PESTICIDE USE AND FOOD SAFETY IN KATHMANDU VALLEY/ NEPAL

MASTER THESIS

Submitted to

Institute of Soil Research
University of Natural Resources and Life Sciences
(BOKU)

By

Nirita Giri
Matriculate Number: h0841180

Vienna, September 2010

TABLE OF CONTENTS

CONTENTS **PAGE NUMBER**

Acknowledgements

Summary

1. Introduction	**1**
1.1 Background	1
1.2 History of pesticide use in Nepal	2
1.3 Actual situation of pesticide use in Nepal	3
2. Objectives and Scope of the study	**5**
2.1 Objectives	5
2.2 Scope	5
3. Literature Review	**6**
3.1 Environmental effects of pesticides	6
3.2 Persistent organic pollutants	6
3.3 Health effects of pesticides	6
3.4 Problems of infrastructure in developing countries	7
3.5 Infrastructure for pesticide use in Nepal	8
3.5.1 Legal and institutional infrastructure	8
3.5.2 Pesticide management and registration system	8
3.5.3 Challenges for the Nepal government	9
3.5.4 Inadequate residue control measures	10
3.5.5 Emerging challenges for trading	11
3.5.6 Classification of pesticides (Guidelines, levels and standard values)	11
3.5.7 Banned and commonly used pesticides	13
3.5.8Former studies on pesticide level	14
3.6 Available methods for the extraction and analysis of pesticides in food	15
3.6.1 High Performance Liquid Chromatography (HPLC)	17
3.6.2 6410 Triple Quadrupole Mass Spectrometer	18
4. Materials and Methods	**20**
4.1 Study area	20
4.2 Study period	21
4.3 Questionnaire survey	21
4.4 Sample collection	22
4.5 Pesticides selected for laboratory analysis	24
4.6 Laboratory analysis of soil and food (tea, fruits and vegetables)	24
4.6.1 Soil analysis	24

4.6.2 Food analysis (tea, fruits and vegetables) 29

5. Results and Discussion **33**
5.1 Survey results 33
5.1.1 Source of information about pesticide use among farmers 33
5.1.2 Storage of pesticide 33
5.1.3 Stages of pesticide use by the farmers 34
5.1.4 Application of pesticides 34
5.1.5 Waiting time after pesticide use 35
5.1.6 Safety measures adopted during pesticide use 36
5.1.7 Knowledge about the health impacts of pesticide application 36
5.1.8 Symptoms of health impacts 37
5.2 Results of laboratory studies 37
5.2.1 Tea 37
5.2.2 Fruits and vegetables 38
5.2.3 Soil 39

6. Conclusions and Recommendations **41**

7. References **43**

8. List of tables, figures and abbreviations **46**
8.1 List of tables 46
8.2 List of figures 46
8.3 List of abbreviations 47

Acknowledgement

I am very pleased to dedicate this work to all the people and organization that provided me moral, technical and financial support and guidance for the successful completion of my study.

First of all, i take my honour to express my gratitude to Austrian Agency for International Cooperation in Education and Research (OeAD-GmbH) for providing me scholarship to carry out Masters Study for two years in University of Natural Resources and Life Sciences (BOKU) Vienna, Austria.

My sincere thanks and gratitude goes to Prof. Dr. Winfried E.H. Blum for his scientific supervision and support despite of his other academic and professional commitments.

I would like to express my respect and heartiest thanks to Ass. Prof. Dipl. Ing. Dr. nat. techn. Axel Mentler of Institute of Soil Research (BOKU) and Ass. Prof. Dr. Phil. Monika Sieghardt of Institute of Soil Ecology (BOKU) for providing me with supervision, inspiration and encouragement throughout the entire course of my research and study programme.

My special thanks to SUNA Group – Scientific Platform at BOKU including the Institute of Analytical Chemistry (Dr. Stefan Hann and Dr. Gunda Koellensperger), Institute of Forest Ecology (Dr. Monika Sieghardt) and the Institute of Forest and Soil Research(Dr. Axel Mentler) for providing me with technical cooperation to conduct my research.

My greatest thanks goes to LVA GmbH, especially Dr. Gartner, Dr. Celine and Dr. Partik for providing me with the technical support to carry out the laboratory analysis of the samples.

I owe my gratitude to all the staffs of Institute of Soil Research (BOKU) for providing me with all the support that i needed during the course of my studies.

I would like to thank the Project Manager of KEF Project 162 (Commission for Development Studies) for providing me opportunity to carry out my research under this project.

My special thanks to Environment and Public Health Organization (ENPHO), Kathmandu Nepal for providing me with all the support that was required for the fulfillment of my studies.

Lastly but most importantly, my heartiest thanks goes to my family members who supported me morally and provided me the best environment for successful completion of my studies.

Summary

Nepal is a country with a very diverse topography with altitudes ranging from 60 m to 8848 m. The economy of the country is mainly based on agriculture. Chemical pesticides are excessively used by the farmers in order to meet the increasing demand for food for the fast growing population. Due to a lack of a proper, continuous and efficient governmental pesticide monitoring programme, Nepalese farmers are facing severe quality related problems when exporting agricultural products like honey, tea and others in European countries. Moreover, Nepal became a member of the World Trade Organization (WTO) in 2004 and can only maintain its membership if it installs an effective monitoring system. Data about pesticide use in various parts of the country have not been collected since many years due to technical and financial constraints. The existing situation urgently demands for the implementation of advanced and fit-for-purpose analytical procedures for accurate monitoring of pesticides in food, soil and drinking water and which would be needed for the management of the existing pesticide problem.

Therefore, the main objective of this study was to develop a method which would be economically feasible for analysing soil and food articles in Nepal and applying this method in selected areas of Kathmandu valley so as to investigate the impacts of commonly used pesticides. Another objective was to investigate the awareness level of the farmers regarding pesticide use and health safety through a questionnaire survey.

A total of 16 vegetables, 4 fruits, 15 soils at 10cm depth and 4 soils at 3 different depths (10cm, 30cm and 50cm) were collected on the farm of the interviewed farmers at neighbouring districts around Kathmandu. 11 tea samples that were grown at different tea estates of Nepal were also collected to study the level of pesticide residue. These samples were brought to Vienna for the laboratory analysis. The tea, fruit and vegetable samples were analyzed in the LVA GmbH, Vienna and the soil samples were analyzed in the laboratory of the Institute of Soil Research at the University of Natural Resources and Life Sciences (BOKU), Vienna. For this purpose, new analytical method was developed. The samples of tea were extracted by following Extraction/Partitioning and Dispersive Solid Phase extraction method QuEChERS and analyzed by Gas Chromatography/mass spectrometry (GC/MS) for food samples and LC-MS/MS (Agilent 6410 Triple Quad) for soil samples. QuEChERS method used for the analysis of food samples which was modified through the optimisation of fragmentor voltage and collision cell energy and was used for the extraction of soil samples.

The questionnaire survey showed that the awareness level among the farmers regarding pesticide use and safety was very low. Out of 30 farmers interviewed, 50% of them stored pesticides in an unlocked room, more than 30% waited less than one week after pesticide application before harvest and more than 50% had problems of eye irritation and about 50% used no safety measures during pesticide handling. The banned pesticide Ethion was found in medium and best quality tea. Also, organic tea was found to be contaminated with pesticides. Some fruits and vegetable samples were also found to be contaminated with pesticides like Ethion, Chloropyrifos, Carbendazim. Almost all the soil samples except that of Kathmandu were found to be contaminated with pesticides.

The data generated from this study could be useful for understanding the present situation of the pesticide contamination level around Kathmandu. However, a detailed study covering different ecological regions of Nepal in order to understand the exact situation of pesticide pollution of soils, food and water should be a topic for further research.

1. INTRODUCTION

1.1 Background

The rapid population growth has resulted in increasing demand for food almost all over the world. In order to fulfill the increasing demand of food, the agricultural productivity needs to be increased. It has been found that many countries of the world have been extensively using chemical pesticides to increase the agricultural productivity so as to fulfill the growing demand for food. About 900 chemical pesticides are used worldwide, legally and illegally, in various food products and for the treatment of crops and soil (Thurman *et. al*., 2008). The application of pesticide for agricultural production is even more severe in developing countries throughout their efforts to eradicate insect-borne, endemic diseases, to produce adequate food and to protect forests, plantations and fibre (wood, cotton, clothing, etc.).

According to FAO (Food and Agricultural Organization), a pesticide is any substance or mixture of substances that are intended for preventing, destroying, controlling and mitigating any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. Pesticides are chemical substances defined as poisons and used in certain circumstances to kill specifically targeted pests (Wassemann, 1972).

Pesticides are often highly stable compounds that can last for years and decades before breaking down. These persistent substances are highly mobile and capable of bioaccumulation. They circulate globally and persistent pesticides that are released in one part of the world can be easily transported to the other part of the world by a repeated process of evaporation and deposition through the atmosphere to regions far away from the original source (Williams, 2000). Although organochlorine insecticides like DDT (Dichloro diphenyl trichloro ethane) and its metabolites (lindane, aldrin) have been banned years ago in many countries because of their mutagenic, carcinogenic and endocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties. Organophosphorous insecticides like chloropyriphos, chloropyriphos-methyl are the most commonly used pesticides around the world; they and their metabolites are detected in the environment although several members of these classes have been banned since years ago. Among the different groups of pesticides, herbicides are more likely to pollute soils. Barcel´o and Hennion (1997) published data about herbicides use: herbicides have been extensively used in the world for over 40 years representing 45% of the total market value in 1993 with more than 80% of herbicide use localised in North America, Western Europe and East Asia; 22% of the total herbicides are also used for non-agricultural purposes.

Pesticides are toxic in nature and do not differentiate between targeted and non-targeted species. These toxic substances can affect the immune system, harm the reproductive system and carcinogenic to humans through direct exposure during application process or exposure through pesticide residues in food, water and soil. Due to injudicious and indiscriminate use of pesticides, many accidents have occurred in different parts of the world, especially in developing countries where the farmers do not have adequate knowledge regarding pesticide use and safety. The farmers in developing countries are steadily increasing their reliance on pesticides as a major means of increasing their harvests. Developing countries account for one third of global pesticide consumption and the vast majority of pesticide poisonings occur in these countries (FAO, 2002).

Like any other countries of the world, Nepal, a developing country, is also confronted with the problems of extensive pesticide use and food security.

1.2 History of pesticide use in Nepal

Pesticides in agricultural sector were introduced in Nepal in the early sixties (Dahal, 1995). Until 1950s, Nepalese were unaware of modern chemical pesticides and were dependent upon traditional organic techniques for killing pests. According to Dahal (1995), chemical pesticides were first introduced to this country in 1955 when Paris Green, gamaxone, and nicotine sulphates were imported from USA (United States of America) for malaria control. DDT (Dichloro diphenyl trichloro ethane) made its first impact in 1956. This was soon followed by a variety of other organochlorines (1950s), organophosphates (1960s), Carbamates (1970s), and Synthetic Pyrethroids (1980s).

The Nepal Malaria Eradication Program (NMEP) in the 1950s was the first major channel to utilize pesticides in Nepal. The chemical pesticides, provided through the grant assistance by USAID (United Nations Association for International Development), were initially in limited quantity, primarily used for the control of vector-borne diseases. As a result of this program, there were ninety nine percent reductions of malaria cases which were completed in early seventies (Giri, 1998).

The establishment of Department of Agriculture, initiated the application of chemical pesticides for crop protection during the mid of the 1960s. Since then, the use of pesticides for plant protection has steadily increased. Increasing demands for chemical pesticides resulted in private dealerships selling and distributing pesticides throughout the country (Dahal *et. al*., 1995).

In 1977, the Nepal Pesticides and Chemical Industries Private Limited. (NEPCIL) was established in Bahadurgunj to supply BHC (Benzene hexa chloride) dust, Malathion, Nepcil Parathion among others locally. The Indian representative such as Crop Health Production Limited, Excel Industries Limited., Cyanamid India Limited., Bharat Pulverizing Mills Limited were the main suppliers of pesticides in Nepal. Organizations like the Cotton Development Board (CDB) and Nepal Malaria Eradication Programs were also authorized to purchase pesticides from foreign distributors (Dahal, 1995). Presently, Indian Pesticide dealers cross the open border freely, selling pesticides in the Terai region and in major towns of Nepal (Palikhe, 2001). At present, the main pesticide industries are; Kishan Agro Chemical, Nepali Krishi Rasayan Products, Pashupati Agro Chemicals, Nepal Pesticides and Chemicals.

Since after the introduction of pesticides in Nepal, its use has been increasing rapidly throughout years for the purpose of improving crop yields, controlling and eradicating vector borne disease, pests, disease control in agriculture and forest crops. The commonly used pesticides are; Malathion, Chloropyriphos, Cypermethrin, Deltametrin, Mancozeb, Methyl Parathion, Fenvelarate, Dichlorvos, Endosulfan, Chlorpyriphos+Cypermethrin, Dimethoate, Carbendazim, Dithane etc. (Palikhe, 2001).

