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Fate and behaviour of pesticides in biobeds

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Fate and behaviour of pesticides in biobeds

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EXECUTIVE SUMMARY

Pesticide residues may be released to the farmyard, and may subsequently be washed off, and hence contribute a significant proportion of the pesticide load being released to surface waters. One approach to minimise releases is to install a treatment system such as a biobed. In its simplest form a biobed is a clay lined hole in the ground filled with a mixture of topsoil, peat and straw and covered with grass. The aim is to create an environment whereby maximum sorption of pesticides is achieved while maintaining bioavailability and optimum conditions for microbial decomposition. Biobeds have been used extensively in Sweden and have been shown to effectively retain and degrade pesticide waste arising from accidental spillages of concentrate and prepared pesticides. However, the suitability of a biobed to treat tank and sprayer washings has not yet been established. The aim of this research was to investigate the use of biobeds for treating the small drips and spills of pesticides that are an unavoidable feature of pesticide use, as well as spray tank and equipment washings under UK conditions.

The degradation and leaching potential of a number of pesticides with a range of physico-chemical properties was investigated in the laboratory and at the semi-field scale. Individual compounds as well as relatively complex mixtures were applied repeatedly to both topsoil and biomix at concentrations up to 20 times the maximum approved rate for soil. All pesticides were degraded in the biomix. The rate of degradation decreased with increasing concentration, however the effects were less significant in biomix relative to soil. Experiments performed using pesticide mixtures showed that interactions between pesticides are possible. However, these effects were again far less significant in biomix relative to topsoil, and with one exception DT_{90} values of < 6 months were calculated for biomix, suggesting that accumulation from one growing season the next should not occur. Degradation in the biomix was significantly quicker than in top soil following repeated applications of the same pesticide mixture. However, the rate of degradation decreased with each application in both matrices, probably a function of the number and frequency of the applications as well as the combined effects of concentration and pesticide interactions on the microbial community.

The relative performance in terms of leaching potential and degradation in lined and unlined biobed was also investigated. Whist the lined system effectively retained the pesticides, only limited degradation was observed, probably due to a number of factors, including low levels of bioactivity in the top of the biobed due to low moisture content and inhibition brought about by the high concentrations of a relatively complex mixture of pesticides. Furthermore, even after the introduction of costly measures to manage water inputs the biobed became waterlogged below 10cm depth within 12months. The use of unlined biobeds removed many of the problems associated with treating large volumes of liquid associated with decontaminating pesticide application equipment. By controlling water inputs and maximising retention time, through increased biobed depth, studies showed that all pesticides other than those classified as very mobile (K_{oc} <15) were effectively retained and subsequently degraded. Even for the highly mobile pesticides leaching losses were <6 %, representing a significant reduction in the amounts of these mobile pesticides reaching ground and surface water bodies.

Biobeds will be built on the farm by the farmer using locally available materials. It is likely that the physical and chemical characteristics of the raw materials and in particular the topsoil will vary. Studies showed there to be no significant difference in either degradation or leaching potential from biobeds when different topsoils were used in the preparation of the biomix. The biobed technology should therefore be readily transferable to the farm scale.

Studies have shown biobeds to able to treat pesticide waste arising from both accidental spillages of concentrate and prepared pesticides as well as the larger volumes of dilute waste associated with decontaminating the application equipment after use and as such would be a useful tool in reducing amounts of pesticide in UK waters. If adopted, they should reduce the concentrations of pesticides measured in environmental waters thus reducing both the risk to the aquatic environment and also the level of treatment required for drinking water supplies.

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GLOSSARY

a.i.	Active ingredient
ANOVA	Analysis of variance
CV	Coefficient of variation
Danish EPA	Danish Environmental Protection Agency
DAT	Days after treatment
DCM	Dichloromethane
DT_{50}	The time for concentrations to decrease to 50% of the initial value
DT_{90}	The time for concentrations to decrease to 10% of the initial value
EC	European Community
EEC	European Economic Community
ES	Electrospray reaction
EU	European Union
GC	Gas chromatograph
HDPE	High Density Polyethylene
HPLC	High performance liquid chromatography
ICM	Integrated crop management
KBr	Potassium bromide
Koc	Sorption coefficient
LC/MS	Liquid chromatography / mass spectrometry
LOQ	Limit of quantification
MEA	Malt extract agar
MEM	Monitoring mode

MWHC	Maximum water holding capacity
PVC-u	Polyvinyl-chloride
SBBR	Sequencing batch biofilm reactor
SC	Suspension concentrate
SE	Standard error
UK	United Kingdom
UV	Ultra Violet

Chapter 1

INTRODUCTION

Pesticides play an important role in the success of modern farming and food production, (*1*) and when used according to label instructions and with appropriate precautions, present minimal risk to the environment.(*2*) However, routine monitoring of surface and groundwaters has shown that contamination with pesticides does occur.(*3,4,5,6,7,8*) Where the water supply serves as a drinking water supply, treatment is often required in order to meet the standards set by the European Drinking Water Directive 80/778/EEC and the revised Directive (98/83/EC) of 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for all pesticides combined. Such treatment can be expensive, with around £1 billion being invested on treatment by the water industry in England and Wales since 1990.(*7*) The origin of these pesticides can be attributed to a number of sources. Diffuse or non point sources of pesticides typically have an indirect entry route into water and include releases from fields during and after the application process. This compares to non-diffuse or a point source whereby the entry route to water is direct, for example leakage from equipment, spillages and incorrect disposal of pesticide waste and washings, Table 1-1, Plate 1-1.(*6,9*)

Table 1-1 Sources of water contamination by pesticides

Diffuse	Point
Spray drift	tankfilling
volatilisation	spillages
surface runoff / overland flow	faulty equipment
leaching	washings and waste disposal
throughflow / interflow	sump, soakaways and drainage
drainflow	direct contamination including overspray
base flow seepage	consented discharges

Clearly, reducing the concentrations of pesticide reaching raw water supplies would be beneficial both in terms of protecting the aquatic ecosystem and also reducing the level of treatment required. Research over recent years has focused on the diffuse inputs of

pesticide residues to water resulting from the application of approved pesticides to land (*10,11,12,13,14*) and also to hard surfaces e.g. concrete and asphalt.(*15,16,17,18*) Optimisation of pesticide use through the adoption of Integrated Crop Management (ICM) and the implementation of environmental protection measures, e.g buffer zones have all helped in reducing these inputs.

Plate 1-1 Empty product containers left adjacent to surface water body potentially allowing direct and rapid contamination

Contamination arising from other sources such as non-approved use, poor practice, illegal operations, accidental releases and inputs of tank and equipment washings have recently been shown to be more significant than previously realised, with between 18 and 84% of the pesticide load measured in some individual catchments attributed to point sources.(*6,19,20,21,22,23,24,25,26,27*) Methods are in place to control some of these inputs for example, in the UK the Code of Practice for the Safe use of Pesticide on Farms and Holdings 1998 (currently under review) makes recommendations as to how pesticides should be used and any associated waste disposed of.(*28*) All filling, washing and disposal activities should be performed on an area so that accidental spillages and waste cannot escape from the area and contaminate soil, surface water or groundwater.

Any dilute pesticide should be disposed of in an environmentally acceptable manner and in accordance with the Groundwater Regulations 1998, either by:

- application of the waste to untreated or under-dosed parts of the field;
- better stewardship in the farmyard;
- storage of the waste pending collection by a licensed disposal contractor;
- improved farmyard design;
- use of equipment to treat the waste;

However, due to the practicalities associated with many the recommended procedures and / or a lack of awareness of the legislation, many users do not comply with the Code guidelines resulting in contamination of raw water, (*7,22*) and whilst treatment equipment is available, this can be costly to buy and maintain. Alternative methods are therefore required that are not only cheap to implement but also easy to manage.

In field mixing and equipment decontamination

The simplest approach would be to transfer all mixing and washing activities to the field, Plate 1-2.(*29*) Sufficient clean water (100 – 200 litres) is normally available in the field to wash the inside of the tank once, with the waste sprayed out in the field. The resultant residues will be intercepted by the crop and soil and will be subject to normal degradation processes. This approach is acceptable to regulators provided that the maximum application rate for the particular pesticide is not exceeded. Should more thorough cleaning be required, to allow for following crop sensitivity, additional clean water will be required. Unless available in the field this will require additional journeys between a clean water supply, normally the farmyard, and the intended disposal site, the field. As the number of ideal spraying days is limited, the additional down time spent travelling to and from the field is unlikely to be looked upon favourably. Furthermore, if the outside of the sprayer is also to be washed in the field a further 100 - 150 litres is required.(*22,30*) The use of modern equipment fitted with injection metering systems reduces the amount of waste generated, as the spray tank itself contains only clean water

and therefore the requirements for decontamination and the disposal of an associated waste are substantially reduced.(*31*) Such equipment undoubtedly reduces the amount of pesticide waste generated. However, the cost of such systems is significant and therefore is unlikely to be adopted by the vast majority of users.

Plate 1-2 Water tanker taken to the field to enable in-field mixing of pesticides

Stewardship

In the UK many of the water companies as well as the pesticide industry as part of their commitment to environmental protection have introduced stewardship campaigns.(*5,8,21,32,33,34*) These schemes are designed to encourage the users of pesticides to consider how their practices impact on the environment and the measures that can be taken to prevent pollution. Better training of sprayer operators and good machinery maintenance can reduce the number of accidental releases.(*35*) However, due to time constraints and other pressures small drips and spills of pesticides are still likely to occur.(*21,22*) Direct inputs from the decontamination of tractors and sprayers (*36*) and residues that remain in the sprayer sump after infield tank rinsing are also considered an unavoidable feature of the spraying operation.(*24,37*) In order to reduce these releases of pesticide to the environment a number of simple management changes have been suggested.(*23,38,39*) These include:

- the transfer of all mixing / handling activities from concrete to permeable hard core, to minimise surface runoff;
- prevention of surface water from the mixing area moving into the farmyard drainage system thus reducing the risk of direct contamination of adjacent surface water bodies;
- washing down away from the farmyard (i.e. in the field);
- not inverting empty containers. Empty spray containers can contain a significant amount of pesticide and unless sealed properly can leak;
- keep foil seals and container lids off the floor (i.e. place into cardboard packaging) as they can hold a significant amount of pesticide;
- store and dispose of empty spray containers and packaging correctly and as soon as possible to reduce the potential for contamination;
- avoid spraying when the soil is cohesive, contaminated soil may stick to tyres and be trafficked back to the farmyard;
- spray headlands last, to avoid driving over / through treated soil or crop
- store sprayer under cover to avoid pesticide residues retained on the external surfaces of the sprayer from being washed off by rainfall

Such stewardship activities have undoubtedly led to a greater understanding of how pesticides might reach water bodies, with the concentrations of pesticides in raw water reported to be reduced by more than 90% in certain catchments following the implementation of modified working practices.(*23,39*) However, in the UK the impact on the concentrations of certain pesticides that are measured in raw water supplies has so far been limited.(*7,8*)

Treatment and storage

The filling of agricultural sprayers and the decontamination of equipment is often performed at the same site year after year due to the convenience of a clean water supply, (*19*) and the location of the pesticide store. The design, management and

operation of these sites is therefore considered a primary target in reducing the amount of pesticide leaving the farmyard.(*40*) Traditionally these areas have been on concrete pads (*35*) which offer little opportunity of sorption and degradation (*17,18*) and which often connect directly to a soakway or water course, resulting in direct and rapid contamination. Modification of the sprayer fill site to allow spills and drips of pesticides as well as equipment washings to be retained and subsequently treated could be one alternative. A number of approaches are available and include:

- storage of the waste pending collection by a licensed disposal contractor;
- use of equipment to treat the waste;

Storage and disposal requires the purchase of at least one UV resistant double skinned tank at £1300 – 5000, with collection charges in the range of £70-80 per 1000 litres. However, if organophosphate compounds are used collection charges increase to £300-400 per 1000 litres. Furthermore, most contractors make an additional charge per collection to cover transport costs and cleaning. Over the course of a normal spray season a typical spray applicator can produce between 3800 and 15000 litres of pesticide contaminated waste water.(*41*) On this basis disposal costs could range form £250 - £6000 depending on the volume of waste. Clearly this approach may be acceptable to those users of pesticides who generate very low volumes of waste or those who can afford it.

Alternatively, environmental protection equipment, for example the Sentinel can be used to treat pesticide waste on the farm. The Sentinel system incorporates a system of chemically induced flocculation, followed by filtration through sand and activated carbon which removes organic substances from wastewater prior to its re-use or disposal. It takes 3 hours for 1000 litres to pass through the filters with complete treatment achieved in approximately 4 hours. Every 3000 litres it is necessary to consolidate the sludge, which accumulates during settlement. Disposal of sludge, waste liquid and exhausted carbon filters should be done through a licensed disposal contractor. The system has been shown to consistently remove > 99.9% of all pesticides tested.(*42*) Even though the Sentinel treatment system has been commercially available for 20 years or more (*42*), uptake has been limited.(*37*) Cost

has probably been the most limiting factor with regards to uptake, with an initial purchase price of £12500 for a standard 1000 litre unit, running costs of £25 per 1000 litres (including sludge disposal), £300 - £400 for an annual service and labour of 1 hour per 1000 litres. The Sentinel is therefore unlikely to be a cost effective way of treating pesticide waste and washing on the majority of farms. Other treatment systems are available for treating pesticide contaminated waste, for example reedbeds (*43*), sequencing batch biofilm reactors (SBBR) (*44*), evaporation beds (*45*) and oxidation systems (*46*), however few systems have shown to be economically viable at the farm scale.

A more cost effective approach to on-farm treatment may be to use a biobed to collect the spillages of pesticide associated with filling of the sprayer.(*23,47*) In its simplest form a biobed is a clay lined hole in the ground filled with a mixture of topsoil, peat and straw and covered with grass (in the volumetric proportions 1:1:2), with the aim of creating an environment whereby maximum sorption is achieved whilst maintaining bioavailability, thus allowing the retained pesticides to be degraded (Figure 1).(*47,48*)

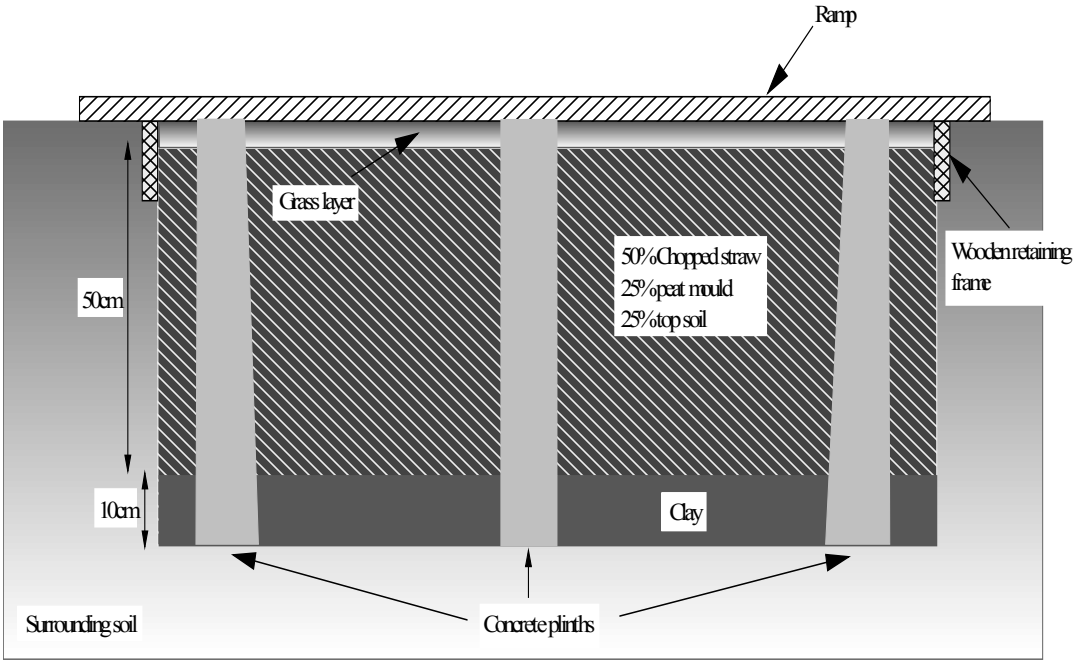

Figure 1-1 Basic biobed design

According to the basic Swedish design (*47*) the topsoil should be rich in humus but have a low clay content thus optimising the potential for sorption whilst maintaining bioavailability. The peat provides additional sites for sorption whilst helping to control the water balance within the system, with the straw acting as a source of lignin. Research suggests that the presence of organisms with ligninolytic potential result in the release of enzyme systems that are capably of degrading a wide range of chemicals including pesticides.(*49*) Increasing the amount of straw will increasing stimulate the degradation of pesticides.(*50*) However, for practical reasons no more than 50% should be incorporated into the biobed system.(*47*) Finally the biobed is covered with grass to help regulate the water balance. Generally, the biobed is equipped with a ramp enabling the tractor and sprayer to be driven over the bed and thus intercept drips and spills. Alternative biobed designs have been suggested, for example connection of the biobed to an adjacent concrete intercept area on which all mixing and washdown activities take place.(*51*) Biobeds have successfully been used in Sweden since 1993 with more than 1000 in use today.(*52*)

Whilst biobeds are being used extensively in Sweden for treating the small drips and spillages of pesticide that arise whilst mixing pesticides, it is likely that in the UK the system will also have to treat the additional pesticide load caused by contaminated runoff from the farmyard and the decontamination of the application equipment. Research is therefore required in a number of areas:

1.) The fate and behaviour in biomix of high concentrations of relatively complex mixtures of pesticides applied repeatedly.

2.) The effect of hydraulic loading on leaching potential and degradation from different biobed systems.

3.) The use of different topsoils in the construction of biomix and the effects on leaching and degradation from biobeds.

4.) Recommendations on the construction of the biobed are needed, such that the required level of treatment is achieved.

This thesis presents the work under taken by the candidate to address these issues and to the further develop biobeds for use under UK conditions.

Aims and Objectives

The overall aim of the PhD was to assess the fate of pesticides in biobeds under UK conditions. The specific objectives of this work were to; i) assess the degradation of pesticides and pesticide mixtures in biomix; ii) investigate the fate of pesticides in different biobed types under 'real world' conditions and iii) provide recommendations on the construction and operation of biobeds in the UK.

Format of presentation

The thesis is presented in the form of a collection of stand alone papers organised in chapters, which between them, address the aims and objectives described above.

Studies to assess the degradability of pesticides in biomix are described in chapters 2 and 3. Chapter 2 focuses on the effects of pesticide concentration and a binary pesticide mixture. More complex mixtures as well as the effects of repeat applications and the issue of bound residues are examined in chapter 3. Experiments investigating the degradability and leaching potential from different biobed systems are described in chapters 4 and 5. Chapter 4 compares the use of lined vs un-lined biobeds as well as performance of biomix relative to topsoil, whilst chapter 5 focuses on the degradability and leaching potential from biobeds when different topsoils are used in their construction. Chapter 6 provides recommendations on how biobeds should be constructed and operated under UK conditions. Finally chapter 7 discusses the findings reported in the preceeding chapters with respect to the uses of biobeds in the UK as an alternative system for treating dilute pesticide waste and washings on the farm. Future research priorities are identified and conclusions are drawn.

Chapters 2 to 6 of the present thesis have been prepared as stand alone papers for submission to international peer-reviewed journals. The status of the different papers with regard to the publication process is presented in Table 1-2. Although submission of the papers to different journals meant that the style of the manuscripts differed, all these documents have been reworked to provide a consistent style across this PhD thesis. For those papers which have been published, copyright rests with the publishers.

Context and disclosure

The work in this thesis was undertaken as a part-time staff candidate while working as a research scientist at Cranfield Centre for EcoChemistry in Shardlow. The body of the work presented was developed through research projects undertaken for the UK Department for Environment, Food and Rural Affairs, (projects PL0527, PL0543, PL0544), the Environment Agency (project P415), the Department of the Environment, Transport and the Regions (project EPG 1/5/104) and the Crop Protection Association (project JA3763E).

All five papers have been written by the candidate as leading author. However, it should also be noted that these papers have gained in quality through suggestions and editing from the co-authors. Chapters 2 – 6 which are in press and published, have also benefited from comments of referees as part of the review process.

Table 1-2 Publication status of the five papers presented in the PhD thesis

Authors	Paper Title	Journal	Status	Corresponding chapter in the thesis
Fogg, P.; Boxall, A.B.A & Walker, A.	Degradation of pesticides in biobeds:The effect of concentration and pesticide mixtures.	Journal of Agriculture and Food Chemistry	Published (Volume **51**; pages 5344-5349)	Chapter 2
Fogg, P.; Boxall, A.B.A, Walker, A. & Jukes A.A.	Pesticide degradation in a "biobed" composting substrate.	Pest Management Science	Published (Volume **59**; pages 527-537)	Chapter 3
Fogg, P.; Boxall, A.B.A, Walker, A. & Jukes A.A.	Degradation and leaching potential from biobed systems.	Pest Management Science	In press	Chapter 4
Fogg, P.; Boxall, A.B.A, Walker, A. & Jukes A.A.	The effect of different soil types on leaching potential and degradation of pesticides in biobeds.	Journal of Agriculture and Food Chemistry	In Press	Chapter 5
Fogg, P.; Boxall, A.B.A, Walker, A. & Jukes A.A.	Leaching of pesticides from biobeds: Effect of biobed depth and water loading.	Journal of Agriculture and Food Chemistry	In Press	Chapter 6

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Chapter 2

DEGRADATION OF PESTICIDES IN BIOBEDS: THE EFFECT OF CONCENTRATION AND PESTICIDE MIXTURES

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Abstract

Biobeds aim to create an environment whereby any pesticide spills are retained and then degraded, thus reducing the potential for surface or ground water contamination. Biobeds may receive high concentrations of relatively complex mixtures of pesticides. The effects of concentration and pesticide interaction on degradation rate were therefore investigated. At concentrations up to 20 times the maximum recommended application rate for isoproturon and chlorothalonil, the rate of degradation in topsoil and biomix decreased with increasing concentration. With the exception of isoproturon at concentrations above 11 mg kg^{-1}, degradation was quicker in biomix (a composted mixture of topsoil, compost and wheat straw) than in topsoil. One possible explanation for faster isoproturon degradation in topsoil as compared to biomix may be that previous treatments of isoproturon applied to the field soil as part of normal agricultural practices had resulted in proliferation of microbial communities specifically adapted to use isoproturon as an energy source. Such microbial adaptation could enhance the performance of a biobed. Studies with a mixture of isoproturon and chlorothalonil

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showed that interactions between pesticides are possible. In biomix the degradation of either isoproturon or chlorothalonil was unaffected by the presence of the other pesticide, whereas in topsoil, isoproturon DT_{50} values increased from 18.5 to 71.5 days in the presence of chlorothalonil. These studies suggest that biobeds appear capable of treating high concentrations of more than one pesticide.

Introduction

The filling and cleaning of agricultural spray equipment is often performed at the same site in the farmyard year after year due to the availability of a clean water supply.(*1,2*) The small drips and spills that can occur at these sites as part of normal agricultural practices (*3*) can result in high concentrations of pesticide being measured in both adjacent water courses and underlying groundwaters.(*1,2,4*) Biobeds aim to trap these drips and spills, and create an environment whereby maximum sorption is achieved while maintaining bioavailability and optimum conditions for microbial decomposition.(*5*) In its simplest form a biobed is a hole in the ground filled with a mixture of topsoil, peat and straw.(*5,6*) The biobed is covered with grass and equipped with a ramp enabling the tractor and sprayer to be parked over the bed while being filled. Studies in Sweden have demonstrated that biobeds can effectively retain and degrade pesticide waste arising from accidental spillages of concentrate and prepared pesticides (e.g. ureas , triazoles, trazines, carbamates), (*7*). Generally persistence increases with increasing concentration,(*2,4,8,9*) and at high concentrations, pesticides have been shown to depress microbial biomass and bioactivity; consequently, degradation may be inhibited.(*9*) In many agricultural situations the use of tank mixes and complex spray programmes is common practice.(*10,1,12*) There is evidence that the persistence of a number of pesticides may be changed when used in combination with other pesticides.(*10,12,13,14*) The objectives of the experiments reported here were, (a) to determine whether biobeds are able to degrade the high concentrations of pesticide that have been measured at spray fill sites, and (b) to study the effects of binary pesticide mixture on degradation rates of individual compounds.

Materials and Methods

Biomix was prepared by mixing topsoil (69% sand, 13% silt, 18% clay, organic mater 1.95%, pH 6.15, maximum water holding capacity 37% w/w), peat free compost (Levington Peat Free Universal), and winter wheat straw in the volumetric proportions of 1:1:2 respectively. The mixture (organic matter 12.36%, pH 7.5, maximum water holding capacity 121% w/w) was composted outside for 80 - 100 days then macerated using a food processor, air dried to approximately 30 - 40% w/w, and refrigerated at a 0-10 °C prior to use. A sample of topsoil, used in the preparation of the biomix was air dried, passed through a 5.4 mm mesh sieve and refrigerated with the biomix prior to use. Disturbed sub-samples of topsoil and biomix were re-packed into 222 cm^3 volumetric tins and the maximum water holding capacity determined by capillary rise.(*15*) The test chemicals were isoproturon and chlorothalonil, which were selected on the basis of their physico-chemical properties, in particular their sorption potential and water solubility and reported degradation rates (Table 2-1), and represent compounds that are of relatively high annual usage,(*16*).

Table 2-1. Study compounds and their reported physico-chemical charactersistics

Active Substance	K_{oc} mL g^{-1}	DT_{50} (days)	Water Solubility (mg L^{-1})
Isoproturon (SC*)	100	6 - 28	65
Chlorothalonil (SC*)	1600 - 14000	6 - 43	0.81

Values taken from Wauchope et al. (1992) (*27*) and Tomlin (2000) (*28*)

* Suspension Concentrate

Effect of concentration on degradation rates

Samples (25 g) of moist topsoil or biomix were weighed into clear glass bottles (125 mL) fitted with bakelite screw cap lids to provide 3 treated replicates and 1 untreated control per sampling time point. Sub-samples of each matrix were taken and moisture contents determined by oven drying at 105°C ±2°C for 24 hours. Formulated isoproturon (Alpha Isoproturon 500™, 43.6 %w/w) and chlorothalonil (Cropgard™,

41.57% w/w) were used to make up stock suspensions in tap water of 5190 and 3118 mg a.i. L^{-1}. Serial dilutions of the stock samples were made in order to achieve final fresh weight concentrations of 11, 23, 46, 91, 228 and 456 mg kg^{-1} of isoproturon and 7, 14, 29, 57, 143 and 287 mg kg^{-1} of chlorothalonil in topsoil and biomix. Topsoil and biomix were treated with either 2.2 mL (isoproturon) or 2.3 mL (chlorothalonil) of the appropriate pesticide suspension in order to achieve a final moisture content of 15 % w/w in topsoil and 105 % w/w in biomix, (40% and 87% of the respective maximum water holding capacities). Tap water was used to adjust the moisture content in untreated samples. Immediately after treatment, three treated replicates and one untreated control were taken for each concentration and frozen (-20°C). The remaining samples were loosely capped and incubated in the dark at 20°C. At intervals of 3, 10, 20, 30, 46, 60 and 90 days after treatment (DAT) three soil and three biomix samples were collected for each chemical treatment, with a single sample from the untreated controls. The samples were stored at –20°C prior to analysis.

Effect of pesticide mixtures on degradation rate

Samples (25 g) of moist topsoil or biomix were weighed into clear glass bottles (125 mL). Individual stock suspensions of 925 and 555 mg a.i. L^{-1} were made up in tap water using formulated isoproturon and chlorothalonil, respectively. For mixture experiments an appropriate isoproturon and chlorothalonil mixture was prepared. Samples were treated with 2.6 mL of the respective stock suspension in order to achieve final fresh weight concentrations of 96 mg kg^{-1} for isoproturon and 58 mg kg^{-1} for chlorothalonil and a moisture content of 15 % w/w for topsoil and 105 % w/w for biomix. Tap water was used to adjust the moisture content in control samples. Following treatment, three treated replicates and 1 untreated control sample were removed and placed immediately into freezer storage. Remaining samples were loosely capped and incubated in the dark at 20°C. At intervals of 3, 10, 20, 31, 60 and 97 DAT three topsoil and three biomix samples were collected from each chemical treatment, with a single sample from the untreated controls. Samples were stored at –20 °C prior to analysis.

Analysis

Isoproturon and chlorothalonil were extracted from topsoil and biomix by shaking for 1 hour with 50 ml methanol on an end over end shaker. The resulting extracts were analysed by HPLC. The extraction efficiencies for isoproturon were >90 % and for chlorothalonil > 82 % in both topsoil and biomix. Concentrations of isoproturon and chlorothalonil were determined using a Kontron Series 320 Pump linked to a Kontron Series 332 UV detector. Samples of extract (20 µl) were injected using a Kontron Series 360 autosampler. Separation was achieved using a Lichrosorb RP18 column (250 mm x 4 mm i.d.) and a mobile phase flow rate of 1 ml/minute. The mobile phase was acetonitrile:water:phosphoric acid (75:24.75:0.25 v/v). The detection wavelength for both compounds was 230 nm and the retention times were 3.18 and 4.56 minutes for isoproturon and chlorothalonil respectively.

Data Analysis

Where possible the first order rate equation was fitted to the observed concentrations, (Equation 1),

$$\frac{dC}{dt} = -kC \qquad \text{(Equation 1)}$$

where C is the concentration (mg kg^{-1} soil), t is the time (days) and k is the degradation rate ($days^{-1}$). The integrated form of this equation (equation 2) was fitted to non-transformed data using the least squares method in order to give the best agreement between calculated and observed concentrations.

$$C_{(t)} = C_0 \exp(-kt) \qquad \text{(Equation 2)}$$

However, the first order rate equation is often considered unacceptable if the determination coefficient (r^2) falls below 0.7 (*17*). Where data indicated increasing rates of degradation with time, DT_{50} and DT_{90} values were calculated using an empirical two-parameter relationship,

$$S/S_0 = \exp\{k_1[1-\exp(k_2 t)]\} \qquad \text{(Equation 3)}$$

where S_0 and S are the concentrations of pesticide at time 0 and time t, respectively. Microsoft Excel Solver was used to estimate parameters k_1 and k_2 using the least squares method in order to give the best agreement between calculated and observed concentrations. The degradation data were summarised by calculating the times to 50% degradation (DT_{50}) and the time to 90% degradation (DT_{90}) from the calculated degradation curves using the relationship;

$$DT_{50} = \ln(1-\ln(0.5)/k_1)/k_2 \qquad \text{(Equation 4)}$$

$$DT_{90} = \ln(1-\ln(0.1)/k_1)/k_2 \qquad \text{(Equation 5)}$$

Similarly where the pattern of degradation was bi-phasic with residue concentrations decreasing slowly after an initial rapid decline, data were fitted to a bi-exponential decay curve. The bi-exponential curve consists of two exponential terms,

$$C_{(t)} = A\exp(-k_1 t)+B\exp(-k_2 t) \qquad \text{(Equation 6)}$$

where $C_{(t)}$ (mg kg^{-1} soil) is the concentration at time *t*, A (mg kg^{-1} soil) and B (mg kg^{-1} soil) are constants, k_1 ($days^{-1}$) and k_2 ($days^{-1}$) determine the decline of the first and second component of the curve, respectively. (*17*)

Results

Effect of concentration on degradation rate

Results from the experiments to investigate the effects of initial concentration are summarised in (Table 2-2) for isoproturon and (Table 2-3) for chlorothalonil. The pattern of isoproturon degradation in topsoil and biomix is shown in (Figure 1-1). At all concentrations in biomix and at concentrations below 46 mg kg^{-1} in topsoil degradation curves were fitted to a simple first order rate equation, (Equation 1). Above 23 mg kg^{-1} concentration in topsoil, the pattern of decline could not be fitted to simple first order kinetics; data indicated increasing rates of degradation with time, with fitted curves showing a short lag phase before the onset of rapid degradation. DT_{50} values for isoproturon in biomix and topsoil ranged from 8.6 to 44.2 days and 9.4 to 34.7 days, respectively. Although a significant ($P < 0.001$) concentration effect was observed in both topsoil and biomix there were no significant differences in the DT_{50} values between substrates. DT_{90} values also highlighted a significant ($P < 0.001$) concentration effect and also a significant ($P<0.001$) difference between degradation rates in topsoil and biomix with DT_{90} values ranging from 29.1 to 51.7 days and 28.5 to 147 days, respectively.

The degradation patterns for chlorothalonil in topsoil and biomix are shown in Figure 2-2). With the exception of biomix treated at 287 mg a.i. kg^{-1} degradation could be interpreted using first-order reaction kinetics, (Equation 2). The pattern of degradation in biomix treated at the highest concentration showed a bi-phasic pattern where residues decreased slowly after an initial rapid decline and persisted at a low levels until the end of the experimental period. Data were therefore fitted to a bi-exponential decay curve, (Equation 6). DT_{50} values ranged from 6.1 to 76.9 days in topsoil and 0.6 to 20.4 days in biomix (Table 2-3). Chlorothalonil degradation was significantly ($P < 0.001$) faster in biomix than in topsoil. However in both matrices, degradation rates decreased with an increase in chlorothalonil concentration ($P < 0.001$). There was a marked increase in the both DT_{50} and DT_{90} values in topsoil up to 57 mg kg^{-1} concentration. At concentrations above 57 mg kg-1, degradation rates showed comparatively lower increases in magnitude.

Table 2-2 DT_{50} and DT_{90}, degradation rate constants (*k*) and determination coefficients (r^2) for isoproturon in topsoil and biomix

Concentration	Topsoil						Biomix					
mg kg^{-1}	DT_{50} (days)	± 1 SE	DT_{90} (days)	± 1 SE	*K* deg (days $^{-1}$)	r^2	DT_{50} (days)	± 1 SE	DT_{90} (days)	± 1 SE	*K* deg (days $^{-1}$)	r^2
11	9.4*	0.5	31.3*	1.5	0.0735	0.98	8.6*	0.2	28.5*	0.8	0.0809	0.99
23	10.8*	0.6	35.9*	2.0	0.0641	0.96	11.1*	0.1	36.9*	0.2	0.0624	0.99
46	19.2**	0.4	29.1**	2.3	*k1*) 0.0888 *k2*) 0.1132	1.00	13.1*	0.2	43.4*	0.8	0.0530	0.99
91	22.1**	3.4	31.8**	6.3	*k1*) 0.0533 *k2*) 0.1192	1.00	16.2*	0.1	53.9*	3.2	0.0427	0.99
228	30.4**	2.7	37.7**	2.3	*k1*) 0.0048 *k2*) 0.1637	1.00	29.2*	1.4	97.1*	4.6	0.0237	0.97
456	34.7**	5.9	51.7**	5.5	*k1* 0.0771 *k2*) 0.0664	1.00	44.2*	1.8	146.9*	5.9	0.0157	0.91

k1 and *k2* determine the decline of the first and second part of the degradation curve respectively.

