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EXTRACTION AND PHOTOLYSIS OF PESTICIDES ON SOIL

LISA JANE FITZPATRICK

BSc. (Hons), GRSC

A thesis submitted in partial fulfillment of the requirements
of the University of Northumbria at Newcastle
for the degree of Doctor of Philosophy

In Collaboration with
AstraZeneca, Brixham, Devon
Avecia, Blackley, Manchester
Zeneca AgroChemicals, Jealott's Hill, Berkshire

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Wise Words

He who learns but does not think is lost,

He who thinks but does not learn is in great danger.

Shall I teach you what knowledge is?

When you know a thing, to recognise you know it,

And, when you do not know a thing,

To recognise you do not know it.

Confucius, b551 BC.

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Last, but definitely not least, huge thank you to my family and friends who have supported me unconditionally during the last three years.

ACN – Acetonitrile

ANOVA - Analysis of Variance

ASE™ - Accelerated Solvent Extraction

ASE™-HPLC-FLU - Accelerated Solvent Extraction – High Performance Liquid Chromatography – Fluorescence Detection

atm - atmospheres

BATNEEC – Best Available Techniques Not Entailing Excessive Costs

BSA - *N, O*-bis-(trimethylsilyl) acetamide

BTEX - Benzene, Toluene, Ethyl benzene, *o*-, *m*-, and *p*-Xylene

Bupirimate – Nimrod, 5-butyl-2-ethylamino-6methylpyrimidin-4-yl disulphamate

CB's – Chlorinated Biphenyls

CCD – Central Composite Design

CEC - Cation Exchange Capacity

CFC's - Chlorofluorocarbons

CRM - Certified Reference Material

DCM – Dichloromethane

DDD – 1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethane

DDE - 1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethylene

DDT - 1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl) ethane

DDX – collective for DDT, DDD and DDE

DEA - Deethylatrazine

DHPSE – Dynamic High Pressure Solvent Extraction

DIA – Deisopropylatrazine

EA – Environment Act

Ethirimol – Milgo, 5-butyl-2-ethylamino-6-methylpyrimidin-4-ol

ETPSE – Elevated Temperature and Pressure Solvent Extraction

EU – European Union

GC – Gas Chromatography

GC/EC-NIMS – Gas Chromatography/Electron Capture – Negative Ion Mass Spectrometry

GC-ECD – Gas Chromatography-Electron Capture Detection

GC-MSD – Gas Chromatography-Mass Selective Detection

HC – Hydrocarbons

HCH – Hexachlorocyclohexane

HpCDF – Heptachlorodibenzo furan

HPLC – High Performance Liquid Chromatography

HxCDD - Hexachlorodibenzo dioxin

i.d. - Internal Diameter

ICP – Integrated Pollution Control

LAAPC – Local Authority Air Pollution Control

LPG vehicles– Light Passenger Goods vehicles

MAE – Microwave Assisted Extraction

MAP – Microwave Assisted Process

MonoCP – Monochlorophenol

MPa - Megapascals

MSDS – Material Safety Data Sheet

OCDD – Octachlorodibenzo dioxin

OCP's – Organochlorine Pesticide

OM – Organic Matter

OPP's – Organophosphorus Pesticides

PAH's – Polycyclic Aromatic Hydrocarbons

PCB's – Polychlorinated Biphenyl's

PCDD's – Polychlorinated Dibenzo Dioxins

PCDF's – Polychlorinated Dibenzo Furans

PCP – Pentachlorophenol

PCT's - Polychlorinated Terphenyl's

PeCDF – Pentachlorodibenzo furan

PFE - Pressurised Fluid Extraction

PLE – Pressurised Liquid Extraction

PLS – Partial Least Squares Regression

PM_{10}'s Particles with diameter less than 10 μm

RSD – Relative Standard Deviation

SEPA – Scottish Environmental Protection Agency

SFE – Supercritical Fluid Extraction

SIM – Selected Ion Monitoring mode

SPE – Solid Phase Extraction

SPM – Suspended Particulate Matter

SRM – Standard Reference Material

SRS – Standard Reference Sediment

SVOC's – Semi-Volatile Organic Compounds

TBA – Terbuthylazine

TCDD - Tetrachlorodibenzo dioxin

TetraCP – Tetrachlorophenol

TiO_2 – Titanium Dioxide

TriCP – Trichlorophenol

UNEP – United Nations Environmental Protection

US EPA – United States Environmental Protection Agency

UV – Ultraviolet light

v/v – volume / volume

VOC's - Volatile Organic Compounds

Abstract

Pressurised fluid extraction (PFE), or under the Dionex tradename, Accelerated solvent extraction (ASE™) is a solvent extraction technique based on high pressure and temperature. PFE has been used to extract a wide range of analytes from both spiked and unspiked soils. The PFE extraction parameters of temperature, pressure, static extraction time, and number of static flush cycles were optimised for each of the chosen analytes; pentachlorophenol (PCP), bupirimate (5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate), ethirimol (5-butyl-2-ethylamoino-6-methylpyrimidin-4-ol), DDT (1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl) ethane), DDD (1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethane) and DDE (1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethylene). The PFE methodology was validated by extracting natively contaminated soil, aged spiked matrices and a certified reference material. Further investigation into the extraction procedure prompted a more detailed investigation into the selection of the extraction solvent, culminating in a model to predict the optimum solvent for extraction. The model is based on the Hildebrand solubility parameter, and has been applied to spiked and aged matrices, a certified reference material, and examples from the literature. The model was determined to be robust for contaminated soil and sediment.

An investigation into the photolysis of selected pesticides, PCP and bupirimate on soil was performed. The soil matrix was deemed to have a significant effect on the rate of degradation. PCP degradation followed first order kinetics, with a soil dependent rate constant between - 8.69×10^{-6} s^{-1} for compost and - 2.00×10^{-6} s^{-1} for mix 2. PLS determined that the sand and organic matter content of the soil was important in the

degradation of PCP. Application of PLS to the results of bupirimate photolysis determined that percentage sand and organic matter content significantly effected the rate of photolysis, and that percentage silt and percentage clay influenced the rate to a lesser extent.

Contents

Aims

The aims of this project were

- To evaluate pressurised fluid extraction as a new technique for the extraction of pesticides from soil.
- To optimise the extraction parameters for a range of analytes.
- To determine the influence of solvent on the extraction procedure.
- To identify whether the soil matrix has any effect on the extraction procedure.
- To determine whether photolysis of analytes occurs on soils in the absence of microbial activity.
- To quantify the photolysis and identify any degradation products.
- To determine which soil parameters influence the rate of photolysis.

Chapter 1

Environmental Legislation

Environmental Legislation

1.0 Introduction

In the past 200 years, advancements in industrial processes have yielded new products and materials. Increased exposure of the environment to chemicals was inevitable. Many chemicals are inert, and pose negligible threat; others such as cadmium and DDT do not. Notable in the past century (1900 - 2000) is the case of DDT [1, 1, 1-trichloro-2, 2-bis-(chlorophenyl)ethane]. DDT is an organochlorine pesticide that had worldwide use until its banning in the West in the 1970's, due to concerns about its prolonged existence in the environment, and implications of adverse effects on human health. DDT has been implicated in the acceleration of breast cancer.[1] DDT and its metabolic breakdown products (DDD and DDE) will be present in the environment well into the next century.[2] Other pollutants, e.g. PAH's, are present from the increase in vehicular emissions.[3] Recently, the problems with particulate and gaseous emissions have been highlighted. High levels of so-called 'nano particles' (PM_{10}'s) and polycyclic aromatic hydrocarbons have been linked to an increase in childhood diseases such as asthma and eczema.[3, 4]

In 1989, a list of priority pollutants was produced in the UK, after a conference on the state of the North Sea. The Red List (table 1.1[5]) is comprised of pesticides, as well as gaseous oxides (oxides of sulphur and nitrogen) and metals. These molecules were found to have a detrimental effect on the health of living organisms. Legislation now governs the limits of these compounds in the environment. Due to the diverse nature of organic analytes in the natural world, distribution into air, land and sea is inevitable.

Hence, separate legislation has been prepared for all three zones of the environment by the European Union (EU) and the United States Environmental Protection Agency (US EPA), amongst others.

Table 1.1 the 'Red List'[5]

Mercury and its compounds	Simazine
Cadmium and its compounds	Tributyltin compounds
DDT	Triphenyltin compounds
Pentachlorophenol and its compounds	Hexachlorobutadiene
Aldrin	Atrazine
Endrin	Trichlorobenzene
Polychlorinated biphenyls	Dichlorvos
Malathion	Fenitrothion
Azinphos-methyl	Dieldrin
Trifluralin	Hexachlorobenzene
γ-Hexachlorocyclohexane	1,2 dichloroethane
Endosulfan	

This chapter does not include legislation on radioactivity, or on the selection of sites for waste disposal site location. A lot of the legislation for pollution from these areas is included in air, water and soil protection.

1.1 Definitions

One widely accepted definition of pollution is;

The introduction by man, directly or indirectly, of substances or energy into the environment resulting in deleterious effects of such a nature as to endanger human health, harm living resources and ecosystems, and impair or interfere with amenities and other legitimate uses of the environment.[6, 7]

Article 2, section 1, part c of the Council Directive of 27 June 1967[8] defines the environment as:

(1) (c) "environment" means water, air and land and their inter-relationship as well as relationships between them and any living organism.

According to the UK Environmental Protection Act of 1990 c 43 part I, section 1 article 3 "Pollution of the environment" means pollution of the environment due to the release (into any environmental medium) from any process of substances, which are capable of causing harm to man or any other living organisms supported by the environment.[9]

1.2 History of European Environmental law

Reduction of the pollution of essential local resources, such as, drinking water and air, was the principal aim of the first legislation dealing with human health and safety. Statutes existed as far back as the fifth century AD in Rome. Complaints about the level of city waste in the River Tiber prompted action from the authorities. More recently, prohibition of the use of open coal furnaces in London was the earliest example of environmental measures in the UK. This was introduced by Edward I in 1306.

As the Industrial Revolution progressed, environmental procedures became more widely employed. The Napoleonic Decree of 1810 concerned with air pollution was enforced in France, Belgium and The Netherlands. In 1863, England adopted health and safety procedures aiming to prevent harm from industrial air pollution. However, only since the end of World War II, and partially due to the international public concern

expressed over the environment, were broader measures to fight pollution of inland waters, air, oceans and soil, introduced. The Treaty of Rome saw the establishment of the European Community in 1957.[10] In 1967 the first major tanker oil spill from the Torrey Canyon off the Southern coast of Britain encouraged both individuals and groups to express their concern about the state of the environment. This and many other incidents, for example, the earlier nuclear bomb on Hiroshima, pressed scientists and the general public to query the after effects of 'Scientific Progress'.[7, 11, 12]

The end of the 1960's saw environmental legislation implemented at national level. The publication of the first documentation on international environmental law was in direct response to the Stockholm Conference on the Human Environment in 1972.[10] The main points of the Stockholm declaration on the human environment were:

- Definition of the environment and the right of humans and animals to live in a pollution free environment.
- To ensure further pollution of the environment was reduced, thus preventing irreversible harm to the environment.
- To conserve the way of life for future generations.
- These goals are to be achieved by both individuals and groups, as all are responsible for the conservation of the planet.

The most important principles, from a pollution aspect, are principles 3 to 9. These state that the responsibility for preservation of wildlife and the prevention of pollution from chemicals, heat etc. is everyone's. Countries unable to accept the full financial / technological burden should be given aid by those countries who can, in a co-operative system. By the 1990's, all of Europe had introduced

environmental legislation at least at National level. In Rio de Janeiro in 1992, on the 20th anniversary of the Stockholm Conference, a large-scale Conference on Environment and Development was held. The Rio 'Earth Summit', heralded a more unified approach to global conservation. During the Conference, several key papers were agreed, that represent a more united effort towards reducing environmental pollution and promoting environmentally sustainable processes.[10]

The main points of the Earth Summit were as follows;

- That the right to development of the planet must not be at the expense of the environment, and vice versa.
- Co-operation between ALL countries is paramount. "Deals" excluding poorer countries were to be avoided.
- All States (countries) should aim to reduce the transport of waste to other countries for disposal. Cost effective measures should be taken to reduce pollution and discharge of substances harmful to the environment, and it is the responsibility of national bodies to promote waste reduction.
- Environmental assessment must be undertaken if any procedure is likely to harm the environment, and, in the case of natural disasters, all efforts should be made to aid afflicted States.

These declarations were adopted on 14 June 1992. Subsequently, the European Community now administrates environmental policy and procedure in the UK.[13]

1.3 Legislation regarding chemicals and other noxious substances.

In 1967, the first EU directive on dangerous substances or chemicals harmful to people or the environment was introduced (Council Directive 67/548/EEC (notification and

labelling). The directive aimed to unify the existing laws that governed dangerous substances, in particular the regulation concerning testing, packaging and labelling. Several updates (>25 to date) of this directive exist. The most noticeable changes to the original directive were made in 1979 (Directive 79/831/EEC)[14] and 1992 (Directive 92/32/EEC).[15] The council adopted directive 92/32/EEC on April 30th 1992 for implementation by October 1993.[15] The first major revision in 1979 dealt mainly with introducing a regime of pre-market testing and notification for new chemicals. Before 1979, new chemicals could be marketed ahead of environmental fate and toxicology study completion. The revision in 1979 required manufacturers of new chemicals, prior to marketing, to supply information on the properties and long term exposure effects of the chemical to the environment and people, as well as emergency procedures in case of spillages. The information was required to be submitted 45 days before the launch date to the EU and other regulatory bodies for approval. Chemicals already subject to EU legislation e.g. pharmaceuticals and low volume chemicals were omitted from this clause. In cases such as this more detailed information on toxicological studies (both human and environmental) were required. Chemicals placed on the market before September 1981, were classed as existing and as such, were exempt from the new legislation.[14] Lists of existing chemicals are found in the European inventory of existing commercial chemical substances (EINECS).[16] The revised directive[14] also included a classification list for noxious substances. The 14 categories ranged from explosive, to corrosive and finally to mutagenic compounds. The directive also introduced standard risk and safety phrases, for use on the MSDS of the substance, as well as a symbol system for the most hazardous property of the substance. Chemicals

classed as hazardous are listed in this directive. The list is regularly updated.[14] The 1992 amendment (Council Directive 92/32/EEC) was a further attempt to harmonise the EU member states' laws regarding hazardous substances. The classifications of hazardous substances were broadened and for the first time included classification criteria for environmental hazards. The supply of MSDS for substances was mandatory.[15] In 1976, the first of thirteen directives Council Directive 76/769/EEC (marketing and use of dangerous substances)[17] controlling the advertising and use of dangerous substances was introduced. Member states were required to guarantee that particular hazardous chemicals were only supplied for use under certain conditions. Chemicals included in this list were PCB's, PCT's, carbon tetrachloride and asbestos. In 1990, probably the most important piece of legislation in the field of environmental law since the control of pollution act of 1974 was introduced. This was the UK Environmental Protection Act. The passage of the act coincided with the increase in public concern about the state of the environment. However this was not seen as a 'cure all' piece of legislation, the strongest criticism of the act was that it did not have a coherent approach to the problem of environmental pollution. The main reason for this is the diverse nature of both the environment and current environmental issues. Part I dealt with integrated pollution control (ICP). There are two pollution control schemes. ICP and LAAPC (Local Authority Air Pollution Control). Before the establishment of industrial processes, authorisation is required from the governing body for processes that are capable of causing harm to the environment. The difference between ICP and LAAPC is in their responsibility and personnel and the local authority governs LAAPC. The most polluting industrial processes are under centralised control with respect to discharges into the environment. Pollution is considered in its entirety before a decision

on the maximum permissible levels. Her Majesty's Inspectorate for Pollution in England and Wales enforces IPC. Scotland has its own environmental bodies.

The aims of IPC were laid down in the white Paper, Our Common Inheritance,[18] and include the following:

- making air cleaner and safer.
- to further improve water quality and the state of the North Sea and coastal waters.
- the establishment of pollution emission levels in both air and recreational waters, and to determine safety tolerances.
- to maintain and strengthen controls over pollution, with a control mechanism based on levels.
- to provide incentive to industry to develop clean technologies and to improve environmental standards.
- to prevent pollution at source, hence decreasing the risk of harm to both humans and the environment by application of the most advanced technology available and to recognise the integrated nature of the environment.
- assess levels of pollutants in the local environment.
- introduce the polluter pays concept.

IPC aimed to harmonise the current rules and legislation on pollution.[9, 19 - 21]

Over the next five years, (up to 1995), an increase in awareness of environmental issues dealing with contaminated land, prompted the publication of the 1995 Environment Act. The act established two new bodies, the Environment Agency (EA), responsible for enforcing legislation in England and Wales, and the Scottish Environmental

Protection Agency, (SEPA). Part II of the act dealt with contaminated land and extended the scope of the 1990 EPA.

Neither of these acts is a full and comprehensive code of practice, as separate legislation exists for pesticide pollution and radiation contamination. In 1996, a unified agency was created for England and Wales with responsibility for IPC, regulation of waste, control of radioactive pollution and the regulation of water pollution. This body is called the Environment Agency.[9, 22]

1.4 Water Pollution

1.4.0 Introduction

There are numerous types of water pollution, including; thermal pollution, chemical pollution (i.e. pesticide run off), radioactive pollution, oil spills, and, siltation. The first three are forms that interfere with reproductive cycles. The last two are more visible sources, such as, oil spills and siltation, which have a larger impact on the public view of pollution. The effect the contamination has depends on several factors, including the actual amount of contaminant and the physico-chemical nature of the molecule. For example, addition of ammonia, a form of nutrient enrichment, causes an increase in the biological oxygen demand.[23] Organisms can cope with small increases of this type of pollution before detrimental effects are seen. On the other hand, a small dose of radioactive material can have a devastating effect in a short time period.[24]

1.4.1 Sources of pollution

Water pollution can be divided into two categories, point sources and non-point sources. Point sources are, as the name implies, derived from single points, and relate

to end of pipe industrial effluents, and sewer and septic systems. Non-point sources are those where the location of the source is not easily definable, such as agricultural run off. Hence pollution from a non-point source often occurs over a wide area and is influenced by the weather e.g., high rainfall etc.

1.4.2 Legislation

The European Union has divided the water legislation into three categories, each relating to different water usage.

Regulations relating to setting water quality objectives for various uses.

Water usage under this title includes that for drinking, bathing and for freshwater fish.

In 1975, the first EEC council directive (75/440/EEC) set the requirements for drinking water of surface water origin. The directive set out standards of treatment that had to be met before water was deemed to be of a suitable quality for human consumption.[25]

In 1980, a new directive (80/778/EEC) superseded that of 1975.[26] This amendment stated maximum permissible levels for 62 microbial, chemical and physical parameters, e.g. pH, turbidity etc. The directive also stated that regular monitoring of water quality must occur. The most recent legislative proposal from 1994 (COM (94) 612) aims to extend Directive 80/778/EEC to all water for domestic use. In addition, the water quality would be subject to stricter guidelines, for example, the maximum permitted lead level per litre of drinking water would be reduced from 50 μg / L to 10 μg / L.

Other directives under this section include those for bathing water and standards for freshwater fish.

Directives which sought to limit or prohibit discharges of dangerous substances into waters by industrial plants.

The 1976 council directive 76/464/EEC identified two lists of substances that were classified as hazardous. The 129 substances located on List 1, also known as the Black List, were those that were to be eliminated from the environment due to their toxicity and bioaccumulation. They included organophosphorus compounds, certain heavy metals and their compounds, (such as, mercury, tin and cadmium), hydrocarbons from petrol distillation processes, as well as persistent synthetic substances, such as vinyl chloride. The limits set on these substances were to ensure pollution by these molecules was drastically reduced. Table 1.2 lists the levels of certain black list substances permitted in both inland and coastal waters over the space of a year.[5]

Table 1.2 Selected Black List substances

Compound	Inland (µg / L)	Coastal and territorial (µg / L)
Aldrin, dieldrin, aldrin and isodrin	0.03 (total)	*a*
Cadmium and its compounds	5 (total)	2.5 (as dissolved metal)
Carbon tetrachloride	12	12
Chloroform	12	12
DDT	0.025	0.025
4,4'-DDT	0.01	0.01
Hexachlorobenzene	0.03	0.03
Hexachlorobutadiene	0.1	0.1
Mercury and its compounds	1 (total)	0.3 (as dissolved metal)
Pentachlorophenol and its compounds	2	2

a – There is no official limit for this compound in coastal and territorial waters.

List II, the Grey List, was separated into two parts. Part (a) contained compounds from List I for which the safety limit values had not yet been established. Part (b) contained compounds that also had a detrimental effect on the environment, for

example, both elemental and inorganic compounds of phosphorus, cyanides and fluorides; however, their contamination could be contained within a given area. This was dependent on the geological characteristics and actual location of that area i.e. discharge of pollutants to a pond. Six daughter directives governing the limits of specific compounds contained in List I were also implemented.[27] These daughter directives included limitations on the discharge of mercury (82/176/EEC and 84/156/EEC),[28, 29] cadmium (83/513/EEC),[30] hexachlorocyclohexane (84/491/EEC),[31] and DDT, pentachlorophenol and carbon tetrachloride (86/85/EEC).[32] More recently, in directives 88/347/EEC[33] and 90/415/EEC[34] limits for the levels of other chlorinated substances, such as aldrin, dieldrin,[33] trichlorobenzene and 1,2-dichloroethane have been added.[34]

Provisions on marine pollution which aim primarily to put an end to pollution, protect the North Sea, the Baltic Sea and the Mediterranean and to prevent pollution from land-based sources.

The Paris Convention proposed to reduce and ultimately prevent marine contamination from land based sources, such as, that from watercourses and submerged pipelines. Council decision 75/437/EEC,[35] approved and adopted this proposal. In 1986, the directive was amended to include marine pollution from atmospheric sources.[32]

Emergency protocols in the case of spillages have been under consideration since 1983, but to December 1999, no guidelines have been published. Dumping of waste into the North Sea has been banned since 1993, but the cessation of PCB dumping was not implemented until the end of last year (1999).

1.5 Air pollution

According to the 1987 Air Pollution Act (Ireland),[36] air pollution is defined as 'a condition of the atmosphere in which pollutants are present in such quantities as to be liable to:

(i) be injurious to public health,

(ii) have a harmful effect on flora or fauna or damage property, or

(iii) impair or interfere with amenities or with the environment.

Primary air pollutants are those produced by industrial process, and fossil fuel combustion, and include nitrous oxides, hydrocarbons, and heavy metals. Secondary air pollutants are mainly comprised of sulphur and nitrogen compounds, and, include pesticidal preparations. They result from the use of chemicals in the general population. The sources of air pollution far exceed the number of air pollutants. The main offenders are vehicular emissions, and those from waste disposal plants.

1.5.1 Legislation

In 1975, the European council instigated procedure for exchange of information between air monitoring associations. This directive mainly dealt with pollution by sulphurous compounds and suspended particulate matter.[37] Further directives concerning air quality and power station emissions were passed by the EC in the 1980s.

Legislation for vehicular emissions.

The first EU directive dealing with vehicular emission appeared in 1970 (70/220/EEC).[38] Emission regulations for hydrocarbons and carbon monoxide from LPG vehicles were set. In 1977, limits of NO_X were added to the directive

(77/102/EEC).[39] The limits of these compounds were further decreased in 1978 and 1983 (directives 78/665/EEC and 83/351/EEC respectively).[40, 41] These levels reduced further to coincide with restrictions imposed in the USA in 1988. Diesel emissions were limited in directive 88/436/EEC and 88/77/EEC.[42, 43]

Lead pollution is a serious health threat. High levels of lead in the blood can lead to poor memory and severe learning difficulties in young children.[44, 45] In 1982, Directive 82/884/EEC set the maximum annual mean concentration of atmospheric lead to 2 $\mu g\ m^{-3}$ [46]. Directive 85/210/EEC reduced the amount of lead in petrol from a maximum of 0.4 g /L[47] to 0.15 g / L.[48] The directive also stipulated that lead free petrol (< 0.013 g / L) should be available to the consumer by the end of 1989. All the laws regarding lead emissions from various classes of vehicles were amalgamated and amended in Directive 91/441/EEC.[49]

In the intervening years, continual reductions in emissions for new cars have been implemented. Particulate matter from diesel engines was reviewed in directive 91/542/EEC.[50] By 1997, all vehicles were subject to strict emission guidelines. It was noted, however, that the quality of the fuel played a large role in vehicular emissions. Hence, in 1996/7, proposals to increase fuel quality were introduced. The directives (COM (96) 248[51] and COM (97) 77)[52] stated that higher standards for fuel would be implemented from year 2000, to guarantee targets for air quality are met by 2010.

Legislation from industrial plant emissions.

The first legislation dealing with emissions from industrial plants was set out in council directive 84/360/EEC.[53] The directive laid down measures aimed at reducing pollution from these sources. Processes listed in Annex I of the directive (Table 1.3) had to seek permission before running the plant from the relevant local and national authorities.

The granting of permission was subject to two stringent criteria;

1. that all suitable precautions had been taken to prevent air pollution, i.e. BATNEEC, and,

2. that the process would not exceed emission limits.

Table 1.3 Prescribed Processes[53]

Industry	Example
Energy	Coke ovens, thermal power stations
Production and processing of metals	Pig iron and crude steel
Manufacture of non metallic mineral products	Cement, ceramic, glass
Chemical Industry	Inorganic and organic chemical production
Waste disposal	Incineration
Other	Paper pulp production

However, in 1988 directive 88/609/EEC[54] set guidelines for atmospheric emissions from large industrial plants. Limits for sulphur dioxides and nitrous oxides (SO_x, NO_x) and dust was set for both new and existing plants. The aim of the directive was to reduce pollution from these discharges by nearly 60 % by 2003. Later, in 1994, smaller industrial plants operating using solid fuels were subject to similar restrictions. SO_x from larger plants were also reduced.

Other Legislation

Protection of the ozone layer.

The first legislation on the protection of the ozone layer the EC brought into force was in 1978.[55] The aim of this resolution was to limit the production of CFC's and to promote alternative compounds. Several directives exist on limiting the production of specific ozone depleting substances, such as Decision EEC/80/372,[56] which capped the production of CFC-11 and CFC-12.

The 1985 Vienna convention[57, 58] highlighted the need for protection of the ozone layer, and laid down the foundation for the Montreal protocol.[59] This further discussed the problem of ozone reduction and obliged countries to state their position on reduction of CFC's and other ozone damaging compounds. The protocol also laid down a time scale for the reduction and phasing out of use of CFC's. The protocol was further amended by the addition of new limits and substances in 1990, 1992 and 1995.[60 - 62] Selected examples from the most recent list of controlled ozone depleting compounds, and a measure of their depleting potential is shown in table 1.4.[63] The depleting potential is an index from 0 to 10, and is a measure of the potential effect each controlled substance has on the ozone layer, 0 having no effect and 10 having the most detrimental effect with respect to current ozone levels. Note, groups VII and VIII are incomplete.

Table 1.4[63] Ozone Depleting Potential of Various Air Pollutants

Group	Molecular Formula	Ozone Depleting Potential
Group I Chlorofluorocarbons	$CFCl_3$ (CFC-11)	1.0
	CF_2Cl_2 (CFC-12)	1.0
	$C_2F_4Cl_3$ (CFC-113)	0.8
	$C_2F_4Cl_2$ (CFC-114)	1.0
	C_2F_5Cl (CFC-115)	0.6
Group II, fully halogenated Chlorofluorocarbons, including isomers	CF_3Cl (CFC-13)	1.0
	C_2FCl_5 (CFC-111)	1.0
	$C_2F_2Cl_4$ (CFC-112)	1.0
	C_3FCl_7 (CFC-211)	1.0
	$C_3F_2Cl_6$ (CFC-212)	1.0
	$C_3F_3Cl_5$ (CFC-213)	1.0
	$C_3F_4Cl_4$ (CFC-214)	1.0
	$C_3F_5Cl_3$ (CFC-215)	1.0
	$C_3F_6Cl_2$ (CFC-216)	1.0
	C_3F_7Cl (CFC-217)	1.0
Group III Halons	CF_2BrCl (Halon-1211)	3.0
	CF_3Br (Halon-1301)	10.0
	$C_2F_4Br_2$ (Halon-2402)	6.0
Group IV, Carbon tetrachloride	CCl_4	1.1
Group V, 1,1,1-trichloroethane	$C_2H_3Cl_3$	0.1
Group VI, Methyl bromide	CH_3Br	0.6
Group VII Hydrobromofluorocarbons	$CHFBr_2$	1.0
	CHF_2Br	0.74
	CH_2FBr	0.73
	C_2HFBr_4	0.8
	$C_2HF_2Br_3$	1.8
	$C_2HF_3Br_2$	1.6
	C_2HF_4Br	1.2
	C_3HFBr_6	1.5
	$C_3HF_4Br_3$	2.2
	C_3HF_6Br	3.3
	$C_3H_2F_3Br_3$	5.6
	$C_3H_2F_4Br_2$	7.5
	$C_3H_2F_5Br$	1.4
Group VIII Hydrochlorofluorocarbons	$CHFCl_2$ (HCFC-21)	0.04
	CH_2FCl (HCFC-31)	0.02
	CH_3FCl_2 (HCFC-141b)	0.110
	C_2H_4FCl (HCFC-151)	0.005
	C_3HF_6Cl (HCFC-226)	0.1
	$C_3H_4FCl_3$ (HCFC-251)	0.01

Table 1.5 shows the phase out dates for each group of compounds[56]

Table 1.5 Phase out dates for Ozone Depleting Compounds

Group	Phase out date
I	1999
II	1999
III	1999
IV	1999
V	1999
VI	2005
VII	1999
VIII	2010

Waste incineration plants.

Regulations limit emissions from waste incineration plants. In 1990, two directives came into force regarding these emissions and both new and existing waste incineration plants.[64, 65]

1.6 Terrestrial Pollution

1.6.1 Soil

Soil is an essential part of the ecosystem. It helps to filter out waterborne and atmospheric pollutants before they reach the groundwater. The soil composition varies according to the soil type and location.[66] For many years it was classed as a renewable resource, requiring little or no protection via legislation. Increased use and abuse of the soil has depleted nutrients and reduced its capability to remove chemicals. A report by UNEP in 1987 estimated that 15 % of global land i.e. 22.2 million sq. km, has deteriorated, an area nearly the size of the entire North American Continent.[67]

Contaminated land is inherently difficult to define. Contamination is a necessary condition for pollution. Contamination can be defined as 'the introduction or presence

in the environment of alien substances or energy, on which we do not wish or are unable to pass judgement on whether they cause, or are liable to cause, damage or harm. Classifying land, as to whether it is contaminated is much harder. There are no set criteria on which to base an evaluation.[9, 21]

1.6.2 Sources of pollution

There are three main sources of terrestrial pollution, those from natural events e.g. PAH's from volcanoes, forest fires etc., and that from industrial sources e.g. Chernobyl in 1985, and that from landfill sites.

1.6.3 Legislation

Legal protection for soil is relatively recent. There are few laws that directly address contamination/pollution of the land. Most of these laws are a result of protecting other compartments of the environment, e.g. water and air. Iceland was the first country to address the subject of soil erosion in 1895 due to the significant problems that the country has. By 1907, Iceland had a national agency to deal with the issue.[7]

In 1972, the Council of Europe adopted the European Soil Charter. The Charter contains guidelines for action with regard to soil protection, as well as focusing on the need for soil conservation. Although very little was actually actioned by the soil charter; it laid the framework for more specific and binding legislation in other sectors.[68] A good example of this is the 1986 directive (86/278/EEC) dealing with the use of sewage sludge for agricultural purposes. The directive states a code of practice for the use of sludge and restricts the planting of certain crops post-application.[69]

Other directives aimed at reducing pollution to other compartments of the environment

by restricting 'dumping' include directive 91/676/EEC,[70] the protection of waters from nitrate pollution, and directive 91/156/EEC[71] on encouraging recycling and making provisions for the harmless disposal of waste.

The 1990 EPA was criticised for almost ignoring the problem of contaminated land, and in 1995; the EA focused on extending the provision of the EPA, and included more detail on the civil liability aspects of contaminated land. The EA of 1995 shifts the emphasis from previous land use, to the presence of substances that may cause harm. However, a major problem still lies in the classification of contaminated land. For example, under the 1990 regulations, two adjacent sites of land, which are not classed as contaminated individually, could not be combined and the whole are designated contaminated. This meant the local authority could not action the landowner to clean up the land. The 1995 regulation changed this and consequently, more land can be cleaned at the owner's expense.

1.7 Summary

The evolution of environmental legislation over the last 40 years is shown in figure 1.1.

The problems faced by the EC in drawing up appropriate protection for the environment are in part due to the diverse nature of both pollution and the ecosystem.

To a certain extent, legislation is governed by the ability of the environment to cope with inputs of energy. The sources of pollution are numerous, and control of the processes causing detrimental effects is key in the conservation of the environment.

While adequate procedures and laws exist for the protection water and air, little is available for protection of the soil. The main reason is that soil was viewed for a long

time as a renewable self-sustaining resource. It has taken time to realise the extent of the damage caused by overuse of farmland and chemical reduction of crop damaging pests.

Figure 1.1 Evolution of Environmental Legislation in Europe[5, 7, 10]

1957 — Treaty establishing the European Economic Community[10]

1967 — EC Directive relating to the Classification, Packaging and Labelling of Dangerous Substances 67/548[8]

1968 — European Declaration of Principles of Air Pollution Control,[72] European Water Charter[10]

1972 — European Soil Charter,[68] Stockholm Conference on the Human Environment and Plan of Action[68]

1973 — EC Directive relating to the Classification, Packaging and labelling of Solvents(73/173/EEC),[73] First EC Programme of Action on the Environment[10]

1975 — EC Directive on the Sulphur Content of Certain Liquid Fuels (75/116),[7] EC Directive Concerning the Quality of Bathing Water (76/160)[74]

1976 — EC Directive on Pollution Caused by Certain Dangerous Substances Discharged in the Aquatic Environment of the Communities (76/464)[27]

1978 — EC Directive on Toxic and Dangerous Wastes (78/319),[75] EC Directive Relating to the Classification, Packaging and Labelling of Pesticides (78/631).[76] EC Directive Concerning the Lead Content of Petrol (78/611)[77]

1980 — EC Directive on Chlorofluorocarbons (80/372),[56] EC Directive Relating to the Quality of Water Intended for Human Consumption (80/778)[26]

1982 — EC Directive on Limit values and Quality Objectives for Mercury Discharges by the Chloro-Alkali electrolysis Industry (82/176),[28] EC Directive on a Limit Value for Lead in the Air (82/884)[78]

1983 — Agreement in Dealing with Pollution of the North Sea by Oil and Other Harmful Substances[10]

1984 — EC Directive on Combating Air Pollution from Industrial Plants (84/360),[53] EC Directive on Limit values and Quality Objectives for Discharges of Hexachlorocyclohexane (84/491)[31]

1985 — Vienna Convention for the Protection of the Ozone Layer,[57, 58] EC Directive on Limits of Lead and Benzene in Petrol (85/581)[79]

1986 — EC Directive on Sewage Sludge used in Agriculture (86/278),[69] EC Directive on Limit Values and Quality Objectives for Discharges of Certain Dangerous Substances (86/280)[80]

1987 — Montreal Protocol on Substances that Deplete the Ozone Layer[59]

1990 — Environmental Protection Act[9, 19]

1991 — EC Directive on Toxic and Dangerous Waste (91/698)[81]

1992 — Rio Declaration on Environment and Development[10]

1995 — Environment Act (Incorporating Integrated Pollution Control)[9, 21]

1996 — Proposal for an EC Directive on Fuel Quality[51]

1997 — Proposal for an EC Directive on the Reduction of Air Pollution from Motor Vehicles[52]

2000

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Chapter 2

Extraction Techniques in Environmental Analysis

Extraction Techniques in Environmental Analysis

2.0 Introduction

There are a wide range of organic analytes in the environment, including polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's), and pesticides.[1-3] Contamination by pesticides is different from that caused by other organic molecules, as the introduction of pesticides into the environment is deliberate. Pesticides tend to partition between all the environmental compartments, air, water and soil.[1, 2] The quantification of pesticides and other carcinogenic compounds in the environment is essential. Health risks for both humans and animals have been highlighted in the last century, notably with DDT, the effects of which will be felt well into the new century.[4] The preliminary and probably the most important stage in any analysis, is the quantitative removal of the analyte from the matrix. This chapter concentrates on the extraction of environmentally relevant organic analytes from natively contaminated soil and sediment. Several methods of solid / liquid extraction are commercially available; these range from simple sequential shaking of the solid with aliquots of solvent, such as Shake Flask extraction, to extraction using high pressure and temperature in a sealed system, as in pressurised fluid extraction. Publication of the use and inter-comparison of these techniques is prolific. However, validation of the proposed analytical method is required to determine the robustness of the procedure. Commonly, validation takes the form of extraction of a material for which the contamination level is known. Suitable environmental solids for the determination of most organic analytes, especially pesticides are scarce. Assessment of the robustness of any extraction techniques is frequently

achieved by the extraction of spiked samples. However, caution should be exercised when analysing the data, as it provides no real indication of the behaviour of the analyte in the matrix. Many researchers try to simulate matrix-analyte interactions by artificially ageing the sample. Ageing can take the form of one or more of the following; extremes of temperature, exposure to UV light, and simply leaving the analyte and matrix for a fixed period of time preceding extraction. Spiked samples are relatively easy to prepare. Two procedures are commonly used: "spot" spiking and "slurry" spiking. Spot spiking involves the introduction of the analyte in a small volume of organic solvent (μL) to a comparatively large quantity of matrix. The solvent is removed by evaporation prior to sample extraction and analysis. Alternatively, slurry spiking is when the analyte is added to the matrix in a large volume of organic solvent (mL). The slurry mixture is then continually stirred while the solvent is evaporating. An additional benefit of stirring is that the sample is as homogeneous as possible.[5 - 7]

Before analysis and hence quantification of the analyte can occur, it is usually necessary to remove the target compound from the matrix it is in, using an organic solvent (or solvent mixture). Energy is also required. Traditional extraction techniques, such as, Soxhlet extraction or Soxtec extraction use heat energy to remove the analyte, whilst sonication or shake-flask extraction use agitation of the solvent-matrix mixture. Nevertheless, these procedures can be time consuming. Recently, automation has played a large role in the development of new extraction techniques. These include supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurised fluid extraction (PFE). These techniques have been applied to a wide range of analytes and matrices.

2.1 What is soil?

Soil is formed through the gradual breakdown of rock, by several mechanisms, including weathering and erosion, the rock is gradually ground down to smaller and smaller particles. Soil can best be defined as a composition of five main components. These are clay minerals, organic matter air, water and a living component. The quantities of these constituents vary according to the soil type and location.[8] As this chapter concentrates on the extraction from soil and sediment, a brief look at the components of soil is beneficial, as this can also give insight into the behaviour of the analyte in 'real' samples.

2.1.1 Clay minerals

The effect of soil clay and organic matter is often cited in the literature as having significant effects on the extraction of pesticides.[9 - 13] The clay minerals for instance have been associated with the difficulties in extracting planar (or nearly planar) molecules e.g. naphthalene from soil.[14] There have been several hypotheses as to why this should be true. The main theory is thought to be due to the structure of the clay minerals.[15] Clay minerals are based primarily on silicates and oxides. They typically have a particle size of less than 0.002 mm. They form a "sticky wet looking" mass when wet and "clump together" when they are dry. There are many types of clay minerals found in soil. They can be based on crystalline structure as in the case of gibbsite, an aluminium oxide, or amorphous (i.e. no regular structure) such as calcite or dolomite. However, by far the most interesting is the behaviour of certain crystalline silicate minerals. Crystalline clay minerals can be further classified as having chain structure or layer structure. The most

interesting are those with layer structure. These come in two categories; 1:1 layers such as kaolinite (figure 2.1) and 2:1 layers such as montmorillonite (figure 2.2).[16]

Figure 2.1 Kaolinite/Kandite group.
1:1 family of layered silicates.

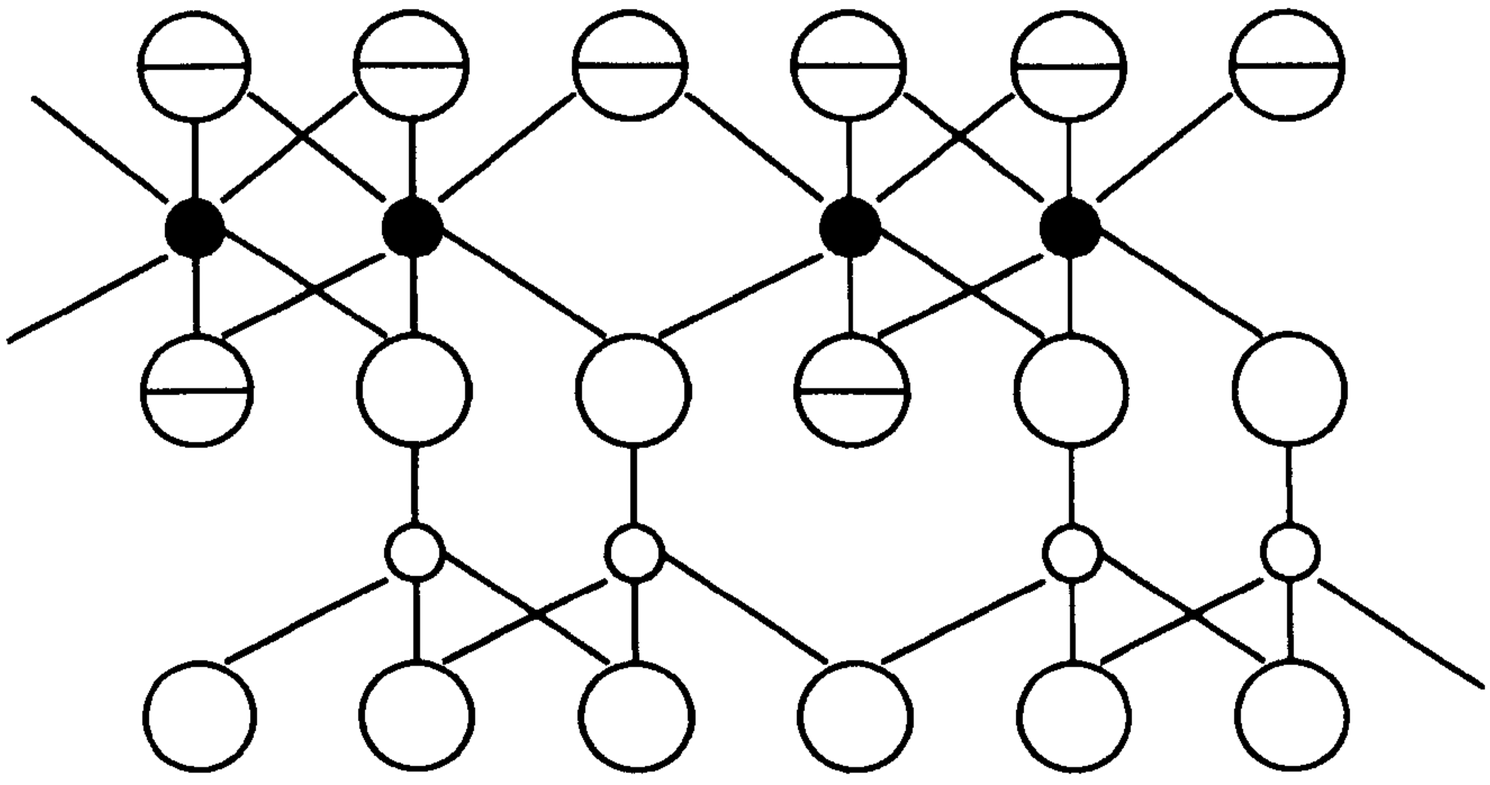
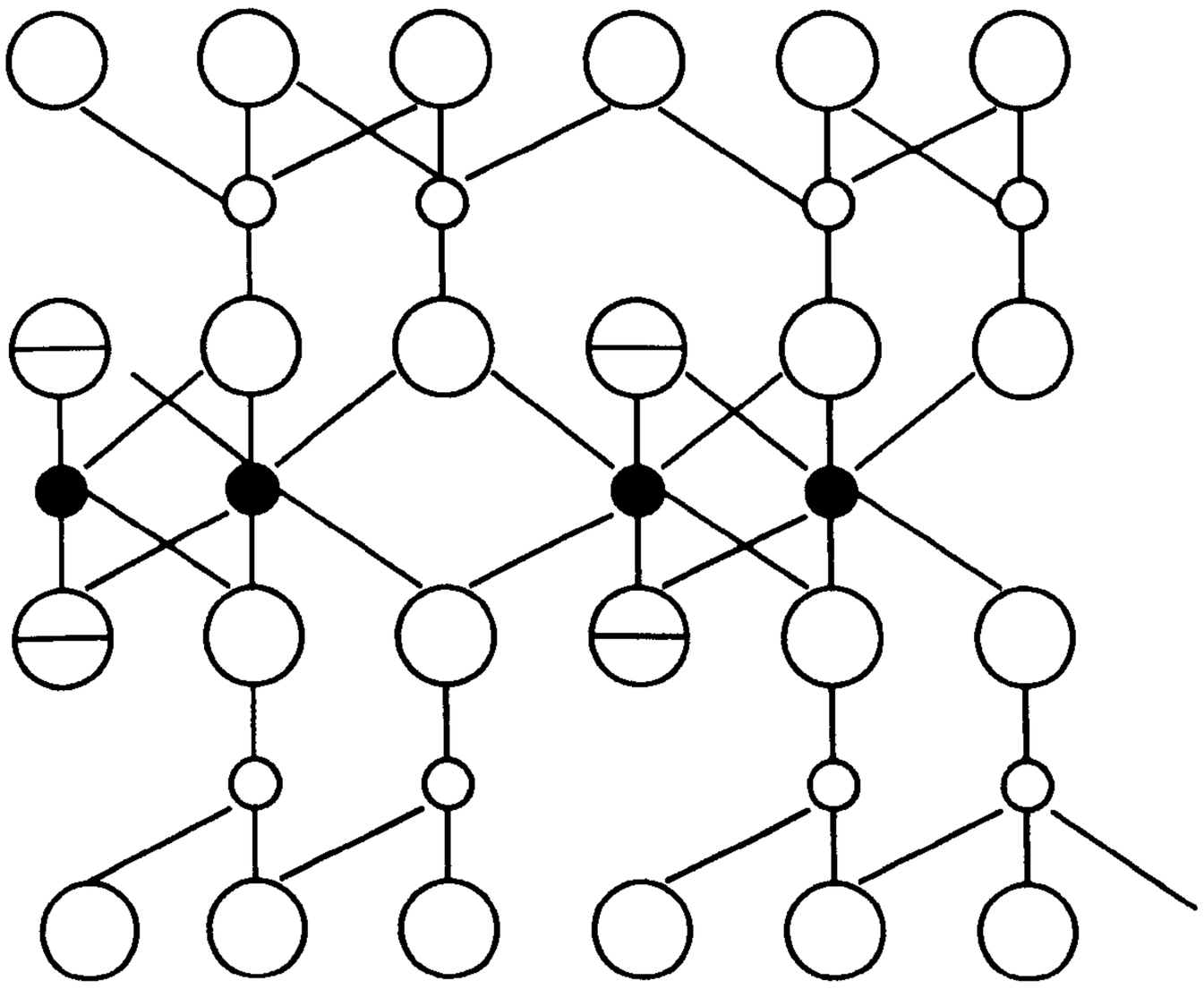


Figure 2.2 Montmorillonite/Smectic group
2:1 family of layered silicates.



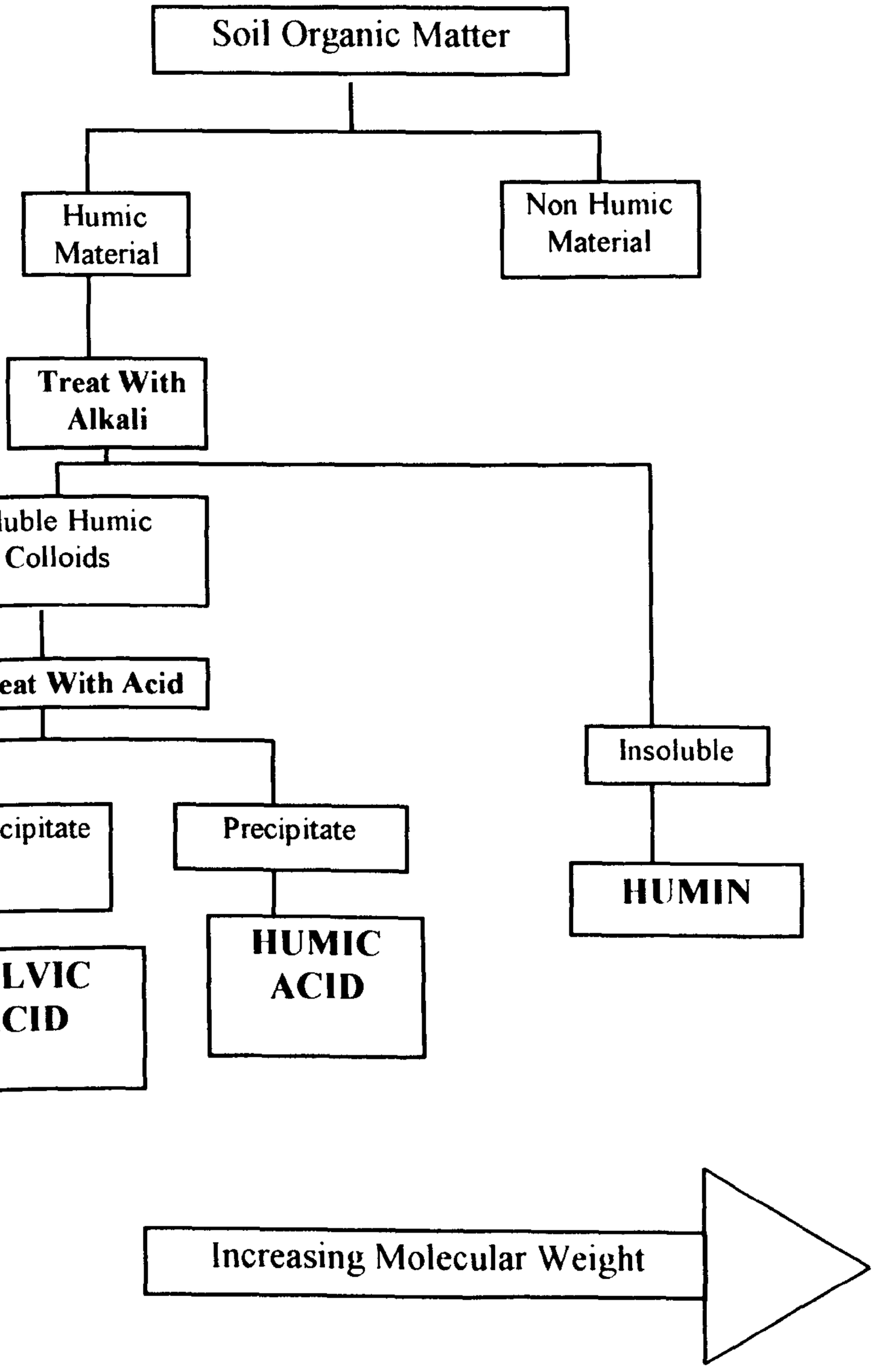
The 1:1 layer structure is based on a repeating pattern of tetrahedrally (four co-ordinated) silicon and octahedral (six co-ordinated) aluminium atoms. The presence of oxygen atoms and hydroxyl groups allows hydrogen bonding, and consequently helps to stabilise the crystal structure. The 2:1 layer is based on an octahedral co-ordinated aluminium atom sandwiched between two layers of tetrahedrally co-ordinated silicon atoms. When water is introduced to the latter of these systems, the polar water molecules can get in between the layers causing swelling. As the clay dries, the layers return to their original interplanar distance. If the water is associated with pesticides, the pesticides are then stuck in the layer structure when the clay is dry. Hence there are implications for extraction.[14, 17] Since the bonds between the layers are relatively strong, the pesticide is unable to move, hence there have been studies performed on the adsorption of pesticides to clay minerals.[16, 18 - 20] These have shown that after the clay has dried, the extraction of the pesticides is very difficult and low extraction efficiencies are obtained. The cation exchange capacity is also fundamental to the behaviour of the clay minerals. It is a measure of the ability of the clay to substitute metal ions from the lattice to the surrounding environment. These metal ions are not directly bonded to the lattice, but they balance out any charges within the crystal structure.[16]

2.1.2 Organic matter

The organic matter content of any soil is dependent upon location. For example, a soil in an area with high vegetation, such as in a forest will have a larger amount of organic matter than a soil with very little flora around. The organic matter can be split into three distinct components, humic acid, fulvic acid and humin. Humin is the decomposing

remains of both plants and animals. Humic and fulvic acids are not well characterised. They are not classed as single substances, but are a mixture of acids with similar properties. For example, humic acid is that fraction of humus that is soluble in dilute alkali, but does not precipitate upon addition of a mineral acid. Figure 2.3[21] summarises the various fractions and their solubilities.

Figure 2.3 Fractionation of Organic Matter



Organic matter has been studied by several groups and they have implied that the higher the organic matter content of a soil, the greater the adsorption of the analyte, hence reduced extraction efficiencies are obtained.[9 - 11]

2.1.3 Water

Water plays a principal role in the soil environment. Not only does it affect plant growth, it can help to create or destroy soil structure. The relationship between soil and water is very complex; it affects the physical properties of a soil, for example, the expansion of the clay fraction (see above), and the transport of nutrients through the soil profile. The water content of a soil can vary immensely, from being totally saturated to completely dry. The maximum amount of water a soil can hold without it draining away is known as the field capacity. This is an important quantity that can greatly influence the extraction of pesticides from field samples.[16]

2.1.4 Micro organisms

The living portion of the soil is composed of microorganisms such as fungi, and bacteria. They are responsible for breaking down dead and decaying matter; they also help to release nutrients into the soil through decomposition.[8] The living population can make up around 5 % of the soil volume. The micro-organisms present in soil do not directly influence the ease of extraction. Frequently, they degrade the parent pesticide to give a wide range of metabolites, that in turn can strongly adsorb to soil, as in the case of atrazine degradation.[22]

As stated earlier, the composition of soils varies from location to location. However, a system of classification of soil types has been in use for many years. This classification is based on the sand, clay and silt content of the soil.[16] The silt fraction is composed of very fine particles and their size is between 0.002 mm and 0.05 mm. The silt fraction has a fine texture when it is dry. Sand has a gritty texture and has a diameter between 0.05 mm and 2 mm. Sand, clays and silt make up the largest volume of soil (around 45 % of the total soil volume), and consist primarily of silicates. Organic matter is the smallest fraction, only making up around 5 % of the soil volume. Water makes up around 25 % of the soil volume and carries the nutrients essential for plant life.[8] The soil textural triangle gives a broad indication of soil type.[8] Commonly, further information is required about the exact composition of a test soil. Methods exist that allow the calculation of several of these parameters, for example, cation exchange capacity (CEC) which is a measure of the ability of a soil to co-ordinate to multivalent species, such as metal ions. pH is a measure of the soil acidity,[10] which can influence the extraction of ionic pesticides; organic matter and clay type and content can also be established, their importance to the extraction of pesticides is highlighted above.

2.2 Microwave-Assisted Extraction

2.2.1 Interaction of microwaves with matter.

Microwaves are short wavelength, high frequency electromagnetic radiation. To prevent interference with radio transmissions, industrial and domestic microwaves function at a wavelength of around 12.2 cm (1.02×10^{-3} eV). Microwaves are made up of two wave components acting perpendicular to each other and the direction of propagation (travel)

and vary sinusoidally. These are a magnetic field component and an electric field component. Like other electromagnetic energy, microwaves are said to have a dual nature, that is, they can act like waves, but also have particulate character (photons). Electrons in the ground state of a molecule absorb photons. The electron is raised to the next energy level. These changes in the levels are discrete and do not occur continuously, as electrons occupy definite energy levels. The energy is quantised. The electric field component interacts only with charged (or polar) particles. The dielectric constant of a material determines the ease of polarisation of the molecule. If charged particles (e.g. electrons) present in the molecule are mobile, a current is set up in the material. However, strongly bound electrons undergo a different phenomenon. The particles reorganise themselves so they are in phase with the electric field. This is called dielectric polarisation. Four components have been identified within the total dielectric polarisation. They represent the four main types of charged particles that are found in matter, electrons, nuclei, permanent dipoles and charges at interfaces.

An equation linking all four constituents of the total dielectric polarisation is stated in equation 2.1.

$$\alpha_t = \alpha_c + \alpha_a + \alpha_d + \alpha_i \qquad \text{Eqn. 2.1}$$

Where α_t is the total dielectric polarisation

α_c is the electronic polarisation

α_a is the atomic polarisation

α_d is the dipolar polarisation

and α_i is the interfacial polarisation.[23]

Frequent changes in the orientation of the electric field cause similar changes in the total dielectric polarisation. Changes in the dipolar polarisation results in heating in the material. Interfacial polarisation (the Maxwell-Wagner effect) only has a significant effect on dielectric heating when conducting particles are suspended in a non-conducting material. The other two components have no effect on heating. Therefore, in order to heat a solvent (or mixture of solvents) part of it must be polar. Sensitisers are molecules that preferentially absorb the microwave radiation and pass it on to other molecules.[24] Non polar solvents, such as, hexane do not absorb microwave energy, but a mixture of hexane and acetone does.

2.2.2 Instrumentation for Microwave-Assisted Extraction:

A microwave extraction system consists of a microwave generator (magnetron), wave guide, resonant cavity and a power supply. The magnetron is a diode in a magnetic field. Indentations in the magnetron act as anode, causing resonance. This resonance behaves as a source for the microwave energy. The wave-guide concentrates the microwave energy onto the sample. Two types of industrial microwave extraction systems are available: Pressurised MAE and Atmospheric MAE. In pressurised systems, the pressure can be controlled up to 200 psi, while the pressure is constant in atmospheric MAE. The power rating for the two systems is also different. Atmospheric instruments have a power rating of 300 watts compared to 950 watts for some pressurised systems. In the pressurised system, up to 12 samples are placed into a carousel in a microwave transparent extraction vessel. The vessels are lined with an inert material. The vessels are irradiated with

microwave energy. The temperature and pressure in one of the cells can be monitored and controlled using an infrared sensor and water manometer, respectively. The controllable parameters are temperature of the extraction, time of extraction, and pressure in each vessel as well as the amount of microwave power the vessels receive. In built safety features of the extraction system, include solvent vapour alarm and rupture membranes in each vessel. Rupture membranes fracture when the pressure exceeds the maximum, allowing the contents to siphon into a central container.[25]

2.2.3 Applications of Microwave-assisted extraction:

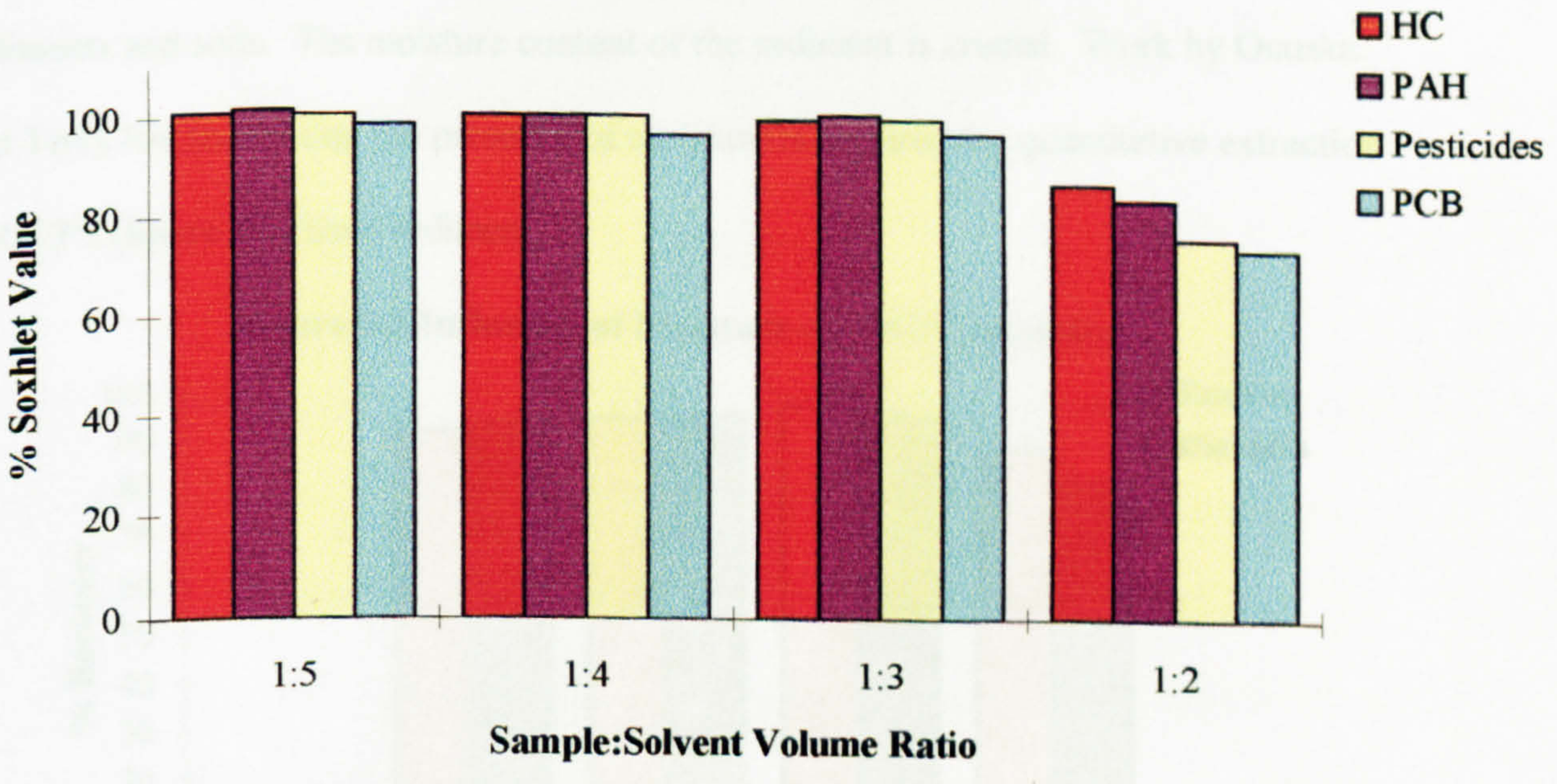
Extraction of Pesticides

Microwave technology has been used to extract pesticides from spiked and real samples. Steinheimer[22] has used microwave technology to extract the herbicide atrazine and its degradation products, deisopropylatrazine (DIA) and deethylatrazine (DEA) from contaminated soil samples. Briefly, the soil sample was extracted with water and then three successive times with dilute hydrochloric acid. The extracts were combined and analysed using HPLC with UV detection. Two soils of differing composition were investigated. The first was a loamy soil (Nashua) and the second was a silty loam (Treynor). Sample clean up was required due to the coloured nature of the extract. Solid phase extraction (SPE) and centrifugation was employed for this. The average recoveries of the degradation compounds (DEA and DIA) were between 85 - 95 % for the loamy soil and 85 - 115 % for the silty loam soil. The recoveries for the parent compound (atrazine) and a surrogate, terbutylazine (TBA) were 65 - 55 % for Nashua soil and 55 - 50 % for

Treynor soil. The decrease in the extraction efficiency was thought to be due to the increased basic nature of the degradation products over the parent compound.

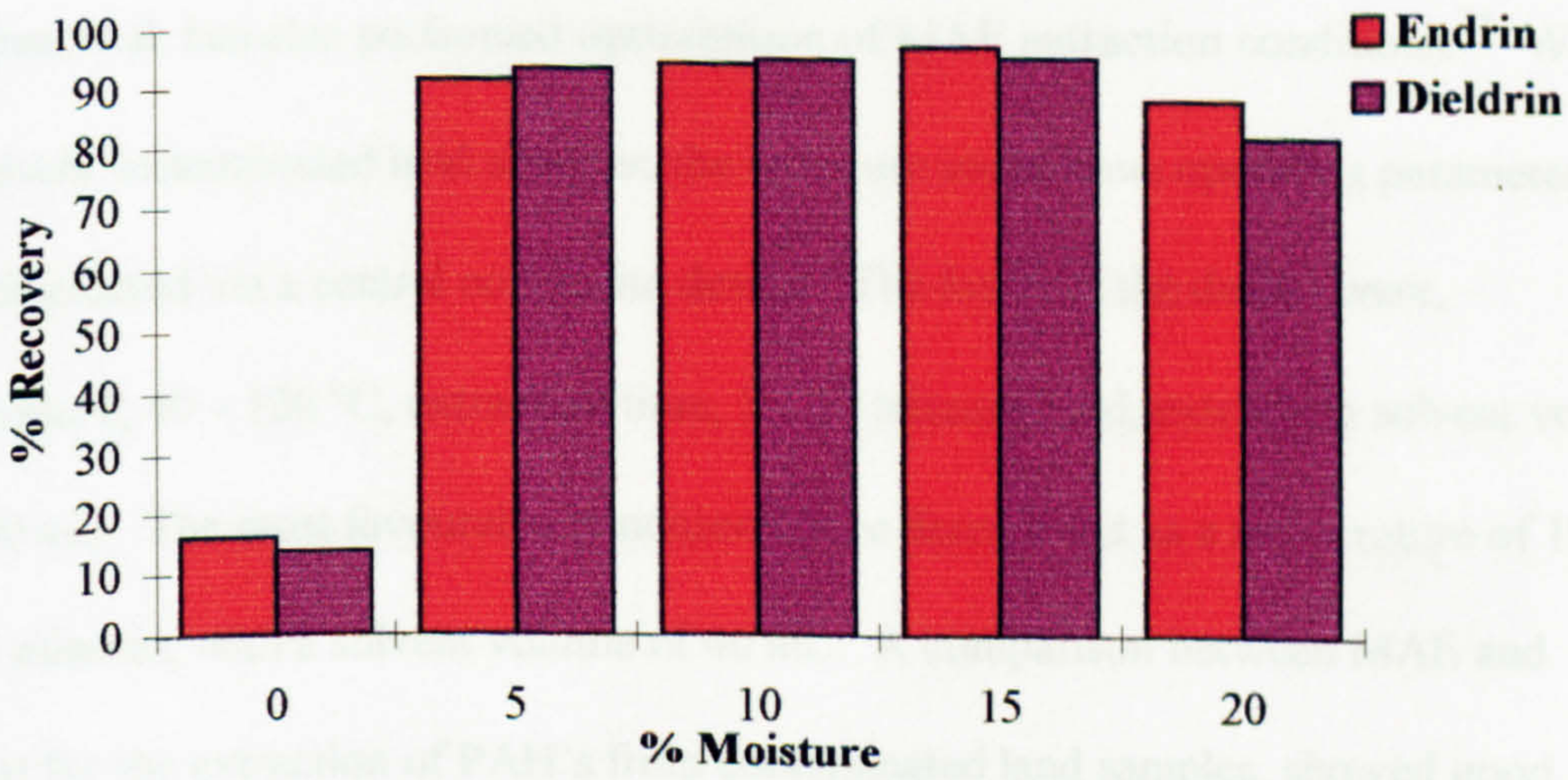
The influence of solvent on the microwave extraction of DDT and it's metabolites, DDD and DDE has been investigated by Pastor et al.[26] This group investigated three solvent systems, acetone:hexane (1:1 v/v), toluene and hexane. The latter two solvents had 10 % water added to allow heating effects to occur. Taking Soxhlet extraction recovery as 100 %, they found that all the solvents performed within the error of the experiment, with recoveries ranging from 97 - 103 %. An investigation into the effect of sample:solvent ratio is represented in figure 2.4. The results indicate that quantitative extraction requires at least a 1:3 sample:solvent volume ratio.

