FDETERMINATION OF PESTICIDE RESIDUES IN SOME VEGETABLES AND HEAVY METALS IN THE VEGETABLES AND SOILS FROM SELECTED FARMLANDS IN MINNA, NIGERIA

BY

ZULAIHAT ABDULHAMID

DEPARTMENT OF CHEMISTRY

AHMADU BELLO UNIVERSITY, ZARIA

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BY

Zulaihat ABDULHAMID, B.Sc (A.B.U ZARIA) 1989, MTech (FUT MINNA) 1999

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NIGERIA

AUGUST, 2016

DECLARATION

I declare that the work in this thesis entitled "Determination of Pesticide Residues in Some Vegetables and Heavy Metals in the Vegetables and Soils from Selected Farmlands in Minna, Nigeria" has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Zulaihat ABDULHAMID . . . . . .

Name of Student Signature Date

CERTIFICATION

This thesis entitled “Determination of Pesticide Residues in Some Vegetables and Heavy Metals in the Vegetables and Soils from Selected Farmlands in Minna, Nigeria” by Zulaihat ABDULHAMID, meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

Professor E.B. Agbaji	. . . . .	. . . . .
Chairman, Supervisory committee	Signature	Date
Professor C. E. Gimba	. . . . .	. . . . .
Member, supervisory committee	Signature	Date
Professor A. S. Agbaji	. . . . .	. . . . .
Member, supervisory committee	Signature	Date
Professor V. O. Ajibola	. . . . .	. . . . .
Head of Department	Signature	Date
Professor K. Bala	. . . . . .	. . . . .
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

To my husband Dr Kasim Uthman Isah for his patience, understanding and prayers.

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ABSTRACT

The aim of this study was to assess the level of pesticide residues and heavy metals in three staple vegetables commonly cultivated in Minna and also determine the physico-chemical properties and heavy metal content of the farm soils where the vegetables were grown. *Amaranthus hybridus* (spinach), *Hibiscus esculentus* (okra), *Telfairia occidentalis* (fluted pumpkin) and soil samples were collected from seven selected farms in Minna, Niger State. The vegetables were analysed for the presence of six pesticides (cypermethrin, carbofuran, lambda-cyhalothrin, aldrin, endrin and heptachlor) using gas chromatography triple quadrupole mass spectrometry and six heavy metals (cadmium, copper, iron, manganese, nickel and zinc) by atomic absorption spectrometry. The soil samples of the farms were also assessed for the heavy metals and physico-chemical properties that include pH, electrical conductivity, particle size, organic carbon, organic matter, exchangeable bases and cation exchange capacity. The soil-plant transfer factors between the level of heavy metals in the soils and their levels in the vegetables were also determined. The transfer factor results revealed no absorption of Cd in the three vegetables of all farms except in Keteren gwari farm. The TFs values for Zn and Cu from Mandela farm were the highest for all vegetables compared to other farms. The results of the pesticide residues analysis revealed that none of the okra and fluted pumpkin leaves showed the presence of any of the pesticides analysed while spinach samples from farms in Chanchaga and Mandela areas contained cypermethrin in the range of 0.60-8.65 mg/kg.

Heptachlor was also detected in the spinach samples of the same farms during full scan but their levels were below the detection of the instrument used. The results for the physicochemical properties of the farm soils indicate: pH; 5.77-7.70, soil organic matter 1.63-3.87 %, electrical conductivity; 17-37 µS/cm. The particle size results was: sand; 65.00-95.80, silt; 1.60-33.60, clay; 0.60-6.00 and exchangeable bases: Na^+; 0.48-1.64, K^+; 0.05-

0.45, Mg^{2+}; 0.11-0.31, Ca^{2+}; 2.30-4.21, cation exchange capacity; 3.68 to 4.85. The mean levels of heavy metals in the soil samples were found to be in the range: Cd (0.18-1.34 mg/kg), Cu (6.71-47.59 mg/kg), Fe (1942.13-2205.90 mg/kg), Mn (182.48-371.78 mg/kg), Ni (0.65-6.90 mg/kg), Zn (15.70-225.20 mg/kg). The result of the vegetables analysed showed that *Amaranthus hybridus* contained: Cd (4.65-4.85 mg/kg); Cu (4.31-30.17 mg/kg); Fe (27.06-683.95 mg/kg); Mn (13.01-81.00 mg/kg); Ni (1.22-1.44 mg/kg); Zn (2.48-164.78 mg/kg), while *Hibiscus esculentus* contained: Cu (11.22-19.90 mg/kg); Fe (114.71-189.86 mg/kg); Mn (33.26-103.61 mg/kg); Ni (1.24-1.54 mg/kg) and Zn (38.81-97.95 mg/kg), *Telfairia occidentalis*: Cd (5.11-7.16 mg/kg); Cu (4.96-36.93 mg/kg); Fe (116.72-592.28 mg/kg); Mn (95.67 to 572.72 mg/kg); Ni (1.36-3.42 mg/kg); Zn (7.25-69.06 mg/kg). The concentration of the metals in the soil samples varied according to the following trend: Fe>Mn>Zn> Cu>Ni>Cd. The average concentration of all the metals in each farm also gave the trend Farm B>Farm G>Farm E>Farm C>Farm F>Farm D>Farm A. The control farm (A) contained lower levels of most of the investigated metals The findings indicate the presence of heavy metals in all the farm soils but only Fe was above the FAO/WHO standards of 1000mg/kg. The trends of the average concentration of all six metals in the different vegetables from all farms were Spinach: Fe>Zn>Mn>Cu>Cd>Ni; okra: Fe>Zn>Mn>Cu>Ni and fluted pumpkin leaves: Fe>Mn>Zn>Cu>Cd>Ni. The result also revealed spinach was the only vegetable sample in this research that was contaminated with pesticides and heavy metals. This implies that continuous monitoring and education of farmers about proper use of pesticides is necessary. Indiscriminate disposal of waste in the environment and citing farms near dumpsites, garages, road side etc should be discouraged.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ADI	Acceptable Daily Intake
ADP	Agricultural Development Project
AOAC	Association of Official Analytical Chemist
ATSDR	Agency for Toxic Substances and Disease Registry
BHC	Beta Hexachloro Cyclohexane
CB	Carbamates
DDT	Dichlorodiphenyltrichloroethane
DDVP	Dichlorvos
DSPE	Dispersive Solid Phase Extraction
EC	Electrical Conductivity
EI	Electron Ionization
ETDI	Estimated Total Daily Intake
FAO	Food and Agricultural Organization
GCB	Graphitized Carbon Black
GC/ECD	Gas Chromatography/ Electron Capture Detector
GC/MS	Gas Chromatography/Mass Spectrometry
GWRTAC	Ground Water Remediation Technologies Analysis Centre
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Kd	Adsorption Coefficient
LD	Lethal Dose
LUI	Local User Interface
MMT	Methylcyclopentadienyl Manganese Tricarbonyl
MPI	Maximum Permissible Intake
MRLs	Maximum Residue Limits
MSD	Mass Selective Detector
NIST	National Institute of Standards and Technology

NOAEL	No Observed Adverse Effect Level
OCPs	Organochlorine pesticides
OP	Organophosphorus
POPs	Persistent Organic Pollutants
PP	Poly Propylene
PSA	Primary Secondary Amine
PVC	Poly Vinyl Chloride
RfD	Acute Reference Dose
SIM	Selective Ion Monitoring
SOM	Soil Organic Matter
SP	Synthetic Pyrethroid
TEPP	Tetra Ethyl Pyro phosphate
TIC	Tentative Identification Compound
TPP	Triphenylphosphate
TOF	Time of Flight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

A pesticide is any substance or mixture of substances intended for controlling any pest, including vectors of human or animal disease and unwanted plants or animals' species. They could interfere with the production, processing, storage, transport or marketing of food and other agricultural commodities. The term also includes substances intended for use as plant growth regulators, defoliants, desiccants or agents for thinning fruit or preventing the premature fall of fruit. In addition, these substances are applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2002).

Pesticides are widely used in many areas of modern agriculture but the hazards they have brought along with them to food safety and human health have become the focus of world attention. Although the benefits of pesticides cannot be overstated, their use raises a number of environmental concerns such as potential toxicity to humans and other animals (Kamrin, 1997). Most pesticides are resistant to physical, chemical and biological degradation and accumulate in both aquatic and terrestrial food webs (Valiyaveettil *et al*., 2010). Over 98 % of sprayed insecticides and 95 % herbicides reach a destination other than their target species, including non- target species, air, water and soil (Vivekanandhan and Duraisamy, 2012). Some pesticides are considered too hazardous for sale to the general public and are designated Restricted-Use Pesticides. Only certified applicators, that have passed an examination on their handling, may purchase or supervise the application of Restricted Use Pesticides. Records of sales and use are required to be

maintained and may be audited by government agencies charged with the enforcement of pesticide regulations (US EPA. 2011).

Though pesticide regulations differ from country to country, pesticides and products on which they have been applied are traded across international borders. To deal with inconsistencies in regulations among countries, delegates to a conference of the United Nations Food and Agriculture Organization (FAO) adopted an International Code of Conduct on the Distribution and Use of Pesticides in 1985 to create voluntary standards of pesticide regulation for different countries. The Code was updated in 1998 and 2002. The FAO claim the code has raised awareness about pesticide hazards and decreased the number of countries without restrictions on pesticide use (FAO, 2002).

More than 700 pesticides are registered for use in the world and more continue to persist in the environment even though they are no longer being applied (Wylie, 1997). According to the Stockholm Convention on Persistent Organic Pollutants (POPs), 10 of the 12 most dangerous and persistent organic chemicals are pesticides (Gilden *et al.*, 2010). Persistent pesticides are highly toxic, causing an array of diverse effects, notably death, diseases and birth defects among humans and animals (Etonihu *et al.*, 2011). Chronic exposure of humans to low doses of pesticides through air, water and food may lead to chronic toxicity due to accumulation of residues in the body over a long period of time. Possible health problems associated with chronic pesticide toxicity include cancers, congenital malformations, neurological disorders, infertility, blood dyscrasias, impotence, immunological disorders, liver and kidney damage, skin alterations and worsening of existing health conditions (Sesline and Jackson, 1994; Jobling *et al.*, 1995). Acute and sub-acute toxicity may also arise from exposure to high doses among people

who are directly involved in the manufacture, formulation, mixing and application of pesticides or in suicide and homicide cases. Human exposure may be through dermal contact, inhalation or accidental ingestion. Symptoms of acute toxicity vary with the individual chemical involved but may generally include dizziness, headaches, sweating, fatigue, numbness, vomiting, cramps, chemical burns of the eye and skin, neurological effects, respiratory tract irritation, liver and kidney damage, coma or death (Koprucu *et al.*, 2006; Turgut, 2007).

1.2 Pesticide Usage

Large-scale use of pesticides began after World War II with the widespread use of organochlorine and organophosphorus compounds. Other chemical groups were subsequently developed and are used in agriculture today (e.g. triazine herbicides, carbamate insecticides and synthetic pyrethroids). However, pesticides are not a new development and have been used for centuries. For example, sulfur was used in classical Roman times for pest control in agriculture (Smith and Secoy 1976). In the 19th century, highly toxic, mainly inorganic compounds of copper, arsenic, lead and sulfur were used for the control of fungal diseases and insects (Hamilton and Crossley, 2004).

There is now overwhelming evidence that pesticides do pose potential risk to humans and other life forms and unwanted side effects to the environment (Igbedioh, 1991). No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world-wide deaths and chronic illnesses due to pesticide poisoning are about 1million per year (Environews, 1999). The estimate of World Health Organization and the UN environment programmes also showed that each year, about 3 million workers in

agriculture in the developing countries experience severe poisoning from pesticides, resulting to about 18,000 deaths; while about 25 million workers suffer mild pesticide poisoning (Jaga, 2003; FAO, 2002).

Rosendahl (2008) reported that the list of pesticides used on vegetables in West African countries include organochlorine and organophosphorus pesticides declared by WHO as obsolete or banned. As at August 2008, Nigeria had registered a total number of 354 pesticides and agrochemicals (Keri, 2009) and the challenges the country is facing in relation to pesticides include:

(i) pesticide mismanagement and handling by unlicensed retailers;

(ii) smuggling of pesticides across the porous land borders on every side of the country;

(iii) non-implementation of the harmonized registration documents and study protocols; and

(iv) exporting products that exceed MRLs, resulting to rejection by importing countries (Keri, 2009).

According to Damalas and Eleftherohorinos (2011) a greater part of pesticides used for killing pests persist in the environment and may be accumulated in human body by many ways such as through drinking water and eating vegetables and fruits. The small quantity of such pesticides gradually increases in the body which becomes the cause of many human diseases like gastric cancer, cytogenetic damage and kidney infections.

1.3 Pesticide Residues in Food

Pesticide residues are very small amounts of pesticides that can remain in or on a crop after harvesting or storage and make their way into the food chain (Food Standards

Agency, 2010). They can remain even when pesticides are applied in the right amount and at the right time. They may need to be on the surface of foodstuffs to protect them from pest during storage and some are applied after harvest for this purpose. The Codex definition of a pesticide residue refers not only to the active ingredient but also to any derivatives of pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance (Hamilton and Crossley, 2004).

Studies of the environmental fate, metabolism and processing of food provide basic information for studying residue levels in food. Chronic and acute consumer intake estimates compare dietary exposure with acceptable intakes derived from the toxicology of food. Natural compounds, for proprietary reasons, have not usually been studied as thoroughly as synthetic compounds and therefore the safety of these compounds is frequently less well known (Hamilton and Crossley, 2004). The risk assessment of residues in food must be acceptable at the international level to protect the consumer and to prevent disruption of the international trade in food.

Most pesticide residues occur in food as a result of the direct application of a pesticide to a crop or farm animal or the post-harvest treatments of food commodities such as grains to prevent pest attack. Residues also occur in meat, milk and eggs from the consumption by farm animals of feed from treated crops. However, residues can also occur in foods from environmental contamination and spray drift at the time of application. In addition, transport of residues and sediment, for example in storm water run-off or leaching through the soil to ground water, may also contaminate drinking water sources (Hamilton and Crossley, 2004).

There has been increased public concern about the impact of organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and dieldrin on the environment. These compounds have both high environmental persistence and high fat solubility which commonly lead to residues occurring in meat, milk and eggs. Most countries have now withdrawn the registration of these persistent organochlorine pesticides. However, residues are occasionally detected in food because of the environmental contamination that remains from historical usage of the chemical. For example, animals grazing on contaminated land readily consume residues, which can be detected in the fat. Grazing cattle may consume 1 kg of soil per head per day and so will ingest the residue directly from the soil as well as residue in the pasture or forage itself. Of the crops grown in soil contaminated with organochlorines, root crops are the most likely to take up residues (Hamilton and Crossley, 2004).

1.4 Heavy Metals in Vegetables and Soil

Heavy metals are important environmental pollutants and their presence in the atmosphere, soil, water and food chain can cause serious problems to living things (Mubofu, 2012). Pollution by heavy metals is of significant ecological and environmental concern. This is due to the fact that they are not easily biodegradable thereby precipitating far reaching effects on the biological system (Otitoju *et al*., 2012).

Heavy metals enter plant, animal and human tissues via air inhalation, diet and manual handling. Motor vehicle emissions are a major source of airborne contaminants including arsenic, cadmium, cobalt, nickel, lead, antimony, vanadium, zinc, platinum, palladium and rhodium (Balasubramanian *et al*., 2009). Water sources can be polluted by heavy metals leaching from industrial and consumer waste; acid rain can exacerbate this process by releasing heavy metals trapped in soils (Worsztynowicz and Mill, 1995). Plants are

exposed to heavy metals through the uptake of water; animals eat these plants; ingestion of plant- and animal-based foods is the largest sources of heavy metals in humans (Radojevic and Bashkin, 1999). Heavy metals can accumulate in organisms as they are hard to metabolize (Pezzarossa *et al*., 2011).

1.5 Justification

A survey of literature reveals that data on pesticide residues in fruits, vegetables and other foodstuffs from Nigerian farms and markets are limited and in some areas lacking. This calls for concern as the toxicological effects of the chemicals which humans and animals are exposed to daily are ever-increasing. In the last few years, emphasis has been placed on a group of chemicals referred to as endocrine disruptors, mostly man-made compounds suspected of interfering with the body's hormone system by blocking or mimicking normal function. One of the avenues for human exposure to these compounds is through the consumption of agricultural products that have been treated with pesticides (Stenerson *et al*., 2010). In recent times, there have also been reported cases of food poisoning and deaths related to pesticide remnants in food materials in Nigeria (Etonihu *et al.,* 2011). In addition, there has been increased level of activities related to metal processing and haphazard disposal of domestic wastes in our major cities. The selected farms are situated near dumpsites, mechanic workshops, car parks and along the town's main drainage system and thus have an elevated risk of potential contamination. It is therefore necessary to determine the levels of pesticide residues and heavy metals in vegetables and soils of the farms since the consumption of pesticide / heavy metals contaminated foods via daily diet is a major source of exposure to these pollutants and poses a potential health threat to humans (Calderon *et al*., 2003; Roychowdhury *et al.,* 2003; Zhang *et al*., 2011). Information obtained from this research will be useful to the Ministry of Agriculture and Agricultural Development Project (ADP) in Niger State by providing them with the level

of pesticide residues and heavy metal level in some vegetables and soil from selected farms in Minna. The results from this project will also inform future researchers in the area of pesticide residues in vegetables in Niger State and Nigeria, since literature on pesticide residues from Nigerian foodstuffs are sparse (Atuma, 1985; Adeyeye and Osibanjo, 1999; Umyimandu and Udochu, 2002; Anyakora *et al.*, 2008; Musa *et al.*, 2010; Nsikak and Aruwajoye, 2011; Etonihu *et al*., 2011).

1.6 Aim and Objectives

The main aim of this study is to determine the level of pesticide residues and heavy metals in three staple vegetables commonly cultivated in Minna. Namely: *Amaranthus hybridus* (spinach), *Hibiscus esculentus* (okra) and *Telfairia occidentalis* (fluted-pumpkin leaves, ugu) from seven selected farms in Minna. The study will also involve the determination of the physico-chemical properties and heavy metal content of the soil samples from the same farms. This study provides baseline information on pesticide residues in the selected vegetables in this area by using one of the latest methods for extracting and analyzing them. The objectives of the study are:

(i) survey of pesticides used on vegetable farms in Minna;

(ii) determination of organochlorine (aldrin, endrin heptachlor) pesticide residues in the vegetables on same selected farms;

(iii) determination of carbamate (carbofuran) and pyrethroid (cypermethrin, lambdacyahalothrin) pesticide residues in the vegetables;

(iv) analysis of the physico-chemical characteristics of the soil from the farms;

(v) assessment of the levels of cadmium, copper, manganese, iron, nickel and zinc in the soil and the vegetable samples;

(vi) calculation of the correlation coefficient between the amount of heavy metals in the soil and the vegetables;

(vii) calculation of soil-plant transfer factors; and

(viii) comparison of the results with the maximum safety limits set by FAO/WHO.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pesticides and Food Safety

The increasing demand for food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins. Food safety issues and potential health risks are of the most serious environmental concerns (Brhane and Shiferaw, 2014).

Pesticides are among the most extensively used chemicals in the world and are also among the most hazardous compounds to humans as well, causing many people to change their lifestyle by opting for organic agricultural products. Though some of these pesticides can be beneficial in decreasing the population of harmful insects, others can be damaging to the environment and can cause serious disturbances. Studies have shown that pesticides can cause both short term and long term damages to the people and the environment, particularly when they run off into water ways or if used indiscriminately (Azmi and Naqvi, 2011). Synthetic pesticides are behind many people's decision to switch to organic products and practices, especially where diet is concerned. The number of people demanding pesticide-free organic food has increased sharply in recent years as more information has been uncovered about the health risks associated with pesticides.

2.2 Classification of Pesticides

Pesticides can be classified by target organism, chemical structure, and physical state. They can also be classified as inorganic, synthetic, or biologicals (AMA, 1997). Plant-derived pesticides, or "botanicals", include the pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside.

Pesticides can also be classified based on their mechanism of biological function or application method. Most pesticides work by poisoning pests ((Paul and Simonin, 2007) and systematically moves inside a plant following absorption by the plant. With insecticides and most fungicides, this movement is usually upward and outward, resulting in increased efficiency. However, systemic insecticides, which poison pollen and nectar in the flowers, may kill bees and other needed pollinators. Synthetic pesticides can be grouped by their active ingredient. Major chemical groups are: carbamates, organochlorines, organophosphates, pyrethroids and chlorophenoxy acids.

In 2009, a new class of fungicides which are safer and greener were developed. They work by inhibiting the action of phytoalexins, the natural defense chemicals released by plants (Eurek, 2009).

Pesticides can be grouped on the basis of the type of pest they control as: algicides or algaecides for the control of algae, avicides for the control of birds, bactericides for the control of bacteria, fungicides for fungi and oomycetes. Others are herbicides for the control of weeds, insecticides for the control of insects, miticides for mites, molluscides for slugs and snails, nematocides for the control of nematodes, rodenticides for rodents and virucides for the control of viruses.

2.3 Types of Synthetic Pesticides.

According to Azmi and Naqvi (2011), the type of synthetic chemical compounds present in a pesticide can be used to classify them as:

2.3.1 Organochlorine (OC)

Organochlorine pesticides are chlorinated hydrocarbons used extensively from the 1940s through the 1960s in agriculture and mosquito control. Representative compounds in this group include DDT, methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone,

lindane, and benzene hexachloride. Organochlorine pesticides accumulate in the environment. They are very persistent and have a long half-life of 15-30 years (Azmi and Naqvi, 2011).

2.3.2 Chlorophenoxy acids

They have been in used since 1930 as herbicides and include 2, 4- Dichlorophenoxy acetic acid. They are toxic for plants especially broad leaf plants or weeds and mimic plant hormones. They are less toxic to animals but may produce eye irritation and gastrointestinal disorder. They are degraded in about two weeks but regular exposure may be teratogenic (capable of interfering with the development of foetus causing birth defects) in animals (Azmi and Naqvi, 2011).

2.3.3 Organo-phosphates (OP)

Organo-phosphates are more toxic to mammals but they are degraded in 2-4 weeks. They came in general use as pesticides in 1950 as a result of the development of resistance against OC and DDT. The common insecticides are malathion, parathion, methyl parathion, dimecron, diazinon, dimethoate, chlorpyriphos, DDVP or dichlorvos, TEPP (Tetraethyl pyrophosphate). They are compounds of phosphorus and sulphur. Basically, they are acetyl cholinesterase inhibitors. Among them TEPP is the most toxic with LD_{50} of 1 mg/kg while malathion is least toxic with LD_{50} of 1500 mg/kg. They have shorter half-life and quickly degrade, but their first metabolite is more toxic e.g. paraoxon, malaoxon (Azmi and Naqvi, 2011).

2.3.4 Carbamates (CB)

They are esters of carbonic acid and first developed in Nigeria by U.K. Scientists. They include propoxur (baygon), carbaryl (sevin), Temik and Zectran. They are also less persistent and degrade in about 4 weeks. They are acetyl cholinesterase inhibitors. They

are of varied toxicity e.g. propoxur has LD_{50} of 30 mg /kg while carbofuran is more toxic with LD_{50} of 8 mg/kg and thiodicarb is less with LD_{50} of 66 mg/kg (Azmi and Naqvi, 2011). Carbofuran as a Carbamate pesticide is a derivative of carbamic acid, $HOC(O)NH_2$. They vary in their spectrum of activity, mammalian toxicity and persistence. It is also a relatively unstable compound that break down in the environment within weeks or months. It is commonly used as surface sprays or baits in the control of pests. Most carbamates are extremely toxic to Hymenoptera, and translocate within plants, making them an effective systemic treatment (Fischer, 2014).

2.3.5 Synthetic pyrethroids (SP)

They are being mostly used these days because they are less toxic to mammals but sufficiently toxic to insects. They also inhibit cholinesterase and have been developed on the basis of natural pyrethroids found in chrysanthemum flower. The natural pyrethrins have immediate knockdown effect but are less toxic and less persistent. The synthetic pyrethroids have more toxic and persistent effect. The examples of these are permethnin, bioallethrin, resmethrin, cypermethrin etc. They also have a half-life of 2 – 4 weeks, are general esterase inhibitors and have effects on other enzymes as well (Azmi and Naqvi, 2011).

2.4 Pesticide Residues in Food Crops

Residues of organochlorine insecticides, especially DDT and hexachlorocyclohexane (HCH) have been detected in man and his environment the world over (Hayes and Laws, 1991) due to injudicious and indiscriminate use. Seventy- five percent of all pesticides in the world are used in developed countries, but use in developing countries is increasing (Lee, 2006).

Rodrigues *et al* (2007) determined the level of organochlorine pesticides (OCPs) in medicinal plants harvested in Brazil using GC/MS in the selected ion monitoring mode. The result revealed the presence of all the pesticides below the maximum residues level (MRL) except dieldrin which was above the European Pharmacopoeia of 50 ng/g. OCPs residues were also investigated in vegetables from Deyang and Yanting areas of Sichuan province China. The study involved 34 samples of 19 varieties of vegetables using a gas chromatograph with electron capture detector (GC-ECD). All vegetable samples had some levels of one or more OCPs with one vegetable (green pepper) having 3 pesticides exceeding the Chinese Extraneous Maximum Residue Limit of 50 ng/g (Owago *et al*., 2009).

Eight fruit samples of apple, guava, orange, grapes, pear, persimmon, banana and pear from local markets of Nawabshah district of Pakistan were analysed for the presence of organophosphate, pyrethroid and organochlorine pesticides. A total of seven fruit samples were found contaminated with pesticides and among those only one out of eight fruit samples was found above the MRL. Some samples were also found to contain multiple pesticide residues (Anwar *et al.*, 2004).

Bempah *et al* (2011) also assessed the concentration of pesticide residues in fruits and vegetables from markets in Kumasi to generate awareness about the lethal effects on human beings as well as estimate the potential health risks associated with the pesticide residues with regard to consumers. A total of 350 locally produced fruits and vegetables from six main markets in Kumasi were analyzed by gas chromatography equipped with electron capture detector. The results showed that 37.5 % of the fruits and vegetables samples analyzed contained no detectable level of the monitored pesticides, 19.0 % of the

samples gave results with levels of insecticides residues above the MRL, while 43.5 % of the samples were below the MRL. The analysis of health risk estimates revealed that none of the pesticides exceeded the reference dose in both fruit and vegetables except endrin which exceeded reference dose in vegetables, suggesting a great potential for systemic toxicity in children considered the most vulnerable population subgroup (Bempah *et al*., 2011).

Nigerian farmers buy and use many pesticides for protecting crops and livestock. Udoh (1998) conducted a study to investigate the conditions of use and impact of pesticides in the six agricultural zones of Akwa Ibom State. It was found that more than 600,000 farming households in the State may have been exposed to various problems due to poor handling and use of pesticides.

To generate a baseline data in pesticide residues in Nigerian cereals, Osibanjo and Adeyeye (1995) analysed the levels of OCPs in rice, maize, millet and sorghum. The result revealed that all cereal samples had some levels of one or more OCPs below FAO's MRL with some percentage having at least one residue level or more above the MRL. Etonihu *et al.,* (2011) also analysed samples of some Nigerian food grains (white maize, white beans and sorghum) purchased randomly from open markets in Nasarawa and Plateau states. Using gas chromatography and titrimetry, the samples were analysed for pesticide residues and nutritional iodine contents. The results showed a slightly higher residue level in the beans samples from Keffi market than beans samples from Akwanga.

In Lagos state, Ogah *et al* (2011a) determined the incidence and quantity of organochlorine pesticide residues in maize samples collected from various markets and

compared values obtained with established safety values in order to highlight possible health hazards. The results showed that 96 % of the maize samples contained residues of one or more organochlorine pesticides. Mean concentrations ranged from 7.9-52.0 µg/kg and maximum residue limits (MRLs) of some pesticides were exceeded in up to 7 % of samples. The estimated total diet intakes (ETDIs) for aldrin and dieldrin exceeded their maximum permissible intakes.

Organophosphates and carbamate pesticides were also assessed in beans samples collected from different markets in Lagos state using GC/MS. All beans samples were found to contain residues of one or more organophosphates and carbamate pesticides. The estimated total diet intake (ETDI) for dichlorvos exceeded its maximum permissible intake (MPI by 131 % (Ogah *et al*., 2011b).

Nsikak and Oruwajoye (2011) reported that in view of compelling evidence of health effects on humans based on weak implementation of government policy on regulation/ ban on pesticides in Nigeria, there is need for evaluation of OCPs and other types of pesticides in food crops. They detected levels of some OCPs in samples of tomato and chilli pepper from local markets in Ota, Ogun state using GC-ECD. The OCPs levels were generally below the FAOs maximum residue limits except for Hexachlorocyclohexane and trans-nonachlor**.**

2.5 Effects of Pesticides on the Environment

The environmental impact of pesticides consists of the effects of pesticides on non-target species. Over 98 % of sprayed insecticides and 95 % of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields (Miller, 2004). Each pesticide comes with a specific set of

environmental concerns, the effects of which have led many pesticides to be banned, while regulations have limited or reduced the use of others. Over time, pesticides have generally become less persistent and more species-specific. However, the global spread of pesticide use, including the use of obsolete pesticides that have been banned in some developed countries has overall increased (Lamberth *et al.*, 2013).

Many of the chemicals used in pesticides are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation (US/EPA, 2007) The use of pesticides decreases the general biodiversity in the soil resulting in lower soil quality (Johnston, 1986) with the additional effect that less organic matter in the soil allows for lower water retention (Kellogg *et al*., 2000). This decreases yields for farms in drought years, when organic farms have had yields 20-40% higher than their conventional counterparts (Lotter *et al*., 2003). A smaller content of organic matter in the soil increases the amount of pesticide that will not stay in the area of application, because organic matter binds to and helps break down pesticides (Kellogg *et al*., 2000). Degradation and sorption are both factors which influence the persistence of pesticides in soil. Depending on the chemical nature of the pesticide, such processes control directly the transportation from soil to water, and in turn to air and food (Kellogg *et al*., 2000).

Nitrogen fixation which is required for the growth of higher plants, is hindered by pesticides in soil (Manuel *et al*., 2008). The insecticides DDT, methyl parathion, and especially pentachlorophenol have been shown to interfere with legume-rhizobium chemical signalling. Reduction of this symbiotic chemical signalling results in reduced nitrogen fixation and thus reduced crop yields (Manuel *et al*., 2008).

Pesticides can kill bees and are strongly implicated in pollinator decline, and the loss of species that pollinate plants. Application of pesticides to crops that are in bloom can kill honeybees, which act as pollinators. The USDA estimate that US farmers lose at least $200 million a year from reduced crop pollination because pesticides applied to fields eliminate about a fifth of honeybee colonies in the US and harm an additional 15 % (Miller, 2004). On the other side, pesticides have some direct harmful effect on plants including poor root hair development, shoot yellowing and reduced plant growth (Walley *et al*., 2006).

According to Liroff (2000), chlorinated hydrocarbons could pollute the tissues of virtually every life form on the earth, the air, the lakes and the oceans, the fishes that live in them and the birds that feed on the fishes. It has been found that atrazine, a popular weed killer feminize frogs, leading to sterility. About 80 million pounds of atrazine is used by farmers annually all over the world except in the EU countries where it has been banned since 2004.

Crisp *et al* (1998), reported that certain environmental chemicals including pesticides termed as endocrine disruptors are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure are increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer.

Pesticides can enter the body through inhalation of aerosols, dust and vapour that contain pesticides; through oral exposure by consuming food/water; and through skin exposure by direct contact. The effects of pesticides on human health depend on the toxicity of the chemical and the length and magnitude of exposure (Lorenz, 2009). Farm workers and

their families experience the greatest exposure to agricultural pesticides through direct contact.

Pesticides that are sprayed can move through the air and may eventually end up in other parts of the environment, such as soil or water. Pesticides that are applied directly to the soil may be washed off the soil into nearby bodies of surface water or may percolate through the soil to lower layers and ground water (Harrison, 1990). Some pesticides, including aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene, are considered persistent organic chemicals (POPs). They are compounds that resist degradation and thus remain in the environment for years. Some POPs have the ability to volatilize and travel great distances through the atmosphere to become deposited in remote regions. Such chemicals may have the ability to bioaccumulate, biomagnify and bioconcentrate up to 70,000 times their original concentrations. POPs can affect non-target organisms in the environment and increase risk to humans by disruption in the endocrine, reproductive, and immune systems (Ritter *et al.*, 1995).

2.6 Types of Exposure and Hazards caused by Pesticides

Different groups and segments of a population are exposed to pesticides in different ways and in different degrees. Exposure could be intentional (suicides or homicides) or unintentional (occupational and non- occupational exposures from water, air and food). The occupational hazards in industrial settings and the ecological repercussions in the environment could be grouped as under:

(i) Operational hazards: - This could be during manufacture and formulation of pesticides in industrial settings and their distribution and use in field conditions.

(ii) Direct toxic effects on non-target animal life such as pollinators, predators, wild life and so on during application of pesticides.

(iii) Post application hazards or indirect toxic effects which involve risk to non-target animals due to toxic residues of pesticides in food or due to pollution of the ecosystem, habitat as a whole such as water bodies or soil (ICMR Bulletin, 2001).

2.7 Assessment of Human Exposure

Before allowing the use of a pesticide on food crops, tolerance or maximum residue level is set. This is the amount of pesticide residue allowed to remain in or on each treated food commodity. If residues are found above that level, the commodity will be subject to seizure by the government. Actual residues are unlikely to exceed this level when a pesticide is applied according to label directions. In setting the maximum residue levels, the following are considered:

(i) The toxicity of the pesticide and its break-down products.

(ii) How much of the pesticide is applied and how often.

(iii) How much of the pesticide residue remains in or on food by the time it is marketed and prepared.

(iv) All possible routes of exposure to that pesticide (residues on each crop use, as well as exposure from drinking water and residential exposure) (US EPA, 2015).

In human beings, the pesticide residue level is an index of exposure, which may be acute, occupational or incidental. In acute exposure, the residue level has a diagnostic potential, and in the occupationally exposed, the residue level merits an insight reflective of industrial exposure. However, in the general population, the residue level is a measure of the incidental exposure and/ or average levels of the persistent pesticides which are mainly through the food chain (ICMR Bulletin, 2001).

2.8 Residues in Food Commodities and Average Daily Intakes

The concentrations of pesticide residues in food samples vary greatly not only from region to region and year to year but also from one specific food item to another within the same food group (ICMR bulletin, 2001). They can even vary substantially from one food sample to the next within the same region of the country.

Perusal of the residue data on pesticides in samples of fruits, vegetables, cereals, pulses, grains, wheat flour, oils, eggs, meat, fish, poultry, cow milk, butter and cheese in India indicates their presence in sizeable amounts. For example, the fungicide hexachlorobenzene (HCB) was identified in water, human milk and human fat samples collected from Faridabad and Delhi. HCB residues were also found in various raw food commodities from some markets in India. DDT and Hexachlorocyclohexane (HCH) residues were detected in groundnut and sesame oil samples collected from Tamilnadu (ICMR Bulletin, 2001).

In a multi-centric study to assess the pesticide residues in selected food commodities collected from different states of India, DDT residues were found in about 82 % of the 2205 samples of cow milk collected from 12 states. About 37 % of the samples contained DDT residues above the tolerance limit of 0.05 mg/kg (whole milk basis) (ICMR Bulletin, 2001). The highest level of DDT residues found was 2.2 mg/kg. The proportion of the samples with residues above the tolerance limit was maximum in Maharastra (74 %) followed by Gujarat (70 %), Andhra Pradesh (57 %), Himachal Pradesh (56 %) and Punjab (51 %) (ICMR Bulletin, 2001). In the remaining states, this proportion was less than 10 %. Data on 186 samples of 20 commercial brands of infants formulae showed the presence of residues of DDT and HCH isomers in about 70 and 94 % of the samples with their maximum level of 4.3 and 5.7 mg/kg (fat basis), respectively (ICMR Bulletin, 2001).

The average total DDT and BHC consumed by an adult were 19.24 mg/day and 77.15 mg/day respectively. Fatty food was the main source of these contaminants. In another study, the average daily intake of HCH and DDT by Indians were reported to be 115 and 48 mg per person, respectively which were higher than those observed in most of the developed countries (ICMR Bulletin 2001). Measurement of chemicals in the total diet provides the best estimates of human exposure and of the potential risk. The risk of consumers may then be evaluated by comparison with toxicologically acceptable intake levels.

2.9 Toxicological Assessment

Toxicity studies aim to determine the nature and extent of toxic effects caused by the pesticide and to find doses that cause no adverse effects in the test animals (No Observed Adverse Effect Level (NOAEL)). A wide range of studies from acute to chronic is necessary on laboratory animals, with dosing regimens and animal examination designed to investigate effects like tumour initiation and production, changed bodyweight gain, increased liver weight, changed blood properties, enzyme inhibition and foetal abnormalities (Hamilton and Crossley, 2004).

