Holistic Assessment of Pesticide Residues in Irish Agricultural Soil

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A thesis submitted for the award of PhD

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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எல்லா புகழும் இறைவனுக்கே

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List of Abbreviations and Acronyms

AChE	acetylcholinesterase
AKGA	α-ketoglutaric acid
ALS	acetolactate synthase
AMPA	Aminomethylphosphonic acid
ASs	active substances
Azo	azoxystrobin
Bos	boscalid
BR	basal respiration
CABI	Centre for Agriculture and Bioscience International
CoV	coefficient of variance
CA	Commonage area
CA	citric acid
CL	Cropland
CLPP	community-level physiological profiles
C-P	carbon-phosphorus
DCM	dichloromethane
DCU	Dublin City University
DLM	Dutch mini-Luke
d-SPE	dispersive solid-phase extractions
DT50	half-life
EGL	Extensive grassland
EPSP	5-enolpyruvoyl-shikimate-3-phosphate synthase
EU	European Union
FAO	Food and Agriculture Organization of the United Nations

Flu	fluroxypyr
GABA	γ-aminobutyric acid
GAL	galactose
GDP	Gross Domestic Product
GDPR	General Data Protection Regulations
GL	glucose
GLSIR	glucose substrate-induced respiration
GUS	Groundwater Ubiquity Score
GVA	Gross Value Added
H’	Shannon functional diversity index
HAc	acetic acid
HPLC	High-Performance Liquid Chromatography
IGL	Intensive grassland
IS	internal standard
K_D	distribution coefficient
K_H	Henry’s constant
K_{OC}	adsorption coefficient
K_{ow}	partition coefficient
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
LQ	lower quartile
LUSSI	Land use and Soil Inventory for Ireland
MA	malic acid
Max	maximum

MC	moisture content
MCPA	2-methyl-4-chlorophenoxyacetic acid
MDL	Method Detection Limit
Me	mean
ME%	Matrix effect
MeCN	acetonitrile
Med	median
MeOH	methanol
MgSO4	magnesium sulphate
Min	minimum
MQL	method quantification limit
MRM	multi-reaction monitoring
MSIR	multiple substrate-induced respiration
n.d.	not detected
NAGA	n-acetyl glucosamine
NI	non-ionisable
PBT	persistent, bioaccumulative, and toxic
pKa	dissociation constant
PPE	personal protective equipment
Pro	prothioconazole
PSA	primary-secondary amine
qCO_2	Metabolic quotient
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
QuPPe-PO	Quick Polar Pesticides method

SANTE	Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed
SD	standard deviation
SIR	substrate-induced respiration
SIS	Irish Soil Information System
SM	Soil Moisture
SOM	Soil organic matter
SOPs	standard operating procedures
SRC	sarcosine
SWHC	Soil Water Holding Capacity
T_0	initial absorbance values
T_1	Second reading absorbance values
TN	total nitrogen
TP	total phosphorus
TPD	Total Pesticide Detected
TPP	triphenyl phosphate
UNECE	United Nations Economic Commission for Europe
UQ	upper quartile
USDA	US Department of Agriculture
UV	ultraviolet
Vp	vapour pressure
WS	water solubility

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Vickneswaran, M., Carolan, J. C., & White, B. (2021). Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch mini-Luke extraction methods. Analytical Methods, 13(46), 5638–5650. https://doi.org/10.1039/d1ay01248g

Vickneswaran, M., Carolan, J. C., Saunders, M., & White, B. (2022). Establishing the extent of pesticide contamination in Irish agricultural soils (**Under review**; Manuscript Number: STOTEN-D-22-25070)

Oral Presentations

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Developing tools for pesticide detection and toxicity testing in agricultural soils*, DCU Chemistry Day 2019, 10th May 2019, Ireland.

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Developing tools for pesticide detection and toxicity testing in agricultural soils*, Irish Pollinator Research Network, 20th January 2021, Ireland.

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke (DML) extraction methods*, 6th Annual DCU Chemical Research Symposium, 4th June 2021, Ireland.

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke (DML) extraction methods*, Environ 2021, 16th – 18th June 2021, Ireland.

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Establishing to what extent Irish agricultural soils are contaminated with pesticides*, Environ 2021, 14th-15th July 2022, Ireland.

Poster Presentations

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke (DML) extraction methods*, EnvChem2021, 25th June 2021, Ireland.

Mathavan Vickneswaran, Jim Carolan, and Blánaid White, *Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke (DML) extraction methods*, Analytical Research Forum 2022, 13th-14th June 2022, Ireland.

Workshops and awards

1. Winter School on Mass Spectrometry, 4th-5th March 2019, Palaiseau, France.
2. Soil Biology Lab Skills Training Course, 30th August-3rd September 2021, Wageningen University & Research, Netherlands.
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Holistic assessment of pesticide residues in Irish agricultural soil

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Abstract

Agricultural industry highly dependent on pesticide usage, for boosting efficiency and productivity, however it can result in irreparable damage to the soil layers. Hence, it is essential to understand the current level of pesticide contamination in the Irish agricultural soil. This work focuses on establishing the level of contamination of ten pesticide compounds in the Irish agricultural soil. To obtain a holistic understanding, the determined pesticide concentrations were then correlated with the soil physicochemical properties and microbial activities. The analytical challenges to quantifying pesticides from soil were addressed by comparing QuEChERS and Dutch mini-Luke, where Dutch mini-Luke was determined to be the preferred extraction method for all the targeted analytes as it provides a superior analytical advantage over QuEChERS.

In 2021, soil samples were collected from 25 fields, 24 agricultural lands and one commonage land. It was determined that 92% of the collected fields exceeded the parametric limit set for Irish drinking water for five pesticides. Interestingly, neonicotinoids we detected in 24 sites, including one with no history of pesticide application. Additionally, the correlation of total pesticides detected, and soil physicochemical properties indicated that pesticides correlate negatively with soil organic matter and positively with the percentage of clay. To further explore the correlation, the potential relationship between the microbial community and the pesticide concentration was assessed using MicroResp. Although the complete interaction between targeted pesticides and the agricultural soils is not fully understood, these results provide the baseline for a more detailed investigation of pesticides in the Irish soil environment.

Chapter 1 :

Introduction

1.1 Introduction to Pesticides

Agricultural practices are crucial in securing global food security and reducing hunger in an ever-increasing global population.[1] However, crop cultivation faces a constant battle against pests such as insects, weeds, and diseases that can negatively impact yield. In this context, introducing synthetic pesticides has significantly contributed to overcoming pest-induced damage and helped farmers ensure improved agricultural yield for centuries.[2] However, the use of pesticides presents a dilemma to the global community. Ideally, a pesticide should only affect the targeted pests, but in reality, chemical pesticide residues may persist in the soil and environment, impacting unintended targets even long after their application.[3]

Once humans started cultivating food crop species for consumption, insects, other plants, and fungi became potential enemies. In large part, these organisms do not negatively impact human development; however, they are classified as pests when they compete for food resources, indirectly affecting the quality of human life.[4] To completely comprehend the competition between humans and pests, a database of pests compiled by Centre for Agriculture and Bioscience International (CABI) recorded the presence of 1901 crop pests, with their distribution reaching as widely as 195 countries, whilst the estimated crop loss of certain crop types to pests can be as high as 80%.[4,5] The need to control these pests and ensure sustainable crop production has resulted in the widespread use of pesticides in modern agriculture. Commercially, there are thousands of pesticide brands available for crop growers to choose from based on various criteria. Pesticides are typically classified based on criteria including target pest, pesticide compound chemical structure, or protection against a health hazard.[6]

Common targeted pests include insects, weeds, fungi, rodents, arachnids, molluscs, and algae.[7]

Generally, pesticide formulations consist of two types of chemical ingredients: active and inactive substances (also known as adjuvants). Currently, there are 1387 active substances (ASs) registered with the European Commission's database, of which 466 are authorised for use in the European Union (EU).[8] The AS in a pesticide formulation is specifically designed to prevent, destroy or control the intended pest.[9,10] Therefore, this is the component of a pesticide formulation typically chemically and/or biologically active, accomplishing the expected results through a specific mode of action.[11] Modern pesticide formulations, in the EU especially, undergo a rigorous regulatory assessment before the approval of the pesticide product,[12] with importance given to avoiding PBT (persistent, bioaccumulative, and toxic) compounds. Nonetheless, these pesticides can be transported away from the initial application site during their persistence period. The extremeness of this transportation is reported in multiple studies where pesticides have been discovered in air and surface water samples in the Arctic.[13,14] Additionally, in agricultural system, Sur and Tork approximated that only 5% of the total percentage of seed dressing or soil-applied pesticide would be available for translocation and pest interaction.[15] It is estimated that upon application, almost all (99.9%) applied pesticides can be subjected to pesticide drift.[16–18] Pesticide drift is a phenomenon where numerous simultaneous routes, including spray drift,[19,20] volatilisation,[21,22] and surface run-off,[23–25] could result in a transfer away from the initial application site of the applied pesticides (Figure 1.2). Of the applied pesticides that are not lost through pesticide drift, approximately 80-90% of AS are estimated to accumulate in soil.[26,27] Depending

on various factors, ranging from cost and targeted pests, formulation of the AS typically involves either emulsifiable concentrates or solid particles (granules, dust, wettable powder, or soluble powder).[28]

The inclusion of inactive substances into the pesticide formulation provides a vector or delivery mechanism for the formulated physical forms of AS.[11,29,30] The role of inactive substances is to enhance the effectiveness of the active component and product performance,[7] and since inactive substances do not have direct chemical or biological effect on the pest, they are labelled as “adjuvants” “co-formulants” or “inerts”.[9,31] The inclusion of inert substances in the pesticide formulation could be in the form of liquid or solid, with objectives including improving the AS’s absorption, dissolution, stability and pesticidal activity, as well as facilitating the handling of the AS to create formulations that are more convenient, safer, easier and more accurate to apply.[9,31,32] Despite its name, the inactive substance can still be chemically active. It is only classified as “inactive” since the substance’s mode of action is not to act directly on the pest.[9] Commonly, inactive substances will be included in commercial pesticide products as co-formulants, ready mixed and ready to be used. However, certain inactive substances can also be used separately and added during the on-site preparation of the pesticide mixture for agricultural application.[9,29,33] The most widely used adjuvants are surfactants. Adding surfactant into the pesticide mixture promotes the formation of micelles, increasing the solubility and the half-life (DT50) of the active substances, with a resultant increase in the active substance efficacy.[30,34,35] To understand the terms better, the term half-life is defined as the length of time required to reduce the concentration by 50% from initial concentration point in time. In contrast to half-life, DT50 is defined as length

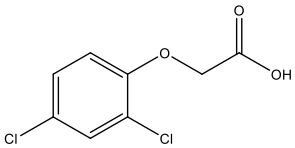
of time required for the concentration to decline to half of the initial concentration. To further complicate matters, substances classed as inactive in one formulation can be classified as active in another.

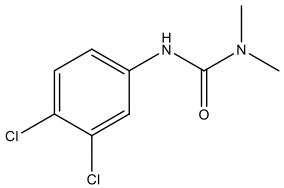
An example of this circumstance is the pesticide product registered for residential and commercial use, citric acid. Citric acid is used either as an inactive substance to reduce the pH of a formulation or as an AS in disinfectants and fungicides.[36] A similar example relates to the formulation of a glyphosate-based herbicide called Glyphogan. Vanlaeys *et al.* reported that the ingredients labelled as “inert” in the formulation of Glyphogan have a higher potency than the AS glyphosate on its own.[37] The author noted that after 24 hours, the “inert” formulant POE-15, from the family of polyethoxylated alkylamines, were observed to contribute to rapid cell mortality of the tested mouse Sertoli cell line. Furthermore, data on the inactive substances’ capability to independently exhibit toxic properties were also observed for pesticides of other classes and mechanisms of action, azoxystrobin and tebuconazole,[38] carbaryl, malathion and imidacloprid,[39] and bifenthrin and fipronil,[40] where the commercial formulations were noted to have higher toxicity compared to ASs alone. Thus, it is essential to consider the formulation entirely rather than just considering the AS when attempting to assess the potential effects of specific pesticides.

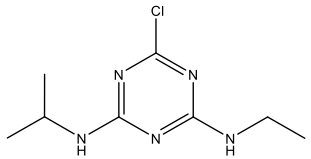
1.2 Pesticide classification

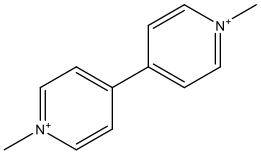
Pesticides are categorised by different classification terms, namely chemical classes, functional groups, mode of action, toxicity, and the targeted pests.[41] In terms of chemical classes, pesticides are routinely divided into two main groups, *chemicals*, namely organochlorines, organophosphates, carbamates, dithiocarbamates, carboxylic acid derivatives and substituted ureas, and *biopesticides*, which contain biocontrol agents derived from natural organisms or substances extracted from natural materials, namely animals, plants, bacteria, or certain minerals.[42,43] Within the chemicals grouping, compounds are further classified based on their target pest, with the three main chemical pesticide groups consisting of herbicides, which target weeds and unwanted plant growth; fungicides, which target fungi; and insecticides, administered to target insects and other arthropods. Other classes within this grouping are molluscicides, acaricides, nematicides, pheromones, plant growth regulators, repellents, and rodenticides.[28,44] Alternatively, pesticides can be chemically categorized further based on the functional groups of their molecular structures.[44] Accordingly, Table 1.1 summarises the main pesticide groups and the pesticides that fall under those groups, described according to their chemical classes, chemical structure, general description and examples of those pesticides, based on global pesticide usage of the year 2017.[45]

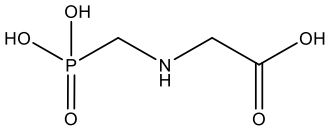
Table 1.1 Chemical classification and description of major pesticide groups, adapted from the Pesticides Use database of the Food and Agriculture Organization of the United Nations (FAO) of global pesticide usage of the year 2017.[45]

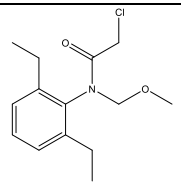
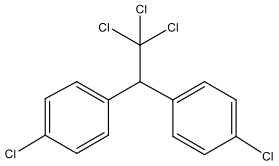
Groups	Chemical Classes	Example of chemical structure	Description	Examples of pesticides
Herbicides	Phenoxy acid	 **2,4-D**	Synthetic auxin is used as a plant growth regulator.[46] Used as systemic, postemergence herbicides and to control annual and perennial broadleaf weeds in cereals, orchards, forestry, grassland, and as well as for crop protection.[47] Major metabolites are phenol, with a chemical structure of a polar carboxylic side chain attached to an aromatic ring and lipophilic phenyl moiety.[48–50]	2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and methylchlorophenox

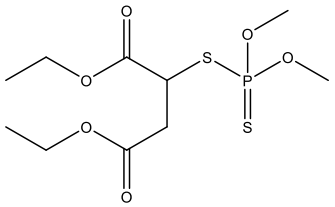
				y-propionic acid (MCPP).[47]
	Urea	 **Diuron**	Photosynthesis inhibiting weed regulator in both agricultural and non-agricultural practices, used as a pre-and post-emergence herbicide.[51] Almost all urea herbicides are trisubstituted ureas with free imino-hydrogen and urea bridge which can be substituted by sulfonyl, phenyl, triazine, benzothiazole, alkyl or other moieties.[52] Two major derivatives of urea are phenyl ureas and sulfonylureas. As sulfonylurea herbicides are recently	Chlorotoluron, diuron, isoproturon, linuron, dimefuron, methabenzthiazuron, neburon, siduron, and tebuthiuron.[51]

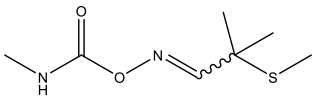
			developed, it has higher herbicidal activity than phenyl ureas.[28]	
	Triazines	 **Atrazine**	Phenoxy derivative herbicides are widely used to control pre- and post-emergence annual grasses and broadleaf weeds by inhibiting the photosynthesis process.[53] Triazine herbicides can be structurally found either asymmetrical or symmetrical. The general structure of triazine has a three-nitrogen aromatic ring. With symmetrical triazines, at the 2-position of the ring could be methoxy, thiomethyl or chlorine group, and alkylamino group at 4- and 6- positions. As for asymmetrical	Atrazine, cyanazine, prometryn, simazine, propazine, terbuthylazine and metribuzin.[28,54]

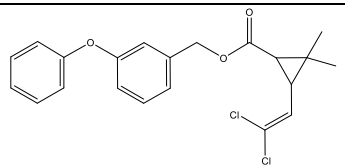
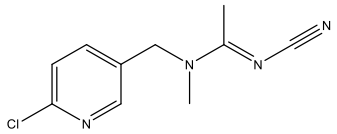
			triazines, the aromaticity of the compound is preserved with the carbonyl group.[11,54]	
	Bipyridyl	 **Paraquat**	Widely used in agriculture and gardening for rapid action to manage a broad spectrum of weeds, ranging from grasses to broad-leafed weeds. Bipyridyl herbicides are most effective when applied topically in the presence of sunlight.[54–56] This herbicide group contains a bipyridyl ring structure and exists as divalent cations linked with anions such as bromide and chloride, with very strong bases due to their quaternary ammonium structure.[28,54,55]	Paraquat and diquat

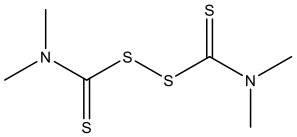
	Organophosphorus	 **Glyphosate**	Broad-spectrum, nonselective and systemic herbicide effective against all annual and perennial plants, extensively used in both agricultural and non-agricultural uses.[11,57] most prevalent organophosphorus herbicides are phosphonomethyl amino acid derivatives[57], produced through a formal oxidative coupling of the methyl group of methyl-phosphonic acid with the amino group of glycine.[54]	Glyphosate and glufosinate.[28,54]
	Amide		Selective and systematic herbicides, used as pre-and post-emergence herbicides to control grasses and broad-leaf weeds, also have foliar contact activity.[54] A key	Alachlor, metolachlor, acetochlor, pretilachlor,

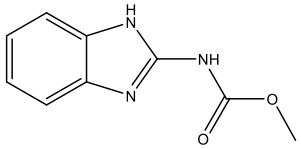
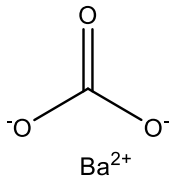
		 **Alachlor**	highlight of the chemical structure of amide herbicides is the N-substituted chloroacetamides and the substituted anilides.[28]	Propachlor, propanil, and butachlor.[28,58]
Insecticides	Organochlorine	 **DDT**	Effective not only against pests in agriculture but also effective against a broad range of insects.[59] Diverse group of chlorinated hydrocarbon derivatives compound contains at least one covalently bonded atom of chlorine, aliphatic or aromatic cyclical structure.[60] These compounds can be characterized by three different chemicals: benzene hexachlorine	Dichlorodiphenyltric hloroethane (DDT), 1,1-dichloro-2,2bis(p-chlorophenyl)ethane (DDD), dichloro diphenyl dichloroethane (DDE), dicofol, endrin, dieldrin,

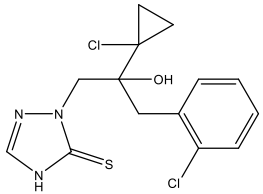
			(BHC) isomers, cyclodiene and DDT analog compounds.[28]	methoxychlor, chlordane, heptachlor and lindane.[61]
	Organophosphate	 **Malathion**	Organophosphates are compounds that contain side chains and other elements attached to phosphorus. Typically, amides, esters or thiol derivatives of phosphoric, phosphorothioic, phosphonic, or phosphinic acids contain three substituents connected to the phosphorus and oxygen group.[54,62] Effective in controlling multiple species of agricultural	Azinphos-methyl, chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, coumaphos, diazinon, dichlorvos, dimethoate, fenitrothion, fenthion and malathion.[28]

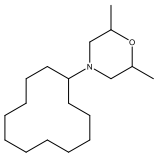
			pests and disease vectors, ranging from insect to mammal pests.[11,62]	
	Carbamates	 **Aldicarb**	Broad group synthetic compound derivatives of physostigmine, the alkaloid of the *Physostigma venenosum* plant[63], composed of esters and thioesters of the carbamic acid. The most common substituent R of insecticidal carbamates is a methyl group.[11] Extensively used to control a broad spectrum of arthropod pests.[64]	Bendiocarb, aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb, methomyl, oxamyl and propoxur.[57,59,65]
	Pyrethroids		It is commonly used in commercial household insecticides and agriculture, as it has strong selectivity and is effective	Acrinathrin, bifenthrin, cyfluthrin, cypermethrin,

		 **Permethrin**	against a wide range of arthropods.[59] Pyrethroids are synthetic derivatives of naturally occurring pyrethrin compounds obtained from pyrethrum extracts.[11,66] Each pyrethroid compound consists of one to three asymmetric carbon atoms, four stereoisomers, and two or four diastereoisomer or enantiomer pairs.[67,68]	deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin, tau-fluvalinate, permethrin.[28,67]
	Neonicotinoid	 **Acetamiprid**	Neonicotinoids are highly effective broad-spectrum and systemic insecticides which target the pest's nervous system.[69] With the chemical association with nicotine, neonicotinoid compounds exist either as cyclic compounds, with five-membered ring systems, or noncyclic compounds.	Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitromethylenes,

			These insecticides can be classified into three chemical groups, N-cyanoamidines, nitromethylenes and N-nitroguanidines.[70,71]	thiamethoxam, and thiacloprid.[70]
Fungicides	Dithiocarbamates	 **Thiram**	Broad-classed fungicides are highly reactive due to their metal-chelating agent. Dithiocarbamates are sulfur-containing carbamates.[72] Dithiocarbamates are prepared from ethylenediamine, and these chemicals are heavy metals salts of ethylenebisdithiocarbamate.[28] These fungicides were applied on fruits and several post-harvested crops,[73] and as seed protectants.[28]	Mancozerb, maneb, metiram, nabam, thiram, zibeb and ziram.[28,74]

	Benzimidazoles	 **Carbendazim**	Benzimidazoles are an extensively used group of broad-spectrum organic systemic fungicides in agriculture for pre- and post-harvest protection of crops against many fungal diseases, such as brown patches, fruit rot, grey mould, or anthracnose.[11,75] Benzimidazole is a bicyclic heteroaromatic compound linked together with both benzene and imidazole.[76]	Benomyl, carbendazim and thiabendazole.[28,74]
	Inorganic	 **Barium carbonate**	Broad-spectrum group of fungicides formulated from copper, potassium, and sulphur, approved to be used as foliar fungicides for both conventional and organic farming.[77,78] In general terms, inorganic fungicides are pesticides that do	Barium carbonate, sodium dichromate, copper sulfate, zinc chloride, zinc phosphide,

			not have carbon as the basis of their molecular structure. Except for copper and sulfur-based compounds, most inorganic compounds, namely arsenic, cyanide and mercury, are banned.[79]	cadmium chloride, and sulfur.[11]
	Triazoles	 **Prothioconazole**	Triazole pesticide derivatives are highly effective and systemic fungicides against a broad spectrum of fungal diseases, namely powdery mildews, rusts, and leaf spotting, while providing excellent protection, curative and exterminating those fungal pests.[80,81] All triazole compounds have a 1,2,4-triazole ring linked to a hydrophobic backbone through position 1. Commonly, the hydrocarbon	Cyproconazole, flusilazole, flutriafol, metconazole, myclobutanil, propiconazole, Prothioconazole, tebuconazole, and tetraconazole.[57,80]

			backbone would have substituted phenyl groups at both ends or an alkyl group at one of the ends.[80]	
	Morpholines	 **Dodemorph**	Derived from tetrahydropyran by replacing one methylene group with NH.[82] Systemic fungicide that is highly effective in protecting and eradicating agricultural crop's pathogenic fungi, such as powdery mildew.[83]	Amorolfine, dodemorph, fenpropimorph, tridemorph.[28,82]

1.2.1 Mode of action of different types of pesticides

The effectiveness of the usage of pesticides to control pests, in the context of both agriculture and horticulture, is highly dependent on a few factors, namely environmental conditions, the crop stage and the mode of action of the pesticide in use.[84,85] In terms of mode of action, it can be generalised as Figure 1.1, where any selection pesticide (herbicide, insecticide or fungicide) is applied to deter specific pests and results in the pests coming into contact and being exposed to the pesticides. Following exposure, the pesticide will affect the pest through a specific mode of action, resulting in various degrees of toxicity, utilising strategies such as endocrine disruptions, neurological disturbance, and hindering reproduction and development processes.[86,87] The further analysis of the mode of action of pesticides, according to their types (herbicides, insecticides, and fungicides), is detailed in the next section. In addition to the discussion of mode of action according to the pesticide types, Table 1.2 highlights in detail the mode of action of the common and widely used pesticide chemical classes.[45]

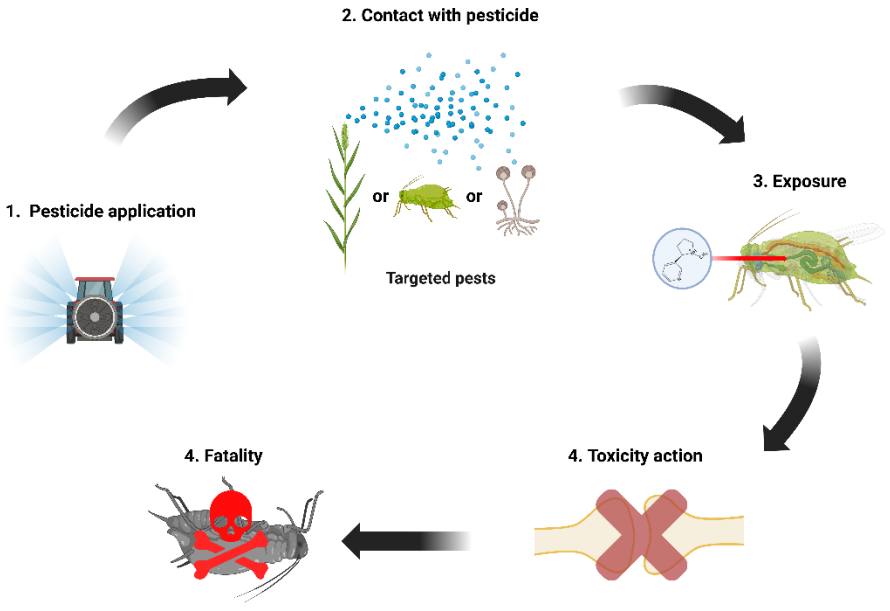


Figure 1.1 Generalised pesticide's mode of action.[86,88]

1.2.1.1 Herbicides

Herbicides, or chemical weed killers, are a class of pesticides that are used in the agricultural industry to protect the crop and agricultural yield by eradicating undesired plants.[88,89] In 2018, it is estimated that without the usage of herbicides in Europe, agricultural production could have been reduced by up to €16.7 billion.[90] To continuously protect and boost agricultural yields, total herbicide usage has been steadily increasing, especially in Europe, with the highest usage recorded in 2019 (the most recent year for which data is available) with 120,000 tonnes.[89] To achieve successful weed eradication, herbicides are designed to interact and interfere with metabolic and biochemical pathways, resulting in tissue injury, irreversible damage, and eventual death of the weeds.[91] The general mode of action of herbicides revolves around disruption, inhibition, interruption or mitigation of the regular pest plant's growth.[92–94] The individual specific modes of action will be further discussed below.

1.2.1.1.1 Amino acid synthesis inhibitors

As amino acids are essential building blocks for healthy plant development and growth, amino acid synthesis inhibitors can be used to inhibit the production of specific amino acids. One of the essential enzymes in the branched-chain amino acid biosynthetic pathway is acetolactate synthase (ALS).[95,96] ALS is involved in the biosynthesis of valine, leucine, and isoleucine, essential branched amino acids for plant growth.[97] To inhibit the activity of ALS, the herbicides commonly used are from the sulfonylurea family, which both the roots and foliages can readily absorb and translocate in the xylem and phloem to the growing points of the plant.[96,98] Considering ALS is an enzyme common in plants and microorganisms but not animals, ALS-inhibiting herbicides are highly selective,

and certain compounds are incredibly active in targeting the plant's active sites.[96] Another vital pathway in plants is the shikimic acid pathway. The aromatic ring amino acids, such as phenylalanine, tryptophan, and tyrosine, are synthesised through this pathway.[96,99] Glyphosate is the only known herbicide whose site of action is the specific and defined target that can effectively inhibit the 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSP).[100] Following the inhibition of EPSP, there is a reduction of aromatic amino acids, which are crucial for protein synthesis and plant growth.[101,102] The herbicide glufosinate can also be used to effectively inhibit the activity of another essential enzyme in supplementing a plant's development, glutaminate synthetase.[103] The role of glutaminate synthetase is to catalyse the conversion of L-glutamate, ATP, and ammonia into L-glutamine, ADP, and PO_4^{3-}. In addition, this enzyme also plays a vital role in the assimilation of nitrogen.[104] Consequently, due to inhibition of the production of amino acids, the plant's ability to produce proteins and essential metabolites will be restricted, eventually leading to the plant's death.[105]

1.2.1.1.2 Cell division inhibitors

Tubulin is an essential protein for building the intracellular skeleton forming the wall of microtubules in the plant.[106] Various chemical classes, such as benzoic acids, dinitroanilines, and pyridines, are mitotic poisons that inhibit cell division in plants. These herbicides, when administered, will bind with tubulin and interfere with normal cell division.[107,108]

1.2.1.1.3 Photosynthesis inhibitors

As photosynthesis is essential for plants to obtain energy, inhibiting this process is a sure way of halting its growth. All the herbicides capable of photosynthesis inhibition actions are nitrogen-containing compounds, such as amides, nitriles,

phenyl ureas, phenyl carbamates, pyridazines, and triazines, with a diverse chemical composition.[108,109] Most herbicides act through one particular means, by causing hindrance to the transfer of electrons by binding to a specific protein that regulates electron transfer. This binding then inhibits the electron transfer to plastoquinone, a low molecule quinone resulting in interference towards the photosynthesis of the pest plant.[108,110] This is not the only mechanism however, as photosynthesis-inhibiting herbicides can also act through the generation of free radicals, blockage of the electron transport system, and inhibition-destruction of protective pigments.[108,111]

1.2.1.2 Insecticides

Globally it is estimated that insect pests reduce agricultural yields by 10-16% before and after harvest.[112] To prevent and eliminate the threat posed by insect pests, insecticides have been used since the Middle Ages.[113] Modern insecticides include novel compounds with various chemical structures capable of interacting with different target and nontarget sites, including the pest's receptors and enzymes.[114] As most developed insecticides are broad neurotoxicants, capable of eradicating a multitude of species of insect pests, and efforts have also been made to develop chemicals that are capable of inhibiting or enhancing the respiration activity, activating the acetylcholine receptor and gamma-aminobutyric acid (GABA) receptor of a selective group of insect pests.[115]

1.2.1.2.1 Nervous system signal interferents

Insecticides that can disrupt the signal transduction in the nerves act rapidly, and they target the many sensitive nervous sites that are vulnerable to even a minor disruption, which may ultimately prove to be potent. Signal-inhibiting actions of insecticides happen through a number of mechanisms[116–118]; including preventing proper closing of the voltage-gated sodium channels, depolarization of calcium channels, causing distortion and excitation of nerve impulse transmission by interacting with pores of the insect's lipoprotein structure, obstructing the GABA gated chlorine channels, and disruption of neural transmission in the nervous system of invertebrates. The signal inhibition action of the insecticide triggers a cessation of feeding and irreversible paralysis of all the affected insect pests.[115]

1.2.1.2.2 Cholinesterase inhibitors

The enzyme acetylcholinesterase (AChE) plays a crucial role in mediating synapses, myoneural functions, and ganglia of the nervous system of both insects and animals.[108] By targeting this enzyme, insecticides can phosphorylate the esteratic active site of the AChE. This action then leads to the AChE being irreversibly inhibited and accumulation of the ester acetylcholine, which disrupts the normal functioning of the nervous system.[117,119]

1.2.1.2.3 Chitin synthesis inhibitors

Chitin is the second-most abundant naturally occurring long-chain polysaccharide[120], and is quite versatile, as it makes up a significant constituent in the exoskeleton of arthropods and the cell walls of fungi.[121] Insecticides with chitin inhibitors are most effective against juvenile insects at the stage of metamorphosis. When administered, those inhibitors will impede the synthesis

of the new cuticle, thereby preventing them from moulting successfully to the next stage.[108,122] Certain insecticides act much sooner than the insect's metamorphosis by causing defects in egg hatching.[123]

1.2.1.3 Fungicides

Like the undesired plant and insect pest infestation, fungi can also negatively affect agricultural yield. In 2011, it is estimated that the agricultural yield losses of the major crops and the fungi are enough to feed 8.5% of the global population.[124] Fungicides are generally used to prevent and eliminate fungal infections by applying on fruits, tubers, and vegetables during storage, or directly applied to crops or soil.[57] Mode of action of fungicides can be broadly grouped into sterol synthesis inhibitors, respiration inhibitors, and multi-site enzyme inhibitors.[108]

1.2.1.3.1 Sulfhydryl group inhibitor

Sulfhydryl groups are reactive and very often crucial in the active sites of many enzymes.[108] Sulfhydryl targeting fungicides react with sulfhydryl-containing enzymes and coenzymes of fungal cells. The fungicide completely immobilises an enzyme through the complex formation of the active substance with the metal atoms of metal-containing enzymes. The sulfhydryl inhibitors are commonly mutagenically active, where they affect the structure and functions of the cell membranes and inhibit the enzyme system, which leads to tumour formation in the fungal mitochondria, leading to eventual death.[28,108]

1.2.1.3.2 Cell division inhibitors

Similarly, to cell division inhibitors employed through herbicides, inhibitors of fungicides target the protein responsible as the building block of the intracellular skeleton in cells, tubulin. The tubulin exists in two forms, α- and β-tubulin, and

together a balance of assembly and disassembly maintains the microtubules. Meanwhile, microtubuli are responsible for the maintenance of cell movement, cell shape, intracellular transport, and secretion. Hence, with tubulin inhibiting fungicide in action, spindling of microtubules will be inhibited, interrupting the balance by binding to various sites of the β-tubulin.[108] As a result, the fungal cell division will be impaired. This would then lead to inhibition of mitosis in fungal hyphae and killing of growing mycelia, and eventual death of the fungi.[108,125]

1.2.1.3.3 Ergosterol inhibitors

Fungicides with ergosterol-inhibitors are effective against many different fungi. All inhibitor fungicides fall into the same group of fungicides, known as the demethylase inhibitors, where they are structurally diverse compounds but have a typical heterocyclic N-containing ring.[126] These demethylase inhibitors target the enzyme cytochrome P450-sterol-14α-demethylase, which is essential for synthesising ergosterol. Ergosterol is the most common sterol in fungi, essential for maintaining cell membranes and fungal growth.[127,128] As the synthesis of sterols is multistage and complex, various groups of fungicides can act on different targets of that synthesis.[108]

Table 1.2 Mode of action of common pesticide chemical classes.[45]

Pesticides Chemical Classes	Mode of Action
Organochlorine	Upon exposure, the insect's sodium gates will be deactivated through the activation of potassium conductance and followed by destabilized negative after-potential when leakage of Na^+ ions happen through the nerve membrane. This action causes the insect to experience hyperexcitability and repetitive discharges in insect nerves, and finally, convulsions.[129–131]
Organophosphorus	Organophosphorus insecticides promote irreversible inhibition of the enzyme acetylcholinesterase (AChE) by binding with the esteratic site of cholinesterases through an electrophilic mechanism. This binding causes inhibition to the synaptic transmission of the nerve impulse in the insect nervous system. Due to the inability of phosphorylated AChE to hydrolyze the increasing concentration of acetylcholine in the synapse, acetylcholine's binding to its postsynaptic receptor is prolonged. Eventually, this leads to signs of intoxication, including hyperexcitability, tremors, paralysis, and finally, death of the insect.[132–134] As for organophosphorus with herbicidal actions, they act as an amino acid synthesis inhibitor. The herbicides target the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase

	enzyme, preventing phosphoenolpyruvate (PEP) from binding into the binding site of the enzyme-shikimate-3-phosphate complex.[135,136] The binding of PEP to the enzyme is necessary for the generation of the EPSP from shikimate-3-phosphate and phosphoenolpyruvate as part of the shikimic acid pathway.[136,137] Without it happening, it will lead to crucial aromatic amino acids for protein syntheses, such as phenylalanine, tyrosine, and tryptophan, being completely exhausted and biosynthetic metabolic pathways to halt, resulting in plant death.[137]
Carbamates	The mode of action is similar to organophosphorus insecticides, and even the signs of intoxication are identical, with interference toward the nerve impulse causing excessive neuroexcitation. However, compared to OPPs, the decarbamylation of acetylcholinesterase is much more rapid, and carbamates insecticides are considered reversible acetylcholinesterase inhibitors.[117,133,138]
Pyrethroids	Affect the nervous system of insects by causing multiple action potentials. Insecticidal actions of pyrethroids depend on their ability to modify the kinetics of voltage-sensitive sodium channels, increasing sodium's permeability into the nerve membrane. This action occurs through the retardation of the kinetics of sodium channel activation,

	inactivation and deactivation.[131] Two types of nerve excitability depending on the structure of the pyrethroid administered. Type I compounds cause continuous burst discharges of an action potential following a single stimulus, while type II compounds cause a use-dependent block of the action potential supplemented by depolarization of the resting potential.[139] However, regardless of the pyrethroid structures, the exposed insect will quickly develop hyperexcitations, convulsions, paralysis, and death upon intoxication.[139,140]
Neonicotinoids	The insecticide neonicotinoid mimics acetylcholine in the insect nervous system. Nicotinic acetylcholine receptors are at each end of insects' synaptic nerve terminal and neurone cell bodies.[141] When administered, nicotinic acetylcholine will act on the postsynaptic receptor, causing an influx of sodium ions and generation of action potentials. Without AChE terminating it, continuous stimulation would result in hyperexcitation, tremors, paralysis and death.[117,142–144]
Phenoxy acid	Phenoxy acid herbicides mimic and act as auxin hormones. Upon application, the herbicides will get absorbed through the leaves or roots and are translocated to the meristemic tissues of roots and shoots. Increased accumulation will stimulate the uncontrolled growth of the meristematic

	tissues. On top of that, it will affect the plant at the molecular level preventing healthy plant growth and development by inhibiting the synthesis of DNA and protein.[145,146]
Chloroacetamide	Chloroacetamide herbicide is a shoot growth inhibitor. The mechanism occurs through the association of acetyl-coenzyme A (CoA) and sulfhydryl-containing molecules via thiocarbamate sulfoxides, inhibiting the biosynthesis of nonsphingolipid long-chain fatty acids.[147,148] The inhibition not only impacts the plasma membrane function but also leads to a lack of synthesis of lipids, lignin and protein, which is crucial during the seedling shoot growth stage of a plant.[88] This impairment retards the weeds' preemergence.
Amide	This class of herbicides inhibits the quinone binding site in photosystem II by binding onto the binding site of the protein complex present in the chloroplast thylakoid membrane.[88] The binding causes disruption to the electron transport system, increasing the production of reactive oxygen species. This action then leads to substantial damage to the DNA, proteins and cellular membranes, resulting in the death of the pest plant.[88,149,150]
Triazole	This group of fungicides act by inhibiting the synthesis of sterol by immobilising the enzyme C14-demethylase.[151] As

	sterols are crucial in maintaining the fungal cell membrane's structure and function, disrupting sterol synthesis would lead to weakened fungal cell membranes. Certain triazole compounds would also result in abnormal fungal growth and death.[28,81]

1.3 The behaviours of pesticides in soil

Although cropland soils are reservoirs for essential elements, minerals and biological components crucial in sustaining crop growth, they are also potential reservoirs of pesticides that have been previously applied.[152] Kosubová *et al.* reported pesticides detected in soil samples, even though their application was not reported by farmers, concluding that up to 69% of quantified pesticide residues could have persisted from the previous growing season(s).[153] Similarly, pesticides have been reported to be detected up to 10 years after application.[154] As highlighted before, most modern pesticide chemicals are developed to not persist in the environment. Therefore, the detection of pesticide residues years after application is concerning. This contradiction between pesticides' expected and real-world persistence could be because most pesticide behaviour studies are conducted in optimal laboratory conditions.[153] In the real-world environment, pesticide compounds are exposed to a variety of conditions, which could modify the behaviour of pesticides to take multiple routes, as highlighted by Figure 1.2. When pesticide compounds are applied in the environment, they can go from their source of application to other components of the environment, namely the atmosphere, water and soil.[155] Pesticide residues, in general, tend to reach soil layers through two pathways; either the residue reaches crop-planted soil during

the period of pesticide application, or the accumulated residues on the aerial parts of the plant are washed off during rainfall and deposited into soil.[156]

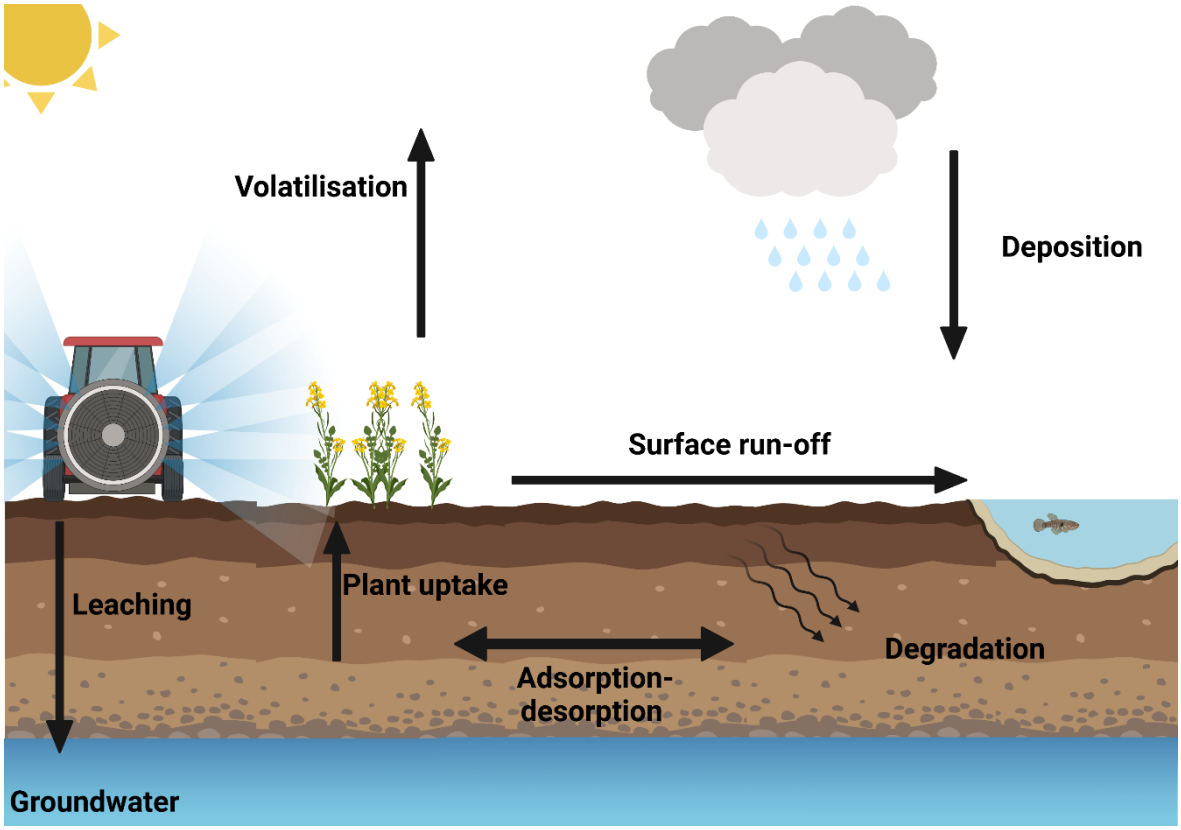


Figure 1.2 *General depiction of pesticide movement and behaviour in the environment.*[157]

Pesticide compounds can exist in multiple forms in the environment, commonly referred to as pesticide residues. Pesticide residues are defined as "*any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities that are considered to be of toxicological significance*".[158] In general terms, pesticide transformation products result from the breakdown process of the parent compound either through chemical or microbiological means, which will be further discussed in Section 1.3.2. Meanwhile, metabolites refer to the conversion

product of the parent compound following cellular metabolic processing, generally by the targeted pests.[159]

Different pesticide groups can interact differently with the immediate soil components they encounter when in soil. Most pesticides will undergo degradation, while others can persist in the soil aggregates in their initial form. The persistence of pesticide compounds in soil, either in their parent compound or their degradation product, is generally measured in terms of half-lives, where half-life is a measure of the pesticide compound reduced by 50% in the soil from any concentration point in time.[160] Consequently, the longer the half-life of a compound, the longer the pesticide compound will stay in the soil in its parent form, therefore prone to continue to take multiple routes through the environment.[161] Pesticide half-life can be categorised in three ways that are best used in estimating their level of persistence in the soil; ranging from low (less than 16 days), moderate (16-59 days), and high (over 60 days).[162] Pesticides with low and moderate persistence levels dissipate faster from the soil layer where there is little to no build-up. Pesticides with high persistence levels present a higher risk of contaminating other components of the environment due to their longer half-lives, which could be the result of pesticide concentration build-up in the soil layers with repeated applications continuously.[163]

In addition to the persistence level, all pesticide compounds fall into two categories, stable and unstable. Unstable pesticide compounds with short half-lives undergo rapid decomposition when exposed to environmental factors representing a low to moderate persistence level. Meanwhile, a stable compound might be retained for longer in the soil layer, with a high half-life representing a high persistence level.[161] Following decomposition, adsorption can occur,

involving binding the pesticide residue to soil organic or inorganic constituents. Adsorption occurs when the soil constituents attract the pesticide compound from its solution, causing the removal of the pesticide compound from the water in the soil, followed by its sequestration on the soil constituent's surface.[164] This sequestration process will then bind the pesticide residue to the soil. Pesticide binding to any soil constituents can happen through covalent and non-covalent bonding. Pesticide compounds or their degradation products which bind with soil constituents through chemical reactions, often form stable associations that increase soil persistence.[6]

An additional process that can occur to pesticides in the soil is desorption, which involves the process where abound pesticide residue is volatilized, leached or removed from the soil.[165] In comparison to adsorption, desorption is hysteretic and related inversely proportional to adsorption.[166,167] Pesticide desorption into the soil solution is mainly initiated to reinstate the soil-aqueous solution equilibrium.[168] For instance, when a pesticide compound is in equilibrium with the adsorbed pesticide and then removed from the soil solution via degradation or transport mechanism, another pesticide compound will desorb from the soil constituents and re-establish the initial solution equilibrium.[167] However, the longer a pesticide compound resides in the soil layers, the harder it will be to desorb. This is not only due to the kinetics of pesticide desorption being slower than adsorption[167], but it is also during the steady state of sorption that the compound is transferred to more restricted sites of soil constituents.[169] In the instance when a pesticide residue binds with soil constituents, it balances the chemical equilibrium between soil constituents and soil water. However, when changes caused by environmental factors result in the desorption of a pesticide,

it will cause an imbalance in soil chemical equilibrium. In exchange for the desorbed pesticide compound, another available pesticide or another soil component with the right functional group will adsorb into the soil layer, again balancing the soil's chemical equilibrium.[164] Hence, adsorption and desorption processes dominate factors that affect the movement of pesticide residues in and out of the soil environment (Figure 1.3).

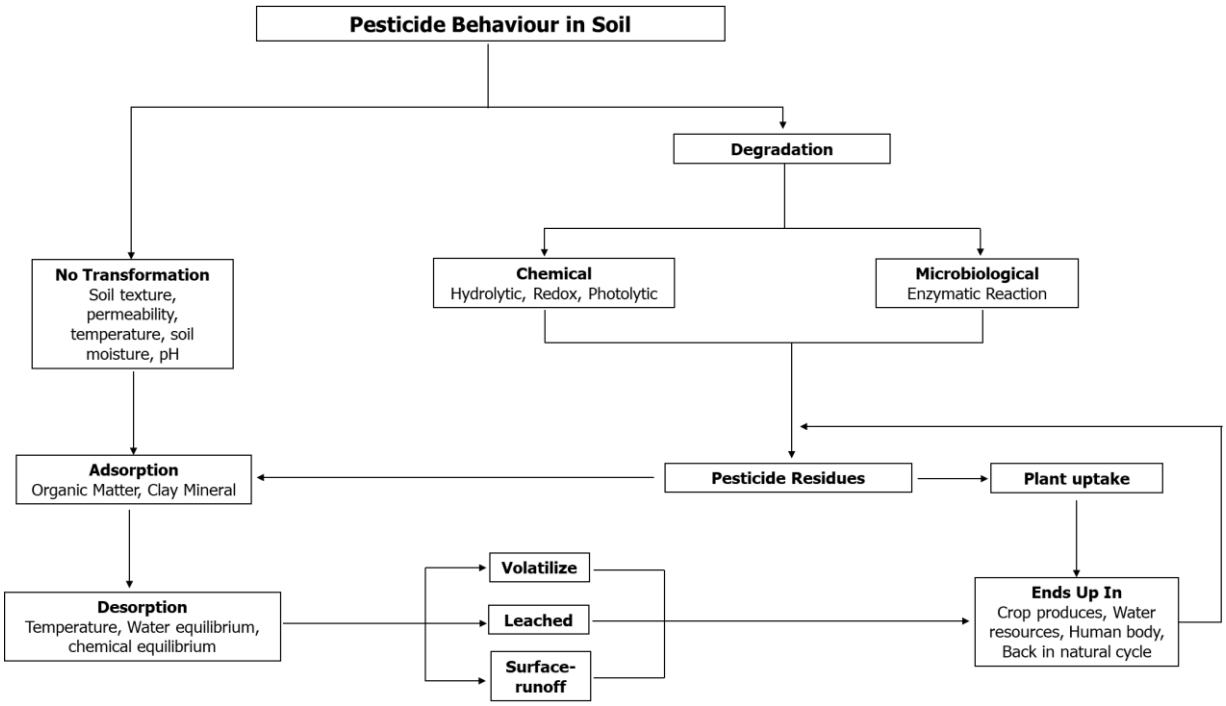


Figure 1.3 *Detailed flowchart of pesticide movement in soil environment; adapted from Langenbach.*[157]

Soil physicochemical properties impact the pesticide behaviour in soil layers. However, physicochemical properties of a particular pesticide, including molecular size, water-solubility, lipophilicity, ionisability, volatility and polarizability, also determine its behaviour in general.[170,171] Adsorption-desorption actions are the primary interactions pesticide compounds undergo with soil constituents. Differences in the molecular size of the pesticide also govern their solubility, even though they have the same polarity. With decreasing

molecular size, pesticides are typically increasingly more water-soluble.[172] Water solubility of a pesticide compound can be used to determine the persistence of the pesticide in the environment, where very soluble pesticides adsorb strongly unto soil constituents and will not be readily available for degradation due to their strong polar nature.[7] To predict the potential behaviour of a pesticide in an environment, several sensitive mathematical models can be used. These include half-life, as mentioned earlier, but in addition, distribution coefficient (K_D), adsorption coefficient (K_{OC}) and partition coefficient (K_{ow}) models are used to determine the persistence level of a pesticide.[173] When pesticides are applied by spraying, airborne pesticide droplets will be vulnerable to evaporation and volatilization. K_D would be beneficial in estimating the losses of sprayed pesticides and vapour pressure, solubility, and physicochemical parameters of the applied pesticide, leading to atmospheric deposition.[174]

Following atmospheric deposition, sprayed pesticides are susceptible to wind conditions, resulting in spray drift and the deposition of the pesticide far from the targeted site.[175] In soil, pesticides with low K_D values will be very mobile and highly soluble, potentially contaminating groundwater through surface run-off or leaching.[176] However, the K_D coefficient is a less sensitive mathematical model as it does not consider the soil's organic content, which is a crucial soil sorbent in the adsorption of pesticides.[177] Contrary to the spray application, pesticides applied through seed dressings are directly inserted into the soil of the specified area. To predict pesticide mobility in this scenario, K_{OC} is best used. K_{OC} is the soil organic carbon-water partitioning coefficient calculated by normalizing soil and water partition coefficient (K_d), which is a ratio of the pesticide concentration in the dry weight of organic matter to its dissolved concentration in the

water.[178,179] As organic matter is one of the dominating sorbents in soil, factoring in the adsorption coefficient could be crucial in predicting the mobility of pesticides in soil.[180,181] Thus, the higher the K_{OC} of a pesticide compound, the lower the concentration of the pesticide in soil solution, and higher K_{OC} valued compounds are less mobile (Table 1.3).[182] Therefore, chemicals with high K_{OC} value are the same as those with high K_D, where the higher the K_{OC}, the greater the adsorption of the chemicals to the soil.[183]

Table 1.3 *Classification of chemical mobility in soil based on K_{OC} value.*[184]

K_{OC}	Mobility in Soil
0-50	Extremely mobile
50-150	High mobility
150-500	Medium mobility
500-2000	Low mobility
2000-5000	Slightly immobile
>500	Entirely immobile

K_{OW} is the correlation coefficient of octanol-water partition and is determined by calculating the ratio of a specific pesticide's concentration in octanol and water.[185] The K_{OW} value helps predict the degree of affinity of a pesticide towards water or other soil constituents.[186] This coefficient is also useful for predicting the environmental fate of a pesticide by measuring the lipophilicity or tendency of the pesticide compounds that exist in the aqueous phase to translocate into the soil organism's lipid layers.[182] Accumulation of increasing quantity of high K_{OW} valued

pesticides in the soil organism's body tissues leads to a phenomenon known as the bioaccumulation.[187]

To account for the broad range of organic compound concentrations in soil, log K_{OW} is generally used, which also has the advantage of being directly proportional to the energy contributions from the molecular structure of chemical compounds.[188] For instance, log K_{OW} value increases with an increasing number of hydrophobic structures such as benzene rings, chlorine atoms and methylene groups in a molecule. Hence, the hydrophilicity or hydrophobicity of a compound can be determined when its log K_{OW} ranges are between -3 (extremely hydrophilic) and +10 (very hydrophobic).[188] Furthermore, if a pesticide has a low log K_{OW} value, it indicates that it is chemically polar and will, therefore, interact well with water and have high-water solubility. Pesticides with high log K_{OW} must be treated cautiously as their low water solubility means they will readily be adsorbed by organic matter.[172,189] Moreover, with increasing log K_{OW} value, there is an increased risk of bioaccumulation occurring. United Nations Economic Commission for Europe (UNECE) noted that a log K_{OW} value of four and above could lead to the pesticide compound persisting longer in the soil, subsequently leading to an increased chance for the chemical compound to be uptaken by the soil organisms.[187] Miller *et al.* noted that log K_{OW} increases when other physical factors, such as pesticide molecular surface area, molecular weight, density and boiling point, increase, which reduces their polarity.[190] Additionally, it is essential to categorise pesticides based on their chemical nature to standardize the classification of pesticides worldwide. When it comes to chemical classification, pesticides are best characterised based on the chemical nature of their active

substance, allowing the user to access the details related to the efficiency and physicochemical nature of the pesticide.[7]

1.3.1 Adsorption-desorption in soil

Pesticides that accumulate in soil may experience transformation. Regardless of the possible transformation outcome, a pesticide compound has the potential to incorporate and adsorb strongly to suitable soil constituents.[191] Adsorption and desorption of pesticides in the soil mainly depend on three key factors; sorbents, sorptive, and environmental (Figure 1.4).[164,192,193] The sorbents in soil represent the organic and inorganic soil constituents which provide suitable polarity and functional group for the adsorption of any pesticide compounds, which represents the sorptives. Meanwhile, the physical and chemical properties of the sorptives, such as concentration, ionic charges and sorptive size, also determine the sorption interactions. Furthermore, environmental factors, namely soil texture, temperature, precipitation and soil pH, alter the sorption processes between the sorbents and sorptives.[166,173,194]

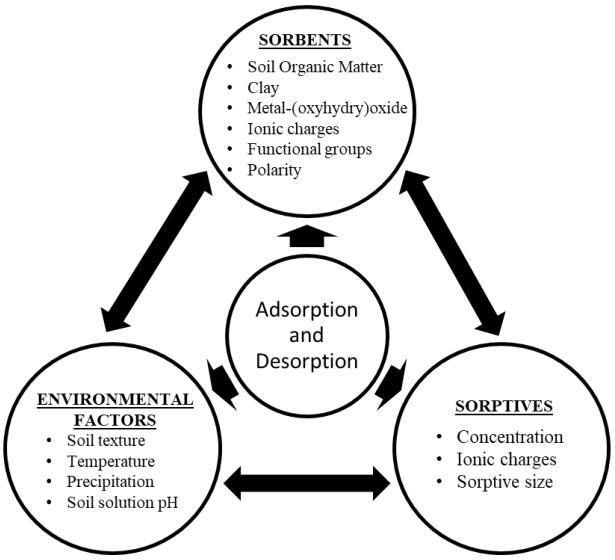


Figure 1.4 *Generalised relationship depicting factors that affect adsorption and desorption in the soil environment.*[166,173,194]

There are three known types of soil sorbents that a chemical compound can adsorb to; metal-(oxyhydrl)oxides, clay and organic matter.[192,195,196] Of the three sorbent classes, soil organic matter and clay minerals are identified as the most critical soil sorbents that play an essential role in pesticide adsorption in the soil layers, as they make up the significant layers of solid-phase materials in soils.[192,197,198] Soil organic matter constituents are heterogeneous, with large components decomposing plant materials and soil organisms. In addition to their heterogeneity, different organic sources will have different functional groups capable of adsorbing diverse pesticide compounds.[180,199] Owing to their heterogeneity, soil organic matter makes up the portion of soil that is an uncharged organic sorbent that exhibits a series of polarities, with extensive dispersal of an electron across the molecules.[192] Kearney *et al.* suggest that due to the presence of numerous functional groups in and around soil organic matter, such as carbonyl, amino, imidazole, sulfonic, sulfhydryl, and carboxyl, it can adsorb pesticide residues well.[164] Due to the heterogeneity of the functional groups in soil organic matter, pesticide compounds can adsorb to soil organic matter through various degrees of strengths, either through van der Waals forces, hydrogen bonding, ion exchange, dipole-dipole interactions, and covalent bonding (Figure 1.5).[164] The chemical affinity of pesticide compounds toward soil organic matter is dictated by their chemical configuration and the soil organic matter's size. With decreasing particle size, organic matter's surface area increases, providing numerous sorption sites and prospects for the chemical compounds to adsorb onto it.[200] Pesticide residue binding to the organic matter could benefit the environment as adsorbed pesticide residues typically have lower toxicity and are less prone to leaching and polluting groundwater.[201,202]

Nevertheless, pesticides and their metabolites bound to the organic matter would not be available from further degradation and dissipation from the environment, therefore increasing their persistence in soil.[203]

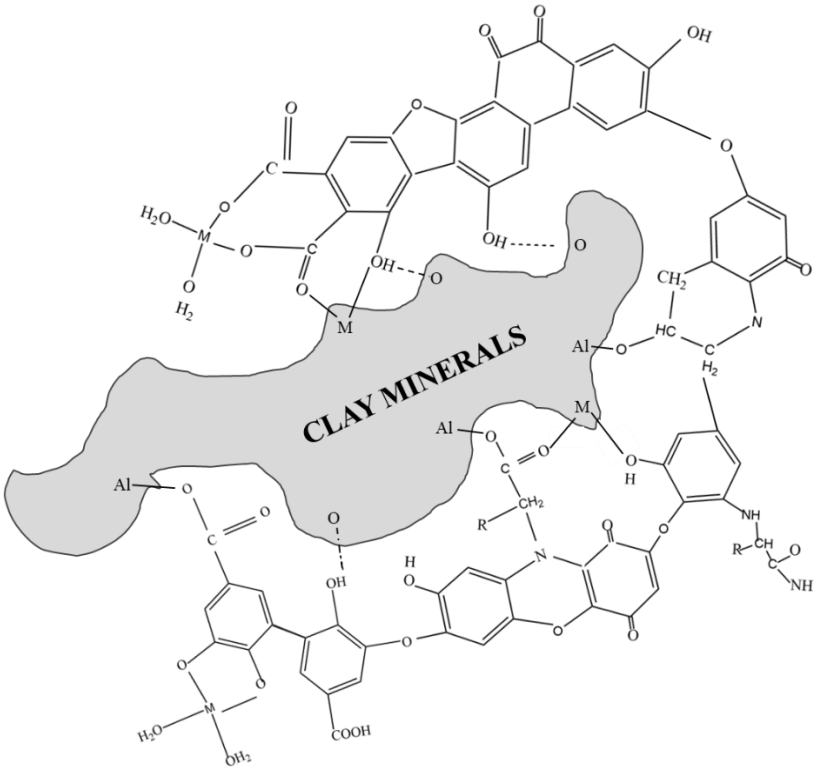


Figure 1.5 *Association of functional groups of soil organic matters with clay minerals; adapted from Kearney et al., and Koskinen and Harper.*[164,167]

The remaining sorbent class, metal-(oxyhydr)oxides, exist predominantly as coatings on clay minerals in soil.[204] This increases the metal-binding sites in soil, increasing its affinity for heavy metals to be significantly higher than clay minerals.[205] Meanwhile, pesticide residues are observed to have higher adsorption affinity for the clay minerals compared to metal-(oxyhydr)oxides.[192,206] These minerals can strongly adsorb dissolved organic matter unto their surface without difficulty, suggesting that pesticide residues also have the potential to bind to the mineral's surface.[207] Clay minerals can be divided into two distinct

types, crystalline and amorphous. However, amorphous minerals, namely silicate layers, provide hydrophobic sites for effective binding of pesticide residues.[203] As with organic matter, clay minerals have key functional groups, siloxane ditrigonal cavities and inorganic hydroxyl, that contribute to pesticide adsorption unto their surface.[164] Clay minerals in soil are predominantly negatively charged due to their layers of aluminium-oxygen, and silicone-oxygen sheets are often chemically substituted by ions of lower valence, making them a source of negative charge in soil.[192] Being anionic sorbents, clay attracts cationic compounds, rendering them immobile. Even though clay minerals are one of the major sorbents in soil, Đurović *et al.* postulated that clay minerals preferentially adsorb pesticide residues only in soil layers with a low percentage of organic matter.[208] This postulation is plausible as dissolved organic matter tends to mask the clay surface layer reducing the accessibility of pesticide residues to the clay mineral surface, thus preventing them from binding (Figure 1.5).[209] Even though it has been observed that soil organic matter performs most efficiently in the adsorption of pesticide residues compared to clay minerals, both of these sorbents are highly associated with each other.[210] For instance, soil organic matter that been mask with clay minerals, will remain protected from mineralisation. Through this masking process, concentration of soil organic matter is directly or indirectly governed by clay minerals. [210] Hence, it can be postulated in the soil layers with high organic matter and clay mineral percentage, has a higher probability of pesticide adsorption. Nevertheless, the sorption of pesticide residues by soil organic matter strongly depends on the pesticide's hydrophilicity, while pesticide residue adsorption by clays depends on the type and properties of pesticide and clay minerals.[211]

With the availability of active adsorption sites on the sorbents, the principal factor that governs sorption in soils is the properties of the pesticide compounds.[192,212] The first determinant of the sorption of a pesticide compound, either onto soil organic matter or clay, is the ionisability of a pesticide compound. Pesticides with ionic equilibrium constants near the soil pH range have a K_D that is quite sensitive to the pH of the sorbing soil since the adsorption of the pesticide will be a combination of both the ionized and non-ionized species. pH also affects the soil surface ionic charges, where each unit fluctuation in pH increases the ratio of adsorption of ionized and non-ionized compounds approximately tenfold.[213–216] Wauchope *et al.* noted one in three commercial pesticides to be either acidic or basic.[217] Ionisation is pH dependent; hence if a pesticide is either acidic or basic, it can dissociate into ions in the soil solution with a pH range of 5-8. Following dissociation, ionized forms of a pesticide compound will behave differently compared to non-ionic pesticides.[218] Acidic and basic compound dissociation can be measured using the pK_a, or the log of acid dissociation constant value, and pK_b, or the log of base dissociation constant value.[183,218] The chances for volatilization of ionisable pesticide compounds are meagre, but there is a higher probability for the dissociated ions to get adsorbed strongly to the soil constituents.[203,217,218] Table 1.4 summarizes the effects of acid and base pK values and their environmental fate. Ionisable pesticide compounds, such as weakly basic compounds, are easily protonated at low soil pH values. Following ionization, cationic pesticide species adsorb to the clay content.[203] However, with increased soil organic matter, cationic species have a higher inclination to adsorb to the soil organic matter due to the presence of carboxylic and phenolic functional groups.[219] As the weakly acidic pesticides exist either as undissociated

molecules or the corresponding anion. This form will result in anionic species repelled by the negative net charge of soil colloids, resulting in low K_d values.[220,221] Nonetheless, acidic pesticides may be sorbed on soil organic matter via an anion exchange mechanism.[11,218] As for the non-ionic pesticides, the compound's polarity largely dictates their adsorption to the soil's organic or inorganic constituents.[222] Polar compounds can adsorb to both organic matter and clay particulate in the soil; however, non-polar compounds will adsorb to organic matter due to their inclination to adsorb through hydrophobic partitioning.[203]

Table 1.4 Environmental fate of acidic and basic pesticides.[183,218]

pKa and pKb value	Ionic pesticide species within normal pH range (5-8)	Potential environmental fate
pKa>10	Neutral	Except in alkaline conditions, it behaves as a non-ionic compound, has lower solubility than an anionic state, and has lower mobility in an anionic state, making volatilisation possible.
pKa<3	Negative charge	High mobility in the soil unless complex compounds are formed, very soluble, high mobility under alkaline conditions, low volatility.

pKa 3-10	Ratio X^-/XH	When soil pH is close to pKa, pH's influence is more significant on solubility, mobility and volatility.
pKb>11	Neutral	Except in acidic conditions, it behaves as a non-ionic compound, with lower solubility than a cationic state, higher mobility in a cationic state, and possible volatilisation.
pKb<4	Positive charge	Very soluble, low mobility, low volatility, strong adsorption to the soil with a long half-life.
pKb 4-11	Proportion $X^+/X(OH)$	When soil pH is close to pKb, there is a strong influence of pH on solubility, mobility and volatility.

