Determination of neonicotinoid insecticides in water, soil and sediment samples: acute and chronic risk assessment

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Determination of neonicotinoid insecticides in water, soil and sediment samples: acute and chronic risk assessment

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Abstract

Neonicotinoids are a type of insecticides pesticides widely used worldwide as a result of their low vertebrates toxicity, relative environmental stabilities, good bioavailability and high level of selectiveness. These insecticides are commonly employed in agricultural activities, in grass management and horticulture as well as in households to control domestic pet flea. Due to neonicotinoids intensive usage, they are continuously introduced to the water bodies where they can adversely affect the aquatic life and accumulate in sediments. Moreover, they can end up in drinking and unintentionally consumed by human beings resulting to health effects. With this regard, this work reports for the first time on the occurrence of neonicotinoids in sediment, soil tap, sludge, wastewater and river water samples from the province of KwaZulu-Natal. Also, the ecological risk of neonicotinoids in water sources was also assessed for the first time in the samples from this province.

The liquid chromatography coupled with a photo-diode array detector (LC-PDA) method was modified and applied for the simultaneous detection of neonicotinoids (clothianidin, thiamethoxam and imidacloprid). Ultrasonic extraction (UE), soxhlet extraction (SE) and solid-phase extraction (SPE) methods were developed and applied for the extraction of nitro-guanidine neonicotinoids in water, soil and sediment samples. The SPE, SE, and UE parameters that influence the recoveries of the analytes were first optimized before application to real samples for the analytes recovery improvement. The SPE was used for the extraction of neonicotinoids in sludge and water samples, while SE and UE were both used to extract soil and sediment samples. The extraction conditions optimized for SPE were conditioning solvent and sample volume. While for the UE were extraction time, extraction solvent, and the solvent volume. And for SE method, extraction solvent and the extraction solvent volume were optimized. The LC-PDA method used for detection was also first optimized to improve peak separation, retention times, detection limits and quantification limits. The optimized parameters for the LC-PDA method were the mobile phase, flow rate, and the PDA detection wavelength.

Optimum water recoveries of the neonicotinoids ranged from 79 to 112%. The detection and quantification limits of the analytes in water samples were 0.013 - 0.031 µg/L and 0.041 - 0.099 µg/L, respectively. The obtained analytes concentration ranged from 0.061 - 0.10 µg/L, 0.077-3.76 µg/L and 0.99 - 15 µg/L in tap, river and wastewater, respectively. Analyte recoveries ranged from 85 - 102% in soil and 92 - 103% in sediment for the ultrasonic extraction method. The neonicotinoid recoveries ranged from 83 to 109% in soil and between 84 to 94% in sediment samples for the Soxhlet extraction method. The method's detection limits and quantification limits in solid samples ranged from 40 - 80 µg/kg and 140 - 270 µg/kg, respectively. The relative standard deviation was less than 4%. The concentration determined in real environmental samples were 47 to 410 µg/kg in soil and 25 to 410 in sediment.

The toxicity studies showed that clothianadin pose a high risk towards daphnia species in the river. Imidacloprid, clothianidin and thiamethoxam posed medium risk against algae, daphnia and fish species in the effluent receiving water bodies. These results imply the necessity to continuously monitor these neonicotinoids in the water sources. In South Africa there is limited data concerning the environmental occurrence of neonicotinoids, therefore this work will contribute towards the information available for the analysis of neonicotinoids. This will assist the policy makers to establish the MRL values that are precise for the African continent.

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Thank you, God for providing me with strength to always achieve my dreams. For bringing me to life and do great things in my life, Lord Almighty you deserve all the Glory. Praises goes higher above the sky!

Dedication

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This work is dedicated to the loving and supportive Ngomane family, more especially my mother who supported me through it all. The MSc project is also dedicated to my future children, as I pave the way to greatness. I grew up in rural areas of Nkomazi in the Mpumalanga province, where people are not really motivated to study higher degrees and I took steps to give them light of what education can do to the youth. Education is a weapon no one can take away from you, it put you in higher places and it brings dignity and respect.

Presentations

1. UKZN Postgraduate Research & Innovation Symposium (Oral Presentation)
   Title: Method development and application for the determination of neonicotinoid insecticides in water samples (SPE-LCPDA)
   Date: 17 October 2019
2. UKZN Department Postgraduate Seminar Series (Oral Presentation)
   Title: Methods development and applications for the determination of neonicotinoids insecticides in soil and sediment samples (UE, SE-LCPDA)
   Date: 18 March 2020

Journals originating from this study

Corresponding Author: Dr P.N Mahlambi
Co-Author: N.C Ngomane
Title: Determination of neonicotinoids in tap, river, wastewater and sludge samples: risk assessment and environmental relevance of neonicotinoids from wastewater
Journal: Journal of Water Process Engineering

Additional modules/training courses attended:

(i) Searching for scientific information for research purposes, Focusing on the efficient use of Chemical Abstract Service (CAS)'s SciFinder

(ii) Basics of finding information via UKZN Library-PMB, Endnote, ICatalogue, Referencing, Google scholar, EbscoHost, Sabin

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List of Abbreviations

Diode array detector	DAD
Dissolved oxygen	DO
Gas chromatography	GC
High-performance liquid chromatography	HPLC
Hydrophilic-lipophilic base	HLB
International Union of pure and applied chemistry	IUPAC
Limit of detection	LOD
Limit of quantification	LOQ
Liquid–liquid extraction	LLE
Mass spectrometry	MS
Maximum residue limits	MRLs
National pesticide information centre	NPIC
Percentage relative standard deviation	%RSD
Photo-diode array detector	PDA
Solid-phase extraction	SPE
Solid-phase micro-extraction	SPME
Soxhlet extraction	SE
Stir bar sorptive extraction	SBSE
Total dissolved solids	TDS
Ultrasonic extraction	UE
Ultraviolet-visible	UV-Vis
United States environmental protectionagency	U.S. EPA
Wastewater treatment plants	WWTPs
University of KwaZulu-Natal	UKZN

Chapter 1: Introduction

This chapter is composed of the introduction, problem statement which leads to the aim of the study and the objectives followed to accomplish the aim. It also covers the research questions that the study meant to answer and the research justification.

1. Introduction

1.1 Background

Clean water and good quality soil are essential requirements for humanity and the entire ecosystem. Therefore, it is important to maintain the quality and availability of these resources, mainly because the social and economic development depends on them for stability (Bashir, 2018). Found; to date that the quality of surface and underground water is declining all over the world due to pollution by various organic compounds including pesticides. The use of pesticides has been increased in order to increase quality and quantity of crops. This is done to meet the increasing demand of food as results of continuous increase in population. However, their intensive usage leads to environmental contamination as they runoff from point source to pollute surface and underground water as well as the soil and sediments (Aydinal, 2008). Pesticides including neonicotinoids are also applied in private homes and gardens to control pests. Therefore, they can enter the sewage system and reach the wastewater treatment plants where they are completely removed. From the WWTPs, pesticide residues are released to the receiving rivers where they can affect aquatic life and they may end up in drinking water. Human exposure to neonicotinoids may lead to respiratory failure, reduced level of consciousness, lethargy, vomiting, diarrhea, and salivation and aspiration pneumonia. Although the usefulness of pesticides cannot be denied, the negative environmental and human health effect cannot be ignored (Quinin, 2011).

1.2 Problem statement

Pesticides are usually employed to manage pests in agricultural fields and thus improve the quality and quantity of agricultural products. However, the pesticide residues in crops and in the environment endangers the well-being of humans, and other living organisms. Hence, it is crucial to assess and monitor pesticides in different environmental compartments to confirm if they are within the acceptable concentrations that are safe for consumption. On the other hand,

pesticides are present at low concentrations in the environmental samples therefore a sensitive and accurate extraction and analysis method is required for their effective determination. Even though neonicotinoids contaminants have been quantified worldwide, there is limited data reported in South Africa (Selahle, 2021). This work therefore aims to modify ultrasonic extraction (UE), solid phase extraction (SPE), soxhlet extraction (SE) and liquid chromatography equipped with a photo diode array detector (LC-PDA) modification and application for the assessment of neonicotinoid in river water, tap water, wastewater, sludge, soil and sediments. Also, the toxicological assessment of neonicotinoids against micro-organisms in Msunduzi River was evaluated for the first time. Furthermore, the elimination rate of neonicotinoids entering the water cycle from KwaZulu-Natal wastewater treatment plants was assessed for the first time.

1.3 Aim and objectives

Aim

To develop LC-PDA, SE, UE and SPE methods for the analysis of neonicotinoids in river water, tap water, wastewater, sludge, sediment and soil samples. Also, to assess the neonicotinoids toxicity studies again aquatic environments as they are released into the receiving rivers.

Objectives

i) To optimize LC-PDA method for the separation of neonicotinoids.

ii) To optimize SPE method for the extraction of neonicotinoids using spiked water samples.

iii) To optimize UE and SE methods for the extraction of neonicotinoids using spiked soil/sediment samples.

iv) To apply the optimized methods for the qualitative and quantitative analysis of neonicotinoids in river water, tap water, wastewater, sludge, soil and sediment samples.

v) To compare the extraction efficiency of SE and UE for the determination of neonicotinoids in soil and sediments.

vi)To assess the effect of seasonal variations on the concentrations of neonicotinoids in the samples.

vi) To calculate the risk quotients, toxicity units from river water samples and environmental relevance of neonicotinoids from wastewater samples in order to evaluate their toxicity against aquatic organisms

1.4 Research questions

i) Which SPE, UE, and SE parameters need to be optimized in order to improve the recoveries of all the analytes?

ii) Which extraction method will give high analyte recoveries from soil between SE and UE?

iii) Are the neonicotinoids present in the selected study areas and what would be their concentration levels?

iv) Which neonicotinoid compound will dominate in water and solid samples?

v) What is the effect of seasonal variations on the concentration levels of neonicotinoids?

vi) Do the neonicotinoids present in the study area possess potential risk to the aquatic organisms and which level?

vii)Which of the neonicotinoid's is more relevant and toxic towards aquatic species?

viii) Which of the three taxons representative (algae, daphnia magna, and fish) of three ecosystem trophic levels is more susceptible towards the neonicotinoids toxicity?

1.5 Research justification

Pesticides are pollutants that have been identified to be amongst the compounds that plays a big role in the pollution of various environmental compartments. However, to the best of our knowledge there are very limited studies that conducted on the determination of pesticides such as neonicotinoid insecticides in South African environmental samples, while no work has been done in KwaZulu-Natal province. As a result, reliable analytical methods need to be developed and applied for the determination of neonicotinoids in South African environmental samples. The analytical methods employed in the analysis of organic pollutants in environmental samples demand a number of steps such including extraction, clean-up and/or pre-concentration steps due to the low levels at which the pollutants are present in the environmental samples. It is therefore essential to develop analytical methods that are sensitive and accurate which is significant for the effective detection of the organic compounds at trace levels. Moreover, it is important to evaluate the toxicity levels of the obtained neonicotinoids concentrations to aquatic organisms (fish, daphnia and fish) to assess if they have the potential to cause any harm. The purpose of this was therefore to modify and apply SPE, UE and SE followed by LC-PDA for the determination of nitro-guanidine neonicotinoids insecticides from river water, tap water, wastewater, sludge, soil and sediment from KwaZulu Natal province. Also, to assess their toxicity levels against fish, daphnia and algae which were all conducted for the first time in this study.

Chapter 2: Literature Review

In this section, the literature on the application, routes of exposure and also environmental and health issues of neonicotinoid is covered. An evaluation of different analytical techniques that have been previously employed for the neonicotinoid determination in water, soil and sediment samples has been emphasized.

2.1 Neonicotinoid insecticides

Neonicotinoids are pesticides compounds which are classified under the insecticides class (Simon-Delso, 2015). They are neuro-active insecticides that share chemical properties with nicotine. Neonicotinoids have been the most preferred insecticides since the 1990s and they are used in more than 120 countries (Lundin, 2015). Although, they were developed to replace carbamate, pyrethroid and organophosphorus insecticides, which were observed to be highly toxic to applicators and other non-target organisms mainly bees, and aquatic animals (Sánchez-Bayo et al, 2012). Neonicotinoids are hydro-heterocyclic guanidine/amidine compounds and they possess active substituents such as NO_2 & CN (Buszewski, 2019). The chemical structures of these compounds have four shared features; elastic bonds, aromatic heterocyclic group, electron withdrawing group and hydro-heterocyclic (guanidine or amidine groups) as shown in figure 2.1. The combination of the substitutions, contribution of free electrons or elasticity features may result to the variation in mode of action and toxicity strength of neonicotinoids. There are two subclasses of neonicotinoid insecticides which are nitro-guanidine and N-cyano-amidine. Nitro-guanidine neonicotinoids (clothianidin, thiamethoxam, imidacloprid) possess an N-nitro group containing oxygen, making it to be reactive and polar. The N-cyano-amidine neonicotinoids (acetamiprid and thiacloprid) have a cyano-amidine group which does not contain oxygen atom and thus they are less polar and less reactive compounds. As a result of the rapid decomposition of the N-cyano-amidine neonicotinoids, they are not suitable to be employed for the treatment of seeds (Buszewski et al, 2019). Therefore, this work investigated the nitro-guanidine neonicotinoids because they are dominantly used in seed treatment. The nitro-guanidine neonicotinoids are mobile, when applied in seed treatment 2 to 20% is absorbed by the plants through roots to all parts of the plants mostly transportation of the plant such as xylem and phloem. In some case 80 to 98% of the active ingredients of the insecticides remain in the soil, this result to leaching and runoff to surface water. High amount of the active

ingredients that is not absorbed by the plant cause water pollution which poses potential risk to aquatic animals and human health (Wood and Goulson, 2013). They also bio magnify and bio accumulate in the environment, which led to an extinction of other predatory birds such as bald eagles and peregrine falcons, which fed on aquatic insects (Sánchez-Bayo et al, 2012). The selected nitro-guanidine neonicotinoids for the current literature review are thiamethoxam, imidacloprid and clothianidin (Figure 2.1).

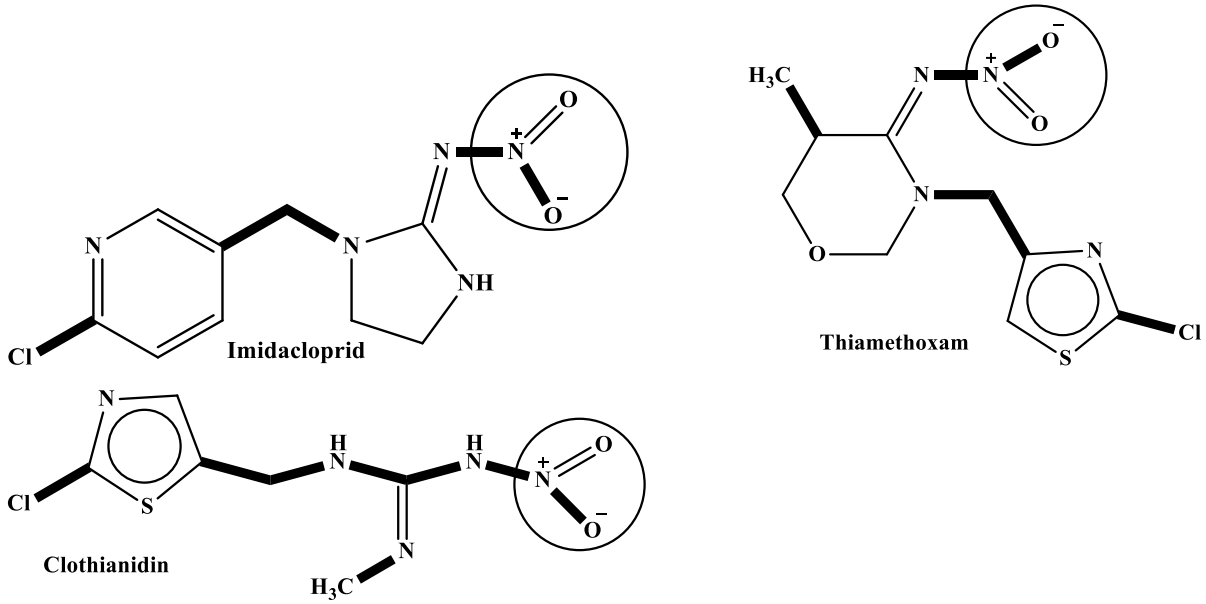

Figure 2.1: Chemical structures of thiamethoxam, imidacloprid and clothianidin nitro-guanidine neonicotinoid insecticides.