The Acceptable Daily Intake (ADI) which is the acceptable level of long-term dietary exposure for humans may be calculated by using a safety factor (usually 100) from the NOAEL for the most sensitive animal species (ADI = NOAEL/100). The ADI is used in the chronic risk assessment and is expressed as an amount of chemical per kilogram of bodyweight (Hamilton and Crossley, 2004). Similarly, the acceptable level of short-term dietary exposure, referred to as the acute reference dose (acute RfD), for humans is calculated, where appropriate, with a safety factor applied to the NOAEL. Short-term toxicity tests provide information on the possible health hazards likely to arise from

repeated exposure over a period of time. The acute RfD is used in the acute risk assessment and is also expressed as an amount of chemical per kilogram of bodyweight (Hamilton and Crossley, 2004).

2.10 The Fate of Pesticides on/in Plants

Pesticide landing on plant surfaces may dissipate through volatilization, photolysis and by microbial activity on the leaf surface, as well as by washing off in rain. Concentrations in or on plant tissue may also decline through growth dilution effects. Many pesticides adsorb to the leaf surface, move into the waxy surface of the cuticle or are absorbed into plant cells, reducing the amount of residues which might wash off and enabling degradation by plant enzymes to occur (Hamilton and Crossley, 2004). Depending on its physical and chemical properties, a pesticide or altered product may become 'systemic,' entering the sap stream of the plant and moving either towards the apex, in the xylem or towards the base, in the phloem. Again depending on their chemical and physical properties, pesticides or their residues in soil beneath plants may be taken up by roots and be moved upwards into the plant in the sap stream (Hamilton and Crossley, 2004). With persistent substances, this may occur well after the original application. Thus, pesticides may move in the sap stream to parts of the plant not directly sprayed, and may leave residues within growing tissues, not just on the plant surface. Pesticide residues remaining in plants may be removed in harvested produce (Hamilton and Crossley, 2004), or may be released in the soil or retained in organic matter as the plant tissue decays.

2.11 Pesticide Metabolism in Crops

When a pesticide is applied to a crop it is subjected to a wide range of biological (enzymes), chemical (hydrolysis) and physical (photolysis) reactions, which may change

its chemical nature. These new chemical structures, called metabolites or degradation products, have different inherent properties to the parent pesticide and the effects of these changes need to be assessed both in terms of environmental and human safety (Hamilton and Crossley, 2004).

2.12 Factors Influencing the Level of Pesticide Residues in Plants

The nature of pesticide residues in plants can be influenced by: adsorption, distribution, metabolism and elimination processes.

2.12.1 Absorption

In a review of the absorption of herbicides, Sterling (1994) concludes that most herbicides are absorbed by plant cells by passive diffusion; however, examples are also given where the evidence suggests that some herbicides, dalapon, 2,4-D, glyphosate and paraquat are absorbed through active transport mechanisms. It is also concluded that weakly acidic herbicides can reach a higher concentration in the cell than that on the outside due to ion trapping in the alkaline components of the cell, the cytoplasm has a pH of approximately 7.5. Weakly basic herbicides are described as accumulating in the more acidic cell compartments such as the vacuole, pH 5.5. The physicochemical properties influencing absorption are lipophilicity, acidity, the cell membrane and the electrochemical potential in the cell.

The uptake of a xenobiotic (a foreign compound in a biological system) by the crop, following a pesticide treatment, depends on the degree of exposure from both the roots and aerial parts of the plant (Hamilton and Crossley, 2004). Pesticides on the surface of the plant or in the soil will be subject to a range of environmental factors, e.g. photolysis and microbial activity, which can result in degradation of the pesticide. These degradation products and the parent pesticide are therefore available for absorption. A further factor

influencing the uptake of xenobiotics from the soil is the interaction of chemicals with the soil (Hamilton and Crossley, 2004). To be absorbed into the roots the xenobiotic needs to be bioavailable or present in the soil–water compartment, a function of the interactions of the chemical with soil organic matter or clay particles. This interaction is measured as the adsorption coefficient, K_d or K_{oc}, and in most cases shows some reversibility (Hamilton and Crossley, 2004). The uptake of compounds by the roots is therefore a factor of the soil adsorption coefficient of the xenobiotic, the concentration gradient between the soil solution and that inside the root, lipophilicity, degree of ionization, and on the mass flow of water. Aerial parts of the plant, i.e. the outer surfaces of the leaf and stem, have layers of cuticular wax above the cell walls which serves as a barrier to water loss and to the entry of xenobiotic compounds (Hamilton and Crossley 2004).

Fantke *et al* (2012) studied the evolution of pesticide residues in crop components harvested for human consumption by taking wheat grains as example. Results show that grain surface and soil are the compartments predominantly influencing the mass evolution of most pesticides in the plant–environment system. Additional influences are associated with substance molecular weight and time span between pesticide application and crop harvest. The uptake and persistence behaviour of the insecticide imidacloprid in tomato plants treated by foliar spray application and soil irrigation was also studied. Total imidacloprid residues were up to five times higher in plants treated by foliar spray application than by soil irrigation. Model calculations suggest that drip-irrigation in a closed hydroponic system minimizes worker and consumer exposure to pesticides and prevents runoff of pesticide by spray drift and leaching into the environment (Juraske *et al*., 2009).

2.12.2 Distribution

Plants have an effective transport system that uses a collection of vascular conduits, xylem and phloem, to distribute nutrients, water and assimilates (Figure 2.1). The flow within these vascular tissues is driven by differences in water potential and osmotic gradients (Hamilton and Crossley, 2004).

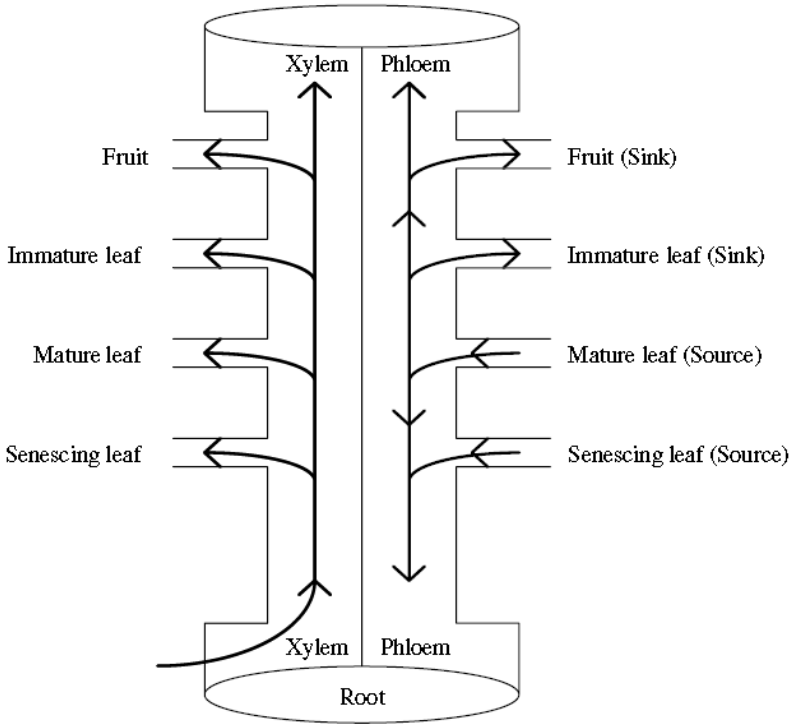


Figure 2.1: A schematic representation of the transport system in plants (Hamilton and Crossley, 2004).

The translocation of xenobiotics within these vascular systems depends on the entry into the system and retention of the compound in the conduits for a sufficient time for them to be transported to other plant tissues. Transport in the xylem depends on the ability of the xenobiotic to be absorbed and on its partitioning behaviour (Briggs *et al*., 1982). Xenobiotic compounds transported in the xylem will be accumulated at the leaf tip and

leaf margins, the extremities of the water flow (Hamilton and Crossley, 2004). A pesticide may be chemically changed during absorption or distribution by enzymes within the plant cells. Any changes are likely to impact the physicochemical characteristics and hence its distribution throughout the plant, e.g. a non-ionized lipophilic compound may enter a plant cell and be conjugated, thus increasing its polarity and hence its ability to diffuse out of the cell (Hamilton and Crossley, 2004).

2.12.3 Metabolism

'Metabolism' in plants refers to processes forming products from chemical reactions on the plant surfaces. These include hydrolysis and photolysis, biological processes which occur outside of the plant like microbiological degradation in soil and bio-transformations of the pesticide or any of the degradation products. The formation of photo-products can be a significant issue in the definition of the residue of concern and may require specific toxicology testing to assess their relevance and analysis of the crop commodities to determine their significance (Hamilton and Crossley, 2004).

The metabolizing capability of plants has been shown in many occasions to be similar to animals with some differences around the catabolism of specific conjugates. Sandermann (1994) went so far as to describe the xenobiotic metabolism in plants as resembling that found in the animal liver based on metabolic patterns and enzyme classes and proposed the 'green liver' concept. The capability of a plant to metabolize a xenobiotic can be influenced by its growth stage at the time of application. It has been shown that the levels of glutathione enzymes were higher in young leaves, which would facilitate the levels of metabolism (Hatton *et al*., 1996).

2.12.4 Elimination

Although plants do not have a classical excretory system, they do effectively eliminate xenobiotic compounds by removing them from active cell processes. They also remove them by storing them in the cell vacuole, (Cole, 1994), by reaction with the structural compartments of the plant, by exudation from the roots into the soil (Walker *et al*., 1994), or through volatilization. Pesticide residues eliminated from cell processes by reaction with lignin and cellulose represent bound residues and require specific assessment of their relevance during risk assessment (Hamilton and Crossley, 2004).

2.13 QuEChERS Extraction

Newly formulated pesticides are very different in their physical properties from their predecessors, such as 4,4'-DDT, they are smaller in molecular weight and designed to break down rapidly in the environment (David, 2008). Therefore, to successfully identify and quantify these compounds in foods, more careful consideration must be placed on sample preparation for extraction, in addition to the instrument parameters for analysis.

Anastassiades *et al* (2003), introduced an improved extraction procedure for the extraction of pesticide residues from food matrices known as **Qu**ick, **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe, the QuEChERS (pronounced “catchers”) based on work done and published by the US Department of Agriculture Eastern Regional Research Centre in Wyndmoor PA. The characteristic points of the QuEChERS method are: (l) extraction with acetonitrile in a disposable tube; (2) single-step salting out and removal of water from acetonitrile by use of anhydrous magnesium sulfate and sodium chloride (NaCl); (3) purification using dispersive-solid-phase extraction (SPE), in which the extract is processed by shaking with primary-secondary amine (PSA) silica gel and then centrifuged to separate the PSA before analysis. The merits of this method include decreased costs of experimental apparatus and solvents as well as reduction in the time

taken to perform the assay without loss of accuracy. In a follow-up study, it was discovered that the method could effectively monitor more than 200 pesticide residues by using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry. It was designed for samples with more than 75% moisture like fruits and vegetables (Kolberg, *et al*., 2011).

It is an extraction method for pesticides in fruits and vegetables, coupled with a clean-up method that removes sugars, lipids, organic acids, sterols, proteins, pigments and excess water (Anastassiades *et al*., 2003). The technique offers a user-friendly alternative to traditional liquid-liquid and solid phase extractions (Anastassiades *et al*., 2003). It employs dispersive solid phase extraction (dSPE), where organic solvent is mixed with a sample, and gram levels of magnesium sulphate are used (to partition water from organic solvent) and primary secondary amine (PSA) adsorbent (to remove sugars and fatty acids), with or without graphitized carbon (to remove pigments and sterols) and/or C18 packing (to remove nonpolar interferences such as lipids) (Anastassiades *et al*., 2003).

QuEChERS is a technique that was developed for multi-class, multi-residue pesticides analysis in fruits and vegetables but more recently has expanded its scope to other trace contaminants in other non-vegetable foods such as meat and fish. Methods for hundreds of pesticides in a variety of fruits, vegetables, meat, and for dry materials such as beans and nuts have been published (Agilent tech, 2009). In the United States, the Association of Official Analytical Chemists (AOAC) has published its 2007.01 method while the European equivalent, the EN 15662 2007, uses similar methodology (QuEChERS, 2010). The technique has resulted in a practical and reliable method for pesticide residues at low detection limits with good precision and accuracy for over 150 pesticides in a wide range of fruits and vegetables (Brown, 2005).

2.14 Gas Chromatography/ Mass Spectrometry

Gas chromatography coupled with quadrupole mass spectrometry detection is considered a powerful technique for the quantitative determination of lower levels of contaminants in complex matrices (Kolberg *et al*., 2011). It is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample.

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer (Karasek and Clement, 1988). The gas chromatograph utilizes a capillary column which depends on the column's dimensions as well as the phase properties. The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amounts of time (called the retention time) to elute from the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately (Karasek and Clement, 1998).

The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. These two components, used together, allow a much finer degree of substance identification than either unit used separately (Karasek and Clement, 1998).

A mass spectrometer is typically utilized in one of two ways: Full Scan or Selective Ion Monitoring (SIM). The typical GC/MS instrument is capable of performing both functions either individually or concomitantly, depending on the set-up of the particular instrument. A schematic diagram of a GC/MS is shown in Figure 2.2.

2.14.1 Full scan MS

In full scan mode, the MS monitors a wide range of mass fragments during a given scan time. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences (Message, 1984). Full scan is useful in determining unknown compounds in a sample. It provides more information than selective ion monitoring (SIM) when it comes to confirming or resolving compounds in a sample (Message, 1984). During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

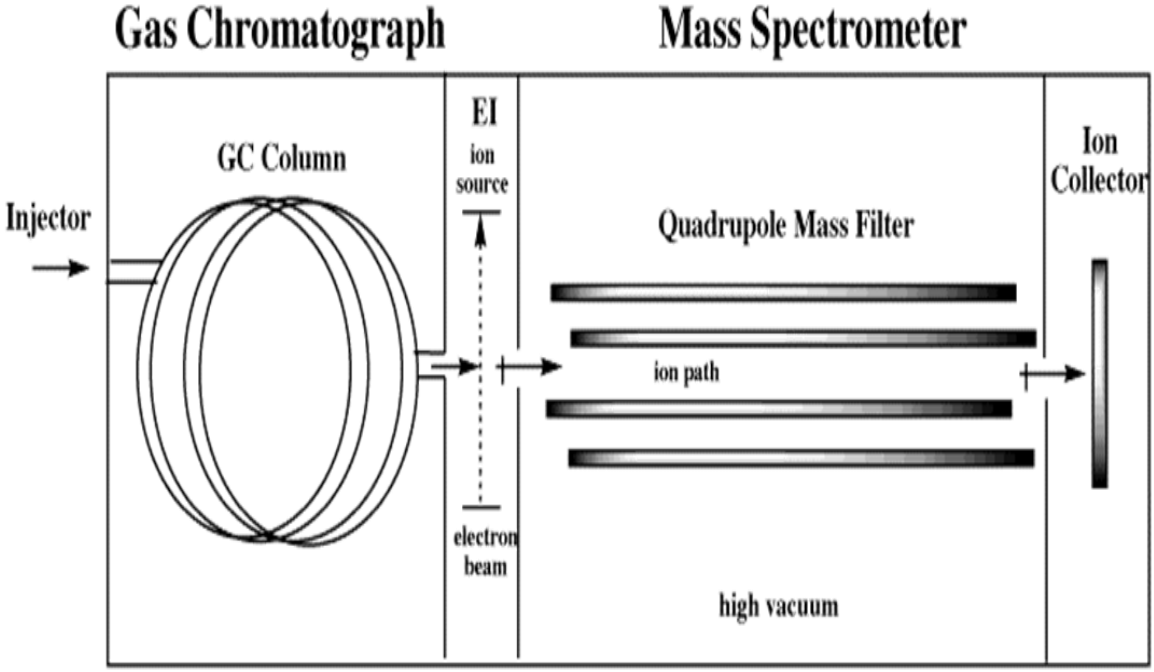


Figure 2.2: Schematic Diagram of GC/MS (University of Colorado, 2011).

2.14.2 Selective ion monitoring

In selective ion monitoring (SIM) mode, the MS monitors certain ion fragments and only those mass fragments are detected by the mass spectrometer (Karasek and Clement, 1988).). The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan (Karasek and Clement, 1988). More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard (Karasek and Clement, 1988).

2.14.3 Electron ionization

The most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS where they are bombarded with free electrons emitted from a filament, the electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way (Message, 1984). This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV. The use of 70 eV facilitates comparison of generated spectra with library of spectra applying algorithmic matching programs (Message, 1984).

2.14.4 Types of Mass Spectrometer Detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer and time of flight (TOF), tandem quadrupoles (MS-MS) (Adlard *et al*., 2001).

2.15 Physico-Chemical Properties of Soil

2.15.1 pH of soil

The pH of soil is a measure of its acidity or alkalinity. It is a valuable variable in soils as it specifically affects plant nutrient availability by controlling the chemical forms of the nutrient. The optimum pH range for most plants is between 5.5 and 7.0. It is the most frequently determined parameter in soil analysis (Alloway, 1995). The measurement of pH in soil is affected by salt concentration and changes in redox potential. Heavy metal cations are most mobile under acid conditions and increasing the pH usually reduces their bioavailability (Alloway, 1995). If the soil pH values are very low, toxic levels can soon be reached and this can damage the roots of plants. Moreover, excessively high pH values quickly leads to a shortage of trace elements because of immobilization.

2.15.2 Electrical conductivity of soil

Electrical conductivity (EC) is the most common measure of soil salinity and is indicative of the ability of an aqueous solution to carry an electric current. The electrical conductivity indicates the amount of dissolved inorganic salts in water and is thus a fast and inexpensive indicator for estimating the extent of contamination (Nwakife, 2015). EC affects crop yields, crop suitability, plant nutrient availability and activity of soil

microorganisms (Nwakife, 2015). Although EC does not provide a direct measurement of specific ions or salt compounds, it has been correlated to concentrations of nitrates, potassium, sodium, chloride, sulphates and ammonia (Nwakife, 2015). EC is higher in low areas or other wet areas where water accumulates than higher-lying, better drained areas. Clay soils that have high cation-exchange capacity (CEC), have higher EC than clays that have low CEC (Nwakife, 2015).

2.15.3 Soil texture/ particle size

Soil texture is a qualitative classification tool used in both the field and laboratory to determine classes for agricultural soils based on their physical texture while size refers to the diameter of individual grains of sediment (Sumaila, 2014). The particle sizes of soil are one of the important factors which determine the level of soil contamination with heavy metals and their amounts in plants (Sumaila, 2014). In general the heavy metal content of the soil increases with decrease in soil particle size (Kabata-Pendias and Pendias, 2001). Soil particles are broadly segregated into three size classes:

(i) Sand comprises quartz and resistant primary minerals such as mica. Sand particles are between 2 mm and 20 microns in size.

(ii) Silt are typically composed of quartz and small mineral particles such as feldspars and mica, and are between 2 and 20 microns in diameter.

(iii) Clay are made up of secondary clay minerals and oxides/oxyhydroxides of iron and aluminium, and are less than 2 microns in diameter.

Particle size analysis is the determination of percentage sand, silt and clay particles, often reported as percentage by weight of oven-dry and organic-matter free soil. The determination of the particle size is based on the fact that particles suspended in water settle depending on the amount of surface per unit volume (Kabata-Pendias and Pendias,

2001). The rate at which they settle can be calculated using Stoke's law "if all other factors are constant, the settling velocity is proportional to the square of the radius of the particle''. Once the percentage sand, silt and clay are measured, the soil is assigned a textural class based on the textural triangle shown below.

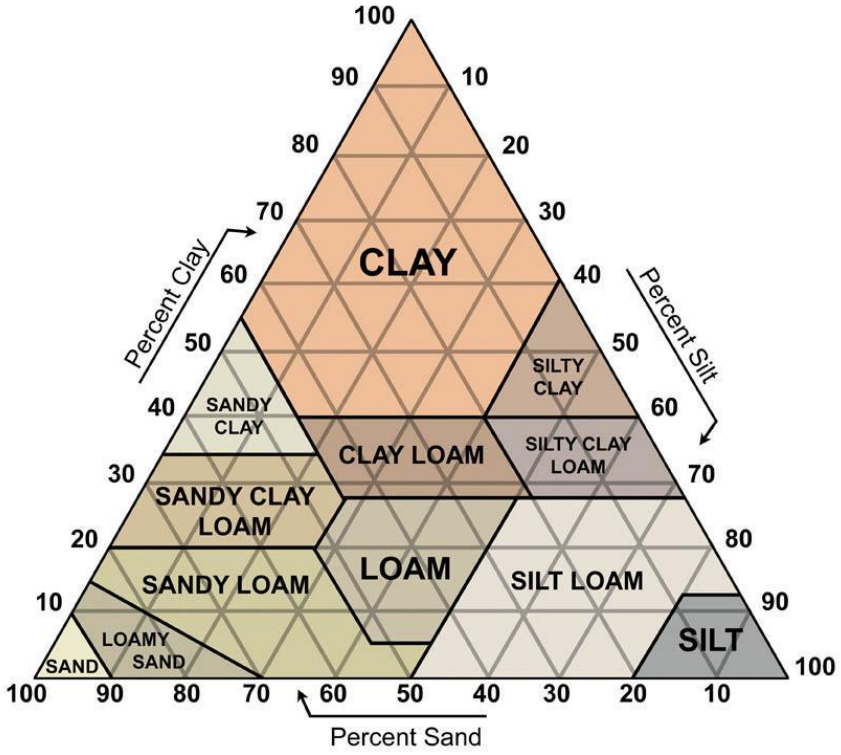


Figure 2.3: Soil textural class triangle (US department of agriculture, 1993)

2.15.4 Soil organic matter (SOM)

Soil organic matter exerts numerous positive effects on soil physical and chemical properties, as well as the soil's capacity to provide regulatory ecosystem services (Brady and Weil, 1999). The concentration of SOM in soils generally ranges from 1 % to 6 % of the total topsoil mass for most upland soils. Soils whose upper horizons consist of less than 1 % organic matter are mostly limited to desert areas, while the SOM content of soils in low-lying, wet areas can be as high as 90 %. Soils containing 12-18 % soil organic carbon are generally classified as organic soils (Troeh and Louis, 2005).

The positive impacts of soil organic matter include: improvements related to soil structure, aggregation, water retention, soil biodiversity, absorption and retention of pollutants, buffering capacity, and the cycling/storage of plant nutrients. Soil organic matter increases soil fertility by providing cation exchange sites and acting as reserve of plant nutrients, especially nitrogen, phosphorus, and sulfur, along with micronutrients, which are slowly released upon soil organic matter mineralization (Nwakife, 2015).

2.15.5 Cation exchange capacity (CEC)

Cation exchange capacity (CEC) is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton and Murphy, 2007). The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force. This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. magnesium, potassium and calcium). In general terms, soils with large quantities of negative charge are more fertile because they retain more cations. However, productive crops and pastures can be grown on low CEC soils (McKenzie *et al*., 2004).

The main ions associated with CEC in soils are the exchangeable cations calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) (Rayment and Higginson 1992), and are generally referred to as the base cations. The CEC of soils varies according the clay %, the type of clay, soil pH and amount of organic matter (Nwakife,2015). Pure sand has a very low CEC, less than 2 meq/100 g, and the CEC of the sand and silt size fractions (2 µm/2 mm) of most soils is negligible. Soils with a low CEC are more likely to develop

deficiencies in potassium (K^+), magnesium (Mg^{2+}) and other cations while high CEC soils are less susceptible to leaching of these cations (CUCE, 2007).

2.16 Heavy Metals

Heavy metals may enter the human body through inhalation of dust, consumption of contaminated drinking water and consumption of food plants grown in metal contaminated soil (Radwam and Salama 2006 ; Brhane and Shiferaw 2014). Studies showed that metals like cadmium, lead, copper, zinc, iron, chromium and manganese are found at toxic levels in soils and vegetables (Qadir *et al*., 2000). Heavy metals are found naturally in the earth, and become concentrated as a result of human caused activities (Qadir *et al*., 2000). Common sources are from mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilisers, paints and treated woods. Lead is the most prevalent heavy metal contaminant (Di Maio, 2001). As a component of tetra-ethyl lead it was used extensively in gasoline during the 1930s-1970s (Lovei, 1998). Lead levels in the aquatic environments of industrialized societies have been estimated to be two to three times those of pre-industrial levels. (Perry and Vanderklein, 1996). Although the use of leaded gasoline was largely phased out in North America by 1996, soils next to roads built before this time retains high concentrations of lead (Perry and Vanderklein, 1996).

Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing, particularly in less developed countries of the world. For instance, cadmium compounds are currently mainly used in re-chargeable nickel-cadmium batteries (Jarup, 2003). Emissions of cadmium have increased dramatically during the 20th century because cadmium containing products are rarely recycled, but often dumped together with household waste. While cigarette is

another source of cadmium exposure in smokers, food is the most important source of cadmium exposure (Jarup, 2003).

Living organisms need some heavy metals for metabolic processes but excess concentrations have a lethal effect (Girisha and Ragavendra, 2009). Due to their importance, the intake of these metals by humans through food chain has been of great concern and has recently been of intense interest by several workers (Bahemuka and Mubofu 1999, Ellen *et al*., 1990). Heavy metals can bind to vital cellular components, such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning (Landis *et al*., 2000). Symptoms and effects can vary according to the metal or metal-compound, and the dose involved. Broadly, long-term exposure to heavy metals can have carcinogenic, central and peripheral nervous system and circulatory effects (Landis *et al*., 2000).

The thresholds of dietary toxicity of heavy metals in soil-crop system is affected by factors such as soil type which includes soil pH, organic matter content, clay mineral and other soil chemical and biochemical properties and crop species. In addition, the interaction of soil-plant root-microbes play important roles in regulating heavy metal movement from soil to the edible parts of crops (Mubofu, 2012).

2.16.1 Cadmium

Cadmium is a heavy metal posing severe risks to human health. It has not yet been shown to have any physiological function within the human body (Ali *et al.*, 2013). Interest has therefore risen in its bio-hazardous potential (Jarup, 2003). Cadmium intoxication can lead to kidney, bone, and pulmonary damages. It is regularly found in ores together with zinc, copper and lead. Therefore volcanic activity is one natural reason for a temporary

increase in environmental cadmium concentrations. Cadmium is widely used in industrial processes, e.g as an anticorrosive agent, as a stabilizer in PVC products, as a colour pigment, a neutron-absorber in nuclear power plants, and in the fabrication of nickel-cadmium batteries. Phosphate fertilizers also show a big cadmium load. Although some cadmium-containing products can be recycled, a large share of the general cadmium pollution is caused by dumping and incinerating cadmium-polluted waste (Jarup, 2003).

The uptake through the human gastrointestinal tract is approximately 5 % of an ingested amount of cadmium, depending on the exact dose and nutritional composition (Jin *et al.*, 2002). Furthermore a high fiber diet increases the dietary cadmium intake. The most important metabolic parameter for cadmium uptake is a person's possible lack of iron. People with low iron supplies showed a 6 % higher uptake of cadmium than those with a balanced iron stock (Godt *et al*., 2006). This is the main reason for the higher cadmium resorption in people with anaemia and habitual iron deficit, such as children or menstruating women (Godt *et al*., 2006). The main organ for long-term cadmium accumulation is the kidney (Orlowski and Piotrowski 2003). Here the half-life period for cadmium is approximately 10 years. A life-long intake can therefore lead to a cadmium accumulation in the kidney, consequently resulting in tubulus cell necrosis. The blood concentration of cadmium serves as a reliable indicator for a recent exposition, while the urinary concentration reflects past exposure, body burden and renal accumulation (Godt *et al.*, 2006).

Excretion of cadmium takes place via faeces and urine. Kidney damage has long been described to be the main problem for patients chronically exposed to cadmium (Barbier *et al*., 2005). Cadmium could also be shown to be associated with occurrences of Itai-Itai, a disease under which patients show a wide range of symptoms such as: low grade of

bone mineralization, high rate of fractures, increased rate of osteoporosis, and intense bone associated pain (Barbier *et al*., 2005). An epidemic occurrence of the Itai-Itai disease was observed in the Jinzu river basin (Japan) in the 1940s. In a study on this occasion, patients were found to show the characteristic symptoms after having eaten rice, grown on fields irrigated with highly cadmium polluted water (Godt *et al*., 2006).

Cadmium can easily move through water movement in the upper layers of soil, from where it is absorbed by plants and is accumulated in leafy vegetables, root crops, cereals and grains as a result of which it enters into food chain (ATSDR, 1999).

2.16.2 Copper

Copper is the third most used metal in the world (Wuana and Okieimen 2011). Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (Abbas *et al*, 2010).

2.16.3 Zinc

Zinc can be found in nearly all soils. It is present in most rocks and is weathered out and deposited into the soil. Zinc is also released by thermal outgassing and other volcanic events. Fallout from such events can be a significant source of zinc in soils and plants.

Anthropogenic release is the primary source of zinc in the environment. Zinc is released from industrial and manufacturing facilities in wastewater effluent or from incinerators. Zinc is used as a constituent in several alloys, including brass, bronze, die-cast metals, and is combined with copper for the production of US pennies. Zinc is also used in

electroplating, smelting, and ore processing (ATSDR 1994). Cobb *et al* (2000) reported that mine tailings and drainage from mines can contain high concentrations of zinc.

The fate and transport of zinc (Zn^{+2}) in the environment is dependent on cation exchange capacity, pH, organic matter content, nature of complexing ligands, and the concentration of the metal in the soil. As pH increases, there is an increase in negatively charged binding sites on soil particles, which facilitates the adsorption of zinc ions and removal from solution (ATSDR 1994). The Zn concentration in the soil and clay content are positively correlated (Lee *et al*., 1998). The most common form of zinc in anaerobic soils is the insoluble zinc sulfide. Therefore, mobility is limited in anaerobic conditions. Zinc mobility increases with low pH (e.g. < 7) under oxidizing conditions and low cation exchange capacity (ATSDR 1994). The presence of competing metal ions and organic ions such as humic material may cause the adsorption of Zn ions to the soil, particularly in soils with an elevated pH, via ligand exchange reactions (ATSDR 1994). These reactions reduce the solubility of zinc in the soil solution and, therefore, reducing its mobility and limit its bioavailability (ATSDR 1994).

2.16.4 Nickel

Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be toxic when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of Ni is an ingredient of steel and other metal products. The major sources of nickel contamination in the soil are metal plating industries, combustion of fossil fuels, and nickel mining and electroplating (Khodadoust *et al*., 2004). It is released into the air by power plants and trash incinerators and settles to the ground after undergoing precipitation reactions. It

usually takes a long time for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all Ni compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic soils, however, Ni becomes more mobile and often leaches down to the adjacent groundwater. Microorganisms can also suffer from growth decline due to the presence of Ni, but they usually develop resistance to Ni after a while. Nickel is not known to accumulate in plants or animals and as a result Ni has not been found to bio-magnify up the food chain (Wuana and Okieimen, 2011).

According to Ismail *et al* (2011), Ni plays its role as a coenzyme in different enzymes and its low content in vegetables and fruits sources can lead to increased blood sugar level, hypertension and deficient growth in human. On the other hand the increased uptake of Ni in fruits and vegetables can lead to reduced blood glucose level, difficulty in breathing and nausea. It is reported that plant growth can be affected if Ni is accumulated above 50 μg/g. Ni is absorbed in plant through root cap and its uptake depends upon environmental factors e.g. temperature, air, pH and so on. Ni absorption is increased as the phosphate contents are increasing and Ni uptake in plant is usually 0.1 % of the dry weight of the plant. It is usually accumulated in vegetative part of the plant body and it is mobile through the plant structure, translocate and become concentrated in the leaves. But after ageing of the leaves it is moved to the seeds for accumulation. Ni and other metal absorption are sometimes affected by fertilizers (Ismail *et al*., 2011).

2.16.5 Manganese

Manganese is a naturally-occurring metal that, in pure form, is silver-coloured with no taste or smell. It is an essential element of the human diet and the second most abundant heavy metal after Iron. Manganese is normally encountered in the environment as a

compound with oxygen, sulfur, or chlorine (ATSDR, 2000). It is an essential nutrient, required in trace amounts for human health and intake is normally sufficient with a balanced diet. However, elevated Mn levels can cause human neurotoxicity. Notably, workers exposed to high airborne Mn levels are at elevated risk of developing a Parkinson's disease -like neurological disorder known as manganism (Cersosimo and Koller, 2006).

There are two forms of manganese in the environment. Inorganic manganese compounds are used in the production of steel, batteries, ceramics, and dietary supplements. These manganese compounds are also generated as combustion products from motor vehicles and coal-burning industrial plants. Organic manganese compounds are used in some pesticides, fertilizers, and in a gasoline additive called methylcyclopentadienyl manganese tricarbonyl (MMT) ((ATSDR, 2000). Manganese compounds can be present as dust particles in the air, and dissolved in ground water or drinking water ((ATSDR, 2000). Walsh (2007) reported that there is significant concern regarding increased human exposure to Mn through use of MMT, and regions using MMT have elevated air Mn levels, particularly near roads with high traffic (Bankovitch *et al.,* 2003).

The primary targets of manganese toxicity are the brain and central nervous system (ATSDR, 2000). Manganese has been shown to be deposited in certain regions of the brain, and exposure to high concentrations in occupational studies was associated with permanent damage, with symptoms of impaired neurological and neuromuscular control, mental and emotional disturbances, muscle stiffness, lack of coordination, tremors, difficulties with breathing or swallowing, and other neuromuscular problems (ATSDR, 2000). Manganese toxicity varies with exposure route. When ingested, manganese may

be among the least toxic of the trace elements (US, EPA, 1988). Inhaled manganese bypasses the gut and can enter the brain in two ways: by olfactory (nasal airway) neural pathways that provide a direct path to brain tissue, and by lung uptake and long residence time that could provide a source of continuing exposure (Weiss, 2006).

2.16.6 Iron

Iron is the second most abundant metal in the earth's crust, of which it accounts for about 5 %. Elemental iron is rarely found in nature, as it combine with oxygen- and sulfur-containing compounds to form oxides, hydroxides, carbonates, and sulphides. Iron is most commonly found in nature in the form of its oxides (Elinder, 1986).

Gurzau *et al.,* (2003) reported that Fe is a necessary metal and is core component of the red blood cells, its deficiency can cause anaemia. Fe is found in two oxidative states ferrous and ferric, only ferrous state is absorbed through the intestinal track and ferric state is reduced by the food constituents (Ismail *et al*., 2011). The absorption of ferrous state can be inhibited by the formation of phosphatides. The daily intake limits is 8-11 mg/day. Fe deficiency is a global problem especially in Asian countries (Brown, 2004). It is reported that about 60 % of the world population is Fe deficient (Ismail *et al*., 2011). It is also needed in the RBC's formation and haemoglobin while its deficiency can lead to anaemia. It is reported that in children iron is needed in more quantity as it increases the blood volume. In muscles, the myoglobin contain iron which gives it the red colour. Green vegetables, fruits, mustard soya bean, liver, kidney, egg yolk are the good sources of Fe (Ismail *et al*., 2011).

Iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, as a result of the formation of free radicals. Disorders of iron metabolism are among the most common diseases of humans, encompassing a broad spectrum of diseases with diverse clinical manifestations ranging from anaemia to iron

overload and, possibly, to neurodegenerative conditions. Understanding iron regulation in the molecular level is critical in identifying the underlying causes for each disease and in providing proper diagnosis and treatments (Evans and Halliwell, 2001; Kuvibidila *et al*., 2001).

Iron has the capacity to accept and donate electrons readily, this capability makes it physiologically essential, as a useful component of cytochromes and oxygen binding molecules. However, iron is also biochemically dangerous because it can damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, protein, and DNA. In the healthy state, this threat is reduced because of the fine regulation of iron metabolism, there is never an appreciable concentration of ‘‘free iron’’. With pathological conditions, however, iron metabolism and superoxide metabolism are clearly interactive. Each can exacerbate the toxicity of the other. Iron overload may amplify the damaging effects of superoxide overproduction in a very broad spectrum of inflammatory conditions, both acute and chronic. Furthermore, chronic oxidative stress may modulate iron uptake and storage, leading to a self-sustained and ever-increasing spiral of cytotoxic and mutagenic events (Emerit *et al*., 2001).

2.17 Heavy Metals in Soils

Soil is a natural body consisting of layers of mineral constituents of variable thickness, which differ from the parent materials in their morphological, physical, chemical and mineralogical characteristics. It is a mixture of minerals or organic constituents that are in solid, gaseous and aqueous states. Soil is formed from weathering of rocks and minerals, the surface rock break into smaller pieces through a process of weathering of rock surface and organic matters (Ojiako and Okonkwo, 2013). The contamination of agricultural soils is often a direct or indirect consequence of anthropogenic activities (McLaughlin *et al.*, 1999). Sources of anthropogenic metal contamination in soils include:

urban and industrial wastes; mining and smelting of non-ferrous metals and metallurgical industries (Singh, 2001). Other ways of metal accumulation in soil is by irrigating farmland with poor quality water. Weathering of rocks, transportation and deposition of heavy metals on farmland and leaching from refuse dumpsite are major sources of soil pollution by heavy metals. (Dosumu, *et al*., 2005).