* The integrated form of the first order rate equation (equation 2) was used to calculate the DT_{50} and DT_{90} values respectively

** The two parameter empirical model (equation 3) was used to calculate the DT_{50} and DT_{90} values respectively

Table 2-3 DT_{50} and DT_{90} degradation rates, degradation rate constants (*k*) and determination coefficients (r^2) for chlorothalonil in topsoil and biomix

Concentration	Topsoil						Biomix					
mg kg^{-1}	DT_{50} (days)	± 1 SE	DT_{90} (days)	± 1 SE	*K* deg (days $^{-1}$)	r^2	DT_{50} (days)	± 1 SE	DT_{90} (days)	± 1 SE	*K* deg (days $^{-1}$)	r^2
7	6.1*	0.1	20.2*	0.5	0.1141	0.99	0.6*	0.3	2.1*	1.0	1.1159	1.00
14	11.5*	0.5	38.1*	1.7	0.0605	0.99	0.9*	0.1	3.0*	0.3	0.7649	1.00
29	23.0**	1.1	105.9**	7.7	*k1*) 0.8949 *k2*) 0.0230	0.99	2.3*	0.3	7.6*	1.1	0.3048	1.00
57	47.9**	1.3	178.3**	6.3	*k1*) 0.0909 *k2*) 0.0123	0.93	3.8*	0.5	12.5*	1.6	0.1845	1.00
143	46.4**	3.7	184.7**	17	*k1*) 0.1259 *k2*) 0.0116	1.00	20.4*	1.2	67.9*	4.0	0.0339	0.99
287	79.6*	1.8	264.3*	6.1	0.0087	0.94	10.0**	9.0	126.5**	9.5	k1 0.0138 k20.5331	0.97

k1 and k2 determine the decline of the first and second component of the degradation curve respectively.

* The integrated form of the first order rate equation (equation 2) was used to calculate the DT_{50} and DT_{90} values respectively

** The bi-exponential model (equation 3) was used to calculate the DT_{50} and DT_{90} values respectively

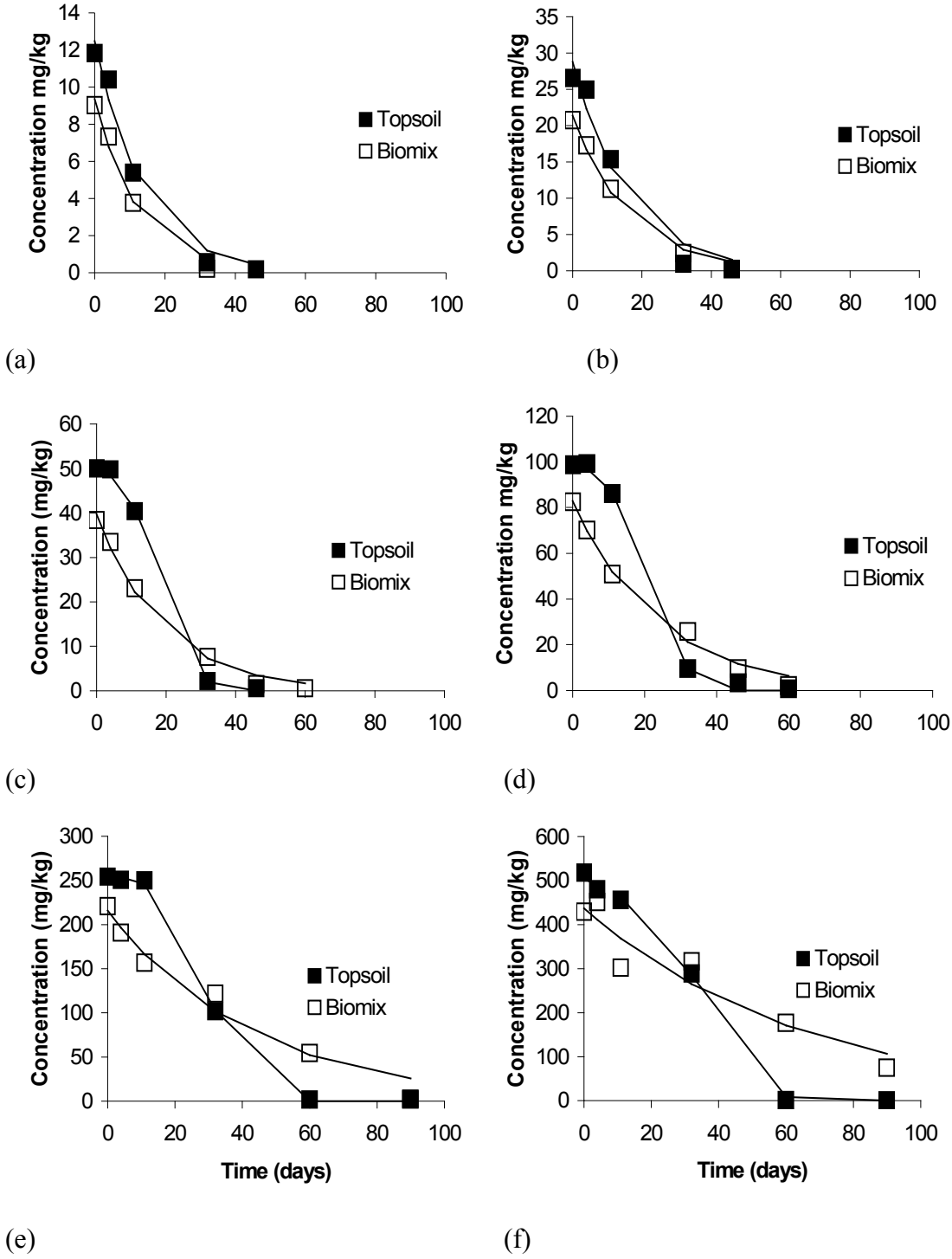


Figure 2-1 Degradation of isoproturon in topsoil and biomix at treatment rates of (a) 11, (b) 23, (c) 46, (d) 91, (e) 228 and (f) 456 mg kg^{-1}

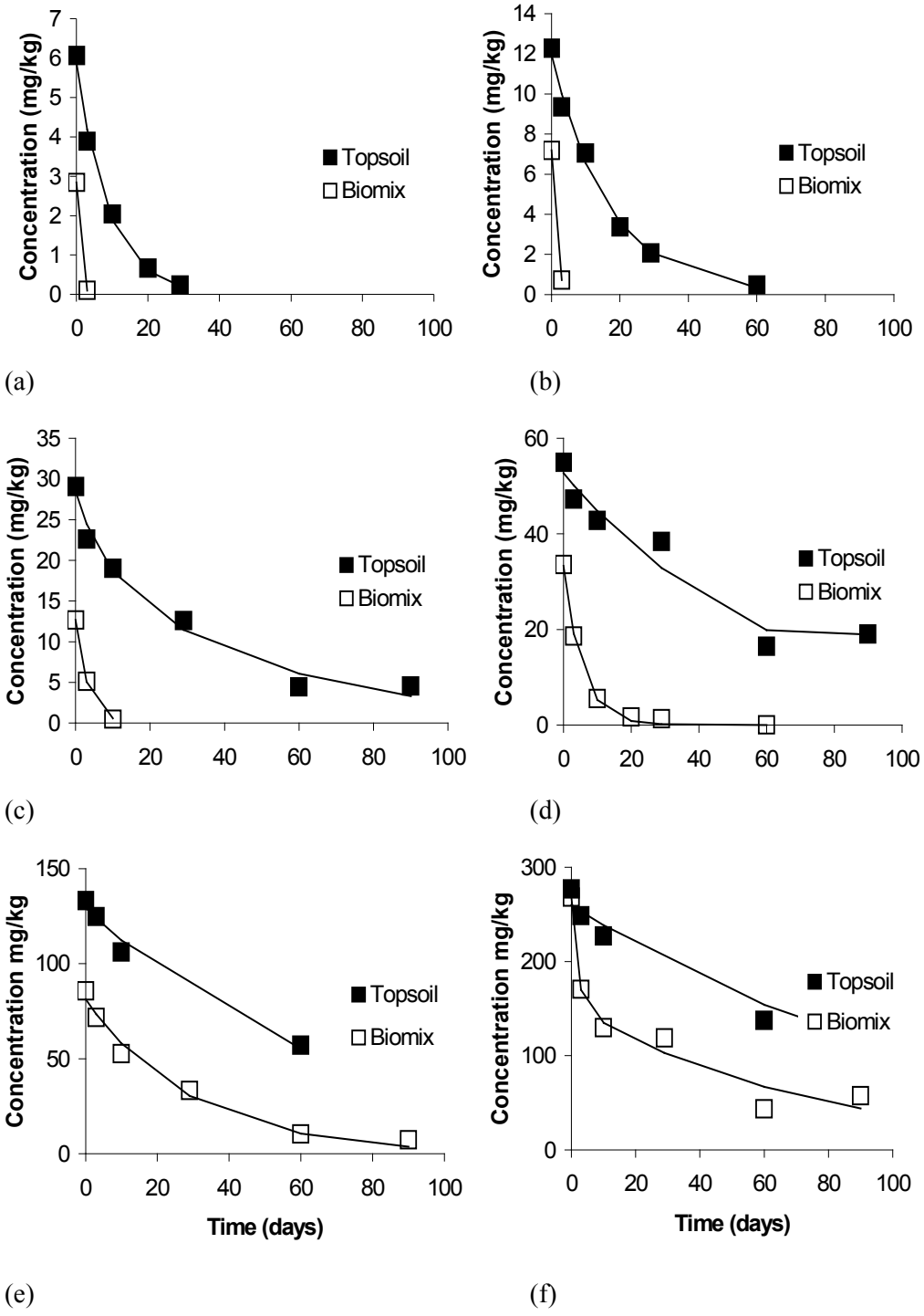


Figure 2-2 Degradation of chlorothalonil in topsoil and biomix at treatment rates of (a) 7, (b) 14, (c) 29, (d) 57, (e) 143 and (f) 287 mg kg^{-1}

Effect of pesticide mixtures on degradation rate

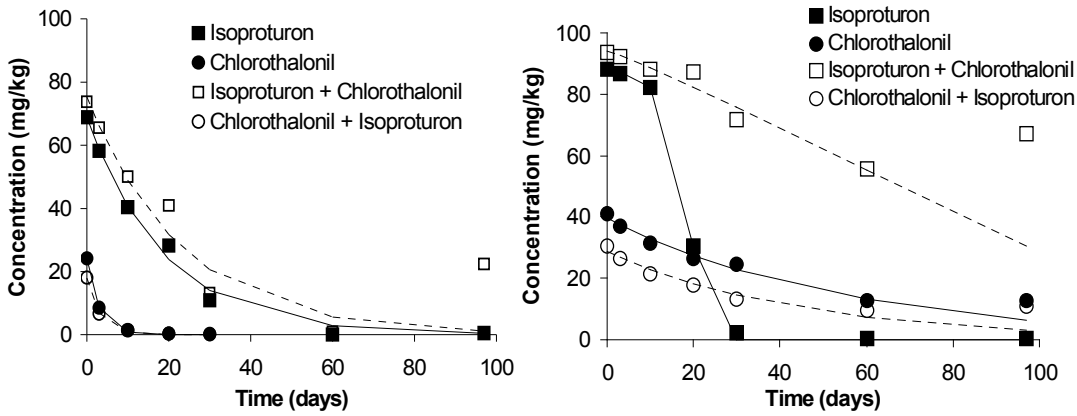
Degradation rates in biomix for either isoproturon or chlorothalonil applied individually or as a mixture were similar (Table 2-4). Degradation data were interpreted by first order kinetics (Equation 2, Figure 2-3a). For isoproturon DT_{50} values of 13.1 days and 16.0 days were calculated for individual and mixture treatments respectively. For chlorothalonil, half-lives of 2.0 days were calculated for chlorothalonil alone and 2.2 days when mixed with isoproturon.

Table 2-4 DT_{50} and DT_{90} degradation rates, degradation rate constants (*k*) and determination coefficients (r^2) for isoproturon and chlorothalonil in topsoil and biomix applied individually and as a mixture

	Topsoil				Biomix			
	DT_{50} (days)	DT_{90} (days)	k deg ($days^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	k deg ($days^{-1}$)	r^2
Isoproturon	18.5	22.8	a) 0.0044 b)0.2744	1.00	13.1	43.4	0.0530	0.99
Isoproturon + chlorothalonil	71.5	140.9	a)0.4868 b)0.0124	0.96	16.0	53.2	0.0433	0.96
Chlorthalonil	37.5	124.4	0.0185	0.98	2.0	6.7	0.3429	1.00
Chlorothalonil + isoproturon	30.0	99.6	0.0231	0.97	2.2	7.2	0.3178	1.00

Patterns of isoproturon and chlorothalonil degradation in topsoil are shown in (Figure 2-3b). The data for chlorothalonil degradation in the presence or absence of isoproturon were fitted to the first-order rate equation, (Equation 2) with similar DT_{50} values of 30.0 days for chlorothalonil alone and 37.5 days in the presence of isoproturon. First order kinetics could not be fitted to the data for isoproturon degradation whether applied alone or in combination with chlorothalonil. As observed previously, at concentrations above 46 mg kg^{-1}, isoproturon degradation rates increased with time, with the curves showing a short lag phase before the onset of rapid degradation, particularly for the individual treatment. Data were therefore fitted to Equation 3. A DT_{50} of 18.5 days was

calculated for isoproturon applied alone to topsoil. In the presence of chlorothalonil there was a significant ($P < 0.01$) increase in DT_{50} to 71.5 days.



(a) (b)

Figure 2-3 Isoproturon and chlorothalonil degradation in (a) biomix and (b) topsoil when applied individually and as a mixture

Discussion

Significant contamination of the spray fill site can occur due to its repeated use and can represent a significant pesticide load even when following best agricultural practices. (*1,18,19*) To minimise the impact of these normal practices on water quality within agricultural catchments biobeds are being developed. The experiments presented herein were made to investigate the ability of biobeds to treat such pesticide waste.

In both topsoil and biomix the rate of isoproturon degradation decreased with increasing concentration. Similar results for isoproturon degradation in topsoil at elevated concentrations have been reported. (*2*) DT_{50} values for isoproturon in biomix and topsoil were similar and were < 45 days for both matrices, which can be classified as moderately persistence. (*20*) However, for biobed treatment systems the DT_{90} measurement may be of more significance in order to determine whether or not compounds are likely to accumulate. DT_{90} values of > 1 year indicate that accumulation may be a problem when routine applications are made. (*21*) For isoproturon DT_{90}

values in biomix were <147 days and for topsoil <52 days. One possible explanation for the higher overall rate of isoproturon degradation in topsoil relative to biomix is the fact that the topsoil used for the experiment had been treated on previous occasions with isoproturon as part of normal agricultural practices. These previous treatments may have resulted in the proliferation of microbial communities specifically adapted to utilise the compound as an energy source, resulting in enhanced biodegradation, as reported by Cox et al. (*22*)

Chlorothalonil at concentrations of 7 to 287 mg kg^{-1} degraded more quickly in biomix than in topsoil at all concentrations, with the amount degraded per unit of time decreasing with increasing concentration. In biomix, the decrease in degradation rate with increasing concentration was linear over the range of concentrations investigated. However, in topsoil, the rate of degradation decreased rapidly up to 57 mg kg^{-1} concentration. Above 57 mg kg^{-1}, the decrease in degradation rate was less pronounced. The differences in chlorothalonil degradation rate may be a consequence of two effects which respond differently in biomix and soil. Firstly chlorothalonil is degraded both biologically and by chemical transformation. (*23*) Secondly there is strong evidence that a metabolite of chlorothalonil (4-hydroxy-2,5,6-trichloroisophtalonitrile, TPN-OH) inhibits the degradation of the parent compound. (*24*) Other studies have shown that microbial activity is depressed in chlorothalonil-treated soils. (*10,24*) Thus, the association of decreasing degradation rates with increasing chlorothalonil concentrations suggested that biodegradation may have been suppressed. The observed degradation of chlorothalonil may have been due to the predominance of the comparatively slower chemical hydrolysis. Chemical transformation may be slower than the rate of biodegradation. However if hydrolysis rate is independent of concentration, this could explain why the relationship between concentration and degradation was not linear above 57 mg kg^{-1} concentration. In biomix it is possible that there is both increased microbiological activity and increased sorption of TPN-OH. Whilst there was a gradual decrease in the rate of chlorothalonil degradation with increased concentration, the effects were less significant than in topsoil over the range of concentrations investigated.

Most studies of the environmental fate of pesticides are done with single applications of one compound. However, in practice repeated applications of tank

mixes containing herbicides, fungicides and insecticides are made. (*10,11,12,14*) Biobeds are likely to receive complex mixtures of more than one active substance applied repeatedly at concentrations far higher than field treatment rates. Studies investigating isoproturon and chlorothalonil degradation when applied as a mixture demonstrated that the rate of degradation of either compound in biomix was similar when applied individually or in the presence of the other pesticide. However, whilst the rate of chlorothalonil degradation in topsoil was similar when applied individually or with isoproturon, isoproturon degradation was inhibited in the presence of chlorothalonil. This inhibition maybe due to a number of factors. The presence of the metabolite (TPN-OH) as reported by Montonaga et al., (*24*) who found that applications of chlorothalonil inhibited the degradation of chlorothalonil. Similar inhibition has been reported for other pesticides Singh et al., (*10*). Alternatively one of the side effects from applying chlorothalonil may have been to suppress the activity of non-target soil micro-organisms, (*25,26*) thus inhibiting the rate at which isoproturon was degraded.

Conclusions

Biobeds are intended to retain and subsequently degrade the pesticide waste originating from spray fill sites. They aim to create an environment whereby maximum sorption is achieved whilst maintaining bioavailability. Due to repeated use of the same filling sites, biobeds are likely to be exposed to high concentrations of more than one pesticide. This study investigated the effects of concentration and mixtures on pesticide degradation rate. At concentrations ranging from half to 20 times the maximum recommended application rate for isoproturon and chlorothalonil, the rate of degradation decreased with increasing concentration. Degradation was generally faster in biomix than in topsoil at all concentrations with the exception of isoproturon at concentrations above 91 mg kg^{-1}. The higher rates of isoproturon degradation in topsoil are thought to be due to previous treatments of isoproturon that resulted in the proliferation of microbial communities adapted to use isoproturon as an energy source (*22*). Studies with a mixture of isoproturon and chlorothalonil showed inhibitory effects of chlorothalonil on isoproturon degradation in topsoil. These antagonistic effects were not apparent in the biomix soil. The results suggested that biobeds are capable of

treating high concentrations of more than one pesticide. However, mixture studies were performed using only a single application of two active substances. We have also examined the effects of applying a mixture of 6 pesticides applied repeatedly to biobeds. The results from these more intensive studies will be presented elsewhere.

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Chapter 3

PESTICIDE DEGRADATION IN A "BIOBED" COMPOSTING SUBSTRATE

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Abstract

Pesticides play an important role in the success of modern farming and food production. However, the release of pesticides to the environment arising from non-approved use, poor practice, illegal operations or misuse is increasingly recognised as contributing to water contamination. Biobeds appear to offer a cost effective method for treating pesticide contaminated waste. This study was performed to determine whether biobeds can degrade relatively complex pesticide mixtures when applied repeatedly. A pesticide mixture containing isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate was incubated in biomix and topsoil at concentrations to simulate pesticide disposal. Although the data suggest that interactions between pesticides are possible, the effects were of less significance in biomix than in topsoil. The same mixture was applied on 3 occasions at 30 day intervals. Degradation was significantly quicker in biomix relative to topsoil. The rate of degradation however decreased with each additional treatment, possibly due to the toxicity of the pesticide mixture to the microbial community. Incubations with chlorothalonil and pendimethalin carried out in sterile and non-sterile biomix indicated that degradation was the main

Fogg P, Boxall ABA, Walker A and Jukes A, Pesticide degradation in a 'biobed' composting substrate. *Pest Manag Sci* **59**: 527-537 (2003)

mechanism responsible for the reduction in recovered residues. Results from these experiments suggest that biobeds offer a viable means of treating pesticide waste.

Introduction

Pesticide contamination of surface waters can arise from a number of sources, including releases from fields during and after the application process, leakage from equipment, spillages, and incorrect disposal of waste pesticide and washings.(*1*) While the movement of pesticides to surface waters from treated fields has been extensively investigated,(*2,3*) only recently has the contamination arising from the other sources been considered.(*4,5,6*) These studies indicate that these sources may make a significant contribution to pesticide contamination of surface waters in the UK.(*4*)

Releases due to incorrect disposal, leakages and spillages can be better controlled through training of sprayer operators and good machinery maintenance.(*7*) Moreover, if the operator follows the Code of Practice for the Safe use of Pesticides on Farms and Holdings (1998, currently under review) (*8*) and the Groundwater regulations (1998),(*9*) releases from tank washings will be minimised. However, due to the practicalities and costs associated with the recommended procedures and the fact that spillages can never be totally avoided, it would be beneficial to employ additional methods of control.

A number of possible approaches are available including: 1) spray equipment could be washed in the field, (*10,11*) thus reducing the requirements for decontamination at the farmyard and the disposal of any associated waste; 2) better design of the farmyard to minimise release of pesticides to nearby surface waters; or 3) treatment systems that are installed on the farmyard to treat any waste arising from spray equipment and during the filling process. Possible treatment systems include the Sentinel,(*12,13*) which combines a chemical treatment process with filtration to remove organic substances from water, or the use of biobeds. Whilst the Sentinel system effectively treats waste and washings, it is costly to install and to maintain.(*14,15*) In contrast, the biobed is a low cost and low maintenance system. In its simplest form a biobed is a hole in the ground filled with a

mixture of topsoil, peat and straw.(*16,17*) The biobed is covered with grass and equipped with a ramp enabling the tractor and sprayer to be driven over the bed.

Studies in Sweden have demonstrated that biobeds can effectively retain and degrade pesticide waste arising from accidental spillages of concentrate and prepared pesticides.(*18*) However, the suitability of a biobed to treat tank and sprayer washings has not yet been established. This study was therefore performed to assess the suitability of biobeds for treating pesticides arising from tank and sprayer cleaning processes and spillages. The specific objectives of the study were to: 1) determine the degradation rates of a wide range of pesticides in the biobed mixture at concentrations that might be expected in the real world; 2) investigate the degradability of mixtures of pesticide in the biobed mixture; and 3) explore the effects of repeated applications on the performance of a biobed.

Materials and Methods

Test matrices and chemicals

The biobed matrix (Biomix) was prepared by mixing topsoil (69% sand, 13% silt, 18% clay), peat free compost (Levington Peat Free Universal) and winter wheat straw in the volumetric proportions of 1:1:2 respectively. Peat free compost was selected as it is a more 'environmentally friendly' alternative to the peat mould that has been used in biobeds in the past. The mixture was composted outside for 80 - 100 days then macerated using a food processor, air dried to approximately 30 - 40% w/w and refrigerated at a 0-10 °C prior to use. A sample of the same topsoil was air dried, passed through a 5.4mm mesh sieve and refrigerated with the biomix prior to use. Disturbed sub-samples of topsoil and biomix were re-packed into 222 cm^3 volumetric tins and the maximum water holding capacity determined by capillary rise.(*19*)

Test chemicals (isoproturon (IPU), chlorothalonil (CT), pendimethalin (PE), chlorpyrifos (CP), epoxiconazole (EP) and dimethoate (DI) were selected to give a

range of physico-chemical properties and reported degradation rates in soil, and to represent compounds that were of relatively wide annual usage, (Table 3-1).(*20*)

Degradation of pesticides in biomix and topsoil

The degradation of each of the test chemicals in soil and biomix was determined over time. Samples of topsoil and biomix were weighed in glass jars (125 mL) to give 24 samples of topsoil (25 g) and 24 samples of biomix (25 g) for each chemical treatment. The topsoil and biomix samples were then treated with 1.9 ml of suspensions made up in tap water containing either 1233 mg L^{-1} IPU, 986.5 mg L^{-1} PE, 354.9 mg L^{-1} CP, 739.8 mg L^{-1} CT, 246.6 mg L^{-1} EP or 167.5 mg L^{-1} DI. This resulted in concentrations of 94 (IPU), 75 (PE), 27 (CP), 56 (CT), 19 (EP) and 13 (DI) mg kg^{-1} fresh soil or fresh biomix. A further 8 samples of soil and 8 samples of biomix were prepared to act as untreated controls.

Table 3-1 Study compounds and their reported physico-chemical charactersistics

Active Substance	K_{oc} mL g^{-1}	DT_{50} (days)	Water Solubility (mg L^{-1})
Isoproturon (SC)	100	6 - 28	65
Pendimethalin (SC)	5000	90 - 120	0.3
Chlorpyrifos (EC)	6000	60 - 120	1.4
Chlorothalonil (SC)	1600 - 14000	6 - 43	0.81
Epoxiconazole (SC)	957 - 2647	60 - 90	6.6
Dimethoate (EC)	16 - 52	7 - 16	22300

Values taken from Wauchope et al. (1992) (*35*) and Tomlin (2000) (*36*)

Immediately following treatment, 3 treated samples and 1 control sample of topsoil and biomix for each active ingredient were removed and stored at -20°C prior to analysis. The remaining samples were loosely capped and incubated in the dark at 20°C. A moisture content of 40% of the maximum water holding capacity was maintained throughout the experiment. Three soil and three biomix samples were removed for each

chemical treatment at 3, 10, 20, 30, 60, 90 and 120 days after treatment (DAT) with a single sample from the untreated controls. The samples were stored at -20°C prior to chemical analysis.

Effect of pesticide mixtures on degradation rate

Samples (24) of topsoil and biomix were prepared as described before (section 3.2) and treated with 1.9 ml of a suspension containing 1233, 986.5, 354.9, 739.8, 246.6 and 167.5 mg l^{-1} of formulated isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate. This gave final concentrations of 94, 75, 27, 56, 19, 13 mg kg^{-1} fresh soil or fresh biomix. The control samples were treated with the sample volume of tap water. The degradation study was then performed using the same sampling timepoints and methodology as described for the single compound studies.

Effect of repeat application on degradation rate

Samples (25g, 63 each of topsoil and biomix) were prepared as described above, and split into three batches (A, B, C) of topsoil and biomix. A further 21 samples each of biomix and topsoil were used as controls. Batches A, B and C were treated with 2.75 ml of a suspension made up in tap water containing 874.3, 702.5, 252.8, 526.8, 43.9 and 119.4 mg l^{-1} of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate respectively in order to achieve final concentrations of 96, 77, 28, 58, 5 and 13 mg kg^{-1} fresh soil or fresh biomix. Three samples from batch A and one control sample were taken immediately after pesticide application and stored at -20°C prior to analysis. All remaining samples (batches A, B and C) were allowed to stand for approximately 30 minutes, before being gently shaken. The bottles were loosely capped, weighed and then incubated in the dark at 20°C. The topsoil and biomix moisture content was made up to 15 and 139 % w/w respectively. These represent 40 and 110 % of the maximum water holding capcity for soil and biomix respectively, although visually the biomix was not saturated. Samples (3 treated and 1

control) were then taken from batch A at 3, 10, 30, 60, 90 and 120 days following this first treatment. These were stored at -20°C prior to analysis.

After 36 d of incubation, lids were removed from batches B and C to allow evaporation of water from the samples. The samples were taken from the incubator 3 days later, weighed, and the weight lost since the first application calculated. A second treatment of 2.75 ml of the pesticide suspension used for treatment 1 was then applied. Tap water was used to make up the moisture balance. Control samples were treated with water only. Immediately after the second treatment, 3 treated samples were taken from Batch B and stored prior to chemical analysis. The remaining bottles were capped, weighed and then returned to 20°C storage. Samples (3 treated and 1 control) were then taken from Batch B at 3, 10, 30, 60, 90 and 120 d after treatment.

After 36 d of incubation following treatment 2, lids were removed from the Batch C samples. These received a third treatment 37 days after treatment 2 with 1.38 ml of a pesticide suspension to give the same total application as used for treatments 1 and 2. Any remaining moisture deficit was corrected with tap water. Untreated controls were treated with water only. Immediately after treatment, samples (3 treated and 1 control) were taken and stored prior to analysis. The remaining samples were then returned to 20°C storage. Samples were then taken from Batch C at 3, 10, 20, 30, 60, 90 and 120 days after the third treatment.

Bound residues

Samples (25g) of biomix were weighed into 125 ml clear glass bottles. Half of the samples were then treated with aliquots (2 ml) of ethanol free chloroform, sealed, and incubated at 30°C for 7 days to fumigate the samples. Following incubation the fumigated samples were evacuated in a vacuum desiccator 6 - 8 times to remove all traces of chloroform.

Aqueous suspensions of pendimethalin and chlorothalonil were prepared in distilled water to give concentrations of 1102 and 827 mg l^{-1} respectively. An aliquot (1.3 ml) of either solution was then applied to the chloroform-treated and untreated biomix samples to give final concentrations of 57 and 43mg kg^{-1} fresh biomix for pendimethalin and chlorothalonil respectively. The biomix moisture content was then made up to 50% w/w (40% of the maximum water holding capacity). Samples were allowed to stand for approximately 30 minutes before lids were attached and the samples were then incubated at 20°C. Three treated samples for each pesticide and for both sterile and non-sterile treatments were removed at 0, 3, 10, 20, 30, 60, 90 and 120 DAT. A single untreated sample for each substrate was taken as a control at each time point.

A further experiment was conducted with chlorothalonil using a more vigorous sterilisation method. Biomix samples (19g and 20g) were weighed into 100 ml Duran bottles. Samples were then autoclaved at 121 °C for 1 hour. Bacterial and fungal sterility was confirmed by spreading a sub-sample (0.1g fresh weight) of the autoclaved material over plates of R2A and malt extract agar (MEA). Plates were maintained at 20°C and checked regularly over a 20 day period for growth of bacterial colonies on R2A and fungal hyphae on MEA. A single sample of the autoclaved biomix was extracted with 50 ml acetonitrile and was analysed using HPLC to check for background interference. The 19 g samples were then re-inoculated with 1 g of non-autoclaved biomix. A 400 mg ai $litre^{-1}$ suspension of chlorothalonil was prepared in sterile distilled water and both un-inoculated and inoculated samples were treated with 3 ml of the prepared solution in order to achieve a final concentration of 60 mg kg^{-1} (fresh weight) chlorothalonil and a moisture content of 50% w/w. Both sterile and non-sterile samples were incubated at 20°C with three treated and one untreated sample from each removed at 0, 5, 10, 20 and 30 DAT.

Analysis

Concentrations of isoproturon and chlorothalonil in samples obtained from the single substance degradation studies were determined by HPLC. Samples were extracted with

50 ml methanol by shaking for 1 h on an end-over-end shaker. Extracts were then analysed by HPLC using a Spectra Physics SP8810 pump linked to a Cecil 1200 UV detector. Samples (20 μl) were injected using a Spectra Physics SP8775 autosampler. Separation was achieved using a Spherisorb C8 column (150 x 4.6 mm). For isoproturon determinations the mobile phase used was acetonitrile:water (40:60) with a flow rate of 1.45 ml min^{-1} to give a retention time of 4.5 min. For chlorothalonil the mobile phase used was acetonitrile:water (60:40) with a flow rate of 1.3 ml min^{-1} to give a retention time of 3.3 min. The detection wavelength was 230 nm for both substances.

Concentrations of pendimethalin, chlorpyrifos, epoxiconazole and dimethoate from the samples from the single substance degradation studies were analysed by GC. Each sample was mixed with anhydrous sodium sulphate (40 g) and extracted with a solvent mixture of 90% dichloromethane and 10% methanol. Soil samples were extracted with 50 mL of solvent whilst 75 mL was used for biomix samples. Concentrations of each pesticide in the resulting extracts were then determined by GC. GC analysis was performed using a Hewlett Packard HP5890 gas chromatograph fitted with a split/splitless injector, 12m x 0.53 mm BPX5 column (SGE), and a nitrogen-phosphorus detector. The carrier gas (helium) flow rate was 7 ml min^{-1} and detector –gas flow rates were 100 ml min^{-1} (air) and 4 ml min^{-1} (hydrogen). Oven temperature was raised from 90 °C to 190 °C (40 °C/min) and then to 220 °C (10 °C/min) and finally to 245 °C (15 °C/min). Samples (2 μl) were injected using a Hewlett Packard HP7673 autosampler. Under these conditions all four pesticides were baseline separated with retention times of 3.1 (dimethoate), 4.2 (chlorpyrifos), 4.7 (pendimethalin) and 7.2 minutes (epoxiconazole). Detector response was linear for all 4 compounds (in dicloromethane/methanol, 9:1) in the range 0.2 to 10 μg/ml. Quantification was achieved by comparison of peak areas with results from external standards.

Samples from the mixture study involving six compounds were analysed by GC as described above. The only difference being that 75 ml of solvent was used in the soil extractions and 100 ml in the biomix extractions. Concentrations of all six pesticides in the extracts were then determined using the GC conditions described above. All six

pesticides were resolved and isoproturon and chlorothalonil had retention times of 3.9 and 3.5 minutes respectively, (Appendix i).

Concentrations of pendimethalin, chlorothalonil and isoproturon in samples obtained from the bound residues study were determined after extraction by HPLC using the same method as used described above for isoproturon and chlorothalonil in the single substance studies. The wavelength for determinations of pendimethalin was 250nm, and the retention time was 6.4 min.

Data analysis

Degradation data were fitted to either first order kinetics or bi-exponential curves where the pattern of residue decline was bi-phasic. Data were summarised by calculating DT_{50} and DT_{90} values from the fitted curves.

Results

Degradation of pesticides in topsoil and biomix

With the exception of epoxiconazole in both topsoil and biomix, the degradation data for all compounds approximated to first order kinetics (Figure 3-1) and appropriate DT_{50} and DT_{90} values were computed from lines of best fit. With epoxiconazole DT_{50} and DT_{90} were estimated by interpolation between data points. Degradation data for all pesticides and treatments are summarised in Table 3-2. With the exception of chlorpyrifos and epoxiconazole, DT_{50} values for the substances in biomix were lower than in topsoil by a factor of between 1.7 (dimethoate) and 5.6 (chlorothalonil). With the exception of epoxiconazole, DT_{50} values in biomix were all less than 50 d whereas the maximum DT_{50} in soil was 225 d (chlorothalonil).

Effect of pesticide mixtures on degradation

With the exception of chlorothalonil, where the values were similar, DT_{50} and DT_{90} of the test compounds when applied in mixture to biomix were higher than DT_{50} and DT_{90} values obtained where substances were applied individually (Figure 3-1, Table 3-2). Generally, DT_{50} and DT_{90} values for the chemicals applied as a mixture to biomix were lower than DT_{50} and DT_{90} values when applied as a mixture to topsoil. The exceptions to this were with chlorpyrifos and epoxiconazole.

