Thesis title:

Occurrence and fate of neonicotinoid insecticides in soils of contrasting characteristics, and in flowers of cultivated and marginal plants from selected sites in Britain

Declaration

This thesis entitled "Occurrence and fate of neonicotinoid insecticides in soils of contrasting characteristics, and in flowers of cultivated and marginal plants from selected sites in Britain" is based upon the work conducted by Adeniyi Kayode Aseperi in the Department of Pharmaceutical and Chemical Sciences in School of Life Sciences, Pharmacy and Chemistry in the Faculty of Science, Engineering and Computing at Kingston University London between March 2015 and Sept 2019. All of the work described herein is original unless otherwise acknowledged in the text or by references. None of the work has been submitted for another degree in this or any other universities.

[Redacted] **03/04/2020**

Adeniyi Kayode Aseperi (Student) Date

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Dr James Barker (Supervisor) Date

Dr Rosa Busquets (Supervisor) Date

Dr Peter Hooda (Supervisor) Date

Dr Philip Cheung (Supervisor) Date

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Finally, I give the glory of this work to the almighty God for his grace that enabled me to complete this programme.

Abstract

Neonicotinoids, popularly known as “neonics”, belong to a class of insecticides commonly used as plant protection agents against several insect pests. After the commercialisation of the first neonic, imidacloprid, in 1991, the sales of neonicotinoids expanded at a rapid pace; presently, the sales of neonicotinoids account for about 28% of the insecticides’ market value.

The efficacy of neonicotinoids as insecticides is attributed to their properties such as high-water solubility, polarity, selective toxicity and being systemic in nature. Neonicotinoids are absorbed by plants and are readily transported via the vascular system due to their systemic nature; this enables their presence in plant’s tissues such as root, stem, leaves, flowers as well as the pollen and nectar. Consequently, the entire parts of the plant are protected from sucking and piercing insect pests.

The mode of action of neonicotinoid insecticides has been recognised as acting as agonist at the postsynaptic nicotinic acetylcholine receptor (nAChRs) sites in the nervous system. The question of bee health and the use of neonics has generated a lot of debate and resulted in a two years moratorium (2013 - 2015) for the limited use of three neonicotinoids on selected crops. Recently, the use of these neonicotinoids, namely, imidacloprid, clothianidin and thiamethoxam has been completely banned in Europe after sustained reports from research and regulatory agencies such as the European Food Safety Authority (EFSA) implicated neonicotinoids as the *killers* of bees.

A number of environmental factors and the physicochemical properties of the chemicals influence their sorption and transport in soils. There is usually negative correlation between mobility factor (M_f) and soil organic matter, however, there are exceptions particularly neonicotinoids eliciting different behaviour in different soil types. The need to understand the behaviour of these insecticides in the environment is desirous in predicting their fate,

occurrence and remediation after their application. There are reports on the determination of different neonicotinoids in soils in UK. However, there is lack of information on the role played by soils with varied characteristics from different geographical location in UK on their adsorption capacities, sorption kinetics and leaching potential.

The primary objective of this work was to assess the sorption and column mobility of neonicotinoid insecticides using field soils (unamended) with varied characteristics in determining their adsorption capacity, partition coefficients, leaching profile, kinetic and isotherm data. To achieve this objective, the work focussed on the investigation of the sorption behaviour of five neonicotinoids, namely dinotefuran, thiamethoxam, thiacloprid, imidacloprid and acetamiprid in five soils with contrasting characteristics. Also, the soil column leaching of the most and least adsorbed neonicotinoids on soils with the least and most organic carbon content was carried out in the laboratory, under control conditions, mimicking field studies.

The presence of neonicotinoids in surface water, contaminated through run off, have been reported as a major source of exposure to aquatic ecosystem. Therefore, the UV photolytic degradation of neonicotinoids in aqueous environment was investigated. Also, the amount of neonicotinoid in flowers of selected cropped and marginal plants and wheat grains, then five months after, their respective soil from the same sites was determined to compare the rate of disappearance with those of UV photolytic degradation in water environment. The use of analytical method and instrumentation analysis was crucial to this study to ensure accurate and reliable data are obtained. Therefore, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) was modified and adapted in the extraction work along with the use of liquid chromatography- tandem mass spectrometry (LC-MS/MS) for high sensitivity and low detection limits in all the sample matrices studied.

The findings from this work suggest that neonicotinoids can accumulate in the soils, particularly after prolonged application. Also, soil types can influence the environmental distribution of neonicotinoids, consequently determining their bioavailability to non-target insects and beneficial soil faunas. The results of the UV photolysis of the neonicotinoids in water showed that neonicotinoids can persist in the environment, especially in temperate regions with lower sun hours. However, the behaviour of neonicotinoids in the aqueous environment can be influenced by their distinct functional groups and their degradation was best modelled with a first-order reaction kinetic with half-life ranging from 11 min to 14 h.

The adapted method for the extraction showed good sensitivity and high recovery (72.24 – 102.67 %) with low LODs (0.22 – 1 µg/kg) and LOQs (0.74 – 3.33 µg/kg) when compared to other methods used in neonicotinoid extraction. The results of this study showed that only thiacloprid insecticide was detected in the flower of oilseed rape (OSR) from Essex farm at a 1.42 µg/g concentration and double the maximum residual limit (MRL) established by the European Commission; a potential risk to honeybee during foraging and nectar collection. Also, less than 0.4 % of thiacloprid insecticide was detected in the soil from the same site where OSR was previously sampled and analysed. The result of the rate of photolytic degradation of the insecticides in aqueous environment was ten-fold lower when compared to the rate of disappearance obtained from the field between the mount of neonicotinoid in oilseed rape flowers and those obtained from the soil.

The sorption and mobility information obtained from this study will enable risk assessment of these insecticides to be conducted and the outcome will be a contribution towards policy decision making, particularly in their field application. Also, the rate, adsorption and isotherm coefficients obtained in this work are key parameters as inputs in multimedia environmental models in improving our understanding of the behaviour and movement of these pesticides in different environmental compartments.

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List of Abbreviations

A

ACE	acetamiprid
ANOVA	analysis of variance
APCI	atmospheric pressure chemical ionisation
ai	active ingredient

C

2-CA	2-chloroaniline
CCC	counter current chromatography
CCD	colony collapse disorder
CEC	cation exchange capacity
CE	collision energy

D

DDT	dichlorodiphenyltrichloroethane
DIN	dinotefuran
DSPE	dispersive solid phase extraction
DT	dwell time

E

ECD	electron capture detector
ED	electrochemical detector
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionisation

F

FERA	Food Environment Research Agency
FLD	fluorescence Detector

FID	flame ionisation detector
Foc	fraction of organic carbon content of soil
G	
GC	gas chromatography
GF	gas flow
GT	gas temperature
GPS	global positioning system
H	
HPLC	high performance liquid chromatography
I	
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IMI	imidacloprid
IS	internal standard
IUPAC	international union of pure and applied chemistry
K	
K_{ow}	octanol-water partitioning co-efficient
K_{oc}	organic carbon-water partitioning co-efficient
L	
LC	liquid chromatography
LC-MS/MS	liquid chromatography coupled to tandem mass spectroscopy
LC-UV	liquid chromatography coupled to UV detector
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
M	

MAE	microwave assisted extraction
MoA	mode of action
MRL	maximum residual limit
MRM	multiple reaction monitoring
MS	mass spectrometry
MSPD	matrix solid phase dispersion

N

nAChR	nicotinic acetylcholine receptor
NEB	nebuliser
NMR	nuclear magnetic resonance
NOEF	no observable effect level

O

OSR	oilseed rape

P

PDP	pesticides data programme
PLE	pressurised liquid extraction
PSA	particle size analysis
PTFE	polytetrafluoroethylene

Q

QuEChERS	quick, easy, cheap, effective, rugged and safe
QqQ	triple quadrupole

R

RIA	radioimmunoassay
RSD	relative standard deviation
tR	retention time

S

SF1	site flower 1
SF2	site flower 2
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SLE	supported liquid extraction
SOC	soil organic carbon
SOM	soil organic matter
SPE	solid-phase extraction
SPME	solid-phase microextraction
SRM	selected reaction monitoring

T

THA	thiacloprid
THX	thiamethoxam
TLC	thin layer chromatography
TRFIA	time-resolved fluorescence immunoassay

U

UAE	ultrasound-assisted extraction
UHPLC	ultra-high performance liquid chromatography
UVDAD	ultraviolet visible diode array detector

Synopsis of the thesis

Chapter 1 introduces the developmental history, physicochemical properties and the insecticidal activity of neonicotinoids. Briefly discussed is the alleged impact of neonicotinoids on bee colonies. Literature on the aspects of neonicotinoids relevant to this thesis are reviewed.

Chapter 2 gives the summary of all the materials used in this study as well as the concluding quality parameters. Details of the materials and methods applied to each experiment in chapters 4, 5 and 6 were presented accordingly.

Chapter 3 describes the steps involved in the development of methods used in the analysis of all the neonicotinoid insecticides in this study. This includes the separation, optimisation, detection and sensitivity of the study compounds using liquid chromatography with ultraviolet-visible (UV-vis) and mass spectrometry (LC-UV and LC-MS). The separation of the study compounds was achieved by the LC-UV system. However, better separation and improved sensitivity were achieved when the method was adapted to the LC-MS system. Also, the recovery in the sample treatment and detection sensitivity were assessed. For all the compounds, quality parameters of the method were assessed with the determination of linearity, LODs, LOQs, repeatability and reproducibility.

Chapter 4 provides the results of the adsorption capacities of the study compounds in soils with divergent organic carbon content obtained from 5 locations in Britain. Further investigation of the kinetics, thermodynamics and leaching characteristics of the most and least adsorbed neonicotinoids, in the soils with the most and least %SOC, was conducted. This is a first step towards unravelling the fate of these insecticides in the natural environment. The results of the UV degradation of neonicotinoids in aqueous medium was provided in this chapter. The rates of decomposition of the four compounds were determined and their half-lives estimated. The effects of water on photolysis was discussed as well as the effect of the degradates on the surrounding aquatic animals. Finally, validity of the developed method was equally tested in the determination

of neonicotinoids residues in environmental samples such as flowers from cultivated and marginal plants, wheat grains (leading to the potential contamination of the surrounding marginal plants attracted by bees from the application of insecticides to wheat farm), honey bee, bee wax and soils. Also, the neonicotinoids in soil and their potential residues in the environment post application was discussed. The effect of the regulations of the EU Commission on the use of pesticide was evaluated.

Chapter 5 Outlines the main findings from the present work. The modified extraction methods developed with acetonitrile solvent producing the highest recovery for the neonicotinoids at lower sample sizes was discussed. The use of neonicotinoids as plant protection with the potential to contaminate ground water, particularly when used in soils low in organic carbon and their possible exposure to bees and soil faunas was assessed. The possibility of photolytic degradation with a UV source lamp at low power was recorded and its benefits highlighted. Also, suggestions for further study and research needs are presented.

The driving force of this doctoral work

The significant role of neonicotinoid insecticides in protecting commercially valuable plants against sucking insects is proven and the efficacies of these chemical compounds cannot be overemphasised. The properties of these insecticides such as high solubility in water, molecular polarity, selective toxicity and systemic nature, together with viable options in modes of their applications, have contributed to their overall success as pesticides.

Allegations of damage of bee colonies leading to collapses have been made against selected neonicotinoid insecticides. Recently, the use of three neonicotinoids, namely, thiamethoxam, imidacloprid and clothianidin has been restricted in the EU member states, while thiacloprid is currently reviewed for its alleged endocrine disrupting properties. As the scientific debate is still in full progress, trade blocs such as the European Union has banned the use of these insecticides based on the study of bees alone. Therefore, the evidence against neonicotinoids remains equivocal as this thesis is being prepared for presentation. It is the story told by Rachel Carlson in her book *Silent Spring* all over again; DDT was banned (apart from malarial hot spots) despite the saving of many lives against malaria parasite.

The author of this thesis believes that fundamental processes such as sorption of insecticides on soils and their propensities to be leached out of soil and transported, cannot be ignored in public discourse and policy decisions regarding the use of pesticides. The doctoral work is done to address these gaps in scientific knowledge.

This work sets out to discover the fate and occurrence of neonicotinoid insecticides in the natural environment, their identification and quantification, followed by studies of their behaviour under some simulated environmental conditions. This work shows that the behaviour of neonicotinoid insecticides in soil environment is largely dependent on soil characteristics. There is great propensity for the insecticides to accumulate. They also persist in soil for long

periods of time given the right conditions. Also, neonicotinoids can be translocated and accumulated in plant parts after uptake from the time of their application with projected negative consequences to food safety.

CHAPTER 1: GENERAL INTRODUCTION

1.0 General introduction

A pesticide is any substance or mixture of substances used to control living organisms that is or are capable of causing damage or economic loss to non-target, plants, animals and humans (Colosio *et al.*, 2016). When insects are targeted for elimination, the chemical agent is known as an insecticide.

The insecticides industry started in the 1940s after the insecticidal properties of earlier synthesised organochloride compounds like dichlorodiphenyltrichloroethane and benzene hexachloride were fully discovered (Lipnick and Muir, 2001). The use of these insecticides was effective against their target pests and widely accepted. However, their persistence in the environment and resistance developed by the target insect pests was noticed which led to the restriction in their usage and subsequent ban in US and Europe in 1972 and 1980 respectively (Lipnick and Muir, 2001).

Since the industrial production of dichlorodiphenyltrichloroethane (DDT) and benzene hexachloride as insecticides, a huge number of different pesticides with high sensitivity and less environmental persistence have been developed and commercialised as pest controls like organochlorines, organophosphates (parathion, malathion, azinophos-methyl), phenoxyacetic acids, captan, carbamates and pyrethroids (Singh *et al.*, 2012 and Jayaraj *et al.*, 2016). However, due to the emergence of resistant insect strains with less sensitive molecular target in their nervous system, the remarkable effectiveness of these insecticides diminished over time (Tomizawa and Casida, 2005). The quest for novel chemicals that are more selective, systemic, environmentally friendly and neuroactive led to the discovery of neonicotinoids (Casida and Quistad, 1998 and Tomizawa and Casida, 2009).

The discovery of neonicotinoid insecticides with selective toxicity and high systemic characteristics as plant protection agents was regarded as a milestone in agrochemical research (Chauzat and Faucon, 2007 and Jeschke *et al*., 2013). The use of neonicotinoids has increased in the last two decades, ever since the first neonicotinoid, namely, imidacloprid, was introduced to the market in 1991. Imidacloprid was manufactured by Bayer Crop Science (Mörtl *et al.*, 2016). Soon afterwards, thiamethoxam, clothianidin and other neonicotinoid insecticides have been produced by various manufacturers in different countries and commercialised (Maienfisch *et al.*, 2001 and Simon-Delso *et al.*, 2014) with newer generations of neonicotinoids being developed (Cutler *et al.*, 2013 and Jeschke *et al.*, 2013).

Neonicotinoids are very popular among farmers all over the world. Distinctive physico-chemical characteristics and broad spectrum of insecticidal activities made them so. They are known to have a unique mode of action and selective toxicity, systemic and translaminar activity, low application rate and pronounced residual activity (Kurwadkar *et al*., 2013). These strengths are the reasons behind the increase of their market share (Jeschke *et al.*, 2010). In 2011, neonicotinoids accounted for 28.5% of the total global insecticide market sales worth US $12.75 million. The insecticides are registered for use in over 120 countries for over 140 crops (Jeschke *et al.*, 2010 and Jeschke *et al.*, 2013).

Increasing introduction of newer chemicals as insecticides, with their agricultural economic importance from plant protection to animal health, have raised their post-application amount in the environment. Large-scale use and wider applications of neonicotinoids in agriculture, veterinary and domestic activities in over 120 countries have been reported with potential to build up in the environment if not degraded (Simon-Delso *et al.* 2014, Goulson 2013 and van der Sluijs *et al.* 2013).

According to FERA (2019), 2 million hectares in the UK, from year 2012 to 2015, were treated with the four registered neonicotinoids; thiamethoxam alone accounted for 74% (Figure 1A). Similarly, thiamethoxam and thiacloprid accounted for 44 and 34 %, respectively, of the total 110,000 kg of the approved neonicotinoids applied in the UK from year 2012 to 2015 (Figure 1B). The chronic accumulation of these insecticides in the environment raises the exposure of non-target organisms such as bees to them. This has been found to be the case in the U.S. as well, with at least one neonicotinoid insecticide detected in 63% of the 48 streams sampled. (Hladik and Kolpin, 2015).

However, there are indications showing a decline in the use of neonicotinoids as an agrochemical for plant protection at the end of the two-year (2013 - 2015) European Union moratorium period. Over 90 % reduction in the use of imidacloprid and thiamethoxam in the total area treated and about a 82 % drop in their total weight applied in UK was observed between years 2015 and 2016 (Figure 1 A & B). However, the amount of acetamiprid and thiacloprid usage during the same years remain relatively unchanged FERA (2019). Therefore, the levels of these insecticides in the environment, long after their effective prohibition date 29th May 2018 by the Commission Regulation (EU) 2018/783 -785 (EU Directives 2018), may be used as an assessment for the scale of exposure to non-target organisms like bees as well as earthworms with residues built up in the soil environment.

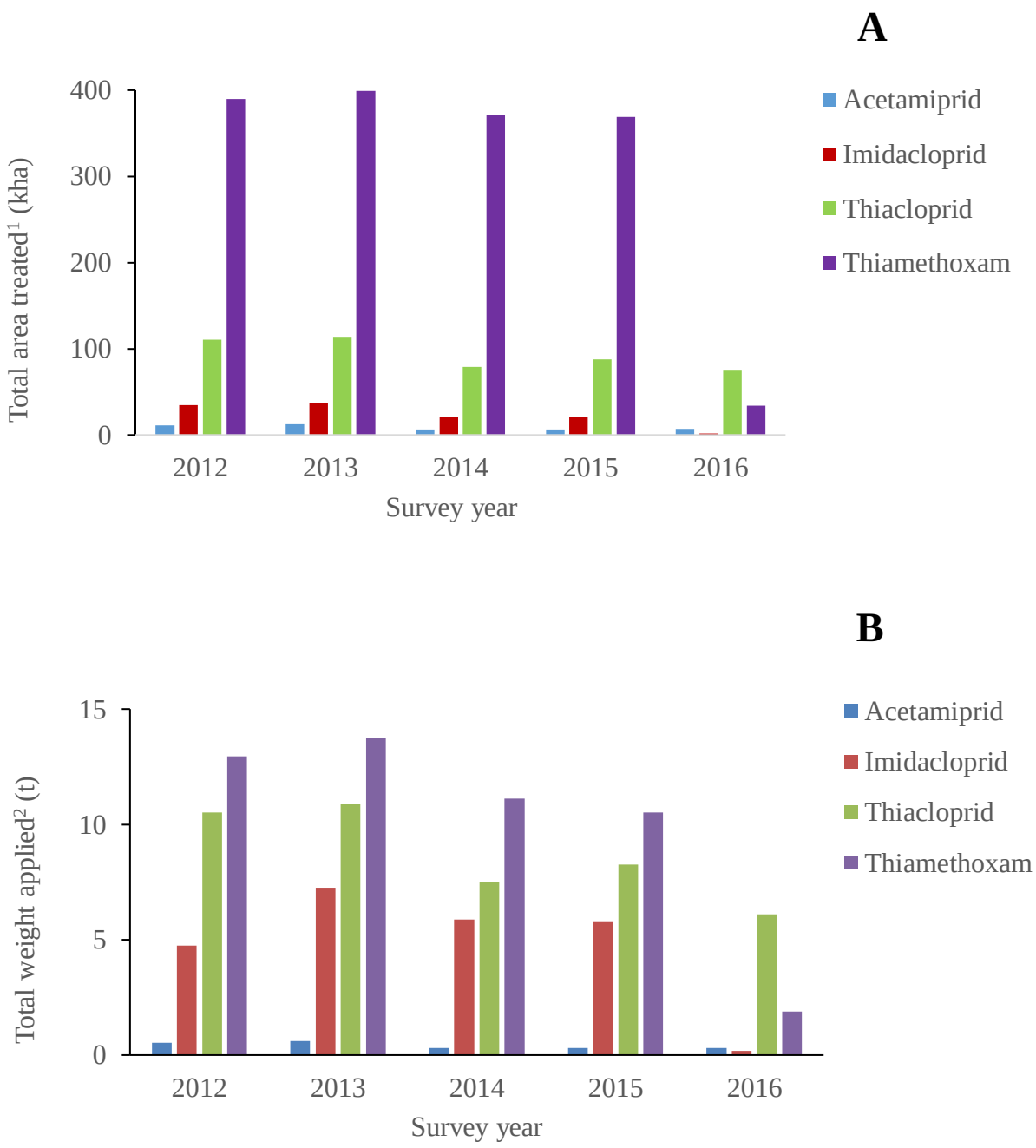


Figure 1. A trend in the agricultural use of neonicotinoid insecticides in UK between 2012 – 2016. (A) - Total area (ha) treated and (B) - Total weight (t) of neonicotinoid applied. 1. This is the basic area treated by each ai, multiplied by the number of times the area was treated. For example, a field of 3 ha is treated 4 times with active X. Therefore, the area treated is 12 ha (3x4). 2. Total weight of ai applied over a survey year. Source: FERA (2019)

1.1. Neonicotinoid insecticides and their role in "colony collapse disorder" (CCD)

1.1.1 Colony collapse disorder (CCD) and its causes

Despite proven success of neonics insecticidal activities, potential ecotoxicities induced by neonicotinoids have been reported. Organisms affected include non-target and beneficial species of bees, butterfly, earthworm, terrestrial predators and aquatic organisms (Chevillot *et al.,* 2017; Butcherine *et al.,* 2018; and Hernando *et al.,* 2018). These contentions have led to ongoing debate among researchers with the emphasis on the urgent re-evaluation of their use (Simon-Delso *et al.,* 2014 and Anderson *et al.,* 2015).

"If the bees disappeared off the face of the earth, man would only have four years left to live", so previsedAlbert Einstein. It is very unlikely Einstein ever made the statement but was reported to have been quoted in a Lancet article entitled "Where have all the bees gone?" (Stindl and Stindl, 2010). It is not known how the Nobel physicist came to that prediction, but the interrelatedness of humans and all other compartments of the ecosystems in which they live and work is undisputable. Bees are generally regarded as the most important pollinator with about 80% of global agricultural pollination services attributed to the domesticated European honeybees (*Apis melifera*) (Breeze *et al.,* 2011). However, the extent of plant pollination by the honeybee is intensely debated as claims of over-estimation with no adequate plant pollination report on the long term studies (that is more than 4 years) with wild bee population dynamics (Aebi *et al.,* 2012).

The environmental health of bees has drawn so much attention in recent years due to the decline of beehives recorded in many parts of the world. This condition has been widely studied in honeybees and led to the global phenomenon described as colony collapse disorder (CCD) (Evans & Schwarz 2011, Cresswell 2011, Paradis *et al.* 2014, vanEngelsdorp *et al.* 2009). The cause of this widespread disorder, registered across North America, Europe and Middle East, is uncertain. However, several possible causes have been put forward to explain the "strange"

disappearance of bees by many researchers. Tapparo *et al.* (2011) and Farooqui (2013) reported that adult bees are unable to return to their hives due to memory loss after coming in contact with plants treated with neonicotinoid insecticides while foraging. Similarly, the exposure of honeybees to neonicotinoids treated plants, through nectar and pollen grains (Paradis *et al.*, 2014) and guttation during pollination (Tapparo *et al.*, 2011) have been reported.

Other causes reported are Israeli acute paralysis virus (IAPV) carried by *Varroa* mites (Di Prisco *et al.*, 2011), pathogens and their corresponding diseases (Genersch, 2010). Combination of many factors including nutritional stress (Naug, 2009), without intermittent feeding on the naturally occurring anti-mite toxins (pyrethrum) producing plant (Sharpe & Heyden, 2009), presence of Entombed pollen (vanEngelsdorp *et al.*, 2009) and many more have been suggested as the potential cause of the disorder.

However, bee colony decline (CCD) is not a new phenomenon as previous situations have been recorded dating back to 1869, between 1905 – 1919, and 1960 – 1975 (Underwood and vanEngelsdorp, 2007). Several names such as "May disease", "Isle of Wight disease" and "disappearing syndrome" have been coined to describe the bee colonies decline circumstances at these periods. Many factors such as poor nutrition, diseases, genetics, Stonebrood caused by the fungus *Aspergillus flavus*, pesticides, cold weather among others were suspected causes. As recent as mid-1999, heavy loses of bee colonies were recorded in several regions in France; 76 % of the apiaries were found to contain one or several serious diseases capable of totally wiping out colonies (Faucon *et al.*, 2002).

Therefore, in view of the recurrence of this condition in the past one hundred years, it will be difficult to conclude on a single causing agent. However, the scientific report submitted to EFSA on the bee mortality and bee surveillance in Europe (EFSA, 2008) concluded that environmental factor, biological factor, beekeeping practice and chemical factors are factors

involved in colony loses; however, the question of the sequence of events that lead to colony mortality needs to be addressed. The report of EFSA, linking overwhelming evidence of neonicotinoid insecticides use to bee toxicity and death, led to the two years moratorium restriction in year 2013 – 2015 (EU Directives, 2013). As recent as 29th May 2018, European Commission Regulation (EU) 2018/783 -785 has now banned the use of these three pesticides on all field crops in Europe (EU Directives, 2018) with limited use in green house farm.

The importance of bees as major pollinators of a wide range of crops, including melons, cucumbers, pears, almonds, cherries, and pears is a reflection of their massive commercial value. Also, the production of honey and honey related products such as dietary pollen, wax, propolis, and bee venom have proved to be of immense benefits to human. However, the use of pesticides to control insect pest for better agricultural yield is desirous for farmers and continued sustenance of life. Therefore, understanding the role of neonicotinoids on bee health and other non-target organisms will rely on further research into their concentration and behaviour in the environment.

1.1.2 Molecular structure and insecticidal activities of neonicotinoids – mode of action

Structural diversity of neonicotinoid insecticides is reflected in the seven widely available neonicotinoids in the market. The diversity is evinced by a six- membered ring system (thiamethoxam), a five-membered (imidacloprid and thiacloprid) ring system and noncyclicity in nitenpyram, clothianidin, dinotefuran and acetamiprid, which are used for crop protection (Jeschke *et al.*, 2010 and Questel *et al.*, 2011)

Considering the presence of a common pharmacophore moiety for all neonicotinoid insecticides:

$$[- N – C (E) = X – Y] \qquad \text{Eq. 1}$$

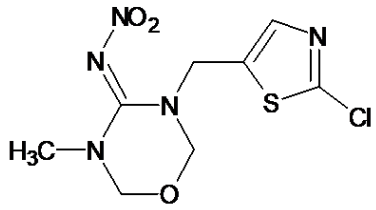
Where the functional group (= X - Y) in Eq.1 is an electron withdrawing group (i.e = N - CN, = N - NO_2 and CH-NO_2) and E is either S, CH_2, NH, NR or O moiety.

The 5 neonicotinoid insecticides in this study (Figure 2) of which 4 are currently licenced for use in UK, are known to possess either a nitromethylene, nitroimine, or cyanoimine group. They are broadly grouped as ring systems and noncyclic neonicotinoids (Matsuda *et al.* 2001 and Jeschke *et al.* 2010).

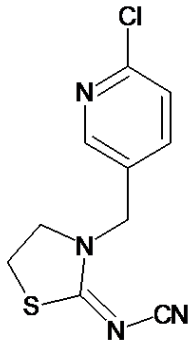
The presence of electronegative moieties on neonicotinoids is identified as the determinant of their reactivity. A number of reactivity studies have supported that neonicotinoids have varying degrees of reactivity with some radicals such as hydroxyl ($HO^{\bullet}$) as well as ubiquitous anions such as carbonate CO_3^{2-}, excited triplet states and singlet oxygen (O^{2-}), sulphate ($SO_4^{\cdot 2-}$) through insecticide-radical charge transfer (Dell'Arciprete, 2010 and Dell'Arciprete *et al.* 2012).

The presence of these naturally occurring radicals, especially the extremely reactive $OH^{\bullet}$, excited triplet states and singlet oxygen (O^{2-}), are effective in reducing the toxicities of neonicotinoids to safe levels. However, the setback that could be encountered is the generation of more toxic metabolites, especially their radical forms than the precursor compounds (Dell'Arciprete, 2009). Therefore, further research into neonicotinoids' reactivity with powerful radicals like oxidizing agents used in advanced oxidation process for the treatment of neonicotinoids contaminated water is encouraged.

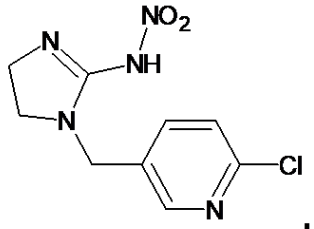
Ring systems



Thiamethoxam[1]

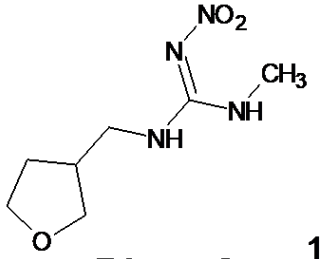


Thiacloprid[2]

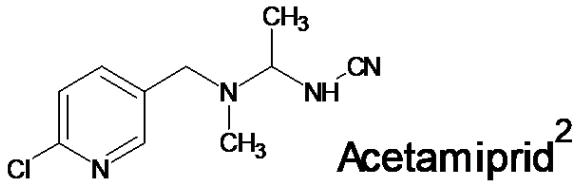


Imidacloprid[1]

Noncyclic neonicotinoids



Dinotefuran[1]



Acetamiprid[2]

Figure 2. Molecular structure of commercially available neonicotinoids in this study. [1]Nitromethylene (nitroimine), [2]Cyanoimine

The potent insecticidal activity of neonicotinoid insecticides with high selectivity and affinity towards the receptor site of target insect is attributable to their chemical structure. The mode of action (MoA) of neonicotinoids against insects has been identified as nicotinergic neuronal pathway blockers at the insect nicotinic acetylcholine receptors (*n*AChR) (Tomizawa *et al.*, 2009). This was supported in the experimental laboratory test performed to verify the impact of active ingredient (imidacloprid) administered as sub-lethal doses whereby the insect showed a decreasing communicative capacity with a subsequent decline in social behaviour (García *et al.*2007).

The current knowledge on the MoA of neonicotinoids causes the accumulation of the neurotransmitter acetylcholine leading to paralysis, cell energy exhaustion and death (Pandey *et al.,* 2009 and Zhang *et al.* 2012). The high selectivity and affinity profile of neonicotinoids to insect *n*AChR against vertebrate is attributed to its 'nitro' and 'cyano' pharmacophore as widely reported by Pandey *et al.*, (2009), Tomizawa *et al.*, (2009) and Farooqui (2013) with low agonist potency at the vertebrate *n*AChR subtypes. However, the nitro- substituted group neonicotinoids were reported to be more toxic to bees while the cyano groups exhibited much lower toxicity to the same target insect in a laboratory study (Iwasa *et al.*, 2004).

Interestingly, the discovery and understanding of neonicotinoid selective MoA as agonist at *n*AChR, located at the central nervous system of insect, has contributed to extending the knowledge and research of the biochemistry of insect nicotinic acetylcholine receptors (Jeschke *et al.*, 2013). Natural compounds, like Spinosyns insecticides, obtained from microorganism and other several new insecticides have been developed while others are in various stages of development based on the knowledge of nAChR as a molecular target (Kirst, 2010 and Jeschke *et al.,* 2013)

1.1.3 Physico-chemical properties of neonicotinoid insecticides

The functional groups of neonicotinoids affect their physico-chemical properties which affect their fate and biological effect in the environment. For instance, the functional groups - NO_2 and - CN are responsible for their hydrogen bonding interaction with nAChRs (Questel *et al.*, 2011); with the nitro group presenting a stronger hydrogen bonding acceptor than the cyano group. This property may be responsible for the higher toxicity of the -NO_2 substituted group of neonicotinoids over the -CN groups. The unique co-planar feature of the nitrogen substituents - NO_2 and - CN with guanidine and amidine yield an electronic conjugation which provides partial negative charge and being part of hydrogen bonding at the receptor subsite (Tomizawa & Casida, 2011, Kagabu, 2011).

The Henry's law constant of a compound is a measure of the ratio of its concentration in the air to its concentration in water; thus, the lower its value, the lower its volatility. Generally, when compared to other pesticides, neonicotinoid insecticides are known to have low vapour pressure, ranging from 3×10^{-7} mPa to 1.7×10^{-3} mPa at 25^{o}C. This implies they are not likely to be found in the vapour state or air. The extremely low Henry's law constant values of neonicotinoids, ranging from 2.9×10^{-16} – 5.30×10^{-8} atm-m^3/mol, makes them a soluble candidate rather than been found in the air. However, neonicotinoids may be found as aerosols for a very short period during spraying (Bonmatin *et al.*, 2014).

A pesticide with high water solubility can leach with ease through the soil column and contaminate ground water. Most neonicotinoids, with molecular weight from 202 to 292 g/mol, are relatively soluble with dinotefuran (39.8 g/L) being the most soluble in water and thiacloprid (0.18g/L) the least water soluble (individual solubility values are given in Table 1). The presence of polar functional groups in the pharmacophore of neonicotinoid increases their

solubility and polarity, from low to high solubility, i.e. [=N–NO_2] < [=N – CN] < [–CH – NO_2] as shown in Table 1.

The high solubility of neonicotinoids in water compared to other classes of insecticides generally promotes their systemic nature. The high solubility characteristics of neonicotinoids improve their uniformity as active ingredient in their formulation, therefore, increasing the homogeneity of the pesticide. When comparing neonicotinoids with other insecticides, fipronil has a low solubility in water (0.004 g/L) lower to formetanate hydrochloride, which is highly soluble (822.0 g/L). The low water solubility of fpronil decreases its soil adsorption with increasing proportion of methanol in the binary water/organic solvent mixture (Bobe *et al.*, 1997). Neonicotinoids, with solubility range of 0.18 – 39.80 g/L, are more soluble than the broad-spectrum insecticide fipronil (0.004 g/L), but less soluble when compared with formetanate (822.0 g/L). The relatively high solubility of neonicotinoids increase their ecotoxicity (10.44 – 64.87 mg/L: 48-h LC_{50}) compared to other poorly water-soluble pesticides such as lindane (0.007 g/L) and pendimethalin (0.0003 g/L) with ecotoxicity range 0.08 - 0.11 mg/L: 48-h LC_{50} (Fliedner, 1997, Hayasaka et al., 2012).

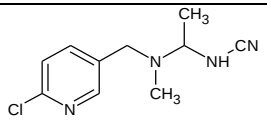
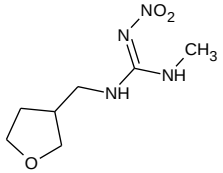
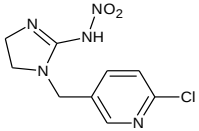
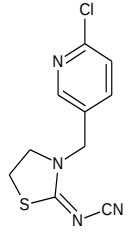
The values of Log K_{ow} for all neonicotinoids are relatively low when compared to other insecticides (Table 2) and this contributes to their low lipophilicity with excellent systemic properties. The lipophilicity of neonicotinoids might have been conferred on it by the 'E' moiety on the pharmacophore [- N – C (E) = X – Y] which may be NH < O < CH_2 < NR < S in increasing order (Jeschke & Nauen, 2008). Neonicotinoids are transported and translocated through the plant tissues (xylem and phloem system) from the roots to the floral parts of the plants due to their systemic characteristics. Consequently, the entire parts of the plants such as the roots, stems, flowers, nectar, and leaves are protected against plant sucking insects (Bonmatin *et al.*, 2003). The systemic nature of neonics increases its dwelling time in the plant

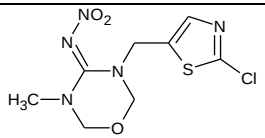
and, therefore, render a longer protection against target insects. This property encourages its agricultural usage in a large scale across the globe.

The generally low values of neonicotinoids' Log K_{ow} and their outstanding plant systemic activity, though shared by organophosphates and methylcarbamates, but with poor selectivity factors, are advantages over the more lipophilic organochlorines and pyrethroids. However, the unique mode of action of neonicotinoids as agonist at nAChR of insects, differs from organochlorines and pyrethroids with Na^+ or Cl^- and Na^+ modulators respectively (Table 2). This ensures the preferential use of neonicotinoids over other insecticides in the market (Tomizawa and Casida, 2005).

The low lipophilicity of neonicotinoids reduces their accumulation in biological systems. However, the combined effect of high solubility, polarity and low Log K_{ow} may enhance their mobility in plants and the environment resulting in greater environmental risks compared to less mobile insecticides.

Table 1. Chemical structure and physicochemical properties of the five pesticides studied.

Pesticides	IUPAC name	Solubility in water (mg/L) at 20°C	log K_{ow}	K_{oc}	[b]GUS leaching potential index (Leachability rating)
 Acetamiprid	((E)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methyl acetamidine)	2,950	0.8	200	0.82 (low)
 Dinotefuran	1-Methyl-2-nitro-3-((tetrahydrofuran-3-yl) methyl)guanidine	39,800	0.549	26	4.95 (very high)
 Imidacloprid	1-[(6-Chloro-3-pyridinyl) methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine	610	0.57[a]	156-960	3.76 (high)
 Thiacloprid	[3-[(6-chloro-3-pyridinyl) methyl]-2-thiazolidinylidene] cyanimide	180	1.26	261-870	1.44 (low)

 Thiamethoxam	3-(2-Chloro-5-thiazolylmethyl) tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine	4,100	0.13[a]	33-117	4.69 (high)

Source: [a]NPIC, (2006); [b]AERU, (2018)

Table 2. Comparison of neonicotinoids with other classes of insecticides. Source: Tomizawa and Casida, (2005)

Class	Log K_{ow}	Nerve target	Potency (LD_{50}, mg/kg)		Selectivity factor*
			Insects	Rats	
Neonicotinoids	-0.7 to 1.3	nAChR	2.0	912	456
Organophosphates	1 to 5.5	AChE	2.0	67	33
Methyl carbamates	-1 to 3	AChE	2.8	45	16
Organochlorines	5.5 to 7.5	Na^+ or Cl^+ channels	2.6	230	91
Pyrethroids	4 to 9	Na^+ channels	0.45	2000	4500

*LD50 in rats/ LD50 in insects

1.2 Distribution of neonicotinoids in the environment

Neonicotinoid insecticides are widely distributed in the environment due to their physicochemical and excellent root systemic properties. Their wider applications ranging from seed treatment (coating), soil drenching, foliar spraying and various soil applications have so contributed to their ubiquity in the ecosystem. In spite of the physicochemical properties of these pesticides, other factors such as soil types, rainfall (erosion), degradations including photolysis, hydrolysis and biodegradations are involved in determining their fate and occurrence in the environment.

Indeed, neonics and their metabolites are considered as ubiquitous in ecosystems with their presence frequently detected in water (Wang et al., 2012, Sánchez-Bayo and Hyne, 2014a, Hladik et al., 2018), soil (Dankyi et al., 2014) and living organisms such as plants (Botías et

al., 2016), insects (Gbylik-Sikorska et al., 2015), birds (Humann-Guilleminot *et al.*, 2019), eel (Zhiming *et al.*, 2013) and rabbit and human body fluids (Kavvalakis *et al.*, 2013, Taira *et al.*, 2013). Although, neonicotinoids are known to have very low vapour pressure ($3 \times 10^{-7} – 1.7 \times 10^{-3}$ mPa) and having poor tendency to volatilise. Their presence in air as drift dust have been rarely reported (Biocca et al., 2015;Krupke et al., 2017) and this particularly occur during foliar spraying by farmers with potential toxicity to nearby foraging bees.

Also, the presence of different neonicotinoids has been reported in several food and food related products (Golge and Kabak, 2015, Gaweł et al., 2019a, Montiel-León et al., 2019) in varying amount with possible harm to animals and humans during their consumption. The presence of pesticides including neonicotinoids along the food chain is controlled and monitored by the establishment of their MRL by relevant authorities; this is to avoid compromising food safety and minimising the risk associated with their ecotoxicity. Therefore, to achieve this task, understanding the fate of this insecticides and their ability to distribute themselves in the environment after their application will be helpful in predicting their presence in different environmental compartment. Also, using sensitive and robust analytical techniques to detect their presence in the various environmental sample matrices in a wide range of concentrations, for example, in soil (ppb – ppm), water (ppt – ppb) and plants (ppb-ppm) is desirous.

1.2.1 Neonicotinoid insecticides in the atmosphere

Neonicotinoids are most likely to be temporarily present in air as an aerosol during their manual or mechanical spraying as folia application. This process makes pesticides particularly neonicotinoids available and at risk to non-target beneficial foraging insects such as honeybees, butterflies and possibly man if close enough to the point of application.

Although, neonics are not likely to volatilised due to their low Henry's constant values, however, the impact of the contaminated planter dust containing neonicotinoids on honey bees' health and other foraging non-target animals has been reported (Goulson 2013; Cresswell 2011 and Sanchez-Bayo & Goka 2014). The assessment of dust drifting of pesticides in aerosols were largely on honeybees and other concerned pollinators with a small foraging area. However, the extent of impact on non-target areas such as waterways, land and run-off water need to be considered.

1.2.2 Neonicotinoid insecticides in soil

Planting seeds which have been coated or treated with neonicotinoids and/or other insecticides and their direct application to soil for plant uptake are a major route of soil contamination. Following their application, insecticides are depleted from soil through plant uptake and natural degradation processes such as photolysis, hydrolysis, etc. (Horwood, 2007), thereby, reducing their ecotoxicity on soil biota.

There are several reports on the rates of degradation of different neonicotinoids in the soil from both laboratory and field studies. Table 3 showed the persistence of different neonicotinoids in soils ranging from 3 days to 19 years (7000 days); this indicates that neonicotinoids have varying degrees of persistence under different conditions. However, The variability in the degradation of insecticides are due to several controlling factors such as soil type (dry, wet, texture and organic matter), UV radiation (surface degradation), moisture or surface water, temperature, pH (Bonmatin *et al.*, 2014) and insecticide dose (Chopra *et al.* 2011).

Dose dependency for degradation of insecticides was reported for other insecticides such as fipronil, a similar solubility characteristic with most neonicotinoids, with half-lives of 23.35 and 24.31 days at relatively low doses of 56-112 g active ingredient/ha (Chopra *et al.* 2011).

However, no such study has been reported for any neonicotinoid insecticides and due to the importance of obtaining this data, there is a need for further research in this area.

Temperature is a factor determining the concentration of these insecticides in the soil as their degradation is temperature dependent. For instance imidacloprid half-life decreased from 547 to 153 days then to 85 days at temperatures of 5, 15 and 25^{o}C respectively (Bonmatin *et al.*, 2014). While, as an operating condition in the degradation of imidacloprid using combined advanced oxidation process (AOP), based on hydrodynamic cavitation, temperature was reported not to have affected its degradation at operating temperatures of 34, 39, and 42^{o} C yielding the same degradation (12.85 %, 12.69 % and 12.54 %, respectively) (Patil *et al*, 2014). These contrasting results need further research to ascertain the role played by temperature during degradation of insecticides and if geographical location is a factor to be considered during the formulation of regulations for pesticides use and control.

Table 3. Degradation (half-life expressed in days) rates of commonly used neonicotinoids in soil

Insecticides	Range of DT_{50} (days)
Imidacloprid	100-1230
Clothianidin	148-7000
Acetamiprid	31-450, 450[a]
Dinotefuran	75-82
Nitenpyram	8
Thiacloprid	3.4 - >1000
Thiamethoxam	7-335

Source: Bonmatin *et al.* (2014); Goulson (2013); PPDB (2007) [a]

The fate of pesticides such as neonicotinoids in soil is generally determined by their sorption characteristic and this has consequently influenced their mobility and leaching potentials (Bonmatin *et al*., 2014). The mobility of pesticides in soil have been largely reported to be determined by soil pH (Cao *et al*., 2008), ionic strength (Peng *et al*., 2009), organic matter (Kasozi *et al*., 2012, Zhang *et al*., 2018), and aquifer depth (Worrall and Kolpin, 2004).

The application of pesticides to soils have led to their detection in many environmental samples, however, the process leading to this contamination is complex and knowledge of their mobility mechanism is still limited (Zhang *et al.*, 2012). For example, negative correlation usually exists between the mobility factor (R_f) of pesticides and soil organic matter. However, Chen *et al.*, (2010) reported a decrease in the sorption of prometryne pesticides to soil and increase in its downward movement in soil columns when a dissolved organic matter fraction was added to the soil. Therefore, it is a matter of great importance to explore the environmental behaviour of neonicotinoids and factors responsible for their fate in soils.

1.2.3 Neonicotinoid insecticides in aquatic systems

Neonicotinoids have the potential to move from their point of application in the field to rivers or other water bodies by leaching through the soil column and run-off water. This increases their exposure to non-target organisms living in water such as fish, crustacean, oysters, etc. Also, humans are exposed to insecticides such as neonicotinoids when contaminated water or poorly treated water are consumed with potential serious health risks.