Soils may also become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan *et al*., 2008 and Zhang *et al*., 2010). Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (GWRTAC,1997). Soils are the major sink for heavy metals released into the environment by aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.,* 2006), and their total concentration in soils persists for a long time after their introduction (Adriano, 2003). Changes in their chemical forms and bioavailability are, however, possible. The presence of toxic metals in soil can severely inhibit the biodegradation of organic contaminants (Maslin and Maier, 2000). Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land

usability for agricultural production causing food insecurity, and land tenure problems (McLaughlin *et al*., 2000a; McLaughlin *et al*., 2000b; Ling *et al*., 2007).

The fate and transport of a heavy metals in soil depends significantly on the chemical form and speciation of the metal (Shiowatana *et al*., 2001). Once in the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity (Shiowatana *et al.,* 2001; Buekers, 2007). This distribution is believed to be controlled by reactions of heavy metals in soils such as (i) mineral precipitation and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake (Levy *et al*, 1992).

Heavy metals in trace amounts are micronutrients needed by soil, however some are not essential and hence do not possess specific mechanisms for their accumulation in the soil (Cervantes, 2001). The uptakes of such type of heavy metals are carried through carriers used for the uptake of essential metals for soil (Cervantes, 2001). The retention capacity of heavy metal by soil also depends upon the oxidation and valence state of the metal. It was found that soil accumulate more chromium (VI) than chromium (III). It has been seen that the maximum quantity of elements contaminant was always contained in the bottom soil and a minimum in the top soil. The sources of the metals could be due to atmospheric deposition from mining activities, industrial, fertilizer or water used for irrigation. The reason for low accumulation in the top soil could be as a result of leaching (Zayed, 1998). The soil particle size could affect the availability of essential metals in the soil, whereby the structure and size of the soil particles determine the surface area that influences the chemical and physical characteristics of the mineral. However the metals content in the soil increases with a decrease in the soil particle size (Sumaila, 2014). The pH of soil

should be around 5.5 – 7.5 for normal soil, so pH of soil below this range caused low availability of most of essential minerals needed by the soil.

In a study to assay the presence of heavy metals and their possible effects on the ecosystem, soil samples were collected at different areas of mechanic workshops of Onitsha metropolis. The findings revealed that the inhabitants of those areas are put in danger due to high concentrations of Pb, Cu, Ni and Cr (Ojiako and Okonkwo, 2013).

Sumaila, (2014) determined the levels of Cu, Mn, Fe, Ni, Zn, Co and Cd in soils obtained from farms in Paiko, Niger State. The result reveals that all the agricultural soils were highly contaminated with iron which could cause serious health problems to people.

Pujar *et al* (2012) expressed a growing concern about the possibility of soil contamination resulting in uptake by plants and the introduction of the elements in vital food chains affecting food safety. These metals can pose a significant health risk to humans, particularly in elevated concentrations above the very low body requirements (Gupta and Gupta, 1998).

Fernandez–Turiel *et al*., (2001) found elevated levels of heavy metals in urban soils located within the vicinity of a lead smelter in Lastenia, Argentina. Other sources of human activities contamination include the addition of manures, sewage sludge, fertilizers and pesticides to soils, with a number of studies identifying the risks in relation to increased soil metal concentration and consequent crop uptake (Whatmuff, 2002).

2.18 Heavy Metals in Vegetables

The consumption of vegetables and fruits as food offer rapid means of providing adequate vitamins supplies, minerals and fibre. Vegetables used as food include those used in making soups or served as integral parts of the main sources of a meal (Ihekeronye, and Ngoddy, 1985). Leafy vegetables occupy a very important place in the human diet, they

also act as neutralizing agents for acidic substances formed during digestion (Yusuf, and Oluwole, 2009) but unfortunately constitute a group of foods which contributes maximally to heavy metals consumption (Akpan *et al*., 2013). In developing countries like Nigeria, rapid and unorganized industrialization and urbanization have contributed to the elevated levels of heavy metals in the environment (Akpan *et al.,* 2013). They are non-biodegradable and persistent environmental contaminants that may be deposited on the surface and then adsorbed into the tissues of vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environment as well as from contaminated soils (Singh *et al*., 2010).

Commercial and residential vegetable growing areas are subject to atmospheric pollution, in the form of metal containing aerosols (Yusuf and Oluwole, 2009). These aerosols can enter the soil and be absorbed by vegetables, or alternatively be deposited on leaves and adsorbed. Studies of vegetables grown in locations close to industry have reported elevated levels of heavy metals. Vousta *et al.,* (1996) studied the impact of atmospheric pollution from industry on heavy metal contamination in vegetables grown in Greece. The results of the study indicated significantly higher levels of metal accumulation in leafy vegetables as compared with root vegetables. Another study also shows accumulation of greater concentrations of cadmium in the edible leafy portions of crops, than the storage organs or fruit (Jinadasa *et al*., 1997; Lehoczky *et al.,* 1998).

The bioaccumulation of metals in vegetables has also been shown to distort the composition of beneficial nutrients in them. For example, the accumulation of chromium has led to decrease in protein and chlorophyll contents of a species of aquatic plant found in fresh water habitats called Najas indica (Dosumu, *et al*., 2005). Other studies also have

revealed that plants have different metal absorption ability and capacity (Sarita, *et al*., 2002). Accumulation of heavy metals by vegetables may depend on plant species as well as concentration of the heavy metal. It may also depend on the efficiency of different plants in absorbing metals, which is evaluated by either plant uptake or soil-to plant transfer factors of the metals (Brhane and Shiferaw, 2014). Contamination of vegetables with heavy metals may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale (Brhane and Shiferaw, 2014). These heavy metals are not abundant in soil, but may be accumulated through disposal of sewage water. Sewage effluents are considered not only a rich source of organic matter and other nutrients but also they elevate the level of Cu, Pb, Cr and Cd in receiving vegetable of soils (Kalaskar M, 2012). Elevated concentration of these metals can consequently affect human vital organs, leading to unwanted side effects (Jarup, 2003; Sathawara *et al*., 2004). Jamali *et al*., (2007) also supported the view that vegetables grown on agriculture land irrigated with waste water or dressed with solid sewage sludge are at high risk of heavy metal contamination.

The uptake of heavy metals by plants grown in polluted soils has been studied to a considerable extent (Yusuf and Oluwole, 2009). Akan *et al.,* (2013) determined the concentration of some heavy metals in spinach, lettuce, cabbage and onions freshly harvested from farms in Biu, Borno state. The results showed that leaves contained much higher concentrations of heavy metals than roots and stems. These values were higher than those recommended by Food and Agricultural Organization (FAO) and the WHO/EU joint limits. The high levels of these heavy metals place the consumers of these vegetable crops grown within the study area at health risk with time unless an urgent step is taken by relevant agencies to address the issue. Ismail *et al* (2011) reported that a work

on heavy metals in seasonal vegetables from Turkey reveals that vegetables collected from urban areas have higher contentment of metals uptake than those from rural areas. The findings of another research showed that heavy metal contamination of edible vegetable is high at road construction sites (Otitoju *et al.,* 2012). In most areas in the world where vegetables are cultivated during dry season, farmers usually depend on water from river for irrigation, and this water might already be contaminated with high amount of trace metals (Ayers and Westcott, 1985).

Maleki and Zarasvand (2008) also agreed on the fact that cultivation of vegetables on contaminated lands leads to accumulation of toxic metals in them which then enter the human body through the consumption of these contaminated plants The plants may also be contaminated during their harvesting and transportation as a result of atmospheric deposition from industries, mining and automobiles discharge (Aljassir *et al*., 2005).

Ladipo and Doherty (2011) analysed the level of Pb, Cd and Zn in fluted-pumpkin leaves from Lagos markets. Results revealed that the levels of Cd and Zn were within the safe limit of WHO/FAO and Pb was not detected in all samples. In another study conducted by Sumaila (2014) to assess the levels of Cu, Mn, Fe, Ni, Zn, Co and Cd in vegetables grown on some farm lands in Paiko, Niger State, the results showed that leafy vegetables had relatively higher manganese contents than non-leafy vegetables probably due to the fact that leaves are food-making house of plants and could have higher mineral contents than the other parts or they might have picked this metal from atmospheric deposition (Ayodele and Abubakar, 2001).

Jarup (2003) reported prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of the metals in the kidney and

liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases.

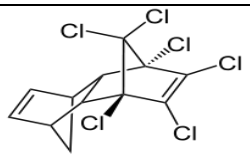
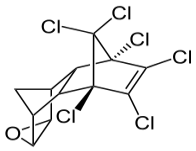
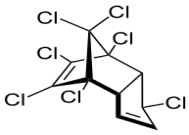
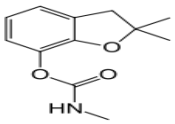
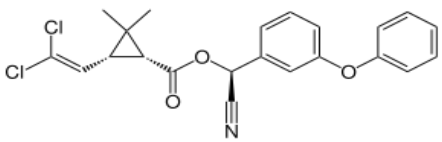
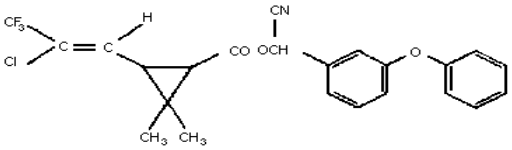
CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Survey

A survey was conducted to determine the types of pesticides used by farmers in the study area. Information was obtained from the State Agricultural Development Project (ADP), the vegetable farmers and pesticide vendors. Selected pesticides for analysis and their chemical structures are shown in Table 3.1.

Table 3.1: Pesticides and their Chemical Structures (NPIC, 1998).

Pesticide	Chemical Structure
Aldrin	
Endrin	
Heptachlor	
Carbofuran	
Cypermethrin	
Lambda-cyhalothrin	

3.2 Samples

The samples used for the research were spinach leaves (*Amaranthus hybridus*), okra fruit (*Hibiscus esculentus*) and fluted pumpkin leaves, (*Telfairia occidentalis*). These vegetables were taken from seven selected farms in some areas of Minna metropolis as shown in Table 3.2. Okada Farm (A) situated about 25 km away from most of the farms and where anthropogenic activities are the least was taken as the control farm for the heavy metals analysis. Figure 3.1 is the map of Nigeria showing Niger State and map of Niger State showing the Local Govt. areas. Figure 3.2 is the map of Minna showing the sampling sites.

Table 3.2: Names of farms and their designation

Farm Name	Designation
Okada Road farm	A_C
Kpakungu farm	B
Dutsen kura farm	C
Keteren gwari farm	D
Chanchaga farm	E
Mandela farm	F
Sauka ka huta farm	G

3.3 Sampling

All three vegetable samples were obtained from each of the seven farms. A systemic approach of sampling was adopted with some random elements (SANCO, 2007). An imaginary **W** was drawn on the particular area in the farm where the samples were to be taken, then primary samples were collected at intervals along the four arms of the **W** following standard procedures reported by Hemingway (1984). 500g of each vegetable was sampled.



Figure 3.1: Map of Nigeria showing Niger State and Map of Niger State showing local government Areas.

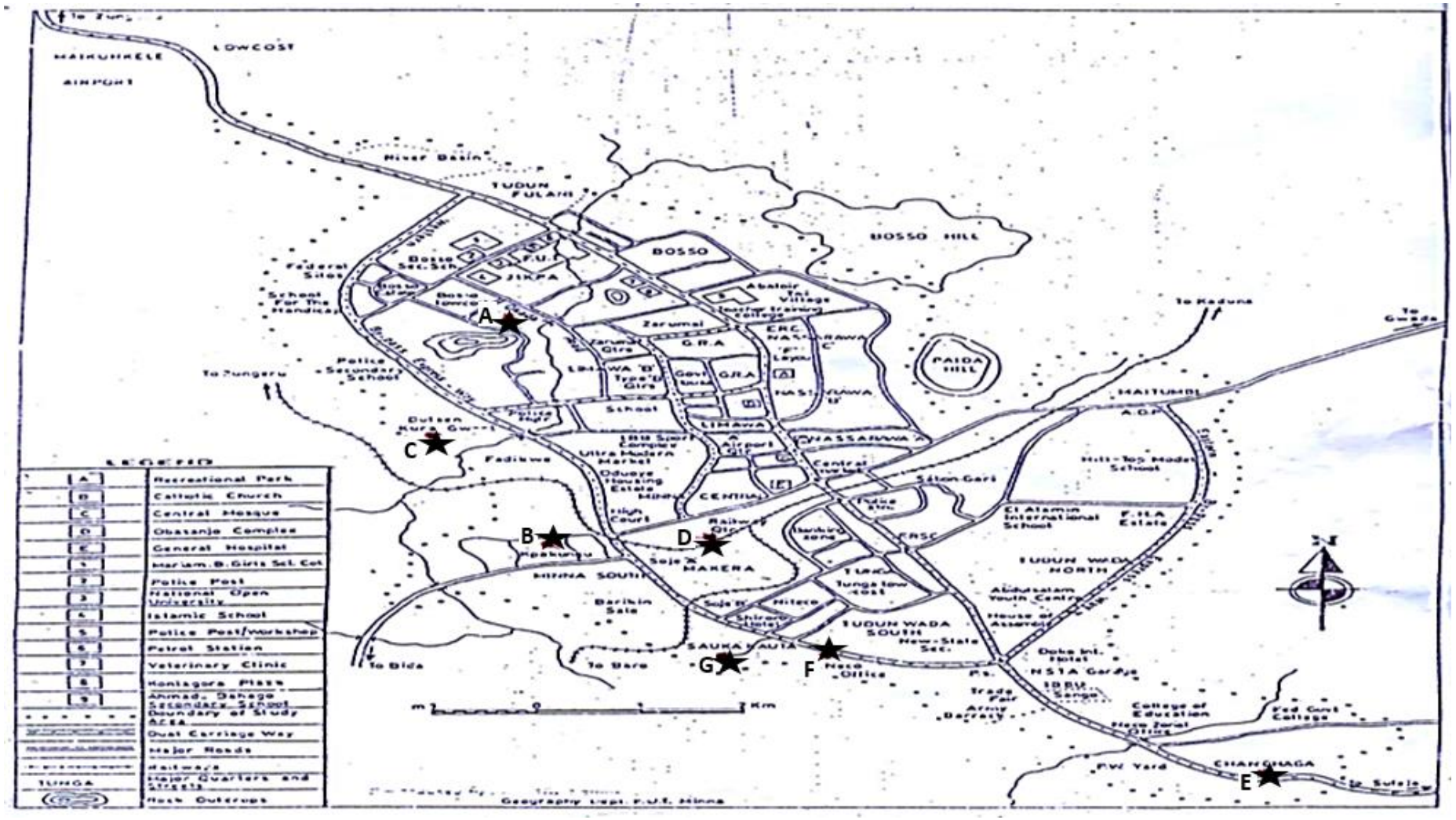


Figure 3.2: Map of Minna showing sampling locations

Samples were identified clearly and marked indelibly in a way that prevents inadvertent loss or confusion of labelling. Samples were taken in the morning in separate clean and ventilated polythene bags and transported to the laboratory on the same day**.** The conditions in which samples were delivered to the laboratory were approximate to that acceptable to a discerning purchaser.

Soil samples were collected from a depth of 0 to 15 cm from the surface (Allen, 1974; Opaluwa *et al.,* 2012). Ten soil samples were collected from ten different spots, which were pooled together to form a composite of each individual sample. They were placed in clean dried plastic containers and transported to the laboratory for analysis.

3.4 Sample Processing

For GC/MS analysis, sample processing was done on fresh vegetables. The vegetables were cut coarsely with a knife and kept in separate labelled polythene bags in a freezer at -18 ^{o}C overnight prior to milling. Freezing the samples facilitated processing, increased homogeneity and thus reduced sub-sampling variation (Anastassiades *et al.,* 2003). Each sample was homogenized by grinding it separately in a blender making sure that one sample was not contaminated by another. Homogenization maximizes the available surface area for better extraction efficiencies (Anastassiades *et al.,* 2003).

To prepare samples for AAS analysis, stem and leaf stalks of the vegetables were removed and the leaves washed with distilled water to remove dust and dirt and then chopped into small pieces (Omojola, 1993). They were subsequently placed on sheets of paper and left to air dry at room temperature to remove excess moisture after which they were weighed and dried at 60 ^{o}C overnight in an oven. Each dried sample was ground in a mortar sufficiently to pass through a 60-mesh sieve. The powder was packed and stored in clean, dry, stoppered glass containers.

Each soil sample was dried on a clean polythene sheet. Stones and other debris were removed and the bulk of the soil was thoroughly mixed by hand. Samples were then air dried for 5 days and ground in a mortar to pass through an 80-mesh sieve (Omojola, 1993). The portion that passes through the sieve was stored for analysis.

3.4.1 Acid digestion for vegetables and soil samples

The digestion procedure used for determining heavy metals was as described by Omojola (1993). From the dried pulverized sample 1 g was weighed and poured into a 100 cm^3 beaker and 10 cm^3 of concentrated nitric acid was added and heated at 40 ºC. After the evolution of brown fumes, heating was continued until a clear solution was obtained. De-ionized water was added at intervals to prevent drying of the digest. The clear solution was diluted to 40 cm^3 and filtered with a whatman filter paper No. 41 into a 100 cm^3 volumetric flask and stored until needed. The beakers were rinsed thoroughly and then the solution was made up to the mark with de-ionized water. It was poured into a 100 cm^3 polypropylene bottle and stored in the refrigerator at 4 ºC for analysis. A blank sample was also prepared by digesting 15 cm^3 of nitric acid with low heat and later transferred quantitatively after which it was made up to the mark with distilled deionized water into 100 cm^3 standard volumetric flask and stored until needed.

3.5 Sample Preparation for Pesticide Residues Determination

Sample preparation is always the major process in the complete analytical procedure for the determination of pesticide residues in food products (Lehotey *et al*., 2005).The methodology employed for pesticide residues analysis was the AOAC International Official method 2007.01 (Lehotay *et al.*, 2007). The process involved two steps: sample extraction and partitioning using an organic solvent and salt solution, then sample clean-up using a dispersive solid phase extraction (dSPE) technique.

3.5.1 Preparation of reagents

(a) Preparation of solvent

The solvent used for the analysis was 1 % (v/v) acetic acid in acetonitrile: 10 cm^3 glacial acetic acid in a 1 litre acetonitrile solution (Anastassiades *et al*., 2003). The choice of solvent is one of the most important decisions to take in pesticide residues analysis (Hercegova *et al*., 2007).

(b) Preparation of pesticide stock solutions (1000mg/L)

Using 99 % pure standards of carbofuran, 608 calibration mix (containing heptachlor, aldrin, endrin) and AOAC QuEChERS spike mix (containing lambda-cyhalothrin, cypermethrin) pesticides, 10 mg was weighed and dissolved in 1% acetic acid in acetonitrile and made up to the mark in a 10 cm^3 volumetric flask. These were then stored in amber coloured bottles at 4 ºC in a refrigerator.

(i) Sample extraction

The homogenized sample was placed in a container after blending and labelled (Plate 3.1a). The sample (15 g) was taken into a 50 cm^3 polypropylene tube (Plate 3.1b). Using a 20 cm^3 syringe 15 cm^3 of 1 % acetic acid in acetonitrile and 0.1 cm^3 of Triphenylphosphate (TPP) was added. A sachet of Restek extraction and buffering salts containing 6 g anhydrous magnesium sulphate and 1.5 g anhydrous sodium acetate was emptied into the extraction tube. The tube was then capped and vortexed for one minute and centrifuged for five minutes at 3000 rpm. 15 g of sample extracted with 15 cm^3 of solvent gave a dilution factor of one (1g sample per cm^3 organic solvent).

(ii) Extract clean up

Using the extracts resulting from the phase separation stage of sample extraction, 1 cm^3 of the supernatant was transferred with a syringe to a 2 cm^3 polypropylene tube containing

pre-weighed dSPE sorbents: 50 mg primary secondary amine (PSA), 150 mg magnesium sulphate ($MgSO_4$) and 50 mg graphitized carbon black (GCB). PSA was to remove sugars and fatty acids, $MgSO_4$ to remove residual water and GCB to remove pigments and sterols (Anastassiades *et al*., 2003). The tube was capped and shaken vigorously for 2 minutes and then placed in a centrifuge that supports 2 cm^3 tubes. It was then centrifuged for 5 minutes at 3000 rpm to separate the solid materials from the liquid extract. The final extract obtained was carefully transferred to amber coloured vials and kept in the refrigerator prior to analysis by GC/MS. The procedure is as shown in plate 3.1 (a-l) (Anastassiades *et al*., 2003).

3.6 GC/MS Analysis

The GC/MS analyses were performed in a Bruker Scion Triple Quadrupole. A Bruker Scion 451 gas chromatograph equipped with electronic pressure control for split/splitless injector was used. A capillary fused silica column Rxi-5ms (30 m length, 0.25 mm i.d and 0.25 µm film thickness) was used due to its low bleed and high inertness characteristics (Sternerson *et al*., 2010). Aliquots of 0.001cm^3 of samples were injected into the gas chromatograph. The GC oven temperature was programmed at 70 ºC for 3 minutes followed by an 8 ºC per minute ramp to 270 ºC (held for 5 minutes). Carrier gas was high purity helium (99.9999% purity) at a constant flow rate of 1.0 cm^3 per minute (Sternerson *et al*., 2010). The mass spectrometer Bruker Scion triple quadrupole was operated using electron impact ionization (EI (+) external ion volume control 70 eV). The mass range was 50-600 m/z and the MS workstation software was used for data acquisition. Since the 451-GC is part of an integrated GC/MS, the local user interface (LUI) and keyboard were not required. The MS system was set in selective ion monitoring (SIM) mode for quantification (Sternerson *et al*., 2010).

Tentative identification compound (TIC) was used for the identification of the pesticides in the vegetable samples by comparing their mass spectra with a mass spectra library (library search). An EPA, NIST/2011 library of about 60,000 stored spectra was used. The computer then provided a short list of the best matches between the library spectra and the measured. The correlation reference, which is a measure of how well the sample peak mass spectrum matched the standard was 999. Equation 3.1 was used t$^{o\ c}$alculate concentration of the detected pesticides (Ogah *et al.,* 2011b).

$$\text{Pesticide Content (mg/kg)} = \frac{\text{AsVf}}{\text{Wts Cf}} \quad (3.1)$$

where,

As = Peak area of sample
Vf = Final volume of clean extract
Wts = Weight of sample extracted
Cf = Calibration factor

$$\text{The Cf of each pesticide} = \frac{\text{Peak Area of Standard}}{\text{Total Amount of Standard Injected}} \quad (3.2)$$



(a)



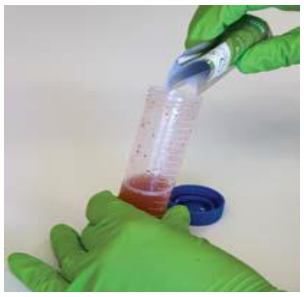
(b)



(c)



(d)



(e)



(f)



(g)



(h)



(i)



(j)



(k)



(l)

Plate 3.1: Pictures of QuEChERS extraction procedure. (a) Homogenized sample (b) Weighing homogenized sample (c) Sachet of Restek extraction salts (d) Tearing the sachet open (e)(f) Adding extraction salts to the sample (g) Mixing extraction salts with sample (h) Centrifuge for 50ml tubes (i) transferring extract to 2ml dSPE tube (j) Mixing extract with dSPE salts (k) Centrifuge for 2ml tube (l) Amber coloured vial.

3.7 Physico-Chemical Analysis of Soil from Farms

Soil samples from the farms were subjected to routine physico-chemical analysis using standard methods.

3.7.1 Determination of Soil particle size (Bouyoucos, 1962)

Preparation of solution

5 % Calgon (sodium hexametaphosphate) solution

For the preparation of this solution, 50.00 g of sodium hexametaphosphate was weighed into a 250 cm^3 beaker. This was dissolved using distilled water, transferred into a 1000 cm^3 volumetric flask and made up to the mark with distilled water.

Procedure

A blank was prepared using 100 cm^3 5 % Calgon solution diluted to 1000 cm^3 with distilled water. Then 50.00 g of soil sample was weighed and transferred into a dispersing cup and 100 cm^3 calgon solution was added. The cup was attached to an electric mixer and mixed for about one minute. The suspension was quantitatively transferred into a 1000 cm^3 measuring cylinder and made up to the mark with distilled water. This was allowed to stand overnight. The temperature and hydrometer reading of the blank were noted and the plunger was inserted into the suspension and carefully mixed for 30 seconds until a uniform suspension was obtained. The reading was taken and the plunger removed. The hydrometer was then gently inserted into the suspension and reading taken after 40 seconds (this was taken as the amount of silt plus clay suspended as the sand had settled to the bottom). This procedure was repeated for each sample after which the equipment was thoroughly rinsed with distilled water. The hydrometer reading was again recorded after six hours 52 minutes. This was taken as the amount of clay in the suspension since silt had settled to the bottom of the cylinder. Determinations were done in triplicate and

the values obtained were used to calculate the percentage of silt, sand and clay in the soil samples.

Calculations

$$\% \text{ Clay} = \frac{\text{Hydrometer reading at 6hrs 52min x 100}}{\text{Weight of sample}} \quad (3.3)$$

$$\% \text{ Silt} = \frac{\text{Hydrometer reading at 40 seconds x 100}}{\text{Weight of sample}} - \% \text{ Clay} \quad (3.4)$$

$$\% \text{ Sand} = 100 - \% \text{ Silt} - \% \text{ Clay} \quad (3.5)$$

3.7.2 Determination of pH (Nwakife, 2015)

Preparation of buffer standard solution

Standard Buffer Solutions (pH 4, 7 and 9.2) were prepared by dissolving each tablet of pH 4, 7 and 9.2 in a 100 cm^3 volumetric flask and made to the mark with distilled water.

Procedure

The prepared standard buffer solutions were used to calibrate the pH meter. The pH measurement was determined using 1:1 ratio of soil sample to distilled water. This was achieved by weighing 20.00 g of the air-dried and sieved sample into 100 cm^3 beaker and 20 cm^3 of distilled water was added. The mixture was stirred for 30 minutes and the pH of the sample measured using a glass-electrode pH meter. The pH meter electrode was thoroughly rinsed with distilled water before /after immersing into each sample and the pH determined.

3.7.3 Determination of electrical conductivity

Preparation of solution

Potassium Chloride Solution 0.01 mol/dm^3

Using an analytical balance, 0.7456 g of dried potassium chloride was weighed and dissolved in distilled water in a 1000 cm^3 volumetric flask and the volume was made up to the mark at 25 ºC. This served as the standard reference solution.

Procedure

From the air-dried sieved samples, 20.00 g was weighed into a 100 cm^3 beaker and 20 cm^3 of distilled water was added and the mixture stirred. The electrode of the conductivity meter was inserted into the supernatant and the electrical conductivity taken (Ajai *et al.*, 2014).

3.7.4 Determination of soil organic matter (OC)

The determination of soil organic matter was carried out using Walkley-Black wet oxidation method (Walkley and Black, 1934; Nwakife, 2015).

Preparation of solutions

(i) Potassium dichromate, $K_2Cr_2O_7$, 1 mol/dm^3

Using an analytical balance, 49.024 g of oven dried $K_2Cr_2O_7$ (at 105 ºC) was weighed into 250 cm^3 beaker and dissolved with distilled water. This was transferred into a 1000 cm^3 volumetric flask and made to the mark with distilled water.

(ii) Iron (II) tetraoxosulphate (VI) acid, 0.4 mol/dm^3

To prepare this solution, 112 g of $FeSO_4.7H_2O$ was dissolved in 800 cm^3 of distilled water containing 15 cm^3 concentrated sulphuric acid. The solution was diluted to 1000 cm^3 with distilled water and stored in a dark bottle.

(iii) Ferroin Indicator, $(C_{12}H_8N_2)FeSO_4$

Ferroin indicator was prepared by weighing 1.485 g of 1,10 ortho-phenanthroline monohydrate ($C_{12}H_8N_2.\ H_2O$) and 0.695 g Iron (II)tetraoxosulphate(VI) heptahydrate,

$FeSO_4.7H_2O$ and dissolved in 80 cm^3 of distilled water in a 100 cm^3 standard flask and made up to the mark. This was then stored in a dark bottle.

Procedure

From the pulverized samples of soil, 1.00 g was weighed into a 250 cm^3 Erlenmeyer flask and 10 cm^3 of 1 M $K_2Cr_2O_7$ was added and swirled gently in order to disperse the soil in solution. To this mixture, 20 cm^3 of concentrated H_2SO_4 was added and the flask was immediately swirled to ensure thorough mixing. A thermometer was inserted into the flask and heating commenced on a hot plate. The mixture was swirled over the hot plate until the thermometer reading attained a temperature of 135 ºC after which the flask was removed and allowed to cool on asbestos sheet in a fume cupboard. The mixture was then diluted to 200 cm^3 with distilled water after cooling for about 30 minutes and titrated with 0.4 mol/dm^3 $FeSO_4$ solution and Ferroin indicator. The endpoint was taken to be near when the solution first assumed greenish appearance which gradually changed to green and finally to brown. Using the concentration of $K_2Cr_2O_7$ solution in mol/dm^3, volume of $FeSO_4$ used in sample titration and volume of $FeSO_4$ in blank titration, the percentage carbon was determined (Walkley and Black, 1934; Nwakife, 2015).

From the equation:

$$2Cr_2O_7^{2-} + 3C + 16H^+ \rightarrow 4Cr^{3+} + 8H_2O + 3CO_2 \qquad (3.6)$$

2 cm^3 of 1 mol/dm^3 dichromate solution is equivalent to 3 mg of carbon

$$OC(\%) = \frac{0.003g \times M \times 10cm^3 \times (1 - \frac{T}{S}) \times 100}{\text{weight of sample}} \qquad (3.7)$$

where:

M = concentration of $K_2Cr_2O_7$ solution in mol/dm^3

T = volume of $FeSO_4$ used in sample titration (cm^3)

S = volume of $FeSO_4$ used in blank titration (cm^3)

3.7.5 Determination of cation exchange capacity of soil samples

Preparation of solutions

(i) KCl, 1.0 mol/dm^3

To prepare this solution, 74.56 g of KCl was dissolved and transferred to a 1000 cm^3 volumetric flask and made to the mark with distilled water.

(ii) Phenolphthalein indicator

Phenolphthalein indicator was prepared by weighing 0.1 g of phenolphthalein dissolved, in 100 cm^3 ethanol mixed with 100 cm^3 distilled water by constant stirring. The solution was then filtered and transferred to a reagent bottle (Sumaila, 2014).

(iii) NaOH, 0.05 mol/dm^3

Using an analytical balance, 2.0 g of NaOH was weighed and dissolved in distilled water, transferred to a 1000 cm^3 flask and made up to the mark.

(iv) Ammonium acetate (pH 7), 1.0 mol/dm^3

Neutral ammonium acetate solution was prepared by dissolving 77.08 g of ammonium acetate in distilled water in a 250 cm^3 beaker. The solution was transferred to a 1000 cm^3 flask and made up to the mark with distilled water (pH adjusted to 7 with acetic acid).

(v) HCl, 0.01 mol/dm^3

To prepare this, 0.80 cm^3 of conc. HCl was measured and added into 500 cm^3 distilled water and made up to the mark in a 1000 cm^3 volumetric flask.

(vi) NaF, 1mol/dm^3

Using an analytical balance, 41.99 g of NaF was weighed and dissolved in distilled water and made up to the mark with distilled water in a 1000 cm^3 volumetric flask.

(vii)NaCl, 1mol/dm^3

From the analytical reagent, 2.5422 g of dried sodium chloride at 105 ºC was accurately weighed and dissolved in distilled water. This was transferred into a 1000 cm^3 volumetric flask and made up to the mark with distilled water.

Determination of exchangeable bases (Na^+, K^+, Mg^{2+}, Ca^{2+})

After air drying and sieving (2 mm), 5.00 g of each sample was weighed into 250 cm^3 conical flask in triplicates. To each of the samples, 100 cm^3 of 1.0 mol/dm^3 ammonium acetate solution (pH 7) was added and the flask swirled gently, covered and allowed to stand overnight. The suspension formed was transferred into a Buchner funnel that had been lined with moistened Whatman paper No. 110. Soil extract filtrate obtained was used for determining Ca and Mg using complexometric titration while Na and K were determined by flame photometry.

(i) Determination of Ca^{2+} and Mg^{2+} (El mahi, *et.al* 1987)

Using the soil extract filtrate, 20 cm^3 was pipetted into a 150 ml conical flask and 50 cm^3 of distilled water was added, followed by 10 cm^3 of $NH_4Cl–NH_4OH$ buffer solution. To each of the samples, 10 drops each of $NH_2OH.HCl$, $K_4Fe(CN)_6$, TEA and EBT indicator were added and titrated with standard EDTA to permanent blue colour. To determine Calcium alone, 5 cm^3 of the extract was pipetted and 10 drops each of $NH_2OH.HCl$, $K_4Fe(CN)_6$, TEA and 10 % NaOH to raise pH to 12. Calcon indicator (5drops) was added and the resulting solution was titrated against standard EDTA. The value obtained for Ca plus Mg and Ca alone were used to calculate the Ca and Mg in the soil samples respectively.

Calculations,

Meq (Ca + Mg) or Ca/100 g

$$= \frac{\text{Vol. of EDTA required x molarity of EDTA (0.01) x 100 cm}^3 \text{ x 100}}{\text{Sample taken for titration (cm}^3\text{) x Weight of soil (g)}} \quad (3.8)$$

Meq (Mg) = Meq (Ca + Mg) – Meq (Ca)

(ii) Determination of Na^+ and K^+

From the soil extract, 5cm^3 was pipetted into 50 cm^3 volumetric flask and diluted to the mark with 1.0 mol/dm^3 NH_4OAc solution. This was sprayed into the flame of the photometer, and a calibration curve was prepared from which the concentration of Na and K were determined (Nwakife, 2015)

Calculation,

K = Graph reading × 10 = mg K/100 g air-dried soil (3.9)

Na = Graph reading × 10 = mg Na/100g air-dried soil (3.10)

Where 10 is the dilution factor

Determination of Exchange Acidity

Using each of the air-dried and sieved samples, 5.00 g was weighed into a beaker and 100 cm^3 of 1.0 mol/dm^3 KCl was added. The content of the beaker was mixed thoroughly then allowed to stand for 30 minutes and filtered with a Buchner funnel to obtain the extract. From the extract 25 cm^3 was pipetted into a 250 cm^3 flask and 100 cm^3 of distilled water was added. To this, 5 drops of phenolphthalein indicator was added and titrated with 0.05 mol/dm^3 NaOH to the first permanent pink end point. The amount of base used is equivalent to Al^{3+} and H^+. To the titrant, 2 drops of 0.01mol/dm^3 HCl was added until it becomes colourless and 10 cm^3 of 1mol/dm^3 NaF and 2 drops of methyl red were added. This was then titrated with 0.01mol/dm^3 HCl to obtain the exchange acidity (Sumaila, 2014).

meq of H^+/100g soil = M x V x 100/25 x 100/5 (3.11)

where,

1 meq/100 g = 1 cmol/kg

M = concentration of the acid (mol/dm^3)

V = final volume of acid used (cm^3)

25 = volume of extract taken (cm^3)

5 = weight of soil used (g)

CEC (cmol/kg) = Exchangeable bases + Exchange Acidity (3.12)

3.8 Atomic Absorption Spectrometric Determination of Heavy Metals

All metals were determined with a Thermo Scientific ICE 3000 series atomic absorption spectrophotometer.

3.8.1 Preparation of 1000mg/L stock solutions

Cadmium stock solution: This was prepared by dissolving 2.0360 g of $CdCl_2$ in 200 cm^3 of deionised water in a beaker. Then 1.5 cm^3 of conc. HNO_3 was added. This was then quantitatively transferred into a 1 litre flask and diluted to the mark with deionised water. Working standards of 1 to 8mg/L were prepared from the stock. Cadmium was determined at a wavelength of 228.8nm.

Copper stock solution: Copper (II) stock solution was prepared by dissolving 3.7980 g of $Cu(NO_3)_2.3H_2O$ in 200 cm^3 of deionised water in a beaker, 1.5 cm^3 of conc. HNO_3 was added. This was then quantitatively transferred into a 1 litre flask and diluted to the mark with deionised water. Working standards of 2 to 8mg/L were prepared from the stock. Copper was determined at a wavelength of 324.8nm.

Iron stock solution: Iron (III) stock solution was prepared by dissolving accurately weighed 4.8400 g of Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) in 200 cm^3 of deionized water in a beaker, 1.5 cm^3 of conc. HNO_3 was added. This was quantitatively transferred into a 1 litre flask and then diluted to the mark with deionized water. Working standards of 2 to 8mg/L were prepared from the stock and iron was determined at a wavelength of 248.3nm.

Manganese stock solution: 3.6077 g of $MnCl_2.4H_2O$ was dissolved in 200 cm^3 of deionized water in a beaker and 1.5 cm^3 of conc. HNO_3 was added. This was diluted to 1 litre in a volumetric flask with deionized water. Working standards of 2 to 8mg/L were prepared from the stock. Manganese was determined at a wavelength of 279.5nm.

