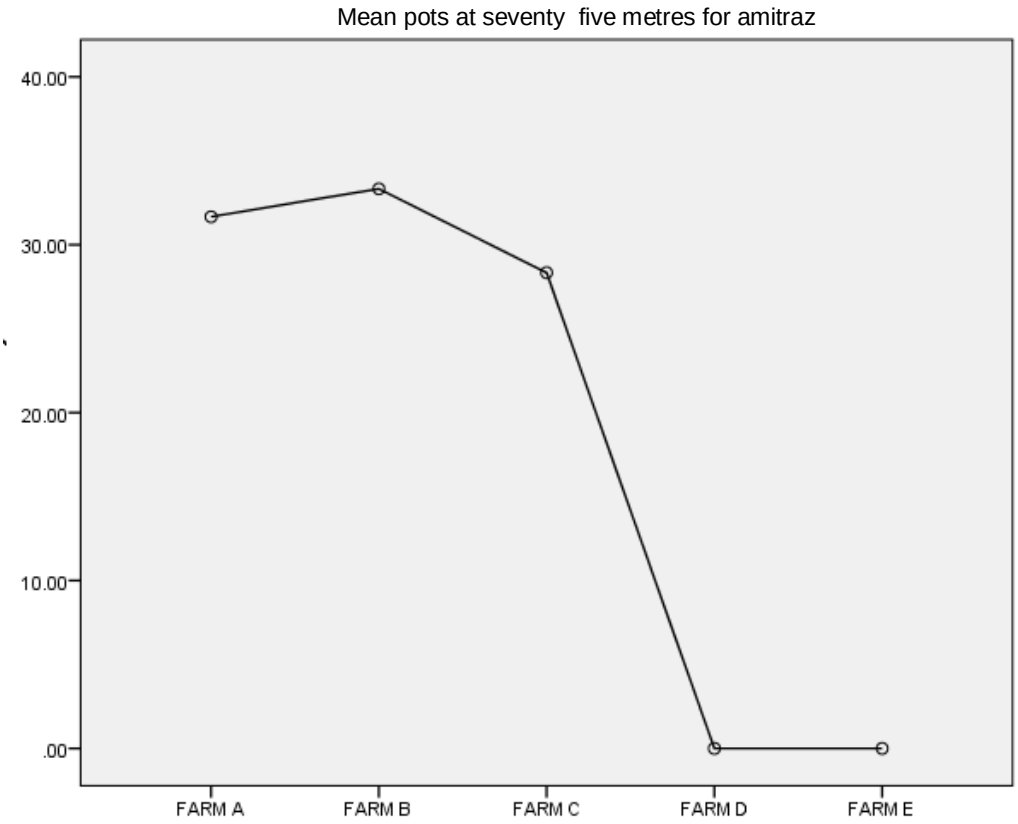


Concentration of amitraz on farms

Mean Plots at fifty metres for amitraz



Concentration of amitraz on farms



Concentration of amitraz on farms

Appendix 12: chlorpyrifos data analysis in soils for farms A, B, C, D and E at investigated distance of 25 m, 50 m and 75 m

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Twenty five	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	193.6667	4.04145	2.33333	183.6271	203.7062	189.00	196.00
	FARM E	3	213.0000	1.73205	1.00000	208.6973	217.3027	211.00	214.00
	Total	15	81.3333	103.31620	26.67613	24.1187	138.5479	.00	214.00
Fifty	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	158.6667	2.30940	1.33333	152.9298	164.4035	156.00	160.00
	FARM E	3	177.6667	4.50925	2.60342	166.4651	188.8683	173.00	182.00
	Total	15	67.2667	85.52399	22.08220	19.9051	114.6283	.00	182.00
Seventy five	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	133.3333	2.08167	1.20185	128.1622	138.5045	131.00	135.00
	FARM E	3	154.0000	3.46410	2.00000	145.3947	162.6053	150.00	156.00
	Total	15	57.4667	73.18164	18.89542	16.9400	97.9933	.00	156.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Twenty five	Between Groups	149400.667	4	37350.167	9659.526	.000
	Within Groups	38.667	10	3.867		
	Total	149439.333	14			
Fifty	Between Groups	102349.600	4	25587.400	4984.558	.000
	Within Groups	51.333	10	5.133		
	Total	102400.933	14			
Seventy five	Between Groups	74945.067	4	18736.267	5735.592	.000
	Within Groups	32.667	10	3.267		
	Total	74977.733	14			

Twenty five

	CONCENTRATION OF CHLORPYRIFOS ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		193.6667	
	FARM E	3			213.0000
	Sig.		1.000	1.000	1.000
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		193.6667	
	FARM E	3			213.0000
	Sig.		1.000	1.000	1.000

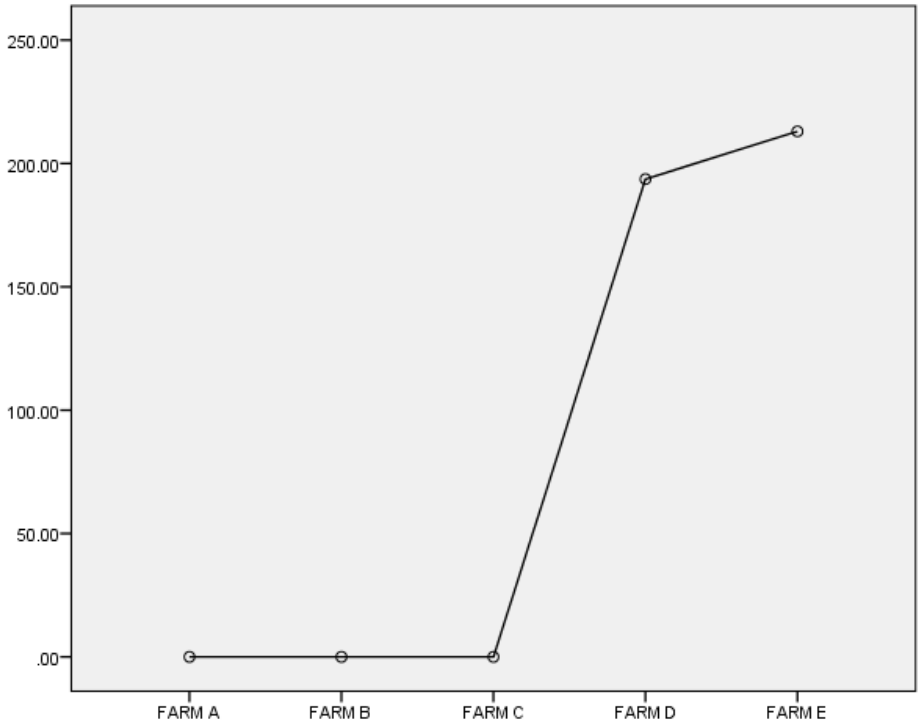
Fifty

	CONCENTRATION OF CHLORPYRIFOS ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		158.6667	
	FARM E	3			177.6667
	Sig.		1.000	1.000	1.000
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		158.6667	
	FARM E	3			177.6667
	Sig.		1.000	1.000	1.000