Neonicotinoids have been detected in many waters, especially drinking water at 0.1μg/L the maximum concentration set by EU for drinking water (Seccia *et al.*, 2005). The increased use and high solubility (Table 1) of neonicotinoids has raised their potential to be found in many water bodies such as lakes (Morrissey *et al.* 2015, Hladik *et al.*, 2018), river (Sánchez-Bayo

and Hyne, 2014a), estuarine (Hano et al., 2019), even in marine sediments (Martín *et al.*, 2017) (Appendix 1); thus, with risk to increased number of populations of non-target aquatic animals.

The persistence, toxicity and metabolite concentration of these pesticides in water are determined by a number controlling conditions such as temperature, pH, duration of sunlight exposure, microbial presence as well as formulation and pesticides doses. The comprehensive knowledge of how these conditions affect pesticides in aquatic environment is pivotal to understanding their fate in that ecosystem. The effect of temperature, pH as well as pesticides' physical state, have been reported to play a role in their solubility and distribution potential (Bonmatin *et al.*, 2014). However, these are yet to be investigated for their uptake by plant before, during and after application of the insecticides.

Photolysis, hydrolysis and microbial activities play major role in the degradation and disappearance of neonicotinoids from the water environment. This degradation mechanisms predict their presence or metabolites in different amount depending on the extent of their exposure to the degradation sources. Varying degree of UV photolytic degradation in water have been reported for different neonicotinoid insecticides. Kurwadkar *et al.*, (2016) showed UV photolytic degradation for dinotefuran, imidacloprid and thiamethoxam with half-life of 3.6, 2.3 and 3.8 h respectively in water. In a different UV photolytic degradation experiment, Chen *et al.*, (2018) reported very low half-life values of 0.62, 1.33, 1.61, 2.48 and 8.2 minutes for five neonicotinoids namely thiamethoxam, clothianidin, imidacloprid, thiacloprid and acetamiprid respectively. In the second author's report, the effects induced by the water matrix species including inorganic anions and natural organic matter were reported to be also non-negligible when photolysis was assessed in other sources of water such as waste and ultrapure water.

These reports suggest that neonicotinoids are able to degrade in water and their removal is possible under the right conditions. However, in the photolysis of neonicotinoids in the water environment, the use of UV lamp sources with a lower power rating against the commonly used high power UV lamp sources is more environmentally realistic, as well as being desirable in order to achieve both a cost reduction and energy efficiency. Therefore, the study of this abiotic degradation mechanism in water can provide information about the environmental stability of neonicotinoids on exposure to *natural* sunlight.

1.2.4 Neonicotinoid insecticides in biological systems

Neonicotinoids are easily taken up and widely distributed throughout the plant, thus rendering protection against many insect pests. Humans and animals are exposed to neonicotinoids and their metabolites through consumption of the treated plants with health implications. Many studies have reported varying amounts of neonicotinoids in plants' pollen, nectar and leaves (Sánchez-Hernández *et al.,* 2016), honeybees (Hou *et al.*, 2019a), rice whole grains and rice straw (Karthikeyan *et al.*, 2019) and human and rabbit urine, hair, breast milk (Kavvalakis *et al.*, 2013 and Taira *et al.*, 2013), eel (Zhiming et al., 2013), birds (Hao *et al.*, 2018 and Humann-Guilleminot *et al.*, 2019) and shrimps (J. Du *et al.*, 2018).

Also, the persistence of these insecticide in the environment have contributed to their possible exposure to plants and animals even long after their application. The lower lipophilicity of neonicotinoids, as reflected in their low Log K_{ow} (Table 1) and poor selectivity in their binding site to vertebrates (Tomizawa & Casida, 2009), is an advantage to their usage with reduced toxicity. However, their bioaccumulation in non-target insects with ecosystem economic importance, particularly honeybees, butterfly and earthworm, is still attracting considerable interest from research and regulatory bodies globally.

The biochemical mode of action of neonicotinoid insecticides acting as agonists at the nAChR sites of insects is in the public knowledge. All neonicotinoids are known to have high binding affinity to insects with I_{50}-values around 1 nM, while thiamethoxam presents a binding affinity of ten thousand fold less when compared to other neonicotinoids (Nauen *et al.,* 2003). Therefore, neonicotinoid insecticides may present different levels of toxicity during their exposure; this requires further research particularly since thiamethoxam is likely to be a neonicotinoid precursor for clothianidin, which acts with high affinity on the same receptor site as imidacloprid and all other neonicotinoids (Nauen *et al.,* 2003)

Neonicotinoids are exposed to plants through different methods of the application by direct trunk/stem injection or indirectly by uptake from soil and treated seeds. Also, during absorption through the leave cuticle, neonicotinoids are taken into plants during their spraying. Although all neonicotinoids are known for their toxicity to insect pests, they, however, show different capacities for their plant uptake. Acetamiprid was reported with 40 % and 80 % plant uptake by cotton and cabbage respectively, while imidacloprid gave 30 and 70 % uptake with the same plants (Bonmatin et al., 2014).

1.3 Review of analytical methods used for the analysis of neonicotinoids

1.3.1 Introduction

In the last two decades, neonicotinoids are widely used as insecticides and this has increased their frequent detection in the environment with possible risk exposure to non-target organisms (Bonmatin *et al.,* 2014, Botías *et al.,* 2015). In recent decades, due to the growing interest in the analysis of neonicotinoids, as well as their risk to bee health, a diverse range of analytical methods have been developed for their determination and quantification from soils, plant tissues, biological media, food, and feeds. However, low levels of these insecticides in the environment cannot, with certainty, be attributed to adverse health effects. To understand the

occurrence and fate of neonicotinoid insecticides in different environmental media such as soil, water, air, plants and animals, the use of sensitive analytical methods for their determination at trace levels is desirous; particularly complying with regulatory requirements.

The physico-chemical properties of neonicotinoids and the matrix characteristics are the main important factors that will be considered in the choice of analytical methods amidst others such as cost, availability of instruments, etc. Chromatographic and spectroscopic analytical techniques, involving either liquid or gas chromatography with varying detection methods are widely used in the analysis of different organic compounds. Prior to the advent of mass spectrometer (MS) as the detector, UV detector, diode array detector (DAD), fluorescence detector (FD), flame ionisation detector (FID) and electron capture detector (ECD) have been widely used in the analysis of pesticide residues including neonicotinoids. However, the need for sensitivity and selectivity of target analytes, particularly from complex matrices, led to the use of MS as preferred choice of detector.

Liquid chromatography and gas chromatography coupling to mass spectrometer as LC-MS and GC-MS have been used in the analysis of various pesticides due to their solubility and vapour pressure respectively. However, the liquid chromatography hyphenated with mass spectrometric working in tandem mode (LC-MS/MS) offers more sensitivity and selective analysis and it is the most used option in recent studies (Jones *et al.*, 2014, Jiang *et al.* 2018, Casado *et al.*, 2019). Notably, the high solubility and low volatility of neonicotinoids (Table 1) means that the use of LC-MS/MS is to be preferred over other instrumental methods of analysis from different sample matrices. The use of LC-MS/MS as an analytical instrument is extensively reflected in the analysis of neonicotinoid insecticides in different matrices using varying analytical methods (Appendix 1).

Prior to the analysis of neonicotinoids with the LC-MS/MS instrument, the need to extract the compounds in its purest form from the, sometimes very complex matrix environment, is most desired. Therefore, extraction and clean-up; separation and determination steps are commonly followed in the analysis of pesticides in environmental samples (Calatayud-Vernich *et al.,* 2016, Kasiotis *et al.,* 2018, Gaweł *et al.,* 2019). The different array of sample matrices, sample preparation steps and various types of instrumental analysis available in the analysis of pesticides involving several activities as shown in Figure 3. The quantification of the pesticides requires the assessment of their recovery during the clean-up in the different types of sample and effect of the matrices on the signal during its determination with LC-MS. Clean-up of extracts may result in the limited loss of some analytes, but inadequate clean-up could compromise the quality of data obtained. The best quantification strategy and quality parameters of the analysis need to be established.

1.3.2 Extraction and clean-up procedures: QuEChERS approach

The extraction of target compounds, such as pesticides, from environmental and biological samples can be challenging. The analyte of interest in the sample matrix is required to be analysed with the analytical instrument. However, the presence of matrix co-extracts in the sample can have profound influence on the outcome of the results obtained (Wilkowska & Biziuk, 2011). Therefore, the success of analysis of neonicotinoids from ranges of environmental samples is hugely dependent on the extraction method employed.

Several extraction and clean-up procedures, as well as their combinations (Figure 3) such as liquid-liquid extraction (LLE) (Farajzadeh & Feriduni, 2016, Timofeeva *et al.,* 2017), solid phase extraction (SPE) (Tao *et al.,* 2019, Hou *et al.,* 2019b), dispersive solid phase extraction (DSPE) (Cao *et al.,* 2018, Hou *et al.,* 2019b) matrix solid phase dispersion (MSPD) (Balsebre *et al.,* 2018), solid-phase microextraction (SPME) ((Liang *et al.,* 2017), stir-bar sorptive extraction (SBSE) (Gorji *et al.,* 2019), dispersive liquid-liquid microextraction (DLLME)

(Moreno-González *et al.,* 2012, Pastor-Belda *et al.,* 2016), microwave assisted extraction (MAE) (N. Li *et al.*, 2015, L. J. Du *et al.,* 2018) , supercritical fluid extraction (SFE) (Gallo *et al.*, 2017, R. Li *et al.*, 2018, Sakai *et al.,* 2019) and QuEChERS (quick, easy, cheap, efficient, rugged and safe) (Paradis *et al.* 2014; Economou *et al.* 2009 and Giroud *et al.* 2013, Montiel-León *et al.,* 2019) have been reported for the determination of pesticides, such as neonicotinoids, from various samples matrices (Appendix 1).

LLE is a traditional method used in the past in the extraction of neonicotinoids from varying sample matrices but has the following disadvantages: time consuming, requires large sample sizes and solvents, tedious and cumbersome purification process, expensive, generates a lot of waste and often produced poor quantification results (Suganthi *et al.,* (2018). The aforementioned challenges were overcome by the development of a DSPE method with reduced solvents and sample size and improved data sensitivity (Wilkowska & Biziuk, 2011).

Although SPE ensures better sample clean-up but requires plastic cartridges containing 250 to 2000 mg of sorbent material, a larger sample and multiple solvents generates solvent waste fractions. However, DSPE has the advantages of ensuring larger and more reproducible recoveries of analytes with acidic or basic properties (e.g. acephate, neonicotinoids thiabendazol) over SPE. Also, DSPE does not require SPE apparatus, cartridges, vacuum, pre-treatment of sorbent, channelling, drying out, collection tube, flow control, elution solvent, dilution of extract or solvent evaporation steps; DSPE is therefore quicker and cheaper using less sorbent, smaller amounts of sample and less equipment. Although it provides better interaction with the extract for clean-up, it has a limitation of only being used when the SPE sorbent removes matrix components and not the analytes (Wilkowska & Biziuk, 2011).

QuEChERS is one of the most widely used methods in the analysis of several pesticides such as neonicotinoids in a wide range of environmental and biological matrices: pollen (Chen *et*

al., 2013, López-Fernández *et al.,* 2015, Jiang *et al.,* 2018); fruits and vegetables (F. Zhang *et al., 2012,* Montiel-León *et al.,* 2019), honey (Calatayud-Vernich *et al.,* 2016, Gaweł *et al.,* 2019) honeybees, beebread and beeswax (Fidente *et al.,* 2005, Kasiotis *et al.,* 2018), water (Lehmann *et al.,* 2017, Jankowska *et al.,* 2019), soil (Dankyi *et al.,* 2014), rice and rice straw (Karthikeyan *et al.,* 2019). The QuEChERS technique, which is based on a salting-out extraction of analytes in acetonitrile, involves the use of liquid-liquid extraction (LLE) and, thereafter, followed by partial purification (or cleanup) by dispersive solid phase extraction (DSPE) (Anastassiades *et al.,* 2003). The method was initially developed and used in the analysis of multi-class residues of pesticides, including neonicotinoids, in fruits and vegetables (Anastassiades *et al.,* 2003, Koesukwiwat *et al.,* 2010).

The flexible approach of the QuEChERS method has led to the development of several modified and adapted procedures; this largely depends on the nature of the analytes and the sample matrix composition (David *et al.* 2015). The removal of pigments and sterols in complex matrices such as pollen, using graphitised carbon black (GCB), C18 and primary and secondary amine exchanged (PSA) sorbent material for the DSPE steps, has been reported by (Chen *et al.,* 2013). However, the recovery of the planar aromatic structure, such as with most neonicotinoids, is hindered because GCB is known to retain pesticides with planar-aromatic structure and substantial adsorption and loss of hydrophobic compounds (Dankyi *et al.,* 2014, Jiao *et al.,* 2016).

Suganthi *et al.,* (2018) used DSPE with acetonitrile as extraction solvent in the analysis of neonicotinoids in sugarcane with recovery ranging from 62 – 130% at spiking level of 0.005µg/g concentration. The use of DSPE sorbent is widely accepted over other methods in that they have the capacity to isolate insecticides from complex matrices without the undesirable co-extraction of lipids and the significance loss of analytes; thereby, improving the sensitivity of their detection (Moreno-González *et al.,* 2018).

Several DSPE sorbents such as primary, secondary amines (PSA), C_{18}, graphitised carbon black, zirconium oxide-based sorbents (Z-Sep), and activated charcoal, have been reported in the analysis of neonicotinoids with varying recoveries (Moreno-González *et al.,* 2018). However, McManus *et al.*, (2019), without using DSPE sorbent, reported recoveries of 86 – 100 %, 71 – 82 % and 56 – 103 % for four neonicotinoids in sediment, laboratory sand and agricultural soil respectively and concluded that the recovery of analyte is largely dependent on its sample matrix.

Despite that, DSPE has a high capacity to remove lipid components in the sample with satisfactory analyte recoveries (73.7%–119.0%, Dias *et al.,* 2016). The shortcomings of the DSPE with Z-Sep include a high capacity to retain some non-polar pesticides and consequently poor recoveries are achieved in their analysis (Hakme *et al.*, 2018).

Robles-Molina *et al.*, (2016) and Parrilla Vázquez *et al.*, (2016) have recently proposed the use of a DSPE sorbent named Enhanced Matrix Removal-Lipid sorbent (EMR-Lipid) to remove major lipid classes from the sample matrix with excellent pesticide recoveries. The versatility and compatibility of DSPE sorbents with different solvents like water, methanol, acetonitrile, hexane, dimethyl ether, dichloromethane and their combination promote its usage in different fields of chemical analysis.

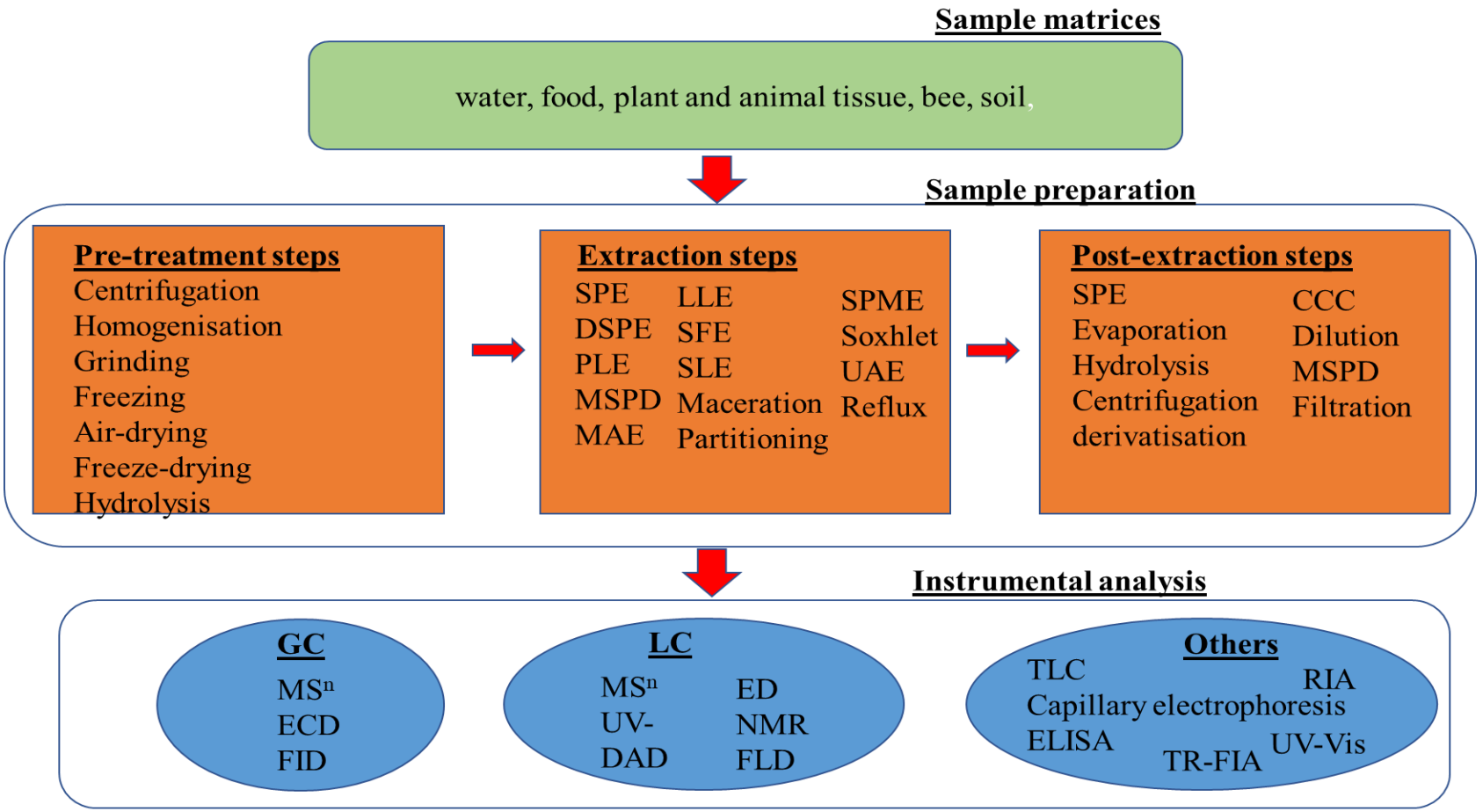


Figure 3. The principal steps involved in the analysis of neonicotinoids from several sample sources using different types of sample preparations (pre-treatment steps; extraction steps and post-extraction steps) and varying instrumental analyses. The full meaning of the abbreviations can be found in the list of abbreviations

The use of Dispersive Liquid-Liquid Microextraction (DLLME) as a sample treatment method tested along with QuEChERS was assessed and found to be a good alternative, see Table 4 (Jovanov *et al.,* 2014). There are numerous advantages of DLLME over other traditional extraction methods including simplicity of operation, low cost, reduced volume of extractor solvent, speed, high enrichment factor and ease of linkage to analytical methods. Besides these advantages, the drawback of DLLME is the prerequisite of the solvent extractor which should have ability to extract the analytes, low water solubility and must be compatible with the analytical instrument of choice; especially when solvents of higher density than water are used (Primel *et al.,* 2017).

Chlorinated solvents such as carbon tetrachloride (18 µL, Yazdi *et al.,* 2008), chloroform (80 µL, (Wu *et al.,* 2009), dichloromethane (70 µL, Chou *et al.,* 2009), etc., though in low volume, are generally used as extractor solvents in DLLME. However, chlorinated solvents are toxic, hazardous and do not comply with the green analytical chemistry principle. Besides the toxicity of these solvents, their non-polar nature is equally a limitation if used for the extraction of more polar compounds like neonicotinoid insecticides. In other to reduce the drawbacks stated earlier, ionic liquids (Aguilera-Herrador *et al.,* 2010) and solvents lighter than water such as alcohols namely; 1-hexanol and 1-octanol (López-Darias *et al.,* 2010, Liu *et al.,* 2010) are used.

The application of DLLME in pesticides' analysis has seen a considerable growth since its introduction in 2006 (Primel *et al.,* 2017). There have been several modifications on the technique aiming to make it faster, less toxic, promote the extraction of more polar compounds, reduce solvent consumption and solvent exposure to the analyst. For every modification, a new acronym was generated leading to a wide range of classifications and terminologies in the circle of dispersive liquid-liquid microextraction (Table 5).

Table 4. Compilation of different types of sample treatment methods in the analysis of seven neonicotinoid in honey matrix between QuEChERS and DLLME (Jovanov *et al.*, 2014).

Steps	QuEChERS	DLLME
Sample size	15 mL	5 mL
Dispersive agent	Buffering salts (4 g of magnesium sulphate, 1 g of sodium chloride, 0.5 g of sodium citrate dibasic sesquihydrate and 1 g sodium citrate tribasic dehydrate)	Dichloromethane (2 mL)
Extraction agent	10 ml of acetonitrile (waster was not added due to high water content of the sample)	Acetonitrile (0.5 mL)
Agitation	Shaking for 1 min and centrifuging at 3000 rpm for 10 min. Vortexing and centrifuged. ACN layer (1.5 ml) was dried under a gentle stream of nitrogen	Shaking for 1 min, sonication for 10 min and centrifugation for 5 min at 2500 rpm
Cleanup	Aliquot added with magnesium sulphate, (0.9 g) and PSA (0.150 g). A subsequent step of vortexing at the cleanup vial for 1 min and centrifuging for 10 min at 3000 rpm and ACN solvent later was evaporated to dryness under the steam of nitrogen.	The dichloromethane was evaporated to dryness under the flow of air
Reconstitution	Dry residue reconstituted in 0.15 ml of the mobile phase and vortex for 2 min	Dry residue reconstituted in mobile phase solvents
Recovery (%)	72 – 95	69 – 113
LOD (µ/kg)	2.0 – 2.5	1.5 – 2.5
LOQ (µ/kg)	5.0 – 10.0	5.0 – 7.5

Table 5. The different types of modification to DLLME and their application with the recorded sensitivities

Abbreviation	Meaning	Extractor solvent	Analytes	LOD (µg/L)	Recovery (%)	RSD (%)	Reference
PDLLME	Partitioned Dispersive Liquid–Liquid Microextraction	dichloromethane	Herbicides from aqueous samples.	0.10 - 0.28	97.4 to 101.7	< 5.9	Chou *et al.,* 2009
UA-DLLME	Ultrasound-Assisted Dispersive Liquid–Liquid Microextraction	carbon tetrachloride	triazines, organophophates, acaricides and pyrethroids	0.09 - 0.57	90.5 - 107.7	< 8%	Cui *et al.,* 2013
UDSA-IL-DLLME	Up-and-down Shaker-assisted Ionic Liquid-based Dispersive Liquid–liquid Microextraction	Ionic liquid	UV filter from water samples	0.2 - 1.3	92 - 120	< 7.1	Ku *et al.*, 2013
AALLME	Air Assisted Liquid–Liquid Microextraction	dibromoethane	5 triazoles from water	0.2 – 1.1	92 - 105	< 4	Farajzadeh *et al.*, 2013

USAEME	Ultrasound-assisted Surfactant-enhanced Emulsification Microextraction	toluene	organophosphate from water samples	1 - 2 ng/L	90.1 - 104.7	< 6.3	Su and Jen, 2010
TIL-DLME	Temperature-controlled Ionic Liquid Dispersive Liquid Phase Microextraction	Ionic liquid	triclosan, triclocarban, and methyl-triclosan from water samples.	1.15 - 5.33 ng/L	58.9 - 92.4	< 20	Guo *et al.*, 2010
DLLME-ISCS	The dispersive liquid–liquid microextraction method using a low-density organic solvent and an improved solvent collection system	1-nonanol	organochlorine from water samples	0.7 - 9.4 ng/L	73 - 119	< 10.8	Chang *et al.*, 2011
VALLME	Vortex Assisted Liquid–Liquid Microextraction	1-octanol	fungicides from water samples	0.73 - 1.33	81.3 - 116.8	< 11.8	Wang *et al.*, 2013

DSPE-DLLME	Dispersive Solid-phase Extraction Liquid–Liquid Microextraction	Acetone and followed by PSA and GCB	organophosphate pesticides from soil samples	0.2 - 0.5 ng/g	79.6 - 106.8	< 8	Wang *et al.*, 2014
SD-DLLME	Solvent-based De-emulsification dispersive liquid–liquid microextraction	1-octanol and Acetone. Water was used as demulsifier	Fifty-eight pesticides and PPCPs were extracted	0.004 - 0.4	60 – 120 for 84% of the compounds,	< 29	Caldas *et al.*, 2016

Despite the high recoveries of analytes (Table 4) recorded in the use of DLLME, solid matrix partition clean-up with Extrelut NT20 columns containing diatomaceous earth in sample treatment for the analysis of four neonicotinoids has been reported, with dichloromethane as the extraction agent and good recoveries ranging from 76% to 99 % (Fidente *et al.,* 2005).

Many solvents, such as acetone (Anastassiades *et al.,* 2003), acetonitrile (Anastassiades *et al.,* 2003, Maštovská & Lehotay, 2004) and ethyl acetate (Banerjee *et al.,* 2007), have been employed in the extraction of neonicotinoids from samples because they produce high analyte recoveries. However, varying challenges associated with each solvent determine their choice by the analyst. For instance, acetone is readily miscible with water, however, its removal from water is not possible without the use of a non-polar solvent. Contrarily, ethyl acetate is only partially miscible with water, therefore, the addition of non-polar solvent makes it difficult to separate in water; consequently, most highly polar pesticides like neonicotinoids do not separate.

Acetonitrile (ACN) is classified as a nitrile in terms of its functional group and possessing perfect bond angle of 180^{o} between the methyl group carbon, the central carbon atom and the nitrogen atom. The properties of ACN, as earlier described, provides its polar nature and makes it readily miscible with water. The extracts of samples in ACN are known to contain fewer interfering co-extracts when compared to the corresponding extracts of acetone and ethyl acetate (Wilkowska & Biziuk, 2011). Also, acetonitrile can equally be easily separated from water with the addition of salts such as $MgSO_4$, NaCl, NaOAc (salting-out). The attributes of acetonitrile encourage its use as a solvent of choice in QuEChERS method in the determination of neonicotinoids from the several samples.

The performance of the selected sample treatments, clean-ups and respective chromatographic methods need to be evaluated and validated based on association of official analytical chemists AOAC official method 2007.01 (AOAC International, 2011).

1.3.3 The use of liquid chromatography hyphenated with mass spectroscopy

Besides the extraction and clean-up procedures for pesticides, which are important steps for good quantification, the techniques required for their separation and detection are essentially important. Technological advancements in the field of mass spectrometry and chromatography, aiming to achieve sensitivity and selectivity, have led to the development and applications of several tandem instruments such as LC-MS and GC-MS (Hecht *et al.,* 2016) (Souza Tette *et al.,* 2016).

These two instruments (LC-MS and GC-MS) have shown great success in the analysis of multi-residues of pesticides, including neonicotinoids from several environmental and biological samples as presented in Annex 1. Information such as retention times of the analytes and two or more transitions to quantify and confirm the identity of each analyte are made possible with the use of these instruments. The instruments are highly sensitive and produce results that are consistent with the maximum residual limit established by international legislation (Bargańska *et al.,* 2013), Kasiotis *et al.,* 2018). However, LC-MS is a suitable technique in the analysis of neonicotinoids, since it does not require any derivatization procedures and the identification and quantification can be performed in a single step (Zuloaga *et al.,* 2012).

Neonicotinoids are relatively soluble and very polar in nature, as earlier stated in Table 1, when compared to other similar pesticides such as fipronil (very high solubility) and formetanate (very low solubility) with solubilities of 3.78 and 822, 000 mg/L respectively. The solubility and polar nature of neonicotinoids promote the choice of LC-MS over GC-MS in their determination in samples of interest (Barbi et al., 2019). Consequently, these properties have contributed to high volume of publications in the analysis of neonicotinoids published in both Chemosphere and Science of the Total Environment reported in years 2017-2019, with 60 to 10 research articles using LC-MS and GC-MS respectively.

The combination of liquid chromatography (LC) and mass spectrometry (MS) presents a major challenge for solvent compatibility. Therefore, for high sensitivity and reproducibility results, the MS detection needs to be optimised for particular sample matrices, composition of mobile phases and analytes. Four major parts of the mass spectrometer may contribute to the sensitivity of the overall instrument performance namely: ionisation, transmission of the ions, fragmentation and detection. The interface of LC/MS is broadly categorised into two: those that use the mobile phase to assist in ionisation (gas-phase ionisation methods) and those where the mobile phase is removed prior to ionisation; these two categorise fall into three areas: gas-phase ionisation, desorption ionisation methods and evaporative ionisation methods.

Two of the commonly used ionisation methods for the analysis of neonicotinoids in LC-MS are electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI); both methods belong to the category of evaporative ionisation methods. However, APCI requires the use of a carefully control heated source (> 120 ^{o}C) which may be destructive to thermolabiles, while ESI, in contrast, is operated without an additional heat source in the desolvation/vaporisation chamber, therefore fragmentation of thermolabile compounds can be prevented (Sjöberg & Markides, 1999). The attribute of the fragmentation of thermolabile compounds by APCI may contribute to the choice and use of ESI as a soft ionisation technique for the analysis of several polar pesticides that are thermolabile, such as neonicotinoids (López *et al.,* 2018).

Other ionisation methods such as atmospheric pressure photoionisation (APPI), fast atom bombardment (FAB) ionisation, electron impact (EI) ionisation, matrix assisted laser desorption ionisation (MALDI) among others are commonly used. The analysis of neonicotinoid insecticides with the ESI mode has been reported in the positive mode, however, some have equally been carried out in the negative mode (Annex 1).

The determination of neonicotinoids insecticides is generally in environmental and biological samples with a complex matrix environment. Therefore, the matrix effect is a huge challenge, particularly when their residue determination at the trace level is required for regulatory and safety purposes. The enhancement or decrease in analyte signal from extracts obtained in the presence of matrix compared to those obtained in solvent is generally termed matrix effect (Souza Tette *et al.,* 2016).

Undoubtedly, the problem of matrix effect is usually reported for LC-MS analysis (Montesdeoca-Esponda *et al.,* 2018). The presence of high concentration of ionisable organic components in the matrix such as natural organic matter, salts, ion-pairing agents and other contaminants can cause interference with the ionisation process of ESI (Zuloaga *et al.,* 2012, Li *et al.,* 2017). The results of these signal interference usually lead to false quantitative results (Jakimska *et al.,* 2013).

In order to compensate for potential matrix interference, particularly when ESI mode of the LC-MS is used, some of the approaches suggested are: standard addition, surrogate labelled standards and the use of matrix-matched calibration (Stüber & Reemtsma, 2004, Zuloaga *et al.,* 2012, Jakimska *et al.,* 2013, Han *et al.,* 2016, Martín *et al.,* 2017). Clearly, there are challenges known with each procedure suggested; standard addition procedure is time-consuming and very tedious with many samples to be prepared; surrogate labelled standards can be expensive and considering the desire of cost-effective analysis while matrix-matched calibration method requires to use matrices that ideally, should not contain any of the selected compounds, which in practice is not always possible (Jakimska *et al.,* 2013, Martín *et al.,* 2017).

Another possible approach to improve the performance for pesticides' recovery, with reduced matrix effect due to signal suppression when analysed in the LC-MS with the ESI mode, is the

use of nano flow. The nano flow reduces the amount of matrix component entering the mass spectrometer at the same time as the analyte (Moreno-González *et al.,* 2017a, Moreno-González *et al.,* 2017b and Rodriguez *et al.,* 2002)**.** This can be achieved by improved sample preparation through selective extracton and cleanup procedures, as previously reported in section 1.3.2. Also, dilution of the extract is another alternative to reduce the amount of matrix extract entering the ESI mode since this reduces the competition beteween the analytes and the components of the matrix for ionisation, Li *et al.,* 2017. However, the sesnsitivity of the analyte may be a challenge due to possible lower amount present for analysis.

The analysis of ionisable analytes in the LC-MS requires a mass analyser with high resolution and sensitivity. Different mass analysers and detectors are commercially available. The commonly-used mass analysers with their varying mass range are: magnetic sector (1 – 15,000 *m/z*); quadrupole (1 – 5,000 *m/z*); ion trap (1 – 5,000 *m/z*); time of flight (unlimited); and Fourier transform (up to 70 kDa). Presently, tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS) allows the detection of neonicotinoids at low concentration for several matrices such as honey and pollen. The analysis of neonicotinoids from complex matrices with LC-MS/MS improves sensitivity, reduces matrix interference and provides structural information about each analyte (Souza Tette *et al.,* 2016). Also, the reliability of the result is increased with the spectrometer analysing only the ions of interest with multiple reaction monitoring (MRM) mode (Tomasini *et al.,* 2012). Therefore, the need for accurate and reliable results of target analytes such as neonicotinoids from complex matrices has led to the increased use of LC-MS/MS methods. In spite of the positive things about the detection and ionisation potential of LC-MS/MS, appropriate and affordable sample preparation is fundamentally important for a reliable analysis and result.

1.4 Objectives of the study

The fate of neonicotinoids in soil are not completely well known with high degree of certainty, despite the much-reported toxic effects that these insecticides cause to bees, other pollinators and aquatic animals. The obtaining of such information on sorption kinetics, leaching profile, degradation data and mobility of neonicotinoid insecticides in determining their occurrence and fate in the environment poses analytical challenges.

Also, the part played by neonicotinoids in the decline of bee colonies, as widely publicised, is still an on-going debate with no conclusive research-based decision regarding their impact among other factors considered.

Therefore, the objectives of this study were:

1. To randomly select soils, having no pre-history of contamination, with varying characteristics to understand the effect of field soil types (unamended) on the uptake of neonicotinoids.
2. To elucidate the sorption kinetics, isotherm and leaching profile of the most and least sorbed neonicotinoids, as a means of predicting their mobility in soils with potential risk to surface and ground water contamination.
3. To assess the effect of UV photolytic degradation of neonicotinoids in water under controlled conditions using a low power UV lamp source.
4. To optimse the QuChERS method for the analysis of neonicotinoids in relevant matrices related with the study of neonicotinoids. The method was tested with spiked and unspiked samples of soil, wheat, flowers of cropped and marginal plants.

CHAPTER 2: MATERIALS AND METHODS

2.0 Materials and methods

2.1 Chemicals and materials

Standard stock solutions (1000 µg/g) of each neonicotinoid insecticide was made from 100 mg each standard in methanol: water at ratio 50:50; acetamiprid (99.9 % purity), imidacloprid (99.9 % purity), dinotefuran (98.6 % purity), thiacloprid (99.9 % purity) and thiamethoxam (99.6 % purity) obtained from Sigma-Aldrich Ltd, Dorset, UK. Similarly, standard stock solutions (1000 µg/g) of the internal standard was made from 2-chloroaniline obtained from Sigma-Aldrich, Germany, 98.8 % purity. Diluted intermediate solutions were prepared with the mixture of all the neonicotinoids at 15 µg/g and followed by calibration standard solutions 1.0 – 9.0 µg/g and 0.001 – 1.0 µg/g for LC-UV and LC-MS/MS analysis respectively. All prepared solutions were wrapped with aluminium foil and refrigerated at 4°C until analysis. HPLC grade methanol (99 % purity) and LC-MS grade formic acid (99 %) were obtained from Fisher Scientific (UK). Ultrapure water (18 MΩ cm at 25ºC) was used throughout the study (Purelab, UK).

2.2 Analysis of neonicotinoids with LC-UV-Vis

The LC chromatograph (Schimadzu, LC-2010AHT, Japan) was equipped with a UV detector. The analytical column used was a Waters Atlantis® (UK) C_{18} (150 mm x 2.1 mm) with a particle size of 5 µm and a C_{18} guard column (5 mm x 2.1 mm) from Waters Atlantis® (UK). The optimal operating separation conditions: 1.0 mL/min flow rate, 20µL injection volume, detection wavelength 244 nm, 55:45 % of methanol: 0.1 % formic acid in water mobile phase in isocratic condition were developed for the LC-UV. Standard stock solutions, 1000µg/g, of each compound were prepared by weight in water – methanol (50:50). Diluted intermediate solutions of 15 µg/g concentration and subsequent multi-level calibration standard solutions

ranging from 1.0 – 9.0 µg/g were prepared in 100 % water. A mixture of the compounds with the internal standard (2-chloroaniline) was prepared.

2.3 Analysis of neonicotinoids with LC-MS

The LC-MS/MS instrument used was Agilent LC-1260 Infinity and MS-6340 Triple Quad, equipped with MassHunter Workstation Software, version B.04.01. The analytical column used was a Waters Atlantis® (UK) C_{18} (150 mm x 2.1 mm), with a particle size of 5 µm and a C_{18} guard column (5 mm x 2.1 mm) from Waters Atlantis® (UK). The optimal working condition developed for the LC-MS/MS was: 0.27 mL/min; 10 µL injection volume; column temperature 40°C; mobile phase was methanol (solvent A) and 0.1 % HCOOH in water (solvent B) under a gradient condition of 0 – 2 min, 10 % solvent A, 2 - 6 min, 10-50 % solvent A, 6 – 9 min, 50 % solvent A, and returned to initial conditions in 4 min, 5 min post run delay. The optimised analysis was in the ESI in positive mode. The acquisition of the five study compounds in Multiple Reaction Monitoring (MRM) is shown in Table 6 with their corresponding collision energies (CEs), quantitation/confirmation ions and source conditions of drying gas (N_2) temp. 325°C; and flow of 12 L/min.; and nebuliser gas (N_2) at 50 psi. All sample extracts and standards included 2-chloroaniline (0.6 µg/g as internal standard). The optimal potential applied in the ion optics were capillary voltage ±4000 *V*, octapole RF 600 V; octapole DC 5 V; Lens 1 DC 4.2 V; Lens 2 DC -6.2 V; Lens 2 DC EF Off -6 V; skimmer 15 V; and chamber current of 0.12 μA and were used for mass spectrometry.

Table 6. MRM transitions for quantitation/confirmation used for each compound studied and their corresponding collision energies (CEs)

Compound	Q1 mass	Q3 mass	CE, eV	Quantitation/Confirmation
acetamiprid	223	126	20	Quantitation
	223	90	20	Confirmation
Ratio MRM1/MRM2 (%)	72			
dinotefuran	203	129	20	Quantitation
	203	113	20	Confirmation
Ratio MRM1/MRM2 (%)	88			
imidacloprid	256	209	45	Quantitation
	256	175	47	Confirmation
Ratio MRM1/MRM2 (%)	84			
thiacloprid	253	126	40	Quantitation
	253	90	40	Confirmation
Ratio MRM1/MRM2 (%)	71			
thiamethoxam	292	211	32	Quantitation
	292	181	45	Confirmation
Ratio MRM1/MRM2 (%)	86			

Dwell time: 0.01 s.

2.4 Soil characterisation

2.4.1 Soil sampling and pre-treatment

In achieving the objectives of this study, the sites labelled BR, TH, EY, TLW and ST (Table 7), were selected to ensure there was no history of previous contamination with the study pesticides based on witnessed information obtained from the local neighbours. In all the soil sampling, the topsoil samples were collected between 0-20 cm deep. Three randomly selected spots, 50–100 cm apart, in each selected geographical location were sampled using an auger and combined together to form a composite sample and labelled, Table 7.

Table 7. Sampling locations of soils and their land use in the UK used in the sorption and leaching studies

Sample label	Location (UK)	Latitude – Longitude	Land use
BR	Brighton (East Sussex)	50.849133, -0.118022	Golf course
TH	Thornton Heath (Surrey)	51.397848, -0.097930	Domestic garden
EY	Eynsford (Kent)	51.374549, 0.213009	Farmland
TLW	Tolworth (Surrey)	51.372305, -0.276660	Farmland
ST	Stornoway (Western Isles, Scotland)	58.205537, -6.353212	Domestic garden

The soils were air-dried in the hood in the laboratory for 4 days in the dark with the removal of plant debris before grinding using a pestle and mortar. Soil particles (< 2 mm diameter) were thoroughly mixed, stored and sealed in sample polythene bags prior to their use in experiments.

2.4.2 Soil characterisation

Soil pH values were determined in soil-water suspensions of weight ratio 1:2.5. After shaking with a rotary shaker for 2 h and allowed to stand for 15 minutes, the pH was measured using a previously calibrated pH meter. Soil organic carbon (SOC) was determined using the Walkley-Black procedure (ISRIC, 2002). Soil particle sizes were determined by the hydrometer method. Cation exchange capacity (CEC) was determined using 1M sodium acetate solution to saturate the soil exchange sites with Na^+ ions at pH 7. The Na^+ ions were displaced with NH_4^+ ions (from ammonium acetate solution). Sodium content was then determined by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Jobin-Yvon Ultima, 2C, France). The methods for the characterisation of the soils pH, CEC and soil particles sizes listed above

(Table 15) were carried out as described in ISRIC (2002). See appendix 11 for the detail description of the soil characterisation procedures.

2.5 Data analysis

The effects of the amount of organic matter (% SOC) on the adsorption of neonicotinoids were evaluated by the statistical t-test. One-way ANOVA, using 3 replicates in Microsoft Excel (2016), was used to detect any significant differences in the average adsorption capacities of all the pesticides within all the soils (see Appendix 7), tested at 95 % confidence limit (α = 0.05).

The quality parameters limit of detection (LOD), limit of quantification (LOQ) were estimated at a signal-to-noise ratio of 3 and 10, respectively. Repeatability and reproducibility were assessed with standards at 0.5 µg/g for LC-UV and 0.001 and 0.005 µg/g for LC-MS/MS. from the injection of the mentioned standards on 6 repeated analyses during the same day; and 2 analyses on 3 non-consecutive days, respectively. Quality controls at a concentration of 0.5 µg/g was run every 6 samples for both the LC-UV and LC-MS/MS analysis.

CHAPTER 3: DEVELOPMENT OF METHODS FOR THE ANALYSIS OF NEONICOTINOIDS

3.0 Development of method for the analysis of neonicotinoids

3.1 Introduction to method development for the analysis of neonicotinoids

The assessment of the fate and distribution of pesticides in soils, water, air, and biological matrices involve the use of sensitive analytical instrument. Also, validated methods of extraction, clean up and analysis of organic contaminant in the environment, according to national and international guidelines with accurate and reproducible results, are desirous in the field of analytical science.

A number of techniques such as gas chromatography (GC) and/or high performance or ultra-performance liquid chromatography (HPLC or UPLC) coupled to ultraviolet-visible (UV-vis) and mass spectrometry (MS) have been used in the identification and quantification of insecticides and their metabolites. The choice of the use of this instrument is largely based on their physicochemical properties such as solubility, vapour pressure, chemical structure as well as their molecular weights from different matrices. Generally, all neonicotinoid insecticides are known to have high solubility with very low vapour pressure (Table 1). Therefore, this property makes liquid chromatography a favoured separation technique over gas chromatography.

The requirement of detection of ultra-low concentrations of neonicotinoids, down to ppt levels, is a challenge amongst the presence of a myriad of components in these complex materials. Several analytical techniques are available for use in environmental chemical analysis, however, recent analysis have been carried out with gas chromatography, liquid chromatography coupled with ultraviolet visible (LC-UV) and mass spectrometer (LC-MS) (Koesukwiwat et al., 2010). The challenges of coupling liquid chromatography technique to a mass spectrometer have been

largely overcome by the use of different effective interface such as atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). Benefits including compatibility, sensitivity, and versatility are associated with the use of these interfaces. Also, the availability of various MS detections such as single or triple quadrupole (González-Mariño et al., 2018), orbitrap (Casado *et al.*, 2019), ion trap (Comtois-Marotte et al., 2017) or time of flight (Taliansky-Chamudis et al., 2017) provides an increasing array of suitable methods available to meet the objective of the analysis.

Previously, in the analysis of neonicotinoids from environmental samples such as bees, beewax, pollen dietary, soil and water, methods of extraction were found to be time consuming ranging from 35 minutes to 1hr, even more. Also, extractions of neonicotinoids were limited to one or two matrices (López-Fernández et al., 2015, Lentola et al., 2017, Karthikeyan et al., 2019) with the use of large amount of extraction solvent and sample size, hence, raising concerns for the generation of waste and waste disposal problem. Therefore, to meet the objective of this study in assessing the fate and distribution of neonicotinoids in different environmental samples, an existing extraction method was adapted to determine neonicotinoids from several matrices including soils, water, plants' flowers, bees, bee wax and wheat grains.

There are different acquisition modes such as full scan, selected ion monitoring (SIM), selected reaction monitoring (SRM) and multiple reaction monitoring (MRM) for various uses within the MS analysis (Appendix 1). Although less selectivity is sometime useful for the separation and optimisation of mixture of analytes, hence, LC-UV was used first prior to the use of LC-MS to help avoid co-elution and signal suppression problem that may be encountered in a more complex technique where the suppression of signals could also be due to other factors. The developed LC-UV method, with well separated chromatographic peaks of the analytes, was adapted to the

LC-MS/MS for better selectivity of the analytes studied and improved sensitivity at very low quantification levels in the study matrices.

3.2 Optimisation of the LC separation of neonicotinoids

Prior to the chromatographic separations of the insecticides, the optimal wavelength (appendix 3) with maximum absorbance was determined using a UV-vis spectrophotometer (Cary100 UV-vis Agilent, Australia). The chromophore of the compounds, nitro and cyano groups, pi bonds or atoms with non-bonding orbitals such as the lone pair ion, oxygen, nitrogen or a halogen, caused the absorption of energy over a certain range of wavelengths in the ultraviolet and the visible light region. The results of the wavelength with maximum absorbance of the selected insecticides and the internal standard are reflected in Table 8.