The dynamic liquid component of soil is known as the soil solution. Soil solution comprises dissolved gases, electrolytes, organic compounds, and labile substances from different soil sources.[223,224] Adsorption and desorption of sorptive compounds to and from the soil sorbents highly depend on the concentration of these chemical compounds in the soil solution. This cause and effect can be explained based on Le Chatelier's Principle of chemical equilibrium, which dictates that an increased chemical concentration in the soil will increase its adsorption rate.[192] For instance, as one of the final degradation products of glyphosate, aminomethylphosphonic acid (AMPA),[225] increased concentration of glyphosate in the soil will lead to an increase of AMPA's concentration. This, in turn, will increase the adsorption of glyphosate or AMPA to the soil sorbents.

Conversely, removing mobile glyphosate or AMPA compounds from the soil solution will cause an imbalance in the soil solution chemical equilibrium, driving the desorption of glyphosate or AMPA compounds from the soil sorbents.[192,226] In this way, there would always be a particular concentration of glyphosate or AMPA persisting in the soil. Subsequently, when sorptive of equivalent ionic charges and concentrations compete for the active sites or functional groups for adsorption, the sorptive's size will be the decisive factor.[192] It is generally observed that adsorption affinity increases with the increasing ion radius of a sorptive compound. An ion that enters the soil solution will form a hydration sphere around the ion. The hydration sphere would become more susceptible to further association with other soil constituents. However, ions with smaller ionic radius have an energetic disadvantage compared to larger ions, as it requires more energy to remove the surrounding water molecule. Consequently, larger ions could form a covalent bond quickly with the sorbents. For instance, Sparks discusses how the adsorption affinity of ions of elements within a single group of periodic tables decreases with the ionic radii.[212]

Soil solution's pH, coupled with chemical degradation hydrolysis, influences the adsorption ability of soil constituents.[227] Weber *et al.* confirmed in their study that soil solution's pH does not necessarily correlate with soil organic matter or clay, making it an essential factor in determining the inclination of pesticide compounds to retain in soil.[228] As the soil solution's pH decreases, hydroxyl and carboxyl functional groups on the sorbents, mainly soil organic matter, will be protonated. This protonation then increases positive charge density on the sorbents, facilitating anion compounds' adsorption while decreasing cation compounds' adsorption. Cation exchange capacity, or the ability of a soil to retain

cations, generally increases with increasing soil pH.[192] Nevertheless, pH alone would not be able to influence the increased adsorption of pesticide compounds; pH, soil organic matter and clay are a collection of soil properties that play their role as a collective. Increasing soil organic matter content and decreasing soil solution's pH are observed to increase the retention time of pesticides in the soil.[228]

Figure 1.5 generalises the close interconnectivity between the sorbents and the sorptives and the environmental factors that play key roles in regulating the fate of a pesticide in the soil environment. These environmental factors can be divided into biotic and abiotic properties. Biotic, or biological, factors consist of living organisms ranging from micro, meso and macro sizes. Environmental abiotic properties include light intensity, temperature, precipitation, and air movement.[166,173,194] While it is essential to consider the biotic and abiotic properties of the environment to determine the behaviour of pesticide residue in the soil, it is also important to take into account soil physicochemical properties such as texture, permeability, soil temperature, soil moisture and soil pH, as all these properties also play a key role in determining the extent of pesticide persistence. For instance, soil structures such as texture and permeability generally affect the movement of air or water through soil layers, further controlling the movement of pesticide residues.[164] Given that an increase in soil aggregates will affect pesticide movement, an increase in soil bulk density will decrease the rate of pesticide adsorption.[229] In particular, soil layers that makeup particles of large size will increase the possibility of leaching occurring, which then eases the pathway for the pesticide to reach groundwater.[221] Following that, temperature also plays a crucial function in the desorption of pesticide residues

from soil constituents. Adsorption of pesticides to soil organic matter or clay minerals is usually an exothermic reaction, where heat is given off when ionic or hydrogen bonds are formed.[230] In general, an increase in soil temperature also increases pesticide solubility in water, which then increases the lability of the pesticide compound through the soil layers.

Furthermore, mobile pesticide compounds have a strong desorption rate, increasing leaching or surface runoff, resulting in surface and ground water contamination.[231] This is supported by observations that even though pesticides tend to remain stable in a lower temperature, desorption and mobility of pesticides increase at a higher temperature.[232] Some pesticides do, however, behave differently by exhibiting endothermic adsorption in soils. This endothermic adsorption indicates that the adsorption of the chemical to soil constituents requires activation energy, also known as chemisorption. Furthermore, suppose the adsorption potential between pesticide and soil constituents increases with temperature. In that case, it could indicate an increase in surface area for adsorption and the creation of reactive and active sites. Nevertheless, exothermic adsorption more or less happens explicitly in nature.[233] Ultimately, the adsorption and desorption of pesticide residue in the soil could not be fully comprehended without considering the linkage and interaction between all three main factors simultaneously.

1.3.2 Degradation of pesticides in soil

1.3.2.1 Chemical degradation

Along with sorption, degradation is the other prominent process for making any pesticide compound benign or dissipate from the environment.[234,235] Pesticide degradation involves the breakdown of the pesticide's chemical structure, which can be initiated either chemically or microbiologically.[156] Chemical breakdown can be initiated by both abiotic and biotic components of the soil, while microbiological breakdown happens entirely through enzymatic activity.[164] However, the degradation mechanisms a pesticide would eventually undergo strongly depend on its vulnerability to chemical structure alterations.[235] Chemically mediated pesticide degradation processes can be generally divided into hydrolytic, redox, or photolytic reactions. In hydrolytic reactions, water is the principal degrading agent, where the combination of water and pesticide at the molecular level results in a complete or partial breakdown of the pesticide compound.[235] As many functional groups in pesticide compounds are vulnerable to changes in pH, degradation of these compounds through hydrolysis is ubiquitous in the environment. During hydrolysis, water molecules will react with the pesticide compound forming two individual and stable labile compounds.[183] For instance, pesticides that are derivatives of carboxylic acids have a carboxyl functional group and pesticide derivatives of carbamates have amide and ester linkages, all of which are vulnerable to hydrolysis. These transformations involve nucleophilic displacement when the organic molecule, RX, reacts with water, forming a carbon-oxygen bond and cleaving a Carbon-X bond in the parent pesticide to form ROH and a leaving group (X-) (Equation 1.1).[156]

$$RX + H_2O \rightarrow ROH + X^- + H^+$$

Equation 1.1

Similar to the adsorption and desorption of pesticide compounds, soil pH plays a vital role in supplementing degradation via hydrolysis. Pesticide stability may be pH dependent and can be easily influenced by fluctuations in soil pH and water, which increases the likelihood of the pesticide dissociating easily and forming separate compounds. An example is the pesticide atrazine, which rapidly degrades when the soil pH decreases as it readily donates electrons.[236] Typically, pesticide compounds vulnerable to degradation through hydrolysis would have a strong polar nature. These compounds produce degraded substances that are water-soluble and susceptible to biodegradation or photolysis. Hence, as the resulting substances are also easily degraded and less persistent, it reduces the potential for the toxic compound to accumulate in the soil even with repeated application.[183]

A redox reaction involves donating or gaining electrons between reactant and product, also known as oxidation or reduction reactions. Redox-mediated pesticide degradations are usually induced by another degradation process, typically a photolytic reaction.[237] Ultraviolet light is quite potent in degrading pesticides in the environment.[238,239] Photolytic reactions in natural environments typically involve either direct or indirect photodegradation. Direct photodegradation occurs when a pesticide compound is excited by absorbing the ultraviolet light, resulting production of a triplet state and breaking down through the means of either of three different processes, homolyses, heterolysis or photoionization.[240] Indirect photodegradation is when another photon-absorbing environmental constituent reacts and transfers energy to a ground-state

pesticide.[237] However, although ultraviolet light is a valuable source of energy in degrading pesticides, it is less effective in the photodegradation of pesticides in soils as sunlight is only able to penetrate the soil to a depth of less than 0.5 mm.[241] Examples of pesticides that are vulnerable to photodegradation are those weakly sorbed to soil constituents and water-soluble, where there is a higher chance for the pesticide to rise with capillary water to the soil surface.[164] For instance, based on the mobility classification based on K_{OC} values (Table 1.3), both clothianidin and thiamethoxam are classified as pesticide compounds with high mobility with K_{OC} values of 60 and 68.4, respectively. Due to their weak interaction with the soil components, these compounds can rise with the water molecules and be exposed to sunlight for rapid degradation. This phenomenon is reported by Li *et al.*, where the authors noted that with exposure to UV light, both in soil, clothianidin and thiamethoxam rapidly degraded within 97 to 112 hours and 88 to 103 hours, respectively.

1.3.2.2 Microbial degradation

Microbiological degradation can be initiated or assisted by numerous groups of microorganisms, mainly bacteria and fungi. The microbial degradation rate in soil is influenced by various environmental properties, namely temperature, moisture, physicochemical properties of the soil, and the presence of other carbon or nitrogen sources.[242] The importance of soil microorganisms for the dissipation of pesticides in the soil environment is widely accepted.[65,242–246] The general biodegradation mechanism can be condensed into three main parts. The first part would be the contact, where the microorganism adsorbs the pesticide compound, which takes place on the cell membrane surface. It is followed by the pesticide compound entering the cell through the cell membrane surface and, lastly, a rapid

enzymatic reaction in the membrane resulting in full or partial degradation of the compound.[247] The pesticides' microbial degradation processes can be further divided into biodegradation, co-metabolism and synthesis.[156] The biodegradation metabolism is the process where, in the effort to obtain nutrients for growth and energy, microorganisms metabolise pesticides into smaller and harmless compounds, namely H_2O and CO_2.[156] This process of conversion of pesticide to completely oxidised end products is called mineralisation.[248]

Biodegradation can be further classified into three different phases.[156] Phase one involves introducing additional functional groups such as hydroxyl, amine, thiol and carboxyl groups through oxidation, reduction and hydrolysis of the pesticide compound. Following that, the products of phase one enter phase two, combining with endogenous molecules, becoming more hydrophilic via a conjugation reaction.[249] During conjugation, a pesticide or its degraded substances form a bond with other available hydrophilic substrates, forming acetylated, alkylated, methylated, glycosidic or amino acid conjugates. Some pesticide compounds may also undergo oligomerization, a process where a pesticide associates with itself or other chemical compounds in the soil, forming a higher molecular weight compound than the parent compounds.[156] Due to their stable nature, the oligomerized compound will remain labile among the soil constituents available for microbiological degradation, or it will eventually be incorporated into soil organic matter, which increases its persistence in soil.[86] For instance, hydrophobic pesticides, such as metolachlor, acetamiprid, prochloraz or thiamethoxam, have very low solubility in water but high adsorption affinity toward organic matter. These pesticide compounds will then adsorb strongly to the surface and crevices of organic matter, becoming inaccessible for

microorganisms to degrade them. Hence, higher persistence in the soil layers.[250,251] Figure 1.6 illustrates the biodegradation phases leading to mineralisation catalysed by microbial process, with the acetamiprid biodegradation pathway as an example.

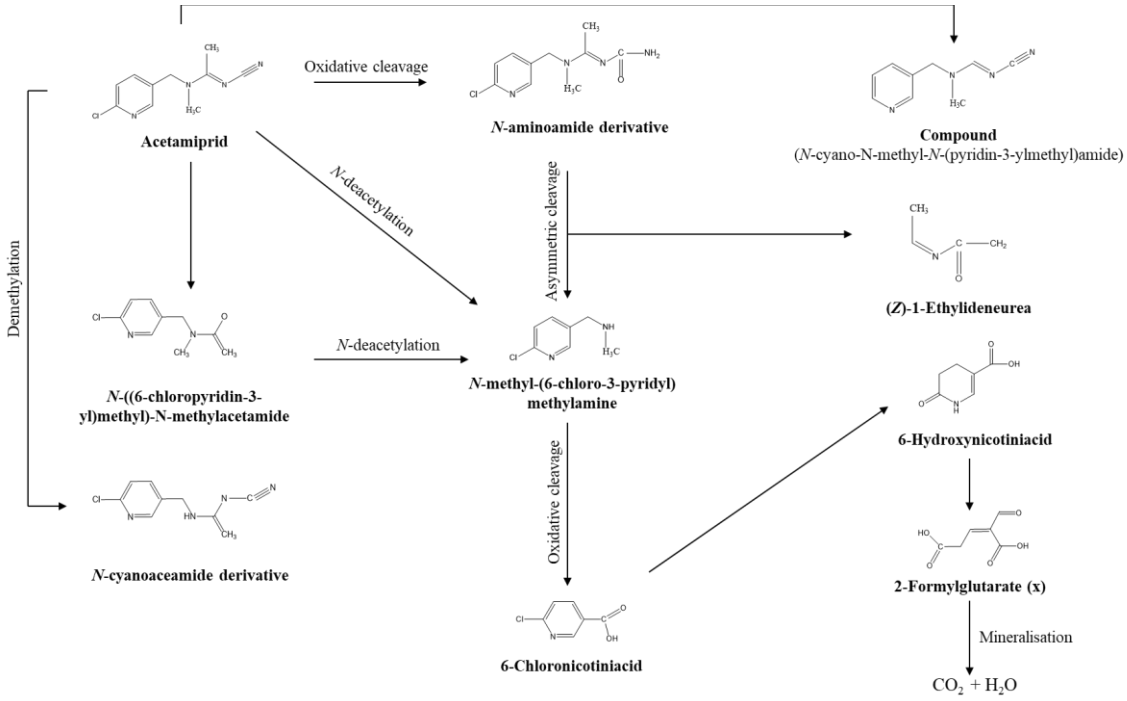


Figure 1.6 Biodegradation pathways of acetamiprid; adapted from Pang et al.[252]

On the other hand, co-metabolism might occur when the available soil microorganisms lack the enzymatic capability to mineralise the pesticide compounds for energy directly.[156] Co-metabolism would result in partial degradation because the enzymes involved lack substrate specificity. As a result, the pesticide compound undergoes minor structural modification without the microorganism deriving any nutrients or energy for growth.[253] Consequently, co-metabolic degradation can result in the mineralisation of the pesticide compound through sequential attacks, or the co-metabolic result of one microorganism is used by another microorganism as an energy and growth substrate.[156] Kumar *et al.* noted that the efficiency of co-metabolic degradation of chlorpyrifos is higher

through the synergistic activities of multiple microorganisms compared to individual microorganisms. The author also reported that the enzymatic activity in a consortium containing three bacterial strains and a fungal strain is higher than individual strain activities, resulting in 100% degradation of chlorpyrifos. The higher pesticide biodegrading capability of co-metabolism through diverse and synergistic action compared to a single strain was also highlighted by Ortiz-Hernández *et al.*[254]

Although chemical degradation predominantly occurs in the natural environment, microbiological degradations are more efficient, and the rate of degradation mediated by microorganisms is more rapid.[164] Most microorganisms degrade pesticide compounds to use degraded products as metabolites or nutrients. Additionally, microorganisms are highly adaptable in obtaining sustenance based on the environment they able to flourish.[255,256] Porto *et al.* noted that soil microorganisms could adapt to utilising high-persisting pesticide compounds in their immediate surroundings for their energy source.[243] For chemical degradation to occur in the soil, numerous environmental factors, such as temperature, moisture, light energy, and organic matter, must be combined and optimal. Meanwhile, many native soil microorganisms can develop complex and efficient metabolic pathways that can effectively biodegrade pesticides that end up in the soil, [209] while actively using the degraded metabolites for survival, which is more advantageous than depending solely on natural chemical degradation.[257–259]

1.4 Transport of pesticides in the soil environment

All the elements in the soil environment, specifically pesticides, water, and soil, simultaneously affect the pesticide transport processes.[221] Depending on the treatment applied to the soil, pesticides may undergo different transport processes that affect their behaviour and fate. There are a few possible transport mechanisms for pesticides to propagate from their applied site; transport by soil organisms, plant absorption, run-off, leaching, and volatilization (Figure 1.2).[164,260] Following their entrance into the soil, pesticide compounds are available for degradation, chemical or microbial, unless these compounds have an affinity toward other soil constituents and get absorbed. The soil matrix or soil biosphere will be a wall for these absorbed chemical compounds, preventing degradation.[261,262] As discussed in the previous sections, adsorbed pesticides persist longer in soil and are excluded from further environmental transport. In certain circumstances, these persistent compounds evade biodegradation, even if they are not absorbed or labile when microorganisms fail to enter between soil microspores, limiting the capacity to promote degradation.[263] However, the fluctuation of multiple biotic or abiotic factors in the soil environment will cause the pesticide residue to be desorbed and become labile.

Following desorption, labile pesticides could pursue a different transport mechanism. Labile pesticides are readily absorbed or degraded by soil organisms.[260] Most pesticide degradation process is undertaken by soil microorganisms, as discussed in Section 1.3.2.2. However, the negative impact of pesticide exposure to beneficial non-target soil meso- and macro-fauna had also been widely previously reported.[175,264–270] Non-target organism refers to any organisms that are not the direct target of the pesticide application,[271] and the

existence of affected non-target beneficial soil organisms are masses, namely soil invertebrates.[266] As most soil organisms spend their life cycle in the soil, there is a probability that due to bioaccumulation, they may contain higher pesticide concentrations than their environment.[260] Depending on the organism's species characteristics, environmental settings, and the physical and chemical properties of both the pesticide compound and soil, the amount of absorbed pesticide will vary. Accordingly, the log K_{OW} coefficient value of pesticides can be used to estimate the probability of that pesticide being absorbed by soil organisms.[173,272] Increasing log K_{OW} value increases the probability of the pesticide compound accumulating in the tissues of soil meso- and macro-organisms.[273,274] These non-targeted soil organisms are often exposed directly to pesticides through either contact or ingestion.[275] As the soil organisms frequently come in contact with pesticide compounds, these compounds can harm the sensitive organisms, resulting in a fatality. However, if the organism is not sensitive to the chemical compound, the absorbed chemical compound could be retained and accumulated in the organism's body over time.[275]

Even though the pesticide compound may not harm the insensitive soil organism, it could still harm animals that are higher on the trophic levels of the food chain.[17,18,276] For example, Senthilkumar *et al.* reported that the concentration of organochlorine pesticides and polychlorinated biphenyls were found in the body tissue and eggs of local and migratory birds that prey on molluscs, earthworms and insects in the agricultural fields.[277] Similarly, Yohannes *et al.* found high accumulation concentrations of the pesticide DDT's metabolite, p,p'-DDE, in four bird species and their fish prey species.[278] The author inferred that the bird species could have bioaccumulated the metabolite from consuming their fish

prey, and at the level p,p'-DDE detected in the liver lipid layers of the bird, it can result in eggshell thinning and reduction in the survival of young birds. This deformation can adversely impact the survival rate of the bird species.[278] Consequently, if the bioaccumulated chemical compounds do not harm the organisms or get metabolised in the body, the pesticide compounds can be eventually returned to the soil through excretion or following the organism's demise.[274,279]

Uptake by a plant is another possible mobility route for labile pesticide residues into crops. For pesticide residues to take this route, it is profoundly influenced by numerous factors, mainly environmental conditions, climate, physicochemical properties of the compounds and soil, degree of pesticide accumulation and crop type.[260,275,280] For instance, Garcinuno *et al.* reported that the probability for a pesticide to be absorbed by crops increases with decreasing soil pH.[281] However, the uptake of pesticides by crops decreases with a higher percentage of organic matter content in the soil.[201,282] From the perspective of the properties of pesticide compounds in soil, pesticide compounds with a log K_{OW} value of less than three, that are stable without any affinity to soil constituents and highly lipophilic, can be absorbed and bioaccumulate in the crop plant.[275,280] With repetitive application and accumulation of pesticides in the agricultural field increases the odds that more compounds will be available for absorption by the plant crops.[280] Moreover, the degree of absorption ranges from one crop species to another. For example, bioaccumulation of pesticides was observed to be higher in the pumpkin compared to the zucchini.[283] Similarly, a comparison between zucchini and cucumber crop species reveals that zucchini can bioaccumulate 1.21% higher concentrations of the metabolite p,p'-DDE compared to the cucumber crop.[284]

Consequently, the uptake of pesticides by crop plants is probably the major route by which pesticide residues bioaccumulate in the food chain, leading to exposure to humans and animals.[18,285] Lipophilic compounds normally bioaccumulate in the crop's roots. However, a small amount of compounds would be translocated to the crop's stems, leaves, and even fruits.[283,286] Even if the pesticide residues are not accumulated in crop production, accumulation in other plant foliage can be released back into the soil when plant litter reaches the soil layer. When the plant litter breaks down, the accumulated pesticide residue will go through the transport cycle again.[268] However, on the other hand, pesticide compounds that are adsorbed strongly into soil or taken up by plants will be unavailable from physical transport processes such as leaching or surface runoff.

Groundwater or surface water contamination with pesticide compounds mainly occurs through leaching or surface runoff. The leaching process is the downward movement of hydrophilic contaminants through the soil layers, facilitated by preferential flow in soil.[287,288] Preferential flow is defined as a physical process where there is fast transport of water and contaminants through small fractions in the soil pore system.[289] These small fractions that enable preferential flows could be formed and transformed through various soil biological activities, including tunnelling activity by earthworms, termites, or ants, as well as through root movements.[290,291] The movement of pesticides along with the leaching process is governed by two main mechanisms, dispersion and mass flow. In the dispersion mechanism, water flow through soil layers creates molecular diffusion, which occurs due to changes in concentration gradient and mechanical mixing. Molecular diffusion then enhances the desorption of pesticides from soil sorbents. Pesticide movement via mass flow happens when the compounds are

either dissolved or suspended, and vertical movement is enabled through the combined effect of gravitational and capillary forces.[292] The leaching process is then further supported by soil composition and texture, where leaching tends to increase in soil layers with a higher percentage of sandy texture, which has large pores, but decreases with higher clay-textured soil, where the particles are compact and capable of holding more water.[293] Additional to soil texture and porosity, soil properties such as organic matter and water content also play a vital role in facilitating the leaching of dissolved pesticide compounds into groundwater. Organic matter in the soil increases the soil's ability to hold water, as well as providing an increased surface area for the adsorption of pesticides and a plethora of bacterial communities available to assist in degradation. Removal of this layer of organic matter significantly increases the leaching process of hydrophilic pesticide compounds.[181,294,295] Moreover, together with leaching, surface runoff is another transport mechanism for pesticide compounds contaminating the nearby water source.

Generally, pesticides from the soil surface leach through soil layers and end up in the groundwater. These chemical compounds contaminate surface water through the drainage processes from the groundwater. However, runoff from the soil surface also significantly increases the accumulation of contaminants in the surface water.[296,297] Pesticide compounds adsorbed on the soil surface are most vulnerable to surface runoff. These compounds are easily extracted into the moving water through various mechanisms, including diffusion of dissolved pesticides from soil constituents into the runoff flow, dissolution of stationary pesticide compounds, or desorption from soil sorbents into the moving water.[292] Accordingly, Burgoa and Wauchope determined that surface runoff contributes

up to 5% of relative losses of applied pesticides, which increases under vulnerable conditions for surface runoff.[298–301] In a natural environment, surface runoff occurs when the soil layers are saturated with water from precipitation, and small laminar flows along the surface lead to the formation of turbulent flows that carry pesticides to nearby water bodies.[302] Alternatively, in anthropogenically modified environments, such as agricultural fields, surface runoff frequently occurs due to the inability of water to penetrate the soil surface. This inability may arise from the soil layer's compaction or increased soil bulk density resulting from field traffic.[303–305] Ankenny *et al.* reported that the infiltration rate decreases as much as 95% in the field wheel tracks compared to uncompacted soil.[306] Consequently, either through leaching or surface runoff, persistent pesticide compounds that end up in the water sources may have an adverse effect on both the organisms in the water and organisms that utilise it.

Another common dissipation pathway for pesticides is atmospheric transport. Through atmospheric transport, pesticides can be transported far from the initial site of application, leading to long-range contamination, a phenomenon known as pesticide drift.[260,307] Atmospheric transport of pesticides begins through the volatilization process from the soil, followed by pesticide drift. Even though a higher percentage of atmospheric pesticide transport happens as soon as the pesticide droplets are aerially sprayed, pesticides that reach the soil can still dissipate through volatilization.[307,308] This will result in volatilization starting as soon as the pesticide is administered, and the process continues for several weeks or months, which results in a significant amount of pesticide lost to the environment.[309,310] Accordingly, Taylor and Spencer concluded that cumulative losses of pesticides from the soil through volatilization could be as high as 90%

of the total application dose.[310] However, the exact percentage of losses would not be expected to be ubiquitous, as the author described, with significant temporal and spatial variation. The properties of the pesticide govern its potential volatility, such as vapour pressure of the compound on the soil solid or liquid surface, concentration in the soil, adsorption affinity with the soil particles, and the properties of the soil, namely texture, porosity, soil temperature, pH and water content.[11,260] Additionally soil water content plays an essential role in volatilization as dissolved pesticides tend to vaporize more easily from moist soil than dry soil.[311,312] Following pesticide application and incorporation into soil, the volatilization rate of the pesticide residues on the surface should be initially high.[313] As the pesticide concentration on the surface depletes, volatilization becomes highly dependent on the pesticide movement from the deeper soil layer to the surface. This movement happens through a convective flow which intermingles with the movement of soil solution upward in response to changes in the concentration gradients and water evaporation.[310,314]

Besides soil water content, soil temperature also governs the volatilisation rate, as higher temperature aids the soil solution transformation into a gaseous state more rapidly.[260,309,315] Nonetheless, Wolters *et al.* reported that volatilisation of pesticides is more interconnected with soil water content. The authors found that higher soil temperature but decreasing soil water content resulted in a low volatilization rate, from which they concluded that water content on the soil top layer was the key driving force in pesticide volatilization.[22] Pesticides can be categorised into different classes of volatility, ranging from low volatility to semi-volatility, and most chemicals' volatilization is considerably affected by the water evaporation from the soil layers.[18,311] Therefore, the rate of a partition of

pesticides in the soil solution and the atmosphere can be most suitably determined using Henry's constant (K_H), which is subjected to Henry's Law.[309,316,317] Pesticides with a K_H value of more than 2.5 x 10^{-5} have a higher tendency to volatilize rapidly from the soil. Meanwhile, soil with a KH value of less than 2.5 x 10^{-5} tends to accumulate on the soil surface as it does not have vapour pressure strong enough to break the layer of stationary air on the soil surface.[18,316] Consequently, persistent airborne pesticides should eventually return to the soil or water surface through precipitation or fallout, which leads to exposure and uptake by humans, plants and animals.[275,279,318]

1.5 Pesticides usage in the Republic of Ireland

In 2016, the total land area for agricultural production of countries in the European Union (EU) came to 173 million hectares, about 39% of the total EU land cover.[319] Whilst the 2016 total land area for agriculture is a slight decrease of six million hectares from the previous year of 2015, in 2018 the utilised agricultural area increased by one million hectares to 174 million hectares.[320] Although the EU utilised agricultural areas were observed to fluctuate between years, in 2019, the EU agricultural industry generated a gross value added (GVA) of €181.5 billion, contributing 1.3% of the EU's GDP.[321] Interestingly, this fluctuation of the utilised agricultural land area also reflects sales of pesticides in the EU, where the total volume sold were noted to increase or decrease by 6%, around 350,000 tonnes, between 2011 and 2020. In 2020, the total volume of pesticides sold was approximately 346,000 tonnes, 12,582 tonnes higher than in 2019 but 2% lower than in 2018.[322] It is worth noting that, even though the total sale of pesticides does not necessarily reflect the total pesticide usage data, due to lack of data on pesticide usage in EU, using the sales data to assess the trend

of usage is a practical alternative. The practicality of this approach of assessment is highlighted by Bekarian *et al.*, where in their study on using point-of-sale data for tracking the usage patterns of residential pesticides, the authors noted that even though utilising just the pesticides sales data has limitations, such as market coverage, it still provides a helpful snapshot of pesticide usage patterns.[323] Additionally, even though there is a fluctuation of pesticide sales volume in the EU, considering the annual sales of pesticides are 6% above or below 350,000 tonnes, this high pesticide sale volume raises environmental concerns.

The most recent comprehensive farm structure survey in the Republic of Ireland was conducted in 2016. The Irish total land area is approximately 6.9 million hectares, and 71% (4.4 million hectares) of the total land area was used for agricultural purposes.[324] The farming area structure can be classified as follows; 4.1 million hectares are used for grass silage, hay and pasture, 0.29 million hectares are devoted to crop production, including fruits, cereals, potatoes, and horticultural production, and finally, 0.016 million hectares is used for rough grazing.[324] Figure 1.7 highlights the distribution of different agricultural land use types in the Republic of Ireland. With a heavy economic dependence on agricultural industry output, in 2019, this industry contributed €14.4 billion or 4.3% of GVA to the Irish national economy.[325] Accordingly, between 2016 and 2019, the total utilised agricultural area in Ireland was noted to increase yearly, with a slight decline in 2020, but still 0.51 million hectares higher than in 2016 (Figure 1.8).[326] Even though there are no significant changes in the volumes of total pesticide use, with an average volume used of 2805 tonnes between 2016 and 2020, the lack of clarity as to the difference in application rates of different classes and types of pesticides limits the overall usefulness of this level of data.

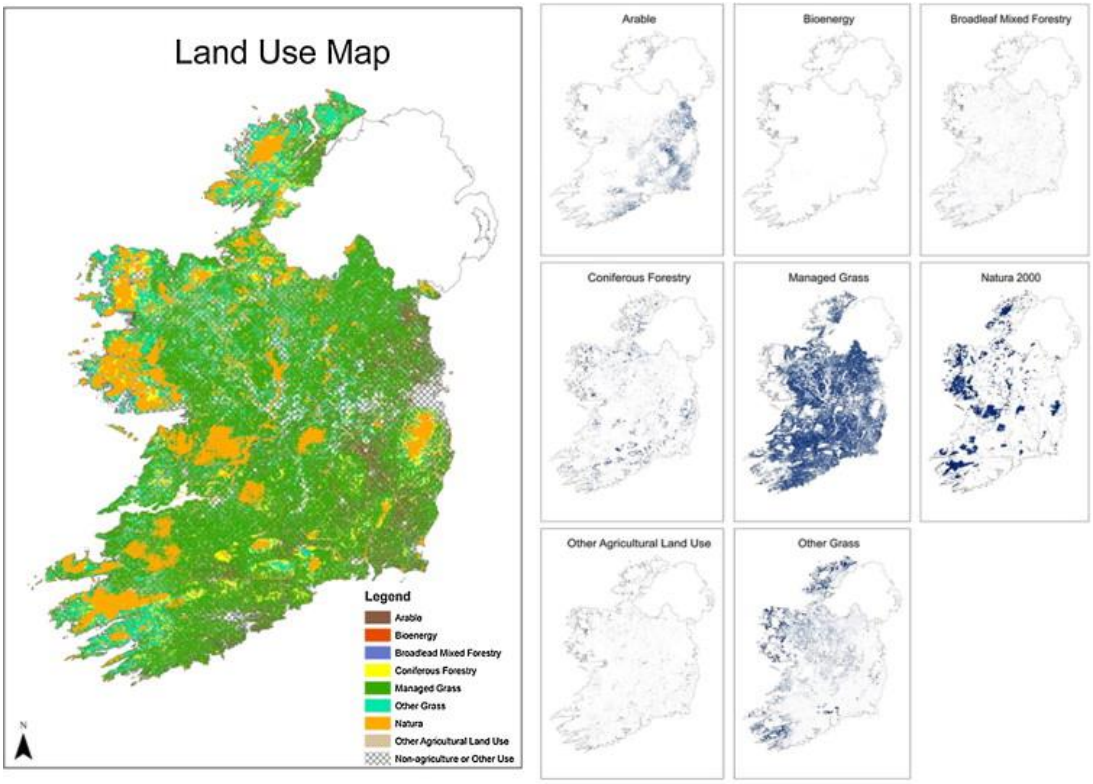


Figure 1.7 Distribution map of different agricultural land use types of the Republic of Ireland of 2015; usage permission obtained from Elsevier.[327]

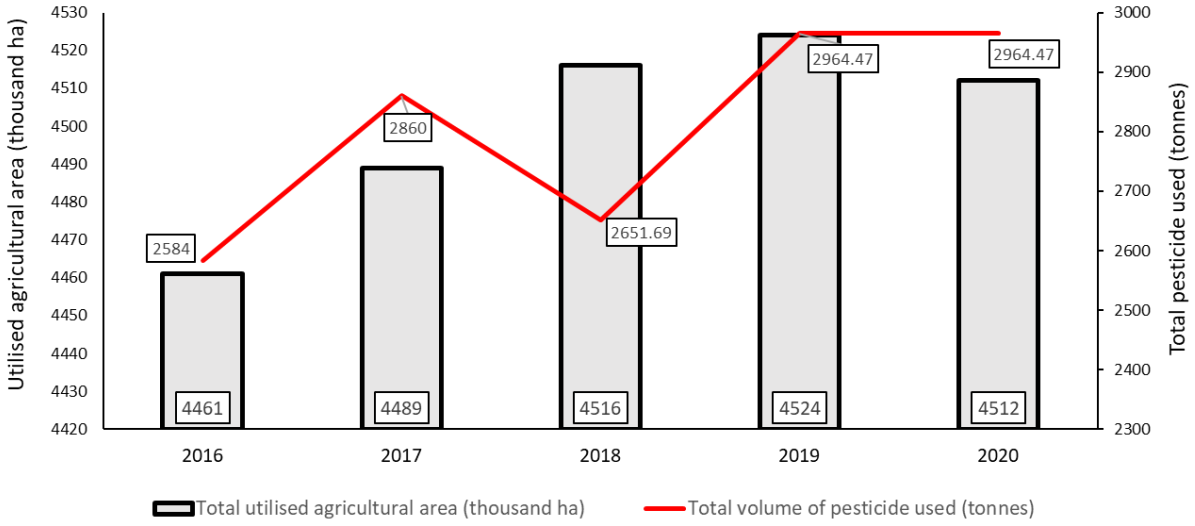


Figure 1.8 Total utilised agricultural area compared with the total pesticide used in the Republic of Ireland from 2009 to 2017, adapted from the FAOSTAT of land use and total pesticide usage from the year 2016 to 2020.[326,328]

As discussed earlier, utilising the sales record of pesticides can indicate the nature of pesticide usage in Irish agriculture. Based on Eurostat's record of sales of pesticides by types in 2018, herbicides are the most substantial pesticide sold in the Republic of Ireland, followed by fungicides, insecticides, and molluscicides are the lowest sold pesticide type (Figure 1.9).[329] This sales record also complements the area of usage and percentage applied in Irish arable crops, where the three main pesticide types are, in increasing usage area and application percentage, insecticides, fungicides and herbicides.[330] However, with the crop types grassland and fodder, the application percentage of pesticide types are slightly different, with fungicides are the widely used pesticide type, followed by the herbicide and insecticides.[330]

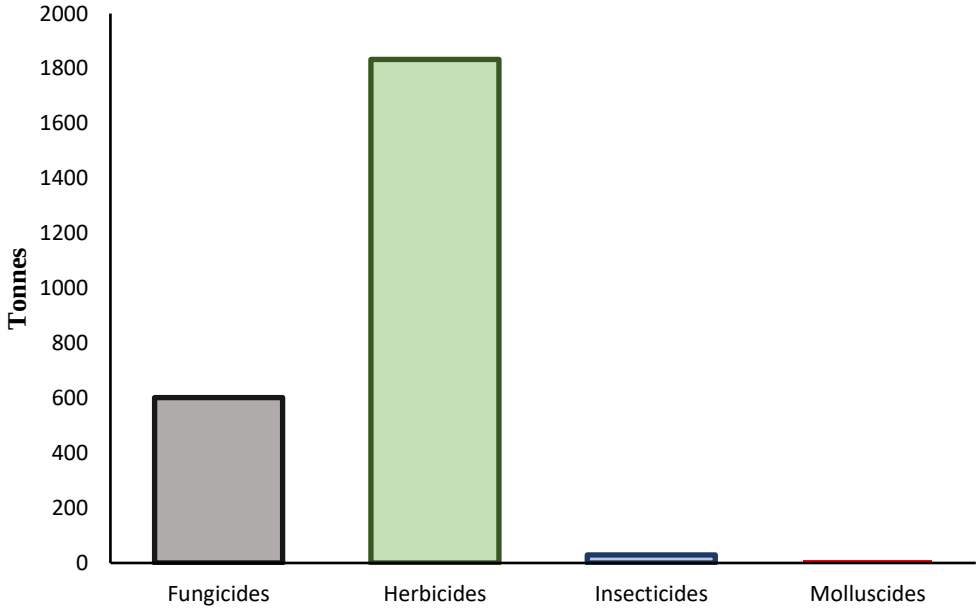


Figure 1.9 Total pesticide sales by types, adapted from the Eurostat database on Ireland's pesticide sales record in 2018.[329]

As herbicides, fungicides and insecticides are the most widely used pesticide types, it is vital to give more attention to these types of pesticides. The five widely used herbicides in the Irish agricultural landscape are fluroxypyr, followed by glyphosate, 2-methyl-4-chlorophenoxyacetic acid (MCPA), tribenuron-methyl, and triclopyr.[330] It is important to note that both fluroxypyr and glyphosate are the most prominently used herbicides, with the estimated usage per national basic area to exceed 200,000 hectares between 2014-2017.[330] Both of these systemic herbicides are very effective against broadleaf weeds, and the application method is the foliar spray. However, compared to fluroxypyr, glyphosate is a non-selective herbicide (Table 1.5). With the widespread usage of glyphosate, the formation of its primary degradation metabolite, aminomethylphosphonic acid (AMPA), is unpreventable.[331,332] Even though MCPA is similar to fluroxypyr in terms of target and selectivity towards the target pest, MCPA is only applied in less than 150,000 hectares of Irish agricultural area.[330]

On the other hand, the most widely used fungicides are chlorothalonil, followed by prothioconazole, epoxiconazole, pyraclostrobin, and prochloraz. The two top fungicides, chlorothalonil and prothioconazole, are mainly used to deter broad-spectrum diseases, non-selective, and the preferred method of application is foliar spray or seed treatment. Regarding systemic properties, prothioconazole is a systemic fungicide, while chlorothalonil is not (Table 1.6). As the European Union (EU) imposed a ban on its sales and distribution in May 2020,[333] this pesticide usage in Ireland has now halted. Although there is no recent data on

prothioconazole usage volume in Irish agriculture, chlorothalonil and prothioconazole had exceeded 200,000 hectares of usage between 2014 to 2017, so Irish farmers might be depending on prothioconazole as the primary fungicide. This increased dependence on prothioconazole could result in higher usage since 2020.

Finally, in Irish agriculture, lambda-cyhalothrin and clothianidin were the most widely used insecticides between 2014 to 2017 (Table 1.7), with lambda-cyhalothrin being non-systemic and clothianidin systemic. In terms of application method, lambda-cyhalothrin and clothianidin can be applied as soil or foliar spray, [334–336] at the same time, clothianidin can also be applied as a seed treatment.[336] However, similar to chlorothalonil, clothianidin has been banned for outdoor use in the EU since 2018.[337] Even though the application of three neonicotinoid pesticides (clothianidin, thiamethoxam and imidacloprid) is currently banned for outdoor use, it is still permitted to be used in permanent greenhouses. In some instances, the banned neonicotinoids remain in use in some EU member states through the emergency derogation provision.[338] Even if some of the neonicotinoid pesticides are currently under strict usage regulation, due to their long half-lives, [26,339–343] their long presence in the environment had been previously observed,[342] hence, they are further discussed in section 1.5.5.

Table 1.5 Overview of widely used herbicides in Irish agriculture.[330]

Active Substances	Target	Type	Chemical Class	Application	Reference
Fluroxypyr	Annual and perennial broadleaf weed and woody brush	Systemic (Selective)	Pyridine carboxylic acid	Foliar spray	[344]
Glyphosate	Annual and perennial broadleaf weeds	Systemic (Non-selective)	Glycine derivative (phosphanoglycine)	Foliar spray	[345]
2-methyl-4-chlorophenoxyacetic acid (MCPA)	Annual and perennial broadleaf weeds	Systemic (Selective)	Phenoxy carboxylic acid	Foliar spray	[346]
Tribenuron-methyl	Postemergence broadleaf weed	Systemic (Selective)	Sulfonylurea	Foliar spray or directly to the soil	[347,348]
Triclopyr	Broadleaf weeds and brush	Systemic (Selective)	Chloropyridine	Foliar, ground, and aerial spray, tree trunk or stump and soil injection	[349]

Table 1.6 Overview of widely used fungicides in Irish agriculture.[330]

Active Substances	Target	Type	Chemical Class	Application	Reference
Chlorothalonil*	Broad-spectrum fungal diseases	Non-systemic (non-selective)	Chlorinated isophthalic acid derivative	Foliar spray	350
Prothioconazole	Broad-spectrum fungal diseases	Systemic (Non-selective)	Triazolinthione family	Foliar spray or seed treatment	351,352
Epoxiconazole	Broad-spectrum fungal diseases	Systemic (Non-selective)	Azoles	Foliar spray	353,354
Pyraclostrobin	Broad-spectrum fungal diseases	Systemic (Non-selective)	Strobilurin	Foliar spray	355–357
Prochloraz	Broad-spectrum fungal diseases	Non-systemic (non-selective)	Urea and imidazole group	Foliar spray or seed treatment	358

*Banned and withdrawn from the market since May 2020.[333]

Table 1.7 Overview of widely used insecticides in Irish agriculture.[330]

Active Substances	Target	Type	Chemical Class	Application	Reference
Lambda-cyhalothrin	Broad-spectrum of invertebrates	Non-systemic (non-selective)	Synthetic pyrethroid	Ground and foliar spray	334,359
Clothianidin*	Broad-spectrum of invertebrates of piercing-sucking pests	Systemic (Non-selective/selective)	Neuro-active	Ground, foliar spray or seed treatments	69,336
Cypermethrin	Broad-spectrum of invertebrates	Systemic (Non-selective)	Synthetic pyrethroid	Foliar spray	360,361
Dimethoate	Broad-spectrum of invertebrates	Systemic (Non-selective)	Organophosphate	Foliar spray	362
Esfenvalerate	Broad-spectrum of invertebrates	Non-systemic (non-selective)	Pyrethroid	Foliar spray	363,364

*Banned for outdoor usage since 2018. [337]

1.5.1 Fluroxypyr

Fluroxypyr is a pyridine derivative and systemic pesticide with two primary metabolites, fluroxypyr-methoxypyridine and fluroxypyr-pyridinol (Table 1.5). As it has a carboxyl group, it is characterised as an acidic compound. Moreover, fluroxypyr is a systemic and selective chemical that mimics the auxin, indoleacetic acid. This auxin plays a crucial role in the plant's root and shoots development.[365] Hence, the mode of action of fluroxypyr would be that upon administration, it will cause uncontrolled growth in the broadleaf weeds. The uncontrolled growth will put the weed under stress, eventually leading to the pest's death.[366] When fluroxypyr is released into the soil, it is estimated that its persistence ranges between 28 and 78 days.[367] Changes in the behaviour of fluroxypyr in the soil are observed when there is a fluctuation of abiotic factors such as pH, temperature or precipitation. Among the mentioned factors, the fluctuation of soil pH presents the most influential factor toward the pesticide compound's ability to adsorb in the soil. In a high pH (6-8) soil, fluroxypyr, whose pKa constant of 2.94, will be very soluble, with low volatility and high mobility unless complex compounds are formed (Table 1.8).[368,369] Consequently, increasing soil pH has been reported to increase the rate of leaching of fluroxypyr through the soil layers. This increased leaching rate is due to increased pH (2-10), decreasing the compound's ability to adsorb into the soil fivefold.[368]

Pastrana-Martinez *et al.* studied how temperature affects the adsorption of fluroxypyr, determining that increasing temperature negatively affects adsorption capacity.[370] Likewise, with K_{OC} values of 136 (Table 1.8), fluroxypyr is expected to have high mobility even in normal soil solution pH (Table 1.3). Nevertheless, even though fluroxypyr is observed to be highly mobile, with low adsorption

capacity and increased leaching rate, biodegradation could attenuate the environmental impact of this compound. Accordingly, research by Tao & Yang suggested that the degrading of fluroxypyr decreased significantly with the removal of organic matter and soil microbes.[259] Hence, it can be postulated that the dissipation of fluroxypyr is highly dependent on the availability of soil organic matter and bacteria. Microorganisms species, namely *Actinomycetes* and *Pseudomonas,* assist immensely in dissipating fluroxypyr as these microbes use fluroxypyr as their nutrient source, degrading the compound in the process.[371] Precipitation had been noted to assist in the dissipation of fluroxypyr from the environment.[372] Pang *et al.* also found that higher precipitation increases the rate of fluroxypyr degradation, even though the reason for changes is not fully understood.[372] However, it can be postulated that higher precipitation increases the soil moisture content enhancing the microbial community's growth and the higher degradation rate of fluroxypyr.[373,374]

In the Irish context, fluroxypyr fate in the soil is hard to predict. The pH of Irish agricultural soil (arable and grassland) is measured to be in the range of 3.5-7.2 (mean=5.5),[375] indicating that fluroxypyr application could result in high mobility of the pesticide through the soil layers. As highlighted above, even though acidic soil would result in higher persistence of fluroxypyr in the soil layers, as the Irish agricultural soil has a high pH range, it could also lead to the leaching of fluroxypyr. On the other hand, Irish agricultural soil is determined to have organic carbon (g/kg) ranging between 15.5-492.7 g/kg (mean=67.3),[375] and in the scale of organic carbon content of European soil, Ireland’s agricultural soil to have medium to high organic carbon content.[376] Consequently, due to the high organic matter content of Irish agricultural soil and the log K_{OW} value of 2.2 (Table 1.8),

fluroxypyr can be predicted to adsorb strongly to the soil organic matter, resulting in accumulation and high persistence of fluroxypyr.

On the other hand, Ireland is noted to have high precipitation, with an average annual precipitation of 1230 mm.[377] Accordingly, as highlighted by Pang *et al.*, higher precipitation would increase the degradation rate of fluroxypyr, which could indicate the half-life of fluroxypyr to be short in Irish agricultural soil.[372] Additionally, multiple studies were able to establish that higher soil organic matter will increase the abundance and diversity of soil microorganism community,[378–380] which would catalyse further the degradation of fluroxypyr in the Irish agricultural soil. On the whole, fluroxypyr can be predicted to undergo rapid degradation in the Irish agricultural soil, due to higher soil microbial activity with high precipitation volume and organic matter content, with a slight possibility of leaching can accumulation.

1.5.2 Glyphosate

Glyphosate is a post-emergence, broad-spectrum and systemic herbicide which, upon degradation in the soil, forms the primary metabolite aminomethylphosphonic acid (AMPA) and minor metabolite, glyoxylate, where both of these metabolites can further degrade to release carbon dioxide (Table 1.6).[381,382] Glyphosate is a glycine derivative that requires foliar spray as the mode of application, where the active substance gathers at the growing points of a plant. Due to glyphosate's mode of action, upon application, the plant will not be able to biosynthesise the essential aromatic amino acids, namely phenylalanine, tyrosine, and tryptophan which is to inhibit the amino acid 5-enolpyruvoyl-shikimate-3-phosphate synthase effectively.[77] Without those amino acids, the plants will not be able to build proteins, leading to death by starvation.

Glyphosate's behaviour in the soil is highly dependent on its form, where an acid form of glyphosate is very active in the soil layers, with the pKa constants of 2.27, 5.57, and 10.86.[383] With this range of pKa, it is postulated that glyphosate will have low mobility, nevertheless, because glyphosate ions always exist as complexes in the form of zwitterions, they will adsorb strongly to soil constituents and practically immobile in soil pH solution ranging between 7 and 8.5 (Table 1.4).[226,384]

It is also noted that increasing soil pH (7-8.5) would increase the negative charge of both the soil minerals and glyphosate, which decreases glyphosate adsorption.[226,385] Glyphosate's strong immobilisation in the soil is also supported by the fact that it has K_{OC} values, which range from 1424 (Table 1.8).[383] Interestingly, the persistence of glyphosate in the soil is quite variable, where half-life is reported by Benniccelli *et al*. to be between 1 and 174 days.[382] Meanwhile, other literature noted glyphosate has a shorter half-life, between 1.5 and 53.5 days.[386] The variation between these reports could result from a difference in soil composition, as Grunewald *et al.* noted that even though glyphosate has a relatively short half-life (2 to 91 days) when mixed with soil particles, glyphosate tends to persist longer, with up to 215 days.[387] As glyphosate's adsorption and persistence in the soil is quite variable and affected by soil properties, such as soil organic matter and soil minerals,[388] or climate conditions,[386] it is challenging to determine glyphosate's half-life in the soil environment. Compared to other transformation pathways, biodegradation is the key dissipation pathway for glyphosate in the soil.[389,390] Biodegradation for glyphosate can happen through two main pathways. Both pathways are bacterially driven and involve C-P lyase to break the C-P bond.[391] It is postulated

that the biodegradation pathways are specific to species, where different species of soil microorganisms would biodegrade glyphosate using either of the two pathways.[391] The first pathway, the carbon-phosphorus (C-P) lyase enzyme, releases sarcosine (SRC) and phosphate, which bacteria use to meet metabolic requirements. Meanwhile, the second pathway requires oxidase to break down the carbon-nitrogen bond in GLP, which produces AMPA. The bacterial genus *Pseudomonas* has been shown to degrade glyphosate to SRC and AMPA, depending on P requirements. This bacterial genus cleaves the C-P bond with the sole purpose of obtaining phosphorus for its metabolic functions.[392] Consequently, a suitable soil environment also affects biodegradation. For instance, unsuitable pH, temperature, humidity or salinity in the soil would lower the degradation rate of glyphosate, as it will slow down the soil microbial activity.[226,386] Even though glyphosate is primarily degraded through biodegradation, it is recently reported that glyphosate also can be dissipated through an abiotic pathway where glyphosate is observed to degrade into its primary metabolite through exposure to metallic ions.[393]

Referring to the K_{OC} value of glyphosate (1424), glyphosate is prone to strongly bind to soil organic matter.[332] As previously established, glyphosate poses a high accumulation and persistence threat with Irish agricultural soil's high organic matter content. The accumulation risk is further supported considering the pH of Irish agricultural soil,[375] with a more acidic to neutral soil environment, which would further increase the immobilisation potential of glyphosate in the soil layers. Additionally, with high polarity (log K_{OW} = -6.28) and high-water solubility,[394] glyphosate also poses a high leachability risk,[226] however, with the GUS index of 0.21, glyphosate is more prone to persist in the soil than leach

through the soil layers. However, Irish agricultural soil's high organic matter content can house a higher abundance of soil microorganisms, leading to rapid biodegradation of the adsorbed glyphosate. Consequently, in terms of toxicity, partial biodegradation of glyphosate, not resulting in mineralisation, increases the risk of higher concentrations of AMPA. AMPA, which will be discussed further below (section 1.5.3), has been demonstrated by multiple studies that, compared to glyphosate, it persists longer in the soil and has higher toxicity.[331,332,395]

1.5.3 AMPA

Although glyphosate's primary metabolite, AMPA, exhibits similar behaviour in adsorption and degradation in soil, it is noted that AMPA persists longer in the soil as it has a longer DT50 than its parent compound.[386,396,397] In optimal laboratory conditions, glyphosate is observed to have a mean DT50 of 49 days; meanwhile, AMPA is more persistent, with a mean of 120 days.[398] In contrast, Annett, Habibi, & Hontel reported that AMPA's half-life might be higher than earlier findings, ranging from 76 to 240 days in soil.[399] The variation in AMPA's half-life is generally dictated by similar factors that affect glyphosate, except for the biodegradation factor. This variation is because AMPA possesses higher biological stability compared to glyphosate.[387,400] As explored in Section 1.5.2, microorganisms actively biodegrade glyphosate to utilise the resulting phosphorus as their energy source. However, compared to glyphosate, AMPA biodegradation generally occurs at a lower rate, likely because AMPA has to be acetylated before a microorganism can utilise phosphorus.[91] This postulation is supported by la Cecilia and Maggi, where the authors determined that even in optimal conditions for bacterial activity, AMPA was still observed to biodegrade at a slower rate.[400] Moreover, the persistence of AMPA is expected to be higher

than glyphosate because it is being formed as the degradation product of AMPA; thus, AMPA has to be more than or similarly persistent to glyphosate.[386] Interestingly, Simonsen *et al.* reported that there were still residues of AMPA detected in their blank soil over two years of incubation. The authors also observed that the aged residues degraded slower than the newly added compounds' degradation rate.[396] In the Irish context, the higher organic matter content of Irish agricultural soil would increase the adsorption rate and accumulation of AMPA, resulting in longer half-lives and persistence of AMPA. Additionally, because Irish agricultural soil pH range between 3.5-7.2 (mean=5.5),[375] could also increase the accumulation of AMPA.[401] Hence, with variation in half-lives of AMPA and continuous application of glyphosate in the Irish agricultural industry, it could lead to higher accumulation in the soil, leading to adverse impact on the environment.[402]

Table 1.8 Chemical properties of widely applied pesticides (>150,000 hectares) in the Irish agricultural industry.[330,394,403–405]

Pesticides	Vp (mPa)	pKa	log *K*ow	Koc	DT50 (days)	WS (mg/L)	GUS index
Fluroxypyr	4.00E-06	2.94	2.2	136	21	91	2.42
Glyphosate	1.31E-02	2.34	-6.28	1424	16.11	100000	0.21
Prothioconazole	4.00E-04	6.9	3.82	1765	1336	300	-0.18
Epoxiconazole	3.50E-04	NI	3.3	280-2647	353.5	7.1	2.09
Lambda-cyhalothrin	2.00E-04	NI	5.3-7.0	283707	175	5.00E-03	-2.09
Clothianidin*	0.00E+00	11.9	0.905	60	1155	327	3.74

*Banned for outdoor use but still used in permanent greenhouses.[337] Notations. Vp = vapour pressure; pKa = dissociation constant; NI = non-ionisable; log K_{ow} = partition coefficient; K_{oc} = soil adsorption coefficient; DT50 = half-life; WS = water solubility; GUS = Groundwater Ubiquity Score.

1.5.4 Prothioconazole

Prothioconazole, one of the less well-studied pesticides, is a systemic triazolinthione fungicide used to deter a broad spectrum of fungal diseases (Table 1.6). The most effective method of prothioconazole application is through seed treatment or foliar spray. Prothioconazole is quite effective in deterring *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes*, which can be economically crippling in cereal farming. CYP51 is a cytochrome enzyme crucial for mediating ergosterol synthesis.[406] Upon application, prothioconazole inhibits CYP51, obstructing ergosterol synthesis in the pest fungi. Without ergosterol production, the fungi experience morphological and functional changes in the cell membrane due to demethylation at C14 of lanosterol or 24 methylene dihydrolanosterol,

which leads to the death of the fungi.[407,408] Prothioconazole is a weak acid active substance with a dissociation constant of 6.9.[405] With that value, prothioconazole's mobility, solubility and volatility are highly dependent on the pH of the soil solution, where the compound will exist as an anion, and anions do not adsorb strongly to soil organic matter or soil mineral constituents (Table 1.4). However, with a K_{OC} value of 1765 (Table 1.8), prothioconazole has low mobility in the soil (Table 1.3).

Furthermore, with a Vp value of 4×10^{-04}, prothioconazole on the soil surface will not be transported through volatilization (Table 1.8). Upon degradation, prothioconazole-desthio is the main metabolite for this pesticide. In plants, when prothioconazole metabolises, it extensively forms several compounds. However, the most prominent is prothioconazole-desthio.[409] Prothioconazole was found quite difficult to hydrolyse in a buffer solution, and it takes 120 days for complete degradation.[352] However, it is observed that prothioconazole undergoes rapid degradation with half-lives below 5.82 days in an optimum aerobic environment with a pH range of 5.9 – 7.9 identified to be the optimal pH for the degradation of prothioconazole.[409] Bayer, a manufacturer of prothioconazole-based herbicides, had stated in their hazards statement that prothioconazole usage in areas where soils are permeable would result in the chemical's main metabolite, prothioconazole-desthio, leaching into groundwater.[410] Microbiological degradation is determined to be the primary degradation mechanism of prothioconazole in the soil environment.[411] As microbiological degradation is the main transformation pathway, soil organic matter can be inferred to affect the degradation rate of prothioconazole. Zhang *et al.* noted that soil samples with the lowest organic carbon content record the lowest prothioconazole degradation

rate.[411] This result further supports the notion that soil microbial communities dominate the degradation rate of prothioconazole. This correlation is because a higher concentration of soil organic matter will increase the labile carbon for the microorganisms to use as an energy source. This increase of labile carbon then, in turn, increases the microorganism community in the soil, which can actively degrade prothioconazole.[409]

As Irish agricultural soil has high organic matter content with organic carbon within the range of 15.5-492.7 g/kg (mean=67.3),[375] prothioconazole was observed to have the same accumulation risk as fluroxypyr, glyphosate and AMPA. Increased accumulation risk and lower leaching risk are further supported by high log K_{OW} (3.82), high K_{OC} value (1765), and low GUS index (-0.18). However, referring to the pH value of Irish agricultural soil ranges between 3.5 to 7.2 (mean=5.5),[375] it approaches the optimal pH (5.9-7.9) for the degradation of prothioconazole.[409] Furthermore, as postulated earlier, with higher microbial abundance and diversity, the potential for rapid biodegradation of prothioconazole in Irish agricultural soil is very high. Consequently, similar to glyphosate and AMPA, prothioconazole's main degradation product, prothioconazole-desthio, had been previously established to be of higher potency than the parent compound.[412,413] Therefore, even though prothioconazole could rapidly degrade in Irish agricultural soil, the resulting prothioconazole-desthio is more persistent (half-life = 16.3-72.3 days), warranting continuous monitoring of both the parent and degradation product in the environment.[414,415]

1.5.5 Neonicotinoids

Clothianidin is the only recorded neonicotinoid that was one of the broadly used insecticides in the Irish agricultural industry between 2014-2017.[330] However, in this section, clothianidin will be discussed together with other neonicotinoids due to their worldwide use.[416] Neonicotinoids are a family of pesticides, with their derivatives including acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam.[142] Neonicotinoid takes effect when a pest comes in contact with any neonicotinoid. The insecticide will act selectively on the insect's nicotinic acetylcholine receptor, which stimulates the postsynaptic receptors. Upon stimulation, the receptors will increase Na^+ ingress and K^+ egress, paralyzing nerve conduction and finally leading to the death of the insect.[69,417] With the estimated half-lives of neonicotinoids ranging between 3 to 6931 days in the soil, the persistence of neonicotinoids is a considerable concern.[26] In the soil layers, the behaviour of neonicotinoids is dominated by various soil properties, namely organic matter, clay minerals, temperature, pH and cation exchange capacity are essential to determine the behaviour of neonicotinoids in the soil.[418,419]

Looking further at the pKa values of the neonicotinoids, it ranges from no dissociation to dissociation constant as high as 12.6 (No dissociation: imidacloprid, thiamethoxam, and thiacloprid; Dissociation: acetamiprid: 0.7, clothianidin: 11.1, dinotefuran: 12.6, and nitenpyram: 3.1).[420] Based on these values, it can be predicted that all neonicotinoids will have high solubility and mobility in anionic form, except in alkaline conditions (Table 1.4). Nevertheless, K_{OC} values of neonicotinoids vary from one pesticide to the other, ranging from low to high values, 60 (clothianidin), 68 (thiamethoxam), 267 (acetamiprid), 800

(imidacloprid), and 1584 (thiacloprid), indicating the range of mobility in the soil (Table 1.3).[394] Even though neonicotinoids are indicated to have a range of mobility, they are not vulnerable to volatilization.[26,420] At the same time, neonicotinoids have high water solubility,[26] which can cause the pesticide to leach through the soil quite easily to surface or groundwater systems. However, the optimum hydrolysis of neonicotinoids in the soil is highly dependent on the soil pH. Todey *et al.* noted that the pH range between 5 to 8.5 is optimal for the hydrolysis of neonicotinoids.[421] However, as soil pH is prone to severe fluctuation in the environment, hydrolysis might not be the primary degradation pathway for neonicotinoid compounds. In terms of other degradation pathways, biodegradation is the most common form of dissipation of neonicotinoids in the environment, assisted by various soil and environmental physicochemical properties, namely, temperature, pH, and initial concentration of application.[418,422–424] In neutral pH (7-8), moisture and temperature conditions, soil microbial activities are boosted. Hence, rapid degradation of bioavailable neonicotinoids is possible; however, such suitable environmental conditions are rarely constant in real field conditions.[246,425–428] Hence, even though rapid degradation is theoretically possible, it is not the reality in most environmental situations.

