UNIVERSITY OF KWAZULU-NATAL

EXTRACTION OF PESTICIDES USING SELECTED ANALYTICAL METHODS FROM SOIL AND MAIZE SEGMENTS: CUMULATIVE AND HEALTH RISKS ASSESSMENT

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SANDISIWE GLADNESS ZONDO

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Sandisiwe Gladness Zondo

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A thesis submitted to the School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg (KZN), for the degree of [DOCTOR OF PHILOSOPHY IN CHEMISTRY].

This thesis has been prepared according to **Format [either 1 or 2]** as outlined in the Information for the guidance of examiners of Higher degrees, which states:

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Dr Precious N. Mahlambi

ABSTRACT

Increased agricultural operations result in increased usage of various pesticides to safeguard agricultural crops, however this is done without paying attention to the effects of the amounting potential harm both humans and the environment are exposed to. In this present study, a structured study was conducted to investigate the uptake of atrazine, mesotrione, 2,4-dichlorophenoxyacetic acid and glyphosate herbicides from contaminated soil and their translocation into different maize segments. Soil profile and quality of irrigation water were also assessed as they are crucial resources required in agricultural crop production due to their ability to influence the yield and quality of the agricultural products. Various physicochemical parameters were measured in an attempt to monitor the soil profile, irrigation water and maize quality harvested from Buhle farm located in Howick, KwaZulu-Natal Province. The irrigation water physicochemical parameters considered were the pH, electrical conductivity, alkalinity and chloride concentration. The soil physicochemical parameters considered were moisture content, pH, electrical conductivity, texture, total nitrogen as well Mg, Na, K, Zn, Mn, P and N elements. Maize was analysed for nutrition content and medicinal health promoting compounds. Based on the attained results, the soil texture contained high clay content (56.4%), followed by sand (40.6%) and silt (2.98%). The concentrations for total nitrogen, phosphorus and potassium which were translated to high soil fertility were 2700, 19 and 222 mg L^{-1}, respectively. These particular elements are essential for agricultural plantation processes and consequently maize quality and maize yield. The levels of sodium, sodium adsorption ratio and electrical conductivity found in irrigation water were 0.05 mg L^{-1}, 2 and 1.81 µS m^{-1}, respectively. The findings showed that maize harvested from Buhle farm had high starch content of 58.6%. Fibre, protein and fat contents in maize were 23.4, 9.01 and 4.55%, respectively. Furthermore, the total anthocyanin, total flavonoids and total phenolic acid content were 8.5, 49.5 and 100 mg L^{-1}, respectively. High amounts of phenolic acid detected indicated therapeutic ability of the maize since phenolic acids are essential for cancer prevention to the consumer. The presence of anthocyanin, flavonols and phenolic acids in maize crop is associated with its quality that could benefit livestock and human after consumption.

The analysis of herbicides in soil and maize samples require sample pre-treatment due to their low concentration and complex matrix hence an ultrasonic extraction, microwave-assisted

extraction (MAE), Soxhlet extraction (SE) and QuEChERS methods were investigated. The optimization and application of ultrasonic extraction, MAE, SE and QuEChERS methods were conducted for the effective extraction of pesticides from maize and their corresponding soil samples. The analysis of pesticides (atrazine, glyphosate, 2,4-dichlorophenoxyacetic acid and mesotrione) was done with gas chromatography-flame ionization detector. Factors influencing the efficiency of the extraction methods such as the extraction solvent, extraction time, solvent volume, sample wetting and spiking concentration were assessed. Under the optimum experimental conditions, the relative standard deviation (RSD), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ), and percentage recoveries were the quantitative characteristics of the current methods assessed. All calibration curves showed a high correlation coefficient (R^2) ≥0.996, indicating good linearity. The LODs and LOQs ranged between 0.22-0.32 µg L^{-1} and 2.0-2.9 µg L^{-1} for SE , 0.1-0.25 µg L^{-1} and 1.1-2.2 µg L^{-1} for MAE, 0.02 – 0.15 µg L^{-1} and 0.2 - 0.5 µg L^{-1} for UE and 0.01 – 0.23 µg L^{-1} and 0.13 – 0.8 µg L^{-1} for QuEChERS. The maize and soil analytes recoveries for SE, MAE, EU and QuEChERS ranged between 62-80% and 70-81%, 80-98% and 85-101%, 100-104% and 91-97 % and 94-115% and 92-101%, respectively with the repeatability, articulated as RSD values of which are within the acceptable range as they are lower than 20%. MAE method showed higher sensitivity compared to SE while, UE and QuEChERS both showed high sensitivity for the extraction and quantification of the target analytes at low concentrations found in soil and maize cob. It was observed that 2,4-dichlorophenoxyacetic acid (2.4-D) was least absorbed by the soil, however, all the studied herbicides showed high absorption in the leafy segment of the maize plant due to the high polarity of the leaf cuticle. Glyphosate showed high absorption rate in soil, roots, stalk and leaves while mesotrione was highly absorbed in corn and tassels in all treatments. The absorption rate of analyte increased with increasing growth days. The higher treatment concentration (0.75 g L^{-1}) showed elevated accumulation with the highest concentration (1.02 µg L^{-1}) observed for glyphosate in leaves after 140 days and high mesotrione in corn (0.51 µg L^{-1}) and tassel (0.42 µg L^{-1}) observed after 120 days. Even though all maize treatment showed a pesticide toxicity index (PTI) values of <1, the health risk index (HI) data were below 100% threshold as well indicating no possible health risk linked with the intake of these crops by both adults and children.

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
Ach	Acetylcholine
AChE	Acetylcholinesterase
BHC	benzene hexachloride
Bt	bacillus thuringiensis
CNS	Central nervous system
ECD	Electron Capture Detector
ECD	Electron capture detector
FID	Flame ionization detector
FPD	Flame photometric detector
GC	Gas Chromatography
HPLC	Higher Performance Liquid Chromatography
LC	Liquid Chromatography
LLE	Liquid-liquid extraction
LOD	Limits of Detection
LOQ	Limits of Quantification
MRLs	Maximum residue limits
MRM	Multiple reaction monitoring
MS	Mass Spectrometry
MSD	Mass selective detector
MSPD	Matrix solid-phase dispersion
NPD	Nitrogen phosphorus detector
OCs	Organochlorines
OPs	Organophosphorus
PDA	Photodiode Arrays
QuEChERS	Quick, easy, cheap, effective, rugged and safe

RSD	Percentage Relative Standard Deviation
SIM	Selective ion monitoring
SPE	Solid Phase Extraction
SPME	Solid phase microextraction
TOF	Time of flight mass analyzer
UE	Ultrasonic Extraction
WHO	World Health Organization

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, **[Sandisiwe Gladness Zondo]** declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References section/s.

Signed ……

[Sandisiwe Gladness Zondo]

DECLARATION 2-PUBLICATIONS

Paper 1 presented as chapter 2: Usage of agricultural pesticides: the environmental and human health impacts.

Submitted to Journal of Comprehensive Reviews in Food Science and Food Safety

Paper 2 presented as chapter 3: Assessment of soil, irrigation water and quality for maize produced in Buhle farm located in Howick, KwaZulu-Natal Province, South Africa.

S. Zondo, PN. Mahlambi. Journal of Agriculture. 68(2) (2022), 87-96. https://doi.org/10.2478/agri-2022-0008.

Paper 3 presented as chapter 4: Comparison of ultrasonic and QuEChERS extraction methods efficiency for the determination of herbicides residues in soil and maize cob

S. Zondo, PN. Mahlambi. Trends in Sciences. 19(24) (2022), 3030. https://doi.org/10.48048/tis.2022.3030

Paper 4 presented as chapter 5: Comparison of Soxhlet and microwave assisted extractions efficiency for the determination of herbicides in soil and maize cob: cumulative and health risks assessment.

Submitted to Journal of Crop Science

Paper 5 presented as chapter 6: Assessment of herbicides uptake and translocation into soil and different maize segments followed by characterization with gas chromatography –flame ionization detector.

S. Zondo, PN. Mahlambi. Journal of Agriculture. 68(2) (2022), 87-96. https://doi.org/10.2478/agri-2022-0008.

From all the above publications, my role included carrying out all the experimental work and contributing to the writing of the publications along with my supervisor. Co-author contribution was also that of an editorial nature, checking on the scientific content in her field, and my correct interpretation of the data in her field. Based on her expertise, she may have added minor parts to the manuscripts.

Signed: … [redacted] ……..

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Chapter 1 Introduction

1.1 Background

Maize is the major feed grain in South Africa and a staple crop countrywide. This makes agricultural sector an important industry to the economy both as an earner of foreign currency (because of its multiplier effects) and as an employer. According to Southern African Grain (2022/2023) South Africa produces on average 15.6 million tons of maize annually where the predominant maize-producing domain is Free State (4 051 500 tons), followed by North West (2 332 500 tons) and Mpumalanga province (2 190 000 tons). However, other provinces produce maize but in small quantities (Figure 1.1). The 59% of the produce is white maize and 41% of the produce is yellow maize (DAFF 2021). Maize raw material is used for the manufacturing of different products such as paper, food, medicine, textiles and paints (Mogala 2020). According to DAFF (2021) humans utilize about 50% of the produced maize in South Africa for consumption, while animal feed industry utilizes 40% and about 10% is utilized for seed industry.

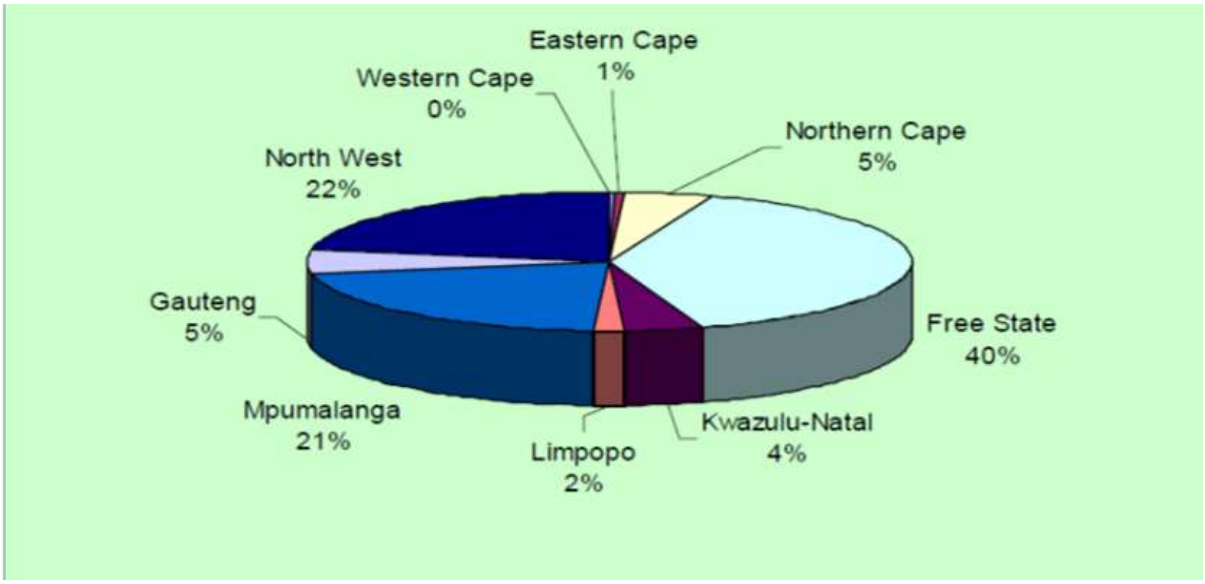

Figure 1.1 Production of maize in nine South Africa provinces (Mogala 2020)

High nutritional composition of maize makes it a primary source of energy when consumed by humans as a vegetable. Livestock industries, snack industries and millers industries depend on

maize crop production. Maize crop production has encountered significant deviations in the past six years, in all the maize producing provinces due to climate change (Table 1.1) (DAFF 2021/2022).

Table 1.1 Maize production (tons) by provinces from 2018/19 to 2020/21 production season

Season	**2018/2019**	**2019/2020**	**2020/2021**
Province			
Western Cape	30600	30600	36000
Eastern Cape	69700	119000	169000
Northern Cape	630750	633900	655000
Free State	1758000	2209000	7016000
KwaZulu-Natal	399600	434500	722000
Limpopo	117000	126000	270000
Mpumalanga	1977300	2347500	3543000
Gauteng	348000	330000	745000
Northern West	399000	522000	2693000

Due to continuous increase in population growth globally, there is an increasing demand for food production in order to accommodate this growth. Therefore, over the years the increase in maize production has been influenced by use of fertilizer and pesticides (Abate *et al.* 2017). Pesticides are compounds that can be synthesized or derived from natural sources such as animals, bacteria, plants and certain minerals (Oguh *et al.* 2019). They are substances mainly applied to crops in order to destroy agricultural pest and unwanted plants that compete with maize crops. Agriculturally, pesticides are important substances of a comprehensive strategy in increasing crop quality and quantity and consequently improve human health. They also prevent pre and postharvest loss of crops to pest-ranging from insects, animal and weed to microorganisms (Aktar *et al*. 2009).

There are more than 500 pesticides registered in South Africa (Quinn *et al.* (2011); Ntombela and Mahlambi (2019)) and South Africa is one of the four largest importers of pesticides in sub-Saharan Africa. Application of pesticides is in both agricultural and non-agricultural sectors including turf on spot pitch, oil industries, golf courses as a preservative, and in wastewater treatment plants as disinfectant. They are also employed in homes to control weed

in premises and gardens yards, for the prevention of algae in swimming pools and as the active ingredient in detergent (Ntombela and Mahlambi 2019). Pesticides applied on the soil are uptaken by the roots and translocated to different crops parts and thus be consumed by human beings. Although the use of pesticides may result in increased crop production as they kill insects, fungi and undesirable plants, they may have detrimental effects on human and animal life. The world-wide deaths and chronic diseases due to pesticide poisoning number are about 1 million per year (Forum 1999), this is because pesticides are both carcinogenic and mutagenic. Thus it is essential that pesticides be continuously monitored in crops.

1.2 Problem statement

The study was conducted in Howick at Buhle agricultural field where studies on maize, tubers and agronomic crops production are conducted to improve food security for KwaZulu-Natal community. Due to large agricultural production conducted, the use of synthesized fertilizers and pesticides have been an alternative to improve crop yield and quality. This is due to that pesticides prevent large crop losses and hence they play an important role in agricultural sector. However, after maize crop harvest, the agriculture sector pays attention only on nutrition quantification and not pesticides toxicities. Hence, the concentration of pesticides residue levels in the planted crops is not known and eventually their health effect upon consumption. This study therefore focused on quantifying pesticides residues in soil and maize crops. Furthermore, to investigate their translocation to leaves, tassel and maize cob in order to evaluate if they are within the maximum residue levels.

1.3 Aim and objective

1.3.1 Aim

To assess the uptake of pesticides by soil and their translocation to the stalk, leaves, tassel and maize cob as well as their effect on crop nutrition.

1.3.2 Objectives

The aim of this work was achieved through the following objectives:

(a) To develop/optimise and apply Soxhlet, ultrasonic, microwave and QuEChERS method followed by Gas Chromatography-flame ionization detector for the determination of pesticides.

(b) To study the uptake of atrazine, 2,4-dichlorophenoxyacetic acid (2,4-D), glyphosate and mesotrione by soil and their translocation to stalk, leaves, tassel and maize crop.

(c) To confirm if pesticides residues are within the guideline levels for beneficial crop usage and below the set MRLs.

(d) To assess the degree of toxicity to human health.

1.4 Hypothesis and Research questions

1.4.1 Hypothesis

The pesticides applied in the soil are taken up by stalk, leaves, tassel, and maize cob.

1.4.2 Research questions

- Which extraction method would be best suitable for the analysis of pesticides residues in crop samples?
- Are the selected pesticides (atrazine, 2,4-D, glyphosate and mesotrione) taken up by soil and do they translocate to stalk, leaves, tassel and maize crop?
- Are these pesticides within the guideline levels for beneficial crop usage and below the set MRLs?
- What is their risk assessment to human health?

1.5 Originality of Research

Maize serves as staple food in many households in South Africa, therefore there is an elevating demand on its production. To maintain its steady supply and economic potential, chemically synthesized pesticides are intensively used in the agricultural sector to increase crops protection and thus the crop yield. However, the misusage or over usage of pesticides for maize production has health effect on consumers as pesticides possess toxicity effect due to their composition (Nacke *et al.* 2013). Pesticides applied on the soil are absorbed by the roots and translocated to the stalk, leaves, tassel as well as the maize cob and thus be unintentionally consumed by human beings. The tassel produces pollen which is transported to the silk for the maize seed development. In the chloroplast of the leaves, photosynthesis process occurs to stimulate maize plant growth (Holding and Streich 2013). The stem is where the growth of the maize cob takes place. Therefore, the contamination of any of these matrices by pesticides may results in the contamination of the maize cob and thus affect human health upon consumption. Therefore, the assessment of pesticides contamination in these maize matrices is important for food quality

assurance. The accumulative and persistence characteristic of pesticides and their toxicity affect the human diet, hence a requirement for the analysis of pesticides in maize crops in comparison with international standard is indispensable. For their effective assessment, reliable analytical method needs to be developed/optimized. Hence, the aim of this study was to optimize, validate and apply Soxhlet, QuEChERS, ultrasonic and microwave assisted extraction methods followed by gas chromatography-flame ionization detector for the determination of atrazine, glyphosate, mesotrione, 2,4-D in soil, stalk, leaves, tassel and maize cob from Buhle Farm located in the Howick, province of KwaZulu-Natal South Africa. To the best of our knowledge the soil health in the field of interest has not been investigated to estimate the fertilizer nutrients needed to supplement those in the soil for maize production, water quality has never been assessed to investigate the salinity and sodicity factors and their influence in maize production. The assessment of selected pesticides uptake and translocation on different maize segments of the maize grown in Buhle Farm to investigate the highly absorbing fragment was conducted for the first time. The extraction efficiencies of the ultrasonic-assisted, QuEChERS, Soxhlet and microwave-assisted extraction methods were compared for the first time for the selected pesticides. Moreover, the potential health risk effect to the maize end user was conducted for the first time on this farm. The maize grown in the Buhle farm is traded for different purposes including human consumption and animal feed. Therefore, the findings from this study will enable maize growers from this farm to be aware of the uptake and translocation capacity of herbicides and their potential health risk to the end user.

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Chapter 2 Usage of agricultural pesticides: the environmental and human health impacts

Abstract

The agroecosystem relies heavily on pesticides to supply the world's growing need for food production and security. Each year, large amounts of chemically synthesized pesticides are used u to increase crop yields. This is extremely hazardous since 98% of pesticides sprayed on targets may have an adverse effect on non-target creatures. According to several studies, the environment is directly contaminated by 80% of pesticides that are sprayed. Pesticide depositions have been shown to compromise the quality of soil, water and crops. The microbial life danger due to pesticide has reduced soil respiration by 35%, and water supplies are pesticide-polluted to approximately 90% of their capacity. Human exposure increases the chance of developing chronic abnormalities which include cancer, reproductive harm, birth defects, neurological and development effects, immunotoxicity and disruption of the endocrine system. The Toxicity Quotient (TQ) which is used to evaluate the toxicity level of the individual pesticide and is calculated from the ratio between each pesticide residue concentration and the corresponding maximum residue levels (MRLs). The pesticides toxicity index (PTI) is a screening approach used to assess the degree of exposure to toxicity of complex pesticide mixtures. The health risk index (HI) characterizes the health risk (HR) assessment of consumers caused by the consumption of pesticide-contaminated food chain. This review therefore discusses the consequences of pesticides on the ecosystem, natural biodiversity, food chains, and subsequent health problems. It also covers the analytical methods that have been employed for the determination of pesticides in crops. Furthermore, the toxicity index and health risk assessment are discussed for human exposure assessment.

Keywords: Pesticides, Environment, Food Chain, Human health, Toxicity

2.1 Introduction

Over the last century, pesticides and agrochemicals have played a significant role in agriculture systems globally, allowing a substantial increase in food production and crops yields. Despite this, the necessity to increase food production is further influenced by the exponentially growing human population. Some conflicts that paralyze food production includes immigrants growth together with climate change effects which compromise food availability in many countries and necessitate more effort in food production (Matsa, 2020). Agriculturally, pesticides and fertilizers have emerged as a crucial instrument for plant production, protection and crop enhancement. Weeds, pests and crops pathogens are responsible for worldwide losses in crops with wheat losses ranging from 10 to 28%, rice losses from 25 to 41%, maize losses from 20 to 41%, potatoes from 8 to 21%, and soybean losses from 11 to 32% (Savary et al., 2019). A closer assessment of pesticide usage reveals that currently crop treatments is more frequent and often overall (Nayak & Solanki, 2021). According to WHO (2019), pesticide usage (measured in tones of active ingredient) increased by 46% between 1996 and 2016, globally. Four million tons of pesticides are utilized annually on a global scale, with herbicides accounting for the majority (56%), insecticides (19%), fungicides (25%) followed by other kinds including nematicides and rodenticides (Maggi et al., 2019). Pesticides usage in Africa has increased and stood at hundred and eight thousand metric tons between 2019 and 2022 for agricultural activities with herbicides accounting for the majority 47.5% of them, insecticides 29.5% and fungicides 5.5%.

Pesticides are used indiscriminately and consequently pollute and detriment the biota (Kumar et al., 2022) while employed to promote agricultural output. The environmental transmission of pesticides originates harm to surrounding non-target organisms. According to previous studies, only 0.1% of pesticides is believed to reach their target species with the remainder polluting and harming the environment (Gill & Garg, 2014). Future paths for improved food production must be selected prudently while science-based methods supported by research are considered. Thus, scientific research is an essential component of this process for advancing food production, improving safety, and protecting the environment. This article reviews the different classes of pesticides residues, their effect on the environment and crops and toxicity impact to the consumer additionally, the extraction techniques and analytical techniques.

2.2 World population and food production

Despite agriculture's effectiveness in managing the growth in the global population, there are serious concerns about the future as to how long can the sustainability of food supply keep up with growing demand. Throughout the whole twentieth century, the world population increased exponentially from 1.65 billion to 7.7 billion and it is anticipated that the world's population would increase to around 8.5 billion by 2030, 9.7 billion in 2050, and 10.9 billion in 2100 (an Estimate, 2022). With such exponential growth, there is still a rising hunger concern globally (Tetzlaff, 2022; UNICEF, 2022). Globally, the proportion of individuals who are undernourished increase in 2019 to 10.6% and in 2020 to 11% (Guo et al., 2021; Saxena et al., 2021). The Food and Agricultural Organization (FAO) of the United Nations estimates that 821 million people, or almost one in nine people, are undernourished globally (UNICEF, 2022). With the increasing food insecurity concerns, the Sustainable Development Goal (SDG) intention of ending hunger by 2030 could not materialize (Molotoks et al., 2021). These factors strain food production practises in the agricultural sector hence the employment of pesticides to promote steady food production, supply as well as human health.

2.3 The role of pesticides in Agriculture

Enormous benefits have been obtained in agriculture using different types and classes of pesticides. Pesticides are necessary and indispensable in agriculture for food production and crop protection. Farmers have employed them to control and manage weeds and insects in agricultural cultivation, and there have been reports of significant increases in agricultural output as a result of pesticide usage (Ren et al., 2021; Singh, 2021). This has promoted agricultural production significantly in order to cope with demographic growth. Improvements in plant varieties, mechanization, and the use of fertilizers have contributed to advance food production, however pesticides have been a primary part of the process since they reduce harvest losses resulting from illnesses, weeds and pests. Figure 1 indicate the mostly used pattern of pesticides globally. Vegetable production would decrease by 54%, grain production by 32% and fruit production by 78% without the usage of pesticides (Lamichhane, 2017). Therefore, pesticides are crucial for reducing weeds and crop disease rates while increasing agricultural yields globally. Consequently, they have exponentially contributed to hunger reduction and provided access to a steady supply of food.

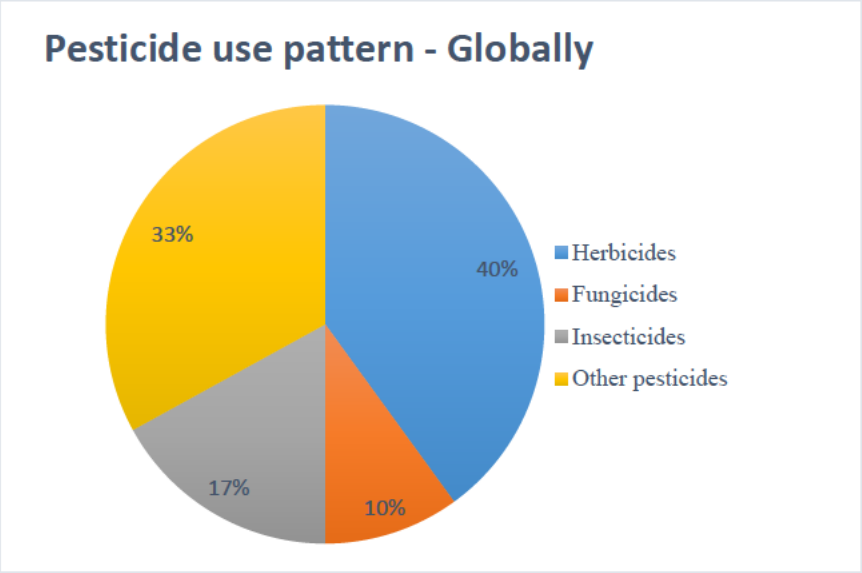

Figure 2.1: Application of different kinds of pesticides globally (Sarkar et al., 2021)

2.4 Classification of Pesticides

Pesticides are described according to the diversity of their characteristics, including their, chemical composition, mode of action and toxicity (Hassaan & El Nemr, 2020; Tudi et al., 2021; Yadav & Devi, 2017). The sources of pesticides origin are divided into two classes which are chemical pesticides and bio-pesticides. Chemical pesticides are characteristically organic substances made through chemical synthesis or from natural sources. Bio-pesticides are organic compounds that use non-toxic processes to control pests (Alengebawy et al., 2021). The chemical composition of pesticides can be categorized into four main classes which includes organochlorines, organophosphorus, carbamates, pyrethrin, and pyrethroids.

Organochlorine (OC): are family of organic compounds bonded to chlorine (Cl^-) atoms through covalent bonding to the carbon structure. These pesticides are lipophilic, and thus they accumulate in the fatty tissue which promotes their toxicity nature since they comprise persisting properties. Organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), heptachlor, aldrin, lindane and endosulfan are widely used in the agriculture production and they have a great level of toxicity (Omeje et al., 2021; Petrović et al., 2022)

Organophosphorus (OP): are chemical compounds with the molecular formula $O=P(OR)_3$ that are produced during the esterification process between alcohol and phosphoric acid forming phosphoric acid esters or thiophosphoric acid esters. OP hydrolyses when alcohol is released

from the ester bond. OP exposure, whether acute or chronic results in different levels of toxicity in plants, animals, insects, and human. A number of agricultural produce, such as rice, strawberries, spinach, peaches, onions, avocados are regularly treated with OP pesticides such as methyl parathion (O,O-dimethyl-O-(p-nitrophenylphosphorothioate), chlorpyrifos, malathion, parathion and diazinon. A combination of two or more insecticides is also applied to improve the effectiveness of insecticides on produce (Kamal et al., 2022; Syafrudin et al., 2021).

Carbamate: are N-methyl carbamates derived from a carbamic acid and cause carbamylation of acetylcholinesterase at neuronal synapses and neuromuscular junctions. They bind to acetylcholinesterase reversibly, while sharing a similar mode of action with irreversible phosphorylation of acetylcholinesterase by organophosphates (Parra-Arroyo et al., 2022; Raffa & Chiampo, 2021). Therefore, carbamates often have a toxicity period of less than 24 hours and a toxicological appearance that is comparable to OP poisonings. Carbonate pesticides such as carbofuran, aldicarb, oxamyl, methomyl, fenobucarb and ethienocarb are thermally unstable and soluble in water (Xiao et al., 2021) moreover, they are hazardous to vertebrates (Bhatt et al., 2021; Mdeni et al., 2022).

Pyrethroids: are derived from pyrethrin and they are extensively used insecticides. The chrysanthemum flower is originally used to extract pyrethrin consisting of two main functional groups (alcohol and acid) (Liu et al., 2021; Patel et al., 2022). They are divided into two types based on their chemical structure and mode of pest control. Permethrin is type I and deficiencies the cyno group while deltamethrin is type II containing a cyano group at the phenyl benzyl alcohol position (Bose et al., 2021). Pyrethroids have a lower degree of persistence and are photodegradable (Rezende-Teixeira et al., 2022).

Biopesticides: are extracted from compounds that occurs naturally with the potential to function as pesticides. They have biodegradable properties and do not harm non-target species. Plant incorporated, microbial and biochemical are the three groups into which they are classified (Souto et al., 2021).

2.5 Effect of pesticides usage on soil

Through the excessive use of pesticides, the soil is being directly or indirectly contaminated and compromised. Factors affecting mobility and persistence of pesticides in the agroecosystem includes water solubility, the octanol/water partition coefficient ($LogK_{ow}$), soil-sorption constant (K_{oc}), half-life in soil (DT_{50}), organic matter, pH and clay content. Metabolic pathways involves transformation through hydrolysis, methylation, and ring cleavage resulting in the production of numerous hazardous phenolic chemicals (Aktar et al., 2009a). The interaction between the soil and pesticides determines the amount of pesticides retained since soil organic matter is the primary influential soil characteristic resulting in the adsorption increase as the organic matter increases (de Rebello et al., 2019). Table 1 shows the comparison of soil organic matter, texture and pesticide concentrations detected in different studies conducted globally. The soil ability to bind to positively charged ion in an exchangeable state is through sorption which includes Van der Waal's forces, hydrogen bonding, hydrophobic bonding and electrostatic interactions which includes charge transfer, ion exchange or ligand exchange as well as covalent bonding or the combinations of these reactions. The pH of the soil is also a significant factor as the adsorption increases with diminishing soil pH for ionizable pesticides (Kah & Brown, 2007). Gondar et al. (2013) studied the effects of ionic strength and pH on the adsorption of metalaxyl and penconazole non-ionic fungicides on four soils samples with different amounts of organic carbon. The adsorption isotherms fitted a Freundlich equation. The study revealed that for pH > 5, segregation of the fungicides between the soil solution and the solid phase did not differ with the pH. This was due to the ionization of the carboxylic groups of the soil organic matter, which favoured water molecule fixation to the negatively charged sites by hydrogen bonding. The existence of water molecules conferred organic matter with hydrophilic nature, which hindered the adsorption of the hydrophobic molecules of the fungicides. At lower pH, the fraction adsorbed on the solid phase increased as the pH decreased. The decrease in the pH gave rise to protonation of the ionized carboxylic groups, thus causing a reduction in the number of negatively charged sites and desorption of water molecules. Resulting in the organic matter becoming more hydrophobic, thus favouring the adsorption of neutral organic molecules of metalaxyl and penconazole.

Table 2.1: Comparison of pesticide concentrations in soil globally

Pesticides	Place and country	Soil type	Soil pH	Soil organic matter	Concentration $\mu g\ kg^{-1}$	Reference
Chlorothalonil	China	Clay	8.1	36.03	76.71	(Wang et al., 2021)
		Sandy	7.71	1.07	1.25	
2.4-D	Mazandaran, Iran	Loam	7.1	2.81	1.6	(Jamshidi et al., 2022)
		Clay loam	6.5	3.62	2.5	
		Sandy clay loam	7.6	2.10	0.38	
Phenazine-1-carboxamide	China	Red clay loam	5.3	1.01	5.75	(Ou et al., 2020)
		Red loam	8.2	1.72	10.4	
		Black soil	7.1	1.98	12.8	
Alachlor	Pakistan	Silty clay loam	7.21	1.35	19	(Ahmad, 2018)
p,p' -DDD	Beijing, China	School yards	-	-	6.47	(Xiaofei et al., 2008)
Carbendazim	Basrah, Iraq	agricultural soil	-	-	1259	(Raheem et al., 2017)
Pendimethalin	Kosjerić, Serbian	Loam	6.72	3.43	14.32	(Đurović et al., 2009)
Endosulfan	India	Clay soil	8.46	2.29	0.45	(Kumar & Philip, 2006)
Chlorpyrifos	Western Cape, South Africa	Black soil	-	-	63.6	(Degrendele et al., 2022)

Pesticide residues enter the ecosystem through their application into the soil during crop production where pesticides can leach from the treated fields (Figure 2), mixing and washing sites, or waste disposal areas and reach surface water. This result to surface water systems, including rivers, lakes, streams, reservoirs, and estuaries, to be exposed to the accumulation of pesticides and other chemicals since they are small captive sinks of the by-products of human activities. Surface water systems are linked to both groundwater and atmospheric water through the hydrologic cycle (Lorenz et al., 2017). Moreover, pesticides in surface water gets transferred into groundwater through seepage of the soil. Additionally, pesticides are released into the atmosphere by transpiration and evaporation.

