# ROLE of RIVERBANK FILTRATION in the ATTENUATION of HERBICIDES

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Luận văn này xin được Kính tặng Ba Mẹ tôi, những người luôn mong mỏi tôi đi xa hơn trên con đường học tập.

# ABSTRACT

This thesis for the first time reports the fate and behaviour of herbicides mecoprop (MCPP) and isoproturon (IPU) in the hyporheic zone of a river bank. Two laboratory studies based on fixed-bed circulation and $^{14}C$-respirometry were developed to investigate the attenuation of the two herbicides in riverbank filtration (RBF), a means of pre-treatment of drinking water obtained from bank-side boreholes.

The first laboratory study investigated the sorption and biodegradation of MCPP and IPU (100 μg $L^{-1}$) in a river water (RW)-riverbed sediment (RS) system with materials obtained from a site on the River Thames at Gatehampton, England. Using a fixed-bed circulation method, approximately 18-20 % of the herbicides were removed by sorption, with the remainder removed by a high rate of biodegradation during 14 circulating days. The RS-borne microorganisms played a primary role in the biodegradation process of these herbicides, while the RW-borne microorganisms contributed very little. In addition, after a period of incubation (by 18 circulation days with IPU) the RS-borne microorganisms were able to immediately mineralise $^{14}C$-IPU (29.4 % $^{14}CO_2$) while the RW-borne microorganisms were not competent to do so (1.6 % $^{14}CO_2$).

The second laboratory study investigated catabolic insights into IPU degradation in river water (RW), groundwater (GW) and riverbed sediment (RS). Very low maximum levels of mineralisation of IPU were observed in RW (0.4 % $^{14}CO_2$) and GW (1.2 % $^{14}CO_2$) while very high maximum level of mineralisation of IPU was obtained in RS (14.5 % $^{14}CO_2$). Furthermore, the catabolic competence with respect to IPU was enhanced with increasing the IPU-dosed concentrations (ranging 1 – 100 μg $L^{-1}$) in RS microcosm. By plotting the maximum mineralisation levels versus the residual IPU concentration (after various periods of incubation), a logarithm linear relation between the maximum mineralisation levels and IPU concentrations was obtained. This relationship suggested that higher mineralisation levels are achieved for higher IPU concentrations. Nonetheless, the catabolic activity not only was not significantly enhanced ($p > 0.05$) after a period of incubation (0 – 10 days) but also was greatly decreased ($p < 0.05$) after 30 incubation days.

Based upon the experimental results, to remove the herbicides from 1 L of RW contaminated with MCPP and IPU (up to 100 μg $L^{-1}$), a required volume of RS (bulk density of 1.25 ± 0.02 g $cm^{-1}$ and porosity of 50.6 %) was determined to be 0.027 $m^3$. Extent in a RBF context, it is suggested that a bank-side borehole with a capacity of 16 x $10^6$ L $day^{-1}$ and 25 % river-fed water could be protected from the river-borne herbicide pollution (up to 100 μg $L^{-1}$) if the borehole is located at a minimum distance (path length) of 400 m from the river with the thickness of a RS layer to be 6 m.

Collectively, the herbicides MCPP and IPU were completely degraded in a hyporheic zone of a river bank. Microorganisms originated from RS played a pivotal role in the degradation. This demonstrated that RBF is potentially a highly efficient pre-treatment method which can totally remove herbicide pollution in river. Hence, bank-side boreholes which are mainly or partly fed by induced RW may be benefit from this natural attenuation process.

## ACKNOWLEDGEMENTS

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# Chapter 1

# INTRODUCTION and BACKGROUND

## 1.1 The Problem Addressed in this Study

The sustainable development of humanity depends on our ability to bring mankind into a lasting equilibrium with nature. Unfortunately, the number of people on the Earth continues to increase, while natural resources remain limited. This places an ever-increasing pressure on human beings to look for innovative technologies to efficiently utilize the existing resources. Fresh water in general and groundwater in particular is one of the most precious natural resources which is becoming scarce as the population grows.

The human boom is not only increasing the demand for water but threatening to pollute its sources as well. Large and small industrial enterprises, the water industry, urban infrastructure, agriculture, horticulture, transport, discharges from abandoned mines, and deliberate or accidental pollution incidents all affect water quality. In particular, a large amount of pesticides has been used, with $1.9 \times 10^4$ tonnes applied on $4.1 \times 10^7$ treated hectares in Great Britain in

2006 (Garthwaite *et al.*, 2006). As a result, pesticides in the aquatic environment have become the focus of much attention. The European Community Drinking Water Directive prescribed a maximum allowable concentration of 0.1 μg $L^{-1}$ for an individual pesticide in drinking water (98/83/EC, 1998). Nine typical pesticides in surface water of England and Wales which most frequently exceeded the threshold of 0.1 μg $L^{-1}$ have been monitored by the Environment Agency since 1998 (Figure 1.1).

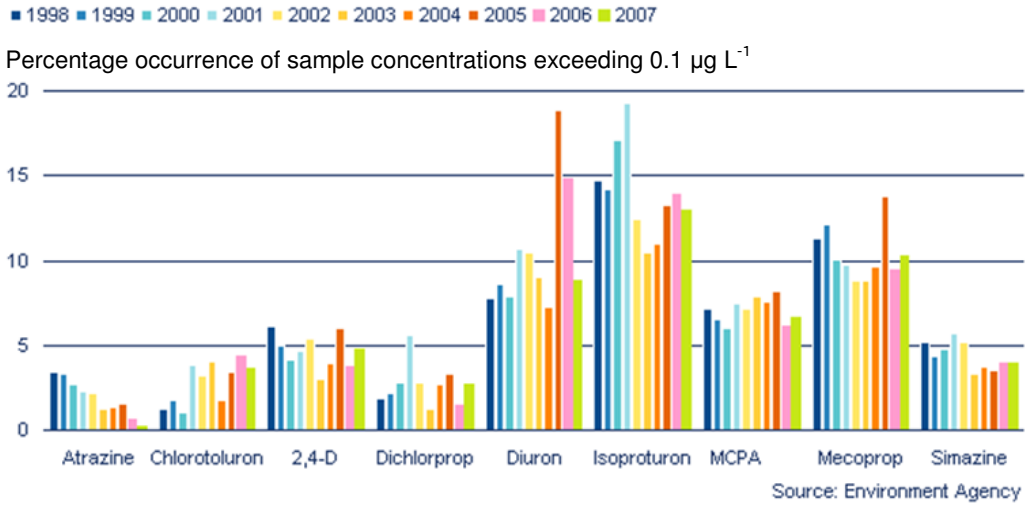



**Figure 1. 1** Pesticides in surface waters by substances in England and Wales, 1998 to 2007 (Environment Agency, 2009).

According to this survey, the frequency exceeding the 0.1 μg $L^{-1}$ threshold of isoproturon and mecoprop in 2006 was notably high at 13.84 % and 9.48 %, respectively (Environment Agency, 2009). Beside the pollution sources caused by daily living activities, the threats from accidents or the incidence of chemical spills into river were also significant. The accidents reported below, by the way of example, provide some contents.

On 1st November, 1986, fire at a Sandoz Ltd. storehouse at Basel, Switzerland resulted in roughly between 6 and 22 tonnes of 20 pesticides to enter the Rhine contained within fire-fighting water. An estimated a half million fish, mostly

eels, were killed as a direct result of the spill. Rapid co-ordinated responses by water supply authorities downstream of Basel (in former West Germany and The Netherlands) resulted in the shutdown of all river water intakes until the pollution plume passed by, so that no polluted water was fed into public supply networks. Fortunately, the induced infiltration sources showed no detectable effects, suggesting that bank filtration processes performed adequately (Deininger, 1987; Capel *et al.*, 1988).

On 6$^{th}$ March, 1990, another fire event occurred in Horsell (near Woking), United Kingdom. The main fire was centred on a structure containing high pressure timber treatment plant and chemical storage tanks which held an estimated 30,000 L of liquid VASCOL MWR working solution of 1% w/v tributyltin oxide (8 g $L^{-1}$) and 0.5% w/v lindane (4 g $L^{-1}$) in a light petroleum distillate. Approximately 25,000 L of wood preservative and a large amount of fire-fighting water ran off into the surface drains. These drains were connected, via a 3 km surface water culvert, to the River Bourne South, a tributary of the River Thames. Control of the incident and the subsequent monitoring was undertaken by the National Rivers Authority, Thames Region but in spite of the installation of absorbent booms to prevent the downstream migration of the pollution plume, a toxic mix of tributyltin and lindane moved downstream, causing a major pollution incident on both the River Bourne South and Thames. Three drinking water intakes operated by the Thames Water Plc and the North Surrey Water Company were closed for a period of 5 – 7 days as a precautionary measure (Dowson *et al.*, 1996). In other instances, disposal of liquid herbicide waste into landfills in former excavations in the Lincolnshire Limestone near Helpston, Lincolnshire (National Grid Reference TF 120 030) has given rise to extensive groundwater pollution (Sweeney *et al.*, 1998). It was estimated that about 40 tonnes of predominantly mecoprop was been leached from this site which was thought to have migrated approximately 2.5 km to a public supply borehole at Etton (where water was treated prior to distribution) (Williams *et al.*, 2004).

The Health and Safety Executive (HSE, 2008) reported that 94 pesticides incidents (complaints) were investigated during 2007/08. Thirty-two complaints involved allegations of ill-health, with the remaining 62 complaints involving other issues to do with pesticides use. The total of 94 incidents was a decrease of 6 from the previous year's figure (2006/07) and 46 % lower than the average for the previous ten years. Figure 1.2 shows the numbers of incidents and complaints compared with previous years.

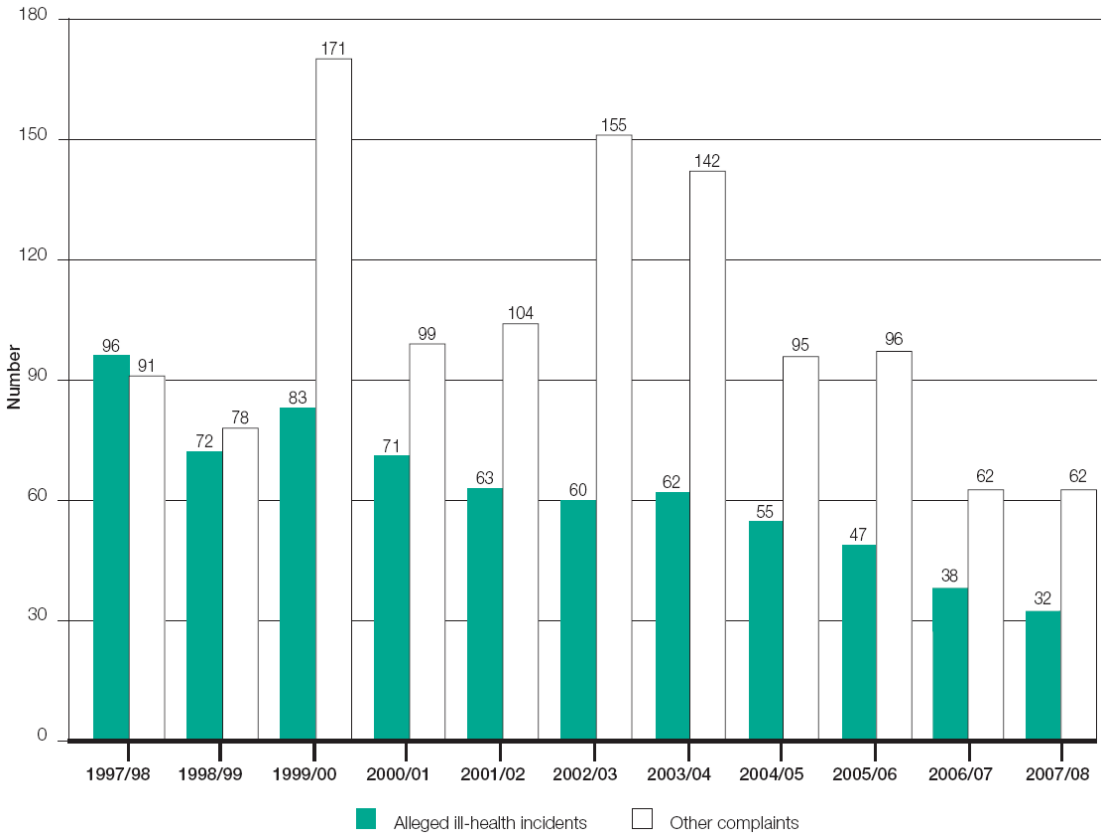



**Figure 1. 2** Field Operation Directorate alleged ill health incidents and other complaints relating to pesticides 1997/98 – 2007/08 (HSE, 2008).

Therefore, under increasing demand of domestic water and threat of pesticide pollution, water supplies have been looking for not only new water resources but also utilizing natural processes to protect existing water sources against pollution. Riverbank filtration is one of these processes which can apply natural microorganisms to mitigate pesticide pollution (Ray *et al.*, 2002b). A brief introduction to this process is presented in the following sections.

## 1.2 Riverbank Filtration

### 1.2.1 Introduction

The abstraction of groundwater as a drinking water resource is commonplace throughout the world. Some abstraction boreholes of this drinking water have been located in alluvial aquifers close to rivers and rely upon riverbank filtration to maintain water potability. The infiltration of pollutants from river water to groundwater is of great interest since many water-works use natural or artificial bank filtration as a first step in the treatment of river water for public water supplies (Piet and Zoeteman, 1980; Sontheimer, 1980). The efficiency of riverbank filtration in removing pollutants has been documented in a number of previous studies. Younger *et al.* (1993) highlighted the important of streambed sediments as a barrier to groundwater pollution by river water. Hiscock (2005) listed the pollutants from surface water such as suspended solids, particles, biodegradable compounds, bacteria, viruses and parasites which could be eliminated as they are filtered through the porous materials of a bank and reach the vicinity of abstraction boreholes. The removal of pollutants by riverbank filtration such as herbicides atrazine, triazine, acetamide (Verstraeten *et al.*, 2002b; Vargha *et al.*, 2005), dissolved organic carbon (Ludwig *et al.*, 1997), aromatic amines (Bornick *et al.*, 2001; Worch *et al.*, 2002), and pathogens (Havelaar *et al.*, 1995; Ray, 2002) have also reported. Besides the advantages of utilising bank filtration, undesirable effects on water quality have been reported. Such effects include the increases in hardness, ammonium and dissolved iron and manganese concentrations and the formation of hydrogen sulphite and other malodorous sulphur compounds as a result of changing redox conditions (Hiscock, 2005). Ray *et al.* (2002a, 2002b) systemised and published existing knowledge of the world-wide application of riverbank filtration. These publications lead readers from the history of riverbank filtration to the latest studies of removal of contaminants in surface water as well as conceptual design and construction of riverbank filtration systems.

Abstraction of drinking water from boreholes adjacent to rivers has been practiced throughout the Europe such as the River Danube in Central Europe (from Austria to Black Sea), the Rivers Rhine and Elbe in Germany, the Rivers Lot and Seine in France, to the United States of America such as the Rivers Columbia, Missouri, Mississippi, Ohio, Colorado, Rio Grande, Russian and Connecticut (Ray *et al.*, 2002b) and in many areas of the developing world such as the Rivers Ganga in India (Dash *et al.*, 2006), the Rivers Nile in Upper Egypt (Shamrukh and Abdel-Wahab, 2008).

In the United Kingdom, the first known utility to use riverbank filtration for water-supply purposes was the Glasgow Waterworks Company built in 1810 (Ray *et al.*, 2002a). Currently, at the Gatehampton site in central-southern England, seven abstraction boreholes constructed along the River Thames in this location are obtaining up to 65 x $10^6$ L $day^{-1}$ of potable supply. To date, no serious chemical or pesticide incident spill has been reported in this area. However, the River Thames is surrounded by agricultural fields in its floodplain. Thus, agricultural pollutants from these areas might be transported to the river by many means, mainly by runoff. Consequently, the vicinity abstraction boreholes could then be fed by the polluted water from the river. Obviously, this raises concern of residue pesticide pollution entering groundwater resources and drinking water supplies.

### 1.2.2 Concept of Riverbank Filtration

Riverbank filtration can occur under natural conditions or induced by abstraction from boreholes proximity to the surface water course. Typical flow conditions associated with different types of riverbank filtration schemes are shown in Figure 1.3. The pumping action creates a difference of "head" pressure between the river and the aquifer. Water from the river then percolates through the pores of the riverbank materials as it migrates to the boreholes. As a consequence of its journey, contaminants from river water can

be partly or wholly removed. Depending upon the required water quality, addition treatments can be used before supplying the water to consumer.

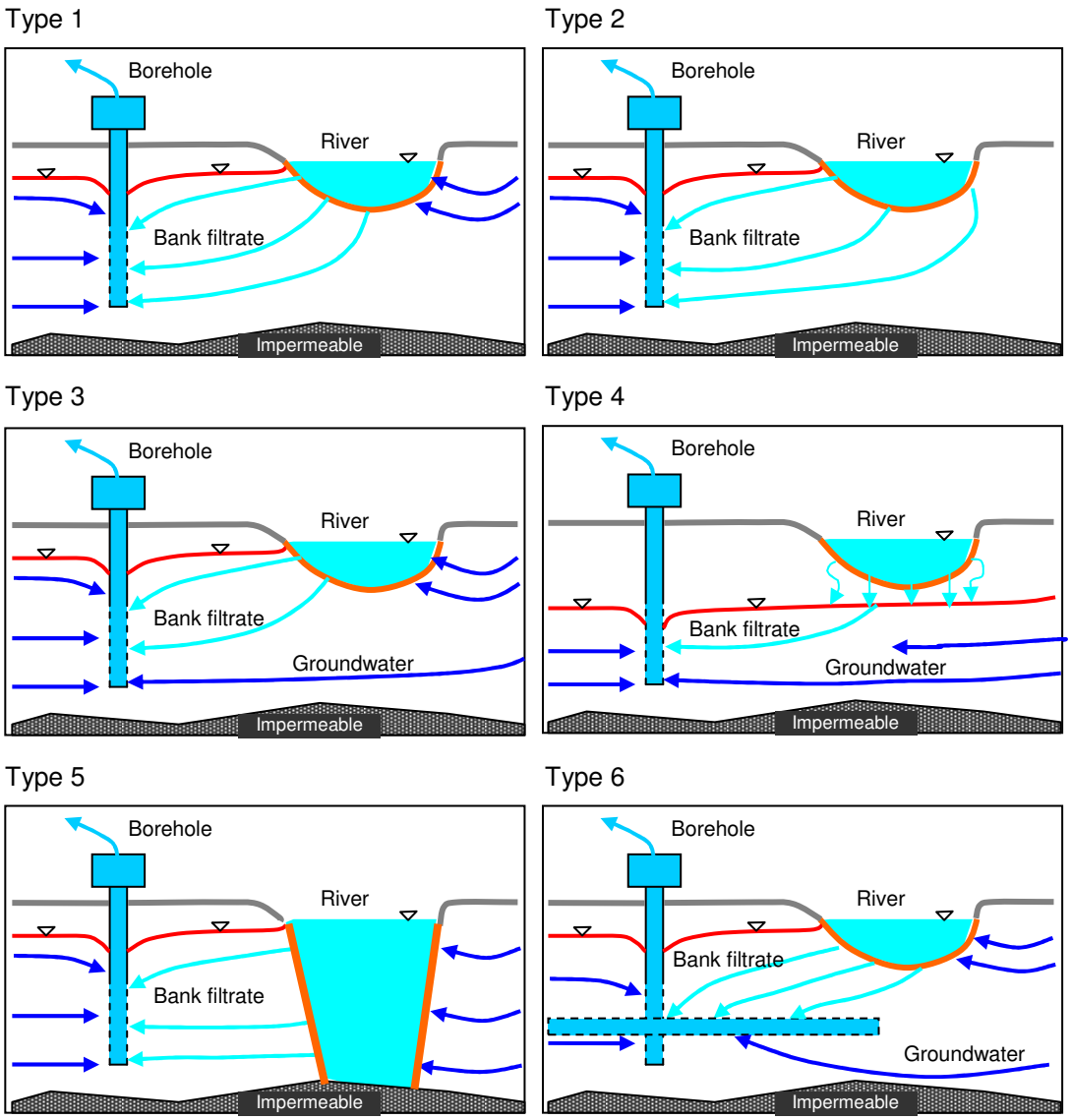



**Figure 1. 3** Schematic representation of types of flow conditions at RBF sites. The majority of RBF schemes (Type 1); groundwater flow beneath the river (Type 3, 4, and 6); the unsaturated zone beneath the river (Type 4); the river bed cut into the confining layer (Type 5); lateral abstraction boreholes affected by RBF (Type 6). After Hiscock (2005).

The capacity of the natural system to fulfil this service will be dependant upon a number of factors such as the activity of indigenous microorganisms, the

loadings of chemicals in the source water (Malzer *et al.*, 1992; Ray *et al.*, 1998; Ray, 2004), the hydrogeological properties (Hoehn, 2001), the hydraulic conductivity of the river bed (Hiscock, 2005), the river water and groundwater levels, the characteristics of the sediment found at the river-aquifer interface (Ray, 2001) and the interaction of all of these factors. The formation of the colmation layer at the interface between the surface water and the riverbed sediment plays an important role that directly affects to the infiltration processes in the riverbank. This layer could be considered as a complex phase consisting of clay minerals, organic matter, and living organisms which develops on the surface of a riverbed by the precipitation of the substances in the river. Particularly, the aerobic microbial activity in the colmation layer plays a pivotal role in the removal of organic contaminants from surface water (Hiscock and Grischek, 2002).

Warren *et al.* (2003) has reviewed that the microcosms in riverbed sediment differs from that in overlying waters containing suspended sediment in several ways. Dissolved $O_2$ diffused from overlying water into the sediment bed largely controls the conditions in riverbed sediment. The concentration of dissolved $O_2$ in the porewaters usually decreases sharply below the water-sediment interface. In the upper few centimetres (or less) of the sediment bed where dissolved $O_2$ is present – the oxic layer – aerobically-respiring bacteria dominate, and oxidation reactions (both biotic and abiotic) occur relatively easily. Below this – the anoxic layer – sediments become anaerobic and progressively less oxidising and more reducing with depth, with anaerobically-respiring bacteria beginning to dominate. Different microbial populations and population sizes will also be characteristics at different sediment depths, according to the shape of the oxic-anoxic sediment depth profile. Another characteristic of riverbed sediments is that sediment porewaters generally contain higher concentrations of dissolve ions and dissolved organic matter than the overlying waters. Thus, the pH may also differ from that in the overlying water and vary with depth.

Most riverbank filtration systems are constructed in alluvial aquifers located along riverbanks. These aquifers may consist of a variety of deposits ranging from sand, to sand and gravel, to larger cobbles and boulders. Ideal conditions typically include coarse-grained, permeable water-bearing deposits that are hydraulically connected with riverbed materials (Hunt *et al.*, 2002). Three types of wells benefiting from riverbank filtration are presented below (Hunt *et al.,* 2002):

(1) *Horizontal Collector Well*: A circular central collection caisson sunk into the ground with horizontal lateral well screens pushed out into unconsolidated aquifer deposits, in many cases into alluvial deposits beneath a river or lake. It is typically used by the United States water utilities to produce drinking-water supplies from groundwater sources or from riverbank through filtration.

(2) *Vertical Well*: a tubular well that is drilled vertically downward into a water-bearing stratum or under the bed of lake or stream.

(3) *Pit Well*: a shallow, large diameter well that, in most instances, is manually dug into the ground. Typically, a pit well is constructed for an individual residential water supply.

Vertical well type has been used at the Gatehampton site, England, as a method to collect groundwater and river water. The next section introduces the pesticide usage as a threat to river water and the proximity boreholes.

## 1.3 Pesticide Usage

Pesticides are substances or a mixture of substances including commercial formulations of plan protection products which are used as acaricides, biological control agents, defoliants, desiccants, fungicides, growth regulators, herbicides, insecticides, molluscicides or nematicides (Lydy *et al.*, 2004; Garthwaite *et al.*, 2006). They can be grouped according to their use and also classified based on the functional group in their molecular structure with some major groups being organohalogen, organophosphorous, organonitrogen, organosulphur (van der Hoff and van Zoonen, 1999).

The benefits of using pesticides are many, such as increased crop production, lower-cost maintenance and control of public health hazards. However the unintended adverse effects can be considerable, particular to the aquatic environment for instance river water and groundwater. Neglectfully these adverse effects, the use of pesticides for pest control has increased over the last five decades, replacing manual or mechanical treatment methods with chemical treatment. The use and number of pesticides have grown steadily worldwide since the 1960s, but declined slightly in Germany by the late 1990s (Verstraeten *et al.*, 2002a). Worldwide, approximately 2.50 x $10^6$ tonnes of pesticides have been applied, mainly in agriculture (van der Werf, 1996). In the United States, total use of conventional pesticides have increased from about 0.30 x $10^6$ tonnes in 1964 to over 0.50 x $10^6$ tonnes in 1979, subsequently remaining fairly constant or decreasing, reaching about 0.44 x $10^6$ tonnes in 1995. Herbicides constitute the largest share of total conventional pesticides used. The total annual use of herbicides remained fairly constant at about 0.27 x $10^6$ tonnes between 1979 and 1995 (Nowell *et al.*, 1999).

In Great Britain, around 0.03 x $10^6$ tonnes pesticides per annum were applied between 1990 and 2005 (Central Science Laboratory, 2008), mainly in

agriculture and horticulture, but products were also used in public and recreational areas, at industrial sites, on highways and railways, and in homes and gardens (Central Science Laboratory, 2008). Statistics reported in 2006 showed that fungicides accounted for 35% of the total pesticide-treated area of arable farm crops; herbicides, desiccants and sulphuric acid 32%; insecticides and nematicides 10%; growth regulator 9%; molluscicides 2%; and sulphur less than one percent (Garthwaite *et al.*, 2006). In contrast, by weight, herbicides, desiccants and sulphuric acid accounted for 57% of the pesticide-active substances applied, fungicides 21%, growth regulators 14%, insecticides and nematicides 3%, sulphur 2%, molluscicides, seed treatments and sulphur one percent each (Garthwaite *et al.*, 2006). The total mass applied of all pesticides to all crops in Great Britain is presented in Figure 1.4.

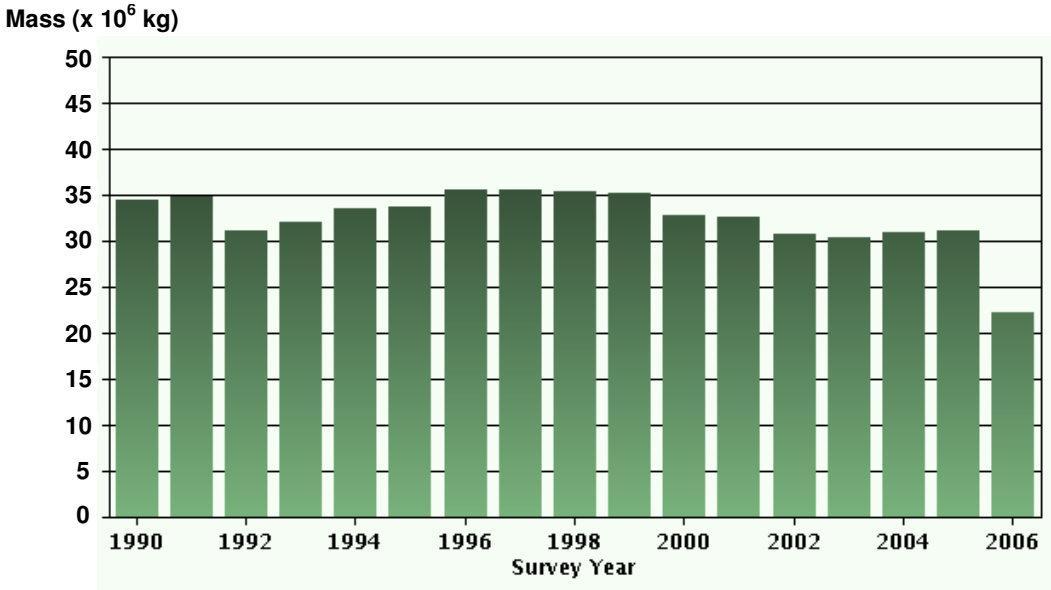



**Figure 1. 4** Total mass applied (kg) of all pesticides to all crops in Great Britain since 1990 to 2006 (Central Science Laboratory, 2008).

It is important to learn that the amount of applied pesticides actually consumed by pests was very small compared with the used amount, less than 0.1% (Jiemba, 2004) or less than 0.3% in most studies (van der Werf, 1996). The use of pesticides has therefore led to their occurrence in many hydrologic systems, including surface water, groundwater, wastewater and drinking water. The

pesticide residues could be transported into rivers by runoff from urban and rural areas, by groundwater discharge, along drainage tiles and by atmospheric deposition (Verstraeten *et al.*, 2002a).

In some reported cases, the presence of herbicides and other organic compounds in groundwater has been attributed to surface water that has been contaminated with herbicides by periodic flooding, bank storage of river water, artificial recharge by impoundment, and induced infiltration (Exner, 1990; Verstraeten *et al.*, 1999; Worch *et al.*, 2002). In other cases, pesticides have been identified in surface water during base-flow conditions and have been attributed to inflow from contaminated groundwater (Barbash and Resek, 1996).

The promulgation of regulations has lagged behind the formulation of new pesticides. The World Health Organisation issued drinking water quality guidelines for 33 pesticides (WHO, 1993). The U.S. Environmental Protection Agency has defined health advisories for 71 pesticides (U.S.EPA, 1994). In Europe, the regulations for pesticides and several other parameters are not based on toxicological aspects, but on the "precautionary principle". Thus, the maximum tolerance levels for pesticide residues in drinking water have been set at 0.1 $\mu g\ L^{-1}$ for an individual compound and its degraded products, and at 0.5 $\mu g\ L^{-1}$ for all pesticides and their degraded products (98/83/EC, 1998).

## 1.4 Behaviour of Pesticides in Water-Sediment Systems

### 1.4.1 Introduction

An understanding of the occurrence and distribution of pesticides in riverbed sediment requires consideration of pesticide sources, transport processes and mechanisms of transformation and removal from the sediment. In general, the movement of a pesticide from the point of application to the river bed is firstly controlled by processes that deliver the pesticide to the stream, and then by processes that deliver the pesticide from the water column to the bed sediment. Once in the sediment, environmental processes continue to act upon the pesticide and contamination via river-fed seeping into the riverbed sediment may occur. This thesis aims to investigate the fate and behaviour of pesticides in the interaction zone of river water and riverbed sediment.

There are several factors which control the distribution of a pesticide in a water-sediment system (Figure 1.5). River water contains suspended matter, dissolved organic and inorganic matter and many kinds of aquatic biota. These fractions interact with a pesticide molecule depending on its physico-chemical properties such as hydrophobicity ($K_{OW}$) and determine the pesticide distribution. Thus the freely dissolved fraction of a pesticide is reduced by association with these substances and/or bioaccumulation. Two dominant processes, sorption and biodegradation processes which determine the occurrence of a pesticide in a water-sediment system, are outlined in the following sections.

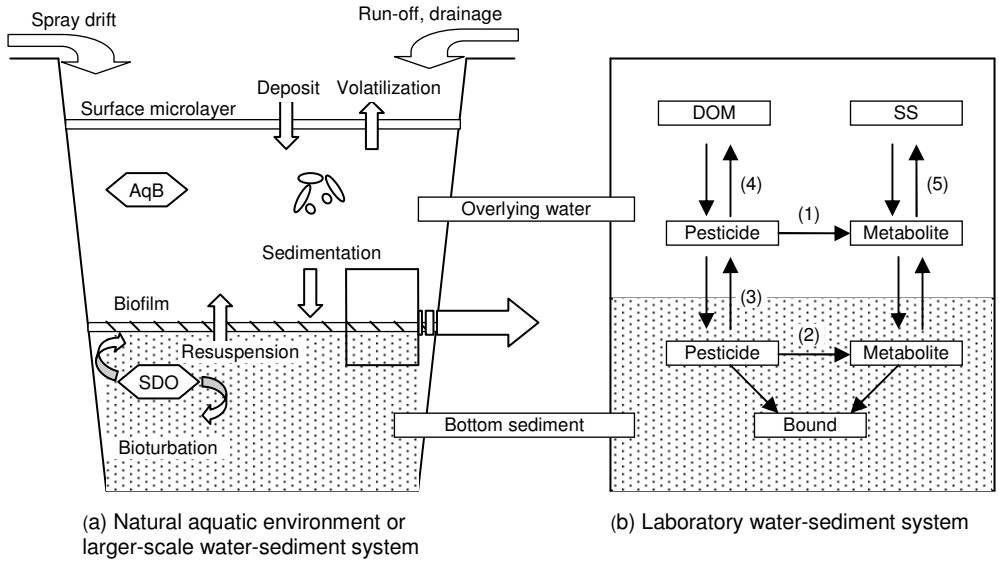



**Figure 1. 5** Transport, distribution and transformation processes of pesticides in a water-sediment system. DOM, dissolved organic matters; SS, suspended solids; AqB, aquatic biota such as fish, invertebrates, plankton, and macrophytes; SDO, sediment dwelling organisms; (1) hydrolysis, photolysis, redox reactions, and biodegradation; (2) hydrolysis, redox reactions, and biodegradation; (3) adsorption, desorption, and diffusion; (4) solubilisation, complex formation, and catalysis; (5) adsorption, desorption, and catalysis. After (Katagi, 2006).

## 1.4.2 Sorption of Pesticides on/into Riverbed Sediment

Sorption is introduced as a process in which chemical associates with a solid phase. Riverbed sediment was interested in this study as a solid phase. Riverbed sediment was considered as a very complex phase consisting of clay minerals, organic matter, and living organisms. Therefore, the sorption capacity of sediment with respect to a pesticide molecule greatly varies. Due to the compositional complexity of sediments, sorption is not, however, the result of a single process, but may result from both adsorption and absorption in/on a range of matrix (Warren *et al.*, 2003).

Pesticides found in riverbed sediment could arise from several sources: the water column, aquatic biota, and groundwater. In water column, a pesticide may be present in the dissolved phase or in association with soil particles. The pesticide will redistribute itself between the water and aquatic (suspended) particles in the water column. The particle-associated pesticide then can be deposited in the riverbed sediment. In aquatic biota, a pesticide may be: (1) in dead or living biotic particles (such as algae and detritus) settling to the sediment-water interface; or (2) in higher organisms settling to the bed sediment where they decompose; or (3) in excretions (such as faecal material) containing pesticide contaminants which are released and then settle to the river bed. In the last source, in groundwater, a pesticide may be in a contaminated alluvial aquifer passing through the bank once the water level of the river is lower than the level of groundwater.

To understand and predict the distribution of pesticides in riverbed sediment, it is crucial to examine both the thermodynamics of the association or sorption of the pesticides with riverbed sediments (which controls the equilibrium state of a system) and the kinetics (i.e. how quickly this state is approached) (Warren *et al.*, 2003). Nowell *et al.* (1999) reported that once a pesticide reaches bed sediment, it can undergo a number of processes that will determine its short-term behaviour and long-term fate. Generally, a hydrophobic pesticide will arrive at a sediment-water interface in association with some type of particular matter. In the particle-rich environment of the riverbed sediment, sorptive processes are critical to the overall behaviour and fate of a pesticide. Simultaneously with the sorption process, a fraction of pesticide will undergo desorption into pore water or overlying water as the pesticide re-equilibrates between the water and sediment in its new environment. The sorption-desorption cycle of the pesticide continues throughout its lifetime in bed sediment as the microcosm continues to change. The physical location of sediment particles, together with their associated pesticides, is also likely change with time.

There are several factors that control distribution of pesticides in a water-sediment system. In water phase, many kinds of dissolved and suspended species such as organic compounds, humic substances, metal oxides, and clay particles can interact with pesticides and cause an increase of their apparent water solubility and sometimes to retard or catalytically accelerate their hydrolysis via sorption or reaction with functional groups therein (Katagi, 2002). In sediment phase, sediment's physical properties such as bulk density, water content, porosity, and particle size (Percival and Lindsay, 1997), redox potential (Bohn, 1971), organic components (Cranwell, 1976) has been reported as factors affecting the sorption of pesticides. In other instances, bubbles generated from biodegradation process such as carbon dioxide or methane can physically affect to the distribution of sediment in water-sediment system.

### 1.4.3 Biodegradation of Pesticides in a Water-Sediment System

After initial deposition on/into riverbed sediment, a pesticide continues to react with the environment and might be degraded. The processes controlling degradation of pesticides in a water-sediment system can be conveniently classified into abiotic and biotic processes (Wolfe *et al.*, 1990; Warren *et al.*, 2003). However, biotic process caused by indigenous microorganisms is especially interesting as it is a major process in the complete mineralisation of aromatic compounds to harmless inorganic products (Alexander, 1981; Aislabie and Lloydjones, 1995; Sorensen *et al.*, 2003). The natural attenuation rate of phenylurea and phenoxy acid herbicides, with respect to mineralization of the aromatic structure to $CO_2$, is either no detectable or very slow in samples from groundwater aquifer (Johnson *et al.*, 1998; Larsen *et al.*, 2000; Kristensen *et al.*, 2001b). The degradation of phenylurea and phenoxy acid herbicides in a water-sediment interaction zone is thus of major interest because most agricultural fields function as recharge zones for aquifers, rivers and lakes, and

thereby serve as biological filters determining the degree to which the herbicides biodegrade before transport by water.

Microbial metabolism of a pesticide is the primary force in its transformation or degradation in a water-sediment system, and bacteria and fungi are the two major groups among microorganisms in pesticide degradation (Katagi, 2006). Paris *et al.* (1981) reported that microorganisms in natural water can play a role in degrading a pesticide. Furthermore, a sediment phase, especially in the oxic layer, should be more important when microbial degradation is considered.

In some cases pesticides are metabolised as an energy source for microbial growth (*biodegradation*) and in others transformed without usage for energy by microorganisms (*cometabolism*). In the former case, a chemical will be finally mineralised to carbon dioxide and inorganic components, while different microorganisms transform a pesticide molecule in the latter by sequential cometabolic attacks. The major reactions observed in microbial transformation of a pesticide consist of oxidative, reductive, and hydrolytic reactions, and some metabolites are known to be further conjugated (Katagi, 2006).

In many respects, the surface of particles at the top layer of a bottom sediment is partially covered with microbes such as hyphae of water molds (Hulbert *et al.*, 2002). In shallow water body, sunlight exposure would enhance algal activity at the water-sediment interface, resulting in formation of biofilms (Katagi, 2006). In additions, biofilm formation by the growth of microorganisms and bioturbation by sediment dwellers such as chironomids and oligochaetes may modify the distribution and degradation of pesticides (Katagi, 2006). Therefore, much of the particulate organic carbon that is delivered to the sediment-water interface is decomposed and re-introduced back into the water column as either dissolved organic carbon or mineralised carbonate species (Cole, 1983; Chiou, 1998). In order to estimate the pesticide behaviour in river water-riverbed sediment interface, knowledge of kinetics and

biodegradation mechanisms of a pesticide through laboratory work is a valuable approach.

## 1.5 Physico-Chemical Properties of the Herbicides Isoproturon and Mecoprop

In the same way that a pesticide is used to get rid an area of unwanted pests, an herbicide is fabricated to get rid of unwanted plant life. Unwanted plants include weeds, shrubs, unproductive bushes or trees, and other growth that takes nutrients away from crops and other useful plants. The following sections introduce the physico-chemical properties of the two herbicides isoproturon and mecoprop which were chosen for investigation in this study.

### 1.5.1 Isoproturon

Isoproturon or IPU is a trade name for 3-(4-isopropylphenyl)-1,1-dimethylurea or 3-p-cumenyl-1,1-dimethylurea with a molecule formula of $C_{12}H_{18}N_2O$ and CAS registry number of 34123-59-6. Isoproturon is a phenylurea herbicide. A structure of isoproturon is depicted in Figure 1.6.

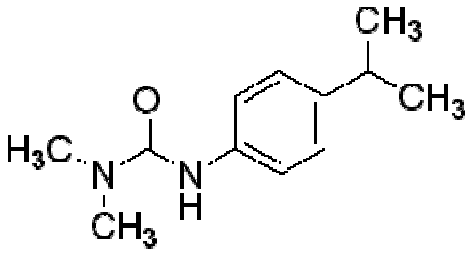



**Figure 1. 6** Structure of isoproturon

As one of the most extensively-used herbicides in Europe (Stangroom *et al.*, 1998) and in England and Wales (Garthwaite *et al.*, 2006), isoproturon was

widely used for pre- and post- emergence control of annual grasses and broad-leaved weeds in spring and winter wheat, spring and winter barley, winter rye, and triticale, at a 1.0 – 1.5 kg $ha^{-1}$ application rate (Tomlin, 2006). In Great Britain, approximately 7.9 x $10^6$ kg of herbicides were applied on 13 x $10^6$ treated area hectares in 2006, including 1.5 x $10^6$ kg of isoproturon used on more than 1 x $10^6$ treated hectares (Central Science Laboratory, 2008).

Below are several physico-chemical properties of isoproturon:

- *Physical chemistry*: Molar mass: 206.29 g $mol^{-1}$; Form: colourless crystals; Melting point: 158 $^oC$; Vapour pressure: 3.15 x $10^{-3}$ mPa (20 $^oC$); 8.1 x $10^{-3}$ mPa (25 $^oC$); Henry's constant: 1.46 x $10^{-5}$ Pa $m^3$ $mol^{-1}$; Density: 1.2 g $cm^{-3}$ (20 $^oC$); Solubility (mg $L^{-1}$, 22 $^oC$): in water 65, in methanol :75, in dichloromethane: 63, in acetone: 38, in benzene: 5, in xylol: 4, in n-hexane: 0.2 (all in g $L^{-1}$, 20 $^oC$); Stability: very stable to light, acids and alkalis; Hydrolytically cleaved by strong alkalis on heating; $DT_{50}$ 1560 d (pH 7) (Tomlin, 2006);
- Octanol/water partition coefficient, log $K_{OW}$ = 2.5 (20 $^oC$) (Tomlin, 2006); 2.25 (Worthing, 1991); 2.537 calculated (Evelyne *et al.*, 1992);
- Sorption partition coefficient, log $K_{OC}$ = 2.66 soil, calculated (Kenaga, 1980); 1.86 soil, HPLC-screening method (Kordel *et al.*, 1993); 2.11 soil (Kordel *et al.*, 1993), (Traubeberhard *et al.*, 1994);
- *Half-lives in the environment*:

  *Air*: 0.743 – 74.3 h, based on estimated rate constant for the vapour phase reaction with hydroxyl radicals in air (Atkinson, 1987);

  *Surface water*: 288 – 864 h, based on observed photolysis on soil plates under summer sunlight (Helling, 1976);

  *Groundwater*: 95 – 5040 h, based on unacclimated aqueous aerobic and anaerobic degradation half-lives (Howard *et al.*, 1991);

  *Soil*: 408 – 2520 h, based on aerobic soil die-away test data for one soil at 15 $^oC$ and 30 $^oC$ (Gingerich and Zimdahl, 1976); 15 – 21 days at 20 $^oC$

in soil (Traubeberhard *et al.*, 1994); estimated half-lives of 14.6 days under conventional tillage, 7.99 days under ridge tillage and 12.17 days with no tillage (Mackay *et al.*, 1997);

- *Environmental fate rate constants or half-lives:*

  *Photolysis*: atmosphere photolysis half-life of 288 – 864 h, based on observed photolysis on soil TLC plates under summer sunlight (Helling, 1976); aqueous photolysis half-life of 288 – 864 h, based on observed photolysis on soil TLC plates under summer sunlight (Helling, 1976); half-life of 1.5 h for 215 μg $mL^{-1}$ to degrade in distilled water under 254 nm light (Kulshrestha and Mukerjee, 1986).

  *Oxidation*: photooxidation half-life of 0.743 – 74.3 h in air, based on estimated constant for the vapor-phase reaction with hydroxyl radicals in air (Atkinson, 1987).

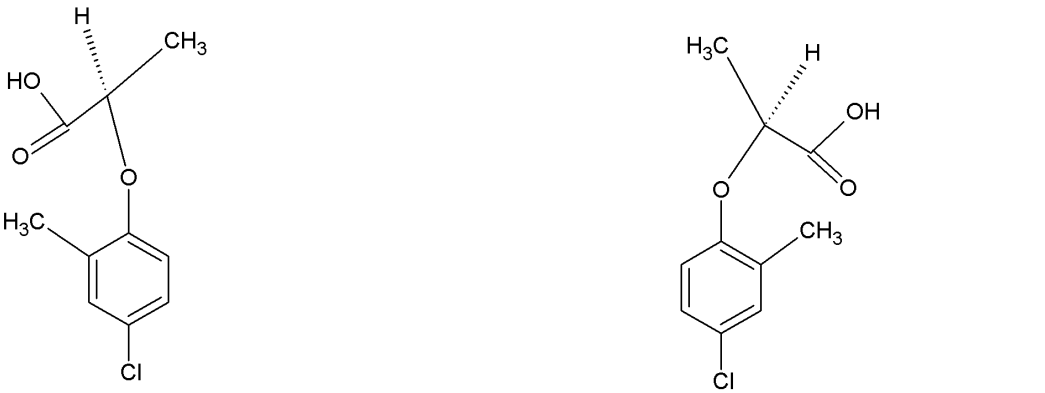
  *Biodegradation*: aqueous aerobic half-life of 408 – 2520 h, based on aerobic soil die-away test data for one soil at 15 $^{o}$C and 30 $^{o}$C (Gingerich and Zimdahl, 1976); aqueous anaerobic half-life of 96 – 360 h, based on anaerobic soil die-away test which tested one soil (Gingerich and Zimdahl, 1976).

## 1.5.2 Mecoprop

Another widely used herbicide is mecoprop. Mecoprop or MCPP is a trade name for (RS)-2-(4-chloro-o-tolyloxy)propionic acid or chemical abstract name of (±)-2-(4-chloro-2-methylphenoxy)propanoic acid with a molecule formula of $C_{10}H_{11}ClO_3$ and CAS registry number of 7085-19-0. It is one of a group of chlorophenoxyalkanoic or phenoxy acids used as a selective, hormone-type herbicide. Herbicide formulations contain mecoprop in the acid form, or as salts (potassium, dimethylamine, diethanolamine, sodium, magnesium) or esters (iso-octyl or 2-ethylhexyl) (Department of the Environment, 1994). However, mecoprop was most commonly applied in the UK in formulations as a salt (Fletcher *et al.*, 1995). Salt formulations of mecoprop are highly water

soluble (500 – 920 g $L^{-1}$ at 20 $^{o}$C) and therefore much more prone to leaching or poorly sorbed (Williams *et al.*, 2004; Buss *et al.*, 2006).

The presence of an asymmetric (chiral) carbon atom in the aliphatic side chain results in two different optically active forms (stereoisomers or enantiomers), the R-isomer and the S-isomer (Williams *et al.*, 2003), which have identical physical and chemical properties but behave differently in biological system. The herbicide mecoprop comprises equal proportions of the R- and S- isomers (known as a racemic mixture). However, only the R-isomer has herbicidal properties (Tomlin, 2006), and the product "mecoprop-P" has been developed containing only the R-isomer. *In this thesis, the term "mecoprop" or "MCPP" means mecoprop-P only unless otherwise specified.* Figure 1.7 illustrates the structure of two enantiomers of mecoprop.




R-mecoprop (herbicidal properties) S-mecoprop (no herbicidal properties)

**Figure 1. 7** Molecule structure of mecoprop, showing the two enantiomers. After (Environment Agency, 2001; Williams *et al.*, 2003).

Mecoprop was frequently applied to control broad-leaved weeds in cereal crop fields, ornamental lawns, sports turf, drainage ditches and banks and for forest site preparation, at 2 – 3 kg $ha^{-1}$ (Buss *et al.*, 2006; Tomlin, 2006). Approximately 0.5 x $10^6$ kg of mecoprop (including both enantiomers) were used in Great Britain on nearly 1 x $10^6$ treated hectares (Central Science Laboratory, 2008). Hence, mecoprop became the most frequently occurring

herbicide detected in United Kingdom groundwater and the second most common herbicide in UK surface water (Buss *et al.*, 2006). As a List 1 substance under the Groundwater Directive (80/68/EEC), by virtue of being an organohalogen, its direct or indirect application to groundwater is prohibited. As a List 2 substance under the Dangerous Substances Directive (76/464/EEC), discharges to surface waters must be minimised and concentrations of mecoprop must not exceed a concentration of 20 μg $L^{-1}$ in fresh, coastal or estuarine waters. In drinking water, the concentration of individual pesticide compounds must not exceed 0.1 μg $L^{-1}$ under the Drinking Water Directive (98/83/EC, 1998). For guidance on quantitative risk assessment of mecoprop transport in groundwater in the United Kingdom, reader is referred to the (Environment Agency, 2003, 2004).

Below lists several primary characteristics of mecoprop:

- *Physical chemistry*: Molar mass: 214.65 g $mol^{-1}$; Form: colourless crystals; Melting point: 93 – 95 $^{o}$C; Vapour pressure: 1.6 mPa (25 $^{o}$C); Henry's constant: 2.18 x $10^{-4}$ Pa $m^3$ $mol^{-1}$ (calculated); Solubility in water: 880 mg $L^{-1}$ (25 $^{o}$C), in acetone, diethyl ether, ethanol >1000, ethyl acetate 825, chloroform 339 (all in g $kg^{-1}$, 20 $^{o}$C); stable under the effects of heating and to hydrolysis, reduction, and atmospheric oxidation. Mecoprop is acidic, and forms salts, many of which are water-soluble; pKa 3.78 (Tomlin, 2006); the molecule will be ionised at neutral and alkaline pH values (Environment Agency, 2004).
- Octanol/water partition coefficient, log $K_{OW}$ = 0.1004 (pH 7); 3.2 (Chamberlain *et al.*, 1996); 3.94 (Dao *et al.*, 1983); 2.83 (Braumann *et al.*, 1983); 0.10 (Worthing, 1991); 0.09; 3.126, (Ilchmann *et al.*, 1993); 3.13 (Hansch *et al.*, 1995).
- Sorption Partition Coefficient, log $K_{OC}$ = 2.11 (Kenaga, 1980; Bottoni and Funari, 1992); 1.30 (Lohninger, 1994).

- *Half-lives in the environment*:

  *Air*: 3.8 – 37.8 h, based on an estimated rate constant for the vapour phase reaction with hydroxyl radicals in air (Atkinson, 1987).

  *Surface water*: 168 – 240 h, based on estimated aqueous aerobic biodegradation half-life (Howard *et al.*, 1991).

  *Groundwater*: 336 – 4320 h, based on aqueous aerobic and anaerobic biodegradation half-lives (Howard *et al.*, 1991).

  *Soil*: 168 – 240 h, based on aerobic soil grab sample data (Kirkland and Fryer, 1972; Smith and Hayden, 1981; Howard *et al.*, 1991); 21 days (Halfon *et al.*, 1996).

- *Environmental Fate Rate Constants or Half-lives*:

  *Oxidation*: photooxidation half-life of 3.8 – 37.8 h in air, based on an estimated rate constant for the vapor-phase reaction with hydroxyl radicals in air (Atkinson, 1987).

  *Biodegradation*: aqueous aerobic half-life of 168 – 240 h, based on aerobic soil grab sample data (Kirkland and Fryer, 1972; Smith and Hayden, 1981); aqueous anaerobic half-life of 672 – 4320 h, based on anaerobic digestor sludge data (Battersby and Wilson, 1989).

## 1.6 Aims, Objectives and Hypotheses

In response to the current interest regarding the potential of riverbank filtration in removing herbicides, this thesis describes research that was undertaken to improve our understanding of physical, chemical and biological interaction between herbicides and materials of a hyporheic zone. In pursuit of this aim, the primary objectives of this research were to:

(1) Investigate the attenuation of the herbicides mecoprop and isoproturon in a river water-riverbed sediment system using a fixed-bed column circulation method. Two dominant processes, sorption and biodegradation, were to be considered;

(2) Investigate the levels of catabolic activity, with respect to isoproturon, in incubated riverbed sediments obtained from the fixed-bed column experiments;

(3) Investigate the catabolic activity using $^{14}C$-radiorespirometry with respect to isoproturon in dosed and undosed treatments for river water, groundwater and riverbed sediment with different periods of incubation time;

(4) Investigate relationship between loss of isoproturon with respect to concentrations of isoproturon and levels of catabolic competence in the treatments;

(5) Comment upon the potential of riverbank filtration for the removal of the herbicides mecoprop and isoproturon in a wider context based on the results from the laboratory experiments. To these ends a river water-riverbed sediment interaction path length was estimated in order to assess

if boreholes at the Gatehampton study site would be protected from river water-born herbicide pollution

The following hypotheses form the foundation of the research:

(1) Herbicides will sorb on/into sediment. The extent to which this sorption takes place will be dependent upon a) herbicide physical and chemical properties, and, b) the properties of the sediment;

(2) Herbicides will be degraded in sediment. The extent of degradation will be dependent upon a) herbicide physical and chemical properties, and, b) microbial catabolic competence;

(3) Loss of herbicides will be most strongly dependent upon microbial catabolic competence in sediment rather than river water and/or groundwater;

(4) The addition of herbicide to sediment and/or river water and/or groundwater will increase the levels of catabolic competence;

(5) Levels of catabolic activity in sediment and/or river water and/or groundwater will be proportional to concentrations of herbicide present; with higher substrate concentrations promoting higher levels of catabolic competence;

## 1.7 Structure of this Thesis

According to the above objectives and hypotheses, a series of experiments was carried out under laboratory conditions. The outcomes of this study are presented in the subsequent six chapters:

- Chapter 2 introduces the characteristics, e.g. location, topography and climate, geology, hydrogeology and surface water-groundwater interaction at the Gatehampton field site where was chosen as the case study for this research. Readers can also find the proportion of river water-fed into the adjacent production boreholes in this chapter;

- Chapter 3 presents the field sampling methods and analytical methods. The HPLC method for measuring herbicide concentrations is also provided. Moreover, the experimental methods using in Chapters 4 and 5, including a fixed-bed column circulation method and a respirometry method, are introduced in this chapter. Development for the fixed-bed column circulation method is also presented;

- Chapter 4 presents two experiments which consider hypotheses 1, 2 and 3 (Section 1.6). Experiment 1 investigated the attenuation of mecoprop and isoproturon in a river water-riverbed sediment system using a fixed-bed column method. Sorption and biodegradation of these herbicides were examined as outcomes of this experiment. Experiment 2 investigated the catabolic competence with respect to isoproturon in incubated riverbed sediments (after 18 recirculation days) extracted from the end of Experiment 1.

- Chapter 5 presents three experiments which consider hypotheses 4 and 5 (Section 1.6). Experiment 1 investigated catabolic activity with respect to

isoproturon (IPU) in the IPU-undosed river water, groundwater and riverbed sediment treatments. Experiment 2 investigated catabolic activity with respect to isoproturon in the IPU-dosed river water, groundwater and riverbed sediment treatments which were dosed with isoproturon to give the final concentrations of 0.1, 1.0 and 100.0 μg $L^{-1}$. Incubation times of 0, 5, 10 and 30 days were also examined in both IPU-undosed and IPU-dosed treatments to investigate the influence of incubation time on the catabolisms of isoproturon. Experiment 3 aimed to determine the $^{12}$C-IPU residual concentrations at the point of the second addition of $^{14}$C-IPU (after periods of incubation) in the treatments with riverbed sediment.

- Chapter 6 places the results of Chapters 4 and 5 within a wider context of river bank filtration application. Context was provided with specific consideration of site constraints (Chapter 2) at the Gatehampton site. A simple model was offered to estimate the shortest path length between a river and a bank side borehole in order to assess whether or not the abstraction borehole could be protected from herbicide pollution from the river. This chapter serves to support the application of riverbank filtration to remove herbicide residues. Further context is provided with respect to water resources beyond the specific study site;

- Chapter 7 provides the conclusions of this study. Further research recommendations are also suggested in this chapter;

- Finally, the appendices are presented at the end of this thesis including the experimental data of Chapters 4 and 5.

# Chapter 2

# FIELD SITE DESCRIPTION

## 2.1 Introduction

To study the fate and behaviour of herbicides in a water-sediment interaction zone, it is necessary to understand the effects of what Tóth (1970) called the "hydrogeologic environment" on groundwater flow systems – that is topographic, climatic, geological, land use, hydrological and hydrogeological characteristics of a site. Indeed, these field characteristics are mutually affected and heavily influence on a water-sediment interaction zone. For instance, topographic and climatic properties control direction of surface water and groundwater flows, geological properties affect not only on the distribution of hydraulic conductivity but also on the physico-chemical properties of groundwater and surface water. Land use may change the direction flows (both surface water and groundwater) and may cause contamination for surface water and groundwater. These factors establish the hydrological and hydrogeological properties in an interaction zone.

This chapter aims to describe the previous field work that was reported to improve our understanding of physical, chemical and biological interaction of river water and groundwater in a hyporheic zone. In order to give a real environmental context to the study, a field site where water abstraction occurs was selected. The site chosen for this study was located at Gatehampton, south-west of Goring in Oxfordshire, England (National Grid Reference SU 600 797) (solid red circle in Figure 2.1a). The Gatehampton site is situated in a steep-sided valley close to the River Thames and consists of seven boreholes drilled through the Thames Gravels into the Chalk (Figure 2.1b). Groundwater in this site has been abstracted by Thames Water since 1990 and the current total pumping rate is approximately 65 x $10^6$ L $day^{-1}$. The peak licence for the source is 105 x $10^6$ L $day^{-1}$, making it one of the largest groundwater abstractions in the United Kingdom. Seven abstraction boreholes (denoted from A1 to A7in Figure 2.1b) are located approximately 100 to 500 m away from the River Thames. Investigation by Jackson *et* al. (2006a) showed that there was a significant contribution from the river to the boreholes. In the following sections of this chapter relevant environmental factors that related to the field site are described for reader's reference.

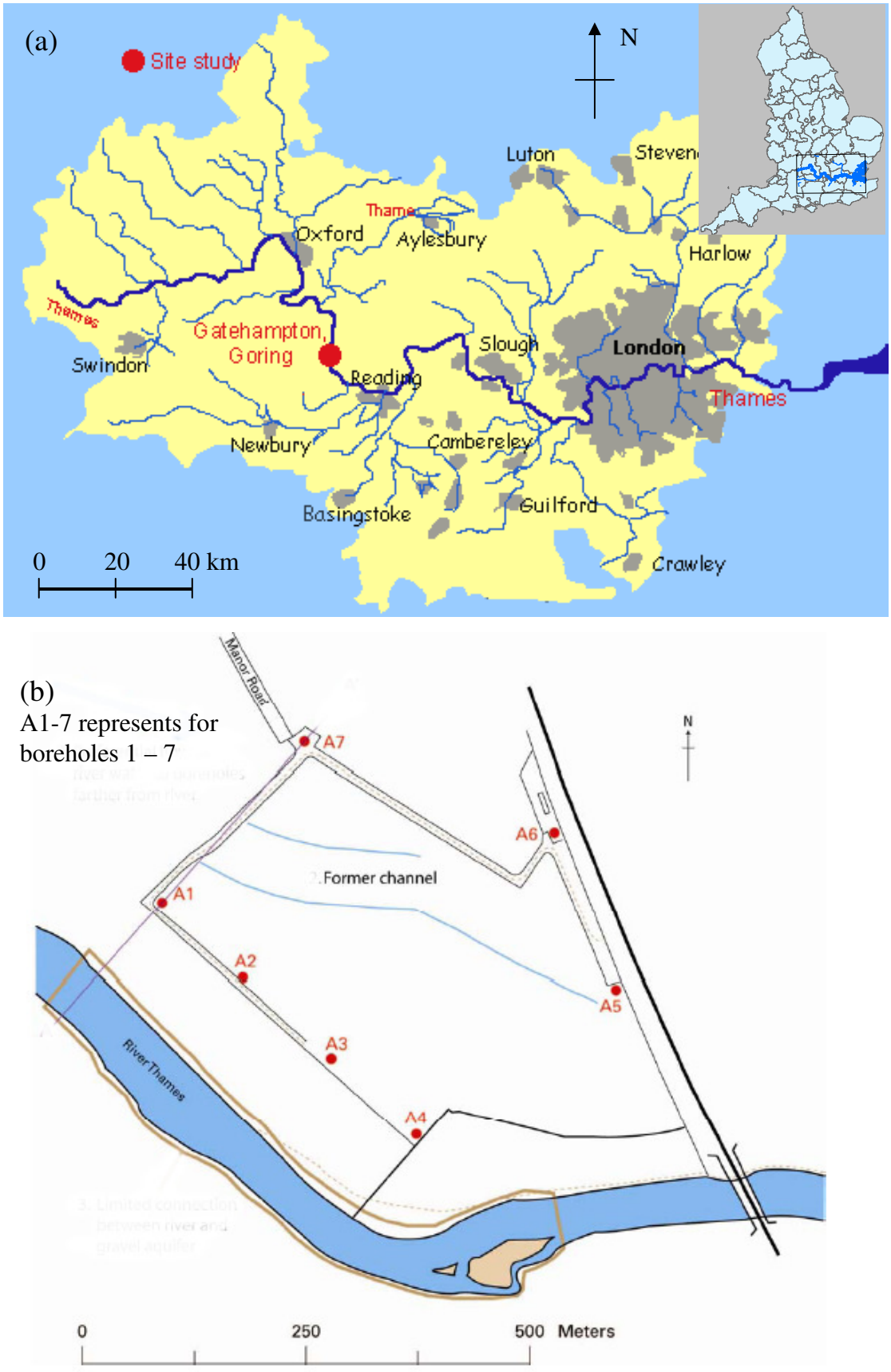



**Figure 2. 1** (a) - Location of the Gatehampton site study, adapted to Environment Agency; (b) - Location of the boreholes at the Gatehampton site.