1.3 Actual situation of pesticide use in Nepal

As in many other parts of the world, Nepal is not an exception in case of misuse and overuse of chemical pesticides in agricultural production. The country is struggling to fulfill the demands of food for growing population. An increasing trend on pesticide use has been found since past years (Figure 1)

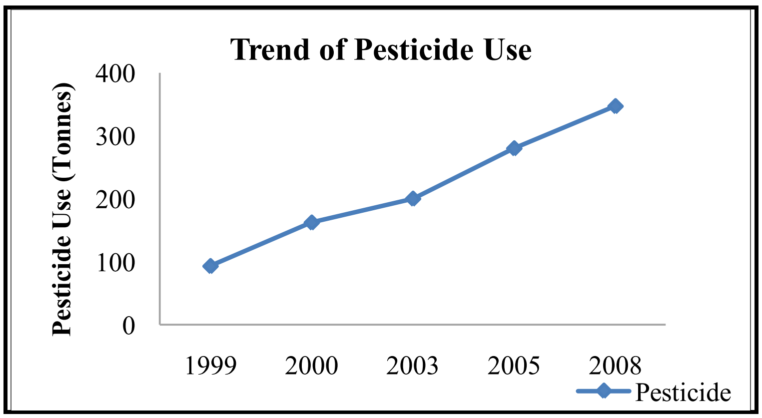


Figure 1: Trend of Pesticide Use in Nepal (DFTQC, 2008)

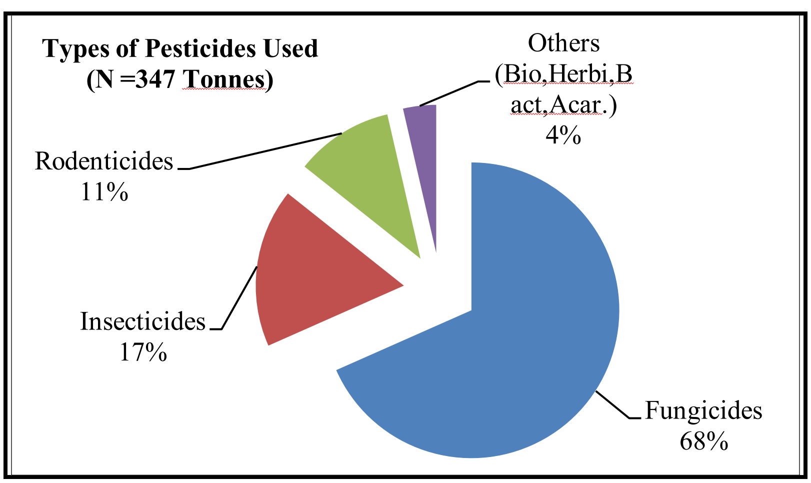


Figure 2: Types of pesticides used in Nepal (DFTQC, 2008)

According to various studies conducted in the past, it has been found that the chemical pesticides are intensively being used in agricultural production in Nepal. From figure 2, it has been clear that Fungicides are used in maximum amount which is sixty-eight percent followed by insecticides seventeen percent, rodenticides eleven percent and four percent of others. As a result, various problems in human, plants, animals and environment are being observed. It has been common to hear in the news or to read in papers about the harmful impact that has been caused by pesticide overuse/misuse to human, plants or animals in some part of the country. For example, death of cattles due to the consumption of pesticide contaminated water and spraying pesticides in mustard field made loss of 2 million rupees due to death of bees and destruction of beehives.

The farmers and retailers of pesticides do not have adequate knowledge regarding pesticide use and health safety (NPC, 1995). Some farmers even dip fruits and vegetables in pesticide solution just before bringing them to the market for selling. Others treat food grain with pesticides against storage pests. Many misuses have been reported by Giri (1998) and Baker *et. al*.,(1995) generally from farmers who do not realize the extent to which pesticides are poisonous and hazardous to humans and the nature. The concept of chronic poisoning and health risks is not fully understood by farmers, distributors and importers.

Furthermore there is no controlling mechanism for the purchase and sell of pesticides within and outside the country. Due to open borders, the farmers can easily go to the neighbouring countries and purchase any pesticide that they want and use them haphazardly in their fields. Although pesticide misuse in the country has been recognized and addressed by National Conservation Strategy, there exists no routine monitoring programme of food quality for pesticide concentration. This has resulted with refusal of many Nepalese products like honey, tea in the international market, which has effected in the economy of the country. Because of haphazard use of pesticides in food, people are consuming pesticides unknowingly that can cause several negative impacts on their health, which is also a drawback for the country with the possibility of losing its human resources.

In the context of developing country like Nepal, there is a lack of technical infrastructures, trained human resources and financial packages for the periodic and routine monitoring of food quality. The analysis of food samples in the laboratory for pesticide concentration requires trained human resources, technical infrastructures and costs. Hence development of feasible methods for the analysis of pesticide residue in food and food products that can be applied and adopted in context of developing country like Nepal by the use of local human resources is a very important aspect for the effective and efficient implementation of monitoring programme on a routinely basis.

Hence this report provides the details of activities that were carried out to gather information so as to investigate the level of awareness about pesticide use and food safety among farming community of the central Nepal. Moreover, the analytical results of various pesticides in food and soil samples as well as development of new method for the analysis of pesticide residue in soil samples have been described in this report.

2. OBJECTIVES AND SCOPE OF THE STUDY

The objectives and scope of the present study are as follows;

2.1 Objectives

The specific objectives of the study were:

- To compile information on the pesticide use among farmers
- To analyse the pesticide residue in food articles
- To analyse the pesticide residue in soil samples
- To develop an economical and feasible method for the analysis of pesticides in soil samples

2.2 Scope

The scope of the study was to analyze the levels of pesticides in different food and soil samples of Kathmandu valley as well as to assess the awareness level of farmers regarding pesticide use and safety.

3. LITERATURE REVIEW

In order to carry out the present study, various reports, research papers, books, magazines were reviewed. The findings from literature review are presented in the following order;

3.1 Environmental effects of pesticides

Use of pesticides can have unintended effects on the environment. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including nontarget species, air, water, bottom sediments, and food (Miller, 2002). Pesticide contaminates land and water when it escapes from production sites and storage tanks, when it runs off from fields, when it is discarded, when it is sprayed aerially, and when it is sprayed into water to kill algae. The amount of pesticide that migrates from the intended application area is influenced by the particular chemical's properties: its propensity for binding to soil, its vapor pressure, its water solubility, and its resistance to being broken down over time (Miller, 2002). Factors in the soil, such as its texture, its ability to retain water, and the amount of organic matter contained in it, also affect the amount of pesticide that will leave the area. Some pesticides contribute to global warming and the depletion of the ozone layer (Pesticide Wikipedia modified on 2010).

3.2 Persistent organic pollutants

Persistent organic pollutants (POPs) are compounds that resist degradation and thus remain in the environment for years. Some pesticides, including aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene, are considered POPs (Department of Pesticide Regulation, 2008). POPs have the ability to volatilize and travel great distances through the atmosphere to become deposited in remote regions. The chemicals also have the ability to bioaccumulate and biomagnify, and can bioconcentrate (i.e. become more concentrated) up to 70,000 times their original concentrations. POPs may continue to poison non-target organisms in the environment and increase risk to humans by disruption in the endocrine, reproductive, and immune systems; cancer; neurobehavioral disorders, infertility and mutagenic effects, although very little is currently known about these chronic effects. Some POPs have been banned, while others continue to be used.

3.3 Health effects of pesticides

Pesticides can be dangerous to consumers, workers and close bystanders during manufacture, transport, or during and after use (USEPA, 2007).The World Health Organization and the UN Environmental Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly (Jeyaratnam, 1990). Organophosphate pesticides have increased in use, because they are less damaging to the environment and they are less persistent than organochlorine pesticides (Jaga *et al*, 2003). These are associated with acute health problems for workers that handle the chemicals, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobichon, 1996). Additionally, many studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions, cancer, depression, neurological deficits, miscarriages, and birth defects (Engel *et al*., 2000).

3.4 Problems of infrastructure in developing countries

In many of the developing countries, there is always a lack of appropriate pesticide approval/registration procedure and/or inadequate resources to implement and enforce existing schemes, lack of legislation on working conditions and lack of post-registration monitoring of pesticides. Access to acutely toxic (cheaper) pesticides is easy (fish drying, committing suicide/perpetrating terrorist attacks). Faulty equipment, poor-quality products and adulteration makes the products more hazardous or ineffective and contribute to over dosing. The several problems faced by developing countries are;

- Lack of capacity (manpower and financial resources) to advice on and enforce national laws, approved codes of conduct.
- Inadequate management and storage of obsolete stocks and used packaging materials.
- Lack of facilities for proper waste management.
- Spray equipment in poor condition, including leaks and blocked nozzles; common use of "informal" application techniques (bucket and brush).
- Lack of washing facilities to shower after spraying and for regular washing of clothes; clothes are usually washed in the sources of drinking water.
- Reuse of containers of pesticide for food and drink storage, no facilities for safe disposal.
- Supply problems caused by: repackaging in small containers without labels and instructions; limited range of products, and; quality of pesticide products.
- Lack of baseline and trend pesticide data in food and water.
- Lack of pesticide resistance monitoring data and resistance strategies to prevent over dosing.
- Overlapping mandates and coordination of the necessary technical resources.

The contributing factors for the above mentioned problems are;

- Poor information leading to a lack of knowledge about pests and pesticides hazards (scientists, analysts, extension workers, decision makers and applicators).
- Complex label instructions, labels not in local languages, poor literacy and understanding of pesticide hazards.
- Lack of information record keeping at the small enterprise level (e.g. farms) on storage, handling, use of pesticides and disposal of waste pesticides and empty containers.
- Application without protection – farmers and farm workers lack of protective clothing, even if available, climatic conditions make it impossible to wear.
- No training in application procedures or hazard awareness, leading to: mixing with bare hands; combining different products; applying on crops for which a product is not intended (cotton pesticides on vegetables, public health insecticides on dried fish).
- Houses near fields, and non-target crops and biodiversity affected by spray drift.

• Inability to recognize pests, predators and to measure economic losses, thus leading to a "pesticide treadmill" effect when no alternatives are available.
• Difficulties for the scientists in developing countries to quickly update their skills and move from a reactive fighting "bush fires" to a proactive prevention

3.5 Infrastructure for pesticide use in Nepal

Given that food safety issues are multi-faceted and multi-disciplinary, it is only through integrated efforts by agriculture, industry and health authorities that quality issues can be resolved successfully. Environmental, health and sanitary standards required by developed countries can be perceived to be non-tariff barriers to trade by developing countries. These trade measures can take various forms, such as technical standards and regulations, sanitary and phytosanitary (SPS) measures, packaging regulations and labeling requirements.

3.5.1 Legal and institutional infrastructure

Monitoring food quality and safety has become more important both in the domestic and export market. Nepal has prioritized food safety and quality in order:

• To prevent the adulteration of foodstuffs;
• To safeguard the rights and well-being of the consumers by effective implementation of the Food Act, thus retaining the standard of quality for food products during production, processing, import and export; and
• To provide laboratory services for food quality control, import/export trade and industrial output.

Nepal faces a significant challenge concerning food adulteration and contamination emanating from primary production, processing, distribution, marketing and preparation. In addition, industrial chemicals are entering the food chain in the production and processing of food products. The main legal instruments regarding Nepal's trade in food products are the Food Act 2023 (1966) and the Food Rules 2027 (1970). The Food Rules contains provisions for food additives, contaminants, inspections, licensing and analysis of food. Standards have been developed for 108 food products.

3.5.2 Pesticide management and registration system

The Pesticide Act, 1991 and the Pesticide Rule, 1993 cover measures to regulate import, manufacture, sale, storage, transport, distribution and use of pesticides. It is mandatory that any pesticide before distribution and importation should be first registered in accordance with the registration procedure adopted by the Pesticide Board. Pesticides other than notified ones are not to be imported, exported, produced, used or distributed. The regulations also prohibit the sale of any pesticide, which is imported for scientific or research purposes. However these rules and laws have not been materialized efficiently and effectively and they are restricted in paper and not into practice due to several technical and financial constraints.

3.5.3 Challenges for the Nepal government

There are many challenges associated with the use of pesticides, including those categorized as Persistent Organic Pollutants (POPs). Collectively with the non-POPs pesticides, many of which also share similar problems. However, depending upon the socio-economic situation, the levels of industrialization, literacy and geographical features, the challenges would vary from one country to another.

3.5.3.1 Aggressive marketing strategies

Dealers take up intensive and aggressive marketing strategies to sell their products with the perception that there are no other alternatives for farmers except the use of chemical pesticides.

3.5.3.2 Misuse of pesticides

Regular misuse of pesticides causes pests to adopt and become resistant to the pesticides. Most pesticides are then required at higher doses to achieve the same level of control, though often these are not as effective.

It is a known fact that farmers do not follow the preharvest waiting period. They apply pesticides near harvest time, some even dipping vegetables in pesticides before selling. Others treat food grain with pesticides against storage pests. Many misuses have been reported by Giri (1998) and Baker et al. (1992) generally from farmers who do not realize the extent to which pesticides are poisonous and hazardous to humans and the nature. The concept of chronic poisoning and health risks is not fully understood by farmers, distributors and importers.

Studies have shown that over 60% farmers using pesticides for over five years wait less than two weeks after spraying pesticides before harvesting the crop (DFTQC, 2004). The negative health effects of pesticides, therefore is a serious problem requiring efforts to reduce pesticide misuse in Nepal. The environmental costs of pesticide misuse are potentially huge. Endosulphan is a broad spectrum and has been restricted in many countries to non-aquatic habitats as it is highly toxic to fish. Farmers place pesticides into rivers and streams in order to catch fish (DFTQC, 2004).

3.5.3.3 Pesticide residues in food

There are numerous reports of excessive pesticide residues in food in Nepal. Annual Bulletins from the Central Food Research Laboratory (CFRL) routinely detected residues from their sampling program. From 1981 to 1986, the residues of organochlorine and organophosphate insecticides were a more serious problem in cereal grains, legumes and vegetables but have declined in recent times. Residues surpassing legal limits set by Nepal Government by the Food Standardization Committee were reported existing in the Annual Bulletins from 1992/1993 through 1995/96 such as tea (Malathion, DDT, BHC), grapes (methyl parathion), rice (BHC), chickpea (DDT) and organophosphates on vegetables (greens, potato, cabbage, chickpea and pumpkin).

3.5.3.4 Illegal transboundary movement

The use of banned or restricted pesticide cannot be prevented effectively because of illegal trans-boundary movement of pesticides. There is little or no information on such illegal movements regarding the name, quantities of chemicals sold.

3.5.3.5 Public awareness

Many farmers/workers are unaware of some of the properties of pesticides, in what conditions they present danger and how to protect oneself from poisoning, The general belief seems to be that if one doesnot die immediately then pesticides present no harm. There is widespread ignorance of the existence of chronic pesticide poisoning. Concept of pesticide resistance/resurgence is not understood by farmers. Importers/Resellers/Farmers refer to pesticides as medicines rather than poisons.

3.5.3.6 Disposal of obsolete pesticides

Nepal's most prominent problem is its stockpiles of obsolete pesticides. The problem of obsolete pesticide stocks is caused by purchasing more pesticides than are needed. If these stocks are not used within 2 years they gradually become unusable due to deterioration in storage. Any pesticide stockpiles are potentially hazardous. There is also a risk of severe environmental impacts in the event of a flood, fire, earthquake & other natural disaster. It is estimated that 74 tons of obsolete pesticides are stored in Nepal at the moment. This amount of pesticide waste generated in Nepal over the last 3-4 decades is not enough for the cost savings.