Apart from degradation, neonicotinoids are mainly retained in the soil layers through adsorption, causing neonicotinoids to be less bioavailable for degrading. For instance, the sorption of imidacloprid and its metabolites are affected mainly by organic matter and clay minerals.[206,423] As highlighted above, adsorbed neonicotinoids could result in the pesticide compound being protected from biodegradation, potentially increasing their persistence in the agricultural soil.

This postulation is supported by the findings of Jones *et al.,* where the author reported the detection of neonicotinoid compounds in the soil three years after application.[429] This finding is also strengthened by Bonmartin *et al.,* where the persistence of neonicotinoids was observed to be highest under cold, dry conditions and low organic matter, with a half-life in the soil exceeding 1000 days.[420] Interestingly, neonicotinoids, especially clothianidin, imidacloprid and thiacloprid, were detected in organic farming land in Switzerland with no neonicotinoids application history of up to 10 years.[430]

Based on the available Irish agricultural soil properties, it can be inferred that neonicotinoids have a high risk of leaching and accumulation. All three banned neonicotinoids, clothianidin, imidacloprid and thiamethoxam, were observed to have low log K_{OW} values, 0.9, 0.6 and -0.13, respectively. [394] Furthermore, these pesticides were also determined to have high GUS index, 3.74, 3.69, and 3.74, respectively, confirming high leaching risk in Irish agricultural soil, further strengthening the need to ban them for outdoor use. On the other hand, thiacloprid is determined to be an accumulation risk in high organic matter content of Irish agricultural soil, with high log K_{OW} and K_{OC} values, 1.26 and 1584, respectively, with relatively low water solubility (185 mg/L) and low GUS index (1.1).

As highlighted, the agricultural land of Ireland is determined to have high organic matter content, with organic carbon ranging between 15.5 and 492.7 g/kg (mean=67.3).[375] As most of the widely used pesticides have high K_{OC} values (Table 1.8), it can be inferred that pesticide accumulation in the soil layers is a significant risk facing Irish agricultural industry. Of all the current widely used pesticides, lambda-cyhalothrin was determined to pose the highest risk of

accumulation. Even though lambda-cyhalothrin is moderately persistent with a half-life of 175 days, it poses a high persistence risk with an extremely high K_{OC} value of 283707, high log K_{OW}, very low water solubility and low GUS index.[394] This postulation is further supported by Ali and Baugh, where they noted that lambda-cyhalothrin is not significantly affected by soil pH, but the percentage of organic matter content dominates adsorption capacity.[431] Similar to lambda-cyhalothrin, epoxiconazole is also observed to have the chemical characteristics of a high-persisting pesticide. In their monitoring study of European agricultural soil, Silva *et al.* noted that epoxiconazole is one of the most frequently detected at the highest concentrations in the soil samples.[152] The frequent detection of epoxiconazole is a testament to their long persistence in the soil, strengthened by the high log K_{OW} and K_{OC} values and their low water solubility (Table 1.8). Together with epoxiconazole, glyphosate and AMPA were also frequently detected at the highest concentrations in European-wide agricultural soil samples by Silva *et al.*, which could lead to the assumption that Irish agricultural soil currently faces a similar level of contamination risks.[432]

1.6 Conclusion

As much as agricultural food production is essential for the continued survival of all living organisms, it cannot happen at scale without soil. The soil layers not only serve as primary natural resources in the agricultural industry but are also essential in recycling and detoxifying organic materials, nutrients and global gases. With the continuous usage of pesticides and soil being the endpoint of most pesticides, accumulating pesticide residues in soil is an ever-increasing risk. Therefore, pesticide concentrations in Irish agricultural field soils must be assessed. Evaluating concentrations of existing and commonly used pesticide residues in soil would not only give an overview of the soil health but also assess the extent of soil contamination. Contamination in soil can have a knock-on effect that potentially damages soil's precious biomass and nutrient cycle, leading to contamination of other natural resources, namely water and air. Additionally, there are possibilities of pesticide residues taken up by crops grown on soil with a high concentration of pesticide residue, ending up in the food chain.

Silva *et al.* noted that in Europe, agricultural soils with multiple mixtures of pesticide residues are common, that out of the sampled 317 agricultural sites, 183 sites were determined to contain two or more pesticide residues. This observation revealed the extent of pesticide contamination in European agricultural soils, raising many concerns. Nevertheless, as the Republic of Ireland was not included in the study by Silva *et al.*, it is impossible to extrapolate the status of pesticide contamination in Irish agricultural soil based on other countries' pesticide detection records. This underscores the need for assessment of national and local conditions, focusing on widely used pesticides in the Irish context, comparison of different soil types and classes of Irish agricultural soils

and considering Irish land use and crop types. Establishing the extent of soil contamination specific to Irish agricultural soil can provide valuable insight, supporting future decisions with regard to future legislative developments and accurate risk assessments of pesticide residues in Irish agricultural soil, ultimately enabling sustainable pesticide use while protecting the soil resources.

1.7 Aims and objectives

Establishing an effective soil policy directive for sustainable pesticide use in the agricultural industry requires a baseline knowledge of the current levels of contamination, an understanding of the soil's physicochemical and biological properties, and what is required to maintain healthy, high-quality agricultural soil. To our knowledge, monitoring or assessing the contamination level of widely used pesticides has not been carried out for Irish soils. Currently, our understanding of pesticide occurrence in the Republic of Ireland is based on studies conducted as meta-analyses of the literature,[330,433] on food and products,[434–436] on groundwater,[437] and even wastewater, [438] but not on Irish agricultural soil. Therefore, it is anticipated that the findings of this thesis will provide novel insight into the current level of pesticide contamination in Irish agricultural soil, providing a baseline reference for future monitoring efforts in the Republic of Ireland. Therefore the main objectives of this thesis are to (a) compare, validate and establish an accurate and reproducible extraction method of a single mixed analysis of neonicotinoids, triazoles, and synthetic auxin pesticides, (b) provide a baseline overview of pesticide contamination in Irish agricultural soil and establish a baseline reference for future monitoring, and (c) baseline understanding of the impact of pesticide application on the microbial functional properties of Irish agricultural soil.

Chapter 2 presents the development of a suitable extraction for the pesticides of interest in Irish agricultural systems. It also includes a systematic comparison of Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and Dutch mini-Luke (DLM) on the effectiveness of quantification of the target seven pesticides (acetamiprid, clothianidin, fluroxypyr, imidacloprid, prothioconazole, thiacloprid, and thiamethoxam). Whilst QuEChERS are the most widely used pesticide extraction techniques globally, their limitations with regard to prothioconazole are highlighted here. DLM is demonstrated to overcome these limitations, enabling prothioconazole's accurate quantification in a variety of soil samples.

Chapter 3 provides a comprehensive insight into the presence and concentration of widely used pesticides in Irish agricultural soil. These widely used pesticides, five pesticides and five neonicotinoids, were elucidated from the soil samples collected from 25 agricultural sites representing different soil types and classes, and the soil samples were collected within 24 hours and a week after pesticide application. Based on the pesticide application record, prothioconazole was highlighted as the most widely applied pesticide, where it was applied in 18 of the 25 studied fields. However, while ten sites had detections above the Limit of Quantification (LOQ), within a week, only four fields had detections above LOQ. In contrast, fluroxypyr was not detected above LOQ in either sampling timepoint. Additionally, neonicotinoids were detected in 96% of the sampled sites, even though it was banned in 2018. Furthermore, whilst theoretical risk assessment indicated that prothioconazole posed an accumulation risk, its concentration decreased by 72% within a week.

Chapter 4 highlights the impact of pesticide application on the fluctuation of soil microbial functional properties. Soil organic matter (SOM) was determined to be

the only soil property that correlated significantly positive with the soil microbial functional properties; hence this study focused on the impact of pesticide application on soil microorganisms in different classes of SOM. The importance of agricultural practices that preserves SOM was highlighted here, where soil microbial biomass and functional diversity were noted to be the highest in the soil samples with high SOM. Additionally, a week after the pesticide application, microbial biomass, respiration activities and functional diversity of the soil class with higher SOM were observed to decline, while the stress indicator (qCO_2) increased significantly.

Chapter 5 concludes the thesis based on the works presented in the thesis. Additionally, as the studies in this thesis aim to provide a baseline knowledge on the current level of pesticide contamination and the effect of pesticide application on microbial functional properties in Irish agricultural soil, future research possibilities were suggested to explore for further development of this thesis's findings.

1.8 References

1 M. F. Bellemare, *Am J Agric Econ*, 2015, **97**, 1–21.

2 C. Damalas, *Scientific Research and Essay*, 2009, **4**, 945–949.

3 J. S. Pettis, E. M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose and D. vanEngelsdorp, *PLoS One*, , DOI:10.1371/journal.pone.0070182.

4 E. C. Oerke, *Journal of Agricultural Science*, 2005, **144**, 31–43.

5 CABI, Plantwise - Lose less, feed more, https://www.plantwise.org/, (accessed 24 September 2019).

6 B. Gevao, K. T. Semple and K. C. Jones, *Environmental Pollution*, 2000, **108**, 3–14.

7 T. J. Zacharia, *Pesticides in the Modern World - Trends in Pesticides Analysis*, , DOI:10.5772/17513.

8 E. Commission, EU Pesticides database, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN, (accessed 27 April 2020).

9 C. Cox and M. Surgan, *Environ Health Perspect*, 2006, **114**, 1803–1806.

10 FIFRA, 7USC13, https://www.govinfo.gov/content/pkg/USCODE-2012-title7/html/USCODE-2012-title7-chap6-subchapII-sec136.htm, (accessed 5 October 2022).

11 J. R. Plimmer, in *Handbook of Pesticide Toxicology Principles*, ed. R. I. Krieger, Academic Press, 2nd edn., 2001, pp. 95–107.

12 European Union, *REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009*
concerning the placing of plant protection products on the market a nd repealing Council Directives 79/117/EEC and 91/414/EEC, 2009, vol. 24.

13 J. E. Balmer, A. D. Morris, H. Hung, L. Jantunen, K. Vorkamp, F. Rigét, M. Evans, M. Houde and D. C. G. Muir, *Emerg Contam*, 2019, **5**, 70–88.

14 G. Zhong, Z. Xie, M. Cai, A. Möller, R. Sturm, J. Tang, G. Zhang, J. He and R. Ebinghaus, *Environ Sci Technol*, 2012, **46**, 259–267.

15 R. Sur and A. S. Tork, *Bull Insectology*, 2003, **56**, 35–40.

16 Jannes Stolte, Mehreteab Tesfai, Lillian Øygarden, Sigrun Kværnø, Jacob Keizer, Frank Verheijen, Panos Panagos, Cristiano Ballabio and Rudi Hesse, *Soil threates in Europe: Status, methods, drivers, and effects on ecosystem services*, 2015.

17 D. Pimentel and M. Burgess, in *Integrated Pest Management: Pesticide Problems, Vol.3*, 2014, pp. 1–474.

18 H. M. G. van der Werf, *Agric Ecosyst Environ*, 1996, **60**, 81–96.

19 D. Nuyttens, W. Devarrewaere, P. Verboven and D. Foqué, *Pest Manag Sci*, 2013, **69**, 564–575.

20 O. Zivan, Y. Bohbot-Raviv and Y. Dubowski, *Chemosphere*, 2017, **177**, 303–310.

21 M. Houbraken, K. Doan Ngoc, F. van den Berg and P. Spanoghe, *Science of the Total Environment*, 2017, **599–600**, 1408–1416.

22 A. Wolters, V. Linnemann, M. Herbst, M. Klein, A. Schäffer and H. Vereecken, *J Environ Qual*, 2003, **32**, 1183–1193.

23 G. Imfeld, F. Meite, C. Wiegert, B. Guyot, J. Masbou and S. Payraudeau, *Science of the Total Environment*, 2020, **741**, 140437.

24 S. Pätzold, C. Klein and G. W. Brümmer, *Soil Use Manag*, 2007, **23**, 294–305.

25 X. Zhang, Y. Luo and K. S. Goh, *Environmental Pollution*, 2018, **234**, 48–58.

26 D. Goulson, *Journal of Applied Ecology*, 2013, **50**, 977–987.

27 C. Druart, M. Millet, R. Scheifler, O. Delhomme, C. Raeppel and A. De Vaufleury, *Science of the Total Environment*, 2011, **409**, 4280–4288.

28 J. L. Tadeo, B. Albero and R. A. Perez, in *Analysis of Pesticides in Food and Environmental Samples*, ed. J. L. Tadeo, CRC Press Taylor and Francis Group, 2nd edn., 2019, pp. 1–40.

29 R. Mesnage and M. N. Antoniou, *Front Public Health*, 2018, **5**, 1–8.

30 M. J. L. Castro, C. Ojeda and A. F. Cirelli, in *Green Materials for Energy, Products and Depollution*, eds. E. Lichtfouse, J. Schwarzbauer and D. Robert, Springer, Dordrecht, 2013, vol. 3, pp. 287–334.

31 I. Travlos, N. Cheimona and D. Bilalis, *Agronomy*, , DOI:10.3390/agronomy7030060.

32 M. Marutani and V. Edirveerasingam, *J Environ Qual*, 2006, **35**, 1994–1998.

33 S. N. M. Yusoff, A. Kamari and N. F. A. Aljafree, *International Journal of Environmental Science and Technology*, 2016, **13**, 2977–2994.

34 C. J. Wang and Z. Q. Liu, *Pestic Biochem Physiol*, 2007, **87**, 1–8.

35 A. Fernández Cirelli, C. Ojeda, M. J. L. Castro and M. Salgot, *Environ Chem Lett*, 2008, **6**, 135–148.

36 U. S. EPA, *Fed Regist*, 2002, **67**, 1925–1931.

37 A. Vanlaeys, F. Dubuisson, G. E. Seralini and C. Travert, *Toxicology in Vitro*, 2018, **52**, 14–22.

38 S. I. L. Gomes, A. Ammendola, S. Casini and M. J. B. Amorim, *Science of the Total Environment*, , DOI:10.1016/j.scitotenv.2020.142098.

39 H. J. Puglis and M. D. Boone, *Arch Environ Contam Toxicol*, 2011, **60**, 145–155.

40 S. Beggel, I. Werner, R. E. Connon and J. P. Geist, *Science of the Total Environment*, 2010, **408**, 3169–3175.

41 M. Tudi, H. D. Ruan, L. Wang, J. Lyu, R. Sadler, D. Connell, C. Chu and D. T. Phung, *Int J Environ Res Public Health*, 2021, 18, 1–24.

42 H. S. Rathore, in *Handbook of Pesticides: Methods of Pesticide Residues Analysis*, 2010, pp. 7–45.

43 M. Sporleder and L. A. Lacey, in *Insect Pests of Potato*, eds. A. Alyokhin, C. Vincent and P. Giordanengo, Elsevier Inc., 2013, pp. 463–497.

44 G.-F. Pang, in *Analytical Methods for Food Safety by Mass Spectrometry*, Academic Press, 2018, pp. 1–9.

45 FAO, Global Pesticide Use till 2017, http://www.fao.org/faostat/en/?#data/RP, (accessed 27 March 2020).

46 N. Jayakody, E. C. Harris and D. Coggon, *Br Med Bull*, 2015, **114**, 75–94.

47 T. Paszko, P. Muszyński, M. Materska, M. Bojanowska, M. Kostecka and I. Jackowska, *Environ Toxicol Chem*, 2016, **35**, 271–286.

48 G. Haberhauer, L. Pfeiffer and M. H. Gerzabek, *J Agric Food Chem*, 2000, **48**, 3722–3727.

49 R. Celis, M. C. Hermosín, L. Cox and J. Cornejo, *Environ Sci Technol*, 1999, **33**, 1200–1206.

50 S. Susarla, G. V. Bhaskar and S. M. R. Bhamidimarri, *Environmental Technology (United Kingdom)*, 1997, **18**, 937–943.

51 B. E. Watt, A. T. Proudfoot, S. M. Bradberry and J. Allister Vale, *Toxicol Rev*, 2005, **24**, 161–166.

52 S. Morais, M. Correia, V. Domingues and C. Delerue-Matos, *Pesticides - Strategies for Pesticides Analysis*, , DOI:10.5772/13126.

53 Homer. M. Lebaron, Janis. E. Mcfarland and Orvin. C. Burnside, in *The Triazine Herbicides 50 years Revolutionizing Agriculture*, eds. Homer. M. Lebaron, Janis. E. Mcfarland and Orvin. C. Burnside, Elsevier B.V., 2008, pp. 1–12.

54 S. Sousa, M. L. Maia, L. Correira-Sá, V. C. Fernandes, C. Delerue-Matos, C. Calhau and V. F. Domingues, in *Controlled Release of Pesticides for Sustainable Agriculture*, Springer, 2020, pp. 59–109.

55 M. Eddleston, in *Critical Care Toxicology*, eds. J. Brent, K. Burkhart, P. Dargan, B. Hatten, B. Megarbane, R. Palmer and J. White, Springer International Publishing AG, 2nd edn., 2016, pp. 1–20.

56 G. Rytwo and M. Tavasi, *Pest Manag Sci*, 2003, **59**, 1265–1270.

57 P. K. Gupta, *Herbicides and fungicides*, Elsevier Inc., 2014.

58 N. Hu, Y. Li and L. Hu, *Agricultural Science and Technology*, 2016, **71**, 1379–1382.

59 R. Vargas-Bernal, E. Rodrguez-Miranda and G. Herrera-Prez, *Pesticides - Advances in Chemical and Botanical Pesticides*, , DOI:10.5772/46227.

60 L. Shen and F. Wania, *J Chem Eng Data*, 2005, **50**, 742–768.

61 R. Jayaraj, P. Megha and P. Sreedev, *Interdiscip Toxicol*, 2016, **9**, 90–100.

62 M. Kazemi, a M. Tahmasbi, R. Valizadeh, a a Naserian and A. Soni, *Agricultural Science Research Journals*, 2012, **2**, 512–522.

63 F. Matsumura, *Toxicology of Insecticides*, Plenum Press, 1985.

64 A. M. Blacker, I. D. Kelly, J. L. Lantz, G. J. Mihlan, R. L. Jones and B. M. Young, in *Hayes' Handbook of Pesticide Toxicology*, ed. R. Krieger, Elsevier Inc., 3rd edn., 2010, pp. 1619–1632.

65 M. Doble and A. Kumar, in *Biotreatment of Industrial Effluents*, eds. M. Doble and A. Kumar, Elsevier Inc., 2005, vol. 6, pp. 89–100.

66 K. Palmquist, J. Salatas and A. Fairbrother, in *Insecticides - Advances in Integrated Pest Management*, ed. F. Perveen, InTech, 2012, pp. 251–278.

67 A. Gajendiran and J. Abraham, *Front Biol (Beijing)*, 2018, **13**, 79–90.

68 D. A. Laskowski, *Review Environmental Contamination and Toxicology*, 2002, **174**, 49–170.

69 M. Watts, *Neonicotinoids*, 2011.

70 P. Jeschke, R. Nauen, M. Schindler and A. Elbert, *J Agric Food Chem*, 2011, **59**, 2897–2908.

71 C. Bass and L. M. Field, *Current Biology*, 2018, **28**, R772–R773.

72 R. C. Gupta, M. Sachana, I. M. Mukherjee, R. B. Doss, J. K. Malik and D. Milatovic, in *Veterinary Toxicology: Basic and Clinical Principles: Third Edition*, 2018, pp. 495–508.

73 L. Ye, J. Guo and R. S. Ge, in *Vitamins and Hormones*, Elsevier Inc., 1st edn., 2014, vol. 94, pp. 349–390.

74 P. E. Russell, *Journal of Agricultural Science*, 2005, **143**, 11–25.

75 A. N. Petit, F. Fontaine, P. Vatsa, C. Clément and N. Vaillant-Gaveau, *Photosynth Res*, 2012, **111**, 315–326.

76 P. K. Singh and O. Silakari, in *Key Heterocycle Cores for Designing Multitargeting Molecules*, ed. O. Silakari, Elsevier Ltd, 2018, pp. 31–52.

77 R. C. Gupta and J. W. Crissman, in *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, Elsevier, Third Edit., 2013, pp. 1349–1372.

78 J. P. Zubrod, A. Feckler, D. Englert, N. Koksharova, R. R. Rosenfeldt, F. Seitz, R. Schulz and M. Bundschuh, *Journal of Applied Ecology*, 2015, **52**, 310–322.

79 P. Karuppuchamy and S. Venugopal, in *Ecofriendly Pest Management for Food Security*, 2016, pp. 651–684.

80 Y. S. Wu, H. K. Lee and S. F. Y. Li, *J Chromatogr A*, 2001, **912**, 171–179.

81 D. S. Mueller, *Integrated Crop Management*.

82 V. Ji Ram, A. Sethi, M. Nath and R. Pratap, in *The Chemistry of Heterocycles*, Elsevier Ltd, 2019, pp. 3–391.

83 E. I. Mercer, *Biochem Soc Trans*, 1991, **19**, 788–793.

84 M. Gavrilescu, *Eng Life Sci*, 2005, **5**, 497–526.

85 R. C. Kuhad, A. K. Johri, A. Singh and O. P. Ward, in *Applied Bioremediation and Phytoremediation*, eds. A. Singh and O. P. Ward, Springer-Verlag, 2004, vol. 1, pp. 35–54.

86 E. Hodgson, in *PESTICIDE BIOTRANSFORMATION AND DISPOSITION*, Elsevier Inc., 2012, pp. 53–69.

87 J. E. Casida, *Chem Res Toxicol*, 2009, 22, 609–619.

88 S. I. Sherwani, I. A. Arif and H. A. Khan, in *Herbicides, Physiology of Action, and Safety*, 2015, pp. 165–186.

89 A. Tataridas, P. Kanatas, A. Chatzigeorgiou, S. Zannopoulos and I. Travlos, *Agronomy*, , DOI:10.3390/agronomy12030589.

90 S. Wynn and E. Webb, *Environ Sci Eur*, 2022, 34.

91 B. Hove-Jensen, D. L. Zechel and B. Jochimsen, *Microbiology and Molecular Biology Reviews*, 2014, **78**, 176–197.

92 J. M. Green and M. D. K. Owen, *J Agric Food Chem*, 2011, **59**, 5819–5829.

93 K. Grossmann, J. Kwiatkowski and S. Tresch, *Auxin herbicides induce H 2 O 2 overproduction and tissue damage in cleavers (Galium aparine L.)*, .

94 V. K. Varanasi, A. S. Godar, R. S. Currie, A. J. Dille, C. R. Thompson, P. W. Stahlman and M. Jugulam, *Pest Manag Sci*, 2015, **71**, 1207–1212.

95 V. K. Rai, *Biol Plant*, 2002, **45**, 481–487.

96 J. S. Holt, S. B. Powles and J. A. M. Holtum, *Annu Rev Plant Physiol*, 1993, **44**, 203–229.

97 R. Ordonio, Y. Ito, Y. Morinaka, T. Sazuka and M. Matsuoka, in *International Review of Cell and Molecular Biology*, Elsevier Inc., 2016, vol. 321, pp. 221–257.

98 T. W. Jabusch and R. S. Tjeerdema, *Chemistry and Fate of Triazolopyrimidine Sulfonamide Herbicides*, 2008.

99 E. Schönbrunn, S. Eschenburg, W. A. Shuttleworth, J. V. Schloss, N. Amrhein, J. N. S. Evans and W. Kabsch, *Proc Natl Acad Sci U S A*, 2001, **98**, 1376–1380.

100 D. R. Geiger and M. A. Fuchs, in *Herbicide Classes in Development*, 2002, pp. 59–85.

101 C. D. S. Tomlin, *The pesticide manual: A World compendium.*, British Crop Production Council, 2009.

102 EPA-738-F-93-011, *R.E.D. FACTS Glyphosate*, U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 1993.

103 C. A. C. G. Brunharo, H. K. Takano, C. A. Mallory-Smith, F. E. Dayan and B. D. Hanson, *J Agric Food Chem*, 2019, **67**, 8431–8440.

104 A. Blanco and G. Blanco, in *Medical Biochemistry*, Academic Press, 2017, pp. 336–393.

105 E. Dor, S. Galili, E. Smirnov, Y. Hacham, R. Amir and J. Hershenhorn, *Front Plant Sci*, , DOI:10.3389/fpls.2017.00707.

106 J. R. Ellis, R. Taylor and P. J. Hussey, *Plant Physiol*, 1994, **105**, 15–18.

107 T. C. C. Fernandes, M. A. and M. A., *Herbicides - Current Research and Case Studies in Use*, , DOI:10.5772/55169.

108 J. Stenersen, *Chemical Pesticides Mode of Action and Toxicology*, CRC, 2004.

109 R. Ricardo, J. Luiz and W. Luiz, in *Applied Photosynthesis*, 2012, pp. 3–22.

110 S. Z. Tóth, G. Schansker and R. J. Strasser, *Biochim Biophys Acta Bioenerg*, 2005, **1708**, 275–282.

111 A. H. Cobb and J. P. H. Reade, *Herbicides and Plant Physiology*, Wiley-Blackwell, Second., 2010, vol. 69.

112 D. P. Bebber, M. A. T. Ramotowski and S. J. Gurr, *Nat Clim Chang*, 2013, **3**, 985–988.

113 T. G. E. Davies, L. M. Field, P. N. R. Usherwood and M. S. Williamson, *IUBMB Life*, 2007, 59, 151–162.

114 R. C. Gupta, I. R. M. Mukherjee, J. K. Malik, R. B. Doss, W. Dettbarn and D. Milatovic, in *Biomarkers in Toxicology, Second Edition*, Elsevier Inc., 2019, pp. 363–375.

115 M. Ghanim and I. Ishaaya, 2011, 385–407.

116 R. T. von Stein, K. S. Silver and D. M. Soderlund, *Bone*, 2005, **23**, 1–7.

117 J. E. Casida and K. A. Durkin, in *Annual Review of Entomology*, 2013, vol. 58, pp. 99–117.

118 J. R. Bloomquist, *Comparative Biochemistry and Physiology. Part C: Comparative*, 1993, **106**, 301–314.

119 J. R. Von Osten, R. Tinoco-Ojanguren, A. M. V. M. Soares and L. Guilhermino, *Arch Environ Health*, 2004, **59**, 418–425.

120 F. Chicatun, G. Griffanti, M. D. McKee and S. N. Nazhat, in *Biomedical Composites*, Elsevier Ltd., Second Edi., 2017, pp. 163–198.

121 R. L. Whistler, in *Industrial Gums: Polysaccharides and Their Derivatives: Third Edition*, 1993, pp. 601–604.

122 F. Meyer, M. Flötenmeyer and B. Moussian, *Pest Manag Sci*, 2013, **69**, 1136–1140.

123 H. Merzendorfer, *Insect Sci*, 2013, **20**, 121–138.

124 M. C. Fisher, D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw and S. J. Gurr, *Nature*, 2012, 484, 186–194.

125 C. Yang, C. Hamel, V. Vujanovic and Y. Gan, *ISRN Ecol*, 2011, **2011**, 1–8.

126 H. Hof, *Antimicrob Agents Chemother*, 2001, **45**, 2987–2990.

127 F. J. López-Ruiz, A. Pérez-García, D. Fernández-Ortuño, D. Romero, E. García, A. de Vicente, J. K. M. Brown and J. A. Torés, *Pest Manag Sci*, 2010, **66**, 801–808.

128 R. Becher and S. G. R. Wirsel, *Appl Microbiol Biotechnol*, 2012, **95**, 825–840.

129 J. R. Coats, *Environ Health Perspect*, 1990, **87**, 255–262.

130 R. K. Gupta and R. C. Gupta, in *Reproductive and Developmental Toxicology*, Elsevier Inc., 2017, pp. 1301–1325.

131 T. Narahashi, *Pestic Sci*, 1976, **7**, 267–272.

132 R. D. O'brien, *J Agric Food Chem*, 1963, **11**, 163–166.

133 T. R. Fukuto, *Environ Health Perspect*, 1990, **87**, 245–254.

134 A. L. Crane, G. Abdel Rasoul, A. A. Ismail, O. Hendy, M. R. Bonner, M. R. Lasarev, M. Al-Batanony, S. T. Singleton, K. Khan, J. R. Olson and D. S. Rohlman, *J Expo Sci Environ Epidemiol*, 2013, **23**, 356–362.

135 E. Schönbrunn, S. Eschenburg, W. A. Shuttleworth, J. V. Schloss, N. Amrhein, J. N. S. Evans and W. Kabsch, *Proc Natl Acad Sci U S A*, 2001, **98**, 1376–1380.

136 H. Maeda and N. Dudareva, *Annu Rev Plant Biol*, 2012, **63**, 73–105.

137 K. M. Herrmann and L. M. Weaver, *Annu Rev Plant Physiol Plant Mol Biol*, 1999, **50**, 473–503.

138 T. Shivanandappa and Y. Rajashekar, in *Advances in Plant Biopesticides*, 2014, pp. 323–345.

139 D. M. Soderlund, in *Hayes' Handbook of Pesticide Toxicology*, Elsevier Inc., Third Edit., 2010, vol. Volume 2, pp. 1665–1686.

140 D. M. Soderlund, *Arch Toxicol*, 2012, **86**, 165–181.

141 B. T. Green, K. D. Welch, K. E. Panter and S. T. Lee, *Chem Res Toxicol*, 2013, **26**, 1129–1138.

142 M. Tomizawa and J. E. Casida, *Annu Rev Pharmacol Toxicol*, 2005, **45**, 247–268.

143 K. Matsuda, S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso, D. B. Sattelle and S. D. Buckingham, *Trends Pharmacol Sci*, 2001, **22**, 573–580.

144 C. Moffat, S. T. Buckland, A. J. Samson, R. McArthur, V. Chamosa Pino, K. A. Bollan, J. T. J. Huang and C. N. Connolly, *Sci Rep*, 2016, **6**, 1–10.

145 K. J. Germaine, X. Liu, G. G. Cabellos, J. P. Hogan, D. Ryan and D. N. Dowling, *FEMS Microbiol Ecol*, 2006, **57**, 302–310.

146 Y. Song, *J Integr Plant Biol*, 2014, **56**, 106–113.

147 C. Lamberth, in *Bioactive Carboxylic Compound Classes: Pharmaceuticals and Agrochemicals*, eds. C. Lamberth and J. Dinges, Wiley-VCH Verlag GmbH & Co. KGaA, First edit., 2016, pp. 293–302.

148 E. P. Fuerst, *Weed Technology*, 1987, **1**, 270–277.

149 A. M. Au, *Encyclopedia of Food Sciences and Nutrition*, 2003, 4483–4487.

150 M. T. Rose, T. R. Cavagnaro, C. A. Scanlan, T. J. Rose, T. Vancov, S. Kimber, I. R. Kennedy, R. S. Kookana and L. Van Zwieten, in *Advances in Agronomy*, Elsevier Inc., 2016, vol. 136, pp. 133–220.

151 F. M. Fishel, *Pesticide Toxicity Profile: Triazole Pesticides*, 2014.

152 V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2018, **653**, 1532–1545.

153 P. Kosubová, L. Škulcová, Poláková, J. Hofman and L. Bielská, *Chemosphere*, , DOI:10.1016/j.chemosphere.2020.126902.

154 C. Bernasconi, P. M. Demetrio, L. L. Alonso, T. M. mac Loughlin, E. Cerdá, S. J. Sarandón and D. J. Marino, *Agric Ecosyst Environ*, , DOI:10.1016/j.agee.2021.107341.

155 EFSA, *EFSA Journal*, , DOI:10.2903/j.efsa.2018.5348.

156 V. Chaplain, L. Mamy, L. Vieuble-Gonod, C. Mougin, P. Benoit, E. Barriuso and S. Nelieu, in *Pesticides in the Modern World - Risks and Benefits*, InTech, 2011, p. 560.

157 T. Langenbach, *Applied Bioremediation - Active and Passive Approaches*, , DOI:10.5772/56418.

158 Y. Picó, in *Encyclopedia of Food and Health*, Elsevier Inc., 2015, pp. 311–318.

159 R. A. Cloyd, *Kansas State University Agricultural Experiment Station and Cooperative Extension Service*, 2012, 1–2.

160 Guidance to Calculate Representative Half-life Values and Characterizing Pesticide Degradation | US EPA, https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-calculate-representative-half-life-values, (accessed 7 October 2022).

161 A. Mandal, B. Sarkar, S. Mandal, M. Vithanage, A. K. Patra and M. C. Manna, in *Agrochemicals Detection, Treatment and Remediation*, Elsevier, 2020, pp. 161–187.

162 D. Hanson, B.; Bond, C.; Buhl, K.; Stone, *National pesticide information center*, 1–4.

163 B. Hanson, C. Bond, K. Buhl and D. Stone, Pesticide Half-life Fact Sheet, http://npic.orst.edu/factsheets/half-life.html.

164 P. C. Kearney, D. R. Shelton and W. C. Koskinen, in *Kirk-Othmer Encyclopedia of Chemical Technology*, John Wiley & Sons, Inc., 2000, pp. 419–451.

165 J. Chorover and M. L. Brusseau, in *Kinetics of Water-Rock Interaction*, eds. S. Brantley, J. Kubicki and A. White, Springer, New York, NY, 2008, pp. 109–149.

166 P. C. Kearney, D. R. Shelton and W. C. Koskinen, *Encyclopedia of Agrochemicals*, , DOI:10.1002/047126363x.agr336.

167 W. C. Koskinen and S. S. Harper, in *Pesticides in the Soil Environment*, 1990, pp. 51–77.

168 M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. C. Mejuto and L. García-Río, *Agric Ecosyst Environ*, 2008, 123, 247–260.

169 M. C. Vagi, A. S. Petsas, M. N. Kostopoulou and T. D. Lekkas, *Int J Environ Anal Chem*, 2010, **90**, 369–389.

170 J. B. Weber, in *In Fate of Organic Pesticides in the Aquatic Environment*, ACS Symposium Series, 1972, pp. 55–120.

171 F. J. Stevenson, in *In Bound and Conjugated Pesticide Residues*, ACS Symposium Series, 1976, vol. 29, pp. 180–207.

172 C. D. Linde, *Physico-Chemical Properties Environmental Fate of Pesticides*, 1994.

173 I. Scheunert, in *Fate and Prediction of Environmental Chemicals in Soils, Plants, and Aquatic Systems*, 1st edn., 2018, pp. 1–22.

174 E. C. for E. and T. of C. ECETOC, *Soil and Sediment Risk Assessment of Organic Chemicals*, 2004.

175 J. Stanley and G. Preetha, *Pesticide toxicity to non-target organisms: Exposure, toxicity and risk assessment methodologies*, 2016.

176 F. Hernandez, J. Beltran, M. Forcada, F. J. Lopez and I. Morell, *Int J Environ Anal Chem*, 1998, **71**, 87–103.

177 C. J. Sinclair, PhD Thesis, University of York , 2009.

178 EPA, *Understanding Variation in Partition (Kd) Values*, 1999, vol. I.

179 Y. Li, P. Su, Y. Li, K. Wen, G. Bi and M. Cox, *Chemosphere*, 2018, **207**, 708–714.

180 R. J. Smernik and R. S. Kookana, *Chemosphere*, 2015, **119**, 99–104.

181 W. R.D., Y. S., L. J.B.H.J., K. R., T. K., R. B., K. A., K. W., G. Z., L. M. and U. J.B., *Pest Manag Sci*, 2002, **58**, 419–445.

182 E. Voutsas, in *Thermodynamics, Solubility and Environmental Issues*, ed. T. M. Letcher, Elsevier B.V., 2007, pp. 205–227.

183 V. J. Pereira, J. P. A. R. d Cunha, T. P. d Morais, J. P. Ribeiro-Oliveira and J. B. d Morais, *Bioscience Journal*, 2016, **32**, 627–641.

184 P. J. McCall, R. L. Swann, D. A. Laskowski, S. M. Unger, S. A. Vrona and H. J. Dishburger, *Bull Environ Contam Toxicol*, 1980, **24**, 190–195.

185 Y. Cohen, W. Tsai, S. L. Chetty and G. J. Mayer, *Environ Sci Technol*, 1990, **24**, 1549–1558.

186 G. Pérez-Lucas, N. Vela, A. E. Aatik and S. Navarro, in *Pesticides - Use and Misuse and Their Impact in the Environment*, Intech Open Access Publisher, 2019, pp. 1–27.

187 UNECE, *Annex 9 Guidance on Hazards to the Aquatic Environment*, 2013.

188 H. Cumming and C. Rucker, *ACS Omega*, 2017, **2**, 6244–6249.

189 E. Yasser and A. Alkareem, *Journal of Environment and Earth Science*, 2013, **3**, 199–210.

190 M. M. Miller, S. P. Wasik, G. Lan. Huang, W. Ying. Shiu and Donald. Mackay, *Environ Sci Technol*, 1985, **19**, 522–529.

191 A. J. Cessna and N. D. Westcott, in *Pesticide Interactions in Crop Production: Beneficial and Deleterious Effects*, ed. J. Altman, CRC Press, 1993, pp. 59–85.

192 A. Thompson and K. W. Goyne, *Nature Education Knowledge*, 2012, **4**, 7.

193 W. R. Roy, in *Handbook of Solvents*, ChemTec Publishing, Volume 2., 2019, pp. 1203–1253.

194 I. K. Konstantinou, A. K. Zarkadis and T. A. Albanis, *Published in J. Environ. Qual*, 2001, **30**, 121–130.

195 P. Wang and A. A. Keller, *Water Res*, 2009, **43**, 1448–1456.

196 L. Cox, M. C. Hermosín, R. Celis and J. Cornejo, *Water Res*, 1997, **31**, 1309–1316.

197 J. E. D. Davies and N. Jabeen, 2003, **46**, 57–64.

198 N. Kumar, I. Mukherjee and E. Varghese, *Environ Monit Assess*, 2015, **187**, 1–10.

199 A. G. Ahangar, R. J. Smernik, R. S. Kookana and D. J. Chittleborough, *Chemosphere*, 2008, **70**, 1153–1160.

200 P. Benoit, I. Madrigal, C. M. Preston, C. Chenu and E. Barriuso, *Eur J Soil Sci*, 2008, **59**, 178–189.

201 S. U. Khan, in *Soil Organic Matter*, eds. M. Schnitzer and S. U. Khan, Elsevier Inc., 1976, pp. 137–171.

202 J. M. Bollag, *Environ Sci Technol*, 1992, **26**, 1876–1881.

203 M. Kah and C. Brown, *Rev Environ Contam Toxicol*, 2006, **188**, 149–217.

204 K. S. Smith, *The Environmental Geochemistry of Mineral Deposits*, 1999, 161–182.

205 X. Wang and Y. Li, *J Hazard Mater*, 2011, **189**, 719–723.

206 L. Cox, W. C. Koskinen, R. Celis, M. C. Hermosin, J. Cornejo and P. Y. Yen, *Soil Science Society of America Journal*, 1998, **62**, 911–915.

207 H. Li, G. Sheng, B. J. Teppen, C. T. Johnston and S. A. Boyd, *Soil Science Society of America Journal*, 2003, **67**, 122–131.

208 R. Đurović, J. Gajić-Umiljendić and T. Đorđević, *Journal Pesticides and Phytomedicine*, 2009, **24**, 51–57.

209 X. Ren, G. Zeng, L. Tang, J. Wang, J. Wan, Y. Liu, J. Yu, H. Yi, S. Ye and R. Deng, *Science of the Total Environment*, 2018, **610–611**, 1154–1163.

210 B. Sarkar, M. Singh, S. Mandal, G. J. Churchman and N. S. Bolan, in *The Future of Soil Carbon: Its Conservation and Formation*, Elsevier Inc., 2018, pp. 71–86.

211 G. Sheng, C. T. Johnston, B. J. Teppen and S. A. Boyd, *J Agric Food Chem*, 2001, **49**, 2899–2907.

212 D. L. Sparks, *Environmental Soil Chemistry: Second Edition*, 2003.

213 D. D. Fontaine, R. G. Lehmann and J. R. Miller, *J Environ Qual*, 1991, **20**, 759–762.

214 L. Ukrainczyk and H. A. Ajwa, *Soil Science Society of America Journal*, 1996, **60**, 460–467.

215 R. N. Stougaard, P. J. Shea and A. R. Martin, *Weed Sci*, 1990, **38**, 67–73.

216 W. O. Werkheiser and S. J. Anderson, *J Environ Qual*, 1996, **25**, 809–814.

217 R. D. Wauchope, S. Yeh, J. B. H. J. Linders, R. Kloskowski, K. Tanaka, B. Rubin, A. Katayama, W. Kördel, Z. Gerstl, M. Lane and J. B. Unsworth, *Pest Manag Sci*, 2002, **58**, 419–445.

218 A. G. Hornsby, R. D. Wauchope and A. E. Herner, *Pesticide Properties in the Environment*, 1996, vol. 1.

219 N. B. Stolpe and M. S. Kuzila, *Soil Sci*, 2002, **167**, 338–345.

220 D. Smith, P. Pivonka, C. Jungnickel and S. Fityus, *Transp Porous Media*, 2004, **57**, 251–277.

221 J. B. Weber, in *Mechanisms of Pesticide Movement into Ground Water*, eds. R. C. Honeycutt and D. J. Schabacker, CRC Press, 2nd edn., 1994, pp. 15–41.

222 Sundaram Baskaran, PhD Thesis, Massey University, 1994.

223 J. Tölgyessy, in *Chemistry and Biology of Water, Air and Soil Environmetal Aspects*, ed. J. Tölgyessy, Elsevier B.V., 1993, pp. 621–698.

224 W. L. Lindsay and K. M. Catlett, in *Future Prospects for Soil Chemistry*, ed. P. M. Huang, SSSA Special Publications, 1998, pp. 123–138.

225 P. Sidoli, N. Baran and R. Angulo-Jaramillo, *Environmental Science and Pollution Research*, 2016, **23**, 5733–5742.

226 O. K. Borggaard and A. L. Gimsing, *Pest Manag Sci*, 2008, **64**, 441–456.

227 G. Palma, R. Demanet, M. Jorquera, M. L. Mora, G. Briceño and A. Violante, *J Soil Sci Plant Nutr*, 2015, **15**, 549–560.

228 J. B. Weber, G. G. Wilkerson and C. F. Reinhardt, *Chemosphere*, 2004, **55**, 157–166.

229 V. Chaplain, A. Brault, D. Tessier and P. De´fossez, *Eur J Soil Sci*, 2008, **59**, 1202–1208.

230 Z. Maznah, M. Halimah and S. Ismail, *Chil J Agric Res*, 2016, **76**, 371–377.

231 P. Bhardwaj and A. Kapley, in *Microbial Factories: Biofuels, Waste treatment: Volume 1*, 2015, vol. 1, pp. 293–314.

232 S. Rani and D. Sud, *Plant Soil Environ*, 2015, **61**, 36–42.

233 D. Broznić and Č. Milin, *J Environ Sci Health B*, 2012, **47**, 779–794.

234 J. J. T. I. Boesten and A. M. A. van der Linden, *Journal of Environment Quality*, 2010, **20**, 425.

235 K. Fenner, S. Canonica, L. P. Wackett and M. Elsner, *Science (1979)*, 2013, **341**, 752–758.

236 D. L. Shaner and W. B. Henry, *Journal of Environment Quality*, 2007, **36**, 128.

237 T. Katagi, in *Reviews of Environmental Contamination and Toxicology*, 2004, pp. 1–190.

238 E. Evgenidou and K. Fytianos, *J Agric Food Chem*, 2002, **50**, 6423–6427.

239 A. C. Gerecke, S. Canonica, S. R. Muller, M. Scharer and R. P. Schwarzenbach, *Environ Sci Technol*, 2001, **35**, 3915–3923.

240 H. D. Burrows, M. Canle L, J. A. Santaballa and S. Steenken, *J Photochem Photobiol B*, 2002, **67**, 71–108.

241 G. C. Miller and S. G. Donaldson, in *Aquatic and Surface Photochemistry*, Taylor & Francis, 1st edn., 1994, pp. 97–109.

242 Ma. Laura, E. Snchez-Salinas, E. Dantn Gonzlez and M. Luisa, in *Biodegradation - Life of Science*, InTech, 2013.

243 A. L. M. Porto, G. Z. Melgar, M. C. Kasemode and M. Nitschke, in *Pesticides in the Modern World – Pesticides Use and Management*, ed. M. Stoytcheva, InTech, 2011, pp. 407–437.

244 H. Zaffar, U. Irshad, A. Pervez and A. Naqvi, *J. Appl. Environ. Biol. Sci*, 2016, **6**, 1–6.

245 M. A. Dar, G. Kaushik and J. F. Villareal Chiu, in *Abatement of Environmental Pollutants: Trends and Strategies*, Elsevier, 2019, pp. 25–66.

246 S. Hussain, C. J. Hartley, M. Shettigar and G. Pandey, *FEMS Microbiol Lett*, 2016, **363**, 1–13.

247 S. Chen, M. Hu, J. Liu, G. Zhong, L. Yang, M. Rizwan-ul-Haq and H. Han, *J Hazard Mater*, 2011, **187**, 433–440.

248 R. L. Crawford, in *Comprehensive Biotechnology, Second Edition*, Elsevier Inc., 2011, vol. 6, pp. 3–13.

249 R. E. Hoagland, R. M. Zablotowicz and J. C. Hall, in *Pesticide Biotransformation in Plants and Microorganisms*, 2000, pp. 2–27.

250 Ma. Laura, E. Sanchez-Salinas, E. Dantn Gonzlez and M. Luisa, in *Biodegradation - Life of Science*, Intech Open Access Publisher, 2013, pp. 251–287.

251 L. J. Shaw, Y. Beaton, S. Sousa, L. A. Glover, K. Killham and A. A. Meharg, *Soil Biol Biochem*, 2002, **34**, 531–539.

252 S. Pang, Z. Lin, W. Zhang, S. Mishra, P. Bhatt and S. Chen, *Front Microbiol*, 2020, 11.

253 Y. Huang, L. Xiao, F. Li, M. Xiao, D. Lin, X. Long and Z. Wu, *Molecules*, , DOI:10.3390/molecules23092313.

254 M. L. Ortiz-Hernández, E. Snchez-Salinas, E. Dantn Gonzlez and M. Luisa, in *Biodegradation - Life of Science*, InTech, 2013.

255 N. T. Joutey, W. Bahafid, H. Sayel and E. El Ghachtouli, in *Biodegradation - Life of Science*, eds. R. Chamy and F. Rosenkranz, IntechOpen, 2013, pp. 471–480.

256 W. Fritsche and M. Hofrichter, in *Biotechnology: Environmental Processes II*, eds. H. J. Rehm and G. Reed, Wiley-VCH Verlag GmbH, Second., 2000, pp. 146–167.

257 M. Iranzo, I. Sainz-Pardo, R. Boluda, J. Sanchez and S. Mormeneo, *Ann Microbiol*, 2001, **51**, 135–143.

258 D. K. Singh, *Indian J Microbiol*, 2008, **48**, 35–40.

259 L. Tao and H. Yang, *Environ Monit Assess*, 2011, **175**, 227–238.

260 S. Navarro, N. Vela and G. Navarro, *Spanish Journal of Agricultural Research*, 2007, **5**, 357–375.

261 R. S. Kookana, S. Baskaran and R. Naidu, *Australian Journal of Soil Research*, 1998, **36**, 715–764.

262 J. J. Pignatello, C. R. Frink, P. A. Marin and E. X. Droste, *J Contam Hydrol*, 1990, **5**, 195–214.

263 T. Langenbach, in *Applied Bioremediation - Active and Passive Approaches*, eds. Y. B. Patil and P. Rao, IntechOpen, 2013, vol. 1, pp. 3017–331.

264 T. Gunstone, T. Cornelisse, K. Klein, A. Dubey and N. Donley, *Front Environ Sci*, 2021, 9.

265 F. Sánchez-Bayo, *Toxics*, 2021, 9.

266 T. T. Iyaniwura, *Rev Environ Health*, 1991, **9**, 161–176.

267 J. Abraham, G. S. Benhotons, I. Krampah, J. Tagba, C. Amissah and J. D. Abraham, *Entomol Exp Appl*, 2018, **166**, 695–702.

268 D. P. Kreutzweiser, D. G. Thompson and T. A. Scarr, *Ecotoxicol Environ Saf*, 2009, **72**, 1053–1057.

269 A. R. Main, E. B. Webb, K. W. Goyne and D. Mengel, *Agric Ecosyst Environ*, , DOI:10.1016/j.agee.2019.106693.

270 J. L. Pereira, T. V. S. Galdino, G. A. R. Silva, M. C. Picanço, A. A. Silva, A. S. Corrêa and J. C. Martins, *J Environ Sci Health B*, 2018, **53**, 447–453.

271 C. Thiour-Mauprivez, F. Martin-Laurent, C. Calvayrac and L. Barthelmebs, *Science of the Total Environment*, 2019, 684, 314–325.

272 D. W. Connell and R. D. Markwell, *Chemosphere*, 1990, **20**, 91–100.

273 T. Katagi and K. Ose, *J Pestic Sci*, 2015, **40**, 69–81.

274 W. Yu-Ting, *IOP Conf Ser Earth Environ Sci*, , DOI:10.1088/1755-1315/199/2/022055.

275 H. S. Rathore and L. M. L. Nollet, *Pesticides: Evaluation of environmental pollution*, 2012.

276 G. L. Stephenson, C. D. Wren, I. C. J. Middelraad and J. E. Warner, *Soil Biol Biochem*, 1997, **29**, 717–720.

277 K. Senthilkumar, K. Kannan, A. Subramanian and S. Tanabe, *Environmental Science and Pollution Research*, 2001, **8**, 35–47.

278 Y. B. Yohannes, Y. Ikenaka, S. M. M. Nakayama and M. Ishizuka, *Environmental Pollution*, 2014, **192**, 121–128.

279 O. Tiryaki and C. Temur, *J Biodivers Environ Sci*, 2010, **4**, 29–38.

280 Shahamat U. Khan, *Pesticides in the soil environment*, Elsevier Scientific Publishing Company, 1980.

281 R. M. Garcinuño, P. Fernández-Hernando and C. Cámara, *Water Res*, 2003, **37**, 3481–3489.

282 X. Y. Yu, G. G. Ying and R. S. Kookana, *Chemosphere*, 2009, **76**, 665–671.

283 M. J. I. Mattina, J. White, B. Eitzer and W. Iannucci-Berger, *Environ Toxicol Chem*, 2002, **21**, 281–288.

284 X. Wang, J. C. White, M. P. N. Gent, W. Iannucci-Berger, B. D. Eitzer and M. I. Mattina, *Int J Phytoremediation*, 2004, **6**, 363–385.

285 S. Paterson, D. Mackay, D. Tam and W. Y. Shiu, *Chemosphere*, 1990, **21**, 297–331.

286 M. J. I. Mattina, B. D. Eitzer, W. Iannucci-Berger, W. Y. Lee and J. C. White, *Environ Toxicol Chem*, 2004, **23**, 2756–2762.

287 L. F. Jørgensen, J. Kjær, P. Olsen and A. E. Rosenbom, *Chemosphere*, 2012, **88**, 554–562.

288 L. Guo and H. Lin, in *Advances in Agronomy*, Elsevier Inc., 1st edn., 2018, vol. 147, pp. 61–117.

289 M. Werkenthin, B. Kluge and G. Wessolek, *Environmental Pollution*, 2014, **189**, 98–110.

290 R. Kretzschmar, *Encyclopedia of Soils in the Environment*, 2004, **4**, 276–284.

291 S. Reichenberger, W. Amelung, V. Laabs, A. Pinto, K. U. Totsche and W. Zech, *Geoderma*, 2002, **110**, 63–86.

292 E. K. Triegel and L. Guo, in *Mechanisms of Pesticide Movement into Ground Water*, eds. R. C. Honeycutt and D. J. Schabacker, Two., 1994, pp. 1–16.

293 A. M. L. Lindahl and C. Bockstaller, *Ecol Indic*, 2012, **23**, 95–108.

294 A. Farenhorst, *Soil Science Society of America Journal*, 2006, **70**, 1005–1012.

295 E. G. Hatzigiannakis, G. K. Arampatzis, P. Paschalis, A. Th, A. D. Tsitouras, A. Ioannis, A. Ch and T. K. Karyotis, 104–114.

296 M. Larsbo, M. Sandin, N. Jarvis, A. Etana and J. Kreuger, *J Environ Qual*, 2016, **45**, 1367–1374.

297 M. Sandin, K. Piikki, N. Jarvis, M. Larsbo, K. Bishop and J. Kreuger, *Science of the Total Environment*, 2018, **610–611**, 623–634.

298 B. Burgoa and R. D. Wauchope, in *Environmental behavior of agrochemical*, eds. T. R. Roberts and P. C. Kearney, John Wiley & Sons, Inc., 1995, pp. 221–255.

299 C. Leu, H. Singer, C. Stamm, S. R. Müller and R. P. Schwarzenbach, *Environ Sci Technol*, 2004, **38**, 3835–3841.

300 M. J. Shipitalo and L. B. Owens, *Environ Sci Technol*, 2003, **37**, 944–950.

301 L. M. Southwick, T. W. Appelboom and J. L. Fouss, *J Agric Food Chem*, 2009, **57**, 1413–1420.

302 M. E. Grismer, *Hydrology*, , DOI:10.3390/hydrology3020018.

303 H. Bormann, L. Breuer, T. Gräff and J. A. Huisman, *Ecol Modell*, 2007, **209**, 29–40.

304 R. W. Vervoort, S. M. Dabney and M. J. M. Römkens, *Soil Science Society of America Journal*, 2001, **65**, 1227–1234.

305 O. Evrard, K. Vandaele, C. Bielders and van Wesemael, *Earth Surf Process Landf*, 2009, **34**, 613–628.

306 M. D. Ankeny, T. C. Kaspar and M. A. Prieksat, *Soil Science Society of America Journal*, 1995, **59**, 200–204.

307 C. A. Damalas, in *Environmental Indicators*, eds. R. H. Armon and O. Hanninen, Springer Science+Business Media Dordrecht, 2015, pp. 251–261.

308 A. Tapparo, D. Marton, C. Giorio, A. Zanella, L. Soldà, M. Marzaro, L. Vivan and V. Girolami, *Environ Sci Technol*, 2012, **46**, 2592–2599.

309 C. Bedos, P. Cellier, R. Calvet, E. Barriuso and B. Gabrielle, *Agronomie*, 2003, **23**, 407–418.

310 A. W. Taylor and W. F. Spencer, in *SSSA Book Series*, 1990, pp. 213–269.

311 T. J. Gish, J. H. Prueger, W. P. Kustas, C. S. T. Daughtry, L. G. McKee, A. Russ and J. L. Hatfield, *J Environ Qual*, 2009, **38**, 1785–1795.

312 R. Reichman, D. E. Rolston, S. R. Yates and T. H. Skaggs, *Environ Sci Technol*, 2011, **45**, 2144–2149.

313 C. L. Davie-Martin, K. J. Hageman, Y. P. Chin, V. Rougé and Y. Fujita, *Environ Sci Technol*, 2015, **49**, 10431–10439.

314 D. E. Glotfelty and C. J. Schomburg, *Reactions and Movement of Organic Chemicals in Soils*, 1983, **Vol. 85**, 73–88.

315 V. Ochoa and B. Maestroni, in *Integrated Analytical Approaches for Pesticide Management*, eds. B. Maestroni and A. Cannavan, Academic Press, 2018, pp. 133–147.

316 W. A. Jury, W. F. Spencer and W. J. Farmer, *J Environ Qual*, 1984, **13**, 580–586.

317 F. Ferrari, M. Trevisan and E. Capri, *J Environ Qual*, 2003, **32**, 1623–1633.

318 A. J. Cessna, F. J. Larney, L. A. Kerr and M. S. Bullock, *Can J Soil Sci*, 2006, **86**, 545–554.

319 Eurostat, Utilised agricultural area by categories, https://ec.europa.eu/eurostat/databrowser/view/tag00025/default/table?lang=en, (accessed 25 May 2020).

320 Land use overview by NUTS 2 regions | Eurostat, https://ec.europa.eu/eurostat/databrowser/view/LAN_USE_OVW__custom_3614309/default/table?lang=en, (accessed 18 October 2022).

321 Eurostat, *Agriculture, forestry and fishery statistics — 2020 edition*, 2020.

322 Pesticide sales statistics | Eurostat, https://ec.europa.eu/eurostat/databrowser/view/AEI_FM_SALPEST09__custom_3612747/default/table?lang=en, (accessed 18 October 2022).

323 N. Bekarian, D. Payne-Sturges, S. Edmondson, B. Chism and T. J. Woodruff, , DOI:10.1186/1476-069X-5.

324 CSO, Farm Structure Survey, https://www.cso.ie/en/releasesandpublications/ep/p-fss/farmstructuresurvey2016/da/lu/, (accessed 14 May 2022).

325 CSO, *Fact Sheet on Irish Agriculture (January 2021)*, 2021.

326 FAOSTAT, Utilised agricultural area of Ireland, https://www.fao.org/faostat/en/#data/RL, (accessed 18 October 2022).

327 L. O'Sullivan, R. E. Creamer, R. Fealy, G. Lanigan, I. Simo, O. Fenton, J. Carfrae and R. P. O. Schulte, *Land use policy*, 2015, **47**, 42–54.

328 FAOSTAT, Pesticides usage of Ireland, 2016-2020, https://www.fao.org/faostat/en/#data/RP, (accessed 18 October 2022).

329 Eurostat, EU Pesticides Sales, https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do, (accessed 25 May 2020).

330 A. López-Ballesteros, A. Delaney, J. Quirke, J. C. Stout, M. Saunders, J. C. Carolan, B. White and D. A. Stanley, *PeerJ*, 2022, **10**, 1–21.

331 S. Daouk, P. J. Copin, L. Rossi, N. Chèvre and H. R. Pfeifer, *Environ Toxicol Chem*, 2013, **32**, 2035–2044.

332 S. Guilherme, M. A. Santos, I. Gaivão and M. Pacheco, *Environmental Science and Pollution Research*, 2014, **21**, 8730–8739.

333 EU, Draft Commission Implementing Regulation concerning the non-renewal of the approval of the active substance chlorothalonil, https://ec.europa.eu/growth/tools-databases/tbt/en/search/?tbtaction=search.detail&Country_ID=EU&num=625&dspLang=en&basdatedeb=&basdatefin=&baspays=&basnotifnum=&basnotifnum2=&bastypepays=ANY&baskeywords=chlorothalonil, (accessed 9 October 2022).

334 NPIC, *Lambda-Cyhalothrin*, 2001.

335 Arysta LifeScience, *LAMBDA EC/26/09/2013*, 2013.

336 T. Xu, D. G. Dyer, L. L. Mcconnell, S. Bondarenko, R. Allen and O. Heinemann, *Environ Toxicol Chem*, 2016, **35**, 311–321.

337 European Union, *Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin*, 2018, vol. 14.

338 Y. Epstein, G. Chapron and F. Verheggen, *Ambio*, 2022, **51**, 1764–1771.

339 M. Rexrode, M. Barrett, J. Ellis, M.-G. Patrick, A. Vaughan, J. Felkel and J. Melendez, *EFED risk assessment for the seed treatment of clothianidin 600FS on corn and canola*, 2003.

340 O. Lundin, M. Rundlöf, H. G. Smith, I. Fries and R. Bommarco, *PLoS One*, , DOI:10.1371/journal.pone.0136928.

341 J. D. Fernández-Bayo, R. Nogales and E. Romero, *J Agric Food Chem*, 2009, **57**, 5435–5442.

342 J. M. Bonmatin, · I Moineau, · R Charvet, · M E Colin, · C Fleche and · E R Bengsch, in *Environmental Chemistry*, 2005, pp. 483–494.

343 P. Jeschke and R. Nauen, in *Comprehensive Molecular Insect Science*, 5th edn., 2005, pp. 53–105.

344 P. R. Durkin, *Fluroxypyr Human Health and Ecological Risk Assessment*, 2009.

345 A. M. Henderson, J. A. Gervais, B. Luukinen, K. Buhl, D. Stone, A. Cross and J. Jenkins, *Glyphosate General Fact Sheet*, 2010.

346 U. S. EPA, *R.E.D Facts (MCPA)*, 2004.

347 FAO, *Tribenuron-Methyl*, 2019.

348 D. PubChem, Tribenuron-methyl, CID=153909, https://pubchem.ncbi.nlm.nih.gov/compound/Tribenuron-methyl.

349 U. S. EPA, *U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC*, 2014.

350 U. S. EPA, *R.E.D. FACTS Pesticide (Chlorothalonil)*, 1999.

351 A. P. and V. M. A. APVMA, *Canberra: Australian Pesticides and Veterinary Medicines Authority*, 2007, 17.

352 U. S. EPA, *United States Environmental Protection Agency*, 2007, 1–55.

353 U. S. EPA, *Pesticide Fact Sheet (Epoxiconazole)*, 2006.

354 D. PubChem, Epoxiconazole, CID=3317081, https://pubchem.ncbi.nlm.nih.gov/compound/Epoxiconazole#section=Mass-Spectrometry, (accessed 22 July 2019).

