**COLLEGE OF SCIENCE AND TECHNOLOGY**

**SCHOOL OF SCIENCE**

# *Assessment of Cypermethrin and Profenofos residues in surface water and water sediments of Nyabarongo river*

*A dissertation submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science (MSc) in Environmental Chemistry*

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**April, 2022**

## DECLARATION

I hereby declare that this dissertation titled "*Assessment of Cypermethrin and Profenofos residues in surface water and water sediments of Nyabarongo river*" is the result of my own original work. It is being submitted for the degree of Master of Science in Environmental chemistry and it has not been submitted for any other degree at the University of Rwanda or at any other institution.

Martin NYANDWI

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Signature ……………………

Date …………………………

I, Associate Professor Theoneste Muhizi, the supervisor of this dissertation titled "*Assessment of Cypermethrin and Profenofos residues in surface water and water sediments of Nyabarongo river"* declare the approval submission of the dissertation for examination.

Supervisor: Associate Professor Theoneste Muhizi

Signature ………………………

# DEDICATION

To

My lovely wife,

My children,

My whole family and,

Friends.

God bless you!

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To all, thank you very much and God bless you!!!

Martin NYANDWI

## ABSTRACT

The study was carried out to assess both Cypermethrin and Profenofos residues in Nyabarongo surface water and water sediments. Surface water and water sediments samples were collected at two different sampling sites on two sides of the river which are located at the nearest the junction of Nyabarongo and Nyabugogo. Both pesticides were analyzed using coupled Gas Chromatography Mass Spectrometer. The obtained results indicated that Cyprmethrin and Profenofos residues were absent in all surface water samples. However, these chemicals were detected in most water sediments samples. For 28 samples (14 on Kamonyi side and 14 on Nyarugenge side) analyzed for Cypermethrin all samples (equivalent to 100%) were contaminated and results were above the detection limits while for 27 samples (12 on Kamonyi side and 15 on Nyarugenge side) analysed for Profenofos only 6 samples (equivalent to 22%) were below the Limit of detection and 21 (equivalent to 78%) were above the limit of detection. Agricultural application of pesticides is the main source of pesticides in the river and may have reached it through spray drift, surface runoff or overland flow, leaching, drain flow and through flow. The results of analysis were ranged from 0.1663 mg/kg to 1.8353 mg/kg and 0.2021 to 1.0373 mg/kg in water sediments for Cypermethrin and Profenofos respectively. Data have shown that the water sediments of Nyabarongo river are most polluted. These pesticides have the potential to accumulate in water sediment because of their low water solubility or high octanol-water partition coefficient (Kow) greater and higher soil half-life at least greater than 30 days [1]. Though the persistence of both pesticides is not so high but their presence in water sediments may affect heath of biota as well as consumers of fish from Nyabarongo river.

***Keywords**: Pesticides, GCMS, Cypermethrin, Profenofos*

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**ABBREVIATIONS**

DDD: Dichlorodiphenyldichloroethane

DDE: Dichlorodiphenyldichloroethylene

DDT: Dichlorodiphenyltrichloroethane

EI: Electron Impact Ionization

FAO: Food and Agriculture Organization

GC: Gas Chromatography

GCECD: Gas Chromatography Electron Capture Detector

GCMS: Gas Chromatography Mass Spectrometer

GPC: Gel Permeation Chromatography

ISO: International Organization for Standardization

$LC_{50}$: Lethal Concentration

LC-MSMS: Tandem Liquide Chromatography Mass spectrometer

$LD_{50}$: Median Lethal Dose

LOD: Limit of Detection

LOQ: Limit of Quantification

MAE: Microwave Assisted Extraction

MINAGRI: Ministry of Agriculture and Animal Resources

MRM: Multiple Reaction Monitoring

OCP: Organo Chlorinated Pesticides

OPP: Organo Phosphorus Pesticides

PSTA 4: Strategic Plan for Agriculture Transformation 4

RSB: Rwanda Standards Board

RSD: Relative Standard Deviation

SIM: Selected Ion Monitoring

SPE: Solid Phase Extraction

UR-CST: University of Rwanda-College of Sciences and Technology

# CHAPTER I. GENERAL INTRODUCTION

## I.1. Background

Water is an indispensable natural resource that is often prodigiously threatened by anthropomorphic activities [2]. Pesticides represent one of the major pollutants that may hinder the required quality of water. The intensification of agricultural production systems in the world has enabled production of a plenty supply in food as well as fiber, but at some cost to the country's quality of water, soil, and air resources. The search for accelerated development and alleviation of a global issue of food security has forced human being to use synthetic chemical most importantly in agricultural sector for wild plants and pest control and thereafter boost increased productivity [3]. Though the application rate would be different country by country but pesticides are now applied everywhere in the world including Rwanda. There are several factors which influence a pesticides' potential to contaminate water: The ability of the pesticide to dissolve in water (solubility), environmental factors, such as, soil, weather, season, and distance to water sources, application methods and other practices associated with the pesticide use [4,5]. Surface waters and water sediments of Nyabarongo river is prone to contamination with pesticides as a result of agricultural activities in its surroundings which implies the application of pesticides all along its flow path. These may reach the river bank though surface runoff or though the wind [6,7]. The presence of pesticides in Nyabarongo water would be associated with direct human health effects such as oncological (cancer), pulmonary and hematological morbidity, as well as on inborn deformities and immune system deficiencies" as well as ecological effects such as bio concentration and bio magnification though the living organism under exposure [6].
The evaluation study of the pesticides in surface water of Nyabarongo river will provide scientific baseline of the level of pollution.

Based on the survey conducted by FAO, whereby 10 pesticides in different formulations were identified as frequently applied in the agricultural activities [7] namely: Cypermetrin (Insecticide), Profenofos (Insecticide), Metalaxyl (Fungicide), Mancozeb (Fungicide), Abamectin (Insecticide), Carbendazim (Fungicide), Sulphur (Fungicide), Lambda Cyhalothrin (Insecticide), Deltamethrin (Insecticide), Imidacloprid (Insecticide).

This study has assessed the presence of Cypermethrin and Profenofos whose application rate is high, with critical toxicity and classified among Highly Hazardous Pesticides (HHPs) that are still in use in Rwanda [8]. It has conducted a quantitative estimate of the above mentioned pesticides in the surface water and water sediments of Nyabarongo in different locations of the river in alongside its flow.

The study was conducted in 731 households by the National Institute for Statistics (NISR) in the 2016/2017 agricultural year provides information on the use of pesticides by households during Season A and B (in 2017) per province. The Surveyed pesticides were Dithane M45/Mancozeb, Dimethoate, Cypermethrin and Dursban and showed that on average the application of Cypermethrin is around 47.4% and that particularly the southern province covered 57.1 and 52.3% respectively in season A and B [9].

## I.2. Problem Statement

In order to fulfil the national agriculture policy, the Rwanda ministry of agriculture and animal resources (MINAGRI) has developed its fourth strategy plan for agriculture transformation (PSTA 4) to promote the growth of the agriculture sector in partnership with the private sector. PSTA 4 has four priority areas including: (i) innovation and extension; (ii) productivity and resilience; (iii) inclusive markets and value addition; (iv) enabling environment and responsive Institutions [10]. However, the intensification of agriculture implies the use of external inputs especially pesticides as well as industrial fertilizers by farmers to increase crops productivity [11]. This results in accelerated environmental pollution. Pesticides in Rwanda are mostly used by local farmers who do not have knowledge on safety measures and adequate usage. This might result in the application of high dosages, irregular dumping of pesticides, high levels of drift to air, environment contamination through rain wash out, soil leaching and deposition [7]. There is also probability of use of banned pesticides in Rwanda as sometimes local farmers are unable to afford the expensive pesticides and prefer to buy cheaper ones from the illegal markets or in the neighboring countries [12].

As the main river in Rwanda, Nyabarongo drains most of water from different locations of the country resulting in the accumulation and gradual concentration of various chemicals all along its flow. Among the chemicals used by farmers, figure Cypermetrhrin and Profenofos to fight against a wide range of insects, especially Lepidoptera, but also Coleoptera, Diptera, Hemiptera, and other classes, in fruit, vines, coffee, vegetables, potatoes, cucurbits, lettuce, capsicums, tomatoes, cereals, maize, soya beans, cotton, coffee, cocoa, rice, pecans, oilseed rape, beet and ornamentals [15,16]. The agriculture in Nyabarongo and its neighboring marchland is dominated by the growth of maize and vegetables including tomatoes, cabbages, onions, eggplant, broccoli, Lettuce, green pepper and carrots which are frequently treated by these pesticides. According to Rwanda ministry of agriculture and animal resources, 2135.7 tons of pesticides were imported in financial year 2019-2020 and has increased to 7667.4 tons in financial year and 2020-2021 [14]. This shows an intense and accelerated application of pesticides. Their fate after use may contribute to rivers contamination. As the main river in Rwanda, Nyabarongo occupies a total length of 351 km and a surface of 4,450 $km^2$ [15], and flows towards different regions of the country where agriculture activities are applied at high rate. All these constitute its risk of contamination by different chemicals and microorganisms. In spite of these contamination risks, the need for using water from this river is increasing. For example, Nzove water treatment plant provides 145,000$m^3$/day which are supplied in many locations of Kigali city with the intent to add 65,000$m^3$/day in near future [16]. In addition, biota such as fish, living in this river risk to be highly exposed to pollutants including pesticides and other chemicals which are released in by both natural and anthropogenic activities. These chemicals may bioconcentrate in the tissue of these organisms and therefore be transmitted to humans through food web inducing different diseases to human [17].

Considering these pollution risks to the rivers, it is necessary to conduct regular assessment on the presence of pollutants in water and water sediments and to advise consumers. Having a scientific baseline on the level of contamination of Cypermethrin and Profenofos in surface water and water sediments of Nyabarongo river, is of interest since these products was reported to cause different human health challenges including genetic damage and reproductive harm and heart disease to some extent [18].

### I.3. Significance of the study

Agriculture is crucial for Rwanda’s growth and reduction of poverty, as the backbone of the economy, it accounts for 39 percent of gross domestic product (GDP), 80 percent of employment, 63 percent of foreign exchange earnings, and 90 percent of the country’s food needs [19]. Though the role of the sector to the life of the country is very important, there are still challenges related to low soil fertility and increasing number of pests that forces the farmers to use the agricultural inputs including Pesticides [20].

In addition, lack of awareness and low level knowledge of farmers on the nature and application measures of pesticides is a challenge for the control of pesticides in the environment [23].

Rwanda's topography with hilly terrain facilitate a quick transport of these agricultural inputs from the area of application to various environmental medium. Particularly, pesticides which can in addition to water can be transported by wind [19].

Water is an indispensable resource that everyone needs in the everyday life but is highly vulnerable to the contamination of these inputs with higher toxicity.

This study has a great impact not only in Rwanda but also in the region and generally on international community as it provides a background information on the current situation of the quality of water of Nyabarongo, the largest river of the country whose water resources are used by a great number of populations. It provides an information on which extent the application of pesticides in agricultural activities contribute to national or even regional water pollution. It extensively explores the contribution level of Cypermethrin and Profenofos to water pollution. All this information provides a baseline to Government of Rwanda decision makers in the area of national environmental protection strategies, status of national and international standards compliance in terms of water quality, and status of implementation of international conventions ratified by Rwanda.

## I.4. Objectives

### I.4.1. Overall objective

This study aims at quantifying the pollution levels of Cypermethrin and Profenofos in both surface water and water sediments of some localities of Nyabarongo river.

### I.4.2. Specific objective

- ✓ To develop an analytical method to analyze Cypermethrin and Profenofos pesticides in surface water and water sediments of Nyabarongo river

- ✓ To determine the level of Cypermethrin and Profenofos pesticide residues in water from different locations of Nyabarongo river alongside its flow path.

## I.5. Research Questions

- ➢ Does the application of pesticides in Rwanda pose a significant threat to water resources?
- ➢ Among the applied pesticides at which level and which one attempt to reach surface of Rwandan water resources?

## I.6. Scope of the study

The scope of this study was limited in space and in time. The study focused on assessment of the level of Cypermethrin and Profenofos pesticides in Nyabarongo river. So as to track the probable source of the detected pesticides, a thorough study was done on water of Nyabarongo tributaries of Cyogo and Nyabugogo rivers.

