UNIVERSITY OF EDUCATION, WINNEBA

ANALYSIS OF PESTICIDE RESIDUES IN PINEAPPLES SOLD IN WINNEBA, GHANA

GRACELOVE EYI-MENSAH

MASTER OF PHILOSOPHY

2022

UNIVERSITY OF EDUCATION, WINNEBA

ANALYSIS OF PESTICIDE RESIDUES IN PINEAPPLES SOLD IN WINNEBA, GHANA

GRACELOVE EYI-MENSAH
(200029325)

A thesis in the Department of Chemistry Education, Faculty of Science,
submitted to the School of Graduate Studies
in partial fulfillment
of the requirements for the award of the degree of
Master of Philosophy
(Chemistry Education)
in the University of Education, Winneba

MARCH, 2022

DECLARATION

STUDENT'S DECLARATION

I, **Gracelove Eyi-Mensah**, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

Signature: …….

Date: …….

SUPERVISOR'S DECLARATION

I hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis/dissertation/project as laid down by the University of Education, Winneba.

Supervisor's name: Dr. Arkoful Sam

Signature: ……

Date: ……

DEDICATION

This work is dedicated to my lovely parents, siblings and the late Madam Mary Edukwei Eyi-Mensah.

ACKNOWLEDGEMENTS

By the Grace of God, I embarked on the journey of pursuing this program and study and by His blessings and favour, I have come this far. This thesis is an outcome of guidance and support of many people and this is an opportunity for me to thank them all.

I express a heartfelt gratitude and appreciation to Dr. Arkoful Sam, my supervisor, for his constructive criticisms, comments, corrections, suggestions, guidance and patience which brought this write-up this far. To him I say thank you and may the almighty God continue to bless and grant him with more favour to reach greater heights.

I also want to extend my heartfelt thanks to all my lecturers at the University of Education, Winneba who taught me various courses from which I acquired all the knowledge which together have brought changes to my life. I am also greatly thankful to Mr. Twumasi Ankrah Kwarteng, sir may God continue to bless you.

I would like to specially thank Mr. Francis Ofosu-Koranteng of GSA for the hospitality, time, guidance and inspiration right from when I first met him and also for encouraging me to keep moving and pursue my aspirations including all the other staff of the pesticide residue analysis laboratory at Ghana Standards Authority. Not forgetting the pineapple sellers at Winneba whom I also thank for their cooperation and information given to make this piece a success.

As said by some wise men, –even a journey of thousand miles begins with the single step". I could not have embarked on this journey without the constant, love, support and motivation I received from my best friends on campus, Mr. Alfred Quansah, Mr. William Kofi Ekpe, Mr. Alfred Acheampong and all my course mates especially Mr.

Felix Akompi, Mr. Thomas Dorpe and Mr. Atsu Jacob who were also excellent colleagues. I would also like to thank my pastor, Rev. Mrs. Joana Arkoful, for her constant prayers, advice and motivation.

Finally, I am particularly indebted to my father (Mr. Nicholas Eyi-Mensah), my mother (Madam Joyce Adisenu) and all my siblings (especially Benedicta, Catherine, Augustina and Keziah) for their valuable suggestions, help, prayers, support, prompt advice and always believed in my potentials and also encouraged me to pursue my dreams and make them real. We rejoice in the Lord for what he has done and will do in the family. And to all who contributed to my success in one way or the other, may God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY / ABBREVIATIONS

AMA	Accra Metropolitan Assembly
ANOVA	Analysis of Variance
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
GSA	Ghana Standards Authority
HCH	Hexachlorocyclohexane
LOD	Limit of Detection
LOQ	Limit of Quantification
Mg/kg	Milligrams per Kilogram
MRLs	Maximum Residue Limits
ND	Not Detected
OCPs	Organochlorine Pesticides
OPPs	Organophosphorus Pesticides
SPE	Solid Phase Extraction
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
QuPPe	Quick Polar Pesticides Methods

ABSTRACT

Pesticides have been known to be extensively used to ensure high crop yields both during production and for post-harvest treatment. This increased use of the pesticides has resulted in pollution of the environment and also has caused many associated short-term and long-term effects on human health. Hence, this study is to analyze the pesticide residues in the pineapples sold by various fruits vendors in Winneba as well as compare the residual levels with EU MRL's. Gas chromatography with selective electron capture detector (GC-ECD), gas chromatography with pulsed flame photometric detector (PFPD), and Liquid chromatography mass spectrometer (LC-MS) were used to detect and determine the amount of organochlorine insecticides, organophosphate insecticides and herbicide and growth regulators respectively. Results from the analysis of the pineapples showed that there were no organochlorine and organophosphate insecticides in any of the pineapple samples. However, some herbicides and growth regulators were detected in some of the pineapple samples with fluazifop having the least mean concentration of 0.0001 ± 0.0001mg/kg and ethephon having the highest mean concentration of 0.0032 ± 0.00102mg/kg. Also, from the results of the detected pesticide residues, the finding shows that none of the pesticides detected exceeded the Maximum Residue Limits (MRLs) established by the European Union (EU). This therefore shows that despite the occurrence of pesticide residues in some of the samples, it could still be considered safe for human consumption. Nevertheless, further investigation and research and continuous monitoring with more strict regulation of pesticide residues in the food commodities is highly recommended.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter focuses on the background to the study, statement of the problem, objectives, hypothesis, purpose of the study, significance of the study and organization of the study.

1.1 Background to the Study

The effort of the global community over the years at producing enough food to meet the demand for food that has tripled over the last 50 years, has brought about new incentives and policies in agriculture. Key among the new incentives is the gross liberalization of the pesticides trade in both developed and developing countries to make pesticides affordable and accessible to famers (Bodirsky et al., 2015). The use of pesticides to control insect pests, which cause damage to crops and result in severe loss in food production in tropical countries like Ghana, has become recognized and accepted as an essential component of modern agricultural practice. However, prolonged use of pesticides along with lack of suitable averting behavior/use of basic protective requisites enhances the probability of accidental intoxication significantly (Ntow et al., 2009).

Pesticide residues in or on plants may be unavoidable even when pesticides are used in accordance with good agricultural practice (PS 1997; Uysal-Pala and Bilisli 2006). Fruits are usually subjected to pre- and post-harvest treatments. Exposure to pesticides through consumption of fruits is almost continuous, either as a result of direct treatment or due to environmental or cross contamination. The pesticide content in fruits except for direct spraying, is also influenced by their presence in the soil, where

the fruits grow, or in the water used for irrigation. Pesticides can also be transported by rain or wind from the points of treatment to the neighbouring crops and areas where they are unwanted or harmful (Hajslova and Zrostlikova, 2003; Stocka et al., 2011). Organophosphates, carbamates and pyrethroids are routinely applied to fruit crops for broad spectrum insect control (Rawn et al., 2004; Rawn et al., 2006); organochlorines and other compounds are mainly used as post-harvest treatments for fungi control, especially in fruits intended for direct human consumption (Park et al., 2004).

Fresh fruits and vegetables are an important part of a healthy diet due to the significant presence of nutrients and minerals. Pineapple *(Ananas comosus* L. Merr.) is one of the most highly appreciated fruits in the world because of its delicious flavour, and the fact that it contains proteolytic enzymes that aid digestion (Bartolome *et al.,* 1995). Cabrera *et al.* (2000) reported that only orange juices are consumed in greater amounts worldwide than pineapple juice which they estimated to be 200,000 metric tonnes annually.

According to Joy (2010), pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits; containing considerable calcium, potassium, fibre, and vitamin C, but low in fat and cholesterol. It is also a good source of vitamin B1, vitamin B6, copper and dietary fibre. Pineapple is a digestive aid and a natural Anti-Inflammatory fruit. Fresh pineapples are rich in bromelain which demonstrates significant anti-inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. Pineapple is an excellent cerebral toner; it combats loss of memory, sadness and melancholy.

Though there are many benefits associated with the consumption of pineapples, there is also a growing concern on its cultivation with agrochemicals such as fertilizers, and pesticides. In Ghana, agro-chemicals are used in cocoa, oil palm, cola nut, coffee and cotton farms, vegetables (e.g., tomato, eggplant, onion, pepper, okra, cabbage, lettuce, carrot) and fruit production (e.g., papaya, citrus, avocado, mango, cashew, pineapple), mixed-crop farming systems involving cereals (e.g., maize, millet, sorghum, rice), tuber crops (e.g., yam, cassava, cocoyam, sweet potato) and legumes (e.g., cowpea, bambara nut, groundnut, soybean). Overall, agrochemical especially fertilizer used on pineapple is fairly high because pineapple is grown on sandy soils (Fianko, Donkor, Lowor & Yeboah, 2011).

Over the years pesticide use has become a common agricultural practice in Ghana and its use in pineapple production is not an exception. However, lack of knowledge of the types, uses, and the effects of these pesticides among small-and large-scale farmers, has resulted in their misuse and consequently, their accumulation in various foods and feed items. Over time, these pesticides can accumulate in the bodies of humans, causing various health related problems, such as disrupting the endocrine system, which can influence development, growth, reproduction, and behavior. Children, in particular, may be more susceptible to these risks owing to their higher overall consumption of fruits and vegetables (National Academy of Sciences, 1993). Also, scientists have found that commonly used pesticide products, which include insecticides and herbicides, can cause long-term health impacts such as cancer, neurological problems, and learning disabilities. Some can even kill people by poisoning them. It is therefore important to know the type of pesticides that are used locally on pineapples and their health effects and thus the need for pesticide residue analyses.

1.2 Statement of the Problem

Pineapple can be used as a supplementary nutritional fruit for good personal health. The pineapple fruits are normally consumed fresh or as fresh pineapple juice. Field ripe fruits are best eaten fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs - dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. Pineapple cropping is dominated by conventional monocropping with high levels of agrochemical inputs (Loeillet, 2013) due to nitrogen (N) and potassium (K) fertilization (Dorey et al., 2015; Teixeira et al., 2011), weed management, crop protection and flowering induction**.**

In Ghana, organochlorine pesticides (OCPs) were prohibited in farming practices because of their persistency, bioaccumulative properties and human health implications. It was enforced in May 2004 by the Stockholm Convention. Even though DDT (an organochlorine pesticide) is highly restricted, its residues have been found in some Ghanaian vegetables and fruits (Ntow, 2001; Amoah *et al.,* 2006). Accordingly, organophosphorus (OP) and synthetic pyrethroid (SP) pesticides are the only registered and most commonly applied pesticides for pest and disease vector eradication. Washing and boiling may not remove the pesticides (especially the organochlorines) completely (Bull, 1982) and this may be a threat to public health if they exceed the maximum permissive levels.

Pesticides mostly used to control foliar pests of pineapple in Ghana include chlorpyrifos, dimethoate, diazinon, cymethoate and fenitrothion while the fungicides maneb, carbendazim, imazil, copper hydroxide are used for post-harvest treatment (Abutiate, 1991; Kyofa-Boamah and Blay, 2000). Kyofa- Boamah and Blay (2000)

cited by Aboagye (2002) recorded that glyphosate, fluazifop-butyl, ametryne, diuron or bromacil are normally employed in land clearing. Dinham (2003) estimates that 87% of farmers in Ghana use chemical pesticides to control pests and diseases on vegetables and fruits. Ntow, Gijzen, Kelderman and Drechsel (2006) gave the proportions of pesticides used popularly on vegetable farms as herbicides (44%), fungicides (23%) and insecticides (33%) which shows that vegetable farmers mostly use herbicides during production.

The residues from the application of these pesticides do not only have direct effect on human health but also on water bodies and other aquatic organisms. Studies conducted by Echeverría-Sáenz et al. (2012) revealed that, different pesticide residues were detected in water samples collected across the Jiménez River watershed with herbicides (ametryn, bromacil, diuron), organophosphorus insecticides (diazinon and ethoprophos) and triazole fungicides resulting from residues in pineapple production which affected the Jiménez River aquatic ecosystems and degraded riparian habitats.

In developed countries, regular monitoring of pesticide residues in food assures conformity with the principles of good agricultural practice (GAP) and consumer risk assessment. However same cannot be said about Ghana as there are limited studies on pesticide residue analysis and human health risk assessment data on fruits, vegetables and other food commodities. Information on residue levels of these pesticides under contemporary agricultural practices by farmers whose products are sold in Winneba will be necessary to evaluate the need for any improvement on the agricultural practices to ensure the safety of consumers and also the acceptance of Ghanaian pineapples on the international markets.

1.3 Objectives

This study is therefore, designed with the following objectives:

1. Identify the types of pesticides used locally in producing pineapples sold in Winneba.
2. Assess the levels of the various pesticides residues found in the pineapples and compare the residue levels with the acceptable international food safety limits (MRLs).
3. Compare the pesticide residue levels in the pineapples collected from the different selling points in Winneba.

1.4 Hypothesis

1.4.1 Null Hypothesis (H_o)

There is no significant difference in mean concentrations of pesticide residues in pineapples from different sample locations in Winneba.

1.4.2 Alternative Hypothesis (H_A)

There is a significant difference in mean concentrations of pesticide residues in pineapples from different sample locations in Winneba.

1.5 Purpose of the Study

The purpose of the study is to analyze the pesticide residues in pineapples sold in the Winneba Municipality.

1.6 Significance of the Study

The results from this study hope to be the baseline data upon which annual or other long-term monitoring studies could be compared with as well as utilizing it in estimating the potential health risks associated with the consumption of pineapples. This assessment is also important to know the actual status of contamination by toxic

pesticide residues for future policies and to ensure confidentiality of consumers in the quality of food. More so, with such data, the Ministry of Food and Agriculture can be assured of compliance of the principles of good agricultural practices and consumer risk assessment. Finally, it can be used when drafting future environmental policies and control programmes for Ghana and taking preventive actions to minimize human health risks.

1.7 Organization of the Study

This research is presented in five chapters. The first chapter deals with the background to the study, statement of the problem, objectives, hypothesis, purpose of the study, significance of the study and organization of the study. The chapter two reviews some relevant literature related to the study while the chapter three focuses on the methodology used in the study. This comprises the laboratory analysis, sample preparation, extraction, extract purification, instrumental analysis and statistical analysis. Chapter four deals with the discussion of the results obtained and finally the chapter five. Chapter five incudes the summary, conclusions and recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter focuses on review of literature under the following headings: pineapple production in Ghana, diseases/ pests in pineapple production, importance of pesticide use, classification of pesticides, the use of pesticides in pineapples, types of pesticides used in pineapple production, consequences of pesticide use, pesticide residue in pineapples and determination of pesticide residues in food. Overall, this chapter carefully examines research works conducted on pesticide residue analysis in food commodities.

2.1 Pineapple Production in Ghana

Agyare (2010) argues that pineapple is by far the most important crop within the horticultural subsector of the Ghanaian economy. Over 15000 individuals are employed by this industry with about 40% of the number being women and generates rural incomes of over six (6) million USD.

Large and medium commercial farms account for about 70% of production with the remaining produced by smallholders. Agyare (2010) also said that pineapple production in Ghana covers over 8000 acres of land and is predominant in the Greater Accra, Eastern, Central and Volta regions of the country. The varieties usually produced are Sugarloaf, Smooth Cayenne and now the MD2. Two basic methods of pineapple production are mostly employed by farmers in the country. These are the organically produced pineapple and the inorganically produced pineapple. In organic production, the use of pesticides or chemical fertilizers are not common as compared to the inorganic type, where chemical fertilizers are commonly used. According to

Kleeman (2011), small scale farmers take advantage of organic farming whilst the large-scale farmers are well suited to engage in the normal conventional farming.

The fruits are harvested throughout the year, however the month of March – April is considered the peak periods. The midyear rainy season—June to September—is a poor time for fruit quality; as a result, planned production and export are lowered at that time. It is for this reason, that the pineapples used for this analysis will be bought in the month of March.

2.2 Importance of Pineapple

Ripe pineapples are eaten fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple is utilized in curries and various meat dishes. The fermented pulp is made into a popular sweetmeat in the Philippines (Morton, 1999).

Morton (1999) also stated that Pineapple juice is prepared as syrup or is utilized in confectionery and beverages, or converted into powdered pineapple extract, which has various roles in the food industry. The juice of the peel can be made into vinegar or mixed with molasses for fermentation and distillation of alcohol. Bromelain, or bromelin, a protein obtained from pineapple peel is used for tenderizing meat and chill proofing beer. Certain cultivars e.g., 'Perolera' are grown especially for fiber production and their young fruits are removed to give the plant maximum vitality. Pineapple crowns and pineapple waste from the processing factories are used to feed cattle, pigs and chickens. Expendable plants from old fields can be processed as silage for maintaining cattle when other feed is scarce (Morton, 1999).

Pineapple juice is taken as a diuretic and to expedite labour, also as a gargle in cases of sore throat and as an antidote for seasickness (Morton, 1999). The pineapple fruit with the crown intact is often used as a decoration and there are variegated forms of

the plant universally grown for their showiness indoors or outdoors. Potted, ethylene treated pineapple plants with fruits have also been used as indoor ornamentals (Morton, 1999).

Hidaka et al. (2008) in a study reported that, the major component extracted from pineapple could reduce CD25 expression (*trans*-membrane protein present on activated T cells) and inhibit cyclooxygenase-2 (COX-2) expression *via* anti-inflammation and antitumor activities. Fresh pineapple juice containing bromelain enzyme according to clinical studies has a healing pathway for HIV/AIDS. In a recent study by Pandjaitan et al. (2014), HIV-positive human serums were incubated with bromelain at different concentrations (4 h, 37°C). These yielded negative results at bromelain concentrations of >10 mg/ml. Following this, seven HIV patients were given two glasses/day of fresh pineapple juice. The results showed that within 4 months, all seven patients achieved substantial improvement in their CD4 + counts with three of them already reaching normal CD4 + counts. Moreover, two of them, showed that the viral counts in their system were below detection limit (<400 copies/mL). A study by Zang et. al (2005) also revealed that the bromelain in pineapple juice was able to correct menstrual disorders and providing relief from painful periods.

Chapple et al (2012) also reported that, Vitamin C found in pineapple juice, helps as a great remedy for oral health and can reduce the risk of gingivitis and periodontal disease. It also helps the body to fight against the bacteria and the toxins that invade human gum tissues and helps in repairing damaged tissues and in keeping the lymphatic system healthy.

Pineapple is a good source of manganese, which is an essential cofactor in a number of enzymes important in energy production and antioxidant defense. This high level of manganese in pineapple benefits the skin, collagen, cartilage, and bone material. Studies have also indicated that pineapple juice is good for the health of pharynx and also the larynx. A combination of glucosamine, chondroitin sulfate, and manganese may significantly improve the symptoms of mild to moderate osteoarthritis of the knee (Orlando, 2017). Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed up tissue repair as a result of injuries, diabetic ulcers, and general surgery. In addition, another important use of pineapple juice is its ability to dissolve mucus and thus help one in a quick recovery from diseases such as tuberculosis (Debjit, Chandira, Jayakar, & Kumar, 2009). With all these benefits associated with pineapples it is an undeniable fact that consumers eat a lot of it and hence there is a necessity for an analysis in its production with agrochemicals such as pesticides.

2.3 Pests/ Diseases of Pineapple

Unfortunately, the yield of pineapple is affected by pests, which necessitates the use of pesticides to control them. Diseases of pineapple are associated with fungi, bacteria, nematodes and viruses.

2.3.1 Fungal Diseases

2.3.1.1 Phytophthora Heart (Top) Rot

The phytophthora heart (top) rot disease is caused by the fungi, *Phytophthora cinnamomi* and *Phytophthora nicotianae,* belonging to oomycetes. Plants of all ages are attacked, but three- to four-month-old crown plantings are most susceptible. Fruiting plants or suckers on ratoon plants may be affected. The colour of the heart

leaves of the diseased plant changes to yellow or light coppery brown. Later, the heart leaves become wilted (causing the leaf edges to roll under), turn brown and eventually die. Once symptoms become visible, young leaves are easily pulled from the plant, and the basal white leaf tissue at the base of the leaves becomes water-soaked and rotten with a foul smell due to the invasion of secondary organisms. The growing point of the stem becomes yellowish-brown with a dark line between healthy and diseased areas (Joy & Sindhu, 2012).

2.3.1.2 Phytophthora Root Rot

The Phytophtora root rot is caused by *Phytophthora cinnamomi*. The symptoms above ground are similar to those caused by nematodes, mealy bug wilt and low levels of soil oxygen and are not diagnostic. Leaves change in colour from a healthy green to various shades of red and yellow. Leaf tips and margins eventually become necrotic, root system dies and plants can easily be pulled from the ground.

Fruits from infected plants remain small and ripened and become unmarketable. If symptoms are recognized early and control measures are taken, plants can recover their growth and development. If roots are killed right back to the stem, the plant often fails to regenerate (Joy & Sindhu, 2012).

2.3.1.3 Base (Butt) Rot

The base or butt rot disease is caused by the fungus, *Chalara paradoxa.* Symptoms are seen only on crowns, slips and suckers before or immediately after planting. A grey to black rot of the soft butt tissue develops, leaving stringy fibers and a cavity at the base of the stem. If affected material is planted, partial decay of the butt severely reduces plant growth. When butt decay is severe, plants fail to establish, wilt rapidly and leaf tissue dies. Unlike Phytophthora heart rot, the young leaves remain firmly

attached to the top of the stem. Infected plants can easily be broken off at ground level (Joy & Sindhu, 2012).

2.3.1.4 Fruit-Let Core Rot (Green Eye) Disease

The pathogens which are responsible for fruitlet core rot (green eye) disease are

Fusarium guttiforme and *Penicillium funiculosum.* This is an internal fruit disease. Smooth Cayenne fruits do not usually show any external symptoms. However, fruit of the rough-leaf (Mauritius) may produce fruitlets that fail to colour – a condition often referred to as ‗green eye'. Severely affected fruitlets may become brown and sunken as the fruit ripens. Internal symptoms consist of a browning of the center of the fruitlets starting below the floral cavity and sometimes extending to the core. The browning, which remains quite firm, varies in size from a speck to complete discolouration of one or more fruitlets (Joy & Sindhu, 2012).

2.3.1.5 Fusariosis

The fusariosis disease is caused by the fungus, *Fusarium guttiforme.* It is sporadic and affects all parts of the pineapple plant but is most conspicuous and damaging on fruit. Fruits exhibit stem resetting and curvature of the plant because portions of the stem are girdled or killed. Rough leaf pineapple cultivars are more susceptible than smooth-leaf varieties (Joy & Sindhu, 2012).

2.3.1.6 Green Fruit Rot

Green fruit rot is caused by the oomycete, *Phytophthora cinnamomi.* Green fruit in contact with the soil are liable to be infected. A water-soaked rot develops internally behind affected fruit lets with no external symptoms. As the disease progresses, a general, water-soaked rot of the green fruit with a distinct brown margin develops in the green fruit (Joy & Sindhu, 2012).

2.3.1.7 Interfruitlet Corking

Interfruitlet corking disease is caused by the fungus, *Penicillium funiculosum*. Fruits affected by inter fruitlet corking often show shiny patches on the shell early in their development, where the trichomes (hairs) have been removed by mite feeding. Externally, corky tissue develops on the skin between the fruitlets, but usually only _patches' of eyes are affected. Fine, transverse cracks may also develop on the sepals and bracts. In moderate to severe cases, corkiness surrounding fruitlets prevent their development and one side of the fruit will be malformed (Joy & Sindhu, 2012).

2.3.1.8 Leathery Pocket

The fungus, *Penicillium funiculosum,* causes leathery pocket disease in pineapple. With this disease, fruits do not usually show any external symptoms. Internally, the formation of corky tissue on the walls of the fruitlets makes them leathery and brown. Miticide application at flower induction and then three weeks after can reduce the disease (Joy & Sindhu, 2012).

2.3.1.9 Water Blister

Water blister disease is caused by the fungus, *Chalara paradoxa,* which also causes butt (base) rot and white leaf spot. This is the major postharvest disease of fruit for the fresh fruit market. The disease takes three to four days to develop after harvest and is therefore not a common problem in fruit used for canning. Water blister can be severe in fresh fruits consigned to distant markets when refrigeration is not available. The disease does not occur in the field unless fruits are over-ripe or injured. Symptoms include water blister, which is also referred to as black rot or soft rot. This causes a soft, watery rot of the fruit flesh and makes the overlying skin glassy, water-soaked and brittle. The skin, flesh and core disintegrate and the fruit leaks through the shell.

In advanced cases, only a fruit shell containing only a few black fibres remains. This shell collapses under the slightest pressure. This can be managed by dipping the base of the fruit in a recommended fungicide within five hours of harvesting and storing the fruit at 9℃ (Joy & Sindhu, 2012).

2.3.1.10 White Leaf Spot

White leaf spot disease is caused by the fungus *Chalara paradoxa,* which also causes water blister and butt (base) rot. The first symptom is a small, brown spot on the leaf, usually where the leaf margin has been rubbed by another leaf during strong winds. These spots lengthen rapidly during wet weather. During prolonged wet periods, spots may reach more than 20 cm in length and spread to the leaf tip. Fine weather rapidly dries the affected area leaving cream coloured or almost white, papery spots; hence the name, ‗white leaf spot'. The margins of the spot often remain brown (Joy & Sindhu, 2012).

2.3.1.11 Fruit Rot by Yeast and Candida Species

Yeasts, *Saccharomyces* spp. and *Candida* spp. are among the most common organisms found in nature which cause yeasty rot in pineapple. The disease mainly occurs during spring in overripe or damaged fruit. Yeasts ferment sugar solution, producing alcohol and releasing carbon dioxide. The first symptom is a bubbling exudation of gas and juice through the crack or injury where infection occurred. The shell then turns brown and leathery and, as the juice escapes, the fruit becomes spongy. Internally, the decaying flesh turns bright yellow and develops large gas cavities. Finally, all that remains of the fruit is the shell and spongy tissue (Joy & Sindhu, 2012).

2.3.1.12 Nematodes Associated Diseases

Root-knot nematode (*Meloidogyne javanica*), the root lesion nematode (*Pratylenchus brachyurus*) and the reniform nematode (*Rotylenchulus reniformis*) are associated with pineapple. Root-knot nematodes produce distinct terminal swellings on the roots, stopping further root development. The root lesion nematode invades the outer root tissues, causing black areas (lesions) of dead or injured plant cells on the root surface. The root lesion nematode can completely encircle the root. Reniform nematodes reduce the number of lateral and fine feeder roots; the remainder elongate normally so that plants retain good soil anchorage. Root-knot nematodes cause stunting, yellowing and dieback of plants (Joy & Sindhu, 2012).