Effect of repeated applications on degradation rate

When the mixture of pesticides was added repeatedly to topsoil and biomix, degradation of the study compounds was significantly more rapid in biomix than in topsoil (Figure 3-2, Figure 3-3; F value = 627, $P<0.001$, df = 1). In both matrices, there was a significant (F value 758; $P<0.001$, df = 2) decrease in the rate of degradation following each additional application of the study compounds (Table 3-3, Table 3-4).

Bound residues

Degradation was significantly ($P<0.05$) quicker in non-sterile biomix than in material fumigated with chloroform, (Figure 3-4). Calculated DT_{50} values for pedimethalin were 81.4 and 124.5 d (Figure 3-4a) in non-sterile and sterile biomix respectively and DT_{50} values for chlorpyrifos were 25.3 and 41.0 d respectively (Figure 3-4b). Chlorothalonil degradation in autoclaved biomix that had been re-inoculated with non-sterile biomix was significantly ($P<0.001$) quicker than in autoclaved sterile biomix. Degradation in both matrices followed first order kinetics (Figure 3-5) with a calculated DT_{50} of 23.4 and 77.7 days for non-sterile and sterile biomix, respectively.

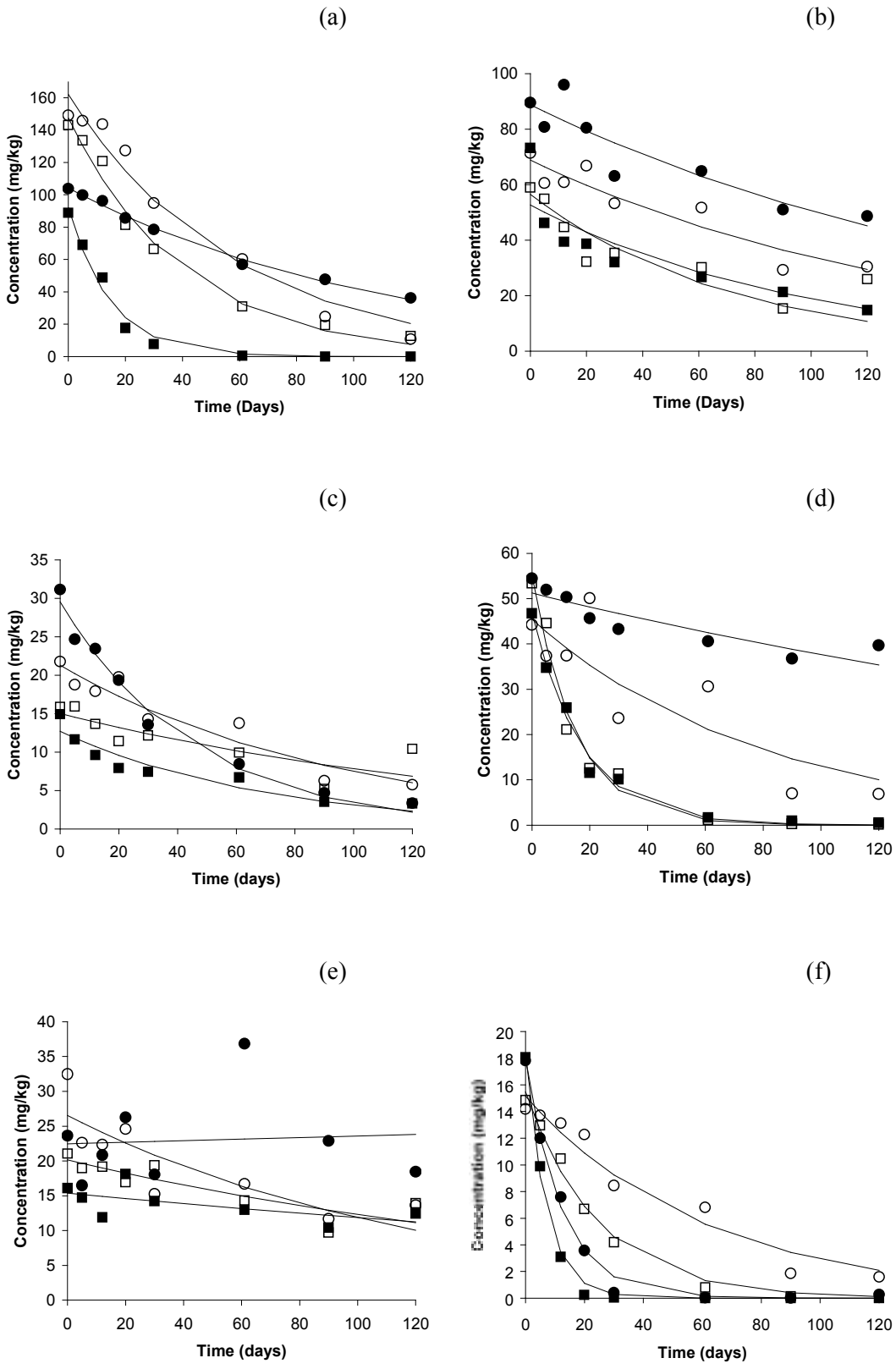

Figure 3-1 Degradation of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate when applied to biomix as an individual treatment (■), topsoil as an individual treatment (●), biomix applied with each of the remaining 5 pesticides (□) and topsoil with each of the remaining 5 pesticides (○).

Table 3-2 DT_{50} and DT_{90} degradation rates, degradation rate constants (k) and determination coefficients (r^2) for isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate when applied individually and as a mixture to topsoil and biomix

	SINGLE TREATMENTS								MIXTURE TREATMENTS							
	TOPSOIL				**BIOMIX**				**TOPSOIL**				**BIOMIX**			
	DT_{50} (days)	DT_{90} (days)	K deg ($days^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	K deg ($days^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	K deg ($days^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	K deg ($days^{-1}$)	r^2
Isoproturon	76.3	253.6	0.0091	1	10.3	34.2	0.0673	0.98	40.2	133.5	0.0173	0.97	28.0	92.9	0.0248	0.99
Pendimethalin	122.9	408.4	0.0056	0.83	50.2	166.9	0.0138	0.79	98.0	325.5	0.0071	0.88	67.2	223.4	0.0103	0.78
Chlorpyrifos	31.8	105.7	0.0218	0.98	49.1	163.2	0.0141	0.88	66.0	219.3	0.0105	0.92	106.0	352.1	0.0065	0.67
Chlorothalonil	225.0	747.4	0.0031	0.80	12.2	40.5	0.0568	0.99	55.1	182.9	0.0126	0.74	10.6	35.1	0.0657	0.98
Epoxiconazole	>120	>120	-	-	>120	>120	-	-	85.8	284.9	0.0081	0.71	140.0	465.2	0.0050	0.74
Dimethoate	8.6	28.6	0.0805	0.99	5.0	16.5	0.1398	0.99	42.0	139.5	0.0165	0.96	17.1	56.8	0.0405	0.99

- value could not be calculated

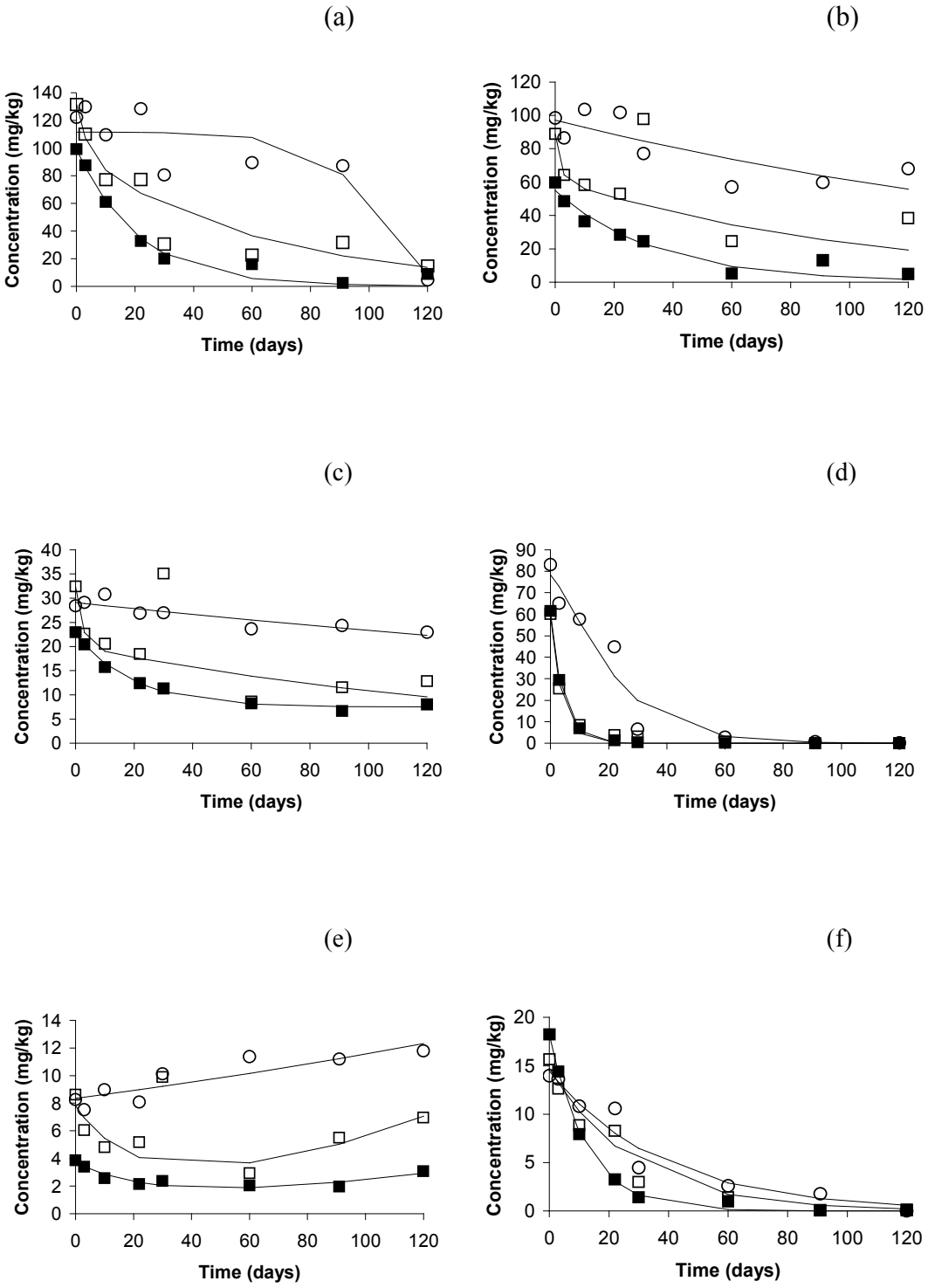

Figure 3-2 Degradation of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate following one (■), two (□) and three (○) applications to biomix of a mixture containing all 6 pesticides.

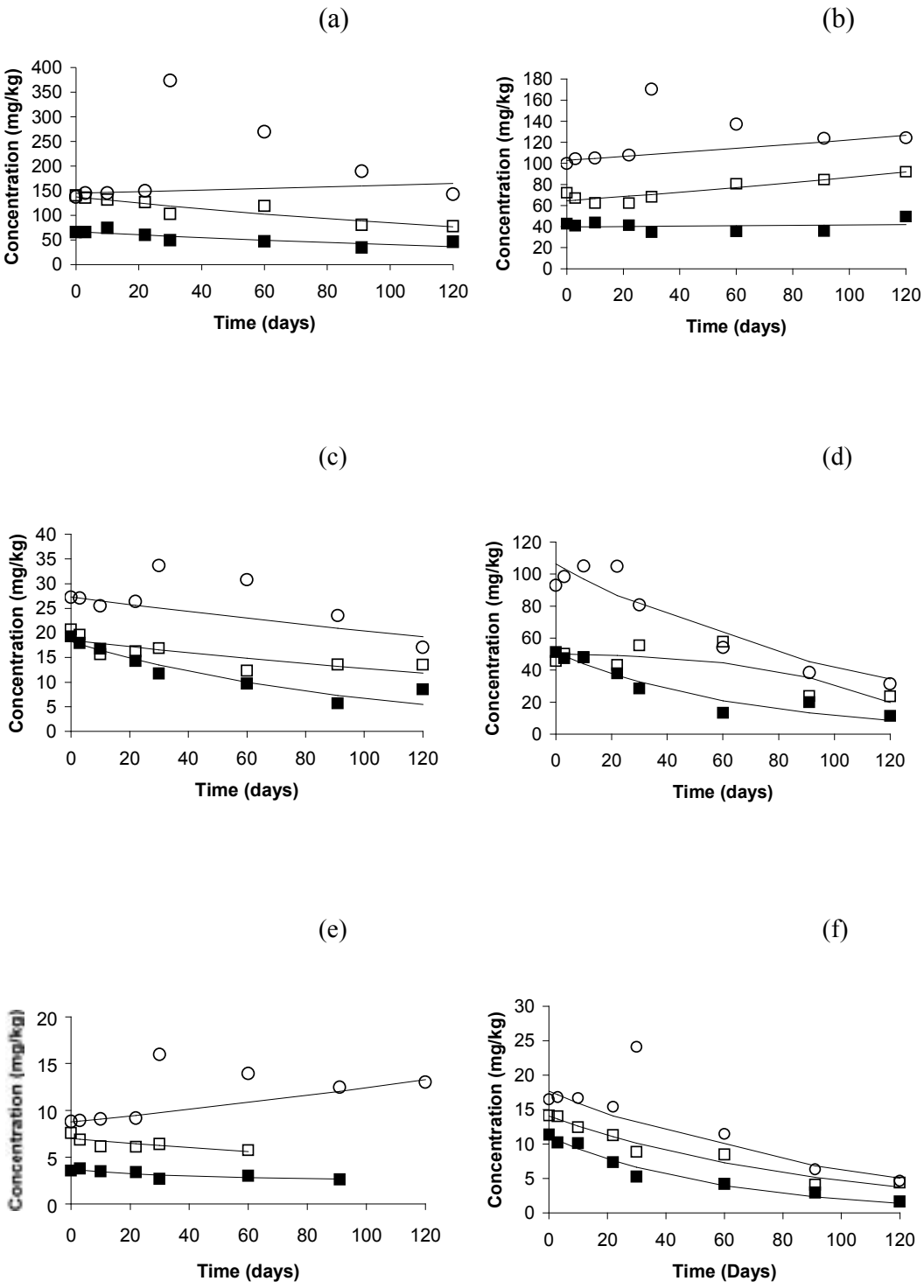

Figure 3-3 Degradation of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate following one (■), two (□) and three (○) applications to topsoil of a mixture containing all 6 pesticides.

Table 3-3 DT_{50} and DT_{90} degradation rates and determination coefficients (r^2) for isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate following three repeat treatments to biomix of a mixture containing all 6 pesticides.

	APPLICATION 1			APPLICATION 2			APPLICATION 3		
	DT_{50} (days)	DT_{90} (days)	r^2	DT_{50} (days)	DT_{90} (days)	r^2	DT_{50} (days)	DT_{90} (days)	r^2
Isoproturon	14.5	48.1	0.98	22.7	122.6	0.96	101.7	118.5	0.97
Pendimethalin	23.5	78.1	0.94	33.5	198.8	0.84	149.8	497.6	0.64
Chlorpyrifos	26.5	115.3	0.99	34.9	297.3	0.89	314.3	1044.0	0.80
Chlorothalonil	2.9	9.8	1.0	2.7	9.1	0.99	17.7	45.5	0.94
Epoxiconazole	61.0	-	0.89	24.4	-	0.79	-	-	-
Dimethoate	8.6	28.4	1.0	19.3	64.1	0.97	25.9	86.0	0.95

- value could not be calculated

Table 3-4 DT50 and DT90 degradation rates and determination coefficients (r^2) for isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate following three repeat treatments to topsoil of a mixture containing all 6 pesticides.

	APPLICATION 1			APPLICATION 2			APPLICATION 3		
	DT50	DT90	r^2	DT50	DT90	r^2	DT50	DT90	r^2
Isoproturon	136.7	453.9	0.71	142.3	472.6	0.86	-	-	-
Pendimethalin	-	-	-	-	-	-	-	-	-
Chlorpyrifos	68.6	228.0	0.90	186.1	618.1	0.66	237.3	788.3	0.83
Chlorothalonil	46.9	155.7	0.92	111.2	147.2	0.66	74.1	246.0	0.89
Epoxiconazole	545.7	-	0.76	187.4	622.6	0.61	-	-	-
Dimethoate	40.3	133.8	0.97	62.7	208.4	0.96	66.3	220.3	0.97

- value could not be calculated

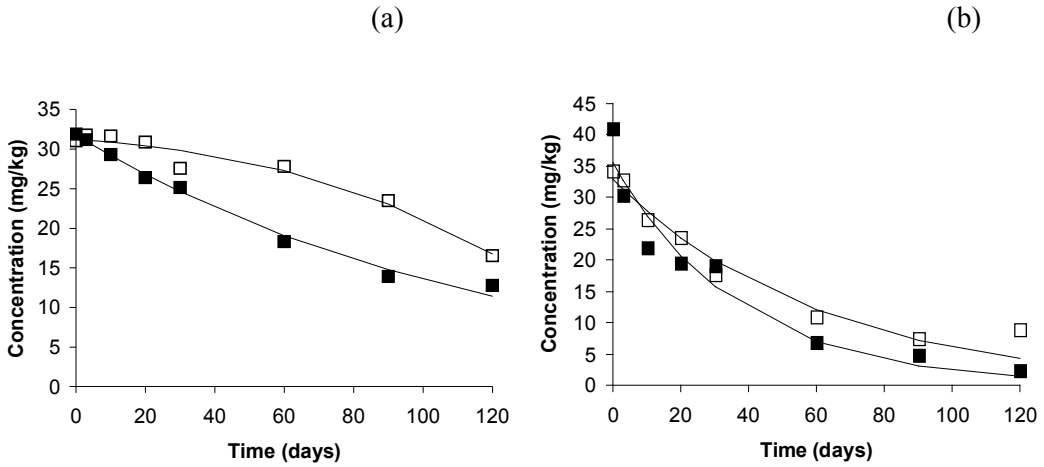

Figure 3-4 Degradation of (a) pendimethalin and (b) chlorothalonil in chloroform fumigated (□) and non-fumigated (■) biomix

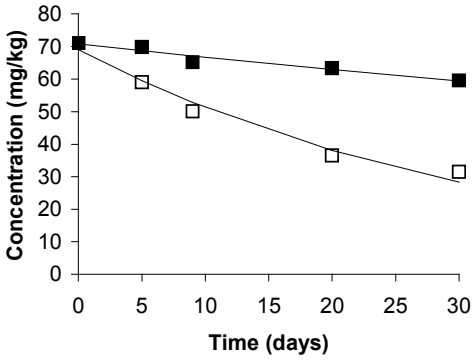

Figure 3-5 Degradation of chlorothalonil in autoclaved 'sterile' biomix (■) and in biomix that has been autoclaved and re-inoculated with non-sterile biomix (□).

Discussion

Biobeds are capable of retaining and degrading small volumes of pesticide waste (*17*) and have been in operational use in Sweden since 1993.(*18*) Concentrations up to 60 mg kg^{-1} have been measured in a number of field biobeds but in general, residue levels are similar to those that would be measured in a field soil following normal agricultural applications.(*17*) However, if such a system is to treat dilute pesticide waste and equipment washings in the UK, it must cope with high concentrations of complex mixtures of pesticide, often applied repeatedly and in large volumes. This study was therefore performed to determine whether biomix is able to degrade the loadings of pesticide that could be applied to a biobed in the UK, and to determine the effects of pesticide mixtures and repeat application on pesticide degradation.

DT_{50} values in biomix, for a range of pesticides covering a range of physico-chemical properties and stabilities and, based on recent research,(*6*) applied at concentrations likely to arise from UK application rates, were generally substantially shorter than DT_{50} values measured in a topsoil sample. With the exception of epoxiconazole, which was not degraded during the test duration, DT_{50} values in biomix ranged from 5 - 50 d. For a treatment system of this type however, the DT_{90} measurement may be of more significance in order to determine whether or not compounds are likely to accumulate. DT_{90} values of > 1 year indicate that accumulation may be a problem particularly when regular treatments are made.(*21*) With the exception of epoxiconazole, all substances tested had DT_{90} values of less than 6 months. Other researchers have obtained similar results. For example, Henriksen et al.,(*22*) demonstrated that isoproturon applied at concentrations from 0.0005 to 25,000 mg kg^{-1} degraded faster in biomix than in topsoil at all concentrations with no sign of toxic effects on the micro-organisms. In the same study, mecoprop was applied to biomix and topsoil and at concentrations below 5000 mg kg^{-1} there was no significant difference between biomix and topsoil. However, above this concentration degradation was only measured in biomix. The results of the current study and previous work therefore indicate that a biobed can degrade pesticides and that accumulation is unlikely to be a problem. There may be substances that cannot

be treated by the biobed (e.g. epoxiconazole) and it may be necessary to control releases of these substances.

Most studies of the environmental fate of pesticides are conducted with single applications of one compound. However, in practice repeated applications of tank mixes containing herbicides, fungicides and insecticides are made.(*23,24,25,26*) Biobeds are therefore likely to receive complex mixtures of more than one active substance often applied repeatedly at concentrations far higher than would occur in soil following normal field treatments. Experiments involving a mixture of 6 active substances showed that, in general, degradation was faster in biomix than in topsoil; the exceptions to this were chlorpyrifos and epoxiconazole. With the exception of chlorothalonil, degradation of the compounds applied to the biomix as a mixture was slower than when the compounds were applied individually. However, DT_{50} values measured in the mixture were generally less than 5 months and the majority of DT_{90} values were less than one year. The biomix therefore appears to be able to better degrade a complex mixture of pesticides than soil and as, with single applications, accumulation of pesticides in the biomix over time is unlikely to be a problem.

Repeated use of certain compounds over a number of seasons can result in enhanced rates of degradation due to adaptation of specific microbial communities which utilise the compound as an energy source and thus degrade the compound very rapidly.(*27,28,29*) In the field, such enhanced degradation can result in reduction or loss of efficacy of a pesticide,(*30*) but in a biobed, enhanced degradation could improve performance. The degradability of three applications, made at 30 day intervals, was therefore investigated. Whilst degradation was quicker in biomix compared to topsoil, the rate of degradation decreased with each additional application. Whilst many agricultural soils possess the necessary ingredients to cause enhanced degradation of a susceptible pesticide, the lack of enhancement in some soils may be due to the absence of responsive microbes or essential cofactors, unsuitable environmental conditions, presence of inhibitory factors or faster reversion to normality.(*31*) These experiments were performed using a mixture of 6 active substances applied at concentrations four times higher than the maximum recommended dose. Whilst the timing and number of

pesticide treatments can effect the rate of pesticide degradation (*29*) it is likely that the negative effects of high concentrations and the interaction between the different active substances masked any increase in microbial activity. Whilst no increase in degradation was observed in these studies, repeated exposure of an agricultural soil to a susceptible pesticide increases the chances that adaptation and enhancement will occur.(*31*) The present experiments used a 30 day interval between treatments. In reality this may not represent real world use conditions. Analysis of pesticide usage data, in particular that for autumn applied herbicides, shows that applications are typically made over continuous 5-10 day periods. Apart from other occasional days, it is likely that the same compounds will not be used again for further 12 months. Experiments performed over this time frame may show results that are different from those reported here.

Organic compounds entering the environment are subject to several fate processes, with the net result being a decline in residual concentrations. However a significant proportion of organic compounds or their degradation products can remain within the soil in the form of bound residues. It is generally observed that the available portion of a compound remaining in the soil decreases with time and the bound residue fraction increases.(*32*) In order for biobeds to gain approval for use in the UK, it is essential that the pesticide residues that are retained within the biomix are degraded and not simply retained within the organic matrix of the system. Experiments were therefore made using chlorothalonil, a compound known to degrade rapidly in biomix, and pendimethalin a more persistent herbicide, in order to determine whether the decline in residues observed in the individual and mixture studies resulted from degradation or sorption to the matrix. Whilst a statistical difference was measured in degradation rates for both pendimethalin and chlorothalonil in biomix sterilised by chloroform fumigation, the data suggested that there was a decrease in the extractable concentration in the sterile matrix. A possible reason for this may be incomplete sterilisation of the biomix by chloroform fumigation,(*33*) or that a microbial community became re-established in the biomix during the time course of the study. Concentrations of pendimethalin remained relatively static for approximately 10 days before a decline was observed. Ingham and Horton (*34*) reported that whilst bacterial and fungal populations were reduced to 37 - 79% of their original populations by chloroform fumigation the

population recovered to their original numbers after two days. In a second experiment using biomix sterilised by autoclave, degradation was minimal in the sterile relative to the non-sterile biomix. These data therefore suggest that degradation was the main process responsible for the reduction in chlorothalonil residues and not irreversible binding to the biobed matrix.

Conclusions

This study was performed to investigate the suitability of biomix for treating pesticide waste and washings. Degradation was generally faster in biomix than in topsoil when a pesticide was applied on its own or in a mixture. Whilst degradation of pesticides applied in mixture to biomix was slower than when applied alone, DT_{90} values indicate that even in a mixture, pesticides will be degraded within a 12 month period. Multiple treatments of a mixture containing six active substances were made to biomix and topsoil at application rates four times higher than the maximum recommended for field use. Whilst degradation was significantly quicker in biomix relative to topsoil, the rate of degradation decreased with each additional treatment possibly due to the toxicity of the pesticide mixture to the microbial community or to a higher proportion of pesticide being available for extraction at higher concentrations. The results suggest that biomix may be capable of treating waste containing a complex mixture of pesticides often applied repeatedly at high concentrations, although control measures may need to be introduced to ensure that certain pesticides are not released to a biobed. Clearly, degradation is only one factor that needs to be considered when assessing the suitability of a biobed system. We have also examined the leaching potential of pesticides in biobeds, and other aspects of biobed management. The results of these studies will be presented elsewhere.

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Chapter 4

DEGRADATION AND LEACHING POTENTIAL OF PESTICIDES IN BIOBED SYSTEMS

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Abstract

Biobeds provide a potential solution to pesticide contamination of surface waters arising from the farmyard. Previous work has shown that biobeds can effectively treat spills and splashes of pesticide. This study investigated the potential for biobeds to treat much larger volumes and amounts of pesticide waste not only arising from spills but also from washing processes. Two systems were assessed using a range of pesticides at the semi-field scale, i.e. a lined biobed system and an unlined system. Studies using the lined biobeds demonstrated that water management was crucial with biobeds needing to be covered to exclude rainwater. Once covered, the top of the biobed became hydrophobic, restricting moisture loss and resulting in saturated conditions at depth. The drying out top layer coincided with a measured decrease in microbial biomass in the treated biobeds. Applied pesticides were effectively retained within the 0-5cm layer. Whilst all pesticides tested degraded, low moisture content and microbial activity meant degradation rates were low. Studies using unlined biobeds showed that only the most mobile pesticides leached, and for these >99% was removed by the system, with a significant proportion degraded within 9 months. Peak concentrations of the two most

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mobile pesticides did however exceeded limits that are likely to be required by regulatory bodies. However, with optimisation of the system it is thought that these limits could be reached.

Introduction

Surface waters and groundwaters have been shown to be contaminated with a range of pesticides.(*1,2*) In order to meet the standards set by e.g. the European Drinking water Directive 80/778/EEC, treatment is required before these water resources can be used for drinking water and such treatment can be expensive. Research over recent years has focused on the non-point sources of pesticide contamination, resulting from application to agricultural land.(*3,4,5,6*) However, contamination arising from other sources such as non-approved use, poor practice, illegal operations, accidental releases from the farmyard and inputs of washings is increasingly recognised as contributing to water contamination.(*7,8,9,10*) For example, a number of workers have indicated that point sources (i.e. the spills and washings from the farmyard) can contribute between 18 and 84 % of the pesticide load measured in individual catchments.(*11,12,13,14,15,16*)

Better training of sprayer operators and good machinery maintenance can reduce the number of spills and by following appropriate codes of practice and regulations, releases to the farmyard due to spray tank washings should be minimised.(*17,18,19*) However, even with well trained operators, small drips and spills are still likely to occur,(*10,11*) and due to time constraints and other pressures, Codes of Practice may not always be followed. Inputs from equipment washings and residues that remain in the sprayer sump after infield tank rinsing are also an unavoidable feature of the sprayer operation.(*7,20*) For example, it is reported that between 0.5 and 25 L of dilute spray solution remains within the sprayer sump after in field tank washing and disposal,(*10,21*) and that over the course of a normal spray season a typical spray applicator can produce between 3800 and 15000 litres of pesticide contaminated waste water.(*22*) The concentration of pesticide can vary greatly, however, and concentrations of up to 450 mg L^{-1} have been reported for tank washings following two internal tank rinses.(*10*)

Additional methods for preventing pesticide waste, washings and spills, arising from farmyards, from reaching both surface and ground waters are therefore required. Biobeds appear to provide a low cost alternative for treating pesticide waste and washings, providing a matrix to absorb the pesticide(s) and facilitate biodegradation. In its simplest form a biobed is a clay lined hole in the ground filled with a mixture of topsoil, peat and straw.(*23,24*) The biobed is covered with grass and equipped with a ramp enabling the tractor and sprayer to be driven over the bed. Studies in Sweden have demonstrated that biobeds can effectively retain and degrade pesticide waste arising from accidental spillages of concentrate and prepared pesticides.(*25*) However, studies performed in Denmark have shown that the clay membrane at the base of the biobed could not retain all of the leachate draining through the biobed.(*26*) Studies have also shown that whilst less mobile pesticides are effectively retained within the biobed matrix significant amounts of the more mobile pesticides can leach from the biobed.(*26, 27, 28*) A number of modifications to the basic biobed design have been suggested in order to remove the leaching risk from biobeds and these included the inclusion of an impermeable membrane underneath the biobed,(*29*) and the use of activated carbon filters to remove any pesticide present in leachate draining from the biobed.(*28*)

Recent laboratory-based studies show that biobeds may be able to degrade the high concentrations and complex mixtures of pesticide that are likely to arise in washings as well as spills,(*30*) the use of biobeds for treating larger amounts of waste (i.e. spills and washings) has not yet been established.

This study was therefore performed to determine whether biobeds could be used to treat pesticides arising from spillages on the farmyard as well as from tank and sprayer washing activities. The specific objectives were to 1) compare the performance of both lined and unlined biobeds; and 2) on the basis of the results, provide recommendations on the construction and operation of a biobed system. Studies were performed at the semi-field scale.

Materials and Methods

Preparation of biomix

Biomix was prepared by mixing topsoil (69% sand, 13% silt, 18%, 1.95% organic carbon, pH 6.15, maximum water holding capacity 37% w/w), peat free compost (levington peat Free Universal) and unchopped winter barley straw in the volumetric proportions of 1:1:2 respectively.

Test chemicals

Test pesticides were selected on the basis of their physico-chemical properties and average annual usage, (Table 4-1).(*31*) Formulated isoproturon (Alpha Isoproturon 500^{TM}), 43.6% w/w, pendimethalin (Stomp 400 SC^{TM}), 36.4% w/w and chlorpyrifos (Dursban 4), 44.65% w/w were used to make up a stock suspension in tap water of 11,140mg AI litre^{-1}, 8,000mg AI litre^{-1} and 5,825mg AI litre^{-1} of isoproturon, pendimethalin and chlorpyrifos respectively. Formulated chlorothalonil (Cropgard^{TM}), 41.57% w/w, epoxiconazole (Opus^{TM}) 12.1% w/w and dimethoate (Rogor L40^{TM}) 37.4% w/w were used to make up a stock suspension in tap water of 6533 mg AI litre^{-1}, 756 mg AI litre^{-1} and 2438 mg AI litre^{-1} of chlorothalonil, epoxiconazole and dimethoate respectively.

Table 4-1 Study compounds and their reported physico-chemical charactersistics

Active Substance	K_{oc} mL g^{-1}	DT_{50} (Soil) (days)	Water Solubility (mg L^{-1})
Isoproturon	100	6 - 28	65
Pendimethalin	5000	90 - 120	0.3
Chlorpyrifos	6000	60 - 120	1.4
Chlorothalonil	1600 - 14000	6 - 43	0.81
Epoxiconazole	957 - 2647	60 - 90	6.6
Dimethoate	16 - 52	7 - 16	22300

Values taken from Wauchope et al. (1992) (46) and Tomlin (2000) (47)

Lined Biobeds

Forty biobed lysimeter cores were prepared using unplasticised polyvinyl chloride (PVC-u) piping (19 cm internal diameter x 75 cm length) with one end of the cut pipe sealed using a socket. Cores were filled with 15cm of washed sand and a 50cm layer of biomix (organic matter 12.36%, pH 7.5, maximum water holding capacity 121% w/w), packed to a density (measured 427 days after construction) of 1.27 g cm^{-3} in the top 0-10cm layer and 0.53 g cm^{-3} for the bottom layer and placed into the ground in 5 groups of 8. The biobed columns were free of any vegetation. Four of the 5 groups of cores were treated with 50 mL of the pesticide mixture containing isoproturon, pendimethalin and chlorpyrifos in December 1998 and January 1999 in order to achieve a final treatment rate of 1114 mg (isoproturon), 800 mg (pendimethalin) and 583 mg (chlorpyrifos). Treatment rates gave nominal concentrations in the 0-5cm layer of 618 mg kg^{-1} (isoproturon), 443 mg kg^{-1} (pendimethalin) and 323mg kg^{-1} (chlorpyrifos). The remaining group of 8 cores was left untreated and acted as a control. The four treated groups of cores were treated with 50 mL of the pesticide mixture containing chlorothalonil, epoxiconazole and dimethoate in April 1999 and June 1999 in order to achieve a final treatment rate of 653 mg (chlorothalonil), 76 mg (epoxiconazole) and 244 mg (dimethoate). Treatment rates gave nominal concentrations in the 0-5cm layer of 361 mg kg^{-1} (chlorothalonil), 42 mg kg^{-1} (epoxiconazole) and 135mg kg^{-1} (dimethoate). Application rates were based on theoretical worst case disposal rates (i.e. 2 applications of 100 litres of full strength dilute pesticide). A roof was constructed over the cores following the first treatments with isoproturon, pendimethalin and chlorpyrifos to exclude rainfall. To simulate runoff from an impermeable pesticide handling area connected to a biobed, artificial irrigation was applied in February, May, July, August and September at the rate of 314 mL per core equivalent to 11.1 mm of rainfall.