355 C. C. Kalebich, M. E. Weatherly, K. N. Robinson, G. M. Fellows, M. R. Murphy and F. C. Cardoso, *Anim Feed Sci Technol*, 2017, **225**, 38–53.

356 R. Zaluski, L. A. Justulin and R. D. O. Orsi, *Sci Rep*, , DOI:10.1038/s41598-017-15581-5.

357 U. S. EPA, *United States Environmental Protection Agency*, 2001.

358 FAO, *FAO Specifications and Evaluations for Agricultural Pesticides (Prochloraz)*, 2006.

359 C. Sieke, *Lambda-Cyhalothrin (146)*, 2007.

360 N. P. I. C. NPIC, *Cypermethrin*, 1998.

361 U. S. EPA, *Reregistration Eligibility Decision (RED) (Cypermethrin)*, 2008.

362 A. P. and V. M. A. APVMA, *Exposure and Risk Assessment Including Occupational Health and Safety Assessment of Dimethoate*, 2012.

363 EFSA, *EFSA Journal*, , DOI:10.2903/j.efsa.2014.3873.

364 U. S. EPA, *U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC*, 2008.

365 R. Prusty, P. Grisafi and G. R. Fink, *Proc Natl Acad Sci U S A*, 2004, **101**, 4153–4157.

366 BLM, *Fluroxypyr Ecological Risk Assessment Final*, 2014.

367 H. Cederlund, E. Börjesson, E. Jonsson and T. Thierfelder, *Journal of Environment Quality*, 2012, **41**, 1884.

368 J. P. Gao, J. Maguhn, P. Spitzauer and A. Kettrup, *Water Res*, 1998, **32**, 1662–1672.

369 M. Kah and P. C. D. Brown, *Environment Department*, 2007, **PhD**, 228.

370 L. M. Pastrana-Martínez, M. V. López-Ramón and C. Moreno-Castilla, *J Colloid Interface Sci*, 2009, **331**, 2–7.

371 H. Jung, K. D. Sohn, B. Neppolian and H. Choi, *J Hazard Mater*, 2008, **150**, 809–817.

372 N. Pang, Y. Cui and J. Hu, *Chemosphere*, 2016, **165**, 320–328.

373 A. Borowik and J. Wyszkowska, *Plant Soil Environ*, 2016, **62**, 250–255.

374 G. Ojeda, J. Patrício, H. Navajas, L. Comellas, J. M. Alcañiz, O. Ortiz, E. Marks, T. Natal-da-Luz and J. P. Sousa, *Applied Soil Ecology*, 2013, **64**, 77–83.

375 A. Orgiazzi, C. Ballabio, P. Panagos, A. Jones and O. Fernández-Ugalde, *Eur J Soil Sci*, 2018, **69**, 140–153.

376 D. de Brogniez, C. Ballabio, A. Stevens, R. J. A. Jones, L. Montanarella and B. van Wesemael, *Eur J Soil Sci*, 2015, **66**, 121–134.

377 Ireland - Climatology | Climate Change Knowledge Portal, https://climateknowledgeportal.worldbank.org/country/ireland/climate-data-historical, (accessed 11 October 2022).

378 T. Ramesh, N. S. Bolan, M. B. Kirkham, H. Wijesekara, M. Kanchikerimath, C. Srinivasa Rao, S. Sandeep, J. Rinklebe, Y. S. Ok, B. U. Choudhury, H. Wang, C. Tang, X. Wang, Z. Song and O. W. Freeman, in *Advances in Agronomy*, Academic Press Inc., 2019, vol. 156, pp. 1–107.

379 J. Tian, N. He, L. Hale, S. Niu, G. Yu, Y. Liu, E. Blagodatskaya, Y. Kuzyakov, Q. Gao and J. Zhou, *Funct Ecol*, 2018, **32**, 61–70.

380 G. Bongiorno, E. K. Bünemann, L. Brussaard, P. Mäder, C. U. Oguejiofor and R. G. M. de Goede, *Applied Soil Ecology*, 2020, **154**, 103596.

381 N. S. Nomura and H. W. Hilton, *Weed Res*, 1977, **17**, 113–121.

382 R. P. Bennicelli, A. Woliñska, Z. Stêpniewska and M. Bogudziñska, *Int Agrophys*, 2009, **23**, 117–122.

383 T. R. Robert, in *Metabolic Pathways of Agrochemicals: Part 1: Herbicides and Plant Growth Regulators*, eds. T. R. Robert, D. H. Hutson, P. W. Lee, P. H. Nicholls and J. R. Plimmer, The Royal Society of Chemistry:Cambridge, UK, 1998, pp. 383–404.

384 P. H. Nicholls and A. A. Evans, *Pestic Sci*, 1991, **33**, 331–345.

385 K. G. I. D. Kumari, P. Moldrup, M. Paradelo, L. Elsgaard and L. W. de Jonge, *Water Air Soil Pollut*, , DOI:10.1007/s11270-016-2867-2.

386 C. P. M. Bento, X. Yang, G. Gort, S. Xue, R. van Dam, P. Zomer, H. G. J. Mol, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2016, **572**, 301–311.

387 K. Grunewald, W. Schmidt, C. Unger and G. Hanschmann, *Journal of plant nutrition and soil science*, 2001, **164**, 65–70.

388 A. J. Al-Rajab, S. Amellal and M. Schiavon, *Agron Sustain Dev*, 2008, **28**, 419–428.

389 R. Schroll, H. H. Becher, U. Dörfler, S. Gayler, S. Grundmann, H. P. Hartmann and J. Ruoss, *Environ Sci Technol*, 2006, **40**, 3305–3312.

390 K. B. Liphadzi, K. Al-Khatib, C. N. Bensch, P. W. Stahlman, J. A. Dille, T. Todd, C. W. Rice, M. J. Horak and G. Head, *Weed Sci*, 2005, **53**, 536–545.

391 H. Zhan, Y. Feng, X. Fan and S. Chen, *Appl Microbiol Biotechnol*, 2018, 102, 5033–5043.

392 R. E. Dick and J. P. Quinn, *FEMS Microbiol Lett*, 1995, **134**, 177–182.

393 J. Ascolani Yael, J. D. Fuhr, G. A. Bocan, A. Daza Millone, N. Tognalli, M. Dos Santos Afonso and M. L. Martiarena, *J Agric Food Chem*, 2014, **62**, 9651–9656.

394 K. A. Lewis, J. Tzilivakis, D. J. Warner and A. Green, *Human and Ecological Risk Assessment*, 2016, **22**, 1050–1064.

395 C. Cosemans, N. van Larebeke, B. G. Janssen, D. S. Martens, W. Baeyens, L. Bruckers, E. den Hond, D. Coertjens, V. Nelen, G. Schoeters, H. W. Hoppe, E. Wolfs, K. Smeets, T. S. Nawrot and M. Plusquin, *Int J Hyg Environ Health*, , DOI:10.1016/j.ijheh.2021.113895.

396 L. Simonsen, I. S. Fomsgaard, B. Svensmark and N. H. Spliid, *J Environ Sci Health B*, 2008, **43**, 365–375.

397 X. Yang, F. Wang, C. P. M. Bento, L. Meng, R. van Dam, H. Mol, G. Liu, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2015, **530–531**, 87–95.

398 E. Capri and A. Vicari, *Environmental fate and behaviour of glyphosate and its main metabolite*, 2010.

399 R. Annett, H. R. Habibi and A. Hontela, *Journal of Applied Toxicology*, 2014, **34**, 458–479.

400 D. la Cecilia and F. Maggi, *Environmental Pollution*, 2018, **233**, 201–207.

401 P. Sidoli, N. Baran and R. Angulo-Jaramillo, *Environmental Science and Pollution Research*, 2016, **23**, 5733–5742.

402 C. M. Benbrook, *Environ Sci Eur*, 2016, **28**, 3.

403 EFSA, *EFSA Journal*, 2011, **9**, 1–91.

404 EFSA, *EFSA Journal*, , DOI:10.2903/j.efsa.2008.138r.

405 PubChem, Prothioconazole | C14H15Cl2N3OS, https://pubchem.ncbi.nlm.nih.gov/compound/Prothioconazole#section=Octanol-Water-Partition-Coefficient, (accessed 28 May 2020).

406 J. Zhang, L. Li, Q. Lv, L. Yan, Y. Wang and Y. Jiang, *Front Microbiol*, 2019, 10.

407 JMPR, *Prothioconazole*, .

408 S. L. Kelly, D. C. Lamb, C. J. Jackson, A. G. S. Warrilow and D. E. Kelly, *Adv Microb Physiol*, 2003, **47**, 131–186.

409 H. F. Lin, B. Dong and J. Y. Hu, *Environ Monit Assess*, 2017, **189**, 1–9.

410 Bayer, 2010, 0–12.

411 Z. Zhang, B. Gao, L. Li, Q. Zhang, W. Xia and M. Wang, *Science of the Total Environment*, 2018, **634**, 875–883.

412 J. E. Parker, A. G. S. Warrilow, H. J. Cools, B. A. Fraaije, J. A. Lucas, K. Rigdova, W. J. Griffiths, D. E. Kelly and S. L. Kelly, *Appl Environ Microbiol*, 2013, **79**, 1639–1645.

413 Z. Zhang, J. Zhang, X. Zhao, B. Gao, Z. He, L. Li, H. Shi and M. Wang, *J Hazard Mater*, , DOI:10.1016/j.jhazmat.2020.122756.

414 EFSA, *EFSA Journal*, , DOI:10.2903/j.efsa.2007.106r.

415 U. S. Epa, 2005, 1–5.

416 D. Klingelhöfer, M. Braun, D. Brüggmann and D. A. Groneberg, *Environ Res*, 2022, 213.

417 S. W. Page, in *Small Animal Clinical Pharmacology*, Elsevier Ltd, Second Edi., 2008, pp. 198–260.

418 K. Banerjee, S. H. Patil, S. Dasgupta, D. P. Oulkar and P. G. Adsule, *J Environ Sci Health B*, 2008, **43**, 151–156.

419 P. Zhang, C. Ren, H. Sun and L. Min, *Science of the Total Environment*, 2017, **615**, 59–69.

420 J. Bonmatin, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. D. Mitchell, D. A. Noome, N. Simon-Delso and A. Tapparo, *Environmental Science and Pollution Research*, 2015, **22**, 35–67.

421 S. A. Todey, A. M. Fallon and W. A. Arnold, 2018, **37**, 2797–2809.

422 F. Flores-Céspedes, E. González-Pradas, M. Fernández-Pérez, M. Villafranca-Sánchez, M. Socías-Viciana and M. D. Ureña-Amate, *J Environ Qual*, 2002, **31**, 880–888.

423 E. Dankyi, C. Gordon, D. Carboo, V. A. Apalangya and I. S. Fomsgaard, *J Environ Sci Health B*, 2018, **53**, 587–594.

424 R. Karmakar, S. B. Singh and G. Kulshrestha, *Bull Environ Contam Toxicol*, 2006, **76**, 400–406.

425 S. Gupta, V. T. Gajbhiye and R. K. Gupta, *Bull Environ Contam Toxicol*, 2008, **80**, 431–437.

426 G. Hu, Y. Zhao, B. Liu, F. Song and M. You, *J Microbiol Biotechnol*, 2013, **23**, 1617–1626.

427 S. S. Phugare and J. P. Jadhav, *Clean (Weinh)*, 2015, **43**, 296–304.

428 N. S. Shaikh, S. v Kulkarni, M. S.Mulani and ,Ulfat I Baig, *Int J Innov Res Sci Eng Technol*, 2014, **03**, 16301–16307.

429 A. Jones, P. Harrington and G. Turnbull, *Pest Manag Sci*, 2014, **70**, 1780–1784.

430 S. Humann-Guilleminot, Ł. J. Binkowski, L. Jenni, G. Hilke, G. Glauser and F. Helfenstein, *Journal of Applied Ecology*, 2019, **56**, 1502–1514.

431 M. A. Ali and P. J. Baugh, *Int J Environ Anal Chem*, 2003, **83**, 923–933.

432 V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2019, **653**, 1532–1545.

433 J. McGinley, J. Harmon O'Driscoll, M. G. Healy, P. C. Ryan, P. E. Mellander, L. Morrison, O. Callery and A. Siggins, *Soil Use Manag*, 2022, **38**, 1162–1171.

434 M. Alexandra and D. Rivadeneira, 2021.

435 R. Tobin, *J Nutrit Health Food Sci*, , DOI:10.15226/jnhfs.2014.00113.

436 S. Kavanagh, M. Henry, J. C. Stout and B. White, *Environmental Science and Pollution Research*, 2021, 28179–28190.

437 S. L. McManus, C. E. Coxon, P. E. Mellander, M. Danaher and K. G. Richards, *Science of the Total Environment*, 2017, **601–602**, 594–602.

438 L. Jones, B. Kinsella, K. Forde, A. Furey and F. Regan, *Analytical Methods*, 2017, **9**, 4167–4174.

Chapter 2 :

Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke extraction methods

Abstract

The expanding nature of the agricultural sector has fuelled the intensification of plant protection products usage, including pesticides. These pesticides may persist in soils, necessitating their accurate determination in a variety of soil types. However, due to their complex nature, the effective extraction of pesticide residues from soil matrices can present challenges to pesticide detection and quantification. This research compared two well-known extraction methods, QuEChERS and Dutch mini-Luke, by assessing their specificity, sensitivity, accuracy, precision and reproducibility in extracting seven distinct pesticides with a range of chemico-physical characteristics from Irish soils. The HPLC-UV conditions were optimised to separate the seven pesticides, and it was shown that both extraction methods successfully extracted neonicotinoids with recovery values ranging between 85 to 115%. Fluroxypyr and prothioconazole could not be efficiently extracted using QuEChERS, however, the recovery values of both the analytes ranged between 59 to 117% using Dutch mini-Luke. Furthermore, with the exception of prothioconazole using Dutch mini-Luke, both extraction methods resulted in reproducibility and precision values below or equal to 20%. Lastly, Dutch mini-Luke is noted to have a lower matrix effect than QuEChERS, except for prothioconazole. The comparison results showed that Dutch mini-Luke resulted in superior method sensitivity, better recovery, and lower matrix effect towards most investigated analytes and was the only extraction technique that successfully extracted all pesticides analysed in soil matrices.

2.1 Introduction

Pesticide usage in the agriculture sector is prevalent as plant protection products are considered integral to increasing agricultural productivity food production.[1,2] It is estimated that in 2019, the global usage of pesticides is approximately 2 million tonnes annually, with an approximate increase of 75% the following year.[3] In Europe alone, pesticide sales volume was 333 418 tonnes in 2019.[4] It is projected that in 2050, the world population will increase to between 9.4 and 10.1 billion people, and arable land use and pesticide application will likely increase accordingly. With extensive and increasing agrochemical use and increased prevalence of crop pests and diseases in combination with inappropriate pesticide use, there exists considerable potential for environmental pollution. The unintended fate of those polluting pesticide compounds occurs through numerous simultaneous transfers, including spray drift,[5,6] surface runoff,[7–9] volatilisation,[10,11] degradation and leaching.[12–14] Even if the pesticide transfer in the environment does not occur during the application of the chemicals, some studies show that pesticides can give rise to contamination through non-direct processes, such as the generation of airborne clouds of dust during the sowing of pesticide coated seeds,[15–17] or during litter breakdown, where pesticide residues, systemically persistent in the plant material are released into the environment.[18,19] As most applied chemicals end up settling on the soil, pesticide residues can get deposited and persist in the soil layers.[20,21] The persistence of pesticide residue depends on how strongly they adsorb to the available soil sorbents, and there are typically three available soil sorbents; soil organic matter, metal-(oxyhydrl)oxide, and clay.[22–24] Clay and organic matter make up the major constituents of soil,[22,25,26] and are associated with numerous functional groups,

such as carbonyl, amino, imidazole, sulfonic, sulfhydryl, carboxyl, inorganic hydroxyl, and siloxane ditrigonal cavities, which increases the affinity of pesticide residues to chemically adsorb to soil components.[13] Once adsorbed, these pesticide residues are usually excluded from further degradation or transport through the environment.[20,21] However, fluctuations of biotic and abiotic factors in the soil environment could induce desorption of the adsorbed pesticide back into the environment again.[27–29] Following their desorption, these pesticide residues can be transported around the environment through multiple routes.

In order for thorough assessments of the levels of chemical residues and their persistence in the soil to be conducted, reproducible and robust methods and protocols for extracting and identifying pesticides are required. However, pesticide residue extraction from the soil remains challenging, and the quantification of persisting pesticide residues is difficult due to the complex interactions between the soil sorbents and the sorptive pesticide compounds.[10,30] A number of extraction methods have proven effective in extracting pesticides from soils, namely single-drop and liquid microextraction,[31] supercritical and pressurised liquid extraction (PLE),[32] microwave-assisted extraction (MAE),[33] solid-phase microextraction (SPME),[34] sorptive-phase extraction,[35] hollow-fibre membrane solvent microextraction,[36] ultrasonic solvent extraction (USE),[37] and Soxhlet extraction.[38] However, these methods have associated disadvantages, including considerable time requirements in their set up, excessive solvent consumption or limited success for specific compounds.[39] The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which significantly simplified pesticide extraction, was welcomed by modern analytical labs to overcome these issues. Anastassiades *et al.* first developed the QuEChERS

extraction method in 2003 to analyse pesticide residues in food products,[40] but since then, it has been adapted to extract pesticides from many other matrices.[41–46] The QuEChERS method is not the only economised version of the previously described extraction methods, but it also has more environmentally friendly procedures that align with current green chemistry and analytical ethics. In addition, this method allows for large sample throughput and consistent reproducibility with high recoveries of the broad spectrum of compounds.[39] QuEChERS extraction only requires three steps to obtain pesticide residue extracts; partitioning, salting-out, and clean-up.[40,47,48]

One of the main challenges associated with the analytical analysis of environmental samples, particularly soils, is the interference encountered in the form of matrix effects. Based on the European Guidelines document (SANTE),[49] the matrix effect can be unfamiliar to the analytical system adopted, inconsistent in presence and intensity, and may not be obvious. As it affects method selectivity and sensitivity, in the form of enhancement of suppression effect on the detection system response, eliminating matrix effects during pesticide residue analysis is critical. A complex matrix like soil requires robust clean-up steps to remove any co-eluting compounds, which are often quite numerous in the soil matrix, whilst still ensuring a reproducible and effective extraction of analytes of interest. The clean-up steps in QuEChERS extraction involve dispersive solid-phase extractions (d-SPE), involving three of the most widely used solid sorbents; primary and secondary amine (PSA), C_{18}, and graphitised carbon black (GCB).[40] Usage of these solid sorbents aids the removal of contaminants and prevents unwanted co-extractants from the matrix, where the addition of PSA enhances the removal of polar compounds, such as sugars, organic acids, and fatty acids;

C_{18} removes lipids, sterols, and other non-polar compounds; and GCB removes pigments.[40,50] Even though these clean-up components are crucial, QuEChERS does not have one fixed procedure for eliminating matrix effects from different matrices. Usage of d-SPE cleaning components in any QuEChERS extraction requires additional optimisation study. An inclusion of d-SPE during clean-up steps requires consideration of their suitability to the analytes of interest,[39,51,52] the particular matrix,[40,53] quantity of d-SPE required,[54,55] the combinations of d-SPE to be used,[39,56] and standing time for the mixture of d-SPE components in sample extracts.[57] Only then can the most appropriate clean-up procedure that maximises the efficiency of pesticide residue recovery be selected.

Compared to d-SPE based clean-up of QuEChERS, dissolution using liquid/liquid extraction methods employed through Dutch mini-Luke[58,59] is one of the oldest and most effective means of reducing matrix effects. Despite liquid/liquid partitioning disadvantages, namely the higher volumes of solvents and waste, the Dutch mini-Luke extraction provides relatively cleaner extracts even without additional clean-up step.[58,60]. Additionally, as Dutch mini-Luke uses a combination of acetone/petroleum ether/dichloromethane (v/v 1/1/1), it presents a lower co-extractive concentration than acetonitrile and ethyl acetate. Lower concentrations of co-extractives result in fewer contaminants being introduced to the instrument systems.[61] Given that there is no requirement for clean-up step optimisation, Dutch mini-Luke represents a robust extraction method that can be successfully employed on multiple matrices effectively without the additional modification.

We present here a systematic comparison of the QuEChERS and Dutch Mini-Luke extraction methods for the quantification of multiple classes of pesticides

from blank soils through fortified recovery experiments using High-Performance Liquid Chromatography (HPLC) coupled with ultraviolet detection. The extraction methods were fully validated and evaluated based on extraction efficiency, limits of detection and quantification, and pesticide recoveries. The target analytes which included five insecticides, one herbicide, and one fungicide, were selected based on their abundance in pesticide usage records for Ireland. To our knowledge, a comparison between QuEChERS and the Dutch mini-Luke pesticide extraction method for soil matrix has not been reported. The comparison provided the most effective way for a single mixed extraction and analysis of widely used pesticides in soil.

2.2 Materials and methods

2.2.1 Reagents and materials

HPLC grade acetonitrile (MeCN), HPLC grade methanol (MeOH), HPLC grade dichloromethane (DCM), HPLC grade acetone, HPLC grade ethyl acetate, reagent grade MeOH, formic acid 98%, acetic acid (HAc) 100%, ammonium formate, anhydrous sodium sulphate, citrate salt extraction tube (sodium chloride: 1 g, sodium citrate dibasic sesquihydrate: 0.5 g, sodium citrate tribasic dihydrate: 1 g), primary-secondary amine (PSA), anhydrous magnesium sulphate ($MgSO_4$), and the certified reference standards, all of >97% purity, of acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, fluroxypyr, prothioconazole and the internal standard of triphenyl phosphate were purchased from Sigma-Aldrich (Ireland). A 25 kg pack of sand of 50-70 mesh particle size were obtained from Lennox (Ireland). HPLC grade petroleum ether was obtained from Fisher Scientific (Ireland). Millipore Millex syringe filters with hydrophilic PTFE membrane (pore size 0.22 µm and 20 mm diameter) and 1.5 mL

autosampler vials were purchased from Sigma-Aldrich. The ultrapure water, deionised to a resistance of < 18 MOhm, used throughout the study was generated using ELGA Purelab Ultra SC MK2 (ELGA, UK).

2.2.2 Preparation of standard solutions

Individual stock standard solutions (1000 ng μL^{-1}) of acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, fluroxypyr, prothioconazole and the internal standard of triphenyl phosphate (TPP) were prepared monthly in HPLC grade acetonitrile and stored glass vials in -20^{o}C. The working standard solutions (10 ng μL^{-1}) were prepared by diluting in 25% HPLC grade MeOH in ultrapure water.

2.2.3 Collection of blank soil samples

Blank soil samples were collected from the Dublin City University (DCU) community garden, a small-scaled pesticide use free biologically intensive vegetable farm. The same extraction methods were performed on the blank soil samples to ensure there was no potential interference.

2.2.4 Soil sample preparation

The surface soil samples were collected at 15 cm to 20 cm depths. The collected blank soil samples were air-dried for 24 h at room temperature, ground and sieved through a 1 mm mesh to remove any plant roots, rocks, etc. Prepared soil samples were then stored in zip-lock bags at -4^{o}C until analysis.

2.2.5 Extractions

2.2.5.1 QuEChERS Extraction

Five grams of each soil sample were weighed into 50 mL PTFE centrifuge tubes and spiked at the required fortification level of pesticide standard solution. Then the centrifuge tube was hand-shaken for homogeneous mixing of the pesticide standard and the soil and left standing for 45 min in a fume hood. After 45 min, 5 mL of deionised water was added to the mixture to hydrate the soil, followed by 10 mL acetonitrile with 1% acetic acid. The mixture then was shaken vigorously for 1 min and sonicated for the next 10 min. Following sonication, the citrate salt mixture (sodium chloride: 1g, sodium citrate dibasic sesquihydrate: 0.5g, sodium citrate tribasic dihydrate: 1g) was added into the centrifuge tube. The centrifuge tube was then vortexed for 1 min before being centrifuged at 4000 rpm for 10 min. Nine mL of supernatant was transferred to a 15 mL PTFE centrifuge tube containing 300 mg PSA and 900 mg $MgSO_4$. The extract was vortexed for 1 min, followed by 10 min centrifugation at 4000 rpm. 5.0 mL of supernatant was then transferred into a silanised glass vial and then concentrated to dryness under a gentle stream of nitrogen. The concentrated extract was then reconstituted in 500 µL of mobile phase solution. Finally, the extract was filtered through a 0.22 µm hydrophilic PTFE syringe filter into an autosampler vial for HPLC-UV analysis.

2.2.5.2 Dutch mini-Luke Extraction

15 g of blank soil samples were weighed into 250 mL PTFE centrifuge tubes and spiked at the required fortification level of pesticide standard solution. Subsequently, 15 mL of deionised water was added and shaken vigorously for 1 min, followed by adding of 30 mL of 1% acetic acid in acetone, and the mixture was then homogenised using IKA Ultra-Turrax T-25 homogeniser for 30 s at 1500 rpm. 30 mL petroleum ether and 30 mL dichloromethane were added to the homogenate, and the sample mixture was homogenised again using the homogeniser for 30 s at 1500 rpm to induce phase separation. After centrifugation at 4000 rpm for 10 min, 60 mL of the obtained supernatant was carefully transferred into a 100 mL conical flask. The extracts were evaporated to reduce the volume before being transferred to a 10 mL volumetric flask made up to volume with ethyl acetate. Following that, 0.5 mL of the ethyl acetate was then diluted into 10 mL volumetric flask topped up with methanol. Finally, 1 mL of methanol extract then filtered through 0.22 µm hydrophilic PTFE syringe filter into an autosampler vial for HPLC-UV analysis.

2.2.6 HPLC-UV analysis

The HPLC-UV analysis was carried out using the Shimadzu SPD-20A Prominence HPLC system coupled with a UV-Vis detector (Tokyo, Japan), set at a wavelength of 254 nm. The chromatographic separation was performed using an XBridge UPLC BEH column (4.6x100 mm i.d., 3.5 µL 3.9 X 5 mm). The analytes were separated using a gradient of 5 mM ammonium formate with 0.1% formic acid in ultrapure water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The mobile phase gradient was as follows: 10% B from 0 to 0.5 min; a gradient increase to 98% B in 28 min; composition maintained

at 98% B for 4 min, followed by returning to the starting mobile phase and re-equilibration time for 4 min. The flow rate used was kept constant at 0.5 mL min^{-1}. The injection volume was 10 µL.

2.2.7 Parameters of method validation and comparison

2.2.7.1 Specificity

Method specificity was validated by analysing blank soil samples (n = 3) to determine whether any interference was occurring for any of the targeted analytes.

2.2.7.2 Linearity

Pesticide standards were injected individually at seven concentrations incrementally. The lowest point of concentration for each analyte was the method quantification limit (MQL). The linearity of each of the targeted analytes was measured based on their response in the solvent and soil calibration ranges (matrix-matched calibration).

2.2.7.3 Accuracy (recovery%) and precision (RSD%)

Recovery and precision assessment were carried out by fortifying blank samples at three concentration levels, MQL, five times MQL and ten times MQL, for three replicates. Recoveries between 70-120% with RSD% lower than 20% were considered satisfactory.[49] Recoveries of the three concentrations were further evaluated using multiple unpaired t-tests of an analyte between both extraction methods to determine significant differences between the means of an analyte's recoveries. The method precision was validated in terms of reproducibility and repeatability, represented by the relative standard deviation (RSD%).

2.2.7.4 Method Detection Limit (MDL) and Method Quantification Limit (MQL)

MDL and MQL evaluated method sensitivity. The MDL was considered acceptable when the signal to noise ratio (S/N) was ≥3. MQL was deemed satisfactory when quantified with acceptable accuracy with the lowest fortification level when recoveries are between 70-120%, with RSD% lower than 20%. The significant difference of a respective analyte's MDL and MQL in both extraction methods was evaluated using multiple unpaired t-tests.

2.2.7.5 Matrix Effect (%)

Matrix effect (ME%) was calculated to assess the influence of co-extracted compounds from the soil on analytical signals. ME% were calculated based on equation 2.1, comparing the slopes in the matrix (Sm) (blank extracts) calibration solutions and the pure solvent (Ss) (in acetonitrile) calibration solutions.

$$ME (\%) = ((Sm/Ss) - 1) \times 100\%)$$

Equation 2.1

2.2.7.6 Quality control

Quality control was carried out by using TPP as an internal standard for each batch of sample analysis and fortified before each extraction to reach a concentration of 1 ng μL^{-1} in the final extract.

2.3 Results and discussion

2.3.1 Comparison of the procedural blanks for the two methods

A series of procedural blank sand and blank soil samples for the two extraction methods were performed to check for potential contamination or co-elutants respective to each extraction procedure. The inclusion of blank sand samples in each extraction method was to assess if any co-elutants arise from the extraction components themselves rather than from the soil matrix. These checks were performed to confirm no co-elutants from the blank samples and the extraction components eluted at the same retention time as the targeted analytes (Appendix A). Overall, the specificity of the method was confirmed, with no other contamination observed at the same retention time as the targeted compounds from all the blanks using the two extraction methods.

2.3.2 Comparison of method detection limit (MDL) and method quantification limit (MQL)

The linearity of the calibration curves was evaluated using seven procedural calibration points (ranging between 0.1 to 3 ng μL^{-1}), which were performed by spiking the blank samples before extraction. Correlation coefficients (r^2) were evaluated for both methods (Tables 2.1 and 2.2). For the QuEChERS extraction, the r^2 value for most analytes ranged between 0.901 and 0.939 in blank sand samples and between 0.914 and 0.961 in soil samples (Table 2.1). For both blank samples, the linearity of the fluroxypyr and prothioconazole could not be determined due to a failure to detect both analytes through the procedural calibration line. For the Dutch mini-Luke extraction, all targeted analytes were successfully resolved using a procedural calibration line, with r^2 values ranging between 0.938 and 0.991 and 0.934 and 0.992 for the sand and soil samples respectively (Table 2.2). Although the r^2

ranges obtained for both QuEChERS and Dutch mini-Luke were acceptable and allowed for the accurate determination of the MDL and MQL in both substrates, the failure to identify fluroxypyr and prothioconazole from the QuEChERS extracted samples highlights a limitation with this method in comparison to Dutch mini-Luke.

The sensitivity of each extraction method was assessed in terms of MDL and MQL, which were estimated based on the standard deviation of the response and slope of the constructed procedural calibration line. The QuEChERS extraction only successfully assessed the MDL and MQL for neonicotinoids in blank sand and soil samples. In the blank sand samples, QuEChERS extraction resulted in MDL values QuEChERS extracted blank sand samples ranged between 0.31 to 0.36 ng μL^{-1}, while the MQL ranged from 0.95 to 1.08 ng μL^{-1}. In the blank soil samples, QuEChERS resulted in MDL values ranging between 0.56 and 0.85 ng μL^{-1} and MQL values ranging between 1.7 and 2.58 ng μL^{-1}. On the other hand, Dutch mini-Luke extractions provided complete information on MDL and MQL for all the targeted analytes. In the blank sand samples, the MDL ranged between 0.18 to 0.48 ng μL^{-1} and the MQL ranged between 0.53 to 1.46 ng μL^{-1}, whereas in blank soil samples, the MDL of analytes ranged between 0.20 to 0.42 ng μL^{-1} and the MQL ranged between 0.60 to 1.27 ng μL^{-1}.

When the MDL and MQL values were compared, it was determined that Dutch mini-Luke allows the detection and quantification at lower pesticide concentration values than the QuEChERS method (Figure 2.1 and 2.2). Even though Dutch mini-Luke's MDL values for most analytes are observed to be lower, only the analytes acetamiprid and thiamethoxam records significant difference (p-value<0.01). On the other hand, the MQL value for all the neonicotinoids were

observed to be significantly different, with either p-value less than 0.01 or 0.001. As the MDL and MQL are estimated through the procedural calibration line, the value obtained could broadly vary from one method to another as it known to be affected by interference from the matrix.[62–64] In the specificity study, analysis of sand blank extract using QuEChERS extraction still shows the presence of co-elutants, while Dutch mini-Luke displays a baseline that indicates low to no presence of co-elutants. Considering, sand blanks should not have any possible elutants, indicating the elutants could be from the QuEChERS extraction component themselves. Hence, the lower MDL and MQL from Dutch mini-Luke ultimately translate to better method sensitivity.

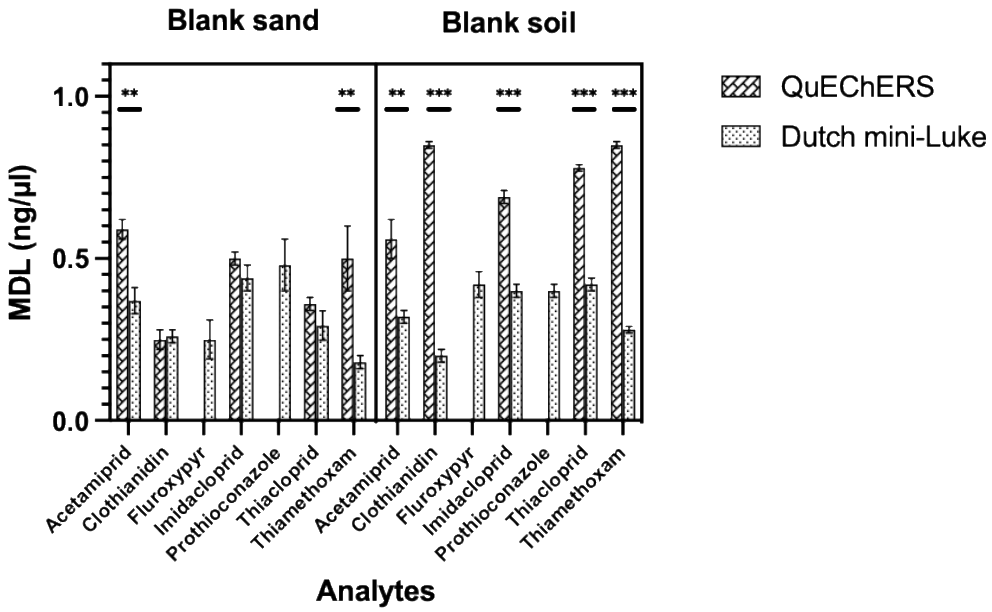


Figure 2.1. Comparison of MDL values obtained through QuEChERS and DML using blank sand and blank soil samples. Asterisks show statistical significance (**p < 0.01, ***p < 0.001)

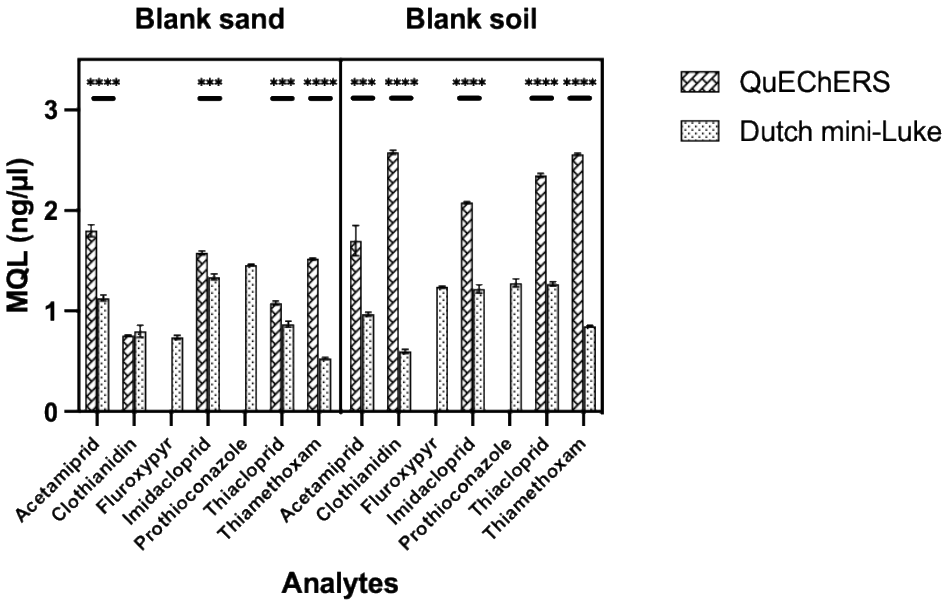


Figure 2.2. Comparison of MQL values obtained through QuEChERS and DML using blank sand and blank soil samples. Asterisks show statistical significance (***p < 0.001, ****p < 0.0001).

Table 2.1. QuEChERS range, correlation coefficient (r^2), MDL and MQL of targeted pesticides through procedural calibration line

Pesticides	Solvent				Sand				Soil			
	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})
Acetamiprid	0.05 – 3	0.999	0.13	0.39	0.1 -3	0.917	0.31	0.95	0.1 -1.5	0.961	0.56	1.7
Clothianidin	0.05 – 3	0.999	0.13	0.38	0.1 -3	0.901	0.32	0.97	0.1 -3	0.914	0.85	2.58
Fluroxypyr	0.1 – 3	0.999	0.22	0.68	ND	ND	ND	ND	ND	ND	ND	ND
Imidacloprid	0.05 – 3	0.999	0.18	0.54	0.1 -3	0.934	0.31	0.95	0.1 -0.7	0.942	0.69	2.08
Prothioconazole	0.05 – 3	0.999	0.15	0.44	ND	ND	ND	ND	ND	ND	ND	ND
Thiacloprid	0.05 – 3	0.999	0.13	0.39	0.1 -3	0.919	0.36	1.08	0.1 -1.5	0.927	0.78	2.35
Thiamethoxam	0.05 – 3	0.991	0.15	0.46	0.1 -3	0.939	0.31	0.95	0.5 - 3	0.915	0.85	2.56

Not Detected (ND)

Table 2.2. Dutch Mini Luke range, correlation coefficient (r^2), MDL and MQL of targeted pesticides through procedural calibration line

Pesticides	Solvent				Sand				Soil			
	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})
Acetamiprid	0.05 – 3	0.999	0.13	0.39	0.05-3	0.962	0.37	1.13	0.05 -2	0.961	0.32	0.97
Clothianidin	0.05 – 3	0.999	0.13	0.38	0.1-3	0.985	0.26	0.80	0.05 -3	0.992	0.20	0.60
Fluroxypyr	0.1 – 3	0.999	0.22	0.68	0.05-3	0.983	0.25	0.74	0.05-1.5	0.935	0.42	1.26
Imidacloprid	0.05 – 3	0.999	0.18	0.54	0.05-2	0.946	0.44	1.34	0.05 -1.7	0.939	0.40	1.22
Prothioconazole	0.05 – 3	0.999	0.15	0.44	0.05-2	0.938	0.48	1.46	0.5-1.7	0.934	0.42	1.26
Thiacloprid	0.05 – 3	0.999	0.13	0.39	0.05-3	0.977	0.29	0.87	0.05 -3	0.952	0.42	1.27
Thiamethoxam	0.05 – 3	0.999	0.15	0.46	0.05-3	0.991	0.18	0.53	0.05 - 3	0.978	0.28	0.85

2.3.3 Comparison of QuEChERS and Dutch Mini Luke recoveries

Recovery efficiency is a critical property of any extraction method as it signifies the methods' accuracy and performance. The recovery experiments were performed by fortifying blank soil and sand samples at three levels, corresponding to low, medium, and high concentrations of pesticide analytes in the soil, based on the MQL values calculated from the linearity. Pesticide recovery was reported as a percentage of the spiked concentration. Both blank soil and sand samples were fortified at the same concentration levels to be comparable. Based on the SANTE guidelines, acceptance criteria of the validation parameters of the method should have an average recovery in the range of 70-120% with RSD% less or equal to 20%.[49] Three different fortification concentrations, the MQL, five times MQL, and ten times MQL, were chosen for each extraction method. The fortification at these three concentrations levels gives a complete evaluation of the method's robustness in efficiently recovering all the targeted analytes over a range of concentrations.

Evaluation of the extraction efficiencies achieved using QuEChERS shows that the recovery percentage from the blank sand matrix had a slightly higher or similar recovery percentage than the recovery in blank soil samples. Recovery of the neonicotinoids from the blank sand and blank soil samples were deemed satisfactory, with recovery values of 85 to 111% (Fig. 2.3). However, the analytes fluroxypyr and prothioconazole were not detected at any of the three fortified levels. Comparing the recovery of analytes in the blank soil, at MQL level, Dutch mini-Luke's extraction method is observed to have significantly higher recovery percentages for all the neonicotinoids compared to QuEChERS, where the multiple unpaired t-tests depict comparison p-value is either between 0.05 or

0.01. Meanwhile, the fortification at five times MQL level in blank soil, only acetamiprid and thiamethoxam were noted to have significantly different recovery percentages ($p<0.05$), and Dutch mini-Luke performed consistently better in the recovery of neonicotinoids from blank soil samples at ten times MQL, with clothianidin, imidacloprid, thiacloprid, and thiamethoxam noted to have significant difference of either $p<0.05$ or $p<0.01$ (Fig. 3).

The failure of QuEChERS to extract detectable fluroxypyr and prothioconazole from the blank soil samples could be due to the presence of organic matter and clay components. The amounts of organic matter and clay in soil matrices are directly proportional to the adsorption of pesticide analytes.[65–67] Fluroxypyr and prothioconazole have log K_{OW} values of 2.20,[68,69] and 4.05,[70,71], respectively, which indicates their greater affinity to organic matter. Therefore, it can be assumed that both residues are adsorbed strongly to the organic matter or clay components in the blank soil samples as soon as they are spiked. Hence, sample preparation remains a crucial step in any extraction method, which presents a challenge for the QuEChERS extraction to trigger the desorption of pesticide analytes from soil constituents. In addition, the soil is a complex matrix that requires extra attention on the clean-up step during an extraction. Hence, the clean-up step in QuEChERS extraction on soil matrix is essential for removing any co-extractants that might also have been extracted. As much as dispersive SPE (d-SPE), namely PSA, is crucial for the matrix clean-up step, it can inhibit the recovery of analytes. Sack *et al.* had shown that PSA inclusion during sample clean-up during acidic pesticide analysis increases the loss of free acids.[52] The results of this study support this finding as we failed to quantify fluroxypyr and prothioconazole using QuEChERS extraction, and it seems that using d-SPE

comes with a trade-off between obtaining a cleaner extract and comprehensive analyte recovery.

In contrast to QuEChERS, the Dutch mini-Luke extraction successfully extracted fluroxypyr and prothioconazole with recovery efficiency values between 59 to 117% and good recovery values (between 102 and 115%) were obtained for the neonicotinoids. The Dutch mini-Luke extraction method also has better recovery efficiencies across all the targeted analytes in comparison to QuEChERS (Fig. 3). Even though, under the SANTE guidelines, fluroxypyr extracted using Dutch mini-Luke with MQL fortification concentration level gives a lower percentage (59%) than the acceptance criteria (70-120%), it still outperforms QuEChERS where fluroxypyr was not quantified at all. Compared to QuEChERS, Dutch mini-Luke has additional advantages. Dutch mini-Luke not only includes mechanical energy through high rpm homogeniser, but it also provides chemical energy, with the inclusion of higher organic solvents such as acetone, dichloromethane, and petroleum ether. Mechanical grinding in immiscible organic solvents breaks the soil constituents into much smaller particles, exposing more extensive surface area for extraction, which helps to expose the adsorbed pesticide analytes in the humic substances or the inter-crystalline layers of clay minerals, subsequently breaking their bonds and improving partitioning into the organic phase.[72]

Furthermore, fluroxypyr and prothioconazole with pKa values of 2.94,[73] and 6.9,[74] respectively, are most stable at low pH values. With this in mind, both of these analytes are expected to be extracted most efficiently using acidified solvents.[75] While salt is required to extract polar analytes, such as neonicotinoids, it inhibits the extraction of fluroxypyr and prothioconazole. QuEChERS cannot offer flexible modification towards the pH of extraction solvents without sacrificing the overall

performance of multi-class pesticide extraction. However, even when using acidified solvents, such as 1% acetic acid in acetonitrile (as used in this study), fluroxypyr and prothioconazole were still not extracted using QuEChERS. The usage of PSA during QuEChERS d-SPE clean-up is crucial as PSA helps to efficient removal of sugars, pigments, and organic acids.[76]

However, as Lehotay *et al.* had shown, the usage of the PSA clean-up step during extraction decreases the acidity of the extracts by 2-3 pH units.[75] This drastic change in pH could lead to loss of fluroxypyr and prothioconazole through degradation or be retained, as PSA interacts with both labile acidic analytes. This reasoning is supported by published studies demonstrating that the recovery of acidic analytes' improves when PSA is not used for clean-up.[44,77,78]

2.3.4 Comparison of QuEChERS and Dutch Mini Luke reproducibility

Due to the failure to quantify fluroxypyr and prothioconazole using QuEChERS, the RSD% of both these analytes were unsuccessfully measured. However, QuEChERS provided good reproducibility for the neonicotinoids with values ranging from 2.6 to 10.1%, below the RSD% acceptance threshold of 20% (Table 2.3). On the other hand, the RSD% percentage of all the targeted analytes was successfully measured using Dutch mini-Luke, with values less than or equal to 20%, except for prothioconazole, where there was a higher variability observed for the fortification level of MQL and ten times MQL. To fully interpret the reason behind this variability, the RSD% percentages of prothioconazole extracted using Dutch mini-Luke were compared in the blank sand and blank soil extracts, where high variability was only observed in the prothioconazole fortified soil sample (Figure 2.4). The higher than acceptable RSD% value could have resulted from

their unpredictable behaviour toward the organic matter components in the soil. This explanation is supported by assessment of the matrix effect, which for prothioconazole was more affected by soil matrix components than for the other analytes (Figure 2.5).

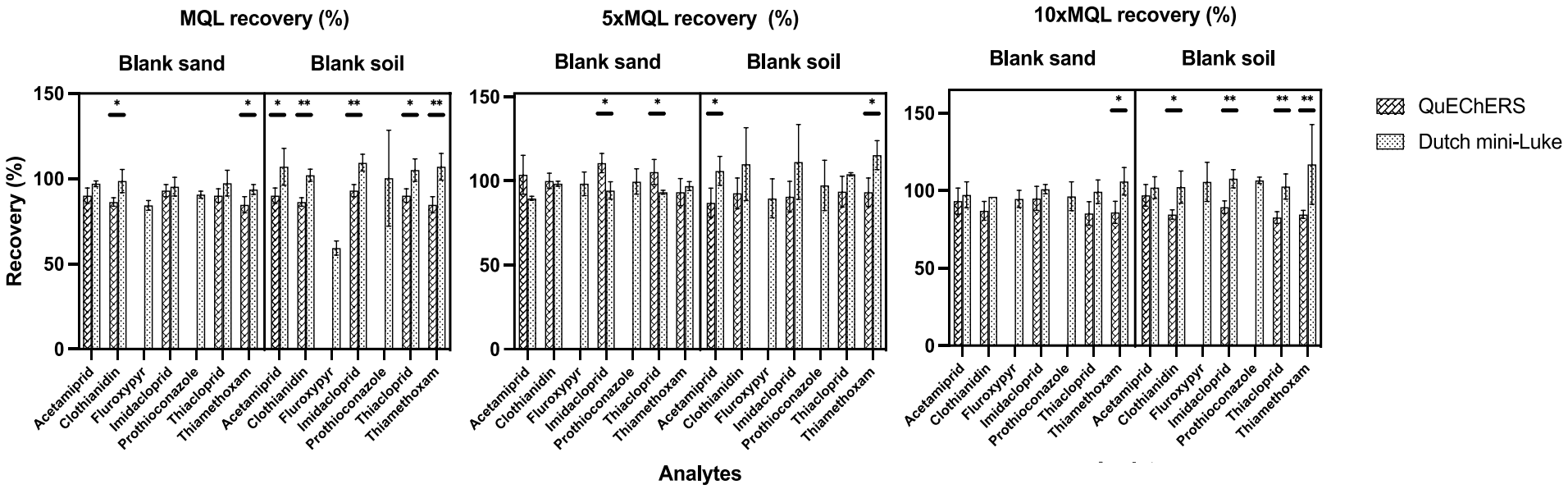


Figure 2.3 Comparison of recovery efficiencies using three different fortification concentrations extracted from blank sand and soil samples. Asterisks show statistical significance (*p < 0.05, **p < 0.01).

Table 2.3. Recoveries and RSD% of the seven targeted pesticides fortified at MQL, five times MQL, and ten times MQL concentrations in blank sand and soil samples using QuEChERS and Dutch Mini Luke extractions.[40,58,59]

Pesticides	Fortification level	QuEChERS % Recovery ± RSD%		Dutch mini-Luke % Recovery ± RSD%	
		Blank sand	Blank soil	Blank sand	Blank soil
Acetamiprid	MQL	90 ± 4.9	90 ± 4.9	97 ± 1.4	107 ± 9.8
	5xMQL	105 ± 11.1	87 ± 9.9	90 ± 1.5	106 ± 8.1
	10xMQL	93 ± 9.3	97 ± 7.0	101 ± 3.1	108 ± 5.8
Clothianidin	MQL	86 ± 2.6	86 ± 2.6	99 ± 6.7	102 ± 3.4
	5xMQL	100 ± 4.9	92 ± 9.6	99 ± 1.4	110 ± 19.6
	10xMQL	87 ± 7.3	85 ± 3.9	96 ± 0.3	102 ± 10.3
Fluroxypyr	MQL	ND	ND	84 ± 2.8	59 ± 6.8
	5xMQL	ND	ND	99 ± 7.1	90 ± 12.5
	10xMQL	ND	ND	99 ± 7.6	103 ± 8.0
Imidacloprid	MQL	93 ± 3.6	93 ± 3.6	95 ± 5.9	109 ± 4.5
	5xMQL	111 ± 5.1	91 ± 10.1	94 ± 5.8	111 ± 20.3
	10xMQL	95 ± 8.4	89 ± 4.3	95 ± 5.8	106 ± 11.9

Prothioconazole	MQL	ND	ND	91 ± 1.9	100 ± 27.8
	5xMQL	ND	ND	99 ± 7.4	97 ± 15.5
	10xMQL	ND	ND	106 ± 8.3	117 ± 21.8
Thiacloprid	MQL	90 ± 4.6	90 ± 4.6	97 ± 8.1	105 ± 6.1
	5xMQL	105 ± 7.2	94 ± 9.9	93 ± 1.2	104 ± 1.0
	10xMQL	85 ± 8.9	83 ± 4.2	96 ± 9.1	107 ± 1.9
Thiamethoxam	MQL	85 ± 5.4	85 ± 5.4	94 ± 3.0	107 ± 7.7
	5xMQL	93 ± 8.5	93 ± 9.5	97 ± 2.6	115 ± 7.3
	10xMQL	86 ± 8.6	85 ± 3.3	97 ± 8.3	102 ± 7.0

ND: Not Detected

RSD%: relative standard deviation

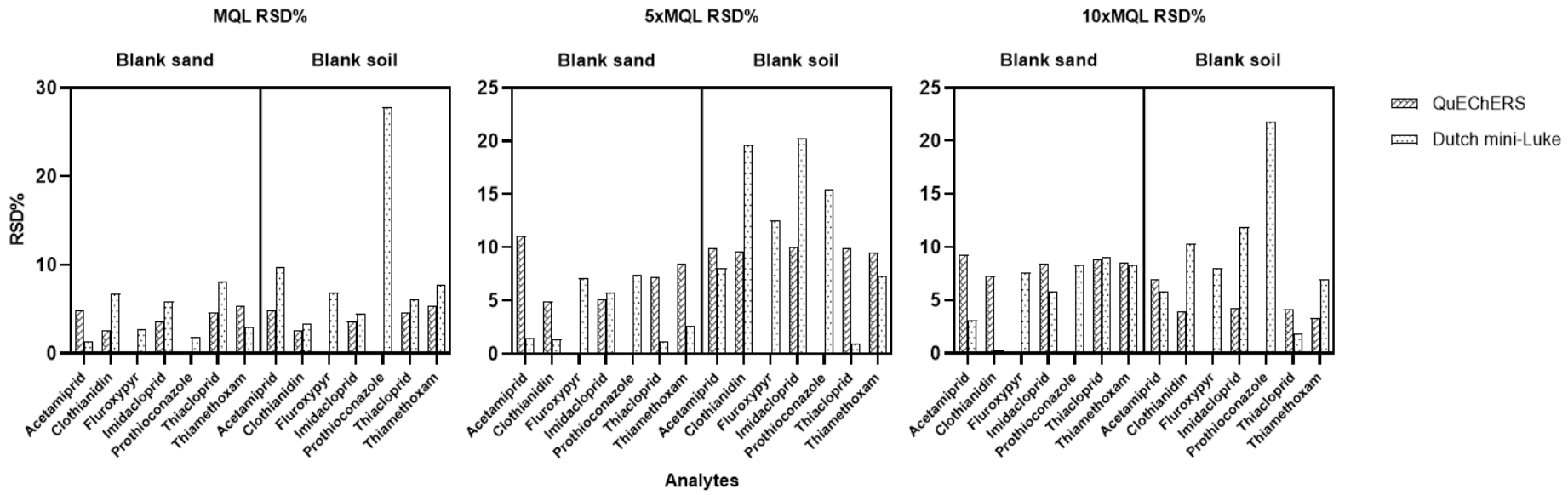


Figure 2.4 Comparison of RSD% value obtained using three different fortification concentrations extracted from blank sand and soil samples.

2.3.5 Comparison of QuEChERS and Dutch Mini Luke matrix effect

Matrix matched calibration was carried out by reconstituting increasing pesticide concentrations in dried sand and blank soil extracts. Matrix effect (ME) was calculated by comparing the slopes of the calibration curves of standards in solvent and samples. MEs with values between +20% and -20% are considered to represent low matrix effects, values between +20% and +50% represent a medium matrix effect, and values less than -50% or higher than +50% represent high matrix effects.[79,80] In the blank sand sample, QuEChERS extraction displayed a low matrix effect consistently across most analytes, except for prothioconazole, which was not detected in both blank sand and blank soil. The highest matrix effect using QuEChERS was exhibited by imidacloprid with 19%, and the lowest is fluroxypyr with 11%. However, in QuEChERS's blank soil extract, fluroxypyr shows a high matrix effect with a value of 291% (Figure 2.5). The failure to detect prothioconazole and the high matrix effect value for fluroxypyr are examples of matrix effects signal suppression (loss in response) and signal enhancement (increase in response). Lin *et al.* also reported signal suppression for prothioconazole but could eliminate the matrix effects by performing calibration using an external matrix-matched standard.[81]

Additionally, the author Kaczyński *et al.* noted that the inclusion of PSA in the clean-up step not only failed to provide the expected recovery range for fluroxypyr from a soil matrix but also the matrix effect could not be reduced.[44] In theirs study, the inclusion of PSA had enhanced the signal of fluroxypyr by 47.2%, compared to not including a clean-up step at all.[44] Due to these matrix effects, signal suppression of prothioconazole could lead to a false-negative measurement,[82] whereas an enhanced signal for fluroxypyr could lead to a false-positive

measurement.[83,84] The ever-present issue with pesticide quantification from complicated matrices such as soil is the presence of co-eluting compounds that negatively affect the extraction method's precision, sensitivity, and accuracy.[85] Under the matrix effect experiment, all the analytes are reconstituted in the dried blank extracts, so failure to detect them is not due to failure to extract them. The specific mechanism of matrix effects is uncertain, although it is thought to arise from competition between an analyte and undetectable matrix components that co-elute.[86] A number of factors can produce signal suppression or enhancement, but they are mainly caused by endogenic compounds already present in the sample and remain in the extract after sample preparation or extraction. Endogenic compounds can be ionic compounds, such as inorganic electrolytes or salts, polar compounds, such as amines, carbohydrates, lipids, peptides or urea.[83] As discussed earlier, soil organic matter and PSA are the main affecting factors for analytes with the QuEChERS extraction method. Even though it is hard to determine which factor plays a more prominent role, the inclusion of a blank sand matrix in the comparison experiments can represent the suppression or enhancement effect presented by the components used during the extraction procedure. The suppression of prothioconazole's signal is an indication that the QuEChERS extraction components, namely PSA, are interfering with the quantification of this base-sensitive analyte.[52,75,77]

Even though the ME values for all the analytes in the Dutch mini-Luke blank sand were observed to be negative in value (Figure 2.5), they still represent low matrix effects. As stated before, as the analytes were reconstituted in dried blank extracts, the only possible factor in the blank sand extract that suppresses the analytes' signal would be the carry-over from the Dutch mini-Luke extraction

components. However, as the analyte's signal suppression values range between -1 and -11%, based on the SANTE guidance document, it is classified as subtle interference, and the extraction method does not require additional modification for sample analysis.[49] In addition, prothioconazole was successfully quantified using Dutch mini-Luke, although a high matrix effect of 140% was observed. The low matrix effects in the blank sand matrix extract allow us to conclude that the high matrix effect for prothioconazole is not due to any of the Dutch mini-Luke extraction components. Therefore, the high matrix effect for prothioconazole is most likely due to the soil matrix itself, with a strong possibility it is caused by the soil organic matter.

Compared to d-SPE clean-up of QuEChERS, Dutch mini-Luke employs a more straightforward means of reducing or eliminating the matrix effect through sample dilution. The main advantage of using sample dilution to reduce or eliminate the matrix effect is that it introduces less matrix load into the chromatographic system with every injection. Ferrer *et al.* had stated that in the analysis of a multi-residue method, the sample extract injection would be similar to the amount of the matrix injection, that is, 1 g of sample per mL.[87] In contrast, this study's Dutch mini-Luke extraction method has a sample dilution factor of 1/20. This means that 1 g of sample extract injection would only introduce the chromatographic system of 0.05 g of matrix load. This translates to better sensitivity and does not require additional extraction components that could compromise the quantitative analysis of the targeted analytes. With reduced levels of matrix components being injected into the analytical system, the life of sensitive equipment can be prolonged.[87–89]

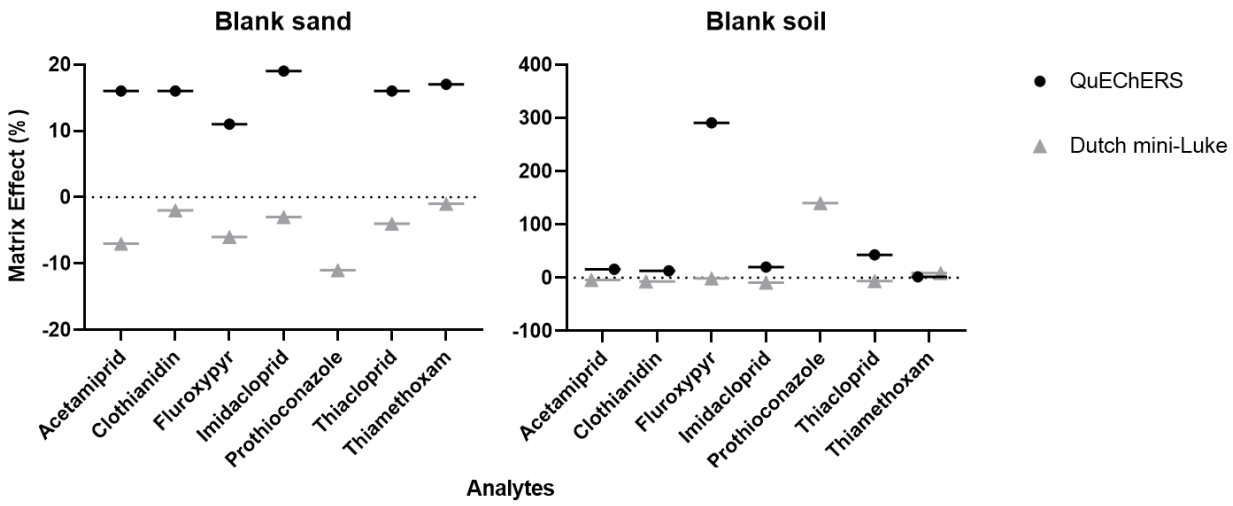


Figure 2.5 Comparison of matrix effect using a matrix-matched calibration curve with blank sand and blank soil samples.

2.3.6 Other parameters for comparison

In addition to the validation parameters described above, a number of additional parameters were considered, such as sample weight requirement, extraction time, number of extraction steps and procedures, and volume of solvent usage (Table 2.4).

The selection of extraction parameters, general characteristics and solvent requirements was based on established protocols, as reported in the literature and standard operating procedures (SOPs) in place in governmental bodies.[40,58] Both methods vary considerably, are advantages to using each method. For example, QuEChERS requires lower amounts of starting sample, lower volumes of solvent, fewer extraction steps, and a more rapid method overall. Due to these differences, the extraction conditions are not directly comparable between these two extraction methods.

The potential shortcomings of Dutch mini-Luke include longer times and higher costs in comparison to QuEChERS. Figure 2.6 depicts the estimated cost for the

dedicated equipment required for both extraction methods. The high cost for employing Dutch mini-Luke extraction comes in the form of homogeniser, and its disperser tool, where during the time of writing this article, the total cost for both of the equipment comes to a total of €3690.50.[90,91] QuEChERS does not require a specific tool to assist in the extraction, and it only requires a vortex mixer to ensure thorough mixing of the extraction components. During the time of purchase in 2019, the vortex mixer cost €153.13.[92] In addition, the use of higher volumes of organic solvents, namely acetone, dichloromethane, and petroleum ether, for Dutch mini-Luke extractions also presents a certain degree of risk to the user and additional waste handling requirements. When using these extraction solvents, the user must practise extra vigilance when handling and changing between solvents in various steps.