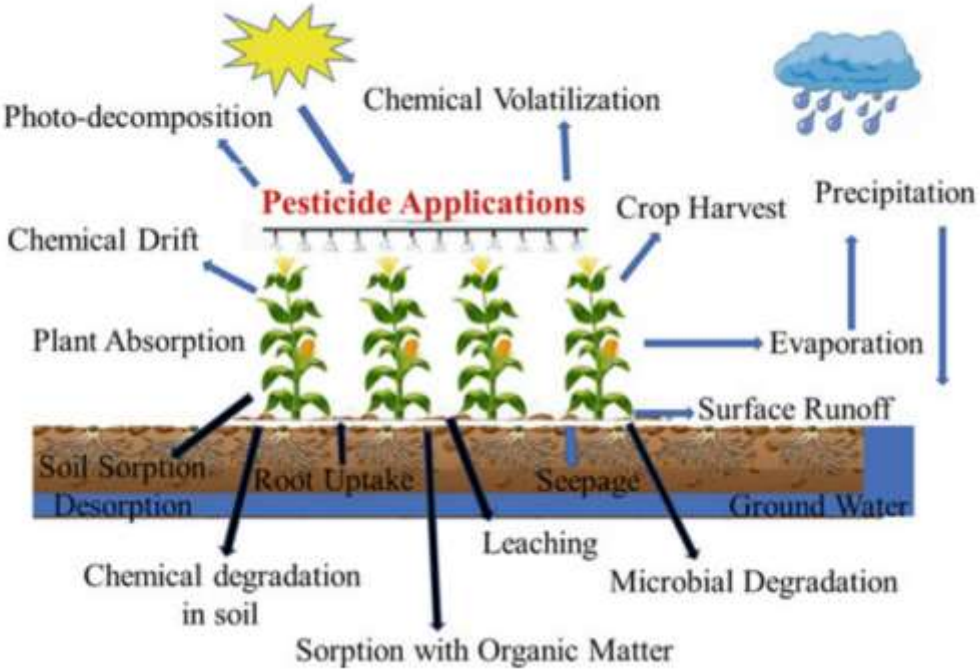

Figure 2.2: Application of pesticides, decomposition, and the entrance of residues through soil, surface water, and ground water into ecosystems (Pérez-Lucas et al., 2019)

From the application occurrence, pesticides undergo biotransformation by the action of the microbes or soil biota. Physical and chemical factors also cause physic-chemical transformation of pesticides. The pesticide transformation products are also accumulated and stored in the soils where they compromise the beneficial soil microbial life. This significantly compromises microfauna microscopic animals essential for decomposition of soil organic matter and stabilization of soil aggregates. Also, extensive soil treatments of pesticides destroy the soil bacteria responsible for nitrogen fixation (Mahmood et al., 2016). Mycorrhizal fungi responsible for colonizing the root system of a host plant, providing increased water and

nutrient absorption capabilities, have also shown degradation effects (Serbent et al., 2021). Continuous declines of useful microbes in the soil compromises the crop productivity hence food security. Pesticides degradation compromises biochemical processes, microbial biodiversity and enzyme activity (Burul et al., 2022) which leads to significant loss in soil fertility and soil ecosystem. Moreover, repeated pesticide application pose a negative impact on the development, colonization potential, and metabolic processes of beneficial root-colonizing soil microorganisms such arbuscular mycorrhiza, fungus, algae and bacteria (Arjumend et al., 2022).

The growth and accumulation of *Pseudomonas* strains in soil structures which is a major component of soil fertility, is reduced due to the usage of herbicides such as thifensulfuron methyl, chlorsulfuron, metsulfuron and sulfonyl-urea (Campillo-Cora et al., 2022). Similar to chlorothalonil, captan and benomyl fungicides reduces the respiration process of soil (a microbial indicator) by 30–50% (Boteva et al., 2022). These toxic residues influence the specific soil microbial species or their enzyme activity, which in turn disrupts significant biochemical processes including nitrification, ammonification and nitrogen fixation (Panneerselvam et al., 2022). Pesticides significantly impact the soil organic matter and mineralization which are two primary important soil characteristics that control and regulate soil production. The application of herbicides like primeextra, glyphosate, paraquat and atrazine drastically reduces the amount of organic matter in the soil (Sebiomo et al., 2011). Surface water bodies develops residue contaminants due to pesticides runoff from the treated soil and plants which could disrupt aquatic life over time. Leaching pesticides from soil surfaces build up in the ground water system with time (Navarro et al., 2021).

2.6 Pesticides Residues and Food Chain Contamination

One of the primary global concern on food safety is pesticide contamination. The exposure of food consumers to toxic residue through food chains is a well-known and ongoing issue (Sumudumali & Jayawardana, 2021). Previous studies have shown the accumulation of pesticides in soils, terrestrial ecosystems, and aquatic environments, and their lethal effects on human and non-human biota. Chemical synthesized pesticides have made it possible to more than double food production output by protecting crops from pest thereby increasing yields. Due to high food demands, new pesticides are synthesized and utilized for higher agricultural

productivity (Chávez-Dulanto et al., 2021). Since pesticides are intrinsically toxic and deliberately spread in the environment and food chains, their production, distribution and use call for strict regulation and control. Regular monitoring of residues in food chain and the environment is also indispensable. Government establishes and uses the acceptable daily intake to establish the maximum residual limits (MRLs) for each pesticide in agricultural crops to warrant crops quality (Wang et al.). Globally, it is projected that 0.35 million people are killed yearly from pesticide poisoning and this is a great concern (Zhai et al., 2022).

Shah (2020b) reported several cases of pesticide residues detected on food that were exceeding the MRLs. A study conducted in India assessed OP, OC and pyrethroid pesticides on crops and 15.3% of samples exceeded the MRLs. In Brazil, pesticides assessment programs were conducted and less than 3% of the samples exhibited residual levels over the MRL. In Poland, 34% of the cereal grains samples showed pesticide residues and 3% of those samples had levels above the permissible limit. Mutengwe et al. (2016), assessed a total of 99.27% fruits and 0.73% vegetables for the presence of 73 pesticides. The pesticides residues were detected on 56.46% of the samples, while 0.78% was above the permissible limits. A study from Maule Region (Talca, Chile) found pesticide residues on the fruits and vegetables that were used to make school children's snack. Detection frequencies for malathion dicarboxylic acid, 3,5,6-trichloro-2-pyridinol, 2-isopropyl-4-methyl-6-hydro-xypyrimidine, and para-nitrophenol were 1.1 µg kg^{-1}, 71.4 µg kg^{-1}, 43.3 µg kg^{-1}, and 98.96 µg kg^{-1}, respectively (Muñoz-Quezada et al., 2019) that the children were exposed to. A study conducted in Bloemfontein (South Africa) detected heptachlor in *Brassica oleracea var. capitata* (cabbage), *Beta vulgaris var. cicla* (swiss chard), and *Solanum tuberosum* (potato). The quantities of pesticide residues found in the samples of cabbage ranged from not detected to 121.6 ng/kg for heptachlor, which was below the acceptable MRLs. While the existing situation indicated that eating vegetables grown in the Bloemfontein province poses only minor health risks, increased amounts of unmonitored consumption of these crops may result in elevated levels (Motshabi et al., 2021). These poisoning have a close link to unnecessary exposure to these toxic compounds and thus their proper usage and continuous monitoring is of importance. The primary and consistent source of pesticides in food chain is soil. Table 2 indicates the detected residues in different foodstuff worldwide with India showing high pesticides residues due to its highest population in the world hence high reliability on pesticides for continuous food supply (Shah & Parveen, 2021).

Table 2.2: Concentrations of residues in foodstuff worldwide.

Foodstuff	Place and country	Detected pesticides	Concentration µg kg^{-1}	Reference
Tomatoes	Johannesburg, South Africa	Boscalid	50	(Mutengwe et al., 2016)
Tomato	Kampala Metropolitan Area, Uganda	Omethoate	12.4	(Ssemugabo et al., 2022)
Cabbage Green onion	Sharkia Governorate, Egypt	Chlorpyrifos	7.33 951	(El-Sheikh et al., 2022)
Cabbage	Monze, Zambia	Monocrotophose	60	(Mwanja et al., 2017)
Carrots	Lucknow, India	Chlorpyrifos methyl	3.44	(Covaciu et al., 2017)
Strawberries Water melon Melons Grapes	Shanghai, China	Fluopyram Dinotefuran Fasthiazate Ethirimol	3.3 15.3 3.1 71.0	(Zhang et al., 2021)
Cucumber,	Croatia, Balkans	Chlorpyriphos	30	(Jurak et al., 2021)
Maize	Howick, South Africa	Atrazine Glyphosate 2,4-dichlorophenoxyacetic acid Mesotrione	11.36 20.65 11.50 24.61	(Zondo & Mahlambi, 2022)

2.7 Effect of pesticides on human and animal health

The toxicity of pesticides can be chronic and acute, depending on the amount of the chemical and how frequent the substance is exposed. Acute toxicity harms the consumer after a single exposure and typically lasts for a short time. Chronic toxicity has a negative impact that surface from partly exposure and have long-term effects. Tongo and Ezemonye (2015)**,** assessed γ-HCH, heptachlor, heptachlor epoxide, aldrin and dieldrin organochlorines residues in edible

tissue of slaughtered cattle in Benin City, Southern Nigeria. Total pesticide residues ranged from 2.38 to 3.86 μg kg^{-1} in muscle, 3.58 to 6.3 μg kg^{-1} in liver, 1.87 to 4.59 μg kg^{-1} in kidney and 2.54 to 4.35 μg kg^{-1} in tongue. Pesticide accumulation in the cow tissues were in the order: liver > tongue > muscle > kidney. Human health risk assessment showed EDI estimations for heptachlor epoxide, aldrin and dieldrin exceeding threshold values, indicating possible carcinogenic health hazard for consumers especially children. Pesticides are transported by the bloodstream throughout the human body and defecated through the skin, urine then after metabolism they are exhaled into the air. The maternal blood placenta, breast milk, and human fat-tissues that easily absorb and accumulate fat soluble pesticides are distinctive toxicity pathways (Prabhu & Lakshmipraba, 2022).

Human exposure manifests in a number of ways, from moderate symptoms like minor allergy symptom or skin irritation to severe symptoms such nausea, dizziness or severe headache. Longer exposures result in chronic abnormalities which include cancer, reproductive harm, birth defects, neurological and development effects, immunotoxicity and disruption of the endocrine system. Three key categories such as genotoxic, carcinogenic and neurotoxic effects, and reproductive impacts are used to classify the long-term consequences of pesticides on human (Shah, 2020a). A genotoxic agent is classified as chemical, biological or physical substance that interrelates with genetic material (DNA) to cause changes, damage, or ruptures as well as substances that obstruct the enzymatic processes of protein synthesis, genesis, or polymerization necessary for the segregation of chromosomal. These modifications may impede embryonic development or mark the beginning of cancer development. Genomic damage can result from pesticide exposure. Pre-mutagenic damage from pesticides, such as DNA strand breaks and DNA adducts, gene mutations, such as insertion, deletion, inversion, and translocation, and chromosomal aberrations, such as whole-chromosome gain or loss of chromosone, breaks or deletion (clastogenicity), and rearrangements of chromosomal, are three broad categories of genetic damage (Mattick & Amaral, 2023). Neurotoxicity is a negative impact on the peripheral or central nervous system emanating from biological, physical and chemical agents. Growing neurological systems in children causes them to be more vulnerable to neurotoxic substances, such as pesticides (Iqubal et al., 2020). Pesticides exposure causes the neuronal cell death through cytoskeleton disruption, calcium excess, oxidative stress induction or mitochondrial damage (Martínez et al., 2020). The majority of currently used synthetic herbicides, insecticides as well several fungicides has neurotoxicants abilities.

Endocrine disrupting chemicals (EDCs) are becoming one of the leading concerns and are understood to pose substantial and immediate dangers to the human health. EDCs have been

shown to impair intrauterine development, shorten gestation, and disarray with metabolic planning (Bou Zerdan et al., 2021). Through alteration of peroxisome proliferator activated receptors, thyroid hormone receptors and primarily estrogen receptors, prenatal exposure to EDCs can have an impact on embryonic neurodevelopment (Basak et al., 2020). Findings of a prospective cohort research with 57310 pesticide applicators in the USA revealed links between bladder cancer and two imidazolinone herbicides (imazethapyr and imazaquin) (Koutros et al., 2016). Another case-control research (881 controls and 953 cases) of male agricultural field workers in Egypt showed increasing bladder cancer cases associated with pesticides contact (Amr et al., 2015)

2.8 Exposure pathways of pesticides to humans

Interactions of pesticides are direct from agriculture, occupation and household activities and indirect from food stuff. Pesticides can enter the human body by any of four routes: through the skin (dermal), eyes (ocular), lungs (respiratory), or mouth (oral) (Kankam, 2021; Lakshitha, 2021; Molpeceres et al., 2021). Pesticides contamination on human body increases as the concentration or dosage increases. A study by Nicolopoulou-Stamati et al. (2016) described how the different pathways exposure develop different health illness such as cancers in humans including brain tumors, leukemia, non-Hodgkin lymphoma (NHL), breast, prostate, stomach, lung, liver, colorectal and bladder cancers.

2.8.1 Oral pathway

The oral exposure is through consumption of foodstuff contaminated with pesticide residues (Reeves et al., 2019). The usual cases occur when pesticides are transferred into an unlabelled soft drink and consumer inadvertent (Kuo et al., 2012). Also, from handling food prior washing hands after pesticides handling or equipment for pesticides application in agricultural field. Koutros et al. (2009) conducted a cohort on pesticides users in the Unite State and imazethapyr, a heterocyclic aromatic amine usage was associated with cancer cases. Significant trends were observed in this study with increasing lifetime exposure for colon cancer and bladder cancer. Rateratios (RRs) increased by 137% for bladder cancer and 78% for colon cancer when the highest exposed were compared to the non-exposed.

2.8.2 Ocular pathway

The ocular exposure is through eye tissues, and when pesticides are absorbed in large quantities by the eyes they cause fatal illness. Eyes are typically harmed by granular pesticides depending on the weight of the individual particles. When pesticides are applied using power equipment, they bounce back at high speed from the vegetables or surfaces causing eye damage (Fareed et al., 2012). Kirrane et al. (2005) reported the association of retinal degradation and fungicide used to the wives of the farmers in the North Carolina. The study revealed that the wives were at high risk of eye disorder. Fungicides promoting this disorder were mancozeb and ziram.

2.8.3 Respiration pathway

The respiratory exposure is through inhalation of pesticides that could cause serious harm to the nose, throat and lungs tissues. The pesticide exposure is low when pesticides application is in large droplets with conventional application equipment. When a low-volume equipment is used for the application of the concentrated material, the potential for respiratory exposure increases due to the smaller droplets production (Amaral, 2014). In addition, as the temperature increases, evaporation levels of pesticides increase and consequently increase this exposure. Hoppin et al. (2002) assessed the pesticides contribution to respiration complications within the farmers between the age of 16 to 88 years in the North Carolina. Farmers showed the wheezing difficulties relating to the usage of atrazine and alachlor herbicides.

2.8.4 Dermal pathway

Dermal exposure is through skin absorption of pesticides from spillages, splashing or spray drift, loading when mixing, pesticides disposal and pesticides cleaning (Saldana et al., 2007). Formulation of pesticides mostly differ in physical and chemical properties which consequently affect its ability on skin absorption (Beard et al., 2014). However, the skin absorption is influenced by the duration and the amount of exposure. Liquid formulated pesticides are readily absorbed in the skin and other body tissues compared to granule form.

The presence of pest in foodstuff can lead to human health risks depending on the extent of toxicity. Kim et al. (2022) assessed the dermal exposure effect on the Korean farmers between the age of 39 and 72 years. Men showed high insulin resistance as compared to female farmers. Adults farmers showed pathophysiology of diabetes due to continuous application exposure without considering the state of the protective gear used and the spraying technique.

2.9 Pesticides in maize

Maize is the most staple food source, especially in Africa. However, the continued cultivation of maize as a staple food source is threatened by a number of factors, including insects, pests, weeds and pathogens (Mabe et al., 2017; Meissle et al., 2010). Most of maize varieties are highly vulnerable to stem borers, armyworms, silkworm, and weevils, weeds; and maize diseases such as downy mildew, maize rust, leaf blight, and leaf spot, which affect maize productivity (Mabe et al., 2017). Extensive use of chemical pesticides by agricultural sector have allowed better pest and weeds control hence, high maize production (Méndez et al., 2011; Tijani & Nurudeen, 2012). Pesticides are applied to the seed prior to planting in a form of seed coating to protect against soil-borne risks to the plant. The spray application is the common form of pesticides application in agriculture fields. Pesticides are either applied during pre-emergent and post-emergent of crops to reduce competitive pressure from newly germinated weeds and to reduce harm to the desired target crop. The contamination of maize may also result from the application of biosolids and sewage sludge are municipal and industrial wastewater treatment by-products that are commonly used as soil conditioner/fertilizer for vegetable crop. This is due to that they rich sources of organic nutrients. Biosolids have high content of macro-and micro nutrients and organic matter which promotes their usage as soil conditioners and thus they contribute towards availability of pesticides in soil (Usman et al. 2012). Some of the commonly used pesticides in maize crops includes atrazine, glyphosate, 2,4-Dichlorophenoxyacetic acid (2.4-D), gramoxone and mesotrione. Pesticides have different chemical structures and they exhibit a wide range of biological modes of action. Their basic feature is toxicity, or the ability to evoke chemical damages and in consequence, the death of the organism. The effect of pesticides involves blocking the activity of certain enzymes or hormones controlling important processes which indicate that pesticides are dangerous for living organisms (Aktar et al., 2009b).

Atrazine is the herbicide which belongs to the triazine family. It is widely utilized in the production of corn as a results of its efficiency in weed removal, low cost as well as crop protection (Gianessi, 2008). It is used in the prevention of pre-emergence broadleaf weeds in maize production The structure of atrazine in given in Figure 3. Atrazine is linked to the potent endocrine disrupter, interfering with the body's endocrine system by producing adverse reproduction, development neurological and immune effect in humans, abnormal growth

pattern and neurodevelopment delays in children (Dabrowski et al., 2014; Rinsky et al., 2012; Vogel et al., 2015).

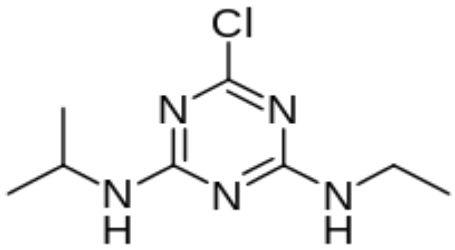

Figure 2.3: Structure of atrazine

Glyphosate is used to prevent pre-emergence broadleaf weeds, grasses, and sedges in various field and row crops around the globe. It is a systematic herbicide that is absorbed through the plant tissue to kill broadleaf plants, weeds and grasses. It is used in corn, cotton and soybeans. In maize production, glyphosate is used in pre-plant burn down of weeds (Gouse, 2014). It is the main ingredient of Roundup herbicide used in agriculture and house hold as weed control (Bolognesi et al., 2009; Organization, 2017). The structure of glyphosate is given in Figure 4. Glyphosate is carcinogenic to humans, also, the increase in micronuclei, a biomarker of chromosomal damage has been observed after spraying with glyphosate (Bolognesi et al., 2009; Organization, 2017).

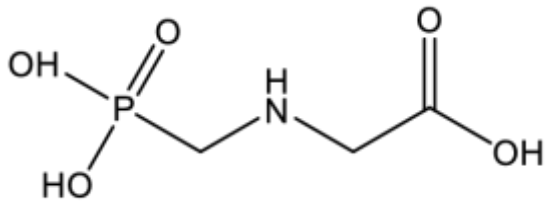

Figure 2.4: Structure of glyphosate

2,4-D is the herbicide which belong to phenoxyalkonates family. It is used to prevent post-emergence broadleaf weeds by causing uncontrolled growth in them. The 2.4-D is used in agriculture and in residential weed insect control (Burns et al., 2011). The structure of 2,4-dichlorophenoxyacetic acid is given in Figure 5. The 2.4-D has been found to be carcinogenic to humans causing non-Hodgkin's lymphoma cancer (Burns et al., 2011).

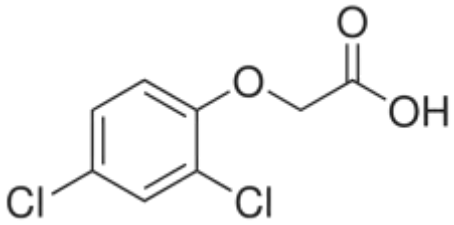

Figure 2.5: Structure of 2,4-D

Mesotrione is a non-selective herbicide developed for the pre- and post-emergence control of a wide range of broad-leaved and weeds in maize (*Zea mays*). It is a member of the benzoylcyclohexane-1,3-dione family of herbicides, which are chemically derived from a natural phytotoxin obtained from the Californian bottlebrush plant, *Callistemon citrinus*. The structure of mesotrione s given in Figure 6.

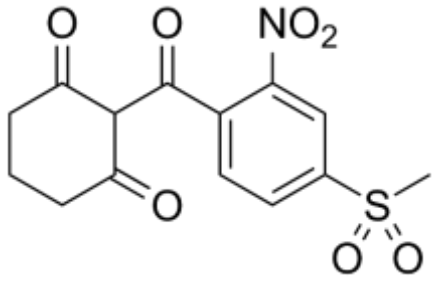

Figure 2.6: Structure of mesotrione

Gramoxone is the herbicide which belong to dipyrids family. It is a non-selective herbicide that is used for control of fibrous rooted gasses and annual broadleaf weeds. It is used globally as weed control in the agricultural sector and for home gardens (Tsai, 2013). The structure of gramoxone is given in Figure 7. Gramoxene causes neurological damage and dysfunctional kidneys and liver in humans, also, fatalities can occur due to irreversible pulmonary fibrosis, inflammation and respiratory failure (Blanco-Ayala et al., 2014; Huang et al., 2019; Shadnia et al., 2018).

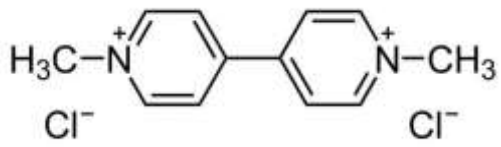

Figure 2.7: Structure of gramoxone

2.10 Uptake of pesticides by crops

Pesticides may enter plants through leaf surface during spraying and roots through absorption from soil. Pesticides can also be carried by wind and be deposited onto aquatic or terrestrial plants. Water transported pesticides enter plants from roots and be carried upward in the transpiration stream by plant during transpiration. When pesticides enter the plant, it is distributed within the plant from cell to cell or through plant vascular system. Uptake by roots from the soil take alternative or simultaneous two pathways (symplastic system and apoplastic system) to reach the xylem vessel where it is moved to the top of the crop with the transpiration stream in the xylem. Symplastic system is a plant tissue bounded by the plasmalemma and connected via plasmodesma which is the reactive environment that places chemicals in proximity to enzymes and other reactant. Movement within the conductive portion of the symplast occurs via mass flow and diffusion. Apoplastic system includes all the non-living portions of the plant. Xylem elements and cell walls form a water-permeable continuum through which both short- and long-distance solute transport occurs by mass flow and diffusion. Diffusive movement plays the primary role in short-distance transport. Balance between the distribution of pesticides in the apoplastic-symplastic compartments determines the overall transport pattern (Donnarumma et al., 2009; Hellström, 2004; Parween et al., 2016).

A study on the distribution of dieldrin in soil and its translocation to roots and aerial parts of vegetable crops grown in greenhouse and fields have been studied by Hwang et al. (2017)**.** Results showed a translocation of residues in cucurbitaceous fruits and flowers at 0.065 mg/kg (zucchini), 0.039 mg/kg (cucumber) and 0.012 mg/kg (melon) of dieldrin which were taken up from contaminated soil. Florence et al. (2015) studied the uptake of chlordecone by root vegetables (yam, dasheen and sweet potato). Chlordecone content in the vegetables ranged from 18 to 336 µg kg^{-1} in dasheen, from 2 to 439 µg kg^{-1} in yam and from 2 to 209 µg kg^{-1} in sweet potato. Yam accumulated more chlordecone than dasheen and sweet potato tubers which indicated that the soil types did not have the same potential to contaminate the root vegetables. The findings were in good agreement with the hypothesis that chlordecone uptake by root vegetables is based on passive and diffusive processes and limited by transport and dilution during growth.

2.11 Pesticide toxicity index (cumulative risk) and health risk assessment

A pesticide undergoes metabolic processes due to which its toxic effects may be modulated when entering a living organism. A search for biomarkers for pesticide toxicity should include indicators of overall health as well as specific indices selected according to the mode of action of the investigated pesticide. The toxicity effect of the pesticides residues in foodstuff is assessed by using the toxicity quotient (TQ) to measure toxicity resulting from an individual pesticide and is derived from ratio between each pesticide residue concentration and the matching MRLs. The pesticides toxicity index (PTI) which is a screening approach to assess the degree of exposure to toxicity of complex pesticide mixtures is then obtained as the sum of the TQ for each pesticide compound as stated in equation 1 and 2.

$$TQ = \frac{C}{MRL} \quad (1)$$

$$PTI = \Sigma\, TQ \quad (2)$$

Where: *C* is the concentrations of the detected individual pesticide residue (µg L^{-1}), and MRL is the maximum residue limits (µg L^{-1}) of the individual pesticide. The PTI acceptable threshold of > 1.00, poses no harm to human health (Shalaby et al., 2021).

The health risk index (HI) is used to characterize the health risk (HR) to the consumers caused by the consumption of pesticide-contaminated vegetables. The HI is determined by dividing the estimated daily intakes (EDI) (mg/kg/day) by the corresponding values of the WHO/FAO-established acceptable daily intake (ADI) (mg/kg/day) (Khan et al., 2020), as indicated in the equation 3 and 4. If the HI is lower that 100%, it indicates minimal harm to human health (Akoto et al., 2016):

$$EDI = \frac{A\ X\ B}{C} \quad (3)$$

$$HI = \frac{EDI}{ADI}\ X\ 100 \quad (4)$$

Where: *A* represent the concentration of detected pesticide residues in vegetable (µg L^{-1}). B represent the average daily intake of vegetables (g). C is the average body weight for South Africans (70.8 kg for adults and 20.8 kg for child), (Walpole et al., 2012).

2.12 The physico-chemical properties of pesticides

The interaction behavior of pesticides with biological parameters is influenced by their physical properties. The more hydrophilic groups a compound possesses, the highly polar the compound is resulting in the greater transportation and distribution of substance through the soil and crop. The lower $LogK_{ow}$ values indicate that the compound is hydrophilic and its active molecules will preferentially partition in the aqueous phase via hydrogen bonds and consequently increase pesticides mobility and absorption by soil and crops (Table 3). Higher pKa relate to high hydrogen ion (low pH) consequently, pesticides become highly soluble and this increase absorption and transmission to the soil and crops (Pereira et al., 2016). Vapor pressure signifies air solubility ability which depends on the size of the organic compound and functional group of the organic compound. Higher vapor pressure promotes pesticides to turn into gaseous state and consequently, inhibit their application effectiveness. A Log of 10^{-3} vapor pressure indicate very high pressure while Log of 10^{-7} indicate very low pressure (Pereira et al., 2016). Mesotrione has higher pKa and low $LogK_{ow}$ is expected to be present at higher concentrations in crops. $LogK_{ow}$ is expressed as a nondimensional base 10 logarithm with a range of -5 to 5. Therefore 2.4-D is a lipophilic substances and has a lower bioaccumulation factor due to higher $LogK_{ow}$ compare to other herbicides (Pereira et al., 2016)

Table 2.3: Physico-chemical properties of pesticides

Pesticides	Water solubility (mg/L)	pKa	XLogP3	$LogK_{ow}$	Vapor pressure (mmHg)
2.4 D	64	3.4	2.8	5.78	$1.86x10^{-7}$
Mesotrione	22	3.12	0.7	0.90	$4.27x10^{-7}$
Gramoxone	100	-	1.7	4.22	$7.5x10^{-6}$
Atrazine	34	1.60	2.60	2.61	$2.89x10^{-7}$
Glyphosate	17	2	4.6	-3.40	$1.84x10^{-7}$

2.13 The maximum allowable concentration limits of pesticides in soil and crops

Pesticide residues are the traces of a chemical remaining in treated food, agricultural commodities or animal feed remaining. They are by-products of pesticide application in conversion products, metabolites, reaction products and impurities considered to be of toxicological significance. Due to their toxicity ability, Maximum Residue Limits (MRLs) have been recommended by the Agricultural Standards Committee (Table 4) to enforce quality assurance for the consumer safety (DAFF, 2019).

Table 2.4: The maximum allowable concentration limits (mg/kg) of pesticides in soil and crops (DAFF, 2019; Quinn et al., 2011).

Pesticide	Grain	Soil
Glyphosate	2	0.1
2,4-D	0.5	0.02
Atrazine	0.05	66
Gramoxone	0.05	0.5
Mesotrione	0.06	1.5

2.14 Sample preparation techniques

Different extraction techniques are used for the determination of pesticides present in the different soil and crops. Sample preparation is an important step since the analytes are extracted from the complex matrices. This is followed by cleaning stage of the analytes from any impurities present in the extracts (Padrón et al., 2006). Typically used extraction techniques includes: Soxhlet extraction (SE), quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction, microwave assisted extraction (MAE), etc., while the sample clean-up is done using the solid-phase extraction (SPE)

2.14.1 Solid phase extraction (SPE)

The SPE involves four essential steps: Conditioning or equilibration stage for wetting the sorbent with a slightly polar or non-polar solvent which activates the functional groups of the sorbent for effective interaction with the analytes. This is followed by sample loading stage for percolating the analyte through the solid phase resulting to allow the analytes to adsorb on the sorbent. Then, the clean-up stage is applied for the removal of undesirable impurities leaving the analytes behind. Lastly, the elution step is done for the collection of the analytes from the

stationary phase using the appropriate solvent (Figure 8). Different adsorbents are utilized for pre-concentration and clean-up of pesticides residues in different matrixes. The C_{18} is used to eliminate brooding of the compound peaks in the on-line solid phase extraction-chromatography system. Silica-bond TMA Chloride (SAX)-PSA cartridge is used for the removal of impurities such as organic acids, sugars and fatty acids leaving target analytes attached to the sorbent. Amino propyl (NH_2) SPE cartridge is used for the elimination of lipid compounds at low temperature (Eticha, 2020) leaving target analytes attached to the sorbent. This method offers significant advantage such as less consumption of organic solvent, shorter analysis time, no phase emulsion, higher method recovery, and more efficient removal of interfering compounds (Nodeh et al., 2017). However, the cartridge plugging may occur due to the different matrices physical properties. Also, its five extraction steps results to the prolonged method optimization time (Andrade-Eiroa et al., 2016).

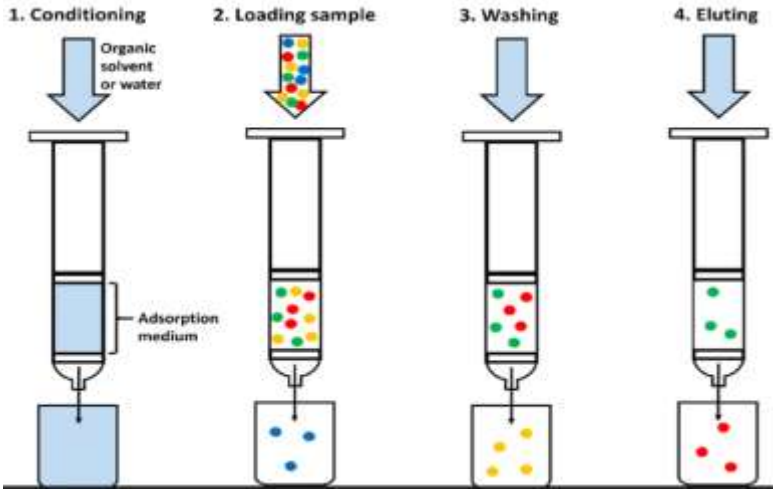

Figure 2.8 Schematic diagram showing four main extraction steps involved in SPE procedure (Abd-Talib et al., 2014).

2.14.2 Ultrasonic extraction (UE)

The UE is based on the extraction of samples with an organic solvent by applying ultrasound radiation ranging from 20 kHz to 2000 kHz (Organization et al., 2008) in an ultrasonic bath. The mechanical effect of ultrasound induces a great penetration of solvent into sample and improves mass transfer, resulting to an enhancement of analyte extraction efficiency. Type of solvent and irradiation conditions (temperature and amplitude of sonication) are factors

influencing extraction efficiency (Fenoll et al., 2009). The UE significant advantages are that it is economical, limited solvent usage and reduced extraction duration (Samsidar et al., 2018).

2.14.3 QuEChERS extraction

QuEChERS is a quick, easy, cheap, effective, rugged and safe technique used in the extraction and clean-up of pesticides residues. The initial introduction of this extraction procedure was on the extraction of pesticides residues in fruits and agricultural crops, however, there has been increased interest in this technique for the extraction of agrochemicals residues from different industrial matrices. QuEChERS is based on the sample treatment with liquid-liquid partitioning and dispersive solid-phase extraction (d-SPE) for the removal of undesirable impurities. Successfulness of the method is due to its flexibility and high efficiency. Moreover, the technique provides more acceptable extraction clean-up of analyte interferences to yield excellent results after chromatographic instrumentation. New developments have been introduced to improve the purification of the extract, different d-SPE sorbents have been introduced for the improvement of the clean-up stage and utilization of buffer additions for the salting-out partitioning step, formation of salts and use of different extraction solvent (Rejczak & Tuzimski, 2015). QuEChERS principle involves homogenizing the sample followed by the addition of the appropriate solvent. The reagents used depend on the type of sample to be analysed. This is followed by the addition of QuEChERS content, vertexed and the mixture is centrifuged. Following this, the sample is put through a d-SPE clean-up prior to analysis. This method offers significant advantage such as small solvent volume, produces minimum hazardous waste (Lehotay et al., 2004)

2.14.4 Soxhlet extraction (SE)

Soxhlet is a widely used traditional method for the extraction of pesticides traces from different environmental complex matrices. It is classified as one of the leading methods due to its ability to extract complex samples such as different plant tissues, soil, waste water sediments, etc. Its principle involves a sample in a solid state being placed in the cellulose thimble followed by placement into a Soxhlet extractor chamber which is then placed into a round bottom flask containing an extracting solvent. Extraction is attained by means of a hot condensate of an organic solvent that is continuously refluxed through the sample distilling in a closed system

SE offers significant advantages which is to conduct the extraction process unattended with the ability to analyse complex environmental solid samples. It has high extraction efficiencies due to repetitively interaction of the sample with a portion of the solvent (Azwanida, 2015). However, exposure to flammable and hazardous organic solvents and longer extraction time is a major drawback for this technique. Moreover, SE is particularly applicable to solid samples only.