## 2.2 Topography and Climate

The topography of the Gatehampton area is dominated by the Chalk escarpment, which forms the Chiltern Hills and the Berkshire Downs, and a number of deep valleys with perennial streams which breach the escarpment. Two such valleys are followed by the Thames and the Lea. In many places, the dry valleys of ephemeral streams occur as tributaries to the main valleys. On the upper high of the Chalk escarpment, elevations up to 300 m above Ordnance Datum Newlyn (AODN) are attained, while the valley floors lie at elevations between 40 m AODN (Goring Gap) down to 20 m AODN (Dorney).

In addition to topographic effects, surface water flow and groundwater flow are affected by climate. Precipitation and evaporation are the primary sources of recharge and discharge of surface water and groundwater. Daily rainfall data obtained for 69 rainfall gauging stations within the regional study area covering the Marlborough and Berkshire Downs and South-West Chilterns. Rainfall varied between approximately 550 and 950 mm $year^{-1}$ across the region (Jackson *et al.*, 2006a). Within the Goring Gap the mean rainfall was approximately 700 mm $year^{-1}$ but over the interfluves of the Marlborough Downs and South-West Chilterns the mean rainfall increased up to 850 mm $year^{-1}$ (Jackson *et al.*, 2006a). Monthly MORECS potential evaporation data collated for MORECS squares (Jackson *et al.*, 2006a) gave a mean monthly value of 50 mm. It was suggested that long term average potential evaporation varied much less over the region than rainfall.

## 2.3 Geology

Geological characteristics at the Gatehampton site have been investigated and modelised by GSI3D model (Jackson *et al.*, 2006a). The results from the model showed that the main component at the Gatehampton site was Chalk including such features from high to low levels as Seaford Chalk, Lewes Nodular Chalk, New Pit Chalk, Hollywell Nodular Chalk, the Zig Zag Chalk and the West Melbury Marly Chalk. The Chalk was the main aquifer from which the majority of groundwater abstraction is drawn. At the immediate borehole positions, the Chalk aquifer comprised the Hollywell Nodular Chalk, the Zig Zag Chalk and the West Melbury Marly Chalk. Figure 2.2 presents the geological cross-section across the Thames Valley and through Gatehampton.

For the purposes of this thesis, the geological investigation is focused on the riverbed and the vicinity of the borehole positions. According to Jackson *et al.* (2006a) and previous work, beneath the river Thames at Gatehampton, the gravels were approximately 3 m thick, possibly increasing to 10 m to the north-east of the ring of abstraction wells. However, there is uncertainty to the nature of the superficial deposits across the site.

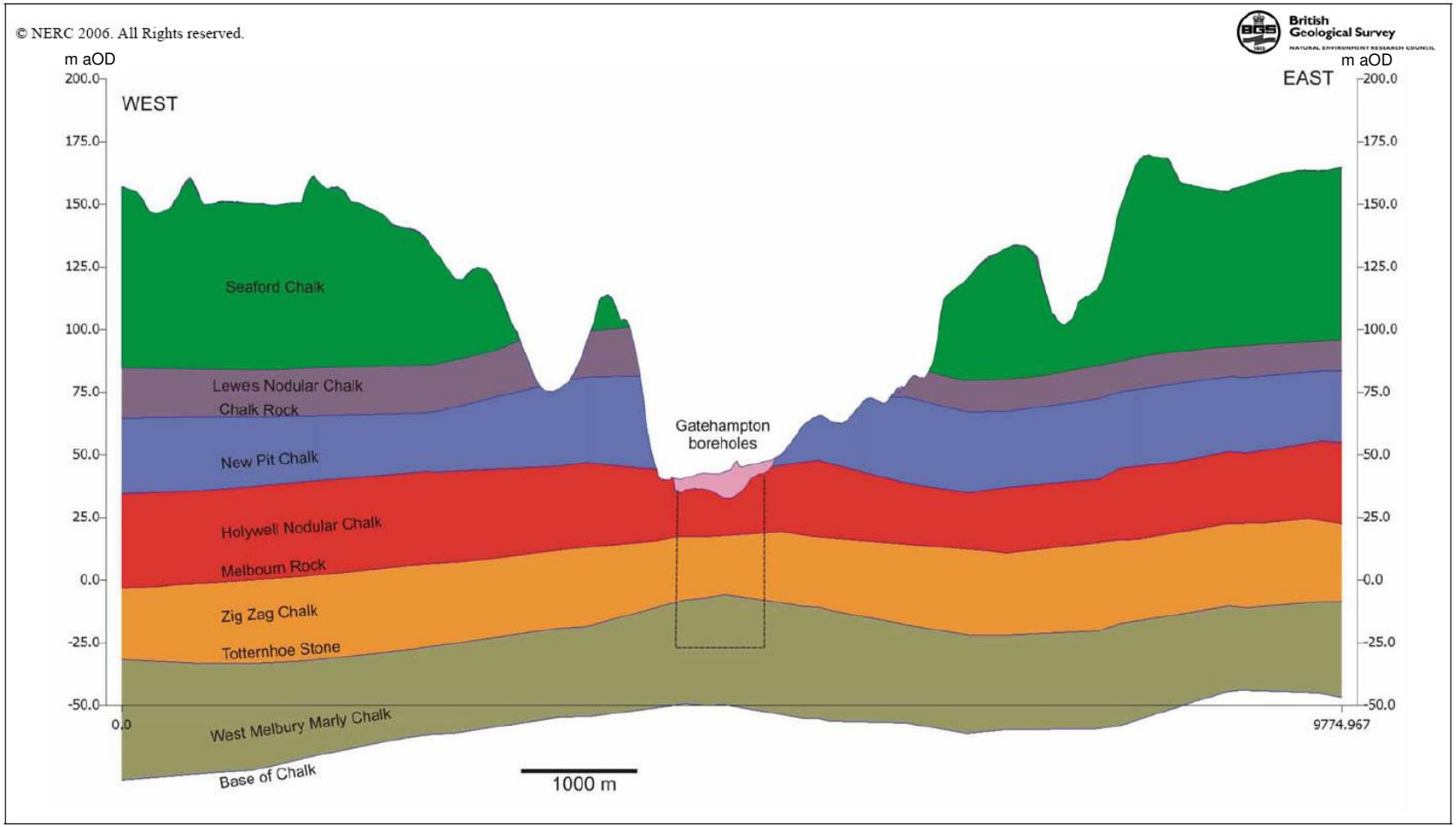



**Figure 2. 2** Geological cross-section across the Thames Valley and through Gatehampton. After Jackson *et al.* (2006a).

## 2.4 Land-use

An investigation performed by the Centre for Ecology and Hydrology resulted in the Land Cover Map 2000 (Release 1, January 2001) (Figure 2.3). Following this map, the land-use of the Thames region was predominantly arable or horticultural land. However, there were significant areas of improved or semi-natural grassland, woodland, and urban areas, particularly in the south-east regarding the latter. The land-use types were specified according to the following types: 1-Broad-leaved/mixed woodland, 2-Corniferous woodland, 3-Arable and horticulture, 4-Improved grassland, 5-Semi-natural grass, 6-Mountain, heath, bog, 7-Buit up area and garden, 8-Standing open water, 9-Coastal and 10-Oceanic seas. It was suggested that residuals of herbicides used in this region might caused pollution to surface water and groundwater sources.

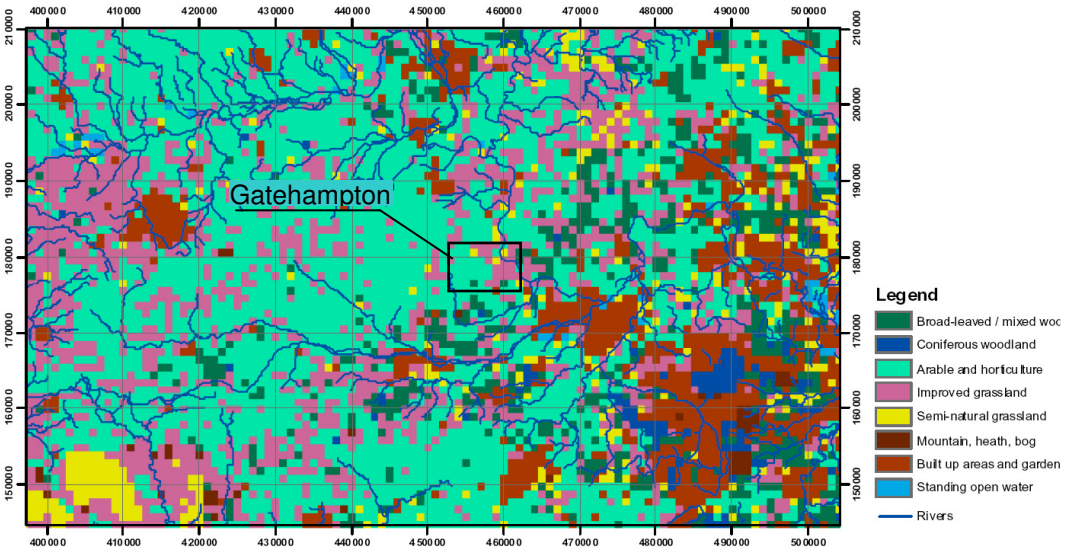



**Figure 2. 3** Predominant land-use types of the Thames Basin within each 1 km grid square (Land Cover Map 2000 Aggregate Class data ©NERC, 2006, quoted by Jackson *et al.* (2006a).

## 2.5 Hydrology

The study site is situated on the north-west of London and consists of the Marlborough and Berkshire Downs together with the South-West Chilterns. Mean total river flow of the River Thames at the Reading gauging station observed from November 1992 to December 2004 was 38.9 $m^3 s^{-1}$. A large number of rivers drain the area. However, the main river is the Thames which flows into the area at Benson and leaves the area at Windsor. The Thames, the major river draining southern-central and south-east England, rises in the Cotswold Hills of Gloucestershire and flows eastward to the North Sea between the counties of Essex and Kent. The Thames is some 346 km in length from its source 110 m above sea level, through its estuary at Southend-on-Sea. The Thames basin downstream to the Gatehampton site represents a rural farming area of approximately 3500 $km^2$ (Neal *et al.*, 2000).

## 2.6 Hydrogeology

Groundwater formed from many surface water bodies acts as reservoirs for storage of water and as conduits for transmission (Todd and Mays, 2005). The storage capacity of groundwater reservoirs combined with small flow rates provides large, extensively distributed sources of water supply.
Groundwater travels slowly for varying distances within the earth's crust until it returns to the surface by action of natural flow, plants or humans. Naturally, rivers in the system are one of the main outflows for groundwater and also control the direction of groundwater flow. However, when pumping action occurs in the boreholes near a surface water bodies (e.g. river, channel or lake), groundwater system become a conduit for transmission of water from the rivers to the boreholes. This is the case at the Gatehampton site where there are several boreholes located along the river Thames.

Hydrogeology at the Gatehampton site had been investigated since 1980s by the Thames Water Inc. and recently work has been reported by Jackson in 2006. According to these investigations, the groundwater system at the Gatehampton site consists of a number of aquifers. The Chalk is the main aquifer from which the majority of groundwater abstraction is drawn. Flows from the River Thames, runoff and septic tanks also recharge to the groundwater system. On the other hand, the main river in the system, the River Thames is the predominant control on groundwater flow. To identify direction of groundwater flow, groundwater level contours has been presented by Jackson *et al.* (2006a).

Following Jackson *et al.* (2006a), a long time-series of groundwater contours was conducted to determine how the pattern of groundwater flow changes in the vicinity of the Gatehampton site. A series of contours between 1 April (end of recharge period) and 1 October (end of recession period) 2004 are presented in Figure 2.4. In general, the groundwater flow for this area remains relatively consistent. Groundwater flow occurs towards the main rivers in the system including the River Thames, River Kennet and River Wye. Seasonal variations in 2004 were not significantly different, although greater variations occur in the interfluve area.

The chalk transmissivity of the area calculated from pumping test data showed that an estimated 25 % of values were less than 380 $m^2$ $day^{-1}$ and 75 % were less than 1500 $m^2$ $day^{-1}$ (Allen *et al.*, 1997). For the Gatehampton site, a mean value of transmissivity was reported of 6480 $m^2$ $day^{-1}$ (Jackson *et al.*, 2006b). Low hydraulic conductivity of approximately 0.002 m $day^{-1}$ was estimated for the bed of the River Thames lined with brown and grey organic-rich silts (Younger *et al.*, 1993). More recent work (Jackson C., *pers. comm.*) based on a numerical groundwater modelling suggested a riverbed hydraulic conductivity of between 0.05 – 1 m $day^{-1}$.

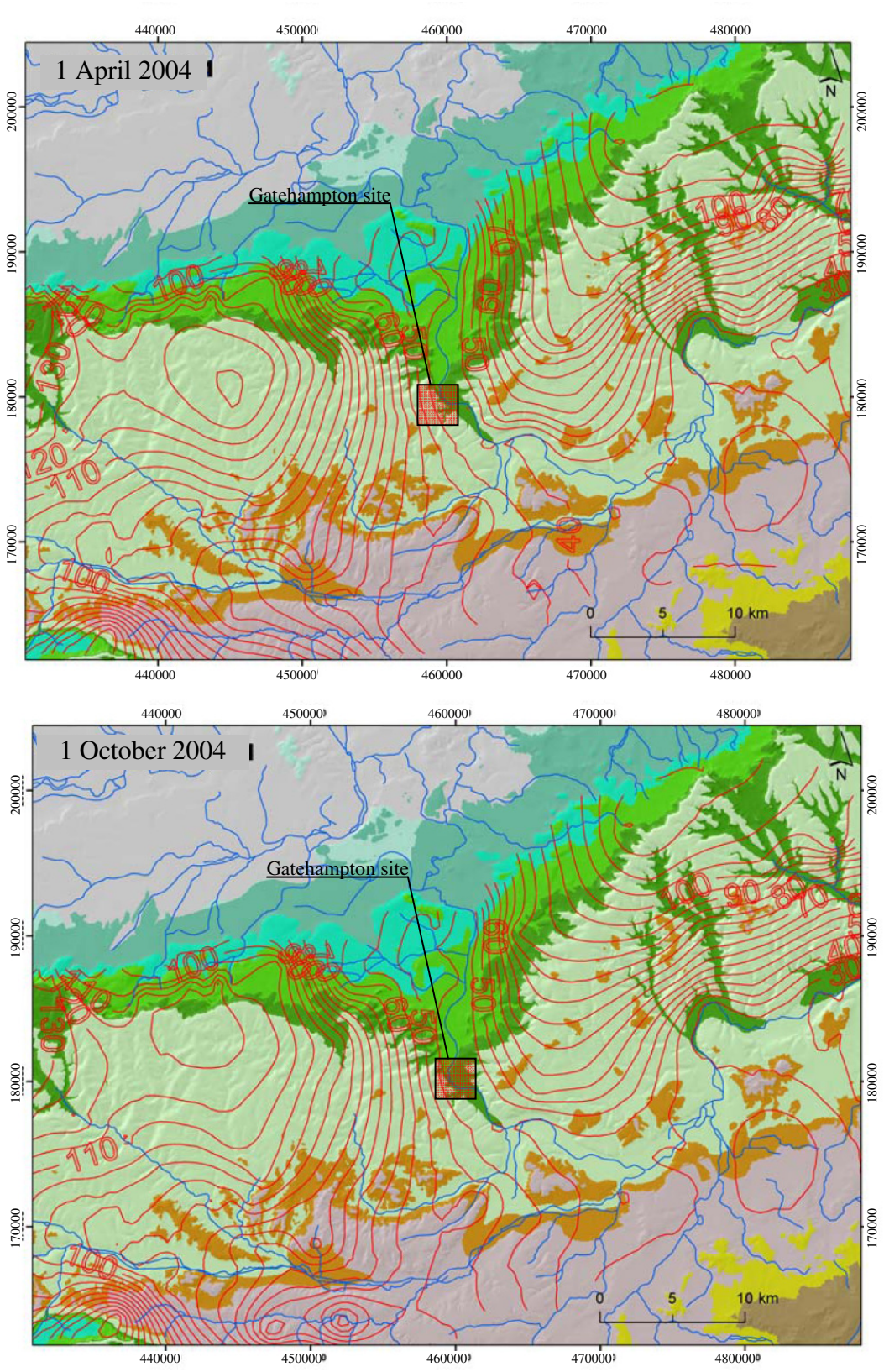



**Figure 2. 4** Groundwater contours at the Middle Thames Basin between 1 April and 1 October in 2004. After Jackson *et al.* (2006a).

## Development of Abstraction Boreholes at Gatehampton

The Gatehampton site was developed in the mid to late 1980s. Initially, seven boreholes were drilled and pumped into the public supply network at around 20 x $10^6$ L $day^{-1}$ in 1990. The total abstraction from the site increased to approximately 60 x $10^6$ L $day^{-1}$ by the end of 2004. The abstraction rate was set to increase further in the near future with the drilling of a new borehole to give an anticipated peak output from the site of over 100 x $10^6$ L $day^{-1}$. The development of output from boreholes in the Gatehampton area is illustrated in Figure 2.5. The water drawn from the boreholes was mainly used to meet demand in the South Midlands and Oxford areas.

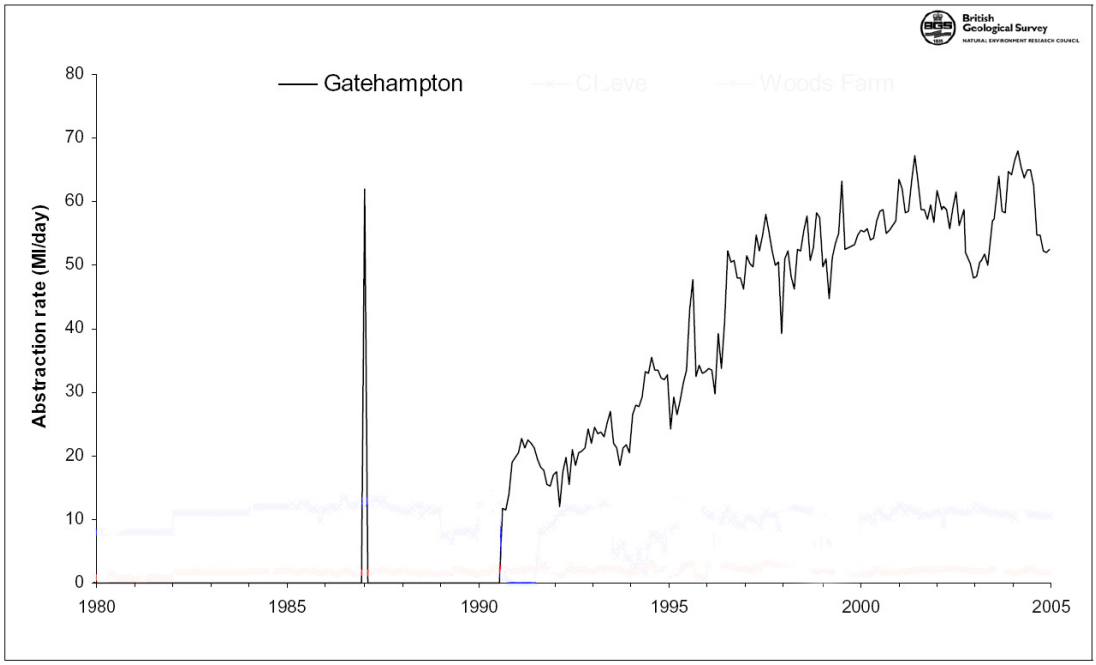



**Figure 2. 5** Time-series of groundwater abstraction at Gatehampton area. After Jackson *et al.* (2006a).

Evidence presented by Jackson *et al.* (2006a) indicated that the Gatehampton boreholes drew water from a combination of the three sources of water: (1) leakage from the River Thames (17 – 45%); (2) flow in the alluvium and valley gravel deposits underlying the Thames; and (3) the regional groundwater flow

in the Chalk aquifer. The connection with the River Thames was spatially variable and, therefore, the contribution of water from the Thames and the groundwater flow could also vary spatially and temporally.

## 2.7 Surface water-Groundwater interaction

Surface water today is groundwater tomorrow and vice verse. Surface water and groundwater are not isolated components of the hydrologic system, but instead interact in a variety of physiographic and climatic landscapes (Sophocleous, 2002). Thus, development or contamination of one commonly affects the other. Recently, attention has been focused on the surface water-groundwater interaction in a hyporheic zone where biogeochemical processes frenquently occur. In addition, many mathematical models have attempted to simulate the pathlines of the groundwater flows in the interaction zone. This section outlines the investigation of Jackson *et al.* (2006a) regarding the interaction between the River Thames and abstraction boreholes at Gathampton and the work of Barkwith (*pers. comm. of the British Geological Survey*) regarding the pathlines and resident time of river water-fed flow to the boreholes.

### 2.7.1 Interaction between the River Thames and the Abstraction Boreholes

At the Gatehampton site, the minimum and maximum groundwater levels of the boreholes were recorded by British Geological Survey to identify the relationship between the measured groundwater levels and river levels. These levels of the abstraction boreholes and the River Thames were plotted on simplified geological logs (Figure 2.6).

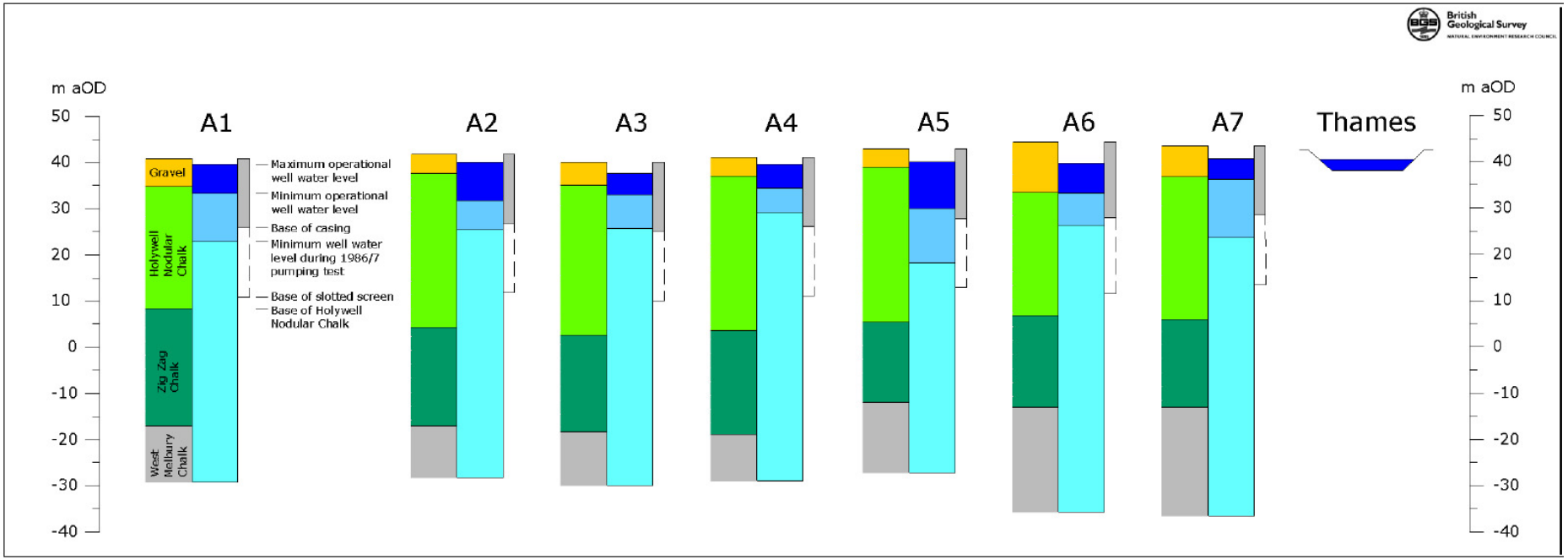



**Figure 2. 6** Schematic of water level variation in Gatehampton Chalk boreholes. After Jackson *et al.* (2006a).

It was noted that all the minimum site operational groundwater levels were below the base level of the River Thames and the majority of the maximum operational groundwater levels (boreholes 1, 3, 4, 5 and 6) were also below the stage of the River Thames. Moreover, examination of time-drawdown curves during the group pumping test (in 1986/7 by Thames Waters) and the River Thames stage hydrograph illustrated the augmentation of the boreholes by river water. Hence, it was suggested that there was a potential for the Chalk abstraction boreholes to draw river water throughout the bank.

British Geological Survey investigated the intrusion of water from the River Thames to the abstraction boreholes using three different approaches including (i) water chemistry, (ii) stable isotopes and (iii) atmospheric trace gases to estimate the proportion of river water in the boreholes. Following the first approach (water chemistry) river water percentages varied from 17% for Borehole 3 to 45% for Borehole 5. Stable isotope methods using $\delta^{18}O$ and $\delta^{2}H$ indicated that the proportion of river water in each borehole altered in a wide range from 5% to 55%. The last method, using the atmospheric trace gases CFCs and SF6, produced a variation of river water percentages from 20% to 55%. However, the mixing between groundwater and river water greatly depends on borehole abstraction rates, groundwater levels and river levels. The percentages of river water in each borehole obtained using these methods are shown in Table 2.1.

**Table 2. 1** Average percentages of river water in the Gatehampton site production boreholes (Jackson *et al.*, 2006a).

| Borehole | Method | | | Average (%) |
|---|---|---|---|---|
| | (i) Chemistry | (ii) Stable isotopes | (iii) Trace gases | |
| BH1 | 30 | 20 | 30 | 27 |
| BH3 | 15 | 10 | 25 | 17 |
| BH4 | 25 | 20 | 20 | 22 |
| BH5 | 45 | 35 | 55 | 45 |
| BH6 | 25 | 5 | 45 | 25 |
| BH7 | 30 | 55 | 45 | 43 |

The average for the river water component in Borehole 6 was identified to be 25%. The average for the river water component in all of the boreholes (excluding Borehole 2, which was not sampled) was determined to be 30%. Every method indicated that Boreholes 5 and 7 contained the highest proportion of river water, even though these boreholes are further from the river than Boreholes 1, 3 and 4. This suggested that boreholes 5 and 7 were better connected to the River Thames either directly thought the Chalk or via the overlying gravels.

### 2.7.2 Pathlines and Resident Time of River Water-Fed Flows to the Abstraction Boreholes

Along with the proportion of river water in the boreholes, flow-path of water from the River Thames to the boreholes and its resident time was estimated by Barkwith A. (*pers. comm. of the British Geological Survey*) using the ZOOPT particle tracking model. Relied upon the geological studies, it is obvious that the geological structure at the Gatehampton site greatly varied. For simplification, however, the model boundaries composed of 3 layers: layer 1 – gravel (3-10 m thick and 10-40% of porosity range); layer 2 – chalk (10-50 m thick and 1-6% of porosity range) and layer 3 – Lower Chalk (1% of porosity).

The mesh spacing in the borehole area is 25 m. Tracking particles in this area were marked from 339 – 402. After being run for 50 days, the paths of individual particles is presented in Figure 2.7

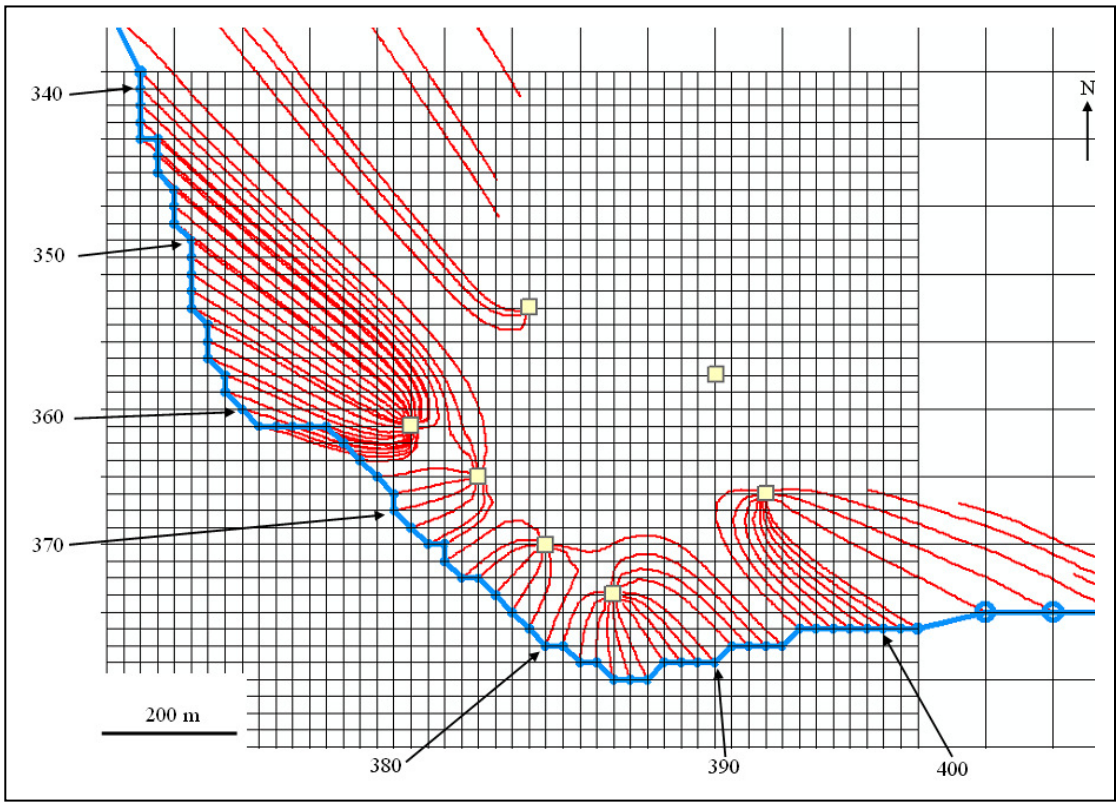



**Figure 2. 7** Numerical flow model (ZOOPT) representation of groundwater flow (red lines) to the chalk abstraction boreholes (yellow boxes) from the River Thames (blue line). After Barkwith A. (*pers. comm. British Geological Survey*).

Relied upon the different porosities of the three layers, the resident times taken for each particle from the River Thames to reach its destination (the boreholes) are presented in Figure 2.8.

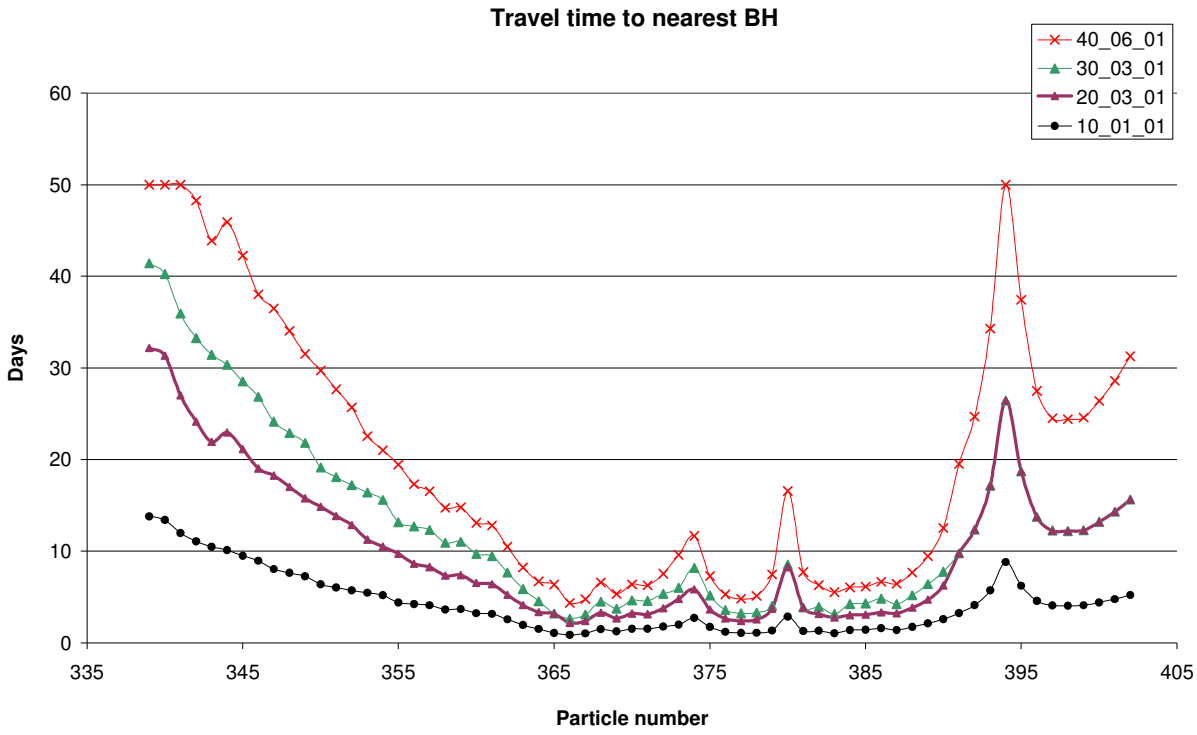



**Figure 2. 8** Travel times for each particle form the river to the borehole (ZOOPT particle tracking model). After Barkwith A. (*pers. comm. British Geological Survey*).

The resident times were determined varying between 0.5 and 50 days, with the lower porosities giving faster transport times. Relied upon this resident time and assumed the distance from the river to the nearest borehole to be 100 m, the velocity of the river water-fed flow was calculated varying from 2 to 200 m $day^{-1}$. Although this result was calculated with respect to an inert particle, it was a very important input to design the flow rate and the volume of a column in the fixed-bed column circulation experiment which will be introduced in the next chapter.

## 2.8 Summary

Examination the above field characteristics, it is provided that Chalk aquifer was the main source of groundwater at the Gatehampton area. There are seven abstraction boreholes operating in this site with a total pumping rate of 65 x $10^6$ L $day^{-1}$ and increasing to 100 x $10^6$ L $day^{-1}$ in the near future. A recent study reported that the abstraction boreholes received between 17 – 45% of river water. This is one of the crucial addresses of this thesis because, with the fraction of river water/groundwater varying in 17 – 45%, the quality of river water will definitely affect to the quality of groundwater. The particle tracking model provided the resident times of a particle varying between 0.5 and 50 days. Relied upon this result, a velocity of the river water-fed flow to a borehole varied between 2 and 200 m $day^{-1}$. Therefore, if river water is polluted by residual herbicides, the question is whether or not groundwater abstracted from the vicinity boreholes will be polluted by the same herbicides originating from the river water? Following chapters try to answer this question.

# Chapter 3

# SAMPLING and EXPERIMENTAL METHODS

## 3.1 Introduction

This chapter provides an account of the field work in which natural samples were collected and the laboratory work in which experiments were carried out. The analytical methods measuring the physico-chemical properties of the field samples are presented following the sampling methods. The principles of the fixed-bed circulation column method and respirometery method using in Chapters 4 and 5 are also introduced. High performance liquid chromatography (HPLC) analytical method is presented as a common method to measure concentration of herbicides mecoprop and isoproturon. Figure 3.1 outlines the data acquisition process for the field and laboratory work.

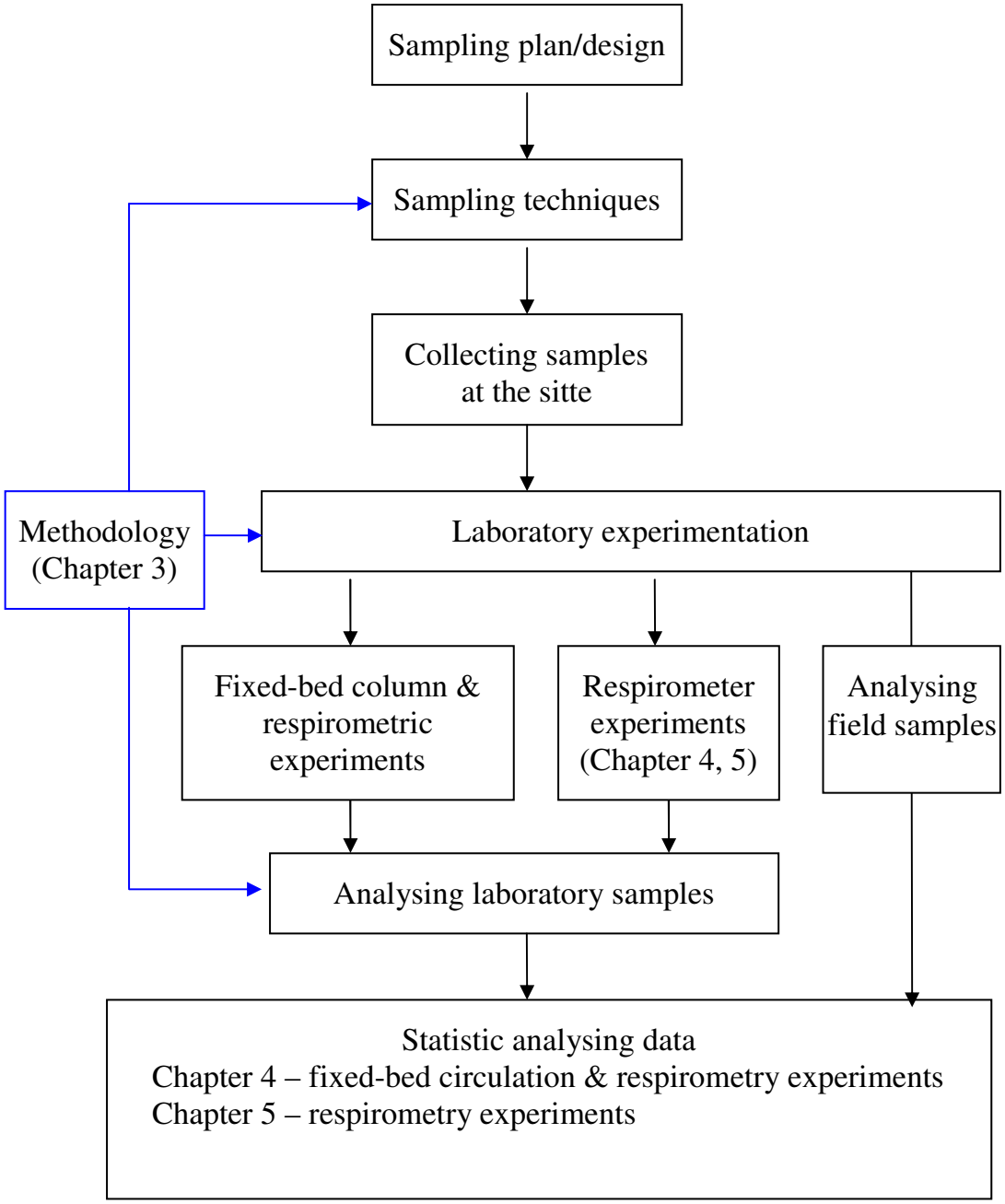



**Figure 3. 1** Data acquisition process for the current study

## 3.2 Sampling and Analytical Methods

The site was visited several times for initial investigation, subsequently two trips planned to collect riverbank materials on: (1) 14 September 2007, and (2) 18 April 2008. Before going into the field for sampling, a plan was made with the following notes:

- *Quantitative*: designed in consideration of the required laboratory analyses and experiments;
- *Position*: designed to obtain representative samples;
- *Time*: designed for transportation and laboratory storage so that no significant change occurs in the samples;
- *Apparatus*: designed for proper collection of samples in consideration of available instruments and resources.

According to the above notes, natural materials including river water, groundwater and riverbed sediment were collected at the Gatehampton site (see Section 2.1 for details). Several parameters were measured at the field site, e.g. pH, temperature, dissolved oxygen and electricity conductivity. Other parameters were conducted in laboratory. The samples were transported to laboratory on the same day. They were stored in a cold room (4 $^{o}$C) and dark for a maximum of 7 days before using for experiment and analysis.

### 3.2.1 River Water Sampling Method

River water samples were collected at the Gatehampton site. The river water was obtained on the surface of the River Thames and approximately 5 m away from the bank (National Grid Reference SU 600 797). Approximately 10 L of river water was collected in a plastic bucket (rinsed by the river water five times before use) and the several parameters (e.g. pH, temperature, dissolved

oxygen and electricity conductivity) were immediately measured. Thereafter, 40 L of river water were obtained in four 10 L-polyethylene containers after rinsing five times. No bubble or headspace was allowed in the containers (to reduce diffusion and volatilization processes). The samples were transported to laboratory in the same day and kept in a cold and dark room (4 $^{o}$C). It was noted that because the river water and the riverbed sediment samples were collected at the same position, thus the river water samples were obtained prior to the riverbed sediment samples in order to minimise the effects from disturbing the riverbed.

### 3.2.2 River Water Analytical Methods

Thermometer, Hanna pH meter and Hanna HI 9142 Dissolved oxygen meter were used to measure the field site parameters including temperature, pH, dissolved oxygen and electrical conductivity (three replicates). Other physico-chemical parameters were measured at laboratory.

Alkalinity was analysed on the following day of the field trip. A volume (50 mL) of the river water sample was added with 5 drops of phenolphthalein indicator and 5 drops of B.D.H 4.5 indicator then titrated by HCl 0.01M to grey colour (from colourless). $HCO_3^-$ concentration was estimated by multiplying the alkalinity value with 61.

Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) were measured using the Thermalox TN, TC analyzer (high temperature combustion method). The stock solution for TN analysis (200 mgN $L^{-1}$) was prepared from dissolving 1.4443 g $KNO_3$ in 1000 mL MiliQ water. The standard solutions for TN analysis of 0, 5, 10, 15, 20 and 25 mgN $L^{-1}$ were automatically prepared by the Thermalox. The stock solution for TC and TOC analysis (1000 mgC $L^{-1}$) was prepared from dissolving 2.1255 g of potassium acid phthalate ($COOHC_6H_4COOK$) in 1000 mL MiliQ water. The standard solutions for TC

analysis of 0, 20, 40, 60, 80 and 100 mgC $L^{-1}$ were automatically prepared by the Thermalox. The TOC of the sample was calculated by subtracting from the TC and its TIC (total inorganic carbon) value (TOC = TC – TIC). The TIC was separately measured by acidified the sample with HCl 10% then analysed using the Thermalox.

Anions $Cl^-$, $NO_3^-$, $SO_4^{2-}$ and cations $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $K^+$ were measured using the Dionex DX600+DX320 Ion Chromatography (IC) system (first audit trail 2002, made in Sunnyvale, USA). The stock solutions for anions were prepared from $NaNO_3$, $K_2SO_4$ and NaCl. The stock solutions for cations were prepared from NaCl, $CaCl_2$, $MgCl_2$ and $K_2SO_4$. The standard solutions were made in appropriate with the estimated concentrations of these anions and cations in river water. The set-up for anion analysis was of column of AG18 & AS18 2x250mm, eluent of 31mM KOH, isocratic mode, temperature of 30 $^oC$. The set-up for cation analysis was of column of CG12A & CS12A 2x250mm, temperature of 35 $^oC$, eluents of MiliQ water (C) and 20 mM methanesulfonic acid (MSA) (D), gradient mode following the ratio minutes:%C:%D of 15:75:25 then 30:0:100 then 6.2:75:25.

### 3.2.3 Groundwater Collection Method

Groundwater sample was collected from borehole 6 at the Gatehampton site (National Grid Reference SU 604 800, see Figure 2.1 for borehole 6) on 18 April 2008. This Chalk abstraction borehole is located approximately 500 m away from the River Thames. The borehole depth is 76 m below the surface. Groundwater was obtained during the borehole was pumping for supply.

Before sampling, groundwater was pumped out for 15 minutes from the sampling tap of the borehole. Parameters e.g. pH, temperature, dissolved oxygen and electricity conductivity were immediately measured in a plastic bucket (rinsed 5 times before use). Then, 40 L of groundwater was collected in

the four 10L-polyethylene containers after five times rinsing by groundwater. The containers were tightened with a strong cap without bubbles or headspace inside. The samples were then transported to laboratory on the same day and kept in a cold (4 $^{o}$C) and dark room. Groundwater samples were used within 3 days for the experiments in Chapter 5.

### 3.2.4 Groundwater Analytical Methods

Analytical methods to determine the groundwater properties were similar to the methods using for river water analysis (Section 3.2.2).

### 3.2.5 Riverbed Sediment Collection Method

Riverbed sediment samples were collected from the bed of the River Thames at the Gatehampton site (National Grid Reference of SU 600 797 – the same position where the river water was collected). Composite samples were designed to obtain the riverbed sediments. Figure 3.2 shows the plan of the nine positions from which riverbed sediments were collected.

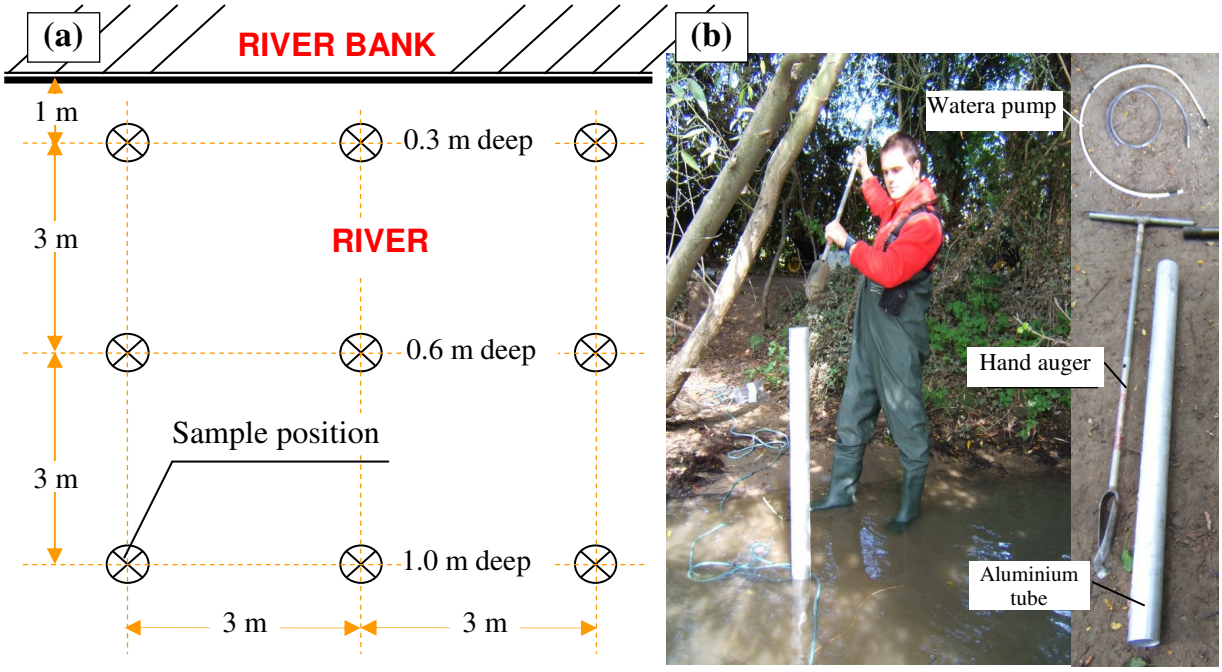



**Figure 3. 2** (a) – collecting positions of riverbed sediments at the River Thames (National Grid Reference SU 600 797); (b) – illustration for sampling the riverbed sediments.

Riverbed sediment was collected using a hand auger. An aluminium tube (10 cm diameter, 120 cm length) was hammered into the bed to keep it stable. A Waterra inertial pump was used to suck the water out. The hand auger was then inserted into the tube to obtain the sediment samples. Approximately 1 kg of sediment was removed at each position. The sediment samples were then thoroughly mixed together by hand in a polyethylene box (100 L). Subsequently, the mixed sediment samples were transported to laboratory on the same day and kept in a cold (4 $^{o}$C) and dark room. The riverbed sediments were used within 3 days for the experiments described in Chapters 4 and 5.

### 3.2.6 Riverbed Sediment Analytical Methods

The density, specific surface area and particle size distribution of the riverbed sediment sample was determined by the Mastersizer 2000 Version 5.30.010 (Malvern Instrument Ltd.). The porosity, *n*, was calculated using equation 3.1:

$$n = \frac{V_v}{V_t} = \frac{m_w}{V_t} = \frac{m_{wet,sed} - m_{dry,sed}}{V_t} \quad (3.1)$$

Where

$V_v$ – volume of void;

$V_t$ – total volume of the sediment;

$m_w$ – mass of water in the sediment sample, was the difference in mass before and after drying;

$m_{wet,sed}$ – mass of wet sediment;

$m_{dry,sed}$ – mass of dry sediment, determined by drying the sediment sample in an oven for 72 hours at 105 $^oC$;

The bulk density of the sediment sample, $\rho_b$, was determined as $\rho_b = \frac{m_{dry,sed}}{V_t}$

The pH value of the sediment sample was determined by dilute the sediment sample in MiliQ water with a ratio of 1:5. Then the pH meter (HANNA pH Meter) was used to measure the pH of the solution.

Parameters such as TN, TC, TOC and sulphur were analysed within 7 days of sample collection. To measure TC, the sediment (approximately 20 g wet) was dried at $40^0C$ for 48 h. Shell, leaf fragments greater than 0.5 cm in size were removed before the sediment was homogenized by mortar and pestle. Sediment was then stored at room temperature in desiccator until analysis. For TOC measurement by direct acidification method, a 5 g of sediment sample was transferred to a glass vial (10 mL), acidified by 1mL of sulphurous acid 6% w/v $SO_2$, and allowed to effervesce. The acidified samples were dried in an

oven at 40 $^{o}$C. This procedure was repeated until the samples cease to effervesce (5 times). The dry samples were also homogenized by mortar and pestle and stored at room temperature (20 $^{o}$C) in desiccator until analysis. C, H, N, and S analysis was performed using a Carlo Erba Instrumemts SHNS-O EA 1108 Elemental Analyzer. The technique used for the determination of CHN and CHNS was based on the quantitative "*dynamic flash combustion*" method. The samples were held in a tin container, placed inside the autosampler drum where they were purged with a continuous flow of helium and then dropped at preset intervals into a vertical quartz tube maintained at 904 $^{0}$C (combustion reactor). When the samples were dropped inside the furnace, the helium stream was temporarily enriched with pure oxygen and the sample and its container melted and the tin promotes a violent reaction (flash combustion) in a temporary enriched atmosphere of oxygen. Under these favourable conditions even thermally resistant substances were completely oxidized. During this process, $CO_2$, $H_2O$, $NO_x$ and $SO_2$ gases were produced. Quantitative combustion was then achieved by passing the mixture of these gases over the catalyst layer. The mixture plug of combustion gases was then passed over copper and removed the excess of oxygen and to reduce the nitrogen oxides ($NO_x$) to elemental nitrogen ($N_2$). The resulting mixture was directed to the chromatographic column (porapak PQS) where the individual components were separated and eluted as Nitrogen ($N_2$), Carbon dioxide ($CO_2$), water and Sulfur dioxide ($SO_2$) with the help of a Thermal Conductivity detector whose signal fed a potentiometric recorder or an Integrator or the automatic workstation known as EAGER 100.

## 3.3 Experimental Methods

In general, there were two types of bioreactors for testing the destruction of organic pollutants applying in this thesis, relying on either immobilised cells or suspended growth of microorganisms. With the first type, microorganisms were fixed on some types of support and so were not removed during the effluent leaf the reactor. This approach was applied for designing a fixed-bed column reactor. With the second type, microorganisms presented in a suspension continuously and they could grow freely in water or attached to soil or sediment that was maintained in suspension. This approach was applied for designing a respirometer. Sections 3.3.2 and 3.3.3 present the principles and the method development of the fixed-bed column circulation method and the respirometry method.

### 3.3.1 Fixed-Bed Column Circulation Method

#### *3.3.1.1 Introduction of a fixed-bed column*

A fixed-bed column or also known as a testfilter has been developed and applied for simulating degradation of organic compounds during bank filtration processes for many of years (Sontheimer, 1988; Malzer *et al.*, 1992; Knepper *et al.*, 1999; Bornick *et al.*, 2001). The conceptual design of the testfilter is relied on the fixed-bed biological reactor principle. The principle of a fixed-bed column circulation system is depicted in Figure 3.3.

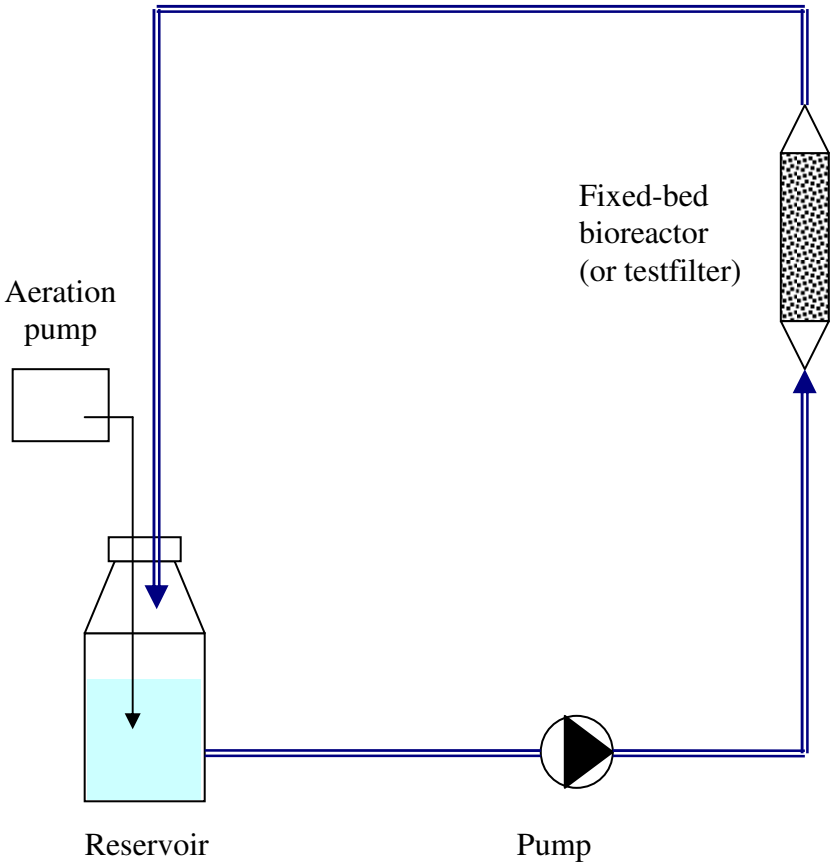



**Figure 3. 3** Principle of a fixed-bed column circulation system or a testfilter system for simulating the degradation of organic compounds during bank filtration. After Knepper *et al*., (1999).

In a fixed-bed column circulation system, the column is filled with loose packing materials e.g. activated carbon (Alexander, 1999; Knepper *et al.*, 1999), inert solid material (pumice stone or Hydrofilt) (Bornick *et al.*, 2001; Worch *et al.*, 2002), alginate beads, diatomaceous earth, hollow glass fibres, polyurethane foam, polyacrylamide beads (Alexander, 1999) or riverbed sediment as in this study. Contaminated water is circulated through the column packing with solid material. Aerobic condition is maintained by an aeration pump. During water is percolated through the fixed-bed column, it is suggested that biofilm is formed on the solid packing material which brings about a rapid biodegradation as account of the high cell density (Alexander, 1999; Knepper *et al.*, 1999; Worch *et al.*, 2002). A modification of fixed-film

treatment employs immobilised or strongly sorbed cells. The cells are immobilised by firmly attaching the organisms or physically embedding them in the solid matrix. Common to many of these systems is the greater tolerance to high chemical concentrations of the cells that are in the films or that are immobilised than cells in suspension. The greater resistance may be associated with sorption of the substrate to the solid or immobilising material, thereby reducing the amount available to suppress the microorganisms, or to some other mechanism (Alexander, 1999).

#### *3.3.1.2 Development of a fixed-bed column*

This section describes the developmental work undertaken during the course of this research to design, build and validate a fixed-bed test system for the assessment of herbicide fate in a river water-riverbed sediment system.

***(1) Designing the column***

Based on the principle of a fixed-bed column bioreactor (Alexander, 1999) and the conceptual design of a testfilter developed by the German people e.g. Sontheimer (1988), Knepper *et al.* (1999), Bornick *et al.* (2001) and Worch *et al.* (2002), a fixed-bed column using riverbed sediment packing material was designed for this study. The column was made by glass in order to minimise the sorption and reaction of chemicals on the column wall. The column has 90 mm long and 40 mm inside diameter. Four versions of the fixed-bed column have been developed during this research. Figure 3.4 illustrates the development of four versions of the fixed-bed column.

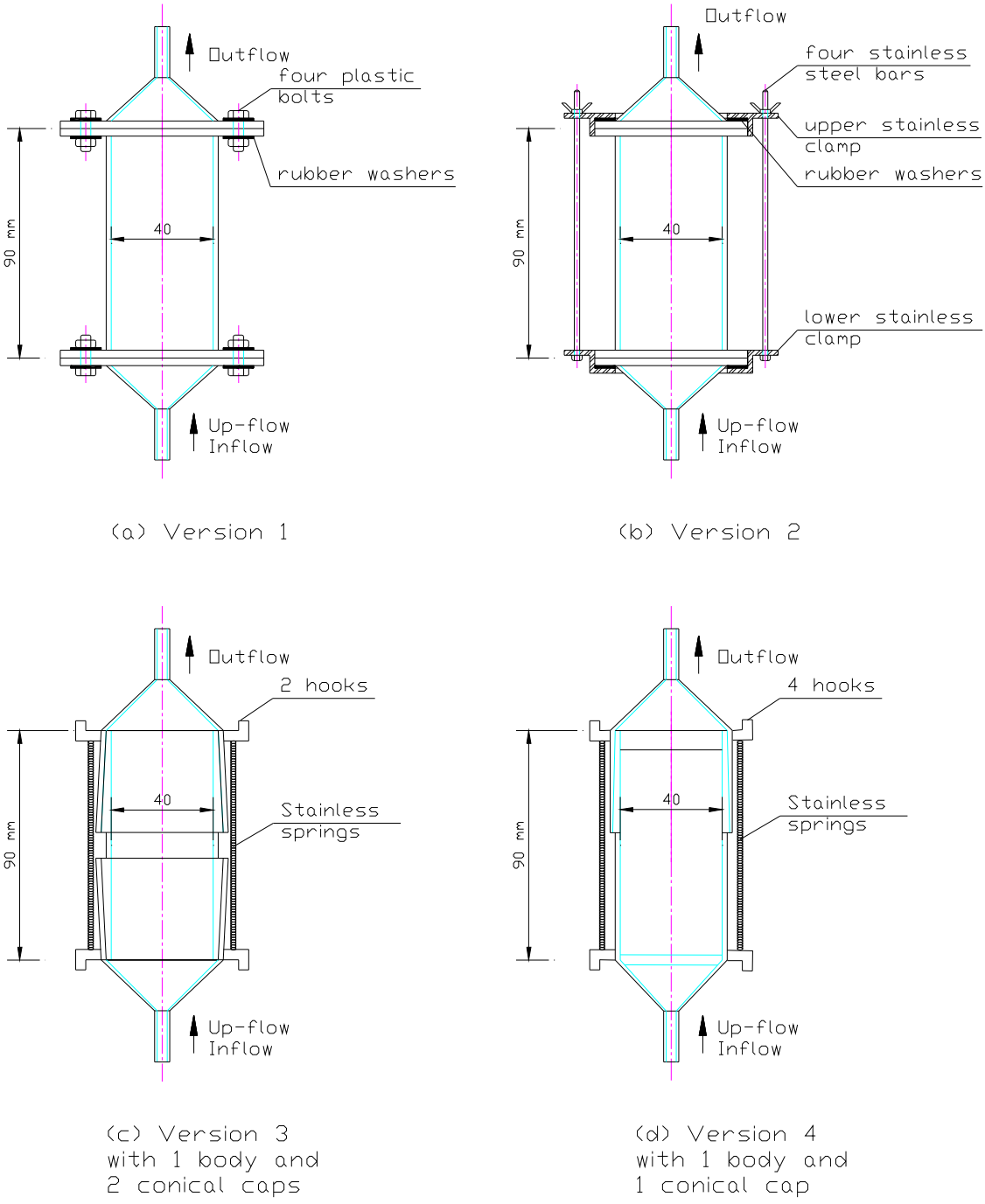



**Figure 3. 4** Developing a fixed-bed column (testfilter).

Version 1 (Figure 3.4a), the body and two end caps were connected together by 8 sets of plastic bolts and nuts (4 for each end). The column was firstly packed with pumice stone (inert material). It worked properly as particle sizes of the pumice stone were large. Hence the pressure inside the column was low. However once riverbed sediment was packed in the column, pressure considerably increased due to fine particle size of the sediment. This resulted in leaking and in several cases the column was broken due to high pressure. In addition to, with this version the cap and the body of the column were easily broken once the plastic bolts were tightened to connect these parts together.