Thiamethoxam is a neonicotinoid that was discovered in 1991 and first registered in New Zealand in 1997 (Maienfisch, 2001). Thiamethoxam is the second generation that belongs to the chemical subclass thianicotinyls. A broad-spectrum neonicotinoid that the plants quickly absorb and transported to aerial parts including pollen, where it prevents insect feeding (Maienfisch, 2001). Thiamethoxam is commonly employed due to its potent towards insects. Due to its ability reach out to aerial tissues of the plants as well its persistence in the environment (Tosi and Nieh, 2017). Imidacloprid is a neonicotinoid in the chloronicotinyl nitroguanidine chemical family. Imidacloprid was discovered in 1984 and was first registered in 1994 in the United State by the United States Environmental Protection Agency (EPA) (Bonmatin et al, 2005). It is the most selling neonicotinoid for veterinary and agricultural application as a result of its great effectiveness in insects. It has also been authorized to be employed as the most accurate product in cats and dogs (Stanneck et al., 2012). Clothianadin was established in 1995 by Takeda chemical industries and Bayer AG (Kagaku, 2006). It was

initially approved for use in 2001 by the Japan Plant Protection Association, and was then conditionally approval in 2003 by the USA EPA. According to the EPA, its main risk of concern is its effect to non-target insects including honey bees. (Kagaku, 2006).

2.2 Physical parameters of neonicotinoid insecticides

The mobility of neonicotinoids in the environment is controlled by their physical parameters such as the octanol-water coefficient (Log K_{ow}), vapour pressure, solubility, melting point, polarity. The solubility of neonicotinoids in water is high due to low octanol-water coefficients (<2.5). Hence, they are more expected to be present in water with low adsorption potential to sediments and soil. Thiamethoxam has the higher water solubility than the other neonicotinoids, and therefore expected to be found in higher concentration in water compared to other compounds. On the other hand, clothianidin has higher log Kow compared to the other neonicotinoids and thus it has better chances of being adsorbed in soil/sediment compared to the other neonicotinoids. Due to their mobility, they are deposited on soil, which leads to leaching to surface waters and sediments. As results of low vapour pressures of neonicotinoids, they are less expected to be present in the air (Buszewski, 2019).

Table 2.1: Physico-chemical parameters of neonicotinoid insecticides (Shimshoni, 2019).

Insecticide	Vapour pressure (Torr at 25 ℃)	Solubility (mg/L at 20 ℃)	Log K_{OW}	Soil Affinity (Log K_{OC})	Half- lives (Aerobic soil metabolism) days
Thiamethoxam	1.36×10^{-9}	4100	-0.13	1.75	25-100
Imidacloprid	5.07×10^{-8}	610	0.57	2.19	40-997
Clothianidin	1.3×10^{-10}	327	0.70	2.08	148-1,155

2.3 The importance of neonicotinoid insecticides

Pests affect agricultural crops hence, pesticides are applied in agricultural lands to prevent diseases to increase production of food for profit (Aktar, 2009). The application of pesticides has been appraised to have avoided over seven million people to die through destroying pests that transfer diseases since 1945 (Aktar, 2009). With this regard, application of pesticides resulted in the decrease on the occurrence of a fatal disease malaria which is transported by infested mosquitoes (Cuervo - Parra, 2016). Furthermore, the bubonic plague disease which is

passed by typhus and rat fleas was also lessened as a results of pesticides application. Neonicotinoids are commonly employed in seed treatment where they travel in all plants aerial parts protecting against insect (Cuervo-Parra, 2016). Imidacloprid is the most employed neonicotinoid in crops such as sunflower, and oilseed corn (Sluijs, 2013). The other commonly used neonicotinoids are thiamethoxam which is used in maize, cotton, cereals, peas, soybeans, sugar beets, while clothianidin is employed in maize, soybeans, leafy greens (Reaves et al, 2020). The application of neonicotinoids has also assisted in the stop the escalation of insect's resistance to organophosphate and pyrethroid pesticides. Neonicotinoids are also used in non-agricultural applications including grass management activities, control of pests and treatment of pet flea in private homes and gardens (Kundoo et al, 2020).

Table 2.2: Uses of nitro-guanidine neonicotinoids (Reaves, 2020)

Pesticides	Uses
Thiamethoxam	Maize, cotton, cereals, peas, soybeans, sugar beets
Imidacloprid	Sunflower, oilseed corn
Clothianidin	Maize, soybeans, leafy greens

2.4 Human exposure pathways to neonicotinoid insecticides

As a result of neonicotinoids usage in agricultural and non-agricultural areas; they are now present in matrices, and dust at homes. Therefore, human beings are exposed to them in various pathways including ingestion, inhalation and dermal. The high exposure to the insecticides is thought to be during application.

2.4.1 Ingestion exposure

Compared to other agrochemicals, neonicotinoids are easily absorbed by the roots and translocated to different parts of the plant owing to their mobility nature (Kundoo, 2020). Therefore, neonicotinoids can be found in the plant pollen and nectar after they have been directly applied to the soil or via seed coating (Sánchez-Bayo, 2012). This neonicotinoids nature promotes their traces to be transferred from the treated plants to crops, fruits, vegetables after harvest leading to their consumption a principal ingestion exposure pathway. (Craddock, 2019). Furthermore, the hydrophilic character of neonicotinoids could result to considerable amount being ingested through drinking neonicotinoids polluted water (Craddock, 2019). This

is more prevalent in water supply that are nearby the agricultural fields with frequent sowing seeds pretreated or direct spraying application of the neonicotinoid (Bonmatin, 2015).

2.4.2 Inhalation exposure

The neonicotinoid polluted pollen can become airborne and thus inhaled by humans upon exposure where it can be absorbed in the lungs and respiratory tracts as neonicotinoids are water soluble (Wood, 2017). Neonicotinoids have been observed to be abundant in pollen collected from insecticides applied areas, therefore attribution of exposure to neonicotinoids intake through inhalation should not be neglect (Wood, 2017).

2.4.3 Dermal exposure

It is a multifaceted process of the skin contamination by a pollutant for a long period which is the major exposure route for farm workers who work in direct contact with the pollutants (Kabata-Pendias, 2004). This complex process can cause significant effects 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. Also, applying pesticides on windy weather can increase chances of exposure. Dermal exposure, especially in developing countries, is due to low regulated safety rules in workplaces, the use of old or leaking machines, and working with pesticides without hand gloves (World Health Organisation, 2015). In general, there are various forms of pesticide formulations such as solid (granules, dust or powders) and aqueous forms which are readily absorbed through the body membrane and tissue.

2.5 Effects of neonicotinoid insecticides on humans and aquatic animals

A number of studies have shown trace levels of neonicotinoids in the environment with their severe effects in several species including mammals (Tomizawa, 2003). While a number of them are harmless to humans, many may cause severe effects when humans ingested or exposed to high concentrations of the analytes. Neonicotinoids were made for crops and plants protection from unwanted plant feeding insects. However, they have the capacity to cause toxic effects to humans, and other non-target organisms. Neonicotinoids interrupt the insect nervous system operation which includes brain areas communication leading to paralyses and deaths (Buszewski, 2019). The approval of neonicotinoids by United State Environmental Agency (EPA) for commercial use was due to their toxic effect to humans and wildlife resulting from their strong chemical affinity for the nicotinic acetylcholine receptors of the insect. Furthermore, they are restricted to pass the barrier of the mammalian between the brain and the

blood (Tomizawa, 2003). Even though neonicotinoids met the registration requirements of the pesticide high toxic effect to insects compared to mammals, they have been observed to have the increasing toxicity in mice cancerous liver tumors (Gibbons, 2015). A Studies of in-vivo and in-vitro revealed their unfavorable effects on mammals at sub-lethal doses (Tomizawa, 2004). The metabolites for some of the neonicotinoids have been observed to possess high toxicity compared to the parental compound (Goulson, 2015). Imidacloprid's metabolite (disnitro-imidacloprid), which is produced in the environment or the mammalian body during metabolism has been found to have high affinity for the nicotinic acetylcholine receptors of the mammalian (Koshlukova, 2006). Even though, neonicotinoid have been found to be more toxic towards arthropods and aquatic insects the alertness of their impacts on aquatic environments and the ecosystem at large is minimal (Sánchez-Bayo, 2014). Different aquatic and terrestrial taxa susceptibility to neonicotinoid is dependent on their detoxification ability, kinetics, and concentration (Escher, 2011). The mode of neonicotinoids action is more effective because their effects are cumulative with time as the neurons do not rebuild (Tennekes, 2013). Apart from death of aquatic organisms, the exposure to neonicotinoid can result to sub-lethal effects, such as reduced body size in mayflies and fish, feeding inhibition, impaired movement, immune-suppression and reduced fecundity in fish (Sánchez-Bayo, 2016).

2.6 The fate of neonicotinoids in the environment

The neonicotinoids contamination by spray drift and surface runoff from Farmland results into excessive levels of pesticides in surface waters compared to groundwater (Starner, 2012). Pesticides reach underground water through seepage of contaminated surface water, improper disposal, accidental spills and leakages (Meybeck, 1996). Neonicotinoids can also be sprayed on the crop or soil, and used as seed treatment or granules on soil (Starner, 2012).

When neonicotinoid is applied as a seed dressing, it is absorbed by the plant roots and translocate to the pollen and nectar where beneficial insects (e.g., bees) are affected (Farouk, 2016). When they are employed as a seed coating it's a small percentage (1.6 to 20%) of the applied active ingredient that enters the crop for its protection while the rest of the ingredient (80-98.4%) remains to contaminate the environment (Farouk, 2016). Hence, leads to the leaching of the neonicotinoid residues in soil and runoff to surface water where they bioaccumulation in pollinators at sub lethal concentrations (Sánchez-Bayo, 2014). The neonicotinoids are accumulated by the aquatic arthropods causing food source depletion to

predatory birds. Also, direct poisoning may occur when the birds directly use the neonicotinoid coated seeds as a source of food (Figure 2.2), (Sánchez-Bayo, 2014).

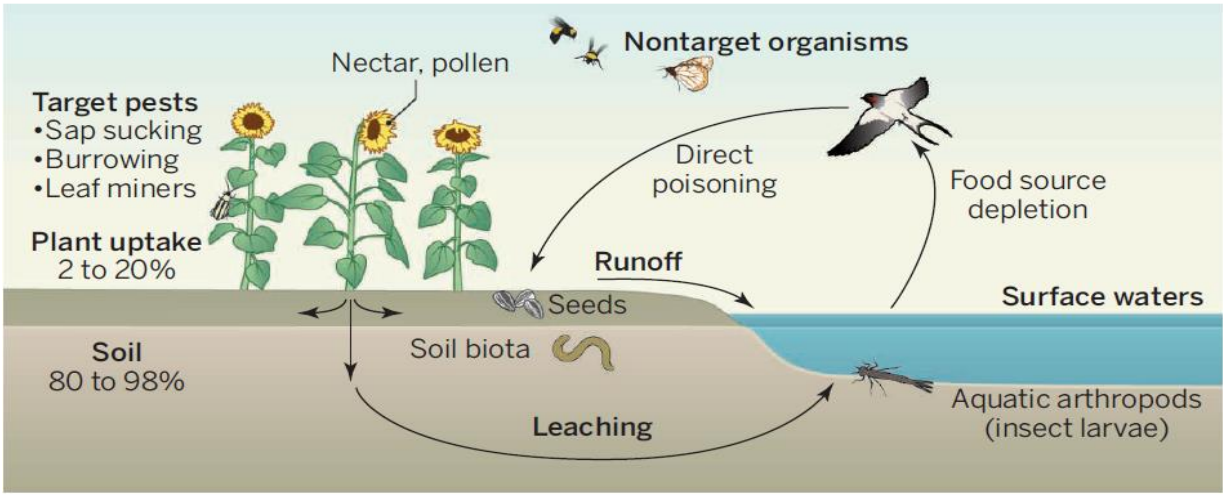

Figure 2.2: Fate of neonicotinoids insecticides and pathways of environmental contamination (Sánchez-Bayo, 2014).

2.6.1 Soil contamination

The main deposit source of exposure to neonicotinoids is during application. Pollution of neonicotinoids in the environment could be due to the climate and relatively developed agricultural activities, grass management and gardening (Bonmatin, 2021). Neonicotinoids are persistent in soils under certain conditions; however, their dissipation with time is increased by low temperature and precipitation, low microbial activity levels as well as poor soil quality. The half-life for clothianidin is 13-1386 days, 7-72 days for thiamethoxam and 104-228 days for imidacloprid. However, they differ in various environmental conditions (MÖrtl, 2016). Neonicotinoids are mostly found in aerobic soils due to dosages at high temperatures can increase their sorption to the soil particles. As a result, transport route of neonicotinoids and their leaching potential to groundwater are mainly encouraged by the type of soil combined with irrigation or rain events intensity. However, the neonicotinoids mobility in soil is influenced by their physico-chemical parameters (i.e., solubility, soil affinity, vapour pressure and Log Kow), and the characteristics of soil (MÖrtl, 2016).

2.6.2 Sediment contamination

Many studies have been conducted on quantifying neonicotinoids concentrations in water compared to sediment, hence there is limited data based on the analysis of neonicotinoids in

sediment samples. As a results of neonicotinoids ubiquity scores in groundwater, they have long half-lives in soil, they have high ability to leach to sediments than other commonly known agrochemicals (i.e., chlorpyrifos, glyphosate, etc), (Maloney, 2020). Maloney, (2020) reported 63% detection of nitro-guanidine neonicotinoids in sediments with an average concentration of 1.19 mg/kg across four sampling periods which sediments retain neonicotinoids. Sediments may act as neonicotinoids sources to the water column as desorption and degradation in sediment samples is slower compared to their photo-degradation in the water column (Kuechle, 2019). Increasing degradation rates as a result of increasing temperatures may result in neonicotinoids lower concentrations in sediment.

2.6.3 Water contamination

The widespread agricultural application of mobile neonicotinoids results to soil contamination followed by the transferal of residues to the aquatic environment. Neonicotinoids may drift out from their area of application, leach in the soil and therefore be transferred to groundwater and surface water (Sanchez Bayo, 2016). Also, neonicotinoids are hydrophilic with a water solubility ranging from 184 - 4100 mg/L which lead to their high transportation to surface water. However, as a result of their persistence and solubility, studies have indicated the presence of neonicotinoids in drinking water due to their incomplete removal by the conventional water treatment technologies. The half-life of neonicotinoids in water through photolysis are 2.7-39.5 days for thiamethoxam, < 1 for imidacloprid and 0.1 Days for clothianidin. Due to a number of effects initiated by neonicotinoids, it is important that they are monitored to ensure that their concentrations are in the range that cannot pose danger to humans and other sources of life (Borsuah, 2020).

2.7 Neonicotinoids degradation

2.7.1 Microbial degradation

The microbial degradation is their main disappearance pathway of neonicotinoids in soil (Farouk, 2016). Pure microbe cultures of *Pseudomonas SSP.G1, FHZ, and Leifsonia SP. PC-21* isolated from soil have proved to be capable of degrading neonicotinoids. However, the physical parameters such as pH, temperature and soil organic content may have a direct impact on neonicotinoids microbial degradation in soil. This is due to that neonicotinoids are stable and gradually hydrolyze at acidic or neutral pH, and they degrade slowly even in alkaline pH (Morrisey, 2015). However, microbes are active at neutral pH 7 (Kunene, 2019), hence, at this pH the microbial degradation of neonicotinoids can be improved.

2.7.2 Photo-degradation

The surface residues of neonicotinoids from foliar spray have been reported to quickly degrade compared to the neonicotinoids translocated inside plant matrix, regardless of the high physiological activity taking place inside the plant matrix (Gupta, 2008). The soil residues containing neonicotinoids have been reported to be highly persisted under sunlight from 11.1 to 25.1 days half-lives compared to 4.4 to 17.7 days of the thin film residue (Gupta, 2008). The solubilization of neonicotinoids to humic substances or adsorption to clay minerals encourages their photo-degradation on soil surface (Katagi, 2004). Fast dissipation of neonicotinoids could be a result of their photolytic ability. Photo-degradation can also be improved by the parameters of the soil such as the pH at neutral level (7), high temperatures; high solubility and low soil affinity, which then contribute to the seasonal variation of neonicotinoids insecticides.

2.8 The chemistry of neonicotinoids insecticides

All neonicotinoids have similar action mechanism where they all act as agonists on the nicotine receptors of acetylcholine because they have the same moiety (Cartereau, 2018). The chemical structures of neonicotinoids have four shared pharmacophores; (1) elastic bonds aromatic, (2) heterocyclic group, (3) hydro-heterocyclic group, (4) electron withdrawing substituent such as NO_2 and CN (Buszewski, 2019). Neonicotinoid insecticides are divided into two sub-classes; cyano-amidine and nitro-guanidine neonicotinoids. Besides the biological activity influenced by the pharmacophores, these pharmacophores are also accountable for physico-chemical properties such as soil degradation, photolytic stability, toxicity in different animals, metabolism in plants and insects. Open-chain compounds such as clothianidin are slightly lipophilic compared to imidacloprid and thiamethoxam which are the corresponding cyclic compounds. Regarding the electron withdrawing groups, the increase in their lipophilicity is S > C > O > NH. The root uptake of neonicotinoids is highly efficient for lipophilic compounds, and thus, the highly lipophilic neonicotinoids are favoured for seed treatment application. Figure 2.3 below represent the four common pharmacophores of neonicotinoid insecticides (Jeschker, 2008).