Two untreated cores were collected prior to the first pesticide treatment and sectioned into approximately 3 equal parts. Sub-samples were obtained from each section for microbial biomass determination. Following treatment cores were collected on 8 occasions over a 12 month period (Table 4-2). On each sampling occasion, 3 treated

cores and one untreated control were collected, the cores were then sectioned (0-5 cm, 5-10cm, 10-20cm, 20-30cm and 30-50cm). Sections down to 20cm depth were homogenised in a food processor and stored at -15°C prior to chemical analysis. With the exception of samples taken at T = 0 and T = 3 sub-samples were collected (0-10 cm, 10-30 cm and 30-50 cm) for biomass and moisture content determinations.

Table 4-2 Sampling time points for lined biobeds

Time Point	Days after application 1	Days after application 2	Days after application 3	Days after application 4
T = 0	1			
T = 1	36			
T = 2	105	68		
T = 3	123	86	1	
T = 4	165	128	43	
T = 5	260	223	138	89
T = 6	322	285	200	151
T = 7	365	328	243	194

Applications 1 and 2 (isoproturon, pendimethalin and chlorpyrifos)

Applications 3 and 4 (chlorothalonil, epoxiconazole and dimethoate)

Unlined Biobeds

Two sets of four lysimeters were prepared using PVC-u piping (19 cm internal diameter x 75 cm length) with one end of the cut pipe sealed using a socket fitted with a drain outlet. Cores were filled with 2-3cm of gravel followed by 15cm of washed sand. A 50 cm layer of either biomix (organic matter 12.36%, pH 7.5, maximum water holding capacity 121% w/w) or topsoil (69% sand, 13% silt, 18%, 1.95% organic carbon, pH 6.15, maximum water holding capacity 37% w/w) was then packed into each lysimeter. A density (measured 316 days after construction) of 1.67 g cm^{-3} in the 0-5cm layer down to 0.21 g cm^{-3} at the base was achieved for the biomix compared to 1.68 g cm^{-3} to 0.59 g cm^{-3} for topsoil. The base of each core drained via the drain outlet through Teflon tubing to a 2.5 litre amber glass bottle located in a central collection pit, Plate 4-1.(*32*) Three of the biomix filled lysimeters and 3 soil filled lysimeters received split applications of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole

and dimethoate. Treatment rates and timings were the same as for the lined biobed cores. A potassium bromide (KBr) tracer was also applied (628 mg $core^{-1}$) to check the hydrological integrity of the lysimeters, as well as to determine breakthrough timing of the infiltrating water. Collection vessels were monitored after all rainfall events and the total volume of leachate recorded. Volumes in excess of 500 mL were collected and stored at 0 - 10°C prior to analysis. Where possible, a 60 mL sub-sample was also taken for KBr analysis. At the end of the study, (254 days after application 1) all cores were sectioned (0-5, 5-10, 10-20, 20-30 and >30 cm), homogenised and stored at -15°C prior to analysis.

Plate 4-1. Unlined topsoil and biomix lysimeters

Plate not included in the submitted paper

Analysis

Water analyses

Water samples were either analysed directly using high performance liquid chromatography (HPLC) or analysed by HPLC or gas chromatography (GC) after liquid / liquid extraction. For leachate collected prior to the application of chlorothalonil,

epoxiconazole and dimethoate, samples (500 ml) were extracted twice into 2 x 30 mL HPLC grade dichloromethane (DCM) in a 1 L glass separating funnel. The DCM extracts were combined and evaporated to dryness using a rotary evaporator at 40°C. The resulting residue was then re-dissolved in 2 mL of a mixture containing 60% acetonitrile and 40% water. Concentrations of isoproturon and pendimethalin were determined by HPLC using a Kontron Series 320 pump linked to a Kontron Series 332 UV detector. Samples (20 µL) were injected using a Kontron Series 360 autosampler. Separation was achieved using a Lichrosorb RP18 column (250mm x 4mm i.d.) and a flow rate of 1 mL min^{-1}. For isoproturon determinations, a 75:25 acetonitrile:water mobile phase was used, for pendimethalin determinations, a 90:10 acetonitrile:water mobile phase was used. The detection wavelength for both compounds was 250 nm. Quantification was achieved by comparing peak areas with results from known standards. For chlorpyrifos determinations, sub-samples (1 mL) of the acetonitrile/water extracts were mixed with 25 mL water and extracted into 1 mL hexane. Concentration of chlorpyrifos were then determined by GC with a nitrogen / phosphorous detector (GC Method 1). Separation was achieved using 3% OV1 on Chromosorb WHP column (1 m x 3mm i.d.), nitrogen flow was 50 mL min^{-1}, hydrogen flow was 2 ml min^{-1} and air flow was 450 mL min^{-1}. The column temperature was 220°C, the injector temperature was 225°C and the detector temperature was 230°C. Quantification was again achieved by comparison of peak areas with results from known standards. Recovery checks for all 3 compounds were > 93%.

For leachate collected following application of all six pesticides, samples (200 mL) were extracted three times into 30 mL DCM in a 500 mL glass separating funnel. The DCM extracts were passed through anhydrous $Na_2 SO_4$ and then evaporated to dryness at 40°C. The resulting residues were re-dissolved into 2 mL of a mixture containing 10% methanol, 90% DCM. Concentrations of each pesticide were determined on a Hewlett Packard HP5890 gas chromatograph fitted with a split/splitless injector, 12m x 0.53 mm BPX5 column (SGE) and a nitrogen-phosphorus detector (GC Method 2). The carrier gas (helium) flow rate was 7 ml min^{-1} and detector –gas flow rates were 100 mL min^{-1} (air) and 4 mL min^{-1} (hydrogen). Oven temperature was raised from 90 ^{o}C to 190 ^{o}C (40 ^{o}C min^{-1}) and then to 220 ^{o}C (10 ^{o}C min^{-1}) and finally to 245 ^{o}C (15 ^{o}C min^{-}

^{1}). Samples (2 µL) were injected using a Hewlett Packard HP7673 autosampler. Under these conditions all six pesticides were baseline separated with retention times of 3.1 (dimethoate), 3.5 (chlorothalonil), 3.9 (isoproturon), 4.2 (chlorpyrifos), 4.7 (pendimethalin) and 7.2 minutes (epoxiconazole). Quantification was achieved by comparison of peak areas with results from external standards. Recoveries with DCM extraction of water spiked at 0.01 mg L^{-1} were > 94% for all compounds.

Concentrations of potassium bromide were determined using ion chromatography. Water samples (0.5 mL) were filtered (0.2 µm) and analysed using a Dionex DX-100. Samples (25 µL) were injected neat with a typical retention time of 2.3 minutes. The system was calibrated using a series of standards with known concentrations of bromide with a limit of detection set at 1.1 mg L^{-1}.

Soil analysis

Following solvent extraction, soil and biomix samples were analysed either by HPLC or GC.

Samples T=0 to T=3 (Table 4-2), solid material (25 g) were mixed with 50 mL methanol. Samples were shaken for 50 minutes using a wrist action shaker and then allowed to stand until the solid material had settled. Aliquots (2 mL) of the clear methanol were transferred directly to glass HPLC vials for determination of isoproturon and pendimethalin using the HPLC method described above. Sub-samples (either 1 ml or 5 mL) of the methanol extract were taken for chlorpyrifos determination. For chlorpyrifos determination, the methanol extracts were mixed with 50 mL water and the methanol/water mixture extracted into 5 mL hexane. The hexane extract was dried using 5 g anhydrous Na_2SO_4 prior to GC analysis using the GC Method 1 described above.

For all other soil and biomix, samples (40 g) were placed into 250 mL glass bottles. Anhydrous Na_2SO_4 (40 g) plus 160 mL of a mixture containing 90% DCM and 10% methanol was added, with samples shaken for 1 hour using an end-over-shaker.

Samples were allowed to stand until clear, with an aliquot of the solution taken for determination of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate using the GC Method 2 described above. With the exception of chlorothalonil (82%) the recovery of all 6 pesticides exceeded 95%.

Microbial biomass

Total microbial biomass was determined by fumigation extraction.(*33*) Chloroform (2 mL) was added to triplicate samples (20 g) of soil and biomix, a control sample was left untreated. Treated and untreated samples were sealed and incubated at 30°C for 7 - 10 days. Following incubation fumigated samples were evacuated 4 - 6 times in a vacuum dessicator to remove the chloroform and then shaken for 50 minutes with 50 mL of 2 M potassium chloride. Samples were then centrifuged, a 1 mL extract taken to which 0.5 mL of ninhydrin was added. The samples were then immersed in a boiling water bath for 20 minutes. After cooling, samples were made up to 10 mL using 50:50 mixture of ethanol and water, transferred to plastic cuvettes and the absorbance measured using a spectrophotometer at 570 nm. The absorbances were corrected for the unfumigated controls and the amounts of ninhydrin reactive N derived from a calibration curve produced using different concentrations of L-lucine. The results were corrected for moisture content and the total biomass C (mg kg^{-1}) calculated.(*33*)

Results

Lined Biobeds

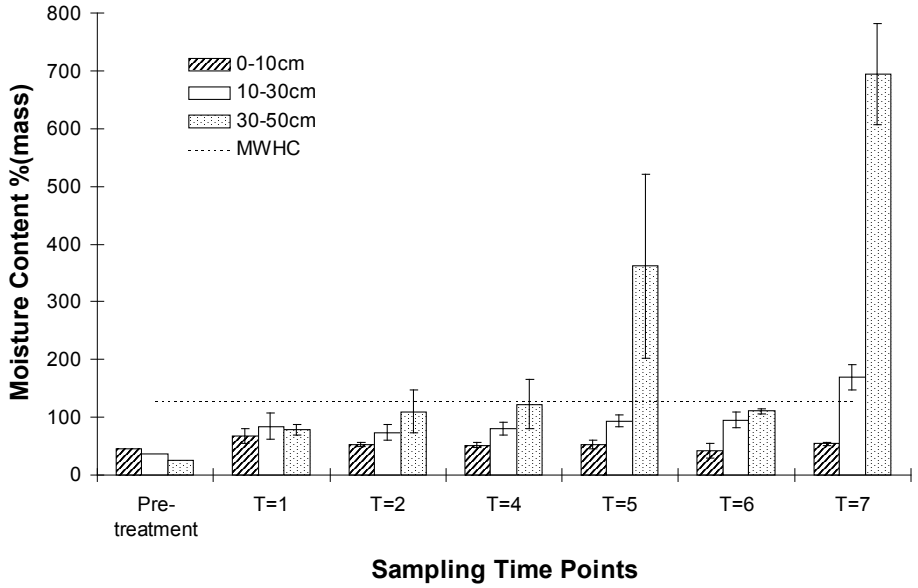
Moisture status and Microbial biomass

Prior to being covered, lined biobeds intercepted 156 mm of rainfall equivalent to 4.42 litres, with an additional 1.5 litres applied in the form or artificial irrigation over the course of the experiment. The measured maximum water holding capacity for the biomix was 127 % w/w, equivalent to approximately 8.2 litres of water per core.

Moisture content in the 0 - 10 cm layer remained relatively static (average 52 %) throughout the study period. Below 10cm depth a gradual increase was measured with saturated conditions observed by the end of the study (Figure 4-1). Total microbial biomass in the untreated cores (0 - 50cm) ranged from 264 to 5310 mg kg^{-1} carbon and in the treated (0 – 50cm) cores 141 to 3164 mg kg^{-1} carbon. Despite considerable variation in measurements in the 0 - 10 cm layer, biomass in the treated cores declined over the study (Anova $P<0.05$, F 13.28, df 1), whereas in the untreated cores the measured biomass remained relatively static, (Figure 4-2). In the 10 - 30cm and 30 - 50 cm layer there was no significant difference between the treated and untreated cores.

Pesticide residues

The highest concentrations of all pesticides were measured in the 0-5cm layer of the biobed, (Figure 4-3). Concentrations in the deeper layers were significantly lower indicating little downward movement of the study compounds, (Figure 4-4). Concentrations of isoproturon, pendimethalin and chlorpyrifos in the 0-5cm layer remained static for the first 100d. During the next 100d rapid degradation was observed after which residues persisted at a low levels until the end of the experimental period, (Figure 4-3a,b,c). For chlorothalonil and epoxiconazole the rate of degradation was slow such that the amount of each pesticide recovered at the end of the study was similar to that measured at the beginning, (Figure 4-3d,e). However, for dimethoate the pattern of degradation was relatively fast and < 10% of the applied dose was recovered at the end of the study, (Figure 4-3f).



MWHC: maximum water holding capacity

Figure 4-1 Measured water contents (+/- 1SE) in lined biobeds

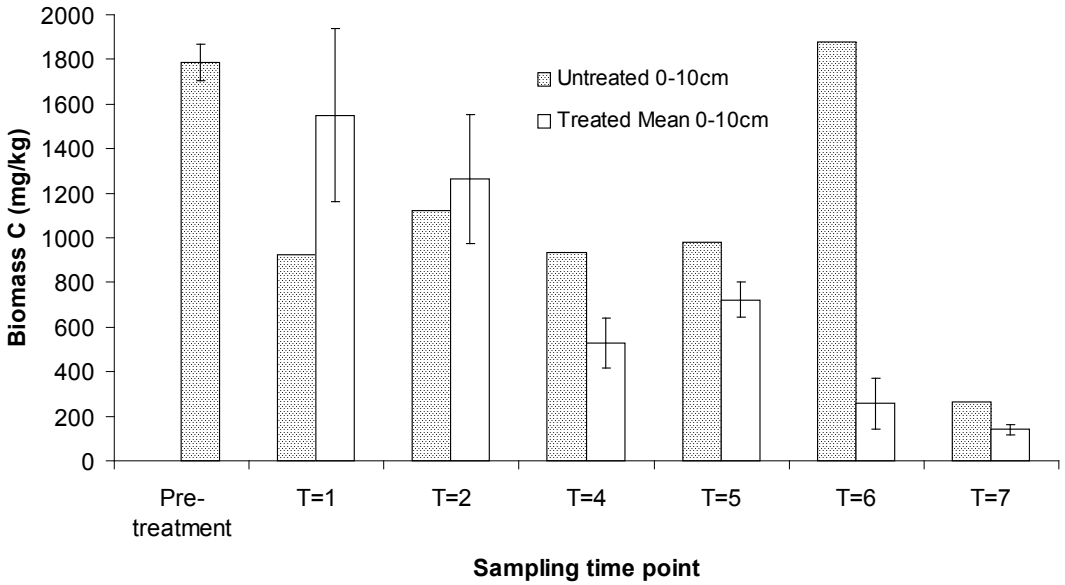
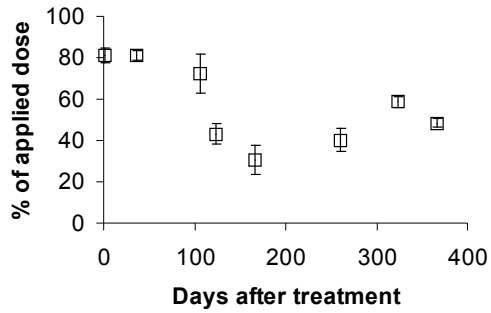


Figure 4-2 Mean microbial biomass (+/- 1SE) in the 0 - 10 cm layer of the lined biobed columns

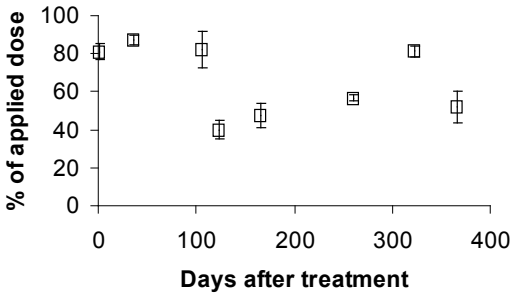
Unlined Biobeds

Unlined topsoil and biomix lysimeters received 116% of the long term average rainfall, equivalent to 16.9 litres of water per lysimeter, with 13 samples of leachate being collected over a 9 month period. With one exception, cumulative leachate volumes were similar with approximately 10 litres collected from both topsoil and biomix lysimeters. The maximum water holding capacity of the biomix lysimeters was approximately twice that of topsoil (8.1 litres compared to 4.1 litres). Rapid breakthrough of bromide was observed from topsoil lysimeters with highest concentrations observed 35 DAT. Movement of bromide through biomix filled cores was much slower with maximum concentrations not being observed until 102 DAT. Generally, data suggest chromatographic water movement in both the topsoil and biomix filled lysimeters indicating hydraulic integrity of the test system. With the exception of pendimethalin concentrations of pesticide in leachate from biomix filled lysimeters were significantly lower than in leachate from topsoil (Figure 4-5). Peak concentrations of active ingredient in leachate from biomix ranged from 0.15 μgLl^{-1} (epoxiconazole) to 127 $\mu g\ L^{-1}$ (isoproturon) whereas from topsoil cores concentrations ranged from 0.47 $\mu g\ L^{-1}$ (pendimethalin) to 3845 $\mu g\ L^{-1}$ (isoproturon). By the end of the study (i.e. 254d after the first application), concentrations of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate in leachate from the biomix columns had dropped to less than 1.0 $\mu g\ L^{-1}$ (Figure 4-5).

With the exception of dimethoate in soil, no pesticide was detected in the soil or biomix matrix below 30 cm depth with the majority being retained in the top 10 cm (Figure 4-6). By the end of the study between 7 % (isoproturon) and 30 % (epoxiconazole) remained in the biomix whereas between 0.7 (isoproturon) and 38 % (pendimethalin) remained in the topsoil cores (Table 4-3). This indicates that in biomix only a small proportion of the applied dose (<1%) is leached and between 70 and 93% is degraded.



(a)*



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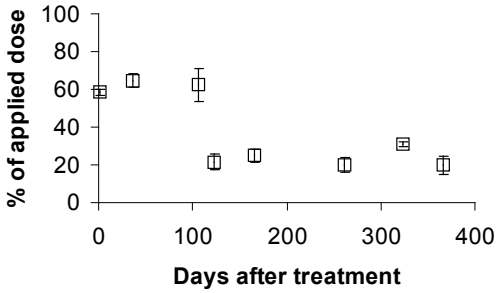

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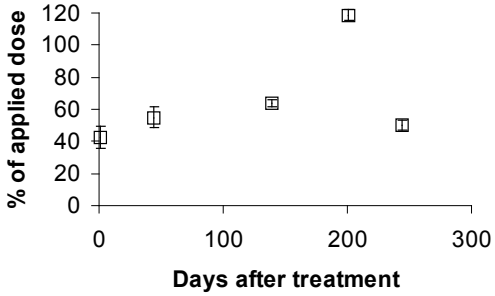
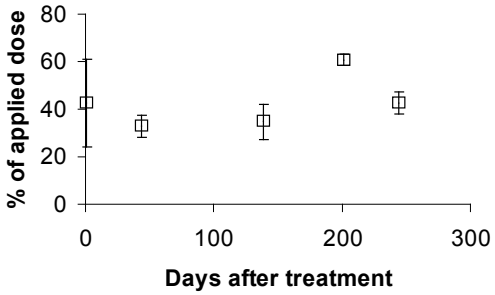
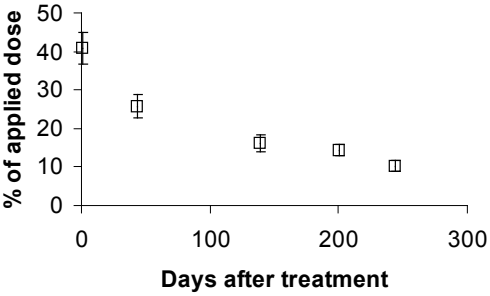


(d)**



(e)**

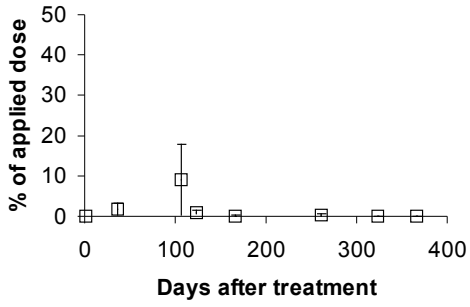


(f)**

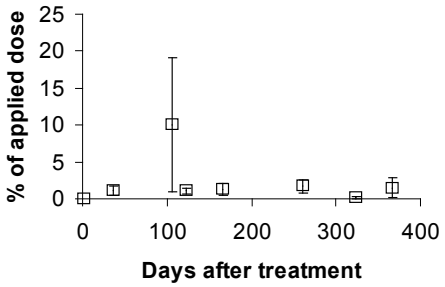
Figure 4-3 Amounts (+/- 1SE) of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (d) dimethoate remaining in the 0-5cm layer of the lined biobeds expressed as % of the applied dose

* For isoproturon, pendimethalin and chlorpyrifos the second application was made 37 days after treatment 1.

** For chlorothalonil, epoxiconazole and dimethoate the second application was made 49 days after treatment 1.



(a)*



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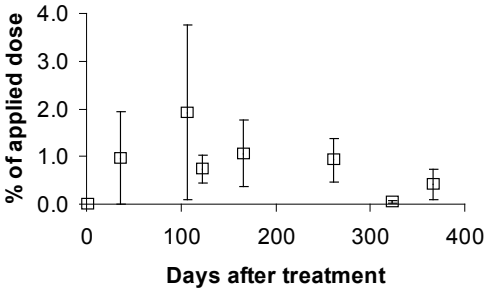

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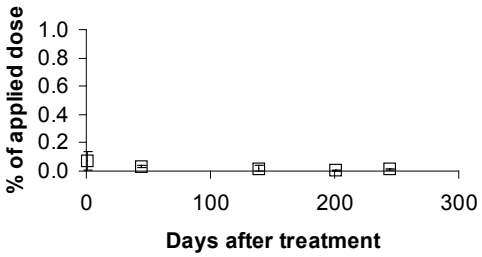
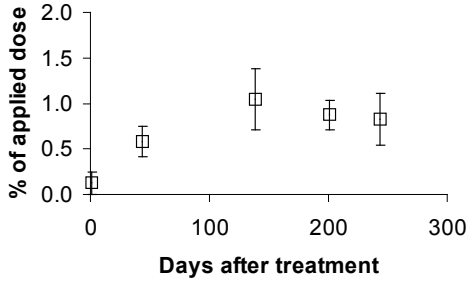
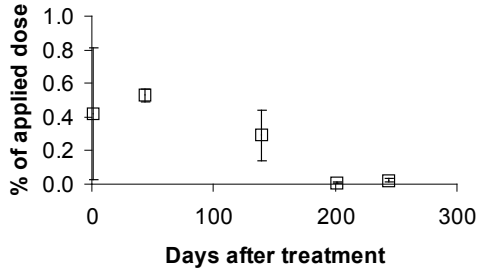


(d)**



(e)**



(f)**

Figure 4-4 Amounts (+/- 1SE) of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate remaining in the 5-10cm layer of the lined biobeds expressed as % of the applied dose

* For isoproturon, pendimethalin and chlorpyrifos the second application was made 37 days after treatment 1.

** For chlorothalonil, epoxiconazole and dimethoate the second application was made 49 days after treatment 1.

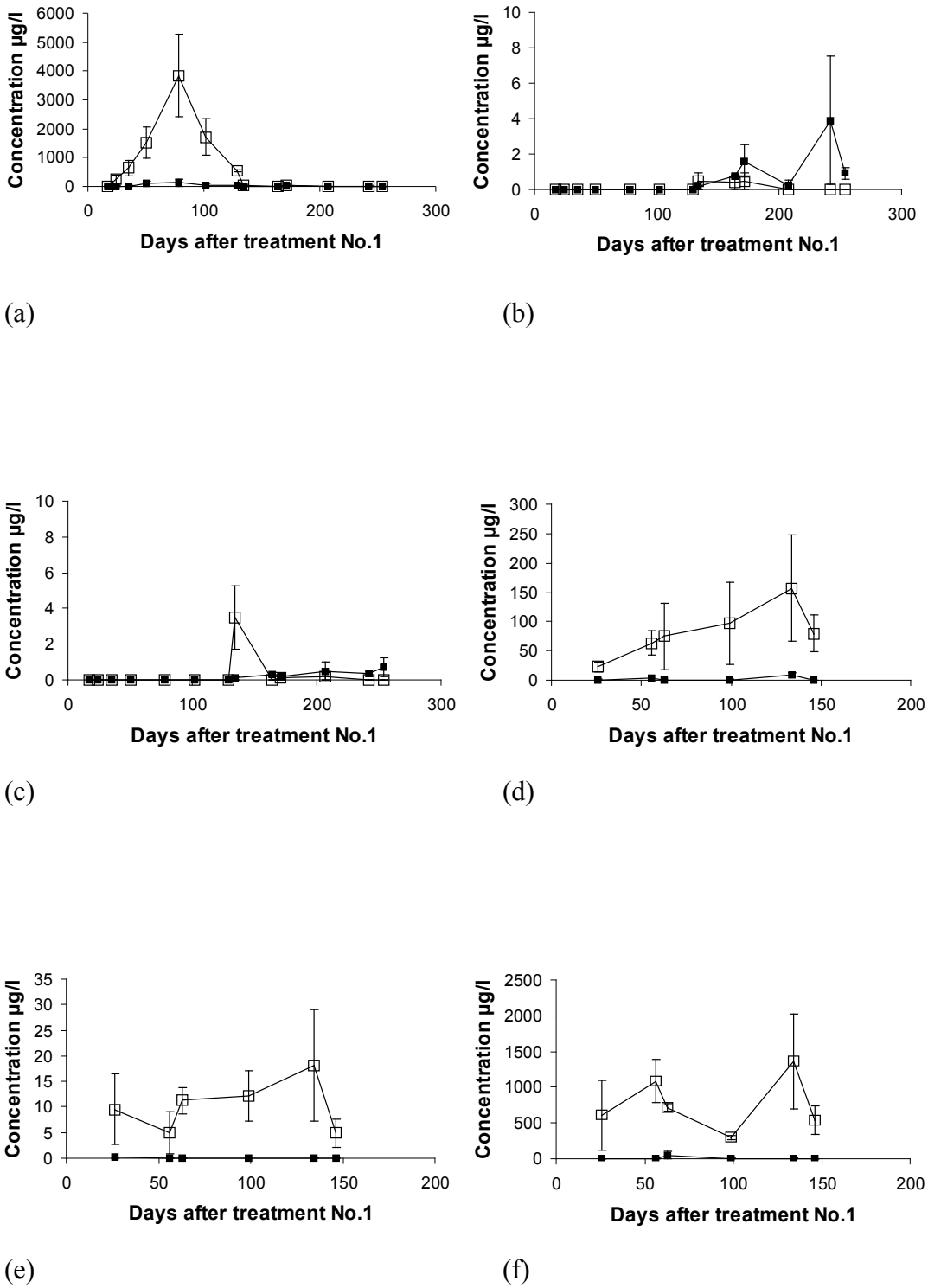


Figure 4-5 Concentrations (+/- 1SE) of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate measured in leachate from soil (□) and biomix (■) filled lysimeters

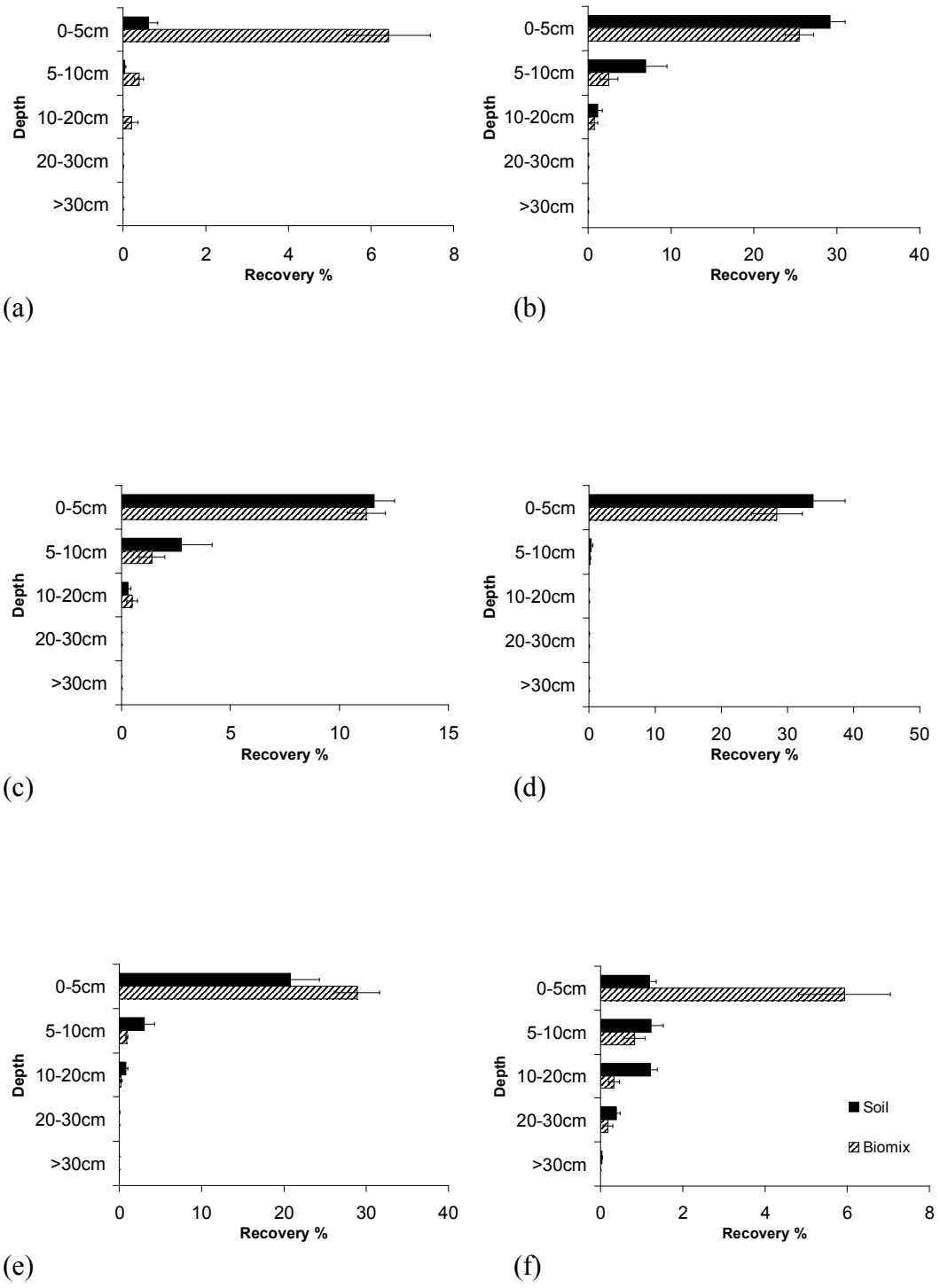


Figure 4-6 Amounts (+/- 1SE) of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate expressed as % of the applied dose remaining in unlined biobeds 254 days after the first treatment

Table 4-3 Mass balance (*) for un-lined topsoil and biomix lysimeters

Pesticide	TOPSOIL Leached %	TOPSOIL Retained %	TOPSOIL Degraded %	BIOMIX Leached %	BIOMIX Retained %	BIOMIX Degraded %
Isoproturon	1.5	0.7	97.8	0.1	7.0	92.9
Pendimethalin	0	37.4	62.6	0	28.8	71.2
Chlorpyrifos	0	14.6	85.4	0	13.1	86.9
Chlorothalonil	0.2	34.2	65.5	0	25.6	71.4
Epoxiconazole	0.3	24.7	75.0	0	30.0	70.0
Dimethoate	8.4	4.0	87.5	0	7.3	92.7

* Mass balance calculated 217 days after last application of isoproturon, pendimethalin and chlorpyrifos and 83 days after application of chlorothalonil, epoxiconazole and dimethoate

Discussion

Biobeds have been in use in Sweden since 1993 with more than 1000 in practical use on farms and other places where pesticide sprayers are filled up.(*25*) The basic design of a 0.6m deep hole lined with clay and filled with biomix with an access ramp has remained largely unchanged,(*24*) with reliable performance being measured for up to 8 years.(*25*) Whilst the Swedish system has been shown to be able to treat pesticide spills, the use of biobeds for treating the large volumes of waste and high amounts of pesticide associated with washings as well as spillages has not yet been established. If a system could be developed to deal with these types of inputs, then it is possible that incidences of contamination of surface waters by pesticides could be greatly reduced.

In this study, two systems were investigated: namely a lined system where the biomix was enclosed in a sealed column and an unlined system where leachate was able to percolate from the bottom of the biomix. The use of a lined system was considered attractive as it minimises the potential for leachate to contaminate groundwaters and is hence likely to be more attractive to regulatory authorities.

The lined biobed columns had to be covered following the first herbicide application to exclude clean rain water from being intercepted by the biobed itself. However, irrigation was applied to each column to simulate runoff from an area of hard standing. A survey of local farms carried out prior to the study concluded that the preferred

location of a biobed would be adjacent to the existing pesticide mixing area. Of the farms surveyed the mixing area was generally constructed from concrete and as such would generate run-off in response to both rainfall and cleaning operations. Once covered, the top 10cm dried out to form a cap. Hydrological connectivity was interrupted severely restricting evaporation from the system. Minimal water loss resulted in saturated conditions below 10cm within 12 months, agreeing with observations reported for covered Swedish Biobeds.(*25*) Microbial biomass was used to assess levels of biological activity within the biobed. Over a 12 month period, biomass decreased in the 0-10cm layer. This was probably a function of low moisture content, but there may also have been inhibition by the high levels of retained pesticide.(*27,34*) Adequate water is essential for microbial activity and thus biodegradation. Generally, experiments have shown an increase in the rate of pesticide loss with increase in soil moisture status up to 5 kPa (field capacity).(*35,36*) At moisture contents below 75%, microbial activity in biobeds can be limited.(*25*) Although pesticides were effectively retained in the lined system, residues levels of ≤ 52% were still recovered after 12 months. Generally, persistence increases with increasing concentration,(*25, 37, 38, 39, 40*) and at high concentrations, pesticides have been shown to depress microbial biomass and bioactivity; consequently, degradation may have been inhibited.(*39*) In many agricultural situations the use of tank mixes and complex spray programmes is common practice.(*34, 41, 42*) There is evidence that the sorption and persistence of a number of pesticides may be changed when used in combination with other pesticides.(*30, 34, 42, 43, 44*) On the basis of the results, it therefore appears that lined biobeds would be unlikely to cope with large volumes of waste associated with tank and sprayer washings as they would become waterlogged and microbial activity would be reduced. Some form of water management might resolve these problems but this would probably result in increased costs and time inputs from the user.

