ASSESSMENT OF ORGANOPHOSPHATE PESTICIDE RESIDUES IN AGRICULTURAL SOIL AND THEIR ASSOCIATED HEALTH RISK IN SIRINKA WOLDIA, ETHIOPIA

A Thesis Submitted to Department of Chemistry, College of Natural and Computational Sciences, Presented in Partial Fulfillment of the Requirements of Master of Science Degree in Chemistry (Analytical).

Office of Graduate Studies

Woldia University

By: Wendifraw Teshome Yimer

Advisor name: Ashenafi Zeleke (PhD)

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Woldia, Ethiopia

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Advisor approval sheet

I, the advisors of the thesis entitled "assessment of organophosphate pesticide residues in agricultural soil with their associated health risk in sirinka woldia, Ethiopia "Submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry in the graduate program of the Department of Chemistry, which has been conducted by Wendifraw Teshome, I hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Name of advisor	Signature	Date
Name of co-advisor	Signature	Date

We, the undersigned members of the Board of Examiners of the thesis by Wendifraw Teshome, have read and evaluated the thesis entitled Determination of organophosphate pesticides in agricultural soil with associated health risk assessment in Sirinka Woldia, Ethiopia" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement for the degree of Master of Science in Chemistry (Analytical).

Chairperson	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and Faculty Graduate Committee (SGC).

Head, Department	Signature	Date
Faculty Dean	Signature	Date
Office of Postgraduate Studies, Dean	Signature	Date

DECLARATION

I declares that this Ms.c thesis on the title, “assessment of organophosphate pesticide residues in agricultural soil with their associated health risk in Sirinka Woldia, Ethiopia**”** is my original work and that all sources of material used for this thesis have been dully indicated and acknowledged with complete references.

_____	_____	_____
Student name:	Signature	Date

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Last but not least, I would like to appreciate and thank all the staff of the departments of soil resource and watershed management that can give me a chance to study my master's degree.

RECOMMENDATION

I, the advisor(s) of this thesis, hereby certify that I have read the revised version of the thesis entitled "assessment of organophosphate pesticide residues in agricultural soil with their associated health risk in sirinka woldia, Ethiopia" prepared under my guidance by Ashenafi Zeleke (PhD), submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry (Analytical). Therefore, I/we recommend the submission of a revised version of the thesis to the department, following the applicable procedures.

_____	_____	_____
Major Advisor	Signature	Date

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LIST OF ABBREVIATION AND ACRONYM

SARC	Sirinka Agricultural Research Center
OCPs	Organochlorine Pesticides
OPPs	Organophosphate Pesticides
GC-MS	Gas Chromatography-Mass Spectrometer
FAO	Food and Agriculture Organization
WHO	World Health Organization
ASEAN	Association of Southeast Asian Nations European Union
MRLs	Maximum Residue Limits
ADD	Average Daily Dose
EU	European Union
USEPA	United States Environmental Protection Agency
IASS	International Agricultural Soil standards
OM	Organic Matter

ABSTRACT

Pesticides are used to eliminate various pests in agriculture, including fungi, insects, rodents, and unwanted plants. To control these pests and improve crop yields, a diverse array of organophosphorus pesticides (OPPs) are frequently employed. In this study, analysis of some selected organophosphorus pesticides was carried out in soil samples collected from two sites in Sirinka, woldia Ethiopia. The soil samples was extracted using the QuEChERS method. Agilent Technologies 7000C gas chromatography coupled with a mass spectrometric detector (GC-MS) was utilized for chromatographic quantitative determination of the pesticides. The instrument calibration curves for each analyte constructed in soil samples were linear in the concentration range of 2–50 μg/kg with $R^2 > 0.99$, and the LODs and LOQs of the method were in the range of 0.06–0.45 μg/kg and 0.2–1.5 μg/kg, respectively. The recovery and precision study results were within acceptable standard guidelines. Three organophosphate residues were detected in the soil samples at varying concentrations. The rank order of OPPs detected in soil samples was malathion (4.42 $\mu g\ kg^{-1}$*) > diazinon (1.24* $\mu g\ kg^{-1}$*) > Chlorpyrifos (1.16*$\mu g\ kg^{-1}$*) from SARC and malathion (2.63* $\mu g\ kg^{-1}$*) > diazinon (1.23* $\mu g\ kg^{-1}$*) > chlorpyrifos (1.15* $\mu g\ kg^{-1}$*) from FAR, respectively. The level of OPP residue in the soil samples was lower than the US MRLs for agricultural soils. Physicochemical analyses of the farming soil were conducted to investigate their potential influence on pesticide accumulation. The results showed that pH (7.91 ± 0.02), electrical conductivity (EC) (0.2 ± 0.003), organic carbon (OC) (0.59 ± 0.11), available phosphorus (4.0 to 15.8 mg* kg^{-1}*), and available nitrogen (0.022 to 0.17 kg/ha) all fell within the permissible limits established by the IASS. Non-dietary health risk assessment was conducted by calculating ADD from ingestion, inhalation, and dermal contact pathways (HQ, HI) and following non-carcinogenic and carcinogenic risks in adults and children. Hence, the hazard index (HI) values obtained for exposure to pesticides in soil in adults and children were* $1.5x10^{-5}$ *and* $7.61x10^{-5}$*, respectively. The findings indicated that the concentrations of OPPs were within the permissible limits of USEPA; thus, the human population in the study area is in safer range.*

Key words: Organophosphate Pesticides, GC-MS, Soil, Health risk,

CHAPTER ONE

1. Introduction

1.1. Background of the study

Pesticides are synthetic chemicals used to control weeds and harmful pests in agricultural fields and lawns. These compounds provide a wider defensive purpose for humans against disease-carrying insects. Additionally, these are employed to shield crop plants from rodents, fungus, and arthropods that degrade them [1] There has been a noticeable surge in the application of several pesticides to agricultural crops in the past few years. Miller (2004) and Chiaia-Hernandez et al. (2017) have documented that a substantial proportion of insecticides and herbicides applied, specifically 98% and 95%, respectively, often deviate from their intended targets, resulting in contamination of land and water bodies [2, 3]. When pesticides are used in large quantities, they destroy not just insects and other invertebrates but also animals, birds, and flowers.

Contamination of the natural environment with pesticides has been a major concern to researchers because of their toxicity and threat to human, soil, water, animal, and plant health [4, 5]. Pesticides that reach the soil after spraying can adversely affect soil quality by harming essential soil organisms such as earthworms, bacteria, and natural enemies, which play crucial roles in decomposition and nutrient cycling [6]. This could reduce the biomass of soil microorganisms by directly affecting or destroying their behavior, reproduction, and metabolism, which could irreversibly damage and change the highly interdependent ecosystem [7, 8]. Pesticide residues in soil can exhibit phytotoxicity, contaminate plant tissues, and ultimately enter the food chain via root uptake by plants. In addition, pesticide residues in soil and the environment have the tendency to pollute groundwater and surface water through leaching and surface runoff, thereby increasing the risk of environmental contamination. There have been reports that increased pesticide residue deposition in the food supply and drinking water poses substantial risks to human health [9, 10]. For instance, thyroid dysfunction, low sperm count in men, birth defects, an increase in testicular cancer, reproductive[11] and immune malfunction[12], endocrine disruption, cancers, immunotoxicity [13], and Neurobehavioural and developmental disorders [8] are all reported to be affected by exposure to pesticide residues in food and drinking water.

These pesticides have such a wide spatial dispersion that their concentration in the soil continues to rise, sometimes even after heavy rain [14]. They permeate deeper soil layers and become persistent, so they exhibit their damaging effects over many years by changing how quickly living things breathe and how much CO_2 they produce [15].

Particularly, organophosphate pesticides are regarded as one of the broad-spectrum pesticides that may effectively control a variety of pests. The growth of germs, pests, and weeds can be efficiently controlled by broad-spectrum organophosphate pesticides, which also have great efficacy and can boost agricultural output. In the other hand their excessive use cause nerve poisons by causing stomach poison, contact poison, and fumigant poison. A class of pesticides known as organophosphates includes several extremely hazardous substances. They were among the most extensively used pesticides until the turn of the 21st century. All of them have the potential to produce acute and sub-acute toxicity, and 36 of them are currently registered for use in the United States [16]. These are now the pesticides that are most commonly used [17], yet 70% of organophosphate pesticides (OPPs) are extremely hazardous. OPPs are suspected of causing or exacerbating a number of human health issues, including cancer, immune system interference, and hormonal disturbance [18]. Chlorpyrifos, parathion, diazinon, famphur, phorate, terbufos, and malathion are examples of extensively used OPPs.

Herbicides and insecticides are used in Ethiopia for both agricultural production enhancement and public health campaigns. OPPs are among the pesticides that are commonly used in the country's rural areas [19]. OCPs and other products of OPP degradation are classified as persistent pollutants on a global scale. Most of these chemicals have restricted uses because of their harmful qualities [20]. Nonetheless, the extensive environmental damage caused by the use of OCPs and OPPs for agricultural and health purposes in underdeveloped countries. Because of these pesticides' low volatility, chemical stability, environmental resilience, lipophilicity, and slow metabolic breakdown, they have bio-accumulated in birds, animals, and the food chain[21]. Pan *et al*. (2018) reported mobility, persistence, and volatility of different pesticides in soil may be dependent on physicochemical properties of soil. For example content of phosphorus and nitrogen, level of organic carbon, electrical conductivity and also pH of soil were affect the nature of pesticide in the soil [22]. Yao et al. (2022) stated that there was a direct relationship between numbers of the pesticides detected in

soils and organic carbon matter, diversity of latitude, the difference in temperature, environment of region [23]. Yadav et al. (2016) stated organic matter of soil can decrease the mobility of different pesticides and effect on the biodegradability, reachability, volatility, persistence, and bioactivity of pesticides in the soil [24]. Due to this reason, the physicochemical properties of soil is assessed in this study to correlate its effect on OPPs accumulation.

The Sirinka Agricultural Research Center (SARC), a major agricultural research institute in northern Ethiopia, has relied on pesticides, particularly organophosphate pesticides, to manage pests and diseases in its crops. While these pesticides have been effective in increasing agricultural productivity, their frequent use raises concerns about potential environmental and health risks. To date, there has been no assessment of organophosphate pesticide residues in the surrounding agricultural soil. This study, therefore, aims to fill a critical knowledge gap by assessing organophosphate pesticide residues in agricultural soils surrounding SARC. This investigation will involve measuring residue levels and evaluating the associated human health risks.

1.2. Statement of the problem

Organophosphate pesticides are widely used in agriculture, making them well-known environmental pollutants. They have been discovered in different levels in vegetables, soil, and surface waters [25]. As a result, productivity in current agriculture has greatly increased due to the usage of pesticides. However, it has also resulted in an extensive increase in the amount of pesticides in food and the surrounding environment, with harmful impacts on human health. Pesticide poisoning occurs in thousands of millions of cases worldwide each year [26]. Environmental scientists have recently expressed serious concerns about the amount of pesticides used, how they are applied, and whether or not they are abused, particularly in agriculture[27].

The idea of soil contamination has turned out to be one of the primary environmental troubles these days. Soils are directly or indirectly contaminated by the effluence of industry and agricultural practices. As a result, pesticides in soil would have a major preoccupation with toxicity to human beings, animals, plants, aquatic life, and the environment. Regular monitoring of the concentrations of pesticides in the soil is therefore necessary to prevent the risk of toxicity. Literatures findings confirmed that contamination of soil with pesticides causes diverse health effects after prolonged

periods of exposure. In this context, a quality assessment of the soil in Habru woreda, especially in SARC and its surrounding is mandatory. It is necessary to evaluate the levels of pesticide residues in the environment because of the importance of pesticide residues for public health and the lack of knowledge about the long-term consequences of low-dose exposure to these pesticides on people and the environment. Because of its widespread use and ease of access, organophosphate is the cause of many deaths globally [28]. In developing nations like Ethiopia, where many farmers lack adequate pesticide safety knowledge, the risks associated with pesticide exposure may be significantly higher. While organophosphate insecticides are widely used in Habru woreda to enhance agricultural yields, farmers often lack awareness of the potential health hazards.

The reason for selecting Sirinka and SARC as the study area is mainly because soil is one of the major resources for crop production, and there is a high consumption of agrochemicals (insecticides and herbicides). In this area, the level of pesticides in the soil has not been studied yet. In this context, a quality assessment of the soil in Habru woreda, especially in Sirinka, is necessary. Hence, this study attempts to show the level of pesticide use and suggest possible solutions or recommendations for improving the quality of soil, reducing the risk to health, and filling these gaps.

1.3. Objective of the study

1.3.1. General objective of the study

The general objective of this study was to assess the levels of organophosphate pesticides with their associated health risks of the agricultural soil collected around Sirinka and SARC.

1.3.2. Specific objective of the study

The following specific objectives were studied to achieve the aforementioned general objective.

- To extract OPPs from selected agricultural soil using organic solvent.
- To determine the levels of OPPs in selected agricultural soil.
- To characterize some physicochemical parameters of the agricultural soil sample.

- To compare the levels of obtained pesticides with acceptable standards for each pesticide.
- To assess possible health risks of selected OPPs for both children and adults ages.

1.4. Significance of the study

The assessment of pesticide levels in agricultural soils in Sirinka were used to sensitize the concerned bodies on the importance of environmental conservation. Therefore, this study was mainly focused on providing adequate information on the levels of OPPs pesticides in selected agricultural soil to ensure the health and dietary safety of society. The findings of this study offer valuable baseline information on the levels of pesticide in soil for further researching regarding the general analysis of pesticide residue in the soil matrix and provide information for the relevant institutions and organizations to act appropriately for the welfare of society in light of the findings. This study report will communicate the level of pesticide pollution in the study region to those responsible for environmental management bodies. It provides baseline data for the creation of guidelines pertaining to Ethiopia's soil's maximum allowable residual level for organophosphates.

CHAPTER TWO

2. Literature review

2.1. General over view of pesticides and its application

Complex chemical and biological systems exist in the environment (atmosphere, hydrosphere, and lithosphere), and they can be transported from one environmental compartment to another by natural processes or human activity. For example, a variety of pollutants, originating from agricultural activities, municipal discharges, industrial wastes, and natural processes such as the decomposition of living organisms, are found in many bodies of water [25, 29]. These substances include organic and inorganic contaminants, minerals, salts, and other substances.

Chemical pesticides are widely utilized for many purposes, such as managing forests, preventing parasite illnesses caused by insects, building railroads, and controlling weeds in agriculture. To stop animals, weeds, insects, and microbes from attacking crops, chemicals referred to as pesticides are used [29, 30].

Pesticides are made up of a large range of chemical classes with distinct physical and chemical characteristics. Certain compounds exhibit persistence, while others break down in the environment to yield varying levels of toxicity and distribution that closely resemble those of their parent compounds. As a result, they may have a negative impact on ecosystems overall and non-target organisms [30]. Pesticide use in agriculture steadily increased after World War II, increasing food production worldwide. The first artificial organic insecticides were organochlorine compounds, including DDT, which were first produced commercially in 1943[25]. There is a constant need for food and food items due to the growing global population. The performance of the current agricultural areas needs to be significantly improved due to the relative scarcity of new regions that are appropriate for agriculture.

