Method development and application: Solid phase extraction (SPE), Ultrasonic extraction (UE) and Soxhlet extraction (SE) for the analysis of pesticides in water, soil, sediment and sludge

UNIVERSITY OF KWAZULU - NATAL

INYUVESI YAKWAZULU-NATALI

Philisiwe Nganaki Kunene

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Declaration

I, Philisiwe Nganaki Kunene, hereby declare that apart from the help from my supervisor, the research reported in this dissertation is my own work. It has never been submitted for examination in any university and degree apart from where it is being submitted at the University of KwaZulu-Natal for the degree of Master of Science.

----------(signature of the candidate)

----------(Supervisor)

School of Chemistry and Physics, University of KwaZulu-Natal, Pietermaritzburg, 2019

Abstract

Ultrasonic extraction (UE), Soxhlet extraction (SE) and solid phase extraction (SPE) have been developed and applied for the simultaneous determination of the five most commonly used triazine pesticides. The extraction parameters that affect the recovery of the analytes for SPE, SE and UE methods were optimized before the application of the methods. The SPE optimized parameters were conditioning solvent and sample volume. The UE optimized parameters were: extraction solvent, the volume of extraction solvent and extraction time. The SE optimized parameters were extraction solvent and sample wetting. The analyses were conducted using a high-performance liquid chromatography-diode array detector (HPLC-DAD) which was also optimized to improve the limit of quantification and detection.

The methods validation was performed using the mixture of triazine pesticides spiked distilled water and soil samples. The recoveries obtained were 107 - 111 %, 75 - 100% and 71 – 87% for SPE, UE, and SE respectively. The limits of detection (LOD) and limits of quantification (LOQ) obtained ranged between 0.67 –1.2 µg/L and 2.0 – 3.5 µg/L for SPE respectively. For UE, they ranged from 1.0-2.0 µg/kg and 3.2 – 6.1 µg/kg and for SE, they ranged from 0.092-0.22 µg/kg and 0.28 – 0.69 µg/kg respectively. A good precision with a relative standard (RSD) less than 20% in all compounds was achieved for all methods.

The developed and validated methods were then applied to river water, wastewater, sludge, soil and sediment samples from around KwaZulu-Natal. The concentrations obtained were 3.0 - 65 µg/L in river water, 2.5 - 49 µg/L in wastewater, 8.4 -2820 µg/kg in liquid sludge, 17 - 1017 µg/kg in soil and 1.1 – 123 g /kg in sediment samples. The most dominant triazine was found to be simazine. In Gilboa Farm soil samples, simazine was found to be above the Maximum Residual Limits (MRLs). In Darvill sludge samples, simazine, atrazine, and ametryn were above MRLs. In Amanzimtoti wastewater samples, atrazine was above MRLs. In Bishopstowe river water samples, simazine was above MRLs.

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Psalms 107:1 "Give thanks to the LORD, for he is good; his love endures forever."

Thank You for strengthening me and for your endless love, I would have not accomplished anything in life without your mercy. You have been so good to me.

Dedication

To my superb mother (Ntombikayise Bahlalile Kunene, my father (Bhekumqondo Elijah Kunene), and the rest of the Kunene Family.

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List of abbreviation

%RSD	Percentage relative standard deviation
DO	Dissolved oxygen
d-SPE	dispersal-solid phase extraction
ECD	Electron capture detector
FI	Fluorescence
FID	Flame ionization detector
GC	Gas chromatography
GPS	Global positioning system
HF-LPME	Hollow fiber-liquid phase microextraction
HLB	Hydrophilic-lipophilic base
HPLC	High-pressure liquid chromatography
IUPAC	International Union of pure and applied chemistry
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave extraction
MRLs	Maximum residue limits
MS	Mass spectrometry
PDA	Photodiode array
SA	South Africa
SE	Soxhlet extraction
SPE	Solid phase extraction
TDS	Total dissolved solids

UE	Ultrasonic extraction
UE	Ultrasonic extraction
UKZN	University of KwaZulu-Natal
USA	United States of America
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
WWTPs	Wastewater treatment plants

Chapter 1

This chapter covers the introduction, the problem at hand that led to the aim of this project as well as the objectives followed to achieve the aim. Research questions that the project was opting to answer as well as research justification have also been covered.

1.1 Introduction

This world is packed with chemicals that contaminate air, water, soil, and food, thus cause profound changes in the quality of the environment in which human beings live. The effects of these chemicals are due to their persistence which allows them to remain for years in the environment, as well as their environmental toxicity and thus they are called persistent organic pollutants (POPs). POPs are organic chemicals containing carbon in their structure. POPs are semi-volatile compounds, which enables them to move all over the atmosphere, dispense the pollutants across the earth, and hence they can be found in places where they never generated or applied. They have low water solubility and highly soluble in fat and hence they bioaccumulate in the living organism`s fatty tissues. They are lipophilic and they biomagnify as they are transferred over the food chain, hence they have been measured in various organisms These chemicals include pesticides, polycyclic aromatic hydrocarbons, pharmaceuticals, etc. (WHO, 2010, Buccini, 2003).

Pesticides are divided into various groups, which are named after their application, these include herbicides, insecticides, fungicides, etc. The target pesticide compounds in this project were triazines such as simazine, atrazine, ametryn, propazine and terbuthylazine which fall under herbicides family. They are widely applied in agricultural (croplands) and non-agricultural sectors (playgrounds, roadsides, and railways) to control weed (Rodríguez-González *et al.*, 2014). They are also used in wastewater treatment plants and in domestic activities as they are the active ingredient (Nyoni, 2011). Pesticides can be introduced in the environment in various ways such as application during farming, manufacturing process, sanitation processes and natural sources such as volcanic eruptions, unauthorized dumping of pesticide products or their containers, accidental spillages during manufacturing and transportation and pesticides drifting and they can contaminate target and non-targets components (Adeyinka, 2014). During pesticide application 20 – 30% of pesticides drift as a result of the environmental conditions, unskilled operators, type of equipment used for the application as well as the preparation of the pesticide solutions (Nascimento *et al*., 2018).

There are more than 3000 registered pesticides in South Africa. Pesticides are applied to soil or crops and from the point where application occurs, they can be transported into the non-targeted environment including surface water and groundwater through leaching from the soil, surface, and crop run-off, volatilization, rain deposition, etc. Hence, they can affect humans, aquatic life and organisms (Damalas and Eleftherohorinos, 2011). Previous studies have indicated the presence of different pesticides in the South African environment (Quinn *et al*., 2011). In South Africa, there are limited studies that have prioritized pesticide threats to environmental and human health (Dabrowski, 2015, Ntow *et al*., 2006).

Pesticides evaluation in the environment requires their extraction from the matrix in order to allow their effective determination. Since environmental matrices are complex, the preparation step is significant. There are common extraction techniques that have been used for the extraction of triazines in water, soil, sediment and sludge including the traditional methods (Soxhlet extraction, liquid-liquid extraction, etc.) and the modern methods (ultrasonic extraction, solid phase extraction, solid phase microextraction, hollow fiber liquid phase extraction, etc.). The use of modern extraction techniques is associated with environmentally friendly solvents, low solvent consumption, and sample size without losing the sensitivity of the instrument. Also, reduced analysis time has been reported which allows for a number of analyses to be done (Nascimento *et al.*, 2018). The extraction step is then followed by the separation and detection of the analytes where the chromatographic techniques have played a major role in the analysis of pesticides. There are diverse groups of chemicals that characterize the variety of pesticides used in modern agriculture. Therefore, it is significant to select a suitable chromatographic technique, which will be able to determine as many pesticides as possible. Gas chromatography (GC) and liquid chromatography (LC) are the most widely used chromatographic techniques associated with different detectors e.g. LC with universal detectors such as such as photodiode array, UV/Vis Absorbance and for GC such as electron capture detector, flame ionization detector, etc. In this work, solid phase extraction, ultrasonic extraction, and Soxhlet extraction methods followed by liquid chromatography with photodiode array detector were developed/modified and then applied for the determination of triazine pesticides in river water, wastewater, soil, sludge and sediment samples.

1.2 Problem Statement

Pesticides are of special interest because of their toxicity and high biological activity (Quinn *et al.*, 2011). Due to their wide usage, they are widely spread in the environment. Pesticides constitute one of the most hazardous groups of contaminants. Some of these compounds are persistent in the environment and resistant to degradation, they are volatile and thus can be found in non-target places. They bio-magnify as they move through the food web and they are lipophilic, thus they bioaccumulate in fatty tissues. Due to these behaviors, they pollute the environment and also pose a potential risk to humans and other life forms. Thus, death and chronic diseases have been reported worldwide as a resulted of pesticide poisoning. In South Africa there is insufficient reliable data on levels and distribution of pesticides and the maximum residue limits (MRLs) used in this work were adopted from other countries (London *et al*., 2005, Bol'shakov *et al*., 2014). These compounds are present in low concentrations that are below detection limits of our instrument of use. Therefore, there is a need to employ sample clean-up and /or preconcentration techniques. Also, continuous analysis is important to generate more reliable data in order for the policymakers to be able to set the MRL values specific for South Africa. Therefore, in this work solid phase extraction, ultrasonic extraction and Soxhlet extraction techniques have been optimized and then applied to river water, wastewater, soil, sediment and sludge samples for the extraction of triazines. The analyses were conducted using liquid chromatography coupled with a photodiode array detector (HPLC-DAD).

1.3 Aim

To develop solid phase extraction (SPE), ultrasonic extraction (UE) and Soxhlet extraction (SE) followed by liquid chromatography coupled with photodiode array detector (HPLC-DAD) methods for the determination of pesticides in water, soil, sediments, and sludge.

1.4 Objectives

- To develop an HPLC-DAD method for the separation and detection of triazine pesticides.
- Validate the developed HPLC-DAD method for the separation and detection of triazine pesticides.

- To optimize SPE, UE and SE methods for the extraction of pesticides using spiked distilled water and soil sample in order to obtain conditions that will permit higher recoveries of all the analytes.
- To apply the optimised methods to water, sediment, soil and sludge samples for the analysis of pesticides.
- To identify and quantify the pesticides present in water, soil, sediment and sludge samples.
- To compare the extraction efficiencies for UE and SE methods.

1.5 Research questions

- What parameters can be optimised to extract pesticides at lower concentration levels in the environment sample?
- Are pesticides under study present in the chosen study areas and at what concentration levels are they present?
- Are the pesticides found in water samples associated with pesticides in sediment samples?
- Are WWTPs able to eradicate pesticides in water during the process of water treatment?
- Are pesticides concentrations in the influent water higher compared to the effluent point of WWTPs?
- Are pesticides concentrations in wastewater effluent higher compared to river water where the effluent is discharged?

1.6 Research justification

The contamination of the environment by pesticides is due to the increase in their usage in pest management and increase of food production. Obtaining applicable and reliable information on the concentrations of pesticides in the environment is important for the formulation of environmental protection policy (Akoto *et al.*, 2016). Micro pollutants are counted as the biggest problem, where the analyst is confronted with several diverse compounds occurring at trace concentrations. As a result, the necessity for reliable information on the occurrence of organic micro pollutants in the environment is the driving force for the introduction and development of the present analytical techniques and procedures.

The analytical method used to determine the organic compound in the environmental samples requires many steps including clean-up and/or pre-concentration steps, this is due to the low

concentration at which the compound exists. Also, the development of extraction techniques that are fast, cheap, consume a small amount of solvents and sample mass, give higher recoveries of the analytes, provide low limits of detection and quantification is of importance for effective determination of the compounds at low concentration levels (Kumar and Vijayan, 2014).

In South Africa there is insufficient information regarding the levels and distribution of pesticides in the environment, therefore, it is important to develop the method of extraction techniques that are supreme for the analysis of pesticides in the environment (Tadeo, 2008). Also, there are very few studies that have been done on the development and application of analytical methods for the determination of pesticides in the South African environment especially KwaZulu Natal. In this project, three methods (SPE, UE, and SE) were developed/ modified then validated and applied for the analysis of pesticides in river water, wastewater, soil, sludge and sediment samples in KwaZulu Natal (Durban and Pietermaritzburg areas).

Chapter 2

Introduction

In this section, findings by other researchers concerning pesticides in the environment are discussed. Discussed aspects include the uses, exposure pathways as well as health and environmental effects of pesticides. A review of the various sample preparation, separation, and detection techniques that have been used worldwide for the analysis of pesticides in liquid and solid samples has been highlighted.

2.1 What are pesticides?

Pesticides are compounds that are used to kill pests and prevent or reduces the damages that pests may cause (Kim *et al*., 2017). Pesticides can contaminate by touch or ingestion and can lead to death immediately or over a long period of time depending on the type of pesticides and concentration. There are different types of pesticides that were made for different purposes, including insecticides, herbicides, fungicides, rodenticides. Triazines are a class of pesticides that fall under herbicides family and they are the most commonly used pesticide compounds. Currently, there are 25 different types of triazines which are commercially available and used herbicides to control weeds or undesirable plants. Other herbicides can destroy any plant they are applied on while others are designed for selected species (Jurewicz *et al*., 2006). Their mode of action is to inhibit the photosynthetic transportation of electrons on the unwanted plants in agricultural and non-agricultural sectors (Waxman, 1998). The commonly used herbicides include simazine, atrazine, ametryn, propazine, etc.

2.1.1 Simazine

Simazine is a white crystalline powder and Its name according to IUPAC is 6-chloro-2-N,4-N-diethyl-1,3,5-triazine-2,4-diamine, with the molecular formula of $C_7H_{12}ClN_5$ (Figure 2.1) and the molecular weight of 201.66 g/mol. This compound was the first produced triazine herbicide. It was registered and sold in 1956 in the United State for noncropland to be used on Swiss railroad, corn and right of way. It was then ratified for the entire vegetation control in noncropland areas. Due to the extended facts on simazine practice, it was recognized by the United State food administration as well as the United States Department of agriculture and drug administrator to be used in corn. It was also approved as an aquatic herbicide since it was

found to be more effective in aquatic conditions and it was used to control algae in swimming pools (LeBaron, 2011). It is more effective when it is applied in summer and later in winter.

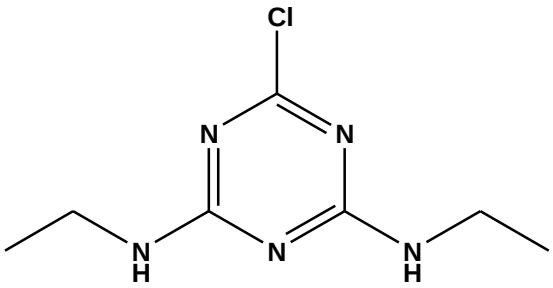

Figure 2. 1: Chemical structure of simazine

Source: (Donati and Funari, 1993)

2.1.2 Atrazine

Atrazine is an odorless white crystalline powder and its name according to IUPAC is 6-chloro-4-N-ethyl-2-N-propan-2-yl-1, 3, 5-triazine-2, 4-diamine with the chemical formula of $C_8H_{14}ClN_5$ (Figure 2.2) and the molecular weight 215.62 g/mol. Atrazine was introduced and registered later in the 1950s at the United State to be used in corn (LeBaron, 2011). Atrazine managed to make the maize growing possible and increased the number of acres in maize farming in the United State and thus improved the economy (Amadori *et al.*, 2013). It is selective for corn as it is metabolised quickly by corn via a conjugation reaction with glutathione. Its effectiveness is independent of agronomic and environmental conditions (Donati and Funari, 1993).

.

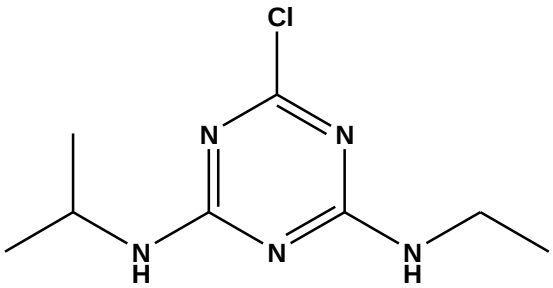

Figure 2. 2: Chemical structure of atrazine

Source: (Kaufman and Kearney, 1970)

2.1.3 Ametryn

Ametryn is a snowy powdered methythiotriazine herbicide that is slightly soluble in water and soluble in an organic solvent. Its molecular formula is $C_9H_{17}N_5S$ with the IUPAC name of 4-

N-ethyl-6-methylsulfanyl-2-N-propan-2-yl-1, 3, 5-triazine-2, 4-diamine (Figure 2.3), with a molecular weight is 227.33 g/mol. It was first introduced in the United State in 1964 and it is extensively used in sugarcane (Santos *et al.*, 2015). The poisonousness of this compound is class (III) meaning that it is moderately poisonous in both mammals and fish. However, it is more toxic to mollusks and crustaceans.

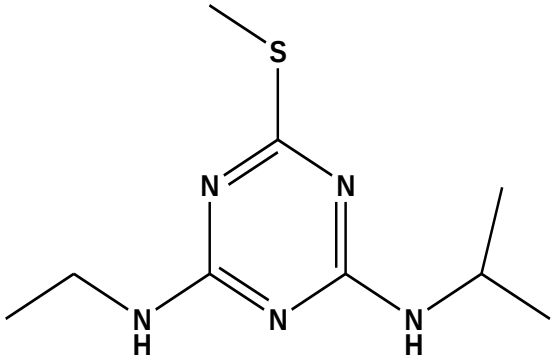

Figure 2. 3: Chemical structure of ametryn

Source: (Farré *et al*., 2002)

2.1.4 Propazine

Propazine is a chlorotriazine herbicide, which is a white powder with a putrid odor. It has low water solubility but highly soluble in organic solvents. Its name according to IUPAC is 6-chloro-2-N,4-N-di (propan-2-yl)-1,3,5-triazine-2,4-diamine with the molecular formula of $C_9H_{16}ClN_5$ (Figure 2.4) and a molecular weight of 229.71 g/mol. United State first introduced propazine known as Milogard. Its spectrum activity was found to be almost the same as that of simazine and atrazine. However, its advantage over simazine and atrazine is the acceptance by Umbelliferae species, which allows it to be used in celery and carrot. It is selective for sorghum and can be the product of choice for sorghum (LeBaron, 2011).

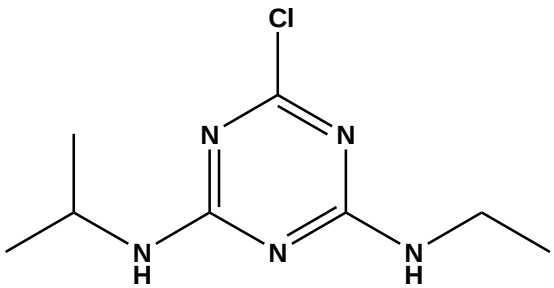

Figure 2. 4: structure of propazine

Source: (Thurman and Scribner, 2008)

2.1.5 Terbuthylazine

Terbuthylazine is a chlorotriazine herbicide characterised by the tert-buthylamino and ethylamino side chain. Its IUPAC name is 2-N-tert-butyl-6-chloro-4-N-ethyl-1, 3, 5-triazine-2, 4-diamine with the molecular formula of $C_9H_{16}ClN_5$ (Figure 2.5) and molecular weight of 229.71 g/mol. It is utilized in more than 45 countries including South Africa. In the United States, it is only registered for use in cooling towers. It was found to be a better replacement for chloro-s-triazine herbicides (atrazine and simazine) to control weed in maize, orchard, and vineyards in Ireland, United Kingdom, Spain, and Portugal, as atrazine usage discontinued since it was found in groundwater at a concentration higher than the arbitrary of 0.1 ppb. Terbuthylazine is normally applied in the winter and spring seasons (LeBaron, 2011).

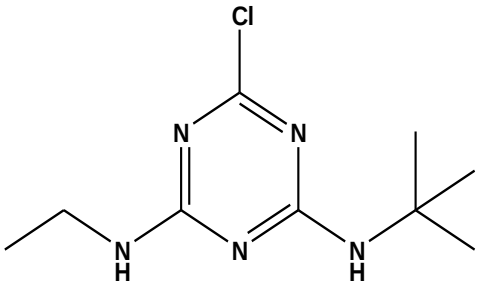

Figure 2. 5: Chemical structure of terbuthylazine

Source: (Du Preez *et al*., 2005)

2.2 Physical properties of triazine herbicides

The behavior of triazines herbicides in the environment dependent on their physical properties mainly the solubility, pKa, polarity (XLogP3), melting point, octanol-water coefficient (log Kow), and vapor pressure (Table 2.1). The water solubility of triazines is low while their octanol-water coefficient values are relatively high above 2.5 (except simazine) thus, their concentration levels are expected to be low in water and high in solid samples (sediment, sludge, and soil) due to their relatively high adsorption (Goodwin *et al*., 2017). Triazines are weak base compounds with pKa values range from 1.60 to 4.10 and they are medium polar, which increases their chances to be present in water. Triazines are unlikely to be found in the air because of their low vapor pressures (Tsai, 2010).

Table 2. 1: Physico-chemical properties of triazine herbicides

Pesticides	Solubility (mg/L)	pKa	XLogP3	Melting Point (˚C)	LogKow	Vapour pressure (mmHg)
Simazine	5.0	1.62	2.2	225	2.38	$2.2x10^{-8}$
Atrazine	34	1.60	2.6	173	2.61	$2.89x10^{-7}$
Ametryn	209	4.10	3	83.6	2.98	$2.74x10^{-6}$
Propazine	8.6	1.7	2.9	229.7	2.93	$1.31x10^{-7}$
Terbuthylazine	9.0	2.0	3.1	175	3.40	$6.75x10^{-7}$

Source: (Oliveira *et al*., 2013; Halmilton *et al*., 2003)

2.3 The uses of pesticides

Pesticides are used in different places for different purposes. Table 2.2 summaries the uses of the targeted triazines under this study.

Table 2. 2: Some uses of major triazine herbicides

Triazine	Uses
Ametryn	Sugarcane, corn, pineapple
Atrazine	Corn, sorghum, sugarcane
Propazine	Sweet sorghum
Simazine	Pear, citrus, filbert, apple, peach, almond, grape, walnut, corn
Terbuthylazine	Grape, sorghum, corn

Source: (LeBaron, 2011)

Pesticides are used in a wide range of settings and mostly in agriculture. They are used virtually in all sides of our daily lives to ensure the quality and the quantity of nutrition we consume and to manage the insect and rodents in household etc., (Nasrabadi *et al*., 2011). The use of pesticides allows more food production in a given area of land (Wanwimolruk *et al*., 2015). Even though a variety of climate changes has an effect on increasing harvests, as of tropical fruit, vegetable to corn and plantations of trees but they are not consistent as pesticides. The

variation of planted crops are not all responsive to one or any type of pesticides applied but they are susceptible to a different host of pests therefore individual crop need a different chemical mixture of pesticides (Buccini, 2003). Pesticides usage also improves farm revenues; therefore, agriculturalists apply pesticides to guard harvests from fungal diseases pests and weeds. They also inhibit mice, rats, fliers and additional inserts from polluting food while they are being stored. Pesticides protect human health by protecting food harvests from pollution by fungi and likewise protect people from disease-carrying organisms (Quinn *et al*., 2011).

In business, pesticides are also employed in many ways unnoticeable such as in paints and plastics, waterproofs may also contain fungicides to prevent mold. Focusing on herbicides, they are used along highways and in road crews to control vegetation for safety reasons. This is done to allow efficient water escape during flooding or downpour to clear the roadsides and to enlarge the visibility for drivers. Also, they are applied in parks, natural areas and wetlands to prevent them from invasive unwanted plants (Damalas, 2009). Herbicides are active ingredients, therefore they are used in some household cleaning materials and other products including sunscreen, ace laundry bleach detergent powder, radical power ultra-dawn hand soap-old product, abhushane, jewelry, absorbine refresh mint natural body wash and leg brace AFM safe choice supper clean (Crittenden *et al.*, 2012, Weinberg and Teodosiu, 2012). Triazines are used as coupling agents for the synthesis of the peptide in a solid phase, also in solution as a side chain of anti-biotic in pharmaceutical industries. They are also used in oil fields for preservatives purposes (Nyoni, 2011).

Pesticides are also extensively used worldwide because of their economic benefits. Farmers use them for the protection of products as well as the increase in yield and quality. Their usage also decreases other expensive inputs such as labor due to that fewer people are employed to apply pesticides than people which could be employed to hand remove weed in farms (Damalas, 2009). The global estimates losses of crops indicated that pest- persuaded losses were above 50%. Therefore, if pesticides are not used the production of food would drop and prices of food would increase thus no competition of major commodities from farmers in the global market (Oerke *et al*., 2012). It has been reported that Taiwan's agricultural environment has applied a large amount of pesticides in fruits and vegetables than in other countries followed by China, where pesticides are widely applied in rice crops (Pariona, 2017 April 25) as presented in Figure 2.6, (Nai *et al*., 2017).

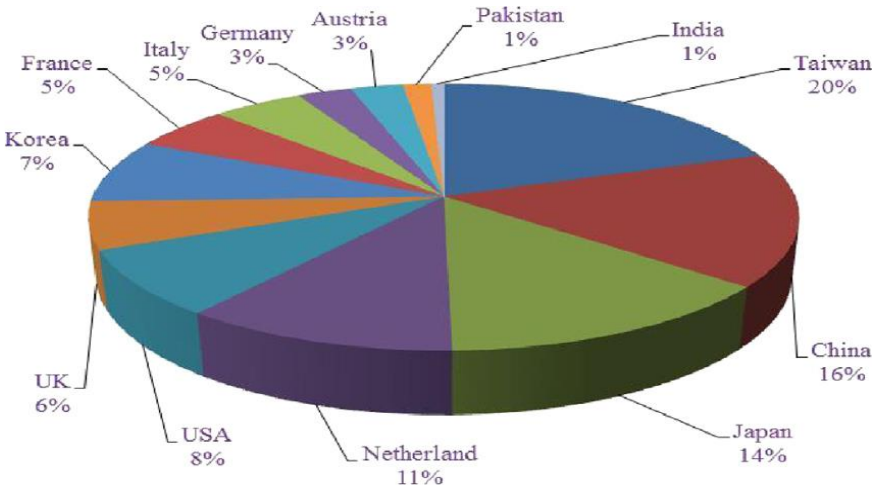

Figure 2. 6: Worldwide consumption of pesticides

Source: (Yadav *et al*., 2015)

2.4 Human's exposure pathways to pesticides

Even-though pesticides protect ornament plants and crops from the harmful organisms and unwanted pests, they have the capacity to harm people as well as other non-targets and the environment. More exposure occurs during pesticide application and 97% of the whole physique is exposed during the application of pesticides. In our everyday lives, we are exposed to pesticides either conventional or incidental. Incidental exposure occurs through both eating and drinking and eating contaminated water and food or the use of insect repellent in our houses or on our body membrane. The conventional exposed occurs via work-related exposure such as a farmer applying a pesticide in non-closed fields and glasshouses, labors in the pesticide industries and exterminators of the pests (Singh *et al*., 2018). The pesticide exposure increases when the agriculturist does not follow the instructions on how to apply pesticides or safety guidelines on protecting the body and fundamental sanitation practice while others are exposed in them due to the nature of their work for instance during loading, transportation, mix and application of formulated pesticides. Pesticides can reach into people in many routes including oral, dermal, respiratory, eye contact (Damalas and Eleftherohorinos, 2011, Kim *et al*., 2017, Singh *et al*., 2018).

2.4.1 Oral exposure

Oral exposure refers to drinking or eating contaminated water or food. It occurs either accidental because of carelessness or intentional for specific motives. Damalas and Eleftherohorinos (2011) reported the increase in the degree of oral exposure where human

poisoned cases occurred due to pesticides transfer from their original labeled container into soft drink containers and thereafter accidentally drunk or drinking water kept in pesticides containers (Damalas and Eleftherohorinos, 2011).