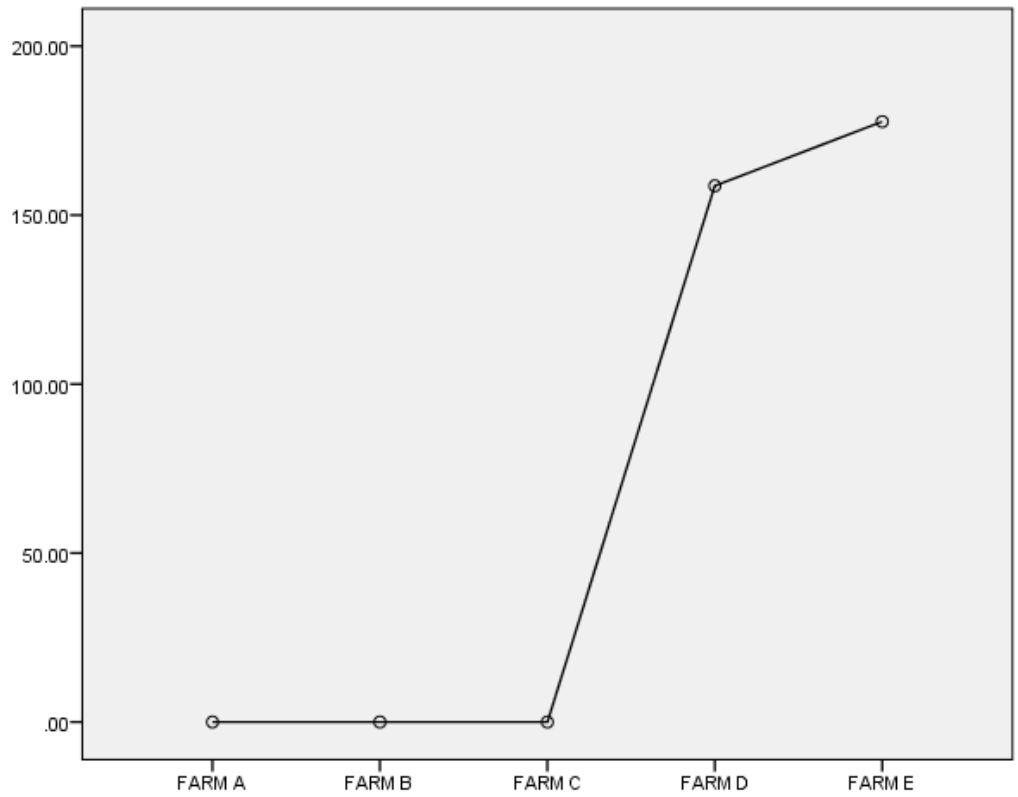
Seventy five

	CONCENTRATION OF CHLORPYRIFOS ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		133.3333	
	FARM E	3			154.0000
	Sig.		1.000	1.000	1.000
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		133.3333	
	FARM E	3			154.0000
	Sig.		1.000	1.000	1.000

Mean plots at twenty-five metres for chloroprifos

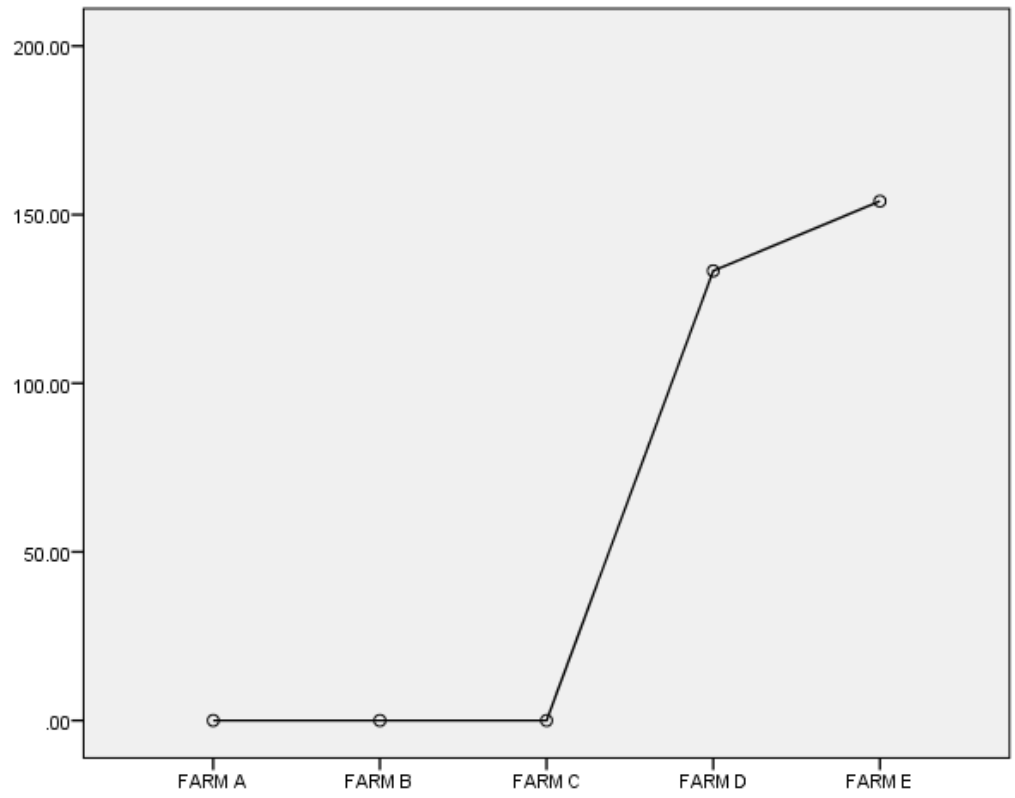


Mean plots at fifity metres for chloroprifos on farms



Concentration of chlorpyrifos on farms

Mean plots at seventy five metres for chloroprifos on farms A,B, C, D and E



Appendix 13: cypermethrin data analysis in soils for farms A, B, C, D and E at investigated distance of 25 m, 50 m and 75 m

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Twenty five	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	18.0000	.00000	.00000	18.0000	18.0000	18.00	18.00
	FARM E	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00
	Total	15	6.6667	8.49930	2.19451	1.9599	11.3734	.00	18.00
Fifty	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	13.6667	1.15470	.66667	10.7982	16.5351	13.00	15.00
	FARM E	3	13.6667	.57735	.33333	12.2324	15.1009	13.00	14.00
	Total	15	5.4667	6.94742	1.79382	1.6193	9.3140	.00	15.00
Seventy five	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	12.6667	.57735	.33333	11.2324	14.1009	12.00	13.00
	FARM E	3	10.3333	.57735	.33333	8.8991	11.7676	10.00	11.00
	Total	15	4.6000	5.88946	1.52065	1.3385	7.8615	.00	13.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Twenty five	Between Groups	1010.667	4	252.667	3790.000	.000
	Within Groups	.667	10	.067		
	Total	1011.333	14			
Fifty	Between Groups	672.400	4	168.100	504.300	.000
	Within Groups	3.333	10	.333		
	Total	675.733	14			
Seventy five	Between Groups	484.267	4	121.067	908.000	.000
	Within Groups	1.333	10	.133		
	Total	485.600	14			