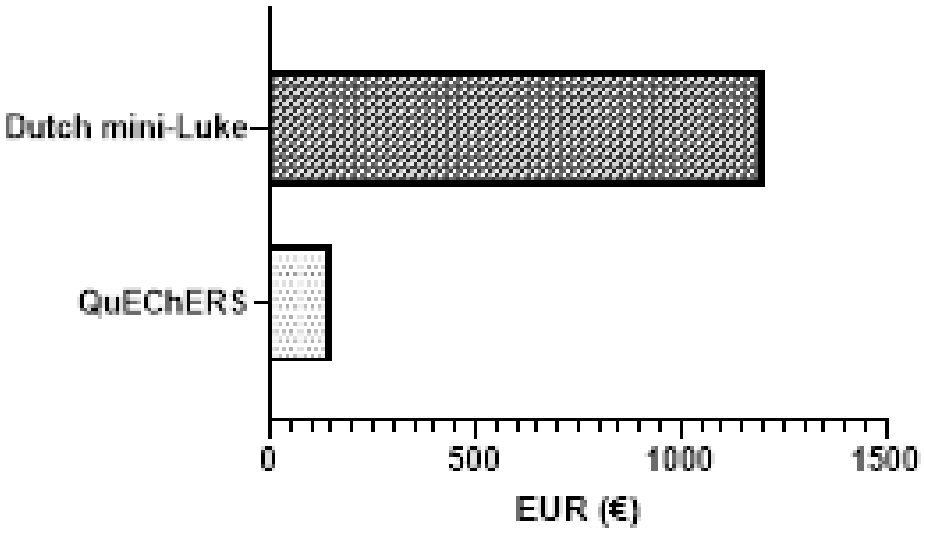


Figure 2.6 Comparison of equipment costs required for QuEChERS and Dutch mini-Luke extraction methods.

Selected examples of the health risks associated with each solvent include; acetone can cause severe eye irritation and toxicity to specific target organ of category three with a single exposure,[92] dichloromethane is suspected of causing cancer,[93] petroleum ether can cause specific target organ toxicity of category two with repeated exposure and can be fatal if swallowed or enters the airway.[94]

Table 2.4 Comparison of additional parameters for the QuEChERS and Dutch mini-Luke extractions

Parameters	QuEChERS extraction	Dutch mini-Luke extraction
Sample weight	5 g	15 g
Extraction time (a batch of four)	1 hour	3 hours
Extraction steps to analysis	Eight steps (Extraction, salting-out, phase partitioning, centrifugation, clean-up, second centrifugation, concentrating, reconstitution)	Six steps (Extraction, phase partitioning, centrifugation, concentrating, dissolutions into ethyl acetate, dissolutions into MeOH)
Sequential or simultaneous procedure	Two sequential extraction containers	Four sequential containers
Solvent usage	15 mL (deionised water and acetonitrile)	105 mL (deionised water, acetone, DCM, and petroleum ether)

These health risks can be avoided if the user is attentive during every extraction while following recommended exposure controls, using the required personal protective equipment (PPE), and disposed of in accordance with the national and local regulations. On the other hand, acetonitrile would be the only non-polar solvent used in QuEChERS extraction, where it can be toxic when in contact with skin, causes serious eye irritation and harmful if inhaled or swallowed, and can be avoided with proper use of PPE and cautiousness was practised. Moreover,

as Dutch mini-Luke requires the usage of additional tools, namely a homogeniser and its attachment, a certain level of technical training is required before the tools can be used efficiently during extraction. For these reasons, carrying out Dutch mini-Luke extractions requires a higher user skill level in comparison to QuEChERS.

2.4 Conclusion

Even though the QuEChERS extraction method complements recent trends toward “green” pesticide extraction techniques by providing faster, more straightforward, and cost-efficient approaches, it is not always suitable for determining pesticides belonging to certain chemical groups. To explore this further, we compared QuEChERS and the traditional Dutch mini-Luke and assessed their extraction efficiencies for seven pesticide analytes representing a number of different chemical groups of insecticide, herbicide, and fungicide. Table 2.5 summarises our findings, demonstrating that all targeted analytes could be successfully recovered from both blank sand and soil samples, with good recovery (59-117%), except fluroxypyr at MQL fortification level with 59%. As for the repeatability of Dutch mini-Luke, all analytes had an RSD% value lower than or equal to 20%, except for prothioconazole at MQL and 10xMQL fortification with 27.8% and 21.8%, respectively.

Table 2.5 Summary of the method validation parameters comparison between QuEChERS and Dutch mini-Luke

Method validation parameters	QuEChERS		Dutch mini-Luke	
	Blank sand	Blank soil	Blank sand	Blank soil
Method Detection Limit (MDL) (ng μL^{-1})	0.31 – 0.36	0.56 – 0.85	0.18 – 0.48	0.20 – 0.42
Method Quantification Limit (MQL) (ng μL^{-1})	0.95 – 1.08	1.7 – 2.58	0.53 – 1.46	0.60 – 1.27
Accuracy (recovery%)	85 - 111	83 - 97	84 - 106	59 - 117
Precision (RSD%)	2.6 – 11.1	2.6 – 10.1	0.3 – 9.1	1.0 – 27.8
Matrix Effect (%)	11 - 19	-11 – -1	2 - 291	-9 - 140

On the other hand, the QuEChERS extraction method had a satisfactory recovery for all the fortified neonicotinoids with percentages ranging between 85 to 111% and RSD% values of 2.6 to 10.1%. However, QuEChERS could recover neither fluroxypyr nor prothioconazole in any blank samples or at any fortification level. Compared to QuEChERS, Dutch mini-Luke does present analytical advantages, where it offers better sensitivity in the form of lower MDL and MQL, better recovery, and lower matrix effects in relation to most analytes. Hence, Dutch mini-Luke was determined to be the preferred extraction method for a single mixed analysis of neonicotinoids, triazoles and synthetic auxin pesticides from soil samples.

2.5 References

1 E. C. Oerke, *J. Agric. Sci.*, 2005, **144**, 31–43.

2 The World Bank, *Agriculture for Development*, 2008.

3 A. Sharma, V. Kumar, B. Shahzad, M. Tanveer, G. P. S. Sidhu, N. Handa, S. K. Kohli, P. Yadav, A. S. Bali, R. D. Parihar, O. I. Dar, K. Singh, S. Jasrotia, P. Bakshi, M. Ramakrishnan, S. Kumar, R. Bhardwaj and A. K. Thukral, *SN Appl. Sci.*, 2019, **1**, 1–16.

4 Agri-environmental indicator - consumption of pesticides - Statistics Explained,https://ec.europa.eu/eurostat/statistics/explained/index.php?title=Agri-environmental_indicator_-_consumption_of_pesticides, (accessed 25 May 2021).

5 O. Zivan, Y. Bohbot-Raviv and Y. Dubowski, *Chemosphere*, 2017, **177**, 303–310.

6 D. Nuyttens, M. De Schampheleire, K. Baetens, E. Brusselman, D. Dekeyser and P. Verboven, *Trans. ASABE*, 2011, **54**, 403–408.

7 S. Pätzold, C. Klein and G. W. Brümmer, *Soil Use Manag.*, 2007, **23**, 294–305.

8 G. Imfeld, F. Meite, C. Wiegert, B. Guyot, J. Masbou and S. Payraudeau, *Sci. Total Environ.*, 2020, **741**, 140437.

9 X. Zhang, Y. Luo and K. S. Goh, *Environ. Pollut.*, 2018, **234**, 48–58.

10 A. Wolters, 2003.

11 M. Houbraken, K. Doan Ngoc, F. van den Berg and P. Spanoghe, *Sci. Total Environ.*, 2017, **599**–**600**, 1408–1416.

12 J. B. Weber, in *Mechanisms of Pesticide Movement into Ground Water*, eds. R. C. Honeycutt and D. J. Schabacker, CRC Press, 2nd edn., 1994, pp. 15–41.

13 P. C. Kearney, D. R. Shelton and W. C. Koskinen, in *Kirk-Othmer Encyclopedia of Chemical Technology*, John Wiley & Sons, Inc., 2000, pp. 419–451.

14 S. Navarro, N. Vela and G. Navarro, *Spanish J. Agric. Res.*, 2007, **5**, 357–375.

15 D. Foqué, B. Beck, W. Devarrewaere, P. Verboven and D. Nuyttens, *Pest Manag. Sci.*, 2017, **73**, 1908–1920.

16 H. F. Schnier, G. Wenig, F. Laubert, V. Simon and R. Schmuck, *Bull. Insectology*, 2003, **56**, 73–75.

17 M. Greatti, R. Barbattini, A. Stravisi, A. G. Sabatini and S. Rossi, *Bull. Insectology*, 2006, **59**, 99–103.

18 D. Kreutzweiser, K. Good, D. Chartrand, T. Scarr and D. Thompson, *Ecotoxicol. Environ. Saf.*, 2007, **68**, 315–325.

19 D. P. Kreutzweiser, D. G. Thompson and T. A. Scarr, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 1053–1057.

20 R. S. Kookana, S. Baskaran and R. Naidu, *Aust. J. Soil Res.*, 1998, **36**, 715–764.

21 J. J. Pignatello, C. R. Frink, P. A. Marin and E. X. Droste, *J. Contam. Hydrol.*, 1990, **5**, 195–214.

22 A. Thompson and K. W. Goyne, *Nat. Educ. Knowl.*, 2012, **4**, 7.

23 P. Wang and A. A. Keller, *Water Res.*, 2009, **43**, 1448–1456.

24 L. Cox, M. C. Hermosín, R. Celis and J. Cornejo, *Water Res.*, 1997, **31**, 1309–1316.

25 J. E. D. Davies and N. Jabeen, 2003, **46**, 57–64.

26 N. Kumar, I. Mukherjee and E. Varghese, *Environ. Monit. Assess.*, 2015, **187**, 1–10.

27 J. P. Bloomfield, R. J. Williams, D. C. Gooddy, J. N. Cape and P. Guha, *Sci. Total Environ.*, 2006, **369**, 163–177.

28 S. Qian, H. Zhu, B. Xiong, G. Zheng, J. Zhang and W. Xu, *Environ. Sci. Pollut. Res.*, 2017, **24**, 11493–11503.

29 P. Wu, W. Z. Wu, Z. H. Han and H. Yang, *Environ. Monit. Assess.*, , DOI:10.1007/s10661-016-5372-6.

30 X. Zhang, Y. Luo and K. S. Goh, *Environ. Pollut.*, 2018, **234**, 48–58.

31 A. Salemi, R. Rasoolzadeh, M. M. Nejad and M. Vosough, *Anal. Chim. Acta*, 2013, **769**, 121–126.

32 C. Gonçalves, J. J. Carvalho, M. A. Azenha and M. F. Alpendurada, *J. Chromatogr. A*, 2006, **1110**, 6–14.

33 P. Herbert, S. Morais, P. Paíga, A. Alves and L. Santos, *Int. J. Environ. Anal. Chem.*, 2006, **86**, 391–400.

34 K. J. James and M. A. Stack, *HRC J. High Resolut. Chromatogr.*, 1996, **19**, 515–519.

35 H. Dabrowska, Dabrowski, M. Biziuk, J. Gaca and J. Namieśnik, *J. Chromatogr. A*, 2003, **1003**, 29–42.

36 S. King, J. S. Meyer and A. R. J. Andrews, *J. Chromatogr. A*, 2002, **982**, 201–208.

37 S. Babić, M. Petrović and M. Kaštelan-Macan, *J. Chromatogr. A*, 1998, **823**, 3–9.

38 H. S. Rathore, in *Handbook of Pesticides: Methods of Pesticide Residues Analysis*, 2010, pp. 7–45.

39 A. Masiá, K. Vásquez, J. Campo and Y. Picó, *J. Chromatogr. A*, 2015, **1378**, 19–31.

40 M. Anastassiades, S. J. Lehotay, D. Štajnbaher and F. J. Schenck, *J. AOAC Int.*, 2003, **86**, 412–31.

41 H. F. Lin, B. Dong and J. Y. Hu, *Environ. Monit. Assess.*, 2017, **189**, 1–9.

42 L. K. Chai, F. Elie and C. Jinang, *Int. J. Environ. Anal. Chem.*, 2014, **94**, 519–530.

43 M. M. McManus, R. P. Oates, S. Subbiah, D. Klein and J. E. Cañas-Carrell, *J. Chromatogr. A*, 2019, **1602**, 246–252.

44 P. Kaczyński, B. Łozowicka, M. Jankowska and I. Hrynko, *Talanta*, 2016, **152**, 127–136.

45 R. Urkude, V. Dhurvey and S. Kochhar, *Pesticide Residues in Beverages*, Elsevier Inc., 2019.

46 N. Pang, T. Wang and J. Hu, *Food Chem.*, 2016, **190**, 793–800.

47 J. L. Tadeo, R. A. Perez, B. Albero, A. I. Garcia-Valcarcel and C. Sanchez-Brunete, *J. AOAC Int.*, 2012, **95**, 5–24.

48 J. Vera, L. Correia-Sá, P. Paíga, I. Bragança, V. C. Fernandes, V. F. Domingues and C. Delerue-Matos, *Sample Prep.*, , DOI:10.2478/sampre-2013-0006.

49 SANTE, *Analytical Quality Control and Method Validation for Pesticide Residues Analysis in Food and Feed*, Document N., 2019.

50 K. Pszczolinska and M. Michel, *J. AOAC Int.*, 2016, **99**, 1403–1414.

51 E. Dankyi, C. Gordon, D. Carboo and I. S. Fomsgaard, *Sci. Total Environ.*, 2014, **499**, 276–283.

52 C. Sack, J. Vonderbrink, M. Smoker and R. E. Smith, *J. Agric. Food Chem.*, 2015, **63**, 9657–9665.

53 T. Rejczak and T. Tuzimski, *Open Chem.*, 2015, **13**, 980–1010.

54 M. Urban and C. Lesueur, *Food Anal. Methods*, 2017, **10**, 2111–2124.

55 R. Pérez-Burgos, E. M. Grzelak, G. Gokce, J. Saurina, J. Barbosa and D. Barrón, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 2012, **899**, 57–65.

56 M. Mei, Z. X. Du and Y. Cen, *Fenxi Huaxue/ Chinese J. Anal. Chem.*, 2011, **39**, 1659–1664.

57 J. Wang, L. Zhao, X. Li, Y. Jiang, N. Li, Z. Qin and C. Pan, *Bull. Environ. Contam. Toxicol.*, 2011, **86**, 326–330.

58 A. de Kok, M. Hiemstra and C. P. Vreeker, *Chromatographia*, 1987, **24**, 469–476.

59 M. Hiemstra and A. de Kok, *J. Chromatogr. A*, 2007, **1154**, 3–25.

60 A. Lozano, B. Kiedrowska, J. Scholten, M. De Kroon, A. De Kok and A. R. Fernández-Alba, *Food Chem.*, 2016, **192**, 668–681.

61 R. J. Fussell, T. F. Scientific and H. Hempstead, *Thermo Sci. White Pap.*, 2016, 1–16.

62 N. Saadati, M. P. Abdullah, Z. Zakaria, S. B. T. Sany, M. Rezayi and H. Hassonizadeh, *Chem. Cent. J.*, 2013, **7**, 1.

63 European Commission, *Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11813/2017*, 2017.

64 V. Andreu and Y. Picó, *TrAC - Trends Anal. Chem.*, 2004, **23**, 772–789.

65 R. Durovic, J. Gajic-Umiljendic and T. Dordevic, *Pestic. i fitomedicina*, 2009, **24**, 51–57.

66 E. Barriuso and R. Calvet, , DOI:10.1080/03067319208027003.

67 J. P. Gao, J. Maguhn, P. Spitzauer and A. Kettrup, *Water Res.*, 1998, **32**, 1662–1672.

68 M. Kah, S. Beulke and C. D. Brown, *J. Agric. Food Chem.*, 2007, **55**, 4487–4492.

69 M. Kah and C. Brown, *Chemosphere*, 2008, **72**, 1401–1408.

70 EFSA, *EFSA Sci. Rep.*, 2007, **106**, 1–98.

71 R. Kashuba and C. Salice, *Environmental Fate and Ecological Risk Assessment for the Registration of Prothioconazole*, 2006.

72 D. A. Lambropoulou and T. A. Albanis, *Anal. Bioanal. Chem.*, 2007, **389**, 1663–1683.

73 EFSA, *EFSA J.*, 2011, **9**, 1–91.

74 PubChem, Prothioconazole | C14H15Cl2N3OS, https://pubchem.ncbi.nlm.nih.gov/compound/Prothioconazole#section=Octanol-Water-Partition-Coefficient, (accessed 28 May 2020).

75 S. J. Lehotay, K. Maštovská and A. R. Lightfield, *J. AOAC Int.*, 2005, **88**, 615–629.

76 M. Oliver, *Appl. Note Mass Spectrom. Appl. Food Saf. Anal.*, 2013, 1–473.

77 M. S. Young, K. Van Tran and J. C. Shia, *Waters - Appl. note*, 2015, 1–6.

78 A. Steinborn, L. Alder, M. Spitzke, D. Dörk and M. Anastassiades, *J. Agric. Food Chem.*, 2017, **65**, 1296–1305.

79 N. Mantzos, A. Karakitsou, I. Zioris, E. Leneti and I. Konstantinou, *Int. J. Environ. Anal. Chem.*, 2013, **93**, 1566–1584.

80 B. Kmellár, P. Fodor, L. Pareja, C. Ferrer, M. A. Martínez-Uroz, A. Valverde and A. R. Fernandez-Alba, *J. Chromatogr. A*, 2008, **1215**, 37–50.

81 H. F. Lin, B. Dong and J. Y. Hu, *Environ. Monit. Assess.*, , DOI:10.1007/s10661-017-5943-1.

82 A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders and Y. Michotte, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 2009, **877**, 2198–2207.

83 F. Gosetti, E. Mazzucco, D. Zampieri and M. C. Gennaro, *J. Chromatogr. A*, 2010, **1217**, 3929–3937.

84 K. Zhang, Matrix Effects in Multi-Residue Pesticide Analysis When Using Liquid Chromatography-Tandem Mass Spectrometry, https://www.restek.com/en/technical-literature-library/articles/matrix-effects-in-multi-residue-pesticide-analysis-when-using-liquid-chromatography-tandem-mass-spectrometry/, (accessed 19 June 2021).

85 W. Zhou, S. Yang and P. G. Wang, *Bioanalysis*, 2017, **9**, 1839–1844.

86 P. J. Taylor, *Clin. Biochem.*, 2005, **38**, 328–334.

87 C. Ferrer, A. Lozano, A. Agüera, A. J. Girón and A. R. Fernández-Alba, *J. Chromatogr. A*, 2011, **1218**, 7634–7639.

88 B. K. Choi, D. M. Hercules and A. I. Gusev, *J. Chromatogr. A*, 2001, **907**, 337–342.

89 IKA® ULTRA-TURRAX® disperser tools, stainless steel dispersing element for T-25 digital disperser, 18 mm, 1/cs, https://www.sigmaaldrich.com/IE/en/product/aldrich/z404586?context=product, (accessed 21 June 2021).

90 IKA® ULTRA-TURRAX® dispersers T-25 Digital, for volumes of 1-2,000 mL, 230 V, 1/cs, https://www.sigmaaldrich.com/IE/en/product/sigma/z722464?context=product, (accessed 21 June 2021).

91 Vortex mixers, CLASSIC and WIZARD | VWR, https://ie.vwr.com/store/catalog/product.jsp?catalog_number=444-0745, (accessed 22 June 2021).

92 Acetone | CH3COCH3 - PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/Acetone#section=GHS-Classification, (accessed 22 June 2021).

93 Dichloromethane | CH2Cl2 - PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/Dichloromethane#section=Safety-and-Hazards, (accessed 22 June 2021).

94 NITE - Chemical Management Field - GHS Information, https://www.nite.go.jp/chem/english/ghs/06-imcg-0810e.html, (accessed 22 June 2021).

Chapter 3 :

Establishing the extent of pesticide contamination in Irish agricultural soils

Abstract

To establish meaningful and sustainable policy directives for sustainable pesticide use in agriculture, baseline knowledge of pesticide levels in soils is required. To address this, five pesticides widely used in Irish agriculture and five neonicotinoid compounds pesticides were screened from soils from 25 fields. These sites represented diversity of soil and land use types. Prothioconazole was detected in 16 of the 18 sites where it had been recently applied, with the highest maximum concentration quantified of 45.66 µg/kg. However, a week after application only four fields had prothioconazole concentrations above the limit of quantification (LOQ). Fluroxypyr was applied in 11 sites but was not detected above LOQ. Glyphosate and AMPA were not detected. Interestingly, neonicotinoids were detected in 96% of all sampling sites, even though they were not reported as recently applied. Excluding neonicotinoids, 60% of sites were found to contain pesticide residues of compounds that were not previously applied, with boscalid and azoxystrobin detected in 15 of the 25 sites sampled. The total pesticide concentrations detected in Irish soils were significantly negatively correlated with clay fraction, while average pesticide concentrations were significantly positively correlated with log K_{ow} values. A comparison to the parametric limit set for Irish drinking water revealed that 17 fields exceeded the limit for total pesticide concentrations, even when recently applied pesticides were removed from calculations. Theoretical environmental risk assessment of quantified pesticides determined that azoxystrobin has high leaching risk, while boscalid, which was detected but not applied, has an accumulation risk. This information provides insight into the current level of pesticide contamination in

Irish agricultural soil and contributes to the European-level effort to understand potential impacts of pesticide contamination in soil.

3.1 Introduction

Plant protection products in the form of herbicides, fungicides, and insecticides have become a ubiquitous component of global agricultural management practices, responsible for increased crop productivity and yield and driving agricultural intensification.[1–3] They can be applied throughout all phases of crop cultivation, including as seed treatments prior to planting or after seed germination,[4] during the crop growth period when pests or diseases are at their highest levels,[5,6] or before the shipment of the crop products.[7–9] Agriculture is vital to the economies of many countries, including Ireland, where it directly contributed 4.3% of gross value added (GVA) or €14.4 billion to the national economy in 2019.[10] The total land area of Ireland is approximately 6.9 million hectares, of which 4.4 million hectares, or 71%, are used for agriculture.[11] The majority of this farmed area (4.1 million hectares) is used for pasture, hay, and grass silage, with 0.016 million hectares used for rough grazing and 0.29 million hectares devoted to crops, including cereals, potatoes, fruit and horticultural production.[11] To sustain and improve the productivity of the Irish agricultural industry, the utilised agricultural area would reflect the amount of pesticide usage. For instance, between 2016 and 2017, the area of Irish land used for agriculture was 28.31 thousand hectares, whereas the total pesticide usage in 2017 was 2861 tonnes, an increase of 323 tonnes from the previous year.[12–14] Despite the increased costs that arise from pesticide use, their use has consistently increased over the past decades. More than 3000 different types of pesticides used in Europe over the past 55 years, raising serious environmental concerns

regarding the intensive release of synthetic chemicals into the environment.[2,15–19]

The assumption that pesticides dissipate from the environment after achieving their function has been shown to be invalid. It is estimated that about 99.9% of applied pesticides are subjected to off-site transfer,[20–22] through numerous simultaneous routes, including volatilisation,[23,24] spray drift,[25,26] surface run-off,[27–29] degradation and leaching,[30–32] while only 0.1% of the applied pesticides reach their intended target [20]. Additionally, Lechenet *et al.* reported that current recommended pesticide application rates exceed what is necessary for efficient crop protection,[33] which could result in higher soil contamination than intended. Pesticide presence in soils can originate from multiple sources; either through aerial spray onto crops where the soil can be exposed to pesticides from drips from the crop,[34,35] direct treatment of the soil,[36,37] or through seed-coating.[37–39] Once the pesticide is incorporated into the soil, it enters a dynamic environment in which it can display highly variable behaviour, depending on one of the four main processes, adsorption, desorption, degradation or transformation, and leaching.[40–42] However, the efficiency of the process is fundamentally influenced by numerous factors, including the physicochemical properties of the pesticide compound and the soil layer, the climatic condition and soil biotic properties.[42–46]

The adsorption and desorption potential of a pesticide residue can be inferred based on their physicochemical properties, namely dissociation constant (pKa), partition coefficient (log K_{ow}), solubility, adsorption coefficient (K_{oc}) and vapour pressure,[47–50] or based on the soil physicochemical properties such as pH, soil organic matter, and the soil texture.[43,47,51] However, organic matter and clay mineral content principally dictate the levels of pesticide persistence.[52–54] These

components are linked,[55] and present multiple functional groups onto which pesticide residues can adsorb. In addition, their relatively large and chemically active surface areas increase their chemical affinity for pesticide residue adsorption,[56] and although soil organic matter is usually thought to represent a better adsorption matrix compared to clay minerals,[57–59] both of these matrices' presence are highly associated with each other.[55] Although they can exist independently, organic matter in the soil is directly or indirectly governed by clay minerals.[55] Once organic matter interacts with clay minerals, it remains protected from mineralisation, consequently increasing the organic matter content in the soil.[55] Hence, it can be postulated that soil with high clay or high organic matter, or both, has a higher risk of pesticide accumulation. On the other hand, high soil organic matter content would be expected to increase microbial activity,[60–63] which increases microbial degradation, the primary degradation route of soil pesticides. Soil microbes can break down pesticides through metabolic processes such as polymerisation, accumulation and conjugation, co-metabolism, and mineralisation.[43] Even though microbial degradation correlates positively with the soil organic matter content,[64] the efficiency of the process is affected by abiotic properties of the soil, such as aeration, pH moisture, and temperature.[47] Additionally, the uncertainties of climatic conditions, such as rainfall, relative humidity, evaporation, air movement, light and temperature, add to the complexity of predicting pesticide mobility in soil.[65]

To date, our knowledge of pesticide occurrence on the island of Ireland derives from studies on groundwater,[66] wastewater,[67] food and products,[68–70] and meta-analyses of the literature.[71,72] Although pesticide contamination of agricultural soils has been assessed for Northern Ireland,[2] no equivalent records or studies

exist for the Republic of Ireland. The principal aim of our study was to provide a baseline overview of pesticide contamination in agricultural soils from 25 sites across the Republic of Ireland and use this information to establish a baseline reference for future monitoring. In total, ten pesticides (five neonicotinoids and five non-neonicotinoids) were selected for analysis. The selected pesticides' structures were further highlighted in Table A1.1. Although neonicotinoids have been banned for outdoor use in Ireland since 2018;[73–75] their long half-lives (ranging from 3-6931 days) and environmental persistence resulted in their inclusion.[76–81] The other pesticides were selected based on their widespread and large-scale use in Irish agriculture.[72] Finally, the glyphosate metabolite aminomethylphosphonic acid (AMPA) was included in the monitoring, given that it can accumulate rapidly and persist in soil environments.[82]

To the best of our knowledge, the characterisation of such widely used pesticides has not been conducted for Irish soils or sites. Our study also evaluates potential correlation between applied pesticide concentration to their chemical properties, the soil physicochemical properties, and the land-use type to provide a broader and more holistic interpretation of the fate of specific pesticides in specific contexts. Finally, a comparison was made between the individual and total pesticide concentrations to the safety limits set for Irish drinking waters and groundwaters and assessed their potential for leaching from agricultural soils based on the properties of the individual chemicals and specific soil types. It is anticipated that the results of our study will offer insights into pesticide contamination levels in Irish agricultural soils and form the basis for future pesticide monitoring and management actions.

3.2 Materials and Methods

3.2.1 Standards and reagents

All salts and solvents were analytical or LC-MS grade. Acetone, acetonitrile, acetic acid (Hac) 100%, dichloromethane, petroleum ether, formic acid 98%, sodium hexametaphosphate, anhydrous sodium sulphate, ammonium bicarbonate, ammonium formate, methanol, Millipore Millex syringe filters with hydrophilic PTFE membrane (pore size 0.22 µm and 20 mm diameter), PTFE centrifuge tubes (15 mL and 250 mL), and total nitrogen and phosphorus cell test kits were purchased from Merck Life Science (Ireland). Ultrapure water, deionised to the resistance of <18 MOhm, was generated using ELGA Purelab Ultra SC MK2 (ELGA, UK). Certified reference analytical standards, >97% purity, of acetamiprid, azoxystrobin, boscalid, clothianidin, fluroxypyr, glyphosate, imidacloprid, prothioconazole, thiamethoxam, thiacloprid, and the internal standards, acetamiprid-d3, clothianidin-d3, imidacloprid-d4, thiacloprid-d4, thiamethoxam-d3, triphenyl phosphate (TPP), MCPA-d6 and malathion-d10 were purchased from Merck Life Science (Ireland). Deuterated MCPA was used as an internal standard for fluroxypyr, while malathion-d10 was used as an internal standard for azoxystrobin, boscalid and prothioconazole. The internal standards Glyphosate-$^{13}C_2$, ^{15}N, and AMPA-^{13}C, ^{15}N were purchased from LGC standards (LGC, UK). Pesticide stock solutions were prepared in LCMS grade acetonitrile, except for glyphosate and AMPA, which were prepared in deionised water. Working standard solution then diluted from stock solution before analysis. All working standard solutions were freshly prepared from stock on the analysis day and filtered through 0.45 µm pore-sized PTFE membrane filters before analysis. 2 mL silanised amber autosampler vials were obtained from Agilent (Germany).

XBridge UPLC BEH column (4.6x100 mm i.d. 3.5 µm particle size) and VanGuard cartridge holder were all purchased from Waters Chromatography (Ireland). HILICpak VT-50 2D column (2.0x150 mm, 5 µm particle size) was purchased from Shodex (Germany).

3.2.2 Soil sampling site selection

Sites were selected to represent the variety of soil conditions and agricultural practices across Ireland using the Irish Soil Information System (SIS),[83,84] and the spatially integrated Land use and Soil Inventory for Ireland (LUSSI).[85] All communications and contact complied with General Data Protection Regulations (GDPR). Soil samples were collected i) from sites within 24 hours of pesticide application and ii) from sites with no recent human or agricultural activities or history of pesticide use to serve as non-pesticide controls.

3.2.3 Soil sampling

In total, 25 soil samples were obtained from 25 sites (Figure 3.1) between April to July 2021. These 25 sites were sampled within 24 hours of pesticide application and the sites revisited a week after for the collection of samples one week after application. Five soil cores (15 cm depth; topsoil) were collected randomly across each field, combined, and homogenised to produce a single sample from each site. Approximately one kilogram of the soil mixture was packed in clean plastic Ziplock bags, placed in an icebox (temperature of approximately 4^{o}C and below), kept in the dark, and transported to the lab. All samples were then stored at -20^{o}C. Prior to further analysis, soils were defrosted, air dried and sieved using a stainless-steel sieve with a mesh size of 2 mm. Large objects such as roots and stones were removed, and where necessary, a pestle

and mortar were used to process soils further. Sieved soil samples were stored in Ziplock bags and restored at -20^{o}C.

3.2.4 Soil physicochemical properties determination

Soil physicochemical properties were determined in triplicate for each site by measuring pH_{CaCl2}, texture, organic matter, moisture content, water holding capacity, total nitrogen (TN) and total phosphorus (TP). Clay, silt and sand content were measured using a standard hydrometer (152H ASTM) after dispersion in sodium hexametaphosphate (50 g/L). Fractions were classified as sand, silt and clay following the US Department of Agriculture (USDA) system to distinguish particle size.[86,87] A pH meter was used to measure the pH of air-dried soil samples mixed with 0.01M $CaCl_2$ (at the ratio of 1:5) and left overnight (ISO, 2005). Moisture content was determined by calculating the difference in weight before and after oven drying fresh soil samples for three days (72 h) or until the soil samples achieved constant weight.[88,89] Water holding capacity was measured using the Haines-funnel system, where the soil samples were saturated with 100 mL of water for 30 min, then the water was drained, and the amount of water retained in the soil was calculated.[90] Oven-dried samples were used for determining total soil organic matter by the loss-on-ignition method. The samples were subjected to 550^{o}C in a muffle furnace for 6 hours and left in the furnace overnight. The total soil organic matter percentage was then calculated from the difference before and after ignition.[91] Cell test kits determined total nitrogen and phosphorus photometrically after digestion. Total nitrogen was determined following Koroleff's digestion method, while total phosphorus was analysed through digestion in sulfuric solution.[92,93]

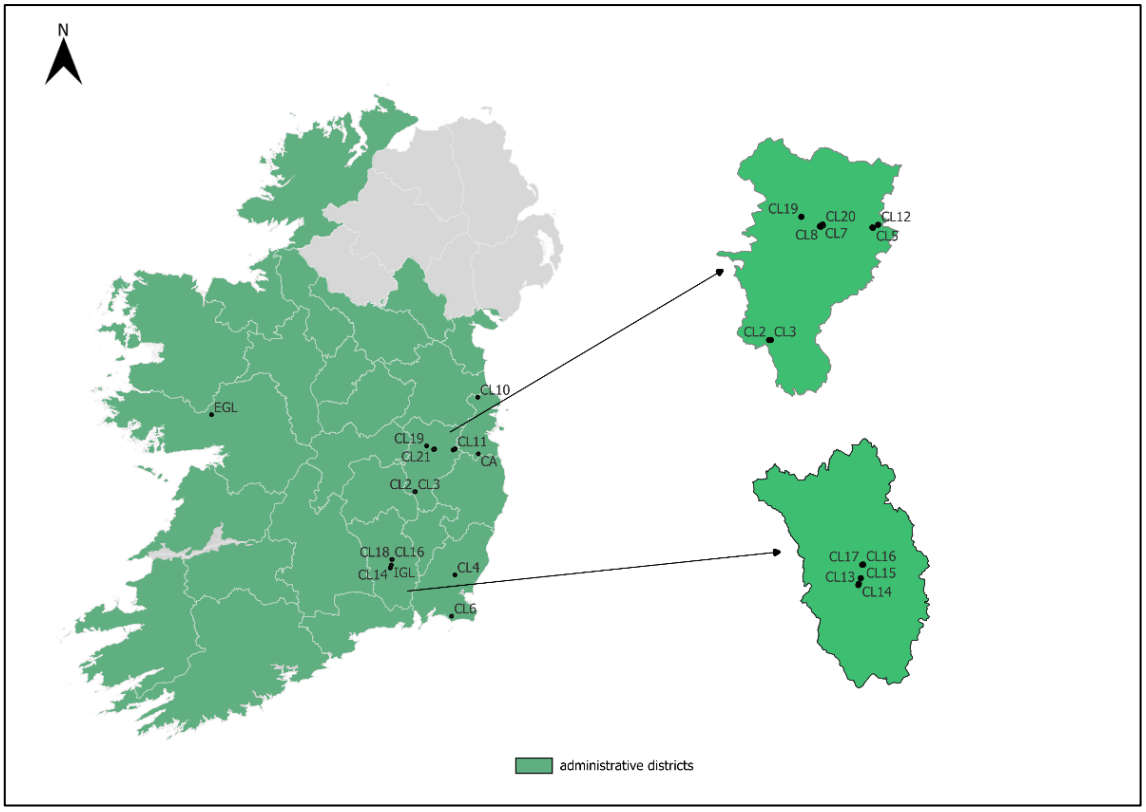


Figure 3.1 Sampling point map of the research area (Republic of Ireland), callouts are for the counties Kildare and Kilkenny. The label for each point indicates the field's label: *EGL:* Extensive grassland.; *IGL:* Intensive grassland.; *CL:* Cropland.; *CA:* Commonage Area.

3.2.5 Soil pesticide extraction methods

The targeted pesticides, acetamiprid, AMPA, azoxystrobin, boscalid, clothianidin, fluroxypyr, glyphosate, imidacloprid, prothioconazole, thiacloprid, and thiamethoxam, were extracted using two different extraction methods, decided based on compound polarity. Glyphosate and AMPA were extracted using the modified QuPPe-PO method,[94] while all the other analytes were extracted using the Dutch mini-Luke method.[95]

3.2.5.1 Dutch mini-Luke method

An aliquot of 15 g air dried and sieved soil sample was weighed into a 250 mL PTFE centrifuge tube, and 15 mL deionised water was added and shaken vigorously for 1 min. Then, 30 mL of 1% acetic acid in acetone were added and homogenised using IKA Ultra-Turrax T-25 homogeniser at 1500 rpm for 30 s. Following the homogenising, 30 mL dichloromethane and 30 mL petroleum ether were transferred to the tube, and the sample mixture was homogenised again using the homogeniser at 1500 rpm for 30 s to induce phase separation. Then, the centrifuge tubes were centrifuged at 4000 rpm for 10 min, and 60 mL of the supernatant was carefully decanted into a 100 mL conical flask. The supernatant then evaporated under N_2 gas flow to circa 2 mL before being transferred to a 10 mL volumetric flask and made up to volume with ethyl acetate. Subsequently, 0.5 mL of the ethyl acetate sample was diluted into a 10 mL volumetric flask made up of volume with methanol. Finally, 1 mL aliquot of the methanol extract was filtered through a 0.22 µm hydrophilic PTFE syringe filter into a silanised autosampler vial for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

3.2.5.2 QuPPe-PO method

Two grams of homogenised air-dried soil samples were weighed into a 15 mL Falcon centrifuge tube. 2 mL of acidified deionised water (1% formic acid) were added and vortex mixing for 1 min, followed by 10 mL of acidified methanol (1% formic acid) addition and 1 min of vortex mixing. The centrifuge tube was then centrifuged at 4500 rpm for 10 min. Approximately 10 ml of supernatant was transferred into a silanised glass vial and concentrated to dryness under an N_2 stream. The concentrated residue was re-dissolved in 1 mL of MeOH: H_2O

(50:50) solution before being filtered through a 0.22 µm hydrophilic PTFE syringe filter into a silanised autosampler vial for LC-MS/MS analysis.

3.2.6 LC-MS/MS methods and conditions

The pesticides, acetamiprid, azoxystrobin, boscalid, clothianidin, fluroxypyr, imidacloprid, prothioconazole, thiacloprid, and thiamethoxam, were analysed with the C18 column method, while glyphosate and AMPA were analysed using a novel HILIC column method. Two multi-reaction monitoring (MRM) transitions were monitored for all the targeted compound, and the data acquisition are detailed in Table 3.1.

3.2.6.1 C18 column method

LC-MS/MS analysis was performed using liquid chromatography (Agilent 1290 Infinity II) coupled with a triple quadrupole mass detector (Agilent 6470A) and XBridge UPLC BEH C-18 analytical column of 4.6 x 100 mm, 3.5 µm particle size. The sheath gas temperature was kept at 340^{o}C, and the sheath gas flow was 11 L/min. 5 mM ammonium formate with 0.1% formic acid in deionised water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) were used for the gradient program, which started at 30% B and increased to 50% in 5 min, was linearly increased to 100% B in 7 min, and it was held at 100% for 2 min before the column reconditioned back to 30% in 2 min. The flow rate was maintained at 0.5 mL/min, the column temperature was kept constant at 30^{o}C, and the injection volume was 10 µL.

3.2.6.2 HILIC column method

Similar LC-MS/MS system used as above, and a Shodex HILICpak VT50-2D analytical column of 2 x 150 mm, 5 µm particle size. 10 mM ammonium bicarbonate in deionised water (mobile phase A) and pure acetonitrile (mobile phase B) were used for the gradient program. The flow rate was constant at 0.1mL/min, with an injection volume of 30 µL. The gradient program started at 80% A and increased to 100% A in 5 min. The A% concentration was held for 8 min before the column was re-equilibrated back to 80% in 3 min. The sheath gas temperature was kept at 350^{o}C, and the sheath gas flow was 12 L/min.

Table 3.1 Acquisition and chromatographic parameters for the targeted pesticides.

No.	Pesticide	T_R (min)	MRM 1	CE 1	MRM 2	CE 2	Polarity
1	Acetamiprid	4.39	223.2 > 126.1	20	223.2> 56.1	20	+
2	AMPA	5.71	110.0 > 63.0	20	110.0> 79.0	36	-
3	Azoxystrobin	9.66	404.0 > 372.0	19	404.0> 344.0	27	+
4	Boscalid	9.89	343.0 > 307.0	20	343.0> 272.0	32	+
5	Clothianidin	3.82	250.0 > 169.0	12	250.0> 132.0	12	+
6	Fluroxypyr	5.90	255.0 > 181.0	24	255.0> 209.0	12	+
7	Glyphosate	12.56	168.0 > 63.0	32	168.0> 150.0	8	-
8	Imidacloprid	4.14	256.2 > 175.2	25	256.2> 209.0	20	+
9	Prothioconazole	10.52	344.0 > 326.0	8	346.0> 328.0	20	+
10	Thiacloprid	5.37	253.0 > 126.0	36	253.0> 90.0	50	+
11	Thiamethoxam	3.27	292.0 > 211.1	15	292.0> 181.0	24	+

3.2.7 Quality control

The extraction method and analytical performance validation were adapted from SANTE Guidance Document on analytical quality control and method validation procedures for pesticide residue analysis in food and feed.[96] Matrix-matched calibration standards for each targeted pesticide were prepared in a composite soil sample encompassing all the collected soil samples, with linearity ranging between 0.05 µg/L to 100 µg/L. Two internal standards (IS) were used, stable isotopically labelled IS for quantitative analysis, while TPP used quality control for extraction performance. The performance of the Dutch mini-Luke extraction method was evaluated using accuracy (recovery%) and precision (RSD%) studies. The sensitivity of the extraction method was assessed by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ), calculated by taking into the slope of the calibration curve and the standard deviation of the slope, and multiplying by 3.3 and 10, respectively. Recovery and precision assessment were measured by fortifying pesticide-free soil samples at two concentrations, 2.66 µg/kg, which is the standardised LOQ for all the pesticides, and five times the standardised LOQ, 13.3 µg/kg. Nearly all the targeted pesticides fulfilled the acceptance criteria of the validation parameters based on SANTE guidelines,[96] where the average recovery was recommended to be in the range of 70-120%, with RSD% less or equal to 20% (Table 3.2), except for prothioconazole at LOQ level (61.7%), and both fortification levels for glyphosate (53.3% and 67.9%, (respectively) and AMPA (59.8% and 68.7%, respectively). The unsatisfactory recovery% and RSD% can be explained based on the instrumental matrix effect (ME%) experienced by AMPA, glyphosate and prothioconazole (Table 3.2). ME% was calculated using the response of targeted

pesticide response and the response of matching isotopically labelled IS, where 100% of the ME% value indicates no instrumental matrix effect. In comparison, values below 100% indicate a loss in response (ion suppression), and value above 100% indicates an increase in response (ion enhancement).[97] Hence, based on the ME% value, AMPA, glyphosate and prothioconazole experience ion suppression from the soil matrix resulting in unsatisfactory recovery% and RSD%. However, as all the targeted pesticides achieved good linearity and reproducibility of calibration curves ($r^2 > 0.890$) and the response compensation with matching isotopically labelled IS, the quantitation analysis of this study is valid.

3.2.8 Statistical analysis

The data management, analysis and visualisation using three software; Microsoft Office Excel, R statistical software (version: 4.2.1) and Graphpad Prism (version: 9.4.1). Only pesticide concentrations quantified at or above LODs were used for data analysis. Soil samples with pesticide concentrations below LOQs but above LODs were recorded based on each method's individual pesticides' LODs to minimise bias of left-censored data.[98–100] Pearson correlation coefficients were used to study the relationship between concentration and frequency of soil pesticides detection to soil physicochemical properties and pesticide properties. The hierarchical cluster heatmap was determined using the *pheatmap* package in R, where it was performed using the log concentration of the quantified pesticides' concentration profile. The dendrogram of the heatmap influences the clustering pattern, where Euclidean distance metrics were used for complete clustering.

Table 3.2 Linearity, Recoveries%, RSD%, LOD, LOQ and ME% for all the target pesticides in the composite soil sample (*n*=3).

No	Pesticides	Linearity (r^2)	Fortification Levels (µg/kg)				LOD (µg/kg)	LOQ (µg/kg)	ME%
			2.66		13.3				
			Recovery%	RSD%	Recovery%	RSD%			
1	Acetamiprid	0.999	105.7	11	96.57	18.9	0.11	0.33	114.9
2	AMPA	0.890	59.8	44.9	68.7	31.1	1.74	5.28	71.5
3	Azoxystrobin	0.996	101.3	14.3	97.7	26	0.3	0.91	94.4
4	Boscalid	0.999	118.1	9.2	99.7	25	0.13	0.39	147
5	Clothianidin	0.999	118.6	3.8	105.7	5.6	0.07	0.23	86.6
6	Fluroxypyr	0.987	74.7	20.1	112	25.3	0.88	2.67	15.6
7	Glyphosate	0.943	53.3	47.1	67.9	27.6	1.03	3.11	58.8
8	Imidacloprid	0.999	108.6	15	96.8	4.6	0.09	0.27	81.6
9	Prothioconazole	0.998	61.7	31.4	95.6	25.9	0.44	1.32	79.2
10	Thiacloprid	0.999	115.6	9.3	98.8	11.7	0.08	0.24	80.7
11	Thiamethoxam	0.992	108.4	7.5	94.7	4.5	0.41	1.23	88.57

3.3 Results and discussion

Pesticide compounds that reside within the soil can behave differently depending on their physicochemical properties, which include sorption coefficient, water solubility, and vapour pressure. In addition, their behaviour may be dictated by the physicochemical properties of the soil itself, such as soil pH, texture, organic matter, moisture, total nitrogen and phosphorus content.[47] Hence, for monitoring purposes, measuring and correlating the pesticide concentrations and physicochemical properties of both components is crucial to understanding the distribution observed here for a number of Irish agricultural soil.

3.3.1 Soil Physicochemical Properties

Most soils studied here ranged from highly acidic to slightly basic, with pH_{CaCl2} values ranging between 3.3 to 7.5 (mean=6) (Table 3.3). Clay percentage ranged between 11 to 29% with a CoV of 27.62% (mean=18.4%), and the maximum silt percentage being 49% with a CoV of 52.53% (mean=16.16%). However, the percentage of sand indicates a high degree of variability between the sites, with a CoV of 13.60% with a range of 36 to 82%. Soil organic matter (%) ranged from 3.583% to 42.91%, with CoV values of 89.92%, indicating high degrees of uniformity. The mean value for % soil organic matter was 9.142 which is deemed to be high (i.e. > 5%; (Durovic *et al.*), total nitrogen values (mg/kg) were more diversified, from 23.27 to 137.1 mg/kg (mean=56.01 mg/kg), CoV=47.11)), compared to total phosphorus (mg/kg), which was more uniform across the sites with a CoV of 96.54% and a range of 1.489 to 52.64 mg/kg (mean=11.06 mg/kg).

Table 3.3 Summary of the physicochemical properties for a selection of Irish soils (*n*= 25).

Soil Properties	min	Max.	Med	Me	SD	CoV	LQ.	UQ
%Clay	11	29	18	18.4	5.083	27.62%	14	21
%Silt	5	49	15	16.16	8.489	52.53%	11.5	19
%Sand	36	82	64	65.6	8.921	13.60%	62	71.5
Soil pH_{CaCl2}	3.303	7.467	6.37	6.031	1.134	18.80%	5.149	7.117
Soil Organic Matter (%)	3.583	42.91	6.225	9.142	8.221	89.92%	5.695	8.696
Soil Moisture (%)	2.841	11.71	5.032	5.395	1.842	34.15%	4.348	5.835
Soil Water Holding Capacity (%)	5	31	9	12.45	6.77	54.37%	7	16.85
Total Phosphorus (mg/kg)	1.489	52.64	9.496	11.46	11.06	96.54%	5.436	11.95
Total Nitrogen (mg/kg)	23.27	137.1	54.32	56.01	26.39	47.11%	36.08	72.88

Notation. Min = minimum; Max = maximum; Med = median; Me = mean; SD = standard deviation; CoV = coefficient of variance; LQ = lower quartile; UQ = upper quartile.

3.3.2 Irish agricultural soil pesticide concentrations within 24 hours of pesticide application

Of the 25 sampling sites, 18 had been sprayed with prothioconazole, 11 with fluroxypyr, 4 with azoxystrobin, and 1 with glyphosate, with 14 sites with more than one active ingredient applied. No sites had been sprayed with boscalid or any of the five neonicotinoids. A summary of the nine pesticide residue concentrations detected at the 25 sampling sites is presented in Table 3.4. Overall, nearly all the targeted pesticides were detected, except for glyphosate and AMPA, even though glyphosate was applied in one of the 24 agricultural fields (Figure 3.2) and both were removed from subsequent analyses. The total pesticide concentrations ranged from below the level of detection (n.d.) to 45.66 µg/kg; the highest concentration detected was the pesticide prothioconazole at 45.7 µg/kg. As for the detection rates, fluroxypyr is the least frequently detected, at 16% of all the sampled sites, while prothioconazole was detected at the rate of 64%. Interestingly, neonicotinoids were detected and quantified in the collected soil samples at detection rates ranging from 28-76%, with the lowest neonicotinoid maximum concentration detected was 0.178 µg/kg, acetamiprid, and the highest maximum concentration detected was thiamethoxam at 1.466 µg/kg.

Based on the spraying record, azoxystrobin, glyphosate, fluroxypyr, and prothioconazole were all applied within 24 hours of sampling. However, in terms of detection, not all pesticides that were reported as being applied at the sampling site were detected in our analysis. As mentioned, glyphosate was not detected in the soil from the site where it was exclusively applied. Azoxystrobin, fluroxypyr and prothioconazole were detected in 50%, 36% and 89% of the sites where they had been reported to be applied, respectively. However, even though fluroxypyr

were detected in four sites, all of them were found to be below the LOQ. Incidentally, prothioconazole was the most frequently and successfully detected pesticide in 16 of the 18 sites where it was applied, with only six sites above the LOQ. The fact that pesticides were not always detected in field soils where they were recently applied could be due to their failure to reach the soil within 24 hours or reflect that the concentrations were below the detection limit in the soil samples collected. In addition, rapid biodegradation of the pesticides to CO_2 and H_2O or other degradation routes to partial metabolites may also result in a failure to detect them, also resulting in decreased toxicity.[101,102]

Table 3.4 Summary of the concentrations of analysed pesticides in Irish soils (*n*=25).

Pesticides (µg/kg)	Min	Max	Me	Med	SD	CoV	Detection rates (all sites) (%)	Relative detection (Sites Detected: Sites Applied)
Acetamiprid	n.d.	<LOQ	<LOQ	<LOQ	-	-	28	7:0
Azoxystrobin	n.d.	2.523	0.823	0.283	0.933	113.4%	24	2:4
Boscalid	n.d.	2.72	1.09	0.987	0.847	77.75%	40	10:0
Clothianidin	n.d.	0.279	0.193	0.201	0.068	35.24%	32	8:0
Fluroxypyr	n.d.	<LOQ	<LOQ	<LOQ	-	-	16	4:11
Imidacloprid	n.d.	0.383	0.177	0.163	0.083	46.79%	36	9:0
Prothioconazole	n.d.	45.66	3.278	0.913	9.546	291.2%	64	16:18
Thiacloprid	n.d.	0.24	0.194	0.2	0.043	22.03%	76	19:0
Thiamethoxam	n.d.	1.466	1.349	1.429	0.195	14.43%	72	18:0

Notation. Min = minimum; Max = maximum; Me = mean; Med = median; SD = standard deviation; CoV = coefficient of variance; n.d. = not detected; and LOQ = Limit of Quantification.

As mentioned previously, Irish soils generally have a high organic matter percentage (Table 3.3), which could contain a more extensive microbial community,[60–63] resulting in rapid pesticide degradation. Even if pesticides were not degraded completely, partial degradation could result in higher mobility of the degraded product in the soil layers, a phenomenon observed for azoxystrobin and its more water-soluble degradation product.[103]

Neonicotinoids were detected in 96% of the sampled fields, despite not being applied to all sites (Figure 3.2). Our findings, although unexpected in the context of what compounds were applied, are consistent with the half-lives reported for neonicotinoids, i.e., that they are exceptionally long, and can differ significantly (31-450 days for acetamiprid, 148-7000 days for clothianidin, 28-1250 days for imidacloprid, 3.4-1000 days for thiacloprid and 7-335 days for thiamethoxam; which highlights the extent of their persistence in terrestrial environments.[79,104] Interestingly the fungicide boscalid was detected in the soils from ten sites, again without any report of recent application. Unlike the neonicotinoids, boscalid is not banned for outdoor use; therefore, the detection of boscalid in soil samples could be due to the persistence of this compound from the previous crop season. The K_{oc} values for boscalid range from 507 to 1110 mL/g,[105] suggesting that strong absorption into the soil, coupled with its low mobility, could result in significant persistence.[106] Such persistence is commonly reported, with an estimated 69% of pesticides detected in soils being attributed to applications from previous crop seasons,[107] with some being reported up to 10 years after application.[108] The identification of non-recently applied pesticides could also be explained through

drift processes from neighbouring other intensively managed agricultural fields (no data was obtained about land use or management in neighbouring sites).

Of the 25 sites included in this study, 23 sites had up to two pesticide products, applied in a single day application within 24 hours of sampling. It was not unexpected therefore that 23 sites were determined to contain at least two or more pesticide residues (Figure 3.3 (a)). However, eighteen fields contained 2-5 residues, with six fields where a large number of residues (>6) were detected. Only one site had no detected pesticides, and no sites had just a single detectable pesticide. Taken together, these findings highlight that more than the recently applied pesticides are present in the soils sampled, which points to a pervasiveness of pesticides in Irish agricultural systems. These findings also indicate that, despite their ubiquitous detection, not all pesticides were present at high concentrations, and not all pesticides had similar accumulation profiles. For instance, both fluroxypyr and acetamiprid were detected in four and seven sites, respectively, but at concentrations below their LOQ values (Figure 3.3 (b)), even though fluroxypyr had just been applied in 11 sites, and acetamiprid had not been applied in any. Another compound not applied in any site was thiamethoxam, but in contrast to acetamiprid, it was detected in 18 fields, with 15 fields above LOQ.

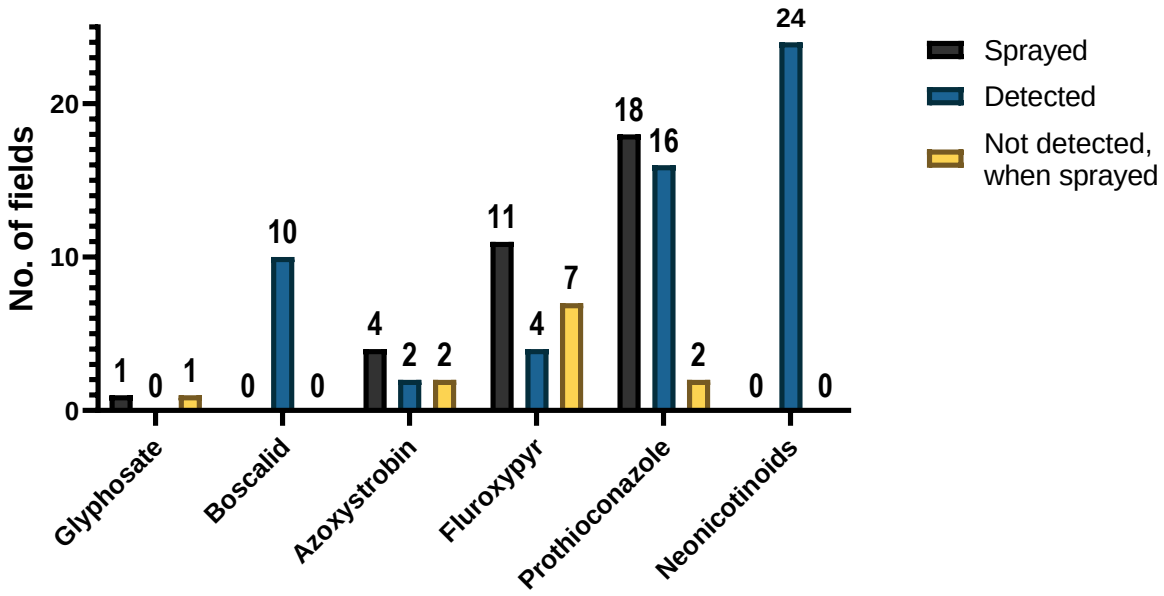


Figure 3.2 Applications and levels of detection for the different pesticide types investigated in this study.

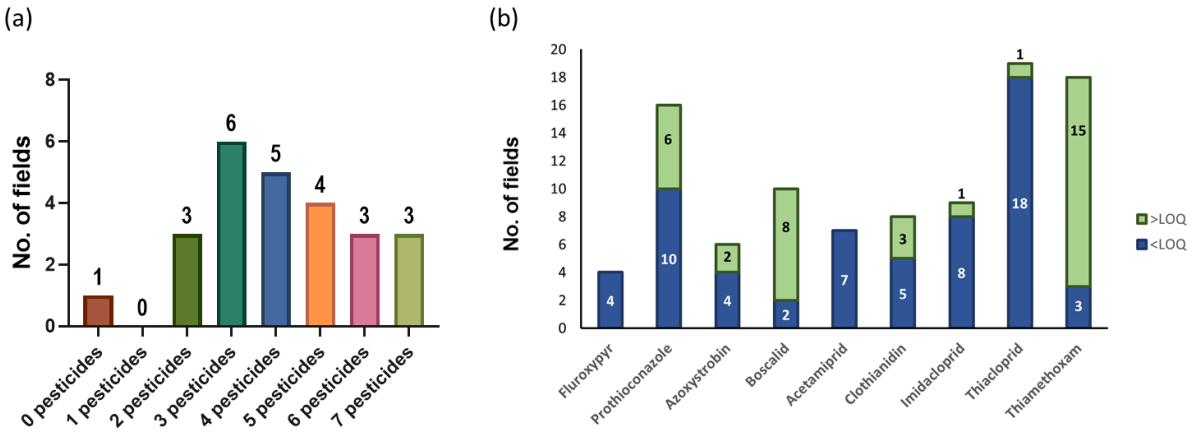


Figure 3.3 Distribution of quantified pesticides in Irish agricultural soils (a) studied fields based on the number of multi-residues (b) pesticide detection frequency and concentration.

3.3.3 Identification of pesticides with land-use characteristics

Hierarchical cluster analysis was used to visualise coherent patterns that emerged based on the concentration profiles of quantified pesticides expressed across the different pesticide types and soil classes (Figure 3.4). Within the studied sites, we show for the first time that hierarchical clustering resulted in two distinctions of Irish agricultural soils, relating to low and high pesticides concentrations. The dendrogram illustrating the clustering of the pesticide classes and types (at the left border of the Figure 3.4) indicated that the cluster with the highest pesticide concentrations is dominated by thiamethoxam, prothioconazole and thiacloprid, which resulted in these pesticide groups being discriminated from other pesticides. In addition to higher pesticide concentrations, the cluster formation was also driven by the detection rate, where prothioconazole, thiamethoxam, and thiacloprid were detected in nearly all the sampled fields, with a detection rate of 64%, 72% and 76%, respectively (Table 3.4).

In addition, the top dendrogram (showing clustering based on the soil classes of the sites and pesticide concentrations) distinguished the two cluster. For instance, the cluster comprising low pesticide concentrations was associated with more diverse land-use types, including cropland, grassland and commonage land, whereas the cluster with high pesticide concentrations was associated with croplands only. Finally, luvisol and surface water gley soil types were largely associated with the high pesticide cluster, while the low pesticide cluster had a more heterogeneous mix of soil types.

Prothioconazole is one of the most widely used fungicides in Ireland and is applied to an estimated 5% of cultivated lands nationally.[72] Of the sites sampled

in this study, 72% were reported to have received a recent application which explains its clustering with the other received a high concentration pesticide. Furthermore, other recently applied pesticides, such as fluroxypyr and azoxystrobin, were detected at a lower rate of 36% and 50% in the sprayed fields, respectively, compared to prothioconazole, which has a high detection rate of 89%, which could be due to the compound's chemical properties. Prothioconazole has an estimated K_{oc} value of 1765,[109] suggesting that this compound has low mobility in the soil layers. In addition, in acidic conditions prothioconazole has a log K_{ow} value of 4.16, indicating that it will readily be adsorbed to the organic matter component of the soil,[110] justifying its failure to dissipate and its high detection levels in this study.

The quantification of high concentrations of neonicotinoids, specifically thiamethoxam and thiacloprid, relative to other recently applied pesticides is highly concerning. Our results indicate that the contamination of Irish agricultural soils with neonicotinoids is highly prevalent. Assuming that neonicotinoids had not been applied after their ban in 2018, their detection could be explained by their lack of degradation and movement through the environment. As soon as neonicotinoids come in contact with soil, multiple factors could influence their behaviour; the soil type, biotic and abiotic properties of the soil, and with the fluctuations of these factors, neonicotinoids can take multiple routes through the environment.[111] This type of mobility may explain the results observed in the extensive grassland (EGL) site where clothianidin, thiacloprid and thiamethoxam were detected. Historically, this site would not have been sprayed with neonicotinoids or planted with neonicotinoid treated seed, all of which were confirmed by the farmer; however, our findings clearly demonstrate the presence

of neonicotinoids. Interestingly the commonage land (CA) site would also have had a history of no pesticide applications, but unlike EGL, no pesticides were detected in CA soil. Given that CA is a natural peatland (Figure 3.4), which is unsuitable for agricultural cultivation; very little human activity has occurred on this land or in the surrounding areas.

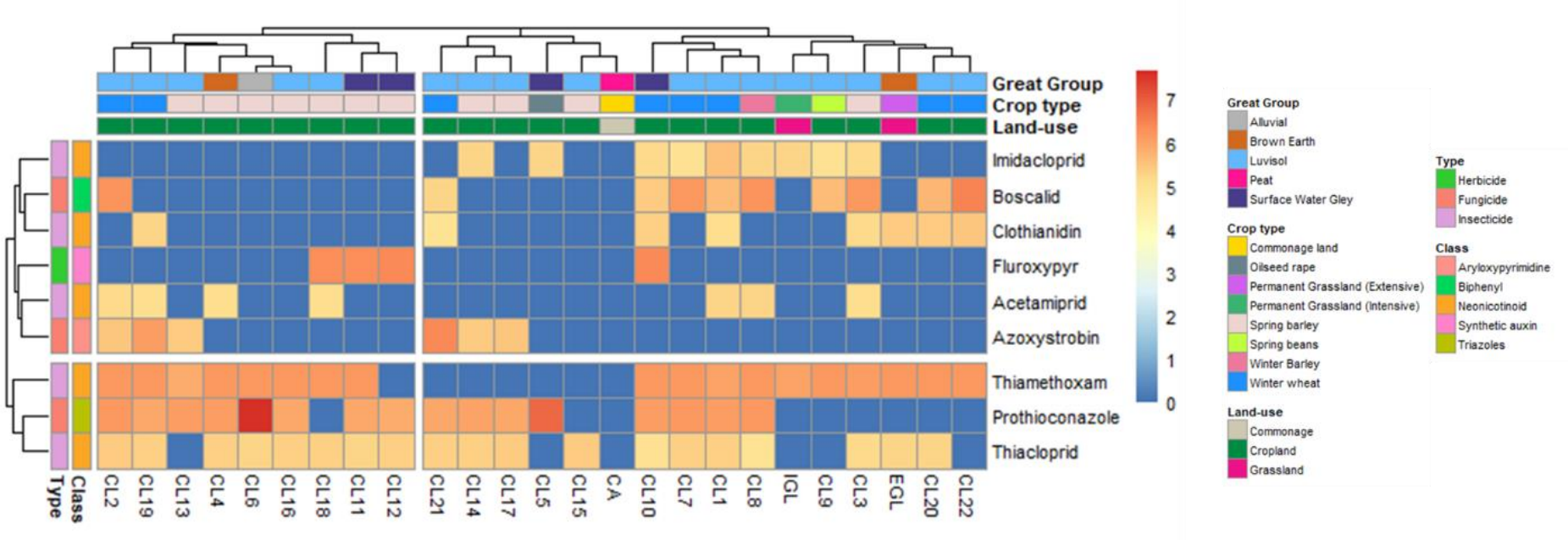


Figure 3.4 Hierarchical cluster heatmap analysis of nine detected pesticides clustered by concentration profiles of targeted pesticides and sampling site details. The colour of each cell represents the $\log_{10}$ pesticide concentrations. The dendrogram was cut to present two clusters of sample sites and two clusters of targeted pesticides. Notation. EGL = Extensive grassland; IGL = Intensive grassland; CL = Cropland; CA = Commonage area.