2.3.2 Bacteria and Phytoplasmas Associated Diseases

2.3.2.1 Marbling

Marbling is caused by bacteria *i.e., Pantoea ananatis* and *Acetobacter* spp. and is a minor problem that occurs sporadically. The disease is serious only in countries where pineapple fruits mature under lowland, tropical conditions. Infected fruits do not show any external symptoms. Internally, the flesh is red-brown and granular in appearance and has a woody consistency (Joy & Sindhu, 2012).

2.3.2.2 Pink Disease

The bacteria, *Pantoea citrea*, *Gluconobacter oxydans* or *Acetobacter aceti* cause pink disease. Infected fruits do not show any external symptoms, even when fully ripe. Internally, the flesh may be water-soaked or light pink and have an aromatic odour, although these symptoms may not be obvious immediately. When sterilized by heat during canning, infected tissue darkens to colours ranging from pink to dark brown. In

some fruits, only one or a few fruitlets may be infected. In highly translucent, low-brix fruits, the entire cylinder can be invaded (Joy & Sindhu, 2012).

2.3.3 Virus Associated Diseases

2.3.3.1 Mealybug Wilt Disease

Mealy bug wilt disease is caused by ampelovirus transmitted by mealy bugs. The early symptoms are a slight reddening of leaves about halfway up the plant. The leaf colour then changes from red to pink and leaves lose rigidity, roll downwards at the margin and the tip of the leaf dies. The root tissue also collapses and the plant appears wilted. Plants can recover to produce symptomless leaves and fruit that are markedly smaller than fruit from healthy plants. Symptoms are most obvious in winter when plant growth and vigour are reduced. Disease development and incidence is affected by plant age at the onset of mealy bug infestation, with younger plants displaying symptoms two to three months following feeding, while older plants may take up to 12 months to develop symptoms. Mealy bug can be controlled by treating the soil with either 2.75kg/ha of chlordane or heptachlor 2.25kg/ha (Joy & Sindhu, 2012).

2.3.3.2 Yellow Spot

Yellow spot is caused by Capsicum chlorosis virus (Tospoviruses). Infection occurs on young crowns when they are still on the fruit or during the first few months after planting. Small (2–5 mm), round, yellow spots appear on the upper surface of the leaves of young plants. These spots fuse and form yellow streaks in the leaf tissue, which soon become brown and die. The virus spreads to the leaves in the plant heart, causing the plant to bend sideways. Infection eventually kills the plant and the virus is not transmitted to subsequent plantings. Infections can occur through open blossoms

causing the development of large, blackened cavities in the side of the fruit (Joy & Sindhu, 2012).

2.4 Pesticides and their Importance

The Food and Agriculture Organization (FAO) has defined a pesticide as; any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (FAO, 2002). Many of the pesticides that are used on crops, gardens or domestic animals, are often a mixture of several chemicals mixed together in desired proportions suspended in appropriate carrier or diluent materials. These chemicals are called active ingredients that are responsible for killing or otherwise, affecting the pests. Apart from the active ingredients, there are other chemicals that are formulated together with the active ingredients that usually do not kill pests. These are called inert ingredients and they serve as carriers, diluents, binders or dispersants which prolong the shelf life of the active ingredients or make the pesticide smell better (Zacharia, 2011).

With the aforementioned diseases and pest that cause damage to crops specifically pineapples, pesticides play an important role in their destruction. The use of pesticides helps in destroying the various organisms which have negative impact on human activities, infrastructure and the materials of everyday life. In most aspects of human activities, pesticides are used to control unwanted organisms, prevent accelerated corrosion of metal constructions, maintain the turf on sport pitches including cricket

grounds and golf courses and help to facilitate a hugely popular pastime that provides fresh air and exercise for millions of people around the world in domestic and ornamental gardening etc. Gattuso (2000) therefore wrote that banning some pesticides would reduce the availability, affordability and overall consumption of fruit and vegetables—a vital protection against cancer.

According to the United State of America Data Programme (2003) cited by Twum (2011) pesticides are used to kill mosquitoes that can transmit deadly diseases like West Nile virus, yellow fever and malaria. They can also kill bees, wasps or ants that cause allergic reactions. Pesticides can protect animals from illnesses that can be caused by parasites such as fleas. Pesticides can prevent sickness in humans that could be caused by mouldy food. Pesticides can be used to clear roadside weeds, farm weeds and weeds that may cause environmental damage. Pesticides are also commonly applied in ponds and lakes to control algae and plants such as wiregrasses that can interfere with activities like swimming and fishing and cause the water to look or smell unpleasant. Pests such as termite which can damage the wooden structures of a house such as ceilings, doors, and window frames may be controlled by pesticides.

2.5 Classification of Pesticides

Pesticides can be grouped by target organism (for examples insecticides, herbicides, fungicides, rodenticides etc), chemical structure (organochlorines, organophosphates, carbamates, phenoxy acids), and physical state (solid, liquid, aerosol).

Plant-determined pesticides, or "botanicals", have been growing rapidly. They include the pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside (Kamrin 1997:15).

The World Health Organization (WHO) has developed a classification system that groups pesticides according to the potential risks to human health caused by accidental contact to human being and they are grouped into the following classes;

Class Ia = extremely hazardous

Class Ib = highly hazardous

Class II = moderately hazardous

Class III = slightly hazardous

Class IV = products unlikely to present acute hazard in normal use.

In this study the chemical classification as well as the target organisms will be used to categorize and discuss the pesticides detected.

2.6 Classification of Pesticides based on the Chemical Composition

Under chemical classification, pesticides are categorized according to the chemical nature of the active ingredients. The chemical classification of pesticides is by far the most useful classification to researchers in the field of pesticides and environment and to those who search for details. This is because, it is this kind of classification that gives the clue of the efficacy, physical and chemical properties of the respective pesticides, the knowledge of which is very important in the mode of application, precautions that need to be taken during application and the application rates. Based on chemical classification, pesticides are classified into four main groups namely; organochlorines, organophosphorus, carbamates and pyrethrin and pyrethroids (Buchel, 1983) but for this study the pesticides to be analyzed are either organochlorines, organophosphates or synthetic pyrethroids.

2.6.1 Organochlorine Compounds

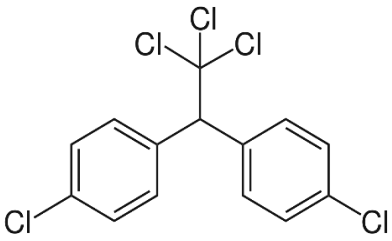
Organochlorines were the first synthetic organic pesticides to be used in agriculture and in public health. Organochlorine insecticides can be divided into two groups: one uses benzene as the raw material and the other uses cyclopentadiene. They are both very stable, therefore their persistence and bioaccumulation are very strong (Rathore, 2012). These pesticides, characterized by their cyclic structure; number of chlorine atoms and low volatility, can be divided into four groups (Anderson *et al.,* 2000). These four groups are:

1. Dichlorodiphenyl ethanes (such as DDT)
2. Cyclodienes (Such as dieldrin, endosulfan and heptachlor)
3. Chlorinated benzenes (Such as hexachlorobenzene) and
4. Cyclohexanes (Such as lindane)

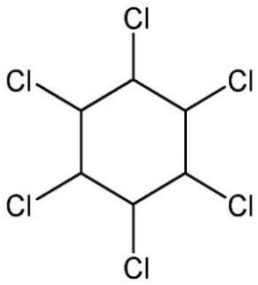
These chemicals were widely used until the mid-1970 when most of them were banned from use in the developed countries. However, one of these insecticides, endosulfan is still widely used throughout the world despite its known adverse effects on humans as an endocrine disrupting compound (Andersen *et al*., 2000).

Kamrin (1997) mentions that organochlorines operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Most organochlorines are widely used as insecticides for the control of a wide range of insects, and they have a long-term residual effect in the environment since they are resistant to most chemical and microbial degradations. Organochlorine insecticides act as nervous system disruptors leading to convulsions and paralysis of the insect and its eventual death. There are many examples of organochlorine pesticides, including, but not limited to: DDT, lindane, endosulfan, aldrin, dieldrin

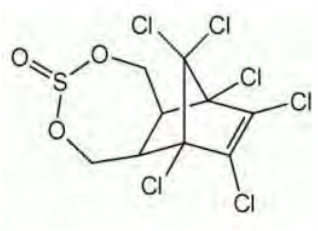
and chlordane which will be analyzed in this study and their chemical structures are presented below.



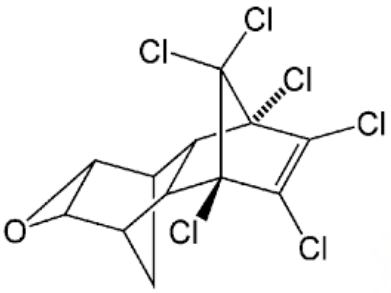
DDT



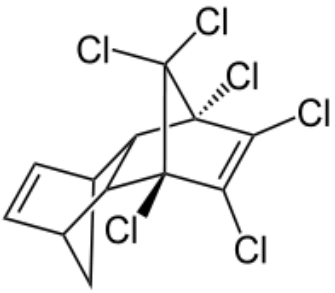
Lindane



Endosulfan



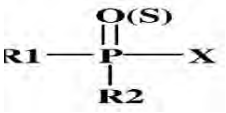
Aldrin



Dieldrin

2.6.2 Organophosphorus Compounds

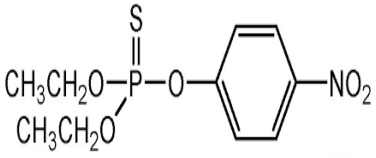
Organophosphorus insecticides contain a phosphate group as their basic structural framework as defined by Schrader's formula:



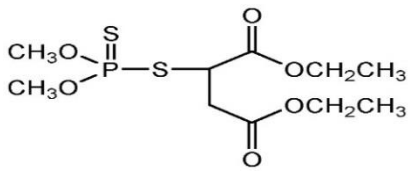
Where, R1 and R2 are usually methyl or ethyl groups, the O in the OX group can be replaced with S in some compounds, whereas the X group can take a wide diversity of forms.

According to Colovic *et al.* (2013) organophosphate and carbamate insecticides have a comparable method of activity. They influence the nervous system of target organisms (and non-target creatures) by disturbing acetylcholinesterase action, the chemical that directs acetylcholine, at nerve neurotransmitters. This restraint causes

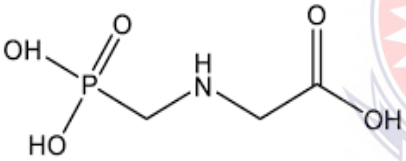
an increase in synaptic acetylcholine and over-stimulation of the parasympathetic nervous system. Hence, nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles and hence paralysis and death. Unlike organochlorines, organophosphorus insecticides are easily decomposed in the environment by various chemical and biological reactions, thus organophosphorus insecticides are not persistent in the environment (Martin, 1968). Some of the widely used organophosphates include parathion, malathion, diazinon and glyphosate and they will also be analyzed amongst other organophosphates in this study.



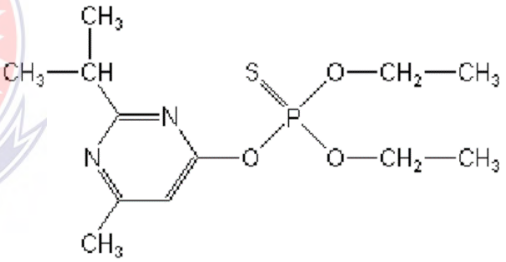
Parathion



Malathion



N-(Phosphonomethyl)-glycine (Glyphosate)



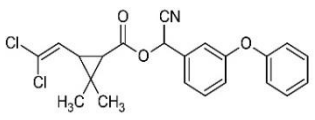
Diazinon

2.6.3 Synthetic Pyrethroids

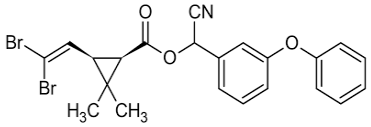
Pyrethroids are synthetic analogues of the naturally occurring pyrethrins; a product of flowers from the pyrethrum plant (*Chrysanthemum cinerariaefolium*). The insecticidal components of pyrethrum flowers are the optically active esters derived from (+)-*trans*-chrysanthemic acid and (+)-*trans*-pyrethroic acid (Zacharia, 2011).

Pyrethroids are known for their fast knocking down effect against insect pests, low mammalian toxicity and facile biodegradation. Although the naturally occurring pyrethrins are effective insecticides, their photochemical degradation is so rapid that

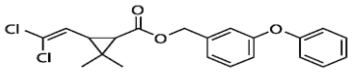
their uses as agricultural insecticides become impractical. The synthetic analogues of the naturally occurring pyrethrins (pyrethroids) were developed by the modification of pyrethrin structure by introducing a biphenoxy moiety and substituting some hydrogens with halogens in order to confer stability at the same time retaining the basic properties of pyrethrins (Zacharia, 2011). Some manufactured pyrethroids are poisonous to the nervous system (Soderlund, 2010). The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin.



Cypermethrin



Deltamethrin



Permethrin

2.7 Pesticide uses in Ghana

The reasons to which farmers use pesticides are many. In a previous work done by Gerken, *et al.* (2001), they suggested that organochlorines are widely used by farmers because of their effectiveness and their broad-spectrum activity. Also, Lindane (Gamma BHC) is widely used in Ghana in cocoa plantations, on vegetable farms and for the control of stem borers in maize. This is evident in a study by Awumbila and Bokuma (1994) who focused their study on 30 organized farms and 110 kraals distributed throughout the 10 regions of Ghana. It was found that, twenty (20) different pesticides were in use with the organochlorine, lindane, being the most widely distributed and used pesticide, accounting for 35% of those applied on farms. Of the 20 pesticides, 45% were organophosphorus, 30% were pyrethroids, 15% were carbamates and 10% were organochlorines (Awumbila & Bokuma, 1994).

DDT is a banned pesticide in Ghana whereas lindane and endosulfan are restricted for the control of capsids on cocoa, stem-borers in maize and pests on coffee. However,

research by Ntow *et al.* (2008) has shown that these agrochemicals are being used in vegetable production because of their potency. Aside using banned pesticides some farmers also mix several pesticides for use in order to increase their potency. Herbicides are the predominant pesticide type used in vegetable production in Ghana probably due to the farmers' perception of weed control. The reason was as long as it is profitable, and no better alternatives are available; the spraying of pesticide is a good investment (Hardy, 1995). This current study will also reveal the intended use of pesticides in pineapple production based on the categories of pesticides that will be detected.

2.8 Review of the various Pesticides used in Crop Production

The results of the investigation carried out by Essumang, Asare and Dodoo (2013) confirmed accumulation of pesticides such as heptachlor 4.0, dieldrin 4.2, aldrin 4.9 and endrin 4.1 µg/kg in the non-target crop (okra grown close to watermelon farm) at elevated levels, a cause of health hazard to humans as their levels were higher than the WHO/FAO safety levels.

Botchway (2000) analyzed pesticide residues in exportable quality cocoa beans collected from selected cocoa growing districts in the middle belt of Ghana and the two shipping ports at Tema and Takoradi. Analysis of the extract by gas liquid chromatography showed detectable amount of lindane residue but the level was about 10% of maximum residue level of 1.0 pg/g permitted by Codex Alimentarius Commission. The results of the research indicate that Ghana's exportable cocoa beans are therefore of no immediate danger of being rejected by any importing country due to presence of lindane residues.

Essumang, Dodoo, Adokoh and Fumador (2008) also evaluated the residue levels of selected pesticides used on tomato crops in Ghana that are likely to have accumulated in the tomatoes during application. The results obtained confirmed that pesticide residues were indeed present in the tomatoes and further analysis quantified the amount present. Analysis of some organochlorine and organophosphorus residue levels in the fruits indicated that chlorpyrifos, which is an active ingredient of pesticides registered in Ghana under the trade name dursban 4E or terminus 480 EC for use on vegetables, has the greatest residue level of 10.76 mg/kg. The lowest residue level observed was that of pirimiphos-methyl with 0.03 mg/kg. This buttresses an earlier study by Ntow (2001) about vegetables on the Ghanaian market such as lettuce, cabbage, tomato and onion, which were found to contain detectable levels of lindane, endosulfan and DDT residues.

Results from similar research conducted by Agyekum, Ayernor, Saalia and Bediako-Amoa (2015), showed that organochlorine, organophosphate and synthetic pyrethroid residues were translocated in all fruit samples analyzed. With respect to tomato fractions, the peels retained more residues compared to the pulp and the central core. In the chemical species, organochlorines were retained more in the peels of tomato than the other fractions of the fruit. Synthetic pyrethroid residues were evenly distributed in the pineapple fruit. In mangoes, the pulp retained more chemical residues than other fractions of the fruit. That is, more organochlorine residues were retained in the pulp of mango than in the other fractions of the fruit. Synthetic pyrethroid residues were evenly distributed throughout the mango fruit.

Laboratory analysis during a study by Baah (2016) confirmed the presence of organochlorine insecticide residues in some of the samples analyzed. Overall, percent

insecticide residues in carrot, lettuce and cabbage samples were 38%, 28% and 34%, respectively, levels which were below the limits set by the EU. Nonetheless the MRLs for heptachlor and mirex exceeded the limits in some cases.

Finally, Fianko *et al.,* (2011) also found disturbing levels of pesticide, heavy metals, microorganisms and mycotoxins contamination in street-vended food samples in Accra.

With this data it is evident that, many researches have been done on pesticide residue analysis in food crops in Ghana. However, most of these researches are done outside Winneba in the Central Region of Ghana, so this study will be conducted in Winneba which is the major town surrounded by areas where pineapple production is common in the region and also where most pineapple farmers bring their produce to sell.

2.9 Common Pesticides used on Pineapples in Ghana

The exposure of people to pesticides in Ghana may be excessive, especially through ground application in cocoa, pineapple, cotton and vegetable farms where compounds of high toxicity are often used (Mensah, Yeboah & Akman, 2004; Yeboah, Lowor & Akpabli, 2003).

According to Aboagye (2002), the pineapple farmers used pesticides either for field application or post-harvest treatment of the fruits. But unfortunately, the pesticides were selected without due cognizance of the provisionally approved pesticides for the production of exportable pineapple in Ghana. Out of twenty-one types of pesticides listed only nine were provisionally approved for production of exportable pineapple. These were diuron, fluazifop-butyl, glyphosate (herbicides) chlorpyrifos, cypermethrin and dimethoate (insecticides); metalaxyl and carbendazim (fungicides) and ethephon (growth regulator).

2.9.1 Ethephon

Ethephon is a plant growth regulator and its use varies with plant species, chemical concentration and time of application. Ethephon regulates phases of plant growth and development by application to various growth sites (Kidd & James, 1991). The active ingredient ethephon is found in a variety of commercial herbicides. Some trade names for products containing ethephon include Arvest, Bromeflor, Etheverse, Flordimex, Flordimex T-Extra, Cerone, Etherel, Chipco Florel Pro and Prep (Anon, 1994) as cited by Proshad *et. al* (2017). Ethephon is used to artificially ripen fruits (Singal *et al*., 2012). It is often considered better than calcium carbide because pineapples treated with 1000 ppm of ethephon required less time (48hours) for ripening than other treated fruits as well as compared with the non-treated fruits. The fruits ripened with ethephon have more acceptable colour than naturally ripened fruits and have a longer shelf life (Ur-Rahman *et al*., 2008). Ethephon comes in ready-to-use, emulsifiable concentrate and aqueous solution formulations. It may also be used in combination with Terpal (with mepiquat-chloride) and Terpal C (chlormequat-chloride). Spraying it on unripe pineapples hasten their ripening time. A batch will ripen almost at the same time, perfect for competitive market demands. Ethephon converts to ethylene after metabolism. Ethylene is a growth and ripening agent naturally produced by plants. It can be added externally to produce a more desirable effect.

2.9.2 Diuron

Diuron, [N-(3, 4-dichlorophenyl)-N, N-dimethyl-urea] is an herbicide belonging to the phenylamide family and the subclass of phenylurea. This substituted urea herbicide inhibits photosynthesis by preventing oxygen production (Wessels & Van der Veen, 1956) and blocks the electron transfer at the level of photosystem II of

photosynthetic micro-organisms and plants. This compound has been used to control a wide variety of annual and perennial broadleaf and grassy weeds, as well as mosses. It has been also used on non-crop areas such as roads, garden paths and railway lines and on many agricultural crops such as fruits, cotton, sugar cane, alfalfa and wheat. However, dispersion of this compound in agriculture leads to pollution of the aquatic environment by soil leaching (Louchart et al., 2000; Thurman et al., 2000). Diuron is considered a Priority Hazardous Substance by the WHO (Malato et al., 2002). A field experiment conducted by Gooddy et al. (2002) in order to investigate the fate and transport of diuron in a calcareous soil and the results suggested that, the continued formation of degradation products exits, as the diuron continues to leach through the soil. Thus, pollution of water and soil by diuron has become a more serious problem due to the formation of 3, 4- dichloroaniline subjected to leaching and bioaccumulation. Diuron is absorbed from the gastrointestinal and respiratory systems. In humans, it is metabolised within hours by hydroxylation and N-dealkylation, then excreted via the urine (Hayes, 1982) as cited in Giacomazzi and Cochet (2004).

2.9.3 Fluazifop

Fluazifop-p-butyl butyl (R)-2-{4-[5-(triuoromethyl)-2-pyridyloxy] phenoxy} propionate is an herbicide with systemic activity in grass weeds and selectivity for *P. vulgaris* plants. This herbicide belongs to the chemical family; aryloxy-phenoxy propionate, which is a strong inhibitor of the enzyme acetyl-coenzyme A carboxylase (ACCase). Inhibition of ACCase by fluazifop, precludes the synthesis of malonyl-CoA, the committed step of fatty acid biosynthesis in plants, thus controlling the grass weeds (Cieslik et al., 2013).

Fluazifop-p-butyl is non-toxic to broadleaf plants and is therefore registered for use in a variety of broadleaf crops, such as soybean, oilseed rape, sugar beet, fodder beet, potatoes, vegetables, cotton, pome fruit, stone fruit, bush fruit, citrus fruit, vines, pineapples, bananas, strawberries, sunowers, alfalfa, ornamentals, and other broadleaf crops.

It was concluded by Horbowicz *et. al* (2013) that fluazifop inhibits maize growth, and the intensity of the effect is positively correlated with the herbicide concentration. However according to Maia (2012), the use of diuron, fluazifop-p-butyl and atrazine + S-metolachlor did not affect growth, yield and fruit quality of pineapple, cultivar 'Pérola'.

2.9.4 Glyphosate

Glyphosate is a popular herbicide used to kill certain plants and grasses that compete with crops, manage how plants grow, get crops ready for harvest, and ripen fruit. It is often used on fruits and vegetables, glyphosate-resistant crops (like canola, corn, cotton, soybeans, sugar beets, and wheat) and lawns, greenhouses, aquatic plants, and forest plantings.

Glyphosate, (*N*-(phosphonomethyl) glycine) is a broad-spectrum systemic herbicide and crop desiccant which affects all vegetation types including trees. However, if over sprayed could damage tree species in active growth hence may only be suitable for clearing vegetation during land preparation (Stringer, 1997; Willoughby et al. 2004). It is an organophosphorus compound, specifically, a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase. In 2015, the World Health Organization reclassified glyphosate as probably carcinogenic to humans (Guyton et al. 2015). Glyphosate may not be

suitable for weeding during early stages of forest plantation development when tree seedlings have been inter-planted with food crops. This is because although targeted at grass and broadleaf weeds, drifts of glyphosate molecules may settle on tree seedlings and crops and may retard their growth, damage or kill them (Willoughby et al. 2004).

2.9.5 Chlorpyrifos

Chlorpyrifos with the IUPAC name O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate and chemical formula, $C_9H_{11}C_{l3}NO_2PS$, is an organophosphate pesticide used on crops, animals, and buildings, and in other settings, to kill a number of pests, including insects and worms. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization based on its acute toxicity (Watts, 2012).

The toxicity of chlorpyrifos has been associated with neurological dysfunctions, endocrine disruption, and cardiovascular diseases. It can also induce developmental and behavioral anomalies, hematological malignancies, genotoxicity, histopathological aberrations, immunotoxicity, and oxidative stress as evidenced by animal modeling.

Due to its endocrine and other toxicological effects, several pharmacological and non-pharmacological approaches have been employed such as the use of antidotes, administration of different drugs and nutritional therapies which include dietary administration of vitamin C, melatonin, and zinc which are capable of ameliorating the toxic effects. Additionally, the microbial degradation/biotransformation can be a useful strategy to mitigate chlorpyrifos-induced toxicity in the environment.

Moreover, food processing methods are quite effective in reducing chlorpyrifos concentrations in foods (ur Rahman et. al, 2020).

2.9.6 Cypermethrin

Cypermethrin is a highly used pesticide for the purpose of killing pests in households, agricultural crops and for several other reasons. Ahmad et .al. (2012) has concluded in their study that the exposure to Cypermethrin leads to the decrease in testicular and epididymal sperm count.

It was also concluded by McDaniel and Moser (1993) that Cypermethrin causes neurobehavioral changes in pawing, burrowing, salivation and it also causes whole body tremor to choreoathetosis, hypothermia, and lower the motor activity.

2.9.7 Diamethoate

Dimethoate is a monocarboxylic acid amide that is N-methylacetamide in which one of the hydrogens of the methyl group attached to the carbonyl moiety is replaced by a sulfanediyl group (dimethoxyphosphorothioyl). It has a role as an EC 3.1.1.7 (acetylcholinesterase) inhibitor, an agrochemical, an acaricide, an EC 3.1.1.8 (cholinesterase) inhibitor, an insecticide, a xenobiotic and an environmental contaminant. It is an organic thiophosphate and a monocarboxylic acid amide, (National Center for Biotechnology Information, 2021). Studies by some researchers have revealed the presence of dimethoate in food including one by Afreh-Nuamah (2016), which showed that the pesticide residual levels (mg/kg) of lambda-cyhalothrin (0.48 ± 0.19), cypermethrin (1.70 ± 1.37) and dimethoate (0.07 ± 0.05) in cabbage samples from Accra Metropolitan Assembly were more than EU MRLs.

2.9.8 Metalaxyl

Metalaxyl is an active ingredient belonging to the phenylamide group (acylalanines). This is one of the most commonly used active ingredients in the fight against mildew worldwide. It has the IUPAC name methyl 2-[(2,6-dimethylphenyl) (methoxyacetyl) amino]propanoate.