2.4.2 Respiratory exposure

Respiratory exposure is where the mist or dust or the fumigant vapor is inhaled. This occurs when prolonged in contact with pesticides or when using inadequate or old pesticides application apparatus (Kim *et al*., 2017). Pesticides have volatile components, as a result, they have the potential for inhalation exposure (Amaral, 2014). The degree of exposure increase when the pesticides are sprayed in the small droplet as there will be more toxic chemicals that are applied in small quantities thus the better are those which are applied in the large droplet. Also, the temperature has an effect on respiratory exposure as pesticide evaporation increase with the increase in temperature (Amaral, 2014, Damalas and Eleftherohorinos, 2011).

2.4.3 Dermal exposure

Dermal exposure can be defined as a multifaceted process of contamination between the skin and pesticide for a long period. This exposure is a dominate route through which farm workers get in contact with these compounds (Anderson and Meade, 2014). This complex process can cause significant impact on fauna and it may result in skin disease such as dermatitis. Dermal exposure predominately results from splashes, drift, and spill of pesticide on uncovered skin, tiring polluted clothes, touching of surface treated with pesticides, and also applying them on windy weather can also increase chances of exposure (Singh *et al*., 2018, Anderson and Meade, 2014, MacFarlane *et al*., 2013). Dermal exposure, especially in developing countries, is due to low regulated safety rules in workplaces, the use of old or leaking machines, working with pesticides without hand gloves, etc (WHO, 2015). In general, there is a various form of pesticide formulation such as solid form (granules, dust or powders) and aqueous form which readily absorbed through the body membrane and tissue (Kim *et al*., 2017).

2.4.4 Eye exposure

Eye exposure occurs when pesticide splash on the eye, use of contaminated hands to rub eyes, application during windy weather and pesticides split back into the eye and also pouring formulation without eye protection (Singh *et al*., 2018). Eye tissues are vulnerable and fragile therefore they are easily injured by chemicals. Most chemicals have been reported to injure eye tissue after the absorption of a sufficient quantity of the chemical. Both powdered/palates and

liquid form of pesticides are potentially hazardous and capable of results in serious disease even mortal illness (Gilden *et al*., 2010). During pesticides, the application is where the exposure is likely to occur usually when they are applied using powerful equipment and at windy weather. Therefore, googles should be worn always to protect the eyes during spraying of pesticides and to protect eyes from dust. The pesticide effect on farm workers was tested in India during and after pesticide application. Ocular symptoms such as swollen eyes, blurry vision, itching and pain in eyes, watering and burning sensation were identified (Mohammed *et al*., 2012).

2.5 Effect of pesticides in the human body

Epidemiology studies have indicated an association of occupational exposure to pesticides with various diseases. The effect of pesticides in the human body includes acute and chronic effects. The pesticide effect in the human body depends on the period and quantity of exposure and also on the properties of the pesticide.

2.5.1 Acute effects

These effects might immediately appear after inhalation, ingestion or skin contact in a day after exposure to pesticides. Acute effects can cause respiratory problems, coughing, sore throat, eyes and skin irritation, loss of consciousness, headache, diarrhea, vomiting, nausea, contact dermatitis, tremendously weakness and neurotoxic effect. Inhaling pesticides can cause serious illness or damages on the lungs, throat, and nose (Amaral, 2014).

2.5.2 Chronic effects

These refer to effects that appear over a long period. The low dose of pesticides does not have an effect at the same time after exposure but over an extended period, they cause serious illness in the human body. These include carcinogenic effects, meaning they have the potential to cause cancer in fauna, mutagenic effect, this refers to genes altering (Dieter, 2018). Pesticides can also cause asthma, a common chronic disease that can present as wheezing, coughing and breathless. In the United States, pesticide poisoning was identified to be linked with asthma (Owens *et al*., 2010, Hernández *et al*., 2011, Amaral, 2014). It can also result in Parkinson's disease, a brain disorder disease, which affects movement, loss balance and cell movement regulator. Epidemiology studies in the French population have suggested that Parkinson`s disease increases due to occupational contact with pesticides (Moisan *et al*., 2015). In

groundwater, it has been found that each 1.0 µg/L of these compounds increases to about a 3% risk of Parkinson`s disease (James and Hall, 2015). Pesticides also have a teratogenic effect and can lead to birth and fatal defects. Owens, (2010) reported the birth and fatal defects rate to be high during the summer and spring which are seasons when pesticides are intensively applied and when their concentrations are high in surface water. The effect includes Down`s syndrome, cleft lip, spine bifida and clubfoot among females who conceive while atrazine, nitrate and other variety of pesticide were in high concentrations (Owens *et al*., 2010).

2.6 The pesticides effect on the environment

Pesticides are used to safely guide human health by preventing food crops from pest and fungi contamination. However, they have an impact on the environment as they can contaminate the environment (turf, water, soil, and other flora). Killing insects, fungi, larvae, bacteria, and weed using pesticides can be poisonous to the host of the other organism, including bird, fish, non-target plants and beneficial insects.

Pesticides may enter the environment from point of application or point source of contamination via crop run off and reach drainages where they can seep and leach to groundwater and pollute it. They can also diffuse via land runoff where they evaporate into the atmosphere. From the atmosphere, they can dissociate by water and sunlight or settle to the earth and precipitate. Depending on the weather conditions, some of the pesticides can be transported to short or long distances away from their point of application. Pesticides, which are dissociated into the atmosphere can stay for a short period while some can last longer. Those that last longer can be deposited by rain into environmental water, which serves as drinking water. This is due to dynamically adsorption and desorption between different environmental samples and water. By environmental sample that refers sediments consisting mud and dead organisms discharged from the underground of the rivers or lakes, algae, marshes, dissolved organic matters such as inorganic compounds counting clay minerals and microorganisms (Tanaka and Katagi, 2008). The distribution cycle of pesticides in the environment is displayed in Figure 2.7.

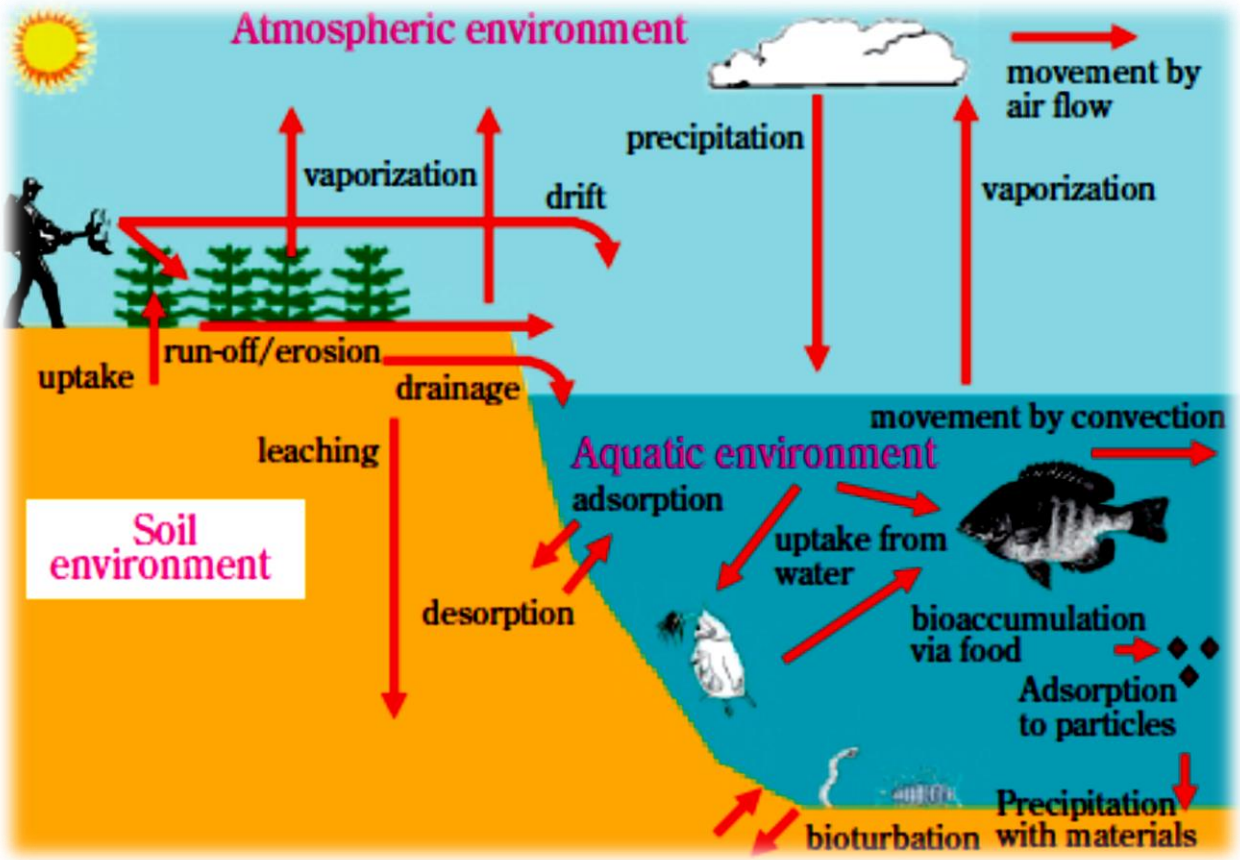

Figure 2. 7: The distribution cycle of pesticides in the environment

Source: (Abhiram *et al*., 2018)

2.6.1 Soil contamination

The major important source of exposure to pesticides is through pesticide-polluted soil (Yadav *et al*., 2015). From the soil, the residues of pesticides leach, get absorbed by the plant's roots or volatilize from the ground into the atmosphere. The residues are found in soil as it acts as a natural basin for different accumulating and intent contaminants, which terminate in the soil from different sources (Ali *et al*., 2014). The amount of accumulated pollutants spreads significant concentration and discharge persistent toxic compounds through photodegradation or microbial degradation resulting in soil pollution. Soil contamination occurs when the application of pesticides surpasses the threshold values. When pesticides are applied to the soil, they undergo various reactions. They may evaporate and vanish to the atmosphere without the chemical change or they can be absorbed by the soil colloids, leach through soil and be degraded by soil microbes. Pesticides have an impact on soil enzymes that are important substances for controlling the value of soil lifetime. Soil enzymes help to control cycles of nutrients, and in turn, fertilization (Riah *et al*., 2014).

2.6.2 Water contamination

Water is essential to all living organisms on earth and its quality is significant to take care of the physical activity of biological cells. Water contamination is a measure problem. Contaminants alter the natural feature of water through an addition of strange substances that may therefore generate some toxic and greenhouse gases, which may subsequently contribute to global warming activities or more severe environmental threats as a result water cannot be consumed or be able to support aquatic organisms including fish, frogs, etc. It is the main cause of worldwide concern as it results in the commencement of various fatal diseases, which are responsible for the death of more than 14000 people every day (Oerke and Dehne, 2004). Due to chemical leaching and chemicals mixing from agricultural practice more than 50% of water get polluted (Damalas, 2009). The pesticide may reach the river through many routes. They may drift outer of the target area during their application, leach over the soil to contaminate groundwater and surface water or they may be accidentally spilled (Singh *et al*., 2018). After application 0.2% is lost per day due to evaporation as a result of precipitation. In several countries, triazines have been quantified in surface and groundwater (WHO, 2003).

Due to many effects caused by triazine herbicides. The maximum residue limits (MRLs) of triazines corresponding to each environmental sample were set by the European Union. MRLs are maximum concentrations that are accepted or legally permitted by the European Union as a standard dose to be detected at certain matrices which has no effect into ecosystem (MacLachlan and Hamilton, 2010). These concentrations are safe, meaning they cannot pose risk to humans and other life forms. MRLs setting is a balancing act: the MRLs are ideally set at a level which are high enough to prevent a rational probability for legally applied of triazines to result in commodity residues that surpass the MRLs yet not too high that there are little chances of sensible likelihood of finding illegal application or misuse (MacLachlan and Hamilton, 2010, Solecki *et al*., 2005). The MRLs of the targeted triazines under study are given in Table 2.3.

Table 2. 3: Triazines allowable concentrations in water and soil.

Triazine herbicides	Maximum Allowable Limits	
	Water (µg/L)	Soil (µg/kg)
Simazine	100	200
Atrazine	20	66
Ametryn	50	200
Propazine	50	200
Terbuthylazine	7	200

Source: *Hamilton et al.,* 2003

2.7 Triazine degradation

Triazine degradation involves biotic and abiotic reactions, which both occur under anaerobic and aerobic conditions and therefore, these processes can be influenced by several factors. Abiotic reactions involve photo degradation, oxidation and hydrolysis. These abiotic reactions can be affected by environment restrictions such as temperature, pH and moisture of the soil (Donati and Funari, 1993). Biotic reactions include a variety of enzymatic reactions, which are catalysed by microorganisms. The environmental conditions that affect the enzymatic reactions are the nature of soil (i.e. organic matter, amount of oxygen and pH), temperature and moisture, and agronomic conditions (i.e. nature and addition of manure), (Donati and Funari, 1993). Hydrolysis and N-dealkylation reactions are the main degradation reactions of triazine herbicides that occur in soil. Both biological and chemical degradation can be relevant in the first soil layers. Whereas there are few pathways of metabolic that seemed possible under anaerobic conditions and also hydrolysis at longer depth does not have the potential to occur.

Atrazine undergoes a transformation in both soil and water. The well-known mechanism of atrazine microbial degradation is an N-dealkylation. The removal of ethyl sidechain is preferential to some microorganisms, while side chain isopropyl is removed by others (Giardina *et al*., 1982). Atrazine metabolites are deisopropylatrazine, deethylatrazine, and hydroxyatrazine. In atrazine bacterial degradation, the first observed products are deisopropylatrazine and deethylatrazine metabolites. Hydroxyatrazine metabolite is absorbed strongly on the soil and it is resistant to degradation in submerging than in aerated soils. The well understood degradation pathway of hydroxyatrazine is N-dealkylation whereas others are not known. Hydroxyatrazine low mobility has been reported; hence, more attention has been

given into the parent pesticide and the other two metabolites (deisopropylatrazin and deethylatrazine). Parent pesticide and metabolites in groundwater and superficial water have been reported to be present (Mahía *et al*., 2008).

Simazine has low water solubility and therefore it is considered as a persistent triazine herbicide. The reported approximation of its persistent in moist soil in summer conditions is about 3-6 months (Reinert and Rodgers, 1987). Simazine undergoes degradation like other triazines via microbial, N-dealkylation and chemical hydrolysis. The metabolites that result in simazine transformation include hydroxysimazine, deisopropylatrazine, and deethyldeisopropylatrazine. Some soil microorganisms use simazine as an energy source (Kaufman and Kearney, 1970). However, simazine does not quickly mineralize (Fournier *et al*., 1977).

Terbuthylazine degradation is via N-dealkylation of the side chain, chlorine group hydrolysis, after dealkylation the amino group and ring cleavage. The terbuthylazine degradation results in deethylterbuthylazine metabolite. In soil, its volatilization seems not to be an applicable dissipation process of the herbicide (Hartley and Kidd, 1987). Ametryn in soil undergoes microbial degradation and it results in two metabolites 1,3,5-triazine-2-amine and N-ethyl-N′-(1-methylethyl)-6-(hydroxy)-1,3,5-triazine-2,4-diamine (Farré *et al*., 2002). Propazine also undergoes degradation in soil and water. Its transformation results in deethylatrazine. It was determined that in sorghum, propazine metabolism takes place by the reactions: N-dealkylation, hydrolytic of the group glutathione with 2-chloro. Conjugation and dehalogenation were the major pathways since the residues of chloro-s-triazines were quantified (Simoneaux and Gould, 2008).

2.8 Chemistry of triazines

2.8.1 Triazine interaction mechanism with soil and water

Nitrogen atoms from the triazine ring donate electrons due to that triazines are Lewis bases compounds. Depending on the pH of the system and pKa of the compounds, triazines can be either be in the protonated or neutral form in the aqueous system. Position 5 in the middle of nucleophiles side chains which are alkylamino is where the site of protonation and basic ring nitrogen are located (LeBaron, 2011). The pH of the scheme is the pKa of an organic base where the compound halves are present in a different form, meaning the other part of the

compound is existing in protonated and another part is present in the neutral form. Most triazines are very weak bases and chloro-s triazines are one of the very weak bases (Fuscaldo *et al*., 1999). They have low pKa values ranging between 1.6 to 1.9 and the methythio-s-triazine and methoxy-s-triazine range between the pKa value of 4.0 to 4.8 whereas hydroxy-s –triazine consists of greater, above 5 pKa values. The chloro-s- triazines in the soil solution are present in the neutral form at pH 4.8 to pH 8. The methythio-s-triazine and methoxy-s-triazine are existing as neutral species in alkaline and neutral soil solution however in acidic soil solution they could be present as protonated or neutral species or both (Fuscaldo *et al*., 1999).

The hydrophobic and hydrophilic characteristics of the triazine compounds are indicated by the microscopic property, which is the water solubility. The position 2, 4 and 6 of the substituents and nature are the ones responsible for compound solubility but in general, the triazines are soluble in neutral water at 20˚C. The formation of hydrogen bond by a water molecule and the nitrogen atom lone pair result to a hydrophilic triazine ring while the nucleophilic side chain alkylamino in position 6 and 4 are hydrophobic. Due to hydrophobic and hydrophilic functionalities of triazines, they display dual solubility equivalent to that displayed by the phospholipid and detergents. According to the sorption, energy is minimalized to allow the interaction of hydrophobic surface and the hydrophobic moiety same thing applies in the water or other polar compound interacts with hydrophilic moiety. The increase of solubility and the triazines protonation occurs when pH approaches the pKa of the compound (LeBaron, 2011).

2.8.2 Fate of triazines in sediment/soil

There are three basic processes that control the fate of triazine herbicides in the soil, these are retention, transformation, and transportation (Bailey and White, 1970). Soil and sediments are very complex mixtures of living organisms. The triazines absorption on the soil surface occurs as a primary means by which they are retained in soil or sediments. There are different types of mineral particles and organic matter consisting of different surface sites namely non-polar, polar and ionic surface sites. The polar triazine molecules functional group interact with the polar and ionic sites and they have a high affinity for water, therefore they turn out to compete with water for this site. However, water out-compete the chlorotriazines for the polar and ionic surface. Other triazines such as methoxymethylthio-triazine and hydroxytriazine are more competitive in polar and ionic sites than chlorotriazines. Alkyl side chain which is a non-polar

side chain of triazine molecule interact with the nonpolar site on the soil surface (LeBaron, 2011). They have a low affinity for water (Kumar *et al*., 2013). The strong interaction between soil and triazine occurs when the triazine functional groups are closely related or matches with the soil surface active site. The sorption of triazines is affected by the pH and aging where the decrease of pH increases the triazine sorption. The longer the period triazines remain in the soil, make it become challenging to extract them because they strongly bound in the soil matrices. The triazines sorption by soil may be affected by various parameters such as concentration, temperature, soil, water content, amount of dissolved organic carbon (LeBaron, 2011). Atrazine is mostly found in groundwater due to its high mobility in soil and because it is frequently used than the other triazine (Fuscaldo *et al*., 1999). Ametryn is a persistent compound, in soil, it can travel both laterally and vertical due to that it is more soluble in water. It may be percolated by high floods, furrow irrigation and rainfall (Briggs, 1992).

2.9 Extraction techniques used for triazines in environment samples

There is a variety of methods used for triazines extraction from water and solid samples. Techniques that are used to extract solid samples include microwave-assisted extraction (MAE) ultrasonic extraction (UE), Soxhlet extraction (SE), etc. For liquid samples, the techniques used are liquid-liquid extraction (LLE), solid phase extraction (SPE), hollow fiber-liquid phase microextraction (HF-LPME), etc., (Trtic-Petrovic *et al*., 2010).

2.9.1 Liquid-liquid extraction (LLE)

LLE is the method which involves the analyte partitioning between the two immiscible phases (organic and aqueous) that are selected to increase differences in solubility, the analyte is then recovered from one of the two phases (Brito *et al*., 2002). LLE is usually used to determine the amount of organochlorine pesticides in sediments and water (Sibali *et al*., 2009). The factors that affect the distribution of the analytes between the two phases include solvent type as well as the pH which is an adjustment to prevent basic and acidic ionization of target compounds (Dean, 2010). This is important as the ionization can hinder effective extraction of the compounds. The advantages of LLE are that it is easy to apply and cheap to perform, also a variety of organic solvent that can be used. It is a multipurpose sample preparation technique, and LLE is recommended in several ordinary analytical techniques. However, the procedure is time consuming and tedious, it requires a larger amount of toxic solvents and prior to analysis, it requires pre-concentration of the sample (Bello-López *et al*., 2012).

2.9.2 Solid phase extraction (SPE)

SPE is the utmost commonly method for the preparation, clean up, pre-concentration, and isolation of target compounds from the matrices. The SPE technique involves retention mechanisms such as adsorption, ion exchange, the partition between the liquid and the solid where the solid materials are the sorbent material. This method involves the passing of a liquid sample containing the analyte into the conditioned SPE sorbent. The target analyte binds onto the sorbent of the cartridge prior to elution (Dean, 2010). SPE is usually used for liquid samples to remove analytes but it also can be used for solid samples that are prior-extracted into solvents (Aznar, 2010). SPE has been used to extract pesticides in different aqueous samples which is due that it can concentrate analytes for better sensitivity, good robustness and high percentage recovery (Donato *et al*., 2015, Radovic *et al*., 2015). Also, it has a fast analysis time, it requires a smaller volume of organic solvent, and it gives high enrichment factors. In addition, it can extract many samples at the same time and it can be applied to a wide variety to sample matrices. However, the plugging of the cartridge may occur. Also, it includes many stages thus it requires a long time for method optimisation (Donato *et al*., 2015).

2.9.2.1 Principle of SPE extraction

SPE consists of four stages which are; the Sorbent conditioning, loading of a sample, washing of the impurity and analyte elution as shown in Figure 2.8 (Zdravkovic, 2015).

Conditioning of the sorbent: This is the first step of SPE, which is also called wetting step, the sorbent is wetted with a solvent to activate its functional groups and thus prepare for a good interaction with the analyte. After this stage, the sorbent is not allowed to dry out before the washing step as it could result in low recoveries (Dean, 2010).

Sample loading: the sample is loaded or passed through the cartridge where the analyte with some interfering compounds is adsorbed in the sorbent bed. In this step, a breakthrough volume is an important parameter to be considered in order to prevent loss of analyte. Breakthrough volume refers to a stage whereby the analyte is no longer absorbed due to no active site available for the analyte to bind as enough sample volume has been loaded (Dujaković *et al*., 2010).

Washing of impurities: it is the removal of additional compounds interfered from the sorbent which is done by passing the suitable solvent through the sorbent. The solvent used must not be too resilient to elute the analyte of the interest or too weak to leave additional compounds

behind. This is an important step to be considered since it ensures that the compounds eluted are only a target analyte with no additional interfering compounds (Berrueta *et al*., 1995).

Elution of analyte: the compound of interest is eluted by a solvent, which is resilient enough to break the bond between sorbent and analyte and thus completely remove all the adsorbed compounds from the cartridge sorbent bed with a small enough volume. It can be determined by mostly on the intermolecular forces formed between the sorbent and the target analyte. Figure 2.8 shows the schematic diagram for SPE (Berrueta *et al*., 1995).

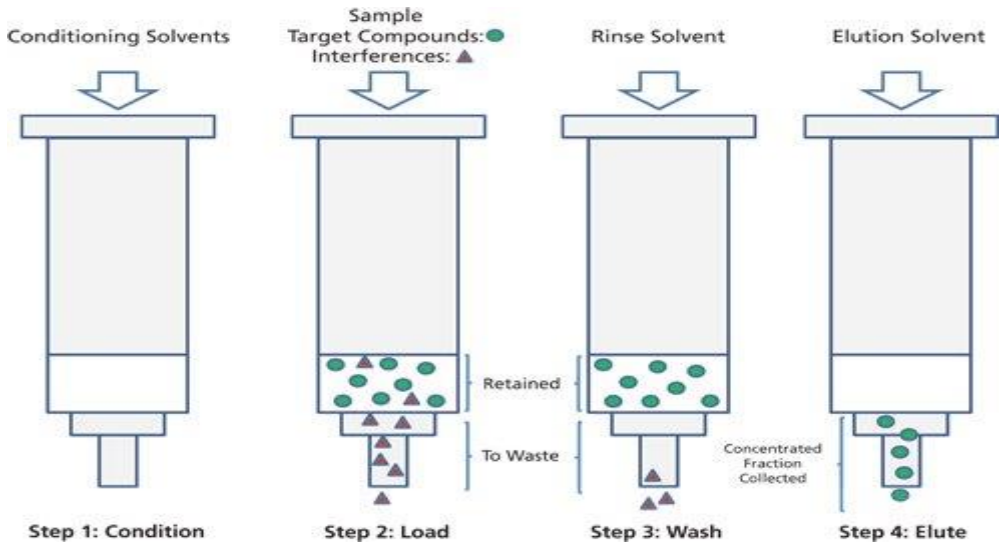

Figure 2. 8: Schematic diagram showing the four stages involved in SPE.

In SPE, the sorbent is packed in in the middle of two fritted disks in a polypropylene cartridge (Berrueta *et al*., 1995). The analyte retention in the sorbent and removal from the sorbent depends on the formed intermolecular forces within an analyte, the matrix, and the SPE. The analyte should have a low affinity for the sample matrix than the SPE sorbent (Masque *et al*., 1998). There are different sorbents used which include, Strata ^{TM}-X sorbent, ENVI-18, Strata $C_{18,}$ and Oasis HLB, etc. The new sorbents (molecularly imprinted polymers and immunosorbents) are made of chemicals together with functional groups including o-carboxybenzoyl, hydroxymethyl, benzoyl, acetyl also extremely cross-linked polymers. These sorbents have shown an improvement on recoveries for most polar compounds including the triazine herbicides. The Strata ^{TM}-X sorbent, Strata $C_{18,}$ and Oasis HLB are all able to extract acidic, neutral and basic compounds due to their properties. Oasis HLB can be used to extract equally non-polar and polar substances due to its chemical composition including hydrophilic

N-Vinylpyrrolidone and combination of lipophilic divinylbenzene polymers (Gros *et al*., 2006). Strata ^{TM}-X has a surface which made up of a pyrrolidone group with styrene (Babić *et al*., 2010). It has been reported that the C_{18} sorbent is good sorbents to extract the triazine and their metabolite efficiently.

2.9.3 Hollow fiber liquid phase microextraction (HF-LPME)

Hollow fiber liquid phase microextraction involves the analyte partitioning between solution and organic solvent. It is a new mode of LPME, which was introduced upon the usage of a cost-effective and disposal hollow fiber. This technique was modified to increase the effectiveness, which resulted in a reduction of extraction time. It also resulted in high enrichment factors, recovery percentage, and extraction throughput. This technique comprises of a capillary porous hydrophilic fiber saturated by organic solvent and its interior filled with the acceptor phase (Figure 2.9) (Sharifi *et al*., 2016). HF-LPME can be used in two of three phases. With two phases, the analyte is extracted from an aqueous to an organic phase immobilized in the membrane pores and in the lumen of the hollow fiber. With three phases an organic phase is placed in the membrane pores and two aqueous are placed at the opposite side of the membrane (Menezes *et al*., 2016). HF-LPME has been used for the clean-up and concentration step of triazines analysis in water (Xiong and Hu, 2008).

HF- LPME was developed by Perdesen-Bjegaard and Rasmussen and has been used by numerous researchers in the latest years because of its advantages such as its simple process and the clean-up step is not necessary. The sample can be stirred without loss of extracting liquid because it is sheltered in HF-LPME. It is very selective and uses a smaller amount of solvent. It is fast, simple and it is inexpensive. It has high enrichment factors. The hollow fiber can prevent interference. However, it is not suitable for a non-polar organic compound. In addition, clogging of the pores for the sample with high dissolved solids may occur (Letseka and George, 2017).