Figure 2.4 Effect of Sample:Solvent Volume Ratio on the Extraction of Pesticides from Soil



MAE has extracted organochlorine pesticides (OCP's) from soil. McMillan et al.,[27] have used MAE for the extraction of Arochlor residues from soil samples. They have compared this approach with Soxhlet extraction and sonication. Microwave extraction consistently extracted greater amount of Arochlor for all the 12 samples investigated. However, they expressed concern at the possible loss of solvent. They completed a study that showed the loss of solvent was less than 2 % of the whole and not deemed to be significant. The major problem encountered with the microwave approach was that additional sample cleanup was required compared to the other two techniques. However for this study, high dilution factors negated the necessity of extensive sample clean up. They also included published data that shows that the bias of microwave extraction is low. Studies by Lopez Avila et al.[28] and Onuska and Terry[29] have shown that extraction time and temperature had no effect on the extraction of 20 OCP's from certified marine sediments and soils. The moisture content of the sediment is crucial. Work by Onuska and Terry has shown that the presence of moisture is required for quantitative extraction of OCP's (figure 2.5) from sediment.

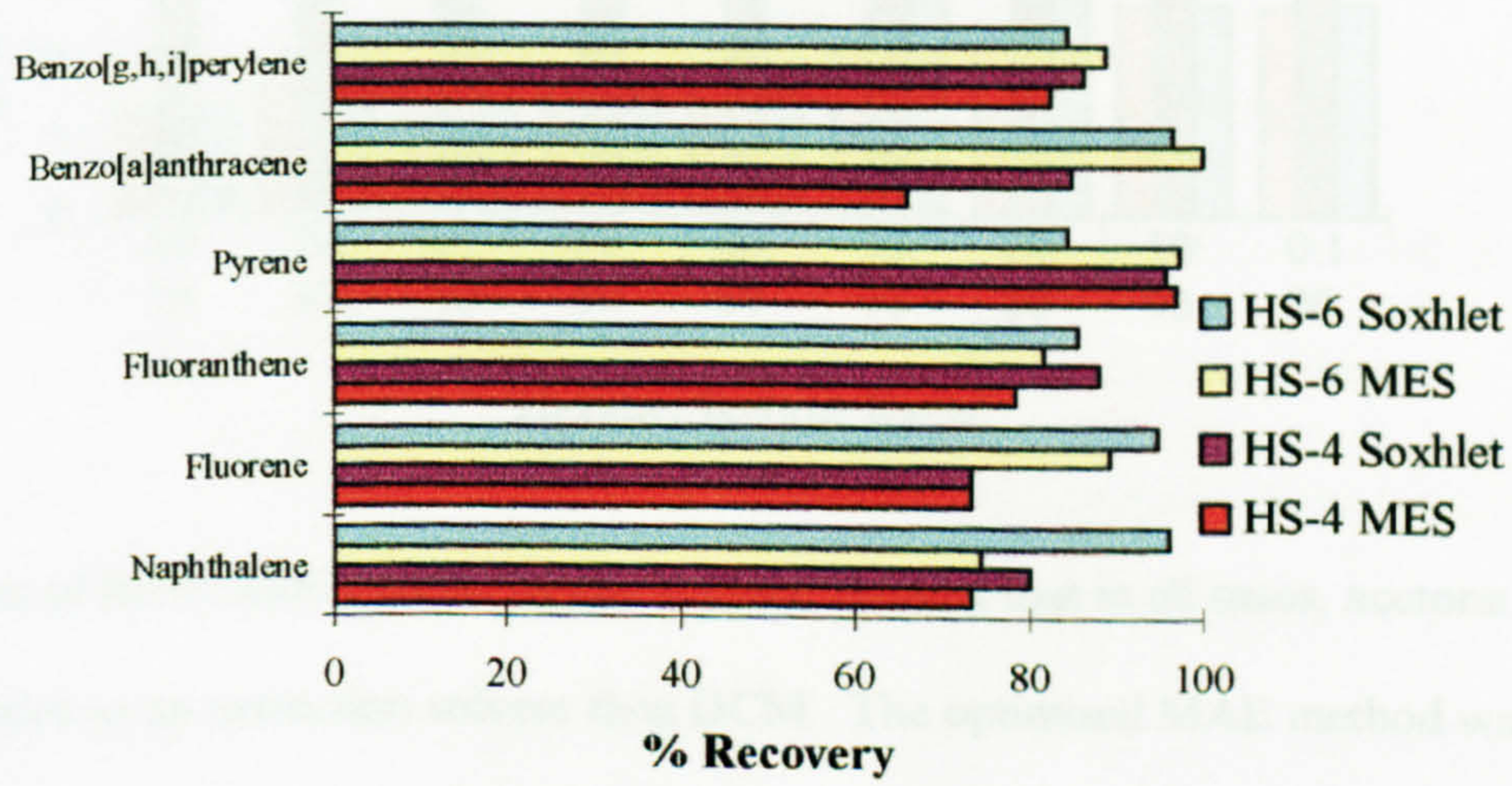
Figure 2.5 Influence of Moisture on OCP Extraction



Extraction of PAH's

MAE has been utilised for the extraction of PAH from two marine sediments by Chee et al.[30] Four solvent systems were assessed, along with various extraction temperatures, extraction times, and volumes of solvent, using a OA_{16} matrix. The optimum extraction conditions were determined as 30 mL acetone:hexane (1:1 v/v), at a temperature of 115 °C for 5 minutes. The results of the optimised microwave extraction were compared with Soxhlet. The results were comparable for both sediments (figure 2.6).

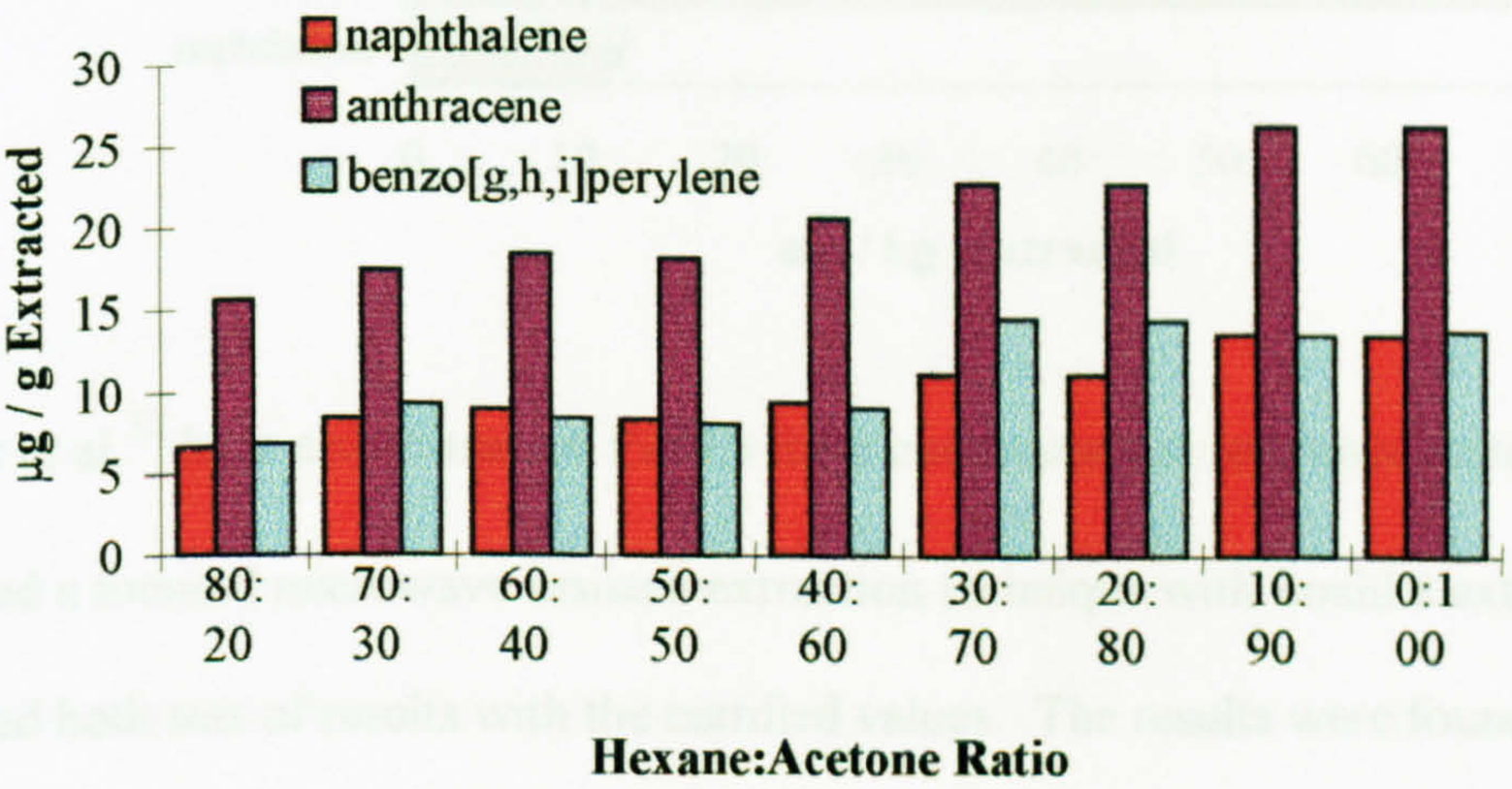
Figure 2.6 Comparison of MAE and Soxhlet of PAH Extraction



Barnabas et al. has also performed optimisation of MAE extraction conditions.[31] Working on natively contaminated land samples, the optimum microwave operating parameters were elucidated via a central composite design. The limits of the design were, temperature; 40 – 120 °C, extraction time; 5 – 20 minutes, and, extraction solvent volume; 30 – 50 mL. The most favourable conditions were determined as a temperature of 120 °C, for 20 minutes, with a solvent volume of 40 mL. A comparison between MAE and Soxhlet for the extraction of PAH's from contaminated land samples, showed good

agreement. The influence of extraction solvent was carried out using one of the soil samples. Various mixtures of acetone and hexane, ranging from 4:1 hexane:acetone (v/v) to 100 % acetone, were used to extract the PAH's. The extraction of PAH's increased as the proportion of acetone increased (figure 2.7).

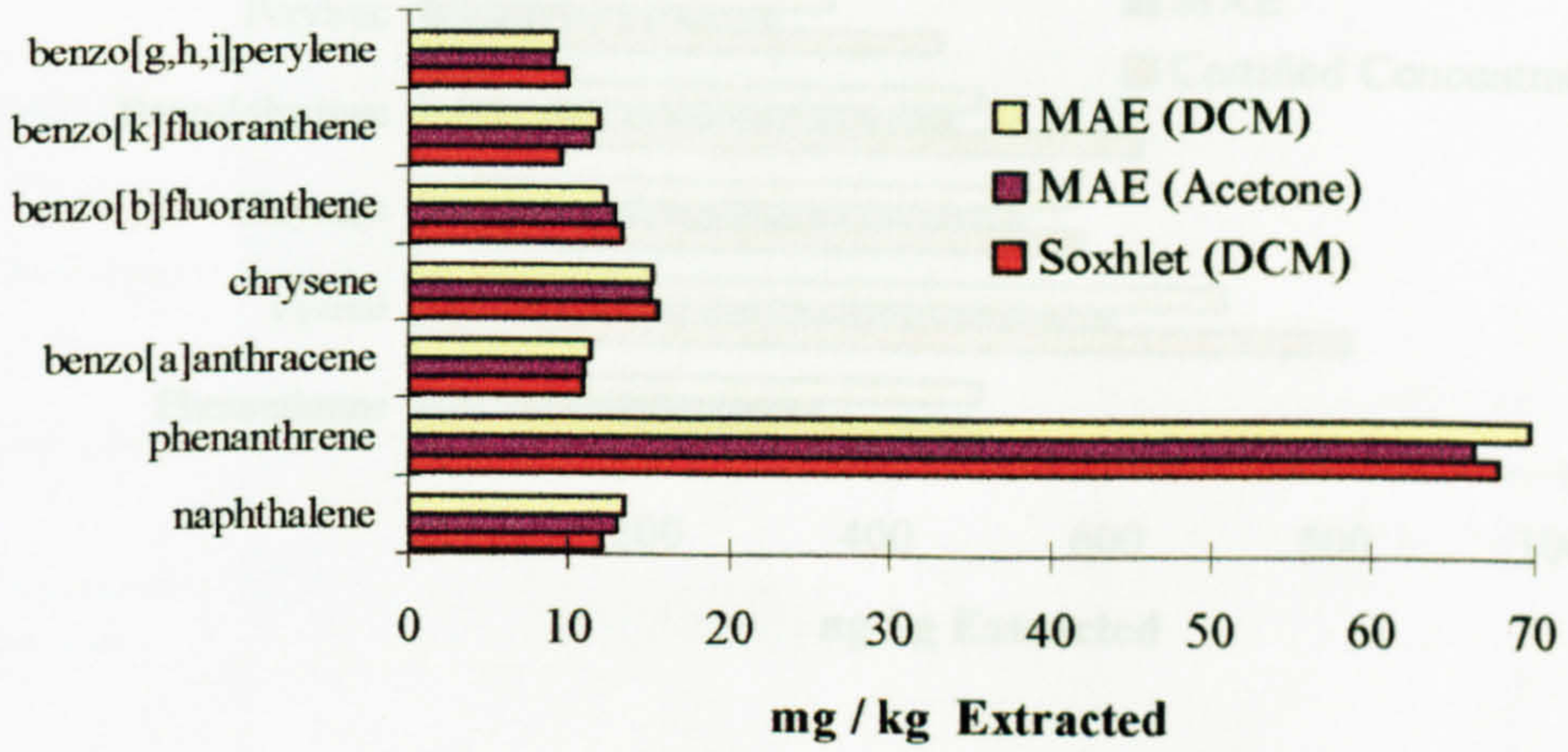
Figure 2.7 Effect of Increasing Acetone Proportion on selected PAH Extraction



Comparison of MAE with both DCM and acetone showed that in all cases, acetone was more effective as an extraction solvent than DCM. The optimised MAE method was applied to CONTEST soil, using both acetone and DCM. The results were compared with Soxhlet. Figure 2.8 shows that MAE is a viable alternative to high solvent consumption techniques, such as Soxhlet extraction.

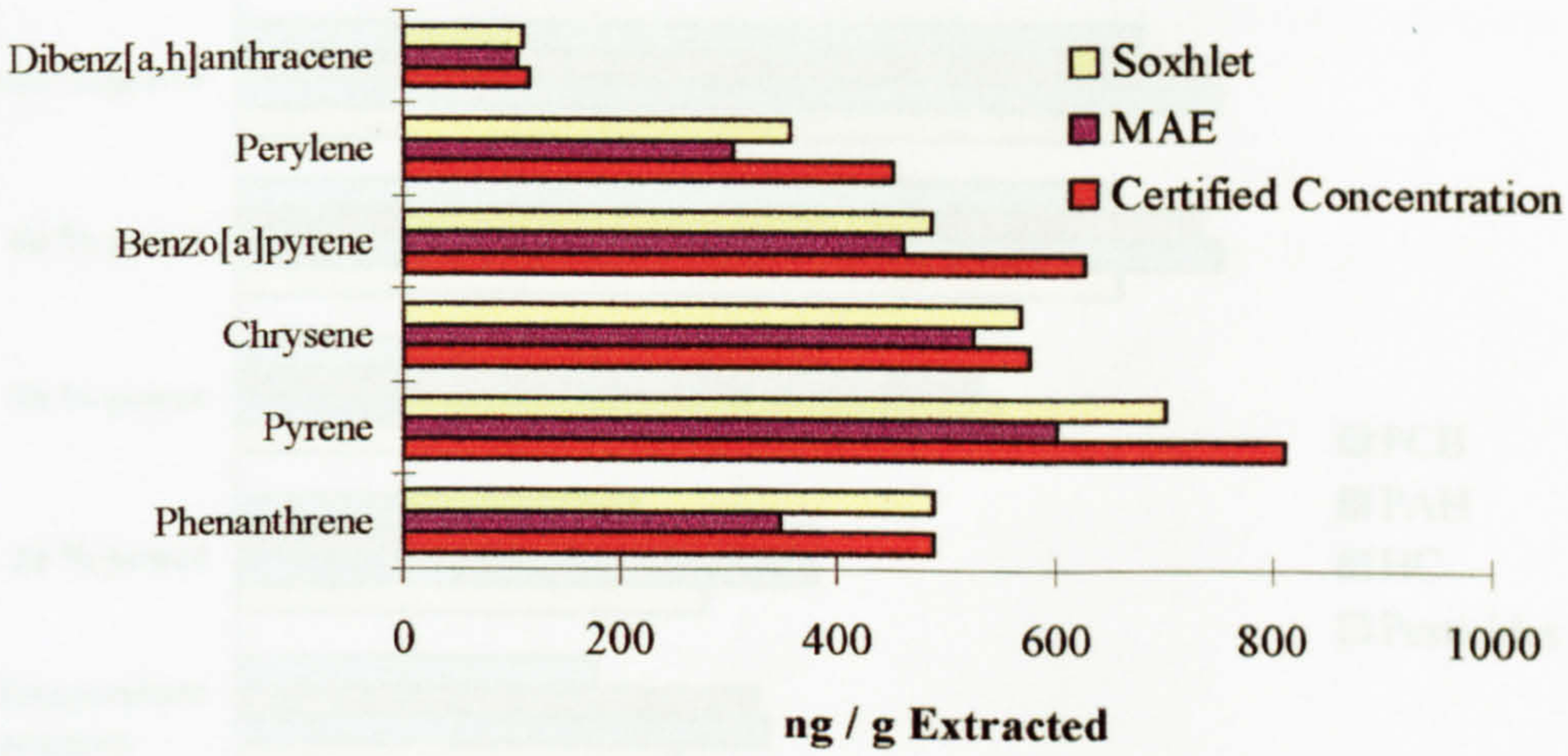
Pastor et al.[26] also investigated the influence of solvent on PAH's extraction. All three systems investigated (acetone:hexane 1:1 v/v, toluene + 10 % water and hexane + 10 % water), gave similar results as Soxhlet and slightly better than ultrasonic extraction with toluene.

Figure 2.8 Comparison of Extraction Techniques for PAH Extraction from CONTEST Soil.



Letellier et al.[32] have also extracted PAH's from environmental reference materials. They compared a focused microwave assisted extraction technique with Soxhlet extraction and compared both sets of results with the certified values. The results were found comparable, and an investigation into matrix dependency determined that no specific matrix optimisation was required. The ratio of MAE to Soxhlet extraction values for two of the marine sediment studies, showed recoveries of 86 – 109 %. Whereas the ratio of MAE to certified values for CRM 524 (soil), and SRM 1649a (Urban Dust), showed recoveries between 83 % and 109 %. Extraction efficiencies for the third marine sediment (SRM 1941a) are compared in figure 2.9.

Figure 2.9 Comparison of Extraction Techniques

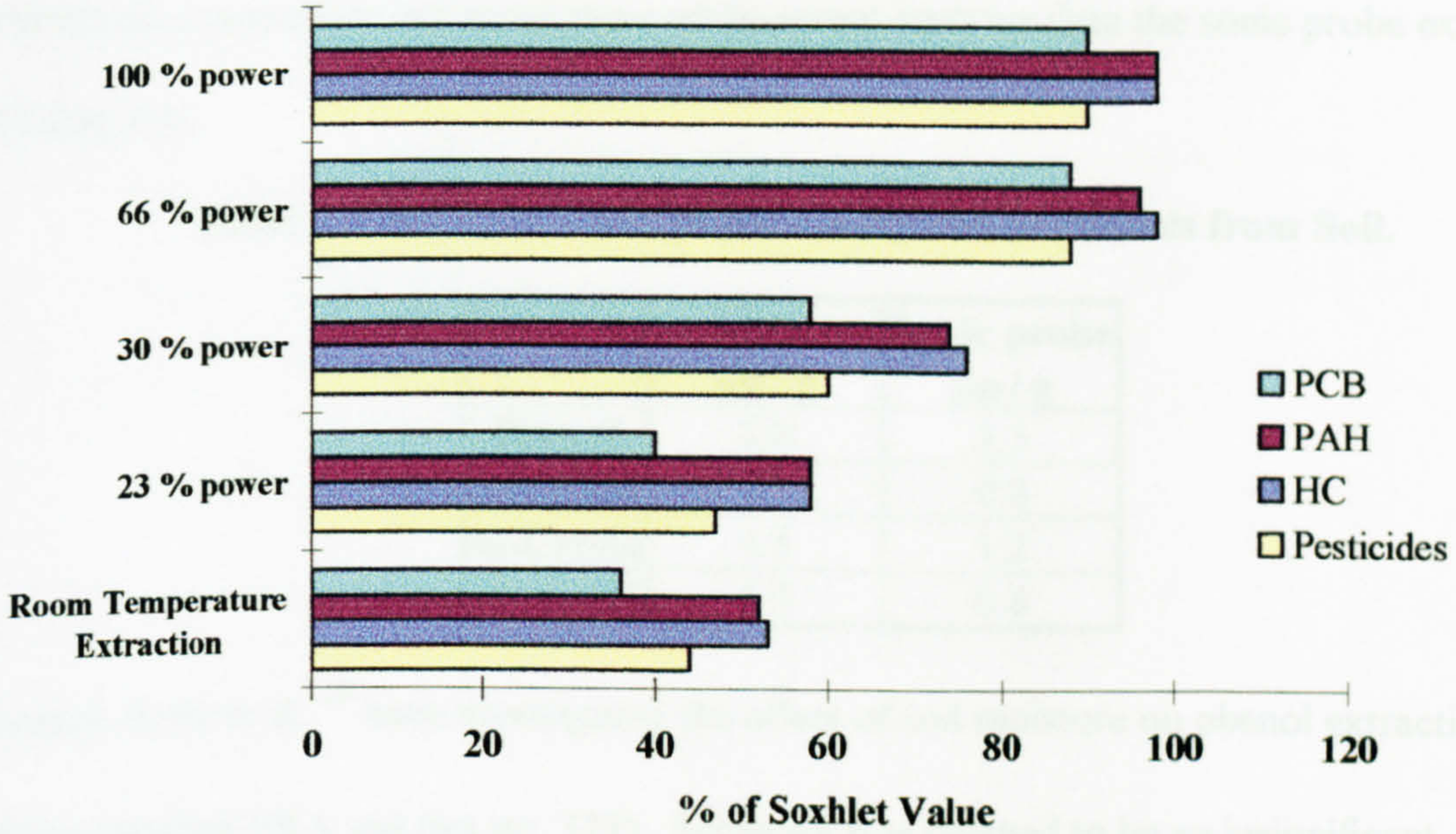


Extraction of PCB's

Lopez-Avila et al.,[33] have investigated the potential of MAE to replace high solvent consumption techniques by extracting PCB's from certified matrices (soil and marine sediments). Comparison of the certified results with MAE yielded a high correlation between the two sets of results, as did comparison of MAE with Soxhlet extraction.

Pastor et al.[26] have compared the room temperature extraction of PCB's with MAE at various power levels and extraction times. Even at only 23 % power for three minutes, MAE was capable of extracting a higher amount of PCB's from the marine sediment investigated than a room temperature extraction (figure 2.10). Comparison of Soxhlet extraction with MAE showed that MAE at 66 % power for 6 minutes gave similar results to those obtained by Soxhlet extraction.

Figure 2.10 MAE vs. Room Temperature Extraction



Extraction of Phenols

Determination of phenol and methyl phenol isomers from soils by a microwave-assisted process (MAP) has been performed by Llompart and co workers.[34] They optimised the extraction procedure using a CCD, and spiked samples of soil. The parameters they investigated were temperature, volume of solvent, and the quantity of acetic acid (derivatising agent) required for maximum extraction efficiency. The extraction temperature and the amount of acetic anhydride was deemed to be significant for all the analytes (phenol, *o*-, *m*-, and *p*-cresol) except *m*-cresol. The volume of extraction solvent (hexane) was also found significant for *o*-, and *m*-cresol. The only interaction term that was significant was the quadratic term for acetic anhydride. From these data, they determined that 10 mL solvent, with 800 μL of acetic anhydride at 130 °C for 5 minutes was the optimum. Using the optimised procedure and real coke plant soil samples, they

compared the *in situ* derivatisation procedure with sonic probe extraction. In all cases, the microwave procedure extracted more of the target analytes than the sonic probe extraction (Table 2.1).

Table 2.1 MAE vs. Sonic Probe Extraction of Phenols from Soil.

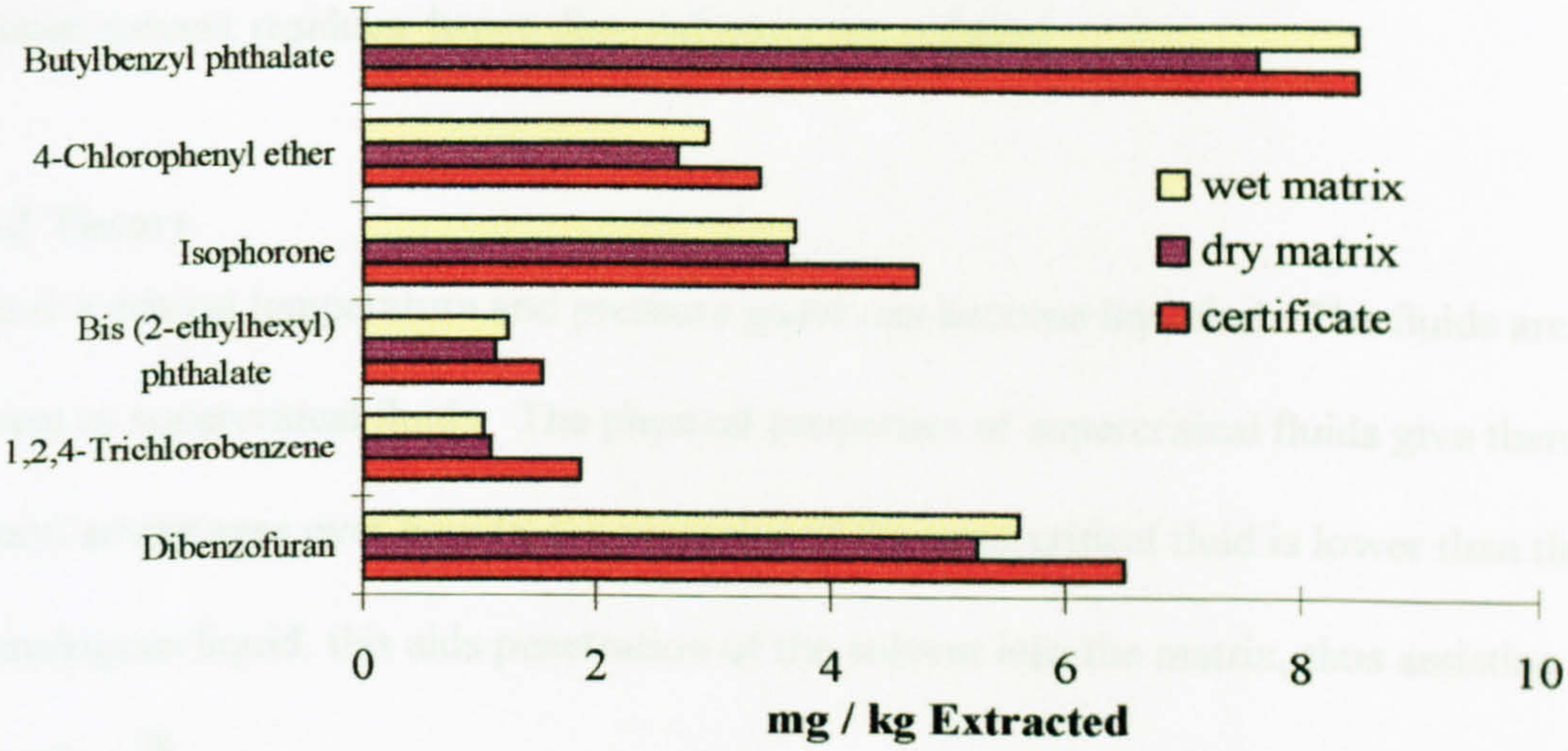
	MAE µg / g	**Sonic probe** µg / g
Phenol	7.0	3.5
***o*-Cresol**	1.5	0.8
***m*-Cresol**	4.5	1.2
***p*-Cresol**	1.5	0.8

Lopez Avila et al.,[28] have investigated the effect of soil moisture on phenol extraction from certified ERA soil (lot no. 323). Moisture was deemed to be an insignificant parameter in the extraction of four of the five compounds investigated. Pentachlorophenol extraction was significantly reduced in the absence of moisture, and 2-methylphenol was poorly recovered irrespective of soil moisture content.

Extraction of other compounds

Several basic and neutral compounds have been extracted from certified materials by Lopez Avila et al.[28] They investigated the effect of water on the extraction recovery and compared the results with those on the certificate. Moisture content was not deemed to have significant effect on the results (figure 2.11). Acceptable recoveries (> 78 %) vs. the certificate were obtained for all the compounds, except the three chlorobenzene compounds. Losses due to volatilisation could account for these poor recoveries, as further investigation showed no significant degradation in solvent, and only 30 % in a soil solvent suspension.

Figure 2.11 Effect of Moisture Content on Extraction



2.3 Supercritical Fluid Extraction (SFE)

2.3.1 Introduction.

In 1879, Hannay and Hogarth[35] noted the enhanced extraction abilities of supercritical fluids. Supercritical fluids were first used commercially in the1960's to produce decaffeinated coffee.[36] Since then, further developments have enabled the use of SFE on an analytical scale. Several solvents can be used in their supercritical state to extract matrices; these include dinitrogen oxide (N_2O), pentane, carbon dioxide, (CO_2) and ammonia (NH_3). However, all except CO_2 have safety problems, such as high reactivity and flammability.[37] The move to supercritical fluids as extraction solvents was prompted by environmental organisations, as typically, the extraction procedure uses environmentally innocuous compounds, such as water and carbon dioxide. Environmental incentives for using supercritical fluids include the fact that it is inert to most materials and

biologically non toxic, on the commercial side, it is also relatively inexpensive and gives minimal solvent residues, hence disposal costs are reduced.

2.3.2 Theory

Above a critical temperature and pressure gases can become liquefied. The fluids are known as supercritical fluids. The physical properties of supercritical fluids give them several advantages over liquids; the viscosity of the supercritical fluid is lower than that of an analogous liquid, this aids penetration of the solvent into the matrix, thus assisting extraction.[38]

2.3.3 Instrumentation

A typical SFE system consists of two pumps, one each for high purity organic modifier and high purity solvent. The extraction cell is enclosed in an oven, and a restrictor controls the carbon dioxide pressure. The extract is collected in a suitable vessel. The most common supercritical fluid used is CO_2. Carbon dioxide is a non-polar solvent, reducing the extraction efficiency of polar analytes. Organic modifiers are added to the carbon dioxide to increase the polarity of the extraction solvent. The organic modifier is usually methanol, although other solvent such as acetone can be used. Liquid carbon dioxide is pumped into the extraction cell where it is raised to its supercritical temperature and pressure (31.1 °C and 74.8 atm). The sample is mixed with a drying agent and placed in the stainless steel extraction cell. As the supercritical fluid passes through the sample, it dissolves the target analytes. The extract is pumped out of the cell and passes through a restrictor into a collection vessel. Whilst in the restrictor the fluid cools enough to returns

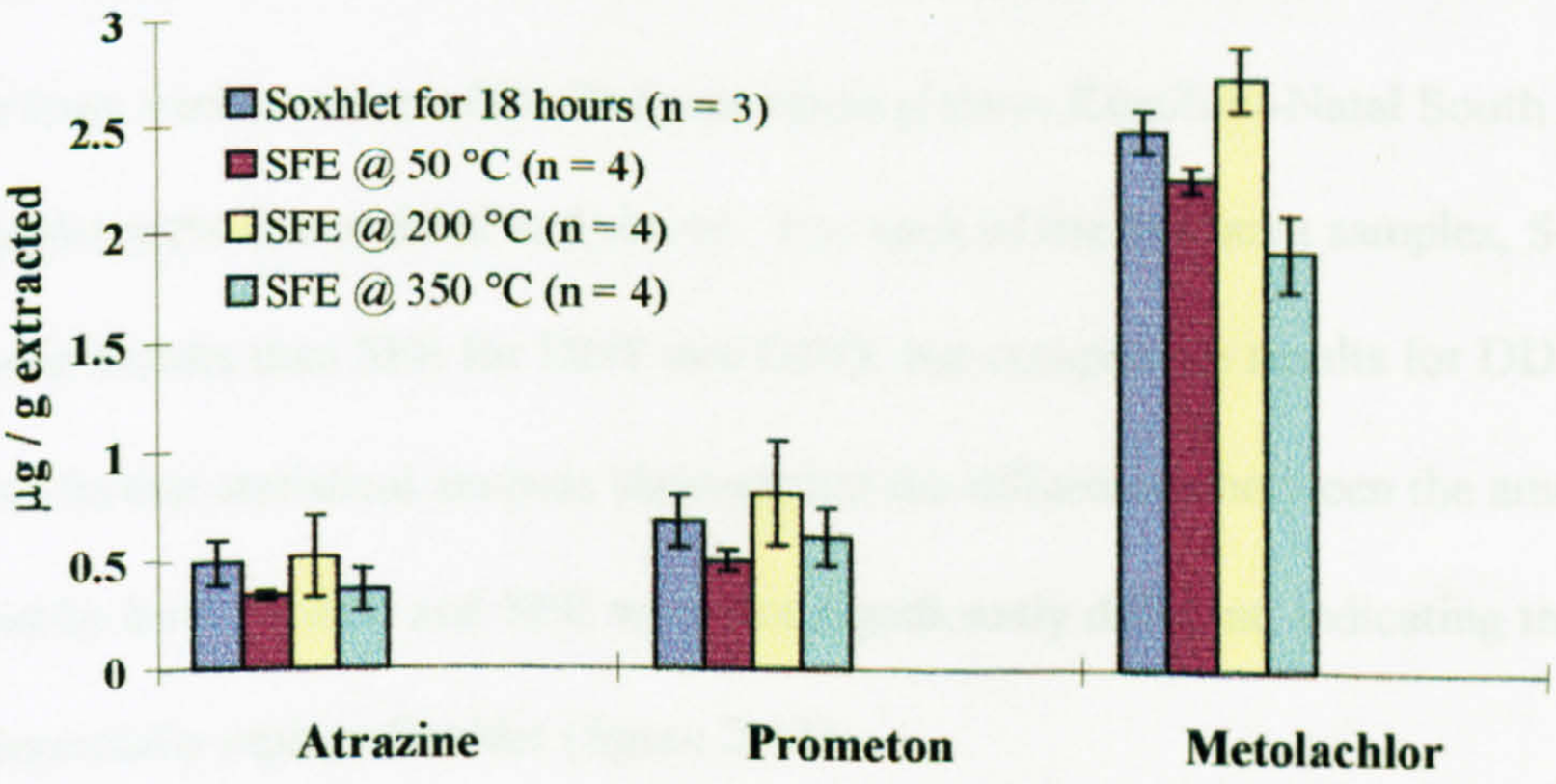
to it's gaseous state, and in order to quantitatively collect the analyte, a few mL of organic solvent (typically the organic modifier) is added to the collection vial. The restrictor maintains supercritical fluid conditions in the extraction cell, as well as allowing the extract to pass into the collection vial. The extract frequently requires further preparative treatment prior to analysis, such as filtration.[37, 25, 39]

2.3.4 Applications of SFE

Extraction of pesticides

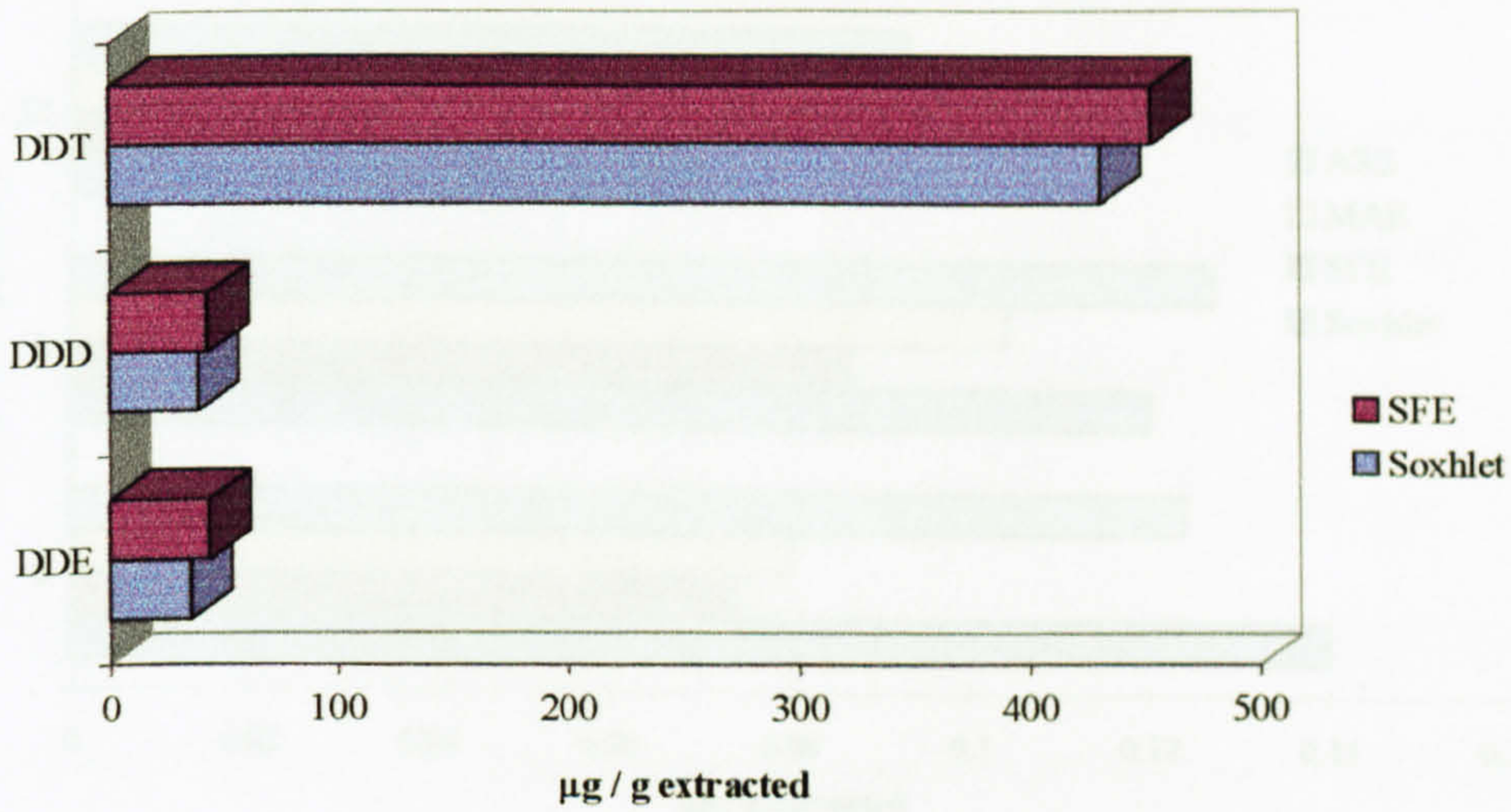
Hawthorne and Miller[40] (39a) have investigated the effect of temperature on the extraction of triazine herbicides and OPP's in real site contaminated soil samples with unmodified CO_2. The soil samples were from various sources; railroad bed soil, industrial site soil, agricultural soil and diesel. Higher recoveries from agricultural soil were obtained at 200 °C vs. 50 °C. A further increase in temperature to 350 °C reduced the recoveries significantly (figure 2.12). SFE at 200 °C gave comparable results to Soxhlet and the precision was similar.

Figure 2.12 Effect of Temperature on OPP Extraction.



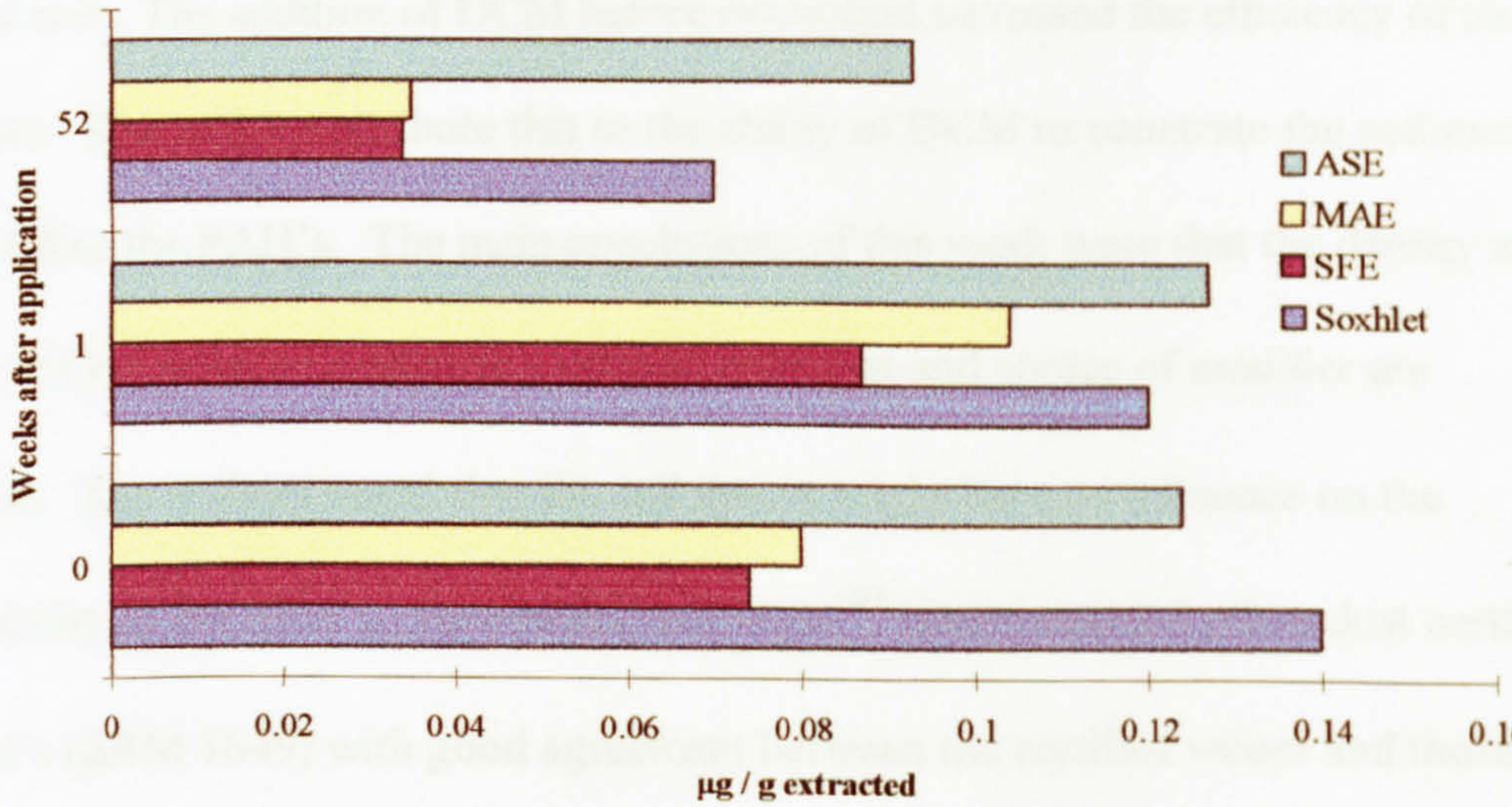
Although real contaminated soils have been investigated, examples pertaining to the extraction of pesticides from real soils and sediments are harder to find. Snyder[10] and Naude[41] have both extracted weathered residues from soils and sediments. The groups came to similar conclusions; that SFE could act as a potential replacement for older high solvent consumption techniques. Snyder et al.[10] extracted three native (real) soils. The first soil was dark topsoil and was contaminated with DDT and it's metabolites. There was no significant difference between the amount extracted by either sonication or SFE. The precision for both techniques was vastly different. The average % RSD for SFE was 6.9 %, compared to 13.5 % for sonication. The other two soils were both found to be contaminated with other organochlorine pesticides, including endrin and endrin ketone. One soil was a sandy loam and the other was a sandy soil. Again, there was very little difference for each pesticide extracted by both SFE and sonication. However for the sandy loam soil, sonication gave better precision than SFE for all the analytes with the exception of endosulfan II. The main conclusions of this work was that sonication vs. SFE for real samples did not yield significantly different results, and SFE gave the best overall precision for the 12 pesticides. Naude,[41] collected real world samples with a grab sampler from various areas of the Pongolo flood plain in KwaZulu-Natal South Africa. The samples were freeze dried and sieved. For each of the four area samples, Soxhlet gave lower results than SFE for DDT and DDD, but comparable results for DDE. However further statistical analysis showed that the differences between the amount extracted by both Soxhlet and SFE were not significantly different, indicating that SFE could potentially replace Soxhlet (figure 2.13).

Figure 2.13 Extraction of DDT and its metabolites by SFE and Soxhlet



Frost et al.,[11] has compared four extraction techniques, SFE, ASE, MAE and Soxhlet to remove weathered hexaconazole residues from two fully characterised soils. Extraction of the soil after 52 weeks application showed that the amount of extractable material had reduced significantly. A time study using SFE was performed but showed no difference between 20, 40 and 60 minutes' SFE extractions. There were differences between the two soil types; the soil with the lower organic matter gave comparable recoveries for all four techniques, whereas the results for the soil with the higher organic matter gave results that are more varied. For example, recoveries by MAE and SFE were half of the target Soxhlet value. ASE however gave comparable results to Soxhlet. Using this data, it was tentatively suggested that ASE is matrix independent to a certain extent (figure 2.14).

Figure 2.14 Comparison of techniques for the extraction of weathered Hexaconazole residues



Extraction of PAH's

Saim et al.[42] used CCD to determine the optimum extraction parameters of ASE of PAH's from natively contaminated soil. When total PAH concentration was considered, the CCD showed that none of the parameters investigated (temp, pressure and static extraction time were significant. Temperature was significant for three individual PAH's; naphthalene, benzo[b]fluoranthene and chrysene. An investigation into the extraction solvent was performed. The solvents were chosen based on their polarity, measured by dielectric constant. Only hexane seemed to have an effect on the recovery of the PAH's, extracting less than the other solvent systems that were investigated. The authors attributed this to its lower polarity.

The influence of extraction parameters has been applied to a standard reference material by Dankers and co workers.[43] SRM 1647b, containing the 16 priority PAH pollutants was extracted by SFE under optimised conditions; 270 atm, 70 °C, CO_2 density of 0.77 g / mL for 30 minutes. The influence of DCM as a static modifier was investigated. Soil

samples were spiked with DCM before extraction and the results were compared with unspiked soil. The addition of DCM before extraction increased the efficiency of the extraction. The authors attribute this to the ability of DCM to penetrate the soil matrix and solubilise the PAH's. The main conclusions of this work were that the density and volume of CO_2 used, as well as the collection solvent and choice of modifier are important. The authors noted that the soil matrix might have an influence on the extractability of the PAH's. Hawthorne and Miller[44] have extracted urban dust certified for PAH's (SRM 1649) with good agreement between the certified values and those obtained by SFE.

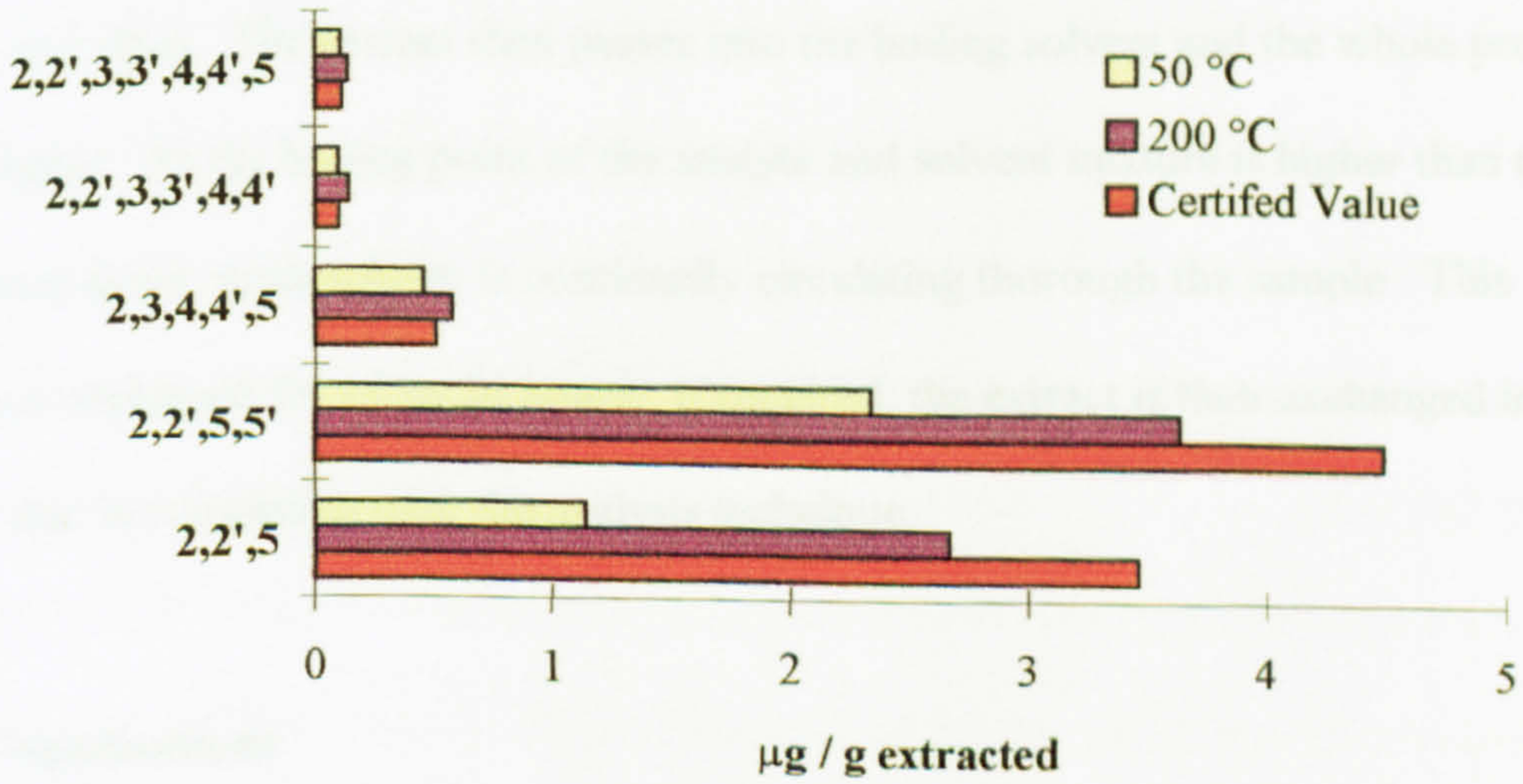
Mineral coal samples were extracted by SFE and compared with Soxhlet and Sonication by Vale and co workers.[45] They investigated the use of isopropanol as a supercritical fluid. Significantly better yields were achieved by SFE at 425 °C and 95 atmospheres in 90 minutes than either 48 hour Soxhlet extraction in DCM or 75 minute sonication extractions in DCM.

Extraction of PCB's

Onuska and Terry[29] have applied SFE to the extraction of PCB's from a certified sediment, EC-1. They compared the SFE results with both Soxhlet and sonication, as well as the certified values. They found that supercritical carbon dioxide with 2 % methanol as the organic modifier at a pressure of 20.7 MPa at 40 °C was comparable to both the Soxhlet and sonication values and was in excellent agreement with the certified values. They also performed leaching experiment to determine if matrix effects were present.

Spiked sediment was extracted at 10.35 MPa, at a temperature of 60 °C. All the PCB's were quantitatively extracted in eight minutes. This experiment was repeated using samples of the certified sediment. Exhaustive extraction of all the PCB's was achieved in 10 minutes. Langenfeld and co workers[46] have investigated the effect of pressure and temperature on the extraction of PCB's from a certified river sediment (SRM 1939) using pure supercritical CO_2. Extraction pressure had no significant effect on PCB recovery, however an increase in extraction temperature increased the recovery of the PCB congeners investigated (figure 2.15).

Figure 2.15 Effect of temperature on PCB recovery at 350 atm



2.4 Other solid/liquid techniques.

Commonly, other liquid/solid techniques are used in comparison with newer instrumental techniques. As a result, literature dealing just with the following techniques is limited.

2.4.1 Soxhlet extraction

2.4.1.1 Instrumentation

Soxhlet extraction is used as the benchmark for any new extraction techniques. Studies use Soxhlet extraction for comparison to new and updated techniques. Basic Soxhlet extraction apparatus consists of a solvent reservoir, Soxhlet extraction body, a heat source (e.g. isomantle) and water cooled reflux condenser. Soxhlet uses a range of organic solvents to remove organic compounds, primarily from solids. The sample is combined with a drying agent, e.g., anhydrous sodium sulphate. The mixture is then placed in a porous extraction thimble, and extracted under reflux conditions. During the extraction process, the solvent is boiled and the vapour passes though the water cooled apparatus and is condensed. The liquid solvent then passes through the sample, removing the analyte as it does. The extract then passes into the boiling solvent and the whole process occurs again. As the boiling point of the analyte and solvent mixture is higher than that of the solvent alone, fresh solvent is continually circulating thorough the sample. This process is continued for 16 to 24 hours. If required, the extract is then exchanged into a solvent that is compatible with the analysis technique.

2.4.1.2 Applications

Studies have been performed comparing Soxhlet extraction with several other extraction techniques, e.g. SFE, MAE for the extraction of pesticides from various spiked and real samples.

Tavares and co workers used Soxhlet extraction to determine the level of DDT and its metabolites DDD and DDE in bay sediment. Aliquots of the sediment were extracted with 180 mL of a mixture of dichloromethane:methanol (2:1 v/v) containing activated copper

wire. The results showed that due to the ratio between DDT and DDE, the pesticide could have been used during the last five years.[47]

2.4.2 Sonication

2.4.2.1 Instrumentation

Sonic extractions can be achieved by the placement of the sample in a solvent in a sonic bath, or via the insertion of a sonic probe into the sample solvent system.

2.4.2.2 Applications

This technique has been taken over by newer automated techniques, but has been used in the past to compare various solvent systems for the extraction of four herbicides from aged, spiked soil samples.[48] The technique was compared with another superseded techniques, shake flask. The soils investigated were of various compositions. Soils were sampled and extracted after both 6 months of weathering and 17 months of weathering. Not surprisingly, in all cases the amount of each herbicide that was extracted decreased the longer the soil was weathered. After six months weathering all the herbicides (no data for triallate) were quantitatively extracted from all the matrices. After 17 months of weathering comparison of sonication and shake flask for the extraction of both nitrofen and profluralin was performed, using ACN:water as the solvent. The results were not significantly different between the two extraction techniques. An average recovery of 87 % by both shake flask and sonication for the extraction of profluralin; and 81 % by sonication, compared with 87 % recovery by shake flask (recovered on two soils only), for nitrofen. After 17 months weathering all the herbicides (no data for benzoylprop-ethyl) were extracted from all the matrices with at least 85 % recovery.

2.4.3 Shake flask

2.4.3.1 Instrumentation

Solid samples, e.g. soil, sediment etc. are placed in a stoppered flask along with the extraction solvent. The entire system is then shaken using a mechanical shaker, for a set period of time, usually for around an hour. Repeat extractions can be performed to quantitatively remove the analyte from the sample and then the extracts are combined before analysis. As the sample is in contact with the solvent, sample cleanup via SPE or similar is normally required prior to analysis.

Although this technique is now rarely used as a definitive method, variations have been used extensively in the past and also when assessing the efficiency of new techniques such as SFE and ASE.

2.4.3.2 Applications

Cotterill[49] has published work that uses the technique to assess the efficiency of solvent systems for the extraction of weathered herbicide residues from soil. Samples of soil of different compositions spiked with herbicides from a range of families. The herbicides included linuron, simazine and propyzamide. The soils were extracted three months after herbicide application. Two different techniques, Soxhlet extraction and shake flask evaluated several solvent systems. Methanol:water (4:1, v/v) consistently gave higher recoveries of selected herbicides from all the soil types using shake flask extraction. A comparison of acetonitrile:water (9:1, v/v) as the extraction solvent for both Soxhlet extraction and shake flask showed that for soil with a high sand content and low organic carbon, there was very little difference between the recovery of the herbicides. Aqueous

methanol extraction of herbicides from a soil with high sand content and pH 7.0 showed that recoveries of greater than 73 % were possible for the pesticides.

2.5 Other Techniques

Matrix solid phase dispersion is a new technique currently being developed for the extraction of organic analytes from environmentally relevant samples, such as soil, as well as biological matrices such as fish tissue. Other techniques that are not mentioned here include the use of SPME for extracting volatile and semi-volatile compounds, such as herbicides from sludges and soils.[50 - 53]

An increase in automation will dramatically change the evolution of extraction techniques. Procedures that reduce solvent consumption and decrease sample preparation time are already widely used. These systems, for example, accelerated solvent extraction and microwave assisted extraction, will rapidly replace the use of other techniques such as Soxhlet and Sonication.

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Chapter 3

Pressurised Fluid Extraction in Environmental Analysis

Pressurised Fluid Extraction in Environmental Analysis

3.0 Introduction

PFE (pressurised fluid extraction),[1] is also known as ASE™ (accelerated solvent extraction)and PLE (pressurised liquid extraction).[2] All three names relate to the process of extraction that utilises common organic solvents under high pressure and high temperature. This approach to the extraction of analytes is not novel. SFE (supercritical fluid extraction) and MAE (microwave assisted extraction) have also been used to enhance extraction efficiency. In SFE, the organic solvent is replaced with carbon dioxide above its critical temperature and pressure (31.1 °C and 74.8 atm). Carbon dioxide in this state has been shown to have superior extraction properties.[3 - 5] Users of both MAE and SFE have a similar problem to overcome. The supercritical carbon dioxide used in SFE is a non-polar molecule; hence, the extraction efficiency of polar molecules is minimal. In MAE, the organic solvent used for the extraction must have a degree of polarity, as only polar molecules absorb microwave energy and contribute to the heating process, which aids extraction. Users of SFE tend to overcome this drawback by the addition of a small amount of polar organic solvent, usually methanol, often referred to as the organic modifier.[6 - 8] Microwave assisted extraction can also use high temperature and pressure to aid analyte extraction.[9] In MAE the heating limitation can be overcome by the addition of a polar solvent to a non-polar one, for example the addition of acetone to hexane.[9, 10] The sample is also in direct contact with the solvent, hence extensive sample clean up is usually required, leading to the loss of the analyte.

In PFE, organic solvent, whether individually, or as a mixture of two or more solvents is used for extraction.[11 - 14] The US EPA has used PFE, since the rate of solvent consumption is low compared to other techniques, e.g. Soxhlet extraction. This requires an assessment of PFE versus

other techniques. As Soxhlet extraction is often quoted as the 'benchmark' for other techniques, the EPA has compared PFE with Soxhlet for a variety of compounds from different solid matrices. The results showed the equivalency of PFE to Soxhlet. Further studies using shake flask and sonication, for the extraction of the same analytes and matrices, showed similar trends.[11]

3.1 Theory of PFE extraction.

Richter et al.[15] and David and Sieber[16] have postulated that the enhanced extraction efficiency seen when using PFE is due to two main effects, disruption of surface equilibrium and solubility and mass transfer effects.

3.1.1 Disruption of Surface Equilibrium.

As temperature increases, the physical properties of the solvent change. For example viscosity decreases figure 3.1 and the surface tension figure 3.2 of the solvent is also reduced.