Nickel stock solution: Ni (II) stock solution was prepared by dissolving accurately weighed 4.9530 g of nickel (II) trioxonitrate (V) hexahydrate ($Ni(NO_3)_2.6H_2O$) and dissolved in 200 cm^3 deionized water with 1.5 cm^3 conc. HNO_3 and made up to the mark with deionized water in a 1 litre volumetric flask. Working standards of 3 to 12mg/L were prepared from the stock. Nickel was determined at a wavelength of 232.0nm.

Zinc stock solution: 1.2450 g of zinc oxide (ZnO) was dissolved in 200 cm^3 of deionized water. 1.5 cm^3 of conc. HNO_3 was added. This was then diluted to 1 litre in a volumetric flask with deionised water. Working standards of 1 to 4mg/L were prepared from the stock and zinc was determined at a wavelength of 213.9nm.

Each sample solution was run in triplicate to ensure the reproducibility of the obtained results. The digest was aspirated into the instrument after all necessary set-up and standardization procedures. For analytical quality assurance, the result of each metal was calculated by subtracting the blank reading from the value using the formula:

$$\text{Concentration (mg/g)} = \frac{\text{C (mg/L)} - C_{Blank} \times \text{Vol. of solution}}{\text{Wt of sample} \times 1000} \quad (3.13)$$

where,

C = Concentration (mg/L)

Vol. of sample = 100cm^3

Weight of sample = 1g

3.9 Soil-Plant Transfer Factor

The transfer factor (TF) was calculated based on the total metal contents of the whole edible part of the plant according to the following equation (Adah *et al*., 2013).

$$TF = \frac{\text{concentration of metal in edible part}}{\text{concentration of metal in soil}} \quad (3.14)$$

CHAPTER FOUR

4.0 RESULTS

4.1 Survey Results

Table 4.1 gives the trade names and chemical names of pesticides commonly used on vegetable farms in Minna metropolis.

Table 4.1: Pesticides used on vegetables farms in Minna

Trade Name	Chemical Name
Bodal	Acetamiprid lambda-cyhalothrin
Delvap (Sniper)	DDVP
Polytrin	Profenofos/ Lambda-cyhalothrin
Furadan	Carbofuran
Karate	Lambda-cyhalothrin
Kombat	Chlorphyphenos
Termex	Chlorpyriphos
Multhrin	Cypermethrin
Instankill	Endosulfan
Best action	Cypermethrin + demethoate
Bioaldrin 40% concentrate	Aldrin
Heptox	Heptachlor
Agrico endrin 20 EC	Endrin

4.2 Pesticide Residues in Vegetables

4.2.1 GC-MS of pesticides standards (Full scan)

The chromatograms of pesticides standards using full scan mode are presented in Figures 4.1- 4.4. Figure 4.1 gives the retention time of heptachlor, aldrin and endrin as peaks 1A (25.531 minutes), 2A (26.393 minutes) and 3A (29.360) respectively. The retention times for the three pesticides were obtained from the full scan of 608 calibration mix standard. The mass spectra corresponding to each pesticide also shows the ion mass for the pesticides.

The chromatogram for carbofuran (Figure 4.2) shows the retention time of 16.496 minutes. The mass spectrum of carbofuran with the ion mass obtained from NIST library search is also shown in the Figure.

Figure 4.3 shows the chromatogram of four cypermethrin isomer peaks that eluted between 35.1minutes and 35.5 minutes. The fragmentation pattern of the various isomers of cypermethrin are shown on the mass spectra.

Full scan of AOAC QuEChERS QC spike mix reference material containing the lambda-cyhalothrin pesticide is shown in Figure 4.4. It had a retention time of 32.985 minutes (peak 1A) and the mass spectrum is also shown in the Figure.

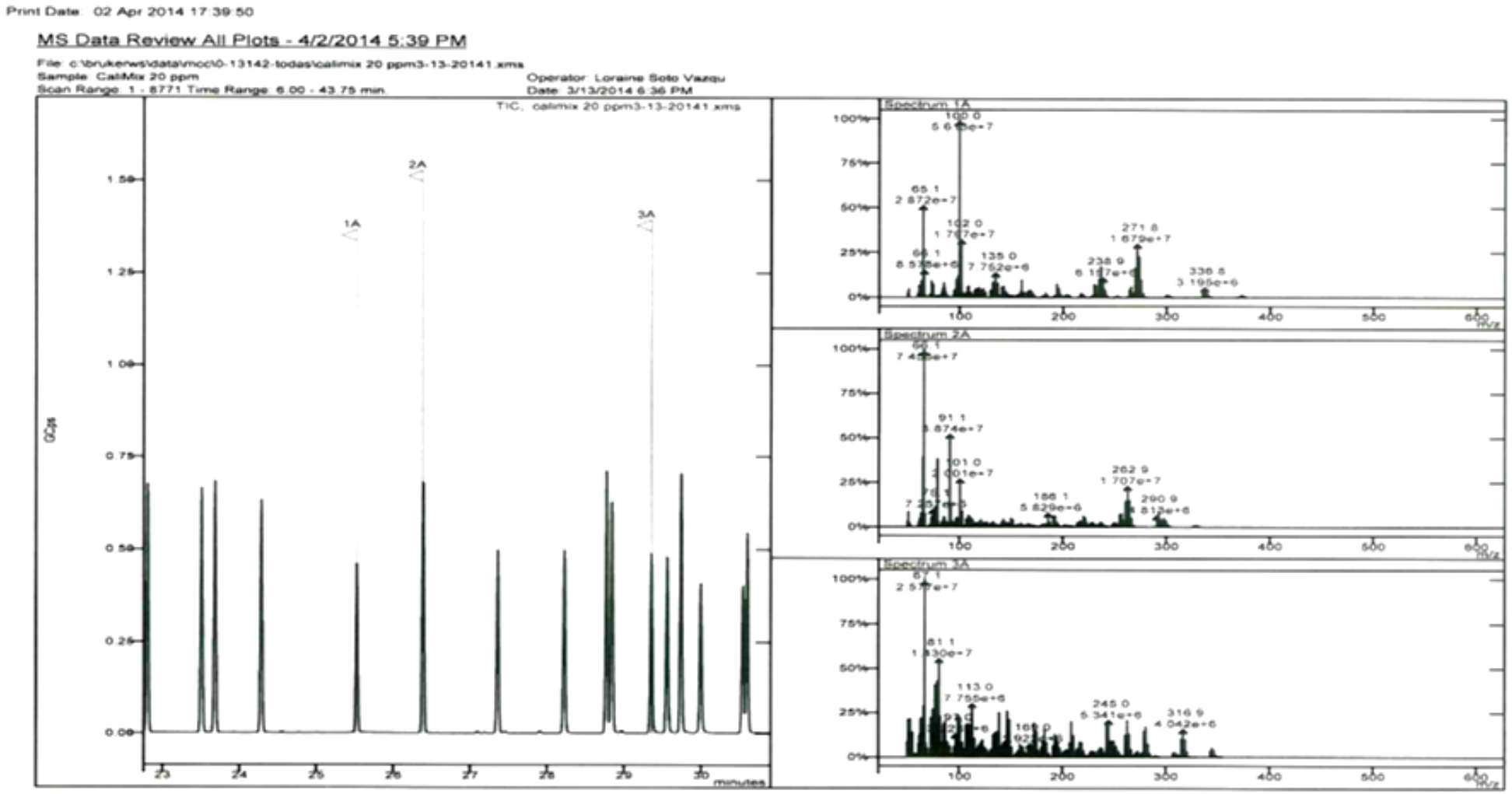


Figure 4.1: Gas chromatogram and mass spectra of heptachlor, aldrin, endrin calibration mix standard

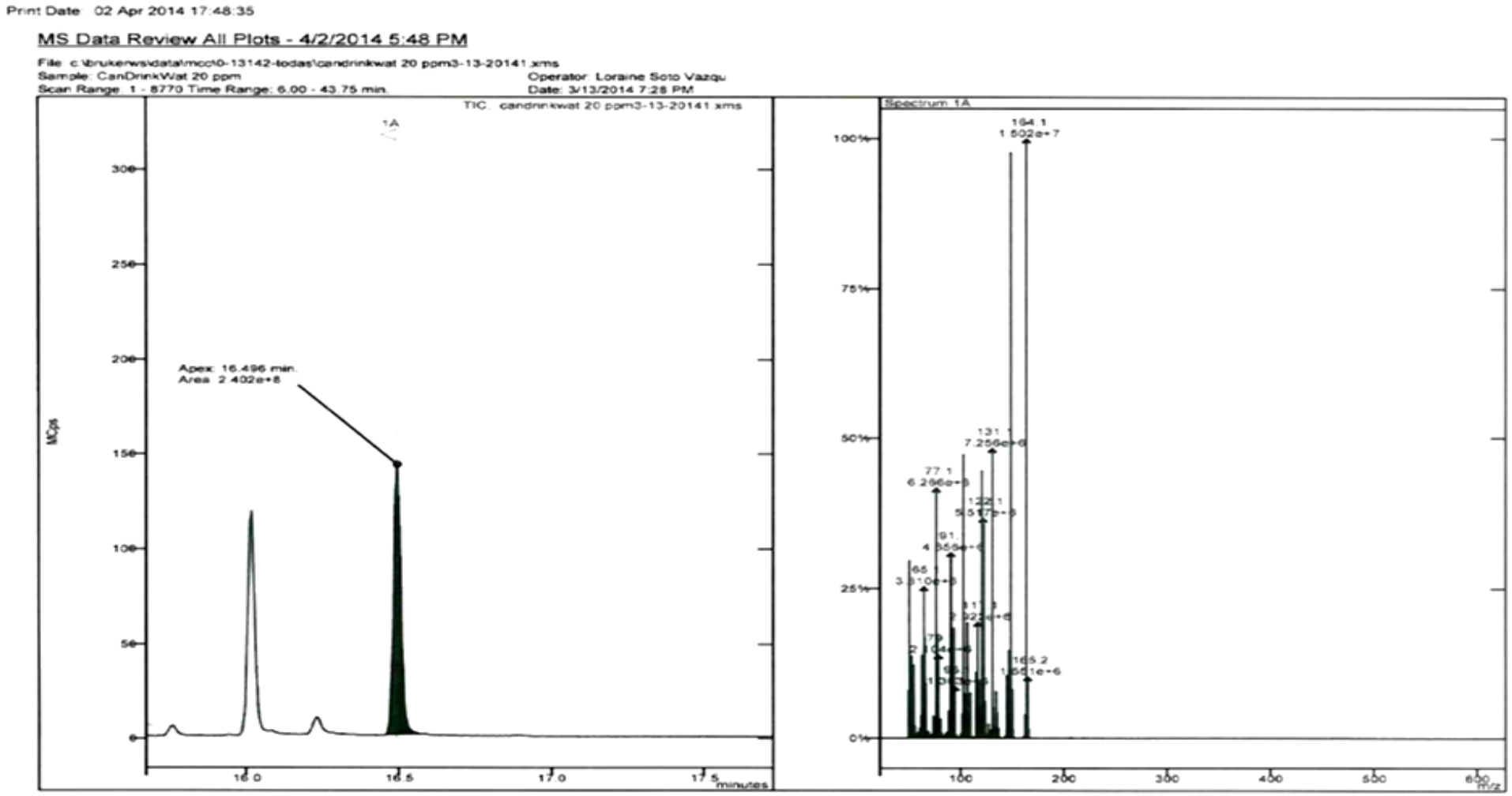


Figure 4.2: Gas chromatogram and mass spectrum of carbofuran standard

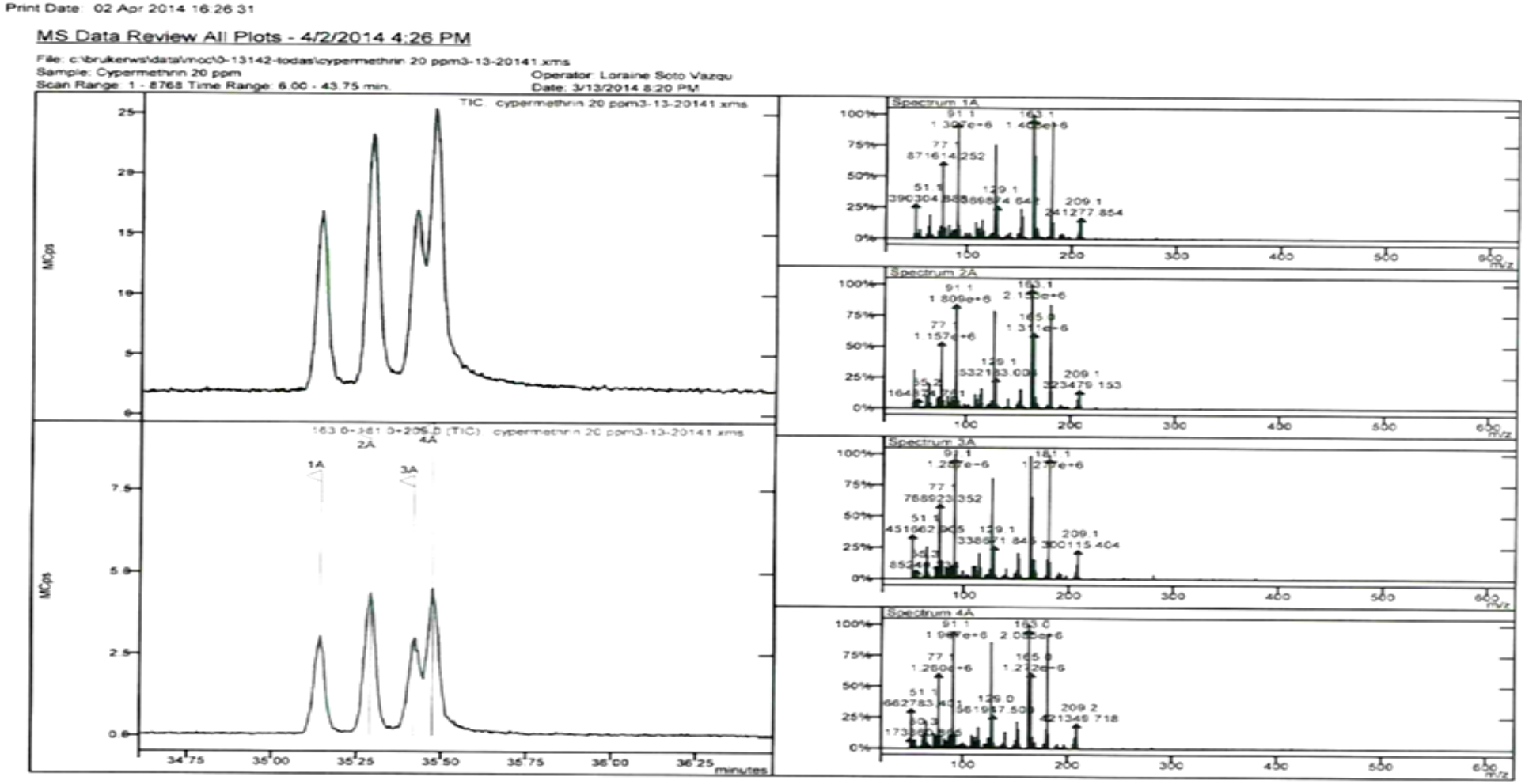


Figure 4.3: Gas chromatogram and mass spectra of cypermethrin isomers mix standard

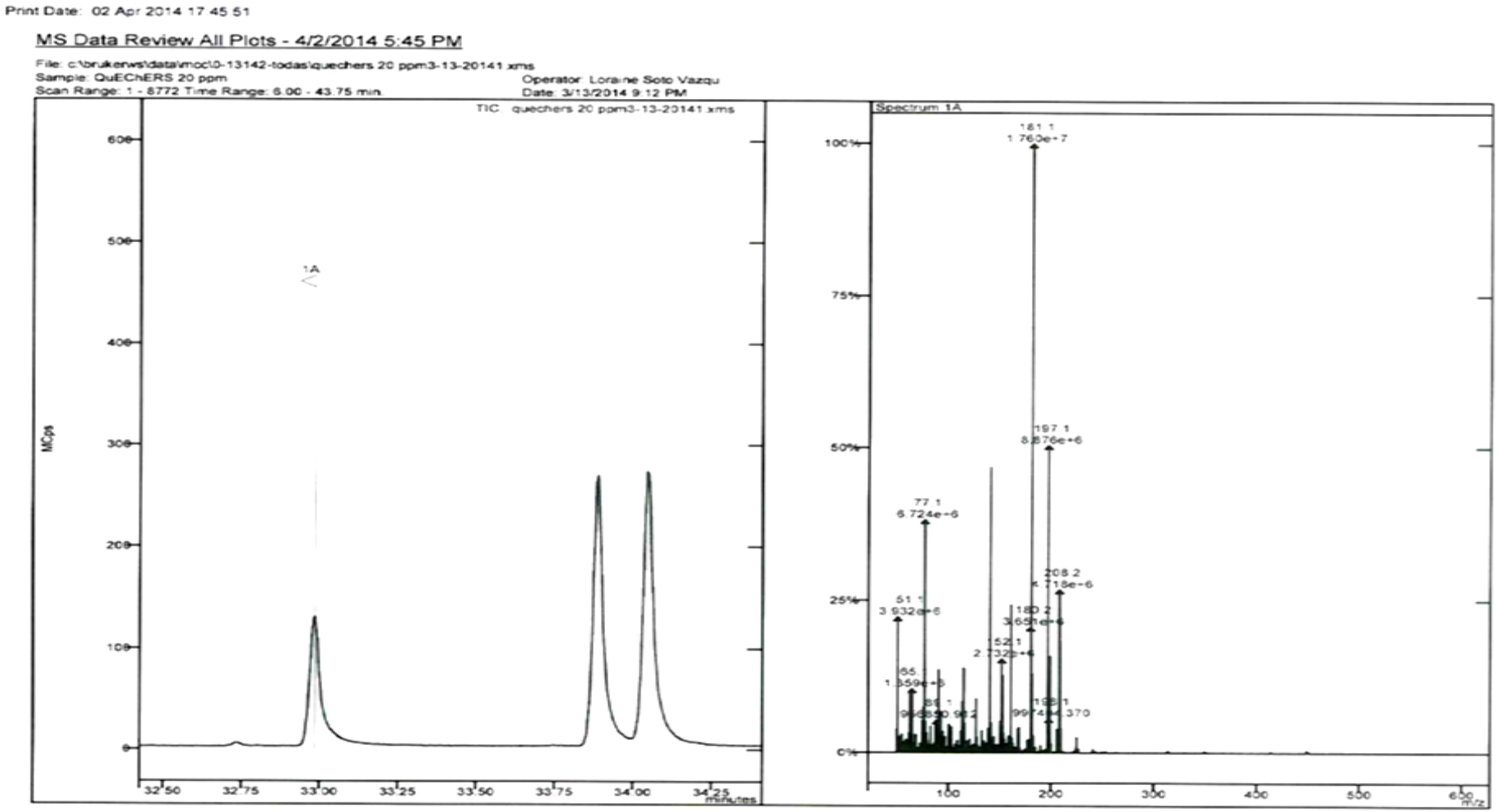


Figure 4.4: Gas chromatogram and mass spectrum of lambda cyahalothrin standard

4.2.2 GC-MS of pesticides standards (SIM mode)

Figure 4.5 gives the elution time of endrin (1A), aldrin (2A) and heptachlor (3A) present in the 608 calibration mix and the ion fragments that were considered for each pesticide in SIM mode. The ion fragments were: heptachlor (65, 100, 272 a.m.u), aldrin (81, 263, 345 a.m.u), endrin (66, 91, 263 a.m.u).

Figure 4.6 shows the time at which carbofuran eluted from the column and the ion fragments entered into the instrument from the many fragments obtained during full scan. The ion fragments were 103, 149 and 164 (a.m.u).

Figure 4.7 also gives the result of SIM analysis of cypermethrin isomers mix showing the elution times of all the isomers and the ion fragments detected: 163, 181 and 209 (a.m.u).

The SIM mode scan of AOAC QuEChERS QC spike mix which is the reference material used for lambda-cyhalothrin is shown in Figure 4.8. The chromatogram gives the retention time of the pesticide and the ion fragments detected. These were 141,181 and 197 (a.m.u).

Table 4.2 gives the retention times and m/z of molecular ions of all the pesticides determined.

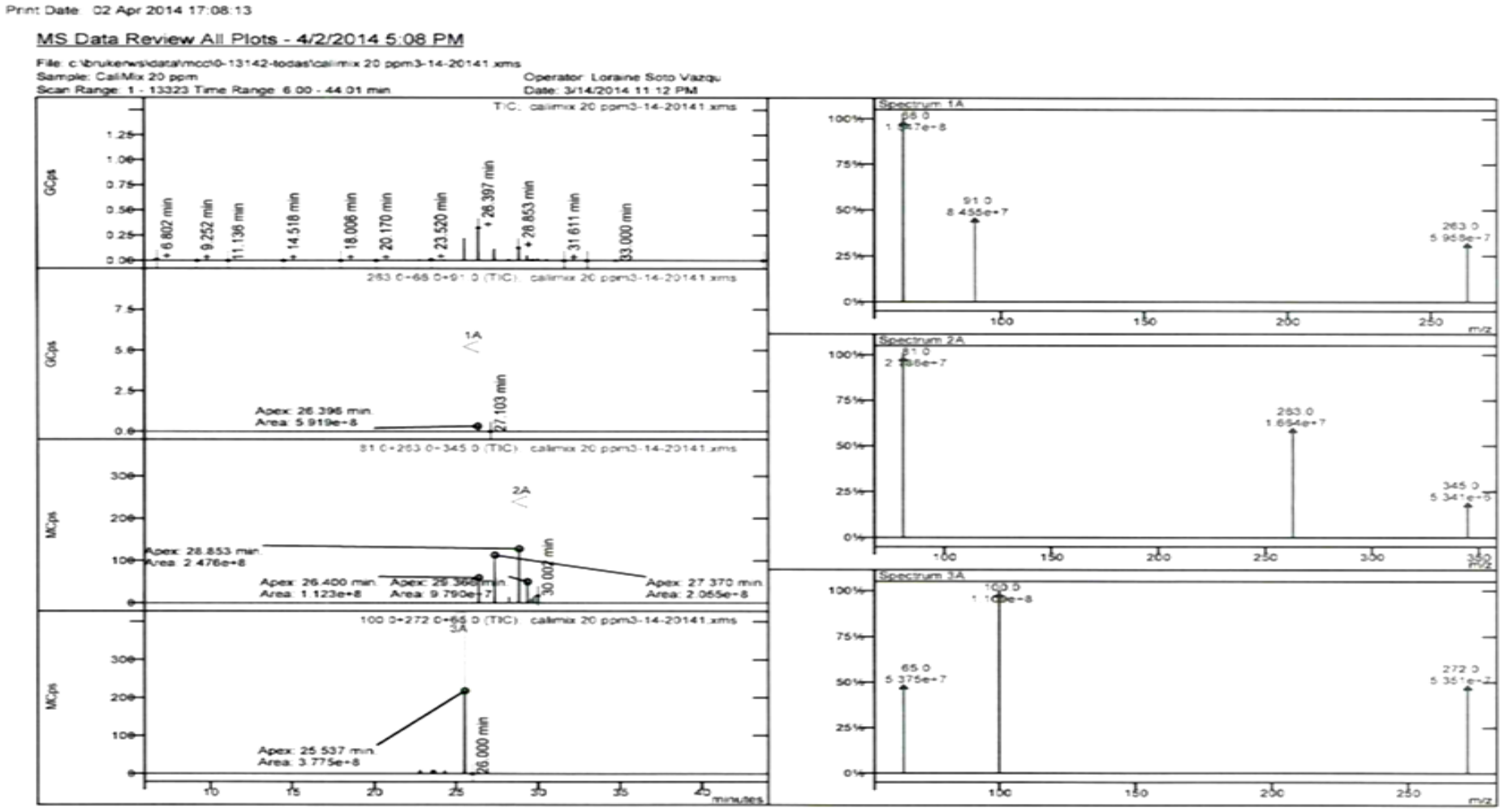


Figure 4.5: Gas chromatograms and mass spectra of 608 calibration mix standard

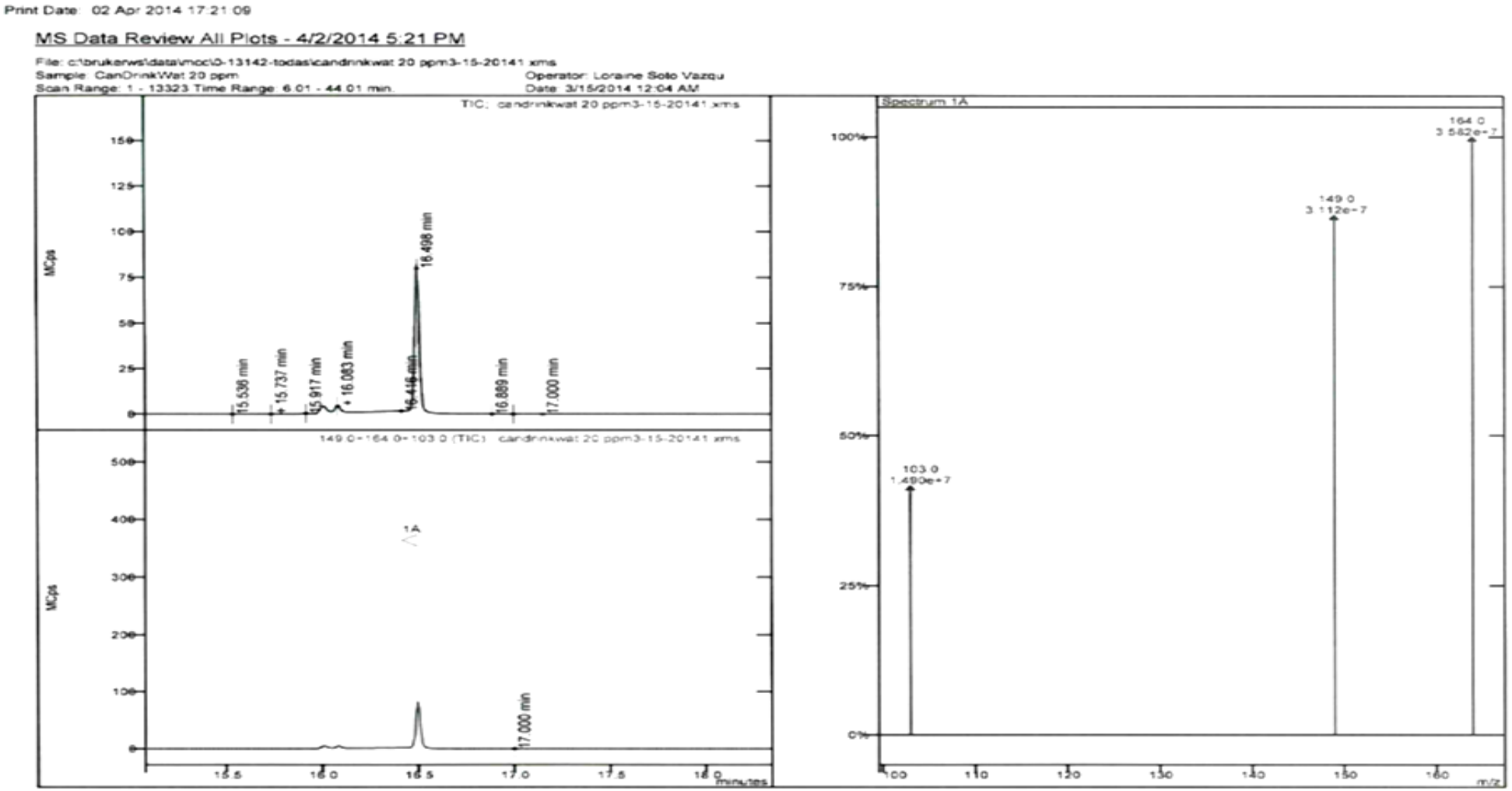


Figure 4.6: Gas chromatogram and mass spectra of Canadian drinking water standard for carbofuran

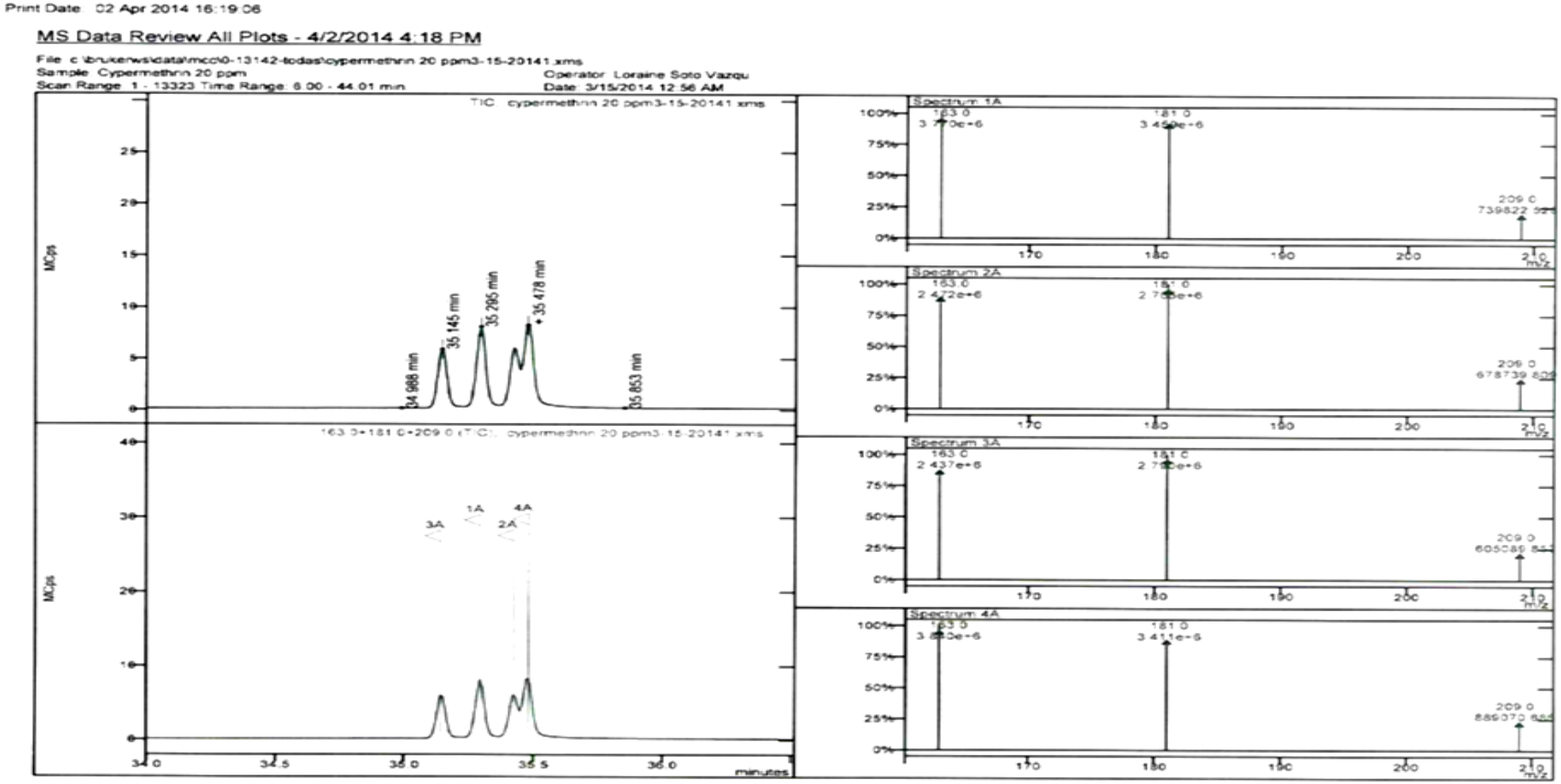


Figure 4.7: Chromatogram and mass spectra of cypermethrin isomers mix standard

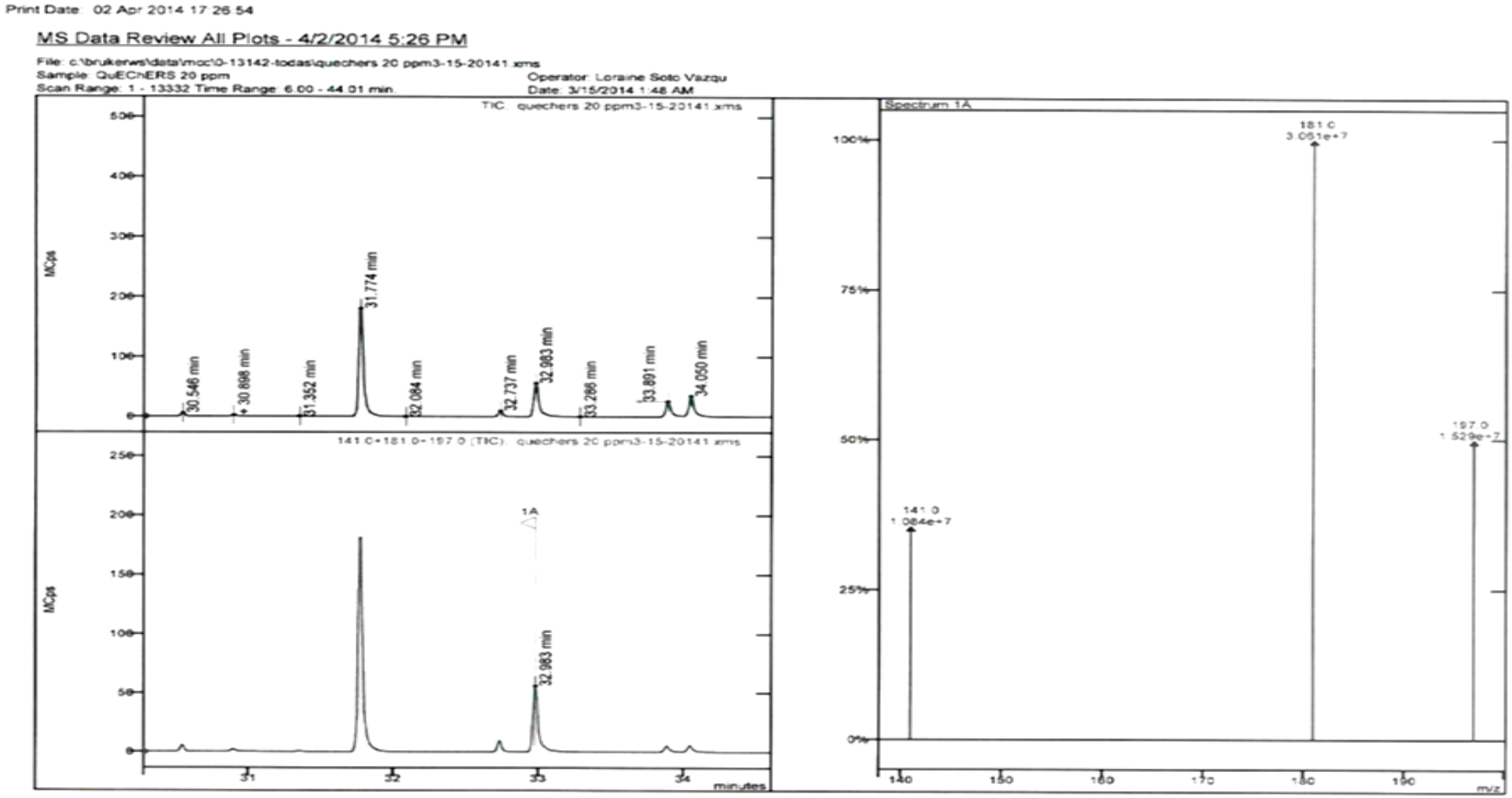


Figure 4.8: Gas chromatogram and mass spectra of lambda-cyhalothrin standard

Table 4.2: Retention time and ion mass of detected pesticides

Pesticides detected	Retention Times (minutes)	Ion mass (a.m.u)
Carbofuran	16.496	103, 149, 164
Heptachlor	25.531	65, 100, 272
Aldrin	26.393	81, 263, 345
Endrin	29.360	66, 91, 263
Lambda-cyhalothrin	32.985	141, 181, 197
Cypermethrin	35.145 35.295 35.478	163, 181, 209

4.2.3 GC-MS of pesticide residues in vegetable samples

The chromatograms and spectra of pesticide residues of some of the vegetable samples are given in Figures 4.9 - 4.16**.** Figure 4.9 gives the chromatogram of pesticide residue in spinach sample from farm Okada road farm showing the most prominent peak eluting at 27.626 minutes. This retention time did not correspond to any of the pesticide standards, indicating the absence of pesticide residue in this sample or if present it was below detection limit of this method.

The chromatogram of pesticide residue in okra sample from Chanchaga farm (Figure 4.10) shows the predominant peak eluting at 24.930 minutes. This again did not correspond to any of the pesticide standards, indicating the absence of pesticide residue in this sample or if present it was below detection limit of this method. Figure 4.11 shows

the most significant peak of pumpkin leaves from the farm on Mandela area at 17.445 minutes. This retention time did not correspond to any of the pesticide standards, indicating the absence of pesticide residue in this sample or if present it was below detection limit of this method. There are however some minor peaks which could not be identified.