The proposed use of LC with a fluorescence detector was substantiated by the examination of the emission of the compounds when excited by UV incident photons. Although, the LC-Fluorescence detector instrument is highly selective and sensitive, poor spectra with weak and very broad peaks was observed for all the insecticides (see Appendix 5). This was because aromatic systems were not present or limited to a benzene ring, in the best cases, and it was substituted by a halogen, atoms that are well known to quench fluorescence.

Table 8. UV maximum absorption wavelengths of selected pesticides and internal standard (2-chloroaniline) in methanol at 5µg/g concentration.

Pesticides	Absorbance	Wavelength, nm
Imidacloprid	0.722	269
	0.443	212
Thiacloprid	0.537	243
Thiamethoxam	0.406	254
	0.263	210
Acetamiprid	0.679	247
	0.431	215
Dinotefuran	0.492	271
	0.177	212
2-Chloroaniline	0.756	292
	2.460	237
	3.021	210

3.2.1 HPLC-UV separation

To achieve good separation for all the compounds, preliminary chromatographic conditions such as different flow rates (0.1, 0.3, 0.8 and 1 mL/min), pH (2.7 and 3.0) and percentage of the organic solvent in different mobile phases (methanol and acetonitrile) were assessed. Also, detection at various wavelengths in UV (Table 8) were optimised for all the study compounds with the chromatogram presented in appendix 4, A - F. Specifically, both isocratic and gradient elution were attempted to resolve the difficulty in their separation of the mixture of the 7 compounds at the same time achieving a run time of less than 10 minutes.

The aim of separating and identifying the study compounds with chromatographic technique was met with some challenges because they eluted at very similar retention times (Figure 4A).

This may be attributed to their very similar physicochemical properties, which are summarised in Table 1. The optimal working condition, detailed in section 2.2, led to the separation of 7 neonicotinoids and the internal standard in two sets as it can be observed in Figure 4B and 4C.

3.2.2 Sensitivity of LC-UV chromatographic conditions

The sensitivity of the method was determined by running the mixture of all the study compounds with the internal standard. Five quantifications levels; 0.5, 1.5, 3.5, 4.5 and 6.0 µg/g, were made by weighing and were used for the calibration curve.

The limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility were determined by dilution a standard at 0.5 µg/g. Specifically, the LOD and LOQ were estimated at a signal-to-noise ratio of 3 and 10 respectively. Repeatability and reproducibility were assessed from the injection of the mentioned standards on 6 repeated analyses during the same day; and 2 analyses on 3 non-consecutive days, respectively. The sensitivity of the LC-UV method is shown in Table 9.

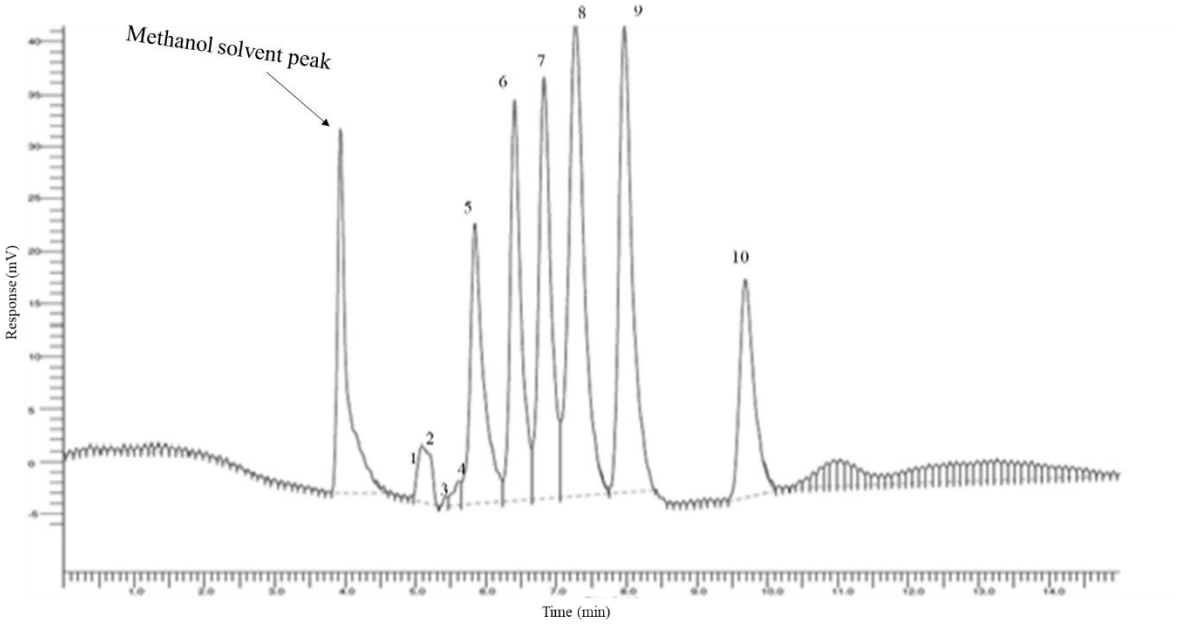


Figure 4A. Chromatogram of 10 compounds' mixture (1 - NTP, 2 - DIN, 3 - CLO, 4 - FH, 5-THX, 6 - IMI, 7 - FIP, 8 - ACE, 9 - THA, 10 - 2-CA) at 15µg/g concentration with some compounds co-eluting. The detection was at wavelength 244 nm, 1 mL/min flow rate and the mobile phase used was 55:45 % (methanol: 0.1 % formic acid in water)

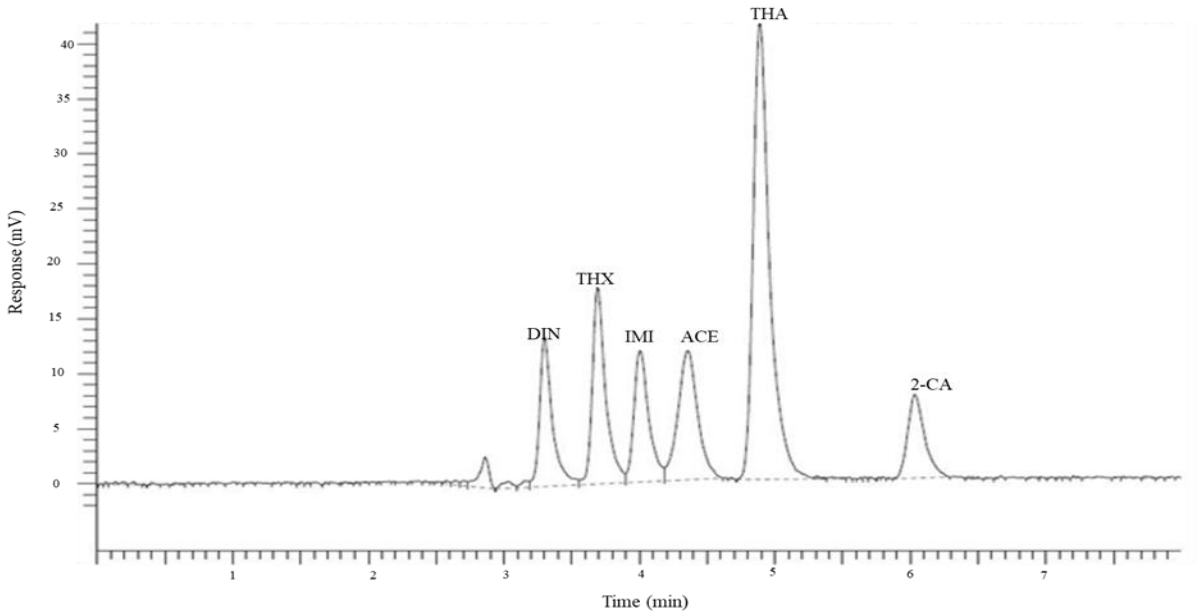


Figure 4B. The separation of DIN (3.21 min), THX (3.51 min), IMI (3.71 min), ACE (3.95 min), THA (4.30 min) and 2-CA (5.18 min) at 15 µg/g concentration. The detection was at wavelength 244 nm, 1 mL/min flow rate and the mobile phase used was 55:45 % (methanol: 0.1 % formic acid in water)

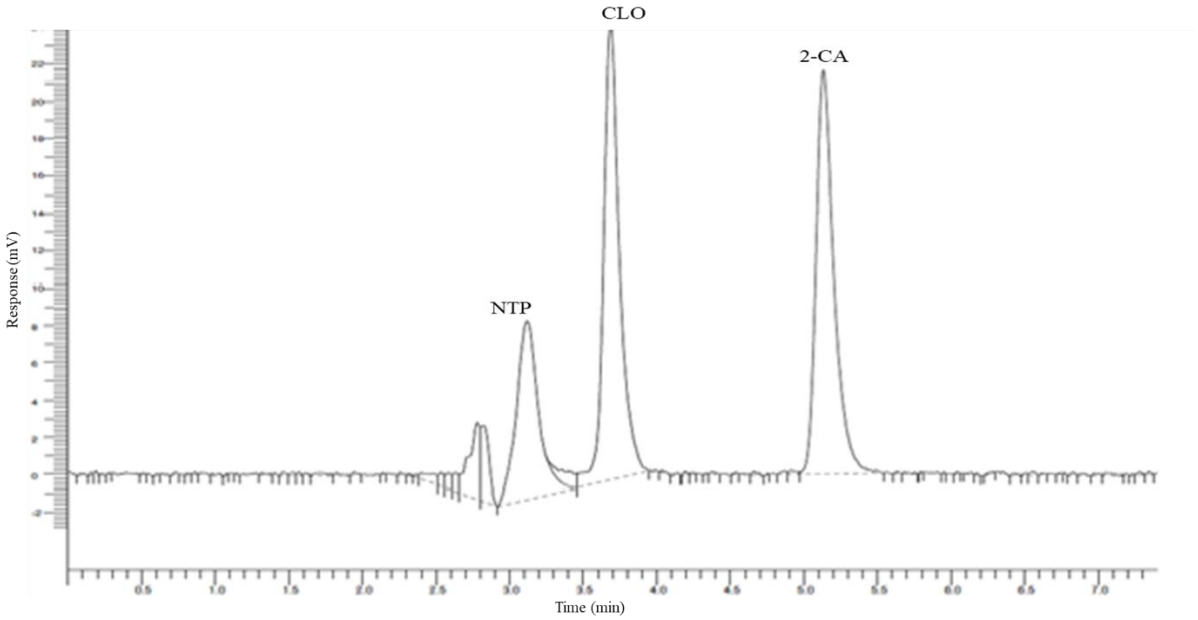


Figure 4C. The separation of NTP (3.12 min), CLO (3.69 min) and 2-CA (5.13 min) at 15 µg/g concentration. The detection was at wavelength 244 nm, 1 mL/min flow rate and the mobile phase used was 55:45 % (methanol: 0.1 % formic acid in water)

3.3 Optimisation and detection sensitivity of the LC-MS/MS method

3.3.1 LC-UV adaptation and optimisation of neonicotinoids with LC-MS/MS

The LC-UV method developed for all the compounds after their separations with the optimal working conditions were adapted to the LC-MS/MS for better sensitivity. A good chromatographic separation of the four compounds (thiamethoxam, imidacloprid, acetamiprid and thiacloprid) including the internal standard (2-chloroaniline) was achieved at 0.001 µg/g in less than 8 minutes without co-elution (Figure 5A) under gradient elution flow. The gradient elution flow used had the following parameters; mobile phase was methanol (solvent A) and 0.1 % formic acid in water (solvent B) under a gradient condition of 0 – 2 min, 10 % solvent A, 2 - 6 min, 10-50 % solvent A, 6 – 9 min, 50 % solvent A, and return to initial conditions in 4 min, 5 min post run delay. However, the optimisation of the compounds in the mass spectrometer was carried out individually at the concentration of 5µg/g. The process of optimisation was carried

out by auto injection of the individual standards into the mass spectrometer without the use of a column (infusion).

The optimised parameters are ESI in the positive mode, fragmentor, gas temperature (GT), dwell time (DT), multiple reaction monitoring (MRM), full scan, nebulizer (NEB), cell voltage (CV), collision energy (CE), gas flow (GF) and mobile phase flow rate. The DT values optimised for all the compounds were at 90s, 100s, 120s and 150s. The optimal value selected for the DT for all the compounds, after optimisation, was 100s due to the average number of scan points acquired for every chromatographic peak greater than 24 for all the compounds. All other parameters were subsequently optimised at DT of 100s. Other parameters optimised were gas temperature (GT) at 300, 325 and 350 ºC; gas flow (GF) at 10 and 12 L/min; and nebulizer (NEB) at 25, 30 and 50 psi. The results of the sensitivity of the optimised parameters, as reflected in the LOD and LOQ estimated by signal-to-noise ratio of 3 and 10 respectively from the instrument response, are shown in Table 10.

Nebulizer at 30 psi produced the least sensitivity results for all the compounds and the internal standard. All the compounds produced the highest sensitivity at NEB of 25 psi. There was, however, not much difference between NEB at 25 and 50 psi (Table 10). The sensitivity of the internal standard was far better in the NEB at 50 psi, therefore, NEB at 50 psi was chosen for the analysis. The best optimised method for all the compounds with the highest sensitivity was reported as DT 100 s, GT 325 ºC, GF 12 L/min and NEB 50 psi.

3.3.2 Sensitivity and reproducibility in LC-MS

The calibration standard mixture at 7 levels; 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 µg/g for all the study compounds with the internal standard 2-chloroaniline (2-CA) at 0.6 µg/g was prepared and analysed with the optimised LC-MS and LC-MS/MS method. The choice of the IS

was due to its similarity with the study compounds in structure and it had distinct elution time and did not react with the study compounds.

The quality parameters LOD, LOQ, repeatability, reproducibility was assessed with standards at 0.001 µg/g and 0.005µg/g as follows: LOD and LOQ were estimated at a signal-to-noise ratio of 3 and 10; respectively. Repeatability and reproducibility were assessed from the injection of the mentioned standards on 6 repeated analyses during the same day; and 2 analyses on 3 non-consecutive days, respectively (Table 11). Quality controls at concentration 0.05 µg/g were run every 6 samples.

A triple quadrupole LCMS/MS method, with acquisition in multiple reaction monitoring (MRM) mode, including the precursor ion, product ions and optimised collision energy was developed (Table 6). According to EU identification criteria (Commission Decision 2002/657/EC, 2002), two MRM transitions (Figure 5B) and their relative abundance ratio are enough to achieve identification of the set target compound. Therefore, the ratio of the calculated results for the two transitions monitored in the MRM were within the required 70 to 130% range for the positive identification of a target compound. However, the transition with the greatest abundance (Table 6) was used for quantitation while the other transition was for confirmation.

Table 9. The instrumental quality parameters in the analysis of the study compounds in standards. UV detection was carried out at 240 nm. LOD and LOQ determined at 500 µg/kg concentration. Repetitivity and reproducibility have been determined by analysis of 500 µg/kg six times (repeatability) and over three days (reproducibility).

LC-UV quality parameter				
Compound	LOD (µg/L)	LOQ (µg/L)	Repetitivity (%)	Reproducibility (%)
Dinotefuran	19.5	64.9	13.2	15.3
Imidacloprid	25.1	83.8	5.0	16.9
Acetamiprid	25.0	83.3	14.5	21.0
Thiacloprid	19.8	66.1	4.1	4.6
Thiamethoxam	20.8	69.4	6.6	7.6

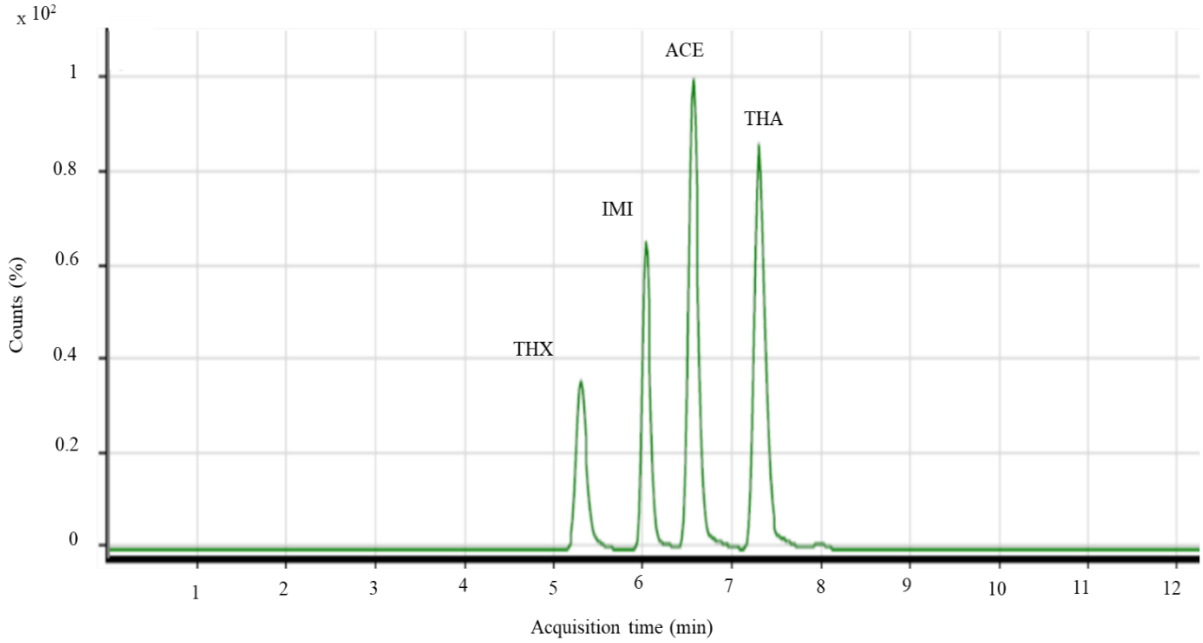


Figure 5A. Total ion chromatogram of the four compounds; thiamethoxam (THX); imidacloprid (IMI); acetamiprid (ACE); thiacloprid (THA) analysed with LC-MS/MS at 1µg/L. The method was developed in section 3.3.1

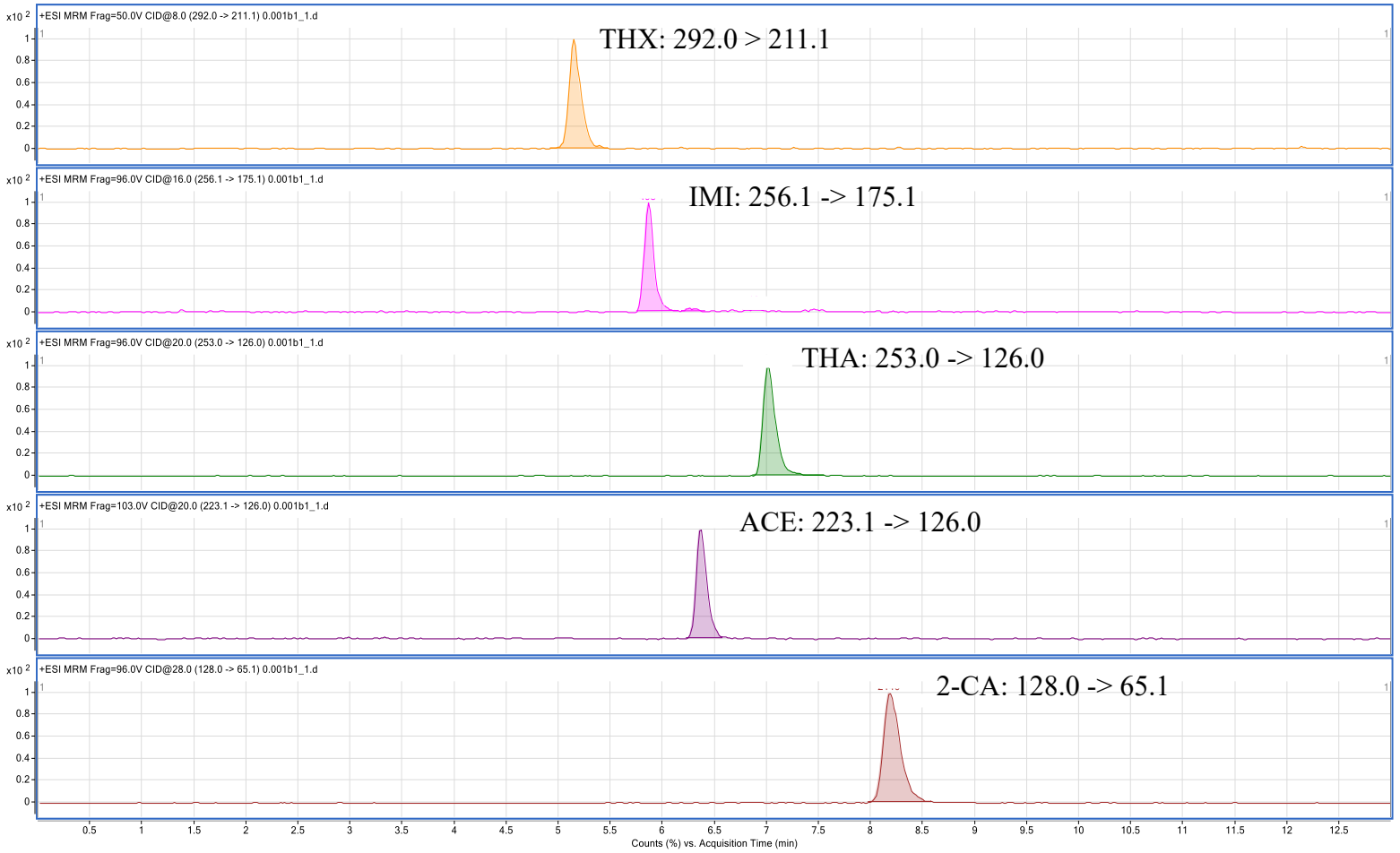


Figure 5B. Total ion chromatogram of 4 compounds and internal standard (2-CA) and their most abundant ions used for confirmation. The method was developed in section 3.3.1

Table 10. The sensitivity of the optimised parameters, gas temperature (ºC), gas flow (L/min), and nebulizer (psi) for the selected neonicotinoids using signal-to-noise (S/N) ratio 3 and 10 for LOD (µg/kg) and LOQ (µg/kg) respectively at 100 µg/kg concentration level

Parameters optimised	Levels	THX		IMI		ACE		THA		IS	
		LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
GT	**300**	0.11	0.40	0.17	0.58	0.05	0.17	0.05	0.18	44.02	146.74
	325	0.09	0.31	0.16	0.53	0.05	0.16	0.05	0.16	46.88	156.25
	350	0.19	0.64	0.31	1.05	0.09	0.29	0.08	0.27	73.06	243.52
GF	**10**	0.12	0.39	0.18	0.61	0.08	0.25	0.08	0.26	47.75	159.17
	12	0.10	0.34	0.16	0.53	0.05	0.16	0.04	0.15	40.75	135.85
	25	0.06	0.19	0.12	0.39	0.03	0.11	0.03	0.11	51.51	171.7
NEB	**30**	0.11	0.35	0.16	0.52	0.05	0.16	0.04	0.15	39.71	132.35
	50	0.09	0.33	0.15	0.50	0.04	0.14	0.04	0.13	32.96	109.87

Table 11. The LC-MS/MS Quality parameters in the analysis of the study compounds in standards. LOD and LOQ have been determined Repetitivity and reproducibility have been determined by analysis of 1 and 5 µg/kg (n = 6) and over three days (reproducibility).

Quality parameters assessed	Concentration level (µg/kg)									
	1					5				
	THX	IMI	ACE	THA	DIN	THX	IMI	ACE	THA	DIN
LOD (µg/kg)	0.20	0.23	0.11	0.10	0.11	0.92	0.99	0.50	0.50	_a
LOQ (µg/kg)	0.68	0.78	0.38	0.34	0.35	3.08	3.30	1.68	1.68	_a
Repetitivity (%)	7.23	7.81	9.54	4.62	_a	11.41	3.74	7.06	11.19	_a
Reproducibility (%)	16.59	13.33	11.66	6.63	_a	14.64	5.33	25.73	13.12	_a

-[a] not determined

3.4 Sample treatment, extraction and detection sensitivity

3.4.1 Sampling and sample preparation

3.4.1.1 Plant flowers and wheat samples

Various cropping histories were noted in the selected fields namely Plumpton, Betchworth, Kiddington, North Weald Bassett, Balcombe and Enysford, with winter wheat, spring barley and oilseed rape being the main crops. The fields were selected, based on collected witness information around the farms, to have been treated, either as seed-treated or foliage spraying, with a range of pesticides including neonicotinoids.

Wheat grains, plant flowers from the foliage of oilseed rape (OSR) and different wild plants of about 0 - 5 metres away from the farmed field were collected during the blossoming period (May – June 2018). All the samples were obtained from selected conventional farm sites located in Plumpton (based on previous research studies on the amount of pesticides exposed to honey bees in the area by David *et al.* 2015), Dorking, Balcombe, Oxford and Essex; mostly in Southeast of England, UK. The samples were collected in the sampling bags and transported to the laboratory where they are prepared for analysis.

The flower parts of daisy plants and white clover (Figure 6) were carefully removed by hands while winter wheat grains were dehusked and air-dried at room temperature in the dark within 3-4 days to a moisture content < 5% to ensure uniformity of sample weight. All dried samples were stored in the freezer at - 20 $^{\circ}$C prior to pesticides extraction and their analysis.

Site 1 Flower 1 (S1F1) - White clover - *Trifolium repens*

Site 1 Flower 2 (S1F2) - Daisy – *Bellis perennis*

Figure 6. Images of the sampled flowers of selected plants with no know pesticide contamination history in Plumpton farm, East Sussex.

3.4.1.2 Honeybees and beeswax samples

Live bees and their waxes were obtained from a beehive housed on private land in Braintree, Essex for which research permission was granted by the owners. Therefore, this study did not involve endangered or protected species. The bees and bee wax were collected in a sterilised container and were transported to the laboratory under a seal tight sampling bag. Also, dead honeybees and the beeswax were collected form a beehive in Kingston Hill, Surrey with the same sampling conditions as described above. The two sampling sites (Braintree and Kingston) are in the South East of England. All the samples were refrigerated at - 20 $^{\circ}$C prior to their analysis.

3.4.1.3 Sampling and pre-treatment of soils for the extraction of neonicotinoids

The sampling and pre-treatment of soils used in the sorption experiment and leaching studies in this work were as described in section 2.4. Also, the sampling locations and their land use were reflected in Table 7 while the characterisation of the soils is shown in Table 15.

3.4.2 Extraction and clean-up for the analysis of neonicotinoids in wheat and marginal plant flowers and its application.

3.4.2.1 Extraction and clean-up recovery of neonicotinoids in wheat and marginal plant flowers

A previously developed method by López-Fernández *et al.*,(2015) on the analysis of neonicotinoid residues from only one sample matrix (dietary bee pollen) was modified for better sensitivity and for wider application to multiple residues of neonicotinoids from several sample matrices. The application of QuEChERS method of extraction and cleanup procedure in the field of chemical analysis from several matrices has been widely accepted (Dankyi et al., 2014, Calatayud-Vernich *et al.*, 2016, Suganthi *et al.*, 2018, Barbi et al., 2019). Currently, the use of QuEChERS methods with varying modifications take between 35 min to 1 hr from

extraction to analysis of sample, and it is usually limited to 1 – 3 sample matrices with large sample size and solvent amount leading to environmental waste.

Therefore, a fast and cost-effective method that can detect residues of neonicotinoids from multiple sample matrices with minimal or no adjustment is desirous. Also, modification of QuEChERS method in the extraction of pesticides from varying sample matrices with smaller sample sizes have been successful (Gómez-Ramírez *et al.*, 2012, Taliansky-Chamudis *et al.,* 2017). Therefore, developing methods that can be applied in the analysis of neonicotinoid residues from multiple environmental samples of complex matrices was a priority in this work.

QuEChERS procedure was initially developed and applied in analysis of analytes in low fat matrices (anastassiades *et al.,* 2003). However, with varying modifications performed on the method, the analysis of analytes from matrices high in fat contents have been reported with good recoveries (choi *et al.,* 2015 and harmon *et al.,* 2013). Also, the use of different buffers like acetate and citrate have been made possible due to its great flexibility (koesukwiwat *et al.,* 2010). Therefore, the flexibility of the QuEChERS method in the analysis of pesticides from varying environmental samples to achieve the desired results is made possible.

Prior to the quantification of neonicotinoids in all the samples, the extraction efficiency of the method was assessed by using different but related spiked uncontaminated samples as blank sample. Samples of flowers of two different marginal plants from uncultivated site (Site 1) in Plumpton, with no recorded history of pesticides application were used as blank. The samples were labelled first and second flower samples from site 1 as S1F1and S1F2 respectively. Also, organic wheat grains with no pesticides was locally sourced and used as blank sample.

The physicochemical properties of the neonicotinoid insecticides to be determined, being polar compounds, and possible signal interference from the sample matrix, were considered in the choice of sample size and centrifuging for the extraction step. Individually, 5 g, as previously

reported in the adapted method (López-Fernández *et al.,* (2015), of each samples of homogenised plant flowers S1F1 and S1F2, was weighed into 50-mL of polypropylene centrifuge tube and labelled "S1F1" and "S1F2" respectively. The content of the centrifuge tubes was spiked with 0.75 mL of the mixture of 1.0 µg/g concentration each insecticide. The spiked sample was allowed to stand in the dark for 24 hours to be stabilised. Thereafter, 10 mL, each of hexane, acetonitrile and water, was added in turn and this was followed by the addition 6 g and 3 g of anhydrous magnesium sulphate and sodium acetate salts respectively. The addition of the two salts was to provide the phase separation of the sample mixture (Anastassiades *et al.*, 2003, Dankyi *et al.,* 2014). The mixture was vigorously vortexed for 1 min, sonicated for 20 min and shook with rotatory shaker at 200 Hub/min for 30 min. The homogenised sample was centrifuged (2264 x *g* for 10 min at 10 ºC).

In order to assess the partitioned liquid phase (hexane, acetonitrile and water) with most recovery of neonicotinoids, 6 mL of each of the solvent layer was carefully separated into SPE Supelclean™ Envi-Carb II/PSA cartridge, initially preconditioned with 6 mL of acetonitrile. The cartridge was thereafter rinsed with a further 3 mL of acetonitrile and the leachates were collected and pooled together and were evaporated to dryness under liquid nitrogen. The dried residue was reconstituted in 0.75 mL of the mobile phase solution with addition of 0.6 µg/g concentration of the internal standard, 2-chloroaniline (2-CA). All the samples were filtered through a 0.22 µm PTFE filter prior to their injection into LC-MS/MS. Each experiment was carried out in triplicate.

Acetonitrile is readily miscible with water but not with hexane. Therefore, acetonitrile is regarded as disperser solvent in extraction process and commonly used in the pesticide extraction and analysis methods (Primel *et al.,* 2017). The extraction of polar compounds such as neonicotinoid is favoured in polar solvent such as acetonitrile. However, the addition of salts (salting-out)

promotes phase separation; this makes neonicotinoids to be less soluble in water and, hence, they are extracted in the acetonitrile phase of the mixture.

The effect of sample size on the recovery efficiency of the modified extraction method was assessed further with sample size 2.5 g following the extraction procedure earlier described. The extraction method at sample sizes of 5 g and 2.5 g produced poor recovery between 4.96 – 8.10 % and 6.69 – 18.47 % respectively for the samples (Table 12). Further assessment of the extraction recovery at sample size of 2.5 g was carried out with several optimising steps to improved recovery as shown in Table 13. Poor recovery between 0.002 and 0.3 % for were recorded for the samples left in the sample vials and the cartridge after their analysis. The pesticides were not left in the cartridge after clean-up and in the sample vials after dryness when they were reconstituted and analysed with no signal detected. Therefore, it was concluded that a large amount of the pesticides, > 99 %, was still in the sample in the extraction tube after the first centrifuge process. The extraction process was repeated by centrifuging the extract in the tube two more times and percentage recoveries of the pesticides were improved 27 – 36 %, 23 – 86 % and 19 – 68 % for S1F1, S1F2 and wheat respectively (Table 13).

Therefore, to have a good recovery for the pesticides from the complex matrices, the extraction was repeated at a lower sample size (1.0 g) and the extraction tube was centrifuged twice prior to being followed by clean-up. The extraction recovery of the compounds from selected marginal plant flowers (S1F1 and S1F2) and wheat grains ranges from 72.24 – 102.67 % (Table 14), after optimisation of the extraction method. The average extraction recovery (%) for each of the pesticides in the three sample matrices (S1F1, S1F2 and Wheat grains) in table 14 for thiamethoxam, imidacloprid, acetamiprid and thiacloprid were in agreement with their corresponding log K_{ow} (Table 1). This explains why thiacloprid, with the highest log K_{ow}, has

the lowest average extraction recovery % due to its strong affinity for non-polar environment of the matrix.

Table 12. Initial extraction recovery assessment of four neonicotinoids in blank of sample matrices

Sample	Sample size (g)	% of recovery			
		THX	**IMI**	**ACE**	**THA**
S1F1	5	5.87	5.39	4.96	5.19
S1F2	5	7.45	8.1	7.41	7.32
S1F1	2.5	6.69	8.85	7.81	9.14
S1F2	2.5	17.49	18.47	16.97	17.71

Table 13. Extraction recovery efficiency assessment of the four pesticides in blank sample matrices at 2.5 g sample size

Extraction steps to improve analyte recovery and consequent % recovery					
Sample	**Extraction improvement steps**	**% recovery**			
		THX	**IMI**	**ACE**	**THA**
S1F1	after washing the sample vial only and analysis	0.08	0.67	0.36	0.49
S1F2	after washing the sample vial only and analysis	0.14	0.88	0.55	0.64
S1F1	after 2nd Cartridge vial wash	0.02	0.15	0.08	0.06
S1F2	after 2nd Cartridge vial wash	0.04	0.30	0.12	0.12
S1F1	after 2nd Cartridge vial wash and vial wash	0.002	0.06	0.03	0.02
S1F2	after 2nd Cartridge vial wash and vial wash	0.01	0.19	0.04	0.04
S1F1	after 2nd Extraction tube centrifuge again	24.47	27.43	17.68	21.79
S1F2	after 2nd Extraction tube centrifuge again	53.64	35.45	19.94	29.04
S1F1	after 3rd Extraction tube centrifuge again	36.32	32.58	28.62	26.74
S1F2	after 3rd Extraction tube centrifuge again	87.57	50.21	30.15	23.25
Winter wheat	extraction tube centrifuge repeated thrice	67.51	54.06	33.55	18.53

Table 14. The final recovery of the neonicotinoids at 1.0 ug/g concentration from 3 matrices at 1 ± 0.0001g of sample as explained in section 3.4.2.1

Sample name	**% of recovery for the neonicotinoids**			
	THX	**IMI**	**ACE**	**THA**
S1F1	92.81	95.94	75.46	72.24
S1F2	102.67	88.91	79.78	72.60
Wheat grains	85.74	88.05	84.71	75.12
Average recovery in 3 sample matrices	93.74	90.97	79.99	73.32

Interestingly, acetonitrile is the simplest organic nitrile that is possible. However, the vast use of acetonitrile as solvent for different polar compounds is in the public knowledge. The structure of acetonitrile showed that the central carbon atom contains a triple bond to the nitrogen atom, therefore, the bond angle between the methyl group carbon, the central carbon atom, and the nitrogen atom is a perfect 180 degree. Because nitrogen is much more electronegative than carbon, therefore, this results in an unequal sharing of the electrons with partial charges. This behaviour enables acetonitrile, classified as polar compound, to accommodate the divergent properties of the study compounds in this work with varying polarities and complexities of their sample matrices.

From the assessment, acetonitrile (Figure 7) was found to recover better all the insecticides in all the matrices with recovery percentage ranges from 75.24 - 93.85 % in S1F1; 72.27 - 101.18 % in S1F2 and 79.37 - 88.76 % in wheat grain while hexane and water solvent produced poor recoveries, approximately < 5 % in each solvent. The extraction recovery of all the pesticides in other matrices (honeybee, bee wax and OSR) gave 75.28 - 85.36 %, 85.21- 94.78 and 83.0 – 98.6% respectively. The results of the adapted method showed better sensitivity with good recovery of the analytes and the application of the method in the extraction of neonicotinoids in more than one matrix is possible.

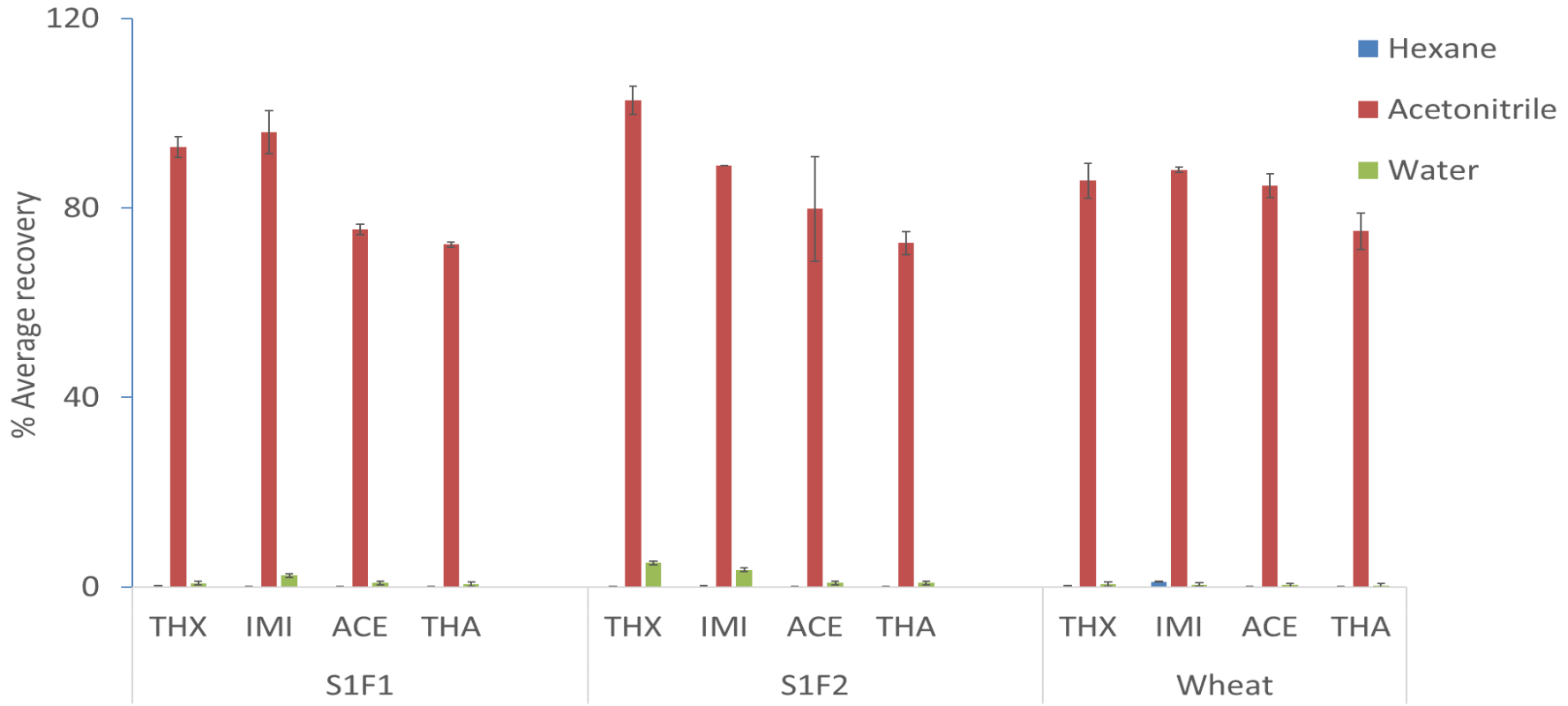


Figure 7. Extraction recoveries assessed for the four neonicotinoids in 3 solvent phases used during sample clean-up: hexane, acetonitrile and water from three sample matrices: S1F1 (site 1 Flower 1), S1F2 (site 1 flower 2) and wheat.

3.4.2.2 Application of developed method to the analysis of neonicotinoids in environmental samples

The application of the developed method in the analysis of neonicotinoid residues in six sample matrices namely; cropped and marginal plant flowers, wheat grains, honeybee, bee wax and soils were described in section 4.3.2.2.

3.4.3 Effect of the soil matrix (suppression/enhancement) on the detection of neonicotinoids by LC-MS

To study the effect of the soil matrix on the analysis of the study compounds in the sample leachates with LC-MS, 0.1 mL of 1.0 µg/g concentration of an individual known standard (thiamethoxam and thiacloprid), was added to 0.9 mL de-ionised water as the control and represented as (A) in the figure 8. Known amount of (A) in the order of 20, 30, and 40 % were added to the Y % (80, 70, and 60 %) corresponding amount of methanol, labelled as (B), to make up to 100 % (1 mL) in volume. The resultant mixture was centrifuge at 2264 x *g* for 10 minutes at 20 °C. Thereafter, the supernatant was decanted and filtered with 0.22 µm PTFE filter prior to analysis. The procedure above was thereafter repeated with drained water, taken from the first pore volume without contamination as mixture (A), as earlier stated and the result was compared with the spiking by ultrapure water instead.

The matrix effect of the BR soil leachates on thiamethoxam, after the addition of 60 % methanol to 40 % of spiked drained leachates produced about 2.2% ion suppression. In contrast, 80 % methanol to 20 % of spiked drained leachates was best for thiacloprid in BR soil leachates producing about 10.3 % ion enhancement. TH soil leachates produced 27.4% enhancement with thiamethoxam in 40:60 leachate/ methanol and 10.1 % suppression was obtained with thiacloprid with the same leachates: methanol ratio.

The reduction in the BR matrix effect on thiamethoxam may be due to its low organic carbon content (Table 15) and, thus, contributing low signal interference. However, in contrast, TH soil is high in organic content and may reduce or increase the signal with high presence of co-extract in the matrix such as humic acid, fluvic acid etc..(Zhou *et al.*, 2018). All the suppressions and enhancement results were considered as correction factors in all the final column soil leachates calculations.

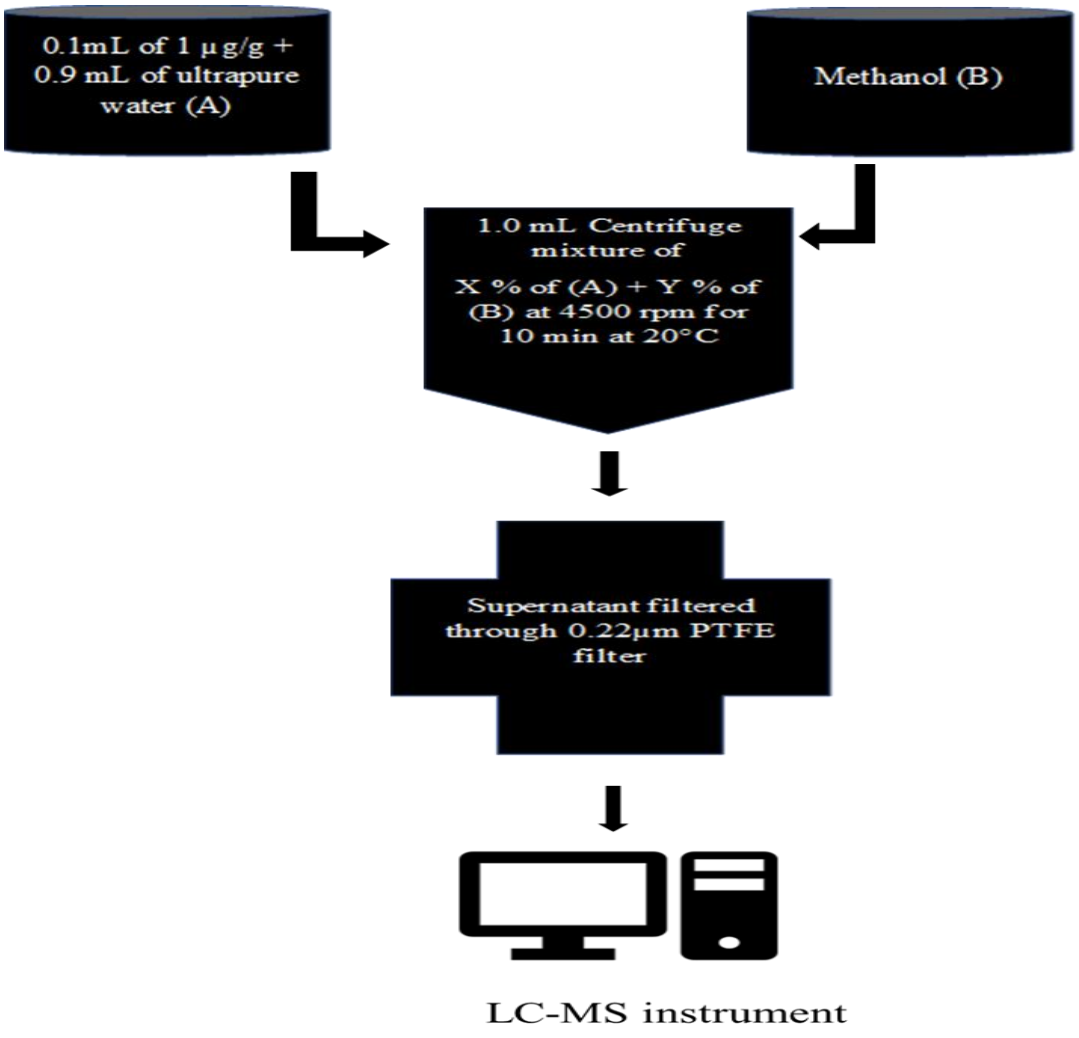


Figure 8. Schematic diagram of testing the removal of macromolecules in soil leachates by different amount of methanol (%) to measure ion suppression in the electrospray for individual neonicotinoids.