Metalaxyl is slightly soluble in water and is translocated readily from roots to the aerial parts of most plants, but its lateral translocation is slight. Because the use of metalaxyl has already resulted in the appearance of strains resistant to it in some pathogens, it is recommended that it be used in combination with other, broad-spectrum fungicides.

2.10 Consequences of using pesticides

The usage of chemicals has occasionally been accompanied by risks to human health and the environment because of their toxic potential, high persistence, bioconcentration, and, especially, their non-specific toxicity (Krauthacker et al. 2001; Barriada-Pereira et al. 2005). Despite the fact that the use of certain organochlorine pesticides in agriculture is prohibited in many countries, these compounds have been detected in the environment due to their persistence worldwide (Rajendran & Subramanian 1997). Pesticides, in particular, are compounds with known inherent toxicity. Their heavy application could result in diffuse pollution that can bring about disturbance of the natural balance, widespread pest resistance, environmental pollution, and hazards to humans and wildlife (Claeys et al. 2011; Hjorth et al. 2011). Studies indicate a decrease in sperm counts have been associated with exposure to organochlorine insecticides (Kannan *et al.*, 1997). Continued exposure to these chemicals for a long period may result in various diseases such as:

- Neurological, psychological and behavioral dysfunctions
- Hormonal imbalances, leading to infertility, breast pain
- Immune system dysfunction
- Reproductive system defects
- Cancers
- Genotoxicity
- Blood disorders.

In a study of boys with cryptorchidism or undescended testes, it was noted that their mothers had higher levels of organochlorine pesticide metabolites in their breastmilk (Damgaard et al., 2006).

2.11 Pesticide Residue in Food

Pesticides, regardless of the means of application, circulate in agro biocenosis and migrate to various elements of the environment, especially to the atmosphere and hydrosphere (Biziuk, 2001; Moreno et al., 2006). Therefore, the pesticide content in fruits and vegetables, except for direct spraying, is also influenced by their presence in the soil, where the fruits grow, or in the water used for irrigation. Pesticides can also be transported by rain or wind from the points of treatment to the neighbouring crops and areas where they are unwanted or harmful (Hajslova & Zrostlikova, 2003; Stocka et al., 2011). They belong to various chemical compound groups (OCP – organochlorine pesticide, ONP – organonitrogen pesticide, OPP – organophosphate pesticide) and can penetrate into the plant in their original form, or in the form of their degradation products which are often more toxic than the starting substance (Jokanović, 2001, 2009; Prasad et al., 2013; Escuderos-Morenas et al., 2003). Pesticides penetrate the plant through the root system and the above-ground parts,

especially through leaves. Features such as the properties of living organisms, chemical structure of the pesticide or soil and atmosphere conditions affect the rate of diffusion. Pesticides also penetrate from the inside of the plant to its other anatomical parts (from the root system to stems and leaves). Plants have small possibilities of waste products excretion (the only way is through leaves transpiring system) for this reason; plants are exposed to a large accumulation of pesticides. Pesticides accumulated in fruits and vegetables may undergo various transformations and processes: physical, chemical as well as photochemical and biochemical ones – leading to irreversible changes and various kinds of damages (Różański, 1998).

2.12 Pesticide Residual Levels in crops grown in Ghana

Many of the world's agricultural activities take place in rural and economically disadvantaged areas where regulations are lacking and health standards are not enforced. For these reasons, pesticides are often improperly used and stored, and workers are often not fully aware of, and protected from the dangers that pesticide exposure and contamination can cause. In addition, due to laxity of regulations, low cost, and the effectiveness of certain hazardous pesticides, sustained use of banned pesticides is an ongoing problem in many low-income rural areas (Wesseling *et al*., 1997).

Farmers around the world including Ghana use pesticides as an insurance policy against the possibility of a devastating crop loss from pests and diseases. Accordingly in Ghana, for decades, pesticides have been employed not only in agriculture to control and eradicate crop pests but also in the public health sector for disease vector control. Nevertheless, there has been a rapid increase in the quantity and use of pesticides (insecticides, fungicides, herbicides, bactericides, rodenticides, and plant

growth regulators) in agriculture over the past years (957,474.2 t in 1992 to 1,912,994 t in 2007) (FAOSTAT 2015). Moreover, this growth trend is expected to heighten for the next decades.

Agricultural pesticides are used in cocoa, coffee, and cotton farming; in vegetable and fruit production; and for other mixed crop farming systems involving cereals (mostly maize), tuber crops (e.g., yam, cassava), legumes (e.g., cowpeas), sugarcane, rice, etc. The majority of these pesticides are employed in the forest areas or farming regions noted for the production of these crops located in Ashanti, Brong Ahafo, Eastern and Western regions of Ghana (Ntow 2005; Amoah et al. 2006). Dinham (2003) estimates that 87 % of farmers in Ghana use chemical pesticides to control pests and diseases on vegetables and fruits.

Ntow et al. (2006) gave the proportions of pesticides used extensively on vegetable farms, in small or large amounts by farmers, as herbicides (44%), fungicides (23%), and insecticides (33%). It has been revealed that the chemical control method is very effective, rapid in curative action, adaptable in most situations, and flexible in meeting changing agronomic, ecological, and economic conditions (Metcalf 1975; Newsom et al. 1976). Among the different types of pesticides known, organochlorine pesticides were extensively used by farmers in the 1980s, because of their cost effectiveness and broad-spectrum activity. DDT, lindane, and endosulfan are mostly employed to control the ectoparasites of farm animals and pets in Ghana (Ntow et al. 2006), but at the moment, these pesticides are not used in agricultural production because of their toxicity and persistence in the environment.

The impact of pesticides have been reported in fruits and vegetables at different intervals throughout Ghana (e.g., Mawuenyegah 1994; Ninsin 1997; Botchway 2000;

Ntow 2001; Aboagye 2002; Amoah et al. 2006; Kotey et al. 2008; Darko and Akoto 2008; Essumang 2008; Armah 2011; Botwe et al. 2011; Bempah et al. 2011a, b, 2012a, b; Akoto et al. 2013). The organochlorines (OCs) appeared to be the most detected and studied in literature revealing either their past usage or persistence in the soils as reflected in the vegetables and fruits indicated earlier. Perhaps farmers clandestinely obtained these chemicals and are currently applying them secretly even though it is prohibited in the Ghanaian market. The organophosphorus (OPs), dichlorvos and chlorpyrifos were also found to be the most popular pesticides applied among the vegetable growers, excluding lambda cyhalothrin. The utilization of synthetic pyrethoids (SPs) is now on the rise by most fruit and vegetable farmers in Ghana. The findings by different groups elucidate this (e.g., Darko & Akoto 2008; Essumang 2008; Armah 2011; Bempah & Donkor 2011; Akoto et al. 2013).

Unfortunately, though fruits and vegetable farming and their consumption are concurrently progressing steadily in the Ghana, yet, the impact of pesticides, particularly OPs and SPs, in the Ghanaian environment has not received the fullest attention with the increase in its usage. This is evident in a report by EPA which showed that, in June 2013, the percentage of OPs and SPs registered was only 23% of the total of 406 and being 50.8 % only of the total insecticides registered (EPA, Ghana 2013).

According to Bempah et al. (2011a, b), the non-conforming results of pesticide residue were detected on the fruits and vegetables because of the different types of pesticides applied by farmers which further suggested a great potential for systemic toxicity in children considering that they are the most vulnerable population subgroup. Moreover, the higher levels observed on the various fruits and vegetables could also

be attributed to the farmers‘ poor knowledge in pesticide application, thus over abusing the chemical. The farmers‘ non-conforming attitude to extension officers‘ advice on the usage of these chemicals could also play a key role.

The high values of pesticide residues in food commodities may also be a consequence of the absence of updated food regulations and also because food laws in Ghana are very old and the authorities in charge keep silent about the levels of pesticide residue in many of these food commodities**.**

2.13 Residual levels in Pineapples

In a research carried out by Agyekum et al. (2015) on the translocation of pesticide residues in tomato, mango and pineapple fruits, it was indicated that more organophosphate and organochlorine residues were detected in pineapple peels compared to the pulp whiles synthetic pyrethroid residues were evenly distributed in the pineapple fruit.

Kyofa-Boamah (2001) cited by Aboagye (2002) analyzed ethephon and triadimefon residue levels in exportable pineapples selected from farms in Ghana. The pineapples were sampled from different farmers' fields in which ethephon was sprayed with different spray concentrations of 200 ml, 90 ml, and 50 ml /15 liters of water. The post-harvest interval observed by these farmers was seven days. Seventy-two fruits sampled from twelfth boxes were sent to a laboratory in Germany by air for residue analysis. The residue levels recorded in fruits sprayed with ethephon concentrations of 200ml, 90ml and 50ml/151iters of water were 3.31mg/kg, 1.13mg/kg and 0.90 mg/kg, respectively. This situation is alarming and the results from farmers practice reveals very high doses applied to immature fruits. However, there was no detection of

residue level of triadimefon (Bayleton 5 %) applied on the stem as post-harvest treatment.

Marchal *et al.* (1999) cited by Pinon (2000) analyzed imazalil and triadimefon residue levels in some pineapples from West and Central Africa. The residue levels recorded for imazalil was higher than the detection threshold of 0.01 mg/kg as set by the European Legislation. In the other cases outside the European Legislation, only 33% of the fruits had triadimefon levels lower than the detection threshold of 0.01 mg/kg.

2.14 Determination of Pesticide Residues in Crops

There have been several studies regarding development and validation of analytical methodologies for pesticide residues analysis in crops and juices (Furlani, Marcilio, Leme, & Tfouni, 2011). Currently, routine methods for the determination of pesticide residues in the environment and food typically require several sample preparations such as extraction, clean-up, and concentration before instrumental analysis. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most useful sample preparation methods for the clean-up procedure (Hernández, Sancho, Pozo, Lara & Pitarch, 2001; Ahmed, 2001).

2.15 Analytical Methods used in Pesticide Residue Analysis

2.15.1 Sample preparation

Sample preparation converts samples into an acceptable form for measurement without loss or unintended alteration. It is an essential aspect of any analytical work and may vary depending on the matrix to be analyzed. Sample preparation starts from the field, storage, preservation, and transportation, all of which must occur without changing the physical and chemical composition of the original sample. When choosing a sample preparation technique, one should try to easily remove

analytes of interest from the sample with a minimum of steps and good recoveries (Wang & Jocelyn Paré, 1997; Santos & Galceran, 2002). Samples such as vegetables, sediment, water etc., are normally stored in inert and airtight containers to prevent them from being exposed to environmental elements or interfering substances. Samples may also be wrapped in aluminum foil. During sample preparation most vegetables are blended. Water samples are filtered over 0.45 µm pore size filters to remove dead leaves and other debris. Soils or sediments are often air dried and ground before extraction (Ntow, 2001; Essumang et al., 2008; Darko et al., 2008; Kuranchie-Mensah et al., 2012).

2.15.2 Extraction

A wide range of extraction techniques are used for the extraction of insecticides from environmental samples. Most authors either use liquid-liquid extraction or solid-liquid extraction techniques. Liquid-liquid extraction is used to determine insecticide residues in water samples. This is a common analytical procedure used by scientists around the globe. An aliquot of water is transferred to a separation funnel followed by the organic solvent of choice. The mixture is shaken for some minutes and allowed to settle for the two separate layers to form. Depending on the density of the solvent used, the organic layer will either be on top of the water or below it. The extracted organic phase is dried by passing it through anhydrous sodium sulfate. This method is very simple and cheap but its disadvantage is a tendency of forming emulsions. Kurachie-Mesah et al. (2012) used this technique to determine OCPs residues in the Densu river by extracting a pre-filtered water sample (1 L) with three times 50 mL of hexane. The recovery varied between 79% and 96%. The technique was also used by Ntow et al. (2008a, 2008b) who extracted unfiltered water (1 L) with three times 25 mL of hexane.

The recoveries varied between 79% and 104%. Even though the volume of the solvent was reduced, the recovery value obtained was very encouraging. Another technique used for insecticide extraction from water is solid phase extraction (SPE). It is straightforward, cost-effective and efficient. OCPs were extracted with SPE bond elute C-18 cartridge using 30% methanol (v/v) to precondition the column and 1.5 mL hexane to elute the analyte. The average recoveries by Ntow (2001) ranged between 85–94%. Darko et al. (2008) also used this technique to analyse OCPs in Lake Bosomtwi. In this study, 5 mL methanol followed by 5 mL acetone and 5 mL of milliQ water was used to condition the column. The OCP residue was eluted with three times 5 mL hexane. The recovery ranged from 85% to 97%. Wylie et al. (2017) also used a modified SPE coupled with high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the extraction of insecticides and herbicide metabolites from urine. A traditional extraction technique used for solid samples is Soxhlet extraction, which has been used since the early 1900s (Luque de Castro & Priego-Capote, 2010).

Although this technique is time-consuming and requires the use of relatively large volumes of solvent, it is cost effective, robust and can be used overnight. This allows for high process efficiency making it still useful in these modern days (Brits et al., 2016). Ntow (2001) used Soxhlet extraction to determine OCPs in crops and sediments using 200 mL of methanol for 8h. The recoveries ranged between 75–90% for crops and 80–110% for sediments. The technique was also used to extract OCPs from sediment and fish using a combination of acetone/hexane (20:80, v/v) at 50 °C for 4h. This was a shorter time compared to Ntow (2001). The recoveries were between 76 ± 4% and 95 ± 5% for sediment and 78 ± 5% and 95 ± 8% for fish (Darko et al., 2008). There is a growing desire for new extraction techniques with

shorter extraction times and minimum solvent use. This has led to the development of new techniques such as the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS), ultra-turax macerators and sonication. These are faster and efficient techniques for extracting organic analytes from solids or semi-solids (Torres et al., 2015). QuEChERS extraction is applied to dry or wet solids or semi-solid samples. For dried samples, water is normally added before the extraction and the amount of water added depends on the ability of the dried matrix to absorb water and move freely in the centrifuge tube. In this technique, the extraction solvent has always been acetonitrile.

The QuEChERS technique has been used in Ghana in the analysis of fruits, vegetables and cereals (Donkor et al., 2016; Fosu et al., 2017; Blankson et al., 2016; Akoto et al., 2015a, 2015b). Donkor et al. (2016) developed and validated the QuEChERS technique for insecticides in pineapple, carrots, lettuces, and tomatoes. Each sample was spiked with the 36 insecticides solution at 0.01, 0.1 and 1 mg/kg respectively. Five replicates were used at each level and for each batch; a matrix blank, method blank and solvent blank were analysed in addition. The average percentage recoveries were 87.2% for pineapple, 90.7% for carrot, 89.0% for lettuce and 83.9% for tomatoes. The same technique was used in the analysis of OCPs in infant cereals. Here, 5 g of each homogenous sample was weighed into a 50 mL centrifuge tube and 10 mL of distilled water was added. The recoveries varied from 69% to 119% (Akoto et al., 2015a, 2015b). The QuEChERS method will also be used in the course of this study due to the fact that pineapple is a high moisture fruit and the use of this method will increase the efficiency and the yield but reduce the time and require small amount of solvents and materials. Aside the QuEChERS method, the QuPPe method will be used for the analysis of highly polar pesticides

like ethephon which are non-amenable to common multiresidue methods like the QuEChERS.

An ultra-turax maceration was used in a number of pesticide residue extractions. A 20.0g weight of the sample was macerated with 40 mL of ethyl acetate. Sodium hydrogen carbonate (5.0 g) and anhydrous sodium sulfate (20.0 g) were added to remove moisture and further macerated for 3 min using the ultra turax macerator. The samples were then centrifuged for 5 min at 3000 rpm (Bempah et al., 2011). Okoffo et al. (2016) also analysed OPPs and SPs using this technique. Ten grams of a homogenous cocoa bean sample was weighed into a 250 mL Nalgene jar (one should avoid using Nalgene plastics when using ECD detectors as it contains plasticizers, which give signal like a sample). A 20 mL volume of distilled water was then added, stirred to form a homogeneous mixture and left to stand for 15 min. Then, 40 mL acetonitrile was added and then homogenized using the Ultra Turrax T25 basic homogeniser for 2 min. They were then centrifuged at a speed of 3000 rpm for 3 min. Recoveries ranged from 70% to 94% for OPPs and 73% –100% for SP residues.

In sonication, an electrical signal is converted to ultrasound energy, which is applied to agitate particles in a sample. It is a good choice for thermally labile analytes. This technique was employed in a single study for the extraction of chlorpyrifos in used protective wear. Each sample was submerged in ethyl acetate and sonicated for 45 min at a temperature of 25 °C. The reported recoveries were between 88% and 96%.

There are still modern extraction techniques developed to reduce both extraction time and solvent use which have not been used by Ghanaian scientists until today. These techniques are easy to automate, increase extraction efficiency with a small amount

of solvent and high temperature and or pressure (Luque de Castro & Priego-Capote, 2010).

Accelerated solvent extraction (ASE) is an example. It uses high temperature and pressure with less solvent of different polarities (Ahmed, 2001). Matrix solid-phase dispersion (MSPD) allows for an extraction and clean-up in the same extraction step, reducing both analytical time and solvent used. It was used in analyzing insecticide residues in fruits and vegetables (Ahmed, 2001; Gilbert-López et al., 2009). All these modern techniques come with an additional cost, which is a limiting factor for a developing country like Ghana.

2.15.3 Clean-up methods

Clean up is important to remove co-extractives and undesired interfering substances from the sample extract before analysis. Soils, fruits, and vegetables require some degree of clean-up, whereas clean-up of a water extract may often be unnecessary. Alumina, florisil and silica gel are mostly used in clean-up of pesticide residue analysis. Sulfur clean-up is required in the case of sediment analysis. Most authors prepare their own silica gel, florisil or alumina columns, with an addition of some Na_2SO_4 which removes the water from the extract. Others use pre-fab columns containing these chemicals. A mixture of alumina and charcoal (12:1, w/w) slurry with 2 cm anhydrous Na_2SO_4 was used by Akoto et al. (2013) in the clean-up of maize and cowpea extracts using 30 mL dichloromethane. Bempah et al. (2016) also used SPE column with a mixture of alumina and florisil. Authors who used the QuEChERS method for extraction also used QuEChERS dispersive solid phase extraction (dSPE) for clean-ups and this study will not be an exception.

2.15.4 Instrumentation

There are many methods that is being used and continually being revised and improved with new and conventional techniques to determine pesticide residues in fruits. The most widely used technique in the analysis of pesticide residues is Gas chromatography because of its high-resolution capacity and the availability of selective detectors in monitoring the pesticides (Fernandez, Pico & Manes, 2001). This technique has the ability to determine a significant number of pesticides and their compatibility in a wide range of food and environmental samples (Frost, 1996). Gas chromatography is also used because of its sensitivity and specific detection to determine several multiclass pesticides in one single analysis (Fernandez et al., 2001; & Sannino et al., 1996).

The main technique used for the determination of insecticide residues in environmental samples is the gas chromatography with electron capture (GC-ECD), mass spectrometer (GC-MS) or pulsed flame photometric (GC-PFPD) detectors. The GC-ECD is very sensitive to halogenated compounds and, therefore, used by many for the analysis of OCPs. The OPPs are normally analysed by GC-PFPD. Most of the SPs, which are of interest to Ghana, also contain halogens, making them easier to be analysed with GC-ECD. GC-MS was often used in the identification or confirmation of an insecticide. All authors used one or a combination of instruments mentioned above for their insecticide residue analysis. In this study the GC-ECD will be used to determine the organochlorines and the synthetic pyrethroids, GC-PFPD will be used for the organophosphates and the LC-MS will be used to determine other pesticides like ethephon, glyphosate amongst others.

Only Wylie et al. (2017) analysed pesticide metabolites using a modification of the SPE isotope dilution LC-MS/MS approach. GC analysis requires a thorough extraction and clean-up procedure, during which analytes may be lost. To ensure accuracy an internal standard is introduced before the extraction process to correct for these losses. The limit of detection (LOD) is the lowest concentration which can be differentiated from the noise, normally three times the noise level. Concentrations measured just above this level have a high degree of inaccuracy, up to 100%. The limit of quantitation (LOQ), normally ten times the noise level, provides a better accuracy, close to the desired 25% which is often used for the analysis of these compounds (Bogdal et al., 2013; Fiedler et al., 2013). The LOD for both OPPs and SPs was found to be around 0.010 mg/kg using GC-PFPD and GC-ECD, respectively (Bempah et al., 2011; Blankson et al., 2016; Okoffo et al., 2017).

2.15.5 Injection

In the analysis of insecticide residues in environmental samples, the preferred injection technique used is the split-splitless injection. Split injection is rarely used because only a small percentage of the sample is injected onto the column, which is a limitation because most insecticide residues in environmental matrixes are found at trace levels. With a split/splitless injection, the entire sample is transferred to the column. From the many articles reviewed, only a few researchers used split injection. On column injection, the third option with which also the entire sample is transferred to the column was not used at all, presumably because it is more complicated.

2.15.6 GC columns

Generally, the capillary columns used in the analysis of insecticide residues in environmental samples are non-polar, in most cases with a stationary phase

composition of 5%-phenylmethylpolysiloxane. The compositions of the stationary phase, length, diameter and thickness of the capillary column all have an influence on the separation. Often, a change in one column parameter enhances some features of column performance and reduces another. All these parameters should be optimized to produce the best separation within a satisfactory time period (Ong & Marriott, 2002; Bhardwaj, 2016). Capillary columns with lengths of 15 m – 60 m are typically used for insecticide residue work. This is because the longer a column, the better its resolution. Meanwhile from literature, the most commonly used capillary column length is 30 m. Due to this, this study will employ a 30 m + 10 m capillary column. Paré et al. (2014) however suggested that the use of a 60 m capillary column in insecticide residue analysis reduces the problem of co-elution. Due to the volatile properties of most insecticides, the best choice for film thickness is 0.25 µm for GC analysis and same will be used for this study. Lower film thicknesses offer lower column bleeding, shorter analysis times and sharper peaks. But too much reduction may cause analyte degradation. The internal diameter (id) of a capillary column has a stronger effect on its separation power than the length. Capillary columns with internal diameters (id) of 0.53mm, 0.32mm and 0.25mm internal diameter are commercially available for insecticide analysis. The 0.53 mm id, which is a wide bore, was used by Ntow (2001) (30 m × 0.53 mm id × 1.5 µm) for the analysis of organochlorines. Though less efficient compared to narrow bores like 0.25mm id, wide bores have a greater sample capacity. To enhance the sensitivity, narrow bores are generally used as they deliver sharper and higher peaks. With this observation, this study will employ a column of internal diameter, 0.25 mm id. Internal diameters of < 0.20mm normally require pressure regulators that can handle higher pressures. Gas flow and temperature also affect the separation capabilities of a capillary

column. When samples are introduced onto a column through the carrier gas depending on the temperature programing, individual components get separated depending on their boiling points, molecular size, polarity and their interaction with the stationary phase (Ong & Marriott, 2002; Bhardwaj, 2016).

Darko et al. (2007) employed an isothermal temperature at 160 °C with a SPB 15m × 0.5mm film column for the analysis of organochlorine pesticides. Most laboratories prefer temperature programing as they can manipulate the temperatures to achieve a perfect resolution. Temperature programming is also needed to make use of the solvent effect when using splitless injection (Ma štovská et al., 2001; Bogdal et al., 2013). Except for Ntow (2001), who started with an initial temperature of 150 °C, the initial temperature ranges for all the other works were from 60°C to 70°C with a final temperature range of 250 –310°C.

Insecticide residues in environmental samples are normally found at trace levels. It is therefore important to install measures to ensure that errors are eliminated or minimized so as to achieve reliable data. All techniques and activities that are undertaken to ensure accurate and reproducible results are known as quality assurance (Bhardwaj, 2016). The author reported the use of reference standards for calibration. These need to be stored under the correct temperature to avoid deterioration and subsequent false positive results. The use of certified reference materials (CRMs) from the right suppliers is also recommended. The use of CRMs e.g., biological materials or sediments, in Ghana has until now not been reported. These materials, e.g., produced by the European Joint Research Centre in Geel (Belgium), or the National Institute for Standards and Technology (NIST) in Gaithersburg (USA) are important to check the overall result of an analysis of

pesticides in vegetables, sediments or other matrices. Though the costs of such materials are modest, it seems most laboratories are unaware of the existence of such materials. Despite this limitation, blank samples of external standards will be used in this study to ensure quality assurance.

The United Nations Environmental Program (UNEP) under the Stockholm Convention organizes biennial interlaboratory assessment studies for POPs (Bogdal et al., 2013; Fiedler et al., 2013). In addition, UN Environment offers on-site training in the POP analysis for selected African laboratories, also such as Ghana, in order to improve their capacity for more efficient laboratory analysis. Since the analysis of environmental samples for insecticide residues is complex, recovery studies are very important for the accuracy of the method used. In this regard, all authors reported on their recovery experiments. To ensure the instrument can detect insecticide residues at low concentrations, the LOD and the LOQ need to be determined. This helps to check the instrument sensitivity and enable one to differentiate between the noise signal and actual sample signals. Only Bempah et al. (2016) reported on both the LOD and LOQ. Just as in this study, some reported only on the LOQ (Ntow, 2005; Ntow et al., 2007, 2008a, 2008b; Osei-Fosu et al., 2014; Fosu et al., 2017; Blankson et al., 2016) while some reported only on LOD (Ntow, 2001; Darko et al., 2008; Bempah et al., 2012; Akoto et al., 2013, 2015; Okoffo et al., 2017) and others were silent on both. This is partly because some authors contract the services of other laboratories in Ghana to do the analysis since they do not have the instrument and capacity (Amoah et al., 2006; Essumang et al., 2008).

2.16 Pesticide Residue Tolerances

A tolerance is the EPA established maximum residue level of a specific pesticide chemical that is permitted in or on a human or animal food in the United States. Residues are trace amounts of pesticide chemicals referred to as ―pesticide chemical residues" that may remain in or on food after applying the pesticide.