3.5.3.7 Infrastructural challenges

Lack of laboratory facilities and technical knowledge for pesticide residue analysis as well as toxicology analysis. Due to the lack of sophisticated lab recognized by Food and Agricultural Organization (FAO) and World Health Organization (WHO), food samples that need vigorous testing have to be sent to India from Nepal (DFTQC, 2010).

3.5.4 Inadequate residue control measures

The control of pesticide and fertilizer residues in Nepal is insufficient to provide the required assurance that products of animal origin meet the standards for MRLs for veterinary drugs and other harmful residues in export markets. A prime example is honey exported to the European market.

These deficiencies are due to lack of:

- A regular and routine residue control programme;
- A competent authority to monitor residue levels throughout the production process, i.e., from the farm to the table;
- Up-dated and comprehensive food safety laws and regulations; and
- Laboratory facilities and analytical capacity to deal with agrochemicals.

As noted above, in order to export animal products to the EU (European Union), there is a need to establish a residue monitoring and control plan according to EU Directive 96/23. This will enable Nepal to be placed on the EU's approved list of third countries and facilitate market access for exports to the EU.

3.5.5 Emerging challenges for trading

Nepal became a member of the World Trade Organisation (WTO) on 23 April 2004. Nepal is one of 50 least developed countries in the WTO, which were granted a transition period until 1 January 2007 to implement certain WTO Agreements, including the Agreements on Sanitary and Phytosanitary (SPS) Measures and Technical Barriers to Trade (TBT) (WTO, 2003).

India is Nepal's main trading partner. Because of Nepal's long and open border with India, it is difficult to track comprehensively all cross-border trade in agricultural products. As a result, significant imports of Indian agricultural products have tended to cross the border without any checks, based in part on the higher production costs in Nepal. This poses a serious challenge for Nepalese products to become competitive with Indian imports. It necessitates that Nepal develop the infrastructure, roads, electricity supply to rural areas, sufficient water systems for irrigation and the timely supply of quality seeds and fertilizers, as well as proper management and storage facilities for agricultural products.

Despite of limited investment in developing agro-processing industries in rural areas, the potential to create employment in agro-enterprises which can substantially reduce the wide prevalence of poverty – 31% in 2003/04 compared with 42% in 1995/96. Between 1995 and 2004, the poverty index in rural areas declined by 8% (from 43% to 35%) and in urban areas by 12% (from 22% to 10%) (Economic Survey 2005, Ministry of Finance).

Nepal's Commerce Policy of 1992 still needs to take fully into account the country's entry into the WTO and its blossoming regional trading systems, such as SAFTA, and the BIMSTEC-EC Free Trade Area. While quality control has become an important element of competitiveness in international trade, Nepal has yet to establish a comprehended food safety regime. The establishment of an agro-food export inspection and certification system is essential to enhance Nepal's access to export market. In Nepal, few industries have attained ISO 9000 certification, which limits its ability to gain access to developed country markets, such as the United States (US), Japan and the European Union (EU). Therefore, adequate infrastructure and mechanisms to assess quality and a certification system for exported products are challenging but essential components for Nepal to increase exports.

3.5.6 Classification of pesticides (Guidelines, Levels, Standard Values)

Different organizations like WHO (World health Organization), EPA (Environment Protection Agency), EU (European Union) as well as different countries have setted different values or guidelines or residue levels for pesticide in food and food products. Some of them have been represented in Table 1 and 2 as follows;

Table 1: WHO Hazard Classification of Pesticides

Description	**Hazard class**	**LD 50 mg/kg body weight**	**Label**	
			color	**Poison**
Extremely hazard	Ia	<5	**Red**	
Highly hazardous	Ib	5-50	**Yellow**	
Moderately hazard	II	50-500	**Blue**	Dangerous
Slightly hazard	III	>500	**Green**	Caution

*Source: WHO/IPCS, 2009

Table 2: Maximum Pesticide Residue Level in selected countries

Country	**Maximum Residue Level (MRL)**
Nepal	Not established
India	Dicofol 5 ppm, Ethion 5 ppm, Fenzaquin 3 ppm, Glyfosinate – ammonium 0.1 ppm, Glyfosate 1 ppm, Quinolphus 0.1 ppm
Pakistan	Not available
Japan	Dichlofluanid 5 ppm, Dichlorvos 0.1 ppm, Dicofol 3 ppm, Dieldrin N D, Diphenoconazole 10 ppm, Diflubenzuron 20 ppm, Emamectin Benzoate 0.5 ppm
EU	Dichlorvos 0.1 ppm, Dicofol 20 ppm, Tetradifone 0.01 ppm, Ethione 2 ppm, Dieldrin 0.02 ppm, Glyphosate 0.1 ppm, Malathion 0.5 ppm, Texaphane 0.1 ppm, Malathion 0.5 ppm, Texaphane 0.1 ppm, MB 0.05 ppm, Permethrin 2 ppm, Phorate 0.1 ppm, Propargite 5 ppm
US	Tetradifone 0.1 ppm, Ethion 2 ppm

*Source: Agro-Enterprise Centre, 2004

3.5.7 Banned and commonly used pesticides

Figure 3: Extremely hazard pesticide used

Figure 4: Different pesticides used

The banned pesticides in Nepal are DDT, Chlordane, Dieldrin, Aldrin, Heptachlor, Mirex, Texofen, BHC, Lindane, Organo Mercuric Fungicide, Phosphamidane, Methyl Parathion, Monocrotophos, Andies. Some of the highly hazardous pesticides that were found to be used by the farmers during the field visit have been represented by Figure 3 and 4.

The pesticides that are commonly used in Terai and hilly regions are represented in Table 3 as follows;

Table 3: Pesticides commonly used in Terai and Hilly regions of Nepal

Terai Region	Hilly Region
Cypermethrin 10%	Mancozeb 75%
Dichlorovous 76 EC	Dichlorovous
Monocrotophous 36%	Carbendazim 50%
Endosulfan	Moliboron
Aluminium Phosphide 56%	Acetamiprid 25%
Ethion	Polytram-metiram 70%
Fumigant	Tricontenol 0.05%
Metacid 50%	Ethion
Methyl paracide	Methyl Parathion
Phorate 10%	Cypermethrin

Fenvelarate	Chloropyrifos
Quinalphos	Endosulfan
Carbofuran 3%	Dimethoate
Methyl Parathion	Omethoate

*Source: DFTQC, 2008

3.5.8 Former studies on pesticide residue level

A study carried out by Food Research Laboratory, Kathmandu found that out of 900 samples of various food products analyzed during 1981 to 1986, 94% were contaminated with DDT (Joshi, 1988).

According to a survey carried out by Dahal (1995) in some parts of eastern, western and central Nepal, organochlorine insecticides like aldrin, endosulfan, BHC dust were found to be used in larger amounts by 95% of interviewed farmers. The study also concluded that farmers used chemical pesticides not only to control pests on crops but also to store the food grains, lentils, vegetables and fruits (Dahal, 1995). The same study also showed that 32% of the interviewed farmers waited less than one week, 31% waited one to two weeks, 25% waited three weeks, 11% waited four weeks and 1% waited five weeks before crop harvesting.

A study carried out by Nepal Government from 1994 to 2004 included 1034 food samples (pulses, fruits, vegetables, water, and weaning food) that were analyzed for organochlorine (DDT, BHC), organophosphorous (Malathion, Parathion, Methyl Parathion) had 3.9% of Malathion, 3.1% of BHC, 2.8% of Methyl Parathion, 1.8% of DDT and 0.3% of Parathion. The levels of pesticides as obtained from the study have been as represented in Table 4 as follows;

Table 4: Year wise sample analysis and detection of pesticides (1994 to 2004)

Year	No. of samples	DDT	BHC	Malathion	Parathion	Methyl Parathion
1995	34	2	13	10	4	3
1996	64			7		2
1997	121			1		10
1998	112			5		8
1999	70			8		4
2000	54		1	10		
2001	134	17	17			2
2002	106					

2003	36					
2004	303		2			
Total	**1034**	**19**	**33**	**41**	**4**	**29**

*Source: DFTQC, 2005

A research conducted to estimate the levels of pesticide residues in different vegetables by Nepal Government in 2005 found that tomato sample from Kavrepalanchowk district (hilly region) was contaminated with 1.64ppm of Mancozeb and 0.840 ppm of Cypermethrin and potato sample from Terai was found to be contaminated with 0.16ppm of Mancozeb (DFTQC, 2004).

3.6 Available methods for the extraction and analysis of pesticides in food

The analysis of pesticide residue in food and environmental samples has been performed by several governmental and private laboratories throughout the world since many years. However the methods adopted by different laboratories are far from ideal. Some residue monitoring laboratories still use the methods that were developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, lengthy procedures, manual labour and less development of technology than today. The introduction of new, more rapid and effective methods is essential for the laboratories to improve the overall laboratory quality and efficiency.

The most efficient approach to pesticide analysis involves the use of multiclass, multi residue methods (MRMs). The first MRM was the Mills method developed in 1960s by U.S. Food and Drug Administration (FDA) (Mills, 1963). In this method, non polar pesticides from non fatty food products were extracted with acetonitril, which was then diluted with water and the pesticides were partitioned into non polar solvent (Petroleum Ether). The drawback of this method was that the polar pesticides were partially lost during extraction process, which was followed with the development of other alternative methods that could determine the compounds that were not extracted by Mills method. The other methods simply modified the Mills method by adding several partitioning, clean up and determinative steps by adopting the initial acetonitril extraction as applied in Mills method.

As mentioned in Anastassiades (2003), Becker (1971), who developed the first Multi residue methods (MRM), added NaCl solution to the first extract, which only partially saturated the water phase with the salt.

In 1980s, Anastassiades and Scherbaum used a mixture of cyclohexane-ethyl acetate (1+1) instead of dichloromethane-petroleum ether (1+1) to induce partitioning. Thier (1999) used solid phase extraction (SPE) to extract pesticides from diluted acetone extracts, thus completely avoiding liquid liquid partitioning. Luke *et al.* (1999) added a combination of fructose, $MgSO_4$ and NaCl to separate water from acetone in the initial extract without using non polar solvents. However, all these separation techniques have one or more disadvantages in practice because acetone is too miscible with water to be easily separated without using non polar solvents (Anastassiades *et al*., 2003).

Acetonitril is easily and effectively separated from water than acetone when salt is added. Another extraction solvent commonly used is ethyl acetate which is partially immiscible with water that makes addition of other non polar solvents to separate water from the extract unnecessary. However a problem with the ethyl acetate is that some of the most polar pesticides do not readily partition into ethyl acetate phase. To increase the recoveries of polar compounds, large amounts of Na_2SO4 are usually added in MRM procedures with ethyl acetate used to bind the water (Anastassiades *et al*., 2003). During 1990s, the increased urgency to reduce the usage of solvent and manual labour in analytical process led to the commercial introduction of several alternative extraction approaches, including superficial fluid extraction(SFE) (Lehotay, 1997), matrix solid phase dispersion (MSPD), microwave assisted extraction (MAE), solid phase microextraction (SPME) and pressurized liquid extraction (PLE). Despite of their advantages, none of the techniques have overcome practical limitations to enable their widespread implementation (Anastassiades *et al*., 2003). Anastassiades *et al*. (2003) developed a method, famous by the name QuEChеRS (Quick, Easy, Cheap, Rugged and Safe), which is widely used for the extraction of food samples all over the world. The recovery of this method for 250 pesticides was found to be 70% to 110 % (Anastassiades *et al*. 2003). The schemetic diagram (Figure 5) of the analytical steps of the QuEChеRS method is as follows;

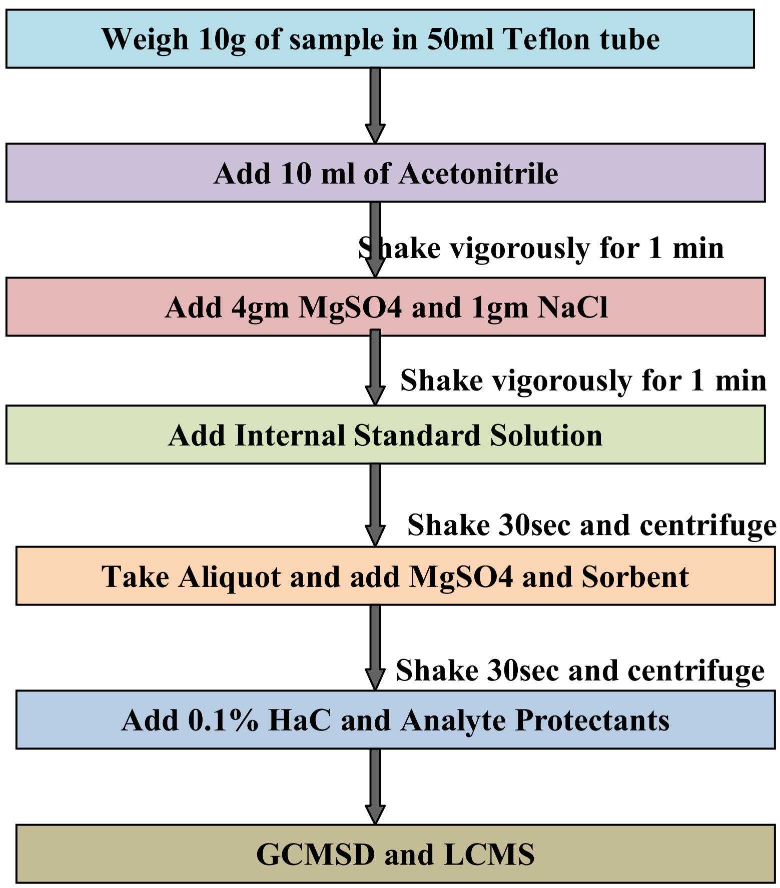


Figure 5: Schematic diagram of the analytical steps of QuEChеRs method

According to a comparative study between QuEChеRs and other methods carried out by Anastassiades, it was found that QuEChеRs method could analyse large number of pesticides with less amount of resources and time. The Figure 6 represents a comparative chart between QuEChеRs and other methods.

