Environmental Fate of Imidazolinone Herbicides and Their Enantiomers in Soil and Water

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Abstract

Imidazolinones represent a new class of herbicides with low mammalian toxicity that can be used at low application rates, either pre- or post-emergence for the control of a wide range of weeds in broadleaf and cereal crops, and non-crop situations. All imidazolinone herbicides are chiral, containing two enantiomers that derive from the chiral centre of the imidazolinone ring. The inhibitory activity of the R(+) enantiomer is nearly eight times greater than that of the S(-) enantiomer. The use of imidazolinone herbicides has increased in recent years in Australia owing to increased popularity of pulses and the introduction of imidazolinone-tolerant canola and wheat. Concerns have been raised about the potential carry over damage to the subsequent crops grown in rotation with legumes and herbicide tolerant crops. Furthermore, the presence of alkaline soils in some regions of Australia may lead to the repellence of imidazolinone herbicides, which are chiefly present in anionic form at high pH values. Thus leaching and potential contamination of ground water may occurr when these herbicides are applied on alkaline soils in certain agroclimatic zones. There is some information in the literature on the degradation, sorption and leaching behaviour of these herbicides in the environment. However, there is little information about the behaviour of these herbicides in alkaline soils found in some areas of Australia. Until now there has been no investigation of enantioselectivity in the degradation of imidazolinone herbicides in soils. Therefore, this study was undertaken to determine the behaviour of three imidazolinone herbicides in solution and Australian soils including enantioselectivity in the degradation of these herbicides in Australian soils.

Analytical method for these herbicides needed to be developed/improved to cater for specific experimental conditions for this study, namely the matrices containing higher

levels of organic carbon and to analyse the two enantiomers of these herbicides. The extraction of imazapyr, imazethapyr and imazaquin was investigated using solid-phase extraction (SPE) procedure. The evaluation of different aqueous solutions (0.1 KCl, 0.5 M NaOH, 0.01M NaOH and 0.5M MeOH:NaOH, (80:20)) showed that the recovery of all three herbicides was greater than 70%. However, the highest level of herbicide recovery was obtained with 0.5M NaOH as the extraction solution. Evaluation of different solid phase extraction cartridges showed that PPL cartridge is most appropriate for the isolation and subsequent quantification of these herbicides in water and humic-amended solutions when used at pH 2. When used with soil extracts, SPE cartridges C_{18} + SCX allowed removal of co-extracting substances, resulting in high levels of herbicide recovery and accurate quantification with HPLC. These improved protocols were used in subsequent studies.

The abiotic degradation of the imidazolinone herbicides imazapyr, imazethapyr and imazaquin was investigated under controlled laboratory conditions. Hydrolysis, where it occurred, and photodegradation both followed first order kinetics for all herbicides. There was no hydrolysis of any of the herbicides in buffer solutions at pH 3 or pH 7; however, slow hydrolysis occurred at pH 9. Degradation of the herbicides in the light was considerably more rapid than in the dark with half lives for the three herbicides of 1.8, 9.8 and 9.1 days for imazaquin, imazethapyr and imazapyr, respectively. The presence of humic acids in the solution reduced the rate of photodegradation for all three herbicides, with higher concentrations of humic acids generally having greater effect. The enantioselectivity of photodegradation was investigated using imazaquin, with photodegradation occurring at the same rate for both enantiomers. Abiotic degradation of imidazolinone herbicides on the soil surface only occurred in the presence of light. The

rate of degradation for all three herbicides on the soil surface was slower than in solution, with half-lives of 15.3, 24.6 and 30.9 days for imazaquin, imazethapyr and imazapyr, respectively.

Sterilizing the soil significantly ($p < 0.05$) decreased the degradation rate of both enantiomers of imidazolinone herbicides, with 81.5 to 89.5% of each enantiomer of the two herbicides remaining unchanged. However, in non-sterilized soils, the degradation of imazapyr and imazethapyr showed enantioselectivity with faster degradation of R(+) enantiomer compared with S(-) enantiomer. There were also some differences in enantioselectivity between different soils, which could be related to variation in microbial populations and enzymes present in different soils. Soil pH had a significant effect on enantioselectivity, which could be due to the effect of this soil property on herbicide sorption and ease of its availability for microbial degradation. This aspect however needs further investigations.

Results from studies on soils receiving organic amendment (lupin residue) showed that degradation of the S(-) and R(+) enantiomers of imazethapyr and imazaquin followed first-order reaction with half-life values of 45.9 to 105 days in non-sterilized soils for S(-) and R(+) enantiomers, respectively. Irrespective of the organic amendment, the degradation rate of the S(-) and R(+) enantiomers of the two herbicides was greater in the Roseworthy (pH 8.2) soil compared with the Clare soil (pH 5.2). Addition of lupin residue as organic amendment (2% w/w) increased degradation rates of both the S(-) and R(+) enantiomers of imazethapyr and imazaquin and significantly ($p < 0.05$) decreased their half-lives in the Clare soil. However, this amendment produced no significant change in degradation of

enantiomers of either of the two herbicides in Roseworthy soil. The enantiomer fraction (EF) values of both herbicides increased over time, which suggested selective degradation of one enantiomer in preference to the other depending on the type of soil and amendment treatment. In the Clare soil, organic amendment increased the EF value at the end of incubation period from 0.61 to 0.76 for imazethapyr and from 0.56 to 0.66 for imazaquin, indicating enantioselective degradation of these herbicides. There was no significant increase in EF values for both herbicides in Roseworthy soil as the result of organic amendment.

In conclusion, photodegradation of imidazolinone herbicides was found to have a major impact on the behaviour of these herbicides in aqueous and soil matrices. The degradation of imidazolinone herbicides in the soil was enantioselective, however, the enantioselectivity tended to be compound-specific and was related to soil types. The findings of this study are expected to be useful for the manufacturers to decrease the amount of chemical load in the environment.

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Chapter 1. General introduction

1.1 Introduction

The imidazolinones are one of five families of herbicides that inhibit the enzyme acetohydroxyacid synthase (AHAS). AHAS is a key enzyme in the pathway responsible for the biosynthesis of branched-chain amino acids in plants (Shaner and Singh, 1997). The imidazolinones were discovered in the 1970s by scientists at the American Cyanamid Company (Novdkov, 1994). All imidazolinone herbicides (imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin) have a chiral imidazole moiety in their molecular structure, but differ in the second heterocycle in the structure (Figure 1.1). Imazapyr and imazethapyr have a pyridine ring and imazaquin has a quinoline moiety (Tan et al., 2005). A common characteristic of all imidazolinone herbicides is the presence of two enantiomers that derive from the chiral centre of the imidazolinone ring. The inhibitory activity of the R(+) enantiomer for AHAS is nearly eight times greater than that of the S(-) enantiomer (Lao and Gan, 2005).

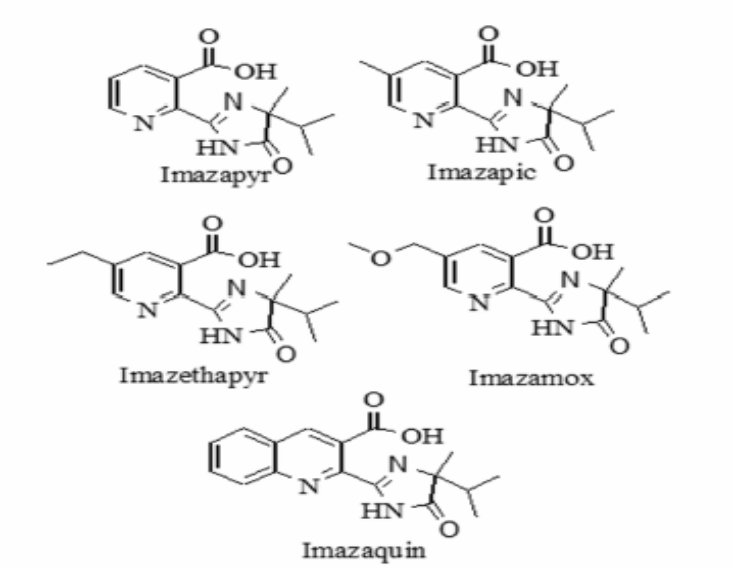


Figure 1.1. Chemical structures of five imidazolinone herbicides.

Imidazolinone herbicides have become widely used because of their low application rates, reduced environmental impact and selectivity in a wide range of cropping systems. They are applied either pre- or post-emergence, as selective herbicides for broad spectrum control of broadleaf weeds and grasses in soybean [*Glycine max* (L.) Merr.] and several other leguminous crops (Barkani et al., 2005; Battaglin et al., 1998). They can also be used as non-selective wide-spectrum herbicides in non-crop situations, as well as in forestry and plantation crops, such as rubber and oil palm (Elazzouzi et al., 1999a; Mallipudi et al., 1991). In addition, the imidazolinone herbicides are used in Clearfield production systems, either singly or in mixtures. The system combines conventionally bred imidazolinone-tolerant crops with imidazolinone herbicides to control weeds. Currently, imazapyr and imazapic are applied with some of the imidazolinone-tolerant crops such as wheat and canola in Australia to control weeds (Tan et al., 2005). Due to increased popularity of

pulses and the introduction of imidazolinone-tolerant canola in recent years, the use of imidazolinone herbicides has increased in Australia (Hollaway and Noy, 1998).

The amphoteric nature (presence of both acidic and basic functional groups) of imidazolinone herbicides allows these herbicides to be in anionic, neutral or cationic states depending upon of the pH of the environment (Pusino et al., 1997; Stougaard et al., 1990). The pKa values of imidazolinone herbicides range from 1.3 to 3.9. When the soil pH is greater than their pKa, these herbicides are usually present mainly in an anionic state ($-COO^-$), while they are in a nonionic (-COOH) form when the soil pH is lower than the pKa (Che et al., 1992; Loux and Reese, 1993). Because of the specific molecular structure of these herbicides, soil factors such as pH, organic carbon content, and ionic strength may affect their persistence in the environment (Johnson et al., 2000; Jourdan et al., 1998). The physiochemical properties of selected imidazolinones are shown in Table 1.1.

Table 1.1. Chemical structure, uses, and selected properties of the imidazolinones herbicides studied (Kah and Brown, 2006).

Herbicide name	Use and application rate (g ha^{-1})	pK_a	K_{oc} (L/kg)	DT_{50} (d)	Water solubility (mg L^{-1})	Log P
Imazapyr	Control of annual and perennial grasses, sedges,and broad-leaved weeds in noncrop area, forestry management,and plantation of rubber trees and oil palms. (100-1700)	1.9 3.6 11.0	4-170	30-150	974	0.11
Imazethapyr	Control of many major annual and perennial grass and broad-leaved weeds in soybeans and other leguminous crops (70-200	2.1 3.9	75-173	30-90	1400	1.04 (pH 5) 1.49 (pH 7) 1.20 (pH 9)
Imazaquin	Pre-plant or pre-emergence control of broad-leaved weeds in soybeans (70-140)	2.0 3.8	13-40	60	60-120	0.34

pK_a, dissociation constant; K_{oc}, distribution coefficient in soil normalised by the organic carbon content; $T_{1/2}$, half-life in soil, time required for 50% of the initial dose to be degraded; log P, octanol: water coefficient (hydrophobicity) of the herbicide.

The imidazolinone herbicides are relatively persistent in soil with half-lives ranging from 30 to 150 days and may have carryover effect to the rotational crops (Curran et al., 1992a; Goetz et al., 1990; Loux et al., 1989c). Moreover, it has been reported that the imidazolinone herbicides show high potential leaching because of their relatively low pK_a values (3.3-3.9) (Carter, 2000; McDowell et al., 1997; Polubesova et al., 2002; Regitano et al., 1997b; Regitano et al., 2002; Vizantinopoulos and Lolos, 1994). Given the persistent nature of these herbicides on some soil types, it is important to investigate the mechanisms responsible for imidazolinone degradation. Such information will assist in developing guidelines to prevent damage by imidazolinone residues due to carryover to sensitive crops grown in the rotation sequence. Furthermore, a sound knowledge of their environmental fate and behaviour would help minimise their off-site transport and impact on non-target organisms.

The use of imidazolinone herbicides for controlling broadleaf and grass weeds in pulse crops has increased in Australia in recent years. However, most research on the behaviour of imidazolinone herbicides has been carried out in other countries where soil types and climate are different to Australia. Because of the presence of the anionic form of imidazolinone herbicides at higher pH values and the alkaline properties of many soils in Australia, these herbicides are poorly sorbed or can be repelled in these soils by the negatively charged colloids. As a result, under high rainfall, leaching of these herbicides may occur, leading to accumulation of these herbicides in subsoils or contamination of groundwater. In addition, there is no information on the behaviour of imidazolinone enantiomers in alkaline soils in Australian environment. In particular, there is no published data available on the relative degradation behaviour of the two enantiomers of these herbicides.

1.2 The objectives of this research

The borader aim of this research was to develop improved understanding of the environmental fate of selected imidazolinone herbicides and their enantiomers in Australian soils. The studies were planned to achieve the following specific objectives:

(1) To develop an improved analytical method based on solid phase extraction (SPE) procedure for separation and concentration of imidazolinone herbicide and their enantiomers, and evaluate its application to soils and waters with high dissolved organic matter concentrations;
(2) To assess the potential of abiotic degradation (hydrolysis and photodegradation) of imazapyr, imazethapyr and imazaquin herbicides in solution and soil in the presence of dissolved organic matter;
(3) To establish the enantioselectiveness of degradation (biotic and abiotic) of imazethapyr and imazaquin in soils amended with organic matter; and
(4) To examine relationship between the enantioselective degradation of imazapyr, imazethapyr and imazaquin and soil characteristics.

1.3 Structure of thesis

The structure of thesis is as follows:

This thesis consists of six chapters. Following a general introduction (Chapter 1), Chapter 2 presents a broad review of published investigations on the fate of imidazolinone and other herbicides in the environment, the enantioselective degradation of chiral pesticides and solid-phase extraction technique for extraction of pesticides from different matrices. The evaluation of imidazolinone extraction from soil solutions using solid-phase extraction

cartridges is discussed in Chapter 3. The next two chapters deal with the abiotic and biotic pathways of degradation of these herbicides and their enantiomers. Chapter 4 discusses the abiotic degradation (photodegradation and hydrolysis) of imidazolinone herbicides in aqueous solutions as well as on the soil surface, whereas the enantioselective biodegradation of imidazolinone herbicides in different soils as well as the impact of organic amendment on enantioselectivity of these herbicides is covered in Chapter 5. Finally, a general discussion on the results of the entire project is provided in Chapter 6, with their agronomic and environmental implication as well as future research needs.

Chapter 2. Review of Literature

2.1 Introduction

Increasing demand of food and fiber in the world has led to greater use of pesticides for production of agricultural crops. It is estimated that without the use of pesticides, approximately 50% of world agricultural production could be lost (Rice et al., 2007). Thus, the use of pesticides is an indispensable tool for the production of high crop yields in modern agricultural systems. At present more than 500 different formulations of pesticides are being used in the environment (Gavrilescu, 2005). It has been reported that less than 1% of the total of applied pesticides reaches the target pests (Gavrilescu, 2005) and without a doubt, a portion of applied pesticides and their transformation products move off-target. There is considerable public concern about the unintentional adverse impact of pesticides on non-target organisms and environmental quality (soil, water and air).

Herbicides account for 50 to 60% of the total pesticides used annually worldwide (Carter, 2000). In Australia, there are over 400 pesticide active ingredients currently registered, 67% of the total pesticide used is herbicides and their usage has increased since the adoption of conservation farming techniques (Kookana et al., 1998). It has also been reported that herbicides account for most of the detected pesticides in ground and surface water (Carter, 1999).

To better understand the behaviour of herbicides in the environment and to avoid their harmful effects on target and non-target organisms, it is essential to consider the physical, chemical, and biological processes that affect herbicide behaviour in the environment.

These processes are complex and dynamic, and are influenced by a range of soil and environmental conditions, such as climatic factors (e.g. temperature, humidity, and aeration), abiotic soil factors (e.g. texture, clay minerals, and pH) and biotic soil factors (e.g. microbial biomass, biological diversity, and plant cover) (Wanner et al., 2005). The main processes influencing the behaviour of pesticides in the environment are sorption, desorption, chemical and biological degradation, volatilization, plant uptake and run-off (Figure 2.1). Information about these processes and the factors that influence their rate is necessary to create more efficient pesticide management practices, to better determine pesticide exposures and threats, and to develop management and remediation approaches where necessary. Furthermore, information on degradation rates and sorption coefficients can also be used to develop predictive tools for the assessment and management of the risks of pesticide contamination of the ground and surface waters.

Canola (oilseed rape, *Brassica napus* L.) and wheat (spring wheat, *Triticum aestivum* L.) are widely grown in Australia. Cultivars of these crops have been bred to be tolerant to imidazolinone herbicides (Brennan and Bolland, 2007). There are concerns about the residual effects of some imidazolinone herbicides on rotational crops with the use of these cultivars. Recommended re-cropping intervals are 10 months for cereals and 34 months for canola to avoid imazethapyr injury, depending on the crop and soil type (Hollaway and Noy, 1998). Imazethapyr residues have damaged rotational crops in Canada 1 year after application (O'Sullivan et al., 1998). Apart from the injury due to carryover of the imidazolinone herbicides, there is also concern about their contamination of ground water (Battaglin et al., 2000). Given the serious impact of imidazolinone residues on subsequent crops, it is important to develop understanding of the behaviour of these herbicides in Australian soils and water environments.

Imidazolinone herbicides are chiral in nature. Chirality is a feature shown by chemical molecules that are asymmetric in structure and results in two species of the molecule that are a mirror image of one another. Approximately 25% of pesticides are chiral, containing two or more enantiomers. It is well known that these enantiomers usually vary in their biological characteristics due to their differential interaction with enzymes or other naturally occurring molecules (Garrison, 2006). Differences in microbial degradation rates may mean that one enantiomer is more persistent in the environment than the other. So far there is little understanding of the enantio-selectiveness of the environmental fate processes for pesticides in general and imidazolinone herbicides in particular. Thus, to predict the behaviour of different enantiomers in the environment, research is needed to investigate how different conditions influence the relative persistence of each enantiomer. For this purpose, degradation studies are necessary with chiral pesticides in a range of different soils to determine relationships between environmental characteristics and enantioselectivity.

2.2 Processes influencing the fate of herbicides in the environment

The behaviour of herbicides in the environment is complex and controlled by inter-dependent physical, chemical and biological processes. The most important processes determining the fate of herbicides in the environment include abiotic degradation (photodegradation and hydrolysis), microbial degradation, volatilization, sorption and transport processes (run-off and leaching) (Figure 2.1). The movement of herbicides from soil into water, air and the food chain is directly controlled by these processes. Different soil and climatic factors determine the relative importance of these processes. These factors include soil texture, soil permeability, soil depth, soil pH and soil organic matter. In

addition, the chemistry of the herbicide, including water solubility and soil sorption, can have an impact on the rate of herbicide degradation. These processes will be discussed in detail in the following sections.

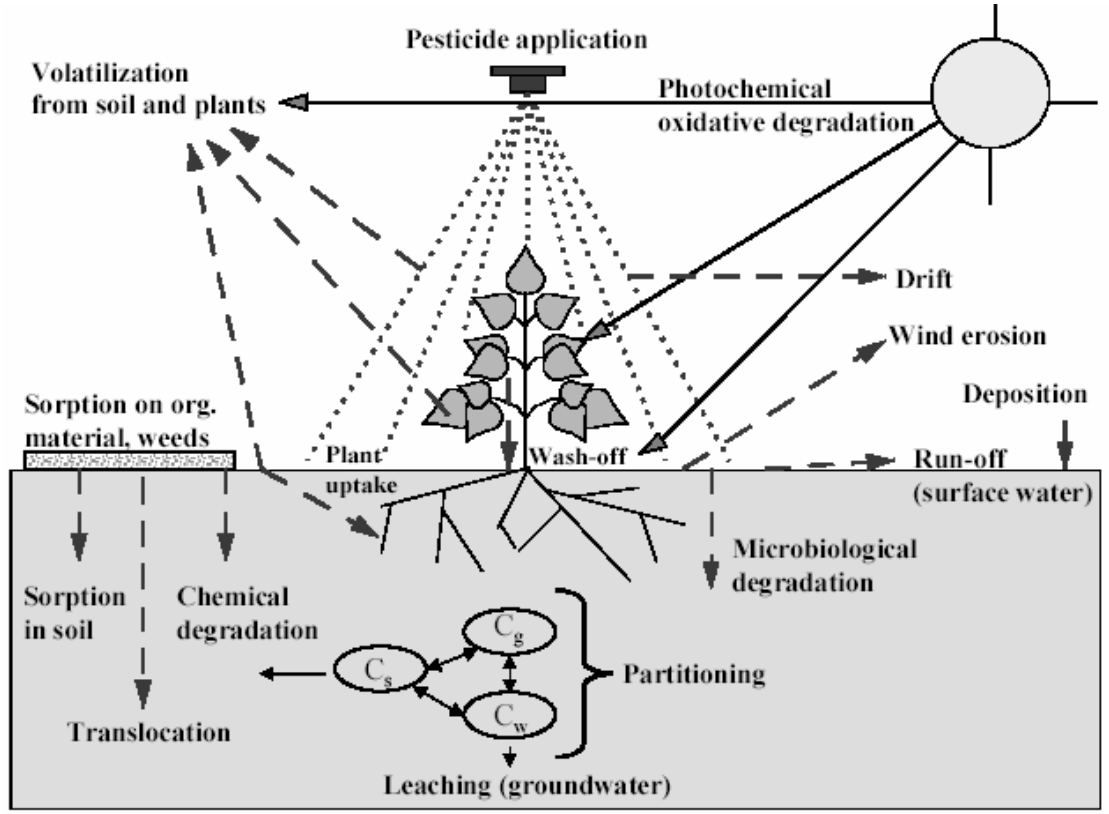


Figure 2.1. The main processes influencing the behaviour of pesticides in the environment. Cg, Cw and Cs represent the pesticide concentrations in the gas, aqueous and solid phases, respectively (Wolters et al., 2004).

2.2.1 Abiotic degradation of pesticides

Understanding degradation (abiotic and biotic) processes of pesticides is not only important for assessing the persistence of residues in environment, but could be crucial because of the formation of by-products that may have lesser or higher toxicity for the environment (Morrica et al., 2001). Abiotic (chemical) degradation refers to the breakdown

of pesticides in the environment that does not involve living organisms. It can occur on the soil surface, in subsoil or in the soil solution. Hydrolysis, oxidation-reduction and photodegradation (direct and indirect) are some of the most important abiotic reactions involved in pesticide degradation. The chemical nature of pesticides, pH and temperature are the most important factors influencing abiotic degradation (Huang et al., 2000).

2.2.1.1 Hydrolysis

Hydrolysis is the addition of a water molecule to the pesticide. A significant factor influencing the hydrolysis of pesticides is solution pH. Different pesticides show acid or alkaline hydrolysis in soil and water depending on the environmental pH. For example, the hydrolysis of chlorpyrifos (Huang et al., 2000; Racke et al., 1996) and flumioxazin (Kwon et al., 2004) appears to be increased under basic conditions. However, hydrolysis of metsulfuron-methyl and most sulfonylurea herbicides is increased under acidic conditions (Li et al., 1999; Morrica et al., 2001). Soil organic matter and clays can enhance the rate of hydrolysis of pesticides by providing a large surface area (Müller et al., 2007). Furthermore, it has also been shown that the hydrolysis of some pesticides is temperature-dependent (Gaynor et al., 1997). To better predict the behaviour of pesticides in the environment, it is important to study hydrolysis of pesticides under the range of pH values typically found in the soil and aquatic environment (5.5-8.0).

The evaluation of hydrolysis of pesticides is not only important for better understanding of their environmental fate, but also allows assessment of their potential for ground and surface water contamination. When pesticides leach into ground water or saturated soil where microbial degradation is limited, abiotic degradation, particularly hydrolysis, is the most important process determining degradation of several pesticides. It is known that

factors such as pH and temperature can affect the hydrolysis of pesticides in the environment. For example, (Dinelli et al., 1997) showed that these two main factors influenced hydrolysis of sulfonylurea herbicides. Hydrolysis is generally not considered important in the degradation of imidazolinone herbicides, although there is only one published report in the literature on this degradation mechanism. This reported that hydrolysis of the acid imidazolinones was negligible over the range of environmental pH range and temperature (Mangels, 1991b). The effect of factors such as presence of dissolved organic matter on hydrolysis of imidazolinone herbicides in water has not been studied.

2.2.1.2 Photodegradation

Photodegradation (photolysis) of pesticides is a chemical reaction that happens only in the presence of light. Most pesticides have UV–Vis absorption bands above 295 nm in the near ultraviolet (Burrows et al., 2002). Pesticide photodegradation occurs in surface waters, on the soil surface and in the atmosphere. In general, photodegradation can be categorised into two processes: direct and indirect photoreaction (Monica et al., 2003). Direct photolysis occurs when a photon is absorbed by a pesticide molecule, producing an excited state that cleaves the molecule. Direct photodegradation is less important in the environment due to the limited amount of UV radiation reaching the Earth's surface (Burrows et al., 2002). Indirect (photosensitized) photodegradation of pesticides occurs due to light absorption of inorganic or organic constituents in water, such as nitrates and dissolved organic matter (DOM), and the transfer of that energy to the pesticide. These components naturally exist at concentrations ranging from 10^{-14} to 10^{-18} M in water (Lam et al., 2005).

2.2.1.2.1 Photodegradation in aqueous solution

Pesticides can be applied directly to the aquatic system or applied to soil and crops with subsequent movement into aquatic environment due to spray drift, run-off, leaching, neglectful dumping of containers, equipment washing etc. (Katagi, 2006). Currently, the contamination of surface and ground water by pesticides and their metabolites is well recognized globally and has become a major concern (Martin et al. 2003). Factors that influence the amount of pesticides transported from field to surface and ground water include soil characteristics, topography, weather, agricultural preparations, and the characteristics of individual pesticides (Konstantinou et al., 2001). Water properties such as pH, the amount of dissolved and suspended constituents and temperature are the most crucial factors that affect photodegradation of pesticides. There are many types of dissolved and suspended components, such as humic substances, metal oxides, and clay particles derived from biota, soil and sediment in natural water. Thurman (1986) reported the concentration of dissolved organic carbon (DOC) is roughly 0.5 mg L^{-1} in ground water and up to 30 mg L^{-1} in coloured water in swamps.

Humic substances constitute approximately 30-50% of the total organic matter in the surface water (Corin et al., 1996; Garbin et al., 2007; Thurman, 1986). Humic substances are natural organic macromolecules that are generally divided into three classes - humic acids, which are soluble in water at pH values above 2, fulvic acids, which are soluble at all pH values and humins, which are insoluble (Zeng et al., 2002). The elemental composition of humic substances comprise carbon, oxygen and hydrogen with small amounts of nitrogen and infrequently phosphorous and sulphur. Humic substances with a molecular weight of approximately 500-5000 consist of a variety of functional groups, such as carboxylic acid, phenolic hydroxyl, carbonyl and hydroxyl (Kulovaara, 1996).

Interaction of pesticides with these components in water can increase the water solubility and affect the abiotic degradation of pesticides. Photodegradation of humic substances at wavelengths >290 to 295 nm leads to the production of the hydroxyl radicals, which are the main reactive oxidant in the environment. In addition, irradiation of humic acid creates hydrogen peroxide, which in turn produces hydroxyl radicals (Graebing et al., 2002). Humic substances also create stable radical species when exposed to light. These stable radicals have a significant role in photodegradation of pesticides and creation of active oxygen species, particularly with the presence of a metal, such as iron (Katagi, 2004). Both clay minerals and humic substances are able to create oxygen-reactive species under UV irradiation (Müller et al., 2007).

While soil components such as humic acids are known to enhance pesticide photodegradation in water bodies due to the production of reactive oxygen intermediates, photodegradation in natural water systems can also be decreased by the UV screening effects of soil particles. Thus, photodegradation of pesticides in natural aquatic systems will be different compared with that in pure water. Both inhibition (Garbin et al., 2007; Kamiya and Kameyama, 1998; Okamura and Sugiyama, 2004; Si et al., 2004) and enhancement (Hustert et al., 1999; Kamiya and Kameyama, 1998) of pesticide photodegradation are known. As the existence of humic substances has important influence on the behaviour of many pesticides in natural water environment, it is essential to investigate the impact of these materials to effectively predict the influence of pesticides on the environment.

2.2.1.2.2 Photodegradation on the soil surface

Soil is a heterogeneous mixture of minerals, organic matter and water with air space. Generally, the solid phase of soil comprises less than 5% organic matter and >95% inorganic matter. More than 90% of inorganic components of soil are crystalline and non-crystalline amorphous minerals with various hydroxyl groups on their surface (Parlar, 1990). Soil clay minerals have a net negative charge and silica tetrahedral and alumina octahedral sheets that bond loosely to each other. Many clay minerals in soil contain iron, which is involved in the creation of reactive species, such as the hydroxyl radicals. These may contribute to the photodegradation of pesticides in soil (Katagi, 2004). Some soil and environmental factors may influence photodegradation of pesticides on the soil surface. These factors include organic matter content, moisture content, texture, mineralogy, pH, and the presence of other organics, which all influence the sorption of organic molecules onto the soil (Frank et al., 2002).

Light can penetrate to a depth of approximately 2 mm in soil, so the condition of this part of soil is influenced by irradiation (Balmer et al., 2000). It has been shown that about 90% of UV spectrum of xenon arc light is attenuated by 0.17 mm of soil (Herbert and Miller, 1990), while (Frank et al., 2002) showed approximately 95% of light is blocked by 0.5 mm of soil. They also demonstrated that the effect of soil depth on photodegradation of niclosamide is more important under dry conditions compared with wet soil. For a variety of pesticides, the estimated depth of direct and indirect photodegradation are approximately 0.23 and 0.28 mm, respectively in controlled environments and 0.32 and 0.62 mm in the field (Katagi, 2004).

There are contradictory results about the impact of soil organic matter on photodegradation of pesticides. (Konstantinou et al., 2001) showed photodegradation of selected herbicides was enhanced with increasing soil organic matter. By contrast, photodegradation of chlorimuron-ethyl, triasulfuron, thifensulfuron-methyl (Albanis et al., 2002) and fipronil (Bobe et al., 1998b) were decreased or screened by the presence of organic matter in soil. Romero et al. (1998) reported a decrease in photodegradation of mecoprop when 10% peat was added to the soil under dry conditions, consistent with a quenching effect. There is some evidence that shows the concentration of soluble iron increases with organic matter in soil (Frank et al., 2002). Soil clay content is another factor that may influence photodegradation of pesticides. An increase of clay content enhanced the photodegradation of metalaxyl (Sukul and Spiteller, 2001). By contrast, Graebing et al. (2002) reported that soil composition had a negligible effect on photodegradation of niclosamide.

Soil pH is a crucial factor in photodegradation of pesticides on the soil surface, particularly for acid-labile pesticides like the sulfonylurea and imidazolinone herbicides (Katagi, 2004). Quan et al. (2005) reported increased photodegradation of *p,p′*-DDT with increasing soil pH. Soil moisture is also important because of its role in producing hydroxyl radicals, which in turn cause photodegrdation of pesticides. Krieger et al. (2000b) reported that photodegradation of florasulam increased in moist soil compared with dried soil. However, there are contradictory results of the effect of moisture on photodegradation of pesticides, particularly in relation to soil depth (Frank et al., 2002).

While imidazolinone herbicides are degraded in soil primarily by microbial metabolisms, photodegradation could be important with these herbicides because they show low sorption to the soil. This increases the risk of off site transport in surface water, where

photodegradation may play an important role. Photodegradation of imidazolinone herbicides including imazaquin, imazethapyr and imazapyr has previously been studied. Photodegradation played a significant role in degradation of these herbicides in the environment (Barkani et al., 2005; Elazzouzi et al., 2002; Elazzouzi et al., 1999b; Etienne et al., 2004; Mallipudi et al., 1991; Mekkaoui et al., 2000; Moorthy et al., 1991; Quivet et al., 2004; Venkatesh et al., 1993). Curran et al. (1992b) studied the photodegradation of imazapyr, imazethapyr, and imazaquin in solution as well as on the soil surface and reported that these herbicides are prone to degrade if they remain exposed to light on the soil surface in the field. They reported that imidazolinone herbicides degraded faster in moist compared to air-dried soil.

Photodegradation of imazaquin is higher at pH values above the p*K*a and in anerobic conditions. Temperature had no significant effect on photodegradation of imazaquin (Barkani et al., 2005). The presence of riboflavin in aqueous solution resulted in decreased photodegradation, leading to an increase in imazaquin half-life compared to pure water (Venkatesh et al., 1993). Imazethapyr losses from photodegradation were found to be 2% and up to 52% from the soil surface and a glass slide, respectively (Goetz et al., 1990). The presence of humic acids delayed photodegradation of imazethapyr, but titanium dioxide (TiO_2) increased the rate of photodegradation (Elazzouzi et al., 2002).

The photodegradation half-life of imazapyr in deionised water (pH =3) was found to be 3.5 days, notably enhanced by the presence of TiO2, humic substances and acetone (Santoro et al., 1999). Mallipudi et al. (1991) reported photodegradation half-lives of imazapyr up to 2.3 days in distilled water and pH 9 buffer, and 2.7 days in pH 5 buffer. Humic acids

decreased the rate of photodegradation of imazapyr compared with deionised water (Bouhaouss et al., 2000; Elazzouzi et al., 1999b).

2.2.2 Sorption

Sorption is a transfer process whereby pesticides are partitioned between the solid and aqueous phases in soil, which determines the behaviour of pesticides in the environment and transport to ground water (Wauchope et al., 2002). In general, several other processes (volatilization, hydrolysis, and photodegradation) determining the behaviour of herbicides in the environment are directly dependent upon sorption (Müller et al., 2007).