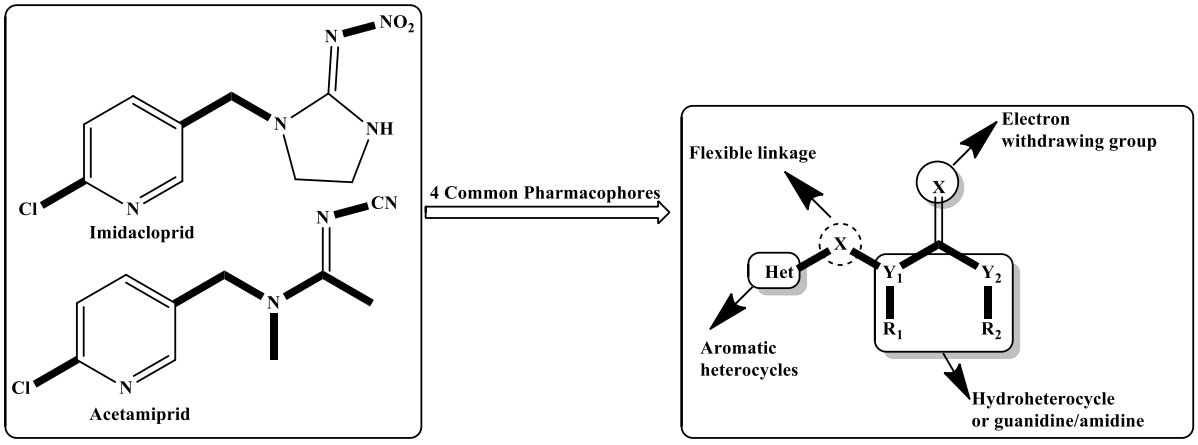

Figure 2.3: Four common pharmacophores of neonicotinoids (Buszewski, 2019).

2.9 Extraction techniques used for neonicotinoids in environmental samples

The liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME), are amongst the extraction techniques used for liquid samples. While Soxhlet extraction (SE), ultrasonic extraction (UE) etc, are used for solid samples.

Table 2.3: Comparison of the extraction techniques used to extract neonicotinoids in environmental samples.

Extraction techniques	Analyte recoveries (%)	Detection limits (µg/L)
LLE	58-88	0.11-0.36
SPE	79-109	0.2-4.4
SPME	86.7-99.2	0.41-0.82
SE	82.6-109	0.06-0.08
UE	84-112	0.15-3.2

2.9.1 Liquid samples extraction methods

LLE involves the analytes dispersal between two immiscible liquids (organic solvent and aqueous sample) in a separating funnel. Thereafter, each layer can be removed and analyzed separately to determine the analytes recovered. For LLE to be effective, solvent with low water solubility, polarity and volatility are required (Klarich, 2017). Even though LLE uses simple steps for effective extraction, its process is tediousness and uses high solvent volumes resulting to environmental contamination. It also often has small analyte enrichment factor and less selective.

The SPE utilizes a liquid-solid extraction separation principle which involves the adsorption of the analytes into solid sorbent (Sandstrom, 2001). The SPE is used to remove interfering matrix components, isolate and preconcentrate the analytes of interest which increases detection sensitivity and thus enables improved qualitative or quantitative analyses by chromatographic techniques (Smith, 2015). SPE uses manifold, which offers excellent sealing and individual control for SPE. The SPE is preferred over LLE as it uses small organic solvent volumes, it has high analytes recovery and produces highly purified extracts. However, clogging of the sorbent may results for turbid samples, it involves lengthy method development and the cartridges are costly (Simpson, 2000). The SPE procedure is composed of four steps which are; conditioning, loading, washing and elution (Figure 2.4). The purpose of the conditioning step is to activate the stationary phase functional groups to allow for maximum interactions between analyte and sorbent. Loading step involves introducing the sample to the sorbent to allow for maximum analyte retention. There is a need to dry the cartridges; the washing step is done to remove matrix interferences from the sorbent with a solvent that will effectively remove the interferences without eluting the analytes of interest. Thereafter, the elution of the adsorbed analytes is conducted using the solvent strong enough to release the analytes from the sorbent thus effectively elute the analytes. This is followed by using HPLC vials to inject the eluents for analysis or they can be dried down and reconstituted in a proper solvent prior to analysis (Wells et al., 2013). In 2020, Banno and Yabuku simultaneously analyzed seven neonicotinoid insecticides in agricultural products involving Solid-Phase extraction and surrogate compensation using Liquid Chromatography-tandem mass spectrometry. The proposed method resulted in excellent recoveries in all tested matrices. Imidacloprid was detected at 0.02 mg/kg and the recoveries calculated in parallel with the analysis were satisfactory.

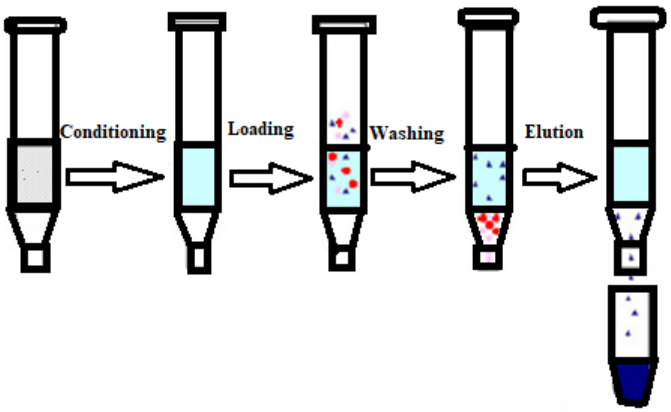

Figure 2.4: Schematic representation of a solid phase extraction.

In SPME, a polymeric fiber coated with a stationary phase is placed in the vapour above a gaseous/liquid sample or directly immersed in a liquid sample and agitated, resulting in the extraction of the targeted analytes (Vas, 2004). These volatile compounds are then desorbed by fiber heating in the GC inlet, while the non-volatile compounds are desorbed by solvent pumping through the SPME-HPLC desorption chamber interface. The SPME is advantageous over SPE and LLE as it requires little or no solvent which makes it a greener extraction method (Wells, 2003). Waleng and co-workers (2022), developed a solid-phase micro-extraction coupled with high-pressure liquid chromatography for the pre-concentration and determination of neonicotinoid insecticides. The analytes of interest were detected and quantified by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD). Under optimum conditions, the limits of detection and quantification were in the ranges of 0.041-0.82 µg/L and 1.4-2.7µg/L, respectively. The linearity ranged from 1.4-700 µg/L with correlation of determination (R^2) values varied between 0.9933 and 0.9987. The intra-day and inter-day precisions were 0.35-0.75% and 1.7-5.5%, respectively. The percentage recoveries ranged from 86.7-99.2%. Therefore, this method showed great potential applicability in pre-concentrating the pollutants from the environment.

2.9.2 Solid samples extraction methods

In SE, the solid sample and the anhydrous sodium sulfate are mixed in a thimble and the extraction is conducted using a suitable solvent in a Soxhlet extractor for 6-24 hours. The extract is then dried, cleaned up and concentrated before chromatographic analysis. The advantages of the SE technique are that there are few variables that can adversely affect extraction efficiency and once loaded it requires no hands-on manipulation and is used as an extraction rate standard for the newly developed extraction method. However, the extraction process takes longer time to achieve greater efficiency of the extraction, it uses large volumes of solvent and it is not appropriate for thermally unstable organic compounds (Moghaddam, 2012). In 2012, Moghaddam and associates successfully applied two extraction methods to study the occurrence of imidacloprid in soil samples. The first method, using a mix of acetone and hexane, was based on soxhlet extraction and the second method, using acetonitrile, methanol and water, was an optimized version of a liquid extraction method. Quantification was performed by reversed-phase High Performance Liquid Chromatography (HPLC) with Diode Array Detection (DAD) at 270 nm using 40:60 (v/v) acetonitrile/water as a mobile phase. The mean recoveries for imidacloprid from soil ranged from 82.6 to 109%, with a relative standard deviation between 1.9 and 5.6% for both extraction methods. The limits of detection

were 0.08 and 0.06 mg/kg for liquid and soxhlet extraction, respectively. Overall, the efficiency of the soxhlet extraction at lower concentrations was better than at higher concentrations, while liquid extraction proved efficient for all spiked levels.

In ultrasonic extraction the solid sample is mixed with the appropriate solvent, and ultrasonicated for a certain time. The centrifugation or vacuum filtration is used to separate the sample and the extract, and then subjected to clean-up, pre-concentration and analyzed. The extraction can be repeated three times to improve the recovery of the analytes (Harrison, 2013). In the ultrasonic extraction process the analytes transfer to the solvent is improved by ensuring the intimate contact between the extraction solvent and the sample matrix. Ultrasonic extraction is preferred over Soxhlet extraction as it uses shorter extraction time, consumes low volumes of organic solvent, energy-efficient, and environmental-friendly (Raina-Fulton, 2016).

2.10 Separation and detection techniques for the determination of neonicotinoids

2.10.1 Separation techniques

Chromatographic techniques especially gas chromatography and high-performance liquid chromatography are commonly used for separation of organic compounds (Coskun, 2016).

The gas chromatography (GC) is used to analyze volatile analytes, however induction can be used to increase volatility for less volatile compounds even though it is cumbersome and introduces possible qualitative errors such as contamination, measurement errors, mechanical/instrumental errors, fractionation errors and loading errors. In GC, a gaseous mobile phase is used to transport sample through a capillary or packed column which contains a polymeric liquid stationary phase (Fiehn, 2017). The separation of the analytes in the sample is achieved based on their affinity differences in stationary phase. Advantages of GC include; highly efficient, quick analysis, sensitive detectors (mg/L), high quantitative accuracy, reliable and rugged technique (Zeeuw, 2015). A simple and sensitive method for the analysis of imidacloprid in water has been developed by Srivastava and associates, (2012) using gas chromatography. Imidacloprid was converted into a volatile imidacloprid-urea by alkaline hydrolysis. The detection of peaks was done with electron capture detector and nitrogen phosphorus detector. The extraction of the compound was conducted using LLE and the mean recovery of imidacloprid-urea in water was found to be 92% with the percent relative standard deviation below 5%. The limit of detection and quantification were obtained to be 20 mg/L and 75 mg/L, respectively (Srivastava, 2012).

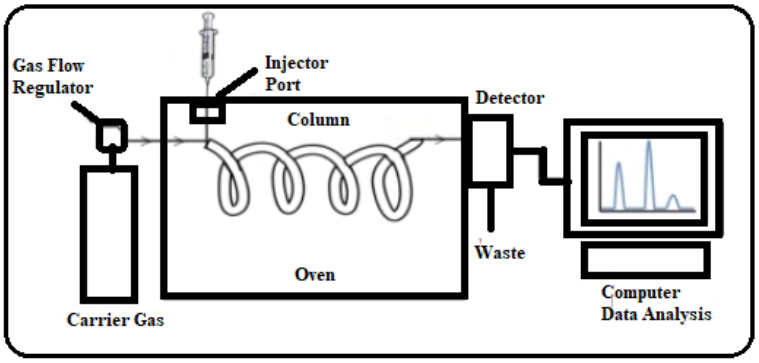

Figure 2.5: The Gas Chromatography set-up diagram

In HPLC, a small sample volume is introduced to the mobile phase (Malviya, 2010). Depending on the physical or chemical interactions the analytes are retarded with the stationary. Elution of the analytes perform under isocratic or gradient mode. Isocratic mode is when single mobile phase composition is used while in gradient the composition of mobile phase is varied during the analysis. The separation of analytes in gradient is a function of their affinity to mobile phase. The commonly used separation mode is the reverse phase where the stationary phase used in less polar than the mobile phase (Unger, 2017). Even though HPLC is highly reproducible and accurate it requires expensive organic solvents in large volumes and can be less sensitive (Hashim, 2016). In 2014, Javanov and colleagues developed and optimized an HPLC-DAD analytical method with dispersive liquid-liquid microextraction (DLLME) and Quick Easy Cheap Effective Rugged Safe (QuEChERS) sample preparation procedures for simultaneous detection of seven neonicotinoids (dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid) in honey samples. Results for accuracy (73.1-118.3%), repeatability (3.28-10.4%) and within laboratory reproducibility (6.45-17.7%), limits of detection (1.5-2.5 µg/kg) and quantification (5.0-10.0 µg/kg) with the use of matrix matched calibration to compensate the matrix effects (Javanov, 2014).

2.10.2 The detection techniques used for neonicotinoids

The commonly used HPLC detectors for the analysis of neonicotinoids are MS and PDA.

Mass spectrometry separates the ions in the gaseous phase based on their mass to charge ratio. The ionization methods such as atmospheric pressure chemical ionization or electro-spray ionization are used to generate the charged species, which can be moved about and manipulated by external electric and magnetic fields (Van Galen, 2016). The stages involved in High-Performance Liquid Chromatography couple with a Mass Spectrometer (HPLC-MS) analysis includes analytes separation by the HPLC column based on their different partitioning between

the stationary phase and the mobile phase. This is followed by spraying the separated analytes into an atmospheric pressure ion source which transform them into ions in the gas phase (Nomura, 2013). The ions are then sorted according to their mass to charge ratio by the mass analyzer such as time of flight quadruple, magnetic sector and ion trap. The detector then counts the ions emergent from the mass analyzer and generate a signal from each ion. The HPLC-MS can simultaneously analyze a sample with many compounds and the data it can produce is excellent, which compensate its high capital and running costs (Lee, 1999).

2.10.2.1 Diode array detector (DAD)

It can provide analytes detection at a single or multiple wavelength. It uses a combination of deuterium and tungsten lamps with radiation emission from 190-850 nm. It consists of a flow cell which collimate the radiation is that is then controlled by a mechanical slit. (Swartz, 2010). Its arrays consist of over 1000 diodes, each of which measures a different narrow-band wavelength range. The high sensitivity and resolution can be attained by programming the entrance slit (Franko, 2010).

2.11 Analysis of neonicotinoids in environmental samples

Lu et al., (2020) assessed the presence of neonicotinoids in China tap water from 71 households where it was observed that each of the collected water samples contained at least one of the assessed neonicotinoids. Thiamethoxam was not detected in all samples, while imidacloprid was detected with a maximum concentration of 0.0106 μg/L and clothianidin was 0.0057 μg/L. Even though these results were found to possess negligible dietary risks of neonicotinoids to adults and children, their presence in drinking water is a public health concern.

Zhang et al., (2020) determined neonicotinoids in water, soil and sediment using HLB-SPE followed by LC-MS/MS. Concentrations up to 0.273 μg/L were obtained for imidacloprid, 0.0687 μg/L for clothianidin, and 0.0037 μg/L for thiamethoxam. The maximum concentrations obtained in agricultural soils were 147, 96 and 42 μg/kg for imidacloprid, clothianidin and thiamethoxam, respectively. These concentrations were higher than those obtained in residential soils which were 28.2, 2.08 and 2.34 μg/kg for imidacloprid, clothianidin and thiamethoxam, respectively. In sediments, maximum concentrations were 0.017, 0.00481, 0.00572 μg/kg for imidacloprid, clothianidin and thiamethoxam, respectively. These results indicate that imidacloprid is the most dominant in all sample matrices. In another

study Bonmatin et al., (2019) evaluated neonicotinoids in soil and sediment using HPLC-MS/MS. The quantification limits were 0.002-0.02 µg/kg and the recoveries were 55-74%. The maximum concentration were 17.1, 4.7 and 1.4 µg/kg in soil and 0.17, 0.5 and 0.11 in sediment, 17.1, 4.6 and 1.49 µg/kg in planted soil for imidacloprid, clothianidin and thiamethoxam, respectively.

The results reported by Zhang et al., (2020) and Bonmatin et al., (2019) showed higher concentrations for imidacloprid in agricultural soil, which is due to that it can be applied as foliar spray or seed treatment. When applied as seed treatment, its higher amount (>80%) remains in soil after application (Zhang et al., 2020). Wang et al., 2019 applied high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) after a combined pretreatment of continuous solvent extraction (CSE) and solid phase extraction (SPE) for the determination of neonicotinoids in river sediments from Jiaozhou Bay of China. The limits of detection quantification obtained were 0.012-0.055 and 0.031-0.091 µg/kg, respectively, while the recoveries were 75 – 98% with a relative standard deviation less than 15%. The concentration in real samples were <LOQ to 0.197 µg/kg.

Zhang et al., 2017 assessed the concentrations of neonicotinoids in water using HPLC/MS/MS, a multi-sorbent solid phase extraction. The recoveries obtained ranged from 76.3% to 107%, the limits of detection were 1.8 – 6.8 ng/L for all analytes. Imidacloprid was detected with concentrations ranging from 32.8 – 193 ng/L, thiamethoxam was detected below the methods quantification limits while clothianidin was not detected.