By contrasting these two sites with a similar pesticide application history, it can be determined that an agricultural field can be contaminated with pesticides even without direct application. There could also be multiple routes of contamination of EGL, such as run-off waters from adjacent fields, off-field dust, or spray-drift during treatment at a nearby field.[25,111–113] Similar findings were also reported by Humann-Guilleminot *et al.* where neonicotinoids, especially clothianidin, imidacloprid and thiacloprid, were detected in extensively managed agricultural land with no history of neonicotinoid application up to 10 years.[114]

To simplify the heatmap the Irish soil great group and pesticide class variables were removed from the hierarchical clustering analysis, allowing us to focus on crop and pesticide types (Figure 3.5 (a)). Even without recent application, insecticides emerge as the most dominant pesticide type distinguished in the clustering as it is the only pesticide type detected in all agricultural fields, followed by fungicide and herbicide. For the land-use types, as the main driver of the clustering is pesticide concentration, and as higher concentrations were observed in the cropland sites, hierarchical clustering analysis yielded croplands clustering, while commonage land and grasslands formed a separate cluster. This clustering pattern also correlates with the recorded percentage of pesticide use in Ireland, where it has been reported that croplands are treated with 95.5% insecticides, 92.6% fungicides and 41% herbicides.[72]

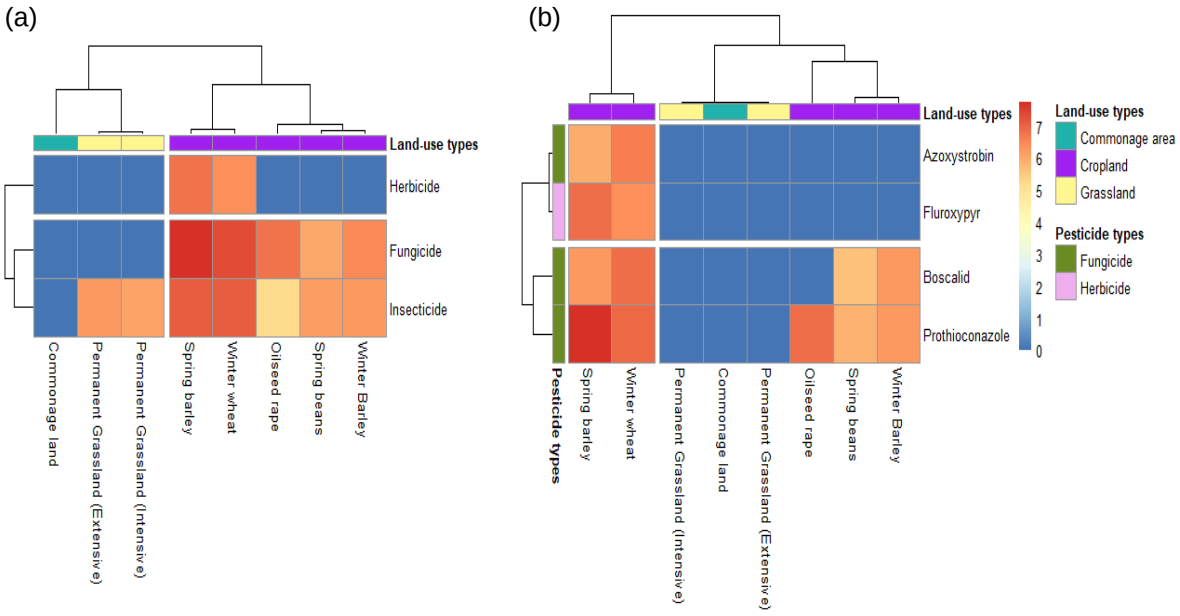


Figure 3.5 Simplified hierarchical cluster analysis heatmap clustered by log10¬ concentration profile (a) Crop types clustered by pesticide types (b) Crop types clustered by targeted pesticides (excluding neonicotinoid).

To further investigate the pesticides that are still used widely in an Irish context, hierarchical clustering was performed after removing neonicotinoids, basing the clustering on crop type and non-neonicotinoid pesticides (Figure 3.5 (b)). This had the result of collapsing the commonage land and grasslands into a single cluster with no targeted pesticides detected. The discrimination of spring barley and winter wheat from other crop types can be explained based on the cereal production area in Ireland. In 2021, of the 356.7 thousand hectares of area utilised for cereal production in Ireland, spring barley, winter barley, and winter wheat are the top three cereals grown, with 42%, 24% and 20% of the total area, respectively.[115]

Additionally, when insecticides were removed from the analysis, it was observed that fungicides and herbicides were predominantly applied to croplands.[72] This is

undoubtedly reflected in the hierarchical clustering where spring barley and winter wheat are grouped in the cluster for high pesticide concentrations, as these crop fields with all the targeted fungicides and herbicides at high concentrations (Figure 3.5 (b)). Notably, azoxystrobin is clustered with fluroxypyr, separated from other fungicides, while boscalid and prothioconazole cluster together as these pesticides are primarily detected in the croplands.

3.3.4 Relationship between pesticides with soil physicochemical and pesticide properties

Pearson correlation analyses were carried out to determine the potential relationship between the soil physicochemical properties and the total pesticides quantified at the respective fields (Figure 3.6 (a)). Only one significant correlation was identified: a positive correlation between total pesticide detected and percentage of clay ($p<0.05$), while weak correlations with the other measured soil physicochemical properties. It is also noted that even though weakly correlated, total pesticide detected correlates negatively with soil organic matter.

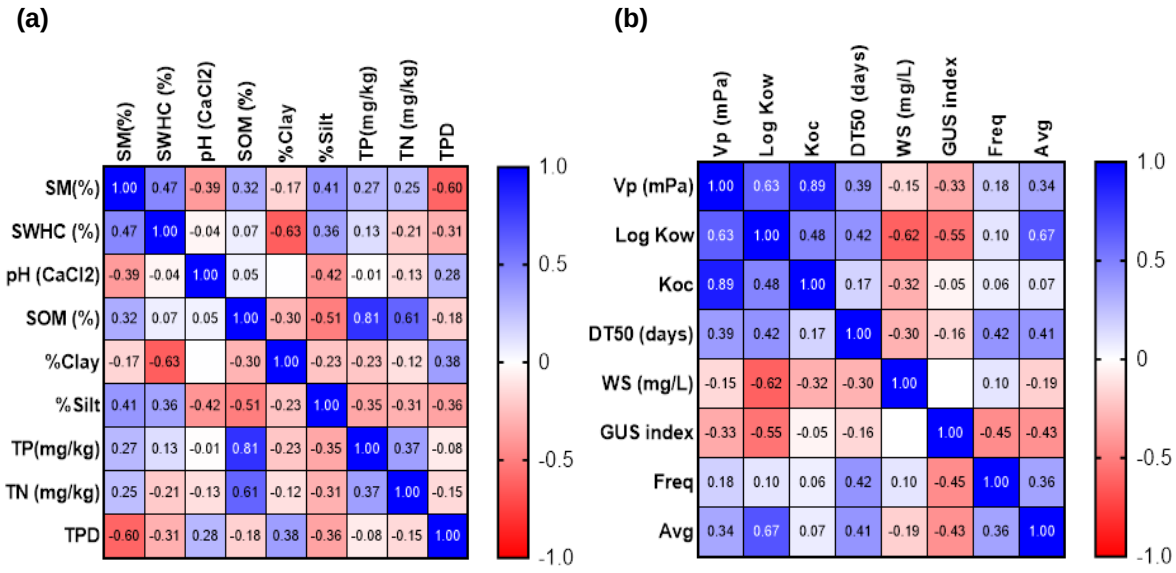


Figure 3.6 Pearson correlations coefficient plot based on the number of soils containing quantifiable pesticide residues (*n*=24): (a) the total pesticide content is represented with the measured soil properties, (b) The frequency of pesticide detection and pesticide average concentrations correlated with their pesticide properties. Notation. SM = Soil Moisture; SWHC = Soil Water Holding Capacity; SOM = Soil Organic matter; TP = Total Phosphorus; TN = Total Nitrogen; TPD = Total Pesticide Detected; Vp = Vapour pressure; DT_{50} = Half-life; WS = Water Solubility; GUS = Groundwater Ubiquity Score; Freq = Frequency of detection; Avg = Average concentration "*N*".

This finding is contrary to many of the reported publications,[2,116–120] where a positive correlation is generally observed for the relationship between pesticides and soil organic matter. However, this observation could be explained based on the Irish soil great group. Luvisol is a soil great group with a subsoil enriched with a higher percentage of clay, and as a more significant number of sampled sites were from the luvisol soil great group, clay particles could be the more dominant component that plays a role in the adsorption of pesticides. Even though the studied sites were also observed to have a high organic matter percentage (>5%), clay particles could be more readily available for pesticide adsorption. This postulation is supported by Durovic *et al.*, where it was stated that even though soil organic matter and clay can both play a role in the adsorption of pesticides, in soils with a higher percentage of clay fractions, pesticide residues would readily adsorb clay component rather than soil organic matter.[121] Similar findings have also been reported in the Czech Republic, where pesticides were reported to correlate negatively with soil organic matter while correlating positively with clay minerals.[122] On the other hand, organic matter and the clay fraction could both exist in high concentrations, and the pesticides would still adsorb more readily to the clay particles. Theoretically, clay can physically coat and encapsulate soil organic matter, resulting in stable aggregate formation,[55] protecting the organic matter from other soil properties, including the adsorption of pesticides. While the organic matter is rendered unavailable for pesticide adsorption due to encapsulation, the larger surface area of clay minerals', with -OH groups and transferable cations, can enhance the adsorption of pesticides.[123] Consequently, it increases pesticide compounds' adsorption to the clay particle, resulting in a positive correlation of pesticide with clay percentage.

The frequency of pesticide detection was weakly correlated with their properties, such as vapour pressure, log K_{ow}, K_{oc}, DT_{50}, water, solubility and Groundwater Ubiquity Score (GUS) index. In contrast, the average concentration of the pesticides had a significant positive correlation with log K_{ow} ($p<0.05$) (Figure 3.6 (b)). This relation indicates that with increasing log K_{ow} values, the pesticide concentration in the soil increases, which conforms to widely reported studies.[123–125]

Soil pH correlates positively with the total pesticide detected. As the pH of the sampled sites was observed to be skewed towards an acidic soil environment (mean=6.37) (Table 3.3), it could result in increased sorbing of pesticides and increased persistence.[126] Chemically, ionic and hydrophilic compounds were observed to efficiently bind to clay minerals,[127] and many of the targeted pesticides are ionisable based on their pKa values (Table 3.6), confirming their high affinity of total pesticide detected towards the clay particles and increased persistence. Interestingly, it is observed in this study that even the non-polar and non-ionic compounds (log K_{ow} >1) (Table 3.6) were detected at high concentrations in soil layers with high clay percentage (Figure 3.4). The findings suggest that even though the basic pesticide properties, such as pKa, log K_{ow}, DT_{50}, and solubility help predict its behaviour and persistence, these properties become less determinant in the real-world scenario where a more significant number of external factors are involved. The fact that most pesticide behaviour studies are conducted under laboratory conditions is an issue often mentioned in the literature,[107] as are the difficulties in transferring this knowledge to highly complex and highly heterogenous soils found in real agricultural sites.

3.3.5 Assessment pesticide concentrations a week after application

Scrutinising the sites for further monitoring purposes, based on the detection and quantification of widely used pesticides, resulted in the selection of 13 sites out of the original 25 sites. Table 3.5 presents the summary of the pesticide concentrations, where the total pesticide concentrations within 24 hours of pesticide application ranged from the level below the detection (n.d.) to 45.66 µg/kg, while the total pesticide concentrations after one week of pesticide application ranged from n.d. to 5.02 µg/kg. Even though the highest total pesticide concentrations decreased by 89%, in both sampling timepoints, the highest concentrations detected were of the pesticide prothioconazole. For the 13 sites, acetamiprid and fluroxypyr both remained under LOQ concentrations during both sampling timepoints, with detection rate increased by 31% for acetamiprid after one week, while there are no changes in detection rate for fluroxypyr.

Table 3.5 Summary of the concentrations of analysed pesticides in Irish soils within 24 hours and one week after pesticide application (*n*=13, that is, the number of sites where pesticides were recently applied).

Pesticides (µg/kg)	Sampling	Min	Max	Me	Med	SD	CoV	Detection rates (13 sites) (%)	Relative detection (Sites Detected: Sites Applied)
Acetamiprid	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	31	4:0
	One week	n.d.	<LOQ	<LOQ	<LOQ	-	-	62	8:0
Azoxystrobin	24 hours	n.d.	2.523	0.319	1.323	0.757	80.51%	23	2:2
	One week	n.d.	2.071	0.159	1.03	0.574	0.00%	8	1:2
Boscalid	24 hours	n.d.	1.59	0.142	0.795	0.437	117.90%	23	3:0
	One week	n.d.	1.034	0.099	0.517	0.284	82.02%	23	3:0
Clothianidin	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	31	4:0
	One week	n.d.	0.271	0.044	0.136	0.094	11.63%	23	3:0
Fluroxypyr	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	31	4:9
	One week	n.d.	<LOQ	<LOQ	<LOQ	-	-	31	2:9
Imidacloprid	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	23	3:0

	One week	n.d.	-	-	-	-	-	0	0:0
Prothioconazole	24 hours	n.d.	45.66	3.851	0.44	12.563	256.50%	85	11:11
	One week	n.d.	5.02	1.06	0.44	1.468	98.84%	85	11:11
Thiacloprid	24 hours	n.d.	0.24	0.086	0.08	0.051	72.74%	92	12:0
	One week	0.194	0.242	0.234	0.238	0.013	5.58%	100	13:0
Thiamethoxam	24 hours	n.d.	1.465	0.872	1.376	0.719	3.70%	62	8:0
	One week	n.d.	1.489	0.91	1.469	0.749	0.60%	62	8:0

Notation. Min = minimum; Max = maximum; Me = mean; Med = median; SD = standard deviation; CoV = coefficient of variance; n.d. = not detected; and LOQ = Limit of Quantification.

Comparing the detection rates of these widely used pesticides over one week indicates that, except for azoxystrobin, all the other pesticides were detected at the same rate. The detection rate of azoxystrobin decreased by 15% (three sites to one site), while the maximum concentration decreased by 18%, from 2.523 to 2.071 µg/kg. While the detection rate did not change for the other compounds, the maximum concentration decreased for boscalid and prothioconazole by 35% and 89%, respectively. Fluroxypyr was detected at the same rate (four sites) for both sampling timepoints, but after one week, it was detected in only two out of nine sites where it was recently applied, in addition to two where it was not.

The detection of neonicotinoids between the one-week sampling timepoint in Irish agricultural soils corresponds with their widely reported long half-lives.[76–81] In contrast to other neonicotinoid analytes, imidacloprid is the only neonicotinoid that was not detected in the 13 sites after a week, while thiamethoxam was detected at the same detection rates (eight sites) between both sampling timepoints. Furthermore, in terms of total pesticide concentrations, nearly all the neonicotinoids were observed to increase in concentrations, with clothianidin, thiacloprid and thiamethoxam increased by 11%, 1%, and 4%, respectively. The increase in neonicotinoid concentrations, even though not applied recently, can be explained by the movement of these persisting analytes upon desorbing from the sites where they had been accumulated, either within the field or from neighbouring fields. This movement can be attributed to the long persistence of neonicotinoids in agricultural soils,[114] and the high mobility of these analytes in the environment.[128–130] Hence, the detection of neonicotinoids during both sampling timepoints further strengthens the necessity for regular monitoring of neonicotinoids in the soil.

Interestingly, even though the detection rates of most of the widely used pesticides were observed to be the same within 24 hours and one week after pesticide application, the total pesticide concentration was observed to decrease between these two-sampling timepoints (Figure 3.7). Prothioconazole were detected in all the fields that it was recently applied (11 sites) with the detection rate of 85%. However, prothioconazole is the only pesticide noted to decrease significantly ($p<0.05$) in total concentration, with a decrease of 72% and only four of the 11 sites being above LOQ. Meanwhile, fluroxypyr were detected in four sites during both sampling timepoints, but with no concentrations above LOQ. Both azoxystrobin and boscalid concentrations decreased by 50% and 30%, respectively, with one site above LOQ for each analyte.

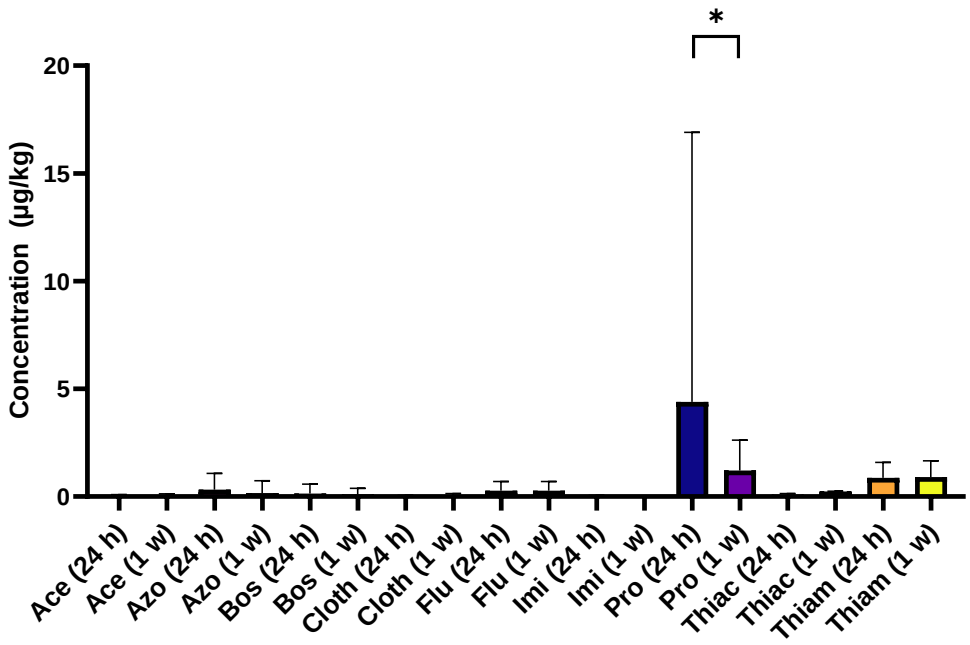


Figure 3.7 Fluctuation of the total concentration of individual pesticide analytes compared within 24 hours and one week after pesticide application (*n*=13). Notation. Ace = Acetamiprid; Azo = Azoxystrobin; Bos = Boscalid; Cloth =Clothianidin; Flu = Fluroxypyr; Imi = Imidacloprid; Pro = Prothioconazole; Thiac = Thiacloprid; Thiam = Thiamethoxam; 24 h = within 24 hours of pesticide application; and 1 w = after 1 week of pesticide application.

Overall, the dissipation of all the widely used pesticides between the two studied sampling timepoints can be attributed mainly to chemical or microbial degradation.[131] In this study, chemical degradation of the pesticides can be influenced by the clay and pH properties, highlighted previously as the only positively correlating soil physicochemical properties to the total pesticide detected in Irish agricultural soil. Similar findings have also been reported by Kah *et al.*, where the author noted a positive correlation between the clay content and the degradation rate of pesticides.[116] The authors highlighted as the clay and organic matter content forms a significantly correlation, the degradation rate of pesticides could be catalysed by both of the soil components rather than just one of them.[116] This finding is also supported by Villaverde *et al.*, where both clay and soil organic matter were noted to have a significant positive correlation with the degradation of dicamba, mesulfuron-methyl, 2,4-D, and flupyrsulfuron-methyl-sodium ($p<0.01$).[132]

Based on the application history of the widely used pesticides, boscalid is the only pesticide that was not applied recently. Although previous studies had noted that boscalid tends to degrade slowly in soil, with half-lives ranging from 31.5 days to 180.1 days,[133,134] up to 96 to 578 days.[105] Interestingly, in this study, the total concentration of this pesticide observed to decrease by 21% within the one week. The initial detection of boscalid in the 24-hour soil samples, even when it is not applied, could be due to repeated application, as noted by Han *et al.*, where degradation rates of boscalid decrease with frequency of treatment.[133] The author also noted that boscalid alters the soil microorganism structure with multiple applications resulting in the specialisation of pesticide-degrading species.[133] However, this shift in soil microbial structure could enhance the

degradation rate of boscalid with time, which could be observed in this study. Similar findings have also been reported by Yu *et al.* with the application of fungicide carbendazim, where the author noted a 90% increase in degradation rate between the first and fourth fungicide application.[135] Nonetheless, even with a high biodegradation rate, due to the high persistence nature of boscalid, the repeated application of boscalid could result in substantial accumulated residues in soil, where its ecological effects in long-term contaminated soil remain uncertain.

Nevertheless, in the Irish agricultural soil, the decrease in total concentration of all the widely used pesticides can result from degradation catalysed primarily by microorganisms. The dominant role of soil microorganisms in the dissipation of pesticide residues in the soil is well known.[50,136] The postulation that microorganisms enhance the decreases is supported by the log K_{OW} values of azoxystrobin, boscalid, fluroxypyr and prothioconazole, 2.5, 2.96, 2.2 and 3.82,[109,137,138] indicating their great affinity to organic matter. As a higher percentage of soil microorganisms are housed in the soil organic matter phase, it increases the probability of contact between the adsorbed pesticides and microorganisms. When microorganisms come in contact with pesticides, the pesticides may be utilised as a source of carbon and energy, rapidly decreasing the pesticide concentrations.[50] This reasoning is supported by various studies,[139–142] where the alteration of organic matter in the soil increases microorganism activity, subsequently escalating pesticide degradation. The lower degradation rate of fluroxypyr compared to the other pesticides can also be linked to soil organic matter and microbial activity. Kah *et al.* had previously highlighted that the degradation rate of fluroxypyr correlates significantly ($p<0.05$)

to the soil organic carbon.[116] Thus, the lowest percentage of decrease of fluroxypyr can be recognised due to the lower organic matter content in Irish agricultural soil.

Despite the fact the pesticide prothioconazole decreases in total concentration significantly ($p<0.05$), it does not necessarily mean the pesticide has dissipated entirely from the environment. It is highlighted by Lin *et al.*, prothioconazole can rapidly degrade to its metabolite prothioconazole-desthio, with half-lives below 5.82 days, and the metabolite is found to persist longer in soils and plants compared to the parent compound.[143] Additionally, prothioconazole-desthio has a higher potency than prothioconazole due to its highly active state.[144] Hence, even though the concentration of the parent compound reduces rapidly, further studies are required to assess the potential unintended impact of the metabolite prothioconazole-desthio in the Irish agricultural soil layers.

3.3.6 Environmental risk assessment

The results of our study indicate that understanding pesticide contamination in Irish agricultural soils is complex - with pesticides applied in sites sometimes, but not always, being detected, and conversely, pesticides not applied being detected. As such, an environmental risk assessment is beyond the scope of this study. However, the findings of this study highlight that potential risks may be involved with pesticide use in Ireland. Assessing pesticide risk in soils is difficult due to the lack of a maximum pesticide limit available for soils. Since pesticides used in agriculture can pose the risk of entering the water bodies via surface run-off or leaching,[145] which would then threaten the drinking water resources, hence, the available European parametric limit for groundwater and drinking waters was used as a proxy for assessing the threat level of the pesticide concentrations in this study. In terms of the compounds applied within 24 hours of sampling – prothioconazole, fluroxypyr, azoxystrobin – the threat level can be interpreted as a “worst case scenario”, with very little time for degradation to occur; however, for the compounds detected that were not recently applied, namely boscalid and the neonicotinoids – this scenario consideration does not apply. The Irish drinking water limit, which is also in agreement with the European drinking water regulation, stipulates individual concentrations of pesticides to be below 0.1 µg/L, while the parametric limit of total pesticide concentration is set to be below 0.5 µg/L,[146] which is similar to the groundwater quality standard.[147,148] These limits are emphasised for treated drinking water, where the potential pesticides in surface waters and groundwaters are reduced during the water treatment.

Nonetheless, the complete removal of pesticides from natural water sources is complex, and the detection of pesticides in drinking waters has been reported on

multiple occasions.[149–152] Findings by Schipper *et al.* highlight that untreated drinking water can exceed the parametric limit set for the total pesticide (>0.1 µg/L).[153] Even though it is difficult to establish the source of pesticide pollution in a water body, run-off and leaching of accumulated soil pesticides could be one of them.[154–156] It is noted here in this study that, based on the total pesticides quantified respective to each sampled site, only one site was established to be below this limit, while 24 sites had pesticide concentrations well above the limit of 0.5 µg/L (Figure 3.8 (a)). As explained earlier; however, simply considering the total pesticide concentrations is not entirely appropriate, given that some compounds were applied within 24 hours of sampling. When these compounds were removed from calculations, such that the only compounds considered were those that were not applied, 17 sites still had pesticide concentrations above the limit of 0.5 µg/L (Figure 3.8 (b)).

The risk of the pesticides leaching through the soil layers and contaminating groundwater can be predicted by other physicochemical characteristics, particularly the percentage of sand content. In this study, an average of 65.6% of the sampled sites were deemed to have high sand content (>45%). Although the soil layers are noted to have high sand content, which usually coincides with high levels of leaching,[71] our detection of multiple pesticides at significant quantities and in addition to the high clay content, allows us to conclude that leaching levels are low for the sites included in this study.

Table 3.6 Chemical properties of all the quantified pesticides.[105,109,137,157,158]

Pesticides	Vp (mPa)	pKa	log K_{ow}	K_{oc}	DT_{50} (days)	WS (mg/L)	GUS index
Acetamiprid	1.73E-04	0.7	0.8	267	8.2	4250	0.94
Azoxystrobin	0.00E+00	NI	2.5	594	181	6	3.10
Boscalid	7.20E-04	NI	2.96	9500	578	4.6	2.66
Clothianidin	0.00E+00	11.9	0.905	60	1155	327	3.74
Fluroxypyr	4.00E-06	2.94	2.2	136	21	91	2.42
Imidacloprid	0.00E+00	1.56	0.57	800	190	610	3.69
Prothioconazole	4.00E-04	6.9	3.82	1765	1336	300	-0.18
Thiacloprid	0.00E+00	NI	1.26	1584	142	185	1.1
Thiamethoxam	7.00E-06	NI	-0.13	68.4	301	4100	3.58

Notation. Vp = vapour pressure; pKa = dissociation constant; NI = non-ionisable; log K_{ow} = partition coefficient; K_{oc} = soil adsorption coefficient; DT50 = half-life; WS = water solubility; GUS = Groundwater Ubiquity Score.

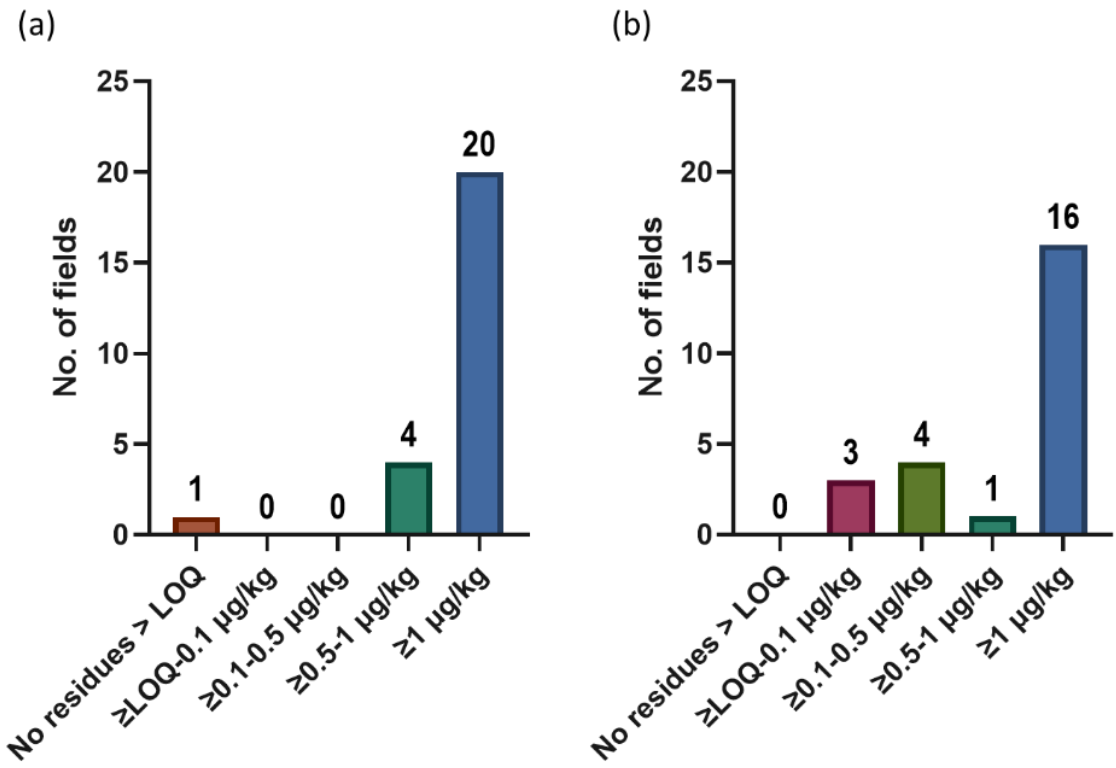


Figure 3.8 Pesticide concentration threshold (a) total pesticide quantified and (b) pesticides that were detected but not applied, (*n*=25), compared with the Irish Drinking Water's and groundwater parametric limit for total pesticide concentrations.[146–148]

Conversely, the risk of pesticide transmission reduces significantly with increasing clay percentage with McGinley *et al.*, reporting that soils with <20% clay have the highest risk of pesticide leaching through their layers and contaminating ground waters.[71] Considering that the mean clay percentage in the sampled sites is 18.4%, it can be established that pesticide movement in the Irish agricultural soil is very low. This statement is further supported by ElGouzi *et al.*, in their study of phenylurea pesticide adsorption potentials, where pesticide leaching decreases and retention increases with increasing clay content.[159] The risk assessment can be further supplemented by considering the pesticide properties. Adsorption coefficients (K_{oc} and log K_{ow}) can be used to predict the

leaching potential, where the smaller the K_{oc} value, the more mobile a pesticide compound would be, while the more hydrophilic a compound is (log K_{ow}) higher the leaching potential. Hence, by linking with the water solubility and GUS index values, the leaching potential of individual pesticides can be isolated.

Based on these pesticide properties, acetamiprid (not applied) and azoxystrobin (applied within 24 hours) were identified as the pesticides with a higher risk of leaching relative to the other pesticides. Even though, both of these compounds have low log K_{ow} and K_{oc} values, with high-water solubility, the GUS value of 0.94 for acetamiprid indicates that this compound has a very low leaching potential,[111] however, azoxystrobin identified to have a very high leaching potential with GUS value of 3.10 (Table 3.6).[137] On the other hand, boscalid (not applied) and prothioconazole (applied within 24 hours) were identified to have a higher probability of persisting in the soil rather than leaching due to their high affinity towards the soil and high hydrophobicity.

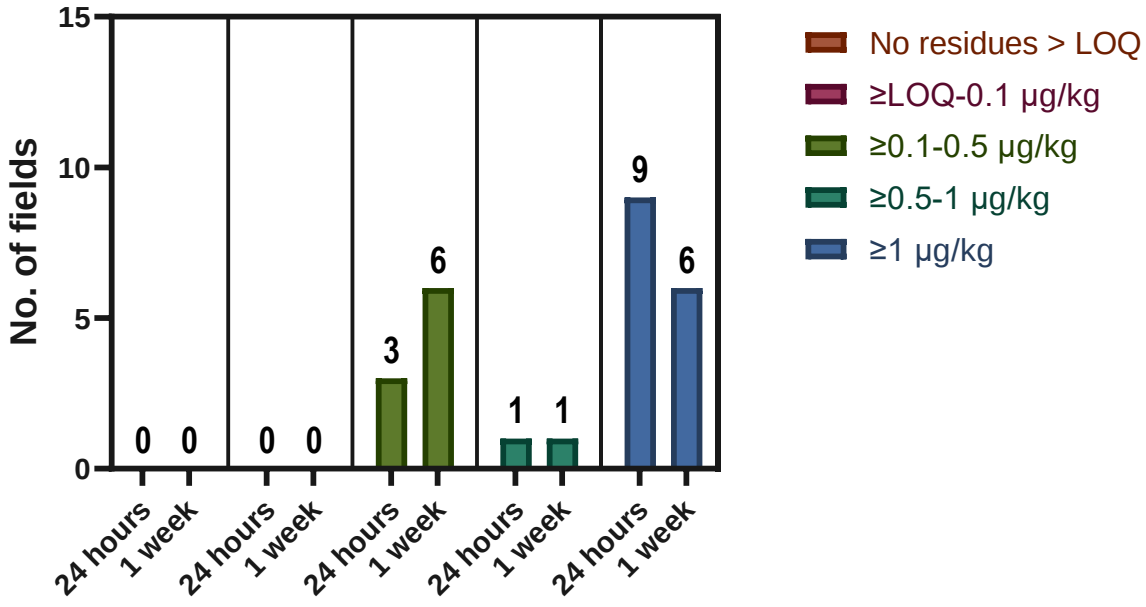


Figure 3.9 Pesticide concentration threshold of widely used pesticides within 24 hours and one week after pesticide application (*n* =13), compared with the Irish Drinking Water's and groundwater parametric limit for total pesticide concentrations [146–148].

Further assessment in the shifts in the widely used pesticide concentrations between the two timepoints (*n*=13), indicated that no fields were found to have total concentrations below 0.1 µg/kg. However, there were an increased number of fields with concentrations of ≥0.1-0.5 µg/kg, while the number of sites between the concentrations of ≥0.5-1 µg/kg remained the same for both sampling timepoints (one site) (Figure 3.9). Interestingly, the sites with total concentrations higher than 1 µg/kg decreased from nine to six sites. It can be inferred that the increasing number of fields in other lower pesticide concentration threshold categories is due to the decreasing total concentrations of the higher pesticide threshold categories. As highlighted before, boscalid and prothioconazole were identified to have a higher risk of persistence based on the physicochemical properties of both the soil and the pesticide. However, we noted that both

boscalid and prothioconazole decrease in concentration, with 30% and 72%, respectively (Figure 3.7). This highlights the difficulty of predicting the behaviour of pesticide compounds in the environment, strengthening the need for continuous pesticide monitoring in the soil.

The pesticide concentrations detected (Table 3.4) may also be used to assess the potential risks of individual pesticides. For example, acetamiprid was detected at levels below the parametric limit of 0.1 µg/kg, with a maximum concentration of 0.178 µg/kg, indicating a lower risk of persistence. By contrast, azoxystrobin, with a concentration of 2.523 µg/kg that could potentially contaminate groundwater.[146] This assessment of potential contamination is supported by McGinley *et al.*, who scored the compound 27 on a transmission risk ranking scale of 9-42.[71] Again, it should be considered that this concentration was detected within 24 hours of application. Even though other targeted pesticides have some leaching potential, not all their properties lead towards it. For instance, clothianidin and imidacloprid both have high water solubility and log K_{ow} values; however, the GUS index of 3.74 and 3.69, respectively, indicates that these pesticide compounds have a very low leaching potential. In summary, therefore, notwithstanding those the total pesticide concentrations in Irish agricultural soils exceeded the parametric limit set for Irish drinking water, the higher leaching potential risk was predictive for only two of the nine quantified pesticides, indicating a higher risk of pesticide accumulation than leaching. In relation to neonicotinoids in particular, acetamiprid has a higher risk of leaching, whereas clothianidin and imidacloprid have higher risks of accumulating. In this study, acetamiprid was the neonicotinoid detected least frequently, and the only one never to exceed the LOD. However, as we have no historical application

information, it is not possible to evaluate if this relative detection pattern is related to extent of leaching or accumulation.

Even though the accumulation of pesticides in the soil layers reduces the transport of those compounds through the environment, this does not automatically lead to the conclusion that they do not pose a potential hazard to human health. For instance, high concentrations of the accumulated pesticides would have a lower degradation rate, as highlighted by Fogg *et al.* The authors reported in their study on biobeds that the rate of degradation decreases with increasing concentrations of the pesticides isoproturon and chlorothalonil.[160] If the availability of pesticides increases in the soil, consequently, it would be expected to lead to biological uptake and bioaccumulation. Wang *et al.* noted that acetamiprid, imidacloprid and azoxystrobin can be translocated from soil to maize leading to bioaccumulation based on their bioavailability.[161] The authors also stated that pesticides with higher log K_{ow} value have a higher risk of accumulating in maize's roots, and pesticides with lower log K_{ow} have a higher risk of translocating to the shoots from the roots. Similar findings were reported by Li *et al.*, where out of five neonicotinoids studied, acetamiprid was identified to have a greater risk of accumulating in the Japanese mustard spinach vegetable shoots, while thiamethoxam was noted to accumulate in the vegetable roots.[162] Ultimately, it needs to be considered that both leaching, or accumulation pose a risk of indirectly increasing the risk to food and environmental safety.

3.4 Conclusion

This study investigated the levels of pesticide contamination in Irish agricultural soils. Soil samples were collected from 25 sites: 22 croplands, two grasslands and one commonage land. Pesticides were applied to 23 of these sites within 24 hours of sampling. From these fields, it was determined that 96% of the soil samples had detectable pesticide concentrations, where the concentrations of pesticides detected ranged from 0.18 to 45.7 µg/kg. Even though there was no recent application history at any of the sites, neonicotinoids were detected in 24 agricultural fields, even in a permanent grassland with no history of pesticide use. Similarly, to neonicotinoids, boscalid was detected in 10 sites even though it was not applied recently. However, glyphosate or AMPA were not detected in the single site where glyphosate was recently applied. It can be postulated that the failure to detect both glyphosate and AMPA could be due to the limitation of the QuPPe-PO method to extract both of these analytes successfully. This limitation is noted in the poor extraction recovery and linearity. Therefore, further studies are required to explore more accurate extraction methods for the successful extraction of glyphosate and AMPA.

Based on the distribution of quantified pesticide residues, 21 sites were shown to have three or more pesticide residues, with three fields having seven residues, against a baseline of no more than two pesticides applied recently at any individual site. Of the pesticides that were applied to the fields sampled, prothioconazole was the most detected pesticide, with the highest concentration quantified is 45.7 µg/kg and a detection rate of 89% in the sites where it was reported to be applied. Based on the hierarchical clustering analysis, luvisol is identified as the soil great group that dominates the highest pesticide

concentrations cluster, croplands discriminate themselves from other land-use types, and spring barley and winter wheat are the crop types of fields that have the highest concentration of fungicides and herbicides. Notably, through Pearson correlation analysis, it was found that the total pesticide detected, and clay fraction forms a significant positive correlation with the total pesticide detected ($p<0.05$). At the same time, log K_{ow} correlated significantly ($p<0.05$) with total pesticide concentrations. Based on the Irish drinking water parametric limit, it was observed that 23 sites with total pesticide concentrations exceeded that limit (0.5 µg/L). When recently applied compounds were removed from calculations, 17 sites still had pesticide concentrations above the limit of 0.5 µg/L. In an effort to assess the environmental risk, by considering both the sampled agricultural soil properties and the individual pesticide properties, acetamiprid and azoxystrobin were identified to have higher leaching potential, while boscalid and prothioconazole to have accumulation risk. However, further monitoring of the sites where widely used pesticides were detected, revealed that all the widely used pesticides decreased in concentrations, in the range of 18-72%, with highest decrease observed in the case of prothioconazole. Even though boscalid was identified to have higher persistence risk, within the one-week timepoint, the pesticide is observed to decrease by 30%. Two main findings emerged from this study: firstly, in 15 of 25 sites analysed, pesticides not applied recently were detected in the soils sampled. Secondly, where pesticides were applied recently, this did not automatically result in quantifiable concentrations of pesticides in the corresponding soils. Fluroxypyr was only detected in 4 of 11 sites where it was applied, and never above the LOQ. Whilst prothioconazole was detected more frequently, in 16 of 18 sites where it was applied, it was below the LOQ in 10 of

these sites. Correlations were observed between pesticide concentrations and the physiochemical properties of both the pesticides and soils. Future studies are needed to evaluate the extent to which applied pesticides degrade, leach or accumulate in soils, and the factors that impact this, and to determine the resultant potential risks of pesticide soil contamination on the Irish environment.

3.5 References

1 M. Tudi, H. D. Ruan, L. Wang, J. Lyu, R. Sadler, D. Connell, C. Chu and D. T. Phung, *Int J Environ Res Public Health*, 2021, 18, 1–24.

2 V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2019, **653**, 1532–1545.

3 S. Jess, S. Kildea, A. Moody, G. Rennick, A. K. Murchie and L. R. Cooke, *Pest Manag Sci*, 2014, 70, 1646–1654.

4 H. W. Jo, M. G. Park, H. J. Jeon, J. K. Moon and S. E. Lee, *Applied Sciences (Switzerland)*, , DOI:10.3390/app11188415.

5 E. C. Oerke, *Journal of Agricultural Science*, 2005, **144**, 31–43.

6 L. Carrasco Cabrera and P. Medina Pastor, *EFSA Journal*, , DOI:10.2903/j.efsa.2021.6491.

7 T. Misumi, N. Ogawa, K. Yamada and T. Shukuya, *Research Bulletin of the Plant Protection*, 2013, **49**, 1–9.

8 M. F. A. Jallow, D. G. Awadh, M. S. Albaho, V. Y. Devi and N. Ahmad, *Int J Environ Res Public Health*, , DOI:10.3390/ijerph14080833.

9 M. G. Park, C. G. Park, J. O. Yang, G. H. Kim, Y. Ren, B. H. Lee and D. H. Cha, *J Econ Entomol*, 2020, **113**, 1711–1717.

10 CSO, *Fact Sheet on Irish Agriculture (January 2021)*, 2021.

11 CSO, Farm Structure Survey, https://www.cso.ie/en/releasesandpublications/ep/p-fss/farmstructuresurvey2016/da/lu/, (accessed 14 May 2022).

12 FAO, Global Pesticide Use till 2017, http://www.fao.org/faostat/en/?#data/RP, (accessed 27 March 2020).

13 Eurostat, Utilised agricultural area by categories, https://ec.europa.eu/eurostat/databrowser/view/tag00025/default/table?lang=en, (accessed 25 May 2020).

14 Eurostat, Agri-environmental indicator - consumption of pesticides - Statistics Explained, https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agri-environmental_indicator_-_consumption_of_pesticides&stable=1#Data_sources, (accessed 31 July 2022).

15 E. Yasser and A. Alkareem, *Journal of Environment and Earth Science*, 2013, **3**, 199–210.

16 P. R. Durkin, *Fluroxypyr Human Health and Ecological Risk Assessment*, 2009.

17 G. Bhandari, K. Atreya, P. T. J. Scheepers and V. Geissen, *Chemosphere*, 2020, **253**, 126594.

18 M. Hvězdová, P. Kosubová, M. Košíková, K. E. Scherr, Z. Šimek, L. Brodský, M. Šudoma, L. Škulcová, M. Sáňka, M. Svobodová, L. Krkošková, J. Vašíčková, N. Neuwirthová, L. Bielská and J. Hofman, *Science of the Total Environment*, 2018, **613–614**, 361–370.

19 FAO, *ITPS Global Assessment of the Impact of Plant Protection Products on Soil Functions and Soil Ecosystems*, FAO:Rome, Italy, 2017.

20 Jannes Stolte, Mehreteab Tesfai, Lillian Øygarden, Sigrun Kværnø, Jacob Keizer, Frank Verheijen, Panos Panagos, Cristiano Ballabio and Rudi Hesse, *Soil threates in Europe: Status, methods, drivers, and effects on ecosystem services*, 2015.

21 D. Pimentel and M. Burgess, in *Integrated Pest Management: Pesticide Problems, Vol.3*, 2014, pp. 1–474.

22 H. M. G. van der Werf, *Agric Ecosyst Environ*, 1996, **60**, 81–96.

23 A. Wolters, 2003.

24 M. Houbraken, K. Doan Ngoc, F. van den Berg and P. Spanoghe, *Science of the Total Environment*, 2017, **599–600**, 1408–1416.

25 O. Zivan, Y. Bohbot-Raviv and Y. Dubowski, *Chemosphere*, 2017, **177**, 303–310.

26 D. Nuyttens, M. de Schampheleire, K. Baetens, E. Brusselman, D. Dekeyser and P. Verboven, *Trans ASABE*, 2011, **54**, 403–408.

27 S. Pätzold, C. Klein and G. W. Brümmer, *Soil Use Manag*, 2007, **23**, 294–305.

28 G. Imfeld, F. Meite, C. Wiegert, B. Guyot, J. Masbou and S. Payraudeau, *Science of the Total Environment*, 2020, **741**, 140437.

29 X. Zhang, Y. Luo and K. S. Goh, *Environmental Pollution*, 2018, **234**, 48–58.

30 J. B. Weber, in *Mechanisms of Pesticide Movement into Ground Water*, eds. R. C. Honeycutt and D. J. Schabacker, CRC Press, 2nd edn., 1994, pp. 15–41.

31 P. C. Kearney, D. R. Shelton and W. C. Koskinen, in *Kirk-Othmer Encyclopedia of Chemical Technology*, John Wiley & Sons, Inc., 2000, pp. 419–451.

32 S. Navarro, N. Vela and G. Navarro, *Spanish Journal of Agricultural Research*, 2007, **5**, 357–375.

33 M. Lechenet, F. Dessaint, G. Py, D. Makowski and N. Munier-Jolain, *Nat Plants*, , DOI:10.1038/nplants.2017.8.

34 M. Tudi, H. Li, H. Li, L. Wang, J. Lyu, L. Yang, S. Tong, Q. J. Yu, H. D. Ruan, A. Atabila, D. T. Phung, R. Sadler and D. Connell, *Toxics*, 2022, **10**, 335.

35 M. Tudi, H. D. Ruan, L. Wang, J. Lyu, R. Sadler, D. Connell, C. Chu and D. T. Phung, *Int J Environ Res Public Health*, 2021, 18, 1–24.

36 R. A. Cloyd and J. A. Bethke, *Pest Manag Sci*, 2011, 67, 3–9.

37 T. Gunstone, T. Cornelisse, K. Klein, A. Dubey and N. Donley, *Front Environ Sci*, 2021, 9.

38 R. Sur and A. Stork, *Bull Insectology*, 2003, **56**, 35–40.

39 A. Alford and C. H. Krupke, *PLoS One*, , DOI:10.1371/journal.pone.0173836.

40 R. M. González-Rodríguez, R. Rial-Otero, B. Cancho-Grande, C. Gonzalez-Barreiro and J. Simal-Gándara, *Crit Rev Food Sci Nutr*, 2011, **51**, 99–114.

41 M. Pateiro-Moure, M. Arias-Estévez and J. Simal-Gándara, *J Hazard Mater*, 2010, **178**, 194–201.

42 P. Wu, W. Z. Wu, Z. H. Han and H. Yang, *Environ Monit Assess*, , DOI:10.1007/s10661-016-5372-6.

43 R. S. Kookana, S. Baskaran and R. Naidu, *Australian Journal of Soil Research*, 1998, **36**, 715–764.

44 J. J. Pignatello, C. R. Frink, P. A. Marin and E. X. Droste, *J Contam Hydrol*, 1990, **5**, 195–214.

45 J. P. Bloomfield, R. J. Williams, D. C. Gooddy, J. N. Cape and P. Guha, *Science of the Total Environment*, 2006, **369**, 163–177.

46 S. Qian, H. Zhu, B. Xiong, G. Zheng, J. Zhang and W. Xu, *Environmental Science and Pollution Research*, 2017, **24**, 11493–11503.

47 O. Tiryaki and C. Temur, *J Biodivers Environ Sci*, 2010, **4**, 29–38.

48 I. Braschi, C. Emanuele and S. Blasioli, *Herbicides and Environment*, , DOI:10.5772/13056.

49 C. D. Børgesen, I. S. Fomsgaard, F. Plauborg, K. Schelde and N. H. Spliid, *Fate of Pesticides - In Agricultural Soils*, 2015.

50 M. Gavrilescu, *Eng Life Sci*, 2005, **5**, 497–526.

51 I. Scheunert, in *Fate and Prediction of Environmental Chemicals in Soils, Plants, and Aquatic Systems*, 1st edn., 2018, pp. 1–22.

52 A. Thompson and K. W. Goyne, *Nature Education Knowledge*, 2012, **4**, 7.

53 J. E. D. Davies and N. Jabeen, *The Adsorption of Herbicides and Pesticides on Clay Minerals and Soils. Part 2. Atrazine* *, 2003, vol. 46.

54 N. Kumar, I. Mukherjee and E. Varghese, *Environ Monit Assess*, 2015, **187**, 1–10.

55 B. Sarkar, M. Singh, S. Mandal, G. J. Churchman and N. S. Bolan, in *The Future of Soil Carbon: Its Conservation and Formation*, Elsevier Inc., 2018, pp. 71–86.

56 E. A. Kerle, J. J. Jenkins and P. A. Vogue, *Report EM8561, Oregon State University Extension Service*, 2007, 1–8.

57 L. Cox, R. Celis, M. C. Hermosin, J. Cornejo, A. Zsolnay and K. Zeller, *Environ Sci Technol*, 2000, **34**, 4600–4605.

58 O. D. Gaonkar, I. M. Nambi and S. K. Govindarajan, *J Soils Sediments*, 2019, **19**, 566–578.

59 M. Mehdizadeh, E. Izadi-Darbandi, M. T. Naseri Pour Yazdi, M. Rastgoo, B. Malaekeh-Nikouei and H. Nassirli, *International Journal of Recycling of Organic Waste in Agriculture*, 2019, **8**, 113–121.

60 E. Karanasios, N. G. Tsiropoulos, D. G. Karpouzas and C. Ehaliotis, *J Agric Food Chem*, 2010, **58**, 9147–9156.

61 M. J. Carpio, M. J. Sánchez-Martín, M. S. Rodríguez-Cruz and J. M. Marín-Benito, *Environments*, 2021, **8**, 32.

62 E. Karanasios, N. G. Tsiropoulos, D. G. Karpouzas and U. Menkissoglu-Spiroudi, *Chemosphere*, 2010, **80**, 914–921.

63 C. García-Delgado, A. D'Annibale, L. Pesciaroli, F. Yunta, S. Crognale, M. Petruccioli and E. Eymar, *Science of the Total Environment*, 2015, **508**, 20–28.

64 L. Tao and H. Yang, *Environ Monit Assess*, 2011, **175**, 227–238.

65 M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. C. Mejuto and L. García-Río, *Agric Ecosyst Environ*, 2008, 123, 247–260.

66 S. L. McManus, C. E. Coxon, P. E. Mellander, M. Danaher and K. G. Richards, *Science of the Total Environment*, 2017, **601–602**, 594–602.

67 L. Jones, B. Kinsella, K. Forde, A. Furey and F. Regan, *Analytical Methods*, 2017, **9**, 4167–4174.

68 M. Alexandra and D. Rivadeneira, 2021.

69 R. Tobin, *J Nutrit Health Food Sci*, , DOI:10.15226/jnhfs.2014.00113.

70 S. Kavanagh, M. Henry, J. C. Stout and B. White, *Environmental Science and Pollution Research*, 2021, 28179–28190.

71 J. McGinley, J. Harmon O'Driscoll, M. G. Healy, P. C. Ryan, P. E. Mellander, L. Morrison, O. Callery and A. Siggins, *Soil Use Manag*, 2022, **38**, 1162–1171.

72 A. López-Ballesteros, A. Delaney, J. Quirke, J. C. Stout, M. Saunders, J. C. Carolan, B. White and D. A. Stanley, *PeerJ*, 2022, **10**, 1–21.

73 European Union, *Commission Implementing Regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as*

regards the conditions of approval of the active substance imidacloprid, 2018, vol. 14.

74 European Union, *Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin*, 2018, vol. 14.

75 European Union, *Commission Implementing Regulation (EU) 2018/ 785 - of 29 May 2018 - amending Implementing Regulation (EU) No 540 / 2011 as regards the conditions of approval of the active sub stance thiamethoxam*, 2018.

76 M. Rexrode, M. Barrett, J. Ellis, M.-G. Patrick, A. Vaughan, J. Felkel and J. Melendez, *EFED risk assessment for the seed treatment of clothianidin 600FS on corn and canola*, 2003.

77 O. Lundin, M. Rundlöf, H. G. Smith, I. Fries and R. Bommarco, *PLoS One*, , DOI:10.1371/journal.pone.0136928.

78 J. D. Fernández-Bayo, R. Nogales and E. Romero, *J Agric Food Chem*, 2009, **57**, 5435–5442.

79 D. Goulson, *Journal of Applied Ecology*, 2013, **50**, 977–987.

80 J. M. Bonmatin, · I Moineau, · R Charvet, · M E Colin, · C Fleche and · E R Bengsch, in *Environmental Chemistry*, 2005, pp. 483–494.

81 P. Jeschke and R. Nauen, in *Comprehensive Molecular Insect Science*, 5th edn., 2005, pp. 53–105.

82 L. Simonsen, I. S. Fomsgaard, B. Svensmark and N. H. Spliid, *J Environ Sci Health B*, 2008, **43**, 365–375.

83 TEAGASC and Environmental Protection Agency (EPA), Irish Soil Information System, http://gis.teagasc.ie/soils/about.php, (accessed 12 May 2022).

84 I. Simo, R. E. Creamer, J. A. Hannam and R. J. A. Jones, *Irish Soil Information System: Final Technical Report 13*, 2014.

85 M. Saunders, G. M. Afrasinei, J. Zimmerman, A. Premrov, K. Black and S. Green, *Soil Organic Carbon and Land Use Mapping (SOLUM)*, 2022.

86 American Society for Testing and Materials, *Standard test method for particle-size analysis of soils D422-63 (2007)*, American Society for Testing Materials, Philadelphia, 2008.

87 F. J. Sikora and K. P. Moore, *Soil Test Methods From the Southeastern United States Southern*, 2014.

88 L. P. van Reeuwijk, *International Soil Reference and Information Centre, Wageningen, NL*.

89 M. R. Motsara, R. N. Roy and Food and Agriculture Organization of the United Nations., *Guide to laboratory establishment for plant nutrient analysis*, Food and Agriculture Organization of the United Nations, 2008.

90 D. S. Jenkinson and D. S. Powlson, *Soil Biol Biochem*, 1976, **8**, 209–213.

91 M. J. J. Hoogsteen, E. A. Lantinga, E. J. Bakker, J. C. J. Groot and P. A. Tittonell, *Eur J Soil Sci*, 2015, **66**, 320–328.

92 Supelco, 2021.

93 Supelco, 2021.

94 M. Anastassiades, ; D I Kolberg, ; E Eichhorn, A.-K. Wachtler, ; A Benkenstein, ; S Zechmann, ; D Mack, ; C Wildgrube, ; A Barth, ; I Sigalov, ; S Görlich, ; D Dörk and ; G Cerchia, *EU Reference Laboratory for pesticides requiring Single Residue Methods*, 2021.

95 M. Vickneswaran, J. C. Carolan and B. White, *Analytical Methods*, 2021, **13**, 5638–5650.

96 SANTE, *Analytical Quality Control and Method Validation for Pesticide Residues Analysis in Food and Feed*, Document N., 2019.

97 J. F. Bienvenu, G. Provencher, P. Bélanger, R. Bérubé, P. Dumas, S. Gagné, É. Gaudreau and N. Fleury, *Anal Chem*, 2017, **89**, 7560–7568.

98 H. Sun, Y. Qi, D. Zhang, Q. X. Li and J. Wang, *Environmental Pollution*, 2016, **209**, 177–185.

99 M. Lambert, C. Inthavong, F. Hommet, J. C. Leblanc, M. Hulin and T. Guérin, *Food Chem*, 2018, **240**, 997–1004.

100 G. P. Dively and A. Kamel, in *Journal of Agricultural and Food Chemistry*, 2012, vol. 60, pp. 4449–4456.

101 E. Hodgson, in *PESTICIDE BIOTRANSFORMATION AND DISPOSITION*, Elsevier Inc., 2012, pp. 53–69.

102 T. Doolotkeldieva, M. Konurbaeva and S. Bobusheva, *Environmental Science and Pollution Research*, 2018, **25**, 31848–31862.

103 R. K. Ghosh and N. Singh, *J Agric Food Chem*, 2009, **57**, 632–636.

104 J. DeCant and M. Barrett, *Revised Assessment for Clothianidin Registration of Prosper T400 Seed Treatment on Mustard Seed (Oilseed and Condiment) and Poncho/Votivo Seed Treatment on Cotton*, 2010.

105 C. Aubee and D. Lieu, *Environmental Fate and Ecological Risk Assessment for Boscalid New Use on Rapeseed, Including Canola (Seed Treatment) Boscalid CAS 188425-85-6 PC Code 128008*, 2010.

106 EPA, Guidance for Reporting on the Environmental Fate and Transport of the Stressors of Concern in Problem Formulations | US EPA, https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-reporting-environmental-fate-and-transport, (accessed 26 July 2022).

107 P. Kosubová, L. Škulcová, Poláková, J. Hofman and L. Bielská, *Chemosphere*, , DOI:10.1016/j.chemosphere.2020.126902.

108 C. Bernasconi, P. M. Demetrio, L. L. Alonso, T. M. mac Loughlin, E. Cerdá, S. J. Sarandón and D. J. Marino, *Agric Ecosyst Environ*, , DOI:10.1016/j.agee.2021.107341.

109 PubChem, Prothioconazole | C14H15Cl2N3OS, https://pubchem.ncbi.nlm.nih.gov/compound/Prothioconazole#section=Octanol-Water-Partition-Coefficient, (accessed 28 May 2020).

110 A. P. and V. M. A. APVMA, *Canberra: Australian Pesticides and Veterinary Medicines Authority*, 2007, 17.

111 J. Bonmatin, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. D. Mitchell, D. A. Noome, N. Simon-Delso and A. Tapparo, *Environmental Science and Pollution Research*, 2015, **22**, 35–67.

112 A. Tapparo, D. Marton, C. Giorio, A. Zanella, L. Soldà, M. Marzaro, L. Vivan and V. Girolami, *Environ Sci Technol*, 2012, **46**, 2592–2599.

113 A. Schaafsma, V. Limay-Rios and L. Forero, *Pesticide Management Science*, 2017, **74**, 323–331.

114 S. Humann-Guilleminot, Ł. J. Binkowski, L. Jenni, G. Hilke, G. Glauser and F. Helfenstein, *Journal of Applied Ecology*, 2019, **56**, 1502–1514.

115 CSO, Area, Yield and Production of Crops 2021 - CSO - Central Statistics Office, https://www.cso.ie/en/releasesandpublications/ep/p-aypc/areayieldandproductionofcrops2021/, (accessed 27 July 2022).

116 M. Kah, S. Beulke and C. D. Brown, *J Agric Food Chem*, 2007, **55**, 4487–4492.

117 M. Kah and C. D. Brown, *Rev Environ Contam Toxicol*, 2006, **188**, 149–217.

118 R. D. Wauchope, S. Yeh, J. B. H. J. Linders, R. Kloskowski, K. Tanaka, B. Rubin, A. Katayama, W. Kördel, Z. Gerstl, M. Lane and J. B. Unsworth, *Pest Manag Sci*, 2002, **58**, 419–445.

119 R. S. Kookana, R. Ahmad and A. Farenhorst, in *ACS Symposium Series*, American Chemical Society, 2014, vol. 1174, pp. 221–240.

120 E. Čadková, M. Komárek, R. Kaliszová, A. Vaněk and M. Balíková, *Soil Sediment Contam*, 2013, **22**, 404–414.

121 R. Durovic, J. Gajic-Umiljendic and T. Dordevic, *Pesticidi i fitomedicina*, 2009, **24**, 51–57.

122 M. Šudoma, N. Neuwirthová, M. Hvězdová, M. Svobodová, Z. Bílková, K. E. Scherr and J. Hofman, *Chemosphere*, 2019, **230**, 347–359.

123 V. Chaplain, L. Mamy, L. Vieuble-Gonod, C. Mougin, P. Benoit, E. Barriuso and S. Nelieu, *Pesticides in the Modern World - Risks and Benefits*, , DOI:10.5772/17035.

124 V. Chaplain, A. Brault, D. Tessier and P. De´fossez, *Eur J Soil Sci*, 2008, **59**, 1202–1208.

125 M. J. Crookes and P. Fisk, *Evaluation of using mobility of chemicals in the environment to fulfil bioaccumulation criteria of the Stockholm Convention*, 2018, vol. 5758319.

126 K. E. Scherr, L. Bielská, P. Kosubová, P. Dinisová, M. Hvězdová, Z. Šimek and J. Hofman, *Environmental Pollution*, 2017, **222**, 283–293.

127 M. J. Sanchez-Martin, M. S. Rodriguez-Cruz, M. S. Andrades and M. Sanchez-Camazano, *Appl Clay Sci*, 2006, **31**, 216–228.

128 M. L. Hladik, A. R. Main and D. Goulson, *Environ Sci Technol*, 2018, **52**, 3329–3335.

129 C. Lu, C. H. Chang, C. Palmer, M. Zhao and Q. Zhang, *Environ Sci Technol*, 2018, **52**, 3175–3184.

130 J.-M. Bonmatin, E. A. D. Mitchell, G. Glauser, E. Lumawig-Heitzman, F. Claveria, M. Bijleveld Van Lexmond, K. Taira, F. Sánchez-Bayo and F.-C. Claveria, *Science of the Total Environment*, , DOI:10.1016/j.scitotenv.2020.143822ï.