The use of unlined biobeds removed the need to manage water inputs whilst at the same time maintaining near optimum conditions for pesticide degradation as rainwater is able to enter and subsequently drain from the system. The studies demonstrated that the concentrations of pesticide leaching from the biomix filled lysimeters were significantly

lower than from soil lysimeters. Only the most mobile compounds leached to any great extent and even for these compounds the system appeared to retain or degrade more than 99% of the applied dose. Whilst > 99% removal was achieved for the 6 compounds tested, maximum concentrations of the two most mobile compounds, isoproturon and dimethoate were 127 and 50.4 $\mu g\ L^1$ respectively. Studies in Denmark using $2m^3$ lysimeters looked at the leaching potential over a 2 year period of isoproturon and mecoprop in both biomix and clay filled lysimeters after receiving two simulated pesticide spills each of 8 g.(*27*) The results showed that total amounts of isoproturon leached were 1947 mg from the soil compared to 32 mg from the biobed; for mecoprop 574 and 175 mg leached from the soil and biobed respectively. Such values may be unacceptable to regulatory authorities. For example, even though the Danish study demonstrated that the biobed system was able to retain a significant amount of the applied dose, pesticide concentrations in leachate were unacceptable to the Danish EPA. In addition, the biobed matrix was classified as hazardous waste. In the UK, the Environment Agency have proposed regulating biobed performance against the Ground Water Regulations (1998). The regulations stipulate that concentrations of pesticide reaching groundwater must be $<0.1\ \mu g\ L^1$.

Henriksen et al., (*27*) proposed that one method of reducing the concentrations of pesticide leaching out of the biobed would be to cover them during the winter period thus excluding excess rain water. In addition they suggested that a closed biobed would remove the issue of pesticides leaching from the system - the work reported here suggests that this may not be a practical solution. The installation of secondary treatment options (e.g. activated carbon) at the outlet of a biobed, has also been investigated and shown to significantly reduce leachate concentrations.(*28*)

In order to prevent the biobed matrix being classified as hazardous waste it is essential that the pesticide retained by the biobed matrix is degraded. In Denmark (*27*) and France (Higginbotham pers. Comm.) studies have shown that biobeds collect, retain and degrade pesticides. However, the regulatory authorities in both countries have classified the matrix as hazardous waste, therefore requiring specialist treatment when requiring disposal. Analysis of the biobed matrix from this study showed that most

pesticide was retained in the top 5cm of the biobed, an observation supported by Toller et al., (*45*), and that after 9 months a significant proportion of the non-leached pesticide had been degraded. With < 30% of the most persistent compound (epoxiconazole) remaining after 9 months (compared to ≤ 52% in the lined systems), accumulation from one growing season should not occur. Laboratory investigations compared pesticide behaviour in sterile and non-sterile biomix and concluded that degradation was the principle mechanism responsible for the reduction in measured concentrations of pesticide.(*30*)

Conclusions

Studies with lined biobeds demonstrated that pesticides with a range of physico-chemical properties were effectively retained. However, monitoring of soil moisture status indicated that lined biobeds needed to be covered in order to exclude rain water from the system. Once covered the surface layer (0-10cm) rapidly dried to form a hydrophobic layer, severely restricting evaporation and thus moisture loss. This resulted in saturated conditions below 10cm depth within 12 months of construction. The drying out of the surface layer was also associated with a decrease in microbial biomass in the treated biobed columns. In the untreated biobeds microbial biomass remained relatively constant indicating that the retained pesticide residues may have an inhibitory effect on the biomix microbial community. Whilst all pesticides tested degraded the rate of degradation for some compounds was slow, a function of low moisture content and microbial activity. Studies with lined biobeds have highlighted that water management is crucial and that accumulation of some pesticides may be possible. Unlined biobed columns were uncovered and leachate was allowed to flow out of the bottom of the column, this removed any need to manage water inputs. Of the 6 pesticides tested only the two most mobile (Koc <100) pesticides leached and for these >99% was retained, and a significant proportion of the retained chemical was degraded within 9 months. Under the controlled conditions of these experiments, unlined biobeds appear capable of treating the pesticide waste and washings that originate from spray fill sites. In order for biobeds to be approved for use it is likely

that the performance of the system will have to improve so that maximum concentrations of pesticide in leachate are close to the 0.1µg L^1 limit. Concentrations of pesticide in leachate will be controlled by a number of factors including, (1) the hydraulic load, (2) the depth of the biobed and (3) the length of time between application and significant rainfall. Experiments are therefore currently been made to investigate the effects of each of these parameters on biobed performance.

Acknowledgements

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Opinions expressed within this paper are those of the authors and do not necessarily reflect the opinion of the sponsoring organisations. No comments should be taken as an endorsement or criticism of any compound or product.

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Chapter 5

THE EFFECT OF DIFFERENT SOIL TYPES ON LEACHING POTENTIAL AND DEGRADATION OF PESTICIDES IN BIOBEDS

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Abstract

Biobeds can be used to intercept pesticide contaminated runoff from the mixing / washdown area, creating optimum conditions of sorption and biodegradation such that the amount of pesticide reaching adjacent water bodies is significantly reduced. The biobed is built on the farm using locally available materials which include, straw, compost and topsoil. The topsoil acts as the inoculum for the system and is likely to vary in terms of its physical, chemical and microbiological characteristics from one farm to another. This study therefore investigated the effects of using different soil types on the degradation and leaching potential from biobeds. Three contrasting topsoils were investigated. Leaching studies were performed using isoproturon, dimethoate and mecoprop-P which were applied at simulated disposal rates to 1.5 m deep biobeds. Annual average concentrations were similar for each soil type with leaching losses of even the most mobile (Koc 12-25) pesticide < 1.64 % of the applied dose. > 98 % of the retained pesticides were degraded in all matrices. Degradation studies investigated the persistence of individual pesticides and pesticide mixtures in the different matrices. DT_{50} values for isoproturon, chlorothalonil, mecoprop-P and metsulfuron methyl applied at 4 times the maximum approved rate were similar across

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the biomix types and were all ≤ reported DT_{50} values for soil treated at approved rates. When applied as a mixture, DT_{50} values in each biomix increased indicating that interactions between pesticides are possible. However, DT_{90} values of < 167 days were obtained in all circumstances indicating a negligible risk of accumulation. Studies therefore indicate that substrate will have little impact on biobed performance so it should be possible to use local soils in the construction process.

Introduction

The presence of pesticides in environmental waters is well documented.(*1,2,3,4,5,6*) These pesticide residues can be attributed to a number of sources including releases from fields during and after application, leakage from equipment, spillages, and incorrect disposal of waste and washings.(4,7) Recent research suggests that the contribution from sources other than those originating from approved applications to agricultural land may be more significant than previously realised.(*8,9,10,11*) Such 'point source' releases can be reduced by modifying handling practices in order to minimise losses.(*12*) However, due to time constraints and other pressures, small drips and spills are still likely to occur.(*8,9*) Additional methods of control are therefore required. A number of possible approaches are available including: 1) spray equipment could be washed in the field (*13,14*) thus reducing the requirements for decontamination at the farmyard and the disposal of any associated waste; 2) better design of the farmyard to minimise release of pesticides to nearby surface waters (*12,15*); or 3) treatment systems that are installed on the farmyard to treat any waste arising from spray equipment and during the filling process. One possible approach is to use a biobed to intercept and treat contaminated runoff from the farmyard and/or drips and spillages arising during the filling process.(*16,17*) In its simplest form, a biobed is a clay lined hole in the ground filled with a mixture of topsoil, peat and straw and covered with grass.(*16,18*) The biobed is equipped with a ramp enabling the tractor and sprayer to be driven over the bed and thus intercept drips and spills. Alternatively the biobed is connected to an adjacent concrete intercept area on which all mixing and washdown activities take place.(*19*) Studies have demonstrated that biobeds can effectively retain

and degrade pesticides, (*15,20,21,22,23,24,25,26,27*), such that the concentrations of pesticide leaving the mixing / washdown area are significantly reduced.
Typically, the constituent components of the biomix (topsoil, peat and straw) are mixed in volumetric proportions of 1:1:2 respectively.(*18*) The peat or compost provides numerous sites for pesticide sorption and also helps maintain aerobic conditions due to its high water holding capacity, whilst the straw acts as an additional food source for the micro-organisms. The topsoil acts as the inoculum for the biomix and should be rich in humus but must have a low clay content.(*16*) However, the model biobed described is generally adapted in order to satisfy site specific conditions (*21*), and to utilise locally available materials, in particular topsoil. There is evidence that soil texture influences the rate at which a pesticide degrades.(*28,29*) Furthermore, water movement is largely controlled by soil texture, with susceptibility to leaching typically associated with low organic matter content, low moisture holding capacity and a relatively sandy texture.(*30*) In a clay textured soil, water movement is much slower however, and can be complicated by large cracks and macropores which may result in by-pass flow and very rapid water movement.(*31*) The objective of the experiments reported here was to assess the impacts of substrate on biobed performance by a) determining whether concentrations of pesticide leaching from biobeds were affected by different topsoils being used to make the biomix and b) investigating degradation in the different biomix types.

Materials and Methods

Preparation of biomix

Three arable topsoils with a range of physical characteristics were collected (Table 5-1). On the basis of texture, these were representative of 46 % of agricultural land in England and Wales.(*32*) Each soil type was mixed separately with peat free compost (Levington Peat Free Universal) and winter wheat straw in the volumetric proportions of 2:1:1 respectively. The mixtures were composted outside for 71 - 97 days prior to use. Biomix for use in the degradation experiments was then macerated using a food processor, air dried to approximately 25 - 40% w/w (depending on topsoil texture), and

refrigerated at a 0-10 °C prior to use. A disturbed sub-sample was then re-packed into 222 cm^3 volumetric tins and the maximum water holding capacity determined by capillary rise.(*33*) The microbial biomass of the three topsoils and the three biomix mixtures was also measured to give an indication of microbial activity.(*34*)

Table 5-1 Characteristics of soils used for leaching and degradation experiments

	Soil Series		
	Wick	Worcester	Blacktoft
% sand (63 µm – 2 mm	65.38	19.63	12.85
% silt (2 µm – 63 µm	18.71	36.05	46.56
% clay < 2 µm	15.39	44.32	40.59
pH (water)	6.15	7.3	7.7
% Organic Carbon	0.9	1.0	3.6
Texture	Sandy loam	Clay	Silty clay
Maximum water holding capacity % w/w	32.99	55.32	64.63

Test chemicals

Test pesticides were selected on the basis of their physico-chemical properties (*35,36,37*), in particular their sorption potential and water solubility, and represent compounds that are of relatively high average annual usage in the UK.(*38*) (Table 5-2)

Table 5-2 Study compounds and their reported physico-chemical charactersistics

Active substance	Product	Concentration % w/w	Koc (mL g^{-1})	Mobility class*	DT_{50} soil (days)	Solubility water (mg L^{-1})
Isoproturon	Alpha Isoproturon 500	43.6	125	Moderately mobile	6-28	65
Chlorothalonil	Cropgard	41.6	1600-14000	Slightly / non-mobile	5-36	0.6-1.2
Dimethoate	Rogor L40	37.4	16–52	Mobile	2-16	23800
Mecoprop	Optica	48	12-25	Very mobile	3-13	860
Metsulfuron-methyl	Jubilee 20 DF	20	4.6-35	Very mobile	7-35	27900

Values taken from Roberts et al., 1998, Roberts et al., 1999 and Tomlin (2000)
* Hollis 1991

Degradation

Samples (112) of each biomix type were weighed out (25 g) into clear glass bottles (125 mL) fitted with bakelite screw cap lids to provide 3 treated replicates and 1 untreated control per sampling time point. Sub-samples of each biomix were taken and moisture contents determined by oven drying at 105 ±2°C for 24 hours. Formulated isoproturon (Alpha Isoproturon 500^{TM}, 43.6 %w/w), chlorothalonil (Cropgard^{TM}, 41.57% w/w), mecoprop (Optica ^{TM}), 48% w/w and metsulfuron-methyl (Jubillee 20 DF), 20% w/w, were used to make up individual as well as mixture stock suspensions in tap water. For the biomix made using the sandy loam topsoil 1233, 824, 571 and 34 mg a.i. L^{-1} of each respective product was used. For the clay textured biomix 2543, 1699, 1177 and 71 mg a.i. L^{-1} were added and for the silty clay, 665, 445, 308 and 18 mg a.i L^{-1}. In order to achieve final dry weight concentrations in the biomix substrate of 100 mg kg^{-1} (isoproturon), 60 mg kg^{-1} (chlorothalonil), 48 mg kg^{-1} (mecoprop-P) and 1.2 mg kg^{-1} (metsulfuron-methyl) and moisture content of 50 % w/w, 3.3 mL of the respective pesticide solution was added to the sand loam biomix, 6.9 mL to the clay biomix and 1.5 mL to the silt clay biomix. Tap water was applied to the remaining untreated samples. Immediately after treatment, three treated replicates and one untreated control were taken for each different biomix type and pesticide treatment and frozen (-20 °C). The remaining samples were loosely capped and incubated in the dark at 20 °C. At intervals of 5, 10, 20, 30, and 60 days after treatment (DAT) three samples were collected from each different biomix and pesticide treatment, with a single sample from the untreated controls. The samples were stored at –20 °C prior to analysis.

Leaching potential

Twelve lysimeters were prepared using PVC-u piping (22.5 cm internal diameter), cut to 165 cm length. Each pipe section was filled with 5cm of washed gravel (10-15 mm diameter) followed by 150 cm of biomix, to give 4 replicates for each of the three biomix types. The base of each core drained via Teflon tubing to a 2.5 litre amber glass collection vessel located in a central collection pit.(*39*) Lysimeters were connected using plastic guttering to 0.16 m^2 concrete slabs. Silicon sealant was placed on three sides of the slab to prevent water loss from the sides. Formulated isoproturon (Alpha Isoproturon 500^{TM}), 43.6% w/w, dimethoate (Rogor L40^{TM}) 37.4% w/w and mecoprop

(Optica ^{TM}), 48% w/w, were used to make up a stock suspension in tap water of 3200 mg a.i. litre^{-1}, 435.2mg a.i. litre^{-1} and 1536 mg a.i. litre^{-1} of isoproturon, dimethoate and mecoprop, respectively. All twelve lysimeters were treated in January 2003 with 50 mL of the pesticide mixture to give a final treatment rate of 298 mg (isoproturon), 40.5 mg (dimethoate) and 143 mg (mecoprop-P). Potassium bromide (KBr) was applied at the same time as the pesticides (314 mg core^{-1}) to check the hydrological integrity of the lysimeters, as well as to determine the breakthrough timing of infiltrating water. Leachate collection vessels were monitored after all rainfall events and the total volume of leachate recorded. Volumes in excess of 200 mL were collected and frozen prior to analysis. Where possible, a 60 mL sub-sample was also taken for KBr analysis. At the end of the study, (115 days after treatment, (DAT) the top 30 cm of the lysimeters was removed and sectioned (0-10, 10-20, and 20-30cm) and the sections were homogenised and frozen prior to analysis. Artificial irrigation was applied to all 12 lysimeters in February, March and April. The cumulative total applied was 91.4mm equivalent to 12.4 litres per lysimeter.

Analysis

Water Extraction

For isoproturon, dimethoate and mecoprop-P added as mixtures, samples (200 mL) were extracted into 3 x 40 mL dichloromethane (DCM) using a glass separating funnel (250 mL). Following extraction, DCM extracts were dried over anhydrous sodium sulphate and then evaporated to dryness using a rotary evaporator at 40°C. The resulting residues were re-dissolved into 2 mL of methanol. Concentrations of isoproturon and mecoprop-P were then determined by HPLC, dimethoate concentrations were determined by GC.

Biomix extraction

Biomix samples (40 g) from the semi-field experiments treated with isoproturon, dimethoate and mecoprop-P added as mixture were placed into glass 250 ml bottles and extracted into 80 ml of methanol for 1 hour using an end-over-end shaker. Following extraction, samples were allowed to stand until clear. An aliquot of the methanol solution was then taken for analysis. Isoproturon and mecoprop-P concentrations were determined by HPLC, dimethoate concentrations were determined by GC. Laboratory samples (25 g) treated with isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl applied individually and as a mixture were shaken for 1 hour on an end over end shaker with methanol (50 mL). Samples were allowed to stand until clear after which an aliquot of the solution was taken for HPLC analysis.

Recoveries for all of the extraction methods were > 94 %.

HPLC analysis

Concentrations of isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl were determined by HPLC using a Spectra Physics SP8810 pump linked to a Kontron 430 UV detector. Samples (20 µl) were injected using a Spectra Physics SP8775 autosampler. Separation was achieved using a hypersil C18 column (250 x 4.6 mm). The mobile phase used was acetonitrile:methanol:0.05M acetic acid (35:30:35) with a flow rate of 1.5 ml min $^{-1}$ which gave retention times of 2.6, 3.4, 4.1 and 5.6 min for metsulfuron-methyl, mecoprop-P, isoproturon and chlorothalonil respectively. The detection wavelength was 230 nm for all three substances. The limit of quantification was 0.05 µg L^{-1} for metsulfuron-methyl and mecoprop-P, 0.03 µg L^{-1} for isoproturon and 0.02 µg L^{-1} for chlorothalonil (Appendix i).

GC analysis

Concentrations of dimethoate were determined on a Hewlett Packard HP5890 gas chromatograph fitted with a split/splitless injector, 12m x 0.53 mm BPX5 column (SGE) and a nitrogen-phosphorus detector. The carrier gas (helium) flow rate was 7 ml min^{-1} and detector –gas flow rates were 100 ml min^{-1} (air) and 4 ml min^{-1} (hydrogen). Oven temperature was raised from 90 ^{o}C to 190 ^{o}C (40 ^{o}C min^{-1}) and then to 220 ^{o}C (10 ^{o}C min^{-1}) and finally to 245 ^{o}C (15 ^{o}C min^{-1}). Samples (2 µl) were injected using a Hewlett Packard HP7673 autosampler. Under these conditions dimethoate had a retention time of 3.1 minutes. Quantification was achieved by comparison of peak areas with results from external standards. The limit of quantification was 0.08 µg L^{-1}.

Bromide

Concentrations of potassium bromide were determined using a Metrohm (Herisau, Switzerland) 790 Personal ion chromatograph and 813 compact autosampler. Analytical columns used were Metrohms', Metrosep RP guard, Metrosep A Supp 4/5 guard, and Metrosep A Supp 4 (250 x 4.0mm). A 20µL injection loop and isocratic eluent of composition 1.8mM sodium carbonate / 1.7mM sodium hydrogen carbonate were used giving a typical retention time of 8.5 minutes. All samples were filtered at 0.45µm (Whatman 13mm polysulphone syringe) prior to loading into the proprietary autosampler cartridges. Limit of quantification was 0.5mgL, with a limit of detection at 0.1mgL^{-1}.

Biomass

Total microbial biomass was determined by fumigation extraction.(34) Chloroform (2 mL) was added to triplicate samples (20 g) of soil and biomix. A control sample was left untreated. Treated and untreated samples were sealed and incubated at 30 °C for 7 - 10 days. Following incubation, fumigated samples were evacuated 4 - 6 times in a vacuum dessicator to remove the chloroform and then shaken for 50 minutes with 50

mL of 2 M potassium chloride. Samples were then centrifuged, and a 1 mL extract was taken to which 0.5 mL of ninhydrin was added. The samples were then immersed in a boiling water bath for 20 minutes. After cooling, samples were made up to 10 mL using a 50:50 mixture of ethanol and water, transferred to plastic cuvettes, and the absorbance measured using a spectrophotometer at 570 nm. The absorbances were corrected for the unfumigated controls and the amounts of ninhydrin reactive N derived from a calibration curve produced using different concentrations of L-lucine. The results were corrected for moisture content and the total biomass C (mg kg-1) calculated.(34)

Data Analysis

Where possible, the first order rate equation was fitted to the observed concentrations, (Equation 1),

$$\frac{dC}{dt} = -kC \qquad \text{(Equation 1)}$$

where C is the concentration (mg kg^{-1} soil), t is the time (days) and k is the degradation rate ($days^{-1}$). The integrated form of this equation (equation 2) was fitted to non-transformed data using the least squares method in order to give the best agreement between calculated and observed concentrations.

$$C_{(t)} = C_0 \exp(-kt) \qquad \text{(Equation 2)}$$

However, the first order rate equation is often considered unacceptable if the determination coefficient (r^2) falls below 0.7 (*40*). Where data indicated increasing rates of degradation with time, DT_{50} and DT_{90} values were calculated using an empirical two-parameter relationship,

$$S/S_0 = \exp\{k_1[1-\exp(k_2 t)]\} \qquad \text{(Equation 3)}$$

where S_0 and S are the concentrations of pesticide at time 0 and time t, respectively. Microsoft Excel Solver was used to estimate parameters k_1 and k_2 using the least squares method in order to give the best agreement between calculated and observed concentrations. The degradation data were summarised by calculating the times to 50% degradation (DT_{50}) and the time to 90% degradation (DT_{90}) from the calculated degradation curves using the relationship;

$$DT_{50} = \ln(1-\ln(0.5)/k_1)/k_2 \qquad \text{(Equation 4)}$$

$$DT_{90} = \ln(1-\ln(0.1)/k_1)/k_2 \qquad \text{(Equation 5)}$$

Similarly where the pattern of degradation was bi-phasic with residue concentrations decreasing slowly after an initial rapid decline, data were fitted to a bi-exponential decay curve. The bi-exponential curve consists of two exponential terms,

$$C_{(t)} = A\exp(-k_1 t)+B\exp(-k_2 t) \qquad \text{(Equation 6)}$$

where $C_{(t)}$ (mg kg^{-1} soil) is the concentration at time t, A (mg kg^{-1} soil) and B (mg kg^{-1} soil) are constants, k_1 ($days^{-1}$) and k_2 ($days^{-1}$) determine the decline of the first and second component of the curve, respectively. (*40*)

Results

Microbial biomass

The microbial biomass was measured to give an indication of microbial activity. Values of 83.47, 229.4 and 185.5 mg kg^{-1} carbon were measured for the sand, silt and clay topsoils respectively. By mixing the three topsoils with straw and compost a significant (Anova $P<0.05$, F 5.01, df 2) increase in microbial biomass was measured with values of 255.4, 416.7 and 388.2 mg kg^{-1} carbon being obtained for the sand, silt and clay biomix respectively (Figure 5-1).

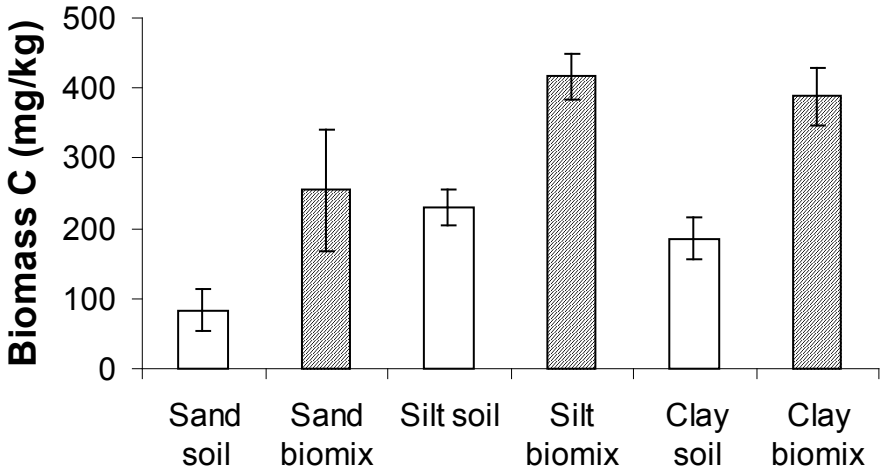


Figure 5-1 Microbial biomass measured in the sand, silt and clay topsoil and biomix

Degradation

Effect of different soils on pesticide degradation

Results from the experiments to investigate the degradation of isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl in biomix made using different topsoil inoculum are summarised in Table 5-3. With the exception of the silt biomix, the pattern of degradation for isoproturon could be fitted to first order kinetics (equation 2), with <5 % of the applied dose remaining in the sand and clay biomix after 20 days. In the silt biomix, after an initial period of rapid degradation, residue levels persisted at low levels until the end of the experiment (Figure 2a). DT_{50} values of 6.3, 13.4 and 5.9 days were calculated for the sand, silt, and clay biomix soils, respectively. The slower rate of isoproturon on the silt biomix resulted in recovered residues of >15 % at the end of the experiment, which were significantly higher (Anova P< 0.05, *F* 40.16, *df* 2) than in the sand and clay biomix. Degradation of chlorothalonil was bi-phasic (equation 6) in all 3 biomix substrates, with similar DT_{50} values measured, ranging from 8.0 days in the sand biomix to 9.4 days in the clay biomix. In the sand and clay biomix < 13% of the applied dose was recovered at the end of the experiment with DT_{90} values of 49.5 days calculated for both matrices.

Table 5-3 DT_{50} and DT_{90} degradation rates, degradation rate constants (*k*) and determination coefficients (r^2) for isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl when applied individually to biomix made using sand, silt and clay topsoils

	SAND				SILT				CLAY			
	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2
Isoproturon	6.3	20.8	0.111	0.99	13.4	52.9	*k*1 1.589 *k*2 0.054	1	5.9	19.5	0.118	0.98
Chlorothalonil	8.0	49.5	*k*1 0.038 *k*2 0.43	0.98	8.2	71.3	*k*1 0.0001 k2 0.10	0.95	9.4	49.5	*k*1 1.953 *k*2 0.082	0.85
Mecoprop-P	6.2	8.6	*a* 0.038 *b* 0.477	1.00	4.3	8.0	*a* 0.343 *b* 0.256	1.00	5.1	7.5	*a* 0.067 *b* 0.476	1.00
Metsulfuron-methyl	13.4	44.4	0.052	0.98	19.5	64.8	0.036	0.99	31.4	104.3	0.022	0.99

Table 5-4 DT50 and DT90 degradation rates, degradation rate constants (*k*) and determination coefficients (r^2) for isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl when applied as a mixture to biomix made using sand, silt and clay topsoils

	SAND				SILT				CLAY			
	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)	*k* deg (days $^{-1}$)	r^2
Isoproturon	21.4	47.7	*a* 0.918 *b* 0.026	0.99	34.7	115.4	0.020	0.98	16.1	30.7	*a* 0.399 *b* 0.062	1.00
Chlorothalonil	15.6	82.0	*k*1 0.024 *k*2 0.23	1.00	19.6	167.0	*K*1 0.011 *k*2 0.15	1.00	14.2	101.9	*K*1 0.017 *k*2 0.17	1.00
Mecoprop-P	6.5	7.6	*a* 0.0008 *b* 1.034	1.00	5.6	8.6	*A* 0.105 *b* 0.365	1.00	6.8	8.8	*a* 0.012 *b* 0.600	1.00
Metsulfuron-methyl	37.4	124.3	0.019	0.99	43.5	66.5	*a* 0.097 *b* 0.048	0.88	58.6	64.7	*a* 0.000008 *b* 0.195	0.96

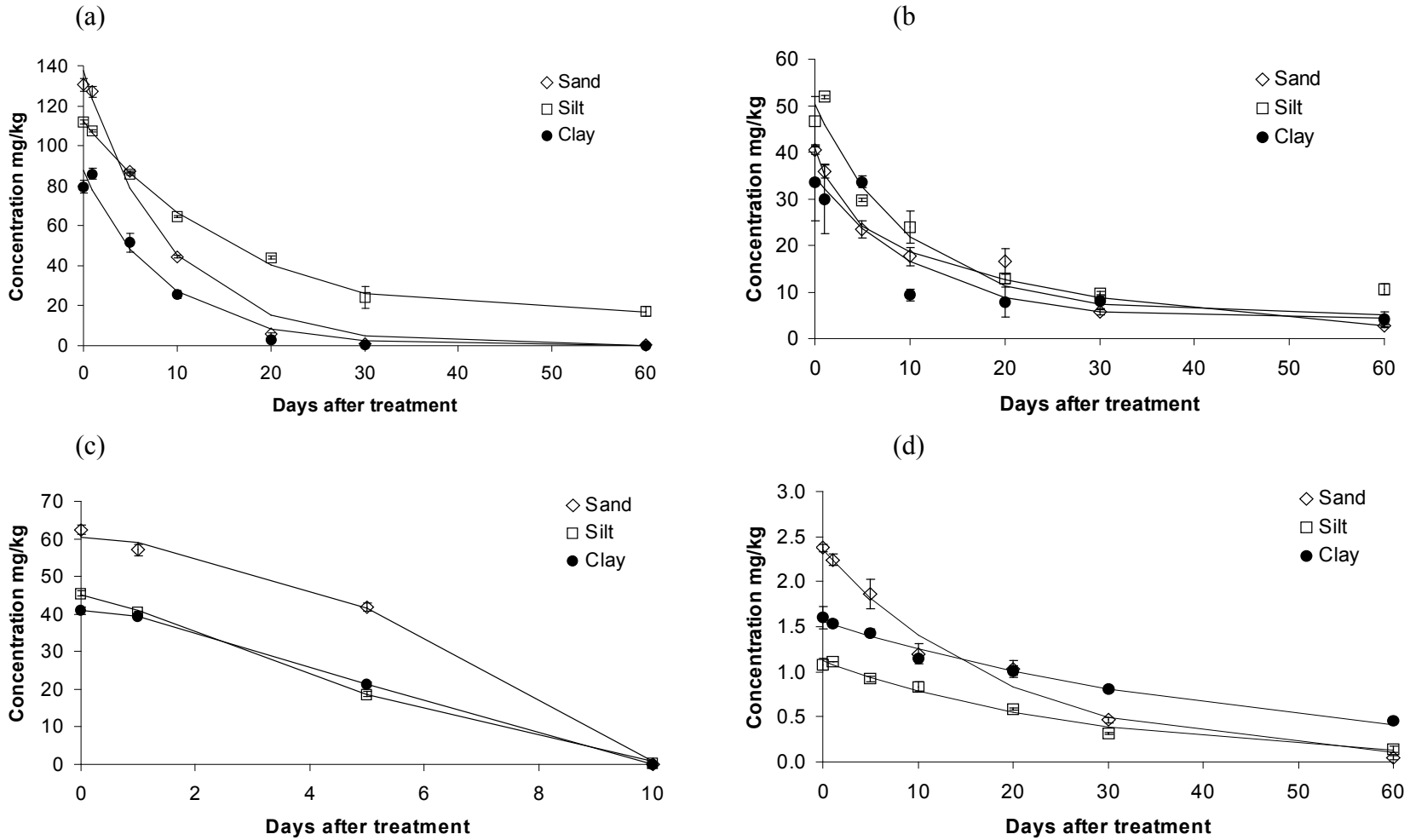


Figure 5-2 Degradation of a) isoproturon, b) chlorothalonil, c) mecoprop-P and d) metsulfuron-methyl in biomix made using three contrasting topsoils

In the silt biomix a DT_{90} of 71.3 days was calculated explaining why significantly (Anova $P < 0.05$, F 7.05, df 2) more (23%) of the applied dose was recovered after 60 days (Figure 5-2b). Mecoprop-P degraded rapidly in all 3 biomix types (Figure 5-2c). The data indicated increasing rates of degradation with time (equation 3). DT_{50} values were between 4.3 days (silt biomix) and 6.2 days (sand biomix) with DT_{90} values of < 9 days in all 3 biomix types. Recovered residues were < 1 % after 10 days. The pattern of metsulfuron-methyl degradation could be fitted to first order kinetics in all three biomix types (Figure 5-2d). The rate of degradation was quickest in the sand biomix (DT_{50} 13.4 days) and slowest in the clay biomix (31.4 days). Similarly DT_{90} values ranged from 44.4 days in the sand biomix to 104.3 days in the clay. Recovered residues at the end of the study significantly different (Anova $P< 0.05$, F 30.11, df 2), with 1.9, 12.7 and 28.3 % of the applied dose measured in the sand, silt and clay biomix soils respectively.

Effect of pesticide mixture on pesticide degradation

Results from the experiments to investigate the degradation of isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl in the different biomix types when applied as a mixture are summarised in Table 5-4. The pattern of isoproturon degradation in the sand and clay biomix was bi-phasic showing increasing rates of degradation with time (Figure 5-3a & c). DT_{50} and DT_{90} values of 21.4 and 47.7 days were calculated for the sand biomix, and 16.1 and 30.7 days for the clay biomix respectively. At the end of the experiment, < 7% of the applied pesticide was recovered. The pattern of isoproturon degradation in the silt biomix also fitted first order kinetics (Figure 5-3b). DT_{50} and DT_{90} values for the silt soil were 34.7 and 115.4 days respectively with 35 % of the applied pesticide recovered after 60 days. For chlorothalonil the rate of degradation was similar in all 3 biomix types. After an initial period of rapid degradation residue levels persisted at relatively low levels until the end of the study, (Figure 5-4a,b & c). DT_{50} values ranged from 14.2 days in the clay biomix to 19.6 days in the silt biomix and DT_{90} values between 82 days (sand biomix) and 167 days (silt biomix) were obtained, (Table 5-4). At the end of the experiment 17, 20 and 31 % of the applied dose was recovered from the sand, clay and silt biomix soils, respectively.