Main and co-workers, (2014) performed LC/MS/MS analysis for the determination of neonicotinoids in water and sediment samples. The limit of quantification was 1.8, 1.2 and 1.1 ng/L while limits of quantification was 20, 4.4 and 17.5 µg/kg for thiamethoxam, clothianidin and imidacloprid, respectively. Mean recoveries from water were 88, 78.9 and 85.9% for thiamethoxam, clothianidin and imidacloprid While in sediments they were 73.6, 72.3 and 73.5% for thiamethoxam, clothianidin and imidacloprid, respectively (Main, 2014).

Sadaria and co-workers, (2016) reported the mass balance assessment for six neonicotinoids during conventional wastewater and wetland. Flow weighted daily composites were extracted using an automatic solid-phase instrument and analyzed by isotope dilution liquid

chromatography tandem mass spectrometry. The concentrations of imidacloprid, acetamiprid and clothianidin in influent were 60.5, 2.9 and 149.7 while they were (58.5, 2.3, and 70.2 in the effluent, respectively.

Dankyi, (2015) reported the analysis of neonicotinoid residues in soils from cocoa plantations using a Quick Effective Cheap Rugged Safe (QuEChERS) extraction procedure and LC-MS/MS. The recoveries ranging from 72.0-104.8% for all analytes with relative standard deviation ≤ 15 were reported. Limit of detection was 1.0, 2.0 and 3.0 µg/kg while limit of quantification was 5.0, 40 and 9.0 µg/kg for thiamethoxam, imidacloprid and clothianidin, respectively. Concentration of neonicotinoids were 4.3 - 251.4 µg/kg for imidacloprid, 12.2-23.1 µg/kg while thiamethoxam was below quantification limits in all samples.

2.12 Risk assessment of pesticides

The concentration levels obtained in the samples does not give a full idea on the toxicity levels of pesticides. Hence, it is important to conduct the risk assessment studies to evaluate the potential risk that the aquatic life is exposed to. Also, the pesticides from wastewater treatment plants needs to be evaluated to assess their persistency during the treatment process as well as their environmental relevance in the aquatic life of effluent receiving waters. For the first time their concentration in wastewater jointly with their toxicity has been proposed. The methods for the pesticides risk assessment in natural waters reported by Köck-Schulmeyer et al., (2013), which is the environmental relevance of pesticides from wastewater index (ERPWI*)* does not consider the presence or elimination in wastewater treatment plants. In contrast, the water cycle spreading index WCSI method proposed by Reemtsma et al., (2006) take into consideration the concentration of the effluent and relative elimination of individual pesticide during the treatment. However, it does not consider the toxicity of the pesticides from the municipal wastewater entering the water cycle and their effect to the aquatic organisms. Therefore, it is important to employ these two risk assessment methods and compare their results to have an idea on the potential risk the aquatic life is exposed to. These can be assessed against three aquatic organisms: algae, daphnia and fish, using equation (2.1 and 2.2). The toxicity levels is then considered based on the ERPWI values as very high if its >10, high for 1-10, medium for 0.01-1, low for 0.001-0.01, negligible for <0.001.

ERPWI = TUp x Srem x 1000

(2.1)

Where TUp = pesticide concentration in WWTP effluent (µg/L)/end point (EC50; LC50) (µg/L). Srem - removal score, while EC50 - represent fifty percent effective concentration in mg/L, LC50 - is fifty percent lethal concentration in mg/L

$$\text{WCSI} = \frac{C_{influent} \times C_{effluent}}{C_{influent} - C_{effluent}}$$

(2.2)

Where $C_{influent}$ and $C_{effluent}$ are concentrations of individual neonicotinoids detected in the WWTP influent and effluent, respectively.

Chapter 3: Methodology

This chapter gives chemicals, instrumentation as well as details of the procedures followed to attain the experimental data. The steps followed to ensure good quality of the results have been explained. The study area and the specific sampling points of this work have been described.

3. Experimental

3.1 Chemicals and reagents

Pure standards of thiamethoxam (97%), clothianidin (97%) and imidacloprid (99.9%) as well as solvents of HPLC grade methanol (99.8%), acetonitrile (99.9%) and acetone (99%) were purchased from Sigma-Aldrich (Steinhem, Germany).

3.2 Instrumentation

The LC-2020 instrument with Shim-Pack GIST C18-HP column (4.6 x 150 mm, 3µm) and a LC-2030/2040 photodiode array detector purchased from Shimadzu (Tokyo, Japan) was used for determination of neonicotinoids in water samples. The detector wavelength was set at 270 nm and the injection volume of 15 µL was used. The mobile phase used contained a mixture of methanol and 0.1% formic acid in water at a ratio of (80:20, v/v) flowing at a rate of 0.3 mL/min. The solid phase extraction (SPE) vacuum manifold used for the extraction of neonicotinoids from water and to clean extracts from Soxhlet and ultrasonic was purchased from Sigma-Aldrich (Steinhem, Germany). The vacuum pump connected to the SPE vacuum manifold was purchased from Edwards (Munich, Germany). The Oasis hydrophobic-lipophilic balance, (HLB) used as SPE cartridges (3 mL, 60 mg) were purchased from Biotage (Uppsala, Sweden). The ultrasonic bath from Science Tech (Durban, South Africa) and the Soxhlet extractor from the University of KwaZulu-Natal Glassblower (Pietermaritzburg, South Africa) were employed to extract neonicotinoids in sediment and soil samples. The furnace used to analyse the organic content of various soil and sediment samples was purchased from United Science (Gauteng, South Africa).

3.3 Working standards preparation

A stock solution mixture (100 mg/L) containing neonicotinoids of interests (thiamethoxam, clothianidin and imidacloprid) was made dissolving 10 mg of each neonicotinoid in a 100 mL

methanol. Working standard solutions (0.1-1 mg/L) were then prepared from the stock solution and analyzed using liquid chromatography with photodiode array to calibrate the instrument.

3.4 Sampling

The study was conducted in the KwaZulu-Natal Province of South Africa, specifically the city of Durban and Pietermaritzburg. The wastewater samples were collected in four wastewater treatment plants (WWTPs) in Durban (Umbilo, Umhlathuzana, Amanzimtoti and Northern WWTPs). River water samples were collected in Pietermaritzburg from five sampling points along Msunduzi River (Wood house, Bishopstowe, YMCA, College Road and Camps Drift) during cold (Autumn) and hot (Spring) seasons. The water from the rivers where the WWTPs discharge their effluent (Umhlathuzana, Mbokodweni, Umbilo, Umngeni River) were also investigated. Tap water samples were collected in five suburbs (Richmond Crest, Boughton, Woodlands, Mkondeni and Scottsville) around Pietermaritzburg area. Camps Drift is located in the upstream of Msunduzi River. This sampling point is nearby a small industrial area where neonicotinoids containing products areused to manage in-house pests, or outdoors against boring, sucking and roof feeding insect or in industry lawns. The YMCA and College Road are in the vicinity of residential areas and closer to turf on spot pitch football ground, while Woodhouse is closer to the golf course and various food manufacturing companies. In these sampling points neonicotinoids is applied to control indoor pests or weeds on the lawns and home gardens and thus run off to the river. Bishop Stowe is situated down Stream of Msunduzi River, and nearby the small holding farms where the farmers use neonicotinoids to control weed on crops (Kunene and Mahlambi, 2019). Umhlathuzana WWTP obtains wastewater from domestic and industrial areas of Shallcross and Marianridge for treatment. This WWTPs discharge its effluent into Umhlathuzana River, which then discharges into the Indian Ocean through the Durban harbour. Amanzimtoti WWTP accepts wastewater Prospecton, Amanzimtoti, Isipingo and KwaMakhutha industrial and residential areas, and its treated effluent is discharged into Mbokodweni River. Northern WWTP discharges its effluent into Umgeni River. Umbilo WWTP is located at the bottom of the nature reserve in Paradise valley and it treated effluent is discharged into Umbilo River (Naidoo, 2013). The WWTPs receive high amounts of wastewater from industries, and households daily which could contain neonicotinoids. However, the wastewater treatment processes are not designed to completely remove these neonicotinoids and thus are discharged to the receiving rivers and end up in drinking water. It was therefore significant to assess the neonicotinoids levels in drinking water, the influent and effluent of WWTPs as well as the rivers where they discharge their effluent.

The GPS coordinates are Camps Drift (-29.630º - 30.365º), College Road (-29.612º - 30.377º), Woodhouse (-29.602º - 30.413º), YMCA (-29.611º - 30.387º), Bishops Stowe (-29.618º - 30.447º), Mbokodweni River (-30.307º – 30.997º), Umhlathuzana River (-29.873º – 30.879º), Umbilo River (-29.845º – 30.891º), Umgeni River (-30.195º – 30.999º), Amanzimtoti WWTP (-30.007º – 30.917º), Umhlathuzana WWTP (-29.876º – 30.881º), Umbilo WWTP (-29.845º – 30.891º), Northern WWTP (-29.795º – 30.995º).

Samples were collected using dark glass 2.5 L bottles to prevent photosensitive reactions. The bottles were pre-washed with soap and rinsed with tap followed by distilled water to prevent sample contamination. Grab sampling technique at a depth of 0-5 cm from the water surface was applied for water collection. Prior sampling, the bottles were first rinsed with the samples and then filled to the brim to avoid oxidation reactions. They were then transported to the lab in a cooler box where they were kept in fridge at 4ºC until further processing and analysis. The soil and sediment samples were collected at a depth of 0 - 15 cm using a core device. Soil and sediment samples were randomly collected at various points and combined to make a 1 kg representative sediment/soil sample in each site. The samples were then transported to the laboratory, air dried at room temperature in a fume hood for 48 hours, followed by grinding using pestle and mortar and sieving through a 1 mm sieve from Endecotts LTD (London, England).

3.5 Sample preparation

Water sample preparation was conducted using solid phase extraction (SPE). The extraction method described by Kunene and Mahlambi, (2019) was adopted. It was then further optimized to improve the percentage recoveries for all analytes of interest from the water samples. The SPE parameters examined were conditioning solvent (methanol, acetonitrile and acetone) and sample loading volume (25, 50, and 100 mL). The other parameters such as washing solvent and eluting solvent were not optimized since acceptable recoveries were obtained. Under optimum conditions, the SPE cartridges were conditioned with 2 mL of methanol and equilibrated using 2 mL of deionized water. Thereafter, 100 mL water sample was loaded at 1 mL/min flowrate in the conditioned cartridge to allow analytes trapping. The impurities were removed by washing the cartridges with 2 mL of deionized water, cartridges were then dried under vacuum. Lastly, the adsorbed analytes were eluted using 5 mL of methanol, reduced to 1 mL by dry nitrogen and analysed with the LC-PDA system.

Soil sample preparation was conducted using ultrasonic extraction (UE) and Soxhlet extraction (SE). Under UE optimum conditions, a 1 g of sample was mixed with 10 mL of acetone and ultrasonicated for 15 minutes. For the SE, 10 g of sample was extracted using 150 mL of acetone and refluxed for 24 hours at 65˚C. The extracts were then reduced 5 mL by nitrogen (for UE) and by the use of a rotary evaporator (for SE) and diluted with ultra-pure water to 100 mL in a volumetric flask prior to the solid phase extraction clean-up step. Thereafter, 100 mL of the sample was loaded in the conditioned cartridge. The contaminating matrixes were removed by washing with 2 mL of deionized water and the cartridges were then dried under vacuum for 15 minutes. Lastly, the adsorbed analytes were eluted using 5 mL of methanol, pre-concentrated to 1 mL by dry nitrogen and injected into the LC-PDA system for analysis. The ultrasonic extraction and Soxhlet methods described by Kunene and Mahlambi, (2019) were employed and further optimized to improve analyte %recoveries. The extraction parameters optimised for both methods were the solvent type, solvent volume, extraction time and sample mass.

3.6 Method Validation

The proposed analytical procedure was validated based on linearity, %recoveries, precision (as %relative standard deviation), limits of detection (LOD) and limits of quantification (LOQ). The linearity was investigated analysing five standard solutions containing neonicotinoids in the concentration range of 0.1-1.0 µg/mL (figure 3.1). The correlation coefficients (R^2) were closer to a unity of 1 which is considered sufficient evidence to conclude that the calibration curves were linear.

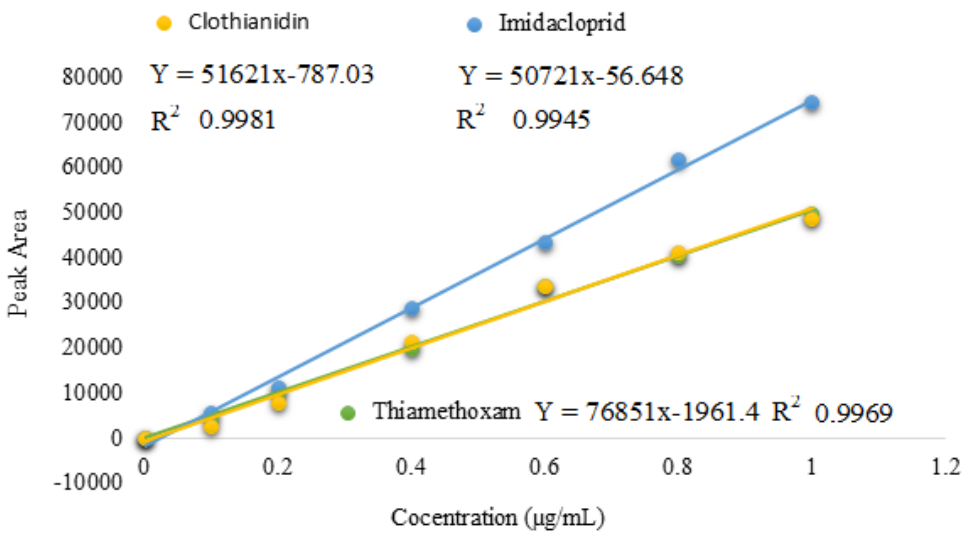

Figure 3.1: The calibration curves of the three nitro-guanidine neonicotinoids.

The LOD and LOQ calculated as 3 and 10 times signal to noise ratios (S/N), respectively were used to measure the methods sensitivity. The accuracy of the method (%recoveries) was assessed using water samples fortified at 10 µg/L with neonicotinoids mixture and subjected to the solid phase extraction prior to LC-PDA analysis. All the analyses were conducted in triplicates and the %percentage relative standard deviation values were calculated to evaluate the precision of the method. The soil samples were fortified at 1 mg/L with neonicotinoids mixture and subjected to ultrasonic or Soxhlet extraction prior to LC-PDA. All the analyses were conducted in triplicates and the RSD values were calculate to evaluate the precision of the method. Figure 3.2 below shows the chromatogram of the neonicotinoids of interest after the liquid chromatographic method optimization. Thiamethoxam elutes first at 6 minutes, followed by imidacloprid 9.5 minutes and clothianidin at 10.9 minutes.

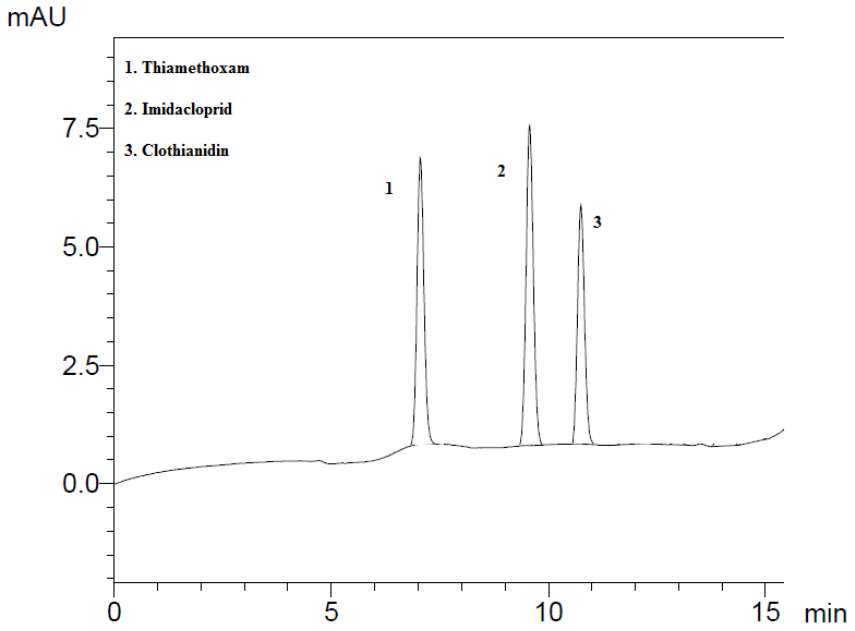

Figure 3.2: The liquid chromatogram peaks of the three nitro-guanidine neonicotinoids.