Version 2 (Figure 3.4b) was made to improve the weaknesses of Version 1. Two stainless clamps were made to house the body and the two end caps by four stainless steel studdings and wing nuts surround the clamps. This design improved the leaking problem and minimised the incidences of breakage. However, this design was not convenient for packing material because the lower end cap and the body were not affixed together once the upper end cap was taken off for packing material. Furthermore, it took a long time to manufacture the stainless clamps and they were high cost (after making the columns from the glass workshop, the mechanic workshop took 6 weeks for making two sets of the clamps).

Version 3 (Figure 3.4c) represented an absolutely new design with two conical end caps. These caps were connected with the body by conical joints and strengthened by stainless springs and hooks surrounded four sides of the column. This design allowed packing sediment quickly and easily. Leaking problem was also solved by the conical joint. After a period for testing, the follow advantages of this version were sustained: (1) easy for packing material, (2) quicker for manufacturing and (3) cheaper for production cost. However, it was also noted that the lower conical cap was not always essential; as a consequence further refinements were made to Version 3 and resulted in Version 4.

With Version 4 (Figure 3.4d), the lower conical joint and cap was removed. The body was connected with one upper conical cap by the conical joint and stainless springs and hooks. This improvement made the material packing and closing the cap becoming easier. This design allowed savings in terms of time and money [(*)]. Figure 3.5 shows a detail design drawing of Version 4 of the fixed-bed column bioreactor.

Although the fixed-bed column was markedly improved, in operation, leaking problem was still possible. It could make interrupting the system. Leaking could be resulted from:

- the conical joint between the body and the cap of the column due to high pressure inside the column;
- the connection positions between the glass and rubber parts of the system;
- breakage of the column because of high pressure inside the column;
- blocking filters which was used at two ends of the body to retain the fine sediment particles in inside the column.

[(*)] However, it might be value to present here that after successfully testing with two columns Version 3, the glass workshop in the UEA had to be refurbished for 3 months. Therefore, in order to produce enough the quantity for the experiments (12 sets of column), I had to persuade the School and work with the Cambridge glass workshop to make the columns. Finally, the columns Version 4 were made.

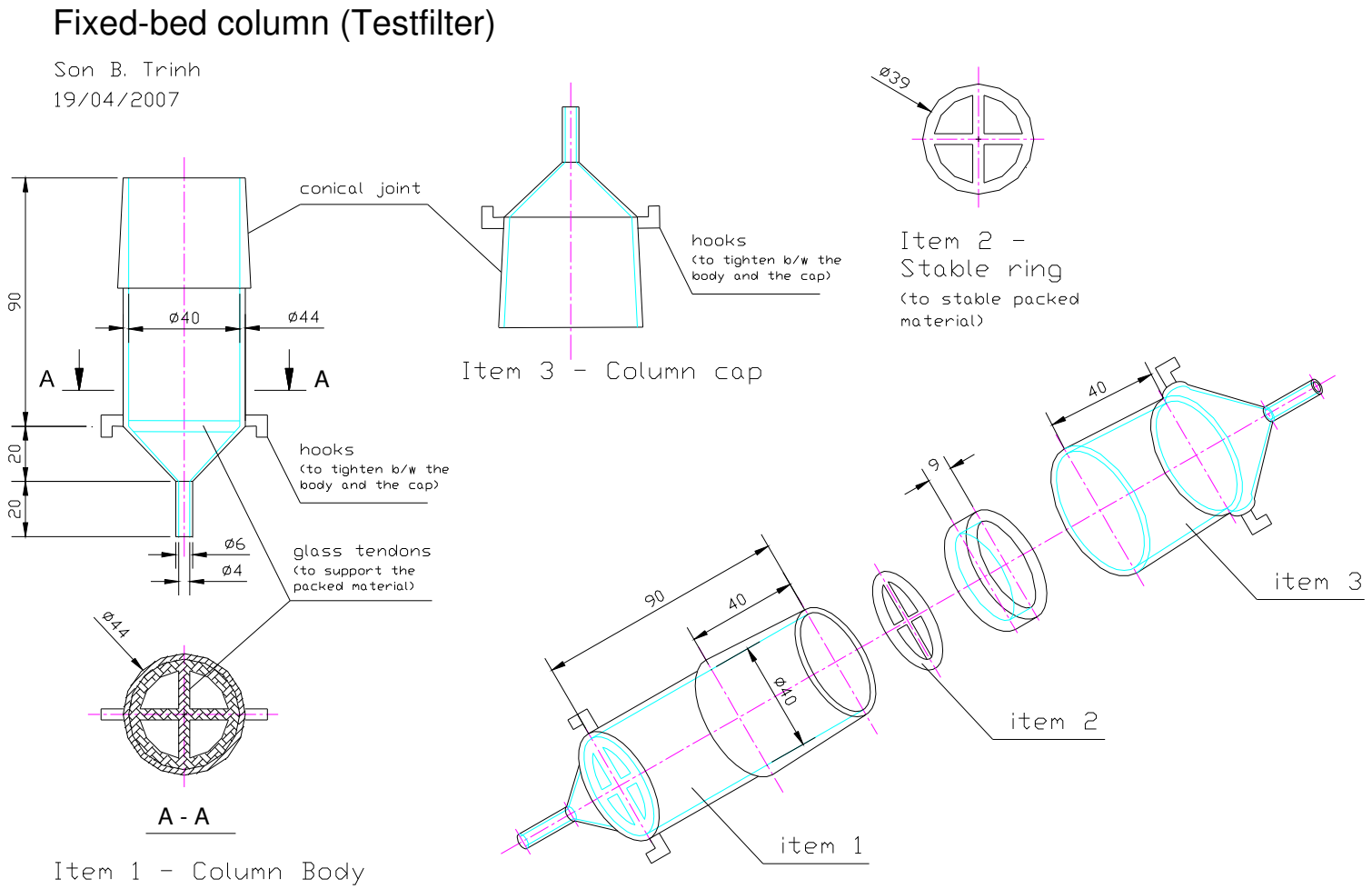



**Figure 3. 5** Detail design for a fixed-bed column (Version 4).

### 3.3.1.3 *Procedure for a fixed-bed column circulation experiment*

After the fixed-bed column was made, it was assembled with other components such as peristaltic pump and reservoir to establish a close system. Figure 3.6 presents a set-up for a fixed-bed column circulation system.

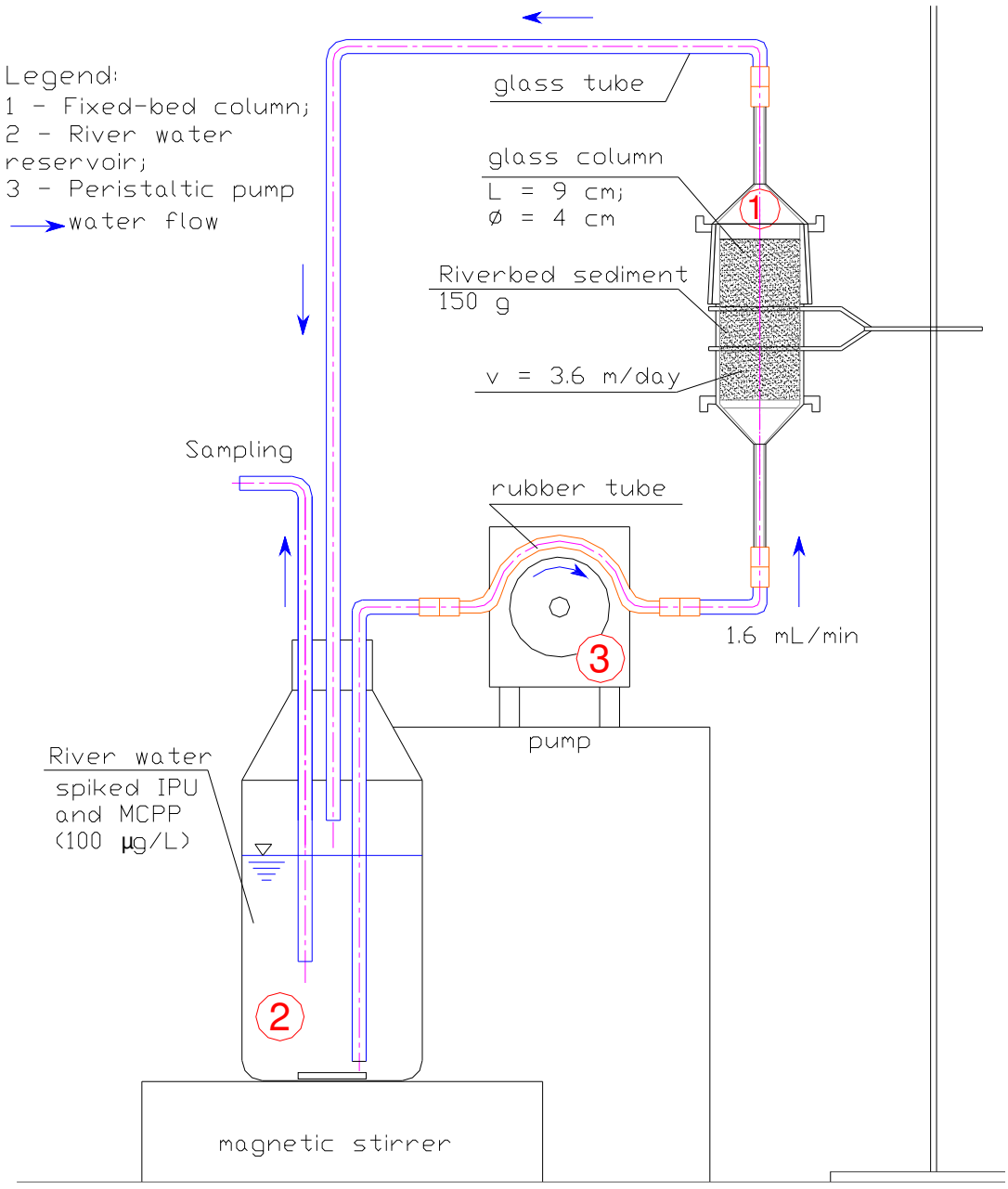



**Figure 3. 6** A set-up for a fixed-bed column circulation system.

Before packing materials (river water and riverbed sediment), all of components of the system such as column, reservoir, glass and rubber tubes were sterilised (121 $^{o}$C, 30 minutes). Then river water (1.5 L) was transferred to the reservoir and riverbed sediment (150 g) was packed into the column. Glass fibre filter papers (Fisherbrand® MF 200, 47mm) were placed at the two ends of the column to retain fine particles. A plastic cap pre-drilled holes was put on the reservoir in order to minimise the volatilisation of river water and microbial contamination from the ambient environment while allowing the recirculation pipe work to be installed. The packing process was performed in a safety cabinet (Herasafe ®, version 02.1999, Kendro Laboratory Product GmbH) to reduce microbial contamination.

The reservoir and the column were then connected together by glass tube and rubber connecters to establish a closed system (Figure 3.6). Recirculation flow through the column was maintained by a peristaltic pump (Watson Marlow 323) with an up-flow mode to ensure that no air bubbles were retained inside the column. The flow rate, $Q$, through the column was determined to be 1.6 mL min$^{-1}$. Relied upon the porosity, $n_e$, of the riverbed sediment of 50.6 ± 2.1 % (see Table 4.3), average linear velocity of the flow driven through the column was calculated to be 3.6 m day$^{-1}$ and specific discharge or Darcy velocity was determined to be 1.8 m day$^{-1}$. Agitation was provided using a magnetic stirrer to ensure a well-mixed solution of herbicides and facilitate oxygen transferred from the headspace to the solution. It was assumed that aerobic condition was adequately maintained during the assay time. The system was operated for the first 60 minutes (without herbicides) for checking leakage. This period was also designed to ensure river water fulfilled the column so that moisture of the riverbed sediment was the same as in the field.

Herbicides mecoprop and isoproturon from the stock solutions was spiked to the reservoir to achieve a designed final concentration. Water samples were

collected from the sampling tube after every designed period of time to observe concentration of the herbicides during the assay time.

It is important to note that aeration, microbial contamination, temperature and light conditions may influence on the biodegradation process occurring inside the column. As the surface of the headspace inside the reservoir (diameter of 18 cm) was large, thus it was assumed that dissolved oxygen was sufficiently supplied by a magnetic stirrer. Microbial contamination may occur by several ways, for instance during the period of transferring materials into the column and installing the system or during the sampling time. Therefore, all of the apparatus including reservoir, column and tubing were sterilised before use. Packing of sediment to the column was performed in a safety cabinet to minimise the microbial contamination. However, sampling might easily microbially contaminate the solution. Hence this step was carried out carefully with a care of microbial contamination. Temperature and light conditions might also affect on the biodegradation process of an herbicide. Nontheless, in order to approach the site conditions, the temperature and light conditions were set as in the laboratory conditions. In addition, the heterogeneous size of the natural riverbed sediment might cause an uneven pressure on the across section of the column. This might lead to the difference of the flow regime among the columns. The contact time between microorganisms attached on the sediment and the chemicals e.g. isoproturon or mecoprop in the water flow might thus be affected. This might result in the differences of the catabolic activity of microorganisms from the systems.

#### *3.3.1.4 Testing the system*

The systems were tested with riverbed sediment and river water collected from the Gatehampton site. Three replicates of the fixed-bed column system were set-up. The procedure of this experiment was described in Section 3.3.1.3.

The stock solution of isoproturon was spiked in the glass reservoirs to give a final concentration of 1000 µg $L^{-1}$. Concentrations of isoproturon in the reservoir were measured at every designed period of time during 14 circulating days. Before analysed by the HPLC system (see Section 3.4.1 for the procedure), the samples were filtered (Millex-GP, 0.22 µm, polyethersulfone, radio-sterilized) and kept in a cold room (4 $^{o}$C, darkness). These concentrations of isoproturon were plotted against the appropriate circulation time. Figure 3.7 presents the attenuation of isoproturon in the testing system.

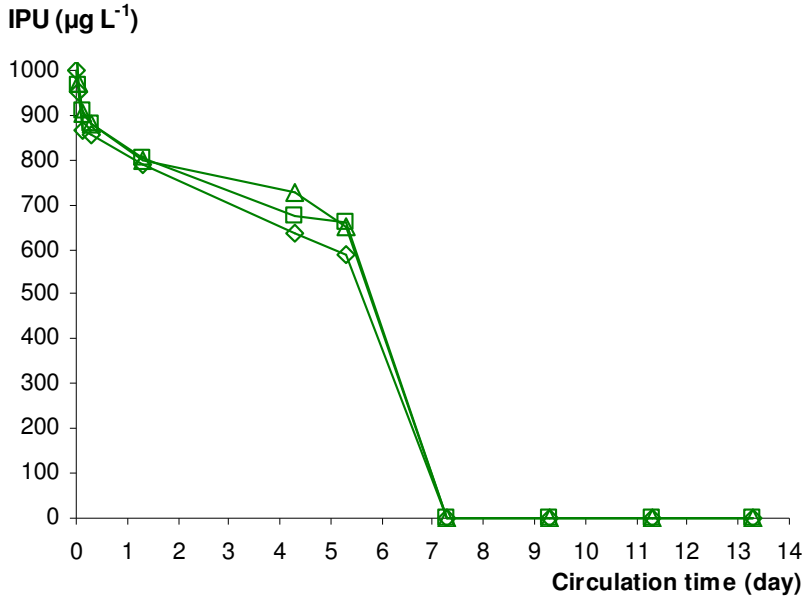



**Figure 3. 7** The attenuation of isoproturon in a river water-riverbed sediment system with the initial concentration of 1000 µg $L^{-1}$.

Observing the three curves (each curve represented for each replication) presenting the concentration of isoproturon (Figure 3.7), they were divided into three phases. The results were reported in associate with these phases as following:

(i) Phase I – rapid sorption phase: occurring during the first 7 hours. Concentration of isoproturon was rapidly decreased from approximately 1000 ± 1 µg $L^{-1}$ to 872 ± 10 µg $L^{-1}$, giving 12.8 ± 0.7 % loss of

isoproturon from the river water. It was assumed that the sorption pseudo-equilibrium of isoproturon was established after 7 hours of circulation. This period were considered as *sorption time* of isoproturon in a river water-riverbed sediment system.

(ii) Phase II – slow attenuation or adaptation phase: occurring from the consecutive period of $8^{th}$ and $127^{th}$ hours. It was observed that, after the rapid sorption phase, isoproturon concentration was slowly decreased, from 872 ± 10 to 632 ± 28 μg $L^{-1}$, giving 23.2 ± 1.8 % of loss during 120 hours of circulation. It was suggested that this period of time were the essential time for adaptation and increase the number of isoproturon degrading organisms.

(iii) Phase III – biodegradation phase occurring from $128^{th}$ to $175^{th}$ hours. Isoproturon was totally removed from river water, from 632 ± 28 μg $L^{-1}$ to below the detection limit of the HPLC (< 1 μg $L^{-1}$), giving 63.2 ± 0.3 % loss of isoproturon during 48 hours of circulation. It was suggested that isoproturon was completely degraded by microorganisms living in the riverbed sediment-river water system.

Over 14 circulation days, isoproturon in the river water was totally removed. This successful experiment supported for the further investigations presented in Chapter 4 with regard to sorption and biodegradation of several herbicides.

#### *3.3.1.5 Advantages and disadvantages of a fixed-bed column circulation system*

There are several advantages of the fixed-bed column circulation system:

- Simulating the attenuation processes occurring at the interaction zone of water and sediment;

- Investigating many organic compounds at the same time by measuring their parent-compound concentrations;
- Investigating the biodegradation of a chemical resulting from both aqueous-borne and solid-borne microorganisms or from the microorganisms born in the individual environment (by sterilising one of them);
- Operating and collecting samples simply during the experiment without interrupt the system;
- Low cost;

Several disadvantages of the fixed-bed column system can also be described:

- The volume of river water recirculating around the system may be considerably changed (decreased) due to sample to measure the concentrations of the testing compounds;
- Leaking or may be broken because the high pressure inside the column;
- The system may be biologically contaminated once packing the solid material into the column and contaminated at the sampling position;
- Chemicals can be adsorbed on the wall of the column or the tube, especially with the rubber tube which is used in a peristaltic pump;

## 3.3.2 Respirometry Method

### *3.3.2.1 Introduction*

Microorganism respiration is a mineralisation process that converts an organic compound to inorganic products, e.g. $CO_2$. The use of $^{14}C$-labelled substrate has been applied to trace the mineralisation of organic contaminants in soil (Bartha and Pramer, 1965; Kunc and Rybarova, 1983; Reid *et al.*, 2001; Reid *et al.*, 2005; Allan *et al.*, 2007). Observing the evolution of $^{14}CO_2$ from the cleavage of the added $^{14}C$-labelled compound, the catabolic potential of the microbial community for that particular compound in such environments can

be determined (Bartha and Pramer, 1965). Furthermore, the extent of impact on both microbial catabolic activity and microbial respiration was dependent on not only the bioavailability of the chemical (Reid *et al.*, 2001) but also the availability of the cells in the particular microcosms. By employing the same $^{14}C$-labelled compound in dissimilar environments such as groundwater, river water or riverbed sediment, mineralisation levels reflected the catabolic potential of the microbial community in the individual environments for the compound. Significantly, the use of $^{14}C$-labelled substrates and measurement of the evolution of $^{14}CO_2$ enabled very much lower substrate concentrations to be used (Neilson and Allard, 2008). It is important to investigate the fate behaviour of herbicides in river water in which the maximum tolerance level for herbicide residues in drinking water is around the threshold of 0.1 $\mu g\ L^{-1}$.

Several devices have been produced for the purpose of absorption of the $^{14}CO_2$ evolved from the $^{14}C$-labelled molecules in both static (Bartha and Pramer, 1965; Buddemey, 1974; Loos *et al.*, 1980; Reid *et al.*, 2001; Rasmussen *et al.*, 2004) and flow-through systems (Huckins *et al.*, 1984). However, the static system has been widely used because it has the advantages of simple design, low cost and fewer uncertainties concerning constant flow rates, leakage and sorption of the $^{14}C$-labelled materials.

Relied upon the static system, Reid *et al.* (2001) successfully designed a simple flask-based $^{14}C$-respirometer system (referred to here as a respirometer) to assess mineralisation of $^{14}C$-labeled substrates under defined conditions. A respirometer is illustrated in Figure 3.8 as a system which was used in the experiments of this thesis. A Schott Duran® bottle (250 mL) with Teflon™-lined screw-threaded lid formed on the basic of the respirometer. The respirometer cap was drilled in the centre, through which a length (30 mm) of stainless steel studding was inserted. The studding was attached at either side of the cap using a washer and a nut. To the section of the rod on the inside of the cap a fine wire stainless steel clip was attached. A $CO_2$ trap, consisting of a

glass scintillation vial (7 mL) containing 1M NaOH (1 mL) loaded on to a GF/A filter paper, was attached to the stainless steel clip. Further details of the respirometer were described by Reid *et al.* (2001).

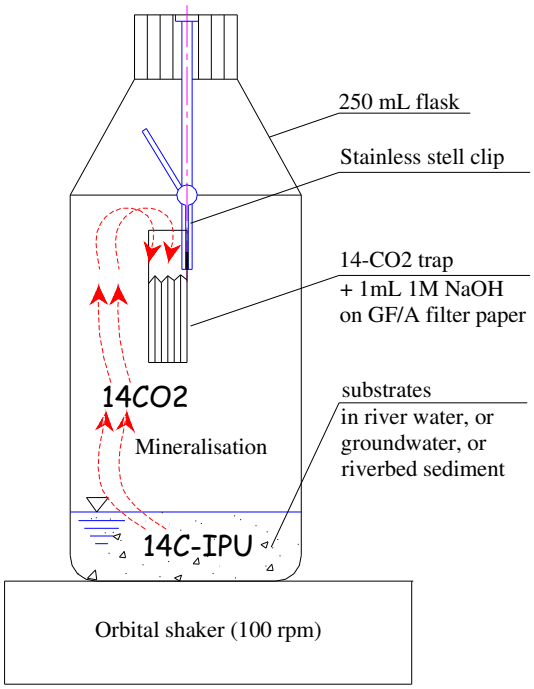



**Figure 3. 8** A respirometer set-up.

### *3.3.2.2 Procedure for a respirometric experiment*

This section presents the common steps of the procedure using respirometer which was applied for the experiments in Chapters 4 and 5. To measure mineralisation, a solid or liquid medium containing $^{14}$C-labelled substrate was transferred to the respirometer. In the case of liquid medium, the substrates were river water or groundwater samples. In the case of solid medium, the substrates were the riverbed sediment sample which was mixed with sterile deionised water. In the case of blank or control treatment, sterile deionised water was used.

The common steps start with the addition of $^{14}$C-IPU. A volume of 100 µL of $^{14}$C-IPU stock solution (10 kBq) was spiked in the respirometer. It was

assumed that isoproturon degrading organisms did not significantly discriminate between radiolabelled $^{14}$C-IPU and non-radiolabelled $^{12}$C-IPU. A vial containing a piece of GF/A filter paper (20 x 40 mm) and 1M NaOH (1 mL) was then suspended inside the respirometer. Then, the respirometer was sealed and agitated by flat-bed shaking at 100 rpm. Continuous shaking could maintain particles and microorganisms in a homogeneous suspension. Agitation also facilitated oxygen transfer from the headspace to the liquid so that aerobic condition was adequately maintained. Any $^{14}CO_2$ evolved as a result of catabolism of $^{14}$C-IPU was trapped in the NaOH vial. After every designed period of time, the lid was unscrewed and the $^{14}CO_2$ trap quickly removed and replaced with a fresh one. The removed vial trap was then wiped with a tissue and liquid scintillation fluid (Ultima Gold) was added (6 mL). The trapped $^{14}CO_2$ activity was determined by liquid scintillation counting (Canberra Packard Tri-Carb 2250CA liquid scintillation analyser) following a 48 h rest period to allow the GF/A filter paper to become transparent.

Catabolic activity with respect to isoproturon was assessed by mineralisation level and mineralisation rate. These parameters were calculated as follows:

*Mineralisation level* or l*evel of catabolic activity (% mineralisation to $^{14}CO_2$)* was illustrated by the percentile of $^{14}CO_2$ evolution. This value was a fraction of the accumulation of dpm (distintegrations per minute) of trapped $^{14}CO_2$ after deducting for the dpm of the "blank" samples and the dpm of the standards. The accumulation data of $^{14}CO_2$ were calculated based on assay time (counted since the addition of $^{14}$C-IPU). The length of assay time was determined to ensure the mineralisation levels reach a plateau. This meant that almost all of the $^{14}$C-subtrate had been mineralised. Maximum mineralisation level was reported as the level over the assay time.

*Mineralisation rate* of an organic compound was obtained from the fitted line which was built from the data points (not averaged) of the mineralisation levels

and the appropriate time during the assay time. The fitted line started at the end point of the adaptation phase (≥ 5% mineralisaion level) and finished at the point which had the mineralisation value of 5 % lower than the mineralisation value after the assay time. Thus, the gradient of the fitted line provided the mineralisation rate and its associated $R^2$ value presents an indication of numerical robustness. In addition, if a replicate has the maximum mineralisation level (after the assay time) less than 10 % $^{14}CO_2$, this means no fitted line could be built and thus no mineralisation rate could be calculated. It is noted that there were three replicates for every treatment, hence the mineralisation rates were presented as the average of the three replicates and the standard errors.

## 3.4 HPLC Analytical Methods

High Performance Liquid Chromatography (HPLC) was used to measure concentration of mecoprop and isoproturon in river water samples. An introduction and the procedure of this method is presented below.

### 3.4.1 Introduction

Chromatography is a general term applied to a wide variety of separation techniques based upon the sample partitioning between a moving phase, which can be a gas, liquid or supercritical fluid and a stationary phase, which can be either a liquid or a solid (Dorsey, 2000). Liquid Chromatography (LC) started to gain more attention in the late 1970s as it was found to be well suited to the demands of a non-destructive selective analytical technique, especially required for new types and classes of thermally unstable or non-volatile and highly polar pesticides and conjugated metabolites, where the application of gas chromatography (GC) often failed (Liska and Slobodnik, 1996; Dorsey, 2000). Although GC and HPLC are widely-used techniques used for analysing pesticides (JunkerBuchheit and Witzenbacher, 1996; Liska and Slobodnik,

1996), HPLC is, however, favoured over GC for acidic pesticides, with high polarities, low volatilities, and thermal instabilities (Liska and Slobodnik, 1996; Pinto and Jardim, 2000). In addition, since the majority of pesticides strongly absorb in the UV region, between 210 – 240 nm, they make excellent compounds for UV detection in LC (Liska and Slobodnik, 1996; Hidalgo *et al.*, 1997; Pinto and Jardim, 2000). Diode array detection (DAD) is also an attractive option for detection of herbicides as it assists the confirmation of peak identity, utilizing a UV spectrum rather than a single wavelength (Aguilar *et al.*, 1996c, b; Galera *et al.*, 1997; Slobodnik *et al.*, 1997).

### 3.4.2 HPLC procedure

A HPLC method was developed to measure concentrations of the herbicides mecoprop and isoproturon in an aqueous solution using a Dionex Summit HPLC system (model 2004, made in Germany) equipped with a solvent rack SOR-100, a P680 HPLC pump, an ASI-100 automated sample injector, a TCC-100 thermostatted column compartment in which was fitted an Acclaim 120 C18 5 μm 120 Å Dionex column (250 x 2.1 mm) with a guard column and a PDA-100 photodiode array detector. Mobile phases were prepared from organic solvent acetonitrile (A) and $NaHPO_4$ (B). A procedure for analysing the herbicides mecoprop and isoproturon was constituted.

The mobile phases of acetonitrile (A) and $NaHPO_4$ (B) were automatically mixed together according to an isocratic mode of 50 % A and 50 % B with a flow rate was set to be 0.4 mL $min^{-1}$; and an injection volume of 180 μL. The temperature of the column was set at 25 $^{o}$C. UV detection of mecoprop and isoproturon was applied at 230 and 242 nm wavelengths, respectively. The Chromeleon software (version 6.8, service package 5) was applied to return the concentrations of the herbicides.

## Noise and drift

The HPLC analysis is a time-dependent process. The appearance of a compound peak is recorded by the deflection of the recorder pen from the baseline. It is necessary to distinguish between the actual component and an artifact caused by pressure fluctuation, bubbles, compositional fluctuation, etc. If the peaks are fairly large, they are easy to distinguish. However, for smaller peaks, it is important that the baseline is smooth and free of noise and drift.

Baseline noise is the short-time variation of the baseline from a straight line caused by electrical signal fluctuations, lamp instability, temperature fluctuations and other factors. Noise usually has a much higher frequency than the actual chromatographic peak. Noise is normally measured "peak-to-peak": i.e., the distance from the top of one such small peak to the bottom of the next. Sometimes, noise is averaged over a specified period of time. Noise is the factor which limits detector sensitivity. In trace analysis, the operator must be able to distinguish between noise peaks and component peaks. Figure 3.9 illustrates noise, component peak and drift.

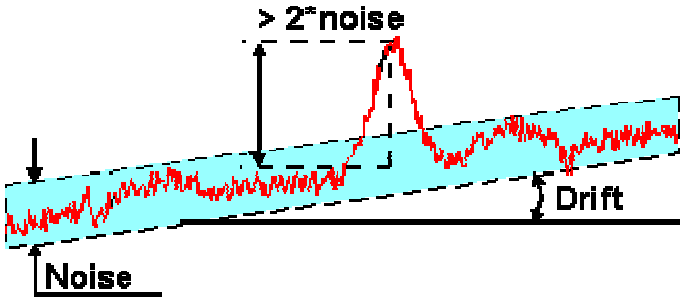



**Figure 3. 9** Noise and drift of a component peak in HPLC analysis

Another parameter related to the detector signal fluctuation is drift. Noise is a short-time characteristic of a detector, but an additional requirement is that the baseline should deviate as little as possible from a horizontal line. It is usually measured for a specified time, e.g. 30 minutes or one hour. Drift is usually

associated with the detector heat-up in the first hour after power-on. Figure 3.6 also illustrates the meaning of drift.

### Limit of detection

The limit of detection (LOD) is the lowest concentration that is just distinguishable from zero or the baseline. There are three different limits: limit of detection (LOD), limit of determination (LODn), and limit of quantification (LOQ). The LOD, LODn, and LOQ are reached when the signal-to-noise ratio is 3, 6 and 10, respectively. This ensures correct quantification of the trace amounts with less than 2% variance.

Applying the above HPLC procedure for analysis of the herbicides mecoprop and isoproturon in standard solutions, the LOQ for mecoprop and isoproturon were determined to be 2 and 1 μg $L^{-1}$, respectively. Figure 3.10 presents the chromatographs for mecoprop and isoproturon standards used to examine the LOD, LODn and LOQ.

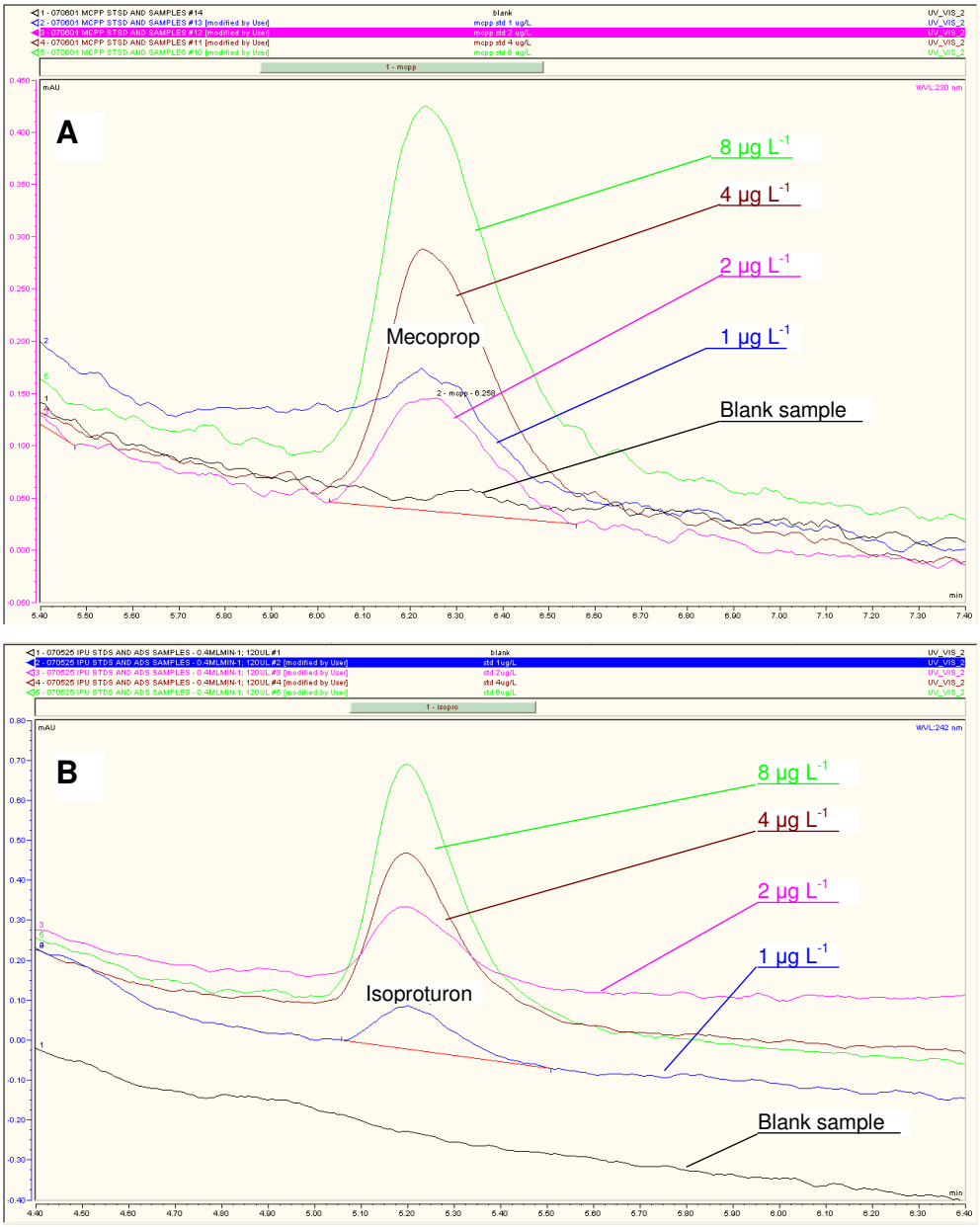



**Figure 3. 10** Limits of quantification for mecoprop (A) and isoproturon (B) using HPLC analysis.

# Chapter 4

# SORPTION and BIODEGRADATION of HERBICIDES in a RIVER WATER – RIVERBED SEDIMENT SYSTEM

## 4.1 Introduction

The role of riverbank filtration with respect to the fate and behaviour of several micro-organic contaminants as well as pesticides from surface water has been outlined in Section 1.2. *In situ*, many processes simultaneously or sequentially, directly or indirectly influence the occurrence of pesticides in a water-sediment system, for instance: adsorption, desorption, diffusion, chemical degradation, photo-chemical degradation, microbial degradation, volatilisation, plant or organism uptake (Bailey and White, 1970; Warren *et al.*, 2003; Katagi, 2006). *In the laboratory*, however, experimental conditions can be controlled so that the effect of an individual process can be investigated. On the other hand, sorption and biodegradation of pesticides in a water-sediment system have been reported as the primary processes resulting in the attenuation of these micro-organic contaminants (Bailey and White, 1970; Karickhoff *et al.*, 1979; Worch *et al.*, 2002; Warren *et al.*, 2003; Katagi, 2006). According to this, under the laboratory conditions, experiments in this chapter aims to investigate the

sorption and biodegradation of the two herbicides mecoprop and isoproturon in a water-sediment system.

### 4.1.1 Sorption of herbicides in a water-sediment system

*Sorption* is defined as the association of micro-organic compounds (known as sorbates) on/in to a solid phase (known as sorbents) e.g. riverbed sediment, soils, activated carbon. In nature, such a solid phase as riverbed sediment is normally a complex mixture of inorganic minerals and natural organic matter. Owing to the compositional complexity of sediments, sorption may result from both two types of processes: (1) *adsorption* – if the molecules attach to a two-dimensional surface (usually in association with a mineral or inorganic-matter surface); (2) *absorption* – if the molecules penetrate into a three-dimensional matrix (usually in association with an organic-matter matrix) (Schwarzenbach *et al.*, 1993; Warren *et al.*, 2003). The *sorption* term used in this thesis includes both *adsorption* and *absorption* processes.

In general, once an herbicide is delivered to a water-sediment interface, a fraction undergoes sorption onto particles of the riverbed sediment, while the remaining fraction undergoes desorption into the river water or groundwater as the herbicide re-equilibrates between water and sediment in the new environment (Nowell *et al.*, 1999). The fraction of herbicides sorbed onto precipitated or suspended sediment particles can be degraded by microbiological activity. Subsequently, the degraded products can be reintroduced back into the water column or groundwater as either dissolved organic carbon or mineralised carbonate species. As a result, a further fraction of herbicide in the water column can continue as sorbed onto the sediment to re-establish a new equilibrium. Additionally, since molecular transfer is a prerequisite for the uptake of organic pollutants by organisms, thus the bioavailability of a given compound, the rate of biotransformation and the toxic effect(s) are affected by sorption processes (Schwarzenbach *et al.*, 1993).

Therefore, in a water-sediment system, sorption is a crucial process for the degradation of a compound. It can control the rates of other processes if the sorption rate is slower than the rates of the others, for example the biodegradation rate.

The sorption-desorption cycle of an herbicide continues throughout its life time in sediments as the ambient environment continues to change. Sorption directly or indirectly influences the magnitude of the effect of other factors. Therefore, it appears as one of the major factors affecting the interactions occurring between the pesticide and the riverbed sediment (Bailey and White, 1970). Indeed, sorption has been presented as a critical process with respect to the overall behaviour and fate of a pesticide (Nowell *et al.*, 1999). Clausen and Fabricius, (2001a) reported that sorption from an aqueous solution to a solid surface is one of the key processes determining the concentration and rate of transport of pesticides in aquifers.

Sorption properties of riverbed sediment are strongly influenced by constituents that have high specific surface area and highly reactive surfaces (Bailey and White, 1970; Clausen and Fabricius, 2001; Clausen *et al.*, 2001). In sediments containing a significant amount of organic matter, sorption of a pesticide is therefore as a rule controlled by the organic carbon content, due to the porous nature and large surface area of humic substances, where a variety of functional groups are present (Bailey and White, 1970; Stevenson, 1976; Chiou *et al.*, 1979). In sediments where organic matter content is low, the association of herbicides with mineral surfaces may become significant (Stevenson, 1976; Brownawell *et al.*, 1990; Schwarzenbach *et al.*, 1993; Celis *et al.*, 1996; Celis *et al.*, 1999). The high degree of variability and complexity in sediment compositions and potential sorptive interactions, however, appears to preclude the possibility of developing a simple, systematic procedure for predicting sorption parameters. A number of environmental factors have been found to influence sorption of the herbicides in riverbed sediment such as: pH,

which controls the electrostatic charges on mineral surfaces, on natural organic matter, and also on weakly basic and acidic pesticides (Schwarzenbach *et al.*, 1993; Gao *et al.*, 1998; Madsen *et al.*, 2000; Clausen and Fabricius, 2001); ionic strength, which reduces pesticides solubility in water, the extent of interaction between charged species and can also influence natural organic matter; and temperature, which increases pesticide water-solubility (Schwarzenbach *et al.*, 1993); clay content and composition, oxides, cation exchange capacity, specific surface area, electrolyte composition, pesticide concentration (Madsen *et al.*, 2000); humus content (Fomsgaard, 1997); organic carbon content (Hamaker and Thomson, 1972; Madsen *et al.*, 2000; Buss *et al.*, 2006); and types of organic matter (Allen-King *et al.*, 2002; Steventon-Barnes, 2002; Huang *et al.*, 2003). Thus, a thorough understanding of sorption is paramount for the prediction of herbicide movement in a RW-RS system.

Many previous studies have investigated the sorption of pesticides to soils (Bailey and White, 1970; Borggaard and Streibig, 1988; Worrall *et al.*, 1996, 1997; Moreau-Kervevan and Mouvet, 1998; Celis *et al.*, 1999; Henriksen *et al.*, 2003; Dores *et al.*, 2009), to natural sediments (Karickhoff *et al.*, 1979), to chalk aquifer material (Johnson *et al.*, 1998) and to mineral components in aquifer sediments and clays (Frissel and Bolt, 1962; Terce and Calvet, 1978; Laird *et al.*, 1992; Sannino *et al.*, 1997; Clausen and Fabricius, 2001a; Clausen *et al.*, 2001b; Clausen *et al.*, 2004). In addition, the sorption of non-ionic or uncharged organic compounds by soils has been shown to be highly correlated with soil total organic carbon (TOC) content (Chiou *et al.*, 1979; Briggs, 1981; Karickhoff, 1984).

With regard to sorption of isoproturon, there were several previous reports e.g. Worrall *et* al. (1996), Pedersen *et al.* (1995), Rae *et al.* (1998). With regard to sorption of mecoprop, a few reports were published e.g. Felding (1997),

Helweg *et al.* (1998), Reffstrup *et al.* (1998). However, very little information is available on the sorption of both isoproturon and mecoprop in a water-sediment system.

### 4.1.2 Biodegradation of herbicides in a water-sediment system

After sorbed on/into a solid phase, some pesticides are readily degraded by microorganisms, while others have proven to be recalcitrant. In fact, degradation can involve biotic and abiotic processes. However, biological degradation has been received an especially attention as it is considered as a major process in the breakdown of aromatic compounds (Alexander, 1981; Bornick *et al.*, 2001). Thus, a definition for microbial degradation or biodegradation is necessary before further discussing on it.

*Biodegradation* is defined as the breakdown of a substance to smaller products caused by microorganisms or their enzymes (Atlas, 1988). While sorption does not alter the structure of an organic molecule and therefore its toxicity may still remain, biodegradation frequently, although not necessarily, leads to the conversion of much of the C, N, P, S and other elements in the original compound to inorganic products. Such a conversion of an organic substrate to inorganic products is known as *mineralisation*. Hence, in the mineralisation of organic C, carbon dioxide ($CO_2$) is released. Plant, animal and particularly microorganism respiration is a mineralisation process that destroys numerous organic molecules. Indeed, microorganisms are frequently the sole means, biological or non-biological, of converting synthetic chemicals to inorganic products (Alexander, 1999). And the major agents causing biodegradation in sediment, river water and groundwater are the microorganisms that inhabit in these environments (Alexander, 1999).

Biodegradation of an herbicide in a river water – riverbed sediment system can occur either in the water column or on the sediment following a sorption process (Warren *et al.*, 2003). In other instances, riverbed sediment can strongly influence on the biodegradation of an herbicide because many herbicides are known to associate strongly with the sediments (Paris *et al.*, 1981; Warren *et al.*, 2003). In the top layer of a riverbed, a high dissolved oxygen concentration often prevails. This results in the domination of aerobically-respiring bacteria in such environment. Under these conditions biotic reactions or biodegradation of an herbicide is often more easily performed (Sophocleous, 2002; Warren *et al.*, 2003). Several reported observations suggested that herbicides will be degraded more slowly below the oxic zone of riverbed sediments, and may therefore be persistent once buried (Oneill *et al.*, 1989; Warren *et al.*, 2003). Furthermore, to understand the biodegradation of an herbicide, it is also important to understand the degradation pathways of an herbicide under environmental conditions. Degradation pathways of herbicides mecoprop and isoproturon are presented in the below sections below.

#### *4.1.2.1 Degradation pathways of mecoprop*

Under anaerobic conditions, the recalcitrance of mecoprop to biodegradation has been widely reported. The presence of phenoxy acids (e.g. mecoprop) in landfill leachate, emanating from six municipal landfills in the USA (Gintautas *et al.*, 1992), suggested that they did not degrade in these usually anaerobic systems. Rugge *et al*., (1999) reported that no degradation of mecoprop was apparent in an anaerobic field injection test, in anaerobic *in situ* microcosms and in anaerobic laboratory batch experiments. No or very little mecoprop degradation was found in anaerobic aquifer samples from Denmark and elsewhere in mainland Europe (Pedersen, 2000; Albrechtsen *et al.*, 2001; Larsen and Aamand, 2001).

In contrast, it is well documented that phenoxy acid herbicides (e.g. mecoprop) degrade in the topsoil where conditions are aerobic and this process is, in the main, microbially mediated (Loos, 1975; Smith, 1989; Buss *et al.*, 2006). It has been observed that under aerobic conditions, mineralisation of only 50% of the total mecoprop content may occur (Heron and Christensen, 1992; Oh and Tuovinen, 1994; Larsen *et al.*, 2000). In one of these studies the remaining 50% was eventually biodegraded after a prolonged period (Heron and Christensen, 1992). It was suggested (but not proven) that this was due to mecoprop having two chiral forms which degrade at different rates or in sequence. In other words the degradation of mecoprop was enantioselective. Johnson *et al.* (2003) reported that a period of acclimation or adaptation is necessary before mecoprop biodegradation takes place at a significant rate. This acclimation period may be the result of the time taken for a microbial population to grow to a size that can degrade the substrate at a clearly measurable rate, or the need for natural genetic and biochemical changes in the microorganims, or both (Roeth, 1986; Smith, 1989). Delayed or ineffective degradation of mecoprop may be a polyauxic effect; that is, the microbial population will preferential degrade other (easier or energetically beneficial) substances in preference to mecoprop (Bitton and Gerba, 1994).

The mechanism and degradation pathways of mecoprop thus have also been widely reported (Tett *et al.*, 1997; Harrison *et al.*, 2003; Williams *et al.*, 2004; Buss *et al.*, 2006). The metabolic pathway involved in degradation of mecoprop is presented in Figure 4.1. Chlorocresol or 4-Chloro-2-methylphenol (4-CMP or PCOC) has been identified as the primary initial transformation product in laboratory culture (Tett *et al.*, 1994; Nickel *et al.*, 1997), soils (Smith, 1989; Klint *et al.*, 1993) and groundwater (Harrison *et al.*, 2003). Further degradation then occurs by hydroxylation at the 6-position of the 4-CMP, followed by cleavage of the aromatic ring. 4-CPM is highly toxic to aquatic organisms (Harrison *et al.*, 2003), and has been confirmed as being a List I substance (organohalogen) for the purposes of the Groundwater Regulation 1998 (JAGDAG, 2001). However, the majority of studies suggested that further transformation of 4-CMP is rapid e.g. (Broholm *et al.*, 2001), and the complete

process resulted in environmentally benign end-products (Nitschke *et al.*, 1999). In other circumstances, degradation of 4-CMP did not occur under anaerobic conditions until all of the mecoprop had been utilized, so it might accumulate under some cases (Harrison *et al.*, 2003). Under such circumstances risk to the environment was elevated due to the increase in concentration of the more toxic 4-CMP intermediate.

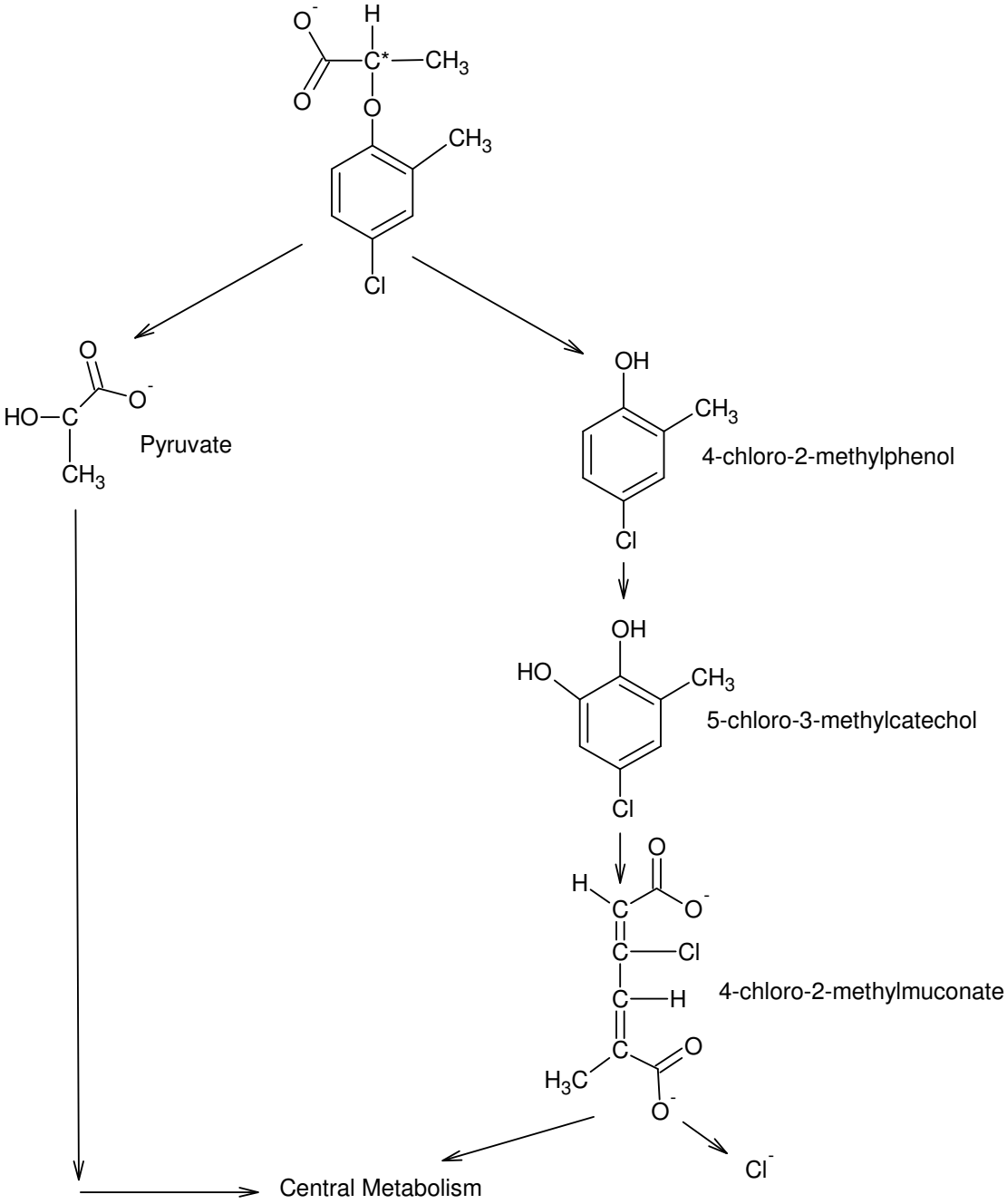



**Figure 4. 1** Biodegradation pathway for mecoprop. Putative metabolic pathway based on (Smith, 1989), (Tett *et al.*, 1994) and (Nickel *et al.*, 1997). The "*" indicates the enantiomeric centre.

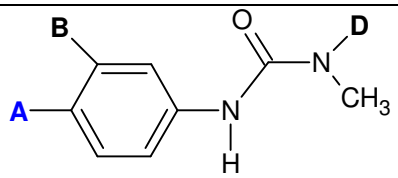
The presence of the two optically active isomers (enantiomers), R- and S-isomers of mecoprop, results in different microbial degradation rates for each isomer (Zipper *et al.*, 1996; Environment Agency, 2001; Romero *et al.*, 2001; Williams *et al.*, 2003). It has been suggested that a change in the ratio of R:S isomers with time could be indicative of biodegradation and could therefore be used as evidence of natural attenuation (Harrison *et al.*, 2003; Buss *et al.*, 2006). However, the literature presents no consistency for which of the isomers is the more rapidly degraded and, selectivity may depend, at least in part, on prevalent redox conditions. For example, in the landfill plume at Helpston, Peterborough (UK), the S-isomer appeared to be preferentially degraded under aerobic conditions (although both isomers were degraded), whereas only the R-isomer degraded under nitrate-reducing conditions (Williams *et al.*, 2003). No biodegradation was observed under iron- or sulphate-reducing conditions. Zipper *et al.* (1998) found preferential degradation of the S-isomer under nitrate-reducing conditions. However, no differential degradation of the R- or S-isomers within the aerobic field injection trial was observed at Vejen in Denmark (Rugge *et al.*, 2002).

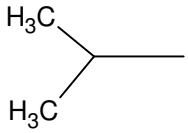
#### *4.1.2.2 Degradation pathways of phenylurea herbicides and isoproturon*

The mechanism and degradation pathways of phenylurea herbicides such as isoproturon have also been well documented (Sorensen *et al.*, 2003; Badawi *et al.*, 2009). Several common phenylurea herbicides and their molecular structure are introduced in Table 4.1. Under moderate temperature and within a pH range of 4 – 10, the common phenylurea herbicides were stable to chemical degradation in aqueous solution (Hill, 1955; Gerecke *et al.*, 2001; Salvestrini *et al.*, 2002). Hence, Sorensen *et al.* (2003) suggested that chemical degradation was of minor importance in most agricultural soils. In contrast, microbial degradation of isoproturon has been established to be more significant in agricultural soils (Cox *et al.*, 1996), diuron (Cullington and Walker, 1999), fluometuron (Bozarth and Funderbu.Hh, 1971), linuron, chlorobromuron and metobromuron (Roberts *et al.*,

1993; El-Fantroussi *et al.*, 2001). Furthermore, microbiological processes frequently mineralised the phenyl ring leading to the ultimate products e.g. $CO_2$ and $H_2O$ (Bending *et al.*, 2001; Reid *et al.*, 2005).

**Table 4. 1** Common phenylurea herbicides and their molecular structures. After Sorensen *et al*., 2003.




| | A | B | D |
|---|---|---|---|
| **Isoproturon** [N-(4-isopropylphenyl)-N',N'-dimethylurea] |  | H | $CH_3$ |
| **Diuron** [N-(3,4-dichlorophenyl)-N',N'-dimethylurea] | Cl | Cl | $CH_3$ |
| **Monuron** [N-(4-chlorophenyl)-N',N'-dimethylurea] | Cl | H | $CH_3$ |
| **Chlorotoluron** [N-(3-chloro-4-methylphenyl)-N',N'-dimethylurea] | $CH_3$ | Cl | $CH_3$ |
| **Fenuron** [3-phenyl-N',N'-dimethylurea] | H | H | $CH_3$ |
| **Fluometuron** [N-(3-trifluoromethylphenyl)-N',N'-dimethylurea] | H | $CF_3$ | $CH_3$ |
| **Metobromuron** [N-(4-bromophenyl)-N'-methoxy-N'-methylurea] | Br | H | $OCH_3$ |
| **Chlorobromuron** [N-(4-bromo-3-chlorophenyl)-N'-methoxy-N'-methylurea] | Br | Cl | $OCH_3$ |
| **Linuron** [N-(3,4-dichorophenyl)-N'-methoxy-N'-methylurea] | Cl | Cl | $OCH_3$ |

In general, the metabolic pathways involving the degradation of phenylurea herbicides are similar in the mechanisms. Figure 4.2 presents general degradation pathways for N-methoxy-N-methyl- and N,N-dimethyl-substituted phenylurea herbicides in agricultural soils. Bacteria and fungus degrade phenylurea herbicides by successive *N*-demethylation of the *N,N*-dimethylurea-substituted compounds, and *N*-demethoxylation of the *N*-methoxy-*N*-methyl-substituted compounds; then hydrolysed these metabolites to substituted aniline products (pathway I, step 1 – 3, Figure 4.2) (Tweedy *et al.*, 1970; Bozarth and Funderbu.Hh, 1971; Field *et al.*, 1997; Badawi *et al.*, 2009). In another way, it was reported that a direct hydrolysis of phenylurea herbicides to their aniline derivatives (pathway II, step 4, Figure 4.2) could be performed by two *A. globiformis* strains (designated D47 and N2) and one *B. sphaericus* strain (ATCC 12123) isolated from soils (Engelhardt *et al.*, 1973; Turnbull *et al.*, 2001b; Tixier *et al.*, 2002). Subsequently, these aniline-based metabolites might be further degraded (El-Fantroussi *et al.*, 2001; Sorensen *et al.*, 2001). Nonetheless, it was also noted that several metabolites of the phenylurea herbicides presented in Figure 4.2 might be more hazardous to non-target organisms than the parent compounds (Remde and Traunspruger, 1994; Tixier *et al.*, 2002). And some of these products have been shown to persist and contribute to contamination of surface and groundwater (Schuelein *et al.*, 1996; Field *et al.*, 1997; Johnson *et al.*, 1998; Thurman *et al.*, 2000).