A significant portion of the pesticides employed in agricultural areas are carried by surface runoff into lakes, rivers, and streams where they either volatilize into the atmosphere or leak into groundwater systems [31]. Therefore, leftovers from these chemicals could be significant contributors to pollution in the environment. They could be in the sky, runoff from severe rains, irrigation, ground water, surface water, or the land where a crop was produced. They could therefore also contaminate food, food items, and biological systems either directly or indirectly. Their persistence has also

shown to be a major problem, especially in systems that contain both surface and ground water [32].

2.2. Definitions of pesticide

In 2003, the Food and Agriculture Organization of the United Nations defined Codex Alimentarius as "any substance intended for preventing, destroying, attracting, repelling or controlling any pest, including unwanted species of plants or animals during the production, storage, transport, distribution, and processing of food, agricultural commodities, or animal feeds, or which may be administered to animals for the control of ectoparasites."[33]

The names are derived from the Latin or scientific name for the group. The ending or suffix -cide means kill or killer. But not all pesticides end with -cide. Examples include: growth regulators, which stimulate or retard the growth of pests; defoliants, which cause plants to drop their leaves; desiccants, which speed the drying of plants for mechanical harvest or cause insects to dry out and die; repellents which repel pests; attractants, which attract pests, usually to a trap; and chemosterilants, which sterilize pests. Pheromones, are scents produced by animals to communicate to other members of the same species, they are used as attractants to monitor or trap insects. Finally, the term biocide is often referred to as a pesticide that kills a wide range of organisms and is toxic to both plants and animal. A pesticide product is actually a mixture of a pesticide as the active component and various inert chemicals as transporters, solvents, or propellants as the inert ingredients. Application of pesticides has significantly reduced crop losses caused by infections (including bacteria and fungus), viruses, animal pests, and weeds. Use of agricultural pesticides has enhanced agricultural output globally and thereby helped ensure food security [33].

2.3. Classification of pesticides

A vast range of chemical substances, including many biological agents, are included in pesticide active components. Since many pesticide structures are quite intricate, they cannot all be categorized into one group. Classification schemes have emerged to account for the expanding spectrum of chemical and biological agents employed in pest management or control[34]. Pesticides are frequently categorized according to their target pest species, chemical makeup, and mechanism of action.

2.3.1. Based on target organism or function

Table 1. Pesticide classification by target pests adopted from [35].

Types of pests	Target pests/function	Examples
Bactericides	Kill bacteria or acts against bacteria	Copper complex
Acaricides	Kill mites that feed on plants and animals	Bifenazate
Rodenticides	Control mice and other rodents	Warfarin
Algaecides	Control or kill growth of algae	Copper sulphate
Larvicides	Inhibits growth of larvae	Methoprene
Repellents	Repel pests by its taste or smell	Methiocarb
Desiccants	Act on plants by drying their tissues	Boric acid
Ovicides	Inhibits the growth of eggs of insects and mites	Benzoxazin
Virucides	Acts against viruses	Seytovirin
Molluscides	Inhibits or kill mollusk's *i.e* snail's usually disturbing growth of plants or crops	Metaldehyde
Nematicides	Kill nematodes that acts as parasites of plants	Aldicarb
Avicides	Kill birds	Avitrol
Moth balls	Stop any damage to cloths by moth larvae or molds	Dichlorobenzene
Lampricides	Target larvae of lampreys which are jawless fish like vertebrates in the river	Triflоromethyl, Nitrophenol
Piscicides	Act against fishes	Rotenone
Silvicides	Acts against woody vegetation	Tebuthiuron

2.3.2. Based on target pest

A. Herbicides

Insecticides and fungicides are the next most often used pesticide classes globally, behind herbicides. They are commonly employed to manage weeds in urban areas, industry, and agriculture. They can offer minimal labor costs and excellent weed control. The continuous use of herbicides has increased crop yield while protecting crops from unnecessary weed competition and enhancing food's nutritional content. In order to reduce weeds, herbicides are usually sprayed to agricultural crops both before and after they emerge [36, 37]. When using a herbicide, the selection of a certain herbicide usually depends on several considerations. The first thing to consider is the approach being taken.

The label of herbicides include usage instructions. For example, a pesticide may be labeled for girdling or injection but not for basal spraying. Herbicides ought to be applied solely for the uses indicated on the label. Ease of use, relative availability, worker exposure, environmental safety, firsthand knowledge, and the herbicide's relative efficacy in controlling the target plant species are the other key factors to take into account when choosing a herbicide. It is always necessary to choose a herbicide that will effectively control the target species, even though the relative relevance of these factors may change based on the situation and the individual [37].

Additionally, plants can become resistant to herbicides. For instance, certain plants have become resistant to the herbicides glyphosate and, more recently, atrazine. One weed that has been resistant to glyphosate is marestail. In the vast majority of soybean, cotton, and corn crops in various U.S. states, glyphosate-resistant weeds are present.

B. Insecticides

Insecticides are substances, either biological or chemical, that are used to control insects. Control might be achieved by eliminating the bug or by taking additional actions to prevent it from operating in a way that is deemed harmful [38]. Pests are managed with insecticides, which come in a range of formulations and delivery systems (sprays, baits, slow-release diffusion, etc.). They may be synthetic or natural.

In recent times, biotechnology has gone so far as to introduce bacterial genes that code for insecticidal proteins into a variety of crop plants, killing off naive bugs that feed on

them [39]. This group of insecticides includes malathion, mercarbam, DDT, aldicarb, carbofuran, pyrethrum, and allethrin.

C. Fungicides

Chemical or biological agents known as fungicides are used to get removal of fungi or fungal spores that significantly reduce agricultural yield, quality, and profitability. They can be administered directly to the soil or sprayed over agricultural areas [40]. A variety of pathogenic fungi can be effectively controlled by broad-spectrum fungicides.
Among the broad-spectrum fungicides include mancozeb, sulfur, and captan. Certain fungicides exclusively work against roomycetes, such as phytophthora; mefenoxam, for instance, has a relatively limited range of activity.

2.3.3. Based on mode of entry

A. Systemic pesticides: Systemic pesticides are pesticides that are absorbed by plants or animals and transferred to untreated tissues. Systemic herbicides move through the plant and can reach untreated areas of leaves, stems, or roots. They are capable of killing weeds with partial spray coverage. They can effectively penetrate the plant tissues and move through the plant vascular system to kill specific pests. Some systemic insecticides are also applied and move through animals to control pests such as warble grubs, lice, or fleas. The movement of pesticides in plant tissues may be unidirectional or multi-directional. Some pesticides may only move in one direction, either up or down within the plant, while others may only move upwards in plants. If applied to the root zone, it will travel throughout the plant, but if applied to the leaves, it will not move throughout the plant. Furthermore, few pesticides are considered locally systemic and move only a short distance in a plant from the point of contact. Examples of systemic pesticides include 2, 4-dichlorophenoxyacetic acid (2, 4-D) and glyphosate [41].

B. **Non-systemic (Contact) pesticides**

non-systemic pesticides only affect the pests they come into touch with, they are also known as contact pesticides. For pesticides to be effective, the pest and the pest must come into physical touch. When bugs come into contact with the pesticide, it enters their bodies through the epidermis and poisons them, killing them. These herbicides may not always reach the plant tissues, and as a result, they are not carried by the plant's

vascular system. Diquat dibromide and paraquat are two examples of contact insecticides [35].

2.3.4. Based on Chemical Nature

The classification of pesticides based on the chemical natures are divided into many classes, of which the main are: Organochlorines, Organophosphorus and Carbamates pesticides.

2.4. Organophosphate pesticide

Organophosphates are phosphoric acid esters, sometimes known as thiophosphoric acid esters. When they were first developed in the 1930s and 1940s, their compounds posed a serious risk to mammals. Since then, organophosphates have been made that are less poisonous to mammals yet dangerous to target organisms like insects[42].

Because OPPs offer safe, affordable, and efficient control of a variety of pests, they are often the treatment of choice despite being less persistent than OCPs. All possible modes of absorption are available for them, including cutaneous, oral, and inhalation. The European Union (EU) has set low maximum residue limits for meat due to the knowledge that OPPs may also concentrate along the food chain. As a result, this kind of chemical needs to be controlled in fatty matrices.

OPPs are thought to be involved in the development or aggravation of several human health conditions, such as immune system interference, cancer, and hormone instability [43]. They are frequently used in fields, gardens, and houses as insecticides. OPPs include things like Malathion, famphur, phorate, terbufos, parathion, and diazinon.

By modifying particular chemical structures, synthetic pyrethroids were developed to surpass natural pyrethrins in terms of biological activity and environmental stability. The foundation of the pyrethroid class of insecticides was formed by natural chemicals called pyrethrins that were taken from plants in the genus Chrysanthemum.

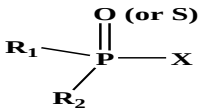
Although natural pyrethrins do have insecticidal activity, they also are inherently unstable when exposed to light. Therefore, the pyrethrin structure was modified to produce more stable compounds that retained the desirable insecticidal and toxicologic properties [44].

Synthetic pyrethroid insecticides have gradually supplanted conventional organophosphate, organonitrogen, and organochlorine pesticides during the past few

decades. Because of its low environmental persistence, selective insecticidal efficacy, and comparatively low mammalian toxicity, this pesticide class has been employed all over the world to manage agricultural pests. Like natural pyrethrins, which are derived from chrysanthemum flowers, they are extremely toxic to fish, aquatic arthropods, and honeybees even at low doses. Repeated exposure, however, does increase the risk of anaphylaxis and allergic responses at extremely low concentrations and should be monitored carefully [45]. A few examples of these pesticides are imidacloprid, deltametrin, acetamiprid, and lambda-cyhalothrin.

2.4.1. Structure of organophosphorus pesticides

In general, OPPs consist of a central phosphorous (P) atom that is double bonded to either oxygen (O) or sulfur (S) atom, and single bonded with either two methoxy (–OCH3) or ethoxy (–OCH_3CH_3) groups [46].



General structure of OPPs compound

Because of their nature, OPPs can bioaccumulate through a range of environmental pathways, especially those involving soil and water, and can only live for a short while. Even though OPPs provide benefits for agriculture, misuse can often have detrimental effects on the environment, especially for humans and animals who come into contact with the chemical through skin absorption, ingestion, or inhalation. Maximum residue limits (MRLs) are established by various regional and international organizations, the World Health Organization (WHO), the Association of Southeast Asian Nations (ASEAN), the European Union (EU), and the Food and Agriculture Organization (FAO), among many other countries, to ensure consumer and environmental safety (Table 2).

Table 2. Maximum residue limits (MRLs) for OPPs in different sources.

Organization	MRLs (mg/kg)	Remarks	Ref.
FAO- WHO	0.3	Human consumption	[47]
EU	0.01- 0.5	Food samples	[48]
	0.02	Soil samples	
ASEAN	0.01- 0.05	Crops	[16]
IFAR	<1	Food samples	[49]

The persistence and stability of OPP residues in agricultural areas, including those used for oil palm [50], rice [51], maize [52], cocoa [53], strawberry [54], and various vegetable farms [55], have altered the soil's composition through absorption, resulting in a wide range of serious pollution issues.

Due to their use during pre- or post-harvest, the chemical elements may collect in trace amounts in the soil matrix, where they may then be changed or dispersed into ecosystems. As a result, the negative consequences of these harmful OPPs and their byproducts can result in soil infertility, acidification, nitrate leaching, enhanced weed species tolerance, and a loss of biodiversity [56]. The OPPs enter the soil matrix through absorption via mineral and soil organic matters, depending on their sorption and mobility properties [57, 58].

2.4.2. List of different types of OPPs and their uses

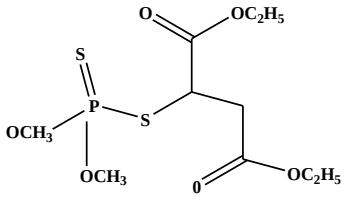
The most important pesticides, from the standpoint of environmental and health, are the persistent ones: Malathion, Diazinon, Dimethoate

A. Malathion

Malathion is a broad-spectrum organophosphate pesticide and miticide, originally registered in 1956. Malathion is used extensively in industry, government, agriculture, and residential settings. The amount of malathion used annually has increased from roughly 11–13 million pounds in 2000 to approximately 15 million pounds at present. Malathion has a boiling point of 156–157 °C and is a colorless to amber liquid with a strong odor. Malathion has a low solubility in aliphatic hydrocarbons but is easily

soluble in most alcohols, esters, aromatic solvents, and ketones. It is also soluble in water. An overview of the chemical substance malathion can be found below [59].

Table 3. The general chemical identification of malathion [60].

Malathion test compound nomenclature		
Chemical structure		
Empirical formula	$C_{10}H_{19}O_6PS_2$	
Common name	Malathion	
IUPAC name	O,O-dimethyldithiophosphate of dimethyl mercaptosuccinate	
CAS registry number	121-75-5	
Chemical class	Organophosphate	
Known impurities of concern	Empirical formula	$C_{10}H_{19}O_6PS_2$
	Common name	Isomalathion
	IUPAC name	Butanedioic acid, [[methoxy(methylthio)phosphinyl]thio]-, diethylester
	CAS registry number	3344-12-5
Molecular weight		330.36 g/mol

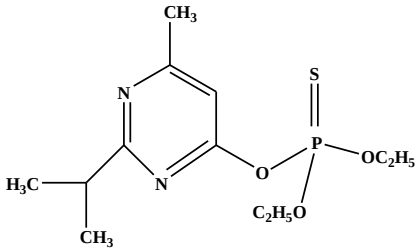
B. Diazinon

As an organophosphate insecticide, acaricide, and nematicide used against a variety of pests, including soil insects and pests of fruit, vegetables, pasture, and field crops, diazinon was initially authorized in the United States in 1956 [61].

The two main advantages of diazinon are: (1) it controls foliar pests on fruits and orchard crops; and (2) it controls soil pests in certain fruit and vegetable crops.

Pure diazinon is a colorless oil which is formulated into “stabilized” technical diazinon. Technical diazinon (> 90% pure) boils at 83–84°C and is a yellow to brown liquid. Technical diazinon has a 40 ppm solubility in water at 20°C. But is completely miscible in acetone, benzene, dichloromethane, ethanol, 1-octanol, toluene, and xylene, and is soluble in petroleum oils [62].

Table 4. The general chemical identification of diazinon [63].

Chemical structure	
Empirical formula	$C_{12}H_{21}N_2O_3PS$
Common name	Diazinon
IUPAC name	O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
CAS registry number	333-41-5
Chemical class	Organophosphate
Molecular weight	304.3 g/mol

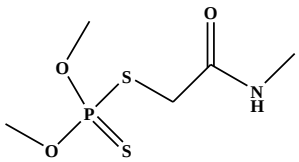
C. Dimethoate

A systemic organophosphate insecticide, dimethoate is applied to a wide range of ornamentals, tree crops, and field-grown agricultural crops. In 1962, it was initially registered in the US [64]

Dimethoate is a white, crystalline substance with a mercaptan odor that melts between 45 and 48 degrees Celsius. It dissolves only slightly in aliphatic hydrocarbons, carbon tetrachloride, and xylenes. At 21 °C, it dissolves in water at a rate of 25 g/L. Additionally, it is highly soluble in alcohols, esters, ketones, benzene, toluene,

methylene chloride, and chloroform. Dimethoate is stable in aqueous solutions at pH 2–7, but hydrolyzes in alkaline environments [64]

Table 5. The general chemical identification of dimethoate.