3.7 Risk assessment

3.7.1 Acute and chronic risk of neonicotinoids in river water

The risk assessment is conducted to evaluate the risk level at which the aquatic environments are exposed to in the rivers. The chronic risk is assessed by calculating the Risk Quotient (RQ) for each neonicotinoid using equation (3.1) while the acute risk is assessed by calculating the Toxicity Unit (TU) using equation (3.2).

$$RQ = EC/PNEC \quad (3.1)$$

Where, EC is the mean or maximum concentration of neonicotinoids detected in river water samples, and PNEC is the predicted no-effect concentration.

The PNEC toxicity is obtained by dividing the lowest short-term LC50; EC50 (acute toxicity) or long-term NOEC

(Chronic toxicity) with an assessment factor (AF). The RQ index value RQ > 1indicates that the presence of the neonicotinoid in water could result to harmful effects while RQ = 0.1-1 indicates medium risk and RQ < 0.1 indicates low environmental risk. The sum of ΣRQ of each neonicotinoid in each site then gives the ecological risk associated with combined neonicotinoids in that site. The ecological risk is negligible if ΣRQsite < 0.01, low ecological risk is predictable for 0.01 < ΣRQsite < 0.1, medium ecological risk is projected for 0.1 < ΣRQsite < 1 while high ecological risk is expected if ΣRQsite >1, (Pérez, et al., 2021).

$$Tu_i(algae, daphnia, fish) = \frac{C_i}{EC50_i\,;\,LC50_i} \quad \text{And} \quad Sum\ Tu_{site} = \sum_{i-1}^{n} Tu_i \qquad (3.2)$$

Where C*i* is the concentration of neonicotinoid *i* in the sample, and LC50*i* or $EC50_i$ is the concentration of neonicotinoid *i* causing a 50% effect in the benchmark organism.

All the individual TU_i of each neonicotinoid detected are summed to get the specific toxic stress of the site (TUsite). This gives an indication of the cumulative toxicity of the residues of neonicotinoids. If the sum of Tus, ΣTu>1, ecological risk is expected, while ΣTu <1 signal no ecological risk (Pérez et al., 2021).

The short and long-term values are obtained from the website http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm.

3.7.2 Environmental relevance of pesticides from wastewater (ERPWI)

The levels of removal in the WWTPs is of importance to identify the neonicotinoids that persist during the treatment process and that are of significance to the aquatic environment. The ERPWI is employed for the identification of relevant neonicotinoids and their toxicity against three aquatic organisms: algae, daphnia and fish using equation (3.3).

ERPWI = TUp x Srem x 1000 (3.3)

Where TUp = pesticide concentration in WWTP effluent (µg/L)/end point (EC50; LC50) (µg/L). Srem - removal score, while EC50 - represent fifty percent effective concentration in mg/L, LC50 - is fifty percent lethal concentration in mg/L (Köck-Schulmeyer, et al., 2013).

The Srem is allocated based on the intervals of the removal rate, while the ERPWI levels of risk are classified as shown on according to Table 3.1. The water cycle spreading index WCSI which is the ratio between the effluent concentration of the compound and its relative removal in a WWTP calculated using equation (3.4) is compared with the ERPWI indexes to allow observation of the toxicity relevance and risk associated to a neonicotinoid (Köck-Schulmeyer, et al., 2013).

$$\text{WCSI} = \frac{C_{influent} \times C_{effluent}}{C_{influent} - C_{effluent}} \qquad (3.4)$$

Where $C_{influent}$ and $C_{effluent}$ are concentrations of individual neonicotinoids detected in the WWTP influent and effluent, respectively.

Table 3.1: Removal intervals, Srem and ERPWI classification (Köck-Schulmeyer, et al., 2013)

% Removal	Srem	ERPWI	Level of risk
75 – 100	0.2	>10	Very high
50 – 75	0.4	1 – 10	High
25 – 50	0.6	0.01 – 1	Medium
0 – 25	0.8	0.001 – 0.01	Low
<0	1.0	<0.001	Negligible

Chapter 4: Results and Discussion

This chapter detail the results obtained in this study. The discussion as well as the comparison of the results with literature is also given.

4.1 Optimization of solid-phase extraction

4.1.1 Effect of the conditioning solvent and sample loading volume

The effect of conditioning solvents was investigated using methanol, acetonitrile, and acetone. The results obtained (Figure 4.1a) showed that higher percentage recoveries (93 – 112%) were achieved for all the analytes when methanol was used. This indicates that methanol was more effective in penetrating through the pores of the sorbent leading to proper activation of the functional groups of the sorbent. This resulted to an increase in surface area available to interact with the neonicotinoids and thus increased the amount extracted from the sample (Kunene and Mahlambi, 2019). All the extraction solvents used are polar and thus were expected to interact effectively with these polar neonicotinoids. However, the efficiency of methanol was further improved by its ability to easily form hydrogen bonding with the analytes of interest. Acetone gave lower percentage recoveries for all the analytes which could be due to that it quickly evaporates resulting to poor sorbent wetting. This led to ineffective activation of the functional groups, and hence lower analytes adsorption. Lower recoveries for thiamethoxam in all solvents used compared to the other neonicotinoid could be due to it high water solubility (Table 2.1). This indicated that it has higher affinity towards the sample matrix compared to the solid phase sorbent which was opposite to the findings for clothianidin by (Walker and Mills, 2002). The statistical analysis done revealed that the mean recoveries are not statistically different as they gave p-values above 0.05. The values obtained were $p>0.58$ for methanol versus acetonitrile, $p>0.32$ for methanol versus acetone and $p>0.64$ for acetonitrile versus acetone (Table S1). Methanol was then selected as the appropriate conditioning solvent due to recoveries above 90% obtained for all neonicotinoids.

The effect of the sample volume was also investigated, as large sample volumes lead to a high pre-concentration factor which subsequently lead to better sensitivity of the analytical method. However, large sample volumes could also result in the sorbent surface saturation therefore reducing the binding sites available for further adsorption of the analytes from the water sample

(Kunene and Mahlambi, 2019). The sample volumes examined were 25, 50 and 100 mL and the results obtained revealed that the neonicotinoids recoveries increased as the sample volume increases (Figure 4.1b). The lower recoveries achieved when 25 mL sample volume was loaded through the sorbent could be due to the limited amount of the neonicotinoids available to interact with the sorbent. The highest percentage recoveries (93-112%) of neonicotinoids were achieved when the sample volume applied was 100 mL. The statistical analysis conducted showed that there is no significant difference in the mean recovery of the assessed sample volumes with the p-values of $p>0.43$ for 25 versus 50 mL, $p>0.17$ for 25 versus 100 mL and $p>0.63$ for 50 versus 100 mL which are more than 0.05 (Table S1). Therefore, 100 mL was considered as the optimum sample loading volume as it gave higher recoveries.

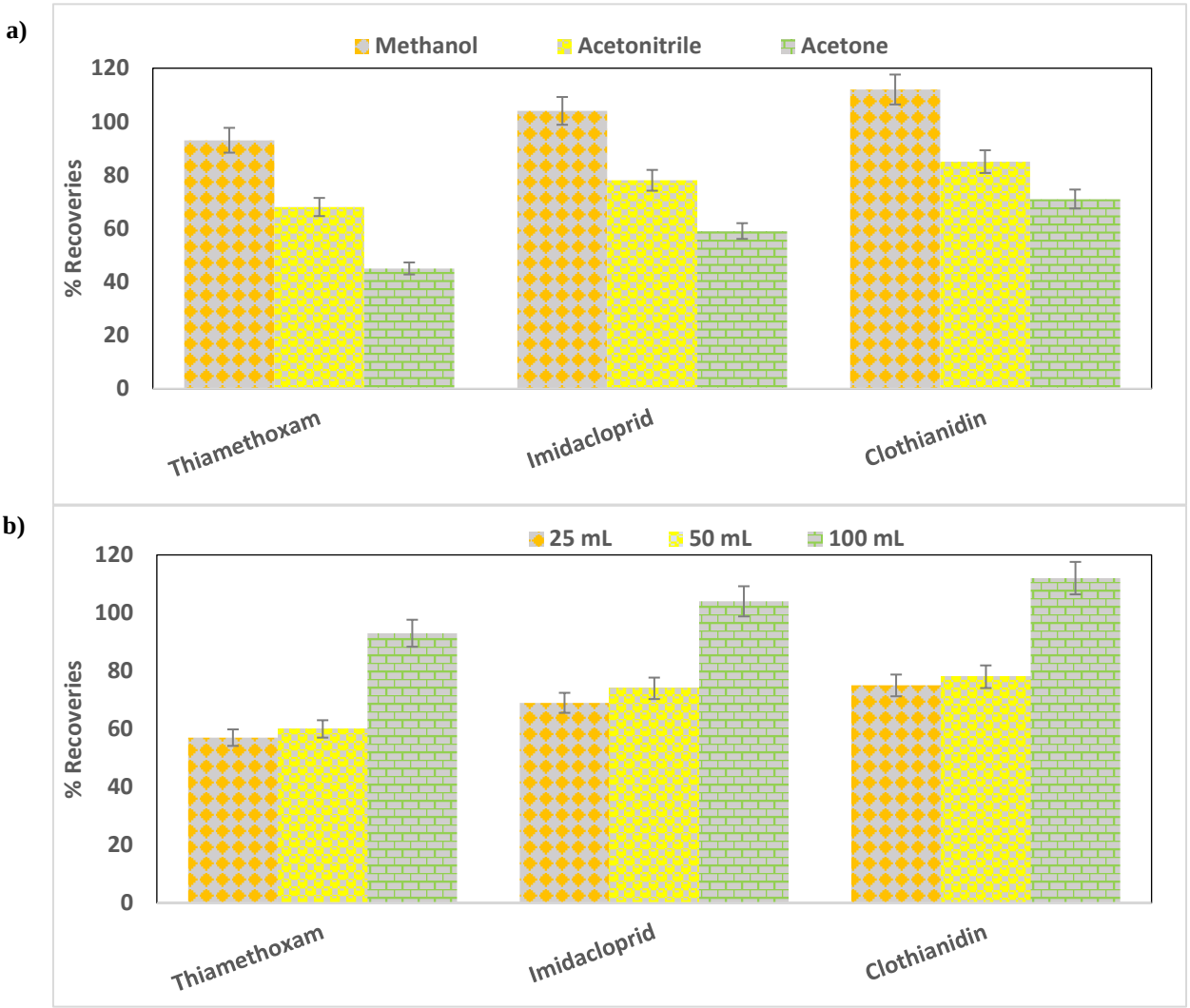

Figure 4.1: The effect of the extraction solvent (a) and sample loading volume (b) on the analyte recoveries

Extraction conditions were – conditioning solvent: 2 mL of methanol, equilibration and washing solvent: 2 mL of deionized water, eluting solvent: 5 mL methanol. River water sample was spiked with neonicotinoids to make final concentration of 10 µg/L.

4.2 Method Validation

The applied method was validated based on linearity, precision and % recoveries, LOD and LOQ. The constructed calibration curves for each neonicotinoid analyte showed good linearity with R^2 values higher than 0.99 in the neonicotinoid's concentration range of 0.1-1.0 mg/L. The LOD and LOQ were found to range between 0.013 to 0.031 µg/L and 0.041 to 0.099 µg/L, respectively, these low values indicated good sensitivity of the optimized method (Table 4.1). The LOQs were also found to be lower than the allowable limit in drinking water (0.10 µg/L) which points out that this method can be effectively applied for the determination of these neonicotinoids. The %recoveries obtained ranged from 79 to 112% which showed good accuracy of the applied analytical procedure in all water matrices. The relative standard deviation (RSD) ranged from 1.1 to 6.5 indicating the good precision of the optimised method (Table 4.1). The recovery and RSD values obtained with the proposed method fulfil the requirements of the guidance document on analytical control and validation procedures, where it was stated that the recoveries for all the analytes should be within a range of 70 – 120% for all spiking levels with RSD ≤ 20% (Fernandez-Alba, 2014). The results obtained indicated good precision and accuracy (Lancu and Radu, 2018).

Table 4.1: Range ((µg/mL)), linear equation, R^2, LODs and LOQs (µg/L) of the analytical method and %Recoveries of analytes and %RSD, (n=3) obtained in tap water, river water and wastewater using Solid Phase extraction.

						%Recoveries		
Compounds	Range	Equation	R^2	LOD	LOQ	Tap water	River water	Wastewater
Thiamethoxam	0.1-1	Y = 76851x-1961.4	0.9969	0.013	0.041	112 ± 1.9	101± 4	91± 4.2
Imidacloprid	0.1-1	Y = 50721x-56.648	0.9945	0.018	0.058	104 ± 6.5	84± 6.5	88 ± 5.0
Clothianidin	0.1-1	Y = 51621x-787.03	0.9981	0.031	0.099	93± 1.1	79 ±1.1	83 ± 3.0

4.3 Application of SPE-LC-PDA in water samples

4.3.1 Physico-chemical parameters of the collected samples

The physico-chemical parameters for all the samples collected were measured before the determination of the neonicotinoid's concentrations. The measured parameters were; chemical oxygen demand dissolved oxygen, dissolved solids, pH, salinity, and conductivity (Table S2-5).

The pH measured was observed to be 6.21 – 7.34, 5.19 – 9.01 and 7.02 – 7.81 for tap water, river water and wastewater samples, respectively. These pH values are within the acceptable range (6.5 – 8.5) for raw water, except for Wood house and College road river water (WHO (2003). The highly acidic pH may result to the protonation of neonicotinoids while highly basic pH may hydrolize them. Also, the neutral pH may activate the microbial degradation, which may all promote the reduction of neonicotinoid concentrations in the water samples. The water salinity was measured to be 0.19 – 0.44, 0.08 – 4.85 and 0.29 – 0.64 psu in tap, river and wastewater which are within the acceptable value of ≤1 psu except in Mngeni, Umbilo and Mbokodweni Rivers. The water samples with higher salinity are expected to have low concentrations of neonicotinoids because high concentration of salinity decreases their solubility in water and increases their sorption to sediments.

The total dissolved solids (TDS) were 106 - 243, 86 - 1972, 304 - 658 mg/L in tap, river and wastewater samples, respectively. These TDS values are within the acceptable amount <1000 except in Northern River water sample where there is a potential negative effect on water body (WHO, 2003). The higher TDS observed in rivers where the WWTP discharge their effluent (105 - 1972 mg/L) compared to water samples from Msunduzi River (86 - 170 mg/L) could be due to the saline industrial effluents and sewage effluent discharge into the rivers (Naidoo, 2013). The conductivity was found to be within the acceptable value of 1700 µS/cm as they ranged between 187 - 758, 172 - 888 and 608 - 1312 µS in tap, river and wastewater, respectively (Wanda et al., 2016). The high values of TDS and conductivity have been reported to indicate high levels of organic pollutants in the water (Nyoni, 2011). The dissolved oxygen (DO) was found to range between 2.4 - 3.6 mg/L, 0.02 - 2.9 mg/L and 0.3 - 2.94 in tap, river and wastewater, respectively which are below the maximum limit of 8.14 mg/L (Munyika *et al*., 2014). The higher DO concentrations in the effluent could be due to aeration process (Madikizela and Chimuka, 2017). Higher DO levels indicate the presence of microbes,

therefore this may result to low especially at a pH of 7. The samples temperature was observed to range between 14-24°C which is within the limit acceptable limit of <35˚C. The higher temperature may result to the decrease in neonicotinoids concentrations due to their possible degradation (Nannou et al., 2015).

4.3.2 Determination of neonicotinoid in tap water

The tap water samples were collected in Richmond Crest, Boughton, Woodlands, Mkondeni and Scottsville. All the assessed neonicotinoids were detected in all the tap water samples collected, even though they were below quantification limits in most samples (Figure 4.2). Imidacloprid was found in higher concentrations than the other neonicotinoids, however, they were all within the acceptable level (0.1 µg/L) of neonicotinoids in drinking water. This is an indication that the water was still safe for consumption. Thiamethoxam was only quantified at Mkondeni tap water (0.062 µg/L), while clothianidin was below quantification limits in all samples. Their low concentrations could be due to base-catalysed hydrolysis which occurs under high pH-conditions during lime softening of the drinking water treatment process (Klarich et al., 2017). These results agree with those obtained by Klarich et al., (2017) in the study where clothianidin, imidacloprid and thiamethoxam were present in all tap water samples with maximum concentrations of 0.057, 0.039 and 0.0041 µg/mL, respectively. However, literature results are lower than those obtained in the current study except for clothianadin. These findings indicate that neonicotinoids are poorly removed via treatment systems leading to their presence in drinking water. This could lead to unplanned consumption by humans resulting to human health effects.