Figure 3.1 Effect of Temperature on the Viscosity of Toluene

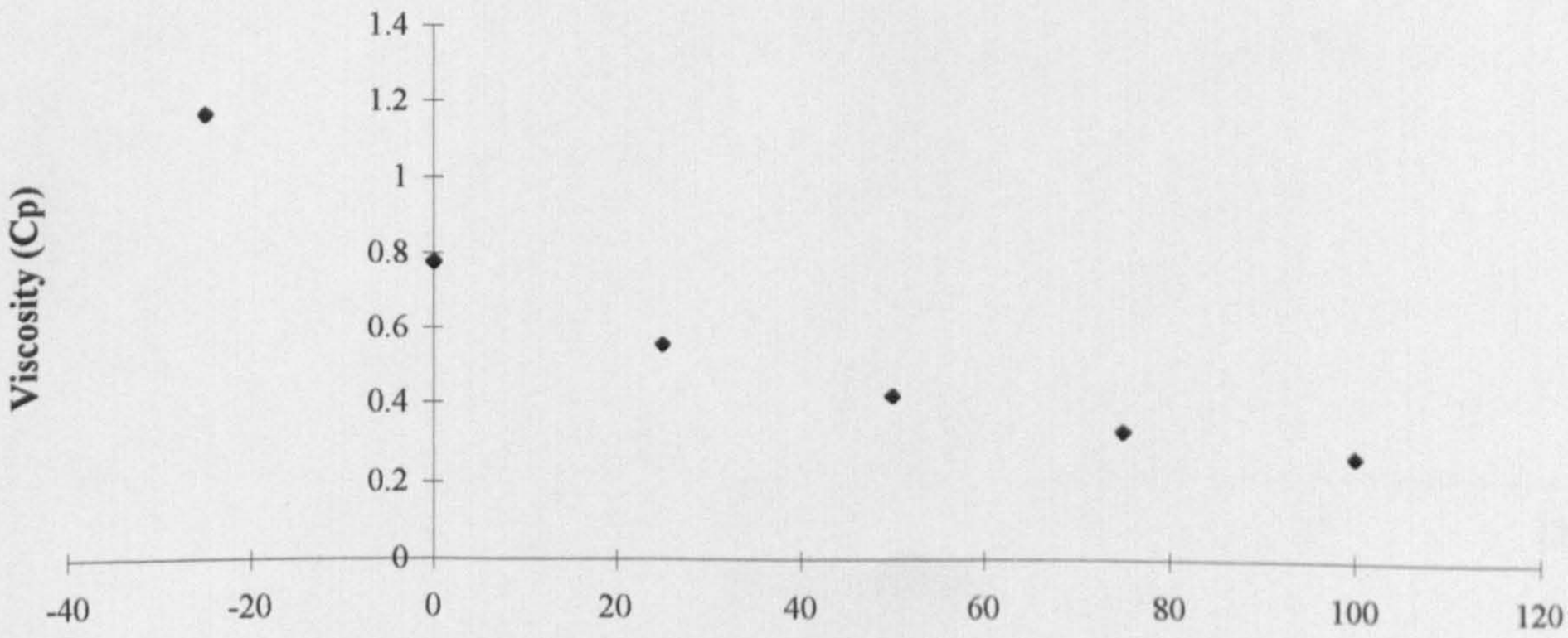
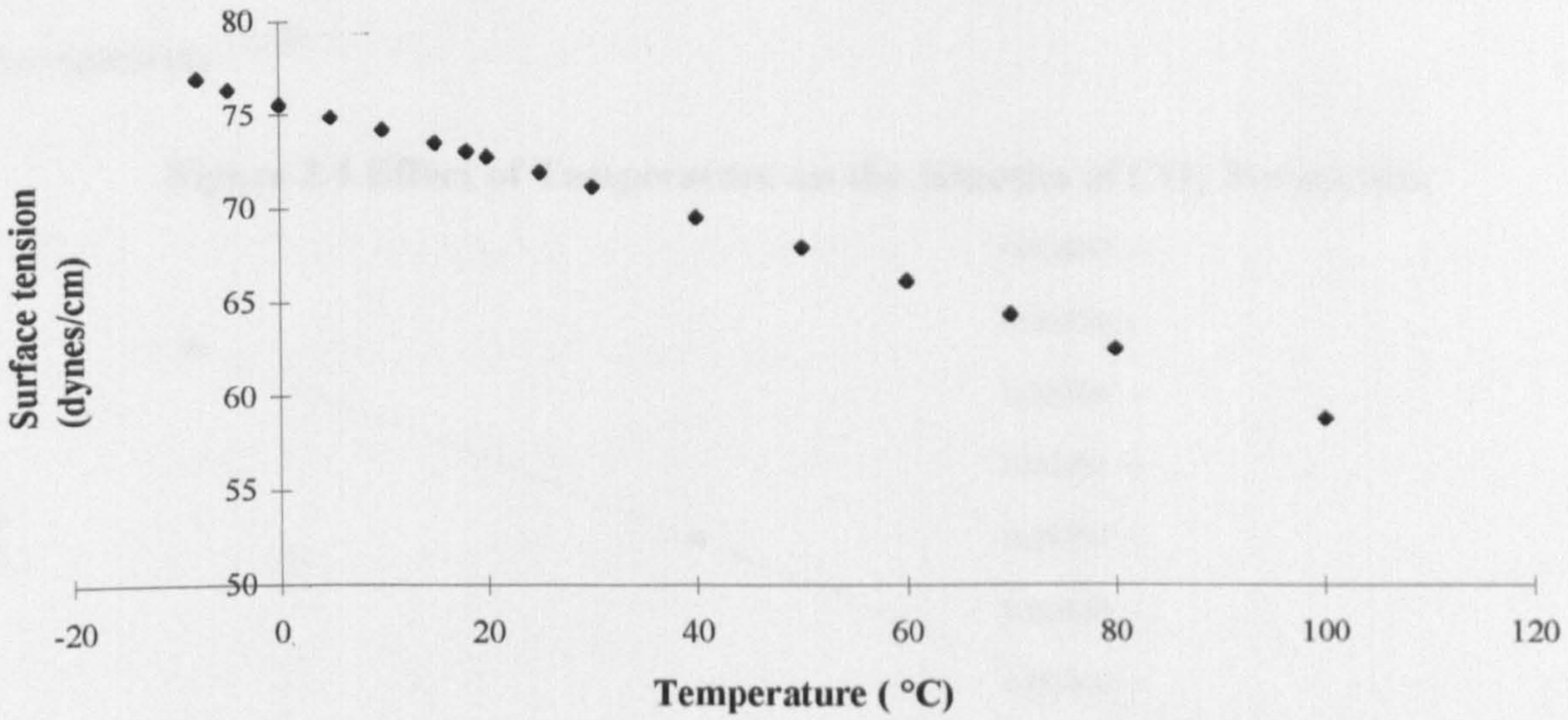
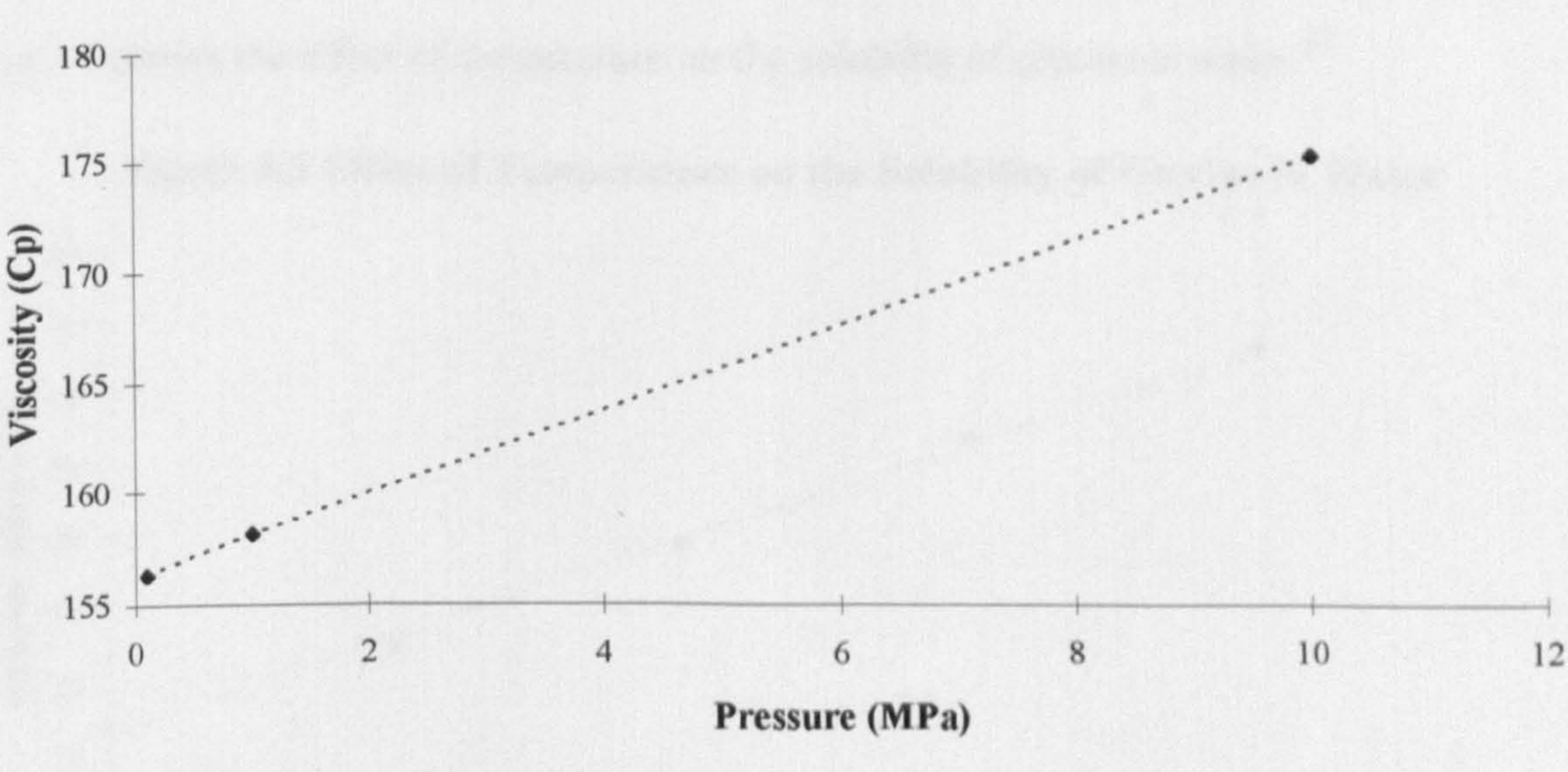


Figure 3.2 Effect of Temperature on the Surface Tension of Water



Pressure also has an effect on the viscosity of a solvent. This most easily measured by use of a gas. The relationship between the viscosity of a gas and pressure is mirrored in a liquid, but the effect of pressure on a gas is easier to measure (figure 3.3).[17]

Figure 3.3 Effect of Pressure on the Viscosity of Methane Gas at 100 °C[18]

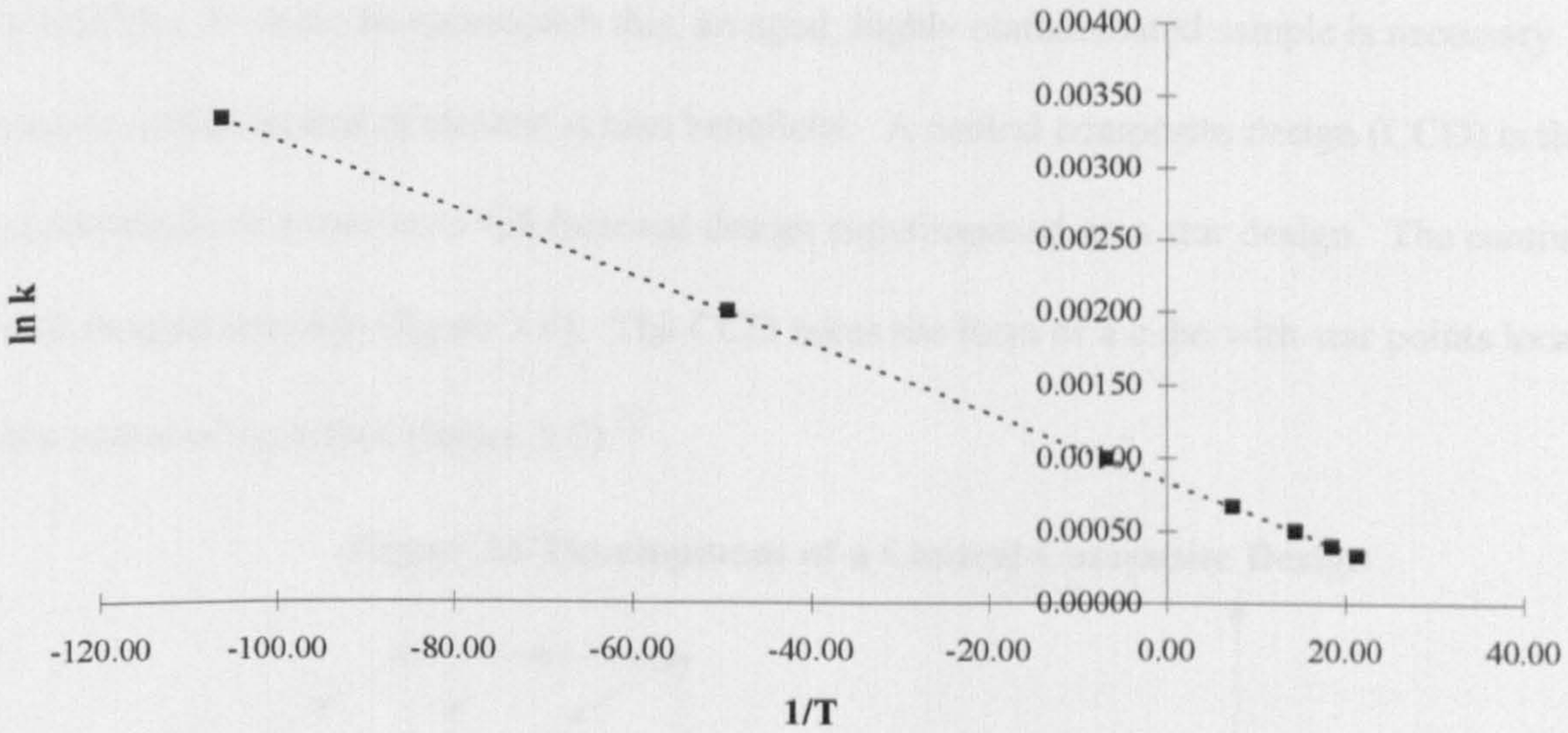


3.1.2 Mass Transfer and Solubility Effects.

Kinetic theory predicts that for every 10 °C rise in temperature the kinetics of a reaction increase. Figure 3.4 shows the effect of temperature on the kinetics of a gas phase reaction. A similar

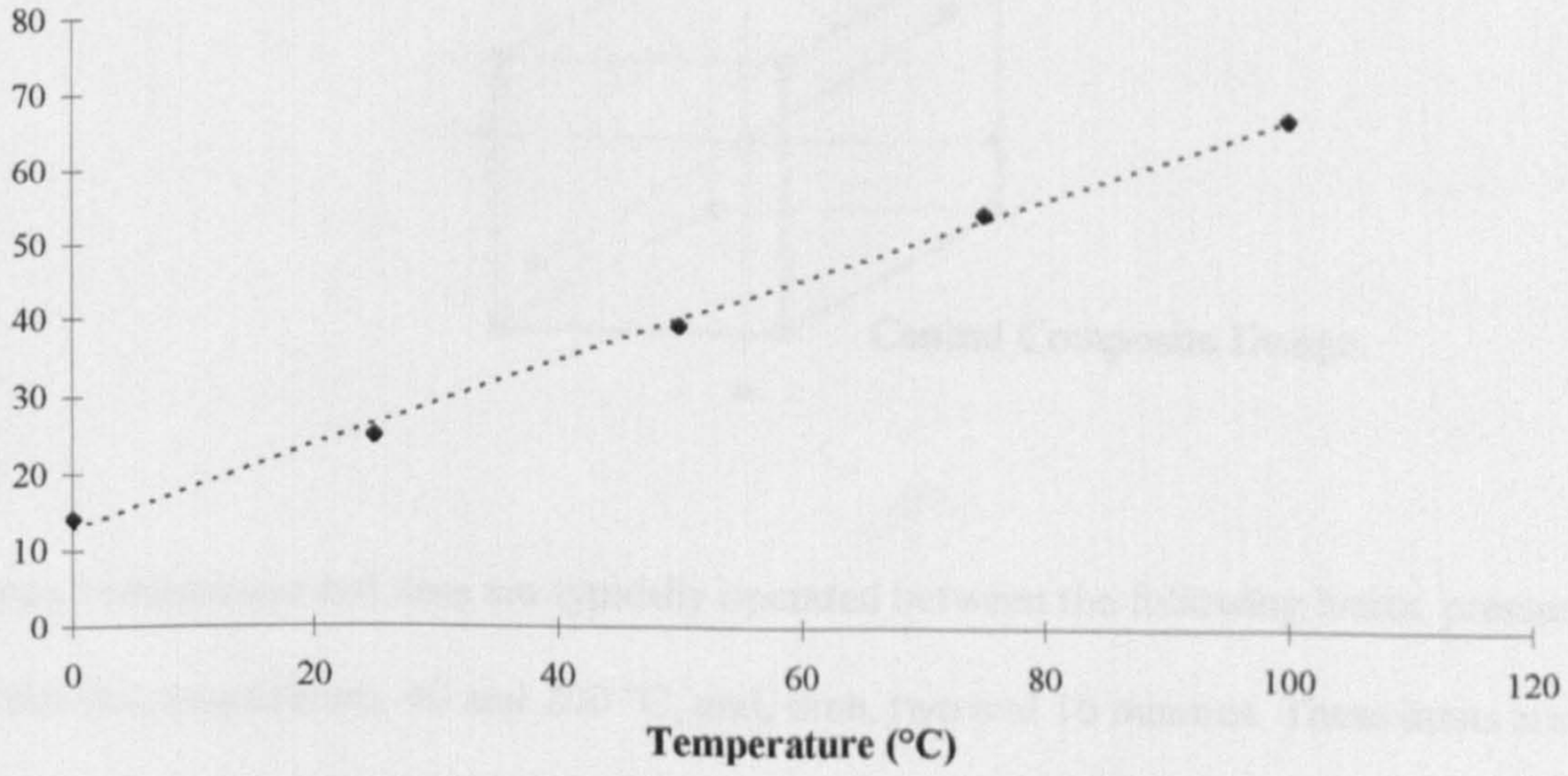
phenomenon occurs for liquid phase reactions. Pressure has minimal effect on the rate of liquid phase reactions.[19, 20]

Figure 3.4 Effect of Temperature on the Kinetics of CO_2 Formation.



Changes also occur with respect to the analyte. It becomes more soluble in the solvent as the temperature increases. Thus for quantitative extraction, there is a reduction in solvent volume. Figure 3.5 shows the effect of temperature on the solubility of glycine in water.[21]

Figure 3.5 Effect of Temperature on the Solubility of Glycine in Water

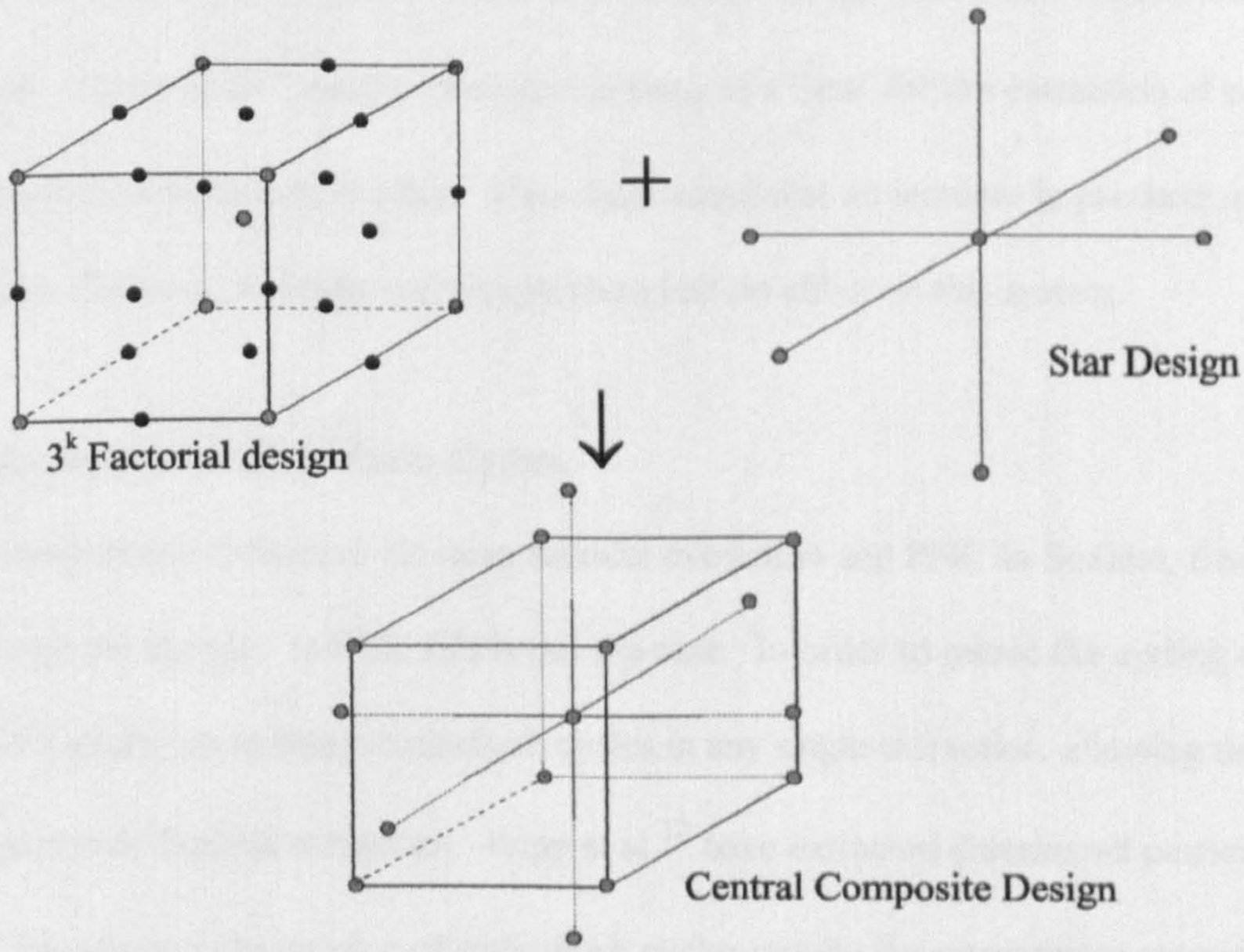


3.2 Method Development

3.2.1 Optimisation of PFE extraction conditions.

Central composite design (CCD) is an efficient method of optimising the PFE extraction conditions. In order to accomplish this, an aged, highly contaminated sample is necessary. A matrix similar to that of interest is also beneficial. A central composite design (CCD) is the combination of a two-level full factorial design superimposed on a star design. The centres of the two designs coincide (figure 3.6). The CCD takes the form of a cube with star points located in the centre of each face (figure 3.6).[22]

Figure 3.6 Development of a Central Composite Design



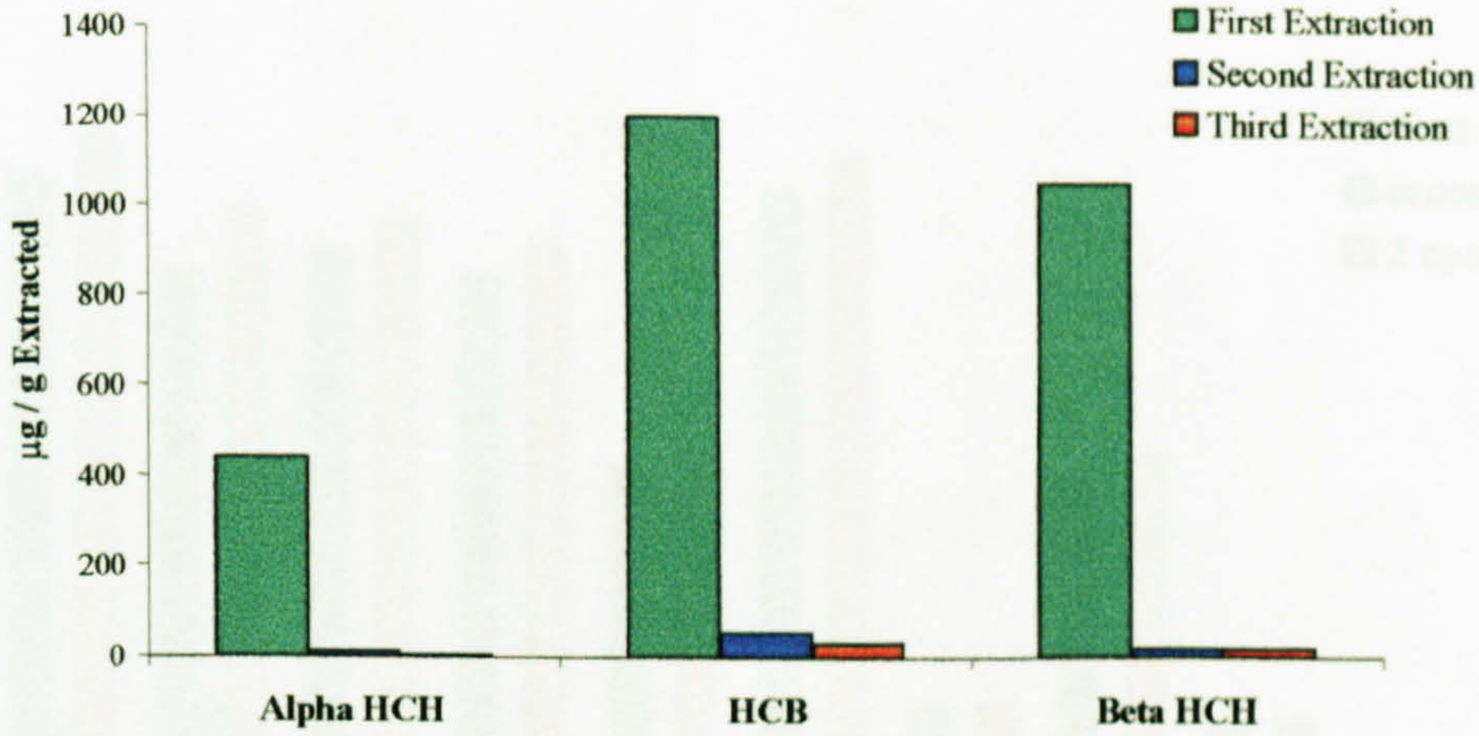
Pressure, temperature and time are typically operated between the following limits: pressure, 1000 and 2400 psi, temperature, 40 and 200 °C, and, time, two and 16 minutes. These limits are based on instrumental constraints and allow the extractions to occur safely. Sixteen experiments are

performed, incorporating replicate experiments to evaluate the reproducibility. Multilinear regression shows the relationship between response and the parameters. The results of the regression analysis are used to plot a response surface. Saim et al.[23] used this approach to determine the optimum extraction conditions of polycyclic aromatic hydrocarbons. They found that none of the parameters investigated had any significant effect on the recovery. A different approach used by Schantz et al.,[20] involved keeping the oven temperature constant (100 °C) and varying the pressure between 1000 and 2200 psi. To optimise the extraction temperature, constant pressure was established, whilst the temperature was varied between 50 and 150 °C. The optimum conditions for PAH's, PCB's and chlorinated pesticides were established as 100 °C and 2000 psi. Obana et al.[13] used a 'change one thing at a time' for the extraction of acephate and methamidophos from orange juice. They determined that an increase in pressure improved the extraction efficiency, but time and temperature had no effect on this system.

3.2.2 Optimisation of Static / Flush Cycles.

There is a fundamental difference between Soxhlet extraction and PFE. In Soxhlet, fresh solvent is cycled through the sample. In PFE, this is not the case. In order to mimic the cycling of solvent, the PFE can perform up to three static-flush cycles in any single extraction, allowing the PFE to mimic the action of Soxhlet extraction. Popp et al.[12] have extracted chlorinated pesticides from soil. They investigated the number of static flush cycles require for quantitative extraction (figure 3.7).

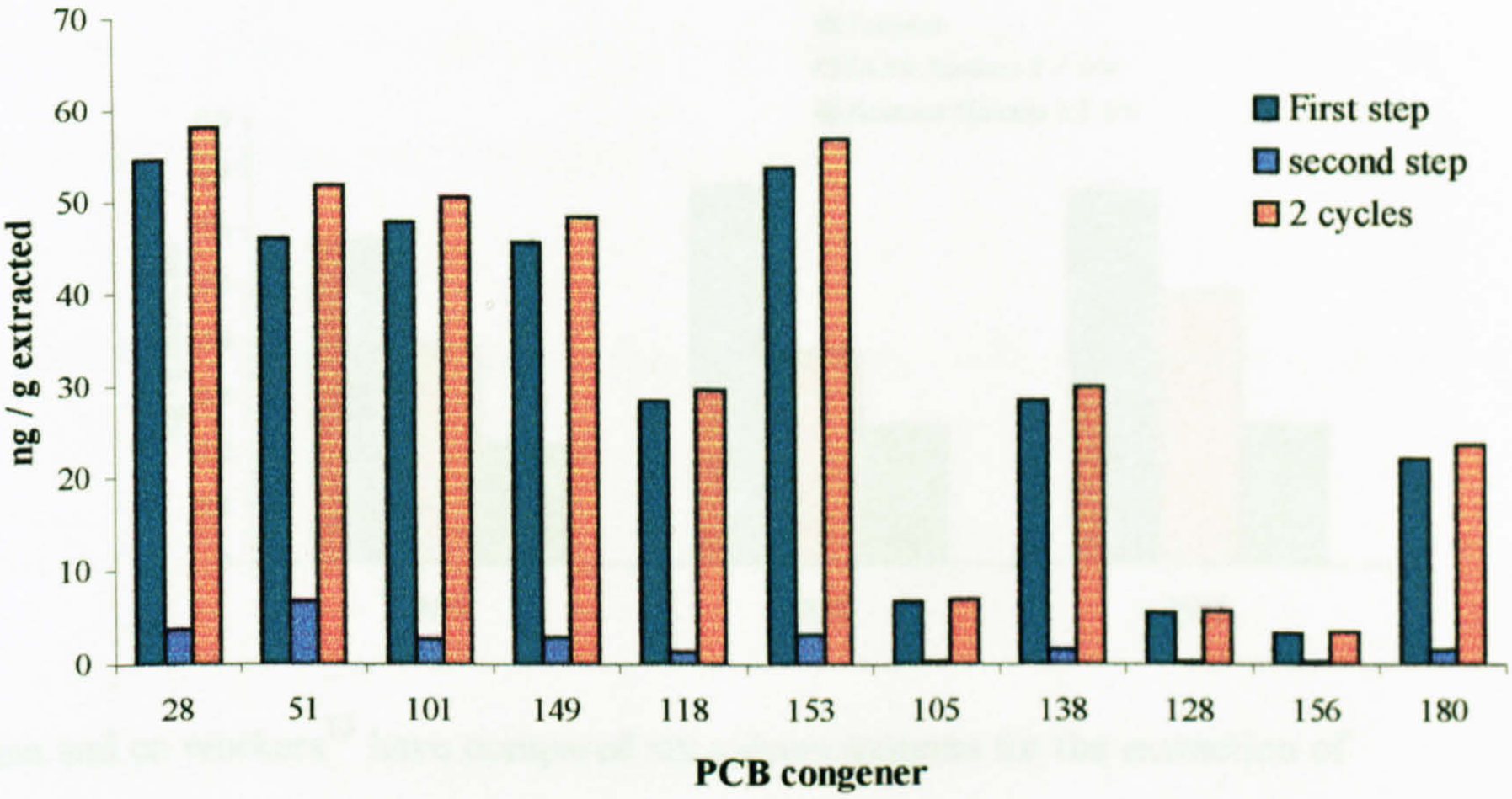
Figure 3.7 PFE Cycle Study



All ensuing extraction work required two cycles.

The EPA method suggests one 5 minute static extraction cycle.[11] Popp et al.[12] compared the EPA method with a static extraction time of 10 and 15 minutes, as well as two static steps of 5minutes. They concluded that there was no significant difference between the 10 and 15 minute extraction steps, but two successive 5 minute cycles extracted more than either of the longer cycles. Bjorklund et al.[24] have seen that one static step is not always able to quantitatively extract the analyte from a matrix. In this study, a harbour sediment (CRM 536) contaminated with PCB's was extracted by PFE. Initial investigation showed that analytes were present in the second extract. Two cycles were superior to two separate extractions. Figure 3.8 summarises the data.

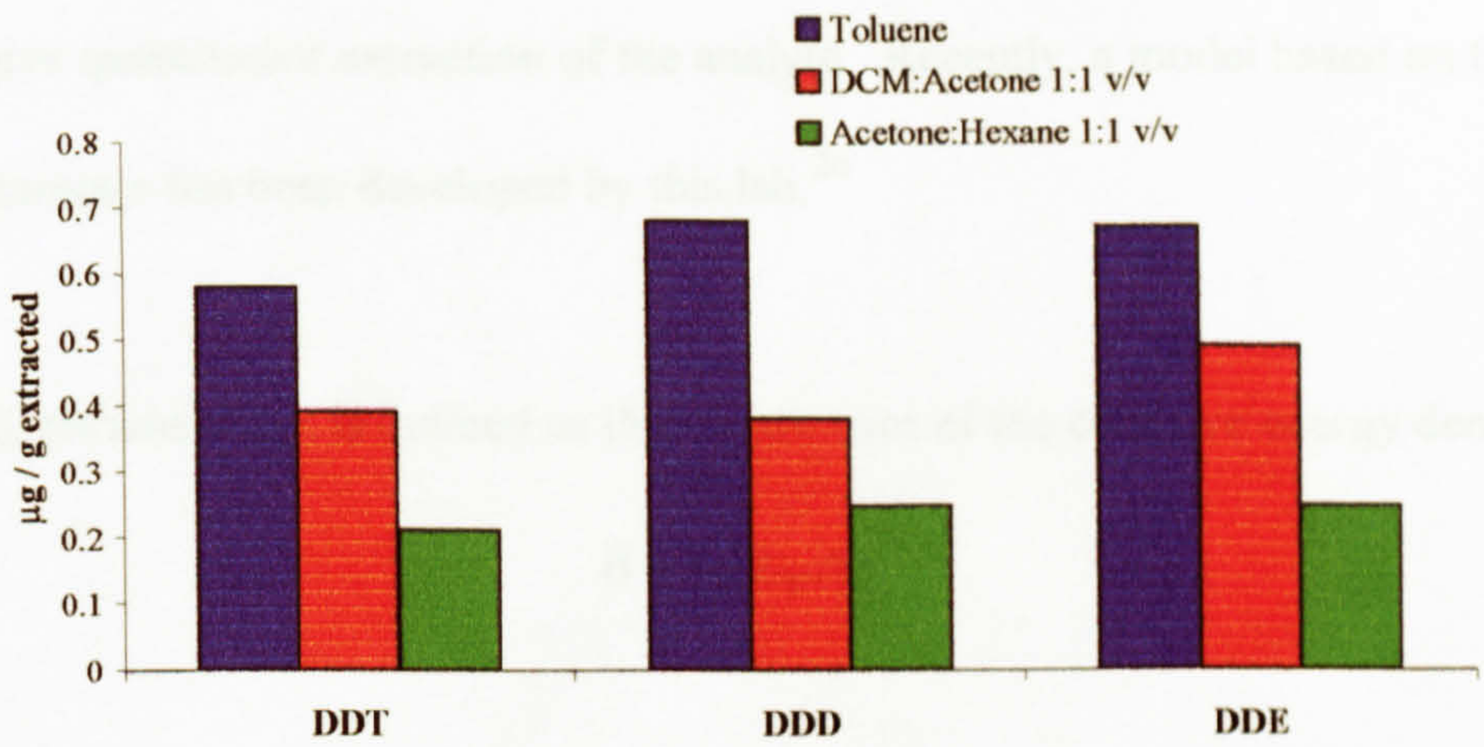
Figure 3.8 Effect of Cycles on PCB Extraction



3.2.3 Optimisation of PFE Extraction Solvent.

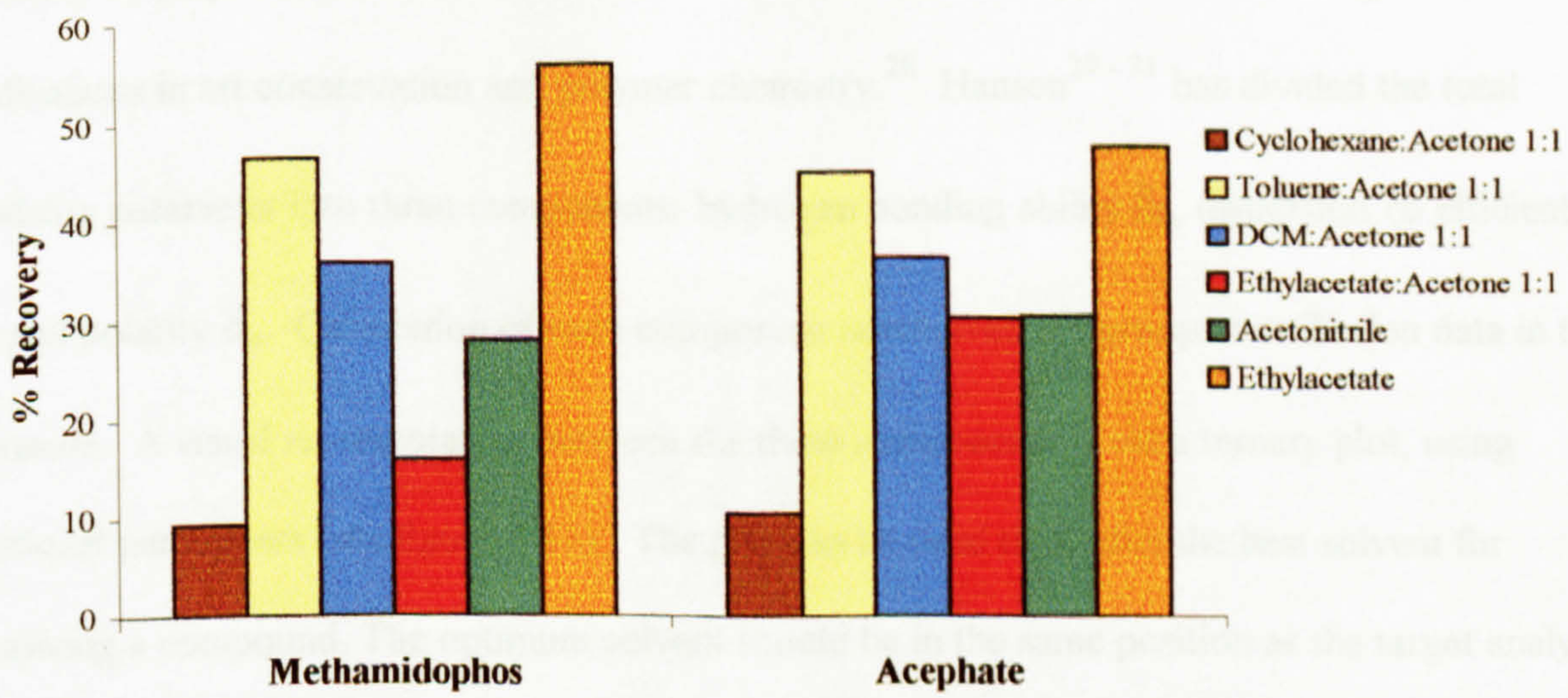
Optimisation of solvent for an extraction procedure is very often time consuming, and many traditional extraction techniques, such as Soxhlet extraction requires large volumes of organic solvent. The current trend is to reduce the consumption of organic solvents used in chemistry, as these have detrimental environmental effects. Legislation has effectively banned the use of chlorinated solvent in the EU. The costs of proper waste disposal are also high, prompting the search for alternative solvents.[25] PFE allows solvent optimisation to occur with smaller volumes of solvent. However even this advantage does not completely compensate for the time required for the extractions. Usually, comparison of several solvent systems is necessary to optimise the extraction solvent. There are copious examples of this approach in the literature. A few examples include Popp et al.,[12] who have used three different solvent systems for the extraction of spiked soil contaminated with DDT and its metabolites, DDD and DDE. They determined that acetone: hexane was the optimum solvent for the extraction (figure 3.9)

Figure 3.9 Comparison of Solvent for OCP Extraction



Obana and co workers[13] have compared six solvent systems for the extraction of organophosphorus pesticides, acephate and methamidophos from orange juice (figure 3.10). They determined that ethyl acetate was the optimum solvent for this extraction.

Figure 3.10 Comparison of Solvent Systems for OCP Extraction



N.B. All mixtures are 1:1 v/v.

Poster et al[14] has compared three solvent systems for the extraction of PCB's from a standard reference material, an urban dust. The solvent systems used were acetonitrile, DCM and

hexane:acetone 1:1 (v/v). A better way of solvent optimisation is to predict the solvent required that would give quantitative extraction of the analyte. Recently, a model based on the Hildebrand solubility parameter has been developed by this lab.[26]

The solubility parameter, δ, is defined as the square root of the cohesive energy density or

$$\delta = (\Delta E_V / V)^{1/2}$$

Where

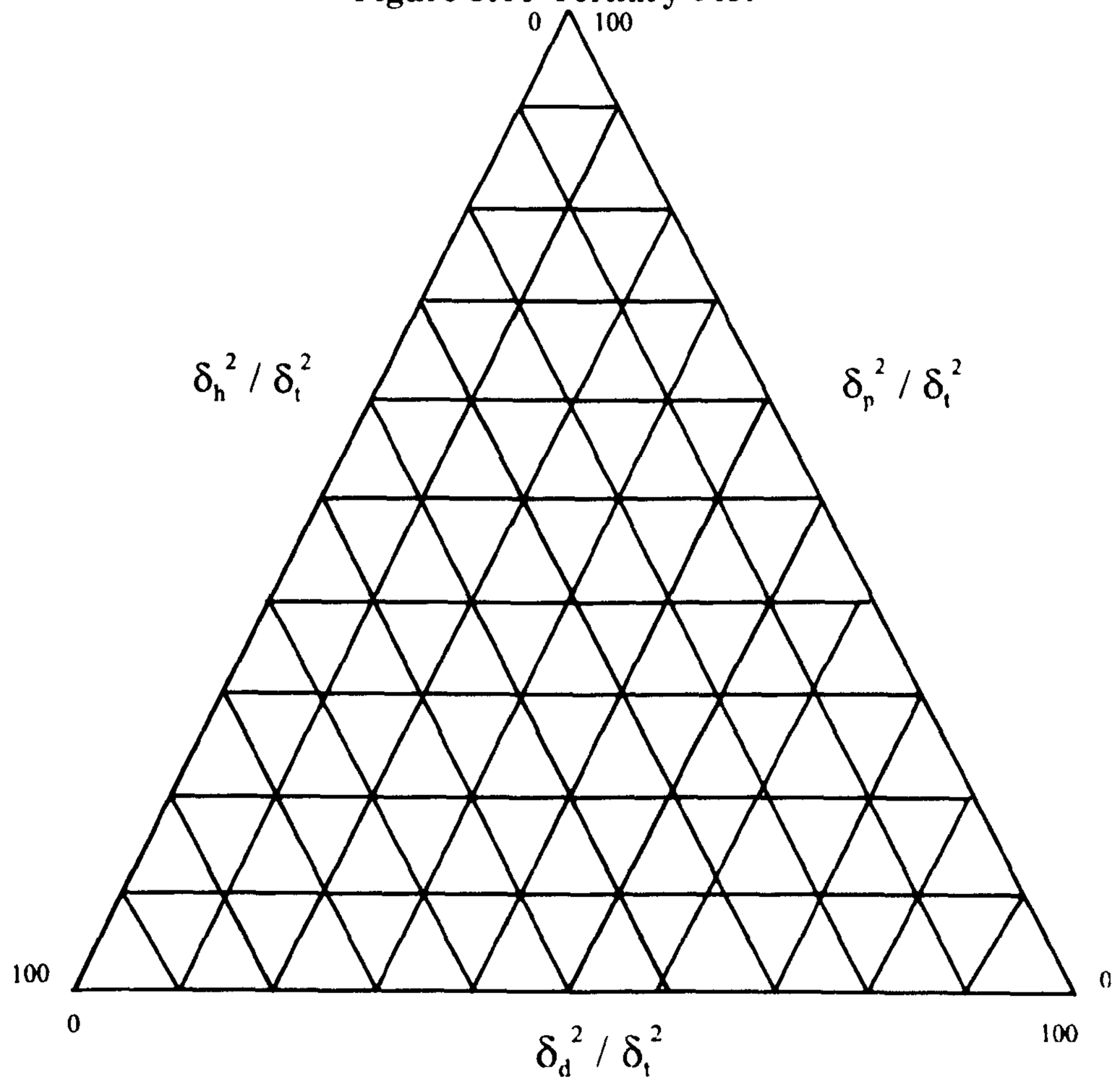
δ = the solubility parameter

ΔE_V = the energy of vaporisation at a given temperature

V = molar volume of the molecule.[27]

It is used as a measure of the solubility of compounds in various solvents. Prediction of the solubility of polymers and resins in various solvents utilised this approach. It has major applications in art conservation and polymer chemistry.[28] Hansen[29 - 31] has divided the total solubility parameter into three components: hydrogen bonding ability δ_h, dispersion co efficient δ_d, and polarity δ_p. Calculation of each component is achieved via group contribution data in the literature. A visual representation between the three components is via a ternary plot, using fractional parameters (see figure 3.11). The plot can be used to predict the best solvent for dissolving a compound. The optimum solvent should be in the same position as the target analyte. This thinking can be applied to extraction techniques. To quantitatively extract an analyte from a matrix, the solvent should have similar properties as the compound, i.e. non-polar solvents are better at extracting non-polar analytes. Using this approach, an informed selection of the most suitable solvent for a particular analyte can then be made.

Figure 3.11 Ternary Plot

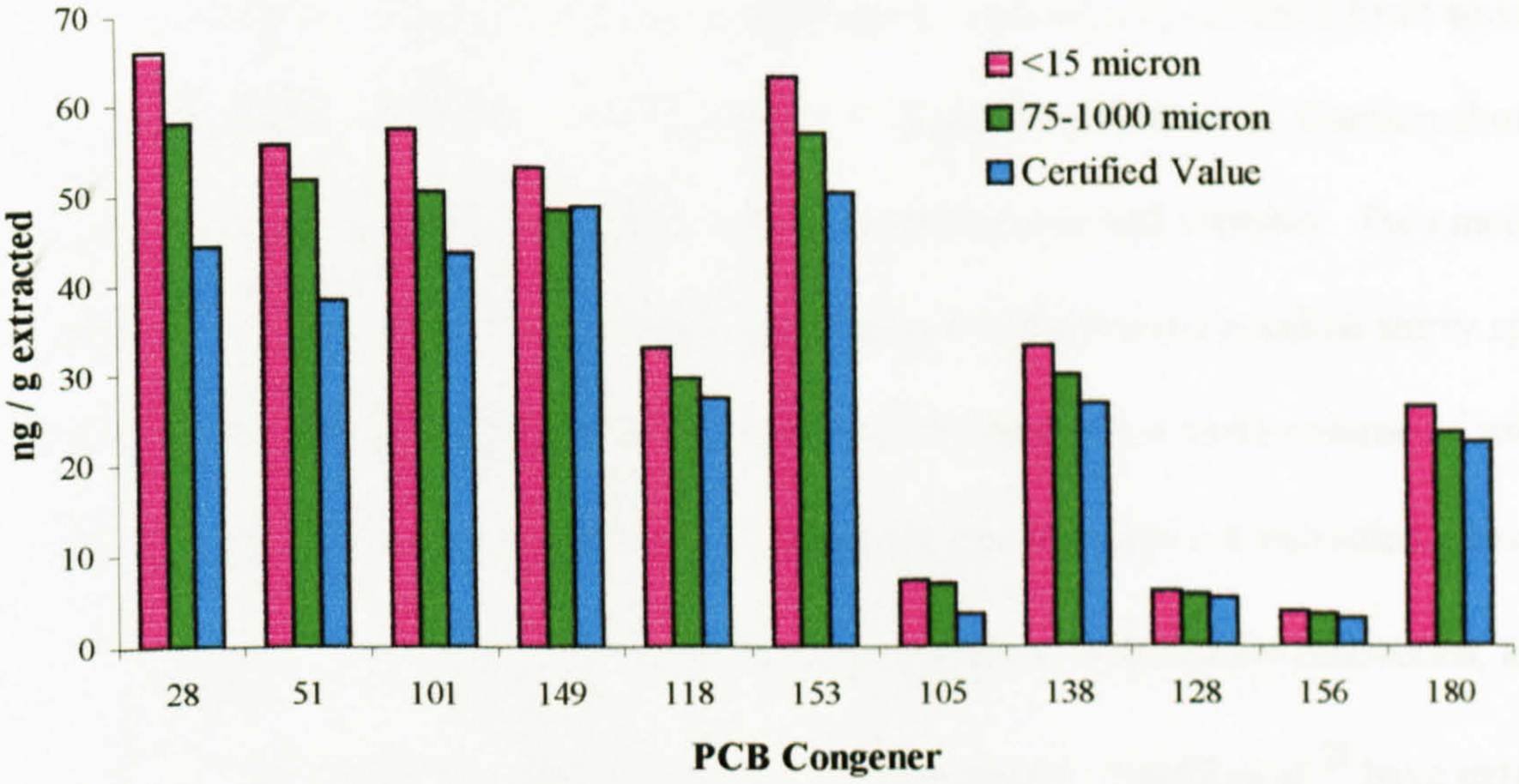


3.2.4 Effect of particle size.

Scant literature exists on the effect of particle size in PFE. Bjorkland et al.[24] have extracted marine sediment contaminated with PCB's. They completed a brief study on the effect of particle size on PFE extraction. The conclusion of this short study was that smaller particle size increased extraction efficiency. Figure 3.12 compares the results of the extraction of CRM 536 (harbour sediment) with a particle size of < 15 μm and a particle size between 75 and 1000 μm.

The authors noted that greater extraction efficiency (versus the certified values) occurred with a smaller particle size. Irrespective of particle size, both sediments yielded greater amounts of PCB's than the stated certified value.

Figure 3.12 Effect of Particle Size on PCB Extraction



3.2.5 PFE Method development procedure

From these studies method development for PFE should follow the procedure laid out below –

1 Determine the identity of the analyte

2 Sample preparation.

Air dry sample for 24 hours.

Grind sample to small particle size (<15 μm), mix well to ensure homogeneity.

Mix sample with a dispersing agent prior to extraction.

3 Optimisation for time, temperature and pressure, via one of the following methods

Central composite design, or other experimental design matrix e.g. factorial design.

Simplex design

4 Solvent selection via prediction model, or, experience

5 Static cycle determination.

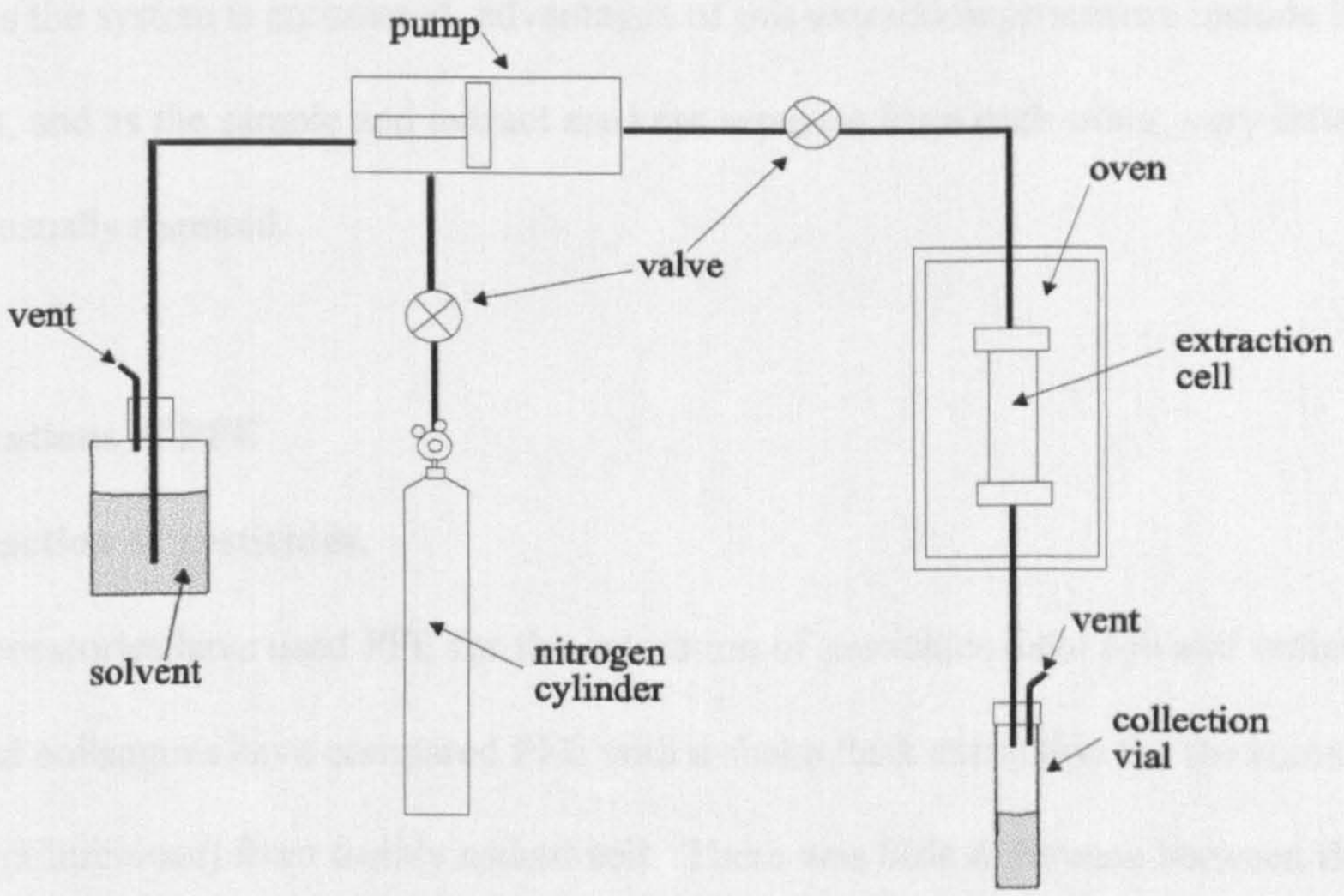
3.2.6 Validation of method.

Once all the extraction parameters have been optimised, recovery experiments from spiked inert matrices are required to determine the robustness of the proposed method. Caution should be exercised at this stage, as spiking rarely reflects what happens in real samples. Two methods of spiking are widely used. The first is called spot spiking and the second is called slurry spiking. The first method is where the analyte is introduced to the matrix in a small volume of solvent (typically μL), The solvent is then allowed to evaporate and the matrix is extracted. Several studies,[32 - 34] have shown that the former type of spiking gives quantitative recoveries, as the analyte does not have sufficient time to interact with the matrix. Ezzell et al.[32] have extracted organophosphorus pesticides (OPP's) and herbicides from spiked soil samples by PFE and compared it with conventional Soxhlet extraction, with good agreement between the two sets of results. This is not surprising as spiked sample rarely reflect real aged samples. To fully assess a new technique, a range of aged samples should be extracted and compared with alternative extraction techniques, such as Soxhlet extraction. The second, more realistic method of spiking is called slurry spiking where the analyte is introduced to the matrix in a large volume of solvent. The solvent is allowed to evaporate and the matrix is left for a significant length of time (ideal minimum 1 month) before extraction. This allows the analyte time to interact with the matrix. Dean et al.[35] and Frost et al.[36] performed two studies of note. These investigations showed that the longer the spiking time, the less of the target analyte was recovered. Once the recovery experiments are completed, application of the technique to various matrices and analytes and comparison with older established methods of extraction e.g. Soxhlet extraction is advisable.

3.3 PFE Instrumentation

An automated ASE™ system is available from Dionex Corporation, and consists of a solvent delivery system, an oven; carousel and computer controlled software. Figure 3.13 shows a schematic for the system.

Figure 3.13 PFE Schematic



Up to 24 samples can be sequentially extracted in stainless steel extraction cells. These consist of two end caps joined by a cylindrical cell body. Each end cap contains a stainless steel frit to help prevent cell blockage. The cells are available in five volumes (1 mL, 5 mL, 11 mL, 22 mL and 33 mL) to allow both wet and dry samples to be extracted efficiently. The sample to be extracted is mixed with an inert matrix to reduce solvent consumption. The sample is quantitatively transferred to the stainless steel extraction cell that has been fitted with a filter to prevent transport of particulate matter through the cell and into the solvent lines. The extraction solvent

is pumped into the cell, where it is heated to the temperature and pressure stated in the method (typically between 100 and 200 °C, 6.9 – 20.7 MPa). The solvent is then kept in contact with the sample for a specified static extraction time, usually between 5 and 20 minutes. The analyte containing solvent is then flushed through the cell to a glass collection vial. A few mL (as a % of the cell volume) of solvent is the used to rinse the cell. The lines are then purged with high purity nitrogen to remove the last residues of the solvent and analyte. The extract is then ready for analysis. As the system is automated, advantages of this extraction procedure include high sample throughput, and as the sample and extract are kept separate from each other, very little sample cleanup is usually required.

3.4 Applications of PFE

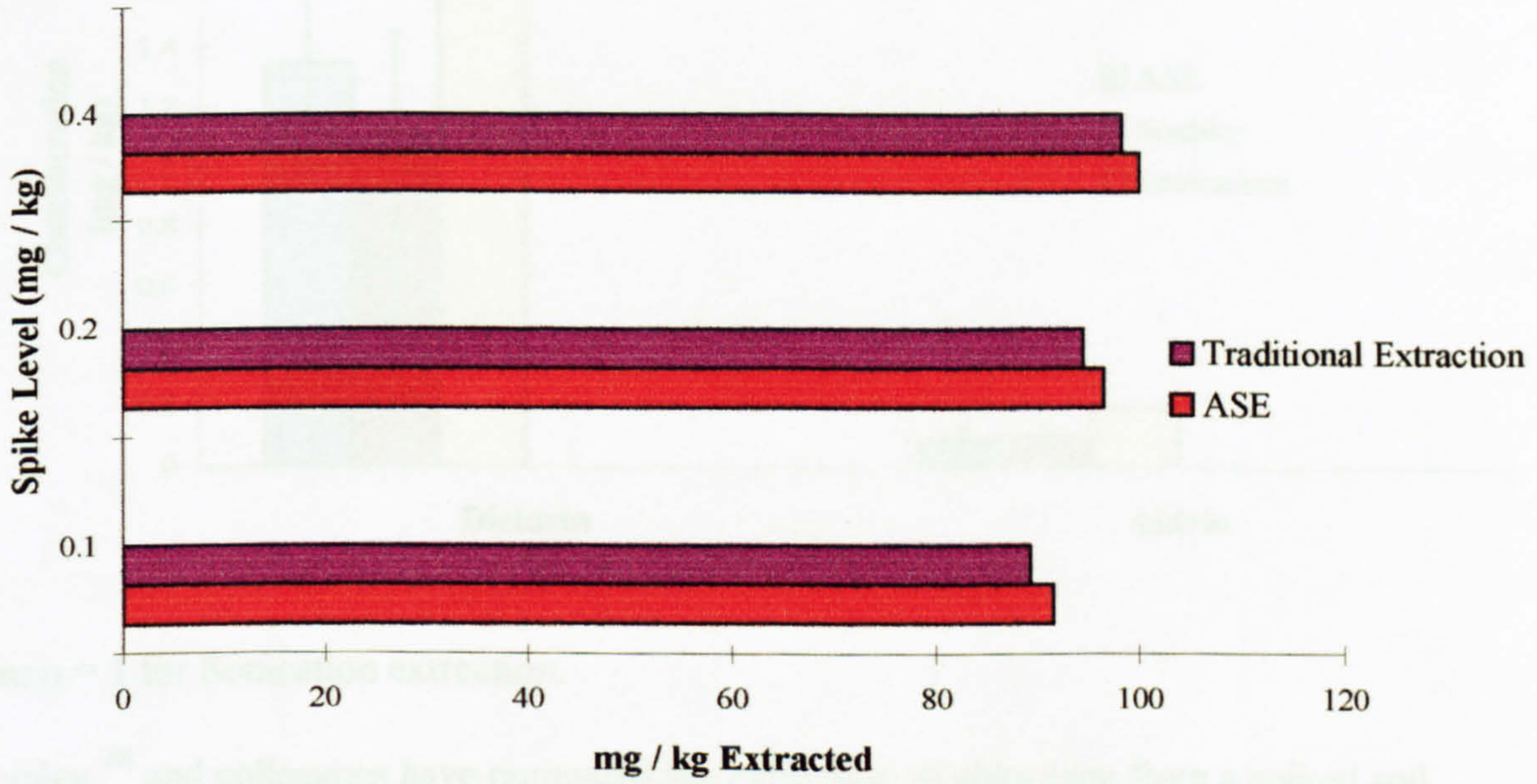
3.4.1 Extraction of pesticides.

Several laboratories have used PFE for the extraction of pesticides from soil and sediments. Conte[34] and colleagues have compared PFE with a shake flask extraction for the recovery of a herbicide (diflufenican) from freshly spiked soil. There was little difference between the amount extracted for the range of spike levels investigated (0.1 - 0.4 mg / kg). Figure 3.14 shows these results.

Ezzell et al.[32] extracted organophosphorus pesticides (OPP's) and herbicides from three different spiked matrices (clay, loam and sand) at two different concentration levels. He compared the results by PFE with conventional Soxhlet extraction, with good agreement between the two sets of results. No matrix dependence on recovery seemed to exist. This is not surprising as spiked samples rarely reflect real aged samples. To fully assess a new technique, a range of aged samples

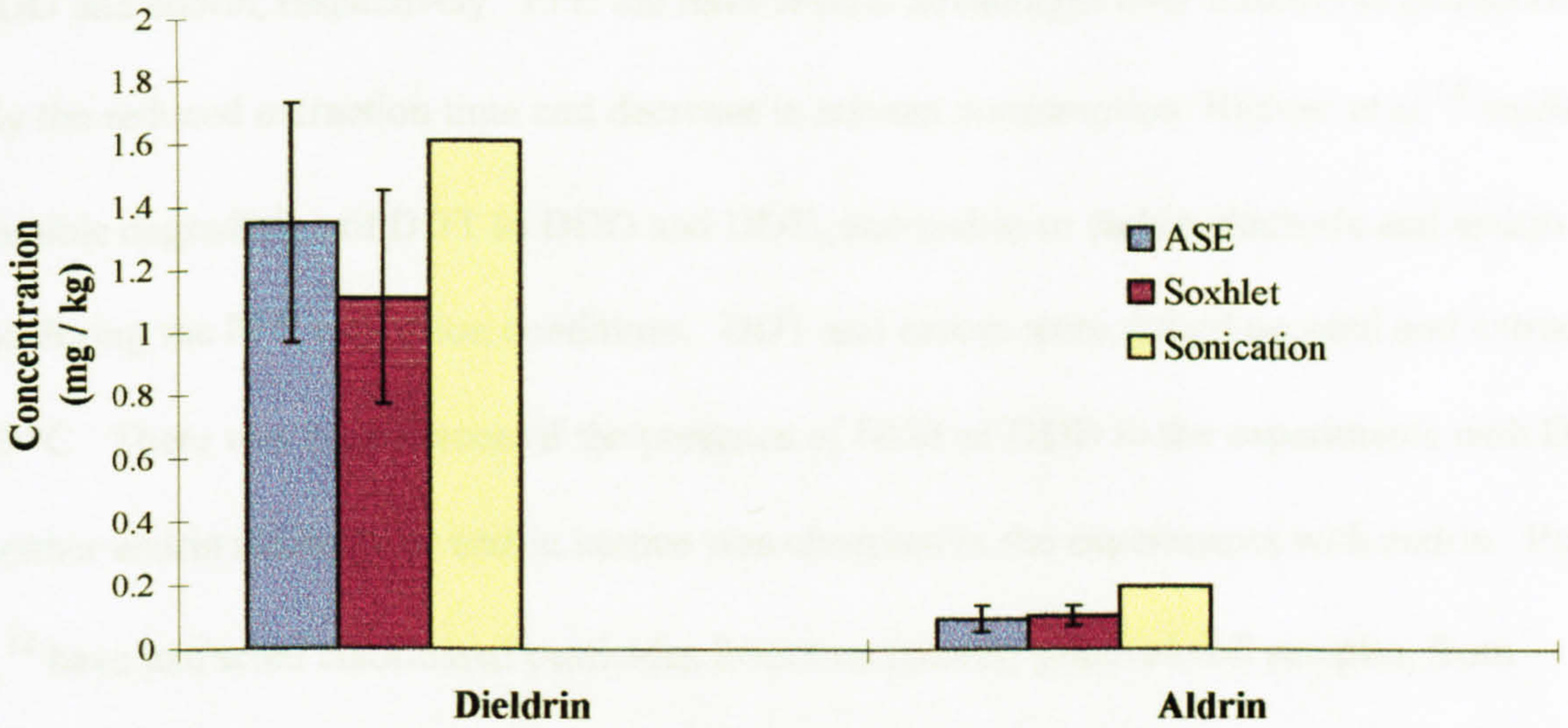
should be extracted and compared with alternative extraction techniques, such as Soxhlet extraction.

Figure 3.14 Extraction of Diflufenican by PFE and Traditional Solvent Extraction



Fisher et al.[37] and Brumley et al.,[38] explored this. The former group extracted soils contaminated with organochlorine pesticides (OCP's) by PFE, Soxhlet extraction, and sonication. The results, presented in figure 3.15, show that PFE is at least as good as Soxhlet extraction for the extraction of dieldrin and aldrin, but both methods give poorer recoveries when compared to sonication. The precision of PFE and Soxhlet extraction were very good (0.04 % - 0.38 % for PFE compared with 0.03 to 0.34 % for Soxhlet). However, it was noted that variation in the amount extracted was probably due to the heterogeneity of the soil sample. The sonication method was optimised for OPP's, whereas the other two techniques were used as screening techniques, this could also account for the apparent reduced efficiency of OPP extraction by PFE and Soxhlet extraction.

Figure 3.15 Extraction of OCP's from Contaminated Soil



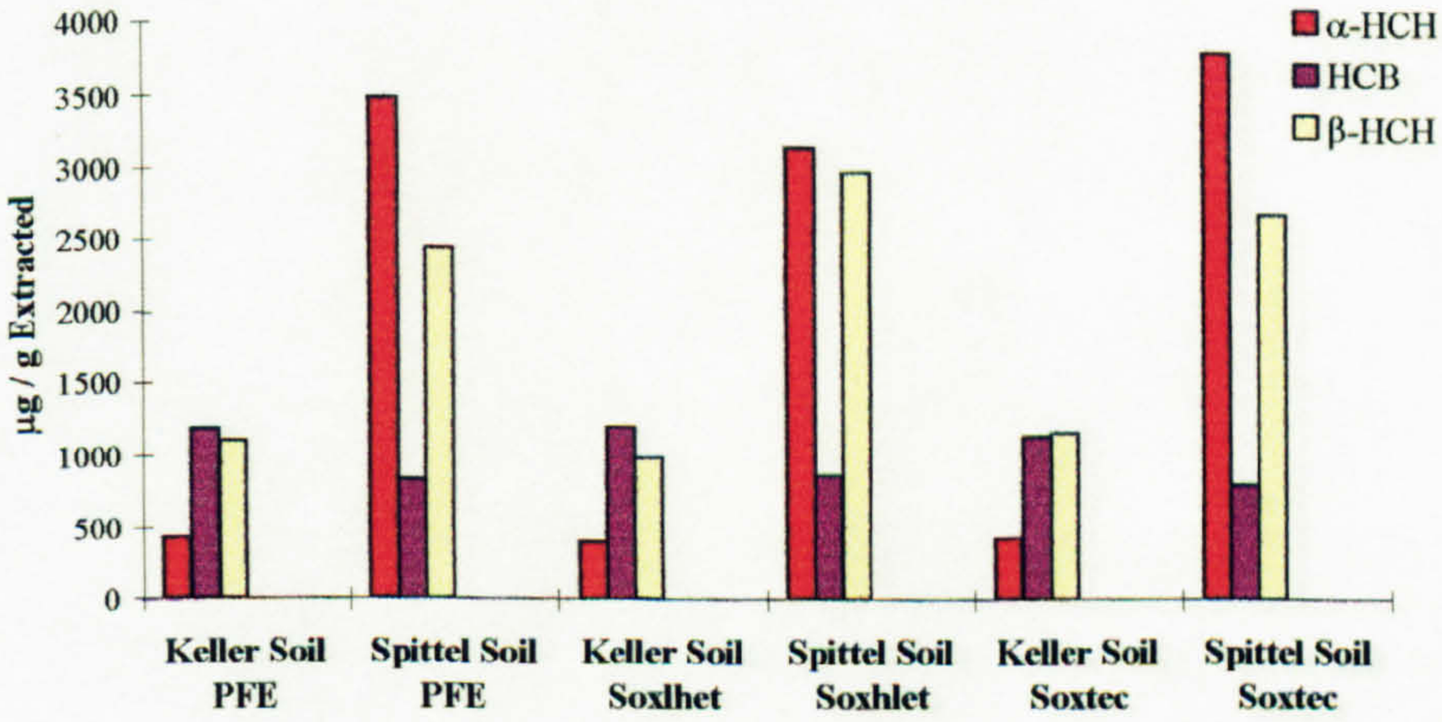
Note n = 1 for Sonication extraction.

Brumley,[38] and colleagues have compared the extraction of chlordane from a spiked soil.

Chlordane is a mixture of polychlorinated compounds, hence more than one peak is obtained in the chromatography. The total amount of chlordane is found by determining the amount for each peak and then adding the results for each peak. The three techniques they chose to study were PFE, SFE and compared the results with Soxhlet. Three spiked levels were chosen, 2.0 μg / g, 0.2 μg / g and 0.02 μg / g. This was done in order to determine the sensitivity of two GC detection techniques, GC-ECD and GC/EC NIMS. The recoveries using the 2 μg / g spike level, showed that both PFE and SFE were comparable to Soxhlet extraction. PFE gave average recoveries of 85 % for all the chlordane component peaks, Soxhlet gave a mean recovery of 82 % and SFE gave an average of 125 %. It was also noted that the PFE extracts did not require further treatment, a distinct advantage, as the other two techniques required SPE clean up before the analysis could be performed. Li et al.,[39] have also extracted spiked and certified soil samples. They extracted soil contaminated with organochlorine pesticides by MAE and compared the

results with Soxhlet extraction. The spiked samples gave recoveries of between 95 % and 155 % for DDD and endrin, respectively. PFE did have several advantages over traditional extraction, namely the reduced extraction time and decrease in solvent consumption. Richter et al.[15] studied the possible degradation of DDT to DDD and DDE, and endrin to endrin aldehyde and endrin ketone during the PFE extraction conditions. DDT and endrin were spiked on sand and extracted at 150 °C. There was no evidence of the presence of DDE or DDD in the experiments with DDT, and neither endrin aldehyde or endrin ketone was observed in the experiments with endrin. Popp et al.,[12] have extracted chlorinated pesticides from two natively polluted soil samples, from floodplains in Germany. This group compared PFE with Soxhlet and Soxtec. Figure 3.16 shows that PFE was equivalent to Soxhlet for 18 hours, or Soxtec for 6 hours.

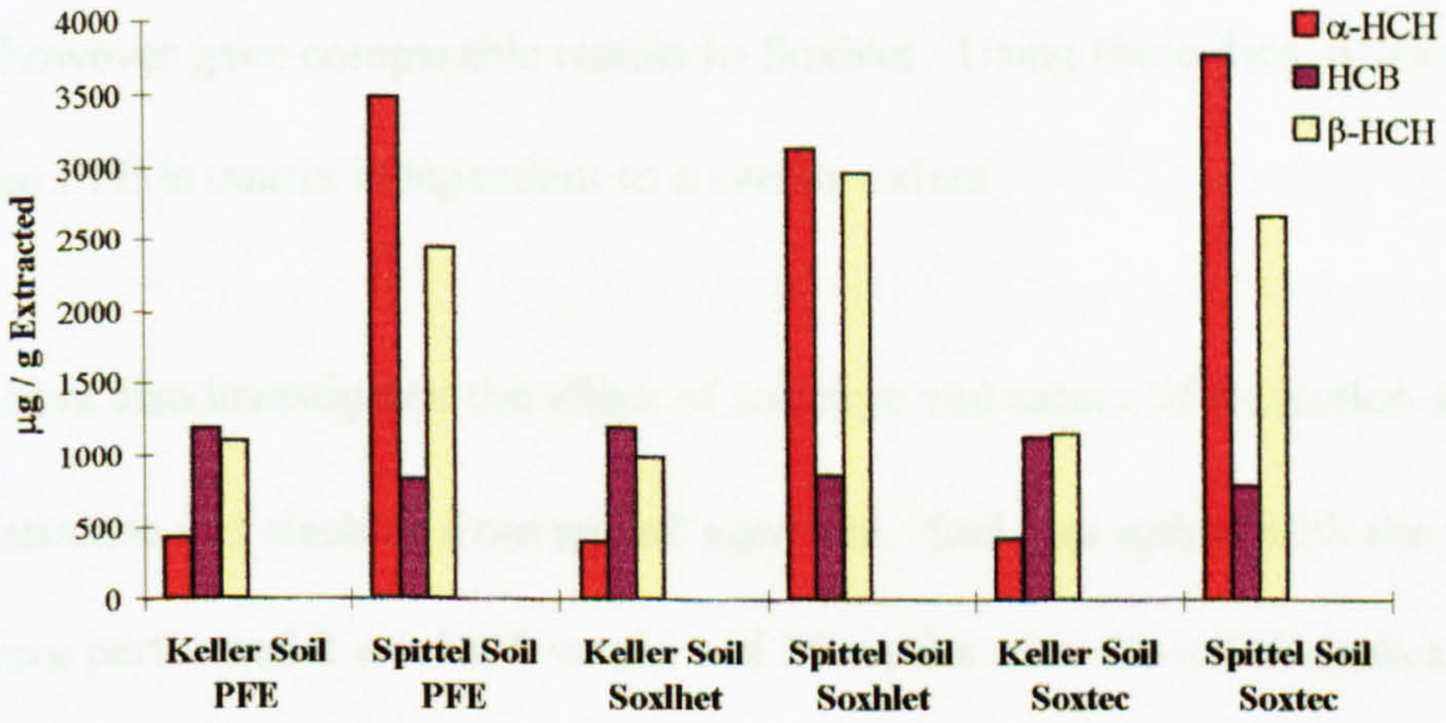
Figure 3.16 Comparison of Extraction Techniques of OCP's from Soil



The number of extractions was varied to determine if the analytes were quantitatively removed with one step. They determined that the predominant part of the contaminants was extracted during the first extraction step. They also noted the yield of the second extraction was much lower but not negligible and that the yield of the third procedure was very low (see figure 3.7). They also completed a solvent study that showed, unlike microwave extraction, PFE does not

results with Soxhlet extraction. The spiked samples gave recoveries of between 95 % and 155 % for DDD and endrin, respectively. PFE did have several advantages over traditional extraction, namely the reduced extraction time and decrease in solvent consumption. Richter et al.[15] studied the possible degradation of DDT to DDD and DDE, and endrin to endrin aldehyde and endrin ketone during the PFE extraction conditions. DDT and endrin were spiked on sand and extracted at 150 °C. There was no evidence of the presence of DDE or DDD in the experiments with DDT, and neither endrin aldehyde or endrin ketone was observed in the experiments with endrin. Popp et al.,[12] have extracted chlorinated pesticides from two natively polluted soil samples, from floodplains in Germany. This group compared PFE with Soxhlet and Soxtec. Figure 3.16 shows that PFE was equivalent to Soxhlet for 18 hours, or Soxtec for 6 hours.