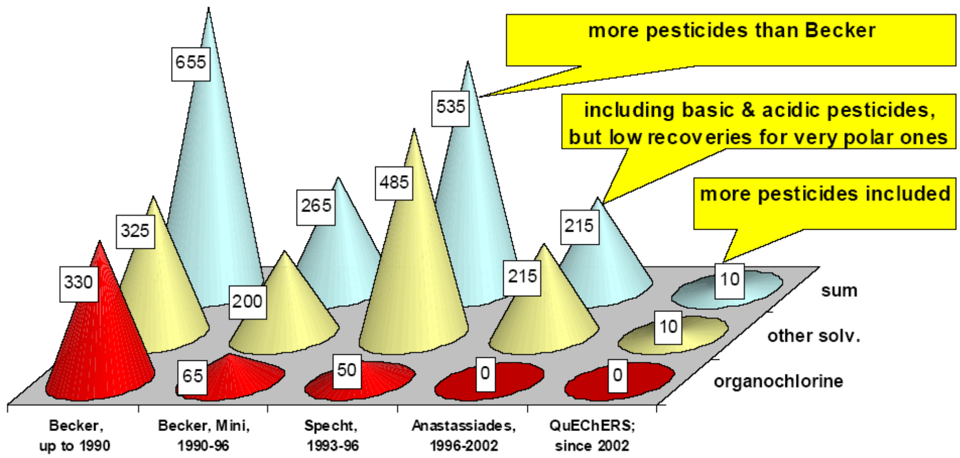


Figure 6: Comparision of QuEChERs with other methods (Anastassiades *et al*., 2003)

3.6.1 High Performance Liquid Chromatography (HPLC)

The principle of HPLC system is that the separation of analytes present in liquid sample takes place by the partition in mobile and stationary phase with the liquid eluents. In stationary phase, there is a separation of organic compounds via reversed phase (stationary phase – non polar substances and mobile phase - polar substances). The mobile phase consists of solvent A, which is water with 10% acetonitrile and solvent B which is acetonitrile with 10% water.

The mixture to be separated is loaded onto the top of the column followed by more solvent. The different components in the sample mixture pass through the column at different rates due to differences in their partioning behavior between the mobile liquid phase and the stationary phase. The compounds are separated by collecting aliquots of the column effluent as a function of time.

The HPLC system consists of a series of interconnected modular structures which are represented in Figure 7. The different components have different functions and features. The function of the mobile phase reservoir and the pump is to ensure an adjustable, constant and reproducible flow of 0.5 to 5 ml/min. The function of the injection valve is to inject a variable sample volume ranging from 1 to 100 µl. The analytical column separates the components in the sample. The detector detects the substances present in the sample and the concentration of the substances present in the sample is represented as peaks in the computer monitor.

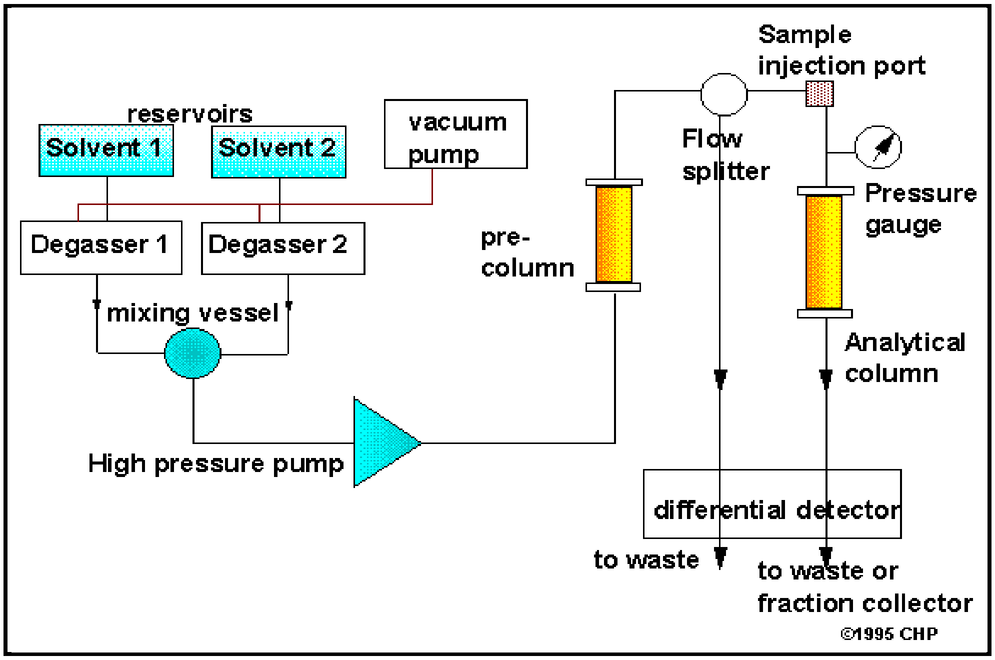


Figure 7: Working principle of HPLC system

3.6.2 6410 Triple Quadrupole Mass Spectrometer

The Triple Quadrupole mass Spectrometer is a LC/MS-MS system with highest sensitivity and excellent reproducibility. This highly advanced LC/MS-MS system consists of three quadrupole (QQQ). Typically, QQQ is used to monitor multiple targets in a single analysis, therefore, the term MRM (Multiple Reaction Monitoring) is used.

For this study, Agilent G6410 Triple Quadrupole Mass Spectrometer was used for the analysis and detection of parameters under study. The parameters optimized for the detection and analysis of pesticides through Agilent G6410 Triple Quadrupole Mass Spectrometer has been described in detail in section 4.6 of Chapter 4.

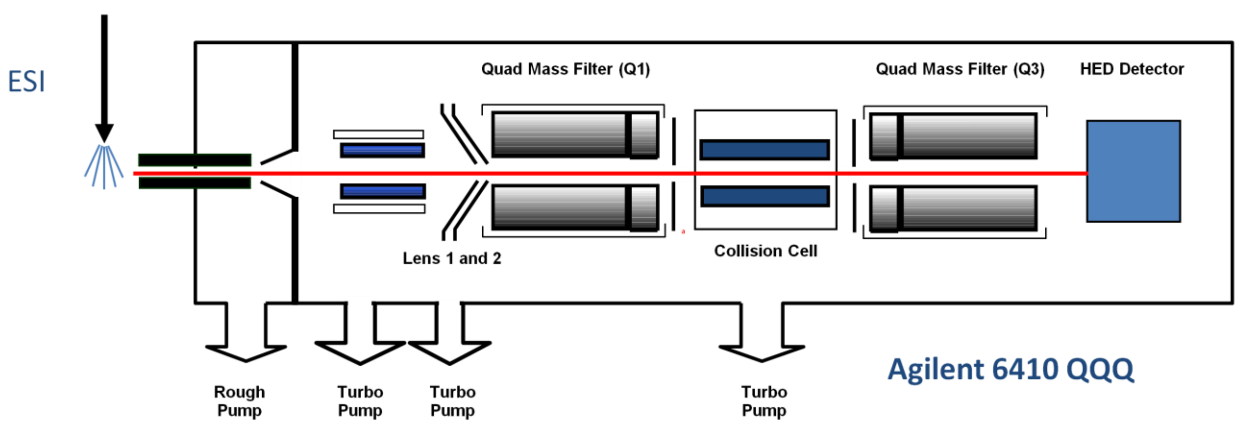
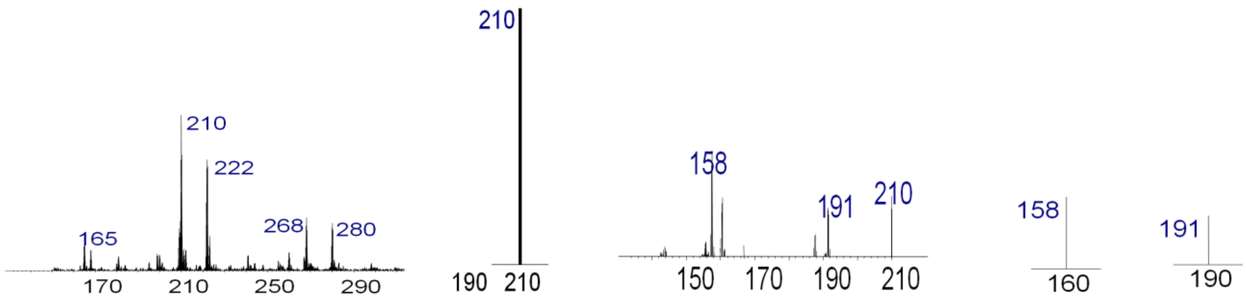


Figure 8: Working Principle of Agilent G6410 Triple Quadrupole Mass Spectrometer

The spectrum with background ions is passed from ESI. Out of all the ions, Quad mass filter 1 (Q1) lets only target ions to pass through it. The function of collision cell is to break ions apart. Quad mass filter (Q3) monitors only the characteristic fragments for qualitative and quantitative analysis.



Background ions from ESI | Fragment ion from Q1 | Collision cell breaking ion 210 | Q3 monitors characteristic fragments 158 & 191 from ion 210

4. MATERIALS AND METHODS

The details of materials and methods that were followed in order to carry out this study have been described as follows;

The study was differentiated into three parts including Desk Study, Field Study and Laboratory Analysis. During the desk study, various literatures, books and papers were reviewed so as to understand and update the information regarding residue analysis in food and its social aspects in Nepal as well as various parts of the world. Field study was carried out in the selected sites to collect the information about various aspects of pesticide use and safety and also to use this information as the baseline data for the laboratory analysis of the collected food samples. Laboratory analysis of the collected samples was carried out to investigate the residue level of the analyzed pesticides.

4.1 Study area

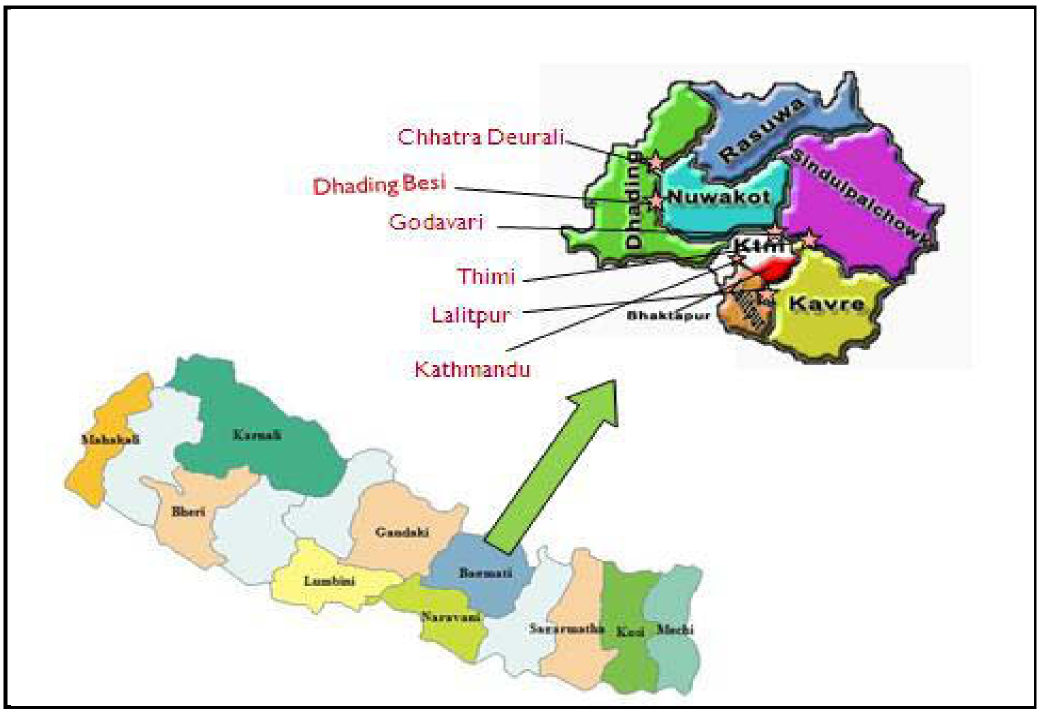


Figure 9: Study area

On the basis of various literatures reviewed, the selected study sites were the neighbouring districts of the capital Kathmandu as well as Kathmandu itself (Figure 9). The neighbouring districts around Kathmandu that were selected for the study purpose were; Dhading (Chhatradeurali and Dhading Besi), Lalitpur, Bhaktapur and Thimi. Due to rapid urbanization and population growth, residents of Kathmandu depend upon the fruits and vegetables that are grown in these neighbouring districts. From these districts, fruits and vegetables are brought to

Kathmandu every morning and sold in the market. It has been announced by the national media and published in national newspapers several times that the fruits and vegetables brought to

Kathmandu for consumption were found to be contaminated with pesticides, when they were occasionally tested by the Food Research Laboratory affiliated to Nepal Government. As there is no facility from the government to analyse the food samples before they are distributed into the market, the residents of Kathmandu have been consuming pesticides unknowingly. In this regard, it was utmost important to study about the pesticide use in these places and their residue level in food and food products and this was the main reason for the selection of these sites in this study.

4.2 Study period

The different activities carried out during different months from 2008 to 2010 have been represented in Table 5 as follows;

Table 5: Study Time Schedule

Activities	**Aug-08**	**Sep-08**	**Oct-08**	**Nov-08**	**Dec-08**	**Jan-09**	**Nov-09**	**Jan-10**	**Feb-10**	**Jul-10**	**Aug-10**
Lietrature Review											
Initial Field Visit											
Questionnaire Survey											
Sample Collection											
Laboratory Analysis of Food samples											
Development of Method for the Soil Analysis											
Laboratory Analysis of Soil samples											
Data Analysis of Questionnaire Survey											
Data Analysis of Laboratory Study											
Report Writing											

4.3 Questionnaire survey

Figure 10: Questionnaire survey in Chattradeurali

Figure 11: Questionnaire Survey in Dhading Besi

Field study was carried out through Questionnaire Survey so as to collect the information on awareness level of farmers regarding pesticide use, the type and amount of pesticide they were using, their knowledge regarding the safety measures to be taken during and after pesticide use (Figure 10 and 11). A total of thirty farmers were interviewed. On the basis of this survey results, different pesticides that they were using in their farm was identified, which were selected for the laboratory analysis of the collected samples to study their residue level.