# CHAPTER II. LITERATURE REVIEW

## II.1. Introduction

Pesticides are all chemicals that are used to kill or control pests [21]. They are used to protect crops against insects, weeds, fungi, and other pests [21]. These products play a significant role in food production by protecting crops against pests and thus increasing production yields. This group of products includes herbicides, insecticides, fungicides, nematocides, and rodenticides [21,22]. There are more than 1,000 pesticides used around the world to ensure food security [22]. The estimate of around 67,000 different crop pest species were identified all over the world, including plant weeds, pathogens, some vertebrate and invertebrates species and they all cause about a 40 per cent reduction in the world's crop productivity [10]. This growing number of pests explain a strict use of pesticides in agricultural sector. In Rwanda, the agricultural sector contributes 33% to the growth domestic product (GDP) [10] . This shows that the use of pesticides is a must. Even though the application rate of pesticides in Rwanda is still low below 1kg/ha [7], which are unlikely to cause major dangers when used and disposed of in a precautionary way but low skills of applicator may result in abnormal and adverse environmental effects such as their presence in water resources [23]. Poor management of agricultural additives such as pesticides, herbicides and fertilizers are the potential agent of water pollution as well as soil degradation and decreasing agricultural yield and reducing resilience to forth coming environmental hazards [24]. Water resource is much vulnerable to this issue. Contamination of water by these chemicals constitutes a concern to living organisms including human and biota [25]. Each pesticide has specific properties and toxicological effects. Pesticides are potentially toxic to humans and can have both acute and chronic health effects, depending on the quantity and the ways in which a person is exposed [21] Some of the pesticides are persistent and can remain in the soil and water for years and although they have been banned in developed countries for agricultural application but are still in use in many developing countries [3]. Note that five millions of people die annually from water-borne diseases [26]. Global contamination by persistent organic pollutants including pesticides is one of the global challenges. Studies of major rivers and streams find in general that 90% of fish, 100% of surface water samples, and 41% of major aquifers contain one or more pesticides at detectable levels [27] . As a result of pesticide contamination of streams, rivers, lakes, and underground water supplies, drinking water is also widely contaminated.

### II. 2. Adsorption and entry of pesticide into soil and water

To reach water, pesticides should pass through different ways. For plant protection many pesticides are sprayed very close to surface water this causes spray drift. This builds a potential route to surface water pollution. Besides, when spread, a part of pesticides reaches the soil and adsorbed. The adsorption means an attraction between a chemical and soil, vegetation, or other surfaces. It most often refers to the binding of a chemical to soil particles. Pesticides that are adsorbed to soil particles are more likely to remain in the root zone where they may be available for plant uptake and microbial or chemical degradation. However, pesticides that are strongly adsorbed to soil usually are less available for microbial degradation and plant uptake. Those that are adsorbed weakly to soil particles are more likely to move through the soil profile with infiltrating water to surface and ground waters [28]. During raining, soil is sometime saturated and then water run off flows together with adsorbed chemicals and reaches water bodies [29]. In addition, soluble pesticides can be transported through leaching phenomenon to ground and surface water [29]. Besides, artificial drainage has been shown to be responsible for the transport of significant quantities of dissolved pesticides or that carried on water sediment particularly when rainfall and subsequent drainage occur shortly after application [29]. As other organic pollutants, transport of pesticides to reach into water bodies are influenced by different characteristics including solubility, adsorption, vapour pressure, weather, topography and ground cover [22]. The water-soluble chemicals tend to reach bigger areas than lipid soluble chemicals. Reason why more pesticides which are mainly hydrophobic tend to be adsorbed in water sediments [30].

### II.3. Existence of pesticides in atmosphere

Pesticide mobility may result in redistribution within the application site or movement of some amount of pesticide off site. After application, part of pesticides may be adsorbed to soil particles, vegetation, or other surfaces and remain near the site of deposition. These adsorbed chemicals can be later moved by wind and reach atmosphere. Through this movement, pesticides can reach areas which are far from the application site. In addition, pesticides which are sprayed may volatilize and be transported by wind becoming airborne [28]. These pesticides may come back to earth through atmospheric depositional processes that can be classified into two categories, those involving precipitation, called wet deposition, and those not involving precipitation, called dry deposition. Either category of processes involves both particle and gaseous transfer to the earth's surface [31].

### II.4. Ecological impacts of pesticides on environment

In general pesticides are toxic, poisonous, potentially hazardous to humans, animals, other organisms, and the environment and affect no targeted organisms. In fact, all chemical quantity sprayed are not reaching the target due to different physical chemical properties of the environment (Figure 1).

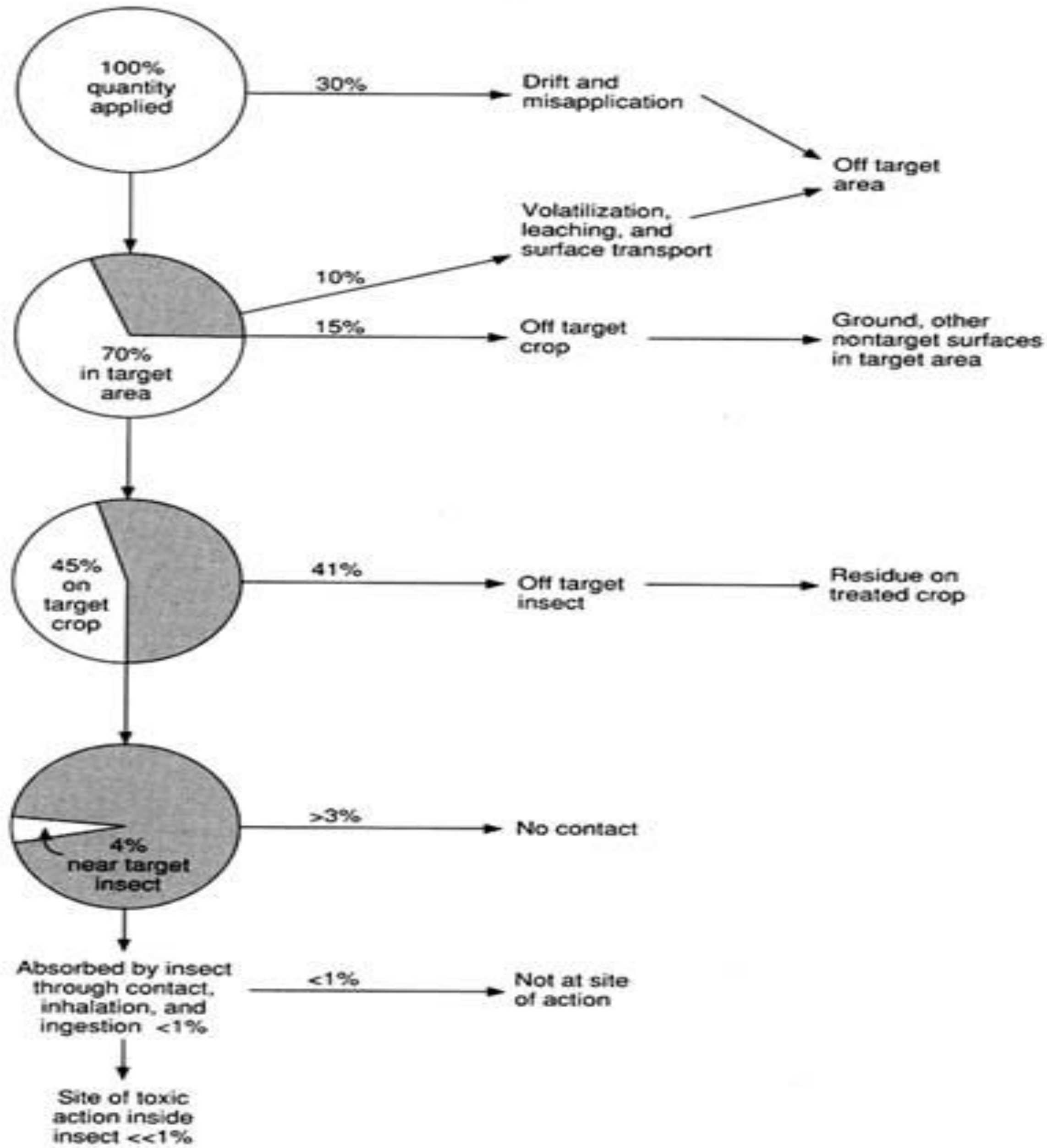


**Figure 1:** Mass balance of a hypothetical aerial foliar-spray application of an insecticide [32]

Therefore, people who use pesticides or regularly come in contact with them must understand the relative toxicity and potential health effects of the products they use. Depending on their effect to ecological composition, pesticides are classified according to their toxicity (Table 1) [7]**.** Toxicity is estimated by using different expression with the main Lethal Dose LD50 which is meaning the concentration of the pesticide which will kill half the test organisms over a specified test period. The lower the LD50, the greater the toxicity. In addition, the impact of these chemicals is directly dependent on the exposure time, amount of pesticides, as well as the toxicity class of chemical as shown by the below expression.

**Risk = Exposure (amount and/or duration) × Toxicity Class** [33]

**Table 1:** Categorization of pesticides according to their toxicity

| **Routes of Exposure** | **Category** | | | |
|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** |
| **Oral LD50** | 50–500 mg/kg | 500–5,000 mg/kg | >5,000 mg/kg | 50 mg/kg |
| **Inhalation LC50** | 0.2–2 mg/L | 2–20 mg/L | >20 mg/L | 0.2 mg/L |
| **Dermal LD50** | 200–2,000 mg/kg | 2,000–20,000 mg/kg | >20,000 mg/kg | 200 mg/kg |
| **Eye Effects** | Corrosive corneal opacity not reversible within 7 days | Corneal opacity reversible within 7 days; irritation persisting for 7 days | No corneal opacity; irritation reversible within 7 days | No irritation |
| **Skin Effects** | Corrosive | Severe irritation at 72 hours irritation | Moderate irritation at 72 hours | Mild or slight at 72 hours |
| **Signal Word** | DANGER POISON  | WARNING | CAUTION | CAUTION |

Some pesticides lie their danger to not only their persistence characters in environment, but also to the side products they release during degradation. Different biotic and abiotic factors intervene in the degradation and may accelerate the process or not. These include chemical reactions, hydrolysis, photolysis, and oxidation initiated by different microorganisms found in environment [32, 33]. The degradation process may lead to formation of products which may have greater, equal or lesser toxicity than the parent compound. DDT degrades to DDD and DDE, while Cypermethrin which is in question in this study, degrades to give 3-phenoxybenzoic acid (PBA) which may cause abnormal heart formation in aquatic living organisms like fish [35] and cyclopropane carboxylic acid (CPA) products whose target organs are central nervous and cardiovascular systems may cause central nervous system depression, cardiac disturbances, eye and skin burns, severe respiratory tract irritation with possible burns, severe digestive tract irritation with possible burns [18, 37]. Like other Organophosphorus pesticides, Profenofos and its products of degradation (Figure 2) presents various health effects like of apoptosis and necrosis, inhibit blood cholinesterase, abnormal development, skeletal defects and altered heart morphology [36]. In 2015, the United States Environmental Protection Agency officially reported Profenofos to be toxic to mammals, birds, fish, and aquatic invertebrates [37].