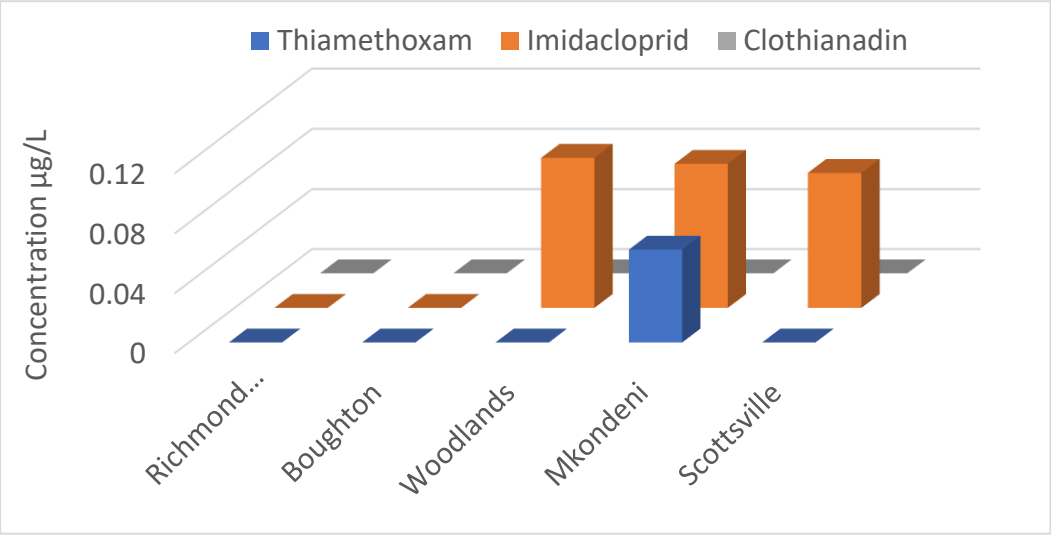

Figure 4.2: The concentrations (µg/L) of neonicotinoids detected in tap water

4.3.3 Determination of neonicotinoid in river water

The effect of seasonal variations in the concentration levels of neonicotinoids was investigated using samples collected along Msunduzi River (Wood house, Bishopstowe, YMCA, College Road and Camps Drift) during cold (Autumn) and hot (Spring) seasons. The evaluated neonicotinoids compounds were detected in all sampling sites during the cold season, however, they were not quantifiable in some sites (Figure 4.3). Higher concentrations of the neonicotinoids (0.081 - 0.20 µg/L) were observed in the cold season compared to hot season. This could be due to low rainfall resulting in low water levels in the rivers during the cold season, causing an increase the neonicotinoids concentrations due to pre-concentration (Kunene and Mahlambi, 2019). Higher concentrations could also be due to lower sunlight levels which result in little or no photo degradation of the compounds. In some sampling points the neonicotinoid were not detected in the hot season. This could be due to lower concentrations at which the compounds are present compared to the methods detection limits and unavailability of the contaminant sources at each sampling point. Woodhouse and Bishop stowe were the most contaminated sampling points. Higher concentrations in Woodhouse could be due to the pollutants run-off from the New-England landfill site which is closer to the sampling point. Also, direct disposals of used materials containing the neonicotinoid from the residents may contribute towards such high concentrations. Contamination in Bishop stowe sampling point may be due to the effluent from the Darville wastewater treatment plant that is discharged before the sampling point. Also, pollutants run-off from agricultural sectors near the sampling point might have contributed to contamination in Bishop stowe (Kunene and Mahlambi, 2019). This suggest a possible intensive usage of these neonicotinoids by farmers in the province of KwaZulu Natal.

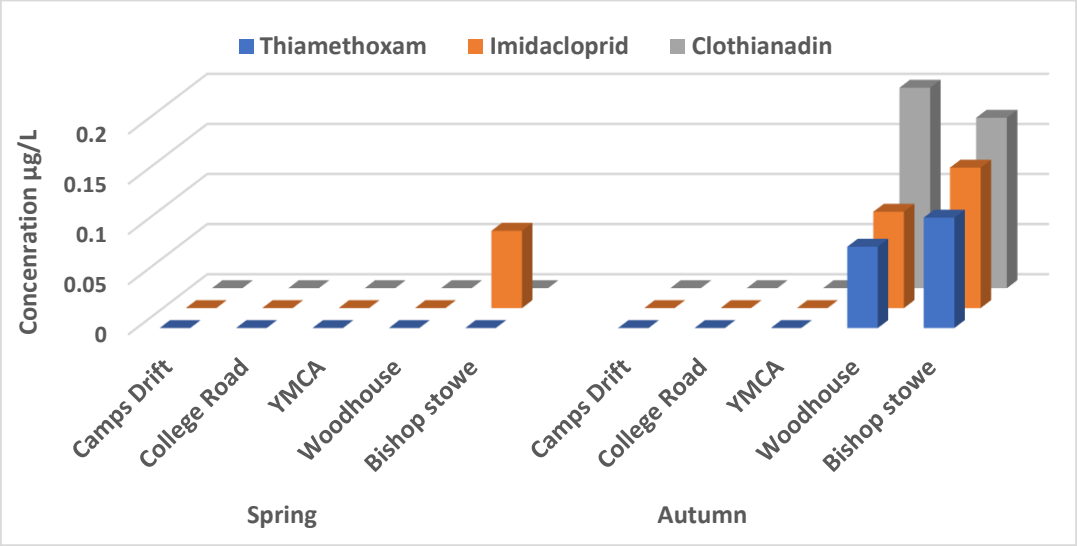

Figure 4.3: The maximum concentrations (µg/L) of neonicotinoids detected along Msunduzi River in autumn and spring

4.3.4 Determination of neonicotinoids in wastewater

The wastewater samples were collected at Umbilo, Umhlathuzana, Amanzimtoti and Northern WWTPs. Imidacloprid and clothianidin reached concentrations up to 15 and 9.4 µg/L, respectively (Table 4.2). This could be due to their high usage in industrial vegetation control, grass management activities, pet flea treatment, and pest control in private homes and gardens (Pietrzak et al., 2019). The highest concentration observed for thiamethoxam was 5.5 µg/L which might have been contributed from its usage against termites, cockroaches, adult flies, ants, fleas (Pietzrak et al., 2019). Since only a small percentage of applied neonicotinoids reach the targeted pests and they are usually not completely degraded in the environment they can be transported to wastewater treatment plants.

In the effluent samples, the neonicotinoids were detected in most samples even though imidacloprid was not quantified in Umhlathuzana and Umbilo WWTPs. Clothianidin was below the quantification limit in Northern and Umbilo, while thiamethoxam was below the quantification limit in Umbilo effluent. The concentrations of the neonicotinoids showed a decrease in all effluent (0.99 – 3.3 µg/L) compared to the influent (2.3 – 15 µg/L) samples. These results indicate a possible full or partial removal of neonicotinoids by microbial degradation, hydrolysis, and oxidation by chlorine during the treatment process (Sadaria, et al., 2016). The concentrations were above the maximum allowable concentrations in most of the effluent samples. This indicates that the WWTPs are contributing towards pollution of the receiving rivers by these neonicotinoids posing a potential risk to the aquatic life. This could

be due to that the WWTPs are not designed to completely remove these compounds. Also, these compounds are water soluble which makes it easily for them to escape the treatment process. Umbilo WWTP was found to be the most polluted treatment plant as all the compounds analysed were detected in both influent and effluent samples. The results obtained agrees with those observed by Sadaria et al., (2016) where the WWTP effluents substantially increased neonicotinoid concentrations in river waters where the effluents are discharged. Sadaria and co-workers reported that thiamethoxam was either detected below the detection limits or not detected in the influent samples. Imidacloprid maximum concentration in the effluent was 0.048 µg/L, while clothianidin was 0.116 µg/L which revealed limited removal of these analytes (Sadaria et al., 2016).

The neonicotinoids were detected in all sludge samples (1.3 – 6.9 µg/L), however, imidacloprid and clothianidin were not quantified in Umhlathuzana WWTP. This indicates that neonicotinoids get adsorbed to sludge sample. Since sludge removal is done in the early stage of the treatment process the adsorbed neonicotinoids get removed with it resulting in lower concentrations in the effluent. In a study conducted by Sadaria and co-workers, the thiamethoxam concentration loads in sludge were below the detection limits. However, imidacloprid had a maximum concentration of 0.033 µg/L and clothianidin was 0.451 µg/L (Sadariaet al., 2016) which are lower than the concentrations obtained in the current study. The concentrations obtained in effluent are lower than those observed in the rivers where they discharge their effluent especially for clothianadin. This indicates that there are other factors that contribute towards pollution by these neonicotinoids in the rivers other than the WWTPs. Thiamethoxam was less frequently detected in river water which could be due to its short half-live (12–14 days). However, clothianidin was frequently detected which could be due to that it is a degradation product of thiamethoxam (Sánchez-Bayo et al., 2016).

The wastewater treatment plants processes showed varying percentage removal of the studied neonicotinoid which was higher for thiamethoxam followed by clothianidin while imidacloprid was least removed signaling its persistence during the treatment process. The %removal were calculated using equation 4. Thiamethoxam was completely removed in all WWPs except in Umbilo where 78% was removed. Imidacloprid was completely removed in Northern and Amanzimtoti WWTPs, while in Umhlathuzana and Umbilo had 95% and 55% removal respectively. Clothianadin was completely removed in Amanzimtoti and Umhlathuzana WWTPs while Northern and Umbilo had 69% and 71%, respectively. These results indicate that Amanzimtoti and Umhlathuzana WWTPs were able to completely remove or reduce all

the neonicotinoids resulting in less than 5% discharged into the receiving rivers. Umbilo had lower removal percentage for almost all the neonicotinoids with the lowest observed for imidacloprid (55%). Lower removal percentage (22%) of imidacloprid had been reported by Lancu and Rabu, (2018) in Romania (Bucharest WWTP). The removal efficiency depends on the processes used and reactions that occurs between the pollutants and other organic compounds which can either enhance, delay or do nothing to the degradation rate of the neonicotinoids (Lancu and Radu, 2018). These results indicated that the, neonicotinoids emissions from WWTPs need to be reduced so that they do not exceed the maximum residue values.

$$Removal\ efficiency = \frac{[Influent]-[Effluent]}{[Influent]} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.4)$$

Table 4.2: The concentrations (µg/L) of neonicotinoids detected in wastewater, receiving rivers and sludge, n = 3

	NT				**AM**				**UH**				**UB**			
	Inf	**Eff**	**SG**	**UR**	**Inf**	**Eff**	**SG**	**MR**	**Inf**	**Eff**	**SG**	**UHR**	**Inf**	**Eff**	**SG**	**UBR**
Thi	2.8±1.4	Nd	2.7±1.3	nd	4.1±1.6	nd	3.2±1.3	nd	5.5±2.5	Nd	2.6±1.3	nd	4.6±2.1	0.99±0.4	3.9±1.3	1.18±1.4
Imi	3.5±1.8	Nd	1.3±1.1	nd	15±4	nq	1.4±3.1	nd	2.3±1.9	0.23±0.1	nq	nd	7.3±2.7	3.3±2.2	2.9±3.1	2.10±1.9
Clo	6.8±4.4	2.1±1.4	2.5±2.5	1.3±0.9	3.2±2.4	nq	2.9±2.5	2.1±1.9	9.4±5.4	Nq	nq	0.18±0.2	6.5±3.2	1.9±1.1	6.4±2.5	3.7±2.0

nd – not detected; nq – below quantification limit; Thi – Thiamethoxam; Imi – Imidacloprid; Clo – Clothianadin; NT – Northern wwtp; UR – Umngeni River; UM – Umbilo wwtp; UBR – Umbilo River; UH – Umhlathuzana wwtp; UHR – Umhlathuzana River; AM – Amanzimtoti wwtp; MR – Mbokodweni River, Inf – Influent; Eff - Effluent; SG - Sludge

4.3.5 Risk assessment in water samples

4.3.5.1 The acute and chronic toxicity of neonicotinoids in Msunduzi River

The risk quotient (RQ) and toxic units (TUs) values were calculated for all neonicotinoids of interest at three taxons representative (algae, daphnia magna, and fish) of three ecosystem trophic levels in order to cover all food chains in the water. The RQ was used for the assessment of chronic toxicity using the maximum concentrations obtained. The chronic toxicity was observed to be in the following order clothianidin>imidacloprid>thiamethoxam (Table 4.3). Its only clothianidin RQ value that was found to be above 1 (RQ = 1.7), which indicates its potential to cause detrimental effects, however, this is more susceptible towards daphnia magna species. The highest RQ for algae and fish was observed for imidacloprid, however it was less than 0.1 indicating low risk against these species. Even though these results indicate low risk, they can still causse an effect on biota particularly, fish that bioaccumulate pesticides and for which it may be lethal even at trace levels (Masiá et al., 2014). These findings agree with those of Masiá et al., (2014) where imidacloprid was found to possess low chronic toxicity effect on the algae, daphnia and fish species in Llobregat River (Spain). The sum of ΣRQ values in the study site revealed that the overall chronic toxicity of the studied neonicotinoids combined possess high ecological risk against aquatic species with ΣRQ value of 1.80. The main contributor towards this risk was clothianidin. These results suggest that the assessment of a single-chemical underestimate the actual risks to aquatic species in water bodies containing a mixture of neonicotinoids.

Table 4.3: PNEC and RQ values calculated for maximum environmental concentrations of neonicotinoids in river water, according to chronic toxicity data measured in algae, daphnia magna, and fish

Neonicotinoid	Fish		Aquatic invertebrate (*Daphnia magna*)		Algae	
	Chronic 21 days NOEC		Chronic 21 days NOEC		Chronic 96 hours NOEC	
	PNEC (µg/L)	RQmax	PNEC (µg/L)	RQmax	PNEC (µg/L)	RQmax
Imidacloprid	9020	0.02	1800	0.1	10000	0.01
Clothianadin	20000	0.01	120	1.7	>100000	0.002
Thiamethoxam	20000	0.01	>100000	<0.001	>100000	0.002

The Tu was used for the assessment of acute toxicity. The sum of the Tus was found to be less than 1 indicating no acute ecological effect of these neonicotinoids against algae, daphnia and fish in the study sites (Table S6). To the best of our knowledge, the combined chronic and acute toxicity effect for these three neonicotinoids was conducted for the first time worldwide against these three-aquatic species (fish, algae and daphnia species).

4.3.5.2 Environmental relevance of neonicotinoids from wastewater treatment plants (ERPWI)

The levels of removal in the WWTPs is of importance to identify the neonicotinoids that persist during the treatment and that are of significance to the aquatic environment. The WCSI was found to be 6.02, 3.8 and 1.26 µg/L for imidacloprid, clothianidin and thiamethoxam respectively, which indicated that imidacloprid is the most relevant neonicotinoid. The ERPWI for these neonicotinoids was calculated for algae, daphnia magna, and fish to assess the toxicity effect of all three ecosystem trophic levels (Table S7). The mean ERPWI for the three studied WWTPs and the three aquatic organisms (algae, daphnia and fish) was 0.17, 0.024 and 0.0019 µg/L for imidacloprid, clothianidin and thiamethoxam, respectively (Figure 4.4). This is classified as medium risk level for all neonicotinoids as it is in the range of 0.01-1 (Köck-Schulmeyer et al., 2013). These results indicated that the studied neonicotinoids do persist during the wastewater treatment process, resulting to them entering rivers

where they can affect aquatic organisms. This agrees with the results obtained for WCSI which suggested that imidacloprid is the most problematic neonicotinoid for the aquatic environment especially the daphnia species.

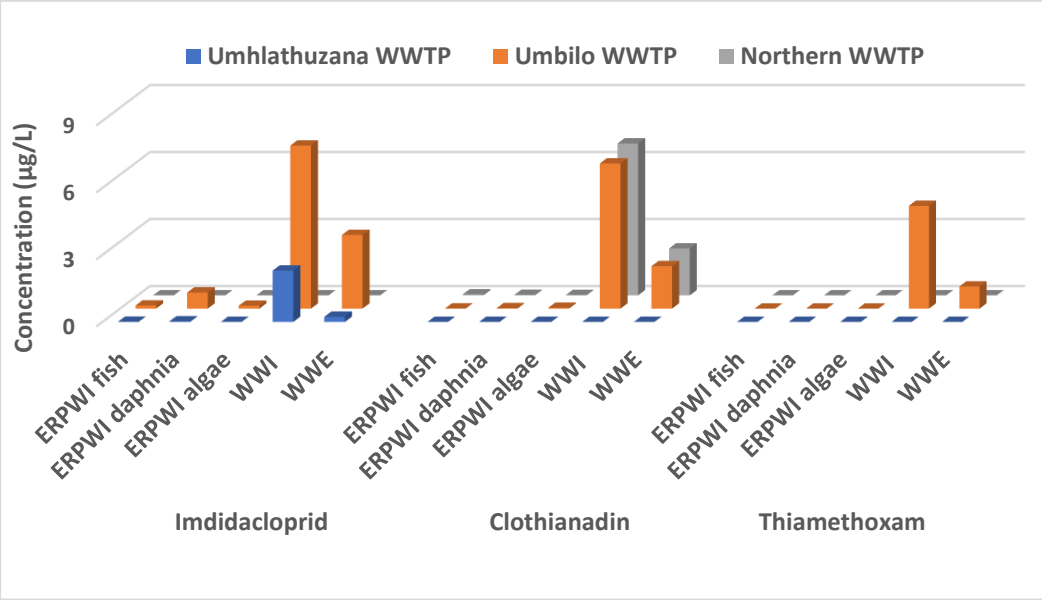

Figure 4.4: ERPWI for neonicotinoids for each aquatic organism in each wastewater treatment plant

4.4 Application in sediment samples

4.4.1 Optimization of ultrasonic extraction method

The optimization of ultrasonic involved assessing the influence of the extraction solvent type, solvent volume, sample mass and extraction time on the recoveries of the neonicotinoids. The extraction solvent type plays an important role in the extraction process as it controls the interaction and dissolution of the anaytes and thus affects the analytes recoveries from the sample matrix (Chaves et al., 2020). The effect of extraction solvent

was investigated using methanol, acetone, and acetonitrile, where these polar solvents were selected to match the polarity if the analytes of interest. Acetone gave comparable recoveries with methanol ranging between 78-88% and 75-81%, respectively while for acetonitrile they were 69-78%, (Figure 4.51a). These results indicate that acetone and methanol were able to penetrate through the soil particles and efficiently desorbed the neonicotinoids adsorbed on or in soil aggregates resulting in high neonicotinoids recoveries. Higher efficiency for methanol and acetone could be as a result of the ability to form hydrogen bond, hence they were able to bind with the neonicotinoids. Also, acetone possess polar and non-polar functional groups and hence, it has the ability to dissolve polar and non-polar compounds and thus was able to properly dissolves all the analytes of interest resulting to their high recovery in the solvent. Acetonitrile forms a dipole dipole interaction which is weaker than the hydrogen bond, also, acetonitrile being a protophilic solvent, it properly dissolves compounds that are less polar thus, it resulted to slightly lower recoveries of the neonicotinoids (Bonventre, 2014).