3.5 Conclusions of chapter 3

The nature of the physicochemical properties of all the study compounds play a significant role in their separations and optimisation. In particular, the relatively close molecular weight, high polarity and low Log K_{ow} posed some affinity challenges as reflected in the similar retention times observed; some of the compounds co-eluted prior to improving the process to obtain their optimal separation. The separation of the five compounds was in less than 7 min on the LC-UV with a run time of 8 min. The separation of the compounds was improved with better peak separations in 8 min in the LC-MS with a run time of 13 min under gradient elution.

The instrument sensitivity of the compounds analysed with LC-UV and LC-MS for improved sensitivity was assessed using signal to noise ratio of each peak response to determine quality control parameters like LOD, LOQ, repeatability and reproducibility. The values of 19 – 25 µg/kg LOD and 65 – 84 µg/kg LOQ were achieved for all the compounds in LC-UV analysis with repeatitivity and reproducibilities of < 14.5 and < 21 % respectively. In the LC-MS analysis, the values of 0.10 – 0.23 µg/kg and 0.5 – 1 µg/kg LODs were obtained at concentration levels 1 and 5 µg/kg respectively for all the study compounds while the values of 0.34 – 0.78 and 1.68 – 3.3 µg/kg LOQs, at the same levels of concentration for all the compounds. Repeatitivity and reproducibility were < 11.4 and < 25.7, at the two levels assayed for all the compounds.

The best extraction solvent for the recoveries of the neonicotinoids in the present study was acetonitrile when compared to other solvents, hexane and water. The recoveries values, 72 – 101 %, was found in acetonitrile while poor recoveries, less than 5 %, was reported in hexane and water. The sensitivity of the quality parameter is sufficient for the analysis of neonicotinoids in flowers and soils according to values previously reported in the literatures (Jiang *et al.* 2018, Daniele *et al.*, 2018). Suppression/enhancement of the soil extract were measured prior to all the neonicotinoids analysis. The values of 2.2 – 10.3 % and 10.1 – 27. 4 % for suppression and

enhancement respectively were recorded for all the compounds and were considered in calculations for all the experiments.

The extraction method was successfully adapted from its application to only dietary bee pollen (a matrix) to seven matrices (cropped and marginal plants' flowers, wheat, honeybees, bee wax, bee comb and soils) as shown in section 4.3 in this study with better sensitivity in the LOD and LOQ ranging from 0.22 – 1 and 0.74 – 3.33 µg/kg respectively. The choice of lower sample size (0.5 and 1 g) and repeating the centrifugation process (n=3) improved the extraction of the analytes into the acetonitrile solvent phase than the other solvents (water and hexane).

CHAPTER 4: FATE AND DISTRIBUTION OF NEONICOTINOIDS IN SOIL, WATER AND AGRICULTURE

4.0 Fate and distribution of neonicotinoids in soil, water and agriculture

4.1 Fate of neonicotinoids in soil (sorption and leaching)

4.1.1 Introduction to the study of the sorption and leaching of neonicotinoids in soil

Soil definition is generally perceived to be difficult due to its multifunctionality; soils serve different purposes to agronomists, farmers, builders, geologists, engineers among others. Historically, many definitions have been proposed to describe soil, however, Certini *et al.*, (2013) defined soil as a centimetric or thicker unconsolidated layer of fine-grained mineral and/or organic material, with or without coarse elements and cemented portions, lying at or near the surface of planets, moons, and asteroids, which shows clear evidence of chemical weathering.

Soils have been largely accepted from the viewpoint of agronomist as the medium through which plants are grown. The vastness of different types of soils naturally available for farmers have been harnessed as potential to produce various types of crops for human and animal consumptions; therefore, soils have been an integral part of ecosystem for its sustainability. However, the needs to increase and improve crops yields to constantly meet the challenges of population growth, especially in the face of militating agent such as pests, has necessitated the use of pesticides in controlling these pests.

Jayaraj *et al.*, (2016) reported that about 0.3 % of the applied insecticides go into the target pests while about 99.7 % disappeared into the environment. Therefore, the continuous use of insecticides has potential to raise their levels in the environment, particularly soils, with risk to

the ecosystem. Soils were initially regarded as inert materials but the interaction or exchange of chemicals between the surrounding environment (air, water, and liquid) and soils have been described as sorption (Sposito, 2008). Sorption can either be described as adsorption or desorption depending on the physiochemical properties of the compounds (sorptives) and the nature of the surrounding environment (sorbents).

The sorptive, that is the chemical that is sorbed to the soil (sorbent), may be categorised as; (i) anionic (i.e. negatively charged due to more electrons than protons), (ii) cationic (i.e. positively charged due to fewer electrons than protons), and (iii) uncharged organic sorptives exhibiting a range of polarities (i.e. non-polar to polar based on the distribution of electrons across the molecules). The major solid phase materials in soils are; (i) silicate clays layers, (ii) metal-(oxyhydr)oxides, and (iii) soil organic matter. In soils, the silicate layers are primarily negatively charged and represent the largest source of negative charge. The metal-(oxyhydr)oxides are variably charged due to their surfaces becoming hydroxylated when exposed to water and thereafter assuming anionic, neutral, or cationic forms based on the degree of protonation which is influenced by the pH of the solution (Thompson *et.al.*, 2012).

The soil organic matter, SOM, consists of the living and partially decayed non-living materials as well as the aggregation of biomolecules and products of transformation of organic residues decay called humic substances. The presence of several reactive sites like anionic hydroxyls (R-OH), carboxylic groups (R-COOH), cationic sulfhydryl (R-SH), and amino groups (R-NH_2), as well as the un-charged and non-polar regions such as aromatic (-Ar-) and aliphatic moieties ($[-CH_2-]_n$) on the soil organic matter are known to play an important role in determining the mobility and bioavailability of both organic and inorganic trace components in the environment.

The nature (size and charges) of soil organic matter and clay minerals as well as temperature, solution pH, and type of pesticides and their concentration are known to determine the soil-water distribution of most pesticides. This is reflected in measurements and interpretations of pesticide soil-solution distribution coefficients (K_d) which indicates the sorption capacity of the chemical by the required soil. Therefore, the measure of K_d values provide information on the mobility and environmental fate of the chemicals (Weber *et al.,* 2004). Another key parameter that influences the transport and mobility of chemicals in soil is the measurement of the fraction of organic carbon content of soil or sediment (f_{oc}). In understanding the fate of chemicals in the environment, K_d values are highly correlated to the f_{oc} of soil or sediment as organic carbon/water (K_{oc}) partition coefficients with exclusive sorption to soil organic carbon content (Franco and Trapp, 2008)

Adsorptions of neonicotinoids and fipronil were enhanced by organic matter and clay mineral content while desorption from soil was reduced by lower temperature and pesticides concentration (Bonmatin *et al.,* 2014). This behaviour, in the presence of organic matter, contributed to their reduced mobility in soil and may be due to the presence of hydrophilic bonding on functional group of the pesticides binding with the phenolic hydroxyl and carboxylic acidic group of the soil organic matter (Bonmatin *et al.,* 2014). This explains their distributions in many water bodies due to their metabolites binding to sediments of several freshwater and marine water bodies (Bonmatin *et al.*, 2014). The effect of different amount of organic carbon content of soils on pesticides sorption mechanism was however yet to be reported. However, fipronil metabolites (desulfinyl, sulfide and sulfone) exhibited different sorption coefficients in which sulfone derivatives witnessed the highest concentration as reported by Ying & Kookana (2006).

Neonics, from laboratory and field studies, may exhibit a wide range of half-lives in soils from 7 to 6931 days. N-nitroguanidine (dinotefuran, imidacloprid, thiamethoxam and clothianidin) have longer DT_{50} than the N-cyanoamidines (thiacloprid and acetamiprid) (Paul, 2016). High water solubility and low K_{ow} with neonicotinoid insecticides means lower tendencies for adsorption to soil particles, rendering the dispersion of the pesticides, and their accumulation, in ecosystems more easily. Thiamethoxam is more water soluble (4,100 $mg \cdot L^{-1}$) than Thiacloprid (180 $mg \cdot L^{-1}$), with lower Log K_{ow} 0.13 when compared to thiacloprid Log K_{ow} 1.26 (Table. 1), these properties being a warning sign for their mobility in the environment.

In spite of highly mobile pesticides due partly to their high solubility, some neonicotinoids have been reported to persist in the environment for many years with their residue being detected in plants many years after their application (Kurwadkar *et al.,* 2013). For instance, the herbicide atrazine (with solubility 34.7 $mg.L^{-1}$ and Log K_{ow} 2.7) is frequently detected in European surface water at levels of 5 - 25 $ng.L^{-1}$ (Hillebrand *et al.,* 2014; Criquet *et al.,* 2017 and Poulier *et al.* 2014) despite its prohibition in Europe since 2004 (European Union, 2004). However, the secondary emission of these pesticides from sources such as sediment and soils are possible because of their re-equilibration among various bulk media (Pan *et al.,* 2019).

Much has been published regarding the sorption of pesticides in a diversity of conditions. However, there is paucity of information on the effect of concentration on their soil adsorption capacity (µg/g). Also, lack of correlation between the amount of insecticides adsorbed and the organic carbon (OC) content has been reported to be attributed to the low values of the OC of the selected soils (Fernández-Bayo *et al.,* 2008), while Zhang *et al.,* (2012), using soil with intentionally and deliberately added organic matter, concluded that soil organic matter was critical to sorption activities of organic chemicals in soil.

Therefore, this discrepancy needs to be further investigated to substantiate the credibility of environmental data important in determining the potential risk of neonicotinoids to both non-target terrestrial and aquatic organisms. Nonetheless, to establish the extent of sorption as a function of organic matter, of the ease of leaching, and of modes of distribution in the soil-water phase are of fundamental importance. These are the focuses of experimental investigation in this work.

Leaching is the downward movement of dissolved chemicals in the soil profile with the percolating water. Chemicals, such as insecticides found in the soil environment after their application, are likely to be rapidly transported through the soil and contribute to ground water contamination in the region with intense agricultural activities. There are hypotheses proposed to explain this transport phenomenon such as preferential flow, co-transport with colloidal matter or a combination of both processes (Vereecken, 2005). However, the rate and magnitude of rapid transport may be determined by boundary conditions, soil properties such as structure, organic matter, clay content, and even farming management including time and extent of application.

Leaching properties of insecticides is known to play a significant role in their temporal and strata distribution in soil. In general, the mobility of pesticides and their risk of leaching have been correlated with a weak adsorption on the soil matrix which is quantified in terms of small soil organic carbon-water portioning coefficients (K_{oc}) values. Leaching can be calculated by Groundwater Ubiquity Score (GUS) using the sorption coefficient (K_{oc}) and the soil half-time (DT_{50}) as $GUS = \log_{10}(DT_{50}) \times (4 - \log_{10}(K_{oc}))$ (Bonmatin *et al.*, 2014).

As shown in Table 1, the leaching potential index for the five neonicotinoids under this study ranges from 1.44 – 4.95 with thiacloprid being the least and dinotefuran the most (AERU, 2018). Evidently, from the same table 1, the insecticides leaching potential index correlated

strongly with their solubilities. The mobility and leaching of neonicotinoid insecticides in amended soils and under different soil conditions have been reported (Gupta, *et. al.* 2008, Kurwadkar *et al.,* 2014 and Rodríguez-Liébana *et al.* 2018). However, to the best of our knowledge, the influence of organic carbon of unamended British soils with divergent characteristics on the soil mobility of neonicotinoids through leaching are yet to be reported anywhere; contributing the answers to this knowledge gap is one of the objectives of this thesis. Also, there is scant information about the metabolites and degradates of these insecticides, since most reports were on the parent compound's residues, which may be more toxic and persist longer in the environment; this, however, needs to be investigated to fully understand their fate in the environment.

Adsorption capacities, sorption kinetics, sorption isotherm, and assessments of leaching potential of the neonicotinoid insecticides were carried out in different soils and models were applied to aid the prediction of their distribution in the soils.

4.1.2 Materials and methods

The studies of adsorption capacities and sorption kinetics of neonicotinoid insecticides in soil were carried out with an HPLC-UV system. LC-MS/MS instrument was used to determine neonicotinoids in isotherm equilibrium and column leaching studies

All the samples were filtered through 0.22 μm PTFE filter (Millex, Millipore, UK) prior to their injection to prevent clogging of the column with particles trapped during sample preparation as well as preserve and prolong the life of the column. Also, the filtering process is to improve sensitivity by removing any peak interference in the chromatogram.

4.1.2.1 Chemicals used

All the chemicals used were described in section 2.1 for all the compounds. The chemicals used for the characterisation of the soils were: Potassium dichromate (0.1667 M); barium diphenylamine sulphonate (0.16 %); ferrous sulphate (1M); standard pH solution 4, 7 and 9; sodium acetate (1 M), ammonium acetate (1 M); ethanol (99% purity), hydrogen peroxide (30 vol. %); concentrated phosphoric acid (85 wt. %), sulphuric acid (96 wt. %); hydrochloric acid (98 wt. %) and 5% (w/v) hexametaphosphate. All the aforementioned chemicals were purchased from Signa-Aldrich Ltd., UK.

4.1.2.2 Soil sampling

To elucidate the distribution mode of neonicotinoids in the soil environment require the use of soils with different properties. Soil samples were collected from different locations, as shown in Table 7, after investigation into their uses. Also, soils that are optimum for arable cropping, with pH within neutral and alkaline were sampled in view of the desired plants' flowers for the analysis. Topsoil, about 0-20 cm, samples were collected from 3 randomly selected spots, 50–100 cm apart, in each selected site using an auger. The soil samples were combined to form a composite sample and labelled with acronyms reflecting the sampling locations (Figure 9). The soil samples were collected in a sampling bag, sealed and transported to the laboratory where they were air-dried for 4 days in the dark and under room temperature. Large lumps were pulverised to ensure consistent and uniform moisture level (< 5%) throughout the soil samples during drying. Plant debris were carefully removed before grinding using a pestle and mortar and the soil sample were sieved, using standard sieves, into different particles sizes for soil characterisation. Soil particles (< 2 mm diameter) were thoroughly mixed, stored and sealed in sample polythene bags prior to their use in experiments.

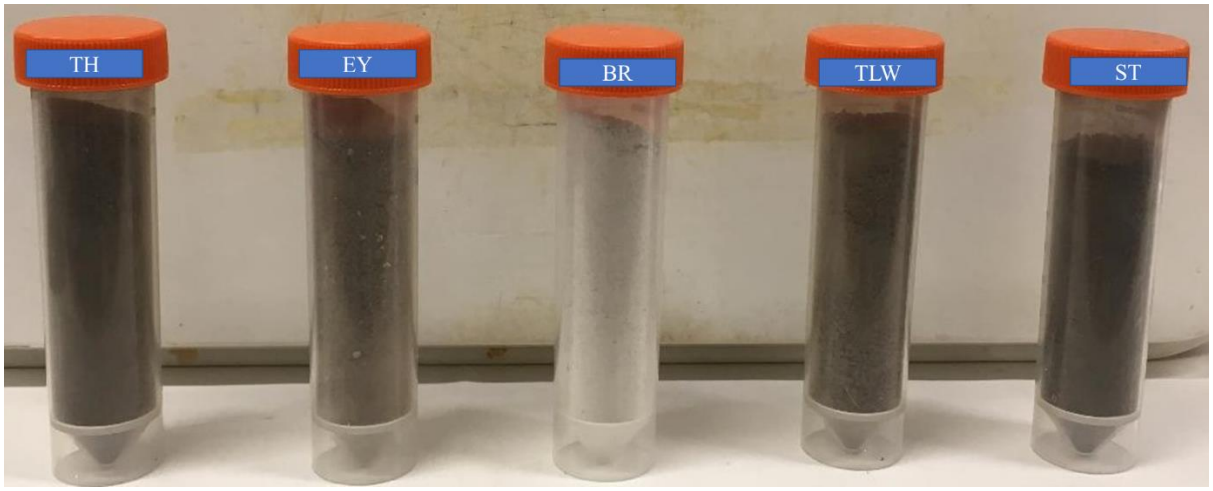


Figure 9. The image of the soil samples used in sorption experiment with varying characteristics obtained from selected locations labelled as TH – Thornton Heath, EY-Eynsford, BR- Brighton, TLW-Tolworth and ST-Stornoway.

4.1.2.3 Soil characterisation

The characterisation of all the soils used for adsorption, sorption kinetics and sorption isotherm experiments was carried out according to ISRIC (2002) and were described in section 2.4.2. The results of the characterised soils are shown in table 15 and a detailed description of the soil characterisation procedures are shown in appendix 10.

4.1.2.4 Separation and detection conditions

The separation and detection for all the study compounds in the experiment in this section were carried out as reported in section 3.2 and 3.3 for the LC-UV and LC-MS respectively. Also, the soil extraction and recovery efficiency for the extraction of neonicotinoids from soil after leaching were reported in section 3.4.2.2 for soil extraction procedure and 3.4.3 for testing effect of the matrices (suppression/enhancement) on the detection of neonicotinoids by LC-MS.

4.1.3 Experimental conditions for the study of sorption, kinetics and leaching

4.1.3.1 Adsorption experiment

Insecticides, at two fortification levels, 2.5 μg/g and 25 μg/g, were independently incubated at 1:5 soil to insecticides solution in a 50 mL polypropylene centrifuge tube in an orbital shaker (Janke & Kunkel, Model HS 500) (100 rpm, 25 ˚C) for 48 hr, expected time to have reached equilibrium, (OECD, 2000). The sample centrifuge tubes were covered with aluminium foil due to the light sensitivity of the insecticides throughout the period of shaken to prevent possible degradation during incubation.

All the soils used in the adsorption studies were previously tested for pesticide levels prior to the experiment by shaking the soil:water solution of 5 g of soil in 20 mL of water for 48 hr in the dark and protected with aluminium foil. The filtered extract, after centrifugation and passing through 0.22 μm PTFE filter, was injected into the LC-MS and showed no presence of chromatographic peak signal.

The supernatants were carefully collected after centrifugation at 2264 x *g* for 10 min at 22 ^{0}C and filtered through 0.22 μm PTFE filters prior to their immediate analysis with HPLC-UV with 2-Chloroaniline (2-CA) as the internal standard. The initial concentration of each neonicotinoid solution was analysed along with a quality control and the blank solution of each soil without the analyte was analysed as a control and to test for possible suppression or enhancement of the pesticides. The amount of pesticides adsorbed was quantified, per mass of soil, from the difference between the amount of pesticide found in the supernatant of each sample (after incubation) and the control solutions where there was not soil. Each experiment was carried out in triplicate.

4.1.3.2 Kinetic sorption and sorption isotherm experiment

In this sorption experiment, the most and least adsorbed neonicotinoids on the soils with the highest and lowest percent organic carbon were assessed at different times and at varying concentrations levels under separate studies according to the standard batch equilibrium method as stated in the Organisation for Economic Co-operation and Development guidelines (OECD, 2000).

For the time dependent sorption kinetics experiment, 4 g of soil samples were placed in 50 mL polypropylene centrifuge tubes and mixed with 20 mL of an aqueous standard solution of the pesticides at 2.5 mg/L in an orbital shaker (25 ^{0}C, 100 rpm). Aliquots (0.18 ml) was taken from the supernatant at each set time for 10, 20, 40 min, and 1, 2, 6, 12, 24, 48, and 72 h. All the tubes were covered with aluminium foil to prevent possible photolytic degradation. A 1.8 mL aliquot was taken from the same sample to ensure that > 90% of the initial solution was maintained throughout the experiment. The sorption isotherm at relatively low concentrations levels, 2 g of soil to 10 mL of pesticides solution at 0.10; 0.25; 0.50; 0.75; 1.0; 1.25; and 1.50 µg/g for 48 h, were assessed on four soils with contrasting SOC at room temperature in the laboratory.

An aliquot from sorption kinetic and sorption isotherm experiments was filtered through a 0.22 µm membrane pore by gravity using micro-centrifuge at 2264 x *g* for 20 min prior to its immediate analysis using HPLC-UV and LCMS/MS respectively. Blank soils without pesticides were used as controls and the initial pesticides solution was analysed as quality controls. The amount of pesticides adsorbed was calculated by the difference between the amount found in the supernatant in each sample at the set times and the control solutions.

4.1.3.3 Leaching column experiment

Soil, less than 2 mm fraction, was packed into a flash chromatograph glass column (4 cm i.d. and 50 cm height) by addition of successive layers of soil to establish uniform bulk density of about 1.1 g/mL (Figure 10). Glass wool was placed at the bottom of the column to avoid soil loss. The soil was pre-wetted with one pore volume of water (175 mL) in order to displace air trapped in the soil pores and, thereafter, the excess water in the soil column was allowed to drain off by gravity. The glass columns, after draining excess water, were covered with aluminium foil to avoid photolytic degradation of the pesticides.

A single 1 mL pulse application of 1000 µg/ml of the standard neonicotinoid solution was evenly applied at the top of the column to obtain a homogenous distribution of the neonicotinoids in the top of layer of the soil column. To avoid disturbance of the soil surface by water droplets, a minimum of 10 cm water-head was constantly maintained while dropping water through the peristaltic pump at a predetermined rate (0.8 mL/min). The soil column was drained using a liquid to solid ratio of 2 L/kg dry matter according to the ISO guideline on soil quality (ISO/TS 21268-1, 2007). The leachates were collected in glass tubes at 60 min pre-set time using a fraction collector. The leachates were mixed with methanol (60:40 methanol/ aqueous leachate) prior to their analysis to precipitate macromolecules in the sample. The methanol-leachate mixture was centrifuged and the supernatant was filtered through 0.22 µm PTFE syringe filter prior to their injection into LC-MS/MS for quantification.

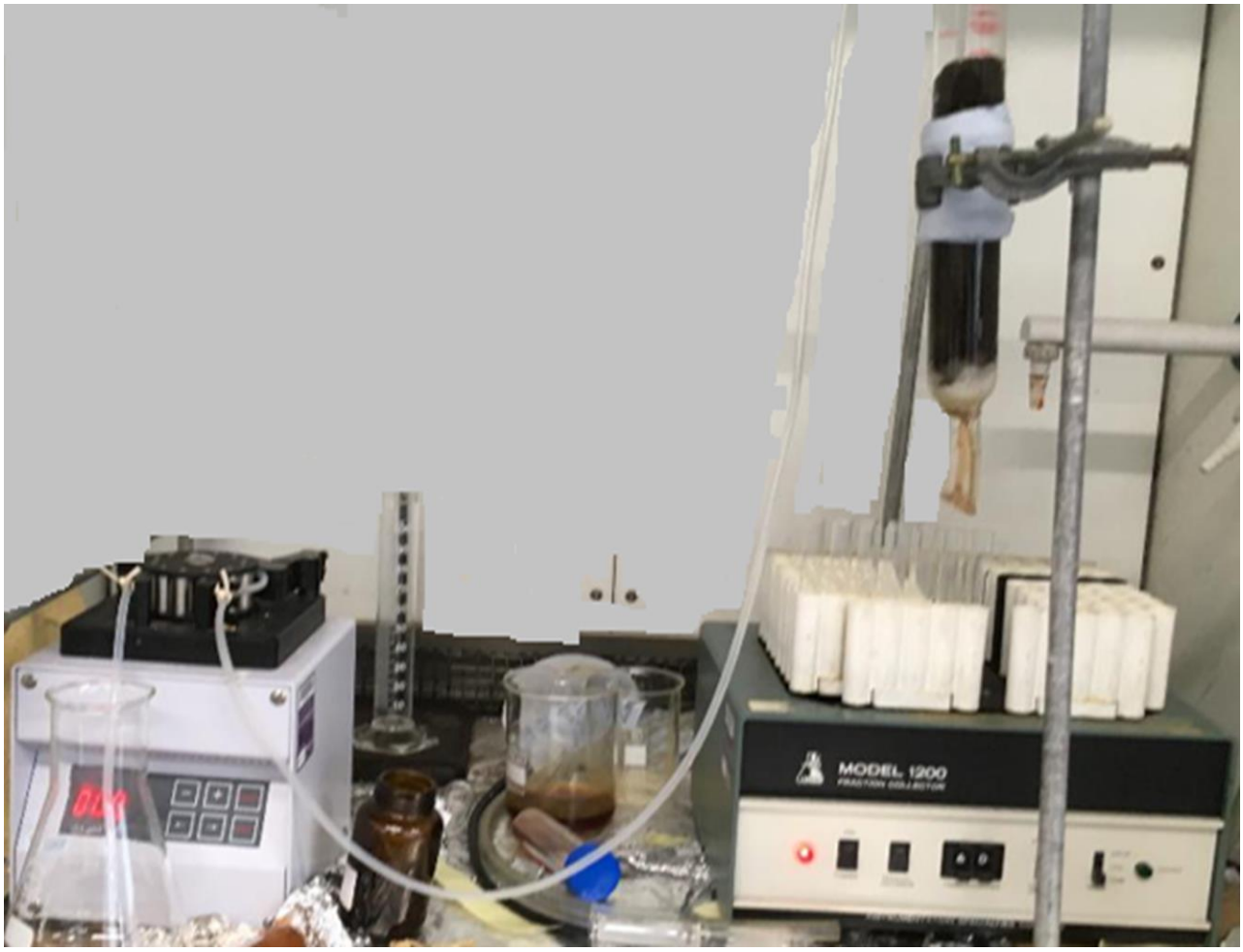


Figure 10. The image of the soil column leaching experiment as set up in the laboratory with the peristaltic pump dropping water at 0.8 mL/min and the fraction collector set to collect the leachates in glass tubes at a pre-set time of 1 hour. To prevent photolytic degradation, after taking this picture and prior to the start of the experiment, the soil column was completely wrapped with aluminium foil and the whole compartment covered with aluminium throughout the experiment.

To assess the effect of the matrix on the analysis of the study compounds in sample leachates with LC-MS, the drained water, taken from the first pore volume without contamination, was spiked with a known concentration of the standards (100 µL of 1 µg/g) as described in section 3.4.3. The result was compared with spiking ultrapure water instead (Figure 8). After leaching was completed, the soil column was drained and carefully divided into three sections (top, 0-5 cm; middle, 5-12 cm; and base, 12-20 cm) and air-dried until constant weight. The dried soil

was finely ground with pestle and mortar and the pesticides residual after the leaching process was determined by extraction, clean-up and LC-MS analysis.

4.1.4 Results and discussion

4.1.4.1 Adsorption capacity

To investigate the effect of organic carbon content of soil on the distribution potential of the study insecticides in soils, the physicochemical properties of the soils were determined. The content of organic carbon (12.5%) for TH soil was highest among the five soils with BR soil the lowest (0.8 %) (Table 15).

In this work, two different concentration levels of the pesticides in soil, at higher levels than the values found in the environment (Jess *et al.*, 2018), were used to simulate a worst-case scenario where, particularly, farmers are likely to apply pesticides at about 30 - 40% higher concentrations than the recommended level (Selvarajah & Thiruchelvam, 2007 and Garthwaite *et al.,* 2016). Neonicotinoid adsorption in 5 soils with adsorption capacities ranged from 0.17 to 11.26 μg/g and from 0.20 to 115.33 μg/g when incubated with 2.5 μg/g and 25 μg/g of each individual neonicotinoids, respectively is presented in Figure 11.

The ANOVA results of the amount of pesticides adsorbed demonstrated that a significant difference occurs among the mean values (n = 3) of the adsorption capacities of the five pesticides in the five soils of contrasting SOC (%), at $\alpha = 0.05$, p-value = 0.005 and 0.0007 at low and high contamination levels tested (see Appendix 7.1, a - d). This suggests that the soil type, particularly the soils rich in organic carbon content, may play a part in pesticides-sorption relationship.

However, assessing the relationship between % SOC and the pesticide adsorption capacities showed a lack of correlation with the insecticides, except for imidacloprid and thiamethoxam following a t correlation test (p 0.05) (see Appendix 7.2, a-b). Also, thiacloprid adsorption

capacities, being the most adsorbed compound, ranged from 5.93 - 10.77 μg/g and 31.93 - 115.33 μg/g at low and high assayed contamination concentrations respectively, while the least adsorbed insecticide, with adsorption capacities ranging from 0.17 – 9.3 μg/g at low and 1.33 – 31.58 μg/g at high concentration, was thiamethoxam (see Appendix 6.1 – 6.5). All the neonicotinoids showed lower affinity for the BR soil, with the lowest %SOC, and the highest for the TH with highest %SOC.

Table 15. Soil characteristics of five soils from different locations in the South East of the UK. The characterisation of the soils was carried out as described in ISRIC (2002).

Soil sampling locations	sand-silt-clay, %	SOC, %	pH (water)	CEC, cmol/kg
BR	31.9- 45.3- 22.8	0.8	8.8	1.4
EY	45.1- 31.5- 23.4	2.6	8.3	5.6
TLW	46.1- 26.5- 27.4	3.2	7.3	10.8
ST	77.9 - 1.5- 21.3	9.2	7.1	14.1
TH	41.0- 37.6- 21.4	12.5	7.1	21.0

Adsorption capacities of the neonics studied, displayed in appendix 6, were in agreement with their individual log K_{ow} values (Table 1) with THX < DIN < IMI < ACE < THA ranging from 0.13 - 1.26. Therefore, soil adsorption of thiamethoxam, with a high solubility in water (4,100 mg/L) did not appear to be influenced by soil organic content, and may be competing with minerals and dissolved organic compounds for binding sites on soils (Jin *et al.,* 2016; P. Zhang *et al.,* 2018). This attribute is important in understanding the role and effect of soil amendment within dissolved organic compounds to cause their build up in the environment (Spark *et al*., 2002). High soil adsorption of thiacloprid (one of the most adsorbed insecticides, see Figure

11) could be caused by not only to its moderate solubility in water (180 mg/L), but also to a high Log K_{ow} of 1.26, and to the presence of chloro-substituted pyridine and thiazole rings in its structure (Figure 2).

The sorption kinetics and leaching using a column experiment of thiacloprid and thiamethoxam insecticides, being the most applied pesticides in UK since 2012 (FERA 2019), were further assessed to study their behaviour and potential to spread in the environment.

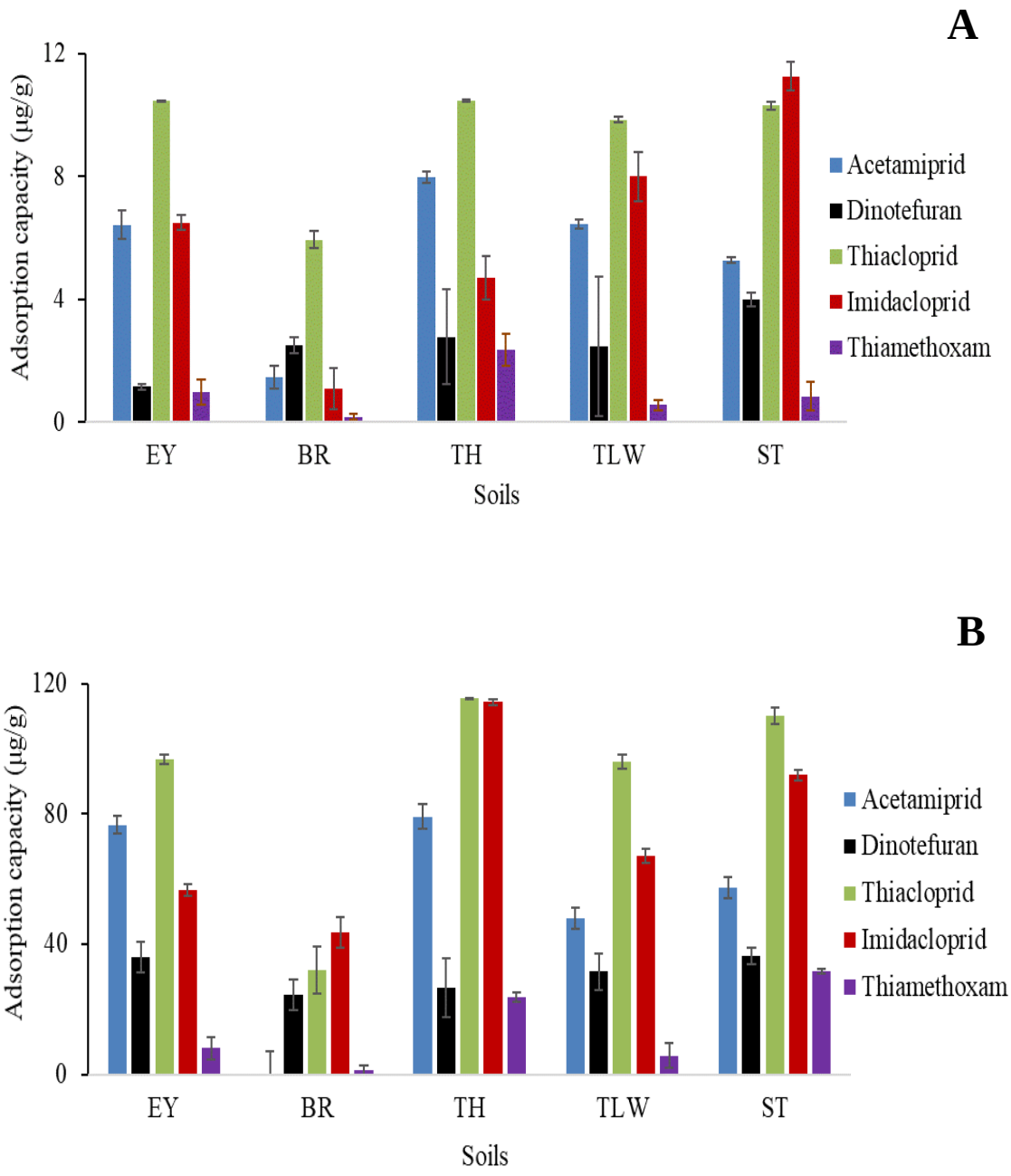


Figure 11. Assessment of the amounts of neonicotinoids sorbed in soils at soil-pesticides solution ratio of 1:5 at two levels of initial pesticide concentrations of 2.5 µg/g (A) and 25 µg/g (B). Results given as average (n = 3) ± SD. The adsorptive capacity is expressed as µg neonicotinoids/g of soil

4.1.4.2 Sorption kinetics

The time dependent sorption behaviours of thiacloprid and thiamethoxam were observed over a period of 72 hours. Sorption of the two pesticides (thiamethoxam and thiacloprid) was akin to higher adsorption capacities of other pesticides in the TH soil than the BR soil (see Figure 11). The two soils, TH and BR soils, were selected being the least and most neonicotinoids adsorbing soils with extreme values of SOC (see Table 15) which largely represent the range of soils with %SOC that is found in England for farming activities. Also, this is supported by the witnessed in the 0 – 300 g/kg range organic carbon content of arable soils distributed across England and Wales as reported by Bellamy *et. al.* (2005).

In the BR soil, thiamethoxam attained equilibrium (in sorption processes) faster than thiacloprid *i.e.,* at 6 h vs 24 h (sorption profile with time shown in Figure 12). It is interesting to note that sorption of both insecticides in the TH soil was rapid: within the first 15 minutes, about 79-82 % adsorption achieved. Then the amount adsorbed did not increase much as time passed. This may indicate a strong adsorption-desorption of the insecticides taking place in soils with higher % SOC over the remaining contact time. The sorption (Figure 12) of the insecticides correlates well with their log K_{ow} and solubility (Table 1) as expected and the organic carbon content of the soil may also be responsible for enhanced sorption, such as reported for imidacloprid and diuron by Fernández-Bayo, *et al.* (2008) and dinotefuran, imidacloprid and thiamethoxam by Kurwadkar, *et al.* (2013).

Although thiacloprid was not studied in the two papers cited and the sorption behaviour was not compared. However, in our study the sorption behaviour of both thiamethoxam and thiacloprid were found to suggest their physicochemical properties as well as the soil type may have to be considered in predicting their fate in the soil environment. This is because, the sorption pattern of the two insecticides (THX and THA) were similar in the soil rich in organic

carbon (TH) contrary to our expectation. Therefore, to better understand the behaviour of these insecticides in soil environment, commonly applied kinetic models were applied.

Established kinetic models (hyperbolic, pseudo-second order rate equations, Elovich and Weber-Morris models) were applied to gain further insight into their sorption phenomena. Interpretations of the parameters of the applied sorption kinetics models are given below:

Hyperbolic Model: The linear form (Eq. 2) of this model provides useful sorption parameters values that can be adjusted to the experimental data as previously used by Fernández-Bayo *et al.* (2008) and Cáceres *et al.* (2010).

$$1/q_t = (B/ q_{max} \text{ X } 1/t) + 1/ q_{max} \qquad \text{Eq. (2)}$$

Where, q_t is the sorbed quantity (μg/g) at time t (h), q_{max} (μg/g) is the maximum sorbed amount, t (h) is the pesticides solution-soil contact time, and B is an empirical constant.

Pseudosecond-Order Kinetic Reaction Model: The application of this model is with the assumption that the sorption capacity could be proportional to the number of active sites on the adsorbent, as reflected in Eq. 3 below. Application of this model was widely reported in the literature to explain the adsorption kinetics mostly effectively (Robati 2013, Fernández-Bayo *et al*. 2008, Eris & Azizian 2017)

$$dq/dt = K^* (q_{max} - q_t)^2 \qquad \text{Eq. (3)}$$

q_{max} and q_t were as defined in the hyperbolic model above and K is the reaction-rate constant (μg/g min).

The model is usually represented by its linear form as shown in Eq. 4

$$t/q_t = 1/(K^* q_{max}) + t/q_{max} \qquad \text{Eq. (4)}$$

Elovich Equation: This equation (Eq. 5) describes second order kinetics with the assumption that the actual solid contact surface are energetically heterogeneous, however, the equation fails to propose any definite mechanism for adsorbent-adsorbate (Yakout *et al*, 2010). Also,

Fernández-Bayo *et al* (2008) suggested the equation reflected two phase of adsorption kinetics; a fast initial reaction due to pesticides movement to the most accessible part of the sorbent, and slower reaction phase due to in and out pesticides' diffusion from the sorbent microspores. The linear form of this equation is given below by:

$$qt = \frac{1}{Y} \ln (X * Y) + \frac{1}{Y} \ln t \qquad \text{Eq. (5)}$$

Weber-Morris Model (Intraparticle diffusion): This equation (Eq. 6) considers a varying degree of proportionality of the sorption processes with $t^{1/2}$ and this is given *by:*

$$q_t = K^* t^{1/2} + C \qquad \text{Eq. (6)}$$

Also, q is the sorbed quantity (μg/g) of pesticides at time t, C is the intercept (μg/g) as shown in the equation (Eq. 6) and K is the intraparticle diffusion rate constant ($\mu g/g min^{-1/2}$)

Among all the models, the linearised form of pseudo-second order kinetic reaction model gave the best fitting with R^2 in the range 0.990 - 1.00, for both pesticides on both soils was observed (Figure 13). The values of q_{max} obtained with the pseudo-second order model (see Table 16) were similar to the hyperbolic model values but with poor regression coefficients. Similar results were obtained by Fernández-Bayo *et al.* (2008), with other pesticides, imidacloprid and diuron, when tested on different soils.

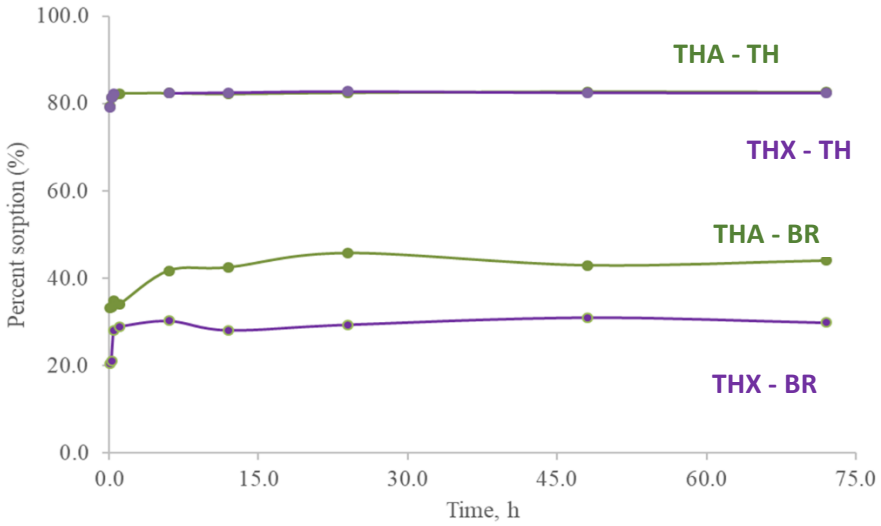


Figure 12. Percentage of the amount of thiamethoxam and thiacloprid sorbed to soils high and low in organic content, TH and BR respectively, when 4g of the soil samples were incubated with 20 mL of 2.5 µg/g pesticides aqueous solution at different time intervals 0 – 72 h.

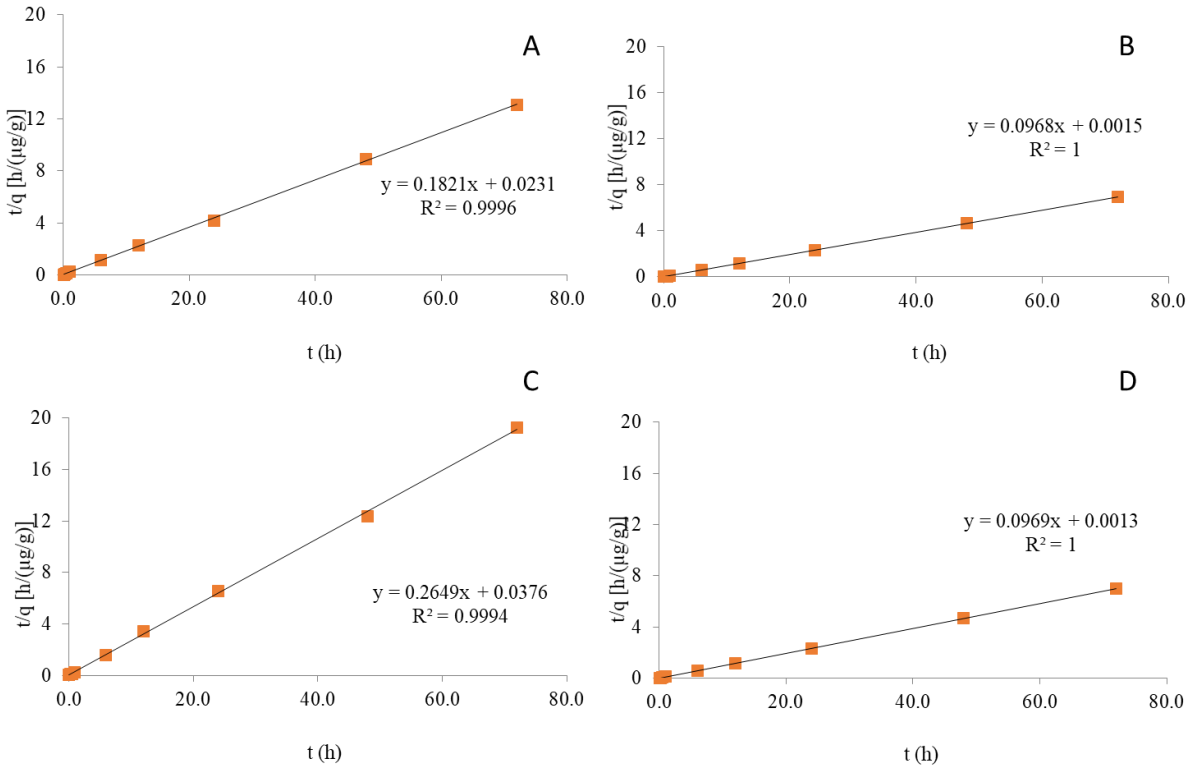


Figure 13. The linear form of pseudo second-order equation of the uptake of thiacloprid in Soil BR (A) and TH (B) and thiamethoxam in BR (C) soil and TH (D) at different contact times.

Thiacloprid presents higher q_{max} values on both soils than thiamethoxam (Table 16). Moreover, the value of q_{max} for thiacloprid in TH-soil was found to be about 95% higher than the corresponding value for thiamethoxam. The values obtained with the pseudo-second order model were in sync with the organic carbon content of the soils with TH-soil having higher SOC content, *i.e. 0.8 vs 12.5 %*, more than the BR-Soil (Table 15). The significant role of SOC in the sorption of pesticides has been propounded by Liyanage *et al.* (2006) and the results obtained in the present study concur with their findings. The values of the kinetic rate constant (k) for the two pesticides are similar in both soils, with the values of the TH-soil more than double of those of the BR-Soil (Table 16).