2.16.1 Maximum Residual Limits

According to Codex Alimentarius international food standards, a maximum residual limit (MRL) is the maximum concentration of a pesticide residue (expressed as mg/kg), to be legally permitted in or in food commodities and animal feeds. They are mostly used to ensure that the pesticides are only being used in accordance with good agricultural practices (GAP). The MRLs may differ for different countries due to differences in food consumption patterns and agricultural practices. In Ghana, there are no set maximum residual levels (MRLs) and therefore MRLs by international bodies such as the Codex Alimentarius Commission and acceptable daily intake (ADI) values established by the World Health Organization (WHO) are often used as bench marks. The ADI is the estimated amount of a chemical in food (mg/kg body weight d^{-1}) that can be ingested daily over a life time without appreciable health risk to the consumer (FAO, 2002). If a residue level exceeds the MRL, it could imply that the crop has not been grown according to good agricultural practice and so the product is not permitted to be sold, imported or exported. The residues of pesticides on crops are being monitored with respect to maximum residual limits and are based on analysis of a given residue remaining on food products.

The maximum residual limit is not a health-based exposure limit and hence exposure to residue in excess of an MRL does not necessarily imply a risk to health (Boobis et

al. 2008). Many studies conducted in Ghana (Aboagye, 2002; Bempah et.al, 2012; Koranteng et. al 2017) compared concentrations of pesticide residues to EU MRLs. This present study will also compare the pesticide residue concentrations to that of the EU MRLs. Other studies have also been conducted outside the country including a study conducted in Ivory Coast by Datte (2020) to quantify 30 commonly used pesticides (eg. metolachlor, parathion-methyl, chlorfenvinphos, diuron, Linuron, Aldicarb, terbutryn, atrazine, propazine, terbuthylazine) in real samples of pineapple juice. The data collected showed that 30% of the investigated pineapple juice samples were free of pesticides residues or had a level below Limit of Detection (<LOD), while 70% (21 samples) of the samples analyzed exceeded the Maximum Residue Levels (MRLs) set by the European Commission for Simazine, Metolachlor, Linuron and Aldicarb.

2.16.2 Acceptable Daily Intake and Acute Reference Dose

The toxicological reference values according to FAO (2021), used in dietary risk assessment are the Acceptable Daily Intake (ADI) and the Acute Reference Dose (ARfD).

The Acceptable Daily Intake (ADI) is the estimate of the amount of a pesticide in food or drinking-water that can be ingested daily over a lifetime without appreciable health risk to the consumer, on the basis of all the known facts at the time of the evaluation. It is expressed in milligrams of the pesticide per kilogram of body weight. The Acute Reference Dose (ARfD) on the other hand is the estimate of the amount of a pesticide in food or drinking-water that can be ingested over a period of 24 hours without appreciable health risk to the consumer, on the basis of all the known facts at

the time of the evaluation. It is also expressed in milligrams of the pesticide per kilogram of body weight.

Dietary intake of the pesticide is estimated by combining national or regional food consumption statistics with the estimated residues in food and/or drinking water. The consumer is considered to be adequately protected when estimated long-term and short-term dietary intake of pesticide residues does not exceed the acceptable daily intake (ADI) and the acute reference dose (ARfD), respectively.

CHAPTER THREE

METHODOLOGY

3.0 Overview

This chapter focuses on the study area of the research, sample collection points, laboratory analysis, sample preparation, extraction, extract purification, instrumental analysis and statistical analysis.

3.1 Study Area

Agyare (2010) noted that pineapple production in Ghana covers over 8000 acres of land and is predominant in the Greater Accra, Eastern, Central and Volta regions of the country. Winneba in the Central Region of Ghana was chosen as a study area because it is one of the major towns in the region with a population of about 55331 and also the major town closer to the areas where pineapple cultivation is high in the region. The town lies between latitude 5º21'00"N and longitudes 0º37'30"W. It has a total land area of 1,658.7 square kilometres. Fig. 1 is a map showing Winneba town and the sample collection points.

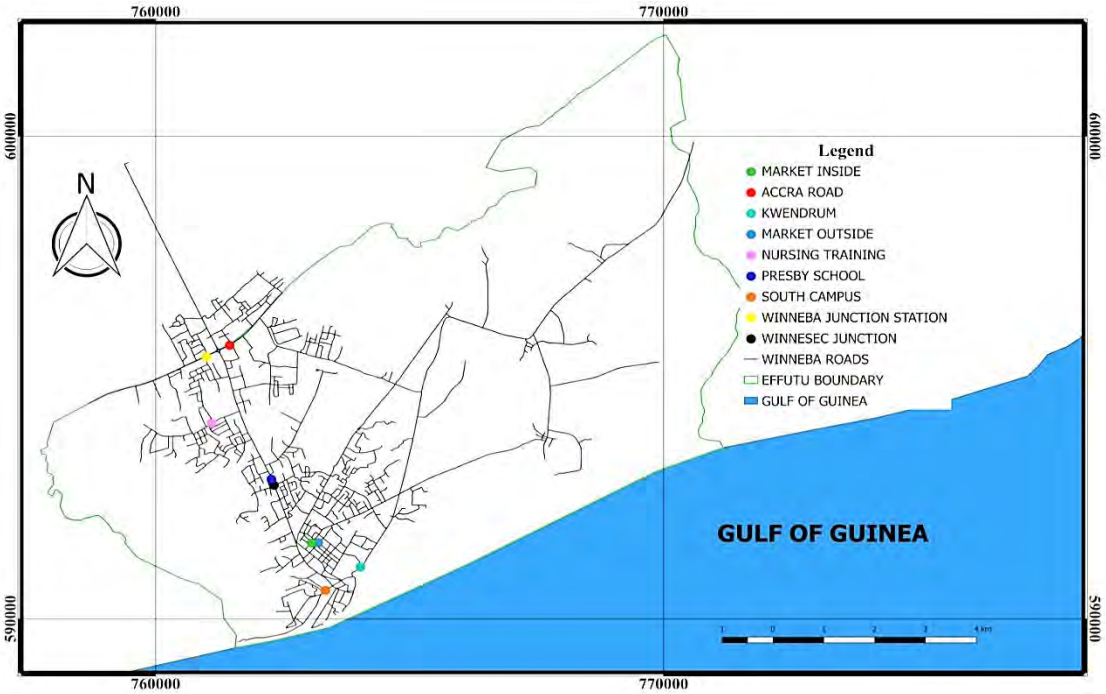


Figure 1: A location map of selected pineapple vendors in Winneba-Ghana.

3.2 Sample Collection

Fresh pineapples are available all year-round, with the peak period being between March and July. The pineapples were therefore bought from ten (10) sellers in Winneba during the month of March, 2021. These pineapples sellers were major pineapple sellers who buy their produce directly from the farmers. Codex Alimentarius (FAO/WHO, 1984) protocols for sampling and for the portions of commodities to be analysed were followed in the survey. Samples were put into boxes and labelled with a unique sample identity. The labelled samples were then transported to the laboratory and worked on immediately. A total of sixty (60) pieces of pineapples were obtained from the sellers.

Table 1: Sampling sites/points of Pineapples

Sample site/ points (Selling locations)	**Sample ID**	**Number of samples**
Winneba Junction	S1	6
Winneba Junction main station	S2	6
Trauma Hospital	S3	6
North Campus	S4	6
Winneba Secondary School Junction	S5	6
South Campus	S6	6
Yepemso Market A	S7	6
Yepemso Market B	S8	6
Royal Spot	S9	6
Winneba main market	S10	6
Total Number of Samples		**60**

3.3 Laboratory Analysis

Laboratory analysis was carried out at the Pesticide Residue laboratory of the Ghana Standards Authority (GSA), Shiashie- Accra.

3.4 Cleaning of Glasswares/ Equipment

Prior to the analysis, glass wares for analysis were washed with detergent and rinsed with tap water. They were further rinsed with acetone to remove organic residues from the glassware and dried overnight in an oven at 150ºC and stored in dust free cabinets until required.

3.5 Sample Preparation

The pineapples in each sample box were cut into pieces with a clean sharp knife on a clean wooden chopping board. It was then poured into the Foss Homogenizer -2096 and blended into a uniform mixture with equal concentration to obtain a homogenous representative sample. The knife, chopping board and homogenizer were washed thoroughly with detergent and rinsed under running water one sample after the other to avoid cross contamination.

3.6 Extraction for Organochlorines and Organophosphates

In this study the method used for extraction and cleanup for the organochlorine and the organophosphate insecticides was the QUECHERS method. 10g each of the comminuted homogenous sample was weighed into a 50ml centrifuge tube. 10ml acetonitrile was added and vortexed for one (1) minute after which a mixture of 4g magnesium sulphate anhydrous, 1g sodium chloride, 1g trisodium citrate dehydrate and 0.5g disodium hydrogencitrate sesquihydrate were also added and immediately vortexed for a further 1minute and centrifuged for 5minutes at 3500rpm.

3.7 Extract Purification

3.7.1 Dispersive Solvent Phase Extraction

Co-extracts were removed from the matrix as follows: 6ml aliquot of each extract was transferred into a 15ml centrifuge tube which contained 150mg PSA and 900mg magnesium sulphate. The tube was then closed and shook vigorously for 30seconds and centrifuged for 5minutes at 3000rpm.

4ml of the cleaned extract was transferred into a round bottom flask and the pH was quickly adjusted to ca.5 by adding 40µL of 5% formic acid solution in acetonitrile (v/v), and the filtrate was concentrated below 40℃ on the rotary evaporator just to dryness. The filtrate was redissolved in 1ml ethyl acetate by pipetting followed by 20µL of 1% poly ethylene glycol in ethyl acetate (v/v). The extract was finally transferred into a 2ml standard opening vial for quantitation by GC-ECD and GC-PFPD.

3.8 Gas Chromatographic Analysis of Residues

3.8.1 Instrument for Analysis of Organochlorine Pesticides

The residues were analyzed using a Varian CP- 3800 GC-ECD with a combiPAL Autosampler and 30m + 10m EZ guard × 0.25mm internal diameter fused silica capillary coated with VF-5ms (0.25µm film) from Varian Inc or equivalent analytical column. The temperatures used for the injector (operating in splitless mode), oven and Electron Capture Detector were 270℃; 70℃ / 2min, 180℃/ 1min, 300℃; and 300℃ respectively. The gas used was nitrogen (carrier) with a flow rate of 1 ml/min constant flow and make-up also with a flow rate of 29 ml/min.

3.8.2 Instrument for Analysis of Organophosphate Pesticides

Varian CP- 3800 GC-PFPD with a combiPAL Autosampler and 30m × 0.25mm internal diameter fused silica capillary coated with VF-1701ms (0.25µm film) from Varian Inc or equivalent analytical column were used for the analysis. The temperature for the injector operating in splitless mode, oven and Detector- PFPD were 270℃; 70℃ / 2min, 200℃/ 1min, 250℃ and 280℃ respectively. The gases used were nitrogen (carrier) 2 ml/min constant flow, Air 1 with flow rate of 17, H_2 with flow rate of 14 and Air 2 with flow rate of 10ml/min.

3.9 Liquid Chromatography Analysis of Residues

The method used for this extraction was the QuPPe method. 10g of each sample homogenate was weighed into 50ml centrifuge tube, after which 10ml methanol containing 1% formic acid was added. The mixture was then vortexed for 1minute and centrifuged for 5minutes at 3000rpm. The supernatant was withdrawn and filtered into a plastic auto sampler vial. It was then introduced into the LC-MS for analysis.

3.10 Instrument for LC-MS Analysis

The ultra-high-performance liquid chromatography, UHPLC – Agilent Infinity 1290 with a Quaternary pump coupled to an Agilent 6400 QQQ mass spectrometer was used together with the electrospray ionization (ESI) interface for the analysis in positive ionization mode.

3.11 Quality Control and Quality Assurance Measures

The efficiency of the method was determined by recoveries of an internally spiked sample. One sample was spiked with a 0.5ml of 1ppm external standard and extracted under the same conditions as the analytes. To check for interferences, a blank sample

containing no detectable pesticides was analyzed along with the samples under the same conditions.

3.12 Statistical Analyses

Inferential statistics was done by excel 2016 to determine the means and standard deviations of the residual levels. Analysis of variance (ANOVA) was used to determine the significance of the differences in the means of the experimental data of pesticide residues from the different sample sites. The statistical Differences were considered significant at $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

Under this chapter, the pesticide residues detected are presented and discussed, the concentrations of the residues are compared with maximum residual levels from the European Union database on pesticide residual limits (https://ec.europa.eu/food/plant/pesticides/eu-pesticidesdatabase/mrls/?event=search.pr), and the residual levels are then compared amongst the different sampling points to ascertain whether there is a significant difference.

4.1 Pesticide Residues Present in Samples

The pesticide residues found in each of the pineapple samples sold in Winneba are presented below.

4.1.1 Levels of Organochlorine and Synthetic Pyrethroid Insecticide Residues Found in Pineapples sold in Winneba

The concentrations of the various organochlorine and synthetic pyrethroids residues in each sample are calculated in mg/kg and presented in Table 2.

Table 2: Levels of Organochlorine and Synthetic Pyrethroid Residues in Pineapples.

PESTICIDES	SAMPLE POINTS									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Beta-HCH	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Gamma-HCH	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Delta-HCH	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Heptachlor	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Aldrin	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Allethrin	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Gamma - chlordane	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
α-endosulfan	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
p,p _– DDE	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Dieldrin	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Endrin	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
B-endosulfan	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
P,p _DDD	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
P,p _-DDT	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Endosulfan sulphate	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Bifenthrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fenpropathrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Methoxychlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Lambda-cyhalothrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Permethrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cyfluthrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cypermethrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fenvalerate	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Deltamethrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Source: Field data, 2021; LOQ of Organochlorines – 0.005mg/kg; LOQ of Synthetic Pyrethroids – 0.010mg/kg

4.1.2 Levels of Organophosphate Insecticide Residues Found in Pineapples sold in Winneba

The concentrations of the organophosphate insecticide residues in each sample are calculated in mg/kg and presented in Table 3.

Table 3: Levels of Organophosphate Insecticide Residues in Pineapples.

PESTICIDES	SAMPLE POINTS									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Methamidophos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Ethoprophos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diazinon	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fonofos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dimethoate	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pirimiphos - methyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Malathion	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fenitrothion	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Parathion – ethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorfenvinphos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Profenofos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Source: Field data, 2021; LOQ of Organophosphates – 0.01mg/kg

4.1.3 Levels of other Pesticide Residues Found in Pineapples Sold in Winneba

The concentrations of the other pesticide residues such as weedicides, fungicides and growth regulators found in each sample are calculated in mg/kg and presented in Tables 4, 5 and 6.

Table 4: Levels of Herbicides or Weedicides in Pineapples

PESTICIDES	SAMPLE POINTS									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Terbutryn	0.001	0.001	0.001	0.001	0.001	0.001	<0.001	0.001	0.001	0.001
Diuron	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.003	0.002
Nicosulfuron	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fluazifop	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Metolachlor	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Tebufenozide	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Aclonifen	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trifloxystrobin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Glyphosate	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.006	0.020	<0.001

Source: Field data, 2021; LOQ of Herbicides – 0.001mg/kg.

Table 5: Levels of Fungicides in Pineapples

PESTICIDES	SAMPLE POINTS									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Carbendazim	0.002	<0.001	<0.001	0.002	0.002	<0.001	0.002	0.002	0.002	0.002
Metalaxyl	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Source: Field data, 2021; LOQ of Fungicides – 0.001mg/kg.

Table 6: Levels of Growth Regulators in Pineapples

PESTICIDES	SAMPLE POINTS									
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Ethephon	0.008	0.005	0.003	0.008	<0.001	0.003	0.005	<0.001	<0.001	<0.001

Source: Field data, 2021; LOQ of Ethephon – 0.001mg/kg.

From Tables 2,3,4,5 and 6; the S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 are pineapple samples collected from different sellers in Winneba. There were no organochlorine, synthetic pyrethroid and organophosphate insecticide residues detected in the pineapple samples. The residual level of organochlorines, synthetic

pyrethroids and organophosphates in the pineapples were not quantified because their concentrations did not exceed the quantification limit of the method used.

There have however been evidences of organochlorine and organophosphate pesticides in selected commodities studied from urban markets and supermarkets in Greater Accra region of Ghana. For example, a study by Bempah et al. (2012) detected pesticides such as lindane, methoxychlor, aldrin, dieldrin, endrin, p,p‘-DDE, p,p‘-DDT, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, profenofos and malathion in papaya, watermelon, banana, mango, pear and pineapples. A similar research was conducted by Armah (2011) to determine the types of pesticides used by vegetables farmers in the cultivation of cabbage in Cape coast. It was reported that, out of the twenty-one-pesticide residues detected, nine (9) were pyrethroids and twelve (12) were organophosphates. This shows the presence of organochlorine pesticides despite the fact that they have been banned for a considerable amount of time in Ghana and therefore suggested the possibility of sporadic use of these pesticides for agriculture or mainly due to the past extensive use of these pesticides for agriculture in Ghana as it has been banned for over a decade ago.

These findings also corroborate the findings of a study conducted in China by Nakata et al. (2002) who found elevated levels of organochlorine pesticides residues in fruits and vegetables collected from Shanghai and Yixing. Similarly, in an investigation carried out by Hura (1999), by monitoring organochlorine residues in fruits and vegetables in Eastern Romania, it was concluded that organochlorine pesticides were found in all analyzed samples. Bempah et al. (2011a, b) have also reported high residue levels of permethrin, cyfluthrin, cypermethrin, fenvalerate and deltamethrin in pear, lettuce, watermelon, pineapple, carrot and onion.

However, the data from this study shows lower concentrations of the residues of organochlorine, pyrethroid and organophosphorus pesticides, in the analyzed samples. This might be due to its ability to degrade rapidly in the environment. Also, the results from this study with respect to the organophosphorus pesticides falls in line with the results obtained by Bempah et al. (2012) which showed that diazinon and malathion were the least predominant pesticide with residues of 0.007mg/kg and 0.006mg/kg in pineapple samples. In Ghana pesticides like aldrin, parathion and dieldrin have been banned because of their high toxicity and persistence in the environment that can produce residue problems in subsequent crops (Gerken *et al.,* 2001). Hence the absence of organochlorines in the samples could be because of the ban on the use of organochlorines in fruit and vegetable production. Armah (2011) and Bempah et al. (2011a, b) reported exceedingly high residual levels of pyrethroids pesticides in fruits and vegetables in Ghana and these results are not comparable to that of this study.

Also, six (6) other pesticides of which four (4) of them are weedicides/ herbicides, one of them is a fungicide and one (1) of them is also a growth regulator were found in the pineapples. This therefore shows that the farmers from whom the sellers buy their produce, use either one or more pesticides in their pineapple production. The detection of more herbicides could be attributed to the fact that the farmers want to reduce labour costs and also to cultivate larger acres. They used the right pesticides (Table 4) for the production of these pineapples with the exception of terbutryn which is not in the list of provisionally approved pesticides to be used on pineapples. It goes to confirm the study by Teisson (2000) that residue problems on pineapple do not only concern fungicides and ethephon, but also include fertilizers, herbicides, nematicides, and other plantation pesticide treatments.

Table 7 provides a list of pesticides provisionally approved for use on pineapples in Ghana.

Table 7: Provisionally approved pesticides for application on pineapple in Ghana.

Trade Name	Active ingredient	Type
Diuron	Diuron	Herbicide
Fusilade	Fluazifop-butyl	Herbicide
Roundup	Glyphosate	Herbicide
Dursban	Chlorpyrifos	Insecticide
Cypermethrin	Cypermethrin	Insecticide
Perfekthion	Dimethoate	Insecticide
Ridomil	Metalaxyl	Fungicide
Goldazim	Carbendazim	Fungicide
Ethrel	Ethephon	Growth regulator

Source: Suglo *et al.* (2001) cited in Aboagye, (2002).

4.2 Comparison of Levels of Detected Pesticide Residues with the EU MRLs.

The concentrations of the detected pesticide residues were compared with EU MRLs to determine whether they were above or below the maximum residual level. This is captured in Table 8.

Table 8: Detected Pesticides Residues in Pineapple Samples with Exceeding EU MRLs Values

Pesticides	Mean (mg/kg)	Standard Error	Minimum Concentration	Maximum Concentration	MRLs (mg/kg)
Ethephon	0.0032	0.00102	0	0.008	2
Carbendazim	0.0014	0.00031	0	0.002	0.1
Terbutryn	0.0009	0.0001	0	0.001	0.01
Diuron	0.0006	0.00034	0	0.003	0.01
Fluazifop	0.0001	0.0001	0	0.001	0.01
Glyphosate	0.003	0.00201	0	0.02	0.1

Source: Field data, 2021; (see MRLs in Appendix E)

All the organochlorine and organophosphate and synthetic pyrethiod insecticides recorded zero (0) concentrations from all the sample points. A research conducted by Akoto et al. (2013) revealed that among the organophosphorus pesticides investigated, ethoprophos and phorate were not detected in both the maize and cowpea samples whereas diazinon, pirimiphos-methyl and fonofos were detected only in the cowpea samples. The organophosphorus pesticide residues detected in both samples (maize and cowpea) however were below the prescribed EU MRL's. In contrast, similar work conducted in Nigeria by Ogah et al. (2011) reported significant concentration of diazinon (0.0278mg/kg), pirimiphos-methyl (0.0925 mg/kg) and chlorpyrifos (0.0982mg/kg) which were above their MRLs in beans (P. vulgaris). Again, organophosphorous pesticide residues such as ethoprophos (1.13544mg/kg), phorate (0.67820 mg/kg) and fenitrothion (0.16500mg/kg) were found in cabbage with their levels exceeding the EU-MRL in a study by Armah (2011).

A study conducted by Darko and Akoto (2008) on vegetables obtained from markets in Kumasi also show relatively high levels of dimethoate and malathion in tomatoes, eggplant and paper. Similarly, Akomea-Frempong et al. (2017) determined the concentrations of pesticides residues in ready-to-eat vegetables (salads) sold in Kumasi and found that the mean concentration of diazinon (0.040 $mgkg^{-1}$) in all the samples exceeded the EU MRLs (0.01 $mgkg^{-1}$) together with concentrations of chlorpyrifos, deltamethrin, fenvalerate, diazinon and permethrin which were all above their respective EU MRLs. Akoto et al. (2013) detected pyrethroid residues in the cowpea which were found to be lower than their respective EU-MRL.

For this study, Ethephon was detected in the samples bought from sample point 1, 2, 3, 4, 6 and 7 with concentrations of 0.008mg/kg, 0.005mg/kg, 0.003mg/kg,

0.008mg/kg, 0.003mg/kg and 0.005mg/kg respectively. The mean concentration of ethephon across all the sample points was 0.0032 ± 0.00102mg/kg. Carbendazim was also detected in samples from sample point 1, 4, 5, 7, 8, 9, 10 with concentrations of 0.002mg/kg, 0.002mg/kg, 0.002mg/kg, 0.002mg/kg, 0.002mg/kg, 0.002mg/kg and 0.002mg/kg respectively with a mean concentration of 0.0014 ± 0.00031mg/kg. Terbutryn was also detected in samples from all the sample points except sample point 7 and all with concentrations of 0.001mg/kg and a mean concentration of 0.0009 ± 0.0001mg/kg. Diuron was detected in samples from sample points 8, 9 and 10 with concentrations of 0.001mg/kg, 0.003mg/kg and 0.002mg/kg respectively. The mean concentration recorded for diuron was 0.0006 ± 0.00034mg/kg.

Fluazifop was detected in samples from sample point 9 at a concentration of 0.001mg/kg and a mean concentration of 0.0001 ± 0.0001mg/kg. Glyphosate was also detected in samples from sample point 6, 8 and 9 with concentrations of 0.004mg/kg, 0.006mg/kg and 0.020mg/kg respectively and its mean concentration was 0.003 ± 0.00201mg/kg. This indicates that the pineapple samples from all the various selling points in Winneba contained at least two residues but the various concentration of the pesticides detected were all below the EU MRL. This implies that the pineapples are safe for all consumer groups and even suitable for exportation. These results can be compared with studies by Acquaah and Darko (2007) who found lower concentrations of organochlorines in meat from the Kumasi and Buoho abattoirs as compared to the maximum limits set by FAO/WHO.

A similar study conducted in Nigeria by Adeyeye and Osibanjo (1999) found that residue levels of organochlorine pesticides in raw fruits, vegetables and tubers from markets were generally low and none were above the FAO‘s maximum residue limits.

Usman et al. (2009) sampled marketed fruits and vegetables from 62 Lahore, Pakistan and found that all had residue levels below the maximum residue limit (MRL) set by WHO. Again, Ripley et al. (2000) reported pesticide residue in cabbage and fruits in Canada; however, the levels were below the MRL's.

It is also worth noting that, Glyphosate recorded a value of 0.020mg/kg in one of the samples which is far above the concentrations of the other concentrations of pesticides detected (Table 5). Though most of the results from this study showed smaller concentration, it effects should however not be overlooked as Karalliedde et al. (2003) explained that, the effect of an insecticide on human health does not depend on the quantity of the insecticide accumulated but also on the duration and frequency of exposure and the health of the person at the time of the exposure.

4.3 Comparison of the Residual Levels of the Pineapples Collected from the Different Sampling points in Winneba (Ho).

The residual levels of pesticides detected were compared across the different sampling points (sellers) to determine whether there was a statistical difference between the mean concentrations from the different locations and this is presented in Figure 2.

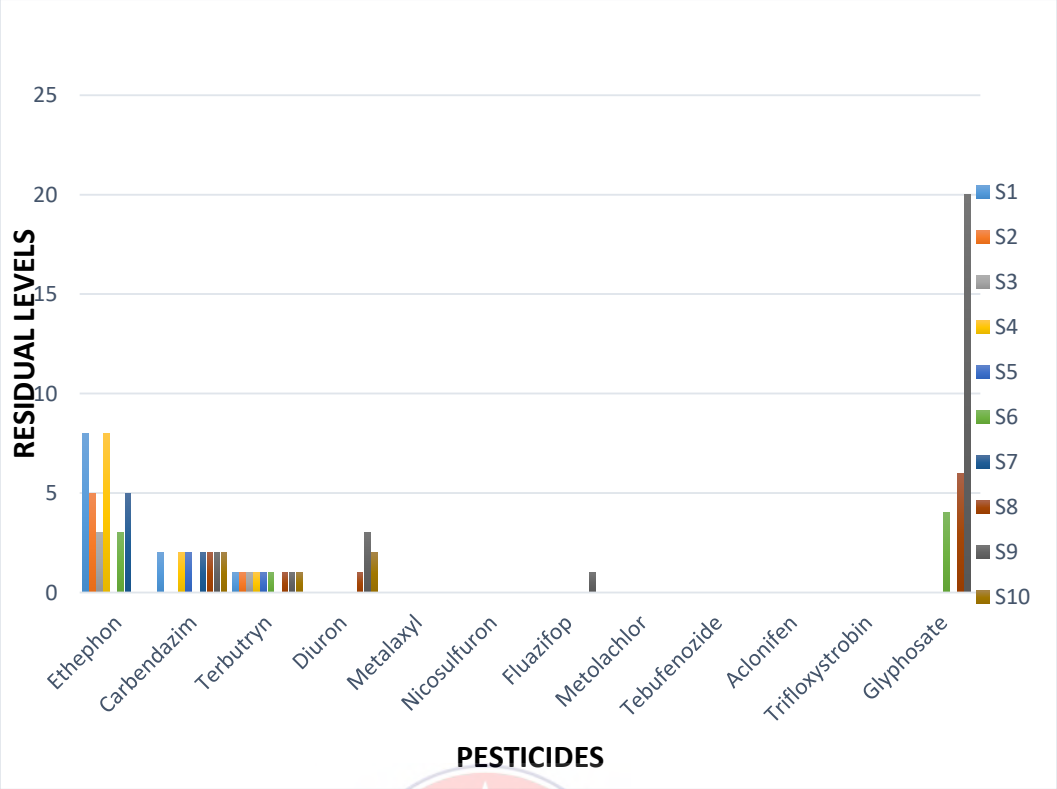


Figure 2: Residual Levels of Pesticides in Pineapples from the Different Sample points.