Soil sorption is usually characterized by a partition constant, K_d, which is a ratio of solid phase to solute concentrations. High values of K_d indicate that a pesticide has a higher affinity to soil particles and is thus less likely to be transported off-site to water bodies and more likely to be resistant to microbial degradation (Zhang et al., 2000). It is known that degradation of many pesticides is largely restricted to the bioavailable portion in the soil solution (Beulke et al., 2004), so when pesticides are sorbed they are less accessible to degrading microorganisms. Reactions between the pesticide and the sorbent may also be time-dependent and the distribution between the dissolved and sorbed state may require days, weeks or even months to reach equilibrium (Cox et al., 1996; Cox et al., 2001; Koskinen et al., 2002). Sorption of some pesticides has also been shown to increase with the length of time the pesticide is in contact with soil (Cox et al., 1998; Pignatello et al., 1993).

Soil factors, such as organic carbon content, texture, pH, moisture and temperature, influence the sorption of pesticides in soil. Pesticides properties, such as the molecular structure of pesticide, hydrophobicity, molecular charge and hydrogen binding, can also affect sorption (Kah and Brown, 2006). As imidazolinone herbicides are ionisable compounds, this section of the literature review focuses on the sorption of these compounds and the soil factors that have an important influence on ionisable pesticides.

Ionisable herbicides have weak acidic and/ or basic functional groups that can become charged within certain pH range (Kah and Brown, 2006). Approximately a third of modern pesticides are a weak or strong acid or base. Ionisable herbicides such as phenoxy acids, triazines, sulfonylureas and imidazolinones, constitute a major groups of soil-applied herbicides and include compounds commonly detected in ground water and surface water (Kah and Brown, 2006). Ionisable pesticides contributed eight out of the 15 main compounds detected in ground water and surface water in France (Kah and Brown, 2006). Ionisable herbicides also contributed six of the nine pesticides detected exceeding threshold concentration (0.1 μg L^{-1}) in surface fresh water and seven out of the 10 pesticides detected in ground water in the UK (Kah and Brown, 2006). Battaglin et al. (2000) studied the occurrence of imidazolinone herbicides in the midwestern United States and found imazethapyr, the most frequently detected imidazolinone herbicide, was above 0.01 μg L^{-1} in 83% of 130 streams and 24% of 25 ground water samples.

The three main factors influencing the sorption of ionisable pesticides in soil are soil properties, climatic factors and the chemical characteristics of the pesticide itself. While the sorption of ionisable pesticides is affected by soil properties, such as organic matter, clay contents, Fe/Al oxide and hydroxides, the most important factor is soil pH (Undabeytia et al., 2004). Temperature and moisture content of soil are the most important

climatic factors that impact on sorption of ionisable pesticides. For example, Goets et al. (1986) showed that the sorption of imazaquin increased with drying and wetting of soil due to decreased thickness of water surrounding soil minerals, which increased the concentration of imazaquin near sorption areas.

Like other polar ionisable pesticides, the sorption of imidazolinone herbicides is highly sensitive to soil pH, because of the amphoteric characteristics of these herbicides. Imidazolinones are sorbed through several mechanisms including ligand exchange, cation and/or water bridging ion exchange, hydrogen bonding, electrostatic interaction or hydrophobic partitioning (Regitano et al., 1997).

Ionisation of the carboxyl group of imidazolinone herbicides increases their solubility in water, resulting in movement in soil. The presence of an anionic form of imazapyr, imazethapyr and imazaquin at soil pH greater than the pK_a, causes these herbicides to be repulsed by soil colloids, leading to the low sorption at neutral or high soil pH (Che et al., 1992; Stougaard et al., 1990). At low soil pH, imidazolinone herbicides mainly exist as uncharged species that can interact with the hydrophobic surfaces of organic matter and negatively charged soil colloids (Wepplo, 1991). The sorption of imazapyr to soil has been noted to be weak (K_d values varying from 0.07 to 0.19), resulting in high mobility in soil (Vizantinopolous and Lolos, 1994).

For soils from temperate regions with permanent negative charges, the literature shows that the sorption of imazaquin is positively related to the organic carbon content, which influences the number of hydrophobic sites, and is negatively related to the soil pH (Regitano et al., 1997a; Regitano et al., 2005). Depending upon the soil type, the persistence of imazethapyr increased with decreasing soil pH (Loux and Reese, 1993),

which can increase the potential carry over damage to non-target crops in rotations in subsequent year (Onofri, 1996). Clay and organic carbon content had a positive effect on the sorption of imazethapyr in soil (Goetz et al., 1990; Loux et al., 1989a).

2.2.3 Volatilization

Volatilization is a physio-chemical process where a pesticide is transferred into the atmosphere as a gas (Müller et al., 2007). Volatilization will decrease the bioavailability of pesticide for the control of pests, as well as the potential for the contamination of ground water. However, the potential for contamination of the atmosphere and surface water will be increased (Wolters et al., 2004). Depending on the properties of pesticides and environmental conditions, volatilization losses can be as high as 90% of the applied dose for pesticides, such as trifluralin or lindane, after one week. For other herbicides volatilization is a minor loss, such as with atrazine where volatilization accounted for 2% of the application dose after 24 days (Wolters et al., 2004). Losses via volatilization can be important for those pesticides that remain on the soil or plant surface (Gavrilescu, 2005). The vapour pressure of the pesticides is the most important factor, although volatilization of pesticides is complex and for a specific compound it can be affected by agricultural practices and environmental conditions, such as soil water content, tillage and soil texture (Cousins et al., 1999). For example, losses of chlordane within 2 days were 50% and 2% in moist silt loam and dry sandy loam soil, respectively (Taylor and Spencer, 1990). The reported rate of pesticide losses via volatilization range from a few g ha^{-1} day^{-1} to 2000 g ha^{-1} day^{-1} on the day of application depending on the pesticide and the application dose (Bedos et al., 2002; Woodrow et al., 1997).

2.2.4 Run-off

There have been concerns about the off-site transport of pesticides to non-target areas since the 1970s when pesticides were detected in surface and ground water bodies (Baker and Mickelson, 1994). Run-off and leaching are two processes where pesticides can enter waterways (Dabrowski et al., 2002; Fawell, 1991). Run-off is the movement of water, which may include suspended colloids, across the soil surface. Run-off often occurs when the rate of precipitation or irrigation is more rapid than the infiltration of water into the soil. Indeed, it can be considered as the most significant factor contributing to surface water contamination in arid areas (Everts, 1997). Loss of pesticides via run-off has been reported to be 2-5% for wettable powders and 1% for other pesticide formulations. Factors influencing pesticide run-off include rainfall features (time period of the first rainfall after application of pesticide, its duration and intensity), surface aspects (topography, soil permeability, and surface cover), agricultural practices (soil-incorporation of pesticides) and the chemical characteristics of the pesticide (Leu et al., 2004; Schrievera et al., 2007). The highest concentrations of pesticides are detected in surface water after heavy rainfall (Domagalski et al., 1997). There is a strong correlation between the amount of pesticide in surface water and their concentration in the top 10 mm of the soil. Consequently, the risk of run-off for those pesticides remaining at the soil surface is higher than those incorporated into the soil. One reason for this is the sorption of pesticides onto soil particles and lack of degradation, which can lead to greater susceptibility to run-off (Larson et al., 1995). The anionic form of imidazolinone herbicides dominates at soil pH of 6 to 9 and this form can be repelled by the negative charges of the soil colloids, leading to low adsorption in neutral and high pH soils. Thus, higher desorption of these herbicides in soils containing low organic content is expected. The study of adsorption and desorption of

imazapyr on eight soils showed a considerable correlation with the organic carbon content of soils (Qiquan and Liu, 1999). The authors suggested that decrease of organic carbon content contributed to increased ease of desorption of imazapyr.

2.2.5 Leaching

Leaching of pesticides to ground water creates a threat to drinking water when it is used as a potable water source and aquatic water resources (Dabrowski et al., 2002). Thus, an understanding of pesticide mobility is considered a pre-requisite to estimating the ground water contamination potential of a pesticide. Pesticides may leach into the unsaturated region of soil either as water-dissolved or particle-associated complexes. Preferential flow is the movement of water and pesticides through a small portion of soil volume and will likely be the more rapid form of leaching (Malonea et al., 2004). Leaching of pesticides occurs mainly from macro-pores (cracks, worm holes, root channels and large voids) through the subsoil to ground water. The pathways of preferential flow are temporary structures and their distribution across the field are not uniform (Ogden et al., 1999). As the transport of pesticides via preferential flow is more rapid compared with matrix flow, the possibility of pesticide retention or degradation on the soil surface is less for pesticides in solution.

Matrix flow is the slower movement of water and pesticides through soil and there is more likelihood of pesticide retention by soil particles (Gavrilescu, 2005). Matrix flow is expected to be the foremost route of pesticide movement in the sandy textured and low organic matter soils, whereas preferential flow occurs mostly in heavy loams and clay soils. There are several reports, showing no leaching of imidazolinone herbicides in soil

(Goetz et al., 1990; Renner et al., 1988; Stougaard et al., 1990). For example, Mangels (1991) reported that leaching of imazethapyr was not important below 15 cm. Similarly, it has been shown that more than 90% of imazethapyr remained in the top 10 cm of soil in column leaching studies (Flint et al., 1989; Loux et al., 1989b; Zeleznik et al., 1992). However, others have shown imazethapyr to leach beyond 30 cm in sandy-textured soils, depending upon the amount of rainfall (O'Dell et al., 1992; Wyk and Reinhard, 2001). Imazethapyr was among the most frequently detected herbicides in rivers and ground water in the mid-west US (Battaglin et al., 2000). It has been suggested that leaching of imidazolinone herbicides is higher in soils in tropical areas compared with temperate areas. For example, 13% and 34% of imazapyr leached in clay and clay loam soil columns, respectively, following artificial irrigation of 40 mm in Brazil (Oliveira et al., 2001).

Persistence and sorption of pesticides in soil are important factors determining the leaching of pesticides through soil. Imazaquin has longer persistence (Basham et al., 1987; Loux et al., 1989b) and lower sorption than other imidazolinone herbicides (Regitano et al., 1997b), giving it greater potential to leach. The leaching of imazaquin has been reported in soil columns (Basham et al., 1987) and in field studies (Sorokina and Thomas, 1997). It has been suggested that the time interval between the application and the first rainfall is a significant factor for the leaching of imazaquin. Regitano et al. (2005) observed no leaching of imazaquin at soil pH less than the pK_a (3.8), but more than 80% of the herbicide was leached at pH values above 5.5.

2.2.6 Biotic (microbial) degradation of herbicides

Microbes have the ability to degrade pesticides either completely or partially and this is a major process of pesticide degradation,, which is affected by both sorption of pesticides and transport in soil (Fomsgaard, 1995). The rate of degradation and sorption values are two of the most powerful parameters for modelling pesticide concentrations in soil, water and the environment. For example, Lehmann et al. (1992) observed a significant positive correlation between sorption and degradation of the weak acid herbicide flumetsulam.

Bacteria, actinomycetes, fungi and algae are the major microorganisms involved in biological degradation. However, the most abundant microorganisms are not necessarily the most important for pesticide degradation. A single gram of soil may contain 5000 to 7000 different bacterial species and approximately 10000 fungal colonies (Gavrilescu, 2005). Also there is variation both spatially and temporarily in soil microorganisms due mainly to the differences in soil type and depth. It is been reported that even the most persistent pesticides can be degraded to at least some extent by microbial cultures.

Pesticide transformation can occur through three types of reactions. The first is growth-linked metabolism (biodegradation) where organisms use the pesticides as a source of energy. This usually requires an acclimation period and a lag phase exists before rapid degradation occurs. The reasons for this acclimation period are unclear at present and may be induction of enzymes required for pesticide metabolism, production of a naturally occurring pesticide-degrading communities, or genetic change in the microbial population (De Lipthay et al., 2002). Sørensen and Aamand (2003) observed acclimation periods of approximately 14 days, followed by a rapid exponential increase in $^{14}CO_2$ release.

Degradation rates of 2,4-D, propham and glyphosate herbicides in soil were slow when herbicides were first applied, but increased with increasing numbers of herbicide-degrading microorganisms (Robertson and Alexander, 1994). The second type of degradation is co-metabolism, which is the most common method of pesticide degradation. With co-metabolism there is no lag phase and pesticides are not used as an energy source (Bending and Sonia Rodriguez-Cruz1, 2007). The last type of degradation is bioaccumulation, characterized by both incorporation and accumulation of pesticide through active or passive processes (Figure 2.2) (Aislabie and Lloyd-Jones, 1995; Müller et al., 2007).

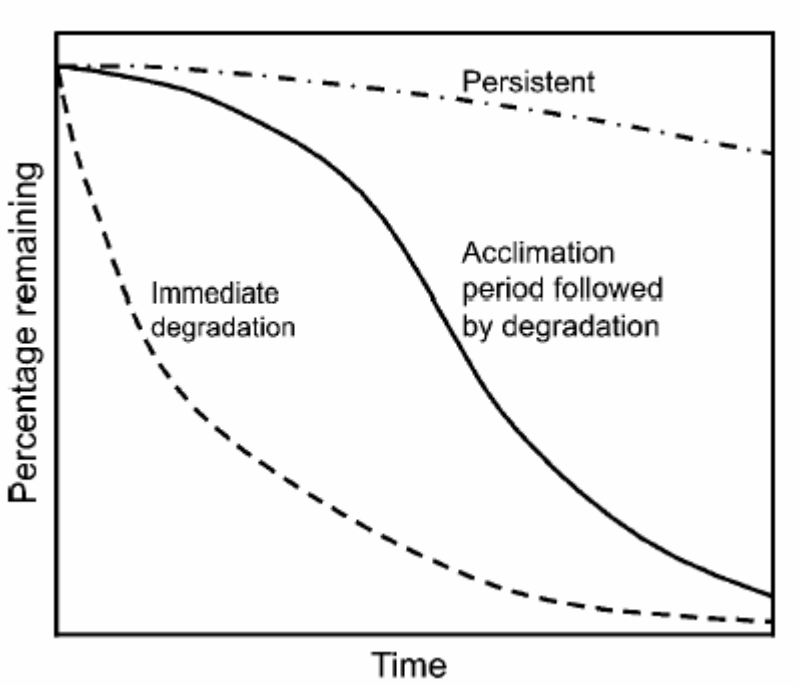


Figure 2.2. Different types of microbial degradation of pesticides.

Biotic degradation of pesticides depends not only upon their bioavailability, but also on the viability and activity of microoganisms in soil. Degradation of the thiocarbamate herbicides diallate and triallate was correlated to microbial biomass (Anderson, 1984).

There are several environmental and pesticide-associated factors that impact on microbial degradation of pesticide. The environmental factors include temperature, soil water content, soil pH, nutrient availability, species composition, and the distribution of organisms and pesticide in the soil. Pesticide properties important in microbial degradation are the chemical structure, molecular weight, the type of functional groups, pesticide concentration, biotoxicity, and water solubility (Aislabie and Lloyd-Jones, 1995).

Environmental conditions can dramatically change the microbial population in the soil. Levy et al. (2007) suggested that the hot and dry conditions in the summer of 2003 in Germany caused significant changes in the microbial population structure and consequently in specific soil functions of the community. This led to a decrease in isoproturon mineralization in a field lysimeter (Levy et al., 2007). The degradation rate of florasulam is affected by temperature, with half-lives for florasulam of 1.0 to 8.5 days at 20-25°C increasing to 6.4 to 85 days at 5°C (Krieger et al., 2000a). Different soil moisture regimes affected the degradation of isoproturon, benazolin-ethyl, and glyphosate with negligible mineralisation of the herbicides at a soil water potential of -20 MPa, increasing mineralization within a soil water potential range of -20 to -0.015 MPa and greatest mineralization at -0.015 MPa. Taylor-Lovell et al. (2002) reported half-lives of 9.6, 2.4, and 1.5 days for isoxaflutole in air-dry soil, soil at -1.5 MPa, and at -0.1 MPa soil water potential, respectively. Too much soil water may decrease herbicide degradation (Schroll et al., 2006). Soil pH can also influence microbial degradation of herbicides. Degradation of atrazine occurs most readily in soils of neutral pH and of cyanazine in neutral to slightly basic soils (Blumhorst and Weber, 1994). A study of sorption and degradation of a weak acidic herbicide (mesotrione) in 15 different soils covering a wide range of soil textures, soil pH values (4.4 to 7.5), and organic carbon contents (0.6 to 3.3%) showed increased

soil pH decreased K_d and decreased half-life of this herbicide in the soil (Dyson et al., 2002). The rate of degradation of 2,4-D was decreased with increasing organic matter content over 10 soils, with increasing sorption of the herbicide. An increase in organic carbon content to 12% increased both sorption and degradation of this herbicide, caused by increased biological activity of the soil (Bolan and Baskaran, 1996). A four-component model shown in Figure 2.3 represents the main mechanisms involved in the incubation of pesticides. Any alterations of this equilibrium can have enormous influences on the overall fate of the pesticide due to the reversibility of both sides of the equilibrium reactions.

NOTE: This figure is included on page 29 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.3. Schematic diagram of probable processes during the incubation of a pesticide in degradation studies (Wanner et al., 2005).

The degradation rate of most pesticides is determined by the first-order kinetics. Degradation rates are recognized to be site-specific and are affected by soil characteristics including organic carbon content, temperature, pH, nutrient availability and environmental conditions. Second-order kinetics describes the degradation rate of pesticides as a function of concentration and the size of microbial community, which is changing as the pesticide is

degraded (Gavrilescu, 2005; Loux et al., 1989d). The most commonly used equation to express degradation rates assumes that degradation rate is proportional to the pesticide concentration (Equation 2.1):

$$\frac{dC}{dt} = kC^h \qquad \text{Equation 2.1}$$

where C is the pesticide concentration, k a degradation rate constant, h the order of the reaction and t is the time. As the degradation of most pesticides can be described by first-order kinetics, the degradation rate constant k can be calculated from the following equation (Equation 2.2):

$$k = \frac{2.303}{t} \log\left(\frac{C_0}{C_t}\right) \qquad \text{Equation 2.2}$$

where C_0 is the initial concentration and C_t is the concentration at time t. Once k is known, the half-live of the pesticide is equal to 0.693/k.

Microbial degradation is the primary process that degrades imidazolinone herbicides in the soil. The rate of microbial degradation is a function of the herbicide concentration in the soil solution (Cantwell et al., 1989a; Flint and Witt, 1997). Soil properties, including the population of microorganisms in soil, temperature, moisture contents, organic matter, pH, and soil particle distribution, have an affect on the persistence of imazethapyr and other imidazolinone herbicides in the soil (Ayeni et al., 1998; Flint and Witt, 1988). Persistence of imidazolinone herbicides decreased with increasing temperature and moisture contents due to enhanced microbial activity in soil (Flint and Witt, 1988; Flint and Witt, 1997;

Goetz et al., 1990; Loux and Reese, 1993). Imazethapy is persistent in soil (Hart et al., 1992). Incubation of imazethapyr at different temperature and moisture regimes produced half-lives ranging from 192 to 318 days in a silty-clay soil and 78 to 270 days in a silty-loam soil (Goetz et al., 1990). A bi-phasic process has been reported for the dissipation of imazethapyr in a silt-loam soil, indicating a rapid stage during the first two months, followed by a phase of slow dissipation (Loux et al., 1989d). Decreasing temperature from 20°C to 10°C caused an increase in imazethapyr half-life by 55, 250, and 140% at concentrations of 0.1, 1 and 10 mg L^{-1}, respectively (Vischetti, 1995).

2.2.7 Influence of organic amendment on the microbial degradation of herbicides

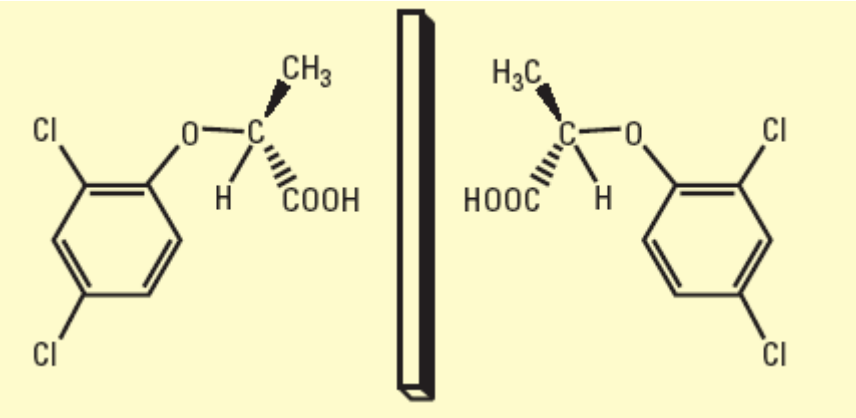
Organic matter content of soil influences the sorption, degradation and movement of pesticides in soil. Hence, the impact of organic amendments on the behaviour of pesticides in soil has been studied extensively (Albarrána et al., 2004; Baskaran et al., 1996; Gan et al., 1998; Sánchez-Camazano et al., 2000). It is well-known that the sorption of both non-polar and ionic pesticides is affected by organic matter. Several studies have demonstrated that degradation of pesticides is limited to the soil solution portion only and the sorbed pesticides are expected to be protected from microbial degradation. For example, residues extracted from olive oil increased the sorption and decreased the biodegradation of simazine (Albarrána et al., 2004). While organic amendments increase pesticide sorption and consequently decrease availability of pesticides for microbial degradation, they also increase microbiological activity by providing a source of energy and food (Cox et al., 2001; Sánchez-Camazano et al., 2000).

The population and activity of microorganisms is greater in soils that contain more organic carbon and nutrients (Böhme et al., 2005; Bosma et al., 1997). Compost amendment at three compost concentrations (1000, 2500 and 5000 ppm) increased microbial degradation of atrazine with no impact on sorption of the herbicide (Getenga, 2003). Microbial degradation of glyphosate in compost amended soil was rapid for 20 days and then slowed down. The first rapid period was suggested to be the result of degradation of free glyphosate in soil solution, while the slower phase was the result of degradation of herbicide that was initially sorbed (Getenga and Kengara, 2004). Wanner et al. (2005) reported stimulation of microorganism activity through corn straw amendment to soil, which led initially to a decrease in the rate of degradation of the fungicide dithianon, but enhanced degradation in the longer term. In another study Thom et al. (1997) incorporated leaf powder into soil and reported increased microbial activity and decreased persistence of the fungicide difenoconazole. Addition of 5% compost, manure or cornstalks significantly increased bacterial populations and dehydrogenase activity, resulting in increased degradation of atrazine (Moorman et al., 2001).

Tillage systems modify the amount of organic carbon in soil and produce changes in soil chemical and physical characteristics, such as biological activity, pH, and moisture (Seifert et al., 2001). Higher respiration rates and enzymatic activity of microorganisms have been found on the soil surface in no-till farming systems (Levanon et al., 1994). Thus, there is the expectation of differences in degradation, sorption and movement of pesticides in different tillage systems. For example, the mineralization rates of atrazine, diazinon and carbofuran are all enhanced in no-till soil (Levanon et al., 1994).

2.3 Chirality of herbicides

The expression chirality is drived from the Greek word *cheir* for handedness. Molecules that exists in more than one form (enantiomers) that are non-superimposable mirror images of each other are called chiral molecules (Imran et al., 2003). An example is given in Figure 2.4. The 1:1 ratio of the enantiomers is called the racemic mixture. Frequently, only one enantiomer has significant biological activity, meaning there is some advantage of using the active enantiomer instead of the racemic mixture.



R-Dichloroprop S-Dichloroprop

Figure 2.4. Two enantiomers (mirror image isomers) of dichlorprop (Garrison et al., 1996).

2.3.1 The importance of chirality in pesticides

For the enantioselective degradation of pesticides, one or more enzyme reactions involved in different degradation steps must be specific or preferential for one of the enantiomers. The enantioselctive degradation in enzymatic reactions is usually explained by the three-point model (Wong, 2006). In this model, the active enantiomer binds more strongly to the active site of the enzyme, while the less active enantiomer binds unsuccessfully resulting in

lack of fit with the active site. Degradation of the enantiomers of chiral pesticides may occur by the following pathways: (1) There are two enantioselective enzymes, each specific for one enantiomer; (2) both enantiomers are concurrently degraded by one enzyme, but at different rates; (3) there is preferential degradation of one enantiomer by the enzyme and degradation of the second enantiomer after the first has been degraded; or (4) enantioselective degradation of one enantiomer by one enzyme with slow isomerization of the other enantiomer by an isomerise (Müller and Kohler, 2004).

Chirality is observed among phenoxypropanoic acid herbicides, acetamide pesticides, organophosphorus pesticides, pyrethroid insecticides and imidazolinone herbicides (Williams, 1996; Wong, 2006) (Table 2.2). Most chiral pesticides are produced and marketed as their racemic mixtures. There are only 28 chiral pesticides that are marketed as single-enantiomers or mixtures enriched by the active enantiomer (Williams, 1996). Recently, regulatory considerations have encouraged pesticide manufacturers to switch from racemic mixtures to single enantiomers or enriched enantiomer pesticide formulations in order to reduce the pesticide load in the environment (Garrison et al., 1996). For example, the total amount of metolachlor used has decreased by about 40% since it has been enriched to have 86% of the active S-enantiomer (O'Connell et al., 1998).

Table 2.1. Commercially important chiral pesticides (Williams, 1996).

Herbicides	**Insecticides**	**Fungicides**
clethodim	abamectin	cyproconazole
dichlorprop	acephate	epoxiconazole
fenoxaprop	cyhalothrin	fenpropimorph
fluazifop	cypermethrin	flutriafol
glufosinate	deltamethrin	metalaxyl
haloxyfop	fenvalerate	propiconazole
imazamethabenz	fipronil	tebuconazole
imazaquin	malathion	triadimefon
imazapyr		
imazethapyr		
mecoprop		
metolachlor		
quizalofop		
sethoxydim		

2.3.2 Enantioselective degradation of chiral pesticides

Enantiomers have identical physicochemical properties, therefore it is expected that abiotic degradation processes will be the same (Müller and Kohler, 2004). However, it is recognized that enantiomers usually behave differently with respect to toxicity and interactions with biological systems. These characteristics of enantiomers may lead to very different microbial degradation rates and different persistence in the environment (Wong, 2006). Enantioselective degradation of pesticides including phenoxypropanoic acid herbicides, acetamide pesticides, organophosphorus and pyrethroid insecticides has been studied (Müller and Kohler, 2004; Wong, 2006).

Among phenoxypropanoic acid herbicides, mecoprop and dichlorprop have two enantiomers, with only the R-enantiomer having significant biological activity (Garrison et al., 1996). In several countries, mecoprop and dichlorprop are currently sold as the pure active R-enantiomer (Müller and Kohler, 2004; Williams, 1996), thereby reducing the pesticide load in the environment. Enantioselective degradation of mecoprop and dichlorprop in soil has been demonstrated under laboratory conditions and in field soils. These studies have demonstrated that one enantiomer is degraded faster than the other depending upon the conditions (Garrison et al., 1996; Lewis et al., 1999; Müller and Buser, 1997; Zipper et al., 1999).

In the case of acetamide pesticides, enantioselective degradation of metalaxyl and metolachlor has been studied (Buerge et al., 2003; Ma et al., 2006). Metalaxyl and metolachlor have two and four enantiomers, respectively. For metolachlor, the S-enantiomers have 10 times more herbicidal effect towards weeds than the R-enantiomers (O'Connell et al., 1998). The faster degradation of S-metolachlor in soil compared with racemic metolachlor indicated that 73.4% of rac-metolachlor and 90% of S-metolachlor were degradaed over the 42 days incubation (Ma et al., 2006). For metalaxyl, the R-enantiomer has 3-5 times more fungicidal effect compared to the S-enantiomer (Buser and Müller, 1995). Marucchini and Zadra (2002) reported the opposite enantioselectivity for metalaxyl in soils and plants, with faster degradation of the R-enantiomer in soil and faster degradation of the S-enantiomer in sunflowers. Metalaxyl, has been confirmed to degrade enantioselectively with favoured degradation of the R-enantiomer (Buser et al., 2002; Buser and Müller, 1995; Monkiedje et al., 2003).

Enantioselective degradation of pyrethroid and organophosphorus pesticides has been less well studied. All the pyrethroids pesticides have two or three chiral centres, so they have several enantiomers. As with other chiral pesticides, enantioselective degradation has been reported (Wong, 2006). Preferential degradation of specific enantiomers of pyrethroid insecticides including *cis*-bifenthrin, permethrin and cyfluthrin was observed under laboratory-controlled conditions and in the field (Qin et al., 2006). It is been reported that 30 out of 70 commercially available organophosphate (OP) pesticides are chiral (Garrison, 2006). The (+) enantiomer of fenamiphos was observed to degrade faster than the (-) enantiomer in soils (Wang et al., 2004). Soil properties including texture, organic matter and pH had no influence on enantioselective degradation of this insecticide. Enantioselective degradation of ruelene was altered by temperature changes and deforestation (Lewis et al., 1999).

Imidazolinone herbicides are currently sold commercially as racemic mixtures. The imidazolinone enantiomers have been shown to have different herbicidal activities, with the R(+) enantiomer eight-times more inhibitory to target weeds than the S(-) enantiomer (Lao and Gan, 2005). Currently little is known about the enantioselective degradation of these herbicides in different matrices. The only published investigation studied the enantioselective degradation of imazaquin in aqueous slurries of soil from two sites in Georgia and Ohio, USA (Jarman et al., 2005), which showed non-selective degradation of enantiomers of this herbicide after 3 months of incubation.

2.3.3 Kinetic analysis of pesticide enatiomers

To determine enantioselectivity in environmental analyses, the concept of enantiomer ratio ($ER=A_+/A_-$) is used, where A_+ and A_- are the concentrations of the (+) and (-) enantiomers, respectively. ER will equal 1 if the different enantiomers are degraded equally. As a result of limitations in ER, such as undefined results when the second enantiomer is not detectable and the lack of linear relationship between the plots of ER against the peak area of first enantiomer, enantiomer fraction (EF) has been proposed as a more useful index. EF is the ratio of the concentration of one enantiomer to the sum of concentrations of all enantiomers (Equation 2.3) (Harner et al., 2000; Imran et al., 2003).

$$EF=ER/(ER+1) \text{ or } EF = 1/(1 + 1/ER) \qquad \text{Equation 2.3}$$

The range of EF is only between 0 and 1, and when a pesticide has only 2 enantiomers an EF value of 0.5 signifies that both enantiomers are degrading at the same rate and behaving as a racemic mixture. The EF value can be applied to the investigation of microbial degradation processes and the fate of chiral pesticides in the environment (Hegeman and Laane, 2002).

2.3.4 Separation of pesticide enantiomers

Separation of the chiral pesticide into its enantiomers can be carried out using a variety of analytical methods. Gas chromatography (GC) and capillary electrophoresis (CE) methods have higher efficiency and faster separation of enantiomers compared with HPLC. Gas chromatography can also separate contaminants and impurities from the compounds and

analyse multiple enantiomers simultaneously. However, the major disadvantage of GC is the conversion of the compound to its volatile species, so at present HPLC is the best option for separation of enantiomers. The major benefit of HPLC is the possibility to separate enantiomers using fraction collection, enabling the evaluation of environmental processes and toxicity (Imran et al., 2003; Lao and Gan, 2006).

Both direct and indirect detection methods are used in HPLC to separate pesticide enantiomers. The indirect method requires derivatization of the chiral pesticides, resulting in some limitations for this technique. Derivatization is time consuming and a suitable agent is not always available. Alternatively, a chiral selector can be used either in the mobile phase or in the stationary phase. Chiral stationary phases (CSPs) are usually available as specialised HPLC columns. Approximately 80% of chiral pesticides can be separated by HPLC, mainly using CSPs (Jackson et al., 2001; Lin et al., 2006). There are several reviews about the techniques used to separate pesticide enantiomers with different matrices (Armstrong et al., 1993; Jackson et al., 2001; Penmetsa et al., 1997).

2.4 Determination of pesticides using solid phase extraction (SPE)

Environmental analysis of pesticides regularly entails a broad variety of matrices, ranging from air to soil samples. Extraction of pesticides and extract clean-up are essential steps in the analytical procedure. A careful selection of appropriate methods is required to obtain the best possible results (Lopez-Avila, 1999). In general, separation of pesticides and subsequent analysis of materials are separate steps. Separation of pesticides from the medium may require one or more of the following procedures. Pre-treatment is used to

improve the uniformity of the matrix and increase the efficiency of extraction of the pesticide. Extraction removes the analyte of interest from the matrix, and this is typically achieved with a solvent. Clean up may often be required to remove interfering co-extracted substances that may interfere with the analysis. There may also be separation of different classes of analytes before analysis (Khan et al., 2005).

There are several extraction methods are used commonly for the removal of pesticides from the soil matrix. These techniques include liquid-liquid extraction (LLE), ultrasonic extraction (UE), soxhlet extraction, pressurized fluid extraction (PFE), microwave assisted extraction (MAE) and supercritical fluid extraction (SFE) (Mitra, 2003); (Khan et al., 2005; Vicente and Yolanda, 2004).

Solid phase extraction (SPE) has developed during the last few decades as the preferred alternative to LLE for the extraction of a wide range of pesticides from water and soil, as it is faster, uses less organic solvents and has higher recovery of more polar pesticides (Redondo et al., 1996). SPE is similar in operation to HPLC separation systems in that the sorbent in the SPE cartridge is the stationary phase and the aqueous solution loaded onto the cartridge and the organic solvent used in the elution step are analogous to the mobile phase in the HPLC (Pichon, 2000). The best recovery is achieved when the sample is firmly retained by the SPE sorbent and easily desorbed by the solvent during the elution step. This allows high throughput of samples. A larger surface area of sorbent provides better retention of analytes. The common surface areas of SPE sorbents are 200 to 800 m^2 g^{-1} (Poole, 2003).