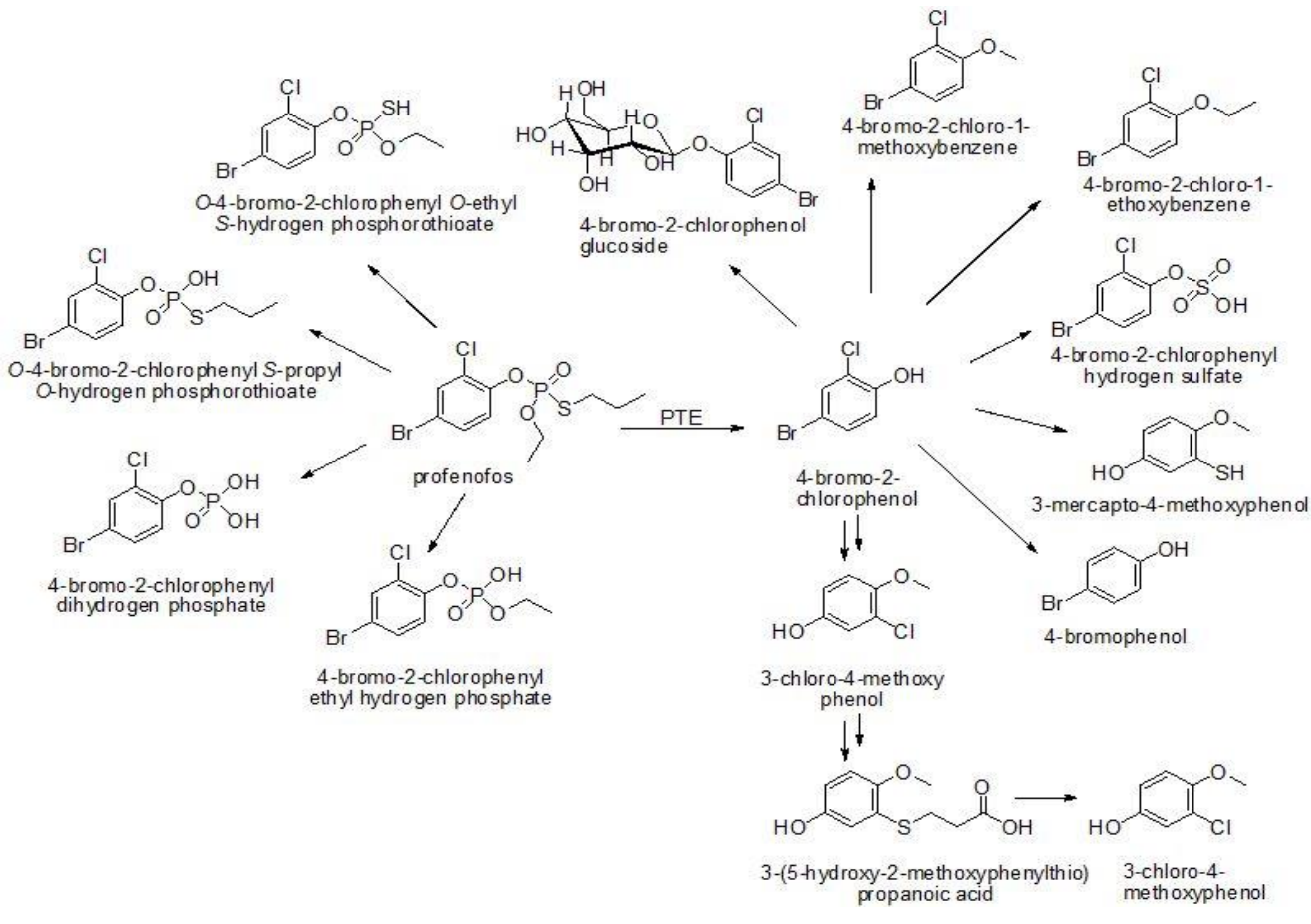


**Figure 2:** Decomposition pattern of Profenofos

During degradation, environmental fungi, bacteria, and other microorganisms use pesticides as food and thus breakdown their bonds leading to smaller products. This process is possible due to different enzymes produced by microorganisms and greatly depends on different environmental characteristics including moisture, temperature, oxygen, and pH [28]. Enzymatic degradation is possible due to different reactions modifying the structure of pesticide. These include hydrolysis, dehydrogenation, dehalogenation, decarboxylation, condensation, and so on [38]. Beside enzymatic degradation, pesticides can also be affected by different other chemicals and physical parameters initiating their degradation. When pesticide reacts with water, oxygen, or other chemicals in the environmental medium, it changes its properties through reactions [39]. Sunlight is another factor that affect pesticides in the environment leading to the formation of other sub-products. The intensity and spectrum of sunlight, length of exposure, and properties of the pesticide affect the rate of photo degradation [28].

### II.5 Particularity of Cypermethrin and Profenofos

Cypermethrin and Profenofos are two main pesticides widely used in Rwanda [8]. According to the data from National Institute of Statistics of Rwanda [40], rocket which is formed by these two active ingredients was used at the rates of 37.8 and 30.4% in 2021 and 2020, respectively (Table 2).

**Table 2:** Current application rate of both pesticides

| Pesticide formulation | Year of application | Agricultural season | Application rate (% of farmers) |
|---|---|---|---|
| **Rocket** | 2021 | Season A | 37.8 |
| | 2020 | Season B | 30.4 |
| **Cypermethrin** | 2021 | Season A | 14.3 |
| | 2020 | Season B | 17.6 |

#### II.5.1. Cypermethrin

Cypermethrin or (+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (Figure 3), is an insecticide of the synthetic pyrethroids family. All of the insecticides in this family have chemical structures that are loosely based on pyrethrins, insecticidal compounds found in chrysanthemum flowers [18].

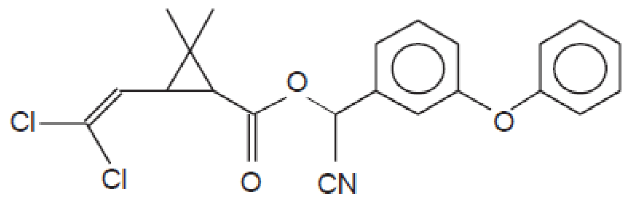


**Figure 3:** Cypermethrin structure

It is an active ingredient in many pesticides' formulations applied in Rwanda (Table 2). Its application is wide and ranges from the control of cockroaches, fleas and other indoor pests at home, restaurants, hospitals, schools and food-processing plants to agricultural pest control in cotton, fruit, vegetable and cereals (maize) growing. Cypermethrin and Profenofos are classified as Category II (Table 3). Unlike the natural pyrethrins, Cypermethrin is relatively stable to sunlight and is adsorbed very strongly on soil particles, especially in soils containing large amounts of clay or organic matter. Movement in the soil is therefore extremely limited and downward leaching of the parent molecule through the soil does not occur to an appreciable extent under normal conditions of use [18].

### II.5.2 Profenofos

Profenofos or (RS)-(O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate) is a pesticide belonging to the family of organophosphate insecticide.

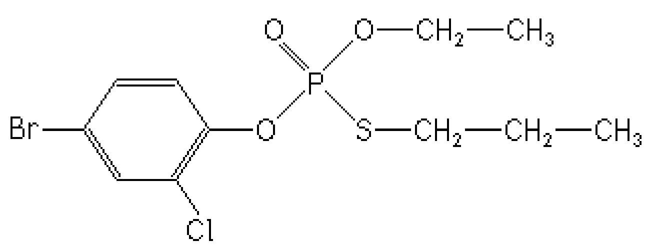


**Figure 4:** Profenofos structure

Like Cypermethrin, Profenofos is as well an active ingredient in many pesticides' formulations applied in Rwanda and is classified as Category II (Table 4) [21]. Profenofos is used to control, whiteflies, spider mites, plant bugs, and fleahoppers in plants like grains fruit and vegetables as well as in cotton to control tobacco budworm, cotton bollworm, armyworm and cotton aphid [13].

When used on crops, residual amounts spread into the environment via air, soil and water. When in water, the hydrolysis is the main route of dissipation and results mainly in products like 4-bromo-2-chlorophenol and o-ethyl-s-propyl phosphorthioate [13].

As an insecticide, Profenofos is a restricted use pesticide to control budworm, bollworm, armyworm, aphids, whiteflies, spider mites, plant bugs, and fleahoppers on various plants such as Maize, Tobacco, Sugarbeet, Soybeans and Potatoes. Most of the time it is used in conjunction with Cypermethrin to enhance their efficacy.

**Table 3:** Pesticide's formulations used in Rwanda that include Cypermethrin and Profenofos

| Formulation | Active Ingredients | Pesticide Category | WHO Classification |
|---|---|---|---|
| **CYPERSCOPE 5 EC** | CYPERMETRIN 50 GM/L | INSECTICIDE | **II** |
| **ROKKET 44 EC** | PROFENOFOS 40% 440-600<br>CYPERMETHRIN 4% EC | INSECTICIDE | **II** |
| **CYPRO 44 EC** | PROFENOFOS 40%<br>CYPERMETHRIN 4% EC | INSECTICIDE | **II** |
| **PROFEX SUPER** | PROFENOFOS 400g/l<br>CYPERMETHRIN 40g/l | INSECTICIDE | **II** |
| **TARGET** | PROFENOFOS 400g/l<br>CYPERMETHRIN 40g/l | INSECTICIDE | **II** |
| **AFRICYPER 5% EC** | CYPERMETHRIN 5% | INSECTICIDE | **II** |
| **SUPA ALPHA** | ALPHACYPERMETHRIN 10% | INSECTICIDE | **II** |

## II.6 Coupled gas chromatography/ mass spectrophotometer as analytical tool for pesticides residues

Gas Chromatographic system coupled to mass spectrometer (MS) is the technique used for identifying and quantifying several pesticides including Cypermethrin and Profenofos in water and water sediments. Samples for injection in the system are prepared via various extraction techniques such as Liquid-liquid extraction and solid phase extraction [41].

## II.7 Description of Solid phase extraction technique

The solid phase extraction cartridges use a technique which is based on selective partition of one or more components between two phases. One phase is a solid phase and acts as a sorbent and another one is a liquid phase with compound of interest. The component of interest may adsorb on a solid phase depending on the properties of the compound of interest and that of the sorbent (Table 4) [42]. Nowadays, the sorbent materials are packed properly in a tube to form a uniform bed with good flow distribution called SPE cartridges. Their working principle is now based on 3 important steps which are: Preconditioning of SPE cartridge, extraction of analyte from sample, adsorption to sorbet of SPE cartridge and elution of the analyte of interest (Figure 5).

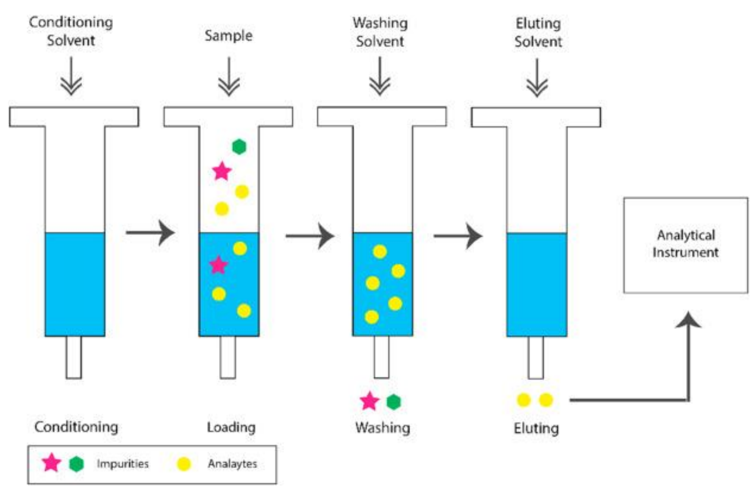


**Figure 5:** Steps of SPE [43]

**Table 4:** Separation optimization criteria in LC

| Mode | Adsorbent | Retention Mechanism | Sample Matrix |
|---|---|---|---|
| **Reversed Phase** | Non-polar<br>C2<br>C8<br>C18<br>Phenyl | Non-polar or hydrophobic interactions | Aqueous samples<br>• Biological fluids<br>• Aqueous extracts<br>• Environmental water samples<br>• Wine or beer |
| **Normal** | Polar<br>• Alumina<br>• Silica (Si-OH)<br>• Diol<br>• cyano<br>• amino<br>• florisil | Polar Interactions<br>• H-bonding<br>• pi-pi<br>• dipole-dipole<br>• induced dipole | Non-polar<br>• Organic extracts of solids<br>• Very non-polar solvents<br>• Fatty oils, hydrocarbons |
| **Ion Exchange** | • Quaternary amine (anion)<br>• Sulfonic acid (cation) | • Electrostatic attraction of charged functional groups of the analyte to oppositely charged functional groups on the sorbent | Aqueous or organic samples of low salt concentration (<0.1M)<br>• Biological fluids<br>• Solution-phase synthesis reactions |

**II.8 Description of the analytical instrumentation**

The separation occurs in the gas chromatographic column (capillary here was used) when vaporized analytes are carried through by the inert heated mobile phase (so-called carrier gas, helium was used). The driven force for the separation is the distinguishable interactions of analytes with the stationary phase (semi-liquid thin layer coating on the inner wall of the column or solid sorbent packed in the column) and the mobile phase respectively. The differences in the boiling points and other chemical properties between different molecules in a mixture separate the components while the sample travels through the length of the column.