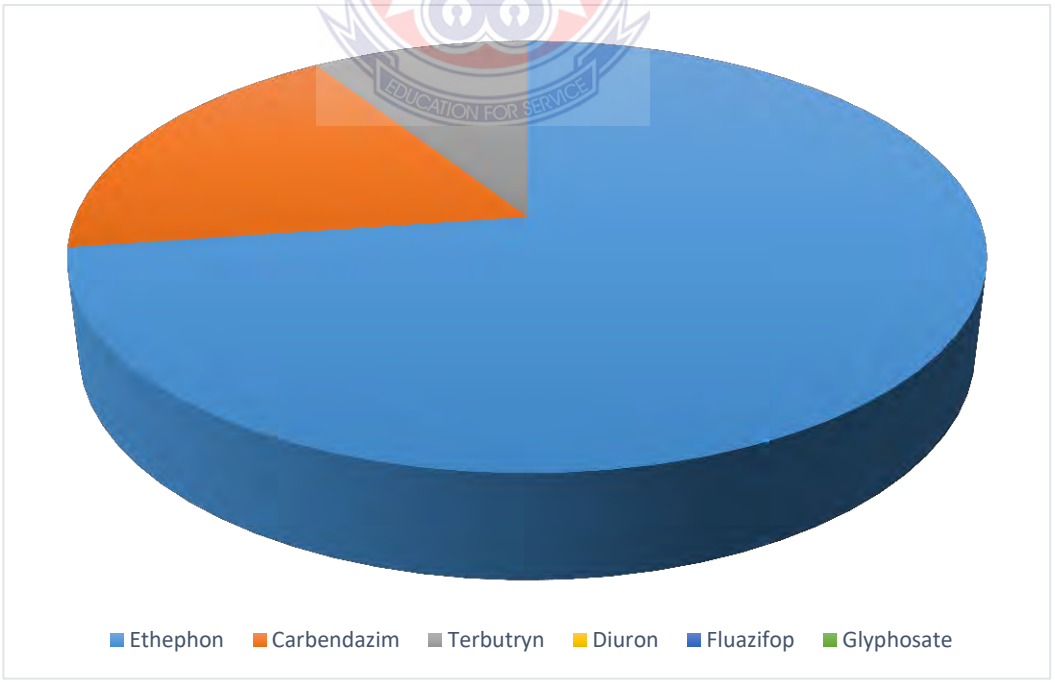


Figure 3: Concentrations of pesticide residues in pineapples from sample point 1.

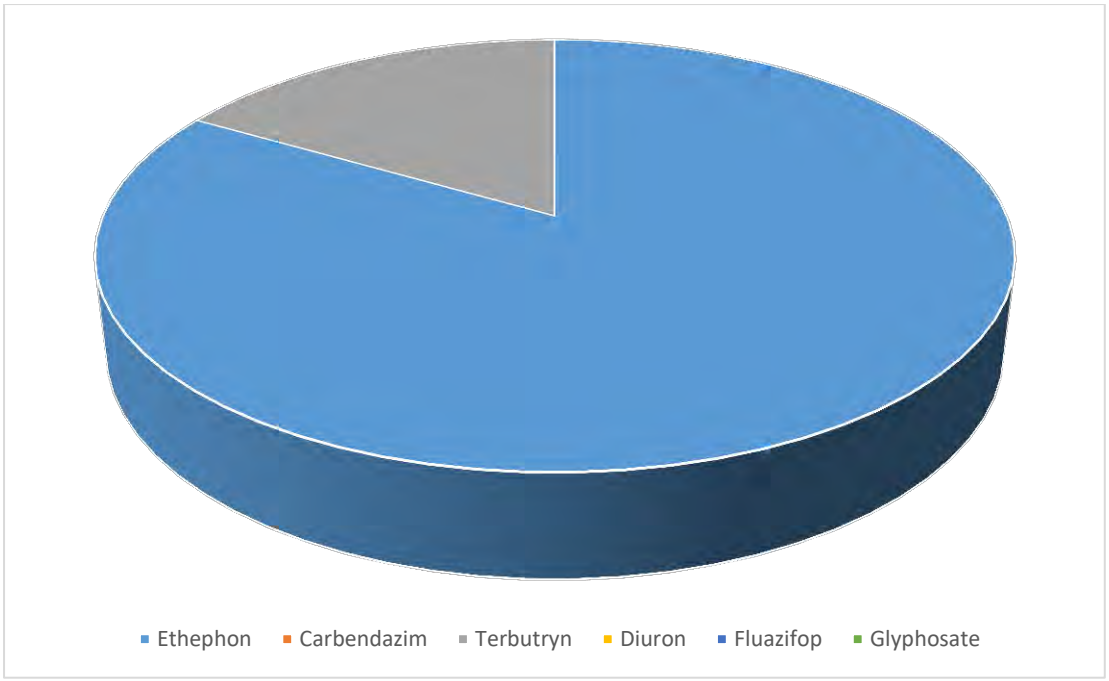


Figure 4: Concentrations of pesticide residues in pineapples from sample point 2.

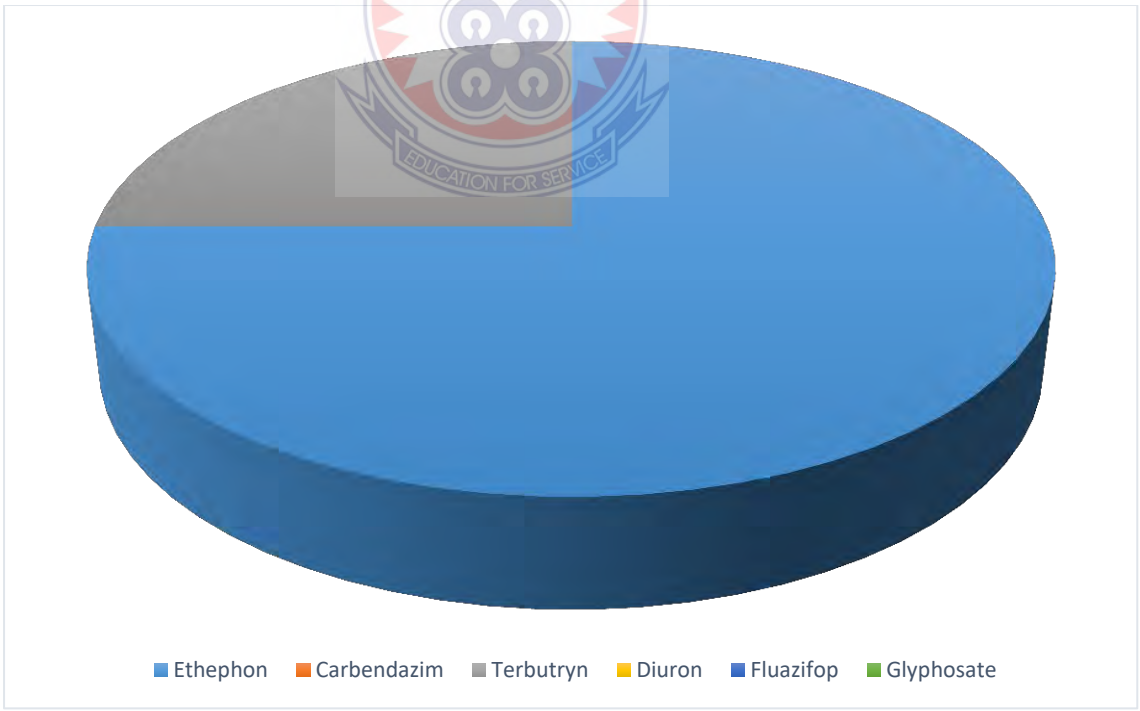


Figure 5: Concentrations of pesticide residues in pineapples from sample point 3.

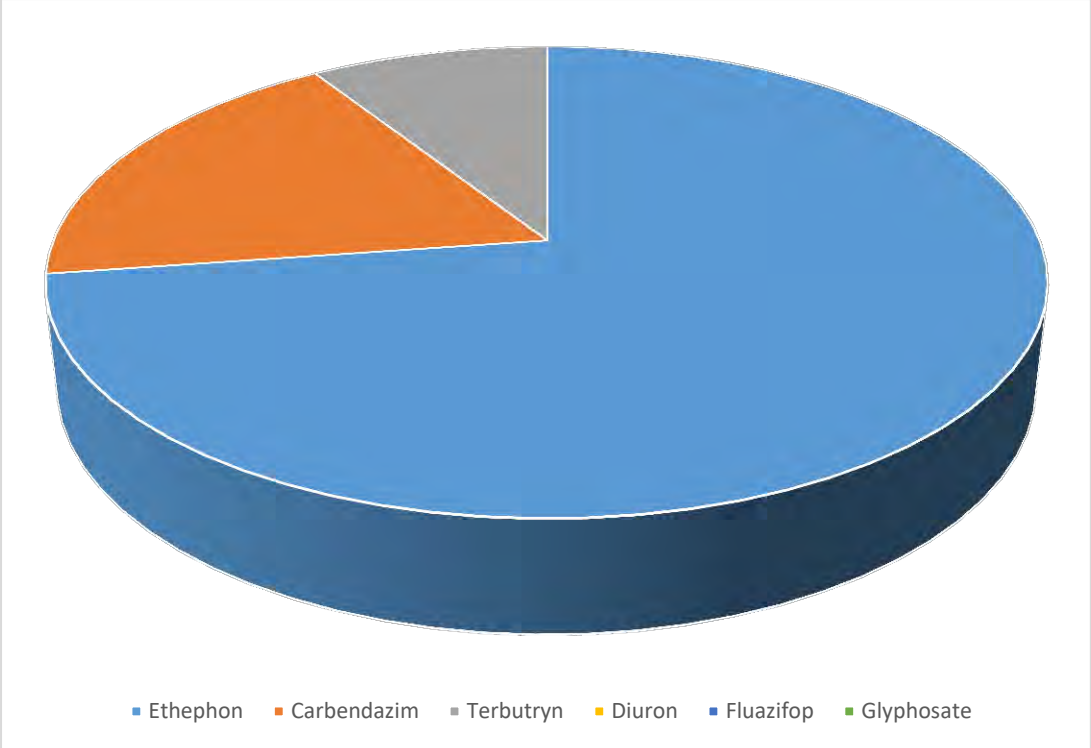


Figure 6: Concentrations of pesticide residues in pineapples from sample point 4.

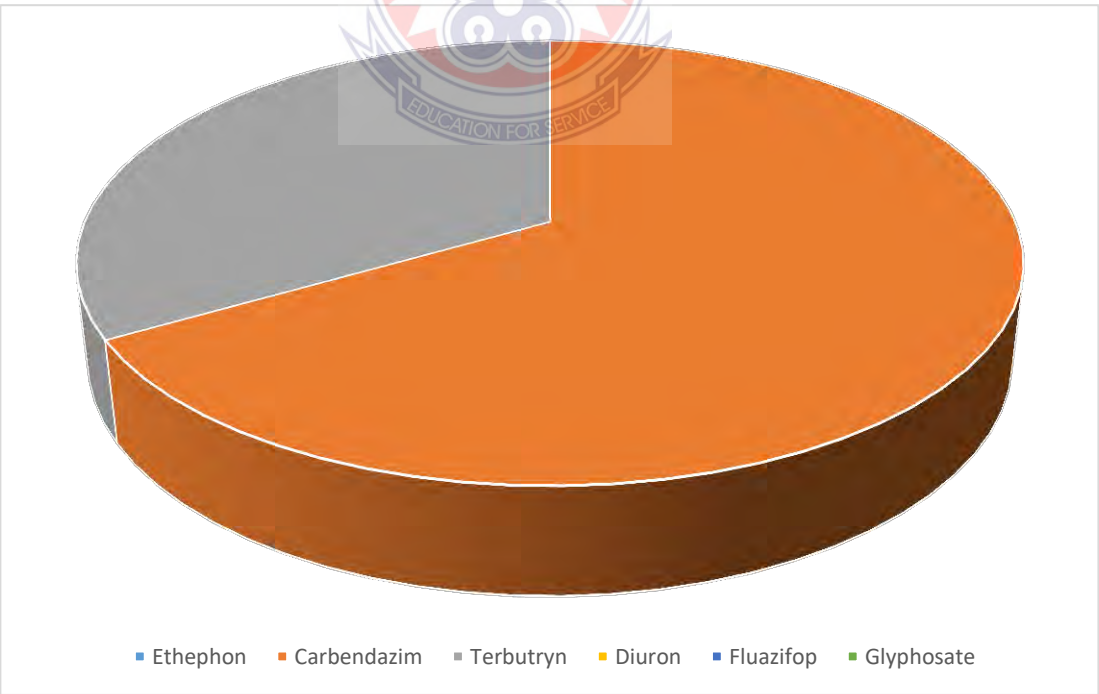


Figure 7: Concentrations of pesticide residues in pineapples from sample point 5.

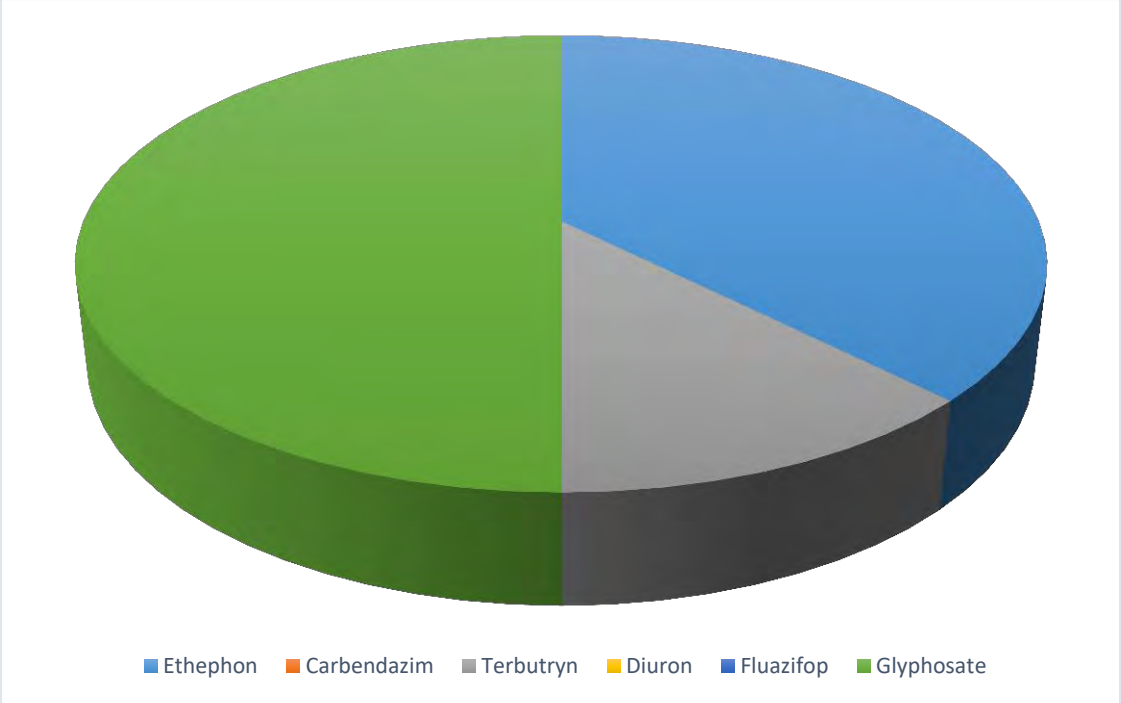


Figure 8: Concentrations of pesticide residues in pineapples from sample point 6.

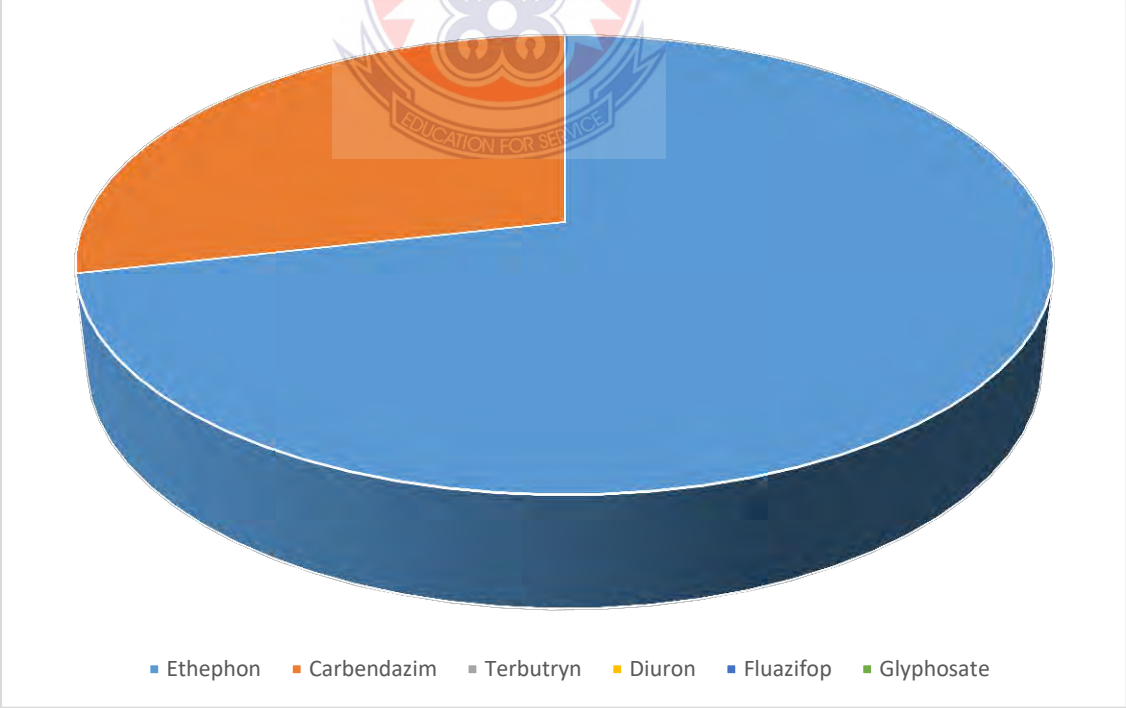


Figure 9: Concentrations of pesticide residues in pineapples from sample point 7.

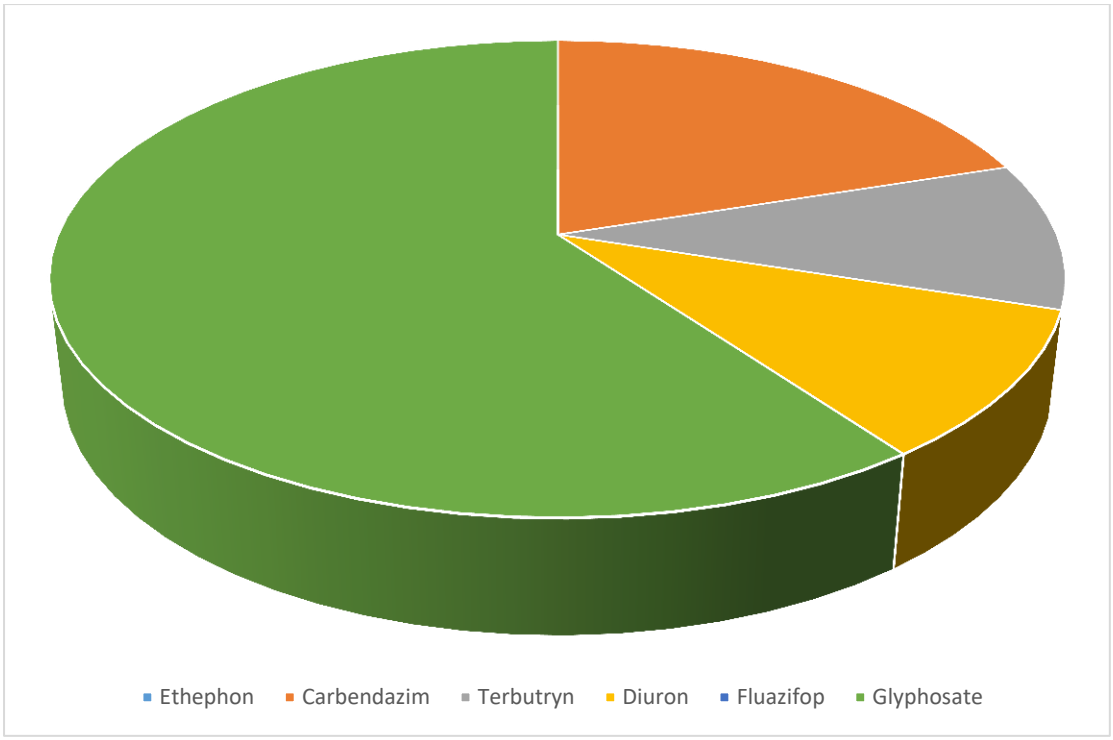


Figure 10: Concentrations of pesticide residues in pineapples from sample point 8.

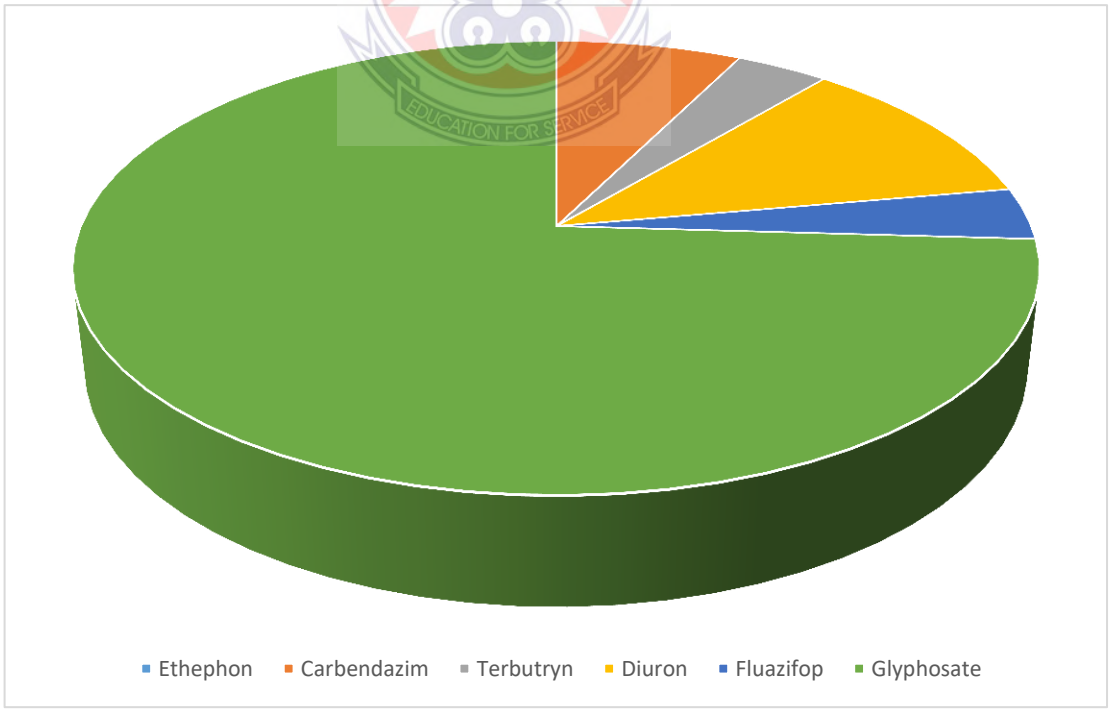


Figure 11: Concentrations of pesticide residues in pineapples from sample point 9.

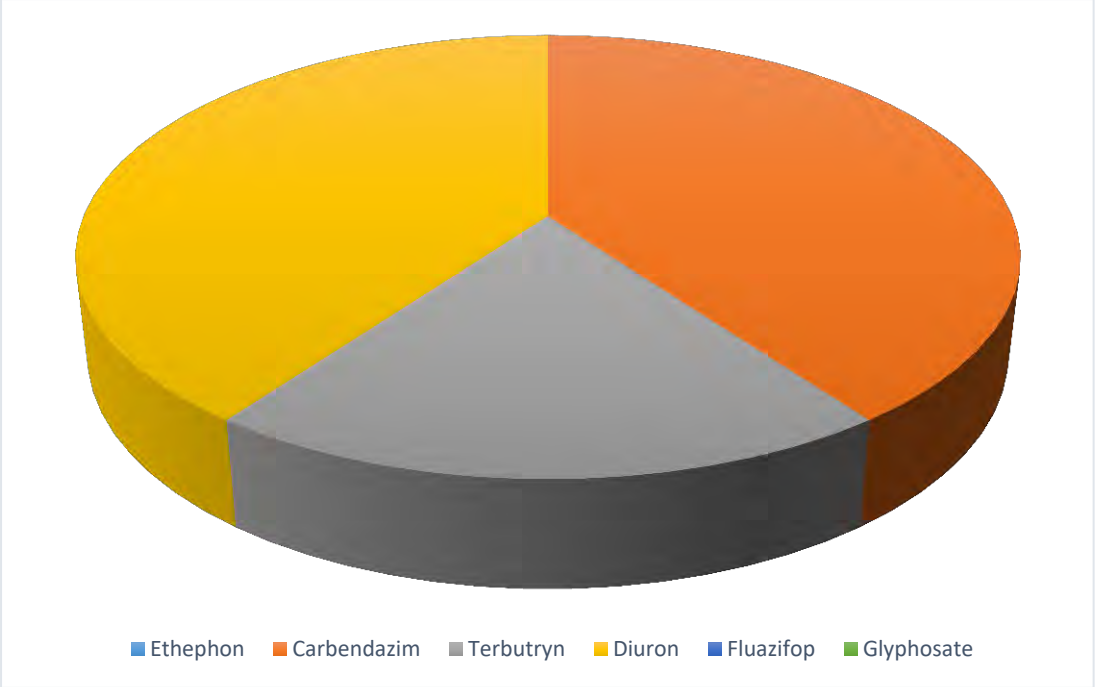


Figure 12: Concentrations of pesticide residues in pineapples from sample point 10.