4.4 Sample collection

Figure 12: Sample of tomatoes

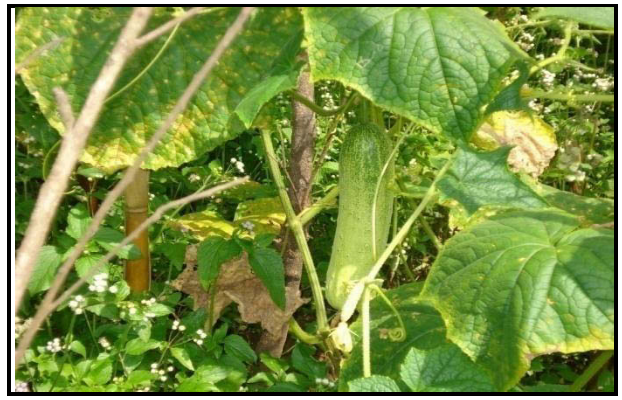


Figure 13: Sample of cucumber

Vegetable samples were collected from the farm of the interviewed farmers. A list of collected vegetable samples along with their place of production has been represented in Table 6 as follows;

Table 6: List of collected vegetable samples

S.N.	Products	Place
1	Potato I (Organic)	Kirtipur
2	Spinanch	Thimi II
3	Brinjal	Ason Market
4	Carrot	Ason Market
5	Cucumber I	Chattradeurali I
6	Fenugrik leaves	Kirtipur
7	Radish I	Dhading Besi II
8	Cauliflower	Kathmandu II
9	Tomato	Dhading Besi I
10	Rayo (green leaves)	Kathmandu I
11	Bittergourd	Bhading Besi II
12	Cabbage	Kalimati Market
13	Cucumber II	Chattradeurali II
14	Salat	Ason Market
15	Radish II	Ason Market
16	Potato II	Ason Market

Some fruit samples were purchased from famous vegetable and fruit markets located in Kathmandu, which were Kalimaket and Ason Market. The samples of banana and apple I were purchased from Kalimati market whereas citrus fruit and apple II were purchased from Ason market, both located in Kathmandu in order to investigate the pesticide residue level on a random basis.

The samples of tea of various qualities that were produced in different tea estates of Nepal from Jhapa, Ilam and Dhankuta were purchased from a shop in Kathmandu to study their pesticide residue levels. The different tea samples collected for the laboratory analysis were;

- Dhankuta Medium Quality Tea
- Dhankuta Better Quality Tea
- Ilam Better Quality Tea
- Ilam Green Tea of Better Quality
- Ilam Black Tea of Better Quality
- Jhapa Medium Quality Tea
- Jhapa Better Quality Tea
- Teenpate Tea Bags of Medium Quality
- Jhapa Best Quality Tea
- Teenpate Jhapa of Medium Quality
- Organic Tea from Jhapa

Samples of soil were collected from the farms of interviewed farmers so as to investigate the amount and type of pesticide present in that soil. The soil samples were collected from the depth of 10cm. In order to study about the pesticide concentration in the soil at different depths, soil

Figure 14: Collection of Soil sample

Figure 15: Picture of Potato farm

samples were also collected from different depths of 10cm, 30cm and 50cm from Godavari of Kathmandu (Figure 14 and 15).

The collected samples were packed each in a clean plastic bag and were labelled with sample identification number and sample name. The samples were stored in an icebox and were brought to the laboratory for the storage at – 4^0C.

A total of sixteen vegetable samples, four fruit samples, eleven tea samples, fifteen soil samples at the depth of 10cm and four soil samples at different depths of 10cm, 30cm and 50 cm were collected for study purpose.

4.5 Pesticides selected for laboratory analysis

The selection of pesticides that were analyzed in food samples (vegetable and fruit) were carried out through the information as obtained from the questionnaire survey. From the survey, it was found that the farmers of the selected sites were using the following pesticides;

Carbendazim, Chloropyrifos, Cypermethrin, Dichlorovous, Dicofol, Dimethoate, Dimethomorph, Diphenylamine, Endosulfan, Ethion, Fenvalarate, Imidacloprid, Methyl Parathion, Omethoate and Phenyl Phenol were the pesticides that were selected for the present study.

4.6 Laboratory analysis of soil and food (tea, fruits and vegetables)

The samples of food (tea, fruits and vegetables) and soil that were collected in Nepal were stored at – 4^0C in the refrigerator. The samples were brought to Vienna, Austria for the laboratory analysis. All the samples were collected just three days before they were brought to Vienna. The samples of food (Tea, Fruits and Vegetables) were brought to a food testing laboratory (LVA GmbH) located in Vienna for the laboratory analysis. The food samples in the laboratory of LVA were stored at – 4^0C till they were analyzed. The development of extraction method for the analysis of soil samples as well as the extraction of soil samples were carried out in the laboratory of Institute of Forest and Soil Research, University of Natural Resources and Applied Life Sciences (BOKU), Vienna. The extracted soil samples were brought to LVA GmbH for the laboratory analysis.

LVA is a pioneer laboratory in Austria which is famous for the microbiological and chemical analysis. It is an accredited institute (EN ISO 17025) and fulfills the function of state authorised institute.

4.6.1 Soil analysis

4.6.1.1 Instrumentation

Agilent 1100 HPLC, LC/MS-MS Triple Quadrupole mass spectrometry, Mettler Toledo balance for weighing the standards, working balance for weighing the reagents, Alfa 1-2 LD freeze dryer for drying the soil samples, Bandelin ultrasonic bath, vortex mixture and centrifuge

4.6.1.2 Reagents and chemicals

Acetonitrile of HPLC grade was used as a solvent for the entire analytical purpose including standard preparation, sample extraction and preparation, volume makeup after dilution. Anhydrous MgSO4, NaCl, Tri Phenyl Phosphate (Internal Standard) and PSA Sorbent

4.6.1.3 Standard preparation

For standard preparation, the required amount of pesticide standards were mixed with required volume of ultrapure water to make the standard solution but this trial could not be successful as most of the standards were not soluble in water. So for making standard solution, a solution of 10% Acetonitrile and 90% water was used (100ml of Acetonitrile with 900ml of water = 1000 ml), which dissolved all the pesticides.

The stock solutions, each of 100 ppm of each pesticide as mentioned in section 4.6 were prepared, labelled and were stored in airtight clean bottles. From this, a single mix standard of 100ppm was prepared, which was diluted firstly to 10ppm and to 1ppm. The 1ppm mix standard was used to make the calibration standards of 0.1ppm, 0.2ppm, 0.5ppm. The 10ppm mix standard was used to make the calibration standard of 2ppm and 5 ppm. In this way a series of calibration standards ranging from 0.1 ppm to 5 ppm was prepared. The single and mixed stock solutions were stored at - 4^{0}C while the calibration standards were made on the day of analysis.The calibration standards, each 2ml, were transferred to HPLC vials to be analysed in HPLC system.

4.6.1.4 Extraction of soil

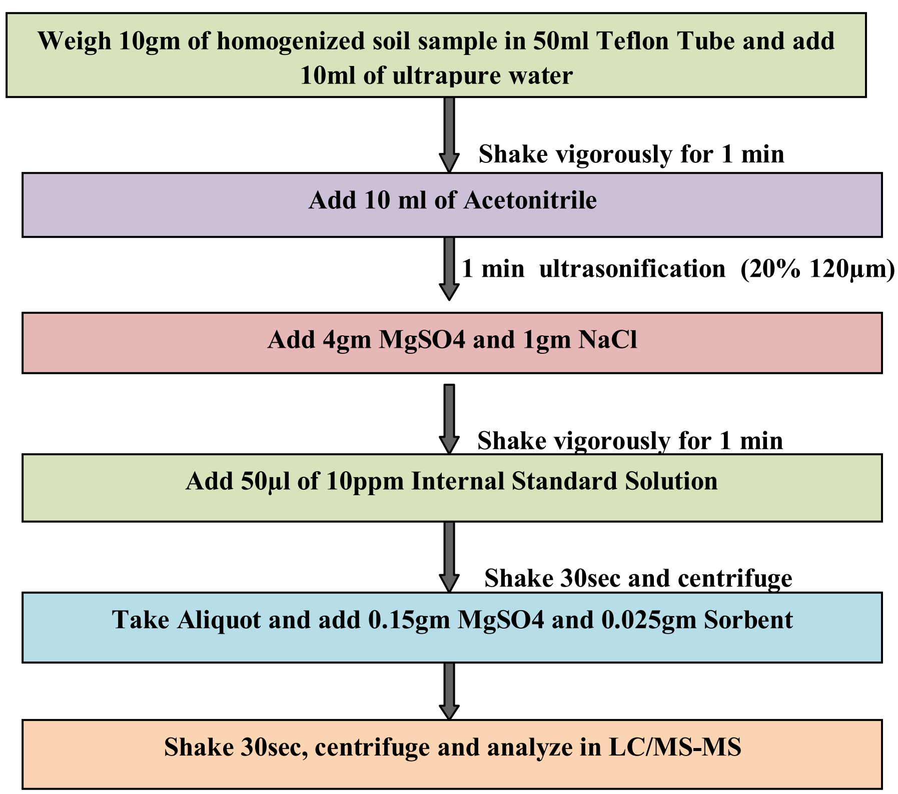


Figure 16: Schematic diagram of QuEChERs method adopted for extraction of soil

The soil samples were dried in freeze dryer for 24 hours and were milled in order to achieve complete homogenization. The extraction of the soil sample was done by following the QuEChERS method that was used so far only for the extraction of food samples. In the present study, the QuEChERS method was modified and then adopted for the analysis of soil samples. The steps followed during the extraction of soil samples by following QuEChERS method is well explained by Figure 16.

The reason for using 10ml of water in 10gm of soil in the first step was to maintain the water content in the soil sample as the soil sample was completely dried before the extraction process.

After the extraction of soil samples, 2ml of each extracted sample was transferred into two HPLC vials. One set of the sample was stored at -4^{0}C for further analysis if necessary and the other set was used for the LC/MS-MS analysis. The following Figure 17 illustrates the steps followed during the extraction of soil samples through QuEChERS method;

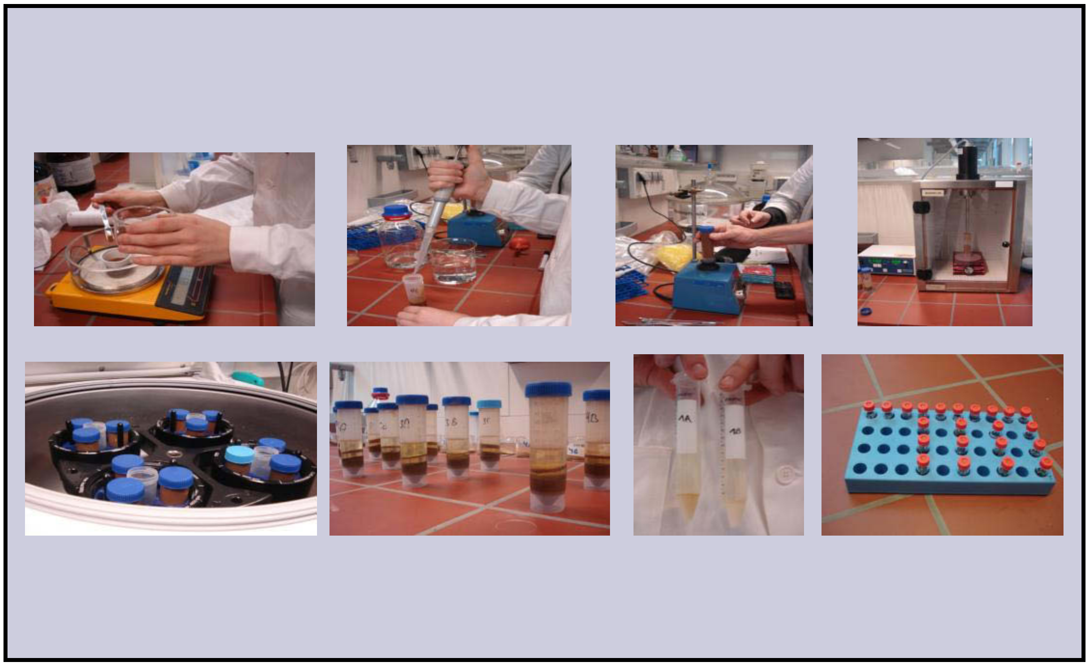


Figure 17: Steps followed during extraction of soil samples

4.6.1.5 Optimization of the method for LC-MS/MS analysis

Before loading the soil samples into the LC/MS-MS system for the extracted soil samples, the LC/MS-MS system needs to be optimized for the parameters (pesticides) to be analyzed. In the present study, the method optimization was carried out as described follows;

In order to investigate the detection possibility of all the pesticides under study by HPLC system as well as to find the retention time of each pesticide, 2ml of each pesticide standard of 10ppm as well as blank (acetonitrile) was injected into the HPLC system. The high-performance liquid chromatography system was an Agilent Technologies HP-1100 Series (Agilent Technologies). Chromatographic separation was achieved using a Zorbax SB-C18 analytical column 2.1×150 mm, 3.5_m 270 particle size from Agilent Technologies at a column flow of 0.6 ml/min, a maximum pressure of 300 bar, injection volume 10µl and Temperature of 40^0C. The Agilent 1100 HPLC had three detectors viz, Diode Array Detector, Variable Wavalength Detector and Fluorescence Detector. The mobile phases consisted of solvent A: 10% Acetonitrile and solvent B: 90% Acetonitrile with 0.1% of HCOOH. The gradient was;

Time	Solvent B
0.0	90.0
28.0	2.0
30.0	0.0
31.0	0.0
31.50	90.0
40.0	90.0

The HPLC was controlled with CHEM 32 system software.