As the separated substances emerge from the column opening, they flow further into the MS through an interface. This is followed by ionization, mass-analysis and detection of mass-to charge ratios of ions generated from each analyte by the mass spectrometer (Figure 6) [44].

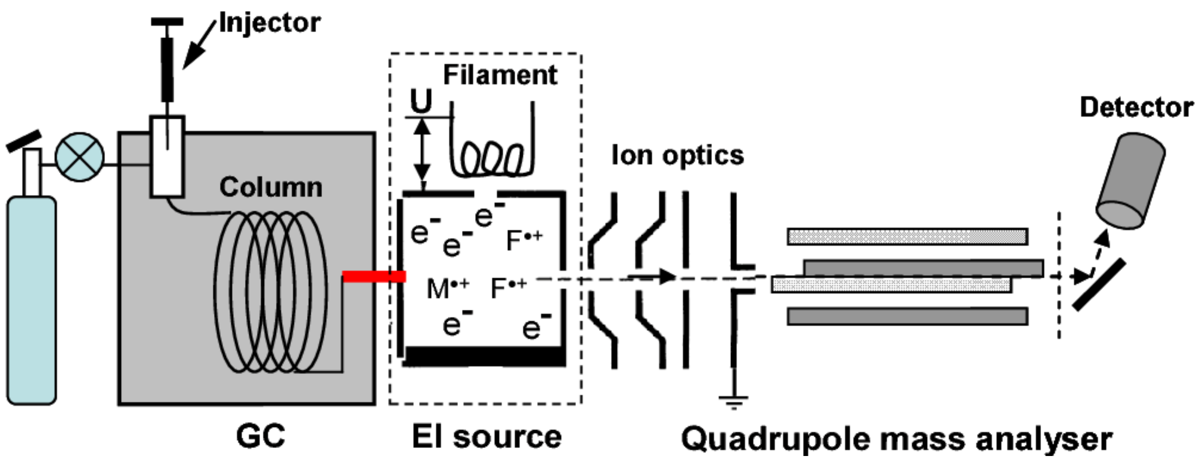


**Figure 6:** GC-MS System

The two well-accepted standard types of the ionization techniques in GC-MS are the prevalent electron impact ionization (EI) and the alternative chemical ionization (CI) in either positive or negative modes. The EI mode was used in this research. When Charged ions generated in the ion source enter the mass analyzer, the quadrupole mass analyzer is scanned sequentially such that only specified ion mass may be passed at one time. All other ions are lost. There are 2 main types of scan modes: Full scan whereby all ions in the specified mass range are allowed to go to the detector and Single Ion Monitoring (SIM)whereby only one mass is allowed and monitored to quantify a target compound (Figure 7).

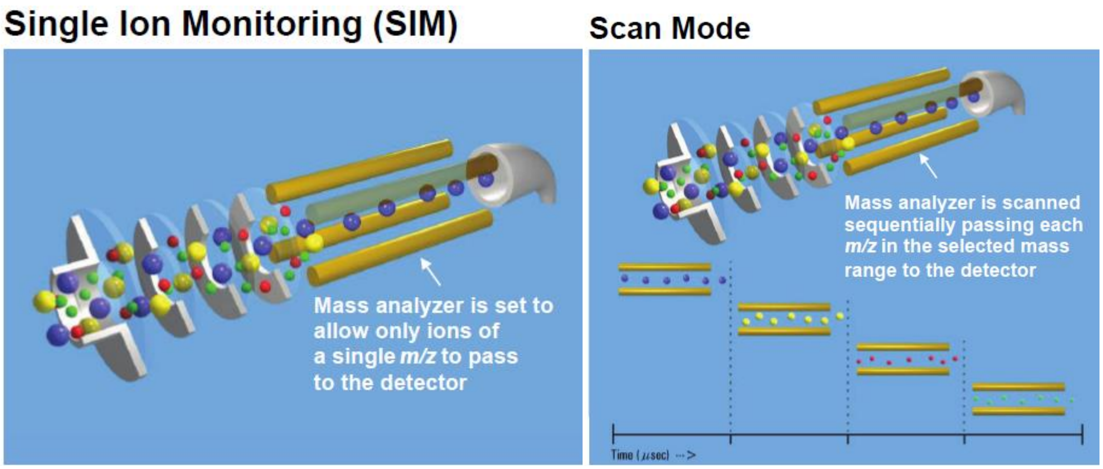


**Figure 7:** SIM Versus Full Scan

Single ion monitoring was used as a scan mode for the quantification of the target compounds [45].

### II.9 Different methods used to detect both Cypermethrin and Profenofos residues in environmental samples

A methods of analysis for the determination of Pyrethroid insecticides which include Cypermethrin in water and water sediment using Gas Chromatography/Mass Spectrometry was developed and validated by other scientists in 2009 [46]. The water samples were prepared by solid-phase extraction and water Sediment samples were extracted by microwave-assisted extraction (MAE), with SPE and HPLC-GPC cleanup of matrix interferences that occur in water sediment extracts. Quantitation for both extracts was achieved with GC/MS and GC/MS/MS [46].

An extraction and analysis of Pyrethroid insecticides in surface water, water sediments and biological tissues at environmentally relevant concentrations was performed whereby sample extracts were analyzed using dual column high resolution gas chromatography (GC) with electron capture detectors (GC/ECD). Water sediment and tissue samples were extracted by automated pressurized fluid extraction (PFE) and cleanup of extracts was accomplished using automated gel permeation (size exclusion) chromatography (GPC) [47].

A method for the determination of organophosphate pesticides in sea and surface water was successfully developed and used where an ultrasound-assisted dispersive liquid–liquid micro-extraction technique was used for sample preparation and it was coupled to GC-MS/MS which performs the identification and quantification [48].

As well a method was optimized for analysis of organophosphate pesticides in surface water. The method consisted of the dispersive liquid-liquid micro-extraction procedure followed by LC-MSMS analysis [48].

# CHAPTER III. MATERIALS AND METHOD

## III.1 Sampling Site

The sampling site was selected with respect to the objectives of the assessment. Considering the Rwandan watershed (Figure 8), Nyabarongo collects most of the river waters of Rwanda, especially when it reaches Kigali city.

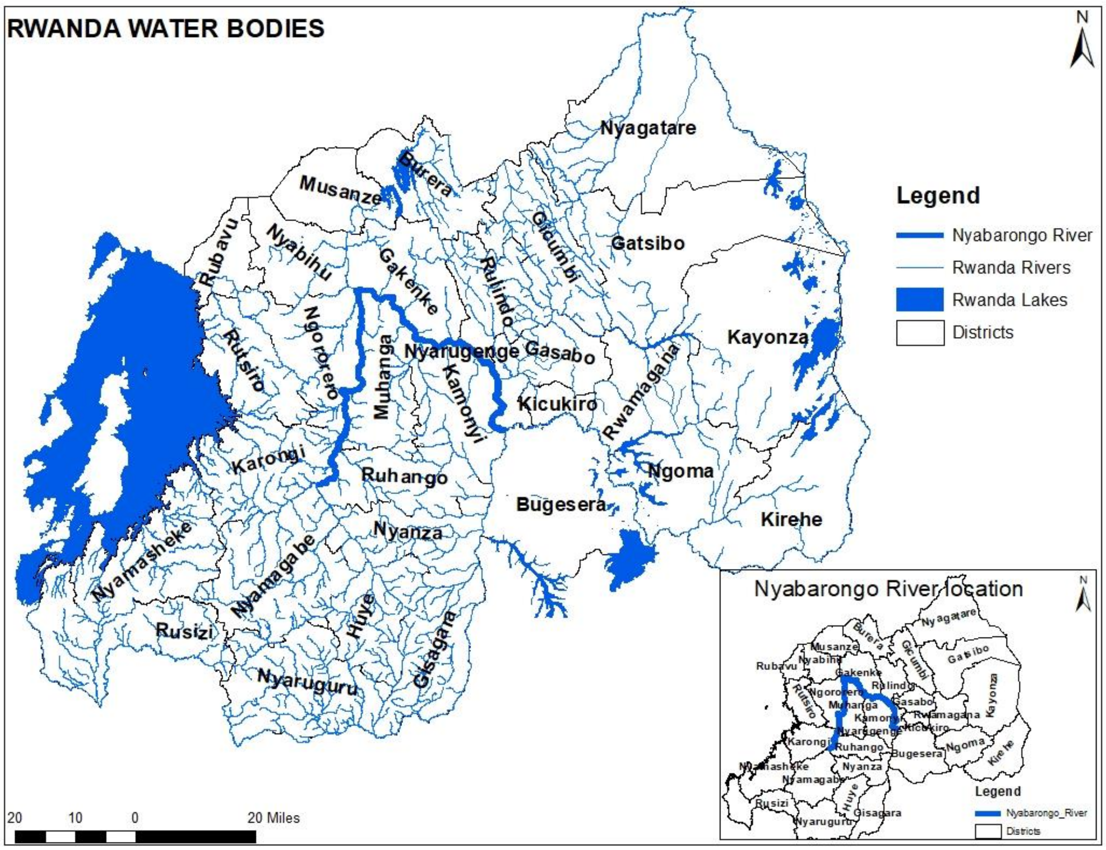


**Figure 8:** Rwanda water bodies

Samples were taken as close as possible to the areas in Kigali city and Kamonyi district where agricultural activities are more engaged and apply as much as possible pesticides to protect crops. In all these cases, samples were taken ensuring that the sampling location is suitable for taking representative samples with due regard for the objective of the sampling by taking into account drainage of water run offs around the river as well as physico-chemical properties of water body (such as temperature, pH and conductivity).

To ensure temporal and spatial variability on the sampling site, 2 parallel locations at the same cross section of the river were sampled, one on the side of Kamonyi district (Southern) and another on the side of Nyarugenge district (Kigali city). This was in order to ensure that all pesticides that are applied on both sides of the river and drained by different rivers that confluent in Nyabarongo are captured (Figure 9).

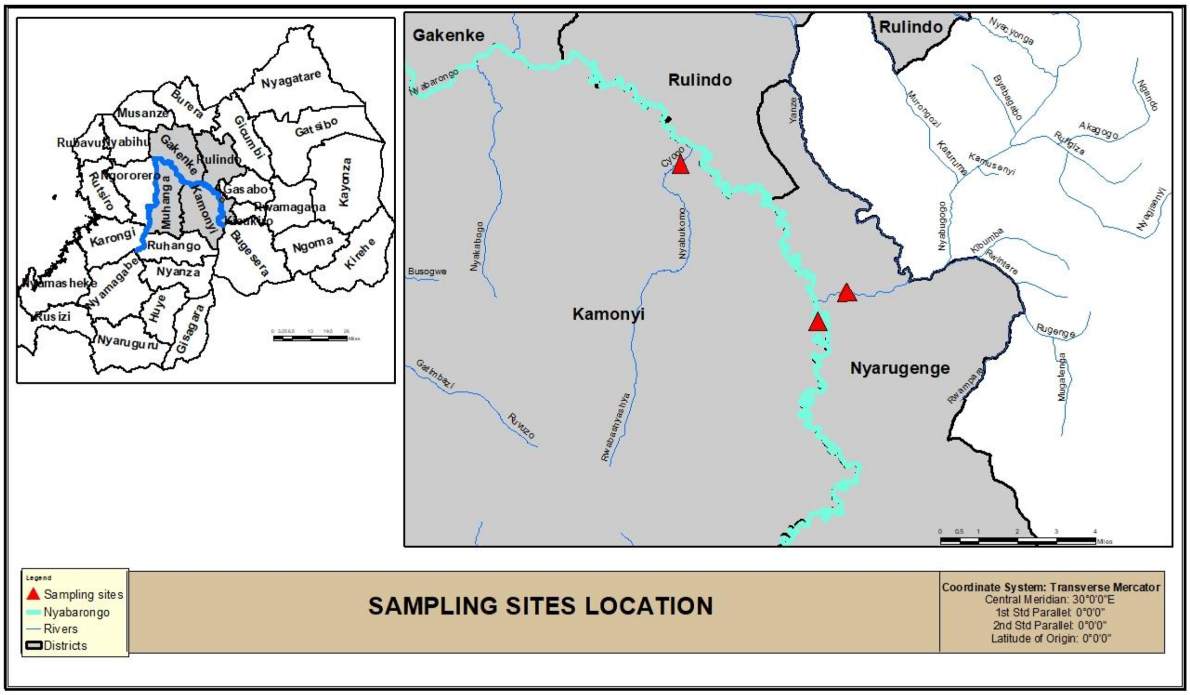


**Figure 9:** Sampling sites localization map

To evaluate contributions of Nyabarongo tributaries to overall pesticides load in surface water and water sediment, samples were also taken from 2 rivers of Cyogo and Nyabugogo (Figure 9). The sampling sites were selected randomly. These sites were chosen in a way that increases probability of detecting pesticides. Therefore, two parallel sites whereby more water sheds and rivers from various locations confluent in Nyabarongo were chosen.