Chemical structure	
Empirical formula	$C_5H_{12}NO_3PS_2$
Common name	Dimethoate
IUPAC name	O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate
CAS registry number	60-51-5
Chemical class	Organophosphate
Molecular weight	229.3 g/mol

The major toxic degradate of dimethoate is omethoate (O,O-dimethyl S-(Nmethylcarbamoylmethyl) phosphorothioate). Omethoate is registered as an active ingredient internationally, but not in the United States.

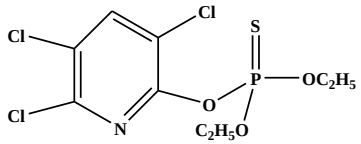
D. Chlorpyrifos

First authorized in 1965, chlorpyrifos is a broadspectrum, chlorinated organophosphate insecticide, nematicide, and acaricide that is used to manage soil- and foliage-borne insect pests on a range of food and feed crops [63].

Chlorpyrifos is an organophosphate insecticide, acaricide, and miticide that is applied to a range of food and feed crops to control pest insects that are soil-borne and foliage-borne. Every year, about 10 million pounds are administered in agricultural settings. People who are exposed to chlorpyrifos at very high levels (such as in accidents or large spills) may experience nausea, vertigo, confusion, respiratory paralysis, and even death

due to cholinesterase inhibition, which is an over stimulation of the neurological system [63].

Table 6. The general chemical identification of chlorpyrifos [65].

Chemical structure	
Empirical formula	$C_9H_{11}Cl_3NO_3PS$
Common name	Chlorpyrifos
IUPAC name	[0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate],
CAS registry number	2921-88-2
Chemical class	Organophosphate
Molecular weight	350.6 g/mol

Technical Chlorpyrifos has a melting point of 41.5– 42.5 °C and is a white, crystalline solid. In both neutral and acidic aqueous solutions, chlorpyrifos is stable; however, stability diminishes with increasing pH. While nearly insoluble in water, chlorpyrifos is soluble in the majority of organic solvents, including methylene chloride, acetone, and xylene. Based on its low vapor pressure of $1.87x10^{-5}$ mm Hg at 20°C, chlorpyrifos is not a very volatile substance (Merck Index, 11^{th} Edition). At 25 °C, the greatest vapor concentration that can be achieved is 25 ppb [65].

2.4.3. Fate, migration, and toxicity impacts of residual OPPs in soil

A. Fate and behavior of residual OPPs in soil

Commonly, adhesion to soil particles (primarily organic matter and clays), degradation by microbes and/or free enzymes, or leaching across the soil systems with water are the

three most likely routes for pesticides to enter soil particles during their application to control pests and vector-borne diseases [66].

OPPs are persistent and mobile, therefore their fate in the environment was dictated by their release, whether accidental or deliberate, which led to higher concentrations and more dangerous levels. Ragnarsdottir, K.V, (2000) and ozkara et.,al (2.16) [67, 68] Studies have also demonstrated that most of the OPPs are considered to possess poor soil mobility and leaching, as they loosely bind to soil particles and their residue is less soluble in water. However, their degradation derivatives indicated otherwise that they are highly persistent in the soil environment [69]. Because of their inherent nonpoisonous and risk to plant and fauna, persistent OPPs are regarded as detrimental. Such OPPs' persistence is affected by a number of processes, including photolysis, hydrolysis, and redox reactivity [70].

B. Migration of residual OPPs in soil

Apart from enhancing crop yield according on food need, OPPs are typically employed in agriculture operations throughout the world to kill, repel, or manage specific types of plant or animal life-type pests [71]. The widespread usage of OPPs in agriculture industries across the globe has become unstoppable due to the endeavor to effectively kill or manage pests together with the goal to boost crop productivity to meet supply and demand for food. Cacers et,.al (2010)[70] Having said that, the OPPs migrate through the soil system after the remaining residual OPPs along with their by-products are transported long distances by air and water (Figure 2). This migration allows OPPs to persist toxically and contaminate soil leading to soil infertility, acidification, nitrate leaching, increased tolerance of weed species and biodiversity depletion. Such evidence emphasizes the concept of OPP movement in the soil system. In this section, we'll talk about the four major migratory flows of sorption by soil, volatilization, leaching, and runoff.

Firstly, sorption of OPPs by soil begins once the analyte compound comes in contact with soil constituents such as soil organic matter, water, and inorganic minerals. The molecular characteristics of the solute, the soil's composition, and the experimental conditions under which the sorption is being studied are the three crucial factors that are frequently coupled in a complex manner. The mineral surfaces in aqueous solutions have a pH-dependent surface charge, which causes OPPs that are less water-soluble to be more hydrophobic and, as a result, are frequently heavily sorbed to soil particles[70].

Secondly, a naturally, pesticide volatilization in the soil system and subsequent dissemination in the environment is possibly the most significant method of pesticide dispersal [72].

Thirdly, pesticides transported via soil surface runoff from the agricultural areas leads to a majority of pesticide residue in water sources. Runoff events are concerning as the aquatic organisms could be affected by the toxicity of residual OPPs that moves through waterways from their site of application [73]. OPPs have a higher propensity to exhibit weak persistence in the environment during the leaching process. The increased leaching capacity may also be influenced by elements like chemical characteristics, adsorption activity, persistency, and other environmental parameters (such rainfall and soil porosity with high precipitation, low temperatures, and sandy soils with low organic matter) [74, 75].

C. Toxicity impact of residual OPPs in soil

Due to their frequent exposure to OPPs, agriculture workers, such as farmers and pesticide sprayers, are in particular categorized as high-risk persons. According to Buchanan et al., Jamal *et al.*, and Farahat *et al.*, (2019) [76], modest levels of regular exposure to OPPs may have long-term consequences on the central and peripheral neurological systems. Moreover, symptoms like dizziness and numbness were also recorded to be substantially more prevalent among the affected worker with additional impairments like blurred vision, paresthesia, headache, vertigo, superficial sensory loss, trophic and vasomotor changes, together with reduced ankle and deep reflexes. Additionally, Pazymino et al [77], manifested low levels (28 Units/ml being optimal level) of erythrocyte acetylcholinesterase(AChE) in the blood as a result of AChE loss through cytogenetic monitoring in a population subjected to fenamiphos and profenofos application at flower plantation in Quito, Ecuador. On the other hand, OPPs have also been categorized as endocrine-disrupting agents (EDs), as they disrupt the functions of the endocrine system, and thus triggering adverse health consequences in intact organisms [78].

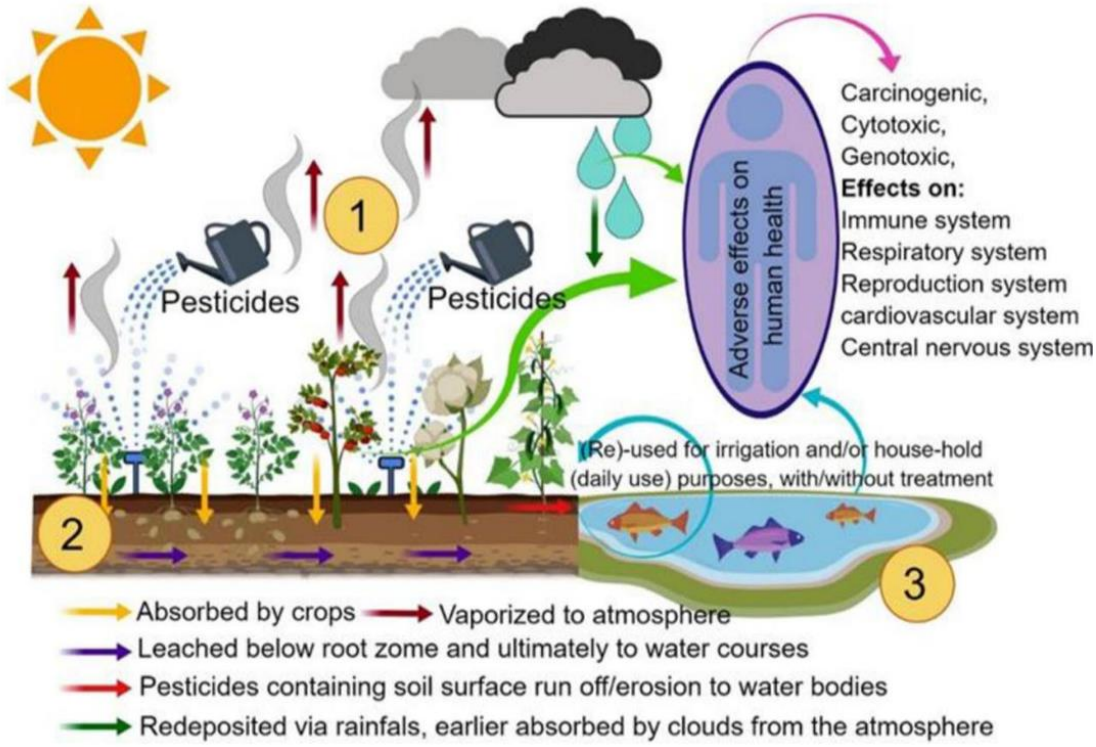


Figure 1. Pesticide Behavior, Fate, and Effects in the Tropics: An Overview of the Current State of Knowledge [79].

CHAPTER THREE

3. Methodology

3.1. Study Area Location

Geographically, the study area lies between 11° 45' 46"N to 11° 43' 53"N latitude and 39° 37' 36"E to 39° 34' 12"E longitude in the North Wollo zone of Amhara national regional state, which is perched on the eastern escarpment of the Ethiopian highlands of north central Ethiopia. The Sirinka agricultural research center is located 510 km from Addis Ababa and 10 km south of the north-western Wollo Zone capital town (Woldia).

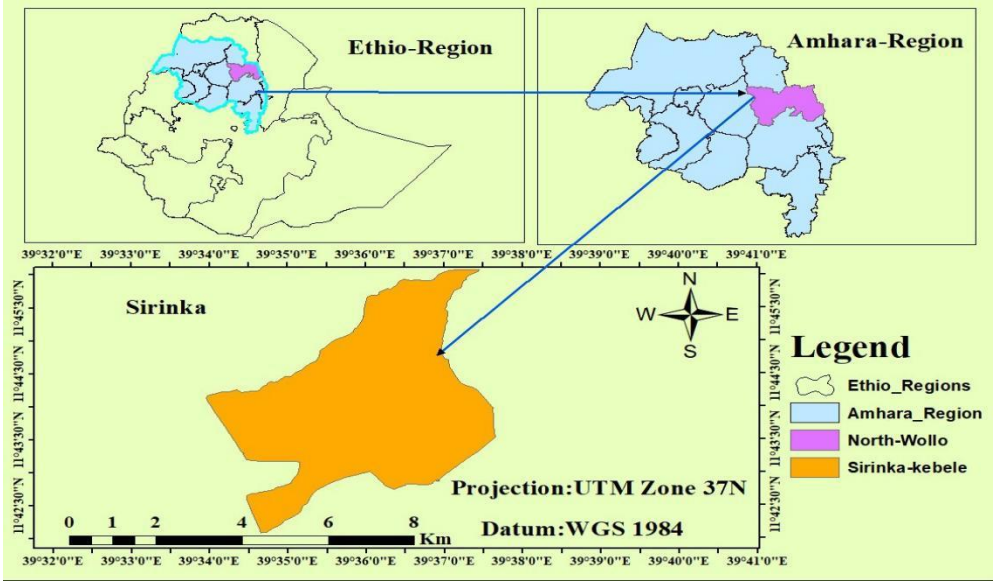


Figure 2. Maps of the study area

3.2. Apparatus and instruments

Agilent Gas Chromatography equipped with a mass spectrometry (GC-MS model; 7000C, triple quad, India), sieve 200µm, beakers, aluminum foils, GPS, Centrifuge (model MAX DREHZAHL: 6000 1/min, type: corning LSE), Oven, Furnace (Nabertherm GmbH, model L15/11/P320), Micro-pipette, Syringes, Measuring Cylinders, Surgical Gloves, Electronic Balance, Vortex-shaker, Muffle Furnace, Teflon Tube, Plastic Bag, Spatula, and Soil Auger were used in this study.

3.3. Chemicals

Chemicals and reagents used for the analysis of soil samples of selected four organophosphate pesticides where, reference standards (diazinon, dimethoate, malathion, and chlorpyrifos) were used by Bless Agri Food Lab in Addis Ababa, Ethiopia. In addition, analytical-grade acetonitrile and all other reagents and solvents used from Bless included anhydrous magnesium sulfate, anhydrous sodium acetate, ethyl acetate, primary secondary amine (PSA), and acetic acid.

3.4. Standard solutions

To prepare 1000 ppb of each pesticide stock standard solution, 10 μg of each pesticide was dissolved in 10 mL volumetric flasks containing acetonitrile. The mixture was then kept in a refrigerator below 6 ◦C. The pesticide standard solutions with concentration ranges of 2–50 ppb (w/w) were prepared form stock solution using serial dilution in acetonitrile, spiked with sample matrix, and kept at 4 ◦C for calibration. Dilutions of intermediate solutions were used to create additional workable solutions.

3.5. Soil sampling

In the study area, there are two sampling sites, which are SARC and around Sirinka (FAR). From each, 13 and 18 core soil samples, about 1 kg each, were randomly taken with a soil auger from the area at a depth of 0 to 20 cm. The samples were combined to create a composite sample. The composite soil samples were well mixed, and sub-samples were taken. These gave two representative soil samples from the study area. The soil samples were kept in well-labeled sampling plastic bags and transported to the laboratory for preparation. The soil samples were dried in an oven at 60 °C over a night to a consistent weight and grounded and sieved through a 2 mm nylon mesh in the lab. After sieving, sub-samples were obtained for the investigation of pesticide residues. The soil sampling procedure was adopted from Fosu-Mensah (2016) [16], with some modifications. Before extraction and analysis, the samples were maintained in a refrigerator at a temperature of -4 °C.

All sample preparation steps were undertaken at Woldia University Department of Chemistry Research Laboratory Room. The final analysis of the prepared samples by GC/MS instrument was done at Bless Agri Food Laboratory Services, Addis Ababa, Ethiopia.