2.14.5 Microwave assisted extraction (MAE)

Its principle involves application of microwave energy to enable analytes separating from the matrix to the extracting solvent (Trusheva et al., 2007). Dipoles of polarized and polar materials interact with microwave radiation producing heat to the materials surface and the heat is transmitted by conduction. Hydrogen bonding becomes disrupted by dipole rotation of the molecules prompted by microwave electromagnetic increasing dissolved ions to migrate and stimulate penetration of the solvent into the sample matrix (Kaufmann & Christen, 2002). The MAE is considered a selective extraction technique that favour high dielectric constant solvent and polar molecules. MAE technique offers limited solvent volume and extraction time. The MAE increases analytes recoveries and reproducibility with caution of utilizing accurate extraction condition to eliminate thermal degradation. However, this extraction technique is limited cycles of MAE (e.g. from 2 × 10 s to 3 × 10 s) results in pesticide residue decrease due to the oxidation of compounds.

2.15 Separation and detection techniques of pesticides

Due to the low concentration of pesticides and the high complexity of the matrix, the use of analytical techniques which provides high selectivity and sensitivity is essential. Gas chromatography (GC) and liquid chromatography (LC) (Figure 2.9) are the mostly used techniques for multi-residue analysis of pesticides (Wiest et al., 2011) . The choice of the separation technique depends on the characteristics of the pesticides of interest. The volatile, semi-volatile and thermally stable compounds can be determined by GC, whereas non-volatile and/or thermally unstable ones should be determined by LC (Debayle et al., 2008; Kujawski et al., 2014).

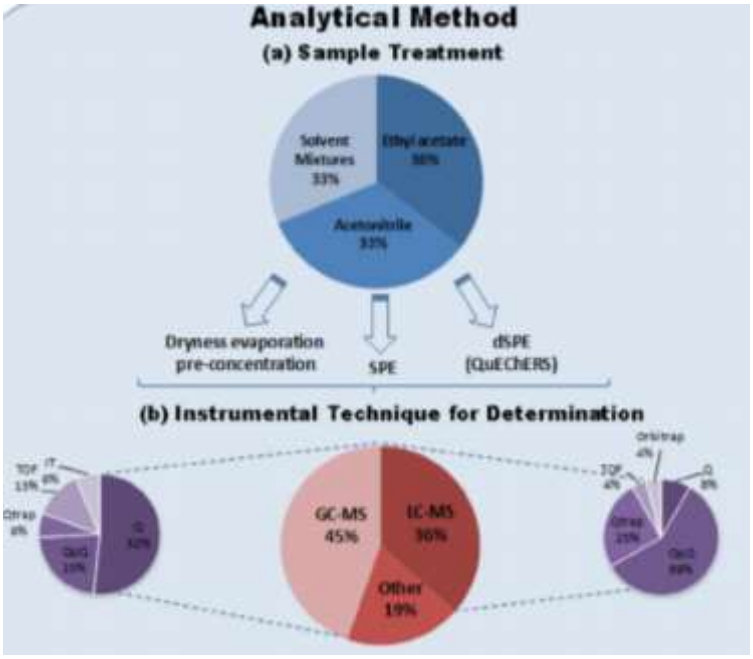

Figure 2.9 Scheme diagram summarising analytical methods information for a). Sample pre-treatment and b). Separating techniques for analysis (Grimalt & Dehouck, 2016).

Separation of pesticides has been carried out by GC coupled with various detectors. Due to their sensitivity, detectors such as an electron capture detector (ECD) and mass spectrometry detector (MSD) (Łozowicka et al., 2017), flame photometric detector (FPD) (Bakırcı & Hışıl, 2012), nitrogen phosphorus detector (NPD) (Łozowicka et al., 2017), Flame ionization detector (FID) (Abd El-Gawad, 2016) are used. Mass detection methods are also employed to improve method sensitivity which are equipped with analyzers such as ion trap (IT) (Tao et al., 2009), quadrupole (de Oliveira et al., 2012), triple quadrupole (QqQ) (Wu, 2017), time of flight mass analyzer (TOF) (Cervera et al., 2012). Additionally, to minimize the matrix interference selective ion monitoring (SIM) (Lima et al., 2017) or multiple reaction monitoring (MRM) (Walorczyk, 2008) are used, where the analyte mass to charge ratio (m/z) are concentrated to achieve a lower limit of detection and quantification with less interference. Liquid chromatographybased techniques have been employed coupled with ultraviolet (UV), photodiode array (PDA), diode array detector (DAD) and mass (MS) detectors.

2.16 Analysis of pesticides

Pesticides residue analysis have been conducted using various extraction, separation and detection methods all over the world. This indicate that pesticides contamination in various

matrices is a worldwide problem. Wang et al. (2021), studied the uptake of imidacloprid, difenoconazole, acetamiprid, azoxystrobin, tricyclazole and tebuconazole pesticides in soil and maize. The results showed accumulation of pesticides by maize from the soil, with imidacloprid (27.73 μg g^{-1}), acetamiprid (17.75 μg g^{-1}), tricyclazole (18.96 μg g^{-1}), azoxystrobin (12.56 μg g^{-1}), tebuconazole (10.66 μg g^{-1}) and difenoconazole (2.13 μg g^{-1}). The pesticides accumulation quantity was correlated negatively with adsorption coefficient and correlated positively with concentration of pesticides in situ pore water (C_{IPW}). Bioaccumulation factor in roots varied from with 0.61 for imidacloprid being the lowest to 974.64 for difenoconazole being the highest. This study revealed that the uptake, translocation and accumulation in soil by maize is influenced by their physicochemical properties, especially log K_{ow}. Stamm et al. (2016), investigated the uptake of insecticides applied on seeds and their translocation to different plant parts. Results showed different uptake and translocation trend in the vegetative stage. Flupyradifurone uptake was higher than clothianidin uptake initially in VC soybean vegetation stage. For VC soybeans, clothianidin and imidacloprid equivalents in the roots were significantly less than in the stems and unifoliate leaves. In V1 soybean vegetation stage the uptake among three insecticides were not detected and unaffected by soil moisture stress. In V2 soybean vegetation stage, the clothianidin was affected negatively by soil moisture stress while flupyradifurone and imidacloprid were not affected. For V2 soybeans, clothianidin equivalents in the roots were significantly greater than in the first and second trifoliate leaves. This investigation increased the understanding of the uptake, accumulation and translocation of insecticides used for soybean seed treatments. The uptake, accumulation and translocation of these insecticides showed different response to soil moisture stress. Santos-Hernández et al. (2018) investigated the presence of 2,4-D and atrazine in corn samples using high performance liquid chromatography with UV detection (HPLC-UV). Prior to their analysis, microwave-assisted extraction was conducted. The analytes were enriched using solid-phase extraction with Strata X sorbent. The LODs obtained were 0.03 and 0.02 mg kg^{-1} for 2,4-D and atrazine, respectively. The extraction recoveries of 2,4-D and atrazine in corn samples were from 82.6 to 98.2% with RSD ranging from 4.0 to 7.2%. Mohammadnia et al. (2020) extracted 2,4-D with micro-solid phase extraction (D-μ-SPE) from water and food (tomato, cucumber, celery, and lettuce) samples and analyses were done using HPLC-UV. Under the optimized conditions, LOD and LOQ values were 0.007 and 0.02 μg mL^{-1}, respectively, recoveries were 88.0-94.0% with RSD less than 7.5%. Residues found in water were 0.89 μg mL^{-1}, lettuce 0.91 μg mL^{-1}, celery 0.91 μg mL^{-1}, tomato 0.94 μg mL^{-1} and cucumber 0.93 μg mL^{-1} with RSD less than 7.5%. Zou et al. (2015) studied the gramoxone in four edible vegetables (cabbage, lettuce,

spinach and Chinese cabbage) using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS-MS). The samples were extracted with ultrasonic extraction and cleaned up by weak cation exchange solid-phase extraction. The recoveries obtained ranged from 43.6 to 73.5% and the LOD was 0.94 ng g^{-1}. Only few samples showed small gramoxone amounts >20 ng g^{-1}. Cabbage sample detected at 19.7 ng g^{-1} gramoxone. Marín et al. (2020) evaluated the residues of glufosinate (GLU), glyphosate (GLY) and its metabolite aminomethylphosphonic acid (AMPA) in three varieties of lettuce (iceberg, romaine and escarole), agricultural soil and irrigation water. The determination was done using ultrasonic extraction followed by liquid chromatography with tandem mass spectrometry triple quadrupole (LC-QqQ-MS/MS). The LOD and LOQ were 0.09 and 0.33 ng mL^{-1}, 0.08 and 0.30 ng $mL^{-1}$and 0.05 and 0.17 ng mL^{-1} for GLY, GLU and AMPA, respectively. The recoveries were 91%, 89% and 103% for GLY, GLU and AMPA, respectively in irrigation water while they were found to be 88%, 109% and 106%, respectively in vegetables and 101%, 89% and 103%, respectively in soil. The absence of interfering peaks at the analyte elution times in blank MS/MS chromatograms of different water, vegetable and soil samples confirmed the selectivity of the method. Al-Rahman et al. (2012) applied QuEChERS method followed by HPLC-DAD to assess the degradation rate of acaricide fenpyroximate in apple, citrus and grape fruits. The results showed 91.41%, 72.7%, 83.6% and 96.0% degradation of acaricide fenpyroximate in apple, citrus, and grape, respectively after 7 days of application. Preharvest interval and concentration found in samples were: 7 days and 0.3 mg kg^{-1} for apple, 3 days and 0.2 mg kg^{-1} for citrus, 3 days and 0.2 mg kg^{-1} for grape fruits, and 14 days and 0.2 mg kg^{-1} for grape fruit.

Study by Santilio et al. (2019) investigated glyphosate in maize and rice by using liquid chromatography triple quadrupole mass spectrometry. Samples were extracted with QuEChERS method. The mean recoveries for both matrices were within 70–105% at three fortification levels. The precision for replicates was <20% for both matrices. The LOD was determined to be 0.002 mg kg^{-1} for rice and 0.004 mg kg^{-1} for maize and LOQ was determined to be 0.01 mg kg^{-1} for both matrixes. Miensah et al. (2015) evaluated presence of atrazine and lindane residues in maize production in Ghana. Samples were extracted using QuEChERS method and residues detected using gas chromatography-electron capture detector (GC-ECD) and gas chromatography-mass spectrometry (GC-MS). Residues of atrazine were below detection limits of 0.010 mg kg^{-1} except in the Ashanti region where atrazine was 0.05 mg kg^{-1} which was within the EU-MRL of 0.1 mg kg^{-1}. Kashyap et al. (2005) extracted 2,4-D in soil samples collected from the agricultural field by acetonitrile in a modified Soxhlet apparatus

and analysed using HPLC–UV. The recoveries obtained varied from 85% to 100%. The LOQ and LOD were 0.010 and 0.005 mg kg^{-1}, respectively. The measured residues of 2,4-D ranged from 0.006 mg kg^{-1} and 0.012 mg kg^{-1} with an average of 0.009 +/- 0.002 mg kg^{-1}. The measured residues of 2,4-D in soil samples are generally low and do not exceed the MRLs. Baranowska et al. (2012) studied the presence of mesotrione, simazine and atrazine in thermal water, sediment and broccoli samples using Soxhlet extraction followed by HPLC-UV and UV-Vis spectrophotometer. The limits of detection were in the range of 0.03-0.10 µg mL^{-1} and the uncertainties of analytical methods were lower than 10%. Simazine and atrazine in the concentration range of 0.03-0.82 µg/g were detected in most of the vegetable samples, whereas mesotrione in the concentration of 2.40 and 4.96 µg mL^{-1} was found only in thermal waters and 0.28 µg/g in a sediment sample. Wu et al. (2010), utilized C18 cartridge for SPE and capillary electrochromatography for the determination of organophosphorus pesticides in vegetables (cabbage, white radish) and fruits (grape, pear, and orange). The detection limits ranged from 0.008 to 0.2 mg/kg, while mean recoveries ranged between 78.9 to 87.2% and 81.4 to 98.6% in vegetables and fruits, respectively. Hu et al. (2010), utilized SPE coupled with capillary gas chromatography and electron capture detector (GC-ECD) for the determination of metribuzin and quizalofop-p-ethyl residues in potato and soil. The LOQ of the method were 0.01 mg kg^{-1}, and the mean recoveries ranged from 72.9 to 109.5% with RSD ranging from 0.7 to 9.2%. Asensio-Ramos et al. (2009), extracted organophosphorus pesticides with ultrasonic using a mixture of methanol and acetonitrile in forestal, ornamental and agricultural soil samples. Recovery values were obtained between 45 and 96% for all the pesticides and soils, except for malathion and malaoxon in forestal and ornamental soils, from which they could not be quantitatively extracted. The LOD of the whole method ranged between 0.48 and 7.78 ng g^{-1}. Obuseng et al. (2013), employed d-SPE, (QuEChERS) method and SPME for the clean-up of pesticides from plant matrices and GC-ECD for characterization. Concentration levels of aldrin, 1,1-dichloro-2,4-bis[chlorophenyl]ethane(DDD), 1,1-dichloro- 2,2-bis[p-chlorophenyl] ethylene (DDE), 1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane (DDT), dieldrin, endosulfan and endrin were studied. The limits of detection ranged from 0.102 to 1.693 µg L^{-1} for all the pesticides. Aldrin and endosulfan were detected in the nymphaea nouchali roots at concentrations of 3 - 21 µg kg^{-1} and 5-3 µg kg^{-1}, respectively. Pentachlorobenzene (PCB) and hexachlorobenzene (HCB) were also detected but were not quantified. Farajzadeh et al. (2019), extracted diazinon, chlorpyrifos, penconazole, oxadiazon, and diniconazole pesticides from fruits and vegetables using combination of QuEChERS and dispersive liquid–liquid microextraction. Gas chromatography–flame ionization detection (GC-FID) separation

technique was employed. The relative standard deviations were ≤ 7% for intra-(n = 6) and inter-day (n = 4) precisions at a concentration of 100 μg L^{-1} of each analyte. The LOD were in the ranges of 0.27–0.48 μg L^{-1} in the solution and 0.68-1.2 μg kg^{-1} in the solid samples. Several fruit and vegetable samples were analyzed by the proposed method, and penconazole was found in grape 4 μg kg^{-1} and other samples were free of the studied pesticides. Liu et al. (2011), extracted triazolopyrimidine sulfonamide herbicide residues in soil, water, and wheat. These residues were cleaned up with an off-line C18 SPE cartridge and analysed with ultra-performance liquid chromatography coupled with tandem mass spectrometry. The LOQ did not exceed 3 μg kg^{-1} in different matrices. Overall average recoveries for this method in water, soil, wheat plants, and seeds at three levels ranged from 75.4% to 106.0%, with relative standard deviations in the range of 2.1–12.5% (n=5) for all analytes. Study conducted by Petrarca et al. (2016), extracted procymidome residues in baby foods using QuEChERS method and analysed by gas chromatography–mass spectrometry. QuEChERS-dSPE provided a more effective removal of matrix co-extractives from extracts, which contributed to lower matrix effects. Twenty-two commercial fruit-based baby food samples were analyzed by the developed method, being procymidone detected in one sample to be 0.05 to 0.1 mg/kg at a level above the legal limit established. Wang et al. (2012), extracted organophosphorus pesticides from broccoli, tomato, green soybean, radish and shallot vegetables using QuEChERS method. Liquid chromatography-mass spectrometry (LC-MS/MS) technique was employed for the determination. Recoveries for all but dibrom at fortification levels of 10, 40, 80 μg/kg in broccoli, tomato, green soybean, radish and shallot ranged from 55 to 122% with relative standard deviations of 1.6-18%. The limits of quantification were 0.1-8 μg/kg. Nurse et al. (2010) assessed the mesotrione doses that would provide at least 90% control of four economically important weeds, without impacting final maize yield by more than 5% in comparison to a weed-free control. The doses required to reduce weed biomass by at least 90% (I90) varied with location and year, and for common lambsquarters and velvetleaf differed by application timing. For lambsquarters, the estimated doses required ranged from 10 to 1984 g ai ha^{-1} for preemergence applications and 15–38 g ai ha^{-1} for postemergence applications. Doses of 45 and 19–243 g ai ha^{-1} were required to effectively reduce the biomass of redroot pigweed. Velvetleaf was effectively controlled preemergence with 288 g ai ha^{-1} and postemergence with 31 g ai ha^{-1} of mesotrione. Final maize yield was only reduced by more than 5% of a weed-free control when a dose of less than 35 g ai ha^{-1} of mesotrione was applied. These results showed that biologically effective weed control with reduced doses of mesotrione is possible depending on the spectrum of broadleaved weed species present in the field. Zhang

et al. (2013) evaluated the weed control efficacy of four post-emergence herbicides nicosulfuron, mesotrione, topramezone and the combination of mesotrione/nicosulfuron when they were applied at reduced doses at different weed growth stages. Experimental results showed that nicosulfuron, topramezone and the combination of mesotrione/nicosulfuron provided better weed control efficacy than mesotrione when they were applied at their label recommended doses at the 2- to 3-leaf and 4- to 5-leaf stages of weeds; nicosulfuron and mesotrione/nicosulfuron could at least be reduced by 33% and topramezone reduced by 67% without sacrificing total weed control efficacy and maize grain yield. Nicosulfuron and its combination with mesotrione could effectively control broadleaved and grass weeds when their doses were reduced by 67% and by 33%, respectively. Topramezone could effectively control broadleaved and grass weeds when its dose was reduced by 67%. Zondo and Mahlambi (2022) assessed the atrazine, mesotrione, 2.4-D, and glyphosate herbicides uptake by soil and their translocation into different maize segments. Due to the strong polarity of the leaf cuticle, all the herbicides studied validated considerable absorption in the leafy section of the maize plant. It was shown that 2.4-D was least absorbed by the soil due to its lipophilic characteristic. While mesotrione was extensively absorbed in corn and tassels, glyphosate demonstrated a high absorption rate in soil, roots, stalks, and leaves due to their hydrophilic nature. The highest concentration of glyphosate (1.0 µg/l) was detected in leaves after 140 days and the highest concentrations of mesotrione in maize (0.51 µg/l) and tassel (0.42 µg/l) was detected after 120 days. The PTI values were greater than 1, but the HI data was below 100%, pointing to limited health concern for both adults and children who consume these crops.

2.17 Difficulties in pesticides analysis and handling

The analysis of pesticides is still challenging especially for complex matrices such as food. The major difficulty is the need to remove chemically unrelated main matrix components (such as proteins, lipids, and organic matter) and other chemically related analytes that interferes with the instrumental determination of the investigated compounds. Some pesticides are fat-soluble non-polar molecules (such as organ chlorine), and tend to concentrate and persist in the fat causing extreme challenge to avoid the co-extraction of fatty material. This results in the inefficient removal of pesticides from the sample matric leading to underestimation of the actual concentration levels present which necessitate the dSPE and SPE cleanup options subsequently to solvent extraction. The difficulties that relates to pesticides handling is due to that, during pesticides application, the majority of farmers wear typical clothes with gloves and

rarely wear coveralls and goggles. This points to the lack of strict monitoring and regular scrutinizing of herbicides residues as well as education on the accumulation and toxicity to the environment, humans and livestock. This indicates that government should regulate strict legislation to ensure complete adherence to pesticides manufacturing and application rules. These gaps needs to be addressed to comply with the global and country's requirements. Moreover, this points for harmless herbicides like bio-pesticides as pest control safe replacement for these harmful herbicides.

2.18 Conclusion and future recommendations

The usage of pesticides for crop management was originated to rescue human-life and will continue in the future due demanding staple food production and food security. Although it will be difficult to completely expel pesticides in the foreseeable future, it is important that they are utilized with extreme caution. The majority of pesticides are potentially hazardous to humans and can have serious health effects, including cancer**.** According to epidemiological data, lymphoma, leukemia and numerous other cancers are more common in farmers and people who uses pesticides. To reduce the hazards to human health, occupational safety regulations as well as adequate pesticide management and storage regulations must be put into place. Users of pesticides should be aware of the risk and dangers associated these chemicals, properly handling and usage, and usage of personal protective equipment effective to minimize harm posed to environmental resources and human health. Reducing the frequency and use the least amount of pesticides, alternative pest management strategies like integrated pest management, which include a variety of control methods such cultivating resistant genotypes, cultural, physical, and mechanical pest control, must be utilized. Furthermore, generating pest-resistant crop genotypes can be made simpler by using progressive cropping techniques like bio and nanotechnology. It is necessary to look for pesticides with less side effects and replace the current pesticides with higher environmental and health dangers. Efforts should be undertaken to develop comprehensive pesticide risk mitigation techniques and treatments to decrease children's exposure in order to guarantee healthy childhood development.

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Chapter 3 Soil and irrigation water quality assessment for maize production in Buhle farm in Howick, KwaZulu-Natal Province, South Africa

Abstract

The continuous monitoring of soil health and irrigation water quality influences the crop yield and the quality of agricultural produce. In this study, various physicochemical parameters were measured to monitor the soil profile, irrigation water and maize quality harvested from the Buhle farm located in Howick in the KwaZulu-Natal Province of South Africa to ensure steady high quality food supply for the consumer. The maize grains were sampled from the maize crop from the maize stalk, the corresponding soil samples were collected from the upper surface of the soil (0-15 cm surface layer) using the soil auger and the irrigation water sample was taken from the irrigation tanks using polyethylene sample bottles. The physicochemical parameters considered for irrigation water were pH, electrical conductivity, alkalinity and chloride due to their ability to affect water quality which consequently affect crop growth and quality. The soil physicochemical parameters considered were moisture content, pH, electrical conductivity, texture, total nitrogen and nutrients (protein, fat, fibre, starch, total mineral matter and elements). These parameters determine the soil quality, water content, the ratio of absorbed and lost energy, concentration of ions and elements present which in turn affect or promote the yield and quality of crops. Maize grains were analysed for nutritional content and medicinal health-promoting compounds to assess the influence of soil and irrigation water on the maize quality and consequently the health of the consumers. The concentrations of total nitrogen (N), phosphorus (P) and potassium (K) in soil, which were translated into high soil fertility were 2700, 19 and 222 mg kg^{-1}, respectively. The results obtained were within the required specification for high quality maize production. The levels of sodium, sodium adsorption ratio and electrical conductivity in the irrigation water were 0.05 mg L^{-1}, 2 and 1.81 µS m^{-1}, respectively, indicating safe water of low salinity. Maize was high in starch (58.6%) while fibre, protein and fat contents in the maize were 23.4, 9.01 and 4.55%, respectively, indicating suitability for consumption. Furthermore, the total anthocyanin, total flavonoids and total phenolic acid content of the maize were 8.5, 49.5 and 100 mg L^{-1}, respectively. Overall, this study showed the presence of health-promoting compounds in the maize crop which is associated with its high quality for consumption. The validity of the analysis methods was tested using certified reference materials. The concentrations of the reference materials were

not statistically different from the certified values, attesting to the validity of the analysis methods.

Key words: Soil profile, water quality, soil fertility, nutrition, antioxidants

3.1 Introduction

Soil is an important natural resource that living things depend on for growth to meet their daily needs. As a growth medium for crop production, the soil has the ability to retain moisture and nutrients (Nwachokor et al. 2009). This natural resource is vitally important for agricultural sector. Soil quality is the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (Sumithra et al. 2013). The composition of soil includes mineral particles, a biological system of living organism as well as organic matter and these are differentiated into horizons (Kinyangi 2007). Interaction of the biological, physical and chemical components of the soil determines its quality and health (Papendick and Parr 1992). Soil quality is assessed for agro-ecosystems where the main, service is productivity. Therefore, knowledge about soil characteristics is essential to predict crop quality and yield.

High soil fertility promotes high nutritious crops enriched with starch, fibre, protein, macronutrients and fat along with micronutrients such as vitamin B complex, ß-carotene, magnesium, zinc, phosphorus and copper which are all essential for human health and development. Soil fertility is essential for plant growth and yield as indicated by nitrogen (N), phosphorus (P), potassium (K), micronutrients and adequate soil moisture. Organic carbon is a significant soil property which determines the level of soil productivity in agriculture (Sumithra et al. 2013, Jain et al. 2014). Thus, soil nutrients deficiency results in limited crop yield (Hartemink, 2010). Soil pH is a primary chemical property that controls the concentration and plant adsorption of solutes in the soil (Akpoveta et al. 2010). Soil moisture promotes nutrients absorption by plants and it is much related to soil texture and soil structure (Yannawar et al. 2013). Soil texture is the relative proportion of clay, sand, and silt particles in the soil volume and it influences soil aeration, soil-water relation, nutritional status and plant root penetration (Saxton and Rawls 2016). Soil temperature effects the biological, chemical and physical interactions related to crop growth (Jain et al. 2014, Nayana and Malode 2021). Soil electrical conductivity (EC) measures amounts of ions in the soil solution and correlates with soil properties that affect cation exchange capacity, drainage condition, organic matter level,

salinity and soil characteristics (Solanki and Chavda 2012). The concentration and composition of soluble salts in the irrigation water determine its quality for human and livestock consumption as well as irrigation of crops. Water quality, therefore, is an important component with regard to sustainable use of water for irrigation in agriculture, especially when salinity development is expected to be a problem in an irrigated agricultural area (Solanki and Chavda 2012).

Food security and quality remains a global concern for humans and livestock and that is highly dependent on soil health. Maize (*Zea mays L*) is the most staple and important agricultural crop in South Africa. About 73 million tons of maize are produced annually in South Africa (DAFF, 2016). However, this volume can increase with continuous monitoring of the soil health and irrigation water quality trends in South African farming and consequently steady food supply (Orfanou et al. 2019). In this regard, maize quality and yield in Buhle farm is influenced by solar radiation, tillage method and unmonitored soil health and irrigation water quality. Therefore, it is imperative to monitor soil conditions, water and maize quality for the benefit of the consumer and consequently, providing knowledge on improving the system management and sustainability for this field. In this context the objective of this study was to assess the soil and irrigation water with the aim of evaluating their effect on the quality of maize quality harvested from Buhle farm located in Howick, KwaZulu-Natal Province.

3.2 Materials and methods

3.2.1 Study area and sample collection

Soil and maize samples were collected from the Buhle experimental field, located in Howick, KwaZulu-Natal Province, South Africa (Figure 1). Buhle Farm is an agricultural field where studies on maize, tubers and agronomic crops production are conducted for the KwaZulu-Natal community. The exact sampling locations can be found through the usage of the Global Positioning System (GPS), represented by the co-ordinates -29.523633, 30.247441. The location is known for its high temperatures in summer (wet season) which normally range from 21°C to 38°C, while these usually drop in winter (dry season) ranging from 10°C-28°C. On average, precipitation is approximately 569 mm annually. For the purpose of the present study, the sampling was conducted in September of 2021 before the treatments were applied as described in chapter 6. The maize crop was taken from the maize stalk, the corresponding soil samples were collected from the upper surface of the soil (0-15 cm surface layer) using the soil

auger. 40 cores were taken in a random zig-zag pattern across the studies sites. The cores were mixed in a packet to make a composite sample. FC 701 white maize cultivar was used throughout the study. A 100 mL of irrigation water sample was taken from the irrigation tanks using polyethylene sample bottles. Maize and soil samples were stored in polyethylene bags while water samples were stored in polyethylene bottles during transportation from the field to the laboratory. The sample collection point was 10 km from the laboratory. Samples were stored at 4ºC until analyses were conducted, in triplicate.

Figure 3.1: Location map of the study sites in Buhle Farm, Howick, South Africa

3.2.2 Chemicals and reagents

Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), nitric acid (HNO_3), potassium chromate (K_2CrO_4), silver nitrate ($AgNO_3$), hydrogen peroxide (H_2O_2), sodium hexametaphosphate $(NaPO_3)_6$, HPLC-grade acetone were purchased from Merck (Pty) Ltd, South Africa. Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for water purification.

3.2.3 Analysis of irrigation water

3.2.3.1 Alkalinity analysis

Irrigation water analyses were performed according to the Official Analytical Chemists (AOAC) official methods (AOAC 2005). The pH and EC were measured using conductivity/pH meter (CPC-505 Elmetron, SA).

Alkalinity was determined by titration of a 20mL water sample with 0.02 N H_2SO_4 using methyl orange indicator and alkalinity was calculated using equation (3.1).

$$\text{Alkalinity } \left(\frac{\text{me}}{\text{L}}\right) = 1000 \text{ x N x (volume of H2SO4)} - \text{mL (blank)} \quad (3.1)$$

where N is the concentration in normality of H_2SO_4.

3.2.3.2 Chloride analysis

Chloride content was also determined by titration. However, this was done using a precipitation titration procedure where 1mL of K_2CrO_4 was added to each alkalinity-titrated sample. The resulting mixture was then titrated with 0.02 N $AgNO_3$ until a slight reddish precipitate of silver chromate (Ag_2CrO_4) was formed. The chloride content was calculated using equation (3.2).

$$\text{Chloride (me/L)} = 1000\text{x N x (mL (AgNO3)} - \text{mL (blank)} \quad (3.2)$$

where N as in 1 above is the normality of the titrant.

3.2.3.3 Analysis of minerals in irrigation water

Macro and micro plant nutrient element analysis of irrigation water samples were conducted using a mixture of 1mL of 1% (v/v) HNO_3 and 1mL of 0.1 % (v/v) lanthanum chloride ($LaCl_3$) in 500mL of irrigation water sample. The HNO_3 and $LaCl_3$ were added to the irrigation water samples to preserve and release the elements in the irrigation water. The resulting mixture was filtered using a 99mm Whatman No1 qualitative filter paper. Five millilitres of the filtrate was then transferred into a 25mL volumetric flask and diluted and made up to volume with deionized water. The elements in the diluted sample were measured and quantified using Inductive Coupled Plasma−Optical Emission Spectroscopy, ICP-OES (Agilent, 5800 ICP-OES, SA).

3.2.4 Soil analysis

3.2.4.1 Moisture content

One (1) gram of the soil sample was weighed into a pre-dried weighing dish and placed in a convection oven set at 105°C and dried for two hours and thereafter allowed to cool to room temperature in a desiccator. The dish containing the oven-dried sample was weighed to the nearest 0.1g and the mass recorded. The above steps were repeated until a constant weight was achieved. The percentage moisture was calculated using equation 3.3.

$$\text{Moisture (\%)} = \frac{\text{Mw}}{\text{Ms}} \text{x } 100 \quad (3.3)$$

where Mw is the mass of water in soil = (Wet mass of soil- dry mass of soil), and Ms, is dry mass of soil = (Wet mass of soil -mass of water in the soil, Mw).

3.2.4.2 Soil pH, EC and Particle size analysis (texture)

A soil sample was mixed with deionized water at 1:2.5 ratio and agitated using a shaker (Labcon, Durban) for 1 hour. The suspension was then filtered using Whatman No. 1 filter paper prior to analysis. The pH and EC electrodes were immersed into the filtrate for 10 minutes at 24 °C to measure the pH and EC.

For soil texture analysis, the soil sample was air dried at room temperature for 96 hours, ground and sieved through a 2mm sieve. About 20g of soil sample was weighed into a 1L beaker and wetted with small amount of deionized water. A 30mL of 30 % hydrogen peroxide (H_2O_2) was added to the sample and allowed to settle at room temperature for 5 minutes. The sample contents were placed in a water bath at 100 °C and allowed to boil to remove any unreacted H_2O_2. A 20mL of dispersing agent was added to the mixture. The dispersing agent was prepared by mixing 20mL of 2% sodium hydroxide (NaOH) with 10mL of 10% sodium hexametaphosphate $(NaPO_3)_6$ and stirred for 10 minutes. The sample was decanted into 1L polyethylene measuring cylinder and filled up to the mark with deionized water and allowed to settle overnight. The following day, the soil sample was brought into suspension by applying 40 firm strokes (up and down) using a plunger. Sand and coarse silt were sampled at 100mm below the surface with the pipette using a 20mL glass pipette and the sample was discharged into the pre-weighed beaker. This was followed by sampling the fine silt at 75mm below the surface of the suspension in the cylinder with the pipette, representing the clay content and the sample was discharged into the pre-weighed beaker. Sample beakers were placed in an oven at 105°C to dry overnight. Texture class was determined using equation 3.4, 3.5, 3.6 and 3.7.

$$\%\ \text{Silt and Clay} = \left(\left(\frac{\text{mass containing dried silt} - \text{mass of empty beaker} - \text{blank}}{\text{mass of the original sample}}\right)(\text{moisture})\right) \quad (3.4)$$

$$\%\ \text{Clay} = \left(\left(\frac{\text{mass containing dried clay} - \text{mass of empty beaker} - \text{blank}}{\text{mass of the original sample}}\right)(\text{moisture})\right) \quad (3.5)$$

$$\%\ \text{Silt} = (\%\ \text{Silt} + \text{Clay}) - \text{Clay} \quad (3.6)$$

$$\%\ \text{Sand} = 100 - (\%\ \text{Silt} + \text{Clay}) \quad (3.7)$$

3.2.4.3 Total N and elemental analysis

3.2.4.1 Total N and elemental analysis

For total N analysis, 1g of the soil sample was weighed into a ceramic boat and 0.5g of vanadium pentoxide was added as a combustion catalyst and placed into the ceramic horizontal furnace at 1100 °C with an autoloader and the percentage of nitrogen was measured. Samples were combusted in an induction furnace in the presence of oxygen to form water, carbon dioxide, sulfur dioxide, nitrogen oxides and nitrogen. Carbon dioxide and sulfur dioxide were removed, and nitrogen oxides was reduced to nitrogen. Total nitrogen was then measured using a thermal conductivity detector. Nitrogen-to-protein conversion factor was then used to calculate the total (crude) protein content of the sample.