### III.2 Sampling method

During this study, both surface water and water sediments were targeted for the analysis of pesticides residues. To proceed, 1 liter and 100 grams of surface waters and water sediments, respectively, were collected from Nyabarongo, Nyabugogo and Cyogo rivers. As the river stream risks not being homogeneous, on Nyabarongo river, samples were each time taken from two parallel sides. At each sampling site, 10 samples of surface waters and water sediments were taken (Figures 9 and 10).

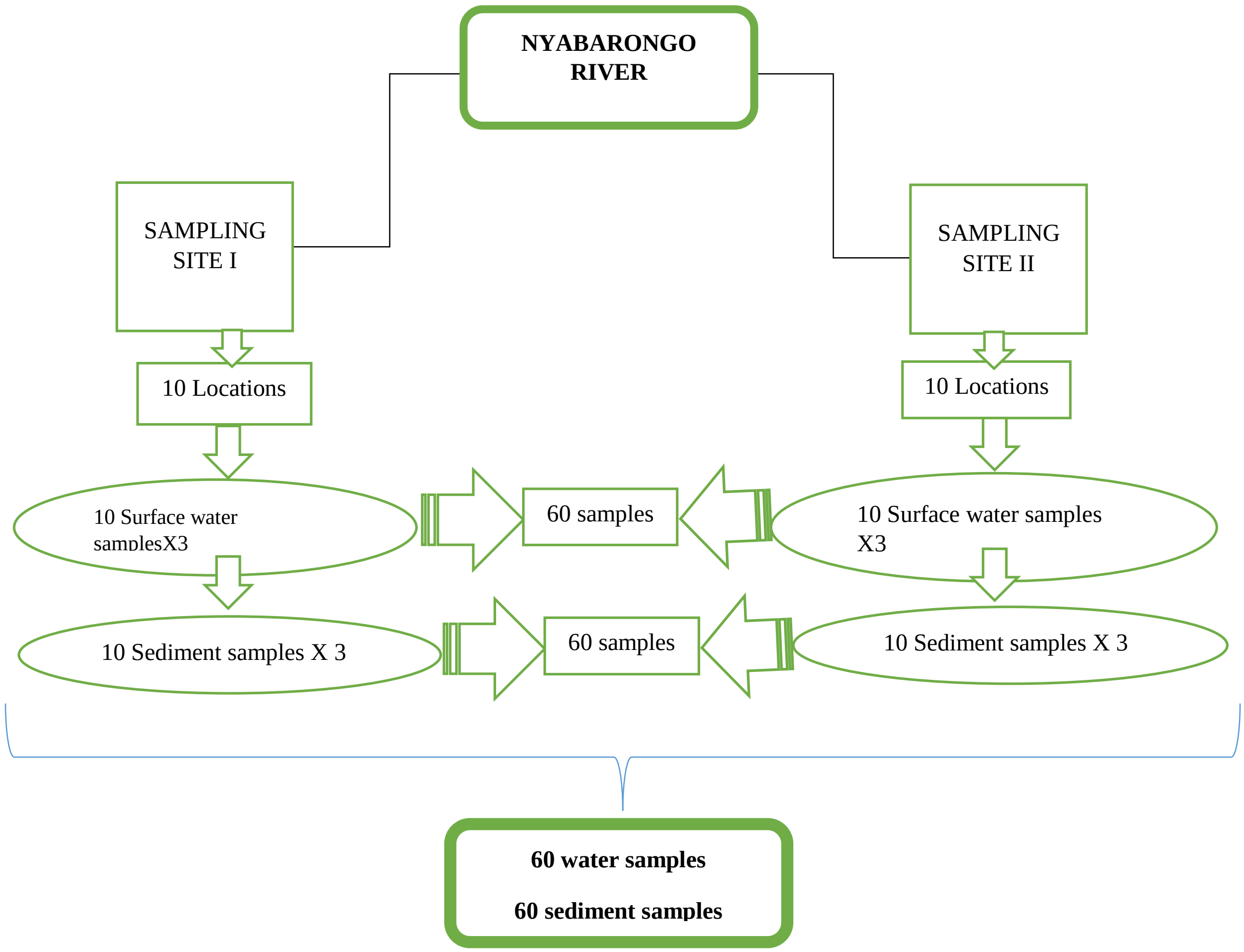


**Figure 10:** Sampling diagram

At Cyogo and Nyabugogo rivers, samples were taken from both sides at four different sites of each side and mixed to assure the homogeneity of the samples. To avoid cross contamination, surface water samples were taken before collecting water sediments. Surface waters and water sediments samples were collected in amber-glass vessels and dark glass vials, respectively. The role of amber-glass containers was to prevent possible photolysis of the pesticides [32, 33]. To avoid biodegradation of dissolved pesticides by microorganisms, samples were treated with $Hg_2Cl_2$ as biodegradation inhibitor [49]. To proceed the methodology already described by other researchers [50] was used. In 1 liter and 100 grams of water sediment samples, 0.2 $cm^3$ (200µL) of saturated mercuric chloride (or 40µL of a 50% saturated solution) were added in sample, just after sampling. Samples were then put in the dark cooler box containing ice and transported to the laboratory. The samples were then kept in a Thermo scientific fridge set at 4ºC for further analysis. The time of storage of the water and water sediment samples sample was reduced to a minimum by performing the extraction within two days from sampling date. During this work, each sample was collected in triplicate.

### III.3 Sample treatment

#### III.3.1 Extraction of Cypermethrin and Profenofos pesticides in water sample

Suspended solid matter were removed by filtration. 500 mL of water were adjusted to pH 4 with 2M $H_2SO_4$ and thereafter 10 g of NaCl was added to increase extraction efficiencies. To start extraction, the C-18 SPE cartridges were preconditioned by eluting with 3 mL of acetone-hexane (1:1 vol/vol), followed by 3 mL of methanol and finally 5 mL of 0.5% (w/vol) ascorbic acid solution (pH 4.5). The ascorbic acid was used to minimize oxidation of the analytes during the drying step. The cartridges were not allowed to dry at any point during conditioning and sample loading in order to keep the sorbent ligands active and prevent air from trapping in the cartridges. Then, the sample which was previously adjusted to pH 4, was passed through the cartridges using a vacuum pump with gentle vacuum (2–3 drops/s, which equals 3–6 ml/min). Samples were then dried by pump for an additional 30 min after the samples were extracted. The sample containers then were rinsed with 5 mL of acetone-hexane and this rinse was subsequently used as eluant. The analytes were eluted from the SPEs with an additional 5 ml of acetone-hexane (1:1 vol/vol) and 5 ml of dichloromethane without vacuum. The resulting extract was reduced to 1.0 ml under a stream of nitrogen, while being heated at 50°C into a sample concentrator (Techne). Finally, Samples were then kept in dark vials for further analysis [51] (Figure 11).

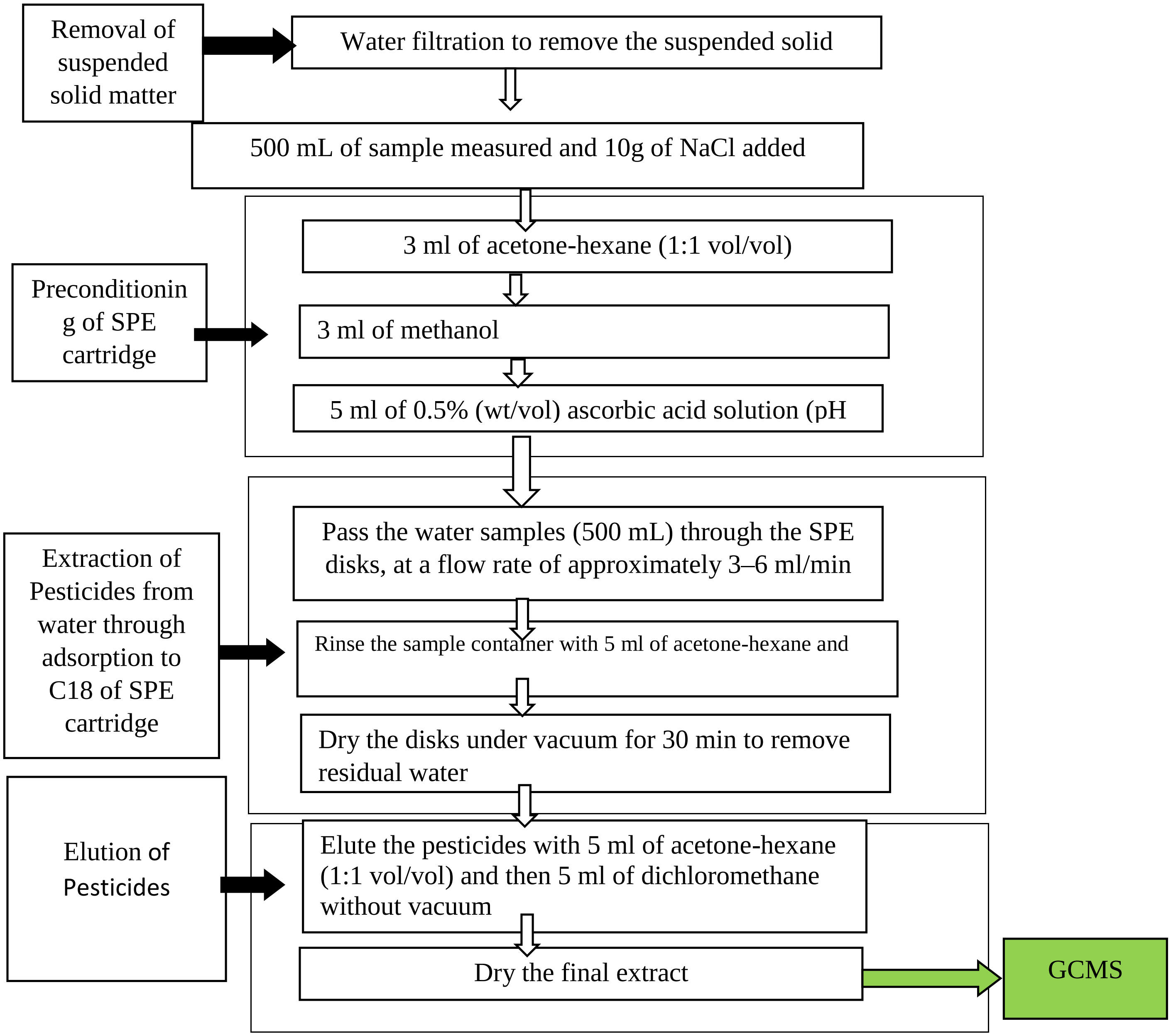


**Figure 11:** Water sample treatment flow chart

### III.3.2 Extraction of Cypermethrin and Profenofos pesticides in water sediments samples

Approximately, 3 g of dried water sediment per sample was ground with anhydrous $MgSO_4$ 1:1 w/w, and transferred to extraction tubes (SPE cartridge bond elut-PPL from Agilent technologies). Samples were extracted using a 10 mL mixture of dichloromethane, acetone, ethyl acetate, and cyclohexane (2:1:1:1 vol/vol/vol/vol) and sonicating using a SONICATOR (SONOREX DIGITAL), for one minute with 3-s pulses. Samples were subsequently vortexed for 5 min and then centrifuged at 495 g (approximately 2102 rpm) for 5 min to separate the extract from the pellet. This procedure was repeated twice more without vortexing, each time with an additional 10 mL of extraction solvent system. The extracts were then combined, evaporated under a nitrogen stream, using peak scientific nitrogen generator and sample concentrator (Techne), to dryness. They were re-dissolved in 1 mL of n-hexane, and kept in amber vials for further analysis using gas chromatography coupled with mass spectrometry (Figure 12).