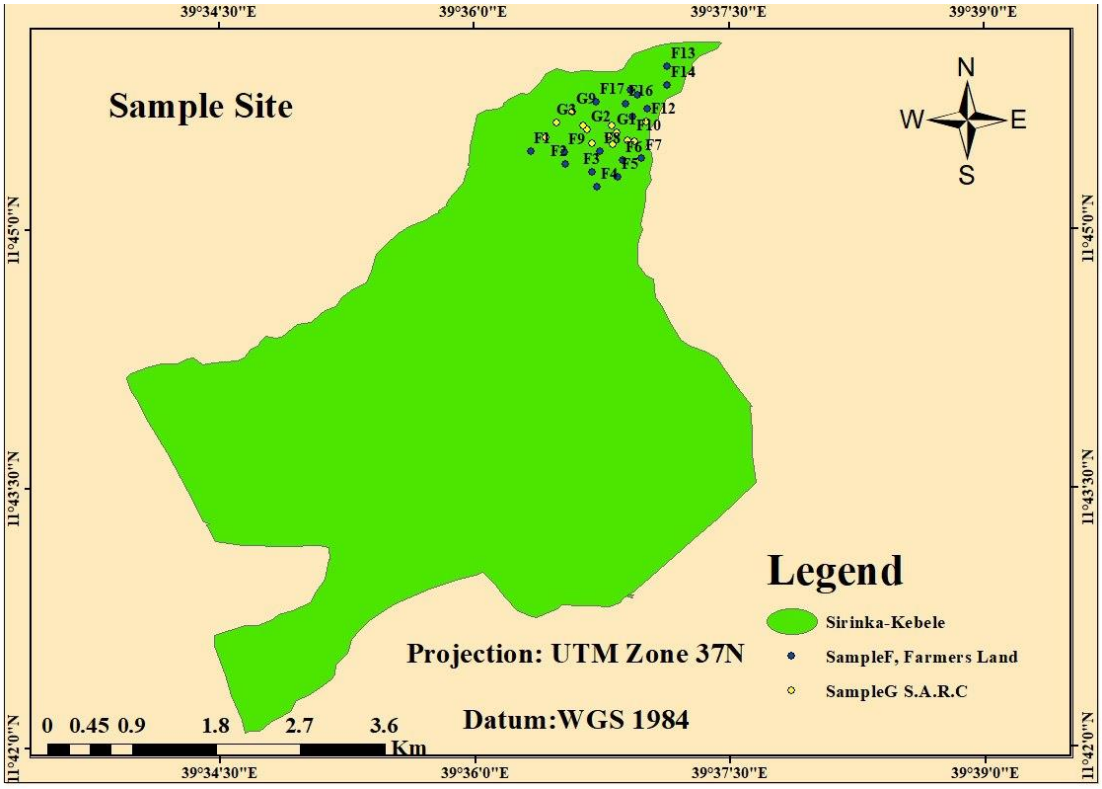


Figure 3. Sampling point of the study area

3.5.1. Extraction and clean-up of OPPs from soil samples

The extraction of the soil samples were carried out using QuECHERS method[80]. 15 g of the representative soil samples were weighed and quantitatively transferred into a 50 mL Teflon tube. Then, 15 mL of 1% acetic acid in acetonitrile and 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous sodium acetate were added to the tube and vortexed (IKA vortex, Genius 3) for 1 minute. Then, the samples were placed on a centrifuge (corning LSE, Germany) and set to centrifuge for 10 minutes at 2000 rpm. The contents were then allowed to stand for 10 minutes to sufficiently separate the phases or layers. Transfer 1 mL of supernatants by using a syringe into a 15 mL centrifuge tube containing 50 mL of PSA and 150 mL of anhydrous MgSO4 and vortexed for 1 min, then centrifuge for 5 min at 1500 rpm. 1 mL of extract was transferred to a GC vial and analyzed using GC-MS (Agilent Technologies, 7000C, triple quad, India).

3.6. Analysis of pesticides residue

Gas chromatography-mass spectrometry examination of samples were used to quantify pesticide residues. The final extract of the soil samples were put into GC vials for

GC/MS analysis, and equation 1shown below were used to calculate the amount of pesticides in soil. Calculations for pesticide residues concentration in soil:

$$\text{Peseticide residue} = \frac{A_s \times I_{std} \times V_f \times C_{Rf}}{A_{std} \times I_s \times M_s} \text{.....equation 1.}$$

Where: A_{sd} = Peak height of the standard; A_s = Peak height of the sample; I_{std} = µl of the standard extract injected; I_s = µl of the sample injected; V_f = Final volume of the sample extract (ml) M_s = Mass in g of the sample; C_{Rf} = Concentration in ppm of the reference standard [81].

3.7. Physico-chemical properties of soil sample

The physicochemical parameters analyzed included pH, electrical conductivity, organic carbon, available phosphorus, and available nitrogen. All the analytical procedures used in the physicochemical analysis of the soil samples were done according to the standard method of soil analysis [82]. Physico-chemical parameters such as pH and electrical conductivity were measured using a Multi-parameter meter (Model YSI 63) manufactured by YSI incorporated, USA. The measuring equipment (Multi-parameter meter Model YSI 63) was first calibrated with standard pH buffers (7 and 10) before immersing the probe into the water. Organic matter was determined by the procedure involving reduction of potassium dichromate ($K_2Cr_2O_7$) by OC compounds and subsequent determination of the unreduced dichromate by oxidation-reduction titration with ferrous ammonium sulfate. This method is referred to as the Walkley-Black method. Available Phosphorus was determined by Bray's P-1 Method. The available (mineralisable) nitrogen was determined using alkaline potassium permanganate method or kjeldahl distillation method. The detail procedure for performing the parameters were placed in appendixes (appendix 3- 7).

3.8. Method validation

The validation study was evaluated for linearity, recovery, limits of detection (LOD) and limit of quantitation (LOQ) in accordance with the European SANCO requirements [83]. By injecting diluted calibration standards, the signal-to-noise ratio of the peaks for each target compound was used to calculate the instrument detection limits. The linearity of the method was studied with calibrations of standard pesticide solutions at 2, 5, 10, 15, 20, 30, 40, and 50 ppb concentration levels for each analyte. The recovery

test was determined by spiking concentrations of pesticide standards with soil sample. Solutions at each spiked level will be prepared in triplicate and were injected. Spiked samples was left to stand for at least 1 h to allow pesticide absorption into the sample. They were then extracted according to the extraction procedures described above.

3.9. Human Health Risks Assessment

3.9.1. The non-carcinogenic risk assessment

The USEPA recommended methodologies were employed to assess the carcinogenic and non-carcinogenic risks associated with the OPPs. The human body is primarily exposed to organic materials found in soil through the respiratory system, skin contact, and food absorption. In order to assess the health risk posed by OPP residues in soil, this study computed and examined the carcinogenic and non-carcinogenic risks in humans by oral intake, skin contact, and respiratory inhalation [84].

The following formulas were used to determine the average daily dosage (ADD, mg kg^{-1} day^{-1}) of pesticides through non-dietary (ingestion, skin contact, and respiratory inhalation) pathways:

$$ADD_{ing} = \frac{Cs\ x\ IR\ x\ CF\ x\ EF\ x\ ED}{BW\ x\ AT} \ldots\ldots\ldots\ldots equation\ 2$$

$$ADD_{der} = \frac{Cs\ x\ SA\ x\ AF\ x\ ABS\ x\ EF\ x\ ED\ x\ CF}{Bw\ x\ AT} \ldots\ldots\ldots equation\ 3$$

$$ADD_{inh} = \frac{Cs\ x\ inhR\ x\ EF\ x\ ED\ x\ CF}{PEF\ x\ BW\ x\ AT} \ldots\ldots\ldots\ldots\ldots\ldots equation\ 4$$

Table 7. Parameters used in exposure no-cancer risk assessments.

Parameter	Unit	Children	Adult	Representative meaning	Ref
ingR	mg day^{-1}	200	100	Soil ingestion rate	[85]
EF	day yr^{-1}	365	365	Exposure frequency	[86]
ED	Year	12	70	Exposure duration	[86]
CF	kg mg^{-1}	10^{-6}	10^{-6}	Conversion factor	[87]
BW	Kg	16	70	Average body weight	[88]
AT	Day	4380	25550	Averaging time	[89]
SA	cm^2	2373	5700	Skin surface area	[90]
AF	mg cm^{-2} day^{-1}	0.2	0.2	Skin adherence factor	[85]
inhR	m^3 day^{-1}	12	15.8	Soil inhalation rate	[91]
PEF	m^3 kg^{-1}	$1.3x10^9$	$1.36x10^9$	Particle emission factor	[89]

Where:

ADD_{ing}, ADD_{der} and ADD_{inh}, are average daily doses taken by oral intake (exposure by non-dietary mouth intake of pesticide, e.g. dust or air-borne particles), ingestion, dermal contact and inhalation of soil dust (in mg kg^{-1} day^{-1}), *C*s are pesticide concentrations in soil (mg kg^{-1}), ABS is the dermal absorption factor, for diazinon, Malathion and Chlorpyrifos is 0.1. [92].

3.9.2. Hazard Quotient and Health Risk Index

To estimate the non-cancer dangers of pesticides in soils through non-dietary pathways, a hazard quotient (HQ) was developed. When the hazard quotient (HQ) is more than one, there is a major risk to human health; however, when the HQ is less than one, there are no significant negative effects on human health [93].

$$\mathrm{HQ} = \frac{\mathrm{ADD}}{\mathrm{RfD}} \quad \text{equation 5}$$

The general risk assessment is called the health risk index (HI). Any index or indicator may be used to calculate the risk index.

$$\mathrm{HI} = \sum \mathrm{HQi} = \sum \frac{\mathrm{ADDi}}{\mathrm{RfDi}} \quad \text{equation 6}$$

Where: HQ_i is hazard quotient in all three exposure per two sample

ADD is the average daily doses of the population in mg day^{-1} kg^{-1} body weight and RfD is the reference dose (mg kg^{-1} day^{-1}) values for each pesticides

3.9.3. Carcinogenic risk assessment

A slope factor (SF) was multiplied by the ADD to estimate the cancer risk assessments for humans. The risk value R of carcinogenic risk is often expressed as [94]:

$$CR = ADD \times SF \quad \text{equation 7}$$

Where: CSF = cancer slope factor in (mg $kg^{-1}day^{-1}$) in three exposure and

ADD = Average daily dose (mg kg^{-1})

The permissible limits of CR for one or more pesticides are $10^{-6} < CR < 10^{-4}$. If the values of CR are greater than 10^{-4}, it indicates the cancer risk affects human health; if CR is lower than 10^{-6}, it did not considered harms to health.

3.10. Statistical analyses

Statistical analysis were performed using Microsoft Excel 2013 software. T-test were used to evaluate the significance of concentration differences between pesticides found from soil samples with that maximum permissible limit value. Paired two sample for mean t-test were used to assess variations of pesticides levels between samples. In all cases, differences were considered significant at probabilistic values of $p < 0.05$. In order to estimate the degree of association among OPPs compounds, Pearson Correlation were also employed with the physicochemical properties of soil and the analyzed pesticides.

CHAPTER FOUR

4. Results and Discussion

4.1. Method validation

The analytical instrument and method performance parameters including linearity, LOD, LOQ, recovery (%R), and precision in terms of %RSD were evaluated as presented in table 8 and 9.

A. Linearity

When using the least squares technique of analysis, the calibration curve's correlation coefficient (R^2) should be greater than 0.99 [95]. Several pesticide calibration standards were examined by GC-MS in order to assess the linearity of the method, and the results were recorded with an R^2 value greater than 0.990, it was discovered that a plot of the pesticides signal vs. concentration was linear for each pesticide in the range of 2 to 50 µg/kg as shown table 8 and appendix 1 . This finding suggests that a linear range of concentrations of these four pesticides were found in soil.

B. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. While the LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the test. In this study, blank solute signal on was used to determine LOD and LOQ [96, 97]. In view of that, the values of the limit of detection and limit of quantification for the detected pesticides using the proposed method are depicted in table 8 below. Limit of detection is expressed as the concentration corresponding to the sample blank value plus three times standard deviation (LOD = 3s) and Limit of quantification is the concentration corresponding to the sample blank value plus ten times standard deviations (LOQ = 10s) where s is the standard deviation of the sample blank.

Table 8. The correlation coefficient, LOD and LOQ of the four pesticides in μg kg^{-1} .

Pesticide	R^2	SARC/FAR	
		LOD	LOQ
Diazinon	0.998	0.09	0.3
Dimethoate	0.999	ND	ND
Malathion	0.995	0.45	1.5
Chlorpyrifos	0.998	0.06	0.2

From the table 8, the limit of detection of the pesticides compared to the international MRL values set by EU MRL, it was lower value. This clearly shows that it is possible to determine the residual levels of the pesticides in these soil samples to lower than the international MRLs. However, the limit of detection and limit of quantification of dimethoate were not determined because the amount were below the detection limit.

C. Precision

Precision is typically reported as % RSD for a number of samples and is a measure of a method's repeatability under typical operating conditions [98]. The relative standard deviation (RSD) of three replicate injections of each pesticide at a concentration of 30 ppb was used to assess the method's precision as shown in table 9. The repeatability of the current method for determining the detected three pesticides was evaluated by calculating the % RSD of the peak areas of three replicate injections of the samples with a concentration of 30 ppb, which were found to be 1.04% to 7.7%. These results show that the current method is better repeatable.

D. Recovery

To evaluate the performance of the analytical procedures and their recovery rate (%), studies were undertaken for each pesticide, as stated in previous literatures reports[99]. By adding pesticide standards to the previously examined samples, recovery studies were conducted [100]. Know concentration of pesticides under investigation were spiked with extracted sample, and then subjected to GC-MS analysis in order to determine the recovery of the pesticides as shown table 9. A standard solution with a concentration of 30 ppb was added to soil samples, respectively, to inquire about the spike recovery percent of the four OPPs. The results have shown that the analysis has

good recovery from (81.67 % to 95.3 % for SARC) and (83.97 % to 94.5 % for FAR) for the four pesticides studied (appendix 2).

$$\%\text{Recovery} = \frac{CS_2 - CS_1}{CS} \times 100 \quad \text{.....equation 8}$$

Where: CS_1 = concentration of pesticide residues in the un-spiked sample,

CS_2 = concentration of pesticide residues in the spiked sample,

CS = concentration of added pesticide standard.

Table 9. Percent recovery, mean and relative standard deviation of the sample.

Compound name	**Sample code**							
	SARC				FAR			
	%R	Mean	SD (n =3)	%RSD	%R	Mean	SD	%RSD
Diazinon	88.03	1.24	0.03	2.4	83.9	1.23	0.02	4.5
Dimethoate	95.3	ND	ND	ND	94.5	ND	ND	ND
Malathion	81.67	4.42	0.15	3.4	86.2	2.63	0.2	7.7
Chlorpyrifos	90.3	1.16	0.02	1.04	88.5	1.15	0.03	2.6

Notation: SD = Standard deviation, ND = Not detected

4.2. The level of organophosphate pesticides residues in soils sample

The levels of OPPs were estimated from the data obtained by GC/MS measurements using the formula shown in equation 1 shown above.

At the two sample sites, SARC and FAR, three different forms of pesticide residues were found in the soil samples at different quantities. Table 10 below reports the results of OPPs in soil samples taken from SARC and on the farmer's land (FAR). With the exception of dimethoate, the others selected pesticides, malathion, diazinon, and chlorpyrifos were found in the soil samples.

The rank order of pesticides in soil based on their concentration was malathion (4.42 μg kg^{-1}) > diazinon (1.24 μg kg^{-1}) > chlorpyrifos (1.16 μg kg^{-1}) for SARC and malathion (2.63 μg kg^{-1}) > diazinon (1.23 μg kg^{-1}) > chlorpyrifos-methyl (1. μg kg^{-1}). According to the results, all the pesticides have been identified in the soil from SARC and FAR, so the highest and lowest of the obtained mean concentrations of pesticides were described. Numerous studies in many nations have been carried out in accordance

with our investigation. As per the findings of Nabhan *et al*. (2018), the most prevalent pesticides found in soil samples from Iraq were diazinon, malathion, and chlorpyrifos [101].

Table 10. Concentration of pesticide residues in soil of farms (μg kg^{-1}).

Pesticides	Mean concentration		EU MRLs	t- value	t- critical
	SARC	FAR			P < 0.05
Diazinon	1.24	1.23	10	0.17	4.3
Malathion	4.42	2.63	20	13.4	4.3
Chlorpyrifos	1.16	1.15	30	0.32	4.3

The measured mean concentrations of malathion ranged from 4.42 μg kg^{-1} at SARC to 2.63 μg kg^{-1} at FAR. Statistically, the concentrations of malathion recorded in the soil samples were significantly ($p < 0.05$) different between the two sites. However, the mean concentration of malathion recorded in soils from the study site was lower than the US MRLs of 20 μg $kg^{-1}$for agricultural soils. Malathion is a broad-spectrum organophosphate insecticide and miticide. In line with prior research, Mahdavi *et al.* (2023) examined pesticide residues in farm soil in the Golestan province, Iran, and found that the average concentration of malathion (0.082 mg kg^{-1}) was significantly lower than the average values (4.42 and 2.63 μg kg^{-1}) found in this study [102].