Twenty five

	CONCENTRATION OF CYPERMETHRINE ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		15.3333	
	FARM D	3			18.0000
	Sig.		1.000	1.000	1.000
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		15.3333	
	FARM D	3			18.0000
	Sig.		1.000	1.000	1.000

Fifty

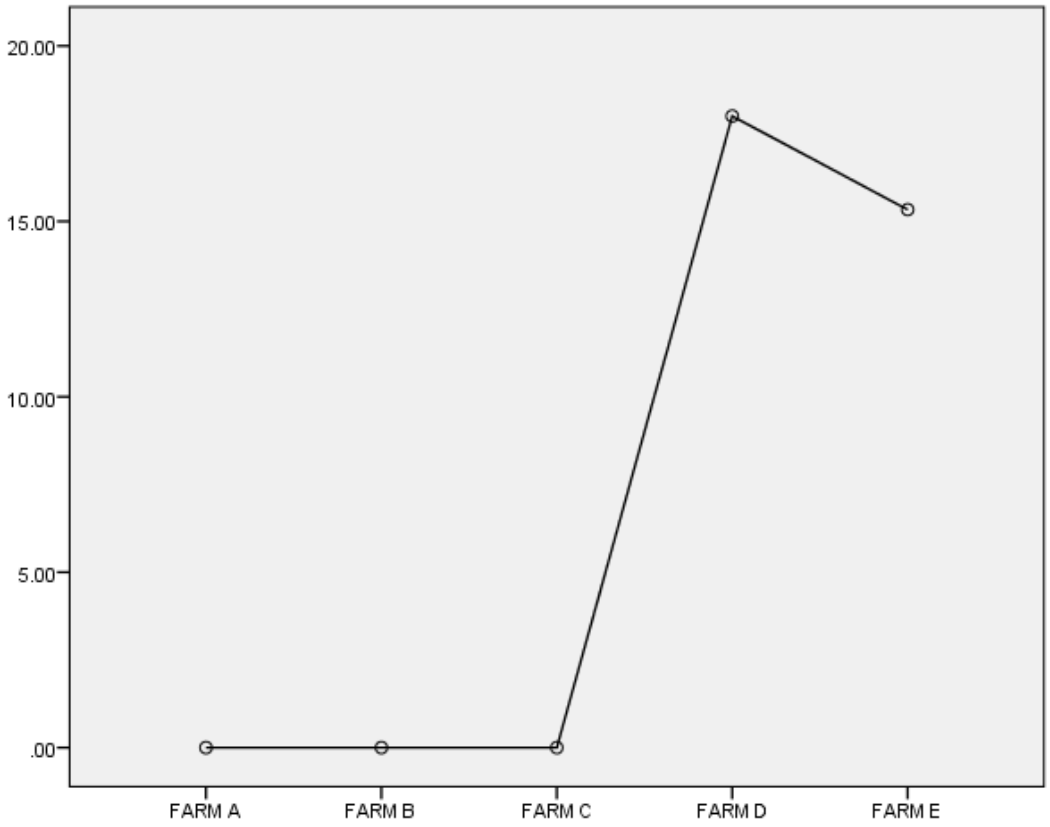
	CONCENTRATION OF CYPERMETHRINE ON FARMS	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	FARM A	3	.0000	
	FARM B	3	.0000	
	FARM C	3	.0000	
	FARM D	3		13.6667
	FARM E	3		13.6667
	Sig.		1.000	1.000
Duncan[a]	FARM A	3	.0000	
	FARM B	3	.0000	
	FARM C	3	.0000	
	FARM D	3		13.6667
	FARM E	3		13.6667
	Sig.		1.000	1.000

.

Seventy five

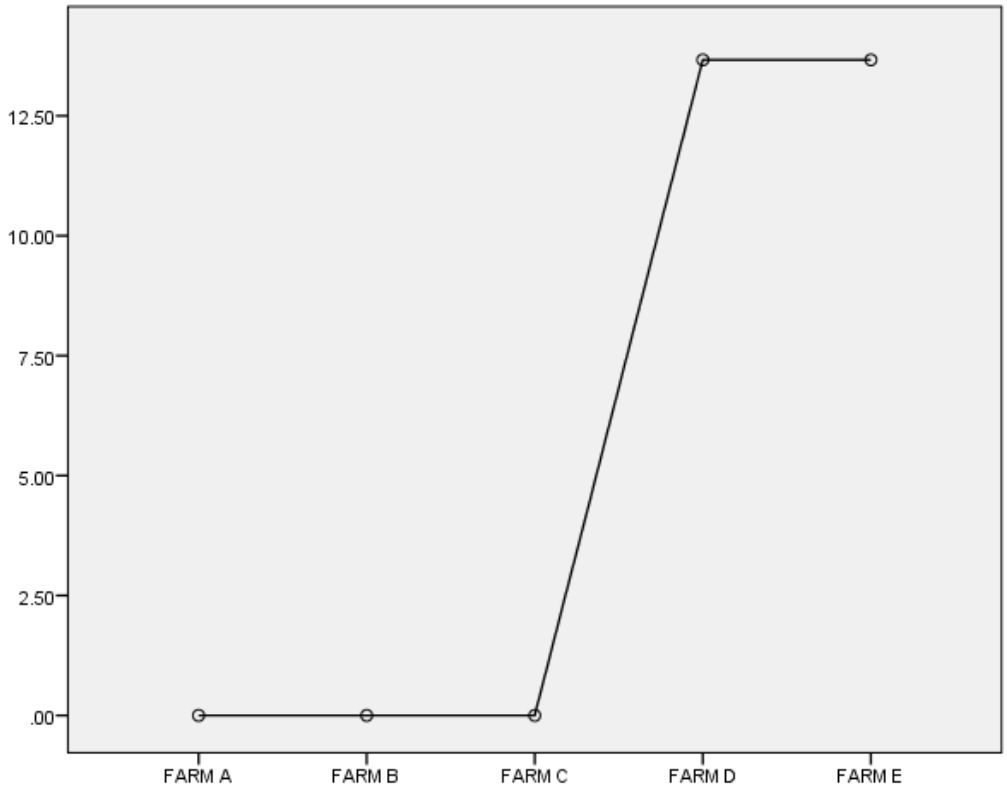
	CONCENTRATION OF CYPERMETHRINE ON FARMS	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		10.3333	
	FARM D	3			12.6667
	Sig.		1.000	1.000	1.000
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		10.3333	
	FARM D	3			12.6667
	Sig.		1.000	1.000	1.000