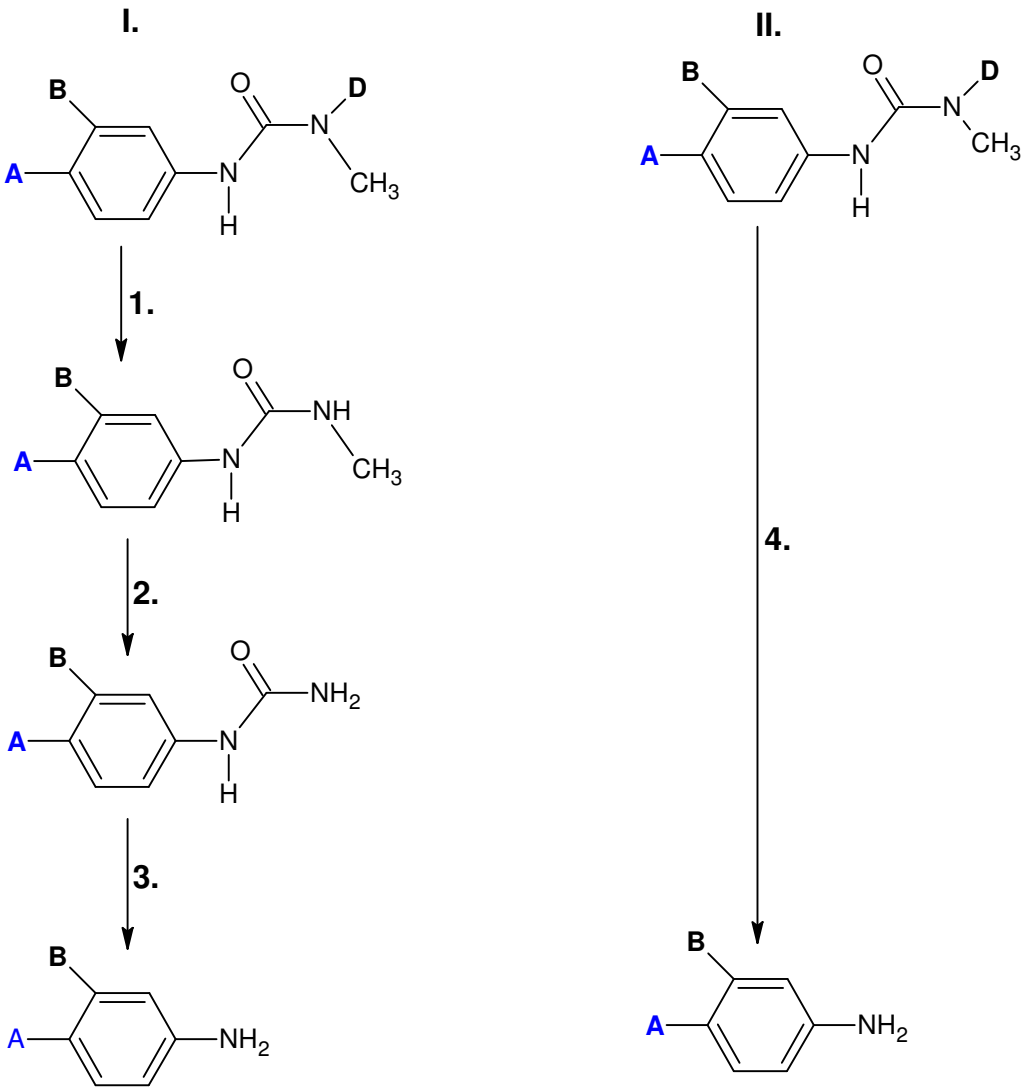



**Figure 4. 2** Proposed general degradation pathways for N-methoxy-N-methyl- and N,N-dimethyl-substituted phenylurea herbicides in agricultural soils. Pathway I: Involving sequential N-dealkylations (step 1 and 2) and hydrolysis to aniline derivatives (step 3). Pathway II: Direct hydrolysis to the aniline derivatives (step 4). See Table 4.1 for identification of substituents A, B and D for each of the phenylurea herbicides. After Sorensen *et al.*, 2003.

Degradation pathways of aniline derivatives from phenylurea herbicides are not well-known (Sorensen *et al.*, 2003). But the metabolism of the similar compounds with the aniline-based metabolites reach the environment from many sources, including paints, dyes, plastics and pharmaceutical products, has been studied e.g. (Parris, 1980; Lyons *et al.*, 1984). Generally aniline

compounds were relatively easily degraded by microorganisms, however the substitutions to the aromatic ring structure, e.g. halogen or nitro groups, might prevent or delay complete mineralisation. The general metabolic pathway for metabolism of aniline involved oxidative deamination to give catechol, which might be further degraded by different ring cleavage pathways at either *ortho*- or *para*- positions (Parris, 1980; Lyons *et al.*, 1984). Other processes such as reductive deamination or dehalogenation might initiate the degradation of aniline metabolites under anoxic conditions (Travkin *et al.*, 2002). Polymerisation process of aniline compounds might also occur (Parris, 1980; Scheunert and Reuter, 2000). A recent study has showed that 4IA (4-isopropyl-aniline) might react with the humic monomer catechol forming a trimer product identified as a distributed *ortho*-quinone (step 14, Figure 4.3) (Scheunert and Reuter, 2000). The polymerisation of 4IA results in the 4,4-diisopropylazobenzene which might accumulate in soils during isoproturon degradation (step 15, Figure 4.3) (Pieuchot *et al.*, 1996; Perrin-Ganier *et al.*, 2001). Occurrence of quinine and azo compounds has also been reported during degradation of chlortoluron (Smith and Briggs, 1978). These polymerisation products probably represented dead-end metabolites, as they have low biodegradability in dissimilar soils (Scheunert and Reuter, 2000; Perrin-Ganier *et al.*, 2001).

Degradation pathways of isoproturon has recently been further elucidated; with further metabolites being identified following isoproturon degradation by both bacteria and fungi. Figure 4.3 presents degradation pathways of isoproturon by the soil bacteria and Figure 4.4 presents degradation pathways by the soil fungi.

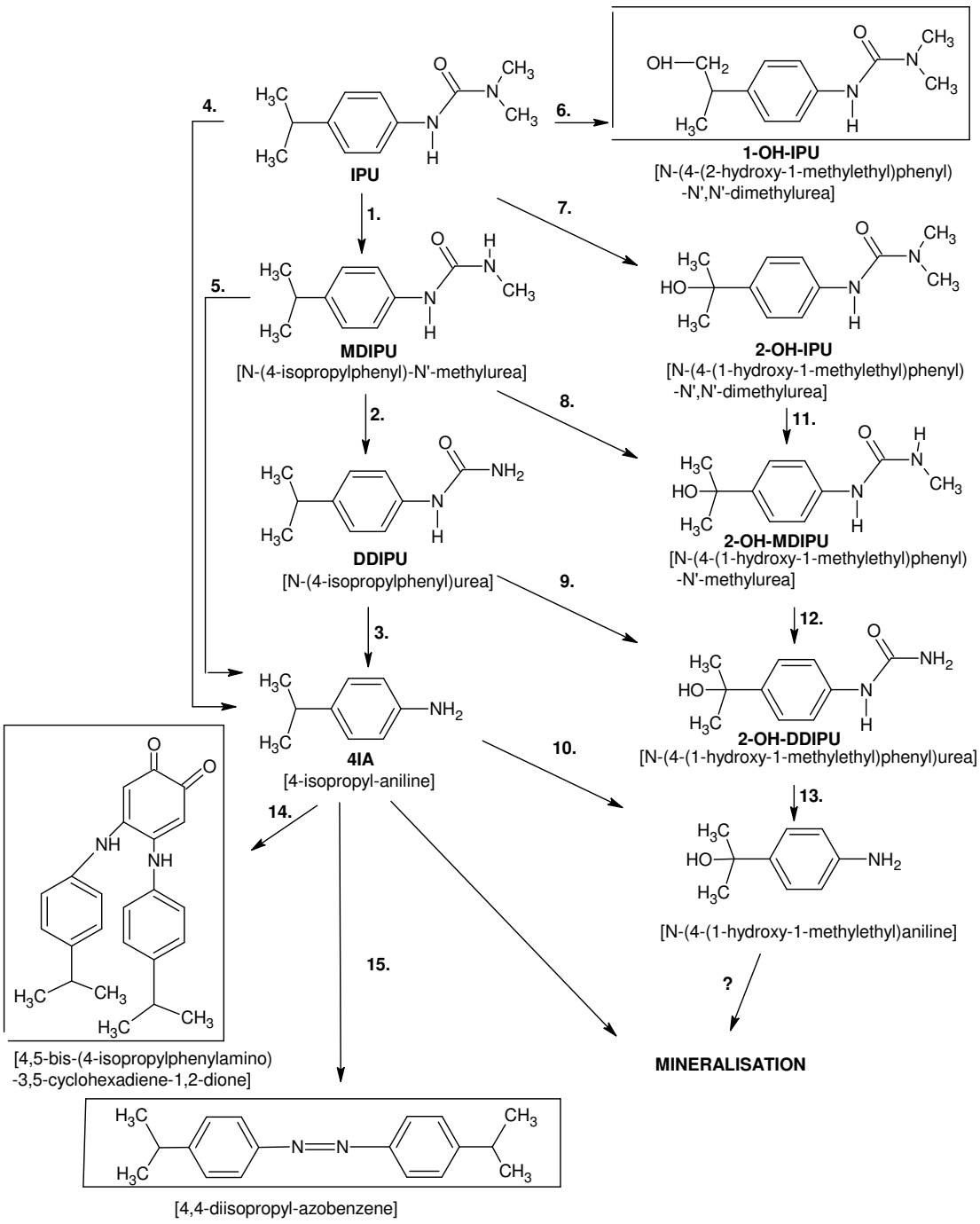



**Figure 4. 3** Proposed degradation pathways of isoproturon in agricultural soil and by defined soil microorganisms. Compounds shown in boxes are dead-end metabolites without any further degradation. After Sorenson *et al.,* 2003.

Steps 1 -3 of Figure 4.3 proposed the metabolic pathways for the mineralisation of isoproturon by *Sphingomonas* sp. Strain SRS2 involving successive *N*-demethylation of IPU to MDIPU, DDIPU and eventually 4IA before completely cleaved to $CO_2$ (Sorensen *et al.*, 2001). A metabolic pathway from

the methylurea group of MDIPU direct to 4IA (Step 5, Figure 4.3) might be active in a mixed bacterial culture (Sorensen *et al.*, 2003). One other interested finding was that the phenylurea herbicides diuron, linuron, monolinuron, metoxuron and isoproturon were able to be degraded directly to their respective aniline derivatives (Step 4, Figure 4.3) by *A. globiformis* strain D47 isolated from the Deep Slade agricultural field (Cullington and Walker, 1999; Turnbull *et al.*, 2001a). *A. globiformis* strain D47 transformed isoproturon by a single step involving hydrolytic cleavage of the *N,N*-dimethylurea side chain to 4IA (Turnbull *et al.*, 2001b). A similar one-step degradation of isoproturon, diuron and chlorotoluron to their respective aniline metabolites has also been reported by *A. globiformis* strain N2 isolated from a French garden soil that had been treated for several years with diuron (Tixier *et al.*, 2002; Widehem *et al.*, 2002). However, none of these strains degraded the phenylurea-derived aniline metabolites produced (Sorensen *et al.*, 2003).

Under laboratory and field environments, MDIPU has been found as a metabolite occurring in the highest concentration following isoproturon treatment of agricultural soils (Mudd *et al.*, 1983; Gaillardon and Sabar, 1994; Cox *et al.*, 1996; Lehr *et al.*, 1996; Pieuchot *et al.*, 1996; Schuelein *et al.*, 1996; Berger, 1999; Perrin-Ganier *et al.*, 2001; Badawi *et al.*, 2009). MDIPU was also the main metabolite occurring during metabolism of isoproturon by pure cultures of soil fungi and bacteria (Roberts *et al.*, 1998; Berger, 1999; Sorensen *et al.*, 2001; Badawi *et al.*, 2009).

Schuelein *et al*. (1996) and Lerh *et al*. (1996) also reported isoproturon could be degraded by hydroxylation of the isopropyl side chain into 1-OH-IPU or 2-OH-IPU (Steps 6 and 7, Figure 4.3) in mixed bacterial cultures and in agricultural soils. 1-OH-IPU has only been detected in mixed bacterial cultures derived from soil and it was reported as a dead-end metabolite without any further degradation (Lehr *et al.*, 1996). Recently, however, Badawi *et al.*

(2009) reported that 1-OH-IPU could be metabolised to 1-OH-MDIPU by agricultural soil fungus *Mortierlla* sp. Gr4 (Figure 4.4).

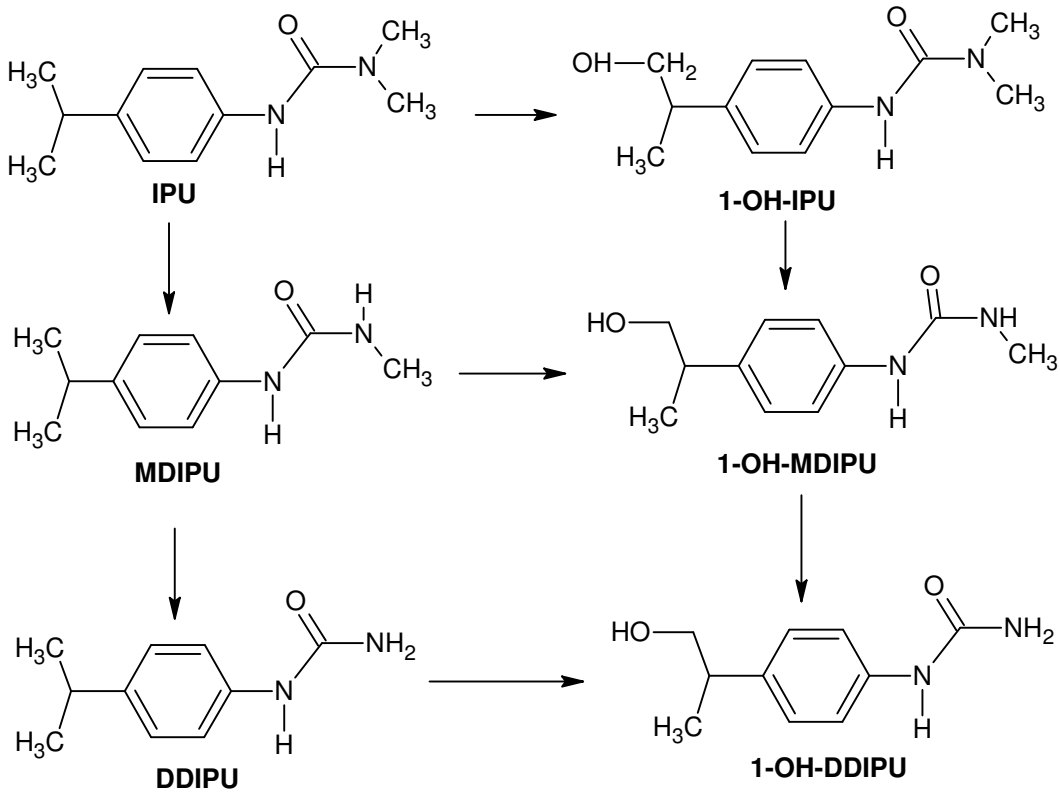



**Figure 4. 4** Proposed degradation pathways of isoproturon by the agricultural soil fungus *Mortierlla* sp. Gr4. After Badawi *et al*. (2009).

In situ, both MDIPU and 2-OH-IPU have been observed in soil porewater and surface runoff following IPU treatment of an agricultural field (Schuelein *et al.*, 1996). Low concentrations of the metabolites DDIPU and 4IA have been detected in IPU-treated agricultural soils (Mudd *et al.*, 1983; Lehr *et al.*, 1996; Sorensen and Aamand, 2001; Badawi *et al.*, 2009) and during the mineralisation of IPU by *Sphingomonas* sp. Strain SRS2 (Sorensen *et al.*, 2001). Several hydroxylated metabolites (Steps 8 – 13, Figure 4.3) have been detected during degradation of isoproturon in different soils (Mudd *et al.*, 1983; Lehr *et al.*, 1996; Pieuchot *et al.*, 1996; Schuelein *et al.*, 1996; Ronhede *et al.*, 2005; Ronhede *et al.*, 2007). Badawi *et al*. (2009) recently reported that 1-OH-

DDIPU (see Figure 4.4) was identified as a new product of the isoproturon pathway and is the final product of two processes shown to be utilised by *Mortierlla* sp. Gr4 in the transformation of isoproturon: *N*-demethylation of the urea group and hydroxylation of the isopropyl ring substituent (Ronhede *et al.*, 2005).

In summary, a number of factors influence the pathways and rates of microbial degradation. Warren *et al.* (2003) listed these factors, including: the type of substrate, temperature, dissolved oxygen, nutrient supply (to enable growth), similarity of the compound to the food sources, environments with previously exposure to the compound or similar compounds, and previous environmental conditions which will control the current population make-up. The concentration of available compound and the sorption of the compound on particles of the sediments therefore become very important.

#### *4.1.2.3 Previous studies for biodegradation of herbicides*

The number of studies for pesticide biodegradation in water - sediment systems is small (Warren *et al.*, 2003). In addition, most of the work has been performed in water-sediment slurries, with very few investigations on degradation in real or simulated sediment beds, where conditions may be very different from those in slurries have been undertaken (Warren *et al.*, 2003). Several reviews have dealt with pesticide behaviours in natural water-sediment systems under laboratory conditions (Bennett, 1990; Wolfe *et al.*, 1990; Muir *et al.*, 1991; Groenendijk *et al.*, 1994; Warren *et al.*, 2003; Katagi, 2006). Other studies have reported the degradation potential of isoproturon in shallow subsurface soils and in groundwater (Cox *et al.*, 1996; Johnson *et al.*, 1998; Issa and Wood, 1999; Bending *et al.*, 2001; Issa and Wood, 2005; Bending *et al.*, 2006). The degradation of mecoprop was also investigated in topsoil (Smith, 1989; Reffstrup *et al.*, 1998; Environment Agency, 2004; Fletcher *et al.*, 2004; Buss *et al.*, 2006), in salt marsh sediment (Fletcher *et al.*, 1995), in

anaerobic surface soil (Department of the Environment, 1994; Harrison *et al.*, 2003), in soil and a chalk aquifer (Johnson *et al.*, 2000; Kristensen *et al.*, 2001a; Williams *et al.*, 2004), in aquifer sediments (Torang *et al.*, 2003), in groundwater (Williams *et al.*, 2001), and in freshwater wetlands (Nilsson *et al.*, 2000). However, the sorption and biodegradation of the herbicides mecoprop and isoproturon in a river water – riverbed sediment system have not been reported yet.

## 4.2 Objectives

To clarify the first three hypotheses described in Section 1.6 and to understand the attenuation, including sorption and biodegradation, of the herbicides mecoprop and isoproturon in a river water – riverbed sediment system (abbreviated RW-RS system), the recirculation fixed-bed approach outlined in Section 3.3.2 was used. In addition, the $^{14}$C-respirometry technique described in Section 3.3.3 was applied to assess levels of catabolic competence in sample taken from the fixed-bed column at the conclusion of the recirculation period. The following objectives are addressed in this chapter:

(1) To investigate the sorption competence of mecoprop and isoproturon on/into the riverbed sediment using a fixed-bed column circulation method;

(2) To investigate the biodegradation properties of mecoprop and isoproturon in a RW-RS system;

(3) To identify whether the river water-born or riverbed sediment-born microorganisms are the main account for the degradation the herbicides;

(4) To establish levels of isoproturon catabolic activity in samples taken from the fixed-bed column upon conclusion of the recirculation.

## 4.3 Materials

### 4.3.1 Field Materials

#### *4.3.1.1 River water*

River water was collected on 14 September, 2007 from the Gatehampton site (see Section 3.2.1). The methods using to analyse the river water samples are presented in Section 3.2.2. Table 4.2 shows the physico-chemical properties of the river water samples.

**Table 4. 2** Measured physico-chemical properties of river water at the Gatehampton site. Value is a means of three replicates ± standard error.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Temp, $^{o}C$ | 16.8 ± 0.1 | $Cl^-$ , mg $L^{-1}$ | 25.8 ± 2.5 |
| pH | 8.12 ± 0.01 | $NO_3^-$ , mg $L^{-1}$ | 25.0 ± 1.2 |
| EC, µS $cm^{-1}$ | 867 ± 1 | $SO_4^{2-}$ , mg $L^{-1}$ | 46.4 ± 3.6 |
| DO, mg $L^{-1}$ | 9.20 ± 0.5 | $Na^+$ , mg $L^{-1}$ | 27.0 ± 1.0 |
| TN, mg $L^{-1}$ | 8.40 ± 0.07 | $Ca^{2+}$ , mg $L^{-1}$ | 100.5 ± 5.1 |
| TC, mg $L^{-1}$ | 53.1 ± 0.4 | $Mg^{2+}$ , mg $L^{-1}$ | 4.98 ± 0.5 |
| TOC, mg $L^{-1}$ | 50.4 ± 0.5 | $K^+$ , mg $L^{-1}$ | 5.60 ± 0.6 |
| Alkalinity, mEq $L^{-1}$ | 3.38 ± 0.08 | | |
| $HCO_3^{-1}$, mg $L^{-1}$ | 260 ± 5 | | |

High temperature (16.8 $^{o}C$) of the river water samples reflected the summer ambient conditions. An alkaline pH of 8.12 was observed in the river water samples. This pH value is consistent with pH values of 7.99 (Jackson *et al.*, 2006a) and 8.14 (Neal *et al.*, 2000) measured for the river Thames close to the Gatehampton site. High dissolved oxygen concentration of 9.20 mg $L^{-1}$ was observed in the water sample.

Calcium of 100.5 ± 5.1 mg $L^{-1}$ and bicarbonate of 260 ± 5 mg $L^{-1}$ dominated the major ions occurring in the river Thames. The high $Ca^{2+}$ and $HCO_3^-$ concentrations in the river water samples reflected that the river water was supplied from predominantly calcareous groundwater sources. The high dissolved oxygen concentration in the river water of 9.20 ± 0.5 mg $L^{-1}$ was a result of the easy diffusion and mixing of oxygen from the atmosphere to surface water. The presence of $NO_3^-$ simultaneously with $SO_4^{2-}$ and $Cl^-$ demonstrated that the river water could be influenced by agricultural activities. High total organic carbon of 50.4 ± 0.5 mg $L^{-1}$ was also measured in these river water samples.

Mecoprop and isoproturon were not detected in both the groundwater and river water samples (the detection limits of the HPLC analytical method are 1 and 2 μg $L^{-1}$ for isoproturon and mecoprop, respectively). However, the average concentrations of mecoprop and isoproturon in river water samples, collected at the Howberry Park, approximately 8 km upstream from the Gatehampton site, were reported to be 0.06 and 0.16 μg $L^{-1}$, respectively (Neal *et al.*, 2000). Minimum values of the herbicides mecoprop and isoproturon were also noted to be less than 0.04 μg $L^{-1}$ and with maximum values of 0.43 and 1.63 μg $L^{-1}$, respectively (Neal *et al.*, 2000).

#### *4.3.1.2 Riverbed sediment*

Riverbed sediment was collected on the same day and at the same position with the river water samples (Section 3.2.3). The riverbed sediments were passed through a 1.7 mm sieve to remove large components such as leaves, rocks, and coarse sediment. Physicol-chemical properties of riverbed sediment samples were analysed and are presented in Table 4.3. The method using to analyse the riverbed sediment samples are presented in Section 3.2.6.

**Table 4. 3** Physico-chemical properties of riverbed sediment at the Gatehampton site study (collected on 14 September, 2007). Value is a means of five replicates ± standard error.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Density (g/cm$^3$) | 2.65 ± 0.00 | S (%) | 0.43 |
| Bulk density (g/cm$^3$) | 1.25 ± 0.02 | N (%) | 0.45 |
| Porosity (%) | 50.6 ± 2.1 | $S_{SA}$* (m$^2$/g) | 0.0713 ± 0.0025 |
| Moisture content (%) | 29.79 ± 0.54 | Particle size distribution (% of weight): | |
| pH | 8.29 ± 0.05 | • 0.020 - 2 μm (clay) | • 1 ± 0.0% |
| TC (%) | 4.42 | • 2 – 50 μm (silt) | • 20 ± 0.2 % |
| TOC (%) | 0.80 | • 50 – 2000 μm (sand) | • 79 ± 0.9 % |

*$S_{SA}$ – Specific surface area

The texture of the riverbed sediment was determined to be a mixture of loamy sand. The sediment was dominated by 79 % sand ( > 50 μm), 20 % silt (2 – 50 μm) and approximately 1 % clay (0.020 – 2 μm). Given the composition of primary sand, a low specific surface area of 0.0713 m$^2$ g$^{-1}$ was obtained. The sediment was found to be alkaline (with a pH value of 8.29), in close agreement with the pH value of river water (pH = 8.12). A low organic carbon content of 8.0 mg kg$^{-1}$ was found in the sediment samples.

### 4.3.2 Chemicals and Laboratory Materials

Isoproturon was purchased from Sigma Aldrich (article/product: 36137, purity of 99.8%). Its physico-chemical properties are outlined in Section 1.5.1. Isoproturon stock solution (10 mg L$^{-1}$) was prepared in deionised water, then sonicated for 30 minutes. The aqueous stock solution was used to spike the appropriate experimental reservoirs.

$^{14}C$ ring-labelled isoproturon ($^{14}C$-isoproturon or $^{14}C$-IPU) was purchased from Amersham Co. Ltd, UK. $^{14}C$-isoproturon stock solution was prepared from isoproturon powder dissolved in ethanol achieving a final concentration of 10 kBq $mL^{-1}$.

Mecoprop was purchased from Sigma Aldrich (article/product: 36147, purity of 99.8%). Its physico-chemical properties are also outlined in Section 1.5.2. Mecoprop stock solution (10 mg $L^{-1}$) was made up in deionised water and was used to spike into the appropriate experimental reservoirs

Ultima Gold Scintillation cocktail was purchased from Perkin Elmer, United Kingdom. All other chemicals were reagent grade and obtained either from Sigma Aldrich or Fisher Scientific (Bishop Meadow Road, Loughborough, Leicestershire, United Kingdom).

## 4.4 Methods

Addressing the above objectives (Section 4.2), two experiments were performed. Experiment 1 aimed to investigate the attenuation including sorption and biodegradation of the two herbicides mecoprop and isoproturon in a RW-RS system. Fixed-bed column circulation method was employed for Experiment 1. Upon conclusion of Experiment 1 a second experiment, Experiment 2, was undertaken to assess the levels of catabolic competence in the fixed bed columns. It was the aim of this experiment to resolve the relative origin of catabolic competence with respect to either river-water associated microorganisms or riverbed-sediment associated microorganisms. To these ends the $^{14}C$-respirometry method was used.

### 4.4.1 Experiment 1 – Fixed-bed Column Circulation with Mecoprop and Isoproturon

The sorption and biodegradation of mecoprop and isoproturon in a RW-RS system were simultaneously investigated using a fixed-bed column circulation method. The development of this method has been described in Section 3.3.2. Four treatments coded Treatments 1, 2, 3 and 4 were set up using the river water and riverbed sediment samples. In every treatment, the materials were sterilised partly (river water only or riverbed sediment only) or wholly (both river water and riverbed sediment) or non-sterile. Sterilisation was performed using an autoclave (PS/QCS/EV150, 2005, Priorclave Ltd.) with parameters were set at 121 $^{o}$C and 30 minutes. Table 4.4 outlines the materials used in the four treatments and purposes of these treatments served within the experimental framework.

**Table 4. 4** Treatments for investigating the sorption and biodegradation of mecoprop and isoproturon in a RW-RS system.

| Treatments | River water (RW) | Riverbed sediment (RS) | Purposes |
|---|---|---|---|
| **1** | Sterile | Sterile | ◦ Abiotic control<br>◦ Sorption |
| **2** | Non-sterile | Non-sterile | ◦ Sorption<br>◦ Biodegradation with attribution from both RW and RS |
| **3** | Non-sterile | Sterile | ◦ Sorption<br>◦ Biodegradation with attribution from RW only |
| **4** | Sterile | Non-sterile | ◦ Sorption<br>◦ Biodegradation with attribution from RS only |

By sterilising the river water or riverbed sediment materials, the microorganisms born in these environments were assumed to be disable to degrade the herbicides. The purposes of the four treatments are presented as following:

- Treatment 1: both river water and riverbed sediment were sterilised before packing in the fixed-bed system. This treatment was designed to preclude biological reactions which could result in the degradation of the two herbicides during the experiment (18 circulation days). Hence, this treatment was used as an abiotic control or "biodegradation free" treatment to compare the biodegradation characteristics of other treatments. Sorption of the two herbicides on/in to the riverbed sediment was also established in this treatment;

- Treatment 2: both river water and riverbed sediment without sterilisation were used in the fixed-bed system. This treatment was designed to investigate both the sorption and biodegradation processes of the two herbicides in a RW-RS system. On account of this treatment using both materials in a non-sterile form, this treatment was anticipated to show the maximum loss compared with the other three treatments (which used the wholly or partly sterile materials);

- Treatment 3: only the riverbed sediment was sterilised while the river water was not. This treatment aimed to investigate the biodegradation of the two herbicides which could result from the river water-born microorganisms only.

- Treatment 4: only the river water was sterilised while the riverbed sediment was not. This treatment aims to examine the biodegradation of the two herbicides resulting from the riverbed sediment-born only.

Every treatment was replicated with three identical fixed-bed column circulation systems. After packing the river water and riverbed sediment into the fixed-bed systems, stock solutions of mecoprop and isoproturon were then spiked in the glass reservoirs to give a final concentration of approximately 100 $\mu g\ L^{-1}$ (for an individual herbicide). The next steps of this experiment are described in Section 3.3.2.2.

River water samples were obtained from the reservoir after a designated period of time, during 18 assay days. At these sampling times three samples were removed from each treatment replicate. The samples were analysed to measure concentration of the herbicides. The samples were filtered (Millex-GP, 0.22 µm, polyethersulfone, radio-sterilized) and kept in a cold room (4 $^{o}$C, darkness) before analysing by the HPLC method (procedure is described in Section 3.4). The value of pH in river water samples was also measured using the Hanna pH meter during the circulation period.

It is important to note that the fixed-bed column systems were operated under the temperature and light conditions inside the laboratory. These laboratory conditions were applied to approach the natural conditions which occur at the water-sediment interaction zone of the field site. The laboratory temperatures were recorded varying in the day time from 15 – 20 $^{o}$C and in the night time from 10 – 15 $^{o}$C. These laboratory temperatures reflected the outdoor temperatures as the laboratory were ventilated (benefited from the four inhaled fans in the fume cupboards and the opened windows). The laboratory light was described as the natural light but without direct sunbeams (to minimise the photochemical degradation). The laboratory had large glass windows (that received light in the day time). The laboratory light was described as the natural light. At night, the laboratory was dark without the illumination of florescent strip lighting.

Figure 4.5 provides a photograph of the experimental apparatus.

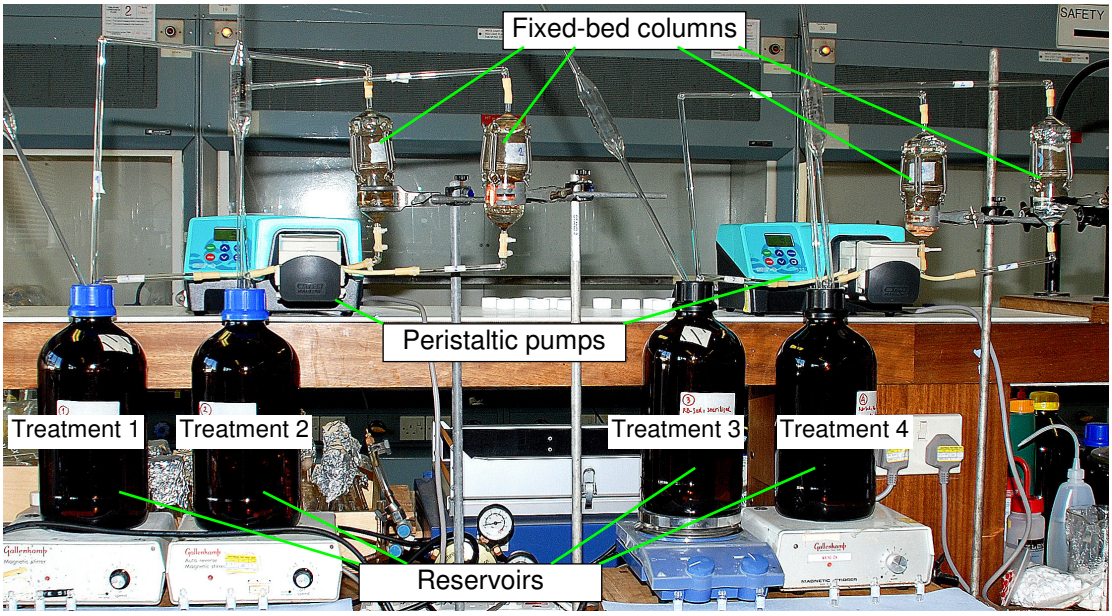



**Figure 4. 5** Fixed-bed column circulation systems for Experiment 1.

### 4.4.2 Experiment 2 – Respirometry Experiment with respect to Isoproturon

The riverbed sediments from Treatments 3 and 4 were removed immediately after Experiment 1 had finished (after 18 circulation days) and transferred to the respirometers in order to investigate the catabolic competence with respect to isoproturon. Three replicates of the riverbed sediment were taken from every column of Experiment 1. Two sets of respirometer were set-up based upon these sediment samples and are described below:

- Set 1: using the riverbed sediment extracted from the columns of Treatment 3 (non-sterile river water and sterile riverbed sediment). Set 1 was designed to investigate the catabolic activity of the river water-borne microorganisms after 18 activated days. As stated above, the riverbed sediment-borne microorganisms were previously disabled by sterilised the riverbed sediment in Treatment 3. Therefore, microorganisms attached to the sediment were assumed to originate from the river water;

- Set 2: using the riverbed sediment extracted from the columns of Treatment 4 (sterile river water and non-sterile riverbed sediment). In contrast to Set 1, Set 2 was designed to investigate the catabolic activity of the riverbed sediment-borne microorganisms after 18 activated days with respect to isoproturon. Again, the river water-borne microorganisms were assumed to be destroyed by sterilising the river water in Treatment 4. Hence, microorganisms attached to the sediment were assumed to originate solely from the sediment.

The respirometry method using $^{14}$C-isoproturon was applied in Experiment 2. Details of the method are presented in Section 3.3.3. The experimental procedure for Sets 1 and 2 were identical. Respirometers contained sterile deionise water (30 mL). Portions of riverbed sediment (10 g) extracted from the columns of Treatments 3 (for Set 1) and 4 (for Set 2) were transferred to the respirometers. $^{14}$C-IPU was immediately spiked to the respirometer. Then, the common consecutive steps were followed the procedure described in Section 3.3.3.2. Figure 4.6 presents a set-up for Experiment 2. Blank respirometers were prepared as described in Section 3.3.3.2.

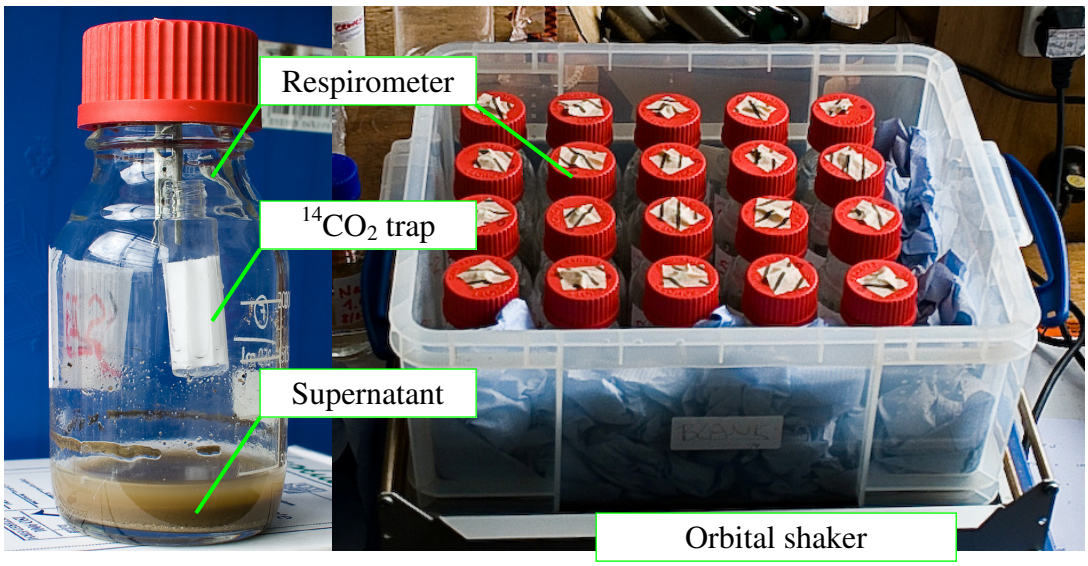



**Figure 4. 6** A respirometer for Experiment 2. Principle of respirometer set-up is shown in Figure 3.5.

Respirometry assay undertaken as Experiment 2, lasted for a duration of 10 days; sufficient time for the levels of mineralisation to reach a plateau. Maximum mineralisation levels reported reflect the extent of mineralisation after 10 days assay time. Method to calculate the maximum mineralisation level and maximum mineralisation rate is presented in Section 3.3.3.2.

### 4.4.3 Statistical Analysis

Experiment 1, the fixed-bed circulation experiment, had three replicates (n = 3) for every Treatments 1, 2, 3 and 4. When samples were removed at the sampling times from the recirculation reservoirs, one sample were removed from each reservoir. Experiment 2, the respirometry experiment, had three replicates (n = 3) for every Sets 1 and 2. When the riverbed sediment samples were removed from the fixed-bed columns of Treatments 3 and 4 (after finishing Experiment 1), three samples were removed from every column. A combination of one-way analysis of variance (ANOVA) and post-hoc tests (Tukey) was used to compare the level of significance among several treatments (more than 3 groups of data). The independent-sample t-test was used to examine the significant difference between two groups. For all tests, a significance p-value of less than 0.05 was used. All statistical analyses were performed using SPSS for Windows® (version 16.0.). Variations in data are given as standard errors of three replicates. Microsoft Excel and Sigma Plot 2000 were used to plot data, calculate the fitted lines and their associations.

## 4.5 Results

### 4.5.1 Experiment 1 – Fixed-bed Column Circulation with Mecoprop and Isoproturon

The attenuation of mecoprop and isoproturon was studied by monitoring the concentration of these herbicides during the recirculation of river water through the riverbed sediment column. The concentrations of the herbicides in the river water of Treatments 1, 2, 3 and 4 were measured after a designated period of circulation up until 18 days. The residual concentration in the reservoirs were then plotted against assay time results were then plotted against the assay time elapsed. Figure 4.7 presents the residual concentrations of mecoprop and isoproturon in all four treatment types with time.

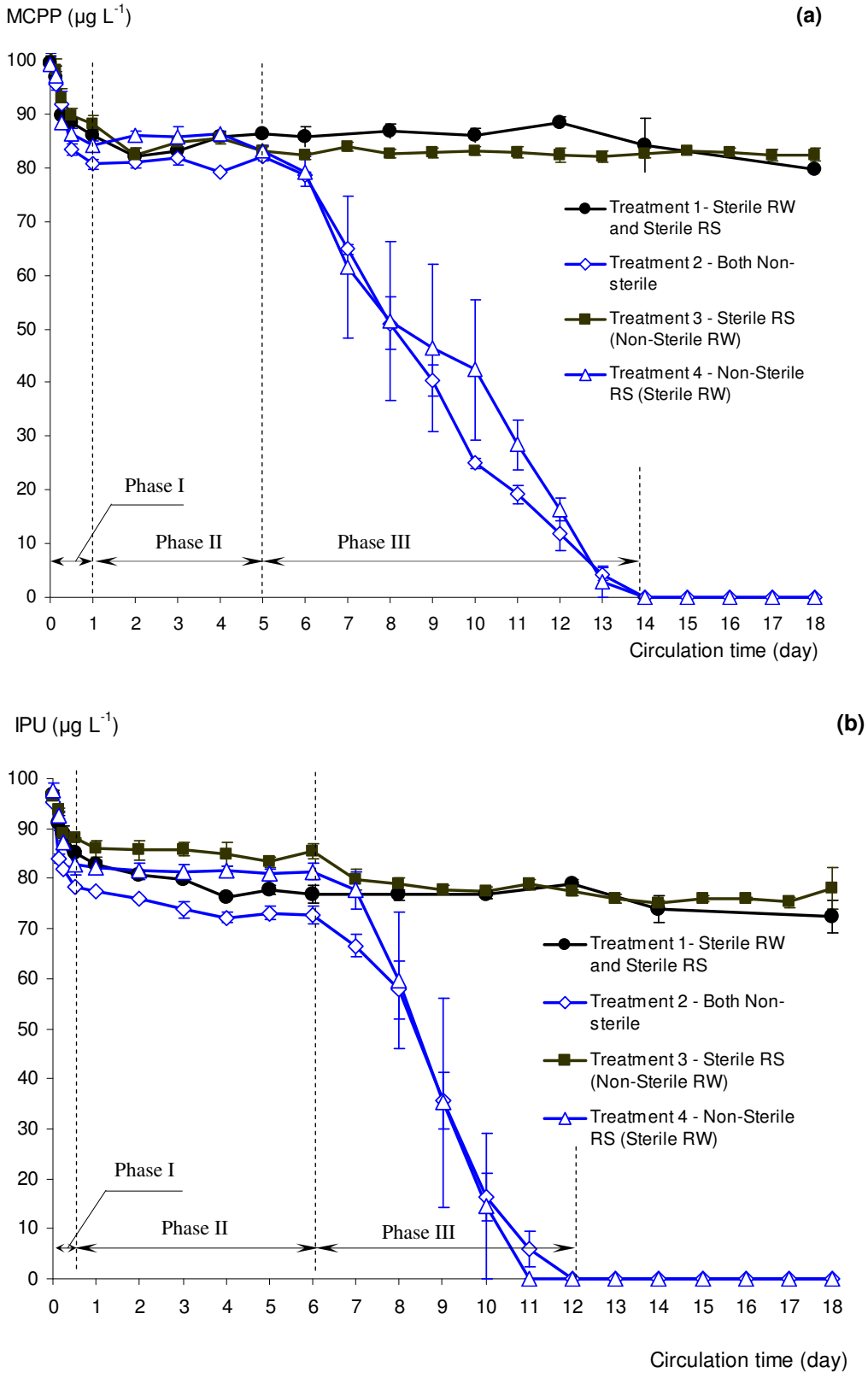



**Figure 4. 7** Attenuation of: (a) - mecoprop (MCPP) and (b) - isoproturon (IPU) in a RW-RS system with sterile and non-sterile RW and RS, error bars present standard error of three replicates. RW: river water; RS: river sediment.

The curves were divided into three phases in order to examine and simulate the attenuation processes of mecoprop and isoproturon in a RW-RS system. Three phases are presented as following:

(i) Phase I considered to be the sorption phase, with:
- Mecoprop: lasting from 0 to 24 hours of circulation, applied to all Treatments 1, 2, 3 and 4;
- Isoproturon: lasting from 0 to 12 hours of circulation, applied to all Treatments 1, 2, 3 and 4;

(ii) Phase II considered to be the lag or adaptation or sometimes acclimation phase, with:
- Mecoprop: lasting for 4 days, from day 2 to day 5, applied to Treatments 2 and 4;
- Isoproturon: lasting for 5.5 days, from day 0.5 to day 6, applied to Treatments 2 and 4;

(iii) Phase III considered to be the biodegradation phase, with:
- Mecoprop: lasting for 9 days, from day 6 to day 14, applied to Treatments 2 and 4;
- Isoproturon: lasting for 6 days, from day 7 to day 12, applied to Treatments 2 and 4.

#### *4.5.1.1 Phase I – Sorption phase*

Concentration of mecoprop and isoproturon in river water were rapidly decreased in all four treatments. With regard to mecoprop, the concentration rapidly declined during the first 24 circulation hours, from approximately 100 ± 1 μg $L^{-1}$ to 86 ± 1, 81 ± 1, 88 ± 1 and 84 ± 1 μg $L^{-1}$ in Treatments 1, 2, 3 and 4, respectively. In the similar way, the concentration of isoproturon was promptly decreased during the first 12 hours, from approximately 100 ± 1 μg $L^{-1}$ to 85 ±

1, 78 ± 0, 88 ± 0 and 83 ± 2 μg $L^{-1}$ in Treatments 1, 2, 3 and 4, respectively. Consecutively, the concentrations of the two herbicides did not significantly change ($p > 0.05$) for a period of several days. For instance, concentrations of mecoprop in Treatments 1, 2, 3 and 4 were determined after 5 circulation days to be 86 ± 0, 82 ± 1, 83 ± 1 and 83 ± 1, respectively; or concentrations of isoproturon in Treatments 1, 2, 3 and 4 were determined after 6 circulation days to be 77 ± 2, 70 ± 1, 85 ± 2 and 81 ± 3, respectively. Thus it was assumed that the pseudo-equilibrium of mecoprop and isoproturon in the RW-RS system was established after 24 and 12 hours of circulation, respectively. These periods of 24 and 12 hours were therefore assumed as the *sorption time* of mecoprop and isoproturon on riverbed sediment, respectively. Based upon these sorption times, the following sorption parameters of mecoprop and isoproturon are defined and calculated as below:

(1) *Percent sorption loss of a pesticide, A (%)*: is the percent loss of a pesticide from the aqueous phase. It is the difference between the initial concentration and the pseudo-equilibrium concentration of the pesticide:

$$A = \frac{C_o - C_e}{C_o} x100 \ (\%) \qquad (4.1)$$

where

$C_o$ – initial concentration of the pesticide, $\mu g\ L^{-1}$;

$C_e$ – pseudo-equilibrium concentration of the pesticide, $\mu g\ L^{-1}$.

In the cases of mecoprop and isoproturon in Experiment 1, $C_e$ was chosen from the concentrations after 24 and 12 hours of sorption times;

(2) *Maximum sorption capacity* on to/into riverbed sediment, $C_{S,max}$ ($\mu g\ kg^{-1}$): the maximum amount of a pesticide (μg) associated with an amount of dry riverbed sediment (kg);

$$C_{S,\max} = \frac{(C_o - C_e) * V_W}{m_{dry,sed}} \quad (\mu g\ kg^{-1}) \qquad (4.2)$$

where

$V_W$ – Volume of the experimental water, *L*. In the case of Experiment 1, $V_w$ = 1.5 L;

$m_{dry,sed}$ – mass of dry sediment, *kg*. In the case of Experiment 1, $m_{dry,sed}$ = 105.105 g (see Session 3.2.6 for more details).

On the other hand, the sorption capacity of a solid phase is strongly dependent on its specific surface area (Clausen *et al.*, 2001). Thus the maximum sorption capacity of a solid phase is actually affected by its specific surface area. A *maximum sorption capacity* which is dependent upon the specific surface area, $C_{S,max,sur}$ ($\mu g\ m^{-2}$), can be calculated from the ratio of the maximum amount of a pesticide sorbed on/into the solid phase and the unit of specific surface area of the solid phase as following:

$$C_{S,\max,sur} = \frac{C_{S,\max}}{S_{SA}} \qquad (\mu g\ m^{-2}) \qquad (4.3)$$

where

$S_{SA}$ – specific surface area of the solid phase, $m^2\ g^{-1}$. In the case of Experiment 1, $S_{SA}$ = 0.0713 ± 0.0025 $m^2\ g^{-1}$ = 71.3 ± 2.5 $m^2\ kg^{-1}$ (Table 4.3).

(3) *Solid-water distribution coefficient, $K_D$ (L $kg^{-1}$)*: the ratio of the maximum amount of a pesticide distributed in a *mass unit* of the solid phase and in the aqueous phase at the equilibrium condition:

$$K_D = \frac{C_{S,\max}}{C_e} \qquad (L\ kg^{-1}) \qquad (4.4)$$

In the same way with the maximum sorption capacity, Clausen *et al.* (2001) suggested a s*olid-water distribution coefficient* normalised to the specific surface area of the solid phase. It can be identified as the fraction of $K_D$ and specific surface area of the solid phase:

$$K_{D,sur} = \frac{K_D}{S_{SA}} \qquad (\text{L m}^{-2}) \qquad (4.5)$$

(4) *Organic carbon-normalised distribution coefficients, $K_{OC}$*: the ratio of the solid-water distribution coefficient of a pesticide and the mass fraction of organic carbon:

$$K_{OC} = \frac{K_D}{f_{OC}} \qquad (4.6)$$

where

$f_{OC}$ – fraction of organic carbon by mass in sediment. In the case of Experiment 1, $f_{OC}$ = TOC (%)/100 = 0.008 (see Table 4.3).

(5) *Retardation factor, $R_D$*: the phenomenon of diminished chemical transport rate relative to the water seepage velocity. Hiscock (2005) introduced the retardation equation, as follows:

$$R_D = 1 + K_D \frac{\rho_b}{n} \qquad (4.7)$$

where

$\rho_b$ – bulk density of the solid phase, kg $L^{-1}$. In the case of Experiment 1, $\rho_b$ = 1.25 ± 0.02 g/$cm^3$ (Table 4.3);

$n$ – porosity of the solid phase, %. In the case of Experiment 1, $n$ = 50.6 ± 2.1 % (Table 4.3).

(6) *Sorption rate constant, $k_{sorp}$ ($h^{-1}$)*: first-order sorption kinetics model was fitted to the observed herbicide concentrations versus sorption time resulting in an estimated sorption rate constant $k_{sorp}$. The mathematical equation is described below:

$$\frac{dC_W}{dt} = -k_{sorp}.C_w \qquad (4.8)$$

Integrating equation (4.8) gives:

$$\ln\frac{C_t}{C_o} = -k_{sorp}.t \qquad (4.9)$$

where

$C_W$ – observed concentration of the herbicides in the aqueous phase during the sorption time (μg $L^{-1}$);

$C_o$ – initial concentration of the herbicides in the aqueous phase (μg $L^{-1}$);

$k_{sorp}$ – first-order sorption kinetic rate constant of the compound ($h^{-1}$);

$t$ – sorption time (h).

### *Sorption isotherms of mecoprop and isoproturon*

Based upon the above equations, sorption isotherm parameters of mecoprop and isoproturon on/into riverbed sediment in Treatments 1, 2, 3 and 4 were determined. As stated in advance, Treatment 2 was considered as the most representative treatment to simulate the sorption process because neither of the materials in Treatment 2 were sterilised. Therefore, sorption values of Treatment 2 were used as a point of reference with which to compare with the values of Treatments 1, 3 and 4. Table 4.5 presents the sorption parameters of mecoprop on/into the riverbed sediment in all four treatments.

**Table 4. 5** Sorption parameters of mecoprop on/into riverbed sediment (means ± standard errors from three replicates).

| Treatments / Parameters | **1** (n=3) | **2** (n=3) | **3** (n=3) | **4** (n=3) |
|---|---|---|---|---|
| A, % | 13.5 ± 1.5 | 19.5 ± 2.0 | 13.2 ± 0.8 | 15.1 ± 0.6 |
| $C_{S,max,}$ μg $kg^{-1}$ | 192 ± 23 | 279 ± 32 | 192 ± 16 | 214 ± 11 |
| $C_{S,max,sur}$, μg $m^{-2}$ | 2.69 ± 0.32 | 3.91 ± 0.45 | 2.69 ± 0.22 | 3.01 ± 0.16 |
| $K_D$, L $kg^{-1}$ | 2.24 ± 0.28 | 3.47 ± 0.43 | 2.17 ± 0.15 | 2.55 ± 0.13 |
| $K_{D,sur}$ , L $m^{-2}$ | 0.031 ± 0.004 | 0.049 ± 0.006 | 0.031 ± 0.002 | 0.036 ± 0.002 |
| $K_{OC}$ | 280 ± 35 | 434 ± 54 | 271 ± 19 | 318 ± 16 |
| Log $K_{OC}$ | 2.44 ± 0.06 | 2.63 ± 0.06 | 2.43 ± 0.03 | 2.50 ± 0.02 |
| $R_D$ | 6.53 ± 0.68 | 9.57 ± 1.07 | 6.36 ± 0.37 | 7.29 ± 0.31 |

In Treatment 2, there were 19.5 ± 2.0 % of mecoprop removed from the river water. The values of the maximum sorption capacities based on the dry mass of the sediment ($C_{S,max}$) and based on the specific surface area of the sediment ($C_{S,max,sur}$) were identified to be 279 ± 32 μg $kg^{-1}$ and 3.91 ± 0.45 μg $m^{-2}$, respectively. The distribution of mecoprop in river water and riverbed sediment was presented by the solid-water distribution coefficient ($K_D$). The

values of $K_D$ (based on the dry mass of the sediment) and $K_{D,sur}$ (based on the specific surface area of the sediment) were calculated to be 3.47 ± 0.43 L $kg^{-1}$ and 0.049 ± 0.006 L $m^{-2}$, respectively. The influence of organic matter in the riverbed sediment on $K_D$ was considered by the organic carbon-normalised distribution coefficient, $K_{OC}$. The values of $K_{OC}$ and $\log K_{OC}$ were determined to be 434 ± 54 and 2.63 ± 0.06, respectively. Based upon the distribution value of mecoprop in water and sediment ($K_D$), the diminished transport rate of mecoprop to the water seepage velocity can be described by the retardation factor ($R_D$). The value of $R_D$ with respect to mecoprop was calculated to be 9.57 ± 1.07.

One-way ANOVA indicated that there was no significant difference ($p > 0.05$) of the sorption parameters of mecoprop in Treatments 2 and 4. However, the sorption parameters in Treatment 2 were significantly higher ($p < 0.05$) than the same parameters in Treatments 1 and 3.

In a similar way with mecoprop, the sorption parameters of isoproturon were obtained from Equations 4.1 to 4.9. Table 4.6 presents the sorption parameters of isoproturon on/into riverbed sediment in all four treatments.

**Table 4. 6** Sorption parameters of isoproturon on/into riverbed sediment (means ± standard errors from three replicates).

| Treatments / Parameters | **1** (n=3) | **2** (n=3) | **3** (n=3) | **4** (n=3) |
|---|---|---|---|---|
| A, % | 11.9 ± 0.8 | 17.6 ± 0.6 | 8.7 ± 1.0 | 15.1 ± 2.8 |
| $C_{S,max,}$ μg $kg^{-1}$ | 165 ± 12 | 240 ± 9 | 119 ± 15 | 212 ± 41 |
| $C_{S,max,sur}$, μg $m^{-2}$ | 2.31 ± 0.17 | 3.36 ± 0.13 | 1.67 ± 0.21 | 2.97 ± 0.58 |
| $K_D$, L $kg^{-1}$ | 1.93 ± 0.14 | 3.06 ± 0.12 | 1.35 ± 0.17 | 2.58 ± 0.55 |
| $K_{D,sur}$, L $m^{-2}$ | 0.027 ± 0.002 | 0.043 ± 0.002 | 0.019 ± 0.002 | 0.036 ± 0.008 |
| $K_{OC}$ | 242 ± 17 | 382 ± 15 | 169 ± 21 | 323 ± 69 |
| log $K_{OC}$ | 2.38 ± 0.03 | 2.58 ± 0.02 | 2.22 ± 0.05 | 2.49 ± 0.10 |
| $R_D$ | 5.77 ± 0.34 | 8.55 ± 0.30 | 4.35 ± 0.42 | 7.38 ± 1.35 |

In Treatment 2, there were 17.6 ± 0.6 % of isoproturon removed from the river water. The values of the maximum sorption capacities based on the dry mass of the sediment ($C_{S,max}$) and the specific surface area of the sediment ($C_{S,max,sur}$) were identified to be 240 ± 9 μg $kg^{-1}$ and 3.36 ± 0.13 μg $m^{-2}$, respectively. The means of $K_D$ (based on mass of the sediment) and $K_{D,sur}$ (based on specific surface area of the sediment) of isoproturon were identified of 3.06 ± 0.12 L $kg^{-1}$ and 0.043 ± 0.002 L $m^{-2}$, respectively. The average values of $K_{OC}$ and log$K_{OC}$ were determined to be 382 ± 15 and 2.58 ± 0.02, respectively. The mean value of $R_D$ with respect to isoproturon was calculated to be 8.55 ± 0.30.

Regarding the sorption parameters of isoproturon in Table 4.6, one-way ANOVA indicated that there was no significant difference ($p > 0.05$) between any of the sorption parameters of isoproturon in Treatments 2 and 4. However, the sorption parameters in Treatments 1 and 3 were significantly lower ($p < 0.05$) than the parameters in Treatment 2.

### *Sorption kinetics of mecoprop and isoproturon*

Sorption kinetics of mecoprop and isoproturon on/into riverbed sediment was investigated by observing concentration of these herbicides during Phase I of Experiment 1 (the first 24 hour for mecoprop and 12 hour for isoproturon). The concentration thereafter did not decrease for several days. It was thus assumed that the equilibrium distribution state of mecoprop and isoproturon on/into riverbed sediment and in river water was reached. It was assumed that the decrease of the herbicide concentration could be simulated using the first-order mathematical model (Equations 4.8). By plotting the natural logarithm of the fraction between the current and the initial concentrations of the herbicides against the sorption time, the sorption rate constants $k_{sorp}$ ($h^{-1}$) were estimated from the gradients of the fit lines.

Figure 4.8 presents the sorption kinetics of mecoprop on/into riverbed sediment from three replicates of Treatments 1, 2, 3 and 4. The average sorption rate constants of mecoprop in Treatments 1, 2, 3 and 4 were estimated to be 0.0073 ± 0.0008 $h^{-1}$, 0.0106 ± 0.0015 $h^{-1}$, 0.0073 ± 0.0005 $h^{-1}$ and 0.0083 ± 0.0003 $h^{-1}$, respectively. One-way ANOVA indicated that there was no significant difference ($p > 0.05$) between the sorption rate constant in Treatment 2 and the sorption rates constants in Treatments 1, 3 and 4.

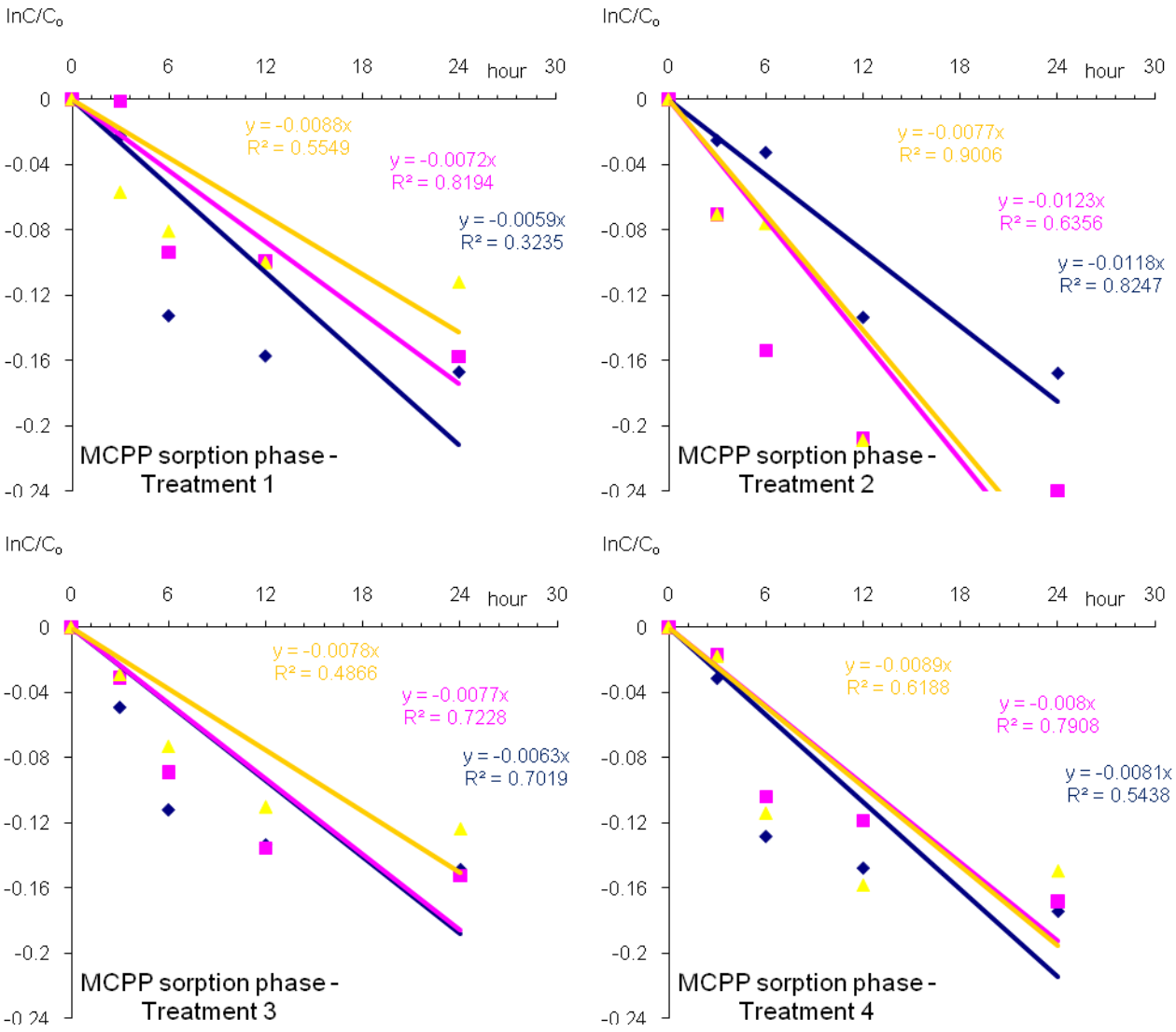



**Figure 4. 8** Sorption kinetics of mecoprop on/into riverbed sediment from three replicates of Treatments 1, 2, 3 and 4. Blue, pink and yellow points represent data of Replicates 1, 2 and 3. Blue, pink and yellow lines represent the fit lines of the appropriate replicates.