Similarly the measured concentrations of diazinon ranged from 1.24 μg kg^{-1} at SARC to 1.23 μg kg^{-1}at FAR. There were no significant site differences ($p < 0.05$) in diazinon concentrations in the two sites. The mean concentrations of diazinon recorded at both sites were below the US MRL of 10 μg kg^{-1} for agricultural soils. In a related investigation of pesticide residues in the soil of farms in the Golestan region, Iran, by Mahdavi *et al.* (2023), the mean value (1.4 μg kg^{-1}) of diazinon concentrations was found to be comparatively higher than the mean values (1.23 μg kg^{-1}) reported in this paper [102].

The measured mean concentrations of Chlorpyrifos ranges from 1.15 μg kg^{-1} at SARC to 1.16 μg kg^{-1} at FAR. Statistically, the concentrations of chlorpyrifos recorded in the soil samples were not significantly ($p < 0.05$) different between the two sites. However, the mean concentration of chlorpyrifos recorded in the soils of farms was lower than the United States (US) maximum residue limit of 30 μg kg^{-1} for agricultural soils.

Chlorpyrifos is a broad-spectrum organophosphorus pesticide. The mean values of chlorpyrifos recorded in this work were lower than the values reported by Fosu-Mensah *et al.* (2016) in soil samples from Dormaa West District, located in the western part of the Brong Ahafo Region of Ghana; its mean concentration was 0.03 mg/kg [16]. In line with other studies, chlorpyrifos residue levels (1.15 μg kg^{-1}) recorded in this study were far lower than the range of 0.52–0.97 mg/kg reported by Mahmud *et al.* (2015) in soil samples from Gashua, Bade Local Government Area Yobe State, in Nigeria [16, 103]. In a similar study, Mahdavi *et al.* (2023) pesticide residues in the soil of farms in Golestan province, Iran, reported that the mean value (6.7 μg kg^{-1}) concentrations of chlorpyrifos were relatively higher than the mean values (1.15 μg kg^{-1}) reported in this work [102].

Considering the presence of organophosphate pesticides in the soil samples, it appears that crop growers at the study site apply these chemicals in considerable amounts. It's possible that these pesticides entered the soil by spray drift during the crop's direct spraying, wash-off from treated fruits and vegetables, improper disposal of leftover spray solution, sprayer wash water, and spent pesticide containers.

4.3. Physicochemical properties of the soil sample

The present investigation focused on the detection of pesticide residues in soils in SARC and around it. To better understand soil physicochemical parameters were determined. These physicochemical soil parameters are displayed in Table 11.

Table 11. The results of physicochemical parameters of soil samples collected from the study area.

Parameters	SARC	FAR	Entire mean	IASS
	Mean ± SD	Mean ± SD		
pH	7.89 ± 0.05	7.92 ± 0.06	7.91 ± 0.02	4 – 8.5
Electrical. Conductivity	0.2 ± 0.004	0.19 ± 0.001	0.2 ± 0.003	4 ms/m
Organic Carbon	0.66 ± 0.23	0.52 ± 0.25	0.59 ± 0.11	0.5 -5.5%
Organic Matter	1.14 ± 0.4	0.95± 0.43	1.05 ± 0.12	0.86 – 9.5%
Available Phosphorus	12.8 ± 0.65	21.43 ± 0.5	17.13 ± 0.12	11-22 kg/ ha
Available Nitrogen	124.8± 1.04	131.7 ± 1.25	128.25 ± 0.15	240-480 kg/ha

Note: IASS = International Agricultural Soil Standards

4.3.1 pH

The hydronium ion (H+) activity in the soil suspension is measured by the pH of the soil. This property influences many aspects of crop production and soil chemistry, including availability of nutrients and toxic substance, activity and diversity of microbial population and activity of certain pesticides [104].

Soil pH is a parameter which influences the bio-availability and transport of pesticides in soil. Average mean pH recorded for SARC and FAR were 7.89 ± 0.05 and 7.92 ± 0.06, respectively. The mean pH recorded for the entire study was 7.91 ± 0.23. Results obtained from the independent sample t-test revealed is not significantly different between SARC and FAR ($p < 0.05$). The average pH of FAR is 0.03 greater than pH of SARC, this indicates FAR is more alkaline than SARC. However, the soil samples

from SARC (7.89) and FAR (7.92) which recorded a mean pH value that was within the IASS acceptable limits of 6.5–8.5 for soil.

4.3.2 Electrical conductivity (EC)

The amount of soluble inorganic salts in the soil is measured by the EC of the soil. Since it indicates the degree to which the soil is appropriate for crop growth, EC is a crucial lab measurement. However, salinity has an impact on plants at every stage of growth, and for many crops, susceptibility varies with different growth phases. Based on a saturation extract, values between 0 and 200 mS/m are safe for all crops; between 200 and 400 mS/m, highly sensitive crop yields are impacted; between 400 and 800 mS/m, many crops are impacted; and only tolerant crops grow fairly well over that threshold. According to Chan et al. (2008), soil EC is the soil's capacity to transfer an electrical charge [105].

Average mean EC recorded for SARC and FAR were 0.2 ± 0.004 mS/m and 0.19 ± 0.001 mS/m, respectively. Results obtained from the paired for two sample mean t-test revealed is not significant different between SARC and FAR ($p < 0.05$). In this study, the mean conductivity value of 0.2 ± 0.003 ms/m was recorded for the entire study. On average the EC of SARC is almost equal to the EC of FAR. The conductivity values recorded during the study were generally low for all the sites and fell within the permissible limit of for soil (EPA 2004). This shows almost negligible effects or safe for all crops.

4.3.3 Organic matter of the soil

The remains of plants, roots, and soil organisms in varying states of synthesis and decomposition are represented by soil organic matter (SOM), which has a diverse composition. Because organic matter is essential for holding soil particles together, soil strength is increased. Two essential features of soil are its ability to retain water and adsorb pesticides, both of which are greatly impacted by soil organic matter. Enhancing the organic content of the soil, be it by applying manure or incorporating cover crops through ploughing, leads to an augmented ability of the soil to retain water and dissolved pesticides within the root zone. This, in turn, facilitates plant accessibility to essential nutrients while also promoting the eventual degradation of the pesticides [106].

Table 10 shows the average means of SOM and SOC recorded for SARC and FAR were (1.14 ± 0.4 % and 0.89 ± 0.43%) and (0.66 ± 0.23 % and 0.52 ± 0.25 %),

respectively. Results obtained from the paired two sample for means t-test revealed is not significantly different between conventional and organic rice ($p < 0.05$). On average, the SOM of SARC was 0.14 greater than that of FAR. In this study, the mean value of SOC and SOM 0.59 ± 0.11 and 1.05 ± 0.12 ms/m was recorded for the entire study. The OC and OM values recorded during the study were generally low to medium values were recorded for all the sites and fell within the permissible limit of for soil.

4.3.4 Available phosphorus (AP)

Due to the very high phosphorus requirements of plants, phosphorous is a very important element that is categorized as a micro-nutrient. Phosphorous is highly soluble and can take on a wide variety of forms in soil. Phosphorus is a naturally occurring substance that is somewhat soluble and found in fertilizers and manure. When phosphate-containing manure and phosphorous fertilizers come into contact with soil, a number of reactions begin that reduce the amount of phosphate that is accessible and soluble. Because most phosphorus is found in organic form, plants can only absorb soluble inorganic and simple forms of phosphate from the soil. As a result, only a tiny amount of inorganic phosphorus is available for plant uptake. Most often, the Bray's P-1 Method is used to determine phosphorus [107].

Average mean AP recorded for SARC and FAR were 12.8 ± 0.65 kg/ha and 0.19 ± 0.5 kg/ha, respectively. Results obtained from the paired for two sample mean t-test revealed is not significant different between SARC and FAR ($p < 0.05$). In this study, the mean AP value of 17.13 ± 0.12 kg/ha was recorded for the entire study. On average the AP of SARC is almost less than to the AP of FAR. The AP values recorded during the study were generally medium for all the sites and fell within the permissible limit of for soil IASS (11-22 kg/ha). This shows almost safe for all plants.

4.3.5 Available nitrogen

Nitrogen (N), essential to plant production and protein synthesis, must be provided to crops either from natural sources or from synthetic fertilizers applied to the soil. In nature, it is only specialized nitrogen-fixing organisms in plants and soil that can take abundant but unusable nitrogen gas from the atmosphere and transform it into a plant-available form. Synthetic chemicals including pesticides have been shown to inhibit nitrogen-fixing rhizobia bacteria, increase dependence on synthetic fertilizers, and reduce overall plant yield. Nitrogenase activity, the key enzyme involved in nitrogen

fixation, has been shown to be less prevalent in soils exposed to pesticides [108].

Average mean (AN) recorded for SARC and FAR were 131.7 ± 1.25 and 124.4 ± 1.04 kg/ha respectively. The mean AN recorded for the entire study was 128.25 ± 0.15 kg/ha. Results obtained from the independent sample t-test revealed is not significantly difference between SARC and FAR ($p < 0.05$). The average AN of FAR is 6.9 greater than AN of SARC, this indicates FAR is higher N contained than SARC. However, the soil samples from SARC and FAR which recorded a mean AN value that was below the IASS acceptable limits of 240 – 480 kg/ha for soil. This is due to Nitrogen deficiencies basically occurs in soil which valve high content of organic matter which is not sufficiently decomposed. Product like sawdust, grass clipping make use of nitrogen from soil as they decompose and hence rob the soil of nitrogen leaching and leaving the soil.

4.4. Relationship between soil Physico-chemical properties and pesticide residues detected

Pearson's correlation was computed to evaluate the relationship between the concentration of detected pesticide and the physicochemical properties of soil as shown in table 12.

Table 12. Values of Pearson's correlation between soil physicochemical parameters and pesticide residues detected.

Sample code	Parameters	Diazinon	Malathion	Chlorpyrifos
SARC	pH	0.56	0.8	0.86
	EC	0.25	-0.98	-0.2
	OM	0.96	-0.34	0.73
	OC	0.98	0.14	0.96
	AP	-0.78	-0.58	-0.98
	AN	0.95	-0.35	0.72

Note: EC = Electrical Conductivity, OC = Organic carbon, AP = Available Phosphorus, AN = Available Nitrogen

A negative significant correlation was observed between EC with (malathion and chlorpyrifos), OM with (malathion), AP with (diazinon, malathion and chlorpyrifos), AN with (malathion) in SARC sample. This negative correlation between parameters and those pesticide residue levels in soil decrease with increase in soil parameters and

vice versa. This could be due to decomposition of diazinon, malathion and chlorpyrifos pesticide with increase in soil pH, EC, OC, OM, AP and AN and vice versa. The finding of this study is in line with a study which reported that residues of soils correlated negatively with organic carbon [109].

The strong positive correlation between pH with (Diazinon, malathion and chlorpyrifos) and OC with (diazinon and chlorpyrifos) indicates that pH of soil could have enhanced the adsorption of these pesticide compounds. Thus, increase in pH and OC resulted in a corresponding increase in concentrations of diazinon, malathion and chlorpyrifos, and diazinon and chlorpyrifos in SARC respectively. In addition OM with (diazinon and chlorpyrifos) and AN with (diazinon and chlorpyrifos) in SARC shows a strong positive correlation. This suggests that these nutrients in soils have significantly increase on the distribution and of pesticides and the level of these nutrients resulted in a corresponding increase in their individual pesticide residues [109].

Also, the positive correlation between EC and (diazinon), OC and (malathion) in SARC sample suggests that these nutrients in the soil have significant influence on the distribution of pesticides and an increase in the level of these nutrients resulted in a corresponding increase in their respective pesticide residues.

4.5. Health risk assessment

4.5.1. Non-Carcinogenic Health Risk Evaluation

Based on the OPP residue concentration observed in the study area, we have assessed the human health risk in children and adults under non-dietary exposure to OPP residues in the agricultural areas of FAR and SARC as shown in tables 13. The health risk of OPPs in the human population was assessed mainly through ADD_{ing}, ADD_{der}, ADD_{inh}, HQ, and HI.

According to Kumar et al. (2014), the hazard quotient is a tool that quantifies the extent of exposure potential that may result in non-carcinogenic health impacts for people over an average exposure time. Hazard quotients of OPPs for children and adult were calculated separately and recorded as $3.4x10^{-7}$ to $3.7x10^{-5}$ and $1.13x10^{-}7$ to $5.4x10^{-6}$, respectively. However, the hazard quotient results of OPPs in the soil samples of Sirinka and SARC were within the acceptable risk level of $HQ \leq 1$ [110].

Table 13. Total non-cancer risks from human exposure via ingestion, dermal absorption, and inhalation (mg kg^{-1} day^{-1}) with its hazard index in children and adult.

Exposure pathways		Children			Adult		
		Diaz	**Mala**	**Chlor**	**Diaz**	**Mala**	**Chlor**
SARC	ADD_{ing}	2.06×10^{-8}	7.36×10^{-8}	1.93×10^{-8}	1.77×10^{-9}	6.3×10^{-9}	1.65×10^{-9}
	ADD_{der}	4.9×10^{-9}	1.75×10^{-9}	4.58×10^{-9}	2.02×10^{-9}	7.2×10^{-9}	1.88×10^{-9}
	ADD_{inh}	1.68×10^{-18}	6×10^{-18}	1.6×10^{-18}	2.05×10^{-19}	7.34×10^{-19}	1.92×10^{-19}
	RfD	7×10^{-4}	7×10^{-2}	5×10^{-3}	0.0007	0.07	0.005
	HQ	3.7×10^{-5}	1.3×10^{-6}	3.4×10^{-7}	5.4×10^{-6}	1.93×10^{-7}	7.1×10^{-7}
FAR	ADD_{ing}	2.05×10^{-8}	4.33×10^{-8}	1.9×10^{-8}	1.75×10^{-9}	3.7×10^{-9}	1.64×10^{-9}
	ADD_{der}	4.86×10^{-9}	1.02×10^{-9}	4.54×10^{-9}	2.0×10^{-9}	4.23×10^{-9}	1.7×10^{-9}
	ADD_{inh}	1.6×10^{-18}	3.52×10^{-18}	1.56×10^{-18}	2.04×10^{-19}	3.72×10^{-19}	1.9×10^{-19}
	HQ	3.62×10^{-5}	7.6×10^{-7}	4.7×10^{-7}	5.35×10^{-6}	1.13×10^{-7}	6.7×10^{-7}
	HI	7.32×10^{-5}	2.06×10^{-6}	8.1×10^{-7}	1.01×10^{-5}	3.06×10^{-6}	1.4×10^{-6}
		CHI = 7.61×10^{-5}				**CHI = 1.5×10^{-5}**	

Notes; CHI = the cumulative hazard index, RfD = reference dose of each pesticide in mg kg^{-1} day^{-1}.

The hazard quotient of OPPs in SARC is higher than the hazard quotient of OPPs on farmer's farmland; this indicates the high consumption of agrochemicals or pesticides, especially OPPs like diazinon, malathion, and chlorpyrifos, in the crop processing area. Similarly, the HI of SARC is higher than FAR in the study. So that Sirinka agricultural and research center is more polluted by pesticides rather than outside the area (FAR).