This was the same for all the samples except spinach sample extract from Chanchaga and Mandela farms which gave retention time corresponding to cypermethrin and heptachlor. The chromatograms of these samples are given in Figures 4.12, 4.13, 4.15 and 4.16.

Figure 4.12 shows the time (35.105-35.515 minutes) at which spinach sample from Chanchaga farm eluted and the peak area of the relevant peak (1A). It also gives the ion fragments detected as 163, 181 and 209 (a.m.u) which confirm the presence of cypermethrin in the sample. Figure 4.13 also gives the time (35.105-35.515 minutes) at which spinach sample extract from Mandela farm eluted and the different peaks including the relevant peak (1A). It also gives the ion fragments detected as 163, 181 and 209 (a.m.u) which confirm the presence of cypermethrin in the spinach sample.

The cypermethrin library search (Figure 4.14) using the EPA/NIST library shows all the ion masses and the structural formula of the cypermethrin molecule. This was used to confirm the presence of cypermethrin in spinach samples from Chanchaga and Mandela farms. Spinach samples from Chanchaga and Mandela farms also gave very weak peaks at 25.531 minutes which is the retention time for heptachlor (Figures 4.15 and 4.16). This indicates the likelihood of the presence of heptachlor in the samples.

Figure 4.17 shows an attempt to quantify the amount of heptachlor in spinach from Chanchaga and Mandela farms. A peak (1A) in the gas chromatogram at the retention time of 25.531 that corresponds to heptachlor is not as prominent as that of heptachlor

standard in 2A. The mass spectrum 1A for the sample, only gave the qualifier ion 65 (a.m.u) instead of the three qualifier ions (65, 100, 272 a.m.u) for the standard as shown in spectrum 2A. The library search for heptachlor is shown in Figure 4.18.

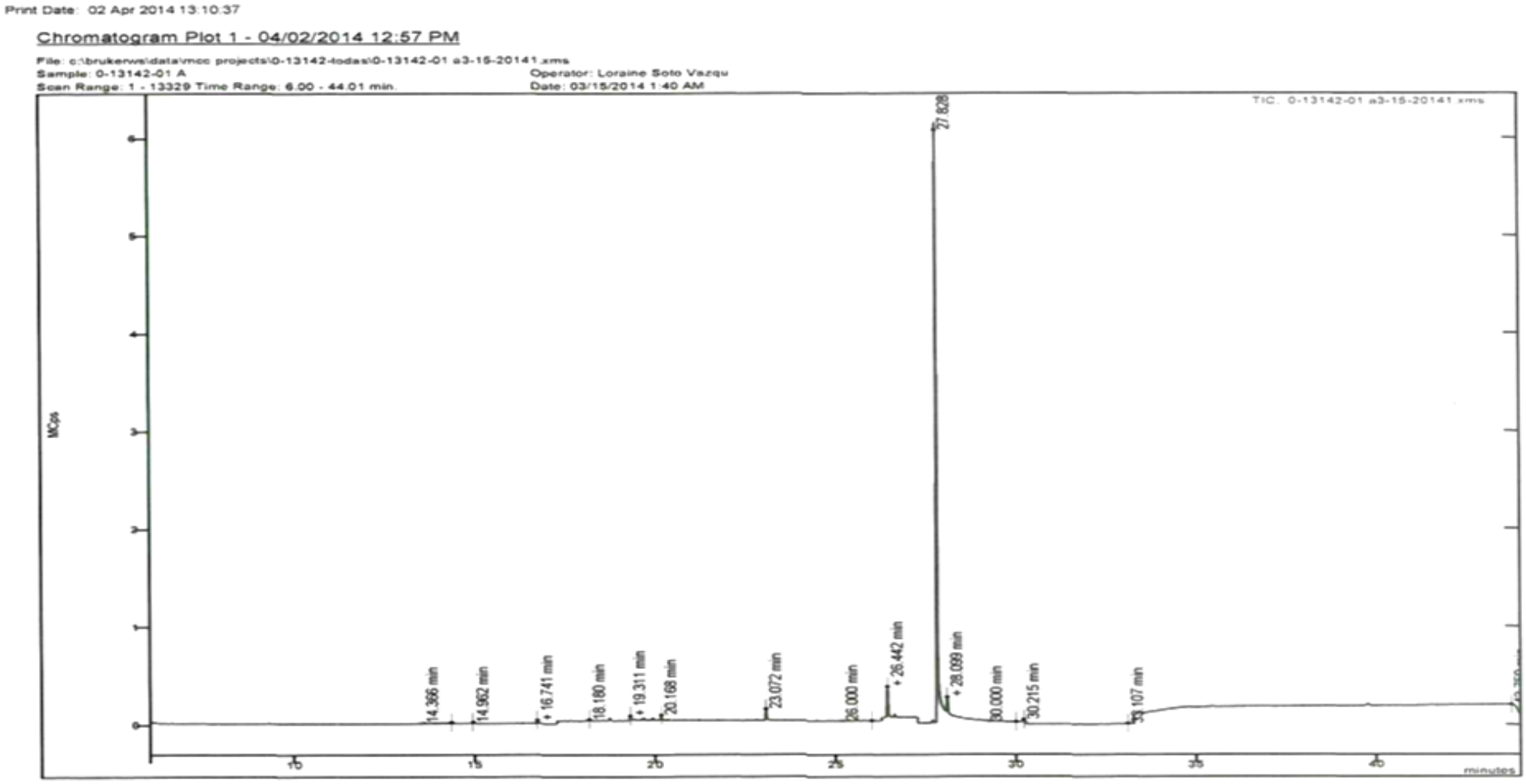


Figure 4.9: Gas chromatogram of pesticide residues in spinach from Okada road farm.

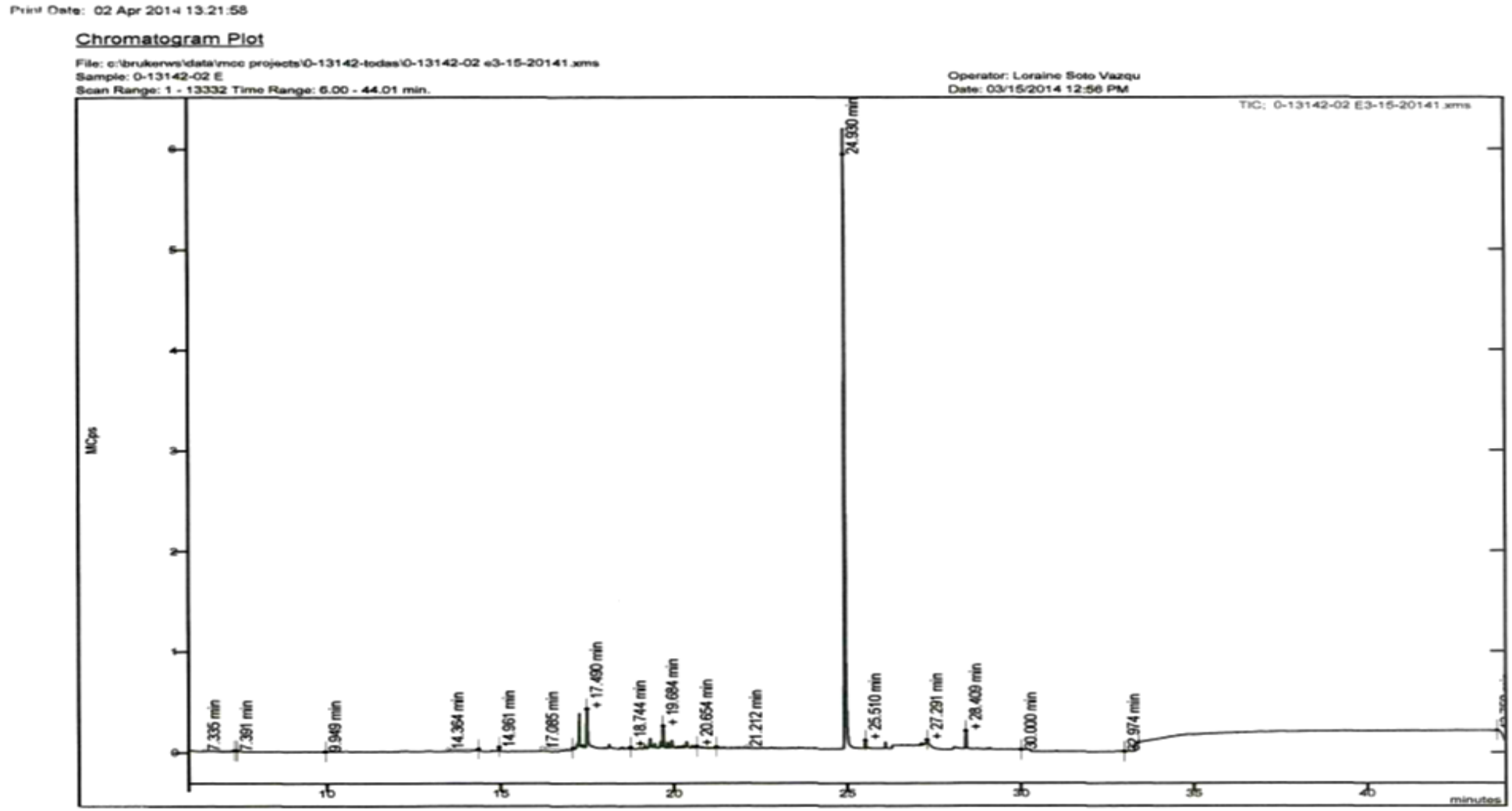


Figure 4.10:Gas chromatogram of pesticide residues in okra from Chanchaga farm.

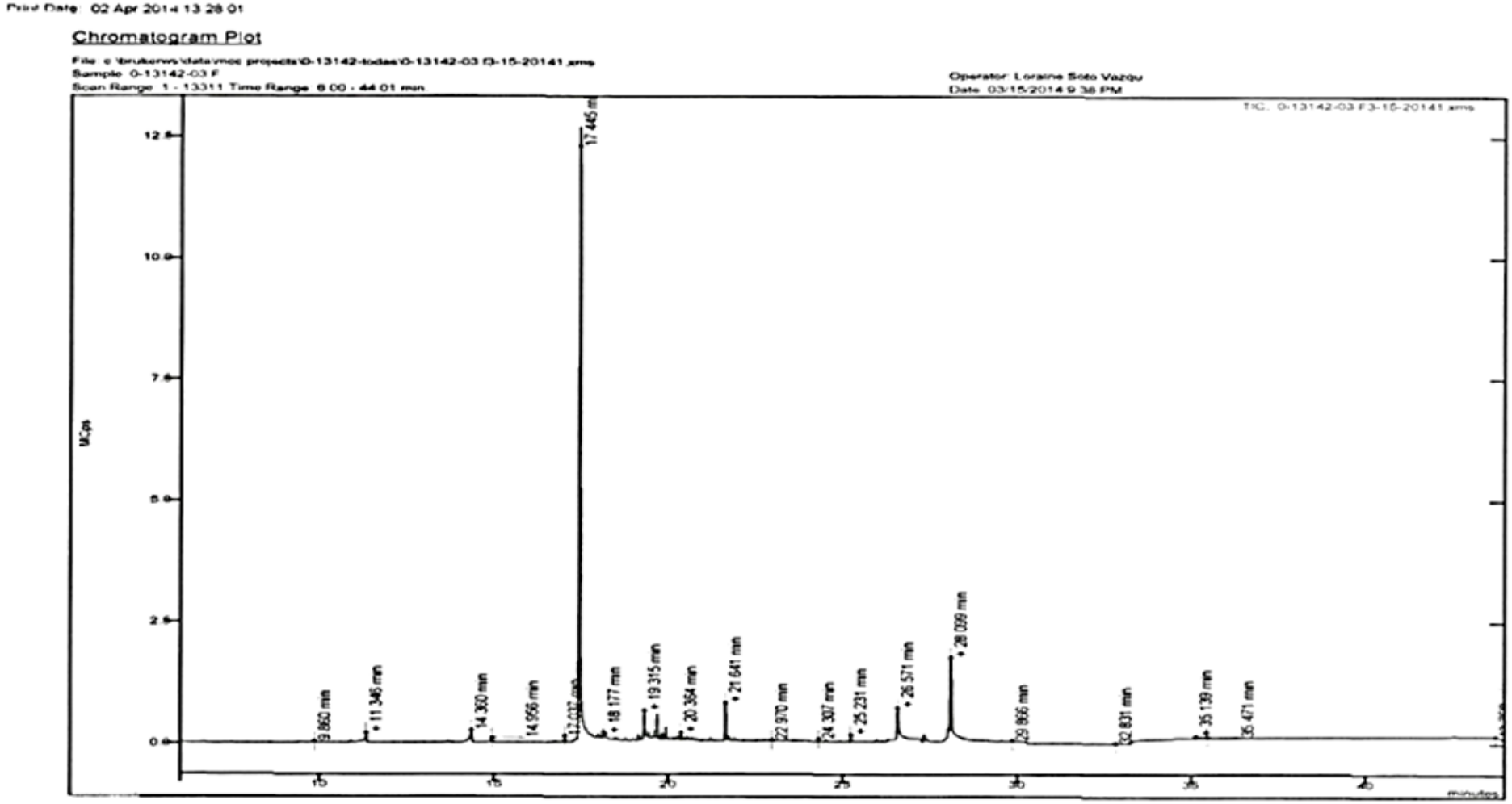


Figure 4.11: Gas chromatogram of pesticide residues in pumpkin leaves from farm on Mandela area.

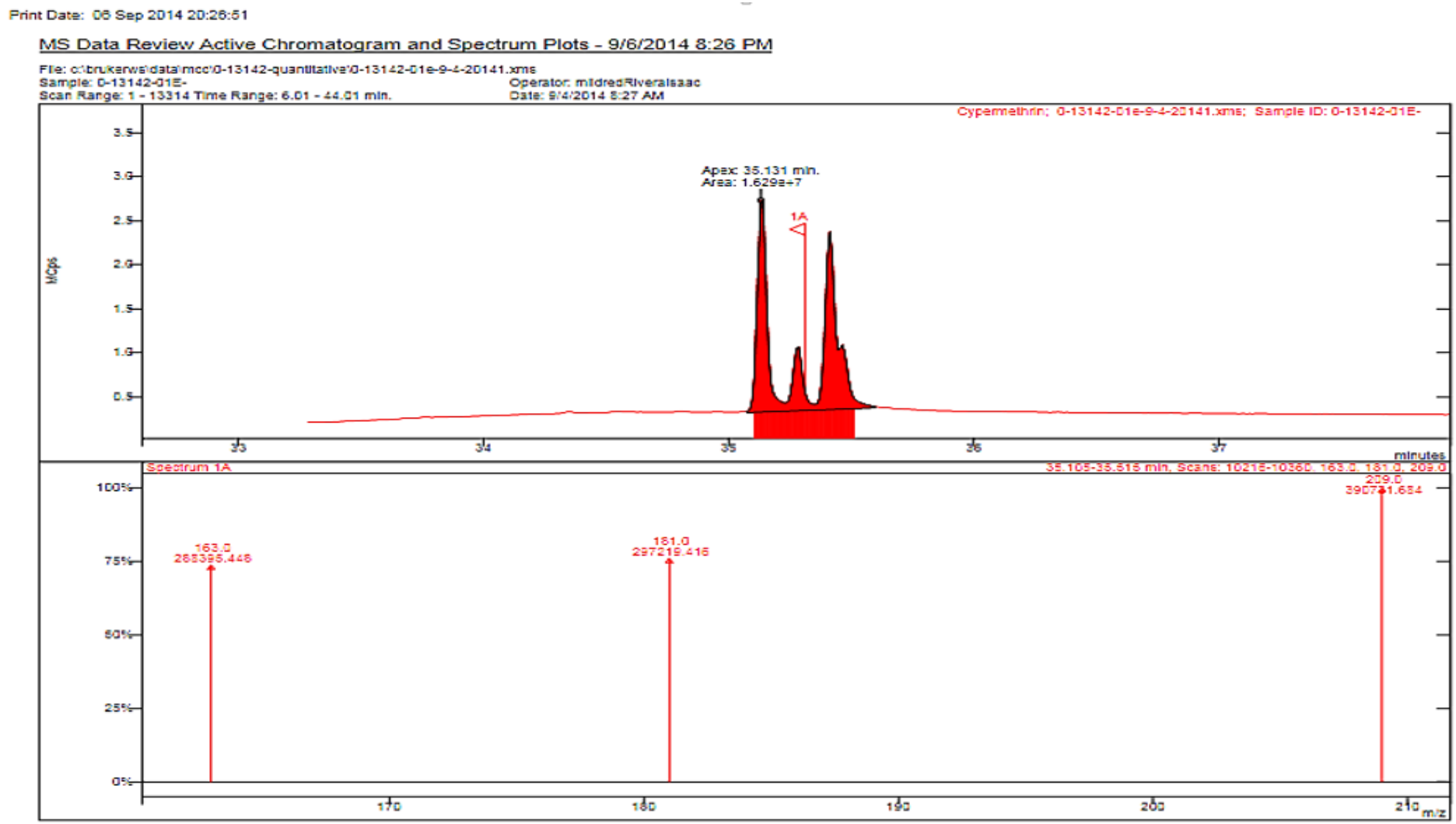


Figure 4.12: Gas chromatogram and mass spectra of pesticide residues in spinach from Chanchaga farm (Cypermethrin)

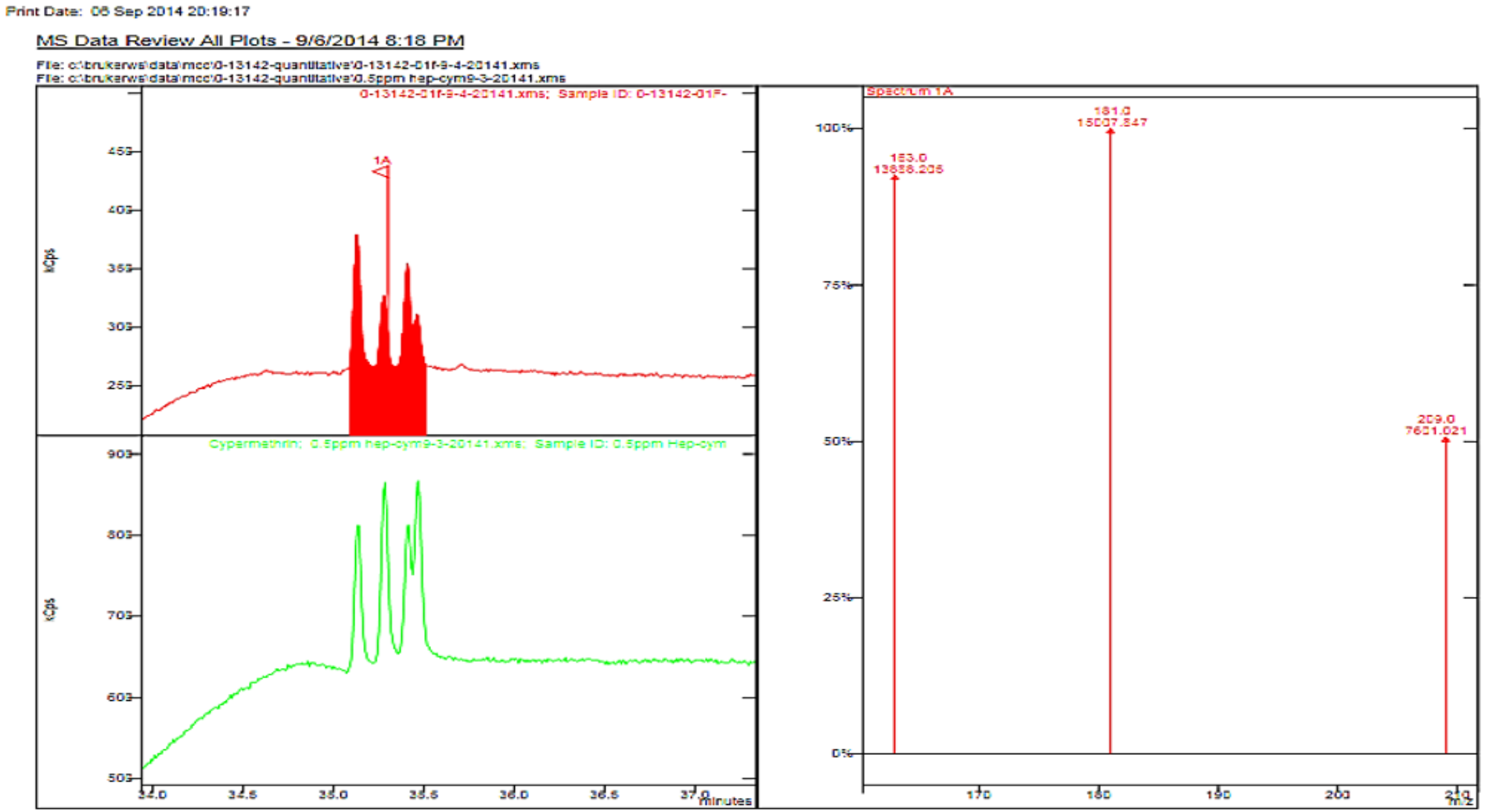


Figure 4.13: Gas chromatogram at lower (red)/ higher (green) intensity and mass spectrum of pesticide residues in spinach from Mandela farm.

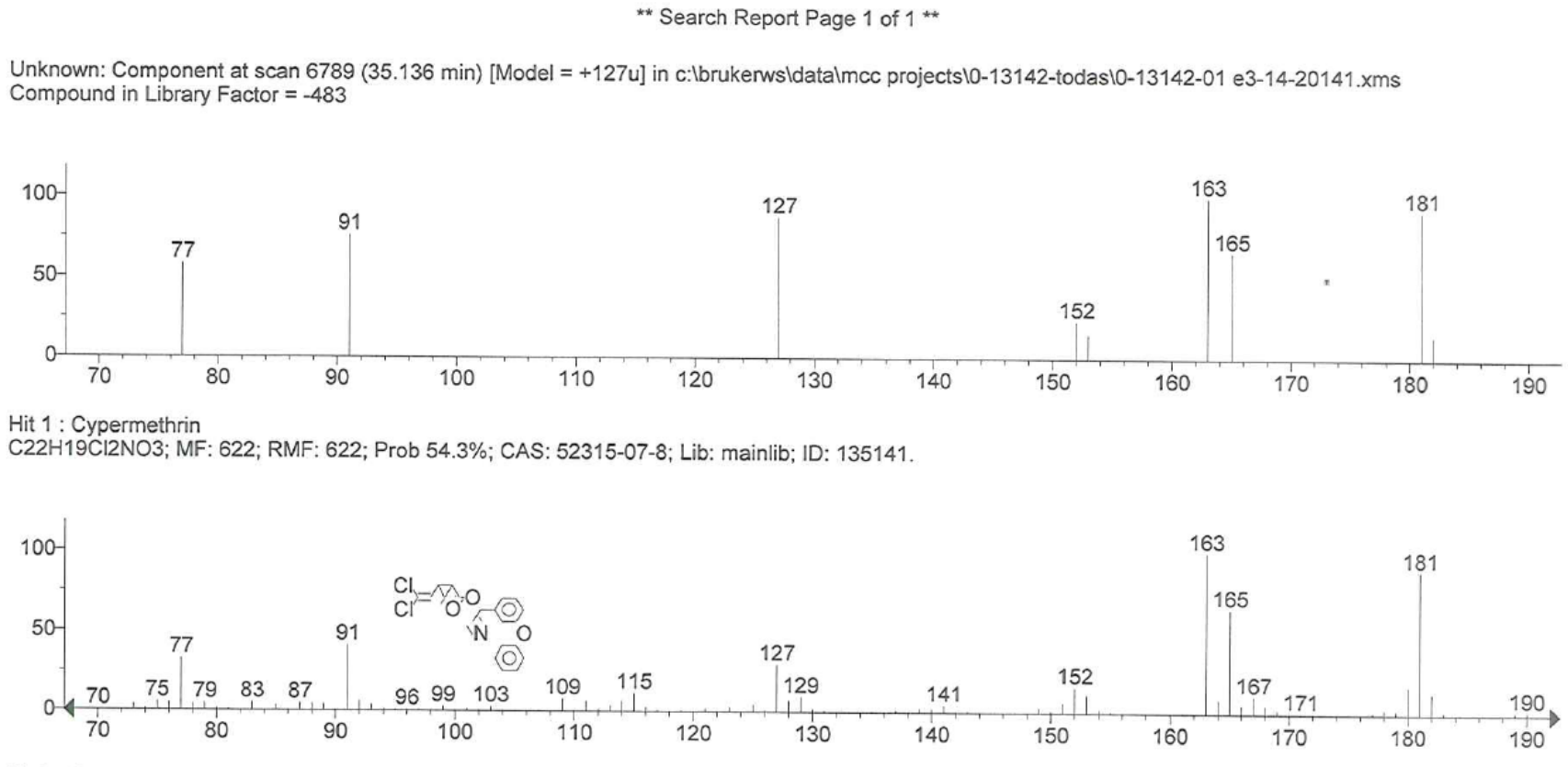


Figure 4.14: Library search for cypermethrin.

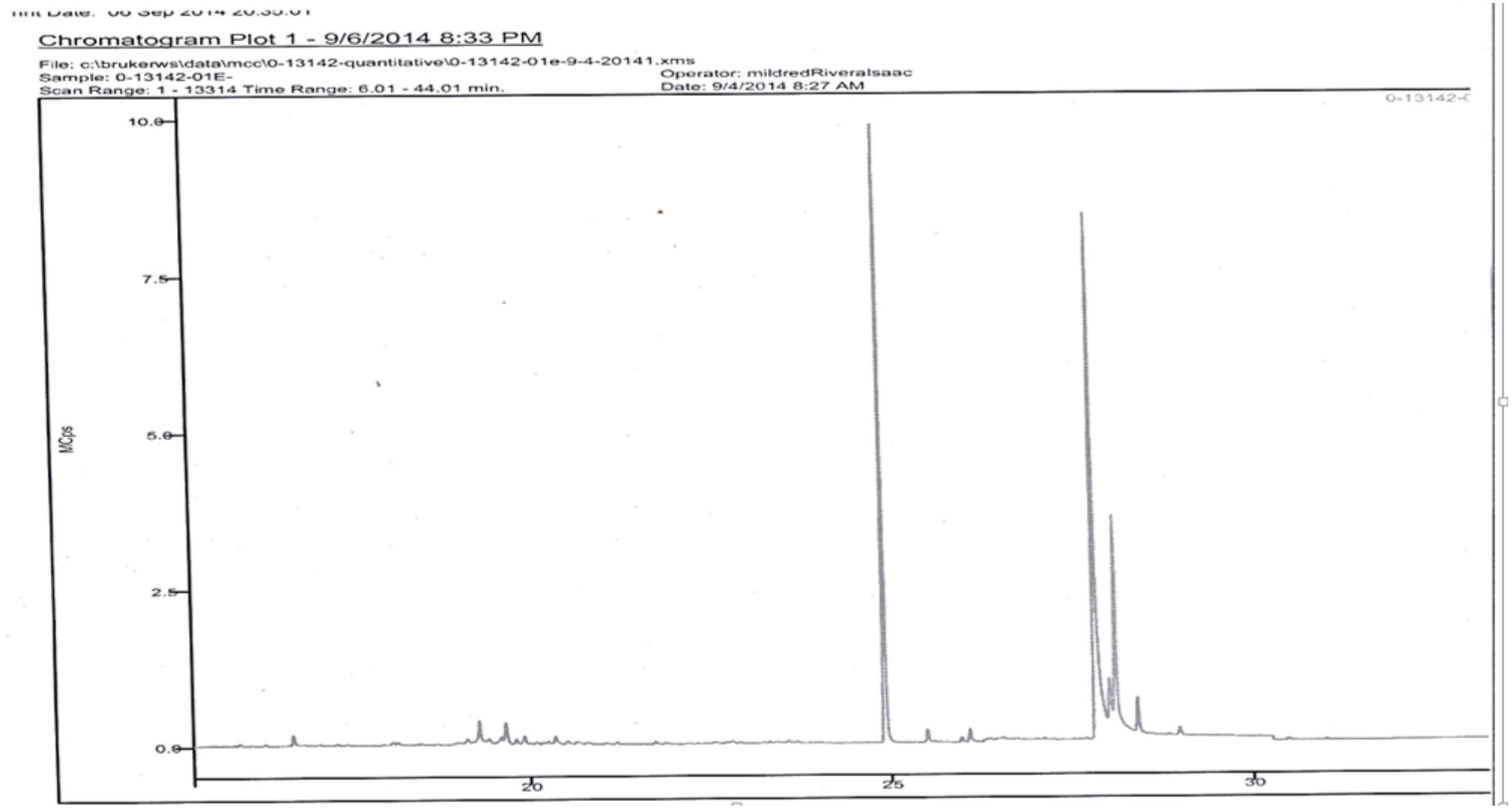


Figure 4.15: Gas chromatogram of pesticide residues in spinach from Chanchaga farm (heptachlor).

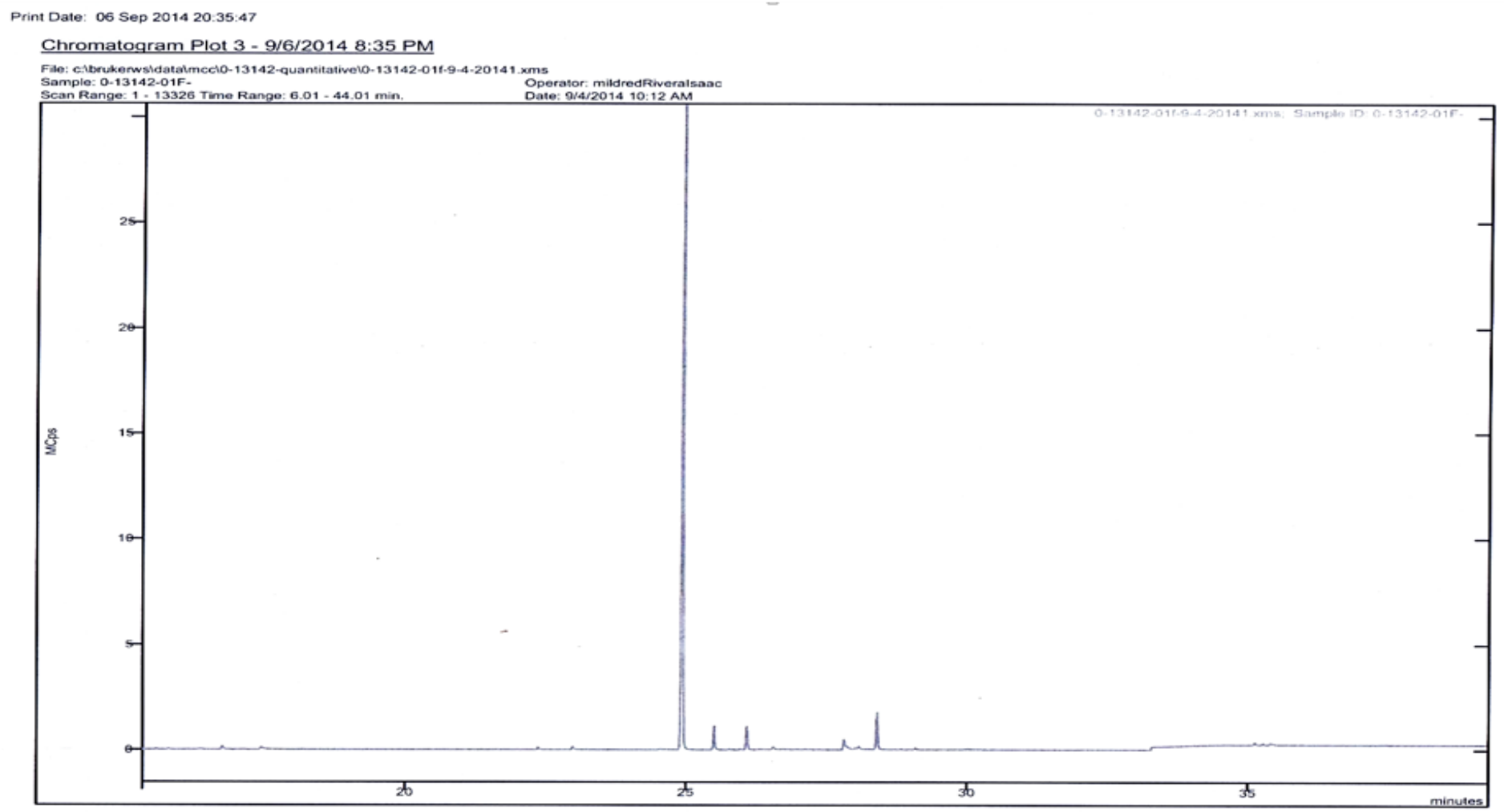


Figure 4.16: Gas chromatogram of spinach from on Mandela road farm (heptachlor).

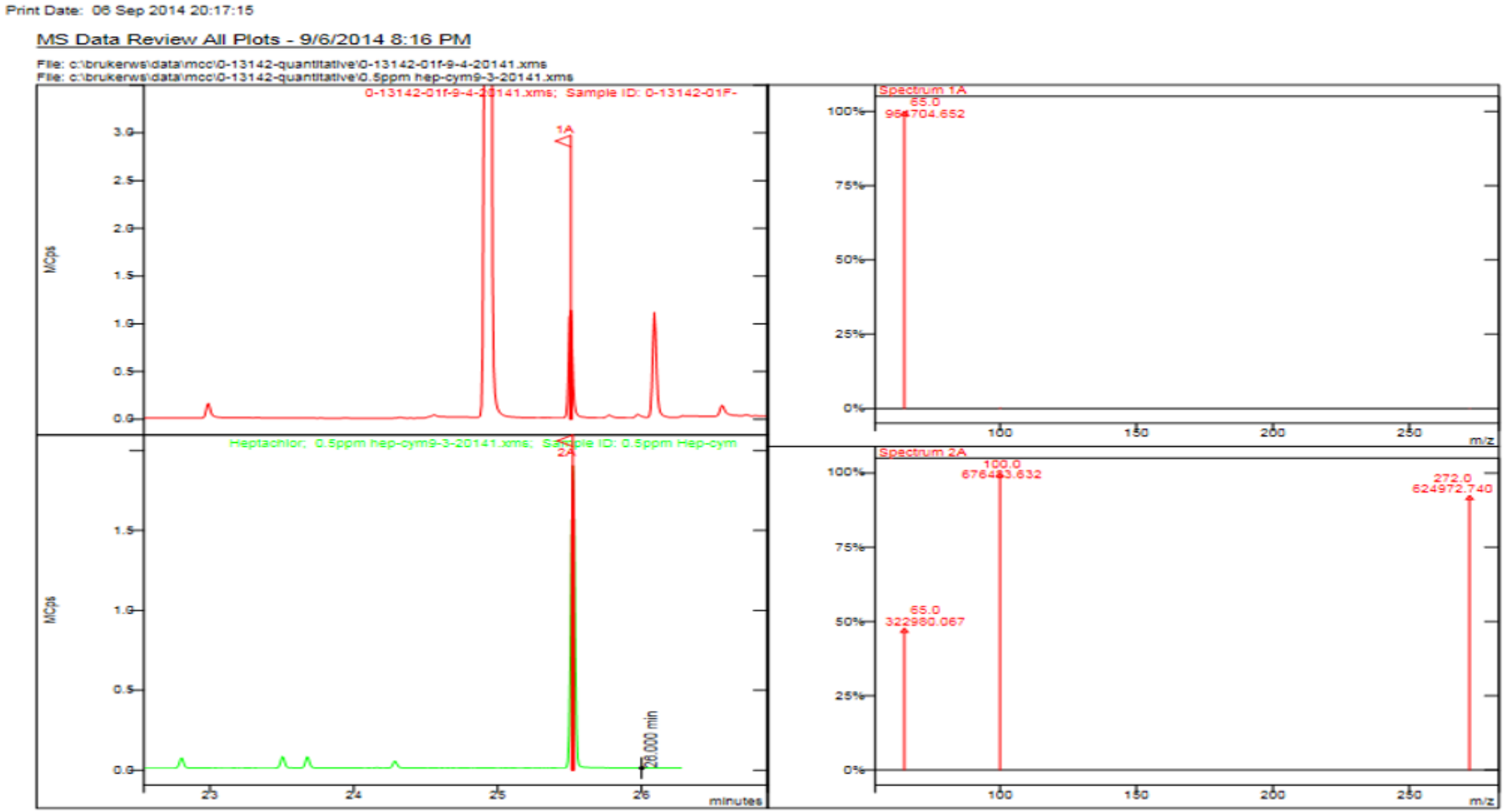


Figure 4.17: Gas chromatograms and mass spectra of pesticide residues in spinach from farm on Mandela road (1A) and heptachlor standard (2A).