Tables 9, 10 and 11 show the different herbicides, fungicides and growth regulators and their concentrations (mg/kg) detected in pineapples sold at different sample locations in Winneba.

Table 9: Detection of Herbicides / Weedicides in Pineapples

PESTICIDES	**SAMPLE POINTS**									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Terbutryn	0.001	0.001	0.001	0.001	0.001	0.001	ND	0.001	0.001	0.001
Diuron	ND	ND	ND	ND	ND	ND	ND	0.001	0.003	0.002
Fluazifop	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND
Glyphosate	ND	ND	ND	ND	ND	0.004	ND	0.006	0.020	ND

ND: Not detected; below the quantification limit of the method used.

Table 10: Detection of Fungicides in Pineapples

PESTICIDES	SAMPLE POINTS									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Carbendazim	0.002	ND	ND	0.002	0.002	ND	0.002	0.002	0.002	0.002
Metalaxyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not detected; below the quantification limit of the method used.

Table 11: Detection of Growth regulators in Pineapples

PESTICIDES	SAMPLE POINTS									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Ethephon	0.008	0.005	0.003	0.008	ND	0.003	0.005	ND	ND	ND

ND: Not detected; below the quantification limit of the method used.

From Figure 3, samples from sample point 1 recorded concentration of Ethephon (0.008mg/kg), carbendazim (0.002mg/kg) and terbutryn (0.001mg/kg). In Figure 4, samples from sample point 2 recorded concentrations of ethephon (0.005mg/kg) and terbutryn (0.001mg/kg). From sample point 3, the detected pesticide residues were ethephon (0.003mg/kg) and terbutryn (0.001mg/kg) as presented in Figure 5. Figure 6 represents the pesticides detected in samples from sample point 4 which recorded concentrations of ethephon (0.008mg/kg), carbendazim (0.002mg/kg) and terbutryn (0.001mg/kg). Samples from sample point 5 recorded concentrations of carbendazim and terbutryn but in small concentrations of 0.002mg/kg and 0.001mg/kg respectively, Figure 7. Samples from sample point 6, as presented in Figure 8 recorded concentrations of ethephon (0.003mg/kg), terbutryn (0.001mg/kg) and glyphosate (0.004mg/kg). Samples from sample point 7 recorded concentrations of

ethephon (0.005mg/kg) and carbendazim (0.002mg/kg) as represented in Figure 9. From Figure 10, samples from sample point 8 recorded concentrations of carbendazim (0.002mg/kg), terbutryn (0.001mg/kg) and diuron (0.001mg/kg) whiles from Figure 11, samples from sample point 9 recorded concentrations of carbendazim (0.002mg/kg), terbutryn (0.001mg/kg), diuron (0.003mg/kg), fluazifop (0.001mg/kg) and glyphosate (0.020mg/kg). Samples from sample point 10 recorded concentrations of carbendazim (0.002mg/kg), terbutryn (0.001mg/kg) and diuron (0.002mg/kg) as represented in Figure 12.

From tables 9 and 10 samples from sample point 9 recorded five different pesticides out of the total of six pesticides that were detected in the samples representing the highest number of residues. It also recorded the highest residue concentration of 0.020mg/kg (glyphosate), Table 9. Samples from sample point 5 had the least level of concentrations which were 0.001mg/kg (terbutryn) and 0.002mg/kg (carbendazim), Tables 9 and 10. The samples from all the sample points recorded at least two pesticide residues.

Similarly, Baker et al. (2002), analyzed pesticide residue data to compare the differences between conventionally grown, integrated pest management (IPM)-grown and organically grown foods. Based on the data collected, the IPM/NDR category, had residues higher than those in organic samples but lower than those in conventionally grown foods. It is however important to note that, Baker et al. (2002) based their comparison on three different market categories of food (conventionally grown, integrated pest management (IPM)-grown/no detectable residues (NDR), and organically grown) and compared using data from three test programmes whereas this study did the comparison based on ten different fruit selling points and compared

using their various pesticide residual levels. Again, Lari et al. (2014) conducted a study to compare the pesticide residues in water bodies. The data collected showed that, higher concentrations of Organochlorine and Organophosphates were found in surface water and hence it was concluded that as compared to ground water, Surface water samples are usually more contaminated.

Statistical analysis showed no significant difference in the mean concentrations of all the detected pesticide residues among the varieties with ($p > 0.05$) as shown in Appendices A and B. This could be because the peels of the pineapples limit the loss of substances from the fruits' internal tissues, protects the fruits against physical, chemical, and biological attacks and protects the fruits against the external environment while the fruit is on the plant as well as after harvest (Antonio et al., 2005). Also, because the pineapple peel acts as a protective shield, that prevents the diffusion of the fungicide and other pesticides (Cabrera et al., 2000). Comparing the different detected groups of pesticides by location with respect to Tables (9,10 and 11), it shows that statistically, there were no significant differences in the mean concentrations of all the herbicide and fungicide residues among the sampling locations.

CHAPTER FIVE

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.0 Overview

In this chapter, the summary of findings is outlined, conclusions and suggestions are made and recommendations are made for further studies.

5.1 Summary of Findings

The study focused on forty-eight (48) pesticides; twenty-four (24) representing 50% of them were organochlorine insecticides analyzed with GC-ECD, twelve (12) representing 25% organophosphate insecticides were analyzed with GC-PFPD and another twelve representing 25% herbicide and growth regulators were analyzed with LC-MS. All the pineapple farmers used pesticides either for field application or post-harvest treatment of the fruits. However, there was no record of farmers using banned pesticides in the study area. Samples from some of the selling points recorded higher levels than others. The residual levels of the pesticides however, were all below EU Maximum Residual Levels.

5.2 Conclusions

The absence of organochlorine, pyrethroid and organophosphate pesticide residues in the samples shows that farmers adhere strictly to the rules governing the safe use of pesticides. Basic reasons for the restricted use of these pesticides could be attributed to the awareness that have been created about the use of banned pesticides in Ghana and their non-availability in the market. However, the small concentrations do not mean that their presence in the pineapples should be completely ignored and taken for granted since they may pose a threat to human health if accumulated for a long time with inappropriate measures. Besides, consumers may also be exposed to other

sources such as vegetables, drinking water, fish, meat and other dairy products such as milk and hence will suffer the cumulative effect of these ubiquitous insecticides.

Finally, the concentrations of the pesticides identified are generally low and below Maximum Residual Levels, hence it can be concluded that the pineapples sold in Winneba are safe for consumption.

5.3 Recommendations

Plant protection and regulatory services division under the Ministry of Food and Agriculture in Winneba and its environs must ensure that farmers are given more training to use pesticides that have low human risk and are environmentally friendly.

Farmers in Winneba and its environs should also be encouraged to consult the nearest extension officer when they are in doubt about the use of a particular pesticide since the use of some pesticides can be withdrawn as they are being replaced by new recommended ones.

Regular monitoring should be done by agricultural extension officers to ensure that farmers use the pesticides only when it is required and that the right pesticides are used in their right proportions. Also, regular monitoring is needed to evaluate human health risk and to identify pest that have developed resistance to any pesticide.

Increasing the rate of studies on the cultivation of variety of fruits in the various agro-climatic regions of Ghana would help limit the risk of human exposure to pesticides in food consumption.

Rules and policies on the import and sale of pesticides in the country must be strengthened and all importers and dealers must be properly trained before being permitted to trade in the sale of pesticides.

REFERENCES

Aboagye, E. (2002). *Patterns of pesticide use and residue levels in exportable pineapple (Ananas comosus L. Merr.)*. Doctoral dissertation, University of Ghana.

Abutiate, W. S. (1991). Guide to the commercial production of pineapple. Papaya and mango in Ghana. Accra, Ghana. *Food Control, 68*, 174–180.

Acquaah, S. & Darko, G. (2007). Levels of organochlorine pesticide residues in meat. *Int. J. Environ. Sci. Tech*., 4 (4), 521-524.

Adeyeye, A., & Osibanjo, O. (1999). Residues of organochlorine pesticides in fruits, vegetables and tubers from Nigerian markets. *Science of the Total Environment*, *231*(2-3), 227-233.

Afreh-Nuamah, K. (2016). High Levels of some insecticides on cabbage samples (Brassica Oleracea L. Var Capitata) from rural and urban farms in Ghana. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). 10,* 97-103.

Agyare, E. A. (2010). Technology for improved income of small-holder pineapple producers in Ghana. *SNV Netherlands Development Organization Case Studies*.

Agyekum, A. A., Ayernor, G. S., Saalia, F. K., & Bediako-Amoa, B. (2015). Translocation of pesticide residues in tomato, mango and pineapple fruits. *Journal of Food Science and Engineering*, *5*, 142-149.

Ahmad, L., Khan, A., Khan, M. Z., Hussain, I., Mahmood, F., Sleemi, M. K. & Abdullah, I. (2012). Toxico-pathological effects of cypermethrin upon male reproductive system in rabbits. *Pesticide Biochemistry and Physiology*, *103*(3), 194-201.

Ahmed, F. E. (2001). Analyses of pesticides and their metabolites in foods and drinks. *TrAC Trends in Analytical Chemistry*, *20*(11), 649-661.

Akomea-Frempong, S., Ofosu, I. W., Owusu-Ansah, E. D. G. J., & Darko, G. (2017). Health risks due to consumption of pesticides in ready-to-eat vegetables (salads) in Kumasi, Ghana. *International Journal of Food Contamination*, *4*(1), 1-11.

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

Akoto, O., Gavor, S., Appah, M. K., & Apau, J. (2015a). Estimation of human health risk associated with the consumption of pesticide-contaminated vegetables from Kumasi, Ghana. *Environmental Monitoring and Assessment*, *187*(5), 1-9.

Akoto, O., Oppong-Otoo, J., & Osei-Fosu, P. (2015b). Carcinogenic and non-carcinogenic risk of organochlorine pesticide residues in processed cereal-based complementary foods for infants and young children in Ghana. *Chemosphere*, *132*, 193-199.

Amoah, P., Drechsel, P., Abaidoo, R. C., & Ntow, W. J. (2006). Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Archives of environmental contamination and toxicology*, *50*(1), 1-6.

Anderson, H. A., & Wolff, M. S. (2000). Environmental contaminants in human milk. *Journal of Exposure Science & Environmental Epidemiology*, *10*(6), 755-760.

Antonio, J., Matas, G. P., Jesu´s, C. and Antonio, H. (2005). Relative humidity and temperature modify the mechanical properties of isolated tomato fruit cuticles. *American Journal of Botany, 92*(3), 462–468.

Armah, F. A. (2011). Assessment of pesticide residues in vegetables at the farm gate: Cabbage (Brassica oleracea) cultivation in Cape Coast, Ghana. *Research Journal of Environmental Toxicology*, *5*(3), 180-202.

Awumbila, B., & Bokuma, E. (1994). Survey of pesticides used in the control of ectoparasites of farm animals in Ghana. *Tropical animal health and production*, *26*(1), 7-12.

Baah, G. G. (2016). *Concentrations of Organochlorine Insecticide residues in selected vegetables in the Sunyani West District of the Brong Ahafo Region of Ghana* (Doctoral dissertation), University of Ghana, Legon.

Baker, B. P., Benbrook, C. M., Iii, E. G., & Benbrook, K. L. (2002). Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets. *Food Additives & Contaminants*, *19*(5), 427-446.

Barriada-Pereira, M.,Gonzalez-Castro, M. J.,Muniategue- Lorenzo, S., Lopez-Mahia, P., Prada-Rodriguez, D., & Fernandez-Fernandez, E. (2005). Organochlorine pesticide accumulation and degradation products in vegetation samples of a contamination area in Galicia (NW Spain). *Chemosphere, 58*, 1571–1578.

Bartolomé, A. P., Rupérez, P., & Fúster, C. (1995). Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars. *Food Chemistry*, *53*(1), 75-79.

Bempah, C. K., & Donkor, A. K. (2011). Pesticide residues in fruits at the market level in Accra Metropolis, Ghana, a preliminary study. *Environmental monitoring and assessment*, *175*(1), 551-561.

Bempah, C. K., Asomaning, J. K., Ansong, D. A., Boateng, J., & Asabere, S. B. (2012). Contamination levels of selected organochlorine and organophosphorous pesticides in Ghanaian fruits and vegetables. *Emirates Journal of Food and Agriculture*, 293-301.

Bempah, C. K., Asomaning, J., & Boateng, J. (2021). Market basket survey for some pesticides residues in fruits and vegetables from Ghana. *Journal of Microbiology, Biotechnology and Food Sciences*, *2021*, 850-871.

Bempah, C. K., Buah-Kwofie, A., Denutsui, D., Asomaning, J., & Tutu, A. O. (2011a). Monitoring of pesticide residues in fruits and vegetables and related health risk assessment in Kumasi Metropolis, Ghana. *Research Journal of Environmental and Earth Sciences*, *3*(6), 761-771.

Bempah, C. K., Buah-Kwofie, A., Enimil, E., Blewu, B., & Agyei-Martey, G. (2012). Residues of organochlorine pesticides in vegetables marketed in Greater Accra Region of Ghana. *Food control*, *25*(2), 537-542.

Bempah, C. K., Donkor, A., Agyei, R., Buah-Kwofie, A., & Boateng, J. (2011). Monitoring of organochlorine pesticide residues in locally produced fruit-based soft drinks in Ghana. *Internet Journal of Food Safety*, *13*, 315-320.

Bempah, C.K., Agyekum, A.A., Akuamoa, F., Frimpong, S., & Buah-Kwofie, A. (2016). Dietary exposure to chlorinated pesticide residues in fruits and vegetables from Ghanaian markets. *J Food Compos Anal, 46*, 103–13.

Bhardwaj, S. K., Dwivedi, K., & Agarwal, D. D. (2016). A review: GC method development and validation. *Int. J. Anal. Bioanal. Chem*, *6*(1), 1-7.

Biziuk, M. (2001) *Pestycydy, Występowanie, Oznaczanie i Unieszkodliwiani*, WNT, Warszawa.

Blankson, G.K., Osei-Fosu, P., Adeendze, E.A., Ashie, D. (2016). Contamination levels of organophosphorus and synthetic pyrethroid pesticides in vegetables marketed in Accra, Ghana. *Food Control, 68*, 174-180,

Bodirsky, B. L., Rolinski, S., Biewald, A., Weindl, I., Popp, A., & Lotze-Campen, H. (2015). Global food demand scenarios for the 21st century. *PloS one*, *10*(11), 139-201.

Bogdal, C., Scheringer, M., Abad, E., Abalos, M., Van Bavel, B., Hagberg, J., & Fiedler, H. (2013). Worldwide distribution of persistent organic pollutants in air, including results of air monitoring by passive air sampling in five continents. *TrAC Trends in Analytical Chemistry*, *46*, 150-161.

Boobis, A. R., Ossendorp, B. C., Banasiak, U., Hamey, P. Y., Sebestyen, I., & Moretto, A. (2008). Cumulative risk assessment of pesticide residues in food. *Toxicology letters*, *180*(2), 137-150.

Botchway, F. (2000). Analysis of pesticide residues in Ghana's exportable cocoa. *A Higher certificate project submitted to Institute of Science and Technology, London, UK*, 44-45.

Botwe, B. O., Ntow, W. J., Kelderman, P., Drechsel, P., Carboo, D., Nartey, V. K., & Gijzen, H. J. (2011). Pesticide residues contamination of vegetables and their public health implications in Ghana. *Journal of Environmental Issues and Agriculture in Developing Countries*, *3*(2), 10-18.

Buchel, K. (1983). *Chemistry of pesticides.* New York, USA: John Wiley & sons, Inc.

Bull, D. (1982). *A growing problem: Pesticides and the Third World poor*. Oxfam.

Cabrera, H. A. P., Menezes, H. C., Oliveira, J. V., & Batista, R. F. S. (2000). Evaluation of residual levels of benomyl, methyl parathion, diuron, and vamidothion in pineapple pulp and bagasse (Smooth cayenne). *Journal of Agricultural and Food Chemistry*, *48*(11), 5750-5753.

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environmental Science & Policy*, *9*(7-8), 685-692.

Chapple, I. L., Milward, M. R., Ling-Mountford, N., Weston, P., Carter, K., Askey, K., & Matthews, J. B. (2012). Adjunctive daily supplementation with encapsulated fruit, vegetable and berry juice powder concentrates and clinical periodontal outcomes: A double-blind RCT. *Journal of Clinical Periodontology*, *39*(1), 62-72.

Cieslik, S., Tuovinen, J., Baumgarten, M., Matyssek, R., Brito, P., & Wieser, G. (2013). Gaseous exchange between forests and the atmosphere. *Dev. Environ. Sci., 13*, 19-36.

Claeys, W. L., Schmit, J. F., Bragard, C., Maghuin-Rogister, G., Pussemier, L., & Schiffers, B. (2011). Exposure of several Belgian consumer groups to pesticide residues through fresh fruit and vegetable consumption. *Food Control*, *22*(3-4), 508-516.

Clarke, E. E. K., Levy, L. S., Spurgeon, A., & Calvert, I. A. (1997). The problems associated with pesticide use by irrigation workers in Ghana. *Occupational Medicine*, *47*(5), 301-308.

Colovic, M. B., Krstic, D. Z., Lazarevic-Pasti, T. D., Bondzic, A. M., & Vasic, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Current Neuropharmacology*, *11*(3), 315-335.

Cudjoe A. R., Kyofa-Boamah M. & Braun M. (2002). –Selected Fruit Crops (Mango, Papaya and Pineapple),” Handbook of Crop Protection Recommended in Ghana: An IPM Approach. *Ministry of Food and Agriculture/Plant Protection and Regulatory Services Director-ate/GTZ, Accra-Ghana, 4*, 60-63.

Damgaard, I. N., Skakkebæk, N. E., Toppari, J., Virtanen, H. E., Shen, H., Schramm, K. W., ... & Nordic Cryptorchidism Study Group. (2006). Persistent pesticides in human breast milk and cryptorchidism. *Environmental Health Perspectives, 114*(7), 1133-1138.

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

Darko, G., & Akoto, O. (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food and Chemical Toxicology*, *46*(12), 3703-3706.

Darko, G., Akoto, O., & Oppong, C. (2008). Persistent organochlorine pesticide residues in fish, sediments and water from Lake Bosomtwi, Ghana. *Chemosphere*, *72*(1), 21-24.

Datte, A. A. Y. E., Paul, G. U., Jean-Marie, K. K., Gains, K. K. K., Henri, B. G., & Lisette, G. H. A. M. (2020). Academic Journal of Chemistry.

De Castro, M. L., & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of chromatography A, 1217*(16), 2383-2389.

Debjit, B., Chandira, R. M., Jayakar, B., & Kumar, K. P. S. (2009). Recent trends of drug used treatment of tuberculosis. *Journal of Chemical and Pharmaceutical Research*, *1*(1), 113-133.

Dinham, B. (2003). Growing vegetables in developing countries for local urban populations and export markets: problems confronting small-scale producers. *Pest Management Science*, *59*(5), 575-582.

Donkor, A., Osei-Fosu, P., Dubey, B., Kingsford-Adaboh, R., Ziwu, C., & Asante, I. (2016). Pesticide residues in fruits and vegetables in Ghana: A review. *Environmental Science and Pollution Research*, *23*(19), 18966-18987.

Dorey, E., Fournier, P., Lechaudel, M., & Tixier, P. (2015). Validity of the pineapple crop model SIMPIÑA across the climatic gradient in Réunion Island. *European Journal of Agronomy*, *62*, 1-12.

Echeverría-Sáenz, S., Mena, F., Pinnock, M., Ruepert, C., Solano, K., De la Cruz, E., & Barata, C. (2012). Environmental hazards of pesticides from pineapple crop production in the Río Jiménez watershed (Caribbean Coast, Costa Rica). *Science of the Total Environment*, *440*, 106-114.

Environmental Protection Agency (EPA) Ghana. (2013). Revised List of pesticides (active ingredients) registered in Ghana as of 30th June 2013.

Escuderos-Morenas, M. L., Santos-Delgado, M. J., Rubio-Barroso, S., & Polo-D ez, L. M. (2003). Direct determination of monolinuron, linuron and chlorbromuron residues in potato samples by gas chromatography with nitrogen–phosphorus detection. *Journal of Chromatography A*, *1011*(1-2), 143-153.

Essumang, D. K., Dodoo, D. K., Adokoh, C. K., & Fumador, E. A. (2008). Analysis of some pesticide residues in tomatoes in Ghana. *Human and Ecological Risk Assessment*, *14*(4), 796-806.

FAO, 2002. Plant production and protection paper 56. Pesticide Residues in food. Rep. Joint meeting on pesticide residues held in Geneva, *Rome 4*(4), 12–20.

FAOSTAT (2015). FAOSTAT Database. Available at: http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/∗/E (Retrieved on April 15, 2021).

Fernandez, M., Pico, Y., & Manes, J. (2001). Pesticide residues in oranges from Valencia (Spain). *Food Additives & Contaminants*, *18*(7), 615-624.

Fianko, J. R., Donkor, A., Lowor, S. T., & Yeboah, P. O. (2011). Agrochemicals and the Ghanaian environment, a review. *Journal of Environmental Protection*, *2*(03), 221.

Fiedler, H., Abad, E., Van Bavel, B., de Boer, J., Bogdal, C., & Malisch, R. (2013). The need for capacity building and first results for the Stockholm Convention Global Monitoring Plan. *TrAC Trends in Analytical Chemistry*, *46*, 72-84.

Fosu, P. O., Donkor, A., Ziwu, C., Dubey, B., Kingsford-Adaboh, R., Asante, I., & Nazzah, N. (2017). Surveillance of pesticide residues in fruits and vegetables from Accra Metropolis markets, Ghana, 2010–2012: a case study in Sub-Saharan Africa. *Environmental Science and Pollution Research*, *24*(20), 17187-17205.

Frost, B.A. (1996). Pesticide analysis: Selecting techniques for analyzing pesticides in fruits and vegetables. *Environnemental Lab. Magazine T004*, Waters Chromatographic Division, Millipore, Milford, Mass, USA.

Furlani, R. P. Z., Marcilio, K. M., Leme, F. M., & Tfouni, S. A. V. (2011). Analysis of pesticide residues in sugarcane juice using QuEChERS sample preparation and gas chromatography with electron capture detection. *Food Chemistry*, *126*(3), 1283-1287.

Gattuso, D. J. (2000). Understanding the Benefits of Pesticides. *Consumers' Research Magazine*, *83*(2), 34-35.

Gerken, A., Suglo, J. V., & Braun, M. (2001). Pesticides use and policies in Ghana. *Pesticide Policy Project, Publication series*, *10*, 90.

Giacomazzi, S., & Cochet, N. (2004). Environmental impact of diuron transformation: A review. *Chemosphere*, *56*(11), 1021-1032.

Gilbert-López, B., Garcia-Reyes, J. F., & Molina-Díaz, A. (2009). Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. *Talanta*, *79*(2), 109-128.

Gooddy, D. C., Chilton, P. J., & Harrison, I. (2002). A field study to assess the degradation and transport of diuron and its metabolites in a calcareous soil. *Science of the Total Environment*, *297*(1-3), 67-83.

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N. & Straif, K. (2015). International Agency for Research on Cancer Monograph Working Group ILF. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol*, *16*(5), 490-491.

Hajšlová, J., & Zrostl kova, J. (2003). Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. *Journal of Chromatography A*, *1000*(1-2), 181-197.

Hardy, B. (1995). Integrated Pest management. *International Potato Centre (CIP) Circular 21*(3), 1-23.

Hernández, F., Sancho, J. V., Pozo, O., Lara, A., & Pitarch, E. (2001). Rapid direct determination of pesticides and metabolites in environmental water samples at sub-µg/l level by on-line solid-phase extraction-liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A*, *939*(1-2), 1-11.

Hidaka, M., Nagata, M., Kawano, Y., Sekiya, H., Kai, H., Yamasaki, K., & Arimori, K. (2008). Inhibitory effects of fruit juices on cytochrome P450 2C9 activity in vitro. *Bioscience, Biotechnology, and Biochemistry*, *72*(2), 406-411.

Hjorth, K., Johansen, K., Holen, B., Andersson, A., Christensen, H. B., Siivinen, K., & Toome, M. (2011). Pesticide residues in fruits and vegetables from South America–A Nordic project. *Food Control*, *22*(11), 1701-1706.

Horbowicz, M., Sempruch, C., Kosson, R., Koczkodaj, D., & Walas, D. (2013). Effect of fluazifop-p-butyl treatment on pigments and polyamines level within tissues of non-target maize plants. *Pesticide Biochemistry and Physiology*, *107*(1), 78-85.

Hura, C., Leanca, M., Rusu, L., & Hura, B. A. (1999). Risk assessment of pollution with pesticides in food in the Eastern Romania area (1996–1997). *Toxicology Letters, 107*(1-3), 103-107.

Jokanović, M. (2001). _Biotransformation of organophosphorus compounds‘. *Toxicology, 166*(3), 139–160.

Jokanović, M. (2009). _Medical treatment of acute poisoning with organophosphorus and carbamate pesticides‘. *Toxicology Letters, 190*(2), 107–115.

Joy, P. P. (2010). Benefits and uses of pineapple. *Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686*, *670*.

Joy, P. P., Anjan, R., & Soumya, K. K. (2012). Insect Pests of Pineapple and Their Management. *Pineapple Research Station, Vazkhulam, Muvattupuzha, India*.

Joy, P.P., & Sindhu G. (2012). Diseases of pineapple (Ananas Comosus): Pathogen systems, infection, Spread and Management. *Pineapple Research Station, Kerala Agricultural University, Muvattupuzha, Ernakulam Vazhakulam, 686*(670).

Kamrin, M. A. (1997). *Pesticide profiles: Toxicity, environmental impact, and fate*. CRC press.