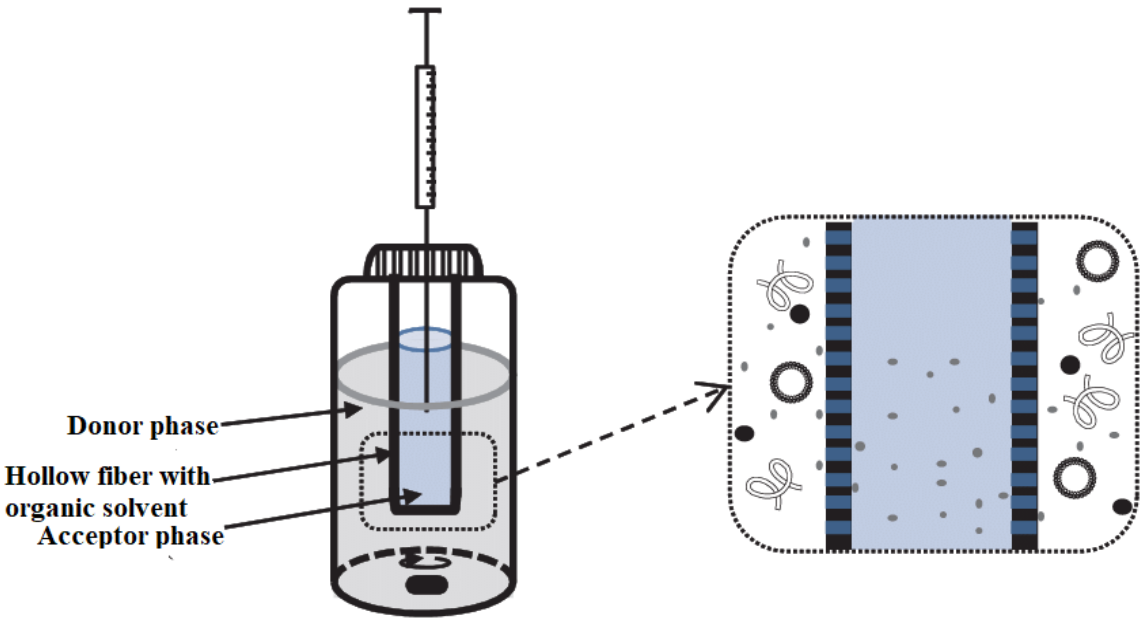

Figure 2. 9: Illustration of hollow fiber liquid phase micro extraction

Source: *(Rodríguez et al., 2013).*

2.9.4 Soxhlet extraction (SE)

Soxhlet is a traditional technique widely used for the extract of persistent organic pollutants from a variety of environmental samples with complex matrices such as soil, sediments, biota`s tissues, dust, etc. The Soxhlet extraction method is one of the leaching methods (Saadati *et al*., 2013). In the Soxhlet extraction method, a solid sample is placed in a thimble. The thimbles then loaded into a chamber of Soxhlet extractor, which is placed into a flask that having an extraction solvent. Soxhlet is fitted out by the condenser and heat is applied to reflux (Figure 2.10). As a solvent vaporise its vapor moves up a distillation arm and overflow into a chamber that loaded with a thimble containing the solid sample. When the solvent is almost full in a chamber it is then removed by the siphon side arm automatically back to a distillation flask. Cycles can be repeated many times and in any cycle quota of solvent which contains a non-volatile compound till the analyte is intense in a distillation flask (Jensen, 2007).

SE method advantages are that it can analyse the larger amount of environmental samples. It can be conducted unattended, it is also a resilient method and considered as well-established as its extraction can only be affected by few parameters (Saadati *et al*., 2013, Guo and Kannan, 2015). SE is still widely used as the sample is repetitively carried into an interaction with a new portion of the solvent, which improves extraction efficiencies (Halfadji *et al*., 2013). The core

drawbacks of conventional SE are; use of non-environmentally friendly solvents that the time for extraction is long. It cannot speed up the process by providing agitation. There is an option of thermal decay of the selected target analytes that cannot be overlooked as extraction usually occurs at the boiling point of the solvent for a prolonged time (Masia *et al*., 2015). A large quantity of solvent is used which then involves vaporization before analysis resulting in the long extraction process (Oluseyi *et al*., 2011).

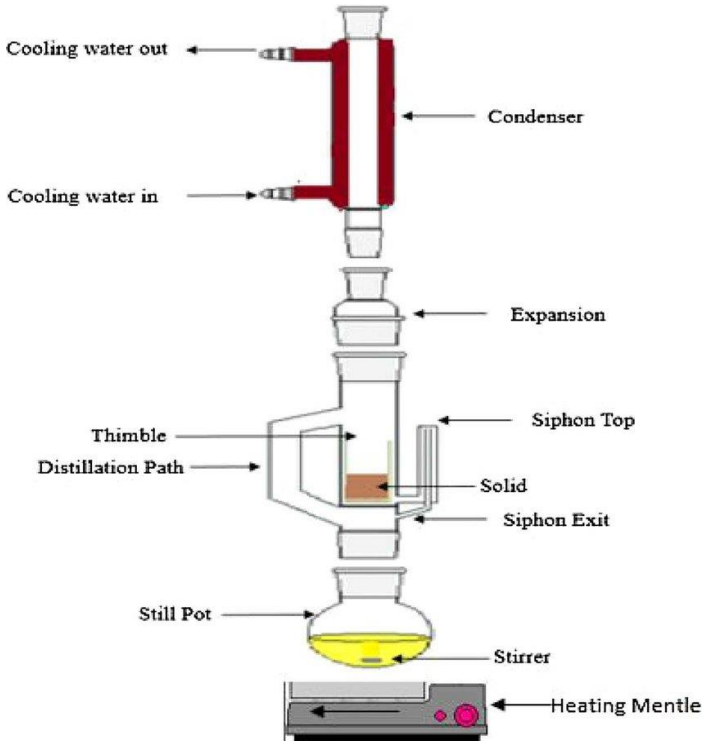

Figure 2. 10: The Soxhlet extraction apparatus

Source: (Azwanida, 2015)

2.9.5 Microwave-assisted extraction (MAE)

In MAE, the sample containing the analytes contained in a vessel with an appropriate solvent and placed in a microwave. The energy from microwaves is used to warm the solvent that is interacting with the sample and thus help the partitioning of the analyte removal from its matrix into the solvent. Microwaves frequency ranges from 0.3 to 300 GHz, which results in a molecular movement by the relocation of ions and dipole rotation. The second application is the straight act of the microwaves on the sample that is able to engross the energy of electromagnet and to convert it into hotness (Sanchez-Prado *et al*., 2015). After extraction, the

vessel is cooled for a few minutes. The solvent is then filtered to remove the matrix and dried out with anhydrous sodium sulphate to remove water (Onuska and Terry, 1993). Figure 2.11 shows the microwave assisted apparatus. The parameters such as extraction time, extraction solvent volume, extraction solvent, etc., need to be optimized for effective extraction of the MAE.

The advantages of MAE close vessel are that the volatile substances are avoided from being lost during the microwave radiation. There is no evaporation that occurs during extraction, therefore, a smaller amount of solvent required (no need for solvent addition unlike open vessel). Contamination is strongly avoided, thus there are few chances of floating contaminants. It uses elevated temperature (which cannot be attained with an open vessel), (Tatke and Jaiswal, 2011). However, it only uses solvents, which can absorb microwaves. It requires time to cool the vessels and a clean-up step is required (Mandal *et al*., 2007, Eskilsson and Björklund, 2000).

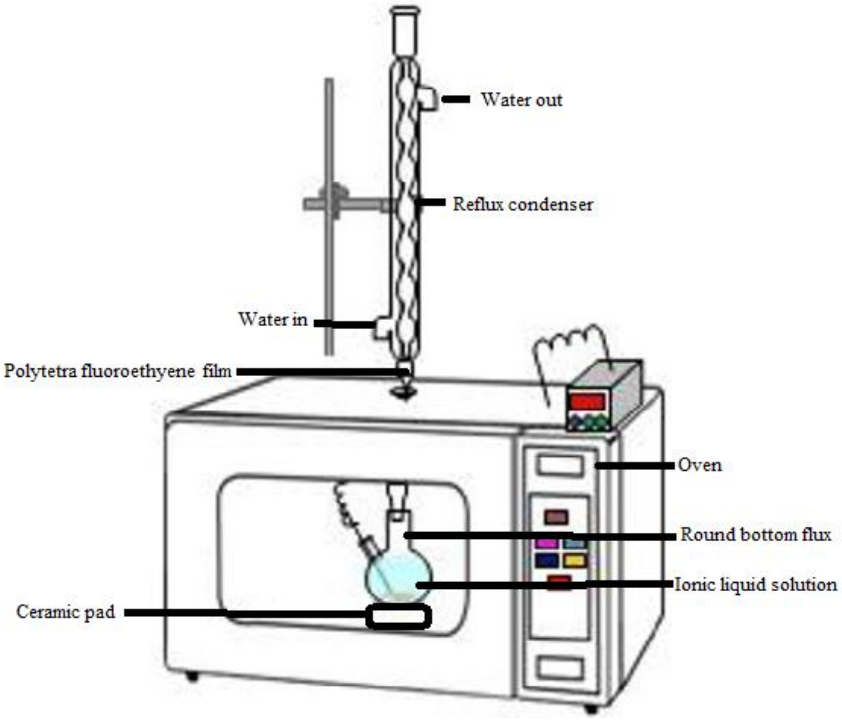

Figure 2. 11: Typical microwave-assisted extraction

Source: (Tatke and Jaiswal, 2011)

2.9.6 Ultrasonic Extraction (UE)

Ultrasonic extraction is a method used to extract the chemical residues in different solid samples. The sample is deepened in the solvent in a glass container and then positioned in the

sonication bath (Eskilsson and Björklund, 2000). It removes the chemical residues by shaking the solid sample containing the target analyte with an appropriately chosen solvent, which is the one that penetrates into solid matrices to disintegrate the solid aggregates. It has been reported to be more efficient in extracting traces of triazines in sediment and soil when compared with other refluxing methods. Ultrasonic extraction has been recommended due to its minimum extraction time and also, it is relatively cheap (Oluseyi *et al*., 2011). Figure 2.12 displays the setup of ultrasonic extraction.

UE advantages are that it is inexpensive, small organic solvent intake and extraction time is reduced. However, it also has some drawback such as the necessity of clean up phase and also, repeated extraction may be required (Eskilsson and Björklund, 2000).

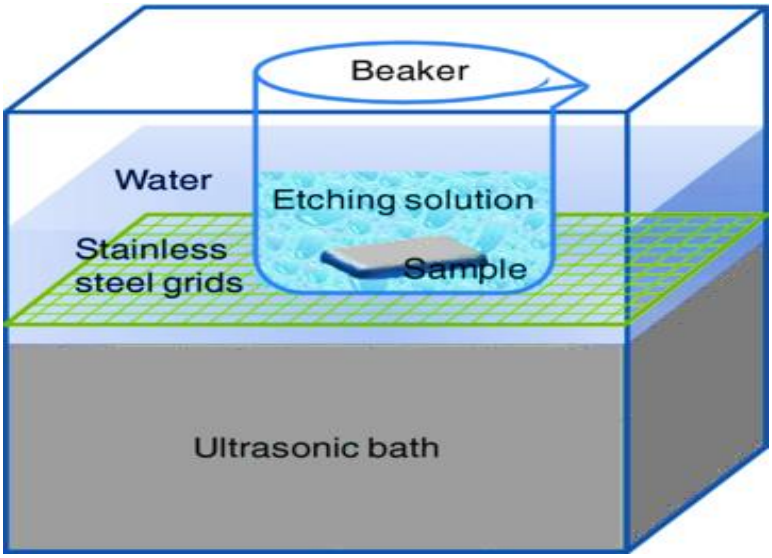

Figure 2. 12: Typical ultrasonic extraction illustration

Source: (González-Centeno *et al*., 2014)

2.9.7 QuEChERS method

QuEChERS which is a quick, easy, cheap, effective, rugged and safe technique has been used for the removal and clean-up of triazines. Initially, the QuEChERS was presented for pesticide residues investigation in high moistness vegetables and fruits, however, it is attaining important approval in the examination of a comprehensive range of analytes in a huge variety of samples. QuEChERS includes liquid-liquid partitioning by means of particular solvent (usually acetonitrile, however, the solvent used to depend on the type of the target analyte is being extracted) and uses dispersive solid-phase extraction (d-SPE) to purify the extract. The wide series of QuEChERS application is likely because of introducing many different modifications

established on the use of different d-SPE sorbents for clean-up step and the use of buffer additions for salting-out partitioning step, salt formulation, and various extraction solvent and (Rejczak and Tuzimski, 2015). The QuEChERS method is shown in Figure 2.13. In principle, a sample is homogenised and the appropriate solvent is added and hand mixed. Thereafter, the QuEChERS content is added and the mixture is vortexed followed by centrifugation. The sample is then washed up using the dispersive solid phase prior to the analysis of the extract. This method is inexpensive, rapid, simple, requires small solvent volume and produces a small amount of hazardous waste (Schenck and Hobbs, 2004).

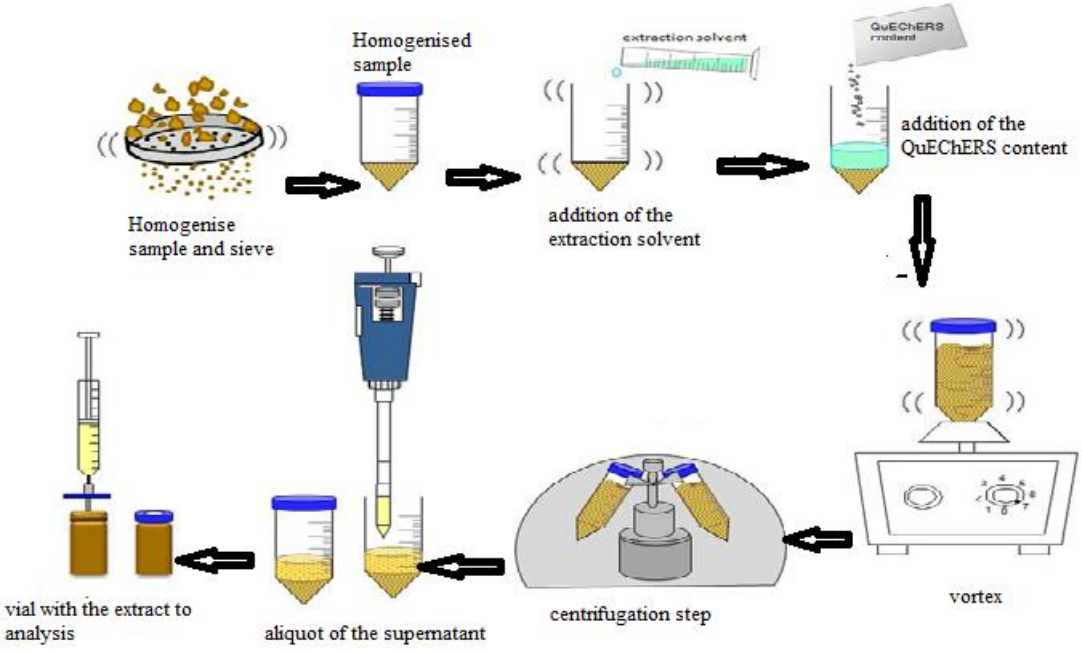

Figure 2. 13: Schematic diagram of the QuEChERS method

Source: (Paíga *et al*., 2015)

2.10 Separation and Detection techniques

Separation techniques are used to separate compounds in a sample mixture. Chromatographic techniques such as liquid chromatography (LC) and gas chromatography (GC) are commonly used for the separation of pesticide mixtures for qualitative and quantitative analysis. Chromatography is an analytical technique, wherein a sample mixture under test is separated into different component under the influence of mobile phase over the stationary phase (Glueckauf and Coates, 1947). After the separation of analytes, a suitable detector identifies them. There are different types of detectors that have been used with these chromatographic

techniques for the determination of pesticides. These includes ultraviolet (UV), fluorescence (Fl), electron capture detector (ECD), mass spectrometry (MS) detector and flame ionised detector (FID) (Giddings, 2002).

2.10.1 Separation techniques

2.10.1.1 High-Pressure Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a form of liquid chromatography, which is used to separate mixtures that are dissolved in a solution. HPLC is used for identification, quantification and to purify the individual components in a mixture. The instrument consists of a detector, solvent reservoir, an injector, a pump, and a separation column. The HPLC detector included ultraviolet (UV), Fluorescence (Fl), Mass spectrometry (MS), DAD detector. In this instrument, the analytes are separated by inserting a socket of the sample mixture on top of the column (Snyder *et al*., 2012).

2.10.1.1.1 Principle of HPLC

In HPLC, a small sample volume is injected into the stream of the mobile phase and slowly moved down the column by a specific interaction with the stationary phase present within the column. The different compounds in the mixture distribute between the stationary phase and the liquid mobile phase. The time at which the eluent is eluted is called a retention time which differs under particular condition (Engelhardt, 2012).

2.10.1.1.2 Normal phase

A normal phase is also known as adsorption chromatography, as the separation of the analytes is based on adsorption to the stationary phase and by polarity. In a normal phase, the mobile phase is non-polar, the stationary phase is polar, hence it effectively works in separating analytes which are decipherable in non-polar solvents (Peng *et al*., 2007). There are few separations carried out in normal phase because its stationary phase is more polar so it results to absorption of more compounds. The strength of adsorption upsurge with the upsurge of analyte polarity, and the interaction between the polar stationary phase and polar analyte increase elution time. Therefore, the polar analytes will be retained as the polarity of the stationary phase, the analyte has the same polarity, and the non-polar analyte will be eluted first. The strength of interaction does not only depend on the functional groups in the analyte molecules but also on the steric factor which allows this method to separate structural isomers.

In a mixture, the too polar solvents tend to deactivate the stationary phase by forming a stationary phase bound water layer on the surface of the stationary phase. This behavior to some extent is unusual to a normal phase because it is the most virtuous and adsorptive mechanism, the hard layer on a surface is preferable in the interaction than the soft layer (Carabias-Martínez *et al*., 2005).

2.10.1.1.3 Reverse phase

In a reverse phase, the stationary phase is modified silica. The silica is derivatised with Me_2SiCl, where R presents a straight chain alkyl group, for instance, C_8H_{17} or $C_{18}H_{37}$. In this phase to increase retention time, more water can be added than the organic phase. The analyte structural properties have an influence on the retention time. The mobile phase is polar therefore non-polar analyte will be retained as a result of the increase in the molecule`s non-polar stationary phase, which is not interrelating with the structure of water. The polar analyte will be eluted first. In a reverse phase, the predominately elution of the analytes is classified into two modes, which are gradient and isocratic elution. Isocratic elution involves the same or continuous mobile phase composition to elute solutes while gradient elution involves changing mobile phase composition with time (solvent programming). In that way, making a strong relative affinity of a hydrophobic stationary phase for the hydrophobic analyte to a mobile phase that is more hydrophilic (Carabias-Martínez *et al*., 2005). Likewise, to decrease the retention time, the more organic solvent should be added to the eluent than water (Peng *et al*., 2007). The schematic diagram of the HPLC instrument in Figure 2.14.

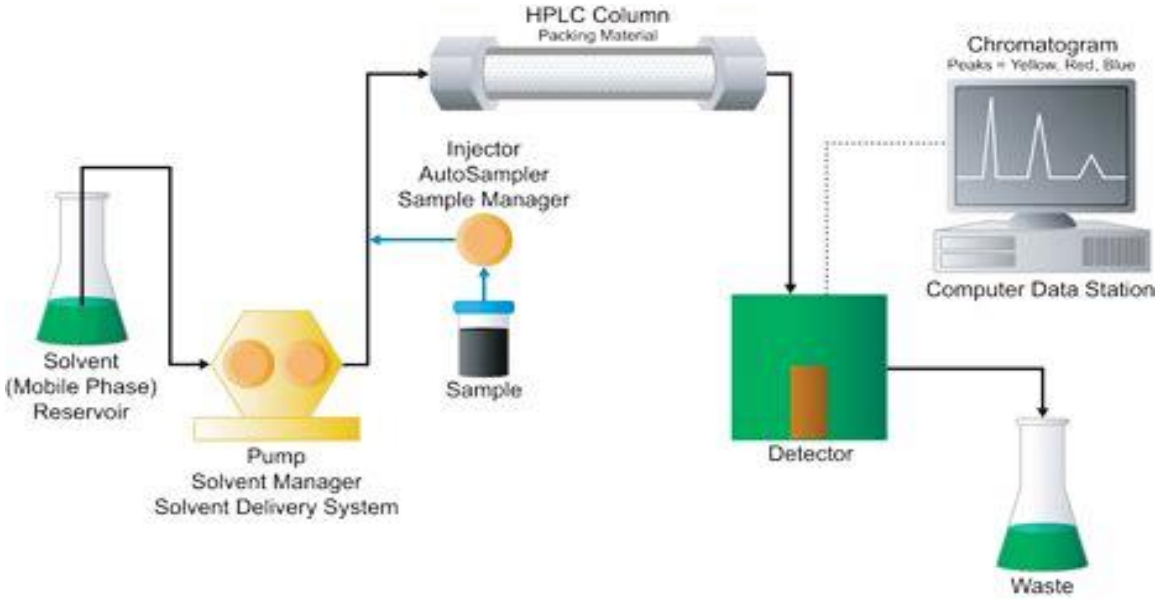

Figure 2. 14: A schematic diagram of the HPLC instrument

Source: (Dobson, 2016 september 09)

UV/Vis absorbance detector

There are three types of HPLC UV detectors which are a single wavelength, variable wavelength and photodiode array detector (PDA) (Snyder *et al*., 2012).

Single wavelength detector: the absorbance of only one given wavelength is monitored by the system at all times. In the 1970s there was only a single wavelength detector and there was no other option. The mercury lamp of a low vapor pressure was used as an optical source of a single wavelength detector and it consists of a strong line at 254 nm. It is a sensitive detector that has the ability to measure the subnanogram amount of an aromatic ring containing components. However in addition of phosphor lamp in the system, there are another two lines observed at a wavelength of 280 nm and 365 nm and at the addition of zinc lamp, the line was observed 214 nm (Dolan, 2016). Single wavelength is cheap and simple when compared to other UV detectors. It is limited in types of compounds that can be monitored and inflexibility (Swartz, 2010). Figure 2.15 shows the typical diagram of single wavelength detector.

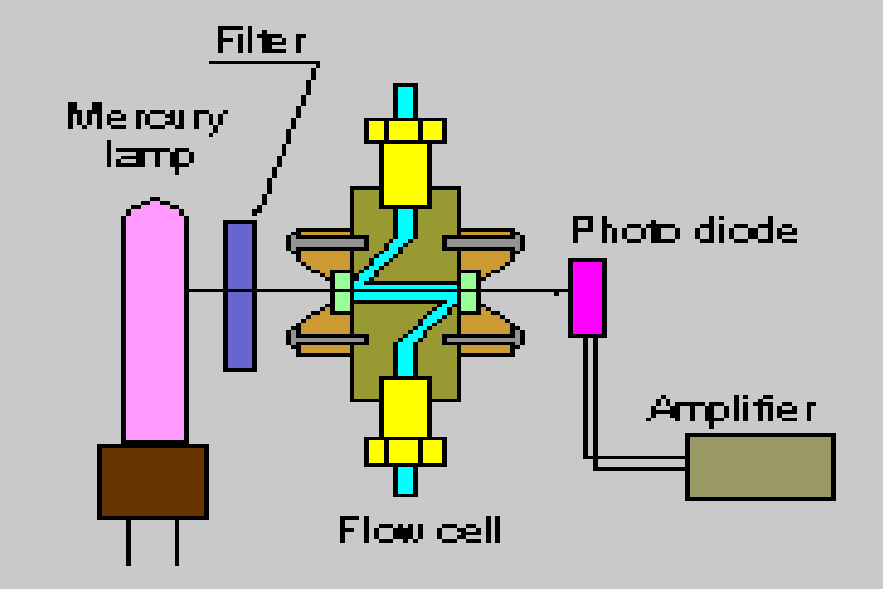

Figure 2. 15: Typical diagram of single wavelength detector

Source: (Dolan, 2016)

Variable wavelength detector at any given time, only a single wavelength is monitored, however, any wavelength in a wide spectral range can be designated. The monitored wavelengths range from 190-900 nm. It requires more advanced optics, used for a wider range of compounds and it is more expensive and versatile (Swartz, 2010). In a variable-wavelength, a deuterium lamp releases a light that passes over the slit onto a movable diffraction grating. From a movable diffraction grating white lights at different wavelength spreads. The rough surface is rotated to direct the chosen portion of the range over the added slit which consists of a slit width approximately 5 nm. From that point, the light is focused through the flow cell onto a photodiode. As the sample passes over the flow cell, the amount of transmitted light to the photodetector is diminished, and this change in transmittance is transformed into the detector output in absorbance units. Ordinarily, a beam splitter is involved, pointing part of the light to the next photodiode. This configuration permits the electronics to create corrections for vacillations in the lamp intensity (Dolan, 2016 August 01). Therefore, improve the instrument optical performance. Figure 2.16 shows the schematic diagram of the variable-wavelength detector.

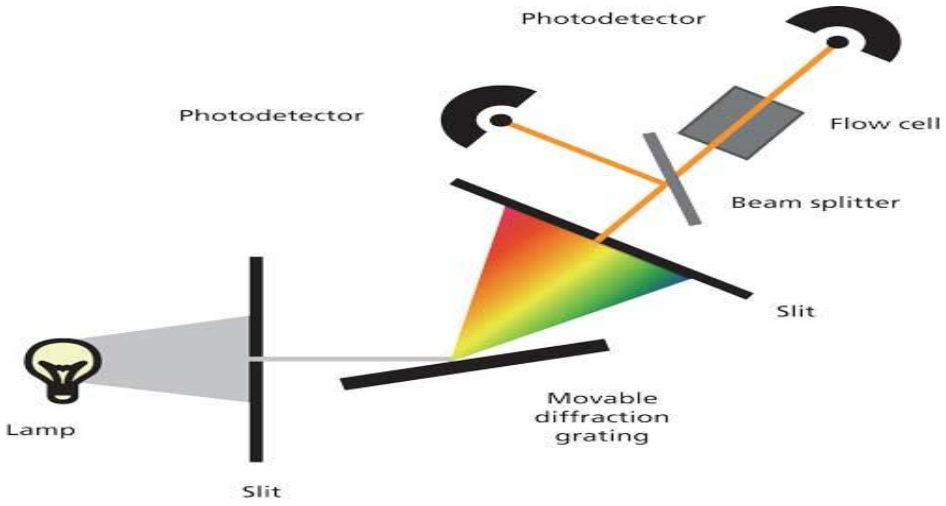

Figure 2. 16: Schematic diagram of the variable-wavelength detector

Source: (Dolan, 2016)

UV-PDA Detector differs from the other two UV-Vis detectors, light from the W and D_2 lamps is excelled straight onto the flow cell, light that passes through the flow cell is spread by the deflection grating, and the amount of the dispersed light is estimated for each wavelength in the photodiode arrays. PDA operates by simultaneously monitoring absorbance of solutes at several different wavelengths. It uses either a series or an array of several detector cells within the instrument, with each responding to changes in absorbance at different wavelengths (Swartz, 2010). Photodiode array detectors provide a good sensitivity throughout the UV/Visible spectral range and highly sensitive at a low light level (Abrahamsson *et al.*, 2018). The PDA detector illustration is shown in Figure 2.17.