Mean plots of cypermethrine at twenty five metres



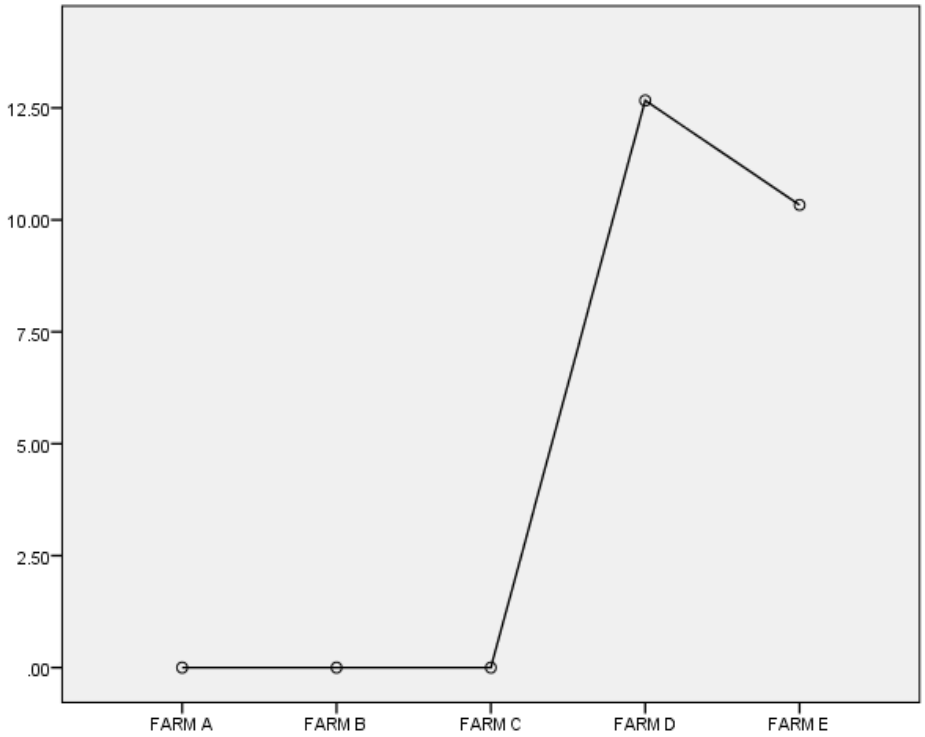
Concentration of cypermethrin on farms

Mean plots of cypermethrine at fifty metres



Concentration of cypermethrin on farms

Mean plots of cypermethrine at seventy five metres



Concentration of cypermethrin on farms

Appendix 14: Amitraz data analysis in water resources for farms A, B, C, D and E at 20 m

Descriptives

TWENTYMETRES

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
farm A	3	73.0000	3.46410	2.00000	64.3947	81.6053	69.00	75.00
FARM B	3	38.3333	1.52753	.88192	34.5388	42.1279	37.00	40.00
FARM C	3	38.6667	1.52753	.88192	34.8721	42.4612	37.00	40.00
FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	15	30.0000	28.55321	7.37241	14.1878	45.8122	.00	75.00

ANOVA

TWENTY METRES

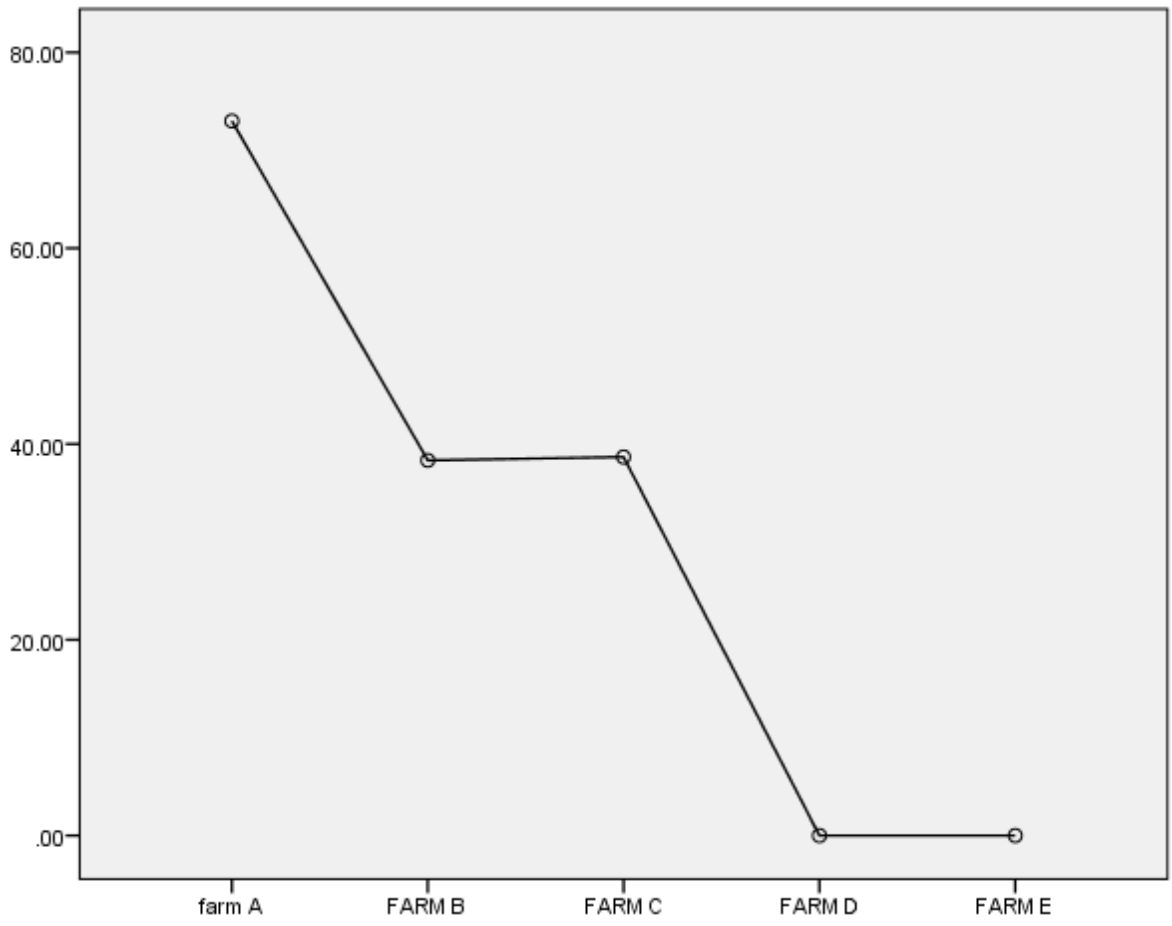
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11380.667	4	2845.167	853.550	.000
Within Groups	33.333	10	3.333		
Total	11414.000	14			

TWENTY METRES

	Concentration of amitraz in water	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM B	3		38.3333	
	FARM C	3		38.6667	
	farm A	3			73.0000
	Sig.		1.000	.999	1.000
Duncan[a]	FARM D	3	.0000		
	FARM E	3	.0000		
	FARM B	3		38.3333	
	FARM C	3		38.6667	
	farm A	3			73.0000
	Sig.		1.000	.828	1.000