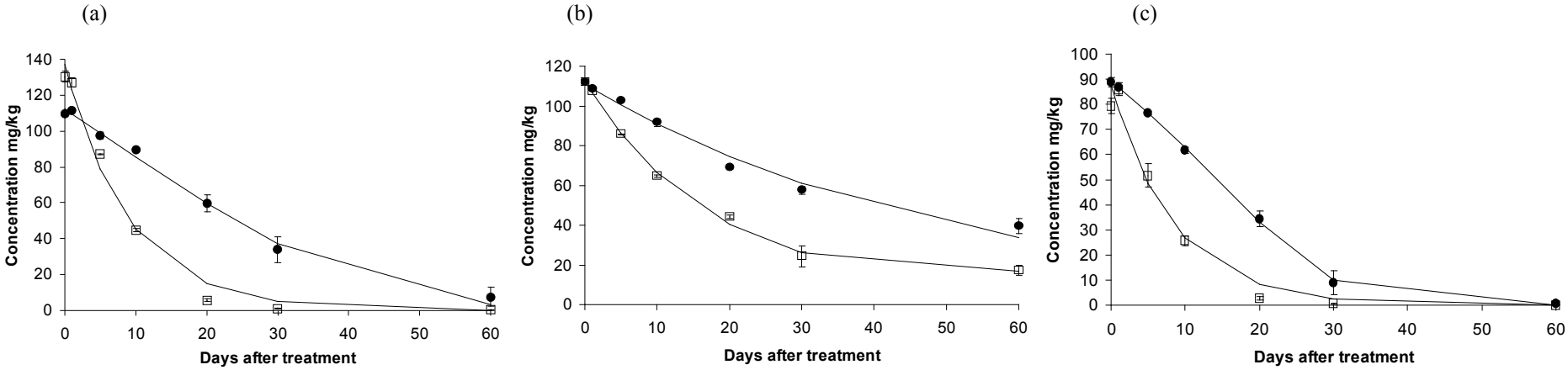


Figure 5-3 Concentrations of isoproturon in biomix made using a) sand b) silt and c) clay topsoil when applied individually □and as part of mixture ● containing isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl

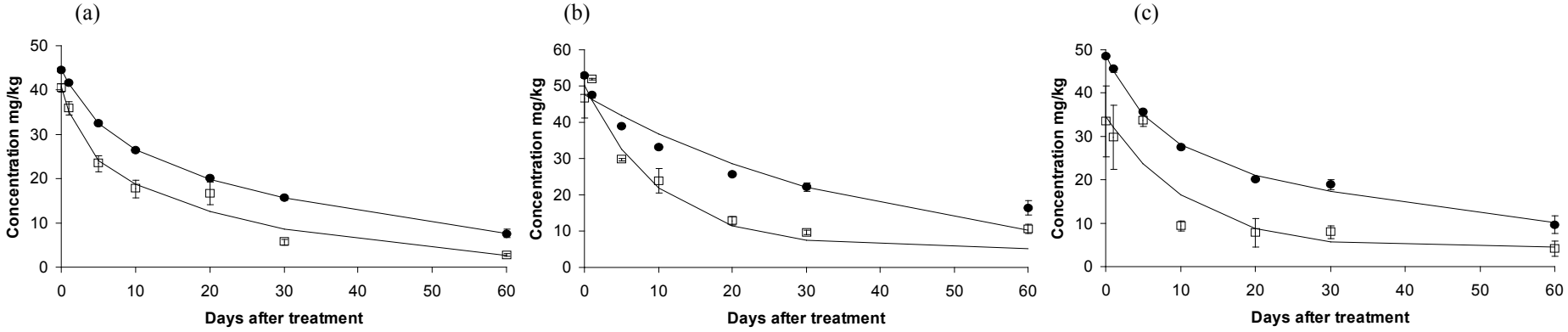


Figure 5-4 Concentrations of chlorothalonil in biomix made using a) sand b) silt and c) clay topsoil when applied individually □and as part of mixture ● containing isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl

Degradation of mecoprop-P was similar to that observed in the individual treatments. The pattern of degradation was the same for all three biomix types showing increasing rates of degradation with time (Figure 5-5a,b & c). DT_{50} values ranged from 5.6 to 6.8 days in the silt and clay biomix, soils respectively, with < 2 % of the applied pesticide remaining in any of the biomix soils after 10 days. For metsulfuron-methyl in the clay and silt biomix soils, very little degradation was observed for the first 30 days following treatment. However, between 30 and 60 days the rate of degradation was much more rapid (Figure 5-6b & c). DT_{50} values of 43.5 days and 58.6 days were calculated for the silt and clay biomix soil respectively. At the end of the study, 23% of the applied dose was recovered from the silt biomix compared with 42 % from the clay. Degradation in the sand biomix soil was fitted to first order kinetics (Figure 5-6a). DT_{50} and DT_{90} vales of 37.4 and 124.3 days were calculated, respectively, with 28 % of the applied dose recovered 60 DAT.

Leaching

Rainfall and leachate volumes

With artificial irrigation (91.4 mm) the total water input for the study period was 201.5 mm, and was 53 % above the long term average for the period January to April inclusive. Leachate samples were collected on 19 occasions providing 228 water samples for analysis. Cumulative leachate volumes ranged from 26.2 – 30.6 litres from the silt biomix lysimeters, from 30.4 – 33.7 litres from the clay biomix lysimeters and from 27.4 – 34.2 litres from the sand biomix lysimeters.

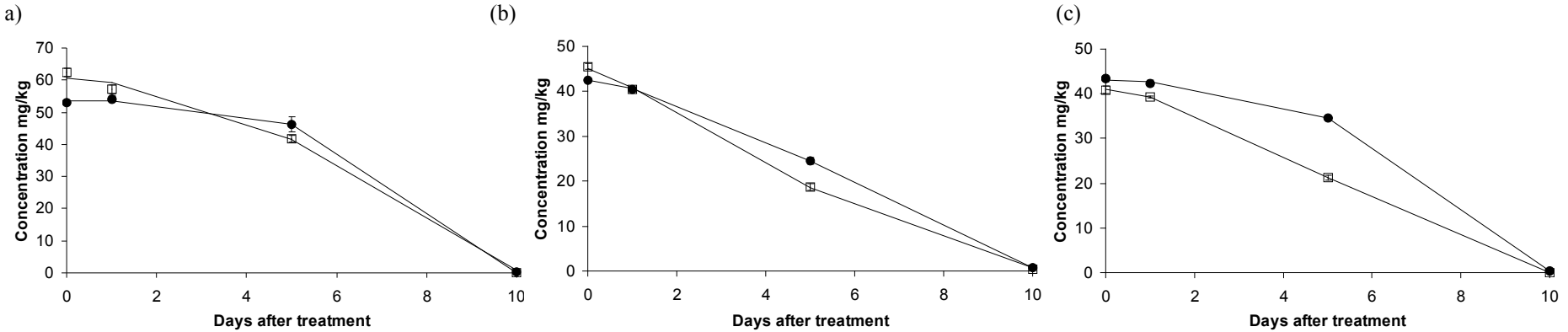


Figure 5-5 Concentrations of mecoprop-P in biomix made using a) sand b) silt and c) clay topsoil when applied individually □and as part of mixture ● containing isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl

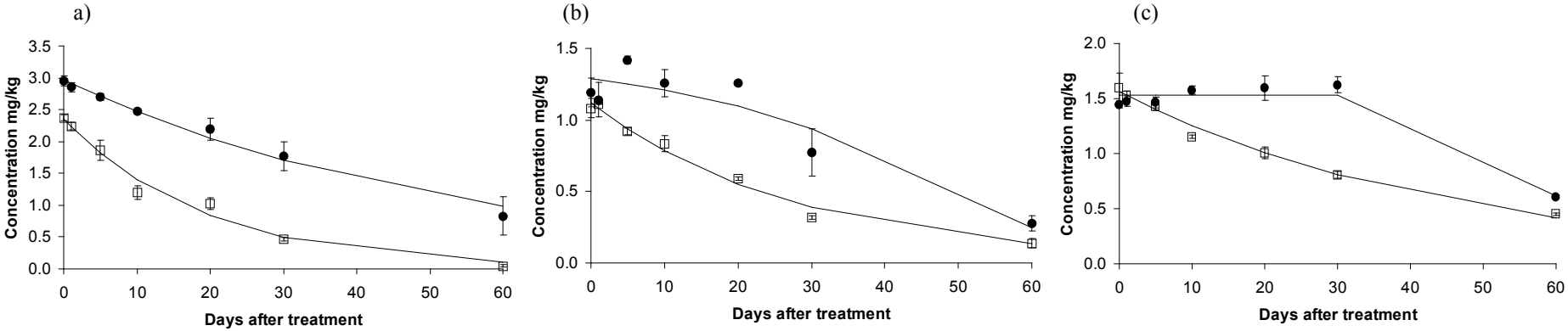


Figure 5-6 Concentrations of metsulfuron-methyl in biomix made using a) sand b) silt and c) clay topsoil when applied individually □and as part of mixture ● containing isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl

Bromide in leachate

Bromide breakthrough curves from the three different biobed mixtures were similar (Figure 5-7). Breakthrough was measured 48 DAT for each of the three biobed mixtures. Maximum concentrations were measured 55 DAT from the sand biomix lysimeters, 79 DAT from the clay biomix lysimeters, and 86 DAT from the silt biomix lysimeters. Concentrations of bromide for the silt and clay biomix lysimeters were below the LOQ (0.5 mg L^{-1}) by the end of the study (108 DAT) and from the sand biomix lysimeters were at 1.7 mg L^{-1}. Cumulative losses from the sand, silt and clay biomix lysimeters were 17, 13 and 12 % respectively.

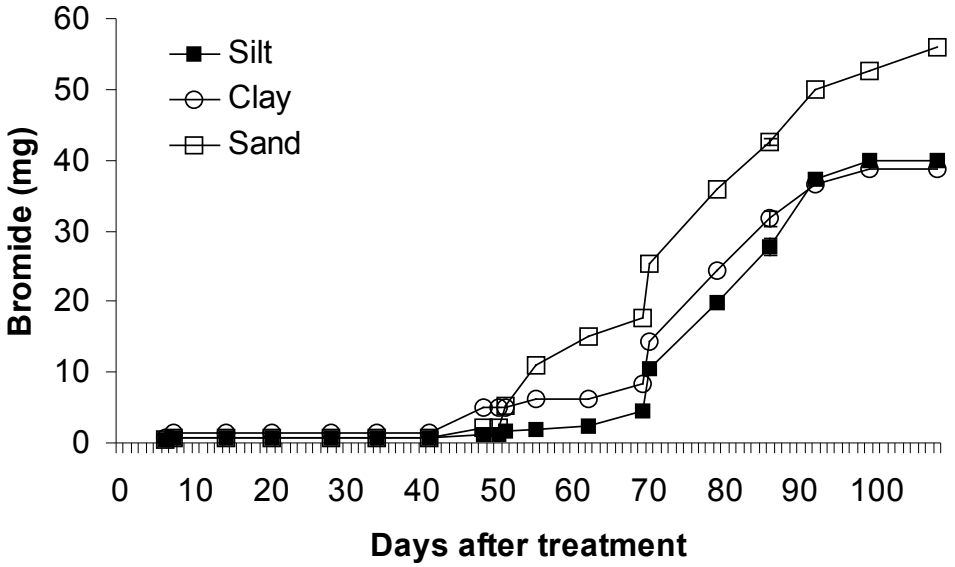


Figure 5-7 Bromide leaching (± 1 SE) from lysimeters filled with different biobed mixtures made using sand, silt and clay textured topsoils

Pesticide residues in leachate

Peak concentrations of isoproturon measured in leachate from the silt, clay and sand biomix lysimeters were 1.62, 2.84 and 6.49 µg L^{-1}, and were measured 50, 70 and 62 DAT respectively (Figure 5-8). Breakthrough from the silt biomix lysimeters occurred 7 DAT, whereas from the clay and sand biomix, breakthrough was much later, i.e. 34 and 50 DAT respectively. Mecoprop-P breakthrough from the silt and clay biomix was

measured 6 DAT and from the sand biomix 14 DAT. Peak concentrations were measured 62 DAT from the silt biomix and 108 DAT from the sand and clay biomix. The maximum measured concentrations were 45.22, 117.7 and 145.3 μg L^{-1} from the silt, clay and sand biomix respectively. Maximum concentrations of dimethoate were measured 50, 70 and 108 DAT from the silt, clay and sand biomix with values of 0.53, 1.06 and 6.27 μg L^{-1} respectively. Breakthrough of dimethoate was measured 34 DAT from the silt biomix, 41 DAT from the clay biomix and 48 DAT from the sand biomix.

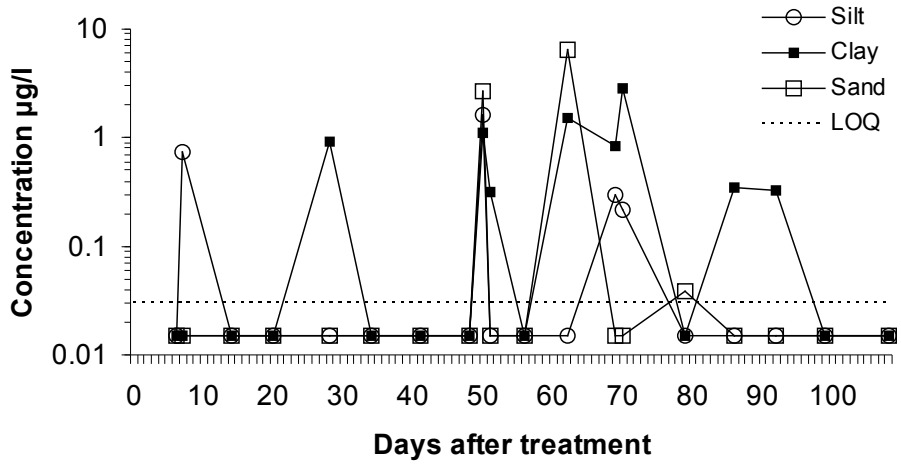
Pesticide residues in biomix

No mecoprop-P was measured in either the sand, silt or clay biomix lysimeters at the end of the study (115 DAT), and no isoproturon or dimethoate was measured below 10 cm depth. For isoproturon, the measured residues (expressed as percentage of the applied dose) remaining in the sand, silt and clay biomix lysimeters were 1.46, 1.53 and 1.13 % respectively. No dimethoate was recovered from the clay biomix lysimeter 0-10 cm layer, with 0.2 % recovered from this layer in the sand biomix and 0.25 % from the silt biomix.

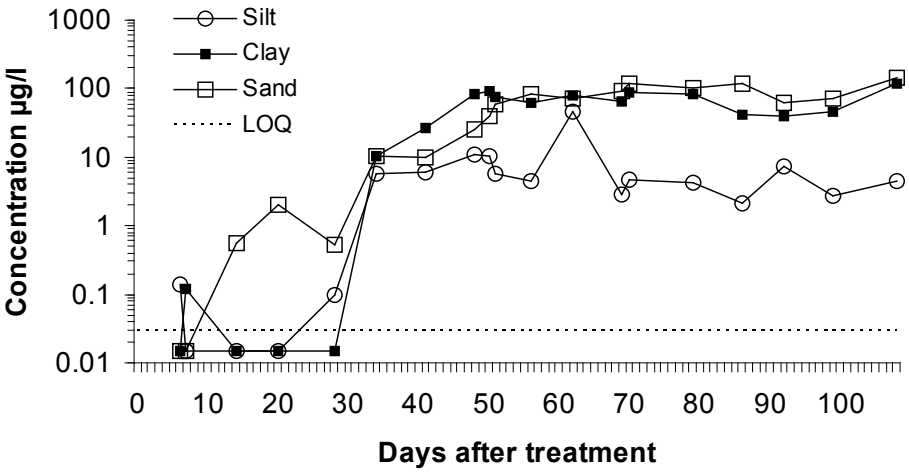
Mass balance

A mass balance was performed to determine the fate of each of the study compounds when applied to the biobed lysimeters filled with the different biomix substrates (Table 5-5). For isoproturon between 0.007% (clay) and 0.002 % (silt) leached, between 0.51 % (silt) and 0.38 % (clay) was associated with the biobed matrix, and between 99.6 % (clay) and 99.5 % (silt) was degraded. For mecoprop-P, between 1.64 % (clay) and 0.04 % (silt) leached, 0 % was recovered from the biobed matrix for either the sand, silt or clay biomix, with between 99.96 % (silt) and 98.36 % (clay) was degraded. For dimethoate between 0.11 % (clay) and 0.004 % (silt) leached, between 0.61 % (silt) and 0 % (clay) was retained in the biobed matrix, and between 99.89 % (clay) and 99.38 % (silt) was degraded.

(a)



(b)



(c)

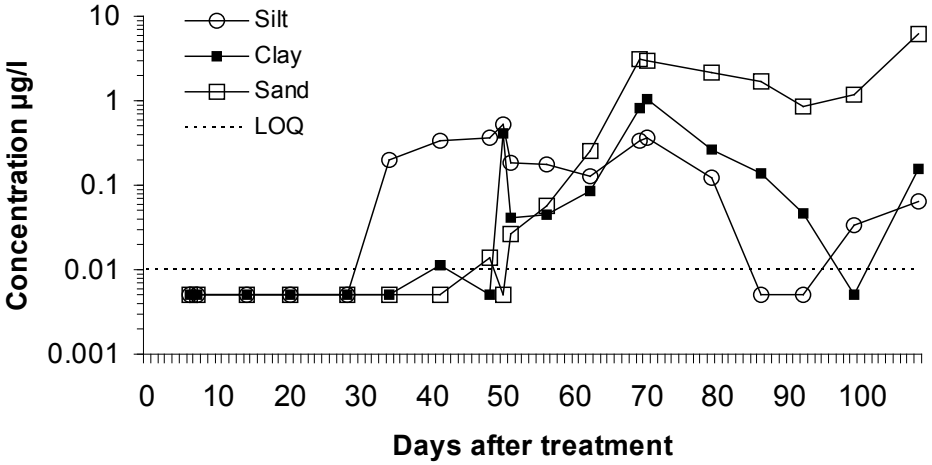


Figure 5-8 Mean concentrations of (a) isoproturon, (b) mecoprop-P and (c) dimethoate from 1.5m deep lysimeters connected to 0.16m^2 concrete slabs and filled with biomix made from either sand, silt or clay topsoil

Table 5-5 Mass balance for lysimeters filled with biomix made using either sand, silt or clay topsoil

Soil Type	% leached	% retained	% degraded	Maximum Concentration ($\mu g\ L^{-1}$)	CV%	Average Concentration ($\mu g\ L^{-1}$)	CV%
Isoproturon							
Sand	0.006	0.50	99.50	6.49	188.8	0.50	129.4
Silt	0.002	0.51	99.49	1.62	96.7	0.16	67.4
Clay	0.007	0.38	99.61	2.84	158.2	0.44	106.7
Mecoprop-P							
Sand	1.36	0	98.64	145	116.9	53	114.6
Silt	0.04	0	99.96	45	154.8	6.15	88.5
Clay	1.64	0	98.36	117	96.1	48	76.4
Dimethoate							
Sand	0.02	0.48	99.50	6.27	128.2	0.98	62.5
Silt	0.004	0.61	99.38	0.53	99.4	0.15	131.6
Clay	0.112	0	99.89	1.06	112.0	0.16	108.3

Discussion

Topsoil is used as the inoculum for the biobed matrix and as biobeds are likely to be built on farms using locally available materials it is likely that the physical and chemical characteristics of the topsoil used will vary considerably. The degradation of pesticides applied to soil is mainly carried out by soil microorganisms, (*41*) therefore those factors which effect microbial activity in soil should also influence rates of pesticide loss.(*42*) In the 3 soils tested here, measured biomass levels were highest in the silt topsoil and lowest in the sand. Mixing each of the soils with compost and straw resulted in a two fold increase in the measured biomass, indicating a significant increase in the levels of microbial respiration. DT_{50} values for individual compounds applied at 4 times the maximum approved rate were ≤ reported DT_{50} values for soil treated at approved rates. However, in practice repeated applications of tank mixes containing herbicides, fungicides and insecticides are made.(*43,44,45,46*) Biobeds are therefore likely to receive complex mixtures of more than one active substance applied repeatedly at concentrations far higher than field treatment rates. When applied as a mixture, DT_{50} values for isoproturon, chlorothalonil, mecoprop-P and metsulfuron-methyl increased indicating that interactions between pesticides applied as a mixture are possible. Similar observations have been reported elsewhere (*22,23,44,46,47*). This inhibition may be due to a number of factors. The application of the fungicide chlorothalonil my have suppressed the activity of non-target soil micro-organisms, (*48,49*) thus inhibiting the rate at which the remaining pesticide were degraded. Singh et al., (*46*) reports that all measured microbial characteristics were adversely affected by chlorothalonil treatment when applied individually or in combination with other pesticides. These findings are supported by a previous study (*50*) where it was reported that soil respiration was suppressed following the application of chlorothalonil. Even though degradation rates of the individual compounds were suppressed when applied as part of a mixture, DT_{90} values were all < 167 days, indicating a negligible risk of carry over from one season to the next.

It is generally accepted that pesticides applied to coarse textured, sandy soils are subject to greater leaching than those applied to soils with higher clay or organic matter content.(51) Studies to investigate the leaching risk from biobeds when different

biomix soils were used showed there to be no significant difference in the amounts of pesticides leaching. Bromide breakthrough curves showed a similar rate of water movement through each of the biomix soils, indicating similar physical characteristics for the three matrices.

Analysis of the biobed matrix from this study showed that all pesticides were retained in the top 10cm and that after 4 months > 98 % of the non-leached pesticide had been degraded. Previous laboratory investigations compared pesticide behaviour in sterile and non-sterile biomix and concluded that degradation by soil micro-organisms was the principle mechanism responsible for the reduction in measured concentrations of pesticide in non-sterile systems and that bound residues were not a significant issue.(*22*)

Conclusions

Pesticides may be released to farmyard surfaces as a result of spillages, leakages and the decontamination of tractors and sprayers, and recent studies have demonstrated that contaminated runoff from the farmyard can contribute a significant proportion of the pesticide load being released to surface waters. Biobeds are one possible approach that can be used to intercept this runoff thus reducing the concentrations of pesticide being released to the environment. The system is cost effective, requires low technical inputs, and utilises materials readily available to the end user. This study has shown that when different topsoils are used, leaching losses and degradation rates were similar. Furthermore, > 98% of the applied pesticide was retained by each of the biomix types. Whilst interactions between pesticides are possible, DT_{90} values suggest that accumulation of pesticides within the biobed should not occur. On the basis of the results presented here the use of different soil types in the construction of the biobed should not effect the level of treatment achieved.

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Opinions expressed within this paper are those of the authors and do not necessarily reflect the opinion of the sponsoring organisations. No comments should be taken as an endorsement or criticism of any compound or product.

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Chapter 6

LEACHING OF PESTICDES FROM BIOBEDS: EFFECT OF BIOBED DEPTH AND WATER LOADING

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Abstract

Pesticides may be released to farmyard surfaces as a result of spillages, leakages and the decontamination of tractors and sprayers. Biobeds can be used to intercept and treat contaminated runoff, thus minimising losses to the environment. Previous studies using lined and unlined biobeds showed that water management was the limiting factor for both systems. Whilst lined biobeds effectively retained pesticides, the system rapidly became water logged and degradation was slow. Studies using unlined biobeds showed that >99% of the applied pesticides were removed by the system, with a significant proportion degraded within 9 months. However, peak concentrations in leachate of certain pesticides (Koc < 125) were unacceptable to the regulatory authorities. These experiments were designed to optimise the design and management of unlined biobeds. Experiments performed to investigate the relationship between biobed depth and water loading showed that biobeds need to have a minimum depth of 1 - 1.5m. The surface area dimension of the biobed depends on the water loading which is controlled by the nature and frequency of pesticide handling activities on the farm. Leaching losses of all but the most mobile (Koc <15) pesticides were < 0.32 % of the applied dose from 1.5 m

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deep biobeds subject to a water loading of 1175 L m^{-2}. These were reduced to <0.06 % when a water loading of 688 L m^{-2} was applied and down to < 0.0001% for a water loading of 202 L m^{-2}. Based on these data a 1.5 m deep biobed, subject to a maximum water loading of 1121 L m^{-2} and with a surface area of 40 m^2 should be able to treat ≤ 44000 litres of pesticide waste and washings such that the annual average concentration of all pesticides, other than those classified as very mobile do not exceed 5 µg L^{-1}. This level of treatment can be improved by further reduction in the hydraulic loading.

Introduction

Routine monitoring of environmental waters has shown that contamination with pesticides does occur.(*1,2,3*) Where the water serves as a drinking water supply, treatment is often required in order to meet the standards set by e.g. the European Drinking water Directive 80/778/EEC. Such treatment can be expensive, with around £1 billion being invested by the water industry in England and Wales since 1990.(*2*) Pesticides are generally applied for agricultural purposes on to land where a microbiologically active soil layer is present and where degradation and dissipation process can take place.(*4*) However, under these normal use conditions losses to the environment can still occur due to processes such as leaching, runoff and drainflow.(*5,6,7,8*) However, contamination arising from other sources such as non-approved use, poor practice, illegal operations, accidental releases and inputs of washings are reported to contribute between 18 and 84 % of the pesticide load measured in some individual catchments.(*9,10,11,12,13,14,15,16,17,18*) Better training of sprayer operators and good machinery maintenance can reduce the number of accidental releases.(*19*) However, due to time constraints and other pressures small drips and spills are still likely to occur.(*15,16*) Direct inputs from the decontamination of tractors and sprayers,(*20*) and residues that remain in the sprayer sump after infield tank rinsing are also an unavoidable feature of the spraying operation.(*12,21*)

The filling and cleaning of agricultural spray equipment is often performed at the same site in the farmyard year after year due to location of the farm pesticide store and the convenience of a clean water supply.(22,23) The design, management and operation of these mixing / handling / washdown areas is therefore considered a primary target in

reducing the amount of pesticide leaving the farmyard.(24) Traditionally these areas have been on concrete pads, which offer little opportunity of sorption and degradation,(*25,26*) and which often connect directly to a soakaway or water course, resulting in direct and rapid transport of pesticides to water bodies. Alternative materials on which pesticides are mixed and equipment decontaminated therefore need to be considered.(*19*) Any alternative should supplement good handling practices that reduce inputs to aquatic systems and also be cheap to use and require low labour and time inputs. One possible approach is to use a biobed to intercept and treat contaminated runoff from the farmyard and/or drips and spillages arising during the filling process. In its simplest form a biobed is clay lined hole in the ground filled with a mixture of topsoil, peat and straw and covered with grass.(*27,28*) The biobed is equipped with a ramp enabling the tractor and sprayer to be driven over the bed and thus intercept drips and spills. The biobed can also be connected to an adjacent concrete intercept area on which all mixing and washdown activities take place,(29) Studies have demonstrated that biobeds can effectively retain and degrade pesticides,(*24,30,31,32,33*), such that the concentrations of pesticides being released from the farmyard are significantly reduced. However, studies have shown the potential risk to ground water from mobile pesticides leaching through the clay layer in the base of the biobed.(*34*) To safeguard against the potential contamination of ground water the UK regulatory authorities insisted that a butyl liner be installed into the base of all experimental biobeds constructed in the UK. However, studies performed at the semi-field scale using lined biobeds showed that whilst pesticides were effectively retained, the biobeds quickly became water logged. Covers had to be placed over the biobeds to exclude clean rainwater. However, once covered the top 10 cm became hydrophobic, forming an impermeable layer which restricted water loss and impeded degradation of the retained pesticides.(*35*) The use of unlined biobeds removed the need to manage water inputs whilst at the same time maintaining near optimum conditions for pesticide degradation. Only the most mobile (Koc <125) compounds leached to any great extent and even for these compounds, the biobed system appeared to retain or degrade more than 99% of the applied dose.(*35*) However, maximum concentrations of pesticide leaching from the biobed were considered unacceptable. In order for biobeds to be approved for use it is likely that the performance of the system will have to improve

such that maximum concentrations of pesticide in leachate are close to the standard of 0.1µg l^{-1} set by the European Drinking water Directive 80/778/EEC.
This study was performed to understand the relationship between biobed size, water load and concentration of a range of pesticides in order to provide guidance on the construction and operation of biobeds in the UK. Experiments were therefore made to examine the effects of (1) the hydraulic load, (2) the depth of the biobed, such that the optimum dimensions and maximum hydraulic loading with respect to concentrations of pesticide in leachate could be determined and regulatory approval for use granted. The studies were performed at the semi-field scale.

Materials and Methods

Preparation of biomix

Biomix was prepared by mixing topsoil (69% sand, 13% silt, 18% clay, organic mater 1.95%, pH 6.15, maximum water holding capacity 37% w/w), peat free compost (Levington Peat Free Universal) and winter wheat straw in the volumetric proportions of 1:1:2 respectively. The mixture (organic matter 12.36%, pH 7.5, maximum water holding capacity 75 - 127% w/w) was composted outside for 71 - 97 days prior to use.

Test chemicals

Test pesticides were selected to cover a range of their physico-chemical properties (*36,37,38*) and which were of high average annual usage in the UK.(*39*) (Table 6-1) Formulated isoproturon (Alpha Isoproturon 500^{TM}), 43.6% w/w, pendimethalin (Stomp 400 SC^{TM}), 36.4% w/w, chlorpyrifos (Dursban 4), 44.65% w/w, chlorothalonil (Cropgard^{TM}), 41.57% w/w, epoxiconazole (Opus^{TM}) 12.1% w/w, dimethoate (Rogor L40^{TM}), 37.4% w/w mecoprop (Optica ^{TM}), 48% w/w, and metsulfuron-methyl (Jubillee 20 DF), 20% w/w, were used to make up stock suspensions in tap water.

Water loading

Twelve lysimeters were prepared using unplasticised polyvinyl chloride (PVC-u) piping (19cm internal diameter x 65cm length) filled with 5cm of washed gravel (10-15mm diameter) followed by 50cm of biomix. The base of each core drained via Teflon tubing to either 10 litre high density polyethylene (HDPE) bottles or a 2.5 litre amber glass collection vessels, (depending on the hydraulic loading) located in a central collection pit.(*40*) Three hydraulic scenarios were investigated. To give a 'high' water loading four lysimeters were connected using plastic guttering to 0.54 m^2 concrete slabs. A further four lysimeters were connected to 0.135 m^2 concrete slabs to give an 'intermediate' loading. The four remaining lysimeters received only direct inputs of rainfall, Plate 6-1. Silicon sealant was placed on three sides of each slab to prevent water loss from the sides. Three lysimeters from each hydraulic loading scenario were treated with 50 mL of the pesticide mixture containing 5100 mg AI $litre^{-1}$, 4080mg AI $litre^{-1}$, 1468mg AI $litre^{-1}$, 3060 mg AI $litre^{-1}$, 1020mg AI $litre^{-1}$ and 694mg AI $litre^{-1}$ of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate respectively in January 2000, in order to achieve a final treatment rate of 255 mg (isoproturon), 204 mg (pendimethalin), 73.4 mg (chlorpyrifos), 153 mg (chlorothalonil), 51 mg (epoxiconazole) and 34.7 mg (dimethoate). Potassium bromide (KBr) was applied (314 mg $core^{-1}$) at the same time as the pesticides to check the hydrological integrity of the lysimeters, as well as to determine the breakthrough timing of infiltrating water. Leachate collection vessels were monitored after all rainfall events and the total volume of leachate recorded. Volumes in excess of 200 mL were collected and frozen prior to analysis. Where possible, a 60 mL sub-sample was also taken for KBr analysis. At the end of the study, (244 days after treatment, DAT) all 12 lysimeters were destructively sampled and sectioned (0-5, 5-10, 10-20, 20-30 and >30cm), sections were then homogenised and frozen prior to analysis.

Plate 6-1 Biobed lysimeters subjected to a range of hydraulic loadings

Plate not included in the submitted paper

Table 6-1 Study compounds and their reported physico-chemical charactersistics

Active substance	Product	Concentration % w/w	Koc (mL g^{-1})	Mobility class*	DT_{50} soil (days)	Solubility water (mg L^{-1})
Isoproturon	Alpha Isoproturon 500	43.6	125	Moderately mobile	6-28	65
Pendimethalin	Stomp 400 SC	36.4	5000-17200	Non-mobile	90-120	0.3
Chlorpyrifos	Dursban 4	44.65	6000	Non-mobile	7-15	1.4
Chlorothalonil	Cropgard	41.6	1600-14000	Slightly / non-mobile	5-36	0.6-1.2
Epoxiconazole	Opus	12.1	957-2647	Slightly mobile	60-90	6.63
Dimethoate	Rogor L40	37.4	16–52	Mobile	2-16	23800
Mecoprop	Optica	48	12-25	Very mobile	3-13	860
Metsulfuron-methyl	Jubilee 20 DF	20	4.6-35	Very mobile	7-35	27900

Values taken from Roberts et al., 1998, Roberts et al., 1999 and Tomlin (2000)
* Hollis 1991

Depth and water loading

A further 18 lysimeters were prepared using PVC-u piping (22.5 cm internal diameter), cut to either, 65 cm, 115cm length or 165 cm length. Each pipe section was filled with 5cm of washed gravel (10-15mm diameter) followed by either 50 cm, 100 cm or 150 cm of biomix. The base of each core drained via Teflon tubing to a 2.5 litre amber glass collection vessel located in a central collection pit.(40) Six lysimeters (2 from each depth) were connected using plastic guttering to 0.32 m^2 concrete slabs. A further six lysimeters were connected to 0.16 m^2 concrete slabs. The six remaining lysimeters received only direct inputs of rainfall. Silicon sealant was placed on three sides of the slabs to prevent water loss from the sides. All eighteen lysimeters from were treated with 50 mL of the pesticide mixture containing 3200 mg AI $litre^{-1}$, 435.2mg AI $litre^{-1}$, 1536 mg AI $litre^{-1}$ and 7.68mg AI $litre^{-1}$ of isoproturon, dimethoate, mecoprop and metsulfuron-methyl respectively in March 2002, in order to achieve a final treatment rate of 298 mg (isoproturon), 40.5 mg (dimethoate), 143 mg (mecoprop), and 0.72 mg (metsulfuron-methyl). Potassium bromide (KBr) was applied (314 mg $core^{-1}$) at the same time to check the hydrological integrity of the lysimeters, as well as to determine the breakthrough timing of infiltrating water. Leachate collection vessels were monitored after all rainfall events and the total volume of leachate recorded. Volumes in excess of 200 mL were collected and frozen prior to analysis. Where possible, a 60 mL sub-sample was also taken for KBr analysis. At the end of the study, (197 days after treatment, DAT) all 18 lysimeters were destructively sampled in the same manner as the lysimeters used in the water loading studies.

Analysis

Water Extraction

For the water loading studies, samples (200 mL) were extracted three times into 30 mL dichloromethane (DCM) in a 500 mL glass separating funnel. The DCM extracts were passed through anhydrous $Na_2 SO_4$ and then evaporated to dryness at 40°C. The resulting residues were re-dissolved in 2 mL of a mixture containing 10% methanol,

90% DCM. Concentrations of all 6 pesticides were determined by GC. For the water loading and depth studies, samples (200 mL) were extracted into 3 x 40 mL dichloromethane (DCM) using a glass separating funnel (250 mL). Following extraction, DCM extracts were dried over anhydrous sodium sulphate and then evaporated to dryness using a rotary evaporator at 40°C. The resulting residues were re-dissolved into 2 mL of methanol. Concentrations of isoproturon and mecoprop-P were then determined by HPLC, dimethoate concentrations were determined by GC and metsulfuron-methyl concentrations were determined by LC/MS.

Biomix extraction

For the water loading studies, samples (40 g) were placed into 250 mL glass bottles. Anhydrous Na_2SO_4 (40 g) plus 160 mL of a mixture containing 90% DCM and 10% methanol was added, with samples shaken for 1 hour using an end-over-endshaker. Samples were allowed to stand until clear, with an aliquot of the solution taken for analysis using GC. With the exception of chlorothalonil (82%) the recovery of all 6 pesticides exceeded 95%. For the water loading and depth studies, samples (40 g) of biomix were placed into glass 250 ml bottles and extracted into 80 ml of methanol for 1 hour using an end-over-end shaker. Following extraction, samples were allowed to stand until clear. An aliquot of the methanol solution was then taken for isoproturon, mecoprop-P and metsulfuron-methyl determination by HPLC.

Recoveries for all of the extraction methods were > 94 %.