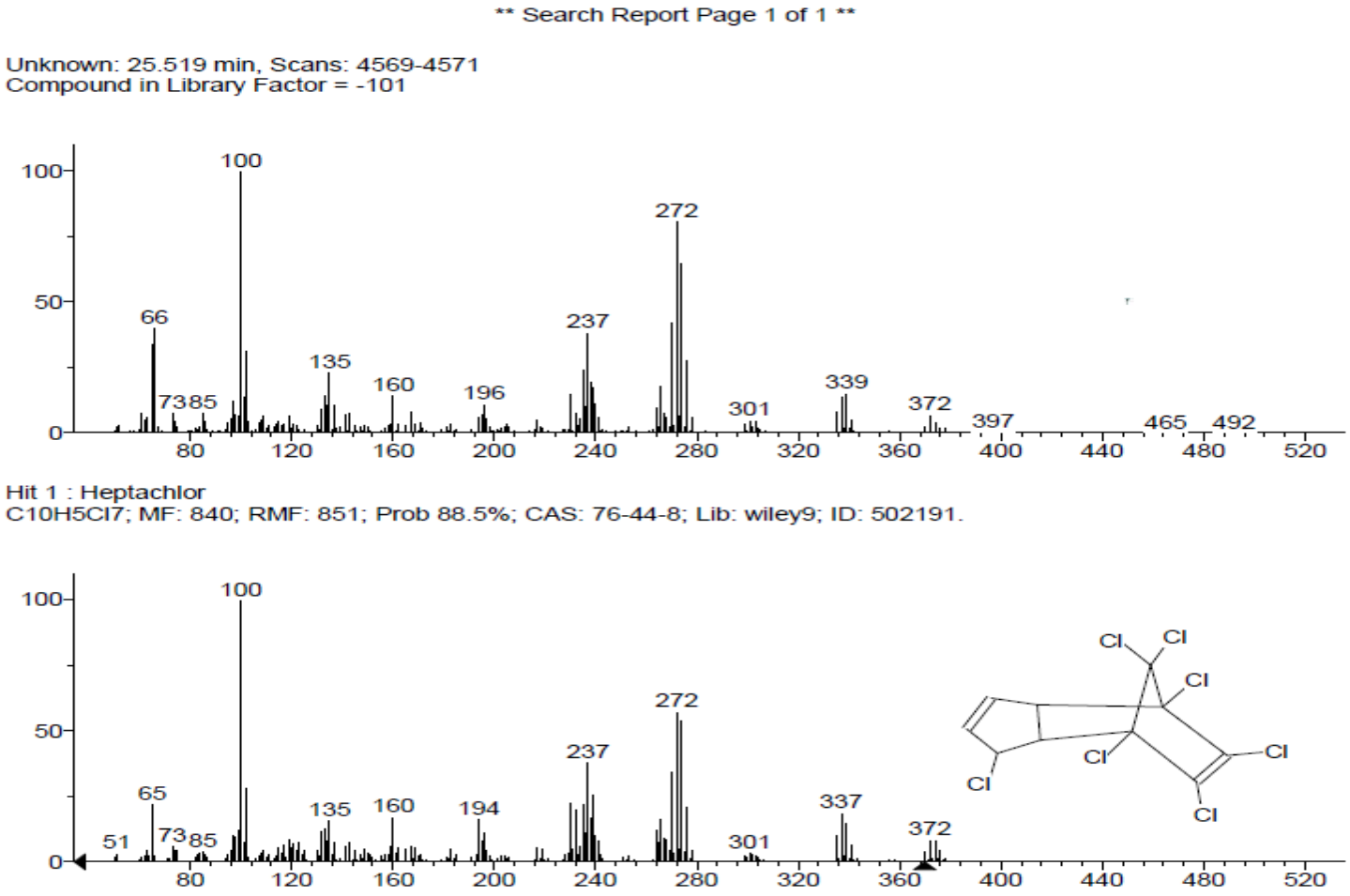


Figure 4.18: Library search for heptachlor.

Table 4.3 gives a summary of results for all spinach samples from all the farms. Spinach samples from Chanchaga and Mandela farms contain cypermethrin pesticide and only one ion fragment of heptachlor was observed.

The summary of results for okra samples from the seven farms is given in Table 4.4. This table shows that none of the okra samples contain any of the pesticides in this research; if present they are below the detection limit of this method.

Analysis results for the pesticide residues in fluted pumpkin leaves of the seven farms is shown in Table 4.5. Clearly, none of the pumpkin leaves contain any of the pesticides in this research; if present they were below the detection limit of this method.

4.2.4 Concentration of detected pesticides in vegetables

Table 4.6 gives a summary of representative quantitative results for spinach samples from Chanchaga and Mandela farms using SIM-MS techniques. The presence of all cypermethrin isomers was observed in samples of spinach from Chanchaga and Mandela farms. The concentration range of 8.65 ±1.30 and 0.60 ±0.09 mg/kg respectively was obtained for each farm. Cypermethrin calibration curve was used for the determination of concentration of the pesticide. The average response factors over the entire calibrations range was $RSD \leq 15\ \%$ demonstrating the method's precision for analysing the pesticides in these samples. The determination coefficient R^2 was 0.9961 with linearity in the compound which allows quantitation using external standardization.

Table 4.3: Pesticide Residues in for Spinach Samples

Farm	Retention Times (Min)	Ion Mass (a.m.u)	Pesticide
A	Nil	ND	ND
B	Nil	ND	ND
C	Nil	ND	ND
D	Nil	ND	ND
E	35.14, 35.41, 35.29 25.52	163, 181, 209 65, 100, 272**	Cypermethrin Heptachlor
F	35.14, 35.41, 35.29 25.52	163, 181, 209 65, 100, 272**	Cypermethrin Heptachlor
G	Nil	ND	ND

a.m.u = atomic mass unit or molecular ion weight units. ND = not detected, if present it is below detection limits. ** only one ion mass (65) detected in full scan and SIM mode.

Table 4.4: Pesticide Residues in Okra Samples

Farm	Retention Times (Min)	Ion Mass (a.m.u)	Pesticide
A	Nil	ND	ND
B	Nil	ND	ND
C	Nil	ND	ND
D	Nil	ND	ND
E	Nil	ND	ND
F	Nil	ND	ND
G	Nil	ND	ND

a.m.u = atomic mass unit or molecular ion weight units. ND = not detected, if present it is below detection limits

Table 4.5: Pesticide Residues in Fluted Pumpkin Samples

Farm	Retention Times (Min)	Ion Mass (a.m.u)	Pesticide
A	Nil	ND	ND
B	Nil	ND	ND
C	Nil	ND	ND
D	Nil	ND	ND
E	Nil	ND	ND
F	Nil	ND	ND
G	Nil	ND	ND

a.m.u = atomic mass unit or molecular ion weight units. ND = not detected, if present it is below detection limits.

Table 4.6: Peak retention time, ion mass detected and pesticides concentration in spinach in farm E and F.

Sample/Farm	Pesticide Identity (Species or Isomer)	Peaks Retention Times (Mins)	Ion Mass Detected (a.m.u)	Pesticide Extract Concentration mg/kg
Spinach (E)	Cypermethrin(a) Cypermethrin(b) Cypermethrin(d) Cypermethrin (e)	35.13 35.28 35.41 35.46	163, 181, 209	8.65 ±1.30
Spinach (E)	Heptachlor	25.51	65	BDL
Spinach (F)	Cypermethrin (a) Cypermethrin (b) Cypermethrin (d) Cypermethrin (e)	35.13 35.28 35.41 35.46	163, 181 209	0.60 ±0.09
Spinach (F)	Heptachlor	25.51	65	BDL

The presence of heptachlor was not confirmed in spinach samples from Chanchaga and Mandela farms only the qualifier ion 65 a.m.u was found, relevant ions (100 and 272) were not detected, if present were below detection limit.

4.3 Physico-Chemical Properties of Soil Samples from Farms

The results of the physico-chemical properties of the soil samples are given in Tables 4.7 to 4.9. The pH values of all the soil samples analyzed from the seven farms ranged from 5.77 to 7.70 (Table 4.7) while keteren gwari farm had the highest pH value Chanchaga farm had the lowest pH value, indicating the acidic nature of the soil. Conductivity measurements show the farm at Sauka ka huta with the lowest value of 17 µS/cm and Dutsen kura farm had the highest value of 37 µS/cm. The soil organic matter and organic carbon were in the range of 1.63-3.87 % and 0.95-2.25 % respectively. The farm at Kpakungu had both the highest organic matter and organic carbon while Sauka ka huta farm had the lowest values. The percentage organic carbon in organic matter was (58 %) in all the soils from different farms (Table 4.7).

The result for particle size and texture of the soil samples is as shown in Table 4.8. The textural class of the soil samples were sandy to loamy sandy, with the exception of Chanchaga farm which was silty loam.

Table 4.9 shows the result for the effective cation exchange capacity (CEC) obtained by summing up the values of the exchangeable bases and exchange acidity. The result from the Table show that Keteren gwari farm had the highest CEC value of 5.15 cmol/kg while Sauka ka huta farm had the least value of 3.68 cmol/kg.

Table 4.7: pH, Conductivity, Organic Matter (OM) and Organic Carbon (OC) content of Soil Samples from the Farms in Minna

Farm	pH	Conductivity (µS/cm)	Organic matter (%)	Organic Carbon (%)	OC in OM (%)
A	7.59±0.07	24.00±1.00	2.44±0.18	1.42±0.37	58.20
B	6.64±0.04	29.00±0.00	3.87±0.19	2.25±0.25	58.14
C	7.25±0.12	37.00±0.00	2.95±0.23	1.72±0.02	58.31
D	7.70±0.05	20.00±1.00	2.06±0.26	1.20±0.21	58.25
E	5.77±0.06	18.00±1.00	3.09±0.17	1.80±0.18	58.25
F	6.73±0.09	21.00±0.00	1.92±0.30	1.12±0.09	58.33
G	7.35±0.11	17.00±0.00	1.63±0.27	0.95±0.18	58.28

Table 4.8: Particle Size (g/kg) and Texture of Soil Samples from the Farms in Minna

Farm	Sand	Silt	Clay	Texture
A	95.80±0.17	1.60±0.26	4.65±0.13	Sand
B	85.60±0.20	12.80±0.36	1.60±0.33	Loamy Sand
C	95.20±0.36	2.80±0.17	2.00±0.10	Sand
D	93.20±0.16	5.00±0.26	1.80±0.17	Sand
E	65.00±0.17	33.60±0.43	1.40±0.26	Silty Loam
F	87.00±0.36	7.00±0.26	6.00±0.20	Loamy Sand
G	89.60±0.26	9.80±0.34	0.60±0.09	Loamy Sand

Table 4.9: Exchangeable Bases and Cation Exchange Capacity (cmol/kg) of Soil Samples from the Farms in Minna

Farm	Na^+	K^+	Mg^{2+}	Ca^{2+}	CEC
A	0.62±0.12	0.23±0.08	0.15±0.03	3.36±0.18	4.65±0.08
B	1.64±0.14	0.05±0.03	0.11±0.02	2.57±0.13	4.33±0.23
C	0.86±0.11	0.45±0.04	0.15±0.03	3.36±0.13	4.85±0.10
D	0.48±0.05	0.10±0.04	0.31±0.04	4.21±0.25	5.15±0.29
E	1.06±0.07	0.12±0.05	0.12±0.02	2.50±0.07	3.83±0.15
G	0.48±0.07	0.08±0.02	0.22±0.01	3.56±0.06	4.37±0.06
F	0.95±0.04	0.29±0.09	0.11±0.02	2.30±0.17	3.68±0.09

4.4 Heavy Metals in Soil from Farms

4.4.1 Level of each heavy metal in all farms

The level of cadmium in the soil samples of all farms analyzed are given in Figure 4.19. From the chart, cadmium was detected in only farms D and G. The Soil samples from other farms did not show the presence of cadmium metal. The highest level of 0.00134 mg/g was recorded in Keteren gwari farm while Sauka ka huta farm had 0.00018 mg/g.

The levels of copper in all the soil samples are presented in Figure 4.20. The soil samples from all farms contained some level of copper. Concentration range of the metal in the

samples was 0.00671 – 0.04759 mg/g. Kpakungu farm had the highest while Mandela farm had the lowest.

Figure 4.21 shows the level of iron in all the soil samples of all farms. From the results, the soil obtained from Chanchaga farm had the highest level (2.2059 mg/g), while the lowest level of 1.94213 mg/g was observed in the sample from Keteren gwari farm.

The level of manganese in all the soil samples are presented in Figure 4.22. From the results, the samples from Kpakunku and Chachaga farms contained almost equal level of the metal 0.36958 and 0.37178 mg/g, respectively. Keteren gwari farm had the lowest level of 0.18248 mg/g.

Figure 4.23 gives the results of nickel in all the soil samples. Okada, keteren gwari and Mandela farms did not indicate the presence of nickel while the samples from Kpakungu farm had the highest level (0.00690 mg/g), followed by Chanchaga farm (0.00433 mg/g). The value of 0.00065 mg/g was the lowest observed level from Sauka ka huta farm. The results of the level of zinc from all the soil samples analyzed is also shown in Figure 4.24. Clearly, all the samples contained zinc. The soil samples from Sauka ka huta farm had the highest level of 0.22520 mg/g while the lowest level of 0.01570 mg/g was obtained in Mandela farm.

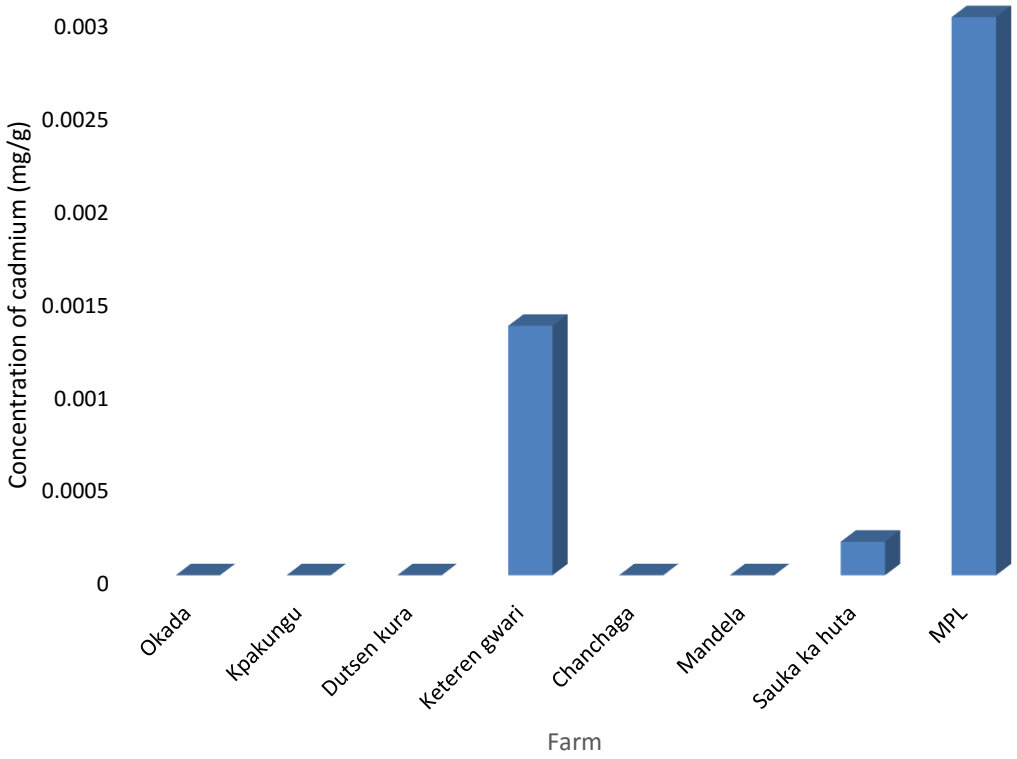


Figure 4.19: Concentrations in of cadmium in soil samples from selected farms in Minna.

MPL = Maximum Permissible Limit (Sumaila, 2014)

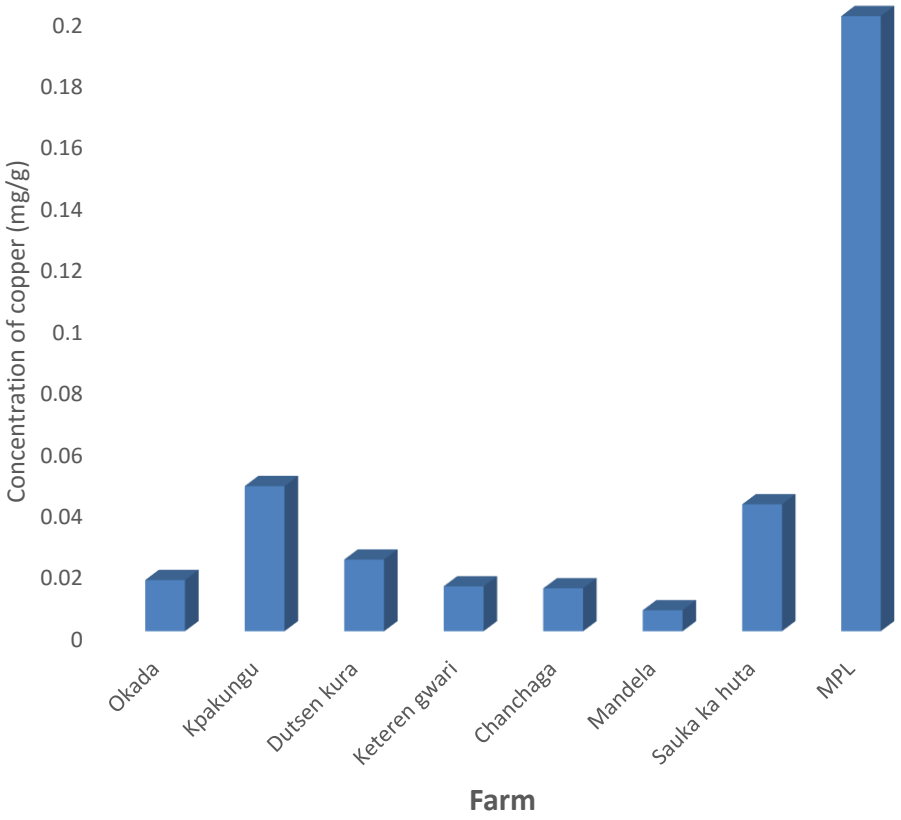


Figure 4.20: Concentrations in of copper in soil samples from selected farms in Minna MPL = Maximum Permissible Limit (Sumaila, 2014)

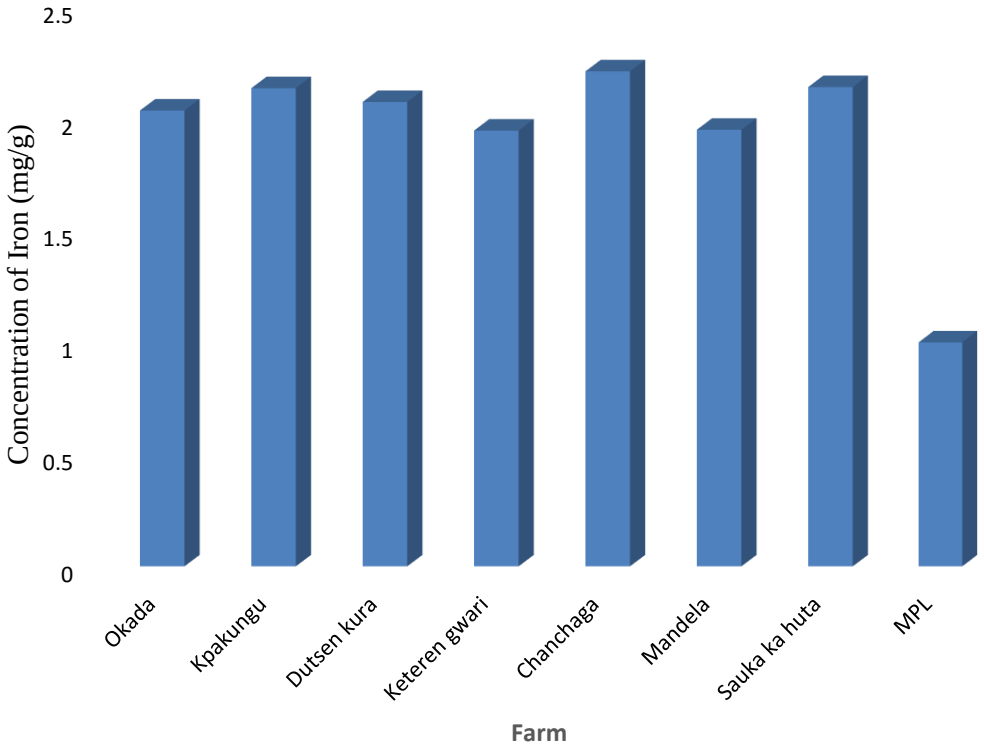


Figure 4.21: Concentrations in of iron in soil samples from selected farms in Minna

MPL = Maximum Permissible Limit (Sumaila, 2014)

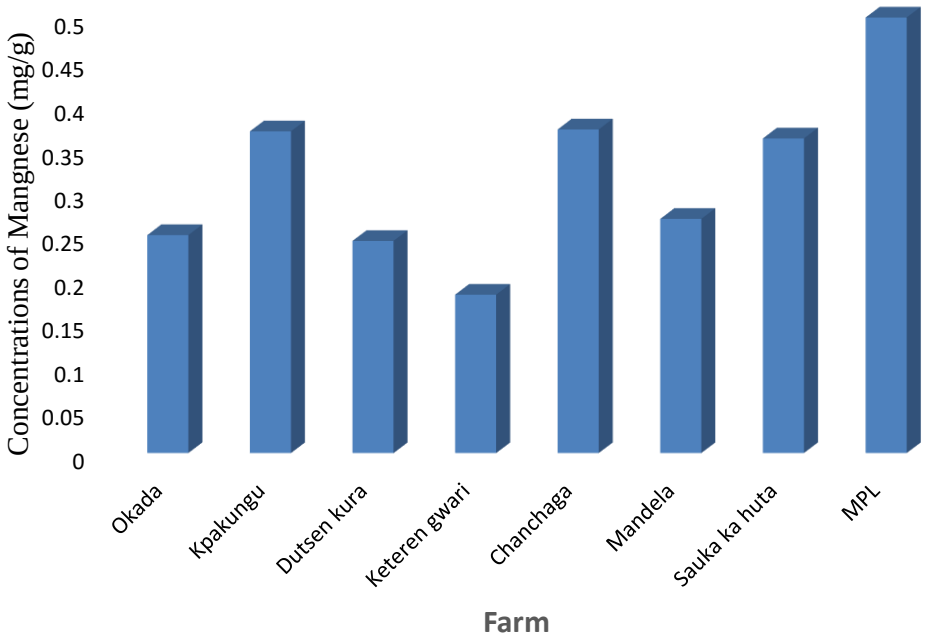


Figure 4.22: Concentrations in of manganese in soil samples from selected farms in Minna

MPL = Maximum Permissible Limit (Sumaila, 2014)

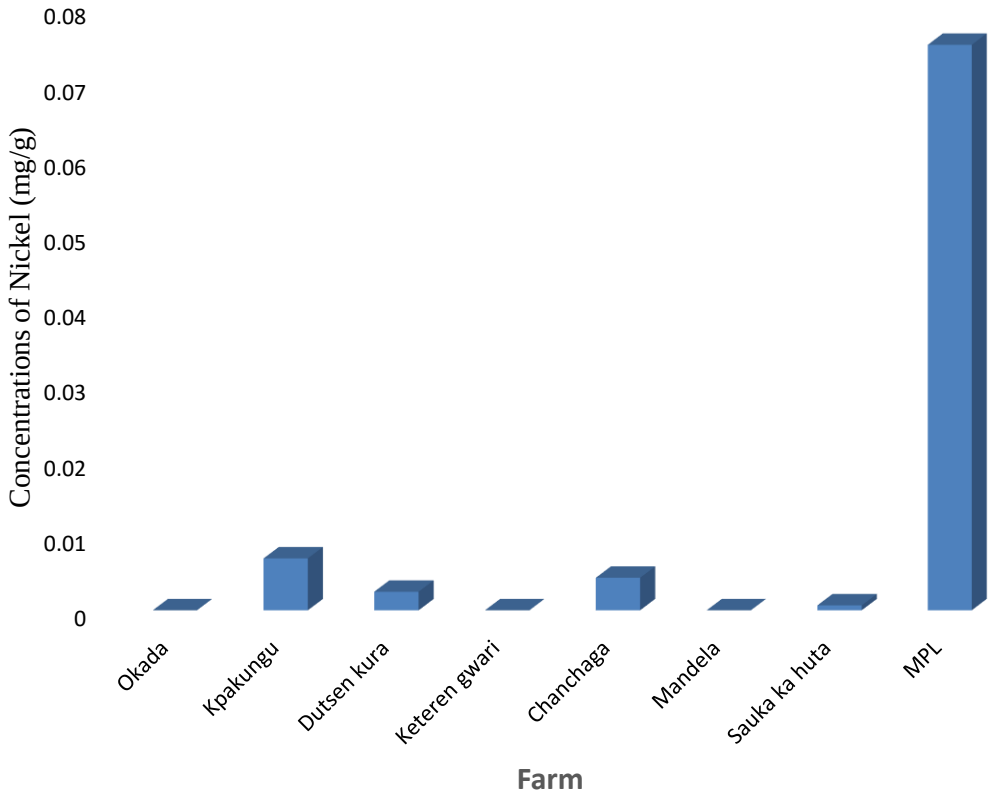


Figure 4. 23: Concentrations in of nickel in soil samples from selected farms in Minna

MPL = Maximum Permissible Limit (Sumaila, 2014)

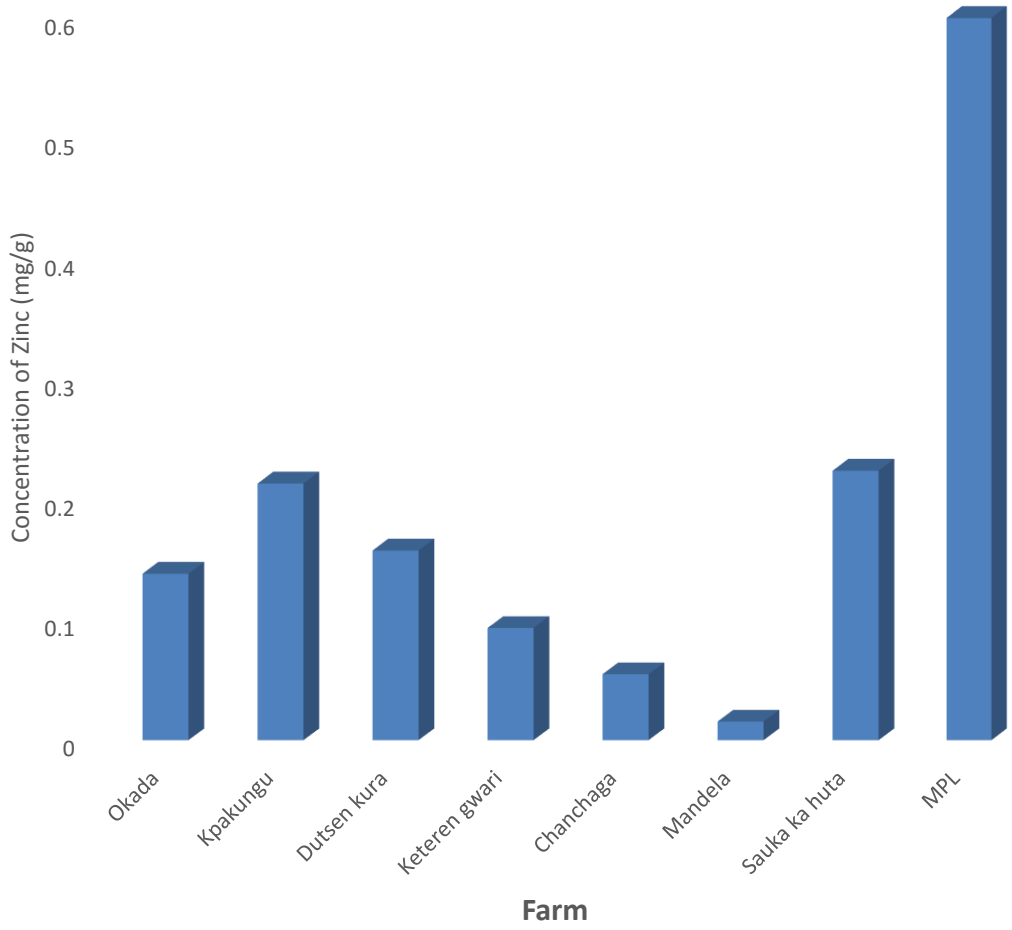


Figure 4.24: Concentrations in of zinc in soil samples from selected farms in Minna

MPL = Maximum Permissible Limit (Sumaila, 2014)

4.4.2 Levels of heavy metals in soil of each farm

The levels of the heavy metals in the soil of the control farm are presented in Figure 4.25. The farm had Fe in the highest concentration of 2.03150 mg/g while Cu was the least (0.01670 mg/g). Mn level recorded in the farm was 0.25070 mg/g. Cd and Ni were either not present or below the detection limit. The level of each heavy metal in Kpakungu farm is shown in Figure 4.26. Fe also had the highest concentration of 2.13080 mg/g in Kpakungu farm. This is followed by Mn (0.3696 mg/g) and then Zn (0.21460 mg/g) while Cu had a low concentration of 0.0476 mg/g and Ni with the least value of 0.0069 mg/g. Cd was also not detected in this farm. Figure 4.27 shows the level of heavy metals present in Dutsen kura farm, with Fe having the highest value of 2.0696 mg/g followed by Mn (0.2440 mg/g) and Zn (0.1591 mg/g) while Cu was low with 0.0235 mg/g and Ni has the least with 0.00247 mg/g. Cd was not detected in this farm.

The levels of the heavy metals in Keteren gwari and Chanchaga farms are shown in Figures 4.28 and 4.29, respectively. The two farms also had Fe in the highest concentration of 1.942 mg/g and 2.206 mg/g respectively. Cd was not detected in Chanchaga farm but very low level was recorded for Keteren gwari farm (0.00134 mg/g). In Keteren gwari farm Ni was below the detection limit while 0.00433 mg/g was recorded in Chanchaga farm. However, the level of Zn in Chanchaga farm (0.05563 mg/g) was lower than that of Keteren gwari farm (0.0947 mg/g). Figure 4.30 shows the levels of heavy metals in Mandela farm. Four metals were detected with Fe having the highest value of 1.9460 mg/g followed by Mn (0.2693 mg/g) while Zn had a value of 0.0157 mg/g and Cu with the least value of 0.0069 mg/g. Cd and Ni were also not detected in this farm. The results for Sauka ka huta farm is presented in Figure 4.31. All the heavy metals except Cd and Ni were detected in this farm. The level of Mn was higher than Zn while Cu was the least and Fe the highest.

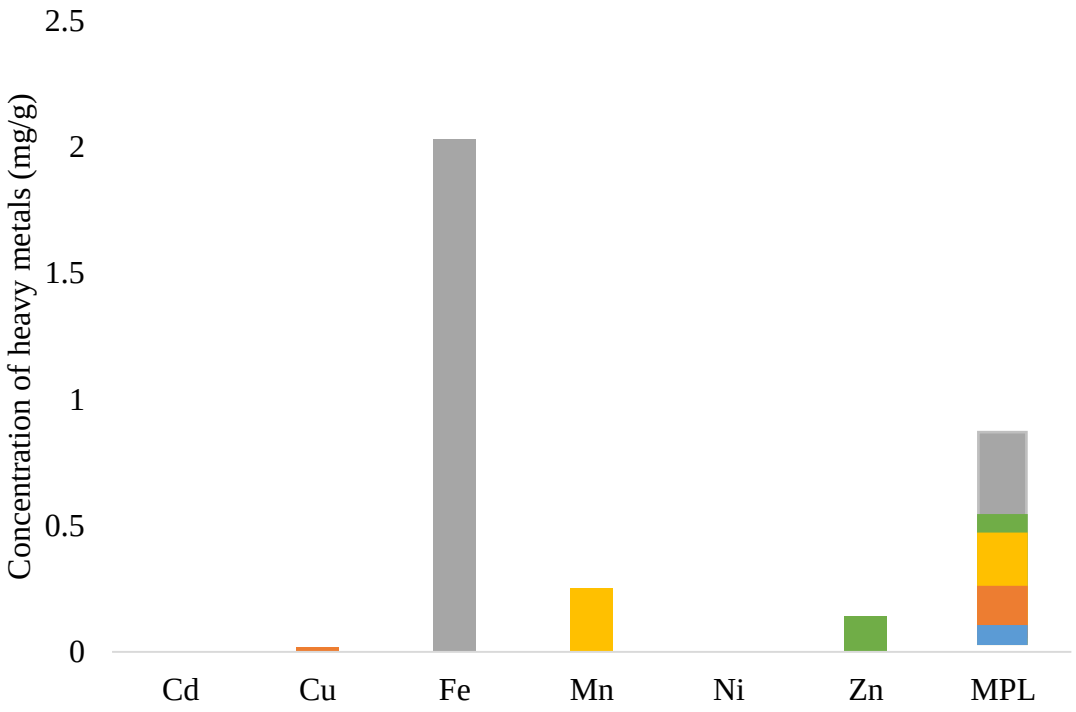


Figure 4.25: Concentration of heavy metals in Okada Road farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

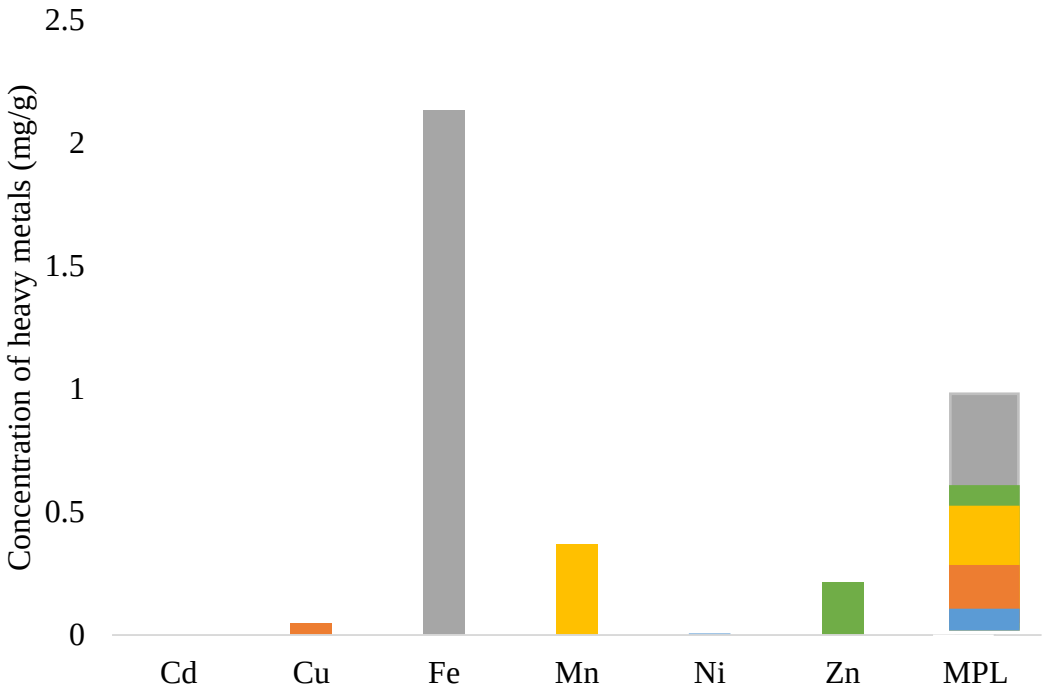


Figure 4.26: Concentration of heavy metals in Kpakungu farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

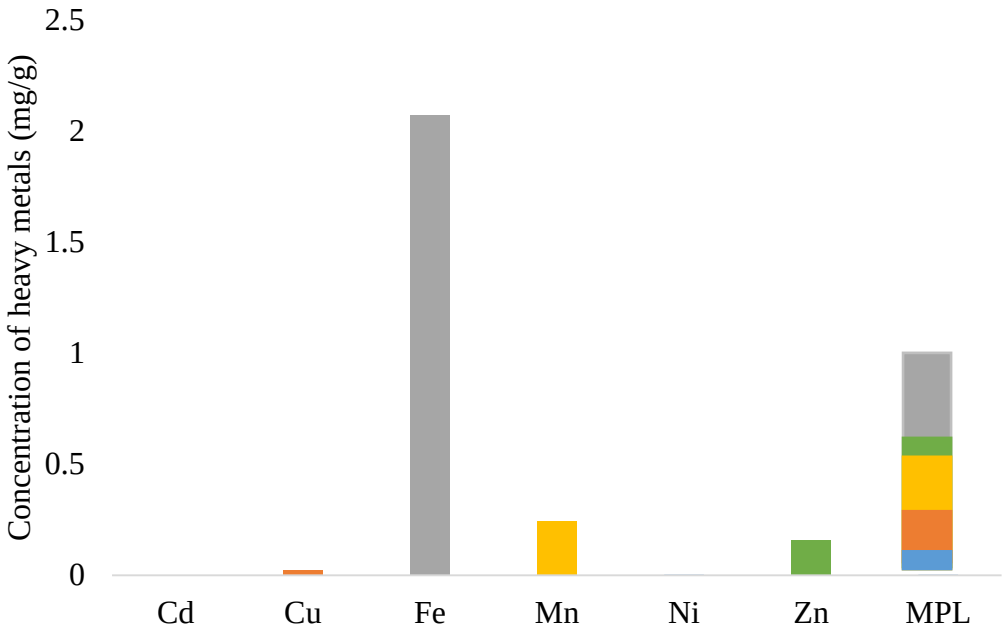


Figure 4.27: Concentration of heavy metals in Dutsen kura farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