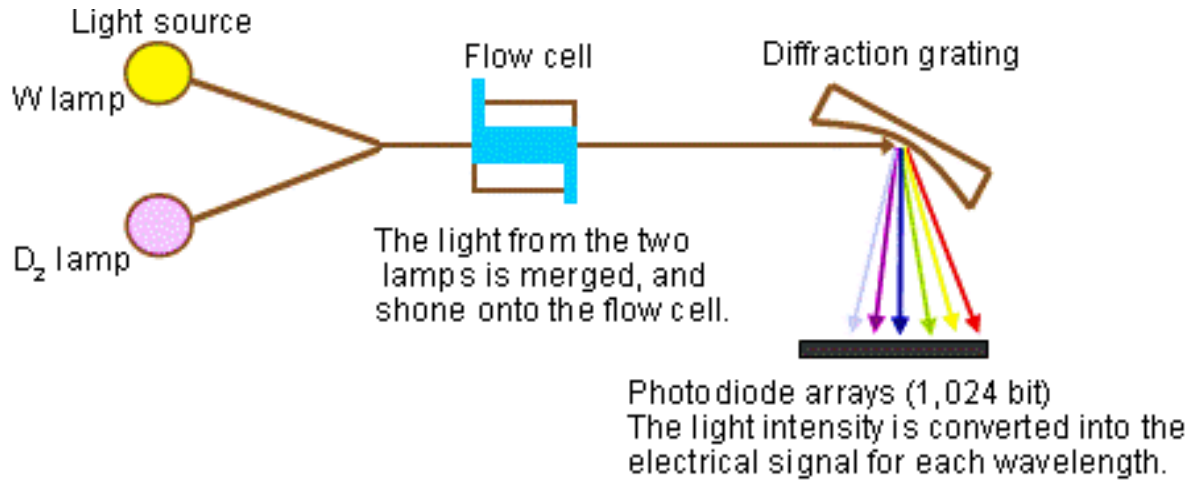

Figure 2. 17: Illustration of a photodiode array detectors detector

Source: (Abrahamsson, 2018)

Fluorescence detector (FLD)

Fluorescence is the most sensitive detector among the other existing detector HPLC detectors, it is approximately 10-100 times more sensitive than the UV-Visible detectors. In the flow cell, this detector can measure even a single analyte molecule. Flourescence is selective and specific among the other optical detectors. It measures the ability of eluting solutes to fluoresce at a given set of excitation and emission wavelengths as it intensity relies on both the emission and excitation wavelength (Dolan, 2016). It is specific for highly condensed molecules with conjugated pi-bonds especially aromatic compounds and others such as alicyclic and aliphatic compounds with highly conjugated double bonds fluoresce and carbonyl groups (Lingeman *et al*., 1985) figure 2.18 show Fluorescence detector.

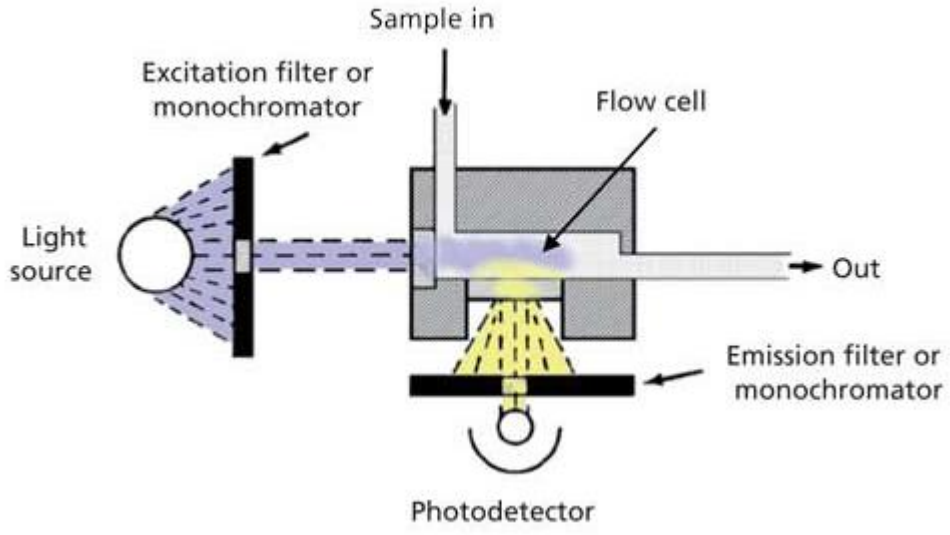

Figure 2. 18: Schematic diagram of fluorescence detector

Source: (Dolan, 2016)

2.10.1.2 Gas Chromatography (GC)

GC is a separation method that is normally used for volatile mixture separation. It is used for several fields including pharmaceuticals and other environmental toxins (Grob and Barry, 2004).

2.10.1.2.1 Principle of gas chromatography

GC is a separation technique where the mobile phase is gaseous and the stationary phase is classified into two, liquid and solid. Separation is achieved via two modes; volatility of the solute, i.e. boiling points or polarity. Helium gas is dominant over other gases because it has

a long range of flow rate, it is a safer gas in comparison with hydrogen and it is well suited for a large variety of detectors. The alternative gases used include Argon hydrogen and Nitrogen, but each is liable upon the detector utilized and the required performance. There are various injectors that are used in GC and the septum injectors are the most used. There are two classification columns for GC, which are the capillary column and packed column. The capillary column has got a coated stationary phase while for packed column consist of a solid substance that is finely inert, divided and which is covered with the liquid stationary phase (Karasek and Clement, 2012).

The separation of compounds is based on the diverse strengths of the interaction of a stationary phase with the compounds. The more the compounds interact with the stationary phase the stronger the interaction. Therefore, the compounds take long to migrate through the column resulting in long retention times. The poor interaction concerning the compounds and the stationary phase results into a quick migration of the compounds and shorter retention times, however, the separation is affected (Piantanida and Barron, 2014). GC can be operated either at isotherm or gradient temperature programs. Temperature programmed improves resolution and also decreases the retention times because it accommodates the separation of compounds with a variety of boiling points as it is consistently ramped. In isotherm, the temperature used is constant therefore it cannot be able to efficiently separate analytes with a broad series of boiling points and different polarities. At a high isotherm temperature, the quality of separation deteriorate and at a low isotherm temperature, the broad peaks are achieved (Karasek and Clement, 2012). The schematic diagram of the GC instrument is presented in Figure 2.19.

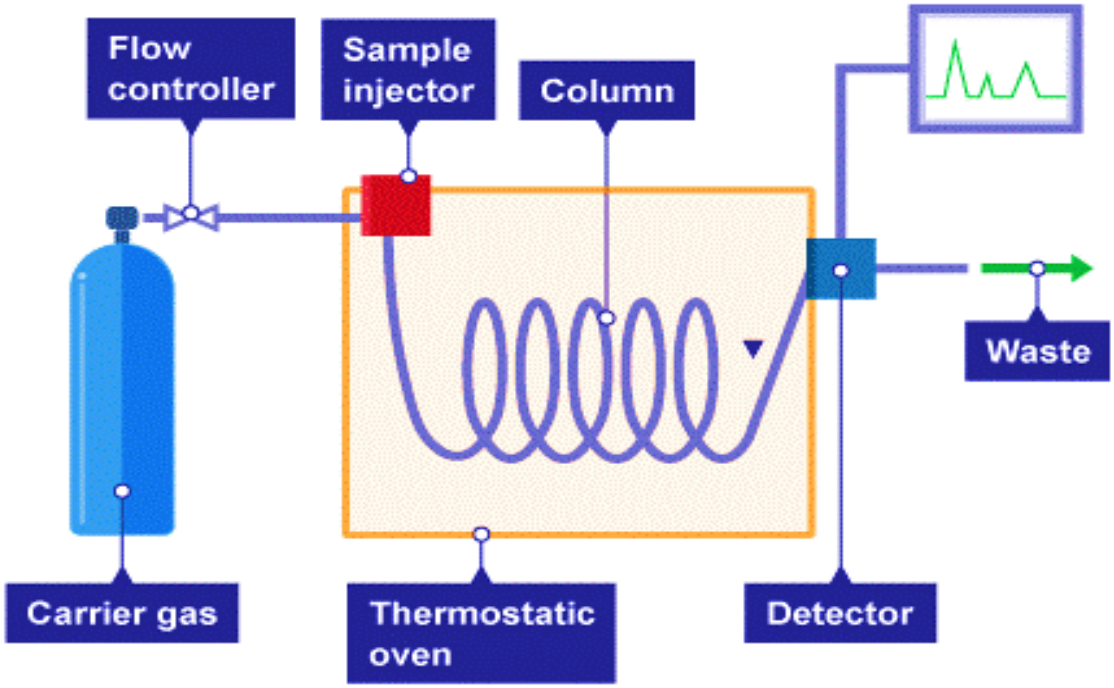

Figure 2. 19: The schematic diagram of the GC instrument

Source: (Dobson, 2016 September 09)

Flame ionization detector (FID)

FID operation is based on the chemical ionization of carbon-based substances burned in the hydrogen diffusion flame. The jet is fed with hydrogen gas and it is enclosed by purified air of high pressure in a coaxial (Zimmermann *et al*., 2002). The combustion decay of a carbon-based compound forms carbon-hydrogen radicals and allows the reaction of chemical ionization. The produced flame-induced is then measured. The flame is directly proportional to the process of the flowing compounds. Usually, the FID flow rate is 30 mL/min for both helium and hydrogen and 300-400 mL/min air (Amirav and Tzanani, 1997). FID has been used for the detection of triazines (Xiong and Hu, 2008). The advantages of FID are that it has a large linear dynamic range, it is a simple, robust operation and it has high sensitivity. However, it consumes a high amount of gas and thus expensive operation costs (Amirav and Tzanani, 1997). Figure 2.20 shows the typical diagram for an FID detector.

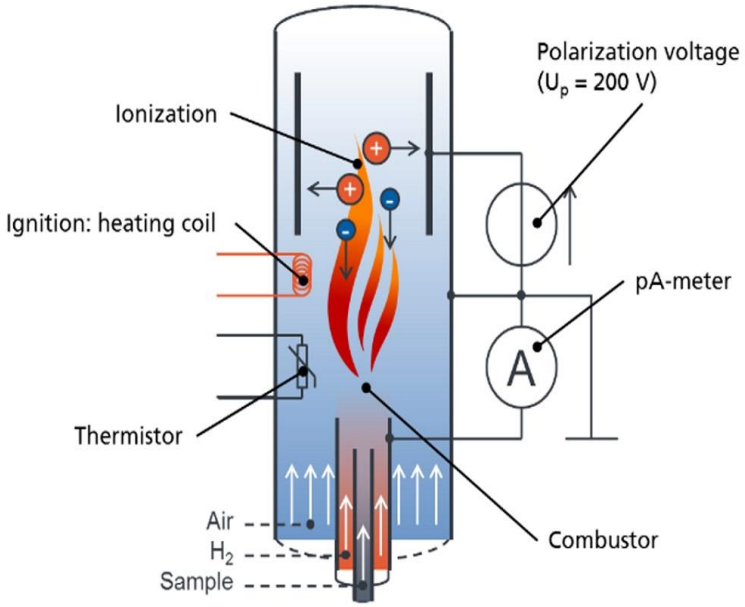

Figure 2. 20: Diagram of flame ionisation detector

Source: IKTS, 2019 February 07

Electron capture detector (ECD)

ECD is used for the detection of halogenated compounds, nitroaromatic compounds and other species containing the electron withdrawing functional groups (Kim *et al.*, 2008b, Poole, 2013). The sample eluate from a column is passed over a radioactive β emitter, usually nickel-63. An electron from the emitter causes the ionization of the carrier gas and the production of a burst of electrons (Poole, 2015). ECD sensitivity is estimated to 1000 times more sensitive than FID thus, it is often used for the analysis of triazines and other compounds in environmental samples (Muendo *et al*., 2012). ECD has been used in complex matrixes for the screening of chlorinated triazines. It has been reported to be sensitive to hydrocarbons, amine, and alcohols (Kim *et al*., 2008b). Figure 2.21 shows the diagram of ECD.

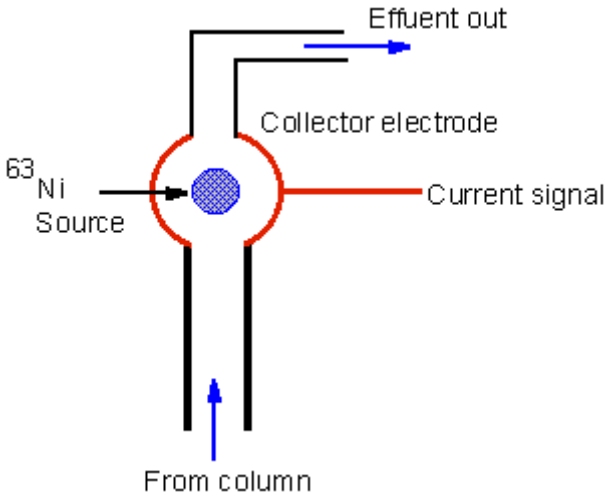

Figure 2. 21: Electron Capture Detector diagram

Source: (Singh, 2017 April)

Mass spectrometry detector (MS)

MS is a technique used to measure the *m/z* ratio of charged particles. MS is known as the better detector over the other GC detectors. In a GC-MS separation of compound is achieved by scanning compound`s mass until the separation is completed. After separation, the sample is then ionized and split into fragments, naturally by an electron-impact ion source or chemical ion source. In. In the process, the energetic electrons are used to bombard the sample. Energetic electrons ionize the molecule by allowing them to drop an electron due to electrostatic repulsion. Additional bombardment changes the ions into fragments. The ions are transferred into an analyser which sorts them according to their mass to charge ratio(*m/z*) (Skoog *et al.*, 2007). This detector has been used in the detection of triazines where better detection limits and improved signal/noise for target triazines were achieved (Cahill *et al.*, 2011). MS advantages include a quick data acquisition, ruggedness and simplicity, and analysis of limitless masses. However, it is not capable of separating compounds with the same *m/z* and molecular formula with low-resolution mass spectrometry also a peak broadening that limits resolution can be achieved due to the difference in ion velocity (Herbert and Johnstone, 2002, El-Aneed *et al.*, 2009).

2.12 Analysis of pesticides

SPE method has been used for the extraction of 20 pesticides in surface water using Oasis HLB cartridges (60 mg, 3 mL), (Peček *et al*., 2013). 3 mL methanol followed by 3 mL water was used as a conditioning solvent. The cartridge was loaded with 100 mL of the sample and the analyte was rinsed with water 3 mL and it was eluted with methanol 10 mL. GC-MS was used for the separation and determination of pesticides. The LOD and LOQ attained ranged between 0.001- 0.5 µg/L and 0.005-1 µg/L, respectively. The concentration obtained ranged between 0.224-3.509 µg/L (Peček *et al*., 2013).

A multiclass method for the determination of 70 pesticides using Strata TM-X SPE sorbent followed by GC–MS/MS has been reported by Donato *et al*., (2015). The cartridge was conditioned by 3 mL of methanol. 3 mL of ultrapure water was used to rinse the column followed by 3 mL of ultrapure water with the pH adjusted to 2.5. For elution of pesticides from cartridge was conducted using the mixture of dichloromethane: methanol (1:1 v/v) 2 mL LOD and LOQ found ranged from 0.006-0.15 µg/L and 0.02-0.5 µg/L respective. The recoveries ranged from 70-117.3% and RSD value of 19.7%. The obtained concentration range between <LOQ - 0.55 mg/L (Donato *et al*., 2015).

Oasis HLB® cartridge has been employed for pesticide determination in ground and surface water in Belgrade, Serbia (Dujaković *at al.,* 2010). The cartridges were preconditioned with methanol: dichloromethane mixture followed by deionised water 10 mL. It was loaded with 250 mL sample volume and washed with 5 mL distilled water. 10 mL methanol: dichloromethane mixture was used to elute the analyte. The SPE extracts were injected into LC-MS to separate the extracted compounds. The obtained recoveries, LOD, and LOQ ranged between 72–129%, 0.0004–0.0055 µg/L and 0.0011– 0.018.2 µg/L respectively. The obtained concentration in surface water range between 0.0059 -0.0178 µg/L and not detected in groundwater (Dujaković *et al*., 2010).

SPE with C_{18} as extraction sorbent followed by LC-MS/MS has been used for the determination of pesticides in wastewater effluent. The cartridge was conditioned with 3 mL methanol followed by 6 mL distilled water. A sample of 10 mL was passed through the cartridge and washed with methanol: water 10/90 v/v 10 mL. Analytes were eluted with methanol 5 mL. The obtained analyte recoveries were within the range of 80–95%, %RSD ranged from 3.2-8.2%. LOD ranged from 0.016 µg/L-0.017 µg/L and LOQ was found to be 0.05 µg/L (Cahill *et al*.,

2011). Demoliner *et al*., (2010) also used SPE with C_{18} sorbent for the extraction of pesticides in groundwater. The cartridge was conditioned with methanol 3 mL followed by ultrapure water 3 mL and acidified pH 3 ultrapure water 3 mL. A sample of 250 mL was loaded into a cartridge and the analyte was eluted with methanol 1 mL. Separation and quantification were performed using LC-DAD and LC-MS/MS. The obtained LOQs, RSD% and recoveries found to be 0.20 µg/L for all compounds, 1-20% and 60.3-107% in LC-DAD respectively. In LC-MS/MS, LOQs, RSD% and recoveries found at a ranged between 0.2-10.40 µg/L, 1-20% and 67-108% respectively (Demoliner *et al*., 2010).

HF-LPME technique has been used for pesticide extraction in tap water followed by GC-FID. The extraction solvent used was o-xylene at a stirring speed of 1200 rpm, extraction time of 35 min and the hollow fiber length of 1 cm. The LOD obtained were between 1.16-48.48 µg/L, RSDs ranged from 3.4-8.0, the enrichment factors were between 27 -530 and the concentrations ranged from 15-150 µg/L (Xiong *et al*. 2008). HF-LPME followed by LC/MS technique has been used for pesticide analysis of natural water. The extraction solvent used was n-octanol at a stirring speed of 100 rpm, extraction time of 120 min and the hollow fiber length of 35 cm. The LOD obtained were between 0.026-0.237 µg/L, RSDs were between 0.2-11.8, enrichment factors were approximately 2000% and the concentrations ranged from 1 to 1.27 µg/L (Trtic-Petrovic *et al*., 2010).

HF-LPME technique has been used for the extraction of pesticides and metabolites in soil and water samples followed by HPLC with fluorescence detection. The extraction solvent used was 1-octanol, agitation speed was 1440 rpm, hollow fiber length of 2.0 cm and extraction time of 30 min. The obtained recoveries ranged from 85-117 %. The LOD ranged from 0.0002 to 0.57 µg/L for water samples and 0.001 to 6.94 µg/kg for soil samples (Asensio-Ramos *et al*., 2012). Determination of atrazine, desethyl atrazine and desisopropyl atrazine in environmental water samples has been done using the HF-LPME technique followed by HPLC with diode array detector (DAD). The extraction solvent used was [bmim]PF_6 and extraction time was 20 min, stirring speed was 1000 rpm and hollow fiber length of 3.5 cm. The obtained recoveries ranged from 93.8 to 104.0% and LOD were 0.0001 µg/L. The concentrations quantified in the fish pond, river, irrigation, and wastewater are 0.00628 µg/L, 0.00439 µg/L, 0.00786 µg/L and 0.00577 µg/L, respectively (Peng *et al*., 2007). HF-LPME has been used for pesticide analysis of river water and sewage. The extraction solvent used was toluene with a magnetic stirring speed of 600 rpm, extraction time of 10 min and the hollow fiber length of 1 cm. GC-FID was

used for analysis. The LOD obtained were between 0.0081 to 0.0169 µg/L, RSDs were between 6.9 to 7.6%, enrichment factors were between 127 to 142%. The target analytes were not detected (Letseka and George, 2017).

UE method has been used to determine pesticides in sediment samples. A solvent mixture of dichloromethane-methanol (1:1) was used for extraction and extraction time was 45 min. It was then centrifuged for 10 min at 4000 rpm. The analysis was done by LC-MS/MS. The obtained recoveries ranged between 77-87% while the LOD and LOQ values ranged from 0.001-0.005 µg/kg and from 0.003-0.1 µg/kg. The obtained concentration ranged between 0.24-0.3392 µg/kg (Radovic *et al*., 2015).

UE and SE were used for the extraction of atrazine herbicide from the soil. Thin layer chromatography was used for separation of analytes and the spots were observed under UV light at 254 nm wavelength. Quantitative analysis was done by measuring absorbance using TLC scanner II UE was carried out with 250 mL of acetone for 15min. The obtained recovery was 103.5% and LOD was 0.005 µg/kg. For SE 250 mL of acetone, solvent was used and the extraction was carried out for 4 hours. The obtained recovery and LOD were 201.9% and 0.005 µg/kg respectively (Babić *et al*., 1998).

SE was used for the determination of atrazine in soil and sediment samples. Extraction was carried out with 250 mL of methanol for 8 hours. Separation and detection were performed by HPLC-UV. The obtained recovery and LOD were 87% and 0.078 µg/kg respectively. The obtained concentration was 0.74 µg/kg (Muendo *et al*., 2012).

Pesticides concentration has been measured in sediment samples by Soxhlet extraction. The extraction solvent used was hexane-acetone 1:1 (150 mL) for 16 hours in the water bath maintained at 60 degrees. The remaining extract was cleaned by a silica gel cartridge prior to GC-MS. The recoveries obtained were greater than 80.7%. The concentration obtained ranged between 0.02- 0.04 µg/kg (Lang *et al*., 2005).

MAE method has been used by Miyawaki *et al* (2017) to extract organochlorine pesticide and polycyclic aromatic hydrocarbons in soil and sediment. The identification/quantification system used was gas chromatography-mass spectrometry. 3 g of the sample was extracted with a 3:2 hexane-water mixture (10 mL) for 30 min at 120°C. The hexane extract was cleaned using

silica gel. The concentration obtained ranged from 9.2 to 408 ng/g. RSD was found at a range of 2.6 to 8.2% (Miyawaki *et al*., 2017).

LC-MS/MS was used for the separation and detection of triazines in soil sediment and sludge. Extraction was carried out by the QuEChERS extraction method. The obtained recoveries ranged between 62-75%, 69-72% and 82-96% in soil, sediment, and sludge respectively. LOQ were found to be 0.0026 µg/kg, 0.003 µg/kg and 0.005 µg/kg for soil, sediment and sludge respectively. The obtained concentration in soil was 0.00948 µg/kg and not detected in sediment and sludge (Masia *et al*., 2015).

GC-MS analysis was used for the separation and determination of pesticides in sediments. The extraction method was carried out with Soxhlet extraction and ultrasonic extraction. Soxhlet extraction was carried out with 250 mL solvent hexane: acetone mixture for 16 h. the extract was cleaned up by silica gel cartridges. The recoveries and concentrations obtained ranged between 80.7-96.1% and 0.12-119.13 µg/kg (Lang *et al*., 2005). For ultrasonic extraction, the 5 g sample was sonicated for 2 h using the 30 mL hexane: acetone solvent mixture. The soil mixture was by centrifuging for 10 min at 200 rpm. The obtained recoveries and concentrations ranged between 81.4-92.0% and the concentrations obtained ranged from 0.13-117.3 µg/kg (Lang *et al*., 2005).

Chapter 3 – Materials and Methods

This chapter presents the material and methods that were used to carry out this study. The sampling areas, sampling procedures as well as the sample pre-treatment, preparation and analysis methods followed are described in details. The quality assurance procedure followed is also discussed.

3.1 Analytical reagents

Simazine (98.7%), atrazine (97.4%), ametryn (98.5%), propazine (99.3%) and terbuthylazine (98.6%) were purchased from Sigma Aldrich (Riedel-de-Haen, Germany). All solvents used were of HPLC grade: acetonitrile (99.9%), acetone (99.8%), dichloromethane (99.8%) and methanol (99.9%) and they were also purchased from Sigma Aldrich and supplied by Honeywell (Steinheim, Germany). Formic acid (≈98%) was purchased from Fluka (Steinheim,

Germany). Oasis hydrophilic-lipophilic balance (HLB) cartridges, (60 mg, 3 mL) supplied by Waters (Milford, USA) were used as solid phase extraction sorbent.

3.2 Instrumentation

SPE vacuum manifolds purchased from Sigma Aldrich (Steinheim, Germany) were used for extraction and pre-concentration of pesticides from water and sludge samples. It was also used for the clean-up of sediments and soil extracts after SE and UE extraction. The vacuum pump connected to the SPE manifold was purchased from Edwards (Munic, Germany). Ultrasonic bath purchased from Science Tech (Durban, South Africa) was used for the extraction of pesticides from soil and sediment. Soxhlet extractor purchased from UKZN Glassblower (Pietermaritzburg, South Africa) was also employed for the extraction of pesticides in solid samples. Centrifuge purchased from Shalom Laboratory (Durban, South Africa) was used for the separation of supernatant liquid and solid. Buchi Rotavapor R114 purchased from Labotec (Flawil, Switzerland) was used to concentrate the extracts. 1 mm sieve purchased from Endecotts LTD (London, England) was used to sieve and homogenise the soil/sediment samples. Furnace purchased from United Science (Gauteng, South Africa) was used to determine the organic matter in soil/sediment samples. The analysis was performed using an LC 2020 system purchased from Shimadzu (Tokyo, Japan). It was connected to a quaternary pump, a degasser, auto-sampler and an LC-2030/2040 PDA detector (Germany, Europe). The chromatographic separation was performed on a Shim-Pack GIST analytical HP column C_{18} (3.5µm, 4.6 mm x 150 mm ID) purchased from Shimadzu (Tokyo, Japan) kept at 40°C. The mobile phase composition used was acetonitrile-water at a flow rate of 0.6 mL/min and the data was acquired at a detection wavelength of 223 nm. The LC gradient program followed was 0 – 10 min (45-55%, acetonitrile:water) and 10 – 25 (30-70%, acetonitrile:water).

3.3 Preparation of stock solution

The stock solution containing pesticides mixture was prepared by dissolving 10 mg of individual powdered standards in acetonitrile to make up a concentration of 100 mg/L. The stock solution was stored away from the sunbeams at 4°C in the refrigerator. Working standards ranging from 0.2 to 1 mg/L were prepared from the stock for calibration curves which were used for quantification purposes.

3.4 High Performance Liquid chromatography –Photodiode array detector (HPLC-DAD) method development

The HPLC-DAD method published by (Caldas *et al*., 2010) was adopted and further optimized. The method was optimised based on mobile phase composition, mobile phase flow rate and detector wavelength. 1 mg/L standard solution of analytes mixture was used for method optimization. After obtaining the optimum analysis conditions, standards with concentration ranging from 0.2 to 1 mg/L were analysed to calibrate the instrument.

The mobile phase composition was tested to achieve a good separation and elution of triazines analytes at a reasonable retention time.

The flow rate was monitored to identify the flow rate which is fast enough to give analytes enough time interaction with the stationary phase and not too fast or too slowly to result into poor separation or broad peaks and long retention time. The investigated flow rates were 0.3 and 0.6 mL/min.

Detector wavelength was investigated to determine the optimum wavelength which appropriately detects all the target analyte. The investigated wavelengths were 220 and 223 nm.

3.5 Sampling

The study area was KwaZulu-Natal which is one of the South African Provinces. The sampling areas were in Pietermaritzburg and Durban which are KwaZulu-Natal cities. KwaZulu-Natal is the second largest populated South African province with approximately 10.27 million people. Durban is the province's industrial and economic centre. It consists of most of KwaZulu-Natal's factories and it is one of South Africa's most important industrial regions. Its factories are mainly for clothing and textiles, food processing, sugar refining, chemicals, and oil refining. Pietermaritzburg is a provincial capital city. It also has a number of industries, including several footwear factories, aluminum plant, and food-processing plants (Britannica, 2017 April 19). Hence, these two cities were targeted to be investigated under this study.
Wastewater samples were collected in five wastewater treatment plants (WWTPs) including Darvill, Amanzimtoti, Umhlathuzana, Umbilo, and Northern. The four WWTPs are situated around Durban while Darvill is in Pietermaritzburg. The river water samples were collected in

the rivers where these investigated WWTPs discharged their treated effluent (Mbokodweni, Umhlathuzana, Umbilo, and Umgeni River) as well as along Msunduzi River (Camps Drift, College Road, Woodhouse, Bishopstowe. Water and sludge samples were collected in amber glass bottles. Soil and sediment samples were collected in an aluminum foil. The sludge samples were collected at Amanzimtoti, Northern and Darvill WWTPs. Sediment samples were collected at Camps Drift, College Road, Woodhouse, Bishopstowe, Mbokodweni River and Umgeni River. Soil samples were collected at Umgeni Valley, Curry Post, Donny Brook and Gilboa Farm which are agricultural lands. Water and sediment samples were collected during the cold season and hot season in order to investigate the seasonal effect on the concentrations of pesticides. Soil samples were collected in the hot season. The Global Positioning System (GPS) system was used to accurately appoint the sampling sites. The coordinates are given in Table (3.1).