For analysis of potassium, sodium and calcium, a 10g of the soil sample was weighed into a polyethylene beaker and mixed with 50mL of extraction solution. The extraction solution was initially prepared by mixing 8.6mL of 37 % HCl and 0.7 mL of 99.7 % H_2SO_4 and made-up to 1L with deionised water. The mixture was agitated for one hour at room temperature using a shaker (Labcon, Durban). The mixture was then filtered using Whatman No. 1 filter paper prior to ICP-OES analysis. For quantification, ICP was calibrated with the respective elements over the concentration range of 1-100 mg L^{-1}. In this study, the extraction solution was used as blank.

Preparative work was slightly altered to allow for the analysis of other metals (P, Zn, Cu and Mn). In this case, a 2.5g soil sample was mixed with 25mL of the extraction solution. The extraction solution was prepared by mixing 0.25M NH_4CO_3, 0.01 M Na_2EDTA, 0.01 M NH_4F and 0.05 g L^{-1} Superfloc (N100). The pH was adjusted to 8 with 1 N NaOH. The mixture was agitated for 10 minutes at room temperature using a shaker (Labcon, Durban) and then filtered using Whatman No. 4, followed by a 4 times dilution. The resulting solution was analyzed on the ICP-OES.

3.2.5 Nutrient composition of maize

3.2.5.1 Protein and fat analysis

The LECO Truspec Nitrogen Analyser (LECO Corporation, Michigan, USA) was employed to measure the content of protein in the samples using Official Analytical Chemists (AOAC) Official Method 990.03. The measurements were conducted in triplicate. The analysis was

done by placing each maize ground sample into a combustion chamber at 1100°C with an autoloader and the percentage of protein was calculated using equation (3.8) as described in another study (AOAC 2005).

$$\%\ \text{crude protein} = \%\ \text{N} \times 6.25 \quad (3.8)$$

where %N is the amount of nitrogen present in the sample.

The Büchi 810 Soxhlet Fat extractor (Büchi, Flawil, Switzerland) was used for the determination of the fat content in the samples with petroleum ether as the extracting solvent. Triplicate analyses were conducted following the AOAC Official Method 920.39 and the percentage of crude fat was determined as explained in equation (3.9) (AOAC 2005).

$$\%\ \text{Crude fat} = \frac{\text{beaker+fat}-\text{beaker}}{\text{sample mass}} \times 100 \quad (3.9)$$

3.2.5.2 Analysis of Fibre

The sample (0.5g) was added into a scintered glass crucible. The marble/buffer beads and 50mL of neutral detergent solution (NDS) (50mL) were added to the glass crucible holder. The NDS was prepared with 124g ethylene diamine tetra-acetic acid, 45.3 g disodium tetraborate, 200g sodium lauryl sulphate, 67mL 2-ethoxy ethanol and 30.4 g disodium hydrogen phosphate. The crucible containing the sample was placed in a glass crucible holder which was thereafter placed into a digestion block set at 110 °C. A 1mL of termamyl (α-amylase) was then added and the container covered with stoppers for 70 minutes. Afterwards, the glass crucible was removed and placed on a draining rack to remove the suspension. The filtration unit connected to the vacuum system was used to suction the samples which were washed three times with boiling water. The sample and sides of the crucible were then rinsed with acetone and the samples were placed in a drying oven at 105 °C for 4 hours. The samples were then cooled in a desiccator, the crucible was weighed and the NDF of the sample was calculated using equation (3.10).

$$\%\ \text{NDF} = \frac{(\text{crucible+dry residue})-(\text{crucible+ash})}{\text{sample mass}} \times 100 \quad (3.10)$$

3.2.5.3 Total mineral matter (ash) and elemental analysis

Ash was determined using the AOAC Method 942.05 [14]. The samples were weighed and placed in a furnace at 550 °C for 24 h. After the volatilisation of the organic matter from the samples, the elemental salts that remained as a residue of ash in the crucibles were calculated using equation (3.11).

$$\% \text{ ash} = \frac{(\text{mass of the sample+crucible after ashing}) - (\text{mass of pre-dried crucible})}{(\text{mass of sample+crucible}) - (\text{mass of pre-dried crucible})} \times 100 \quad (3.11)$$

The mineral elements were analysed using the Agricultural Laboratory Association of Southern Africa (ALASA) Method (Palic et al. 1998). Samples were ashed at 550 °C in a furnace for 12 hours. The samples were dissolved in HCl followed by addition of HNO_3 and then analysed using the ICP–OES.

3.2.5.4 Analysis of starch

The starch content was determined by weighing 1g of the sample into a test tube and 5mL of 80 % ethanol added to the sample in the test tube. The mixture was vortexed and incubated at 80 °C for 30 minutes to completely evaporate the ethanol. Then 10mL of acetate buffer was added to the mixture in the test tube followed by 200µL of Termamyl α amylase enzyme. The mixture was vortexed and incubated for 30 minutes at 90 °C after which the mixture was allowed to cool. After cooling, 200µL of amyl glucosidase was added to the mixture in the test tube and gently shaken followed by incubation at 60 °C for 8 hours. The sample was diluted in a 200mL volumetric flask using deionized water and filtered through Whatman No. 1 filter paper. Five (5) milliliters of copper reagent was added to 3 mL of the filtrate in the test tube followed by addition of the arsernomolybdate reagent (5 mL). The test tube was then shaken and allowed to stand for 90 minutes. The starch content of the sample was determined by UV absorption at 750nm wavelength and the starch content calculated using equation (3.12).

$$\% \text{ Starch} = \frac{0.4555 \times \text{Absorbance of sample} \times 0.9}{\text{sample weight} \times \text{ Absorbance of glucose standard}} \quad (3.12)$$

where the factors 0.4555 = starch to glucose factor and 0.9 = glucose to starch factor

3.2.6 Analysis of Antioxidants

About 30mg of samples was weighed and transferred into a 1 mL falcon tube and 400µL distilled water was added. The samples were boiled in a water bath at 100 °C for 30 minutes followed by addition of extraction buffer (2mL). The 100mL buffer was made by mixing 2mL distilled water, 94.8mL of 95 % EtOH and 3.2mL of 37 % HCl. The sample solutions were vortexed and agitated overnight on a shaker (Labcon, Durban). The samples were centrifuged at 13,000rpm for 15 minutes and the first supernatants were collected. One (1) milliliter extraction buffer was added to each sample pellet, vortexed and agitated for two hours. The samples were then centrifuged at 13,000rpm for 15 minutes and the supernatant was collected and mixed with the first one. A 3 mL of supernatant collected from each sample was centrifuged again at 13,000rpm for 30 minutes. The absorbance was measured spectrophotometrically (Cary 50, Germany) at 530nm, at 350nm and 280nm respectively for anthocyanins, flavonols and phenolic acids, using the extraction buffer as blank. The anthocyanin content was calculated as cyanidin 3-glucoside equivalents [molar extinction coefficient (ε) 26,900 Lm^{-1} mol^{-1}, MW 484.82], the amounts of flavonols and phenolic acids were calculated as quercetin 3-glucoside (ε 21,877 Lm^{-1} mol^{-1}, M.W 464.38) and ferulic acid (ε 14,700 Lm^{-1} mol^{-1}, MW 194.18) equivalents (Lago et al. 2015).

3.2.7 Methods validation and statistical analysis

The accuracy of the methods was validated by analysing certified reference materials (CRMs). Maize flour (FCNC21-AFE16) was purchased from Fera Science proficiency testing Ltd, soil CRM and water CRM were purchased from Merck (Pty) Ltd, South Africa.

The Statistical Package for Social Science (SPSS version 25.0 SPSS Inc, Chicago, IL, USA) was used for the analysis data. The standard deviations and mean values of the irrigation water, soil and maize samples were calculated for all replicate measurements. The significant differences in soil, irrigation water and maize samples were determined using Kruskal Wallis non-parametric test. Where significant differences in results were recorded, the Mann-Whitney U test was employed to determine the specific differences. Significance in the results was measured at the 5 % level.

3.3 Results and discussion

3.3.1 Irrigation water

Water alkalinity/sodicity, salinity and presence of toxic ions are indicators of the quality of irrigation water. The pH, electrical conductivity, and total dissolved solids classify the concentration of soluble salts. In this study, the pH of irrigation was found to be 7.5, indicating weak alkalinity. Irrigation water pH ranging from 6-8 is considered suitable for irrigation purposes **(**Husien et al. 2017). The measured electrical conductivity was 1.81 µS m^{-1} showing low content of soluble salts and thus high purity of water for irrigation purposes. The recommended electrical conductivity in irrigation water is <2.50 µS m^{-1} (Zaman et al. 2018). Due to the low content of soluble salts, irrigation water class was found to be C1-S1, which meant low salinity and low sodicity content. Previous studies reported 7.79 pH, 0.49 µS m^{-1} EC and 180 mg L^{-1} TDS (Zaman et al. 2018). The sodium (Na) hazard in water, represented by the sodium adsorption ratio (SAR) was 2, indicating low sodium toxicity to crop. The CRM values of water (Table 3.1) were not only found to be statistically different compared to certified values, but also confirmed the validity of the method used for analysis.

Table 3.1: **Irrigation water analysis and method validation**

Parameter	Water CRM (certified)	Water CRM (obtained)	Irrigation Water sample	***P value***
pH	6.12	6.89	7.50	***0.042***
EC (µS m^{-1})	7	9	1.81	***0.012***
TDS (mg L^{-1})	41	50	113.00	***0.021***
Mg (mg L^{-1})	2.78	3.45	0.45	***0.032***
Na (mg L^{-1})	0.54	0.69	0.05	***0.045***
K (mg L^{-1})	0.41	0.64	0.25	***0.011***
Zn (mg L^{-1})	0.06	0.01	0.54	***0.044***
Mn (mg L^{-1})	0.06	0.02	0.11	***0.031***
P (mg L^{-1})	NA	0.01	0.08	***0.009***
N (mg L^{-1})	-	-	-	
SAR			2.05	
Water class			C1-S1	

NA-not analysed

3.3.2 Soil quality

The soil and irrigation water quality can be used to predict crop quality and yield. These results represent the average of four sites studied. Soil quality is equated with organic carbon, fertility, and total nitrogen content. In this study, soil organic carbon was 4.4 %, which is in the range of organic carbon in agricultural soil (4-6 %), (Malobane et al. 2020). The concentration of nitrogen (2700 mg kg^{-1}) was found to be above the minimum total nitrogen content considered adequate in agricultural soil, which is 2000 mg kg^{-1} (Hofman and Van 2004). This is the most critical element obtained by plants from the soil and it is a constraint in crop growth. Organic carbon to nitrogen (C:N) ratio significantly attributes to microbial biomass which influence nutrients transformation and soil ability to store and recycle energy and nutrients (Ravindran and Yang 2015). The concentrations of P and K were found to be 19 mg kg^{-1} and 222 mg kg^{-1} (Table 2), respectively. Phosphorus is an important element present in any living cell and responsible for seed germination and promotes root growth while K plays a significant role in physiological process of the plant and resistance of the plant from the diseases (Macabiog et al. 2020). Soil profile had high total cation of 11.93 cmol kg^{-1}, consequently, high cation exchange capacity (CEC), which determines the nutrient ion retention capacity movement of nutrients through the soil profile. This result is indicative of high fertility status of the soil associated with the high clay content (56.41%) (Hendershot et al. 1993). Clay soil is more fertile due to its ability to retain nutrients. Due to the good quality of the soil profile in this study (Table 3.3), agricultural fertilizers were not applied for maize growth. The statistics analysis showed that the results obtained for the certified reference material are significantly different ($p<0.05$) from those of the certified values.

Table 3.2: Soil analysis and method validation

Parameter	Soil CRM (certified)	Soil CRM (found)	Soil sample	***P value***
pH	6.50	6.10	5.95	***0.034***
EC (µS m^{-1})	349.3	282.5	476.1	***0.033***
Mg (mg kg^{-1})	284	274	283	***0.027***
Na (mg kg^{-1})	0.34	0.12	0.06	***0.051***
K (mg kg^{-1})	289	314	222	***0.045***
Zn (mg kg^{-1})	3.5	3.90	27.7	***0.036***
Mn (mg kg^{-1})	66	75.00	23.0	***0.021***

P (mg kg^{-1})	10	11.0	19	***0.030***
N (mg kg^{-1})	NA	6400	2700	***0.043***
Texture class			Clay	

NA-not analysed.

Table 3.3: **Mechanical strength of the soil profile**

Sample	Density	Exchangeable acidity cmol kg^{-1}	Total cation cmol kg^{-1}	Acid saturation (%)	Organic carbon (%)	Clay (%)	Sand (%)	Silt (%)
Soil	1.03	0.11	11.93	1	4.4	56.41	40.61	2.98

3.3.3 Nutrient content of maize

The major nutrient components in maize are carbohydrates (Zondo and Mahlambi 2020). The maize nutrient content results (Table 3.4) showed that starch was present in higher amounts (58.62%), which could be due to endosperm mutant known as amylose-extender that influences growth in the amylose proportion of the starch (Paraginski et al. 2014). Protein content was 9.01 % which is comparable to 10.10 % reported by Nkosi *et al*. (2010). Protein is derived from plant cells of the crop and it is influenced by the plant's genotype and agronomic practices (Zilic et al 2011). The maize flour CRM values obtained from the analysis in this study were comparable to those of certified values. The maize flour is the material that is used for quality control. The fat content (4.5 %) obtained in this study is comparable to the previously reported study in white maize, which was 4.06 % (Bathla et al. 2019). Presence of fat in maize is responsible for its flavour, texture and high palatability (Cantaluppi et al. 2017). The fat in the maize kernel is in the germ of the maize kernel, and it is genetically influenced. The macro and micro minerals obtained in this work were 2.42 % and 1.79 %, respectively which is expected as the macro and micro nutrient elements in maize are generally lower compared with other cereal grains (Bathla et al. 2019). The soil is deficient in micronutrients particularly zinc and this is a global concern (Kihara et al. 2020).

Table 3.4: **Nutrition analysis of the maize crop**

Parameter (%)	Maize flour CRM (certified)	Maize flour CRM (found)	Maize sample	***P value***
NDF	25.72	26.42	23.43	***0.032***
Fat	5.79	6.49	4.55	***0.042***
Ash		1.32	1.67	***0.021***
Protein	8.42	9.24	9.01	***0.021***
Starch	61.42	60.21	58.62	***0.032***
Total macro minerals	-	3.52	2.42	***0.012***
Total micro minerals	-	1.03	1.79	***0.030***

3.3.4 Analysis of Antioxidants

Antioxidants are important as they inhibit oxidation of free radicals in human cells, consequently, protecting the consumer from numerous degenerative diseases. The high phenolic compounds in maize found in this study (Table 3.5) directly indicate higher antioxidant strength (Lago et al. 2015). The total phenolic acid showed high quantity (100.42 mg L^{-1}) which is beneficial to the consumer since phenolic acids have cancer prevention ability. The maize CRM showed high concentration of total flavonoids and total phenolic acid compared to the maize sample, while the total anthocyanin was higher in the maize sample than the CRM. However, there was significant difference in all the concentrations obtained (p<0.05). These chemical compounds containing antioxidant properties have correlation with the biosynthetic pigments in crops (Bathla et al. 2019).

Table 3.5: **Phytochemical analysis of the maize crop**

Parameter (mg L^{-1})	Maize CRM	Maize sample	***P value***
Total anthocyanin	5.21	8.52	***0.051***
Total flavonoids	66.02	49.52	***0.048***
Total phenolic acid	112.42	100.42	***0.020***

3.4 Conclusion

In this study, soil quality, irrigation water and maize grain quality planted in the Buhle farm was evaluated. The cation of interest for irrigation water was Na^+ and the results showed that the concentration was low (0.05 mg L^{-1}) and so it did not pose any sodium hazard. The low sodium adsorption ratio of 2 corroborated the results of sodium hazard of the water. The low EC (1.81 µS m^{-1}) is indicative of a safe water of low salinity. The soil texture high in clay content (56.4%), sand (40.6%) and slit (2.89%) indicated high fertility potential of the soil profile. It was then concluded that soil quality and irrigation water quality were suitable for producing good quality maize. The results of the analysis of key nutrition parameters of maize were consistent with the good quality soil and irrigation water. The results showed that the maize contained high amounts of total anthocyanin, total flavonoids and total phenolic acid compounds. These compounds are considered essential for good health and consumers can be assured of medicinal benefit from consuming this maize.

3.5 References

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Chapter 4 Comparison of ultrasonic and QuEChERS extraction methods efficiency for the determination of herbicides residues in soil and maize grain

Abstract

In this study, the optimization and application of ultrasonic extraction and QuEChERS methods was conducted for the effective extraction of pesticides from maize and their corresponding soil samples. The characterization pesticides (Atrazine, glyphosate, 2,4-dichlorophenoxyacetic acid and mesotrione) was done with gas chromatography-flame ionization detector. Factors influencing the efficiency of the extraction methods such as the extraction solvent, extraction time, solvent volume and spiking concentration were assessed. Under the optimum experimental conditions, the recoveries of analytes ranged between 100-104% in maize grain and 91-97% in soil for ultrasonic method, while they were 94-115% in maize grain and 89-101% in soil for QuEChERS method. The repeatability, articulated as RSD values were less than 5 % for all analytes in both methods. The limits of detection and limit of quantification ranged from 0.02 – 0.15 µg L^{-1} and 0.2 – 0.5 µg L^{-1} for ultrasonic method and 0.01 – 0.23 µg L^{-1} and 0.13 – 0.8 µg L^{-1} for QuEChERS method. The lower limits of detection and quantification with higher recoveries suggests that both methods can be accurately applied for the determination of the assessed pesticides in soil and maize grain. Herbicides concentrations ranged between 0.33 - 45.10 µg L^{-1} in soil and 6.15 – 34.2 µg L^{-1} in maize grain. In all studied herbicides, maize residues detected were found to be lower compared to maize allowable maximum residue limits however, atrazine in all soil samples was found to be lower compared to soil allowable maximum residue limits. This indicates no health effect to the consumer however, the importance of continuous monitoring the herbicides residues in maize to ensure continuous food quality is indispensable to safeguard health for the consumers.

4.1 Introduction

Crops compete with lots of undesired plants that naturally grows along with and compete for carbon dioxide, space, nutrients, sunshine and water. This competition has a negative effect on crop growth and crop yield. Therefore, agrochemicals and herbicides are used to increase crop production while controlling undesired plants growth. Generally, herbicides are categorised as selective and nonselective synthesized chemicals. Selective herbicides disrupt and destroy hormones of the target weeds while nonselective disrupts and damage all growth from soil. Glyphosate is a non-selective herbicide that prevents the plants from making certain proteins that are needed for crop growth, therefore it is applied before maize has emerged. The 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine and mesotrione are selective auxin herbicides that do not affect desired crop. However, they inhibit particular target zone within the target plant physiological and/or biochemical pathway, resulting to lethal and catastrophic magnitudes (Song 2014).

In sub-Saharan Africa, South Africa has the high usage of pesticides for crops protection and crop management (Horn 2019) essential to meet increasing population demands to date and in years to come. Agricultural sector plays a significant role in food security, production and steady supply by employing herbicides and agrochemicals to achieve high crop yield in a short space of time (Burns 2012, Garabrant and Philbert 2012). This efforts consequently promotes the introduction of these compounds in the food structures of the consumer, e.g. seeds, crops and grains (Dong 2016, Karapınar 2022). Herbicides are applied on the soil and taken-up by plant roots and translocate to other plant tissues, including the edible part of the plant. These compounds build-up in the edible part of the crop in low concentration. With the aim of food quality assurance, the maximum permissible levels for different herbicides have been set by the World Health Organization (WHO) as well as Food and Agriculture Organization (FAO) (WHO 2018). Agricultural crops are important components in the diet for human, hence, crop quality assurance should be evaluated from herbicides toxicity as herbicides contains toxic chemicals that poses a risk to the human health (Islam et al. 2018).

Different preparation/extraction techniques are used for the determination of pesticides present in the matrix. Sample preparation is important for analytes extractions from different matrix followed by cleaning stage which removes impurities present in the extracts (Padrón et al. 2006). Typically used extraction techniques for solid samples includes: Soxhlet extraction

(SE), ultrasonic extraction (EU), quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction, microwave assisted extraction (MAE), while the sample clean-up is done using the solid-phase extraction (SPE) (Karapınar and Bilgiç 2022). The UE is based on the extraction of samples with an organic solvent by applying ultrasound radiation ranging from 20 kHz to 2000 kHz (Organization 2008) in an ultrasonic bath. The mechanical effect of ultrasound induces a great penetration of solvent into sample and improves mass transfer, resulting to an enhancement of analyte extraction efficiency. This can be followed by SPE clean-up which is significant for the removal of undesirable impurities leaving the analytes behind. The QuEChERS method is based on the extraction with solvent at room temperature and separation by addition of salt followed by dispersive solid phase extraction (d-SPE) clean-up step to promote pure extract (Anastassiades et al. 2003). These methods are studied to assess and compare their sensitivity and accuracy for the determination of the studied residues. In this current study, ultrasonic and QuEChERS extraction methods were optimised and applied to assess the uptake of 2,4-D, atrazine, mesotrione and glyphosate by roots from their corresponding soil and their translocation to the edible maize grain. Their efficiency to extract the studied herbicides was also compared.

4.2 Methods and materials

4.2.1 Chemicals, and analytical reagents

The 2.4-D (97%), atrazine (97.4%), glyphosate (98.5%) and mesotrione (97.5%) were purchased from Sigma Aldrich (Durban, South Africa). All used solvents were of HPLC grade: acetonitrile (99.9%), acetone (99.8%), dichloromethane (99.8%), ethyl acetate (99.9%), acetic acid (<98%), isopropyl alcohol (98%) and methanol (99.9%) and were bought from Merck (Durban, South Africa). Oasis hydrophilic-lipophilic balance (HLB) cartridges, (60 mg, 3 mL) supplied by Waters (Milford, USA) were used as solid phase extraction sorbent.

4.2.2 Instrumentation

The extraction of pesticides from soil and maize grain was conducted using ultrasonic bath purchased from Prestige (Durban, South Africa). The solid phase extraction (SPE) vacuum manifolds obtained from Sigma Aldrich (Steinheim, Germany) was used for the clean-up of maize and soil extracts from ultrasonic and Soxhlet extraction. The vacuum pump connected to the SPE manifold was purchased from Edwards (Munic, Germany). The lyophilizer from Antech Scientific (Durban, South Africa) was employed to freeze dry the maize samples. The

chromatographic studies were conducted using a Bruker scion 436 GC from Gibbs Technologies (Durban, South Africa) coupled with a flame ionization detector (FID). The separation of herbicides was done on a capillary column, VF-5ms (a length of 30 m × 0.25 mm i.d. × 0.25 m film thickness) supplied by Gibbs Technologies. The injector temperature and detector temperatures were both 250ºC. The temperature program was as follows: initial temperature of 60ºC and held for 1 min, a rate of 30ºC min^{-1} to 150ºC held for 4 min, followed by 15ºC to 250ºC and held for 5 min. The nitrogen was used as a carrier gas and the injection was split/splitless with a purge time of 0.10 min and split of 1:50. The FID was fed with hydrogen (30 mL min^{-1}) and synthetic air (300 mL min^{-1}) and the samples injection volume was 1µL (de Souza Pinheiro and de Andrade 2009)

4.2.3 Working standard solutions

The working standard solutions (0-100 µg L^{-1}) were prepared form 100 mg L^{-1} stock solution in 95% n-hexane containing a mixture of herbicides (2.4-D, atrazine, glyphosate and mesotrione). These were then used for the calibration of gas chromatography within the concentration range 0-100 µg L^{-1} and for spiking samples used for validation studies. The calibration standards were stored at 4°C until analyses were conducted.

4.2.4 Study area

Soil and maize samples were collected from four maize sites where maize production is conducted in Buhle experimental field. The study site is located in the Howick, province of KwaZulu-Natal and district of UMgungundlovu where studies on maize, tubers and agronomic crops are conducted for the KwaZulu-Natal community. The herbicides such as atrazine, glyphosate, 2.4-D and mesotrione are used for weed management. It was therefore important to assess the uptake of these herbicides in the study area to ensure crop quality hence, consumer safety. The exact sampling location can be found through the usage of the Global Positioning System (GPS), represented by the co-ordinates -29.524161, 30.247682. The sampling location is known for its high temperatures in summer (wet season) which normally range from 21°C to 38°C, while these usually drop in winter (dry season) ranging from 10°C-28°C. On average, precipitation is approximately 569 mm annually. For the purpose of the present study, the samples were collected in the month of March 2021 from other maize production sites in the farm for the extraction optimization purposes. The 12 maize crops were sampled from the maize stalk, and their corresponding soils were sampled on the topsoil surface of the soil (0-5,

5-10 cm) and subsoil 10-15 cm surface layer in all studied sites. The soil and maize samples were stored in polyethylene bags and transported to the laboratory.

4.2.5 Sample preparation

Soil samples were air dried for 48 hours, pulverized and passed through 2.0 mm sieve. The maize grains were freeze dried on Lyophilizer and milled into fine powder and passed through 100 mm screen mesh. Samples were then stored at room temperature until analysis were conducted. The herbicides were extracted using ultrasonic extraction followed by SPE clean-up and QuEChERS extraction followed by d-SPE clean up.

4.2.5.1 Optimization of UE

The ultrasonic extraction method published by Asensio-Ramos et al. (2009) was adopted with further optimization to improve the extraction efficiency for all the herbicides studied. In this study the extraction time, extraction solvent, extraction solvent volume and spike concentration were parameters studied to assess their effect on herbicides recoveries from the maize and soil The extraction time was studied to evaluate the optimum sonication time needed for complete extraction without reaching the degradation point. The extraction time studied were 15, 30, 45 and 60 minutes. Different extraction solvents (methanol, acetonitrile, and mixture of methanol: dichloromethane) were studied to evaluate the solubility ability of the studied analytes. The investigated solvent volumes were 15 mL, 25 mL, and 40 mL. Solvent volume is significant for sufficient interaction of sample mass with solvent for complete and effective extraction and to achieve high recoveries.

Under optimum conditions, a 1 g sample (soil and maize cob) was wetted with 5 mL of distilled water to hydrate the active sites. The samples mixture was shaken up with ultrasonic waves for 15 minutes followed by the addition of 25 mL of dichloromethane (DCM): methanol (MeOH) (1:1 v/v) solvent and further ultrasonicated for 15 minutes at 40°C. The sample and supernatant liquid were separated by centrifuging the mixture for 5 minutes at 3000 rpm. The supernatant liquid was reduced to 1 mL by rota-vapor. Then, 1 mL of the extract was subjected to clean up step by SPE.

4.2.5.2 SPE cleaning method

A batch of sample was subjected to cleaning by SPE while the other batch was not cleaned which was done to investigate the cleaning effect. The SPE sorbent used was Oasis HLB (60 mg, 3mL) cartridge. A 500 µL of methanol was used to condition the SPE sorbent for effective interaction with the analyte. This was followed by addition of 500 µL of water to equilibrate the SPE sorbent. The extracted sample was then loaded into the SPE sorbent to trap the analyte on the SPE sorbent. A 500 µL of 5% methanol was used to wash the impurities and the analyte trapped on the sorbent were eluted with 500 µL methanol (Bernardi et al. 2016).

4.2.5.3 Optimization of QuEChERS

The method published by Petrarca et al. (2016) was used with further optimization. The extraction solvents, solvent volume and vortexing time were parameters studied for their effect on recoveries. Different extraction solvents including acetonitrile, methanol, acetone and isopropanol. The investigated solvent volumes were 7 mL, 15 mL, and 30 mL. The vortex agitation time was investigated to allow the homogeneous distribution of the analyte to the axtractant where 0.5 minutes, 1 minute and 3 minutes were investigated.

Under optimum conditions, a 15 g sample (soil and maize cob) was weighed into 50 mL falcon tube. A 15 mL of acetronitrile acidified with 1% acetic acid (v/v) was added and mixed with the vortex for 1 minutes. Then, 1.5 g of anhydrous sodium acetate (NaOAc) and 6.0 g of anhydrous magnesium sulphate ($MgSO_4$) were added to the tube, and this mixture was vortexed for 1 minutes followed by centrifugation at 4000 rpm for 5 minutes at room temperature. This was followed by d-SPE cleaning. An 8 mL of QuEChERS extract, 50 mg of C18-bonded silica (particle size 55–105 µm) sorbent, 50 mg of primary secondary amine (PSA; particle size 50 mL) sorbent and 150 mg of $MgSO_4$ were added to a 15 mL polypropylene centrifuge tube, vortexed for 30 seconds, and then the mixture was centrifuged at 5000 rpm for 5 minutes at room temperature. The supernatant was collected and 1 µL was injected into a GC–FID system.

4.2.6 Statistical analysis

The residue data was analyzed using the Statistical Package for Social Science (SPSS version 25.0 SPSS Inc, Chicago, IL, USA). For all repeat measurements, the mean values and standard deviations of all samples were computed and the Kruskal Wallis nonparametric test was used to detect the significant differences in the residue concentration of samples. The Mann-Whitney

U test was used to determine the specific differences when the significant difference was identified at a 95% confidence limit.

4.3 RESULTS and DISCUSSION

4.3.1 Optimization of EU method

4.3.1.1 Selection of the extraction solvent

The effectiveness of the UE extraction solvent was studied using, acetonitrile (ACN), methanol, mixture of methanol: ethyl acetate (EA) (1:1) and mixture of methanol: dichloromethane (1:1). The mixture of methanol: dichloromethane (1:1) gave higher recoveries ranging from 99.9-102% and 95-98% in cleaned and uncleaned maize, 89-97% and 85-94% in cleaned and uncleaned soil, respectively (Figure 4.1 and 4.2). This point out that less polar and more polar analytes solubilized better in the methanol: dichloromethane mixture (as methanol is polar while dichloromethane is nonpolar), resulting in high extraction efficiency for the target analytes from the sample (Farajzadeh et al. 2019). This is due to that the polarity of the extraction solvent influence the interaction with the sample consequently allow complete elucidation of the target analyte from the matrix, hence high recoveries (Annegowda et al. 2012). Hence, methanol: dichloromethane (1:1) was selected as the optimum extractant for UE in this study. Comparable recoveries were obtained before and after application of the clean-up stage, indicating that it had no effect. This revealed that the target analytes solubilized well in the extraction mixture carrying no impurities. It also confirmed that the target analyte can be analysed without clean-up step, hence it was not considered in the other extractions.