The SPE process involves four separate steps. Conditioning is where the sorbent is exposed to an organic solvent, followed by a solution with similar properties to the sample in relation to polarity, ionic strength and pH. Sample loading is where the sample is passed through the sorbent by gravity or a gentle vacuum. It is critical to control the flow rate in this step, depending on the particle size distribution of the stationary phase, the dimension of the column and sorbent properties. Washing removes compounds likely to interfere with analysis from the sample matrix. Elution transfers the analytes from sorbent bed to a small volume of solvent (Simpson, 2000). The selection of the SPE sorbent is determined by the sample matrix (aqueous or organic) and the properties of pesticide (non-polar, polar or ionized (Poole, 2003). The major SPE sorbents are reversed-phase (i.e., analyte and sorbent are nonpolar, and the sample is polar), normal-phase (i.e., the sample and analyte are nonpolar, and the sorbent is polar) or ion-exchange (i.e., analyte and sorbent have opposite charge) systems (Simpson, 2000).

For pesticide extraction from soil or water, the most important cartridge beds are silica based sorbents like octadecyl (C_{18}) and octyl (C_8) bonded, organic polymer-based sorbents such as polystyrene-divinylbenzene (PS-DVB), and carbonaceous sorbents such as graphitized carbon blacks (GCBs) and porous graphitic carbon (PGC) (Möller, 2006; Pichon, 2000). C_{18} is the most extensively used silica based sorbent. A drawback of this sorbent is its instability at pH values outside 2-8. The extraction of moderately polar to non-polar analytes has been reported using this kind of sorbent (Poole, 2003). Extraction of triazine, phenylurea, organophosphorus and chlorophenoxyacids pesticides that involve adjusting the sample pH to 3 to enhance their retention has been reported in the literature (Matamorosa et al., 2007). The advantage of polymer-based sorbents is stability over the entire pH range and a higher surface area. These sorbents are applicable for polar

compounds not extracted on C_{18} silica, such as metabolites of triazine herbicides. The graphitized carbon sorbents are used for the extraction of very polar pesticides (Möller, 2006; Pichon, 2000; Poole et al., 2000; Redondo et al., 1996; Simpson, 2000).

2.5 Extraction of imidazolinone herbicides from soil and water

Imidazolinone herbicides are present as ions over most of the environmental pH range. This property influences the extraction and analysis of these herbicides from water and soil (D'Ascenzo et al., 1998; Furlong et al., 2000). Extraction of these herbicides is possible in both the charged and uncharged states. The dissociation of imidazolinone herbicides is pH dependent and it is a function of the pK_a (Mangels, 1991; Stout et al., 1996). Therefore, to obtain the best recovery, the pH of the sample needs to be different depending on the type of SPE used.

According to the literature (D'Ascenzo et al., 1998; Lagana et al., 1998; Marchese et al., 2001), existing analytical techniques for the determination of imidazolinone herbicides, particularly in soil, are usually very tedious and time-consuming. Most of these methods, follow a similar method, starting with shaking soil samples in several volumes of 0.5 N NaOH, then processing the soil by a series of precipitations and centrifugations and subsequent partitioning with dichloromethane (CH_2Cl_2). Following evaporation of the CH_2Cl_2, SPE cartridges are used to clean up the sample (Curran et al., 1992a; Loux and Reese, 1992). There are several reports in the literature of the use of SPE cartridges for the purpose of extraction of some imidazolinone herbicides from water and soil (Börjesson et al., 2004; Helling and Doherty, 1995; Lagana et al., 1998; Rodriguez and Orescan, 1998).

However, there is a need to develop improved SPE analysis especially for matrices containing high dissolved organic matter.

2.6 Conclusion

The extensive use of pesticides for agriculture inevitably results in pesticides and their degradation products in the environment. Thus, it is essential to understand the environmental behaviour of pesticides and determine their potential risks to human and animal health and the environment. The introduction and release of imidazolinone herbicides by American Cyanamid as a new class of herbicides with reduced environmental risk due to their low application rates has potential to have major environmental benefits. Owing to the amphoteric characteristics of these herbicides, having both acidic and basic functional groups, the quantification and determination of the behaviour of these herbicides in the environment is usually pH-dependent. These herbicides are in an ionic form at the pH values of 6 to 8, and therefore leaching is expected, particularly in the alkaline soils of Australia. Microbial degradation of the imidazolinone is the main route of degradation in soils but microbial activity is likely to be lower in deeper layers of the soil profile. The imidazolinone herbicides are chiral in nature, having two enantiomers with different herbicidal activities. In spite of the several studies on microbial degradation of imidazolinone herbicides in soils, little is known about the enantioselective degradation of these herbicides. Enantioselective studies could provide valuable information for the manufacturers and environmental protection agencies for decreasing the load of herbicides into environment without decreasing the level of weed control for the farmers.

Chapter 3. Evaluation of imidazolinone extraction from soil solutions using different solid -phase extraction cartridges

3.1 Introduction

As a result of the extensive use of pesticides in agriculture, imidazolinone herbicides can sometimes move off-site and contaminate environmental matrices like soil, sediments and water (Cohen et al., 1995). Due to the toxicity of many pesticides for humans or negative effects on environmental quality, there is concern about their presence in the environment. Extraction of pesticides from environmental matrices is frequently the most time-consuming step in residue analysis. A wide variety of methods and solvents have been used to extract pesticides from different matrices, such as water and soil (Vicente and Yolanda, 2004). The traditional extraction methods, such as liquid-liquid extraction (LLE), have some disadvantages over more recent techniques, such as solid-phase extraction (SPE). For example, LLE utilizes large volumes of solvents and is generally a more time-consuming process than SPE. Additionally, the recovery of many polar pesticides attained by LLE is low due to their relatively high water solubility. Over recent decades, there has been a trend toward SPE techniques as a substitute for the difficult and time-consuming LLE process. More recently, SPE has progressed in automation and introduction of efficient sorbents capable of extraction of polar compounds (Poole et al., 2000; Simpson, 2000).

Using SPE techniques for the extraction of pesticides is more rapid, simple, and economical compared with the usual LLE. Since large volumes of samples can be loaded onto SPE cartridges, it is possible to concentrate the analyte and increase the likelihood of detecting compounds. Method development and the choice of sorbent are two key factors in the use of SPE for the extraction of pesticides from water and soil extracts. The different physico-chemical properties of pesticides need to be considered in method development. This process can be considered as a simple liquid chromatographic process where the sorbent acts as the stationary phase and the mobile phase is the aqueous sample or the organic solvent during the extraction and elution steps, respectively. The best results using SPE cartridges are obtained when pesticides are strongly adsorbed by the sorbent from the aqueous sample, allowing the percolation of a large volume of sample and no or little retention in the elution steps (Wells and Yu, 2000).

The recommended field application rates for imazapyr, imazethapyr and imazaquin range from 100 to 200 g ha^{-1} (Kah and Brown, 2006). These low application rates in field situations makes residue analysis of these herbicides from soil problematic. Extraction of imidazolinone herbicides, particularly from soil, is difficult because of co-extraction of many interfering substances, which produce some interference peaks in the chromatogram, (Vicente and Yolanda, 2004), properties of the herbicides such as low pK_a values and low hydrophobicity, and the low detection limits required. Current methods for the extraction of imidazolinone herbicides are time-consuming, labour-intensive, and costly. The published methods for the extraction of imidazolinone herbicides from water and soil matrices are summarized in Table 3.1. A number of these methods were used for the extraction of imidazolinone herbicides from water. Most of the methods used for the

extraction of soil samples evaluated only one compound. However, the method used by (Lagana et al., 2000) had good recovery for the extraction of three herbicides (imazapyr, imazethapyr and imazaquin) from soil. These researchers developed this method using 1 g of soil for the extraction and liquid chromatography tandem mass spectrometry (LC-MS/MS) for the quantification of the herbicides. However, this method was difficult to employ in the current study, due to the larger soil volume (25 g) involved to enable development of a method that could be used to study the degradation of imidazolinone enantiomers in Australian reference soils .

In this study the amendment of soils with organic materials added further complexity to the detection of imidazolinone herbicides. It became necessary to develop an extraction procedure that removed the additional organic material (humic acids) to achieve a clean chromatogram with no significant exogenous peak interferences for the herbicides. The main objectives of this research were therefore to investigate and evaluate: (1) the efficiency of different solvents for the extraction of the three imidazolinone herbicides from soil; (2) the Varian PPL cartridge (PPL) for pre-concentration and extraction of imidazolinones from humic acid solution and soil; and (3) the application of different types of SPE cartridges for the clean-up of the aqueous and soil extracts.

Table 3.1. **Methods for the extraction of imidazolinone herbicides from water and soil using SPE cartridges** [a].

Matrix	Extraction solution	SPE cartridges (Sorbent bed)	Instrument and detector	Recovery ± RSD (%) from SPE	Herbicides	References
Water	N/A	Carbograph-1	HPLC -UV	89 ± 5.1	Imazapyr Imazethapyr Imazaquin	(Lagana et al., 1998)
water	N/A	Polystyrene divinylbenzene, SAX & alumina	LC-MS	114 ± 9	Imazapyr Imazethapyr Imazaquin	(Rodriguez and Orescan, 1998)
Water	N/A	SAX + RP-102 styrene-divinyl benzene	LC-MS	73 ± 20	Imazapyr Imazethapyr Imazaquin	(Furlong et al., 2000)
Soil	NH_4HCO_3 (0.1 M, pH 5)	SAX + C_{18}	HPLC -UV	N/A	Imazapyr	(Helling and Doherty, 1995)
Soil	KH_2PO_4 (0.01 M pH=8)	Carbograph-1	LC-MS/MS	78 -92 ± 4-5	Imazapyr Imazethapyr Imazaquin	(Lagana et al., 2000)
Soil	0.5 M NaOH	C_{18} + SCX	HPLC -UV	N/A	Imazamox	(Bresnahan et al., 2002)
Soil	$Ca(OH)_2$	SPE disk + C_{18}	HPLC -UV	N/A	Imazethapyr	(Pace et al., 1999)

[a]N/A = not available; HPLC-UV= High performance liquid chromatography-ultraviolet detection ; LC-MS = Liquid Chromatography mass spectrophotometry; LC-MS/MS = Liquidchromatography-massspectrophotometry/mass pectrophotometry; SAX= strong anion exchange; SCX = strong cation exchange.

3.2 Material and Methods

3.2.1 Chemical, reagents and apparatus

Standards of imazapyr, imazethapyr and imazaquin (99% purity) were purchased from Sigma-Aldrich (NSW, Australia). High performance liquid chromatography (HPLC) grade solvents including hexane, 2-propanol, and AR grade solvents of methanol (MeOH), acetonitrile (ACN), and dichloromethane (DCM) were purchased from Biolab (Adelaide, Australia). All solutions were prepared with ultra pure water using a Millipore-Milli Q system (Milli-Q water). The humic acids sample was purchased from Fluka Chemie (Buchs, Switzerland). A 20-port-SPE manifold was purchased from Supelco (NSW, Australia). Whatman GF/C (70 mm, 1.2 mm pore) filters were purchased from Biolab (Adelaide, Australia). A range of SPE cartridges including Varian Bond Elut- C_{18} (C_{18}), Varian Bond Elut SCX (SCX), Varian Bond Elut PPL (PPL), NH_2 and X-AW (AW) were assessed and the suppliers and sorbent properties of the cartridges are listed in Table 3.2. Phosphate buffer solutions were prepared by mixing appropriate stock solutions of K_2HPO_4 and KH_2PO_4.

Table 3.2. Structure and some properties of the sorbents of the SPE cartridges assessed for efficacy of extraction of imidazolinone herbicides from soil solutions.

SPE cartridge	**Supplier**	**Sorbent structure**	
		Based structure	Area (m^2 g^{-1})[a]
PPL	Varian	Polymeric	700
C_{18}	Varian	Octadecyl bonded silica	462
SCX	Varian	Silica	503
X-AW	Phenomenex	Styrene-divinylbenzene	N/A
NH_2	Phenomenex	Aminopropyl bonded silica	N/A

[a] N/A = not applicable

3.2.2 Preparation of standard stock solutions

Stock solutions of the three herbicides were made individually in acetonitrile at a concentration of 1000 µg mL^{-1}. A 100 µg mL^{-1} herbicide standard solution was prepared by diluting 10 mL of the 1000 µg mL^{-1} stock solution into 100 mL with acetonitrile. The diluted solutions of 0.5, 1, 2.5, 5 and 10 µg mL^{-1} were made from the 100 µg mL^{-1} stock for preparing the fortification and calibration solutions. The solutions were stored at -4°C and made freshly every two months.

3.2.3 Fortification of humic acid solution and soil samples

3.2.3.1 Fortification of humic acid solutions

A humic acid stock solution (30 μg mL^{-1}) was prepared by dissolving standard humic acid in Milli-Q water and kept in the dark at 4°C. The pH of the humic acid solution was 6.8. These stock solutions were used for fortification of the imidazolinone herbicides by adding 200 μL of the 100 μg mL^{-1} stock solution of imazapyr, imazethapyr and imazaquin to 100 mL of humic acid to investigate the extraction and concentration of these herbicides with PPL cartridges. Three replicates of imazapyr, imazethapyr and imazaquin (200 μL) were fortified with Milli-Q water and humic acid solutions (30 μg mL^{-1}).

3.2.3.2 Fortification of soil samples

Unless otherwise indicated, the following procedure was used for all extraction studies. For each experiment, three replicates of soil (10 g) were weighed into separate centrifuge tubes and fortified with each herbicide separately by adding 100 μL of 100 μg mL^{-1} to give the required fortification concentration of 1 μg g^{-1}. A blank or unspiked sample, to which 200μL of Milli-Q water was added, was also run in triplicate with each experiment. After fortification, solvents were allowed to evaporate and then soils homogenised using a vortex mixture for 1 min and kept at 4°C for 48 h before extraction.

3.2.4 Evaluation of the efficacy of various extraction solutions

Different extraction solutions (extractants) have been reported in the literature for the extraction of imidazolinone herbicides from soil. In a preliminary experiment the efficacy of a range of extractants including 0.1M potassium chloride (KCl), 0.5M sodium hydroxide (NaOH), 0.01M sodium hydroxide and 0.5M sodium hydroxide:methanol (80:20)(NaOH:MeOH) to extract imazapyr, imazethapyr and imazaquin from soil was investigated. Triplicate soil samples (5g) that were either amended with 2 g lupin residue or non-amended were each spiked with imidazolinone herbicides at 1μg g^{-1} and kept at 4°C for 48 h before extraction. Different ratios of soil and extractants including 1:2, 1:3, and 1:4 were used for extraction of soil samples to find the optimal conditions for maximum recovery of the herbicides from the soil.

3.2.5. Solid-phase extraction clean up

3.2.5.1. Optimizing solution pH for herbicide extraction from water and humic acid solutions using PPL cartridges

PPL cartridges are packed with highly cross-linked and chemically modified styrene divinyl benzene co-polymer. The choice of this cartridge for the extraction of imidazolinone herbicides was based on previous results where 12 phenols (weak acids) were successfully extracted from drinking water (Environmental Protection Agency, 2000). This method is now recommended by the EPA for the determination of phenols in water. In a preliminary experiment the optimal solution pH for maximum recovery was

determined in triplicate by spiking 100 mL of water with 1 μg mL^{-1} of only imazapyr with pH adjusted to 2, 5.5, 7 and 9 with HCl and NaOH. The PPL cartridges were primed with two rinses of 3 mL of DCM followed by two rinses with 3 mL of MeOH, then three rinses with 2 mL of Milli-Q water (pH=2) before use. The cartridges were not allowed to dry out during this process. Spiked water samples (100 mL) adjusted to various pH values were loaded onto the cartridge and upon completion the cartridge was dried under vacuum. The herbicides were eluted with two rinses of 3 mL of DCM and evaporated to near dryness under a gentle stream of nitrogen. An aliquot of 2-propanol (4 mL) was added and evaporated slowly such that the final volume was 2 mL. The herbicide concentration in the final extracts was analysed using HPLC.

To examine the effect of humic acid on the recovery of the herbicides on the PPL cartridges, triplicate solutions of 30 μg mL^{-1} (100 mL) of humic acids and Milli-Q water were spiked with appropriate volume (100 μL) of the working standard solution. After blending for 2 min, samples were acidified to pH 2 with 6N HCl and kept at 4°C for extraction 48 h later. The extraction procedure was the same as outlined above for the preliminary experiment. PPL cartridges were also evaluated for extraction of the imazaquin enantiomers from Milli-Q water and humic acid solutions using the same method. Reverse-phase HPLC was used to quantify herbicides and normal-phase HPLC to quantify the enantiomers of imazaquin (Section 3.5.2).

3.2.5.2 Clean-up of soil extracts with PPL cartridge

Soil samples (10 g) spiked separately with imazapyr, imazethapyr and imazaquin herbicides at the concentration of 1μg g^{-1} were kept at 4°C for one week. The soil samples were shaken with 40 mL of 0.5 M NaOH in an end-over-end shaker for 1 h. The extracts

were decanted and centrifuged at 6000 rpm (5860 ×*g*) for 20 min, then filtered through 70-mm-diameter glass microfiber filters (GF/C, pore size 1.2 μm). The pH of the extracts was adjusted to approximately 2 using 6N HCl before passing through the cartridges. The other steps of the method were as described above (Section 3.2.5.1). Three different solvents were used for elution of the herbicides from the PPL cartridge: DCM; DCM:isopropanol (80:20); and ethyl acetate:isopropanol (80:20). Two sizes of PPL cartridge were assessed: 6 mL, 500 mg cartridge; and 3 mL, 200 mg cartridge. Apart from PPL, a variety of SPE cartridges were examined for the extraction of imidazolinone herbicides (Table 3.2).

A schematic diagram of the spiking method and cleanup of the soil extracts with different SPE cartridges is shown in Figure 3.1. Triplicate soil samples were prepared by spiking 100 μL of standard herbicide solution (100 μg mL^{-1}) separately to 10 g of soil to give a herbicide concentration in soil of 1 μg g^{-1}. When the solvents were completely evaporated, the soil samples were homogenized by Vortex for approximately 1 mins and left overnight at 4°C before extraction. The soil samples were shaken with 0.5M or 0.01M sodium hydroxide with an orbital mechanical shaker for 1 h. The supernatants were decanted and centrifuged at 6000 rpm (5860 × *g)* for 10 min and then the 0.5M NaOH solutions were acidified with HCl to pH 2. The extract solutions of 0.01M and 0.5M NaOH were filtered using a Whatman glass fibre filter (GF/C, 1.2 μm). The 0.01M NaOH solution was directly loaded onto the pre-conditioned cartridge X-AW and the 0.5M NaOH solutions were used to evaluate the three SPE cartridges namely PPL, NH_2 followed by PPL and C_{18} followed by SCX, to assess efficacy of extraction of the imidazolinone herbicides from the NaOH soil extract.

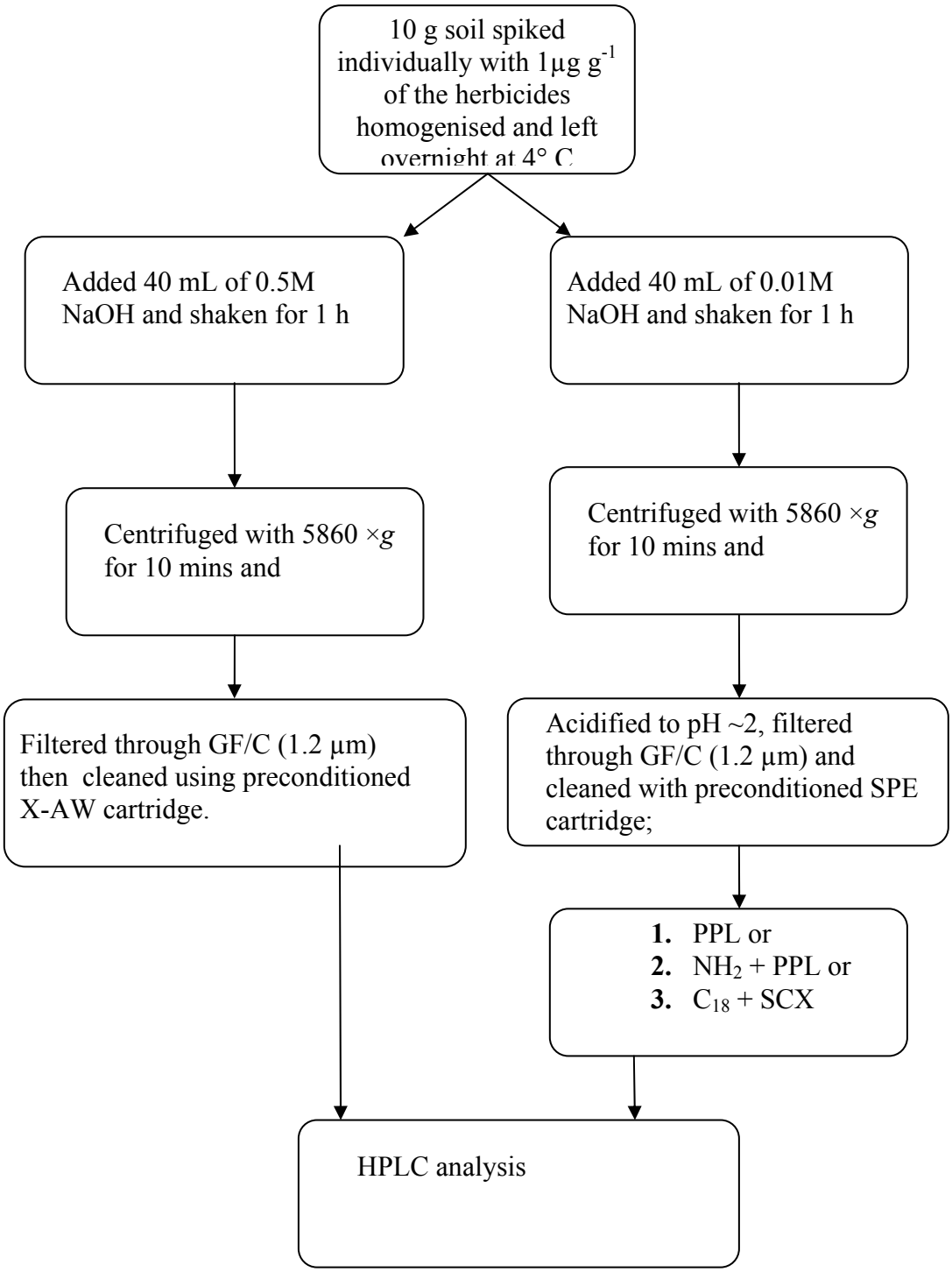


Figure 3.1. Flow chart of methodology for the extraction of imidazolinone herbicides from soil using different SPE cartridges.

3.2.5.3 Clean-up of soil extracts using X-AW cartridge

Due to the poor recovery of herbicides and the presence of interfering peaks near the retention time for imazapyr when the PPL cartridge was used, the X-AW cartridge was tested. This cartridge is a polymer-based anion exchange sorbent with base particles of styrene divinylbenzene, which is modified with a diamine group. The supplier recommends this cartridge for extraction of weak acids, which can be loaded at the pH range of 6-8, as these sorbents are more retentive for polar acidic herbicides than silica (Wells and Yu, 2000). X-AW cartridges were conditioned with 5 mL MeOH: formic acid (98:2) followed by 2 mL Milli-Q water at a flow rate of 3 mL $mins^{-1}$. Approximately 50 mL of soil extract (0.01M NaOH) containing the herbicides was loaded onto the cartridges and after the solution had passed through, the cartridge was washed with 5 mL Milli-Q water and 5 mL MeOH. The SPE cartridges were dried under vacuum for about 5 mins and the herbicides were eluted with 6 mL of MeOH:NH_4OH (98:2). The eluates were dried under a gentle stream of nitrogen gas and re-dissolved into ACN:H_2O (50:50) for analysis.

3.2.5.4 Clean-up of soil extracts using NH_2 and PPL

Owing to interferences by co-extracted macromolecules (the 'humics") in the soil extracts, it was necessary to incorporate an additional SPE cartridge to remove these interfering compounds. For this experiment, a NH_2, anion exchange resin cartridge was used in series with the PPL cartridge. Soil samples (10 g) were extracted as outlined in Figure 3.1. Approximately 50 mL of the 0.01M NaOH soil extract was passed through a NH_2 SPE cartridge (500 mg, 3 mL) that had been conditioned with 5 mL of 1% acetic acid in MeOH and then 5 mL of 1% acetic acid in water. The clear eluate from the NH_2 cartridge was then

passed through the PPL cartridge. All steps in preparing the second cartridge in this experiment were as described in section 3.2.5.1.

3.2.5.5 Clean-up of soil extracts using C_{18} + SCX cartridges

As the recovery of herbicides was relatively low and the collection of eluate from the NH_2 cartridge made this method difficult, a two cartridge method using a C_{18} cartridge followed by an SCX cartridge was tested. This method has been used for the extraction of imazamox from soil (Bresnahan et al., 2002). In the current experiment, C_{18} and SCX cartridges were used in series for cleaning up soil extracts. Approximately 15 g of ceilite was added to the extracts to facilitate filtration and then the acidified solutions were filtered through glass fibre filter paper (Whatman GF/C, 1.2 µm, 70 mm). Initially the C_{18} cartridge was conditioned with 5 mL MeOH followed by 5 mL water and the SCX cartridge was conditioned with 5 mL hexane then 5 ml MeOH and 5 mL water. About 50 mL of 0.5M NaOH soil extracts was passed through the C_{18} cartridge. Next the C_{18} cartridges were stacked on top of the SCX cartridges and the herbicides were eluted with MeOH:water (1:1, 20 mL). The C_{18} cartridges were discarded and the SCX cartridges were rinsed with 5 mL water. The herbicides were then eluted from the SCX cartridges using 20 mL phosphate buffer (pH 6.5). The buffer solution was acidified to pH 2 and the herbicides partitioned with three vigorous washes with 15 mL DCM. The solvent was evaporated under a gentle stream of nitrogen gas to dryness and the herbicides were re-dissolved in 2-propanol and then analysed using HPLC.

3.2.6 HPLC analysis of imidazolinone herbicides

3.2.6.1 Reverse-phase HPLC

The herbicides dissolved in ACN were analysed using reverse-phase HPLC, using an Agilent 1100 HPLC equipped with a quaternary pump, vacuum degasser, diode array detector, autosampler, and a column oven. Data were processed using the commercially available Agilent ChemStation software. Reverse-phase HPLC was performed using an Altima C_{18} column (250 mm × 4.6 mm ID, 5 μm particle size). The HPLC conditions were: an isocratic mobile phase of 55:45 acetonitrile:1% acetic acid in HPLC grade water; a flow rate of 1 mL min^{-1}; a column oven temperature of 25ºC; and a UV-Vis detector set at 240 nm. The retention times of imazapyr, imazethapyr and imazaquin in this system were 2.92, 4.39, and 5.53 mins, respectively. The detection limits of imazapyr, imazethapyr and imazaquin were 0.5 μg mL^{-1}.

3.2.6.2 Normal-phase HPLC

Normal phase HPLC was used for the detection of the imazaquin enantiomers. For normal-phase separation, the final samples were dissolved in *n*-hexane:2-propanol (1:1, v/v). The Agilent 1100 HPLC was used for normal phase analyses with the following conditions: a Chiralcel OJ [cellulose tri(4-methylbenzoate)] column (250 mm × 4.6 mm I.D., 10 μm particle size); an isocratic mobile phase of 65:35:0.1 *n*-hexane:2-propanol:trifluoroacetic acid (TFA); a flow rate of 1 mL min^{-1}; and a UV-Vis detector set a wavelength of 240 nm. The retention times of S(-) and R(+) enantiomers of imazaquin were 9.3 and 10.5 min,

respectively, in this system. The detection limit for both enantiomers of imazaquin was 0.5 μg mL^{-1}.

3.3 Statistical analysis

Percentage recovery data were log-transformed to normalize their distribution before analysis of variance (ANOVA) but the untransformed data is shown in the Results section. ANOVA was used to evaluate differences between recovery rates (%) of extraction solutions and SPE cleaning methods. The significant differences of recovery means were compared using least significant difference (LSD) methods at a confidence level of $p < 0.05$ with GenStat (version 8.2, Rothamsted Experimental Station).

3.4 Results and Discussion

3.4.1 Calibration curves

The concentration of imazapyr, imazethapyr and imazaquin over the range of 0.5 to 10 μg mL^{-1} was plotted against the mean peak area of the herbicides eluting from the reverse-phase HPLC column. The standard calibration curves (Figure 3.2) of imazapyr, imazethapyr and imazaquin were linear from 0.5 to 10 μg mL^{-1} with coefficient of determination (R^2) of above 0.99 for the three herbicides. The linear regression equations for the calibration curves for the three herbicides was:

Imazapyr: $y = 17.42x - 24.9$

Imazethapyr: $y = 16.10x - 17.2$

Imazaquin: $y = 38.80x - 43.5$

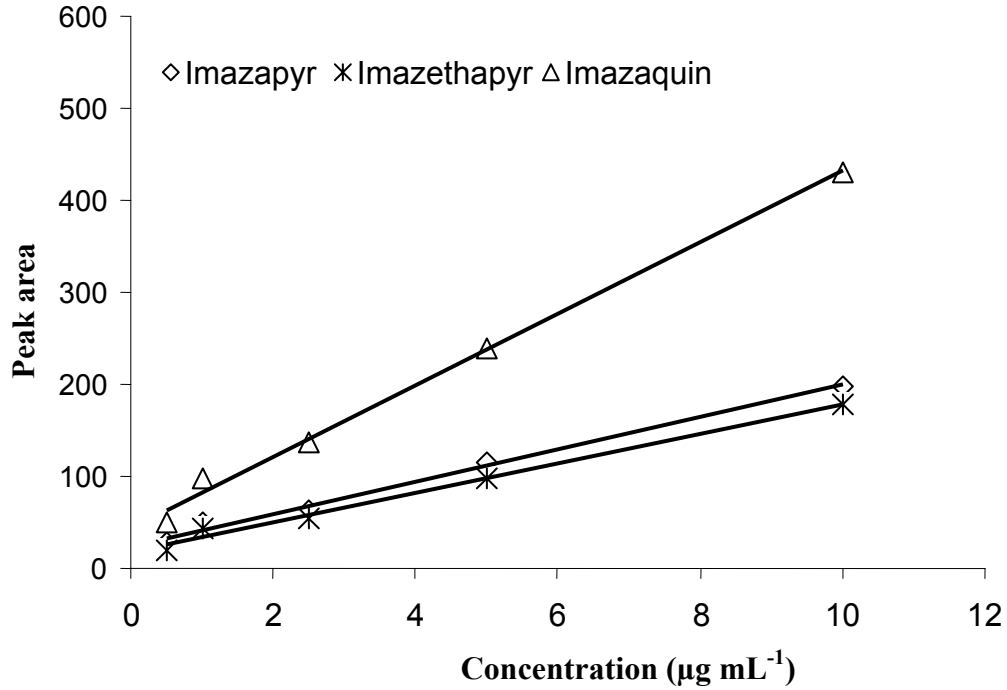


Figure 3.2. Calibration curves for imazapyr, imazethapyr and imazaquin at the concentration level of 0.5 to 10 µg mL^{-1}.

3.4.2 Evaluation of the efficacy of extraction solutions to recover imidazolinone herbicides from soil

The aim of this experiment was to determine the efficacy of different extraction solutions to remove imidazolinone herbicides from soil, particularly when the soil had been amended with organic material, the lupin crop residue in this case. As a result of their polar nature and high water solubility, aqueous solutions were used for extraction of the

imidazolinone herbicides. The mean recoveries of herbicides were statistically analysed to determine any significant differences between the four extraction solutions. The following extraction solutions were assessed; 0.1 M KCl, 0.5 M NaOH, 0.01M NaOH and 0.5M NaOH:MeOH (80:20) for their efficiency to extract imazapyr, imazethapyr and imazaquin from amended and non-amended soils (Table 3.3). There was no significant difference ($p < 0.05$) between amended and non-amended soils for the extraction of imazapyr, imazethapyr and imazaquin herbicides, therefore, the average percent recovery of soils are shown in Table 3.3. All four extraction solutions gave more than 70% recovery of the herbicides from soil (Table 3.3). There were significant differences ($p < 0.05$) between extraction solutions in herbicide recovery from soil. The mean recovery of imazapyr, imazethapyr and imazaquin was 77.2, 78.5 and 89.9%, respectively, which showed a good reproducibility with R.S.D. values between 9.1 and 12.7% with 0.1M KCl. For all three herbicides the lowest recovery was found with 0.1M KCl and this is probably due to the low pH (5.9) of this extraction solution. Others have also found low extraction efficiency of water for the extraction of imidazolinone herbicides from soil, which was attributed to the low pH of the extraction solution (Lagana et al., 2000). The pKa values of the selected imidazolinone herbicides range from 2.2 to 3.8, so extraction solutions with alkaline pH would increase solubility by protonating the herbicides. Hence, at this pH the anionic form of these herbicides are repulsed from the colloids (Rodriguez and Orescan, 1998). The use of alkaline aqueous solutions for the extraction of acidic herbicides is desired because it does not involve use of harmful solvents and is simple to perform. In this study the use of aqueous solution as the main extraction solution for the extraction of imidazolinone herbicides was preferable since it allowed the use of SPE cartridges for clean up of the extracts without the extra step of dilution or evaporation of solvents.

The recovery obtained with KCl was significantly lower ($p < 0.05$) than the 0.5 M NaOH, 0.5M NaOH:MeOH (80:20) and 0.01m NaOH for imazapyr and imazaquin. No difference was observed between the recoveries obtained using 0.5M NaOH:MeOH (80:20) and 0.5M NaOH for imazapyr, although the highest recovery of imazaquin was obtained with 0.5M NaOH:MeOH (80:20) which was significantly ($p < 0.05$) different from the other solutions.

There was no significant difference between the extraction efficiency of 0.01M and 0.5M NaOH for imazethapyr, but higher reproducibility of extraction efficiency was observed when 0.5M NaOH used as extraction solution. The major difficulty encountered using 0.5M NaOH was co-extraction of humic and fulvic acids from the soil, which generated a large unresolved peak at the beginning of the chromatograms. The optimal conditions for the extraction of imazapyr, imazethapyr and imazaquin from soil was a soil:solution ratio of 1:4 using 0.5M NaOH (data not shown).