Table 3.1: GPS coordinates for the sampling sites along Msunduzi River, Durban Rivers, WWTPs and agricultural areas around Pietermaritzburg

Sampling areas	Sampling points	GPS Coordinates
Msunduzi River (PMB)	Camps Drift	-29.630º - 30.365º
	College Road	-29.612º - 30.377º
	Woodhouse	-29.602º - 30.413º
	YMCA	-29.611º - 30.387º
	Bishopstowe	-29.618º - 30.447º
Durban Rivers	Mbokodweni	-30.307º - 30.997º
	Umhlathuzana	-29.873º - 30.879º
	Umbilo	-29.845º - 30.891º
	Umgeni	-30.195º - 30.999º
	Donny Brook	-29.885º - 29.905º

Agricultural areas (PMB)	Curry Post	-29.419º - 30.200º
	Gilboa farm	-29.260º -30.334º
	Mgeni Valley	-29.490° -30.274°
WWTPs	Darvill	-29.601º – 30.428º
	Amanzimtoti	-30.007º - 30.917º
	Umhlathuzana	-29.876º - 30.881º
	Umbilo	-29.845º - 30.891º
	Northern	-29.795º - 30.995º

3.5.1 Sampling areas

Msunduzi River

Msunduzi River has a length of 21.55 Km and it is meandering between the residential areas with a high population, therefore, lots of places along the Msunduzi River are polluted due to illegal disposal of waste as well as chemicals used in households (Openstreetmap Organisation, 2012 November 27). It has been reported that the Msunduzi River has many items floating in the water such as logs, plastic bottles, empty condom wrappers, twigs, headless chickens and shoes (Shamase, 2010 January 04). Another thing which has the influence in the cleanness of the Msunduzi River is the sewer pipes which burst and leak into the river (WWF Organisation, 2016 February 29). Camps Drift is situated up of Msunduzi River and it is located near the steel company (Hulamin) and other small companies. College Road is mainly residential areas and it is also surrounded by places like the football ground, turf on spot pitch and it is used for canoeing (WWFOrganisation, 2016 February 29). YMCA is located next to the bridge in the middle of the residential area, there are a Gym and petrol filling station around it. Woodhouse is near the golf course and many companies including the Tiger brand, Meadow feeds, Albany bakery, urban connate, etc. Bishopstowe is located down the Msunduzi River and it near the smallholding farms and residential areas. The Agriculturist might be using the triazine to control weed on the farm and residents might be using them in the household premises which could result in their presence in the river and WWTP. They can also be contributed by illegal dumping of containers that may be used to contain triazines from households. Figure A3.1 shows points along the Msunduzi River.

Umgeni Valley is the smallest area located in Pietermaritzburg. It is filled with a high population, as well as smallholding agricultural areas. It consists of shacks. Donny Brook, Carry Post, and Gilboa Farm are pine trees and timber farms. In Gilboa farm, there are other activities taking place including informal agricultural areas and timber farming(White, 2012 October 22)

Figure 3. 1: Sampling points along the Msunduzi River

Source: (Kunene, 2018)

In each and every treatment plant samples were collected in the influent (where water from domestic and companies' sources to be treated in WWTP comes in) and in the effluent (where treated water from treatment plant discharged out of the WWTP to the nearby river) to investigate the removal efficiency of triazines during the treatment process. The rivers where the effluents are discharged in were also investigated because it has been reported that triazines are not completely removed with the solid sludge during water treatment and they are resistant to biodegradation, hence they are discharged with the treated effluent to the rivers (Monteith *et al.*, 1995). Therefore, the aim was to investigate loads of pesticides contributed to the environment water from WWTPs. Also, sludge from WWTPs is used as bio-solid in croplands, therefore sludge samples were also analysed to determine the pesticides that can be transferred into croplands by bio-solids when they are applied (Kinney *et al.*, 2006).

Amanzimtoti WWTP

It is located in the middle of Southern N2 North and Mbokodweni in Isipingo, which is occupied by residential and industrial areas. This WWTP receives water from the industries (South gate and Prospecton) and semi-Urban areas (Isiphingo, Amanzimtoti, Folweni, KwaMakhutha, and Athlone park) (Madikizela *et al*., 2014). The treated effluent from Amanzimtoti is discharged in the Mbokodweni River (Madikizela and Chimuka, 2017). The sampling points are shown in Figure 3.2.

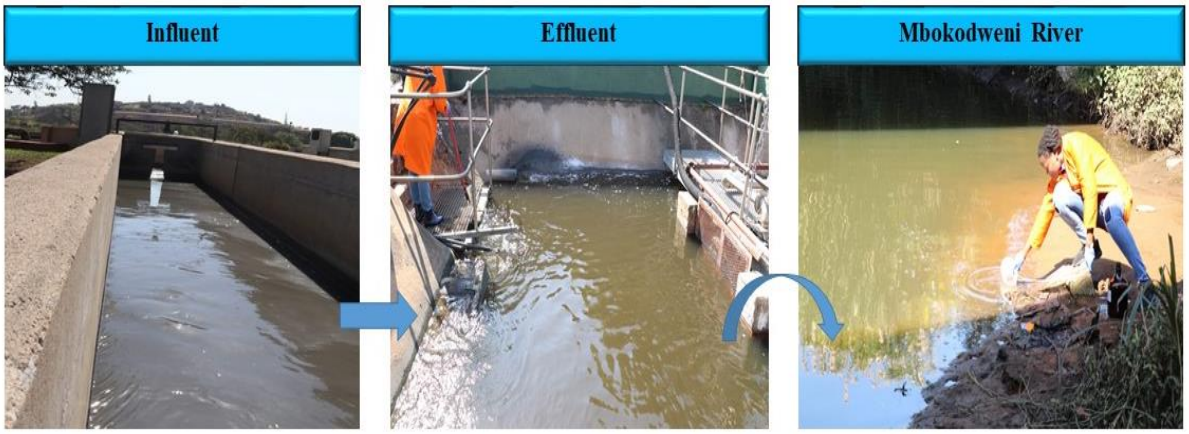

Figure 3. 2: Sampling spot in Amanzimtoti WWTP and the nearby river

Source: (Kunene, 2018)

Umhlathuzana WWTP

It is located along the Umhlathuzana River. This WWTP consists of two influent points, which are Marianridge and Shallcross. After the influents have been treated they are combined as one effluent which is then discharged into the Umhlathuzana River. Marianridge influent receives about 8 000 m^3/d wastewater from both sources. Marianridge receives wastewater from both domestic (70%) and industrial (30%). Whereas Shallcross receives about 2 000 m^3/d of 100% domestic wastewater (Madikizela and Chimuka, 2017). The Umhlathuzana River length and catchment areas are 50 Km and 113 Km^2, respectively. The sampling points are shown in Figure 3.3.

Figure 3. 3: Sampling point in Umhlathuzana WWTP and river

Source: (Kunene, 2018)

Umbilo WWTP

It is located in Pinetown, which one of the suburbs of Durban City. The treatment plant was designed to treat a capacity of approximately 10 000 m^3/day. After the treatment of wastewater, the effluent discharged into the Umbilo River, which is meandering in between the WWTP. Umbilo River sources are around Richmond Farm and they are joining the suburban area of Ashley and then meandering through Durban and Pinetown Queensburgh before canalized to Umbilo River. There are many industries around Umbilo WWTP including two mental finishing companies, two large textile companies, printing companies, storing dyes companies. The waste discharged from these companies can possibly disturb the performance of a WWTP as their discharged wastewater contains enormous amounts of organic compounds. Therefore, there are higher chances for the organic compounds not to be completely removed by WWTP (Pitts, 1993). The sampling points are shown in Figure 3.4.

Figure 3. 4: Sampling point in Umbilo WWTP

Source: (Kunene, 2018)

Northern WWTP

It is occupied by the industries (textile, detergents, pharmaceutical, constructions, petrochemical, and cosmetic) and domestic sources (Nzimande, 2014). It was designed to treat about 53 000 m^3/d wastewater. This WWTP discharge effluent to Umgeni River which has a catchment of 4416 Km^2 and a length of 225 Km. Umgeni River has undergone modifications to accommodate human activities such as commercial, large scale urbanization, and modification of river course (Abafe *et al*., 2018). The effluent is discharged in the Umgeni River. The sampling points are shown in Figure 3.5.

Figure 3. 5: Sampling point in Northern WWTP and Umgeni River

Source: (Kunene, 2018)

Darvill WWTP

Darvill WWTP is located in Pietermaritzburg near the New England landfill site and the sugarcane farms. It has a length of 1.58 Km (Eddy, 2010 December 12). WWTPs receive industrial and domestic wastewater, there is a high possibility of receiving amounts of triazines since they are active ingredients used in a household cleaning product and in other industrial processes. Darville WWTP discharges the effluent in the Msunduzi River which could transfer triazines into the river via effluent discharge. Also, sugarcane farms could possibly be treated with triazines to remove unwanted plants therefore, via crop runoff triazine residues can be transported into the river. The waste from the landfill site could also contribute via surface runoff. They can also be contributed by illegal dumping of containers that may be used to contain triazine from houses. The sampling points are shown in Figure 3.6.

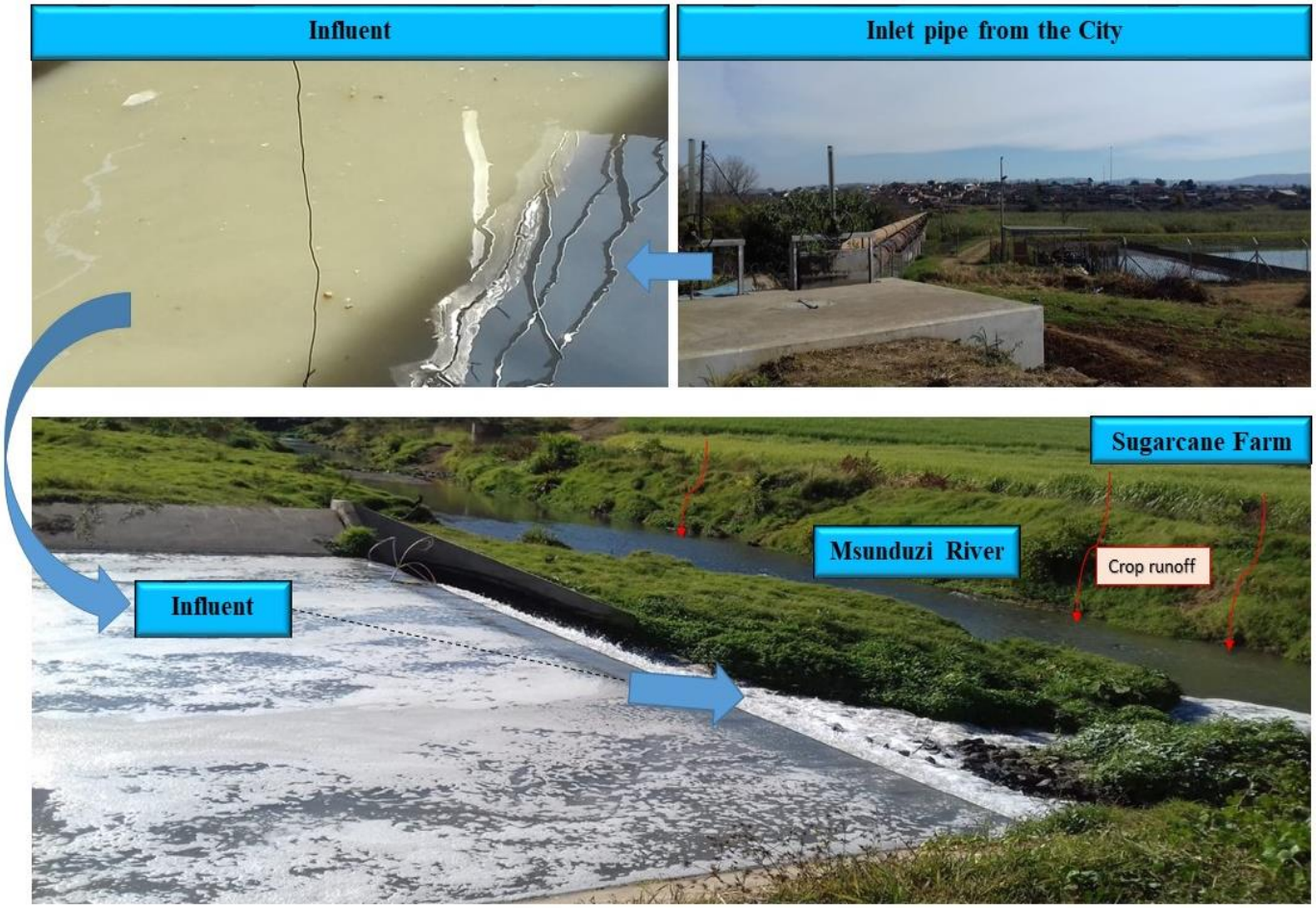

Figure 3. 6: Sampling spots in Darvill WWTP

3.6 Sample pre-treatment

Water samples were filtered through a 55 mm filter paper using a vacuum frit filter to remove particulate matter from the background matrix. Hence prevent blockage of SPE sorbent. Soil and sediment samples were air dried in a fume hood to remove moisture. They were then grounded using pestle and mortar and sieved using 1 mm sieve to remove the plants, roots, gravel and other wreckage and to remix the soil or sediment sample to guarantee homogeneity. This was done to increase the surface contact between the extraction solvent and sample (Azwanida, 2015).

3.7.1 Solid phase extraction (SPE) procedure

The Oasis HLB cartridge (60 mg, 3 mL) were used as SPE sorbent. The SPE sorbent was conditioned with 3 mL of methanol to allow effective interaction with the analytes. 100 mL of water sample spiked with pesticide mixture to make a final concentration of 7 µg/L was loaded into the cartridge to allow the analytes to be trapped by the sorbent. The impurities were washed

with 3 mL of distilled water and the trapped analytes were eluted with 7.5 mL of methanol. The eluent was reduced to 1 mL under a nitrogen stream and then analysed using LC-PDA.

Optimization of SPE

The method reported by Peček, (2012) was used as a starting point and further optimised to improve the efficiency of all the analyte of interest. The SPE parameters that were optimized were conditioning solvent and sample loading volume.

Conditioning solvent

Conditioning solvent was optimised to activate the functional groups of the sorbent and thus ensure consistent interaction between the sorbent and the analyte. The investigated conditioning solvents were methanol, dichloromethane, and acetonitrile.

Sample volume

A sample loading volume was investigated because pre-concentration factors should be as high as possible, however, there was a high possibility of losing the analyte when it is no longer retained by the sorbent. This occurs when the sorbent is saturated, the non-adsorbed analytes is washed away leading to error in the results. This shows that the breakthrough volume has been reached (Donato *et al*., 2015). The sample volumes investigated were 50 and 100 mL.

3.7.2 Ultrasonic extraction (UE) procedure

5 mL of water was added to 1 g of soil/sediment sample for hydration of the active site thus allowing the analyte to evenly dispense over the soil and interrelate with the active sites (Zambonin and Palmisano, 2000). The sample was then ultrasonicated in an ultrasonic bath for 15 minutes to allow the pesticide penetration into soil matrixes. 25 mL of the solvent was then added and further ultrasonicated for 15 minutes. The mixture was centrifuged for 5 minutes, the supernatant liquid was rota-vapored to 1 mL and then diluted to 100 mL with distilled water. Thereafter, the analytes clean-up was done using SPE.

Optimization of UE

The method published by Asensio-Ramos *et al* (2009) was used as a starting point and further optimized (Asensio-Ramos *et al*., 2009). The effect of parameters such as type of extraction solvent, solvent amount and the extraction time was examined. The recovery experiments were used to investigate the efficiency of the extraction procedure.

The extraction solvent was investigated because the extraction efficiency is influenced by the solubility of the target analytes into a solvent used. To determine the effective extraction, methanol, acetonitrile, and mixture of methanol: dichloromethane (1:1 v/v) were investigated.

Solvent volume is one of the parameters that need to be considered to ensure a good interaction of solvent and soil sample which results in effective extraction and hence higher recoveries of the analytes. The investigated volumes of a solvent were 15 mL, 25 mL, and 40 mL.

The extraction time was investigated to determine the optimum sonication time which is long enough to extract analyte completely but not degrade it. The investigated extraction times were 15 minutes, 30 minutes and 45 minutes.

3.7.2 Soxhlet extraction (SE) procedure

10 g soil sample was placed in a thimble which was then loaded into a chamber of Soxhlet extractor and placed into a flask containing 100 mL of methanol. Soxhlet was fitted with the condenser and refluxed at 85˚C for 24 hours. Thereafter, the extract was reduced to 1 mL using a roto-evaporator. It was then transferred into a 100 mL volumetric flask then top up with distilled water. SPE was then applied under optimum conditions for analytes clean up.

Soxhlet extraction optimization

Soxhlet extraction was adopted from Mutua *et al*, (2015) and further optimized. The optimised parameters were extraction solvent and sample wetting(Mutua *et al*., 2015).

Selection of extraction solvent

Extraction solvent was investigated to determine the solvent which can penetrate into soil matrices and dissolve target analyte to increase extraction efficiency. The investigated solvents

were methanol, methanol: acetonitrile mixture (50:50 v/v), methanol: acetone mixture (80:20 v/v) and the mixture of methanol: acetone (50:50 v/v).

Effect of sample wetting

The effect of sample wetting was investigated to determine if the addition of water can improve analyte transportation. Two experiments were done, in the first experiment the soil sample was extracted without water added. In the second experiment, 5 mL of the water sample was added before extraction.

3.8 Method validation

The optimized analytical method was validation based on linearity, precision, the limit of quantification (LOQ), the limit of detection (LOD), and recovery.

Linearity was estimated through the coefficient determination (R^2) of the analytical curves at concentration levels 0.2- 1.0 mg/L. The precision of the method was investigated with regards to repeatability and reproducibility and expressed as percentage relative standard deviation (%RSD). The precision of the extraction method was determined by repeating (n=3) extraction and the analysis of the same standard/extract. LOD and LOQ, which is defined as the lowest concentration of the analyte that can be detected or quantified with accuracy and precision were calculated using a signal to noise ratio (S/N) ratio of 3 and 10, respectively. Recovery investigation was done using distilled water or soil samples spiked with a known concentration of triazines.

3.9 Application to real samples

The methods were then applied to river water, wastewater, sludge, soil and sediment samples after optimization and validation.

Chapter 4- Results and discussion

Introduction

This chapter reports on the results and discussion of the HPLC-DAD technique, SPE, UE, and SE methods optimization, validation as well as their application to river water, wastewater, soil, sediment, and sludge samples.

4.1 Optimization of LC-PDA instrument

The analysis was performed using an HPLC-DAD instrument. The HPLC-DAD method was adopted from Caldas *et al*., (2010) and further optimized. Instrumental parameters such as the mobile phase composition, detector wavelength and flow rate were optimized in order to improve the instrument's limit of detection and quantification. The isocratic mode was first applied, where the flow rate of 0.3 mL/min, mobile phase composition (acetonitrile: water, 42:58%) and a detector wavelength 220 nm were used. Under these conditions, ametryn and propazine peaks did not completely separate and the analysis time was too long (47 minutes). Therefore, the gradient mode was applied and the mobile phase was programmed as 0 - 15 minutes (acetonitrile: water, 60:40%), 15 - 45 minutes (acetonitrile: water, 40:60%). The flow rate and detector wavelength were kept constant. These conditions resulted in the reduction of the analysis time to 26 minutes, however, the peaks of ametryn and propazine were co-eluting. The LC program was then changed to 0 - 12 minutes (acetonitrile: water, 40:60%), 12 - 16 minutes (acetonitrile: water, 50:50%) and 17 - 30 minutes (acetonitrile: water, 30:70%). The flow rate and detector wavelength were changed to 0.6 ml/min and 223 nm, respectively. These conditions separated all the peaks with the analysis time of 24 minutes. However, the first peak (simazine) eluted at 10 minutes which is a longer retention time for the first peak to elute, also the gap between simazine and atrazine retention times was longer ≈ 6 minutes. The conditions were changed to improve their separation, 0 - 10 minutes (acetonitrile: water, 45:55%) and 10 - 25 minutes (acetonitrile: water, 30:70%). The flow rate of 0.6 mL/min and 223 nm detector wavelengths were used. This resulted in better peak separation at a reasonable retention time of 25 minutes and was thus taken as the optimum instrumental conditions. The obtained chromatogram is shown in Figure A4.1.

4.2 Optimization of SPE method

4.2.1 The effect of conditioning solvent on the recoveries of the analytes

The conditioning step is where the selected solvent is passed through the SPE cartridge in order to wet the sorbent bed. This results in the activation of the sorbent's functional groups and thus increase the surface area available for the analytes to bind. Conditioning is a very essential step for the SPE method as it has an effect on the interaction of analytes with the SPE sorbent and hence affects the amount of analytes recovered. Therefore, the effect of the conditioning solvent was investigated using dichloromethane, acetonitrile, and methanol. 100 mL was used as the sample volume. The recoveries above 80% were achieved for all compounds with all the solvents investigated (Figure 4.1). Highest recoveries (107-111%) were obtained when methanol was used which indicated that it was more effective in activating the functional groups of the sorbent than dichloromethane and acetonitrile. This could be due to higher polarity and less viscosity of methanol compared to both other solvents. These properties resulted in it being more effective in penetrating through the sorbent to open up the pores which resulted in effective interaction with the analytes and thus increased the amount of analytes recovered (Masque *et al.*, 1998). Therefore, methanol was then selected as the optimum conditioning solvent.

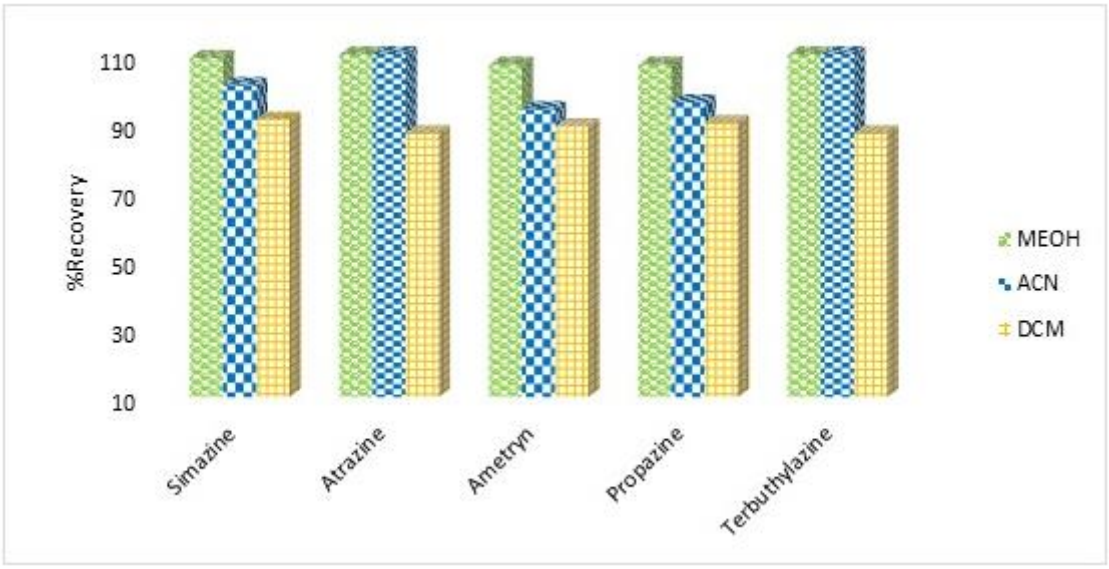

Figure 4.1: Effect of conditioning solvent on triazines recoveries.

4.2.2 The effect of sample loading volume on the analytes recoveries

During the sample loading, a liquid sample containing analyte is passed through the cartridge in order for the analyte to be trapped into the sorbent bed. Sample loading volume was therefore optimised to determine the breakthrough volume because when the breakthrough is reached the SPE sorbent can no longer retain the analytes. As a result, the analyte could be removed in the sorbent before the eluting step due to sample overloading (Dujaković *et al*., 2010). The sample loading volumes of 50 mL and 100 mL distilled water spiked at 7 µ were used in order to examine its effect on the recoveries of the analytes. Higher recoveries (107 – 111%) were obtained. The lower recoveries obtained when 50 mL sample volume was percolated through the sorbent could indicate that there was an insufficient amount of triazines available for interaction with the sorbent hence lower amount was recovered. The results are shown in Figure 4.2. Therefore, 100 mL sample volume was taken as a sufficient sample volume.

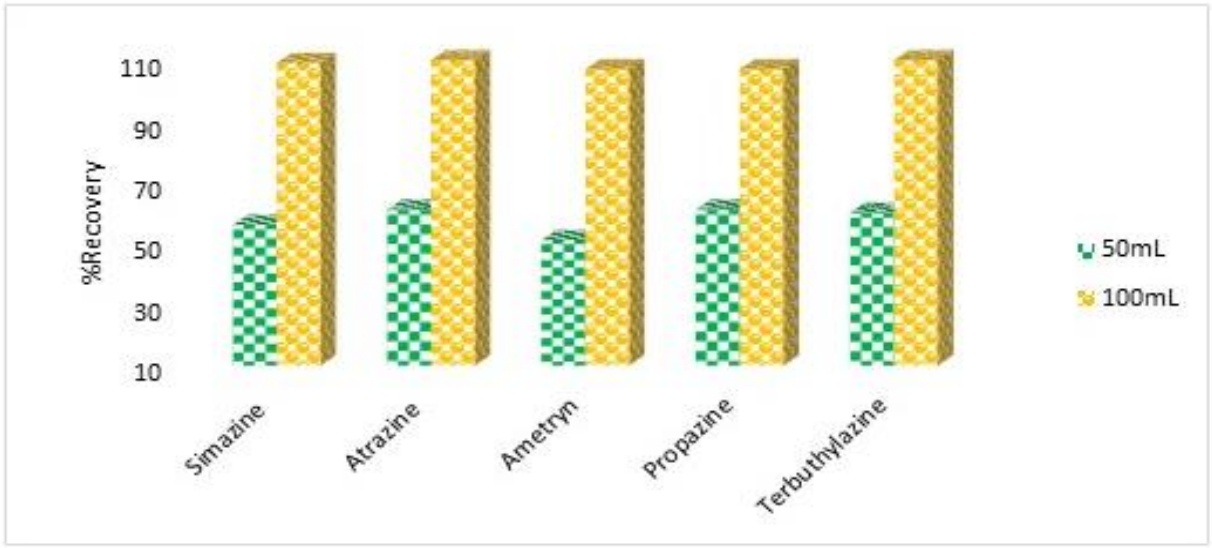

Figure 4. 2: Effect of sample loading volume on triazines recoveries.

4.3 Validation of the analytical method

To validate the optimized SPE/HPLC-DAD analytical method, linearity, LOD, LOQ, precision and recoveries were evaluated to ensure accurate quantification. The calibration curves for all analytes showed a good level of linearity with correlation coefficients (R^2) ranging from 0.9987 - 0.9995. The typical calibration curves are shown in Figure A4.2. The LOD and LOQ obtained ranged from 0.67 - 1.2 µg/L and 2.0- 3.5 µg/L. The recoveries were between 107-111% with the RSDs values of less than 6% which indicated good precision of the optimized method (Donato *et al*., 2015). The obtained results are shown in Table 4.1.