Figure 3.16 Comparison of Extraction Techniques of OCP's from Soil



The number of extractions was varied to determine if the analytes were quantitatively removed with one step. They determined that the predominant part of the contaminants was extracted during the first extraction step. They also noted the yield of the second extraction was much lower but not negligible and that the yield of the third procedure was very low (see figure 3.7). They also completed a solvent study that showed, unlike microwave extraction, PFE does not

Pyle et al.[40] have also extracted chlorinated pesticides from the same marine sediment (SRM 1941), and a natively polluted soil sample using PFE. Additional clean up was required owing to the co-extraction of interfering compounds. Further preparation of the samples was required in the form of centrifugation, as solids precipitated out as the extract cooled.

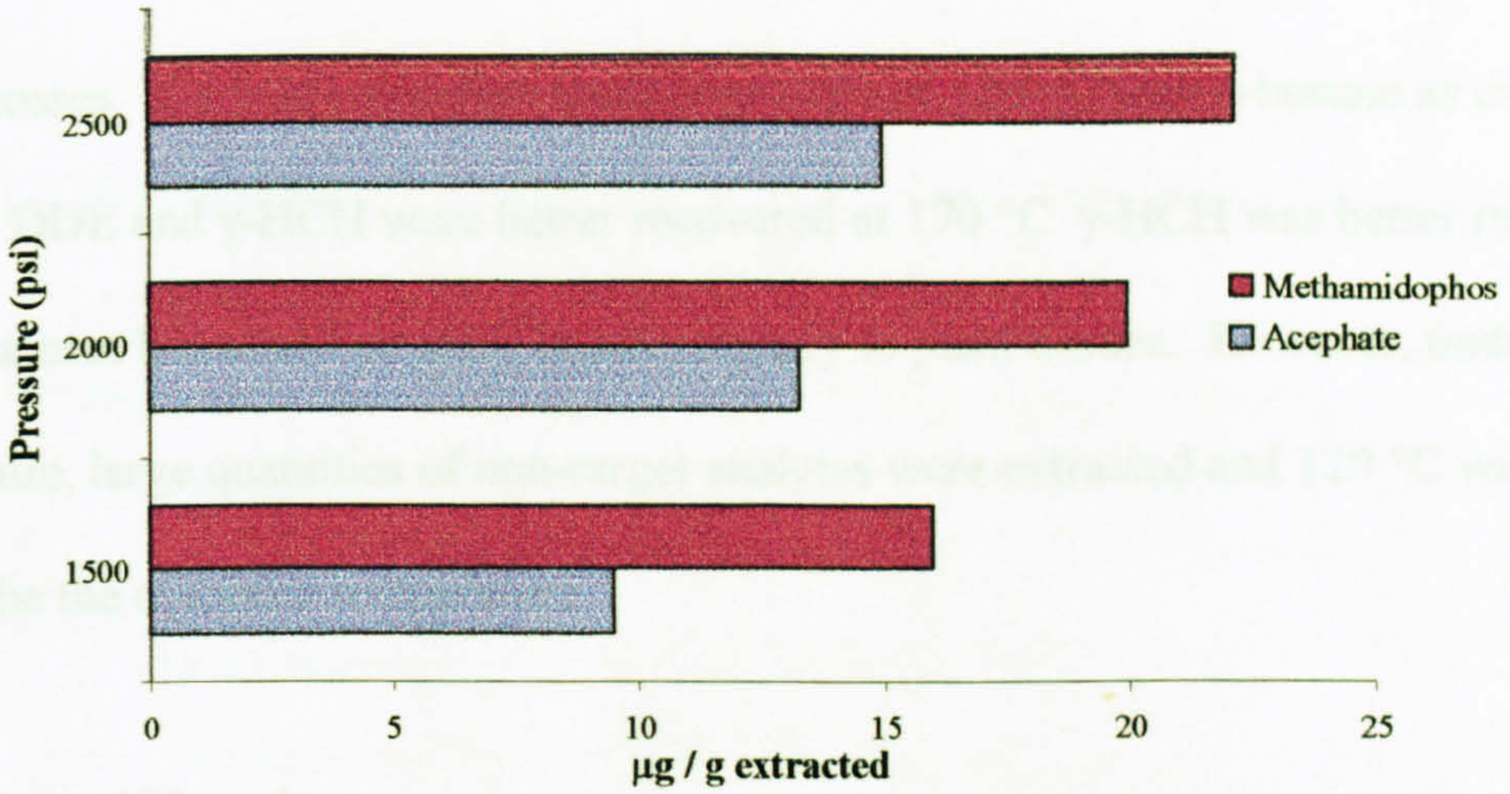
Frost et al.[36] have compared PFE to three other extraction techniques, SFE, MAE and Soxhlet to remove weathered hexaconazole residues from two fully characterised soils. Extraction of the soil after 52 weeks application showed that the amount of extractable material had reduced significantly. SFE extraction time was not deemed significant on the recovery of hexaconazole. Differences between the soils were observed. The soil with the lower organic matter gave comparable recoveries for all four techniques, whereas the soil with the higher organic matter gave varied results. For the latter, recoveries by MAE and SFE were half of the target Soxhlet value. PFE however gave comparable results to Soxhlet. Using these data, it was tentatively suggested that PFE is matrix independent to a certain extent.

Gan et al.[41] have also investigated the effect of soil type and nature of extraction solvent for the recovery of atrazine and alachlor from spiked aged soil. Soil was spiked with the pesticides and extraction were performed 2 weeks, 8 weeks and 26 weeks after the initial application. Of the three solvents investigated (DCM:acetone 1:1 v/v, hexane and methanol), DCM:acetone 1:1 v/v consistently extracted more of the compounds from every soil than either of the other solvents. The results by PFE were compared with those obtained from Soxhlet extraction and solvent – shake extraction. After two weeks, PFE was comparable to the other techniques, however as the ageing period increased, PFE was able to extract more residues than either Soxhlet or sonication.

The only exceptions were the extraction of atrazine from Arlington soil, and the extraction of alachlor from Linne soil, where all three extraction methods performed comparably.

In addition to more common environmental matrices, e.g. soil and sediment, PFE has also been applied to food stuffs and plant matter. Obana et al.[13] extracted organophosphorus pesticides in foods using PFE. Three different solvent mixtures (cyclohexane-acetone (1:1, v/v), dichloromethane-acetone (1:1, v/v) and ethyl acetate-acetone (1:1, v/v)) were evaluated for the extraction of organophosphorus pesticides from flour. The low water content of the matrix meant that no drying agent was required. The recoveries were good with the three solvent mixtures except for dichlorvos whose recovery was ~ 40 %. The low recoveries of dichlorvos were attributed to samples losses during the spiking procedure. Ethyl acetate-acetone (1:1, v/v) mixture gave high RSD values (19 - 34 %). Wet samples, were mixed with a drying agent (diatomaceous earth, particle size 160-800 μm), ground in a mortar with a pestle until the mixture became homogeneous prior to extraction. The influence of extraction time, temperature and pressure were studied for the extraction of methamidophos and acephate from orange juice. Extraction time and temperature seemed to have no effect on extraction yields whereas higher pressures gave better recoveries (see figure 3.19).

Figure 3.19 Effect of Pressure on Extraction from Orange Juice.



Ethyl acetate, toluene-acetone (1:1, v/v), cyclohexane-acetone (1:1, v/v), dichloromethane-acetone (1:1, v/v), ethyl acetate-acetone (1:1, v/v) and acetonitrile were studied for the extraction of the same pesticides from orange juice. Ethyl acetate gave the best recoveries (56 and 47 %). PFE was compared with hexane extraction of some of this pesticides in different foods and although PFE gave slightly lower recoveries, precision was better. Okihashi et al.[42] determined *N*-methylcarbamate pesticides in foods using PFE. Recoveries of the majority of the pesticides ranged from 70 % to 100 %, with RSD values between 0.1 and 11 %. The *N*-methylcarbamate pesticides were found to be stable under the temperature and pressure conditions used in the PFE extractions. Nemoto et al.[43] extracted herbicides in soybeans using PFE. Due to the polar mature of the herbicide analytes and their high solubility in water, water was initially used as solvent. However, when 100 % water was used low and variable extraction volumes were obtained. The high viscosity of water coupled with the high levels of carbohydrates and proteins in soybeans made PFE difficult unless an organic solvent was added. The influence of pH on extraction efficiency was studied by adding hydrochloric acid (HCl) into the aqueous fraction of a 70 % acetonitrile extraction solution. 0.05 M and 0.01 M HCl gave good recoveries for all the

pesticides studied. Wenzel et al.[44] extracted chlorobenzenes, DDX and HCH isomers from pine needles and mosses. The best extraction conditions were at 120 °C with n-hexane as extraction solvent. Only DDE and γ-HCH were better recovered at 170 °C. γ-HCH was better recovered at higher temperatures because it possibly bonds strongly to plant tissues. However, under such high temperature, large quantities of non-target analytes were extracted and 120 °C was concluded to be the optimum temperature.

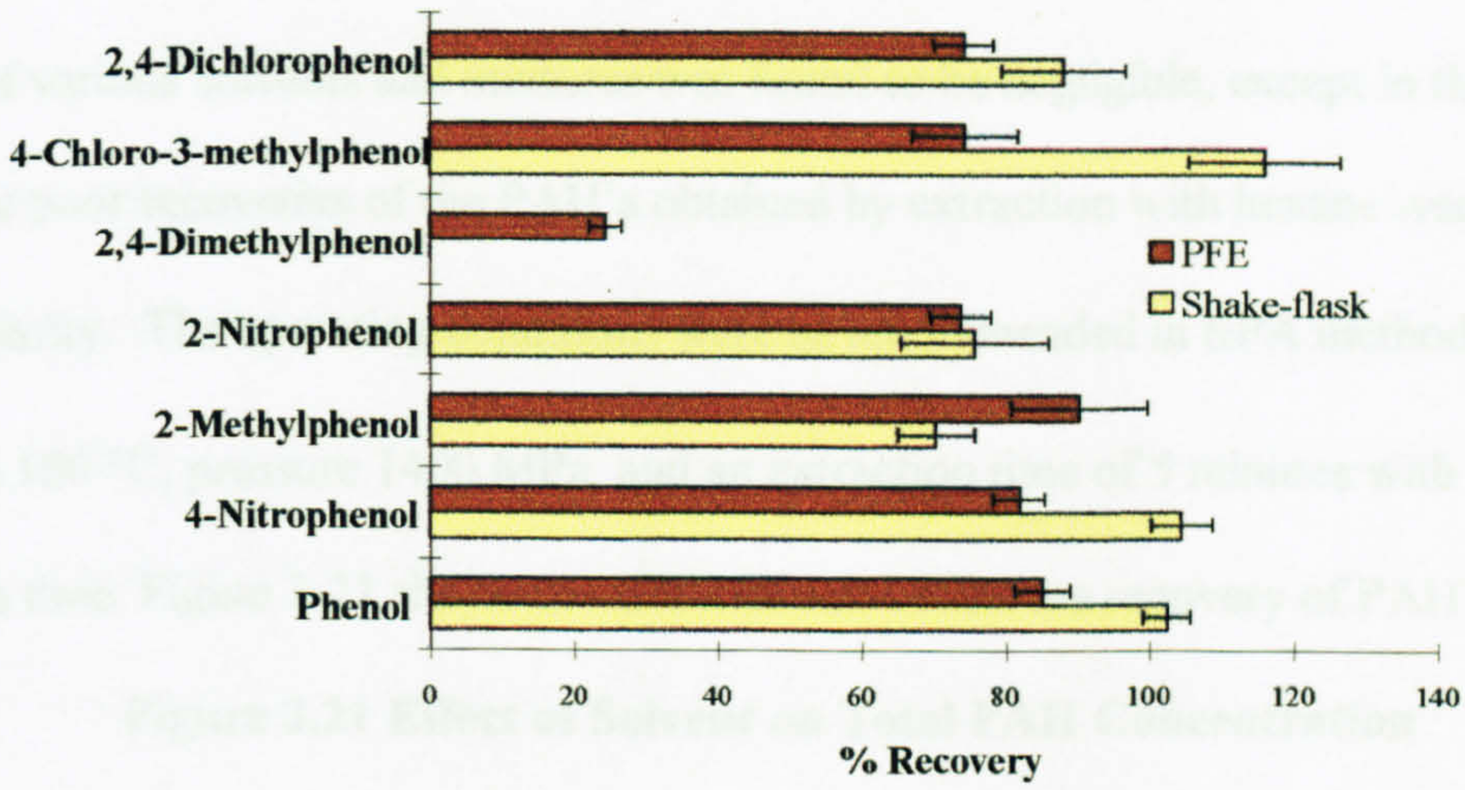
3.4.2 Extraction of Phenols

The use of phenolic compounds in industry is widespread and diverse. They are used in the manufacture of polymers and resins, and are by products in some dye manufacturing processes. Certain phenolic derivatives, such as chlorinated phenoxy acids, exhibit pesticidal properties and can readily degrade in the environment to yield nitro and chlorinated phenols. Some of the more persistent compounds, pentachlorophenol, for example, are included in the US EPA list of priority pollutants, as well as on the UK Red List (table 1.1).[25] Strict European regulations control their use and disposal.[35, 45 - 48]

Dean et al. has used central composite design (CCD)[35] to optimise the accelerated solvent extraction parameters of seven phenols from a slurry spiked soil. The limits investigated were (pressure, 4 - 20 MPa; temperature, 30 - 70 °C; and extraction time 5 - 25 minutes). None of the investigated variables were found to be significant upon the recovery of the phenols, with the exception of 2-methylphenol. Intercept, pressure and extraction time, as well as the interaction between pressure and extraction time and temperature and extraction time was found to have a significant effect upon the recovery of this molecule. The results were compared with shake - flask extraction. Recoveries obtained by both PFE and shake flask extraction were comparable

for all the phenols studied (see figure 3.20). 2,4-Dimethylphenol, however was not recovered by shake flask extraction, and PFE only recovered 24 %.

Figure 3.20 Extraction of Phenols from Soil by PFE and Shake Flask Extraction



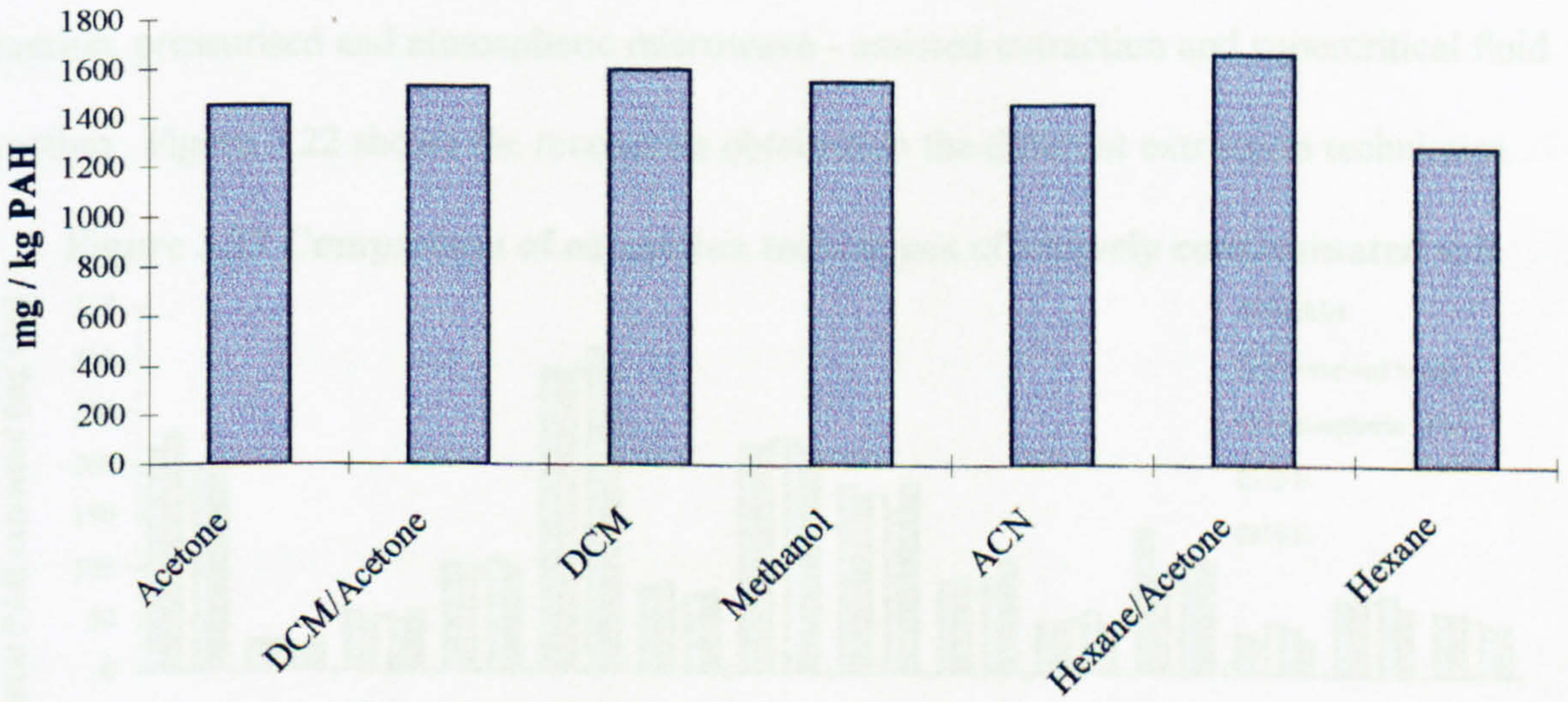
3.4.3 Extraction of PAH's

Polycyclic aromatic hydrocarbons (PAH's) are a group of organic pollutants that exhibit carcinogenic and/or mutagenic properties. The US EPA Environmental Protection Agency and the European Community list them as priority pollutants.[25] PAH's are abundant in the ecosystem, largely due to incomplete combustion of fossil fuels. They also exist naturally due to forest fires.[49, 50] As a result they enter the environment from a wide variety of sources,[51] and therefore are present in all compartments of the environment (atmosphere, soil, water).[52] Several studies in the literature discuss the extraction of these compounds from a variety of solid matrices, such as soil, sludges and sediment.

Saim et al.[23] studied the influence of accelerated solvent extraction variables (pressure, temperature, extraction time and extraction solvent) for the removal of PAH's from a native

contaminated soil. Central composite design was used to study the influence of pressure (1000 - 2400 psi), temperature, (40 - 200 °C) and extraction time (2 - 16 minutes). This group found no significant influence on the recovery of the studied compounds within the limits of the design. The effect of various solvents and mixtures was found to be negligible, except in the case of hexane. The poor recoveries of the PAH's obtained by extraction with hexane were attributed to its lower polarity. The operating conditions were as recommended in EPA method 3545,[11] i.e., temperature 100 °C, pressure 1400 MPa, and an extraction time of 5 minutes with 5 minutes equilibration time. Figure 3.21 shows the effect of solvent on the recovery of PAH's.

Figure 3.21 Effect of Solvent on Total PAH Concentration



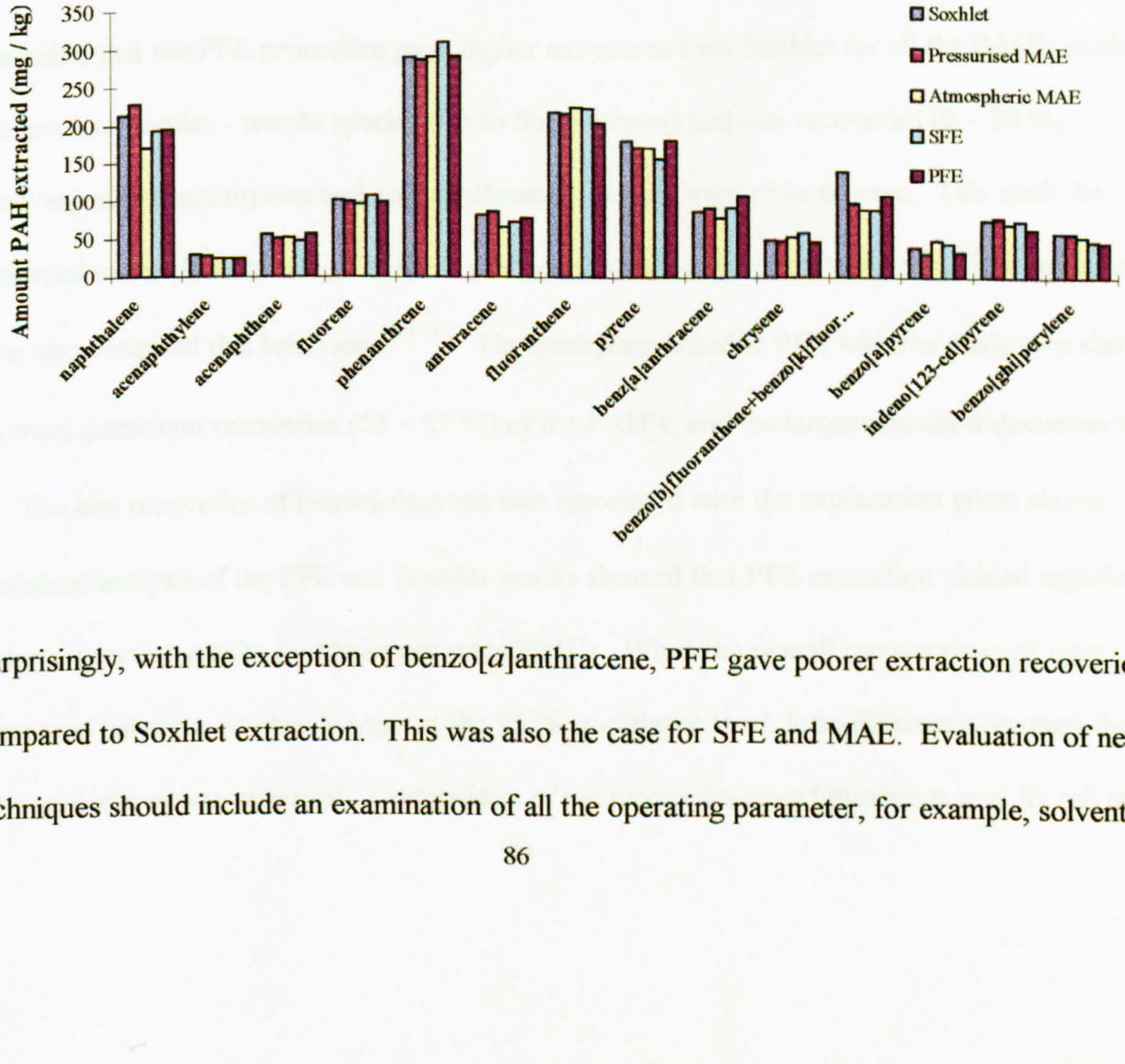
Comparison of PFE with other techniques is also prolific in the literature. Several studies have investigated the potential of PFE compared to Soxhlet extraction, SFE, MAE[12, 20, 53 - 55] as well as other less well used techniques such as methanolic saponification.[52, 56]

Heemken et al.[52] extracted PAH's and alkanes from a reference marine sediment (HS-6, 3 wt. % water content) and air - dried suspended particulate matter (SPM, 5.3 wt. % water content) from

the rivers Elbe and Weser. They compared PFE, SFE, methanolic saponification and Soxhlet. The results were not significantly different. In the same work, the influence of water on extraction efficiency was studied SPM samples containing 56 wt. % water. PFE and SFE of the wet SPM samples gave decreased recoveries. High water content seemed to impair the extraction efficiency. Wet SPM was mixed with anhydrous sodium sulphate (~4 wt. %) as a drying agent, and extracted. The recoveries of the PAH's and alkanes were almost quantitative when compared to air - dried samples. The disadvantages of mixtures with sodium sulphate are reduction of the sample volume and risk of sample contamination.

Saim et al.[53] compared PFE extraction of PAH's from a natively contaminated soil with Soxhlet extraction, pressurised and atmospheric microwave - assisted extraction and supercritical fluid extraction. Figure 3.22 shows the recoveries obtained in the different extraction techniques.

Figure 3.22 Comparison of extraction techniques of natively contaminated soil

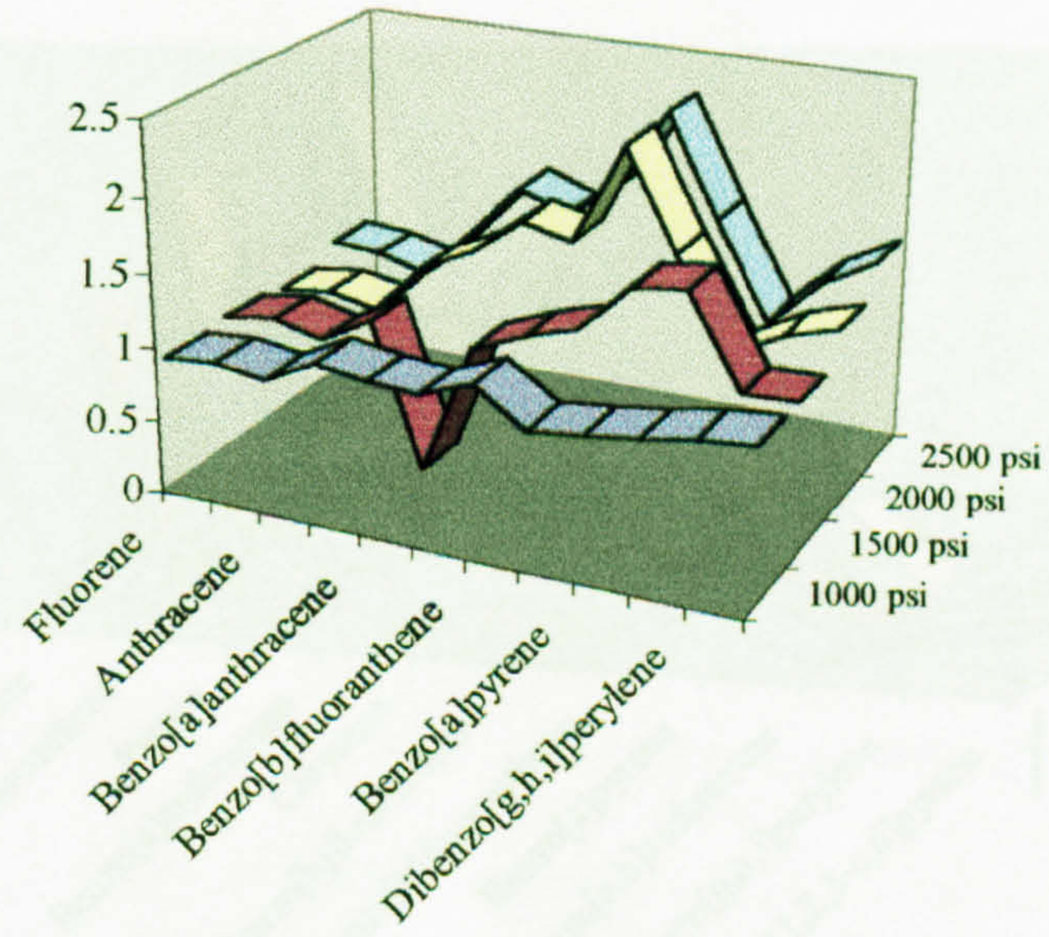


Surprisingly, with the exception of benzo[*a*]anthracene, PFE gave poorer extraction recoveries compared to Soxhlet extraction. This was also the case for SFE and MAE. Evaluation of new techniques should include an examination of all the operating parameter, for example, solvent

consumption, ease of use, speed of extraction etc. Although PFE on this occasion did not meet the extraction efficiency criteria, it does offer reduced solvent consumption, and a level of automation that allows completion of alternative tasks in the laboratory. Kenny et al., have extracted[54] from an aged bituminous coal fly ash. High carbon content (15.5 wt. %), and small particle size, 6 - 16 μm, has the potential ability to strongly adsorb any organic analyte.[36, 57, 58] Three different solvents were assessed; methylene chloride, methanol and toluene. The influence of a static step and extraction temperature using PFE was also investigated. The results were compared with SFE (methanol modified CO_2 and unmodified CO_2), enhanced fluidity solvents (EFS) and Soxhlet extraction. Three PAH's with a range of molecular weight were used to assess the efficiency of each PFE extraction solvent. Fluorene, pyrene and indeno[1,2,3-c,d]pyrene were spiked to the bituminous coal fly ash and extracted under the optimum PFE conditions. In each case, toluene gave the highest recovery from the spiked matrix. Comparison of PFE with Soxhlet concluded that the PFE procedure gave higher extraction than Soxhlet for all the PAH's studied. The low - molecular - weight species (up to fluoranthene) had low recoveries (0 – 70 %). Recovery of acenaphthylene and acenaphthene, however, were close to zero. This could be attributed to the decomposition of these compounds after adsorption to fly ash. Other researchers have also observed this behaviour.[59 - 61] The methylene chloride PFE with the static step showed the most consistent recoveries (53 – 57 %) of the PAH's, and the largest standard deviation was 9 %. The low recoveries of benzo[*a*]pyrene was associated with the explanation given above. Statistical analysis of the PFE and Soxhlet results showed that PFE extraction yielded significantly higher extraction results for the larger mass PAH's. When the overall recovery means were compared using the Student's *t*-test at the 95 % confidence level, little difference between the PFE procedures was observed. Comparison of the recoveries from bituminous coal fly ash and a

lignite fly ash used in a previous study[62] showed that the recoveries obtained from the bituminous fly ash were poorer than the ones obtained from the lignite fly ash. Both fly ash matrices were similar in composition, apart from their carbon content (0.2 % and 15 % for the lignite and bituminous fly ash matrices, respectively). This highlights the inherent difficulty in applying a particular extraction method to different matrices. Richter et al.[15] have studied the effect of PFE extraction parameters (pressure, temperature and solvent volume) in the extraction of PAH's and TPH's from spiked silica's, and reference materials (SRM 1649 and HS-3). Pressure had no effect on the recovery of PAH's from spiked dry silica. However, higher pressures (1500 - 2500 psi) show improved recovery over the use of lower pressures when wet silica with 300 Å pore material were extracted. This is shown in figure 3.23.

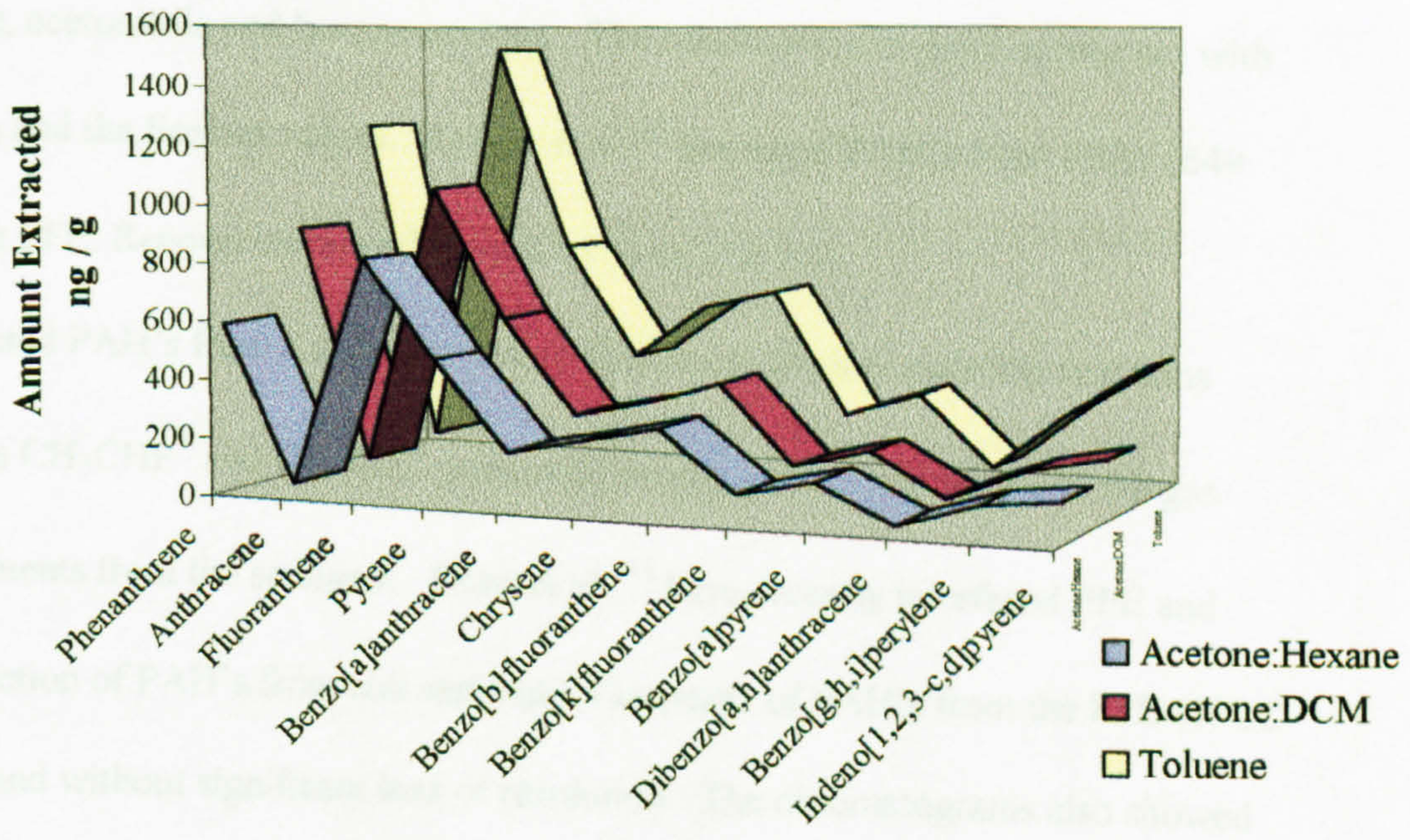
Figure 3.23 Effect of Pressure on PAH Extraction



Elevated pressure and temperature can force the solvent into the pores, aiding extraction of adsorbed analytes.[15, 16] Increased extraction temperature improved precision and recovery of TPH's from ERA certified soil. The volume of solvent required for the quantitative extraction of TPH's from the ERA certified soil was determined to be 1.2 – 1.5 times the sample volume.

PAH's were extracted from SRM 1649 (urban dust), HS-3 (Canadian marine sediment) and the ERA soil under the optimised extraction conditions. Good recoveries and RSD values were obtained for the reference urban dust (88.5 - 125 % recovery and 2.0 - 6.7 % RSD), and for the marine sediment (HS-3). Extraction of eighty five samples (4 - 8 g) of ERA soil assessed the PFE reproducibility. The average recovery was 103 % with 2.7 % RSD. Popp et al.[12] evaluated three different solvents or solvent mixtures (acetone-hexane, acetone-methylene chloride and toluene) to extract PAH's from two real soil samples. Toluene provided the best yields. Figure 3.24 shows the results from one of the soil samples. PFE, with toluene as the solvent, was used to extract other natively polluted samples. In all cases, the yields obtained by PFE were better than those obtained by Soxhlet extraction.

Figure 3.24 Effect of Solvent on PAH Extraction from Greppen Soil



Schantz et al.[20] extracted selected PAH's, the optimum extraction temperature was determined as 100 °C. The effect of extraction solvent was studied at the optimised conditions, i.e. 100 °C and 2000 psi on different certified reference materials. Three solvents were used to extract SRM

1649a (urban dust). The three solvents tested (methylene chloride, hexane-acetone (1:1, v/v) and acetonitrile) gave comparable results to Soxhlet extraction values. For SRM 1650 and SRM 2975 (diesel particulate material) methylene chloride, toluene and toluene-methanol were evaluated as extraction solvents. The results from the three different solvents were comparable. It was noted that PFE seemed to be more efficient than Soxhlet extraction at removing high molecular weight PAH's; such as (benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,j*]anthracene, picene and benzo[*b*]chrysene) in SRM 2975. For SRM 1650, comparison of the PFE extraction results with the certified values yielded good agreement, the only exception being benzo[*ghi*]perylene. This seemingly anomalous result was checked by re-extracting a portion of the material by Soxhlet. The results obtained agreed with those obtained by PFE. The authors' explanation was that there was an error in the original certification results. SRM 1941a was extracted by PFE using methylene chloride, acetonitrile and hexane-acetone. The results were in good agreement with the certified values and the Soxhlet values. Richter et al.[63] extracted PAH's from SRM 1649 (Urban Dust) using PFE. Recoveries ranged from 83.5 % to 126.9 %.

Mosi et al.[64] extracted PAH's from a sediment soil to then study the ion-molecule reactions between the cations CH_3CHF^+ and $CH_3CF_2^+$ generated from 1,1-difluoroethane and the gas chromatographic eluents from the sediment. Chen et al.,[65] have recently interfaced PFE and HPLC for the extraction of PAH's from soil samples. The peaks of PAH's from the PFE-HPLC system were sharp and without significant loss of resolution. The chromatograms also showed similar retention times for loop injection and on-line injection. QCS 345, a certified standard soil was extracted and analysed using PFE-HPLC-FLU and the results obtained were within the limits of the certified values. Jensen et al.[55] extracted PAH's from several reference materials using

PFE. SRS 103-100 was extracted with both PFE and Soxhlet and the results were expressed as a percentage of the Soxhlet value. The recoveries ranged from 83.4 to 142.3 %. Four of the PAH's extracted (anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and dibenz[*a,h*]anthracene) from HS-3 were different from the certified values. It was the authors' opinion, the higher recoveries obtained for the last compounds were due to co-elution with interfering analytes.

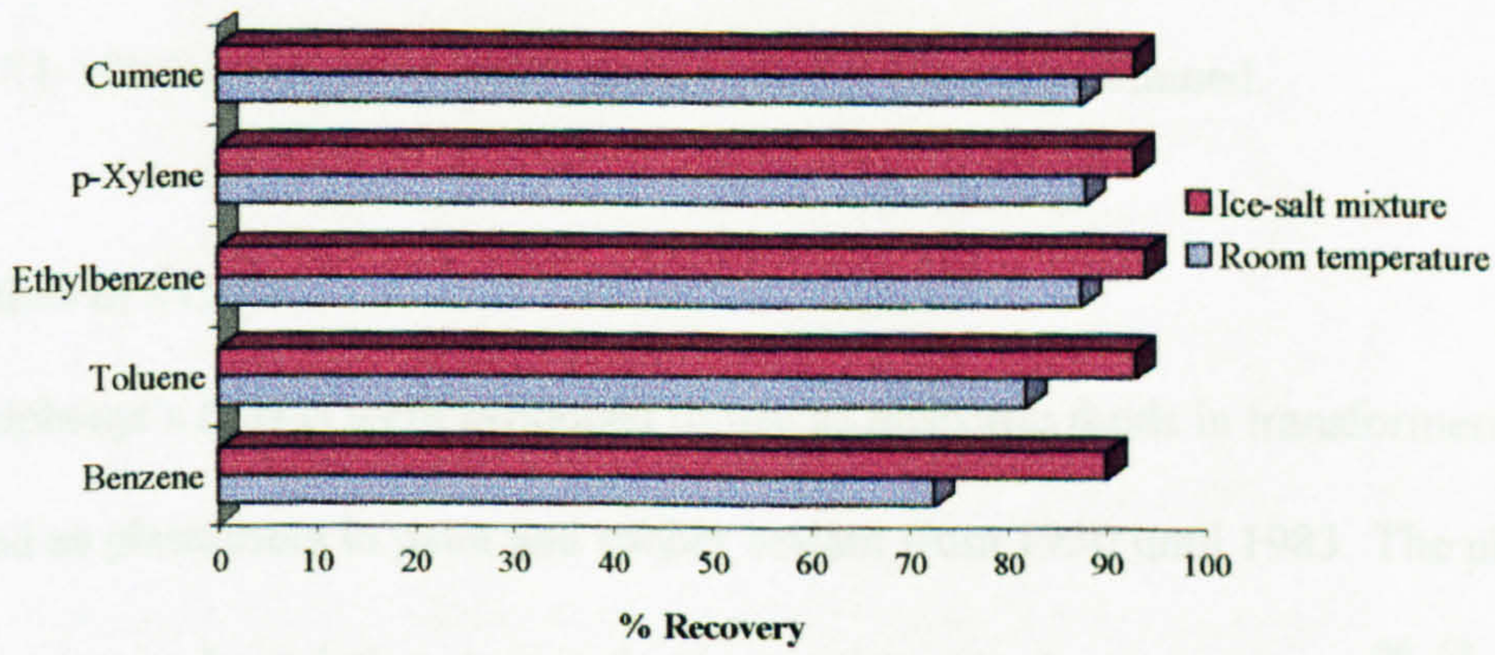
Berset et al.[56] compared different drying, extraction and detection techniques for the extraction of PAH's from natively contaminated soil samples. Soxhlet extraction, alkaline saponification, SFE, PFE, ultrasonic extraction and extraction by shaking. PFE extractions were performed at 13.8 MPa and a temperature of 100 °C with a mixture hexane:acetone:toluene (10:5:1, v/v/v). All the extraction techniques studied were more effective at extracting the PAH's than extraction by sonication. The least variable extraction technique was found to be Soxhlet extraction. However, Soxhlet extraction implied long extraction times (18 hours) and large solvent volumes (160 mL). PFE was the quickest extraction method (0.25 h) and PFE and SFE consumed less solvent than the other extraction methods (30 and 9.3 mL, respectively). However, these latter techniques require more time for method development and higher costs.

3.4.4 Extraction of VOC's

Volatile organic compounds (VOC's) is a term that includes a wide range of naturally occurring and synthetic compounds which have the potential to enter the atmosphere under normal conditions of use. VOC's are found in everything from paint, fuel and coatings to underarm deodorant and cleaning fluids.[66] Many industries, including printing, chemicals, pharmaceuticals, metal cleaning, and photographic supplies, rely on the use of solvents at intermediate points in

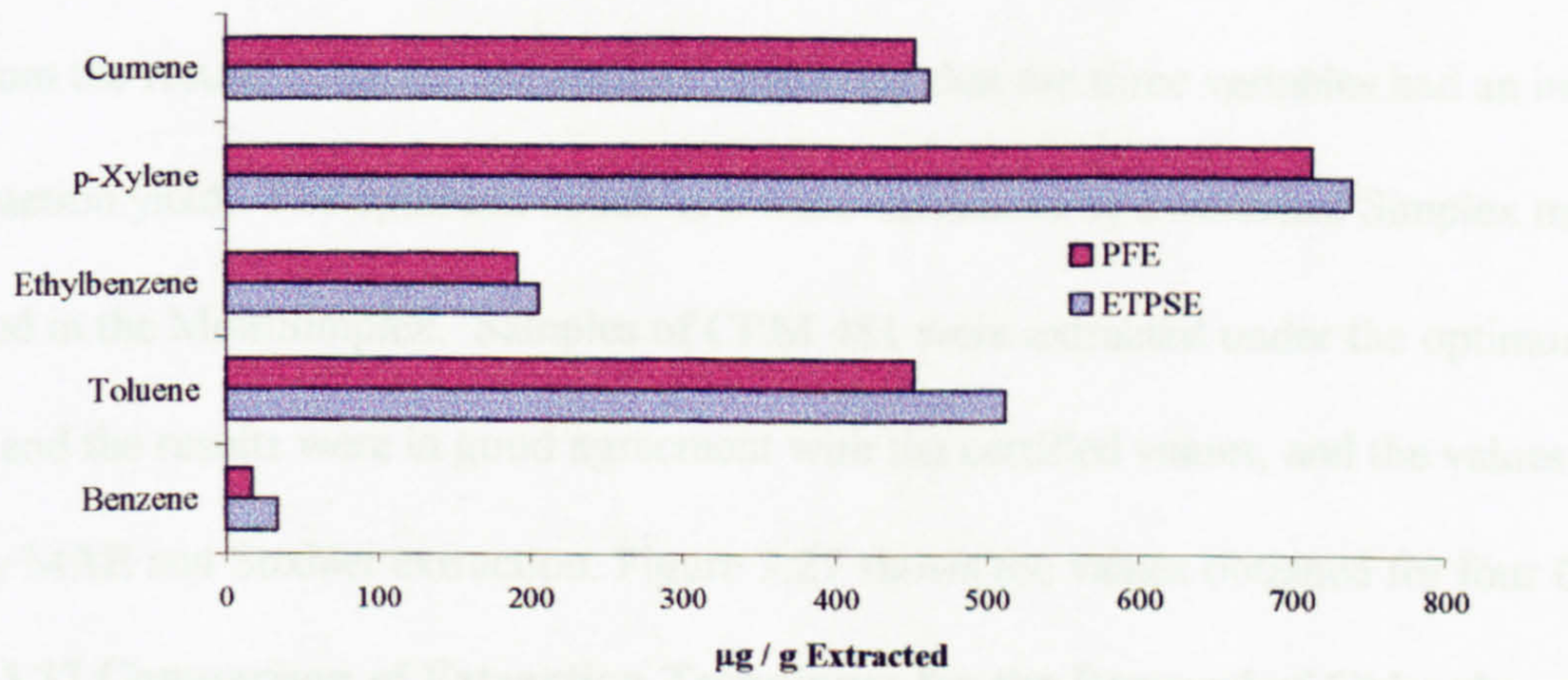
their production processes and these solvents end up as VOC's in the atmosphere. These chemicals often contribute to the production of low-level ozone, which is harmful to animal and plant life and is also a major constituent of photochemical smog, and some of them (benzene, e.g.) are carcinogens. Benzene, toluene, ethylbenzene, xylene and cumene were extracted from a vapour-fortified clay soil (pH 5.3, OM 16 %) by Meney et al.[67] Methanol at elevated temperature (100 or 150 °C) and pressure (100 or 150 bar) for 5 or 30 minutes was used in a home-made system created from parts of HPLC and GC instruments. Analyte extraction increased at the lower temperature and pressure, however this advantage was offset by a loss in precision. Extraction time did not seem to have a significant effect upon extraction recovery. Using this information, the operating conditions were selected. An operating pressure of 150 bar; temperature, 150 °C; and an extraction time of 5 minutes. Due to the volatile nature of the analytes, cooling the collection vial in an ice-salt mixture (temperature ~ - 3 °C) reduced losses. Figure 3.25 shows how lower recoveries (up to 30 %) were obtained when the collection vial was not cooled.

Figure 3.25 Comparison of Collection Efficiencies in High Pressure Solvent Extraction.



The developed "elevated temperature and pressure solvent extraction" (ETPSE) method was compared with a commercial ASE™ system working on the manufacturer's recommended conditions (2000 psi = 143 bar; 100 °C; 5 minutes). Similar levels of ethylbenzene, p-xylene and cumene were extracted with the two systems (see figure 3.26) but the recoveries and, in particular, the precision for more volatile analytes, namely benzene and toluene were significantly improved with ETPSE. The reduced performance of PFE for the volatile analytes was attributed to a lack of a cooling system for the collection vials.

Figure 3.26 Comparison of Commercial and 'Home-Made' Extraction Systems



Richter et al.[15] performed the extraction of BTEX's from a spiked clean sand with methylene chloride at 60°C at 2500 psi and 5 minutes equilibration and 5 minutes static heat. Good recoveries (97.1-100 %) and good RSD values (0.9-3.7 %) were obtained.

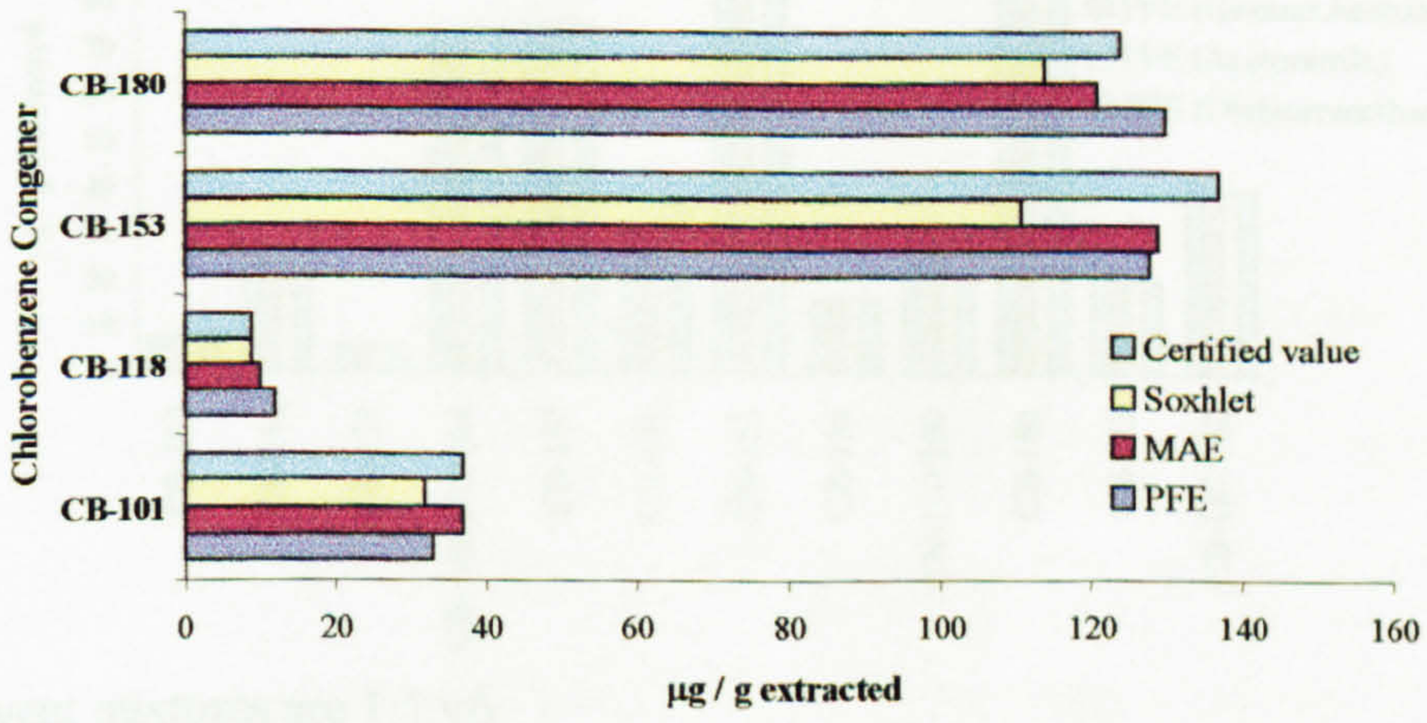
3.4.5 Extraction of PCB's

Chlorinated biphenyl's (CB's) were produced to use as dielectric fluids in transformers and capacitors, and as plasticisers in paint and rubber sealant from 1930 until 1983. The physical and chemical resistance to degradation, means they're persistent in the environment.[66, 68] Several

studies have extracted these ubiquitous pollutants from several environmental matrices, including sludges,[15] soils,[63, 69] and sediment;[14, 20] as well as other biological matrices.[44]

Zuloaga et al.[69] optimised the PFE of PCB's from a natively contaminated soil. The optimised method was applied to the extraction of PCB's from certified industrial soil (CRM 481). The extraction yields were compared with those obtained by MAE and Soxhlet. A central composite design was built to optimise pressure (1000-2400 psi), temperature (70-180 °C) and extraction time (2-16 minutes), while a mixture of acetone:hexane (75:25, v/v) was used as extraction solvent. From the results obtained, the authors concluded that the three variables had an influence on the extraction yield. The optimum conditions were calculated by a modified Simplex method[70] implemented in the MultiSimplex. Samples of CRM 481 were extracted under the optimum conditions and the results were in good agreement with the certified values, and the values obtained by MAE and Soxhlet extraction. Figure 3.27 shows the values obtained for four CB's.

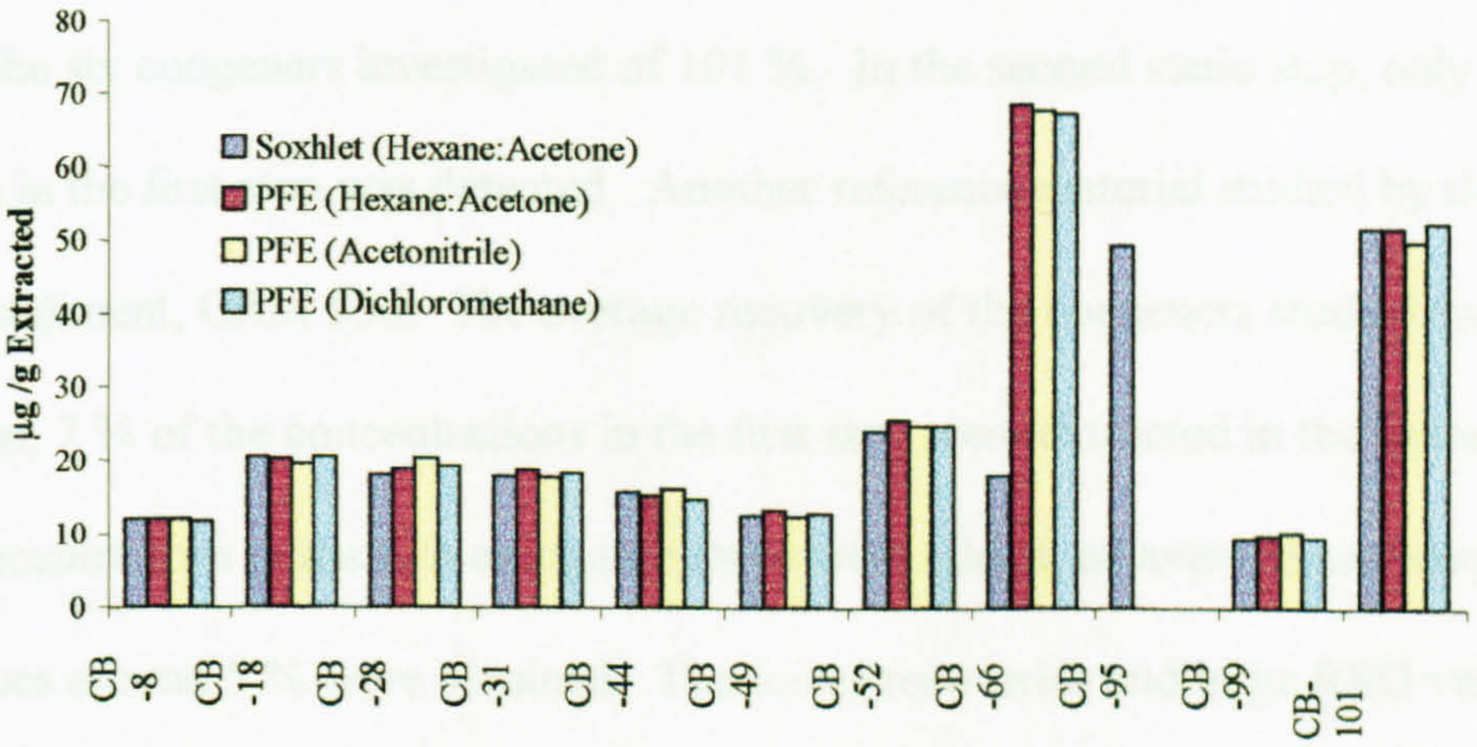
Figure 3.27 Comparison of Extraction Techniques for the Removal of Chlorobenzenes



Richter et al.[15] extracted PCB's from a sewage sludge sample. Recoveries based on the results by Soxhlet ranged from 86.3 % to 90 %. Schantz et al.[20] extracted PCB's from SRM 1649a (urban

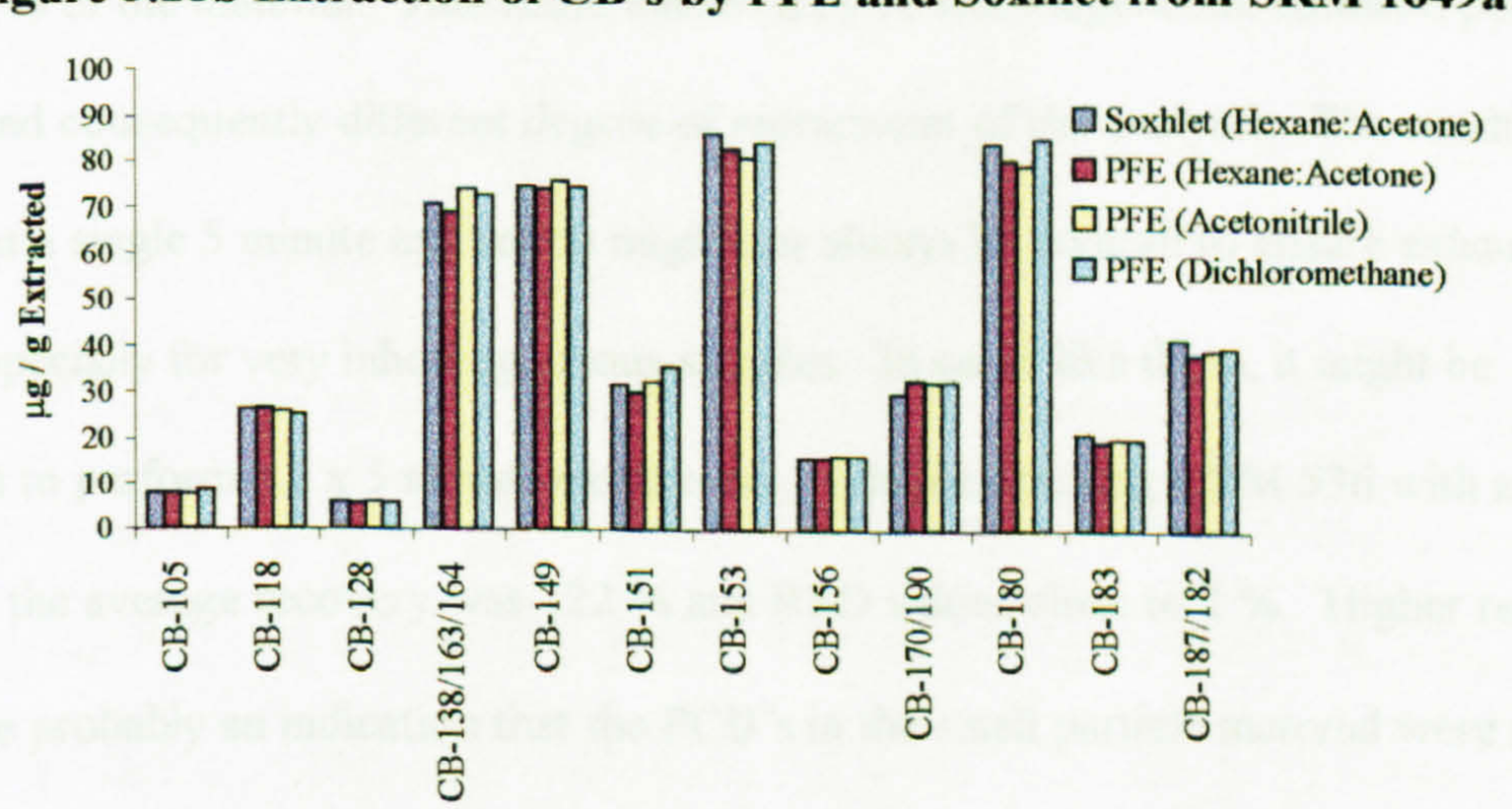
dust), SRM 1941a (marine sediment) and SRM 1944 (waterway sediment) using PFE and three different solvents (hexane-acetone, acetonitrile and methylene chloride). Figures 3.28a and 3.28b shows the results obtained for SRM 1649a are in good agreement with the Soxhlet values.

Figure 3.28a Extraction of CB's by PFE and Soxhlet from SRM 1649a



N. B. All solvent Mixtures are 1:1 v/v

Figure 3.28b Extraction of CB's by PFE and Soxhlet from SRM 1649a



N. B. All solvent mixtures are 1:1 v/v

Richter et al.[63] extracted Aroclor 1254 from a standard reference material (CRM 911-050). Average recoveries of 99.1% and RSD of 3.71% were obtained. Poster et al.[14] used PFE for the certification of PCB's and chlorinated pesticides in SRM 1649a.

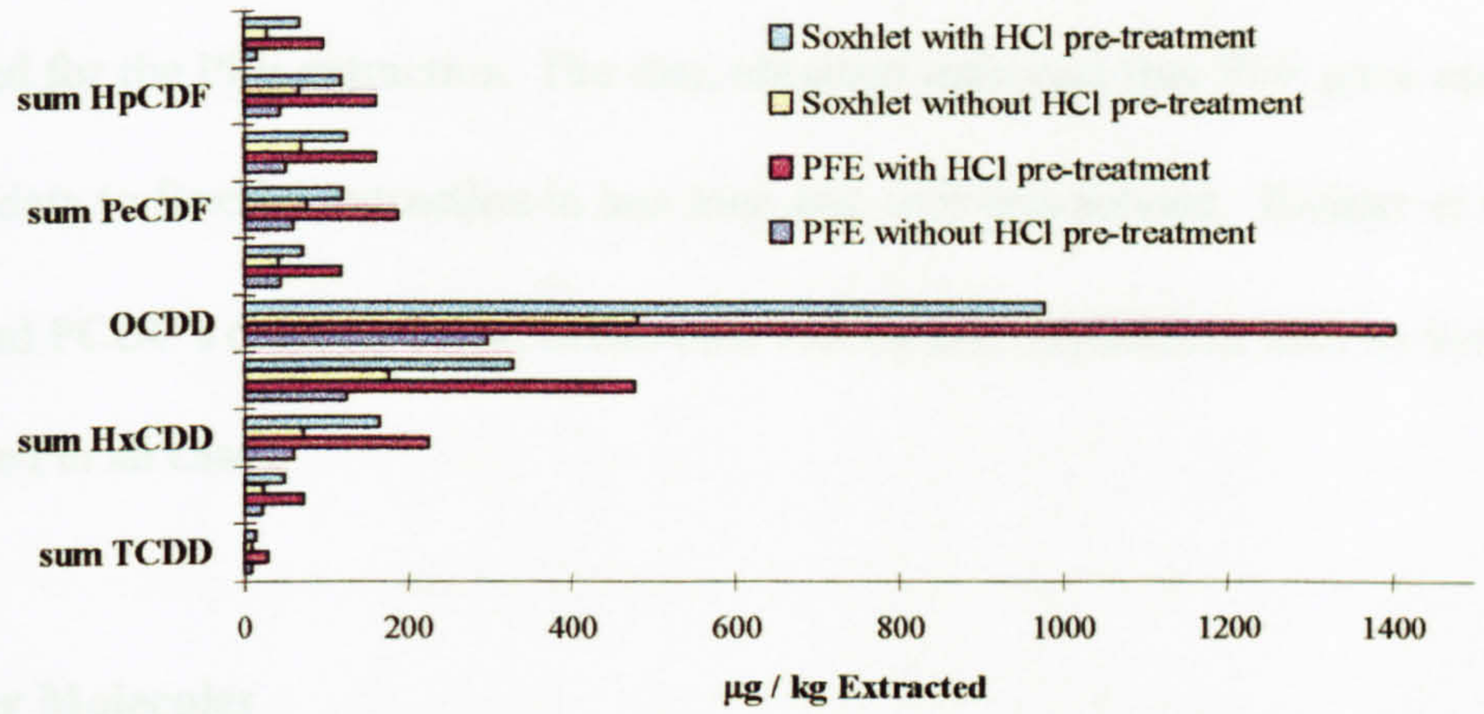
Björklund et al.[24] used PFE to extract PCB's from marine sediment (SRM 1944). Mean recovery for all investigated congeners was 99 % compared to certified values after a 5 minute static step at 100 °C. The average concentration of PCB's found in the second static step was 0.8 % and therefore negligible. PCB's were also extracted from sewage sludge (BCR 392), with an average recovery for the six congeners investigated of 101 %. In the second static step, only 1.4 % of the concentration in the first step was detected. Another reference material studied by this group, was harbour sediment, CRM 536. The average recovery of the congeners studied was of 107 % but in this case, 7 % of the concentrations in the first step were extracted in the second step. When the concentrations in the two extraction steps were added, an average recovery of 114 % and RSD values around 5 % were obtained. The lower recoveries and large RSD values were attributed to differences in particle size. The particle size distribution for this reference material is; 75-1000 μm for 80 % of the material, >1000 μm for 10 % of the material and <75 μm for the remaining 10 % of the material. This could lead to a more inhomogeneous diffusion path distribution and consequently different degree of entrapment of the analytes. The results suggested that a single 5 minute extraction might not always be enough to ensure exhaustive extraction, especially for very inhomogeneous samples. In cases like these, it might be advantageous to perform a 2 x 5 minute extractions. When extracting CRM 536 with a particle size <15 μm, the average recovery was 122 % and RSD values close to 2 %. Higher recoveries obtained were probably an indication that the PCB's in the small particle material were more accessible. PFE was also compared to SFE and higher recoveries were obtained for PFE. The authors noted that the extracts from SFE were cleaner than those from PFE. The higher recoveries found by PFE could be due to interference's.

Wenzel et al.[44] have extracted PCB's from pine needles and mosses. When applying the conditions suggested by the EPA method 3545,[11] (n-hexane-acetone 1:1 v/v, as solvent 100 °C as extraction temperature, 1 x 5 minute extraction time), lower recoveries than those obtained by ultrasonic extraction were obtained. The solvent was changed to n-hexane, and an investigation into the effect of temperature on recovery determined that the best recoveries were obtained at 40 °C. Under these conditions, PCB's that could not be detected by ultrasonic extraction were measured. Similar results were obtained with toluene. However, the long blow down time of toluene prompted use of n-hexane as the extraction solvent. Placing a mixture of Florisil / Al_2O_3 into the extraction vessel also had a positive effect since it served as a preliminary clean-up.

3.4.6 Extraction of PCDD's and PCDF's

Polychlorinated dibenzo-*p*-dioxins (PCDD's) and dibenzofurans (PCDF's) are well known environmental pollutants of high toxic potential.[2] They are by-products of the synthesis of some pesticides (e.g. pentachorophenol) and PCB's and they also appear in incineration processes. Popp et al.,[12] tested the PFE for the extraction of PCDD's / PCDF's from a fly ash originating from a municipal incineration plant. Extraction times of two x 5 minutes and two x 10 minutes were investigated using toluene at 200 °C. Extraction yields increased with extraction times but were still lower than the recoveries obtained by Soxhlet extraction, especially when the degree of chlorination increased. HCl pre-treatment prior to extraction by Soxhlet or PFE increased the recovery. Comparison of this data showed that PFE removed more of the target analytes than Soxhlet extraction (see figure 3.29).