Figure 4.9 presents the sorption kinetics of isoproturon on/into riverbed sediment from three replicates of Treatments 1, 2, 3 and 4. The average sorption rate constants of isoproturon in Treatments 1, 2, 3 and 4 were estimated to be 0.0116 ± 0.005 $h^{-1}$, 0.0191 ± 0.009 $h^{-1}$, 0.0087 ± 0.0013 $h^{-1}$ and 0.0149 ± 0.0027 $h^{-1}$, respectively. One-way ANOVA indicated that there was no significant difference ($p > 0.05$) between the sorption rate constant of Treatments 2 and 4. However, there were significantly different ($p < 0.05$) between the sorption rate constant of Treatment 2 and the sorption rate constants of Treatments 1 and 3.

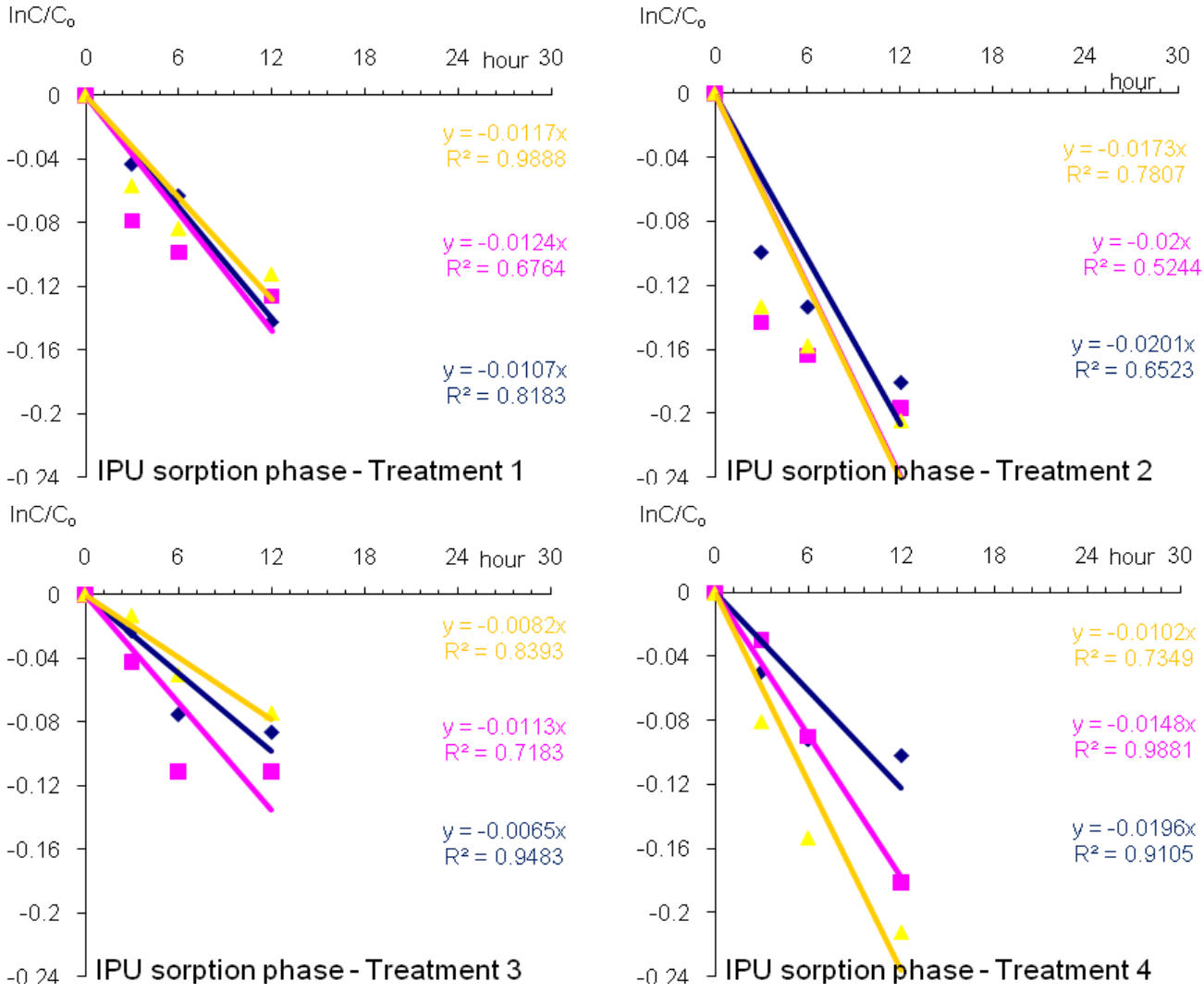



**Figure 4. 9** Sorption kinetics of isoproturon on/into riverbed sediment from Treatments 1, 2, 3 and 4. Blue, pink and yellow points represent data of Replicates 1, 2 and 3. Blue, pink and yellow lines represent the fit lines of the appropriate replicates.

#### *4.5.1.2 Phase II – Adaptation phase*

After the sorption phase, concentration of mecoprop and isoproturon did not significantly change ($p > 0.05$) for a period of several days. Regarding Treatment 1, it was considered as an abiotic control because both river water and riverbed sediment were sterilised. Thus, it was assumed that no microbial degradation occurred in this treatment. Indeed, after the sorption phase, concentration of the both mecoprop and isoproturon was stable over the remaining assay time (18 circulation days including 1 day for the sorption). With respect to mecoprop, the concentration was stable for 17 days, from 86 ± 1 μg $L^{-1}$ at day 1 to 80 ± 2 μg $L^{-1}$ at day 18. With respect to isoproturon, the concentration was measured of 85 ± 1 μg $L^{-1}$ at a half of day 1 and 72 ± 3 μg $L^{-1}$ at day 18. There was a decrease in concentrations of both mecoprop and isoproturon over 18 recirculation days. However, independent t test indicated that there was no significant decrease ($p > 0.05$) between the concentrations of both these herbicides at day 1 and day 18.

Regarding Treatment 2, both river water and riverbed sediment used in this treatment were not sterilised. Thus microorganisms present in both of the environment media had the possibility to degrade the herbicides. After Phase I or the sorption phase, during the consecution of 4 and 5.5 days, the concentrations of mecoprop and isoproturon, respectively did not significantly change. With respect to mecoprop, the concentration was stable for 4 days, of 81 ± 1 μg $L^{-1}$ at day 5 and 80 ± 2 μg $L^{-1}$ at day 5. Independent t test indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 1 and day 5. With respect to isoproturon, the concentration was stable for 5.5 days, 78 ± 0 μg $L^{-1}$ at a half of day 1 and 73 ± 2 μg $L^{-1}$ at day 6. Independent t test indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 0.5 and day 6. Thereafter, concentrations of mecoprop and isoproturon were considerably decreased. The period of

unchanged concentration was assumed as the adaptation time for microorganisms. It is suggested that the adaptation time was essential for microorganisms to adapt to the herbicides and then degrade these compounds as a source of C and energy to proliferate their population. Biofilms on the riverbed sediment was suggested to be established during this period of time.

Regarding Treatment 3, riverbed sediment-born microorganisms were deactivated due to sterilisation of the riverbed sediment. Therefore, degradation in this case, if possible, could be brought about by river water-borne microorganisms. The results showed that, however, the concentrations of the two herbicides in Treatment 3 were not significantly changed during the remaining assay time after the sorption phase. Concentration of mecoprop in Treatment 3 lightly decreased approximately 5 %, from 88 ± 1 to 82 ± 2 $\mu g\ L^{-1}$ during the time from day 1 to the end of day 18, respectively. Independent t test indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 1 and day 18. Similarly, concentration of isoproturon decreased approximately 10 %, from 88 ± 0 to 78 ± 4 $\mu g\ L^{-1}$ during the time from after a half of day 1 to the end of day 18. Independent t test indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 0.5 and day 18. Comparison to the abiotic control treatment (Treatment 1), independent t test showed that no significant difference ($p > 0.05$) of the concentrations of both mecoprop and isoproturon was found between Treatments 1 and 3. This proved that river water-borne microorganisms were not competent to degrade mecoprop and isoproturon in a RW-RS system. The light decrease of the herbicides in Treatment 3 might be explained by the long-term slower sorption processes occurring in this treatment.

Regarding Treatment 4, river water-borne microorganisms were disabled due to sterilisation of the river water. Thus, degradation in this treatment, if possible, could be resulted from the riverbed sediment-borne microorganisms. Similar to the case of Treatment 2, during the period of 4 and 5.5 circulation days, the

concentrations of mecoprop and isoproturon did not significantly change, respectively. With respect to mecoprop, the concentration was stable for 4 days, from 84 ± 1 μg $L^{-1}$ at day 2 to 83 ± 1 μg $L^{-1}$ at day 5. Independent t test indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 1 and day 5. With respect to isoproturon, the concentration was stable for 5.5 days, from 83 ± 2 μg $L^{-1}$ at a half of day 1 to 81 ± 2 μg $L^{-1}$ at day 6. Independent t test also indicated that there was no significant difference ($p > 0.05$) between the concentrations of day 0.5 and day 6. Then, concentrations of mecoprop and isoproturon were considerably decreased. These periods of time were considered as the adaptation times for riverbed sediment-born microoragnisms to grow the population before degrading the herbicides.

#### *4.5.1.3 Phase III – Biodegradation phase*

After a period of adaptation (5 days for mecoprop and 6 days for isoproturon, including the sorption time), concentrations of mecoprop and isoproturon in Treatments 2 and 4 were considerably decreased. In contrast, concentrations of these herbicides in Treatments 1 and 3 remained largely unchanged. Therefore, the investigation of microbial degradation with respect to mecoprop and isoproturon, as stated above, was focused on in Treatments 2 and 4 only.

To investigate the biodegradation of mecoprop and isoproturon in a RW-RS system, degradation kinetics of these compounds were firstly considered. Several mathematical degradation models have been proposed to simulate the kinetics of biodegradation for an organic compound. Collectively, a zero-order model was applied in this study because it was the best fit model to the empirical curves of Treatments 2 and 4 (Phase III, Figure 4.7). The differential form of a zero-order model is presented as below:

$$\frac{dC_t}{dt} = -k_{bio} \qquad (4.10)$$

the integral form is

$$C_t = -k_{bio}.t + C_{0,bio} \qquad (4.11)$$

where

$C_t$ – concentration of the compound in the aqueous phase after assay time, $t$, (μg $L^{-1}$);

$C_{0,bio}$ – initial concentration of the compound (μg $L^{-1}$) in the aqueous phase (μg $L^{-1}$) immediately prior to Phase III commencing;

$k_{bio}$ – zero-order biodegradation kinetic rate constant of the compound (μg $L^{-1}$ $day^{-1}$);

$t$ – retention time of the biodegradation process, (day). In the case of Experiment 1, $t$ varied from day 6 to 14 for mecoprop and from day 7 to 12 for isoproturon.

*Half-life of biodegradation phase*, $t_{1/2,\,bio}$: the time of which the concentration of a compound decreases to a half of the initial concentration. From Equation 4.11, the half-life of biodegradation phase is presented as below:

$$t_{1/2,bio} = \frac{C_{0,bio}}{2k_{bio}} \qquad (4.12)$$

Basing on this approach, biodegradation kinetics of mecoprop and isoproturon were investigated. Figure 4.10 presents the biodegradation kinetics of mecoprop in a RW-RS system.

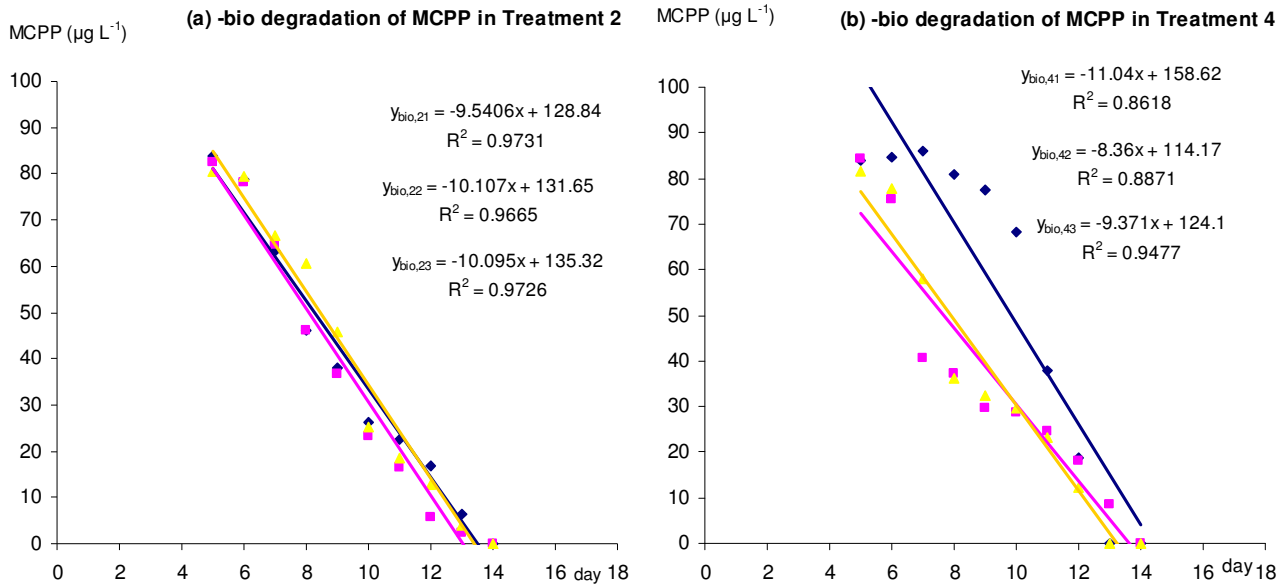



**Figure 4. 10** Biodegradation kinetics of mecoprop in the RW-RS system from Treatments 2 (Figure a) and 4 (Figure b). Blue, pink and yellow points represent data of Replicates 1, 2 and 3. Blue, pink and yellow lines represent the fit lines of the appropriate replicates.

During Phase III from day 6 to day 14 of circulation, concentration of mecoprop in Treatments 2 and 4 rapidly decreased from 82 ± 1 and 83 ± 1 µg $L^{-1}$, respectively, to lower than the detection limit of the HPLC (< 2 µg $L^{-1}$). Using the zero-order model (Equation 4.11), biodegradation rates of mecoprop in Treatments 2 and 4 were determined to be 9.91 ± 0.19 µg $L^{-1}$ $day^{-1}$ (ranging from 9.54 to 10.11 µg $L^{-1}$ $day^{-1}$) and 9.59 ± 0.78 µg $L^{-1}$ $day^{-1}$ (ranging from 8.36 to 11.04 µg $L^{-1}$ $day^{-1}$). In addition, half-lives of mecoprop within the biodegradation phase in Treatments 2 and 4 were determined to be 4.1 ± 0.1 days (ranging from 4.0 to 4.4 days) and 4.4 ± 0.3 days (ranging from 3.8 to 4.9 days). Independent-sample t test also indicated that the biodegradation rate constants of mecoprop in Treatment 2 were not significantly different ($p > 0.05$) to the rate constants in Treatment 4.

Figure 4.11 presents the biodegradation kinetics of isoproturon in a RW-RS system.

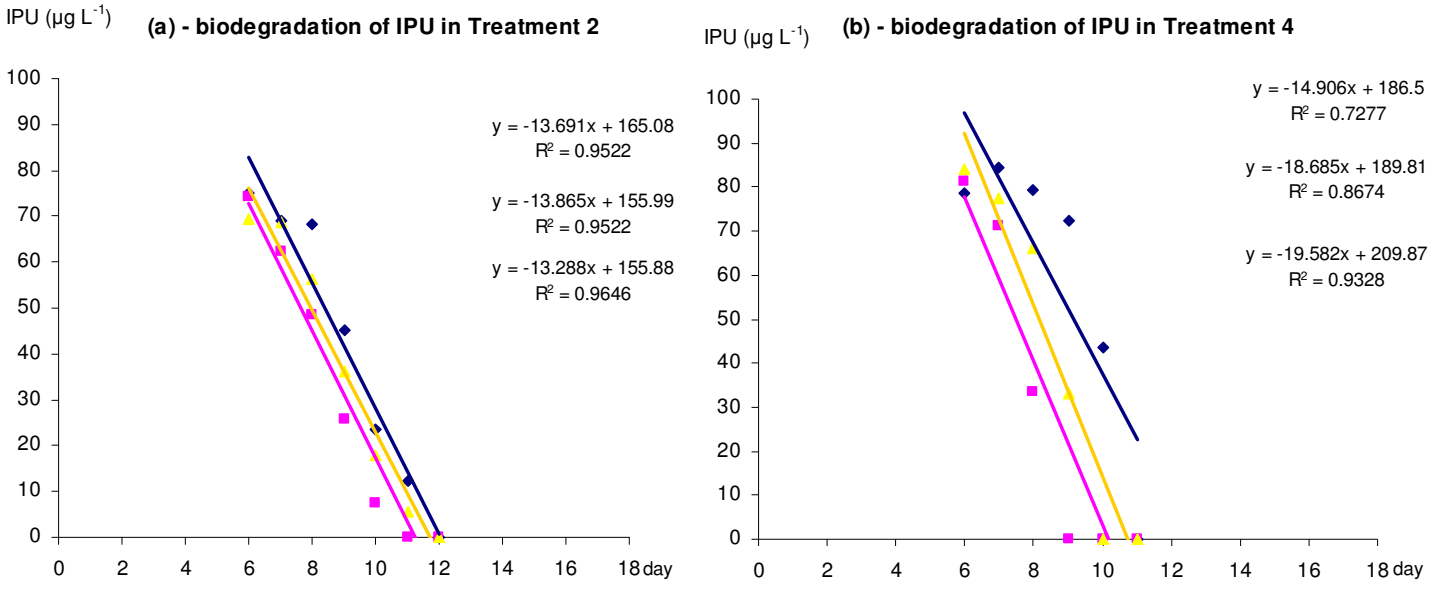



**Figure 4. 11** Biodegradation kinetics of isoproturon in the RW-RS system from Treatments 2 (a) and 4 (b). Blue, pink and yellow points represent data of Replicates 1, 2 and 3. Blue, pink and yellow lines represent the fit lines of the appropriate replicates.

The biodegradation phase or phase III of isoproturon lasted from day 7 to day 12 of circulation. Concentration of isoproturon in Treatments 2 and 4 decreased from 70 ± 1 and 81 ± 2 µg $L^{-1}$, respectively, to lower than the detection limit of the HPLC (< 1 µg $L^{-1}$). Using the zero-order model (Equation 4.11), biodegradation rates of isoproturon in Treatments 2 and 4 were determined to be 13.62 ± 0.17 µg $L^{-1}$ $day^{-1}$ (ranging from 13.29 to 13.87 µg $L^{-1}$ $day^{-1}$) and 17.72 ± 1.43 µg $L^{-1}$ $day^{-1}$ (ranging from 14.91 to 19.58 µg $L^{-1}$ $day^{-1}$), respectively. Using Equation 4.12, half-lives of isoproturon within the biodegradation phase in Treatments 2 and 4 were determined to be 2.7 ± 0.0 days and 2.5 ± 0.3 days (ranging from 2.1 to 3.0 days). Independent-sample t test showed that the biodegradation rates of isoproturon in Treatments 2 and 4 were not significantly different ($p > 0.05$).

### *pH values in Experiment 1*

pH of river water were measured during the 18 circulation days. The average value of pH in all four treatments of Experiment 1 ranged from 7.99 to 8.40. However, no trend of increase or decrease of pH was observed during the experimental time. This suggested that sorption and biodegradation of mecoprop and isoproturon did not affect on the pH of the river water.

## 4.5.2 Experiment 2 – Respirometry Experiment with respect to Isoproturon

Levels of mineralisation from Experiment 2 were plotted against the assay time during 10 days and are presented in Figure 4.12. Two contrast sets of data were observed: Set 1 - (sterile riverbed sediment) with very low mineralisation levels was observed, and Set 2 - (non-sterile riverbed sediment) with mineralisation of isoproturon began immediately. The curves for the evolution of labelled $^{14}CO_2$ were biphasic without a lag period.

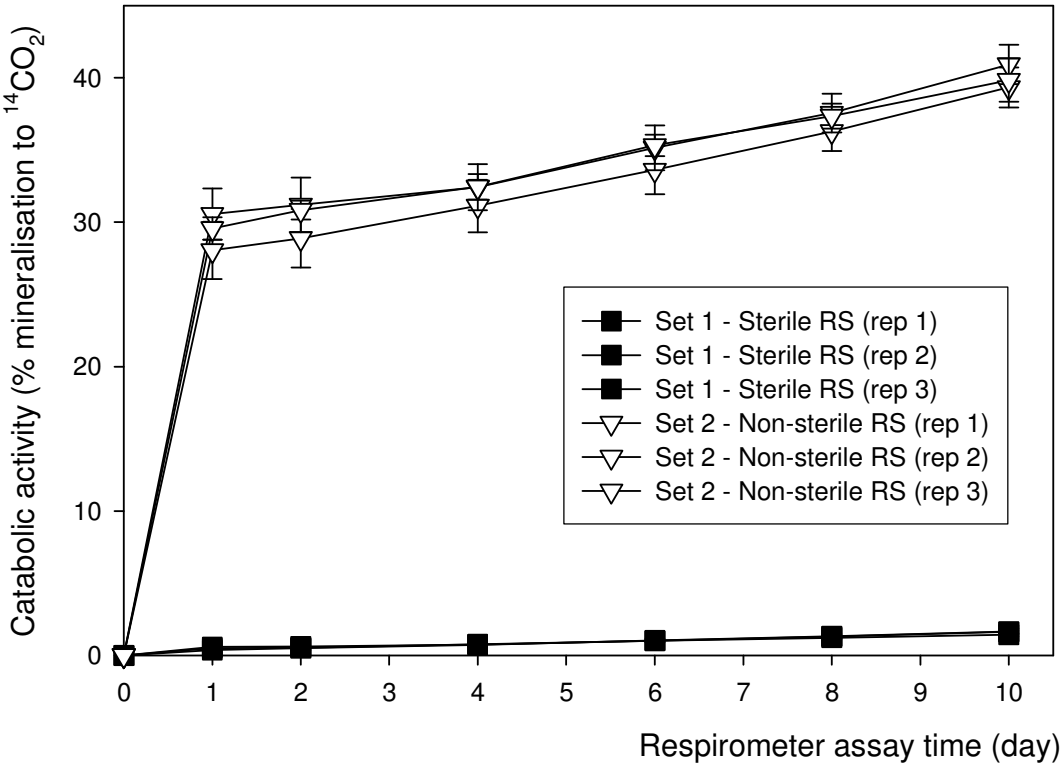



**Figure 4. 12** Catabolic activity with respect to isoproturon in Set 1 – sterile riverbed sediment (RS) and Set 2 – non-sterile riverbed sediment. Error bars represent standard error (n=3).

In Set 1, very low maximum levels of catabolic activity were observed, varying from 1.4 ± 0.1 % to 1.7 ± 0.2 % after 10 assay days. In Set 2, two obvious compartments of the catabolic activity curves were found. The first compartment was defined within the first assay day with a considerable increase of the mineralisation levels. These levels increased from 0 % at the beginning of the experiment to 29.4 ± 1.5 % after one assay day. The maximum mineralisation rate of $^{14}$C-isoproturon was also calculated to be 29.4 % $^{14}CO_2$ day$^{-1}$ from the gradient of the best fit line based on the levels of catabolic activity during day 1. Consecutively, the second compartment of the curves gave a little increase over the remaining 9 days respirometry assay. The maximum level of catabolic activity in Set 2 was 40.0 ± 1.4 %. The high levels of mineralisation in Set 2 provided persuasive evidence that biodegradation was responsible for the loss of the herbicides over Phase III of Treatments 2 and 4 in Experiment 1.

## 4.6 Discussion

As noted above, only a very little number of studies exist that relate to the degradation of mecoprop and isoproturon in the RW-RS interface. The ensuing sections are thus directed towards discussion the results of Experiments 1 and 2 and comparison of these results with those related to agricultural soil, subsoil and aquifer materials.

### 4.6.1 Sorption of the Herbicides Mecoprop and Isoproturon in River Water – Riverbed Sediment Interface

In river water, according to Warren *et al*. (2003) the herbicides mecoprop and isoproturon could exist in several forms of a freely dissolved phase or a colloidal phase or associated with sedimentary materials. The distribution of the herbicides between these various phases was a crucial issue that governed herbicide fate. Once the herbicides were transported to the interface of a water-sediment system, they could be retained on the surface of a particle (adsorption) or diffused into a porous material and then sorbed (absorption). Then the equilibrium between the associated and freely dissolved phases of the pesticides should be set-up. According to this hypothesis, in Experiment 1, the pseudo-equilibrium of mecoprop and isoproturon was established when the concentrations of these herbicides in the circulated river water became stable after Phase I (see Figure 4.7). The period of time to reach the equilibrium state was assumed as the sorption time. Both the sorption isotherms, which controlled the equilibrium state of a system, and the sorption kinetics, i.e. how quickly this state were approached, are discussed below with respect to mecoprop and isoproturon in a RW-RS system.

Before starting to discuss the sorption characteristics of mecoprop and isoproturon on/into the riverbed sediment, it is necessary to consider the significant difference of the sorption parameters of the two herbicides, e.g.

percent sorption loss (A, %), maximum sorption capacity ($C_{S\ max}$, μg $kg^{-1}$), etc, between the treatments in Experiment 1. One-way ANOVA indicated that no significant difference of the sorption parameters ($p > 0.05$) was found between Treatment 2 (materials without sterilisation) and Treatment 4 (sterile river water and non-sterile riverbed sediment). This suggested that sterilisation of river water might not affect the sorption processes of the herbicides. Although the sorption parameters of both mecoprop and isoproturon in Treatments 2 were greater than those in Treatment 4, for example, the percent sorption loss of mecoprop and isoproturon in Treatment 2 were 19.5 ± 2.0 % and 17.6 ± 0.6 % compared with the same parameter in Treatment 4 that were 15.1 ± 0.6 % and 15.1 ± 2.8 %, respectively.

One-way ANOVA also illustrated that significant differences ($p \leq 0.05$) were found between Treatments 2 and 1 (both sterile materials) and between Treatments 2 and 3 (sterile riverbed sediment). This suggested that sterilisation of both materials or of riverbed sediment only resulted in the decline of sorption ability of the sediment to mecoprop and isoproturon.

It was suggested that sterilisation (121 $^{o}$C, 30 minutes) destroyed the structure of the sorbents on/in the riverbed sediment (e.g. polar organic matter or cells) which favoured to adsorb the ionic and organic molecular compounds such as mecoprop and isoproturon. Thus, the sorption capacity in Treatments 1, 3 and 4, which were wholly or partly sterilised, might be anticipated to be lower than in Treatment 2. This result was consistent with the finding of Beck and Jones (1996) that sorption of herbicide isoproturon by the whole soil treatments (non-sterile sediment) may be slightly greater than the sorption of the sterile treatments. Furthermore, microbial degradation may occur in Treatments 2 and 4 and resulted in the greater 'loss' when compared with Treatments 1 and 3.

Given that Treatment 2 continued both riverbed sediment and river water in a non-sterilised form this treatment was used as a point of reference with which

to make comparison of sorption parameters in this research with results from other studies.

#### *4.6.1.1 Sorption isotherm of mecoprop and isoproturon in a river water – riverbed sediment system*

Over the sorption phase (24 hours for mecoprop and 12 hours for isoproturon), 19.5 ± 2.0% of mecoprop and 17.6 ± 0.6% of isoproturon were removed from the river water and sorbed on/into the sediment in Treatment 2. There were several mechanisms which explained the sorption of ionic organic compound such as mecoprop and non-ionic organic compound such as isoproturon. Within a natural environment such the RW-RS interface, sorption is not very often an exchange between one pesticide and a single solid medium. Rather, some combination of interactions may govern the association of the pesticide with any particular solid or mixture of solids. Since mecoprop is an ionisable in the aqueous solution, then electrostatic attraction to specific surface sites exhibiting the opposite charge will promote sorption of the ionic species. For the non-ionic molecules like isoproturon, this organic compound may escape the water by penetrating the natural organic matter present in the system. Additionally, such a non-ionic molecule may displace water molecules from the region near a mineral surface to some extent and be held there by London dispersive and polar interactions (Schwarzenbach *et al.*,1993, p.277). Finally, should the herbicide and the sediment exhibit mutually reactive moieties, for instance, an amino group on isoproturon may bonded to a carbonyl group on the sediment or a halogen ($Cl^-$) group on mecoprop may bonded to a amino group on the sediment. All these interaction mechanisms operate simultaneously, and the combination that dominates the overall solution-solid distribution will depend on the structural properties of the herbicides and the riverbed sediment (Schwarzenbach *et al.*, 1993, p.277).

Schwarzenbach *et al.* (1993) presented that most surfaces of inorganic sorbents such as sand, silt, or clay compositions in riverbed sediment were polar and exposed a combination of hydroxyl- and oxy-moieties to their exterior. These polar surfaces are especially attractive to substances like water that form hydrogen bonds. It is known that mecoprop is an acidic herbicide ($pK_a$ of 3.78). In water, it is hydrolysed and becomes an ionic herbicide (solubility in water of 880 mg $L^{-1}$ at 25 $^{o}$C). Therefore, the adsorption of ionic compounds such as mecoprop was favourable from an energetic point of view.

It is noted that the river Thames, where the samples were collected, perched on a Chalk aquifer (see Chapter 2). Thus, the river water held a high concentration of calcium (100.5 ± 5.1 mg $L^{-1}$, Table 4.2). Calcium concentration in an aqueous solution may affect on the solid-water distribution coefficient of a pesticide. Indeed, in the presence of calcium salts, Clausen *et al.* (2001b) reported that the sorption capacity of mecoprop increased onto kaolinite surfaces and suggested this was due to the formation of clay-calcium-organic acid complexes. On the other hand, an ionic herbicide such as mecoprop could interact with the surface site through, for instances, electrostatic interactions, ion-exchange reactions. And for this herbicide, adsorption to mineral surfaces might be significant (Brownawell *et al.*, 1990; Schwarzenbach *et al.*, 1993; Celis *et al.*, 1996; Celis *et al.*, 1999; Celis *et al.*, 2001).

In proportion to the percent sorption loss of mecoprop, the *maximum sorption capacity ($C_{S,max,sur}$)* of mecoprop on/into the riverbed sediment of 3.91 ± 0.45 μg $m^{-2}$ (at pH 8.2) was much smaller than on/into the goethite of 1224 μg $m^{-2}$ (at pH 4.0) and on/into the ferrihydrite of 3220 μg $m^{-2}$ (at pH 4.7) (goethite and ferrihydrite are types of iron oxides in soils or sediments, reported by Clausen and Fabricius, 2001a). High specific surface areas of goethite (60 $m^2$ $g^{-1}$) and of ferrihydrite (230 $m^2$ $g^{-1}$) against low specific surface area of the riverbed sediment (0.0713 $m^2$ $g^{-1}$) could be the primarily reason accounting for this

difference. In addition, sorption of mecoprop was influenced by the pH of the environment. Indeed, Clausen and Fabricius (2001a) reported that the adsorption capacity increased with decreasing of pH. In a study with aquifer sediment, Madsen *et al.* (2000) also found that the sorption of mecoprop was high with the low pH environment (pH < 6.7) and vice versa with the case of high pH environment (pH > 7.4). For other instances, in near-neutral pH solutions, mecoprop disassociated into a negatively charge and therefore would be expected to be repelled by negatively charged silicate mineral surfaces (Stumm, 1992). Furthermore, the sorption of mecoprop on/into the mineral surfaces occurred at low pH values when there were some positively charged sites available (Clausen *et al.*, 2001). Therefore, low sorption capacity of mecoprop on/into the riverbed sediment in Experiment 1 could result from the high pH value of the river water (pH = 8.2).

On the other hand, it was reported that the $K_D$ value of mecoprop considerably depends on the total organic carbon (TOC) composition in the surface and subsurface soil environments: $K_D$ varying from 0.0 – 0.07 (with 0.2 – 1% TOC), 0.4 – 0.8 (with 2 – 4% TOC) and especially 2.6 – 2.8 (with 4.7 – 5.1% TOC) (Fomsgaard, 1995). Additionally, in a study with a supernatant of aquifer sediment and groundwater, Madsen *et al.* (2000) reported values of $K_D$ ranging from 0.07 ± 0.01 to 0.26 ± 0.03. The values of $K_D$ were reported as 1.6 L $kg^{-1}$ in biobed material (50% chopped straw, 25% sphagnum peat, 25% soil) and ranging from 0.07 to 0.6 L $kg^{-1}$ in natural soil (17% clay, 60% sand, 2% humus) (Henriksen *et al.*, 2003). However, no adsorption of mecoprop was also observed in Danish soil samples with an organic content of 1.3% (Kristensen *et al.*, 2001a).

The solid-water distribution coefficient of isoproturon was reported in the experiments with surface soil samples (from 10 to 90 cm below the surface, at Worcester, U.K.) with 0.8% of TOC to be 1.2 L $kg^{-1}$ (Worrall *et al.*, 1996). This value was smaller than the $K_D$ value of isoproturon in Treatment 2 which

has the same 0.8% of TOC. The soil samples in the Worrall's experiment were collected at the depth of approximately 50cm, however, no other properties i.e. particle-size distribution was reported. A variation of $K_D$ of isoproturon in the topsoil samples ranging from 2.73 to 4.39 L $kg^{-1}$ was also reported by Johnson *et al.,* (1998). Henriksen *et al.* (2003) found that the $K_D$ value of isoproturon in biobed soil (50% straw, 25% sphagnum, 25% soil, with 4.6% of TOC) was 5.2 L $kg^{-1}$. In other instances, Clausen *et al.* (2001b) normalised $K_D$ to specific surface area (under units of L $m^{-2}$) to account for the influence of the solid surface area. After normalisation to the specific surface area, the $K_D$ value of isoproturon on kaolinite was reported as 50 ± 9 L $m^{-2}$. This value was approximately 1160 times higher than the value of $K_D$ in Experiment 1 (to be 0.043 ± 0.002 L $m^{-2}$). This may be resulted from the specific surface area of the kaolinte (8.5 $m^2$ $g^{-1}$) was approximately 120 times higher than the surface area of the riverbed sediment in Experiment 1 (to be 0.0713 $m^2$ $g^{-1}$). Indeed, Bailey and White (1970) stated that the adsorption properties of soils and sediments are strongly influenced by constituents that have high specific surface area and highly reactive surfaces.

In addition, organic carbon content was a significant constituent affecting distribution of pesticides, especially in relation to hydrophobic organic compounds such as isoproturon (Hamaker and Thomson, 1972; Chiou *et al.*, 1979; Karickhoff, 1984; Worrall *et al.*, 1996; Alexander, 1999; Henriksen *et al.*, 2003). It is known that most surfaces of inorganic sorbent are polar, and these polar surfaces are consequently attractive to hydrophilic substances that form hydrogen bonds. Hence, it is quite unfavourable from an energetic point of view if a hydrophobic organic compound sorbs on to the surfaces of inorganic sorbent by displacing the water molecules at such a surface. However, absorption of organic chemicals into natural organic matter or adsorption to a hydrophobic organic surface does require displacement of tightly bound water molecules (Schwarzenbach *et al.*, 1993). Hence, such a non-ionic organic sorbate as isoproturon may successfully compete for

association with solid-phase organic matter. Worrall *et al.* (1996) reported that the adsorption was controlled by the TOC of the soil, at least for values of the TOC exceeding 27 g $kg^{-1}$.

Normalised distribution coefficients on an organic carbon basis have been calculated to reduce the variation in sorption characteristics of different soils or sediments. The *organic carbon-normalised distribution* coefficient ($K_{OC}$) of mecoprop and isoproturon were determined in Experiment 1 as 434 ± 54 (or log $K_{OC}$ = 2.63 ± 0.06) and 382 ± 15 (or log $K_{OC}$ = 2.58 ± 0.02), respectively. This finding was consistent with several other workers. Worrall *et al.* (1996) reported log $K_{OC}$ of isoproturon varying from 2.08 to 2.14 in agricultural soil samples. Beck and Jones (1996) showed that the log $K_{OC}$ for all seven soil treatments investigated ranged from 1.89 ± 0.78 to 2.06 ± 1 L $kg^{-1}$ indicating that organic matter exerted a strong influence on the sorption of isoproturon by clay soil. Kenaga (1980) calculated the log $K_{OC}$ of mecoprop and isoproturon as 2.11 and 2.66, respectively, which was based on the solubility of isoproturon in water.

Although destructive degradation processes are generally regarded as being the most important of natural attenuation processes, retardation may be important in slowing the migration of a pollutant plume within an aquifer and decreasing pollutant concentrations (Smith and Lerner, 2007). Retardation has the effect of slowing the apparent pollutant velocity, and thereby increasing retention time of the pollutant in an aquifer. This may provide additional time for biodegradation processes. And in some instances it is clear that sorption actually enhances biodegradation (Warren *et al.*, 2003). The retardation factor, $R_D$, values of mecoprop (9.6 ± 1.1) and isoproturon (8.6 ± 0.3) in Treatment 2 was considered as a weak retardation (belongs to the range of $5 < R_D < 50$ reported by (Smith and Lerner, 2007). This suggested that the mobility property of these herbicides in riverbed sediment was rather high. The retardation factor of isoproturon in different land uses such as (i) conventional

wheat/maize rotation (CP), (ii) 10-year-old grassed strip (GS) and (iii) 80-year-old oak/chestnut forest (FS) was reported varying from (i) 2.06 – 2.42 , (ii) 2.35 – 3.70, and especially high (iii) 8.40 – 10.75, respectively (Vincent *et al.*, 2007). The higher organic carbon content (1.43%, 1.88%, and 7.11% of organic content versus 0.8%) and finer particle texture (56.4%, 68.4%, and 72.5% of clay and silt versus 21% of clay and silt) in CP, GS and FS samples versus riverbed sediment in Experiment 1, respectively, may account for the above differences. Conversely, no significant mecoprop retardation was observed in the continuous field injection studies in the sand aquifer at Vejen (Broholm *et al.*, 2001) or Borden, Ontario (Agertved *et al.*, 1992) or in the anaerobic landfill plume in a sandy aquifer at Grindsted (Rugge *et al.*, 1999). By assuming some parameters of aquifer sediment such as surface area of 0.48 $m^2\ g^{-1}$, bulk density of 1.6 g $cm^{-3}$ and porosity of 0.3, Clausen and Fabricius (2001a) calculated the retardation factor of Mecoprop as 2.2 which is a little smaller than our finding in Experiment 1 (7.78 ± 0.89). However, mecoprop was retained in groundwater discharging to a freshwater wetland by 25 – 75% compared with the conservative bromide tracer employed (Nilsson *et al.*, 2000). A low retardation value of mecoprop by sorption has been explained by its the water-soluble property (Environment Agency, 2004).

Eventually, the previous studies reported a considerable variation for isotherm sorption characteristics of mecoprop and isoproturon on/into high specific area materials goethite and ferrihydrite (Clausen and Fabricius, 2001), on/into agricultural soils with different concentrations of TOC (Fomsgaard, 1995; Beck and Jones, 1996; Worrall *et al.*, 1996; Kristensen *et al.*, 2001a), supernatant of aquifer sediment and groundwater (Madsen *et al.*, 2000) and "biobed" materials (Henriksen *et al.*, 2003). In addition, there were many environmental factors affecting on the sorption of these herbicides. Hence, it is difficult to compare appropriately the sorption capacity of the riverbed sediment in this study and the other studies. Nevertheless, the isotherm sorption of mecoprop and isoproturon on/into the riverbed sediment are generally low. This implies

that the aquifer and the boreholes along the riverbank may be contaminated by these herbicides.

#### *4.6.1.2 Sorption kinetics of mecoprop and isoproturon in a river water – riverbed sediment system*

Sorption kinetics of mecoprop and isoproturon on riverbed sediment was observed in Phase I of Experiment 1. One-way ANOVA showed that the sorption rate constant of mecoprop in Treatment 2 were not significantly different ($p > 0.05$) to the sorption rate constants in Treatments 1, 3 and 4. It was suggested that sterilisation did not affect to the sorption kinetics of mecoprop. On the other hand, one-way ANOVA also showed that the sorption rate constant of isoproturon in Treatment 2 was not significantly different ($p > 0.05$) to the sorption rate constant in Treatment 4 but significantly higher ($p < 0.05$) than the sorption rate constants in Treatments 1 and 3. It was suggested that sterilisation of river water only did not affect to the sorption kinetics of isoproturon but sterilisation of riverbed sediment significantly decreased the sorption rate constants of isoproturon.

The sorption times of mecoprop and isoproturon in Experiment 1 were determined to be 24 and 12 hours, respectively. Applying the first-order sorption kinetics model, the sorption rate constants of these herbicides in Treatment 2 of Experiment 1 also found to be 0.0106 and 0.0191 $h^{-1}$, respectively. The hydrophilic characteristic of mecoprop and the hydrophobic property of isoproturon were proposed to account for the slower sorption rate of mecoprop than the sorption rate of isoproturon.

The sorption times of mecoprop and isoproturon in Experiment 1 were supported by the review of Warren *et al*. (2003) that the interaction of micro-organic compounds with sediments was conducted over relatively short timescales of 24 and 48 hours. However, most of the previous studies reported

that the sorption times or the sorption rate constants of mecoprop, isoproturon or other organic compounds were shorter or faster than the sorption times or the sorption rate constants of mecoprop and isoproturon found in this thesis. The sorption time of isoproturon in different components of clay soil estimated from a batch laboratory experiment was determined within 1 hour (Beck and Jones, 1996). Worrall *et al.* (1996) presented the value of isoproturon sorption rate constant in top soil (0 – 10 cm) as 0.156 $h^{-1}$. De Wilde *et al.* (2008) using the first-order model reported the sorption rates of isoproturon with initial concentrations of 10 and 1000 mg $L^{-1}$ in different materials varying from 0.13 to 1.45 $h^{-1}$. Sorption kinetics of mecoprop has not been reported yet. Warren *et al.* (2003) reported that sorption rate constants obtained from shake-batch experiments of organic contaminants in different sediments ranged from 0.1 to 1.0 $h^{-1}$.

In most of the previous studies, sorption kinetics of pesticides was studied using sorption batch method. The batch sorption experiment in the study of Beck and Jones (1996) might cause faster sorption rate than the column circulation experiment in this study because the batch was shaken and therefore the movement or transportation of the herbicide from the aqueous phase to the solid phase occurring in the batch should be faster and more complete than occurring in the column. Hence, sorption rate of an organic compound in a shake-batch experiment was usually faster than that in a fixed-bed column experiment. In addition, particle sizes of the sediment in the study of Beck and Jones (1996), reported as 12.3% sand, 31.4% silt and 56.3% clay, were far smaller than particle sizes of the sediment in this thesis, determined as 79% sand, 20% silt and 1% clay. It is learnt that the smaller particle size the larger the specific surface area. Consequently, the larger the specific surface area is, the faster the sorption rate should be. Smaller rate constants indicate a slower sorption process and therefore increase the risk of pesticide leaching (de Wilde *et al.*, 2008). In other instances, de Wilde *et al.* (2008) reported that no correlation was found between the organic matter content of the substrates and

the corresponding sorption rate constants but concluded that specific area or particle size of the substrates influenced the sorption rate.

### 4.6.2 Biodegradation of the Herbicides Mecoprop and Isoproturon in a River water – riverbed sediment System

Following the sorption phase, a period of adaptation or acclimation time was observed in Treatments 2 and 4. This suggested that microorganisms could use this period to adapt and/or proliferate their population before the loss of herbicides becomes rapid and complete. It was suggested (although not confirmed empirically) that biofilms could be formed on the sediment particle during this period of time. Before considering the biodegradation of the herbicides, it is necessary to discuss the adaptation time in the treatments of Experiment 1.

#### *4.6.2.1 Adaptation of lag phase (Phase II)*

The adaptation or acclimation or lag time of mecoprop and isoproturon in Treatments 2 and 4 was proposed to occur immediately after Phase I (sorption phase). It lasted for 4 and 5.5 days for mecoprop and isoproturon, respectively. No significant decline ($p > 0.05$) in concentrations was detectable in this phase as indicated by the independent t test (presented in Section 4.5.1.2). The lag time may be related to proliferation of small populations. Indeed, it is well-known that natural riverbed sediment typically contains a small population of microorganisms acting on the herbicides (mecoprop and isoproturon) that are capable of supporting growth (Katagi, 2006). The population is so small that one, two, three, or several more doubling of the cell number would not bring about an appreciable loss of the herbicides. Only when the bacteria have undergone many cell divisions would a decline in concentration of the parent compound be detected (Spain *et al.*, 1980; Ventullo and Larson, 1986; Alexander, 1999; Bending *et al.*, 2001). Bending *et al.* (2001) also added that

the acclimation period (or lag phase) was unlikely to reflect the time required for development of novel degradative genes or change to existing genes. Several other works have proposed that it is a result of the time needed for enzymes as induced (Torstensson *et al.*, 1975; Stephenson *et al.*, 1984) or for mutation or genetic exchange to occur (Walker and Newman, 1956; Schmidt *et al.*, 1983). Other possible explanations include an insufficient supply of inorganic nutrients (Vashon *et al.*, 1982; Lewis *et al.*, 1986) and the preferential utilization of other organic compounds before the chemical of interest (Kuiper and Hanstveit, 1984; Lewis *et al.*, 1986).

The acclimation time of mecoprop in enrichment environments was reported to be 30 – 37 days (Lappin-Scott *et al.*, 1986). In the study of aerobic degradation of mecoprop with initial concentrations of 65 – 1400 μg $L^{-1}$ in laboratory batch studies of sandy sediments collected from an unpolluted aquifer in Vejen site, Denmark, the acclimation times of mecoprop ranged from 20 to 110 days (Heron and Christensen, 1992). Lag periods also varied with the initial concentration of mecoprop, but with no consistent pattern. At the field scale, (Broholm *et al.*, 2001) demonstrated rapid aerobic degradation of injected mecoprop (at 40 μg $L^{-1}$) in the otherwise unpolluted Vejen aquifer following an initial lag period of 80 – 120 days. It is noted that the length of the acclimation period varies enormously. From 1 hour to many months (Alexander, 1999). Several environmental factors may affect the lag time such as temperature (Atlas and Bartha, 1972), pH and aeration and concentration of N and P (Lewis *et al.*, 1986; Wiggins *et al.*, 1987).

Bending *et al.* (2001) reported that the lag time of isoproturon lasted for approximately 5 – 6 days (during which time almost 40 % of the isoproturon applied was degraded) in soil samples collected from a field receiving regular isoproturon applications over 20 years. A spatial variation of lag time of isoproturon with different soils was also found (Bending *et al.*, 2006), varying from 8 to 18 weeks in samples from the Kirton site (England) and from 0 to 18

days in the samples from Wellesbourne (England). Similar adaptation periods have been reported during degradation of other pesticides in soil (Robertson and Alexander, 1994).

#### *4.6.2.2 Biodegradation in river water (Treatment 3)*

Degradation of the two herbicides mecoprop and isoproturon in a RW-RS system can occur either in the water column or in the riverbed sediment following deposition. However, Warren *et al.* (2003) presented that the situation was complicated in natural river waters by the occurrence of varied microbial populations, suspended sediments, dissolved ions, dissolved organic matter and the sediment bed. Riverbed sediments, in particular, have the potential to strongly influence degradation, because many micro-organic compounds are known to associate strongly with sediments and microbial populations are also known as largely associated with surfaces in the environment, rather than living free in solution. By the sterilization of individual river water or riverbed sediment or both, biodegradation resulting from microorganisms in the appropriate microcosms was controlled. This section discusses biodegradation of the herbicides mecoprop and isoproturon in a RW-RS system and determines which microcosms between the river water and the riverbed sediment were responsible for biodegradation.

In Treatment 3, only the riverbed sediment was sterilised while the river water was not. Thus, degradation of these herbicides, if possible, could result from the microbial communities associated with river water microcosm. However, concentrations of both mecoprop and isoproturon in Treatment 3 were stable after the sorption phase. Furthermore, concentrations of both mecoprop and isoproturon in Treatment 3 were not significantly different ($p > 0.05$) with the concentrations in the control (Treatment 1) over the assay time. This suggested that microbial communities in river water were not a significant vector through which the herbicides were degraded.

Studies for biodegradation of mecoprop and isoproturon in river water have been limited. Hence, a reference to the degradation of a similar organic compound in a similar environment is presented in order to approach an understanding of the above herbicides in river water. In a column experiment (without circulating water) with water and sediment from the River Danube (Hungary), Vargha *et al.* (2005) reported that the herbicide atrazine was not significantly degraded following 10 days subsequently to compound loading. In a circulation column experiment with water from the River Elbe (Germany) and inert solid material, Worch *et al.* (2002) reported that highly chlorinated anilines and nitroanilines were poorly degradable, but unsubstituted aniline was completely degraded after approximately 3 hours. Nevertheless, these authors did not clearly address the degradation of the organic compounds resulting from river water-borne or riverbed sediment-borne microorganisms.

#### *4.6.2.3 Biodegradation in riverbed sediment (Treatment 4) and in a river water – riverbed sediment system (Treatment 2)*

In Treatment 2, neither river water nor riverbed sediment were sterilised. Hence, degradation of the herbicides mecoprop and isoproturon in this treatment could be contributed by microorganisms borne in both river water and sediment. In Treatment 4, only river water was sterilised. Thus, degradation in this treatment was accounted for microorganisms borne in the riverbed sediment only. Results from both Treatments 2 and 4 indicated that the two herbicides mecoprop and isoproturon were completely degraded.

Regarding the concentration of mecoprop in Treatments 2 and 4 during Phase III, independent-sample t test provided that no significant difference ($p > 0.05$) of the mecoprop concentrations between Treatments 2 and 4 was recorded. The similar result was also found for the concentration of isoproturon in Treatments 2 and 4. This suggested that only microorganisms in the riverbed sediment, not those in the river water, were responsible for the degradation of

these herbicides during the biodegradation phase (Phase III). This result is in agreement with the biodegradation result finding in Treatment 3 that the river water-born microorganisms were not competence to degrade the two herbicides (see Section 4.6.1.1 for details). Regarding to the hypotheses stated in Section 1.6, this finding supports for the statement that loss of herbicides will most strongly dependent upon microbial competence in sediment but not in water. In addition, Warren *et al.* (2003) has elucidated that many micro-organic compounds were known to associate strongly with sediments and that microbial populations were known as largely associated with surfaces in the environment, rather than living free in solution.

Using zero-order kinetic model (Equations 4.10 and 4.11), in the same Treatment 2, biodegradation rate constant of isoproturon ($13.62 \pm 0.17$ μg $L^{-1}$ $day^{-1}$) was found to be significantly larger ($p < 0.05$, independent t test) than of mecoprop ($9.91 \pm 0.19$ μg $L^{-1}$ $day^{-1}$). The difference may result from the fact that hydrophobic isoproturon can be transported easier and faster from the aqueous phase and sorbed on to/into the surface of riverbed sediment than that of the hydrophilic mecoprop (sorption rate constant of isoproturon of 0.019 $h^{-1}$ was faster than that constant of mecoprop of 10.6 $h^{-1}$).

Using zero-order kinetics model indicated that the biodegradation rates of mecoprop and isoproturon should be evident in processes and effected by non-growing cells. In this case, the substrates mecoprop and isoproturon might be insufficient to support the growth of the population. The low flow of river water (1.6 mL $min^{-1}$) through the column could be account for this limitation. Furthermore, the nutrients in the system that might limit the growth of the active population became available at a rate constant, but the rate did not fully meet the demand of the herbicide degrading organisms. For example, the herbicide degrading bacteria grew linearly in the fixed-riverbed sediment columns of both Treatments 2 and 4 when oxygen entered the river water (by agitating the reservoirs) at a rate that limited their further multiplication. With

the high initial concentrations of mecoprop and isoproturon in Treatments 2 and 4, using zero-order kinetic model was in agreement with the statement that such oxygen limitation to biodegradation was likely to occur at high substrate concentrations (Alexander, 1999, p. 83). However, in agricultural soil environment, Bending *et al.* (2003) reported that rapid degradation of isoproturon was associated with proliferation of isoproturon-degrading organisms and slow degradation of isoproturon was linked to either a delay in the proliferation of isoproturon-degradaing organisms or apparent cometabolic degradation.

Several previous studies for mecoprop kinetics have applied a zero-order model. It was reported in a study with groundwater that: (i) under the nitrate-reducing microcosm, S-mecoprop did not degrade but R-mecoprop degraded with zero-order kinetics (650 μg $L^{-1}$ $day^{-1}$), and (ii) in aerobic conditions (S)- and (R)-mecoprop degraded with zero-order kinetics at rates of 1900 and 1320 μg $L^{-1}$ $day^{-1}$, respectively (Harrison *et al.*, 2003). These rates are faster than our findings for mecoprop (9.91 ± 0.19 μg $L^{-1}$ $day^{-1}$). The difference may be attributed to the method of experiment (batch experiment versus the fixed-bed circulation column experiments in this study) and different initial concentrations of mecoprop (approximately 10,000 μg $L^{-1}$ in the experiments by Harrison *et al.* (2003) against 100 μg $L^{-1}$ in this study).

The literature half-lives for mecoprop under different conditions in topsoil have been reported to vary from a minimum value of 1.3 days to a maximum value of 102 days, with most cases typically less than 15 days (Environment Agency, 2004). Summarising a number of studies in the United States of American, Canada and Europe, it was concluded that mecoprop degrades rapidly in soil under aerobic laboratory conditions and half-lives generally ranged from 7 to 19 days at 20 $^{o}$C (Department of the Environment, 1994). The time for 50% degradation of mecoprop in surface soil, at concentrations of 2 – 5 mg $kg^{-1}$, has been reported as 1 to 11 days (Amrein *et al.*, 1981; Lindholm *et al.*, 1982;

Helweg, 1993). At concentrations higher than 5 mg $kg^{-1}$ the time for 50% degradation increases with concentration from 6 days at 10 mg $kg^{-1}$ to 42 days at 250 mg $kg^{-1}$ (Amrein *et al.*, 1981). Degradation of mecoprop under anaerobic condition has not been observed (Environment Agency, 2004).

In field study, the potential for biodegradation of mecoprop has been observed varying significantly between sites where mecoprop was applied at typical agriculture use rates, and those sites where mecoprop represented a point source of pollution (Environment Agency, 2004). In the subsurface, degradation of relatively low concentration mecoprop "diffuse" sources have often been rapidly degraded in shallow aerobic soils, whilst higher concentration "point sources" have been less amenable to biodegradation.

A number of factors appear to affect the kinetics of mecoprop and results in a spatial variation of mecoprop kinetics can be listed here: initial concentration (Smith, 1989; Helweg *et al.*, 1998; Reffstrup *et al.*, 1998); pre-exposure of soil or sediment to mecoprop (Smith, 1989); temperature (Smith, 1989; Helweg, 1993); depth of microcosm (Helweg, 1993; Larsen *et al.*, 2000); redox conditions (Department of the Environment, 1994; Nilsson *et al.*, 2000); pH (Smith, 1989; Fomsgaard and Kristensen, 1999; Brady and Weil, 2002); and enantiometric effects (Tett *et al.*, 1994; Romero *et al.*, 2001; Harrison *et al.*, 2003).

With respect to isoproturon in Treatment 2, complete degradation of the parent compound was observed within 12 days with the rate constant of 13.84 ± 0.39 μg $L^{-1}$ $day^{-1}$ and the half-life of biodegradation phase to be 2.5 ± 0.1 days. The bacteria *Sphingomonas* spp. accounted for the degradation of isoproturon and a pH above 7 was generally necessary for a rapid growth-linked degradation (Bending *et al.*, 2003). A spatial variation in the biodegradation rate of isoproturon within an agricultural field has been reported (Johnson *et al.*, 1998; Bending *et al.*, 2001; Walker *et al.*, 2001; Bending *et al.*, 2006). In the soil

samples receiving regular applications of isoproturon, degradation rates of isoproturon varied from complete degradation occurring within 14 days to small amounts (8 – 23 % of isoproturon applied) of intact isoproturon still remaining after 65 days (Bending *et al.*, 2001). Time to 25% dissipations (DT25) of isoproturon were 4.2 and 30.8 days for the field sites with and without previously applied isoproturon, respectively (Bending *et al.*, 2006). While time to 50% dissipation (DT50) of isoproturon in the top soil (0 – 30 cm) and previous exposure with isoproturon was reported as varying from 6.5 to 30 days (Walker *et al.*, 2001). In the unsaturated zone of upper chalk, 3 m below the soil surface, very little degradation of isoproturon has been observed (Johnson *et al.*, 1998). In contrast, no degradation of isoproturon in a groundwater-sandstone system was observed (Ministry of Agriculture-Fisheries and Food, 2000). However, biodegradation of isoproturon in a groundwater-chalk system varied with half-life from 111 to 273 days, while the half-lives of isoproturon in groundwater-topsoils were shorter, varying from 15 to 34 days (Ministry of Agriculture-Fisheries and Food, 2000). In most of the case, it is found that biodegradation of isoproturon in the riverbed sediment in Treatment 2 of this study occurred faster than in the above soils.

Variability in degradation rate of pesticides between different microcosms of riverbed sediment, soils, groundwater, or river water was expected because of the variability in properties of these microcosms. The biodegradation rate was influenced by numerous factors such as organic matter content, pH and nutrient status (Walker *et al.*, 2001). Bending *et al.* (2001) assumed that the pH of soils could reflect direct effects on growth of isoproturon-degrading communities, or on the exchange of degradative genes between components of the soil microbial community, or upon competition between degrading and non-degrading organisms. Moreover, comparison of biodegradation kinetics in different environments of the river water-sediment interface should be carried out with respect to the effect of flow and sedimentation regimes on transport of pesticides to riverbed sediment. The effects of flow rate might also be very

important in the transport of pesticides in river waters (Warren *et al.*, 2003). In relatively slow-flowing rivers such as the Rivers Aire and Calder in Yorkshire (UK), bed and suspended sediments have been found to contain relatively high concentrations of a range of pesticides, notably including the synthetic pyrethroids (Long *et al.*, 1998; House *et al.*, 2000). In contrast, some fast-flowing rivers have a high self-purification ability against pollution, with high water-discharge and sediment loads (Warren *et al.*, 2003). Furthermore, technical error associated with pesticide extraction, analysis and lack of model fit can also account for the variability in degradation rate (between 5.3 and 25.8 % of the variability of isoproturon) (Bending *et al.*, 2006).

Collectively, the herbicides mecoprop and isoproturon were completely degraded by microbial communities living in the riverbed sediment. Approximately 85 % of the applied mecoprop and 80 % of the applied isoproturon were totally lost from the river water within 9 and 6 days, respectively. The biodegradation process occurred after a period of adaptation time (5 days for mecoprop and 6 days for isoproturon including the sorption time). The biodegradation rate of isoproturon ($13.84 \pm 0.39$ μg $L^{-1}$ $day^{-1}$) was found to be faster than that of mecoprop ($9.91 \pm 0.19$ μg $L^{-1}$ $day^{-1}$). Furthermore, biodegradation of mecoprop and isoproturon in riverbed sediment was found to be faster than most of the biodegradation of these herbicides in agricultural soils.

However, there is no warranty that the toxicity of these herbicides was totally destroyed although their parent concentrations in Treatments 2 and 4 were determined as below the detection limit of the HPLC (2 and 1 μg $L^{-1}$ for mecoprop and isoproturon, respectively). This is because the intermediate compound of these herbicides could be produced and accumulated in the riverbed sediment even though microorganisms are frequently the sole means of converting synthetic chemicals to inorganic products such as $CO_2$ (Alexander, 1999). This is considered in Experiment 2.

### 4.6.3 Mineralisation with respect to Isoproturon in Riverbed Sediment

Experiment 2 aimed to investigate the catabolic activity with respect to $^{14}$C-isoproturon in the riverbed sediments removed from the columns of Treatments 3 and 4 (Experiment 1) following the 18 day recirculation period. The original microbial communities in the riverbed sediment removed from the columns of Treatment 3 had been disabled by sterilising, however, the river water in this treatment was not sterilised. Therefore, the riverbed sediment after 18 circulation days in Treatment 3 could carry the river water-borne microorganisms only. Consequently, when the river water was circulated throughout the fixed-sediment columns (18 days), the river water-borne microorganisms might develop on surface of the sediments.