Table 4.1: The LOD, LOQ and recoveries, R^2 and %RSD values (n = 3) attained for SPE/HPLC-PDA

Compound	LOD (µg/L)	LOQ (µg/L)	% Recoveries and (RSD)	R^2
Simazine	0.77	2.3	109 ± 4	0.9993
Atrazine	0.67	2.0	111 ± 4	0.9995
Ametryn	1.2	3.5	107 ±4	0.9987
Propazine	1.1	3.2	107 ± 5	0.9988
Terbuthylazine	0.94	2.9	110 ± 5	0.9989

3.7 Sample preparation

Sample preparation is the most vital step in any analytical procedure. This step has an impact on accuracy and precision. The purpose of extraction is to remove analyte from the original matrix to an appropriate medium that can be easily introduced into the analytical instrument for analysis. Analytes are found at a very small concentration thus pre-concentration step is essential for the analyte quantification and detection. Also, the sample might have background components, therefore, the clean-up step is essential for the separation of the analyte of the interest. The extraction techniques used were solid phase extraction (SPE) for water and sludge samples, while ultrasonic (UE) and Soxhlet extractions (SE) were used for sediment and soil samples. SPE was also used as a clean-up step of extracts from SE and UE. All the extraction techniques were optimised before application to real samples in order to improve their extraction efficiencies.

4.4 Application to liquid samples

The optimised and validated SPE/HPLC-DAD method was then applied to wastewater and sludge samples collected from Darvill, Amanzimtoti, Umhlathuzana, Northern and Umbilo WWTPs and river water samples collected at Mbokodweni, Umhlathuzana, Umbilo and Umgeni River where the investigated WWTPs discharge their effluents as well as in five sampling points along Msunduzi River (Camps Drift, College Road, YMCA, Woodhouse, and Bishopstowe).

Physico-chemical properties

The physicochemical properties for all samples were measured using Bante900P multi-parameter water quality purchased from Bante instruments (Shanghai, China). The physicochemical properties of all samples were measured due to their effect on concentrations of triazines. Measured properties were conductivity, salinity, pH, dissolved oxygen, temperature and total dissolved solids.

Physico-chemical properties of water samples

The pH has an effect on the presence and concentration levels of triazines in water. A neutral pH does not have an effect on the triazines concentrations, however, the microbes do as their activity occurs at pH 7 which results in concentration reduction or absence of triazines, rather than that neutral pH has no effect. At an acidic media, the triazines protonate, and at a basic pH, the triazines hydrolyse which results in the reduction of triazines concentration or absence of triazines (LeBaron, 2011). The measured pH for all samples ranged between 5.9 - 8.2 (Table 4.2 – 4.4) which is within the acceptable limit of 5.5 - 9.5 (Weinberg and Teodosiu, 2012). Instability of pH has an effect on aquatic organisms, at alkaline pH (9), the fish membrane is denatured while at an acidic pH (below 4.5) the fish eggs do no hatch. Also, at a low pH, the organic substance decomposes and the metals that may contain toxins are released from rocks and all over the river, hence pose health risks in aquatic organisms (Dallas and Day, 2004).

The measured sample temperatures ranged between 10-24°C indicating that they were all within the acceptable limits as they are less than 35˚. High temperature has the ability to increase the degradation of triazines as a result triazine concentrations decreases (Nannou *et al*., 2015). High levels of temperature in water also stimulate the sludge decomposition, saprophytic bacteria multiply, fungi and sludge gas formation and the ingestion of oxygen due to decomposition processes as a result affecting the aesthetic value of waterway, therefore decreases triazines concentrations (Nannou *et al*., 2015).

Total dissolved solids (TDS) measured ranged between 566 - 829 mg/L which was within the allowable limit (< 1000 mg/L) and conductivity was between 1140 - 1657 µS/cm which was below the limits of 1700 µS/cm (Pitts, 1993, Wanda *et al*., 2016). High TDS and conductivity concentrations indicate high concentrations of pollutants (Nyoni, 2011). Water with high concentrations of TDS has been reported to cause mortality, coronary heart disease, cancer, arteriosclerotic heart disease, cardiovascular disease (Crittenden *et al*., 2012).

The salinity in (psu) unit was measured to determine the amount of dissolved organic compounds and salt (Zhang *et al*., 2012). It was found to range between 0.3-1.6 psu and were within the acceptable limit (≤ 1 psu) except in the Mbokodweni River (1.5 psu) and Amanzimtoti sludge (1.6 psu) where they were slightly higher. The high concentration of salinity increases the sorption of triazines and decreases their water solubility. Thus, the concentrations of triazines are expected to decrease in water due to being highly absorbed in sediments (Mao and Ren, 2010, Shen and Lee, 2002).

The measured dissolved oxygen (DO) ranged between 8 -19 mg/L which was found to be above the maximum limit of 8.14 mg/L as reported by South Africa water quality in most of the samples (Munyika *et al*., 2014). In all the investigated WWTPs it was observed that DO in influent samples was higher than in the effluent samples which could be due to the courtesy of the aeration process (Madikizela and Chimuka, 2017). High DO indicate a high population of microorganisms available, thus triazines concentrations are expected to be low due to microbial degradation, however, this can be dominant at pH 7 which is where the microbes are active, (Benvenuto *et al*., 2010). Low DO in water results into unsustainable aquatic life as well as the extreme algae growth caused by phosphorus and decomposition of submerged plants (Kramer, 1987).

Table 4.2: Physical properties of wastewater samples collected during cold seasons in Pietermaritzburg and Durban

Sampling Point		DO (mg/L)		Temp (˚C)		Salinity (psu)		TDS (ppm)		pH		Conductivity (µS)	
		Inf	**Eff**	**Inf**	**Eff**	**Inf**	**Eff**	**Inf**	**Eff**	**Inf**	**Eff**	**Inf**	**Eff**
Northern		10	18	18	19	0.4	0.3	450	276	8	7	899	568
Amanzimtoti		18	18	11	12	0.8	0.5	829	566	7	8	1657	1140
Umbilo		15	15	14	14	0.4	0.4	468	414	7	8	935	830
Darvill		13	14	24	23	0.4	0.3	387	312	7	8	773	751
Umhlathuzana	M	17	19	12	10	0.4	0.3	456	298	7	7	915	597
	S	15		15		0.3		262		7		528	

Eff – Effluent

Inf – Influent

M-Marianridge

S-Shallcross

Table 4.3: Physical properties of sludge samples collected during the cold season in Pietermaritzburg and Durban

Sampling Point	DO (mg/L)	Temp (˚C)	Salinity (psu)	TDS (ppm)	pH	Conductivity (µS)
Northern	16	12	0.4	466	6	932
Amanzimtoti	12	13	1.6	1551	7	3
Darvill Activated sludge	-	22	-	-	7	758
Darvill Digested Sludge	-	31	-	-	7	5

Table 4.4: Physical properties of river water samples collected during hot and cold seasons in Pietermaritzburg and Durban

Sampling Areas	Sampling Point	pH		Temp (˚C)		Salinity (psu)		TDS (ppm)		Conductivity (µS)		DO (mg/L)	
		Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Pietermaritzburg	Camps Drift	8	7	18	12	0.2	0.1	191	124	188	248	15	17
	College Road	8	8	19	145	0.1	0.1	82	118	194	235	16	17
	YMCA	8	7	18	13	0.1	0.1	89	120	190	241	14	16
	Woodhouse	8	8	15	19	0.1	0.1	76	110	201	220	14	12
	Bishopstowe	8	8	18	20	0.2	0.2	160	200	351	404	14	8
Durban	Mbokodweni	8	-	11	-	1.5	-	1503	-	3	-	17	-
	Umhlathuzana	8	-	11	-	0.2	-	225	-	451	-	18	-
	Umbilo	7	-	12	-	0.4	-	367	-	733	-	16	-
	Umgeni	7	-	12	-	0.6	-	639	-	1276	-	18	-

- = No sampling was done

4.4.1 Application to wastewater samples

Wastewater and sludge samples were collected in four WWTPs in Durban (Amanzimtoti, Umhlathuzana, Umbilo, and Northern) and Darvill WWTP in Pietermaritzburg during the winter season. The obtained results are given in Table 4.5. In most wastewater samples, simazine was found to be present and was quantified at higher concentrations than the other compounds. Simazine's highest concentration (28 µg/L) was found in Darvill WWTP effluent however, it was below the allowable limits (100 µg/L), (Edition, 2011). Simazine quantification could be due to its selective usage in the aquatic environment as it is used in the swimming pool to prevent the formation of algae (LeBaron, 2011). Higher concentrations of triazines obtained in Darvill WWTP could also be contributed by the agricultural activities that are taking place as the runoff from croplands results to higher concentrations of triazines in WWTPs (Ji *et al*., 2008). Also, the industries nearby Darvill that might be using triazines could contribute towards higher concentrations obtained. The second most detected compound was atrazine with the maximum concentration of 49 µg/L in Amanzimtoti effluent, which is above the allowable limit of 20 µg/L (London *et al.*, 2005). The high pollution in Amanzimtoti WWTP was also predicted by the higher amounts of TDS and conductivity measured in the sample as these parameters indicate the high concentrations of pollutants (Mahananda *et al*., 2010). WWTPs receive loads of domestic wastewater for treatment, therefore the presence of atrazine could be highly influenced by the products containing atrazine that are used in households such as sunscreen, ace itch bleach detergent powder, abhushane (Crittenden *et al*., 2012), jewelry, absorbine refresh mint natural body wash and leg brace AFM safe choice supper clean (Loraine and Pettigrove, 2006, Weinberg and Teodosiu, 2012). In all the samples analysed propazine was not detected. This could have been triggered by its selective usage. It was only registered for use in sorghum, however, it is also applied in other crops but not that much as those crops have their own standard pesticides used. For instance, propazine can be used in carrot, however, the specific herbicide which works well in carrot is terbuthylazine (LeBaron, 2011) and there are no/fewer farms that plant sorghum in KwaZulu-Natal, as a result, low concentration of propazine is expected.

Amanzimtoti WWTP was found to be the most polluted plant. It high pollution could be caused by the high yield of raw water received from the Nungwane dam which is 9.04 MI/day which is way greater than expected from each water resource (Umngeniwater, 2016). The concentration obtained in this work for simazine (28 µg/L) in Darvill WWTP and atrazine (49

µg/L) in Amanzimtoti WWTP are higher than those reported by Odendaal *et al.,* (2015) from the same treatment plants. The previously reported concentrations for simazine and atrazine ranged between 0.01 – 0.04 µg/L and 0.03 – 0.045 µg/L, respectively (Odendaal *et al*., 2015).

High concentrations are expected from the influent as it is receiving from different resources that may contain massive loads of these compounds. However, in this study high concentrations were obtained in the effluent than in the influent which was not expected. Therefore, the industrial and urban areas can end up receiving substantial loads of these compounds as they use the effluent from WWTPs (Petrović *et al*., 2003). The reason for the high concentration attained in effluent could be due to atmosphere deposition (Köck-Schulmeyer *et al*., 2013). However, some compounds were detected in the influent and not detected in the effluent. Lower concentrations in the effluent could be due to their retention in solid sludge as triazines have high Log Kow, which results in their high adsorption capacity to solid sludge. Hence they are removed during the treatment process when the sludge is removed (Goodwin *et al*., 2017). Higher concentration in the effluent (0.075-19.9 µg/L) than in the influent (0.00174 – 0.24 µg/L) has been reported by (Köck-Schulmeyer *et al*., 2013) which they associated sample preservation, sampling, atmosphere deposition and method biases (Köck-Schulmeyer *et al*., 2013). This indicated that herbicides are poorly removed in wastewater treatment plants compared to other compounds such as pharmaceuticals which are removed at a high rate (Kermia *et al*., 2016, Stamatis *et al*., 2010). It has been reported that the agricultural areas and WWTPs affect the natural aquatic environment as pesticides that escape from croplands or discharged by wastewater treatment plants enter the aquatic environment. Hence, it was significant to also analyse river water where the treatment plants discharge their effluent (Petrović *et al*., 2003).

The maximum concentrations of triazines that have been previously reported in WWTPs were 0.24 µg/L in the influent and 19.9 µg/L in the effluent in South Africa at Johannesburg (Köck-Schulmeyer *et al*., 2013). 0.026 µg/L in the effluent was reported in Germany (Münze *et al*., 2017), 0.020 µg/L in the effluent was reported in Italy, 0.210 µg/L and 0.29 µg/L in the effluent and influent, respectively were obtained in Spain (Benvenuto *et al*., 2010). The maximum concentrations of triazines quantified in South African WWTPs are higher than triazines concentrations quantified worldwide. However, they were below the MRLs except for atrazine in Amanzimtoti WWTP obtained in this work. The results reported indicate that triazines resist biodegradation during the water treatment process, hence they are discharged into the

environment with the effluent. These findings also imply that triazines need to be continuously monitored in the environment.

The removal rates of triazines were calculated using the following formula:

$R = \frac{Ci-Ce}{Ci}100$…… (1)

Where R is the removal of the rate (%), Ci is influent concentration and Ce is an effluent concentration in mg/L (Campo *et al*., 2013). Elimination refers to the change of a load of triazines in the inlet (influent) compared to a load of triazines in the outlet (effluent). The negative elimination efficiencies result if a load of triazines obtained in the inlet is low compared to the load obtained in the outlet (Kovalova *et al*., 2012). In this case, most of the triazines were detected at high concentrations in the effluent and therefore the negative elimination efficiencies were obtained. The highest negative values of removal rate (%) were obtained in Amanzimtoti WWTP where they were found at this range from -106 to -1920. Simazine was found to have negative elimination in all WWTPs and that could be due to that simazine does not readily absorb in organic matter and hence has higher chances to remain in water (Heri *et al*., 2008). Many compounds resulted in negative elimination efficiencies which indicated that the WWTPs are underperforming on removing these triazines, as a result of their ability to resist degradation. However, some removal of triazines occurred for terbuthylazine (65%) and ametryn (100%) in Amanzimtoti and Umhlathuzana, respectively. This indicated that some terbuthylazine and ametryn residues received in the influent were eliminated during the treatment process. The undefined results were obtained in cases where the compounds were quantified in effluent and not detected or quantified in influent. This occurred in Umbilo and Northern WWTPs, where simazine was detected in the effluent and not in the influent.

Table 4. 5: Concentration of triazines obtained in wastewater (µg/L) collected during the cold season (n = 3)

	Simazine		Atrazine		Ametryn		Propazine		Terbuthylazine	
Sampling sites	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Darvill	9.7±10	28 ±4	7.8±1	9.0±0.6	nd	nd	nd	nd	nd	nd
Amanzimtoti	8.2±1	17±0.3	2.5±1	49±1	6.2±6	17±2	nd	nd	8.0±2	2.9±0.1
Umbilo	nq	12.3±2	nq	nd	nd	nd	nq	nd	nd	nd
Northern	nd	5.0±1	nd	nd	nd	nd	nq	nd	nd	nd
Umhlathuzana	nq		nd		nd		nd		nd	
Marianridge		25±2		13±2		nd		nd		nd
Umhlathuzana Shall cross	17±3		nd		11±4		nd		nd	

Inf – influent, eff - effluent

4.4.1 Application to liquid sludge samples

The liquid sludge samples were collected at Darvill, Amanzimtoti and Northern WWTPs. The results are shown in Table 4.6. Simazine was present in all investigated WWTPs with the highest concentration at Darvill (2820 µg/L) which was above the MRL value (200 µg/L). Simazine and atrazine presence could be due agricultural activities around Darvill WWTP which results in a firm loads contributed from runoff and hence they could end up swiped into WWTPs (Heri *et al*., 2008). Simazine and atrazine presence in refractory to activated sludge has been previously reported (Monteith *et al*., 1995). It has also been reported that the triazines absorption into solid sludge during sewer purification is less than 40% hence, not all the triazines residues are expected to be removed with the solid sludge and hence they were detected in liquid sludge (Monteith *et al*., 1995).

Darvill WWTP was found to be the most polluted WWTP as all the compounds were detected, however, propazine and terbuthylazine were below the quantification level. This could be due to that they are not used as much as the other triazines as a result of their selective use (LeBaron, 2011). The concentrations for all quantified compounds in Darvill WWTP were above MRLs range which is between 66 - 200 µg/L. Since the sludge is removed at an earlier stage during wastewater treatment that could results in eliminating some triazines with it, which could be the reason for high concentrations detected in sludge samples (Benvenuto *et al.*, 2010).

Table 4.6: The concentration (µg/L) of triazine detected in activated sludge sample, n=3

Compounds	Darvill WWTP	Sampling sites	
		Amanzimtoti WWTP	Northern WWTP
Simazine	2820 ± 7	8.4 ± 4	nq
Atrazine	1380 ± 4	nd	nq
Ametryn	1070 ± 5	nq	nd
Propazine	nq	nd	nd
Terbuthylazine	nq	nd	nd

4.4.3 Application to river water samples

In sampling points along the Msunduzi River (Pietermaritzburg), Bishopstowe was found to be the only contaminated point with simazine (27 µg/L) and atrazine (65 µg/L), Table 4.7. This could be due to that Bishopstowe sampling point is after Darvill WWTP and the dumping site (New England Landfill site) which could play a role in increasing the pesticide concentration levels in the river via effluent discharge and runoff. Also, the agricultural areas around Bishopstowe could contribute to pesticides contamination via crop and surface runoff. In Durban, Rivers simazine was detected in all sampling sites, while atrazine was detected only in Umgeni and Mbokodweni (Table 4.7). More compounds were detected in the Mbokodweni River followed by the Umgeni River which could be due to illegal dumping of waste which could contain triazines near or in these rivers. Also, there many industrials and residential sites around the Mbokodweni River which could contribute these compounds. The results obtained agree with those previously reported by Rimayi *et al.,* (2018) in Johannesburg, where a higher concentration of atrazine was obtained in Jukskei River (923 µg/L), Kylami (0.210 µg/L) and N14 (0.923 µg/L). However, the concentrations obtained in this work are higher than the concentration obtained in Johannesburg. The triazines concentrations detected in the effluent were higher than those obtained in the rivers, where the WWTPs discharged their effluent into which could be due to dilution.

4.4.3.1 Seasonal effect on the detected concentrations of triazines

The seasonal effect on the levels of triazines concentrations was investigated in the samples collected along the Msunduzi River during the hot and cold seasons. High concentrations were detected in a cold season than in a hot season and this trend has been previously reported by

(Stamatis *et al.*, 2010). Only simazine and atrazine were quantified in Bishopstowe during the cold season. High concentrations of triazines in a cold season could be due to the cold weather ability to decrease triazines degradation (Nannou *et al*., 2015). Also, the water level is low during the cold season that could also increase the triazines concentrations due to pre-concentration (Du Preez *et al*., 2005). The increase in triazines concentrations in Bishopstowe could also be explained by the measured high conductivity and total dissolved solids which is one of the signals of pollution (Gakuba *et al*., 2018, Mahananda *et al*., 2010). Simazine was detected in all sampling points during the hot season while atrazine was detected in College Road and Bishopstowe, however, their concentration levels were below the quantification limits. Propazine, terbuthylazine, and ametryn were not detected in all sampling points. The reason for low concentrations could be due to rain dilutions (Masiá *et al*., 2013, Du Preez *et al., 2005)*.

The other reasons for low quantification could be due to triazines transformation which results in a decrease in concentration. Triazine compounds undergo degradation processes such as biodegradation, oxidation, hydrolysis, and photolysis, resulting to a dealkylation of the amino groups, dechlorination and consequent hydroxylation (Thurman *et al*., 1994). The dominant triazines transformation is via the abiotic and biotic mechanisms. The transformation products in surface and groundwater through biotic mechanisms are the dealkylated chloro metabolites, for example, desethyl-terbuthylazine, deisopropyl-atrazine, desethyl-atrazine, and desethylterbumeton. The major abiotic degradation product in water and soil are hydroxy-simazine, hydroxy-atrazine and hydroxy-terbuthylazine (Benvenuto *et al*., 2010, Gasser *et al*., 2007). The recorded pH for all samples was approximately 7 indicating a neutral pH. At neutral pH the microbes are active, therefore there are higher chances for microbial degradation to occur, which could result in the reduction or absence of triazines (Oh *et al*., 2016, LeBaron, 2011).

Table 4. 7: Triazines concentrations (n = 3) obtained in river water from Pietermaritzburg during cold and hot seasons.

Triazines	Concentration (µg/L)									
	Camps Drift		College Road		YMCA		Woodhouse		Bishopstowe	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Simazine	Nd	nq	nd	nq	nd	nq	nq	nq	27 ± 1	nq
Atrazine	Nd	nd	nd	nq	nd	nd	nq	nd	65 ±5	nq
Ametryn	Nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
Propazine	Nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
Terbuthylazine	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Higher concentrations were obtained in Durban Rivers (Table 4.8) than in Pietermaritzburg Rivers (Table 4.7). In Durban Rivers, simazine was quantified in all sampling points and it was followed by atrazine. The possible reason for high concentration obtained could be due to many industries situated near the rivers, more activities taking places such as commercial, modification of river course, the large scale of urbanization and quarrying operations. Hence, they could contribute the pollutants into the rivers.

The maximum concentration of triazines (65 µg/L) obtained in river water in this work is lower than the concentrations obtained in Johannesburg (923 µg/L) by (Rimayi *et al*., 2018). However, it is higher than the levels reported in Nigeria (0.43 µg/L) by (Ogbeide *et al*., 2015) and in Kenya (0.14 µg/L) by (Muendo *et al*., 2012). In Europe, the reported concentration is 0.008 µg/L (Radovic *et al*., 2015), while in Italy it is 0.10 µg/L (Benvenuto *et al*., 2010) which are lower than the results obtained in African countries. The obtained results indicated that African countries are more polluted than in overseas countries. This could be triggered by that Africa is a developing country and it has a high population, therefore larger amounts of triazines are applied in croplands in order to obtain high yield and to protect crops from pests.

Table 4.8: Triazines concentrations obtained in river water from Durban during the cold season (n = 3)

Sampling Sites	Concentration(µg/L)				
	Simazine	Atrazine	Ametryn	Propazine	Terbuthylazine
Mbokodweni	3.0 ± 0.09	5.9 ± 1	nq	nd	nd
Umhlathuzana	9.4 ± 2	nd	nd	nd	nd
Umbilo	3.2 ± 1	nd	nd	nd	nd
Umgeni	18 ± 2	5.2 ± 1	nd	nd	nd

4.5 Optimization of ultrasonic extraction

4.5.1 The effect of extraction solvent on the recoveries of the analytes

The solubility of the target analytes in the solvent used for the extraction process is known to influence the recoveries of the analytes from the solid samples, also the analyte and solvent polarities play a role in the recoveries (Annegowda *et al*., 2012). Therefore, it is important to investigate the type of solvent that could allow high recoveries of the analytes. The extraction solvents that were examined for their effect in the recoveries of the analytes were methanol, acetonitrile, and mixture of dichloromethane: methanol (1:1 v/v). Based on the results obtained, the mixture of dichloromethane: methanol showed to be more effective and gave recoveries ranging from 62-71% (Figure 4.3). These higher recoveries could be due to the mixture of solvents polarities as methanol is more polar and dichloromethane is least polar, hence their different polarities were able to accommodate the various polarities of the analytes. This allowed the efficient extraction for both more and less polar analytes. The mixture was therefore taken as the ideal extraction solvent.

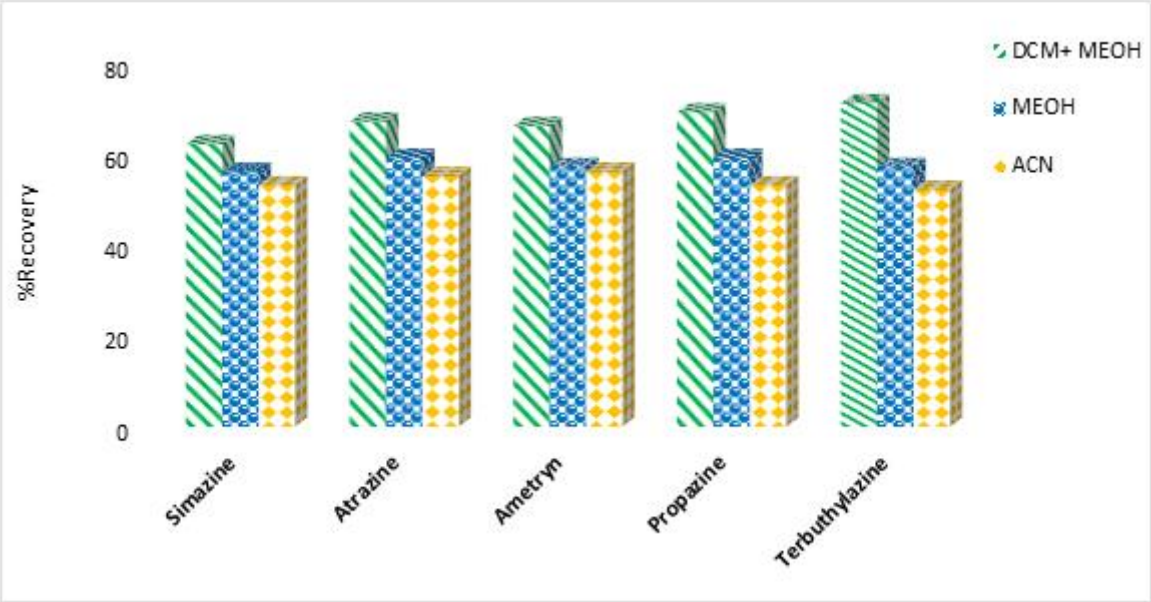

Figure 4.3: Effect of extraction solvent on the recoveries of triazines

4.5.2 The effect of extraction solvent volume on the recoveries of the analytes

The aim of the extraction method's optimization is to obtain the high extraction efficiency for the analytes with the small amount of solvent and at a minimum period of extraction time. Therefore, the effect of dichloromethane: methanol (1:1 v/v) solvent volume was studied using 15 mL, 25 mL, and 40 mL, the other parameters were kept constant. From the results obtained 15 mL solvent volume gave lower analytes recoveries which could be due to poor mass transfer of the analytes from the sample to the solvent as a result of a small quantity of the solvent (Silva *et al*., 2005). The increase in solvent volume has been reported to increase the analytes mass transfer from the soil sample to the solvent, however, the contact between the soil sample and the solvent need to be considered. The increase in solvent volume from 15 mL to 25 mL resulted in an increase in the recoveries of the analytes. However, the recoveries decreased with a further increase to 40 mL solvent volume which could be due to poor interaction between the soil sample and the extraction solvent. The soil sample settled at the bottom of the flask while the solvent impartially floats which caused the ineffective interaction between the soil and the solvent. This resulted in the poor transfer of the analytes from the sample to the solvent and hence low analytes amount was recovered. This effect could be the reason why multiple extractions in each sample using smaller portions of solvent are performed to allow good interaction between the solvent and the soil (Kim *et al*., 2008a). To confirm this statement, 40 mL solvent volume was halved into two portions (20 mL×2) and the same procedure was used to carry out the extractions for both portions. Recoveries between 48 - 71% were obtained

which were higher than those achieved by 1 x 40 mL of solvent volume (45 – 51%). However, they were not higher than the recoveries obtained by 25 mL (Figure 4.4). This observation is in agreement with the previously reported study where the increase in extraction solvent volume increased the recoveries of the analytes but further increase in solvent volume resulted in decreased recoveries (Babić *et al*., 1998). Therefore, 25 mL was taken as the best solvent volume.