Mean plot of amitraz concentration in water resources at twenty metres

Mean plots at twenty metres for amitraz in water sources for all farms



Concentration of amitraz in water

Appendix 15: chlorpyrifos data analsis in water resources for farms A, B, C, D and E

Descriptives

TWENTY METRES

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
farm A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM D	3	179.3333	2.88675	1.66667	172.1622	186.5044	176.00	181.00
FARM E	3	196.6667	.57735	.33333	195.2324	198.1009	196.00	197.00
Total	15	75.2000	95.50856	24.66021	22.3091	128.0909	.00	197.00

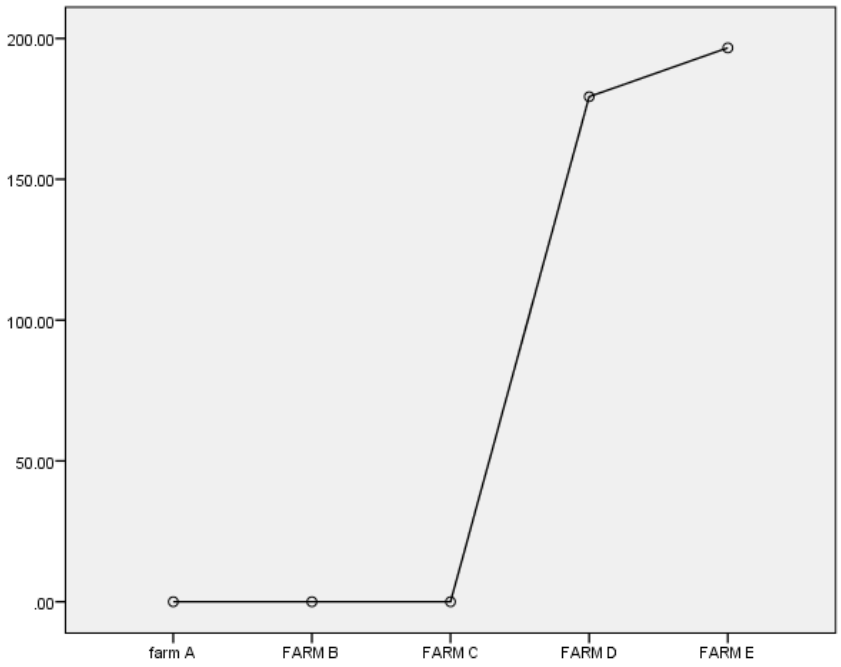
ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	127689.067	4	31922.267	18416.692	.000
Within Groups	17.333	10	1.733		
Total	127706.400	14			

TWENTY METRES

	Concentration of Chlorpyrifos in water	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		179.3333	
	FARM E	3			196.6667
	Sig.		1.000	1.000	1.000
Duncan[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		179.3333	
	FARM E	3			196.6667
	Sig.		1.000	1.000	1.000

Mean plots of chlorpyrifos in water sources at twenty metres



Concentration of chlorpyrifos in water

Appendix 16: cypermethrin data analysis in water resources for farms A, B, C, D and E

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
farm A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
FARM D	3	13.3333	.57735	.33333	11.8991	14.7676	13.00	14.00
FARM E	3	15.3333	.57735	.33333	13.8991	16.7676	15.00	16.00
Total	15	5.7333	7.30427	1.88595	1.6884	9.7783	.00	16.00

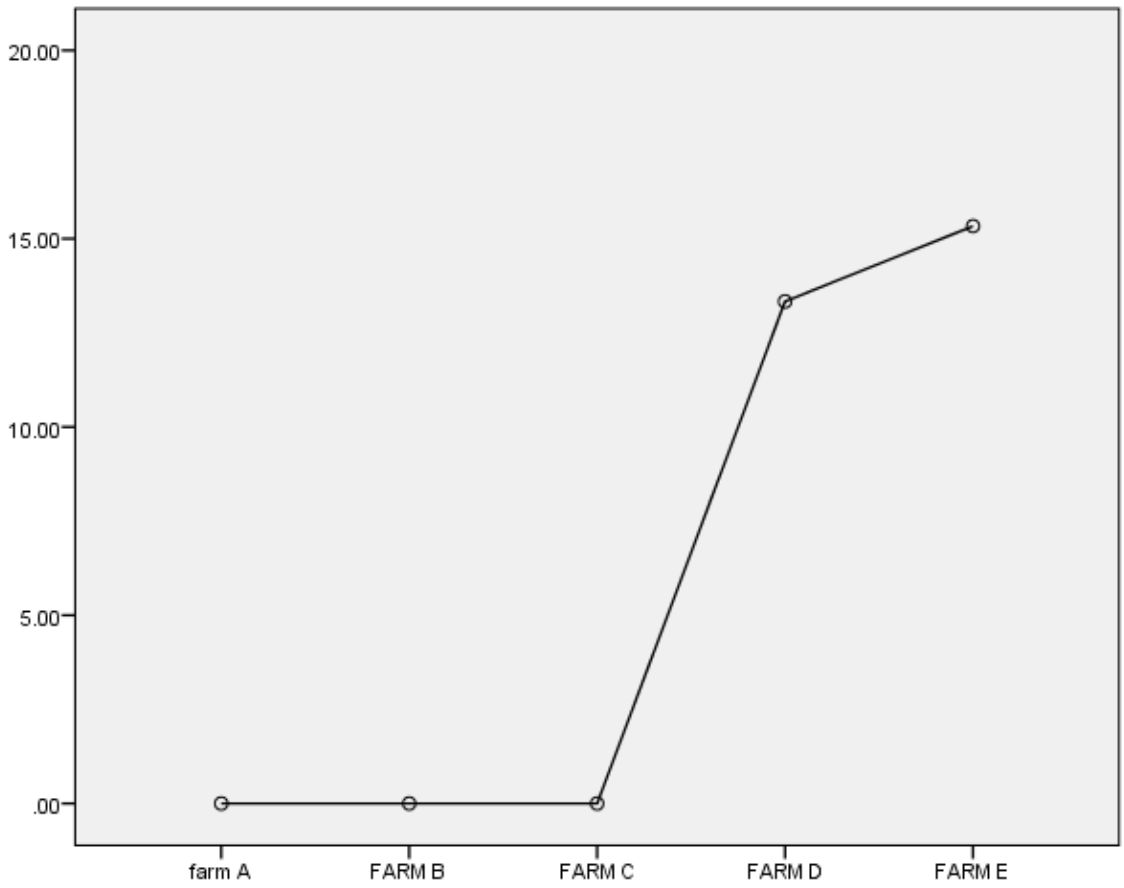
ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	745.600	4	186.400	1398.000	.000
Within Groups	1.333	10	.133		
Total	746.933	14			

TWENTY METRES

	Concentration of CypermethrIne in water	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		13.3333	
	FARM E	3			15.3333
	Sig.		1.000	1.000	1.000
Duncan[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		13.3333	
	FARM E	3			15.3333
	Sig.		1.000	1.000	1.000

Mean plots of cypermethrine in at twenty metres for water resources



Concentration of cypermethrine in water

Appendix 17: Amitraz, chlorpyrifos and cypermethrin data analysis in cattle dip tanks for farms A, B, C, D and E