Figure 3.29 Comparison of PFE and Soxhlet for PCDD / PCDF extraction



Bautz et al.[2] used dynamic high pressure solvent extraction (DHPSE) to extract PCDD's / PCDF's from fly ashes from several municipal waste incinerators, from particles from a dust collector of metal mills and from a soil sample from an industrial area. DHPSE continuously cycles fresh solvent under elevated temperatures and pressures through the sample. Generally, the concentration of PCDD's / PCDF's determined by DHPSE exceeded that of Soxhlet extraction. To exclude the possibility of PCDD's / PCDF's formation potential precursors present in the sample at high temperatures and pressures chlorophenols, chlorobenzenes, and PCB's were spiked on a clean sample free of PCDD's / PCDF's. The spiked sample was extracted by DHPSE. Formation of dibenzodioxins or furans was not observed. From this investigation, it could be concluded that the DHPSE gave better recoveries than the Soxhlet extraction procedure. The results from acid pre-treated samples and untreated sample were comparable. Inconsistent results were obtained when toluene-glacial acetic mixture suggested by Richter[15] was used. In the authors' opinion, for screening of unknown samples, DHPSE may be employed without time-consuming acid pre-treatment. Static and dynamic extractions were also compared in this work. Static extraction required several cycles to achieve satisfactory recoveries. Richter et al.[71] extracted PCDD's / PCDF's from chimney brick, urban dust, fly ash, one soil and three sediment

samples. As an alternative to acid pretreatment, glacial acetic acid at 5 % (v/v) was added to the toluene used for the PFE extraction. The data obtained indicated that PFE gave essentially equivalent data to Soxhlet extraction in less time and with less solvent. Richter et al.[72] extracted PCDD's and PCDF's chimney brick, urban dust and fly ash. Equivalent data to Soxhlet extraction was obtained in all cases.

3.4.7 Other Molecules

Other molecules extracted by PFE include semi volatile organic chemicals (SVOC's), non pesticidal organophosphorus and organosulphur compounds, as well as surface active compounds, from soils and biological matrices. Fisher et al.[37] extracted some SVOC's (o-xylene, phenol, 2,4-dichlorophenol, naphthalene, diethyl phthalate, heptadecane, pyrene, endrin and DDT) from soil samples using PFE and Soxhlet extraction. Blanks contained interfering peaks that co-eluted with certain target analytes. Some of these interferences could not be avoided using SIM mode since the target analyte and the interfering compound had major fragment ions in common. PFE blanks contained some peaks that were absent in the Soxhlet blanks. This suggested that reactions involving the soil organic matter were more important under the high temperature and pressure conditions of PFE. It has been suggested[73] that dissolved oxygen in the system might be responsible for some of the reactions and that the PFE blank might be reduced if the extraction solvent were degassed just prior to use. Tomkins et al[74] organosulphur analytes and phosphorus containing analytes from spiked soil and concrete samples. The recovery of organosulphur compounds was good (95 %), whereas lower recoveries were obtained for organophosphonates (60-80 %). The authors suggested that organophosphonates were either more difficult to extract from both matrices than organosulphur species or they were partially degraded on sample surface.

David et al.[16] studied the influence of temperature (25-200 °C), pressure (700-2500 psi) in the PFE extraction of non-pesticidal organophosphorus hydraulic fluids from soil samples, and compared it with Soxhlet extraction and SFE. Temperature had a minor effect on the extraction yields and best recoveries were obtained at 100 °C. In the authors opinion high values of density and diffusivity improved recoveries, better repeatability was obtained at lower temperatures and since higher temperatures required several minutes between runs to allow the system to cool, 100 °C was chosen as extraction temperature. They concluded that higher pressures increased extraction yields. At higher pressure, the solvent is able to invade better the solid matrix and diffusivities and densities are higher. These three facts are supposed to improve extraction efficiencies. Both SFE and PFE provided recoveries similar to those obtained by Soxhlet; shorter extraction times and smaller solvent volumes were needed with PFE and SFE, although better precision was obtained with Soxhlet extraction.

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Section A

Method Development

Section A

Method Development.

This section concentrates on the optimisation of PFE extraction parameters for a range of pesticide molecules from soils, Hyde Farm, 18 Acres, Chalgrove Farm and Chamberlain soils were supplied by Zeneca AgroChemicals, Jealott's Hill Research Station, Berkshire, UK. Garden soil was collected from a local garden. Compost was purchased from a local garden centre (John Innes compost No. 2). Mixes 1 to 3 were prepared in-house by mixing compost with three other soils in varying proportions, as shown in table 1. The soils were mixed thoroughly. Garden soil, Compost and the three mixed soils were analysed by National Resources Management (NRM, Berkshire, UK) for part6icle distribution, organic matter content, pH, and cation exchange capacity.

Table 1

Soil Mixture	Amount Compost (kg)	Amount Hyde Farm Soil (kg)
Mix 1	0.50	0.50
Mix 2	0.25	0.75
Mix 3	0.75	0.25

Chapter 4 concentrates on the evaluation of extraction techniques for the extraction of two pyrimidine pesticides; bupirimate and ethirimol from spiked inert hydromatrix. Four techniques are compared; Shake Flask, sonication, Soxhlet and PFE. The influence of PFE temperature and pressure are assessed, and the optimum extraction parameters are chosen using this data. The results of this study are applied to three spiked standard soils

(supplied by Zeneca AgroChemicals). The influence of soil matrix for these compounds is briefly investigated.

Chapter 5 optimises extraction parameters for pentachlorophenol, using both inert matrices, spiked soils and a certified reference material. The influence of extraction solvent is investigated, as is the influence of soil matrix on the efficiency of pentachlorophenol extraction.

Chapter 6 investigates the optimum extraction parameters and solvents for the extraction of three closely related organochlorine pesticides, DDT, DDD and DDE from a natively contaminated soil supplied by AstraZeneca. The results of PFE are compared with Soxhlet extraction.

Chapter 4

Bupirimate and Ethirimol

Bupirimate and Ethirimol

4.0 Introduction

This chapter assesses the effect of PFE extraction pressure and temperature, and compares PFE with other extraction techniques. The optimised PFE extraction parameters were applied to the extraction of bupirimate and ethirimol to soils of various compositions. The influences of both extraction solvent type and soil composition were investigated. Bupirimate and ethirimol are manufactured by Zeneca AgroChemicals and are marketed under the trade names Nimrod and Milgo, respectively.[1]

4.1 Experimental

4.1.1 Instrumentation.

An ASE™ 200 Accelerated Solvent Extractor (Dionex (UK) Ltd., Camberley, Surrey) with 11 mL extraction cells was used to perform the extractions. The derivatised extracts were analysed on a GC-MSD (Hewlett-Packard, Palo Alto, USA) in selected ion monitoring mode.

4.1.2 Soil

Various standard soils were provided by Zeneca AgroChemicals, Jealott's Hill, Berkshire. They were spiked in-house with bupirimate and ethirimol pesticides. After spiking, the soil was stored in the dark at room temperature.

4.1.3 Chemicals

The solvents used in this study were certified analytical reagents (Fisher Scientific, Loughborough, Leicestershire). Hydromatrix (Varian Ltd., Surrey, UK) was used to fill the head space of the PFE extraction cells (Dionex), and as an inert matrix for the

spike recovery experiments. Anhydrous sodium sulphate (Merck, Poole, UK) was mixed with the soil sample during Soxhlet extraction. Bupirimate and ethirimol standards were supplied by Zeneca AgroChemicals.

4.1.4 GC-MSD Analysis

The GC-MSD (HP G1800A GCD system, Hewlett Packard, Palo Alto, USA) was operated in selected ion monitoring mode with a splitless injection volume of 1.0 μL. The column used was a DB-5ms (J & W Scientific, Folsom, California, USA), with dimensions of length 30 m x 0.25 mm i.d. x 0.25 μm film thickness. The temperature program used for the analysis was 120 °C, held for 2 minutes to 290°C at a rate of 5 °C/ minute, with a final hold time of 2.5 minutes. The injection port and detector temperatures were set at 250 °C and 280 °C, respectively.

4.1.5 Fortification Procedures

Slurry spike procedure.

Hydromatrix, (45 g) was slurry spiked with bupirimate and ethirimol (10 μg / mL of 2000 μg / mL stock) in DCM (25.00 mL). The solvent was allowed to evaporate and the soil was left to age in the dark for a period of one month. Hydromatrix (1.00 g accurately weighed) was extracted under the conditions stated in the experimental section. Six replicates were performed by each extraction method.

Spot spike procedure

Hydromatrix, (1.00 g) was spot spiked with bupirimate and ethirimol (10 μg / mL of 2000 μg / mL stock) in DCM. The solvent was allowed to evaporate and the soil was

extracted under the conditions stated in the experimental section. Six replicates were performed by each extraction method.

4.1.6 Extraction procedures of fortified matrices

PFE Extraction

Hydromatrix or soil (1 g, accurately weighed) was placed in a stainless steel PFE extraction cell (11 mL capacity) on top of a filter to prevent cell frit blockage. Hydromatix was used to fill the head space to reduce solvent consumption. The cell was placed in the carousel and extracted used the following conditions: pressure; 2000 psi (1 psi = 6894.76 Pa), temperature, 100 °C, with a static extraction time of 5 minutes. Three static / flush cycles were used. The total extraction time was 35 minutes per sample.

Sonication

Hydromatrix or soil (1 g accurately weighed) was sonicated (10 minutes) with DCM (2 x 5.00 mL) and quantitatively transferred to a volumetric flask (10.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer. Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

Shake flask

Hydromatrix or soil (1 g accurately weighed) was shaken (10 minutes) with DCM (2 x 5.00 mL) and quantitatively transferred to a volumetric flask (10.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer.

Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

Soxhlet extraction.

Hydromatrix or soil (2 g accurately weighed) was Soxhlet extracted (24 hours) with DCM (20 mL) and quantitatively transferred to a volumetric flask (25.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer. Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

4.1.7 Effect of PFE temperature and pressure

Effect of pressure

Hydromatrix, (1 g accurately weighed) was extracted by PFE (40 °C, 5 minutes) with at 1000, 2000 and 3000 psi. The extract was quantitatively transferred to a volumetric flask (25.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer. Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

Effect of Temperature

Hydromatrix, (1 g accurately weighed) was extracted by PFE (2000 psi, 5 minutes) at the following temperatures 40 °C, 70°C, 100 °C and 150°C. The extract was quantitatively transferred to a volumetric flask (25.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer. Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

4.2 Results and Discussion.

4.2.1 Chromatography and analyte identification

Figure 4.1 Pyrimidine Chromatogram

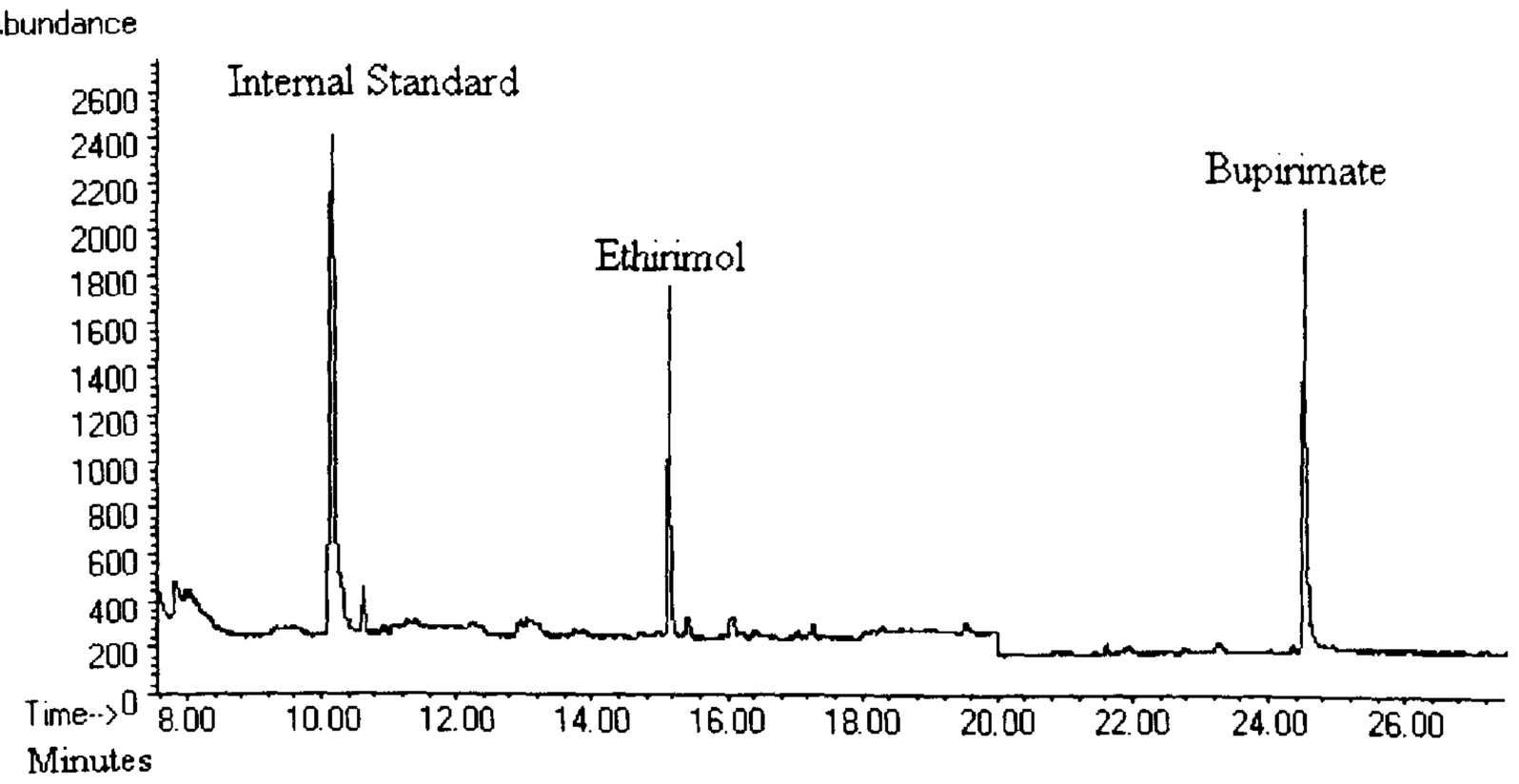


Figure 4.1 shows a chromatogram of the pyrimidine pesticides used in this chapter. The internal standard was hexadecane (rt. = 10.17 minutes). The retention times of bupirimate and derivatised ethirimol were 24.5 minutes and 15.7 minutes, respectively.

The mass spectrum for bupirimate is shown in figure 4.2. Using the mass spectrum, the ions chosen for SIM for bupirimate were m / z 316, m / z 273, and m / z 208.

Figure 4.2 Mass Spectrum for bupirimate

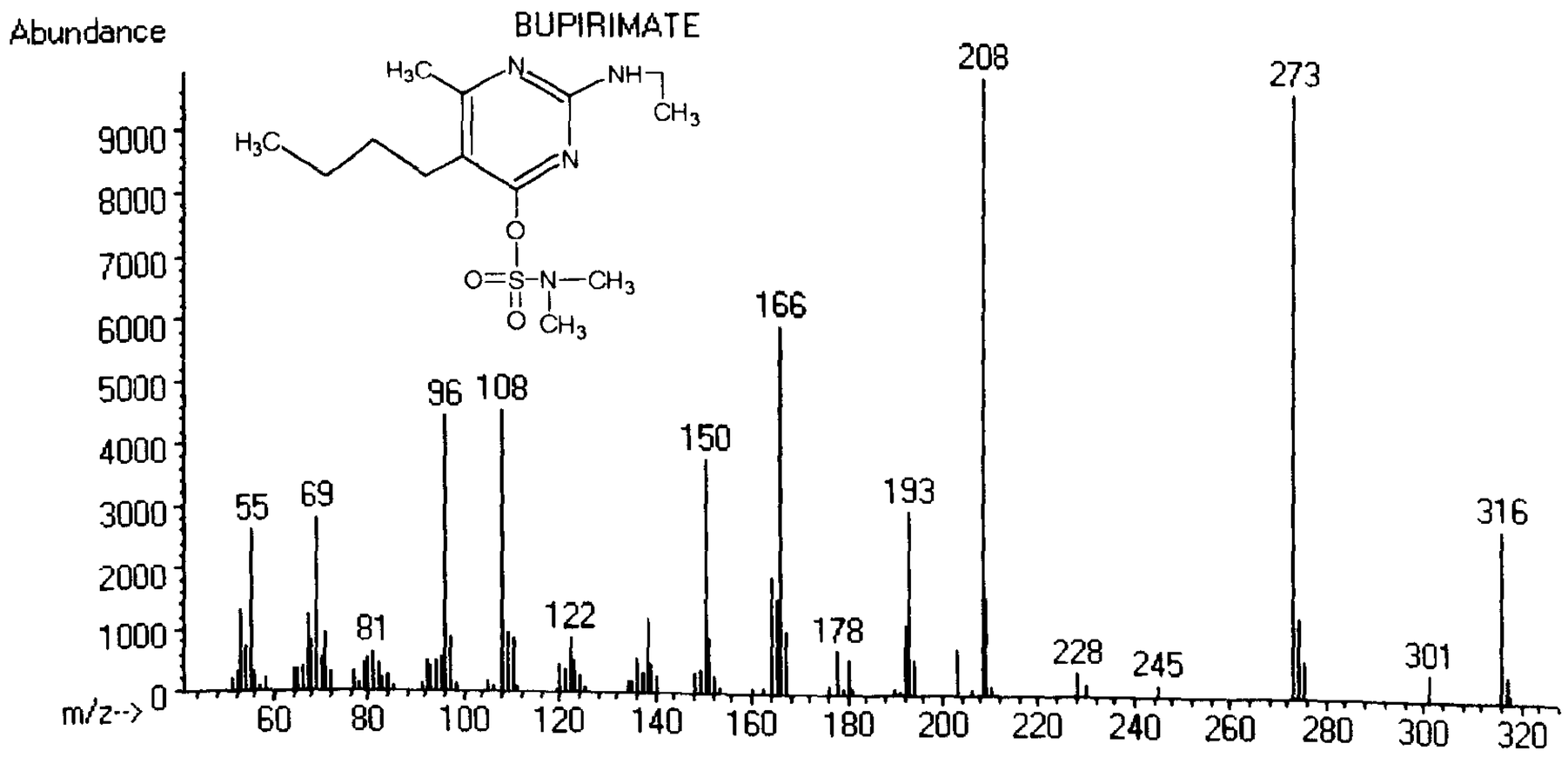


Figure 4.3 shows the mass spectrum for underivatised ethirimol. The SIM ions chosen for derivatised ethirimol were m / z 266 and m / z 238

Figure 4.3 Mass Spectrum for underivatised ethirimol

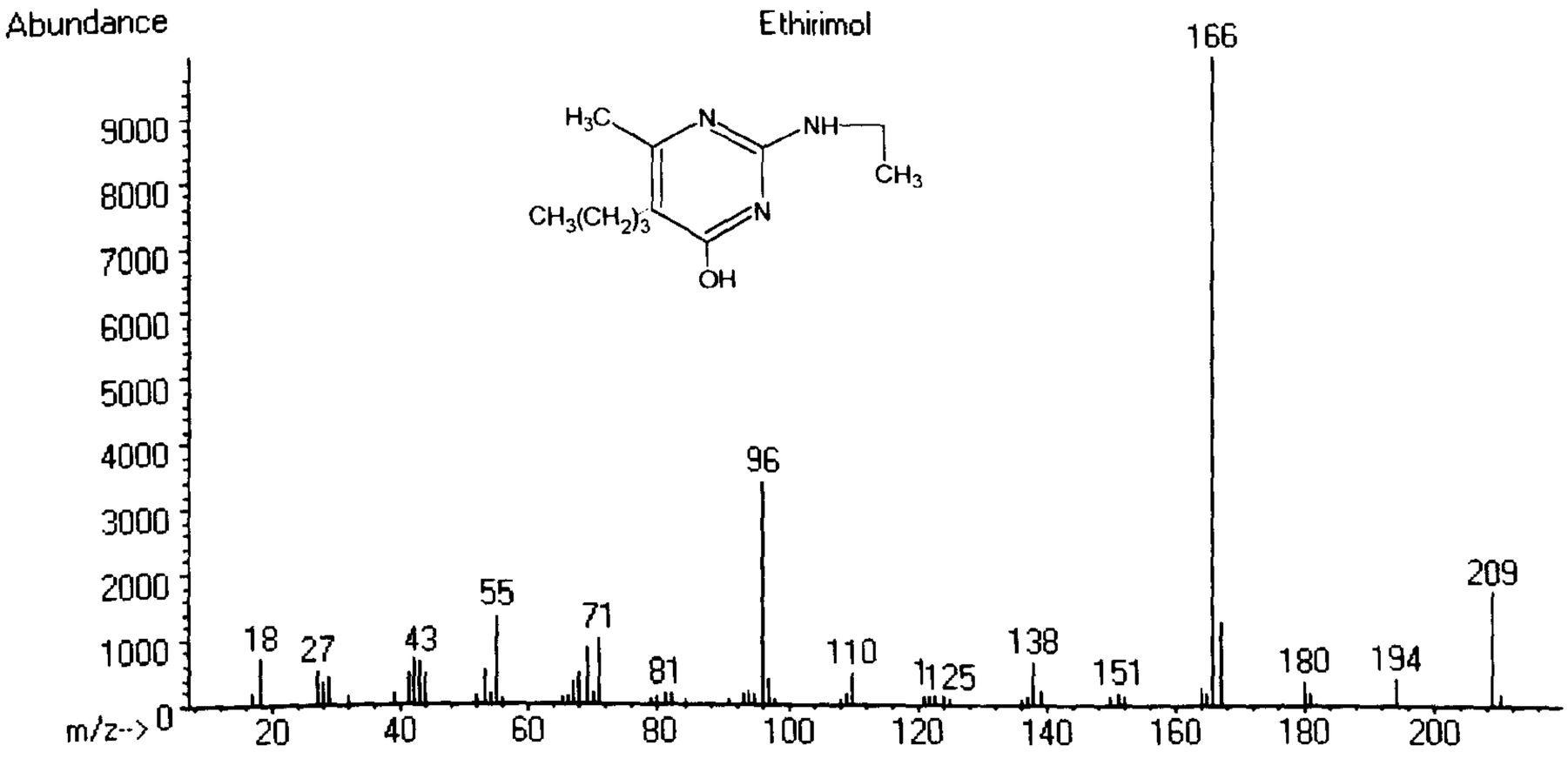


Table 4.1 shows the calibration data for bupirimate and ethirimol. Both correlation coefficients are excellent, indicating linear behaviour over the calibration range (0 $\mu g\ mL^{-1}$ to 4 $\mu g\ mL^{-1}$).

Table 4.1 Calibration data

Compound	Calibration range	Number of data points	Equation	Correlation coefficient, R^2
bupirimate	0 $\mu g\ mL^{-1}$ to 4 $\mu g\ mL^{-1}$	9	$y = 0.3475x + 0.0503$	0.9924
ethirimol	0 $\mu g\ mL^{-1}$ to 4 $\mu g\ mL^{-1}$	9	$y = 0.3201x + 0.0057$	0.9961

4.2.2 Initial studies on bupirimate and ethirimol.

The chromatographic process required derivatisation of the ethirimol, to a silyl ether. Hydroxyl molecules disrupt the derivatisation process; hence, careful selection of the solvent is essential. A study was undertaken to ascertain the losses incurred to bupirimate and ethirimol during acetone removal and subsequent dissolution in DCM. Table 4.2 clearly shows that the blowdown procedure does not effect the recovery of

bupirimate. Quantitative recovery was achieved for all the solvents. However, the blowdown procedure required for acetone removal caused a 40 % loss for ethirimol. An alternative solvent mixture was suggested. This was acetonitrile:dichloromethane (1:1, v/v). A study into the effect of ACN on the derivatisation process was undertaken and proved that the presence of ACN did not have a detrimental effect on the derivatisation process. Table 4.2 summarises the results of the derivatisation study.

Table 4.2 derivatisation study

	% Recovery (% RSD)	
	bupirimate	**ethirimol**
Acetone	92.2 (9.2)	46.5 (10.5)
Dichloromethane	90.0 (4.1)	92.5 (6.6)
Acetonitrile	91.0 (2.4)	80.1 (7.4)
Acetonitrile : dichloromethane 1:1 v/v	90.0 (8.9)	89.4 (5.6)

Effect of pressure and temperature on pyrimidine extraction

In order to assess the effect of pressure and temperature on the recovery of bupirimate and ethirimol, an extraction study on spiked hydromatrix was performed. To remove the effect of pressure and temperature, spot and slurry spiked hydromatrix was extracted with DCM by sonication and shake flask. To remove the effect of pressure, but to determine the effect of temperature, the hydromatrix was also extracted using Soxhlet extraction. To determine the effect of pressure, the PFE was used at 40 °C and a pressure study was performed.

4.2.3 PFE pressure Study

Table 4.3 and figures 4.4 and 4.5 shows the effect of pressure on the PFE extraction of slurry and spot spiked Hydromatrix.

Table 4.3 Effect of pressure on the extraction from spiked Hydromatrix, n = 6.

	Spot Spike (% Recovery)		Slurry Spike (% Recovery)	
	bupirimate	**ethirimol**	**bupirimate**	**ethirimol**
PFE 1000 psi	101.8	93.3	83.2	81.7
PFE 2000 psi	98.9	90.3	83.2	80.7
PFE 3000 psi	93.9	86.7	79.5	78.0

Figures 4.4 and 4.5, and table 4.4 show that quantitative recovery was achieved from spot spiked hydromatrix, and near quantitative recovery was achieved from slurry spiked hydromatrix. Analysis of variance, ANOVA, showed that the results for each pressure were statistically similar, and hence pressure did not have a detrimental effect on the recovery of either molecule. For convenience, 2000 psi was used in subsequent extractions.

Figure 4.4 Effect of pressure on pyrimidine extraction from spot spiked Hydromatrix

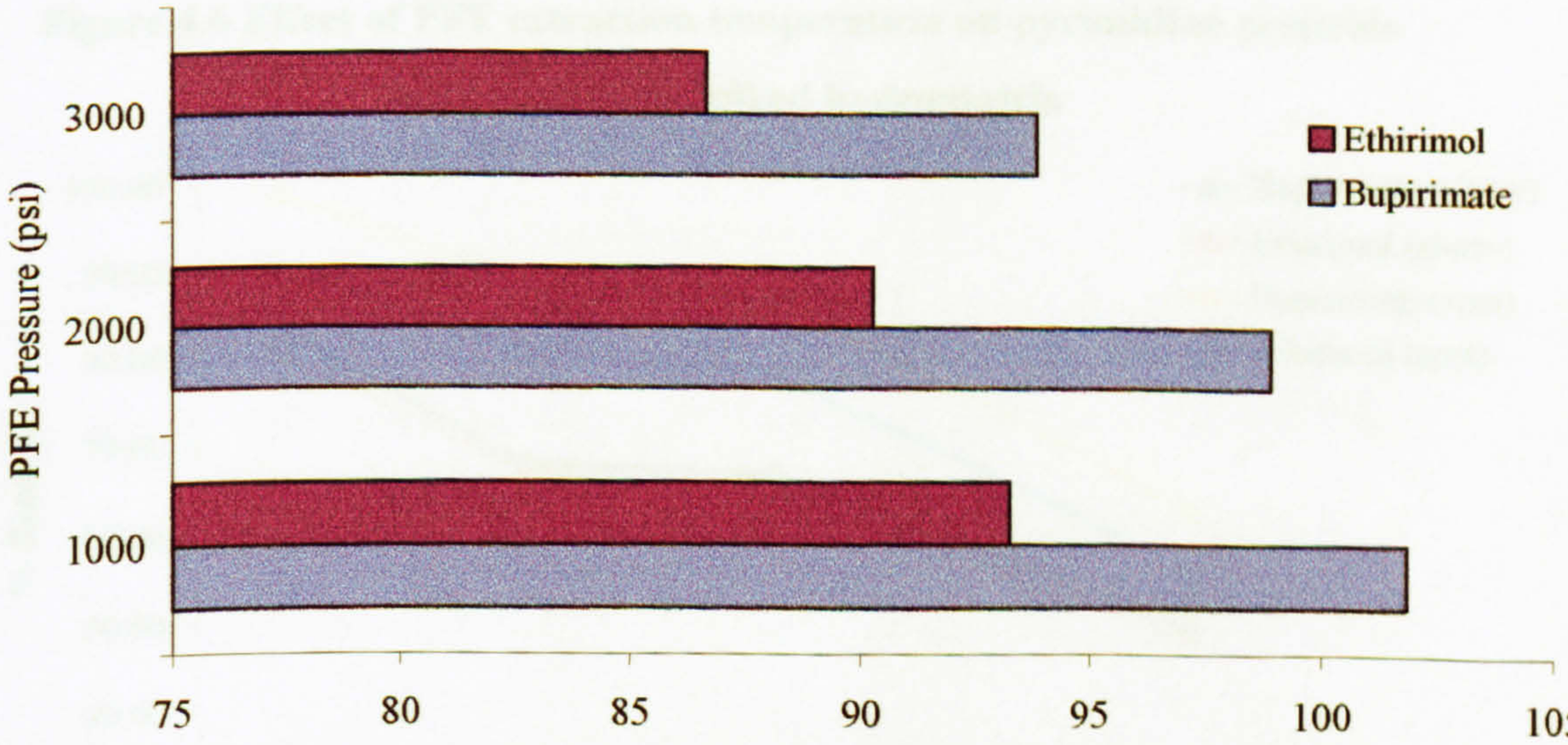
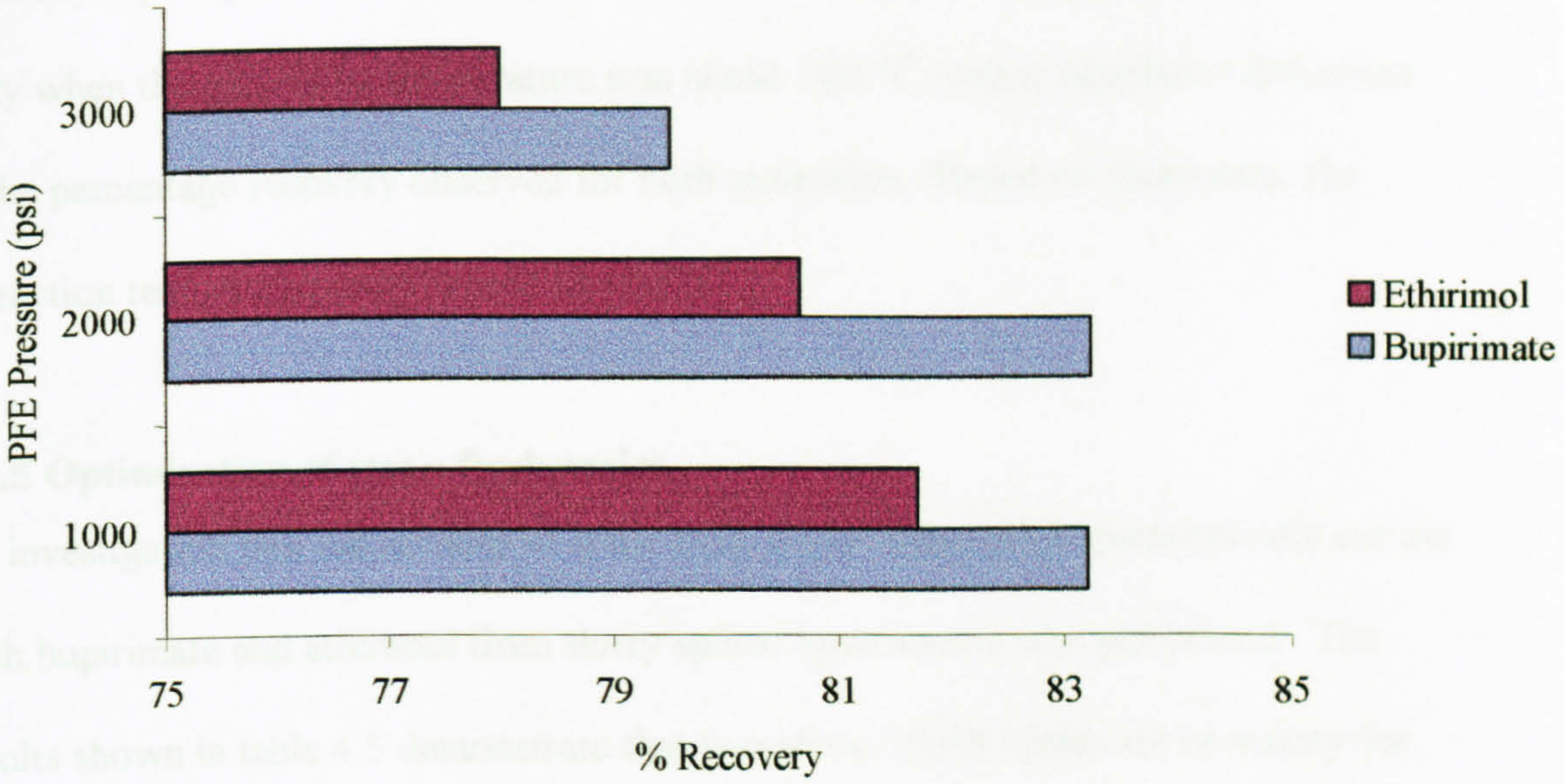


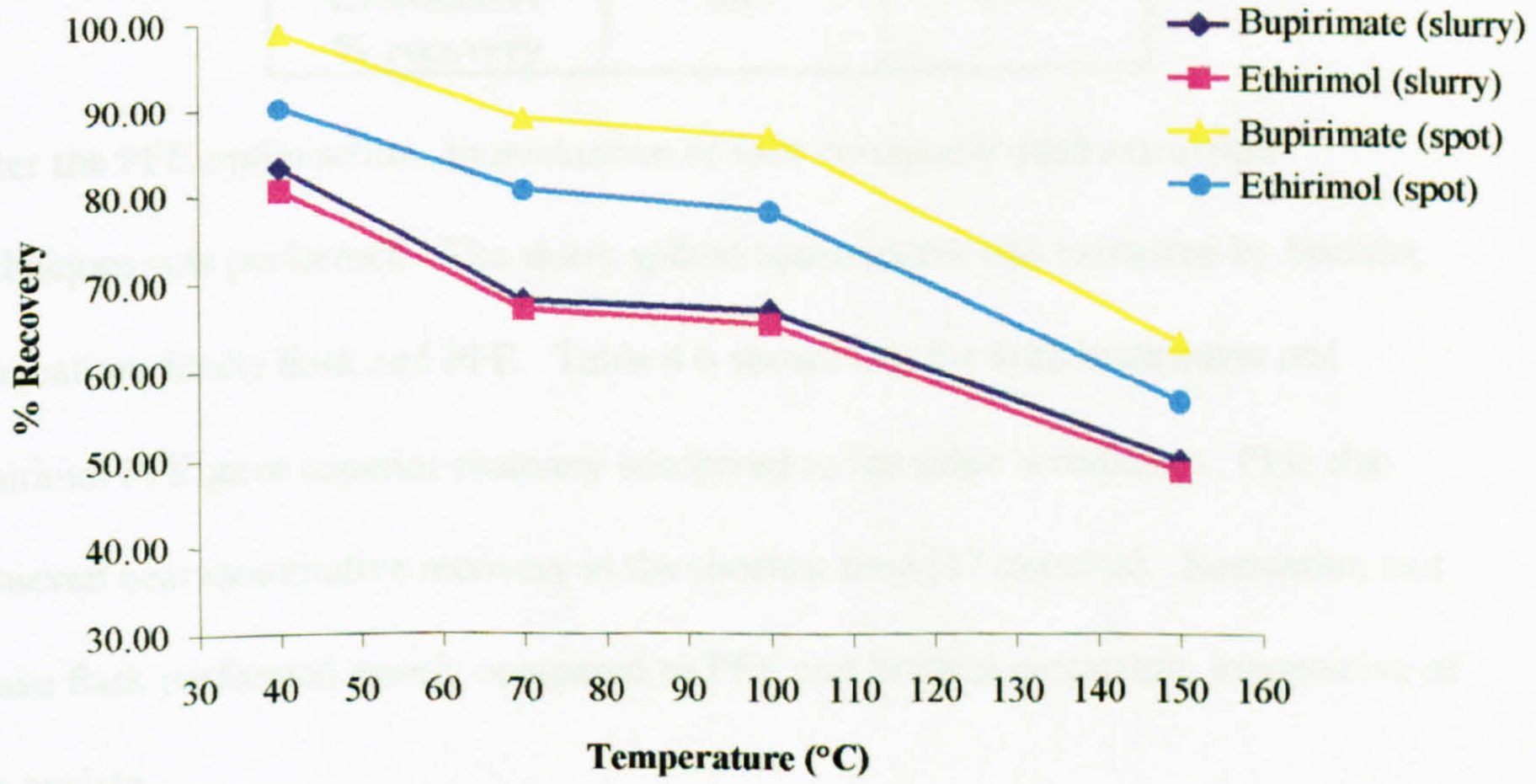
Figure 4.5 Effect of pressure on pyrimidine extraction from slurry spiked Hydromatrix



4.2.4 PFE temperature Study

Figure 4.6 shows the effect of temperature on the recovery of the two molecules from spiked hydromatrix by PFE.

Figure 4.6 Effect of PFE extraction temperature on pyrimidine pesticide extraction from spiked hydromatrix



Relatively high recoveries (> 70 %) of both bupirimate and ethirimol were achieved from both spot spiked and slurry spiked hydromatrix up to a temperature of 100 °C. Only when the extraction temperature was above 100 °C, was a significant difference in the percentage recovery observed for both molecules. Based on these data, the extraction temperature was chosen as 100 °C.

4.2.5 Optimisation of static flush cycles

An investigation into the number of static flush cycles required to quantitatively extract both bupirimate and ethirimol from slurry spiked hydromatrix was performed. The results shown in table 4.5 demonstrate that two static / flush cycles are necessary for the maximum extraction of bupirimate and ethirimol from slurry spiked hydromatrix.

Table 4.5 Cumulative total of the cycle study
(extraction from slurry spiked hydromatrix)

Number of cycles	bupirimate (% recovery)	ethirimol (% recovery)
1	47.6	43.0
2	18.7	21.0
3	0.4	0.3
Cumulative % recovery	66.7	64.3

After the PFE optimisation, an evaluation of four commonly used extraction techniques was performed. The slurry spiked hydromatrix was extracted by Soxhlet, Sonication, Shake flask and PFE. Table 4.6 shows that for both bupirimate and ethirimol PFE gave superior recovery compared to the other techniques. PFE also achieved near quantitative recovery in the shortest time (17 minutes). Sonication and shake flask performed poorly compared to PFE and Soxhlet extraction, irrespective of the analyte.

Table 4.6 Comparison of extraction techniques

Technique	Extraction Time	% Recovery (% RSD)	
		bupirimate	ethirimol
Soxhlet	24 hours	76.6 (8.1)	75.9 (8.5)
Sonication	2 x 10 minutes	70.1 (5.3)	69.2 (4.4)
Shake Flask	2 x 10 minutes	71.3 (3.2)	69.9 (3.4)
PFE	17 minutes	83.2 (4.9)	80.7 (4.9)

Application to slurry spiked soil

To determine the efficiency of PFE on soil samples, three soils were slurry spiked with both bupirimate and ethirimol at the 20 μg / g level. The soil composition is shown in table 4.7. Tables 4.8 and 4.9 compare PFE extraction with the three other techniques used in the hydromatrix spiking study.

Table 4.7 Soil composition

Soil	% Silt	% Clay	% Sand	pH	CEC	% OM
Hyde farm	23	19	58	6.7	17.4	3.2
Chamberlain	4	9	87	7.3	11.0	4.5
18 Acres	24	20	56	6.3	14.0	4.7
Chalgrove Farm	29	37	34	7.4	29.7	5.8
Garden	18	11	71	7.2	16.5	9.8
Mix 2	3	11	86	5.9	12.7	17.5
Mix 1	22	25	53	5.3	32.1	31.3
Mix 3	21	30	49	5.2	41.7	59.4
Comp	22	48	30	5.0	17.6	82.7

Table 4.8 Comparison of extraction techniques for bupirimate (% recovery).

	Chamberlain		18 Acres		Hyde Farm	
	Spot	Slurry	Spot	Slurry	Spot	Slurry
Soxhlet	77.6	72.1	78.6	74.4	88.0	81.8
Sonication	65.0	58.0	66.2	57.6	73.0	71.0
Shake flask	67.0	60.0	68.1	56.0	75.0	67.8
PFE	79.0	73.5	81.3	75.5	89.1	82.0

Table 4.9 Comparison of extraction techniques for ethirimol (% recovery).

	Chamberlain		18 Acres		Hyde Farm	
	Spot	Slurry	Spot	Slurry	Spot	Slurry
Soxhlet	68.7	63.8	69.6	65.8	80.0	74.4
Sonication	57.5	51.3	58.6	51.0	66.4	64.5
Shake flask	59.3	53.1	60.3	49.6	68.2	61.6
PFE	69.9	65.0	71.9	66.8	81.0	74.5

These data show that extraction recovery from spot spiked soil is higher than the extraction of slurry spiked soil. This is due to analyte-matrix interactions that form upon aging. Again, this data shows that PFE and Soxhlet are comparable techniques, whilst sonication and shake flask are consistently poorer. The extraction of bupirimate and ethirimol are highest on Hyde Farm soil, and comparable on both 18 acres and Chamberlain soil. The common factor is organic matter content of the soil (table 4.7). Hyde Farm soil has the lowest organic matter (3.2 %), whilst 18 Acres soil and Chamberlain soil have similar organic matter contents, 4.7 % and 4.5 %, respectively. This indicates that soil composition has a direct influence on the extraction efficiency. To investigate this further a study into the influence of the soil matrix and effect of the extraction solvent was performed.

4.2.6 Soil and solvent study

To investigate the effect of soil composition nine soils of various compositions (table 4.7) were spiked with bupirimate and ethirimol. The soils extracted with six different solvents covering a range of polarities and classes, to determine the influence of solvent type. The soils were extracted by PFE under the optimum conditions stated above.

The results of this study are shown in tables 4.10 and 4.11

Table 4.10 Soil / solvent study results for bupirimate extraction (as % recovery, (%RSD))

Soil	DCM	Iso-hexane	ACN	ACN:DCM 1:1 v/v	ACN:DCM 1:4 v/v	Iso-hexane: (ACN:DCM 1:1 v/v) 2:1 v/v
Hyde farm	67.4 (4.1)	55.3 (4.3)	54.8 (5.2)	79.4 (3.3)	75.4 (3.3)	61.2 (5.1)
Chamberlain	65.7 (3.8)	51.8 (3.2)	53.3 (4.4)	77.7 (3.2)	73.8 (3.7)	60.0 (5.0)
18 Acres	65.2 (4.5)	53.0 (3.5)	52.6 (4.3)	75.8 (4.5)	72.0 (4.5)	59.4 (4.7)
Chalgrove Farm	64.1 (5.0)	50.9 (3.3)	51.4 (3.7)	73.6 (4.2)	69.9 (4.4)	58.6 (4.9)
Garden	63.8 (3.7)	45.8 (4.2)	49.8 (3.6)	70.1 (3.9)	67.6 (5.3)	53.8 (4.2)
Mix 2	63.3 (4.2)	49.0 (4.4)	47.4 (3.2)	69.8 (4.0)	67.0 (4.7)	51.0 (3.7)
Mix 1	62.8 (2.9)	49.2 (3.3)	45.4 (5.4)	69.5 (3.6)	66.3 (4.5)	49.0 (3.3)
Mix 3	62.3 (3.1)	38.9 (3.9)	43.2 (5.2)	69.0 (4.7)	65.50 (3.4)	47.6 (4.7)
Compost	61.8 (3.4)	36.8 (5.1)	38.6 (4.8)	68.7 (5.2)	65.2 (4.7)	45.4 (3.9)

Table 4.10 shows that a mixture of acetonitrile:dichloromethane 1:1 v/v gives the highest extraction results, with nearly 80 % recovery for Hyde Farm soil, and 69 % recovery for compost soil. The results also show that three other solvents, acetonitrile:dichloromethane 1:1 v/v and Iso-hexane:(acetonitrile:dichloromethane 1:1, v/v) 2:1 v/v, and dichloromethane also gave relatively high extraction recovery. Iso-hexane gave the lowest extraction recoveries, 37 % on compost, and 55 % on Hyde Farm soil. This implies that both extraction solvent and soil type influence the recovery of bupirimate.

Table 4.11 Soil/solvent study results for ethirimol extraction, % recovery, (%RSD))

Soil	DCM	Iso-hexane	ACN	ACN:DCM 1:1 v/v	ACN:DCM 1:4 v/v	Iso-hexane: (ACN:DCM 1:1 v/v) 2:1 v/v
Hyde farm	63.8 (5.4)	48.5 (4.8)	51.5 (5.4)	76.6 (3.4)	67.3 (4.3)	58.9 (3.7)
Chamberlain	64.0 (4.7)	50.9 (3.7)	54.6 (4.3)	77.1 (4.2)	71.4 (4.4)	58.3 (4.5)
18 Acres	62.8 (4.30	46.8 (5.4)	50.1 (4.2)	76.5 (3.2)	68.1 (5.1)	58.0 (4.7)
Chalgrove Farm	61.9 (4.7)	49.1 (4.4)	52.4 (5.1)	76.2 (5.1)	65.0 (5.2)	61.9 (4.1)
Garden	60.7 (3.9)	45.9 (3.2)	48.4 (4.0)	76.0 (3.5)	64.1 (5.5)	55.6 (5.0)
Mix 2	60.2 (4.1)	45.0 (4.0)	47.0 (3.4)	75.9 (4.1)	62.1 (3.9)	45.0 (4.1)
Mix 1	59.6 (5.0)	43.5 (4.5)	45.0 (3.8)	75.7 (4.0)	60.7 (4.8)	43.5 (3.2)
Mix 3	59.4 (4.0)	40.8 (4.2)	41.7 (4.4)	75.5 (5.1)	59.4 (4.3)	40.8 (4.4)
Compost	59.0 (3.5)	39.8 (3.6)	40.9 (3.2)	75.4 (4.8)	59.2 (4.7)	47.5 (3.1)

With regard to ethirimol extraction, table 4.11 clearly shows that a mixture of acetonitrile:dichloromethane 1:1 v/v is the optimum extraction solvent, and iso-hexane gives the poorest extraction recoveries, extracting between 40 % and 49 % recovery, depending on soil type. To determine which soil parameters have a direct influence on the extraction, multiple linear regression was performed on the data. Multiple linear regression is a technique that assesses the significance of the individual soil constituents to the overall extraction recovery. Equation 4.1 shows the general multiple linear regression equation.

$$y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \ldots + \beta_{1.2} x_{1.2} + \beta_{1.3} x_{1.3} \ldots \beta_i x_1 \qquad \text{Eqn 4.1}$$

Where β_o is the value of the intercept

x_i are the individual soil parameters, and

β_i are the regression coefficients for the parameters

Due to the high degree of correlation between the soil parameters (table 4.12), only three soil parameters are can be investigated at any one time.

Table 4.12 Correlation data.

	Sand	Silt	Clay	pH	% OM	CEC
Sand	1.00					
Silt	-0.87	1.00				
Clay	-0.94	**0.66**	1.00			
pH	**0.35**	**-0.09**	**-0.48**	1.00		
% OM	**-0.53**	**0.16**	0.71	-0.83	1.00	
CEC	**-0.54**	**0.52**	**0.48**	**-0.40**	**0.41**	1.00

These combinations are

1. % silt, % OM and CEC
2. % clay, pH and CEC
3. % OM, % sand and CEC, and
4. % sand, pH and CEC

Each of these combinations were regressed for both compounds against each solvent. The results of the multiple linear regression determined that the organic matter content of the soil had a direct influence on bupirimate extraction when using either acetonitrile, iso-hexane, or iso-hexane:(acetonitrile:dichloromethane 1:1, v/v) 2:1 v/v. Table 4.13 shows an example of the multiple linear regression of % OM, % sand and CEC for the extraction of bupirimate from compost soil using Iso-hexane as the extraction solvent. Components with a p-value of < 0.05, are considered to have a significant effect on the recovery of bupirimate at the 95 % confidence level.

Table 4.13 Multiple Linear regression result for bupirimate extraction from compost soil using Iso-hexane

	Intercept	% OM	% Sand	CEC
Regression Coefficient	56.6	-0.21	-0.04	-0.05
Standard error	6.02	0.04	0.07	0.12
P-value	$2.3x10^{-4}$	*0.01*	0.55	0.71

N. B. $R^2 = 0.86$ for the analysis.

The influence of organic matter decreased for solvents that gave good extraction recoveries, i.e., acetonitrile:dichloromethane 1:1 v/v, acetonitrile:dichloromethane 1:4 v/v and dichloromethane. Organic matter content and pH of the soil influenced ethirimol extraction when acetonitrile, iso-hexane, or iso-hexane:(acetonitrile:dichloromethane 1:1, v/v) 2:1 v/v were used for the extraction. The influence of these soil parameters decreased when acetonitrile:dichloromethane 1:1 v/v, dichloromethane and acetonitrile:dichloromethane 1:4 v/v were used as the extraction solvent (table 4.14)

Table 4.14 Comparison of % OM P-values for various extraction solvents used for ethirimol Extraction.

Solvent	P-value for % OM	P-value for pH
DCM	0.05	0.06
Iso-hexane	0.02	0.03
ACN	0.01	0.03
ACN:DCM 1:1 v/v	0.07	0.09
ACN:DCM 1:4 v/v	0.05	0.06
Iso-hexane: (ACN:DCM 1:1 v/v) 2:1 v/v	0.03	0.04

4.3 Summary.

PFE has shown the ability to quantitatively extract both pyrimidine and organochlorine pesticides from both inert matrices and real matrices (various soils), with a minimal volume of solvent and operator interaction. Optimisation of the extraction parameters temperature, time and pressure was achieved in a relatively small amount of time (c.f. Soxhlet). The only significant extraction parameter was temperature of extraction in the bupirimate and ethirimol study. Pressure was not deemed to have any effect in either study. The number of static flush cycles that are required for quantitative recovery of pyrimidines from aged spiked soil is two. PFE was shown not to degrade any of the investigated analytes within the extraction parameters that were chosen.

Reference

1. The Pesticide Manual, C. Tomlin (Ed.), 10th edition, The Royal Society of Chemistry, Cambridge (1994).

Chapter 5

Pentachlorophenol Extraction from Soil

Pentachlorophenol extraction from soil.

5.0 Introduction

The extraction and analysis of organic contaminants currently on the US EPA 'Red List'[1] of high priority pollutants is important. Pentachlorophenol, for example, has high toxicity and persistence in the environment,[2] hence levels need to be monitored carefully.

Pentachlorophenol is deposited in the environment through several channels: from the petroleum industry;[3] as a by-product of the dye manufacturing process;[2] as a means of termite control;[2] and, as a herbicide.[2] Derivatives of pentachlorophenol are also used as fungicides to protect against fungal rot of wood.[2]

The aim of this chapter is to determine the optimum PFE extraction parameters, using spiked hydromatrix. The optimised parameters were then applied to a certified reference material (CRM 524), and the influence of extraction solvent was investigated. The influence of soil composition and extraction solvent was applied to a range of spiked, aged soils.

5.1 Experimental

5.1.1 Instrumentation

An ASE™ 200 Accelerated solvent extractor (Dionex (UK) Ltd., Camberley, Surrey) was used to perform the extractions. 11 mL cells were used for all the extractions. The system is automated and capable of 24 sequential extractions. Typical extraction

conditions are based on a pressure of 2000 psi (1 psi = 6894.76 Pa), a temperature of 100 °C and a total extraction time of 10 minutes.

5.1.2 GC-MSD Analysis

The GC-MSD (HP G1800A GCD system, Hewlett Packard, Palo Alto, USA) was operated in selected ion monitoring mode with a splitless injection volume of 0.5 μL. The column used was a DB-5 (J & W Scientific, Folsom, California, USA), with dimensions of length 30 m x 0.25 mm i.d. x 0.25 μm film thickness. The temperature program used for the analysis was: 90 °C for 2 minutes to 250 °C at 10 °C / minute with a final hold time of 12 minutes. The injection port temperature was set at 250 °C, and the detector temperature was set at 280 °C. GC-MSD in selected ion monitoring mode was used to determine the presence of derivatised pentachlorophenol. The ions monitored were m/z = 323 and m/z = 321. Selected standards were run on a daily basis to assess analytical performance.

5.1.3 Soil

The certified reference material (CRM 524), an industrial site soil, contaminated with PCP was supplied from the Laboratory of the Government Chemist (LGC, Teddington, London). Nine soils of various compositions were supplied by Zeneca AgroChemicals, Jealott's Hill, Berkshire.

5.1.4 Chemicals

Solvents were obtained from Fisher Scientific (Loughborough, Leicestershire), and were certified analytical grade. The head space of the extraction cells were filled with

Hydromatrix (Varian Ltd., Surrey, UK). Anhydrous sodium sulphate (BDH, Poole, UK) was mixed with the soil sample during Soxhlet extraction. PCP was purchased from Aldrich Chemical Co., Gillingham. Derivatising agent, *N, O*-Bis-(trimethylsilyl)acetamide (BSA) was purchased from Aldrich Chemical Company.

5.1.5 Fortification procedures

Spot spike procedure

Hydromatrix (5 x 1 g), was spiked with pentachlorophenol in 25 μL dichloromethane at the 20 μg mL^{-1} level. The solvent was allowed to evaporate and the hydromatrix was extracted immediately.

Slurry spike procedure

Hydromatrix (3 x 5 g), was spiked with pentachlorophenol in 25 mL of dichloromethane to give a final concentration of 20 μg mL^{-1}. The solvent was allowed to evaporate overnight and then left for approximately four days before extraction to allow some interaction of the pentachlorophenol with the matrix. Portions (1 g) of the hydromatrix were extracted each time.

5.1.6 Extraction procedures of fortified matrices

Procedure for pressurised fluid extraction

Soil (0.5 - 2 g, accurately weighed) was placed in a stainless steel extraction cell on top of a filter to prevent cell frit blockage. Hydromatrix was used to fill the head space to reduce solvent consumption. The cell was placed in the carousel and extracted using the conditions outlined by the EPA, namely 100 °C, 2000 psi with a static extraction time of

5 minutes. Hexachlorobenzene at the 5 μg mL^{-1} level was used as the internal standard. In the case of PCP, an aliquot of the extract (1.00 mL) was placed in a tapered tube (10 mL), and derivatising agent (BSA, 150 μL), and internal standard (hexachlorobenzene, 10 μL of 1000 μg mL^{-1} stock) was added. The mixture was vortex mixed for 15 seconds prior to chromatographic analysis.

Soxhlet extraction.

CRM soil (1 g accurately weighed) was mixed with anhydrous sodium sulphate (1 g) and Soxhlet extracted (24 hours) with DCM (20 mL) and quantitatively transferred to a volumetric flask (25.00 mL). An aliquot (1.00 mL) was removed and placed in a tapered tube (10 mL). BSA derivatising agent (100 μL) was added and the mixture was mixed (10 seconds) using a vortex mixer. Internal standard (50 μL) was added and the derivatised extract was analysed on the GC-MSD.

5.2 Results and discussion

5.2.1 Chromatography and analyte identification

Figure 5.1 shows the chromatography used in this study, the internal standard, hexachlorobenzene has a retention time of 15.7 minutes, and PCP has a retention time of 17.5 minutes. Figure 5.2 shows the mass spectrum for underivatised PCP.

Figure 5.1 PCP chromatography

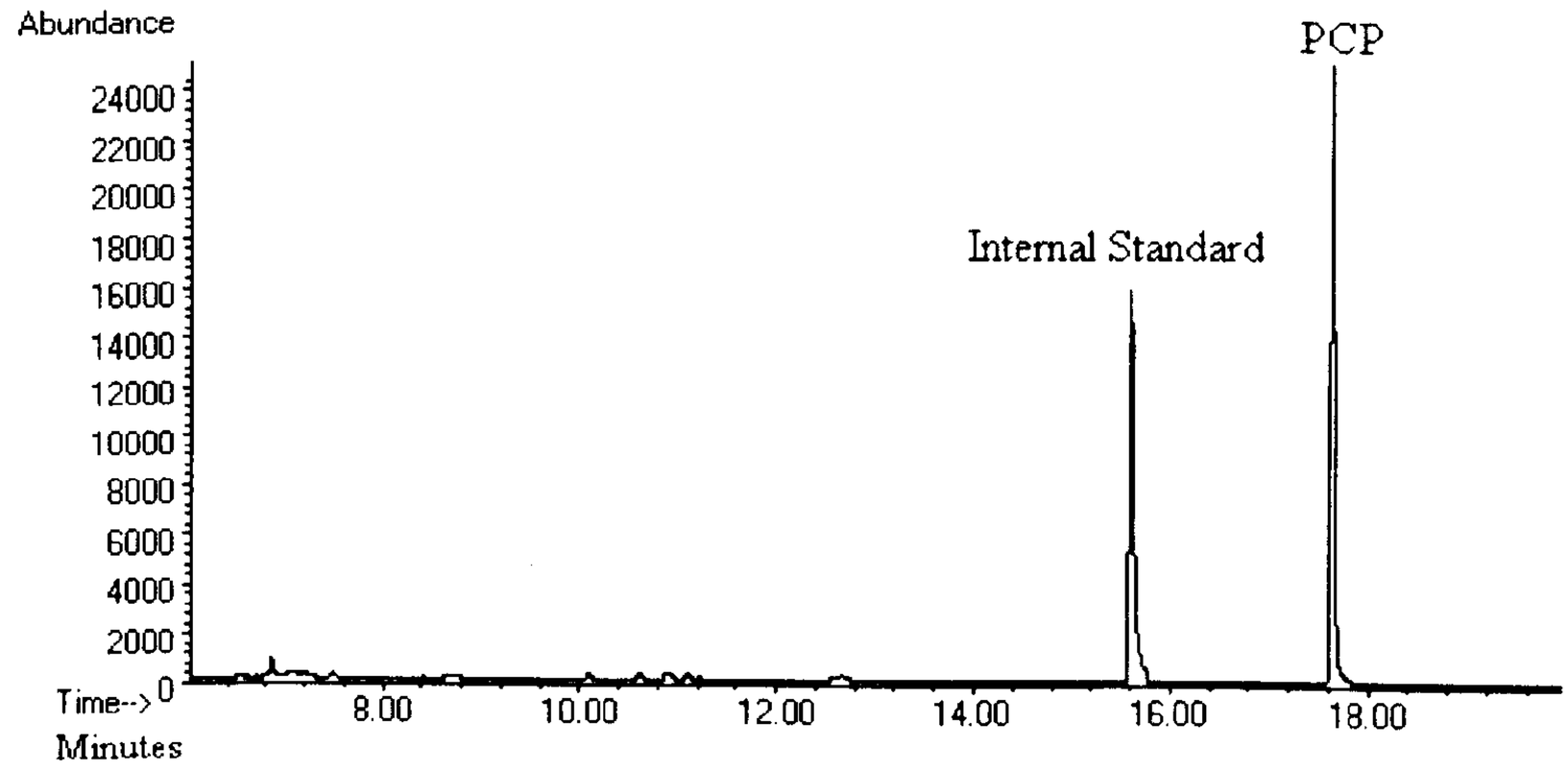
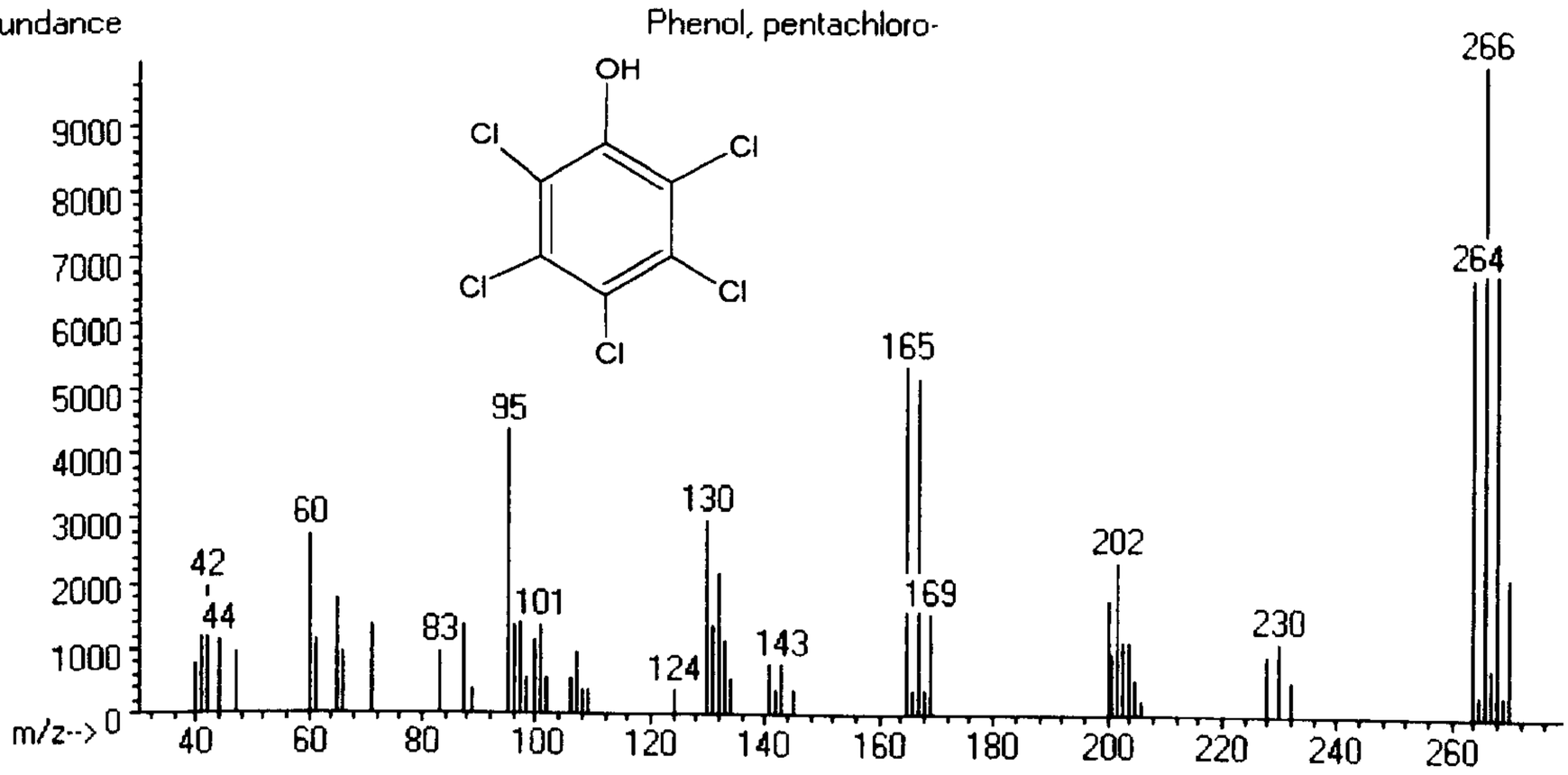


Figure 5.2 PCP mass spectrum



The SIM ions chosen for derivatised PCP quantification were m/z 321 and m/z 323.

Table 5.1 shows the calibration data. Using this chromatography, a linear calibration was achieved between 0 μg /mL and 2 μg / mL, with excellent linearity.

Table 5.1 PCP calibration data

Compound	Calibration range	Number of data points	Equation	Correlation coefficient, R^2
PCP	0 - 2 μg mL^{-1}	8	y = 0.2640x + 0.0301	0.9980

5.2.2 Recovery experiments

The recovery experiments (table 5.2) from the hydromatrix demonstrated that near quantitative recovery occurred irrespective of the spike level and method (spot and slurry) investigated. The spot spike gave average recoveries of 92.6 % (n = 3) from hydromatrix, while the slurry spike gave average recoveries of 89.3 % (n = 3). Good precision was achieved by both methods (RSD < 3.8 %). This showed that the analytical procedure was robust, and that hydromatrix did not influence the extraction of PCP.

Table 5.2 PFE of Pentachlorophenol from Inert Hydromatrix at the 20 μg / g level (n = 6).

	PFE		Soxhlet	
	Spot	**Slurry**	**Spot**	**Slurry**
Mean (μg / g)	18.7	18.4	18.5	18.0
% RSD	3.5	1.5	4.2	5.1

5.2.3 PCP extraction (CRM 524)

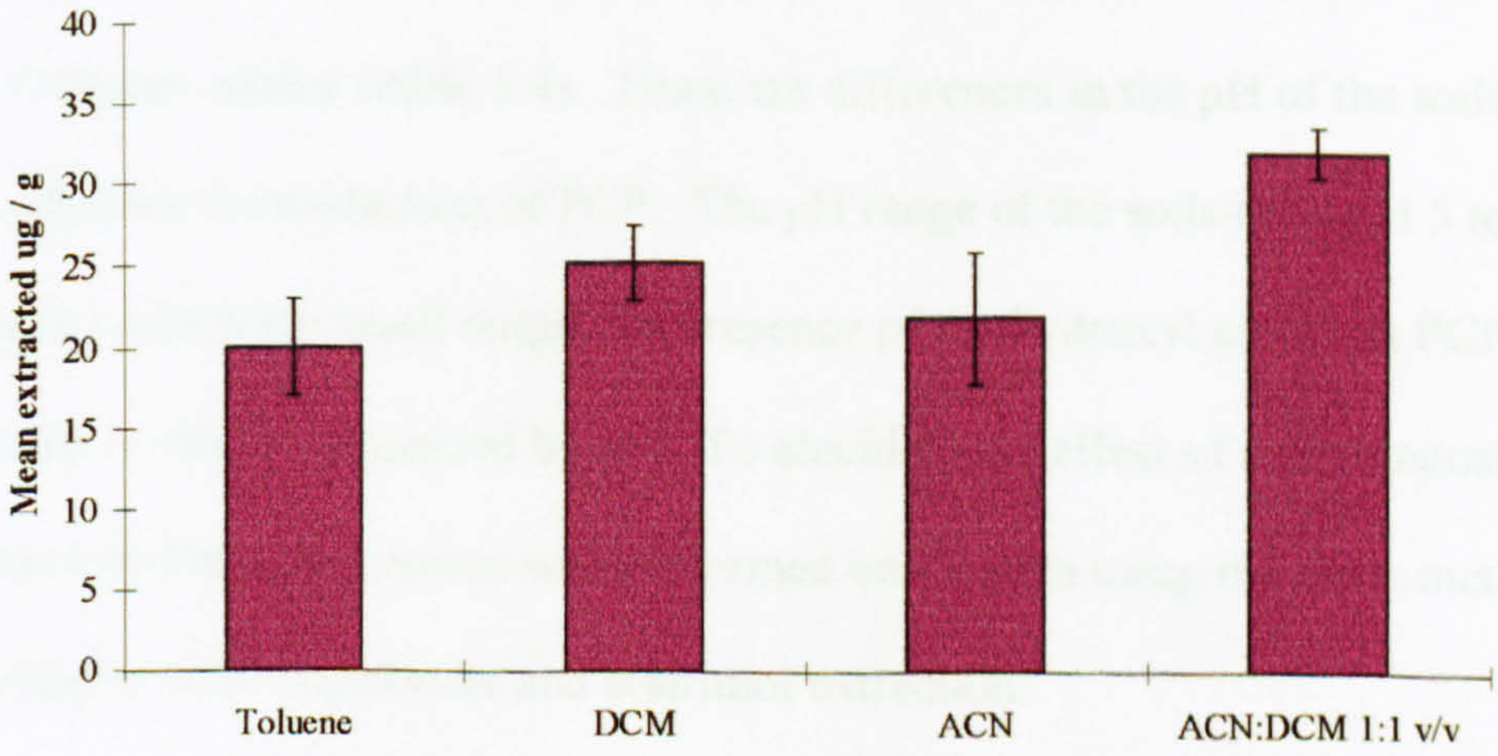
As with bupirimate and ethirimol optimisation, an investigation into the influence of static flush cycles was performed. Table 5.3 shows the results. To assess the effect (if any) of solvent on the extraction, the study incorporated three single extraction solvents, and one mixture. Three static flush cycles were required irrespective of the solvent used.