For the two pesticides in both soils, there is poor correlation between the determined coefficients of the Elovich equation (Table 16), the values of R^2 fell in the range 0.219 - 0.890. Also, the amount of sorbates at the end of the initial rapid phase (at 6 h), compared to that at the end of 24 h, were observed to be higher in thiacloprid for both soils than thiamethoxam (Table 16). This is an indication that thiamethoxam may have a higher tendency to accumulate and linger in ecosystems than thiacloprid. Although the Elovich equation did not appear to be a perfect fit for linearity, the results are congruent with the two-phase principle of sorption mechanism proposed by the Elovich model.

The values of 1/Y were lower in the soils with higher soil organic content, indicating that sorption equilibria of insecticides were probably attained within the first 6 h of application. However, this result was dissimilar to that of Fernández-Bayo *et al*. (2008), who examined soils with similar SOC levels, and reported that the low OC content of the soils correlated neither with the sorbed amount nor with the kinetic parameters for both pesticides; implying that other factors may be controlling the sorption process such as clay content. However, in this work, the clay contents are similar across the soils examined (Table 15).

With the Weber-Morris model, it is known that linearity is observed when intra-particle diffusion is involved in the adsorption process. Usually, a linear graph is obtained when sorbed quantity (µg /g of pesticides at time t (q_t) is plotted against the square root of time ($t^{1/2}$), on condition that intra-particle diffusion is the dominant rate-controlling mechanism (Yakout *et al,* 2010). The unit of k is µg/g min Intra-particle diffusion is a mass transport phenomenon whereby the chemical species of interest traverses inside a solid particle by sole means of diffusion through the water contained in the interstitial spaces of the particle. The process is known to be one of the significant rate-determining steps in sorption. For the two soils, the results obtained show poor linearity with both pesticides, with R^2 falling between 0.04 and 0.72 as the Weber-Morris equation was applied.

Fernández-Bayo, *et. al.* (2008) and Yakout *et. al*. (2010) both reported similar adsorption results with imidacloprid on agricultural soils and strontium adsorption on low cost rice-straw based carbons respectively. In the strontium case, linearity hinted at intra-particle diffusion, although the exact speciation of strontium in the specific soil environment is unknown.

Thermal diffusion of molecular and ionic species in water, governed by Fick's Law, is present in any aquatic system. In this work, the lack of linearity observed with the Weber-Morris equation suggests that Fickian diffusion in the bulk aqueous phase is accompanied by other attenuations to the overall rate of mass transfer, such as the nature and thickness of the nominally stagnant liquid film at the solid-liquid interface, often referred to as the boundary layer. The strength of Wan der Waals forces between the pesticide moiety and the surface of a soil particle also plays an important part in all sorption processes. In terms of interpretation, the higher the value of the intercept C (see Table 16), the greater the thickness of the boundary layer (Kannan & Sundaram, 2001). Calculated values of the intercept C (with units of μg/g), listed in Table 16 for both pesticides were significantly higher than the corresponding values of K (intraparticle diffusion rate constant).

Several mathematical models have been applied to explain the sorption kinetics of pesticides in soil over the range of the contact time. The time required for the pesticide-sorbent to reach equilibrium and the mechanism involved are key parameters used in understanding their sorption behaviours (Fernández-Bayo *et al*., 2008; Cáceres *et al*., 2010; Eris *et al.*, 2017). The incremental sorption of pesticides to soil particles is time dependent and this has been related to pesticide sorption kinetics and diffusion processes. Therefore, the study of kinetics is crucial to understanding the mechanism related to the situation and persistence of pesticides in soils and the sorption parameters measured in this work will contribute to a computerised differential mass balance model for neonics.

4.1.4.3 Adsorption isotherm

Thermodynamics of sorption of thiacloprid and thiamethoxam was studied in four soils with contrasting soil organic carbon content. The two neonicotinoid insecticides were chosen being the most and least adsorbed insecticides in the study compounds. Removal of the insecticides from the environment requires good understanding of how the adsorbate (neonicotinoids) particles distributes between the liquid phase and the solid surface of adsorbent at the equilibrium. The data obtained from the sorption isotherm experiment was fitted into Langmuir and Freundlich models; two of the commonly applied adsorption models.

Langmuir Adsorption Isotherm is the quantitative formation of a monolayer adsorbate on the outer surface of the adsorbent and no further adsorption occurs after that: critical is that this fits into a surface with a finite number of identical sites. However, the Langmuir model assumes the adsorbent surface consists of a specific number of uniform active sites proportional to the surface area at which only one molecule may be adsorbed. Therefore, adsorption energy is uniform on the surface of the adsorbent and independent of the extent of coverage of the surface area of the adsorbent. Also, the adsorption is localised with adsorbed molecules remaining at the site of adsorption until desorbed. Finally, the Langmuir model, as represented in Eq. 7, assumes only a monolayer is formed and no further deposit of adsorbate on sorbed adsorbate molecules except on free adsorbent surface only (Figure 14).

Base on the assumptions stated above, the Langmuir equation is represented as:

$$qe = \frac{Qo\ KL\ Ce}{1+KLCe} \qquad \text{Eq. (7)}$$

However, Eq. (7) is transformed into linear equation to obtain the Langmuir parameters

$$1/\ qe\ = \frac{1}{Qo} + \frac{1}{Qo\ KL\ Ce} \qquad \text{Eq. (8)}$$

Where:

C_e = the equilibrium conc. of adsorbate (amt. adsorbed in µg/g)

q_e = the amount of compound adsorbed per g of the soil (adsorptive capacity in µg/g of soil)

Q_o= maximum monolayer coverage capacity (µg/g)

K_L= Langmuir isotherm constant (L/g) related to energy of adsorption.

Also, the Langmuir equilibrium parameter (R_L) was computed as follows:

$$R_L = 1/[1+(1+K_L C_o)] \quad \text{Eq. (9)}$$

Where:

C_o = initial concentration.

The Eq. (9) indicates the adsorption nature to be either

(1) unfavourable if $R_L > 1$

(2) linear if $R_L = 1$

(3) favourable if $0 < R_L < 1$

(4) irreversible if $R_L = 0$

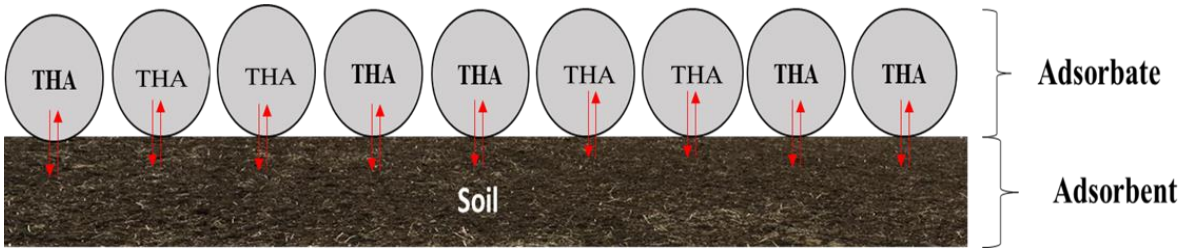


Figure 14. Monolayer model of Langmuir adsorption of neonicotinoids on soils with different characteristics

The values of Q_o and K_L were both derived from the slope and intercept of the plot of $1/ q_e$ against $1/ C_e$ while the regression coefficient, R^2, was obtained from the regression equation of the plot (Table 17).

The Freundlich Adsorption Isotherm is commonly used to describe the adsorption characteristics for the heterogeneous surface with the proposed empirical equation for the adsorption isotherm as follow:

$$Q_e = K_f C_e^{1/n} \quad \text{Eq. (10)}$$

The transformation into linear equation by taking log of both sides of the Eq. (9) is:

$$\text{Log } Q_e = \log K_f + 1/n \log C_e \quad \text{Eq. (11)}$$

Where

1. K_f = Freundlich isotherm constant (µg/g)
2. n = adsorption intensity
3. C_e = the equilibrium conc. of adsorbate (µg/g)
4. Q_e = Adsorptive capacity

The constant K_f is an approximate indicator of adsorption capacity, while 1/n is a function of the strength of adsorption in the adsorption process. If

1. n = 1 then the partition between the two phases are independent of the concentration.
2. 1/n < 1, it indicates a normal adsorption
3. 1/n > 1, it indicates cooperative adsorption

From the data in table 17, all the Freundlich values of 1/n for the thiacloprid and thiamethoxam on the soils tested were found to be less than 1 except for thiamethoxam on BR soil with 1/n of 1.32. According to the Freundlich model, this is a cooperative adsorption type where adsorbates react with other adsorbates to synergistically enhance their adsorption (Liu, 2015); this behaviour may be influenced by the reactive nitro-functional group of

thiamethoxam, which needs to be further investigated. However, in the same BR soil, the value of 1/n for thiacloprid was reported to be 0.93, relatively close to thiamethoxam behaviour. This indicates that the sorption of both neonicotinoids is favourable in the soil tested. The low adsorption intensity of thiamethoxam on BR soil may be attributed to lower content of the organic carbon of the soil; this may possibly influence the extent of removal from the environment or increase the potential to run off and raise their deposit in the nearby rivers or lakes.

The Langmuir equilibrium parameter, R_L, was generally low ranging from 0.20 – 0.48 for both neonicotinoids on all the soils tested. According to Langmuir description, favourable if $0 < R_L < 1$, adsorption of the thiacloprid and thiamethoxam on all the soils with contrasting characteristic, is likely. However, the values seem to be very low and may suggest low adsorption intensities or not adequately represented by the model; although thiacloprid, with the highest value of R_L (Table 17), was adsorbed most (Q_o = 1.84 µg/g) in the TH soil, with the most %SOC.

Generally, from the results of the adsorption isotherm (Table 17), both Langmuir and Freundlich fitted very well into the adsorption of thiamethoxam on BR soil only with high values of regression coefficients. However, both models fitted very well in thiacloprid adsorption to three of the four soils while TH soils presented the poorest regression coefficients for both neonicotinoids in the two models fitted. There is, therefore, a good possibility that these compounds may be adsorbed on adsorbent for the purpose of their removal from the environment. However, the extent of removal may be influenced by the nature of the adsorbent surface and chemistry. This is an area to be investigated under different conditions.

Table 16. Sorption kinetics of thiacloprid and thiamethoxam on two soils with contrasting organic carbon obtained from four models

Soil	Thiacloprid												Thiamethoxam											
	Hyperbolic model		Pseudo-second-order reaction			Elovich			W-M				Hyperbolic model		Pseudosecond-order reaction			Elovich			W-M			
	q_{max}[a]	R^2	q_{max}	K^b x 10^{-3}	R^2	%[c]	1/Y	R^2	C[d]	K[e]	R^2		q_{max}	R^2	q_{max}	K x 10^{-3}	R^2	%	1/Y	R^2	C	K	R^2	
BR	5.08	0.467	5.49	7.88	0.999	88	0.25	0.890	4.32	0.180	0.717		3.66	0.762	3.78	7.05	0.999	66	0.16	0.666	3.10	0.100	0.408	
TH	10.27	0.984	10.18	18.53	1	99	0.03	0.616	10.18	0.008	0.354		0.51	0.06	0.52	19.09	1	96	0.003	0.219	0.50	0.003	0.217	

[a]q_{max} unit in µg/g; K^b unit in g/µg/min; %[c] Percent sorbed during the initial phase (6 h) with respect to the sorbed amount at 24 h; C^d units in g/µg; K^e unit in (µg/g/$min^{1/2}$)

Table 17. Sorption isotherm of thiamethoxam and thiacloprid on four soils with contrasting organic carbon obtained from two models (Freundlich and Langmuir adsorption isotherm)

Thiamethoxam

TLW						Ey						BR						TH					
Freundlich			Langmuir			Freundlich			Langmuir			Freundlich			Langmuir			Freundlich			Langmuir		
K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2
7.32	0.43	0.803	1.16	0.31	0.797	7.32	0.43	0.748	1.16	0.31	0.710	7.51	1.32	0.968	1.14	0.41	0.995	5.16	0.13	0.010	1.40	0.20	0.393

Thiacloprid

TLW						Ey						BR						TH					
Freundlich			Langmuir			Freundlich			Langmuir			Freundlich			Langmuir			Freundlich			Langmuir		
K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2	K_f	1/n	R^2	Q_o	R_L[a]	R^2
1.00	0.58	0.976	0.86	0.31	0.934	1.00	0.50	0.979	0.86	0.29	0.940	11.35	0.93	0.931	0.95	0.36	0.872	3.50	0.23	0.280	1.84	0.48	0.033

a: computed by averaging the individual R_L values (n = 7) obtained from C_o (0.1 - 1.5 µg/g) initial concentrations.

4.1.4.4 Soil column mobility

The breakthrough curves for leaching of thiacloprid and thiamethoxam through soil with the least and most %SOC are shown in Figure 15. The elution of thiamethoxam from the column with BR-soil was at approximately 0.16 bed volume (bv) and 0.29 bv for thiacloprid. Similar elution order was observed in TH-soil, with 0.75 bv and 14.0 bv for thiamethoxam and thiacloprid respectively.

The leaching behaviour of the two pesticides was found to be similar to the sorption pattern as reported in the sorption section of this work with good link to the characteristics of the soil type. Hence, leaching from the soil with poor binding capacities may lead to the migration of these pesticides in the soil environment. Therefore, a good understanding of their column mobility property may influence their choice of multiple application in selected soil types to reduce their accumulation.

Both THA and THX have very limited interaction with the BR soil. Specifically, the THA band in the BR-soil was broader when compared to THX (see Figure 15). This indicates that THA has somewhat more interaction with the BR soil than THX. The asymmetrical curve of the two pesticides, particularly in BR soil, with a longer extended tail in THX curves, may be due to the existence of more than one mechanism involved in the retention; or limited interactions with the soil as reported by Rodríguez-Liébana *et al*. (2018). When the mobility of both insecticides was assessed in TH soil, characterised by being high in organic carbon, the elution was delayed but the same elution order was observed. The bands were broader in this soil which may indicate a range of unspecific interactions with the soil and overloading of the active sites of the soil taking part in the adsorption process (Figure 15). The later elution of the neonicotinoids when compared with BR soil indicates that there was more interaction of both THA and THX with the TH soil than with the BR soil. The gaussian nature of the THX in the TH soil indicates similar interaction of the neonicotinoid molecules migrating through the

column with the soil. However, a diminished non-symmetrical elution profile was observed with THA pesticide in TH soil indicating a stronger affinity for the soil high in %SOC.

The analysis of residues of the pesticides present in the soil after the leaching study showed that the amount of THX eluted from TH-soil was five times more than THA and it was almost twice in BR soils. This shows that THX is less retained in the soils, implying that it can migrate more. However, longer times of abode of THA in the soils (especially with high % SOC) may result in greater exposure to soil faunas due to corresponding longer contact times (Cláudia *et al.*, 2017).

The leaching behaviour of the two insecticides (THA and THX) in the soils with contrasting organic contents correlates well with the GUS leaching index and their solubilities (Table 1), with THX having a high leaching index of 3.82 and appreciable solubility, while THA has a low leaching index of 1.44 and relatively lower solubility. Therefore, both pesticides are leachers but THX may leach faster in soils poorer in organic carbon and with high potential to contaminate ground water. With the smallest value of CEC (Table 15), the BR-soil environment is also strongly alkaline (see Table 15), hinting the existence of high levels of exchangeable cations. Therefore, with greater tendency for clay to disperse and producing poor soil structure, a hydrophilic pesticide like thiamethoxam, in this environment, leaches through the soil with very limited interaction with the substrate.

The degree of soil column mobility of thiamethoxam was observed to be faster than thiacloprid. If we know the binding affinity of a pesticide, we can better determine the risk of using it in sensitive areas. It is therefore a characteristic to be considered in environmental risk assessments. It is part of a cost-benefit-risk assessment. Therefore, the chemical structure of these pesticides, their leaching properties and soil types are important parameters to be considered in determining their fate in the environment including soil.

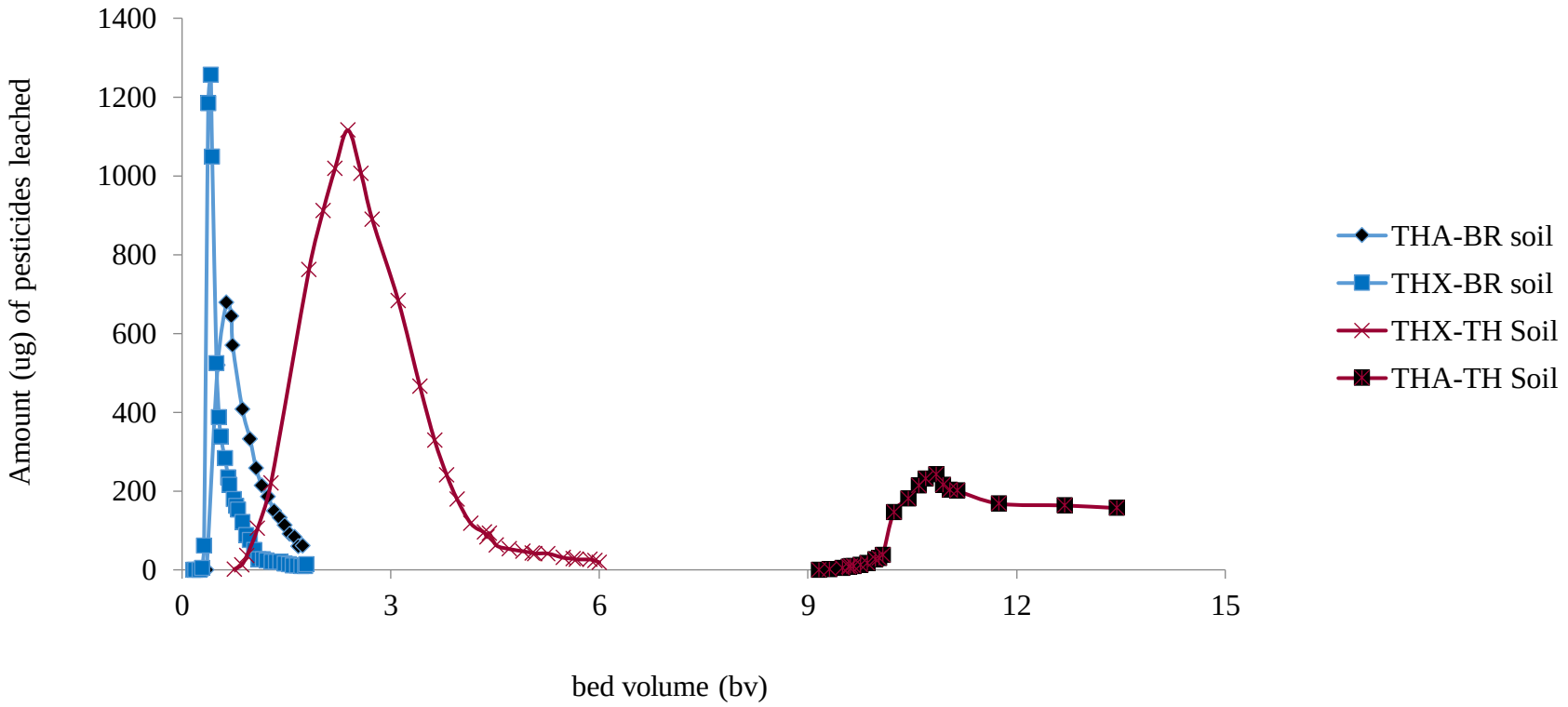


Figure 15. Breakthrough curves corresponding to the leaching of thiacloprid (THA) and thiamethoxam (THX) in 2 equivalent soil columns (4 cm i.d. and 14 cm height) where the neonicotinoids were spiked onto soils (1 mL of 1000 µg/g of pesticide were added to 192 g of soil which was deposited on a layer on the top of the column) with the soils with 0.8 and 12.5 % SOC (BR and TH). NB: 1 bed volume (bv.) = 175 mL of the total soil packed column volume (mL).

The amount of pesticides left in the soil column was determined after the leaching experiment was completed. Prior to the extraction of neonicotinoids from the soil samples after the soil column leaching experiment, the recovery efficiency of the extraction method developed in section 3.4.2.1 was tested on the two soils (TH and BR) used in the leaching study. A good recovery was obtained for both neonicotinoids (THA and THX) in the selected soils tested. The recovery of thiamethoxam and thiacloprid in BR soil were 85.0 and 85.2 % respectively while in TH soil, the recovery of thiamethoxam and thiacloprid were 72.2 and 86.0 % respectively.

Insecticide residues were retained in both soil columns with BR-soil harbouring less residues after leaching, compared to TH soil, see Table 18. Interestingly, most residues of the insecticides were extracted from the upper layer in the BR-soil column, with least amount from the lower layer; this informs remediation strategies for this type of soil when contaminated with the study neonicotinoids. In the TH-soil column, more residues were extracted from the middle and lower layers than the upper part of the column. It is likely that the high level of binding organic matter in TH soil, washed down during continuous flow of water, may be responsible for the differential adsorption behaviour of the insecticides in the soil column operating under gravity. The different behaviour in column mobility of these pesticides, as reflected in the amount of the residues left in different sections of the soil column after the leaching experiment, is reported for the first time in this work and, to the best of our knowledge, has not been reported anywhere.

Table 18. Amount of (µg/g) of THX and THA pesticides retained in different sections of the soil column after leaching. Results given as average (n = 3) ± SD.

Soil	Thiacloprid			Thiamethoxam		
	Upper layer [a]	Middle [b]	Lower layer [c]	Upper layer [a]	Middle [b]	Lower layer [c]
BR	10.34 ± 0.91	6.95 ± 0.78	4.59 ± 0.29	1.21 ± 0.23	1.01 ± 0.26	0.57 ± 0.06
TH	26.36 ± 1.56	85.62± 38.79	83.87 ± 15.67	6.55 ± 0.26	9.58 ± 0.45	7.79 ± 0.81

[a] 0-4 *cm*; [b] 4-9 *cm*; [c] 9-14 *cm*

The amount of pesticides recovered from both soil columns were 0.71 % for thiacloprid and 0.09 % for thiamethoxam in BR-soil column. In the TH-soil column, it was with 29.8 % for thiacloprid and 0.69 % for thiamethoxam of the amount initially applied. The leaching of thiamethoxam in BR-soil with 0.8 % SOC gave similar results to that obtained by Gupta, *et. al.* (2008), with 0.5 % SOC soil and recovering about 66-79 % of applied THX from leachate, with no residue detected in soil, after draining with 2.5 litres of water. The inability of THX to bind strongly with soil, even with high SOC, may be due to its ionised form through the protonated nitrogen and nitro group and stay in aqueous solution instead. This property may be responsible for its ease-of-leaching which leads to enhanced mobility; with potential pollution consequences for both ground water and run-off.

Although these pesticides are highly mobile, some neonicotinoids have been reported to persist in the environment with their residue being detected in plants years after their application (Wood *et. al*., 2017; Jiang *et al.*, 2018) and this positions thiacloprid and their metabolites, with stronger affinities for the binding organic carbon content of the soil, to be a risk to the health of soil faunas.

4.1.5 Conclusions of fate of neonicotinoids in soil

Soil is an important part of the ecosystem and serves as potential sources of natural resources for plant growth. Also, due to the presence of constant climatic changes and anthropogenic activities, biogeochemical transformation occurrence in the soil predominantly influences the distribution of pesticides. This is possible because soil is the predominant sink for environmental pollution including pesticides due to its strong binding capacity. Therefore, monitoring the fate of these pesticides such as neonicotinoids in the soil requires a reliable understanding of their sorption and leaching behaviours.

The sorption and leaching behaviour (two of the commonly studied parameters in understanding the fates of organic pollutants in the environment) of some widely used neonicotinoid insecticides in soils of widely contrasting organic contents (0.8 – 12.5 %) were examined and juxtaposed. The soils originate from four locations in the South-East of England and one from Scotland. Soils with high concentrations of organic carbon retain insecticides better than those of lower organic carbon content.

Thiamethoxam was found to be the least adsorbed insecticide in all soils, and it is one of the most widely neonicotinoids used in the UK. The implication is that it has the greatest potential to contaminate ground water especially when used in a soil with relatively low organic carbon. In contrast, thiacloprid, the most adsorbed insecticide, is expected to be more retained in soils with high organic carbon content. The good sorption characteristics of thiacloprid, as reported in this work, may be exploited in the removal process in order to ensure green environment while the poor sorption of thiamethoxam can be investigated further if the need for its removal from the environment is to be improved. Neonicotinoids which exhibit higher water solubility and lower solubilities in organic media (*i.e.*, low $\log K_{ow}$ values) are less inclined to be adsorbed onto the surfaces of soil particles. Data of adsorption kinetics of neonicotinoids on soils with

different organic content is well represented by a pseudo second order kinetic model (R^2 >0.999)**.**

In flow-through experiments, the high amount of organic carbon content in TH soil prolonged the elution of the pesticides, four times with thiamethoxam and forty-eight times more with thiacloprid. This has two implications: (i) if not degraded, thiacloprid will be rapidly available in the soil environment; (ii) soil faunas may be damaged. The least and most adsorbed neonicotinoids (THX and THA) tend to leave greater residues in the first half of the soil column (from a 20 cm section of a soil column) with the least adsorbing soil rich in silt (BR); in contrast, a soil rich in organic matter (TH) presented most residues from below 20 cm. This has implications in the bioavailability of the neonicotinoids by plants and soil organisms.

4.2 Fate of neonicotinoid insecticides in water

4.2.1 Introduction

The water bodies are constantly contaminated with numerous substances such as pharmaceuticals, plasticisers, pesticides and other harmful chemicals due to several anthropogenic activities (Casado *et al.,* 2019, Fu *et al.,* 2019). Their occurrence and fate in the water bodies are a concern to the health and safety of the ecosystem (Flandroy *et al.,* 2018, Jurado *et al.*, 2019). When pesticides are applied as crop protection agents, Wang *et al.,* (2016) reported that only five percent of their active ingredient reach the target organism and most are retained in the soil/water environment as contaminant for a longer period of time.

The constant application of large volumes of organic compounds has led to a vast majority of them finding their way into the surrounding water bodies leading to a greater pollution challenge from agriculture (Banić *et al.,* 2014). Therefore, the main routes of pesticides contamination of these water bodies are through runoff from agricultural fields and leaching into groundwater, which consequently leads to subsurface discharge into wetlands and surface water (Morrissey *et al.,* 2015). However, other sources of water contamination of neonicotinoids such as the deposition or decay of treated plants, treated seed and soils in water are a possibility considering the solubility of the compounds.

Generally, neonicotinoid insecticides are known to be polar compounds with high solubilities in water. This property may have contributed to their propensities to be found everywhere in the environment, particularly water bodies. The presence of these organic micropollutant neonicotinoids in the water environment has been often reported (Hladik *et al.,* 2014, Klarich *et al.,* 2017, Struger *et al.,* 2017, Hladik *et al.,* 2018) and they are regarded as a threat to aquatic animals (Sánchez-Bayo and Hyne, 2014b). However, their removal from the environment has only been the focus of attention in the last decade or so. Also, the European Commission has now listed four of the neonicotinoid insecticides in this study in the watch list of EU (Decision

2015/495) as substances to be monitored in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament (Barbosa *et al*., 2016).

Pesticides decompose in the soil/water environment due to chemical, physical and microbiological activities, with different resistances to these forces of nature (Divito *et al.* 2007 and Kumar *et al.* 2012). However, degradation is decreased in the higher latitudes and temperate regions due to reduced sun-hours, sunlight intensity and temperature compared to the tropical region. The decomposition of pesticides in the environment can be broadly categorised as biodegradation, photolysis and hydrolysis, which are linked to the criteria considered for pesticides registration and environmental safety evaluation. More consideration is, however, focussed on photolysis in this work.

4.2.1.1 Biodegradation

Soil microbes catabolize neonicotinoids by their enzymes. Ge *et al*. (2014) reported the hydrolysis of 91% of the cyanoimine group of thiacloprid, to thiamide, by the bacteria *En. MelilotiGCMCC 7333*. Aerobicity and Anaerobicity are known to affect biodegradation. Clothianidin's half-life (DT_{50}) in soil fell in the range 148-1155 days under anaerobic conditions, and about 27 days in the aerobic state.

Fipronil insecticides have been reported to be biodegraded. Kumar *et al.* (2012) confirmed the use of two different bacterial cultures, namely, *Paracoccus* sp. and Gamma proteobacteria to degrade fipronil in three different types of soil. According to the same report of Kumar *et al.* (2012), a good correlation was found to exist between the microbes and degradation giving grounds to propose a method of bioremediation. However, degradation by *Paracoccus* sp. showed a better result with reduced residence time of the pesticide in loamy soil compared with that of sandy and clay soils. This may be due to the absence of organic carbon serving as nutrient to sustain the microbes during the reaction time.

The report of Kumar *et al*. (2012), however, only monitored the residual fipronil, while the use of Bacillus *firmus* for fipronil biodegradation as well as its degradates (fipronil sulfide, fipronil sulfone, fipronil amide desulfinyl) in clay loam soil were reported (Mandal *et al.* 2014). In both reports, biodegradation of fipronil was found to be concentration dependent and decreases with increase in concentration. Hussain *et al.,* (2016) reported the fastest rate of bacterial biodegradation for imidacloprid and followed by thiamethoxam, thiacloprid and acetamiprid with more strains of bacteria available for imidacloprid and acetamiprid transformation when compared to others. It has to be noted that the biodegradation of formetanate hydrochloride has not been reported to the best of our knowledge.

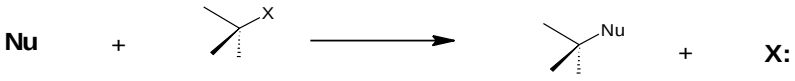
4.2.1.2 Hydrolysis

Hydrolysis is a type of decomposition reaction, as shown in Eq. 11, where the water molecule is employed in the breaking of chemical bonds in the other reactant, as depicted in this equation:

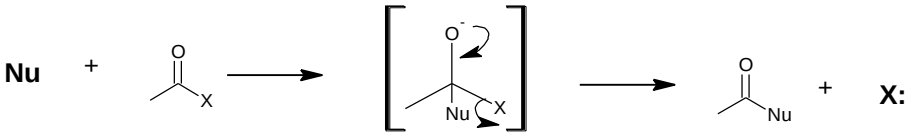
$$AB + H_2O \longrightarrow AH + BOH \qquad \text{Eq. (11)}$$

Notably, a hydrolytic reaction takes place via two classes of mechanisms, namely, Nucleophilic substitution (S_N1 and S_N2) and Addition – Elimination.

(i) Nucleophilic substitution reaction takes place when the leaving group is attached to a sp^3 hybridised carbon centre such as epoxides, phosphate esters and as shown below:



(ii) Addition – Elimination reaction process occurs when the leaving group is attached to the sp^2 hybridised acyl carbon centre found in carboxylic acid derivatives such as esters, anhydrides, amides, urea and carbamates, as shown below:



Pesticides, particularly the ionic ones, can be hydrolysed because they are organic compounds with exchangeable ions leading to either their disappearance or transformation.

Degradation of formetanate hydrochloride [m-(((dimethylamino)methylene)-amino) phenylmethylcarbamate hydrochloride], with the two functional groups formamidine and carbamate, is particularly interesting and has been reported at high pH values. Hydrolysis was rapid, regioselective and tended to occur in aquatic environments (Divito *et al.*, 2007). The presence of two functional groups has meant two possible pathways of formetanate hydrochloride degradation, leading to formation of methylamine and dimethylamine.

The formamidine group of formetanate hydrochloride, being the more labile functional group than the carbamate group, was reported with a half-life of 3.9 to 14.4 hrs under strong to mild basic conditions of pH 12 to 7.6, while the carbamate group has a longer degradation of over six months (Divito *et al.*, 2007). Mandal & Singh (2013) reported that amides are a major metabolite during the hydrolytic degradation of fipronil. The experimentation was on clay loam soil and sandy loam soil. Degradation kinetics of fipronil followed a first order rate equation with half-life (DT_{50}) of total fipronil reported to be 33.4, 33.4 and 30.1 days at 100, 200 and 400 mg/kg for sandy loam soil and 37.6 days at the same application amount for clay loam soil (Mandal & Singh, 2013). This shows that fipronil is more persistent in clay loam soil than sandy loam soil probably due to the clay loam soil's stronger adsorption capacity providing protection against biodegradation compared to the sandy loam soil. However, the percentage of fipronil amide metabolite formed was the least thereby making the hydrolysis of fipronil less important compare to other metabolites by other routes of degradation.

The hydrolytic degradation of neonicotinoids is controlled by pH, temperature and prevailing soil conditions. Pitam *et al* (2013) reported the importance of pH in pesticides' degradation and

concluded that the hydrolysis of acetamiprid in water remains very sluggish in acidic and neutral pH conditions but increases with increasing pH (Table 19).

Table 19. Persistence of acetamiprid in water at different pH values

Days / pH	Concentration remaining in water (µg/mL) at different days							
	0	**3**	**5**	**10**	**15**	**20**	**30**	**45**	**60**
4	9.75	8.68 (10.9)	6.98 (28.4)	3.84 (60.6)	2.02 (79.3)	1.14 (88.3)	0.34 (96.51)	0.08 (99.2)	ND
7	9.87	8.22 (16.7)	6.12 (37.9)	5.22 (47.1)	2.89 (70.7)	1.82 (81.6)	0.69 (93.00)	0.14 (98.6)	ND
9	9.82	7.85 (20.1)	4.87 (50.4)	3.17 (67.7)	1.21 (87.7)	0.74 (92.5)	ND	ND	ND

( ) Percent dissipation, ND not detected. Source: Pitam *et al.,* (2013)

4.2.1.3 Photolysis

Neonicotinoids can be photolysed by different pathways, depending on prevailing environmental conditions. The nitroguanidine functional group of thiamethoxam and imidacloprid (Table 1) is not resistant to photolysis, while the cyanoimine functional group of acetamiprid and thiacloprid was resistant during UV irradiation (Banić *et al.*, 2014). The combination of different sources of photolytic agents has proven to be beneficial in the elimination of these insecticides from environmental sources thereby reducing their ecotoxicity effects.

The use of electron transfer between TiO_2 and different oxidants such as O_3 and H_2O_2 or transition metal ions i.e. Fe^{3+}, Cu^{2+} and Ag^{+} in photolytic degradation of different neonicotinoids has been reported. The effect of pH and ozone dosage on TiO_2 photocatalysis for the degradation of thiacloprid in aqueous medium (Table 20) was investigated by Černigoj *et al.,* (2007) with a good synergistic effect at acidic and neutral pH reported, but less at basic pH due to faster self-decomposition of ozone under alkaline conditions.

The effect of ozonation coupled with different photochemical advanced oxidation processes achieves notable degradation of insecticides as a form of water treatment. However, the effect of the synergy between removal techniques is better pronounced with increased TiO_2 surface area, not the amount, due to adsorption of ozone on the TiO_2 surface (Cernigoj *et al.,* 2010). Other methods of photolysis such as UV/H_2O_2 (Abramović et al., 2010), high pressure mercury lamp and xenon lamp (Zhao *et al.,* 2010) and electro-catalytic degradation using Er- doped Ti/SnO_2-Sb electrode (S. Li *et al.,* 2015) with different modifications have been employed in the photolytic degradation of neonicotinoids and producing relatively similar effects of water/soil remediation.

Table 20. The influence of pH on the formation of Nitrate (v) in thiacloprid degradation

AOP	Time for 10% conversion of organic nitrogen to NO_3 (min)		
	pH 3.2	pH 8.1	pH 11.0
O_3	>10,000	96	53
O_3/UV	130	52	48
O_3/UV/TiO_2	47	38	37
O_2/UV/ TiO_2	300	800	8600

Source: Černigoj *et al.,* (2007)

The use of pesticides, particularly neonicotinoids, as agents of pest control in agriculture has continue unabated. Therefore, to reduce the risk of water pollution by these pesticides, their removal by the use of technologies that will promote their degradation is desirable (Abramović *et al*., 2010). There are several ways to remove organic pollutants from water; either by physical or chemical methods. The chemical treatment methods including activated carbon adsorption, membrane filtration, chemical coagulation, ion exchange on synthetic adsorbent resins, and others known to produce wastes that require additional steps and costs

(Banić *et al.*, 2014). However, to solve the aforementioned challenges, the use of advanced oxidation processes (AOPs) has been proposed to be effective, even providing total degradation of the organic compounds (Pera-Titus *et al.*, 2004).

Direct photolysis depends on the ability of the compounds to absorb the UV photons and their quantum yield, consequently promoting the target molecule to their excited triplet state. The commonly used AOP in the removal of recalcitrant organic pollutants from water is UV photolysis (Banić *et al*., 2014). However, the combination of UV and other photolytic methods such UV/ H_2O_2, UV/Chlorine, UV/7.2Fe/TiO_2/H_2O_2, and Vis/7.2Fe/TiO_2/H_2O_2 and UV/$S_2O_8^{2-}$ have been widely reported under varying experimental conditions (Dell'Arciprete, 2009, Abramović *et al.*, 2010, Cernigoj *et al*., 2010, Banić *et al*., 2014, S. Chen *et al.*, 2018, Acero *et al*., 2019); although a low mineralization rate of some of these methods has been indicated (Kah *et al*., 2018).

Several photolytic degradations of neonicotinoids have been carried out involving the use of UV irradiation in the laboratory. The condition of irradiation has always been in the extreme, with the use of a UV source lamp with high power such as 300 W (Zhao *et al.*, 2010), 125 W (Abramović *et al*., 2010), and 100 W (Dell'Arciprete *et al.*, 2012). However, there is an increase in the use of UV source lamps with lower power ratings, as recently attempted at 65 W (Kah *et al*., 2018), 55 W (L. Chen *et al*., 2018), 15 W (S. Chen *et al*., 2018) and even 8 W (González-Mariño *et al*., 2018) with good degradation results. Although the use of a UV source lamp with high power increases the light intensity contact between the photons and the analytes in the solution, the use of a UV lamp with lower power is regarded to be cost effective and much more desirable in the face of energy management. Naeem and Ouyang, (2009) reported that horizontal positioning of the UV lamp source was better than vertical positioning and enhanced degradation was recorded with increased in power of UV lamp in the degradation of phenol compounds. The importance of the position of the UV source is

rendered with little significance because the 2 components of the incident solar radiation (direct incident light and scattered light), under natural conditions, can enter a substrate at various angles (Kurwadkar *et al.*, 2016). Therefore, the photodegradation of organic compounds directly depends on its UV absorption profile.

With the aim to advance our understanding of the fate of neonicotinoids in the water environment and to support the development of robust exposure assessment procedures covering varying weather conditions, we have decided to evaluate the photolytic degradation of four neonicotinoids under UV source lamp with a very low power in this work.

4.2.2 Materials and methods

4.2.2.1 Chemicals and materials

The concentration of each pesticide (ACE, IMI, THA and THX,) used in this set of experiments was 5 ppm. The chemicals used in this work were as described in section 2.1. The volume of solution irradiated was 5 mL. Glass petri dishes were used to hold the aqueous sample in position to be irradiated. The light source was the "GL-58" hand-held UV lamp, which emits photons with wavelengths in the range 254-365 nm, a total power output of 6 watts, operated at 0.16A/230V. The method of the LC-MS system used in the analysis of the analytes in this work was as described in section 2.3. Nanopure water (18.2MV.cm at 25 °C), processed through a Millipore Simplicity water purification system was used throughout this experiment.

4.2.2.2 Photolysis of samples

All the prepared pesticides solutions, at concentrations of 5 ppm, were covered and wrapped with aluminium foil in sample bottles and kept refrigerated prior to their irradiation. At the start of the experiment, 5 mL of each insecticide solution at 5 ppm concentration level was placed individually in glass petri dishes in triplicate and was continuously irradiated for a period of 6 hours with the surface of the sample directly open to the UV source in an enclosed UV lamp (Figure 16) box. Prior to the start of the experiment, the lamps were equilibrated (warmed up)

for 30 min. The distance between the UV source and the surface of the sample was measured at 15 cm and this was kept the same in all the experiments. The course of photolysis of the pesticides in aqueous solution was followed.

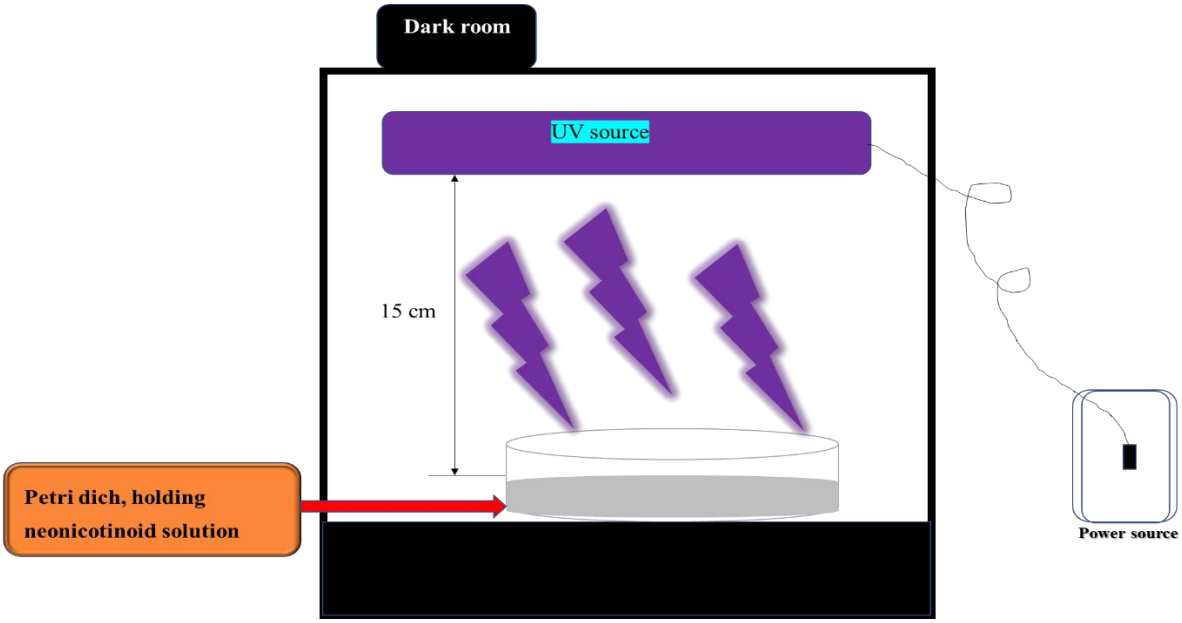


Figure 16. Photocatalytic degradation set up experiment in a dark system using UV source lamp with 6W

From each of the triplicate dishes, 50 μL aliquots were taken after UV exposure of 0, 5, 15, 30 minutes, then 1, 2, 3, 4 and 6 hours. The total amount of sample taken was 8 % of the total initial volume of sample exposed to UV light. The stability of the neonicotinoid insecticides in aqueous solutions in the dark at room temperature was assessed to confirm that degradation was only limited to the UV source exposed during the experiment. Triplicate samples of the solution (2 mL at 1 ppm concentration of the individual insecticide) was kept in the dark at room temperature and in amber sample vials for three days. An aliquot sample was taken each day from each vial and was analysed with the LCMS.

The amount of neonicotinoids loss due to evaporation was assessed by weighing each sample petri dish before and immediately after exposure and this is followed by weighing the same petri dish after taking out each aliquot before the exposure again. This procedure was repeated throughout the duration of the experiment. The weight difference of each sample at every set exposure time was considered as correction factor (Appendix 8) and was applied in all the calculations of the final concentrations.

4.2.2.3 LC-MS/MS analysis of neonicotinoids

The neonicotinoid insecticides left in solution after UV exposure was determined by the addition of internal standard and filtered through 0.22 μm PTFE filter prior to their injection into the LC-MS/MS instrument. Analysis of the neonicotinoids was done with the method devised by Agilent LC-MS instrument as described in section 3.3.1 – 3.3.2 for the optimisation and detection sensitivity of the LC-MS method. The amount of pesticides, after degradation, was quantified from the difference between the amount of pesticide in the initial solution at time = 0 minutes and the amount of pesticides left in the solution at various set times, 0 – 6 hr.

4.2.3 Results and discussion

4.2.3.1 Degradation of neonicotinoids and the effect of water on photolysis

4.2.3.1.1 The stability of neonicotinoids in aqueous solution

The stability of the four compounds was evaluated in the dark at ambient temperature to confirm that the only degradation that occurred during the sample degradation study was because of exposure to UV source. The results showed that the four compounds were stable when kept out of light as shown in figure 17. The average percentage loss over the three days for each insecticide was 1.39, 1.43, 1.65 and 1.73 % for ACE, IMI, THX and THA respectively. Therefore, the compounds stay indefinitely stable in aqueous solution when protected from UV light. Similar results were reported by Banić *et al.*, (2014) and González-Mariño *et al.*, (2018) with the

concentration of the tested neonicotinoids remaining constant, proving the absence of thermally-related degradation processes.