Out of fourteen pesticide standards selected for the study, only eleven pesticides were detected and their retention time was calculated, which is represented in Table 7 as follows;

Table 7: List of pesticides with their retention time

Retention Time	Sig	Lvl	Amount (ppm)	Area	Amt/Area	Ref	Group Name
11.740	1	1	10	1.33273	7.50342		Omethoate
11.993	1	1	10	4.96010	2.01609		Dimethoate
18.027	1	1	10	11.919	$8.38988e^{-1}$		Metalaxyl
22.219	1	1	10	210.68517	$4.74642e^{-2}$		Methyl Parathion
23.186	1	1	10	1.24712	8.01846		Dichlorovous
23.192	1	1	10	7.66647	1.30438		Alfa endosulfan
25.258	1	1	10	24.03247	$4.16104e^{-1}$		Internal Standard
26.040	1	1	10	2.28569	4.37504		Endosulfan
26.392	1	1	10	18.43536	$5.42436e^{-1}$		Methyl; Chloropyrifos
27.836	1	1	10	3.18208	3.14260		Beta endosulfan
30.380	1	1	10	513.32269	$1.94809e^{-1}$		Cypermethrin a
30.604	1	1	10	176.12755	$5.67770e^{-2}$		Cypermethrin b
30.682	1	1	10	231.96342	$4.31102e^{-2}$		Cypermethrin c
30.904	1	1	10	50.89310	$1.96490e^{-1}$		Fenvalarate

The predominant precursor ions (mass) of substances that were to be detected and the product scans were used to identify the main fragments. The instrumental parameters like Collision Cell Energy as well as Fragmentor Voltage were optimized in the Multiple Reaction Monitoring mode, the values of which are represented in Table 8 as follows;

Table 8: Optimization of instrumental parameters

Compound Name	**ISTD?**	**Precursor Ion**	**MS1 Res**	**Product Ion**	**MS2 Res**	**Dwell**	**Fragmentor Voltage(V)**	**Collision Energy (V)**	**Polarity**
Chloropyrifos	¨	321.9	Unit	289.6	Unit	30	80	12	Positive
Chloropyrifos	¨	321.9	Unit	124.8	Unit	30	80	20	Positive
Metalaxyl	¨	280.2	Unit	219.9	Unit	30	90	8	Positive
Metalaxyl	¨	280.2	Unit	191.9	Unit	30	90	12	Positive
Methyl Parathion	¨	264	Unit	231.7	Unit	30	110	12	Positive
Methyl Parathion	¨	264	Unit	124.8	Unit	30	110	16	Positive
Dimethoate	¨	230	Unit	198.7	Unit	30	70	4	Positive
Dimethoate	¨	230	Unit	170.8	Unit	30	70	8	Positive
Dichlorovous	¨	221	Unit	144.8	Unit	30	130	12	Positive
Dichlorovous	¨	221	Unit	108.9	Unit	30	130	16	Positive
Omethoate	¨	214	Unit	182.8	Unit	30	80	4	Positive
Omethoate	¨	214	Unit	154.8	Unit	30	80	20	Positive

The above information about column flow, stop time (gradient), pressure, volume of solvent, temperature, ratio of solvent and retention time was adopted to set up the method in LC/MS-MS QQQ mass spectrometry.

4.6.1.6 LC/MS-MS instrumentation

LC Conditions

Column: Agilent SB-C-18

Column Temperature: 40^0C

Mobile Phase: Solvent A; 10%ACN and Solvent B; 10%H_20

Flow Rate: 0.6ml/min

Gradient:

Time	Solvent B
0.0	10.0
28.0	98.0
30.0	100.0
31.0	100.0
31.50	10.0
40.0	10.0

Injection Volume: 5µl

MS Conditions

Mode: Positive ESI (Agilent 6410) Triple Quadrupole Mass Spectrometer

Nebulizer (psi): 25

Drying Gas Flow: 8l/min

V Capillary: 4000

Drying Gas Temperature: 300^0

Fragmentor Voltage: 70 – 130V

Collision Energy: 4 – 20V

4.6.2 Food analysis (tea, fruits and vegetables)

The food samples were extracted by the Extraction-Partitioning and Dispersive Solid Phase Extraction (QuEChERS) method. Hence the apparatus, chemicals, reagents, solvent needed for the extraction of food samples were same like that is required for soil sample, which has been well explained in above section. Te analysis of extracted food samples were carried out with GC/MS.

The steps followed for the extraction of food samples have been represented in Figure 20 as follows;

Figure 18: Steps followed during extraction of food samples

The samples of tea were extracted according to the DFG S19 method. This conventional method consists of an extraction step with acetone followed by partitioning with ethylacetate/cyclohexane (1:1), clean-up by Gel Permeation Chromatography (GPC) and analysis with GC-MS. Aldrin, forbidden for use since over 20 years, was used as internal standard spiked at the partitioning step. This extraction presents the advantage of providing clean samples concentrated by a factor 20 through the different extraction and cleaning steps.

The lists of the retention time (min), the target ion and the qualifier ions of the pesticides to be analyzed for the full scan screening were selected. Each analyte was provided either from Sigma-Aldrich or from Ehrenstorfer with the highest available purity. The standard salts were at first dissolved in acetone to reach concentrations around 1000 ng/µl and the single standard solutions were then mixed up altogether to reach final concentrations of 10 ng/µl, 5 ng/µl, 2 ng/µl, 1 ng/µl and 0.2 ng/µl.

The GC-MS analyses were performed on a Hewlett-Packard (Agilent Technologies, Waldbronn, Germany) GC-MS Model 6890N Series gas chromatography coupled to a 5973N mass selective detector. A HP 5 MS (30m×0.25mm i.d.) (Agilent Technologies, Waldbronn, Germany) fused silica capillary column with a 0.25 _m film thickness was used with helium as carrier gas at a constant pressure daily adjusted (chlorpyriphos- methylRT relocked to 16.596 min). One microliter of the sample was injected in the splitless mode at 280 ˚C with a splitless time before opening the injector valve of 2 min. The GC oven was operated with the following temperature program: initial tem- Temperature 70 ˚C held for 2 min, ramped at 25 ˚C/min to 150 ˚C not held, followed by a ramp of 3 $^{\circ}$C/min to 200 $^{\circ}$C not held, followed by another ramp of 8 $^{\circ}$C/min to 280 $^{\circ}$C held for 10 min and finally ramped to 320 $^{\circ}$C at 15°C/min held for 2.47 min. The total run time was 47 min, the interface was kept at 320 $^{\circ}$C, the ion source at 250 $^{\circ}$C, the quadrupole at 150 $^{\circ}$C and the mass spectra were obtained at electron energy of 70 eV. The analyses were operated in simultaneous full scan/SIM mode method. The Agilent Chemstation Software G1701DA version D.02.00.237 was used for data analysis. The Gas Chromatograph and Mass Spectrometer parameters are represented in Table 9 as follows;

Table 9: GC and MS parameters

GC	**Agilent Technologies 6890N-5973N**	**Agilent Technologies 6890N-5973N**
Autosampler	CTC Combi Pal	Agilent Technologies 7683B Auto Injector
Auto sampler control software	CTC CombiPal Cycle Composer version 1.5.2	
Inlet	EPC Split/Splitless	
Mode	Splitless, 1.0µl injected	
Inlet Temperature	280^0C	
Pressure	22 to 27psi (Chloropyrifos-methyl RT relocked to 16.596 min)	
Purge Flow	50ml/min	
Purge Time	2min	
Total Flow	55.9ml/min	
Gas saver	15ml/min	
Gas Type	Helium	
Inlet Liner	Agilent Technologies liner splitless, single-taper, glass woll, deactivated, p/n 5062-3587	
Oven		
Initial Temperature	70^0C for 2 min	
Ramp 1	25^0C/min to 150^0C not hold	
Ramp 2	3^{o}C/min to 200^0C not hold	
Ramp 3	8^0C/min to 280^0C hold for 10min	
Ramp 4	15^0C/min to 320^0C hold for 2.47 min	
Total Run Time	47min (last standard elutes around 37 min)	
Equlilibration Time	0.5 min	
Column	Agilent technology HP5MS	
Length	30m	
Diameter	0.25mm i.d.	
Film Thickness	o.25µm	
Mode	Constant Pressure	
Nominal Initial Flow	2.5ml/min	
MSD	Agilent Technology 5973N inert MSD	Agilent Technology 5975 inert MSD
Tune File	Atune.U	
Mode	Simultaneous full scan-SIM mode	
Electron Energy	-70eV	
Solvent Delay	3.2min	
EM Voltage	Atune voltage	
Low Mass (m/z)	50	
High mass (m/z)	550	

Threshold	
Sampleing	2
A/D samples	
Scan/s	
Quad temp	150^0C
Source temp	250^0C

5. RESULTS AND DISCUSSION

The present study was carried out to in two parts; field study to gather information regarding the awareness level of farmers regarding pesticide use and safety while the laboratory study was carried out to investigate the residue level of different pesticides in the food (fruits, vegetables and tea) and soil samples.

5.1 Survey results

A total of thirty farmers from the studied sites were interviewed for the present study. The results of field survey have been described in detail as follows;

5.1.1 Source of information about pesticide use among farmers

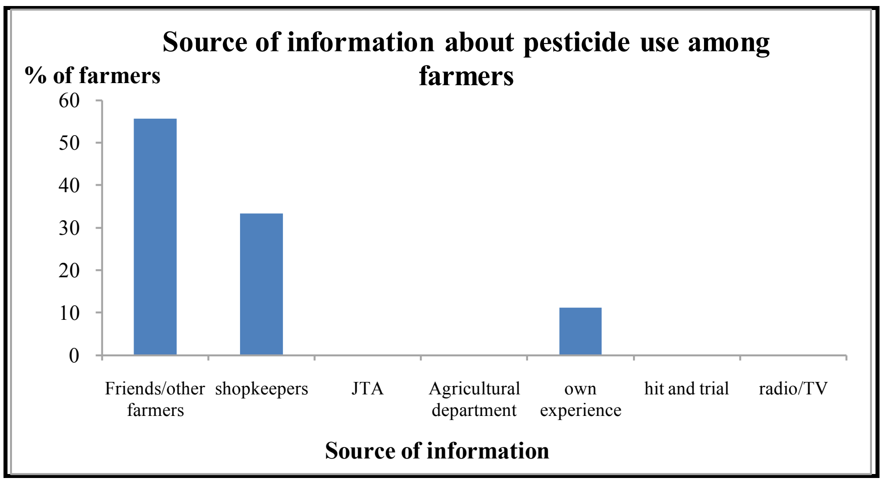


Figure 19: Source of information about pesticide use among farmers

Out of 30 farmers interviewed, more than 50% got information about pesticide use from friends, 30% from shopkeepers while 10% from own experience (Figure 19). Among the interviewed farmers none of them got information about pesticide use from authentic sources like JTA (Junior Technical Assistant) or Agricultural Department. This has showed that the activity of Nepal Government to spread awareness in proper pesticide selection and use among farmers from every corner of the country is not efficient and effective.

5.1.2 Storage of pesticide

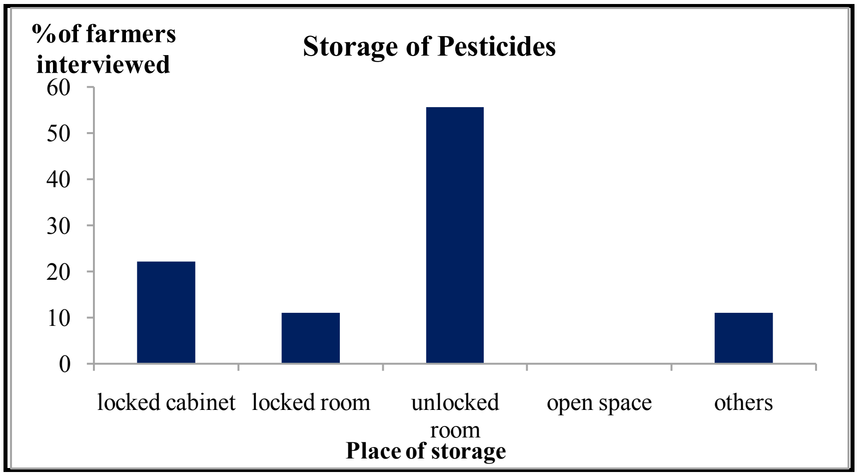


Figure 20: Storage of Pesticide

The survey revealed that more than 50% of the interviewed farmers (30) stored pesticides in an unlocked room while 20% and 10% in locked cabinet and locked room respectively (Figure 20). It is obvious that negligence in storing pesticides may lead to unwanted fatal accidents, especially where there are little children in the family.

5.1.3 Stages of pesticide use by the farmers

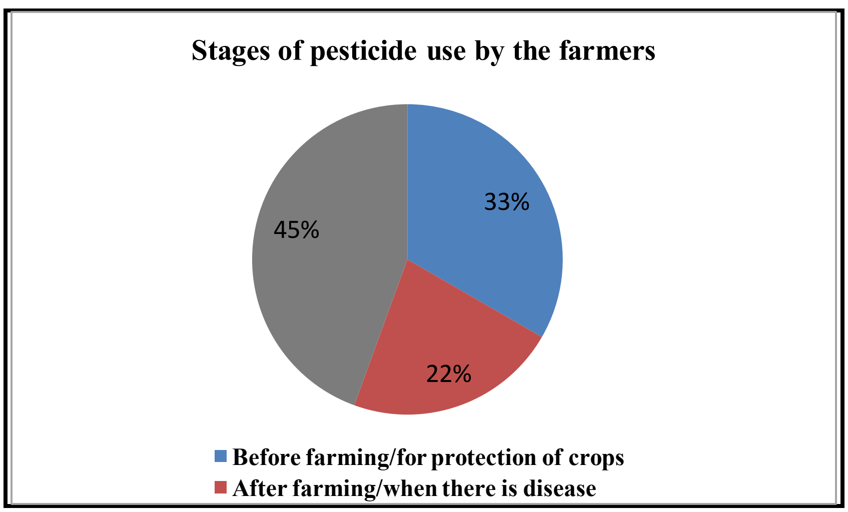


Figure 21: Stages of pesticide use by farmers

Out of 30 farmers interviewed, 33% used pesticides before farming for the protection of crops, 22% used after farming when there is disease and 45% used in both cases (Figure 21). Preharvest application of pesticide is danger practice because it may lead to excessive amount of residues in food crops, especially in vegetables causing adverse health impacts (Dahal, 1995).