GC analysis

Concentrations of isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate from the water loading studies were determined on a Hewlett Packard HP5890 gas chromatograph fitted with a split/splitless injector, 12m x 0.53 mm BPX5 column (SGE) and a nitrogen-phosphorus detector. The carrier gas (helium) flow rate was 7 ml min^{-1} and detector –gas flow rates were 100 ml min^{-1} (air)

and 4 ml min $^{-1}$ (hydrogen). Oven temperature was raised from 90 ^{o}C to 190 ^{o}C (40 ^{o}C min^{-1}) and then to 220 ^{o}C (10 ^{o}C min^{-1}) and finally to 245 ^{o}C (15 ^{o}C min^{-1}). Samples (2 μL) were injected using a Hewlett Packard HP7673 autosampler. Under these conditions all six pesticides were baseline separated with retention times of 3.1 (dimethoate), 3.5 (chlorothalonil), 3.9 (isoproturon), 4.2 (chlorpyrifos), 4.7 (pendimethalin) and 7.2 minutes (epoxiconazole). Quantification was achieved by comparison of peak areas with results from external standards. Recoveries with DCM extraction of water spiked at 0.01 mg L^{-1} were > 94% for all compounds. The limit of quantification was 0.23 μg L^{-1} for isoproturon, 0.12 μg L^{-1} for pendimethalin, 0.11 μg L^{-1} for chlorpyrifos, 0.22 μ g L^{-1} for chlorothalonil, 0.10 μg L^{-1} for epoxiconazole and 0.08 μg L^{-1} for dimethoate.

HPLC analysis

Concentrations of isoproturon, mecoprop and metsulfuron-methyl in extracts from the water loading and depth studies were determined by HPLC using a Spectra Physics SP8810 pump linked to a Kontron 430 UV detector. Samples (20 μl) were injected using a Spectra Physics SP8775 autosampler. Separation was achieved using a Genesis C8 column (250 x 4.6 mm). The mobile phase used was acetonitrile:methanol:0.05M acetic acid (27:28:45) with a flow rate of 1.7 ml min $^{-1}$ which gave retention times of 3.4, 5.0 and 7.5 min for metsulfuron-methyl, isoproturon and mecoprop-P, respectively. The detection wavelength was 230 nm for all three substances. The limit of quantification was 0.05 μg L^{-1} for metsulfuron-methyl and mecoprop and 0.03 μg L^{-1} for isoproturon.

LC/MS analysis

Concentrations of metsulfuron-methyl in water were determined by liquid chromatography / mass spectrometry, operated in positive ion electrospray reaction monitoring mode (ES +MRM). Separation was achieved using a Spherisorb C8 3μ

ODS2 column (150 x 1.0 mm). The mobile phase used was methanol:10 mN ammonium formate:acetonitrile (47.5:47.5:5) with a flow rate of 50 µl min $^{-1}$ and an injection volume of 2.5 µL. Quantification was achieved by comparison between the two transitions (m/z 382/167) quantification and (m/z 382/199) confirmation. Metsulfuron-methyl was reported if both transitions were present at around the correct ratio (10:1). The estimated limit of detection was 0.6 ng mL^{-1}.

Bromide

Concentrations of potassium bromide were determined using two methods of ion chromatography. For the water loading experiment, water samples (0.5 mL) were filtered (0.2 µm) and analysed using a Dionex DX-100. Samples (25 µL) were injected neat with a typical retention time of 2.3 minutes. The system was calibrated using a series of standards with known concentrations of bromide with a limit of detection set at 1.1 mg L^{-1}. For the depth and water loading experiments, a Metrohm (Herisau, Switzerland) 790 Personal ion chromatograph and 813 compact autosampler were used. Analytical columns used were Metrohms', Metrosep RP guard, Metrosep A Supp 4/5 guard, and Metrosep A Supp 4 (250 x 4.0mm). A 20µL injection loop and isocratic eluent of composition 1.8mM sodium carbonate / 1.7mM sodium hydrogen carbonate was used giving a typical retention time of 8.5 minutes. All samples were filtered at 0.45µm (Whatman 13mm polysulphone syringe) prior to loading into the proprietary autosampler cartridges. Limit of quantification was 0.5 mg L^{-1}, with a limit of detection at 0.1 mg L^{-1}.

Results

Water loading

Rainfall for the study period (January to September 2000) was 11% above average and totalled 486.3 mm. Leachate samples were collected on 28 occasions over the 244 day monitoring period. Cumulative leachate volumes from lysimeters receiving only direct inputs of rainfall ranged from 3.4 to 5.1 L. From lysimeters connected to the 0.135 m^2

concrete slabs, leachate volumes ranged from 45.2 to 56.4 L and from those connected to the 0.54 m^2 concrete slabs the volume recorded ranged from 103.7 to 177.6 L. Rapid breakthrough of bromide was observed 7 DAT for the lysimeters connected to the 0.54 and 0.135m^2 concrete slabs compared to 57 DAT for the lysimeters receiving only direct inputs of rainfall, (Figure 6-1).

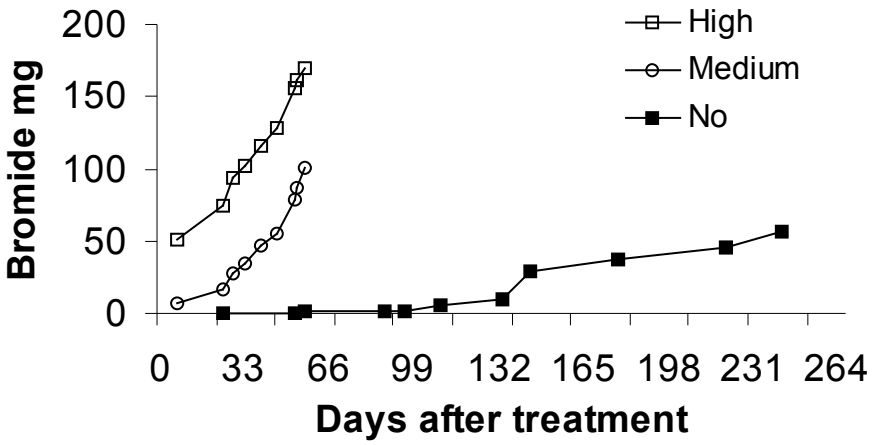


Figure 6-1 Cumulative amounts of bromide measured in leachate collected from lysimeters filled with 0.5m of biomix and subjected to 3 different hydraulic loadings

Maximum concentrations of bromide were measured 7 DAT from lysimeters connected to the 0.54 m^2 slabs, 29 DAT from those connected to the 0.135 m^2 slabs and 221 DAT from those receiving only direct inputs of rainfall. With the exception of chlorpyrifos in lysimeters connected to 0.135 m^2 slabs the highest concentrations of pesticide were measured in leachate collected from lysimeters connected to 0.54 m^2 slabs (Figure 6-2). For lysimeters receiving the highest hydraulic loading, concentrations of pesticide ranged from 1.21 µg l^{-1} for epoxiconazole to 1167 µg l^{-1} for isoproturon. For lysimeter receiving the water loading from 0.135 m^2 slabs, the highest concentrations of pesticide ranged from to 0.35 µg l^{-1} for epoxiconazole to 258 µg l^{-1} for isoproturon and for lysimeters receiving only direct inputs of rainfall, highest concentrations of pesticide ranged from 0.57 µg l^{-1} for epoxiconazole to 1.65 µg l^{-1} for chlorpyrifos.
Cumulative losses of isoproturon and dimethoate from lysimeters connected to 0.54 m^2 slabs were 6.37 % and 6.08 % of the amount applied, respectively, with losses of each of the remaining pesticide being < 0.2%. From lysimeters connected to 0.13 m^2 slabs, losses of isoproturon and dimethoate were 0.2 % and 0.61 % respectively with losses of

the remaining pesticides all below 0.02%. Cumulative pesticide residues in leachate from lysimeters receiving only direct inputs of rainfall were below 0.005% for all 6 pesticides.

In biomix from lysimeters connected to 0.54 m^2 concrete slabs 47% of the total applied pesticide remained within the biobed matrix 244 DAT. No pesticide was measured below 30 cm depth with 39% of the retained pesticide measured in the 0-5 cm layer. In lysimeters connected to the 0.135 m^2 slabs 51% of the applied pesticide was recovered from the biomix with 48% retained within the 0-5 cm layer. No pesticide was measured below 20 cm depth. In lysimeters receiving only direct inputs of rainfall no pesticide was measured below 10 cm depth, with 72% of the applied pesticide retained within the biomix of which 71% was in the 0-5 cm layer. A mass balance was performed to determine the overall environmental fate of the six pesticides under the three hydraulic scenarios investigated, (Table 6-2, Table 6-3 and Table 6-4). For lysimeters with a high hydraulic loading between 0.04% (chlorpyrifos) and 6.37 % (isoproturon) leached, between 0.02% (dimethoate) and 34% (epoxiconazole) was associated with the biomix matrix and 87 % (pendimethalin), to > 99.5% (chlorpyrifos) was degraded. The total amount of pesticide either retained or degraded by the system was > 93%. For lysimeters with a medium hydraulic loading between 0.002% (epoxiconazole) and 0.61 % (dimethoate) leached, 0.02% (dimethoate) and 34% (epoxiconazole) was associated with the biomix matrix and 85 % (pendimethalin), to > 99.7% (isoproturon) was degraded . The total amount of pesticide either retained or degraded by the system was > 99.3%. For lysimeters with no additional hydraulic loading < 0.004% of each chemical applied leached, between 0.11 % (dimethoate) and 33 % (epoxiconazole) was retained within the biomix and 67 %- 99.9% was degraded. More than 99.99% of the applied pesticide was either retained or degraded by the biobed.

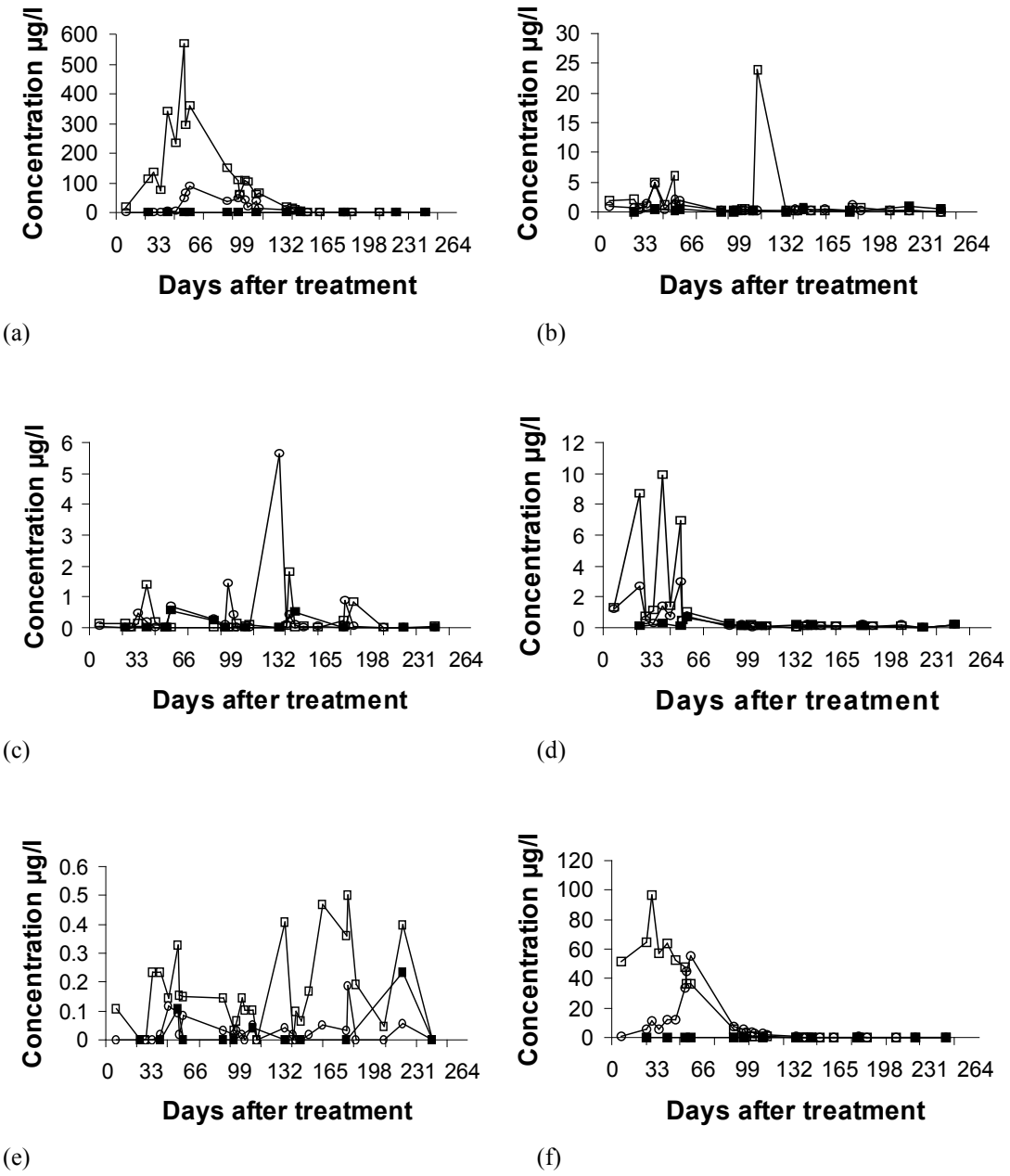


Figure 6-2 Average concentrations of (a) isoproturon, (b) pendimethalin, (c) chlorpyrifos, (d) chlorothalonil, (e) epoxiconazole and (f) dimethoate measured in leachate collected from 0.5 m long biobeds subjected to a high □ and medium ○ water loadings and those receiving only direct inputs of rainfall■

Table 6-2 Mass balance for 0.5 m long biobed columns subjected to a hydraulic loading of 9747 l m^{-2} (connected to 0.54m^2 concrete slabs)

Pesticide	% leached	% degraded	% retained	Maximum concentration µg/l*	Annual average concentration µg L^{-1}
Isoproturon	6.37	93.53	0.10	568.03	101.63
Pendimethalin	0.12	87.08	12.80	23.82	1.78
Chlorpyrifos	0.04	99.52	0.44	1.81	0.19
Chlorothalonil	0.11	98.04	1.85	9.90	1.20
Epoxiconazole	0.05	66.41	33.54	0.50	0.17
Dimethoate	6.08	93.90	0.02	96.84	18.71

* This is highest concentration averaged across the three treated replicates

Table 6-3 Mass balance for 0.5 m long biobed columns subjected to a hydraulic loading of 2797 l m^{-2} (connected to 0.135m^2 concrete slabs)

Pesticide	% leached	% degraded	% retained	Maximum concentration µg/l*	Annual average concentration µg L^{-1}
Isoproturon	0.20	99.71	0.09	89.38	17.78
Pendimethalin	0.01	85.06	14.93	4.58	0.69
Chlorpyrifos	0.01	99.27	0.71	5.61	0.39
Chlorothalonil	0.01	98.30	1.70	2.99	0.47
Epoxiconazole	0.002	66.08	33.92	0.19	0.03
Dimethoate	0.61	99.37	0.02	55.00	7.56

* This is highest concentration averaged across the three treated replicates

Table 6-4 Mass balance for 0.5 m long biobed columns subjected to a hydraulic loading of 486 l m^{-2} (direct inputs of rainfall only)

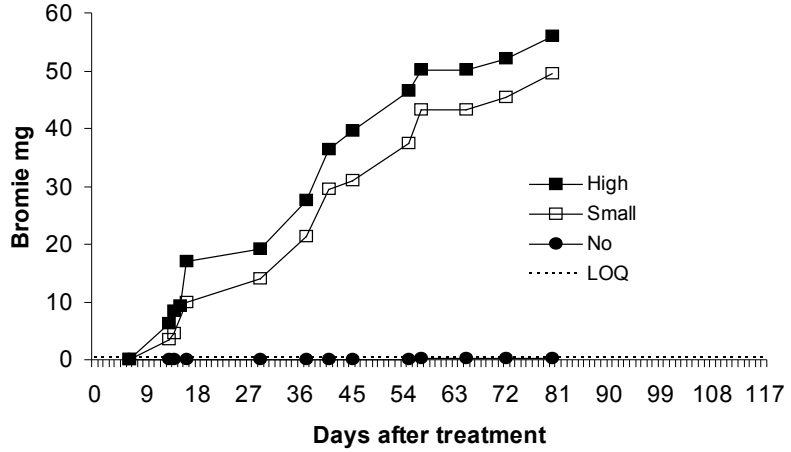
Pesticide	% leached	% degraded	% retained	Maximum concentration µg/l*	Annual average concentration µg L^{-1}
Isoproturon	0.000	98.81	1.19	0.59	0.09
Pendimethalin	0.002	82.80	17.20	1.00	0.31
Chlorpyrifos	0.002	96.51	3.49	0.55	0.11
Chlorothalonil	0.001	90.55	9.45	0.65	0.18
Epoxiconazole	0.001	67.15	32.85	0.24	0.03
Dimethoate	0..004	99.88	0.11	0.22	0.11

* This is highest concentration averaged across the three treated replicates

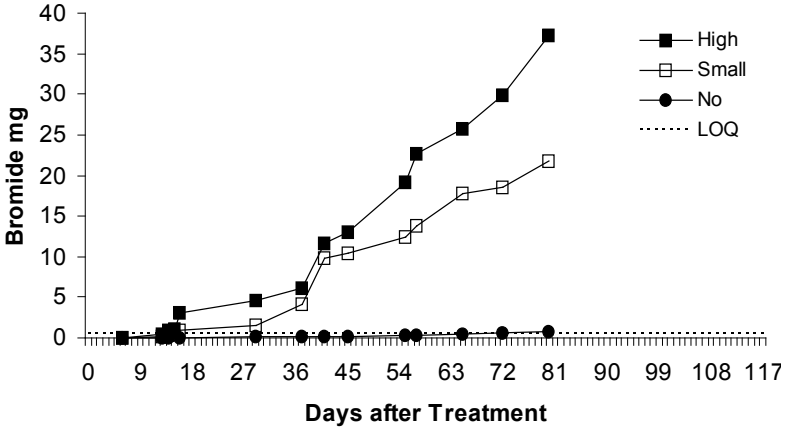
Depth and water loading

Including irrigation, rainfall for the period March to July 2002 was 7 % above average and totalled 201.5mm between application (05/03/02) and collection of the last water samples (09/07/02). Leachate samples were collected on 17 occasions providing 293 water samples for analysis. Cumulative leachate volumes ranged from 2.9 – 3.1 L for the lysimeters receiving only direct inputs of rainwater, from 19.8 – 22.6 L for lysimeters connected to the 0.16 m^2 concrete slabs and from 23.7 – 29.8 L from those receiving the highest water loading (0.32m^2 slabs).

Breakthrough of bromide from all lysimeters receiving high (i.e. connected to 0.32 m^2 slabs) and medium (i.e. connected to 0.16 m^2 slabs) water loads generally occurred 13 – 16 days after treatment. In contrast, breakthrough from the 1.0 and 1.5 m lysimeters receiving only direct water inputs occurred much later (41-55 DAT), (Figure 6-3a & b). No bromide leached from the 0.5 m lysimeters that received only direct rainfall inputs (Figure 6-3c). In all 1.5 m columns and the 1.0 m column receiving only direct rainfall inputs, peak bromide concentrations were observed 80 DAT. Peak concentrations were observed 41 DAT in the 0.5 and 1.0 m columns receiving a medium water loading. Highest concentrations from the 0.5 m and 1.0 m columns receiving a high water loading were observed 16 and 65 DAT respectively. The total amount leached was related to the water loading, and highest amounts of bromide were leached from columns receiving a high water loading whereas lowest amounts leached from the columns receiving only direct rainfall inputs. There appeared to be no relationship between the length of the columns and the amount of bromide leached.



(a)



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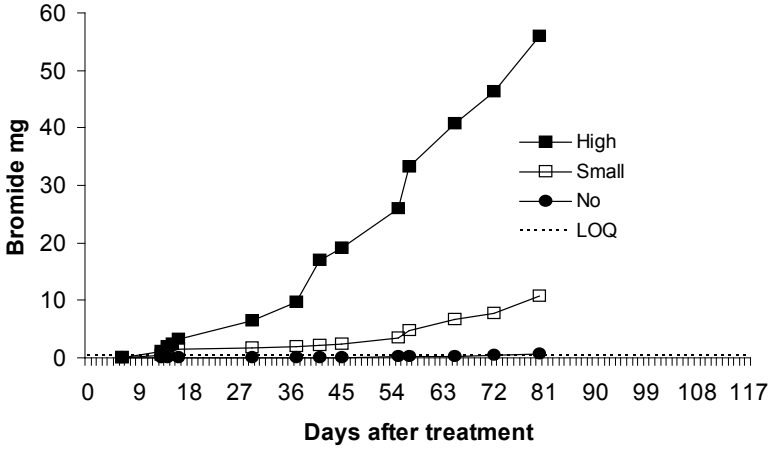


(c)

Figure 6-3 Cumulative amounts of bromide leached from (a) 0.5m, (b) 1.0m and (c) 1.5m deep biobed lysimeters when subjected to No (direct input of rainfall)), low (0.16m^2) and high (0.32m^2) water loadings

Maximum concentrations of pesticide were measured in leachate collected from lysimeters with a high water loading (Figure 6-4). Generally by increasing the depth of the lysimeter up to 1.0 m and controlling water inputs the concentrations of pesticide in leachate were significantly (Anova $P<0.05$, F 6.38, df 1) reduced (Figure 6-5).
From lysimeter subject to the highest water loading, concentrations of isoproturon were 370 .6 µg L^{-1} from 0.5 m lysimeters, 22.9 µg L^{-1} from 1.0 m lysimeters and 30.0 µg L^{-1} from the 1.5 m lysimeters. Breakthrough from the 0.5 m lysimeters was measured 13 DAT with peak concentrations measured 1 day later. Breakthrough from the 1.0 and 1.5m lysimeters was measured 16 DAT. Peak concentrations were measured 55 DAT from the 1.0m lysimeters and 65 DAT from the 1.5 m lysimeters. Cumulative losses of isoproturon were 0.4%, 0.04% and 0.06% for the 0.5 m, 1.0 m and 1.5m deep lysimeters respectively. Maximum concentrations of mecoprop-P were 2217.2 µg L^{-1} from the 0.5 m lysimeters, 157.4 µg L^{-1} from the 1.0 lysimeters and 515.2 µg L^{-1} from the 1.5 m lysimeters and were measured 14, 41 and 101 DAT respectively. Breakthrough was measured 6, 13 and 16 DAT from the 0.5, 1.0 and 1.5m lysimeters respectively. Cumulative losses were 3.4%, 1.0 % and 2.1% for the 1.5 m, 1.0 m and 0.5 m lysimeters respectively. Breakthrough of dimethoate for all depths was measured 6 DAT. Maximum concentrations of 255.8 µg L^{-1}, 2.2 µg L^{-1} and 21.6 µg L^{-1} were measured 14, 87 and 80 DAT for the 0.5, 1.0 and 1.5m deep lysimeters respectively. Dimethoate losses were 1.4%, 0.04% and 0.3% for the 0.5, 1.0 and 1.5m deep lysimeters. Metsulfuron-methyl peak concentrations were 183.0 µg L^{-1}, 28.6 µg L^{-1} and 29.9 µg L^{-1} from the 0.5, 1.0 and 1.5 m lysimeters respectively, with breakthrough measured 13, 14 and 16 DAT respectively. Peak concentrations from the 0.5 m lysimeters were measured 14 DAT and 101 DAT from the 1.0 and 1.5 m lysimeters. The cumulative losses were 100% for the 0.5 m deep lysimeters, 19% for the 1.0 m lysimeters and 15% for the 1.5 m lysimeters.

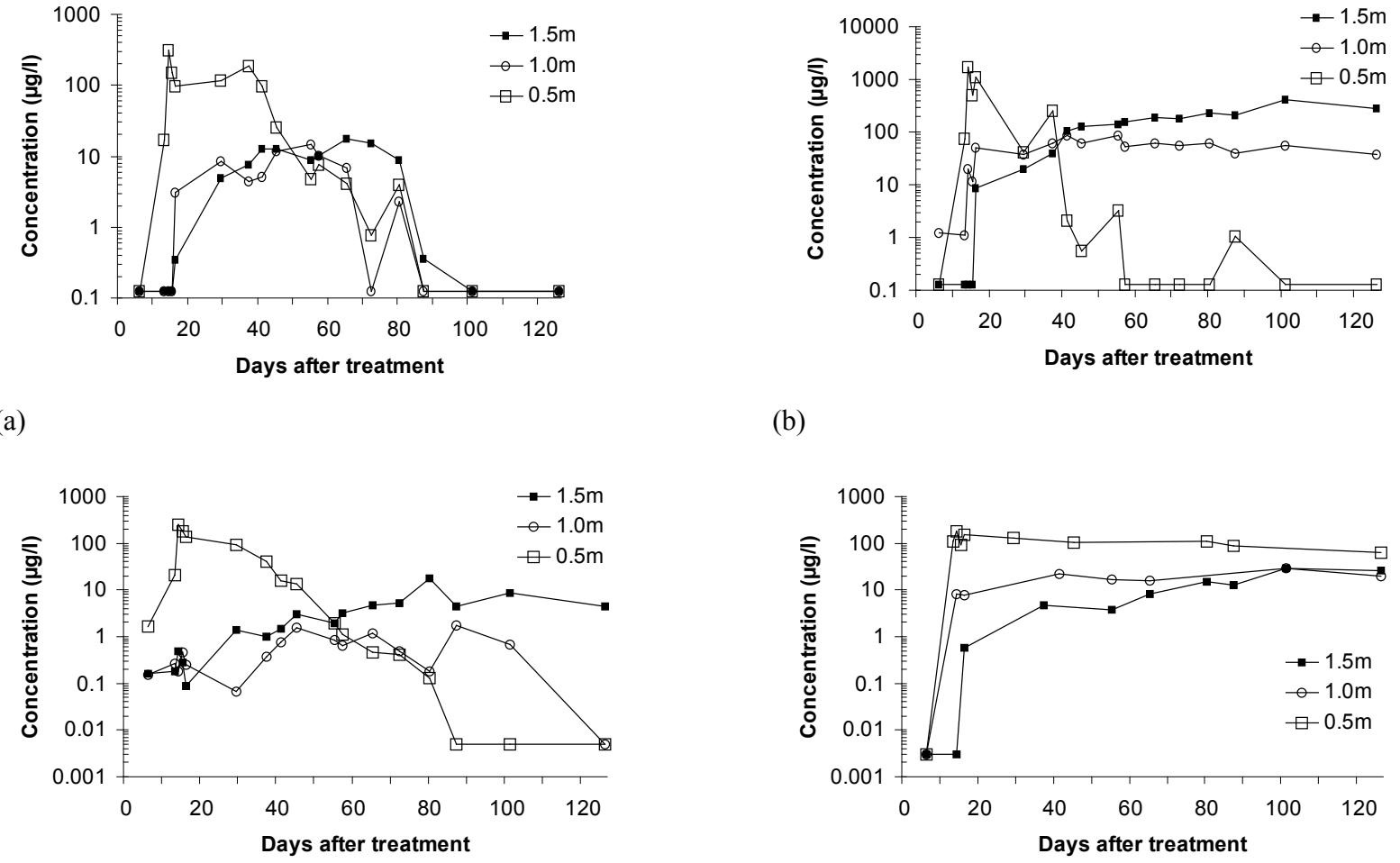


Figure 6-4 Mean concentrations of (a) isoproturon, (b) mecoprop-P, (c) dimethoate and (d) metsulfuron-methyl from different length lysimeters connected to 0.32m^2 concrete slabs

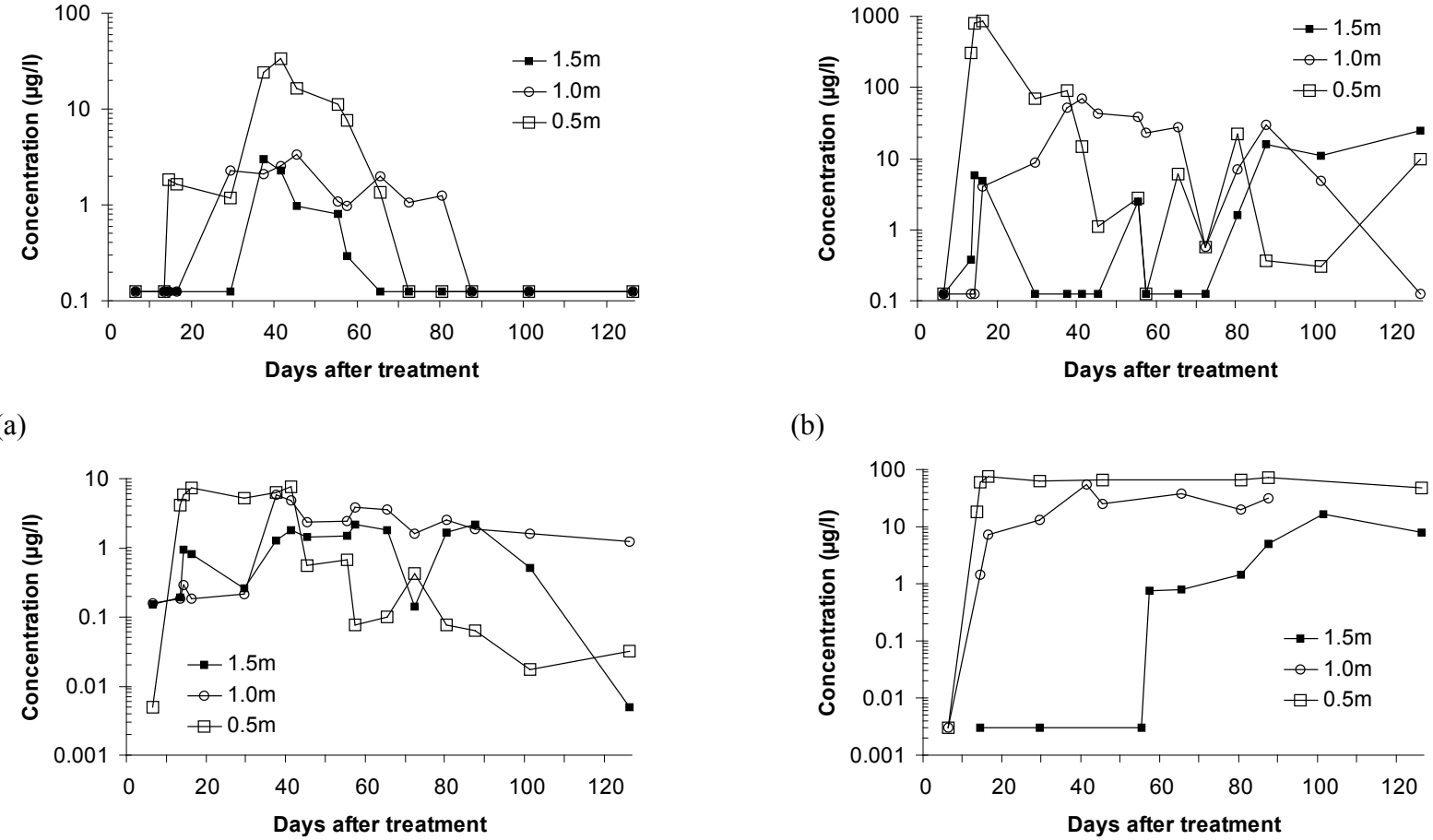


Figure 6-5 Mean concentrations of (a) isoproturon, (b) mecoprop-P, (c) dimethoate and (d) metsulfuron-methyl from different length lysimeters connected to 0.16m^2 concrete slabs

From lysimeters subject to an intermediate water loading maximum concentrations of isoproturon were 45.81 μg L^{-1} from the 0.5 m lysimeters, 5.0 μg L^{-1} from the 1.0 m lysimeters and 2.96 μg L^{-1} from the 1.5 m lysimeters and were measured 41, 45 and 37 DAT respectively. Breakthrough was measured 14, 29 and 37 DAT from the 0.5, 1.0 and 1.5m deep lysimeters. Cumulative losses of isoproturon were 0.05%, 0.006% and 0.001% from the 0.5 m, 1.0 m and 1.5 m lysimeters. For mecoprop-P breakthrough at 0.5, 1.0 and 1.5 m depth was measured 14, 16 and 13 DAT respectively. Maximum concentrations were 1434.3 μg L^{-1} from the 0.5 m lysimeters, 140.7 μg L^{-1} from the 1.0 m lysimeters and 49.46 μg L^{-1} from the 1.5 m lysimeters and these were measured at 16, 41 and 126 DAT respectively, equivalent to cumulative losses of 1.54% for the 0.5 m lysimeters, 0.34% for the 1.0 m lysimeters and 0.12% for the 1.5 m lysimeters. Breakthrough of dimethoate occurred 6 DAT from the 1.0 and 1.5 m lysimeters and 6 DAT from the 0.5 m depth. Maximum concentrations of 12.02, 9.46 and 2.87 μg L^{-1} were measured from 0.5 m, 1.0 m and 1.5 m depth, 37, 41 and 87 DAT respectively. Cumulative losses from the 0.5 m and 1.0 m lysimeters were 0.1% of the applied dose and from the 1.5 m lysimeters 0.06%. For metsulfuron-methyl, breakthrough was measured 13 DAT from 0.5m deep lysimeters, 14 DAT from the 1.0 m deep lysimeters and 57 DAT from the 1.5 m deep lysimeters. Maximum concentrations for each depth (0.5 m to 1.5 m) were measured 16, 41 and 101 DAT and were 75.3, 54.2 and 16.6 μg L^{-1} respectively. Cumulative losses were 48%, 18% and 6 % for the 0.5, 1.0 and 1.5m lysimeters respectively

For lysimeters receiving only direct inputs of rainfall, no concentrations of isoproturon were measured above the LOQ of 0.03 μg L^{-1}. Cumulative losses were estimated to be ≤ 0.0002% of the applied dose for all depths. Maximum concentrations of mecoprop-P were 2.05 μg L^{-1} at 0.5 m depth, 1.23 μg L^{-1} at 1.0 m depth and 4.98 μg L^{-1} at 1.5 m depth. Breakthrough and maximum concentrations coincided and were measured 41 DAT at 0.5 m and 1.5 m depth and 126 DAT at 1.0 m depth. Cumulative losses were ≤ 0.0007% for all depths. At 0.5 m depth concentrations of dimethoate were all below the LOQ. Breakthrough at 1.0 m and 1.5 m was measured 41 DAT with a maximum concentration of 1.23 μg L^{-1} measured at 1.0 m depth, 87 DAT and at 1.5 m depth 0.13

μg L^{-1}, 41 DAT. As for mecoprop-P, losses of dimethoate from the biobed lysimeters receiving only direct inputs of rainfall losses were all ≤ 0.0007%. Concentrations of metsulfuron-methyl were below the LOQ in leachate collected form 1.0 and 1.5 m depth. At 0.5 m, maximum concentrations coincided with breakthrough and were measured 101 DAT at 4.51 μg L^{-1}. Cumulative losses of 0.2% were measured for the 0.5 m lysimeters and ≤ 0.0003% for the 1.0 and 1.5 m deep lysimeters.