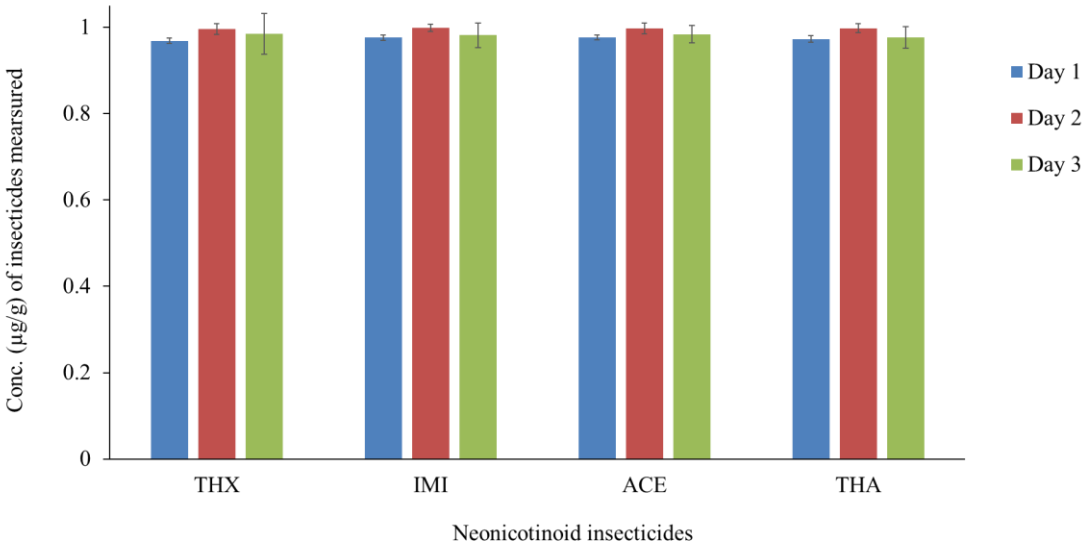


Figure 17. The stability of the 4 neonicotinoids, at 1 µg/g concentration spiking level, in aqueous solution when kept in the dark at room temperature and examined each day for three days (n = 3).

4.2.3.1.2 Photolytic degradation of neonicotinoids under UV source

To date, not much has been published regarding the photodegradation of neonicotinoids in the natural environment. Measurement of the photolytic degradation of pesticides, including neonicotinoids in the natural water environment such as rivers and lakes is almost near impossible. Therefore, results of the photolysis of neonicotinoids in the laboratory environment is desirable in understanding and predicting their fate in such environments with almost certainty. In this work, the UV photolytic degradation of four neonicotinoid insecticides were assessed under a UV source lamp at a low power rating and they all showed different amounts degraded.

The UV photolytic degradations of the four neonicotinoids were assessed with considerations for the loss on evaporation (LoE). This was achieved by applying the correction factors to the

concentration of the compound assayed at each time the aliquots were taken throughout the duration of the experiment. The corrected results (Corrected due to LoE) against the normal experiment without the correction factor (Normal with LoE) are shown in Figure 18 for all the compounds tested. From the results, there was no effect of evaporation on the THX, probably due to its fast degradation, since most of the loss on evaporation happened between the 4 to 6 hr exposure period and almost 100 % of the compound had degraded. However, for THA, ACE and IMI, a noticeable loss due to evaporation, albeit less than 10 %, happened after 6 hr of exposure. Therefore, we can conclude that future UV degradation studies of insecticides in laboratory conditions above 6 hr probably needs to allow for compensation for the loss due to evaporation in order to obtain reliable and reproducible results.

Acetamiprid and imidacloprid residues were detected in the solution at 6 hr (Figure 18A & B) of exposure to the UV source while, in less than 1 hr, thiamethoxam showed 100 % degradation (Figure 18D). Thiamethoxam is less resistant to photolysis compared to thiacloprid, which lasted for about 4 hr before complete degradation (Figure 18, C and D). Interestingly, after about 1 hr of exposure of imidacloprid to the UV source, the photolytic degradation rate was fast, and equilibrium appeared to be established at almost less than an hour with the same amount of the compound remaining in the solution throughout the exposure time (Figure 18B). Perhaps, what is more interesting is that the compound remained almost the same throughout the entire experiment with no further visible degradation taking place. This imidacloprid behaviour is probably attributed to the presence of a transformed product that resists further UV degradation. Therefore, the bio-refractory and recalcitrant nature of these compounds suggests that they cannot be totally removed by solely conventional bio-chemical treatment of wastewater.

The refractory nature of these compounds may be attributed to the electronic distribution and spatial arrangement of functional groups, with nitroguanidine demonstrating lower resistance

than cyanoimine (Banić *et al.*, 2014). Therefore, thiacloprid and acetamiprid may persist in the environment longer than other neonicotinoids, with a better chance of killing target insects. However, their presence in the food chain may raise toxicity concerns. Also, the transformed product of neonicotinoid photolytic and photocatalytic degradation, particularly imidacloprid, may pose additional environmental stress that requires further studies; however, this may depend on other influencers.

The presence of plants, soil and water will influence the rate of photolysis and therefore their individual persistence (Schippers & Schwack, 2010). Also, natural organic matter such as humic acid, and inorganic anions including bicarbonates, nitrates, nitrites, and chloride are known to affect the rate of photolysis in aquatic ecosystems (Abramović *et al.*, 2010, Acero *et al.*, 2019). While some dissolved substances promote photodegradation by generating reactive free radicals, others attenuate or inhibit photolysis by scavenging free radicals such as $OH^{\bullet}$, and, in anaerobic environments, Fe^{2+} will be certainly oxidized to Fe^{3+} (the so-called Fenton reaction).

A kinetic study of the four compounds in aqueous solution was evaluated by plotting the residues of the compounds against time, and the regression equation of the best fitting curve was estimated by the value of the square of the correlation coefficient (R^2). The dissipation kinetics of the compounds were calculated from the first-order equations (Eq. 12) which appear to describe the kinetic data well (see Figure 19):

$$\ln C = \ln C_o - kt \qquad \text{Eq. (12)}$$

where ln C is the natural log of the amount of residue in solution after a given time, t (min) and $\ln C_o$ is the natural log of the initial amount of the compound in solution at time zero, while k is the rate constant, (s^{-1}).

The effect of loss due to evaporation was evaluated on the linearity of the applied first order rate equation results as shown in (Figure 19). The R^2 values, calculated with a correction factor

due to loss of evaporation, ranged from 0.928, 0.987 and 0.997 for ACE, THX and THA respectively. However, IMI showed very poor regression correlation when a first order kinetic model was applied with R^2 of 0.167 (Figure 19 B). Similarly, the R^2 values that were calculated without consideration for loss on evaporation ranged from 0.936, 0.988 and 0.997 for ACE, THX and THA respectively. Also, a poor R^2 value of 0.422 was recorded for IMI. First-order reaction kinetics is demonstrated by dinotefuran, imidacloprid and thiamethoxam in aqueous and soil media, so reported Kurwadkar et al., 2016. This was also reported for thiacloprid in spring onions (Dasenaki et al., 2016). All the compounds, except imidacloprid, showed a good fit with first-order equations; thus, for this reason, the R^2 value for imidacloprid was not presented in the graph (Figure 19).

The lack of good linearity in the imidacloprid data (Figure 19 B) showed that the degradation of the insecticide cannot be described with the use of a single first order kinetic model. The European Commission (European Commission, 2000) and Organisation for Economic Co-operation and Development (OECD, 2002) regard a coefficient of determination ($R^2 < 0.7$) as questionable and invalid. Thus, an attempt to fully describe the imidacloprid data with a non-linearity model, following the exponential description as stated in Eq. 13, was undertaken.

$$Y = Ao.[1 - \exp(-kt)] \qquad \text{Eq. 13}$$

where Y is the response variable in concentration, t is the explanatory variable (time), Ao is the maximum Y value, k is a rate constant that determines the steepness of the curve and was computed based on Excel Solver (Microsoft 2016). The result of the non-linearity model, as applied to the imidacloprid data, did not produce a good fit in both conditions, with or without the consideration of loss on evaporation. Similarly, the residual plots of the two conditions showed a very poor homoscedasticity with lack of good distribution of the data points around the origin of the graph. In view of these poor results, the nonlinearity model was discarded.

The degradation profile exhibited by imidacloprid in this work may be due to its biphasic photodegradation nature. It is possible that imidacloprid, due to its chemical structure, may have degraded into products that are more resistant to photodegradation in the water environment, a behaviour that is different when studied in the soil environment (Kurwadkar et al., 2016).

Degradation of pesticides in the environment cannot always be described by Single First Order (SFO) kinetics (Whitmyre et al., 2004). A fast initial decrease in pesticide concentrations is often followed by a slower decline. This is usually referred to as a bi-phasic pattern of pesticide degradation. Therefore, the imidacloprid data was re-evaluated considering the possibility of a two phase kinetics (Figure 20). The first (usually the fast step) phase kinetic gave a good regression linearity (R^2 = 0.9414) and the second phase regression linearity was R^2 = 0.8771, for the compound when loss of evaporation was not considered (Normal). However, both the first and second phase regression linearity for the same compound, when loss on evaporation was considered (Corrected), were excellent with R^2 values of 0.9906 & 0.9995 respectively. The good fit observed in the biphasic kinetics of imidacloprid data may be due to the presence of a "change point" caused by more than one mechanism in its degradation. Therefore, biphasic kinetics provided a better description of the form of the dissipation curve for imidacloprid, specifically where the SFO and nonlinearity models failed to describe the data.

The persistence of a chemical in environment can be characterized by its half-life (t1/2), which is the time required for a concentration of a chemical to be halved (Wang et al., 2016). The first phase of imidacloprid degradation was very fast, with a half-life of 20 min while its second phase degradation showed the highest half-life of about 28 hrs, while thiamethoxam quickly degraded to half its original amount in about 11 min (Table 21). Considering the high polarity and solubility of thiamethoxam, the compound may be removed from the environment faster with less environmental risk when compared to other neonicotinoid insecticides. However, the degradates

of imidacloprid in the environment may persist longer with risk to the aquatic animals. The photochemistry of the chromophores, =N – CN, =N–NO_2, and =CH – NO_2, in neonicotinoids are marked by different pathways, such as photoisomerization, photocyclization, cycloaddition, and intramolecular hydrogen abstraction. They continue to generate interest in research, particularly in the area of photoswitchable insecticides (Xu et al., 2015, Li et al., 2016).

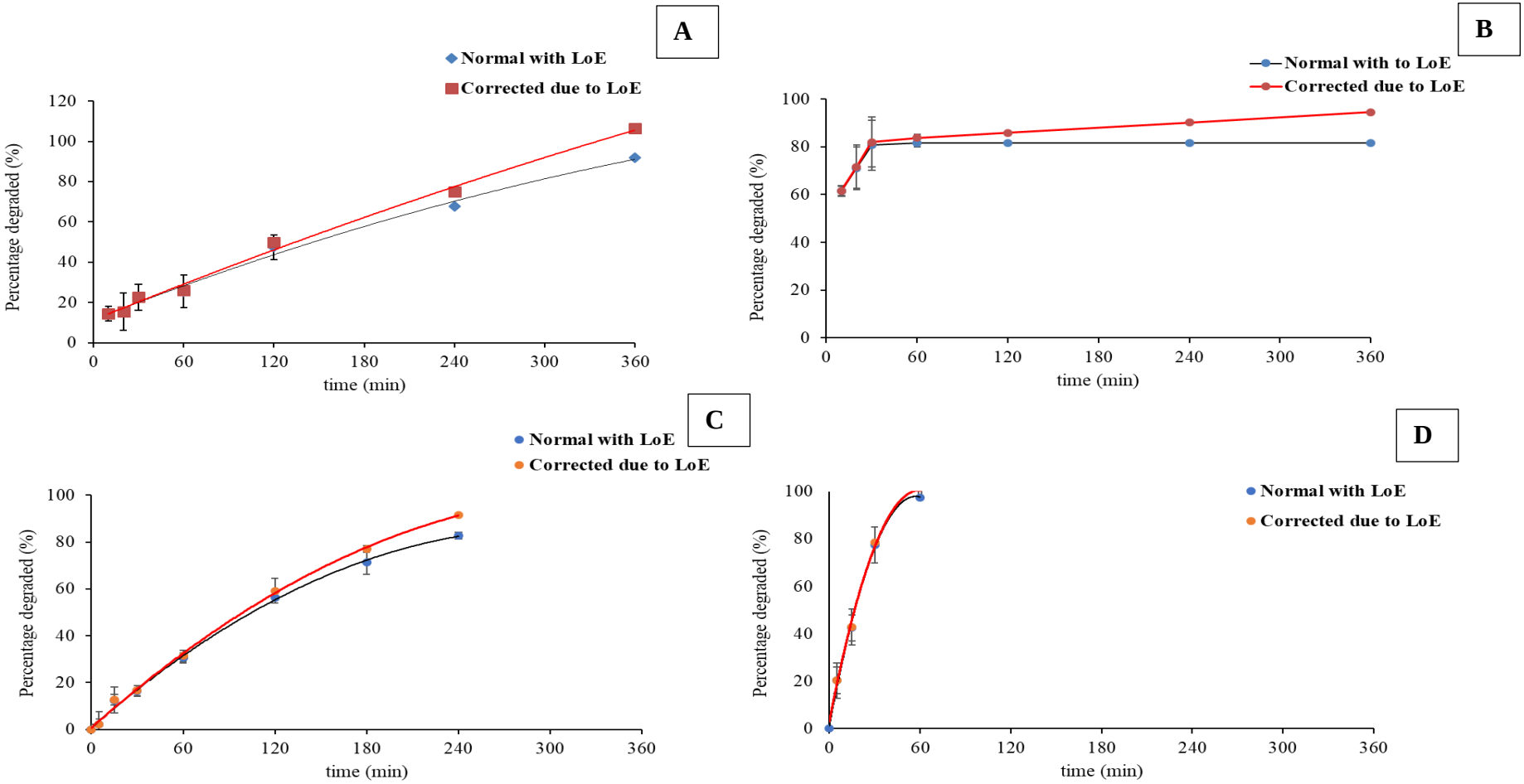


Figure 18. The percentage degraded for (a) acetamiprid, (b) imidacloprid, (c) thiacloprid and (d) thiamethoxam when exposed to UV light in aqueous medium for 6 h.

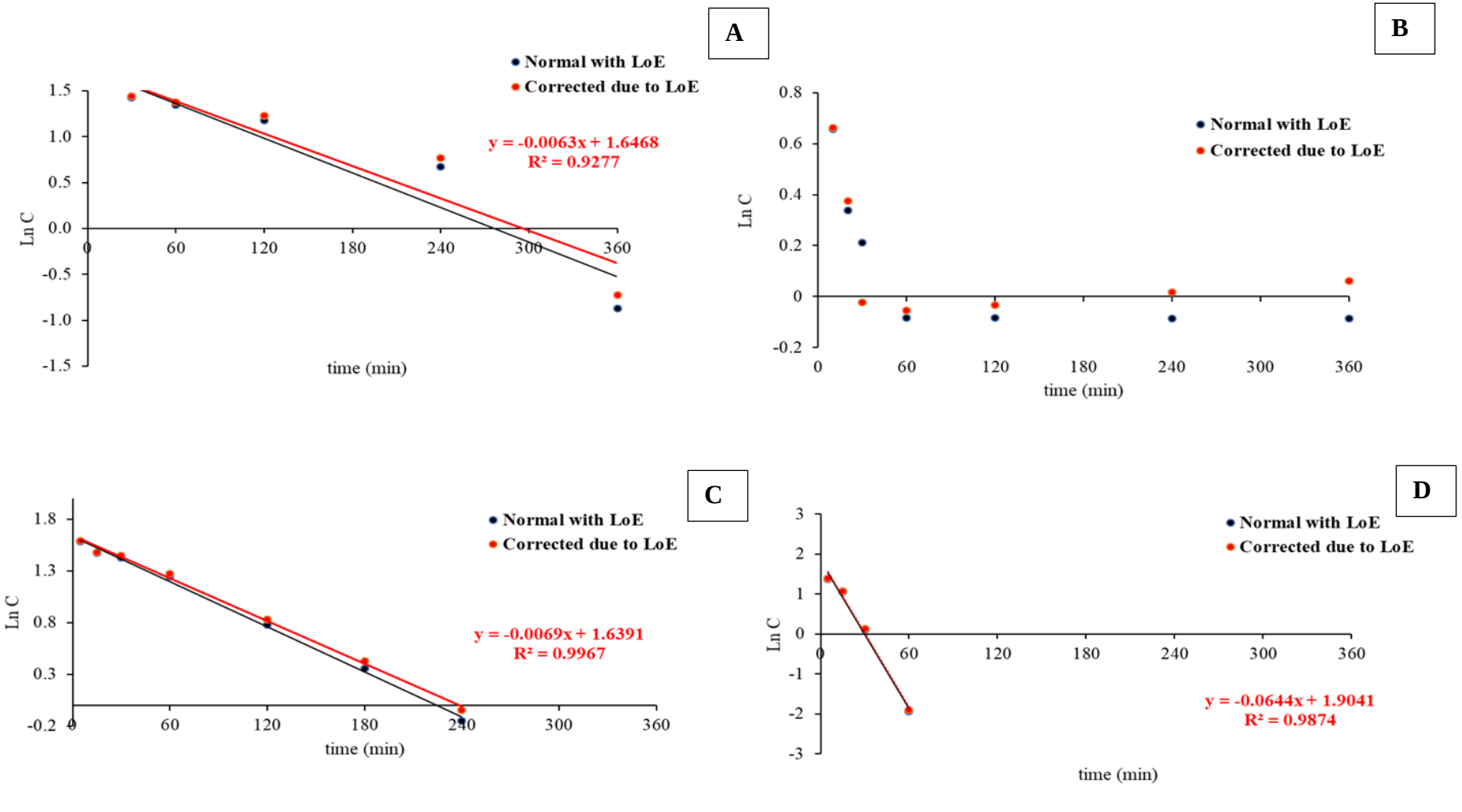


Figure 19. Linear plot of the first order kinetics for dissipation of (a) acetamiprid, (b) imidacloprid (not following a first order kinetic model), (c) thiacloprid and (d) thiamethoxam when exposed to UV light in aqueous medium for 6 h

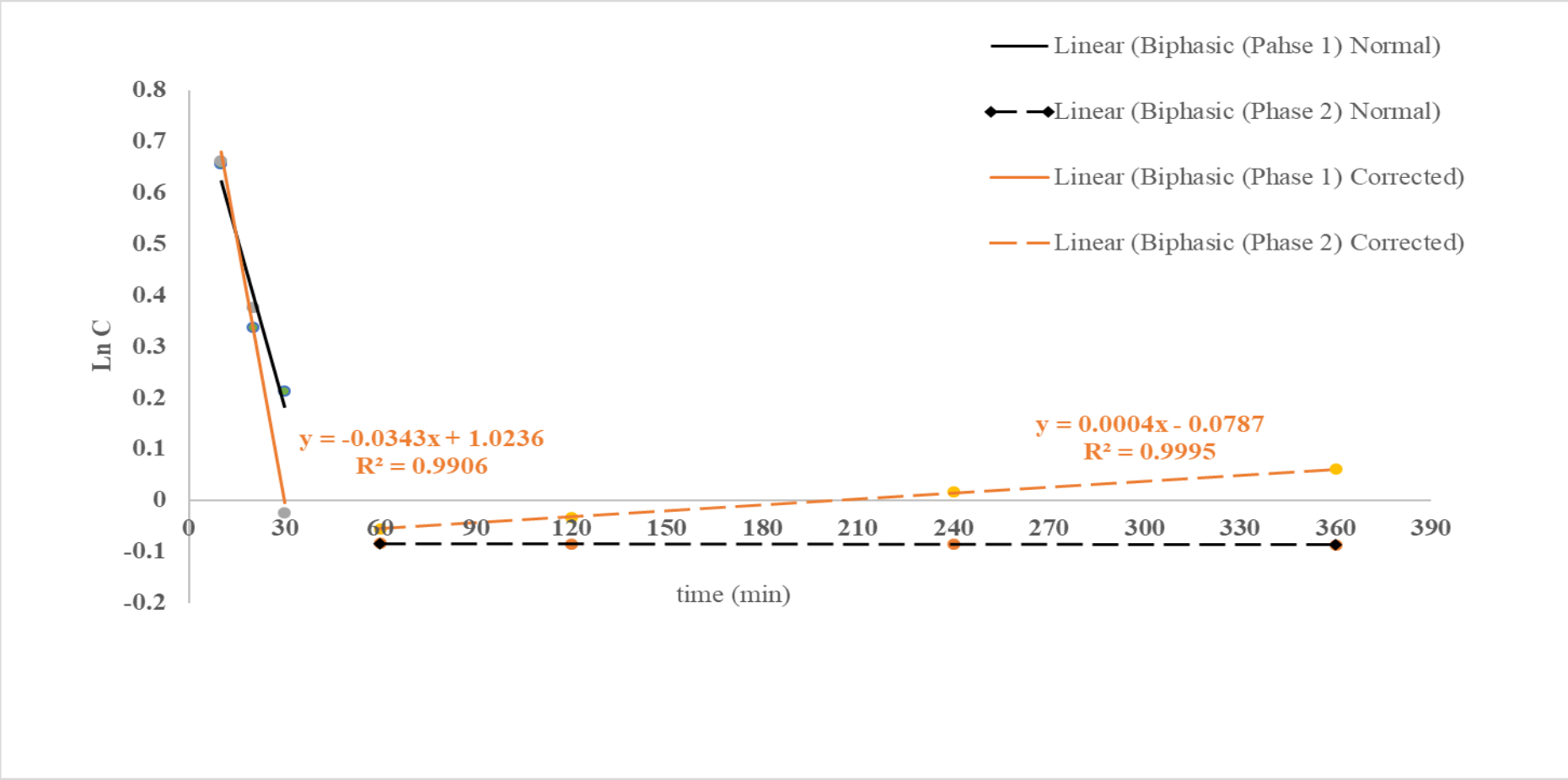


Figure 20. Biphasic dissipation kinetics for linear regression of natural log-transformed imidacloprid data with time (min) after UV light exposure (Normal) and its corrected value on loss on evaporation (Corrected). Description of cases: first phase of biphasic kinetics, time 0 through 30 min in black (Normal) and orange solid lines (Corrected), second phase of biphasic kinetics, time 60 min through 360 min in black (Normal) and orange (Corrected) broken lines.

Table 21. The parameters of first-order rate reaction: rate constant, half-life, and regression coefficients for four neonicotinoids

Neonicotinoid	R^2	Rate constant, $K\ (min^{-1})$	$t_{½}$ (min)
Acetamiprid	0.9277	0.0063	110.02
Imidacloprid	0.9906 (biphasic phase 1)	0.0343	20.21
	0.9995 (biphasic phase 2)	0.0004	1732.87
Thiacloprid	0.9967	0.0069	100.46
Thiamethoxam	0.9874	0.0644	10.76

4.2.4 Conclusions to the degradation of neonicotinoids in water

The photodegradation of the four neonicotinoid insecticides, thiamethoxam, imidacloprid, acetamiprid and thiacloprid, under UV irradiation have been investigated in water solutions in order to assess their persistence in the environment and possible transformation into other potentially more toxic species. This radiolysis study revealed that all the four neonicotinoids studied are photodegradable in aqueous medium when exposed to a UV source under laboratory conditions at low UV power source. All the compounds tested, except for imidacloprid, followed first-order model and with half-lives varying from 11 min to about 2 hr.

However, a biphasic kinetic reaction was applied to describe the degradation profile of imidacloprid with a good R^2 value reported for each of the two phases. The first phase of the imidacloprid degradation showed a very fast half-life of 20 min and the compound showed the possibility of persisting in the water environment for over 28 hrs. In contrast, thiamethoxam was rapidly photodegraded with the lowest half-life value of less than 11 min and showed itself to be the least stable; an indication that it may be less persistent in the environment. However, the

degraded metabolites and intermediate compounds of thiamethoxam may need to be reviewed for persistence as well as their toxicity against non-target organisms in the environment. The behaviour of the photolytic degradation of imidacloprid showed that a more resistant product to degradation may be produced in the water environment with potential to persist longer in the environment than the parent compound. This is a possible exposure to aquatic animals and even in the drinking water available to humans and requires further research.

We have been able to report considerable degradation of these compounds with a UV source at a low power rating and with the possibility to manage cost and energy efficiently. To achieve total degradation of these compounds with high mineralisation, a more advanced oxidation process of photolytic and photocatalytic degradation is suggested. However, the use of a UV source at low power rating is hereby encouraged.

4.3 Occurrence of neonicotinoids in environmental and food samples

4.3.1 Introduction

The extensive applications of neonicotinoid insecticides have led to global reports of their detection in plants (Zhang *et al.,* 2012 and Jiang *et al.,* 2018a), soils (Dankyi *et al.,* 2014), honeybees and bee products (Kasiotis *et al.,* 2014, Daniele *et al.,* 2018), drinking and surface waters (Klarich *et al.,* 2017 and Sánchez-Bayo *et.al.,* 2014), urine & hair in rabbits and humans (Kavvalakis *et al.,* 2013), and guttation drops (Tapparo *et al.,* 2011). Honeybees, bumblebees and butterflies that are attracted by flowers of commercial crops and wild plants are at risk of exposure when collecting nectar and pollen from plants that were previously treated with neonicotinoid insecticides (Botías *et al.,* 2016). The residues of these insecticides and their metabolites, if found above the required MRL, probably due to excessive use or their lack of mobility may be detrimental and toxic to existing soil fauna like earthworm and millipedes that are of immense benefit to the ecosystem.

However, the implementation of the two-year (2013 – 2015) moratorium by the European Union on the limited use of neonicotinoids, particularly imidacloprid, thiamethoxam and clothianidin, on the flowers of plants which attract bees has significantly led to reduction in their use as insecticides. One year after the moratorium, no change was reflected in the amount of imidacloprid and acetamiprid used as insecticides in the UK. However, a significant decline in the amount of thiamethoxam use as insecticide was recorded when compared with thiacloprid during the same period of 2015 – 2016 (Figure 1A &B). Therefore, the levels of neonicotinoids in the environment, long after their effective prohibition date 29 May 2018 designated by the Commission Regulation (EU) 2018/783 -785 (EU Directives 2018), may be assessed and used as possible means of predicting the potential scale of exposure to bees.

The MRL, usually 100 times below the no observable effect level (NOEF), is established to minimise risks to consumers and for trading standards purposes, hence, any food with pesticides level above its established MRL is removed from trade channels. Therefore, monitoring the residual levels of these insecticides in crops and flowers of marginal plants like sunflowers, white clover and daisy where non-target bees collect pollen and nectars for making honey and wax that are consumed by humans, is important to human health risk assessment.

Several publications report the determination of neonicotinoid insecticides in honey, wine, ginger, tea, drinking and river water, honey bees, beeswax, pollen, guttation drops, cotton seed, eels and soils (Mohan *et al.*, 2010, Tapparo *et al.*, 2011, Jovanov *et al.*, 2015, Kasiotis *et al.* 2014, Yáñez *et al.* 2013, Hladik *et.al.* 2014, Dankyi *et al.* 2014, David *et al.* 2015, *Giroud et al*. 2013, Botías *et al.* 2016 and Jiang *et al.* 2018). However, fewer methods have been developed to determine the trace residual level of neonicotinoid insecticides in plants' flowers, wheat, beeswax, honeybees and soil simultaneously at trace levels below their European Union (EU) established MRL (Dankyi *et al.* 2014, Jiang *et al*., 2018).

Different extraction and pre-treatment methods have been employed in the extraction and clean-up of neonicotinoid insecticides in plants and soil matrices with little or no matrix effect. Solid-phase extraction (SPE) was found to be commonly applied in sample pre-treatment with good rapidity, convenience and clean-up efficiency, therefore, suitable for routine analysis. However, no simple, fast and robust extraction and SPE methods has been developed for the determination of thiamethoxam (THX), imidacloprid (IMI), acetamiprid (ACE) and thiacloprid (THA) residues by LC-MS/MS in five different samples namely flowers of cultivated and marginal plants, wheat grains, honeybee, bee wax and soil matrices.

In the present work, this is the first time a method is applied to the determination of four neonicotinoid insecticides in six species of plant's flowers that attract bees, winter wheat

grains, honeybee, beeswax, honeycomb and soil samples that were obtained from eight sites in South East of England. In addition, the amount of neonicotinoid residual left in the soil five months after harvesting rapeseed crops that were previously treated with neonicotinoids were determined and compared with the amount detected in the plant' flower. This is to possibly establish their potential to be retained and how much may be introduced into the food chain.

The objective of this section is to modify and improve the extraction method of an existing method with better recovery to establish the presence of selected neonicotinoid insecticides in multiple environmental samples such as plant flowers that attract bees, wheat grains, honeybee and honey wax. The soils from the site, where neonicotinoids are detected in the flowers of plants previously sampled, were assessed to possibly determine their persistence in the environment and their level of contamination after use. Also, an additional objective was to assess the impact of the Regulation on the usage of the neonicotinoid insecticides with possible effect on risk of bee health.

4.3.2 Materials and methods

4.3.2.1 Collection of samples of wheat, plant flowers, honeybees, beeswax and soil

All the samples used in the extraction work in this study were collected as previously described in sections 3.4.1.

4.3.2.2 Extraction of neonicotinoids from wheat, plant flowers, honeybees and beeswax

About 1 ± 0.001g of the homogenised wheat and plant flowers samples was weighed into polypropylene centrifuge tube and 10 mL, each of hexane, acetonitrile and water, was added in turn. Thereafter, the extraction steps in section 3.4.2.1 were followed as describe in Figure 20. In the case of honeybee and bee wax, 0.5 ± 0.001g of their homogenised sample was weighed to reduce signal interference because of their complex matrices. Also, the extraction steps as explained in section 3.4.1 were followed.

The extraction recovery of neonicotinoids in the matrices of marginal plant flowers and wheat grains had been previously assessed as detailed in section 3.4.2.1. Also, prior to the quantification of neonicotinoids in honeybees, bee wax and OSR (cropped plant), the extraction recovery of the method was assessed at 0.5 ± 0.001 for each honeybee and bee wax and 1 ± 0.001g for OSR of the homogenised samples following the extraction steps as outlined in section 3.4.2.1 and shown in figure 21.

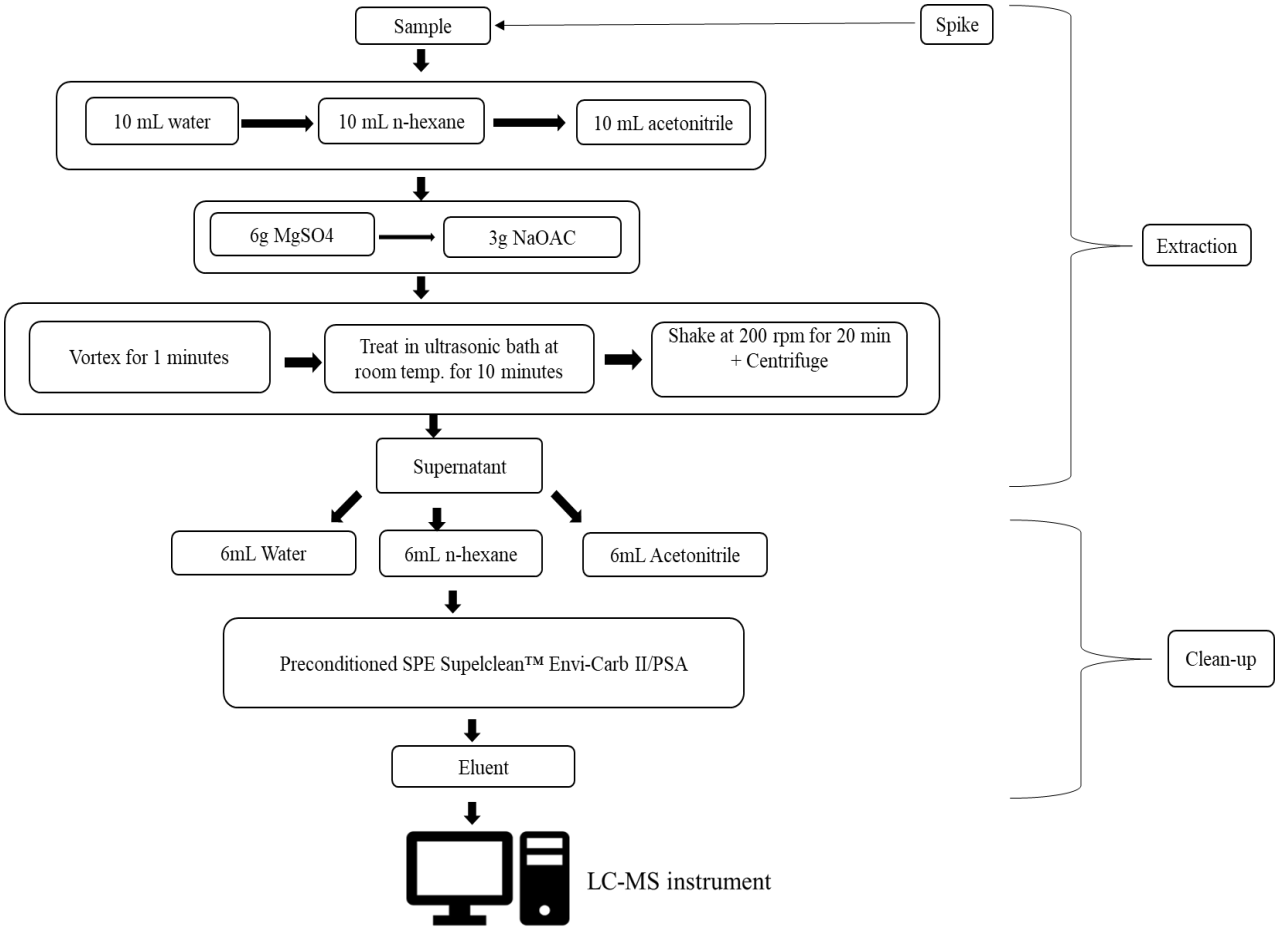


Figure 21. Flow diagram of the modified QuEChERS procedure for sample extraction and clean-up showing the spiking level for recovery estimation.

4.3.2.3 Extraction and analysis of neonicotinoids from soil

4.3.2.3.1 Soil sampling from Essex farmland and characterisation

Upon the detection of neonicotinoid insecticide in the OSR flower in one of the sites, samples of soil from the same site was taken 5 months after the flowers were sampled. The soil samples were collected using auger and sample bags in 4 locations, about 100m apart (to ensure good coverage of the entire site), from 0 – 20 cm deep and labelled as location 1 – 4 (see Appendix 9). The GPS for the farm location could not be provided due to GDPR guidelines. The soils were air-dried in the dark in the laboratory for 4 days with the lumps and plant debris carefully removed. Soil (< 2mm) was thoroughly mixed prior to pesticides extraction and their analysis.

The characterisation of the Essex soil, being samples from the same farm site with no morphological and geological difference, was done by thoroughly mixing representative sample weight (100 g) from each labelled location (L1, L2, L3 and L4) together until uniform mixture of a single soil sample is achieved and characterised as 38.5 % sand; 33.3 % silt and 28.2 % clay, % SOC 3.4, pH (in water) 6.6 and CEC 35.5 cmol/kg. The characterisation of the soil was carried out as described in ISRIC (2002).

4.3.2.3.2 Extraction of neonicotinoids from soils

Prior to the extraction of neonicotinoids from the soil samples, soils with no previous pesticides' contamination was used as blank after it was previously assessed by shaking the soil: water solution of 5 g of soil in 20 mL of water for 48 hr in the dark and protected with aluminium foil. The filtered extract, after centrifugation and passing through 0.22 µm PTFE filter, was injected into the LC-MS and showed no presence of chromatographic peak signal.

The recovery efficiency of the extraction method was evaluated by weighing about 1 ± 0.001g of finely ground dry soils, with no contamination (used as blank), in 50-mL of polypropylene centrifuge tube and spiked with 0.75 mL of 1.0 µg/g level of the insecticides. The spiked sample

was allowed to stand in the dark for 24 hours. Thereafter, the extraction steps, as stated in Section 3.4.2.1, were followed. Similarly, the validated extraction method, as previously described in section 3.4.2.1, was applied to determine neonicotinoid residues in the real field soil sample labelled ESS soil.

4.3.3 Results and discussion

4.3.3.1 Validation of the determination: sample treatment and analysis

The complexities of sample matrices as well as the trace amount of pesticides present have presented challenges in analyte recovery. The determination of neonicotinoids from a wide range of environmental samples requires a method that is flexible, robust and effective with excellent sensitivity. Therefore, the QuEChERS procedure, with high flexibility for wider range of analytes and matrices (Koesukwiwat *et al.,* 2010), have been largely applied to determine neonicotinoids from numerous samples.

To assess the sensitivity of the instrument and the insecticides selectivity, the quality parameters LOD, LOQ were estimated at a signal-to-noise ratio of 3 and 10, respectively at 1.0 µg/g concentration. The results of the LOD for all the compounds in the 7 matrices assessed ranges from 0.22 – 1.0 µg/kg and the LOQ values for the same compounds were reported in the range of 0.74 – 3.33 µg/kg in the study matrices (Table 22). Good recoveries of all the insecticides were reported for the matrices of honeybees, bee wax, OSR and soils and ranges from 75.28 – 101.05 % when tested at 1 .0 µg/g spiking concentration levels for all the pesticide (Table 23). Similar recoveries of the study insecticides were previously recorded for the matrices of marginal plant flowers (S1F1 and S1F2) and wheat grains as shown in table 14, after optimisation of the extraction method.

Table 22. The instrument signal sensitivity, for recovery efficiency of 4 neonicotinoids in 7 different matrices at concentration of 1.0 µg/g, was assessed with LODs and LOQs (µg/kg) and used as the quality parameters

Matrix assessed	THX		IMI		ACE		THA	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
S1F1	0.74	2.48	0.56	1.88	0.23	0.76	0.23	0.77
S1F2	0.80	2.65	0.28	0.93	0.25	0.83	0.29	0.96
Wheat	0.89	2.97	0.67	2.22	0.22	0.74	0.25	0.83
OSR	0.89	2.97	0.66	2.21	0.22	0.74	0.24	0.79
HBW	0.93	3.09	0.66	2.19	0.22	0.74	0.25	0.84
HB	0.83	2.75	0.74	2.48	0.22	0.74	0.26	0.86
BA-soil	1.00	3.33	0.71	2.38	0.22	0.74	0.27	0.89

OSR (oilseed rape); HBW (honeybee wax); HB (honeybee); BA-soil (soils from site with abandoned farming activities in the last 10 years)

Table 23. The extraction recovery (%) of the neonicotinoids at 1.0 ug/g concentration from 4 matrices at 0.5 ± 0.0001g for bee wax and honeybee and 1 ± 0.0001g for OSR-Dor and BA-soil samples as explained in sections 4.3.2.2 and 4.3.2.3.

Sample name	% of recovery for the neonicotinoids			
	THX	IMI	ACE	THA
OSR	98.60	89.61	94.20	83.00
HBW	94.78	92.71	91.74	85.21
HB	75.28	79.72	85.36	83.83
BA-soil	96.75	92.08	101.05	89.81
Average recovery in 4 sample matrices	91.35	88.53	93.09	85.46

4.3.3.2. Determination of neonicotinoids in plant flowers, wheat grains, honeybees, bee wax and comb

The pollen and nectar of plants are platforms for encounter between neonicotinoids and pollinators. The degree to which insects are exposed is largely dependent on the modes with which neonics are applied, e.g., on seed coating or on foliar. The amount of insecticides detected in wild plants in close geographical propinquity to cultivated farms, contrary to popular expectation, were reported to be high, about ten-fold higher; this is an important source of exposure to pollinators, especially at post-harvest period (Botías *et al.,* 2016).

The eleven samples tested for the presence of the residues of neonicotinoids, spread across seven sites in the southeast of England, were shown in Table 24. From all the samples tested, only thiacloprid insecticide was detected at 1.42µg/g in the rapeseed flower (OSR) from the Essex farm. Other neonicotinoids were not detected and therefore, were not reported. The lack of detectable neonicotinoid insecticides in 12 of the 13 samples, even below the LOD, may be attributed to their physicochemical properties such as the ease of degradation, mobility and non-persistence in the environment. Pesticides' degradation is one of the desired properties of pesticides that determines their occurrence and fate in the environment. Thiacloprid insecticide is reported to be stable in the environment with a high affinity for soil organic carbon (Bonmatin *et al.,* (2014) and this may encourage its persistence in the environment. Also, the regulation of the EU Commission on the restriction of the use of three of the neonicotinoid insecticides, as initially observed after the moratorium period in 2015 – 2016 (Figure 1), as well as the persistent outcry by environmentalists, particularly bee keepers, may have contributed to the reduction in their detection in the environmental samples.

In this study, only thiacloprid insecticide was found in the flower of (OSR) plants after their foliar application. About five months later, less than 0.5% of the amount of thiacloprid insecticide

quantified in the oilseed rape plants was detected in the soils from the same location after these were harvested. The lack of information regarding the amount of the insecticides applied and the final amount of the insecticide in the processed OSR seeds did not allow us to evaluate the amount of this insecticide that may be available in the food chain and their health risk to human.

In the US, the analysis of residue data for seven neonicotinoids collected between 1999 and 2015 by the US Department of Agriculture's Pesticide Data Program (PDP) from several food commodities, including fruit, vegetable, grain, honey among others indicates that low levels of neonicotinoids are present in commonly-consumed fruits and vegetables sold in the US with low detection frequencies (Craddock *et al.,* 2019). The food processing that raw food materials like harvested OSR undergo may significantly lower the residue of any pesticides, usually below the MRL, in the finished food products that is available for consumption. In the same survey conducted by Craddock *et al.,* (2019), neonicotinoids were below the limit of detection in all the honey sampled within the sixteen years of the survey. This is contrary to the popular reports around Europe with the presence of neonicotinoids in honey and honey products (Jovanov *et al.,* 2014, Calatayud-Vernich *et al.*, 2016, Gaweł *et al.*, 2019). There is therefore the need to carry out a comprehensive survey of the presence of neonicotinoids in food commodities in Europe with sensitive and robust analytical equipment devoid of discrepancy.

The influence of the two years moratorium period may be linked to the reduction in the application of neonicotinoids to cultivated plants which may have consequently impacted on their low or non-detection in wild plants growing at their close margins. Therefore, the non-detectable levels of neonicotinoid in flowers of wild plants may consequently reduce the prolonged risk of exposure to pollinators like bees that forage during the crops' post-harvest season during the spring and summer (Botías *et al.,* 2015).

Thiacloprid insecticide is not among the banned neonicotinoids, therefore, this may be the reason for its detection as the only neonicotinoids in the study compounds (Table 24); this is speculative due to the limited detection of thiacloprid in a single plant in our study. However, it is with such as concern that the concentration of thiacloprid detected was at such a high concentration; more than twice the 0.6 mg/kg concentration established as the MRL for thiacloprid in OSR (see Appendix 2).

The detection of thiacloprid insecticide above the established MRL in the OSR may suggest the possible abuse of their use as crop protection are carried out at levels above the recommended application rate by farmers. Also, part of the insecticides that were previously applied to crops in the previous seasons without degradation but were retained in the soil may be available to subsequent cultivated plants that were not treated with insecticides.

This situation warrants further investigation and surveying of this agricultural locale with much larger sizes of plant samples from different spots, over more than one planting and harvesting cycle.

Table 24. Sample description, categorisation and site locations.

Matrix assessed	Category of flower/bees and bee products	Sample name description	Location	GPS	THX	IMI	ACE	THA
White clover	Uncultivated	White clover	Streat, Plumpton, East Sussex	50.910869, - 0.079524	-	-	-	-
Daisy	Uncultivated	Daisy	Streat, Plumpton, East Sussex	50.910869, - 0.079524	-	-	-	-
Wheat	Cultivated	Wheat grains	Streat, Plumpton, East Sussex	50.910869, - 0.079524	-	-	-	-
OSR-Dor	Cultivated	Oilseed rape from Dorking	Strood Green, Betchworth, Dorking	51.217743, - 0.281266	-	-	-	-
OSR-Oxf	Cultivated	Oilseed rape from Dorking	Kiddington, Woodstock Oxford	51.868262, - 1.378852	-	-	-	-
OSR-Ess	Cultivated	Oilseed rape from Essex	North Weald Bassett, Essex	51.740982, 0.132019	-	-	-	1.42 ± 0.09
APF-Enysford	Cultivated	Apple flower from Eynsford	Enysford, Kent	51.374549, 0.213009	-	-	-	-
HC-Braintree	Farmed	Honey comb from Braintree	Shalford, Braintree	51.928167, 0.514070	-	-	-	-
BW-New	Farmed	New bee wax from Kingston	Kingston	51.429423, - 0.262500	-	-	-	-
BW-old	Farmed	Old bee wax from Kingston	Kingston	51.429423, - 0.262500	-	-	-	-
HB	Farmed	Honeybee	Kingston	51.429423, - 0.262500	-	-	-	-

The LOD of each neonicotinoid in every sample can be found in Table 22

4.3.3.3 Neonicotinoids in soil and their residues in the environment post- application

Following the detection of thiacloprid insecticides in the oilseed rape flowers from the Essex farm, soil samples were taken from 4 different locations within the farm 5 months (May – Oct 2018) after the OSR were harvested. The soil samples were analysed separately as described in section 6.2.3 to determine the presence and the amount of neonicotinoids distribution between the plants and soils after harvest. The extraction was in acetonitrile solvent with NaOAc and $MgSO_4$ salts, clean-up was carried out using SPE Supelclean™ Envi-Carb II/PSA cartridge and analysed with LC-MS/MS.