The average non-cancer risk of Diazinon was the highest (HI = 7.32×10^{-5} for children and 1.01×10^{-5} for adults), followed by malathion (HI = 2.06×10^{-6} for children and 3.06×10^{-6} for adult) and chlorpyrifos (HI = 8.1×10^{-7} for children and 1.4×10^{-6} for adult). In general, children had comparatively greater non-cancer risks than adults. A possible reason could be that children are more vulnerable to a particular OPP dosage and more prone to unintentionally swallow a large quantity of contaminated soil due to hand or finger sucking and pica behavior [22].

According to the USEPA, in terms of cumulative non-carcinogenic risk is comparatively small or negligible if OPPs have HI < 1. Conversely, there is non-

carcinogenic risk and negative health impacts if the cumulative non-carcinogenic risk value of OPPs is HI > 1 [111].

Tables 13 present the evaluation results of the study, which assessed the non-carcinogenic risk of OPPs in agricultural soils in Sirinka from defferent age stages and exposure routes. The evaluation results indicated that there was no non-carcinogenic risk associated with OPPs for either children or adults, based on the cumulative non-carcinogenic risk (CHI = $7.61x10^{-5}$ for children and CHI = $1.5x10^{-5}$ for adults). Both malathion and diazinon posed comparatively more non-carcinogenic effects than chlorpyrifos.

OPPs in Sirinka agricultural soils do not pose a non-carcinogenic harm to the bodies of adults and children, as shown by the non-carcinogenic risk values of the soils which was lower than the USEPA in both children and adults (HI < 1). Children and adults in Sirinka do not exhibit numerous health issues despite the presence of OPP residues in the soil tests.

For this investigation, a large number of soil samples were gathered from farmlands around farmers. These soils may be often touched by people. To determine the precise amount of OPPs in human bodies and assess the hazards involved, more research is needed.

4.5.2. Carcinogenic Health Risk Evaluation

The risk via dietary pathway was considered in this study, but the OPPs determined in this study does not available in carcinogenicity.

Table 14. The carcinogenic properties of the selected pesticides

Pesticides	Carcinogenicity	CSF	Reference
Diazinon	Not classifiable as to human carcinogenicity" based on the lack of evidence of carcinogenicity	NA	[112]
Malathion	The U.S. EPA classifies malathion as "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential by all routes of exposure".	NA	[113]
Chlorpyrifos	Evidence of non-carcinogenicity for humans, by the U.S. EPA. No human data were found regarding carcinogenic effects of Chlorpyrifos.	NA	[114]

Notes: CSF = Cancer slope factor, NA = Not available at this time

The selected pesticides in this study is not in the levels to poses cancer to human.

CHAPTER FIVE

5. Conclusion and recommendation

5.1. Conclusion

The determination of the target pesticides analysis were validated to test whether or not validated analytical method was employed for the determination of four target organophosphate pesticides (Diazinon, Dimethoate, Malathion, and Chlorpyrifos) in soil samples. The method, utilizing QuEChERS sample preparation and GC-MS detection, demonstrated excellent linearity ($R^2 \geq 0.990$), low limits of detection (LODs) and quantification (LOQs) (ND – 0.09 µg/kg and ND – 1.5 µg/kg, respectively), and acceptable precision and accuracy (%RSD ≤ 15, recoveries 81.65 - 90.6 %). These results confirmed the suitability of the method for the intended purpose.

Analysis of soil samples from the study area revealed the presence of three organophosphate pesticides: Malathion (4.42 µg/kg in SARC, 2.63 µg/kg in FAR), Diazinon (1.24 µg/kg in SARC, 1.23 µg/kg in FAR), and Chlorpyrifos (1.16 µg/kg in SARC, 1.15 µg/kg in FAR). All detected concentrations were below the maximum residue limits (MRLs) established by the European Union.

Soil physicochemical properties between sampling sites were examined. Notably, soil pH was predominantly neutral to slightly alkaline, while organic matter and available nitrogen content were low. This suggests a need for soil improvement through organic amendments and integrated soil fertility management to enhance soil health. Available phosphorus levels indicated the importance of phosphorus fertilization. Statistical analysis revealed significant correlations between the detected pesticides and specific soil parameters: pH and organic carbon (OC) in SARC, and electrical conductivity (EC) in FAR.

A non-dietary health risk assessment was conducted for the three detected pesticides. The results indicated that the potential health risk to both adults and children from exposure to these pesticides in the study area was negligible (Hazard Index, HI < 1), falling within the acceptable non-carcinogenic risk range. This suggests that non-dietary exposure pathways from these farm soils do not pose a significant health threat to the local population.

5.2. Recommendation

The study of pesticide residues in the research area necessitates further investigation of the following aspects:

- A comprehensive assessment of seasonal fluctuations in organophosphate pesticide residues within the soil.
- Evaluation of pesticide residue levels in other environmental matrices, such as water, air, vegetables, irrigation sources, and fruits in Sirinka, to accurately determine the associated risks.
- Further research to establish the correlation between pesticide levels in the soil and crop produced in the area.

References

1. Onuwa, P., et al., *Determination of pesticide residues in edible crops and soil from University of Agriculture Makurdi Farm Nigeria.* 2017. **3**(3): p. 1-17.
2. Miller, G.T.J.I.P.G., California, *Sustaining the Earth, Thompson Learning.* 2004. **9**: p. 211-216.
3. Chiaia-Hernandez, A.C., et al., *Long-term persistence of pesticides and TPs in archived agricultural soil samples and comparison with pesticide application.* 2017. **51**(18): p. 10642-10651.
4. Afful, S., et al., *Assessment of synthetic pyrethroids residues in the waters and sediments from the Weija Lake in Ghana.* 2013.
5. Fianko, J.R., et al., *Agrochemicals and the Ghanaian environment, a review.* 2011. **2**(03): p. 221.
6. Ntiamoah, A. and G.J.J.o.c.p. Afrane, *Environmental impacts of cocoa production and processing in Ghana: life cycle assessment approach.* 2008. **16**(16): p. 1735-1740.
7. Aktar, W., D. Sengupta, and A.J.I.t. Chowdhury, *Impact of pesticides use in agriculture: their benefits and hazards.* 2009. **2**(1): p. 1-12.
8. Gill, H.K. and H.J.P.-t.a. Garg, *Pesticide: environmental impacts and management strategies.* 2014. **8**(187): p. 10-5772.
9. Akan, J., et al., *Organophosphorus pesticide residues in vegetables and soil samples from alau dam and gongulong agricultural areas, Borno State, Nigeria.* 2013. **3**(6): p. 58-64.
10. Agbeve, S., P. Osei-Fosu, and D.J.I.J.A.A.R. Carboo, *Levels of organochlorine pesticide residues in Mondia whitei, a medicinal plant used in traditional medicine for erectile dysfunction in Ghana.* 2014. **1**: p. 9-16.
11. Mesnage, R., et al., *Two cases of birth defects overlapping Stratton-Parker syndrome after multiple pesticide exposure.* 2010. **67**(5): p. 359-359.
12. CM, T.J.E.H.P., *Rotenone, paraquat, and Parkinson's disease.* 2011. **119**: p. 866-872.
13. Lemaitre, M., et al., *Dietary exposure to polychlorinated biphenyls (PCB) and risk of Non-Hodgkin's lymphoma: Evidence from the French E3N prospective cohort.* 2021. **197**: p. 111005.

14. Bento, C.P., et al., *Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness.* 2016. **572**: p. 301-311.
15. Musilek, K., et al., *Design, evaluation and structure—Activity relationship studies of the AChE reactivators against organophosphorus pesticides.* 2011. **31**(4): p. 548-575.
16. Fosu-Mensah, B.Y., et al., *Organophosphorus pesticide residues in soils and drinking water sources from cocoa producing areas in Ghana.* 2016. **5**: p. 1-12.
17. Dirbaba, N.B., et al., *Organochlorine pesticides, polybrominated diphenyl ethers and polychlorinated biphenyls in surficial sediments of the Awash River Basin, Ethiopia.* 2018. **13**(10): p. e0205026.
18. Dessie, G. and P.J.G.A.S.B. Kinlund, Human Geography, *Khat expansion and forest decline in Wondo Genet, Ethiopia*. 2008. **90**(2): p. 187-203.
19. Debelo, S., et al., *Determination of selected pesticide residues in diga water reservoir, Eastern Wollega Zone, Ethiopia.* 2020. **15**(2): p. 90-98.
20. Mossissa. T., *An Assessment of the Utilization of Pesticides and Safety Practices of the Floriculture Industries: The Case of Three Floriculture Farms at Sebeta Town, Oromia Region, Ethiopia*. 2011, St. Mary's University.
21. Haylamicheal, I.D. and M.A.J.E.i. Dalvie, *Disposal of obsolete pesticides, the case of Ethiopia.* 2009. **35**(3): p. 667-673.
22. Pan, L., et al., *Organophosphate pesticide in agricultural soils from the Yangtze River Delta of China: concentration, distribution, and risk assessment.* Environmental Science and Pollution Research, 2018. **25**: p. 4-11.
23. Yao, S., et al., *Levels, distribution and health risk assessment of organochlorine pesticides in agricultural soils from the pearl river delta of China.* International journal of environmental research and public health, 2022. **19**(20): p. 13171.
24. Yadav, I.C., et al., *Occurrence, profile and spatial distribution of organochlorines pesticides in soil of Nepal: Implication for source apportionment and health risk assessment.* Science of the Total Environment, 2016. **573**: p. 1598-1606.
25. Tanweer Ahmad, T.A., et al., *Removal of pesticides from water and wastewater by different adsorbents: a review.* 2010.
26. Kongphonprom, K. and R.J.A.L. Burakham, *Determination of carbamate insecticides in water, fruit, and vegetables by ultrasound-assisted dispersive*

liquid–liquid microextraction and high-performance liquid chromatography. 2016. **49**(6): p. 753-767.

27. Battaglin, W., J.J.W.S. Fairchild, and Technology, *Potential toxicity of pesticides measured in midwestern streams to aquatic organisms.* 2002. **45**(9): p. 95-103.
28. Jaiswal, D.K., et al., *Toxicity of organophosphate pesticide on soil microorganism: risk assessments strategies.* 2021: p. 257-295.
29. Saraji, M. and N.J.J.o.S.S. Tansazan, *Application of dispersive liquid–liquid microextraction for the determination of phenylurea herbicides in water samples by HPLC‐diode array detection.* 2009. **32**(23‐24): p. 4186-4192.
30. Zhou, Q., et al., *Determination of atrazine and simazine in environmental water samples by dispersive liquid–liquid microextraction with high performance liquid chromatography.* 2009. **25**(1): p. 73-76.
31. De Troyer, N., et al., *Water quality assessment of streams and wetlands in a fast growing east African city.* 2016. **8**(4): p. 123.
32. Mahmood, I., et al., *Effects of pesticides on environment.* 2016: p. 253-269.
33. Heiligmann, R.B. and D. Krause, *Relative effectiveness of herbicides commonly used to control woody vegetation in forest stands*. 2002, Ohio State Univ. Sch. Nat. Resources, Extension Fact Sheet F-51-02.
34. Nakao, T., S.J.P.B. Banba, and Physiology, *Minireview: Mode of action of meta-diamide insecticides.* 2015. **121**: p. 39-46.
35. Yadav, I.C., N.L.J.E.s. Devi, and engineering, *Pesticides classification and its impact on human and environment.* 2017. **6**(7): p. 140-158.
36. Merdassa, Y., J.-f. Liu, and N.J.A.M. Megersa, *Development of a one-step microwave-assisted extraction procedure for highly efficient extraction of multiclass fungicides in soils.* 2014. **6**(9): p. 3025-3033.
37. Diggle, A., P. Neve, and F.J.W.r. Smith, *Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations.* 2003. **43**(5): p. 371-382.
38. Monaco, T.J., S.C. Weller, and F.M. Ashton, *Weed science: principles and practices*. 2002: John Wiley & Sons.

39. Ortiz-Hernández, M.L. and E.J.R.i.d.c.a. Sánchez-Salinas, *Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México.* 2010. **26**(1): p. 27-38.
40. Pandey, P., R. Raizada, and L.J.J.o.E.B. Srivastava, *Level of organochlorine pesticide residues in dry fruit nuts.* 2010. **31**(5): p. 705-707.
41. Abubakar, Y., et al., *Pesticides, History, and Classification. Natural Remedies for Pest, Disease and Weed Control, 29-42*. 2020.
42. Chowdhury, M.A.Z., et al., *Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh.* 2012. **9**(9): p. 3318-3329.
43. Marasinghe, J., et al., *Organophosphate pesticide residues in food commodities in Sri Lanka: a review.* 2011. **13**: p. 81-94.
44. Kristensen, P., et al., *European waters assessment of status and pressures 2018.* 2018.
45. Ambrus, A., Y.Z.J.J.o.a. Yang, and f. chemistry, *Global harmonization of maximum residue limits for pesticides.* 2016. **64**(1): p. 30-35.
46. Suzuki, O. and K. Watanabe, *Drugs and poisons in humans*. 2005: Springer.
47. Kuntom, A., et al., *Pesticide application in the oil palm plantation.* 2007. **54**: p. 52.
48. Yu, R., et al., *Human health risk assessment of organophosphorus pesticides in maize (Zea mays L.) from Yushu, Northeast China.* 2018. **24**(3): p. 642-652.
49. Fernandes, V.C., et al., *Magnetic dispersive micro solid-phase extraction and gas chromatography determination of organophosphorus pesticides in strawberries.* 2018. **1566**: p. 1-12.
50. Farina, Y., et al., *Pesticides residues in agricultural soils and its health assessment for humans in Cameron Highlands, Malaysia.* 2016. **20**(6): p. 1346-1358.
51. Alfonso, L.-F., et al., *Adsorption of organophosphorus pesticides in tropical soils: the case of karst landscape of northwestern Yucatan.* 2017. **166**: p. 292-299.
52. Mulla, S.I., et al., *Organophosphate pesticides: impact on environment, toxicity, and their degradation.* 2020: p. 265-290.
53. Broznić, D., et al., *Sorption and leaching potential of organophosphorus insecticide dimethoate in Croatian agricultural soils.* 2021. **273**: p. 128563.