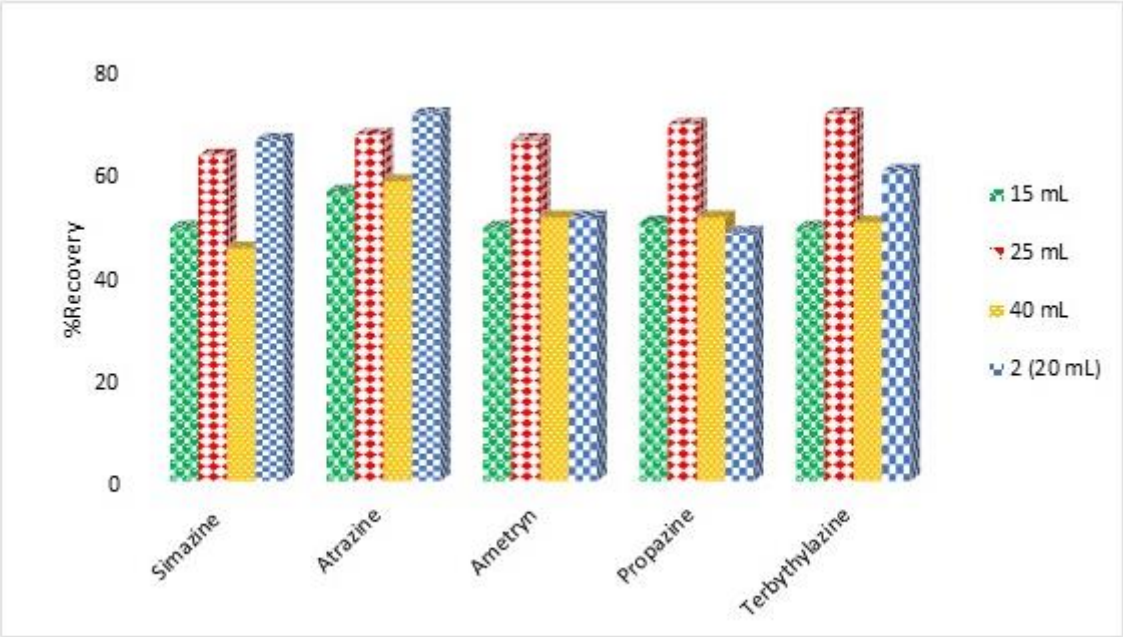

Figure 4. 4: Effect of solvent volume on the recoveries of triazines

4.5.3 The effect of extraction time on the recoveries of the analytes

The extraction time has an influence on the amount of analyte extracted, however, when extraction time is too long it can result in the degradation of the analytes. 15 minutes, 30 minutes and 40 minutes were therefore employed to investigate the effect of extraction time on the analytes recoveries. The results showed an increase in the recoveries with an increase in extraction time from 15 - 30 minutes and then decreased at 40 minutes (Figure 4.5). The lower recoveries obtained at 15 minutes could be due to that it was not long enough to permit effective interaction between the soil and the solvent and a hence lower amount of the analytes was transferred to the solvent. The higher recoveries obtained at 30 minutes could be due to the extraction time was long enough to allow the solvent to penetrate into soil matrices and breakdown the soil aggregates and thus removed the analytes into the solvent. The lower recoveries at 40 minutes could be due to degradation of the analytes as a result of prolonged

extraction time (Naczk and Shahidi, 2006). A similar trend was observed in the previous study where longer extraction time resulted in degradation of the analytes and hence lower recoveries (Babić *et al*., 1998). At 30 minutes extraction time, all analytes were attained at high recoveries ranging between 75 - 100%, and it was therefore chosen as the optimum extraction time.

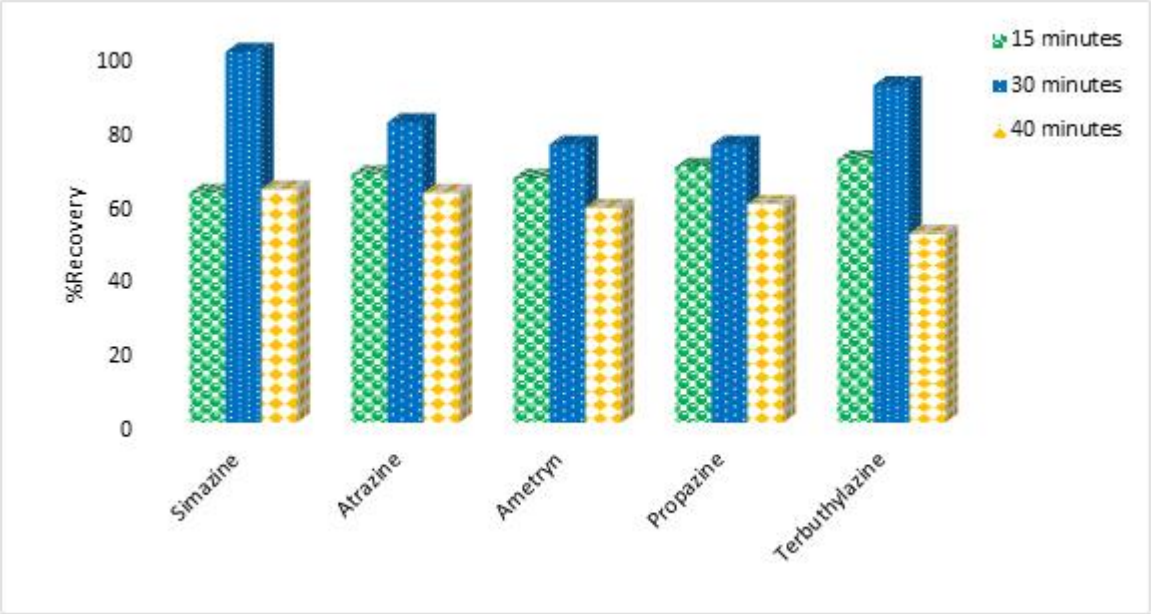

Figure 4.5: Effect of extraction time on the recoveries of triazines

4.6 Optimization of Soxhlet extraction

4.6.1 The effect of extraction solvent on the recoveries of the analytes

The extraction solvent was investigated in order to obtain the appropriate solvent that will effectively leach into the soil matrix and adequately remove the analytes of interest and thus increase the analytes recoveries. The solvents that were explored are methanol, acetonitrile: methanol (1:1 v/v), methanol: acetone (1:1 v/v) and methanol: acetone (4:1 v/v). Methanol alone gave recoveries between 71 - 87%. Lower recoveries were obtained with a mixture of acetone: methanol (20:80 v/v) and acetonitrile: methanol (50:50 v/v) extraction solvents (Figure 4.6). The lower recoveries obtained with the mixture of acetone: methanol (20:80 v/v) could be due to lower vapor pressure which results from the mixture of two organic solvent. Soxhlet extraction principle considers the vapor pressure and boiling point of the solvent for effective extraction and low vapor pressure increases chances to obtain low recovery of the analyte. In the case of mixture acetonitrile: methanol (50:50 v/v) low recoveries could be due to slightly low polarity and high viscosity of acetonitrile compared to methanol, this could be the reason for low removal of triazines from the soil matrix. This is due to that triazines strongly

bind to soil due to their high octanol-water partitioning coefficient (Kotowska *et al*., 2012), therefore, the viscous solvent could result in low analyte transportation and hence lower recoveries (Elbashir and Aboul-Enein, 2015). Methanol was then used as the optimum extraction solvent.

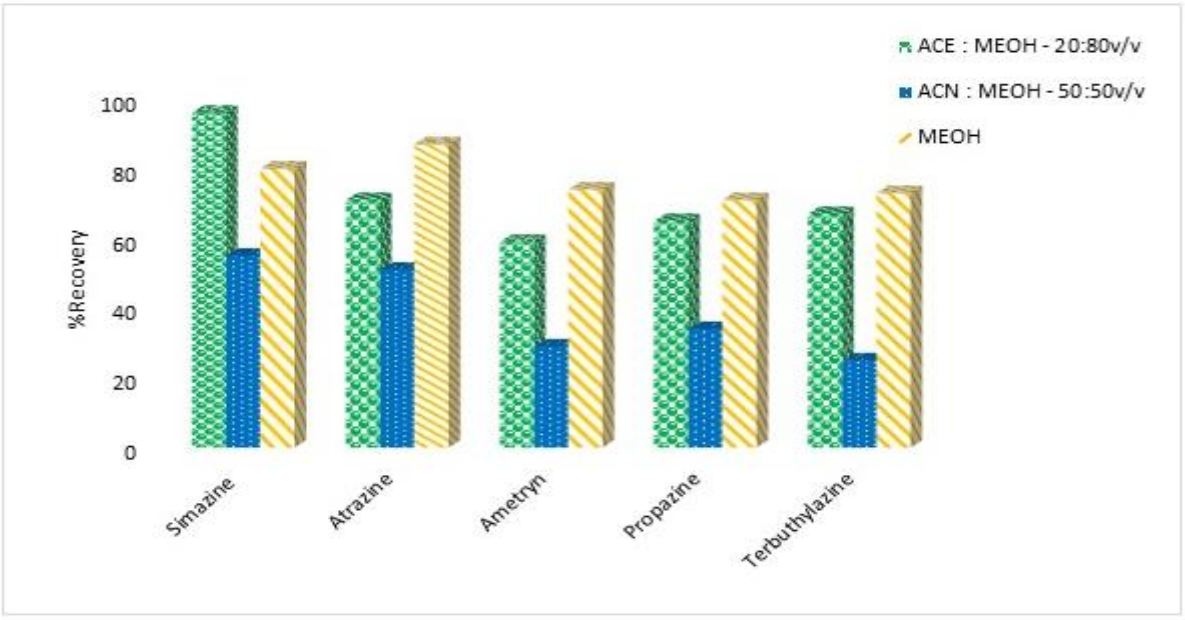

Figure 4.6: Effect of extraction solvent on the recoveries of triazines

4.6.2 The effect of sample wetting on the recovery of the analytes

The wetting step was done in order to hydrate the active site of the soil thus allowing the analyte to evenly dispense over the soil and interrelate with the active sites (Zambonin and Palmisano, 2000). The effect was examined by adding 5 mL of distilled water before transferring the soil sample into the thimble. The sample with added water gave lower recoveries of the analytes which could be due to higher polarity of water that was used for wetting compared to methanol solvent that was used for extraction, (Figure 4.7). This indicated that triazines preferred to remain in water than being transferred to methanol. The hydrophobic and hydrophilic characteristics of the triazine compounds are indicated by water solubility. The position 2, 4 and 6 of the substituents are accountable for the solubility of the triazines but generally, the triazines are soluble in neutral water. In the sample with water added, the formation of hydrogen bonds by water molecules and the nitrogen atom lone pair occurred, resulting into the hydrophilic triazine ring and hydrophobic nucleophilic alkylamino side chain in position 4 and 6. This resulted in the strong binding of triazines to the soil and hence the water molecules

outcompete the methanol as water is more polar than methanol and the investigated triazines are also polar (LeBaron, 2011).

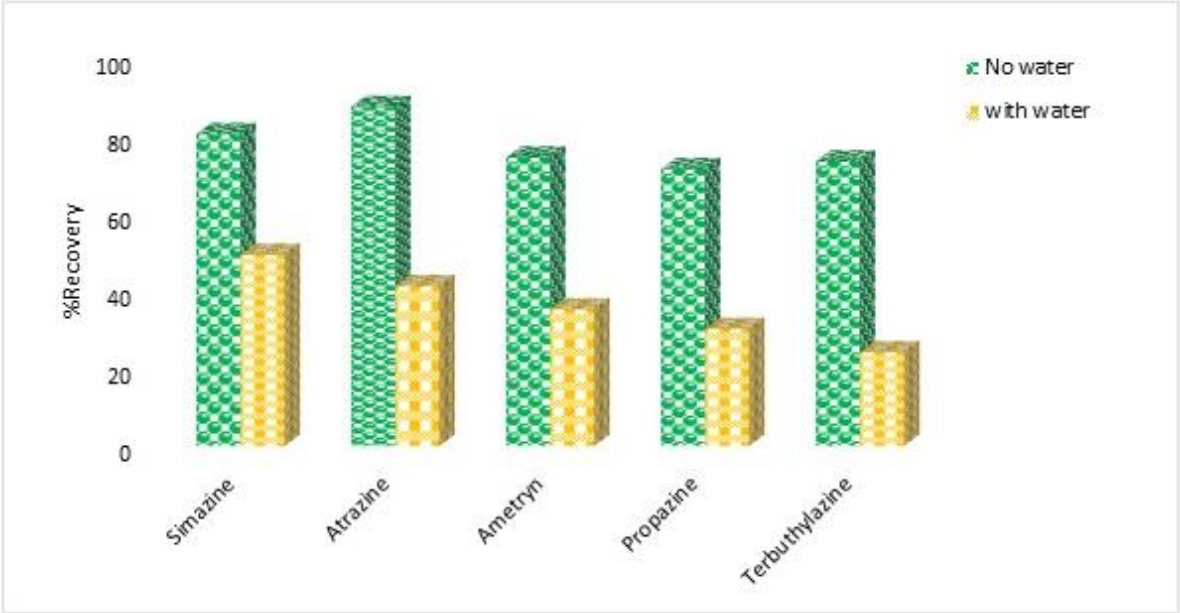

Figure 4. 7: Effect of sample wetting on the recoveries of triazines

4.7 Methods validation

The intra-day precision was achieved by performing three replicate analyses on the same day. The inter-day precision was achieved by performing three replicate the analysis in three different days. Standard deviation (% RSD) ranging between 0.1 to 5 and 2 to 7 for intra and inter-day, respectively were obtained for UE and 2-7 for SE which are in the desirable range as they are less than 20% (Radovic *et al*., 2015). The obtained recoveries of the methods were between 71 - 87% and 75- 100%, for SE and UE method, respectively. The recoveries obtained for SE and UE are comparable and only simazine and terbuthylazine were high in UE. This could indicate that the mixture of dichloromethane and methanol was more effective in extracting these compounds than methanol alone that was used in SE. The LOD and LOQ ranged between 1.0 - 2.0 µg/kg and 3.2 - 6.1 µg/kg, 0.092- 0.22 µg/kg and 0.280- 0.69 µg/kg, for UE and SE, respectively. The LOD and LOQ obtained indicated that the developed methods are sensitive and hence will be able to detect the target analytes at lower concentration levels real samples. In comparison, the LOD and LOQ obtained for SE are lower compared to those of UE, indicating that SE is more sensitive than UE. The results are summarised in Table 4.9

Table 4. 9: The LOD, LOQ, recovery and RSD% value (n=3) obtained from soil samples

Compounds	Ultrasonic extraction					Soxhlet extraction				R^2	MRLs
	LOD	LOQ	%Recovery	%RSD Intra-day	%RSD Inter-day	LOD	LOQ	%Recovery	%RSD Intra-day		
Simazine	1.8	3.7	100	5	0.1	0.12	0.37	80	6	0.9993	200
Atrazine	1.0	3.2	81	4	0.1	0.092	0.28	87	4	0.9995	66
Ametryn	2.0	6.1	75	1	0.3	0.20	0.63	74	7	0.9987	200
Propazine	1.8	5.6	75	0.1	0.7	0.22	0.69	71	2	0.9978	200
Terbuthylazine	1.1	3.5	91	3	1	0.18	0.55	73	4	0.9989	7

4.8 Application of the optimized UE method to soil samples

The optimized UE method was applied for the extraction of triazines in soil samples from agricultural lands (Umgeni Valley, Gilboa Farm, Curry Post, and Donny Brook) as well as along the Msunduzi River (Camps Drift, College Road, Woodhouse, and Bishopstowe).

Physical properties soil and sediment samples

The measured Physico-chemical properties of soil and sediments are presented in Table (4.10 and 4.11). The pH of the soil samples from Curry Post, Donnybrook and Umgeni Valley and sediment sample from Mbokodweni were found to be at acidic media with pH less than 6. Hence, triazine concentration levels were expected to be low due to protonation. Whereas in other soil and sediment samples pH was neutral, therefore, concentrations were expected to be found at their original levels unless if microbial degradation occur. The temperature measured in all the samples was between 19 - 23˚C and they were within the acceptable range between 10 ˚C and 35.6˚C (Florides and Kalogirou, 2005). A temperature range of -28 ˚C to 10 ˚C has been reported to have an influence in the respiration of microbial. It increases decomposition and extracellular enzyme activity that enhance breakdown organic matter and increases mineralization of nitrogen and rate of microbial respiration in soil, as a result, the concentration of triazines decreases (Onwaka, 2016). High-temperature soil improves plant roots growth due to an increase of plants metabolite activities, whereas at the low temperature they behave otherwise. Hence at low soil temperature triazines concentrations increase due to low metabolite activities that can play a role in reducing their concentrations (Onwaka, 2016).

The concentration of salt and other inorganic compounds which are expressed as salinity was practically measured and found to be between 0.01-0.45 psu which indicated that there was less dissolve salt in all of the investigated samples. Salinity results in flocculation which is a positive effect in terms of stability, root growth, and soil aeration. However, at high concentrations (8-15 psu), it can have a lethal effect (Warrence *et al*., 2002).

The TDS measured was found at a range of 24-476 mg/L, which were below the highest range of 750-1500 mg/L. TDS concentration is used to indicate the broad arrays of pollutants in water as they are transported into the rivers through run off to where they result in the death of microorganisms (DeZuane, 1997). This can result in the presence of high concentrations of triazines in sediment samples due to limited microbial degradation (Donati and Funari, 1993).

The conductivity of the soil was found at a range of 46-581 µS, which was within the acceptable limits of 14-1288 µS (Visconti and de Paz, 2016). The high concentration of conductivity above the limits has been reported to have an effect on soil texture, the productivity of crops and organic matter levels. Thus decreases the concentration of triazines in soil and sediment as it affects the organic matter (Jimoh and Mohammed, 2014).
DO in the soil is crucial for plant and animal health as the depletion of dissolved oxygen in soil results in micro-organism suffocation (Morgan, 2000). The measured DO was found at a range of 3 - 10 mg/L. In Donny Brook, Woodhouse, Umgeni Valley, and Curry Post, the DO was found to be below the acceptable range which is 8 - 35 mg/L, (Scott and Evans, 1955).

Organic matter refers to carbon-based compounds that are generated from organism remains such as animals, plants and their waste products. It is essential for nutrient (nitrogen, potassium, and phosphorus) movement in the environment and also plays an important role in retaining water in the surface of the earth (Shahid and Hussain, 2019). The organic matter measured in the collected samples ranged between 6-37 and 1-5 for soil and sediment samples, respectively. The organic matter has an effect on the sorption capability of triazines in soil/sediment. Therefore, the soil/sediment samples with a higher amount of organic matter are expected to have a higher amount of triazines.

Table 4. 10: The physical properties of the soil samples collected in the hot season

Sampling Point	pH	Temp (˚C)	Salinity (psu)	TDS (ppm)	Conductivity (µS)	DO (mg/L)	Organic content
Camps Drift	8	23	0.14	145	291	9	6
College Road	6	23	0.17	175	351	8	6
Woodhouse	7	23	0.11	115	230	2	2
Bishopstowe	7	23	0.11	114	229	8	7
Donny Brook	5	22	0.02	23	52	4	12
Gilboa Farm	8	18	0.10	26	401	10	37
Umgeni Valley	4	21	0.01	25	45	4	11
Curry Post	5	20	0.03	24	46	3	19

Table 4.11: Physical properties of river sediment samples collected during hot and cold seasons in Pietermaritzburg and Durban

Sampling Point	pH		Temp (°C)		Salinity (psu)		TDS (ppm)		Conductivity (µS)		DO (mg/L)		Organic content
	Cold	Hot	cold	Hot	cold	Hot	cold	Hot	Cold	hot	Cold	hot	
Woodhouse	7	6	21	22	0.07	0.04	75	34	206	68	10	10	5
Bishopstowe	7	7	21	23	0.5	0.04	476	39	1057	77	10	8	4
Umgeni	7	-	21	-	0.1	-	105	-	222	-	10	-	5
Mbokodweni	6	-	22	-	0.2	-	303	-	581	-	10	-	1

- Samples were not collected

In most of the sampling points, simazine was detected with the highest concentration in Gilboa Farm (1017 µg/kg) which was above the maximum residue level (MRL) value of 200 µg/kg (Bol'shakov *et al*., 2014). This high concentration could be due to the pre and post emergency application of simazine (LeBaron, 2011). These results were predicted by the low temperature measured in the Gilboa Farm sample which indicated that there was no or low photolysis that occurred resulting in no degradation of triazines and hence high concentrations were observed. High pollution was also indicated by the high conductivity (401 µS) measured which was higher than in other sampling points. However, the conductivity was within the acceptable limit of 700 µS, (Mahananda *et al*., 2010). The other reason for high concentration could be the high organic matter which was measured in Gilboa Farm soil sample as it has been reported that triazines retain in the soil with high organic matter (Shahid and Hussain, 2019). Also, the sampling was done in a hot season (spring) which is when these compounds are often applied. The obtained results for the analyzed samples are given in Table 4.12.

In a study conducted to examine the absorptivity of atrazine and simazine in soil, it was observed that a high amount of triazines were adsorbed in the soil with organic matter than that without organic matter (Amadori *et al*., 2013, Dunigan and McIntosh, 1971). In sampling points along the Msunduzi River, Bishopstowe and by Woodhouse had higher concentrations of triazines that were detected. In Bishopstowe, higher concentrations may be possibly due to contribution from agriculture lands and a dumping site (New England fill site) around the sampling point could contribute high concentrations in. In addition, simazine is applied in the vineyard during the hot season, which could be the reason for its high concentration quantified in soil. Atrazine and simazine quantification at a high concentration than the other analytes could also be due their often used during the hot season. Atrazine has been recognized as one of the best two herbicides that play a major role in the production of corn during the hot season in South Africa. This could, therefore, be the reason for the high concentration of atrazine detected in Woodhouse and Bishopstowe (Du Preez *et al*., 2005).

Ametryn, propazine, and terbuthylazine were not detected in all the samples analysed. The reason for triazines not to be detected in the hot season could be that in a hot season the UV light levels are higher and hence the soil temperature is high due to the long sunshine period. High-temperature soils results in the increase in pesticides breakdown and their persistence in the soil are reduced (Ehrig *et al*., 1991). The other reason for ametryn not to be detected could be due to that its low absorption in soil (Lin *et al*., 2018). Triazines undergo a different transformation in the soil which results in complex metabolites, hence their concentrations

decrease. The pH of the samples collected in Donny Brook, Umgeni Valley, and Curry Post were acidic (Table 4.10), therefore the reason for no triazines detected in these samples could be due to their protonation (Fuscaldo *et al*., 1999). In addition, the soil surface site could also be the reason for not detected, as triazines tend to bind strongly into soil matrices if the functional groups of the compound match with a surface site of the soil. For instance, polar functional groups of the compound strongly bind if they are in match with the polar or ionic surface sites of the soil (LeBaron, 2011).

The maximum concentration reported for terbuthylazine in Germany is 0.056 µg/kg (Modrá *et al*., 2018), whereas in Spain the maximum concentration reported for terbuthylazine is 0.00948 µg/kg (Masia *et al*., 2015). In China, the maximum concentration obtained for atrazine was 0.00230 µg/kg (Wang *et a.,l* 2011), while the other compounds were not detected. These reported concentrations are lower compared to those obtained in this current study which indicates the importance for continuous monitoring of these compounds in South Africa and to have the MRL values set specifically for SA.

Table 4.12: The concentrations of triazines detected in soil samples (n = 3)

Sampling sites	Sampling sites	Detected concentration (µg/kg)				
		Simazine	Atrazine	Ametryn	Propazine	Terbuthylazine
Agricultural areas (PMB)	Donny Brook	nd	nd	nd	nd	nd
	Umgeni Valley	nd	nd	nd	nd	nd
	Gilboa Farm	1017±7	nd	nd	nd	nd
	Curry Post	nd	nd	nd	nd	nd
Msunduzi River (PMB)	Camps Drift	17±6	nd	nd	nd	nd
	College Road	nd	nd	nd	nd	nd
	Woodhouse	87±9	34±7	nd	nd	nd
	Bishopstowe	245±5	19±4	nd	nd	nd

nd – not detected

4.9 Application of the optimized UE and SE methods to sediment samples

The optimized UE and SE methods were applied for the extraction of triazines in sediment samples collected from Mbokodweni, Umgeni, Bishopstowe and Woodhouse Rivers. Samples were collected in winter and spring seasons referred to as cold and hot, respectively.

During the cold season, the concentrations of triazines detected in sediment ranged between 1.1 - 123 µg/kg, whereas in a hot season they were between 4.3 - 35 µg/kg (Table 4.13). The presence of triazines in sediments could be due to that triazines are likely to bind to organic matter since they have lower water affinity (high lipophilic and hydrophobic character) due to the stronger carbon to chlorine bond of the chlorinated pesticides (Kumar *et al*., 2013).
In this study, triazines exhibited higher concentrations in the cold season compared to those quantified in the hot season. Triazines concentrations in the environmental media have been reported to be dependent on desorption and absorption in sediment based on temperature changes. Low temperature and low rainfall in the cold season result to low water flow which could be the measure influence of the high concentrations in cold season as no photo-degradation and dilution occurs (Cheng *et al*., 2007). Also, the application of triazines is recommended during the hot period due to high temperatures, which increases the triazine breaking down reaction resulting in a low risk of accumulation. These could be the reasons for more compounds quantified during the hot season, as corn and vineyard which are normally treated with triazines are planted in the hot season (Ehrig *et al*., 1991).

Maximum concentrations of simazine were detected in Mbokodweni sediments during the cold season, while Woodhouse and Bishopstowe were found to be the most polluted sampling points. The reason for high concentrations could be due to illegal dumping near the rivers as the dumped garbage could contain triazines contaminants as they are used in different cases as the active ingredient. Triazines are used as coupling agents for the synthesis of the peptide in the solid phase, also in solution as the side chain of anti-biotic in Pharmaceutical industries. They are also used in WWTPs as disinfectants (Nyoni, 2011), and hence they are discharged with the effluent into the rivers. They are used as an industrial deodorant, disinfectant, and biocide and hence can find their way into the rivers (Weinberg and Teodosiu, 2012). Higher concentrations in the Umgeni River could also be added by discharges and runoff from the companies around the river as they could be using triazine herbicides or oil that is contaminated by triazines, as these triazines are used in oil fields for preservatives purpose (Nyoni, 2011).

In a study conducted by Modrá *et al*., (2018) in Germany, atrazine (7.3 µg/kg) and simazine (4.6 µg/kg) were detected while terbuthylazine was not detected during the cold season. In Nigeria and Europe, atrazine was found at a maximum concentration of 0.94 µg/kg (Ogbeide *et al*., 2015) and 0.74 µg/kg (Muendo *et al*., 2012), respectively in a cold season. The presence of these compounds during the cold season was reported to be due to the absence of photolysis as a result of low temperatures which leads to low degradation of triazines (Modrá *et al*., 2018). The results obtained in this work are higher than those reported in Nigeria (African country) and worldwide (Europe and Germany) for simazine, however, for atrazine they are comparable but lower compared to those obtained in Germany. This could be suggesting that simazine is widely used in SA. Ametryn was not detected in this study, however, it was quantified in a sediment sample in Australia at a concentration of 0.002 µg/kg (Lin *et al*., 2018).

In this study, the performance of SE and UE methods was compared using sediment samples. The trend of quantified concentrations, especially for simazine, terbuthylazine, and atrazine was similar for both methods. However, low concentration levels for more compounds were detected by SE and not detected by UE. This could be due to that SE has lower LOD and LOQ values which make it be more sensitive than UE. Also, high temperature is used in SE, which could result in more compounds being extracted compared to UE (McGlamery *et al*., 1967).

Table 4.13: The concentration (µg/kg) of triazines detected in sediment samples (n = 3)

Compounds	Soxhlet Extraction								Ultrasonic Extraction							
	WH		BS		MK		UG		WH		BS		MK		UG	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Simazine	39 ±1	24±5	47±8	30±7	116±0.8	-	65±2	-	31±8	35±5	44±11	37±4	123±9	-	94±1	-
Atrazine	nq	18±8	1.6±9	4.3±4	nd	-	1.1 ± 1	-	nd	25±5	nd	29±6	nd	-	nd	-
Ametryn	5.2±5	nd	nd	7.2±6	nd	-	nd	-	nd	nd	nd	nd	nd	-	nd	-
Propazine	18±9	nd	28±7	4.3±3	nd	-	nd	-	nd	nd	nd	nd	nd	-	nd	-
Terbuthylazine	nd	nd	nd	nd	nd	-	nd	-	nd	nd	nd	nd	nd	-	nd	-

nd – not detected; nq –not quantified

WH- Woodhouse River, BS-Bishopstowe River

MK- Mbokodweni River, UG- Umgeni River

- = No sampling was done

A comparison of triazines concentration levels in different matrices was conducted and the obtained results agree with the expected trend. The highest concentration of simazine was obtained in soil followed by sediment matrices were expected because simazine has low water solubility. Therefore, it is likely to adsorb in soil/sediment than to be found in the water matrix (Bol'shakov *et al*., 2014). Also, the concentrations of atrazine were expected to be high in water followed by sediment matrices because it is highly water soluble (Benito *et al*., 2019). The analytes ametryn, propazine, and terbuthylazine were not detected in all the investigated matrices and that could be explained by their limited application as they are selectively used. The observed trend is shown in Figure 4.7.