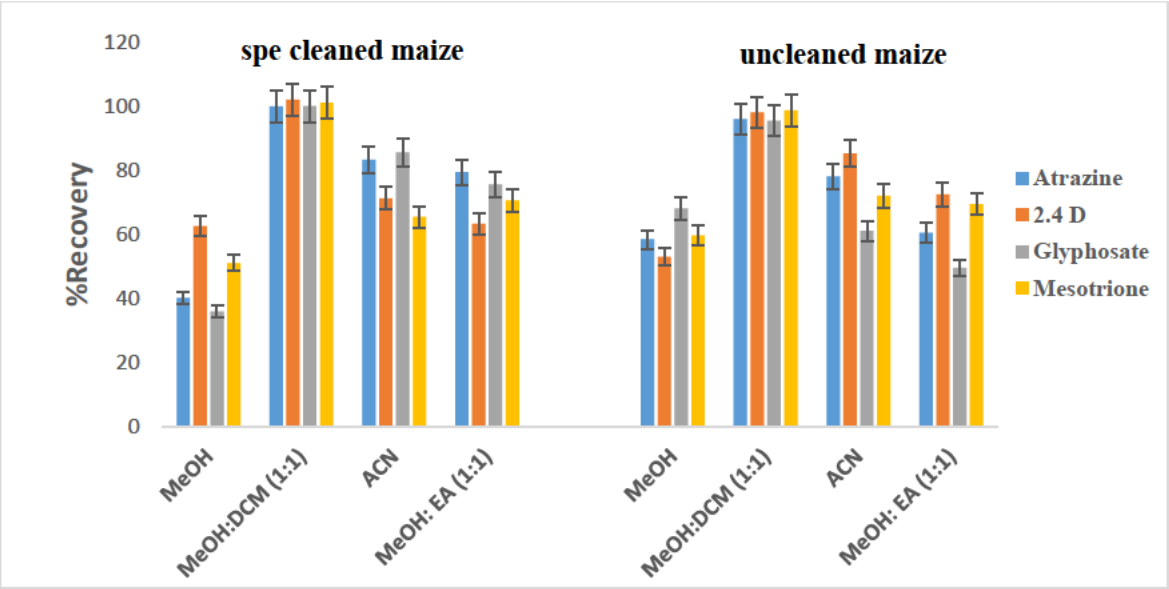

Figure 4.1: Effect of extraction solvents using UE with SPE clean-up and UE without SPE clean-up methods on the pesticides recoveries from maize

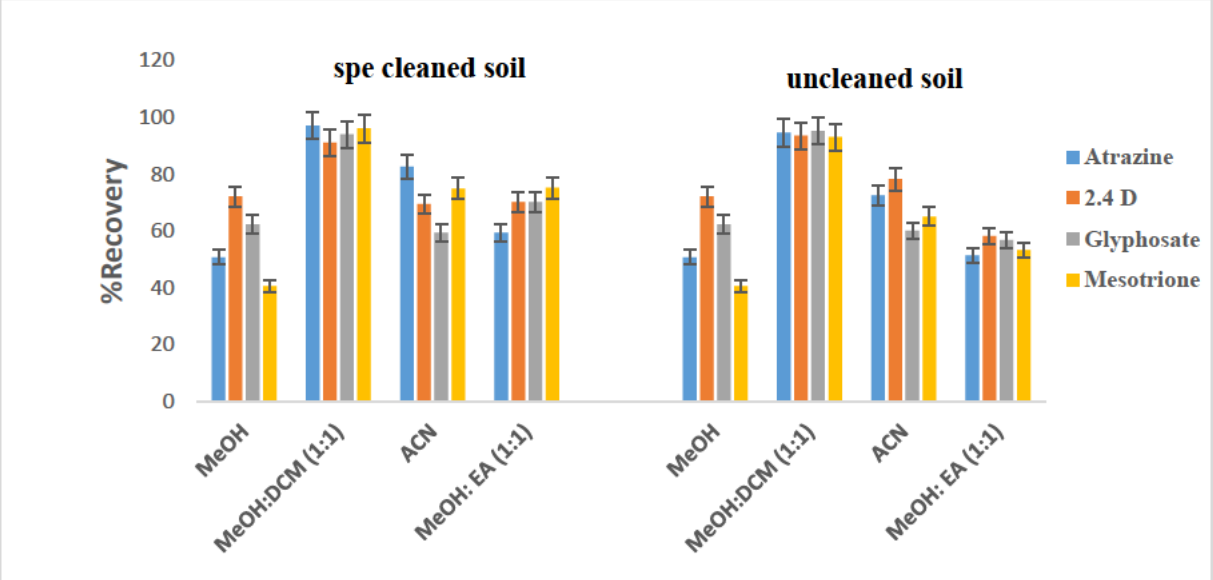

Figure 4.2: Effect of extraction solvents using UE with SPE clean-up and UE without SPE clean-up methods on the pesticides recoveries from soil

4.3.1.2 Effect of the extraction solvent volume

The extraction solvent volume is the element that influence extraction efficiency of the target analytes from the maize and soil samples. To study this factor, different volumes (15, 25 and 40 mL) of methanol: DCM (1:1) were examined. The obtained results (Figure 4.3) showed an increased in extraction efficiency with the increase in extraction solvent volume up to 25 mL and then reduced by further increasing solvent volume. This might be due to the fact that, at

low volume of the extraction solvent (less than 25 mL), extraction of analyte was incomplete hence, due to low mass transfer which led to low recoveries (Silva et al. 2005). Meanwhile, at more than 25 mL extraction volume, the dilution of target analyte surfaced and led to reduced extraction efficiency. The recoveries obtained with 25 mL ranged from 95-98% in maize and 85-94% in soil. This resulted to 25 mL methanol: DCM (1:1) being selected as an optimum volume. The increase in recoveries with an increase in extraction solvent volume followed by a decrease with further increasing volume is in agreement with the study reported by (Farajzadeh et al. 2019).

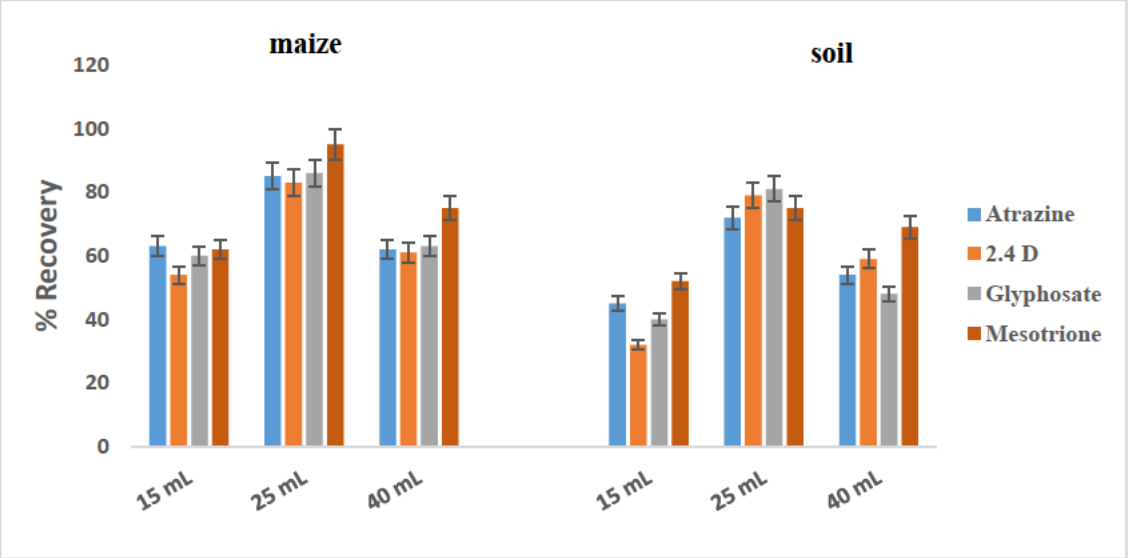

Figure 4.3: Effect of extraction solvent volume on the recoveries of herbicides from maize and soil samples

4.3.1.3 Effect of extraction time

The extraction time is an important parameter as it impacts the quantity of the target analyte recovered. The shorter extraction time prevents the complete extraction process while longer extraction time results into analyte degradation. The effect of extraction time on the analyte recoveries was investigated by employing 15, 30 and 60 minutes. The recoveries increased from 15 to 30 minutes and then decreased with further increasing time to 60 minutes (Figure 4.4) probably due to the degradation of the target analyte. This indicates that 30 minutes extraction time allowed enough diffusion of the solvent into the sample matrices while breaking matrices membranes and elucidating target analytes. The 30 minutes yielded 95-98% on maize and 85-94% on soil samples and consequently selected as the optimum extraction time for the UE method. Babić et al. (1998), also reported the increase in recoveries with an increase in

extraction time however, prolonged sonication time caused degradation of compounds resulting to decreased recoveries.

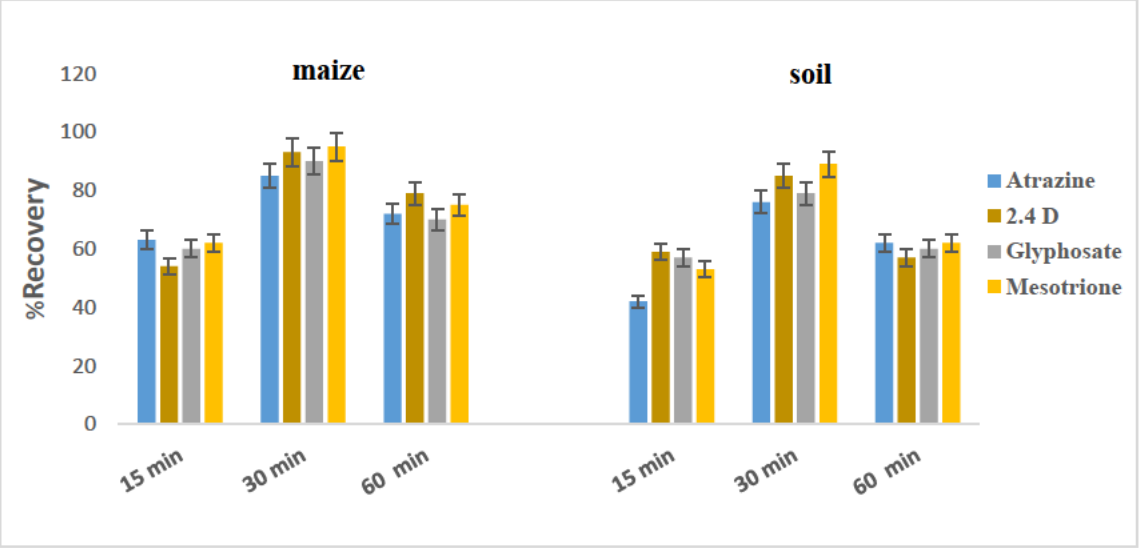

Figure 4.4: Effect of extraction time on the recoveries of herbicides from maize and soil samples

4.3.1.4 Effect of spike concentration

The validity of the studied method was assessed by spiking both maize and soil samples with different spiking concentration 60 and 100 µg L^{-1}. Considering the obtained results (Figure 4.5), the optimum conditions showed recoveries ≥ 99 on both different spiking concentrations in maize and soil samples which determined well developed method.

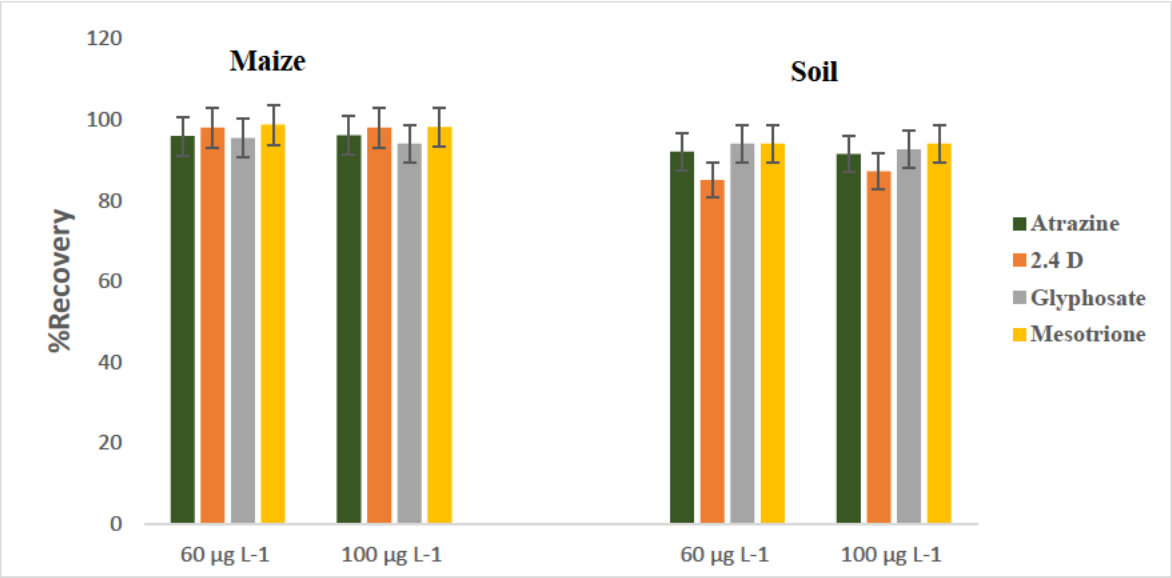

Figure 4.5: Effect of the spiking concentration e on herbicides recoveries from maize and soil samples

4.3.2 Optimization of QuEChERS method

4.3.2.1 Effect of extraction solvent

The effect of extraction solvent on the recovery of analytes were investigated using methanol, acidified acetonitrile, isopropanol and acetone. The Acidified acetonitrile was found to be the optimum solvent with higher recoveries ranging from 94-115% and 92-101% in maize and soil, respectively (Figure 4.6). The acidified acetonitrile showed the ability to extract the studied herbicides with their different polarities nature. This is due to that the addition of NaOAc together with 1% acetic acid allows the buffering of the medium which improves acetonitrile extraction ability. Also, the use of $MgSO_4$ salt during the partitioning step reduced the volume of the aqueous phase by hydration and saturates the molecular spaces with the aqueous solvent, resulting in an increase solubility the analytes (Anastassiades et al. 2003).

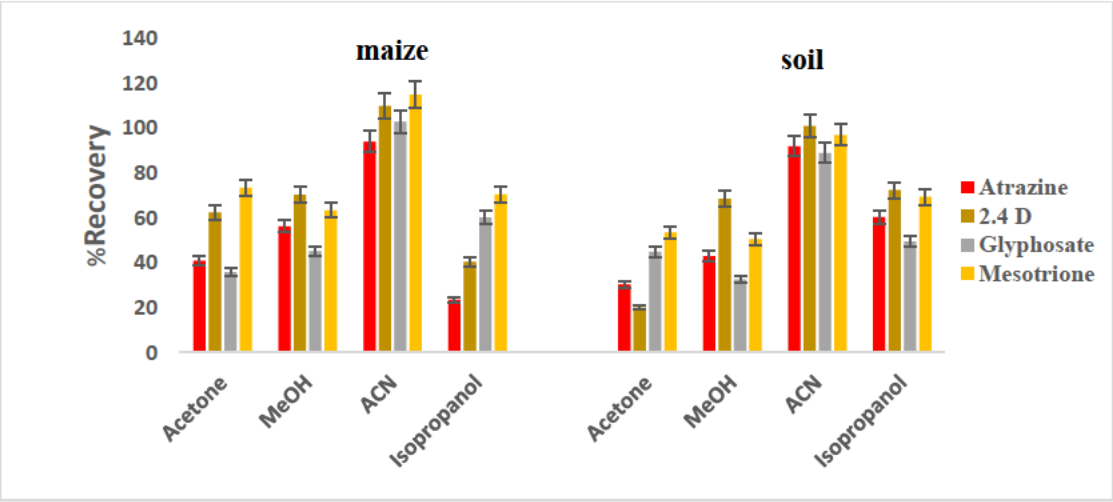

Figure 4.6: Effect of extraction solvents using QuEChERS with dSPE clean-up method on the pesticides recoveries from maize and soil

4.3.2.2 Effect of extraction solvent volume

The influence of extraction solvent volume was examined using 7, 15 and 30 mL. The obtained results (Figure 4.7), showed that 7 mL gave lower recoveries due to incomplete mass transfer of the analyte from the matrix resulting from minimum solvent volume. The increase in volume from 7 mL to 15 mL promoted complete mass transfer of the analyte hence, increase in extraction recoveries. The recoveries obtained for 15 mL ranged from 94-115% and 92-101% in maize and soil respectively. Petrarca et al. (2016) also reported 15 mL as optimum volume for the extraction of pesticides using QuChERS method in baby food samples.

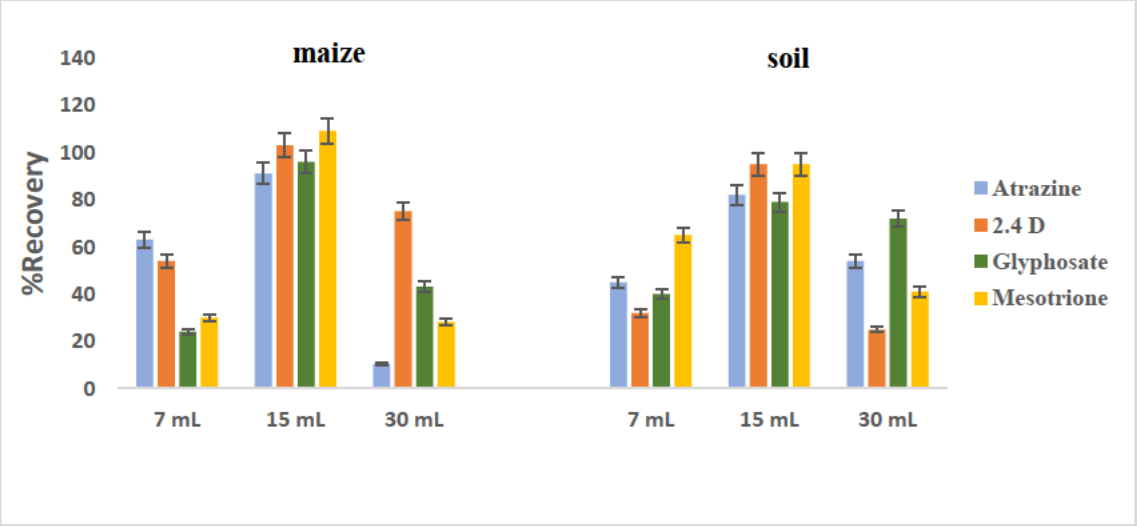

Figure 4.7: Effect of extraction solvent volume on the recoveries of herbicides from maize and soil samples

4.3.2.3 Effect of vortexing time

Vortexing time (0.5, 1 and 3 minutes) was studied to assess the agitation effect on the recoveries. The obtained results (Figure 4.8), showed no effect of the agitating time on the extraction recoveries of the target analyte which agrees with Farajzadeh and co-workers finding (Farajzadeh et al. 2019). These results indicated that all the vortexing times used were adequate to allow interaction and transportation of the analyte bound from the matrix into the extraction solvent. Thus, 1 minutes was selected as a vortexing time for this method.

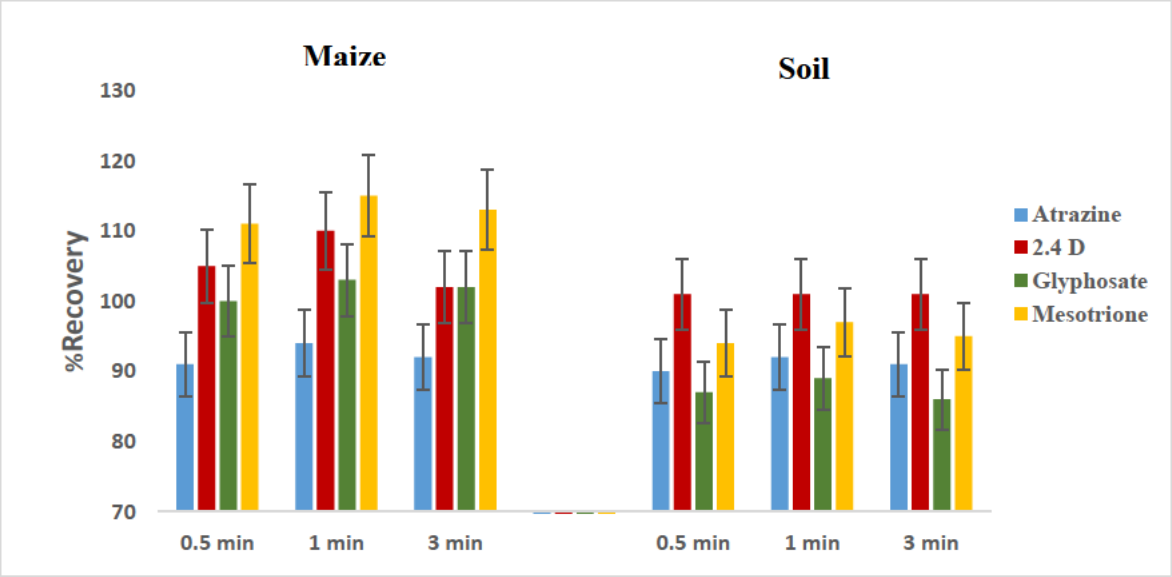

Figure 4.8: Effect of vortexing time on the recoveries of herbicides from maize and soil samples

4.3.3 Method validation

The quantitative characteristics of the present method such as relative standard deviation (RSD), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ) and recoveries were evaluated. The obtained results are listed in Table 1. The linearity of all calibration curves showed a good correlation coefficient (R^2) ≥0.995. The UE LODs and LOQs ranged from 0.02 – 0.15 µg L^{-1} and 0.2 - 0.5 µg L^{-1}, respectively (Table 1) while QuEChERS LODs and LOQs ranged from 0.01 – 0.23 µg L^{-1} and 0.13 – 0.8 µg L^{-1}, respectively (Table 1). The obtained LODs and LOQs indicated that that the developed methods are sensitive and hence will be able to detect the target analyte at concentration of the real sample, also this indicate good sensitivity of both methods. The UE maize and soil analytes recoveries were 100-104% and 91-97 %, respectively, while QuEChERS maize and soil analytes recoveries were 94-115% and 92-101%, respectively with the repeatability, articulated as RSD

values of less than 6% for all analyte which indicated good precision of the introduced methods. Zou et al. (2015), assessed the gramoxone in four edible vegetables (cabbage, lettuce, spinach and Chinese cabbage) with ultrasonic extraction and cleaned up by weak cation exchange solid-phase extraction. The recoveries they obtained (43.6 to 73.5%) were lower compared to those obtained in the current study, while their LOD (0.94 µg L^{-1}) was higher compared to what was obtained in this study, indicating better accuracy and sensitivity of the our method compared to Zou et al. (2015).

Table 4.1: The maize and soil recoveries (%Rec) and RSD% value (n=3), LOD, LOQ and R^2 obtained from QuEChERS and UE methods

Analyte	Maize		Soil		Maize		Soil		R^2
	% Rec, RSD				LOD		LOQ		
	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	Ultrasonic	QuEChERS	
Atrazine	100±2.1	94±3.1	97±0.9	92±2.0	0.02	0.01	0.2	0.4	0.9988
2.4 D	104±1.0	110±0.9	91±3.5	101±1.6	0.15	0.23	0.5	0.8	0.9985
Glyphosate	100±2.0	103±1.1	94±1.9	89±0.8	0.09	0.12	0.3	0.4	0.9990
Mesotrione	101±0.5	115±2.1	96±4.2	97±1.0	0.02	0.04	0.3	0.13	0.9991

4.3.4 Analysis of real samples

The modified UE and QuEChERS methods were then applied to subsoil (0-15 cm), topsoil (0-10 cm and 0-5 cm) and maize grain samples. Residues of atrazine, 2.4-D, glyphosate and mesotrione herbicides were detected in all topsoil and maize samples. In the subsoil structures, only traces of 2.4-D were detected with the UE method with higher concentration (2.4 µg L^{-1}) in site 1 (Table 4.2), while 2.4-D and atrazine were detected with the QuEChERS method with higher concentrations of 1.86 µg L^{-1} and 0.6 µg L^{-1}, respectively. All atrazine detected residues were lower than recommended MRLs. The statistical analysis showed that there was no significant difference between the concentrations obtained with UE and QuEChERS methods. Availability of the 2.4-D and atrazine herbicides to the subsoil is due to their high solubility properties which resulting to their leaching to subsoil structure (Mackay et al. 2006) as compared to the other studied compounds. More pesticides were detected in the subsoil through QuEChERS method as compared to UE method (Table 4.3). Ntombela and Mahlambi (2019) studied the atrazine herbicide in soil and sediments samples from KwaZulu-Natal. The concentrations detected ranged between 0.032-0.93 µg L^{-1} in sediments, while in soil samples they were between 0.12-1.03 µg L^{-1}. In both methods, detected concentrations of the herbicides were higher in the topsoil (0-5 cm) compared to other soil segments which could be due to the high hydrophobicity properties (low solubility), low log K_{ow} of < 10 and presence of functional group containing O, N, and S (Mackay et al. 2006, Bilgic et al. 2022). This allows connection to soil water molecules through hydrogen bonds hence, bioaccumulation of herbicides to the soil and translocation to the edible crop (Martins et al. 2013).

In both methods, glyphosate traces were higher 30.52 µg L^{-1} and 34.2 µg L^{-1} in maize crop sampled from site 1 for UE and QuEChERS method, respectively. However, the obtained maize concentrations are lower than MRLs in all studied sites which makes this finding a less concern since the presence of higher herbicides traces pose a danger to the environment and the consumer. All detected concentrations were different but there were not significantly different. Farajzadeh et al. (2019), extracted diazinon, chlorpyrifos, penconazole, oxadiazon, and diniconazole pesticides from fruits and vegetables using combination of QuEChERS and dispersive liquid–liquid microextraction with GC-FID. Penconazole was found in corn (4 µg kg^{-1}) and other samples were free of the studied pesticides and QuEChERS was found to be effective than dispersive liquid–liquid microextraction. The availability of the herbicides in the tested soil and maize crops maybe due to the application of herbicides to remove growing

competing pest during growth and management processes. This impact greatly food security, therefore, different herbicides were applied to the soil to protect maize crop quality and quantity against competing weeds.

Table 4.2: Concentrations (µg g^{-1} dry weight) of pesticides in soil and maize crop determined using UE method

Analyte	Maize MRL (µg g^{-1})	Site 1				Site 2			
		Soil depth (cm)			maize	Soil depth (cm)			maize
		0-5	0-10	0-15		0-5	0-10	0-15	
Atrazine	50	10.6±11.21	11.62±3.23	ND	6.15±0.52	5.42±2.05	10.45±1.06	ND	11.36±6.11
2.4 D	500	16.5±1.13	6.58±9.12	2.45±1.73	10.28±0.21	10.41±4.66	5.64±0.1.92	1.33±2.89	20.65±2.53
Glyphosate	2000	26.1±8.50	9.45±5.23	ND	30.52±2.99	19.5±0.63	15.42±2.53	ND	11.50±1.05
Mesotrione	60	8.4±2.32	8.1±0.35	ND	11.23±0.87	30.18±3.17	29.44±3.17	ND	24.61±2.98
	Soil MRL	**Site 3**				**Site 4**			
Atrazine	66000	36.30±0.62	20.42±0.91	ND	15.8±1.43	19.41±7.20	5.44±4.36	ND	24.1±4.26
2.4 D	20	28.12±4.63	16.86±0.94	0.33±1.66	18.8±3.55	21.45±3.78	15.26±0.92	1.65±28	18.45±2.64
Glyphosate	100	15.66±3.18	11.86±1.62	ND	9.81±2.14	45.10±2.02	30.79±1.29	ND	9.15±0.68
Mesotrione	1500	19.40±1.01	20.45±2.17	ND	8.05±1.69	15.4±1.36	8.92±0.61	ND	6.15±0.97

*ND - Not detected

Table 4.3: Concentrations (µg g^{-1} dry weight) of pesticides in soil and maize crop determined using QuEChERS method

Analyte	Maize MRL (µg g^{-1})	Site 1				Site 2			
		Soil depth (cm)			maize	Soil depth (cm)			maize
		0-5	0-10	0-15		0-5	0-10	0-15	
Atrazine	50	11.4±8.51	8.62±3.25	0.6±0.90	7.65±2.43	5.42±2.02	10.4±1.01	ND	11.36±0.16
2.4 D	500	10.4±3.15	9.45±9.12	1.86±1.25	8.4±2.94	7.45±1.63	4.52±3.52	ND	15.42±0.12
Glyphosate	2000	28.6±5.86	11.3±0.23	ND	34.2±1.06	15.12±0.64	10.45±2.02	2.56±4.22	18.45±1.66
Mesotrione	60	7.48±1.14	10.4±2.15	ND	10.52±0.95	21.4±0.97	17.41±3.16	ND	14.57±2.34
	Soil MRL	**Site 3**				**Site 4**			
Atrazine	66000	33.4±3.15	18.42±0.9	0.2±2.61	18.4±2.02	23.4±2.54	8.42±5.34	ND	19.15±3.56
2.4 D	20	30.5±6.04	23.5±1.32	0.42±0.96	15.2±3.24	28.52±1.93	11.45±3.90	1.6±20	22.45±3.25
Glyphosate	100	12.56±0.91	8.46±3.61	ND	6.52±1.26	38.45±6.28	29.42±2.34	ND	16.52±1.32
Mesotrione	1500	18.42±2.36	15.45±0.56	ND	9.54±3.68	17.52±4.37	10.32±3.67	ND	7.45±3.64

*ND - Not detected

4.3.5 Comparison of pesticides concentrations obtained in this work with those from literature

Information presented in Table 4.4 clearly shows that atrazine is the commonly studied pesticide. A summary is presented in Table 4.4. Information presented in Table 3 clearly shows that the levels of these pesticides in the investigated matrixes are much higher than those reported in previous studies. This was also the case for the same studies conducted in other South African locations. Such an observation applied to all the investigated soil and maize crop. Moreover, a common distribution of the studied pesticides of concern was observed in the soil and crops. In this study, the concentrations found in soil and maize cob did not show any trend. Based on Table 3, it can be concluded that the distribution of these pesticides is site specific. Factors that could influence the occurrence of pesticides in soil and maize cob and their concentration include the application source and different physicochemical properties of the pesticides. In a South African study, the authors observed that the site location affects the levels of uptake and translocation in plant tissues due to differences in pesticides physicochemical properties that control the mobility of pesticides at different study location and adsorption capacity of residues by plants.

Table 4.4: Concentration ranges for the pesticides detected in this study against those from previous studies

Study site	Analytes	Matrixes	Extraction method	Detection method	Concentration	References
Heilongjiang, China	Atrazine	Soil		GC-FID	8.14 μg kg^{-1}	(Chen et al. 2019)
Bahia, Brazil	Atrazine	Environmental water samples	Micro-solid phase extraction (d-μ-SPE)	GC-MS	< 0.51 μg L^{-1}	(Nascimento et al. 2021)
Turkey	Acetamiprid	Sour cherry	QuEChERS	LC-MS / MS	4,0 μg L^{-1}	(Balkan and Kara 2020)
Lahore, Pakistan	Glyphosate	Spinach and turnip	Solvent extraction	LC-UV/V	0.29 μg L^{-1} and 0.93 μg L^{-1}	(Khan et al. 2020)
Pietermaritzburg, South Africa	Atrazine	Soil	UE	LC-PDA	37 8.14 μg kg^{-1}	(Kunene 2019)
Pietermaritzburg, South Africa	Atrazine	Avocado and carrot	QuEChERS	LC-PDA	4 and 38 8.14 μg kg^{-1}	(Mnyandu and Mahlambi 2021)
Pietermaritzburg, South Africa	Atrazine	Vegetables (bell pepper)	UE	LC-PDA	628.14 μg kg^{-1}	(Mnyandu and Mahlambi 2022)
Howick, South Africa	Atrazine, glyphosate, 2,4-D and mesotrione	Soil and maize	Ultrasonic extraction	GC-FID	Soil 5.42-36.30 μg L^{-1} Maize 6.15-30.52 μg L^{-1}	This study
	Atrazine, glyphosate, 2,4-D and mesotrione	Soil and maize	QuEChERS method	GC-FID	Soil 0.6-38.45 μg L^{-1} Maize 6.52-34.2 μg L^{-1}	This study

4.4 Conclusion

In the present study, the modified UE-SPE and QuEChERS extraction methods showed effectiveness in sample preparation and pre-concentration of the selected pesticides prior to their characterization by GC-FID. However, the results showed that the UE method can be effectively applied without the additional SPE clean up step, making the method cheaper and more available. The experimental data proved that the proposed methods have low LODs and LOQs, good repeatability and high recoveries, and thus can be presented as applicable for the extraction, pre-concentration and quantification of the selected pesticides in the studied pesticides. However, UE showed higher sensitivity accuracy as compared to QuEChERS method. All detected residues in maize and soil were not exceeding the MRLs however the concentrations obtained results from the continuous application of these pesticides in the agricultural fields. Based on the obtained results, the minimum human health risk was observed however, it is suggested that a supported program for the assessment of herbicides traces in soil and crops be developed to achieve sustainable farming systems, food quality and promote continuous consumer's wellbeing.

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Chapter 5 Comparison of Soxhlet and microwave assisted extractions efficiency for the determination of herbicides in soil and maize cob: cumulative and health risks assessment

Abstract

The effectiveness of microwave-assisted extraction (MAE) and Soxhlet extraction (SE) was compared for the determination of herbicides (atrazine, 2,4-D, mesotrione and glyphosate) in soil and maize grain followed by gas chromatography with flame ionization detector. The recoveries of herbicides in maize grain and soil were 62-80% and 70-81%, respectively for SE, while they were 80-98% and 85-101%, respectively for MAE. The analysis repeatability, represented as relative standard deviations were less than 20% for all herbicides in both methods. All the herbicides calibration curves showed a good correlation coefficient (R^2) ≥0.996, indicating good linearity. The SE limits of detection and quantification ranged between 0.22-0.32 µg $L^{-1}$and 2.2-3.2 µg L^{-1}, respectively, while they were between 0.1-0.29 µg L^{-1} and 1.0-2.9 µg L^{-1}, respectively for MAE. These findings showed that MAE method is more accurate and sensitive than SE, thus can be accurately applied for the determination of the assessed herbicides in soil and maize grain. Herbicides concentrations obtained ranged from 2.7 – 20.4 µg g^{-1} in maize and 1.2 - 30.5 µg g^{-1} in soil samples. The concentrations obtained in maize grain were lower than the maximum residue limits suggesting that health effect may not occur upon continuous consumption. The herbicides toxicity index further confirmed the low toxicity effect of the studied maize crop as it did not exceed the threshold value of 1. However, the health risk index was lower than 100% limit and did not exceed the acceptable daily intake of the maize crop in both adult and children indicating no health effect.

Keyword: Soxhlet, MAE, maximum limits, pesticide toxicity index, health risk index, maize.

5.1 Introduction

Pesticides are synthesized chemicals with non-selective or selective properties and widely used in agricultural fields to protect crops against pests and eventually increase the crop yield (Zhang et al. 2012). The herbicides are the commonly used pesticides in agriculture for pest control including atrazine, glyphosate, 2.4 D, mesotrione which interact differently with soil upon application. Atrazine get adsorbed to the soil through cation bridging, involving the formation of an inner sphere complex between H^+ cation and the atrazine-NH group. The NH groups of atrazine get involved in the hydrogen bonding with the water molecules, forming the $N–H\cdots O_w$ hydrogen bonds (Czaplicka et al. 2018). Glyphosate adsorb through the carboxyl, amine and phosphate functional groups that can be sorbed on minerals and organic matter in the soil forming bidentate and tridentate complexes (Duke et al. 2012). The 2.4-D adsorb weekly on soil through the carboxylic group, with sorption being negatively connected with soil pH and positively correlated with organic matter strength (Paszko et al. 2016). Mesotrione adsorb on soil through the hydrogen of the aromatic ketone and the adsorption is mostly higher in acidic soils, where mesotrione primarily exists as a neutral molecule rather than anion (Pintar et al. 2020). The physical properties of the herbicides such as water solubility and log K_{ow} also have an effect on their adsorption in soil as lower solubility and lower log K_{ow} results to higher adsorption of herbicides in soil.