Table 3.3. Preliminary evaluation of different extractants for extraction of imidazolinone herbicides from soil. [a]

	Average Recovery (%) ± SD [a]		
Extractants	**Imazapyr**	**Imazethapyr**	**Imazaquin**
0.1 M KCl	77.2 ± 9.2 a	78.5 ± 12.7 a	89.9 ± 9.1 a
0.01M NaOH	83.7 ± 2.8 b	87.1 ± 3.1 bc	94.2 ± 4.1 b
0.5 M NaOH//MeOH (80:20)	91.6 ± 2.4 c	80.3 ± 2.9 ab	104.2 ± 3.2 d
0.5 M NaOH	95.1 ± 3.6 c	92.3 ± 5.6 c	97.4 ± 4.8 c

[a] Mean recoveries with different letters within columns are significantly different ($p < 0.05$).

3.4.3 Optimization of solution pH for the extraction of imidazolinone herbicides from water and humic acids solution using PPL cartridges

The optimum solution pH at which imidazolinone herbicides are in the appropriate form for retention by the PPL cartridge was determined initially using imazapyr in water and later all three herbicides were assessed at the optimal pH in 100 mL of 30 μg mL^{-1} of humic acid solution. The retention of imazapyr on PPL cartridges rapidly decreased to less than 10% with increasing pH from 2 to 5.5 and decreased further at the pH value 9 (Figure 3.3). This indicates that maximum retention of the imazapyr on the PPL cartridge was when the sample solution was acidified to approximately 2 units below the herbicide pKa (3.6).

The recoveries of three herbicides from acidified humic acid solutions ranged from 94.3% to 123.4% with average RSD of values 6.9% (data not shown). The percent recoveries above 100% were related to a matrix enhancement effect, which has been reported for some pesticides including imidazolinone herbicides. For example, (Furlong et al., 2000) reported that sample matrix effects increased recovery by as much as 200% greater than the spiked concentration for sulfonylurea, imidazolinone and sulfonamide herbicides. This impact is greater for polar pesticides and is influenced by many factors including pesticide nature, the kind of matrix, and the concentration of matrix and/or pesticides (Kumar et al., 2000). The chromatogram of the three imidazolinone herbicides obtained after clean-up with PPL cartridge shows the interfering peaks that made resolution of imazapyr difficult (Figure 3.4).

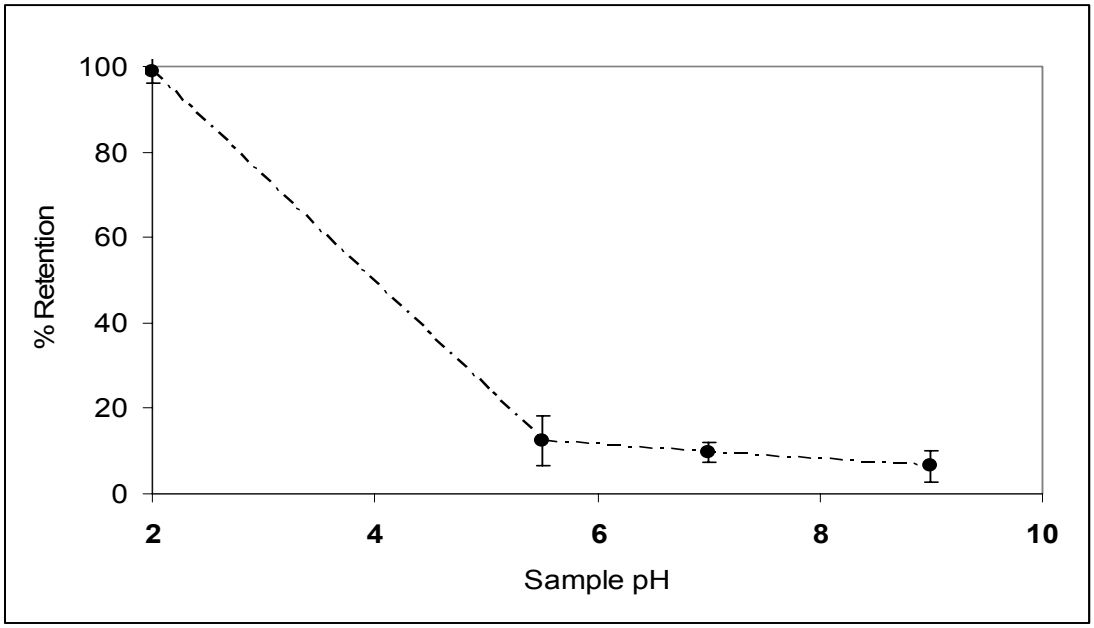


Figure 3.3. Effect of sample pH on retention of imazapyr by PPL cartridge (n=3).

Extraction recovery was also determined for each enantiomer of imazaquin in Milli-Q water and humic acids solution. The recovery of the of S(-) enantiomer was 98.7% in Milli-Q water and 103.5% in 30 μg mL^{-1} of humic acid solution (data not shown). The recovery of the R(+) enantiomer was 99.8% from Milli-Q water and 107.8% from 30 μg mL^{-1} of humic acid solution. The RSD values of R(+) and S(-) enantiomers was 8.2 and 11.3%.

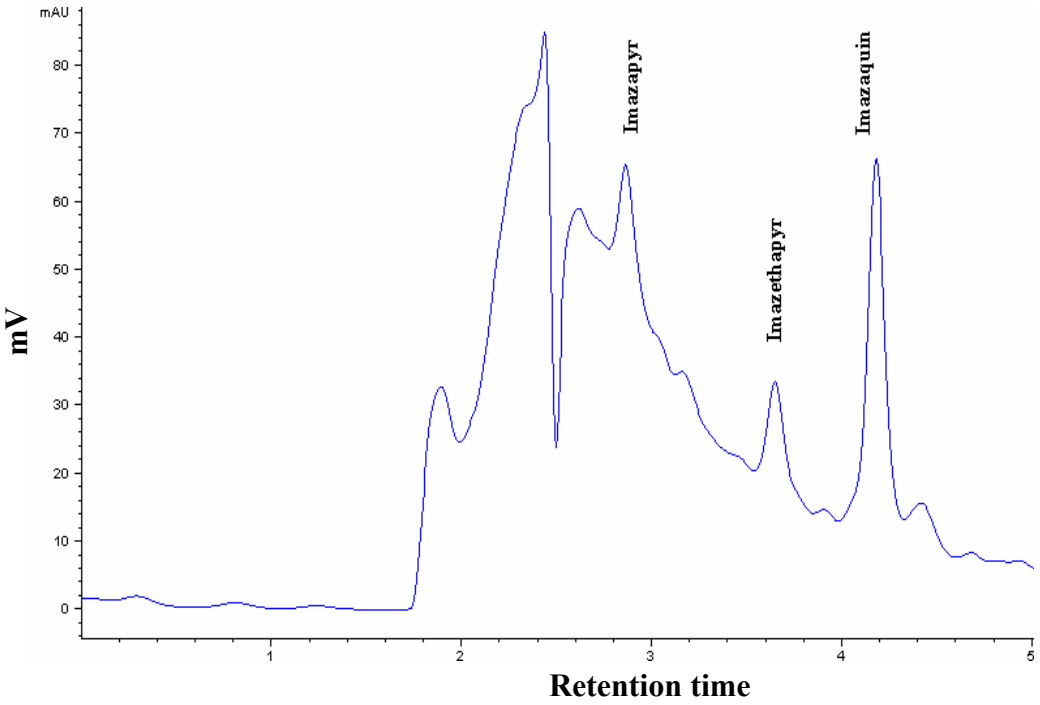


Figure 3.4. Chromatogram of imazapyr, imazethapyr and imazaquin after SPE clean-up with the PPL cartridge of 30 µg mL^{-1} of humic acids solution.

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3.4.4 Optimization of PPL cartridge for extraction of imidazolinone herbicides from soil

Since the extraction procedure based on PPL cartridge from water and humic acid solutions was found appropriate, this cartridge was tested for the extraction of imidazolinone herbicides from soil. Thus, after extraction of the herbicides from soil with 0.5M NaOH as outlined in section 3.2.52, the clear supernatants (0.5M NaOH) were passed through the PPL cartridges and then eluted using 5 mL DCM. The chromatograms for the 0.5M NaOH soil extract (spiked at 1µg g^{-1}) and 10µg mL^{-1} of standard solution of herbicides are shown in Figure 3.5. The chromatogram had interfering peaks between 2 and 3.8 mins where imazapyr eluted (Figure 3.5), which made quantification of this

herbicide difficult. The colour of the 0.5M NaOH soil extracts before passing through the PPL cartridge was yellow and after passage was light yellow indicating that DCM removed a considerable amount of coloured substances from the cartridge. The recovery of imazapyr, imazethapyr and imazaquin from 0.5M NaOH soil extracts using PPL cartridge was significantly different ($p < 0.05$) and was 10.9, 69.3 and 75.4%, respectively.

To reduce the effect of soil co-extractives on imazapyr resolution and to improve herbicide recovery, several solvents were tested in the elution step. Owing to the polar nature of the three herbicides, solvents with increasing polarity were assessed for the recovery efficacy. These solvents were DCM:isopropanol (80:20), ethyl acetate:isopropanol (80:20) and hexane:isopropanol (80:20). None of these solvent mixtures improved the recovery for the three herbicides, which was similar to that obtained using 100% DCM and the coloured substances that caused the interfering peaks on chromatogram were still present (data not shown). Likewise, there was no improvement in the recovery when the PPL cartridge with bigger sorbent bed (6 mL, 500 mg) was used compared with the smaller PPL cartridge (3 ml, 200 mg) (data not shown). Due to the existence of interfering compounds on the chromatograms obtained with the PPL cartridge, particularly with the imazapyr herbicide, and the low recovery of herbicides, a variety of SPE cartridges were examined for the extraction of imidazolinone herbicides, as described below.

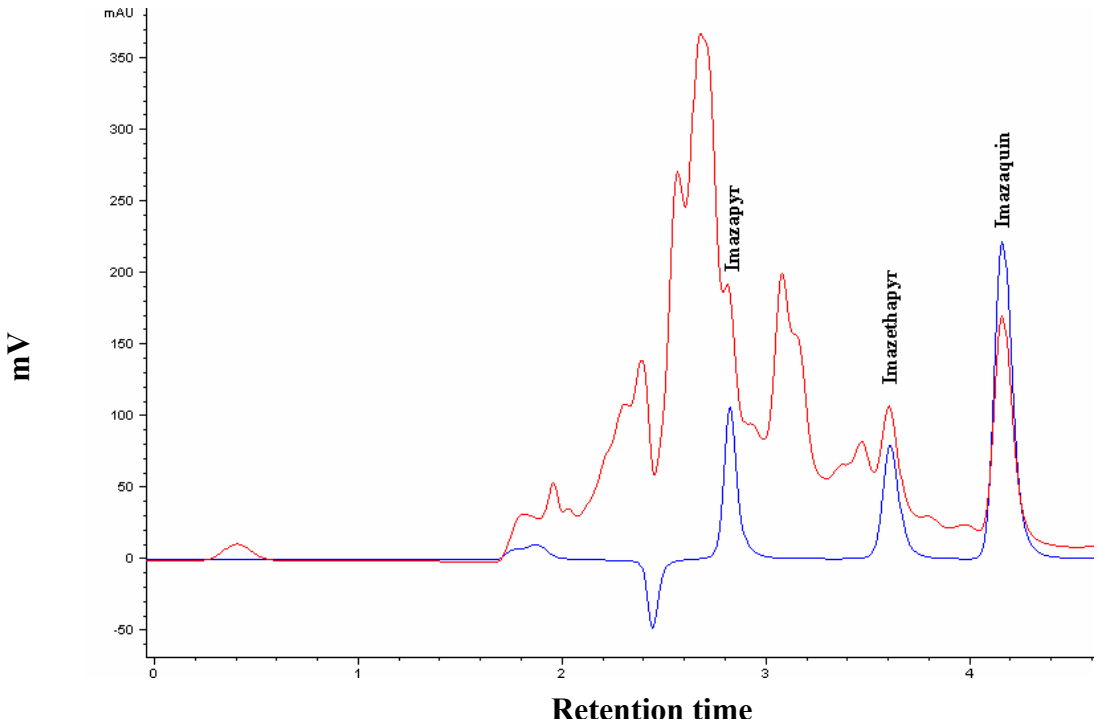

Figure 3.5. Chromatogram of imazapyr, imazethapyr and imazaquin in a standard solution (10µg mL^{-1}) (blue line) and in a 0.5M NaOH soil extract passed through a PPL cartridge (red line).

3.4.5 Extraction of imidazolinone herbicides from NaOH soil extracts using X-AW cartridges at alkaline pH

Some researchers have reported anion-exchange sorbents for the extraction of acidic herbicides with no acidification at neutral pH (Wells and Yu, 2000). Furlong et al. (2000) used strong anion exchange resin cartridges for isolating 12 herbicides including imazapyr, imazethapyr and imazaquin from surface and ground water. They indicated that this cartridge removed much of the dissolved organic carbon from the sample and imidazolinone herbicides were retained on the cartridge. Many other extraction procedures for removing imidazolinone herbicides from soil involve acidifying the extraction solution to approximately pH 2 for the herbicides to be retained on the SPE cartridges (Bresnahan

et al., 2002; Rodriguez and Orescan, 1998). Acidifying the solution pH is an additional step in the extraction procedure that is time-consuming and can cause the precipitation of co-extracted substances, leading to loss of resolution of the imidazolinone herbicides in chromatography.

Owing to large interferences close to the retention time of imazapyr and low recoveries of imazapyr (10.9%) and imazethapyr (69.3%) using the PPL cartridge, an anion-exchange cartridge (X-AW) was assessed for its efficacy to retain the herbicides. The main objective of this experiment was to develop a method to use for the extraction of imazapyr, imazethapyr and imazaquin with no pH adjustment before loading onto the SPE cartridge. Therefore, this experiment used 0.01M NaOH instead of 0.5M NaOH for extraction of the herbicides. The soil extracts were loaded directly onto the X-AW cartridge with no pH adjustment because the pH value of 0.01M NaOH was always less than 10. Although the size of the interfering peaks at the retention time of imazapyr decreased when X-AW cartridge was used, there was still a significant unresolved peak at the beginning of the chromatograms after the clean-up (data not shown). The recovery results of three imidazolinone herbicides using the X-AW cartridge were significantly different ($p < 0.05$) and were 15.6 $\pm$ 12.3, 43.8 $\pm$ 9.8 and 52.8 $\pm$ 11.8% for imazapyr, imazethapyr and imazaquin, respectively (Table 3.4). The RSD for recovery of imazapyr, imazethapyr and imazaquin was lower using the X-AW cartridge than for the PPL cartridge.

The recovery of the three herbicides, particularly imazapyr, was greatly increased by decreasing the sample loading flow rate from 3 mL $mins^{-1}$ to 0.5 mL $mins^{-1}$. For imazapyr recovery increased from 15.6% to 32.2%, whereas for imazethapyr recovery increased from 43.8% to 56.8% and for imazaquin from 52.8% to 61.3% (data not shown).

Decreasing the flow rate would lead to more contact time between the polar herbicides and the sorbent. Although the use of the X-AW cartridge was found to be a critical step for the cleanup of the imidazolinone herbicides, using a single cartridge may be insufficient for effective extraction and cleanup of imidazolinone herbicides from soil solutions. Consequently two SPE cartridges in series were assessed for their efficacy for removing the imidazolinone herbicides from the soil extracts.

Table 3.4. Mean recoveries of imidazolinone herbicides in the extracts of soils spiked at 1μg g^{-1} (n=3) after passing through different SPE cartridges.

SPE cartridge(s)	Imazapyr		Imazethapyr		Imazaquin	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PPL	10.9 a	9.2	69.3 a	8.4	75.4 a	8.9
X-AW	15.6 a	12.3	43.8 b	9.8	52.8 b	11.8
NH_2 + PPL	64.5 b	10.6	76.6 c	12.3	82.7 c	9.7
C_{18} + SCX	85.4 c	7.2	89.7 d	5.8	92.6 d	8.2

Mean recoveries with different alphabet within column are significantly different ($p < 0.05$).

3.4.6 Extraction of imidazolinone herbicides from NaOH soil extracts using NH_2 and PPL cartridges in series

A previous study using NH_2 for the extraction of a sulfonylurea herbicide, triasulfuron, from amended soils found that this cartridge eliminated much of the co-extracted organic

carbon from the sample (Said-Pullicino et al., 2004). The authors reported average recovery for this method of 86.8 ± 0.9% for extraction of triasulfuron from compost-amended samples. In the current study the NH_2 cartridge retained co-extracting humic substances from 0.5M NaOH solution at pH 2, while the imidazolinone herbicides were not retained on this cartridge. Therefore, 0.5M NaOH soil extracts were initially loaded onto the NH_2 cartridge and then passed through the PPL cartridge. Although there was a considerable extraneous peak at the beginning of the chromatograms when this clean-up was used no interfering peaks were observed on the chromatogram at the retention times of the herbicides of interest (data not shown). The recovery of imazapyr at the concentration of 1 μg g^{-1} was 64.5%. Recovery of imazethapyr was higher at 76.6% and the highest for imazaquin at 82.7% (Table 3.4). Furthermore, the recoveries were found to be reproducible indicated by low RSDs. Therefore, no additional attempt was conducted to improve the recovery for the three herbicides.

3.4.7 Extraction of imidazolinone herbicides from NaOH soil extracts using C_{18} and SCX cartridges in series

The use of the C_{18} and SCX cartridges in series to extract imidazolinone herbicides from 0.5M NaOH soil extracts was effective in eliminating coextracts (Figure 3.6). The mean recoveries after using C_{18} and SCX in series were 85.4 ± 7.2% for imazapyr, 89.7 ± 5.8% for imazethapyr and 92.6 ± 8.2% for imazaquin (Table 3.4). This strategy was found to be the most effective at extracting imidazolinone herbicides from soils without co-extracting substances.

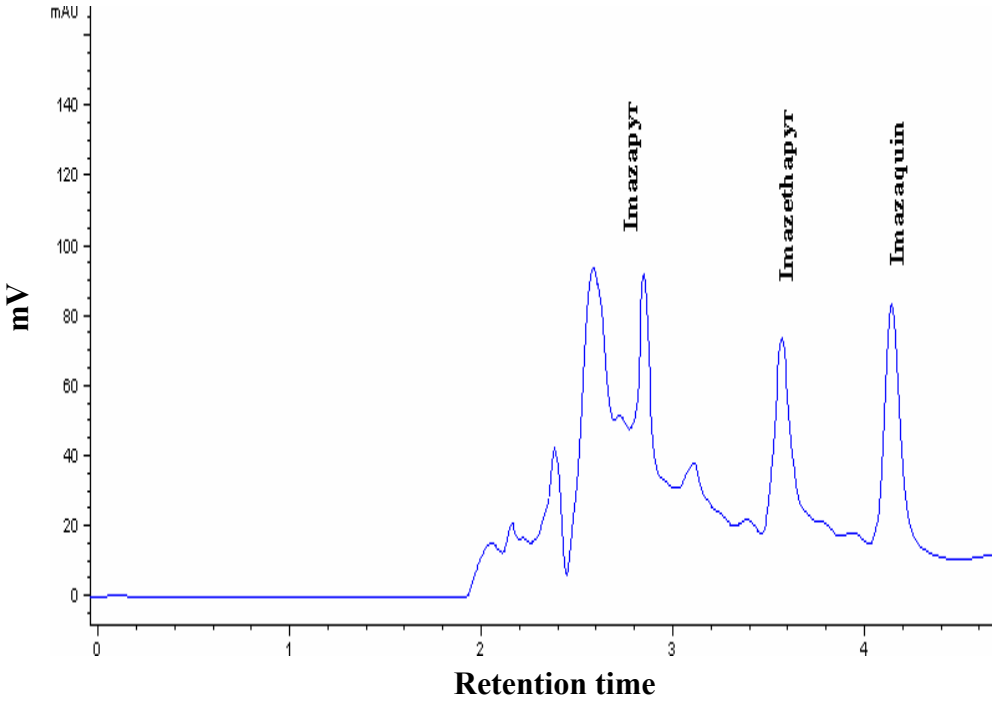

Figure 3.6. Chromatogram of spiked soil sample (1 µg g^{-1}) after passing the soil extract through a C_{18} and then a SCX cartridge.

3.5 Conclusion

In this study the efficiency of different aqueous solutions for extraction of imidazolinone herbicides from soils was evaluated. The recovery of imazapyr, imazethapyr and imazaquin was greater than 70% in the different extraction solutions assessed and the best results were obtained with 0.5M NaOH as the extraction solution. The recoveries obtained from 0.1 M KCl, 0.5 M NaOH, 0.01M NaOH and 0.5M NaOH:MeOH (80:20) were in the range of 77.2–89.9%, 92.3–97.4%, 83.7–94.2% and 80.3-104.2% for imidazolinone herbicides, respectively. Evaluation of different solid phase extraction cartridges showed that the PPL cartridge is an efficient cartridge for isolation and quantification of these herbicides in water and humic-amended solutions when used at pH 2. This cartridge can

be used for the extraction of imidazolinone herbicides from aqueous solutions including those with high concentrations of humic substances. However, when used with soil extracts, a number of co-extracted substances were also retained on the PPL cartridge, which decreased the recovery of imazethapyr and imazaquin and made quantification of imazapyr difficult. Using two SPE cartridges allowed the removal of the co-extracting substances, high recovery of herbicides and clear quantification. The recovery results obtained useing C_{18} + SCX cartridges in series were 85.4 $\pm$ 7.2, 89.7 $\pm$ 5.8 and 92.6 $\pm$ 8.2% for imazapyr, imazethapyr and imazaquin, respectively, which could be used with confidence in the quantification of imidazolinone herbicides. This scheme also allowed the analysis of the two enantiomers of the three herbicides. The method of imidazolinone extraction overcomes limitations and obstacles such as the use of organic solvents, the formation emulsions and the tedious procedures. However, because the damage threshold for some sensitive crops like maize after imazaquin and sugar beets after imazethapyr is less than 5 ng g^{-1}, it is evident that systematic studies are necessary to increase the limit of detection of these herbicides in soil.

Chapter 4. Abiotic degradation (photodegradation and hydrolysis) of imazapyr, imazethapyr and imazaquin herbicides*

4.1 Introduction

The degradation processes of pesticides in the environment may cause the formation of new compounds with reduced toxicity or, in some cases, increased toxicity, to aquatic biota. For this reason, various aspects of pesticide degradation need to be studied to characterize these processes. To assess the behaviour of pesticides in the environment, the impacts of both abiotic and biotic degradation processes should be considered. Among abiotic degradation factors influencing the fate of pesticides, photodegradation and hydrolysis are the most important (Bobe et al., 1998b). In addition, for the prediction of the behaviour and transport of pesticides, determination of hydrolytic as well as photolytic degradation pathways and kinetics of pesticide degradation in the normal pH range of the aquatic environment (5.5-8.0) is essential (Environmental Protection Agency, 1996).

It is also well known that photodegradation of pesticides can play a major role in the degradation of pesticides in aquatic systems (Aikaterini et al., 2005; Lam et al., 2005). Due to the attenuation of light wavelengths >290 nm by the atmosphere, the direct photodegradation of pesticides is expected to be minor (Corin et al., 1996). However, humic substances suspended in aquatic systems may result in different rates of photodegradation of pesticides in natural waters compared with pure water. It has been

* This chapter has been accepted for publication.
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reported that the concentration of dissolved organic carbon (DOC) is roughly 0.5 mg L^{-1} in groundwater and up to 30 mg L^{-1} in coloured water in swamps (Thurman, 1986). As organic and soil constituents suspended in water absorb sunlight, they can play a considerable role in photodegradation of pesticides in aquatic systems (Corin et al., 1996; Garbin et al., 2007). Humic acids are a component of biologically-derived substances that are ubiquitously present in soil and surface water and comprise approximately 30-50% of the total organic matter in the surface water (Corin et al., 1996; Garbin et al., 2007; Thurman, 1986). Humic substances with a molecular weight of approximately 500-5000 consist of a variety of functional groups, such as carboxylic acid, phenolic hydroxyl, carbonyl and hydroxyl (Kulovaara, 1996). Humic acids have a significant impact on the behaviour of pesticides in the environment because of their ability to absorb solar energy in the ultraviolet region (Zeng et al., 2002). Therefore, the fate of pesticides may be different in the presence of humic substances compared with pure water. Both inhibition and enhancement effects of humic substances on the photodegradation of some pesticides have been reported (Bachman and Patterson, 1999; Hustert et al., 1999; Konstantinou et al., 2001).

Photodegradation on the soil surface can be important in the degradation of a range of pesticides applied to soils (Balmer et al., 2000). Photodegradation is likely to be more important when pesticides are spread on the soil surface by application and not incorporated into the soil or in arid and semi-arid regions where they are less likely to be leached into the soil. The soil is a heterogeneous medium and photodegradation processes are expected to be different on soils compared with aqueous systems. Depending upon the depth of soil, direct and indirect photodegradation occurs in soil (Herbert and Miller, 1990). It has been demonstrated that the direct photodegradation of pesticides occurs at a

depth of roughly 0.2-0.3 mm, while the indirect photodegradation occurs at depths up to 0.7 mm in field studies (Balmer et al., 2000). Some soil factors including soil moisture, temperature and humic substances significantly affect pesticide degradation on the soil surface during solar irradiance (Cox et al., 1996). Frank et al. (2002) reported that niclosamide in moisture-maintained irradiated soils showed a steady photodegradation pattern at all studied depths, while on air-dried soil photodegradation decreased with increasing depth. It is well known that the hydroxyl radical, which is the main reactive oxidant in the environment, can be produced when humic substances are exposed to sunlight. Moreover, irradiation of humic acids in soil can also generate hydrogen peroxide in soil (Katagi, 2004). These radicals are also generated by the presence of water on the soil surface (Frank et al., 2002; Graebing et al., 2002).

The imidazolinone herbicides (imazapyr, imazethapyr and imazaquin) are reported to completely degrade in aqueous solutions after two days under ultraviolet light (Curran et al., 1992b). Mallipudi et al. (1991) reported a photodegradation half-life of <3 days for imazapyr in Milli-Q water or buffer solutions of pH 5 or 9. A number of studies have shown a decrease in photodegradation of imidazolinone herbicides in the presence of humic acids compared with pure water (Elazzouzi et al., 1999a; Elazzouzi et al., 1999b), while others have observed the opposite or no effect (Santoro et al., 1999). For example Elazzouzi et al. (2002) showed that the photodegradation of imazethapyr was enhanced in the presence of humic acids compared with a pure aqueous solution. Photodegradation of the imidazolinone herbicides could be important because they show low sorption to the soil (Regitano et al., 1997a), which could increase the risk of offsite transport in surface water,

where the photodegradation process may play an important role in removing the herbicide from the environment.

Photodegradation is also an important process of herbicide loss from soils. (Curran et al., 1992b). Mangels (1991) reported that imazapyr, imazethapyr, and imazaquin are prone to degrade if they remain exposed to light on the soil surface in the field. They reported that imidazolinone herbicides degraded faster in moist compared to air-dried soil. Photodegradation of imazaquin is higher at pH values above the p*K*a and under anerobic conditions. Temperature had no significant effect on photodegradation of imazaquin (Barkani et al., 2005). Imazethapyr losses from photodegradation were found to be 2% and up to 52% from the soil surface and a glass slide, respectively (Goetz et al., 1990). Photodegradation may be a particularly important degradation pathway in Australia where there are relatively high levels of incident UV radiation compared with other regions. To date there is limited information on the hydrolysis of these herbicides (Mangels, 1991).

The common characteristic of all imidazolinone herbicides is the presence of two enantiomers that derive from the chiral centre of the imidazolinone ring. The inhibitory activity of the R(+) enantiomer is nearly eight times greater than that of the S(-) enantiomer on target weeds (Lao and Gan, 2005). At present, it is unclear whether the photodegradation of the imidazolinone herbicides is enantioselective in nature or affected by the presence of dissolved organic carbon.

The aim of this study was to investigate the hydrolysis of imidazolinone herbicides at different pH values, as well as the photodegradation of these herbicides in the presence of humic acids and on the soil surface. In the case of the soil study, our work focused on dry-

soil (considering that soil humidity throughout the experimental time was not controlled) because this is how these herbicides usually used. Studies were also undertaken to determine whether photodegradation of imidazolinones herbicides was enantioselective in nature.

4.2 Materials and Methods

4.2.1 Aqueous buffer solutions

Three buffer solutions were made based on the chemical fate testing protocol from US Environmental Protection Agency (EPA) (Environmental Protection Agency, 1996) (Table 4.1). The pH of buffer solutions was checked with a pH meter at the beginning and at the end of the experiment. The pH values measured at the end of the hydrolysis experiment showed no change from the initial values. Buffer solutions and all glass apparatus were heat-sterilized by autoclaving. A separate stock solution of each imidazolinone herbicide was prepared as detailed in Chapter 3, section 3.2.2.

Table 4.1. Buffer solutions used for the aqueous hydrolysis experiments.

Buffer solution	pH	Preparation for 500mL of buffer solution
A	3	250 mL of 0.1 M potassium hydrogen phthalate + 111 mL of 0.1M hydrochloric acid
B	7	250 mL of 0.1 M potassium dihydrogen phosphate + 145 mL of 0.1M sodium hydroxide
C	9	250 mL of 0.025M borax ($Na_2 B_4O_7$) + 23 mL of 0.1M hydrochloric acid

4.2.2 Incubation of imazapyr, imazethapyr, and imazaquin in aqueous buffers

The hydrolysis of the three herbicides was investigated by incubating herbicides in the three buffer solutions in the dark at 30^{o}C. A 5 mL aliquot of 100 μg mL^{-1} solution of the three individually imidazolinone herbicides prepared in acetone was added aseptically to 50 mL of each heat-sterilized buffer solution to obtain 10 μg mL^{-1} initial concentration of the herbicide. Three replicate solutions were prepared for each herbicide. One unspiked (control) sample was also prepared and incubated for each pesticide at each buffer solution. The pH of the solutions was measured after adding herbicide, and a sample (1 mL) was removed from each solution aseptically and analysed by HPLC at time zero. The herbicide solutions were wrapped in aluminium foil and maintained in the dark at 30ºC. A digital thermometer was put inside the chamber to check for temperature variation during the experiment. An aliquot (2 mL) was taken from each solution every day for the first 7 days and then every 2nd day for the next 20 days. Samples were withdrawn in a laminar flow cabinet to maintain sterility. Herbicide concentrations in the samples were determined by HPLC and compared with standards made fresh each day.

4.2.3 Photodegradation experiments

4.2.3.1 Photodegradation of imidazolinone herbicides and the effect of humic acids

Photodegradation of imazapyr, imazethapyr and imazaquin was studied in the presence of different ratios of herbicide:humic acids (1:1, 1:2, and 1:3) and in sterilised Milli-Q water. The elemental composition of the purified humic acids from Fluka Analytical, similar to

the one used in the experiment, was reported to be 53.1% C, 4.7% H, 37.6% O and 4.6 % N. The functional groups present included -OH, -CH_2, -COOH, -NH_2 and ->C-H, as shown by their fourier transform infrared (FTIR) spectra (Palladino et al., 2007). The aqueous solutions (40 mL) containing each of the individual imidazolinone herbicides in glass tubes were exposed to simulated solar radiation. There were three replicates for each herbicide. The simulated solar irradiation was provided using a high-pressure mercury vapour lamp positioned 30 cm above the containers and with irradiation intensity of 550 W/m^2 throughout the experiments. The herbicide solutions were adjusted initially to pH 7 and maintained above pH 5.5 during the experiment. The temperature was kept constant at 25ºC throughout of the experiment. In addition, a set of all treatments was kept in the dark at the same temperature as control treatments to confirm that any degradation process was the result of photochemical reactions. A 2 mL aliquot of sample was pipetted into vials at 0, 1, 3, 5, 8, 10, 16 and 24 days for imazapyr and imazethapyr and 0, 0.1, 0.3, 0.4, 1.1, 2.3, 4 and 5.3 days for imazaquin. The samples were directly analysed with HPLC without any further clean up.

4.2.3.2 Photodegradation of imazaquin enantiomers

An investigation of the photodegradation of the enantiomers of imazaquin was also undertaken. The set up was similar to the previous study, with different concentrations of imazaquin:humic acid ratios (Milli-Q water, 1:1, 1:2, 1:3) irradiated for 5 days under the same conditions as described in section 4.23.1.

4.2.3.3 Photodegradation of imidazolinone herbicides on the soil surface

A sandy loam soil was collected from the surface layer (0-10 cm) in the field. The soil was air dried at room temperature, sieved (< 2 mm) and autoclaved at 121°C on two separate occasions for 1 h each time. Particle size analysis showed the soil to contain 65.1% sand (> 0.02 mm), 20.3% silt (0.002-0.02 mm) and 14.6% clay (< 0.002 mm) with an organic carbon content of 1.2% and a pH value of 6.7 in water (Merry and Spouncer, 1988).

Sterilized soil (10 g) was transferred to 48 petri dishes (15 cm diameter) and spread evenly to give a depth of approximately 1 mm. Imidazolinone herbicide solution was applied uniformly to the top of the soil sample in the petri dishes to obtain the initial concentration of 10 μg g^{-1}. After evaporation of the solvent from the soil surface, 24 petri dishes were exposed to light in the chamber and the other 24 dishes were wrapped in aluminium foil to maintain darkness (controls). Both irradiated and non-irradiated samples were maintained at 25ºC. The dark samples were set up to evaluate possible effects of chemical and microbiological degradation throughout the irradiation experiment. Soil samples were withdrawn at 0, 1, 5, 10, 17, 27 and 37 days after herbicide addition. At each sampling time, three irradiated and three control samples were taken and transferred to centrifuge cartridges for extraction of the herbicides.