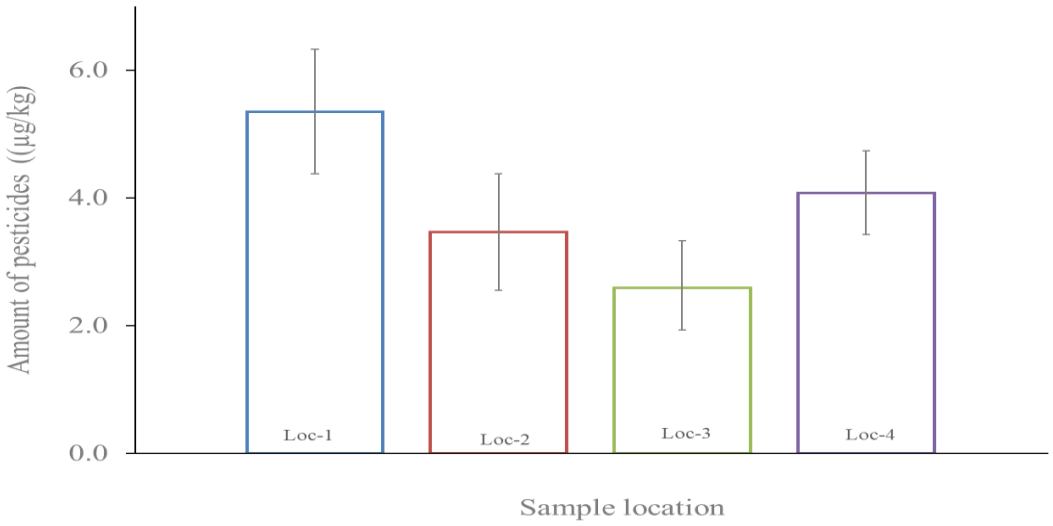


Figure 22. Amount of thiacloprid per gram of soil (µg/g) detected in 4 different locations within a farm site in Essex after 5 months of harvesting OSR planted in the same site.

The results of the analysis show that only thiacloprid was detected in the soil from each location tested in ESS soil and no other neonicotinoids was detected (Figure 22). The range of the concentration of thiacloprid detected was 2.59 - 5.35 ug/kg with the most and least amount detected in location 1 and 4 (L-1 and L-4), as shown in the "Google" image of the farm, where the positions of the respective locations are indicated, have been presented in Figure 23. The highest amount of thiacloprid was detected in L1, followed by L4, with the least in L3, shown in

Figure 22. It can be hypothesized that there is a little mobility of thiacloprid from high region to lower down, and across the farm to the lower region of the farm; this result is in tandem with what we earlier reported in our flow through column studies in the laboratory. However, in view of the limited sampling in this work, this inference may need further research to validate the conclusion.

Thiacloprid may not leach easily through the soil to contaminate ground water or by surface run-offs to areas adjacent to the farm. This result was in agreement with our previous work of assessing and contrasting the leaching potential of thiacloprid and thiamethoxam in soils with divergent %SOC as reported in chapter 4 of this thesis (Section 4.4.3). Thiacloprid showed reduced mobility and low potential to contaminate ground water especially when used in soil high in organic carbon content.

Bonmatin *et al.,* (2014) indicated that neonicotinoids could persist in soil for more than 1000 days, and even for several months, especially when the conditions are unfavourable to degradation and transport. On the other hand, Sanchez-Bayo & Hyne (2014) suspected that neonicotinoids may contaminate the surrounding aquatic ecosystem due to their high solubility. This is so much in consonant with our previous work on leaching where thiamethoxam, high in solubility, readily leach through the soil column, especially in soils low in organic carbon content. The topography of the contaminated soil may promote leaching and mobility of some insecticides such as thiamethoxam, as has been demonstrated in the present work (Section 4.4.3), with low affinity for soil organic content.

The prolonged accumulation of insecticides in the soil post-application may pose a health risk to non-target plants through uptake consequently, increasing the risk their entry into the food-chain (Rundlöf *et al.*, 2015, Botías *et al.,* 2016). Several groups of researchers have reported different concentrations of neonicotinoids in the pollens of target and non-target plants (Girolami *et al.,*

2009, Sánchez-Bayo and Hyne, 2014, Ge *et al*., 2016). However, little is known regarding the differences between their uptake and translocation.

Li *et al.,* (2018) reported the difference between uptake, translocation and accumulation of the five neonicotinoids thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid in a vegetable (Brassica rapa var. perviridis). The report showed no correlation between the uptake of these pesticides and their octanol/water partition coefficient (log K_{ow}), water solubility or dissociation contestant (pK_a). However, the pesticides' molecular weights were significantly correlated with their plant uptake from the soil; more of thiamethoxam and thiacloprid, with higher molecular weight, were sparingly detected in the shoot of the plants than the roots and soil. Therefore, the unique uptake, translocation and accumulation behaviour of these insecticides in plants, with no specific correlation with their log K_{ow} (Li *et al.,* (2018)) need further investigation especially the effect of pesticides concentration, soil pH, moisture etc to effectively evaluate their risk and food safety.

When pesticides are slow to degrade or not at all, risk to some soil faunas that have a role to play in the success of agriculture is increased. Cláudia de Lima e Silva *et al.,* (2017) reported that some of the five species of soil invertebrates studied showed high sensitivity to neonicotinoids while others do not. In addition, Chevillot *et al.,* (2017) proved that exposure of earthworm to neonicotinoids for long periods of time in the soil may affect their reproduction.

As discussed earlier, biodegradation, photolysis and hydrolysis are the chief means for the degradation of pesticides. The role of soil microorganism in neonicotinoid biotransformation have been widely reported (Pandey *et al.,* 2009, Ge *et al.,* 2014, Hussain *et al.,* 2016). However, the toxicity of their metabolites on soil invertebrates as well as their uptake, translocation and accumulation in target and non-target plants is still largely unknown.

To the best of our knowledge, this is the first time, since the prohibition of the neonicotinoid insecticides (thiamethoxam, clothianidin and imidacloprid) by the EU regulation from 30th May 2018, the field determination of four neonicotinoids in pre- and post-harvest in cropped and uncultivated fields is presented as well as the possible impact of the regulation assessed. In this case, only about 0.18 - 0.38 % of the amount in the OSR flowers earlier sampled before their harvest were in the soils from the same field five months after OSR crops were harvested.

The downward trend in the agricultural use of neonicotinoids as insecticides after their ban by the EU commission may be responsible for the reduction in the occurrence of these insecticides unlike their prevalence prior to year 2015 with frequent detection in the environment.

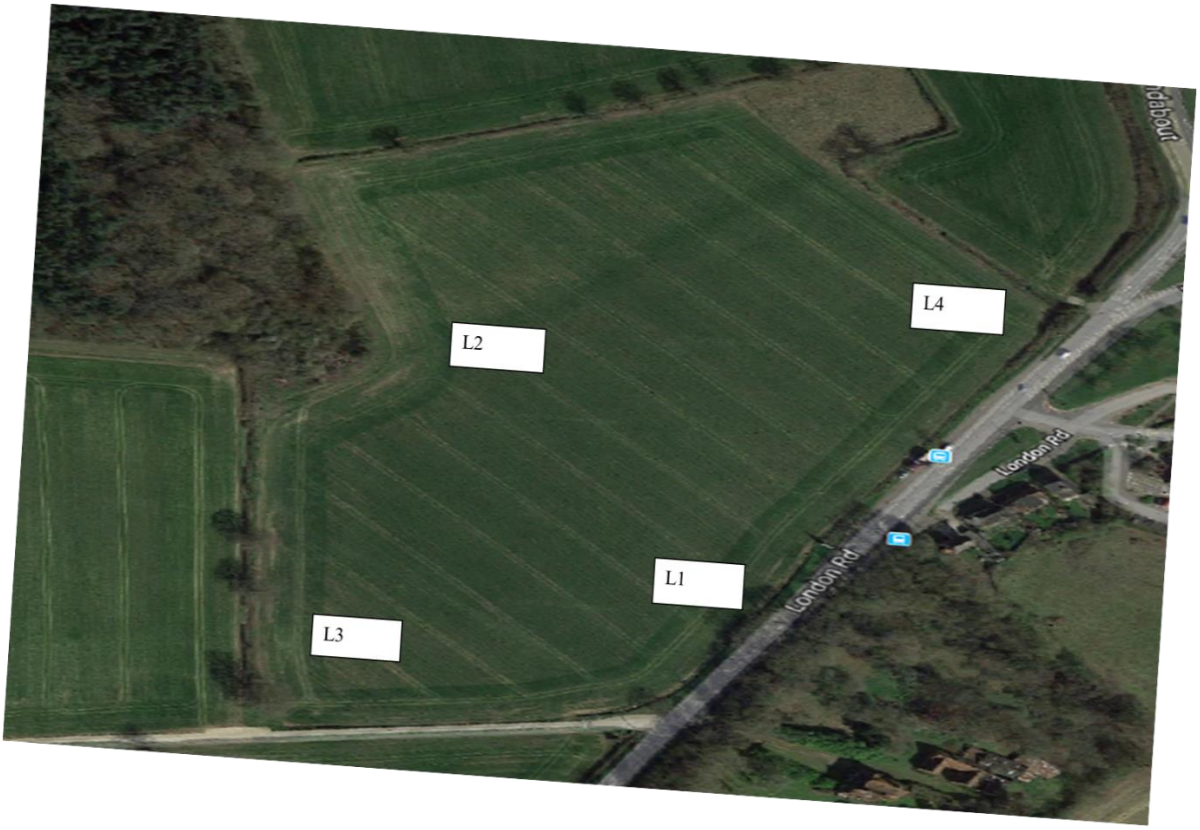


Figure 23. Aerial view of the Essex farm where OSR sampling was made and reflecting the four locations L1, L2, L3 and L4 where soils were sampled 5 months after OSR flowers sampling in May 2018.

4.3.4 Conclusions

The flexibility in the application of the QuEChERS method in extraction and cleanup of complex matrix for better and optimum sample analysis is being exploited in the analysis of samples with complex matrices. The adapted method for the extraction of neonicotinoids presented suitable recoveries (72.24 – 102.67 %) with no chromatographic interference. The LODs of the study pesticides in soil, bee, flowers and wax matrices were in the range of 0.74 – 1 µg/kg; 0.28 – 0.74 µg/kg; 0.22 – 0.25 µg/kg and 0.23 – 0.29 µg/kg for thiamethoxam, imidacloprid, acetamiprid and thiacloprid respectively. The method was successfully applied to all the samples (daisy flower, white clover flower, apple flower, meadow flower, OSR, wheat, honeybee, and bee wax) and only thiacloprid was detected in oilseed rape plants at a concentration double the MRL established by the European Commission for neonicotinoids in oilseed rape plants.

The detection of neonicotinoids in the soil, supposedly months after their application, may be due to their persistent nature which may contribute to the frequency of their detection in soil and aquatic environment many years ahead. Therefore, thiacloprid being the most adsorbed neonicotinoid in our adsorption studies and with low mobility through the soil column test, has the potential to last long in the environment several days after their application. This is similar to atrazine insecticide that was prohibited in year 2004 and was still being detected in surface water about a decade later. Therefore, further monitoring is required to estimate the uptake, trace the translocation, and account for the accumulation of these insecticides in food and the environment.

The effect of the two years of moratorium imposed by the EU which limited the use of selected neonicotinoids from year 2013 to year 2015, and their subsequent prohibition by 30th May 2018, may have contributed to likely drop or non-detection in the occurrence of neonicotinoids in the environmental samples we have reported in the study. The gradual decline in the use of neonicotinoids as plant protection agents suggest that there may be improvement in the quality

of life of pollinators; this is an assertion that requires further investigation in the near future to provide more information on the role of neonicotinoids in the decline of the colonies of bees.

CHAPTER FIVE: CONCLUSIONS OF THIS STUDY AND IDEAS FOR FURTHER STUDIES

5.0 Conclusions of this study and ideas for further studies

5.1 Conclusions from studying extraction and analysis procedure

The developed chromatographic separation of structurally similar neonicotinoids was successful, providing fully resolved narrow chromatographic peaks with minimum tailing. The run time for the separation of the five compounds, including the internal standard was less than 7 min with the LC-UV system (Figure 4B). However, the separation of the compounds was better improved with high resolution in less than 8 min with the LC-MS system (Figure 5A). The developed LC-UV method was adapted successfully to LC-MS/MS and gave excellent sensitivity results with 0.10 – 0.99 µg/kg LODs and 0.34 – 3.30 µg/kg LOQs carried out at 0.001 and 0.005 µg/kg concentration levels. Good repeatabilities (< 12 %) and reproducibilities (< 26 %), at the same level of concentrations, were obtained for the analysis of all the compounds studied.

The challenge of using different methods of extractions and clean ups for the analysis of neonicotinoids from varying samples of complex matrices was addressed in this work. The flexibility of QuEChERS method, with the use of SPE sorbent for clean-up, enabled the development and use of a single method for the extraction of neonicotinoid analytes from flowers, wheat, bees, wax and soil samples by the optimisation and adaptation of the existing method. The centrifugation steps, twice more than the original methods, as well as smaller sample sizes, five times lower than the original method, were optimised.

The adapted method produced better recovery, ranging from 72.24 – 102.67 % in all the seven sample matrices tested when compared to the 81.0 – 98.9 % in only single dietary bee pollen assessed in the original method (López-Fernández *et al.*, 2015). Also, the sensitivity of the method with 0.22 – 1.0 µg/kg recorded as the LOD and 0.74 – 3.33 µg/kg as the LOQ was reported for the neonicotinoids in all the sample matrices assayed. Out of the three solvents tested in the extraction procedure, acetonitrile solvent gave superior recovery for all the insecticides in all the matrices (72 – 101 %) while hexane and water produced poor recoveries less than 5%. The adapted method was sensitive in the determination and analysis of the residues of neonicotinoids from multiple sample matrices namely OSR, white clover, Daisy, wheat grains, apple flower, honeybee, wax and soils. The results of the method agreed with the established MRL for neonicotinoids in the studied matrices by the European Union commission.

The results of extraction and analysis of neonicotinoids from different matrices in this work is an indication of their systemic nature with the ability to be transported by the vascular systems of plants to the anterior parts such as stems and flowers, hence, raising their potential risk to non-target insects and humans. Neonicotinoid insecticides are generally considered to be high in polarity and solubility when compared to other insecticides and are likely to be removed from the soil environment through leaching and run off of surface water. However, they equally have potential to persist in the environment and may degrade to more toxic degradates depending on the condition of the surrounding environment. Therefore, the availability of the degradates of these insecticides in the environment with their potential to enter into the biotic system of the ecosystem requires monitoring to ensure environmental safety in view of their persistent nature.

5.2 Conclusions of studying the fate of neonicotinoid insecticides and their occurrence in the environment

In this work, the sorption and leaching behaviour of some widely used neonicotinoid insecticides namely dinotefuran, thiamethoxam, thiacloprid, imidacloprid and acetamiprid were investigated in five soils of widely contrasting organic contents (0.8 – 12.5 %). The soils originate from four locations in the South-East of England and one from Scotland. Thiamethoxam, the most widely-used neonicotinoid in UK (Figure 1) was the least adsorbed, while thiacloprid, the second most widely-used neonicotinoid in UK during the same period, was the most adsorbed with the highest adsorption capacity (0.17 and 31.58 µg thiamethoxam /g soil and 5.93 and 115.33 µg thiacloprid /g soil) in all the five soils tested at doses 2.5 and 25 µg/g in solution respectively.

The adsorption capacities of the study neonicotinoids in the contrasting soils were significantly different (p 0.05). Except for imidacloprid and thiamethoxam, correlation between organic content of the soil and their uptake by the soil was not apparent; however, correlation was only noticed for imidacloprid and thiamethoxam at higher concentrations. The implication is that these compounds, particularly thiamethoxam with low log K_{ow}, have great potential to contaminate ground water especially when used in a soil poor in organic carbon. In contrast, thiacloprid, the most adsorbed insecticide, is expected to be more retained in soils high in organic carbon.

In the flow-through experiments conducted, based on the neonicotinoids with the most and least adsorption capacities on two soils with divergent organic carbon contents (TH and BR), as expected, the soil high in organic carbon obtained from a garden in Thornton Heath (Surrey), prolonged the elution of the pesticides, four times with thiamethoxam and forty-eight times more with thiacloprid. Therefore, the results show that thiacloprid will be rapidly available in

the soil environment if not degraded and soil faunas may be damaged. In the aqueous photolysis studies carried out in this work under UV light, thiacloprid was found to have a half-life of less than 2 hours. Therefore, thiacloprid is likely to persist longer than other neonicotinoids in the temperate region if the soil environment is favourable; though imidacloprid showed a half-life above 14 hr.

Also, in the column leaching studies, we found out that thiamethoxam and thiacloprid tend to leave greater residues in the first half of the soil column (from a 20 cm section of a soil column) with the least adsorbing BR soil, rich in silt, while, in contrast, a soil rich in organic matter (TH) presented most residues from below 20 cm. This has implications in the bioavailability of the neonicotinoids by plants and soil organisms.

The observation that thiamethoxam leached out of soil readily, together with its relatively low affinity for the organic component in soil, indicates a high risk of ground water contamination and ecological impact when applying this widely used neonicotinoid in soils with low organic matter. When studying the fate of thiamethoxam and thiacloprid in contrasting soils with markedly different properties in a soil column, after 13 bed volumes of water had been filtered through, it was observed that the soil with the lowest organic matter had most of the residue of neonicotinoids in the sub-surface (15 cm) of a soil column whereas the soil richer in organic matter presented most of the contamination deeper. This result support the hypothesis that neonicotinoids with low log K_{ow} are less inclined to be adsorbed onto the surfaces of soil particles and with greater potential to contaminate ground water under increase rain fall.

Established kinetic and isotherm models such as hyperbolic, pseudo-second order rate equations, Elovich, Weber-Morris models, Freundlich and Langmuir models were applied to gain further insight into the sorption kinetic and isotherm phenomena of neonicotinoids. Among the kinetic models applied to the sorption of neonicotinoids on soils with different

organic content, a pseudo second order kinetic model (R^2 >0.999) describes the experimental data well. The values of the maximum amount of neonicotinoid sorbed, q_{max}, obtained with the pseudo-second order model were similar to those obtained by the hyperbolic model, but with poor regression coefficients. These results are congruent with those obtained by Fernández-Bayo *et al.,* (2008), whose work on other pesticides, imidacloprid and diuron, with pseudo second order kinetic model R^2 >0.98, was done on different soils.

The sorption isotherm for four of the compounds with dissimilar soil characteristics informs of a low adsorption intensity of thiamethoxam on soil of low organic carbon content (BR soil). This may influence the extent of removal from the environment. Freundlich and Langmuir models fitted very well for the adsorption of thiamethoxam in all the soils tested and with similar results for thiacloprid except in the soil high in organic carbon content. It is projected that these compounds can accumulate in the soils, particularly after prolonged application, with serious health risk to the environment. Also, the sorption results show that the neonicotinoids may be removed from soils by a process well known as "soil-washing", and the water percolated through adsorption columns.

Generally, studies of the environmental fate of pesticides including neonicotinoids are commonly carried out in a single soil type or soils from the same site (Laabs and Amelung, 2005, Gupta, *et. al.,* 2008, Kurwadkar *et al.,* 2014). However, this is the first time, to the best of our knowledge, where the sorption and soil column mobility of five neonicotinoids in five soils from different sites were assessed. This brings more insight into understanding the behaviour of these insecticides in wider environmental conditions such as extreme soil types.

The parameters of neonicotinoid insecticides measured in this work are based on laboratory studies and are intended to simulate field conditions with good indication of their behaviour in soil and to contribute to a computerized differential mass balance model for neonics. Although

sorption coefficients in tropical soils tend to change significantly between laboratory and field conditions, particularly for polar pesticides including neonicotinoids (Laabs and Amelung, 2005). Therefore, to provide more realistic long-term knowledge of neonicotinoid behaviour in the soils, field experimental studies are recommended.

The potential to disintegrate four of the neonicotinoid insecticides in a water environment at pH of about 7 using very low power rating UV source lamp was investigated. The results show that both thiamethoxam and thiacloprid, the most commonly used neonicotinoid in UK, have half-lives of < 2 hr when irradiated by UV. However, imidacloprid evinced a higher half-life of > 14 hr. The degradation of the insecticides was best modelled with simple first-order reaction kinetics. All the compounds gave good regression coefficients (0.928 – 0.997), however, in the case of imidacloprid, the regression coefficient was poor. The degradation behaviour of imidacloprid indicated that a transformed product is yielded, which may be resistant to further UV attack; hence remain stable over the course of the experiment. Also, due to the absence of the parent compound available for degradation, since first-order reaction kinetics are dependent on the initial concentration, a poor regression coefficient was observed. Therefore, the behaviour of these insecticides in aqueous solution may be attributed to their distinct functional group as well as the surrounding environment.

In this work, using a UV source with a low energy of 6W (the lowest lamp source ever attempted) to initiate photolytic degradation of four of the commonly used neonicotinoids, namely acetamiprid, imidacloprid, thiacloprid and thiamethoxam in a water environment showed positive results. However, for complete mineralisation of the target micropollutant, the use of advanced oxidation photolysis is realistic with a UV source lamp at low wattage.

5.3 Suggestions for further studies

In view of the work carried out in this study and the conclusions made, the following suggestions are hereby reported for further studies:

1. In UK, having a relatively low annual average temp, there is the need to comprehensively assess sorption studies of neonicotinoids in soils under divergent temperature in order to monitor the residue levels of neonicotinoids using sensitive and robust analytical equipment and to ascertain the possible dietary exposure of these pesticides as well as the scale of their contaminations.
2. The assessment of dust drifting of pesticides in aerosol were largely on honeybees and other concerned pollinators with small foraging area. However, the extent of impact on non-target areas such as waterways, land and run-off water need to be considered.
3. The effect of temperature, pH as well as pesticides' physical state, have been reported to play a role in their solubility and distribution potential. However, these are yet to be investigated for their uptake by plant before, during and after application of the insecticides. Therefore, the unique uptake, translocation and accumulation behaviour of these insecticides in plant medium, needs further investigation, especially the effect of pesticides' concentration, soil pH, moisture etc. to effectively evaluate their risk and food safety.
4. There is paucity of information about the metabolites and degradates of neonicotinoid insecticides, particularly imidacloprid, since most reports were on the parent compound's residues, which may be more toxic and persist longer in the environment; this, however, needs to be investigated to fully understand their fate in the environment.
5. The need to further assess the effectiveness of advanced oxidation processes with UV lamp source at lower wattage to investigate complete mineralisation of neonicotinoid

insecticides in water and soils under this condition is desirous in improving the efficiency of degradation processes.

6. Soil quality standards have not yet been established for various pesticides in the UK. Therefore, this study is very important for understanding the risk of agricultural soil pollution and will help in developing effective remediation strategies. It also could provide basic data for the establishment of relevant soil quality standards.

Appendix

1.0 Neonicotinoids Insecticides in different matrices and analytical methods employed.

Matrix	Analytes detected	Sample treatment	Levels found (ng/mL or ng/g)	Quantification strategy	Recovery (%)	Column Xtics	Mobile phase/ Flow rate/ Injection vol	Interface-Detector	Acquisition mode	LOD/ LOQ (ng/g)	Reference
Soil	Imidacloprid Thiamethoxam Clothianidin Acetamiprid Nitenpyram Thiacloprid Imidacloprid Dinotefuran	DSPE and QuEChERS	0.27 - 231	NS	80 – 100	Shim-pack XR-ODS C-18, 50 x 2.0 mm, i.d., 2.2 µm. Guard column (30 x 0.18 mm)	Gradient mobile phase Water: ACN/water (95:5, v:v) with (5mmol/L Ammonium formate and 0.1 % formic acid)/ 200µL/min/ ()	LC-MS/MS ESI^{+}@ 500^{0}c @ 5kV	MRM	0.01 – 0.84/0.05 – 2.79	(Maha F. ɔdel-Ghany *et al.*, 2018)

Sunflower seed	Imidacloprid Thiamethoxam Acetamiprid Thiacloprid	Sample homogenised and extracted with ACN and water with NaCl, cleaned up with modified graphene as SPE	0.034 – 0.048	Standard addition (8 levels)	74 - 119	C-18 column (2.1×100 mm i.d., 1.7 µm, Guard column 2.1×5 mm i.d., 1.7 µm	Gradient mobile phase 0.1 % formic acid in Water: ACN 10 µL	UHPLC-MS/MS ESI^+@ 550 ^{0}C @ 5kV	MRM	0.05 – 5.7/0.2 – 19.1	(Shi *et al.*, 2017)
Honey	Imidacloprid Thiamethoxam Clothianidin Acetamiprid Nitenpyram Thiacloprid Dinotefuran	DLLME and QuEChERS	29.12 (Thiacloprid)	Standard addition (6 levels)	70-120	ZORBAX XDB C-18 50 x 4.6 mm x 1.8µm @30^{0}C	ACN: 0.2% HCOOH H_2O, gradient (10:90) 0.7mL/min	UHPLC, ƛ (244-269nm) DAD	MRM	1.5-2.5/ 5.0-10.0	(Jovanov *et al.*, 2015)
Honey	22 Pesticides in three different families (Neonics,	QuEChERS	0.2-0.5	Standard addition (5 levels)	63-139	Uptisphere C18 15cm x 2.1 mm i.d with inert	H_2O,0.1% HCOOH:	LC-MS/MS ESI^+@ 500^{0}c @ 5kV	MRM	0.07-0.2/0.2-0.5	(Paradis *et al.*, 2014)

	pyrazole n pyrethroids) Imidacloprid Thiamethoxam Clothianidin Acetamiprid Thiacloprid					guard column C18 @40^{0}C	ACN 0.35mL/min 20µL				
Honey	Imidacloprid Thiamethoxam Clothianidin Acetamiprid Nitenpyram Thiacloprid	QuEChERS limited to one purification step with d-SPE citrate extraction tube	0.10-4.0	Standard addition (6 levels)	75-114	Thermo hypersil Gold @30^{0}C C-18 50 x 2.1mm x 1.9µm	0.05% HCOOH & $HCOONH_4$ 2mM in Water: 0.05% HCOOH & $HCOONH_4$ 2mM in MeOH 0.4mL/min 5µL	UHPLC/MS-MS ESI$^+$ @270^{0}C @ 4kV,	SRM	<2.5/4.0	(Proietto Galeano *et al.*, 2013)

Wine	Imidacloprid Acetamiprid Thiacloprid	SPE with Oasis HLB Cartridges percolate @ 5mL/min and vacuum dried 10min and elute with MeOH. And sample clean up	1.1-9.9	Standard addition (8 levels)	70-110	Xterra MS C18 2.1 x 150 mm x 3.5µm with MS C18 2.1 x 10 mm x 3.5µm @28^{0}C	H2O, 0.1% HCOOH: 0.1%HCOOH ACN 0.2mL/min 20µL	LC-TQ MS/MS ESI$^+$ @350^{0}C @4kV,	SRM	0.3-3/1-10	(Economou *et al.,* 2009)
chestnut, shallot, ginger and tea	Imidacloprid Thiamethoxam Clothianidin Acetamiprid Thiacloprid Dinotefuran	Freeze dried samples, homogenised extracted with ACN with $NaSO_4$, cleaned up with Activated carbon and HLB SPE Cartridges	10-20	Standard addition (6 levels)	82.1-108.5	ZORBAX Eclipse XDB @ 30 ^{0}C C-8 150 x 4.6 mm x 5µm	ACN: H_2O, 0.1% HCOOH (30:70) 0.4mL/min	LC-TQ MS/MS ESI$^+$ @540^{0}C @ 4.8kV	MRM	NS*/10-20	(Xie *et al.,* 2011)

Honeybee, honey, pollen	115 pesticides of different groups including **Acetamiprid** and **Thiacloprid** from Neonics grp	Modified QuEChERS	Honeybee (0.3-81.5) Honey (1.6) Bee pollen (6.1-1273)	Standard addition (3 levels)	59-117	ZORBAX Eclipse XDB C-18 Agilent 2.1 x 150 x 3.5µm	H_2O with 5mMHCOONH$_4$ 0.1%HCOOH in both 0.02%ACN: MeOH, 0.3mL/min, 10µL	LC-MS/MS ESI$^{+/-}$	MRM	0.03-23.3/ 0.1-78	(Kasiotis *et al.*, 2014)
Guttation drops	Fipronil Imidacloprid Thiamethoxam Clothianidin Acetamiprid Thiacloprid	Direct injection of sample solution (addition of Guttation drops to 50% Water-MeOH) after filtration on a Millex HV 0.45µm (Millipore) syringe filter	15-18	Standard addition (levels)	91-108	Schimadzu XR-ODS II @45^{0}C C-18 2.2µm x 2 x 100mm with guard C-18 column 2 x 100mm	H_2O: ACN gradient (77/23) 0.4mL/min 5µL	UHPLC-(@ ʎ 244-269nm) DAD		4.5-5.4/15-18	(Tapparo *et al.*, 2011)

Honeybee	Imidacloprid and 6-chloronicotinic acid)	Acetone under ultrasound condition prior to LL partition with dichloromethane	0.3-10 (imidacloprid) 5-30 (6-CNA)	Standard addition (6 levels)	80.2-91.7	50mm x 4.6mm i.d short column packed with 5µm Aquasil C18 post column photochemical reactor	ACN: H_2O 6min Iso-cratic (10:90) @flow rate 1mL/min, switched to 6min linear gradient ACN:H_2O (30:70) 1.4ml/min 1.0mL/min sample in 0.1mol/L of H_3PO_4/KH_2PO_4 in1000µL (pH 3)	LC-PIF-FD		NS/0.3-5	(García *et al.*, 2007)
Spinach, cucumber, apple, pomelo	Imidacloprid Thiamethoxam Acetamiprid Nitenpyram Thiacloprid	QuEChERS with GCB (graphitised carbon) for clean up	5.3-7 (Imidacloprid and imidaclothiz)	NS	73.7-103.8	ZORBAX C-18 50mm x 2.1mm x 1.8µm	MeOH: H_2O Isocratic 25:75	ESI^+ @350^0C	MRM	0.20-0.85/0.66-2.84	(F. Zhang *et al.*, 2012)

Cotton Seed Cake	Imidacloprid Acetamiprid Thiacloprid	Extracted with aqueous acetone under vacuum and loaded onto SPE unit, preconditioned with ACN. Washed with hexane and eluted with ACN finally	5,000, 10,000, 20,000	Standard addition (4 levels)	65.47-110	SupelcosilLC-8-DB column (150 × 4.6 mm, 5 µm).	ACN: H_2O (25:75) 1.2 ml/min 20 µl	HPLC-UV Detector 254nm		5-20/	(Mohan *et al.*, 2010)
Soil from Cocoa plantation field	Imidacloprid Thiamethoxam Acetamiprid Clothianidin Thiacloprid	QuEChERS extraction with NaCl and MgSO4 in acidified acetonitrile followed by cleanup with primary	8-80 while Imidaclopri (4.3-251.4)	Standard addition (10 levels)	72.0-104.8	BDS Hypersil reversed-phase C-18 (250 mm × 2.1 mm; 5 µm) @30°C	MeOH: H_2O (50:50 v/v) Mobile phase A and B consisted of 99% 10 nM ammonium acetate, with 1% methanol	(LC-MS/MS ESI^+	MRM	2-9	(Dankyi *et al.* 2014)

		secondary amine (PSA)					and 90% methanol with 10% 10 nM ammonium acetate respectively 200 µL/min 10 µL.				
Beebread	Pyrethroids and Neonicotinoids and some of their metabolites	QuEChERS limited to one purification step with d-SPE citrate extraction tube	0.03-177.1	Standard addition (6 levels)	53- 119	Kinetex Phenomenex Phenyl-Hexyl (100 × 2.1 mm, 2.6µm) column @60°C	The mobile phases were (A) 0.01% acetic acid with 0.04 mmol/L ammonium acetate in water:(B) MeOH 0.4 mL/min, 2µL	UHPLC–MS/MS ESI^+ @450^0C @3.2kV	MRM	<1-1.7/NS	(Giroud *et al.*, 2013)

Beeswax	acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam	Beeswax melted and diluted in an hexane/isopropanol (8:2, v/v) mixture. Extraction with Water, clean-up on diatomaceous material based cartridges and eluted with acetone. Resulting solution evaporated until dry, reconstituted with a mixture of water and	11-153	Standard addition (6 levels)	85-105	C18 reverse-phase fused-core column (Kinetex2.6µm, 150 mm × 4.6 mm i.d.) with C18 guard column (4 mm × 2.0 mm i.d.)	0.1% formic acid in water: acetonitrile 0.5 mL/min. 15 µL	HPLC–MS/MS ESI^+ @340^0C @2.5kV	SIM	0.4-2.3/1.5-7.0	(Yáñez *et al.*, 2013)

		acetonitrile 50:50 (v/v)									
Eels	dinotefuran, nitenpyram, thiamethoxam, imidacloprid, clothianidin, acetamiprid, and thiacloprid	a pressurized solvent extraction system fast PSE equipped with an automated solvent dispenser from Applied Separations Corporation (Allentown, PA, USA) was used to perform the Subcritical water extraction (SWE)	0.42–1.12	Standard addition (3 levels)	84.6-102.0	Acquity BEH C18 column (50 mm × 2.1 mm, I.D., 1.7 µm particle size).	0.1% formic acid in acetonitrile:0.1 % formic acid in water 0.3 mL/min 10 µL	UHPLC–MS/MS ESI^+ @300^0C @3.3kV	MRM	0.12–0.36 /0.42–1.12	(Zhiming et al., 2013)

		Using 2.0 g diatomaceous earth in a 100 mL mortar compared with the conventional ultrasonic and shaking extraction									
Oranges	115 pesticides including Formetanate Imidacloprid Acetamiprid Thacloprid Thiamethoxam	Modified QuEChER procedure with ACN based extraction followed by d-SPE clean up using PSA	Pesticides of interest was not detected within the range	Standard addition (5 levels)	81.7-111	Inertsil1 ODS-4 column (50 mm x 2.1 mm i.d., 3 µm connected to a Fusion-RP guard column (4 mm x 2.0 mm i.d., 4µm	Water (5 mM ammonium formate: MeOH (5 mM ammonium formate) 5:95% 0.5 ml/min 15µL	HPLC–MS/MS $ESI^{+/-}$@500^0C @4.5kV	MRM	1–11/2-30	(Golge & Kabak, 2015a)

						@30°C					
Tomatoes	109 pesticides including Formetanate Imidacloprid Acetamiprid Thacloprid Thiamethoxam	Modified QuECHER procedure with ACN based extraction followed by d-SPE clean up using PSA	Only Acetamipid detected 15-370	Standard addition (5 levels)	77.1-113.2	Inertsil1 ODS-4 column (50 mm x 2.1 mm i.d., 3 µm connected to a Fusion-RP guard column (4 mm x 2.0 mm i.d.,4µm @30°C	Water (5 mM ammonium formate:MeOH (5 mM ammonium formate) 5:95% 0.5 ml/min 15µL	HPLC–MS/MS $ESI^{+/-}$@500^{0}C @4.5kV	MRM	0.5-10.8/ 1.3-30.4	(Golge & Kabak, 2015b)
Tomatoes	57 pesticides including Imidacloprid Acetamiprid Thacloprid	QuECHER procedure with ACN based extraction followed by d-SPE clean up using PSA	2-10	Standard addition (8 levels)	87-116	Agilent Zorbax C18 column (50 mm x 2.1 mm, 1.8-µm particle size).	23.3µmol/L formic acid in water: 23.3µmol/L formic acid in ACN. 0.35min/L	HPLC–MS/MS ESI^+ @300-400^{0}C @3.5kV	MRM	15-50	(Andrade *et al.*, 2015)

	Thiamethoxam Fipronil					@30°C	2µL				
Urine & hair specimen (rabbit and man)	Imidacloprid and its metabolite, 6-Chloronicotinic acid (6-CINA)	Solid-Liquid extraction with Methanol (hair) & Liquid-Liquid extraction with Methanol (Urine)	27000	Standard addition (7 levels)	81.92-112.34	Discovery C18 column 25cm x 4.6mm x 5µm @30°C	0.1% Formic acid in water: MeOH. 0.6mL/min/ 10µL	LCMS-APCI @200-400^{0}C @3.1.5kV	SIM	Hair (Imi-0.02, 6-CINA-0.01) Urine (Imi-0.02, 6-CINA-0.008)/ Hair (Imi-0.06, 6-CINA-0.04) Urine (Imi-0.006, 6-CINA-0.020)	(Kavvalakis *et al.,* 2013)
Drinking water	Acetamiprid	Solid-Phase Extraction (SPE)	0.1	Standard addition (7 levels)	95-104	LichroCart 125-4 Lichrosphere	Water (0.01% Acetic acid): MeOH (0.01% Acetic	LCMS ESI^{+} 3.5KV	SIM	0.01/0.03	(Seccia *et al.,* 2005)

	Imidacloprid, Thiamethoxam Thiacloprid	LiChrolut EN catridge				100 (5 m) @40°C	acid)/1mL/min /20µL	@300			
Soil from province of Ciudad Real, Spain	Thiamethoxam, Acetamiprid, and Imidacloprid) and the metabolite 6-chloronicotinic acid	Matrix Solid-Phase Dispersion (MSPD) was applied with C18 used as the dispersant	170 - 370	Standard addition (5 levels)	63-99	Uncoated fused-silica capillaries 58 cm (49.5 cm effective length) and 75 m id × 375 m od.	5 mM borate (pH 10.4), 40 mM SDS, and 5% MeOH, 25 kV, 25C, injection pressure (10 s, 20 mbar) and detection 254 nm.	MEKC CE-DAD	-	166.0 - 375.0/489.0 -1249.0	(Ettiene e*t al.*, 2012)
Hawaiian soils	Thiamethoxam and Indoxacarb	Premix with clean Ottawa sand to reduce soil aggregation during extraction with ACN:Methanol (1:1) at 1500psi, 25-	-	-	75-121 (Thiamethoxam) 77-113 (Indoxacarb)	XDB-C8 (4.6 x 150 mm i.d., 5 µm particle size) with a guard column.	Water (1% of 2 propanol) : Methanol, @230nm/1.0mL/min/10µL	HPLC-UV LCMS ESI^+ 3.5KV @300	-	-	(Campbell *et al.*, 2005)

		100oC. Extract concentrated with a gentle flow of Nitrogen and filtered through 0.45um membrane syringe filter.								
River water	Thiamethoxam, Acetamiprid, and Imidacloprid) and the metabolite 6-	off-line SPE with a sorptive material such as Strata-X (polymeric hydrophobic sorbent) and octadecylsilane (C18) was carried out to clean up and preconcentrate	10 – 70	Standard addition (5 levels)	92	Uncoated fused-silica capillaries 58 cm (49.5 cm effective length) and 75 m id × 375 m od.	MEKC CE-DAD	-	103000-810000/340000-267000 ng/mL	(Ettiene *et al.*, 2012)

		the insecticides									
Water from Stream	Imidacloprid Thiamethoxam Clothianidin Acetamiprid Thiacloprid Dinotefuran	An Oasis HLB SPE catridge eluted with 10mL of 50:50 DCM: Acetone	0.002-0.257	Standard addition (5 levels)	71-120	Zorbax eclipse XDB-C18 2.1mm x 150mm x 3.5mm	5Mm HCOOH: ACN/ 0.6min/L	Bio-inert LCMS/MS ESI^+	MRM	0.0032-0.0062	(Hladik *et al.*, 2014)

6-CNA (6-chloronicotinic acid); NS (Not stated); DMRM (Dynamic MRM)

2. Neonicotinoids EU legislation status (EU Pesticides Database website, 2016)

Compounds	EC Status	Approval period	UK Approval	MRL (mg/Kg)	ADI (mg/Kg bw per day)	ARfD	AOEL (mg/Kg bw)	Products to which MRL apply (MRL in mg/Kg)

Acetamiprid Annex II	Approved	01/03/2018 28/02/2033	–	Yes	5.0 – 0.01	0.025	0.025	0.025	➢ Cotton seed (0.7) ➢ Wheat (0.1) ➢ Honey and honey products (0.05) ➢ Spinach (5.0), ➢ Citrus fruits (0.9) ➢ Rapeseed (0.4) ➢ Soil (-[a])
Imidacloprid Annex IIIA	Approved	01/08/2009 31/07/2022	–	Yes	10.0 – 0.05	0.06	0.08	0.08	➢ Cotton seed (0.5) ➢ Wheat (0.1) ➢ Honey and honey products (0.05) ➢ Spinach (0.05), ➢ Citrus fruits (1.0) ➢ Rapeseed (0.1) ➢ Soil (-[a])
Thiacloprid	Approved	01/01/2005 30/04/2020	–	Yes	50.0 – 0.01	0.01	0.03	0.02	➢ Cotton seed (0.15)

Annex II Annex IIIB								➢ Wheat (0.1) ➢ Honey and honey products (0.2) ➢ Spinach (0.15), ➢ Citrus fruits (0.01) ➢ Rapeseed (0.6) ➢ Soil (-[a])
Thiamethoxam Annex IIIA Annex IIIA	Approved	01/02/2007 – 30/04/2019	Yes	20.0 – 0.01	0.026	0.5	0.08	➢ Cotton seed (0.02) ➢ Wheat (0.05) ➢ Honey and honey products (0.05) ➢ Spinach (0.01) ➢ Citrus fruits (0.15) ➢ Rapeseed (0.02) ➢ Soil (-[a])
Clothianidin	Not approved	-	Yes	0.7 – 0.01	0.097	0.1	0.1	➢ Cotton seed (-[a]) ➢ Wheat (0.01)

Annex IIIA Annex IIIA								➢ Honey and honey products (0.05) ➢ Spinach (0.01) ➢ Citrus fruits (0.06) ➢ Rapeseed (0.02) ➢ Soil (-[a])
Dinotefuran	Not Approved (Never notified and authorised in the EU. Also, no toxicological information)	-	No	10.0 – 0.05	0.06	0.08	0.08	➢ Cotton seed (0.5) ➢ Wheat (0.1) ➢ Honey and honey products (0.05) ➢ Spinach (0.05), ➢ Citrus fruits (1.0) ➢ Rapeseed (0.1) ➢ Soil (-[a])

Note**:** ADI (Acceptable Daily Intake); ARfD (Acute Reference Dose); AOEL (Acceptable Operator Exposure Level

Source: EU Pesticides database (2016

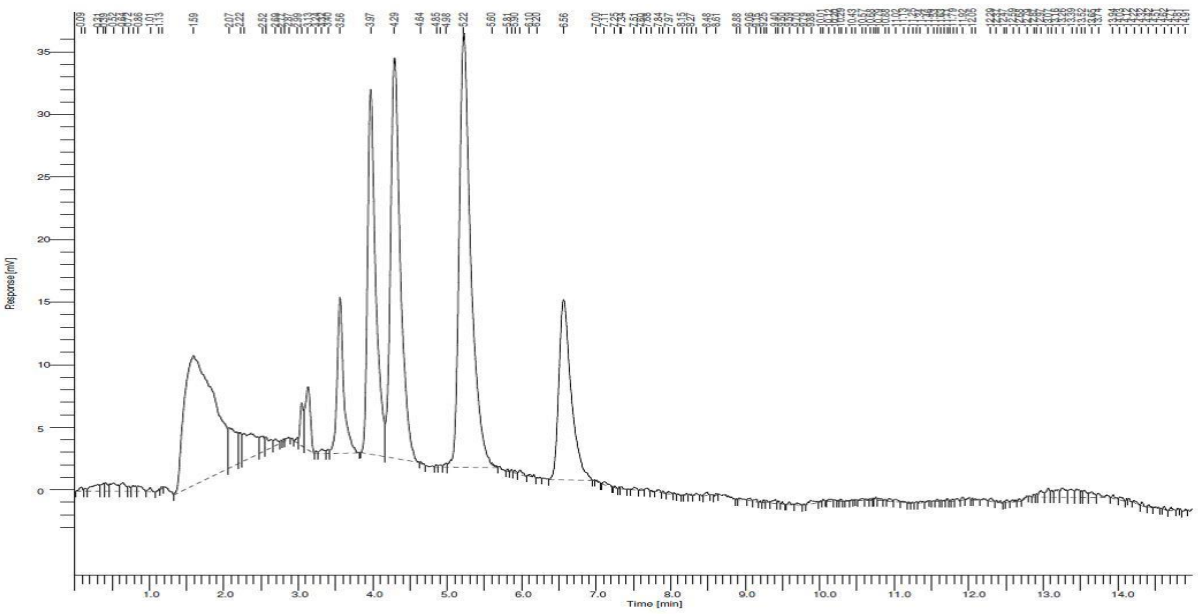
3. UV-visible spectrophotometer results of the 5 neonicotinoids and 2-Chloroaniline assayed at 5 µg/g concentration

Compounds	Abs (amu)	λ_{max} (nm)
Acetamiprid	0.679, 0.431	247, 215
Dinotefuran	0.492, 0.177	271, 212
Imidacloprid	0.722, 0.443	269, 212
Thiacloprid	0.537	243
Thiamethoxam	0.406, 0.263	254, 210
2-Chloroaniline	0.073 0.167	292, 237

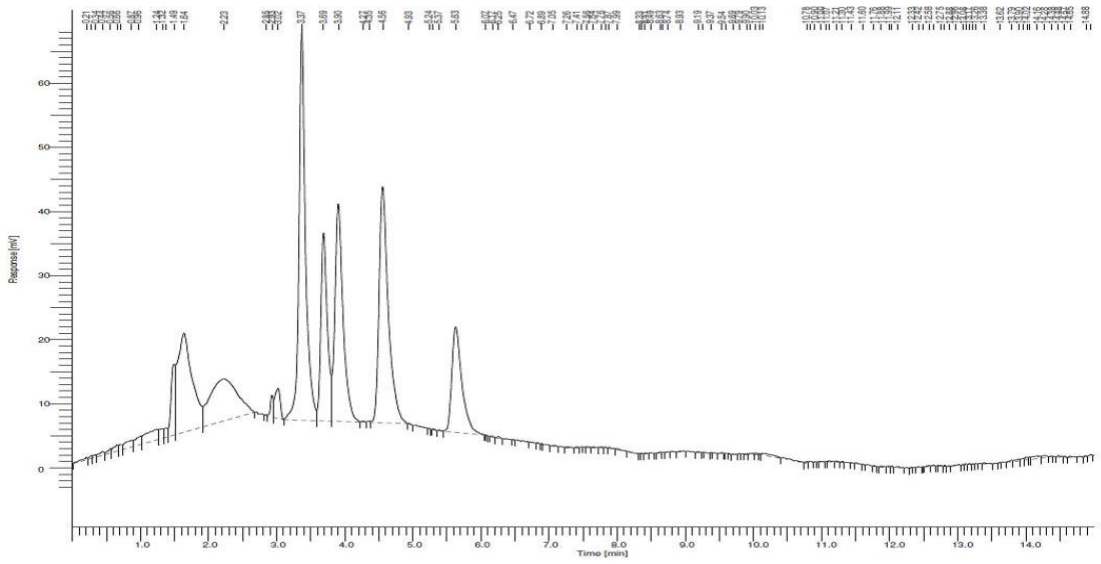
4. HPLC chromatograms for the compounds' separated and peaks identification.