Table 5.3 Cycle study

Number of cycles	Toluene (µg / g per cycle)	DCM (µg / g per cycle)	ACN (µg / g per cycle)	ACN:DCM 1:1 v/v (µg / g per cycle)
1	11.0	15.6	12.4	19.2
2	8.0	7.5	8.1	10.6
3	1.0	2.0	1.3	2.2
Cumulative total (µg / g)	20.1	25.2	21.8	32.0

The results of the solvent study, based on the mean concentration of six replicate extractions per solvent, are shown in Figure 5.3.

Figure 5.3 PCP solvent graph



Typical % RSD's range from 3.1 - 8. The results show that acetonitrile:dichloromethane (1:1, v/v) gave the highest recovery (32 +/- 1 µg / kg), and this was in good agreement with the certificate value (34 +/- 5 µg / kg). The PFE data implies that solvent has an effect on the quantity of PCP extracted from the CRM.

5.2.4 Soil and solvent study

The study of the CRM showed that solvent was an important factor to consider in PCP extraction. To determine if soil composition also affects the extraction of PCP, an

investigation into the effect of soil composition was performed. Nine soils of varying composition (table 5.4) were slurry spiked at the 20 μg /g level with PCP and left to age for 2 weeks in the dark. Table 5.4 shows the results of the soil / solvent study. The results in table 5.4 confirm that the extraction solvent used has a large influence on the recovery of PCP obtained. For example, for compost soil, the recovery ranges from 38 % when using iso-hexane, to 73 % with a mixture of acetonitrile:dichloromethane 1:1 v/v. It is also clear that soil composition has an influence on PCP extraction. When using iso-hexane, the recovery of PCP ranges from 38 % recovery from compost soil, to 57 % from Hyde Farm soil. The major difference between these two soils is their organic matter content. Hyde farm contains only 3.2 % organic matter, whereas compost soil contains over 80 % organic matter (table 5.4). There are differences in the pH of the soils, which can also influence the extraction of PCP. The pH range of the soils from pH 5 to pH 7.4, whilst this is a relatively small range, the presence of the hydroxyl group on PCP indicates that it will be influenced by pH. To elucidate the effect of soil composition further, multiple linear regression was performed on the data using the same method stated in chapter 4 for bupirimate and ethirimol extraction.

Table 5.4 results of the soil/ solvent study (% recovery)

Soil	% Silt	% Clay	% Sand	% OM	pH	CEC	DCM	Iso-hexane	ACN	ACN:DCM 1:1, v/v	ACN:DCM 1:4, v/v	Iso-hexane:(acetonitrile: dichloromethane 1:1, v/v) 2:1, v/v
Hyde farm	23	19	58	3.2	6.7	17.4	70.73	57.36	56.83	84.97	79.88	63.63
Chamberlain	4	9	87	4.5	7.3	11	69.00	53.72	55.28	83.15	78.19	62.41
18 Acres	24	20	56	4.7	6.3	14	68.44	54.96	54.57	81.05	76.31	61.81
Chalgrove Farm	29	37	34	5.7	7.4	29.7	67.35	52.75	53.34	78.75	74.14	60.93
Garden	18	11	71	9.8	7.2	16.49	67.03	47.44	51.65	75.00	71.62	55.94
Mix 2	3	11	86	17.5	5.9	12.7	66.41	50.81	49.10	74.70	71.03	53.04
Mix 1	22	25	53	31.3	5.3	32.1	65.98	51.01	47.12	74.40	70.26	50.98
Mix 3	21	30	49	59.4	5.2	41.7	65.44	40.33	44.81	73.85	69.43	49.52
Compost	22	48	30	82.7	5	17.62	64.90	38.13	40.04	73.51	69.07	47.22

Multiple linear regression showed that soil organic matter and pH have a significant effect on the extraction. Tables 5.5a and 5.5b show an example of the multiple linear regression of % OM, % sand and CEC (table 5.5a) and pH, % sand, and CEC (table 5.5b) for the extraction of PCP from compost soil using Iso-hexane as the extraction solvent. Components with a p-value of < 0.05, are considered to have a significant effect on the recovery of PCP at the 95 % confidence level.

Table 5.5a Multiple Linear regression result for compost soil using Iso-hexane

	Intercept	**% OM**	**% Sand**	**CEC**
Regression Coefficient	62.7	-0.25	-0.04	-0.06
Standard error	6.25	0.07	0.10	0.13
P-value	$3.4x10^{-4}$	*0.02*	0.55	0.71

N. B. $R^2 = 0.84$ for the analysis

Table 5.5b Multiple Linear regression result for compost soil using Iso-hexane

	Intercept	**pH**	**% Sand**	**CEC**
Regression Coefficient	23.7	3.78	-0.06	-0.06
Standard error	8.45	0.05	0.07	0.13
P-value	0.28	*0.04*	0.57	0.85

N. B. $R^2 = 0.76$ for the analysis

P-values of <0.05 indicate a component is > 95 % significant.

The influence is more apparent when extraction solvents giving poor recovery are used, i.e. acetonitrile, iso-hexane, and iso-hexane:(acetonitrile:dichloromethane 1:1 v/v) 2:1 v/v, than when solvents yielding greater quantities of PCP were used (table 5.6).

Table 5.6 Comparison of % OM and pH P-values for various solvents used for PCP Extraction.

Solvent	P-value for % OM	P-value for pH
DCM	0.05	0.08
Iso-hexane	0.02	0.04
ACN	0.01	0.05
ACN:DCM 1:1 v/v	0.09	0.08
ACN:DCM 1:4 v/v	0.07	0.07
Iso-hexane: (ACN:DCM 1:1 v/v) 2:1 v/v	0.03	0.06

5.3 Summary.

PCP has been successfully extracted from spiked matrices (soil and hydromatrix), as well as a certified reference material (CRM 524) using PFE. Optimisation of the PFE extraction procedure determined that the EPA method[4, 5] extraction parameters were adequate to quantitatively extract PCP. An investigation into the number of static flush cycles required determined that three cycles were necessary. Application of the optimised method to the CRM showed it robust. Various solvents were used in the extraction, and showed that solvent selection is an important part of the extraction process. Soil composition was also found to influence the recovery of PCP, with organic matter and pH having a significant influence on the recovery.

5.4 References

1. Chemical Principles of Environmental Pollution, B. J. Alloway and D. C. Ayres, 2nd Edition, Edward Arnold, London (1997).

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4. Test Methods for Evaluating Solid Waste, Method 3540C, Soxhlet Extraction, Revision 3, USEPA, Washington, DC, December 1996.
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Chapter 6

DDT, DDD, and DDE Extraction from Soil

DDT, DDD and DDE extraction from soil.

6.0 Introduction

This chapter concentrates on the optimisation of PFE extraction parameters for OCP extraction from a natively contaminated soil. The influence of solvent was briefly investigated, and showed that contrary to the EPA recommended method, dichloromethane gave a greater extraction that a mixture of acetone:dichloromethane 1:1 v/v.

6.1 Experimental

6.1.1 Instrumentation.

An ASE™ 200 Accelerated Solvent Extractor (Dionex (UK) Ltd., Camberley, Surrey) with 11 mL extraction cells was used to perform the extractions. The extracts were analysed on a GC-MSD (Hewlett-Packard) in selected ion monitoring mode.

6.1.2 Soil

Zeneca Environmental Laboratories, Brixham, provided soil contaminated with DDT and its metabolites (DDE and DDD). After being air-dried and sieved (<2 mm) it was characterised as follows: pH 2.5, organic matter content 7.2 % and cation exchange capacity, 12 mequiv. per 100 g. It was shipped and stored at - 18 °C.

6.1.3 Chemicals

The solvents used in this study were certified analytical reagents (Fisher Scientific, Loughborough, Leicestershire). Hydromatrix (Varian Ltd., Surrey, UK) was used to fill

the head space of the PFE extraction cells (Dionex), and as an inert matrix for the spike recovery experiments. Anhydrous sodium sulphate (BDH, Poole, UK) was mixed with the soil sample during Soxhlet extraction. A pesticide standard comprising of twenty organochlorine pesticides was purchased from Supelco, Walton-on-Thames, UK.

6.1.4 GC-MSD Analysis

The GC-MSD (HP G1800A GCD system, Hewlett Packard, Palo Alto, USA) was operated in selected ion monitoring mode with a splitless injection volume of 0.5 µL. The column used was a DB-5ms (J & W Scientific, Folsom, California, USA), with dimensions of length 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The temperature program used for the analysis was 120 °C, held for 2 minutes up to 290°C at a rate of 5 °C / minute, with a final hold time of two minutes. The injection port and detector temperatures were set at 280 °C.

6.1.5 Fortification Procedures

Spot Spike

Hydromatrix (1 - 2 g, accurately weighed) was placed in a stainless steel extraction cell (11 mL capacity). Stock solution (100 µL of a 2000 µg / mL standard) was added directly to the Hydromatrix to give a final concentration of 8 µg / mL. The solvent (dichloromethane) was allowed to evaporate before additional Hydromatrix was used to fill the head space of the cell. The cell was capped and placed in the carousel prior to extraction. The spiked Hydromatrix was extracted as follows: temperature, 100 °C; pressure, 2000 psi; and a static extraction time of 10 minutes with one static flush cycle.

Slurry Spike

Hydromatrix (6 g, accurately weighed) was placed in a glass beaker. Stock solution (600 µl of 2000 µg / mL) of the target analytes were added to a 25 mL volumetric flask, and made up to the mark with dichloromethane. This was added to the Hydromatrix. The slurry was stirred and the solvent was allowed to evaporate. Forty-eight hours after solvent evaporation, portions of the spiked Hydromatrix (~1 g) were placed in the stainless steel extraction cell and the head space was filled with Hydromatrix. The cell was placed in the carousel and extracted under the same conditions as the spot spiked samples.

6.1.6 Extraction Procedures of fortified matrices

PFE Extraction

Soil (1 g - 2 g, accurately weighed) was placed in a stainless steel extraction cell (11 mL capacity) on top of a filter to prevent cell frit blockage. Hydromatrix was used to fill the head space to reduce solvent consumption. The cell was placed in the carousel and extracted using the following conditions: pressure; 2000 psi [1 psi = 6894.76 Pa], temperature, 100 °C, with a static extraction time of 10 minutes (preceded by a 5 minutes heat-up time). Initial work was done with a single static flush cycle. With additional time required for rinsing with fresh solvent and N_2, the total extraction time was approximately 17 minutes per sample.

Soxhlet Extraction

Soil (1 - 2 g, accurately weighed) was placed in a cellulose extraction thimble with an equivalent quantity of anhydrous sodium sulphate, which had been previously dried at 60 °C for 48 hours. A round-bottomed flask was filled with 40 mL of solvent (dichloromethane). The extraction was performed for either six or 24 hours. The liquid extract was quantitatively transferred to a volumetric flask. DDE content was determined prior to dilution using the GC-MSD. A 1/20 dilution of the extract was prepared for determination of DDD and DDT content by GC-MSD. In addition, the soil was also extracted, according to the above scheme, with a 1:1 v/v mixture of dichloromethane:acetone in accordance with solvent choice as recommended in EPA Method 3540C.[2]

6.2. Results and Discussion.

6.2.1 Chromatography and analyte identification

Figure 6.1 shows a chromatogram for DDX separation on a DB-5 column. Table 6.1 shows the retention times of the analytes and the internal standard.

Table 6.1 Retention times for the chosen analytes

Analyte	Retention time (minutes)
Hexachlorobenzene (Internal standard)	10.07
DDE	14.8
DDD	15.3
DDT	15.8

The separation of all the analytes was achieved in approximately 16 minutes

Figure 6.1 OCP Chromatogram

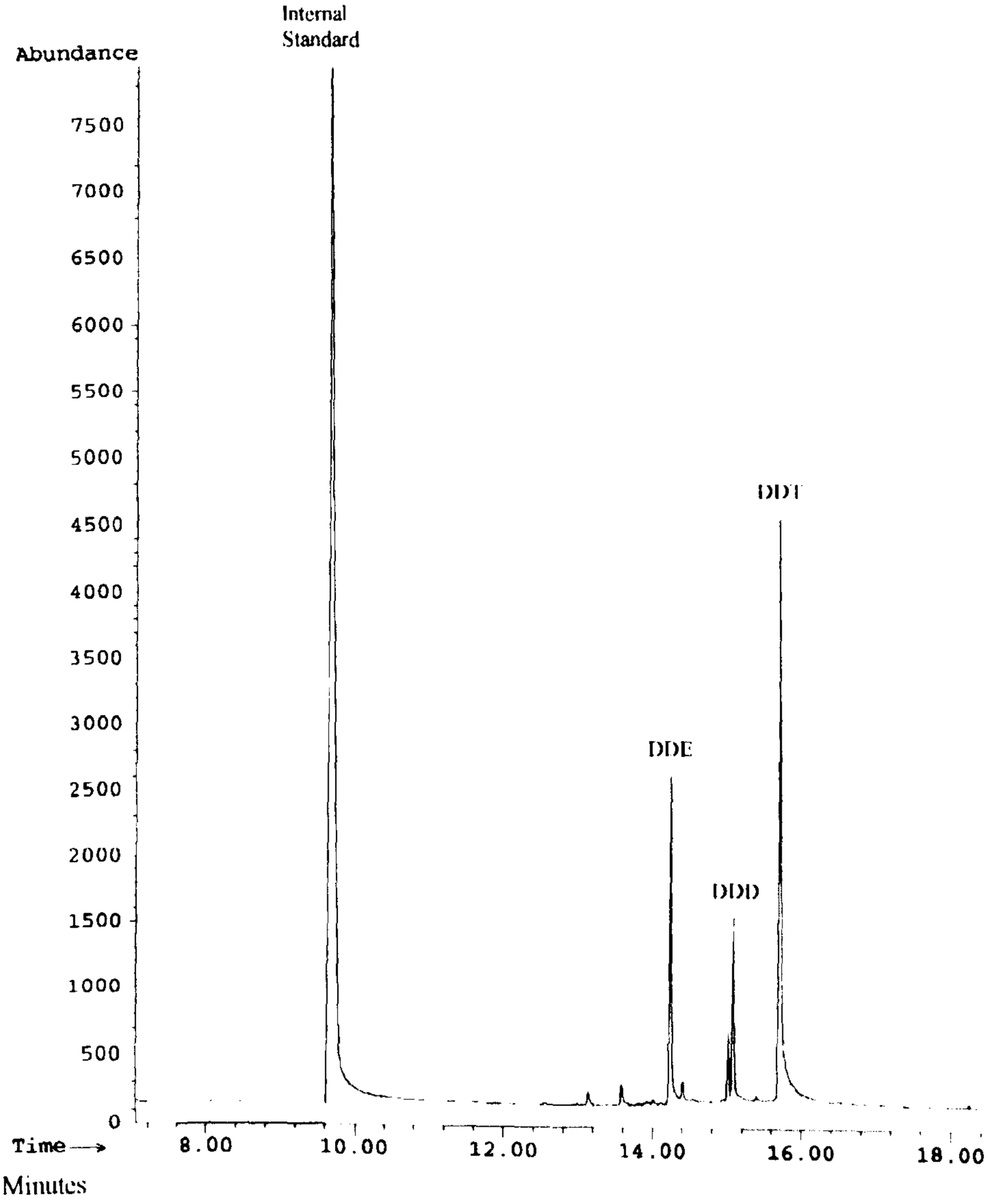
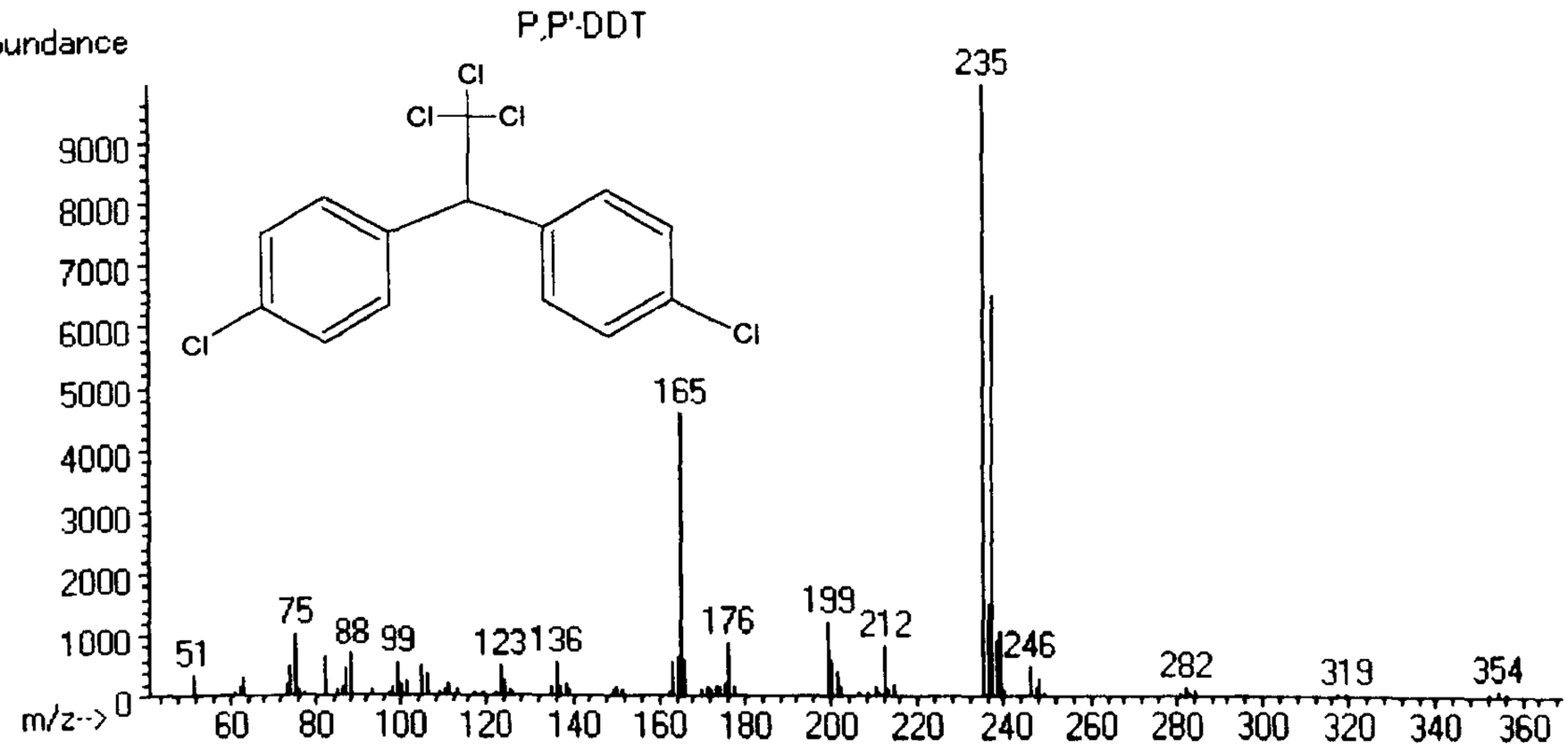
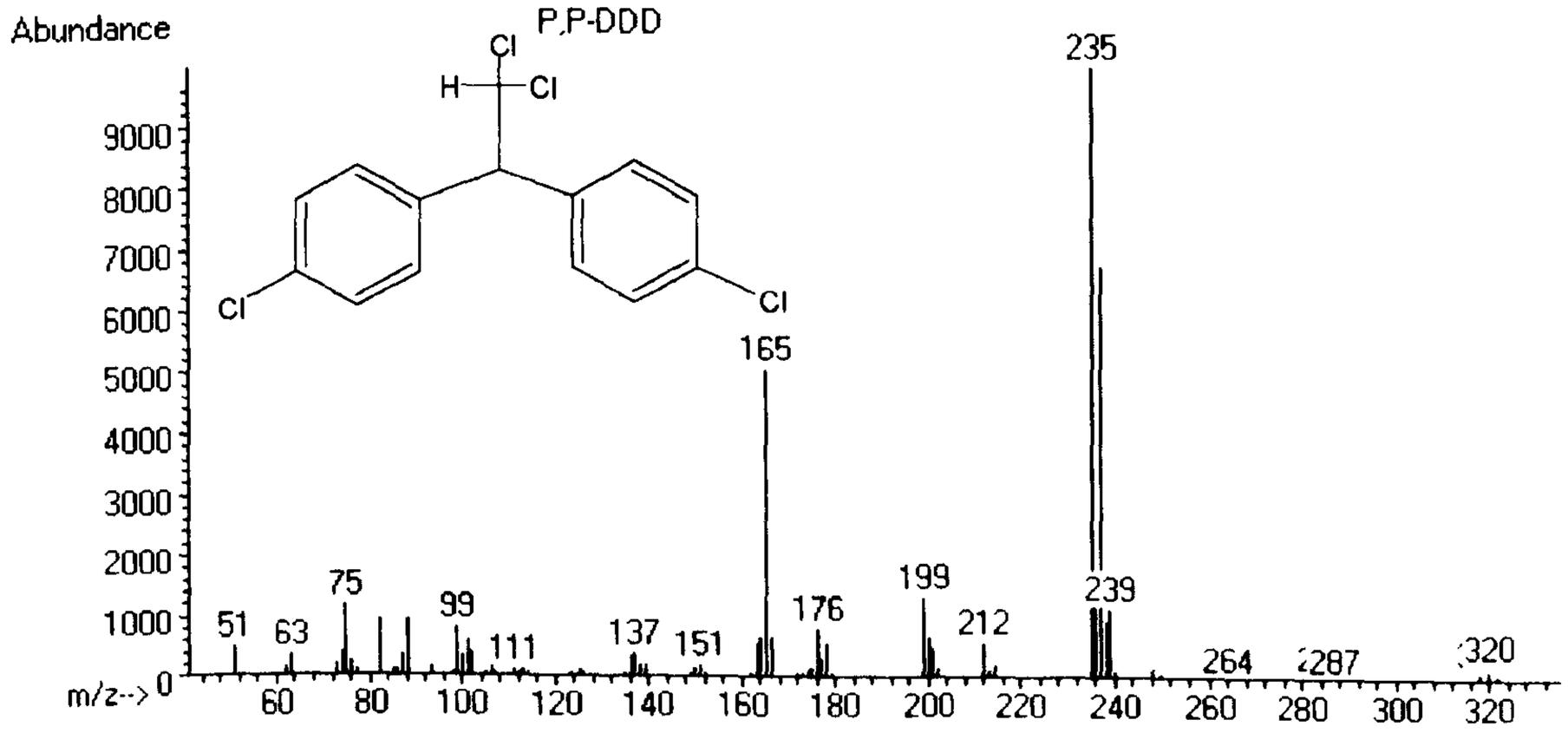


Figure 6.2 Mass spectrum for DDT



The SIM ions chosen for DDT quantification were m/z 235 and m/z 237. They were chosen from the mass spectrum of DDT shown in figure 6.2.

Figure 6.3 Mass spectrum for DDD



The SIM ions from the mass spectrum of DDD (figure 6.3), chosen for DDD quantification were m/z 235 and m/z 237. Figure 6.4 shows the mass spectrum for DDE.

The SIM ions chosen from the mass spectrum for DDE quantification were m/z 246 and m/z 318.

Figure 6.4 Mass spectrum for DDE

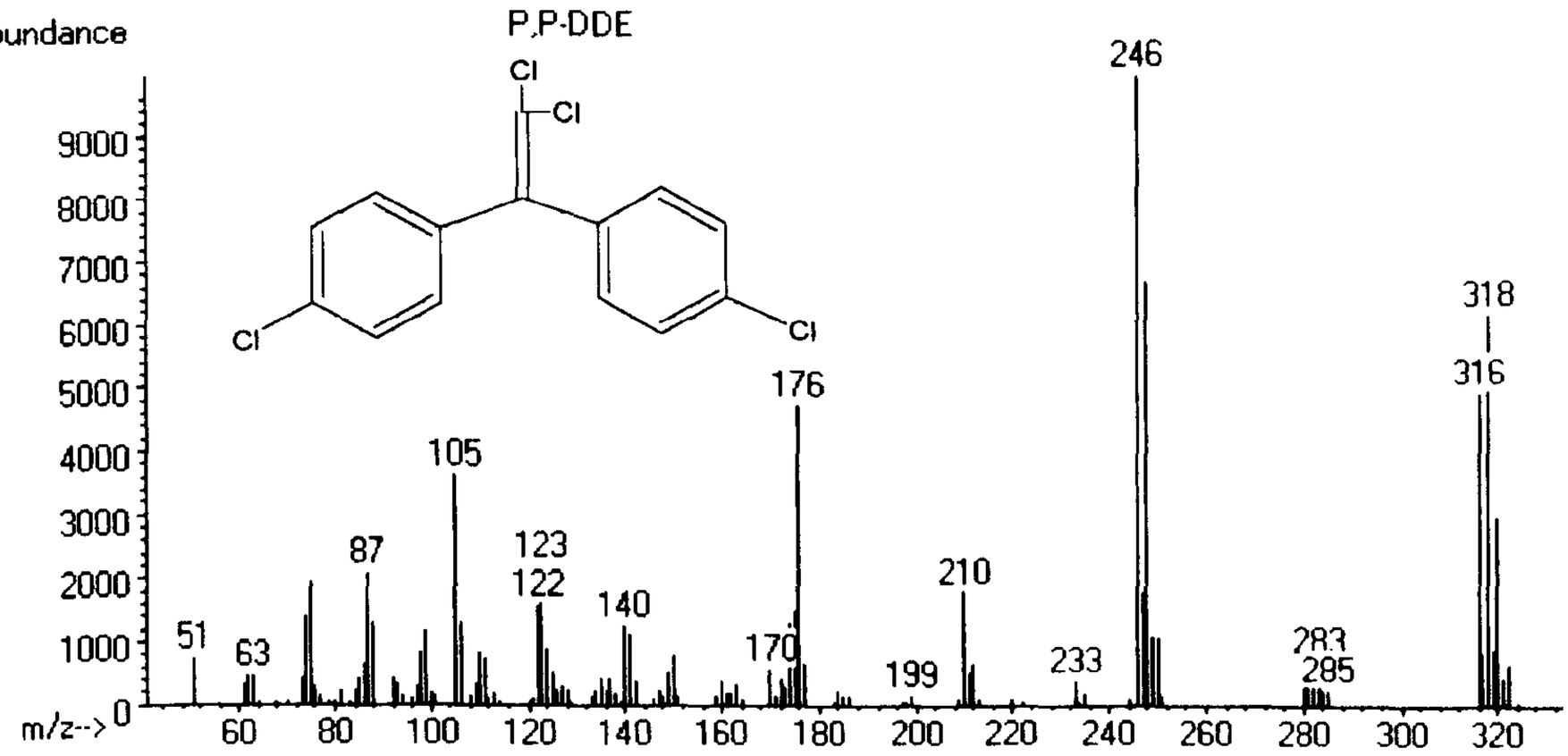


Table 6.2 Calibration Data

Compound	Calibration range	Number of data points	Equation	Correlation coefficient, R^2
DDT	0 - 5 μg mL^{-1}	7	y = 0.2309x - 0.0417	0.9945
DDD	0 - 5 μg mL^{-1}	7	y = 0.7480x - 0.0733	0.9957
DDE	0 - 5 μg mL^{-1}	7	y = 0.2505x + 0.0127	0.9974

Table 6.2 shows the calibration data gained from this chromatography. All the calibration had excellent linearity over the chosen range, 0 μg / ml to 5 μg / ml, with correlation coefficients greater than 0.99.

6.2.2 Recovery experiments

Initial experiments were based on recoveries from spiked (spot and slurry spiked) Hydromatrix, an inert support material. This was done to investigate the effectiveness of PFE and to assess the sample work-up procedure. Results from recovery experiments from spot and slurry-spiked Hydromatrix are shown in Table 6.3. Average recoveries of

91 % for spot spiking and 85 % for slurry spiked for the three analytes, coupled with precisions of < 5.1 % RSD indicated appropriate extraction / sample work-up based on a single static flush cycle.

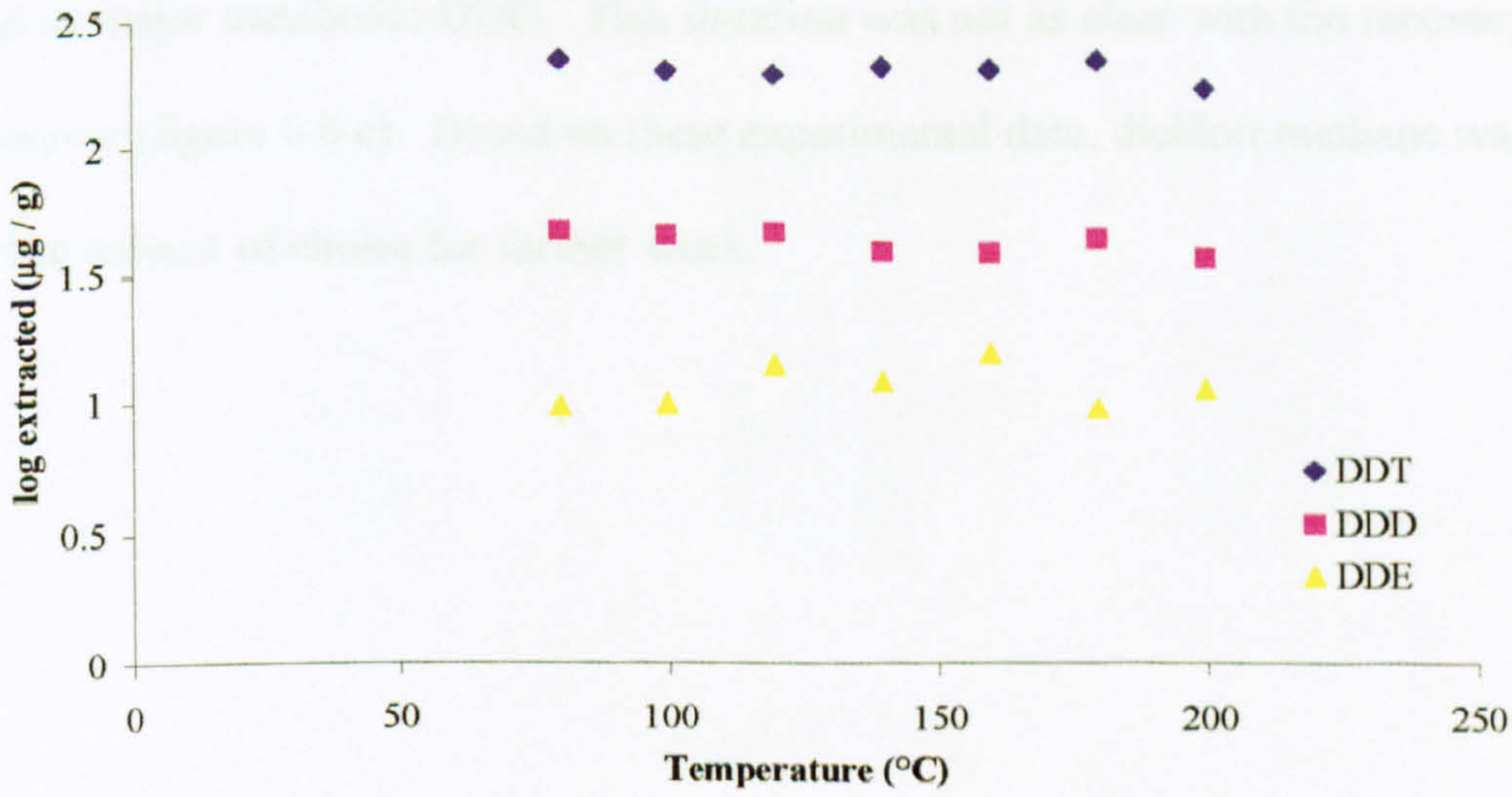
Table 6.3 Results of PFE recovery Extractions (n = 6).

	DDE		DDD		DDT	
	Spot	Slurry	Spot	Slurry	Spot	Slurry
Mean µg / g (% Rec.)	6.7 (84)	7.1 (88)	7.4 (93)	7.4 (92)	7.3 (91)	7.3 (91)
% RSD	3	1.7	4.3	4.8	5.1	2.5

6.2.3 Optimisation of Pressurised liquid Extraction

Initial studies were undertaken to assess the influence of temperature on the recovery of DDT, DDD and DDE from aged, contaminated soil. Seven temperatures were chosen to investigate in the range 80-200 °C in 20 °C increments. Pressure was maintained at 2000 psi and a static extraction time of 10 minutes. Duplicate extraction/analyses were performed at each temperature. The results are shown in Figure 6.5.

Figure 6.5 Effect of temperature on DDT, DDD and DDE



The average extraction efficiencies (n = 14) for DDT, DDD and DDE were 205 (8.6 % RSD), 42.9 (10.2 % RSD) and 10.8 (14.9 % RSD) $\mu g\ g^{-1}$, respectively over the temperature range. It was concluded that an increase in temperature does not significantly alter the amount of analyte extracted. A temperature of 100 °C was chosen as the extraction temperature for further work.

Another important variable in PFE is solvent choice. The ability to select an appropriate solvent for extraction is often neglected, in favour of instrumental variables. Little attempt is often made to select the most appropriate solvent for the analyte. Acetone-DCM (1:1 v/v) is recommended in EPA method 3545A[1] for the extraction of organochlorine pesticides by PFE. Comparison of acetone, DCM, and acetone:DCM, 1:1 v/v, was performed. Figures 6.6 (a-c) [Each determinant was extracted six times with each solvent or solvent combination; error bars represent one standard deviation of the mean.] It is observed (Figure 6.6 a and b) that dichloromethane gave the highest recovery for DDT and its major metabolite DDD. This situation was not as clear with the recovery of DDE however (figure 6.6 c). Based on these experimental data, dichloromethane was selected as the solvent of choice for further work.

Figure 6.6a Influence of solvent on DDT

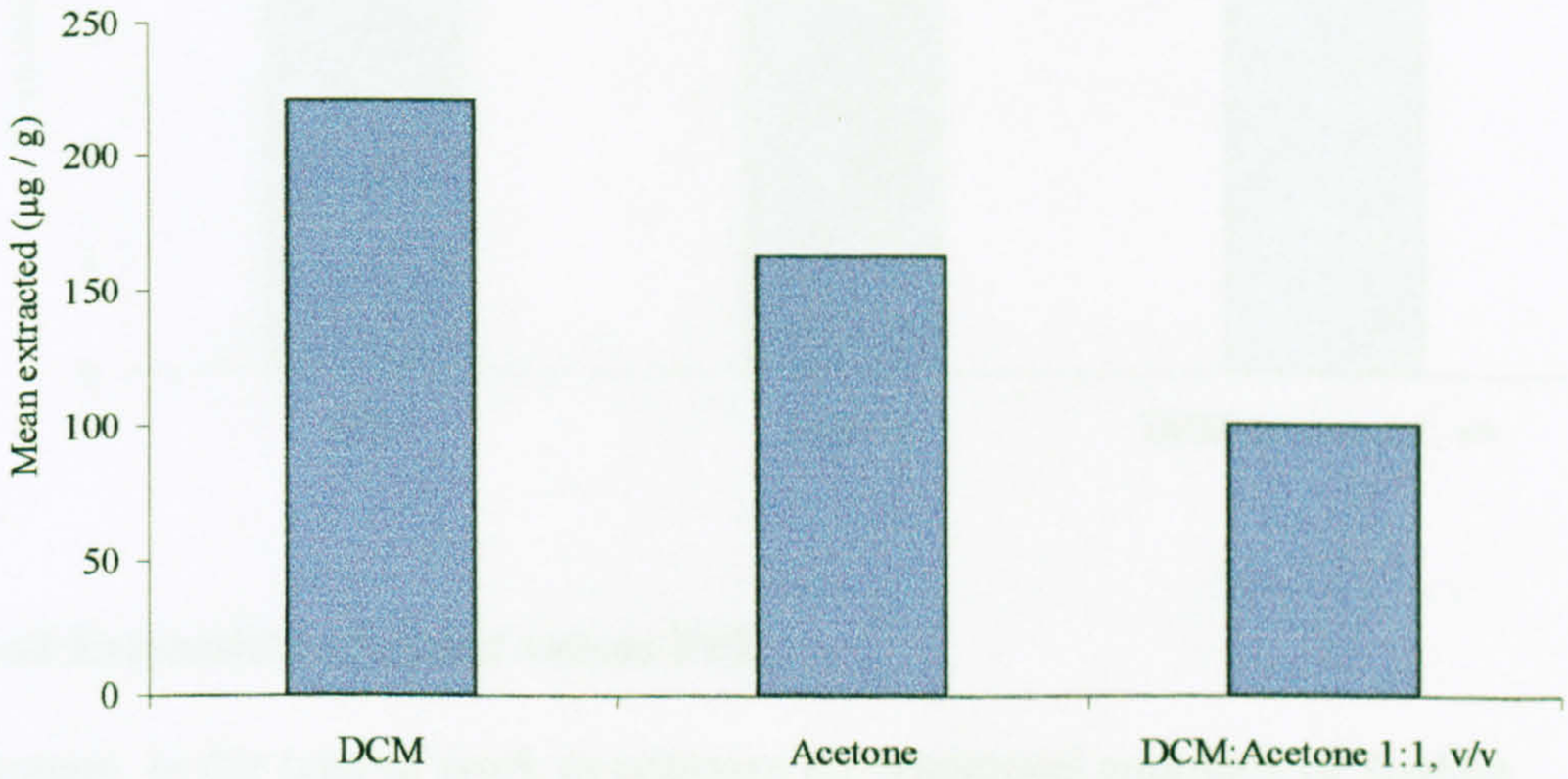


Figure 6.6b Influence of solvent on DDD

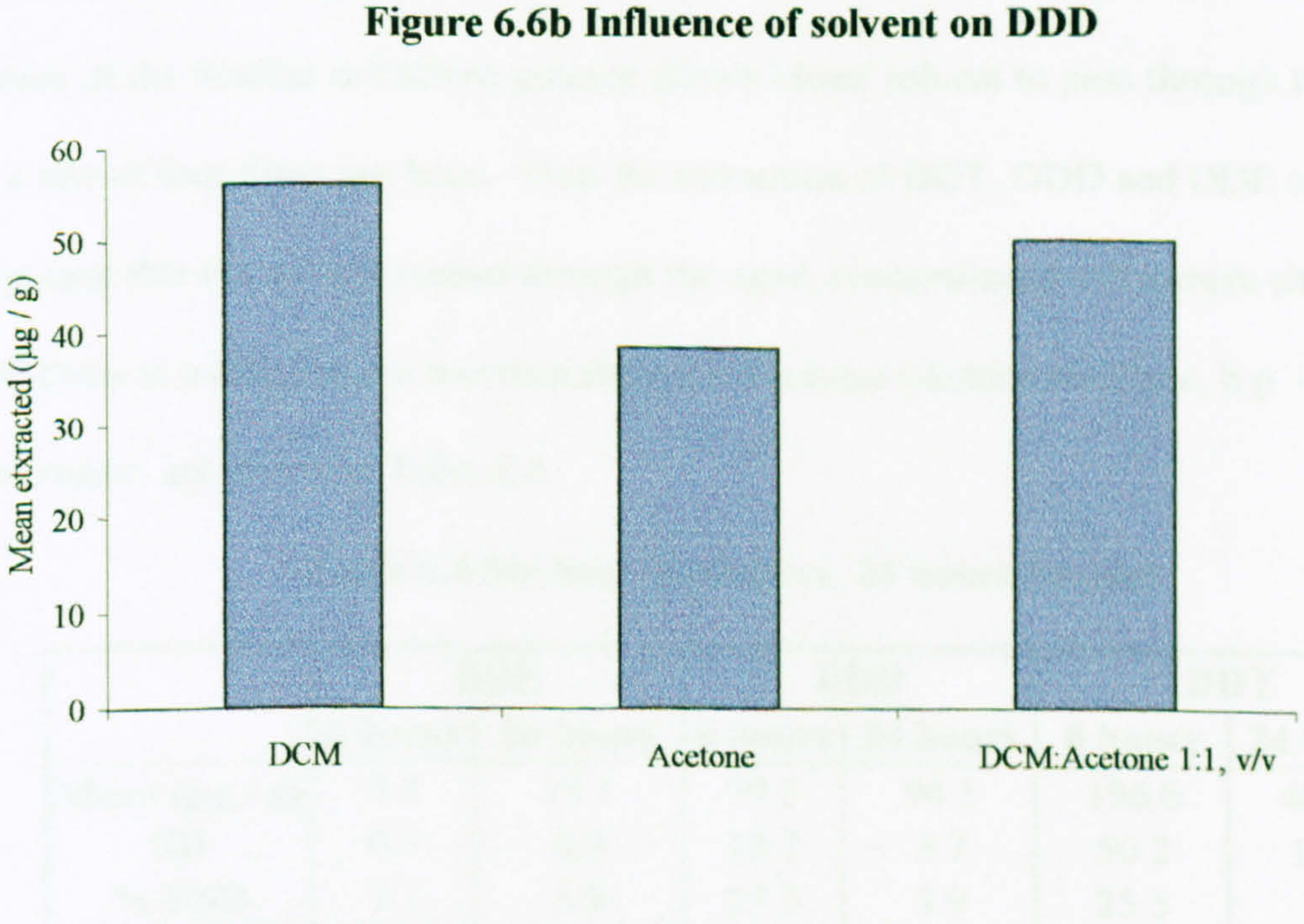
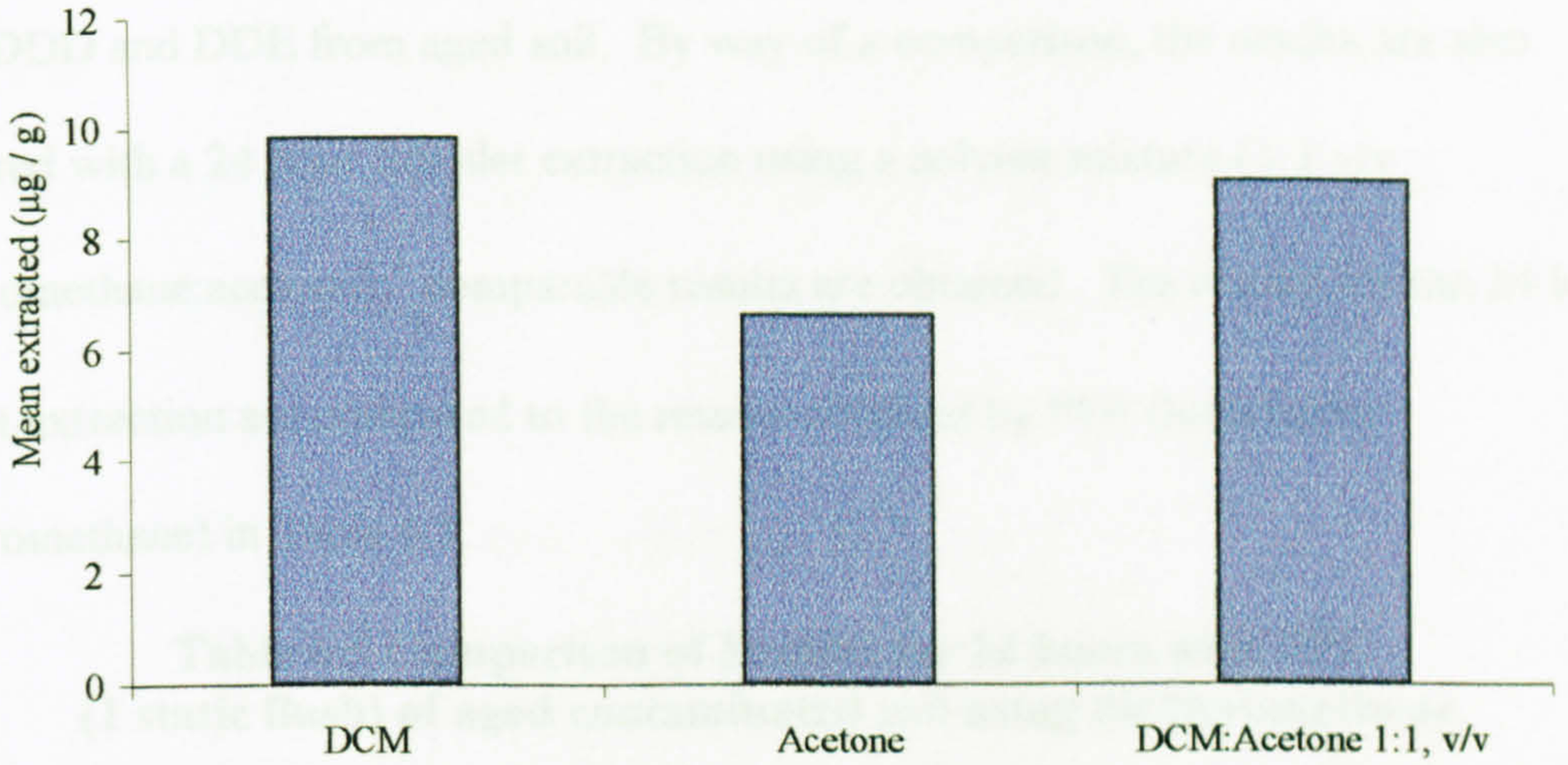


Figure 6.6c Influence of solvent on DDE



6.2.4 Soil Extractions: Soxhlet versus PFE.

It is common, in this type of work to compare the traditional approach of Soxhlet extraction with the newer extraction technique. Using dichloromethane as the extraction solvent, Soxhlet was performed over two different extraction times, six and 24 hours. The nature of the Soxhlet extraction process allows 'clean' solvent to pass through the sample at a rate of four times per hour. Thus for extraction of DDT, DDD and DDE it would be expected that the solvent passed through the aged, contaminated soil sample either 24 or 192 times at a temperature less than that of the solvent (dichloromethane, b.p. 40 °C). The results are shown in Table 6.4.

Table 6.4 Six hour Soxhlet vs. 24 hours Soxhlet

	DDE		DDD		DDT	
	6 hours	24 hours	6 hours	24 hours	6 hours	24 hours
Mean (µg / g)	4.8	13.1	79.5	96.1	196.6	468.4
SD	0.5	0.8	18.7	3.7	50.2	14.5
% RSD	9.7	5.9	23.5	3.9	25.5	3.1

The data shows that 24 hours with dichloromethane is required for maximum extraction of DDT, DDD and DDE from aged soil. By way of a comparison, the results are also compared with a 24 hour Soxhlet extraction using a solvent mixture (1:1 v/v dichloromethane:acetone);[1] comparable results are obtained. The results for the 24 hours Soxhlet extraction are compared to the results obtained by PFE (both using dichloromethane) in Table 6.5.

Table 6.5 Comparison of Soxhlet for 24 hours with PFE (1 static flush) of aged contaminated soil using dichloromethane.

	DDE		DDD		DDT	
	PFE	**Soxhlet**	**PFE**	**Soxhlet**	**PFE**	**Soxhlet**
Mean (µg / g)	10.9	13.1	40.0	96	226	421
% RSD	5.7	5.9	5.0	3.9	8.1	10.7

The surprising results show that Soxhlet for 24 hours is more efficient at removing DDT, DDD and DDE from the aged, contaminated soil. Further investigation of PFE was required.

The fundamental difference between Soxhlet extraction and PFE is the fact that in Soxhlet, a large volume of fresh organic solvent is re-cycled through the sample. However, this is not the case in PFE. The volume of fresh solvent cycled through the sample is minimal (~ 5 mL). During the heat-up period in PFE, solvent in the extraction cell expands causing the pressure to increase. To prevent over pressurisation of the cell, the static valve opens and closes automatically allowing a small volume of solvent to vent. To maintain pressure fresh solvent is pumped in to the cell. It is estimated that the volume of solvent vented is

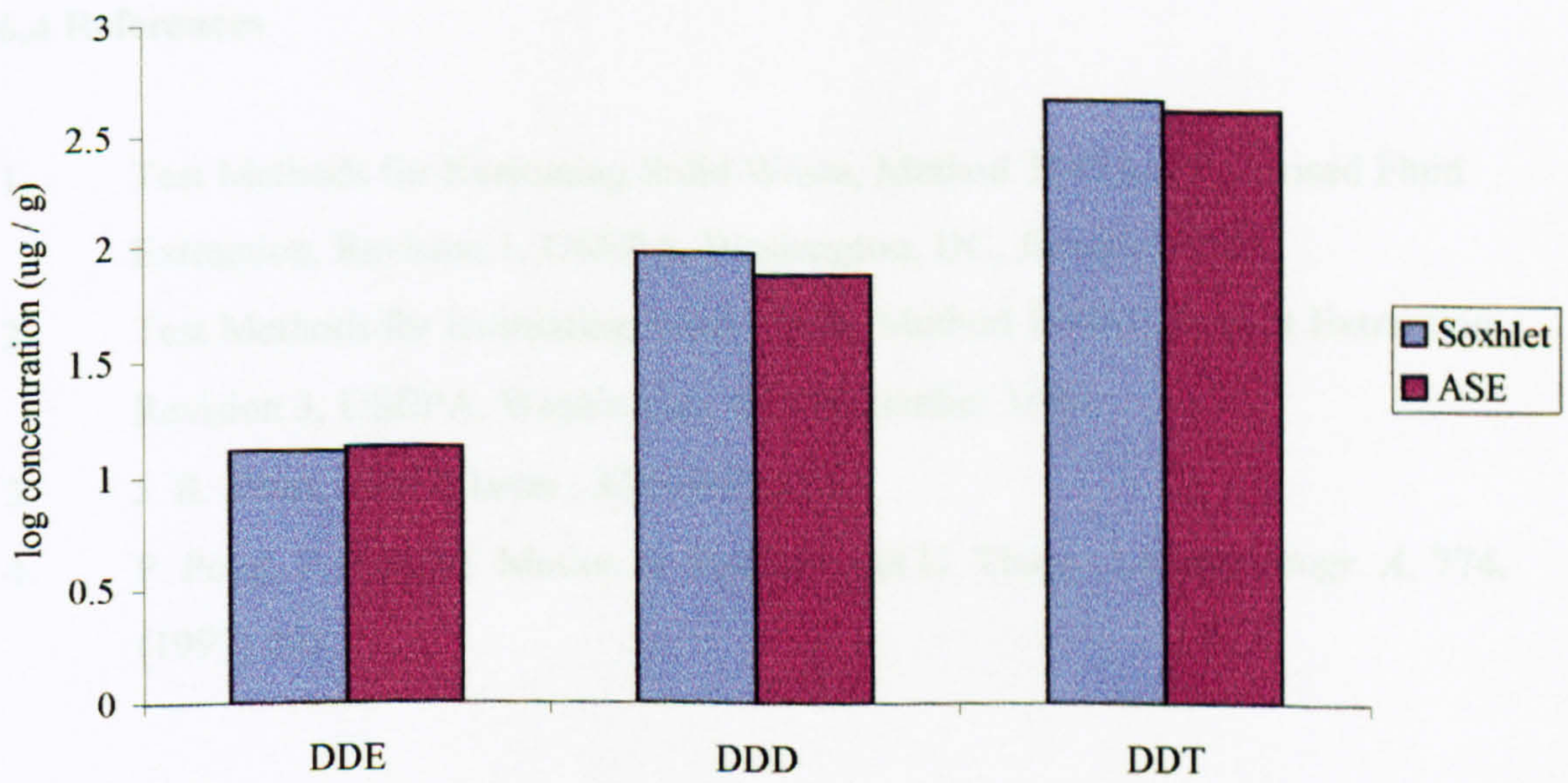
of the order of 0.1-0.2 mL / cycle of the static valve.[3] In order to determine if solvent re-cycling was the reason for the apparent poorer recovery of DDT, DDD and DDE from the aged, contaminated soil in PFE, an assessment of the static flush cycles was undertaken. The results of this study are shown in Table 6.6. In each case a new soil sub-sample was extracted and analysed.

Table 6.6 Static flush study

No Cycles	DDE µg / g (% RSD)	DDD µg / g (% RSD)	DDT µg / g (% RSD)
1	10.9 (5.7)	40.0 (5.0)	226 (8.1)
2	12 (5.2)	59.2 (7.2)	336 (2.6)
3	13.2 (5.2)	79.9 (3.4)	405 (6.7)

The data shows that three static flushes are required to quantitatively remove DDT, DDD and DDE from aged, contaminated soil using a chlorinated solvent. Results from the cumulative total via PFE are compared with 24 hour Soxhlet extraction in figure 6.7.

Figure 6.7 Comparison of PFE and Soxhlet



Good agreement is achievable between the two techniques. It has clearly been shown that the number of static flush cycles required for quantitative recovery from aged, contaminated soil samples is three. A similar study, with similar findings, was reported by Popp et al.[4] for a series of chlorinated pesticides from contaminated soil.

6.3 Summary.

PFE has shown the ability to quantitatively extract organochlorine pesticides from both inert matrices and real matrices (natively contaminated soil), with a minimal volume of solvent and operator interaction. Optimisation of the extraction parameters temperature, time and pressure was achieved in a relatively small amount of time (c.f. Soxhlet). It has clearly been shown that the number of static flush cycles required for quantitative recovery from aged, contaminated soil samples is three. A similar study, with similar findings, was reported by Popp et al.[4] for a series of chlorinated pesticides from contaminated soil.

6.4 References

1. Test Methods for Evaluating Solid Waste, Method 3545A, Pressurised Fluid Extraction, Revision 1, USEPA, Washington, DC, January 1998
2. Test Methods for Evaluating Solid Waste, Method 3540C, Soxhlet Extraction, Revision 3, USEPA, Washington, DC, December 1996.
3. J. R. Dean, *Anal. Comm.*, **33** (1996) 191.
4. P. Popp, P. Keil, M. Moder, A. Paschke and U. Thuss, *J. Chromatogr. A*, **774**, (1997) 203.

Section B

Solvent Selection

Section B

Solvent selection.

This section concentrates on the development of a model to predict the optimum extraction solvent in environmental analysis. Several methods are discussed, along with their inherent problems. The model is applied to work carried out in the previous section i.e. to bupirimate, ethirimol, PCP, DDT, DDD and DDE, before examples are taken from the literature.

The two examples chosen are those that apply to real samples. The first is the extraction of PCDD / F's from fly ash. This work was carried out by Bautz et al.[1] The second example is the extraction of PCB's from a certified reference material. This was performed by Hawthorne et al.[2]

Examples in the literature are scarce, due to the lack of real samples that are extracted using several solvents, one or more of which give poor recovery of the target analyte.

References

1. H. Bautz, J. Polzer and L. Steigliez, *J. Chromatogra. A*, **815,** 1992, 231.
2. S. B. Hawthorne, J. J. Langenfeld, D. J. Miller, and M. D. Burford. *J. Chromatogra. A*, **64,** 1992, 1614.

Chapter 7

Extraction Solvent Selection in Environmental Analysis

Extraction Solvent Selection in Environmental Analysis

7.0 Introduction.

In order to determine the level of contamination of industrial contaminated land sites requires, after appropriate sampling, extraction of the pollutants from the soil. A variety of techniques are available for extraction of organic pollutants from solid environmental matrices.[1] Techniques available, range from the traditional (e.g. Soxhlet extraction, shake-flask and sonication), through to instrumental extraction techniques (e.g. supercritical fluid extraction, microwave-assisted extraction and pressurised fluid extraction). However, irrespective of the sophistication of the technique, each approach has a common feature i.e. choice of solvent. The choice of solvent is largely dependent upon past experience, manufacturers guidelines or recommendations in standard methods (e.g. EPA methods). Typically, as is the case with Soxhlet extraction, a large volume of organic solvent is required. Often the solvents recommended are chlorinated as in the use of dichloromethane. Prediction of the optimum solvent would therefore be advantageous.

There are a few approaches used to try to predict the best solvent for chromatography. Rohrschneider[2] has classed gas chromatography column stationary phase on the basis of the retention time of a similar n-alkane in the system. This retention index is independent of flow rate and the physical dimensions of the column. Snyder[3, 4] extended the work of Rohrschneider and developed a polarity index (P') where P' is used to describe the properties of the solvent. This technique involves the experimental determination of the distribution coefficient, Kg, for the test solutes, ethanol (e), n-octane (o), dioxane (d) and nitromethane (n) in various solvent systems. Correction for the solvent and solute molecular weight gives Kg' and log Kg'',

respectively. The polarity index (P') is then calculated by adding the log Kg'' values for ethanol, dioxane and nitromethane. The contribution to proton acceptor ability, the extent of dipole moment, and the proton donation ability (x_e, x_n and x_d, respectively) are calculated from the ratio log Kg'' / P' for each solute. The approach attempts to mirror the particular interaction properties that are peculiar to that solvent. Other approaches to describe solvent properties include: the use of $E_T(30)$ values, a measure of the ability of a solvent to ionise a molecule at a set temperature[5]; *Z* values which are similar to $E_T(30)$ values, but use a different analyte[5]; and, Acity (*A*) and Bascity (*B*) of the solvent.[5] However none of these methods (to date) have been applied to extraction solvent systems.

The solvent prediction scheme used in this paper is based on the Hildebrand solubility parameter (δ_t).[6, 7] This quantity has been applied to the dissolution of polymeric substances in various solvents, and is commonly used by art conservators in the restoration of old paintings.[8] The solubility parameter is a measure of the internal energy of cohesion in the solvent / solute. Solvents with similar solubility parameters form mixtures,[7] hence an analyte and a solvent that have similar solubility parameters, should also form mixtures.

δ_t is defined as the square root of the cohesive energy density[6, 7] or

$$\delta_t = (\Delta E_V / V)^{1/2} \qquad \text{(Eqn 7.1)}$$

δ_t = the total Hildebrand solubility parameter

ΔE_V = the energy of vaporisation at a given temperature

V = molar volume of the molecule.

Calculations of this sort require knowledge of the heat of vaporisation at various temperatures as well as the molar volume of the substance. However, these values are not widely available for pesticides. Several groups have also developed comparable quantities as the Hildebrand solubility parameter including the addition of group contributions. Fedors[9] and others have used this approach to calculate the total Hildebrand solubility parameter. Blanks and Prausnitz[6] have used a homomorph concept to describe the total solubility parameter. The method is based on the assumption that the polar heat of vaporisation of a molecule can be calculated from the difference in the total energy of vaporisation and the energy of vaporisation of a non-polar liquid that has molecules of nearly the same size and shape as those of the liquid under investigation. Procedures such as this do not give the contribution of each type of interaction commonly found in matter e.g. the polarity, dispersion and hydrogen bonding ability of the solvent; each of which can be vital in the extraction of pesticides. The total solubility parameter has been divided by Small, van Arkel and Prausnitz[6] into two portions, a polar contribution and a non-polar contribution. This procedure does not address the induced and hydrogen bonding ability of the liquid. Hansen[7, 10] has taken this work further and assumes the total cohesive energy is a linear addition of three components: δ_h, hydrogen bonding ability contribution; δ_d, dispersion co-

efficient contribution; and, δ_p, polarity contribution. They are linked by the following equation.

$$\delta_t^2 = \delta_h^2 + \delta_p^2 + \delta_d^2 \qquad \text{(Eqn 7.2)}$$

Hansen based this work on semi empirical equations describing the entropy and enthalpy of mixing of polymers and solvents in solution.[10] Null and Palmer have also used this approach,[11] although their work was based on the findings of Wiehe and Bagley[12] who investigated the magnitude of activity coefficient of alcohol in various inert solvent solutions and developed equations that described the entropy and enthalpy of associations within the solution.

Hoy[6] determined the individual components of the solubility parameter using the following methodology:

1 Determination of the total solubility parameter using the Clausius-Clapeyron equation, which is a measure of the change in vapour pressure of a substance at various temperatures.

2 Regression analysis of molar volume as a function of temperature, molecular weight and density, allowing the calculation of an aggregation number that is an estimate of δ_h.

3. δ_p is then calculated by a group molar attraction method, using equation 7.3, where F_p is the individual group contribution.

$$\delta_p = ({}_z\Sigma^z F_p) / V \qquad \text{(Eqn 7.3)}$$

4. Determination of δ_h is then achieved by rearranging equation 7.1, and solving for δ_h.

van Krevelen and Hoftzyer[13] have also determined the individual components using a group contribution additive method, not unlike that of Fedors.[9] This chapter outlines a procedure for predicting the optimum extraction solvent for the removal of analytes from aged soils. Matrix-analyte interactions are not taken into account, although they will influence the extraction procedure.

7.1 Experimental

All the experimental used in the chapter has been described earlier in chapters 4 – 6. Experimental procedures for bupirimate and ethirimol are detailed in chapter 4, details of PCP are in chapter 5, and details of DDX are in chapter 6.

7.2 Results and Discussion

7.2.1 Limitations of Solvent Prediction.

There are limitations predicting the extraction solvent. The major limitations of the Hansen and Teas systems have been highlighted by Michalski,[16] Blanks and Stavroudis,[17] and Huyskens.[18] It was noted that although Teas was the first to visually represent the solvent selection parameters via a ternary plot, he also manipulated his

data to fit experimental observations.[17] This is shown by the non linear relationship between Hansen's data and Tea's data (figures 7.1 – 7.3).

Figure 7.1 Hansen vs. Teas dispersion contribution values.

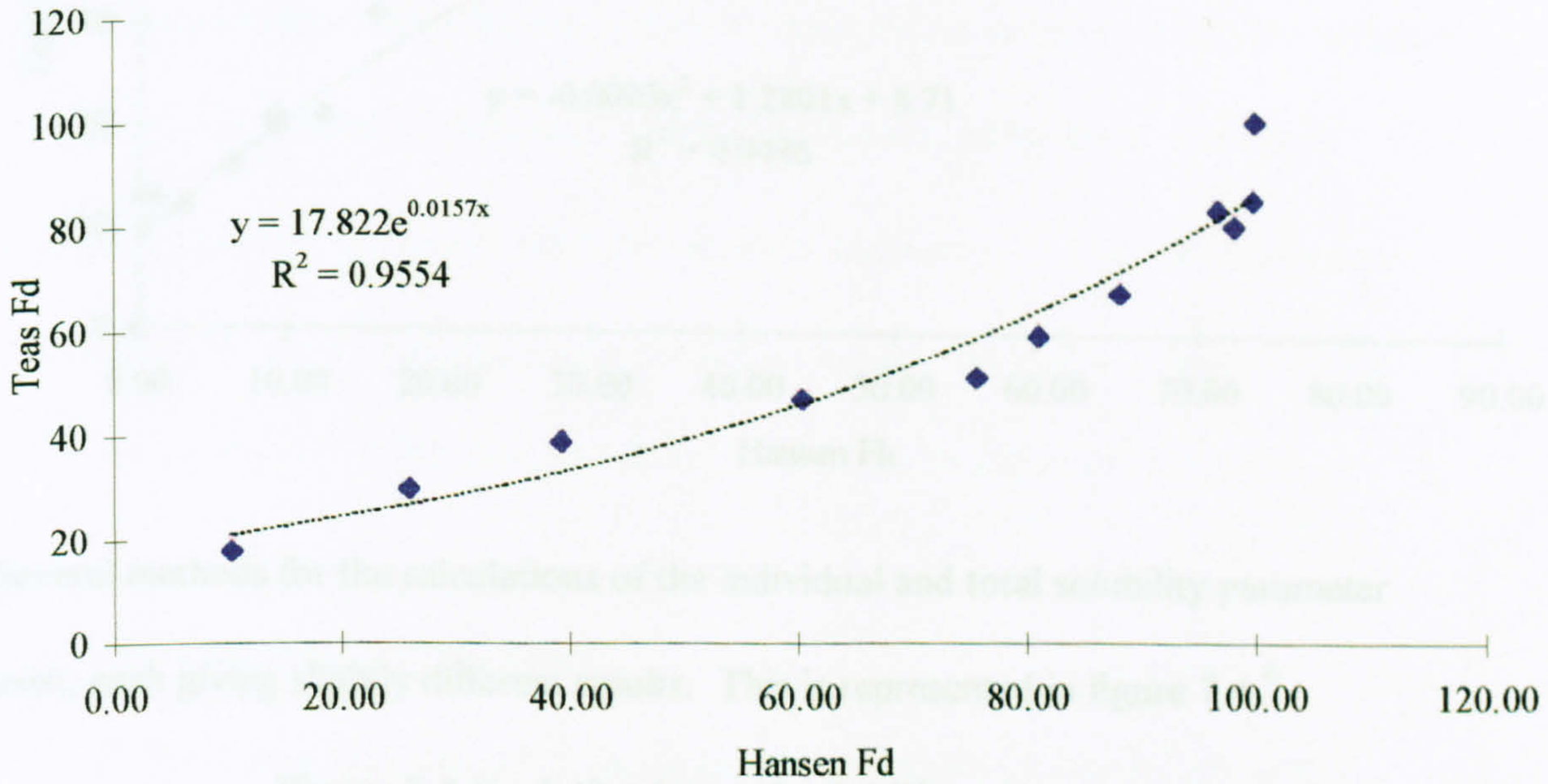


Figure 7.2 Hansen vs. Teas polarity contribution values.