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
AMITRAZ	farm A	3	935.0000	3.46410	2.00000	926.3947	943.6053	931.00	937.00
	FARM B	3	816.0000	1.73205	1.00000	811.6973	820.3027	815.00	818.00
	FARM C	3	285.0000	1.73205	1.00000	280.6973	289.3027	283.00	286.00
	FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	407.2000	412.03124	106.38601	179.0247	635.3753	.00	937.00
CHLORPYRIFOS	farm A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	970.0000	5.19615	3.00000	957.0920	982.9080	967.00	976.00
	FARM E	3	990.0000	5.19615	3.00000	977.0920	1002.9080	987.00	996.00
	Total	15	392.0000	497.00158	128.32526	116.7697	667.2303	.00	996.00
CYPERMETHERIN	farm A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	89.6667	3.51188	2.02759	80.9427	98.3907	86.00	93.00
	FARM E	3	98.6667	4.16333	2.40370	88.3244	109.0090	94.00	102.00
	Total	15	37.6667	47.88627	12.36418	11.1481	64.1852	.00	102.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AMITRAZ	Between Groups	2376740.400	4	594185.100	165051.417	.000
	Within Groups	36.000	10	3.600		
	Total	2376776.400	14			
CHLORPYRIFOS	Between Groups	3458040.000	4	864510.000	80047.222	.000
	Within Groups	108.000	10	10.800		
	Total	3458148.000	14			
CYPERMETHERIN	Between Groups	32044.000	4	8011.000	1350.169	.000
	Within Groups	59.333	10	5.933		
	Total	32103.333	14			

AMITRAZ

			Subset for alpha = 0.05			
	DIPTANKSONFARM	N	1	2	3	4
Tukey HSD[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM C	3		285.0000		
	FARM B	3			816.0000	
	farm A	3				935.0000
	Sig.		1.000	1.000	1.000	1.000
Duncan[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM C	3		285.0000		
	FARM B	3			816.0000	
	farm A	3				935.0000
	Sig.		1.000	1.000	1.000	1.000

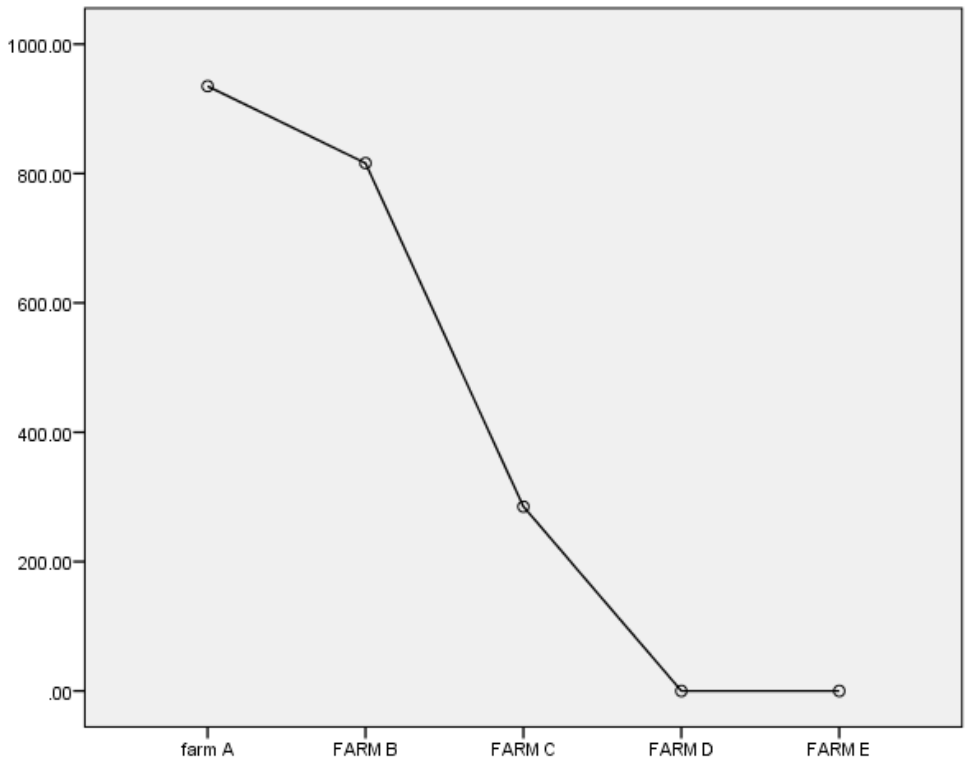
CHLORPYRIFOS

			Subset for alpha = 0.05		
	DIPTANKSONFARM	N	1	2	3
Tukey HSD[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		970.0000	
	FARM E	3			990.0000
	Sig.		1.000	1.000	1.000
Duncan[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		970.0000	
	FARM E	3			990.0000
	Sig.		1.000	1.000	1.000

CYPERMETHERIN

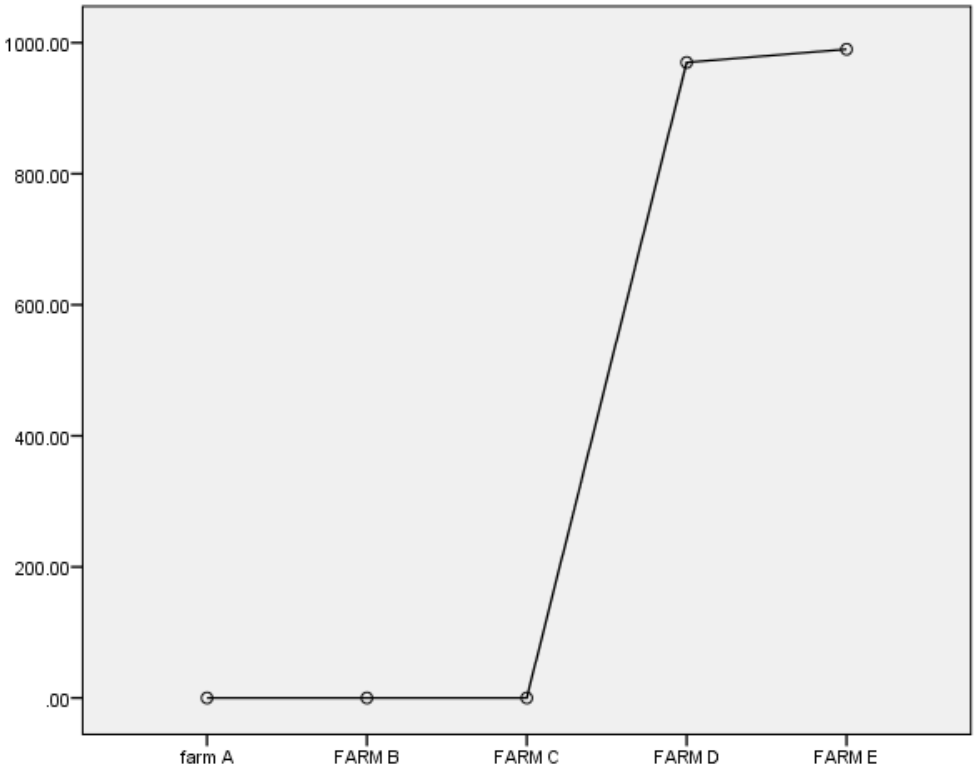
	DIPTANKSONFARM	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		89.6667	
	FARM E	3			98.6667
	Sig.		1.000	1.000	1.000
Duncan[a]	farm A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM D	3		89.6667	
	FARM E	3			98.6667
	Sig.		1.000	1.000	1.000