Very low maximum mineralisation level of 1.7 ± 0.2 % was found in Set 1 which contained the riverbed sediment removed from Treatment 3 indicated that the river water-borne microorganisms were not competence with respect to the mineralisation of isoproturon. This result is consistent with the results of Treatment 3 in Experiment 1 of which the concentrations of isoproturon did not change over 18 circulation days following the sorption phase.

In contrast, very high level of the maximum catabolic activity of 29.4 ± 1.5 % was observed in Set 2 which contained the riverbed sediment removed from Treatment 4. This indicated that isoproturon was immediately mineralised by sediment-borne microorganisms. The levels of mineralisation with respect to $^{14}$C-IPU in these treatments considerably increased after the first day of the experiment without a period of lag time. During days 2 to 10, mineralisation levels in these treatments did not significantly increase. This could result from the exhaust of the substrate isoproturon, nutrients or dissolved oxygen. Furthermore, the high level of catabolic activity in Set 2 was totally consistent with the results found in Treatments 2 and 4 in Experiment 1 of which the

concentration of isoproturon completely disappeared over 18 circulation days. This result again proved that microorganisms in riverbed sediment were catabolically competent to mineralise isoproturon.

It is clear that the average maximum isoproturon mineralisation level with respect to $^{14}$C-IPU in Set 2 of 29.4 ± 1.5 % was higher than the levels in several previous reports. Reid *et al.* (2005) reported that low levels of catabolic activity, ranging from 3.6 ± 0.4 % to 5.9 ± 0.2 % following 10 assay days, were found in the undosed-isoproturon agricultural soil treatments; and high levels of catabolic activity, ranging from 6.9 ± 2.6 % to 25.9 ± 9.5 % following 10 assay days, were found in the dosed-isoproturon agricultural soil treatments. In the field receiving regular applications of isoproturon, Bending *et al.* (2001) reported that levels of catabolic activity varied from approximately 3 to 27 % after 10 days and 5 to 30 % after 22 days. Particularly high levels of catabolic activity (35 %) after 3 days was observed in the enriched soil samples (Bending *et al.*, 2001). A higher level of catabolic activity in the riverbed sediments when compared to the level in agricultural soils demonstrates the higher potential for mineralisation of isoproturon in riverbed sediment than in agriculture soils.

In addition, the maximum mineralisation rate with respect to $^{14}$C-IPU in Set 2 of 29.4 % $^{14}CO_2$ day$^{-1}$ ($R^2$ = 1.00) was higher than mineralisation rates reported elsewhere. Reid *et al.* (2005) reported that low mineralisation rates, ranging from 0.7 % $^{14}CO_2$ day$^{-1}$ ($R^2$ = 0.71) to 1.3 % $^{14}CO_2$ day$^{-1}$ ($R^2$ = 0.76), were found in the undosed-isoproturon agricultural soil treatments; and faster mineralisation rates, ranging from 7.9 % $^{14}CO_2$ day$^{-1}$ ($R^2$ = 0.88) to 9.8 $^{14}CO_2$ day$^{-1}$ ($R^2$ = 0.96), were found in the dosed-isoproturon agricultural soil treatments. The maximum mineralisation rate of isoproturon in enriched soils were found of 11.7 % $^{14}CO_2$ day$^{-1}$ (calculated from enriched soil samples with 35 % of the $^{14}$C applied being mineralised within the first 3 days) and 1.4 % $^{14}CO_2$ day$^{-1}$ (calculated from the transect 1 samples with 30 % of the $^{14}$C

applied being mineralised within 22 days) (Bending *et al.*, 2001). These results indicated that the mineralisation rate in riverbed sediment in Experiment 2 were faster in comparison to many agricultural soils. The elucidation for the rapid mineralisation in riverbed sediment can be the developed isoproturon-degrading microorganisms. These microbial communities became established during Phase III of the recirculation period. Thus, when sediment was removed and transferred to the respirometer, no acclimation time was necessary before the onset of rapid mineralisation.

## 4.7 Conclusions

Sorption, biodegradation and mineralisation of the herbicides mecoprop and isoproturon in a RW-RS system have been studies. Several conclusions have been drawn based upon the results from Experiments 1 and 2.

- Regarding the sorption of mecoprop and isoproturon on/into the riverbed sediment, the sorption times of theses herbicides (24 and 12 hours, respectively) investigated by a fixed-bed column method were longer than the sorption times investigated by a shake-batch method.
- The sorption capacity of riverbed sediment with respect to mecoprop and isoproturon was relatively low (approximately 19.5 % of mecoprop and 17.6 % of isoproturon were sorbed on/into the riverbed sediment during the sorption time). This suggested that the herbicides could easily seep through the riverbed.
- Sorption rate constants of isoproturon were faster than the sorption constants of mecoprop.
- Sterile river water can not affect to the sorption kinetics of isoproturon but sterile riverbed sediment can decrease the sorption rate constants of isoproturon.

- Low specific surface area and low organic matter in riverbed sediment can lead to low sorption capacity of riverbed sediment with respect to mecoprop and isoproturon.
- High pH value and high $Ca^{2+}$ in river water may decrease the sorption characteristics of mecoprop and isoproturon.

In general, sorption of mecoprop and isoproturon on/into riverbed sediment were relatively low. Collectively, this 'body of evidence' suggests that herbicide contamination in groundwater abstracted from boreholes adjacent to the river have the potential to be contaminated if abiotic sorption processes are the only mechanisms active in the removal of herbicides.

Regarding the biodegradation of mecoprop and isoproturon in a RW-RS system, several following conclusions are presented:

- Riverbed sediment-borne microorganisms rapidly can degraded mecoprop and isoproturon after several days of lag time. Conversely, river water-borne microorganisms were not competent to degrade these herbicides.
- The lag times of mecoprop and isoproturon in a RW-RS system was found to be shorter than in agricultural surface and subsurface soils previously reported.
- Mecoprop and isoproturon can be completely removed from river water over 9 and 6 circulation days, respectively, through a fixed-bed column.
- Using a zero-order kinetic model, the biodegradation rate constants of mecoprop and isoproturon were calculated to be $9.91 \pm 0.19$ μg $L^{-1}$ $day^{-1}$ and $13.84 \pm 0.39$ μg $L^{-1}$ $day^{-1}$, respectively. It is noted that biodegradation rate of isoproturon was higher than the rate of mecoprop. Furthermore, biodegradation rates of these herbicides in a RW-RS system were higher than in agricultural soils without previously applied herbicides (no

enhanced biodegradation) but are lower than the rates in agricultural soil environments with previously applied herbicides (enhanced biodegradation). These conclusions support the hypothesis that herbicides mecoprop and isoproturon will be degraded in sediment.

Regarding catabolic activity with respect the $^{14}$C-IPU in riverbed sediment, it is concluded that isoproturon can be completely mineralised by indigenous microorganisms born in riverbed sediment. It was also observed that 18 incubation days with circulating of river water through the sterile riverbed sediment did not impart catabolic competence with respect to isoproturon in the river sediment matrix. These results support the assertions made in section 4.5.1.3 that Phase III (the phase of rapid loss of herbicide) can be attributed to biotic factors. Furthermore, these result support the suggestion that it is microbes borne in river sediment, and not those borne in river water, that are responsible for catabolic competence with respect to isoproturon.

# Chapter 5

# CATABOLIC INSIGHTS into ISOPROTURON DEGRADATION in RIVER WATER, GROUNDWATER and RIVERBED SEDIMENT

## 5.1 Introduction

The fate and behaviour of the herbicides mecoprop (MCPP) and isoproturon (IPU) in a river water-riverbed sediment system has been investigated in Chapter 4. It is clear that, following the rapid sorption phase, both herbicides were completely degraded within 14 days. However, the contribution to degradation competence originating in the different microcosms such as river water, groundwater and riverbed sediment remains unresolved. Furthermore, assessment of catabolic competence in response to more environmentally representative herbicide concentrations (les than 100 $\mu g\ L^{-1}$) remains to be established. In this chapter, isoproturon was chosen for further investigation of the catabolic insights into the degradation of this herbicide in different riverbank materials. Isoproturon was chosen as it was available as a $^{14}C$-analogue while mecoprop was not.

Data presented in Chapter 4 has indicated that, prior to degrading isoproturon, a period of lag time or adaptation time was required before herbicide degradation occurred. Thereafter herbicide disappearance became evident and the rate of destruction became rapid. In other instances, Alexander (1999, p.19) stated that if a second addition is made during the time of active metabolism, the loss of the second increment characteristically occurs with little or no acclimation time; because the organisms responsible for the transformation have become numerous following the first addition of herbicide.

The rate of mineralisation of the second addition may be the same as the rate of the first addition (Kaufman and Kearney, 1965) or, far more commonly, have a greater rate than the first addition (Alexander, 1999, p.21). This enhancement of rate upon repeated additions has been reported frequently for isoproturon added to soils, e.g. by Walker and Welch (1992), Cox *et al.* (1996), Walker *et al.* (2001), Sorensen *et al.* (2003), El-Sebai *et al.* (2005), Reid *et al.* (2005) and Bending *et al.* (2006). Alexander, (1999, p.21) explained that the greater rate on subsequent additions probably results from increases in the number of degrading organisms following repeated treatment with the chemical. Once the indigenous community of microorganisms has become acclimated to the degradation of a chemical and the activity becomes marked, the community may retain its active state for some time (Alexander, 1999, p.21).

Many previous studies on catabolic activity with respect to isoproturon have been reported for soils (Bending *et al.*, 2001; Perrin-Ganier *et al.*, 2001; El-Sebai *et al.*, 2005; Reid *et al.*, 2005), for sandy aquifers and groundwater (Johnson *et al.*, 1998; Larsen *et al.*, 2000). However, as stated in Chapter 4, studies for degradation of isoproturon in river sediment are limited.

To understand catabolic insights into isoproturon degradation in riverbank environments including river water, groundwater and riverbed sediment, several questions were posed:

(1) Can isoproturon be degraded in different environments including river water, groundwater or riverbed sediment?

(2) Can catabolic activity with respect to isoproturon be enhanced with the second addition of $^{14}$C-isoproturon?

(3) Can catabolic activity with respect to isoproturon be enhanced after a period of incubation time?

(4) After a period of incubation time, what is the residual concentration of isoproturon at the point of the second $^{14}$C- isoproturon addition? Can a relationship between this residual concentration and the level of catabolic activity with respect to isoproturon be established?

## 5.2 Objectives

Relied upon the results in Chapter 4 and endeavouring to answer the questions in Section 5.1. The objectives for this chapter were:

(1) Identify the intrinsic catabolic activity with respect to isoproturon in different "free pesticide" environments including river water, groundwater and riverbed sediment;

(2) Identify the induced catabolic activity with respect to isoproturon in different "isoproturon added" environments including river water, groundwater and riverbed sediment (added isoproturon to achieve final concentrations of 0.1, 1 and 100 μg $L^{-1}$);

(3) Identify the influence of incubation time (0, 5, 10 and 30 days) on the intrinsic and induced catabolic activity of isoproturon in the above treatments;

(4) After a period of incubation, identify the $^{12}$C-isoproturon residual concentrations in above riverbed sediment treatments; then determining the relationship between the $^{12}$C-isoproturon residual concentrations and the catabolic activity of isoproturon.

In order to address these objectives, the respirometry method (Section 3.3.3) was applied. Maximum mineralisation levels and maximum mineralisation rates of isoproturon were used to assess the catabolic activity with respect to this compound. The $^{12}$C-isoproturon residual concentrations were measured using a solid phase extraction (SPE) method (Section 5.4.3 below) and a HPLC method (Section 3.4.1).

## 5.3 Materials

### 5.3.1 Natural Riverbank Materials

The natural riverbank materials including river water (RW), groundwater (GW) and riverbed sediment (RS) were independently used as a microcosm to investigate catabolic activity with respect to isoproturon. They were collected from the Gatehampton site on 18 April, 2008. Description of the site is presented in Chapter 2. Methods for collection and analysis these samples are shown in Section 3.2. Physico-chemical properties of the RW, GW and RS are presented below.

#### *5.3.1.1 River water*

River water was collected on 18 April, 2008 at the Gatehampton site. Table 5.1 shows the physico-chemical properties of the sample.

**Table 5. 1** Physico-chemical properties of the river water sample at the Gatehampton site (collected on 18 April, 2008). Value is a means of three replicates ± standard error.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Temp, $^{o}C$ | 10.6 ± 0.1 | $Cl^-$, mg $L^{-1}$ | 30.5 ± 1.4 |
| pH | 8.35 ± 0.01 | $NO_3^-$, mg $L^{-1}$ | 25.2 ± 0.3 |
| EC, µS $cm^{-1}$ | 770 ± 24 | $SO_4^{2-}$, mg $L^{-1}$ | 50.2 ± 3.3 |
| DO, mg $L^{-1}$ | 9.09 ± 1.1 | $Na^+$, mg $L^{-1}$ | 19.1 ± 0.8 |
| TN, mg $L^{-1}$ | 9.19 ± 0.39 | $Ca^{2+}$, mg $L^{-1}$ | 84.6 ± 4.3 |
| TC, mg $L^{-1}$ | 31.7 ± 0.6 | $Mg^{2+}$, mg $L^{-1}$ | 3.78 ± 0.5 |
| TOC, mg $L^{-1}$ | 26.84 ± 0.7 | $K^+$, mg $L^{-1}$ | 3.11 ± 0.3 |
| Alkalinity, mEq $L^{-1}$ | 4.44 ± 0.03 | $HCO_3^{-1}$, mg $L^{-1}$ | 256 ± 23 |

Moderate temperature (10.6 ± 0.1 $^{o}$C) of the river water sample reflected the spring ambient conditions. A light basic pH of 8.35 ± 0.01 was observed in the river water samples. This pH value was consistent with a pH value of 8.12 ± 0.01 of the previous sampling (Section 4.3.1.1). High dissolved oxygen content of 9.09 ± 1.1 mg $L^{-1}$ represented the aerobic condition of the water sample.

Calcium of 84.6 ± 4.3 mg $L^{-1}$ and bicarbonate of 256 ± 23 mg $L^{-1}$ dominated the major ions occurring in the river Thames. High $Ca^{2+}$ and $HCO_3^-$ concentrations in the river water samples reflected that the river water was supplied from predominantly calcareous groundwater sources (see Chapter 2 for details). The presence of $NO_3^-$ simultaneously with $SO_4^{2-}$ and $Cl^-$ demonstrated that the river water could be influenced by agricultural activities. The total organic carbon of 26.84 ± 0.7 mg $L^{-1}$ was also measured in these river water samples. Similar to the previous sampling, isoproturon were not detected in the river water samples (the detection limits of the HPLC analytical method are 1 μg $L^{-1}$ for isoproturon. Thus, it was considered that there were no isoproturon significant occurring in the river water samples.

#### *5.3.1.2 Groundwater*

Groundwater samples were collected on the same day with river water samples, on 18 April, 2008 at the Gatehampton site. Methods for collection and analysis of groundwater samples are presented in Section 3.2. Table 5.2 shows the physico-chemical properties of the groundwater samples.

**Table 5. 2** Physico-chemical properties of the groundwater sample at the Gatehampton site (collected on 18 April, 2008). Value is a means of three replicates ± standard error.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Temp, $^{o}C$ | 12.4 ± 0.1 | $Cl^-$, mg $L^{-1}$ | 18.3 ± 0.5 |
| pH | 7.10 ± 0.01 | $NO_3^-$, mg $L^{-1}$ | 19.5 ± 0.3 |
| EC, µS $cm^{-1}$ | 711 ± 12 | $SO_4^{2-}$, mg $L^{-1}$ | 22.0 ± 2.1 |
| DO, mg $L^{-1}$ | 6.70 ± 0.8 | $Na^+$, mg $L^{-1}$ | 13.0 ± 0.4 |
| TN, mg $L^{-1}$ | 4.47 ± 0.26 | $Ca^{2+}$, mg $L^{-1}$ | 99.3 ± 3.5 |
| TC, mg $L^{-1}$ | 38.3 ± 0.3 | $Mg^{2+}$, mg $L^{-1}$ | 1.74 ± 0.2 |
| TOC, mg $L^{-1}$ | 0.61 ± 0.05 | $K^+$, mg $L^{-1}$ | 1.53 ± 0.2 |
| Alkalinity, mEq $L^{-1}$ | 4.46 ± 0.06 | $HCO_3^{-1}$, mg $L^{-1}$ | 272 ± 15 |

A moderate temperature (12.4 ± 0.1 $^{o}C$) of the groundwater reflected the spring ambient conditions. A neutral pH of 7.10 ± 0.01 was measured. A relatively low dissolved oxygen content of 6.70 ± 0.8 mg $L^{-1}$ reflected a mildly anaerobic condition. Calcium of 99.3 ± 3.5 mg $L^{-1}$ and bicarbonate of 272 ± 15 mg $L^{-1}$ dominated the major ions reflecting that the groundwater samples is derived predominantly from the Chalk aquifer (see Chapter 2 for details). Isoproturon were not detected in the groundwater samples (the detection limits of the HPLC analytical method are 1 µg $L^{-1}$ for isoproturon. It was assumed that there were no significant isoproturon in the groundwater samples.

#### *5.3.1.3 Riverbed sediment*

Riverbed sediment was collected on the same day and at the same position with the river water samples, on 18 April, 2008 (sampling method is described in Section 3.2.3). Methods for collection and analysis of riverbed sediment samples are presented in Section 3.2. Physico-chemical properties of the riverbed sediment samples are presented in Table 5.3.

**Table 5. 3** Physico-chemical properties of riverbed sediment at the Gatehampton site study (collected on 18 April, 2008). Value is a means of five replicates ± standard error.

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Density (g/cm$^3$) | 2.72 ± 0.08 | S (%) | 0.78 |
| Bulk density (g/cm$^3$) | 1.31 ± 0.02 | N (%) | 0.49 |
| Porosity (%) | 51.3 ± 1.8 | $S_{SA}$* (m$^2$/g) | 0.0786 ± 0.0040 |
| Moisture content (%) | 30.22 ± 0.51 | Particle size distribution (% of weight): | |
| pH | 8.35 ± 0.06 | • 0.020 - 2 μm (clay) | • 1 ± 0.0% |
| TC (%) | 6.21 | • 2 – 50 μm (silt) | • 23 ± 0.2 % |
| TOC (%) | 0.94 | • 50 – 2000 μm (sand) | • 76 ± 0.8 % |

*$S_{SA}$ – Specific surface area

The texture of the riverbed sediment was determined to be loamy sand. The sediment was dominated by 76 ± 0.8 % sand (> 50 μm), 20 ± 0.2 % silt (2 – 50 μm) and approximately 1 % clay (0.020 – 2 μm). Given the composition of primary sand, a low specific surface area of 0.0786 m$^2$ g$^{-1}$ was obtained. The sediment was found to be alkaline (with a pH value of 8.35), in close agreement with the pH value of river water (pH = 8.35). A low organic carbon content of 9.4 mg kg$^{-1}$ was found in the sediment samples. Due to the absence of detectable herbicide isoproturon was detected in the river water samples where the riverbed sediments samples were collected, it was assumed that no isoproturon was initially present in the riverbed sediment samples.

### 5.3.2 Chemicals and Analytical Instruments

Isoproturon (IPU or $^{12}$C-IPU) was purchased from Sigma Aldrich (article/product: 36137, purity of 99.8%). Its physico-chemical properties are outlined in Section 1.3.1. Stock solutions of isoproturon were prepared from isoproturon powder dissolved in ethanol achieving final concentrations of 100 mg $L^{-1}$; 1 mg $L^{-1}$; 0.1 mg $L^{-1}$. These stocks were used for isoproturon-dosed treatments in this chapter.

$^{14}$C ring-radiolabelled isoproturon ($^{14}$C-isoproturon or $^{14}$C-IPU) was purchased from Amersham Co. Ltd, UK. $^{14}$C-isoproturon stock solution was prepared from isoproturon powder dissolved in ethanol achieving a final concentration of 10 kBq $mL^{-1}$.

Cartridges used for the solid phase extraction were purchased from Sigma Aldrich (SupelClean ENVI-Carb 3 ml tubes, 250 mg, cat. no. 57088). The cartridge was graphitized non-porous carbon with surface area to be 100 $m^2$ $g^{-1}$. An Ultima Gold Scintillation cocktail was purchased from Packard, UK. All other chemicals were reagent grade and obtained either from Sigma Aldrich or Fisher Scientific (Bishop Meadow Road, Loughborough, Leicestershire, United Kingdom).

Chromatography was performed using a Dionex Summit HPLC system (see Section 3.4.2 for details). The Liquid Scintillation Counter instrument was a Canberra Packard Tri-carb 2250CA (see Section 3.3.3 for details).

## 5.4 Methods

Based upon the objectives presented in Section 5.2, three experiments were designed to investigate the catabolic insights into isoproturon degradation in river water, groundwater and riverbed sediment environments. Experiment 1 investigated the intrinsic (IPU-undosed) catabolic activity with respect to isoproturon in GW, RW and RS environments. Experiment 2 investigated the induced (IPU-dosed) catabolic activity with respect to isoproturon in GW, RW and RS environments. Four periods of incubation times (0, 5, 10 and 30 days) were also associated in Experiments 1 and 2. Experiment 3 aimed to determine residual concentrations in riverbed sediment treatments after periods of incubation time. SPE and HPLC techniques were used to measure the $^{12}$C-IPU concentrations.

The terms "*IPU-undosed*" used in Experiment 1 indicates $^{12}$C-IPU was not added to these treatments *prior to* spiking with $^{14}$C-IPU to establish catabolic activity. The terms "*IPU-dosed*" used in Experiment 2 indicated that the treatment was *firstly* spiked with $^{12}$C-IPU before *secondly* spiked with $^{14}$C-IPU for investigation of induced catabolic activity. The term "*incubation*" is defined as a process by which, after adding an amount of $^{12}$C-IPU, the treatments were placed on a flat bed orbital shaker (100 rpm) for a period of time (0, 5, 10 and 30 days), at room temperature and under the laboratory light. Then second addition of $^{14}$C-IPU was made to investigate the catabolic activity. For example, a period of *zero* incubation days means the $^{14}$C-IPU was spiked *immediately,* after the first addition of $^{12}$C-IPU, and periods of 5, 10 or 30 days explain that the $^{14}$C-IPU was spiked after 5, 10 or 30 days since the first addition of $^{12}$C-IPU. It was assumed that, as stated previously, isoproturon degrading organisms do not significantly discriminate between the $^{14}$C-IPU and $^{12}$C-IPU.

It is of value to note that the laboratory temperatures were recorded to vary in the day time from 15 – 20 $^{o}$C and in the night time from 10 – 15 $^{o}$C. The laboratory light was described as the natural light but without direct sunbeams on to the respirometer in order to minimise photochemical degradation. In addition, no neon lights were used during the day and no other students worked at night during the experimental time, thus there was no fluorescent light either during the day or night.

For abbreviation, the following order for a treatment coding system was used: matrix types denoted RW, GW and RS reflect river water, groundwater and riverbed sediment, respectively. The $^{12}$C-IPU concentration at the time of dosing was then provided and finally, in parentheses, the incubation period prior to $^{14}$C-IPU addition was given. Thus, a treatment coded *GW 0.1 (30)* is a *groundwater* matrix spiked with $^{12}$C-IPU of *0.1* μg $L^{-1}$ and incubated for *30* days prior to $^{14}$C-IPU addition and the commencement of catabolic activity assessment.

The length of assay time for Experiments 1 and 2 was defined as a period of time between the second addition of $^{14}$C-IPU and up to the mineralisation levels reach a plateau. In this chapter, the assay time was determined to be 30 days. Therefore, the maximum levels of catabolic activity were calculated relying upon 30 assay days. The method to calculate the maximum level of catabolic activity has been described in Section 3.3.3.2.

*Adaptation time* was identified by plotting the curves of the mineralisation levels against the assay times. Then the length of a period of adaptation was determined between the starting point of the curve (0 assay days) and the point in which the curve reached the threshold of 5 % mineralisation.

### 5.4.1 Experiment 1 – Intrinsic Catabolic Activity in IPU-undosed Treatments

This experiment aimed to investigate the intrinsic catabolic activity with respect to isoproturon in IPU-undosed treatments with different riverbank microcosms including RW, GW and RS and different incubation periods of time including 0, 5, 10 and 30 days. Based upon the experimental procedure, Experiment 1 was divided into two groups: Group 1 – IPU-undosed treatments with river water and groundwater; Group 2 – IPU-undosed treatments with riverbed sediment.

*Group 1 – IPU-undosed treatments with RW and GW* (RW and GW were treated as different microcosms but using the same procedure): an aliquot (30 mL) of the RW (or GW) was transferred to a respirometer. Two sets of treatments were set-up with 0 and 30 incubation days. Regarding the treatments with 0 incubation days, $^{14}$C-IPU was immediately spiked to the respirometer. Regarding the treatments with 30 incubation days, $^{14}$C-IPU was spiked after 30 days. Then, the common consecutive steps were performed following the procedure described in Section 3.3.3.2. For statistical analysis, every treatment was made with three replicates. Blank treatments were prepared from aliquots (30 mL) of sterile distilled water. The procedure for these treatments is also described in Section 3.3.3.2.

*Group 2 – IPU-undosed RS treatments*: a portion of RS (10 g) was transferred to a respirometer containing sterile distilled water (130 mL) (sterilisation using an autoclave PS/QCS/EV150, 2005, Priorclave Ltd. at 121 $^{o}$C for 30 minutes). Four sets of experiments with 0, 5, 10 and 30 incubation days were set-up. After these periods of incubation, a volume of the solution (100 mL) was removed (the respirometers were kept without shaking over-night before removing in order to preserve the riverbed sediment inside the respirometer). These solutions were then used in Experiment 3 to measure $^{12}$C-IPU residual

concentrations. The remaining volume (30 mL) of the supernatant in the respirometers was used to investigate the intrinsic catabolic activity with respect to isoproturon. $^{14}$C-IPU was spiked in the above four sets after 0, 5, 10 and 30 days and after the removal of the solutions. The common consecutive steps followed the procedure described in Section 3.3.3.2. Three replicates were set-up for every treatment.

### 5.4.2 Experiment 2 – Induced Catabolic Activity in IPU-dosed Treatments

Experiment 2 investigated the catabolic activity of isoproturon in IPU-dosed treatments with RW, GW and RS and different incubation periods. These treatments were also divided into two groups: Group 3 – IPU-dosed RW treatments and IPU-dosed GW treatments; Group 4 – IPU-dosed RS treatments.

*Group 3 – IPU-dosed treatments with RW and GW* (RW and GW were treated as different microcosms but sharing the same procedure): an aliquot (30 mL) of the RW (or GW) was transferred to a respirometer. Then, the first addition of $^{12}$C-IPU stock solution was performed to give the final concentrations of isoproturon in the respirometers to be 0.1, 1.0 and 100.0 μg $L^{-1}$. The resultant treatments were incubated at room temperature with orbital shaking (100 rpm) for 30 days. After this incubation time, the second addition of $^{14}$C-IPU was made. The consecutive steps were copied from the procedure described in Section 3.3.3.2. Three replicates were set-up for every treatment.

*Group 4 – IPU-dosed RW treatment*: a portion of RS (10 g) was transferred to the respirometer containing sterile distilled water (130 mL). Three sets of respirometers were spiked with the first addition of $^{12}$C-IPU stock solution to give the final concentrations of 0.1, 1.0 and 100.0 μg $L^{-1}$. In a similar way to Group 2, four sets of experiments with 0, 5, 10 and 30 incubation days were

set-up. After these periods of incubation, a volume of the solution (100 mL) was removed. These solutions were also used in Experiment 3 to measure $^{12}$C-IPU residual concentrations. The remaining volume (30 mL) of the supernatant in the respirometes was used to investigate the intrinsic catabolic activity with respect to isoproturon. The second addition of $^{14}$C-IPU was spiked in the above four sets after 0, 5, 10 and 30 days and after the removal of the solutions. The common consecutive steps were followed the procedure described in Section 3.3.3.2. Three replicates were set-up for every treatment.

### 5.4.3 Experiment 3 –$^{12}$C-IPU Residual Concentrations after Periods of Incubation

This experiment aimed to determine $^{12}$C-IPU residual concentrations at the points of the second additions of $^{14}$C-IPU in the solutions removed from Group 4. The solutions removed from Group 2 were used as the *control* or *blank* treatments for measurement because they were not spiked with $^{12}$C-IPU but they had the same incubation times of 0, 5, 10 and 30 days with Group 4.

The solutions (100 mL) from Groups 2 and 4 of were passed through syringe filter units (Millex-GP, 0.22 µm, polyethersulfone, radio-sterilised) to remove particles larger than 0.22 µm. Then the solutions were concentrated using the SPE technique (up to a concentration 100 times higher). A SPE procedure was developed and is presented below.

#### *Introduction of SPE method*

Solid phase extraction (SPE) is frequently used as a useful sample preparation technique for pre-concentration and extraction of herbicides from environmental samples, mainly water (Aguilar *et al.*, 1996a; Balinova, 1996; Pinto and Jardim, 2002). With SPE, many of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations,

less-than-quantitative recoveries, use of expensive breakable specialty glassware, and disposal of large quantities of organic solvents. SPE involves the partitioning of solutes between two phases: a liquid (sample matrix) and a solid (sorbent) phase (Camel, 2003). The selection of the type of sorbent able to solve the trace-analysis problem becomes a key decision for analysts. Several types of sorbents for trapping analytes have been introduced in the market e.g. highly cross-linked co-polymers and their new functionalised form, graphitised carbons, as well as *n*-alkylsilicas (Hennion, 1999). A graphitised carbon-based packing was used as sorbent as the SPE method in the current study.

Carbonaceous adsorption media, such as the ENVI-Carb materials (a trademark of the graphitized carbon-based materials of Sigma-aldrich Supelco), consist of graphitic, nonporous carbon that has a high attraction for organic polar and nonpolar compounds for both polar and nonpolar matrices (Supelco, 1998). The carbon surface is comprised of atoms in hexagonal ring structures, interconnected and layered in graphitic sheets. The hexagonal ring structure demonstrates a strong selectivity for planar aromatic or hexagonal ring-shaped molecules and hydrocarbon chains with potential for multiple surface contact points. Retention of analytes is based primarily on the analyte's structure (size and shape), rather than on interactions of functional groups on the analyte with the sorbent surface. Elution is performed with mid- to nonpolar solvents. The unique structure selectivity of ENVI-Carb materials, compared to bonded alkyl-silicas, make them an excellent alternative when the bonded silicas will not work for an application.

### *SPE procedure*

A SPE procedure was developed to enrich concentrations of the herbicide isoproturon in an aqueous solution before analysis using the HPLC technique. The SPE method consisted of four successive steps: (1) conditioning of the

sorbent; (2) application (and percolation) of the sample; (3) cleaning of the sample and (4) desorption and recovery of the analytes. Firstly, the cartridges were conditioned using methanol-acetone (3:2 v/v; 6 mL) then methanol (3 mL) and deionized water (3 mL). Secondly, the samples were loaded on the conditioned cartridges at a flow rate of approximately 10 mL $min^{-1}$, and 5 mL of MiliQ water was used to wash the wall of the beaker and syringe. Thirdly, the loaded cartridges were eluted with 4 mL and 2 mL of methanol-acetone (3:2 v/v). Lastly, the eluate was evaporated to dryness using a gentle stream of nitrogen gas (approximately 60 minutes). A volume of 1 mL acetonitrile was added into the vial (7mL). The contents were shaken thoroughly to re-dissolve the residual and transferred to the HPLC vial. Samples of the re-dissolved residual were finally analysed by an HPLC system. The HPLC procedure was also developed and is presented in Section 3.4.1.

### 5.4.4 Statistical Analysis

A combination of one-way analysis of variance (ANOVA) and post-hoc tests (Tukey) was used to compare the level of significance among several treatments (more than 3 groups of data). The independent-sample t-test was used to compare the data between two treatments (2 groups of data). For all tests, a significance p-value of less than 0.05 was used. All statistical analyses were done using SPSS for Windows® (version 16.0) with graphs plotted using Microsoft Excel and Sigma Plot 2000.

## 5.5 Results

### 5.5.1 Catabolic Activity of Isoproturon in River Water (RW) and Groundwater (GW) treatments

#### *5.5.1.1 Intrinsic catabolic activity in IPU-undosed treatments for RW and GW (Group 1 of Experiment 1)*

The intrinsic catabolic activity with respect to isoproturon in both IPU-undosed RW treatments and IPU-undosed GW treatments was observed from Group 1 of Experiment 1. Very low maximum mineralisation levels (less than 5%) were observed in these treatments. In the RW treatments without incubation, RW 0 (0), the maximum mineralisation level was identified to be 0.4 ± 0.1%. After 30 incubation days, the same value of 0.4 ± 0.1% of the maximum mineralisation level was also obtained in the treatment RW 0 (30).

In the GW 0 (0) treatments, the maximum mineralisation levels were also very low (less than 5%), at only 1.2 ± 0.1%. Compare the maximum mineralisation levels between RW 0 (0) and GW 0 (0), the independent-samples t-test showed that the level of mineralisation in the GW 0 (0) was significantly greater ($p < 0.05$) than the level in RW 0 (0). After 30 incubation days, the maximum mineralisation level of GW 0 (30) was determined to be 1.8 ± 0.9%. However, the independent-samples t-test showed that no significant increase ($p > 0.05$) was found between the maximum mineralisation levels of GW 0 (0) and GW 0 (30). Table 5.4 presents the maximum mineralisation levels in the RW and GW IPU-undosed treatments with 0 and 30 incubation days.

**Table 5. 4** Maximum mineralisation levels with respect to $^{14}$C-IPU in RW and GW IPU-undosed treatments with 0 and 30 incubation days.

| Treatments | Maximum mineralisation levels, (% $^{14}CO_2$, n =3, ± SD) |
|---|---|
| RW 0 (0) | 0.4 ± 0.1 |
| RW 0 (30) | 0.4 ± 0.1 |
| GW 0 (0) | 1.2 ± 0.1 |
| GW 0 (30) | 1.8 ± 0.9 |

#### *5.5.1.2 Induced catabolic activity in IPU-dosed treatments for river water and groundwater (Group 3 of Experiment 2)*

The induced catabolic activity with respect to isoproturon in IPU-dosed RW treatments and IPU-dosed GW treatments was not enhanced after 30 incubation days. The results showed that, in the RW (30) treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ IPU, the maximum mineralisation levels were identified to be 0.3 $\pm$ 0.1 %, 0.3 $\pm$ 0.0 % and 0.3 $\pm$ 0.0 %, respectively. In the GW (30) treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ IPU, the maximum mineralisation levels were identified to be 0.7 $\pm$ 0.2 %, 1.1 $\pm$ 0.2 % and 0.6 $\pm$ 0.2 %, respectively. These levels were lower than the levels in GW 0 (30) (of 1.8 ± 0.9%). However, one-way ANOVA with Tukey tests showed that no significant difference ($p > 0.05$) was found between GW 0 (30) and GW 0.1 (30) ($p = 0.093$); and GW 1 (30) ($p = 0.320$), and GW 100 (30) ($p = 0.064$). Furthermore, one-way ANOVA also indicated no significant difference ($p > 0.05$) between the above IPU-dosed GW treatments. Table 5.5 presents the maximum mineralisation levels in RW and GW IPU-dosed treatments with 30 incubation days.

**Table 5. 5** Maximum mineralisation levels with respect to IPU in RW and GW IPU-dosed treatments with 30 incubation days

| IPU-dosed treatments | Maximum mineralisation level (% $^{14}CO_2$, n=3, ±SD) |
|---|---|
| RW 0.1 (30) | 0.3 ± 0.1 |
| RW 1 (30) | 0.3 ± 0.0 |
| RW 100 (30) | 0.3 ± 0.0 |
| GW 0.1 (30) | 0.7 ± 0.2 |
| GW 1 (30) | 1.1 ± 0.1 |
| GW 100 (30) | 0.6 ± 0.2 |

### 5.5.2 Catabolic Activity of Isoproturon in Riverbed Sediment (RS) treatments

The maximum mineralisation levels of isoproturon in the IPU-undosed and IPU-dosed (0.1, 1, and 100 μg $L^{-1}$) RS treatments was determined over 30 assay days. The mineralisation levels were plotted against the assay time and are presented in Figure 5.1A, B, C and D in accordance to the four periods of 0, 5, 10 and 30 incubation days, respectively. Based upon the shape of the empirical curves in Figure 5.1 and the principle for the mineralisation of an organic compound, the curves were divided into two phases as following:

(i) Phase I was considered as an adaptation phase or lag phase or acclimation phase. The adaptation phase was defined as a stage in which the mineralisation level of a compound was still less than 5 %. The method used to calculate the adaptation time is presented in Section 5.4.

(ii) Phase II was considered as an acceleration phase when the rate of $^{14}$C-IPU destruction became rapid. This phase was defined as the stage in which the mineralisation level of a compound exceeded the threshold 5 % of the adaptation phase and was less than 5 % of the maximum mineralisation level. The method used to calculate the maximum mineralisation level and maximum mineralisation rate is presented in Section 3.3.3.2.

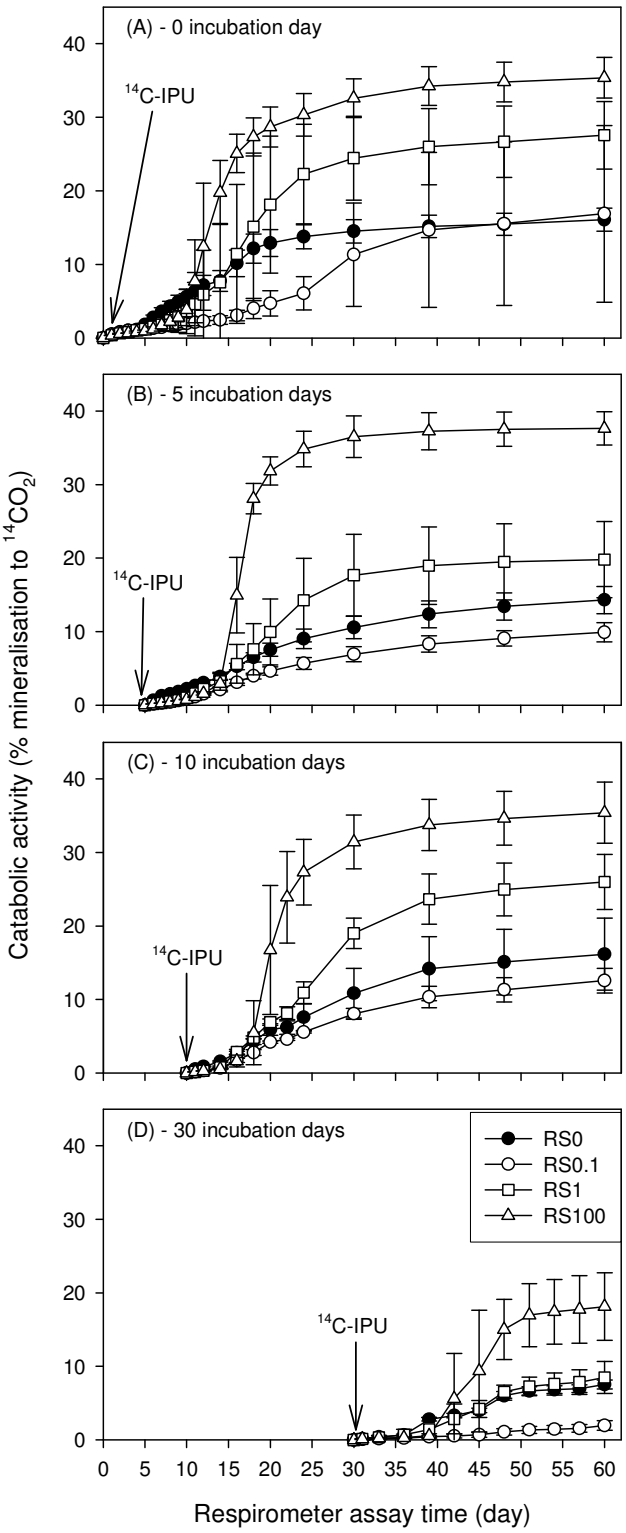



**Figure 5. 1** Catabolic activity with respect to IPU in the riverbed sediment (RS) treatments with IPU-undosed (close-circle – RS 0) and IPU-dosed of 0.1 µg $L^{-1}$ (open-circle – RS 0.1), 1 µg $L^{-1}$ (open-square – RS 1) and 100 µg $L^{-1}$ (open triangle – RS 100) after incubation periods of 0 (Fig. A), 5 (Fig. B), 10 (Fig. C) and 30 days (Fig. D). Error bars represent standard error (n=3) of % mineralization to $^{14}CO_2$.

### 5.5.2.1 Phase I - Adaptation phase in the IPU-undosed and IPU-dosed treatments with riverbed sediment

As stated above, adaptation time was determined by the period of minerlisation which was less than the threshold of 5 %. The results were calculated using Excel. Figure 5.2 shows adaptation times in IPU-undosed and IPU-dosed RS treatments.

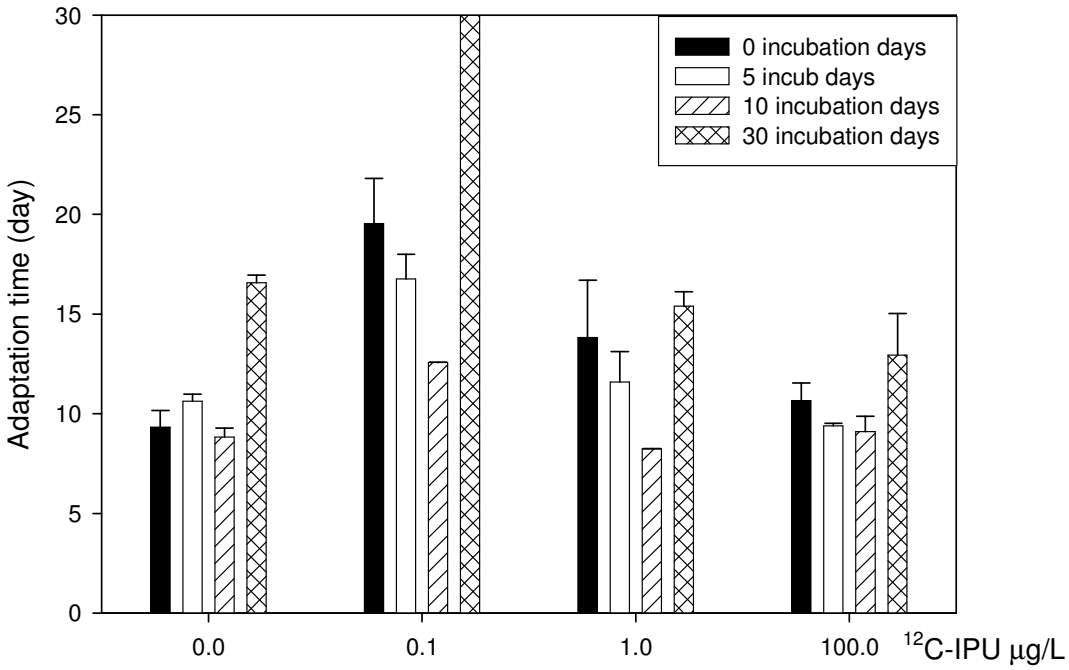



**Figure 5. 2** Adaptation times in IPU-undosed and IPU-dosed riverbed sediment (RS) treatments. Note: maximum mineralisation levels in treatment RS 0.1 (30) were very low; as a consequence a definitive adaptation time could not be established as mineralisation never exceeded 5%. Error bars represent standard error (n=3) of adaptation time (day).

Regarding the IPU-undosed RS treatments (Group 2, Experiment 1), one-way ANOVA with Tukey tests indicated that the adaptation times in the treatments with 0, 5 and 10 incubation days were not significantly different ($p > 0.05$), with values of RS 0 (0), RS 0 (5) and RS 0 (10) were determined to be 9.3 ± 0.8, 10.6 ± 0.4 and 8.8 ± 0.4 days, respectively. However, the adaptation times in the treatments with 30 incubation days (determined to be 16.6 ± 0.4 days)

were significantly different ($p < 0.05$) to the other three IPU-undosed treatments.

Regarding the RS treatments dosed with 0.1 µg $L^{-1}$ of IPU (Group 4, Experiment 2), the adaptation times were high (19.6 ± 2.2 days) in the treatments without incubation time RS 0.1 (0). Then, the adaptation times were shortened in the treatments with 5 and 10 incubation days. They were determined to be16.8 ± 1.2 and 12.6 ± 0.0 days in the treatments RS 0.1 (5) and RS 0.1 (10), respectively. However, in the treatments with 30 incubation days, RS 0.1 (30), no adaptation time was detected because the maximum mineralisation levels in this treatment were very low (less than 5%). Hence, in Figure 5.2, the cross bar which is higher than 30 days is plotted to represent the adaptation time of Treatments RS 0.1 (30). One-way ANOVA with Tukey tests indicated that there was no significant difference ($p > 0.05$) between Treatments RS 0.1 (0) and RS 0.1 (5) but there was significant difference ($p < 0.05$) between Treatments RS 0.1 (0) and RS 0.1 (10).

Regarding the RS treatments dosed with 1 µg $L^{-1}$ of IPU (Group 4, Experiment 2), the same trend with the treatments dosed with 0.1 µg $L^{-1}$ was observed. The adaptation times were decreased from 13.8 ± 2.9 to 11.3 ± 1.5 to 8.2 ± 0.0 days according to the increase of incubation times from 0 to 5 to 10 days, respectively. Thereafter, the adaptation time increased to 15.4 ± 0.9 days after 30 incubation days. However, one-way ANOVA with Tukey tests indicated no significant difference ($p > 0.05$) among these RS 1 treatments.

Regarding the RS treatments dosed with 100 µg $L^{-1}$ of IPU (Group 4, Experiment 2), again, the same trend with the treatments dosed with 0.1 µg $L^{-1}$ and 1 µg $L^{-1}$ was observed. The adaptation times were decreased from 10.7 ± 0.9 to 9.4 ± 0.1 and to 9.1 ± 0.8 days according to the increase of incubation times from 0 to 5 to 10 days, respectively. Thereafter, the adaptation time increased to 13.0 ± 2.6 days after 30 incubation days. One-way ANOVA with

Tukey tests indicated no significant difference ($p > 0.05$) was recorded among these RS 100 treatments.

#### *5.5.2.2 Phase II – Acceleration phase in the IPU-undosed and IPU-dosed riverbed sediment (RS) treatments*

Figure 5.1 indicates that the catabolic activity of isoproturon in the RS treatments considerably increased after adaptation phase. Mineralisation levels and mineralisation rates were examined as the primary parameters to describe catabolic activity in the treatments.

##### *(1) Maximum mineralisation levels in IPU-undosed RS treatments (Group 2, Experiment 2)*

The intrinsic catabolic activity with respect to isoproturon in the IPU-undosed RS treatments was determined after 30 assay days (excluding the incubation time). With the treatments RS 0 (0), RS 0 (5), RS 0 (10) and RS 0 (30), the maximum mineralisation levels did not increase after 0 incubation day with 14.5 $\pm$ 1.6 %, 5 incubation days with 11.5 $\pm$ 1.7% and 10 incubation days with 14.3 $\pm$ 4.4 %, but markedly decreased after 30 incubation days with 7.6 $\pm$ 0.6 %. One-way ANOVA with Tukey tests showed that there was no significant difference ($p > 0.05$) in levels of mineralisation between the treatments RS 0 (0), RS 0 (5) and RS 0 (10), but significant difference ($p < 0.05$) was indicated between the treatments RS 0 (30) versus RS 0 (0) and RS 0 (30) versus RS 0 (10). In addition, comparison of the intrinsic maximum mineralisation levels between the treatments RS and RW or between RS and GW indicated that the mineralisation levels in the RS treatments were significantly higher ($p < 0.05$) than in RW and GW treatments.

### *(2) Maximum mineralisation levels in IPU-dosed RS treatments (Group 4, Experiment 2)*

Regarding the RS treatments dosed with 0.1 µg $L^{-1}$ of IPU, RS 0.1 (0), RS 0.1 (5), RS 0.1 (10) and RS 0.1 (30), the maximum mineralisation levels were determined to be 11.3 ± 4.1 %, 7.7 ± 1.2 %, 10.5 ± 1.3 %, and 2.0 ± 0.7 %, respectively. It is observed that the maximum mineralisation levels decreased after 5 incubation days but increased again after 10 incubation days and considerably decreased after 30 incubation days. One-way ANOVA with Tukey test indicated no significant difference ($p > 0.05$) of the maximum mineralisation levels between treatments RS 0.1 (0), RS 0.1 (5) and RS 0.1 (10) but significant difference ($p < 0.05$) between the treatments RS 0.1 (30) and the other three treatments.

Regarding the RS treatments dosed with 1 µg $L^{-1}$ of IPU, RS 1 (0), RS 1 (5), RS 1 (10) and RS 1 (30), the maximum mineralisation levels were determined to be 24.5 ± 5.7 %, 18.3 ± 5.4 %, 23.8 ± 3.4 % and 8.5 ± 2.2 %, respectively. The same trend was observed with the treatments dosed with 0.1 µg $L^{-1}$, one-way ANOVA with Tukey tests indicated no significant difference ($p > 0.05$) between treatments RS 1 (0), RS 1 (5), RS 1 (10) but a significant decrease ($p < 0.05$) between treatments RS 1 (30) compared with the other three treatments.

Regarding the RS treatments dosed with 100 µg $L^{-1}$ of IPU, coded RS 100 (0), RS 100 (5), RS 100 (10) and RS 100 (30), the maximum mineralisation levels were determined to be 32.6 ± 2.6 %, 36.9 ± 2.7 %, 33.8 ± 3.5 % and 18.1 ± 4.6 %, respectively. It is of value to note that the highest maximum mineralisation level was achieved in the treatments RS 100 (5). In a similar way to the treatments dosed with 0.1 µg $L^{-1}$ and 1 µg $L^{-1}$, one-way ANOVA with Tukey tests indicated no significant difference ($p > 0.05$) between treatments RS 100 (0), RS 100 (5) and RS 100 (10) but a significant decrease ($p < 0.05$) between treatments RS 100 (30) and the other three treatments.

### *(3) Mineralisation kinetics of isoproturon in IPU-dosed and IPU-undosed RS treatments (Group 4, Experiment 2)*

The maximum mineralisation rates of isoproturon in RS were determined after the adaptation phase (Phase I). By plotting the data points of mineralisation levels against the assay time, a linear fit to theses points was determined. The maximum mineralisation rate was obtained from the gradient of the fitted line. Rates were calculated across data points where mineralisation was > 5% and up until the point where rapid mineralisation slowed down. Figure 5.3 presents mineralisation kinetics of isoproturon in IPU-dosed and IPU-undosed RS treatments.

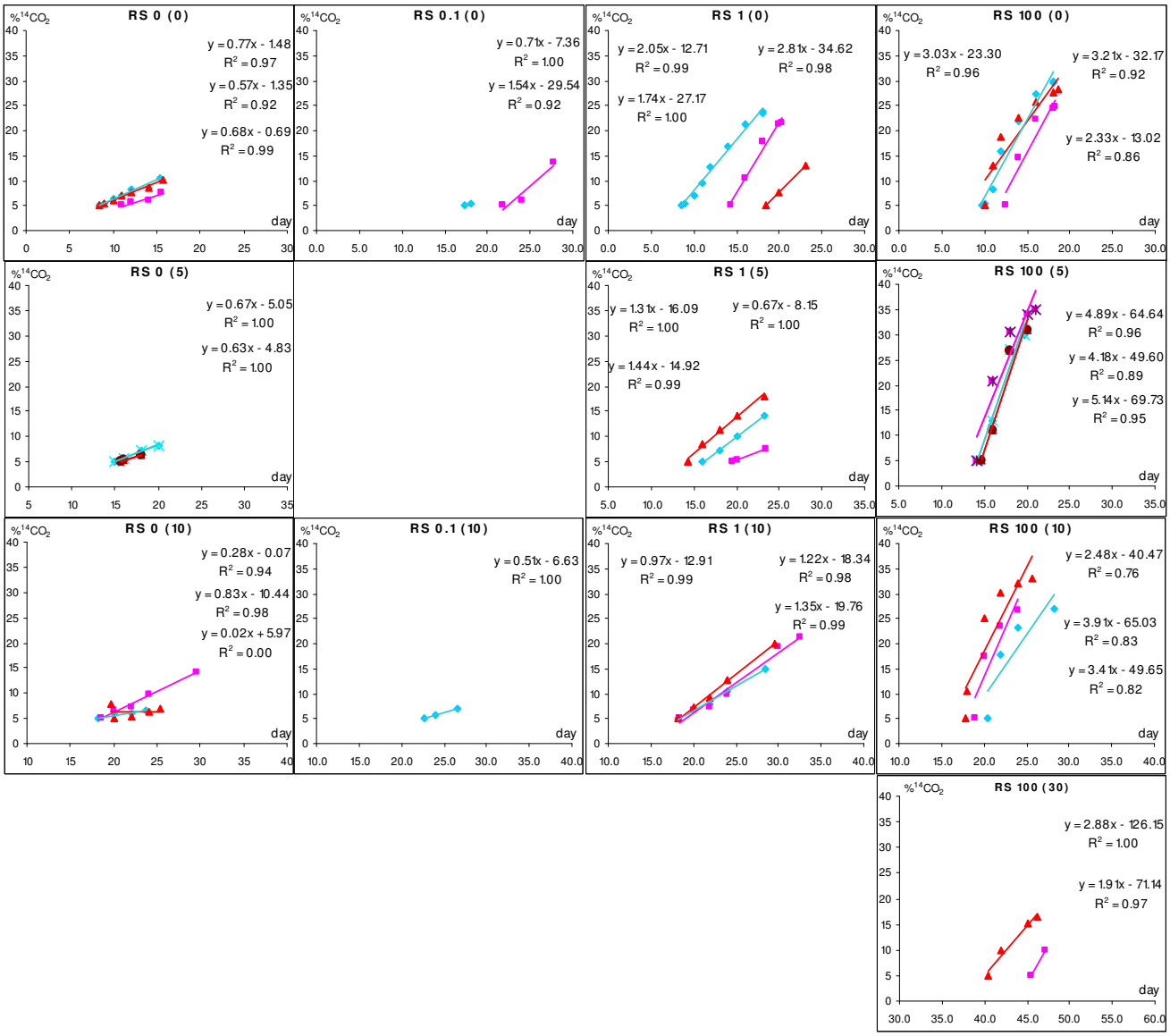



**Figure 5. 3** Mineralisation kinetics of isoproturon in IPU-dosed and IPU-undosed riverbed sediment (RS) treatments from 3 replicates. Missing fitted line in several treatments indicates no mineralisation rate can be detected.

Regarding the IPU-undosed RS treatments with 0, 5 and 10 incubation days, RS 0 (0), RS 0 (5) and RS 0 (10), the averages of the maximum mineralisation rates of three replicates were identified to be 0.67 ± 0.10, 0.65 ± 0.02 and 0.38 ± 0.24 (% $^{14}CO_2$ $day^{-1}$), respectively. It was noted that no maximum mineralisation rate of the treatment RS 0 (30) could be determined because the maximum mineralisation level of this treatment was less than 10 %. One-way ANOVA with Tukey test indicated no significant difference ($p > 0.05$) between treatments with 0, 5 and 10 incubation days.

Regarding the RS treatments dosed with 0.1 µg $L^{-1}$ of IPU, the maximum mineralisation rates of Treatments RS 0.1 (0) was determined to be 1.13 ± 0.42 % $^{14}CO_2$ $day^{-1}$. The maximum mineralisation rate of Treatment RS 0.1 (10) was determined from only one replicate to be 0.51 % $^{14}CO_2$ $day^{-1}$ (the maximum mineralisation rates of the other two replicates could not be detected because their maximum mineralisation levels were less than 10%). The maximum mineralisation rates of the treatments RS 0.1 (5) and RS 0.1 (30) also could not be detected because the maximum mineralisation levels of these treatments were less than 10 %. One sample t-test was used to compare the value of mineralisation rate of Treatments RS 0.1 (10) and RS 0.1 (0). No significant difference ($p > 0.05$) was observed in this comparison.

Regarding the RS treatments dosed with 1 µg $L^{-1}$ of IPU, RS 1 (0), RS 1 (5) and RS 1 (10), the maximum mineralisation rates were determined to be 2.20 ± 0.32, 1.14 ± 0.24 and 1.18 ± 0.11 % $^{14}CO_2$ $day^{-1}$, respectively. It was also noted that the maximum mineralisation rates of Treatments RS 1 (30) could not be detected because the maximum mineralisation levels of this treatment was less than 10 %. One-way ANOVA with Tukey tests indicated significant difference ($p \leq 0.05$) between RS 1 (0) and the other two treatments RS 1 (5) and RS 1 (10). However, no significant different ($p > 0.05$) between treatments RS 1 (5) and RS 1 (10) was indicated.

Regarding the RS treatments dosed with 100 µg $L^{-1}$ of IPU, RS 100 (0), RS 100 (5), RS 100 (10) and RS 100 (30), the maximum mineralisation rates were determined to be 2.86 ± 0.27, 4.74 ± 0.29, 3.27 ± 0.42 and 2.40 ± 0.4 % $^{14}CO_2$ $day^{-1}$, respectively. It is of value to note that the highest maximum mineralisation rate was achieved in Treatment RS 100 (5). One-way ANOVA with Tukey tests showed that the maximum mineralisation rates of Treatment RS 100 (5) are significantly higher ($p < 0.05$) than the rates of Treatments RS 100 (0) and RS 100 (30). However, there was no significant difference ($p = 0.07 > 0.05$) between RS 100 (5) and RS 100 (10).

### 5.5.3 Residual $^{12}$C-Isoproturon in RS Treatments after a Period of Incubation (Experiment 3)

Before presenting the residual $^{12}$C-IPU concentration in the treatments RS 0.1 (0), RS 1 (0) and RS 100 (0) following a period of incubation times, it was necessary to determine the recovery factor of the solid phase extraction step. The recovery factor, *R*, was calculated as following:

$$R = \frac{C_{IPU,extract}}{C_{IPU,0}} x\,100 \qquad (5.1)$$

where:

$C_{IPU,extract}$ – IPU concentration after extraction by SPE method;

$C_{IPU,0}$ – IPU concentration before extraction (concentration of the standards).

The recovery factors for the RS treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ of IPU are presented in Table 5.6. It is noted that, with the treatment RS 0.1 (0), three replicates were prepared. Unfortunately, there was an accident while extracting the solutions (100 mL) from the respirometers and transferring them into the cartridges. Two replicates of the solutions of RS 0.1 (0) treatments were broken. Therefore, the recovery factor for Treatment RS 0.1 (0) was reported without replication (one sample only).

**Table 5. 6** Recovery results for isoproturon in riverbed sediment treatments

| Treatments | *R* (%) | RSD (%) | N |
|---|---|---|---|
| RS 0.1 (0) | 41.4 | - | 1 |
| RS 1 (0) | 91.8 | 1.2 | 3 |
| RS 100 (0) | 86.2 | 1.7 | 3 |

*RSD – relative standard deviation; N – number of replicates*

The recovery factors were used to correct the residual concentrations of $^{12}$C-IPU in the RS treatments of Groups 2 and 4 (Experiments 1 and 2) at the points of $^{14}$C-IPU addition. Table 5.7 presents the residual $^{12}$C-IPU in these RS treatments.

**Table 5. 7** $^{12}$C-IPU residual concentrations in the RS treatments after a period of incubation time.

| Incubation time (day) | $^{12}$C-IPU residual concentration, μg $L^{-1}$ (n =3, ± standard error) | | |
|---|---|---|---|
| | RS 0.1 | RS 1 | RS 100 |
| 0 days | 0.04 ± 0.01 | 0.92 ± 0.01 | 86.20 ± 0.86 |
| 5 days | 0.03 ± 0.01 | 0.61 ± 0.07 | 76.31 ± 2.10 |
| 10 days | 0.03 ± 0.01 | 0.48 ± 0.05 | 57.79 ± 2.72 |
| 30 days | BDL | BDL | 31.31 ± 4.42 |

*BDL = below detection limit*

Regarding Treatments RS 100 (0), RS 100 (5), RS 100 (10) and RS 100 (30), the residual $^{12}$C-IPU concentrations were determined to be 86.20 ± 0.86, 76.31 ± 2.10, 57.79 ± 2.72 and 31.31 ± 4.42 μg $L^{-1}$, respectively. One-way ANOVA with Tukey tests showed that the residual concentrations in Treatments RS 100 (30) were significantly different ($p < 0.05$) to the other three treatments. In addition, the residual concentrations in Treatments RS 100 (10) were also significantly different ($p < 0.05$) to the Treatments RS 100 (0) and RS 100 (5).