Kannan, K. C., Tanabe, S. Giesy, J. P. & Tatsukawa, R. (1997). Organochlorine Pesticides and Polychlorinated Biphenyls in Food Stuffs from Asian and Oceanic Countries. *Rev. Environ. Contam Toxicology* 152: 1- 55.

Karallieddde, L.D., Edwards, P. & Marrs, T.C (2003). Variables influencing the toxic response to organophosphates. *Food and Chemical Toxicology*. 41: 1-13

Kidd, H., & James, D.R. (1991). "The agrochemicals handbook"@eng. *Cambridge (UK) Royal Society of Chemistry.*

Kleemann, L. (2011). *Organic pineapple farming in Ghana: A good choice for smallholders?* (No. 1671). Kiel Working Paper.

Koranteng, S. S., Darko, D. A., Ameka, G. K., & Owusu, E. O. (2017). Residues and risk assessment of organochlorine pesticides in surface sediment of Afram River of Ghana. *Int J Res Chem Environ*, *7*(1), 30-37.

Kotey, D. A., Gbewonyo, W. S. K., & Afreh-Nuamah, K. (2008). High chlorpyrifos levels on vegetables in Ghana. *Pesticide News*, *80*, 1-6.

Krauthacker, B., Romanica, S. H., & Reiner, E. (2001). Polychlorinated biphenyls and organochlorine pesticides in vegetation samples collected in Croatia. *Bulletin of Environmental Contamination and Toxicology, 66*, 334–341.

Kuranchie-Mensah, H., Atiemo, S. M., Palm, L. M. N. D., Blankson-Arthur, S., Tutu, A. O., & Fosu, P. (2012). Determination of organochlorine pesticide residue in sediment and water from the Densu river basin, Ghana. *Chemosphere*, *86*(3), 286-292.

Kyofa-Boamah, M., & Blay, E. (2000). A Study on Pineapple Production and Protection Procedure in Ghana. *Ministry of Food and Agriculture, Plant Protection and Regulatory Services Directorate, Accra, Ghana*.

Lari, S. Z., Khan, N. A., Gandhi, K. N., Meshram, T. S., & Thacker, N. P. (2014). Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. *Journal of Environmental Health Science and Engineering*, *12*(1), 1-7.

Loeillet, D. (2013). Le marché mondial de l'ananas. *Fruitrop*, *215*, 32-39.

Louchart, X., Voltz, M., & Andrieux, P. (2000). Temporal variation in the availability of diuron to transport by overland flow. *Comptes Rendus de l'Academie des Sciences Series IIA Earth and Planetary Science*, 475-481.

Maia, L. C. B., Maia, V. M., Lima, M. H. M., Aspiazú, I., & Pegoraro, R. F. (2012). Growth, production and quality of pineapple in response to herbicide use. *Revista Brasileira de Fruticultura*, *34*, 799-805.

Malato, S., Blanco, J., Cáceres, J., Fernández-Alba, A. R., Agüera, A., & Rodr guez, A. (2002). Photocatalytic treatment of water-soluble pesticides by photo-Fenton and TiO2 using solar energy. *Catalysis Today*, *76*(2-4), 209-220.

Martin, H. (1968). Pesticide manual. *Pesticide manual.*

Maštovská, K., Hajšlová, J., Godula, M., Křivánková, J., & Kocourek, V. (2001). Fast temperature programming in routine analysis of multiple pesticide residues in food matrices. *Journal of Chromatography A*, *907*(1-2), 235-245.

Mawuenyegah, G. K. (1994). *The life and toxicity of insecticide residues applied to cabbage in the farm* (Doctoral dissertation, Dissertation) University of Ghana.

McDaniel, K. L., & Moser, V. C. (1993). Utility of a neurobehavioral screening battery for differentiating the effects of two pyrethroids, permethrin and cypermethrin. *Neurotoxicology and Teratology*, *15*(2), 71-83.

Mensah, F. O., Yeboah, F. A., & Akman, M. (2004). Survey of the effects of aerosol pesticide usage on the health of farmers in the Akomadan and Afrancho farming community. *Journal of the Ghana Science Association*, *6*(2), 44-48.

Metcalf, R.L. (1975). Insecticide pest management. In: Metcalf RL, Luckmann WH (eds) *Introduction to insect pest management*. John Wiley and Sons, New York, London, Sydney, Toronto, pp. 235–277

Ministry of Agriculture (1990). Ghana medium term agricultural development programme (MTADP): An agenda for sustainable agricultural growth and development. *Directorate General for Agriculture, 70,* 35-95.

Moreno, J. F., Liébanas, F. A., Frenich, A. G., & Vidal, J. M. (2006). Evaluation of different sample treatments for determining pesticide residues in fat vegetable matrices like avocado by low-pressure gas chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1111*(1), 97-105.

Morton, J. (1999). Pineapple. Fruits of warm climates (Julia F. Morton. Ed.). *Creative Resources System Inc., Miami, FL.33*,189. 18-28.

Nakata, H., Kawazoe, M., Arizono, K., Abe, S., Kitano, T., Shimada, H., & Ding, X. (2002). Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from China: status of contamination, historical trend, and human dietary exposure. *Archives of Environmental Contamination and Toxicology*, *43*(4), 0473-0480.

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 3082, Dimethoate. Retrieved May 19, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Dimethoate.

National Research Council. (2000). National Academy of Sciences. 1993. Pesticides in the Diets of Infants and children. *National Academy Press, Washington, DC, 184,* 185.

Newsom, L., Smith, R., & Whitcomb, W. (1976). Selective pesticides and selective use of pesticides. *In Theory and practice of biological control*, 565-591

Ninsin, K. D. (1997). *Insecticide use patterns and residue levels on cabbage, Brassica olerecea var. capitata L., cultivated within the Accra-Tema metropolitan area of Ghana* (Doctoral dissertation), University of Ghana.

Ntow, W. J. (2005). Pesticide residues in Volta Lake, Ghana. *Lakes & Reservoirs: Research & Management*, *10*(4), 243-248.

Ntow, W. J., Ameyibor, J., Kelderman, P., Drechsel, P., & Gijzen, H. J. (2007). Dissipation of endosulfan in field-grown tomato (Lycopersicon esculentum) and cropped soil at Akumadan, Ghana. *Journal of agricultural and food chemistry*, *55*(26), 10864-10871.

Ntow, W. J., Drechsel, P., Botwe, B. O., Kelderman, P., & Gijzen, H. J. (2008a). The impact of agricultural runoff on the quality of two streams in vegetable farm areas in Ghana. *Journal of environmental quality*, *37*(2), 696-703.

Ntow, W. J., Gijzen, H. J., Kelderman, P., & Drechsel, P. (2006). Farmer perceptions and pesticide use practices in vegetable production in Ghana. *Pest Management Science: formerly Pesticide Science*, *62*(4), 356-365.

Ntow, W. J., Tagoe, L. M., Drechsel, P., Kelderman, P., Gijzen, H. J., & Nyarko, E. (2008). Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. *Environmental research*, *106*(1), 17-26.

Ntow, W. J., Tagoe, L. M., Drechsel, P., Kelderman, P., Nyarko, E., & Gijzen, H. J. (2009). Occupational exposure to pesticides: Blood cholinesterase activity in a farming community in Ghana. *Archives of Environmental Contamination and Toxicology*, *56*(3), 623-630.

Ntow, W.J (2001). Organochlorine pesticide in water, sediments, crops and human fluids in a farming community in Ghana. *Archives of Environmental Contamination and Toxicology: 557*-563.

Ogah, C. O., Coker, H. B., & Adepoju-Bello, A. A. (2011). Organophosphate and carbamate pesticide residues in beans from markets in Lagos State, Nigeria. *Journal of Innovative Research in Engineering and Science*, *2*(1), 50-61.

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), 1-11.

Okoffo, E. D., Fosu-Mensah, B. Y., & Gordon, C. (2017). Contamination levels of organophosphorus and synthetic pyrethroid pesticides in cocoa beans from Ghana. *Food control*, *73*, 1371-1378.

Ong, R. C., & Marriott, P. J. (2002). A review of basic concepts in comprehensive two-dimensional gas chromatography. *Journal of chromatographic science*, *40*(5), 276-291.

Orlando, L. (2017). The Amazing Pineapple, Hawaii's Natural Health Booster, 2006. *Published online at: http://www. buzzle. com/editorials/4–2–2006–92514. asp (Retrieved on May, 20, 2021)*.

Osei-Fosu, P., Donkor, A. K., Nyarko, S., Nazzah, N. K., Asante, I. K., Kingsford-Adabo, R., & Arkorful, N. A. (2014). Monitoring of pesticide residues of five notable vegetables at Agbogbloshie market in Accra, Ghana. *Environmental monitoring and assessment*, *186*(11), 7157-7163.

Pandjaitan, M., Nugraha, T., & Pamudja, K. H. (2014). Bromelain enzyme in fresh pineapple juice as a healing pathway for HIV/AIDS. *Advanced Science, Engineering and Medicine*, *6*(1), 119-123.

Paré, S., Kaboré, B., Stechert, C., Kolb, M., Bahadir, M., & Bonzi-Coulibaly, L. Y. (2014). Agricultural practice and pesticide residues in soils and pool sediments from the Pendjari biosphere reserve area in Benin, West Africa. *Clean–Soil, Air, Water*, *42*(11), 1593-1603.

Park, D. S., Peterson, C., Zhao, S., & Coats, J. R. (2004). Fumigation toxicity of volatile natural and synthetic cyanohydrins to stored-product pests and activity as soil fumigants. *Pest Management Science: formerly Pesticide Science*, *60*(8), 833-838.

Pinon, A. (2000). Residues in pineapple from West Africa on arrival in Europe. *Fruitrop,* 74, 15-17.

Prasad, R., Upadhyay, N., & Kumar, V. (2013). Simultaneous determination of seven carbamate pesticide residues in gram, wheat, lentil, soybean, fenugreek leaves and apple matrices. *Microchemical Journal*, *111*, 91-96.

Proshad, R., Islam, M. S., Islam, M. N., Hossain, M. R., Kormoker, T., & Billah, K. M. M. (2017). Promiscuous application of toxic agrochemicals on pineapple: health hazard implications in Bangladesh. *Food Research*,2(2),139-145

PS (Processing Studies) (1997). (Appendix E). Commission of the European Communities. *J. Food Compos. Anal., 46,* 103–113.

Rajendran, B., & Subramanian, A. N. (1997). Chlorinated pesticide residues in water from River Kaveri, South India. *Chemistry and Ecology, 13*, 225–236.

Rathore, H. S., & Nollet, L. M. (Eds.). (2012). *Pesticides: evaluation of environmental pollution*. CRC press.

Rawn, D. F. K., Roscoe, V., Krakalovich, T., & Hanson, C. (2004). N-methyl carbamate concentrations and dietary intake estimates for apple and grape juices available on the retail market in Canada. *Food Additives and Contaminants*, *21*(6), 555-563.

Rawn, D. F., Quade, S. C., Shields, J. B., Conca, G., Sun, W. F., Lacroix, G. M., ... & Bélanger, A. (2006). Organophosphate levels in apple composites and individual apples from a treated Canadian orchard. *Journal of Agricultural and Food Chemistry*, *54*(5), 1943-1948.

Ripley, B. D., Lissemore, L. I., Leishman, P. D., Denommé, M. A., & Ritter, L. (2000). Pesticide residues on fruits and vegetables from Ontario, Canada, 1991–1995. *Journal of AOAC International*, *83*(1), 196-213.

Różański, L. (1998) *Przemiany Pestycydów w Organizmach Zywych i Srodowisku*, Agra-enviro Lab, Poznań.

Sannino, A., Mambriani, P., Bandini, M., & Bolzoni, L. (1996). Multiresidue method for determination of organochlorine insecticides and polychlorinated biphenyl congeners in fatty processed foods. *Journal of AOAC International*, *79*(6), 1434-1446.

Santos, F. J., & Galceran, M. T. (2002). The application of gas chromatography to environmental analysis. *TrAC Trends in Analytical Chemistry*, *21*(9-10), 672-685.

Singal, S., Kumud, M. & Thakral, S. (2012). Application of apple as ripening agent for banana. *Indian Journal of Natural Products and Resources*, *3*(1), 61-64.

Soderlund, D. M. (2010). Toxicology and mode of action of pyrethroid insecticides. In *Hayes' handbook of pesticide toxicology* (1665-1686). Academic Press.

Stocka, J., Tankiewicz, M., Biziuk, M., & Namieśnik, J. (2011). Green aspects of techniques for the determination of currently used pesticides in environmental samples. *International Journal of Molecular Sciences*, *12*(11), 7785-7805.

Stringer, W. J. (1997). Herbicides For Small Scale Forestry Operations. Department of Forestry Fact Sheet FORFS 99-9.

Teisson, C. (2000). Research and evolution of the pineapple industry. *Fruitrop, 74,* 17-19.

Teixeira, L. A. J., Quaggio, J. A., Cantarella, H., & Mellis, E. V. (2011). Potassium fertilization for pineapple: effects on plant growth and fruit yield. *Revista Brasileira de Fruticultura*, *33*(2), 618-626.

Thurman, E. M., Bastian, K. C., & Mollhagen, T. (2000). Occurrence of cotton herbicides and insecticides in playa lakes of the High Plains of West Texas. *Science of the Total Environment*, *248*(2-3), 189-200.

Twum, B. T. (2011). *Concentration of organochlorine insecticide residues in tomato (lycopersicon esculentum) fruit: A case study at Akumadan in the Offinso North District of Ashanti Region* (Doctoral dissertation).www.ir.knust.edu.gh.

ur Rahman, H. U., Asghar, W., Nazir, W., Sandhu, M. A., Ahmed, A., & Khalid, N. (2020). A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies. *Science of the Total Environment*, 142-649.

Usman, M., Tahir, S.I.N., Salma, R. and Shahzad, M. (2009): A Quantitative Analysis for the Toxic Pesticide Residues in marketed fruits and vegetables in Lahore, Pakistan. D: *Biomedica, 25,* 171 – 174.

Uysal-Pala, C., & Bilisli, A. (2006). Fate of endosulfan and deltamethrin residues during tomato paste production. *Journal of central European agriculture, 7(*2), 343-348.

Wang, Z., & Paré, J. J. (1997). Gas chromatography (GC): Principles and applications. In *Techniques and Instrumentation in Analytical Chemistry* (Vol. 18, pp. 61-91). Elsevier.

Watts, M. (2012). Chlorpyrifos as a possible global persistent organic pollutant. *Pesticide Action Network North America, Oakland*.

Wesseling, C., McConnell, R., Partanen, T., & Hogstedt, C. (1997). Agricultural pesticide use in developing countries: health effects and research needs. *International journal of health services*, *27*(2), 273-308.

Wessels, J. S., & Van Der Veen, R. (1956). The action of some derivatives of phenylurethan and of 3-phenyl-1, 1-dimethylurea on the Hill reaction. *Biochimica et biophysica acta*, *19*(3), 548–549.

Willoughby, I., Evans, H., Gibbs, J., Pepper, H., Gregory, S., Dewar, J., & Trout, R. (2004). *Reducing pesticide use in forestry: practice guide.* Handbook of Crop Protection Recommended in Ghana.

Wylie, B. J., Ae-Ngibise, K. A., Boamah, E. A., Mujtaba, M., Messerlian, C., Hauser, R., ... & Asante, K. P. (2017). Urinary concentrations of insecticide and herbicide metabolites among pregnant women in rural Ghana: A pilot study. *International journal of environmental research and public health*, *14*(4), 354.

Yeboah P. O., Lowor S., & Akpabli C. K. (2003). –Comparism of Thin Layer Chromatography and Gas Chromatography Determination of Propoxur Residues in a Cocoa Ecosystem". *African Journal of Science and Technology, 4*(2), 24-28.

Zacharia, J. T. (2011). Identity, physical and chemical properties of pesticides. *Pesticides in the modern world-trends in pesticides analysis*, 1-18.

Zhang, C. Z., Wang, S. X., Zhang, Y., Chen, J. P., & Liang, X. M. (2005). In vitro estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms. *Journal of Ethnopharmacology*, *98*(3), 295-300.

APPENDICES

APPENDIX A

ANALYSIS OF VARIANCE (ANOVA) FOR HERBICIDE CONCENTRATIONS ACROSS DIFFERENT LOCATIONS

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000129	9	1.43E-05	1.463719	0.206551	2.210697
Within Groups	0.000294	30	9.8E-06			
Total	0.000423	39				

APPENDIX B

ANALYSIS OF VARIANCE (ANOVA) FOR FUNGICIDE CONCENTRATIONS ACROSS DIFFERENT LOCATIONS

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.2E-06	9	4.67E-07	0.333333	0.943338	3.020383
Within Groups	0.000014	10	1.4E-06			
Total	1.82E-05	19				

APPENDIX C

PICTURES OF ACTIVITIES CARRIED OUT DURING LABORATORY ANALYSIS

Figure 13: Chopping of pineapples for homogenizing

Figure 14: Transferring of pineapples into the foss homogenizer

Figure 15: Addition of solvent to analytes

Figure 16: Evaporation of Analytes under the rotary evaporator

Figure 17: Vortexing of matrix

APPENDIX D

SAMPLE RESULTS OBTAINED FROM LABORATORY ANALYSIS

Print Date: Sun Mar 14 07:33:05 2021 Page 1 of 1

Title :
Run File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\272-pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\spk_pes2-21-rear.mth
Sample ID : 272-pes2-21

Injection Date: 3/7/2021 5:14 PM Calculation Date: 3/12/2021 11:11 PM

Operator : Francis Detector Type: 3800 (10 Volts)
Workstation: OS Bus Address : 44
Instrument : Varian CP-3800 GC Sample Rate : 10.00 Hz
Channel : Rear = PFPD Run Time : 15.685 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

Peak No.	Peak Name	Result (mg/kg)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
Totals:		0.0000		0.000	0			

Total Unidentified Counts : 0 counts

Detected Peaks: 0 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 4 Unidentified Peak Factor: 0

Baseline Offset: 2 microVolts LSB: 1 microVolts

Noise (used): 727 microVolts - monitored before this run

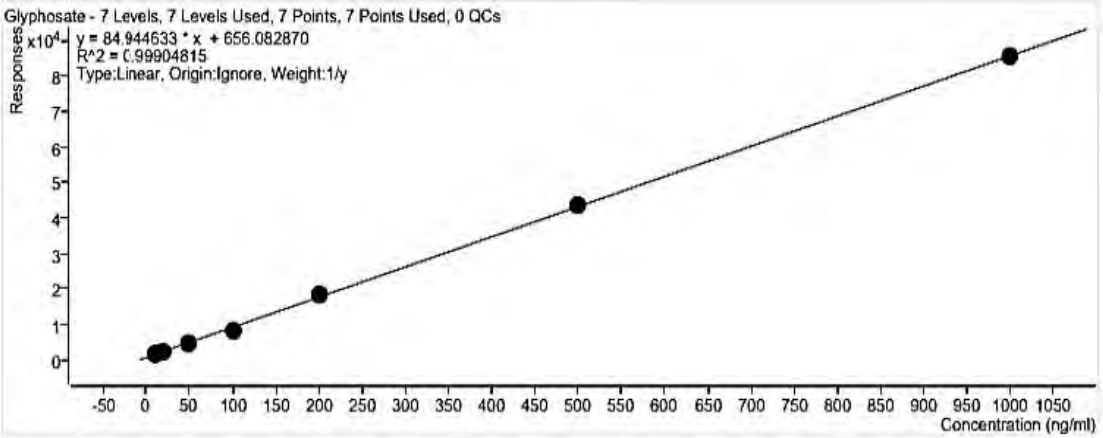
Tray: 1 Vial: 58 Injection Number: 1 Volume: 2.00 uL

Data Handling: No peaks

****

Batch Name	F:\Grace\No. 1 (2021-06-04)Glyphosate\QuantResults\2021-06-07.batch.bin		
Analysis Time	6/7/2021 8:44:28 AM	**Analyst Name**	Admin
Report Time	6/7/2021 8:44:33 AM	**Reporter Name**	Admin
Last Calib Update	6/7/2021 8:44:28 AM	**Batch State**	Processed
Quant Batch Version	B.07.00	**Quant Report Version**	B.07.00

Glyphosate

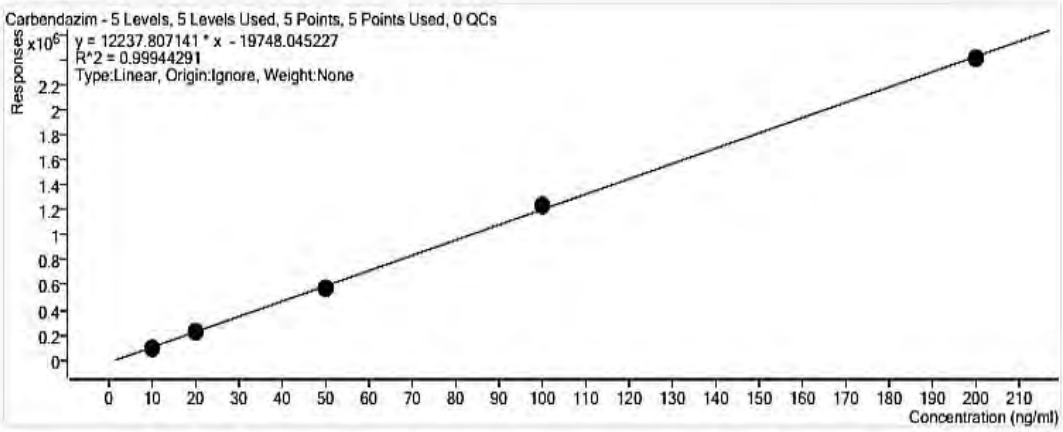


Quantifier

Data File	Sample Name	Sample Type	RT	Transition	Area	Height	Final Conc
Methanol.d	Methanol	Sample	1.471	168.1 -> 150.1	18	4	0 ng/ml
10ppb Glyphosate Std.d	10ppb Glyphosate Std	Calibration	1.560	168.1 -> 150.1	1638	105	12 ng/ml
20ppb Glyphosate Std.d	20ppb Glyphosate Std	Calibration	1.560	168.1 -> 150.1	2448	148	21 ng/ml
50ppb Glyphosate Std.d	50ppb Glyphosate Std	Calibration	1.573	168.1 -> 150.1	4508	251	45 ng/ml
100ppb Glyphosate Std.d	100ppb Glyphosate Std	Calibration	1.548	168.1 -> 150.1	8511	434	92 ng/ml
200ppb Glyphosate Std.d	200ppb Glyphosate Std	Calibration	1.535	168.1 -> 150.1	18312	984	208 ng/ml
500ppb Glyphosate Std.d	500ppb Glyphosate Std	Calibration	1.535	168.1 -> 150.1	43486	2520	504 ng/ml
1000ppb Glyphosate Std.d	1000ppb Glyphosate Std	Calibration	1.522	168.1 -> 150.1	85509	5947	999 ng/ml
Methanol 1.d	Methanol 1	Sample	1.586	168.1 -> 150.1	0	78	0 ng/ml
Blank Sample.d	Blank Sample	Sample	1.573	168.1 -> 150.1	58	11	0 ng/ml
272-pes2-21.d	272-pes2-21	Sample	1.573	168.1 -> 150.1	72	9	0 ng/ml
273-pes2-22.d	273-pes2-21	Sample	1.560	168.1 -> 150.1	53	6	0 ng/ml
899-pes2-21.d	274-pes2-21	Sample	1.598	168.1 -> 150.1	72	7	0 ng/ml
899-pes2-22.d	275-pes2-21	Sample	1.548	168.1 -> 150.1	380	35	0 ng/ml
899-pes2-23.d	276-pes2-21	Sample	1.510	168.1 -> 150.1	94	8	0 ng/ml
899-pes2-24.d	277-pes2-21	Sample	1.548	168.1 -> 150.1	960	65	4 ng/ml
899-pes2-25.d	278-pes2-21	Sample	1.865	168.1 -> 150.1	0	62	0 ng/ml
899-pes2-26.d	279-pes2-21	Sample	1.548	168.1 -> 150.1	1136	66	6 ng/ml
899-pes2-27.d	280-pes2-21	Sample	1.484	168.1 -> 150.1	2374	109	20 ng/ml
899-pes2-28.d	281-pes2-21	Sample	1.560	168.1 -> 150.1	300	27	0 ng/ml
899-pes2-29.d	spk sample	Sample	1.510	168.1 -> 150.1	5445	281	56 ng/ml

Batch Name	C:\Users\DELL\Documents\GC-LCAnalysis\Grace\No. 5 (2021-02-05)DCF\1st run\QuantResults\20210312 LC.batch.bin		
Analysis Time	3/12/2021 10:13:47 PM	**Analyst Name**	DESKTOP-30LR6CL\DELL
Report Time	3/12/2021 10:14:07 PM	**Reporter Name**	DELL
Last Calib Update	3/12/2021 10:13:46 PM	**Batch State**	Processed
Quant Batch Version	B.07.00	**Quant Report Version**	B.07.00