Grouping compounds as suspected from the mixture chromatogram in Figure 4 above

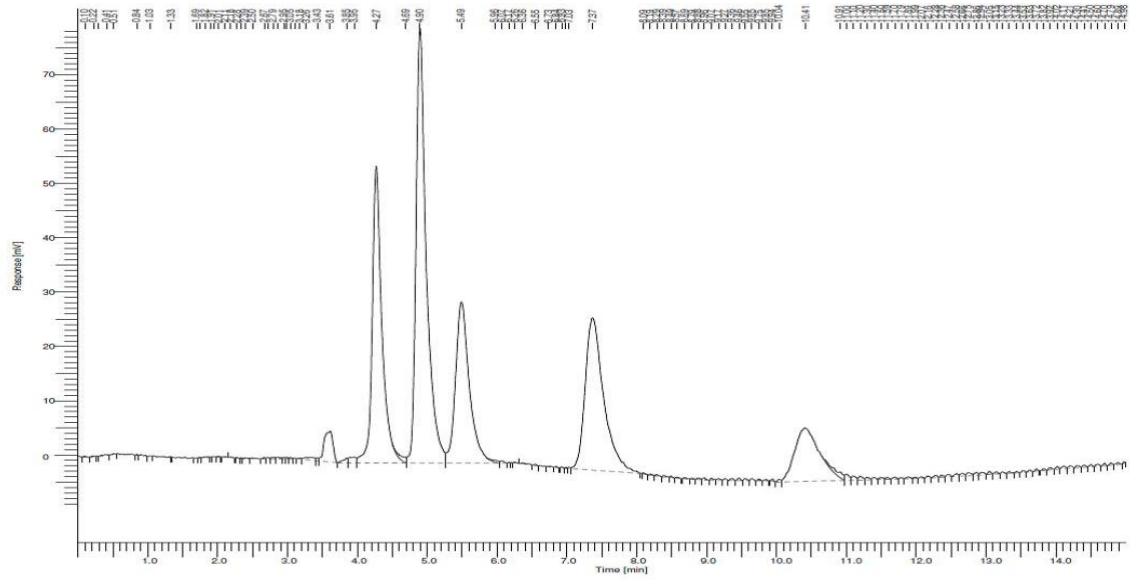
GR 1	GR 2	GR 3	GR 4
Dinotefuran (DIN)	Formetanate (FH)	Formetanate (FH)	Formetanate (FH)
Thiamethoxam (THX)	Clothianidin (CLO)	2-Chloroaniline (2-CA)	Fipronil (FP)
Clothianidin (CLO)	Thiacloprid (THA)		
Thiacloprid (THA)	Acetamiprid (ACE)		
Imidacloprid (IMI)	Nitenpyram (NTP)		
2-Chloroaniline (2-CA)	2-Chloroaniline (2-CA)		



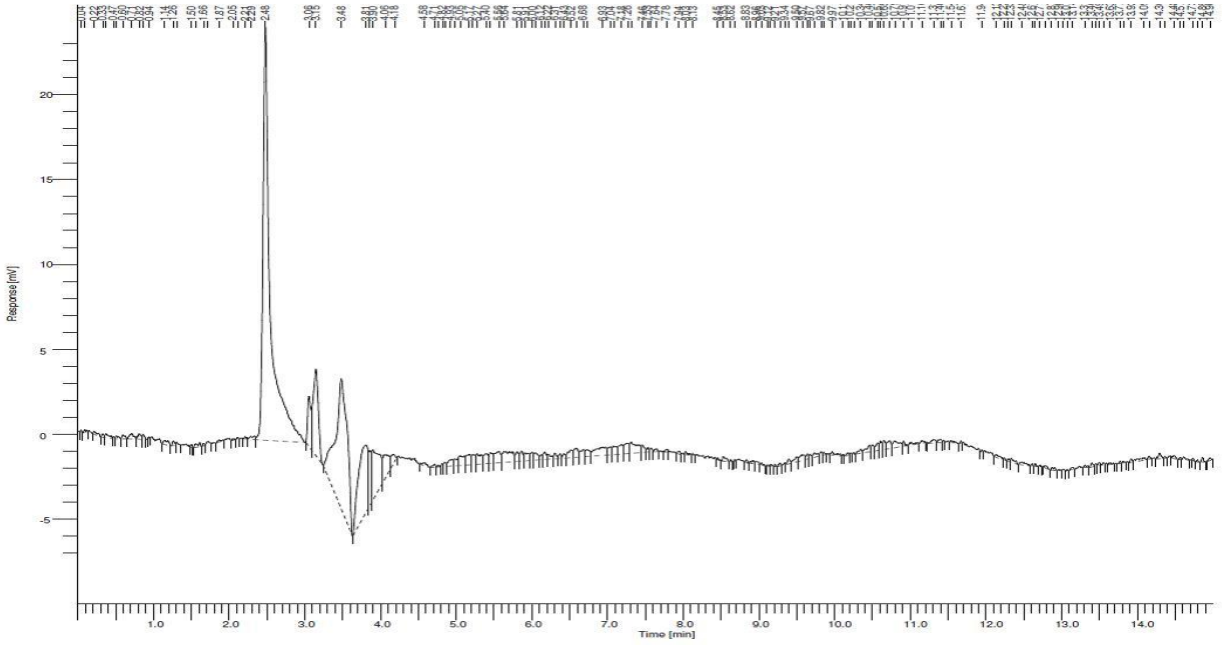
A. Group 1, Only 5 peaks came out, so possibility of co-elution was suspected especially between THX and CLO was suspected.



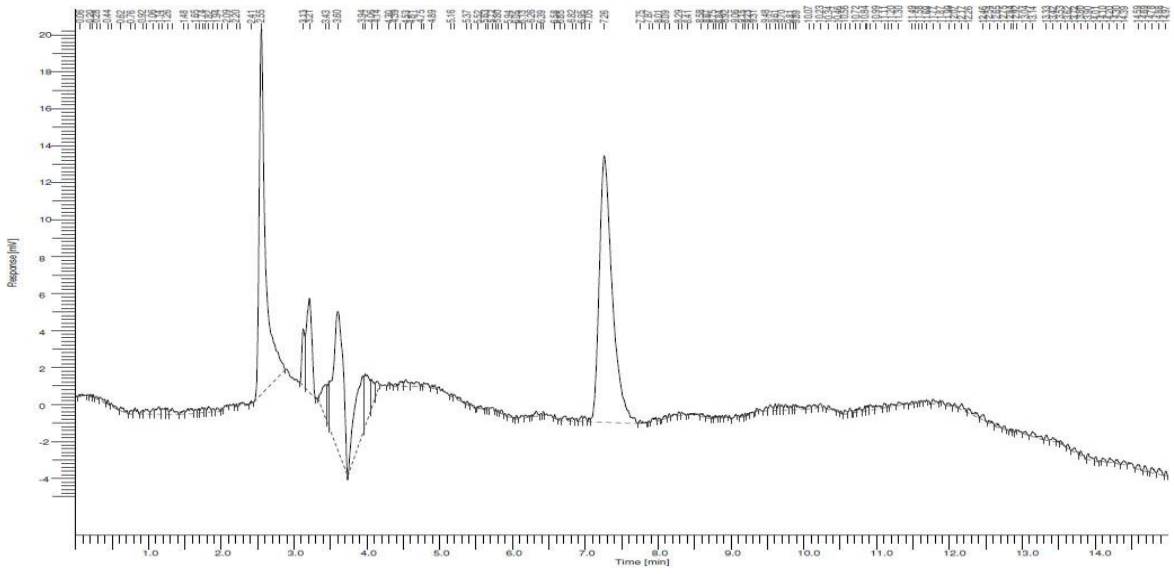
B. Group 1 spiked with DIN stock to confirm DINOTEFURAN position and retention (Rt).



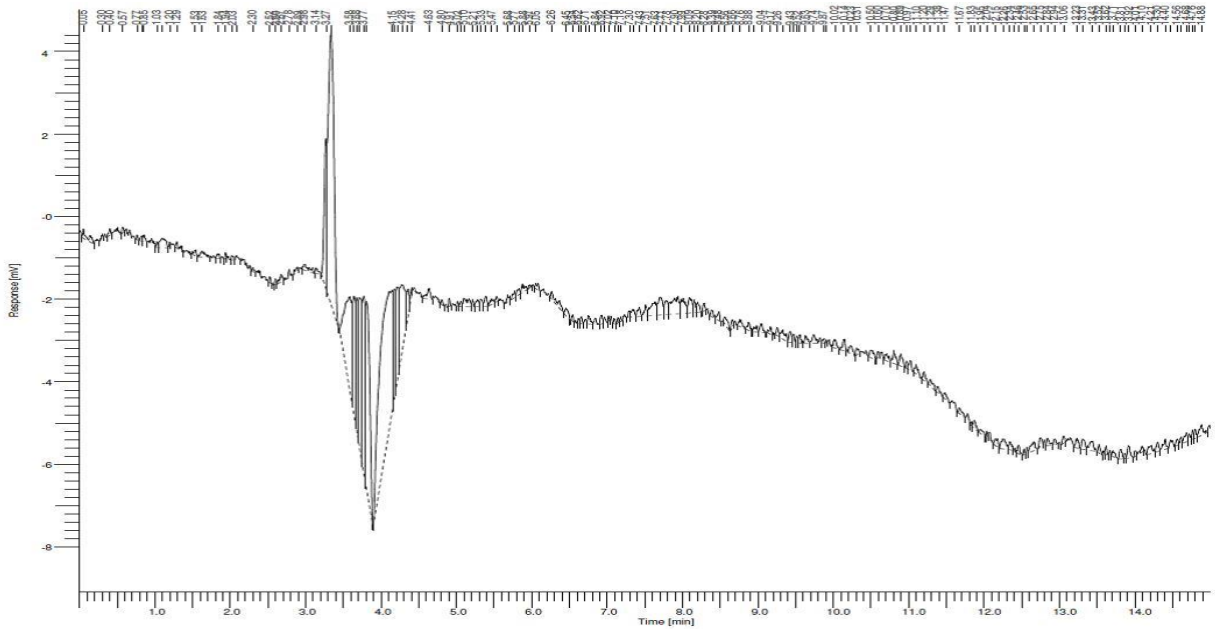
C. Group 1 further Spiked with THX stock to confirm THIAMETHOXAM position and Rt.



D. Group 4 containing both FH and FP in the solution only and were not detected as the peak in the chromatogram is for methanol.

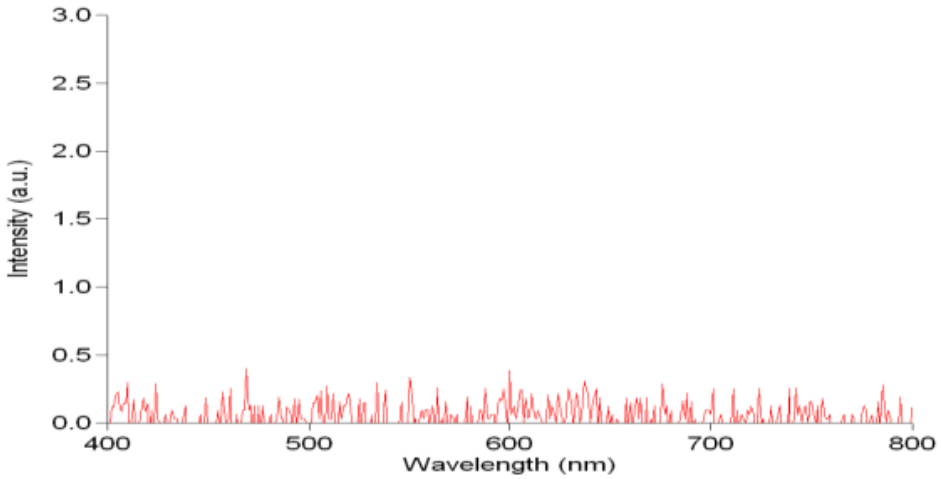


E. Group 3 containing only 2-CA and it came out distinctly showing that FH and FP were not detected in the mixture compared with appendix 4D above.

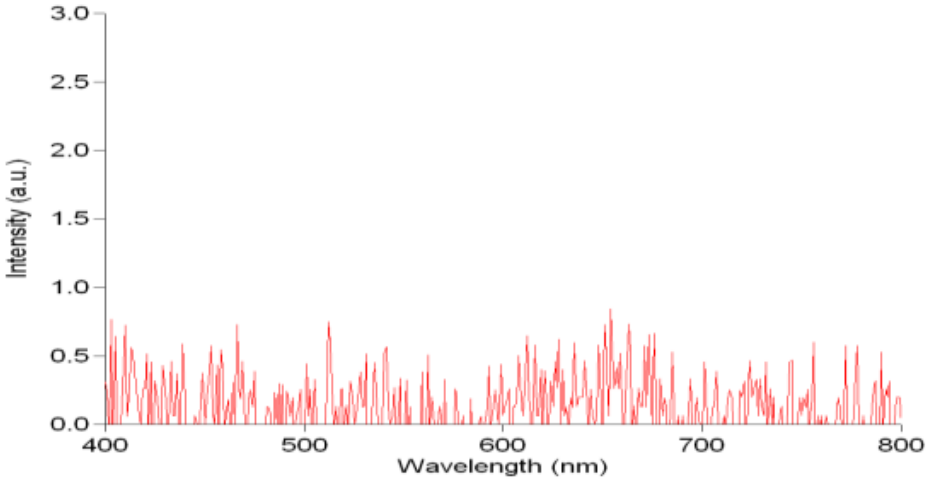


F. Chromatogram for Methanol only

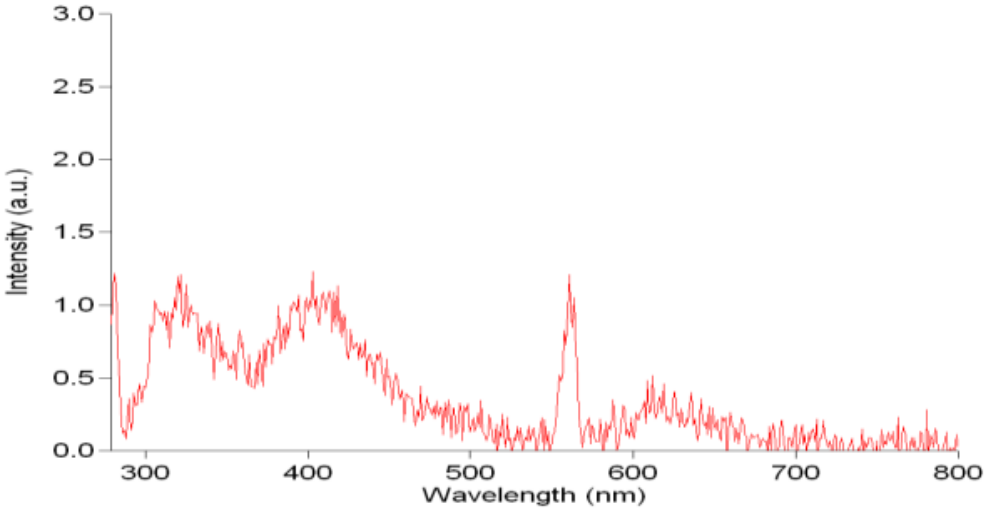
5. The fluorescence spectrum for selected neonicotinoids, fipronil and formetanate HCl



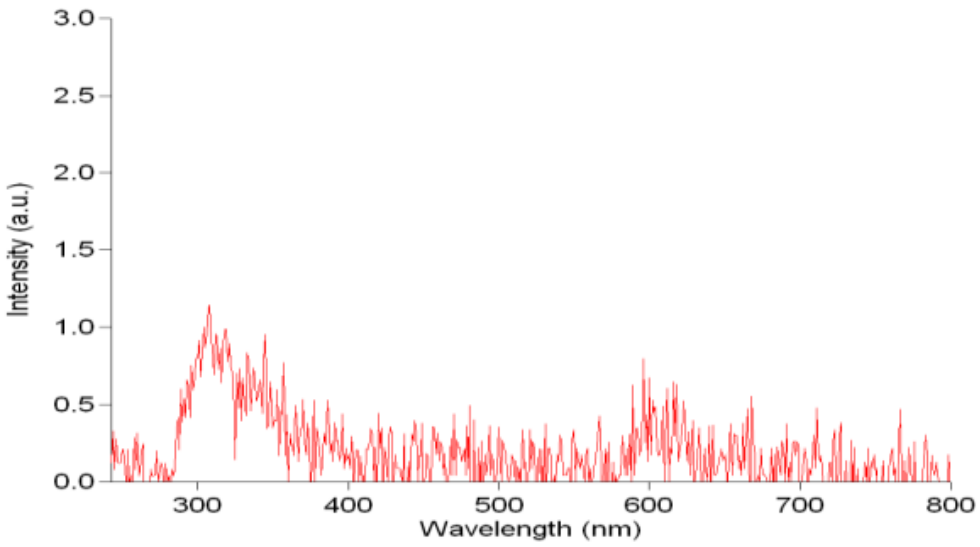
Acetamiprid



Formetanate HCl



Fipronil



Thiacloprid

6. Results of the individual pesticide adsorbed on the 5 soils at two different concentration levels

6.1. Acetamiprid adsorption capacity, K_d and K_{oc} in 5 different soils assayed at low and high concentrations of insecticide: 2.5 µg/g and 25 µg/g. All values are given as mean ± SD

Soil type	Insecticide conc. (µg/g)	Adsorption capacity (µg/g soil)	K_d	K_{oc}
EY	Low	5.40 ± 0.39	5.37 ± 0.77	204.00 ± 29.32
	High	50.75 ± 1.74	6.89 ± 0.55	261.39 ± 20.98
BR	Low	1.21 ± 0.31	0.66 ± 0.19	80.37 ± 23.63
	High	0.13 ± 4.53	0.02 ± 0.26	1.99 ± 31.54
TH	Low	6.70 ± 0.16	8.89 ± 0.59	71.42 ± 4.75
	High	52.42 ± 2.45	7.47 ± 0.90	60.04 ± 7.22
TLW	Low	5.42 ± 0.12	5.36 ± 0.25	166.43 ± 7.70
	High	31.71 ± 2.10	2.84 ± 0.29	88.14 ± 9.00
ST	Low	4.42 ± 0.08	3.66 ± 0.11	39.96 ± 1.24
	High	37.94 ± 2.07	3.82 ± 0.36	41.75 ± 3.93

6.2. Dinotefuran adsorption capacity, K_d and K_{oc} in 5 different soils assayed at low and high concentrations of insecticide: 2.5 µg/g and 25 µg/g. All values are given as mean ± SD.

Soil type	Insecticide conc. (µg/g)	Adsorption capacity (µg/g soil)	K_d	K_{oc}
EY	Low	1.52 ± 0.13	0.51 ± 0.05	19.37 ± 1.84
	High	46.64 ± 6.11	1.88 ± 0.33	71.17 ± 12.48
BR	Low	3.29 ± 0.36	1.26 ± 0.17	152.99 ± 20.45
	High	31.65 ± 6.23	1.14 ± 0.28	138.63 ± 34.13
TH	Low	3.66 ± 2.03	1.55 ± 1.14	12.48 ± 9.19
	High	34.41 ± 11.73	1.29 ± 0.56	10.32 ±4.53
TLW	Low	3.24 ± 3.00	1.425 ± 1.30	44.224 ± 40.31
	High	40.84 ± 7.28	1.573 ± 0.36	48.831 ± 11.09
ST	Low	5.28 ± 0.28	2.37 ± 0.19	25.86 ± 2.02
	High	47.17 ± 3.25	1.90 ± 0.18	20.71 ± 1.95

6.3. Imidacloprid adsorption capacity, K_d and K_{oc} in 5 different soils assayed at low and high concentrations of insecticide: 2.5 µg/g and 25 µg/g. All values are given as mean ± SD.

Soil type	Insecticide conc. (µg/g)	Adsorption capacity (µg/g soil)	K_d	K_{oc}
EY	Low	6.49 ± 0.25	5.46 ± 0.45	207.31 ± 16.88
	High	56.53 ± 1.79	3.73 ± 0.21	141.49 ± 7.88
BR	Low	1.07 ± 0.67	0.48 ± 0.32	58.90 ± 38.43
	High	43.57 ± 4.82	2.47 ± 0.41	300.51 ± 50.45
TH	Low	4.69 ± 0.71	3.07 ± 0.70	24.63 ± 5.65
	High	114.37 ± 0.95	31.74 ± 1.99	254.97 ± 15.96
TLW	Low	7.99 ± 0.81	9.25 ±2.36	287.21 ± 73.27
	High	66.99 ± 2.23	5.13 ± 0.35	159.10 ± 10.91
ST	Low	11.26 ± 0.48	53.98 ± 24.73	589.41 ± 269.97
	High	91.83 ± 1.79	11.33 ± 0.72	123.75 ± 7.84

6.4. Thiacloprid adsorption capacity, K_d and K_{oc} in 5 different soils assayed at low and high concentrations of insecticide: 2.5 µg/g and 25 µg/g. All values are given as mean ± SD.

Soil type	Insecticide conc. (µg/g)	Adsorption capacity (µg/g soil)	K_d	K_{oc}
EY	Low	11.65 ± 0.00	26.58 ± 0.034	1008.84 ± 1.29
	High	93.47 ± 0.16	13.98 ± 0.09	530.81 ± 3.50
BR	Low	6.77 ± 0.02	4.78 ± 0.03	582.69 ± 3.59
	High	29.08 ± 9.09	1.51 ± 0.60	184.47 ± 73.75
TH	Low	11.67 ± 0.03	26.88 ± 0.47	215.99 ± 3.81
	High	110.50 ± 0.47	33.69 ± 1.10	270.65 ± 8.83
TLW	Low	10.97 ± 0.12	19.12 ± 0.96	593.41 ± 29.87
	High	91.85 ± 2.79	13.16 ± 1.45	408.43 ± 44.89
ST	Low	11.46 ± 0.19	24.14 ± 2.31	263.60 ± 25.26
	High	104.41 ± 2.03	23.35 ± 2.56	254.94 ± 28.15

6.5. Thiamethoxam adsorption capacity, K_d and K_{oc} in 5 different soils assayed at low and high concentrations of insecticide: 2.5 µg/g and 25 µg/g. All values are given as mean ± SD.

Soil type	Insecticide conc. (µg/g)	Adsorption capacity (µg/g soil)	K_d	K_{oc}
EY	Low	0.98 ± 0.42	0.36 ± 0.17	13.52 ± 6.31
	High	8.55 ± 3.61	0.27 ± 0.12	10.27 ± 4.65
BR	Low	0.17 ± 0.10	0.06 ± 0.04	6.95 ± 4.30
	High	1.43 ± 1.66	0.04 ± 0.05	5.23 ± 6.08
TH	Low	9.35 ± 0.51	15.29 ± 3.58	122.86 ± 28.75
	High	25.37 ± 1.48	1.09 ± 0.08	8.74 ± 0.62
TLW	Low	0.55 ± 0.16	0.19 ± 0.06	5.99 ± 1.83
	High	6.13 ± 4.11	0.19 ± 0.13	5.95 ± 4.15
ST	Low	0.85 ± 0.46	0.18 ± 0.11	2.01 ± 1.15
	High	33.87 ± 0.73	0.78 ± 0.02	8.55 ± 0.24

7. Data analysis for the assessment of uptake of neonicotinoids by the study soils.

The results of treating the study soils with neonicotinoids and corresponding adsorption capacities are given. The statistical treatment of the data is reported. Every adsorption capacity is given as µg neonicotinoid/g soil from the mean of a triplicate study.

7.1a. Adsorption capacities (µg neonicotinoid/g soil) assayed at low concentration of neonicotinoids (2.5µg/g).

Soil	ACE	DIN	THA	IMI	THX
EY	6.43	1.15	10.48	6.49	0.97
BR	1.44	2.49	5.93	1.07	0.17
TH	7.96	2.77	10.47	4.69	9.32
TLW	6.44	2.45	9.84	7.99	0.55
ST	5.26	4.00	10.30	11.26	0.84

7.1b. ANOVA table (two-way without replication) from the study of the adsorption of neonicotinoids at low concentration (2.5µg/g).

Summary	*Count*	*Sum*	*Average*	*Variance*
EY	5	25.52137	5.104274	16.3075
BR	5	11.10397	2.220794	4.986834
TH	5	35.21621	7.043242	10.38982
TLW	5	27.27627	5.455255	14.91125
ST	5	31.66334	6.332669	19.19192
ACE	5	27.53117	5.506234	6.080391
DIN	5	12.87168	2.574336	1.027477
THA	5	47.00989	9.401978	3.842231
IMI	5	31.51001	6.302001	14.36374
THX	5	11.85842	2.371684	15.1887

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit.
Rows	68.12793	4	17.03198	2.902697	0.05549	3.006917
Columns	169.267	4	42.31676	7.211886	0.00161	3.006917
Error	93.88224	16	5.86764			
Total	331.2772	24				

7.1c. Adsorption capacities (µg neonicotinoid/g soil) assayed at high concentration of neonicotinoids (25µg/g)

Soil	ACE	DIN	THA	IMI	THX
EY	76.64	35.97	96.77	56.53	7.97
BR	0.20	24.41	31.93	43.57	1.33
TH	79.16	26.53	115.33	114.37	23.66
TLW	47.89	31.49	96.02	66.99	5.72
ST	57.30	36.38	110.04	91.83	31.58

7.1d. ANOVA table (two-way without replication) from the study of the adsorption of neonicotinoids at high concentration (25µg/g)

Summary	*Count*	*Sum*	*Average*	*Variance*
EY	5	273.8796	54.77593	1197.147
BR	5	101.4356	20.28712	364.4383
TH	5	359.0571	71.81141	2032.09
TLW	5	248.1161	49.62321	1178.357
ST	5	327.1271	65.42543	1185.659
ACE	5	261.1877	52.23754	1018.298
DIN	5	154.7822	30.95645	29.31851
THA	5	450.0923	90.01847	1124.351

IMI	5	373.2898	74.65795	806.4269
THX	5	70.26347	14.05269	166.9517

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit.
Rows	7955.281	4	1988.82	6.878606	0.002024	3.006917
Columns	19204.66	4	4801.166	16.60549	1.5E-05	3.006917
Error	4626.101	16	289.1313			
Total	31786.04	24				

7.2a. t-correlation test of pesticide adsorption capacities at low concentration 2.5 µg/g.

	Acetamiprid		Dinotefuran		Thiacloprid		Imidacloprid		Thiamethoxam	
Soil	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC
EY	6.43	2.63	1.15	2.63	10.48	2.63	6.49	2.63	0.97	2.63
BR	1.44	0.82	2.49	0.82	5.93	0.82	1.07	0.82	0.17	0.82
TH	7.96	12.45	2.77	12.45	10.47	12.45	4.69	12.45	9.32	12.45
TLW	6.44	3.22	2.45	3.22	9.84	3.22	7.99	3.22	0.55	3.22
ST	5.26	9.16	4	9.16	10.3	9.16	11.26	9.16	0.84	9.16
t exp.	1.39		1.19		1.28		0.67		2.29	
t critical at p 0.05	3.18									

7.2b. t-correlation test table of pesticide adsorption capacities at high concentration 25 µg/g

	Acetamiprid		Dinotefuran		Thiacloprid		Imidacloprid		Thiamethoxam	
Soil	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC	adsorption capacity (µg/g soil)	% SOC
EY	76.64	2.63	35.97	2.63	96.77	2.63	56.53	2.63	7.97	2.63
BR	0.20	0.82	24.41	0.82	31.93	0.82	43.57	0.82	1.33	0.82
TH	79.16	12.45	26.53	12.45	115.33	12.45	114.37	12.45	23.66	12.45
TLW	47.89	3.22	31.49	3.22	96.02	3.22	66.99	3.22	5.72	3.22
ST	57.30	9.16	36.38	9.16	110.04	9.16	91.83	9.16	31.58	9.16
t exp.	1.37		0.07		1.90		12.52		3.42	
t critical at p 0.05	3.182									

8. The calculations of correction factors for consideration of loss due to evaporation during the duration of UV photolysis experiment

Time	Container 1	Wt loss on evaporation (Green) and aliqout taken (Yellow)	% of loss due to evaporation	Cumm % of Loss due to evaporation	Correction factor	Container 2	Wt loss on evaporation (Green) and aliqout taken (Yellow	% of loss due to evaporation	Cumm % of Loss due to evaporation	Correction factor	Container 3	Wt loss on evaporation (Green) and aliqout taken (Yellow	% of loss due to evaporation	Cumm % of Loss due to evaporation	Correction factor	**Ave of correction factors (n = 3)**
Empty container	35.4563					45.6231					35.5506					
Wt of solution + Container	40.4777					50.6300					40.5561					
0	5.0214					5.0069					5.0055					
5 min	40.4577	0.0200	0.3983	0.3983	**1.0040**	50.6099	0.0201	0.4014	0.4014	**1.0040**	40.5350	0.0211	0.4215	0.4215	**1.0042**	**1.0041**
5 (after taken aliquot)	40.3924	0.0653				50.5502	0.0597				40.4815	0.0535				
15 min	40.3744	0.0180	0.3647	0.7630	**1.0076**	50.5307	0.0195	0.3958	0.7972	**1.0080**	40.4540	0.0275	0.5577	0.9792	**1.0098**	**1.0085**
15 (after taken aliquot)	40.3115	0.0629				50.4730	0.0577				40.4003	0.0537				
30 min	40.2853	0.0262	0.5396	1.3026	**1.0130**	50.4438	0.0292	0.6021	1.3993	**1.0140**	40.3622	0.0381	0.7856	1.7649	**1.0176**	**1.0149**
30 (after taken aliquot)	40.2218	0.0635				50.3839	0.0599				40.3089	0.0533				
1hr	40.1695	0.0523	1.0975	2.4001	**1.0240**	50.3272	0.0567	1.1910	2.5903	**1.0259**	40.2372	0.0717	1.5068	3.2717	**1.0327**	**1.0275**
1 hr (after taken aliquot)	40.1065	0.0630				50.2688	0.0584				40.1840	0.0532				
2hr	40.0010	0.1055	2.2687	4.6688	**1.0467**	50.1513	0.1175	2.5292	5.1195	**1.0512**	40.0535	0.1305	2.8165	6.0882	**1.0609**	**1.0529**
2 hr (after taken aliquot)	39.9362	0.0648				50.0936	0.0577				40.0005	0.0530				
3hr	39.8282	0.1080	2.4108	7.0795	**1.0708**	49.9813	0.1123	2.5120	7.6315	**1.0763**	39.8733	0.1272	2.8585	8.9467	**1.0895**	**1.0789**
3 hr (after taken aliquot)	39.7650	0.0632				49.9171	0.0642				39.8199	0.0534				
4hr	39.6563	0.1087	2.5228	9.6023	**1.0960**	49.8066	0.1105	2.5734	10.2049	**1.1020**	39.6872	0.1327	3.1082	12.0549	**1.1205**	**1.1062**
4 hr (after taken aliquot)	39.5941	0.0622				49.7470	0.0596				39.6343	0.0529				
6hr	39.4055	0.1886	4.5580	14.1603	**1.1416**	49.5268	0.2202	5.3396	15.5445	**1.1554**	39.3940	0.2403	5.8844	17.9393	**1.1794**	**1.1588**

9. The image of Essex farm after OSR has been harvested and prior to soil sampling in May 2018

10. Images of the wheat grains sampled from winter wheat plants in Plumpton farm, East Sussex.

11. Detail procedure of soil characterisation according to ISRIC 2000.

11a. Determination of Moisture content and moisture correction factor

Material: Soil less than 2mm

Chemicals: Not applicable

Apparatus:

1. Drying oven
2. Moisture tins or flasks with fitting lid

Procedure:

1. 5g of soil less than 2mm was transferred into a tarred moisture tin and weighed with 0.001 g accuracy (***A gram***).
2. Dried overnight at 105^{o}C (lid removed)
3. The tin was removed from oven, closed with lid, cool in a desiccator and weighed (***B gram***).

Calculation:

The moisture content in wt. % (m/m) is obtained by:

Moist (wt. %) = {A –B/B-tare tin} x 100

The corresponding moisture correction factor *(mcf)* for analytical results or the multiplication factor for the amount of sample to be weighed in for analysis is:

Moisture correction factor (mcf) = (100 + % moist)/100

11b. Determination of soil Organic Carbon content: Walkley-Black procedure

Material: Soil less than 0.25mm

Chemicals:

1. Potassium dichromate standard solution (0.1667M) was made by weighing out 49.04g of $K_2Cr_2O_7$ into 1L volumetric flask and made to volume with water
2. Conc. Sulphuric acid (96%)
3. Conc. Phosphoric acid (85%)
4. Barium diphenylamine sulphonate, 0.16% (indicator) was made by dissolving 1.6 g of barium diphenylamine sulphonate in 1L of water.
5. Ferrous sulphate solution ($FeSO_4.7H_2O$), 1M (approx.) was made by dissolving 278 g of $FeSO_4.7H_2O$ in ca. in 750 mL of water and 15 mL of conc. H_2SO_4 was added. The content was transferred into a 1L volumetric flask and made to volume with water

Apparatus:

1. Burette
2. Pipette
3. Stirrer
4. Measuring cylinder (25 mL)

Procedure:

1. About 1 g of each soil (less than 0.25 mm) was weighed into 500mL wide-mouth Erlenmeyer flask. A control sample was included.
2. 10 mL of dichromate solution was added. Two blanks (Erlenmeyer flask without soil) was included to determine the molarity of the ferrous sulphate soln.

3. 20 mL Sulphuric acid was carefully added with a measuring cylinder. The flask was swirled and allowed to stand on a pad for 30 mins (in fume cupboard).
4. About 250 mL water was added and 10 mL Phosphoric acid was added thereafter with a measuring cylinder and allowed to cool.
5. 1 mL indicator solution was added and titrated with ferrous sulphate solution while the mixture is being stirred. Near the end-point the brown colour became purple or violet-blue and the titration was slowed down. At the end-point the colour changed sharply to green.

Calculation:

The carbon content of the soil was obtained by:

$\% C = M \times (V1-V2/s) \times 0.39 \times mcf$

Where

M = Molarity of ferrous sulphate solution (from blank titration)

V1 = ml ferrous sulphate solution required for blank

V2 = ml ferrous sulphate solution required for sample

S = weight of air-dry soil in gram

$0.39 = 3 \times 10^{-3} \times 100 \times 1.3$, Note (3 = equivalent weight of carbon)

Mcf = moisture correction factor

11c. Particle size analysis (PSA) of soil

1.0 Particle size analysis: hydrometer method

There are numerous particle size classification scales in world usage. The particle size system described below is the International Scale adopted by the International Soil Science Society (ISSS):

Grade	Particle Diameter (mm)	Particle Diameter (µm)
Sand	0.02-2.0	20 - 2000 µm
Silt	0.002-0.02	2 - 20 µm
Clay	<0.002	<2 µm

Particles > 2 mm are labelled 'gravel' on the International Scale.

Another scale in common usage is the Wentworth classification scheme:

Grade	article Diameter (µm)
Sand	63 - 2000 µm
Silt	2 - 63 µm
Clay	<2 µm

1.1 Reagents

5% (w/v) of calgon 50 g sodium hexametaphosphate in 1 L was made, also added was 7 g of anhydrous sodium carbonate.

Addition of this reagent disperses the soil particles by (i) adding sodium ions to increase the exchangeable sodium and cause a repulsion between particles, (ii) by adding hexametaphosphate which is adsorbed on to positive electrical charges on the sesquioxides and kaolinite clay, so preventing attraction to negatively charged clay, and (iii) adding carbonate to raise pH of the solution and so remove positive charges.

This method of fractionation is generally used for finer particles (< 0.02 mm) and is based on the dispersion and settlement of the particles in water. This process depends on the application of Stoke's Law to sediment:

$v = Kr^2$

v = velocity of the fall of a particle through liquid

r^2 = square of the particle radius

K = the K factor can only be accepted as a constant for a fixed or corrected temperature.

Two major assumptions are made if this law is applied to soil particles. The first assumes that all particles behave as perfect spheres and, the second, that they all have the same density (these assumptions are acceptable in most circumstances).

A hydrometer was inserted in the sample suspension to indicate the stage of settlement of the particles after a specified time

1.2 Method

1. About 50 g of soil (< 2 mm fine earth fraction) was weighed out into a tall 800 ml beaker and 60 ml of hydrogen peroxide solution was added. The content was gently warmed on a hotplate, swirling occasionally, until frothing has finished and then boiled for a few minutes to destroy any remaining H_2O_2. This was allowed to cool for 10 mins.

2. About 25 ml of 5% (w/v) hexametaphosphate (Calgon) solution was added to the beaker and transferred into a 500 ml bottle. The bottle lid was securely closed.

3. The contents was shaken for 1 hour - the bottle contained approximately 250 ml of the liquid to ensure the process is sufficiently vigorous. The content of the bottle was transferred into a 1000 ml graduated cylinder using a jet of water from a wash bottle to remove all traces of sediment remaining in the bottle. The cylinder was thereafter filled to the 1000 ml mark with water. The content of the cylinder was thoroughly mixed for 3 mins to ensure a complete

agitation is achieved. Immediately after agitation, the clock was started, and the temperature of the mixture was recorded with a thermometer. 20 seconds before each measurement, the hydrometer was gently lowered into the cylinder and allowed to settle. The reading on the scale (0-60 g per litre) was read from the **top** of the meniscus (to the nearest 0.5g). Readings were taken at 40 seconds (finer than 50 µm fraction - near enough to give an estimate of the 63 µm fraction), 4 minutes 48 seconds (finer than 20 µm fraction) and after 5 hours (finer than 2 µm fraction). After each reading, the **temperature of the suspension was recorded.**

1.3 Calculation of results

The hydrometer graduations refer to a temperature at 20°C. For every 1°C above 20°C, 0.3 was added to the reading, whilst for every 1°C below 20°C, 0.3 was **subtracted**

i.e. if the temperature was 16.5°C: 20°C - 16.5°C = 3.5°C difference, temperature correction therefore = 3.5 °C x 0.3 = 1.05, so 1.05 was subtracted from the hydrometer reading.

A further correction was made to eliminate the effect of the Calgon solution, *i.e.* 1.5 was subtracted from the temperature corrected readings. The resulting figure was the true value, as Z gm/litre of material in suspension. Hence:

% of the particular size fraction =

$$\frac{Z \text{ (hydrometer reading + or - temp. correction - Calgon correction)}}{\text{soil wt.}} \times 100$$

The reading at 40 seconds gives the % Clay + Silt using the U.S. Department of Agriculture size limits of 50 µm (or 63µm) and that at 5 hours the % Clay only. % Silt is found by the difference.

The reading at 4 min 48 sec seconds gives the % Clay + Silt using the ISSS size limit for silt.

The **sand fraction** was determined directly by taking the % clay + silt fraction from 100%.

1.4 Results

(Wentworth size limits)	Your results	(ISSS size limits)	Your results
% Sand (>63 µm)		% Sand (>20 µm)	
% Silt (2 - 63µm)		% Silt (2 - 20µm)	
% Clay (<2µm)		% Clay (<2µm)	
Soil textural class (see below)			

11d. Analysis of soil cation exchange capacities (CEC)

Analysis of CEC is a routine procedure for most soil investigations. CEC reflects the soil's ability to bind and supply nutrients and pollutants.

Analysis involves three stages:

1. Saturation with chosen 'index' cation, to displace those originally on the clays, etc.
2. Removal of excess saturating solution with alcohol.
3. Displacement of exchanged index ion and measurement of the amount of index ion exchanged.

The exchange sites of the soil are first saturated with Na^+ ions at pH7 and the Na^+ ions are then displaced with $NH4^+$ ions. Sodium is then determined by flame emission spectrometry.

Procedure

1. About 4 g of soil (< 2mm) was weighed into a 50-ml polyethylene centrifuge tube and labelled accordingly.

2. Using measuring cylinder, 33 ml of 1M sodium acetate solution was added to the centrifuge tube. The tube was sealed and shaken for 10 minutes on a shaker.

3. The content of the tube was centrifuged, and the supernatant was decanted.

4. The sample was treated with 3 additional 33-ml aliquots of sodium acetate solution. Each time the sample was re-suspended before putting on to the shaker and discarding the supernatant after each centrifugation.

5. At the end of step 4 above, the sample was suspended in 33-ml ethanol and shaken for 5 minutes.

6. The content of the mixture was centrifuged, and the supernatant discarded. This washing procedure was repeated for another two times.

7. At the end of step 6 above, about 33 ml of 1M ammonium acetate was added and shaken for 10 minutes.

8. The mixture was centrifuged, and the supernatant decanted into a 100 ml volumetric flask

9. The extraction procedure was repeated twice more (i.e. to a total of 99 ml ammonium acetate). The content of the flask was carefully made to the 100 ml mark with de-ionised water.

10. The Na content of the solution (y) in the flask was determined by flame emission spectrometry. 10 ml of the solution was diluted to 100 ml and the dilution factor was accounted for while calculating the results.

Determination of Na (589 nm or the Na filter) by flame emission spectrometry

The determination of Na was carried out by using inductively coupled plasma atomic emission spectroscopy (ICP-AES)

Example Calculation

The concentration of the extract y = 4.6 mg/L, so the Na present in 100 ml of extract solution prepared = 0.460 mg

This 0.460 mg came from 4 g soil. Therefore from 1 kg of soil, 0.460 x (1000/4) = 115 mg Na would be extracted per kg of soil.

The molar mass of Na is 23 g/mol or 23 mg/mmol, and so the exchangeable Na is 115 mg/ 23 mg/mmol = 5 mmol/kg soil or 0.5 cmol/kg (10 mmol is 1 cmol). The exchangeable Na is therefore 0.5 cmol/kg. The CEC of the soil is therefore 0.5 cmol/kg

Interpretation

The range of CEC values commonly found for mineral soils

CEC (cmol/kg)	Remarks
<5	Very Low
5-10	Low
10-20	Medium
20-30	High
>30	Very High

Note: CEC increases with organic matter content regardless of soil texture

11e. Determination of pH in water: Potentiometric method 1:2.5 soil: liquid mixture

Material: Soil less than 2mm

Chemicals:

1. *Buffer solution, pH4.00, 7.00 and 9.00 (or 10.00)*
2. *De-ionised water*

Apparatus:

1. pH meter
2. Reciprocating shaker

Procedure:

1. About 10 g of soil less than 2mm was weighed into a 100mL polythene wide-mouth bottle. The blank was included.
2. About 25 mL of water was added and the bottle was capped.
3. The bottle was shaken for 2 hrs.
4. Before opening the bottle for measurement, this was shaken by hand twice.
5. The electrode of the pH meter, previously calibrated with buffer solutions, was immersed in the upper part of suspension.
6. Reading of the pH was taken when the reading has stabilised (accuracy 0.1 unit).

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