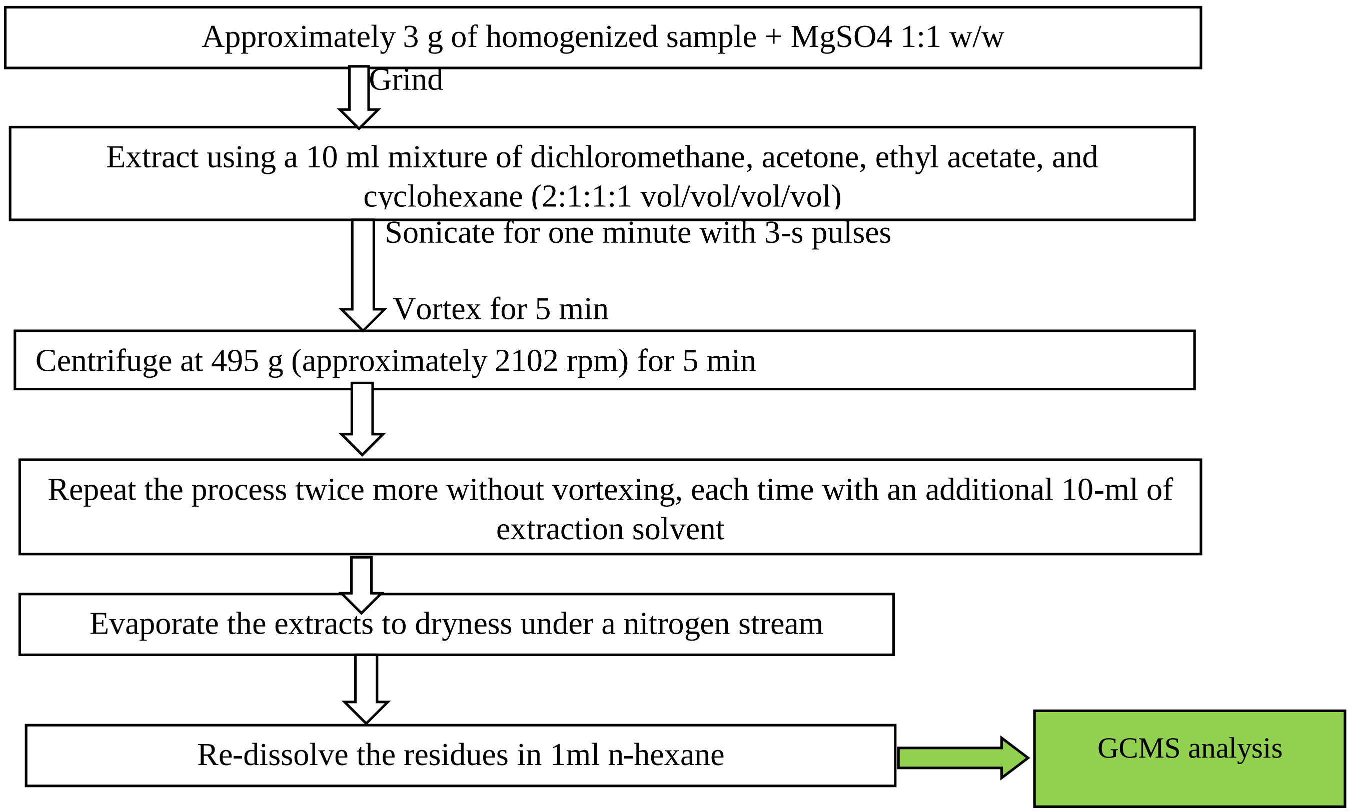


**Figure 12:** Water sediments sample treatment flow chart

### III.4 Chemical analyses of Cypermethrin and Profenofos residues

#### III.4.1 Instrumentation and calibration

Gas Chromatography coupled with Tandem Mass spectrometer with Electron impact ionization (EI), Agilent GC 7890A and MS 7000 Triple quad with 7693 Autosampler was used in Selected Ion Monitoring (SIM) scan mode with Mass hunter software. To start, the equipment was calibrated using standard solutions of Cypermethrin and Profenofos both from Restek whereby different levels were prepared as described in section IV.1. The GCMS instrument was optimized to conditions as described below (Table 5).

**Table 5:** GC and MS conditions

| Compound | MS conditions | | | GC conditions |
|---|---|---|---|---|
| | **Characteristic ion** | **Ionization mode** | **Ionization energy** | |
| Cypermethrin | 181.1 | EI | **70 ev** | Injection volume: 1 mL |
| Profenofos | 337.1 | EI | **70 ev** | Injection mode: Splitless<br>Carrier gas flow: 1.2 mL/min<br>Flow mode: constant flow<br>Inlet temp: 300 °C<br>Initial Temp: 50$^0$C Initial Hold: 1.5 min<br>Ramp 1: 30$^0$C / min to 180$^0$C, hold for 0 min<br>Ramp 2: 10$^0$C / min to 2800C, hold for 0min<br>Ramp 3: 20$^0$C / min to 300$^0$C, hold for 0min |

To standardize our equipment, ten different concentrations of the standards for Cypermethrin which were 30, 40, 50, 60, 70, 90, 100, 300, 400, and 500 µg/L and eleven for Profenofos 10, 20, 30, 40, 50, 60, 100, 200, 300, 400, and 500 µg/L were prepared from a commercial reference standards of 100mg/L, purchased from Chemlab and were analyzed by GC-MS.

### III.4.2 Validation of the analytical method

To ensure the fitness of the analytical methods and that it is consistent with what the application requires, different performance characteristics such as linearity, accuracy and precision were extensively evaluated.

The linearity, which is then method ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [52], was studied by using different standard concentrations levels. Nine concertation levels were used for Cypermethrin (Figure 13) and 11 concentration levels for Profenofos (Figure 14) were used. The obtained coefficient ($R^2$) as a results of correlations of the found concentration versus the expected concentration was used to evaluate the method linearity.

To ensure the closeness of agreement between the true value and the obtained results, the accuracy parameter of the study methods was evaluated through recovery analysis and compared to the acceptance criteria (Table 6). Concentrations of 60 µg/L and 50 µg/L for Cypermethrin or Profenofos, respectively were spiked in sample matrix and the results of both non-spiked and spiked samples were used to calculate the percent recoveries of these pesticides whereby:

$$\% \ \mathbf{recovery} = \frac{(\textbf{concentration of spiked sample} - \textbf{concentration of non spiked sample}) * \mathbf{100}}{\textbf{\textit{Spiked ammount}}}$$ **(1)** [53].

**Table 6:** Recoveries acceptance criteria [54]

| **Concentration** | **Recovery [%]** |
|---|---|
| ≤ 0.01 ppm (≤10 ppb) | 40-120 |
| 0.1-0.01 ppm (100-10 ppb) | 60-110 |
| ≥ 0.1 ppm (≥ 100 ppb) | 80-110 |

To evaluate the closeness of agreement (or degree of scatter) between a series of measurements obtained from replicates of the same homogeneous sample, the precision under repeatability conditions was studied through the estimation of the Relative Standard Deviation (RSD) of the results obtained by duplicate testing of each of the analyzed sample. The minimum and maximum achievable precision was then estimated and compared to the acceptance criteria (Table 7).

$$\mathrm{RSD} = \frac{(\textbf{Standard Deviation}) * \mathbf{100}}{\textbf{Mean}}$$ **(2)** [53].

**Table 7:** Acceptance criteria for precision [54]

| **Analyte concentration** | **RSD [%]** |
|---|---|
| 100 ppm | 8 |
| 10 ppm | 11.3 |
| 1 ppm | 16.0 |
| 100 ppb | 22.6 |

To evaluate the lowest concentration of the analyte that can be reliably detected and quantified, the method limit of detection and Limit of Quantification (LOQ) were estimated by using the technique of blank analysis as well as signal to noise ratio. For blank analysis approach, a series of 9 replicates of blank were analyzed and the results were used to estimate LOD and LOQ for Profenofos as follows:

LOD= Mean + 3SD **(3)** and LOQ= Mean+10SD **(4)** [53]

Signal to noise ratio is also a technique used for LOD and LOQ The signal to noise ratio approach was used for Cypermethrin. Different standard dilutions were injected. The resulting ratio from comparison of measured signals of standard dilutions with known low concentrations of analyte with those of blank sample was used to estimate the LOD and LOQ of Cypermethrin [52]

LOD corresponds to concentration whose S/N ratio is 3:1

LOQ corresponds to concentration whose S/N ratio is 10:1

### III.4.3 Quantification of Profenofos and Cypermethrin residues levels in surface water and water sediment using GC/MS

Samples from sections III.3.1 and III.3.2 were collected in amber vials put on GC/MS described in section III.4.1 with conditions as stated in Table 5. The mass hunter software was used to operate the equipment and data processing. This has started from data acquisition, qualitative analysis of obtained data and quantification of the detected Cypermethrin and Profenofos pesticides. The results of analysis are presented below in Chapter IV.

## CHAPTER IV RESULTS AND DISCUSSION

### IV.1 Linearity, Accuracy, LOD and LOQ of the method used

To evaluate the method linearity, the expected different standard concentrations (section III.4.1) were plotted against the detector response to obtain calibration curves (Figure 13 and 14) usable for quantification of the detected pesticides. The obtained correlation coefficients ($R^2$) of 0.9995 and 0.9989 (Table 8) for Cypermethrin and Profenofos, respectively, were closer to 1 and this confirmed the linear response of the instrument detector to the analyte concentration as previously reported [53].