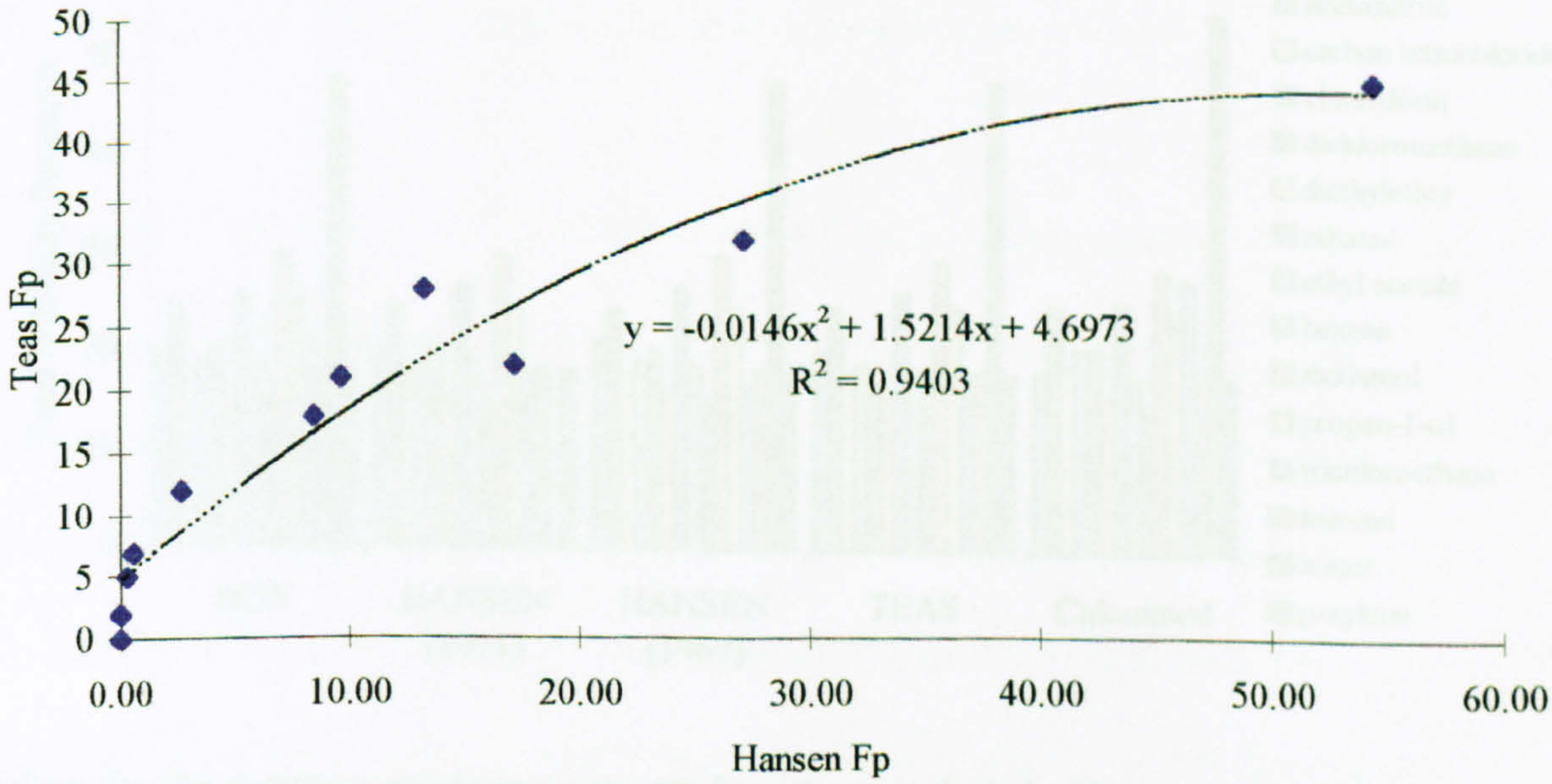
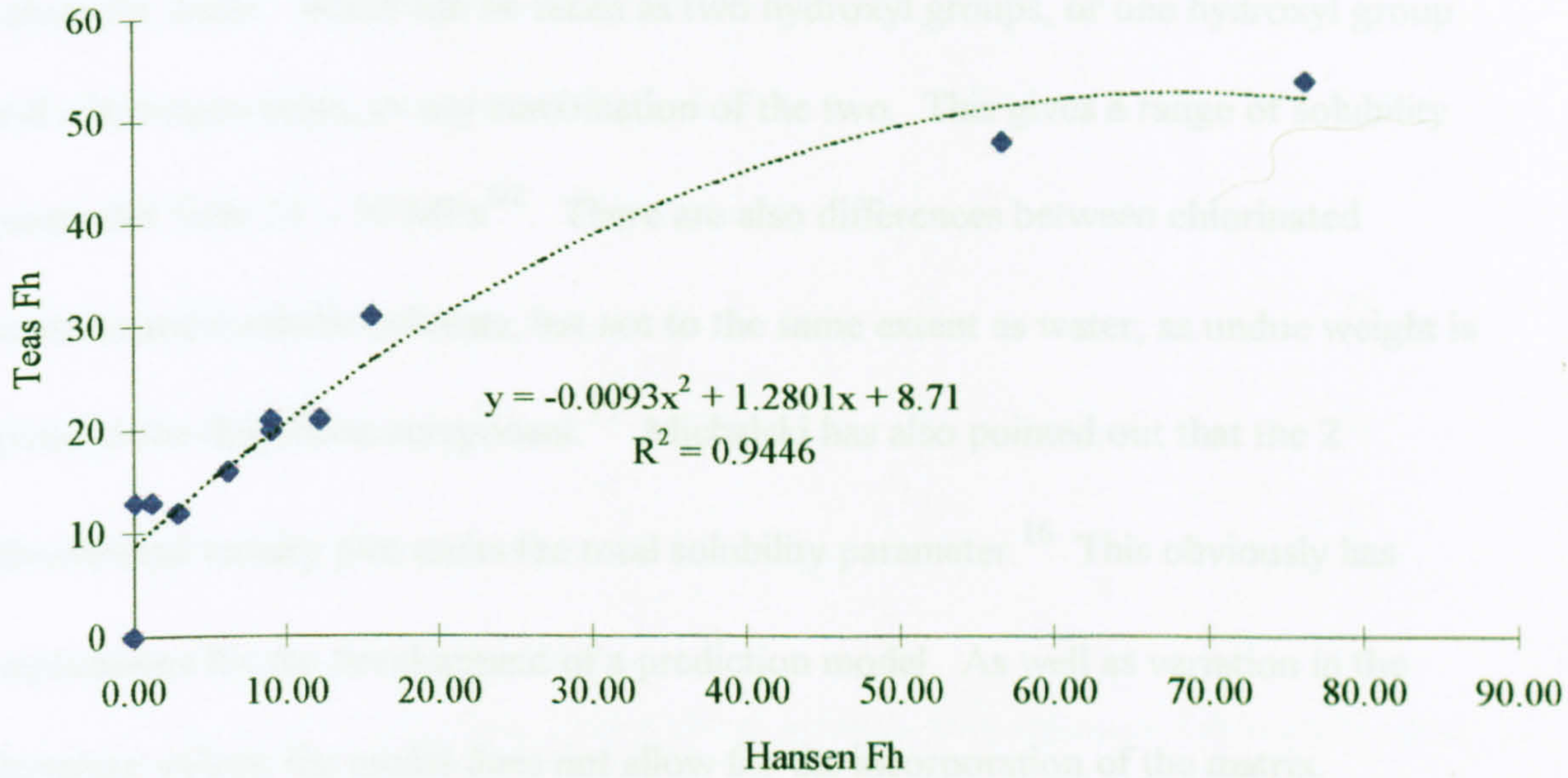
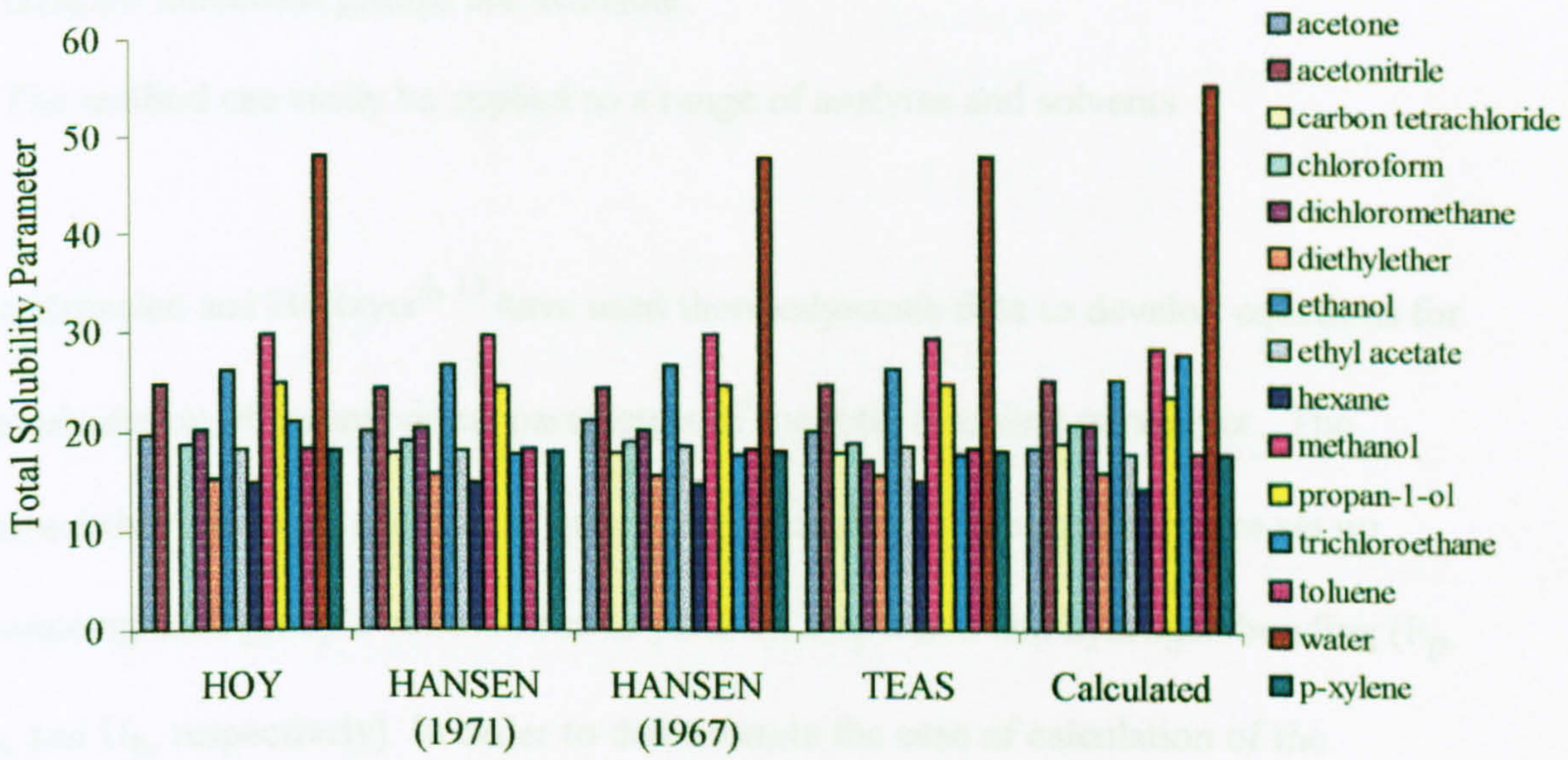


Figure 7.3 Hansen vs. Teas hydrogen bonding contribution values.



Several methods for the calculations of the individual and total solubility parameter exist, each giving slightly different results. This is represented in figure 7.4.[6]

Figure 7.4 Variation in total solubility parameter.



Values for the common extraction solvents have been included. However, there is a large discrepancy between the values for water. This has been attributed to the various

adjustments made by Hoy[6] and Teas,[8, 16] and the actual calculation of the individual values for water. Water can be taken as two hydroxyl groups, or one hydroxyl group and a hydrogen atom, or any combination of the two. This gives a range of solubility parameter from 33 – 70 $MPa^{1/2}$. There are also differences between chlorinated solvents and aromatic solvents, but not to the same extent as water, as undue weight is given to the dispersion component.[17] Michalski has also pointed out that the 2 dimensional ternary plot omits the total solubility parameter.[16] This obviously has implications for the development of a prediction model. As well as variation in the literature values, the model does not allow for the incorporation of the matrix.

7.2.2 Calculations of parameters

The van Krevelen and Hoftzyer[6, 13] approach was selected for calculation of the individual Hansen parameters for the following two main reasons:

1. Data for numerous groups are available.

2. The method can easily be applied to a range of analytes and solvents.

van Krevelen and Hoftzyer[6, 13] have used thermodynamic data to develop equations for the calculation of the individual parameters of the total solubility parameter. The method they used was addition of group contributions. Tables of data were set up containing each group's contribution to polarity, dispersion and hydrogen bonding (F_p, F_d, and U_h, respectively). In order to demonstrate the ease of calculation of the individual group contributions a solvent (methanol) and an analyte (DDT) have been

selected, tables 7.1 and 7.2. Using the following equations (7.4 – 7.7) δ_p, δ_h, and δ_d can be calculated.

$$\delta_d = ({}_z\Sigma^z F_d) / V \qquad (7.4)$$

$$\delta_p = ({}_z\Sigma^z F_p) / V^* \qquad (7.5)$$

$$\delta_p = ({}_z\Sigma^z F_p{}^2)^{1/2} / V \qquad (7.6)$$

$$\delta_h = (({}_z\Sigma^z U_h) / V)^{1/2} \qquad (7.7)$$

* for molecules with more than 1 polar group present, then equation 7.6 must be used instead of equation 7.5, to take into account the interactions between the polar groups[13].

Table 7.1. Calculation of individual group contributions for Methanol

Group	Group contribution to dispersion (F_d) $J^{1/2}\ cm^{3/2}\ mol^{-1}$	Group contribution to polarity (F_p) $J^{1/2}\ cm^2\ mol^{-1}$	Group contribution to hydrogen bonding (U_h) $J\ mol^{-1}$	Molar volume (V) $cm^3\ mol^{-1}$
CH_3	420	0	0	33.5
OH	210	500	20000	10.0
Total	**630**	**500**	**20000**	**43.5**

Appendix A1 shows the calculation of the individual parameters for methanol while appendix A2 shows the calculation for the individual parameters for DDT. The values for the group contributions were taken from reference 15.

Note: 1 MPa = 1 J cm^{-3}; 1 J = 1 Nm

Table 7.2. Calculation of individual group contributions for the analyte, DDT

Group	Group contribution to dispersion (F_d) $J^{1/2}\ cm^{3/2}\ mol^{-1}$	Group contribution to polarity (F_p) $J^{1/2}\ cm^{2}\ mol^{-1}$	Group contribution to hydrogen bonding (U_h) $J\ mol^{-1}$	Molar volume (V) $cm^3\ mol^{-1}$
2 x -Ph-	2540	48400	0	104.8
2 x Cl-CH=	900	1210000	800	48
3 x Cl	1350	2722500	1200	72
1 x CH	80	0	0	-1.0
>C<	-70	0	0	-19.2
Total	**4800**	**3980900**	**2000**	**204.6**

The total Hildebrand solubility parameter, δ_t, was calculated for methanol and found to be 28.31 $MPa^{1/2}$ using equation 7.1. This compared favourably with the literature value of 29.6 $MPa^{1/2}$.[6] Table 7.3 shows the individual calculated parameters for each of the solvents used in the experimental study.

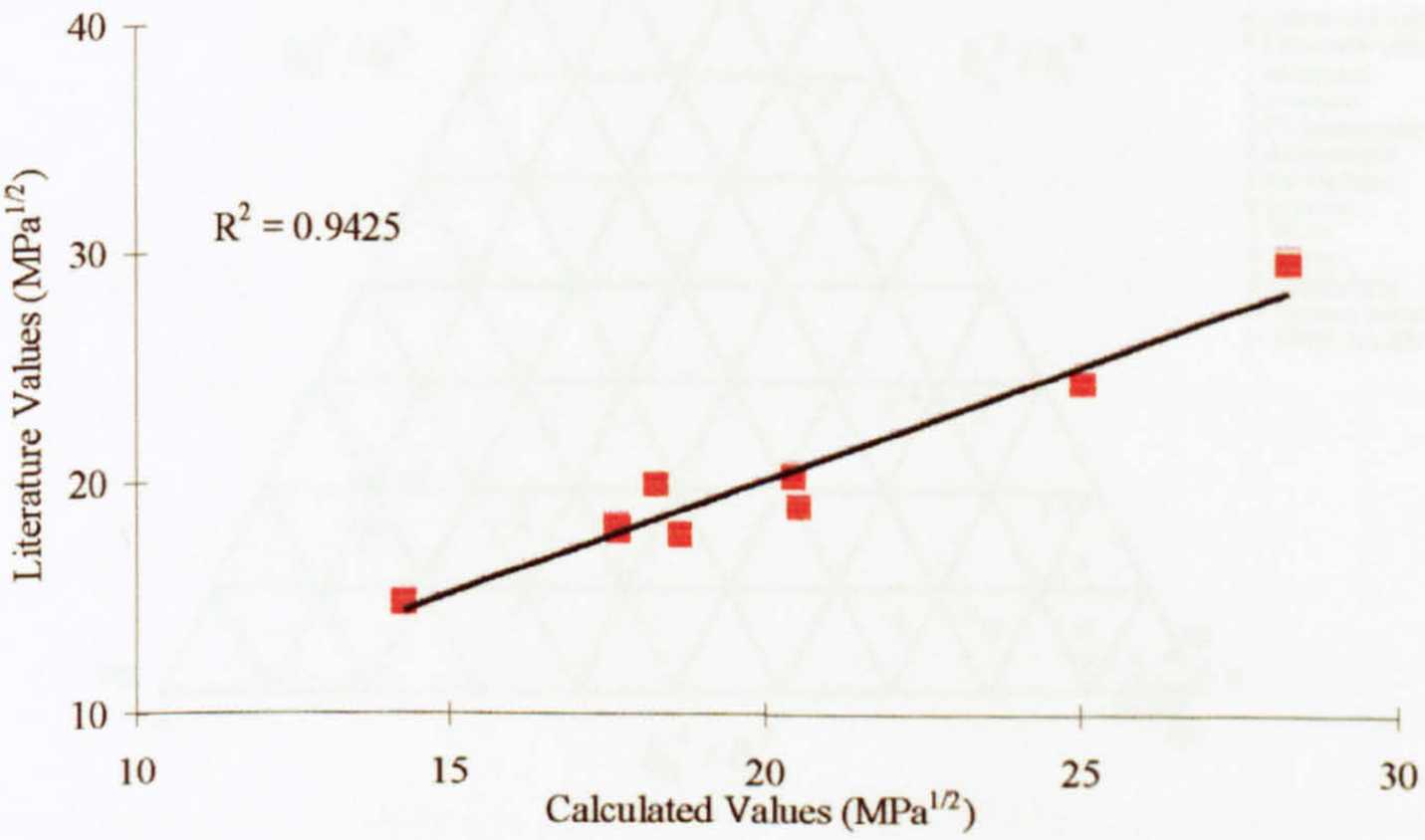
Table 7.3. Total Hildebrand Solubility Parameter and its individual components used in the extraction study

	Dispersion coefficient, δ_d ($MPa^{1/2}$)	Polarity, δp ($MPa^{1/2}$)	Hydrogen bonding, δ_h ($MPa^{1/2}$)	Total Hildebrand Solubility Parameter, δ_t ($MPa^{1/2}$)
Methanol	14.48	11.49	21.44	28.31
Toluene	17.64	1.05	0.00	17.67
Acetonitrile	14.78	19.13	6.59	25.06
Acetone	14.52	9.90	5.07	18.29
Dichloromethane	18.25	8.58	3.53	20.48
Iso-Hexane	14.27	0.00	0.00	14.27
Acetonitrile:dichloromethane (1:1, v/v)	16.52	13.86	5.06	22.77

A comparison between the total Hildebrand solubility parameter[6] and the total calculated solubility parameter, using the approach described above, for ten common extraction solvents (methanol, acetone, dichloromethane, acetonitrile, iso-hexane,

toluene, xylene, chloroform, carbon tetrachloride and ethyl acetate) is shown in Figure 7.5. A good correlation (correlation coefficient, $R^2 = 0.9425$) is observed between the values.

Figure 7.5 correlation between calculate and literature values



Fractional parameters of the Hildebrand solubility parameter can be calculated using equations 7.8 – 7.10 and plotted on a triangular graph in order to give a visual representation of the extent of contribution from the three components (polarity, dispersion and hydrogen bonding).

$$(\delta_d^2 / \delta_t^2) \times 100 = Fr_d \quad (8)$$

$$(\delta_p^2 / \delta_t^2) \times 100 = Fr_p \quad (9)$$

$$(\delta_h^2 / \delta_t^2) \times 100 = Fr_h \quad (10)$$

Table 7.4 shows the calculated (van Krevelen and Hoftzyer data) and literature values[6] of these fractional (Fr) parameters. The numbers have been multiplied by 100 for ease of plotting. Figure 7.6 compares the literature values with the calculated values.

Where no literature value is given, the calculated value and the literature value overlap.

Figure 7.6 Comparison of literature and calculated values

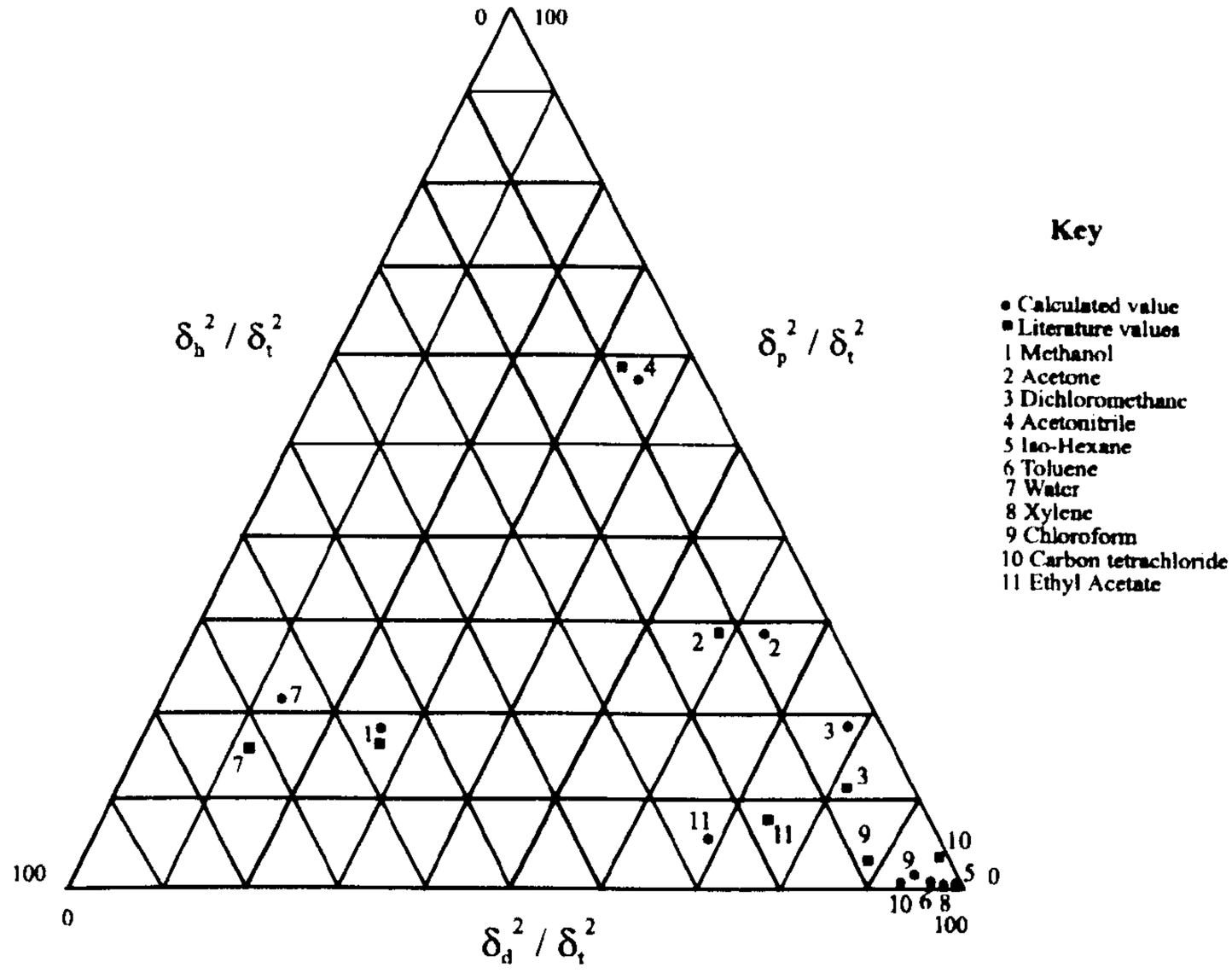


Table 7.4. Literature and Calculated Values of Fractional Parameters for dispersion, polarity and hydrogen bonding of selected solvents.

	$100Fr_d$		$100Fr_p$		$100Fr_h$	
	Calculated value	**Literature value**	**Calculated value**	**Literature value**	**Calculated value**	**Literature value**
Methanol	26.2	26.0	16.5	17.3	57.4	56.7
Acetone	63.0	60.5	29.3	27.2	7.7	12.3
Dichloromethane	79.5	81.2	17.6	9.7	3.0	9.1
Acetonitrile	34.8	39.3	58.3	54.4	6.9	6.3
Iso-hexane	100.0	100.0	0.0	0.0	0.0	0.0
Toluene	99.6	98.2	0.4	0.6	0.0	1.2
Xylene	99.7	96.8	0.3	0.3	0.0	2.9
Chloroform	96.0	88.3	0.0	2.7	4.0	9.1
Carbon tetrachloride	95.2	99.9	0.0	0.0	4.8	0.1
Ethyl acetate	69.4	75.7	4.5	8.5	26.1	15.7

There are some variations, especially for chlorinated and aromatic solvents. This is due to multiplication factors that need to be included in the calculation of δ_p for

chloroform, carbon tetrachloride and dichloromethane in order to take into account identical groups (chlorine) in symmetrical positions.

7.2.3 DDT and metabolites (DDD and DDE) from natively contaminated soil.

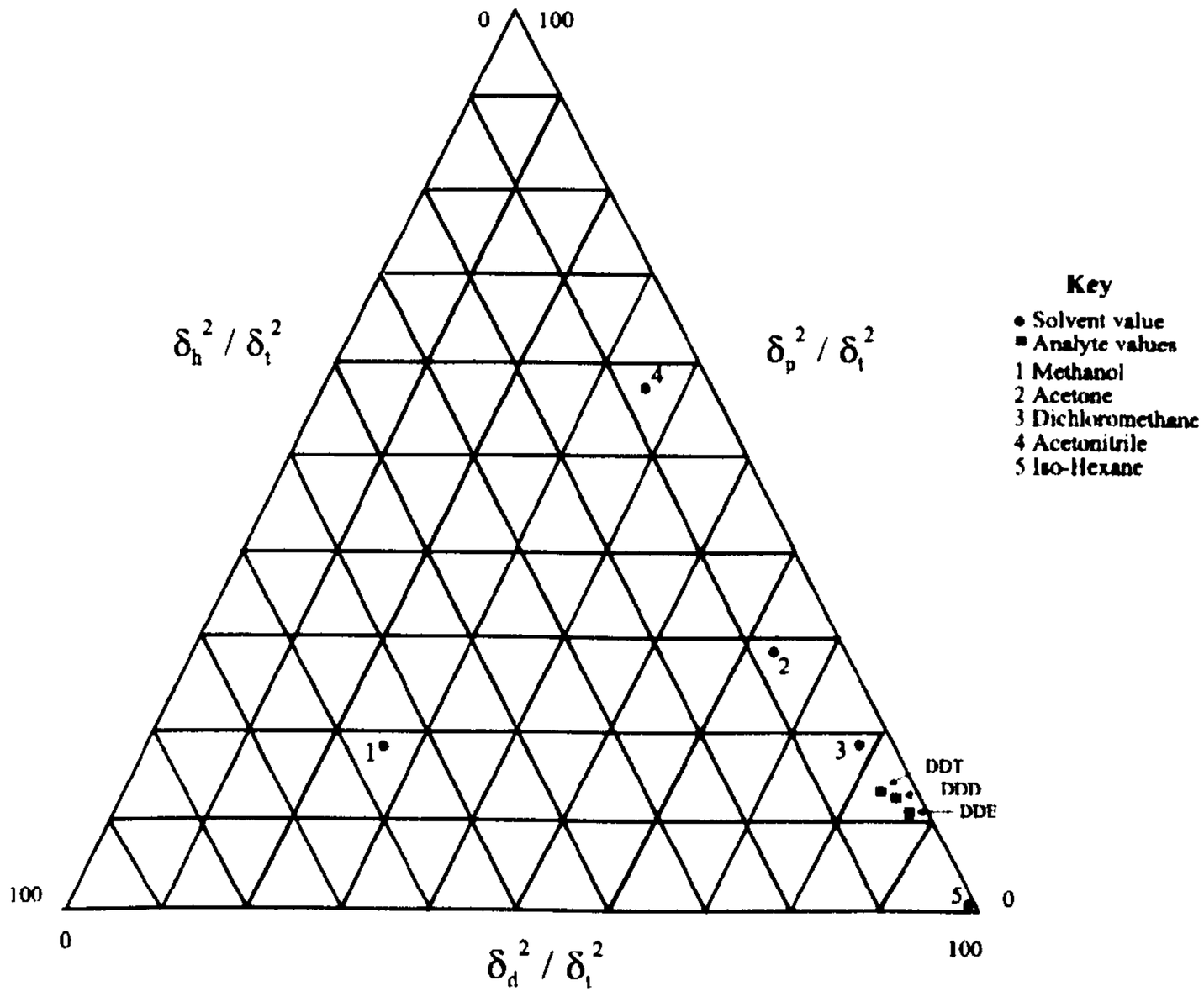
Table 7.5 shows the calculated fractional parameters for the analytes.

Table 7.5. Calculated Values of Fractional Parameters for Dispersion, Polarity and Hydrogen Bonding of DDT, DDD and DDE.

Analyte	$100Fr_d$	$100Fr_p$	$100Fr_h$
DDT	84.0	14.5	1.5
DDD	87.1	11.4	1.5
DDE	89.0	9.8	1.2

Figure 7.7 shows the position of the analytes DDT, DDD and DDE compared to various solvents. Using this plot, DCM is predicted to be the optimum solvent for extraction.

Figure 7.7 Prediction of optimum solvent for DDT, DDD and DDE



Experimental results, based on the mean concentration of six extractions per solvent, for the PFE of DDT, DDD and DDE from a contaminated soil using a selection of organic solvents (five) are shown in figures 7.8 (a-c). Typical percentage relative standard deviations (% RSD) for the PFE of DDT ranged from 3.7 - 11.3; 4.1 - 13.0 for DDD; and, 0.4 - 3.9 for DDE. The PFE extraction results confirm that DCM removes the largest amount of the target analytes from the aged soil matrix. It is observed that the highest recoveries of DDT, DDD and DDE are obtained with DCM. From figure 7.7 however it would also be expected that both iso-hexane and acetone would remove significantly more of the DDT and its metabolites than methanol and acetonitrile. Experimental data (Figures 7.8a-c) also confirms this finding.

Figure 7.8a DDT solvent graph

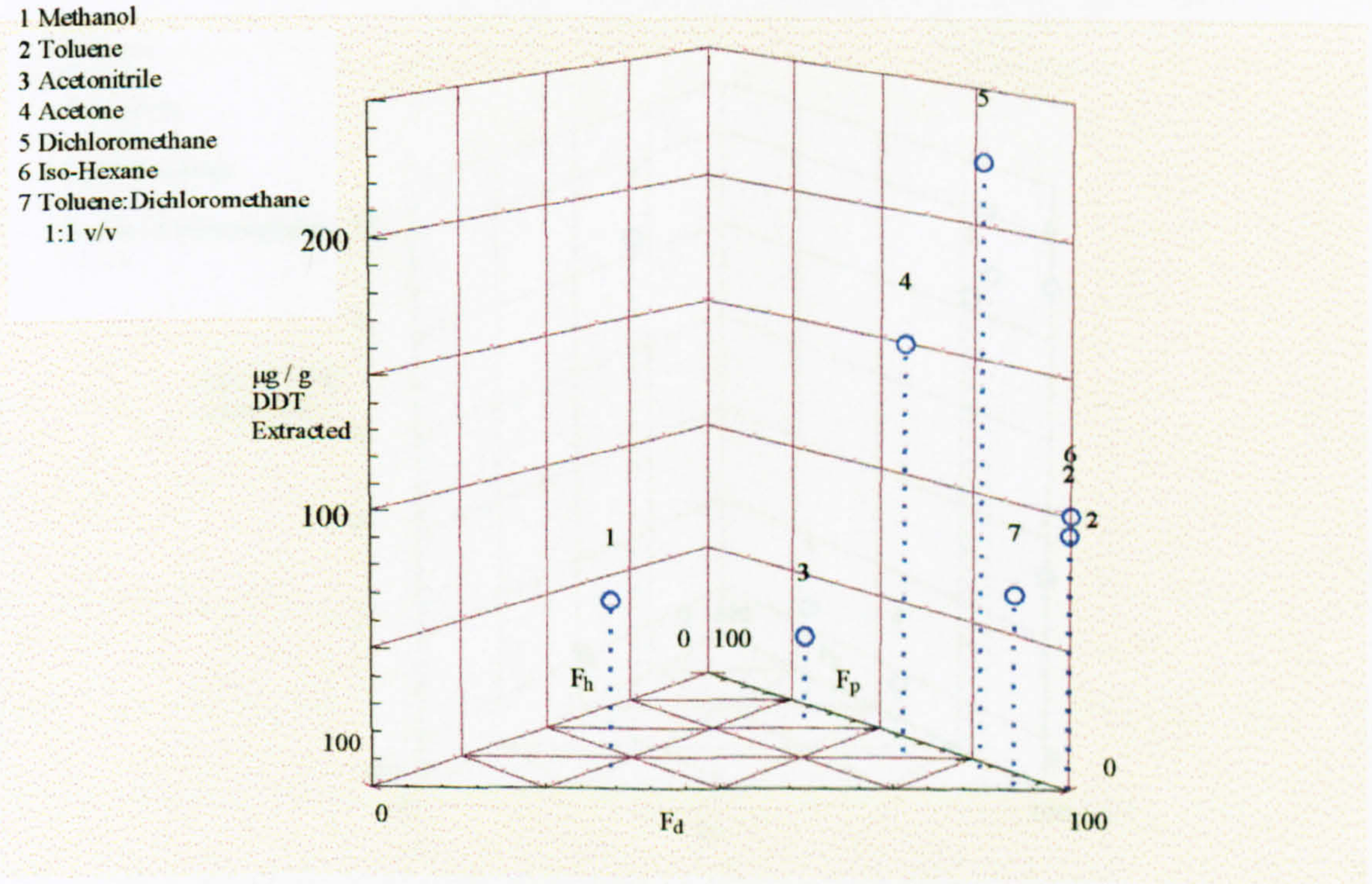


Figure 7.8b DDD solvent graph

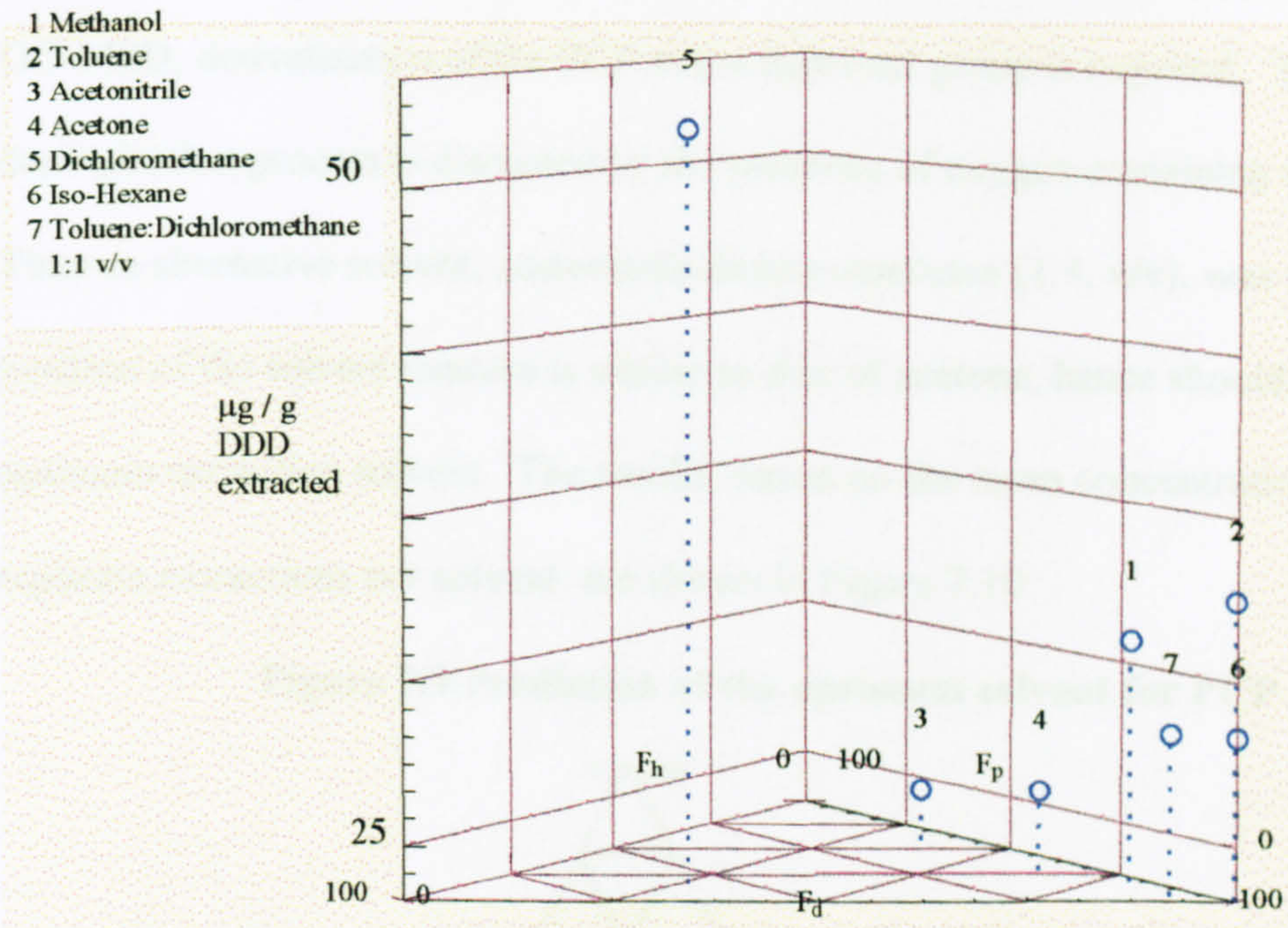
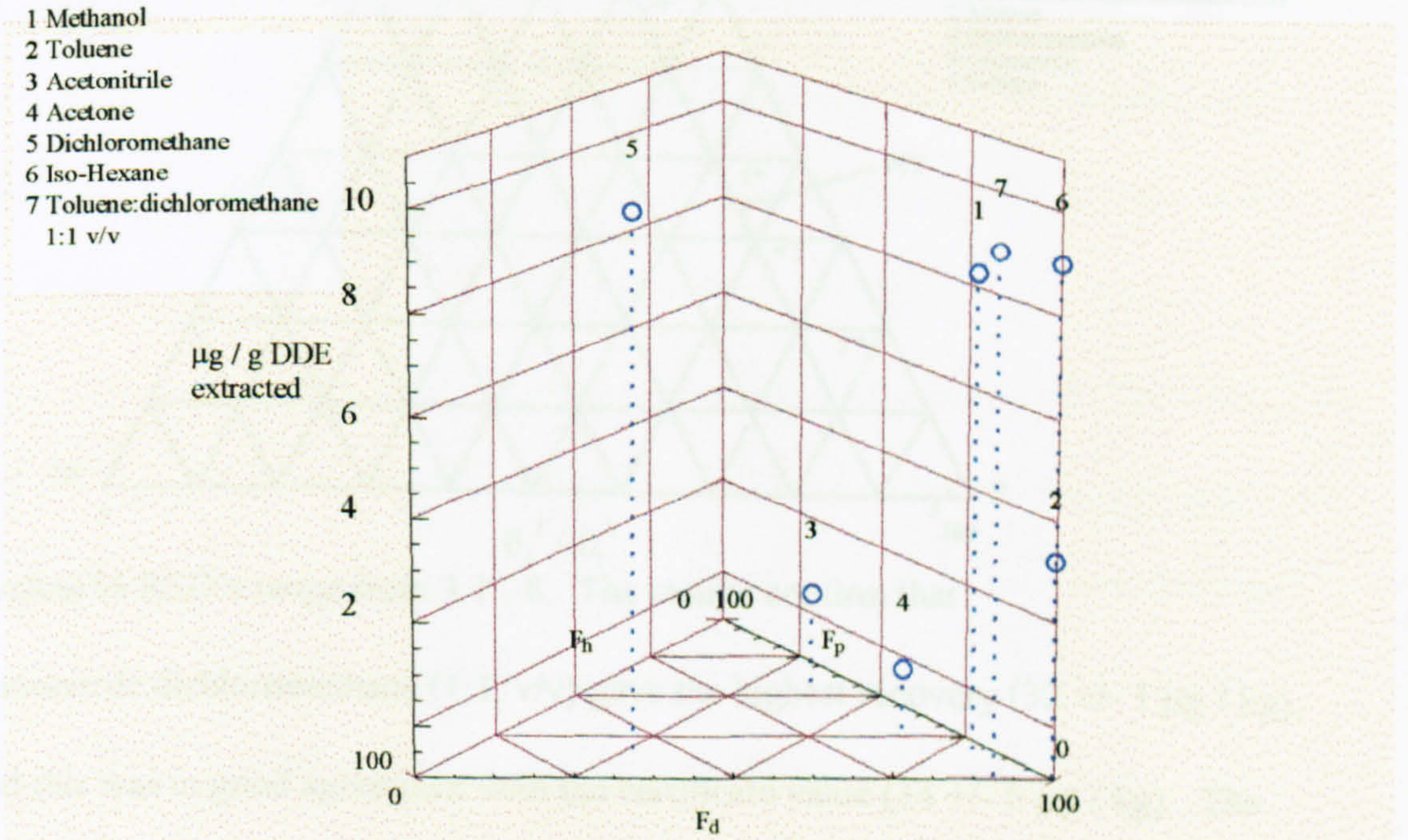


Figure 7.8c DDE solvent graph

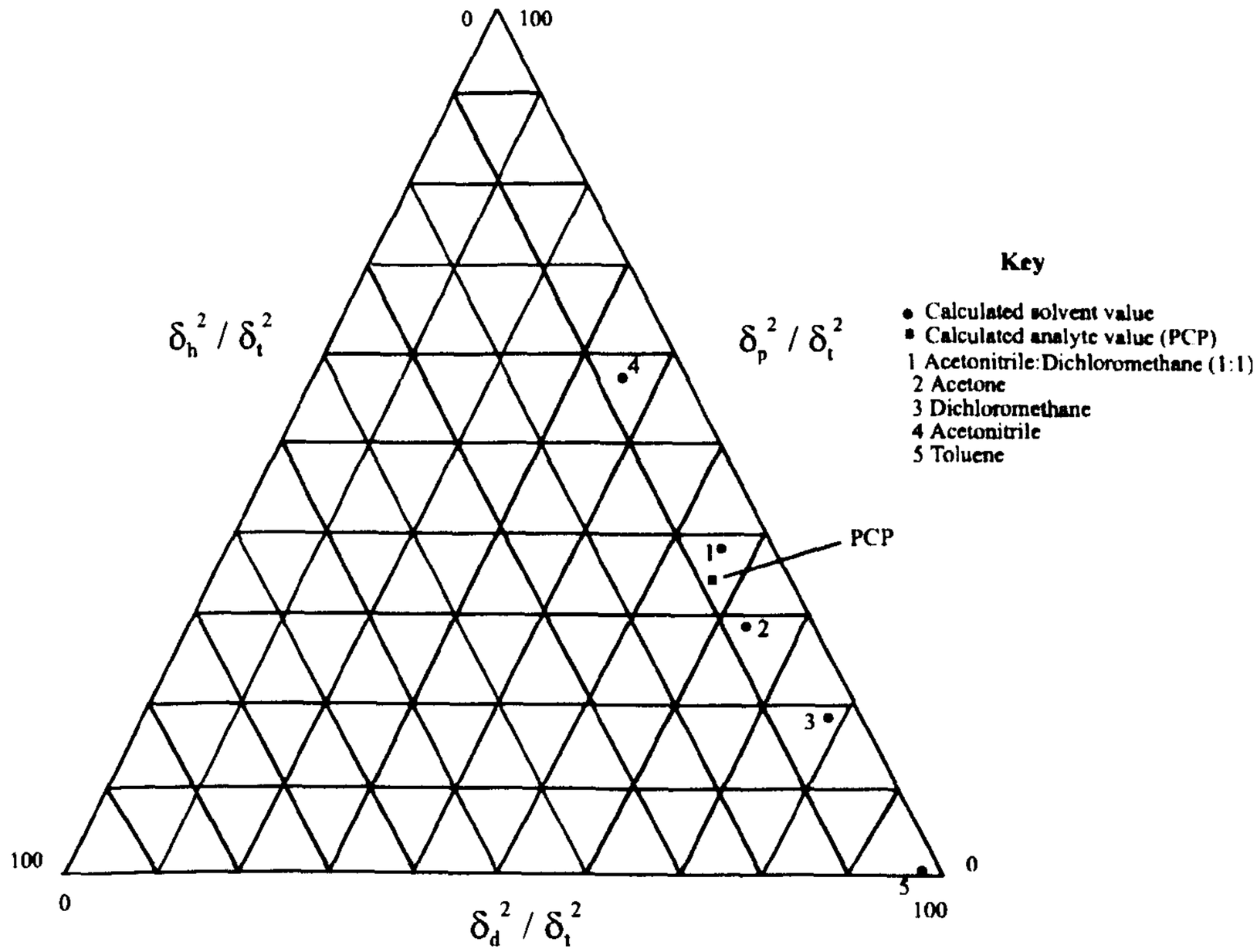


7.2.4 PCP from CRM 524

A similar strategy was applied to the extraction of pentachlorophenol from a certified reference material (CRM 524). It is predicted (Figure 7.9) that acetone would be the

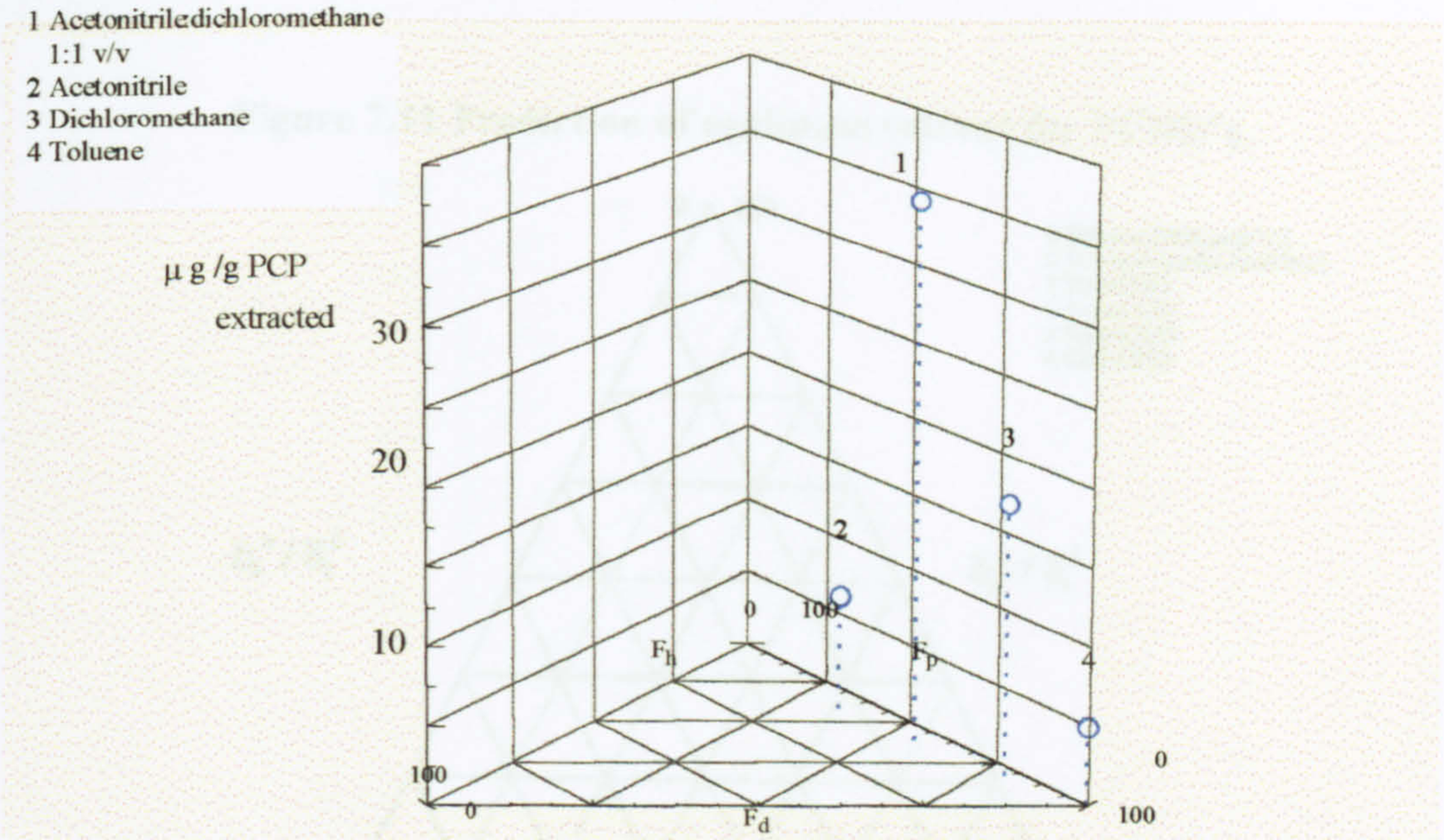
optimum extraction solvent. However, to obtain a separation and analysis of PCP by GC-MSD, derivatisation of the PCP at the hydroxyl group is required. This derivatisation process is disrupted by the presence of oxygen containing molecules. Thus an alternative solvent, acetonitrile:dichloromethane (1:1, v/v), was used. The position of the solvent mixture is similar to that of acetone, hence should be the optimum extraction solvent. The results, based on the mean concentration of six replicate extractions per solvent, are shown in Figure 7.10.

Figure 7.9 Prediction of the optimum solvent for PCP



Typical % RSD's range from 3.1 - 8. The results confirm that acetonitrile:dichloromethane (1:1, v/v) gave the highest recovery (32 +/- 1 µg / kg), and this was in good agreement with the certificate value (34 +/- 5 µg / kg). The model also predicts that toluene would give significantly lower extraction recovery than any of the other solvents, figure 7.10 confirms this.

Figure 7.10 PCP solvent graph



7.2.5 Literature examples.

To validate the model further, two examples of extraction from real samples were taken from the literature and incorporated into the model. The first example is the extraction of polychlorinated dibenzo dioxins from fly ash.[14]

Figure 7.11 shows the relative positions of solvents and analytes for PCDD extraction. From the data it can be seen that the analytes and the solvent used for their extraction are all in the same area on the ternary plot. Therefore, the two solvents should yield similar extraction results. Table 7.6 and figure 7.12 shows that this is the case. Neither solvent appears to give a superior extraction of these analytes.

Figure 7.11 Prediction of optimum solvent for PCDD's.

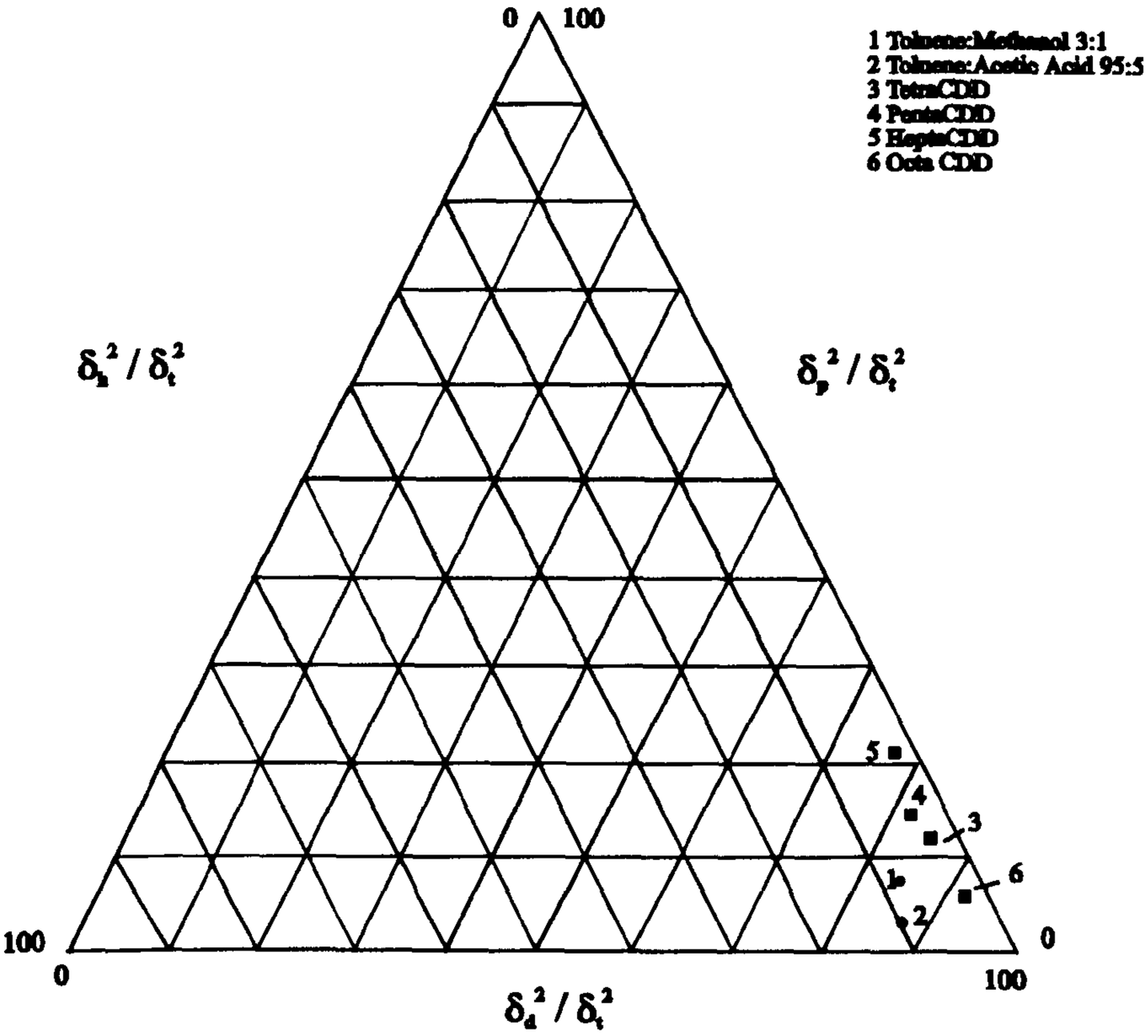
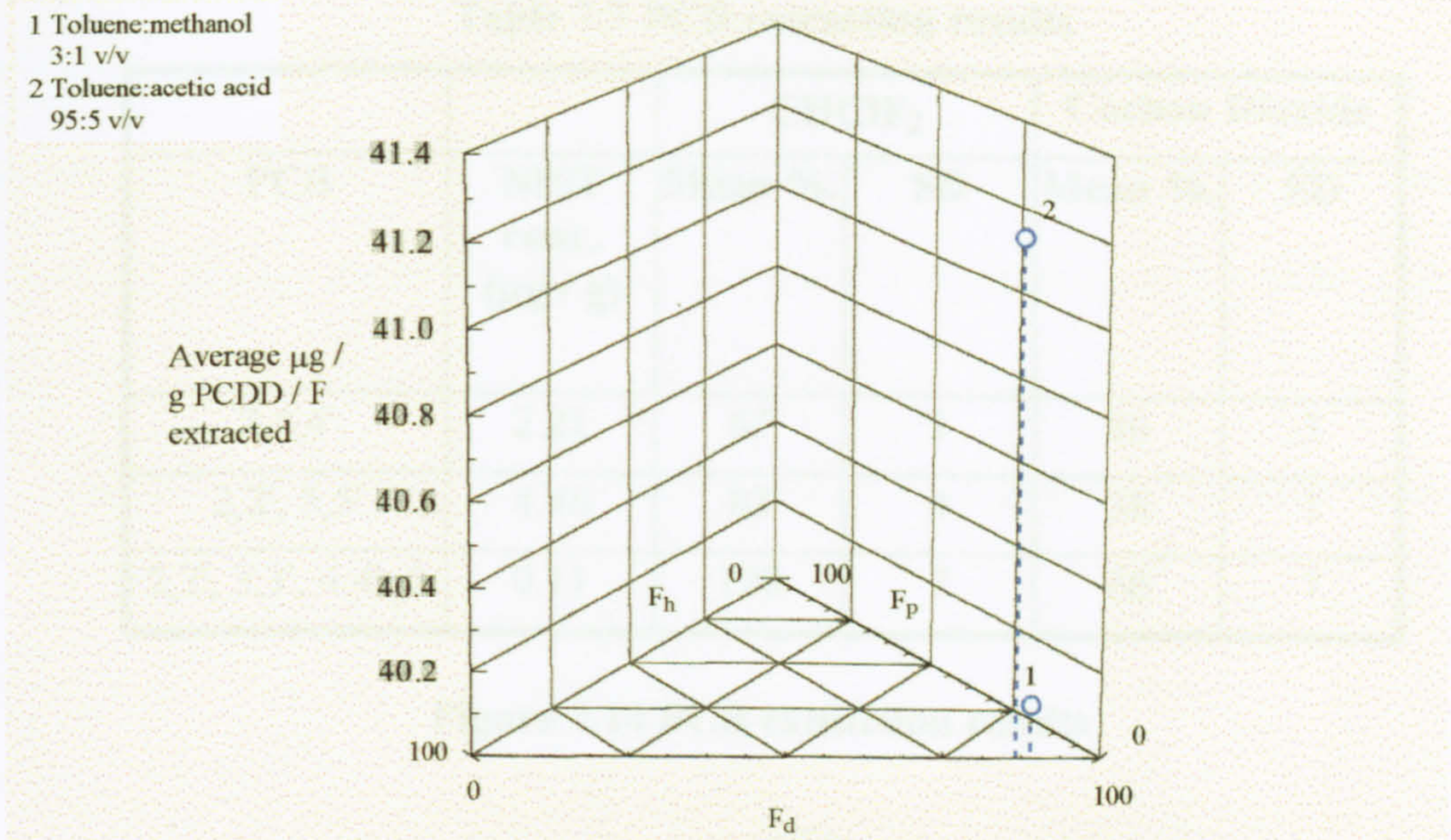


Table 7.6 PCDD Extraction from Fly Ash

	2,3,7,8 tetraCDD (ng / g)	1,2,3,7,8 pentaCDD (ng / g)	1,2,3,4,6,7,8 heptaCDD (ng / g)	OctaCDD (ng/ g)
Toluene: Methanol 3:1	0.25	2	59.7	158
Toluene: Acetic Acid 95:5	0.28	2.7	71	154

Figure 7.12 Recovery of PCDD's



The second example is the extraction of polychlorinated biphenyls from a certified reference material.[15] The ternary plot (figure 7.13) predicts that $CHClF_2$ should give significantly better extraction results that carbon dioxide.

Figure 7.13 Prediction of the optimum solvent for PCB's

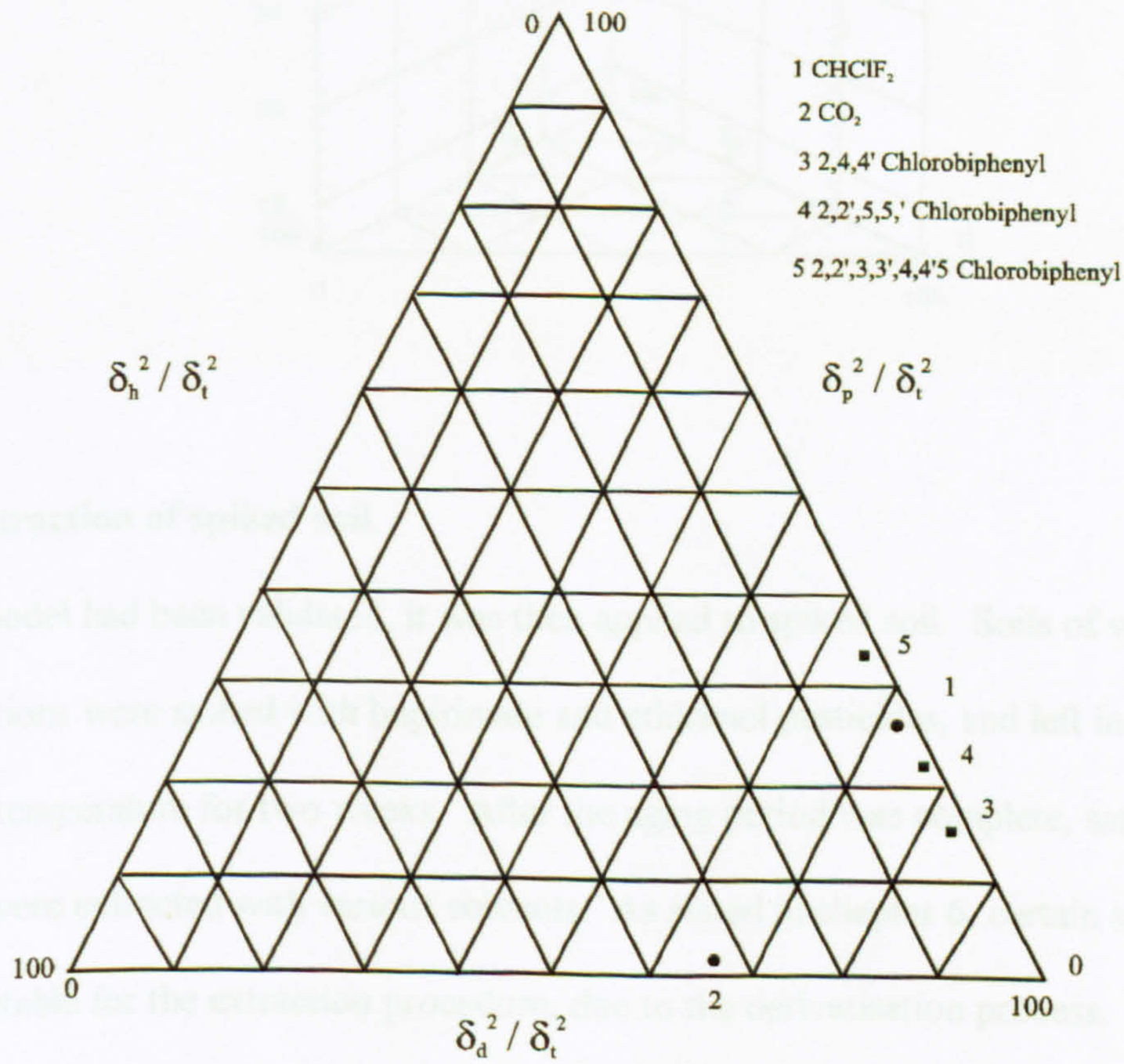
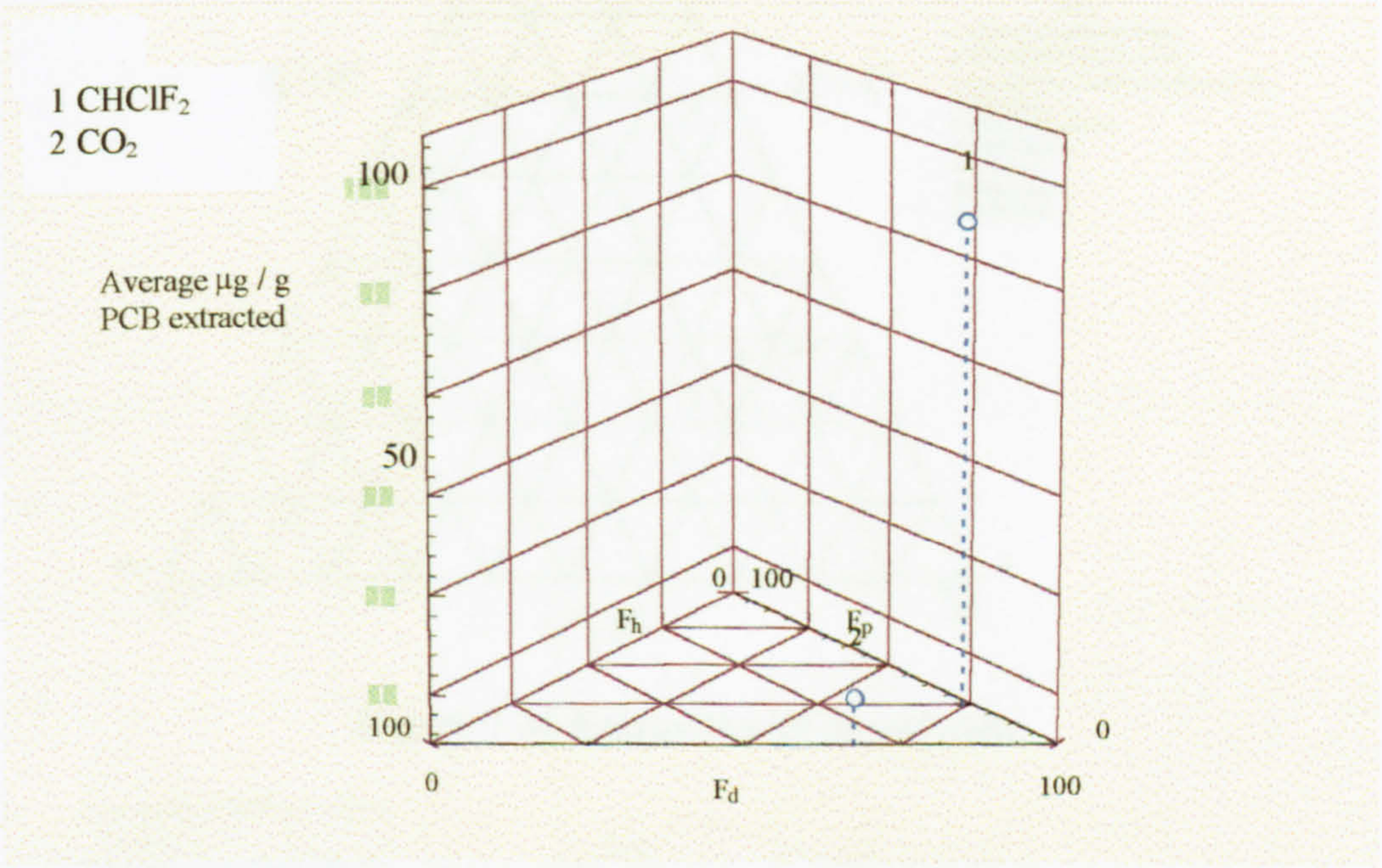


Table 7.7 and figure 7.14 confirms that the optimum solvent is $CHClF_2$.

Table 7.7 PCB extraction results

		$CHClF_2$		Carbon Dioxide	
PCB	**NIST conc. (µg / g)**	**Mean %.**	**SD**	**Mean %.**	**SD**
2,4,4'	2.21	63	3	36	3
2,2', 5,5'	4.48	83	4	38	1
2,2', 3,3', 4,4', 5	0.11	128	7	66	7

Figure 7.14 PCB extraction results



7.2.6 Extraction of spiked soil.

As the model had been validated, it was then applied to spiked soil. Soils of various compositions were spiked with bupirimate and ethirimol pesticides, and left in the dark at room temperature for two weeks. After the aging period was complete, samples of the soil were extracted with various solvents. As stated in chapter 6, certain solvents are unsuitable for the extraction procedure, due to the derivatisation process.

Alternative solvents were suggested for acetone, acetone:dichloromethane 1:1 v/v, and

acetone:iso-hexane 1:1 v/v. Figure 7.15 shows a two dimensional representation of the relative positions of the solvents and analytes.

Replicate extractions showed that the predicted solvent was the experimental optimum for these analytes. Figure 7.16 shows the results of the extraction of bupirimate from compost. Analogous situations were found on all nine soils.