No mecoprop-P or metsulfuron-methyl was measured in the biomix at the end of the study (197 DAT). No isoproturon or dimethoate was measured below 10 cm depth under either of the water loading scenarios investigated with between 92 and 100% retained in the top 5 cm. For isoproturon the measured residues (expressed as % of the applied dose) remaining in the biobed lysimeters were 0.41, 3.51 and 0.13 % for the 0.5 m , 1.0 m and 1.5 m lysimeters respectively and for dimethoate 0.07, 0.53 and 0.08%.

A mass balance was performed to determine the fate of each of the study compounds under the three hydraulic scenarios investigated. For the lysimeters connected to the 0.32 m^2 concrete slabs (high water loading), between 100 % (metsulfuron-methyl) and 0.39 % (isoproturon) leached from the 0.5 m lysimeters, between 0.41 % (isoproturon) and 0 % (metsulfuron-methyl) was associated with the biobed matrix and between 0 % (metsulfuron-methyl) and 99.2 % (isoproturon) was degraded. For the 1.0 m lysimeters between 19.34 % (metsulfuron-methyl) and 0.04 % (isoproturon and dimethoate) leached, between 3.51 % (isoproturon) and 0 % (metsulfuron-methyl) was associated with the biobed matrix, and between 81 % (metsulfuron-methyl) and 99.4 % (dimethoate) was degraded. For the 1.5 m lysimeters, between 15.29 % (metsulfuron-methyl) and 0.06% (isoproturon) leached, between 0.13 % (isoproturon) and 0 % (metsulfuron methyl) was associated with the biobed matrix, and between 85 % (metsulfuron-methyl) and 99.8 % (isoproturon) was degraded (Table 6-5).

For the lysimeters connected to the 0.16m^2 slabs (low water loading), between 48.3 % (metsulfuron methyl) and 0.05 % (isoproturon) leached from the 0.5 m lysimeters, between 0.55 % (isoproturon) and 0 % (metsulfuron-methyl) was associated with the

biobed matrix, and between 52 % (metsulfuron-methyl) and 99.6 % (dimethoate) was degraded. For the 1.0 m lysimeters, between 18.38 % (metsulfuron methyl) and 0.01 % (isoproturon), leached between 0.47 % (isoproturon) and 0 % (metsulfuron-methyl) was associated with the biobed matrix, and between 82 % (metsulfuron-methyl) and 99.7 % (dimethoate and mecoprop-P) was degraded. For the 1.5m lysimeters, between 5.94 % (metsulfuron-methyl) and 0.002 % (isoproturon) leached, between 0.29 % (isoproturon) and 0 % (metsulfuron methyl) was associated with the biobed matrix, and between 94 % (metsulfuron-methyl) and 99.9 % (mecoprop-P) was degraded (Table 6-6).

For the lysimeters receiving only direct inputs of rainfall, between 0.24 % (metsulfuron methyl) and 0 % (dimethoate) leached from the 0.5 m lysimeters, between 0.55 % (isoproturon) and 0 % (metsulfuron-methyl and mecoprop-P) was associated with the biobed matrix, and between 100 % (mecoprop-P) and 99.9 % (dimethoate) was degraded. For the 1.0 m lysimeters between 0.0007 % (dimethoate) and 0.0001 % (isoproturon) leached, between 0.44 % (isoproturon) and 0 % (metsulfuron-methyl and mecoprop-P) was associated with the biobed matrix, and between 99.6 % (isoproturon) and 100 % (mecoprop-P and metsulfuron-methyl) was degraded. For the 1.5 m lysimeters, between 0.0009 % (mecoprop-P) and 0.0001 % (isoproturon and dimethoate) leached, between 1.06 % (isoproturon) and 0 % (metsulfuron-methyl and mecoprop-P) was associated with the biobed matrix and between 99.7 % (dimethoate) and 100 % (mecoprop-P and metsulfuron-methyl) was degraded (Table 6-7).

Table 6-5 Mass balance for 0.5 m, 1.0 m and 1.5 m deep biobed lysimeters subjected to a high water loading (0.32 m^2 concrete slabs)

	% leached			% retained			% degraded			Maximum Concentration (µg L^{-1})			Average concentration (µg L^{-1})		
Pesticide	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**
Isoproturon	0.06	0.04	0.39	0.13	3.51	0.41	99.81	96.45	99.20	17.31	14.92	310.9	5.87	4.01	60.23
Dimethoate	0.32	0.04	1.41	0.08	0.53	0.07	99.60	99.43	98.52	18.16	1.77	253.4	3.46	0.58	44.47
Mecoprop-P	3.37	1.02	2.07	0	0	0	96.63	98.98	97.93	423.1	88.40	1687.2	123.7	45.78	216.1
Metsulfuron-methyl	15.29	19.34	100	0	0	0	84.71	80.66	0	29.90	28.60	183.0	10.09	14.90	103.1

Maximum concentrations are based on the mean from duplicate lysimeters

Table 6-6 Mass balance for 0.5 m, 1.0 m and 1.5 m deep biobed lysimeters subjected to a small water loading (0.16 m^2 concrete slabs)

	% leached			% retained			% degraded			Maximum Concentration (µg L^{-1})			Average concentration (µg L^{-1})		
Pesticide	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**
Isoproturon	0.002	0.01	0.05	0.29	0.47	0.55	99.71	99.52	99.40	2.96	3.34	33.35	0.54	1.09	6.24
Dimethoate	0.06	0.12	0.10	0.20	0.21	0.24	99.74	99.67	99.66	2.20	5.93	7.74	1.06	2.06	2.42
Mecoprop-P	0.11	0.33	1.54	0	0	0	99.89	99.67	98.46	24.79	70.39	877.9	4.27	19.45	137.2
Metsulfuron-methyl	5.94	18.38	48.34	0	0	0	94.06	81.62	51.66	16.60	54.20	75.30	3.64	21.23	52.76

Maximum concentrations are based on the mean from duplicate lysimeters

Table 6-7 Mass balance for 0.5 m, 1.0 m and 1.5 m deep biobed lysimeters receiving only direct inputs for rainfall

	% leached			% retained			% degraded			Maximum Concentration (µg L^{-1})			Average concentration (µg L^{-1})		
Pesticide	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**	**1.5m**	**1.0m**	**0.5m**
Isoproturon	0.0001	0.0001	0.0003	1.06	0.44	0.07	98.94	99.56	99.93	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Dimethoate	0.0001	0.0007	0	0.30	0.13	0.06	99.70	99.87	99.94	0.13	0.62	0.05	0.01	0.06	<0.01
Mecoprop-P	0.0009	0.0006	0.0005	0	0	0	100	100	100	4.98	0.98	1.96	0.94	0.20	0.36
Metsulfuron-methyl	0.0002	0.0003	0.24	0	0	0	100	100	99.76	<0.0006	<0.0006	4.51	<0.0006	<0.0006	0.90

Maximum concentrations are based on the mean from duplicate lysimeters

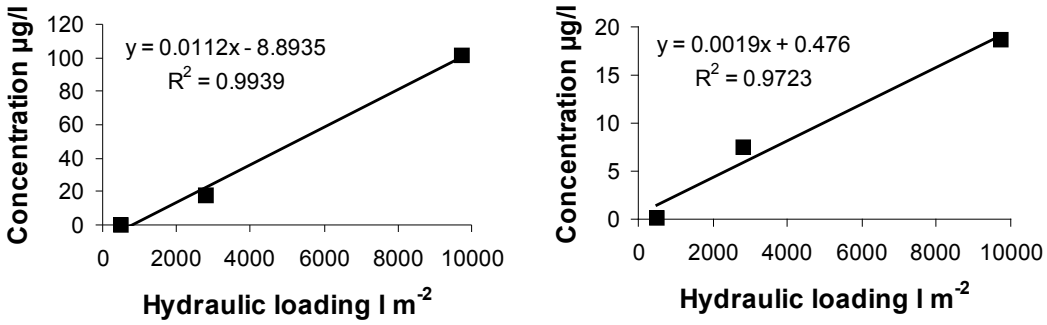
Discussion

Biobeds have been in use in Sweden since 1993 with more than 1000 in practical use on farms and other places where pesticide sprayers are filled up.(*30*) The basic design (*27,28*) has been shown to be able to treat small drips and spills of pesticide originating from the spray fill site. However, if such a system is to treat dilute pesticide waste and equipment washings in the UK, it must cope with large volumes of relatively complex mixtures of pesticide, often applied repeatedly. This study was therefore performed to understand the relationship between biobed size, water loadings and pesticide concentrations in order to provide guidance on the construction and operation of biobeds in the UK.

Lysimeters (0.5 m) connected to 0.54 m^2 and 0.135 m^2 concrete slabs and those receiving only direct inputs of rainfall received a hydraulic loadings equivalent to 9747, 2797 and 486 l m^{-2} respectively. Pesticide leaching potential was clearly affected by hydraulic loading. Amounts of pesticide leaching from lysimeters receiving the highest water loading were < 6.4 % of the applied whereas amounts from lysimeters with a medium water loading were < 0.7 %. From lysimeters receiving only direct inputs of rainfall the leaching losses were <0.004 %. With one exception (pendimethalin at the highest water loading) only the two most mobile pesticides (Koc <125) leached to any great extent and even for these > 93 % was retained by the biobed lysimeters receiving the highest water loading and >99 % from lysimeters receiving a medium water loading. On the basis of the reported physico-chemical properties for pendimethalin (Table 6-1) it would not be expected to represent a leaching risk. The result is therefore considered an experimental artefact. All pesticides were degraded within the biobed with < 35% of the retained pesticide remaining within the biobed matrix after 244 days.

Performance of the biobed with respect to the maximum and annual average concentrations of isoproturon and dimethoate in leachate for both the high (Table 6-2) and medium (Table 6-3) water loading scenarios was unacceptable to the regulatory authorities. Annual average concentrations of both isoproturon (Figure 6-6a) and dimethoate (Figure 6-6b) were therefore correlated against hydraulic loading to enable

the maximum hydraulic loading for a given maximum concentrations in leachate to be calculated.



(a) (b)

Figure 6-6 Annual average concentrations of (a) isoproturon and (b) dimethoate measured in leached from 0.5 m deep lysimeters correlated against hydraulic loading

In order to achieve annual average concentrations of both compounds of, e.g. < 0.1 µg L^{-1} the maximum hydraulic loading to 0.5 m deep biobed should not exceed 200 L m^{-2}. Over the course of a normal spray season, a typical spray applicator can produce between 3800 and 15000 litres of pesticide contaminated waste water, (*41*), not including clean rainwater and on that basis, a biobed of 0.5 m depth would need to have a surface area of between 19 – 75 m^2. Whilst an area of up to 40 m^2 is likely to be acceptable to most sprayer operators, anything larger may be seen as impractical. Methods of optimising biobed performance were therefore investigated. Lysimeters (0.5 m, 1.0 m and 1.5 m) connected to 0.32 m^2 and 0.16 m^2 concrete slabs and those receiving only direct inputs of rainfall received hydraulic loadings equivalent to 1175, 688 and 202 L m^{-2}, respectively. By controlling water inputs and increasing the retention time within the biobed through increasing depth, studies showed that for mobile (Koc 15 – 74, (*42*)) and moderately mobile (Koc 75 – 499, (*42*)) pesticides, < 1.41% of the applied pesticides leached from 0.5 m deep biobeds receiving the highest water loading, compared with < 0.32 % from 1.5m biobeds. For lysimeters subject to a water loading of 688 l m^{-2} < 0.1 % of the applied pesticide leached from the 0.5 m deep biobed compared with < 0.06 % from the 1.5 m biobeds, and those receiving only direct inputs of rainfall (202 L m^{-2}), < 0.0007 % of the applied pesticide leached. At this low water loading annual average concentrations of both isoproturon and dimethoate from

0.5 m deep biobeds were < 0.03 µg L^{-1} supporting the predictions based on data from the previous experiment discussed. For the two very mobile (Koc <15, (*42*)) pesticides tested, mecoprop-P and metsulfuron-methyl, amounts of pesticide leaching from the biobed lysimeters were higher. However, by controlling water inputs and maximising the opportunity for sorption and degradation, the amount of pesticide leaching from the biobed was reduced. For example, at the highest water loading, 100 % of applied metsulfuron–methyl leached from the 0.5 deep biobeds compared with only 15 % from the 1.5 m biobeds. Isoproturon, dimethoate, mecoprop-P and metsulfuron methyl are classified as slightly or moderately persistent, DT_{50} < 60 days (*42*). In these experiments > 96.5 % of the retained pesticide was degraded within 197 days. Currently to gain approval for use in the UK and the EU, the annual average concentrations of a pesticide predicted to reach ground water should not exceed 0.1 µg L^{-1}. Surface water concentrations may be predicted to exceed this value (subject to an ecotoxicological assessment) on the basis that surface waters will require more than minimal treatment in order to obtain suitable quality for human consumption. However, in the future, it is possible that the water Framework Directive may have impacts in catchments where surface waters are abstracted for drinking water. Whilst the performance of the biobed is not subject to the same strict criteria, it does provide a useful framework in which to assess the level of treatment being achieved by the biobed. By manipulating the data generated in experiments investigating the combined effects of biobed depth and hydraulic loading, it is possible to calculate the minimum depth of the biobed and the maximum hydraulic loading such that the annual average concentration in leachate does not exceed a given maximum concentration, for example 0.1 µg L^{-1}. Data for isoproturon (Figure 6-7) and dimethoate (Figure 6-8) clearly demonstrate the combined effects of hydraulic loading and biobed depth on concentrations of pesticide leaching from the biobed, and for these two compounds, data suggest a minimum depth of 1.0 m is required. To establish a maximum water loading for the biobed, annual average concentrations of both isoproturon and dimethoate were correlated separately against hydraulic loading. Lines of best fit were used to calculate hydraulic loadings of 184 L m^{-2} for isoproturon and 469 L m^{-2} for dimethoate such that from a 1.5 m deep biobed concentrations of each pesticide, respectively, should not exceed 0.1 µg L^{-1}.

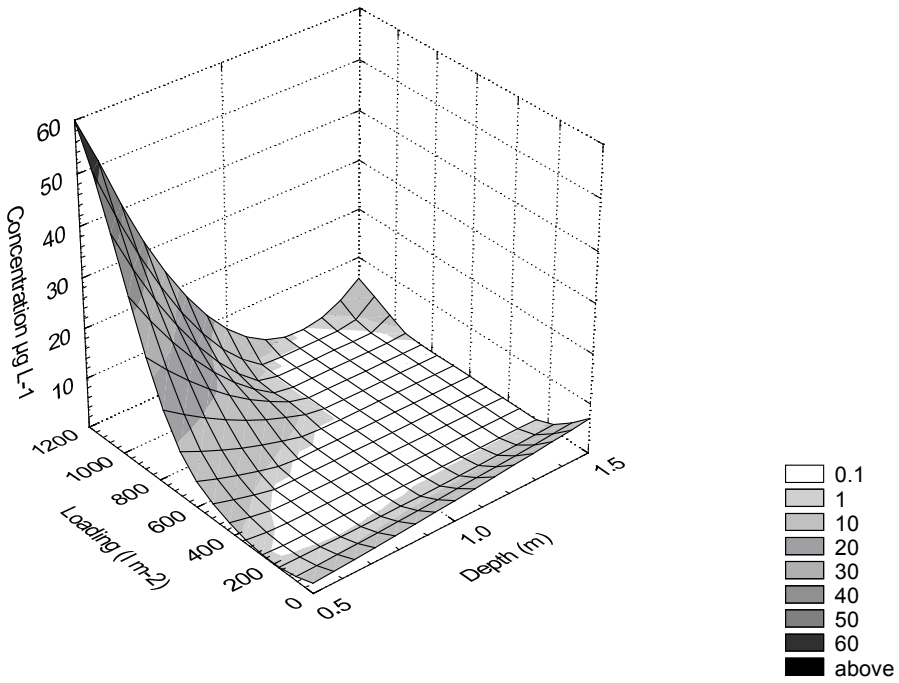


Figure 6-7 Surface area plot showing the combined effects of biobed depth and hydraulic loading on annual average concentrations of isoproturon in leachate

These data can be used to calculate the minimum surface area of a 1.5 m deep biobed in order to treat any given volume of pesticide waste and washings. For example, if the farm had a bunded spray fill area of 40m^2, generated 10,000 litres of tank and equipment washings, and is located in an area where the annual average rainfall is 650mm, then the total volume of liquid entering the biobed would be 36,000 litres. By dividing this figure by the maximum hydraulic loading (184 L m^{-2}) it can be calculated that the surface area of a 1.5m deep biobed would need to be 196 m^2 in order to achieve a maximum average concentration of 0.1 µg L^{-1}. Such physical dimensions are clearly impractical on most agricultural holdings. However, at present, biobeds do not have to comply with EU and UK legislation with respect to predicted concentrations of pesticide reaching ground and surface water bodies. Therefore if a higher maximum annual average pesticide concentration threshold is set, (5 µg L^{-1} for example) the maximum hydraulic loadings increases significantly to 1161 and 1121 L m^{-2} for isoproturon and dimethoate respectively.

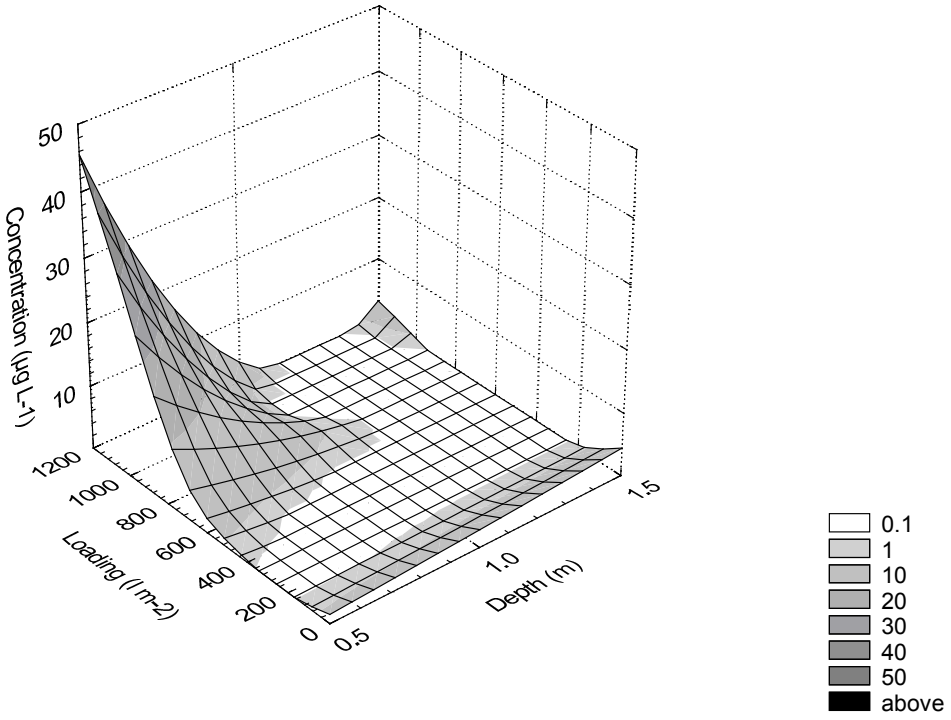


Figure 6-8 Surface area plot showing the combined effects of biobed depth and hydraulic loading on annual average concentrations of dimethoate in leachate

By using these data (1121 L m^{-2}) the surface area of the biobed decreases to only 32m^2 in order to treat the same volume of pesticide waste. Data for mecoprop-P (Figure 6-9) and metsulfuron-methyl (Figure 6-10) show that extremely mobile pesticides (Koc <15, (*42*)) are likely to leach through the biobed. Controlling water inputs does appear to reduce the amount of pesticide leaching from the system, however increasing biobed depth does not appear to give the same level of improvement in performance as observed for isoproturon and dimethoate. In order to achieve annual average concentrations of ≤ 5 µg L^{-1} for mecoprop-P and metsulfuron-methyl, the biobed would have to be at least 1.5 m deep and the hydraulic loading would have to notexceed 387 and 726 L m^{-2} respectively.

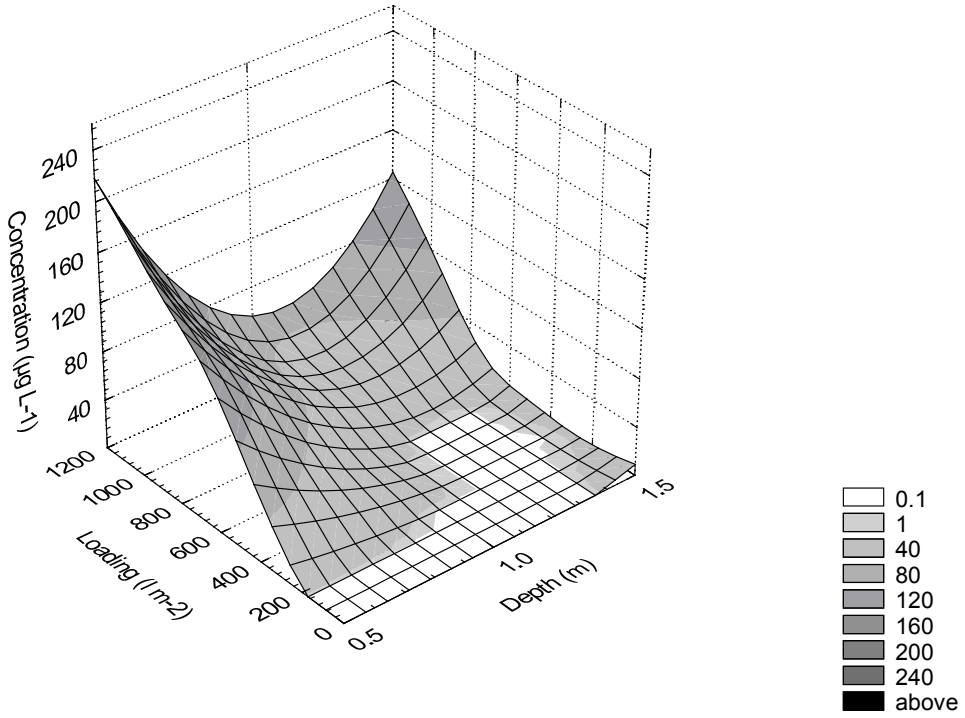


Figure 6-9 Surface area plot showing the combined effects of biobed depth and hydraulic loading on annual average concentrations of mecoprop-P in leachate

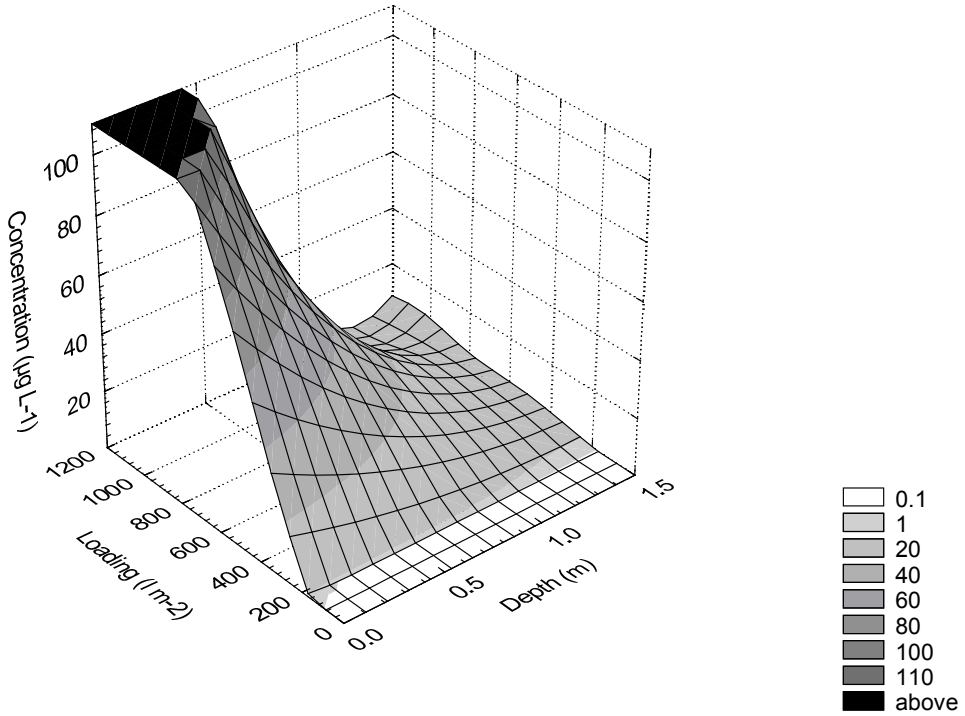


Figure 6-10 Surface area plot showing the combined effects of biobed depth and hydraulic loading on annual average concentrations of metsulfuron-methyl in leachate

Based on recent research (*19,24,31,32,35*) the Environment Agency has issued interim guidance on the use of biobeds in the UK. Unlined biobeds may be used for treating the unintentional spillages that occur during the filling, mixing and handling of pesticides, provided the system is operated in accordance with good agricultural practice. Where the biobed is also used to intercept equipment washings, the biobed will need to lined with all effluent collected for subsequent appropriate disposal and would also require an authorisation under the Ground water Regulations 1998, including prior investigation of the site and possible monitoring of groundwater. Previous studies (*35*) with lined biobeds highlighted that water management is crucial and that accumulation of some pesticides may be possible. The data presented here suggest that unlined biobeds may be able to achieve the required level of treatment, such that approval for use for treating equipment washings can also be granted.

Conclusions

Pesticide leaching from biobeds is clearly affected by the volume of liquid entering the system. By controlling water inputs and maximising the opportunity for sorption, biobeds appear able to treat all pesticides other than those classified as very mobile (Koc <15), such that the risk to both surface and ground water should be acceptable. However, even for highly mobile pesticides, biobed treatment would result in a significant reduction in the amounts of these pesticides reaching surface and ground water. All pesticides tested degraded within 12 months, therefore accumulation from one growing season to the next should not occur. Data suggest a minimum biobed depth of 1.0 – 1.5 m. The surface area of the biobed is dependent on the volume of waste and level of treatment required. However, as a guide, a 1.5 m deep biobed with a surface area of 30 -40 m^2 should be able to treat ≤ 44000 L of pesticide waste such that annual average concentrations of all but those pesticides classified as very mobile are < 5 µg L^{-1}.

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Opinions expressed within this paper are those of the authors and do not necessarily reflect the opinion of the sponsoring organisations. No comments should be taken as an endorsement or criticism of any compound or product.

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Chapter 7

GENERAL DISCUSSION & CONCLUSIONS

Significant contamination of the spray fill site can occur due to its repeated use and can represent a significant point source even when following best agricultural practices. (*1,2,3*) To minimise the impact of these sites on water quality within agricultural catchments biobeds are being developed. Biobeds have been in use in Sweden since 1993 with more than 1000 in practical use on farms and other places where pesticide sprayers are filled up.(*4*) The basic design of a 0.6m deep hole lined with clay and filled with biomix with an access ramp has remained largely unchanged,(*5*) with reliable performance being measured for up to 8 years.(*4*) Whilst the Swedish system has been shown to be able to treat pesticide spills, the use of biobeds for treating the large volumes of waste and high amounts of pesticide associated with washings as well as spillages has not yet been established. If a system could be developed to deal with these types of inputs, then it is possible that incidences of contamination of surface waters by pesticides could be greatly reduced. The experiments presented herein were performed to investigate the ability of biobeds to treat such waste.

The degradation of a number of pesticides with a range of physico-chemical properties was investigated. Individual compounds as well as relatively complex mixtures were applied repeatedly to topsoil and biomix at concentrations up to 20 times the maximum approved field application rate. Results from these experiments are reported in Chapters 2 and 3. In both topsoil and biomix the rate of degradation decreased with increasing concentration, and was faster in biomix than in topsoil. Experiments made using a simple pesticide mixture containing isoproturon and chlorothalonil (Chapter 2) showed that interactions between pesticides are possible. However the effects were far less significant in biomix relative to topsoil. Experiments using a more complex pesticide mixture showed similar results (Chapter 3), and with one exception DT_{90} values for all pesticides tested were < 6 months, suggesting that accumulation for one growing season to the next should not occur. Biobeds are likely to be built on farms

using locally available materials. It is therefore likely that the physical and chemical characteristics of the raw materials and in particular the topsoil will vary. Experiments were therefore made to investigate the degradation and leaching potential from biobeds when three contrasting topsoils were used in the preparation of the biomix, (Chapter 5). Mixing each of the soils with compost and straw resulted in a significant increase in the levels of microbial respiration. Degradation rates for the individual compounds applied at high rates were ≤ reported DT_{50} values for soil treated at approved rates.

Whilst degradation was generally faster in biomix than in topsoil, in experiments specifically investigating the effect of concentration, the herbicide isoproturon degraded more slowing in biomix compared to topsoil. One possible explanation is the fact that the topsoil used for the experiment had been treated on previous occasions with isoproturon as part of normal agricultural practices, which may have resulted in the proliferation of microbial communities specifically adapted to utilise the compound as an energy source, resulting in enhanced biodegradation, as reported by Cox et al.(*6*). In the field such enhanced degradation can result in reduction or loss of efficacy of a pesticide, (*7*) but in a biobed enhanced rates of degradation would improve the performance of the system. The degradability of three pesticide applications, made at 30 day intervals, was therefore investigated (Chapter 3). While degradation was again quicker in biomix compared to topsoil, the rate of degradation decreased with each additional application. Whilst, the timing, as well as the number of pesticide treatments can affect the rate at which a pesticide is degraded, (*8*) it is more likely that the effects were due to the high concentration and the interaction between different active substances.

The degradation and leaching potential from lined and unlined biobeds were compared (Chapter 4). In the lined biobed the biomix was enclosed in a sealed column. This approach was considered attractive to the regulatory authorities as it minimised the potential for leachate to contaminate groundwater. Whilst the lined biobed system effectively retained the pesticides, only limited degradation was observed. Furthermore costly water control measures had to be introduced. The poor performance of the lined biobed was probably due to a number of factors, low levels of bioactivity in the top

10 cm due to low moisture content and inhibition brought about by the high concentrations of a relatively complex mixture of pesticides are considered to be the main reasons.

The use of unlined biobeds removed the need to manage water inputs whilst at the same time maintaining near optimum conditions for pesticide degradation as rainwater was able to enter and subsequently drain from the system. Studies demonstrated that the concentrations of pesticide leaching from the biomix filled lysimeters were significantly lower than from soil lysimeters. Only the most mobile compounds leached to any great extent and even for these compounds the system appeared to retain or degrade more than 99% of the applied dose. Furthermore, studies to investigate the leaching risk from biobeds when different topsoils were used in the biomix showed there to be no significant difference in the amounts of pesticides leaching. Concentrations of the two most mobile compounds (Koc < 100) were however considered unacceptable to the UK regulators, ranging from 50 – 127µg L^{-1}. Experiments were therefore performed to determine whether the biobed system could be optimised to lower concentrations still further (Chapter 6). Pesticide leaching was clearly affected by hydraulic loading and depth. By controlling water inputs and increasing the retention time within the biobed by increasing depth, studies showed that for all pesticides other than those classified as very mobile (Koc <15) could be treated by the biobed such that annual average concentrations from biobeds were < 5µg L^{-1}.

Studies have shown that biobeds can retain and subsequently degrade high concentrations of relatively complex mixtures of pesticides even when applied repeatedly. Water management is crucial in terms of construction costs, performance and the level of management required. The use of unlined biobeds removed many of the problems associated with treating large volumes of liquid, such that all pesticides other than those classified as very mobile (Koc <15) were effectively retained and subsequently degraded. Even for the highly mobile pesticides leaching losses were <6 %, representing a significant reduction in the amounts of these mobile pesticides reaching ground and surface water bodies. Studies have shown that biobeds would be a useful tool in reducing amounts of pesticide in UK waters. If adopted they should

reduce the concentrations of pesticides measured in environmental waters and thus reduce water treatment costs. However, there are still a number of questions to answer.

Future work

1.) Selected substances have been shown to persist in the biobed matrix and, in particular, some fungicides have the potential to accumulate in a biobed over time. Further studies are therefore required to identify the implications of this on the overall performance of the biobed. Factors to be considered should include: 1) which pesticides or groups of pesticides are likely to accumulate? 2) is there any risk of these substances becoming mobile during the lifetime of the biobed? and 3) when a biobed is dismantled, can the substances be degraded and, if so, over what time-frame? The use of manipulation methods e.g. addition of appropriate microbial systems to enhance the degradation of these substances could also be explored.

2.) Although a significant proportion of each pesticide applied to the biobed is removed, data suggests that concentrations in leachate of certain highly mobile pesticides (Koc <25) exceed acceptable levels. It is possible that some form of adsorbent could be included within the biobed, thus reducing the concentrations in leachate. Experiments should be performed to assess the feasibility of using such materials in the biobed.

3.) The long-term operation of the biobed system has not been considered. Prototype biobeds should be constructed and operated in accordance with normal agricultural practice to enable, a) the level of performance achieved by the biobed when used over several growing seasons to be determined and b) the management inputs required by the system.

4.) At some stage complete replacement of the biobed matrix is likely to be necessary. The options for disposal of the spent biomix will depend on the amount of pesticide retained within the biobed matrix. Research suggests that the biomix can be heaped in the farmyard, and left to compost for approximately 12 months prior to disposal to

land.(*5*) Heaping the biomix above ground on an impermeable surface may result in mobile pesticides leaching from the biomix and possible contamination of adjacent surface waters. In order to prevent this, the researchers suggest covering the heap to exclude rainfall. However, once covered the biomix is likely to dry out, inhibiting microbial activity and reducing the rate of degradation. The preferred option for the disposal of spent biomix would be a direct application to land. However, the potential impact on soil and water quality must be quantified through the use of a suitable risk assessment techniques.

Disposal options for study could include:

- Storing a covered heap.
- Storing an uncovered heap.
- Storing a covered heap with microbial inocula added
- Storing an uncovered heap with microbial inocula added
- Actively composted using a range of methodologies
- Direct application to soil at different application rates.

5.) Studies to date have focused on the fate and behaviour of different active substances in both leachate and matrix material. However certain degradation products may be more persistent, more mobile and more toxic than the parent product. It is therefore necessary to identify any relevant metabolites and investigate their fate and behaviour within the biobed.

6.) There are still data gaps which require further investigation in order to fully understand / optimise the biobed system. These include:

- The specific contribution that the straw fraction of the biomix has on the overall performance of the biobed.
- The influence nitrogen has on the degradation of pesticides within the biobed. In particular the nitrogen originating from the compost used to make the biomix, as this can contain significant levels of nitrogen.

- The relative importance of microbial vs fungal degradation of pesticides within the biobed.
- The significance, if any of using chopped straw within the biomix and impact, if any of macerating the biomix.
- To further investigate the relative significance of pesticide concentration vs toxic inhibition.

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Appendix i

Example chromatrograms