4.2.4 Extraction of imazaquin by solid phase extraction (SPE) cartridge

The analytical method developed for the extraction of imazaquin enantiomer using the SPE cartridge described in Chapter 3 (section 3.2.5) was used for this study.

4.2.5 HPLC analysis of imidazolinone herbicides

After extraction of the aqueous and soil samples, the analysis of the extracts was performed using reverse- and normal-phase HPLC-UV described in Chapter 3, section 3.5. A representative chromatogram of imidazolinone herbicides is shown in Figure 4.1.

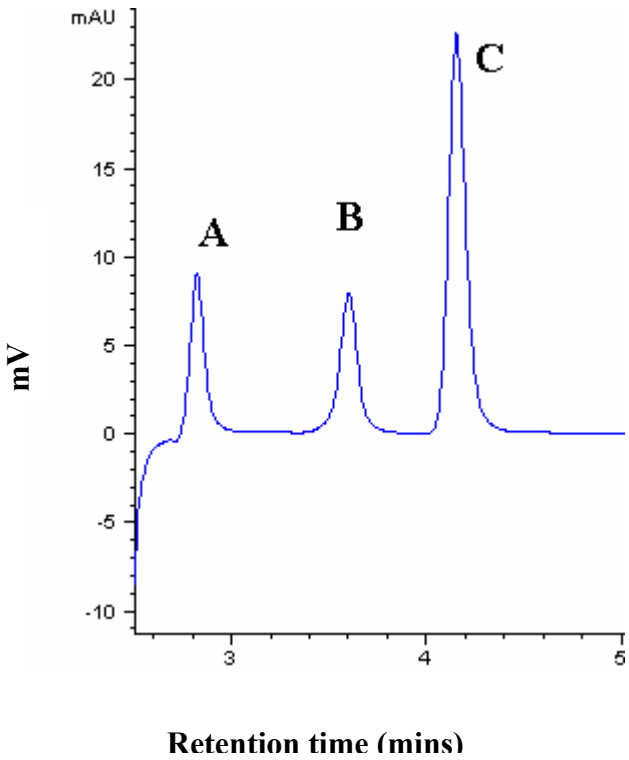

Figure 4.1. Separation of imazapyr (A), imazethapyr (B), and imazaquin (C) in mixed standards of 1 µg mL^{-1} water by HPLC on a reverse phase C_{18} column.

4.2.6 Quality control

A number of factors were taken into account for quality control for the method, including limit of detection and limit of quantification. The limit of detection (LOD), based on a signal to noise (standard deviation of the blank sample = background noise) ratio of three, was 0.5 μg mL^{-1} for all three herbicides. Quantification was performed by comparing peak areas to standard herbicide solutions. All standards were made in solutions to match the matrix of the experimental samples. The limit of quantification (LOQ) was described as the sample concentration needed giving a signal-to-noise ratio of 6:1 and characterized two-fold the LOD for each herbicide. Linearity of the detector response was assessed at 240 nm by injecting 20 μL of solutions of a mixture of the three herbicides at the same concentrations, in which the concentration of herbicides varied from 0.5 to 20 μg. The UV-detector response to the herbicides was linear in this range of sample concentrations. Lack of interference in chromatograms due to matrix effects was confirmed by injecting blank buffer solutions that contained no imidazolinone herbicides.

4.2.7 Statistical analyses

Since the plots of natural log of imidazolinone herbicides against time were linear, a first-order equation was used to determine the rate constants (k) using Equation 4.1:

$$Ln\ C_{(t)} = \text{Ln}\ C_0 - kt \qquad (4.1)$$

where C is the concentration of imidazolinone herbicide at time t, C_0 is the initial concentration and t is the sampling time in days. The half-life, was calculated by $t_{1/2}=0.693/k$

Significant differences between the slopes (and thus the half-lives) were assessed by ANOVA of regression data using GenStat (6th edition, VSN International Ltd) at significance level of $p < 0.05$.

4.3 Results and Discussion

4.3.1 Hydrolysis of imidazolinone herbicides

No hydrolysis of any of the imidazolinone herbicides occurred at 30°C in solutions buffered at pH 3 or pH 7. Slight hydrolysis occurred at pH 9 with extrapolated half-lives ($t_{1/2}$) of 6.5, 9.2 and 9.6 months for imazaquin, imazethapyr and imazapyr, respectively (data not shown). There was no significant difference in the degradation of the herbicides at the three pH values (3, 7 and 9) at constant temperature (30°C). Currently there is only one research publication related to the hydrolysis of imidazolinone herbicides (Mangels, 1991), which demonstrated these herbicides are not hydrolysed at pH values 5, 7 or 9. It has been reported that imazamethabenz-methyl is the only imidazolinone herbicide sensitive to hydrolysis (Jensen et al., 1997). In general, pesticides that are derivatives of carboxylic acids have functional groups that are subject to hydrolysis (Schwarzenbach et al., 2005). The study confirmed that the results from the susbsequent photodegradation experiments are not likely to be confounded by any losses due to hydrolysis and are expected to be solely due to photodegradation of herbicides.

4.3.2 Photodegradation of imidazolinone herbicides in water and in the presence of humic acids

The photodegradation of all three herbicides followed first-order kinetics and the presence of humic acids significantly ($p < 0.05$) decreased the rate of photodegradation of imidazolinone herbicides (Figures 4.2A; 4.2B; 4.2C). The rate constant for imazapyr photodegradation was 0.075 day^{-1} in Milli-Q water giving a half-life of 9.1 days and it decreased significantly ($p < 0.05$) with increasing concentrations of humic acids, reaching 0.037 day^{-1} and a half-life of 18.2 days in a 1:3 ratio of humic acids:imazapyr (Table 4.2). Photodegradation of imazethapyr was less sensitive to humic acids, with the rate constant decreasing from 0.07 day^{-1} (a half-life of 9.8 days) in pure water to 0.051 day^{-1} (a half-life of 13.4 days) in a 3:1 ratio of humic acids:imazethapyr in water. The decrease in photodegradation of imazethapyr was only significant ($p < 0.05$) at the highest humic acid:imazethapyr ratio (3:1). Imazaquin was the most sensitive of all the imidazolinone herbicides to the effect of humic acid on photodegradation. In water the rate constant for this herbicide was 0.369 day^{-1} and the half-life was 1.8 days, but at the highest humic acid ratio it decreased down to 0.246 day^{-1} and the half-life increased to 2.8 days (Table 4.2).

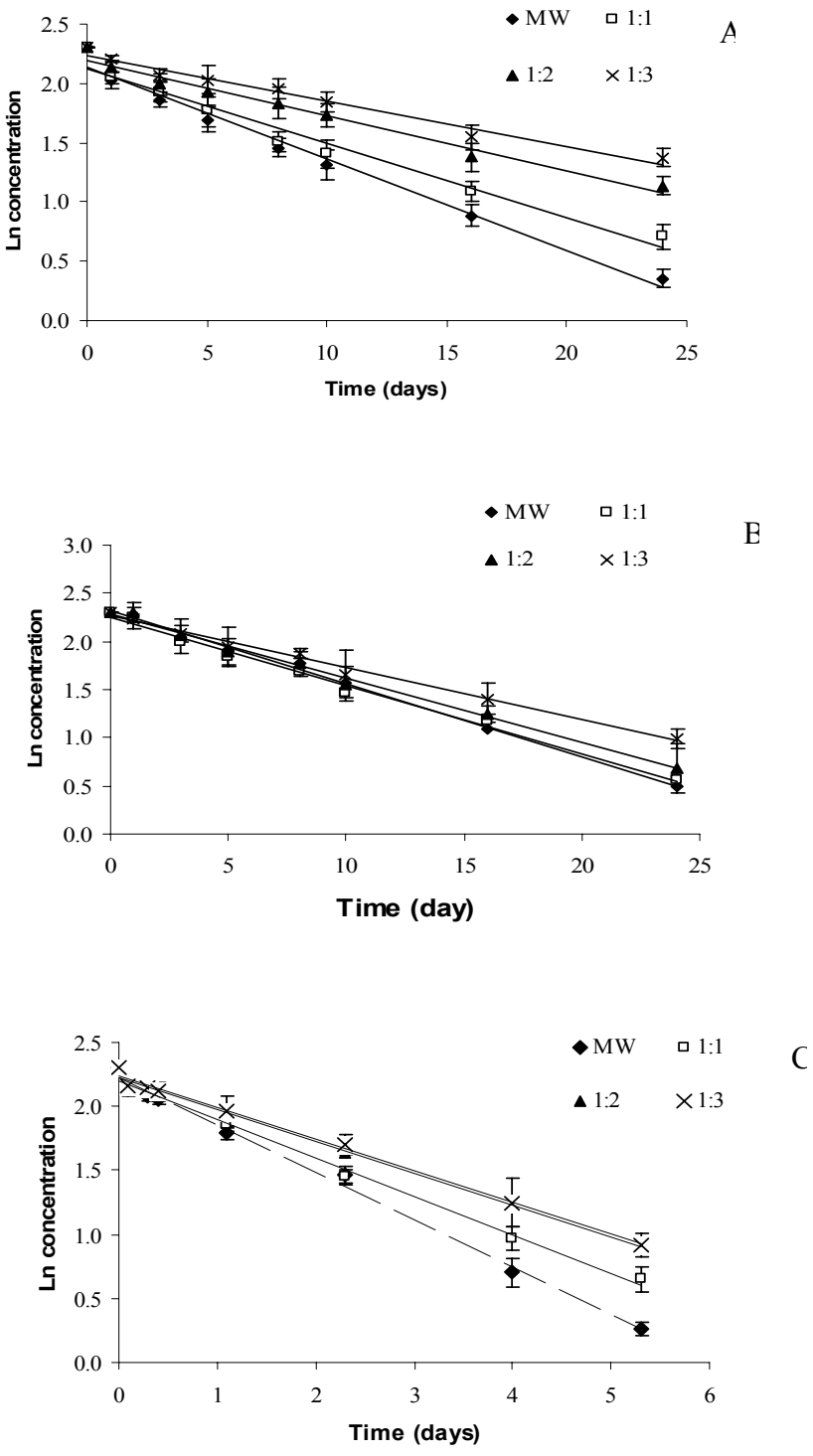

Figure 4.2. First-order rate plots for photodegradation of (A) imazapyr, (B) imazethapyr, and (C) imazaquin in different ratios with humic acids and in Milli-Q water. The ratios of 1:1, 1:2 and 1:3 corresponds to different ratios of herbicide:humic acids concentration and MW is Milli-Q water.

Table 4.2. Photodegradation kinetics of imazapyr, imazethapyr and imazaquin in water and in the presence of humic acids (n =3).

Compounds	**Herbicide:Humic acid ratios**	***k* (day^{-1})**	***t*$_{1/2}$(day) [a]**	***R*^{2}**
Imazapyr	Milli-Q water	0.075	9.1 a	0.98
	1:1	0.063	10.9 b	0.97
	1:2	0.046	14.9 c	0.97
	1:3	0.037	18.2 d	0.97
Imazethapyr	Milli-Q water	0.070	9.8 a	0.99
	1:1	0.067	10.2 a	0.98
	1:2	0.060	11.4 a	0.99
	1:3	0.051	13.4 b	0.99
Imazaquin	Milli-Q water	0.369	1.8 a	0.99
	1:1	0.302	2.2 b	0.99
	1:2	0.249	2.7 c	0.99
	1:3	0.246	2.8 c	0.99

[a] For any one herbicide, half-lives ($t_{1/2}$) followed by the same letter in the column were not significantly different at p <0.05.

Photodegradation of the imidazolinone herbicides was found to be compound-specific in that imazaquin was degraded much faster than either imazapyr or imazethapyr (Table 4.2 and Figure 4.2C). Curran et al. (1992b) also reported imazquin was much more sensitive to photodegradation compared to the other imidazolinone herbicides. Although, the photodegradation of the three herbicides was decreased by the presence of humic acids, the extent of the impact varied with the compound. Photodegradation of imazapyr and imazaquin was significantly ($p < 0.05$) decreased by the lowest concentration of humic acids (Figure 4.2A; 4.2C), whereas photodegradation of imazethapyr was only significantly ($p < 0.05$) decreased by the highest concentration of humic acids (Figure 4.2B).

Due to the UV screening effect of humic substances on chemicals, it has been reported that the transfer of energy and charge among the humic acids and chemicals is capable of inactivation of the excited molecules of some pollutants (Cox et al., 1996; Kamiya and Kameyama, 1998), but the extent of the process might be compound-specific. It has been demonstrated that organic substances in solution can absorb light, resulting in decreased photodegradation of pesticides. This phenomenon is usually known as the optical filter effect (quenching) (Aikaterini et al., 2005). It has been previously suggested that the adsorption of some herbicide molecules to the humic matrix and the protective role of humic acids may be one reason for the prolonged half-life of herbicides in the presence of humic acids in comparison to the half-life in Milli-Q water (Elazzouzi et al., 1999a; Elazzouzi et al., 1999b). This finding has implications for the persistence of these herbicides in aquatic ecosystems, such as lakes and streams, which often contain appreciable amounts of dissolved organic matter. This study shows that while humic acids will decrease the rate of photodegradation of imazapyr and imazaquin, but such effect for

imazethapyr was only possible at unrealistically high concentrations of humic acids in natural aquatic systems.

Photodegradation may be an important pathway for degradation of imidazolinone herbicides in surface water bodies. Imazethapyr has been detected at concentrations exceeding maximum residue limits in 71% of 212 samples in stream and river water samples tested in Midwestern US (Battaglin et al., 2000). The relatively short half-lives of these herbicides in water have been reported in the literature (Barkani et al., 2005; Curran et al., 1992b). For example, a half-life of approximately 2 days has been shown for the imidazolinone herbicides exposed to UV light in aqueous solution (Mallipudi et al., 1991). However, as the results from this study show, the presence of dissolved organic carbon can increase the persistence of these herbicides (by up to a factor of 2) in aquatic environments.

4.3.3 Photodegradation of the herbicides on the soil surface

The concentration of imidazolinone herbicide in the dark-incubated control samples did not change during the period of the experiment, showing the imidazolinone herbicides are stable under the temperature conditions (25°C) and time frame (37 days) of this study. This observation plus the results form the hydrolysis studies assured that any observed degradation can confidently be ascribed to photodegradation process. The natural log concentration of three herbicides plotted against time was linear with an R^2 of 0.96 to 0.98 (Table 4.3, Figure 4.3), indicating that photodegradation process on the soil surface was a first-order reaction. Imazaquin was the most sensitive herbicide to photodegradation with a rate constant of 0.045 day^{-1} and a half-life of 15.3 days. Imazapyr was the most stable of the imidazolinone herbicides with a rate constant of 0.022 day^{-1} and a half-life of 30.9

days. There was a significant ($p < 0.05$) difference in rate constant for photodegradation and the half-lives for three herbicides ($p < 0.05$) (Table 4.3).

Table 4.3. Photodegradation kinetics of imazapyr, imazethapyr and imazaquin on the soil surface. (n=3).

Compounds	***k* (day^{-1})**	***t*$_{1/2}$(day)** [a]	***R*^{2}**
Imazapyr	0.022	30.9a	0.96
Imazethapyr	0.028	24.6b	0.98
Imazaquin	0.045	15.3c	0.97

[a] Values followed by different letters indicate significant differences at $p < 0.05$

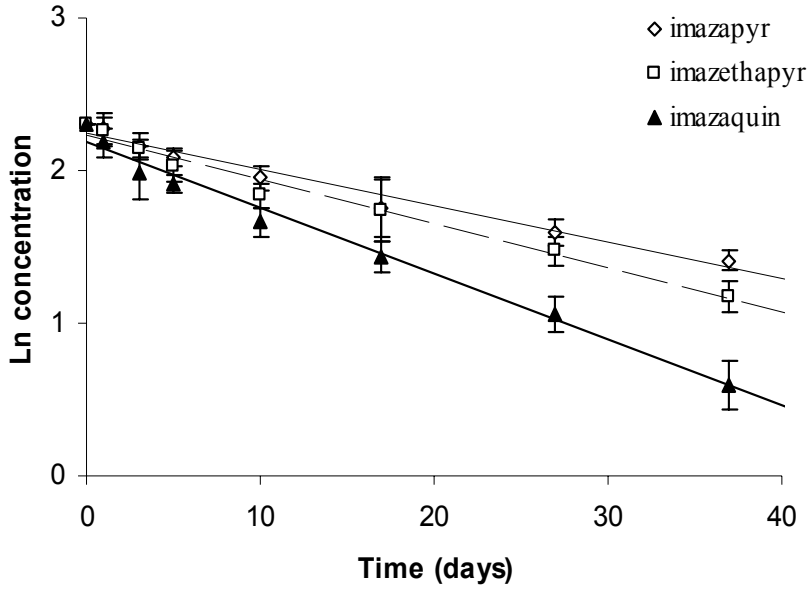

Figure 4.3. First-order rate plots for photodegradation of imidazolinone herbicides on the soil surface.

4.3.4 Photodegradation of imazaquin enantiomers

The imidazolinone herbicides consist of two enantiomers of which one has significantly greater biological activity than the other (Lao and Gan, 2005). Differential degradation of the two enantiomers in the environment will affect the biological activity of soil residues of these herbicides. Therefore, the photodegradation of the two imazaquin enantiomers was investigated in both water and in the presence of humic acids. The results of the experiment showed that both enantiomers of imazaquin were degraded at a similar rate in water and enantioselectivity was not changed during the experimental period (Figure 4.4). EF values are characterized as: area of the S(-) enantiomer divided by the sum of the areas of the both S(-) and R(+)enantiomers eluting from chiral column. EF>0.50 indicate a more rapid degradation of the R(+) enantiomer, EF<0.50 indicate a more rapid degradation of the S(-) enantiomer, and at an EF value of 0.50, degradation is non-enantioselective. In the case of the imazaquin enantiomers peak height rather than peak area was used for determining the EF values due to the incomplete baseline resolution of enantiomers. The EF value of imazaquin was close to 0.5 (range: 0.49 - 0.51) and showed no enantioselectivity during the experiment. Likewise, there was no difference in the rates of photodegradation between the two enantiomers in the presence of humic acids and EF was close to 0.5 (data not shown). These results are consistent with other work that shows that photodegradation of chiral pollutants is not enantioselective (Imran and Aboul-Enein, 2004). In general, it has been shown that the abiotic processes cause no significant change in the enantioselectivity of chiral pesticides (Müller and Kohler, 2004). For example, it has been reported that the change of EF of a-hexachlorocyclohexane, mecoprop, *cis*-chlordane, *trans*-chlordane, heptachlor, *exo*-epoxide and oxychlordane in abiotic conditions are close to 0.5, while the biotic component has a much higher variation from 0.5 (Hegeman and

Laane, 2002). Clearly the abiotic behaviour of the imidazolinone chiral herbicides is consistent with other pollutants.

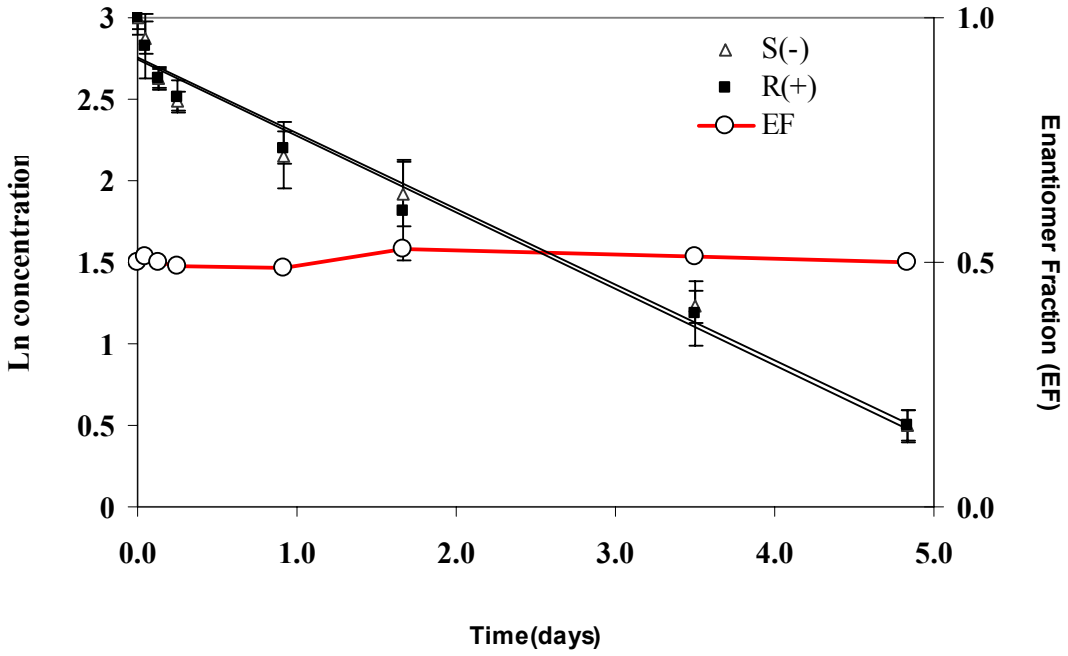

Figure 4.4. Photodegradation of the imazaquin enantiomers (*Ln* concentration) in water. The concentrations of the enantiomers and the enantiomer fraction, EF (the area of the S(-) enantiomer/sum of areas of both enantiomers); values are the mean of triplicate samples and vertical bars represent SEM.

4.4 Conclusions

Hydrolysis of the imidazolinone herbicides imazapyr, imazethapyr and imazaquin was negligible at all solution pH values studied. Therefore, hydrolysis is not expected to play an important role in the degradation of these pesticides in the environment. The photodegradation of imidazolinone herbicides in solution proceeded as first-order

reactions. The rate constants of photodegradation decreased up to 2-fold in humic acid solutions compared with those determined in Milli-Q water. The rate constants ranged from 0.07 to 0.36 per day in the Milli-Q water compared to 0.24 to 0.67 per day in different concentration of humic acids. Photodegradation could play a role in the degradation of these herbicides in the environment, such as when transported off-target in run-off water. In addition, the presence of suspended organic substances in aqueous solutions decreased the photodegradation of all three herbicides by the screening effect, which is in agreement with previous research (Konstantinou et al., 2001). Thus the presence of dissolved organic carbon and turbidity of water is likely to play an important role in determining the persistence of these herbicides in natural water bodies. This study found no evidence for enantioselectivity of photodegradation for imazaquin, with photodegradation occurring at the same rate for both enantiomers.

Chapter 5. Enantioselective degradation of imazapyr, imazethapyr and imazaquin in soil

5.1 Introduction

Approximately one quarter of all pesticides are chiral in nature, consisting of two or more stereoisomers named enantiomers (Hegeman and Laane, 2002; Liu et al., 2005a). This proportion is increasing as pesticides with more complex structures are being introduced and many modern pesticides have chiral structures (Liu et al., 2005b). Enantiomers have identical physical-chemical properties, but may show differences in interactions with enzymes or other biological systems (Garrison, 2006). At present, many pesticides are used as racemic mixtures in the environment despite the fact that the pesticide activity is conferred largely by one enantiomer. More recently, in agriculture there has a been tendency toward increasing use of only the active or enriched enantiomer of chiral pesticides with the aim of decreasing the amount of pesticide applied in the environment (Garrison, 2006; Garrison et al., 1996). For example, S-metolachlor, which is the enriched (88% S- and 12% R-enantiomer) form of metolachlor, has 1.4 to 1.6-fold greater activity than the racemic metolachlor formulation. Since 1997 the switch from racemic metolachlor to S-metolachlor has occurred in many countries including the United States, Canada, South Africa and Australia (Ma et al., 2006). Furthermore in the European Union, approximately 75% of dichlorprop and mecoprop are sold as single enantiomer formulations (Saari et al., 1999). The behaviour of these single enantiomer formulations and the enantioselectiveness of their environmental fate processes need to be established.

The extent of microbial degradation of pesticides is affected by soil parameters such as physicochemical properties, organic matter contents and the characterestics of the pesticide

molecule (Aislabie and Lloyd-Jones, 1995). Soil properties including organic matter, pH, moisture, texture and activity of soil fauna can substantially influence the microbial processes of soil. The addition of organic amendments to the soil can improve the activity of microbial communities in soil as well as affect soil physical, chemical and biochemical characteristics (Cox et al., 2001; Moorman et al., 2001). The consequence of organic amendment on the fate of pesticides in soil depends upon the nature of the amendment and the impact on microbial activity (Briceño et al., 2007; Wanner et al., 2005). Incorporation of organic amendments into soil can increase soil microbiological activity by increasing the availability of simple organic molecules, such as sugars, amino sugars and amino acids. Enhancement of microbial activity, however, does not always result in an increase the rate of degradation of pesticides in the amended soils. Variable effects on microbial activity of soils have been reported in the literature following the addition of organic amendments (Cox et al., 2001; Moorman et al., 2001). It has also been shown that soil factors such as organic matter content and pH accounted for approximately 56% of the difference in microbial processes responsible for N_2O fluxes (EI Sebai et al., 2007). Others have observed that there is a correlation between soil pH and the degradation rate of pesticides, indicating the preference of microbes for higher soil pH (Suett et al., 1996).

As the enantioselective degradation of pesticides has been attributed to biological processes (Buerge et al., 2003), there is the expectation of different degradation rates of pesticide enantiomers with changes in microbial activity caused by organic amendment. There is some evidence showing degradation of enantiomers varies with organic amendment of the soil. Romero et al. (2001) reported the addition of peat changed the enantioselectivity of mecoprop and dichlorprop in soil. Another study showed the enantioselective degradation of dichloroprop-methyl shifted to preferential degradation of

the S-enantiomer with organic amendment of the soil (Lewis et al., 1999). Buerge et al. (2003) observed variation in enantioselective degradation of metalaxyl with changes in soil pH. They showed preferential degradation of the R-enantiomer at soil pH greater than 5, similar degradation of both enantiomers at pH range of 4-5, and reversed enantioselectivity at soil pH less than 4. In another study, the highest enantioselectivity of benalaxyl was reported at the highest pH value of 8.6 and non-enantioselective degradation at the pH values of 4.8 and 5.3 (Wang et al., 2007).

It has been demonstrated that biological systems are responsible for the degradation of imidazolinone herbicides in soil (Basham and Lavy, 1987; Cantwell et al., 1989b; Flint and Witt, 1997). Increased degradation of imazethapyr and imazaquin was observed as soil moisture increased from 15% to 75% of the field capacity (Flint and Witt, 1997). These authors also showed that factors influencing microbial activity also affected the rate of degradation of these herbicides. There was increased persistence of both herbicides in soil at 15°C as compared with 30°C. The higher dissipation of imidazolinone herbicides (including imazamox, imazethapyr, and imazaquin) at soil pH 7 compared with the soil pH 5 has been suggested to be due to greater bioavailability and decreased sorption of these herbicides at the higher pH value (Aichele and Donald, 2005). It is known that soil pH is an important factor affecting the degradation of imazapyr under aerobic conditions but not under anaerobic conditions (Wang et al., 2006).

Despite several studies on the degradation (biotic and abiotic) of imidazolinone herbicides in different environmental matrices, studies on enantioselective degradation of this herbicide group are rare. A study of enantioselective degradation of imazaquin in aqueous slurries of soil from two sites from Georgia and Ohio in the US (Jarman et al., 2005),

showed no enantioselective degradation of this herbicide after 3 months of incubation. As imidazolinone herbicides are currently manufactured and marketed as racemic mixtures, investigations of enantioselectivity of these herbicides could be important for understanding the fate of these herbicides in the environment. The results from these studies would be important for the manufacturers as well as regulatory agencies.

In the present study, three experiments were conducted to achieve the following objectives: (1) to assess enantioselective degradation of imidazolinone herbicides in different soil types with a view to determine any relationship between soil properties with enantioselective degradation; (2) to compare the degradation rate of imidazolinone enantiomers under sterilized and non-sterilized conditions to determine whether microbial degradation has a role in enantioselectivity of these herbicides; (3) and to determine if stimulation of microbial activity through addition of an organic amendment can cause enantioselective degradation of imidazolinone herbicides.

5.2 Materials and Methods

5.2.1 Soil samples

Two soils used in one of the experiments were collected from cropping fields at Roseworthy and Clare townships in South Australia. Four additional soils used in a part of the study were selected from a Soils Archive of CSIRO, which contains soils from field sites across Australia. The selection of soils was based on differences in soil pH, organic matter, and soil texture to attain a broad range of soil properties known to affect the degradation of pesticides. At Roseworthy and Clare, the top 15 cm of the soil was collected

and passed through a 2-mm sieve and stored at 4°C until used. Maximum water-holding capacity (MWHC) was measured after saturation of the soil (20 g) with deionised water in a funnel lined with Whatman No. 1 filter paper. The soil was allowed to drain for 24 h after which the moisture content was determined by oven-drying the samples at 110°C for 24 h. Soil pH was measured in a 1:5 soil:water extract (Rayment and Higginson, 1992). Total organic carbon (TOC) was measured according to the method of (Matejovic, 1997). Particle size analysis was used to determine the proportion of clay, silt, and sand in each soil type and was conducted according to the USDA method (USDA, 1996). Selected physico-chemical properties of soils used in this research are shown in Table 5.1.

Table 5.1. Selected physico-chemical properties of soils used in this study.

Soil	**pH_w[a]**	**MWHC (%)**	**Organic C (%)**	**Particle size%**		
				Clay <0.002 mm	**Silt 0.002-0.02 mm**	**Sand 0.02-2 mm**
Alo-Kingaroy (AK)	8.7	26.1	2.8	35.6	14.1	49.2
Collie (CO)	6.0	21.7	4.4	4.8	5.2	85.1
Jacka (JA)	7.6	38.7	2.9	29.6	38.3	27.4
Otterbourne (OT)	5.0	30.1	3.0	10.3	19.4	64.2
Roseworthy (RC)	8.2	22.4	1.7	19.8	4.7	75.6
Clare (CL)	5.2	22.7	1.9	19.2	13.0	67.8

[a]Soil pH was measured in a 1:5 soil:water extract; MWHC- maximum soil water-holding capacity.

5.2.2 Soil incubation experiments

Unless otherwise indicated, the following procedures were used in this study. To determine the effect of organic amendment on degradation of imazethapyr and imazaquin

enantiomers, lupin (*Lupinus albus* L.) residue from mature plants was ground through a 1 mm screen then mixed into two soils (Roseworthy and Clare only) at 2% (w/w). Lupin residue was used as an organic amendment because it had previously been shown to increase the activity of microorganisms in different soils (data not shown). The properties of lupin residue were as follows: total P (0.6 g kg^{-1}); total C (438.8 g kg^{-1}); total N (14.4 g kg^{-1}); water soluble carbon (32.4 g kg^{-1}); C/N ratio 30. Non-amended soils were included to compare the effect of amendment on degradation of the herbicides. The amended and non-amended soils were split into two portions each with one portion sterilized by autoclaving as described earlier (Chapter 4, section 4.2.3.3). The amended soils were prepared by thoroughly mixing lupin residue and soil in a plastic bag and the moisture adjusted to 50% MWHC with deionised water. The soil (25 g dry wt) was then added to containers with a fine mesh at the bottom (Figure 5.1). Three replicates of each treatment were incubated at 30°C in covered plastic containers along with another small container with approximately 15 mL of deionised water to maintain high relative humidity to minimize water loss from the soil surface.

To minimise the detrimental effect of herbicide solvent on the microbial activity of the soils, the solution volume was kept low and each container was spiked with only 40 µL solution (200 µg mL^{-1}) of imazapyr, imazethapyr and imazaquin, to achieve a herbicide concentration in the soil of 0.3 µg g^{-1} (dry wt). This concentration is equivalent to an application dose of approximately double the maximum field application rate of imazethapyr and imazaquin and recommended dose of imazapyr, assuming uniform incorporation to a depth of 10 cm and the bulk density of 1.5 g cm^{-3}. The higher herbicide concentration was used to allow better quantification of herbicide residues in soil extracts. Soil samples were allowed to equilibrate and solvents allowed to evaporate for

approximately 2 h before mixing with a stainless steel spatula. Each container was weighed weekly and deionised water added to maintain 50% MWHC measured at -33 kPa throughout the incubation period. A set of three replicates was prepared for each sampling date. The covers of the containers were kept loose to enable air exchange during the experiment. Samples were checked regularly to maintain the water content and were also mixed at the time of sampling for aeration. After spiking with the herbicides, soil samples for time zero were collected and stored at 4°C and extracted and analysed within a day. These samples were used to assess the recovery of the extraction method and the homogeneity of herbicide spiking. Soil samples were removed at regular incubation periods and stored at 4°C until analysed using HPLC.

Figure 5.1. Set up used for incubation of soil.

5.2.3 Enantioselective degradation of imidazolinone herbicides

5.2.3.1 Experiment 1: Enantioselective degradation of imidazolinone herbicides in different soils

The experimental conditions of this study were identical to the above experiment except the temperature was maintained at 25°C. At the beginning of this experiment and one month later, 1 mL of a sucrose:potassium nitrate solution (2:02 g kg^{-1} soil, organic C: inorganic N) was added to the soil to supply additional carbon and nitrogen (Flint and Witt, 1997).

5.2.3.2 Experiment 2: Enantioselective degradation in sterilized and non-sterilized soils

This experiment was set up to investigate the enantioselective degradation of herbicide enantiomers in sterilized and non-sterilized soil samples in Roseworthy and Clare soils only. Lupin-amended and non-amended soils were sterilized by autoclaving at 121°C for 1 h twice with a 48 h interval between the first and second autoclaving. Both sterile and non-sterile soils were spiked with the herbicides exactly as described in section 5.2.2. Soil samples were taken at regular intervals of 0, 5, 10, 15, 25, 45, 65 and 90 days, and stored at 4°C until analysed.

5.2.3.3 Experiment 3: Effect of organic amendment on enantioselective degradation of imidazolinones

To study the influence of organic amendment on enantioselectivity of imazethapyr and imazaquin, two weeks before the start of the experiments, lupin-amended and non-amended soils as described in section 5.2.2, were moistened to 50% MWHC, and pre-incubated for two weeks at 25°C in the dark to recover soil microbial activity.