Mean plots of amitraz inside cattle dip tanks



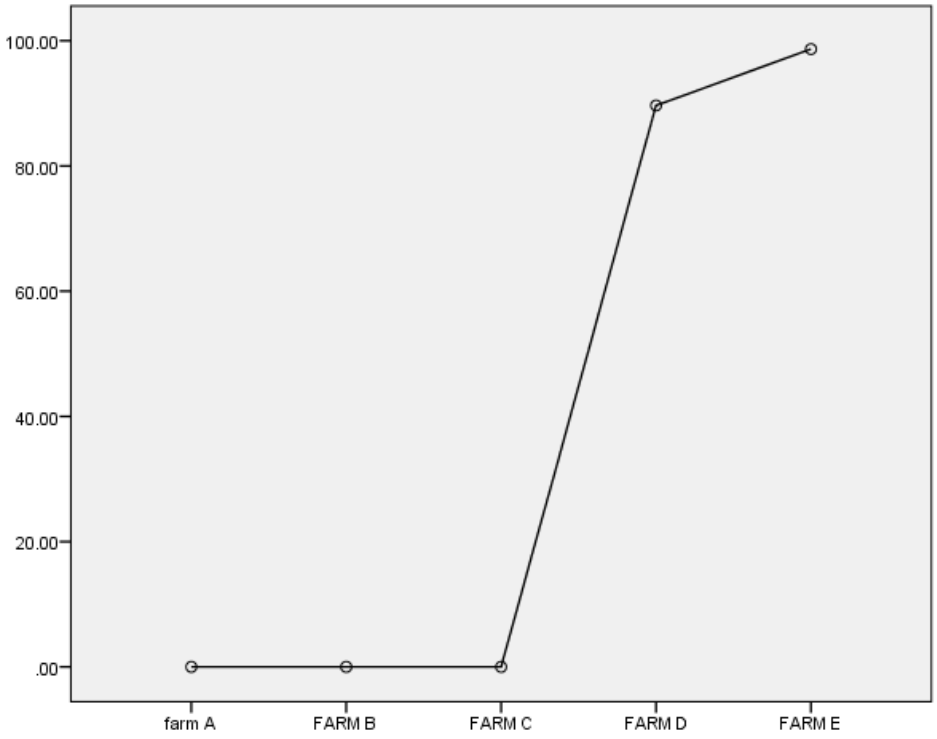
Dip tanks on farms

Mean plots of chloropyrifos inside cattle dip tanks



Dip tanks on farms

Mean plots of cypermrthrin inside cattle dip tanks



Dip tanks on farms

Appendix 17: Amitraz, chlorpyrifos and cypermethrin data analysis in soak pits for farms A, B, C, D AND E

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
AMITRAZZ	FARM A	3	109.3333	4.04145	2.33333	99.2938	119.3729	107.00	114.00
	FARM B	3	56.6667	.57735	.33333	55.2324	58.1009	56.00	57.00
	FARM C	3	68.6667	2.08167	1.20185	63.4955	73.8378	67.00	71.00
	FARM D	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM E	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	46.9333	43.62251	11.26328	22.7760	71.0907	.00	114.00
CHLOROPYRIFO	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	257.6667	1.52753	.88192	253.8721	261.4612	256.00	259.00
	FARM E	3	254.0000	3.46410	2.00000	245.3947	262.6053	250.00	256.00
	Total	15	102.3333	129.74462	33.49992	30.4832	174.1835	.00	259.00
CYPERMETHRINE	FARM A	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM B	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM C	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	FARM D	3	21.0000	1.73205	1.00000	16.6973	25.3027	19.00	22.00
	FARM E	3	20.3333	.57735	.33333	18.8991	21.7676	20.00	21.00
	Total	15	8.2667	10.50487	2.71235	2.4493	14.0841	.00	22.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AMITRAZZ	Between Groups	26598.933	4	6649.733	1583.270	.000
	Within Groups	42.000	10	4.200		
	Total	26640.933	14			
CHLOROPYRIFO	Between Groups	235642.667	4	58910.667	20550.233	.000
	Within Groups	28.667	10	2.867		
	Total	235671.333	14			
CYPERMETHRINE	Between Groups	1538.267	4	384.567	576.850	.000
	Within Groups	6.667	10	.667		
	Total	1544.933	14			

AMITRAZZ

			Subset for alpha = 0.05			
	soakpitsonfarms	N	1	2	3	4
Tukey HSD[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM B	3		56.6667		
	FARM C	3			68.6667	
	FARM A	3				109.3333
	Sig.		1.000	1.000	1.000	1.000
Duncan[a]	FARM D	3	.0000			
	FARM E	3	.0000			
	FARM B	3		56.6667		
	FARM C	3			68.6667	
	FARM A	3				109.3333
	Sig.		1.000	1.000	1.000	1.000