The assessment of the sample mass is important as increasing sample mass increases the amount of the analytes available to interact with the extraction solvent. However, this effect is also dependent on the extraction solvent volume which can controls the solvent saturation stage (Chaves et al., 2020). The sample mass effect was examined using 1, 5, and 10 g soil sample. The neonicotinoids recoveries were observed to decrease with the increase of the sample mass, hence, 1 g sample gave the highest recoveries ranging between 81-92% (Figure 4.5b). This suggests that the solvent volume used was able to fully disperse over the 1 g sample, leading to maximum penetration and optimum interaction with the neonicotinoids, thus improved their recoveries. The lower recoveries obtained when 10 g was used could be due to poor analytes interaction with the solvent leading to poor sample aggregate disintegration.

The effect of extraction solvent volume was explored using 10, 20, and 30 mL and the 10 mL gave higher analyte recoveries ranging between 83-94%. This indicates that this solvent volume was an appropriate ration with the quantity of the sample and thus the extraction process was maximised resulting to improved neonicotinoids recoveries in the extraction solvent (Vilkhu et al., 2011). A sample:solvent ration between 1:5 and 1:10 has been found to give high recoveries for ultrasonic bath extraction where bioactive compounds were analysed. This was reported to

influence the ultrasonic cavitation phenomena and thus the analytes extraction (Vinatoru et al., 2017). The 30 mL solvent volume gave the lowest recoveries ranging between 72-79%, (Figure 4.5c). This could be due to that higher solvent volume may results to a dissolution of the analytes and decrease in analyte mass transfer from the solid sample to the solvent, hence limit the amount of analyte recovered.

The effect of extraction time was explored using10, 15 and 20 minutes. The 15 minutes gave optimum recoveries ranging between 85-102% (Figure 4.5d), indicating that 15 minutes was the adequate to allow neonicotinoids mass transfer from the sample medium to the extraction solvent. The lower neonicotinoids recoveries obtained at 10 minutes could be due to insufficient time to allow effective interaction between the sample and the solvent, thus the neonicotinoids were left in the sample. However, when the extraction time is too long it can result in the degradation of the analytes which could be the reason for lower recoveries obtained at 20 minutes (Chaves et al., 2020).

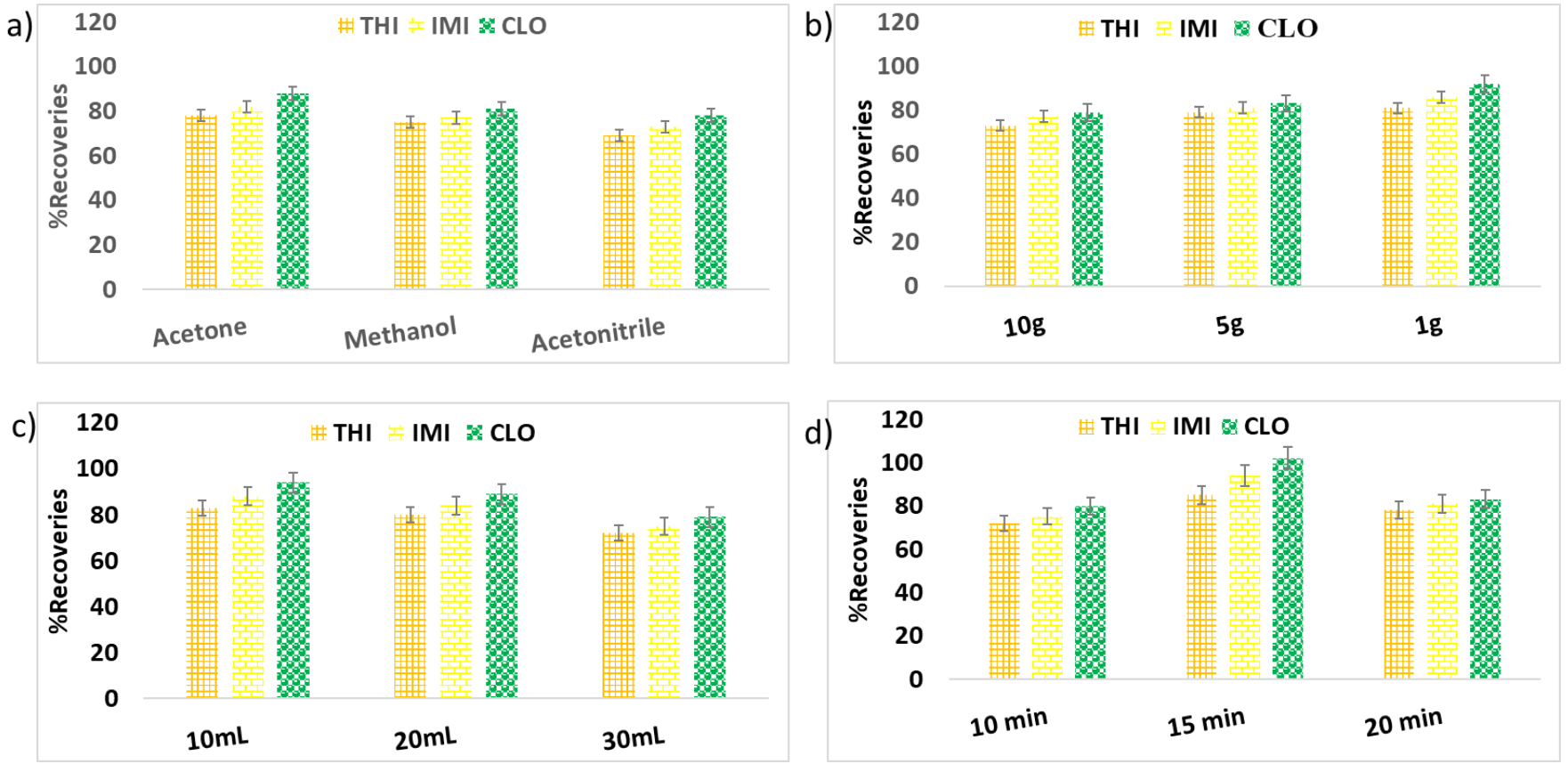

Figure 4.5: The effect of extraction solvent (a), sample mass (b), solvent volume (c) and extraction time (d) on the recoveries of neonicotinoids by ultrasonic extraction

4.4.2 Optimization of Soxhlet extraction method

The effect of extraction solvent type was examined using methanol, acetone, and acetonitrile. The higher neonicotinoids recoveries (79-86%) were obtained for acetone, while acetonitrile gave the lowest recoveries (Figure 4.6a). This agrees with the findings of Ahmad et al. (2017), where higher detected concentration in various solvents were in the following order, acetone>methanol>acetonitrile. This observation was reported to be due to the dielectric constant of solvent where acetonitrile with higher dielectric constant resulted in a slow rate of the extraction process and thus lower amount of the compounds was transferred to the extraction solvent. The results obtained in this work could also be as a results of acetone's low boiling point compared to the other solvents used, thus the temperature used was high enough to speed up the reaction between the neonicotinoids and the acetone leading to higher amount dissolved in the solvent (Kumoro et al., 2009). Even though, the employment of high temperature could improve solubility and diffusion of the analytes, it could also encourage their possible degradation during the extraction process which could be the reason for slightly lower recoveries obtained with acetonitrile (Zhang et al., 2018).

The higher volume of solvent may solubilise high amount of the analytes, however, when it is too high it may dilute the analytes leading to lower recoveries. The effect of extraction solvent volumes was studied using 100 mL, 150 mL, and 200 mL. The 150 mL was the optimum volume with recoveries ranging between 85-93%, this indicates that if was efficient to constantly wet the sample, leading to effective distillation (Figure 4.6b). The lower recoveries obtained with the 100 mL could be due to limited unsaturated fresh solvent portion to be constantly supplied tothe sample and dissolve the neonicotinoids from the sample (Figure 4.6b). A higher sample:solvent ratio of 1:50 and higher have shown to promote complete removal of the analytes from the sample matrix to the extraction solvent which could be the reason why 200 mL also gave acceptable recoveries (Chaves et al., 2020).

To examine the effect of sample mass 5, 10 and 20 g dried soil samples were used. The recoveries showed an increase with an increase in sample mass and then decrease with further increasing mass. This could be due to that there was an increase in the neonicotinoids that were available to dissolve in the solvent with increased sample mass (Figure 4.6c). However, the extraction process of soil samples involves the solvent penetration into the soil matrix, allowing the analytes to dissolves in the solvents and thus their diffusion out of the soil matrix (Zhang et al., 2018). Therefore, if the solvent amount employed is not enough to allow complete

dissolution of the analytes in the sample, the analytes will be left in the samples, which might the reason for lower recoveries observed when 20 g was used. The 10g of the sample showed higher analyte recoveries ranging between 89-107%, this indicates that the sample matrix was properly hydrated and thus permitted proper analytes solubilization (Chaves et al., 2020).

The influence of extraction time was assessed conducting the Soxhlet extraction for 8, 16, 24 hours. Comparable recoveries were obtained when 8 and 16 hours of extraction, however the increase was observed at 24 hours of extraction (87 – 109%), (Figure 4.6d). This could be due to that longer extraction time increases with the increase in the extraction efficiency due to optimum interaction of the analytes and the solvent provided the equilibrium of the analytes is not reached inside and outside the soil matrix (Zhang et al., 2018). However, the extraction process is also controlled by the analytes ability to be easily extracted from the soil matrix which influenced by the analytes interaction with the matrix as this will allow or hinder its removal rate and efficiency (Chaves et al., 2020).

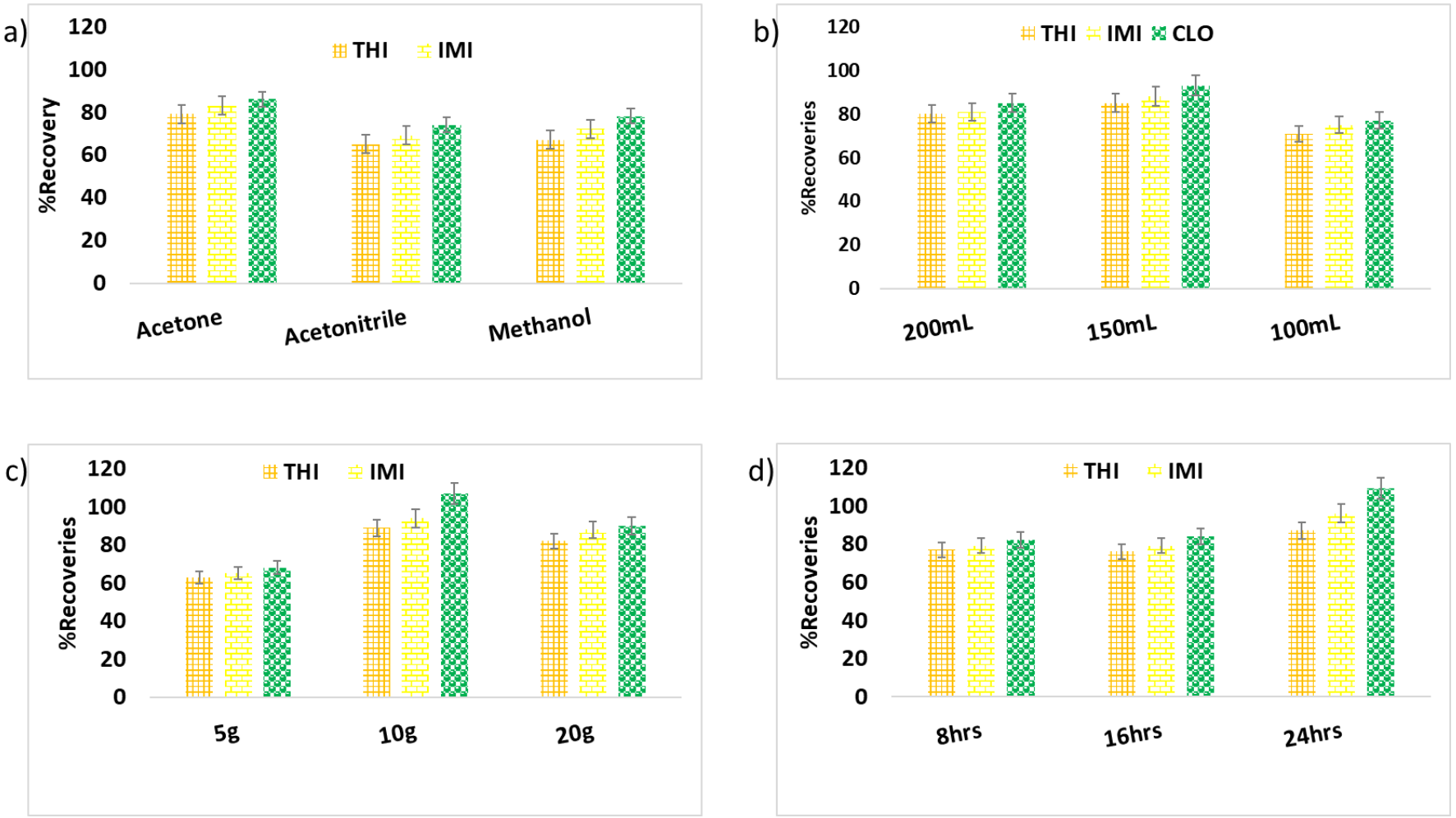

Figure 4.6: The effect of extraction solvent (a), sample mass (b), solvent volume (c) and extraction time (d) on the recoveries of neonicotinoids by Soxhlet extraction

4.4.3 Method Validation in sediment samples

The method validation of the developed analytical procedure was validated based on linearity, %recoveries, precision, limit of detection (LOD) and limit of quantification (LOQ). The linearity was investigated by analysing five standard solutions containing neonicotinoids in the concentration range of 0.1-2.0 mg/L. The LOD and LOQ calculated as 3 and 10 signals to noise ratios (S/N), respectively were used to measure the methods sensitivity. The %recoveries were calculated using fortified soil and sediment samples at 1.0 mg/L of neonicotinoids mixture and subjected to UE, SE and SPE sample-clean up prior to LC-PDA. All the analyses were conducted in triplicates and the RSD values were calculated to evaluate the precision of the method. Both UE and SE methods showed acceptable recoveries indicating that they both be employed to accurately extract the neonicotinoids from soil and sediment samples (Table 4.4).

Table 4.4: The linearity (R^2), methods LODs, and LOQs (µg/kg), rrecoveries of neonicotinoids (n=3) for the optimized UE and SE methods.