5.2.4 Evaluation of soil microbial activity (respiration)

Activity of microorganisms in the soil was monitored during the experiment. The rates of respiration in the herbicide-spiked soils for these experiments were measured with an infra-red gas analyzer (Infra-red Gas Analyzer Servomex 1450, Servomex Group Ltd, Crowborough, East Sussex, TN6 3DU, England). There were three replicates for each individual treatment. Soil samples (25 g soil at 50% MWHC) were placed in a special polyethylene tube fitted to a very fine nylon mesh across the bottom of each container to let the air penetrate through the soil samples. Soil samples were incubated with a 20 mL container of deionised water (to minimise the loss of water from soil samples) in plastic containers in closed glass jars placed under dark at a constant temperature (25°C). The jar cap was sealed with a rubber septum. The CO_2 evolved from the soil was measured by injecting a needle connected to the gas analyzer into the glass jar. Headspace gas was withdrawn through the gas analyzer by a vacuum pump until a maximum value on the LCD indicator was reached. The CO_2 measurement in eight standard gas jars was also included during each measurement to achieve the correlation between CO_2 concentration and voltage. All jars were aerated at each extraction time to maintain adequate O_2 levels. Soil humidity was kept constant at 50% MWHC throughout the experiment by adding deionised water to maintain the initial weight of the containers. After closing the jars, the initial CO_2 in the sample jars and standard gas jars was determined for time zero (T_0). The CO_2 evolved from the soil was calculated from the difference between each sample and the previous sampling time. The amounts of CO_2 respired were measured at pre-determined days after incubation. Figure 5.2 shows the experimental setup including the infra-red gas analyzer and the jars used in this study.

Figure 5.2. Image of infra-red gas analyser with the standard jars used in this study to measure CO_2.

5.2.5 Extraction of herbicides from soils

The extraction of imidazolinone herbicides from soils in this experiment was conducted using a method developed by (Seifert et al., 2001). Details of the method for the extraction of imazapyr, imazathapyr and imazaquin from soil are given in Chapter 3, Section 3.2.5.5.

5.2.6 Separation of the herbicide enantiomers by HPLC using a chiral column

The method used for HPLC analysis of imidazolinone enantiomers (see Chapter 3, Section 3.5.2 for details of method) was developed by (Lao and Gan, 2006). The method was optimised as described in Chapter 3 using a Chiralcel OJ [cellulose tri(4-methylbenzoate)] column (250 mm × 4.6 mm I.D., 10 μm particle size). The concentration of herbicide

enantiomers in extracts from the soil samples was calculated using the freshly generated calibration curves for each run.

5.2.7 Quality control

Calibration curves were generated by plotting the peak area against the known concentrations of each enantiomer. Linear regression analyses were performed in Microsoft Excel. Since the baseline resolution of imazaquin enantiomers was not achievable, the peak heights were used for the quantification of the enantiomers of this herbicide rather than peak areas. Representative chromatograms of imazapyr, imazethapyr and imazaquin standards are shown in Figure 5.3. The elution order of five imidazolinone herbicides including imazapyr, imazethapyr and imazaquin herbicides was determined by (Lao and Gan, 2007) and showed the same elution order with the S(-) enantiomer being first eluted, followed by the R(+) enantiomer.

The relative standard deviations (RSD= SD/mean × 100) were calculated at the range of 0.5 - 10 μg mL^{-1}. As shown in Table 5.2, the calibration curves obtained for the S(-) and R(+) enantiomers of imazapyr, imazethapyr and imazaquin were linear ranging from 0.5 to 10 μg mL^{-1} (Table 5.2). The racemic standards of imidazolinone herbicidess were injected every eighth sample to determine the reproducibility in assessing Enantiomer Fractions (EFs). EF values are characterized as: area of the S(-) enantiomer divided by the sum of the areas of the both S(-) and R(+) enantiomers eluting from the chiral column. EF values were used as a descriptor for evaluating enantioselectivty of herbicides (Harner et al., 2000). The peak areas for imazapyr and imazethapyr deviated slightly from the standards injected at the beginning of the batches when soil extracts were injected. Thus, EF values of these

herbicides were adjusted with a correction factor to achieve racemic EF values in samples at time zero. There were no interfering peaks observed in the chromatograms with the extracted soils as shown in Figure 5.3.

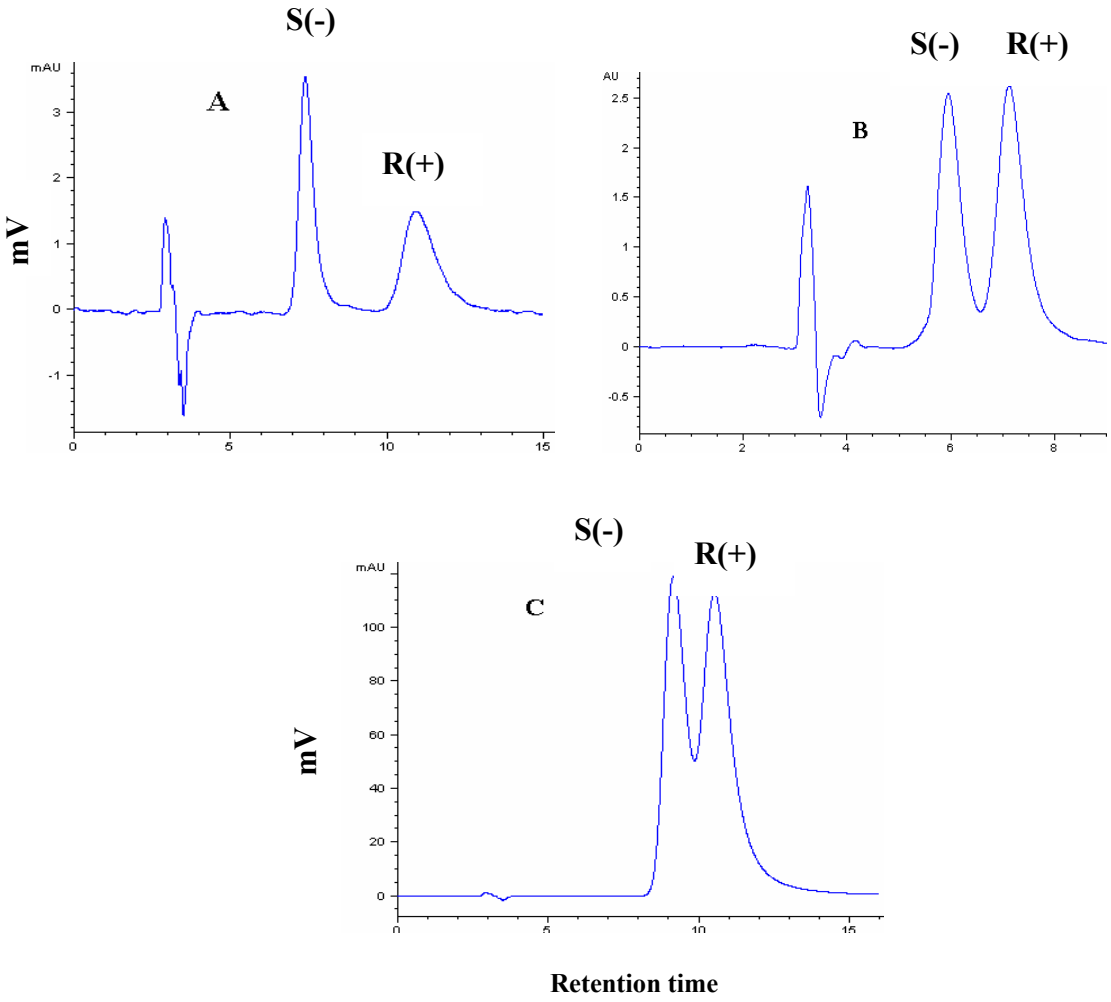

Figure 5.3 Representative HPLC chromatograms of imidazolinone enantiomers separated by the chiral column, (A) imazapyr, (B) imazethapyr, and (C) imazaquin.

Table 5.1. Linearity of the UV detector response and regression coefficients (R^2) for the enantiomers of the three imidazolinone herbicides at 254 nm.

Herbicide	**Enantiomer**	**Equation**[a]	R^2
Imazapyr	S(-)	A= 17.38x - 6.82	0.99
	R(+)	A= 7.397x - 7.39	0.99
Imazethapyr	S(-)	A = 1.8038x - 2.96	0.99
	R(+)	A = 1.8087x - 3.75	0.99
Imazaquin	S(-)	A=0.2964x + 0.43	0.99
	R(+)	A= 0.9009x + 0.42	0.99

[a] A is the peak area/height of enantiomer

Herbicide standard solutions (200 µg mL^{-1}) were prepared in 2-propanol and stored at 4°C in the dark until used. The limit of detection (LOD), based on a signal to noise (standard deviation of the blank sample = background noise) ratio of three, was defined with an accuracy of 20%. In earlier studies (Chapter 3, Section 3.5.1), the LOD was shown to be 0.5 µg mL^{-1} for imazapyr, imazthapyr and imazaquin. There were no significant differences in the background noise between the chromatograms of the standards in 2-propanol and those of the samples. Therefore, the limits of quantification for the samples were considered to be the same as those observed for the standards.

5.2.8 Statistical analyses

The concentration of imidazolinone herbicides remaining (expressed as % of initial concentration) was calculated at each of the sampling times using ln ($C/C_0 \times 100$), where C

is the concentration of the herbicides at each sampling times and C_0 is the initial concentration of the herbicides at time zero (T_0). These percentages were fitted to a first-order curve to obtain the rate constant (k), half-lives ($t_{1/2}$) and coefficient of determination (R^2) of degradation equations. The effect of time, soils, and interaction of soil by time was charactrized using analysis of variance (ANOVA). Data means were compared using least significant difference (LSD) at a confidence level of $p < 0.05$ with Genstat (version 8.2, Rothamsted Experimental Station). GenStat software was also used for multiple linear regression analysis to determine the correlation of soil properties with the degradation rate of herbicide enantiomers and EF data. EF values were calculated as the mean ± SD. Student's paired *t*-test was used to compare the significant differences of the two enantiomers for each herbicide.

5.3 Results and Discussion

5.3.1 Experiment 1: Enantioselective degradation of imazapyr, imazethapyr and imazaquin herbicides in different soils

5.3.1.1 Microbial activity (respiration rate)

Respiration data presented in Figure 5.4 indicated that microorganisms were active in the soils throughout the experiment period. The evolved CO_2 reached a maximum, ranging from 3.06 to 6.72 μg CO_2 g^{-1} soil h^{-1} after 5 days of incubation. Cumulative respiration was highest in AK and CO soils and lowest in CL soils (Figure 5.4). There was no significant difference in cumulative respiration between CL, RC, OT and JA soils.

Multiple linear regression analysis showed that soil respiration had a weak positive correlation with soil pH (p=0.007, R^2=0.16) and stronger positive correlation with organic carbon content (p=0.001, R^2=0.47) (data not shown). Simple regression analyses showed no significant relationship between the soil respiration rate and the rate of degradation of herbicide enantiomers, indicating that soil microbial activity (respiration) may not be a good indicator of the degradation rate of enantiomers of the imidazolinone herbicides considered in this study.

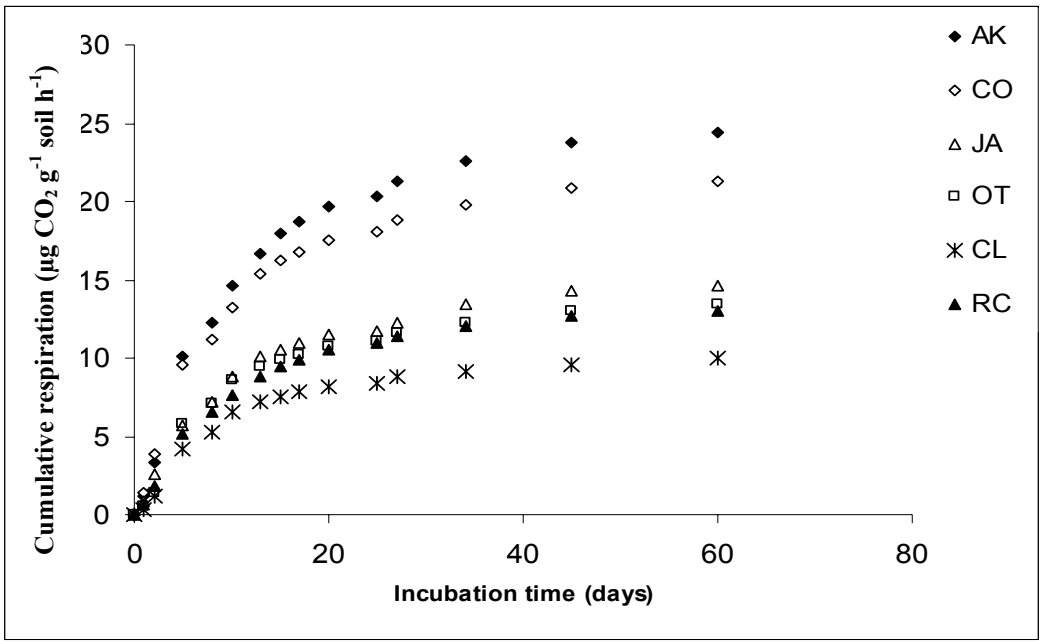

Figure 5.4. Cumulative respiration (µg CO_2 g^{-1} soil h^{-1}) in different soils over 60 days. AK- Alo-Kingaroy; CO-Collie,JA–Jacka; OT-Otterbourne; RC-Roseworthy, CL-Clare.

5.3.1.2 Kinetics of degradation of enantiomers of imazapyr, imazethapyr and imazaquin

An acceptable fit to first-order reaction kinetics was obtained for degradation of the enantiomers of imazapyr, imazathepyr and imazaquin over the incubation time as indicated by regression coefficients ranging from 0.86 to 0.99. However, in some cases, there was a

slight deviation from linearity and a bi-phasic degradation was apparent. This was obvious for the enantiomers of imazapyr and imazethapyr in JA soil (Figures 5.5 and 5.6). The rate constants (day^{-1}) and enantiomer half-lives ($t_{1/2}$) were estimated by fitting the percent $ln(C_0/C)$ against incubation time and are shown in Figures 5.5, 5.6 and 5.7; Tables 5.3, 5.4 and 5.5. In all 6 soils investigated, the half-life of S(-) enantiomer was significantly greater ($p < 0.05$) than that of the R(+) enantiomer for imazapyr, imazethapyr and imazaquin (Tables 5.3, 5.4 and 5.5). For these soils the half-life for the two imazapyr enantiomers ranged from 22.1 to 49.5 days (Table 5.3). This is comparable with the values previously reported in the literature for racemic imazapyr. The half-lives of racemic imazapyr in different soils have been reported to be 25 to 58 days (Azzouzi et al., 1998), 19 to 22 days (Ismail and Ahmad, 1994), 22.5 to 35.7 days (Wang et al., 2005), and 26 to 44 days (Wang et al., 2006).

The degradation rate of the imazethapyr enantiomers was somewhat different with that of the imazapyr enantiomers in identical soils. In this study, the half-life values of imazethapyr enantiomers ranged from 27.3 to 42.8 days in different soils (Table 5.4), whereas the values reported in the literature for racemic imazethapyr are 30 days under laboratory conditions (Flint and Witt, 1997), and 2.6 to 10.6 months in field conditions (Goetz et al., 1990). In another investigation under field conditions the half-life of racemic imazethapyr was 60 days (Mills and Witt, 1989). For the 6 different soils used in this study, the half-life of imazaquin enantiomers ranged from 18 to 49.2 days (Table 5.5), which was consistent with half-life values previously reported in the literature for racemic imazaquin, which ranged from 30 to 60 days (Ahrens, 1994; Baughman and Shaw, 1996; Mills and Witt, 1991; Mills and Witt, 1989). There is no data in the literature on the half-lives of the individual imidazolinone enantiomers in soil.

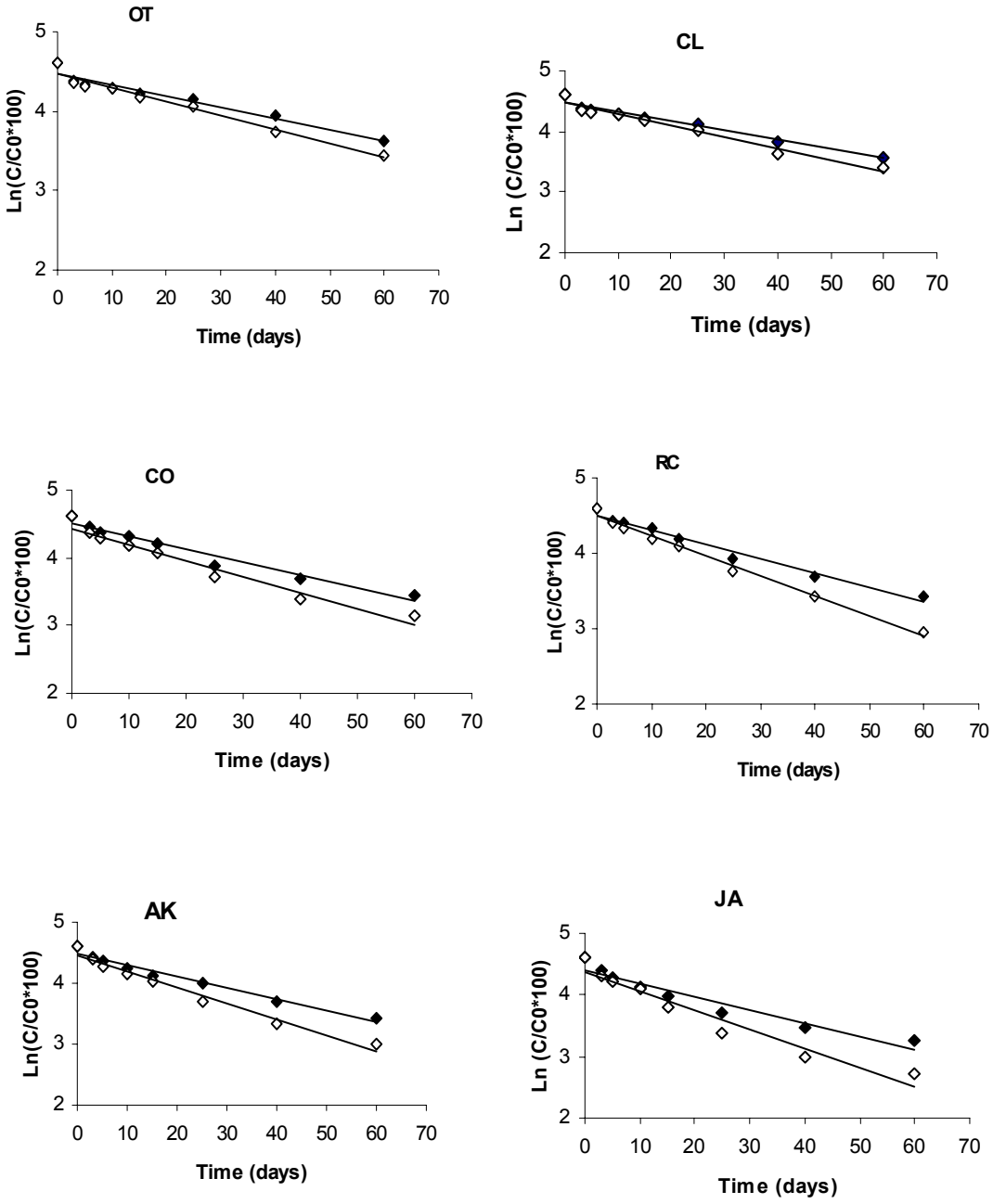

Figure 5.5. Degradation kinetics for (♦) S(-) and (◊) R(+) enantiomers of imazapyr in 6 different soils. C: unknown concentration of the enantiomers at sampling times(t); C_0: the initial concentration at time zero (T_0), AK-Alo-Kingaroy, CO-Collie, JA–Jacka, OT-Otterbourne, RC-Roseworthy, CL-Clare.

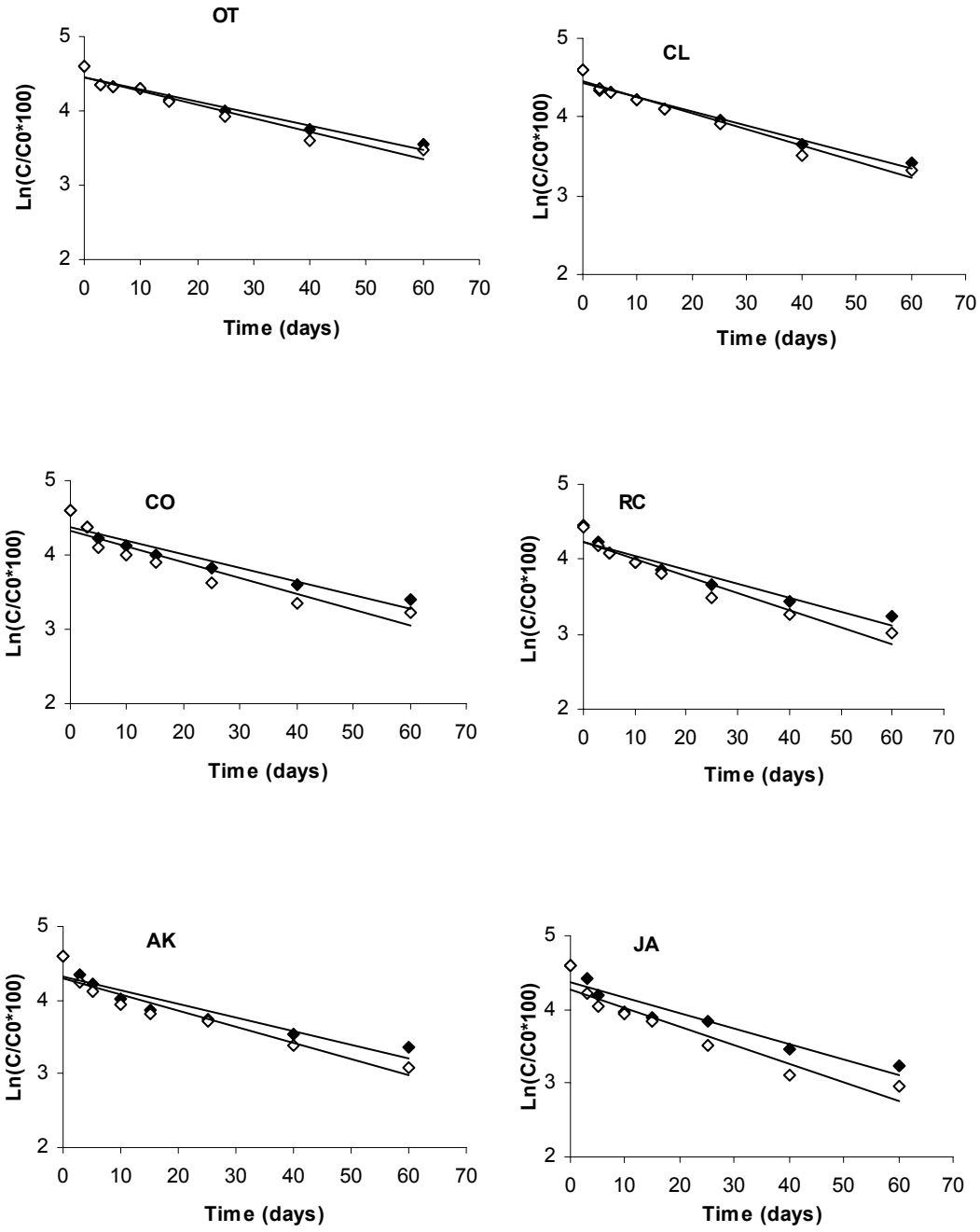

Figure 5.6. Degradation kinetics for (♦) S(-) and (◊) R(+) enantiomers of imazethapyr in 6 different soils. C: concentration of the enantiomers at sampling times (t) and C_0: the initial concentration at time zero (T_0), AK-Alo-Kingaroy; CO-Collie, JA–Jacka; OT-Otterbourne; RC-Roseworthy, CL-Clare.

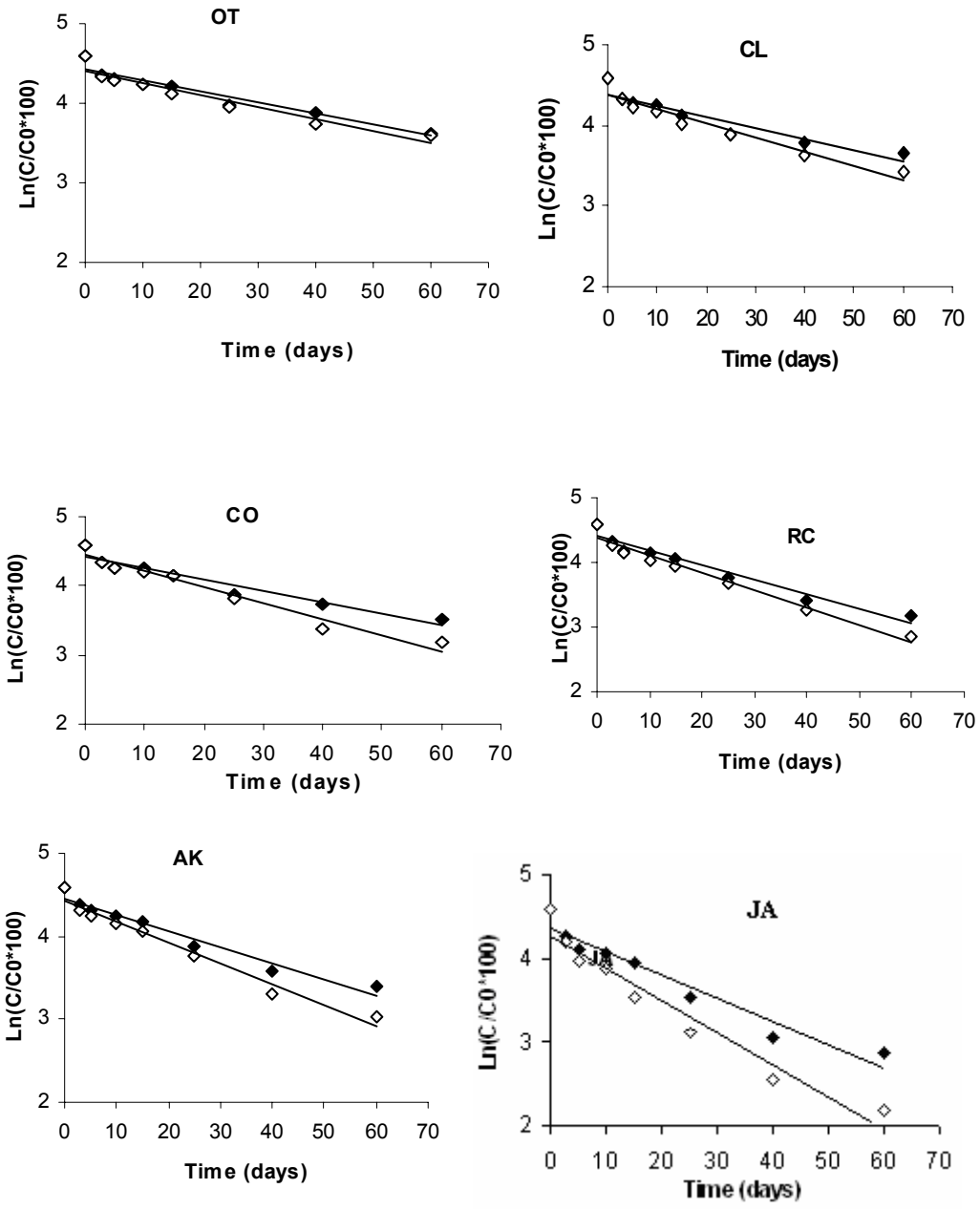

Figure 5.7. Degradation kinetics for (♦) S(-) and (◊) R(+) enantiomers of imazaquin in 6 different soils. C: unknown concentration of the enantiomers at sampling times(t) and C_0: the initial concentration at time zero (T_0), AK-Alo-Kingaroy, CO-Collie, JA–Jacka, OT-Otterbourne, RC-Roseworthy, CL-Clare.

Table 5.2. First-order rate constants (k), half-lives ($t_{1/2}$) and correlation coefficient (R^2) values as derived from the regression models for the degradation of imazapyr enantiomers in different soils.

Soil	**Enantiomer**	**Degradation parameters (n=3)**		
		$t_{1/2}$ **(day)**[a]	k**(day^{-1})**	R^2
Otterbourne (OT)	S(-)	49.5 a	0.014	0.95
	R(+)	39.4 b	0.017	0.97
Clare (CL)	S(-)	44.7 a	0.015	0.96
	R(+)	36.7 b	0.018	0.96
Collie (CO)	S(-)	36.3 a	0.019	0.96
	R(+)	29.1 b	0.023	0.96
Roseworthy (RC)	S(-)	35.9 a	0.019	0.97
	R(+)	26 b	0.026	0.99
Alo-Kingaroy (AK)	S(-)	36.9 a	0.018	0.97
	R(+)	26.6 b	0.026	0.97
Jacka (JA)	S(-)	32.5 a	0.009	0.92
	R(+)	22.1 b	0.031	0.94

[a] Statistical significant differences ($p < 0.05$, Student's pair t-test) within the same soil type are shown with different letters.

Table 5.3. First-order rate constants (k), half-lives ($t_{1/2}$) and correlation coefficient (R^2) values as derived from the regression lines for the degradation of imazethapyr enantiomers in different soils.

Soil	**Enantiomer**	**Degradation parameters (n=3)**		
		$t_{1/2}$ **(day)**[a]	k**(day^{-1})**	R^2
Otterbourne (OT)	S(-)	42.8 a	0.016	0.95
	R(+)	38.5 b	0.018	0.93
Clare (CL)	S(-)	38.5 a	0.018	0.95
	R(+)	33.8 b	0.020	0.96
Collie (CO)	S(-)	37.9 a	0.018	0.91
	R(+)	32.5 b	0.021	0.86
Roseworthy (RC)	S(-)	37.5 a	0.018	0.91
	R(+)	30.8 b	0.022	0.93
Alo-Kingaroy (AK)	S(-)	37.3 a	0.018	0.86
	R(+)	31.9 b	0.021	0.90
Jacka (JA)	S(-)	33.2 a	0.020	0.89
	R(+)	27.3 b	0.025	0.90

[a] Statistical significant differences ($p < 0.05$, Student's pair t-test) within the same soil type are shown with different letters.

Table 5.4. First-order rate constants (k), half-lives ($t_{1/2}$) and correlation coefficient (R^2) values as derived from the regression lines for the degradation of imazaquin enantiomers in different soils.

Soil	Enantiomer	Degradation parameters (n=3)		
		$t_{1/2}$ (day) [a]	k(day^{-1})	R^2
Otterbourne (OT)	S(-)	49.2 a	0.014	0.93
	R(+)	45.6 b	0.015	0.91
Clare (CL)	S(-)	49.5 a	0.014	0.87
	R(+)	39.6 b	0.017	0.92
Collie (CO)	S(-)	42.3 a	0.016	0.93
	R(+)	29.7 b	0.023	0.96
Roseworthy (RC)	S(-)	31.4 a	0.022	0.94
	R(+)	26.1 b	0.026	0.96
Alo-Kingaroy (AK)	S(-)	35.9 a	0.019	0.95
	R(+)	27.6 b	0.025	0.96
Jacka (JA)	S(-)	24.8 a	0.028	0.93
	R(+)	18.0 b	0.038	0.94

[a] Statistical significant differences ($p < 0.05$, Student's pair t-test) within the same soil type are shown with different letters.

5.3.1.3 Enantioselective degradation of imazapyr

The R(+) enantiomer of imazapyr degraded more rapidly than the S(-) enantiomer in all soils (Figure 5.5). The half-lives of the two enantiomers of imazapyr were significantly different ($p < 0.05$) in all soils. Therefore, the EF value of this herbicide was above

EF=0.50 over the experimental period. Both enantiomers of imazapyr degraded much faster in the Jacka (JA) soil with half-lives of 22.1 and 32.5 days for S(-) and R(+) enantiomers, respectively, compared with the other soils. Degradation of imazapyr enantiomers was also relatively rapid in the AK and RC soils. The slowest degradation rate of imazapyr enantiomers was observed in the OT soil with values of 49.5 and 39.4 days for S(-) and R(+) enantiomers, respectively (Table 5.3).

The EF value for imazapyr consistently increased with time of incubation (Figure 5.8). Irrespective of the soil type, the EF for imazapyr increased from the initial 0.50 to 0.59 after 60 days. Assuming the first-order kinetics mechanism for the degradation of enantiomers of imazapyr with the rate constants $k_{(S)}$ and $k_{(R)}$ for the S(-) and R(+) enantiomers, respectively, the following equation can be used to describe EF values as a function of incubation times:

$$ln(\mathrm{EF}) = ln(\mathrm{EF0}) + \Delta k. \qquad \text{equation (5.1)}$$

where EF_0 is the initial value of EF and Δk is the differences between $k_{(S)}$ and $k_{(R)}$. This equation shows the rate at which EF values deviate from initial EF values. This function can be used in its linear form after logarithmic transformation of EF values to determine the differences between enantioselectivity rate versus incubation time. In the case of imazapyr, the relationship of *ln* transformed EF values versus time was bi-phasic with an initial phase rate constant k=0.0069 for the first 5 days compared with k=0.0029 for the second phase from days 10 to 60, indicating the rate of enantioselectivity change during the initial phase is 2.4 times greater than in the second phase (Figure 5.8). The decrease in enantioselectivity during the second phase may be the result of decreased soil microbial

activity. A similar pattern of rapid change in enantiomer ratio (ER) followed by a phase of slower change at later incubation time was also observed for some chiral insecticides (Liu et al., 2005b). These authors suggested that the slower change in ER in the later phase of incubation compared with the initial phase could be due to early depletion of the more accessible portion of the insecticides.