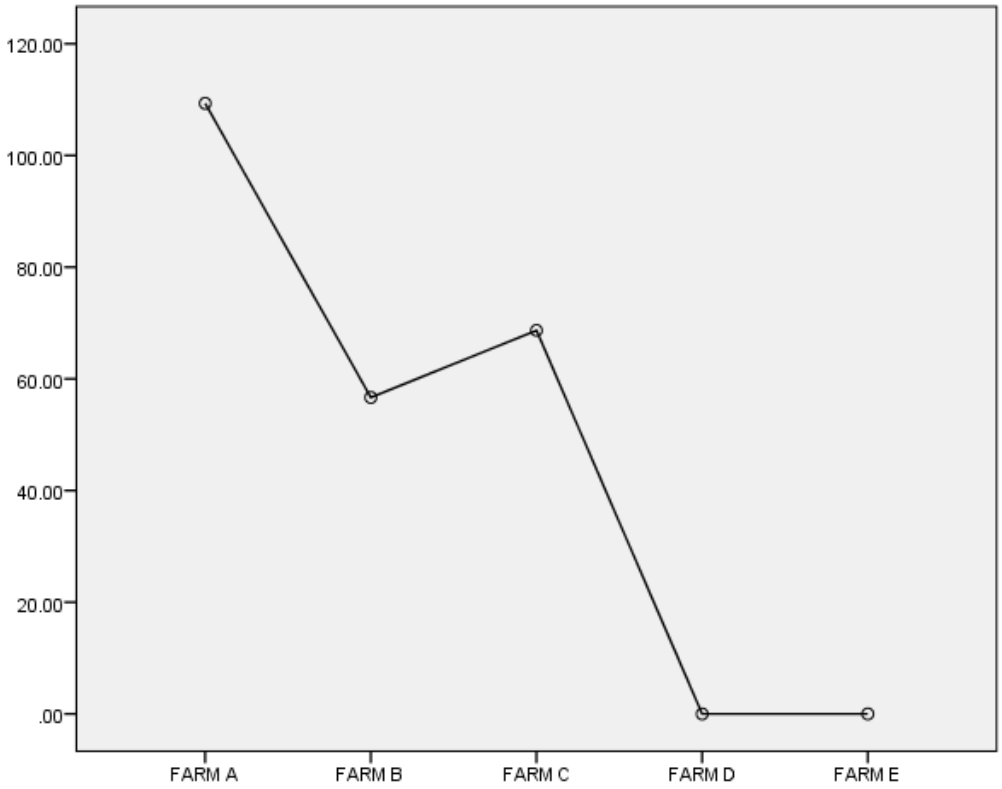
CHLOROPYRIFOS

			Subset for alpha = 0.05		
	soakpitsonfarms	N	1	2	3
Tukey HSD[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		254.0000	
	FARM D	3		257.6667	
	Sig.		1.000	.133	
Duncan[a]	FARM A	3	.0000		
	FARM B	3	.0000		
	FARM C	3	.0000		
	FARM E	3		254.0000	
	FARM D	3			257.6667
	Sig.		1.000	1.000	1.000

CYPERMETHRINE

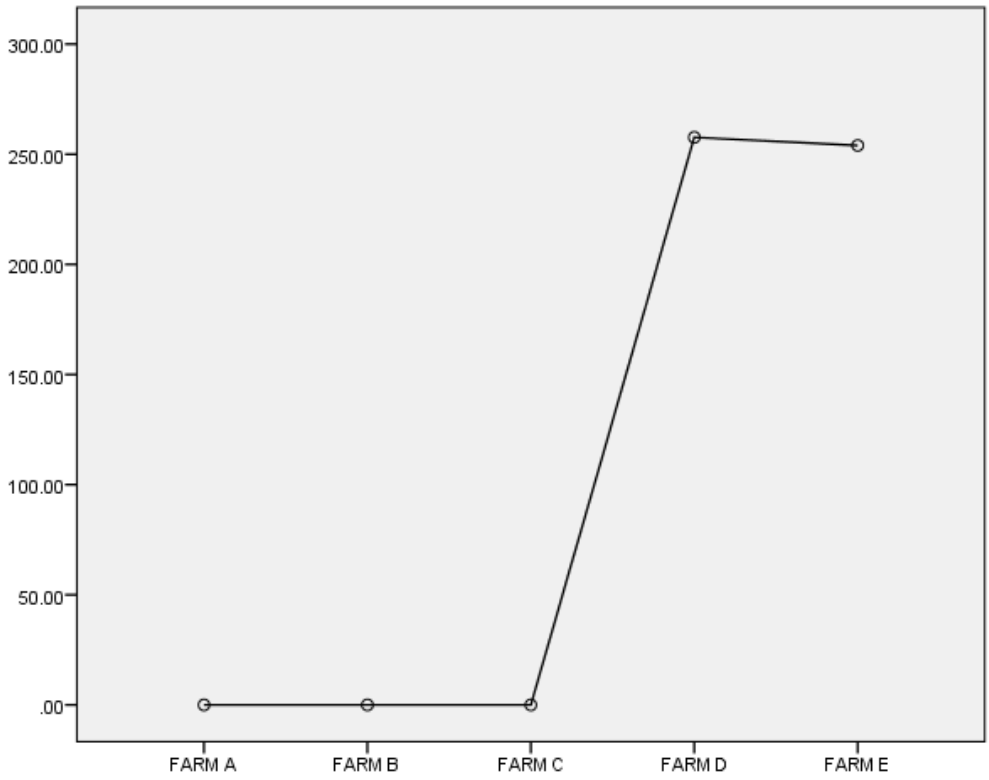
	Soak pits on farms	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	FARM A	3	.0000	
	FARM B	3	.0000	
	FARM C	3	.0000	
	FARM E	3		20.3333
	FARM D	3		21.0000
	Sig.		1.000	.850
Duncan[a]	FARM A	3	.0000	
	FARM B	3	.0000	
	FARM C	3	.0000	
	FARM E	3		20.3333
	FARM D	3		21.0000
	Sig.		1.000	.341

Mean plots of amitraz for soak pits



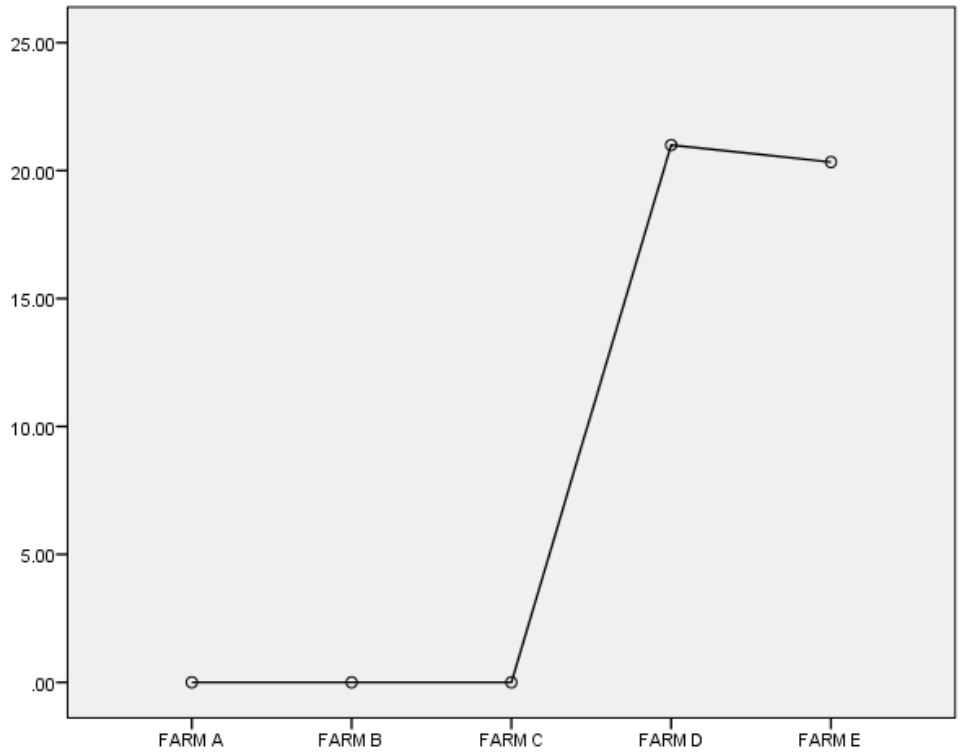
Soak pits on farms

Mean plots of chlorpyrifos for soak pits on cattle dip tanks



Soak pits on farms

Mean plots of cypermethrin for soak pits on cattle dip tanks



Soak pits on farms