131 Y. Huang, L. Xiao, F. Li, M. Xiao, D. Lin, X. Long and Z. Wu, *Molecules*, 2018, 23.

132 J. Villaverde, M. Kah and C. D. Brown, *Pest Manag Sci*, 2008, **64**, 703–710.

133 L. Han, M. Xu, X. Kong, X. Liu, Q. Wang, G. Chen, K. Xu and J. Nie, *Environmental Pollution*, 2022, 120060.

134 Y. He, M. Meng, W. K. Yohannes, M. Khan, M. Wang, A. M. Abd El-Aty, F. Hacımüftüoğlu, Y. He, L. Gao and Y. She, *J Environ Sci Health B*, 2020, **55**, 388–395.

135 Y. Yu, X. Chu, G. Pang, Y. Xiang and H. Fang, *Journal of Environmental Sciences*, 2009, **21**, 179–185.

136 A. Walker, M. Jurado-Exposito, G. D. Bending and V. J. R. Smith, *Environmental Pollution*, 2001, **111**, 407–415.

137 K. A. Lewis, J. Tzilivakis, D. J. Warner and A. Green, *Human and Ecological Risk Assessment*, 2016, **22**, 1050–1064.

138 M. Kah and P. C. D. Brown, *Environment Department*, 2007, **PhD**, 228.

139 R. Abdelhafid, S. Houot and E. Barriuso, *Soil Biol Biochem*, 2000, **32**, 389–401.

140 M. E. Sánchez, I. B. Estrada, O. Martínez, J. Martín-Villacorta, A. Aller and A. Morán, *Chemosphere*, 2004, **57**, 673–679.

141 L. Cox, A. Cecchi, R. Celis, M. C. Hermosín, W. C. Koskinen and J. Cornejo, *Soil Science Society of America Journal*, 2001, **65**, 1688–1695.

142 T. K. James, H. Ghanizadeh, K. C. Harrington and N. S. Bolan, *Sci Rep*, , DOI:10.1038/s41598-021-83052-z.

143 H. F. Lin, B. Dong and J. Y. Hu, *Environ Monit Assess*, 2017, **189**, 1–9.

144 J. E. Parker, A. G. S. Warrilow, H. J. Cools, B. A. Fraaije, J. A. Lucas, K. Rigdova, W. J. Griffiths, D. E. Kelly and S. L. Kelly, *Appl Environ Microbiol*, 2013, **79**, 1639–1645.

145 E. Lewan, J. Kreuger and N. Jarvis, *Agric Water Manag*, 2009, **96**, 1633–1640.

146 EPA and HSE, 2019.

147 European Parliament, *Official Journal of the European Union*, 2000.

148 European Parliament, *Official Journal of the European Union*, 2006.

149 S. Mekonen, R. Argaw, A. Simanesew, M. Houbraken, D. Senaeve, A. Ambelu and P. Spanoghe, *Chemosphere*, 2016, **162**, 252–260.

150 N. Elfikrie, Y. bin Ho, S. Z. Zaidon, H. Juahir and E. S. S. Tan, *Science of the Total Environment*, , DOI:10.1016/j.scitotenv.2020.136540.

151 K. L. Klarich, N. C. Pflug, E. M. DeWald, M. L. Hladik, D. W. Kolpin, D. M. Cwiertny and G. H. LeFevre, *Environ Sci Technol Lett*, 2017, **4**, 168–173.

152 C. Skaarup, K. Wodschow, D. D. Voutchkova, J. Schullehner, O. Raaschou-Nielsen, H. R. Andersen, B. Hansen and A. K. Ersbøll, *Int J Environ Res Public Health*, , DOI:10.3390/ijerph19020823.

153 P. N. M. Schipper, M. J. M. Vissers and A. M. A. van der Linden, *Water Science and Technology*, 2008, 57, 1277–1286.

154 C. Aydinalp and M. M. Porca, *Journal of Central European Agriculture*, 2004, **5**, 5–12.

155 M. A. Dar, G. Kaushik and J. F. Villareal Chiu, in *Abatement of Environmental Pollutants: Trends and Strategies*, Elsevier, 2019, pp. 25–66.

156 N. Eevers, J. C. White, J. Vangronsveld and N. Weyens, *Adv Bot Res*, 2017, **83**, 277–318.

157 EFSA, *EFSA Journal*, 2011, **9**, 1–91.

158 EFSA, *EFSA Journal*, , DOI:10.2903/j.efsa.2010.1542.

159 S. ElGouzi, M. D. Mingorance, K. Draoui, E. H. Chtoun and A. Peña, *Chemosphere*, 2012, **89**, 334–339.

160 P. Fogg, A. B. A. Boxall and A. Walker, *J Agric Food Chem*, 2003, **51**, 5344–5349.

161 F. Wang, X. Li, S. Yu, S. He, D. Cao, S. Yao, H. Fang and Y. Yu, *J Hazard Mater*, , DOI:10.1016/j.jhazmat.2020.124269.

162 Y. Li, L. Long, H. Yan, J. Ge, J. Cheng, L. Ren and X. Yu, *Chemosphere*, 2018, **200**, 603–611.

Chapter 4 :

Fluctuation of microbial functional properties in Irish agricultural soil: during and after pesticide application

Abstract

Pesticide application has become synonymous with agricultural production. However, the impact of pesticide use on the soil microorganism population remains poorly understood, despite the importance of microorganism communities in maintaining soil function. In this research, we report the response of soil microbial biomass, respiration activities, and functional diversity to the quantified concentration of four widely applied pesticides, azoxystrobin, boscalid, fluroxypyr and prothioconazole, during and a week after pesticide applications in the agricultural fields across the Republic of Ireland. Relationships between soil physicochemical and microbial properties were assessed through Pearson correlation. In particular, soil organic matter (SOM) was shown to correlate positively with microbial biomass and functional diversity. From this, the microbial properties differences influenced by three distinct SOM classes were further studied. We found that increasing SOM content in the soil resulted in increased microbial biomass, respiration activities, and functional diversity. In soils with higher microbial biomass and respiration activities, prothioconazole total concentration was found to decrease significantly, with a 72.49% reduction in a one-week period, and the mean total pesticide concentrations in humic soil class decreased substantially, with 69% decrease in mean concentration. Furthermore, in different SOM classes, microbial properties responded distinctively following pesticide application. It is hypothesised that the lower SOM class may have a higher abundance of specialised microorganisms adapted to the pesticide compounds, resulting in proliferation following pesticide application. In contrast, the SOM class with higher organic matter content experienced higher qCO_2 levels after the pesticide application activity, indicative of increased stress

on the microbial population. This stress therefore decreased microbial biomass, respiration activities, and functional diversity. Overall, we conclude that the SOM content of agricultural land is crucial in preserving the soil's microbial properties. This insight into the changes in microbial biomass and activities provides a baseline understanding of the impact of pesticide application in Irish agricultural soil.

4.1 Introduction

Agricultural yield and food security rely heavily on the productivity of agriculture. Meanwhile, this agricultural productivity is dependent on the high quality of the soil in which the crops are grown. The term "soil quality" can be defined as the ability of soil to support animal and plant throughput while regulating the quality of water and air.[1] Soil quality is dependent on the intricate interaction between the soil's biotic and abiotic properties, which is also a good indicator of sustainable land management.[1] Soil abiotic properties, namely soil physical properties, defines the soil quality based on static indicators, such as soil texture, porosity and bulk density, and dynamic indicators, namely water retention capability, leaching potential and erosion potential. On the other hand, chemical properties that characterise soil qualities are soil organic carbon, nutrient availability, soil acidity, and salinity.[2] Additionally, soil layers are also inhabited by a great diversity of micro and macro fauna that plays a crucial role in decomposing, nutrient cycling, transforming, and modifying soil structure, directly affecting the soil quality,[3] in particular, soil microorganisms. Many soil processes, such as decomposition, nutrient cycling, carbon sequestration, water cycling and retention, and pest and pathogen population control, are catalysed by soil microorganisms.[3–6] However, due to the sensitive nature of microorganisms, they

are impacted by physical or chemical changes in their environment,[7] making them an effective indicator of soil quality.

As a result of anthropogenic activities, the degradation of the world's agricultural lands is increasing, which has been attributed to land misuse and soil mismanagement.[8] With the soil's physical and chemical properties experiencing degradation, namely the depletion of the soil's organic carbon pool, biological degradation is an inevitable consequence.[8] Just as the soil microorganisms influence the essential soil processes, the land use and the soil ecosystems also affect the diversity of the soil microorganism. This influence is reflected in studies by Rodrigues et al., and Tin et al., where changes in the natural land use to agricultural land use are noted to induce changes to the soil microorganism's community structure, in some cases, it initiates the loss of microbial biodiversity.[9,10] Interestingly, Drenovsky *et al.* demonstrated that microorganism communities shift to distinct specificity in heavily managed soils compared to natural soil.[11] This shift could be the result of differences in management practices, such as shifting soil pH, supplementation of soil nutrients through fertilising, and monocultures of crops and animals, influencing the structure of soil microbial communities.[6,11–14]

Intensification of agricultural production has been successful achieved for the past decades, mainly due to the development and usage of pesticides, which raises concerns about environmental contamination that increases the rate of soil degradation and decline of soil quality.[15] With constant usage of pesticides, pesticide residues tend to accumulate and persist in the soil layers,[16] eventually encountering soil microorganisms. In the soil layers, pesticide compounds can take multiple routes. However, degradation of pesticides metabolised by

microorganisms, or biodegradation, is the primary mechanism of pesticide degradation in the soil environment.[17,18] Even though biodegradation is the process where a group of microorganisms utilises pesticide compounds as a nutrient source to facilitate growth,[19] the effects of pesticides on microorganisms, in general, can vary.[20] For instance, Al-Ani et al. reported a significant decrease in microbial community and activities with the addition of the pesticides malathion, alphacypermethrin and glyphosate at different concentrations and over multiple periods of incubation.[21] Similar findings also report that microbial biomass and microbial community diversity decrease upon pesticide application.[20] However, it was noted that an initial decrease in diversity would be compensated by increased microbial biomass of resistant microbial communities, resulting in a further shift in the microorganism's community.[20] On the other hand, Medo *et al.* reported that even though the recommended doses did not unfavourably affect the microorganism community, repeated usage or accumulation of pesticides in the soil could enhance the community shifting effects.[22] In this study, it was also observed that when the pesticide concentrations were at 100-fold of the recommended doses, it increased microbial biomass and activities. Since pesticide application and soil pesticide residue concentrations affect the soil microorganisms' composition and activity, it is crucial to assess soil microbes in relation to soil quality and potential function.

Multiple methods can be used to assess soil microbial diversity, which is generally divided into two main approaches, determination using DNA-based approaches,[23] and assessment based on community-level physiological profiles (CLPP).[24] Even though DNA-based approaches can establish taxonomic information on individual microbe or groups of microbial community with great

sensitivity, it does poorly in assessing microbial physiological state. Blagodatskaya *et al.* have summarised soil microbial communities' physiological state into four states: active, potentially active, dormant, and dead.[25] Even though all the microbial states contribute toward the soil's total microbial biomass, the active microorganisms are involved in the current essential soil processes, and hence are crucial for soil health monitoring. The main downside of using DNA-based is that species detection does not differentiate between currently active and those dormant and dead,[23,25] reducing the effectiveness of soil quality assessment. On the other hand, assessing soil microbial communities and related essential soil processes, such as decomposition and nitrogen mineralisation, can be postulated using a phenotypic method such as the CLPP method.[26,27] MicroResp™ is one of the widely used CLPP measurement systems, which provides catabolic profiling through the measurement of CO_2 production of a whole soil microbial community through the utilisation of different carbon sources from whole soils.[28] The substrate-induced respiration (SIR) method of MicroResp™ is an efficient soil assessment based on an active microbial community that dynamically decomposes carbon sources, providing a more informative assessment of the agricultural soil quality compared to just DNA-based methods.

Shifts in land use types and management, especially fluctuation of soil organic matter, have previously been established to alter the microbial functional diversity,[29–34] however little is known about the distinct effects of pesticide application on the microbial community in a real-world setting, especially in Ireland. The previous soil microbial biomass and respiration study in Ireland by Richter et al. only reported the effect of different Irish soil physicochemical

properties and pedogenetic processes on microbial community structure and activity.[33] However, the impact of pesticide usage on microbial functional diversity and respiration activities was not studied. Hence, this study aimed to utilise MicroResp™ thus to (i) identify the relationships of soil physicochemical properties with microbial functional diversity, (ii) use the diagnostic categories used by Richter et al. to correlate the differences in SOM classes to microbial respiration activities, [33] and (iii) assess the changes of microbial functional diversity a week after pesticide application.

4.2 Materials and methods

4.2.1 Soil sampling and sample preparation

The soil samples used for this study are those from the previous chapter. As discussed therein, soil samples were collected from 25 different sites, i) within 24 hours and one week of pesticide application and ii) with no recent agricultural activities or pesticide use history, throughout Ireland (Figure 4.1). The soil samples collected for physicochemical analysis were stored at 4^{o}C until further analysis, while the soil samples collected for pesticide and MicroResp™ analysis were stored at -20^{o}C. For pesticide analysis, the soil samples were defrosted, air dried and sieved to 2 mm, and stored at -20^{o}C until further analysis. Meanwhile, for the microbial functional diversity analysis, the soil samples were defrosted, sieved through 2 mm and stored at 4^{o}C for no longer than one week.

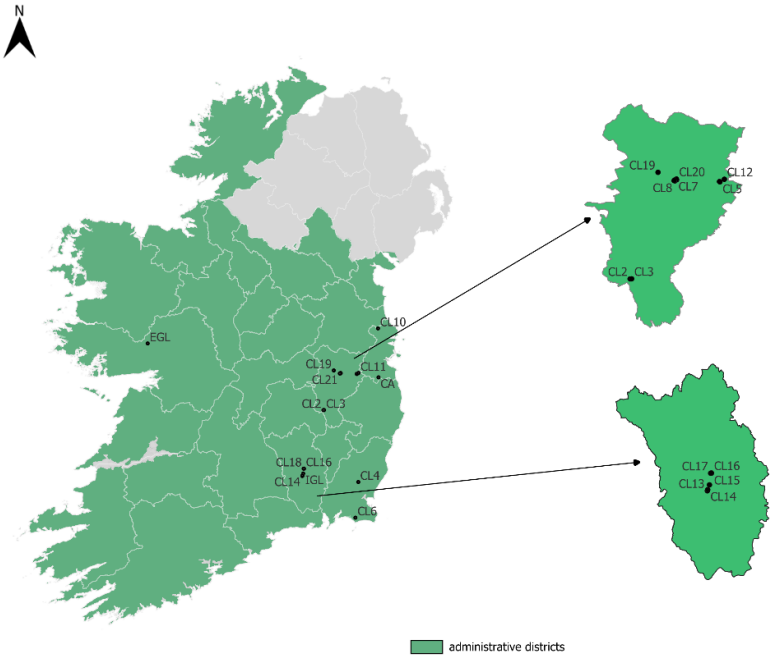


Figure 4.1 Sampling map of the study area (Republic of Ireland); callouts are for Kildare and Kilkenny counties. The label for each point indicates the site's labels: *EGL:* Extensive grassland.; *IGL:* Intensive grassland.; *CL:* Cropland.; *CA:* Commonage Area.

4.2.2 Soil physicochemical properties characterising

The complete characterisation of the measured soil physicochemical properties for all 25 sites is presented in Table 4.1. The soil textures were assessed and classified based on fractions, classified as sand, silt and clay, following the US Department of Agriculture (USDA) system to distinguish particle size.[35,36] The percentage of sand, silt and clay were measured using a standard hydrometer (152H ASTM) following dispersion in sodium hexametaphosphate (50 g/L). Soil pH was measured in a 0.01M $CaCl_2$ (at the ratio of 1:5) that had been previously mixed and left overnight (ISO, 2005). Haines-funnel systems were used to measure water holding capacity through saturation of soil samples.[37] SOM was calculated using weight loss on ignition. Firstly, soil moisture content was determined by calculating the difference in weight before and after oven drying

of fresh soil samples for three days (72 h).[38,39] The oven-dried samples were subjected to 550°C in a muffle furnace for 6 hours and left in the furnace overnight. The weight loss following ignition was calculated and presented as SOM.[40] Total nitrogen (TN) and total phosphorus (TP) were determined using cell test kits following Koroleff's and sulfuric solution digestion, respectively, and the concentrations of TN and TP were determined photometrically.[41,42]

Table 4.1 Summary of Irish soils' physicochemical (data from previous chapter), and microbial respiration properties (*n*=25).

Properties analysed	Me	Med	Min	Max	SD	CoV
Physical-chemical properties						
Clay (%)	18.4	18	11	29	5.08	27.62%
Silt (%)	5	49	15	16.16	8.49	52.53%
Sand (%)	36	82	64	65.6	8.92	13.60%
pH (CaCl2)	6.03	6.37	3.30	7.47	1.13	18.80%
SOM (%)	2.19	1.42	0.83	10.93	2.09	95.69%
MC (%)	5.4	5.03	2.84	11.71	1.84	34.15%
WHC (%)	12.5	9	5	31	6.77	54.37%
TP (mg/kg)	11.46	9.5	1.49	52.64	11.06	96.54%
TN (mg/kg)	56.01	54.32	23.27	137.1	26.39	47.11%
Microbial respiration properties						
Water (BR) (μg CO_2-C g^{-1} dry soil h^{-1})	0.75	0.6	0.08	3.08	0.59	79.24%
GAL (μg CO_2-C g^{-1} dry soil h^{-1})	1.34	1.15	0.04	6.66	1.11	83.05%
GL (μg CO_2-C g^{-1} dry soil h^{-1})	2.54	2.04	0.05	12.69	2.21	87.24%
MA (μg CO_2-C g^{-1} dry soil h^{-1})	4.18	3.29	0.84	17.93	3.35	80.04%
AKGA (μg CO_2-C g^{-1} dry soil h^{-1})	6.27	4.98	0.39	21.4	4.46	71.14%
CA (μg CO_2-C g^{-1} dry soil h^{-1})	4.82	4.17	0.28	16.12	3.2	66.48%
GABA (μg CO_2-C g^{-1} dry soil h^{-1})	1.15	0.94	0.06	4.28	0.97	83.79%

NAGA (μg CO_2-C g^{-1} dry soil h^{-1})	1.34	0.96	0.15	7.34	1.37	101.9%
MSIR (μg CO_2-C g^{-1} dry soil h^{-1})	129.9	101.7	27.23	437.4	91.43	70.41%
GLSIR-Biomass (μg C g-1 soil)	102	81.45	2.28	508.5	88.9	87.18%
Metabolic quotient (qCO_2)	1.15	0.27	0.06	46.72	6.03	524.0%
Shannon Functional Diversity Index (H')	1.69	1.75	0.58	1.87	0.25	14.57%

Notation. Min = minimum; Max = maximum; Med = median; Me = mean; SD = standard deviation; CoV = coefficient of variance; SOM = soil organic matter; MC = moisture content; WHC = water holding capacity; TP = total phosphorus; TN = total nitrogen; BR = basal respiration; GAL = galactose; GL = glucose; MA = malic acid; AKGA = α-ketoglutaric acid; CA = citric acid; GABA = γ-aminobutyric acid; NAGA = n-acetyl glucosamine; MSIR = multiple substrate-induced respiration; GLSIR = glucose substrate-induced respiration.

4.2.3 Soil pesticide extraction methods and analysis

The targeted pesticides (acetamiprid, AMPA, azoxystrobin, boscalid, clothianidin, fluroxypyr, glyphosate, imidacloprid, prothioconazole, thiacloprid, and thiamethoxam) were quantitatively determined based on the extraction methods described in the previous chapter. In brief, neonicotinoids, azoxystrobin, boscalid, fluroxypyr and prothioconazole were extracted using the Dutch mini-Luke method,[43] while the pesticide glyphosate and its metabolite, AMPA, were extracted using QuPPe-PO method.[44] Extractants of both extraction methods were analysed using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis method, with two columns, C18 column for analytes other than glyphosate and AMPA, while glyphosate and AMPA were analysed using the HILIC column method. In total, 25 sites were sampled (24 agricultural sites and one commonage), and the total pesticide concentration for the 25 sites was reported in the previous chapter.

4.2.4 Microbial respiration and functional diversity (MicroRespTM)

The measurement of microbial respiration activities and functional diversity was measured using the MicroResp^{TM} method. This CLPP method was employed as described by Campbell et al. and Creamer et al.[28,30] In brief, the fresh soil samples were sieved through a 2 mm sieve and corrected to a water-holding capacity of between 30 and 60% before dispensing into the deep-well plates using the MicroResp^{TM} filling device. The sample-filled deep-well plates were incubated for six days at 25ºC before the analysis to allow the microbial community to stabilise after the disturbance of sampling and sample preparation.[30,45] A spectrum of seven carbon substrates was selected based on their natural availability as nutrient content or chemical recalcitrance in agricultural soils, existing as root exudates, microbes or remains of microbes of plants.[32] The selected carbon substrates cover a range of carbohydrates, carboxylic acids, and one amino acid and amide, which are D-glucose (GLU), D-Galactose (GAL), L-malic acid (MA), α-ketoglutaric acid (AKGA), citric acid (CA), γ-aminobutyric acid (GABA) and n-acetyl glucosamine (NAGA) were used to assess SIR. Additionally, deionised water was used as a control to measure basal respiration. The substrates were prepared to a concentration of 30 mg g^{-1} soil water. The colourimetric indicator plate was prepared four days in advance by mixing 150 µL purified agar (1%), potassium chloride (150 mM), sodium bicarbonate (2.5 mM) and cresol red (12.5 µL l^{-1}). Subsequently, 150 µL aliquots were dispensed into each column of the detection plate.

After the soil incubation, 25 µL aliquots were dispensed at random into each well of the deep well plate containing soil samples and left open for 30 min in the dark to allow the release of CO_2 from carbonates.[29,30] Before the incubation with the

carbon substrate dispensed deep well plate, the colourimetric values of the detection plates were read at 570 nm (Tecan Infinite M200 plate reader, Tecan Austria GmbH, Grödig, Austria) and recorded as initial absorbance values (T_0). The deep well plates were sealed tightly and incubated for 6 h at 25^{o}C. Finally, after the 6 h incubation, the absorbance (A_{570}) of the detection plates was read a second time (T_1). The final absorbance values at T_1 were normalised using the initial absorbance values at T_0 before the CO_2 concentrations were calculated using a calibration curve: $\%CO_2 = 0.02 \times A_{570}^{-3.11}$ ($R^2 = 0.93$), which was adapted from,[46] where the $\%CO_2$ is the concentration of CO_2 in the headspace after incubation and A_{570} are the normalised absorbance values. In line with practice accepted elsewhere, calibration curves were adapted from the literature.[29,34] Using the formula provided in the MicroResp^{TM} technical manual (Cameron et al.), each substrate's respiration rates (µg CO2-C g-1 hour -1) were estimated from the $\%CO_2$. Subsequently, the respiration rates were corrected for basal respiration (deionised water).[47]

Microbial indices were calculated as follows: basal respiration was estimated based on the respiration rates (µg CO_2-C g^{-1} hour $^{-1}$) when only deionised water was added, respiration rates (µg CO_2-C g^{-1} hour $^{-1}$) of GL carbon substrate were used as GLSIR, multiple substrates induced respiration (MSIR) were calculated as the total sum of all the respired substrates per sample to represent the total microbial respiration activities.[31] The total microbial biomass was estimated based on the respiration rate of GLSIR, the calculation taken from Anderson et al.: µg C g^{-1} soil = GLSIR (µg CO_2-C g^{-1} hour $^{-1}$) x 40.04 + 0.37,[48] and it was recorded as GLSIR-biomass. The biomass-specific basal respiration quotient (qCO_2) is the ratio value of GLSIR to basal respiration.[29,33] The relative substrate

utilisation was calculated by dividing the SIR value of respective substrates by the MSIR. Calculating the relative utilisation is a more accurate representation of the metabolic preferences of the soil microbial communities while removing the bias of difference in microbial biomass due to agricultural management.[49] Finally, the microbial functional diversity was assessed using the Shannon functional diversity index (H').[50] In the case of substrates utilisation, as all the studied substrates had a response from all the sampled sites, the Shannon diversity index reflects the evenness or distribution of respiration activities across the sites.[29,51] Deployment of H' as a measure of diversity related to the microbial respiration activities induced by the heterogeneity of availability of soil organic substrates.[52] H' was calculated using the Equation 4.1:

$$H' = -\sum P_i \, LN \, (P_i)$$

Equation 4.1

Where P_i denotes the respiration induced by the *i*th carbon substrate expressed as a proportion of the total sum of absolute respiration rates.

4.2.5 Statistical analysis

Two software were used for data management: Microsoft Office Excel and Graphpad Prism (version: 9.4.1). Pearson correlations were used to study the relationship between soil physicochemical properties and microbial properties' significant differences between the microbial properties were tested using the Kruskal-Wallis non-parametric test. The rate of changes (%) in pesticide concentrations was determined respective to the sites, using this equation: ($Conc_{24hours}$ - $Conc_{1week}$/ $Conc_{24\ hours}$) x 100, where $Conc_{24\ hours}$ represents pesticide concentration quantified within 24 hours of pesticide application and

$Conc_{1week}$ indicates pesticide concentration quantified a week after pesticide application. For statistical purposes and to minimise bias of left-censored data,[53–55] only the pesticide concentrations above or at limit of detection (LOD) were considered, and for pesticide concentrations below limit of quantification (LOQ) but above LODs, the LOD values of each individual pesticides were used.

4.3 Results and discussion

The 25 sites were selected based on the recency of pesticide application. The targeted pesticides were neonicotinoids to study their current concentrations in the soil following the restriction for outdoor usage,[56–58] and selection of current widespread and large-scale usage in the Irish agricultural industry, azoxystrobin, boscalid, fluroxypyr, glyphosate, and prothioconazole.[59] The complete profile of pesticide concentration for all 25 sites was discussed in detail in the previous chapter. Acknowledging the widespread neonicotinoids concentrations in 96% of all the sampled sites, even though they were not applied recently and their long half-lives of 3-174 days,[60] the focus for this study was given to the sites where the current widely used pesticides (azoxystrobin, boscalid, fluroxypyr and prothioconazole) were detected and quantified, which accounted to 13 out of 24 agricultural sites. Correspondingly in this study, the microbial respiration, activities and functional diversity for all the 25 sites were studied and recorded as baseline data for the Irish agricultural soils and followed by the comparison of the microbial respiration activities and functional diversity within 24 hours and a week after pesticide application of the 13 sites. The most defining categories were determined by correlating soil and microbial properties using Pearson correlations. Accordingly, the soil diagnostic categories of organic matter were selected for soil microbial properties comparison, based on,[33] and the sampled sites into three distinct groups, mineral (0-3.5% SOM), humic (3.5-12% SOM) and histic (>12% SOM).

4.3.1 Relationship between soil physicochemical and microbial respiration properties

The effect of soil physicochemical properties on the microbial community is well established, and in each instance, the soil properties of organic matter are determined to be the main driver of microbial community structure.[33,61] Accordingly, in this study, a significant positive correlation ($p<0.05$) was observed between the SOM and the functional diversity index (H') and between microbial biomass and total nitrogen (Figure 4.2). Interestingly, SOM was also observed to correlate positively with the microbial biomass, even though not significantly. Similarly, microbial biomass correlates positively with clay percentage and soil pH, however, the correlations were not significant ($p>0.05$).

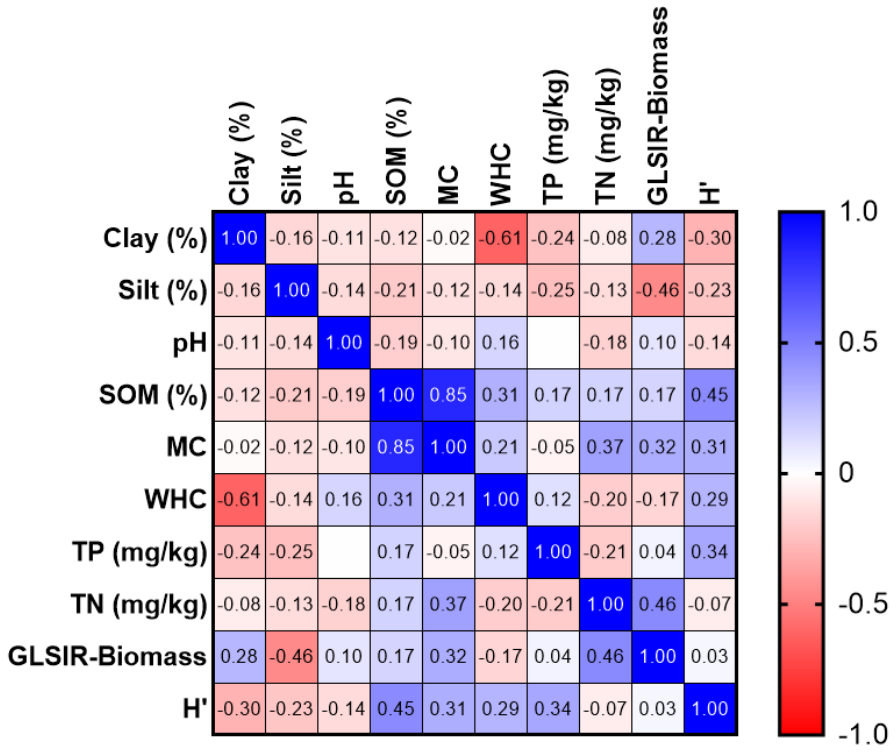


Figure 4.2 Pearson's correlation coefficient heatmap for each soil physicochemical and microbial functional properties.

The positive correlation of H' to the SOM was anticipated. The importance of SOM in agricultural soil cannot be understated. SOM plays a crucial role in supporting the soil's physical structure and facilitates chemical function in the soil, such as nutrient cycling and micronutrient availability improved through chelation.[62] Organic matter in soil can originate from heterogenous sources,[63,64] and as the soil microorganisms are involved in the decomposition of SOM, the more diverse labile carbon pool will steer the growth of specific microbial individuals required for the decomposition of organic matter, which in turn, give rise to diverse soil microorganism community.[65,66] A similar finding to this study was also reported by,[29] where the author observed that in the reduced-tillage agricultural management, the microbial respiration activities were determined to be more diverse due to more substantial organic matter input.

4.3.2 Relationship of SOM diagnostic classes and microbial respiration activities and functional diversity

Based on Pearson's correlation of this study, the SOM content of Irish agricultural soil was determined as the most influential soil property on microbial respiration activities and diversity. Hence further analysis was carried out on the effect of different SOM classes on the microbial respiration activities and diversity. Even though land management is not within this study's scope, it can be interpreted based on the division of SOM diagnostic classes. For instance, extensive agricultural land management, namely reduced tillage, is noted to increase the SOM content.[67–69] In some instances, converting intensively managed agricultural land to extensively managed land can drive a substantial accumulation of SOM content.[68,70] Therefore, considering the correlation between the management and SOM content, in this study, the soil diagnostic

classes can also be categorised; mineral class as intensive management, humic as less intense and histic as extensive management.

4.3.3 Effect of SOM classes on microbial metabolic preference

The SIR rate of individual carbon substrates is represented in Figure 4.3 (a), where there is a proportionate increase of all SIR rates relative to the increasing SOM classes. GAL and GL are simple carbon substrates which can easily oxidised by soil microorganisms as sources of energy.[30,71] Therefore, the utilisation rate of these two carbon substrates reflects the catabolic capacity of the studied soil microbial community. In this study, the absolute and relative utilisation rate of GAL and GL were noted to increase across the SOM classes, indicating that increasing SOM content results in proliferation of overall soil microbial community. Additionally, the carboxylic acid-based carbon substrates MA, AKGA and CA were observed to have consistently high respiration rates between the carbon substrates. Organic acids are key molecules of crop root exudates released into the soil layers as a response to environmental stress rather than for the decomposition of organic matter.[72–75] Hence, a higher level of carboxylic acid-based substrates utilisation indicates that agricultural sites in this study are intensively managed, resulting in a high reproductive rate of *r*-strategist soil microorganisms adapted to degrade the labile organic acid rapidly.[76] Similar findings had also been reported by,[29] where their study of ten European long-term field experiments revealed that AKGA is the most utilised substrate in terms of absolute and relative respiration in sites with intensive agricultural management compared to less intensive conventional agricultural practices. However, contrary to the findings of,[29] in this study, carboxylic based-carbon substrate's respiration rate was observed to increase with SOM class, but the

relative percentage of substrate utilisation was noted to decrease with SOM class (Figure 4.3 (b)).

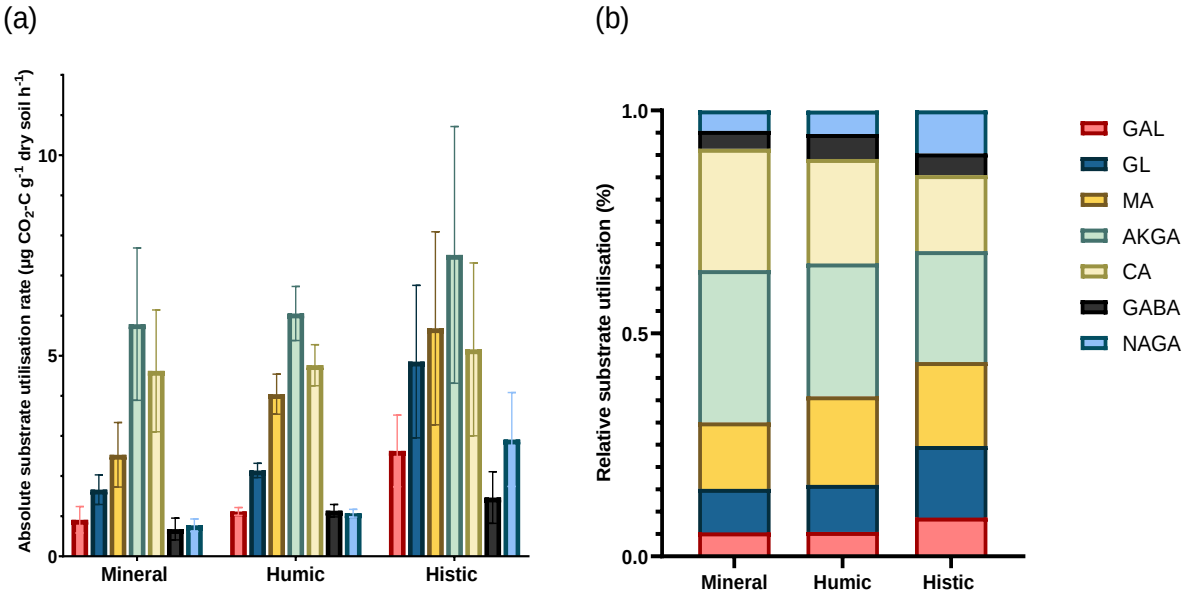


Figure 4.3 Metabolic preferences of the soil microbial communities in three SOM classes (*n* = 25; mineral = two sites, humic = 19 sites, and histic = four sites) expressed as (a) absolute substrate utilisation of seven substrates and (b) relative substrate utilisation rate (%). The error bars represent 95% confidence intervals.

On the other hand, even though GABA and NAGA induced the lowest respiration rate, these carbon substrates' respiration rate was shown to increase with increasing SOM class (Figure 4.3 (b)). GABA and NAGA are amino acid-based carbon substrates. Hence, the respiration rate induced by these substrates represents the fraction of microorganisms capable of utilising amino acids as nitrogen sources. Even though ammonium nitrogen is the desired source of nitrogen, nitrogen can be obtained through assimilatory nitrate reduction, and capable microorganisms can utilise amino acids through deamination/transamination, peptide synthesis or deamination through oxidases

enzymatic processes.[77] Hence, based on the absolute respiration rate, it can be postulated that the community of microorganisms that can utilise amino acid as a nitrogen source was lower in abundance in the mineral and humic class but higher in the histic class of Irish agricultural soils. On the other hand, lower GABA and NAGA activities in mineral and humic classes compared to histic class could be due to the removal of amino acids as a result of sorption to soil exchange sites, reducing their availability for utilisation by soil microorganisms.[78] Based on the relative utilisation, organic acid utilisation was observed to decrease with increased OM classes. This response could indicate that the stress indicator decreased as the OM content increases or intensity decreases.[29]

4.3.4 Effect of SOM classes on microbial respiration activities, biomass, and functional diversity

In addition to the absolute substrate utilisation rate of different carbon substrates in Irish agricultural soils, this study also reports the disparity in the level of microbial respiration activities, biomass, and functional diversity in the same sites. Based on the results obtained, it can be concluded that the SOM drove the microbial respiration activities. Overall, all the measures of microbial biomass, respiration activities, and functional diversity were found to increase with increasing SOM class, except for the qCO_2 measure. Basal respiration represents the general soil microbial respiration activities, representing the level of activity in a real-world setting (Figure 4.4 (a)).[79] Comparison between the three SOM classes indicates that the histic class had significantly higher microbial respiration activities than the mineral class ($p<0.01$); however, it was not significantly higher than the humic class, even though the basal respiration was observed to be 21% higher. On the other hand, the humic class had a 53% higher basal respiration rate than the mineral class, with a significant difference of

p<0.0001. Even though the differences between the SOM classes were not significant, the MSIR, microbial biomass and functional diversity index followed the same trend, where the histic class had the highest value and the mineral class has the lowest value. In this study, the qCO_2 measure between the three SOM classes had contrasting results to other microbial measures, where the humic class has a significantly higher qCO2 value than other classes (p<0.0001).

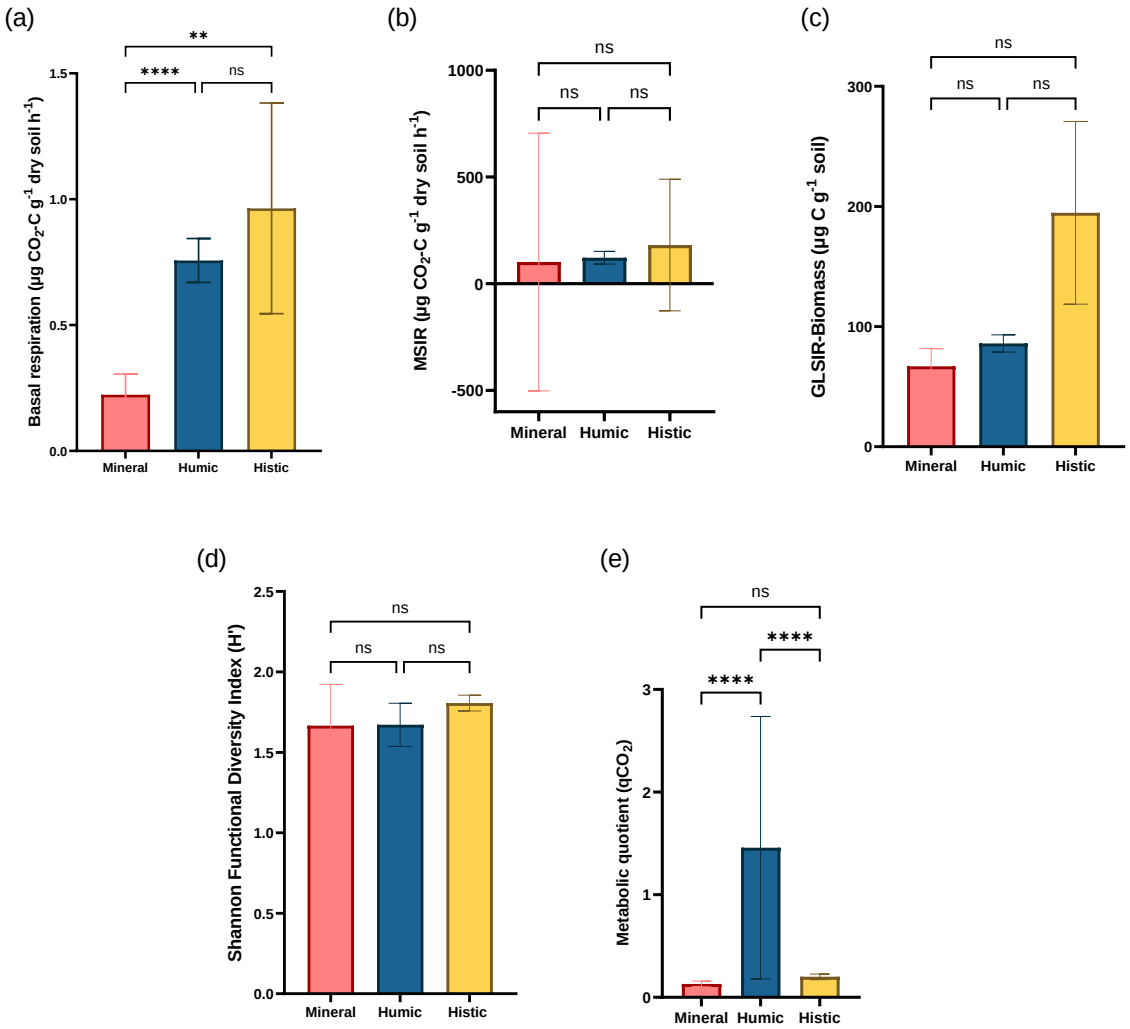


Figure 4.4 Microbial respiration, biomass, and functional diversity, of three distinct SOM diagnostic classes (a) basal respiration, (b) GLSIR-biomass, (c) MSIR, (d) Shannon functional diversity index (H') and (e) metabolic quotient (qCO_2). The error bars represent 95% confidence intervals. Asterisk denotes significance (** p < 0.01, ****p < 0.0001) and ns indicates no significance.

Interestingly, no significant degree of functional diversity changes was observed in the three SOM classes. However, thus finding is in line with other studies that did not find an effect on microbial functional diversity with varying content of SOM.[29,80,81] Microbial biomass represents the total microbial abundance, representing the living and dead microorganisms.[82] This study estimated microbial biomass through GL respiration rate to represent the total microbial abundance. As glucose substrate can easily be oxidised by the soil microorganism,[71] it can accentuate the active and living fraction of soil microorganisms.[48,83,84] As noted previously, even though it is not significantly different between the SOM classes, the microbial biomass is noted to increase with increasing SOM class, with the humic class having 22% and histic having 66% times higher biomass than the mineral class (Figure 4.4 (c)). Similar findings have also been reported by,[33,85] where the authors noted a directly proportional response of microbial biomass to increasing SOM. A higher concentration of SOM would result in increased labile organic carbon, considered the primary energy source for soil microbes, hence supplementing the growth of the microbial community.[62,86,87]. Moscatelli et al. concluded that less intensive land management increases soil microbial biomass and improves SOM stabilization and nutrient mineralization.[31] With a broader heterogeneous range of labile organic carbon available with higher SOM content, this would enhance the MSIR, representing the total soil microbial functional capacity. Similar to the microbial biomass, the histic class in the study reported here was observed to have the highest MSIR, with 44% higher than the mineral class and the humic class to have 17% higher MSIR than the mineral class (Figure 4.4 (b)). These differences in MSIR indicate that histic and humic classes had a microbial community that

can utilise a broader range of substrates compared to the mineral class. Ultimately, the distinctive response of the soil microbial properties respective to the SOM classes strengthens the land management discriminative capability of MicroResp™.

4.3.5 Comparison of microbial biomass, respiration activities and functional diversity to pesticide concentrations

The comparison of microbial respiration activities and functional diversity was driven based on the quantification of the targeted pesticide in all the studied sites, and out of the 25 sites, 13 sites were selected (Table 4.2). Interestingly, discrimination of the 13 sites based on the SOM diagnostic only resulted in two categorisations, mineral with one site and humic with 12 sites, with no pesticide of interest detected in the histic soil sites. It can be postulated that considering histic soil sites have higher microbial biomass and respiration activities (Figure 4.4), this could have led to the rapid degradation of the applied pesticides, resulting in no detection of the targeted pesticides.

Table 4.2 Summary of the concentrations of analysed pesticides in Irish soils within 24 hours of pesticide and one week after pesticide applications (*n*=13), data from previous chapter.

Pesticides (µg/kg)	Sampling	Min	Max	Me	Med	SD	CoV	Rate of Change (%)
Acetamiprid	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	
	One week	n.d.	<LOQ	<LOQ	<LOQ	-	-	100
Azoxystrobin	24 hours	n.d.	2.523	0.319	1.323	0.757	80.51 %	
	One week	n.d.	2.071	0.159	1.03	0.574	0.00%	-50.05
Boscalid	24 hours	n.d.	1.59	0.142	0.795	0.437	117.9 0%	

	One week	n.d.	1.034	0.099	0.517	0.284	82.02 %	-30.05
Clothianidin	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	
	One week	n.d.	0.271	0.044	0.136	0.094	11.63 %	104.32
Fluroxypyr	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	
	One week	n.d.	<LOQ	<LOQ	<LOQ	-	-	0
Imidacloprid	24 hours	n.d.	<LOQ	<LOQ	<LOQ	-	-	
	One week	n.d.	-	-	-	-	-	-
Prothioconazole	24 hours	n.d.	45.66	3.851	0.44	12.563	256.50%	
	One week	n.d.	5.02	1.06	0.44	1.468	98.84 %	-72.49
Thiacloprid	24 hours	n.d.	0.24	0.086	0.08	0.051	72.74 %	
	One week	0.194	0.242	0.234	0.238	0.013	5.58%	171.38
Thiamethoxam	24 hours	n.d.	1.465	0.872	1.376	0.719	3.70%	
	One week	n.d.	1.489	0.91	1.469	0.749	0.60%	4.37

Notation. Min = minimum; Max = maximum; Med = median; Me = mean; SD = standard deviation; CoV = coefficient of variance.

Table 4.2 summarises the concentrations of the targeted pesticides detected at two sampling time points, 24 hours and one week after. The negative value of the rate of change (%) indicates a decrease in concentrations, while the positive value indicates an increase in concentrations between the studied time points. The widely used pesticides, azoxystrobin, boscalid and prothioconazole were observed to decrease in concentration within a week, with prothioconazole decreased significantly ($p<0.05$) with 72.49% lower total concentrations, followed by azoxystrobin and boscalid, 50.05% and 30.05%, respectively. Meanwhile, the other widely used pesticide, fluroxypyr, was only detected below LOQ, in both

sampling timepoints. Interestingly, all the studied neonicotinoids, except for imidacloprid, were observed to have increased in concentration, with the highest increase seen with thiacloprid, with 171.38% increase, followed by clothianidin with 104.32%, acetamiprid with 100%, and thiamethoxam with just 4.37%. Any observed decreases in concentration were attributed to likely dissipation or degradation of the pesticide between these two time points, though the increases in concentration are reasoned based on the sampling design. Even though the sampling effort was to represent the sites as a whole by sampling at random points in the respective sites, it could have led to a homogenised sample with a distinct concentration of pesticides between the two sampling periods. Additionally, considering the neonicotinoids were not applied recently, the increase in concentration might also be attributed to movement of pesticide through the environment. Considering that neonicotinoids have an exceptionally long half-life, 31-450 days for acetamiprid, 148-7000 days for clothianidin, 28-1250 days for imidacloprid, 3.4-1000 days for thiacloprid and 7-335 days for thiamethoxam,[88,89] these pesticides are prone to pesticide accumulation either through surface runoff or deposition of spray drift from nearby fields.[90–93] This reasoning is strengthened based on the log K_{OW} values of neonicotinoids, where the log K_{OW} trend goes as thiamethoxam<clothianidin<acetamiprid<thiacloprid,[94] which coincides with the rate of concentration increase of neonicotinoids. Therefore, the higher the log K_{OW} value, the more inclined the neonicotinoid to accumulate in the soil following deposition.

Comparison of the fluctuation of the mean concentration of the widely used pesticides between the studied two SOM classes revealed no significant differences in concentration shift (Fig. 4.5 (a)). In both SOM classes, the mean

concentration of the all the widely used pesticides were observed to decrease between the studied two timepoints, except for fluroxypyr in the mineral class, where fluroxypyr was not detected in the mineral class within 24 hours of pesticide application, but it was detected in the one week after sample. However, the quantified concentrations of fluroxypyr were below LOQ, therefore the LOD value (0.88 µg/kg) of fluroxypyr were used for reporting. The concentration increases of fluroxypyr a week after can be explained based on the fluroxypyr's K_{OW} value of 2.20,[95] where the compound is deemed to have reduced mobility due to its affinity toward SOM. However, fluroxypyr will be more labile in the mineral class where the SOM is low, reducing the contact with soil microorganisms for degradation to take place. On the other hand, in the humic class, fluroxypyr will readily adsorb onto SOM, resulting in rapid degradation supported by microorganism. Furthermore, the increase in fluroxypyr's concentration in the mineral class can be explained based on degradation conditions. Fluroxypyr is an aminopyridine fungicide compound which is vulnerable to degradation through co-metabolism by soil microorganisms,[96] which could release labile nitrogen as an energy source. Hence, the absence or lower biomass of microorganisms capable of oxidising fluroxypyr, results in a lower pesticide degradation rate. This postulation was demonstrated by Tao et al., where the authors demonstrated that by removing both soil microorganisms and SOM, the degradation of fluroxypyr were slowed.[96]

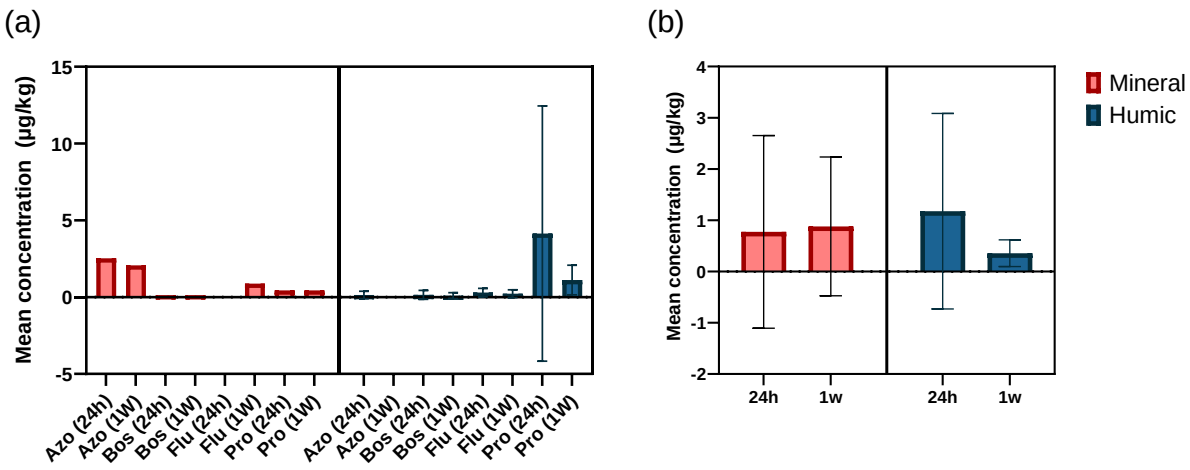


Figure 4.5 Changes in mean pesticide concentrations of widely used pesticides compared between 24 hours and one week after pesticide application, in two SOM classes (a) individual pesticide concentrations and (b) total pesticide concentrations, (*n*=13; mineral=1 site, and humic=12 sites). Notation. Azo = azoxystrobin; Bos = boscalid; Flu = fluroxypyr; and Pro = prothioconazole.

The comparison of the mean concentration of widely used pesticides between the two time points and the two SOM classes indicates that in the mineral class, mean pesticide concentration slightly increase from 0.77 to 0.88 µg/kg. In contrast, in the humic class, mean pesticide concentration decreased extensively, but not statistically significant, from 1.18 to 0.36 µg kg^{-1} (Figure 4.5 (b)). All the widely used pesticides in this study, azoxystrobin, boscalid, fluroxypyr and prothioconazole, had been previously established to be prone to biodegradation.[96–100] The humic class was shown to have 29% higher microbial biomass and 20% higher MSIR compared to the mineral class (Figure 4.4 (b) and (c)). This supports and strengthens the evidence that the decrease in the total pesticide concentration in the humic class can be attributed to higher microbial biomass and respiration activities. Interestingly, looking further into the changes in soil microbial community following pesticide application revealed shifts in the community structure in a week (Figure 4.6). There was contradicting trend

between the mineral and humic class, where in the mineral class, metabolic preference rates were observed to increase following pesticide application, while a slight decrease in humic class was seen. Most notably in the mineral class, the substrate utilisation rate of all the carboxylic acid-based substrates, AKGA, MA and CA, increased by 40%, while the simple carbohydrates, GL, and GAL, increased by 51% and 60%, respectively. A considerable utilisation rate increase observed was for the amino acid and amide, GABA and NAGA, with 61% and 67%, respectively. Meanwhile, the substrate utilisation rate of all the substrates declined in the humic class, with GAL having the highest decline with 42% and the lowest decline was CA with 5%, however, AKGA has a slightly increased utilisation rate of 2%.

Interestingly, looking further into the changes in soil microbial community following pesticide application reveals shifted in the community structure between the two sampling times (Figure 4.6). There was contradicting trend seen between the mineral and humic class, where in the mineral class, metabolic preference rates were observed to increase following pesticide application, while a slight decrease was observed in the humic class. Most notably in the mineral class, the substrate utilisation rate of carboxylic acid-based substrates, AKGA, MA and CA, increased by 40%, while the simple carbohydrates, GL, and GAL, increased by 51% and 60%, respectively. A considerable utilisation rate increase observed was for the amino acid and amide, GABA and NAGA, with 61% and 67%, respectively. Meanwhile, the substrate utilisation rate of all the substrates declined in the humic class, with GAL having the highest decline with 42% and the lowest decline was CA with 5%, however, AKGA had a slightly increased utilisation rate of 2%.

Further analysis of the microbial biomass, respiration activities, and functional diversity conforms to the trend of absolute substrate utilisation, where the mineral class was observed to have a significant increase in microbial biomass ($p<0.001$), increased MSIR, and increased functional diversity. In contrast, the humic class displayed the inverse trend (Figure 4.7 (a), (b), and (c)). Interestingly, the qCO_2 is the only measure where the humic class indicated a significant increase ($p<0.0001$) (Fig 4.7 (d)). qCO_2 as indicator stress on the microbial community, indicating distinctly that there was increased stress in the humic class. This observation was congruent with the overall results taking all the microbial respiration activities and diversity into account, as microbial biomass significantly decreased ($p<0.0001$), as MSIR decreased, and the diversity significantly decreased ($p<0.05$). The stress on the microbial community was apparent with the reduction of all these microbial respiration activities, biomass, and diversity. Furthermore, the increased stress was confirmed based on the previous postulation based on the increased AKGA substrate utilisation and qCO_2 levels (Figure 4.6 and Figure 4.7 (d)). However, despite the decrease in microbial properties in the humic class, the microbial biomass and MSIR were still higher than in the mineral class, which reflected the substantial decrease in total pesticide concentration in the humic class compared to the mineral class.

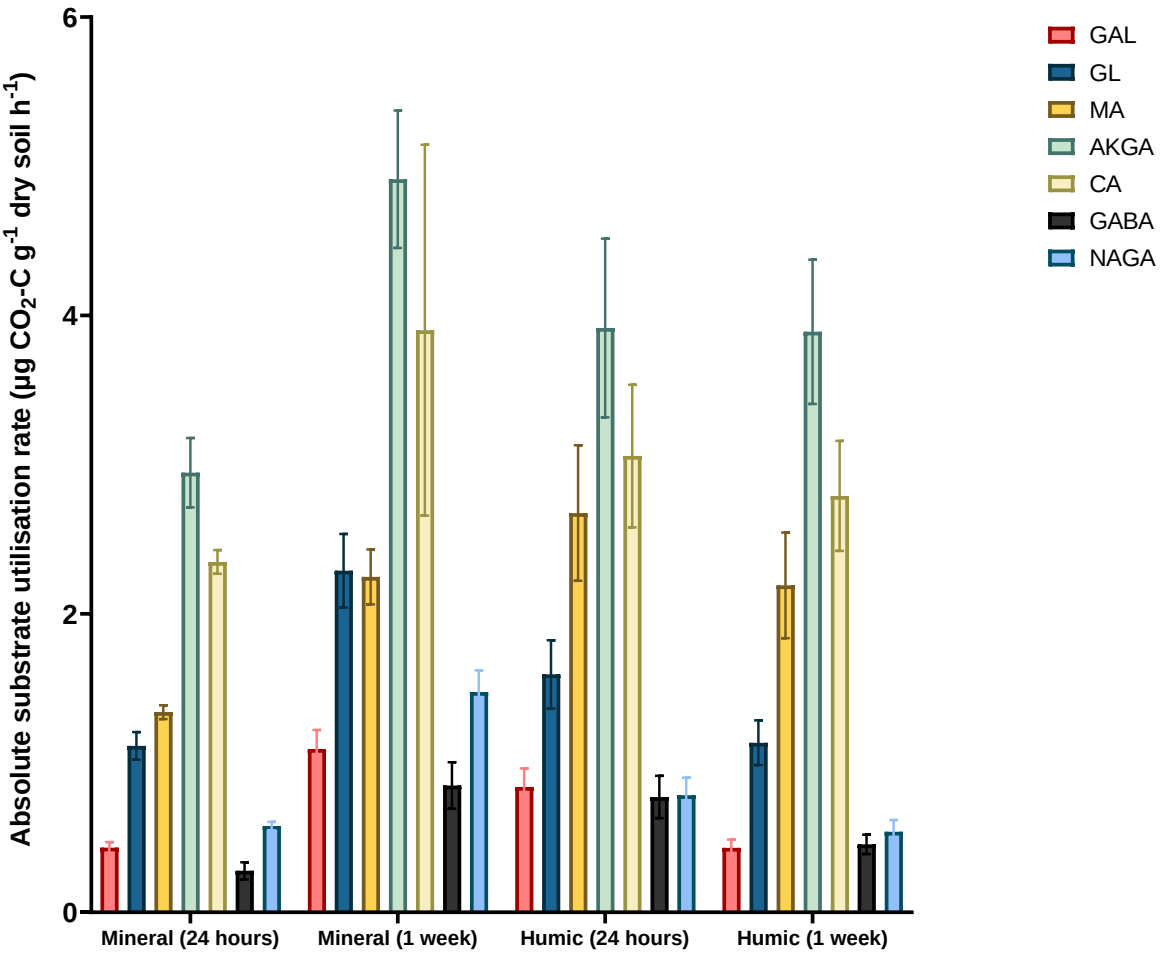


Figure 4.6 Absolute substrate utilisation of seven substrates in two SOM classes (*n* = 13; mineral = 1 site, and humic= 12 sites). The error bars represent 95% confidence intervals.

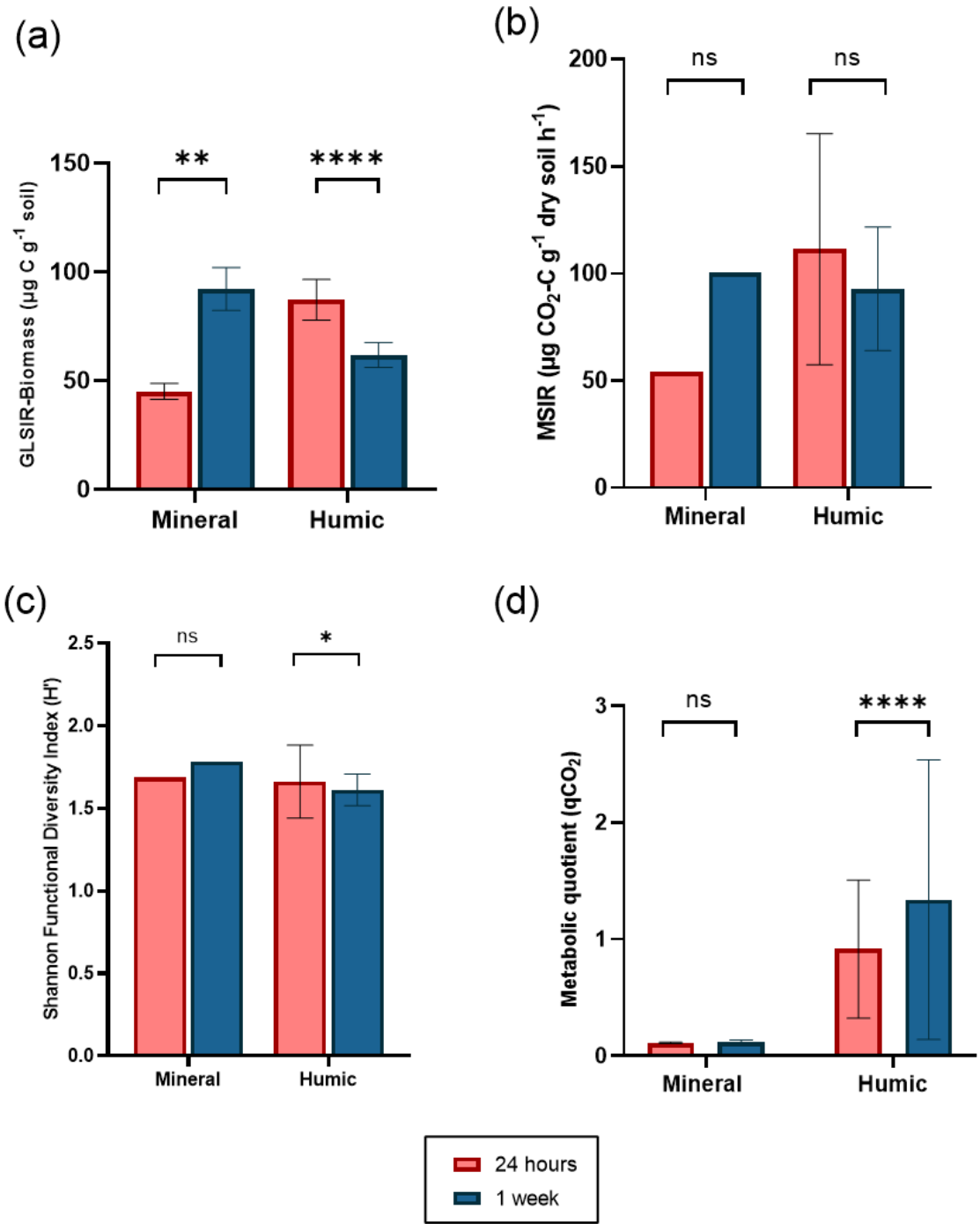


Figure 4.7 Comparison of all microbial respiration activities and functional diversity of two SOM diagnostic classes between 24 hours and one week after soil samples (a) GLSIR-biomass, (b) MSIR, (c) Shannon functional diversity index, and (d) metabolic quotient. The error bars represent 95% confidence intervals. Asterisk denotes significance (* $p < 0.05$, ** $p < 0.01$, ****$p < 0.0001$) and ns indicates no significance.