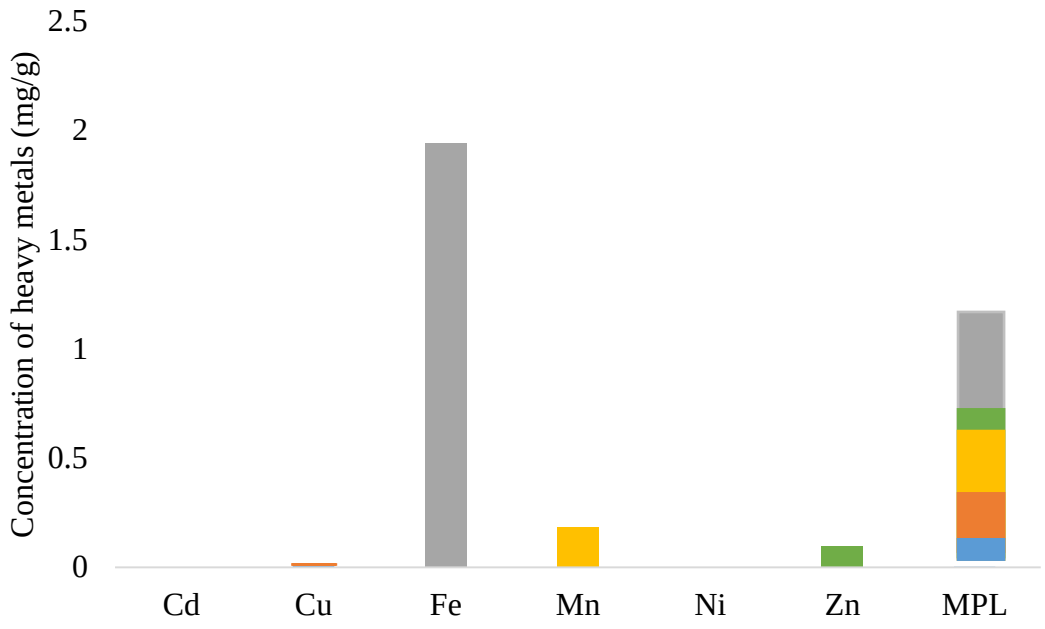


Figure 4.28: Concentration of heavy metals in Keteren gwari farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

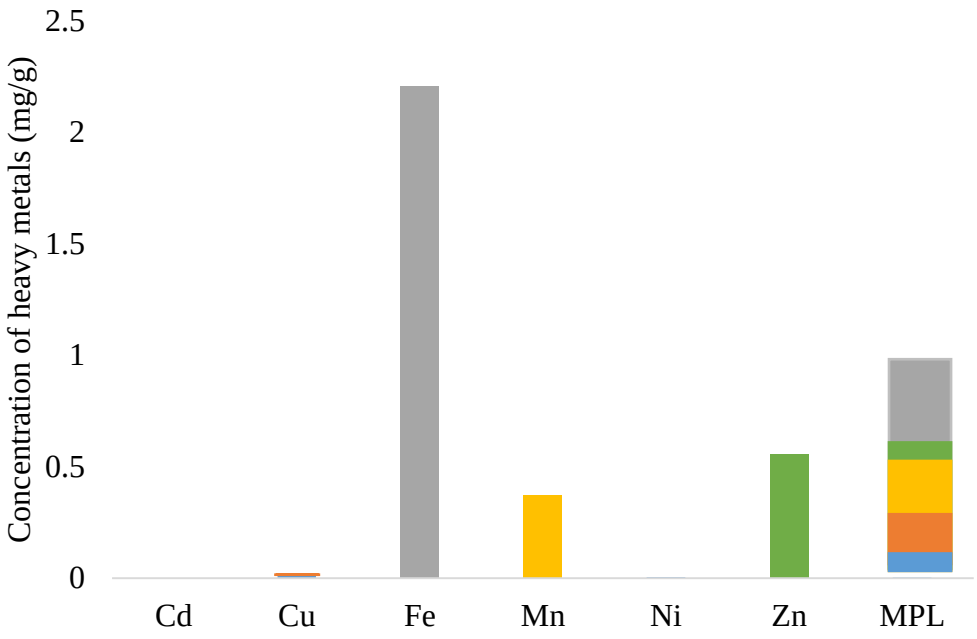


Figure 4.29: Concentration of heavy metals in Chanchaga farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

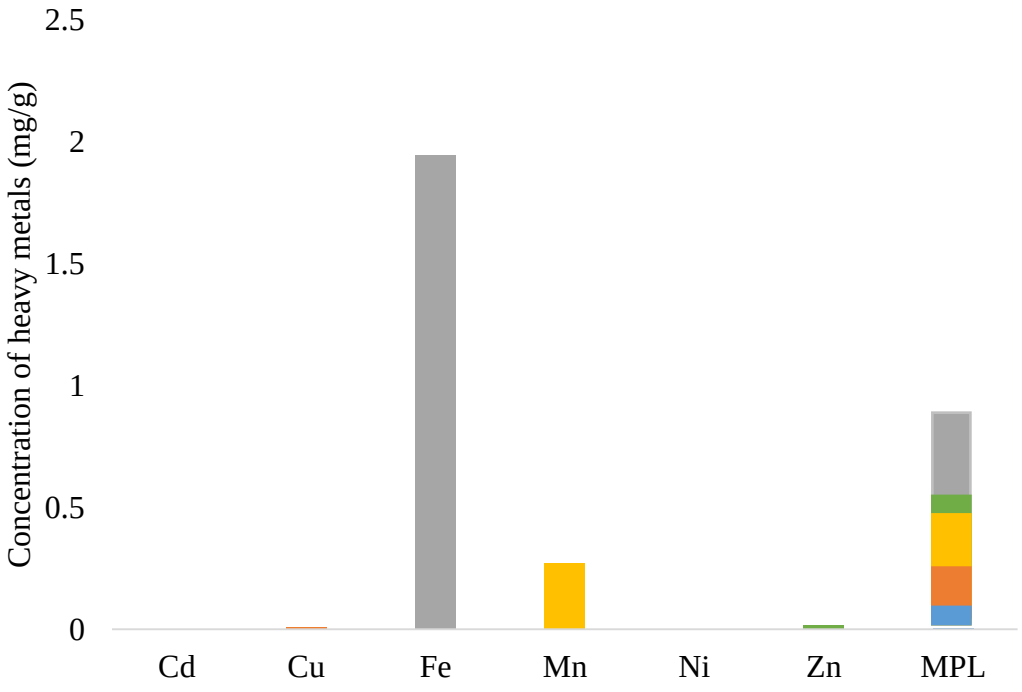


Figure 4.30: Concentration of heavy metals in Mandela Area farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

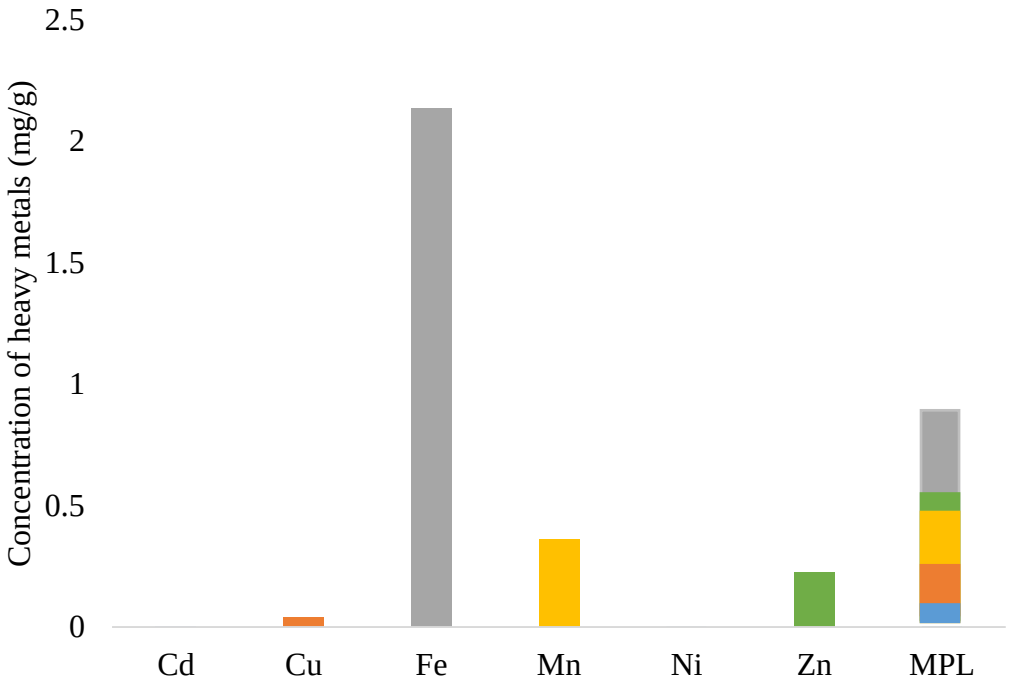


Figure 4.31: Concentration of heavy metals in Sauka ka huta farm

MPL = Maximum Permissible Limit (Sumaila, 2014)

4.5 Concentration of Heavy Metals in the Vegetable Samples

4.5.1 Levels of each heavy metal in vegetables from all farms

The level of cadmium in the three vegetable samples from the farms analyzed are as shown in Figure 4.32. Cadmium metal was not detected in all vegetables from Okada, Kpakungu, Dutsen kura, Chanchaga and Sauka ka huta farms. Only spinach and fluted pumpkin samples from Keteren gwari and Mandela farms had some levels of the metal. The okra samples from the same farms had none. The concentration range of the metals in Keteren gwari farm was 0.00465 to 0.00511 mg/g, and that of Mandela farm was 0.00485 to 0.00716 mg/g. Higher Cd was absorbed by both plants in Mandela farm than Keteren gwari farm. However, the level of Cd in fluted pumpkin was consistently higher than that of spinach in both farms.

The level of copper metal in the three vegetable samples obtained from all farms is presented in Figure 4.33. The samples contained various levels of copper, with pumpkin leaves from Kpakungu farm having the highest value of 0.03693 mg/g and the lowest value of 0.00496mg/g recorded for fluted pumpkin in Mandela farm. The levels of copper in spinach samples ranged from 0.00431 to 0.03017 mg/g that of okra samples ranged from 0.0112 to 0.0199 mg/g and 0.00496 to 0.03693 mg/g for fluted pumpkin samples. The samples from the control Mandela farm had lowest levels of Cu metal.

Figure 4.34 show the level of iron in the three vegetable samples obtained from all farms. Fe was detected in all samples, with fluted pumpkin from Keteren gwari farm having the highest value of 0.78409 mg/g and the lowest value of 0.02706 mg/g recorded for spinach in Okada farm. The level of iron in spinach samples ranged from 0.02706 to 0.68395 mg/g that of okra samples ranged from 0.11471 to 0.18986 mg/g and 0.11672 to 0.78409 mg/g for fluted pumpkin samples**.** Iron is least absorbed by okra irrespective of farm and

with the exception of Dutsen kura farm, fluted pumpkin and spinach absorbed about the same amount of iron.

The level of manganese in the three vegetable samples is presented in Figure 4.35. The highest concentration of manganese was recorded in fluted pumpkin samples from all farms with the exception of Keteren gwari farm. The level of manganese in all samples ranged from 0.01301 to 0.57272 mg/g. The concentration range in spinach samples was between 0.01301 to 0.08100 mg/g; that of okra samples was 0.03326 to 0.10361 mg/g while the range 0.09567 to 0.57272 mg/g was obtained for fluted pumpkin samples.

The results obtained for nickel is as shown in Figure 4.36. Nickel was detected in Kpakungu and Chanchaga farms only. Fluted pumpkin have the highest level of nickel in the two farms while spinach has the least. The level of nickel in all samples ranged from 0.00122 to 0.00342 mg/g. The concentration range in spinach samples was between 0.00122 to 0.00144 mg/g; that of okra samples was 0.00124 to 0.00154 mg/g and 0.00136 to 0.00342 mg/g for fluted pumpkin samples**.**

The concentration of zinc in the three vegetable samples is presented in Figure 4.37. Zinc metal was detected in all samples with the highest level (0.16478 mg/g) recorded in spinach samples from Dutsen kura farm, and the lowest (0.01246 mg/g) recorded in spinach samples from Mandela farm. The concentration range in spinach samples was between 0.01246 to 0.16478 mg/g, that of okra samples was 0.03881to 0.09795 mg/g and 0.01725 to 0.08546 mg/g for fluted pumpkin samples.

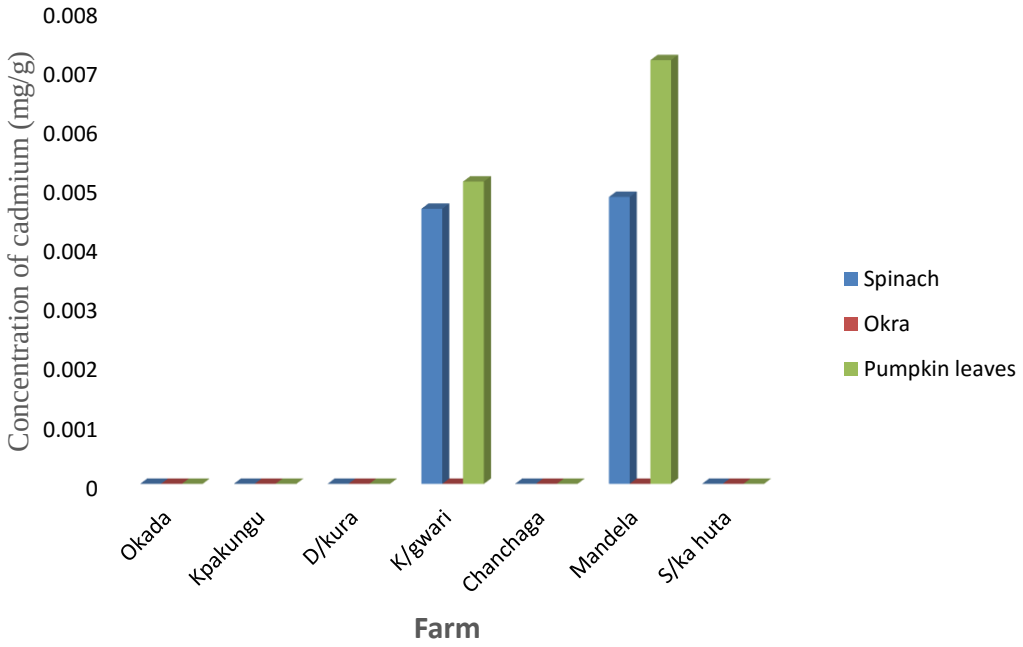


Figure 4.32: Concentration of cadmium in Spinach, Okra and Pumpkin leaf samples from different farms.

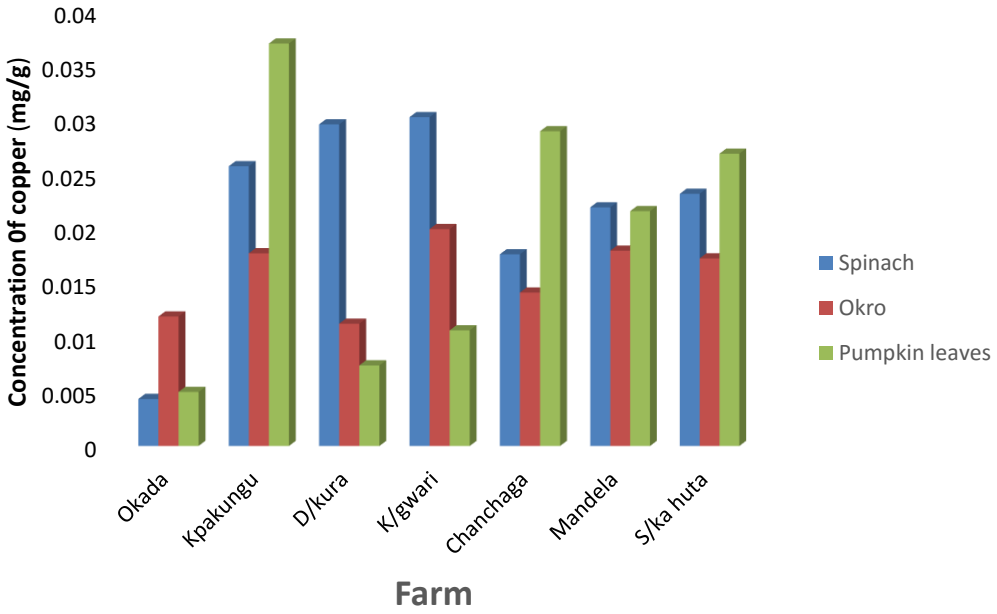


Figure 4.33: Concentration of copper in Spinach, Okra and Pumpkin leaf samples from different farms.

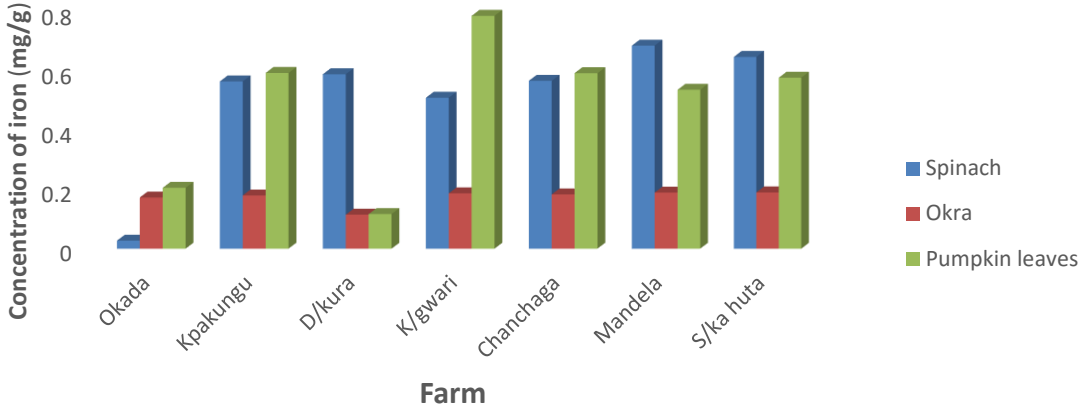


Figure 4.34: Concentration of iron in Spinach, Okra and Pumpkin leaf samples from different farms.

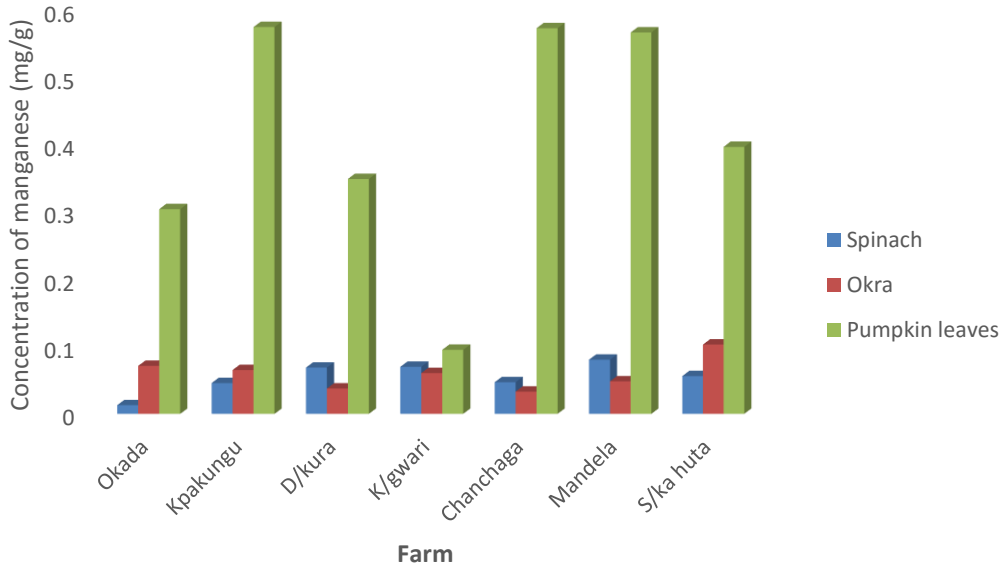


Figure 4.35: Concentration of manganese in Spinach, Okra and Pumpkin leaf samples from different farms.

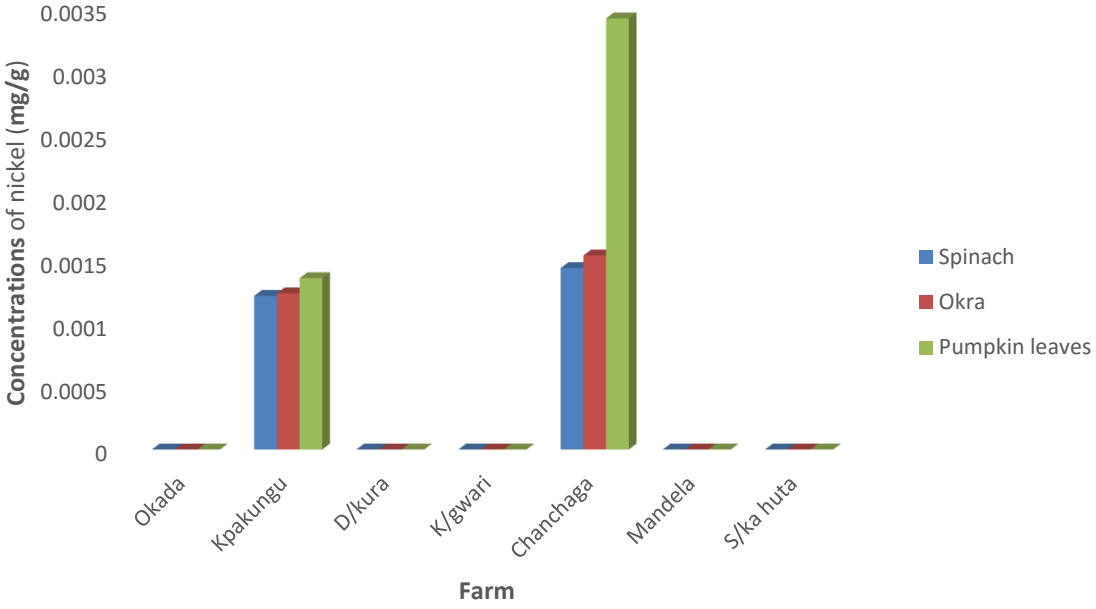


Figure 4.36: Concentration of nickel in Spinach, Okra and Pumpkin leaf samples from different farms.

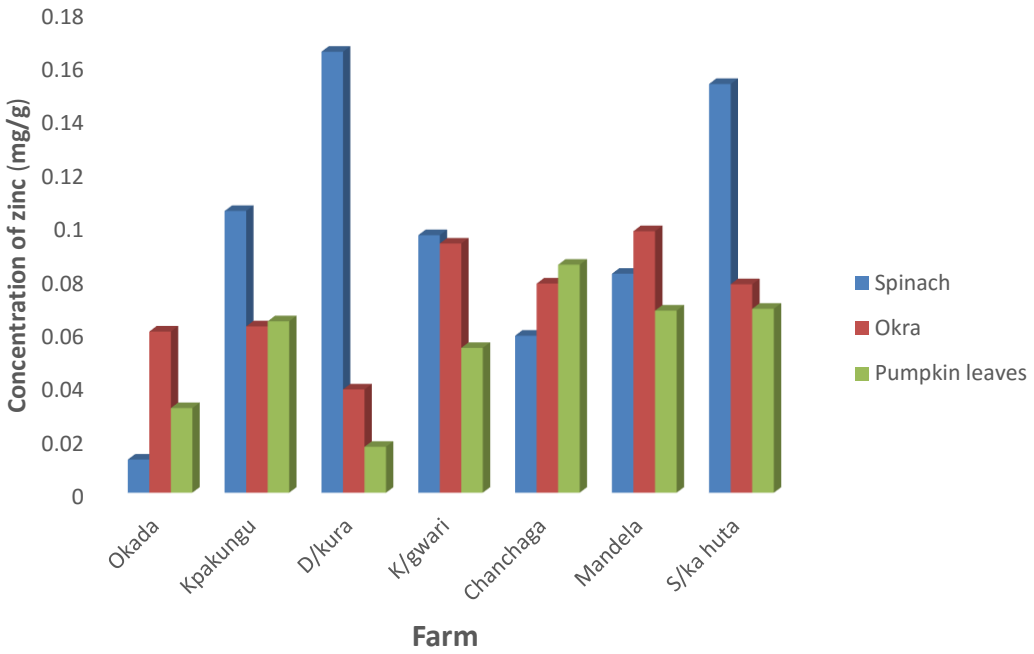


Figure 4.37: Concentration of zinc in Spinach, Okra and Pumpkin leaf samples from different farms.

4.5.2 Level of heavy metals in each vegetable from all farms

The levels of all the heavy metals in spinach samples from all farms analyzed are shown in Figure 4.38. The figure reveals that Fe had the highest concentration in all the spinach samples from all farms, followed by Zn and Mn. The concentration of Zn and Mn however were about the same in Mandela farm: 0.082 mg/g and 0.081mg/g respectively, and Cu was at a low level in all farms. The levels of Ni and Cd were considered negligible in all spinach samples. The general trend of the metals in spinach samples revealed that: Fe>Zn>Mn>Cu>Cd>Ni.

Figure 4.39 gives the levels of heavy metals in okra samples from all the farms. All the okra samples had higher concentrations of Fe than the other metals. In Mandela, Kpakungu and Sauka ka huta farms Mn was the second highest while Zn was second highest in Keteren gwari, Chanchaga and Mandela farms. Concentration of Zn and Mn are almost the same in Dutsen kura farm. Okra samples from all farms had some levels of Cu. Cd was not detected in any of the okra samples while Ni was detected in two farms at very low levels of 0.00124 mg/g in Kpakungu farm and 0.00154 mg/g in Chanchaga farm. The general trend of the metals in okra samples showed that: Fe>Zn>Mn>Cu>Ni.

The levels of the heavy metals determined in fluted pumpkin from the farms are shown in Figure 4.40. The result revealed that Mn and Fe were the most abundant metals in this vegetable from all farms. Zn, Cu and Ni were at a lower concentration with Cd having the least amount in few samples. Fluted pumpkin had the highest average concentration of Mn (0.408 mg/g) compared to spinach (0.055 mg/g) and okra (0.060 mg/g). The general trend of the metals in fluted pumpkin samples showed that: Fe>Mn>Zn>Cu>Cd>Ni.

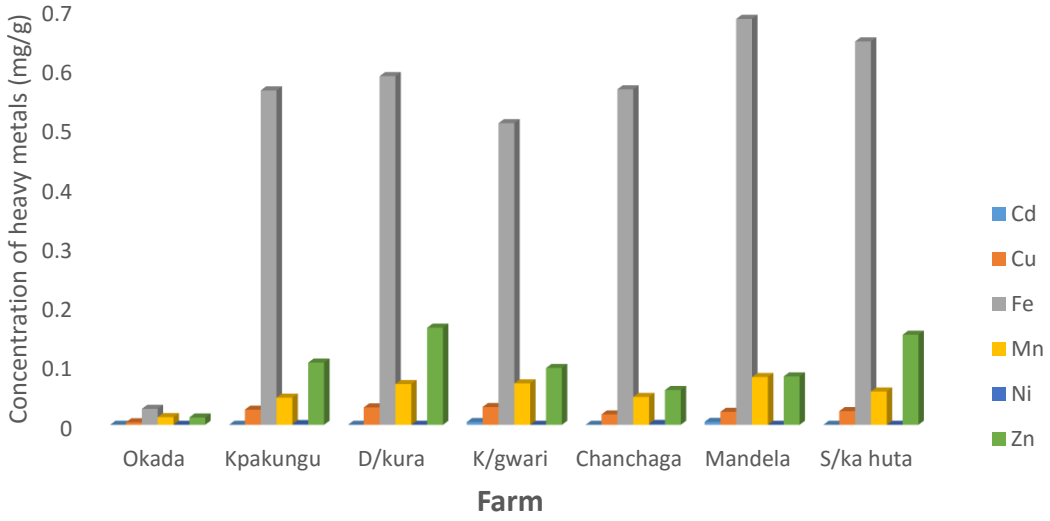


Figure 4.38: Concentration of heavy metals in Spinach samples from all farms

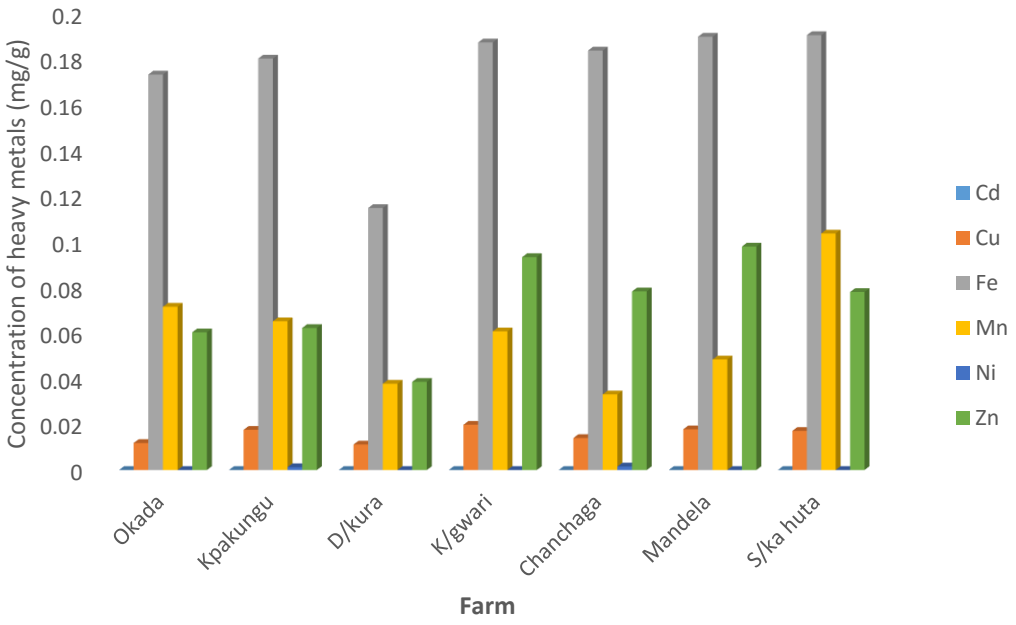


Figure 4.39: Concentration of heavy metals in Okra samples from all farm

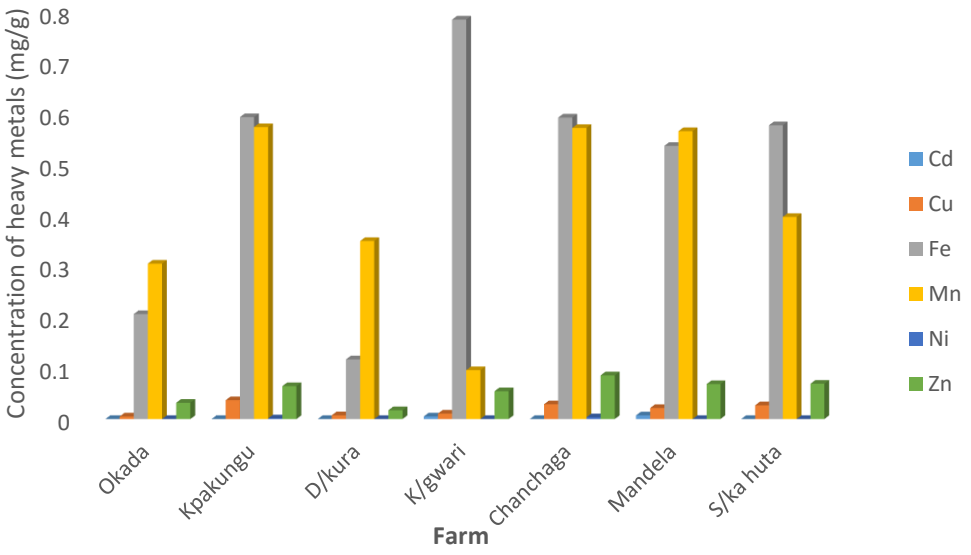


Figure 4.40: Concentration of heavy metals in pumpkin leaves from all farms

4.6 Correlation of Heavy Metal Levels in Vegetables with Soils

The correlation coefficients between the amount of heavy metals in the soils and vegetables of each farm were computed as shown in Tables 4.10 to 4.16. Table 4.10 gives the correlation coefficients for amount of heavy metals in the soil and vegetable samples of Okada farm. A significant positive correlation was obtained for Fe:Cu and there was a significant positive relationship at ($p<0.05$) for Zn:Cu and Zn:Fe. A negative correlation was obtained between Ni:Fe, Mn:Fe, Ni:Mn and Zn:Ni. Cd could not be computed since it was not detected in the soil and vegetables of this farm. The correlation coefficients for amount of heavy metals in the soil and vegetables of Kpakungu farm are shown in Table 4.11.The following pairs of metals showed a significant positive correlation: Fe:Cu, Mn:Cu, Ni:Cu and Zn:Cu. A significant positive correlation at ($p<0.05$) or 95 % confident level was obtained for Zn:Fe, Ni:Fe and Zn:Ni.

Table 4.12 shows the correlation between soil and vegetable samples of Dutsen kura farm. Zn:Fe, Ni:Fe and Zn:Cu gave a significant positive correlation at 95 % confident level. A negative correlation was obtained between Mn:Cu and Zn:Mn in this farm.Table 4.13 gives the correlation between the soil and vegetable samples of Keteren gwari farm. A significant positive correlation was observed for Mn:Fe at 99 % confident level. The correlation coefficient for Chanchaga farm is shown in Table 4.14 with significant positive correlation obtained for Ni:Fe, Ni:Mn and Mn:Cu. Fe:Cu, Zn:Fe and Zn:Ni gave negative correlation. Table 4.15 is Mandela's farm correlation coefficient result showing significant positive correlation between the following: Cu:Cd, Fe:Cu and Zn:Cu with a 95 % confident level for Zn:Fe. Table 4.16 gave the correlation between the amount of heavy metals in the soil and vegetable samples of Sauka ka huta farm. A significant positive correlation was obtained with Fe:Cd, Fe:Cu and Ni:Fe having a significant

positive correlation ($p<0.05$) at 95 % confident level. Ni:Cd gave a positive correlation at 99 % confident level.

Table 4.10: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Okada Farm

Farm A	Cd	Cu	Fe	Mn	Ni	Zn
Cd	.[a]					
Cu	.[a]	1				
Fe	.[a]	.835	1			
Mn	.[a]	.225	.489	1		
Ni	.[a]	.268	-.306	-.421	1	
Zn	.[a]	.957*	.952*	.431	-.007	1

*. Correlation is significant at the 0.05 or 95% confident level (2-tailed).
a. Cannot be computed because at least one of the variables is constant.

Table 4.11: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Kpakungu Farm

Farm B	Cd	Cu	Fe	Mn	Ni	Zn
Cd	.[a]					
Cu	.[a]	1				
Fe	.[a]	.889	1			
Mn	.[a]	.742	.382	1		
Ni	.[a]	.808	.978*	.300	1	
Zn	.[a]	.756	.967*	.136	.957*	1

*. Correlation is significant at the 0.05 or 95% confident level (2-tailed).
a. Cannot be computed because at least one of the variables is constant.

Table 4.12: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Dutsen kura Farm

Farm C	Cd	Cu	Fe	Mn	Ni	Zn
Cd	.[a]					
Cu	.[a]	1				
Fe	.[a]	.572	1			
Mn	.[a]	-.383	.209	1		
Ni	.[a]	.361	.971*	.313	1	
Zn	.[a]	.977*	.732	-.255	.549	1

*. Correlation is significant at the 0.05 or at 95% confident level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.13: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Keteren gwari Farm

Farm D	Cd	Cu	Fe	Mn	Ni	Zn
Cd	1					
Cu	.078	1				
Fe	-.091	-.439	1			
Mn	-.183	-.484	.994**	1		
Ni	.[a]	.[a]	.[a]	.[a]	.[a]	
Zn	-.573	.684	.067	.079	.[a]	1

**. Correlation is significant at the 0.01 or 99% confident level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.14: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Chanchaga Farm

Farm E	Cd	Cu	Fe	Mn	Ni	Zn
Cd	.[a]					
Cu	.[a]	1				
Fe	.[a]	-.290	1			
Mn	.[a]	.714	.401	1		
Ni	.[a]	.218	.821	.836	1	
Zn	.[a]	.642	-.660	.346	-.133	1

a. Cannot be computed because at least one of the variables is constant.