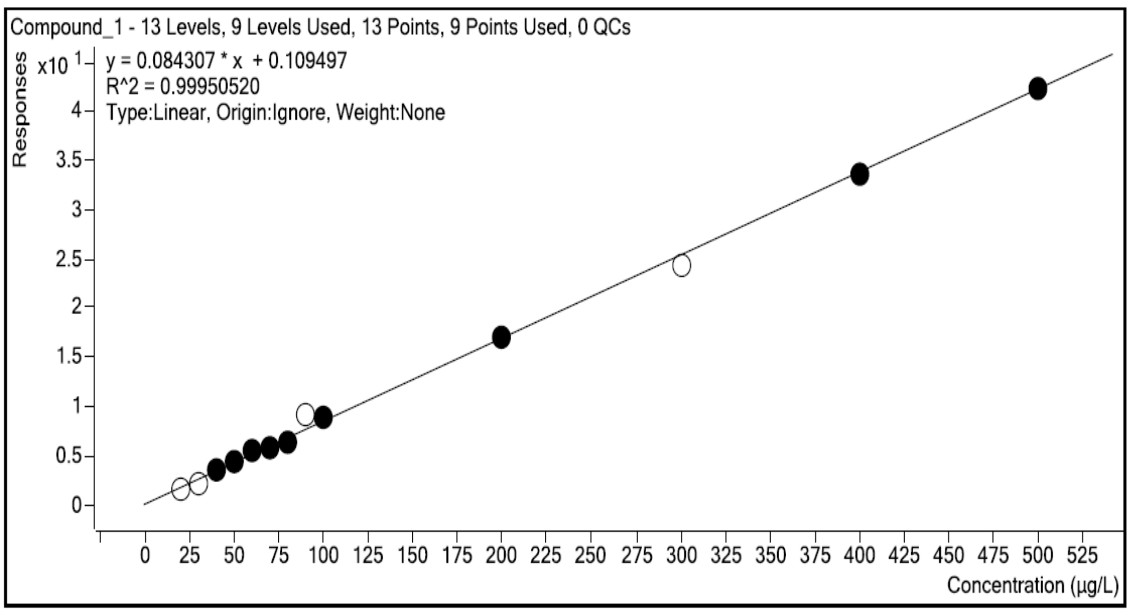


**Figure 13:** Cypermethrin Calibration curve

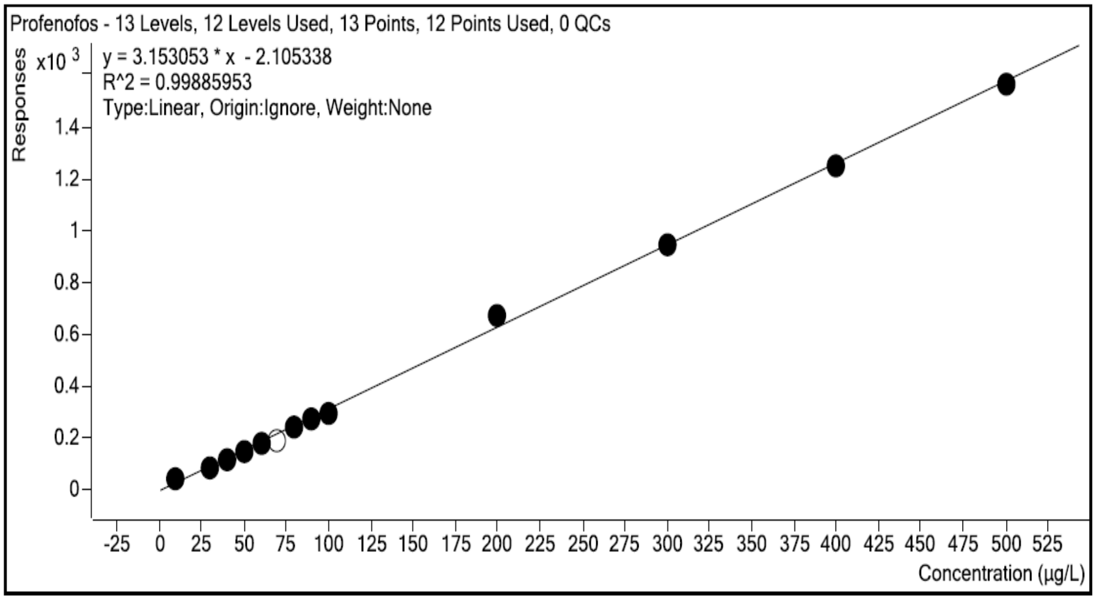


**Figure 14:** Profenofos Calibration curve

Moreover, the accuracy of the study method was evaluated through recovery analysis. Water sediment sample was spiked with 60 µg/L and 50 µg/L of the standards of Cypermethrin and Profenofos, respectively, and the observed recovery according to the formula (1) was ranged between 101.8-114.5% for Cypermethrin analysis and 88-100.4% for Profenofos (Table 8). Considering that the spiked amounts of 0.06 ppm and 0.05 ppm for Cypermethrin and Profenofos, respectively, were in the range of 0.1-0.01 ppm and according to Bratinova et al [54] the corresponding recovery range would have been 60-110%. In this study, the above described obtained data were found to be in acceptable range of recovery and this confirms the accuracy of both analytical methods.

**Table 8:** Linearity and accuracy

| **Compound** | **Linearity** | **Accuracy (% recovery)** |
|---|---|---|
| Cypermethrin | 0.9995 | 101.8-114.5 |
| Profenofos | 0.9989 | 88-100.4 |

Furthermore, the evaluation of the signal to noise ratio for Cypermethrin analysis, was used to study the limit of detection (LOD) and limit of quantification (LOQ). The software masshunter was used to calculate the signal to noise ratio (S/N) of different standards levels starting from 10 µg/L and at 20 µg/L equivalent to 0.13 mg of Cypermethrin per kg of water sediment a S/N ration of 3:1 was obtained corresponding to its LOD. Also at the standard concentration of 40 µg/L equivalent to 0.27 mg of Cypermethrin per kg of water sediment S/N ration of 10:1 was obtained corresponding to its LOQ (Table 9). Beside this, blank analysis approach was used for Profenofos. Both the mean of 9 blank samples results (23.02) and its standard deviation (1.89), were used to calculate LOD and LOQ according to the formula number (3) and (4) respectively. Thus, the estimated LOD was found to be 28.7 µg/L equivalent to 0.19 mg of Profenofos per kg of water sediment, while LOQ was found to be 41.9 µg/L equivalent to 0.28 mg of Profenofos per kg of water sediment (Table 9).

**Table 9:** Limit of Detection (LOD) and Limit of Quantification (LOQ)

| **Compound** | **LOD (**µg/L) | **LOQ (**µg/L) |
|---|---|---|
| Cypermethrin | 20.0 | 40.0 |
| Profenofos | 28.7 | 41.9 |

The optimized conditions used for GC/MS (Table 5) standard analysis enabled to obtain peaks of the chromatograms with retention times of 11.92 and 8.24 minutes for Cypermethrin and Profenofos, respectively. The repeatability and correlation of the obtained peaks (Figures 15 and 16) confirmed the good conditions chosen to be used in this study and thus, the quality of the method used. In addition, the repeatability of the obtained results between duplicates was also assessed and the relative standard deviation (RSD), calculated according to the formula number (2), of each analyzed sample was found to be within 1.84 - 5.25% and 1.63 - 5.17% Cypermethrin and Profenofos, respectively. This showed good precision of the analysis of both pesticides as it is even acceptable that the precision (RSD) reaches 8% for concentrations that are greater or equal to 100 ppm, 11.3% for 10 ppm, 16.0% 1 ppm and 22.6% for 100 ppb (Table 7)**.**

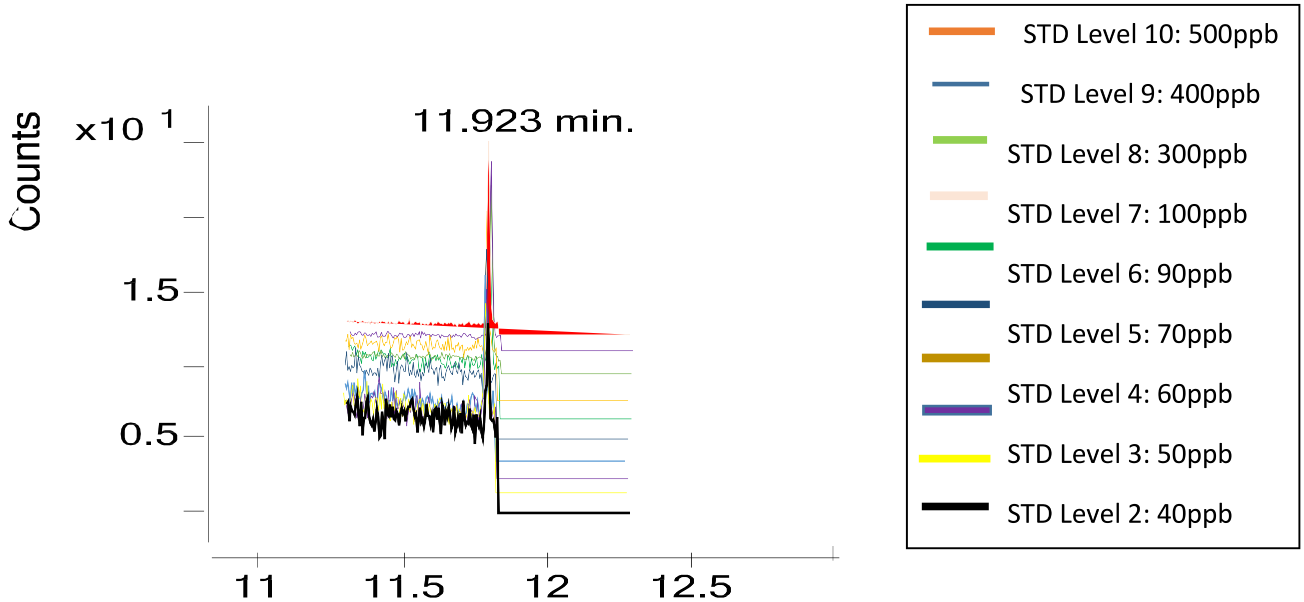


**Figure 15:** Cypermethrin Chromatograms for GCMS Calibration

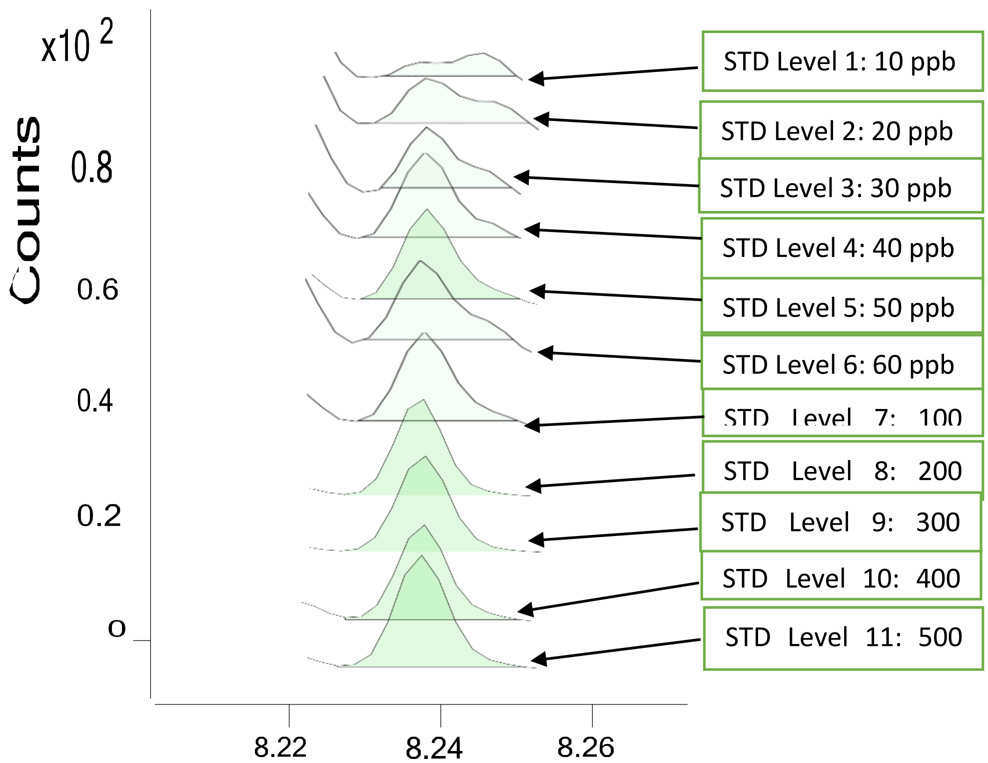


**Figure 16:** Profenofos Chromatograms for GCMS Calibration

## IV.2 Presence of Cypermethrin and Profenofos residues in surface water from Nyabarongo river and their effluents Nyabugogo and Cyogo rivers.

### IV.2.1 Level of Cypermethrin and Profenofos in Nyabarongo water

For both Cypermethrin and Profenofos, none of them was present at a concentration high enough to be quantified in Nyabarongo. Concentration of these two pesticides was found to be below detected limits which were discussed above in section IV.1. This may be due to the fact that, like other pesticides, Cypermethrin and Profenofos bind strongly to the soil [25, 55, 57] and tend to be more likely found in sediments. In addition, due to different waters received by Nyabarongo, concentration of these two chemicals may be diluted and become undetectable. Moreover, both chemicals fairly degrade mostly when they are present in water. Therefore, their presence in water was not at detectable level. Although these pesticides were not detected in water of Nyabarongo, they were however detected in waters of its tributaries of Cyogo and Nyabugogo. This may be due to the fact that water for these rivers are much closer to farms where pesticides were used. In this situation, a part of these chemicals may be not adsorbed to the soil and clay particles, directly reached waters and thus increased their concentrations in surrounding surface waters. In addition, during application pesticide, wind drift, water runoff and leaching may be a factors to accelerate contamination of water by these chemical active ingredients [17]. When pesticides reach the river they are adsorbed to soil and clay particles and then gradually deposited to water sediment. According to previous work [57], such pesticides may be identifiable in water when samples are collected immediately after the application and before their half-lives from the time of application. This may explain the presence of both pesticides in waters of Cyogo and Nyabugogo rivers, tributaries of Nyabarongo river.