54. Vagi, M.C., et al., *Adsorption and desorption processes of the organophosphorus pesticides, dimethoate and fenthion, onto three Greek agricultural soils.* 2010. **90**(3-6): p. 369-389.
55. Pérez-Lucas, G., et al., *Environmental risk of groundwater pollution by pesticide leaching through the soil profile.* 2019. **17**: p. 1-28.
56. Børgesen, C.D., et al., *Fate of pesticides in agricultural soils*. 2015: DCA-Nationalt center for fødevarer og jordbrug.
57. Caceres, T.P., et al., *Sorption of fenamiphos to different soils: the influence of soil properties.* 2008. **43**(7): p. 605-610.
58. Hussaini, S., *Potential of entomopathogenic nematodes in integrated pest management*, in *Integrated pest management*. 2014, Elsevier. p. 193-223.
59. Jensen, I.M. and P.J.H.H.o.P.T. Whatling, *Malathion: A review of toxicology*. 2010: p. 1527-1542.
60. Rashid, R.A., et al., *Determination of malathion levels and the effect of malathion on the growth of Chrysomya megacephala (Fibricius) in malathion-exposed rat carcass.* 2008.
61. Aggarwal, V., et al., *Diazinon—chemistry and environmental fate: a California perspective.* 2013: p. 107-140.
62. Burgess, P., et al., *Toxicological profile for diazinon.* 2008.
63. Bailey, H.C., et al., *Development of procedures for identifying pesticide toxicity in ambient waters: Carbofuran, diazinon, chlorpyrifos.* 1996. **15**(6): p. 837-845.
64. Brady Jr, U. and B.J.J.o.E.E. Arthur, *Biological and chemical properties of dimethoate and related derivatives.* 1963. **56**(4): p. 477-482.
65. Testai, E., F.M. Buratti, and E. Di Consiglio, *Chlorpyrifos*, in *Hayes' Handbook of Pesticide Toxicology*. 2010, Elsevier. p. 1505-1526.
66. Wauchope, R.D., et al., *Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability.* 2002. **58**(5): p. 419-445.
67. Ragnarsdottir, K.V.J.J.o.t.G.S., *Environmental fate and toxicology of organophosphate pesticides.* 2000. **157**(4): p. 859-876.
68. Özkara, A., D. Akyıl, and M. Konuk, *Pesticides, environmental pollution, and health*, in *Environmental health risk-hazardous factors to living species*. 2016, IntechOpen.
69. Koskinen, W.C., S.S.J.P.i.t.s.e.p. Harper, impacts, and modeling, *The retention process: mechanisms.* 1990. **2**: p. 51-77.

70. Cáceres, T., et al., *Fenamiphos and related organophosphorus pesticides: environmental fate and toxicology*. 2010: Springer.
71. Eaton, D.L., et al., *Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment.* 2008. **38**(sup2): p. 1-125.
72. Farahat, T., et al., *Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides.* 2003. **60**(4): p. 279-286.
73. Buchanan, D., et al., *Estimation of cumulative exposure to organophosphate sheep dips in a study of chronic neurological health effects among United Kingdom sheep dippers.* 2001. **58**(11): p. 694-701.
74. Paz-y-Miño, C., et al., *Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador.* 2002. **110**(11): p. 1077-1080.
75. Bergman, Å., et al., *The impact of endocrine disruption: a consensus statement on the state of the science.* 2013. **121**(4): p. a104-a106.
76. Liu, L., et al., *Mitigation of environmental pollution by genetically engineered bacteria—current challenges and future perspectives.* 2019. **667**: p. 444-454.
77. Munn, M.D., et al., *Pesticide toxicity index for freshwater aquatic organisms*. 2001: US Department of the Interior, US Geological Survey Washington, DC, USA.
78. Belden, J.B., et al., *Relative toxicity and occurrence patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean production.* 2007. **3**(1): p. 90-100.
79. Tudi, M., et al., *Agriculture development, pesticide application and its impact on the environment.* 2021. **18**(3): p. 1112.
80. Agroindustriais, P.J.C., Propagação E Melhoramento Genético De Pitaya Comercial E Nativa Do Cerrado, *AOAC. Official methods of analysis of the Association of Official Analytical Chemists.* 2013. **26**(74): p. 62.
81. Muhammad Arif, A., et al., *Organochlorine pesticide residues in raw milk samples collected from dairy farms and urban areas of Lahore district, Pakistan.* Journal of Food Science and Technology, 2021. **58**: p. 129-137.
82. Jones, J., *Soil analysis handbook of reference methods*. 2018: CRC press.
83. Díez, C., et al., *Evaluation of quantification methods to compensate for matrix effects in the analysis of benzalkonium chloride and didecyldimethylammonium chloride in fruits and vegetables by LC-ESI-MS/MS.* 2016. **9**: p. 485-499.

84. Yao, S., et al., *Levels, distribution and health risk assessment of organochlorine pesticides in agricultural soils from the pearl river delta of China.* 2022. **19**(20): p. 13171.
85. EPA, U.J.P.R.D., OSWER, *Supplemental guidance for developing soil screening levels for superfund sites.* 2001. **9355**: p. 4-24.
86. Assessment, P.R.J.P.f.C.P.R.A.U.S.E.P.A.W., *Risk Assessment guidance for superfund: volume III-part a.* 2001.
87. Kicińska, A., M.J.H. Mamak, and E.R.A.A.I. Journal, *Health risks associated with municipal waste combustion on the example of Laskowa commune (Southern Poland).* 2017. **23**(8): p. 2087-2096.
88. China, M.o.E.P.o.t.P., *Technical guidelines for risk assessment of contaminated sites, HJ 25.3-2014*. 2014, China Environmental Science Press Beijing.
89. Ge, J., et al., *Composition, distribution and risk assessment of organochlorine pesticides in soils from the Midway Atoll, North Pacific Ocean.* 2013. **452**: p. 421-426.
90. Qu, C., et al., *Risk assessment and influence factors of organochlorine pesticides (OCPs) in agricultural soils of the hill region: A case study from Ningde, southeast China.* 2015. **149**: p. 43-51.
91. Wcisło, E., et al., *Human health risk assessment in restoring safe and productive use of abandoned contaminated sites.* 2016. **94**: p. 436-448.
92. Canada, H., *Federal Contaminated Site Risk Assessment in Canada Part I: Guidance on Human Health Preliminary Quantitative Risk Assessment (PQRA)*. 2004, Health Canada Ottawa, ON, Canada.
93. Wang, B., et al., *Levels and patterns of organochlorine pesticides in agricultural soils in an area of extensive historical cotton cultivation in Henan province, China.* 2016. **23**: p. 6680-6689.
94. Ali, N., et al., *Human health risk assessment through consumption of organophosphate pesticide-contaminated water of Peshawar basin, Pakistan.* 2018. **10**: p. 259-272.
95. Huber, L., *Validation of analytical methods and processes*, in *Pharmaceutical process validation*. 2003, CRC Press. p. 550-567.
96. Bliesner, D.M., *Validating chromatographic methods: a practical guide*. 2006: John Wiley & Sons.

97. Haile, T., et al., *Determination of Organochlorine and organophosphorus pesticide residues in tomato, potato, and pineapple samples of selected farmlands in Southwest Ethiopia.* Bulletin of the Chemical Society of Ethiopia, 2023. **37**(1): p. 23-33.
98. González, A.G. and M.Á.J.T.T.i.A.C. Herrador, *A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles.* 2007. **26**(3): p. 227-238.
99. Mekonen, S., et al., *Effect of household coffee processing on pesticide residues as a means of ensuring consumers' safety.* 2015. **63**(38): p. 8568-8573.
100. Williams, A.B.J.A.J.o.E.S. and Technology, *Residue analysis of organochlorine pesticides in water and sediments from Agboyi Creek, Lagos.* 2013. **7**(5): p. 267-273.
101. Nabhan, K.J., et al., *Assessment of multiresidue pesticides in agricultural soils from Ledang, Malaysia and related potential health risks.* 2018. **17**(1): p. 99-106.
102. Mahdavi, V., et al., *Concentration and non-dietary human health risk assessment of pesticide residues in soil of farms in Golestan province, Iran.* 2024. **34**(2): p. 968-978.
103. Mahmud, M.M., et al., *Assessment of organophosphorus and pyrethroid pesticide residues in watermelon (citrulus lanatus) and soil samples from Gashua, bade local government area Yobe State, Nigeria.* 2015. **3**(3): p. 52-61.
104. Osman, K.T. and K.T. Osman, *Chemical Properties of Forest Soils.* Forest Soils: Properties and Management, 2013: p. 45-61.
105. Estefan, G., R. Sommer, and J. Ryan, *Methods of soil, plant, and water analysis.* A manual for the West Asia and North Africa region, 2013. **3**(2013): p. 65-119.
106. Estefan, G., *Methods of soil, plant, and water analysis: a manual for the West Asia and North Africa region*. 2013, International Center for Agricultural Research in the Dry Areas (ICARDA).
107. Sims, J.T., *Soil test phosphorus: Bray and Kurtz P-1.* Methods of phosphorus analysis for soils, sediments, residuals, and waters, 2000: p. 13.
108. Ghare, P. and A. Kumbhar, *Study on physico-chemical parameters of soil sample.* International Advanced Research Journal in Science, Engineering and Technology, 2021. **8**(9): p. 171-187.

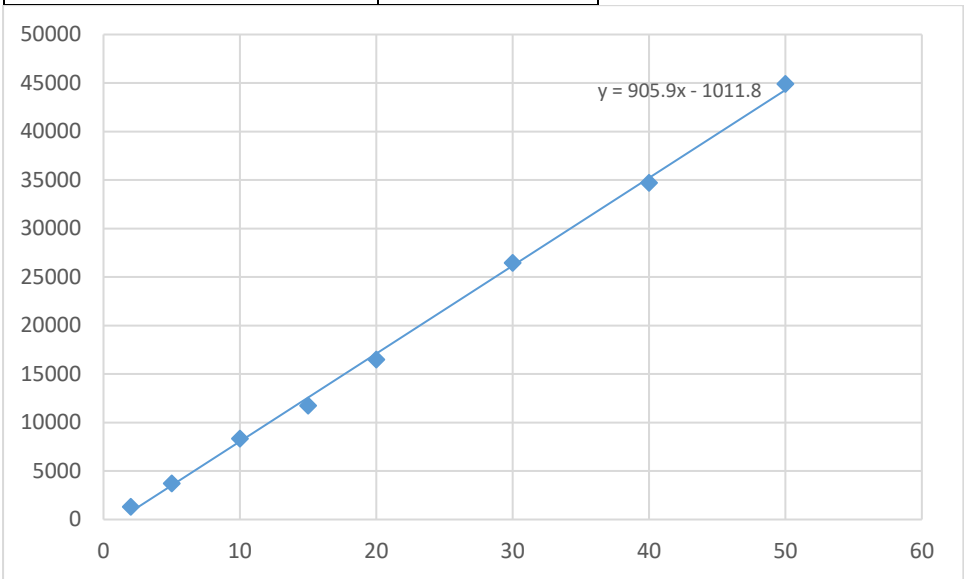
109. Fosu-Mensah, B.Y., et al., *Organophosphorus pesticide residues in soils and drinking water sources from cocoa producing areas in Ghana*. Environmental Systems Research, 2016. **5**: p. 1-12.
110. Kumar, B., et al., *Persistent organic pollutants in residential soils of North India and assessment of human health hazard and risks.* 2014. **96**(2): p. 255-272.
111. Pelletier, M., et al., *Chemical-by-chemical and cumulative risk assessment of residential indoor exposure to semivolatile organic compounds in France*. 2018. **117**: p. 22-32.
112. http://npic.orst.edu/factsheets/archive/diazinontech.html (assessed at September 1)
113. http://npic.orst.edu/factsheets/archive/malatech.html (assessed at September 4)
114. http://npic.orst.edu/factsheets/archive/chlorptech.html (assessed at September 3)

Appendixes

Appendix 1: Calibration of the pesticides

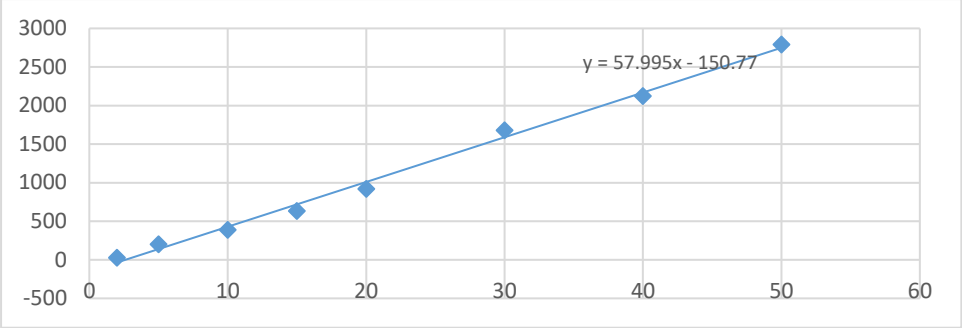
Target compound; Diazinon

Concentration (ppb)	Response
2.0000	1323
5.0000	3733
10.0000	8347
15.0000	11729
20.0000	16503
30.0000	26464
40.0000	34724
50.0000	44897



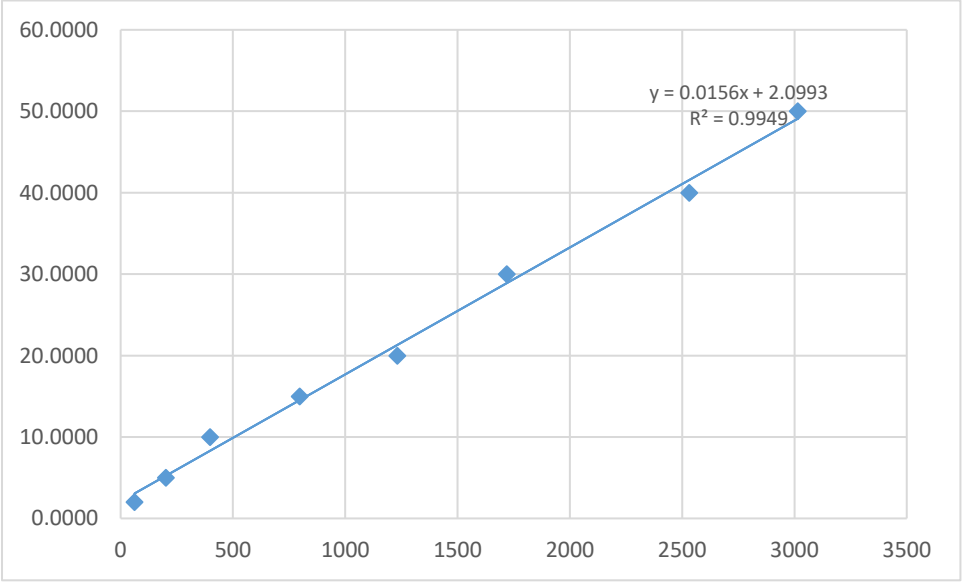
Target; malathion

Concentration (ppb)	Response
2.0000	28
5.0000	203
10.0000	390
15.0000	634
20.0000	920
30.0000	1680
40.0000	2121
50.0000	2793



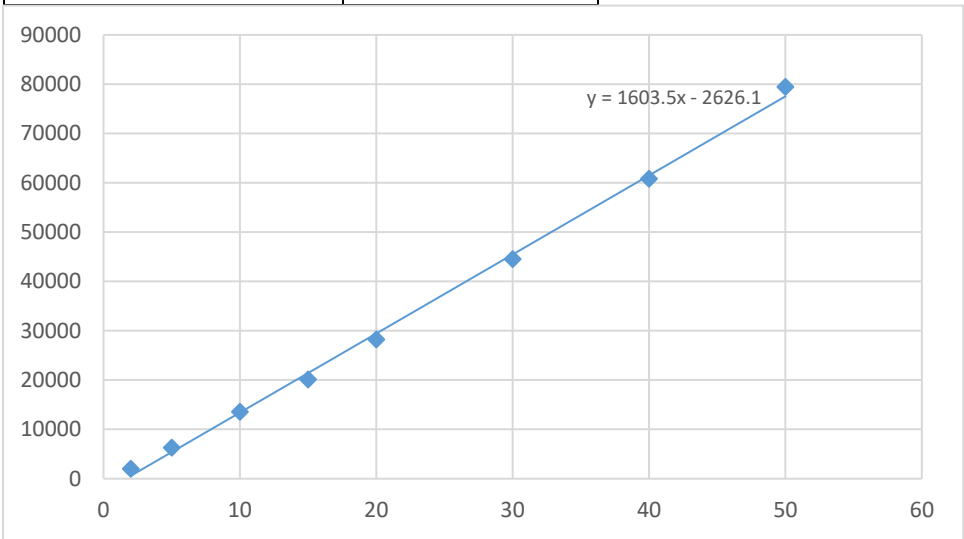
Target compound; dimethoate

Concentration (ppb)	Response
2.0000	62
5.0000	202
10.0000	398
15.0000	797
20.0000	1232
30.0000	1720
40.0000	2532
50.0000	3015



Target compound; chlorpyrifos

Concentration (ppb)	Response
2.0000	1952
5.0000	6277
10.0000	13529
15.0000	20065
20.0000	28217
30.0000	44532
40.0000	60808
50.0000	79414



Appendix 2: Recovery test value

Sample code	Compound name	Spike amount	Spike result	Non-spike result	Recovery
SARC	Dimethoate	30	28.58	0	95.3
FAR	Dimethoate	30	28.34	0	94.5
SARC	Malathion	30	28.89	4.42	81.66
FAR	Malathion	30	28.48	2.63	86.2
SARC	Diazinon	30	27.65	1.24	88.03
FAR	Diazinon	30	26.72	1.23	83.97
SARC	chlorpyrifos	30	28.24	1.15	90.3
FAR	chlorpyrifos	30	27.7	1.16	88.5

Appendix 3. Determination of pH

Soil pH is a crucial soil indicator, and is defined as the negative log of the hydrogen ion activity. Since pH is logarithmic, the H-ion concentration in solution increases ten times when its pH is lowered by one unit. The pH range normally found in soils varies from 5 to 9.