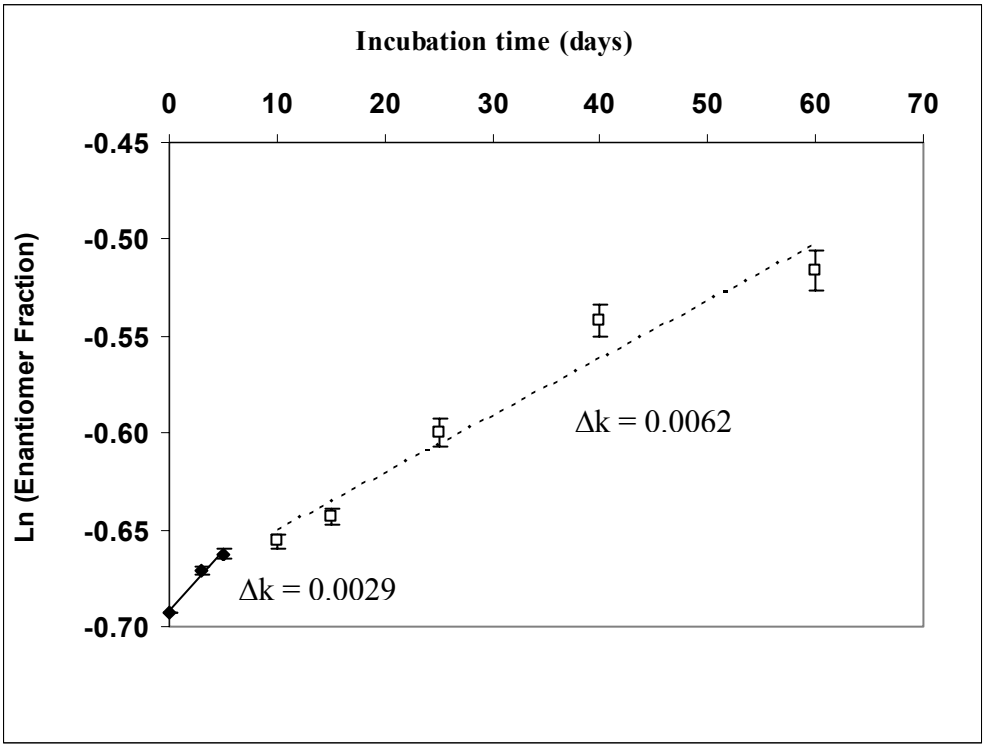

Figure 5.8. Relationship of *ln* of EF values for imazapyr versus incubation time. Vertical bars are standard errors of means (n=18).

5.3.1.4 Enantioselectivity in imazethapyr

The R(+) enantiomer of imazethapyr was also preferentially degraded in all soils compared with the S(-) enantiomer, with EF values ranging from 0.52 to 0.57 (Table 5.6). The degradation rate of the imazethapyr enantiomers was somewhat slower than that of imazapyr in the same soils, although the order of imazethapyr degradation rate among soils

was essentially the same. The general ranking of half-lives of imazethapyr enantiomers, from low to high, in the soils studied was JA < AK< RC < CO < CL< OT soils. This order was similar to that for imazapyr, showing that the factors affecting the degradation of enantiomers of both herbicides in these soils are similar. The EF value of imazethapyr was significantly different from racemic (EF=0.55-0.57) in all soils except OT and CL, where it was similar to racemic (EF=0.52) (Table 5.6). There was an increase in EF value for imazethapyr over time following a biphasic response. The rate of increase for the initial phase was 3.4 times greater than that of the second phase (Figure 5.9).

Table 5.5. Enantiomer fraction values of imazapyr, imazethapyr and imazaquin in different soils.

Soil	pH	Herbicide EF value (n=24) * [a]		
		Imazapyr	**Imazethapyr**	**Imazaquin**
OT	5.02	0.51^{nsA}	0.52^{nsA}	0.51^{nsA}
CL	5.20	0.52^{nsA}	0.52^{nsA}	0.51^{nsA}
CO	6.07	0.53^{*B}	0.55^{*B}	0.52^{*B}
RC	8.20	0.54^{*B}	0.54^{*B}	0.52^{*B}
AK	8.70	0.54^{*B}	0.54^{*B}	0.53^{*C}
JA	7.60	0.55^{*C}	0.57^{*C}	0.56^{*D}

*Shows significant differences of EF values from racemic at the level of $p < 0.05$; ns=no significant difference. [a]Values in the same column followed by the same letter are not significantly different based on LSD at $p < 0.05$. AK-Alo- Kingaroy; CO-Collie, JA–Jacka; OT-Otterbourne; RC-Roseworthy, CL-Clare.

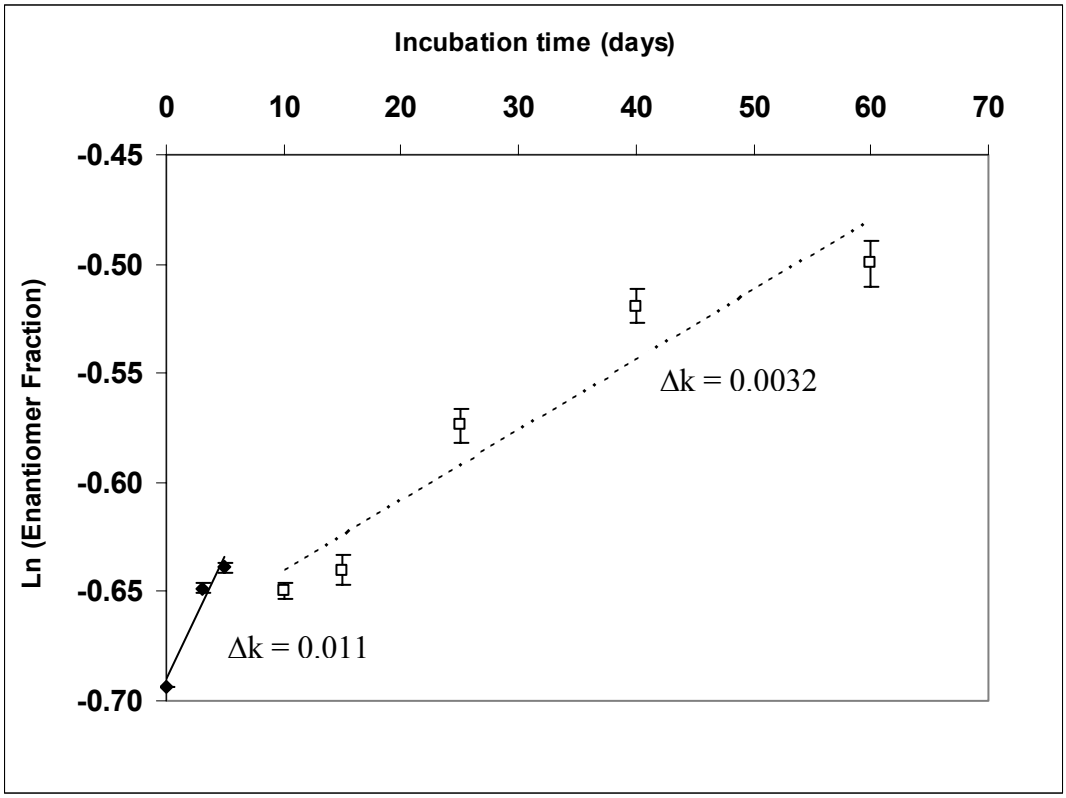

Figure 5.9. Relationship of *ln* of EF values for imazethapyr versus incubation time. Vertical bars are standard errors of means (n=18).

5.3.1.5 Enantioselectivity in imazaquin

The R(+) enantiomer of imazaquin was preferentially degraded in most soils. The EF values for imazaquin in different soils ranged from 0.50 to 0.56. There was no significant difference ($p < 0.05$) between the EF values for CL, OT CO, and RC soils (Table 5.6), while the EF values for the AK and JA soils (0.53 and 0.56, respectively), were significantly different ($p < 0.05$). As with the other two imidazolinone herbicides, enantioselectivity was found to be the most pronounced (0.56) for the JA soil compared with the other soils. As with imazapyr and imazethapyr, the EF values for imazaquin increased steadily in a bi-phasic pattern from the initial 0.50 to 0.57 over the 60 days incubation period (data not shown). The initial phase lasted for 15 days and was 2.15 times faster than the second phase (Figure 5.10). For the imazaquin, the half-life of the S(-)

enantiomer ranged from 24.8 to 49.5 days, while the half-life of the R(+) enantiomer ranged from 18.0 to 45.6 days (Table 5.5).

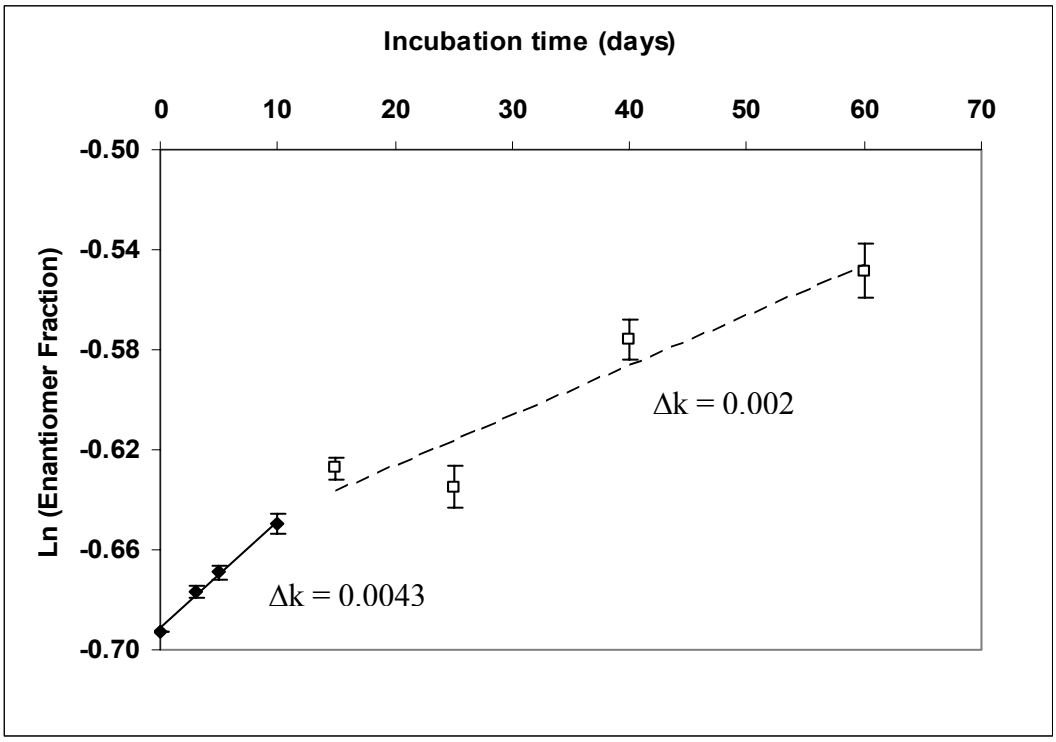

Figure 5.10. Relationship of *ln* of EF values for imazaquin versus incubation time. Vertical bars are standard errors of means (n=18).

5.3.1.6 Effect of soil characteristics on enantioselective degradation of the herbicides

The degradation of imazapyr, imazethapyr and imazaquin in this study showed no lag phase and fitted the first order model with significant correlation coeffecients in all soils. The absence of a lag phase is normally attributed to the involvement of a consortium of microbial species in the degradation of pesticides (Kah et al., 2007). The possible relationship between soil properties including soil pH, microbial activity as indicated by respiration rate, organic carbon and clay content with enantiomer degradation rate as well as EF values of the three herbicides were analysed by using multiple linear regression. Despite the limited soil pH range investigated, the analysis showed soil pH to be the main

soil characteristic correlating with the degradation rate of both enantiomers of all three herbicides. For both enantiomers of all three herbicides the half-lives decreased with increasing pH from 5.0 to 6.1. At the higher pH values there was a slight increase in half-lives. The half-life of the imazapyr and imazethapyr S(-) and R(+) enantiomers decreased from pH 5.0 to 7.6 and then increased at the highest soil pH of 8.7. The effect of soil pH on the half-life of imazaquin enantiomers was even more pronounced (Figure 5.11). It has been previously determined that soil pH was one of the important factors affecting the degradation of imazapyr under aerobic conditions (Wang et al., 2006). There was no significant relationship between other soil properties and the half-lives of imazapyr, imazethapyr and imazaquin enantiomers.

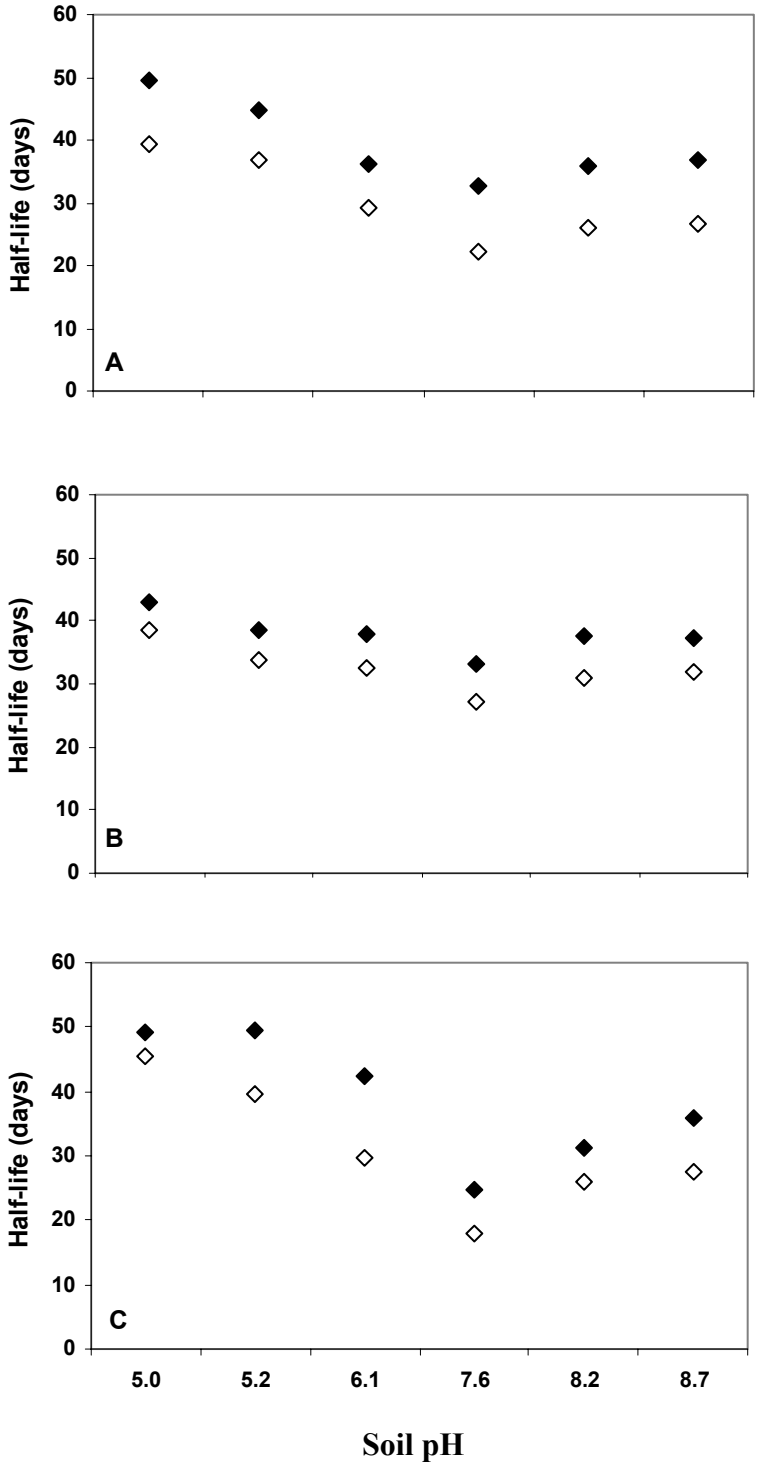

Figure 5.11. The effect of soil pH on half-lives of (♦) S(-) and (◊) R(+)enantiomers of imazapyr (A), imazethapyr (B) and imazaquin (C) in 6 soils.

Significant positive correlations of EF values of imazapyr ($p < 0.001$, $R^2 = 0.41$), imazethapyr ($p < 0.002$, $R^2 = 0.47$) and imazaquin ($p < 0.001$, $R^2 = 0.54$) were found over the soil pH range from 5.02 to 7.6. A similar effect of soil pH on the degradation of racemic imazapyr has been observed by other researchers (Wang et al., 2005; Wang et al., 2006). It has been reported that soil pH can affect the structure of soil microbial communities including fungus:bacteria ratio and the distribution of functional and taxonomic groups (Bending and Sonia Rodriguez-Cruz1, 2007). A higher degradation rate of insecticides, dicarboximide fungicides, substituted urea herbicides and triazine herbicides with increasing pH has been demonstrated by (Singh et al., 2003). For ionizable herbicides such as imidazolinones, an increase in pH may enhance degradation due to decreased strength of sorption and higher bioavailability of these herbicides at higher values (Kah and Brown, 2006). (Aichele and Donald, 2005) showed that imazamox, imazethapyr, and imazaquin degraded faster in soil at pH 7 than pH 5. Increased herbicide sorption at pH 5 and lesser bioavailabity in the soil solution is likely to decrease the extent of microbial degradation of imidazolinone herbicides (Curran et al., 1992a).

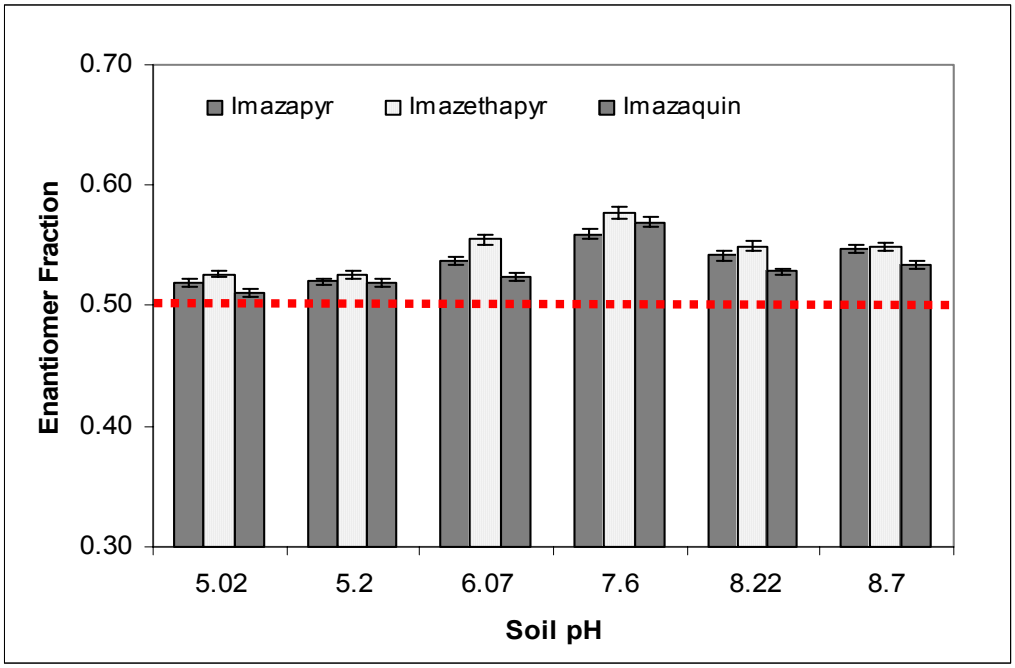

Figure 5.12. The average EF values of imazapyr, imazethapyr and imazaquin over the whole incubation period in 6 soils of different pH values.Vertical bars show the standard errors of means (n=24).

5.3.2 Experiment 2: Degradation of imazethapyr and imazaquin enantiomers in sterilized versus non-sterilized soils

Both enantiomers of imazethapyr and imazaquin degraded significantly ($p < 0.05$) faster in active (non-sterilized) soils as compared with that in the sterilized soils. This shows that sterilization of the soils inhibited degradation and that degradation of the enantiomers of both herbicides was mainly a result of microbial degradation (Figure 5.13). There were negligible differences (Figure 5.13) between degradation rates of S(-) and R(+) enantiomers of both herbicides in sterilized soils. Sterilization caused a 4 to 8-fold increase in half-lives of the S(-) and R(+) enantiomers of imazethapyr and imazaquin compared with non-sterilized soils (data not shown). The small amount of degradation of both enantiomers in the sterile soils could be either due to incomplete sterilization of soils by

autoclaving or contamination of soil samples during the incubation period. Imidazolinone herbicides are known to be resilient to chemical degradation (as shown in Chapter 4) and photodegradation would have been prevented since this experiment was conducted in the dark.

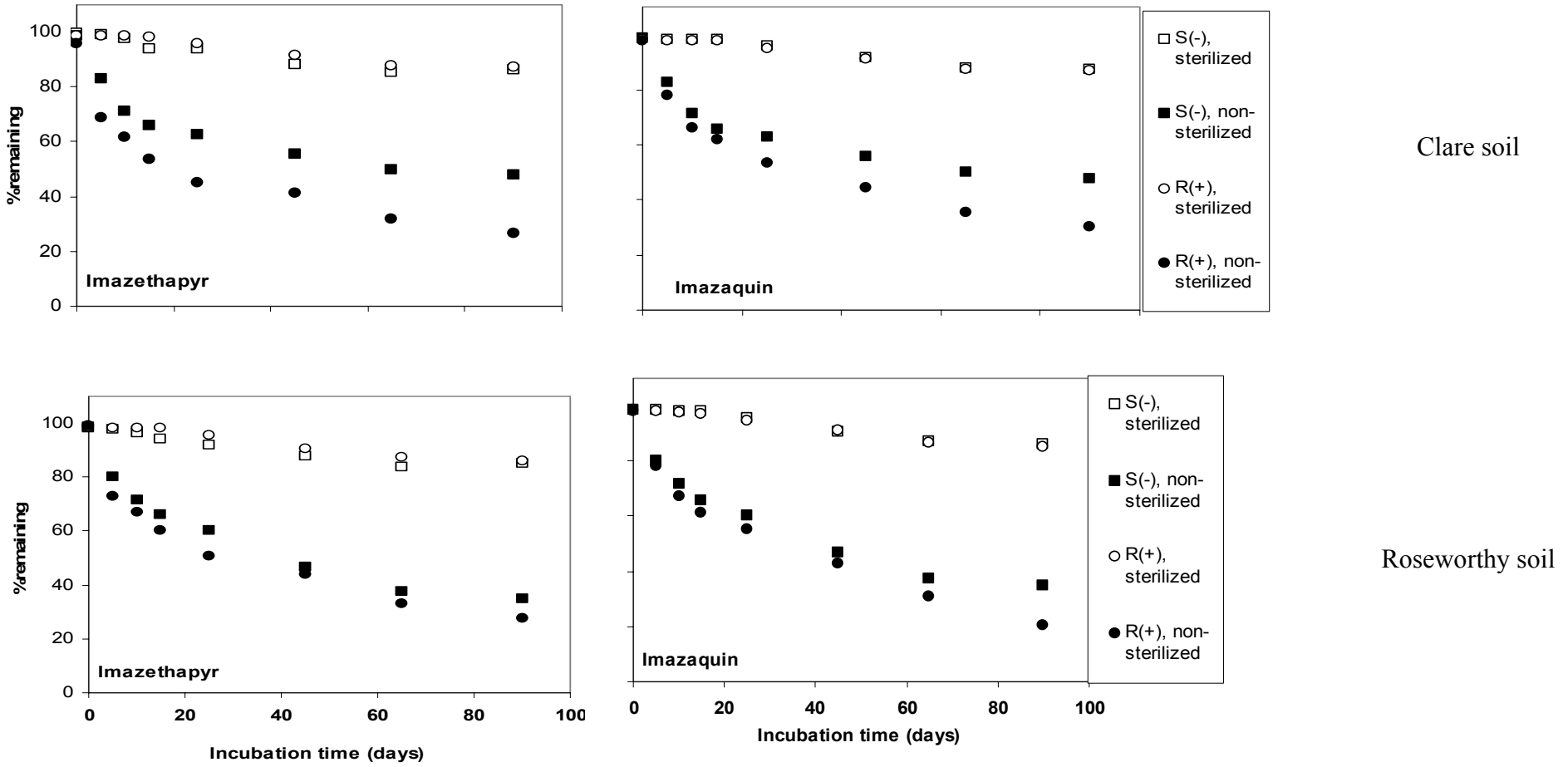

Figure 5.13. Percent of imazethapyr and imazaquin enantiomers remaining in Clare and Roseworthy soil under sterilized and non-sterilized conditions

5.3.3 Experiment 3. Selective degradation of enantiomers of imazethapyr and imazaquin herbicides in soil: effect of organic amendment

5.3.3.1 Microbial activity of soils

The respiration rate of Roseworthy and Clare soils was not significantly different when no plant residue was added to these soils (Table 5.7). When lupin residue (2%, w/w) was added to the soils cumulative soil respiration (μg CO_2 g^{-1} soil h^{-1}) increased significantly ($p < 0.05$) in both soils but the response was much greater (116%) in Clare soil (Table 5.7). Irrespective of soil type, the CO_2 respired decreased over the incubation time (Figure 5.14). The respired CO_2 in sterilized treatments was negligible during the first 30 days of the incubation, but there was some respiration from the sterilized soils after one month of incubation (data not shown), which would suggest that sterilization of the soils was only effective for the first 30 days. It is almost impossible to completely sterilize soils even after 2-3 repeated autoclave cycles (Qin et al., 2006). It is also possible that contamination of incubated soil could have occurred during sampling, which may have revived microbial activity in sterilized soil samples.

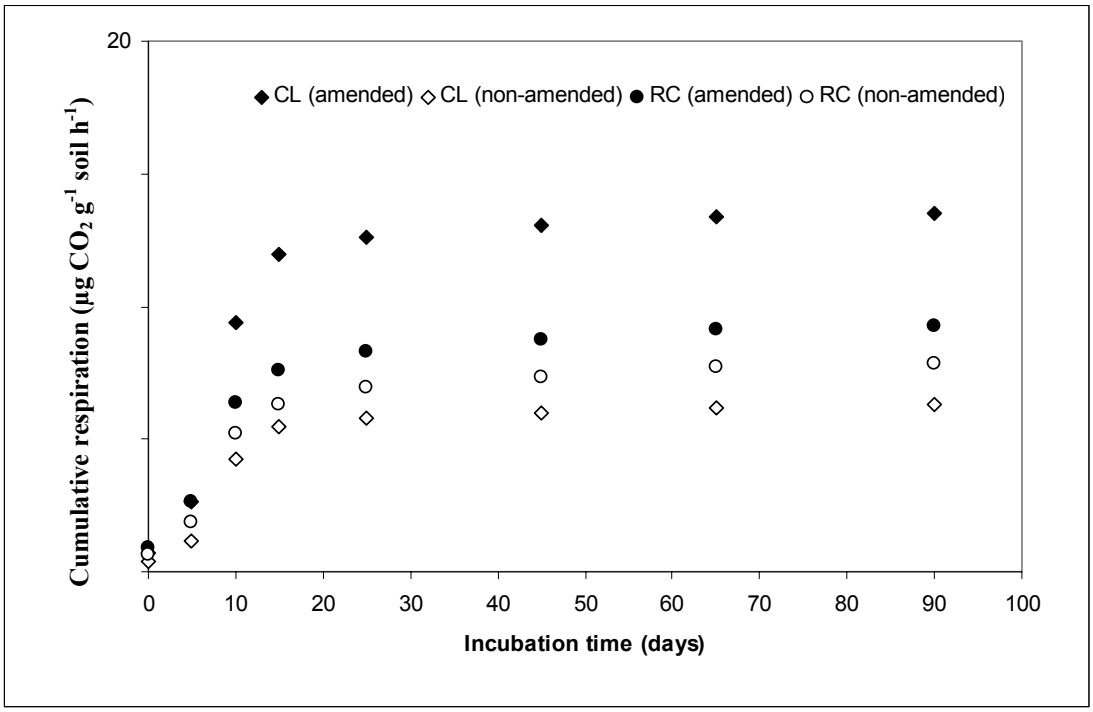

Figure 5.14. Cumulative respiration (µg CO_2 g^{-1} soil h^{-1}) over 90 days incubation from Clare (CL) and Roseworthy (RC) soils with and without organic amendment.

Table 5.6. Cumulative respiration (µg CO_2 g^{-1} soil h^{-1}) of Clare and Roseworthy soils with or without addition of lupin residue after 90 days incubation.

Soil	**Treatment**	**Cumulative respiration (µg CO_2 g^{-1} soil h^{-1})[a]**
Clare	Non-amended	6.2a
	Organic amended	13.4c
Roseworthy	Non-amended	7.8a
	Organic amended	9.2b

[a] Mean values within column followed by the same letter are not significantly different based on LSD at p <0.05

5.3.3.2 Effect of organic amendment on degradation rate of imazethapyr and imazaquin enantiomers

The degradation of the S(-) and R(+) enantiomers of imazethapyr and imazaquin in both the amended and non-amended soils followed first-order kinetics. The half-lives and rate constants were estimated by linear regression analysis for each herbicide enantiomer. In the absence of the organic amendment, the half-lives of the S(-) and R(+) enantiomers of imazethapyr were 90.0 and 64.2 days, respectively, in the Clare soil and 77.9 and 51.0 days, respectively, in the Roseworthy soil (Table 5.8). The half-lives of S(-) and R(+) enantiomers of imazaquin were 105.0 and 72.2 days, respectively, in the Clare soil and 63.0 and 43.3 days, respectively, in the Roseworthy soil (Table 5.9). For both herbicides the half-life of the R(+) enantiomer was significantly ($p < 0.05$) shorter than the S(-) enantiomers in all soil and treatment combinations.

Shorter half-lives of both enantiomers of these two herbicides in a previous study (Experiment 1) compared with the values obtained in this experiment could be related to differences in the incubation conditions particularly the temperature effect on degradation. Experiment 1 was run at 30 ^{o}C while this experiment was run at 25 ^{o}C. This is the first report of differences between enantiomer half-lives for imidazolinone herbicides.

The amount (%) of imazethapyr and imazaquin enantiomers remaining in Clare and Roseworthy soils at the end of incubation time (day 90) with and without organic amendment are summarized in Table 5.10. The degradation rates of the S(-) and R(+) enantiomers of imazethapyr and imazaquin were significantly different ($p < 0.05$) under amended and non-amended conditions for both soils. When there was no amendment in the Clare soil, 44.1 and 32.6% of the S(-) and R(+) enantiomers of imazethapyr remained after

90 days, respectively (Table 5. 10). Addition of lupin residue to the Clare soil significantly increased ($p < 0.05$) the degradation rate of R(+) enantiomer of imazethapyr relative to the non-amended soils. The amount of R(+) enantiomer of imazethapyr remaining after 90 days incubation time was 20.5% with the half-life of 48.5 days in the amended Clare soil compared with 32.6% with the half-life value 64.2 days in non-amended Clare soil (Tables 5.8 and 5.10). For imazethapyr in the Roseworthy soil, 30.7% of the R(+)enantiomer remained after 90 days with a half-life of 58.2 days in the amended soil compared with 24.6% with the half-life of 51 days in the non-amended soil (Tables 5.8 and 5.10). The results showed that the addition of organic amendment had a positive impact on degradation rate of R(+) enantiomer of imazethapyr in Clare soil and a negative effect in the Roseworthy soil. No significant difference was observed in degradation rate of the S(-) enantiomer of imazethapyr as a result of organic amendment to both soils (Table 5.10).

The degradation rate of imazaquin enantiomers was also different with 50.8 and 38.7% of initial herbicide remaining at the end of incubation period in the non-amended Clare soil for the S(-) and R(+) enantiomers, respectively (Table 5.10). In the non-amended Roseworthy soil, 35.8% of the S(-) enantiomer and 22.7% of the R(+) enantiomer of imazaquin remained after 90 days. The degradation rate of the enantiomers of imazaquin was also significantly different ($p < 0.05$) in amended soils, which indicates the preferential degradation of R(+) enantiomer of both herbicides in Clare and Roseworthy soils with and without amendment. Organic amendment significantly ($p < 0.05$) increased the degradation rate of R(+) enantiomer of imazaquin in the Clare but not in the Roseworthy soil. These findings indicate that the organic amendment used in this experiment had no distinct impact on the degradation of S(-) and R(+) enantiomers of imazaquin or the S(-) enantiomer of

imazethapyr in Roseworthy soil. Thus, the effect of organic amendment on degradation of imidazolinone herbicides is both compound-specific and affected by soil type.

Table 5.7. First-order rate constants (k), half-lives ($t_{1/2}$) and correlation coefficient (R^2) values derived from linear regression for the degradation of imazethapyr enantiomers in amended and non-amended soils.

Soil	**Treatment**	**Enantiomer**	**Imazethapyr**		
			$t_{1/2}$ (day)[a]	k(day^{-1})	R^2
Clare	Amended	S(-)	81.5 a	0.008	0.91
		R(+)	48.5 b	0.014	0.92
	Non-amended	S(-)	90.0 a	0.007	0.85
		R(+)	64.2 b	0.010	0.93
Roseworthy	Amended	S(-)	85.6 a	0.008	0.81
		R(+)	58.2 b	0.011	0.91
	Non-amended	S(-)	77.9 a	0.008	0.86
		R(+)	51.0 b	0.013	0.94

[a]Statistical significant differences ($p < 0.05$, Student's pair t-test) within the same soil type are shown with different letters.

Table 5.8. First-order rate constants (k), half-lives ($t_{1/2}$) and correlation coefficient (R^2) values as derived from the linear regression for the degradation of imazaquin enantiomers in amended and non-amended soils.