Table 4.15: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Mandela Farm

Farm F	Cd	Cu	Fe	Mn	Ni	Zn
Cd	1					
Cu	.735	1				
Fe	-.353	-.863	1			
Mn	.604	.045	.152	1		
Ni	.[a]	.[a]	.[a]	.[a]	.[a]	
Zn	.246	.836	-.971[*]	-.370	.[a]	1

*. Correlation is significant at the 0.05 or 95% confident level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.16: Correlation Coefficients between Heavy Metals in the Soil and Vegetables of Sauka ka huta Farm

Farm G	Cd	Cu	Fe	Mn	Ni	Zn
Cd	1					
Cu	.925	1				
Fe	.972*	.976*	1			
Mn	.506	.699	.542	1		
Ni	.995**	.925	.972*	.506	1	
Zn	.858	.800	.899	.131	.858	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.7 Soil- Plant Transfer Factors

Transfer factor (TF) results are shown in Tables 4.17 – 4.23. Table 4.17 shows the transfer factors (TF) for vegetables in Okada farm. The Table revealed that Mn had the highest transfer factor of 1.23 in fluted pumpkin. For spinach and okra samples, Cu had the highest transfer factors of 0.26 and 0.71 respectively while Cd and Ni had 0.00 TF in the three vegetables.

The TF for vegetables in Kpakungu farm is shown in Table 4.18. Cu had the highest TF of 0.54 in spinach followed by Zn (0.49). This is the same for okra samples where Cu had the highest TF (0.37) followed by Zn (0.29). In fluted pumpkin 1.55 was the highest TF for Mn followed by 0.78 for Cu. The TF for Cd in all vegetables from this farm was 0.00.

Table 4.19 gives the TFs for the farm in Dutsen kura. It reveals that Cd and Ni had TFs of 0.00 in all the vegetables of the farm while Mn had TF > 1 in fluted pumpkin. Spinach samples also had TFs > 1 in Cu and Zn. Cu and Zn had the highest TFs of 0.48 and 0.24 respectively in okra.

The TFs for Keteren gwari farms is shown in Table 4.20. Ni had TFs of 0.00 in all vegetables from the farm. The highest TFs for Cd in this study was (3.47) and (3.81) observed in spinach and fluted pumpkin of this farm respectively. Cu had 2.05 in spinach while okra had a TF > 1 in Cu and Zn.

Chanchaga farm's TFs are shown in Table 4.21. Cu had the highest TF in fluted pumpkin while TFs ≥ 1 was calculated for Cu in spinach and okra and Mn in fluted pumpkin. Others had TFs < 1.

Table 4.22 gives the TFs for the vegetables in Mandela farm. The highest TFs for this study in the three vegetables was observed for Zn in this farm. The values were 6.25 in okra, 5.23 in spinach and 4.35 in fluted pumpkin. Cu also had high TFs in spinach (3.17), fluted pumpkin (3.12) and okra (2.60). Cd and Ni had TFs of 0.00.

The TFs for Sauka ka huta farm is shown in Table 4.23. The Table revealed that only Mn had a TF > 1 in fluted pumpkin while the rest had TF < 1. Cd and Ni also had TFs of 0.00 in this farm.

Table 4.17: Soil-Plant Transfer Factor for Vegetables in Okada road farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	0.26	0.01	0.05	0.00	0.09
Okra	0.00	0.71	0.09	0.29	0.00	0.43
Fluted pumpkin	0.00	0.30	0.10	1.23	0.00	0.23

Table 4.18: Soil-Plant Transfer Factor for Vegetables in Kpakungu farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	0.54	0.27	0.13	0.18	0.49
Okra	0.00	0.37	0.08	0.18	0.18	0.29
Fluted pumpkin	0.00	0.78	0.28	1.55	0.20	0.30

Table 4.19: Soil-Plant Transfer Factor for Vegetables in Dutsen kura farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	1.26	0.28	0.28	0.00	1.04
Okra	0.00	0.48	0.06	0.16	0.00	0.24
Fluted pumpkin	0.00	0.31	0.06	1.43	0.00	0.11

Table 4.20: Soil-Plant Transfer Factor for Vegetables in Keteren gwari farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	3.47	2.05	0.26	0.39	0.00	1.02
Okra	0.00	1.35	0.10	0.33	0.00	0.10
Fluted pumpkin	3.81	0.72	0.40	0.52	0.00	0.58

Table 4.21: Soil-Plant Transfer Factor for Vegetables in Chanchaga farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	1.25	0.26	0.13	0.33	0.11
Okra	0.00	1.00	0.08	0.09	0.36	0.14
Fluted pumpkin	0.00	2.05	0.27	1.54	0.79	0.15

Table 4.22: Soil-Plant Transfer Factor for Vegetables in Mandela farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	3.17	0.35	0.30	0.00	5.23
Okra	0.00	2.60	0.10	0.18	0.00	6.24
Fluted pumpkin	0.00	3.12	0.27	2.10	0.00	4.35

Table 4.23: Soil-Plant Transfer Factor for Vegetables in Sauka ka huta farm

Vegetable	Cd	Cu	Fe	Mn	Ni	Zn
Spinach	0.00	0.56	0.30	0.16	0.00	0.68
Okra	0.00	0.41	0.09	0.29	0.00	0.35
Fluted pumpkin	0.00	0.64	0.27	1.10	0.00	0.31

CHAPTER FIVE

5.0 DISCUSSION

5.1 Pesticide Residues Analysis

This study investigated the presence of six pesticides: a carbamate (carbofuran), two pyrethroids (lambda-cyhalothrin and cypermethrin) and three organochlorines (aldrin, endrin and heptachlor) that farmers used on spinach, okra and fluted pumpkin commonly harvested in Minna.

5.1.1 Carbamate pesticide

The retention time of carbofuran (16.496 minutes) as shown in Table 4.2, is the time taken for the carbofuran reference material to travel through the column to the detector. This time was measured from the time at which the standard was injected to the point at which the display shows a maximum peak height for the compound. Carbofuran's identity was confirmed using full scan mass spectra United States, Environmental Protection Agency/National Institute for Standards (EPA/NIST) library search. This is a large library of mass spectra from known compounds, nearly 60,000 stored spectra. The computer then provided a short list of the best matches between the library spectra and the measured one. This procedure was done for all pesticides analyzed. Table 4.2 also gave the m/z values of 103, 149, 164 (a.m.u) as the optimal ion mass that were selected from the full scan spectra of carbofuran in order to perform detection in the single ion monitoring (SIM) mode. The SIM mode was carried out in order to obtain lower detection limits for quantitative analysis. Sensitivity was found to be in tens to hundreds times better and matrix interferences were also low.

None of the samples in this study gave retention times that corresponded to that recorded for carbofuran standard, indicating the absence of carbofuran or if present it was below

the detection limit of this procedure. Another reason could be that carbamate pesticides are relatively unstable compounds that break down in the environment within weeks (Azmi and Naqvi, 2011).

5.1.2 Pyrethroid pesticides

The gas chromatograms and mass spectra of spinach samples from Chanchaga and Mandela farm (Figures 4.12 and 4.13 respectively) gave the retention times of 35.145, 35.295 and 35.478 minutes which corresponded to the retention time range for the cypermethrin isomers (Table 4.2). To confirm the presence of cypermethrin, the mass spectra gave the ion mass of three cypermethrin fragments as shown in the cypermethrin reference material library search using the EPA/NIST library (Figure 4.14). The fragments detected were 163, 181 and 209 a.m.u. The ion ratio (quantifier/qualifier) were found to be consistent with the reference material for this pesticide. The cypermethrin concentration in the spinach samples from Chanchaga farm was found to be 8.65 ±1.30 mg/kg and that from Mandela farm to be 0.60 ±0.09 mg/kg. The average response factors over the entire calibrations range have relative standard deviation, RSD $\leq$ 15 %, demonstrating the method's precision for analyzing the pesticides in these samples.

The FAO/WHO Codex Alimentarius commission gave the maximum residue level (MRL) for cypermethrin on leafy vegetables as 0.7 mg/kg (FAO/WHO, 2013). The spinach samples from Chanchaga farm had value above the MRL, but the value from Mandela farm was below the WHO maximum residue limit. The result from Chanchaga farm calls for concern since it well exceeded the WHO limit. This may be due to non-observance of certain recommended agricultural practices like respecting the safety interval between pesticide applications and harvest or using recommended rate of pesticide (Lee, 1998). The low value (0.60 ±0.09mg/kg) of cypermethrin in the spinach sample of Mandela farm is also significant and should not be neglected since spinach is

a vegetable eaten regularly in this part of the country. It however, appears to be no immediate danger in consuming spinach from other study areas.

The result for this study is similar to that obtained by Zawiya *et al* (2007) where only 38 out of 508 vegetables and fruit samples showed the presence of pesticides but differ in the highest value for cypermethrin obtained (1.48 mg/kg). The highest value recorded for this research was higher than the value (0.012 mg/kg) reported by Chandra *et al*., (2010) for the determination of cypermethrin in Indian vegetables by gas chromatography-electron capture detector (GC-ECD). This difference could be due to the pesticides regulations in the countries involved, the type of vegetables and the method used in the determination of the residues. It could also be due to pesticide application method and the frequency of spraying. Gas chromatography/mass spectrometry with selected ion monitoring (SIM) used for this analysis has more capability for sensitive and specific detection and has been used in confirmation studies of pesticide residue in fruits and vegetables (Zawiya *et al*., 2007).

None of the samples in this study gave retention times that corresponded to that recorded for lambda-cyhalothrin standard, indicating the absence of the pesticide or if present it was below the detection limit of this procedure.

5.1.3 Organochlorine pesticides

The gas chromatograms and mass spectra (Figure 4.5) showed the retention time and ion mass detected for the three organochlorine pesticides in 608 calibration mix standard. None of the samples in this study gave retention times that corresponded to that recorded for aldrin and endrin, indicating the absence of these two pesticides or if present they were below the detection limit of this procedure.

Figures 4.15 and 4.16 showed the chromatograms of spinach samples from Chanchaga and Mandela farms. Very weak peaks observed in the chromatogram at 25.531 minute which is the retention time for heptachlor, indicated the likelihood of the presence of heptachlor in the samples. However the presence of heptachlor was not confirmed during the SIM mode quantitation analysis of these samples. Only the qualifier ion 65 was found at the retention time that corresponded to heptachlor reference material. Other relevant ions (100 and 272) were not detected. The reason for this result could be the fact that the use of organochlorine pesticides has been scaled down due to the ban on their use in response to the Stockholm Convention on the use of persistent organic pesticides (POPs) (Nsikak and Aruwajoye, 2011).

In general, though literature on pesticide residues in vegetables and fruits in Nigeria are limited (Musa *et al.*, 2010), the result of this study is similar to many researches on pesticide residues in vegetables from other parts of the world. For example, in a study conducted to investigate the organochlorine and pyrethroid pesticide residues in fruits and vegetables from markets in Malaysia, only 7.5 % of vegetable samples showed the presence of cypermethrin and none of the 206 fruits gave positive results (Zawiya *et al*., 2007). Also, in the GC-MS determination of organochlorine pesticides in medicinal plants harvested in Brazil, twelve out of forty five samples gave positive results (Rodrigues *et al*., 2007).

In another set of studies in which the results differ from the results of this research, residual levels of OCPs were determined in 34 samples of 19 varieties of vegetables collected from selected sites around Sichuan Province China, using GC-ECD. The results indicated that all the vegetable samples had some levels of one or more OCPs in them

with only one sample exceeding the MRLs (Owago *et al.*, 2008). Nsikak and Oruwajoye (2011) investigated the presence and levels of (OCPs) in some vegetables from Ota Ogun state, OCPs were detected in all the samples analysed using GC-ECD, but the levels were generally below the FAOs maximum residue limits. Also in monitoring insecticides residues in vegetables and fruits from Mauritius, the data showed that most of the vegetable and fruit samples analysed did not contain residues of the monitored insecticides above the accepted maximum residue limit (MRL) as adopted by the FAO/WHO Codex Alimentarius Commission. Furthermore, the result also revealed that 61.5 % of the vegetable and fruit samples analysed contained no detectable level of the monitored insecticides, 36.2 % of the samples gave results with levels of insecticide residues below the MRL, while 2.3 % of the samples showed results above the MRL. Only 3 out of 128 samples contained levels of insecticide residues above the MRL (Lee, 1998). The outcome of this study is similar to what is available in the limited literature available on pesticide residues. Pesticide residues are usually in very small amounts and their analysis have the challenge of extraction from complex matrixes, cleaning interfering matters co-extracted with the pesticides and the mode of detection (Kolberg, 2011).

5.2 Physico-chemical Properties of Soil Samples

5.2.1 pH

The pH value for normal soil is 5.5–7.5. pH of soil below this range can cause low availability of some essential minerals needed by the soil (Brady and Weil, 1999). The pH values of all the soil samples analysed from the seven farms ranged from 5.77 to 7.70 showing slightly acidic to slightly basic (Table 4.7). Such pH values are characteristic of soils in areas where leaching is less pronounced due to low precipitation (Ajai *et al.*, 2014). This also shows the soil samples are within the range for normal soil pH. The

significant difference between the pH of various farms was at ($p < 0.05$). These values are in the same range of 5.0-7.5 reported by Iyaka and Kakulu (2009) and 6.20-6.70 reported by Sumaila, (2014) for soils of Bida and Paiko respectively. The pH values are also lower than values 7.2 - 8.3 for soil samples in India (Kiran, 2013). Chanchaga Farm with the lowest pH value of 5.77 may have greater solubility for metals, therefore becoming more readily available to plants. The pH of a soil has a major effect on metal dynamics controlling adsorption and precipitation, which are the main mechanisms of metal retention in soils.

5.2.2 Electrical conductivity

Conductivity measurements showed Sauka ka huta farm with the lowest value of 17 µS/cm and Dutsen kura farm had the highest value of 37 µS/cm. These values indicate the relative water-soluble salt content of the soil. Electrical conductivity depends on the amount of dissolved minerals and gives the ability of a substance to conduct an electric current at a specific temperature (25 ^{o}C). There is no regular pattern of variation in conductivity between sandy, loamy sand or silt loam soil samples. Boulding, (1994) classified EC of soils as: non saline <2; moderately saline 2-8; very saline 8-16; extremely saline >16. Based on this classification and from the EC results for this study the farm soils may be classified as extremely saline. The EC values in this study were lower than 165-201µs/cm reported for Delta State soils (Akpoveta *et al.*, 2010) and 225-520 µs/cm for soils around paint industries in Kaduna (Ajai *et al.*, 2014).

5.2.3 Soil organic matter (SOM)

The presence of SOM is regarded as being critical for soil function and soil quality. It increases soil fertility by providing cation exchange sites and acting as reserve of plant nutrients like N, P, and S, along with micronutrients. SOM also acts as the major sink and source of soil carbon. Although the carbon content of SOM is known to vary considerably

(Perie and Ouimet, 2008, Jain *et al.,* 1997). SOM is typically estimated to contain 58 % soil organic carbon. This study confirms this value (Table 4.7). The concentration of SOM in soils generally ranges from 1 % to 6 % of the total topsoil mass for most upland soils. Soils whose upper horizons consist of less than 1 % organic matter are mostly limited to desert areas, while the SOM content of soils in low-lying, wet areas can be as high as 90 %. Soils containing 12-18 % soil organic carbon are generally classified as organic soils (Troeh and Louis, 2005). The SOM for the soil samples analysed was 1.63-3.87 %, which is within the range for upland soils. The organic carbon is in the range of 0.95-2.25 % which is lower than 12-18 % for organic soils and higher than 0.38-0.54 % for soils of Delta State (Akpoveta *et al*., 2010). Ajai *et al* (2014) reported 2.18-3.10 % SOM for soils around paint industries in Kaduna. The SOM values (1.72-2.82 %) recorded for soils in Sokoto State (Atiku and Noma, 2011) were within the range obtained for this research.

5.2.4 Particle size

The results for particle size and texture of the soil samples as shown in Table 4.8 reveal that the textural class of the soil samples were sandy to loamy sandy, with the exception of Chanchaga farm which was silty loam. The particle size distribution of the soil showed that the soil contained higher composition of sand than silt and clay in all the sampling sites. This result differs from the textural class of soils of Tangaza, Sokoto state in which most of the soils where clay to clay loam (Atiku and Noma, 2011) but agrees with the values reported by Ajai *et al* (2014) where 61-89 % of the soils were sandy, 12-29 % silt and 0-10 % clay. Trace metals have preferential accumulation in the clay and silt fraction of soil. Okada Farm (control) had the least average concentration of heavy metals and physico-chemical analysis results also reveal that it had the highest % of sand particles. The concentration of heavy metal in soil decreases with increase in the sizes of the soil

particles (Ajai *et al.,* 2014). Dutsen kura and Keteren gwari farms which are predominantly sandy also had lower concentration of heavy metals.

5.2.5 Cation exchange capacity

The exchangeable bases and cation exchange capacity (CEC) of the soil samples was given in Table 4.9. The result from the Table shows that Keteren gwari farm had the highest CEC value of 5.15 cmol/kg while Sauka ka huta farm had the least value of 3.68 cmol/kg. CEC is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilisers (Hazleton and Murphy, 2007). The CEC of soils varies according to the percentage of clay in the soil, the type of clay, soil pH and amount of organic matter. The result of this study showed that Keteren gwari farm with the highest pH value had the highest CEC. Organic matter has a very high CEC ranging from 250 to 400 meq/100 g (Moore *et al.,* 1998). Soils with a low CEC are more likely to develop deficiencies in potassium, magnesium and other cations while high CEC soils are less susceptible to leaching of these cations (CUCE 2007). A higher CEC usually indicates more clay and organic matter is present in the soil resulting in greater water holding capacity. The result of this study shows that Keteren gwari, Dutsen kura, Okada, Mandela and kpakungu fams with higher CEC values had high clay content. The soil of Keteren gwari farm with the highest value of CEC will therefore be less susceptible to leaching of cations (CUCE, 2007). The significant difference between CEC values for various farms was at $p<0.05$. The CEC values for this study area was within the range (2.52- 2.72 cmol/kg) recorded for the soils of Wassaniya forest reserve in Tangaza, Sokoto state (Atiku and Noma, 2011). The values (7.70-9.30 cmol/kg) recorded for soils around selected oil well heads in Bayelsa State (Nwakife, 2015) were however higher. The difference could be due to oil exploration activities taking place in the areas. Van

Herk, (2012) obtained higher CEC values of 20.45-23.54 cmol/kg for soils of Gongulon agricultural sites in Maiduguri, Borno State. The difference may be due to the geography of the areas.

5.3 Heavy Metals in Soil

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Zhang *et al.*, 2010).

The concentrations of Cd, Cu, Fe, Mn, Ni and Zn in soil samples showed that all the studied metals were present in all soil samples except Cd and Ni. Cd was detected in only two farms while Ni was absent in three farms (Figures 4.19 - 4.24). This result is in agreement with the study conducted by Sumaila (2014) and similar to that reported by (Pujar *et al.*, 2012) for Bijapur taluka soils in India where out of the ten farms analyzed, Ni and Cd were not detected in two farms. This could be due to the distribution of heavy metals in soils, which is continuously altered due to various soil processes and is under the influence of environmental factors. Knowledge of the total metal concentration provides limited information about their potential mobility and bioavailability (Bashir *et al.*, 2014; Adah *et al.*, 2013).

The concentration of all the heavy metals detected in the soil samples were below the permissible limits. The particle size distribution of the soil showed that the soil contained higher composition of sand than silt and clay in all the sampling sites. Trace metals have preferential accumulation in the clay and silt fraction of soil. Generally, the concentration of heavy metal in soil increase with decrease in the sizes of the soil particles (Ajai *et al.*,

2014). But the iron contents of the samples which ranged from 1942.13 to 2205.9 mg/kg (Figure 4.18 to 4.23) were above the permissible limits of 1000 mg/kg. The high level of Fe could be as a result of the natural basic rock material of the soil (Qian, *et al.*, 1996) and Fe has been found to occur at high concentrations in Nigerian soil (Adefemi *et al.*, 2007). The range for Cu level (6.71 – 47.59 mg/kg) obtained in this study was found to be higher than the range of 5.67 – 15.17 mg/kg for Paiko reported by Sumaila, (2014) and 6.8 – 29 mg/kg for Minna reported by Iyaka and Kakulu, (2011). It is also higher than 11.0 – 26.0mg/kg for Bida obtained by Iyaka and Kakulu, (2011). This difference could be due to the season this study was conducted (dry season). Concentration of metals in soil vary in dry season and wet season due to the run off effect that is capable of removing heavy metals from the road side soil and the effect of rain may also facilitate the leaching of the soil and contributes to the dilution of the soil solution (Yahaya *et al*., 2009; Anhwange, 2009).

The range for Ni contents (0.65 – 6.90 mg/kg) obtained in this study was found to be significantly lower than the range of 19.17 – 41.33 mg/kg reported by Sumaila (2014) and 15 – 47 mg/kg reported by Yoshinori *et al*(2010) in the study of heavy metal contamination of soils and sediments in Zambia. The result of the study reveals that Ni was absent in Okada farm, the control, and also in Keteren gwari and Mandela farms. This may be attributed to the fact that Ni usually takes longer time for it to be removed from air to other parts of the environment and it often leaches down to adjacent groundwater (Wuana and Okieimen, 2011).The highest concentration (107.13 mg/kg) of nickel (6.90 mg/kg) obtained from this study is lower than the highest concentration recorded for Yauri (Yahaya *et al.*, 2009) and 42.73 mg/kg recorded for Lagos (Olukanmi and Adeoye, 2012).

This study also gave a range of 182.48 – 371.78 mg/kg for Mn and 15.70 – 225.2 mg/kg for Zn which were higher than 130 – 290 mg/kg for Mn reported by Sumaila (2014) and 15 – 154 mg/kg for Zn in Minna soils (Iyaka and Kakulu, 2011). The range for Mn in this study was lower than 651.80mg/kg and 953.52 mg/kg reported by Olukanmi and Adeoye (2012) for Lagos farmland soils. It is also lower than 608.11 mg/kg recorded for Yauri soils (Yahaya *et al.,* 2009).

In general the concentration of the metals in the soil samples varied according to the following trend Fe>Mn>Zn>Cu>Ni>Cd, indicating that all the soil samples had highest level of iron while Cd was the lowest. The average concentration of all the six metals in each farm also gave the following trend B>G>E>C>F>D>A, showing that Kpakungu farm (B) had the highest average concentration of all the metals combined while Okada farm (A) the control, had the least. Kpakungu area of Minna is one of the most densely populated areas with motor parks, petrol stations, vehicle repair garages and dumpsites, which suggest possible mobility of metals from the dumpsites to farmlands through leaching and runoffs (Opaluwa *et al.,* 2012). Aerial deposition could also contribute to metal contents of the soil since the farm is by the busy Minna – Bida road. This is because there is a positive correlation between atmospheric metal deposition and elevated concentrations of heavy metals in top soil (Arora *et al.,* 2008; Debopam *et al.*, 2010). The control farm around Okada road is located in a less densely populated area very close to the GRA and government house where anthropogenic activities are expected to be lower.

It has been reported that top soil and road side soil near heavy traffic in urban areas are likely to have heavy metal contamination from atmospheric deposition. Metals such as Cd, Cu, Pd and Zn are good indicators of contamination in soils because they appear in gasoline, car component, oil lubricants and industrial incinerator emissions (Popoola *et al.*, 2012, Olukanmi and Adeoye 2012).

5.4 Heavy Metals in Vegetables

5.4.1 Cadmium

Cd was detected only in spinach and fluted pumpkin from Keteren gwari and Mandela farms (Figure 4.25). There are dumpsites near the two farms which may contain used electronic parts due to activities of electronic repairs shops around the area. The burning of the dumpsites may be associated with the level of Cd in the samples. None of the okra samples gave a positive result for Cd. This may be due to the fact that green leafy vegetables have more ability to absorb metals deposited on plant surfaces exposed to polluted environments than fruits and stems (Thilini *et al.*, 2014). The result also resembles values recorded for a similar study on heavy metals in Sri Lanka vegetables where Cd exceeded the FAO/WHO limit (Thilini *et al.*, 2014).

This study gave a range of 4.65 – 4.85 mg/kg for Cd in spinach and 5.11 -7.16 mg/kg for fluted pumpkin leaves. These were the only two samples that showed presence of Cd and they are all above the FAO/WHO maximum permissible limits (2007) of 0.20 mg/kg. These results differ from that obtained for vegetables from Paiko town in Niger state by Sumaila (2014) in which Cd was below detection limits in all the samples but similar to a study conducted on Kaduna vegetables where Cd exceeded the permissible limit (Jacob and Kakulu, 2012). The value of 0.090 mg/kg was reported for fluted pumpkin from selected farms in Lagos by Sobukola *et al.*, (2010), which is much lower than the value obtained for the same vegetable in this study. The mean values of Cd from all farms was 4.75 mg/kg and 6.14mg/kg for spinach and fluted pumpkin respectively. A large share of general cadmium pollution is caused by dumping and incinerating cadmium-polluted waste (Jarup, 2003), these activities may be present around the two farms.

5.4.2 Copper

The concentration of Cu was in the range 4.31- 30.17 mg/kg for spinach, 11.22 – 19.90 mg/kg for okra and 4.96 – 36.93 mg/kg for fluted pumpkin leaves. None of the samples exceeded 40 mg/kg which is the FAO/WHO (2007) maximum permissible limits for Cu in vegetables. These values are however higher than the 4.09 – 15.81 mg/kg recorded for Paiko vegetables (Sumaila, 2014). Elbagermi *et al.*, (2012) also gave a value of 5.32 mg/kg for spinach from Misrata, Libya which is similar to some spinach samples in this study. The average levels obtained for Cu in samples from all farms were spinach (21.76 mg/kg), okra (30.97 mg/kg) and fluted pumpkin (19.59 mg/kg). Adah *et al* (2013) reported 0.251 mg/kg and 0.0803 mg/kg for fluted pumpkin and spinach samples from bank of river Benue. In another research conducted in Colombo, Sri Lanka, 7.05 – 18.44 mg/kg was obtained for Cu metal in vegetables of that area (Thilini *et al.*, 2014), this value is similar to some samples in this study.

5.4.3 Iron

All the spinach samples except those of the control farm, all the okra samples and 72 % of fluted pumpkin contained Fe above the FAO/WHO (2007) limits of 450mg/kg. The concentration of Fe in the soil, difference in absorption capacity by the different vegetables may account for the differences in the contents (Mobofu, 2012). This result is however lower than 148.33 – 840.50 mg/kg recorded by Sumaila (2014). The values for Fe also differ from 0. 260 mg/kg average recorded for wild jute, tossa jute and kenaf from Katsina market (Shuaibu *et al*., 2013). The mean values for Fe in spinach, okra and fluted pumpkin leaves from all farms were: 512.0 mg/kg, 173.7 mg/kg and 486.0 mg/kg respectively, showing that spinach samples had the highest Fe content followed by fluted pumpkin while okra was the least. Okra, a fruit vegetable is expected to contain lower levels of heavy metals compared to leafy vegetables (Thilini *et al.*, 2014). The control

farm (A_c) also contained higher level of Fe compared to other metals. This may be due to the fact that Fe usually has relatively high natural concentrations and is therefore not expected to be from anthropogenic sources.

5.4.4 Manganese

Mn had a range of 13.01-81.00 mg/kg for spinach, 33.26 – 103.61mg/kg for okra and 95.67 – 572.72 mg/kg) for fluted pumpkin. These values were higher than the 0.23 – 3.43 mg/kg recorded for Mn in spinach and lettuce from Biu, Borno state (Akpan *et al.*, 2013). The difference between the values could be geographical. All the spinach and okra samples contained Mn values below the FAO/WHO permissible limit of 500 mg/kg. However 43% of fluted pumpkin leaves exceeded the permissible limit. This pattern was also observed in a study on heavy metals in vegetables from Uyo where the fluted pumpkin had higher values than other vegetables (Otitoju *et al.*, 2012). Computing the average concentration of Mn from all farms gave 54.74 mg/kg, 60.25 mg/kg, and 407 mg/kg for spinach, okra and fluted pumpkin respectively.

5.4.5 Nickel

The results showed that only Kpakungu and Chanchaga farms contained Ni. The other farms either did not contain Ni or was below the detection limit. Ni usually takes long time for it to be removed from air to other parts of the environment and often leaches down to adjacent groundwater (Wuana and Okieimen, 2011). Ni gave a range of 1.22 – 1.44 mg/kg for spinach, 1.24 – 1.54 mg/kg for okra and 1.36 – 3.42 mg/kg for fluted pumpkin which are similar to 0.25 – 3.54 mg/kg reported by Akpan *et al* (2013). Only 16% of fluted pumpkin exceed the FAO/WHO limit of 1.5mg/kg. The mean level of Ni in spinach, okra and fluted pumpkin leaves from all farms was 1.33 mg/kg, 1.39 mg/kg and 2.39 mg/kg respectively. These values are different from those reported by Adah *et al* (2013) in a study to determine heavy metals in soil and vegetable samples from bank

of river Benue where an average of 0.2047 mg/kg was obtained for Ni in fluted pumpkin and 0.0714 mg/kg for spinach.

5.4.6 Zinc

Zn levels of 2.48 -164.78 mg/kg for spinach, 38.81 – 97.95 mg/kg for okra and 7.25 – 69.06 mg/kg for fluted pumpkin exceeded the FAO/WHO permissible limit of 60 mg/kg. The average concentration of Zn in spinach, okra and fluted pumpkin from all farms was 91.15 mg/kg, 72.80 mg/kg and 55.80 mg/kg respectively. These values are higher than the 0.67 – 18.89 mg/kg reported by Lawal and Audu (2011) for Zn in spinach, okra, onions and tomatoes from vegetable farms in Kano. High levels of heavy metals in some of the vegetables might be closely related to the pollutants from irrigation water or from soil (Mubofu, 2012). This may be due to the fact that mineral uptake from soil, bioaccumulation and aerial deposition on the leaves all contribute to the mineral levels in the vegetables (Otitoju *et al*., 2012). The high levels observed in the vegetables may also be because most of the farms are located near the source of contamination like tyre wear, motor oil, grease, brake emissions, corrosion of galvanized parts, fuel burning, and batteries e.t.c.

Generally, the results showed that fluted pumpkin contained higher levels of heavy metals followed by spinach and then okra. This agrees with a report by Van Herk, (2012) that in vegetable parts the concentrations of heavy metals are in the order of leaf> stem> root> tuber> bulb> fruit> seed.

Organic matter is known to form chelates with metals and thus do reduce the availability of heavy metals in plants (Epstein, 2002). Physico-chemical properties results revealed that most of the farm soils had high levels of organic matter. This may be the reason why

some vegetables grown in soils that contained some metals gave lower values for the same metal

The level of the heavy metals in each vegetable from all farms as expressed in Figures 4.38 to 4.40 showed that all the vegetables contained high concentration of Fe, while Mn was present in high quantity in fluted pumpkin. Okra samples from all farms had some levels of Cu. The average concentration of all six metals in the different vegetables from all farms varied according to the following trend, Spinach: Fe>Zn>Mn>Cu>Cd>Ni; okra: Fe>Zn>Mn>Cu>Ni and fluted pumpkin leaves: Fe>Mn>Zn>Cu>Cd>Ni. This showed that there is a relationship between the level of metals in the soil samples and in the vegetables. Fe had the highest concentration in the soil and it was also the highest in each vegetable. Ni had the least level in most of the farm soil samples and it also had the lowest concentration in all the vegetables considering that Cd was detected in two farms only.

The mean level of each metal from all farms also gave the following trend: (Cd) F>D; (Cu) B>G>F>D>E>C>A; (Fe) D>G>F>E>B>C>A; (Mn) B>F>E>G>C>A>D; (Ni) E>B; (Zn) G>F>D>B>E>C>A. From this trend it is clear that Okada road farm (A), which served as the control had the lowest levels of all metals.

5.5 Correlation of Heavy Metal Levels in the Vegetables and soils

A correlation matrix for the investigated heavy metals in the soil and vegetables of all farms (Tables 4.10-4.16) showed a significant positive correlation for Zn:Cu, Zn:Fe, Ni:Fe, Zn:Ni, Fe:Cd and Fe:Cu at ($p<0.05$) level of significance. This strong positive correlation means that the probability of obtaining such a correlation coefficient by chance is less than five times out of 100. This indicates the metals in the soil and those in the vegetables are closely associated, thus suggesting their common origin. There was also a negative correlation between Zn:Mn, Ni:Mn, Mn:Cu, Mn:Cd, Zn:Cd. The negative

correlation found between Cd, Cu, Mn and other metals may suggest that their concentration in the vegetables may be not depend exclusively on the amount in the soil.

5.6 Soil-Plant Transfer Factors

Soil to plant transfer of heavy metals is the major path way of human exposure to metal contamination (Lato *et al*., 2012). Higher transfer coefficients reflect high soil contents or greater potentials of plants to absorb metals and bio-accumulate into tissues (Abah *et al*., 2012). High TFs (TF >1) indicate that the plant species is a hyper accumulator of the metal (Baker and Brooks, 1989). However, low transfer coefficients (TF < 1) have been reported to indicate strong sorption of the metals to soil colloids (Kachenko *et al.*, 2006).

The soil-plant transfer factor (TF) differed between the vegetable types and sampling farms as shown in Tables 4.17- 4.23. Table 4.17 revealed highest absorption and bio-accumulation of Mn by fluted pumpkin leaves (TF > 1) in Okada road farm while other metals gave a TF < 1 in all vegetables. The Table also showed no absorption of Cd and Ni in the three vegetables. Cd and Ni were not detected in the soils and vegetables of this farm. Mn was also the only metal with a TF > 1 in fluted pumpkin from Kpakungu and Dutsen kura farms (Table 4.18 and 4.19). Fluted pumpkin may therefore be regarded as a hyper accumulator of Mn. The high TF (TF > 1) for Cd in spinach and fluted pumpkin of Keteren gwari farm may be due to the fact that spinach and fluted pumpkin are hyper-accumulators i.e, they accumulate quantities of metals from the environment regardless of the concentration of the metal in the soil (Prasad and Freitas, 2003).

The TFs values for Zn and Cu from Mandela farm were the highest for all vegetables compared to other farms. Jacob and Kakulu (2012) reported that TFs could differ in sampling farms for the same vegetable due to differences in soil properties. The average TFs for all metals in the vegetables showed that fluted pumpkin generally had the highest

followed by spinach, then okra had the lowest. This may be attributed to fact that leafy vegetables have higher transpiration rate to sustain the growth and moisture content of plants (Lato *et al*., 2012).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The results of the pesticide residues analysis revealed the presence of cypermethrin in spinach samples from two farms. The cypermethrin concentration in one of the samples was above the WHO maximum residues level (MRL) while the other was below. Heptachlor was also detected in these two samples but not quantified. None of the okra and fluted pumpkin leaves gave positive result for any of the pesticides under study. This result compared favourably with some literature on pesticide residues studies conducted in other parts of the world. It also implied that some vegetables produced from the research area were contaminated with pesticide residues above the MRL.

The physico-chemical properties results for the soil samples of the farms showed the pH values to be slightly acidic to slightly basic indicating that the soil samples were within the range for normal soil pH. The conductivity measurements revealed the soil sample to be high in salinity. The organic matter for the soil samples analysed gave values that were within the range for upland soils and the textural class of the soil samples were sandy to loamy sandy. The result for cation exchange capacity indicated the soil samples would have greater water holding capacity and will therefore be less susceptible to leaching of metallic ions. These results agree with literature on physico-chemical properties of soils from the same region.

The soil samples from all farms contained all the heavy metals analysed but only Fe was above the permissible limit set by WHO. The heavy metals analysis results for the vegetables revealed the presence of the metals with the control farm having the lowest average concentration of all metals. Only few of these metals however have concentration

above the WHO limit. These results indicate the possible effect of pollution from dumpsites, mechanic workshops and the water used for irrigation.

The average concentration of all six metals in the different vegetables from all farms varied according to the following trend, Spinach: Fe>Zn>Mn>Cu>Cd>Ni; okra: Fe>Zn>Mn>Cu>Ni and fluted pumpkin leaves: Fe>Mn>Zn>Cu>Cd>Ni.

6.2 Conclusion

The results of the study revealed that some spinach samples were contaminated with pesticide residues while all the vegetables were found to contain the tested heavy metals with pumpkin leaf samples having the highest percentage. The soil samples of the farms were also contaminated with the heavy metals but below the FAO/WHO permissible limits. The control farm contained lower levels of most of the investigated metals suggesting the pollution of most farms soil and vegetables to be as a result of human activities such as indiscriminate use of pesticides/fertilizers, dumping of refuse, exhaust fumes from vehicles and machinery, burning of fossil fuels, irrigating farms with contaminated water etc. The absence of Cadmium and Nickel in most samples suggests that activities producing these two metals in the study area were at low levels. Continuous pollution of the environment will lead to cumulative effects that might expose people to health risks.

6.3 Recommendations

From the results of the study, the following have been recommended

i. A continuous assessment of environmental pollutants should be done in order to obtain detailed information on the level of contamination.

ii. Further studies should be conducted on pesticide residues on a larger scale to ascertain the extent of pesticides contamination on vegetables

iii. Other farm produce and stored ones should also be investigated to know the level of pesticide residues in them.

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