Carbendazim

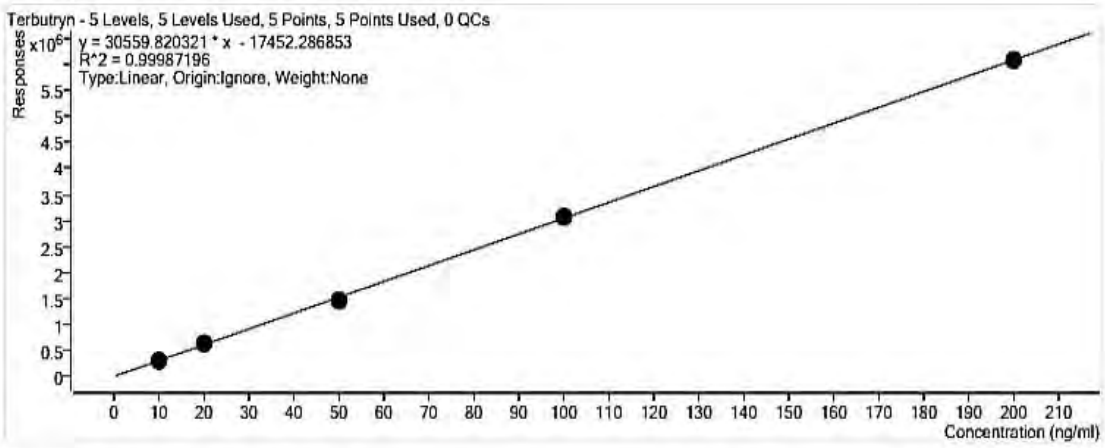


Quantifier

Data File	Sample Name	Sample Type	RT	Transition	Area	Height	Final Conc
acetonitrile 2.d	acetonitrile 2	Sample	1.575	192.2 -> 160.1	0	71	0 ng/ml
10 ppb pest std.d	10 ppb pest std	Calibration	1.692	192.2 -> 160.1	94600	7446	9 ng/ml
20 ppb pest std.d	20 ppb pest std	Calibration	1.692	192.2 -> 160.1	226468	18042	20 ng/ml
50 ppb pest std.d	50 ppb pest std	Calibration	1.709	192.2 -> 160.1	575191	46890	49 ng/ml
100ppb pest std.d	100ppb pest std	Calibration	1.709	192.2 -> 160.1	1242089	98577	103 ng/ml
200ppb pest std.d	200ppb pest std	Calibration	1.709	192.2 -> 160.1	2413279	189869	199 ng/ml
Matrix Blank.d	Matrix Blank	Sample	1.425	192.2 -> 160.1	0	998	0 ng/ml
272-pes2-21.d	272-pes2-21	Sample	1.709	192.2 -> 160.1	748	60	2 ng/ml
273-pes2-21.d	273-pes2-21	Sample	1.275	192.2 -> 160.1	0	61	0 ng/ml
274-pes2-21.d	274-pes2-21	Sample	1.609	192.2 -> 160.1	0	61	0 ng/ml
275-pes2-21.d	275-pes2-21	Sample	1.709	192.2 -> 160.1	3921	332	2 ng/ml
276-pes2-21.d	276-pes2-21	Sample	1.726	192.2 -> 160.1	10724	959	2 ng/ml
277-pes2-21.d	277-pes2-21	Sample	1.442	192.2 -> 160.1	0	75	0 ng/ml
278-pes2-21.d	278-pes2-21	Sample	1.742	192.2 -> 160.1	643	51	2 ng/ml
279-pes2-21.d	279-pes2-21	Sample	1.726	192.2 -> 160.1	3668	305	2 ng/ml
281-pes2-21.d	281-pes2-21	Sample	1.559	192.2 -> 160.1	157	16	2 ng/ml
280-pes2-21.d	280-pes2-21	Sample	1.726	192.2 -> 160.1	2809	216	2 ng/ml
spk_pes2-21.d	spk_pes2-21 b	Sample	1.709	192.2 -> 160.1	568601	44861	48 ng/ml

Batch Name	C:\Users\DELL\Documents\GC-LCAnalysis\Grace\No. 5 (2021-02-05)DCF\1st run\QuantResults\20210312 LC.batch.bin		
Analysis Time	3/12/2021 10:13:47 PM	**Analyst Name**	DESKTOP-30LR6CL\DELL
Report Time	3/12/2021 10:14:10 PM	**Reporter Name**	DELL
Last Calib Update	3/12/2021 10:13:46 PM	**Batch State**	Processed
Quant Batch Version	B.07.00	**Quant Report Version**	B.07.00

Terbutryn

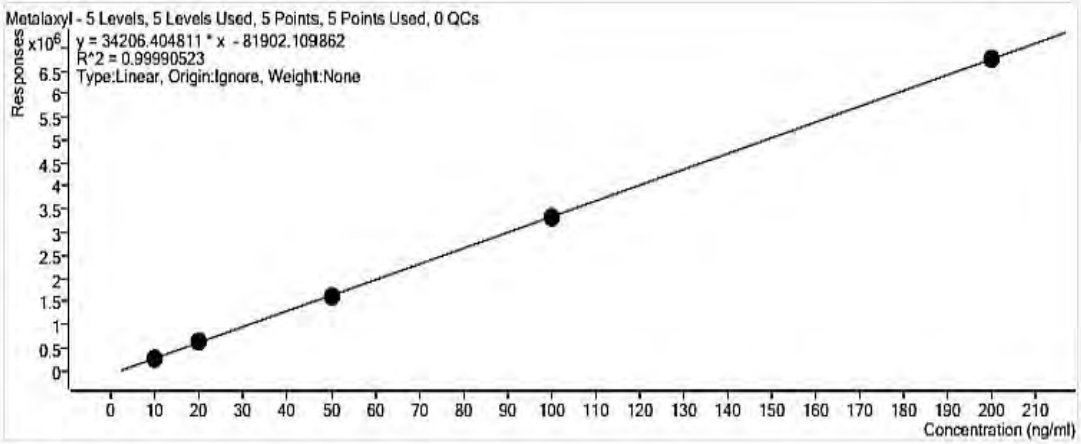


Quantifier

Data File	Sample Name	Sample Type	RT	Transition	Area	Height	Final Conc
acetonitrile 2.d	acetonitrile 2	Sample	18.999	242.2 -> 186.2	0	465	0 ng/ml
10 ppb pest std.d	10 ppb pest std	Calibration	18.798	242.2 -> 186.2	291041	15956	10 ng/ml
20 ppb pest std.d	20 ppb pest std	Calibration	18.647	242.2 -> 186.2	611764	32382	21 ng/ml
50 ppb pest std.d	50 ppb pest std	Calibration	18.664	242.2 -> 186.2	1467675	79407	49 ng/ml
100ppb pest std.d	100ppb pest std	Calibration	18.698	242.2 -> 186.2	3064888	161331	101 ng/ml
200ppb pest std.d	200ppb pest std	Calibration	18.681	242.2 -> 186.2	6090102	316276	200 ng/ml
Matrix Blank.d	Matrix Blank	Sample	18.714	242.2 -> 186.2	0	632	0 ng/ml
272-pes2-21.d	272-pes2-21	Sample	18.731	242.2 -> 186.2	15851	770	1 ng/ml
273-pes2-21.d	273-pes2-21	Sample	18.765	242.2 -> 186.2	5293	344	1 ng/ml
274-pes2-21.d	274-pes2-21	Sample	18.781	242.2 -> 186.2	6780	442	1 ng/ml
275-pes2-21.d	275-pes2-21	Sample	18.714	242.2 -> 186.2	8739	488	1 ng/ml
276-pes2-21.d	276-pes2-21	Sample	18.731	242.2 -> 186.2	13663	729	1 ng/ml
277-pes2-21.d	277-pes2-21	Sample	18.765	242.2 -> 186.2	6245	437	1 ng/ml
278-pes2-21.d	278-pes2-21	Sample	18.446	242.2 -> 186.2	0	1945	0 ng/ml
279-pes2-21.d	279-pes2-21	Sample	18.748	242.2 -> 186.2	14918	815	1 ng/ml
281-pes2-21.d	281-pes2-21	Sample	18.731	242.2 -> 186.2	6611	401	1 ng/ml
280-pes2-21.d	280-pes2-21	Sample	18.664	242.2 -> 186.2	19164	1012	1 ng/ml
spk_pes2-21.d	spk_pes2-21 b	Sample	18.597	242.2 -> 186.2	1392140	73644	46 ng/ml

Batch Name	C:\Users\DELL\Documents\GC-LCAnalysis\Grace\No. 5 (2021-02-05)DCF\1st run\QuantResults\20210312 LC.batch.bin		
Analysis Time	3/12/2021 10:13:47 PM	**Analyst Name**	DESKTOP-30LR6CL\DELL
Report Time	3/12/2021 10:14:14 PM	**Reporter Name**	DELL
Last Calib Update	3/12/2021 10:13:46 PM	**Batch State**	Processed
Quant Batch Version	B.07.00	**Quant Report Version**	B.07.00

Metalaxyl

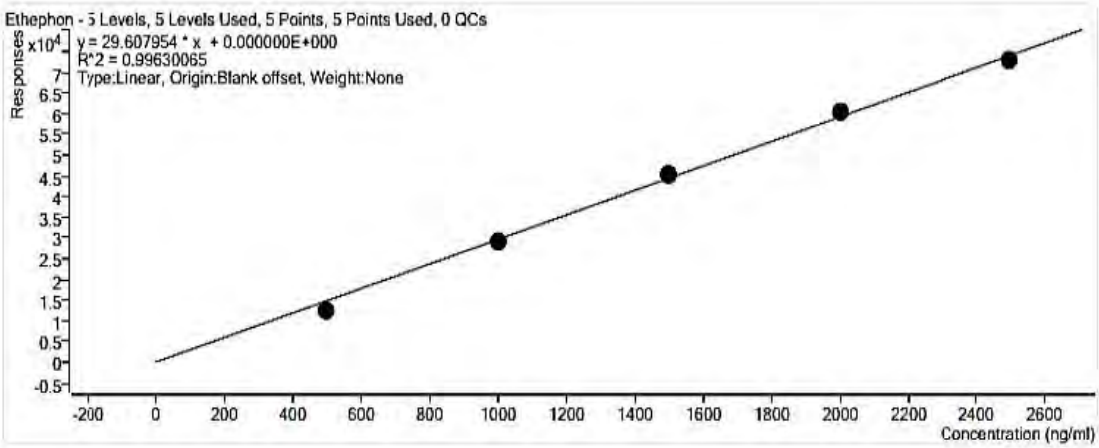


Quantifier

Data File	Sample Name	Sample Type	RT	Transition	Area	Height	Final Conc
acetonitrile 2.d	acetonitrile 2	Sample	19.549	298.4 -> 144.2	0	50	0 ng/ml
10 ppb pest std.d	10 ppb pest std	Calibration	19.499	298.4 -> 144.2	260183	13323	10 ng/ml
20 ppb pest std.d	20 ppb pest std	Calibration	19.549	298.4 -> 144.2	632710	36005	21 ng/ml
50 ppb pest std.d	50 ppb pest std	Calibration	19.566	298.4 -> 144.2	1587357	91442	49 ng/ml
100ppb pest std.d	100ppb pest std	Calibration	19.600	298.4 -> 144.2	3345418	186970	100 ng/ml
200ppb pest std.d	200ppb pest std	Calibration	19.583	298.4 -> 144.2	6763255	375166	200 ng/ml
Matrix Blank.d	Matrix Blank	Sample	19.381	298.4 -> 144.2	0	71	0 ng/ml
272-pes2-21.d	272-pes2-21	Sample	19.448	298.4 -> 144.2	0	60	0 ng/ml
273-pes2-21.d	273-pes2-21	Sample	19.499	298.4 -> 144.2	0	59	0 ng/ml
274-pes2-21.d	274-pes2-21	Sample	19.197	298.4 -> 144.2	0	57	0 ng/ml
275-pes2-21.d	275-pes2-21	Sample	19.398	298.4 -> 144.2	0	55	0 ng/ml
276-pes2-21.d	276-pes2-21	Sample	19.836	298.4 -> 144.2	0	69	0 ng/ml
277-pes2-21.d	277-pes2-21	Sample	19.633	298.4 -> 144.2	0	52	0 ng/ml
278-pes2-21.d	278-pes2-21	Sample	19.870	298.4 -> 144.2	0	92	0 ng/ml
279-pes2-21.d	279-pes2-21	Sample	20.173	298.4 -> 144.2	0	54	0 ng/ml
281-pes2-21.d	281-pes2-21	Sample	19.515	298.4 -> 144.2	0	55	0 ng/ml
280-pes2-21.d	280-pes2-21	Sample	19.499	298.4 -> 144.2	0	54	0 ng/ml
spk_pes2-21.d	spk_pes2-21 b	Sample	19.515	298.4 -> 144.2	1636823	91124	50 ng/ml

Batch Name	C:\Users\DELL\Documents\Lab Analysis\Grace\Ethephon\QuantResults\20210320.batch.bin		
Analysis Time	3/20/2021 10:38:42 AM	**Analyst Name**	DESKTOP-30LR6CL\DELL
Report Time	3/20/2021 10:38:48 AM	**Reporter Name**	DELL
Last Calib Update	3/20/2021 10:38:41 AM	**Batch State**	Processed
Quant Batch Version	B.07.00	**Quant Report Version**	B.07.00

Ethephon



Quantifier

Data File	Sample Name	Sample Type	RT	Transition	Area	Height	Final Conc
Matrix Blank.d	Matrix Blank	Blank		143.0 -> 107.1			ND
500ppb Eth Std.d	500ppb Eth Std	Calibration	2.774	143.0 -> 107.1	12638	1053	427 ng/ml
1000ppb Eth Std.d	1000ppb Eth Std	Calibration	2.795	143.0 -> 107.1	29335	2904	991 ng/ml
1500ppb Eth Std.d	1500ppb Eth Std	Calibration	2.815	143.0 -> 107.1	45106	4482	1523 ng/ml
2000ppb Eth Std.d	2000ppb Eth Std	Calibration	2.835	143.0 -> 107.1	60699	6057	2050 ng/ml
2500ppb Eth Std.d	2500ppb Eth Std	Calibration	2.845	143.0 -> 107.1	72959	7589	2464 ng/ml
272-pes2-21.d	272-pes2-21	Sample	2.714	143.0 -> 107.1	241	19	8 ng/ml
273-pes2-21.d	273-pes2-21	Sample	2.744	143.0 -> 107.1	150	11	5 ng/ml
274-pes2-21.d	274-pes2-21	Sample	2.724	143.0 -> 107.1	78	7	3 ng/ml
275-pes2-21.d	275-pes2-21	Sample	2.643	143.0 -> 107.1	233	10	8 ng/ml
276-pes2-21.d	276-pes2-21	Sample	2.784	143.0 -> 107.1	0	51	0 ng/ml
277-pes2-21.d	277-pes2-21	Sample	2.784	143.0 -> 107.1	94	7	3 ng/ml
278-pes2-21.d	278-pes2-21	Sample	2.703	143.0 -> 107.1	138	12	5 ng/ml
279-pes2-21.d	279-pes2-21	Sample	2.703	143.0 -> 107.1	0	52	0 ng/ml
280-pes2-21.d	280-pes2-21	Sample	2.865	143.0 -> 107.1	0	52	0 ng/ml
281-pes2-21.d	281-pes2-21	Sample	2.855	143.0 -> 107.1	0	54	0 ng/ml
500ppb_spk-pes2-21.d	spk-pes2-21	Sample	3.048	143.0 -> 107.1	12459	1094	421 ng/ml

Print Date: Sun Mar 14 17:10:57 2021 Page 1 of 1

Title :
Run File : C:\Users\DELL\Documents\GC-LCAnalysis\Grace\OC-OP\No.1(2021-03-05) OC\280-pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1(2021-03-05) oc\spk_pes2-21-middle.mth
Sample ID : 280-pes2-21

Injection Date: 3/7/2021 3:17 AM Calculation Date: 3/13/2021 12:12 AM

Operator : Francis Detector Type: 3800 (10 Volts)
Workstation: OS Bus Address : 44
Instrument : Varian CP-3800 GC Sample Rate : 10.00 Hz
Channel : Middle = ECD Run Time : 36.463 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

Peak No.	Peak Name	Result (mg/kg)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
Totals:		0.0000		0.000	0			

Total Unidentified Counts : 0 counts

Detected Peaks: 0 Rejected Peaks: 0 Identified Peaks: 0

Multiplier: 1 Divisor: 1 Unidentified Peak Factor: 0

Baseline Offset: 24 microVolts LSB: 1 microVolts

Noise (used): 402 microVolts - monitored before this run

Tray: 1 Vial: 66 Injection Number: 1 Volume: 2.00 uL

Peak not split: event not in an existing peak
Peak not split: event not in an existing peak
Data Handling: No peaks

****

```
Print Date: Sun Mar 14 07:48:22 2021            Page 1 of 1

Title        :
Run File     : C:\Users\DELL\Documents\GC-LCAnalysis\Grace\OC-OP\No.1 (2021-03-05)op\blnk.run
Method File  : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\spk_pes2-21-rear.mth
Sample ID    : Blnk

Injection Date: 3/7/2021 4:53 PM    Calculation Date: 3/12/2021 11:16 PM

Operator    : Francis               Detector Type: 3800 (10 Volts)
Workstation: OS                     Bus Address  : 44
Instrument : Varian CP-3800 GC      Sample Rate  : 10.00 Hz
Channel    : Rear = PFPD            Run Time     : 15.685 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode         : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

                                Ret.    Time                      Width
 Peak      Peak       Result    Time   Offset      Area    Sep.   1/2   Status
  No.      Name      (mg/kg)    (min)   (min)    (counts)  Code  (sec)  Codes
 ---- ------------ ---------- ------- ------- ---------- ---- ------ ------
 ---- ------------ ========== ======= ======= ========== ---- ------ ------
      Totals:          0.0000             0.000          0

Total Unidentified Counts :          0 counts

Detected Peaks: 0          Rejected Peaks: 0      Identified Peaks: 0

Multiplier: 1          Divisor: 4          Unidentified Peak Factor: 0

Baseline Offset: 209 microVolts          LSB:     1 microVolts

Noise (used): 953 microVolts - monitored before this run

Tray: 1  Vial: 57       Injection Number: 1    Volume: 2.00 uL

Data Handling: No peaks

********************************************************************************
```

```
Print Date: Sun Mar 14 07:33:05 2021            Page 1 of 1

Title       :
Run File    : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\272-pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\spk_pes2-21-rear.mth
Sample ID   : 272-pes2-21

Injection Date: 3/7/2021 5:14 PM    Calculation Date: 3/12/2021 11:11 PM

Operator    : Francis               Detector Type: 3800 (10 Volts)
Workstation: 03                     Bus Address  : 44
Instrument : Varian CP-3800 GC      Sample Rate  : 10.00 Hz
Channel     : Rear = PFPD           Run Time     : 15.685 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode         : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

                                 Ret.    Time                      Width
 Peak      Peak       Result     Time   Offset      Area     Sep.  1/2   Status
  No.      Name       (mg/kg)    (min)   (min)     (counts)  Code (sec)  Codes
 ---- ------------ ---------- ------- ------- ---------- ---- -----  ------
 ---- ------------ ========== ------- ======= ========== ---- -----  ------
      Totals:          0.0000            0.000          0

Total Unidentified Counts :          0 counts

Detected Peaks: 0           Rejected Peaks: 0      Identified Peaks: 0

Multiplier: 1          Divisor: 4          Unidentified Peak Factor: 0

Baseline Offset: 2 microVolts          LSB:     1 microVolts

Noise (used): 727 microVolts - monitored before this run

Tray: 1  Vial: 58       Injection Number: 1    Volume: 2.00 uL

Data Handling: No peaks

********************************************************************************
```

Print Date: Sun Mar 14 17:15:05 2021 Page 1 of 1

Title :
Run File : C:\Users\DELL\Documents\GC-LCAnalysis\Grace\OC-OP\No.1(2021-03-05) OC\spk pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1(2021-03-05) oc\spk_pes2-21-middle.mth
Sample ID : spk_pes2-21

Injection Date: 3/7/2021 4:40 AM Calculation Date: 3/13/2021 12:04 AM

Operator : Francis Detector Type: 3800 (10 Volts)
Workstation: OS Bus Address : 44
Instrument : Varian CP-3800 GC Sample Rate : 10.00 Hz
Channel : Middle = ECD Run Time : 36.463 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

Peak No.	Peak Name	Result (mg/kg)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1	beta-HCH	0.0412	13.228	0.008	447745	BV	0.0	C
2	gamma-HCH	0.0390	13.258	-0.005	109272	VB	2.5	C
3	delta-HCH	0.0307	14.741	0.010	298112	GR	0.0	C
4	heptachlor	0.0366	15.205	0.009	400168	BB	2.6	C
5	aldrin	0.0362	16.329	0.002	392980	BV	2.9	UC
6	Allethrin	0.0399	17.529	0.002	177668	BB	3.3	C
7	gamma-chlord	0.0393	18.434	-0.005	387943	BB	2.9	C
8	A-endosulfan	0.0565	18.868	-0.004	483551	BB	3.0	C
9	p,p'-DDE	0.0569	19.538	-0.002	536094	BB	3.0	C
10	Dieldrin	0.0515	19.791	0.002	460220	BB	3.0	C
11	Endrin	0.0368	20.532	-0.010	330388	BB	2.9	C
12	B-endosulfan	0.0505	21.012	0.012	326839	BV	3.3	UC
13	p,p'DDD	0.0474	21.083	-0.027	406102	VB	3.9	UC
14	p,p'-DDT	0.0398	22.330	0.024	115010	BV	0.0	C
15	Endosulfan s	0.0485	22.341	-0.019	259188	VB	3.1	C
16	Bifenthrin	0.0430	23.740	0.010	114483	BB	3.2	C
17	Fenpropathri	0.0340	24.037	-0.013	81066	BV	3.7	UC
18	Methoxychlor	0.0368	24.211	0.003	64369	VB	4.5	UC
19	Lambda-cyhal	0.0370	25.800	0.111	226465	GR	0.0	UC
20	Permethrin	0.0404	27.378	-0.060	27591	GR	0.0	UC
21	Cyfluthrin	0.0411	28.522	0.004	158688	GR	0.0	C
22	Cypermethrin	0.0436	29.295	-0.030	180585	GR	0.0	C
23	Fenvalerate	0.0430	31.531	0.079	213133	GR	0.0	C
24	Deltamethrin	0.0441	33.358	-0.184	169401	BB	6.0	C
	Totals:	1.0138		-0.083	6367061			

Status Codes:
U - User-defined peak endpoint(s)
C - Out of calibration range

Total Unidentified Counts : 42595 counts

Detected Peaks: 36 Rejected Peaks: 0 Identified Peaks: 24

Multiplier: 0.6 Divisor: 4 Unidentified Peak Factor: 0

Baseline Offset: 32 microVolts LSB: 1 microVolts

Noise (used): 390 microVolts - monitored before this run

Tray: 1 Vial: 68 Injection Number: 1 Volume: 2.00 uL

Calib. out of range; No Recovery Action Specified

****

```
Print Date: Sun Mar 14 17:04:36 2021           Page 1 of 1

Title       :
Run File    : C:\Users\DELL\Documents\GC-LCAnalysis\Grace\OC-OP\No.1(2021-03-05) OC\277-pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1(2021-03-05) oc\spk_pes2-21-middle.mth
Sample ID   : 277-pes2-21

Injection Date: 3/7/2021 1:13 AM    Calculation Date: 3/13/2021 12:15 AM

Operator    : Francis               Detector Type: 3800 (10 Volts)
Workstation: OS                     Bus Address  : 44
Instrument : Varian CP-3800 GC      Sample Rate  : 10.00 Hz
Channel    : Middle = ECD           Run Time     : 36.463 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode        : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

                                 Ret.    Time                      Width
 Peak     Peak       Result      Time   Offset      Area    Sep.  1/2   Status
  No.     Name       (mg/kg)     (min)   (min)     (counts) Code (sec)  Codes
 ---- ------------ ---------- ------- ------- ---------- ---- -----  ------
 ---- ------------ ========== ======= ======= ========== ---- -----  ------
      Totals:          0.0000            0.000          0

Total Unidentified Counts :          0 counts

Detected Peaks: 0          Rejected Peaks: 0      Identified Peaks: 0

Multiplier: 1          Divisor: 1          Unidentified Peak Factor: 0

Baseline Offset: 78 microVolts          LSB:     1 microVolts

Noise (used): 411 microVolts - monitored before this run

Tray: 1  Vial: 63       Injection Number: 1    Volume: 2.00 uL

Peak not split: event not in an existing peak
Peak not split: event not in an existing peak
Data Handling: No peaks

*******************************************************************************
```

```
Print Date: Sun Mar 14 07:46:12 2021           Page 1 of 1

Title       :
Run File    : C:\Users\DELL\Documents\GC-LCAnalysis\Grace\OC-OP\No.1 (2021-03-05)op\280-pes2-21.run
Method File : c:\users\dell\documents\gc-lcanalysis\grace\oc-op\no.1 (2021-03-05)op\spk_pes2-21-rear.mth
Sample ID   : 280-pes2-21

Injection Date: 3/7/2021 10:45 PM    Calculation Date: 3/12/2021 11:15 PM

Operator   : Francis                 Detector Type: 3800 (10 Volts)
Workstation: OS                      Bus Address  : 44
Instrument : Varian CP-3800 GC       Sample Rate  : 10.00 Hz
Channel    : Rear = PFPD             Run Time     : 15.685 min

** GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

Run Mode         : Analysis
Peak Measurement: Peak Area
Calculation Type: External Standard

                                  Ret.    Time                      Width
 Peak      Peak        Result     Time    Offset      Area     Sep.  1/2   Status
  No.      Name       (mg/kg)     (min)   (min)     (counts)  Code (sec)  Codes
 ---- ------------ ----------  ------- ------- ----------  ---- -----  ------
 ---- ------------ ==========  ======= ======= ==========  ---- -----  ------
      Totals:          0.0000              0.000          0

Total Unidentified Counts :          0 counts

Detected Peaks: 0           Rejected Peaks: 0      Identified Peaks: 0

Multiplier: 1          Divisor: 4          Unidentified Peak Factor: 0

Baseline Offset: 44 microVolts          LSB:     1 microVolts

Noise (used): 271 microVolts - monitored before this run

Tray: 1  Vial: 66       Injection Number: 1    Volume: 2.00 uL

Data Handling: No peaks

********************************************************************************
```

APPENDIX E
EU MRLS FOR VARIOUS PESTICIDES

Pesticides	MRL
Beta-HCH	0.01
Gamma-HCH	0.01
Delta-HCH	0.01
Heptachlor	0.01
Aldrin	0.01
Allethrin	0.01
Gamma - chlordane	0.01
α-endosulfan	0.05
p,p _- DDE	0.05
Dieldrin	0.01
Endrin	0.01
B-endosulfan	0.05
P,p _DDD	0.05
P,p _-DDT	0.05
Endosulfan s	0.05
Bifenthrin	0.01
fenpropathrin	0.01
Methoxychlor	0.01
Lambda-cyhalothrin	0.01
Permethrin	0.05
Cyfluthrin	0.02
Cypermethrin	0.05
Fenvalerate	0.02
Deltamethrin	0.01
Methamidophos	0.01
Ethoprophos	0.02
Diazinon	0.3
Fonofos	0.01
Dimethoate	0.01
Pirimiphos -methyl	0.01
Chlorpyrifos	0.01
Malathion	0.02
Fenitrothion	0.01
Parathion – ethyl	0.05
Chlorfenvinphos	0.01
Profenofos	0.01
Ethephon	2
Carbendazim	0.1
Terbutryn	0.01
Diuron	0.01
Metalaxyl	0.01
Nicosulfuron	0.01
Fluazifop	0.01
Metolachlor	0.05
Tebufenozide	0.01
Aclonifen	0.01
Trifloxystrobin	0.01
Glyphosate	0.1