When herbicides are applied in root agricultural soils, they penetrate through plant tissues and the xylem carries ions and water with the transpiration stream from the into the leaf. This process dislocates the metabolism of the plant and uses systematic action to kill the recipient (Singh et al. 2020). Pesticides are of importance for crops protection resulting to them being the bestselling agricultural chemicals with 0.6-1.2 million tons application annually for agricultural weed management (Cuhra 2015). However, pesticides particularly herbicide have been found to have complex and life threatening effect on the environment, field workers and to the consumer (Samsel and Seneff 2013; Robinson et al. 2013). Herbicides have been reported to cause effect ranging from acute (headache, dermal problems) to chronic effect (hormone functioning and endocrine system disruptions, birth defect, carcinogenic), (Mnyandu and Mahlambi 2021). Hence, residues of herbicides in agricultural products raises concerns as they maybe indirectly consumed, and result to serious health hazard for humans and livestock (Bøhn and Millstone 2019; Agostini et al. 2020). The presence of pesticides in crops indicate

the importance of their continuous monitoring to determine if they are within the levels that cannot cause harm to human health.

The determination of pesticides from complex matrices such as soil and maize grain samples require sample preparation, clean-up and pre-concentration prior their analysis to remove impurities and to improve analytes detection limits (Zhang et al. 2012). Soxhlet extraction (SE), microwave assisted extraction (MAE), ultrasonic extraction (UE), quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction, and solid-phase extraction (SPE), have been used for extraction and clean-up of pesticides in the environmental, soil and crops samples (Samsidar et al. 2018). Soxhlet extraction is based on the continuous process which allows the solvent cycles of solvent extraction through reflux that is repeated until the extraction of the organic contaminants is complete (Yusup et al. 2015). Even though Soxhlet uses large amount of organic solvent and longer extraction times, it presents simplicity, ease of visual monitoring of the extraction process and reuse of the solvent after stripping and distillation which improves the analytes recoveries from the sample matrix. The MAE involves the adsorption electromagnetic waves by solid material and converts them to heat energy. Then as the pressure is applied to the solid cell wall, it causes the cell to expand, as the pressure increases, the cell ruptures, and the organic contaminants in the solid cell leaches into the organic solvent (Moret et al. 2019). The MAE offer advantages including shorter extraction process, minimum solvent required, and practically eliminate the concentration stage improving the overall productivity and convenience of the procedure. However, its availability can be limited in other laboratories since it requires an expensive instrument.

There are no studies that investigated the highly absorbing maize fragment due to pesticides translocation in the maize grown in Buhle Farm as there are consumed by humans and livestock. Also, no their risk assessment to human health has not been conducted. The aim of this study was therefore to close the gap by assessing the uptake of atrazine, 2,4-D, mesotrione and glyphosate by soil and its translocation to maize grain. The extraction of herbicides was conducted using Soxhlet and MAE methods followed by SPE clean up and the determination was done by gas chromatography-flame ionisation detector. The solubility (mg/L) and log K_{ow} for 2.4 D is 64 and 5.78, for atrazine its 34 and 2.61, for mesotrione its 22 and 0.90, and for glyphosate its 17 and -3.40, respectively (Farajzadeh et al. 2019), hence their absorption in soil is expected to follow glyphosate>mesotrione>atrazine>2.4-D sequence.

5.2 Methods and materials

5.2.1 Chemicals, and analytical reagents

The 2.4-D (97%), atrazine (97.4%), glyphosate (98.5%) and mesotrione (97.5%) were purchased from Sigma Aldrich (Durban, South Africa). All used solvents were of HPLC grade: acetonitrile (99.9%), acetone (99.8%), dichloromethane (99.8%), ethyl acetate (99.9%), ethanol (<99%), hexane (99.9%) and methanol (99.9%) and were bought from Merck (Durban, South Africa).

5.2.2 Instrumentation

The chromatographic studies were conducted using a Bruker scion 436 GC from Gibbs Technologies (Durban, South Africa) coupled with a flame ionization detector (FID). The separation of herbicides was done on a capillary column, VF-5ms (a length of 30 m × 0.25 mm i.d. × 0.25 m film thickness) supplied by Gibbs Technologies. The injector temperature and detector temperatures were both 250ºC. The temperature program was as follows: initial temperature of 60ºC and held for 1 min, a rate of 30ºC min^{-1} to 150ºC held for 4 min, followed by 15ºC to 250ºC and held for 5 min. The nitrogen was used as a carrier gas and the injection was split/splitless with a purge time of 0.10 min and split of 1:50. The FID was fed with hydrogen (30 mL min^{-1}) and synthetic air (300 mL min^{-1}) and the samples injection volume was 1µL (de Souza Pinheiro and de Andrade 2009). The extraction of herbicides from soil and maize crops was conducted using Soxhlet extractor bought from Anatech (Durban, South Africa), and microwave assisted (Multiwave 5000) obtained from Anton Paar (Durban, South Africa). Centrifuge purchased from Anatech (Durban, South Africa) was employed for the supernatant separation of solid and liquid. Mild steel Rotavapor bought from Chem Lac (Johannesburg, South Africa) was employed to evaporate solvents from the extracts. Advantec thimble filters (ID 19mm OD 22mm L 90mm) supplied by Prestige (Durban, SA) were used as sample holder for Soxhlet extraction. The clean-up of maize and soil extracts from Soxhlet extraction was conducted using solid phase extraction (SPE) vacuum manifolds bought from Sigma Aldrich (Steinheim, Germany). The SPE was connected to a vacuum pump from Edwards (Munic, Germany). Oasis hydrophilic-lipophilic balance (HLB) cartridges, (60 mg, 3 mL) supplied by Waters (Milford, USA) were used as solid phase extraction sorbent. The maize samples were freeze dried using lyophilizer from Antech Scientific (Durban, South Africa).

5.2.3 Working standard solutions

The working standard solutions (20-100 µg L^{-1}) were prepared from a 100 mg L^{-1} stock solution in 95% n-hexane containing a mixture of herbicides (2.4-D, atrazine, glyphosate and mesotrione). These were then used for the calibration of gas chromatography within the concentration range 20-100 µg L^{-1} and for spiking samples used for validation studies.

5.2.4 Study area description and sampling

The study site was Buhle experimental field which is located in the Howick, Province of KwaZulu-Natal (UMgungundlovu district). The Global Positioning Systemco-ordinates for the exact sampling locations are site 1: -29.524161, 30.247682, site 2: -29.523865, 30.247548, site 3: -29.523705, 30.247658 and site 4: -29.523633, 30.247441. The sampling location reaches high temperatures in summer (wet season) which range from 21°C to 38°C, and drop in winter (dry season) ranging from 10°C-28°C. Maize grows well in the wet season since it is the summer crop, rain and sunshine promote its growth as compared to the dry season. On average, precipitation is approximately 569 mm annually. For this study purpose, the samples were collected in the month of March 2021 from other maize production sites in the farm for the extraction optimization purposes. The 12 maize crops were sampled from the maize stalk, and their corresponding soils were sampled on the topsoil surface of the soil (0-5, 0-10 cm) and subsoil 10-15 cm surface layer in all studied sites The samples we collected. The polyethylene bags were used for sample storage and transported to the laboratory where they were air dried in a fume hood for 48 hours. Thereafter, they were pulverized and sieved using 2.0 mm sieve (Prestige, South Africa). The maize grain samples were freeze dried on Lyophilizer followed by milling into fine powder and then sieved through 100 mm screen mesh and stored at room temperature until extraction process.

5.2.5 Sample preparation

5.2.5.1 Soxhlet extraction procedure

The method published by Kunene and Mahlambi (2020) was used with further optimization to improve recoveries for the additional herbicidess. Factors such as the extraction time (8, 12, 24 hours) and extraction solvents (methanol, methanol: acetonitrile (1:1 v/v), ethanol: acetone (8:2 v/v) and the mixture of methanol: ethanol (1:1 v/v)) were optimized.

Under optimum conditions: a 10 g of the soil or maize corn sample was weighed in a cellulose thimble and placed in a Soxhlet chamber fitted with a condenser and connected to a round

bottom flask containing 100 mL of methanol solvent and refluxed for 24 hours at 85°C. The extract was concentrated by a rota-evaporator to 1 mL and then diluted with deionized water to 100 mL and subjected to clean-up by SPE prior chromatographic analysis.

5.2.5.2 Microwave extraction procedure

The method published by (Merdassa et al. 2013) was used with further modification. The parameters optimised were extraction time (2, 8 and 15 minutes) and extraction solvent volume (5, 12, and 25 mL) and extraction solvents (hexane: acetone (1:2 v/v), acetonitrile: acetone (1:2 v/v) and the mixture of methanol: ethyl acetate (1:1 v/v).

Under optimum conditions: a 1 g sample was weighed into the extraction vessel followed by the addition of 12 mL of hexane-acetone mixture (1:2, v/v). The vessels were closed and hand agitated for 1 minute. Extraction was conducted at 400 W (100% output) irradiation power and the temperature program used was ramp from 0 to 160°C for 2 minutes, holding at 160°C for 8 minutes. After extraction, the vessels were allowed to cool for 15 minutes at room temperature before opening. The supernatant was filtered through a Buchner funnel packed with a GF/C grade glass microfiber filter obtained from Whatman (Maidstone, UK) for the removal of suspended solids particulates. The sample was concentrated to dryness under a gentle stream of nitrogen over heating water at 40°C. The residues were re-dissolved in 100 µL dichloromethane, and 5 µL of the extracted sample was injected into the GC-FID for analysis without a need of further cleanup procedure (Merdassa et al. 2013).

5.2.6 Pesticide toxicity index (cumulative risk)

The Toxicity Quotient (TQ) is used to assess the toxicity resulting from an individual pesticide and is derived from ratio between each pesticide residue concentration and the matching MRLs. The pesticides toxicity index (PTI) which is a screening approach to assess the degree of exposure to toxicity of complex pesticide mixtures is then obtained as the sum of the TQ for each pesticide compound as stated in equation 5.1 and 5.2.

$$TQ = \frac{C}{MRL} while \tag{5.1}$$

$$PTI = \Sigma\, TQ \tag{5.2}$$

Where: *C* is the concentrations of the detected individual pesticide residue (µg g^{-1}), and MRL is the maximum residue limits (µg g^{-1}) of the individual pesticide. The PTI acceptable target of less than 1.00, poses no harm to human health (Ramadan et al. 2020; Shalaby et al. 2021).

5.2.7 Health risk assessment

The health risk index (HI) was developed to characterize the health risk (HR) assessment of consumers caused by the consumption of pesticide-contaminated vegetables. The HI is computed by dividing the estimated daily intakes (EDI) by the corresponding values of the WHO/FAO-established acceptable daily intake (ADI) (Khan et al. 2020), as indicated in the equation 5.3 and 5.4. If the HI is lower that 100%, it indicates no possible harm to human health (Akoto et al. 2016):

$$EDI = \frac{A\ X\ B}{C} \tag{5.3}$$

$$HI = \frac{EDI}{ADI}\ X\ 100 \tag{5.4}$$

Where: *A* represent the concentration of detected pesticide residues in vegetable (µg L^{-1}). B represent the average daily intake of vegetables. C is the average body weight for South Africans (70.8 kg for adults and 20.8 kg for child), (Walpole et al. 2012).

5.2.8 Statistical analysis

The Statistical Package for Social Science (SPSS version 25.0 SPSS Inc, Chicago, IL, USA) was used for the analysis of LOD and LOQ concentrations (µg L^{-1}) of four pesticides. The mean values and standard deviations were calculated for all replicate measurements. The statistical differences between treatments will be determined by an analysis of variance (ANOVA) employing the MSTAT-C statistical software, Version 2.10 (MSTAT, Michigan State University). Comparisons between means will be carried out according to Duncan's test. Differences will be considered significant at a P level of <0.05.

5.3 Results and discussion

5.3.1 Optimization of Soxhlet extraction method

5.3.1.1 Selection of extraction solvent

The extraction solvents studied were acetonitrile: methanol (1:1 v/v), acetone: methanol (1:1 v/v), ethanol and methanol. Methanol showed high recoveries for maize and soil ranging between 62-80% and 70-81%, respectively (Figure 5.1). This indicated that methanol can effectively penetrate into soil and maize matrix and adequately remove the analytes of interest from soil and maize and thus increase analyte recoveries. The lower recoveries obtained with acetone: methanol could be due to the lower polarity resulting from the mixture of two organic solvents which affect the effectiveness extraction of these polar compounds based on the " like dissolves like" rule (Nawaz et al. 2020; Porevsky et al. 2014). The lower recoveries for acetonitrile-methanol mixture could be due to the high viscosity and slightly lower polarity of acetonitrile resulting to low elucidation of analytes on interest from the soil and maize matrix to the solvent. The low octanol-water partitioning coefficient of the herbicides could also enforce them to strongly bind into the soil and maize structures (Elbashir and Aboul-Enein 2015). Methanol was then taken as the optimum extraction solvent.

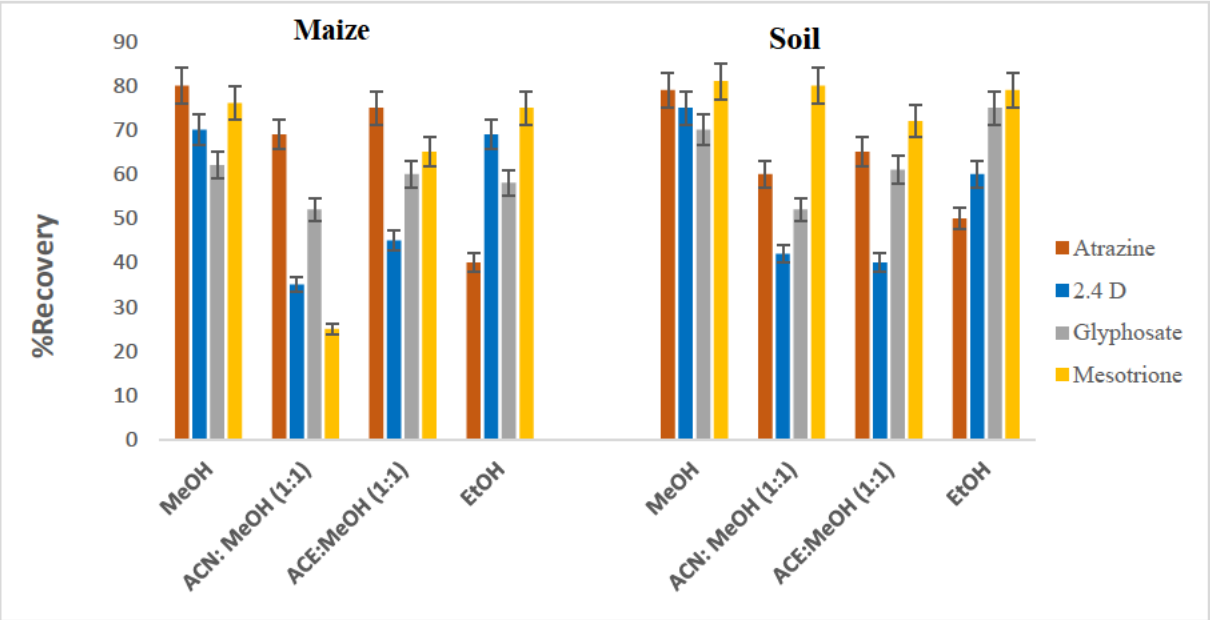

Figure 5.1: Effect of extraction solvent on the analyte recoveries.

5.3.1.2 Effect of the surface wetting

The wetting stage was aimed to evenly distribute the analyte across the soil and its active sites (Dodd et al. 2015). The effect was studied by adding 5 mL of water into the maize and soil samples prior the extraction process. The results showed that wetting the sample resulted in the reduction of the herbicides recoveries (Figure 5.2). This is because there was no steric hindrance and surface tension between these solvents due to high polarity of water. This phenomenon resulted into loss of analyte due to solvent dilution and thus lower amount was transferred to the methanol leading to lower recoveries. The subsequent extractions were then conducted without the wetting of the samples (Deziel et al. 2011).

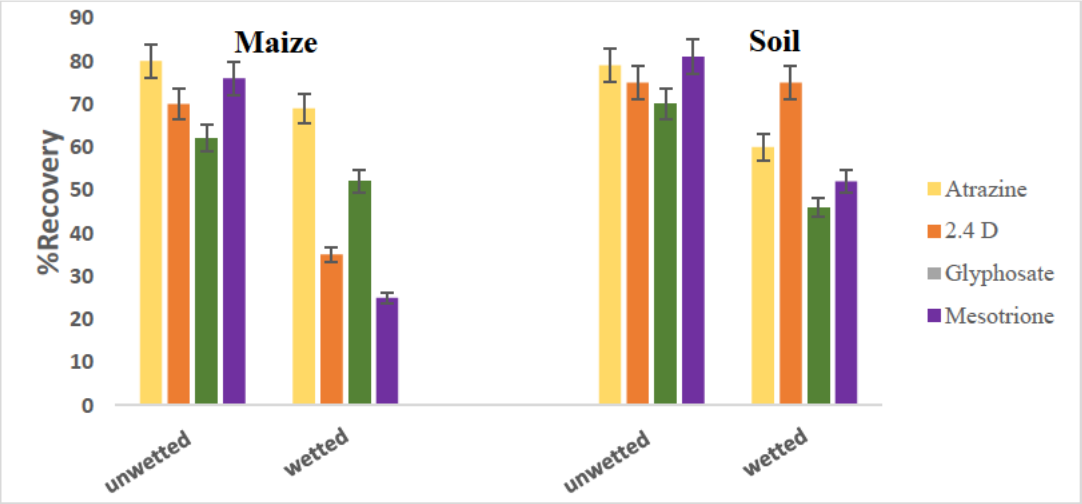

Figure 5.2: Effect of surface wetting on the analyte recoveries.

5.3.1.3 Effect of extraction time

This effect was examined using 8, 12 and 24 hours. The results showed growth in the recoveries as the extraction time increased from 8 to 12 hours however, they decreased with further increased time from 12 to 24 hours (Figure 5.3). The lower analyte recoveries obtained at 8 hours could be due to the limited interaction between the samples and extraction solvent, which then lowered the analyte transfer to the solvent. Higher analyte recoveries obtained at 12 hours could be due to the optimum extraction time which permitted solvent penetration into soil and maize matrices allowing sample aggregates to breakdown hence optimum removal of the analyte into the solvent. The lower recoveries at 24 hours could be an indication of a possible analyte degradation resulting from prolonged extraction time, hence solvent saturation (Naczk and Shahidi 2006). Therefore 12 hours was selected as the appropriate extraction time.

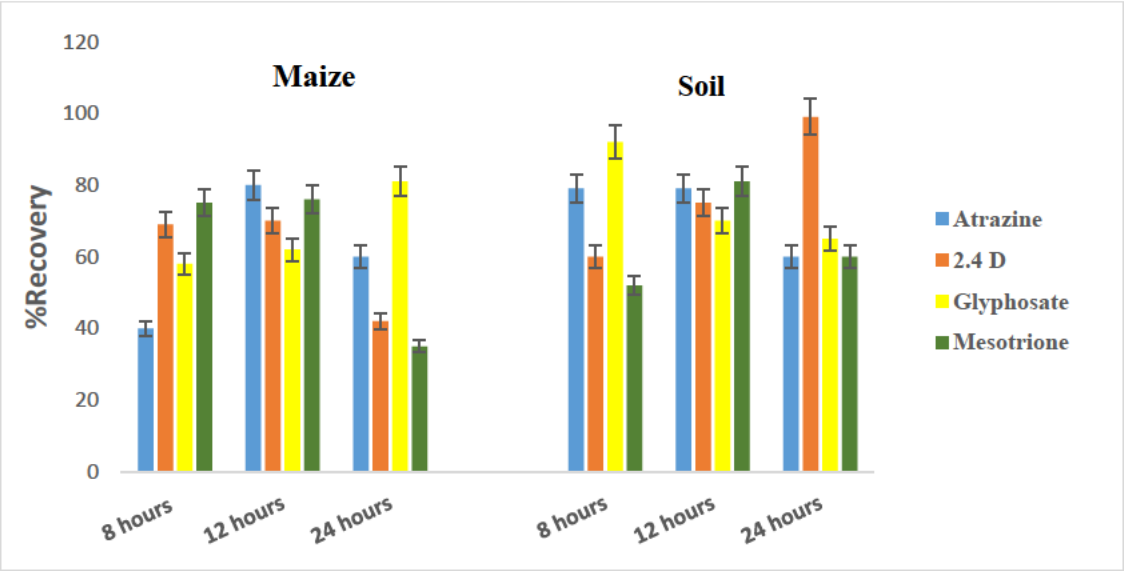

Figure 5.3: Effect of extraction time on the analyte recoveries.

5.3.2 Optimization of MAE methods

5.3.2.1 Selection of extraction solvent

It is imperative to employ the solvent type that will allow for high analyte recovery. Hexane: acetone (1:1 v/v), acetonitrile: acetone (1:1 v/v) and methanol: ethyl acetate (1:1 v/v) were used as extraction solvents to investigate their influence on herbicides recovery. Based on the results (Figure 5.4), the hexane: acetone (1:1 v/v) mixture showed to be more effective, with recoveries ranging from 80-98% for maize and 70-101% for soil. These increased recoveries might be attributable to a mixing of solvent polarities, as hexane least polar and acetone is the semi polar, allowing their differing polarity to accommodate the analytes' polarities. This enabled effective solubility and thus extraction of target analytes. This was due to that polarity of the solvent and the analytes affects the analytes solubility into the solvent resulting to analytes lower recoveries from solid samples (Moret et al. 2019). As a result, the mixture was selected as the ideal extraction solvent. Otake et al. (2008) found hexane: acetone to be the optimum solvent for the extraction of organochlorine pesticides using MAE which was suggested to be due to higher dipole moment (index of polarity) and high boiling temperature.

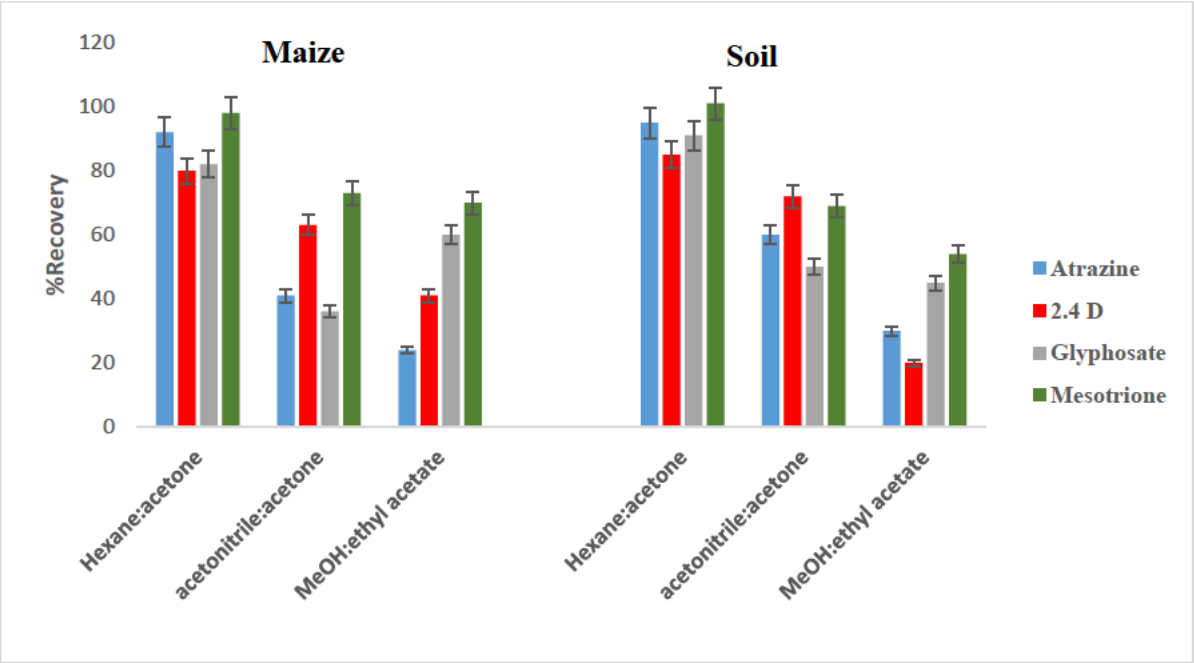

Figure 5.4: Effect of extraction solvent on the analyte recoveries.

5.3.2.2 Effect of extraction time

To evaluate the influence of extraction time on analytes recoveries, 2, 8, and 15 minutes were used. The recoveries showed as increase as the extraction period was increased from 2 to 8 minutes, then declined at 15 minutes (Figure 5.5). The higher recoveries at 8 minutes indicates optimum interaction time to allow the solvent to permeate into the soil matrix and break down the soil aggregates, result in complete extraction of analytes into the solvent without any degradation (Naczk and Shahidi 2006). All analytes were obtained at high recoveries ranging from 80-98% for maize and 70-101% for soil after 8 minutes of extraction time, and this was chosen as the efficient extraction time.

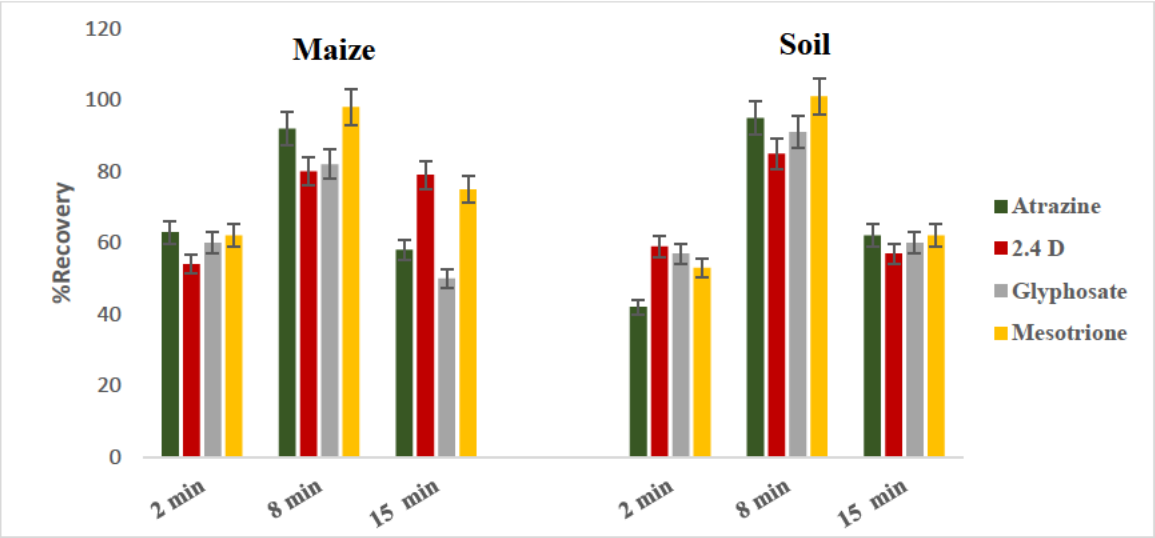

Figure 5.5: Effect of extraction time on the analyte recoveries

5.3.2.3 Effect of extraction volume

The primary aim of optimizing the extraction procedure is to achieve optimum extraction efficiency for analytes with a little amount of organic solvent and a short extraction time. As a result, the effect of a hexane: acetone (1:1 v/v) solvent volume (5, 12, and 25 mL) was investigated. According to the results, a 5 mL solvent volume resulted in poor analytes recoveries, which could be attributable to inadequate mass transfer of the analytes from the sample to the solvent due to the little amount of solvent used (Silva et al. 2005). The mass transfer of analytes from the soil sample to the solvent has been observed to rise as the solvent volume is increased; however, the interaction between the soil sample and the solvent must be considered. In a study conducted by Farajzadeh et al. (2019), an increase in analytes recoveries was observed when the solvent volume was increased from 15 mL to 25 mL. However, further increase in solvent volume resulted in the recoveries decline which was related to inadequate interaction between the soil sample and the extraction solvent. The soil sample sank to the bottom of the flask, while the solvent floats freely, resulting in an inefficient interaction between the soil and the solvent. As a result of the poor transfer of analytes from the sample to the solvent, and thus only a small amount of analytes were recovered.

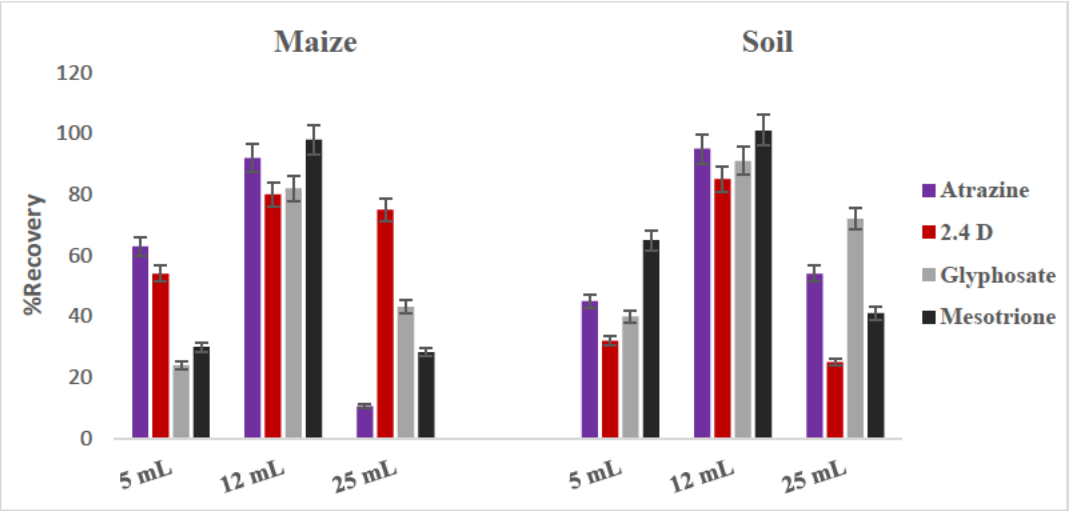

Figure 5.6: Effect of extraction volume on the analyte recoveries

5.3.3 Method validation

The relative standard deviation (RSD), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ), and percentage recoveries were the quantitative characteristics of the current methods assessed. All calibration curves showed a high correlation coefficient (R^2) ≥0.996, indicating good linearity. The LODs and LOQs ranged between 0.22-0.32 µg L^{-1} and 2.0-2.9 µg L^{-1}, respectively for SE (Table 5.1), while they were between 0.1-0.25 µg L^{-1} and 1.1-2.2 µg L^{-1}, respectively for MAE. The LOD and LOQ values obtained suggested that MAE is more sensitive than SE, hence, it is expected to be more effective to quantify the target analytes at low concentrations found in the real sample. However, statistical analysis revealed that they were not significantly different with p-values of LOD ($p<0.023$), LOQ ($p<0.001$) and recoveries ($p<0.019$) in maize while in soil LOD ($p<0.014$), LOQ ($p<0.032$) and recoveries ($p<0.044$) for SE and MAE, respectively. The recoveries ranged between 62-80% and 70-81%, respectively for maize and soil using SE, while for microwave they were 80-98% and 85-101% for maize and soil, respectively. This indicate high accuracy for MAE compared to SE but no significant differences ($p<0.044$) and ($p<0.019$), respectively were found in recoveries for both methods. These findings agree with those of Silva et al. (2010), where SE method gave lower recoveries of atrazine in soil samples as compared to the MAE, which were 65% and 93%, respectively. The analysis repeatability, represented as RSD ranged from 0.7 to 8 for Soxhlet and 0.2 to 5.4 for MAE which are within the acceptable range as they are lower than 20% (Radović et al. 2015).

Table 5.1: The herbicides recoveries in soil and maize and RSD% value (n=3), LOD, LOQ obtained from Soxhlet (SE) and microwave (MAE) methods and the linearity of the method (R^2)

Herbicide	Maize		Soil		Maize		Soil		R^2
	% Rec ± RSD				LOD (µg L^{-1})		LOQ (µg L^{-1})		
	SE	MAE	SE	MAE	SE	MAE	SE	MAE	
Atrazine	80±0.11	92±41.02	79±0.93	95±5.41	0.32	0.12	2.9	1.4	0.9973
2.4 D	70±43.12	80±0.21	75±0.68	85±2.22	0.26	0.10	2.3	1.1	0.9953
Glyphosate	62±25.52	82±2.02	70±0.90	91±3.13	0.30	0.25	2.6	2.2	0.9961
Mesotrione	76±89.59	98±3.53	81±0.21	101±4.85	0.22	0.09	2.0	1.6	0.9990

5.3.4 Analysis of the real samples

The effective usage of MAE and SE methodologies were proven in the extraction of four organic contaminates residues (atrazine, 2.4-D, glyphosate and mesotrione) from agricultural maize grain, top soil (0-0.5 cm and 0-10 cm) and subsoil soil (0-15 cm) samples. The adsorption of herbicides in the soil occurs primarily due to the existence of organic matter (OM) and clay content of the soil which results into translocation to the maize crop. The OM posses low polarity groups that have high affinity for herbicides while the small clay particles have high superficial area where the herbicides can be absorbed (Xiong et al. 1999; Silva et al. 2010).