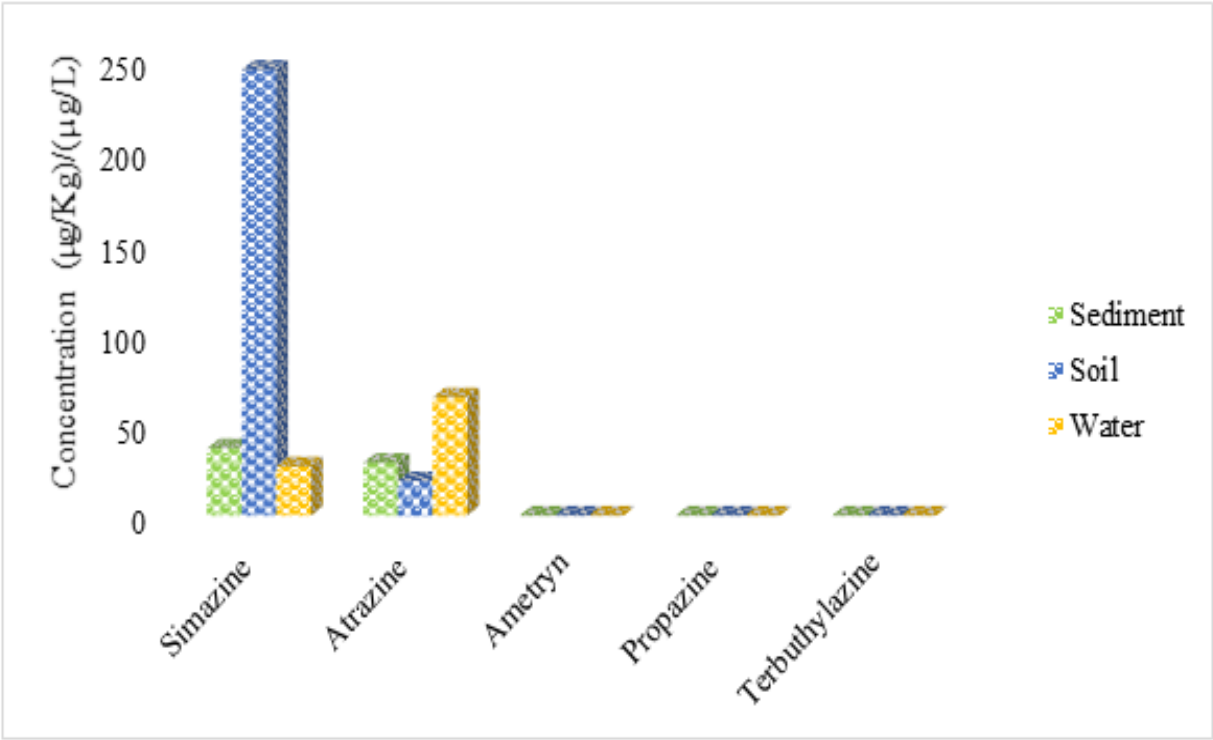

Figure 4.8: Comparison of triazines concentrations in different sample matrices

Chapter 5-Conclusions and recommendations

5.1 Conclusions

The proposed SPE, UE, SE and LC-PDA methods were successfully developed and were found to be appropriate for the determination of pesticides in soil, sediment, water, and sludge samples. The LOD and LOQ obtained for the SPE method ranged between 0.67 - 1.2 and 2.0 - 3.5 µg/L with the recoveries ranging from 107-111%. For UE, the LOD and LOQ ranged from 1.0-2.0 µg/kg and 3.2 – 6.1 µg/kg with recoveries between 75 - 100%. For UE, LOD and LOQ were 0.092-0.22 µg/kg and 0.28 – 0.69 µg/kg with the recoveries between 71 – 87%. In comparison between UE and SE, the obtained recovery results were comparable. However, SE was considered as more efficient than UE due to more compounds quantified which could be due to its lower LOD and LOQ than UE.

The concentrations quantified in the effluent (2.9 - 49 µg/L) were high than those found in the influent (2.5-17 µg/L) which indicates that high concentrations of triazines were discharged from WWTPs into the rivers. All the obtained concentrations were below the MRLs except atrazine in the Amanzimtoti wastewater effluent sample. However, the concentrations obtained in the corresponding river water were below concentration obtained in the effluent which could be due to dilutions in the rivers. The rate removal (%) results for most compounds were negative which indicated that the WWTPs are underperforming and hence almost all of the target compounds were discharged with the effluent. Therefore, it was concluded that the applied treatment technologies are not effective in removing triazines. The concentrations obtained in river water were between 3.0 - 65 µg/L and were all below the MRLs except atrazine and in Bishopstowe. The concentrations obtained in liquid sludge were between 8.4 - 2820 µg/kg and they were above the MRLs. Simazine was found to be present in most water sampling points and Amanzimtoti WWTP was found to be the most polluted WWTP as more analytes were detected. The obtained concentrations in WWTPs were below the MRLs except for atrazine in Amanzimtoti WWTPs effluents, which could be contributed by domestic, industrial and agricultural sources.

Concentrations ranging between 1.1 – 123 µg/kg were obtained in sediment samples with the maximum concentration obtained for simazine in Mbokodweni River sediment. The analytes concentration levels obtained in sediments and river water were higher in samples collected in

cold season than those obtained in the hot season. This could be due to the no/low degradation process because of low temperatures as well as low dilution and low water flow due to low rain. The concentrations obtained in soil were between 17 - 1017 µg/kg and Bishopstowe was the most polluted sampling point. The obtained concentrations were all below the MRLs except for simazine and atrazine in Gilboa Farm. In a comparison of matrices (soil, water, and sediment), it was found that atrazine exhibit high concentrations in water whereas simazine exhibit high concentration in soil. This could be due to the high solubility of atrazine in water as well as the high affinity of simazine to the soil which results in high adsorption.

The obtained concentrations in this work were above the previously reported concentrations worldwide, which indicate the importance of continuous monitoring of these compounds in the environment. This will help to draw a valid conclusion on triazines concentration levels in KwaZulu-Natal environmental samples. Also, to generate more reliable data in the order set the specific MRLs for South Africa and look for a reliable method for their removal in the environment.

5.2 Recommendation for future work

- The use of micro extraction techniques because they require small samples and solvent volume, hence they are more environmentally friendly. However, most of the micro extraction techniques are expensive thus, the use of hollow fiber liquid phase micro extraction (HF-LPME) is recommended because it is more efficient, fast and cheaper.
- Continuous monitoring of triazines in various parts of KwaZulu Natal in order to draw a valid conclusion on their overall overview of triazines pollution in KwaZulu Natal as a whole.
- Continuous monitoring of triazines in WWTPs to monitor their rates of removal as WWTPs have been reported to be the main source of pollutants into the environment.
- Studies on triazines in soil, water, sludge and sediments and other environmental samples in all seasons have to be conducted in order to have an idea of the seasonal effect on the triazines levels.
- More studies have to be conducted in river water especially in rural areas as they use rivers as the main source water in order to investigate human health risks associated with triazines.

List of presentations emanating from the work:

1. P.N. Kunene, P.N. Mahlambi. Method development and application for analysis of pesticides in soil, sludge, and sediment. UKZN Postgraduate research day, 25 October 2018, University of KwaZulu-Natal (Westville), Durban (South Africa), *Oral presentation.*
2. P.N. Kunene, P.N. Mahlambi. Method development and application for analysis of triazine pesticides in sediment: A comparison of extraction methods and seasonal variation. SACI Postgraduate Colloquium, 06 March 2019, Durban University of Technology, Durban (South Africa), *Poster presentation.*
3. P.N. Kunene, P.N. Mahlambi. Method development and application for analysis of pesticides in soil and sediment. SACI Conference, 02 December 2018 CSIR, Pretoria (South Africa), *Oral presentation.*
4. P.N. Kunene, P.N. Mahlambi. Method development and application: ultrasonic extraction, Soxhlet extraction and solid phase extraction for analysis of pesticides in water, sludge, sediment, and soil. Chemistry department Seminar, University of KwaZulu-Natal (PMB), 22 May 2019, Pietermaritzburg (South Africa), *Oral presentation.*

List of papers originating from this project:

1. P.N. Kunene, P.N. Mahlambi. Development and application of the SPE-LC-PDA method for the determination of triazines in water and liquid sludge samples (Accepted in Journal of Environmental Management).
2. P.N. Kunene, P.N. Mahlambi. Optimization and application of ultrasonic extraction and Soxhlet extraction followed by Solid phase extraction for the determination of triazine pesticides in soil and sediment (Submitted to Journal of Environmental Toxicology and Chemistry).

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APPENDIX

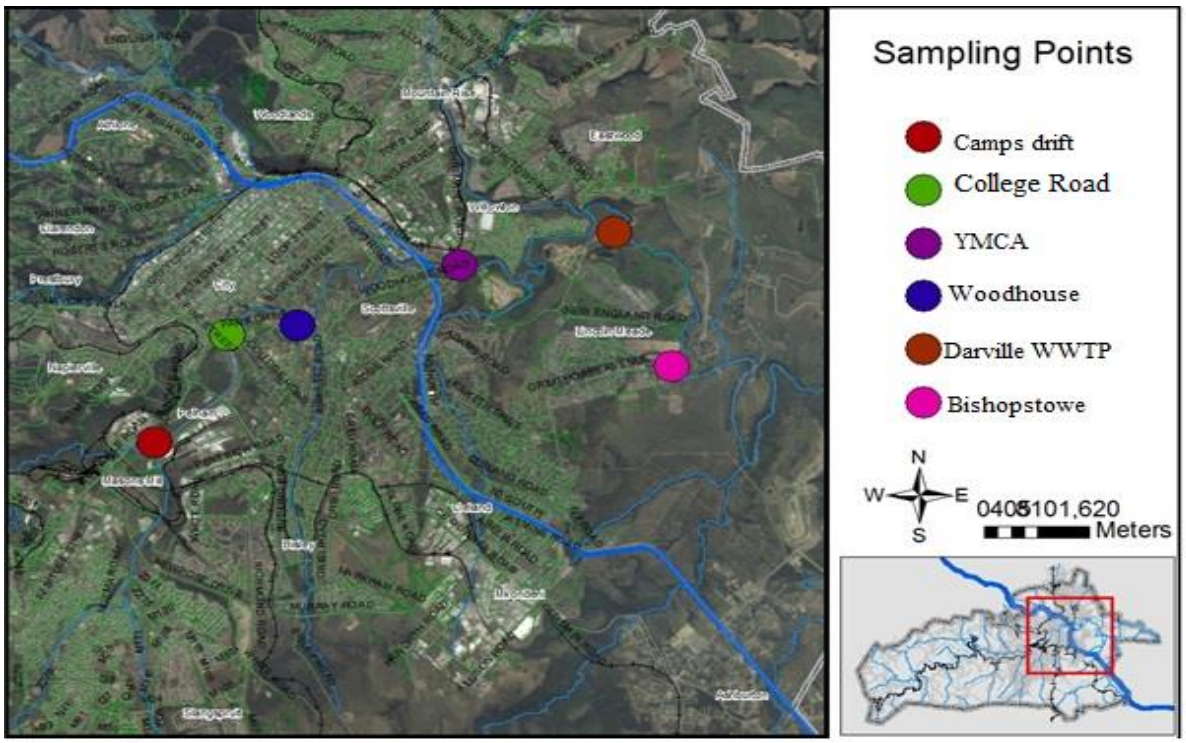

Figure A 3.1: A map of sampling spots for water and sediment around Pietermaritzburg

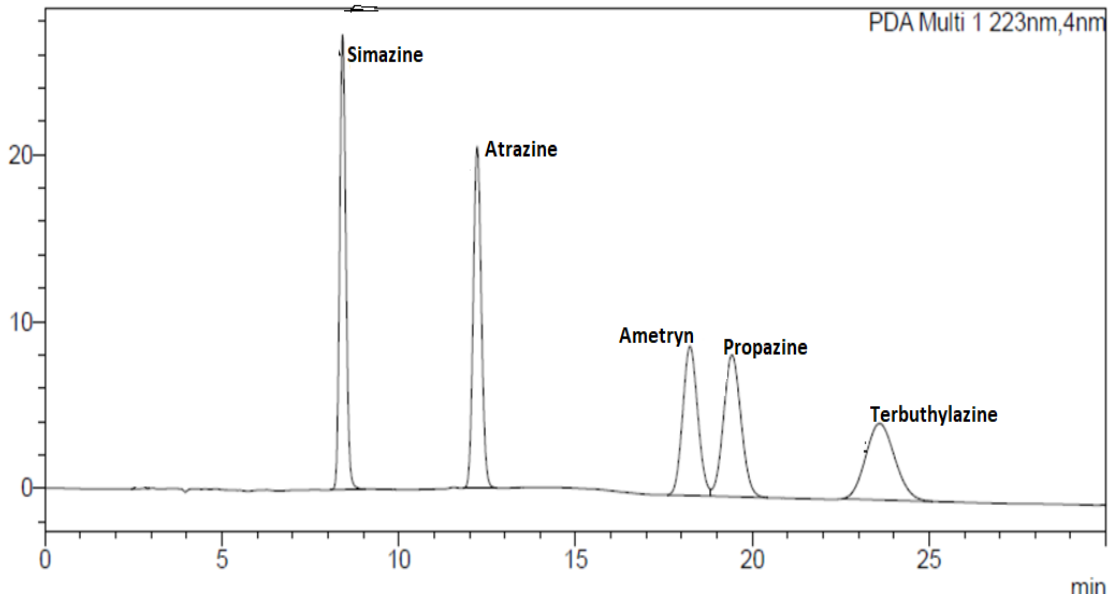

Figure A 4. 1: Chromatogram of the 1ppm mixture of triazines standard solution.
Simazine (A), Atrazine (B), ametryn (C), Propazine (D) and Terbuthylazine (E). Mobile phase: acetonitrile - water. LC gradient program was from 0-10 min 45:55 v/v, and 10-25 min 30:70 v/v, Column: C_{18} (150 mm x 4.6 mm ID), flow rate: 0.6 mL/min, injection volume 10 µL, detection wavelength: 223 nm.

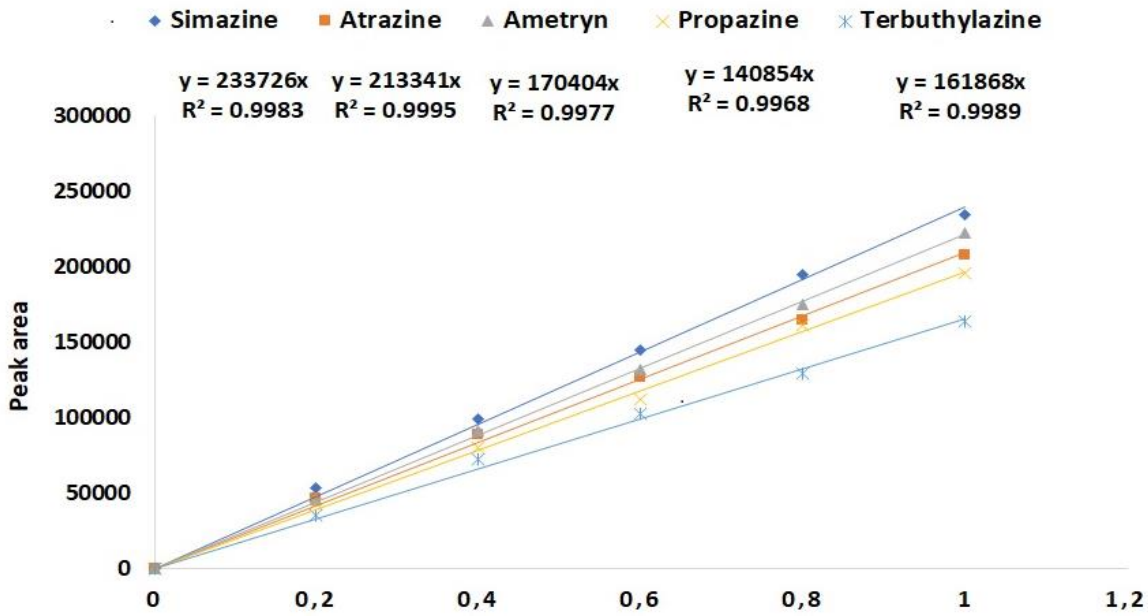

Figure A 4. 2: Typical calibration curves for analytes obtained using LC-UV-PDA.

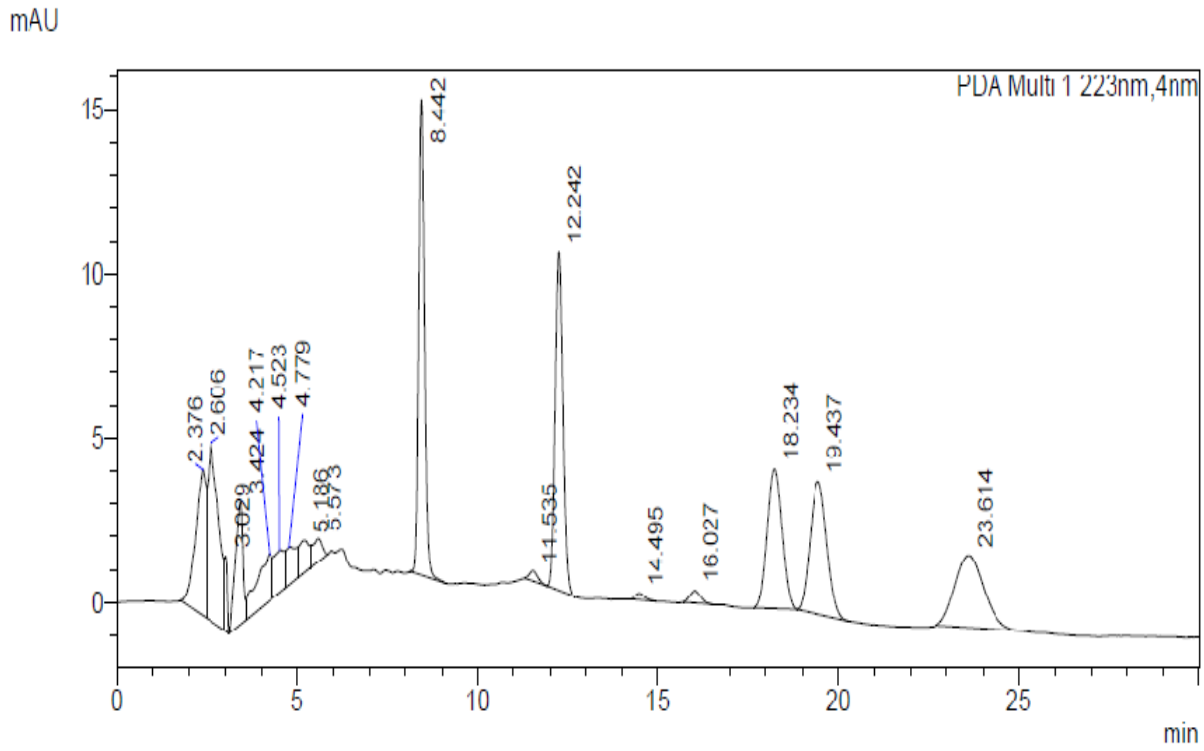

Figure A 4. 3: Chromatogram of Mbokodweni River spiked water sample

Mobile phase: acetonitrile - water. LC gradient program was from 0-10 min 45:55 v/v, and 10-25 min 30:70 v/v, Column: C_{18} (150 mm x 4.6 mm ID), flow rate: 0.6 mL/min, injection volume 10 µL, detection wavelength: 223 nm.

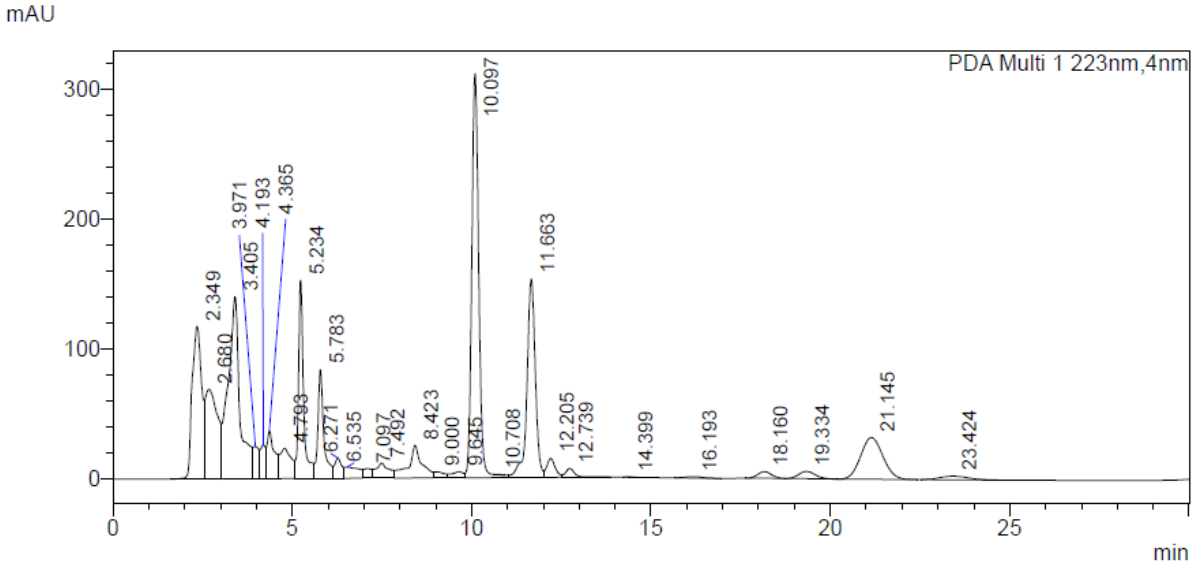

Figure A 4. 4: Chromatogram of Darvill sludge sample.

Mobile phase: acetonitrile - water. LC gradient program was from 0-10 min 45:55 v/v, and 10-25 min 30:70 v/v, Column: C18 (150 mm x 4.6 mm ID), flow rate: 0.6 mL/min, injection volume 10 µL, detection wavelength: 223 nm.

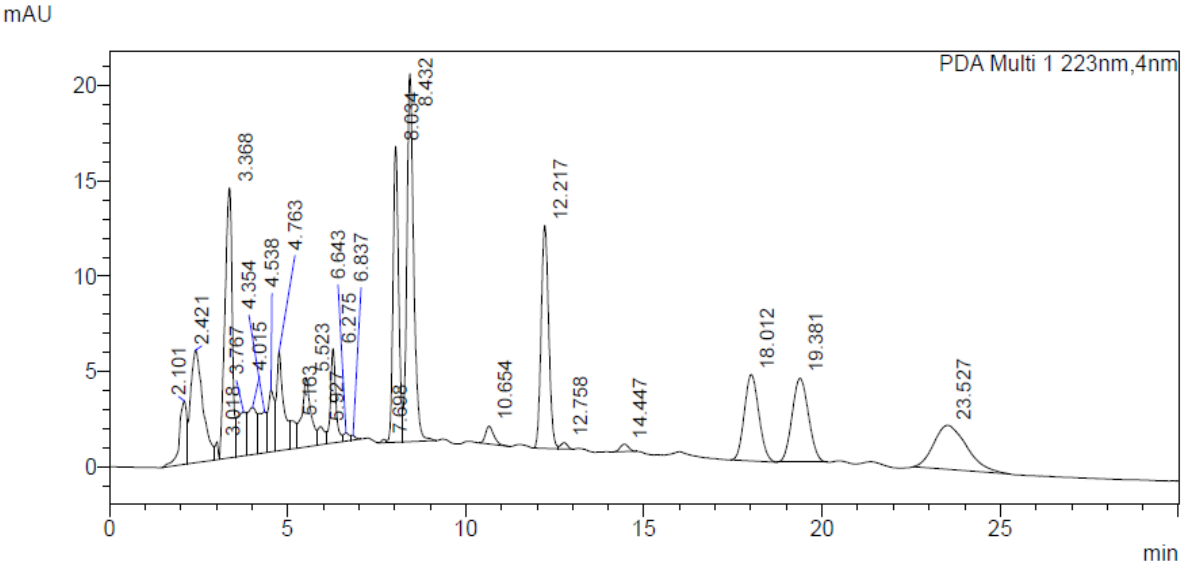

Figure A 4. 5: Chromatogram of Bishopstowe spiked soil sample.

Mobile phase: acetonitrile - water. LC gradient program was from 0-10 min 45:55 v/v, and 10-25 min 30:70 v/v, Column: C_{18} (150 mm x 4.6 mm ID), flow rate: 0.6 mL/min, injection volume 10 µL, detection wavelength: 223 nm.

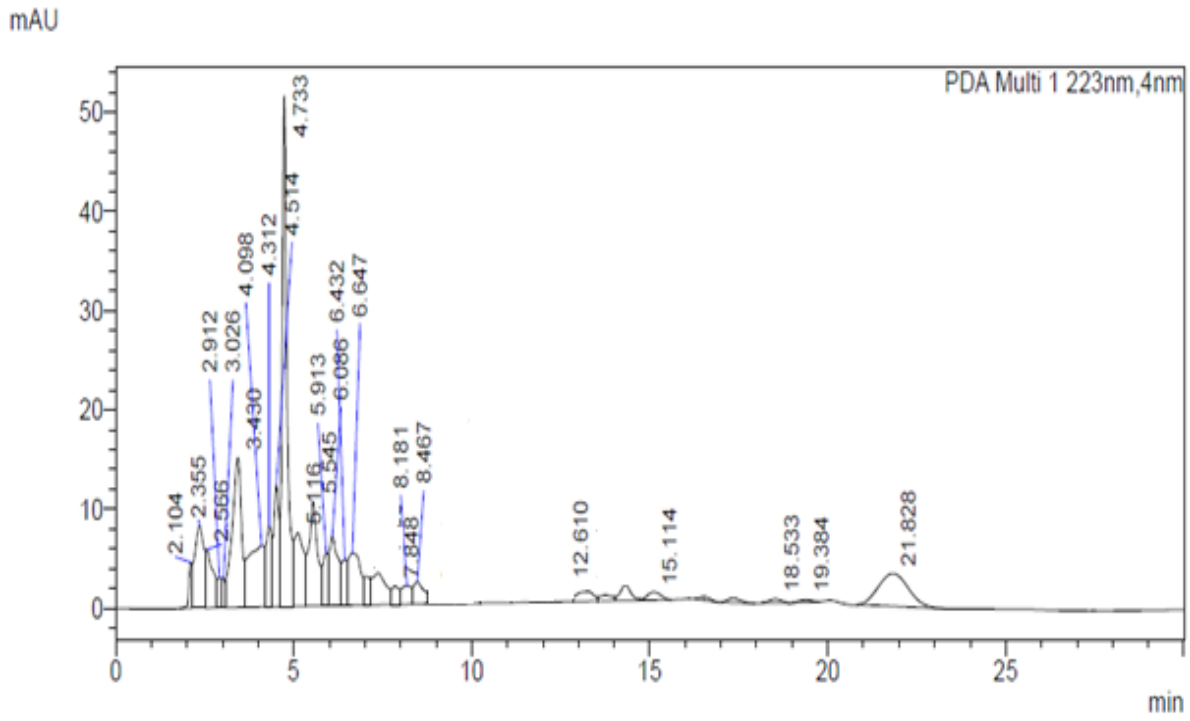

Figure A 4. 6: Chromatogram of Bishopstowe spring sediment sample

Mobile phase: acetonitrile - water. LC gradient program was from 0-10 min 45:55 v/v, and 10-25 min 30:70 v/v, Column: C_{18} (150 mm x 4.6 mm ID), flow rate: 0.6 mL/min, injection volume 10 µL, detection wavelength: 223 nm.