However, no significant difference ($p > 0.05$) of the residual isoproturon concentrations was found between the Treatments RS 100 (0) and RS 100 (5).

Regarding the treatments RS 1 (0), RS 1 (5), RS 1 (10) and RS 1 (30), the residual $^{12}$C-IPU concentrations were identified to be 0.92 ± 0.01, 0.61 ± 0.07, 0.48 ± 0.05 μg $L^{-1}$ and below the detection limit of the HPLC, respectively. One-way ANOVA with Tukey tests showed that the residual concentrations in Treatments RS 1 (0) were significantly different ($p < 0.05$) to Treatments RS 1 (5) and RS 1 (10). However, no significant difference ($p > 0.05$) of the residual concentrations was found between the Treatments RS 1 (5) and RS 1 (10).

Regarding the treatments RS 0.1 (0), RS 0.1 (5) and RS 0.1 (10), the residual $^{12}$C-IPU concentrations were identified to be 0.04 ± 0.01, 0.03 ± 0.01 and 0.03 ± 0.01 μg $L^{-1}$, respectively. However, no isoproturon (below the detection limit of the HPLC) was detected in the treatments with 30 incubation days, RS 0.1 (30).

After 30 incubation days, $^{12}$C-IPU residual concentrations in the RS treatments dosed with 0.1, 1 and 100 μg $L^{-1}$ were significantly decreased ($p < 0.05$, compared with values of the treatments with 0 incubation days), e.g. below the detection limit in RS 0.1 (30) and RS 1 (30) and 31.31 ± 4.42 μg $L^{-1}$ in RS 100 (30). This result suggests isoproturon was degraded in all of these treatments. In particular, isoproturon was degraded with very low initial concentration treatments (0.1 and 1 μg $L^{-1}$).

## 5.6 Discussion

### 5.6.1 Catabolic Activity of Isoproturon in River Water (RW) and Groundwater (GW)

The very low levels (less than 5%) of intrinsic catabolic activity in river water and groundwater (Group 1, Experiment 1) suggested that very limited catabolism with respect to isoproturon occurred in river water or groundwater microcosms. This result is consistent to the findings in Chapter 4 in which no biodegradation occurred in the treatments with sterile riverbed sediment and non-sterile river water (Treatment 3, Section 4.5.1). It is also suggested that indigenous RW-borne or GW-borne microorganisms were not competent to mineralise isoproturon. Another possible explanation for non-biodegradation of isoproturon could be that microorganisms in river water or groundwater had no fixed habitat to attach and proliferate their population. Indeed, Johnson *et al.* (2000b) reported that bacteria require a surface for attachment, before the multiplication and/or production of enzymes capable of degrading isoproturon can occur. Similarly, the importance of sediment as a colonising surface for the development of groundwater bacteria in a shallow sandy aquifer was also presented (Albrechtsen *et al.*, 1997). In other instances, the toxicity of isoproturon might prevent the catabolic activity of microorganisms in river water and groundwater. Low nutrients, i.e. a source of nitrogen or phosphate, or inappropriate pH value in river water or groundwater might cause low-growing of the isoproturon degrading microorganisms. Furthermore, regarding the treatments dosed with 0.1, 1 and 100 μg $L^{-1}$ and incubated for 30 days, the results of no enhancement of catabolic activity suggested that RW-borne and GW-borne microorganisms could not adapt to isoproturon within 30 days.

On the other hand, although at low levels of mineralisation, it is important to noted that the maximum mineralisation levels in GW treatments was significantly higher than those in RW treatments, for instance, 1.2 ± 0.1 % of

GW 0 (0) versus 0.4 ± 0.1 % of RW 0 (0) or 1.8 ± 0.9 % of GW 0 (30) versus 0.4 ± 0.1 % of RW 0 (30). Higher alkaline conditions in river water (pH 8.77) compared to the moderate alkaline conditions in groundwater (pH 7.10) could account for this difference.

Referring to previous studies, biodegradation of isoproturon in surface water and groundwater has received little attention. The persistence of isoproturon was reported in groundwater (in the absence of a solid matrix) (Johnson *et al.*, 1998; Johnson *et al.*, 2003) and in surface water (Ronnefahrt *et al.*, 1997). However, with the presence of solid matrix, GW-borne microorganisms have been shown to be able to degrade isoproturon (Johnson *et al.*, 1998). In agreement with our findings, the low degradation of isoproturon was also observed in groundwater samples from chalk, sandstone and limestone field sites (Ministry of Agriculture Fisheries and Food, 2000). Moreover, Larsen *et al.* (2000) reported that no mineralisation of isoproturon was observed in the presence of nitrate in a sandy aquifer sediment.

### 5.6.2 Adaptation Period in Riverbed Sediment (RS)

Regarding the IPU-undosed RS treatments, the adaptation periods of the treatments with 0, 5 and 10 incubation days were not significantly different ($p > 0.05$) but significantly increased ($p < 0.05$) in the treatments with 30 incubation days. This suggests that, during the first ten days, the substrates, nutrients and dissolved oxygen in the RS environment were sufficient for the development of the bacteria before they started to degrade isoproturon. However, experiencing 30 days, the preferential substrates, nutrients or dissolved oxygen may have become exhausted.

The above suggestion is also applicable to the IPU-dosed RS treatments. Indeed, no significant difference ($p < 0.05$) was observed in the treatments dosed with 0.1, 1 and 100 $\mu g\ L^{-1}$ within 10 incubation days. Nonetheless, a

significant increase in adaptation period was observed in the treatments dosed with 0.1 µg $L^{-1}$ after 30 incubation days. Regarding the treatments with 1 and 100 µg $L^{-1}$, ANOVA showed that the adaptation periods of these treatments with 30 incubation days were not significantly different from the treatments within 10 incubation days although the adaptation times of the treatments with 30 incubation days were greater than the adaptations of the treatments with 10 incubation days, e.g. 8.2 ± 0.0 days of Treatment RS 1 (10) versus 15.4 ± 0.9 days of Treatment RS 1 (30), or 9.1 ± 0.8 days of Treatment RS 100 (10) versus 13.0 ± 2.6 days of Treatment RS 100 (30). This illustrated that bacteria adapted to isoproturon within 10 incubation days. Up to 30 incubation days, the substrates and/or nutrients could be exhausted.

In other instances, the adaptation periods of the IPU-dosed treatments were shortened when the incubation periods were prolonged from 0 to 10 days. For example, the adaptation periods of the treatments RS 1 (0), RS 1 (5) and RS 1 (10) were shortened from 13.8 ± 2.9 to 11.3 ± 1.5 to 8.2 ± 0.0 days, respectively. This is explained that during the incubation time, microorganisms could be exposed and adapted to the available isoproturon. Therefore, once the second addition was spiked into the incubated treatments, the microorganisms required a shorter period for adaptation to degrade this compound.

Several previous studies for adaptation of isoproturon have been reported. However, there is limited information about the adaptation period of isoproturon in riverbed sediment environments. Thus this discussion relied on the comparison of the adaptation of isoproturon in other environments such as groundwater or different agricultural soils.

In groundwater and sterile chalk environments, no adaptation phase was observed in the degradation of isoproturon (Johnson *et al.*, 2000). Johnson *et al.* (2000) explained that perhaps soil microorganisms had penetrated to the groundwater and caused degradation without a lag phase. In the top soil treated

at 15 – 20 $^{o}$C, an adaptation period of isoproturon was reported to be approximately 4 days (Cox *et al.*, 1996). On the other hand, Bending *et al.* (2001) reported that wide variation of adaptation times: from 0 days (no adaptation time) in the soil samples enriched in isoproturon metabolising organisms, to 5 – 6 days in soil samples that had received regular application of isoproturon. A lag phase which lasted for between 8 and 18 weeks was observed in most soil samples from Kirton in Lincolnshire (England) which had not received previous isoproturon application (Bending *et al.*, 2006).

Several hypotheses have been offered to explain the observed adaptation period of an aerobic biodegradation process (Spain *et al.*, 1980; Lewis *et al.*, 1986; Wiggins *et al.*, 1987). The most likely hypotheses include the time for microbial population to: (i) grow to a size sufficient to achieve detectable biodegradation rates (Spain *et al.*, 1980; Ventullo and Larson, 1986; Wiggins *et al.*, 1987); (ii) induce new enzymes (Spain *et al.*, 1980; Stephenson *et al.*, 1984); (iii) undergo genetic changes, e.g., mutation, gene exchange, or rearrangement (Kellogg *et al.*, 1981; Schmidt *et al.*, 1983); and (iv) exhaust preferential substrates before switching to the xenobiotic substrate i.e., a diauxie pattern (Lewis *et al.*, 1986). Other explanations for a delay in biodegradation include the lack of nutrients (Lewis *et al.*, 1986), lack of dissolved oxygen, temporarily inhibitory environmental conditions (e.g., unfavourable pH or temperature or a toxin), and predation by protozoa or other microbial grazers (Wiggins *et al.*, 1987). Furthermore, concentrations and structure of the xenobiotic compound itself probably influence the acclimation period (Alexander and Aleem, 1961; Alexander, 1965; Boethling and Alexander, 1979; Spain *et al.*, 1980; Paris *et al.*, 1981; Rubin *et al.*, 1982; Boyd and Shelton, 1984; Lewis *et al.*, 1986; Wiggins *et al.*, 1987).

### 5.6.3 Maximum mineralisation Level of Isoproturon in Riverbed Sediment

High levels of the intrinsic catabolic activity with respect to isoproturon in the IPU-undosed RS treatments suggested that isoproturon was mineralised by RS-borne microorganisms. Additionally, the levels of intrinsic catabolic activity in the RS treatments were significantly higher ($p < 0.05$) than the levels in GW and RW treatments. For example, the mineralisation levels in the treatments RS 0 (0) of 14.5 $\pm$ 1.6 % were significantly higher than the levels in the treatments RW 0 (0) of 0.4 ± 0.1 % and GW 0 (0) of 1.2 ± 0.1 %. This is because the RS-borne microorganisms were competent to mineralise isoproturon while RW-borne and GW-borne microorganisms were not. This finding is consistent with the results found in Chapter 4 (Treatments 2, 3 and 4 of Experiment 1) that isoproturon was completely degraded by RS-borne microorganisms but not by RW-borne microorganisms. This result again supported the hypothesis (2) and (3) (Section 1.6) that indigenous microbial communities in riverbed sediment environment can play a key factor for mineralisation of isoproturon rather than river water-borne microorganisms.

On the other hand, within 10 incubation days, no significant difference ($p > 0.05$) of the mineralisation levels in IPU-dosed and IPU-undosed RS treatments was observed. But a significant decrease ($p < 0.05$) of the mineralisation levels was recorded in these treatments after 30 incubation days. The result indicated that RS-borne microorganisms were competent to mineralise isoproturon within 10 days. And following 30 days, the catabolic activity of these bacteria with respect to isoproturon considerably decreased ($p < 0.05$). It is suggested that, within 10 incubation days, the substrates, nutrients and dissolved oxygen were still satisfactory for the activity of the microorganisms. However, after 30 incubation days, the substrates, nutrients and dissolved oxygen could be exhausted and causing the decrease of the levels of catabolic activity.

It is important to note that the mineralisation levels in the treatments dosed with 0.1 µg $L^{-1}$ were significantly lower ($p < 0.05$) than the levels in IPU-undosed RS treatments. For example, the maximum mineralisation level in the treatments RS 0 (5) of 11.5 ± 1.7 % was significantly different from the level in RS 0.1 (5) of 7.7 ± 1.2 %. The toxicity with a low amount of isoproturon might result in the decrease in mineralisation. Notwithstanding this, an enhancement of mineralisation was observed in the RS treatments dosed with a high amount of isoproturon of 1 and 100 µg $L^{-1}$. Indeed, the maximum mineralisation levels increased according to the increase of isoproturon dosing. For instance, with 5 incubation days, the mineralisation levels of Treatments RS 0.1 (5), RS 1 (5) and RS 100 (5) significantly increased ($p < 0.05$) from 7.7 ± 1.2 % to 18.3 ± 5.4 % and 36.9 ± 2.7 %, respectively. This result indicated that previous exposure to isoproturon or the first addition enhanced the mineralisation of this compound. An increased catabolic activity with respect to isoproturon was reported in three different arable soils which were augmented with isoproturon (Reid *et al.*, 2005). An extremely rapid degradation of isoproturon (complete degradation within 2 days) in soils which were enriched in isoproturon metabolising organisms by two sequential applications of isoproturon was reported by (Bending *et al.*, 2001). El-Sebai *et al*. (2005) reported that repeated application of isoproturon on the field of Le Souich (France) contributed to the adaptation of soil microflora which became able to rapidly biodegrade this herbicide.

No previous studies for mineralisation of isoproturon in riverbed sediment have been published in the primary literature. Thus a reference to the studies for mineralisation of isoproturon in other environments such as agricultural soils was considered. Typically, in laboratory microcosm experiments with agricultural soils, 5 – 25 % of added $^{14}$C-IPU was mineralised to $^{14}CO_2$ within 2 – 3 months at about 20$^{o}$C (Kubiak *et al.*, 1995; Lehr *et al.*, 1996; Pieuchot *et al.*, 1996; Larsen *et al.*, 2000; Scheunert and Reuter, 2000; Reid *et al.*, 2005). Reid *et al*. (2005) reported that the intrinsic catabolic activity levels of

isoproturon were determined to be 11.5 ± 3.6% in the organic agricultural soil (pesticide free) while in the conventional agricultural soil (treated with isoproturon annually over the previous 6 years and within 5 months prior to sample collection) in Beccles (England) to be 31.4 ± 1.8%. However, recent studies have shown a rapid and extensive mineralisation of isoproturon in some previously field-treated soils, with 40 – 50% of mineralisation levels being achieved within 1 month at 15 – 20$^{o}$C (Bending *et al.*, 2001; Sorensen *et al.*, 2001; Sorensen and Aamand, 2003) suggesting an in situ microbial adaptation to isoproturon metabolism following repeated application at the same field (Sorensen *et al.*, 2003). Bending *et al.* (2001) determined mineralisation levels of isoproturon varying from approximately 15 % to 45 % after 65 assay days in the soil samples which had received regular application of isoproturon. In an aquifer sediment environment, 14 % $^{14}CO_2$ evolution from $^{14}$C-IPU was observed over 267 assay days at 10 $^{o}$C (Larsen *et al.*, 2000). However, no mineralisation of isoproturon was detected in different aquifer sediments under denitrifying, sulphate-reducing or methanogenic conditions following incubation for 312 days at 10 $^{o}$C (Larsen and Aamand, 2001).

Degradation of other phenylurea herbicides, e.g. diuron, linuron and fluometuron, has been reported to be very slowly mineralised in agricultural soils (Bozarth and Funderbu.Hh, 1971; Berger, 1999; Zablotowicz *et al.*, 2000). The mineralisation level of $^{14}$C-phenyl-labelled fluometuron was reported to be less than 3% in agricultural soil over 25 days of incubation at 28$^{o}$C (Zablotowicz *et al.*, 2000). Berger (1999) compared the mineralisation of different $^{14}$C-phenyl-labelled phenylurea herbicides, including linuron, metobromuron, chlorotoluron and isoproturon, in three arable soils but found no production of $^{14}CO_2$ within 56 days at 20$^{o}$C or 30$^{o}$C.

### 5.6.4 Maximum mineralisation Rates of Isoproturon in Riverbed Sediment

Regarding the IPU-undosed RS treatments, no significant difference ($p > 0.05$) of mineralisation rates was observed in the treatments with 0, 5 and 10 incubation days, however the mineralisation rate could not be determined in the treatments with 30 incubation days because of low catabolic activity in these treatments (< 10%). This suggested that mineralisation rates in the IPU-undosed RS treatments were not enhanced during 10 days and significantly decreased ($p < 0.05$) after 30 days. In a similar way to mineralisation level, the mineralisation rate decreased after 30 incubation days could be account for by the exhaustion of the preferential substrates, nutrients or dissolved oxygen in that environment. On the other hand, the isoproturon mineralisation rates found in the IPU-undosed RS treatments, varying from 0.38 ± 0.24 to 0.67 ± 0.10 % $^{14}CO_2$ $day^{-1}$, are in agreement with the previous reported mineralisation rates found in agricultural soils; for instance Reid *et al.* (2005) reported that the mineralisation rates varied from 0.26 to 0.48 % $^{14}CO_2$ $day^{-1}$ in the arable cultivation soils (Oxfordshire, UK) and were 0.74 % $^{14}CO_2$ $day^{-1}$ in the intrinsic organic soil (pesticide free).

It was observed that the maximum mineralisation rate of RS 100 (5) of 4.74 % $^{14}CO_2$ $day^{-1}$ was significantly greater ($p < 0.05$) to that of RS 100 (0) of 2.86 % $^{14}CO_2$ $day^{-1}$. Thus Treatment RS 100 (0) was considered to have a single addition event wherein both $^{12}C$ and $^{14}C$-IPU were added at the same time; while Treatment RS 100 (5) was considered to have 2 IPU- additions (first addition of $^{12}C$-IPU and second addition of $^{14}C$-IPU after 5 days). This indicated that the rate of mineralisation in the RS 100 treatments was significantly enhanced. It is explained that, after the first addition, the degrading organisms was exposed and adapted to isoproturon.

No significant difference of the rate between RS 100 (5) and RS 100 (10) suggested that the isoproturon degrading community retained its active state after 10 incubation days. This finding was supported by the maximum mineralisation levels of these treatments, for example, the highest of the maximum mineralisation level was recorded in the treatment RS 100 (5) with 36.9 ± 2.7 % compared to 33.8 ± 3.5 % in the treatment RS 100 (10). Nevertheless, the number of the isoproturon degrading communities may decrease after 30 incubation days due to the exhaustion of the substrate, nutrients or dissolved oxygen. Indeed, the maximum mineralisation rate and maximum mineralisation level of Treatment RS 100 (30) significantly decreased ($p < 0.05$) to the value of 2.40 ± 0.4 % $^{14}CO_2$ $day^{-1}$ and 18.1 ± 4.6 % after 30 incubation days, respectively.

Regarding the IPU-dosed RS treatments, mineralisation rates of isoproturon increased upon increasing the addition of $^{12}$C-IPU. For instance, upon increasing the addition of $^{12}$C-IPU from 0.1 to 100 µg $L^{-1}$ for the treatments with 10 incubation days, RS 0.1 (10), RS 1 (10) and RS 100 (10), mineralisation rates of these treatments significantly increased ($p < 0.05$) from 0.51 to 1.18 ± 0.11 and to 3.27 ± 0.42 (% $^{14}CO_2$ $day^{-1}$), respectively. It is suggested that mineralisation rates of isoproturon in RS microcosm was significantly enhanced by adding $^{12}$C-IPU into the supernatant treatments (up to 100 µg $L^{-1}$).

In agreement with the enhancement of mineralisation rate by adding isoproturon, Reid *et al.* (2005) reported that the mineralisation rates of the conventional arable cultivation soils dosed with isoproturon (0.05 µg IPU $kg^{-1}$ dry weight of soil) varied from 0.79 to 5.04 % $^{14}CO_2$ $day^{-1}$. Furthermore, Bending *et al.* (2006) reported that isoproturon was degraded faster in Wellesbourne soil (in UK, this field was applied with isoproturon twice in 1999 and 2001 before sampling in 2002) with DT25 (time to 25% dissipation) of 0.56 weeks than from Kirton soil (in UK, this field was not applied with

isoproturon) with DT25 of 4.4 weeks. El-Sebai *et al.* (2005) reported that most of soil samples treated twice with isoproturon showed a maximum rate of mineralisation after 1.5 days while the same maximum rate for samples treated once with isoproturon to be 2.5 days. Many previous studies have shown that accelerated degradation of isoproturon in soils can be induced by repeated application of the herbicide (Cox *et al.*, 1996; Cullington and Walker, 1999; Bending *et al.*, 2001). Nonetheless, several studies for soils and subsurfaces reported that relatively slow isoproturon biodegradation rates, or without any cleavage of the phenyl-ring structure, were observed (Pieuchot *et al.*, 1996; Johnson *et al.*, 1998; Berger, 1999; Larsen *et al.*, 2000; Kristensen *et al.*, 2001; Sorensen and Aamand, 2001). A later study failed to detect any mineralisation of isoproturon in different aquifer sediments under denitrifying, sulphate-reducing or methanogenic conditions following for 312 days at 10$^{o}$C (Larsen and Aamand, 2001). It is crucial to bear in mind that beside the field characteristics, technical errors associated with sampling and analysis and model lack of fit can make a significant contribution to measured within-field variability in pesticide degradation (Bending *et al.*, 2006).

After 30 incubation days, in the same way to mineralisation level, the mineralisation rates in the IPU-dosed and IPU-undosed RS treatments significantly decreased. Indeed, mineralisation rates in Treatments RS 0 (30), RS 0.1 (30) and RS 1 (30) could not be detected due to low catabolic activity (less than 10%). The mineralisation rates of the treatments dosed 100 µg $L^{-1}$ decreased from 3.27 ± 0.42 of RS 100 (10) to 2.40 ± 0.4 % $^{14}CO_2$ $day^{-1}$of RS 100 (30). This could result from the decrease of the population size of isoproturon degrading organisms due to lack of substrates, nutrients and oxygen after 30 incubation days.

### 5.6.5 Relationship between Catabolic activity of Isoproturon and $^{12}$C-IPU Residual Concentration in Riverbed Sediment

Results presented in the preceding section highlighted that catabolic activity with respect to isoproturon was enhanced by the addition of $^{12}$C-IPU. Where catabolic competence was presented and/or promoted it was anticipated that IPU degradation would be taking place. Thus, as incubation periods protracted the opportunity for IPU degradation should become greater. In order to explore relationships between levels of catabolic competence and substrate (IPU) levels residual IPU concentration in the incubation were established immediately prior to the addition of the $^{14}$C-IPU. These concentrations, rather than the original spiking concentrations, were subsequently used to this anticipated mutual relationship.

#### *5.6.5.1 Relationship between maximum mineralisation level and $^{12}$C-IPU residual concentration*

The relationship between maximum mineralisation levels in the IPU-dosed RS treatments and the residual $^{12}$C-IPU concentrations immediately prior to $^{14}$C-IPU addition (determined in Experiment 3) was explored by cross-plotting and regression. A linear relationship between maximum extent of $^{14}$C-IPU mineralisation and the log $^{12}$C-IPU concentration immediately prior to $^{14}$C-IPU addition was established. This relationship has a gradient of 2.72 and an $r^2$ value of 0.77.

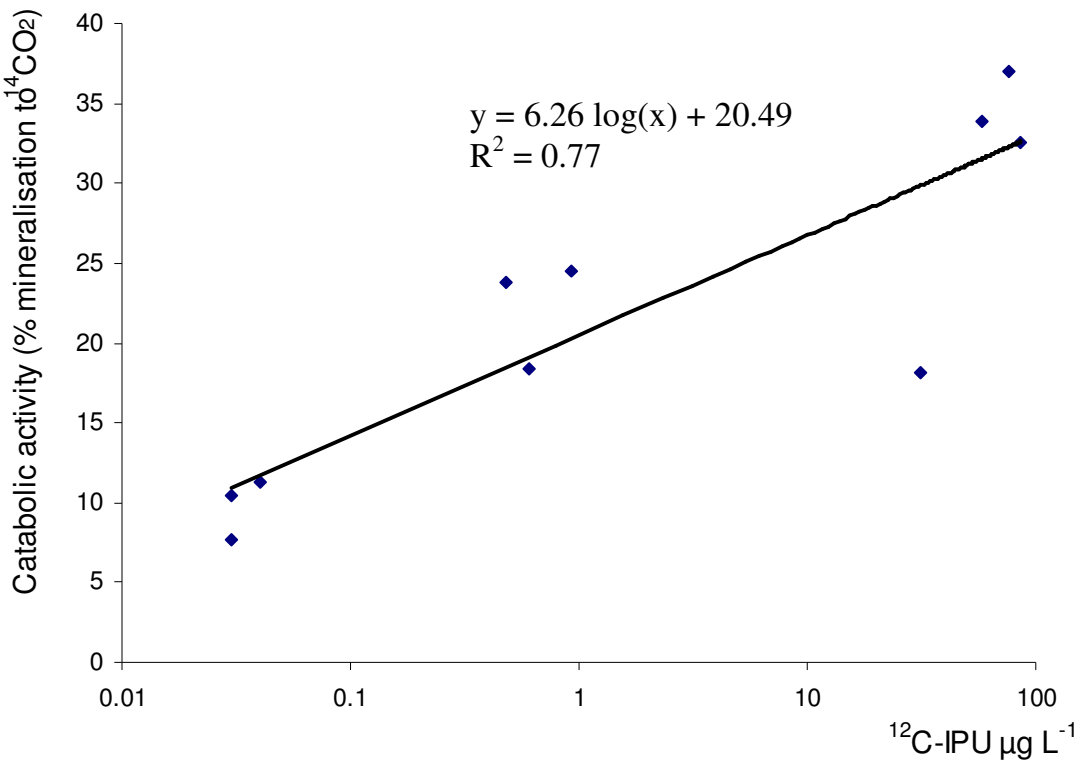



**Figure 5. 4** Catabolic activity as a function of solution phase isoproturon concentration

This evidence of the catabolic enhancement with respect to isoproturon was linked directly to the $^{12}$C-IPU residual concentrations across all the treatments during 30 incubation days. The result suggested that isoproturon can be easily mineralised with high concentration of the substrate isoproturon (approximately 1 to 100 µg $L^{-1}$); in contrast, isoproturon was not easily mineralised at low concentration (less than 1 µg $L^{-1}$).

#### *5.6.5.2 Relationship between maximum mineralisation rate and $^{12}$C-IPU residual concentration*

A relationship between mineralisation kinetics and isoproturon residual concentration was found by plotting the maximum mineralisation rates against the logarithm(10) of residual $^{12}$C-IPU (determined in Experiment 3). Figure 5.5 presents the relationship between the mineralisation rate of isoproturon and log $^{12}$C-IPU residual concentration at the point of the second $^{14}$C-IPU addition.

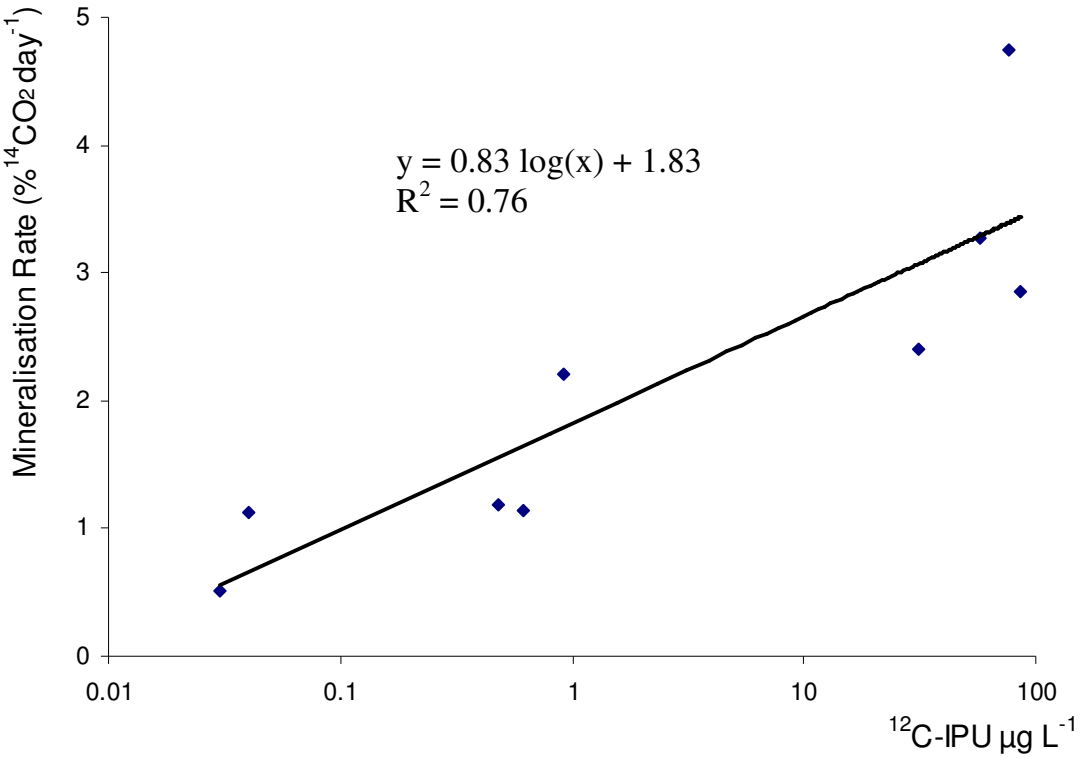



**Figure 5. 5** Maximum mineralisation rate as a function of solution phase isoproturon concentration

In agreement with the maximum mineralisation level, the evidence illustrated in Figure 5.5 suggested that the mineralisation kinetics of isoproturon was enhanced in a solution with high concentration of the substrate isoproturon (approximately 1 to 100 µg $L^{-1}$) and, conversely, the extent of mineralisation was very low where solution IPU concentrations were low (less than 1 µg $L^{-1}$). It is suggested that IPU will persist at low concentrations because IPU catabolic competence may not develop. This is of significance because environmental concentrations of IPU are more typically at the lower end of the concentration scale used in these experiments.

## 5.7 Conclusions

Catabolic insights into isoproturon degradation in river water, ground water and riverbed sediment environments have been studied. Several experiments have elucidated relationships between levels of IPU catabolic activity, IPU concentration (0, 0.1, 1 and 100 μg $L^{-1}$) and incubation times (0, 5, 10 and 30 days). Based on these experiments several conclusions have been drawn:

(1) RW-borne microorganisms and GW-borne microorganisms were not competent to degrade isoproturon;

(2) RS-borne microorganisms were competent to degrade isoproturon;

(3) Catabolic activity with respect to isoproturon was enhanced in the RS microcosm augmented with concentrations of isoproturon varying from 1 to 100 μg $L^{-1}$. However, in the RS environment augmented with lower concentrations of isoproturon (0.1 μg $L^{-1}$), no enhancement in catabolic activity was observed;

(4) Catabolic activity with respect to isoproturon was significantly decreased in the RS treatments incubated after 30 days;

(5) Levels of catabolic activity in RS treatments was established to be proportional to concentrations of isoproturon present; with higher isoproturon concentrations promoting higher levels of catabolic activity;

(6) Isoproturon could be mineralised at low initial concentration of isoproturon (varying from 0.1 to 100 μg $L^{-1}$) in the RS treatments.

Collectively, this chapter provides compelling evidence that microorganisms in riverbed sediment were an important factor responsible for the mineralisation of isoproturon in such an environment. Isoproturon were mineralised at an initially low concentration (varying from 0.1 to 100 μg $L^{-1}$). The catabolic competence with regard to isoproturon was also enhanced in response to herbicide addition.

# Chapter 6

# POTENTIAL for RIVERBANK FILTRATION: LABORATORY RESULTS in a WIDER CONTEXT

## 6.1 New Results

In keeping with the structure of this thesis, the new results presented in Chapters 4 and 5 are summarised in this chapter. The first section reviews the sorption and biodegradation processes of the two herbicides mecoprop and isoproturon in a river water-riverbed sediment system. The second section reviews the catabolic insights into isoproturon degradation in river water, groundwater and riverbed sediment environments. Relied upon these results and the site characteristics at the Gatehampton (described in Chapter 2), a simplified model is offered to simulate the attenuation of mecoprop and isoproturon in a context of riverbank filtration.

### 6.1.1 Sorption and Biodegradation of Mecoprop and Isoproturon in a River Water-Riverbed Sediment System

Sorption and biodegradation of mecoprop and isoproturon in a river water-riverbed sediment system for the first time are reported. Below are the results summarised from Chapter 4.

Sorption of mecoprop and isoproturon on/into riverbed sediment was assessed using a fixed-bed column circulation method. Relied upon the initial concentrations and the pseudo-equilibrium concentrations of these herbicides, their sorption characteristics in a river water-riverbed sediment system were identified as below.

(1) Regarding sorption of mecoprop, during the first 24 hours of sorption time, approximately 19.5 ± 2.0 % of mecoprop were sorbed on/into the riverbed sediment. Several other sorption parameters of mecoprop were also calculated, for example, the maximum sorption capacities to be 279 ± 32 μg $kg^{-1}$ or 3.91 ± 0.45 μg $m^{-2}$, the solid-water distribution coefficients to be 3.47 ± 0.43 L $kg^{-1}$ or 0.049 ± 0.006 L $m^{-2}$, the organic carbon-normalised distribution coefficient to be 434 ± 54 and the retardation factor to be 9.57 ± 1.07. The sorption rate constant of mecoprop was also identified to be 0.0106 ± 0.0015 $h^{-1}$.

(2) Regarding sorption of isoproturon, during the first 12 hours of sorption time, approximately 17.6 ± 0.6 % of isoproturon were sorbed on/into the riverbed sediment. Several other sorption parameters of isoproturon were also calculated, for example, the maximum sorption capacities to be 240 ± 9 μg $kg^{-1}$ or 3.36 ± 0.13 μg $m^{-2}$, the solid-water distribution coefficients to be 3.06 ± 0.12 L $kg^{-1}$ or 0.043 ± 0.002 L $m^{-2}$, the organic carbon-normalised distribution coefficient to be 382 ± 15 and the retardation

factor to be 8.55 ± 0.30. The sorption rate constant of isoproturon was identified to be 0.0191 ± 0.009 $h^{-1}$.

Following the sorption phase, concentrations of mecoprop and isoproturon were not significantly decreased ($p > 0.05$) during the consecutive times of 4 and 5.5 days, respectively. These periods of time were considered as a adaptation or lag phase before acceleration phase.

The acceleration or biodegradation phase was observed right after the adaptation phase. Relied upon the concentrations after the adaptation phase, a zero-order degradation model was applied to simulate the kinetics of the herbicides during this phase. The biodegradation rates and half-lives of mecoprop and isoproturon were determined and summarised below:

(1) Regarding the biodegradation of mecoprop, during a period of 9 days, biodegradation rate of mecoprop in the river water-riverbed sediment system was determined to be 9.91 ± 0.19 μg $L^{-1}$ $day^{-1}$. The half-life of biodegradation phase of mecoprop was identified to be 4.1 ± 0.1 days.

(2) Regarding the biodegradation of isoproturon, during a period of 6 days, in a similar way with mecoprop, biodegradation rate of isoproturon in a river water-riverbed sediment system was determined to be 13.84 ± 0.39 μg $L^{-1}$ $day^{-1}$ and the half-life was also identified to be 2.5 ± 0.1 days.

After circulating for 18 days, the riverbed sediments from the fix-bed column experiments were extracted to investigate the catabolic activity of the microorganisms in these environments with respect to isoproturon. Very low maximum level (1.6 ± 0.2 % $^{14}CO_2$) of catabolic activity with respect to

isoproturon was observed in the riverbed sediment treated with recirculation of the non-sterile river water and the sterile riverbed sediment. Conversely, high maximum level (29.4 ± 1.5 % $^{14}CO_2$) of catabolic activity with respect to isoproturon was recorded in the riverbed sediment treated with recirculation of the sterile river water and the non-sterile riverbed sediment. No acclimation or adaptation phase was observed in this case. The maximum mineralisation rate in this experiment was also calculated to be 29.4 % $^{14}CO_2$ $day^{-1}$ ($R^2$ = 1.00).

### 6.1.2 Catabolic Insights into Isoproturon Degradation in River Water, Groundwater and Riverbed Sediment

Developing the outcomes regarding biodegradation of the herbicide isoproturon in river water-riverbed sediment interaction (Chapter 4), catabolic insights into isoproturon biodegradation were investigated in the different microcosms including river water, groundwater and riverbed sediment. These experiments were carried out using the respirometry method. New results from Chapter 5 are summarised as below.

#### *6.1.2.1 Catabolic activity in river water microcosm*

Regarding the intrinsic catabolic activity in river water microcosm, very low maximum mineralisation level was observed. It was identified to be 0.4 ± 0.1% in the IPU-undosed RW treatments without incubation. In the IPU-undosed RW treatments with 30 incubation days, the maximum mineralisation level did not vary, to be 0.4 ± 0.1%. On the other hand, regarding the RW treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ IPU and incubated with 30 days, the maximum mineralisation levels were not significantly different ($p > 0.05$) from the intrinsic maximum mineralisation levels. It is suggested that no catabolism and enhancement was observed in the river water microcosm.

#### 6.1.2.2 Catabolic activity in groundwater microcosm

Regarding the intrinsic catabolic activity in groundwater microcosm, very low maximum mineralisation level was also obtained. It was identified to be 1.2 ± 0.1% in the IPU-undosed GW treatments without incubation. In the IPU-undosed GW treatments with 30 incubation days, the maximum mineralisation level did not significantly increase ($p > 0.05$), to be 1.8 ± 0.9%. On the other hand, regarding the GW treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ IPU and incubated with 30 days, the maximum mineralisation levels were not significantly different ($p > 0.05$) from the intrinsic maximum mineralisation levels. It is also suggested that no catabolism and enhancement was observed in the groundwater microcosm.

#### 6.1.2.3 Catabolic activity in riverbed sediment microcosm

In the riverbed sediment microcosm, high levels of mineralisation with respect to isoproturon were observed in both IPU-undosed and IPU-dosed treatments. Before accelerating the catabolic activity, a period of time with low mineralisation level (less than 5 %) was observed in all of the treatments with un-dosed and dosed IPU (0.1, 1 and 100 µg $L^{-1}$), without and with incubation time (5, 10 and 30 days). This period of time was considered as the adaptation or lag or, sometimes, acclimation time for the adaptation and growth of the isoproturon degrading organisms.

Regarding the IPU-undosed RS treatments, the following parameters were observed:

(1) The adaptation time: in the treatments with 0, 5 and 10 incubation days, the adaptation times were not significantly different ($p > 0.05$), varying from 8.8 ± 0.4 to 10.6 ± 0.4 days. However, the adaptation time, of 16.6 ± 0.4 days, in the treatments with 30 incubation days was significantly

longer ($p < 0.05$) than the times of the above three IPU-undosed treatments;

(2) The intrinsic maximum mineralisation level: in the same trend with the lag time, the intrinsic maximum mineralisation levels in the treatments with 0, 5 and 10 incubation days were not significantly different ($p > 0.05$), varying from 11.5 $\pm$ 1.7 % to 14.5 $\pm$ 1.6 %, but markedly decreased ($p < 0.05$) after 30 incubation days with the value of 7.6 $\pm$ 0.6 %. In addition, comparison of the intrinsic maximum mineralisation levels between the treatments RS and RW or between RS and GW indicated that the maximum mineralisation levels in the RS treatments are significantly higher ($p < 0.05$) than in RW and GW treatments;

(3) The intrinsic maximum mineralisation rate: there was no significant difference ($p > 0.05$) of the maximum rates in the treatments with 0, 5 and 10 incubation days, varying from 0.38 ± 0.24 to 0.67 ± 0.10 % $^{14}CO_2$ $day^{-1}$. It is noted that no maximum mineralisation rate of the treatment RS 0 (30) could be determined because the maximum mineralisation level of this treatment was less than 10 %.

Regarding the IPU-dosed RS treatments, the following results were observed:

(1) The adaptation time: regarding the RS treatments dosed with 0.1 µg $L^{-1}$ of IPU, the adaptation times were not significant difference in the treatments with 0 and 5 incubation days, of 19.6 ± 2.2 and 16.8 ± 1.2 days, respectively, but significantly decreased ($p < 0.05$) in the treatments with 10 incubation days, of 12.6 ± 0.0 days. However, after 30 incubation days, no adaptation time was detected because the maximum mineralisation levels in this treatment were very low (less than 5%). Regarding the RS treatments dosed with 1 µg $L^{-1}$ of IPU, the adaptation times were decreased from 13.8 ± 2.9 to 11.3 ± 1.5 to 8.2 ± 0.0 days

according to the increases of incubation times from 0 to 5 to 10 days, respectively. Thereafter, the adaptation time increased to 15.4 ± 0.9 days after 30 incubation days. Regarding the RS treatments dosed with 100 µg $L^{-1}$ of IPU, the adaptation times were also decreased from 10.7 ± 0.9 to 9.4 ± 0.1 and to 9.1 ± 0.8 days according to the increase of incubation times from 0 to 5 to 10 days, respectively, and increased to 13.0 ± 2.6 days after 30 incubation days. However, no significant difference ($p > 0.05$) was recorded among these treatments.

(2) The maximum mineralisation level: there was no significant difference among the treatments with 0, 5, and 10 incubation days, for instance, the maximum mineralisation levels varied in the RS treatments dosed with 0.1 µg $L^{-1}$ from 7.7 ± 1.2 to 11.3 ± 4.1 %, or in the RS treatments dosed with 1 µg $L^{-1}$ from 18.3 ± 5.4 and 24.5 ± 5.7 %, or in the RS treatment dosed with 100 µg $L^{-1}$ from 32.6 ± 2.6 to 36.9 ± 2.7 %. But significant difference was observed in the treatments with 30 incubation days, for example the maximum mineralisation levels were determined in the treatments dosed with 0.1, 1 and 100 µg $L^{-1}$ to be 2.0 ± 0.7 %, 8.5 ± 2.2 % and 18.1 ± 4.6 %, respectively.

(3) The maximum mineralisation rate: regarding the RS treatments dosed with 0.1 µg $L^{-1}$, no significant difference ($p > 0.05$) of the maximum mineralisation rates was observed in the treatments with 0 and 10 incubation days, to be 1.13 ± 0.42 and 0.51 % $^{14}CO_2$ $day^{-1}$, respectively. Regarding the RS treatments dosed with 1 µg $L^{-1}$, the maximum mineralisation rate of the treatment with 0 incubation days, of was significantly higher ($p < 0.05$) than the rates of treatments with 5 and 10 incubation days, of 1.14 ± 0.24 and 1.18 ± 0.11 % $^{14}CO_2$ $day^{-1}$, respectively. Regarding the RS treatments dosed with 100 µg $L^{-1}$, the maximum mineralisation rate of the treatment with 5 incubation days, of 4.74 ± 0.29 % $^{14}CO_2$ $day^{-1}$, was significant higher ($p < 0.05$) than the rates

of the treatments with 0 and 30 incubation days, of 2.86 ± 0.27 and 2.40 ± 0.4 % $^{14}CO_2$ $day^{-1}$, respectively. However, no significant difference ($p > 0.05$) between the treatment with 5 and 10 incubation days, 3.27 ± 0.42 % $^{14}CO_2$ $day^{-1}$.

#### *6.1.2.4 Relationship between the catabolic activity of isoproturon and the $^{12}$C-IPU residual concentrations in riverbed sediment environment*

A logarithmic relationship between the levels of catabolic activity and the $^{12}$C-IPU concentration at the point of the second $^{14}$C-IPU addition was obtained to be the logarithmic fit line with the gradient of 6.26 and the association factor of 77%. In addition, a logarithmic relationship between the mineralisation rate of isoproturon and the $^{12}$C-IPU residual concentrations at the point of the second $^{14}$C-IPU addition was also determined to be the logarithmic fit line with the gradient of 0.83 and the association factor of 76%.

## 6.2 A Wider Context for River Bank Filtration – Attenuation of Herbicides over a River Water-Riverbed Sediment Interaction Path Length

Relied upon the above new results (Section 6.1), it is clear that the herbicides mecoprop and isoproturon can be completely degraded in a river water-riverbed sediment interaction system. Hence, if a production borehole could be constructed alongside the bank of a river, a question emerged is how far the borehole should be located from the river in order to make sure the borehole will be protected from the pesticide pollution, particularly with the case of the concentrations of the pesticides mecoprop and isoproturon up to 100 µg $L^{-1}$. This section tries to offer a simple model to estimate an essential path length to remove the pesticide pollution from river water. An application for a wider context at the Gatehampton site is also considered.

### 6.2.1 One-dimensional Flow Case

Relied upon the results from the fixed-bed column circulation experiments (Experiment 1, Section 4.4.1), this section offers a simplified one-dimensional flow model. The model can be used to simulate the attenuation of an herbicide by riverbank filtration and estimate the essential path length for the herbicide which is totally filtrated throughout a riverbank.

Findings in Chapter 4 illustrated that the herbicides mecoprop and isoproturon required at least 14 circulation days to be totally decomposed. As described above, after the first 6 days of sorption and adaptation, mecoprop and isoproturon required at least for 9 days to be microbially degraded. It was shown that biodegradation played a primary role in the degradation of these herbicides. Therefore, a period of 9 days was chosen here to calculate the essential path length of riverbed to ensure both mecoprop and isoproturon could be totally removed from the river water. The calculation was undertaken as below.

Riverbed sediment (150 g with bulk density of 1.31 g $cm^{-3}$ and porosity of 50.6 %, see Table 4.3) was packed in the glass column with a diameter of 4 cm. A volume of 1.5 L of river water contaminated by 100 μg $L^{-1}$ mecoprop was then circulated. The system is depicted in Figure 6.1.

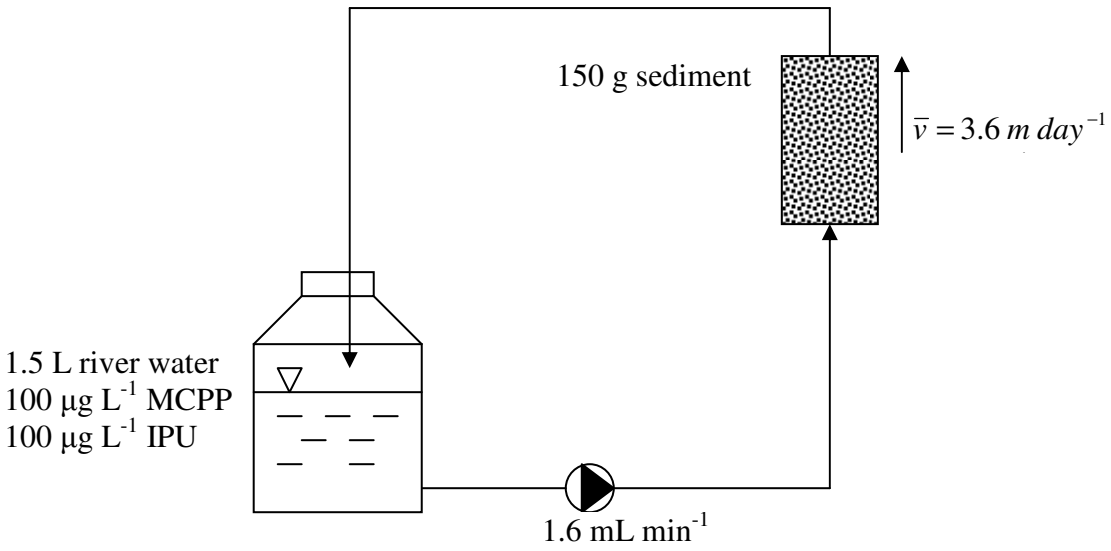



**Figure 6. 1** Diagram of the fixed-bed column circulation experiment

The volume of riverbed sediment in the column, $v_{sed\ col}$, was:

$$v_{sed,col} = \frac{150\ g}{1.31\ g\ cm^{-3}} = 115\ cm^3 = 1.15 * 10^{-4}\ m^3 \qquad (5.1)$$

The height or path length of the sediment layer in the column (diameter of 4 cm), $l_{path,col}$, was:

$$l_{path,col} = \frac{115}{\pi * 2^2} = 9.2\ cm \qquad (5.2)$$

In the circulation experiment, mecoprop in 1.5 L of river water requires 9 circulation days to be completely removed. Thus, a volume of 1.5 L of river water was circulated during 9 days. *In situ*, contaminated river water flowing to a borehole cannot be circulated (one-dimensional flow only). In order to apply the results of the fixed-bed circulation column experiments, a one-dimensional model was formed to simulate this process. Figure 6.2 shows the one-dimensional model to treat the herbicide pollution from the river water.

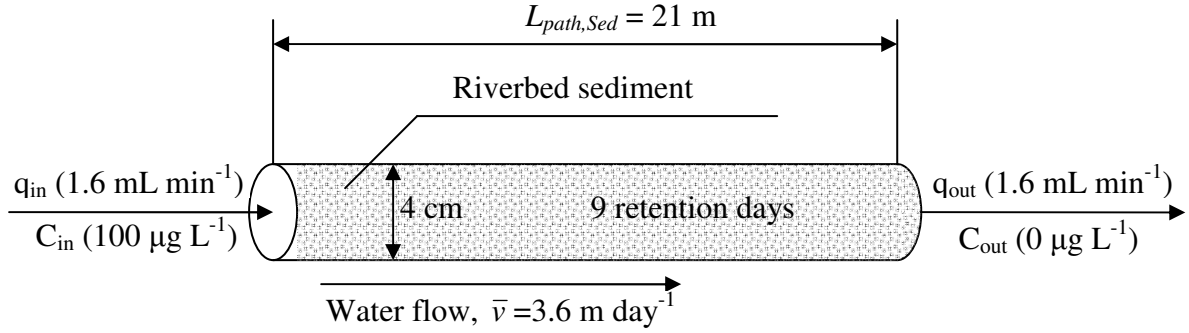



**Figure 6. 2** One-dimensional model for treatment of herbicide pollution in a river water-riverbed sediment system.

A total volume of river water, $V_{water}$, passed through the column (non-circulated) over 9 days (with the total flow $q_{col}$ = 1.6 mL min$^{-1}$) was calculated as follows:

$$V_{water} = 1.6mL/\min * 60\min * 24h * 9day = 20736\,cm^3 = 20.736\,L \qquad (5.3)$$

It was assumed that only the water could flow through the pore space of the riverbed sediment. Thus, the void volume (or pore space) of the riverbed sediment, $V_{void,\,Sed}$, required to treat 1.5 L of contaminated river water was equal to the total volume of the river water, $V_{water}$:

$$V_{void,\,Sed} = V_{water} = 20.736\,L \qquad (5.4)$$

Hence, the void volume of the riverbed sediment required to treat 1 L of the river water, $V_{void/L,\,Sed}$, was:

$$V_{void\,/\,L,\,Sed} = \frac{20.736\,L}{1.5L} = 13.824\,L \qquad (5.5)$$

The volume of the riverbed sediment (with porosity of 50.6 %) required to treat 1 L of the river water, $V_{sed/L}$, was:

$$V_{sed/L} = \frac{13.824\ L/L}{0.506} = 27.320 \approx 0.027\ m^3 \quad \text{riverbed sediment} \qquad (5.6)$$

The above calculation presents that, in order to treat 1 L of herbicide-contaminated river water (from 100 μg $L^{-1}$ depletion to zero), it is required a volume of 0.027 $m^3$ of riverbed sediment (porosity of 50.6 %) with the conditions such as water velocity of 3.6 m $day^{-1}$, retention time of 9 days, cylinder profile flume with diameter of 4 cm.

The essential path length, $L_{path,Sed}$, of required riverbed sediment was calculated as follows:

$$L_{path,Sed} = \frac{0.027}{\pi * 0.02^2} = 21 \quad m \qquad (5.7)$$

### 6.2.2 General Field Case

In *situ*, the total flow to a borehole was very much higher than the $q_{col}$ value of 1.6 mL $min^{-1}$. Considering the example of a borehole at the Gatehampton site, exploiting a total flow of approximately 16 x $10^6$ L $day^{-1}$ of which 25 % was assumed to be fed by the river (Jackson *et al.,* 2006a), then, the total flow from the river to the borehole, $Q_{RW, BH6}$, was:

$$Q_{RW,BH6} = 16*10^6 * 0.25 = 4*10^6 \ L\ day^{-1} \quad (5.8)$$

If the velocity of groundwater flow towards a borehole is similar to the velocity of the flow circulated in the fixed-bed column experiment of 1.6 mL $min^{-1}$ or 3.6 m $day^{-1}$, then, according to Equation (5.6), the volume of riverbed sediment required to treat the above total flow (4 x $10^6$ L $day^{-1}$) during 9 biodegradation days, $V_{sed,bio}$, was:

$V_{sed,bio}$ = 4*$10^6$ (L/day) * 9 day * 0.027 ($m^3$/L) = 972*$10^3$ $m^3$ sediment (5.9)

Generally, a total flow of 4 x $10^6$ L $day^{-1}$ of river water requires a volume of 972 x $10^3$ $m^3$ of riverbed sediment in order to completely remove mecoprop and isoproturon from 100 μg $L^{-1}$ to zero over 9 days. The problem now is how far from a river should a borehole be located to be protected from herbicide pollution.

In *situ*, the flow path from a river to a borehole does not have a cylinder-profile with a diameter of 4 cm as in the above one-dimensional model. It is assumed that the groundwater flow from a river to a borehole was described in Figure 6.3, then the path length was calculated as follows:

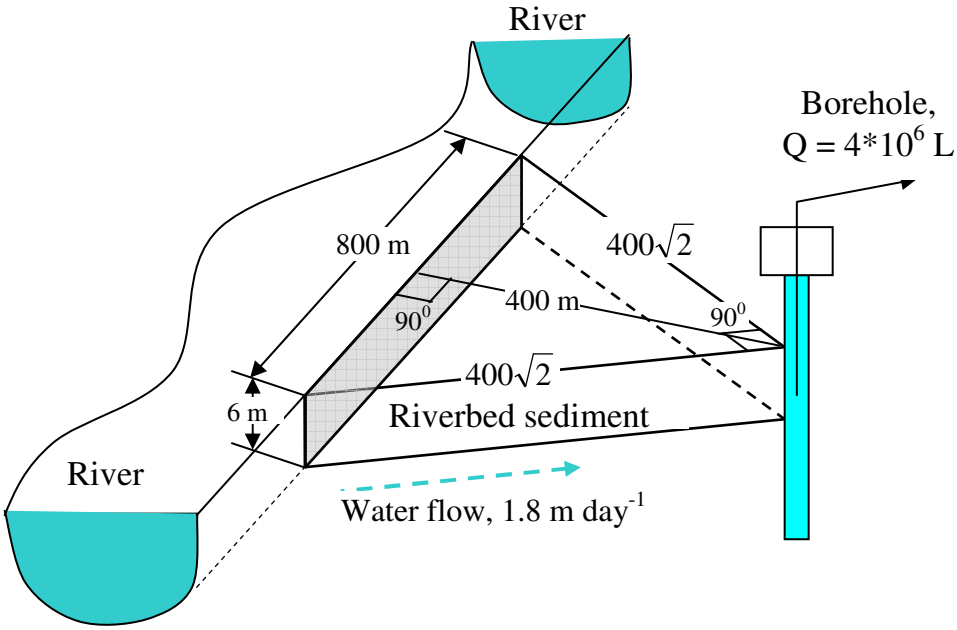



**Figure 6. 3** Simplified model of the path length from a river to a bank-side borehole.

The borehole water capture area was assumed to be an equal square triangle (Figure 6.3) with a side of $400\sqrt{2}$ m. The path length, $L_{sed,\ in\ situ}$, from the river to the borehole was:

$$L_{sed,in\ situ} = \frac{400\sqrt{2}}{\sqrt{2}} = 400m \qquad (5.10)$$

The thickness of the sediment layer, $d_{sed,\ in\ situ}$, was calculated as follow:

$$d_{sed,in\ situ} = \frac{972 * 10^3}{1/2 * 800 * 400} = 6.075\ m \approx 6\ m \qquad (5.11)$$

Assuming that the greater benefit is obtained in terms of river water capture, the closer the distance between a borehole and a river, then the above calculation suggested that a borehole with 4 x $10^6$ L $day^{-1}$ of river-fed water

should be protected from pollution by the herbicides mecoprop and isoproturon up to a concentration of 100 μg $L^{-1}$, if a borehole is located at a position with a minimum distance of 400 m from the river and a minimum thickness of riverbed sediment layer of 6 m.

It is noted that the biodegradation of mecoprop and isoproturon occurs after a period of 6 days of sorption and adaptation (Section 4.4.1). During the sorption and adaptation phases, the herbicides were not degraded. Therefore, the herbicides may appear in groundwater or in boreholes and contaminate groundwater source. Fortunately, when these herbicides pass through the riverbed sediment, they were sorbed on/into the riverbed sediment. The problem was whether or not the herbicides mecoprop and isoproturon could be transferred from the river through the above cubic triangle of riverbed sediment to the borehole.

To solve this problem, the maximum sorption capacities, $C_{S,max}$, of the riverbed sediment of mecoprop and isoproturon to be 279 ± 32 and 240 ± 9 μg $kg^{-1}$ of dry sediment, respectively (Tables 4.5 and 4.6) were known. As the maximum sorptive capacity of the riverbed sediment for isoproturon was lower than that for mecoprop, then the value of the sorption capacity of dry riverbed sediment to isoproturon was used for the following calculation (183 ± 24 μg $kg^{-1}$). The moisture content of riverbed sediment was approximately 30 % (Table 4.3), thus the maximum capacity of riverbed sediment to isoproturon based on a unit of wet sediment, $C_{S\ max,\ IPU,\ wet\ RS}$, was:

$$C_{S\ max,\ IPU,\ wet\ RS} = 183*(1 - 0.3) = 128 \ \mu g\ kg^{-1} \text{ wet sediment} \qquad (5.12)$$

The problem was whether or not the volume of riverbed sediment, $V_{sed,\ biod}$ = 972 x $10^3$ $m^3$, was able to absorb the required amount of herbicides during a 6-

day adaptation period under a pumping capacity of 4 x $10^6$ L $day^{-1}$ river-fed water obtained from a borehole. This problem was solved as follows:

The volume of contaminated river water needed to be treated over 6 days, $V_{water,\ adsorpt}$:

$$V_{water,\ adsorpt} = 4*10^6 * 6 = 24*10^6\ L \qquad (5.13)$$

Assuming that this volume of water contains isoproturon with a concentration of 100 µg $L^{-1}$, then the amount of isoproturon in the above volume, $M_{IPU}$, was:

$$M_{IPU} = 100\ \mu g\ L^{-1} * 24*10^6\ L = 2400*10^6\ \mu g\ isoproturon \qquad (5.14)$$

With reference to Expression (13), the weight of wet riverbed sediment, $M_{sed}$, was:

$$M_{sed} = \frac{2400*10^6}{128} = 18.75*10^6 \text{ kg sediment} \qquad (5.15)$$

Table 2.3 provides the bulk density of the riverbed sediment to be 1.31 g $cm^{-3}$ or 1.31 kg $L^{-1}$. Thus, the volume of the riverbed sediment requiring to sorb the isoproturon, $V_{sed,\ adsorp}$, was:

$$V_{sed,adsorp} = \frac{18.75*10^6}{1.31} = 14.31*10^6\ L = 14.31*10^3\ m^3 \qquad (5.16)$$

Comparing Equations (5.16) and 5.(9), it is found that the volume $V_{sed,\ adsorp}$ (14 x $10^3$ $m^3$) was very much smaller than the volume $V_{sed,biodeg}$ (972 x $10^3$ $m^3$).

This result suggested that if a borehole is situated at a minimum path length of 400 m from a river, the herbicides mecoprop and isoproturon should be sorbed in/onto the riverbed sediment before the water flow reaches the borehole during the first 6 days of their sorption and adaptation phases. Hence, although the herbicides cannot be cleaved by the microorganisms during the initial adaptation time, they are also not able to pollute the borehole. Returning to Borehole 6 at the Gatehampton site, it is located at a distance of approximately 500 m from the River Thames, thus it should be free from mecoprop and isoproturon herbicide pollution in river water if the thickness of the sediment layer is greater than 6 m.