**Table 10:** Level of Cypermethrin and Profenofos in water samples from Cyogo and Nyabugogo river

| Sample ID | Reportable results(µ/L) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cypermethrin in Cyogo | | | | Profenofos in Cyogo | | | | Cypermethrin in Nyabugogo | | | | Profenofos in Nyabugogo | | | |
| | **R. 1** | **R.2** | **Mean** | **SD%** | **R.1** | **R.2** | **Mean** | **SD%** | **R.1** | **R.2** | **Mean** | **SD%** | **R.1** | **R.2** | **Mean** | **SD%** |
| Water S.1 | 52.36 | 48.13 | 50.24 | 4.21 | 91.42 | 87.08 | 89.25 | 2.43 | 18.14 | 17.02 | 17.58 | 3.19 | 36.2 | 32.64 | 34.42 | 5.17 |
| Water S.2 | 32.34 | 30.11 | 31.23 | 3.58 | 73.06 | 70.71 | 71.88 | 1.63 | 27.46 | 26.34 | 26.9 | 2.09 | 25.17 | 23.61 | 24.39 | 3.20 |
| Water S.3 | 24.48 | 22.24 | 23.36 | 4.78 | 61.66 | 56.32 | 58.99 | 4.53 | 21.38 | 19.36 | 20.37 | 4.97 | 36.52 | 34.96 | 35.74 | 2.18 |
| Water S.4 | 30.25 | 29.02 | 29.63 | 2.08 | 83.24 | 77.9 | 80.57 | 3.32 | 25.68 | 24.56 | 25.12 | 2.24 | 42.1 | 39.54 | 40.82 | 3.14 |

*With: Water s. : water sample; R.: replicate; SD: standard deviation*

Results from the 4 samples collected in Cyogo river showed the presence of Cypermethrin in the range of 24.48-52.36 µ/L and Profenofos ranged between 58.99-89.25 µ/L (Table 10). This may be due to higher application rate of rocket pesticide with the two active ingredients, Profenofos and Cypermethrin, which is used at the rate of 54.9% comparatively to other pesticides applied in the district of Kamonyi in seasonal agricultural report of 2021 [9].

Besides, four water samples collected from Nyabugogo river have also showed the presence of Cypermethrin and Profenofos respectively in the range of 20.37-25.12 µg/L and 24.39 - 40.82 µg/L (Table 10). Their presence may be due to the application of rocket pesticides in agricultural activities of vegetables mainly in its part of Gatsata.

Though rocket was found to be the most applied formulation, it is however likely to note that it is sold in more than 6 different types of trade names, Rocket, Roket, Rokatt, Jacket, Profexsuper and Loket [55]. The higher concentration range of Profenofos when compared to Cypermethrin may be due to higher proportion of Profenofos in rocket pesticide applied [8].

The Cyogo river passes through valley which is dominated by agricultural activities predominantly of vegetables such as eggplant, cabbage, onions and tomatoes cultivated in agricultural season C (starting from June to September) which alternate with maize cultivated in Season A (starting from mid-September to mid-December) [56] the time when samples were collected. In addition, this valley communicates with another valley of Nyabuvomo and also surrounded by valley of Mugera valley, which are dominated by the use of these pesticides to protect either vegetables, maize in seasons A and C. The higher proportion of Profenofos in the applied pesticide formulation may explain why Profenofos was detected in higher level than Cypermethrin [8].

In this study, Cyogo river was found more contaminated than Nyabugogo river. The higher proportions were observed from samples collected from Cyogo at the maximum concentrations of 52.36 µg/L and 89.25 µg/L, against the maximums of 25.12 µg/L and 40.82 µg/L in Nyabugogo river, for Cypermethrin and Profenofos, respectivelly. This may be due to observed higher farming activities in Cyogo valley and its surroundings than Nyabugogo valley which is located crossing the city where farming activities are not extensive. The presence of these pesticides in waters of both Cyogo and Nyabugogo indicated their contribution to pesticide pollution in Nyabarongo river.

### IV.2.2 Presence of Cypermethrin and Profenofos residues in water sediments from Nyabarongo river.

Water sediments from Nyabarongo river have shown the presence of both Cypermethrin and Profenofos at the maximum concentrations of 1.5 and 0.77 mg/kg respectively. In fact, Nyabarongo river is characterized by silt and clay particles fractions which settle gradually on its bottom to form water sediments. It is known that silt and clay particles are mostly reactive because of their higher surface area and adsorption capacity [59]. In addition, Cypermethrin and Profenofos are slightly denser than water with 1.24 and 1.46 g/mL, respectively and therefore tend to settle below water [60]. Nyabarongo water body, like other surface waters, contains many kinds of dissolved and suspended species, such as organic compounds, humic substances, metal oxides, and clay particles originating from many kinds of biota, soil, and water sediment. Suspended water sediment particles are the main components. They usually form a complex matrix and tend to exist as larger flocculated particles that settle down on the bottom of the surface water [29]. Particularly, Cypermethrin is adsorbed very strongly on soil particles, especially in soils containing large amounts of clay or organic matter [18]. These pesticides might have been adsorbed to clay and soil particles of the river and then settled down in the water sediments and the remaining residues in surface water may have been degraded by photodegradation and transformed into other particles. This can explain the reason why both pesticides studied were detected in water sediments and not detected in surface water of Nyabarongo river (Table11).

**Table 11:** Level of Cypermethrin and Profenofos in water Sediments of Nyabarongo

| Sample ID | Reportable results (mg/kg) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cypermethrin at Kam.side | | | | Cypermethrin at Nyar. side | | | | Profenofos at Kam. side | | | | Profenofos at Nyar. side | | | |
| | R.1 | R.2 | Mean | SD% | R.1 | R. 2 | Mean | SD% | R.1 | R.2 | Mean | SD% | R.1 | R.2 | Mean | SD% |
| Sediment S.1 | 0.89 | 0.82 | 0.86 | 3.88 | 0.25 | 0.23 | 0.24 | 4.92 | 0.14 | 0.13 | 0.13 | 2.8 | 0.18 | 0.17 | 0.17 | 2.15 |
| Sediment S. 2 | 0.47 | 0.43 | 0.45 | 3.71 | 0.19 | 0.18 | 0.19 | 4.57 | 0.08 | 0.07 | 0.07 | 5.04 | 0.1 | 0.09 | 0.1 | 3.89 |
| Sediment S.3 | 0.51 | 0.47 | 0.49 | 4.14 | 0.85 | 0.8 | 0.83 | 3.37 | 0.67 | 0.64 | 0.65 | 2.07 | 0.39 | 0.36 | 0.37 | 3.62 |
| Sediment S.4 | 0.98 | 0.92 | 0.95 | 3.51 | 0.26 | 0.23 | 0.25 | 4.8 | 0.17 | 0.16 | 0.17 | 4.24 | 0.15 | 0.14 | 0.14 | 2.56 |
| Sediment S.5 | 0.8 | 0.74 | 0.77 | 4.34 | 0.6 | 0.54 | 0.57 | 4.99 | 0.32 | 0.3 | 0.31 | 4.4 | 0.2 | 0.19 | 0.2 | 1.82 |
| Sediment S.6 | 1.53 | 1.46 | 1.5 | 2.21 | 0.99 | 0.93 | 0.96 | 2.94 | 0.28 | 0.26 | 0.27 | 3.86 | 0.29 | 0.29 | 0.29 | 1.26 |
| Sediment S.7 | 1.84 | 1.77 | 1.8 | 1.84 | 0.87 | 0.81 | 0.84 | 3.36 | 0.78 | 0.75 | 0.77 | 1.74 | 0.23 | 0.23 | 0.23 | 1.58 |
| Sediment S.8 | 0.54 | 0.5 | 0.52 | 4.55 | 1.39 | 1.33 | 1.36 | 2.06 | 0.63 | 0.6 | 0.61 | 2.21 | 0.58 | 0.54 | 0.56 | 3.63 |
| Sediment S.9 | 0.9 | 0.83 | 0.86 | 3.88 | 1.38 | 1.33 | 1.35 | 2.06 | 0.46 | 0.43 | 0.45 | 3.06 | 0.46 | 0.42 | 0.44 | 3.84 |
| Sediment S.10 | 1.47 | 1.4 | 1.44 | 2.32 | 0.88 | 0.82 | 0.85 | 3.32 | 0.42 | 0.39 | 0.4 | 3.38 | 0.3 | 0.29 | 0.29 | 1.25 |

*Sediment S.: Sediment sample; Kam.: Kamonyi; Nyar.: Nyarugenge; R.: replicate; SD: standard deviation*

According to the results obtained, Cypermethrin and Profenofos were detected at higher level in water sediments of Nyabarongo (Table 11) and might be from a gradual deposition of soil and clay particles from various agricultural areas where Nyabarongo passes through. Tributary rivers like Cyogo and Nyabugogo and probably others which were not covered in this study and confluent in Nyabarongo like Mwogo, Rukarara, Nyarubugoyi, Mbirurume, Mashyiga, Kiryango, Munzanga, Miguramo and Satinsyi [61] may largely contribute to this remarked pollution. In fact all these cited rivers are passing through valleys in which they are intense agriculture activities applying pesticides, including rocket as protective means of their crops [8]. Again in this study, was detected in higher amount than Profenofos. It was detected at concentrations of 0.45-1.8 mg/kg and 0.19-1.36 mg/kg against 0.07-0.77mg/kg and 0.1-0.56 mg/kg of Profenofos (Table 11) for sites of Kamonyi and Nyarugenge, respectively. The Strategic Stock of pesticides 2018/2019 fiscal year, around 2,200 litres of pesticides including Cypermethrin 5% EC and Profenofos 40% EC among others [8]. Particularly, the 2019 NISR report has shown that in 2019, Cypermethrin was used at the rate of 21% while in 2017, it was used at the rate of 47.4% [9]. These high percentages of use, adding to their relative stability character may explain why both pesticides were detected in this study in water sediments [9]. The values of Cypermethrin residues obtained in this study, in Nyabarongo river, was a bit higher than what observed in surface water and water sediments of Ebro River Delta, Guating reservoir and in the Mekong Delta, Vietnam [62, 64]. This indicated the intensive application of this pesticide in Rwanda.

Though there was no significant variation both considered sampling site sides. The maximum detection was 1.8 and 0.77 mg/kg for the site of Kamonyi side and 1.36 and 0.56 mg/kg for the site of Nyarugenge side (Table 11), respectively for Cypermethrin and Profenofos. A slight difference from Kamonyi versus Nyarugenge site may be due to the abundance of farm lands around Kamonyi site and to the sharp slopes toward this part of the river favoring the easy entrance of pesticides and other chemical materials in that region of the river. Moreover, the lowest concentration on the other side of Nyarugenge may be related to slow current toward this part hindering the rapid accumulation of these pesticides, but leading to their degradation before reaching the site [65].

Due to its instability, the detection of the Profenofos residues in either samples may be implied to its recent application in the agricultural fields located in the vicinity of the study areas [66, 67].

These findings are similar to those observed in a study conducted in tomato fields of Owiro Estate in Tanzania, where the detection of organophosphorus was attributed to its recent use [67].

# CHAPTER V: CONCLUSION AND RECOMMENDATIONS

## V.1 Conclusion

The present study aimed at assessing pesticide residues in Nyabarongo river water and water sediments. It has extensively evaluated the concentration level of Cypermethrin and Profenofos. Samples were collected at well demarked sampling units, preserved, prepared and analysed as per validated method. The results of analysis have shown no evidence of studied pesticides residues in waters of Nyabarongo river but residues of these pesticides were detected in the water sediments above the level of detection. The presence of these pesticides in Nyabarongo was confirmed by their detection in 2 sampled Nyabarongo tributaries of Cyogo and Nyabugogo rivers. Based on these results, we can conclude that the application of pesticides in Rwanda contribute to water pollution and this may affect human's health and aquatic organisms' normal life.

## V.2 Recommendations

- ✓ Agriculture is the main economic activity in Rwanda with 70% of the population engaged in the sector, more awareness on safe use of pesticides is required for pesticides applicators.
- ✓ Research on a regular basis is required so as to assess the level of contamination of water and other environmental media such as soil, water sediments and air.
- ✓ Import of pesticides in the country should be strengthened through an enforced registration scheme.
- ✓ Category II pesticides (to which Cypermethrin and Profenofos belong) are toxic, if an alternative can be found to replace them it would be adopted or clear caution procedures for their application to the end users should be established.

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