Herbicide concentrations were found to be greater in the topsoil than in subsoil segments in both methods, which might be owing to the high hydrophobicity (low solubility <100 mg L^{-1}). The present functional groups comprises double bonded N, S, O rings and Cl atoms, as well as alcohols, amines and ketones in pesticides and low log K_{ow} < 10 (Mackay et al. 2006). Functional groups permitted herbicides to form hydrogen bonds with soil water molecules, allowing bioaccumulation in the soil and transfer to edible crops. Glyphosate is expected to be higher in topsoil due to lower log K_{ow} and high hydrophobicity compared to the other studied herbicides. Also, glyphosate contains functional groups such as carboxyl, ammine and phosphate that can sorbed on minerals and organic matter in the soil forming bidentate and tridentate complexes. The residues of the studied herbicides were not detected in the subsoil structures (Table 5.2) using SE method while only 2.4-D was detected using MAE with the highest concentration of 3.2 µg g^{-1}. The high solubility qualities of 2.4-D herbicide result in it leaching from the topsoil structure, making it available to the subsoil (Dionisio and Rath 2016; Farenhorst et al. 2009).

The concentrations of herbicides varied in both the SE (1.2 – 10.5 µg g^{-1}) and MAE (4.5 – 30.5 µg g^{-1}) techniques depending on the sampling sites (Table 5.3) due to the possible loss of target compounds as a result of Soxhlet longer refluxing time which is limited by the boiling point of the polar solvent (Numata et al. 2004; Otake et al. 2008). This could be the other reason why 2.4-D was not detected by SE but detected by MAE. Higher concentrations in MAE could be due to the fact that it execute the extraction process in closed pressure-resistant containers, hence, the extraction temperature rises above the boiling point of the extraction solvent under atmospheric pressure (Numata et al. 2004). High temperatures reduce the viscosity of the

extraction solvent and aid in the disruption of solute-matrix interactions, resulting in increased extraction efficiency. The physical-chemical qualities of the specific herbicide, application technique, interaction with the extraction solvent, interaction with the physical-chemical properties of the growth medium, and temperature all have a role in bioaccumulation (Martins et al. 2013).

All the studied herbicides were detected in all the grain samples and higher concentration were also obtained with MAE. Glyphosate traces were greater in the maize grain from site 1 (15 µg g^{-1}), while higher concentration for atrazine was observed in site 2 (18.4 µg $g^{-1)}$ and 2.4-D (20.4 µg g^{-1}) in site 4. Santos-Hernández et al. (2018) studied 2.4-D and atrazine in corn samples using MAE coupled SPE and the residues were undetected. In a study conducted by Li et al. (2020) on organophosphorus pesticides in rice, maize and wheat samples using MAE with QuEChERS method, none of the target compounds was detected in all samples. In this study, all sites tested showed concentrations lower than herbicides MRLs in maize, this indicated less consumer health effect. The MRLs for atrazine, 2.4-D, glyphosate and mesotrione are 50, 500, 2000 and 60 µg g^{-1} (WHO 2019), respectively. The availability of herbicides in the tested soil and maize grain are due to these herbicides being highly used to manage growing weeds that compete with maize plants, compromising effective crop development and nutrient levels. This fact has a significant influence on food security, resulting in these herbicides being applied to the growing media soil to preserve maize crop quality and quantity against competing weeds.

Table 5.2: Concentrations (µg g^{-1} dry weight) of pesticides in soil and maize crop determined using Soxhlet extraction method

Analyte	Site 1				Site 2				Site3				Site 4			
	Depth (cm)				Depth (cm)				Depth (cm)				Depth (cm)			
	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize
Atr	1.2±0.9	5.9±0.5	ND	3.5±2.3	3.5±3.6	2.1±0.1	ND	5.8±1.0	0.4±0.1	4.2±6.5	ND	9.4±3.4	6.4±3.2	3.4±6.3	ND	4.1±3.2
2.4-D	2.9±1.1	1.4±3.1	ND	8.4±6.4	8.4±2.0	9.4±4.3	ND	4.3±0.9	2.5±2.8	1.8±0.9	ND	3.8±3.9	8.2±2.2	6.5±1.9	ND	6.5±4.6
Gly	6.8±2.9	4.9±4.6	ND	2.7±2.1	6.8±0.1	2.5±0.3	ND	4.0±3.5	8.1±1.8	9.2±2.4	ND	6.7±2.4	9.5±2.9	8.0±0.2	ND	11.0±1.6
Mes	4.6±8.7	2.5±0.6	ND	3.1±0.3	1.2±2.8	5.6±6.8	ND	6.4±5.1	4.4±3.5	5.8±2.9	ND	8.8±2.2	10.5±1.9	7.9±0.6	ND	6.1±0.5

Note: Atr - atrazine, 2.4-D – 2.4 dichlorophenoxyacetic acid, Gly – glyphosate, Mes - mesostrione

Table 5.3: Concentrations (µg g^{-1} dry weight) of pesticides in soil and maize crop determined using MAE method

Analyte	Site 1				Site 2				Site3				Site 4			
	Depth (cm)				Depth (cm)				Depth (cm)				Depth (cm)			
	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize	0-5	0-10	0-15	maize
Atr	12.4±1.5	7.6±3.2	ND	5.4±1.4	8.4±6.8	5.6±1.5	ND	18.4±0.1	26.0±2.3	30.5±8.9	ND	12.4±5.0	20.5±7.2	10.2±4.3	ND	15.1±3.9
2.4-D	8.4±1.1	4.5±9.1	0.6±0.9	5.1±1.9	12.4±2.4	8.2±6.3	3.2±2.2	11.5±5.6	20.0±3.0	8.5±3.3	1.2±3.9	8.5±6.1	18.5±5.9	9.4±7.9	ND	20.4±4.2
Gly	20.5±2.8	18.3±3.1	ND	15.0±1.6	19.5±1.9	19.5±7.1	ND	13.6±4.3	11.2±4.1	9.6±4.2	ND	4.4±2.2	29.5±3.0	26.2±4.0	ND	11.2±8.0
Mes	9.48±3.1	8.0±1.1	ND	11.5±5.3	21.4±3.0	18.4±3.1	ND	17.5±1.8	19.4±6.5	12.5±2.5	ND	7.4±4.8	11.5±1.3	9.3±3.6	ND	7.0±6.3

Note: Atr - atrazine, 2.4-D – 2.4 dichlorophenoxyacetic acid, Gly – glyphosate, Mes - mesostrione

5.3.5 Pesticide toxicity index (PTI) values (cumulative risk)

The toxicity quotient (*TQ*) was used to assess the toxicity for a single herbicide, while the pesticide toxicity index (*PTI*) assessed toxicity for a mixture of all the herbicides studied. The *PTI* obtained values were <1 from all studied sites indicating no health hazard is likely to result (Khan et al. 2020), Table 5.4. The results obtained in this study are lesser compared to those obtained by Shalaby et al. (2021) who reported *PTI* of 128.44 in potatoes crops collected from the agricultural fields in Dakahlia (Egypt), this is highly influenced by geographic properties of the studied site, crop and a cultivar of interest.

5.3.6 Health risk assessment

The pesticides level of exposure over time and the prospective health effect is considered by assessing chronic exposure of the consumer. The health of the consumers exposed to chronically hazardous pesticide residues will only be at risk if the HI exceeds the ADI value of 222 g/person/day and consequently above 100% (Shephard et al. 2002). The HI data for both adults and children ranging from 7.3 – 29% and 22-92%, respectively (Table 5.4). The risk assessment results of the studied maize crops showed that there is no potential health risk associated with consuming these crops for both by adult and children since the HI is below the threshold limit of 100%. These findings agree with those of Shalaby et al. (2021), who reported *HI* <100% in potatoes, pepper, cucumber and eggplant crops collected from the agricultural fields in Dakahlia (Egypt). Although consumption of these maize crop may not cause instant health issues, caution should be exercised since these pesticides are known to be hazardous and deadly to the consumer. For example, atrazine has adverse effect on the consumer's health which is likely to cause tumors, breast, ovarian, and uterine cancers as well as leukaemia and lymphoma cancer in the body (Pathak and Dikshit 2011). 2.4-D is likely to cause blood cancer and sarcoma (a soft-tissue cancer) to the consumer (Kumar 2019)**.** Glyphosate is likely to pose a carcinogenic hazard to humans (Gillezeau et al. 2019) while mesotrione can cause ocular effects, kidney and liver effects (Jabłońska-Trypuć et al. 2019).

Table 5.4: Toxicity Quotient and health risk assessment detected in maize crops

Herbicides	Site 1			Site 2			Site 3			Site 4		
	TQ	*HI* (%) adult	*HI* (%) children	*TQ*	*HI* (%) adult	*HI* (%) children	*TQ*	*HI* (%) adult	*HI* (%) children	*TQ*	*HI* (%) adult	*HI* (%) children
Atrazine	0.11	7.7	27	0.36	26.3	92	0.25	17.7	62	0.30	21.6	75.5
2.4 D	0.01	7.3	25.5	0.02	16.4	57.5	0.02	12.1	42.5	0.04	29.1	91
Glyphosate	0.01	21.4	75	0.01	19.4	68	0.00	6.3	22	0.01	16	56
Mesotrione	0.19	16.4	57.5	0.29	25	87.5	0.12	10.5	37	0.12	10	35
PTI	0.32			0.68			0.39			0.47		

Note: *PTI* – pesticides toxicity index; *TQ* – toxicity quotients; *HI* – health index

5.4 Conclusion

The modified Soxhlet and MAE extraction methods showed effective sample preparation and pre-concentration of the studied herbicides prior to GC-FID characterisation in the current study. The adsorbed analytes of interest on the soil and maize matrixes were efficiently desorbed by the hexane: acetone (1:1) in the MAE method and by the hexane in Soxhlet method. Even though MAE instrument is expensive, the experimental results showed that the MAE procedures is more advantageous compared to conventional Soxhlet method. This is due to its high recoveries, lower amounts of extracting solvent, increased sensitivity, and precision for herbicides detection and measurement which are all significant for pesticides residue determination in agricultural matrices. Pesticide residues exceeded the maximum residue limits in the soil and maize samples analysed, owing to the usage of these pesticides in agricultural sector. The pesticides toxicity quotients did not exceed the threshold value of 1, indicating no health hazards effects to the end user. The health risk index was found to be lower than 100% limit, however, it did not exceed the acceptable daily intake of the maize crop in both adult and children indicating no danger to the consumer. Based on these findings, it is recommended that a funded program for assessing herbicide residues in soil and crops be implemented in order to achieve sustainable agricultural systems, food quality, and consumer well-being. Furthermore, farmers need to be educated to adhere to appropriate agricultural practices, pesticides proper dosages and how to limit pesticide use before harvesting.

5.5 References

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Chapter 6 Assessment of Herbicides Uptake by Soil and Translocation to Different Maize Segments

Abstract

Increased agricultural operations result in increased usage of various pesticides to safeguard agricultural crops, however this is done without paying attention on the effects of the amounting potential harm both humans and the environment. In this present study, a structured study was conducted on the uptake of atrazine, mesotrione, 2,4-dichlorophenoxyacetic acid and glyphosate herbicides from contaminated soil and translocation into different maize segments. It was observed that 2.4-D was least absorbed by the soil, however, all the studied herbicides showed high absorption in the leafy segment of the maize plant due to the high polarity of the leaf cuticle. Glyphosate showed high absorption rate in soil, roots, stalk and leaves while mesotrione was highly absorbed in grain and tassels in all treatments. The absorption rate of analyte increased with increasing growth days. The higher treatment concentration (0.75 g L^{-1}) showed elevated accumulation with the highest concentration (1.02 µg L^{-1}) observed for glyphosate in leaves after 140 days and high mesotrione in grain (0.51 µg L^{-1}) and tassel (0.42 µg L^{-1}) observed after 120 days. All maize treatment showed a PTI values of <1 with the HI data below 100% which indicated no possible health risk linked with the intake of these crops by both adults and children.

Keyword: Herbicides, toxicity index, health risk assessment, maize, GC-FID.

6.1 Introduction

The cropping system that sustains agricultural field without reducing soil fertility and controlling of pests, weeds and disease problem is essential for agricultural practices. The increasing population globally increases the demand in the agricultural production and to meet these increasing demands, the cropping system must improve crop yields on both commercial farms, small scales farms and maintain them. However, uncontrollable weed growth is a hindering factor and also mechanical control is difficult and time consuming with machinery as well as laborious by hands (Monteiro & Santos, 2022). Consequently, usage of chemical weed controller has received great interest in the agricultural sector due to minimal cultivation of crops. This in turn reduces soil erosion, power necessary to work agricultural soil and improves microbial biomass, soil structure and organic matter maintenance in the agricultural soils (Hellerstein & Vilorio, 2019).

The application of herbicide on the agricultural field is during pre-planting, pre-emergence and initial and late post emergence (Hossain et al., 2019). Maize is herbicide tolerant crop and the use of the herbicides has intensified in the maize production. Herbicides are applied on the soil, absorbed by the maize roots and translocate to the stalk, leaves, tassel as well as the maize cob. Tassel produces pollen which is transported to the silk for the production of maize seed, in the leaves is where photosynthesis occurs which produce for the maize plant while stem is where the cob grows. Therefore, the contamination of any of these matrices by pesticides may results in the contamination of the maize cob and thus be consumed by human. It is believed that herbicides has threatening effect on humans due to their non-biodegradable nature in the soil (Gill & Garg, 2014). It is therefore importance monitor the uptake of herbicides by agricultural matrices to ensure that the concentration in the final product in within the levels that cannot cause harm to human health after consumption.

The absorption and uptake of organic compounds from the soil by crop roots is a crucial pathway for them to accumulate chemicals. In general, the availability of an organic compound in soil and its ability for diffusion over the root surface depends on its availability in the growing media (Wang et al., 2021) . The bioavailability of chemicals in soil are primary absorbed by soil structures followed by uptake and storage in plant roots, then translocated to aboveground tissues through plant transpiration. Many factors governs the influence of the uptake and transport of organic chemicals in soil, including cultivation conditions, plant species

soil type, and the physicochemical characteristics of compounds (Li et al., 2019). However, under the same experimental conditions, physicochemical factors such as the molecular weight, octanol/water partition coefficient (log K_{ow}) and water solubility influence the absorption and translocation capabilities of compounds by the same plant. Li et al. (2021) studied the uptake, translocation, and subcellular distribution of triadimefon, tebuconazole, and epoxiconazole pesticides in rice. The findings showed that rice roots could take up the three triazole pesticides, although their distribution in plant tissues differed. In rice roots, the three pesticides accumulated in the following order: epoxiconazole (4.26 µg L^{-1}) > tebuconazole (2.63 µg L^{-1}) > triadimefon (1.37 µg L^{-1}), but in rice shoots, the order was reversed: triadimefon (0.48 µg L^{-1}) > tebuconazole (0.40 t µg L^{-1}) > epoxiconazole (0.21 µg L^{-1}).

The assessment of herbicides residues in soil and other agricultural matrices has been conducted using microwave assisted extraction (MAE) method (Feng et al., 2022; Kalogiouri et al., 2021). The advantage of this approach is the ability to extract analytes from different matrixes using lesser solvent and extraction duration (Sharma & Zalpouri, 2022). Its simplified operation procedure converts electromagnetic waves absorbed by solid materials into thermal energy. The cell then magnifies as pressure is applied to the solid cell wall, the cell ruptures as pressure rises, and the organic residues in the solid cell leach into the organic solvent. The most popular chromatographic technique is gas chromatography (GC), which primarily separates substances based on their volatilities. Polar, non-polar, volatile, and readily vaporized substances can be studied using the GC technique. Pesticide characterization using GC are frequently coupled with particular detectors such as an electron capture detector (ECD), a flame photometric detector (FPD), a nitrogen phosphorus detector (NPD), and a flame ionization detector (FID) (Karapınar, 2021). In this study, the mostly used herbicides (atrazine, 2,4-D, glyphosate and mesotrione) in maize production in the KwaZulu-Natal Department of Agriculture were studied. The MAE technique was utilized extract the herbicides from sample matrix to study their uptake and translocation into different maize parts (roots, stalk, tassel, leaves and maize grain). The extracted herbicides were then characterized with gas chromatography-flame ionization detector. To the best of our knowledge, the assessment of herbicides uptake, the translocation to different maize segments and their extraction using MAE was conducted for the first time. Moreover, the potential health risk effect to the maize end user was conducted for the first time on this farm.

6.2 Methods and materials

6.2.1 Chemicals and materials

Analytical grade acetone (70%), n-hexane (95%), dichloromethane (99%) were sourced from Sigma-Aldrich (Steinheim, Germany). The deionized water was produced using Aqua Max Basic 360 Series water purification system (Trilab, Durban, SA).

6.2.2 Maize plantation

The 0.25 g L^{-1}, 0.50 g L^{-1} and 0.75 g L^{-1} (w/v) treatment solutions of Glyphosate, 2,4-D, atrazine and mesotrione herbicides were prepared in water. Experiments plots were designed as randomized block, in the one-hectare experimental field, 12 plots were prepared by 6 m^2 each for the three replicates of each of the four treatments including control. The FC 701 white maize cultivar was used throughout the study. Three prepared treatments were applied at the post emergent while the control was left untreated, and nine treatments were conducted. The Knapsack pressure spray (TWK, Howick, SA) with a four nozzles bar was used with a constant pressure of 200 KPa providing, a 1 ms^{-1} application velocity with the nozzles positioned at a 70 cm height from the soil. In each plot, six rows were prepared with 100 cm spacing in between and the seeds were sown at a depth of 1 inch in October 2021. The maize was allowed to grow for 5 months to allow full maturity. The plant inter distance was 24 cm and the sprinkler irrigation system was conducted weekly for irrigation purposes. Hoeing and weeding was conducted after three weeks at the early stages of seeding establishment.

6.2.3 Sampling and sample storage

Soil and plant samples were collected from each sample plot. Soil samples were sampled on the upper surface of the soil (0-15 cm surface layer) using an auger. 40 cores were taken in a randomized zig-zag pattern across each plot. The cores were mixed in a packet to make a composite plots sample. The plant samples removed from the soil using shovel and were collected every 4 weeks after plantation until harvesting stage. Samples were stored in polyethylene bags and transported to the laboratory. Upon arrival, soil samples were air dried at room temperature for 36 hours then pulverized and passed through 2.0 mm sieve. The maize plant samples were washed with tap water to remove soil particles and finally rinsed with deionized water. The water was then wiped out with paper towel then left overnight to dry on the pre-cleaned bench top while ensuring no contact between the sample and bench top surface. The plants were then sub-divided into different plants segments (roots, stalk, tassel, leaves and

maize grain). Thereafter, all plant samples were freeze dried on Lyophilizer (Antech scientific, South Africa), followed by microwave digestion.

6.2.4 Microwave extraction procedure

A 1 g sample was weighed into the extraction vessel followed by the addition of 12 mL of hexane-acetone mixture (1:2, v/v). The vessels were closed and hand agitated for 1 minute. Extraction was conducted using microwave set eat 400 W (100% output) irradiation power and the temperature program used was ramped from 0 to 160°C for 2 minutes, holding at 160°C for 8 minutes. After extraction, the vessels were allowed to cool for 15 minutes at room temperature before opening. The supernatant was filtered through a Buchner funnel packed with a GF/C grade glass microfiber filter obtained from Whatman (Maidstone, UK) for the removal of suspended solids particulates. The samples were concentrated to dryness under a gentle stream of nitrogen over heating water at 40°C. The residues were re-dissolved in 100 µL dichloromethane, and 1 µL of the extracted sample was injected into the GC-FID for analysis without a need of further cleanup procedure (Merdassa et al., 2013).

6.2.5 Gas chromatography – flame ionization detector conditions

The chromatographic studies were assessed using a Bruker scion Model 436 GC from Gibbs Technologies (Durban, South Africa) along with a flame ionization detector (FID). Herbicide separation was performed on a Gibbs Technologies VF-5ms capillary column (length 30 m, 0.25 mm i.d, 0.25 m film thickness). The injector and detector temperature were both set at 250ºC. The temperature program was as follows: a starting temperature of 60ºC was maintained for 1 minute, ramped at a rate of 30ºC min^{-1} to 150ºC min^{-1} and held for 4 minutes, then ramped at a rate of 15ºC to 250ºC and held for 5 minutes. The carrier gas used was nitrogen, and the injection mode was split with a purge duration of 0.10 min and a split of 1:50. The sample injection volume was 1µL and the FID was supplied with 30 mL min^{-1} hydrogen and 300 mL min^{-1} synthetic air.

6.2.6 Pesticide toxicity index (cumulative risk)

Cumulative risk assessment is an imperative way to study hazards related to various residues. The Toxicity Quotient (TQ) which is used to evaluate the toxicity level of the individual pesticide and is calculated from the ratio between each pesticide residue concentration and the corresponding maximum residue levels (MRLs). The pesticides toxicity index (PTI) which is

a screening method for determining the degree of toxicity exposure to complex pesticide mixtures, is then calculated as the total of the TQ for each pesticide compound, as shown in equation 6.1 and 6.2.

$$TQ = \frac{C}{MRL} \quad (6.1)$$

$$PTI = \Sigma\, TQ \quad (6.2)$$

Where: C represents the measured individual pesticide residue concentrations (µg g^{-1}), and MRL represents the individual pesticide's maximum residue limits (µg g^{-1}). PTI tolerable target of less than 1.00, stances no detriment to the consumer (Ramadan et al., 2020; Shalaby et al., 2021).

6.2.7 Health risk assessment

The health risk index (HI) is used to characterize the health risk (HR) assessment of consumers posed by the intake of pesticide-contaminated vegetables. As shown in equation 6.3 and 6.4, the HI is calculated by dividing the estimated daily intakes (EDI) by the corresponding values of the WHO/FAO-established acceptable daily intake (ADI), (Khan et al., 2020). If the HI is less than 100%, it means that there is no risk to human health (Akoto et al., 2016).

$$EDI = \frac{A\ X\ B}{C} \quad (6.3)$$

$$HI = \frac{EDI}{ADI}\ X\ 100 \quad (6.4)$$

Where: *A* is the concentration of the pesticides residue detected in the crop (µg g^{-1}). B represent the average daily vegetable consumption. C represents the average body weight for South Africans (70.8 kg for adults and 20.8 kg for child) (Walpole et al., 2012).

6.2.8 Statistical analysis

The statistical differences between treatments will be determined by an analysis of variance (ANOVA) employing the MSTAT-C statistical software, Version 2.10 (MSTAT, Michigan

State University). Comparisons between means will be carried out according to Duncan's test. Differences will be considered significant at a P level of <0.05.

6.3 Results and discussion

6.3.1 Herbicides uptake assessment

In each sampling day, the maize plants were sampled with their corresponding soils in each treatment plot. The soil, root, stalk, leaves samples were analysed on the first three months until maize and tassel started shooting on the fourth and fifth month of the planting season. The varying concentrations were observed in each plot. In all treatments, the pesticides showed the ability to be taken up by roots thereby translocated into different parts of the maize plant because of the direct contact between soil and roots. The absorption of glyphosate by soil was observed to be higher than the other herbicides in all treatments (0.25, 0.50 and 0.75 g L^{-1}) with concentrations ranging between 0.23-0.53 µg g^{-1} (Table 6.1), 0.55-0.78 µg g^{-1} (Table 6.2) and 0.73-0.99 µg g^{-1} (Table 6.3), respectively. There were significant differences observed in the glyphosate mean concentrations in all treatments as 0.25 g L^{-1} showed ($p<0.021$), while 0.50 g L^{-1} showed $p<0.002$ then 0.75 g L^{-1} showed $p<0.035$. This could be due to the glyphosate ability to bind to minerals and soil organic matter in the soil through carboxyl, amine, and phosphate functional groups thereby generating bidentate and tridentate complexes with soil structures (Duke et al., 2012). Also, it was observed that 2.4-D absorption by the soil was the least in all treatment (0.25, 0.50 and 0.75 g L^{-1}) yielding 0.05-0.25 µg g^{-1}, 0.30-0.56 µg g^{-1} and 0.51-0.72 µg g^{-1}, respectively. This could be due to its intermediate solubility resulting to its lower absorption ability by clay through the carboxylic group, with sorption being negatively connected to the amounts of hydrogen ions (H^+) present on clay soil (pH 5.95), (Paszko et al., 2016). There were significant differences observed in 0.25 g L^{-1} treatment, soil showed $p<0.002$**,** stalk $p<0.012$, leaves $p<0.043$, corn $p<0.039$ and tassel $p<0.045$. The most important physicochemical properties of the herbicides such as low solubility and log K_{ow}, affect the rate of absorption in the soil thus, affecting their translocation to different maize segments. In the studied herbicides, glyphosate has lower solubility (mg/L) and lower log K_{ow} (17 and -3.40) followed by mesotrione (22 and 0.90), atrazine (34 and 2.61) then 2.4-D (64 and 5.78), (Farajzadeh et al., 2019). The lower solubility and log K_{ow} of glyphosate and mesotrione favoured their rapid absorption in soil and crops as compared to atrazine and 2.4-D. As a result,

their absorption in soil showed ascending order of glyphosate>mesotrione>atrazine>2.4-D in all treatments.

Pandey et al. (2020) reported significant leaching of 21.99 µg L^{-1} and surface runoff of 42.25 µg L^{-1} losses of chlorantraniliprole insecticide due to high rain which occurred after 4 hours of pesticides application. In 2021/2022 planting season, the province of KwaZulu-Natal (current study area) experienced a high volume of rain which might have resulted to surface run-off and leaching of herbicides leading to their loss to non-target areas due to water stagnation and high intensity rains that happened immediately after pesticide application. Thus, lesser herbicides were available in the soil for uptake and translocation, which could be the reason for lower concentrations found in different parts of the plants. The wet growing season influenced mesic (moist) conditions which resulted in a thinner and more polar cuticle as compared to crops produced under xeric (dry) conditions (Comas et al., 2013). The cuticle is the part of a leaf that consists of plates and protuberances of wax imbedded in different layers of cutin- a mixture of polymers of dicarboxylic and hydroxycarboxylic acid ester. This explains the high absorption of all four analyte in the leafy segment of the maize plant based on the "like dissolve like" phenomenon as compared to other maize segment. The exterior region of the cuticle allows non-polar herbicides to enter, whereas the inner portion allows polar herbicides to be absorbed (Matzenbacher et al., 2014). The corn and tassel detected lower residues when compared to other plant parts which could be due to the maize sending less residues to the most prominent part of the plant (Dlamini, 2015).

The detected concentration showed increasing trend as the treatment concentration increased with time, which was observed in all treatments. The 0.25 g L^{-1}, 0.50 g L^{-1} and 0.75 g L^{-1} showed increasing mesotrione concentration after 120 and 140 days with 0.34 µg g^{-1} and 0.42 µg L^{-1}, 0.42 µg g^{-1} and 0.51 µg g^{-1}, and 0.74 µg g^{-1} and 1.71 µg g^{-1}, respectively. However, atrazine showed 0.34 µg g^{-1} after 120 which decreased to 0.28 µg g^{-1} after 140 days. This could be due to the fact that atrazine has a shorter lifetime in acidic soil resulting in it degradation on clay structures as the growth time increases consequently affecting the translocation (Pérez et al., 2022).

Glyphosate and mesotrione both showed high absorption rate in soil, roots, stalk and leaves while mesotrione was also highly absorbed in corn and tassels in all treatments. This is due to the fact that mesotrione adsorb on soil through the hydrogen of the aromatic ketone and the

adsorption is mostly higher in acidic soils, where mesotrione primarily exists as a neutral molecule rather than anion (Pintar et al., 2020). The corn concentrations did show significant difference at 0.75 g L^{-1} treatment with atrazine showing $p<0.044$, 2.4-D ($p<0.025$), glyphosate ($p<0.035$) and mesotrione $p<0.030$ in soil. The tassel concentrations did show significant difference at 0.75 g L^{-1} treatment with atrazine showing $p<0.016$, 2.4-D ($p<0.052$), glyphosate ($p<0.044$) and mesotrione $p<0.025$ after 140 days. Fauzi and Ngawit (2021), studied the effect of herbicides residues (glyphosate, atrazine, alachlor, oxadiozon and 2,4-D amine) on maize growth and yield. Glyphosate and atrazine were observed to be highly efficient against weeds management in maize, while alachlor, oxadiozon, and 2,4-D amine were efficient against broadleaf weeds. In this study, glyphosate and atrazine residues were 0.086 µg g^{-1} and 0.088 µg g^{-1} in soil.

Table 6.1: Concentrations (µg g^{-1} dry weight) of residues from 0.25 g L^{-1} treatment plot

Days	Herbicide	Concentration					
		Soil	Roots	Stalk	Leaves	Grain	Tassel
30		0.27 ± 0.29	0.20 ± 0.42	0.10 ± 0.45	0.25 ± 0.35	-	-
60		0.31 ± 0.42	0.19 ± 0.89	0.22 ± 0.76	0.28 ± 0.75	-	-
90	Atrazine	0.25 ± 0.68	0.31 ± 0.23	0.25 ± 0.90	0.36 ± 0.21	-	-
120		0.42 ± 0.04	0.32 ± 0.11	0.45 ± 2.12	0.61 ± 0.01	0.34 ± 0.81	0.12 ± 0.97
140		0.39 ± 0.08	0.36 ± 2.41	0.40 ± 1.56	0.42 ± 0.98	0.28 ± 0.11	0.26 ± 1.21
P value		***0.009***	***0.046***	***0.001***	***0.032***	***0.022***	***0.035***
30		0.10 ± 0.86	0.09 ± 3.42	0.11 ± 0.74	0.15 ± 2.14	-	-
60		0.05 ± 1.34	0.11 ± 1.42	0.19 ± 0.21	0.36 ± 1.12	-	-
90	2.4-D	0.15 ± 0.95	0.15 ± 0.88	0.29 ± 0.89	0.31 ± 0.56	-	-
120		0.20 ± 0.10	0.19 ± 0.56	0.32 ± 1.45	0.42 ± 0.97	0.24 ± 4.21	0.09 ± 0.25
140		0.26 ± 0.29	0.22 ± 0.05	0.42 ± 0.32	0.45 ± 0.81	0.29 ± 2.12	0.16 ± 0.12
P value		***0.002***	***0.003***	***0.012***	***0.043***	***0.039***	***0.045***
30		0.23 ±1.36	0.25 ±0.64	0.22 ±0.87	0.28 ±0.19	-	-
60		0.36 ±2.42	0.32 ±0.15	0.39 ±0.46	0.30 ±2.04	-	-
90	Glyphosate	0.42 ±0.45	0.39 ±0.97	0.32 ±0.32	0.46 ±0.86	-	-
120		0.46 ±0.68	0.33 ±0.68	0.38 ±0.12	0.40 ±0.72	0.30 ± 0.16	0.20 ± 1.45
140		0.53 ±0.70	0.42 ±0.89	0.39 ±0.05	0.48 ±0.69	0.38 ± 0.98	0.28 ± 1.05
P value		***0.021***	***0.001***	***0.042***	***0.008***	***0.007***	***0.041***
30		0.28 ± 0.89	0.28 ± 2.89	0.26 ± 3.45	0.32 ± 2.56	-	-

60		0.25 ± 4.12	0.42 ± 2.00	0.35 ± 0.15	0.31 ± 1.48	-	-
90	Mesotrione	0.36 ± 0.15	0.44 ± 0.74	0.49 ± 0.01	0.53 ± 0.23	-	-
120		0.30 ± 0.36	0.49 ± 0.91	0.43 ± 0.34	0.49 ± 0.05	0.34 ± 0.24	0.11 ± 2.45
140		0.48 ± 1.78	0.59 ± 0.82	0.60 ± 0.31	0.65 ± 0.75	0.42 ± 1.45	0.25 ± 1.11
P value		***0.032***	***0.002***	***0.040***	***0.039***	***0.051***	***0.030***

- Not detected

Table 6.2: Concentrations (µg g^{-1} dry weight) of residues from 0.50 g L^{-1} treatment plot

Days	Herbicide	Concentration					
		Soil	Roots	Stalk	Leaves	Grain	Tassel
30		0.44 ± 1.56	0.25 ± 0.77	0.36 ± 0.86	0.32 ± 0.75	-	-
60		0.35 ± 0.46	0.39 ± 0.65	0.38 ± 4.52	0.31 ± 0.49	-	-
90	Atrazine	0.38 ± 0.97	0.31 ± 0.87	0.49 ± 0.89	0.53 ± 0.68	-	-
120		0.46 ± 1.15	0.41 ± 0.44	0.43 ± 0.17	0.49 ± 0.47	0.28 ± 3.42	0.22 ± 0.99
140		0.43 ± 0.59	0.40 ± 1.24	0.60 ± 1.86	0.65 ± 0.05	0.22 ± 0.47	0.30 ± 1.26
P value		***0.032***	***0.045***	***0.030***	***0.001***	***0.050***	***0.028***
30		0.30 ± 0.45	0.18 ± 0.64	0.21 ± 0.86	0.28 ± 0.47	-	-
60		0.48 ± 1.56	0.38 ± 2.56	0.42 ± 5.42	0.48 ± 5.20	-	-
90	2.4-D	0.32 ± 6.45	0.28 ± 1.45	0.38 ± 1.52	0.45 ± 1.56	-	-
120		0.38 ± 2.14	0.33 ± 0.08	0.28 ± 2.32	0.36 ± 0.46	0.15 ± 3.20	0.20 ± 0.89
140		0.56 ± 0.48	0.42 ± 1.45	0.32 ± 1.03	0.62 ± 1.89	0.30 ± 1.56	0.26 ± 0.18
P value		***0.021***	***0.035***	***0.015***	***0.028***	***0.048***	***0.012***
30		0.55 ± 2.45	0.35 ± 0.56	0.41 ± 0.86	0.48 ± 0.03	-	-
60		0.65 ± 1.45	0.52 ± 5.12	0.39 ± 2.35	0.49 ± 0.25	-	-
90	Glyphosate	0.68 ± 0.56	0.49 ± 2.36	0.42 ± 2.10	0.56 ± 0.45	-	-
120		0.76 ± 0.68	0.63 ± 1.20	0.58 ± 0.25	0.65 ± 0.02	0.42 ± 0.35	0.33 ± 0.56
140		0.78 ± 0.95	0.62 ± 1.45	0.59 ± 1.56	0.68 ± 1.02	0.31 ± 2.15	0.29 ± 0.11
P value		***0.002***	***0.009***	***0.035***	***0.038***	***0.049***	***0.031***
30		0.62±5.23	0.30 ± 0.45	0.20 ± 0.62	0.38 ± 1.26	-	-

60		0.51±0.25	0.48 ± 2.36	0.42 ± 0.11	0.40 ± 1.00	-	-
90	Mesotrione	0.56±4.23	0.48 ± 0.65	0.50 ± 2.56	0.62 ± 2.30	-	-
120		0.58±0.54	0.50 ± 0.52	0.49 ± 2.45	0.52 ± 0.45	0.42 ± 0.56	0.31 ± 0.35
140		0.72±1.25	0.59 ± 1.11	0.62 ± 2.65	0.69 ± 5.62	0.51 ± 2.51	0.42 ± 1.45
P value		***0.052***	***0.026***	***0.035***	***0.014***	***0.018***	***0.033***

- Not detected.