5.1.4 Application of pesticides

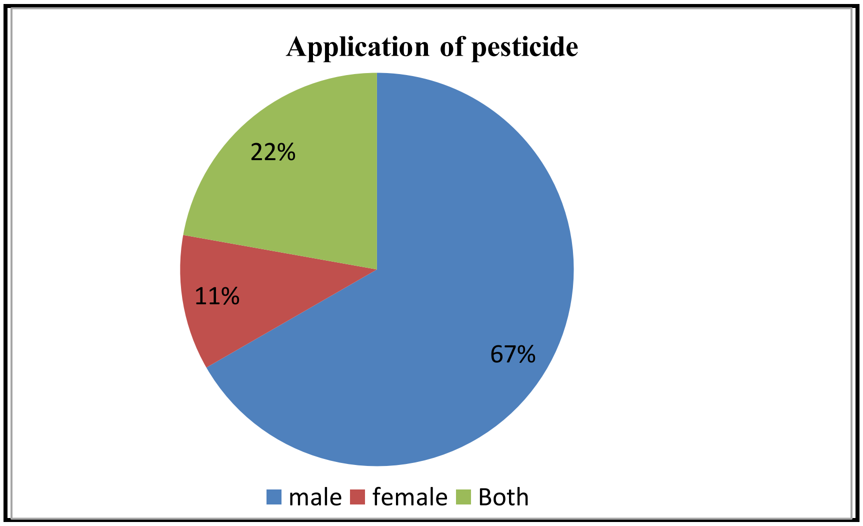


Figure 22: Application of pesticide

In this study, it was found that 67% of the interviewed farmers (30) applied pesticides in their field by both male and female while 22% and 11% applied by male and female respectively (Figure 22). Application of pesticide by female can be more dangerous because if the female doesn't take protective measures during and after pesticide application, her skin can be contaminated with pesticides that can be transferred to other members of family (like baby) through various ways or contamination of food cooked by her. So, taking protective measures during and after pesticide application is very important for the prevention of pesticide hazard.

5.1.5 Waiting time after pesticide use

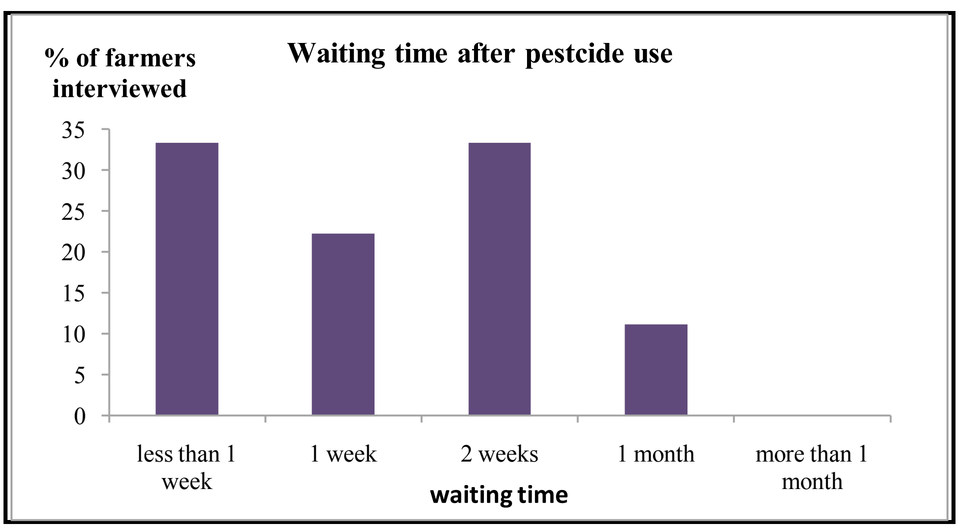


Figure 23: Waiting time after pesticide use

Waiting time between pesticide application and harvesting of crops is very important point to be considered by farmers. The waiting time is different for different pesticides and this should be strictly followed by the farmers. But due to illiteracy, carelessness and lack of awareness, some farmers even apply pesticides on the day of harvesting. In the present study, out of 30 farmers, nearly 35% used to wait less than 1 week and only 10% waited for 1 month (Figure 23). The application of pesticide close to harvesting time could be the main reason for the contamination of fruits and vegetables brought to Kathmandu from surrounding districts.

5.1.6 Safety measures adopted during pesticide use

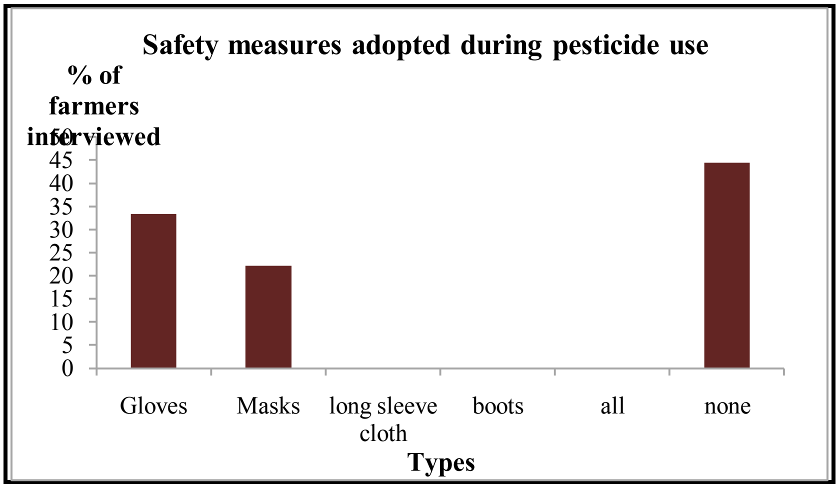


Figure 24: Safety measures adopted during pesticide use

The adoption of safety measures during and after pesticide application is very important factor for preventing against harmful impacts of pesticide. The various safety options could be use of gloves, masks, long sleeved cloth, glass, long boots etc. The present study showed that 45% of total interviewed farmers (30) used none of the safety measures while 30% used gloves and 20% used masks (Figure 24).

5.1.7 Knowledge about the health impacts of pesticide application

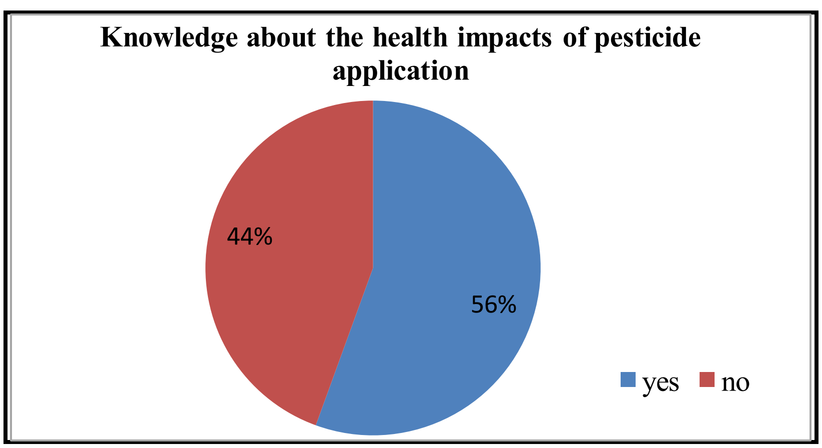


Figure 25: Knowledge about the health impacts of pesticide

Out of 30 farmers interviewed, 56% had knowledge about the health impacts of pesticide while 44% did not have (Figure 25). Although 56% farmers had knowledge about the impacts of pesticides, none of them used any protective measures during and after pesticide application. So, this shows that the farmers were careless to adopt protective measures and did not have willingness to change their behaviour.

5.1.8 Symptoms of health impacts

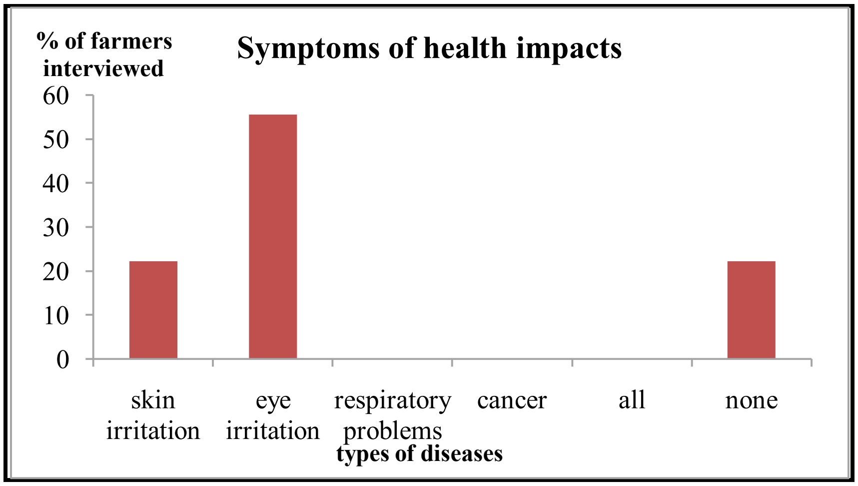


Figure 26: Symptoms of health impacts

Out of 30 farmers interviewed, more than 55% had the problems of eye irritation, 22% had skin irritation while 22% had no symptoms at all (Figure 26). The pesticides can also have severe effect after some years of continuous and unprotective application.

5.2 Results of laboratory studies

The analytical results of the laboratory tests to investigate the amount of pesticide residues in food (tea, fruits and vegetable) and soil samples have been described as follows;

5.2.1 Tea

A total of eleven tea samples were analyzed to study about the residue level of pesticides. Tea samples were purchased from different shops located in Kathmandu. The following graph shows the levels of pesticide residues in different quality and type of tea samples.

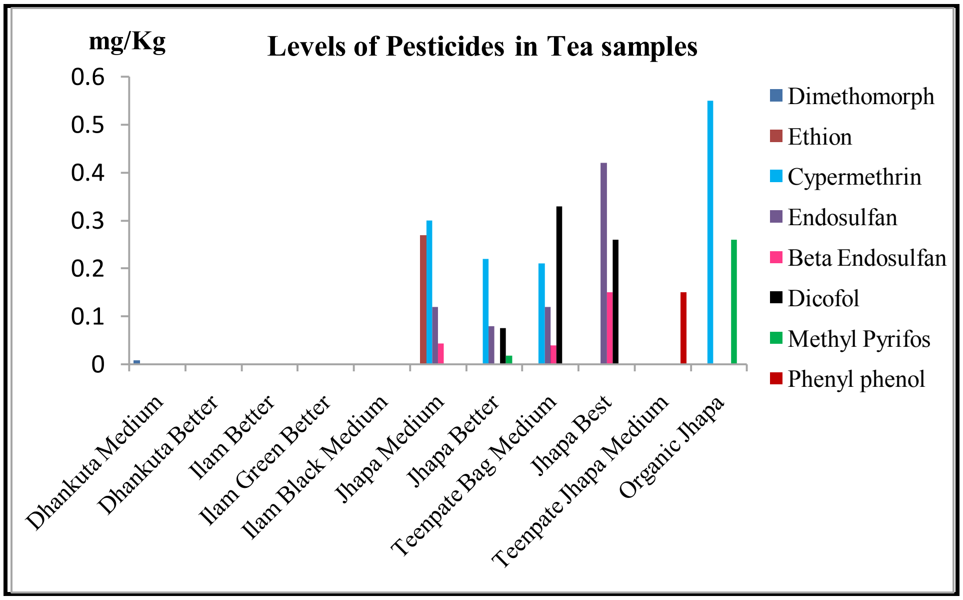


Figure 27: Levels of pesticides in tea samples

The above graph (Figure 27) shows that more than 50% of the tea samples were found to be contaminated with different pesticides. The contaminated teas were medium and best quality tea from Jhapa. However the pesticide concentration in the tea samples from Ilam and Dhankuta were below the limits of detection. Ethion, which is a banned organophosphorous pesticide, was found at the level of 0.27mg/Kg in medium quality tea. This showed that some farmers are still using the banned pesticide in food products, which can cause detrimental health impact to public health. Even the best quality tea was found to be contaminated with 0.57mg/Kg of Endosulfan Sulfate (chlorinated insecticide) and organic tea with 0.55mg/Kg of Cypermethrin (Organo phosphorous pesticide).

5.2.2 Fruits and Vegetables

The levels of pesticides found in the fruits and vegetable samples have been represented in Table 10 as follows;

Table 10: Levels of pesticides in fruits and vegetable samples

Food Article	Place	Pesticide	Unit	Concentration
Spinach	Thimi II	Chloropyrifos	mg/Kg	**0.007**
Cucumber	Chattradeurali I, Dhading	Metalaxyl	mg/Kg	**0.025**
Tomato	Dhading Besi I	Cypermethrin	mg/Kg	**0.84**
Apple I	Kalimati Market	Carbendazim	mg/Kg	**0.018**
Apple II	Ason Market	Diphenylamine	mg/Kg	**0.002**
Citrus fruit	Ason Market	Ethion	mg/Kg	**0.085**

Out of sixteen different vegetables analyzed, three were found to be contaminated with pesticides while in case of fruits, two samples were contaminated out of four.

5.2.3 Soil

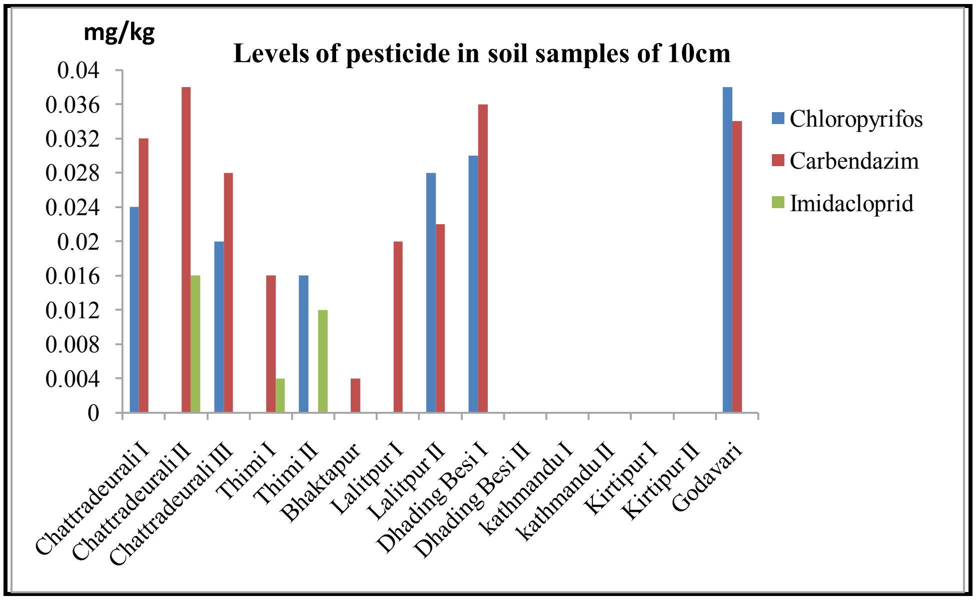


Figure 28: Levels of pesticides in soil samples of 10cm depth

The above graph (Figure 28) shows the levels of pesticide residues in soil samples collected from the farm of interviewed farmers at a depth of 10cm. All the soil samples except that from Kathmandu and Kirtipur were found to be contaminated with pesticides like Chloropyrifos, Carbendazim and Imidacloprid. The graph also revealed that almost all the contaminated sample contained Carbendazim up to 0.038mg/Kg followed by Chloropyrifos up to 0.038mg/Kg.