Neonicotinoids	%Recoveries				MLOD	MLOQ	R^2
	Soil		Sediment				
	UE	SE	UE	SE			
Thiamethoxam	8.5±1.5	87±2.6	92±3.1	84±3.9	0.4	1.5	0.9969
Imidacloprid	94±2.1	96±3.0	96±2.6	90±2.1	0.4	1.4	0.9945
Clothianidin	102±2.4	109±1.9	103±2.4	94±1.4	0.8	2.7	0.9981

4.5 Application of UE-LC-PDA in soil and sediment samples

4.5.1 The effect of seasonal variations on the concentrations of neonicotinoids in soil samples

The effect of seasonal variations in the concentration levels of neonicotinoids was determined using soil and sediment samples collected during Autumn (cold) and Spring (hot) seasons along Msunduzi river (Camps drift, Woodhouse, Bishopstowe) in Pietermaritzburg. There was no clear trend of seasonal effect on the concentration of neonicotinoids in soil as some compounds has higher concentrations in the spring season while some were higher in the autumn season. This could be due to that the presence of neonicotinoids is dependent on their source of contamination in the sampling point while their detection depends on the methods detection limits. Neonicotinoids were detected in all samples during the spring season with the highest concentration of 410 μg/kg observed for clothianadin in Bishopstowe (Figure 4.7). The clothianidin and thiamethoxan were not detected in the autumn in Bishopstowe and Camps Drift, respectively. Imidacloprid was detected at a higher concentration (390 μg/kg) in Bishopstowe followed by thiamethoxam (270 μg/kg) in Woodhouse sample. High concentration of neonicotinoids was observed in autumn could be due to the neonicotinoids limited photodegradation as there is a low sunlight during the cold season. Also, there is low rainfall, thus the neonicotinoids concentration dissolution in soil, and also their transfer to water which results from erosion of frequent rainfall is reduced. The higher concentrations of neonicotinoids detected in Woodhouse could be a result of their run-off from the nearby New England Landfill site or illegal dumping of neonicotinoids containing waste. The high concentrations of neonicotinoids in Bishopstowe sampling area may be due to the effluent disposal by the Darville wastewater treatment plant or their residues run-off from the nearby agricultural areas. These results agree with the findings of Zhang et al., (2020), where higher concentrations of imidacloprid were observed to be higher in soils from agricultural areas (147 μg/kg) compared to other areas. However, their results are lower than the concentration obtained in this work. In general, the neonicotinoids presence in soil samples could be due to soil's high ability to deposit pesticides thus affecting their overall dispersion to other environmental compartments (Vryzas et al., 2018). Also, the mobility and persistence of pesticides is controlled by their adsorption into soil, organic matter, and clay content/composition, soil minerals, soil pH and temperature (Arias-Estévez, 2008). The organic matter

promotes the sorption affinity of neonicotinoids in soil/sediment samples, hence the samples with a higher amount of organic matter are expected to have a higher concentration of neonicotinoids.

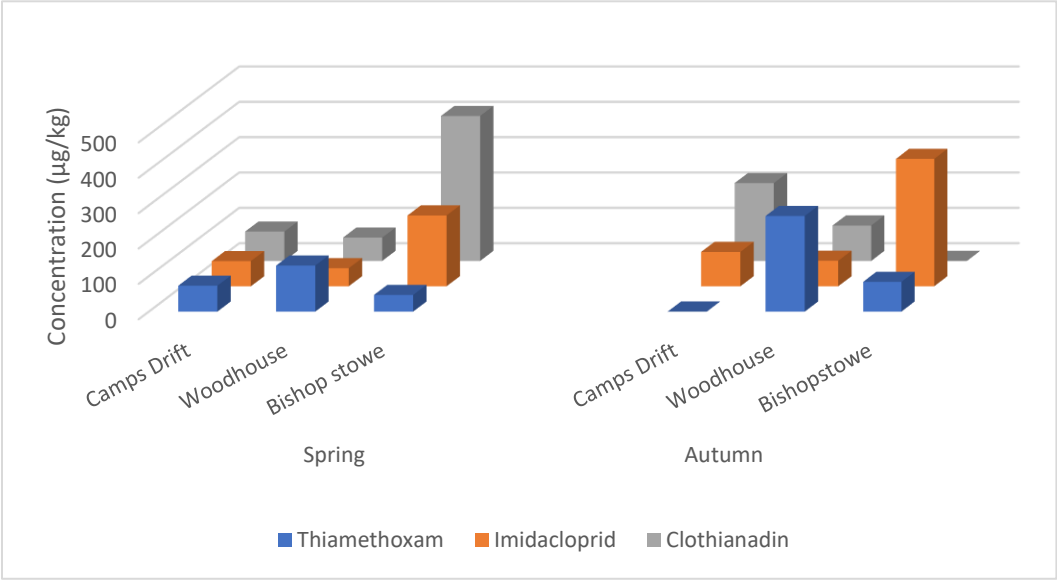

Figure 4.7: Concentrations of neonicotinoids detected in soil during spring and autumn seasons

4.5.2 Comparison of UE and SE efficiency on the extraction of neonicotinoids from sediment samples

The neonicotinoids were detected in all sediment samples (Figure 4.8). Neonicotinoids have high environmental risk in water environment and they frequently move between water and sediment through precipitation and run-off. Therefore, it can be inferred that neonicotinoids in sediment samples can have considerable negative effects on sensitive invertebrate species (Zhang et al., 2020). It was observed that UE was generally more effectiveness for the extraction of neonicotinoids than SE, even though slightly high concentrations for imidacloprid were observed for SE. The higher concentrations obtained with ultrasonic could be due to its mechanical force that permits effective solvent penetration into the sample matrix and consequently increase the contact surface area between the solid matrix allowing the transfer of analytes to the solvent (Mnyandu and Mahlambi, 2022). Higher concentrations obtained from soxhlet could be due to that its process involves a repeated contact of the sample with the fresh portions of solvent and cause the equilibrium transfer to shift leading to improved recoveries of the analytes into the solvent.

The clothianadin was generally higher in sediment samples than the other neonicotinoids with the highest concentration of 410 µg/kg in Bishop stowe sediment. This could be due to its low hydrophilicity and also long persistence in sediments (Zhang et al., 2020). The clothianidin concentrations are lower than those found by Bonmatin et al., (2019) in sediment from France which were 0.05 µg/kg.

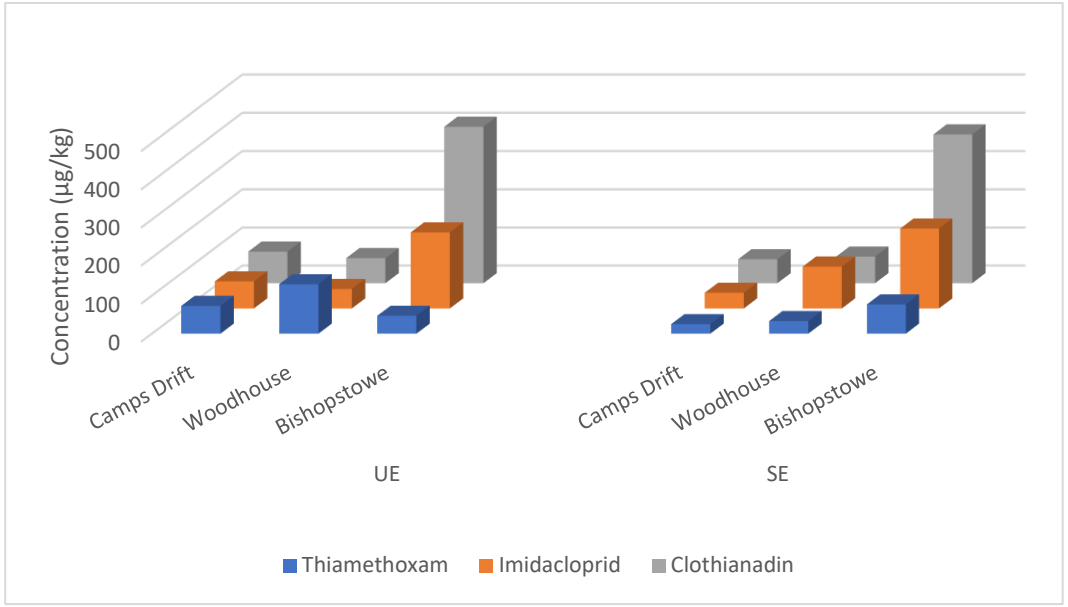

Figure 4.8: Concentrations of neonicotinoids detected in sediment using UE and SE methods.

Chapter 5: Conclusion and Recommendations

5.1 Conclusion

The SPE, UE, SE followed by LC-PDA method was optimized and applied successfully to tap, river, wastewater, sludge, soil and sediment samples. Low concentrations of neonicotinoids were observed in drinking water suggesting that they are safe for consumption. However, neonicotinoids presence indicates that they are unintentionally consumed by humans. This emphasises the importance of their continuous monitoring in water sources to safeguard human health. The neonicotinoids concentrations found in wastewater effluents are higher than the allowable levels in drinking water in all samples. This indicate that the wastewater treatment plants were not effective in removing the neonicotinoids. As a result, they are transferred into the rivers which put aquatic life in high potential risk.

In sediment and soil samples, clothianidin was the dominant insecticide while Bishopstowe and Woodhouse were the highly polluted areas. There were high concentration levels of neonicotinoids in the autumn compared to the spring season and in some samples, the neonicotinoids exceeded the maximum residue limit. Further studies are needed to fully understand the fate of neonicotinoids in South Africa, to increase food safety and decrease environmental risks.

The toxicity unit values showed that the studied neonicotinoids currently have no acute risk towards the aquatic species in the river water. However, risk quotient values suggested high ecological risk of clothianidin especially against daphnia species which may result in the decline of these valuable species. The environmental relevance of imidacloprid from wastewater treatment plants revealed that it has medium risk against algae, daphnia magna and fish species. These findings emphasise the need to search for reliable methods to improve the wastewater treatment processes to reduce their contribution towards pollution of water sources. Furthermore, these results will contribute towards the limited database on the occurrence and toxicity of neonicotinoids in Africa and thus allow the policy makers to establish the allowable limits that are precise for the African continent.

5.2 Recommendation for future work

- Continuous assessment of neonicotinoids in different parts of KwaZulu-Natal to conclude precisely on the pollution level of these compounds within the province.
- Assessment of neonicotinoids in all South African provinces to have an idea on South African continent pollution resulting from the neonicotinoids since they are registered to be used in South Africa.
- Assessment of micro extraction techniques for the assessment of neonicotinoids in various water bodies as they employ small volumes of samples and organic solvent making them environmentally friendly techniques.
- Assessment of agricultural soils and crops as neonicotinoids are widely used in agricultural field, to have an idea on human health upon crops consumption.
- Assessment on crops irrigated with effluent water or sludge from wastewater treatment plants
- Monitoring of neonicotinoids in sediment, water, plants, soil, sludge, in all four seasons to assess their impact on the neonicotinoids level.
- Assessment of fish samples to evaluate accumulation of neonicotinoids in various tissues

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Appendix

Table S1: Significant difference of mean recoveries for the effect of extraction conditioning solvent and sample volume

Significant difference of mean recoveries for the effect of extraction conditioning solvent

t-Test: Two-Sample Assuming Equal Variances		
	Methanol	*Acetonitrile*
Mean	68,66667	80,333333
Variance	576,3333	530,33333
Observations	3	3
Pooled Variance	553,3333	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0,60743	
P(T<=t) one-tail	0,288179	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,576358	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Equal Variances		
	Methanol	*Acetone*
Mean	68,66667	89,33333
Variance	576,3333	434,3333
Observations	3	3
Pooled Variance	505,3333	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1,12597	
P(T<=t) one-tail	0,16158	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,323161	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Equal Variances		
	Acetonitrile	*Acetone*
Mean	80,33333	89,33333
Variance	530,3333	434,3333
Observations	3	3
Pooled Variance	482,3333	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0,5019	
P(T<=t) one-tail	0,321054	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,642108	
t Critical two-tail	2,776445	

Significant difference of mean recoveries for the effect of sample volume

t-Test: Two-Sample Assuming Equal Variances		
	25 ml	*50ml*
Mean	67	79,333333
Variance	84	505,33333
Observations	3	3
Pooled Variance	294,6667	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0,87996	
P(T<=t) one-tail	0,214288	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,428575	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Equal Variances		
	25 ml	*100ml*
Mean	67	88,33333
Variance	84	422,3333
Observations	3	3
Pooled Variance	253,1667	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1,64211	
P(T<=t) one-tail	0,087956	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,175913	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Equal Variances		
	50ml	*100ml*
Mean	79,33333	88,33333
Variance	505,3333	422,3333
Observations	3	3
Pooled Variance	463,8333	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0,51181	
P(T<=t) one-tail	0,317873	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,635746	
t Critical two-tail	2,776445	

Table S2: The physical properties of wastewater samples

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		0.48	2.61	15.1	13.2	0.40	0.32	427	339	7.27	7.46	858	675
Amanzimtoti		0.39	1.56	14.0	18.5	0.59	0.46	623	475	7.12	7.81	1241	945
Umbilo		0.52	2.94	14.2	17.7	0.47	0.32	501	338	7.02	7.22	1002	676
Umhlathuzana	M	0.33		17.6		0.64		658		7.23		1312	
	S	0.56	2.30	14.9	24.0	0.29	0.34	304	352	7.05	7.62	608	707

Table S3: The Physical properties of tap water samples

Sampling Point	DO (mg/L)	Temp (˚C)	Salinity (psu)	TDS (ppm)	pH	Conductivity (µS)
Richmond Crest	3.48	17.4	0.44	168.8	6.75	421
Boughton	2.89	19.3	0.35	120.2	6.87	316
Woodlands	2.85	22.6	0.28	243	7.39	758
Mkondeni	3.66	20.2	0.19	106	7.34	187
Scottsville	2.41	18.8	0.33	206.4	6.21	229

Table S4: Physical properties of Pietermaritzburg River water samples during the Autumn Season

Sampling point	**DO (mg/L)**	**Temp (˚C)**	**Salinity (psu)**	**TDS (ppm)**	**pH**	**Conductivity (µS)**
Camps Drift	0.05	15.8	0.15	170	7.55	330
College Road	0.05	16.2	0.08	87	7.37	173
YMCA	0.05	14.4	0.08	86	6.82	172
Wood house	0.06	14.1	0.08	86	5.91	173
Bishop stowe	0.02	16.5	0.13	138	6.95	278

Table S5: Physical properties of Pietermaritzburg and Durban River water samples during the Spring Season

Sampling Point	**DO (mg/L)**	**Temp (˚C)**	**Salinity (psu)**	**TDS (ppm)**	**pH**	**Conductivity (µS)**
Camps Drift	2.66	17.1	0.15	163	8.01	328
College Road	2.90	23.2	0.10	112	9.01	223
YMCA	2.50	17.5	0.10	108	7.98	212
Wood House	1.39	17.2	0.10	105	7.59	210
Bishop stowe	1.29	22.1	0.15	163	7.25	325
Mngeni	0.76	16.6	2.03	1972	7.47	395
Umhlatuzana	2.12	17.3	0.23	237	7.61	474
Umbilo	0.64	15.8	3.18	398	7.38	515
Mbokodweni	1.93	16.3	4.85	445	7.55	888

Table S6: PNEC and Toxic units (Tu) of neonicotinoids in river water, according to acute toxicity data measured in algae, daphnia magna, and fish

Neonicotinoid	Fish	Aquatic invertebrate (*Daphnia magna*)	Algae		
	Acute 96 hour LC_{50}	Acute 48 h EC_{50}	Acute 72 hour EC_{50}	ΣTu	
	PNEC (µg/L)			BS	WH
Imidacloprid	> 83000	85000	>10000	E-	0.002
Clothianadin	>104200	>40000	>40000		
Thiamethoxam	>125000	>100000	>100000		
Tu_i (BS)	E-	E-	E-		
Tu_i (WH)	E-	0.001	0.001		

E- = more than 4decimals, BS – Bishopstowe site, WH – Woodhouse site

Fish acute: 96 h LC_{50} for *Oncorhynchus mykiss*. Invertebrates acute: 48 h EC_{50} for *Daphnia magna*. Algae acute: 72 h EC_{50}, growth. *Scenedesmus quadricauda* for imidacloprid, *Pseudokirchneriella subcapitata* for clothianidin and thiamethoxam were used. Fish chronic: 21 days NOEC for *Oncorhynchus mykissas latipes* for imidacloprid, *Pimephales promelas* for clothianidin. Invertebrates chronic: 21 days NOEC for *D. magna.* Algae chronic: 96 h EC_{50}, growth. *Scenedesmus quadricauda* were used. Data obtained from the Pesticide Properties Database (PPDB, 2021), and P'erez et al., 2021.

Table S7: Toxic units (Tu) and ERPW of neonicotinoids in wastewater, according to acute toxicity data measured in algae, daphnia magna, and fish

Neonicotinoid	Fish			Aquatic invertebrate (*Daphnia magna*)		Algae	
		Tup	ERPWI	Tup	ERPWI	Tup	ERPWI
Imidacloprid	UH	2.5x10-5	0.0051	1.28x10-4	0.026	2.3x10-5	0.0046
	UB	3.6x10-4	0.14	0.0018	0.72	3.3x10-4	0.13
Clothianadin	NT	1.1x10-4	0.042	5.2x10-5	0.021	3.9x10-5	0.015
	UB	1.8x10-5	0.0073	1.1x10-5	0.038	3.4x10-5	0.038
Thiamethoxam	UB	7.9x10-6	0.0016	9.9x10-6	0.020	9.9x10-6	0.0020

UB – Umbilo WWTP, UH - Umhlathuzana WWTP, NT – Northern WWTP