Although MicroResp^{TM} can be used to determine the physiological profiles of soil efficiently, it lacks the specificity to provide the breakdown of soil microorganisms' taxonomic composition. However, the taxonomic composition can be postulated based on the soil's physicochemical properties (Table 4.1). In terms of microbial decomposer community, fungi and bacteria are the two groups that overshadow in terms of biomass. Nevertheless, based on the soil's physicochemical properties, it can be determined either it is the bacterial or fungal community that flourishes. For instance, the range of pH of the studied agricultural sites ranged between 3.3 to 7.5 (mean: 6.0), which can be classified as acidic soil, and in acidic soil, fungal biomass dominates other soil microorganisms owing to their tolerance to low pH.[101,102] Accordingly, considering most of the widely used pesticides monitored in this study are fungicides, a significant decline in the microbial' respiration activity and biomass can be attributed to the loss of fungal biomass. Consequently, the application of azoxystrobin fungicide had been established to cause havoc to the microbial community by altering the community structure, inhibiting the soil enzyme activity, and reducing the microbial functional diversity.[103–105] Azoxystrobin is broad spectrum, and with direct soil application, the deleterious effect can be experienced by both targeted and non-targeted fungal communities. The findings of this study certainly reflect this reasoning. For instance, the microbial community were not significantly impacted in the mineral class where azoxystrobin is mobile. However, in the humic layer where the pesticide compound accumulated (Figure 4.5 (a)), there is a significant reduction in microbial respiration activities, biomass, and functional diversity (Figure 4.7 (a)). Similar findings have also been reported by Sopeña et al., where the soil

microbial enzymatic activities significantly decrease with azoxystrobin application.[106]

In addition to the type of pesticides applied, the disparities in soil microbial response between the mineral and humic class can be hypothesised based on the microbial adaptation to pesticide compounds. Kearney et al. had noted that when the soil microorganisms first come into contact with pesticide compounds, there will be a lag phase, followed by a rapid decline in the pesticide concentrations.[107] The lag phase is the initial phase where the soil microorganisms have reduced activity or slowly adapted to the degradation of newly available pesticide compounds.[108] It is recognised that the lag phase varies greatly between different applied pesticides and different soil properties,[107] which can result in a different range of lag phase between four days to 80 days.[109,110] Following the lag phase, specific microorganisms capable of producing enzymes that can degrade the pesticide compounds would benefit from deriving energy to grow, resulting in the proliferation of the particular microorganism community.[20,111] This phenomenon is reflected by the findings of this study on the response of soil microorganisms in the mineral class, where the soil microbial population had adapted to the pesticides applied, which could have been applied previously on the same site or has chemical similarities to the pesticide compounds applied on the same crop,[107] resulting in increased biomass, MSIR, and functional diversity. For instance, Yun et al. reported that the application of the fungicide chlorothalonil only significantly reduced the soil microorganism population during the first two applications, however, further applications did not alter the overall population densities.[112] A similar finding was also reported by Yu et al., where the author noted a 90% increase in the degradation rate of fungicide

carbendazim compared to the first and fourth applications.[113] On the fourth application, the author stated the application did not impact the richness of the soil microbial community, however, the population is enriched with soil microorganisms that had adapted to specifically degrade carbendazim.[113]

In contrast, the soil microorganisms in the humic class, which is established to have higher microbial biomass and MSIR than the mineral class, might not have the adapted and specialised microbial population, resulting in decreased biomass and respiration activity while entering the lag phase. This postulation is further solidified by referring to the increased qCO_2 levels of the humic class ($p<0.0001$) (Figure 4.7 (d)). An increased qCO_2 level indicates that the disturbance to the ecosystem resulted in the soil microorganisms shifting energy from growth to maintenance. This shift is due to the inability of the soil system to replenish the carbon demand,[114] which increases the stress on the soil system. This stress on the soil microorganisms can be caused by natural stress conditions such as a limitation on the space and nutrients for growth, and not only from the exposure to the applied pesticides alone.[115] A similar qCO_2 response was also reported by da Rocha et al., where the author noted a significant increase in qCO_2 levels with $p<0.001$ after seven days of pesticide application.[116]

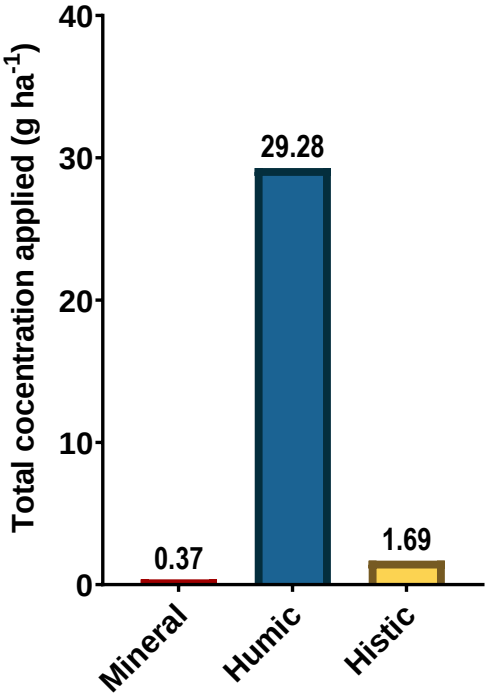


Figure 4.8 Total concentration of the active ingredients applied respective to SOM classes (based on the farmer's pesticide application record).

If the assessment of qCO_2 levels is taken further, as previously mentioned, the comparison between all three classes, the humic class is observed to have significantly higher qCO_2 levels ($p<0.0001$) compared to either mineral or histic (Figure 4.4 (e)). Interestingly, the recent pesticide application record mirrors the soil microbial stress response, respective to each SOM class (Figure 4.8), with the humic class having the highest qCO_2 levels, followed by the histic class and mineral class. A similar finding has also been reported by Prado *et al.*, where the author stated that even a small amount of the pesticide diuron application concentration, 1.67 µg g^{-1}, could have a strong toxic effect on the soil microorganisms, resulting in total inhibition of microbial respiration activities and total microbial biomass, which could potentially increase the stress levels.[117] The findings of this study, where the qCO_2 levels increases with pesticide application

concentrations, indicate that qCO_2 measurement can be used as an effective indicator to evaluate and detect the early onset of environmental disturbance.

4.4 Conclusion

This study describes the fluctuation of microbial properties in Irish agricultural soil during and a week after pesticide application. The Pearson correlation between the soil physicochemical and microbial properties indicated that SOM correlated significantly positively with microbial biomass and functional diversity. SOM's positive correlation to the microbial properties prompted the segregation of the sampled sites into three distinct soil diagnostic classes, mineral, humic and histic. The adaptation of the SOM diagnostic classes revealed that the carboxylic acid absolute utilisation rate was highest in Irish agricultural soil, while the response was directly proportional to the increasing SOM content. The amino acid and amide substrates were noted to increase with the SOM class, even though these groups of substrates were the lowest utilised substrate. Further analysis of the soil microbial biomass highlighted those activities and functional diversity increased with increasing SOM class, even though the humic class was noted to have enhanced qCO_2 levels compared to other classes. Total pesticide concentration comparison between the classes revealed that the histic class was identified as the only class with no pesticide residues, indicating the rapid degradation of pesticide compounds catalysed by higher microbial biomass and activities. Meanwhile, humic and mineral classes were shown to have mean pesticide concentrations of 0.77 and 1.18 $\mu g\ kg^{-1}$, respectively, within 24 hours of pesticide application. However, within one week, the total pesticide concentrations decreased by 69% in the humic class, corresponding to higher microbial biomass and MSIR. Incidentally, the qCO_2 levels of microbial

communities in the humic class were significantly higher than in the mineral class, suggesting higher stress levels and the soil microbes entering the lag phase.

These results lead to the following conclusions: (i) MicroResp^{TM} presents an effective means of discrimination of land management intensity based on SOM diagnostic classes, (ii) SOM are drivers of crucial soil microbial properties and activities in Irish agricultural soil, (iii) pesticides degradation in Irish agricultural soil is catalysed by soil microorganism, based on fluctuation of microbial biomass, respiration activities and functional diversity, and (iv) land use with higher SOM content are sensitive to pesticide application activity reflected by increased qCO_2 levels.

This study presents a baseline knowledge of the effect of pesticide application on the soil microbial population. However, the results reported in this study lacks robustness due to the limitation of replicates of similar soil diagnostic classes. This limitation presents the uncertainties of the observed pesticide application impact on the soil microbial functional properties, which is highlighted in the wide span of the error bars. Therefore, to fully comprehend and utilise this knowledge to its full potential, further scientific investigations are warranted to improve the comprehensive interpretation of the fluctuation of soil microorganisms to pesticide application by incorporating additional soil class replicates and pesticide and microorganisms' parameters.

4.5 References

1 J. W. Doran and M. R. Zeiss, *Soil health and sustainability: managing the biotic component of soil quality*, 2000, vol. 15.

2 S. M. Hermans, H. L. Buckley, B. S. Case, F. Curran-Cournane, M. Taylor and G. Lear, *For Ecol Manage*, 2000, **138**, 335–356.

3 M. Pulleman, R. Creamer, U. Hamer, J. Helder, C. Pelosi, G. Pérès and M. Rutgers, *Curr Opin Environ Sustain*, 2012, 4, 529–538.

4 B. C. Nwachukwu, A. S. Ayangbenro and O. O. Babalola, *AMB Express*, , DOI:10.1186/s13568-022-01388-9.

5 E. Barrios, *Ecological Economics*, 2007, **64**, 269–285.

6 S. M. Hermans, H. L. Buckley, B. S. Case, F. Curran-Cournane, M. Taylor and G. Lear, *Microbiome*, , DOI:10.1186/s40168-020-00858-1.

7 E. K. Bünemann, G. D. Schwenke and L. van Zwieten, *Australian Journal of Soil Research*, 2006, 44, 379–406.

8 R. Lal, *Sustainability (Switzerland)*, 2015, **7**, 5875–5895.

9 J. L. M. Rodrigues, V. H. Pellizari, R. Mueller, K. Baek, E. D. C. Jesus, F. S. Paula, B. Mirza, G. S. Hamaou, S. M. Tsai, B. Feiglf, J. M. Tiedje, B. J. M. Bohannan and K. Nuẅsslein, *Proc Natl Acad Sci U S A*, 2013, **110**, 988–993.

10 H. S. Tin, K. Palaniveloo, J. Anilik, M. Vickneswaran, Y. Tashiro, C. S. Vairappan and K. Sakai, *Microb Ecol*, 2018, **75**, 459–467.

11 R. E. Drenovsky, K. L. Steenwerth, L. E. Jackson and K. M. Scow, *Global Ecology and Biogeography*, 2010, **19**, 27–39.

12 N. Fierer, C. L. Lauber, K. S. Ramirez, J. Zaneveld, M. A. Bradford and R. Knight, *ISME Journal*, 2012, **6**, 1007–1017.

13 N. A. Cassman, M. F. A. Leite, Y. Pan, M. de Hollander, J. A. van Veen and E. E. Kuramae, *Sci Rep*, , DOI:10.1038/srep23680.

14 E. L. M. Figuerola, L. D. Guerrero, D. Türkowsky, L. G. Wall and L. Erijman, *Environ Microbiol*, 2015, **17**, 678–688.

15 P. M. Kopittke, N. W. Menzies, P. Wang, B. A. McKenna and E. Lombi, *Environ Int*, 2019, 132.

16 X. Wang, M. U. Sial, M. A. Bashir, M. Bilal, Q. U. A. Raza, H. M. A. Raza, A. Rehim and Y. Geng, *Sustainability (Switzerland)*, 2022, 14.

17 M. Gavrilescu, *Eng Life Sci*, 2005, **5**, 497–526.

18 M. Waldman and Y. Shevah, *Pure and Applied Chemistry*, 1993, **65**, 1595–1603.

19 Ma. Laura, E. Snchez-Salinas, E. Dantn Gonzlez and M. Luisa, in *Biodegradation - Life of Science*, InTech, 2013.

20 A. Kalia and S. K. Gosal, *Arch Agron Soil Sci*, 2011, 57, 569–596.

21 M. A. M. Al-Ani, R. M. Hmoshi, I. A. Kanaan and A. A. Thanoon, in *Journal of Physics: Conference Series*, Institute of Physics Publishing, 2019, vol. 1294.

22 J. Medo, J. Maková, J. Medová, N. Lipková, R. Cinkocki, R. Omelka and S. Javoreková, *Sci Rep*, , DOI:10.1038/s41598-021-91755-6.

23 P. Nannipieri, J. Ascher-Jenull, M. T. Ceccherini, G. Pietramellara, G. Renella and M. Schloter, *Pedosphere*, 2020, **30**, 5–17.

24 R. Calbrix, K. Laval and S. Barray, *Eur J Soil Biol*, 2005, **41**, 11–20.

25 E. Blagodatskaya and Y. Kuzyakov, *Soil Biol Biochem*, 2013, 67, 192–211.

26 S. Krause, X. le Roux, P. A. Niklaus, P. M. van Bodegom, J. T. Lennon T., S. Bertilsson, H. P. Grossart, L. Philippot and P. L. E. Bodelier, *Front Microbiol*, 2014, 5.

27 S. A. Wood, D. S. Karp, F. DeClerck, C. Kremen, S. Naeem and C. A. Palm, *Trends Ecol Evol*, 2015, 30, 531–539.

28 C. D. Campbell, S. J. Chapman, C. M. Cameron, M. S. Davidson and J. M. Potts, *Appl Environ Microbiol*, 2003, **69**, 3593–3599.

29 G. Bongiorno, E. K. Bünemann, L. Brussaard, P. Mäder, C. U. Oguejiofor and R. G. M. de Goede, *Applied Soil Ecology*, 2020, **154**, 103596.

30 R. E. Creamer, D. Stone, P. Berry and I. Kuiper, *Applied Soil Ecology*, 2016, **97**, 36–43.

31 M. C. Moscatelli, L. Secondi, R. Marabottini, R. Papp, S. R. Stazi, E. Mania and S. Marinari, *Pedobiologia (Jena)*, 2018, **66**, 36–42.

32 R. Murugan, R. Loges, F. Taube, A. Sradnick and R. G. Joergensen, *Microb Ecol*, 2014, **67**, 907–918.

33 A. Richter, D. Huallacháin, E. Doyle, N. Clipson, J. P. van Leeuwen, G. B. Heuvelink and R. E. Creamer, *Eur J Soil Sci*, 2018, **69**, 414–428.

34 L. B. Martínez-García, G. Korthals, L. Brussaard, H. B. Jørgensen and G. B. de Deyn, *Agric Ecosyst Environ*, 2018, **263**, 7–17.

35 American Society for Testing and Materials, *Standard test method for particle-size analysis of soils D422-63 (2007)*, American Society for Testing Materials, Philadelphia, 2008.

36 F. J. Sikora and K. P. Moore, *Soil Test Methods From the Southeastern United States Southern*, 2014.

37 D. S. Jenkinson and D. S. Powlson, *Soil Biol Biochem*, 1976, **8**, 209–213.

38 L. P. van Reeuwijk, *International Soil Reference and Information Centre, Wageningen, NL*.

39 M. R. Motsara, R. N. Roy and Food and Agriculture Organization of the United Nations., *Guide to laboratory establishment for plant nutrient analysis*, Food and Agriculture Organization of the United Nations, 2008.

40 M. J. J. Hoogsteen, E. A. Lantinga, E. J. Bakker, J. C. J. Groot and P. A. Tittonell, *Eur J Soil Sci*, 2015, **66**, 320–328.

41 Supelco, 2021.

42 Supelco, 2021.

43 M. Vickneswaran, J. C. Carolan and B. White, *Analytical Methods*, 2021, **13**, 5638–5650.

44 M. Anastassiades, ; D I Kolberg, ; E Eichhorn, A.-K. Wachtler, ; A Benkenstein, ; S Zechmann, ; D Mack, ; C Wildgrube, ; A Barth, ; I Sigalov, ; S Görlich, ; D Dörk and ; G Cerchia, *EU Reference Laboratory for pesticides requiring Single Residue Methods*, 2021.

45 J. Bloem, D. W. Hopkins and A. Benedetti, *CABI publishing*, 2006.

46 K. M. Brolsma, J. A. Vonk, E. Hoffland, C. Mulder and R. G. M. de Goede, *Biol Fertil Soils*, 2015, **51**, 913–922.

47 C. M. Cameron, C. D. Campbell, S. J. Chapman and D. White, 2007, 2962.

48 J. P. E. Anderson and K. H. Domsch, *Soil Biol Biochem*, 1978, **10**, 215–221.

49 J. Yu, A. Unc, X. Zhang and Y. Steinberger, *Applied Soil Ecology*, 2016, **101**, 124–131.

50 A. C. Kennedy and K. L. Smith, *Plant Soil*, 1995, **170**, 75–86.

51 G. D. Bending, M. K. Turner, F. Rayns, M. C. Marx and M. Wood, *Soil Biol Biochem*, 2004, **36**, 1785–1792.

52 S. Marinari, E. Bonifacio, M. C. Moscatelli, G. Falsone, L. V. Antisari and G. Vianello, *Geoderma*, 2013, **192**, 437–445.

53 H. Sun, Y. Qi, D. Zhang, Q. X. Li and J. Wang, *Environmental Pollution*, 2016, **209**, 177–185.

54 M. Lambert, C. Inthavong, F. Hommet, J. C. Leblanc, M. Hulin and T. Guérin, *Food Chem*, 2018, **240**, 997–1004.

55 G. P. Dively and A. Kamel, in *Journal of Agricultural and Food Chemistry*, 2012, vol. 60, pp. 4449–4456.

56 European Union, *Commission Implementing Regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance imidacloprid*, 2018, vol. 14.

57 European Union, *Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin*, 2018, vol. 14.

58 European Union, *Commission Implementing Regulation (EU) 2018/ 785 - of 29 May 2018 - amending Implementing Regulation (EU) No 540 / 2011 as regards the conditions of approval of the active sub stance thiamethoxam*, 2018.

59 A. López-Ballesteros, A. Delaney, J. Quirke, J. C. Stout, M. Saunders, J. C. Carolan, B. White and D. A. Stanley, *PeerJ*, 2022, **10**, 1–21.

60 PPDB, Pesticide Properties Database, http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm, (accessed 5 September 2022).

61 F. Garciá-Orenes, A. Morugań-Coronado, R. Zornoza and K. Scow, *PLoS One*, , DOI:10.1371/journal.pone.0080522.

62 M. Wander, in *Soil Organic Matter in Sustainable Agriculture*, 2004.

63 J. Rethemeyer, P. M. Grootes, F. Bruhn, N. Andersen, M. J. Nadeau, C. Kramer and G. Gleixner, in *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms*, 2004, vol. 223–224, pp. 521–527.

64 B. T. Christensen, in *Advances in Soil Science*, 1992, pp. 1–90.

65 J. Tian, N. He, L. Hale, S. Niu, G. Yu, Y. Liu, E. Blagodatskaya, Y. Kuzyakov, Q. Gao and J. Zhou, *Funct Ecol*, 2018, **32**, 61–70.

66 T. Ramesh, N. S. Bolan, M. B. Kirkham, H. Wijesekara, M. Kanchikerimath, C. Srinivasa Rao, S. Sandeep, J. Rinklebe, Y. S. Ok, B. U. Choudhury, H. Wang, C. Tang, X. Wang, Z. Song and O. W. Freeman, in *Advances in Agronomy*, Academic Press Inc., 2019, vol. 156, pp. 1–107.

67 M. B. MacHmuller, M. G. Kramer, T. K. Cyle, N. Hill, D. Hancock and A. Thompson, *Nat Commun*, , DOI:10.1038/ncomms7995.

68 A. S. Grandy and G. P. Robertson, *Ecosystems*, 2007, **10**, 58–73.

69 A. Fließbach, H. R. Oberholzer, L. Gunst and P. Mäder, *Agric Ecosyst Environ*, 2007, **118**, 273–284.

70 R. T. Conant, J. Six and K. Paustian, *Biol Fertil Soils*, 2003, **38**, 386–392.

71 J. Holatko, T. Hammerschmiedt, R. Datta, T. Baltazar, A. Kintl, O. Latal, V. Pecina, P. Sarec, P. Novak, L. Balakova, S. Danish, M. Zafar-Ul-hye, S. Fahad and M. Brtnicky, *Sustainability (Switzerland)*, 2020, **12**, 1–19.

72 M. E. Schutter, J. M. Sandeno and R. P. Dick, *Biol Fertil Soils*, 2001, **34**, 397–410.

73 S. Sharma, A. Rangger and H. Insam, *Effects of Decomposing Maize Litter on Community Level Physiological Profiles of Soil Bacteria*, 1998.

74 A. Canarini, C. Kaiser, A. Merchant, A. Richter and W. Wanek, *Front Plant Sci*, 2019, 10.

75 D. L. Jones, *Organic acids in the rhizosphere-a critical review*, 1998, vol. 205.

76 R. Romaniuk, L. Giuffré, A. Costantini and P. Nannipieri, *Ecol Indic*, 2011, **11**, 1345–1353.

77 D. Geisseler, W. R. Horwath, R. G. Joergensen and B. Ludwig, *Soil Biol Biochem*, 2010, 42, 2058–2067.

78 L. C. Vinolas, J. R. Healey and D. L. Jones, *Kinetics of soil microbial uptake of free amino acids*, Springer-Verlag, 2001, vol. 33.

79 M. Ebrahimi, M. R. Sarikhani, A. A. Safari Sinegani, A. Ahmadi and S. Keesstra, *Catena (Amst)*, 2019, **174**, 371–382.

80 R. Tao, Y. Liang, S. A. Wakelin and G. Chu, *Applied Soil Ecology*, 2015, **96**, 42–51.

81 R. Calbrix, S. Barray, O. Chabrerie, L. Fourrie and K. Laval, *Applied Soil Ecology*, 2007, **35**, 511–522.

82 R. A. Houghton, in *Encyclopedia of Ecology*, 2008, pp. 448–453.

83 A. Sradnick, M. Oltmanns, J. Raupp and R. G. Joergensen, *Organic Agriculture*, 2018, **8**, 29–38.

84 R. G. Joergensen and M. Raubuch, *Soil Biol Biochem*, 2002, **34**, 1317–1324.

85 J. Hofman, L. Dušek, J. Klánová, J. Bezchlebová and I. Holoubek, *Environ Int*, 2004, **30**, 19–30.

86 R. J. Haynes, *Advances in Agronomy*.

87 M. H. Chantigny, in *Geoderma*, Elsevier, 2003, vol. 113, pp. 357–380.

88 J. DeCant and M. Barrett, *Revised Assessment for Clothianidin Registration of Prosper T400 Seed Treatment on Mustard Seed (Oilseed and Condiment) and Poncho/Votivo Seed Treatment on Cotton*, 2010.

89 D. Goulson, *Journal of Applied Ecology*, 2013, **50**, 977–987.

90 J. Bonmatin, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. D. Mitchell, D. A. Noome, N. Simon-Delso and A. Tapparo, *Environmental Science and Pollution Research*, 2015, **22**, 35–67.

91 A. Schaafsma, V. Limay-Rios and L. Forero, *Pesticide Management Science*, 2017, **74**, 323–331.

92 A. Tapparo, D. Marton, C. Giorio, A. Zanella, L. Soldà, M. Marzaro, L. Vivan and V. Girolami, *Environ Sci Technol*, 2012, **46**, 2592–2599.

93 O. Zivan, Y. Bohbot-Raviv and Y. Dubowski, *Chemosphere*, 2017, **177**, 303–310.

94 D. Pietrzak, J. Kania, E. Kmiecik, G. Malina and K. Wątor, *Chemosphere*, , DOI:10.1016/j.chemosphere.2020.126981.

95 M. Kah, S. Beulke and C. D. Brown, *J Agric Food Chem*, 2007, **55**, 4487–4492.

96 L. Tao and H. Yang, *Environ Monit Assess*, 2011, **175**, 227–238.

97 Y. Shi, Z. Ye, P. Hu, D. Wei, Q. Gao, Z. Zhao, J. Xiao, M. Liao and H. Cao, *Ecotoxicol Environ Saf*, , DOI:10.1016/j.ecoenv.2020.111203.

98 C. C. Howell, K. T. Semple and G. D. Bending, *Chemosphere*, 2014, **95**, 370–378.

99 Y. Feng, Y. Huang, H. Zhan, P. Bhatt and S. Chen, *Front Microbiol*, 2020, 11.

100 E. Wołejko, B. Łozowicka, P. Kaczyński, M. Jankowska and J. Piekut, *Environ Monit Assess*, 2016, **188**, 1–14.

101 R. G. Joergensen and F. Wichern, *Soil Biol Biochem*, 2008, **40**, 2977–2991.

102 J. Rousk, P. C. Brookes and E. Bååth, *Appl Environ Microbiol*, 2009, **75**, 1589–1596.

103 P. Guo, L. Zhu, J. Wang, J. Wang, H. Xie and D. Lv, *Environ Earth Sci*, 2015, **74**, 1353–1361.

104 D. W. Daniel, L. M. Smith, J. B. Belden, S. T. McMurry and S. Swain, *Science of the Total Environment*, 2015, **514**, 290–297.

105 E. M. Adetutu, A. S. Ball and A. M. Osborn, *J Appl Microbiol*, 2008, **105**, 1777–1790.

106 F. Sopeña and G. D. Bending, *Chemosphere*, 2013, **91**, 1525–1533.

107 P. C. Kearney and S. T. Kellogg, *Pure and Applied Chemistry*, 1985, **57**, 389–403.

108 E. Noshadi and M. Homaee, *Soil Tillage Res*, 2018, **184**, 37–44.

109 A. Qureshi, M. Mohan, G. S. Kanade, A. Kapley and H. J. Purohit, *Pest Manag Sci*, 2009, **65**, 798–804.

110 A. A. Pourbabaee, S. Soleymani, M. Farahbakhsh and E. Torabi, *International Journal of Environmental Science and Technology*, 2018, **15**, 1073–1084.

111 D. I. Bakalivanov, in *Adaptation Of Soil Microorganisms to Decomposition Of Some Herbicides*, eds. N. S. Margaris, M. Arianoustou-Faraggitaki and W. C. Oechel, Springer Netherlands, Dordrecht, 1984, vol. 13.

112 L. Y. Yun, M. Shan, H. Fang, X. Wang and Q. C. Xiao, *J Agric Food Chem*, 2006, **54**, 10070–10075.

113 Y. YU, X. CHU, G. PANG, Y. XIANG and H. FANG, *Journal of Environmental Sciences*, 2009, **21**, 179–185.

114 T. H. Anderson and K. H. Domsch, *Soil Biol Biochem*, 2010, **42**, 2039–2043.

115 V. B. Menezes-Oliveira, J. J. Scott-Fordsmand, A. Rocco, A. M. V. M. Soares and M. J. B. Amorim, *Science of the Total Environment*, 2011, **409**, 3370–3374.

116 A. G. da Rocha, L. M. Pitombo, J. D. Bresolin, W. T. L. da Silva, E. L. G. Espindola and V. B. de Menezes Oliveira, *SN Appl Sci*, , DOI:10.1007/s42452-020-3175-4.

117 A. G. S. Prado and C. Airoldi, *Pest Manag Sci*, 2001, **57**, 640–645.

Chapter 5

Conclusions and future work

5.1 Conclusions

Across the globe, it is increasingly clear that the benefits of pesticide usage in the agricultural industry comes at the cost of contamination of all components of the environment, especially soil.[1–5] Even though modern pesticides undergo stringent approval and registration process, in which they are risk assessed, and it is typically demonstrated that they do not pose a persistence risk in the environment,[6] with soil half-lives ranging from only a few days to weeks, a body of scientific research illustrates that residues of various pesticides cause contamination. In multiple instances, pesticides have detected in sites where it was not applied or even in the soil samples that had not been applied pesticides in the past ten years.[7,8] As such, there is a need to understand the factors that impact pesticide residues persistence, the extent of pesticide accumulation, and to assess the potential risks of persistence and accumulation, of likely multiple pesticide active substances. However, an effective regulatory effort to achieve a sustainable pesticide usage requires a large body of information specific to local pesticide usage scenarios and environmental conditions, which is limited in the Republic of Ireland.

The main objective of this thesis is to provide an insight into the levels of pesticide contamination in the main agricultural soil classes and land uses across the Republic of Ireland and to understand the impact of pesticide application on the soil microorganism's abundance and activities. Ten pesticides were selected for analysis to ensure this monitoring effort represents the most widely used active substances in the Irish agricultural industry. To enable accurate determination and quantification of these pesticide compounds, establishing an accurate extraction method was the first crucial step undertaken, and to achieve this, two

different extraction methods were compared and validated. After ensuring the soil sampling sites were selected to represent the different soil and land use types in Ireland, soil samples were collected within 24 hours and one week after pesticide application. The soil samples were then analysed for targeted pesticide concentrations for both time points, and the risk of the quantified pesticides was further assessed in the Irish agricultural soil. Finally, using the same soil samples, the microorganism's functional properties were measured and compared between the two timepoints. This enabled an evaluation of the fluctuation of soil microorganism abundance and activities during and a week after pesticide application.

Figure 5.1 highlights the structure and primary outputs of the thesis. The main aim of this research was to determine the level of pesticide contamination specific to Irish agricultural soil. Therefore, the current literature was assessed to identify knowledge gaps, with a specific focus on types and chemical classes of pesticides, soil properties dominating pesticide behaviour in soil, and identifying the most widely used pesticides and postulating their likely potential behaviour in the Irish context. Chapter 1.0 reviewed the published literature surrounding the main pesticide groups, chemical classes, mode of action of different pesticide types and classes, and possible behaviour of pesticide compounds in the soil. Additionally, pesticide usage in the Republic of Ireland was scrutinised by identifying the widely used herbicides, fungicides, and insecticides. The possible behaviour of these pesticides in the Irish agricultural soil was postulated based on the literature-reported values on soil organic matter content, pH and precipitation levels.

Subsequently, based on the review of the literature on pesticide usage in Ireland, five widely used pesticides were selected (azoxystrobin, boscalid, glyphosate, fluroxypyr, and prothioconazole), together with a selection of neonicotinoid pesticides (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) were selected for targeted analysis. Even though three of the five neonicotinoids are currently banned for outdoor usage, due to their long half-lives and environmental persistence, they were included in this study. Due to the differences in the polarity and their adsorption strength in soil, establishing a robust and effective method was the first step for accurate pesticide detection and quantification (Chapter 2.0). Accordingly, two extraction methods, QuEChERS and Dutch mini-Luke, were assessed for their extraction efficiencies for seven pesticide analytes, five neonicotinoids, fluroxypyr and prothioconazole. On the whole, the Dutch mini-Luke extraction method was analytically advantageous as it provided both the physical and chemical parameters to extract strongly bound pesticides in the soil effectively. Dutch mini-Luke also offered better sensitivity, recovery, and lower matrix effects to most targeted pesticides.

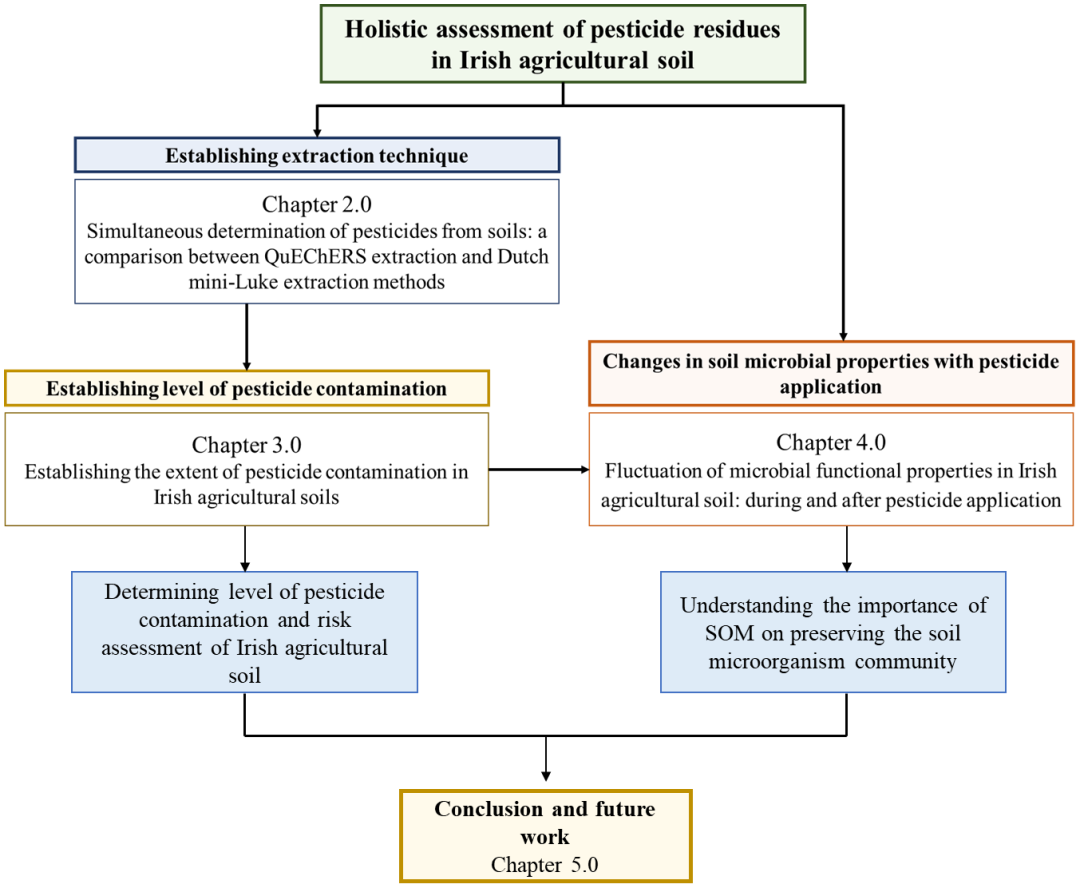


Figure 5.1 Flowchart of the studies and outputs of this thesis

Following the determination of Dutch mini-Luke as the preferred extraction method, soil samples were collected within 24 hours and one week after pesticide application from 25 agricultural sites, namely 23 croplands, two grasslands and one commonage land (Chapter 3.0). Of the 25 fields, 23 of these sites had either azoxystrobin, fluroxypyr, prothioconazole, or a combined application, a maximum of two pesticides, simultaneously applied. From these agricultural sites, widespread pesticide contamination was detected, with 24 sites determining pesticide concentrations in the range of 0.18 to 45.7 µg/kg. Even though not all sites with recent application of pesticides, namely azoxystrobin, fluroxypyr, and prothioconazole, resulted in quantifiable concentrations, due to very little time for degradation to occur, the quantification level of these pesticides can be

interpreted as the highest threat level it might impose, or a “worst case” scenario. On the other hand, this scenario does not apply to the quantifiable detection of pesticides that were not applied recently, specifically boscalid and neonicotinoids. Neonicotinoids were detected in 96% of the sampled sites, even though these pesticides were not applied recently. Even though the extensively managed grassland site has no history of pesticide use, neonicotinoids were detected in that soil sample. Considering that three of the five targeted neonicotinoids have been banned since 2018, the widespread detection of neonicotinoids strengthens the understanding of their long half-lives and the concerning risk of their high mobility through the environment.

In the worst-case scenario, where all pesticide residues, including those recently applied, are considered, of 25 sites, only one site was revealed to have no pesticide residues, and as high as 21 sites had three or more pesticide residues. Based on the hierarchical clustering analysis, croplands discriminated from other land-use types, with spring barley and winter wheat being the crop types with the highest detected concentrations of the targeted pesticides. Risk assessment based on the Irish Drinking Water's and groundwater parametric limit for total pesticide concentrations (0.5 µg/L) was used to establish the risk associated with the detected pesticides and the total pesticide concentrations for each site as a proxy for risk assessment. Accordingly, 23 sites were quantified with total pesticide concentrations exceeding the set limit. Moving from the worst case scenario, by removing the recently applied pesticides from the assessment, 17 sites still had total pesticide concentrations above the limit of 0.5 µg/L. Even though prothioconazole and boscalid were inferred to have accumulation risk based on the pesticide properties, prothioconazole significantly decreased in

concentration (72% decrease) a week after it was applied, while the total concentration of boscalid decreased by 30%. These contradictory findings between the expected pesticide risk and real-world monitoring results of these pesticides highlight the difficulty of predicting pesticide behaviour in a highly complex and heterogenous matrix such as soil.

To understand the impact of pesticide application on the soil environment, the biotic properties, in the form of soil microorganism functional properties, were measured in the soil samples collected within 24 hours and one week after pesticide application. These soil samples were used to determine the microbial biomass, respiration activities and functional diversity during pesticide application, and the changes were catalysed a week after. SOM was determined to be the most dictating soil physicochemical property of the measured soil microbial properties. Hence, the microbial properties differences and fluctuation between the two sampling time points were studied in relation to three distinct SOM classes (mineral, humic and histic). In the soil samples collected during pesticide application, proportional to the increasing SOM classes, the soil microbial biomass, activities and functional diversity were shown to increase. In both sampling timepoints, the carboxylic acid absolute utilisation rates were highest in the Irish agricultural soil, while amino acid and amide substrate utilisation rates were lowest. Consequently, histic was noted as the only SOM class with no pesticide residues a week after application, highlighting the rapid degradation of pesticide compounds in the soil layers with high organic matter content. This observation is further supported by a 68% decrease in total pesticide concentrations in the humic class. Even though in the one week after samples, the humic class recorded a higher total pesticide concentration decline

than the mineral class, it also recorded higher soil microbial stress levels in the form of higher qCO_2 levels. This further highlights the importance of soil microorganisms in efforts to remediate pesticides in agricultural systems, where higher pesticide degradation rate corresponds with higher microbial biomass and respiration activities of humic class. However, with higher abundance, functional diversity and respiration activity, humic soil is more sensitive to sudden exposures, such as pesticide application, forcing soil microorganisms to shift energy from growth to maintenance, resulting in higher stress levels.

5.2 Main contributions of the study

This thesis and the studies within were carried out to contribute towards monitoring the level of widely used pesticide contamination, and the impact of pesticide application on the soil microorganisms in Irish agricultural soil. As a result, this thesis contributes towards novel findings as follows:

- The development and validation of a robust extraction method, Dutch mini-Luke, for a single mixed analysis of neonicotinoids, triazoles, and synthetic auxin pesticides from soil samples. This extraction method performed effectively and reproducibly without additional clean-up steps or modification, and it was validated in HPLC-UV and HPLC-MS/MS.

- A novel report on the widespread contamination of pesticides in Irish agricultural soil, with targeted analysis of five widely used pesticides in the Irish agricultural industry and five neonicotinoid pesticides. Statistical analyses were coupled with pesticide quantification to identify the soil physicochemical properties contributing to pesticides' persistence in the Irish agricultural soil. A theoretical environmental risk assessment specific

to Ireland was determined based on the pesticide properties and the dissipation level within one week of pesticide application. The risk assessment was further supplemented by monitoring the targeted pesticides' dissipation in one week, which indicated that even though a widely used pesticide were anticipated to have accumulation risk, in the real-world setting, they registered the most significant decrease in concentration.

- The impact of pesticide application on the Irish agricultural soil microorganism was investigated, where the soil microbial properties between two sampling timepoints were collated with the shift in pesticide concentrations between the same sampling timepoints. The microbial fluctuation resulting from pesticide application was further studied based on the differences in SOM classes, highlighting the importance of SOM classes in preserving the soil microorganisms' abundance and activities.

5.3 Future work

This thesis reported multiple novel studies and findings on the level of pesticide contamination in Irish agricultural soil, which presented several areas for future research that can be further expanded and built upon, as follows:

- Even though the Dutch mini-Luke extraction method presented the best recovery, reproducibility, sensitivity, and lower matrix effect compared to the QuEChERS extraction method, QuEChERS is more environmentally friendly and aligns with the current need for green chemistry and analytical ethics. Hence, further research needs to be explored on improving Dutch mini-Luke extraction; (a) by reducing the volume of the organic solvent

used and (b) by introducing additional clean-up steps to overcome the matrix effect that might arise from the miniaturisation of the procedure.

- It is estimated that more than 3000 different types of pesticides have been in use in Europe over the past 55 years.[9–14] Even though a targeted analysis of the ten pesticides used in this thesis is a good starting point for establishing the level of pesticide contamination in Irish agricultural soil, there is much space to enhance the study further. The main avenue to explore would be to increase the number of the targeted pesticides and their metabolites. Therefore, the focus should be on the pesticides that are widely used in Ireland.[15] This thesis reports the occurrence of three widely used fungicides (azoxystrobin, boscalid, and prothioconazole), and two herbicides (fluroxypyr and glyphosate), but no currently widely used insecticides were included in the analysis. Hence, future studies should include more diverse chemical classes and pesticide types, to improve the comprehension of the level of contamination in Irish agricultural soil. Additionally, as has been highlighted in some instances, the resulting metabolites of pesticides can be more persistent and potent compared to the parent compounds, such as AMPA and prothioconazole-desthio, [16–20] inclusion of metabolites in future studies could further improve the monitoring and risk assessment of widely used pesticides in the soil system.

- The site selection process for obtaining permission and cooperation from the farmers was arduous and complicated. However, we did succeed in securing the maximum number of sites for which we had funding (25 agricultural fields) for the work in this thesis. Based on the findings of this

thesis, spring barley, winter barley and winter wheat had been identified as the crop type fields to contain higher concentrations of pesticides, mainly fungicides. This crop types are the top three cereals grown in Ireland, thus the occupy up to 356.7 thousand hectares of area. [21] Therefore, further monitoring efforts should be taken to assess the management and pesticide application practices in these crop types, to ensure the practices does not further increase the level of pesticide contamination in the environment.

- One of the main limitations of the thesis is the lower opportunity to carry out comparison studies between similar soil and land use properties. Therefore, to further strengthen the findings of this thesis, future studies should expand by obtaining multiple replicate sites for each soil class and type, land use type, and crop type, which is lacking in this study. Additionally, this thesis had highlighted the importance of SOM content in agricultural sites for the maintenance of soil quality, hence, future studies could focus on the differences in land management (intensive, extensive and organic farming), and the resulting effect on the soil physicochemical and microbiological properties.

- Two sampling timepoints, within 24 hours and a week after pesticide application, were used to elucidate the pesticide concentrations and study the fluctuation of the soil microbial functional properties. Future research could improve the monitoring effort further by including additional sampling timepoints that span the reported half-lives for the pesticides being analysed, for instance, a month, six months, and a year after pesticide application, for a thorough assessment of the fate of the widely applied

pesticides. Additionally, with the suggested sampling timepoints, a more comprehensive risk assessment of the widely applied and high-persistence pesticides can be completed.

- The impact of pesticide application on the biotic soil properties was established by assessing the soil microorganisms' abundance and functional properties fluctuation. Future research could explore more in-depth measurements of the same group of organisms by including high-throughput sequencing methods. The inclusion of these methods would allow a more detailed analysis of pesticide application on shifts in the soil microorganism community structures down to the species taxonomical level. Jacobsen and Hjelmsø reviewed multiple studies that reported the impact of a particular type of pesticide application on microbial diversity.[22] By conducting similar studies on widely used pesticides and soil classes specific to the Republic of Ireland would significantly improve Irish agricultural soil quality monitoring and preservation efforts. In addition to assessing the status of soil microorganisms following pesticide application, the impact on other biotic components of the soil, such as invertebrates, would increase the robustness of soil monitoring as this group of organisms had been used in monitoring studies globally.

In conclusion, this work achieved firstly an effective and reproducible soil pesticide extraction method, which then enabled a multifaceted insight into pesticide contamination in Irish agricultural soils of different class and utilised for different land uses, and the impact of pesticide application on the soil microorganisms' structure and activities. Soil is one of earth’s non-renewable resources that is the foundation of agricultural industry, and food and produces

system, hence preserving the quality and health of soil should be a global priority. Even though the usage of pesticides results in higher productivity and crop yield, efforts need to be taken to monitor and establish potential detrimental impacts of this practice, to ensure overall environmental, and food and health safety. However, this effort cannot be done by adapting to the level of pesticide contamination reports of other countries. Therefore, this thesis's reported knowledge provides novel findings in the Irish context and serves as a reference for future monitoring. Additionally, EU member states have recently adopted proposals aimed at reducing hazardous pesticides and general pesticides use by 50% over the next eight years.[23] The findings of this thesis will be particularly useful for the negotiations on how these proposals can be achieved, contributing towards European-level efforts for sustainable pesticide use in the agricultural industry.

5.4 References

1 M. Lechenet, F. Dessaint, G. Py, D. Makowski and N. Munier-Jolain, *Nat Plants*, , DOI:10.1038/nplants.2017.8.

2 M. Tudi, H. Li, H. Li, L. Wang, J. Lyu, L. Yang, S. Tong, Q. J. Yu, H. D. Ruan, A. Atabila, D. T. Phung, R. Sadler and D. Connell, *Toxics*, 2022, **10**, 335.

3 M. Tudi, H. D. Ruan, L. Wang, J. Lyu, R. Sadler, D. Connell, C. Chu and D. T. Phung, *Int J Environ Res Public Health*, 2021, **18**, 1–24.

4 R. A. Cloyd and J. A. Bethke, *Pest Manag Sci*, 2011, 67, 3–9.

5 T. Gunstone, T. Cornelisse, K. Klein, A. Dubey and N. Donley, *Front Environ Sci*, 2021, 9.

6 European Union, *REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAM ENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market a nd repealing Council Directives 79/117/EEC and 91/414/EEC*, 2009, vol. 24.

7 C. Bernasconi, P. M. Demetrio, L. L. Alonso, T. M. mac Loughlin, E. Cerdá, S. J. Sarandón and D. J. Marino, *Agric Ecosyst Environ*, , DOI:10.1016/j.agee.2021.107341.

8 P. Kosubová, L. Škulcová, Poláková, J. Hofman and L. Bielská, *Chemosphere*, , DOI:10.1016/j.chemosphere.2020.126902.

9 E. Yasser and A. Alkareem, *Journal of Environment and Earth Science*, 2013, **3**, 199–210.

10 P. R. Durkin, *Fluroxypyr Human Health and Ecological Risk Assessment*, 2009.

11 G. Bhandari, K. Atreya, P. T. J. Scheepers and V. Geissen, *Chemosphere*, 2020, **253**, 126594.

12 V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema and V. Geissen, *Science of the Total Environment*, 2019, **653**, 1532–1545.

13 M. Hvězdová, P. Kosubová, M. Košíková, K. E. Scherr, Z. Šimek, L. Brodský, M. Šudoma, L. Škulcová, M. Sáňka, M. Svobodová, L. Krkošková, J. Vašíčková, N. Neuwirthová, L. Bielská and J. Hofman, *Science of the Total Environment*, 2018, **613–614**, 361–370.

14 FAO, *ITPS Global Assessment of the Impact of Plant Protection Products on Soil Functions and Soil Ecosystems*, FAO:Rome, Italy, 2017.

15 A. López-Ballesteros, A. Delaney, J. Quirke, J. C. Stout, M. Saunders, J. C. Carolan, B. White and D. A. Stanley, *PeerJ*, 2022, **10**, 1–21.

16 S. Daouk, P. J. Copin, L. Rossi, N. Chèvre and H. R. Pfeifer, *Environ Toxicol Chem*, 2013, **32**, 2035–2044.

17 S. Guilherme, M. A. Santos, I. Gaivão and M. Pacheco, *Environmental Science and Pollution Research*, 2014, **21**, 8730–8739.

18 C. Cosemans, N. van Larebeke, B. G. Janssen, D. S. Martens, W. Baeyens, L. Bruckers, E. den Hond, D. Coertjens, V. Nelen, G. Schoeters, H. W. Hoppe, E. Wolfs, K. Smeets, T. S. Nawrot and M. Plusquin, *Int J Hyg Environ Health*, , DOI:10.1016/j.ijheh.2021.113895.

19 J. E. Parker, A. G. S. Warrilow, H. J. Cools, B. A. Fraaije, J. A. Lucas, K. Rigdova, W. J. Griffiths, D. E. Kelly and S. L. Kelly, *Appl Environ Microbiol*, 2013, **79**, 1639–1645.

20 Z. Zhang, J. Zhang, X. Zhao, B. Gao, Z. He, L. Li, H. Shi and M. Wang, *J Hazard Mater*, , DOI:10.1016/j.jhazmat.2020.122756.

21 CSO, Area, Yield and Production of Crops 2021 - CSO - Central Statistics Office, https://www.cso.ie/en/releasesandpublications/ep/p-aypc/areayieldandproductionofcrops2021/, (accessed 27 July 2022).

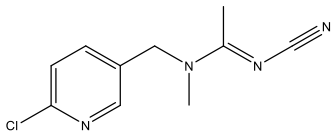
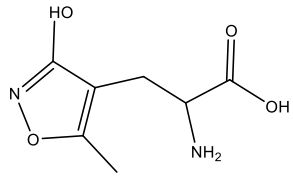
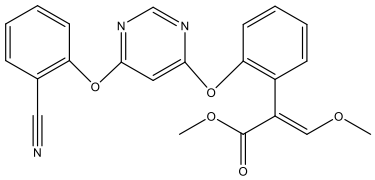
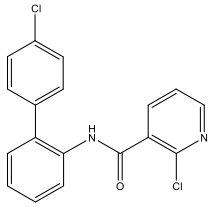
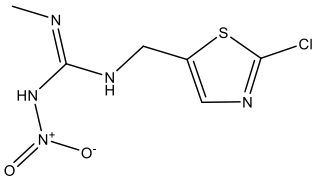
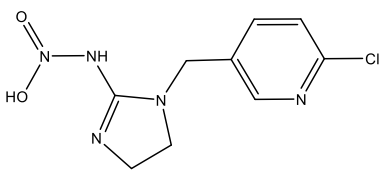
22 C. S. Jacobsen and M. H. Hjelmsø, *Curr Opin Biotechnol*, 2014, 27, 15–20.

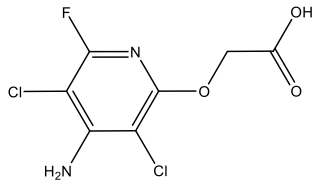
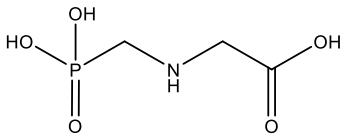
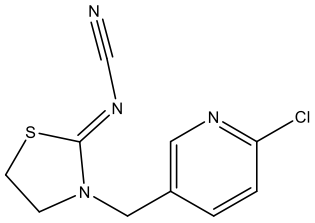
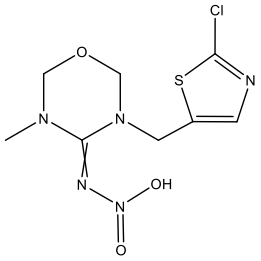
23 C. EU, *Proposal for a Regulation of The European Parliament and of The Council on the sustainable use of plant protection products and amending Regulation (EU) 2021/2115*, 2022.

Appendix

Appendix A: Chemical structures of the targeted pesticide compounds

Table A1.1. Targeted pesticide compounds and their chemical structures

No.	Pesticide compounds	Chemical structures
1	Acetamiprid	
2	AMPA	
3	Azoxystrobin	
4	Boscalid	
5	Clothianidin	
6	Imidacloprid	

7	Fluroxypyr	
8	Glyphosate	
9	Thiacloprid	
10	Thiamethoxam	

Appendix B: Simultaneous determination of pesticides from soils: A comparison between QuEChERS extraction and Dutch Mini-Luke extraction methods

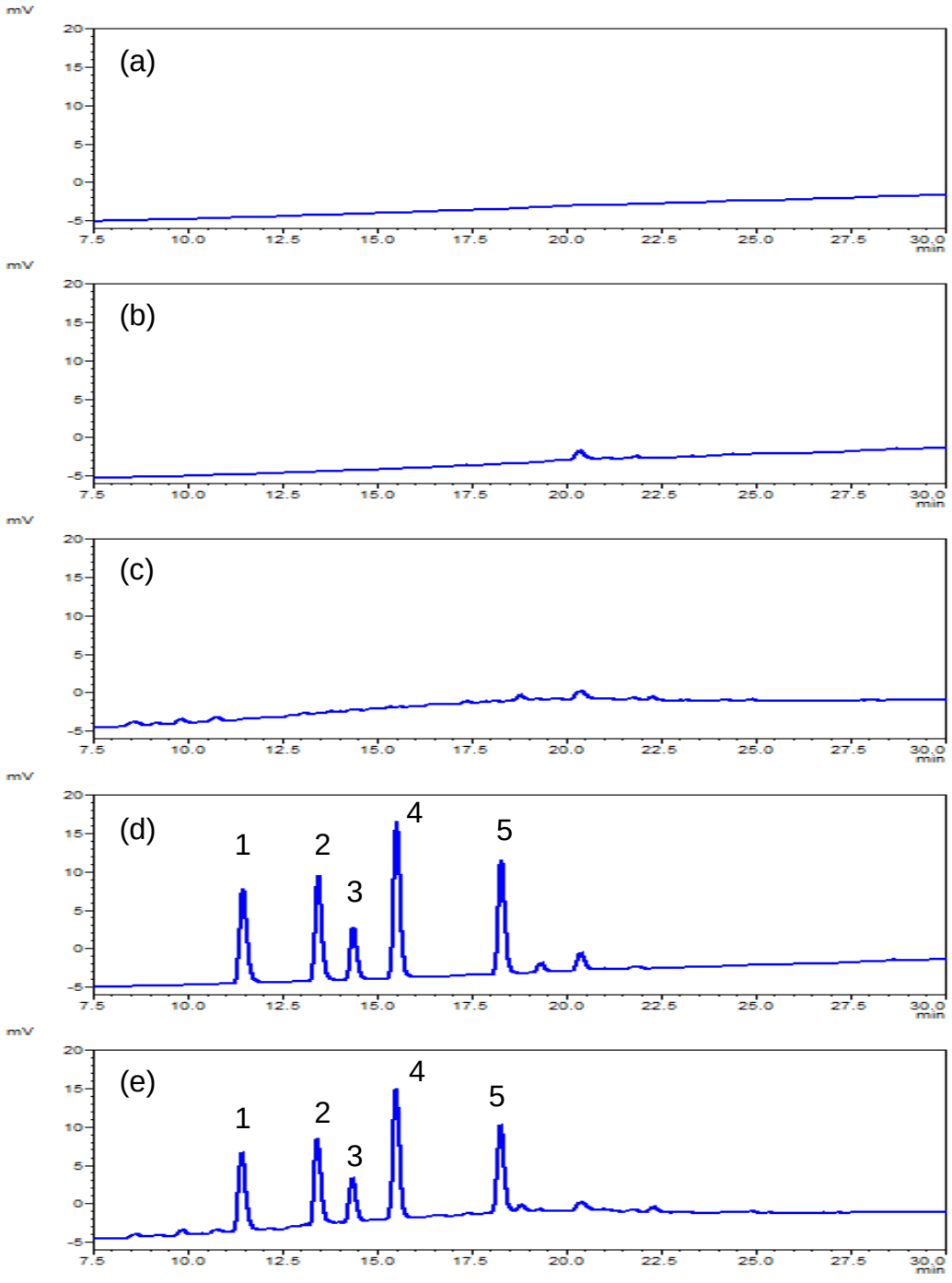


Fig. B2.1. Representative of obtained chromatograms with QuEChERS extraction method: (a) solvent blank; (b) blank sand extract; (c) blank soil extract; (d) blank sand pesticide mixture extract; and (e) blank soil pesticide mixture extract. Analyte peaks are labelled as following: (1) thiamethoxam, (2) clothianidin, (3) imidacloprid, (4) acetamiprid, and (5) thiacloprid.

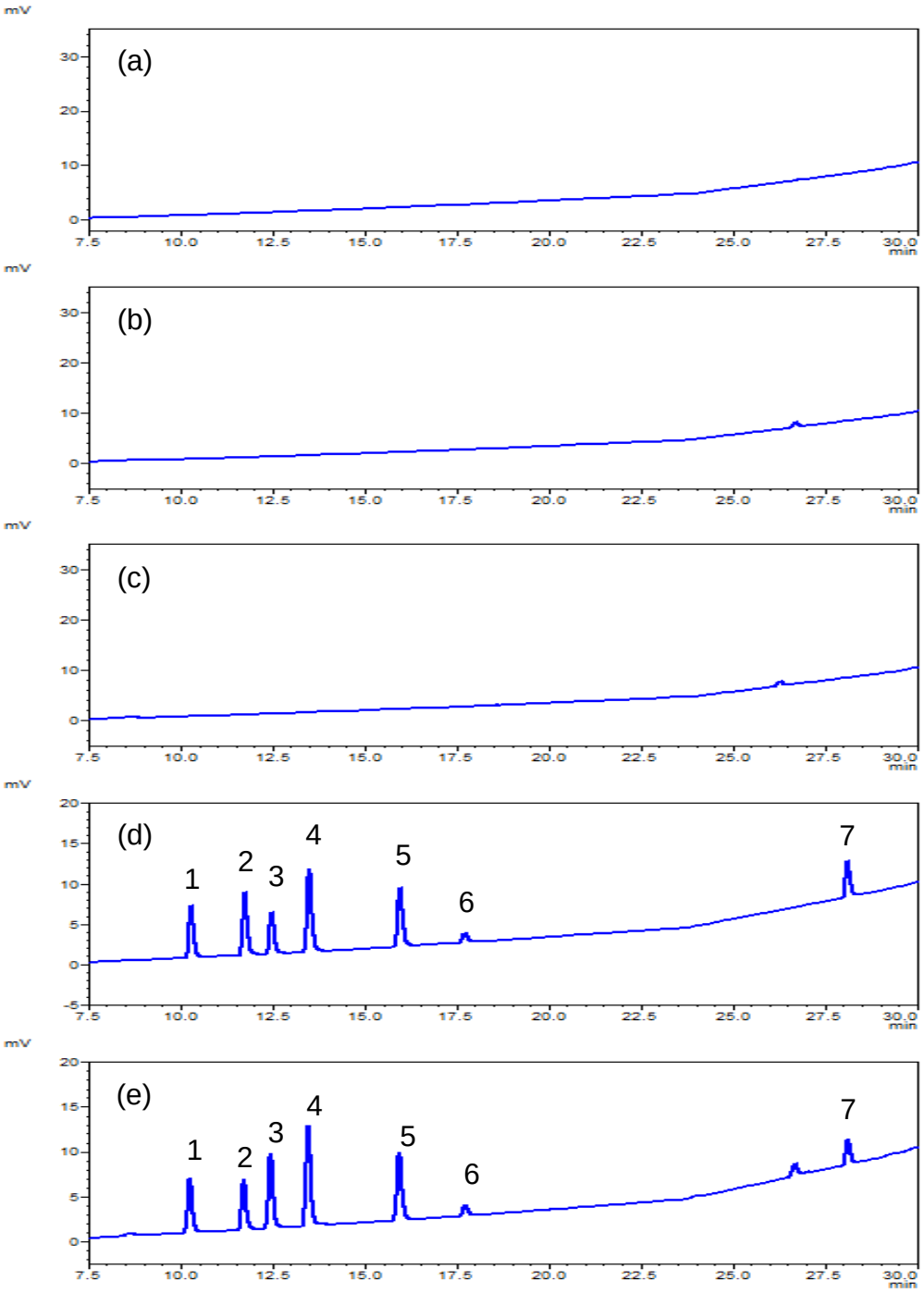


Fig. B2.2. Representative of obtained chromatograms with Dutch mini-Luke extraction method: (a) solvent blank; (b) blank sand extract; (c) blank soil extract; (d) blank sand pesticide mixture extract; and (e) blank soil pesticide mixture extract. Analyte peaks are labelled as following: (1) thiamethoxam, (2) clothianidin, (3) imidacloprid, (4) acetamiprid, (5) thiacloprid, (6) fluroxypyr, and (7) prothioconazole

Table. B2.1. Summary of the method validation parameters comparison between QuEChERS and Dutch mini-Luke based on analytes

Analytes	Acetamiprid		Clothianidin		Fluroxypyr		Imidacloprid		Prothioconazole		Thiacloprid		Thiamethoxam	
	Blank sand	**Blank soil**	**Blank sand**	**Blank soil**	**Blank sand**	**Blank soil**	**Blank sand**	**Blank soil**	**Blank sand**	**Blank soil**	**Blank sand**	**Blank soil**	**Blank sand**	**Blank soil**
Method Detection Limit (MDL) (ng μL^{-1}) **(QuEChERS)**	0.31	0.56	0.32	0.85	ND	ND	0.31	0.69	ND	ND	0.36	0.78	0.31	0.85
Method Detection Limit (MDL) (ng μL^{-1}) **(Dutch mini-Luke)**	0.37	0.32	0.26	0.20	0.25	0.42	0.44	0.40	0.48	0.42	0.29	0.42	0.18	0.28
Method Quantification Limit (MQL) (ng μL^{-1}) **(QuEChERS)**	0.95	1.7	0.97	2.58	ND	ND	0.95	2.08	ND	ND	1.08	2.35	0.95	2.56
Method Quantification Limit (MQL) (ng μL^{-1}) **(Dutch mini-Luke)**	1.13	0.97	0.80	0.60	0.74	1.26	1.34	1.22	1.46	1.26	0.87	1.27	0.53	0.85
Accuracy (Recovery%) **(QuEChERS)**	90-105	90-97	86-100	85-92	ND	ND	93-111	89-93	ND	ND	85-105	83-94	85-93	85-93

Accuracy (Recovery%) **(Dutch mini-Luke)**	90-101	106-108	96-99	102-110	84-99	59-103	94-95	106-111	91-106	97-117	93-97	104-107	94-97	102-115
Precision (RSD%) **(QuEChERS)**	4.9-11.1	4.9-9.9	2.6-7.3	2.6-9.6	ND	ND	3.6-8.4	3.6-10.1	ND	ND	4.6-8.9	4.6-9.9	5.4-8.6	3.3-9.5
Precision (RSD%) **(Dutch mini-Luke)**	1.4-3.1	5.8-9.8	0.3-6.7	3.4-19.6	2.8-7.6	6.8-12.5	5.8-5.9	4.5-20.3	1.9-8.3	15.5-27.8	1.2-9.1	1.0-6.1	2.6-8.3	7.0-7.7
Matrix Effect (%) **(QuEChERS)**	16	16	16	13	11	291	19	20	ND	ND	16	43	17	2
Matrix Effect (%) **(Dutch mini-Luke)**	-7	-4	-2	-7	-6	-1	-3	-9	-11	140	-4	-6	-1	9
Optimal Extraction Method?	**Dutch mini-Luke**		**Dutch mini-Luke**		**Dutch mini-Luke**		**Dutch mini-Luke**		**Dutch mini-Luke**		**Dutch mini-Luke**		**Dutch mini-Luke**	