Various categories of soil pH may be arbitrarily described as follows: -

- ✓ Strongly acid (pH < 5.0)
- ✓ Moderately to slightly acid (5.0-6.5)
- ✓ Neutral (6.5-7.5)
- ✓ Moderately alkaline (7.5-8.5), and
- ✓ Strongly alkaline (> 8.5)

Equipment and reagent

- pH meter
- distilled water
- pH 7 and 4 buffer solutions for calibrating the pH meter
- glass beaker
- digital balance

Procedure

1. Calibrate the pH meter by buffer
2. Measure a 5g soil sample into the beaker
3. Add 25ml distilled water to the sample
4. Stir vigorously for 5 seconds and let stand for ten minutes
5. Place the electrode in the slurry, swirl carefully and read the pH immediately.

Appendix 4: determination of soil Electrical Conductivity

Equipment and apparatus

- pH meter
- distilled water
- digital balance

Procedure

1. 30 g of soil sample was place into the beaker and 30 ml of distilled water was added into the beaker
2. The lid was putted onto the beaker and shake vigorously.
3. The EC pocket meter was inserted into the soil-water mixture.
4. The reading was taken while the soil particles are still in suspended in solution and allowed the reading to stabilize.

Appendix 5: Determination of Soil Organic Matter/ Organic Carbon

Organic matter/organic carbon can be estimated by volumetric and colorimetric methods. However, the most common procedure involves reduction of potassium dichromate ($K_2Cr_2O_7$) by OC compounds and subsequent determination of the unreduced dichromate by oxidation-reduction titration with ferrous ammonium sulfate. This method is referred to as the Walkley-Black method.

Apparatus

- Magnetic stirrer
- Glassware and pipettes for dispensing and preparing reagents
- Titration apparatus (burette)
- Beaker
- Volumetric flask
- Conical flask

Reagents

A. Potassium Dichromate Solution ($K_2Cr_2O_7$), 1N
    - Dry $K_2Cr_2O_7$ in an oven at 105 °C for 2 hours. Cool in a desiccator (silica gel), and store in a tightly stoppered bottle.
    - Dissolve 49.04 g $K_2Cr_2O_7$ in DI water, and bring to 1-L volume.

B. Sulfuric Acid (H_2SO_4) concentrated (98 %, sp. gr. 1.84)

C. Orthophosphoric Acid (H_3PO_4), concentrated

D. Ferrous Ammonium Sulfate Solution [$(NH_4)_2SO_4.FeSO_4.6H_2O$], 0.5 M Dissolve 196 g ferrous ammonium sulfate in DI water, and transfer to a 1-L flask, add 5 mL concentrated H_2SO_4, mix well, and bring to volume.

E. E. Diphenylamine Indicator $(C_6H_5)_2NH$ Dissolve 1 g diphenylamine indicator in 100 mL concentrated H_2SO_4.

Procedure

1. 1 g of air dried was weighed into a 500-mL beaker.
2. Add 10 mL 1 N potassium dichromate solution using a pipette, add 20 mL concentrated H_2SO_4 using a dispenser, and swirl the beaker to mix the suspension.
3. Allow to stand for 30 minutes.
4. Add about 200 mL DI water, then add 10 mL concentrated H_3PO_4 using a dispenser, and allow the mixture to cool.
5. Add 15 drops diphenylamine indicator, add a Teflon-coated magnetic stirring bar, and place the beaker on a magnetic stirrer.
6. Titrate with 0.5 M ferrous ammonium sulfate solution, until the color changes from violet-blue to green.
7. Prepare two blanks, containing all reagents but no soil, and treat them in exactly the same way as the soil suspensions.

Calculations

$$M = \frac{10}{V_{blank}}$$

$$oxidizable\ Oranic\ Carbon\ (\%) = \frac{[V_{blank} - V_{samble}] \times 0.3 \times M}{Wt}$$

$$Total\ Organic\ Carbon\ (\%) = 1.334\ \times oxidizable\ Oranic\ Carbon(\%)$$

$$Organic\ Carbon\ (\%) = 1.724 \times Total\ Organic\ Carbon(\%)$$

Where:

M = Molarity of [$(NH_4)_2SO_4.FeSO_4.6H_2O$] solution (about 0.5 M)

V_{blank} = Volume of [$(NH_4)_2SO_4.FeSO_4.6H_2O$] solution required to titrate the blank (mL)

V_{sample} = Volume of $[(NH_4)_2SO_4.FeSO_4.6H_2O]$ solution required to titrate the sample (mL)

W_t = Weight of air-dry soil (g)

Appendix 6: Determination of available phosphorus in a soil sample

Bray's p-1 method

In Bray's method a mixture of ammonium fluoride and hydrochloric acid (NH_4F + HCl) act as the extracting agent. It works in two ways, firstly, the hydrogen ions from HCl have a solubilising effect, especially on Ca-P, which makes phosphate get extracted. Secondly, the fluoride ions (from ammonium fluoride) complex with the Al3+ and Fe3+ ions, thereby releasing the Al and Fe bound phosphorous into the extract. The liberated phosphorous (present as orthophosphates) in solution is treated with ammonium molybdate under acidic conditions. It forms a complex called ammonium phosphomolybdate. The complex is then treated with a reducing agent like, stannous chloride or ascorbic acid to obtain intense blue colour compound called molybdenum blue. The intensity of colour of this complex is proportional to the concentration of phosphate and can be read with the help of a photoelectric colorimeter at a wavelength of 660 nm or 880 nm depending on the reducing agent used.

A number of standard solutions containing known concentration of the phosphorus are prepared. The colour is developed under conditions used for the soil extract and their absorbance is measured at a suitable wavelength. A plot of absorbance as a function of concentration is drawn and the concentration of the phosphorus in the soil extract is found out using this standard curve. This method is suitable for acidic soils and does not give good results with clay soils and calcareous soils. These soils have high degree of base saturation and neutralise the acid in the extractant, thereby reducing its solubilising effect.

Apparatus

- Conical flask (100 cm^3)
- Horizontal shaker
- Whatman No.1 filter paper

- Pipette
- Volumetric flask (25 ml, 100 ml, 1L)
- Photoelectric colorimeter (UV-VIS spectrophotometer)

Reagents and solution provided

1. Bray's P-1 extractant : Mix 30 ml of 1M NH4F solution (prepared by dissolving 3.7 g of solid ammonium fluoride in water and making up the volume to 100 L) and 50 ml of 0.5M HCl (prepared by diluting 2.2 ml of concentrated HCl to 50 ml) in a 1L volumetric flask. Make up the volume with distilled water. Store the solution in a polyethylene bottle.
2. Dickman and Bray's reagent (1.5%): Dissolve 15 g of ammonium molybdate (in powdered form) in about 300 ml of distilled water taken in a 1L volumetric flask and warm it to ˜ 50°C. Cool and add 350 ml of 10 M HCl to it with constant stirring. Make up the volume with distilled water.
3. Stannous chloride (stock solution): Dissolve 10 g of $SnCl_2.2H_2O$ in 25 ml of conc. HCl in a 100 ml beaker. Warm if necessary. Cool, add a piece of pure metallic tin (or Zn) to it so as to prevent oxidation and store the solution in a glass stoppered brown bottle. The solution is stable for short time.
4. Stannous chloride (working solution): Dilute 1ml of the stock solution to 66.0 ml by distilled water just before use.
5. Phosphorous standard (100 mg P L): Dissolve 0.4394 g of oven dried monobasic potassium phosphate in some distilled water. Add 25 ml of ~ 3.5 M H_2SO_4 (prepared by diluting 5 ml of concentrated sulphuric acid to 25 ml) and make up the volume.
6. Phosphorous standard (working solution, 2 mg P L or 2 ppm P): Pipette out 1 ml of 100 ppm standard solution of phosphorous in a 50 ml volumetric flask and make up the volume with distilled water.

Procedure

The procedure involves three steps and each step further involves a sequence of activities as per the following details.

a) **Extraction**

- Weigh 2g of air dried soil sample and put into a 50 ml extraction vessel or flask.
- Pipette 20 ml of the Bray's P-1 extraction reagent and add to the vessel.

- In another flask (marked as blank) take 20 ml of the Bray's P-1 extraction reagent without the sample of the soil. This would act as a blank or reference. Treat this solution in exactly the same way as for the test solution.
- Shake the mixture for 5 minutes on a mechanical shaker.
- Quickly filter the extract through Whatman No. 1 filter paper. Ensure that the filtrate is collected within 10 minutes and save the extract.

b) Development of color

- Transfer 5 ml of the filtrate to a 25 ml volumetric flask.
- Add 5 cm3 of ammonium molybdate solution.
- Gently shake the flask for some time and dilute the mixture to about 20 ml with distilled water (add water along the sides of the flask so as to wash them.)
- Add 1ml of working solution of $SnCl_2$, shake the solution and make up the volume with distilled water.
- Transfer an aliquot to a spectrophotometric tube or cuvette and measure the absorbance at 660 nm using a UV-VIS spectrophotometer. Use the 'blank' solution as the reference (for zero absorbance setting). Record the readings in the observation table given below.

c) Calibration

- Take six volumetric flasks of 25 ml each and label them as 0, 1, 2 , 3 , 4 and 5 µg respectively.
- Transfer 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the working standard solution of 2 mg/L respectively to the flasks labelled above and add 5 ml of the extractant to each of them.
- Develop the colour as with the soil extract. Follow steps 2 to 4 as given under development of colour.
- Measure the absorbances of these solutions one by one using a spectrophotometer and record them in the table given below. Use blank as the reference.

Calculations

Plot a graph (Calibration curve) between the concentrations of phosphorous (x-axis) in the standard solutions and the corresponding absorbance values (y-axis) as recorded.

Mark the absorbance reading for the soil sample on the calibration curve and read the corresponding value of concentration on the X- axis.

The value of available phosphorous (in kg/ha) can be obtained from the following formula:

$$Available\ PHosphorus\left({}^{kg}/_{ha}\right) = \frac{Z\ x\ V\ x\ 2.24}{A\ x\ W}$$

Where:

Z = the amount of P (in µg) as read from the graph

V = the volume (in ml) of the extracting agent used

A = the volume (in ml) of the aliquot used in colour development

W= the mass (in g) of the soil sample

Substituting the values, we get:

Available P = Z x 4.48 kg/ha

Appendix 7: Determination of Available Nitrogen

The easily hydrolysable and oxidisable fractions of organic nitrogen present in a soil sample can be extracted out using alkaline $KMnO_4$ - a mild oxidising agent. For this purpose a known amount of soil sample is boiled with a measured quantity of alkaline $KMnO_4$. It leads to evolution of ammonia which is absorbed in a known volume of standard sulphuric acid. A part of the acid is neutralised by ammonia and the excess is titrated with a standard solution of alkali using methyl red as indicator. This method gives the amount of available nitrogen content as the percent of the soil. This value is also expressed in terms of Kg/ha. For this purpose it is assumed that 1 ha of furrow slice (0-15cm) of the soil weighs 2.24 million kg. If the amount of available nitrogen is found to be less than 272 kg/ha. The sample is rated low, if it is 272- 544 kg/ha, it is rated medium and if the available nitrogen content is more than 544 kg/ha it is rated as high.

Apparatus

- Kjeldahl flask (800 ml)
- Distillation apparatus
- Measuring cylinder
- Pipette

- Burette
- Conical flask

Reagents

1. 0.32% $KMnO_4$: Weigh 3.2 g $KMnO_4$ and transfer it to a 1 L or 1000 ml volumetric flask. Add some distilled water to the flask and dissolve it by gently swirling the flask. Carefully make up the volume to 1 L.
2. 2.5% NaOH: Weigh 25 g of NaOH pellets and transfer to a 1 L graduated beaker or measuring cylinder. Dissolve it in distilled water and carefully make up the volume to 1 L.
3. 0.02 M NaOH: Dissolve about 1.0 g of NaOH pallets in water in a standard flask or a measuring cylinder. Standardise by titrating against 0.01 M oxalic acid using methyl red indicator. Adjust the strength to 0.02 M.
4. 0.01 M H_2SO_4: Carefully add 0.6 cm3 of concentrated H_2SO_4 (sp. gr. 1.84) to one L of distilled water. Standardise by titrating against a standard alkali solution using methyl red indicator. Adjust the strength to 0.01 M.
5. 0.15% methyl red indicator: Dissolve 0.15 g of methyl red powder in 50 ml of ethyl alcohol and make up the volume to 100 ml with water.

Procedure

1. Set up the distillation apparatus with the help of your counsellor.
2. Weigh 20 g of given soil sample and transfer it carefully into Kjeldahl distillation flask.
3. Moisten the sample with about 10 ml of distilled water. Wash down the soil adhering to the neck of the flask.
4. Add 100 ml of 0.32% $KMnO_4$ solution and 100 ml of 2.5% NaOH solution and a few glass beads or broken pieces of glass rod to avoid bumping to the above sample and immediately stopper the flask.
5. Take 25 ml of 0.01 M H_2SO_4 in a 150 cm3 conical flask and add 3-4 drops of methyl red to it. Dip the end of the delivery tube of the distillation apparatus into it.
6. Heat the distillation flask steadily to distill 100 ml of liquid ammonia in about 30 minutes time.
7. Titrate the excess of standard H_2SO_4 left in the conical flask with 0.02 M NaOH and note the volume used (X ml) in the observation table.

Observation

Weight of soil taken = 20 g

Volume of 0.01 M H_2SO_4 = 25 ml

Volume of 0.02 M NaOH required for bank titration = X ml

Volume of 0.01 M H_2SO_4 used by NH_3 involved = (25 – X) ml

Calculations

1 ml of 0.01 M H_2SO_4 = 0.00028 g of Nitrogen

Amount of Nitrogen in 20 g of soil = (25 – X) x 0.00028 g = Zg

Available Nitrogen in kg/ha = Z x 22400