Another study to investigate the concentration of pesticide residue at different depths viz. 10cm, 30cm and 50cm was carried out so as to assess the distribution pattern of pesticides at different depths.

The following graph (Figure 29) explains about the distribution of pesticide residue at different depths of 10cm, 30cm and 50 cm in the soil sample of the studies site. The soil profile study revealed that there was homogenous distribution of pesticides in all the studies depths. This showed that the farmers were using the same type of pesticide since past and were continuing to use the same. From this study, it was also found that a highly carcinogenic organophosphorous insecticide "Methyl Parathion" was also found to be used by the farmers and its distribution was homogenous at the three different depths.

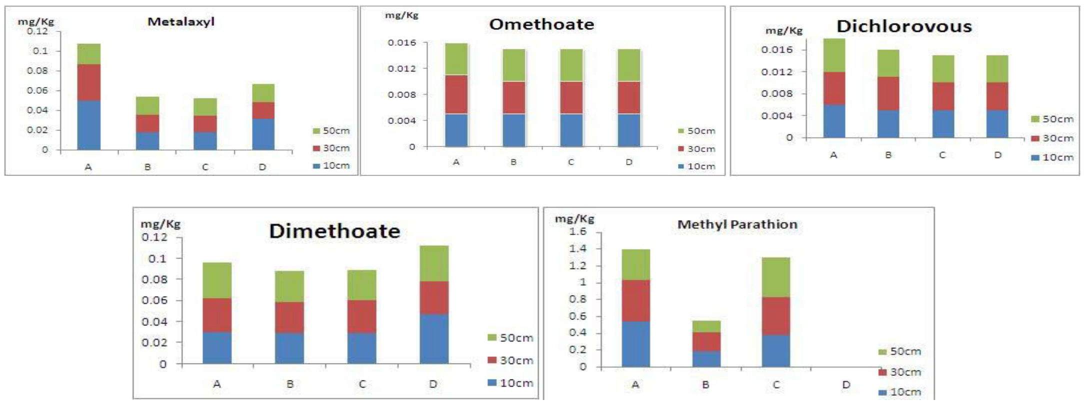


Figure 29: Distribution of pesticide in soil samples at depths of 10cm, 30cm and 50cm

6. CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study are based on small sample size: Field study - thirty farmers interviewed and Laboratory study - (fruits - fourteen, vegetables -sixteen, tea - eleven and soil - fifteen (10cm) and 4 (10cm, 30cm and 50cm). A small sample size decreases the probability of finding contaminated samples. However, it has been believed that the present sample size was sufficient to study and get idea about the pesticide use and safety among some farmer communities of central Nepal. Nevertheless, for better understanding and drawing meaningful conclusion, an in depth study covering all the ecological divisions of Nepal (Terai, Hills and Mountain) is very important.

The followings are the conclusions drawn from the present study;

- The awareness level of interviewed farmers regarding pesticide use and health safety was very less. From the field study, as expressed by farmers themselves, it was difficult for them to change their behaviour in which they were adopted. This was the reason that they were careless regarding the adoption of safety measure during and after pesticide application.
- As announced by different media in the past, the findings of the present study also showed that some tea samples were found to be contaminated with pesticides. Among them, one was banned pesticide too. The medium, best and even the organic tea was found to be contaminated with pesticides.
- Cypermethrin, Endosulfan Sulphate, Beta Endosulfan, Methyl Pyrifos and Phenyl Phenol, Ethion were the pesticides found in tea samples in different amounts.
- Some vegetable and fruit samples were also found to be contaminated with pesticides. The pesticides found in vegetable samples were Chloropyrifos, Metalaxyl, Cypermethrin and in fruits were Carbendazim, Ethion and Diphenylamine.
- The soil samples were found to be contaminated with different pesticides (Chloropyrifos, Metalaxyl, Methyl Parathion, Omethoate, Dimethoate, Dichlorovous and Imidacloprid) at different concentrations.
- The concentration of pesticides was found to be homogenously distributed in all the depths of 10cm, 30cm and 50cm. This showed that the farmers were continuing to use the same type of pesticides since past as revealed from the present study.
- A new modified QuEChERS method for the analysis of soil samples has been developed in the present study, which could be affordable and feasible in context to Nepal for further studies.

Based on the findings of the present study, the following recommendations have been made so as to improve the present situation of pesticide use in Nepal;

- Education on safety precautions regarding pesticide use to the farmers (male and female) should be made more efficient, covering all regions of Nepal. As long as farmers are not far away from awareness, the problem of pesticide pollution cannot be solved.

- The use of different media like radio, television could be effective in order to spread awareness to the farmers. Also, the local community clubs in association with government could provide training to farmers through public lectures, street theatres in local language etc.

- Farmers should also be well informed of the fact that using pesticide in recommended amount does not result with harmful impacts. It brings hazard only when farmers don't follow the recommendation and use it haphazardly.

- The activity of pesticide residue monitoring should be made effective and regular by the government. If the government laboratory doesn't have sufficient technology and manpower, it should work in close collaboration with private or semi-governmental laboratory that has facility (human and technological resource) and resources for pesticide residue monitoring.

- Pesticides are the potential health hazards, which have drawn attention of food quality control agencies, certification bodies, international community and trading partners. Due to the lack of regular monitoring scheme of MRL on pesticides, it has affected the export of tea, honey and other food commodities in the recent years. Similarly, the certification of organic foods has faced problem due to the absence of this monitoring scheme.

- There should be strict regulation that should be implemented for stopping the import of banned chemical pesticides especially from India and China due to open borders.

- Scientific evaluation of potential adverse effects resulting from human exposure to food borne hazards. Risk analysis is relatively new applied science based on sound scientific footing to protect consumer's health by Appropriate Level of Protection (ALOP). Necessary data and information for the food standardization should be base on risk analysis of particular food commodity.

7. REFERENCES

D. Barcel´o, M.C. Hennion (1997) Introduction, in Trace determination of Pesticides and their Degradation Products in Water, Elsevier, Amsterdam

Dahal L. (1995) A Study on Pesticide Pollution in Nepal, IUCN, Nepal

Dahal, L., Baker, S.L. and Gyawali, B.K. (1995) Promoting Proper Use of Pesticides use in Nepal, Ministry of Agriculture / Winrock International, Nepal

DFTQC (2004) Annual Bulletin of Department of Food Testing and Quality Control, Kathmandu Nepal

DFTQC (2005) Annual Bulletin of Department of Food Testing and Quality Control, Kathmandu Nepal

DFTQC (2008) Annual Bulletin of Department of Food Testing and Quality Control, Kathmandu Nepal

E.M. Thurman, Y. Fany, P. Zavitsanos and J.A. Zweigenbaum (2008) Multiresidue analysis of 100 pesticides in food samples by LC/Triple Quadrupole Mass Spectrometry, Agilent technologies Publication 5989-5469EN

Ecobichon D.J. (1996) Toxic Effects of Pesticides, 5th Edition New York: MacMillan, 643-689

Economic Survey (2005) Ministry of Finance, Kathmandu, Nepal

Engel L.S., O' Meara E. S., Schwartz S.M. (2000) Maternal occupation in agriculture and Risk of limb defects in Washington State, 1980-1993, Scand J Work Environmental Health 26 (3): 193-8

Food Act (1967) Government of Nepal, Kathmandu, Nepal

Food and Agricultural Organization (2002) International Code of Conduct for the distribution and use of pesticides. Retrieved on 18th July 10

Food and Agricultural Organization (2004) International Code of Conduct for the distribution and use of pesticides. Retrieved on 18th July 10

Food Regulation (1970) Government of Nepal, Kathmandu, Nepal

Giri N. P. (1998) Pesticide pollution in vegetable crop in Kathmandu valley, Tribhuwan University, Nepal

Jaga K., Dharmani C (2003) Sources of exposure and public health implications of organophosphoric pesticides 14 (3): 171-85

Jeyaratnam J. (1990) Acute Poisonong: a major health problem, World Health Stat Q 43 (3):139-44 PMID 2238694

Knutson R. (1999) Economic Impact of reduced pesticide use in the United States

Luke M., Froberg, J.E. and Masumoto H.T. (1999) Analytical Chemistry 58, 1020-1026

M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J.Schenck (2003) Fast and easy multiresidue method employing extraction/partitioning and dispersive solid phase extraction for the determination of pesticide residues in produce, 86, 412 – 431

M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J.Schenck (2003) Validation of a simple and rapid multiresidue method and its implementation in pesticide residue analysis, poster presented at MGPR Symposium, France

Miller G.T. (2002) Living in the Environment (12^{th} Edition) Wadsworth/Thomson Learning. ISBN 0-534-37695-5

Miller G.T. (2004), Sustaining the Earth, 6th edition, Thompson Learning, Inc. Pacific Grove, California. Chapter 9, Pages 211-216.

Mills P.A., Onley J.H. and Guither R.A. (1963) Analytical Chemistry 46, 186-191

National Planning Commission Report (1995), Nepal Government, Kathmandu, Nepal

Palikhe, B.R. (2001) Pesticides pollution management in Nepal: In harmony with nature, Agriculture and Environment communication issue, Ministry of Agriculture and Cooperative, Kathmandu, Nepal

Palmer W.E., Bromley P.T. and Brandenburg R.L.(2007) Wildlife & pesticides, North Carolina Cooperative Extension Service. Retrieved on 22^{nd} July, 2010.

Pesticide Act (1991) Nepal Government, Kathmandu, Nepal

Pesticide Rule (1993) Nepal Government, Kathmandu, Nepal

Pesticides Wikipedia, The Free Encyclopedia, Retrieved on 20th July 2010

Science Daily (2006), Retrieved on 20^{th} July 2010

Tea Policy Nepal (2000) Nepal Government, Kathmandu, Nepal

US Environmental Protection Agency (2007) www.epa.gov. Retrieved on 15^{th} July, 2010

Wassemann M. and Wasserman D., (1972) Effects of organochorine insecticides on homeostatic and immunologic process, fate of pesticides in the environment, Gordon and Breach London

Williams Michael (2000) Nutrition and the Environment, ACC SCN 21 Report, Geneva Switzerland

WHO (2009), Recommended classification of pesticides by hazard, International Programme for chemical safety (IPCS), WHO

WTO Report (2003), Retrieved on 20^{th} July 2010

8. LIST OF TABLES, FIGURES AND ABBREVIATIONS

8.1 List of tables

1. WHO Hazard Classification of Pesticides
2. Maximum pesticide residue level in selected countries
3. Pesticides commonly used in Terai and Hilly regions of Nepal
4. Year wise sample analysis and detection of pesticides (1994 to 2004)
5. Study time schedule
6. List of collected vegetable samples
7. Optimization of instrumental parameters
8. Levels of pesticides found in fruits and vegetable samples

8.2 List of figures

1. Trend of pesticide use in Nepal
2. Types of pesticide used in Nepal
3. Extremely hazard pesticide used
4. Different pesticides used
5. Schematic diagram of the analytical steps of QuEChеRS method
6. Comparison of QuEChеRS with other methods
7. Working principle of HPLC system
8. Working principle of Agilent G6410 Triple Quadrupole Mass Spectrometry
9. Study area
10. Questionnaire survey in Chhatradeurali
11. Questionnaire survey in Dhading Besi
12. Sample of tomatoes
13. Sample of cucumber
14. Collection of soil samples
15. Potato farm
16. Schematic diagram of QuEChеRS method adopted for soil sample extraction
17. Steps followed during extraction of soil samples
18. Steps followed during extraction of food samples
19. Source of information about pesticide use among farmers
20. Storage of pesticides
21. Stages of pesticide use by the farmers
22. Application of pesticides
23. Waiting time after pesticide use
24. Saftey measures adopted during pesticide use

25. Knowledge about health impacts of pesticide application
26. Symptoms of health impacts
27. Levels of pesticide in tea samples
28. Levels of pesticides in soil samples of 10cm depth
29. Distribution of pesticide in soil samples at the depth of 10cm, 30cm and 50cm

8.3 List of abbreviations

ACN	Acetonitrile
BHC	Benzene Hexa Chloride
BOKU	University of Bodenkuluture Wien
C FRL	Central Food Research Laboratory
CBD	Cotton Development Programme
CTC	Cut, Tear and Curl
DDT	Dichloro diphenyl trichloro methane
DFTQC	Department of Food Testing and Quality Control
EEA	European Economic Area
EPA	Environmental protection Agency
EQS	Environmental Quality Standards
EU	European Union
FAO	Food and Agricultural Organization
FSB	Food Standardization Board
GC/MS	Gas Chromatography Mass Spectrometry
GDP	Gross Domestic Product
HPLC	High Performance Liquid Performance
ILO	International Labour Organization
ISO	International Standard Organization
LC/MS-MS	Liquid Chromatography Mass Spectrometry
MDG	Millennium Development Board
MIC	Methyl Isocyanate
MRL	Maximum Residue Level
MRLs	Maximum Residue Levels
MRM	Multi Residue Methods
MSPE	Matrix Solid Phase Extraction
NEPCIL	Nepal Pesticides and Chemical Industries Private Limited
NIH	National Institutes of Health
NMED	Nepal Malaria Eradication Programm
NPTN	National Pesticide Telecommunication Network
PBT	Persistent Bioaccumulative and Toxic
PLE	Pressurized Liquid Extraction
PMRA	Pesticide Management Regulatory Agency
POPs	Persistent Organic Pollutants
SAFTA	South Asian Free Trade Association
SDME	Solid Phase Micro Extraction
SPE	Solid Phase Extraction
SPS	Sanitary and Phytosanitary
TBT	Technical Barriers to Trade
UK	United Kingdom
UN	United Nations

USA	United States of America
USAID	United States Association for International Development
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WTO	World Trade Organization