### 6.2.3 Summary

The crucial role of riverbed sediments as a barrier to groundwater pollution at the Gatehampton has been reported by Younger *et al.* (1993). This section again underlines the importance of riverbank sediment filtration as a valuable, natural pre-treatment for exploiting drinking water from bank-side boreholes. Such boreholes can be protected from river-borne pesticide pollution caused by a spill or over-application in agricultural areas (up to 100 $\mu g\ L^{-1}$ of herbicide concentration in river water). In reality, the concentration of herbicides in river water was usually much lower than a level of 100 $\mu g\ L^{-1}$. In addition, the influence of previous exposure of the microorganisms to the introduced herbicides can also enhance the biodegradation processes for herbicides. Hence, the potential of riverbank filtration and, in particular, riverbed sediment filtration in the context of bank filtration schemes for the removal of the herbicides mecoprop and isoproturon is very high.

# Chapter 7

# CONCLUSIONS

# and RECOMMENDATIONS for FURTHER WORK

## 7.1 Conclusions

A fixed-bed column circulation method has been successfully developed (Chapter 3) to facilitate the study of herbicide interactions in a river water-riverbed sediment system. Sorption and biodegradation of the herbicides mecoprop and isoproturon in a river water-riverbed sediment system have been investigated using this fixed-bed column circulation method (Chapter 4). The recirculation of the river water containing the herbicides mecoprop and isoproturon through a fixed-bed column system has revealed the following:

- Mecoprop and isoproturon were sorbed on/into the riverbed sediment within 1 day and that this sorption accounted for approximately 18-20 % reduction of these herbicides from the recirculated river water. The sorption rate constants of mecoprop and isoproturon were estimated to be $0.0191 \pm 0.009\ h^{-1}$ and $0.0106 \pm 0.0015\ h^{-1}$, respectively.

- Following the sorption phase, an adaptation or lag phase was observed during a period of 6 circulation days.

- After the lag phase, mecoprop and isoproturon completely destroyed during the period of 9 circulation days. The extensive and rapid decrease in the herbicide concentrations was not observed in the treatments with sterile riverbed sediment.

- The biodegradation rate constant of isoproturon was determined to be $13.62 \pm 0.17$ μg $L^{-1}$ $day^{-1}$, which was significantly higher than the biodegradation rate of mecoprop determined to be $9.91 \pm 0.19$ μg $L^{-1}$ $day^{-1}$.

In general, where non-sterile riverbed sediment was used in experimental treatments, herbicides removal from the recirculation water was completed by the time of 14 circulation days. In contrast, where sterile riverbed sediment was used in experimental treatments, residual herbicide concentrations in the recirculation water remained similar to concentration established following the initial sorption phase; in all cases the residual concentrations in recirculation water (after 14 days), where sterile riverbed sediment was used, were greater than 78 %.

These observations support the hypotheses below (framed in Chapter 1):

Hypothesis (1):

Herbicides will sorb on/into sediment. The extent to which this sorption takes place will be dependent upon a) herbicide physical and chemical properties, and, b) the properties of the sediment;

Hypothesis (2):

Herbicides will be degraded in sediment. The extent of degradation will be dependent upon a) herbicide physical and chemical properties, and, b) microbial catabolic competence;

Hypothesis (3):

> Loss of herbicides will be most strongly dependent upon microbial catabolic competence in sediment rather than river water;

The riverbed sediment removed from the fixed-bed system following a recirculation period of 18 days was screened, with respect to isoproturon catabolic competence, using $^{14}$C-respirometry. This experiment revealed that:

- High levels of isoproturon catabolic competence in riverbed sediment removed from treatments containing non-sterile riverbed sediment (extent of $^{14}$C-isoproturon mineralisation was 29.4 ± 1.5 %). In this case, no lag time was observed. In contrast, very low levels of isoproturon catabolic competence was observed in the riverbed sediment removed from treatments containing sterile riverbed sediment but non-sterile river water (extent of $^{14}$C-isoproturon mineralisation was 1.4 ± 0.1 %);

These observations further support Hypothesis (3):

> Loss of herbicides will be most strongly dependent upon microbial catabolic competence in sediment rather than river water;

A separate set of $^{14}$C-respirometry studies (as presented in Chapter 5) explored the relationship between levels of isoproturon catabolic competence in river sediment, river water and groundwater with respect to isoproturon concentration and time given for its accommodation. These experiments revealed the following:

- Both river water and groundwater had low levels of catabolic competence with respect to isoproturon. Mineralisation of $^{14}$C-isoproturon in these materials was never found to be greater than 1.2 ± 0.1%. Furthermore, no enhancement was noted following isoproturon addition to these microcosms.

- In contrast, riverbed sediment was found to have significantly higher levels of catabolic activity (14.5 $\pm$ 1.6 %). Additionally, significant enhancements in levels of catabolic competence were noted following isoproturon addition to riverbed sediment treatments. This enhancement was noted to be dependent upon isoproturon concentration present in the flasks at the beginning of $^{14}$C-isoproturon mineralisation assessment. The concentration dependency of level of catabolic competence (ascribed as extent of $^{14}$C-isoproturon mineralisation) was described by the equation [$y = 6.26 \lg(x) + 20.49$] and $R^2 = 0.77$ (with y represents for % mineralisation and x represents for $^{12}$C-isoproturon, $\mu g\ L^{-1}$).

These observations support again Hypothesis (3):

Loss of herbicides will be most strongly dependent upon microbial catabolic competence in sediment rather than river water and/or groundwater;

In addition, these observations also support Hypotheses 4 and 5 with respect to riverbed sediment. However, these hypotheses are not supported with respect to river water and groundwater:

Hypothesis (4):

The addition of herbicide to sediment and/or river water and/or groundwater will increase the levels of catabolic competence;

Hypothesis (5):

Levels of catabolic activity in sediment and/or river water and/or groundwater will be proportional to concentrations of herbicide present; with higher substrate concentrations promoting higher levels of catabolic competence.

Relied upon results from the fixed-bed column circulation experiment, a simplified one-dimensional model was used to estimate a shortest pathway for a borehole which induces herbicide contaminated water from a river. The model provided that a volume of 0.027 $m^3$ riverbed sediment was required to clean 1 L of river water contaminated with the herbicides mecoprop and isoproturon (up to 100 $\mu g\ L^{-1}$). This model was extended to the riverbank filtration context in the Gatehampton site. Assuming that a bank-side borehole with a capacity of approximately 16 x $10^6$ L $day^{-1}$ and 25 % river-fed water and velocity of groundwater flow of 3.6 m $day^{-1}$ (Chapter 2), the borehole will be protected from the herbicide pollution up to 100 $\mu g\ L^{-1}$ if it is located at a minimum distance (path length) of 400 m away from the river in a hyporheic zone with a 6 m thickness of the riverbed sediment layer. These conditions enable the retention time of contaminated river water was long enough for biological degradation.

In general, this thesis provides encouragement for the potential to use riverbank filtration in removal of herbicide pollution from river water. Microbial communities in a riverbed sediment environment can play a pivotal role in the degradation of the herbicides mecoprop and isoproturon. The outcomes of this research provide mechanistic insight into the capacity and responsiveness of the riverbank materials to remove the herbicides. Moreover, degradation of mecoprop and isoproturon in a river water-riverbed sediment interaction zone can extend to other phenoxy acid herbicides and phenyl-urea herbicides.

## 7.2 Recommendations for Further Work

To comprehensively understand the fate and behaviour of the herbicides in a river water-riverbed sediment interaction zone, further laboratory and *in situ* field studies should be carried out. Developing the laboratory work undertaken in this thesis, the prospects for further work are outlined below:

(1) Investigate the fate and behaviour of herbicides with low concentration (less than 1 μg $L^{-1}$) using a fixed-bed column circulation model in order to approach the frequent concentration of herbicides in surface water environments;

(2) Investigate the influence of flow rate through a fixed-bed column on the attenuation of the herbicides;

(3) Investigate the formation of biofilm on the surface of sediment during the biodegradation time;

(4) Investigate the mutual influence of herbicides on their degradation in a river water-riverbed sediment system;

(5) Investigate catabolic activity with respect to mecoprop in riverbank materials using a respirometry method;

(6) Investigate the degradation of herbicides under low temperature conditions, e.g. 5 or 10 $^{o}$C;

(7) Investigate the population dynamics of the degrading microorganisms in response to changes in substrates;

(8) Improve the sampling methods to collect riverbed sediment samples in order to preserve the structure of the bed and give a better simulation.

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# APPENDICES

## A.1 Results from the sorption and biodegradation experiments (Chapter 4)

### A.1.1 Concentration of mecoprop in Experiment 1

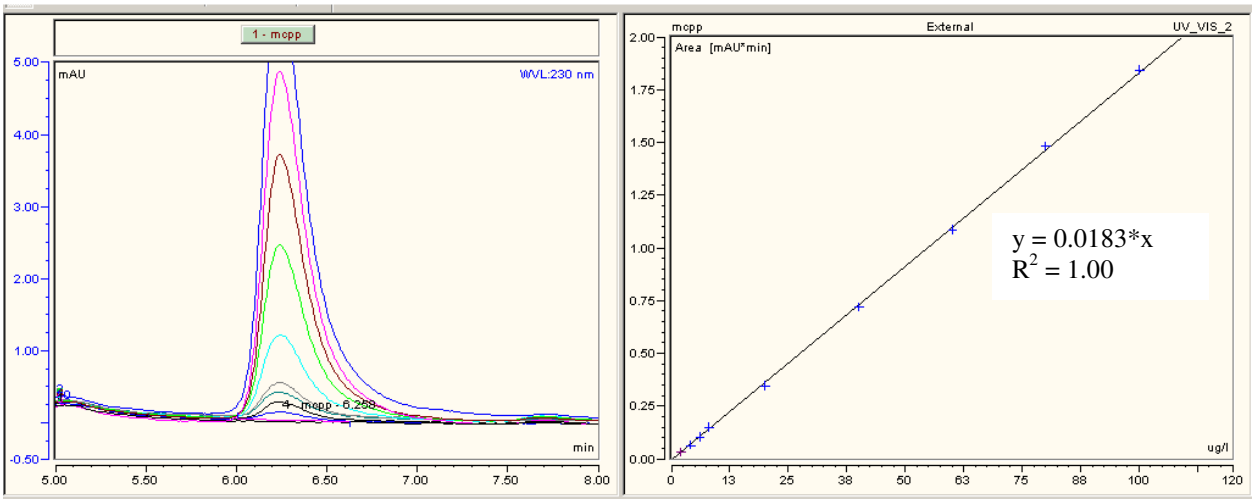


**Figure A.1.1** Calibration line of mecoprop for Experiment 1 in Chapter 4

**Table A.1.1** Concentration of mecoprop (μg $L^{-1}$) in Treatment 1 (sterile river water and sterile riverbed sediment)

| Retention time (day) | T1.1 | T1.2 | T1.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 102.07 | 98.32 | 97.94 | 99.44 | 2.28 | 1.32 |
| 0.125 | 99.77 | 98.21 | 92.53 | 96.84 | 3.81 | 2.20 |
| 0.25 | 89.42 | 89.52 | 90.34 | 89.76 | 0.50 | 0.29 |
| 0.50 | 87.25 | 89.03 | 88.62 | 88.30 | 0.93 | 0.54 |
| 1.00 | 86.40 | 84.00 | 87.56 | 85.99 | 1.82 | 1.05 |
| 2.00 | 85.06 | 80.5 | 80.92 | 82.16 | 2.52 | 1.46 |
| 3.00 | 84.09 | 82.25 | 83.33 | 83.22 | 0.92 | 0.53 |
| 4.00 | 84.89 | 86.37 | 85.76 | 85.67 | 0.74 | 0.43 |
| 5.00 | 86.46 | 85.7 | 86.68 | 86.28 | 0.51 | 0.30 |
| 6.00 | 85.62 | 82.72 | 88.86 | 85.73 | 3.07 | 1.77 |
| 8.00 | 87.72 | 88.56 | 84.35 | 86.88 | 2.23 | 1.29 |
| 10.00 | 86.93 | 83.57 | 87.49 | 86.00 | 2.12 | 1.22 |
| 12.00 | 89.89 | 88.93 | 86.26 | 88.36 | 1.88 | 1.09 |
| 14.00 | 92.28 | 85.56 | 75.03 | 84.29 | 8.69 | 5.02 |
| 18.00 | 83.99 | 79.11 | 75.60 | 79.57 | 4.21 | 2.43 |

**Table A.1.2** Concentration of mecoprop (μg $L^{-1}$) in Treatment 2 (non-sterile river water and non-sterile riverbed sediment)

| Retention time (day) | T2.1 | T2.2 | T2.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 102.97 | 101.80 | 100.95 | 101.91 | 1.01 | 0.59 |
| 0.25 | 97.68 | 94.86 | 94.09 | 95.54 | 1.89 | 1.09 |
| 0.5 | 94.55 | 87.27 | 93.57 | 91.80 | 3.95 | 2.28 |
| 1 | 85.47 | 82.71 | 81.95 | 83.38 | 1.85 | 1.07 |
| 2 | 82.60 | 80.08 | 79.16 | 80.61 | 1.78 | 1.03 |
| 3 | 80.49 | 79.55 | 82.63 | 80.89 | 1.58 | 0.91 |
| 4 | 83.36 | 79.27 | 82.74 | 81.79 | 2.20 | 1.27 |
| 5 | 78.77 | 78.8 | 80.01 | 79.19 | 0.71 | 0.41 |
| 6 | 83.68 | 82.4 | 80.45 | 82.18 | 1.63 | 0.94 |
| 7 | 78.78 | 77.96 | 79.47 | 78.74 | 0.76 | 0.44 |
| 8 | 63.25 | 64.75 | 66.52 | 64.84 | 1.64 | 0.95 |
| 9 | 46.05 | 46.26 | 60.71 | 51.01 | 8.40 | 4.85 |
| 10 | 38.17 | 36.86 | 45.92 | 40.32 | 4.90 | 2.83 |
| 11 | 26.37 | 23.33 | 25.29 | 25.00 | 1.54 | 0.89 |
| 12 | 22.46 | 16.53 | 18.67 | 19.22 | 3.00 | 1.73 |
| 13 | 16.95 | 5.72 | 12.95 | 11.87 | 5.69 | 3.29 |
| 14 | 6.35 | 2.50 | 4.18 | 4.34 | 1.93 | 1.11 |
| 15 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 16 | 0 | 0.00 | 0 | 0.00 | 0.00 | 0.00 |
| 17 | 0 | 0.00 | 0 | 0.00 | 0.00 | 0.00 |
| 18 | 0 | 0.00 | 0 | 0.00 | 0.00 | 0.00 |

**Table A.1.3** Concentration of mecoprop (μg $L^{-1}$) in Treatment 3 (nonsterile river water and sterile riverbed sediment)

| Retention time (day) | T3.1 | T3.2 | T3.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 101.66 | 105.77 | 97.63 | 101.69 | 4.07 | 2.35 |
| 0.25 | 96.78 | 102.56 | 94.85 | 98.06 | 4.01 | 2.32 |
| 0.5 | 90.88 | 96.79 | 90.77 | 92.81 | 3.45 | 1.99 |
| 1 | 88.95 | 92.36 | 87.47 | 89.59 | 2.51 | 1.45 |
| 2 | 87.61 | 90.81 | 86.30 | 88.24 | 2.32 | 1.34 |
| 3 | 83.75 | 83.54 | 79.97 | 82.42 | 2.13 | 1.23 |
| 4 | 85.41 | 83.32 | 85.08 | 84.61 | 1.13 | 0.65 |
| 5 | 87.42 | 85.40 | 83.65 | 85.49 | 1.89 | 1.09 |
| 6 | 84.54 | 81.50 | 82.92 | 82.99 | 1.52 | 0.88 |
| 7 | 83.16 | 82.55 | 80.90 | 82.21 | 1.17 | 0.67 |
| 8 | 85.56 | 83.66 | 82.68 | 83.97 | 1.47 | 0.85 |
| 9 | 84.12 | 81.84 | 81.82 | 82.59 | 1.32 | 0.76 |
| 10 | 84.09 | 83.40 | 80.70 | 82.73 | 1.79 | 1.03 |
| 11 | 84.16 | 81.57 | 83.65 | 83.13 | 1.37 | 0.79 |
| 12 | 83.95 | 83.93 | 80.91 | 82.93 | 1.75 | 1.01 |
| 13 | 84.77 | 80.31 | 82.09 | 82.39 | 2.25 | 1.30 |
| 14 | 82.99 | 80.06 | 83.15 | 82.07 | 1.74 | 1.00 |
| 15 | 80.67 | 83.74 | 83.62 | 82.68 | 1.74 | 1.00 |
| 16 | 83.00 | 83.02 | 83.09 | 83.04 | 0.05 | 0.03 |
| 17 | 82.05 | 82.20 | 84.45 | 82.90 | 1.34 | 0.78 |
| 18 | 81.62 | 84.55 | 80.82 | 82.33 | 1.96 | 1.13 |

**Table A.1.4** Concentration of mecoprop (μg $L^{-1}$) in Treatment 3 (nonsterile river water and sterile riverbed sediment)

| Retention time (day) | T4.1 | T4.2 | T4.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 101.75 | 98.09 | 97.99 | 99.28 | 2.14 | 1.24 |
| 0.25 | 98.6 | 96.48 | 96.26 | 97.11 | 1.29 | 0.75 |
| 0.5 | 89.48 | 88.42 | 87.43 | 88.45 | 1.02 | 0.59 |
| 1 | 87.76 | 87.12 | 83.65 | 86.18 | 2.21 | 1.28 |
| 2 | 85.46 | 82.90 | 84.39 | 84.25 | 1.28 | 0.74 |
| 3 | 87.10 | 86.27 | 84.42 | 85.93 | 1.37 | 0.79 |
| 4 | 85.20 | 89.22 | 82.58 | 85.67 | 3.35 | 1.93 |
| 5 | 86.93 | 86.89 | 84.76 | 86.19 | 1.24 | 0.72 |
| 6 | 83.80 | 84.40 | 81.43 | 83.21 | 1.57 | 0.91 |
| 7 | 84.61 | 75.48 | 77.67 | 79.25 | 4.77 | 2.75 |
| 8 | 86.06 | 40.66 | 57.99 | 61.57 | 22.91 | 13.23 |
| 9 | 80.84 | 37.11 | 36.11 | 51.35 | 25.54 | 14.75 |
| 10 | 77.34 | 29.65 | 32.34 | 46.44 | 26.79 | 15.47 |
| 11 | 68.40 | 28.81 | 29.84 | 42.35 | 22.57 | 13.03 |
| 12 | 37.71 | 24.51 | 23.16 | 28.46 | 8.04 | 4.64 |
| 13 | 18.75 | 18.03 | 12.38 | 16.39 | 3.49 | 2.01 |
| 14 | 0.00 | 8.56 | 0.00 | 2.85 | 4.94 | 2.85 |
| 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

## A.1.2 Concentration of isoproturon in Experiment 1

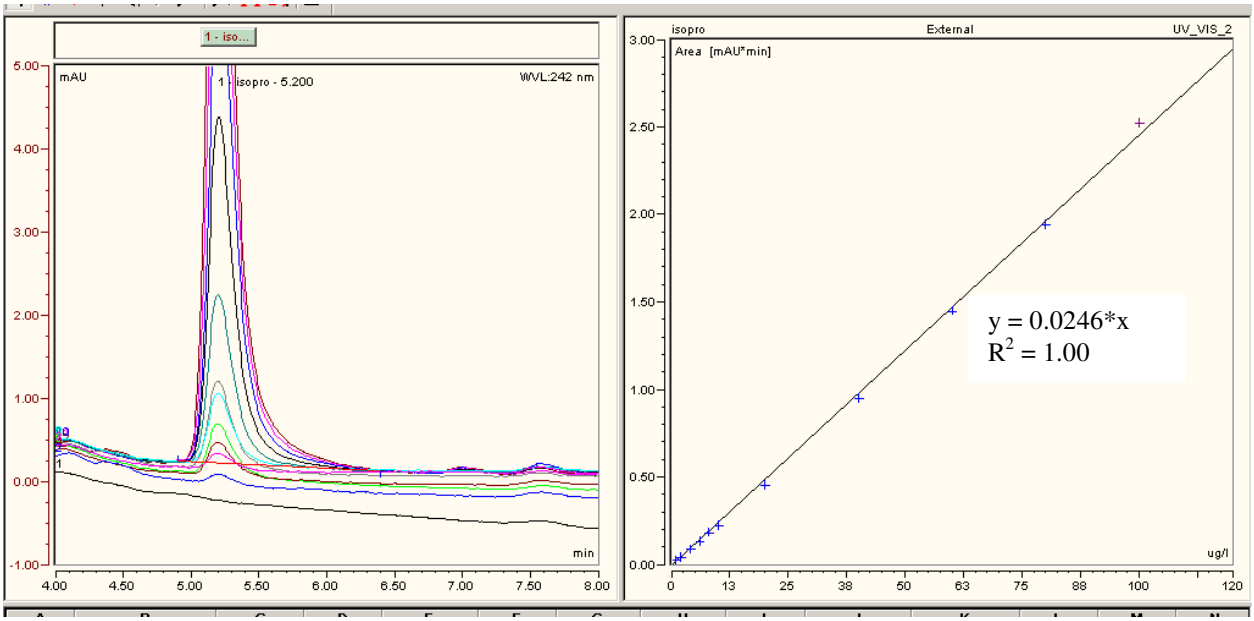


**Figure A.1.2** Calibration line of isoproturon for Experiment 1 in Chapter 4

**Table A.1.5** Concentration of isoproturon (μg $L^{-1}$) in Treatment 1 (sterile river water and sterile riverbed sediment)

| Retention time (day) | T1.1 | T1.2 | T1.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 98.12 | 96.85 | 94.94 | 96.64 | 1.60 | 0.92 |
| 0.125 | 93.97 | 89.49 | 89.69 | 91.05 | 2.53 | 1.46 |
| 0.25 | 92.12 | 87.77 | 87.31 | 89.07 | 2.65 | 1.53 |
| 0.50 | | 85.38 | 84.84 | 85.11 | 0.38 | 0.27 |
| 1.00 | 84.31 | 81.55 | 82.19 | 82.68 | 1.44 | 0.83 |
| 2.00 | 81.44 | 80.39 | 80.26 | 80.70 | 0.65 | 0.37 |
| 3.00 | 80.11 | 80.21 | 79.23 | 79.85 | 0.54 | 0.31 |
| 4.00 | 77.12 | 75.97 | 76.1 | 76.40 | 0.63 | 0.36 |
| 5.00 | 79.17 | 78.01 | 76.05 | 77.74 | 1.58 | 0.91 |
| 6.00 | 79.52 | 73.55 | 77.34 | 76.80 | 3.02 | 1.74 |
| 8.00 | 78.21 | 74.65 | 77.36 | 76.74 | 1.86 | 1.07 |
| 10.00 | 77.43 | 74.93 | 78.15 | 76.84 | 1.69 | 0.98 |
| 12.00 | 80.52 | 76.76 | 79.18 | 78.82 | 1.91 | 1.10 |
| 14.00 | 77.18 | 68.70 | 75.99 | 73.96 | 4.59 | 2.65 |
| 18.00 | 71.92 | 66.88 | 78.33 | 72.38 | 5.74 | 3.31 |

**Table A.1.6** Concentration of isoproturon (μg $L^{-1}$) in Treatment 2 (non-sterile river water and non-sterile riverbed sediment)

| Retention time (day) | T2.1 | T2.2 | T2.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 94.45 | 95.38 | 95.79 | 95.21 | 0.69 | 0.40 |
| 0.125 | 85.52 | 82.63 | 83.84 | 84.00 | 1.45 | 0.84 |
| 0.25 | 82.65 | 80.96 | 81.81 | 81.81 | 0.85 | 0.60 |
| 0.5 | 78.84 | 78.34 | 78.06 | 78.41 | 0.40 | 0.23 |
| 1 | 77.6 | 76.83 | 77.61 | 77.35 | 0.45 | 0.26 |
| 2 | 76.77 | 74.92 | 75.89 | 75.86 | 0.93 | 0.53 |
| 3 | 74.89 | 75.62 | 70.77 | 73.76 | 2.62 | 1.51 |
| 4 | 72.12 | 74.2 | 70.39 | 72.24 | 1.91 | 1.10 |
| 5 | 73.56 | 74.99 | 70.83 | 73.13 | 2.11 | 1.22 |
| 6 | 74.97 | 74.25 | 69.27 | 72.83 | 3.10 | 1.79 |
| 7 | 69.02 | 62.25 | 68.52 | 66.60 | 3.77 | 2.18 |
| 8 | 68.21 | 48.61 | 56.41 | 57.74 | 9.87 | 5.70 |
| 9 | 45.23 | 25.69 | 36.28 | 35.73 | 9.78 | 5.65 |
| 10 | 23.42 | 7.63 | 17.87 | 16.31 | 8.01 | 4.62 |
| 11 | 12.2 | 0 | 5.67 | 5.96 | 6.11 | 3.52 |
| 12 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 13 | | | | | | |
| 14 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |

**Table A.1.7** Concentration of isoproturon (μg $L^{-1}$) in Treatment 3 (non-sterile river water and sterile riverbed sediment)

| Retention time (day) | T3.1 | T3.2 | T3.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 96.35 | 98.31 | 94.41 | 96.36 | 1.95 | 1.13 |
| 0.125 | 94.17 | 94.25 | 93.18 | 93.87 | 0.60 | 0.34 |
| 0.25 | 89.39 | 87.98 | 89.79 | 89.05 | 0.95 | 0.55 |
| 0.5 | 88.40 | 87.97 | 87.65 | 88.01 | 0.38 | 0.22 |
| 1 | 88.36 | 82.75 | 86.77 | 85.96 | 2.89 | 1.67 |
| 2 | 89.50 | 83.03 | 84.50 | 85.68 | 3.39 | 1.96 |
| 3 | 88.77 | 85.05 | 83.89 | 85.90 | 2.55 | 1.47 |
| 4 | 89.66 | 83.62 | 81.66 | 84.98 | 4.17 | 2.41 |
| 5 | 85.10 | 83.54 | 81.25 | 83.30 | 1.94 | 1.12 |
| 6 | 86.64 | 87.45 | 82.45 | 85.51 | 2.68 | 1.55 |
| 7 | 83.85 | 77.63 | 77.95 | 79.81 | 3.50 | 2.02 |
| 8 | 76.39 | 80.99 | 79.18 | 78.85 | 2.32 | 1.34 |
| 9 | 79.46 | 77.52 | 76.45 | 77.81 | 1.53 | 0.88 |
| 10 | 79.36 | 76.42 | 76.96 | 77.58 | 1.57 | 0.90 |
| 11 | 80.82 | 78.76 | 76.96 | 78.85 | 1.93 | 1.12 |
| 12 | 79.37 | 77.94 | 75.41 | 77.57 | 2.00 | 1.16 |
| 13 | 77.68 | 75.29 | 74.97 | 75.98 | 1.48 | 0.85 |
| 14 | 75.48 | 74.30 | 75.72 | 75.17 | 0.76 | 0.44 |
| 15 | 76.54 | 76.79 | 74.69 | 76.01 | 1.15 | 0.66 |
| 16 | 75.29 | 76.43 | 76.24 | 75.99 | 0.61 | 0.35 |
| 17 | 76.22 | 76.49 | 73.09 | 75.27 | 1.89 | 1.09 |
| 18 | 70.75 | 85.22 | 78.41 | 78.13 | 7.24 | 4.18 |

**Table A.1.8** Concentration of isoproturon (μg $L^{-1}$) in Treatment 4 (sterile river water and non-sterile riverbed sediment)

| Retention time (day) | T4.1 | T4.2 | T4.3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0 | 96.33 | 96.50 | 100.28 | 97.70 | 2.24 | 1.29 |
| 0.125 | 91.68 | 93.67 | 92.52 | 92.62 | 1.00 | 0.58 |
| 0.25 | 87.86 | 88.18 | 86.02 | 87.35 | 1.17 | 0.67 |
| 0.5 | 86.99 | 80.51 | 81.08 | 82.86 | 3.59 | 2.07 |
| 1 | 85.08 | 80.87 | 80.89 | 82.28 | 2.42 | 1.40 |
| 2 | 84.21 | 78.47 | 81.69 | 81.46 | 2.88 | 1.66 |
| 3 | 84.13 | 79.33 | 80.67 | 81.38 | 2.48 | 1.43 |
| 4 | 82.95 | 80.36 | 81.66 | 81.66 | 1.29 | 0.75 |
| 5 | 79.46 | 80.19 | 83.06 | 80.90 | 1.90 | 1.10 |
| 6 | 78.53 | 81.44 | 84.08 | 81.35 | 2.78 | 1.60 |
| 7 | 84.41 | 71.13 | 77.30 | 77.61 | 6.65 | 3.84 |
| 8 | 79.55 | 33.37 | 66.10 | 59.68 | 23.75 | 13.71 |
| 9 | 72.53 | 0.00 | 33.01 | 35.18 | 36.32 | 20.97 |
| 10 | 43.74 | 0.00 | 0.00 | 14.58 | 25.25 | 14.58 |
| 11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

## A.1.3 Mineralisation levels of isoproturon in Experiment 2

Mineralisation levels of samples from the respirometers were determined by a scintillation counter. The instrument returned the results as disintegration per minute (dpm). The results are presented in the following table:

**Table A.1.9** Disintegration per minute (dpm) of $^{14}CO_2$ in the respirometer samples in Experiment 2

| Samples \ Assay day | | 1 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| M3.1 | Repl. 1 | 23 | 112 | 88 | 133 | 131 | 126 |
| | Repl. 2 | 181 | 38 | 105 | 106 | 104 | 108 |
| | Repl. 3 | 242 | 30 | 93 | 162 | 143 | 163 |
| M3.2 | Repl. 1 | 226 | 28 | 74 | 132 | 129 | 129 |
| | Repl. 2 | 230 | 37 | 35 | 96 | 102 | 135 |
| | Repl. 3 | 216 | 34 | 79 | 109 | 134 | 139 |
| M3.3 | Repl. 1 | 155 | 34 | 86 | 96 | 88 | 107 |
| | Repl. 2 | 212 | 38 | 131 | 107 | 104 | 91 |
| | Repl. 3 | 203 | 41 | 88 | 101 | 86 | 91 |
| M4.1 | Repl. 1 | 10912 | 308 | 706 | 757 | 772 | 1112 |
| | Repl. 2 | 8524 | 278 | 945 | 933 | 1143 | 1082 |
| | Repl. 3 | 9784 | 341 | 738 | 958 | 943 | 1030 |
| M4.2 | Repl. 1 | 9386 | 178 | 653 | 995 | 1006 | 1151 |
| | Repl. 2 | 11067 | 189 | 343 | 979 | 811 | 1248 |
| | Repl. 3 | 11373 | 362 | 328 | 921 | 767 | 1158 |
| M4.3 | Repl. 1 | 10638 | 396 | 507 | 1016 | 738 | 999 |
| | Repl. 2 | 9736 | 544 | 413 | 1105 | 652 | 456 |
| | Repl. 3 | 10415 | 450 | 821 | 911 | 791 | 1199 |
| Blank samples | Repl. 1 | 18 | 20 | 19 | 21 | 24 | 21 |
| | Repl. 2 | 23 | 17 | 21 | 23 | 25 | 21 |
| | Repl. 3 | 19 | 20 | 18 | 18 | 26 | 19 |
| Standard | Repl. 1 | 34838 | | | | | |
| | Repl. 2 | 34960 | | | | | |
| | Repl. 3 | 34160 | | | | | |

## A.1.4 Concentration of isoproturon in Experiment 3

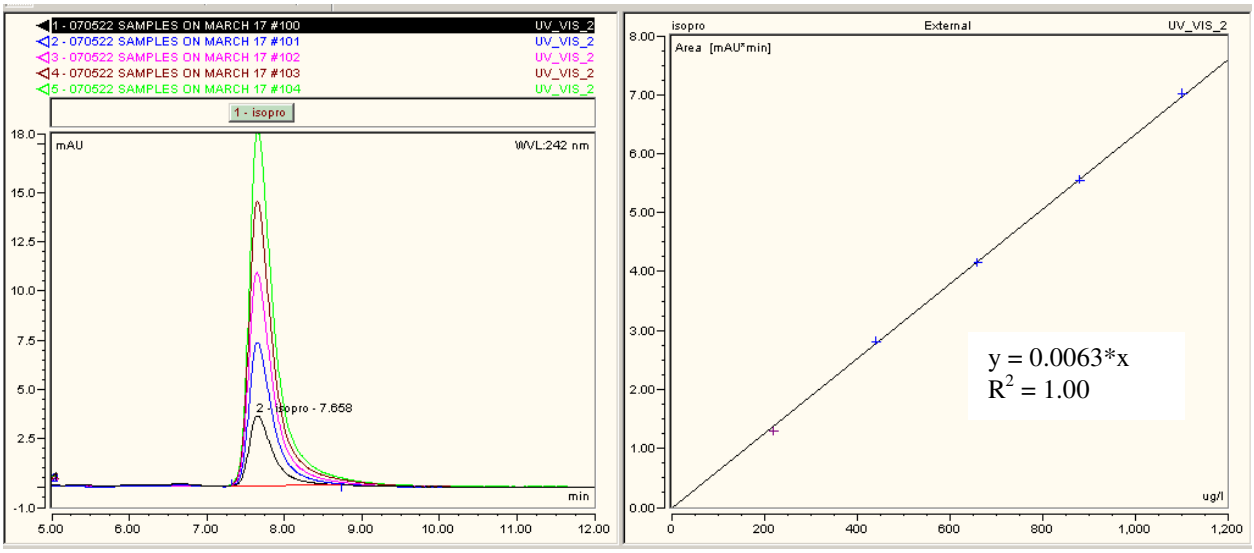


**Figure A.1.3** Calibration line of isoproturon for Experiment 3 in Chapter 4

**Table A.1.10** Concentration of isoproturon (μg $L^{-1}$) in the riverbed sediment treatments with higher initial concentration (1000 μg $L^{-1}$) in Experiment 3 (three replicates)

| Retention time (day) | H1 | H2 | H3 | Mean | SD | Standard error |
|---|---|---|---|---|---|---|
| 0.00 | 999.8032 | 1053.8 | 1055.58 | 1036.40 | 31.70 | 18.30 |
| 0.04 | 953.5782 | 965.349 | 971.822 | 963.58 | 9.25 | 5.34 |
| 0.13 | 865.2857 | 908.746 | 903.36 | 892.46 | 23.69 | 13.68 |
| 0.29 | 855.3795 | 879.682 | 881.035 | 872.03 | 14.44 | 10.21 |
| 1.29 | 787.5767 | 802.275 | 798.256 | 796.04 | 7.60 | 5.37 |
| 4.29 | 634.6328 | 676.049 | 728.85 | 679.84 | 47.22 | 33.39 |
| 5.29 | 587.4812 | 658.935 | 650.824 | 632.41 | 39.12 | 27.66 |
| 7.29 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 9.29 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 11.29 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |
| 13.29 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 |

## A.2 Results from the catabolic experiments (Chapter 5)

### A.2.1 Mineralisation of $^{14}$C-isoproturon in IPU-undosed and IPU-dosed treatments with 0 incubation days

**Table A.2.1** Dpm* of $^{14}CO_2$ in the respirometer samples in IPU-undosed and IPU-dosed treatments with 0 incubation days

| Assay day | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 16 | 18 | 20 | 24 | 30 | 39 | 48 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | R** | | | | | | | | | | | | | | | | | | | | | |
| RS 0 (0) | 1 | 330 | 281 | 206 | 65 | 519 | 801 | 725 | 606 | 581 | 588 | 692 | 758 | 415 | 2155 | 1656 | 368 | 480 | 451 | 477 | 194 | 411 |
| | 2 | 326 | 275 | 138 | 88 | 442 | 585 | 454 | 363 | 373 | 380 | 371 | 475 | 407 | 1484 | 1385 | 662 | 762 | 595 | 578 | 214 | 443 |
| | 3 | 334 | 272 | 233 | 87 | 621 | 799 | 653 | 574 | 570 | 522 | 620 | 537 | 568 | 1672 | 1387 | 670 | 694 | 628 | 472 | 263 | 619 |
| RS 0.1 (0) | 1 | 293 | 193 | 149 | 119 | 79 | 167 | 251 | 297 | 245 | 177 | 179 | 157 | 129 | 608 | 1253 | 835 | 1273 | 1592 | 1070 | 385 | 582 |
| | 2 | 536 | 307 | 211 | 72 | 105 | 112 | 77 | 104 | 66 | 59 | 89 | 146 | 106 | 388 | 479 | 410 | 1340 | 9363 | 5537 | 1168 | 1827 |
| | 3 | 295 | 162 | 110 | 123 | 64 | 79 | 59 | 66 | 110 | 130 | 135 | 111 | 143 | 421 | 390 | 318 | 418 | 631 | 777 | 400 | 660 |
| RS 1 (0) | 1 | 288 | 186 | 125 | 122 | 257 | 604 | 766 | 863 | 953 | 1188 | 1800 | 2426 | 2841 | 3364 | 1716 | 1295 | 1605 | 864 | 824 | 317 | 459 |
| | 2 | 304 | 195 | 138 | 316 | 85 | 81 | 95 | 151 | 238 | 234 | 283 | 324 | 667 | 4781 | 5255 | 2745 | 2403 | 1477 | 1078 | 421 | 707 |
| | 3 | 307 | 186 | 130 | 240 | 77 | 81 | 56 | 74 | 53 | 73 | 89 | 121 | 174 | 400 | 1150 | 2629 | 5133 | 2459 | 1553 | 794 | 882 |
| RS 100 (0) | 1 | 267 | 135 | 108 | 262 | 101 | 268 | 789 | 766 | 603 | 806 | 2209 | 5406 | 4587 | 3812 | 1783 | 1062 | 1294 | 1454 | 1386 | 478 | 530 |
| | 2 | 280 | 145 | 94 | 60 | 59 | 75 | 46 | 74 | 151 | 206 | 297 | 613 | 8881 | 5461 | 1720 | 893 | 1061 | 1854 | 1288 | 388 | 438 |
| | 3 | 295 | 186 | 64 | 71 | 131 | 345 | 410 | 306 | 503 | 1362 | 5993 | 4300 | 2697 | 2407 | 1444 | 1110 | 1273 | 1715 | 977 | 439 | 405 |
| RW 0 (0) | 1 | 28 | 28 | 29 | 25 | 28 | 28 | 28 | 23 | 39 | 38 | 41 | 36 | 38 | | 57 | | 52 | | 66 | | 60 |
| | 2 | 33 | 27 | 27 | 26 | 39 | 43 | 43 | 38 | 45 | 36 | 37 | 36 | 37 | | 58 | | 52 | | 86 | | 106 |
| | 3 | 28 | 27 | 25 | 24 | 29 | 47 | 55 | 44 | 37 | 32 | 26 | 30 | 28 | | 41 | | 44 | | 71 | | 82 |
| GW 0 (0) | 1 | 107 | 76 | 57 | 54 | 55 | 54 | 53 | 49 | 50 | 50 | 46 | 42 | 58 | | 114 | | 134 | | 200 | | 431 |
| | 2 | 102 | 81 | 65 | 56 | 61 | 53 | 53 | 50 | 47 | 48 | 47 | 42 | 54 | | 117 | | 163 | | 255 | | 217 |
| | 3 | 116 | 85 | 69 | 67 | 71 | 64 | 64 | 50 | 57 | 48 | 43 | 44 | 55 | | 137 | | 128 | | 261 | | 461 |
| Blank | 1 | 18 | 19 | 18 | 18 | 19 | 20 | 16 | 16 | 16 | 18 | 19 | 16 | 17 | 18 | 19 | 18 | 19 | 18 | 17 | 17 | 18 |
| | 2 | 23 | 19 | 18 | 19 | 18 | 19 | 18 | 16 | 16 | 17 | 17 | 15 | 18 | 20 | 19 | 19 | 18 | 17 | 20 | 18 | 93 |
| | 3 | 19 | 17 | 20 | 18 | 18 | 19 | 19 | 18 | 17 | 18 | 18 | 16 | 16 | 18 | 18 | 19 | 18 | 19 | 18 | 14 | 15 |

Dpm* – disintegration per minute; R** - Replicate

## A.2.2 Mineralisation of $^{14}$C-isoproturon in IPU-undosed and IPU-dosed treatments with 5 incubation days

**Table A.2.2** Dpm* of $^{14}CO_2$ in the respirometer samples in IPU-undosed and IPU-dosed treatments with 5 incubation days

| Assay day | | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 16 | 18 | 20 | 24 | 30 | 39 | 48 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | R** | | | | | | | | | | | | | | | | |
| RS 0 (5) | 1 | 538 | 492 | 193 | 338 | 349 | 360 | 343 | 621 | 1083 | 1073 | 886 | 1457 | 1339 | 1581 | 862 | 753 |
| | 2 | 465 | 408 | 167 | 239 | 251 | 260 | 267 | 608 | 1022 | 777 | 526 | 839 | 993 | 1168 | 886 | 849 |
| | 3 | 432 | 564 | 155 | 231 | 252 | 325 | 337 | 689 | 964 | 927 | 900 | 1042 | 1070 | 1254 | 629 | 440 |
| RS 0.1 (5) | 1 | 133 | 99 | 84 | 157 | 194 | 224 | 264 | 387 | 655 | 658 | 432 | 637 | 845 | 1063 | 555 | |
| | 2 | 118 | 101 | 82 | 144 | 172 | 273 | 279 | 403 | 667 | 699 | 446 | 698 | 830 | 923 | 659 | 359 |
| | 3 | 133 | 119 | 143 | 186 | 208 | 248 | 292 | 551 | 861 | 655 | 666 | 958 | 1128 | 1103 | 517 | 435 |
| RS 1 (5) | 1 | 133 | 102 | 127 | 145 | 239 | 530 | 461 | 554 | 1602 | 1543 | 2183 | 3898 | 2366 | 812 | 258 | 180 |
| | 2 | 153 | 128 | 132 | 154 | 222 | 217 | 269 | 441 | 805 | 805 | 765 | 2009 | 2653 | 1214 | 493 | 325 |
| | 3 | 139 | 120 | 156 | 214 | 316 | 522 | 655 | 1366 | 2838 | 2053 | 2243 | 3615 | 2466 | 874 | 408 | 322 |
| RS 100 (5) | 1 | 139 | 102 | 107 | 134 | 162 | 351 | 445 | 975 | 7264 | 10152 | 2607 | 2004 | 941 | 657 | 300 | 160 |
| | 2 | 127 | 87 | 117 | 141 | 145 | 290 | 345 | 1262 | 12778 | 7083 | 2622 | 2650 | 1554 | 302 | 59 | 45 |
| | 3 | 138 | 107 | 104 | 152 | 158 | 202 | 267 | 762 | 6323 | 11517 | 3081 | 1920 | 1202 | 764 | 289 | 147 |
| Blank | 1 | 20 | 16 | 16 | 16 | 18 | 19 | 16 | 17 | 18 | 19 | 18 | 19 | 18 | 17 | 17 | 18 |
| | 2 | 19 | 18 | 16 | 16 | 17 | 18 | 15 | 18 | 20 | 19 | 19 | 18 | 17 | 20 | 18 | 93 |
| | 3 | 19 | 19 | 18 | 17 | 18 | 17 | 16 | 16 | 18 | 18 | 19 | 18 | 19 | 18 | 14 | 15 |

Dpm* – disintegration per minute; R** - Replicate

## A.2.3 Mineralisation of $^{14}$C-isoproturon in IPU-undosed and IPU-dosed treatments with 10 incubation days

**Table A.2.3** Dpm* of $^{14}CO_2$ in the respirometer samples in IPU-undosed and IPU-dosed treatments with 10 incubation days

| | Assay day | 11 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 30 | 39 | 48 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | R** | | | | | | | | | | | | |
| RS 0 (10) | 1 | 357 | 279 | 576 | 913 | 1087 | 1176 | 164 | 588 | 1483 | 1994 | 393 | 310 |
| | 2 | 453 | 269 | 517 | 965 | 1322 | 1488 | 347 | 1822 | 3725 | 3283 | 759 | 1121 |
| | 3 | 364 | 299 | 442 | 816 | 985 | 939 | 181 | 695 | 1984 | 2077 | 865 | 1048 |
| RS 0.1 (10) | 1 | 142 | 117 | 218 | 616 | 687 | 1241 | 327 | 948 | 2338 | 2225 | 916 | 994 |
| | 2 | 109 | 144 | 304 | 830 | 797 | 1075 | 267 | 636 | 1741 | 1613 | 703 | 777 |
| | 3 | 126 | 147 | 327 | 876 | 876 | 870 | 355 | 560 | 1439 | 1208 | 611 | 1045 |
| RS 1 (10) | 1 | 132 | 128 | 464 | 1724 | 1222 | 1442 | 842 | 1748 | 4702 | 2201 | 824 | 689 |
| | 2 | 128 | 94 | 266 | 1467 | 1770 | 1140 | 603 | 1819 | 6972 | 4991 | 1165 | 970 |
| | 3 | 135 | 135 | 372 | 1441 | 1331 | 2074 | 1236 | 2635 | 6057 | 3022 | 937 | 704 |
| RS 100 (10) | 1 | 136 | 109 | 194 | 685 | 934 | 3625 | 7472 | 3952 | 4033 | 2414 | 675 | 412 |
| | 2 | 143 | 100 | 184 | 337 | 1701 | 10337 | 4526 | 2186 | 2470 | 1123 | 516 | 383 |
| | 3 | 110 | 153 | 246 | 1406 | 5830 | 10698 | 3757 | 1340 | 2591 | 1574 | 815 | 1008 |
| Blank | 1 | 19 | 16 | 17 | 18 | 19 | 18 | 18 | 19 | 18 | 17 | 17 | 18 |
| | 2 | 18 | 15 | 18 | 20 | 19 | 19 | 19 | 18 | 17 | 20 | 18 | 93 |
| | 3 | 17 | 16 | 16 | 18 | 18 | 19 | 21 | 18 | 19 | 18 | 14 | 15 |

Dpm* – disintegration per minute; R** - Replicate

## A.2.4 Mineralisation of $^{14}C$-isoproturon in IPU-undosed and IPU-dosed treatments with 30 incubation days

**Table A.2.4** Dpm* of $^{14}CO_2$ in the respirometer samples in IPU-undosed and IPU-dosed treatments with 30 incubation days

| Sample \ Assay day | R** | 31 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RS 0 (30) | 1 | 81 | 181 | 197 | 1812 | 440 | 487 | 1698 | 476 | 114 | 103 | 552 |
| | 2 | 122 | 217 | 225 | 1596 | 389 | 547 | 1453 | 517 | 141 | 118 | 464 |
| | 3 | 146 | 255 | 228 | 1332 | 313 | 510 | 1250 | 531 | 169 | 115 | 395 |
| RS 0.1 (30) | 1 | 51 | 72 | 53 | 104 | 38 | 62 | 217 | 147 | 62 | 68 | 237 |
| | 2 | 91 | 90 | 76 | 246 | 169 | 187 | 380 | 212 | 78 | 65 | 221 |
| | 3 | 62 | 91 | 72 | 162 | 81 | 116 | 359 | 255 | 153 | 153 | 536 |
| RS 1 (30) | 1 | 64 | 100 | 83 | 276 | 606 | 1460 | 2780 | 721 | 379 | 306 | 768 |
| | 2 | 218 | 304 | 677 | 1297 | 725 | 565 | 660 | 345 | 140 | 108 | 246 |
| | 3 | 104 | 83 | 52 | 73 | 43 | 54 | 96 | 122 | 41 | 38 | 66 |
| RS 100 (30) | 1 | 67 | 97 | 71 | 86 | 49 | 64 | 100 | 78 | 47 | 72 | 137 |
| | 2 | 138 | 81 | 66 | 124 | 616 | 1653 | 6295 | 1329 | 290 | 158 | 322 |
| | 3 | 155 | 106 | 80 | 372 | 6645 | 3851 | 1981 | 1529 | 418 | 356 | 311 |
| RW 0 (30) | 1 | 93 | 65 | 50 | 51 | | 61 | | 56 | | | 60 |
| | 2 | 92 | 59 | 46 | 44 | | 57 | | 54 | | | 75 |
| | 3 | 94 | 64 | 58 | 60 | | 75 | | 60 | | | 96 |
| RW 0.1 (30) | 1 | 32 | 48 | 47 | 48 | | 55 | | 48 | | | 46 |
| | 2 | 50 | 37 | 34 | 37 | | 43 | | 48 | | | 55 |
| | 3 | 86 | 53 | 48 | 49 | | 57 | | 63 | | | 69 |
| RW 1 (30) | 1 | 47 | 47 | 50 | 52 | | 67 | | 59 | | | 81 |
| | 2 | 45 | 48 | 49 | 50 | | 63 | | 64 | | | 71 |
| | 3 | 33 | 46 | 41 | 41 | | 62 | | 56 | | | 68 |
| RW 100 (30) | 1 | 56 | 42 | 39 | 39 | | 50 | | 56 | | | 60 |
| | 2 | 53 | 42 | 41 | 38 | | 48 | | 53 | | | 62 |
| | 3 | 54 | 52 | 53 | 47 | | 64 | | 52 | | | 56 |
| GW 0 (30) | 1 | 89 | 75 | 95 | 105 | | 211 | | 280 | | | 289 |
| | 2 | 111 | 107 | 152 | 163 | | 312 | | 515 | | | 859 |
| | 3 | 120 | 88 | 112 | 149 | | 183 | | 173 | | | 188 |
| GW 0.1 (30) | 1 | 147 | 121 | 77 | 61 | | 89 | | 90 | | | 106 |
| | 2 | 125 | 108 | 65 | 51 | | 55 | | 59 | | | 80 |
| | 3 | 129 | 128 | 106 | 80 | | 112 | | 113 | | | 137 |
| GW 1 (30) | 1 | 161 | 120 | 94 | 82 | | 118 | | 115 | | | 169 |
| | 2 | 99 | 136 | 108 | 102 | | 152 | | 152 | | | 201 |
| | 3 | 163 | 121 | 99 | 79 | | 116 | | 157 | | | 271 |
| GW 100 (30) | 1 | 130 | 104 | 72 | 52 | | 69 | | 73 | | | 87 |
| | 2 | 131 | 141 | 82 | 67 | | 97 | | 106 | | | 114 |
| | 3 | 114 | 97 | 62 | 48 | | 44 | | 56 | | | 73 |
| Blank | 1 | 18 | 19 | 18 | 17 | 17 | 19 | 17 | 17 | 19 | 18 | 18 |
| | 2 | 17 | 17 | 15 | 20 | 19 | 16 | 18 | 17 | 16 | 21 | 93 |
| | 3 | 18 | 17 | 18 | 18 | 18 | 14 | 14 | 19 | 19 | 17 | 15 |

Dpm* – disintegration per minute; R** - Replicate

## A.2.5 Concentration of residual isoproturon in IPU-dosed riverbed sediment treatments (Experiment 3)

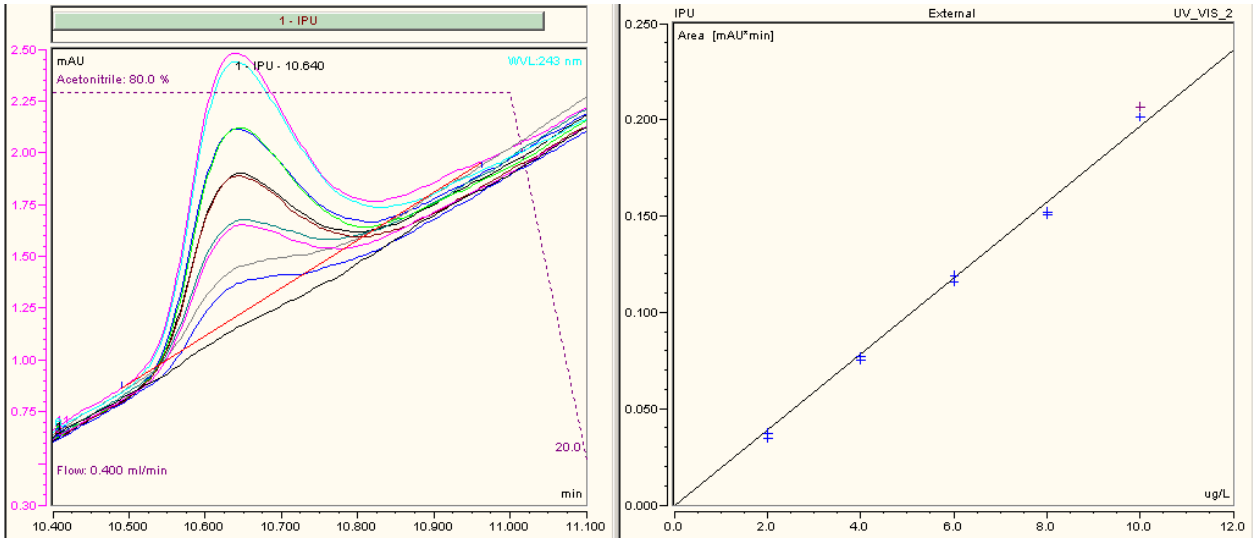


**Figure A.2.1** Calibration line for the samples from solid phase extraction (SPE) experiment with the concentration approximately 0.1 → 10 µg $L^{-1}$

**Table A.2.5** Concentration of isoproturon (µg $L^{-1}$) in IPU-dosed riverbed sediment treatments with 0.1 µg $L^{-1}$ after concentrating 100 times by SPE method

| | R0.1 1 | R0.1 2 | R0.1 3 | mean | SD | RSD (%) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| 0 incub | 4.14 | | | 4.14 | | | 41.4 |
| 5 incub | 3.10 | 3.08 | 4.21 | 3.46 | 0.65 | 18.7 | |
| 10 incub | 3.47 | 2.59 | 3.58 | 3.21 | 0.54 | 16.9 | |
| 30 incub | 0.00 | 0.00 | 0.00 | 0.00 | | | |

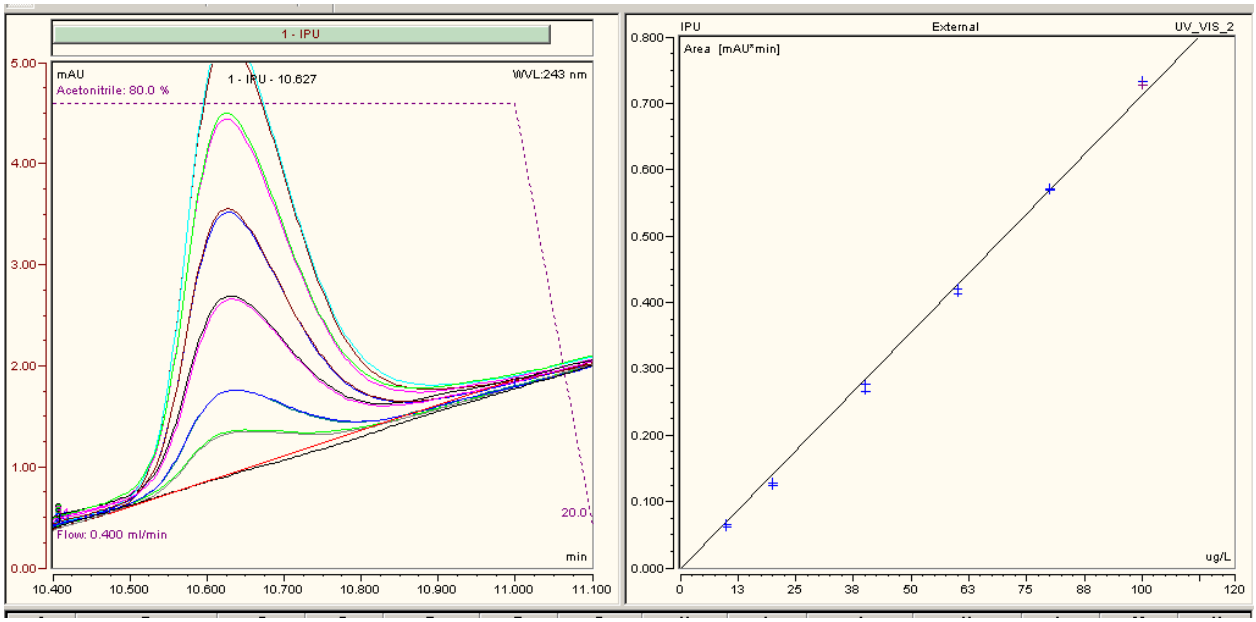


**Figure A.2.2** Calibration line for the samples from SPE experiment with the concentration approximately 1 → 100 µg $L^{-1}$

**Table A.2.6** Concentration of isoproturon (µg $L^{-1}$) in IPU-dosed riverbed sediment treatments with 1 µg $L^{-1}$ after concentrating 100 times by SPE method

| | R1 1 | R1 2 | R1 3 | mean | SD | RSD(%) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| 0 incub | 90.6 | 92.7 | 92.0 | 91.8 | 1.06 | 1.1 | 91.8 |
| 5 incub | 47.6 | 39.6 | 57.6 | 48.2 | 9.01 | 18.7 | |
| 10 incub | 51.1 | 58.1 | 73.2 | 60.8 | 11.29 | 18.6 | |
| 30 incub | 0.0 | 0.0 | 0.0 | 0.0 | | | |

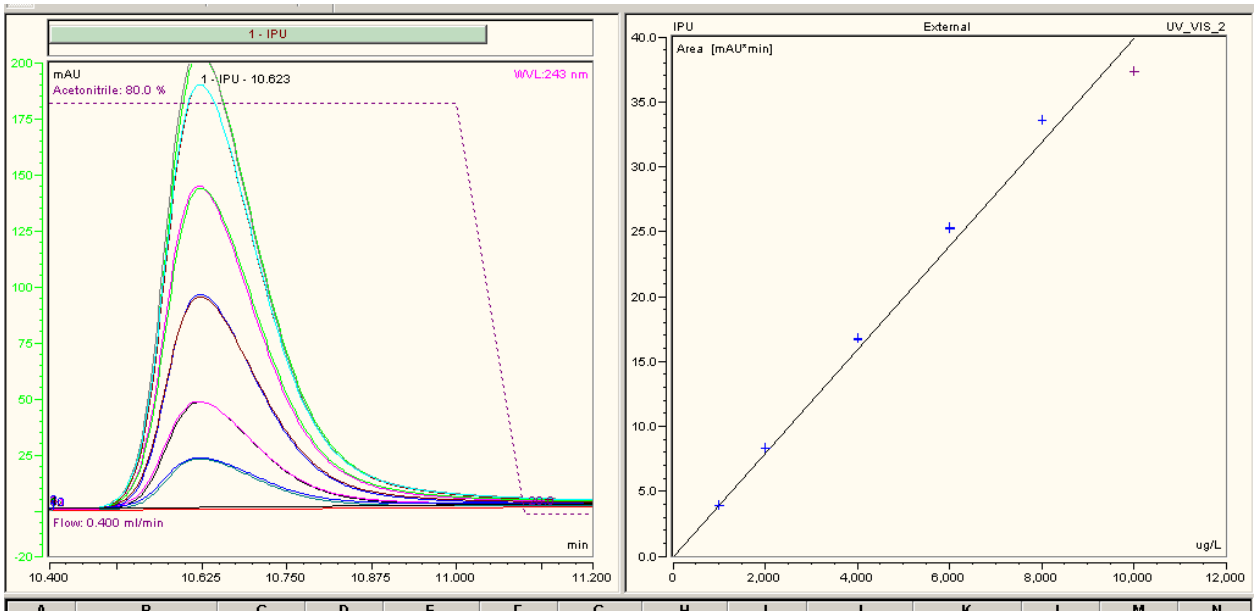


**Figure A.2.3** Calibration line for the samples from SPE experiment with the concentration approximately 100 → 10000 µg $L^{-1}$

**Table A.2.7** Concentration of Isoproturon (µg $L^{-1}$) in IPU-dosed riverbed sediment treatments with 100 µg $L^{-1}$ after concentrating 100 times by SPE method

| | R100 1 | R100 2 | R100 3 | mean | SD | RSD(%) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| 0 incub | 8639.0 | 8758.0 | 8463.0 | 8620.0 | 148.42 | 1.7 | 86.2 |
| 5 incub | 6128.0 | 5967.0 | 5242.0 | 5779.0 | 471.97 | 8.2 | |
| 10 incub | 8049.0 | 7401.0 | 7442.0 | 7630.7 | 362.87 | 4.8 | |
| 30 incub | 3658.0 | 2252.0 | 3482.0 | 3130.7 | 766.02 | 24.5 | |