Soil	**Treatment**	**Enantiomer**	**Imazethapyr**		
			$t_{1/2}$ (day)[a]	k(day^{-1})	R^2
Clare	Amended	S(-)	92.4 a	0.007	0.92
		R(+)	45.9 b	0.015	0.93
	Non-amended	S(-)	105.0 a	0.006	0.86
		R(+)	72.2 b	0.009	0.91
Roseworthy	Amended	S(-)	65.4 a	0.010	0.90
		R(+)	47.5 b	0.014	0.96
	Non-amended	S(-)	63.0 a	0.011	0.93
		R(+)	43.3 b	0.016	0.97

[a]Statistical significant differences ($p < 0.05$, Student's pair t-test) within the same soil type are shown with different letters.

Table 5.9. Imazethapyr and imazaquin enantiomers remaining (%) in Clare and Roseworthy soils with and without organic amendment after 90 days of incubation.

Soil	**Treatment**	**Imazethapyr (% remaining)[a]**	
		S(-)	R(+)
Clare	Amended	40.2 aA	20.5 bA
	Non-amended	44.1 aA	32.6 bB
Roseworthy	Amended	40.5 aA	30.7 bB
	Non-amended	38.6 aA	24.6 bA
		Imazaquin (% remaining)	
		S(-)	R(+)
Clare	Amended	44.7 aA	21.6 bA
	Non-amended	50.8 aB	38.7 bB
Roseworthy	Amended	34.9 aC	20.3 bA
	Non-amended	35.8 aC	22.7 bC

[a] Values in the same column and row followed by different letters are significantly different at $p < 0.05$ by LSD. Means in each row and column followed by different letters are significantly different ($p< 0.05$). Small and capital letters show statistical differences for data in rows and columns, respectively.

5.3.3.3 Effect of organic amendment on soil properties

In this experiment, the addition of lupin residue increased the pH of the Clare soil by 0.9 units to pH 6.1 at the end of incubation time (Table 5.11). In contrast, the change of pH value of Roseworthy soil was negligible. This finding is consistent with literature about the effects

of organic amendment on soil pH. Previous research has shown the addition of plant residues may increase pH which may increase microbial activity particularly in acidic soils (Clark et al., 2007; Xu et al., 2006a; Xu et al., 2006b).

Table 5.10. The effect of organic amendment on soil pH over the incubation times.

Soil	Treatment	Initial pH[a]	Measured pH after incubation time (days)		
			5	65	90
Clare	Non-amended	5.2	5.2	5.2	5.2
	Amended	5.2	6	6.2	6.1
Roseworthy	Non-amended	8.2	8.2	8.2	8.2
	Amended	8.2	8.3	8.3	8.2

[a]Soil pH values were measured in a 1: 5 soil: water extract

The previous experiment showed that the rate of degradation of imazethapyr and imazaquin enantiomers was correlated with soil pH (Experiment 1, Section 5.4.1.6), which is in agreement with the known effect of soil pH on microbial activity (Motavalli et al., 1995). The greater influence of organic amendment on the degradation of the R(+) enantiomers of both imazethapyr and imazaquin in the Clare soil compared with the Roseworthy soil may be related to the increase of soil pH, and consequently microbial activity, after the addition of lupin residue. Organic amendment of the Clare soil significantly ($p < 0.05$) increased the respiration rate (> 2 times) over that of the non-amended soil, while no significant increase of respiration rate was observed in the Roseworthy soil following organic amendment (Table 5.11). The increased pH in Clare soil may also have contributed to greater bioavailability of

the compounds to microorganisms as sorption of compounds is expected to decrease with increases in pH.

5.3.3.4 Enantioselective degradation of imazethapyr and imazaquin

To compare the enantioselective degradation of the herbicides in different soils, EF values of both herbicides were calculated as described earlier (in Section 5.2.6) for both non-sterilized as well as sterilized soils. In the sterilized soils, EF values did not deviate from the EF value for a racemic mixture (0.49-0.51) for both imazethapyr and imazaquin (data not shown). By contrast, in all non-sterilized soils, the degradation of imazethapyr and imazaquin was enantioselective. Irrespective of organic amendment, the EF values of imazethapyr increased from the initial value of 0.50 to 0.69 and 0.64 after 90 days of incubation time in Clare and Roseworthy soils, respectively, (Figure 5.15). The variation of EF values of imazethapyr was higher in Clare soil compared with the Roseworthy soil with significant differences ($p <$ 0.05) from the the EF value for a racemic mixture at day 5 after incubation (Table 5.12). For imazaquin, the EF increased from an initial value of 0.50 at day zero to 0.61 and 0.62 at day 90 in Clare and Roseworthy soils, respectively (Figure 5.16). There were smaller differences between Clare and Roseworthy soils in the EF values of imazaquin. These results clearly indicate that the rate of degradation of the R(+) enantiomer was faster than that of the S(-) enantiomer for imazethapyr and imazaquin in both soils.

Table 5.11. EF values of imazethapyr and imazaquin in Clare and Roseworthy soils over the whole incubation period.

Incubation time	**EF value (n=6)**[a]			
(d)	**Clare**		**Roseworthy**	
	Imazethapyr	Imazaquin	Imazethapyr	Imazaquin
0	0.51 a	0.50 a	0.51 a	0.50 a
5	0.55 b	0.51 ab	0.53 ab	0.50 a
10	0.56 b	0.52 ab	0.52 ab	0.51 a
15	0.59 c	0.51ab	0.52 ab	0.52 b
25	0.62 d	0.54 cb	0.53 cb	0.52 b
45	0.62 d	0.55 c	0.56 d	0.52 b
65	0.66 e	0.58 c	0.63 e	0.54 c
90	0.69 e	0.61 d	0.64 e	0.62 d

[a] EF Values in the same column followed by different letters are significantly different at $p < 0.05$ by LSD. EF = (area of S(-) enantiomer)/(area of S(-) + area of R(+) enantiomer))

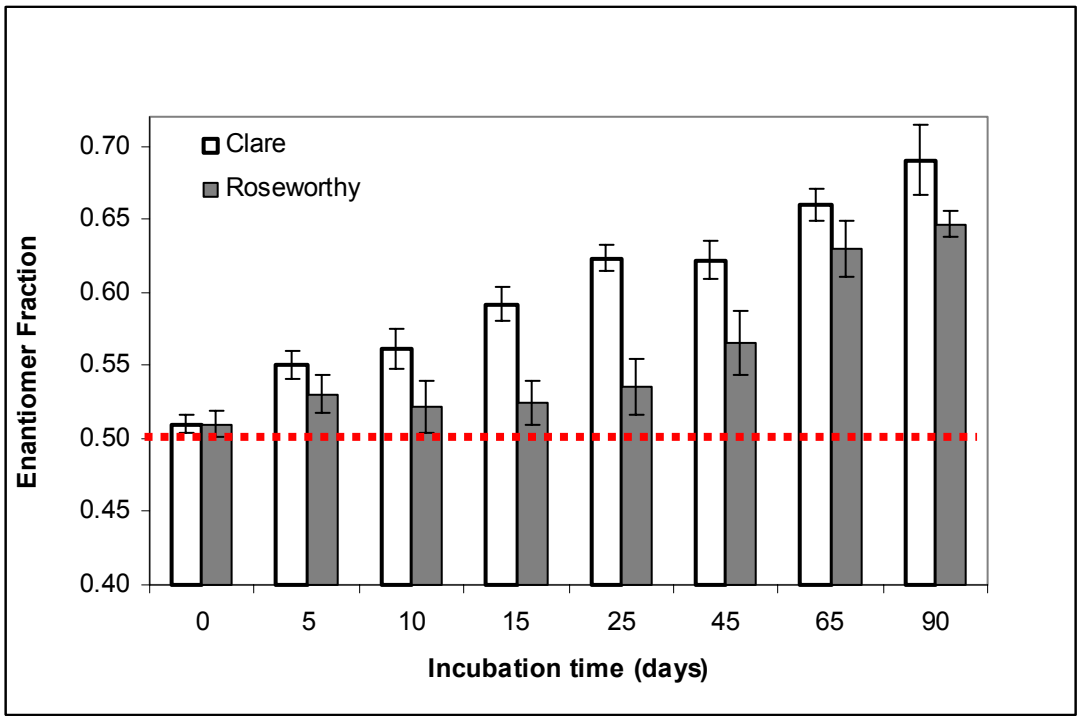

Figure 5.15. The EF values for imazethapyr in Clare and Roseworthy soils over time. Vertical bars are standard errors of means (n=6).

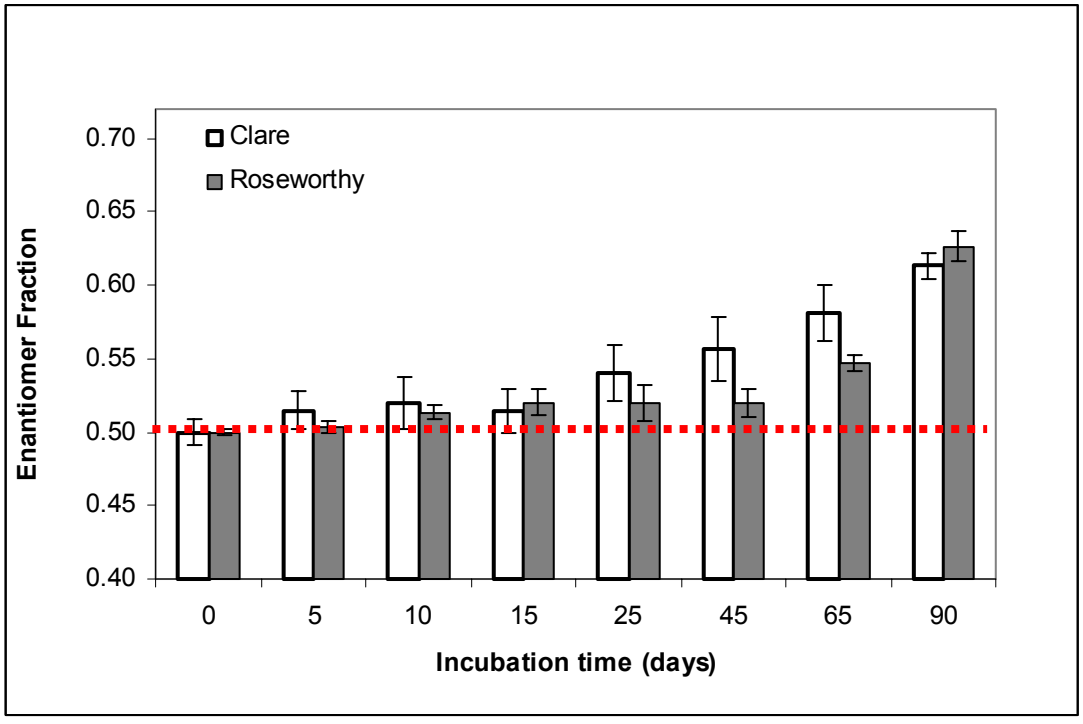

Figure 5.16. The EF values for imazaquin in Clare and Roseworthy soils over time. Vertical bars are standard errors of means (n=6).

5.3.3.5 The effect of organic amendment on enantioselectivity of imazethapyr and imazaquin

With organic amendment of the soils, the variation in enantioselectivity (as indicated by the EF values) was dependent upon the type of soil. The EF values of imazethapyr in the amended Clare soil increased significantly ($p < 0.05$) from the initial value of 0.51 to 0.76 at the end of 90 days incubation period (Figure 5.17, Table 5.13). The greatest influence of amendment in this soil was found at the later phase (> 65 days) of incubation (Figure 5.17). The EF value of imazaquin also increased with organic amendment of the Clare soil from the initial value of 0.50 to 0.66 over the incubation period (Figure 5.18, Table 5.13). By contrast, enantioselectivity of imazethapyr was significantly ($p < 0.05$) inhibited by organic amendment of the Roseworthy soil. The EF value of imazethapyr decreased from 0.68 to 0.61 with organic amendment of the Roseworthy soil. However, organic amendment of the Roseworthy soil appeared to have no effect on EF values for imazaquin. Without organic amendment, the Roseworthy soil has higher potential for microbial activity, which may explain this observation (Table 5.13). In an earlier study (Experiment 1, section 5.4.1.6), there was a significant positive correlation between EF of imazethapyr ($p < 0.002$, $R^2 = 0.47$) and imazaquin ($p < 0.001$, $R^2 = 0.54$) and soil pH ranging over the range 5.02 to 7.6. Therefore, the higher EF values observed in Roseworthy soil in the absence of organic amendment could simply be due to its higher soil pH compared with Clare soil.

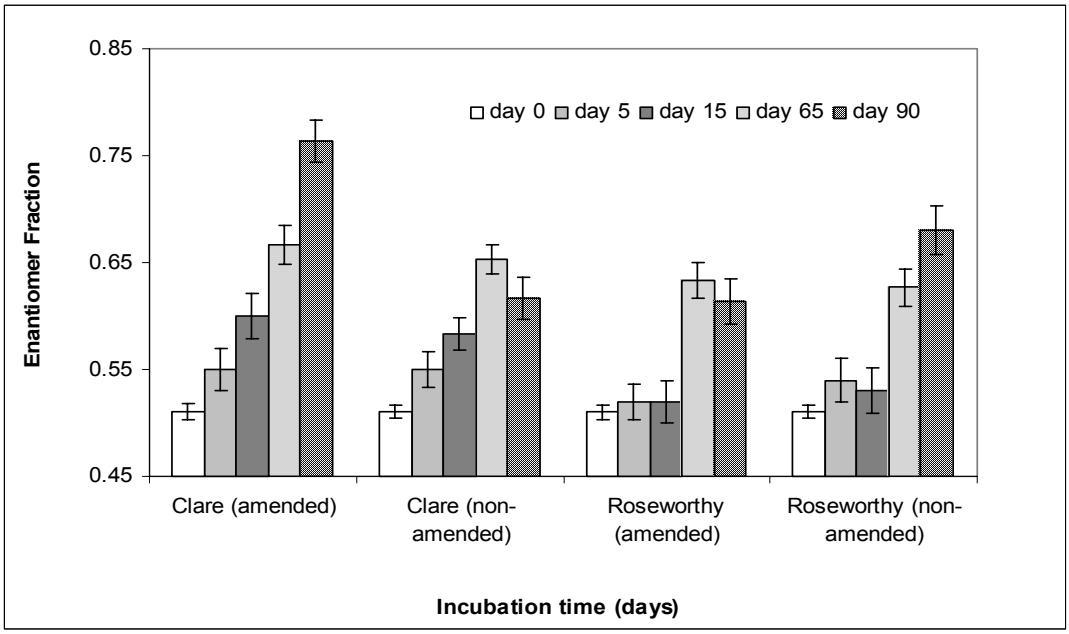

Figure 5.17. Enantiomer fraction (EF) of imazethapyr in different soils with and without amendment over 90 days of incubation; values represent means ± standard deviation of 3 replicates.

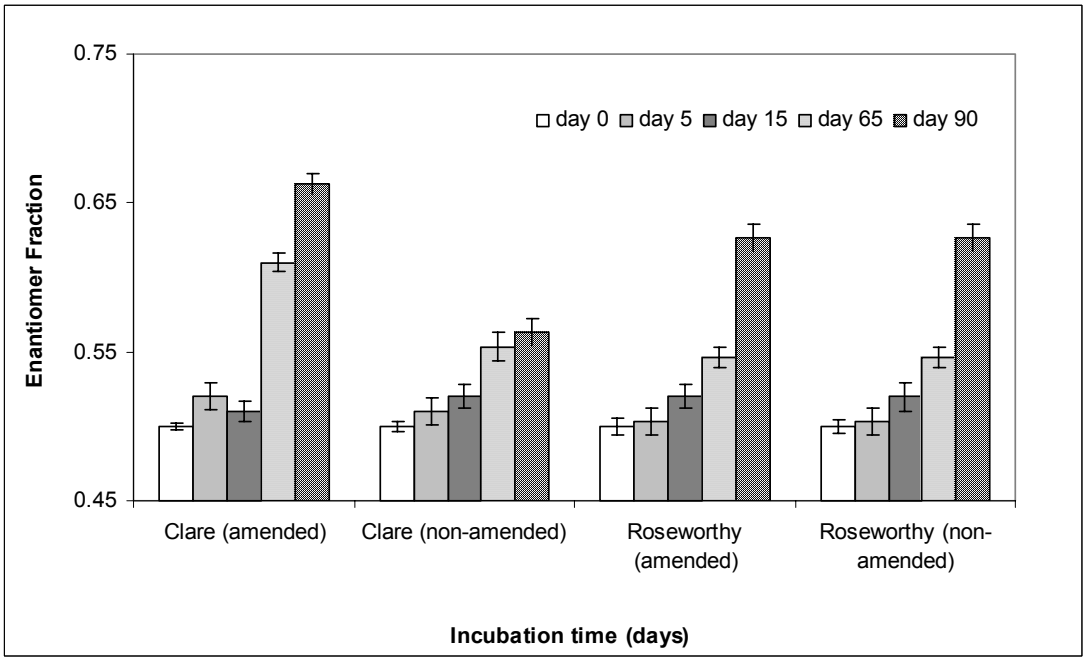

Figure 5.18. Enantiomer fraction (EF) of imazaquin in different soils with and without amendment over 90 days of incubation; values represent means ± standard deviation of 3 replicates.

Table 5.12. EF values of imazethapyr and imazaquin in Clare and Roseworthy soils with and without organic amendment at 90 days after incubation.

Soil	**Treatment**	**EF value (n=3)**[a]	
		Imazethapyr	Imazaquin
Clare	Amended	0.76 a	0.66 d
	Non-amended	0.61 b	0.56 c
Roseworthy	Amended	0.61 b	0.62 b
	Non-amended	0.68 c	0.61 b

[a] Values in the same column and row followed by different letters are significantly different at $p < 0.05$ by LSD.

In this study (Experiment 1), there was no correlation between enantioselectivity and other soil properties such as microbial activity (as indicated by respiration rate), texture and organic carbon contents. The effect of soil factors on degradation rate of pesticides is probably a result of a combination of these factors instead of different factors acting individually. While, this study clearly demonstrated faster degradation of the active R(+) enantiomer of three imidazolinone herbicides in 6 different Australian soils, more information on the factors influencing enantioselective degradation of imidazolinone herbicides could be obtained with other soils of contrasting physico-chemical properties. In particular, the soils chosen in this study had a limited variation in soil properties other than pH. These findings are biologically and environmentaly significant because the R(+) enantiomer of imidazolinones has been previously shown (Lao and Gan, 2005) to have 8-fold greater herbicidal effects on target organisms than the S(-) enantiomer. Occurrence of enantioselectivity in both degradation and toxicity can have environmental significance

The addition of lupin residue caused an increase in soil respiration rate. Previous work has shown that the amendment of soils with organic material can enhance microbial activity (Gan et al., 1998). Even though organic amendment can influence soil microbial activity, it may not always affect enantioselective degradation of pesticides in all soils. Other associated factors such as soil pH, could interact with the organic matter content of soils and affect the degradation of herbicide enantiomers in the soil. In a previous report, the addition of peat to a clay loam soil changed the degradation of mecoprop and dichlorprop from preferential degradation of the S-enantiomer to the R-enantiomer (Romero et al., 1998). Soil properties, such as soil moisture and organic carbon content, are factors that affect the degradation of pesticides in soil and any changes in these factors can lead to variations in enantioselectivity (Buerge et al., 2003; Lewis et al., 1999). In the current study, two physico-chemical properties, soil pH and organic carbon content, influenced soil respiration rate, while soil pH had the most influence on enantioselective degradation of imidazolinone herbicides. Additional studies with various organic amendments and soils with different physico-chemical properties will help to identify further processes influencing enantioselective degradation of these herbicides. The limitation of this type of research is that extrapolation from measurements on a set of incubation to predict the half-life values is a common problem. A longer incubation of the pesticides that meet more than 90 percent of degradation is required to support this extrapolation and thus ensure the acceptable estimation of half-life values in microbial degradation studies.

5.4 Conclusion

There was evidence for enantioselective degradation of imidazolinone herbicides imazethapyr and imazaquin resulting from faster degradation of the R(+) enantiomer in comparison with the S(-) enantiomer in non-sterilized soils, but not in sterilized soils, as expected. This demonstrated the involvement of the soil microbial populations in the preferential degradation of the R(+) enantiomer of imidazolinone herbicides. The degradation of the enantiomers of all three herbicides investigated followed first-order kinetics with half-lives of different herbicides in different soils ranging from 18 to 49.5 days, depending on the herbicide and soil. The rapid degradation of the R(+) enantiomer of the three herbicides caused EF values to be significantly greater than 0.50 for all soils. Furthermore, there was a steady increase in the EF value with time, but the rate of change in EF was influenced by soil type. It took only 3 days in the JA soil for there to be a significant deviation ($p < 0.05$) in EF values from that observed for a racemic mixture, while this change only became significant after 25 days for CL and OT soils. There were significant differences in enantioselectivity of all three herbicides in different soils, indicating diversity of microbial populations present in these soils. Soil pH had a strong correlation with ennantioselectivity of the three herbicides, which could be realted to the impact of pH on herbicide sorption. The rate of deviation of the EF values from that observed for a racemic mixture showed a biphasic pattern for all three herbicides, with a rapid initial phase followed by a slower second phase of biodegradation.

Irrespective of the addition of organic amendment, degradation of S(-) and R(+) enantiomers of imazethapyr and imazaquin followed first-order kinetics with half-lives ranging from 51 to 64.2 for imazethapyr and 63 to 105 days for imazaquin in different soils. Addition of the

organic amendment to the soils significantly ($p < 0.05$) enhanced the degradation rate of both S(-) and R(+) enantiomers of imazethapyr and imazaquin in the Clare soil, with no significant effect on the degradation of enantiomers in the the Roseworthy soil. The EF values of the two herbicides were not different to 0.50 for sterilized soils, while the addition of lupin residue resulted in increased preferential degradation of the R(+) enantionmer over the S(-) enantiomer. The effect of organic amendment on enantioselectivity differed between the two soils investigated. The EF values in Clare soil increased from racemic to 0.61 and 0.76 for imazethapyr and 0.56 and 0.66 for imazaquin over 90 days for imazethapyr in non-amended and amended soils, respectively. However, organic amendment to the Roseworthy soil did not affect EF values for the herbicides relative to the non-amended treatment. It appears that organic amendment of soil influences enantioselectivity of imidazolinone herbicides by altering soil pH; however, further work is required to understand fully the effect of soil pH on enantioselectivity of these herbicides.

Chapter 6. General Discussion

Imidazolinone herbicides strongly inhibit the enzyme acetohydroxyacid synthase (AHAS), and provide effective weed control at relatively low application rates (Shaner and Singh, 1997). These herbicides mainly degrade in soil through microbial degradation and the rate of degradation depends on the herbicide, temperature, moisture, soil texture and pH (Flint and Witt, 1997). Under low soil pH conditions, the imidazolinone herbicides will actually bind to soil particles, so they are more prone for carryover. Imidazolinone herbicides are chiral in nature, having two enantiomers with very different herbicidal activity (Lao and Gan, 2006) and possibly different environmental fate too. There is currently no information on the relative degradation of the enantiomers of these herbicides in soils. Therefore, there is a need to investigate the degradation of the two enantiomers individually rather than the total herbicide residue as racemic mixture. In this study, methods were developed for extracting and quantifying imidazolinone herbicides from soil with different levels of organic matter arising from soil amendments. These methods were used to examine abiotic and biotic degradation of the two enantiomers for each of the three imidazolinone herbicides, imazapyr, imazethapyr and imazaquin.

6.1. SPE extraction of imidazolinone herbicides

Several investigations were conducted to improve the performance of the analytical method by enhancing extraction and cleanup efficiency for aqueous samples with high concentrations of humic acids. For this purpose a newer solid phase extraction (SPE) cartridge PPL, a cartridge with a polymeric stationary phase based on a polystyrene divinylbenzene resin, was used for the separation and clean-up of imazapyr, imazethapyr

and imazaquin from aqueous samples. This SPE cartridge has been successfully employed for the extraction of weak acid phenols from drinking water in the USA (Environmental Protection Agency, 2000). In the present study, the PPL cartridge was found to be effective for the extraction and pre-concentration of these herbicides from water and humic acid solutions.

The use of PPL cartridges to extract imazapyr, imazethapyr and imazaquin from water and humic acids solution revealed that: (i) humic acids were effectively retained by PPL cartridges at neural and alkaline pH, but herbicide recovery was unacceptably low; and (ii) imidazolinone herbicides were retained by the PPL cartridge and when eluted with dichloromethane at pH 2, in the presence of humic acids, herbicide recovery was high (94 to 123%). The use of the SPE cartridge for the extraction of the herbicides from water is simpler than from soil samples due to three reasons (i) easy evaporation of dichloromethane in the elution step of the procedure, (ii) the ability to use a more appropriate solvent for the analytical method and (iii) the ability to re-dissolve the final extracts in this solvent. Satisfactory results were achieved with the PPL cartridge from soil extracts, with recoveries ranging from 69 and 75% for imazethapyr and imazaquin, respectively and RSD values less than 10%. This procedure avoids the need for a second SPE cartridge as suggested by other workers (Furlong et al., 2000), which increases time and cost of extraction of these herbicides from soil. In this study, changing the solvent to more polar mixtures had no effect on the recovery of these herbicides. Of the cleanup procedures examined in this study, NH_2 and PPL cartridges used in series retained much of the humic substances, but not imidazolinone herbicides, which is consistent with the observations by (Said-Pullicino et al., 2004). This allowed high and reproducible recovery of the three herbicides which ranged from 85 to 92%, and eliminated interference from

humic substances. This study demonstrated that PPL is the most appropriate SPE cartridges to clean-up and retain imidazolinone herbicides and remove the interference by humic acids in the analysis of imidazolinone herbicides with HPLC-UV method.

6.2 Degradation of imidazolinone herbicides

The studies of abiotic degradation (hydrolysis and photodegradation) conducted on selected imidazolinone herbicides in aqueous solution and/or on soil surface have enhanced our understanding of the behaviour of these herbicides in the environment. The hydrolysis of imazapyr, imazethapyr and imazaquin was studied in aqueous solutions of different pH in the absence of light at 30°C. These three herbicides were found to be stable in acid (pH values of 3 and 5) and neutral (pH 9) buffer solutions, with more than 85% of herbicides remaining unchanged after 21 days. This finding means that hydrolysis is an insignificant pathway for loss of these herbicides and the subsequent photodegradation experiments were not confounded by any hydrolytic losses.

The photodegradation experiments revealed that imidazolinone herbicides were susceptible to photodegradation, but this effect was compound-specific. For many pesticides, direct photodegradation plays a relatively minor role in degradation due to lack of absorption of sunlight photons by these compounds. Humic substances are invariably present in aquatic environments, but when present in abundance they can “screen out” reactive photons in natural light (Miller and Chin, 2002). Thus, indirect photodegradation of pesticides mediated by the natural components of aquatic systems may become more important. The latter becomes more significant for imidazolinone herbicides when taking into account the potential leaching of these herbicides into the water bodies. In this study, photodegradation

rates of imazapyr, imazethapyr and imazaquin in the presence of humic acids were found to be significantly lower leading to the increase of half-life relative to Milli-Q water, indicating a strong dependence of this process on the composition of the aqueous matrix. The greater persistence of pesticides in the presence of humic acids owing to the "optical filter effect (quenching)" has been reported in previous studies (Elazzouzi et al., 1999b). An important outcome of this study is the possibility of higher persistence of imidazolinone herbicides in aquatic ecosystems, such as lakes and streams, especially those that contain significant amounts of dissolved organic matter.

The studies on photodegradation of three imidazolinones at the soil surface showed that the screening effect of soil particles caused a decrease in the rate of photodegradation. The slow degradation of imidazolinones in the soil compared with that in aqueous systems is likely to be the result of light-shielding by the soil (Katagi, 2004). The results of this research on the photodegradation of imidazolinone herbicides has implications for situations where herbicide remains on the soil surface after post-emergence application, provided there is no rainfall after application to leach the herbicides residues into subsoil. If there is any off-target movement of these herbicides into aquatic environments, photodegradation could also be an important pathway of their loss from water bodies. It can also be an important process when these herbicides remain on plant leaves (not verified here). Any practice that increases the chances of these herbicides to remain on the soil surface can increase photodegradation of the herbicides. Conversely, the incorporation of imidazolinones into the soil will reduce photodegradation of these herbicides compared with post-emergence application to the soil surface. Therefore, the half-lives of these herbicides may differ significantly among different agroclimatic zones. Given that in Australia there are relatively high levels of incident UV radiation compared with other

regions, overseas data would need to be used with caution. More work is needed to make a comparative assessment on the rates of degradation of these herbicides in different regions. In the experiment on ennatiomers, the photodegradation of imazaquin was not found to be enantioselective. As the abiotic reactions are known to be not enantioselective (Garrison, 2006), any enrichment of one enantiomer over the other in aquatic systems such as lakes or rivers is likely to result from biodegradation of these herbicides.

Given the chiral nature of these herbicides, the importance of biodegradation in determining their fate in soils and lack of knowledge on the behaviour of enantiomers, a major emphasis in this thesis was placed on the relative biodegradation of the two enantiomers. Three experiments were conducted to assess enantioselectivity of degradation of imidazolinone herbicides in a range of soil types under sterilized and non-sterilized conditions and to establish if stimulation of microbial activity through addition of an organic amendment can cause enantioselective degradation of imidazolinone herbicides. These experiments revealed that degradation of imidazolinone herbicides in the soil was indeed enantioselective in nature, with significant deviation observed in EF from 0.50 as degradation proceeded. However, this change in EF only took place in in microbially-active soils. Interestingly, the R(+) enantiomer, which has much greater biological activity than the S(-) enantiomer, was degraded at a faster rate.

Soil pH was the only soil property that showed any relationship with enantioselectivity. This association could be due to greater availability of these herbicides at high pH for microbial degradation or it could be related to the composition of microbial community at high soil pH. The dependence of enzymatic reactions on pH has been demonstrated

previously and positive correlation of soil pH with enantioselectivity of other chiral pesticides has also been reported (Buerge et al., 2003).

In this experiment, higher EF values were found in the Roseworthy soil (pH 8.2) compared with the Clare soil (pH 5.2) for imazethapyr and imazaquin. By contrast, enantioselectivity of imazethapyr and imazaquin were increased in the Clare soil by organic amendment, but not in the Roseworthy soil. Thus, the effect of organic amendment on degradation of imidazolinone enantiomers was both compound-specific as well as soil-dependent. The increased use of no-tillage systems in Australia has led to appreciable enhancement of organic matter in soil (Reeves, 1997). Therefore, further work is needed to investigate the effect of increased organic carbon on degradation of these herbicides. The enhanced microbial and enzyme activity attributed to soils characterized by increased plant residues or enhanced organic carbon can potentially assist the degradation of herbicides like the imidazolinones (Locke and Bryson, 1997). Based on the observed differences in the rates of degradation of the two enantiomers, it may be desirable for the manufacturers to alter the formulation of these herbicides in favour of the more biologically active but less persistent R(+) enantiomer. Such a change could decrease the amount of chemical load in the environment without sacrificing weed control for the user. Since the active enantiomer of these herbicides was found to be preferentially degraded, it could be argued that pure enantiomer instead of racemic compounds need to be used in toxicity studies to prevent the overestimation of toxicity. The result of this study along with the determination of occurrence of enantioselectivity in toxicity can have significant implications for the environment. In the case of other pesticides, relative sensitivity to degradation could be very different to that observed for imidazolinone herbicides in this experiment. For example, for MCPP and DCPP as well as mecoprop and dichloroprop (Garrison et al.,

1996), the active enantiomer were degraded more slowly than the inactive enantiomer in the environmental matrices. Clearly, there is need for more comprehensive research on environmental fate and effects of enantiomers of these and other similar pesticides.

6.3 Future research

At present, the extraction methods for imidazolinone herbicides are time-consuming, labour-intensive, and costly. Therefore, further research on the extraction of imidazolinone herbicides is necessary to improve the recovery and reduce the cost of extraction. Future research should focus on improving the SPE extraction of imidazolinone herbicides from different soils with a single SPE cartridge to reduce the extraction time and the cost. The use of a PPL cartridge obtained satisfactory results for two imidazolinone herbicides (imazethapyr and imazaquin), but this method may need further improvement for the extraction of all imidazolinone herbicides. Moreover, for the imidazolinone herbicides which persist in soil and carryover to the next growing season (Hollaway and Noy, 1998), this method may not be sufficient to detect the herbicides residue in soil. Thus, it is essential to lower the detection limit and improve the sensitivity of the extraction.

Research on photodegradation process should be conducted on different soils of contrasting physico-chemical properties. Soils with high organic carbon contents should also be included in these experiments, because it has been shown that the contribution of the photodegradation processes to the dissipation rate of some herbicides is greater in soils with higher organic carbon content (Scranoa et al., 2004). Establishing a laboratory system for soil photodegradation in which the moisture and temperature could be controlled is essential to better simulate the photodegradation rate and predict the fate of herbicides in

the environment. Moreover, much overseas research on abiotic degradation of the imidazolinone herbicides has been in a different agro-ecological regions with different soil and climatic properties. Comparison of photodegradation in relation to UV levels in Australia to other countries could be an interesting future project. Due to many factors involved and lack of abundant data, some of the observations reported in these regions are rather provisional. Thus, in order to be definitive about the finding, comparative assessment of degradation behaviour need to be used with caution.

Future research on the enantioselective degradation should take into account the impact of environmental conditions on the relative degradation of enantiomers and focus not only on their efficacy as herbicides but also on their environmental toxicity and impact. Since enantioselectivity was noted to be both compound specific and dependant on soil properties, more work needs to be conducted in relation to what properties actually determine enantioslectivity and to establish whether the herbicidal active enantiomer is always degraded more rapidly or not? Several other question need to be addressed in future research on enantiomers. Which mechanism underlies the enantioselectivity and its variation? Secondly, under what conditions the R(+) enantiomer would persist longer? As adoption of minimum tillage can lead to increase in total organic carbon and microbial activity, what would be the implications of this variation for enanatioselectivity? Environmental fate and effects of chiral pesticides remains a fertile area for future research.

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