Table 6.3: Concentrations (µg g^{-1} dry weight) of residues from 0.75 g L^{-1} treatment plot

Days	Herbicide	Concentration					
		Soil	Roots	Stalk	Leaves	Grain	Tassel
30		0.55 ±0.52	0.45 ±5.32	0.41 ±0.65	0.48 ±0.45	-	-
60		0.65 ±4.52	0.62 ±2.15	0.59 ±0.75	0.49 ±0.65	-	-
90	Atrazine	0.68 ±0.65	0.49 ±1.52	0.42 ±1.25	0.56 ±0.68	-	-
120		0.76 ±1.45	0.63 ±0.25	0.58 ±2.65	0.65 ±0.52	0.52 ±0.65	0.43 ±2.35
140		0.70 ±2.56	0.62 ±0.62	0.50 ±0.95	0.68 ±3.25	0.50 ±1.56	0.39 ±0.52
P value		***0.042***	***0.032***	***0.042***	***0.012***	***0.032***	***0.016***
30		0.62 ±0.65	0.30 ±0.65	0.20 ±2.65	0.38 ±0.35	-	-
60		0.51 ±2.56	0.48 ±2.65	0.42 ±1.30	0.40 ±5.23	-	-
90	2.4-D	0.56 ±1.36	0.48 ±0.35	0.50 ±2.65	0.62 ±2.30	-	-
120		0.58 ±1.01	0.50 ±1.52	0.49 ±0.82	0.52 ±1.65	0.42 ±2.45	0.31 ±3.45
140		0.72 ±0.56	0.59 ±0.62	0.62 ±1.55	0.69 ±0.25	0.62 ±1.25	0.57 ±4.12
P value		***0.025***	***0.036***	***0.024***	***0.006***	***0.041***	***0.052***
30		0.73 ± 3.56	0.65± 4.21	0.58 ± 0.48	0.68 ± 2.32	-	-
60		0.66 ± 1.56	0.72± 2.65	0.51 ± 0.25	0.69 ± 0.65	-	-
90	Glyphosate	0.82 ± 0.85	0.69± 2.39	0.55 ± 0.62	0.76 ± 5.62	-	-
120		0.86 ± 4.51	0.78± 0.32	0.92 ± 3.21	0.98 ± 0.35	0.62 ± 0.65	0.50 ± 0.65
140		0.99 ± 0.65	0.82± 0.28	0.79 ± 0.45	1.02 ± 1.5	0.78 ± 0.81	0.68 ± 0.58
P value		***0.035***	***0.029***	***0.048***	***0.036***	***0.028***	***0.044***
30		0.68±2.45	0.78±2.56	0.66 ±4.56	0.69 ±2.32	-	-

60		0.75± 6.52	0.62±3.65	0.55 ±3.56	0.71 ±0.18	-	-
90	Mesotrione	0.78±0.32	0.64±5.21	0.79 ±2.65	0.83 ±0.98	-	-
120		0.80±6.24	0.71±0.24	0.73 ±0.65	0.89 ±2.65	0.74 ±0.35	0.51 ±0.65
140		0.88±2.35	0.81±1.25	0.89 ±5.26	0.75 ±1.45	0.82 ±2.36	0.71 ±1.45
P value		***0.030***	***0.042***	***0.036***	***0.025***	***0.015***	***0.025***

- Not detected

6.3.2 Pesticide toxicity index (PTI) values (cumulative risk)

The pesticide toxicity index (PTI) was used to estimate toxicity for a combination of all herbicides investigated, whereas the toxicity quotient (TQ) was used to assess toxicity for the individual herbicide. All maize treatment showed a PTI values of <1 (Table 4), thereby suggesting the no possibility of the health hazard to the consumer of this maize in all studies treatments (Khan et al., 2020). Chaikasem and Roi-et (2020) reported a high PTI value of 31.20 on maize grain collected from River Basin area (India) that was irrigated with contaminated surface water. Shalaby et al. (2021), reported a PTI value of 128.44 in potatoes harvested from Dakahlia (Egypt).

6.3.3 Health risk assessment

The potential health effect and the residue levels of exposure over time is considered by assessing health risk status. When the HI exceeds the ADI value of 222 g/person/day and hence exceeds 100% will expose the consumer to the chronical harmful pesticides residue (Shephard et al., 2002). For all the treatments, the HI data for both children and adult were below 100% (Table 6.4) hence, the risk assessment findings for the tested maize crops revealed that there is no possible health risk linked with the intake of these crops by both adults and children. On the 0.75 µg L^{-1} treatment, it was observed that glyphosate and mesotrione showed higher HI in children compared to other treatments in 120 and 140 sampling days with 3.10, 3.90 and 3.70, 4.10, respectively. This is due to the lower average body weight for children compared to adults, thereby posing high risk to children than in adults. Hossain et al. (2015) reported HI for carbaryl in tomato (1.09), chlorpyrifos and carbofuran in brinjal were 1.97 and 1.17, respectively. Shalaby et al. (2021) reported <100% HI in potatoes, pepper, cucumber, and eggplant crops obtained from Dakahlia's agricultural areas. Although ingestion of these maize crops is unlikely to produce immediate health problems, continuous exposure can lead to an adverse health effect due to the toxicity and carcinogenicity of these herbicides to consumers (Gandhi et al., 2021).

Table 6.4: Toxicity Quotient and health risk assessment detected in maize crops

Days		0.25 g L^{-1} treatment			0.50 g L^{-1} treatment			0.75 g L^{-1} treatment		
	Herbicides	TQ	HI (%) adult	HI (%) children	TQ	HI (%) adult	HI (%) children	TQ	HI (%) adult	HI (%) children
120	Atrazine	0.01	0.49	1.70	0.01	0.40	1.40	0.01	0.74	2.60
140	Atrazine	0.01	0.40	1.40	0.00	0.50	1.75	0.01	0.89	3.10
120	2.4 D	0.00	0.34	1.20	0.00	0.21	0.75	0.00	0.60	2.10
140	2.4 D	0.00	0.41	1.45	0.00	0.43	1.50	0.00	0.89	3.10
120	Glyphosate	0,00	0.43	1.50	0.00	0.60	2.10	0.00	0.89	3.10
140	Glyphosate	0.00	0.54	1.90	0.00	0.44	1.55	0.00	1.11	3.90
120	Mesotrione	0.01	0.49	1.70	0.01	0.60	2.10	0.01	1.06	3.70
140	Mesotrione	0.01	0.60	2.10	0.01	0.73	2.55	0.01	1.17	4.10
	PTI	**0.03**			**0.03**			**0.05**		

Note: PTI – pesticides toxicity index; TQ – toxicity quotients; HI – health index.

6.4 Conclusion

This study concludes that all the assessed herbicides are taken up by the soil and translocated to different maize segments particularly the leafy segment. These findings suggested that both livestock and humans could not be harmed by the daily intake of maize grains containing these herbicides residues. The absorption showed ascending order of glyphosate>mesotrione>atrazine>2.4 in all treatments. The PTI of all residues showed no potential health risk to humans more than individual risk index, however, the HI revealed that the maize grain is considered acceptable for consumption. Nevertheless, the results suggest a need for strict monitoring and regular scrutinizing of herbicides residues and their accumulation in all agricultural farms in South Africa. Moreover, a need for harmless herbicides like bio-pesticides should be investigated as pest control safe replacement for these

harmful herbicides. Furthermore, the results showed that maize can be used for phytoremediation of the studied herbicides.

6.5 References

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Chapter 7 Conclusion and future recommendations

7.1 Conclusion

In this study, soil quality, irrigation water quality was assessed before the plantation of maize in Buhle farm. This was followed by the pesticides translocation assessment, maize crop quality and toxicity studies on the planted maize. The cation of interest for irrigation water was Na^+ resulting from the geologic materials which the waters have been in contact and the results showed that the concentration was low (0.05 mg L^{-1}) and so it did not pose any sodium salt hazard. The low sodium adsorption ratio of 2 corroborated the results of sodium hazard of the water. The low EC (1.81 µS m^{-1}) is indicative of a safe water of low salinity. The soil texture high in clay content (56.4%), sand (40.6%) and slit (2.89%) indicated high fertility potential of the soil profile. It was then concluded that soil quality and irrigation water quality were suitable for producing good quality maize. The results of the analysis of key nutrition parameters of maize were consistent with the good quality soil and irrigation water. The results showed that the maize contained high amounts of total anthocyanin, total flavonoids and total phenolic acid compounds. These compounds are considered essential for good health and consumers can be assured of medicinal benefit from consuming this maize. The modified UE-SPE, Soxhlet, MAE and QuEChERS extraction methods showed effectiveness in sample preparation and pre-concentration of the selected pesticides prior to their characterization by GC-FID. The UE and QuEChERS were compared and the results showed that the UE method can be effectively applied without the additional SPE clean up step, making the method cheaper and more available. The MAE and Soxhlet were compared and the results showed that the MAE method can be effectively applied without the additional SPE clean up step. The experimental data proved that the proposed methods have low LODs and LOQs, good repeatability and high recoveries. However, UE and MAE showed higher sensitivity accuracy as compared to QuEChERS and Soxhlet methods. The adsorbed analytes of interest on the soil and maize matrixes were efficiently desorbed by the hexane: acetone (1:1) in the MAE method and by the hexane in Soxhlet method. Even though MAE instrument is expensive, the experimental results showed that the MAE procedures is more advantageous compared to other methods. This is due to its automated process, high recoveries, lower amounts of extracting solvent, increased sensitivity, and precision for herbicides detection and measurement which are all significant for pesticides residue determination in agricultural matrices. Pesticide residues did not exceed the maximum residue limits in the soil and maize samples analysed. This study concludes that all the assessed herbicides are absorbed by the soil and taken up by the plant roots then

translocated to different maize segments particularly the leafy segment. These findings suggested that both livestock and humans could not be harmed by the daily intake of maize crop containing these herbicides residues for a short term. However, harmful effects may occur from small doses ingested over a period of time causing chronic effects including birth defects, toxicity to a fetus, production of benign or malignant tumors, genetic changes, blood disorders, nerve disorders, endocrine. However, their availability in the maize grain will prime to continuous ingestion, potentially leading to harmful long-term consequences on individuals. The absorption showed ascending order of glyphosate>mesotrione>atrazine>2.4 in all treatments. The PTI of all residues showed no potential health risk to humans more than individual risk index, however, the HI revealed that the maize corn is considered acceptable for consumption.

7.2 Recommendations and Future work

The results suggest a need for strict monitoring and regular scrutinizing of herbicides residues and their accumulation in all agricultural farms in South Africa. Moreover, a need for harmless herbicides like bio-pesticides should be investigated as alternative pest control for these harmful herbicides.

APPENDIX

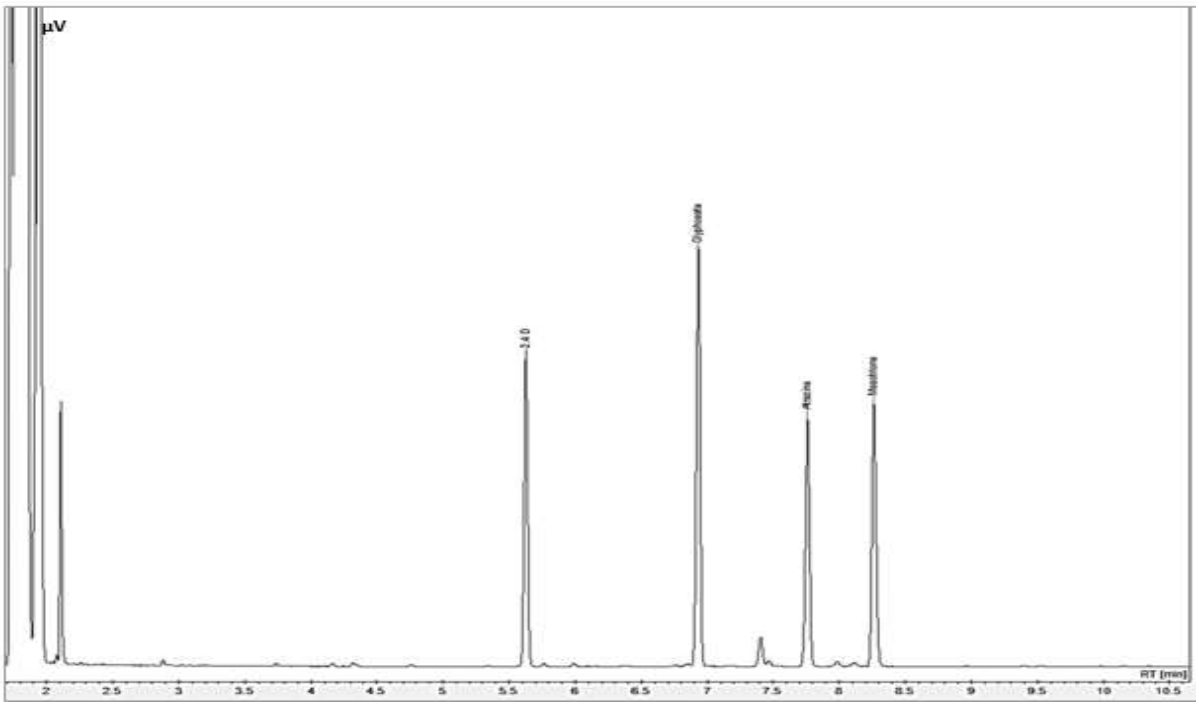

Figure 1: Chromatogram of spiked maize sample.

Mobile phase: nitrogen, Column: VF-5ms capillary column (length 30 m, 0.25 mm i.d, 0.25 m film thickness). The temperature program: a starting temperature of 60ºC was maintained for 1 minute, ramped at a rate of 30ºC min^{-1} to 150ºC min^{-1} and held for 4 minutes, then ramped at a rate of 15ºC to 250ºC and held for 5 minutes.

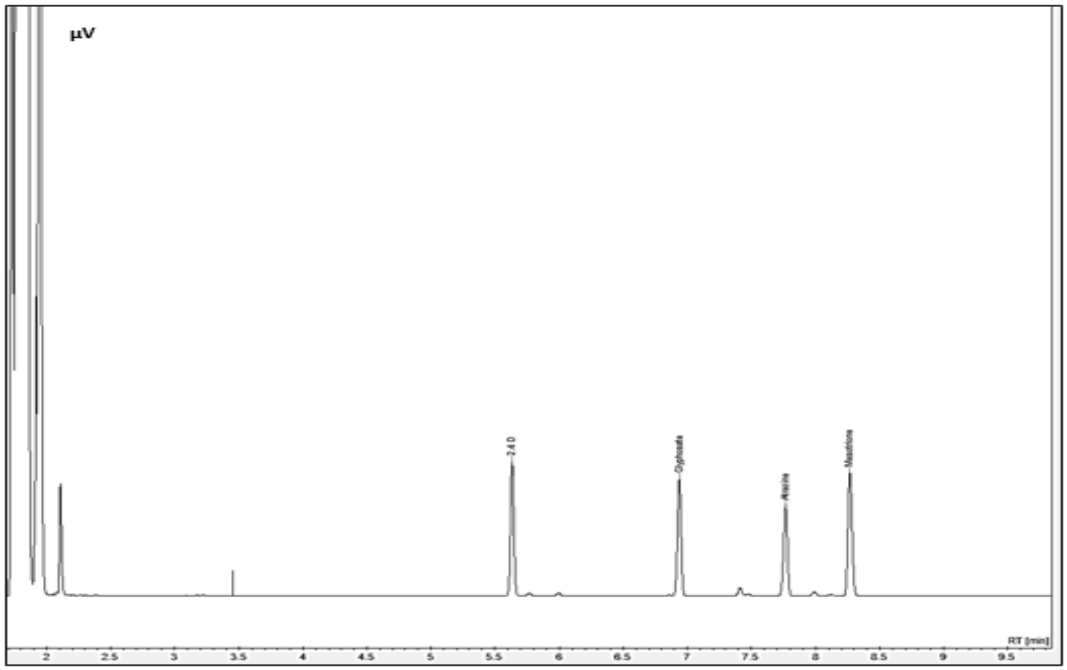

Figure 2: Chromatogram of 0.25 g L^{-1} treatment on maize sample.

Mobile phase: nitrogen, Column: VF-5ms capillary column (length 30 m, 0.25 mm i.d, 0.25 m film thickness). The temperature program: a starting temperature of 60ºC was maintained for 1 minute, ramped at a rate of 30ºC min^{-1} to 150ºC min^{-1} and held for 4 minutes, then ramped at a rate of 15ºC to 250ºC and held for 5 minutes.

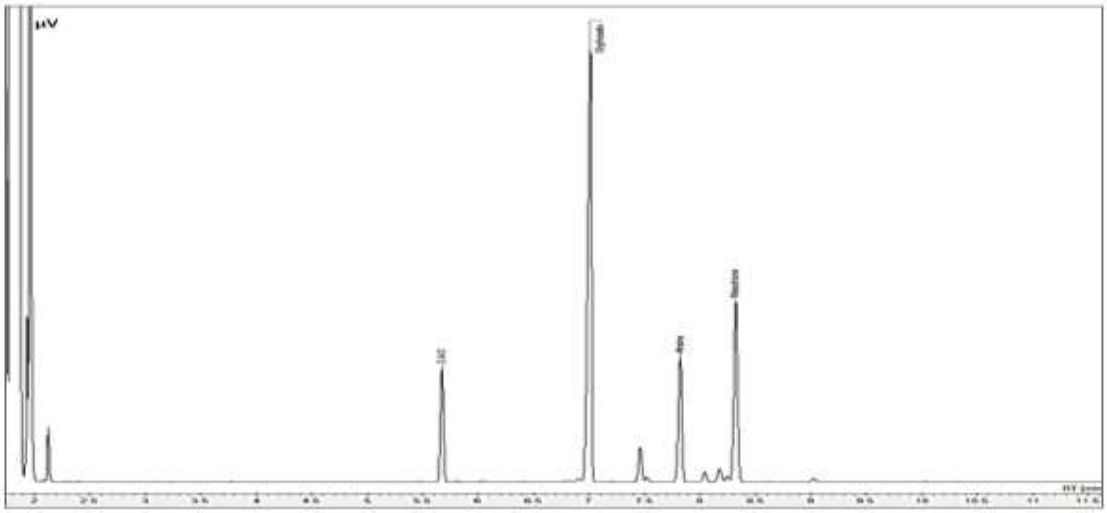

Figure 3: Chromatogram of 0.75 g L^{-1} treatment on maize sample.

Mobile phase: nitrogen, Column: VF-5ms capillary column (length 30 m, 0.25 mm i.d, 0.25 m film thickness). The temperature program: a starting temperature of 60ºC was maintained

for 1 minute, ramped at a rate of 30ºC min^{-1} to 150ºC min^{-1} and held for 4 minutes, then ramped at a rate of 15ºC to 250ºC and held for 5 minutes.

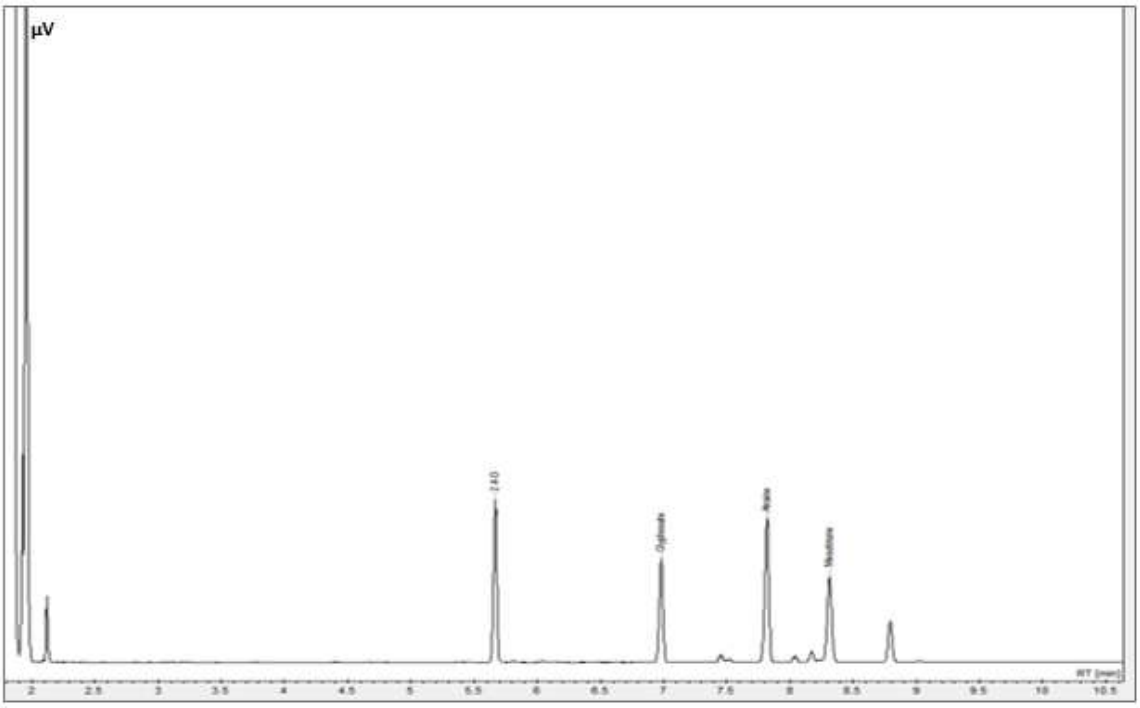

Figure 4: Chromatogram of 0.50 g L^{-1} treatment on soil sample.

Mobile phase: nitrogen, Column: VF-5ms capillary column (length 30 m, 0.25 mm i.d, 0.25 m film thickness). The temperature program: a starting temperature of 60ºC was maintained for 1 minute, ramped at a rate of 30ºC min^{-1} to 150ºC min^{-1} and held for 4 minutes, then ramped at a rate of 15ºC to 250ºC and held for 5 minutes.

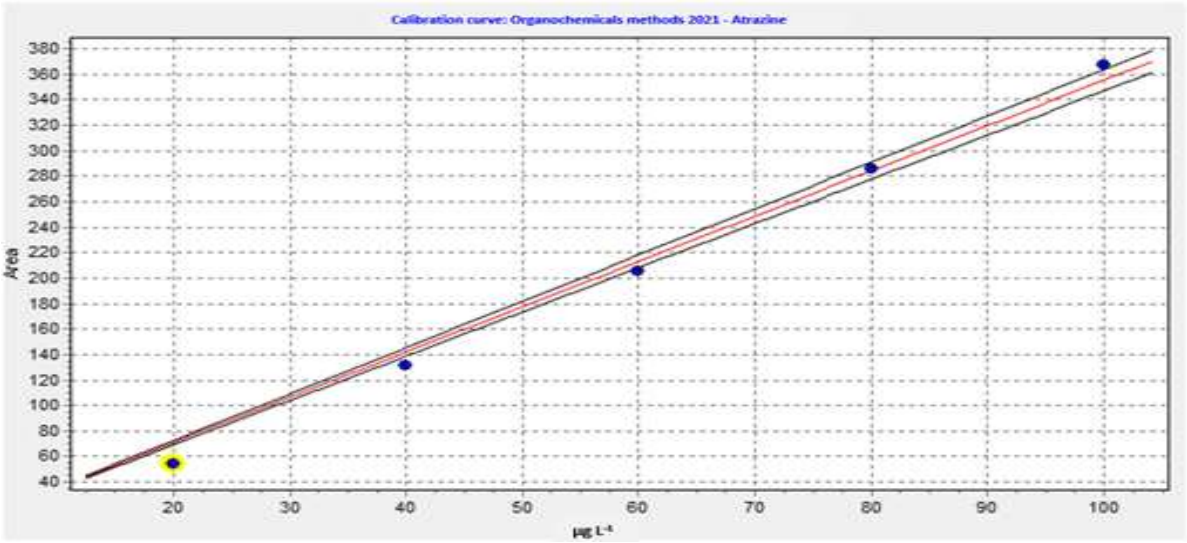

Figure 5: Calibration curve for atrazine obtained using GC-FID.

Vhfb nmm,

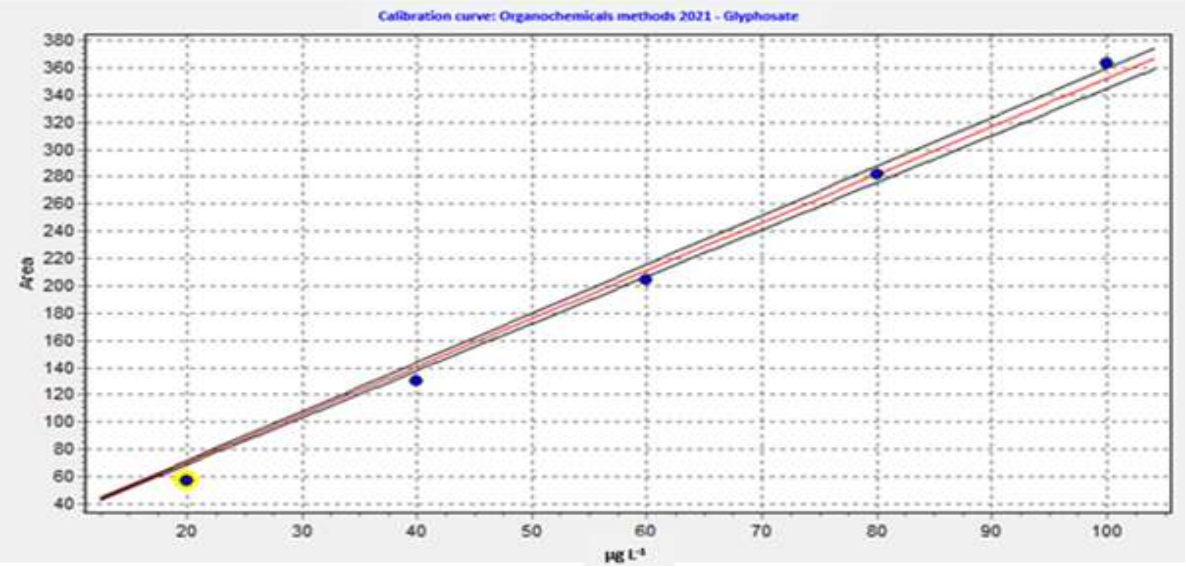

Figure 5: Calibration curve for glyphosate obtained using GC-FID.

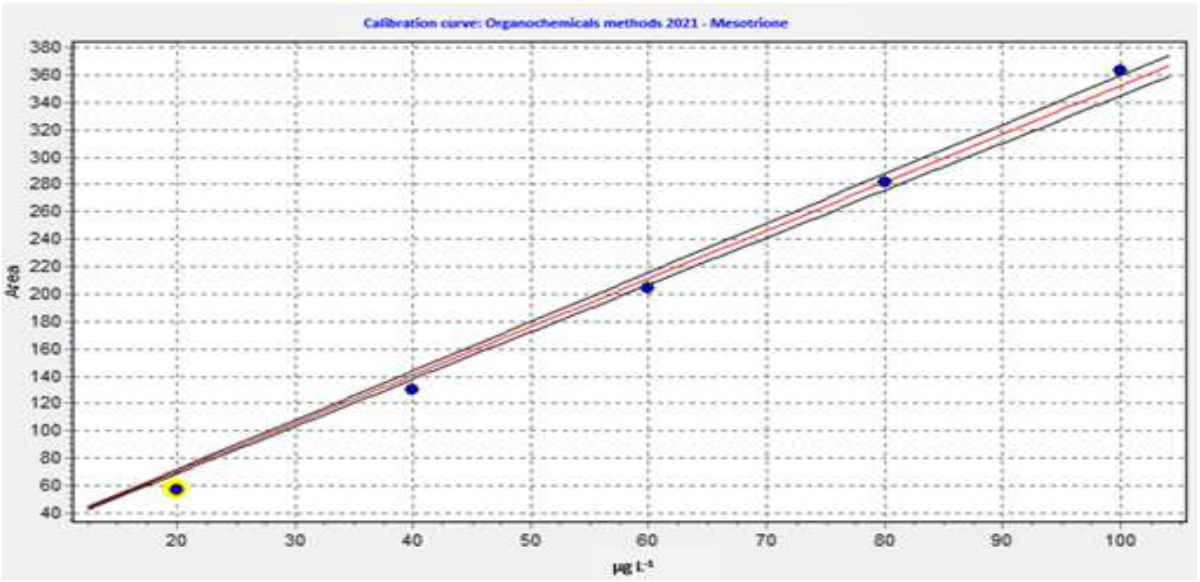

Figure 5: Calibration curve for mesotrione obtained using GC-FID.

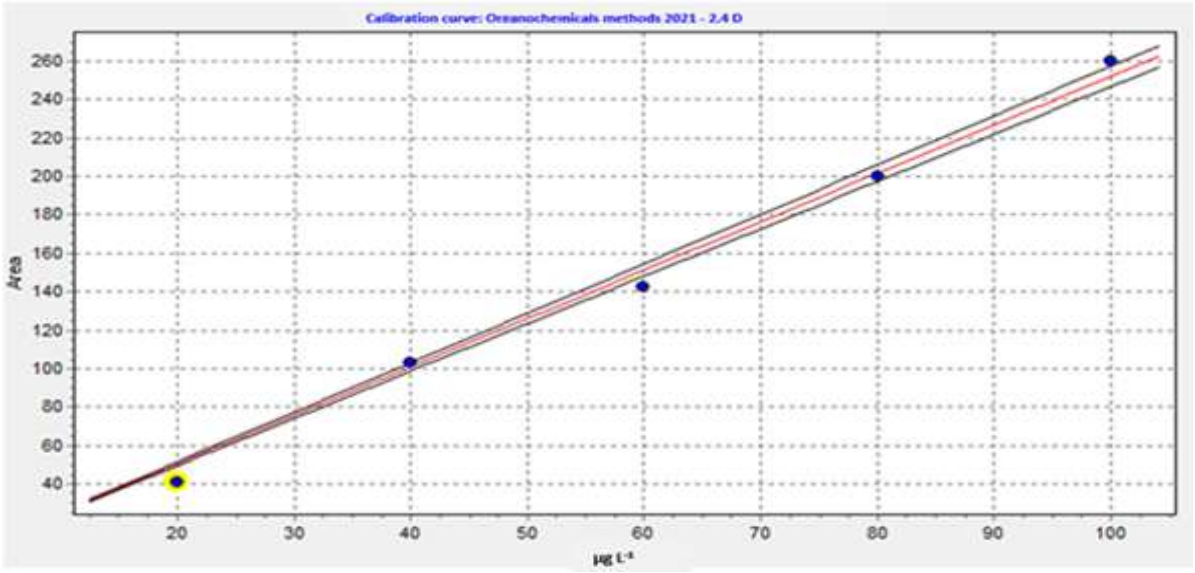

Figure 5: Calibration curve for 2.4-D obtained using

PTI calculation for 0.25 g L^{-1} treatment

$TQ = \frac{Conc}{MRL} while$

$TQ = \frac{0.34}{50} = 0.01$

$PTI = \Sigma\, TQ$

$PTI = 0.01 + 0.01 + 0.00 + 0.00 + 0.00 + 0.00 + 0.01 + 0.01$

$PTI = 0.03$

	0.25 g L^{-1}			0.50 g L^{-1}			0.75 g L^{-1}		
	CONC	MRLS	TQ	CONC	MRLS	TQ	CONC	MRLS	TQ
Atrazine	0,34	50	0,01	0,28	50	0,01	0,52	50	0,01
Atrazine	0,28	50	0,01	0,22	50	0,00	0,5	50	0,01
2.4 D	0,24	500	0,00	0,15	500	0,00	0,42	500	0,00
2.4 D	0,29	500	0,00	0,3	500	0,00	0,62	500	0,00
Glyphosate	0,3	2000	0,00	0,42	2000	0,00	0,62	2000	0,00
Glyphosate	0,38	2000	0,00	0,3	2000	0,00	0,78	2000	0,00
Mesotrione	0,34	60	0,01	0,42	60	0,01	0,74	60	0,01
Mesotrione	0,42	60	0,01	0,51	60	0,01	0,82	60	0,01
PTI			**0,03**			**0,03**			**0,05**