Figure 7.15 Prediction of optimum solvent for bupirimate and ethirimol

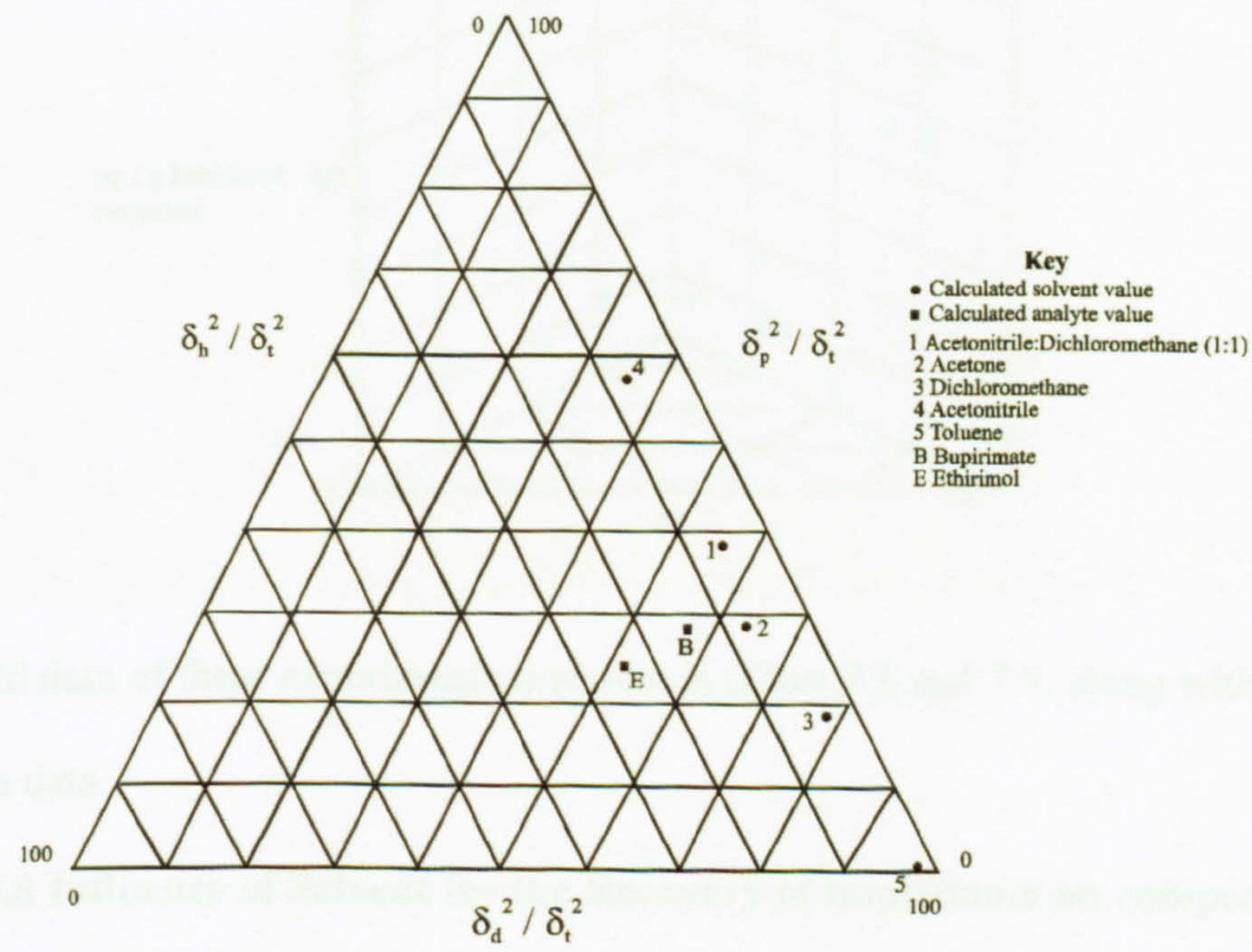


Figure 7.16 Extraction of bupirimate

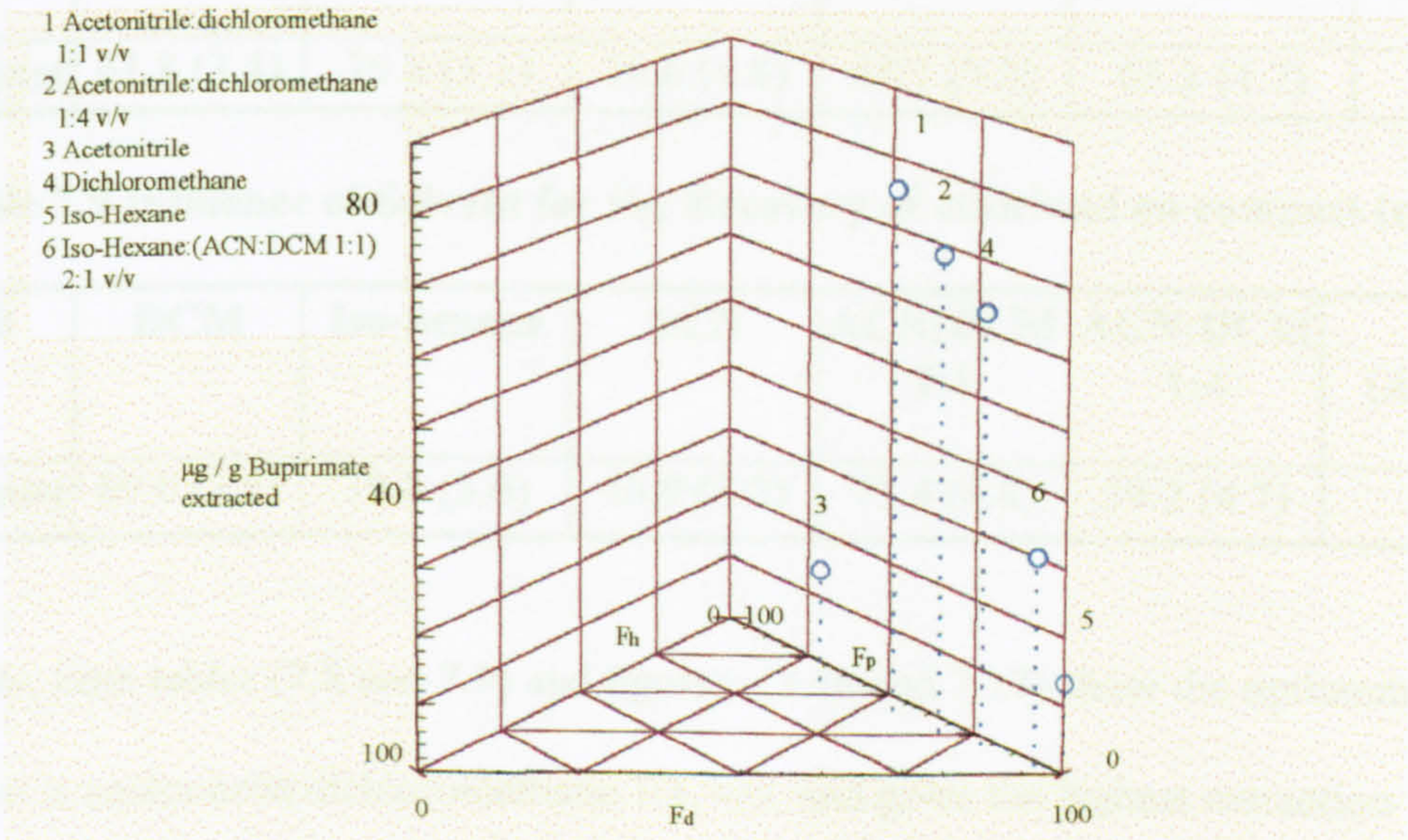
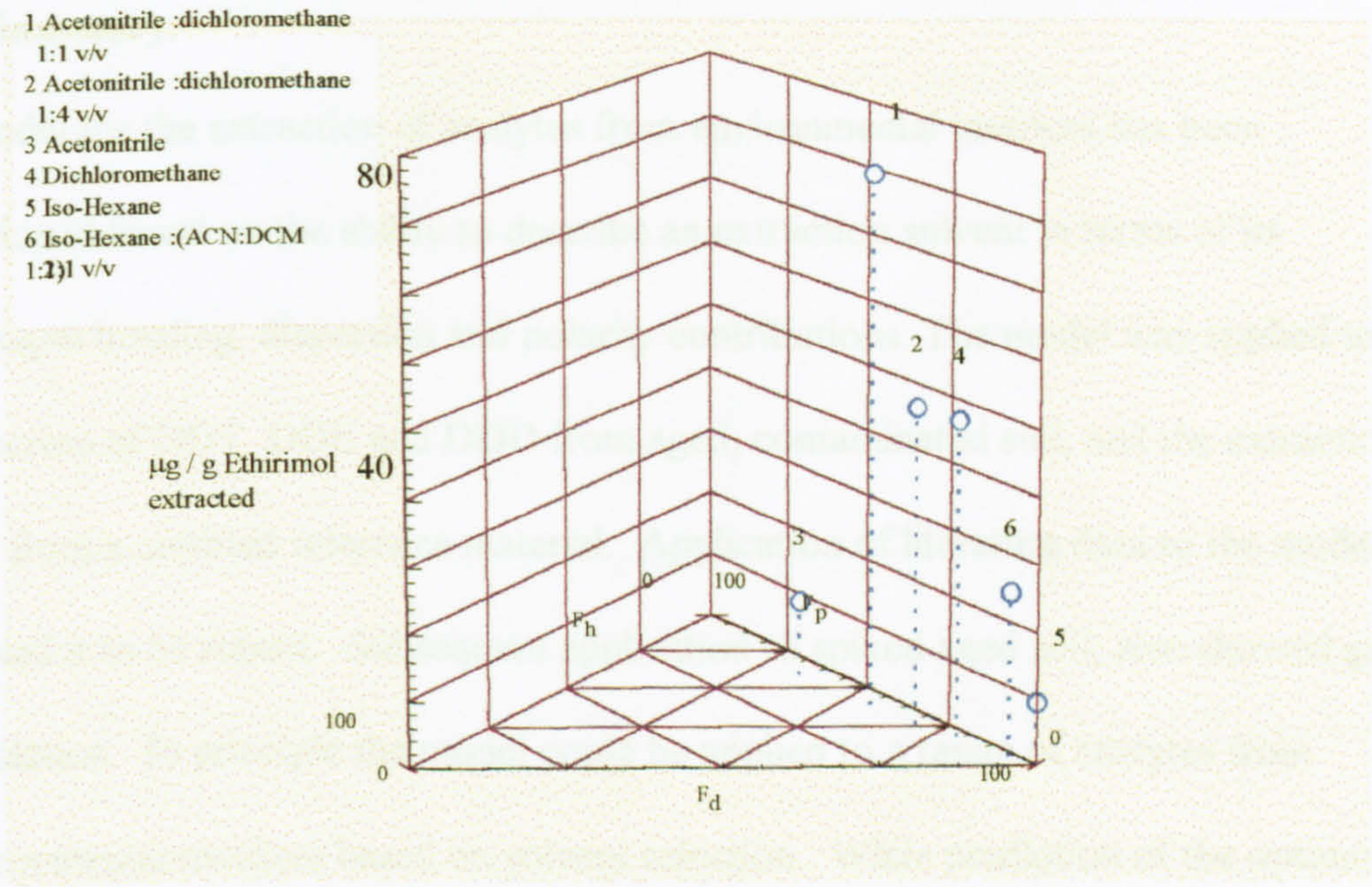


Figure 7.17 shows the extraction results for ethirimol from compost soil, again, analogous situations were found for all nine soils.

Figure 7.17 Extraction of ethirimol



Tabulated data of these experiments is shown in tables 7.8 and 7.9, along with precision data.

Table 7.8 Influence of Solvent for the Recovery of bupirimate on compost (n = 6)

Soil	DCM	Iso-hexane	ACN	ACN:DCM 1:1	ACN:DCM 1:4	Iso-hexane: (ACN:DCM 1:1, v/v) 2:1 v/v
Compost	61.8 (3.4)	36.8 (5.1)	38.6 (4.8)	68.7 (5.2)	65.2 (4.7)	45.4 (3.9)

Table 7.9 Influence of Solvent for the Recovery of ethirimol on compost (n = 6)

Soil	DCM	Iso-hexane	ACN	ACN:DCM 1:1	ACN:DCM 1:4	Iso-hexane: (ACN:DCM 1:1, v/v) 2:1 v/v
Compost	59.0 (3.5)	39.8 (3.6)	40.9 (3.2)	75.4 (4.8)	59.2 (4.7)	47.5 (3.1)

Clearly, both tables (7.8 and 7.9) and figures (7.16 and 7.17) show the optimum solvent is acetonitrile:dichloromethane 1:1, v/v, and gives the highest extraction recovery for both bupirimate and ethirimol. Figure 7.15 also implies that both toluene

and acetonitrile should give poorer extraction recoveries than the optimum. Tables 7.8 and 7.9 show this is the situation.

7.3 Summary.

A model for the extraction of analytes from environmental matrices has been developed based on the ability to describe an extraction solvent in terms of its hydrogen bonding, dispersion and polarity contributions. The model was applied to the extraction of DDT, DDE and DDD from aged, contaminated soil, and the extraction of PCP from a certified reference material. Application of literature data to the model showed it to be robust. Subsequent application to spiked aged soil, also showed good correlation. In principle the model could be applied to a range of analytes from environmental matrices based on solvent selection. While prediction of the optimum organic solvent is desirable it is only one part of the extraction process. The extraction of an organic compound is a more complicated process, a process that is dependent upon the interaction of the analyte with the soil matrix. While a particular extraction technique may be able to remove the "easily extractable" fraction in a short time period with any organic solvent there may remain a "non-extractable" fraction. This latter fraction relates to the chemical interactions that may occur between an analyte and the matrix. For example, an organic compound may become chemically altered, undergo polymerisation or covalent binding with humic substances present in the soil.[19] An appreciation of the complexity of the problem should not be ignored. In fact, knowing if, why and how a pollutant associates itself within the soil matrix is an important development in understanding the extraction process.

7.4 References

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Section C

Pesticide Degradation on Soil

Section C

Pesticide degradation on soil.

This section discusses the photolysis of two UV light sensitive pesticides, bupirimate and pentachlorophenol on soils of varying composition. All the soils used are described in Section A; Method Development. The soils were slurry spiked with the analytes and subjected to UV light. As a control, samples were kept in the dark. No microbial action was apparent. Samples of the exposed soil were taken at different time intervals, extracted under the optimised PFE conditions, and analysed on the GC-MSD. Both molecules showed evidence of photolysis, with evidence of soil composition dependency i.e. the rate and quantity of photolysis was soil dependent. Partial least squares multiple regression was used as a tool to elucidate the soil components that were influencing the photolysis of the two molecules.

Chapter 8

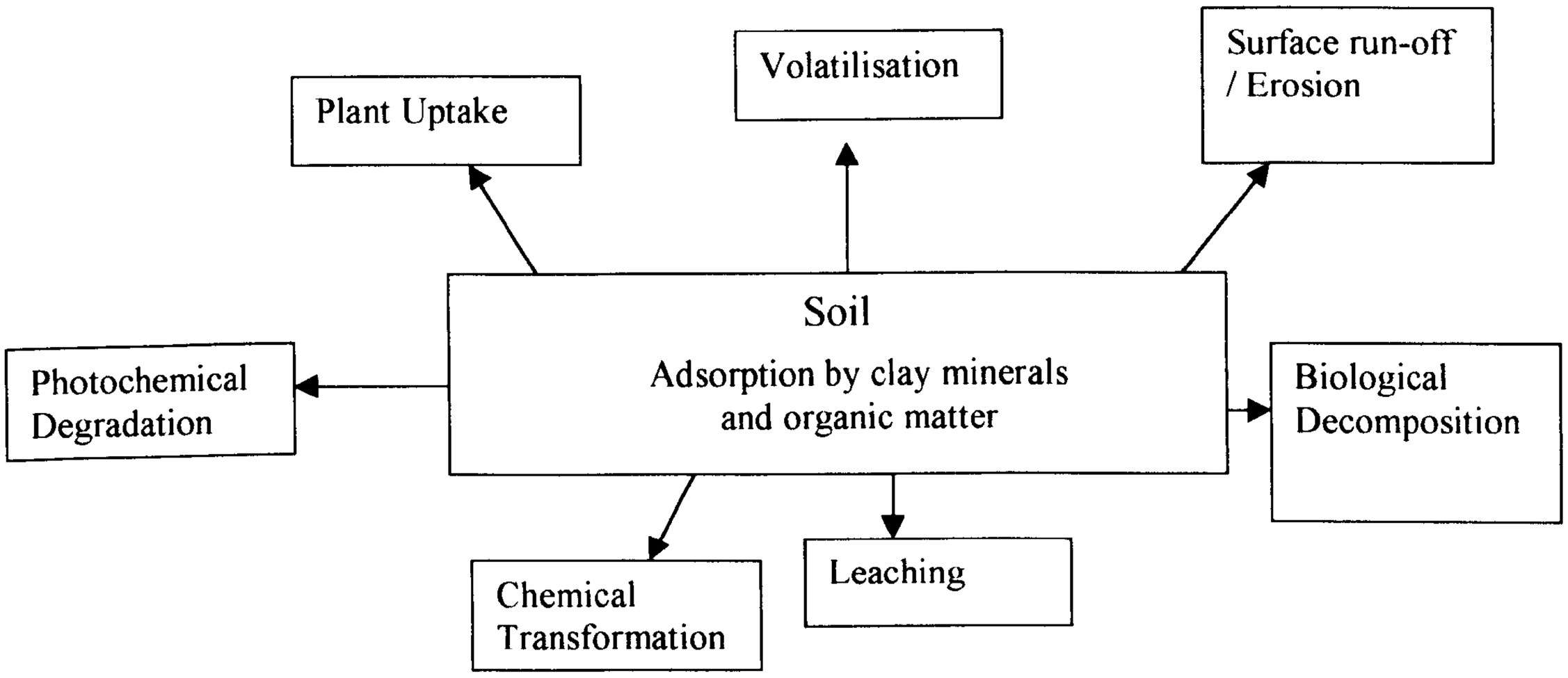
Pesticide Photolysis on Soil

Pesticide photolysis on soil.

8.0 Introduction

The extraction and analysis of organic contaminants currently on the US EPA 'Red List'[1] of high priority pollutants is important. However, also essential is the determination of the fate of these molecules in the environment. There are numerous routes that pesticides can take once in the environment. Figure 8.1 summarises the most common pathways.[2]

Figure 8.1 pesticide routes to the environment



Extensive literature exists on the degradation of pesticide molecules on soil and in water. Several mechanisms have been postulated for the degradation of molecules on soil. These include chemical,[3, 4] microbial and photo induced degradation.[5] Nearly all of the mechanisms that do not involve microbial action, rely on the presence of oxygen, usually in the form of water as a source of hydroxyl radicals.[3 - 5] In particular, this chapter focuses on the photolysis of pentachlorophenol and bupirimate on soil.

Pentachlorophenol is highly toxic and persistent in the environment[6] hence levels need to be monitored carefully. Pentachlorophenol is deposited in the environment through several channels: from the petroleum industry;[7] as a by-product of the dye manufacturing process;[6] as a means of termite control;[6] and, as a herbicide.[6] Derivatives of pentachlorophenol are also used as fungicides to protect against fungal rot of wood.[6]

As with most aromatic compounds, PCP is readily biodegraded[8 - 10] and sequestered (incorporated) into the actual soil matrix.[11 - 13] However, PCP is also subject to chemical degradation, and photolysis. Photolysis of PCP has been widely investigated in aqueous systems.[14 - 16] Limited literature exists on the photolysis of PCP on soil compared to the vast amount on photolysis in water. The literature that does exists is contradictory; Combrisson and Monrozier state in their work that photolysis of PCP on soil is negligible when compared to biodegradation,[17] whilst Goshal states that photolysis accounts for 40 % of the degradation of PCP on thin soil films.[18] Work by Wong and Crosby,[19] Hwang et al.,[20] and Donaldson and Miller[21] also support the finding that PCP is readily degraded by UV light.

Bupirimate is degraded to ethirimol in soil both microbially and photochemically.[6] Adsorption and degradation of ethirimol has been studied by Cancela et al., on various soil components, in particular peat and various types of montmorillonite clay.[22] Scant literature studying the degradation of bupirimate exists. The aim of this chapter is to

determine if photolysis of PCP and bupirimate occurs in the absence of biologically active species on soil, and if the soil composition has an effect on the rate of photolysis.

8.1 Experimental

8.1.1. Instrumentation

An ASE™ 200 Accelerated solvent extractor (Dionex (UK) Ltd., Camberley, Surrey) was used to perform the extractions. 11 mL cells were used for all the extractions.

A hand built light box, dimensions 158 cm x 30 cm x 38 cm, fitted with two Bellarium-S UV tubes (80 watts power each) was used for the photolysis experiments.

8.1.2. Soil

Zeneca AgroChemicals, Jealott's Hill Research Station, Berkshire supplied standard soils covering a range of compositions (table 8.1) as discussed at the beginning of section A.

Table 8.1 Soil Composition

Soil	% Silt	% Clay	% Sand	% OM	pH	CEC
Chamberlain	4	9	87	4.5	7.3	11.0
Hyde Farm	23	19	58	3.2	6.7	17.4
18 Acres	24	20	56	4.7	6.3	14.0
Chalgrove Farm	29	37	34	5.6	7.4	29.7
Garden	18	11	71	9.8	7.2	16.49
Mix 2	3.0	11	86	17.5	5.9	12.7
Mix 1	22	25	53	31.3	5.3	32.1
Mix 3	21.0	30	49	59.4	5.2	41.7
Compost	22	48	30	82.7	5	17.62

8.1.3. Software

TableCurve 2D (version 4, SPSS, Inc., Chicago, USA) was used to determine the equations of the degradation curves. Statistica was used to perform partial least squares

multiple regression (CSS Statistica/W, Release 5.0 with Industrial units, Statsoft UK, Letchworth, UK). ACD/Chemsketch (version 4.55, Advanced Chemistry Development Inc., Ontario Canada) was used to produce the 3-dimensional structures, and degradation schemes.

8.1.4. Chemicals

The solvents used in this study were certified analytical reagents (Fisher Scientific, Loughborough, Leicestershire). Hydromatrix (Varian Ltd., Surrey, UK) was used to fill the head space of the PFE extraction cells (Dionex). Anhydrous sodium sulphate (Merck, Poole, UK) was mixed with the soil sample during Soxhlet extraction. Bupirimate was supplied by Zeneca AgroChemicals. Hexachlorobenzene, pentachlorophenol and *N,O*-bis(trimethylsilyl)acetamide derivatising agent was purchased from Aldrich Chemical Company, Gillingham, Dorset, UK.

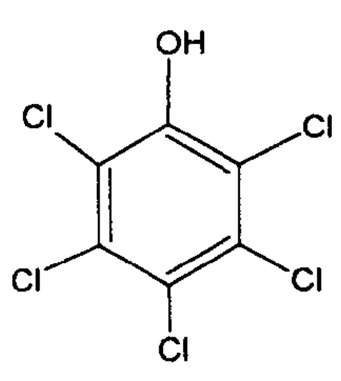
8.1.5. GC-MSD Analysis

8.1.5.1 Pentachlorophenol

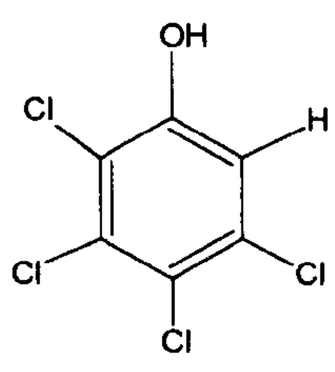
The GC-MSD (HP G1800A GCD system, Hewlett Packard, Palo Alto, USA) was operated in selected ion monitoring mode with a splitless injection volume of 0.5 µL. The column used was a DB-5 (J & W Scientific, Folsom, California, USA), with dimensions of length 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The temperature program used for the analysis was 90 °C for 2 minutes to 250 °C at 10 °C / minute with a final hold time of 12 minutes. The injection port temperature was set at 250 °C, and the detector temperature was set at 280 °C.GC-MSD in selected ion monitoring mode was used to determine the presence of derivatised pentachlorophenol. The ions monitored

were m/z = 323 and m/z = 321. Quantification was achieved by use of a ten point calibration curve from 0 μg mL^{-1} to 5 μg mL^{-1} with a regression coefficient in excess of 0.97. Selected standards were run on a daily basis to assess analytical performance. Figure 8.2 shows the structures of possible degradation products of PCP. The relevant ions used for their determination are shown in table 8.2. Diphenols are formed during microbial and aqueous degradation.[23]

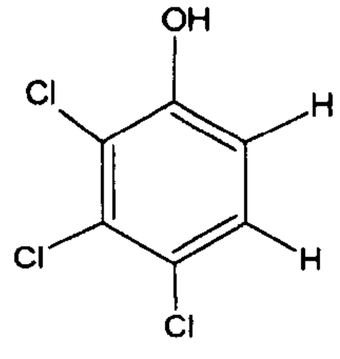
Figure 8.2 Degradation products of PCP



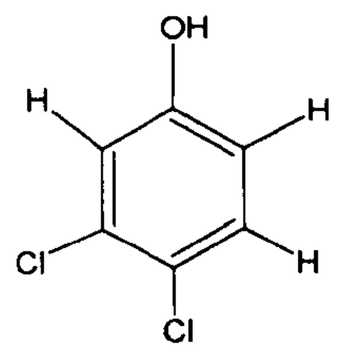
Pentachlorophenol



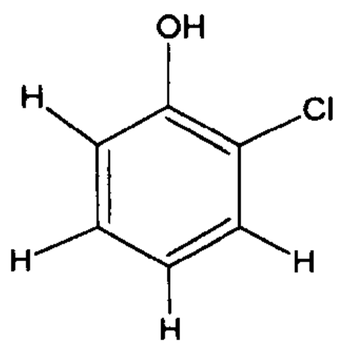
Tetrachlorophenol



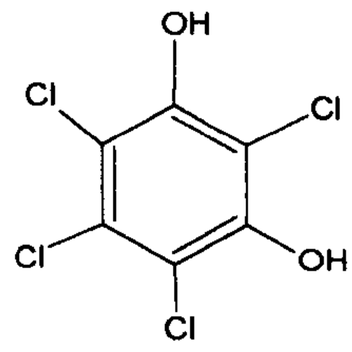
Trichlorophenol



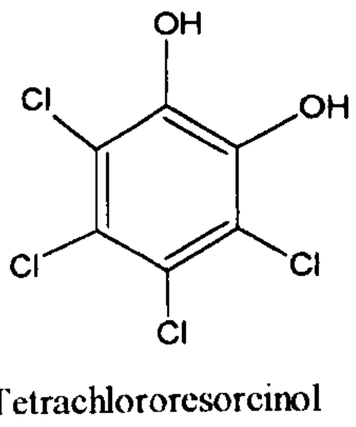
Dichlorophenol



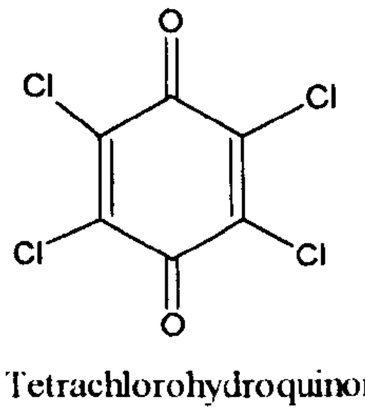
Monochlorophenol



Tetrachlorocatechol



Tetrachlororesorcinol



Tetrachlorohydroquinone

8.1.5.2 Bupirimate

The GC-MSD (HP G1800A GCD system, Hewlett Packard, Palo Alto, USA) was operated in selected ion monitoring mode with a splitless injection volume of 1.0 μL.

The column used was a DB-5ms (J & W Scientific, Folsom, California, USA), with dimensions of length 30 m x 0.25 mm i.d. x 0.25 μm film thickness. The temperature program used for the analysis was 120 °C, held for 2 minutes to 290°C at a rate of 5 °C / minute, with a final hold time of 2.5 minutes. The injection port and detector temperatures were set at 250 °C and 280 °C respectively.

GC-MSD in selected ion monitoring mode was used to determine the presence of each of the analytes. Table 8.2 shows the ions of the derivaised species that were selected for monitoring. Quantification was achieved by the use of an eight-point calibration curve from 0 μg mL^{-1} to

10 μg mL^{-1}. R^2 values for each of the analytes were in excess of 0.99. A selected standard (5 μg / mL) was run every day to assess analytical performance.

Table 8.2 Ions used in identifying derivatised degradation products

Compound	Quantifying Ion (m/z)	Qualifying Ion (m/z)
Bupirimate	273	208
Ethirimol	266	238
Pentachlorophenol	323	321
Tetrachlorophenol	289	287
Trichlorophenol	255	253
Dichlorophenol	221	219
Monochlorophenol	185	187
Tetrachlororesorcinol	322	307
Tetrachlorocatechol	392	304
Tetrachlorohydroquinone	246	228

8.1.6 Fortification Procedure

Soil (9 x 200 g), was subjected to UV light for 24 hours as a sterilisation process. The sterilised soil was then spiked with either pentachlorophenol or bupirimate in 100 mL of

dichloromethane to give a final concentration of 20 μg g^{-1}. The solvent was allowed to evaporate overnight and then left for two weeks in the dark prior to extraction. Each 200 g of spiked soil was then halved, and 100 g was left in the dark, and the other 100 g was used in the photolysis experiments.

8.1.7 PFE Extractions

8.1.7.1 PCP

Using the results of the solvent selection (see chapter 5), and due to the derivatisation process, the solvent used for the extractions was acetonitrile:dichloromethane 1:1 v/v. The extraction parameters are as follows; temperature, 100 °C, at a pressure of 2000 psi with a static extraction time of 5 minutes (3 cycles), giving a total extraction time of 18 minutes per sample. Six samples per soil were extracted at various time intervals.

8.1.7.2 Bupirimate

Using the solvent selection information (see chapter 4), the optimum solvent was determined to be acetonitrile:dichloromethane 1:1 v/v. The extraction parameters were temperature, 100 °C, at a pressure of 2000 psi, with a static extraction time of five minutes (2 cycles), giving a total extraction time of 14 minutes per sample. Six samples per soil were extracted at various time intervals.

8.1.8 Photolysis procedure

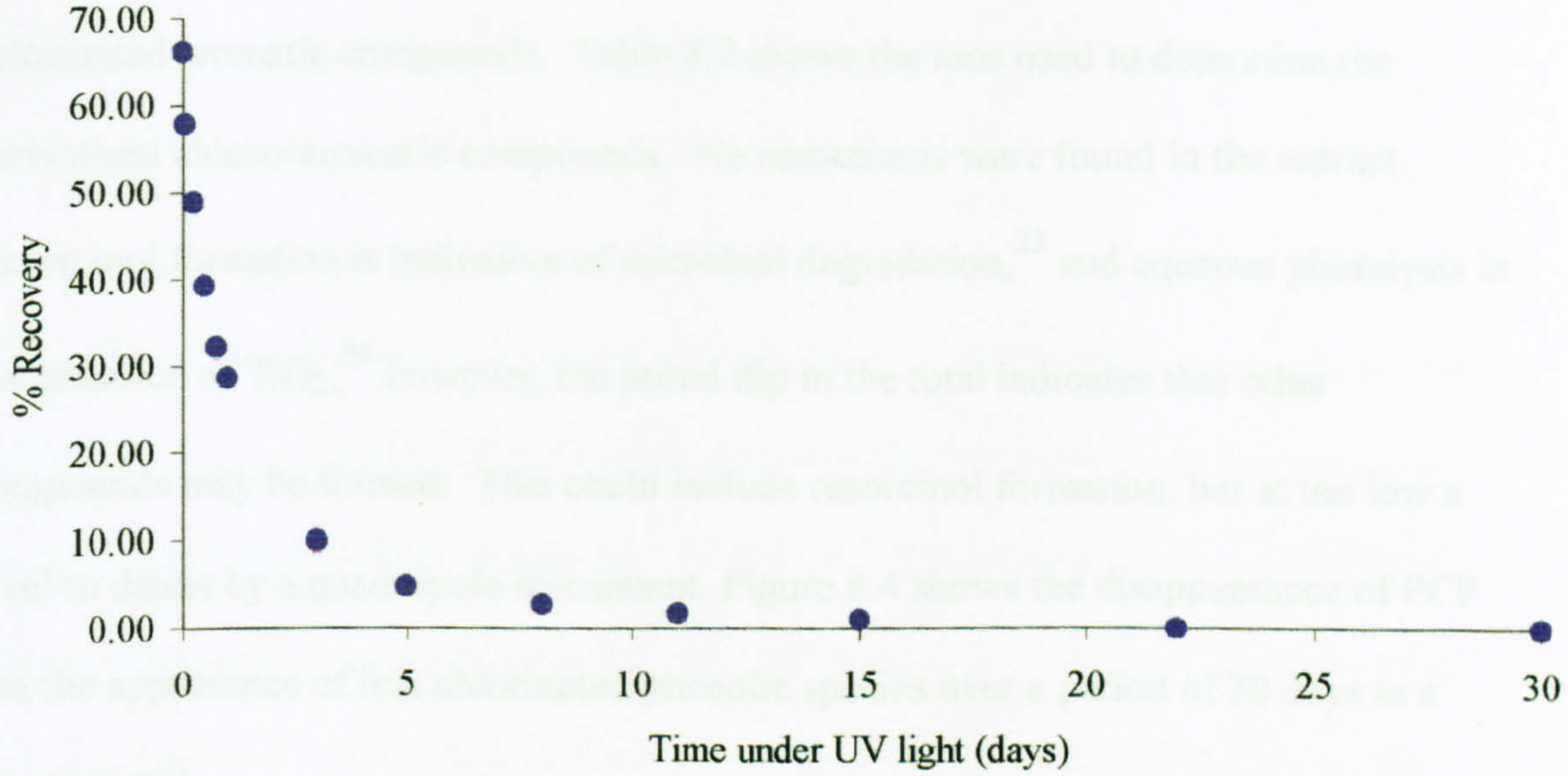
Samples of soil (1.0000 g), were placed on a watch glass (6 cm diameter), and placed in the light box. Six samples per time interval were prepared. The samples were at constant temperature and 8 cm from the source. At set time intervals, six replicate soil samples were extracted by PFE, under the optimum conditions.

8.2 Results and discussion

8.2.1 PCP

Six samples per soil per time interval were extracted by PFE and analysed on the GC-MSD. Figure 8.3 shows the exponential disappearance of PCP on compost over the course of the study.

Figure 8.3 Disappearance of PCP on compost



After approximately 0.5 days, half of the PCP has gone. Table 8.3 shows the measured half lives for PCP degradation. The half lives were determined by interpolation of the graph at 50 % loss.

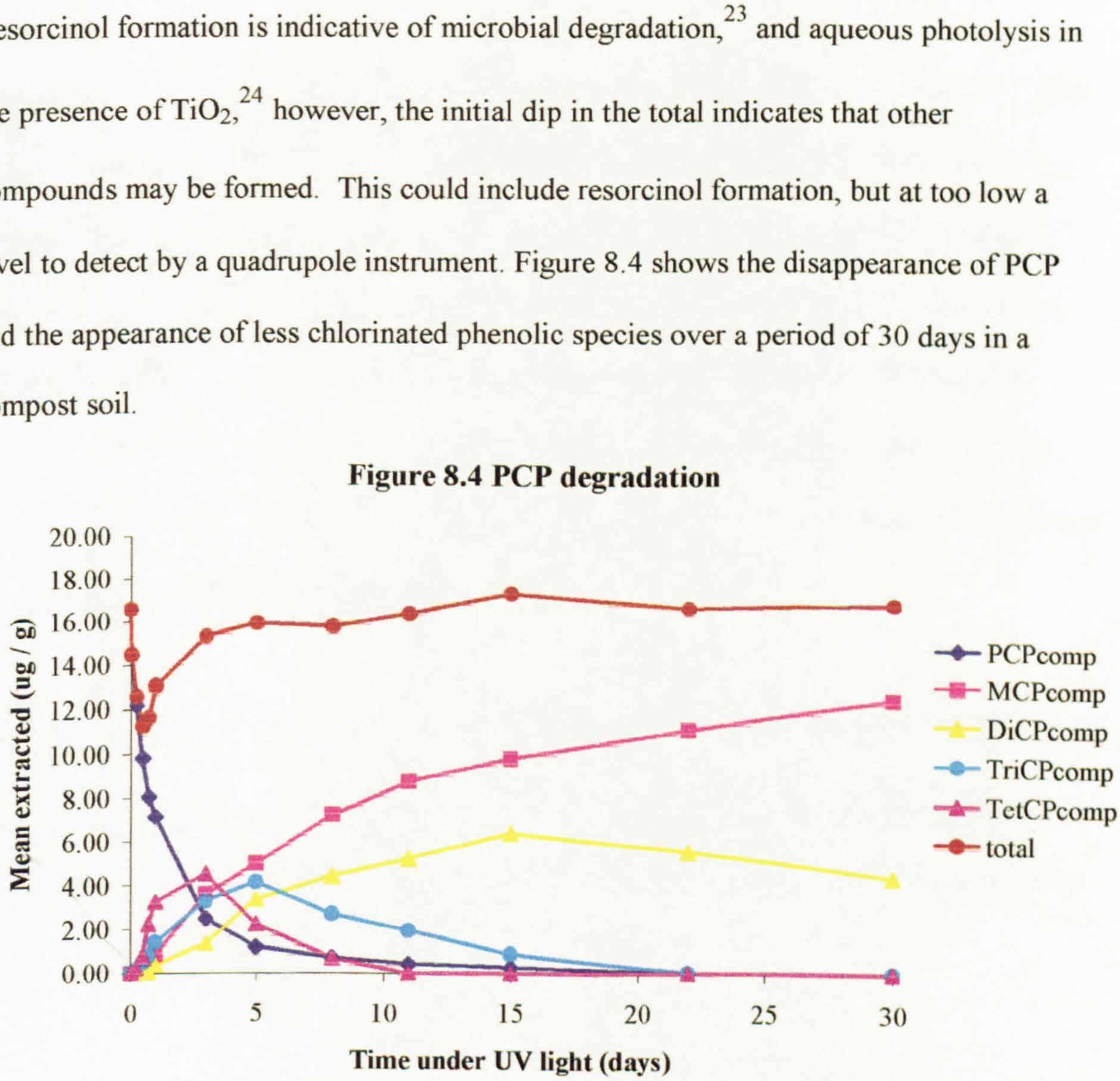
Table 8.3 Measured half lives for PCP

Soil	Time (days)
Hyde Farm	1.25
Chamberlain	3.0
18 Acres	1.25
Chalgrove Farm	0.75
Garden	2.4
Mix 2	2.25
Mix 1	1.25
Mix 3	1.0
Compost	0.5

Soil sterilised under UV for 24 hours is deemed to be free of microbes.[25] The dip occurs in the first 24 hours, and therefore could be evidence of microbial activity. Soil samples taken after 24 hours were spiked onto a nutrient agar plate. Minimal soil flora / fauna were found.

Analysis of the extract on the GC-MSD in total ion mode, showed the presence of other chlorinated aromatic compounds. Table 8.2 shows the ions used to determine the derivatised chloro-aromatic compounds. No resorcinols were found in the extract. Resorcinol formation is indicative of microbial degradation,[23] and aqueous photolysis in the presence of TiO_2,[24] however, the initial dip in the total indicates that other compounds may be formed. This could include resorcinol formation, but at too low a level to detect by a quadrupole instrument. Figure 8.4 shows the disappearance of PCP and the appearance of less chlorinated phenolic species over a period of 30 days in a compost soil.

Figure 8.4 PCP degradation



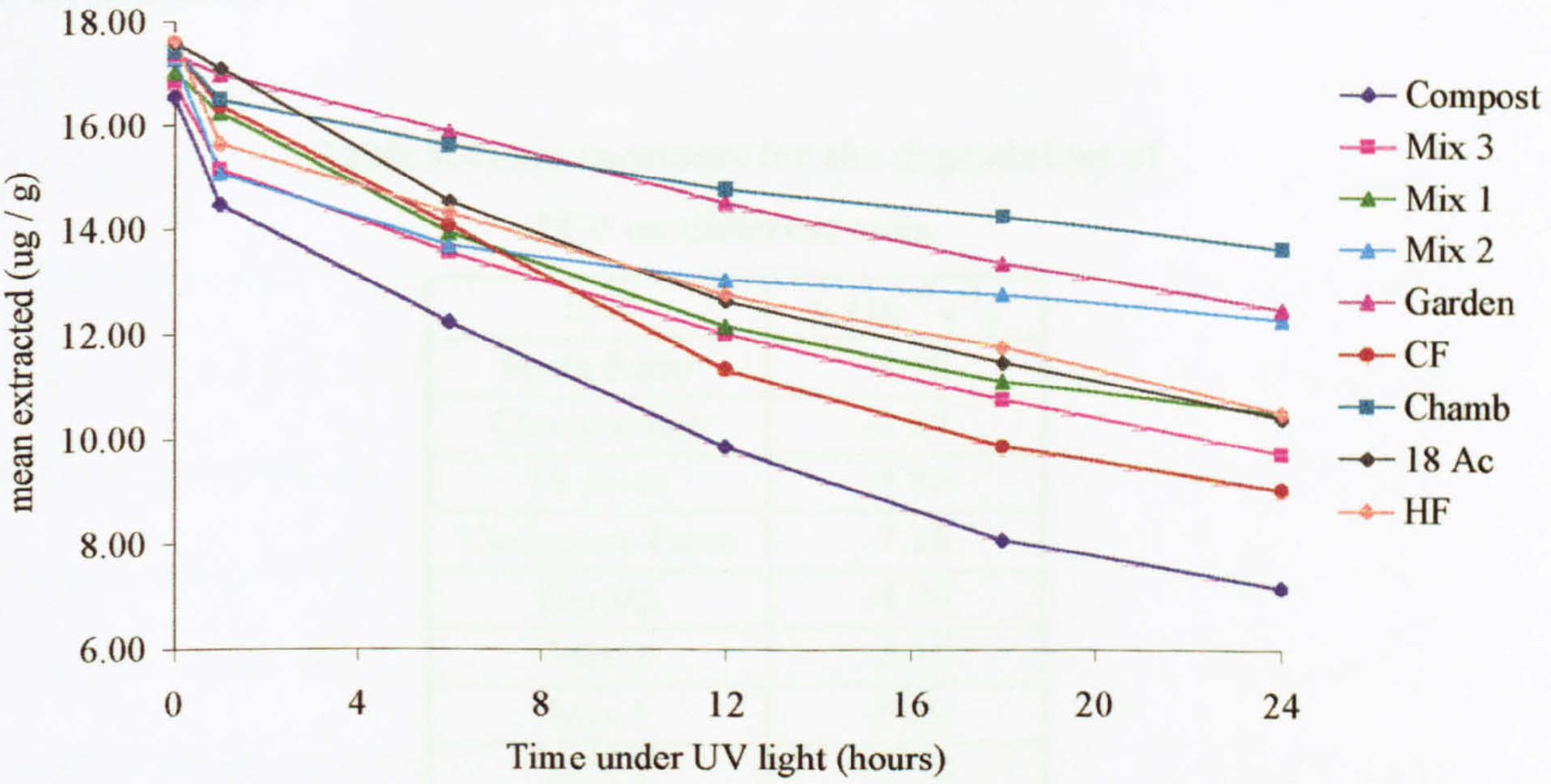
NB 20 µg / g is 100 % extraction

Analogous situations were found in the other eight soils. The amount of degradation products varies between the soils, implying soil composition has a significant effect on the degradation of PCP. Appendix A3 shows the loss of PCP for the other eight soils. It is clear from the data in appendix A3, that more degradation occurs on soils containing high organic matter content, e.g. compost, mix 3, mix 2 and mix 1. Soils such as Chamberlain and Hyde Farm which have a lower organic matter content, have less PCP degradation.

Appendix A4 shows a series of chromatograms for PCP degradation on compost. After 6 hours, the quantity of PCP has decreased, and there is clear evidence of the presence of tetrachlorophenol and trichlorophenol. After a further six hours, there is evidence of dichlorophenol and monochlorophenol. At eight days under UV light, the quantity of PCP has been reduced so much that it is near the limit of detection of the GC-MSD. After a further three days under UV light, tetrachlorophenol has disappeared, and the quantity of trichlorophenol has been significantly reduced, with a corresponding increase in both dichlorophenol and monochlorophenol.

The rate disappearance of PCP in the first 24 hours of on each soil is different (figure 8.5). Generally, the more clay is present in the soil the greater the rate of degradation. Previous studies in the literature show that PCP degradation follows first order kinetics with respect to PCP.[26 - 28]

Figure 8.5 Rate of PCP photolysis



For a first order reaction, the rate of reaction is directly proportional to the concentration of the reactant (equation 8.1)

$$A \xrightarrow{k} \text{products} \qquad \text{Eqn. 8.1}$$

Where k is the rate constant for the reaction.

Hence,

$$\frac{-d[A]}{dt} = k[a] \qquad \text{Eqn. 8.2}$$

This equation (8.2) can be integrated to give equation 8.3

$$\ln [A] = \ln[A]_o - kt \qquad \text{Eqn. 8.3}$$

Where [A] is the concentration of A at time t, and $[A]_o$ is the initial concentration of A. A plot of ln[PCP] vs. time will give a linear plot, with a gradient equal to the rate constant, -k, and intercept equal to the initial concentration of PCP. The smaller the rate

constant, the slower the degradation. Table 8.4 compares the values of the rate constants for the nine soils.

Table 8.4 rate constants for the degradation of PCP on different soils.

Soil	k (10^{-6} s^{-1})
Hyde Farm	-4.76
Chamberlain	-2.25
18 acres	-5.86
Chalgrove Farm	-7.36
Garden	-4.00
Mix 2	-2.35
Mix 1	-5.20
Mix 3	-5.35
Compost	-8.69

The rate of degradation is greatest on compost soil ($k = -8.69 \times 10^{-6}$ s^{-1}), and very low on Chamberlain soil ($k = -2.25 \times 10^{-6}$ s^{-1}). The general trend follows the percentage organic matter in the soil, the more organic matter, the greater the degradation. These data imply that soil composition has an effect on the degradation rate of PCP.

Further investigation into the effect of soil components was performed. Multiple linear regression can determine the soil components that are involved in PCP degradation.

Table 8.5 shows the extent of correlation between the six measured soil components.

Table 8.5 Correlation data

	Sand	Silt	Clay	pH	% OM	CEC
Sand	1.00					
Silt	-0.87	1.00				
Clay	-0.94	**0.66**	1.00			
pH	**0.35**	**-0.09**	**-0.48**	1.00		
% OM	**-0.53**	**0.16**	0.71	-0.83	1.00	
CEC	**-0.54**	**0.52**	**0.48**	**-0.40**	**0.41**	1.00

Non-correlated variables are in bold. Due to the high degree of correlation, multiple linear regression (as discussed in section 4.2.5) would not give an overall impression of which soil components are important. The equation of the degradation curve was determined in TableCurve, using an equation of the form $y = a + b \exp(-x/c)$. Where a is the intercept on the y axis, b is the pre-exponential constant and the value of c indicates the amount of degradation of bupirimate (or PCP) on the soil matrix. Partial least squares multiple regression is a mathematical technique that allows elucidation of significant parameters despite high correlation. PLS can take into account the variability of the replicates. Partial least squares regression was performed to determine of any of the measured components had an influence on the rate of degradation. Partial least squares regression examines both X and Y data and extracts components which are directly relevant to the variables. These are extracted in decreasing order of relevance. So, to form a model, the correct numbers of components are extracted to model relevant underlying effects.

The general equation for multiple partial least squares regression is

$$y = x_1.comp_1 + x_2.comp_2 + x_3.comp_3 + x_4.comp_4 \ldots\ldots x_i.comp_i \qquad \text{Eqn 8.4}$$

Where y is the dependent variable, $comp_i$ is component in and x_i is the weight of component i. Each component is comprised of a multi linear regression equation of the form

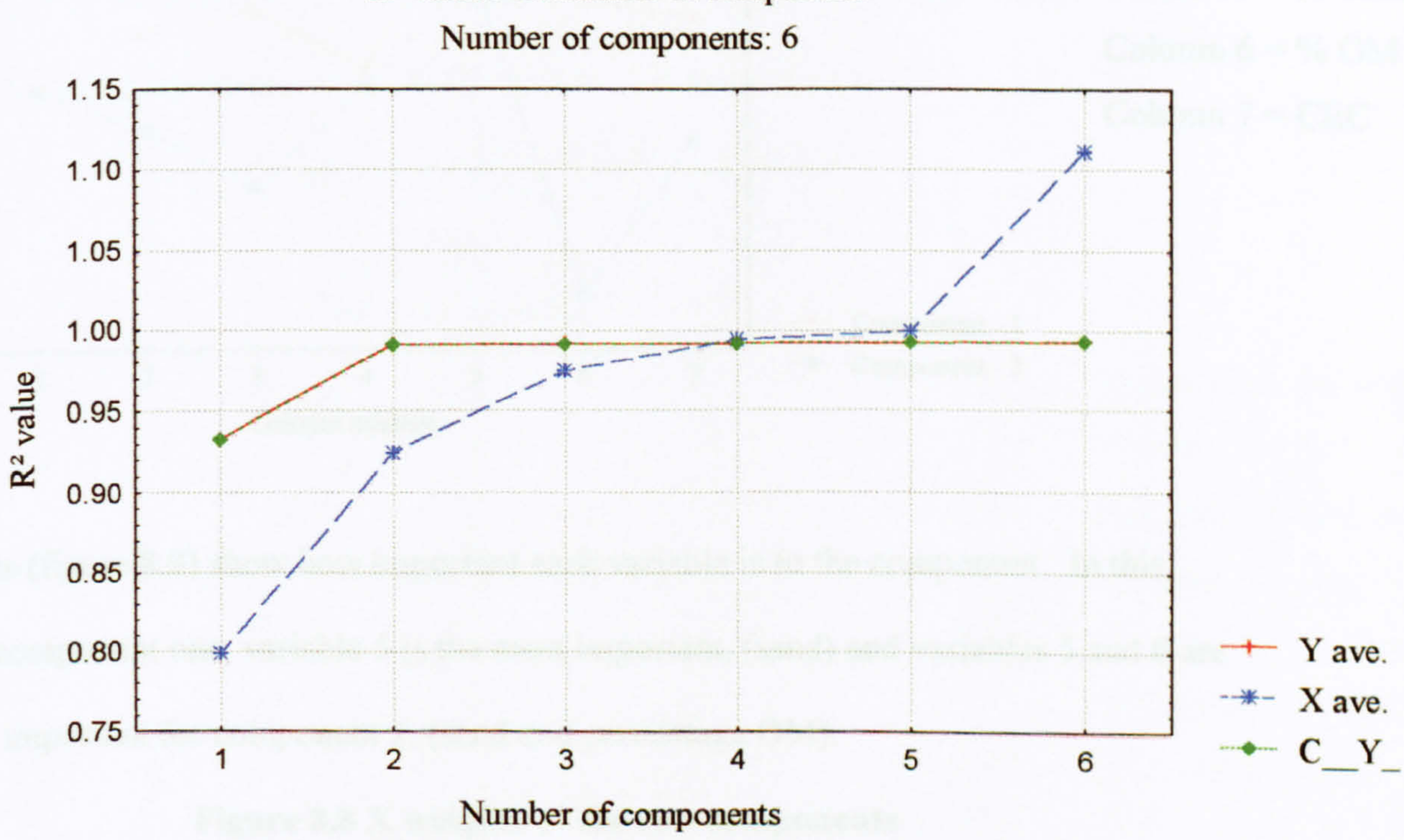
$$PLScomp1 = \beta_0 + \beta_1.V_1 + \beta_2.V_2 + \beta_3.V_3 + \beta_4.V_4 \ldots\ldots \beta_i V_i \qquad \text{Eqn 8.5}$$

Where V_1 to V_i are the independent variables, and β_0 to β_i are the variable coefficients.

Initially, PLS extracts the same number of components as there are variables, however a

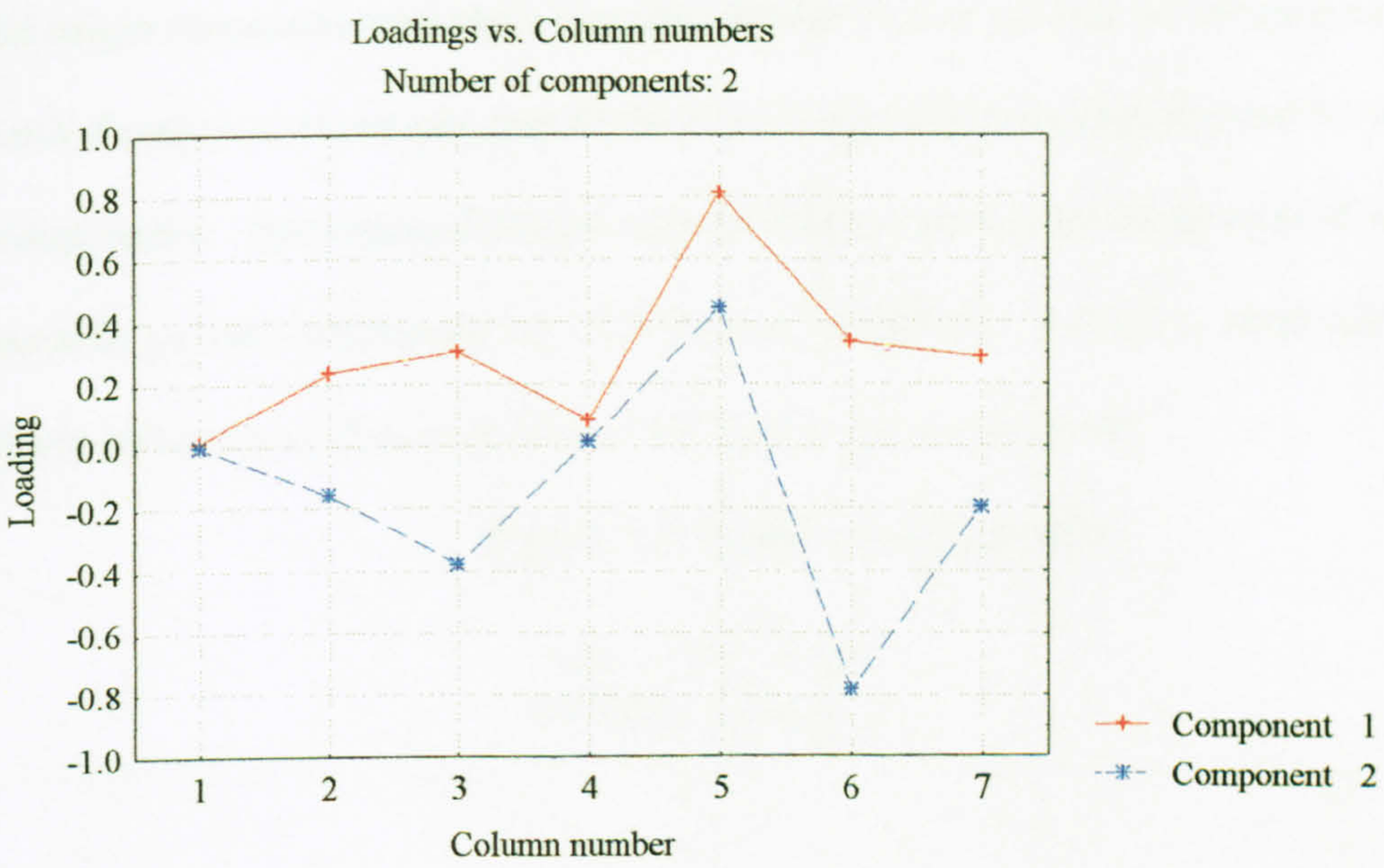
plot of correlation coefficient vs. number of components can be used to reduce the number of components. A figure 8.6 show that two components are required to model the data, as the variation in the R^2 value is minimal.

Figure 8.6 Determination of PLS components



There are seven variables, intercept, % silt, % clay, pH, % sand, % organic matter (% OM), and cation exchange capacity (CEC). Examination of the loadings (figure 8.7) of the two components, which determines which variables are the most influential on each component, it can be seen that variables 4 and 5 (i.e. pH and % sand) are the most influential variables that define component 1, and variables 5 and 6 (i.e. % sand and % OM) define component 2.

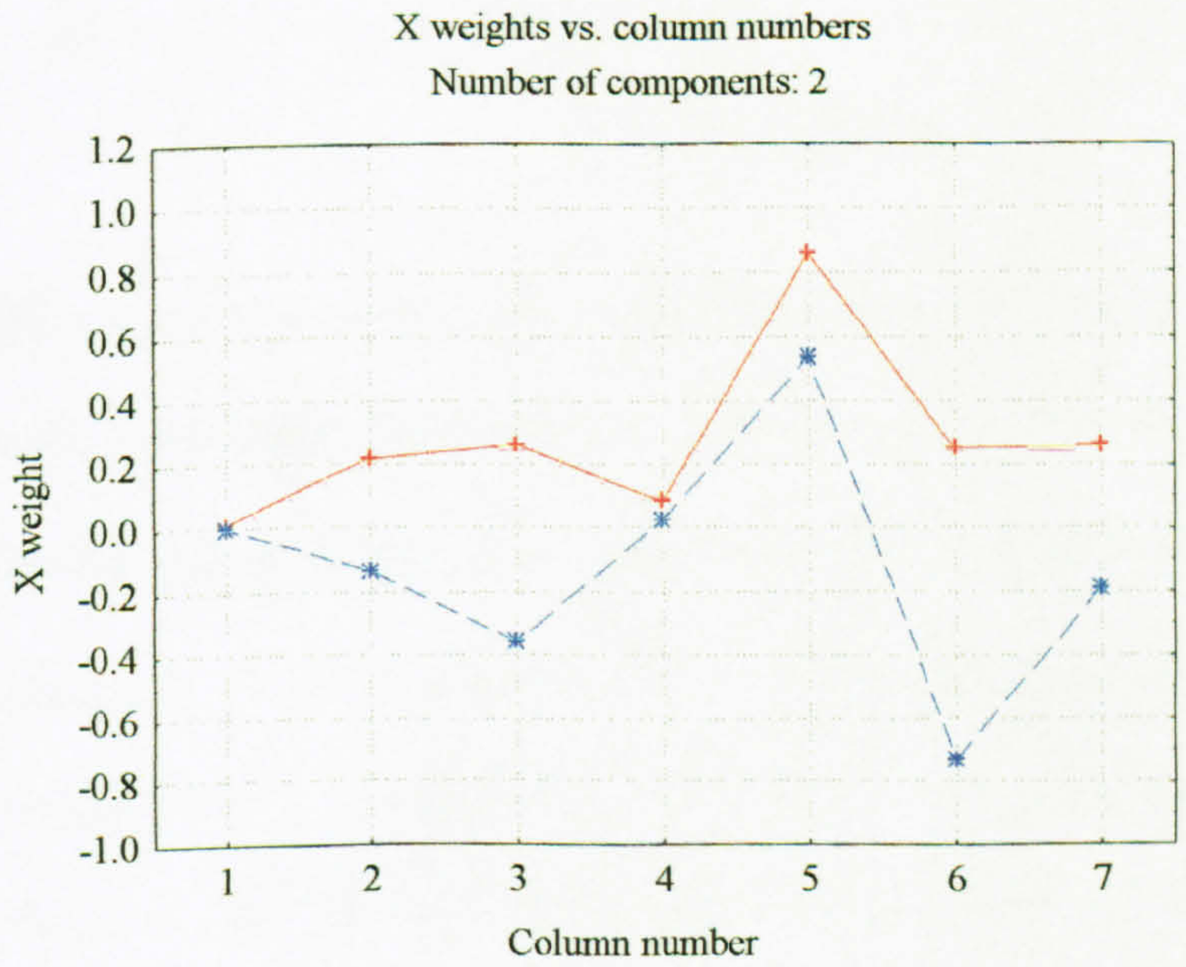
Figure 8.7 Loadings of the two components



Column 1 = Intercept
Column 2 = % Silt
Column 3 = % Clay
Column 4 = pH
Column 5 = % Sand
Column 6 = % OM
Column 7 = CEC

X weights (figure 8.8) show how important each variable is to the component. In this case for component one, variable 5 is the most important, (sand) and variables 5 and 6 are the most important for component 2, (sand and percentage OM).

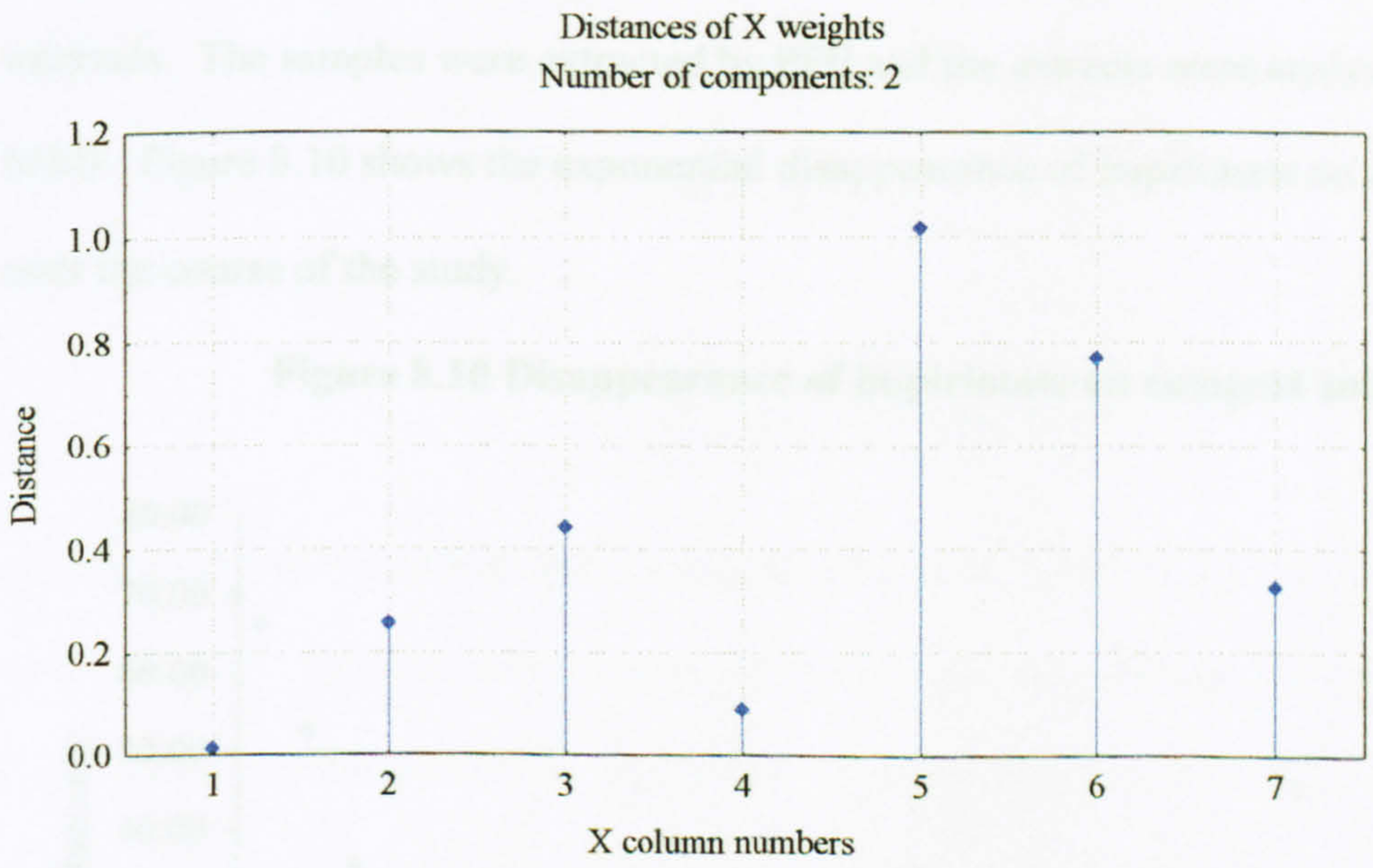
Figure 8.8 X weights of the two components



Column 1 = Intercept
Column 2 = % Silt
Column 3 = % Clay
Column 4 = pH
Column 5 = % Sand
Column 6 = % OM
Column 7 = CEC

The distances of x weights (figure 8.9), is the Euclidean distances of the variables from the origin computed from the x weights divided by the number of components i.e. 2. Each distance is the square root of the sum of squared x weights divided by the number of components. The largest distances are the major contributors to the overall conceptual variable i.e. rate of degradation. In this case, variables 5, and 6, i.e. sand and OM have a direct influence on the extraction of PCP from the various soils.

Figure 8.9 Distances of X weights



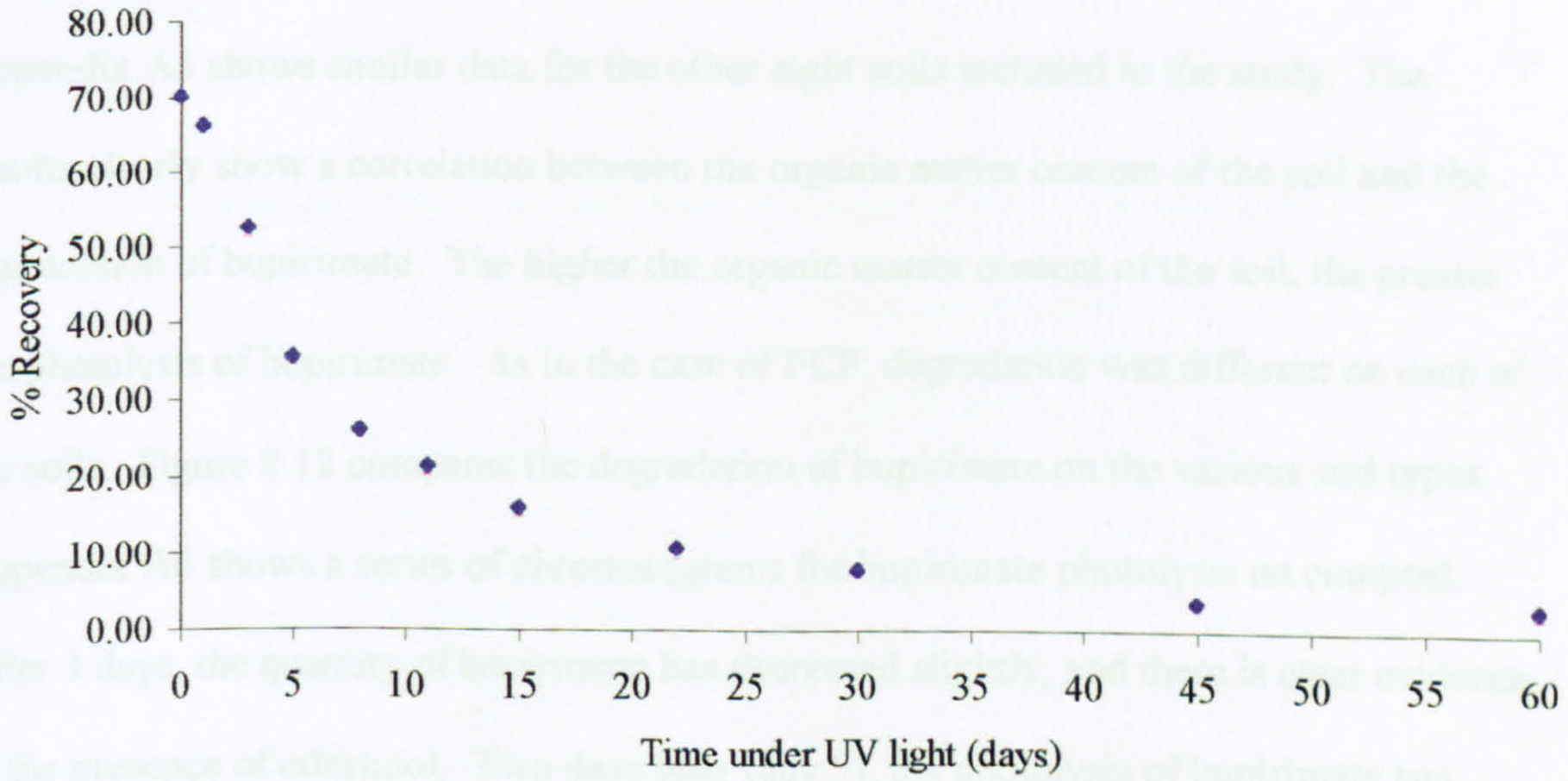
The components of the soil matrix that are significant upon extraction are percentage sand, percentage organic matter content, and to a lesser extent, percentage clay. Organic matter has been implicated by Goshal[18] and Donaldson and Miller[21] in the photolysis of PCP. They found that the higher the amount of organic matter, the greater the degradation. Comparison of Hyde farm soil (3.2 % OM) with compost (80 % OM) (figure 8.5 and table 8.1) shows that as the proportion of organic matter increases, the less

phenol is extracted. Sand is mainly comprised of silicates, which contain silanol groups.[29] These are able to interact with the hydroxyl groups of the chlorophenols, holding them on the silica surface.[29] As stated in chapter 2, certain forms of clay are able to swell in the presence of suitable solvents, those capable of hydrogen bonding. As the clay dries, there is potential for some of the PCP to be trapped irreversibly in the soil matrix.[29 - 32]

8.2.2 Bupirimate and Ethirimol

Slurry spiked soil were subjected to UV light and samples were taken at various time intervals. The samples were extracted by PFE and the extracts were analysed by GC-MSD. Figure 8.10 shows the exponential disappearance of bupirimate on compost soil over the course of the study.

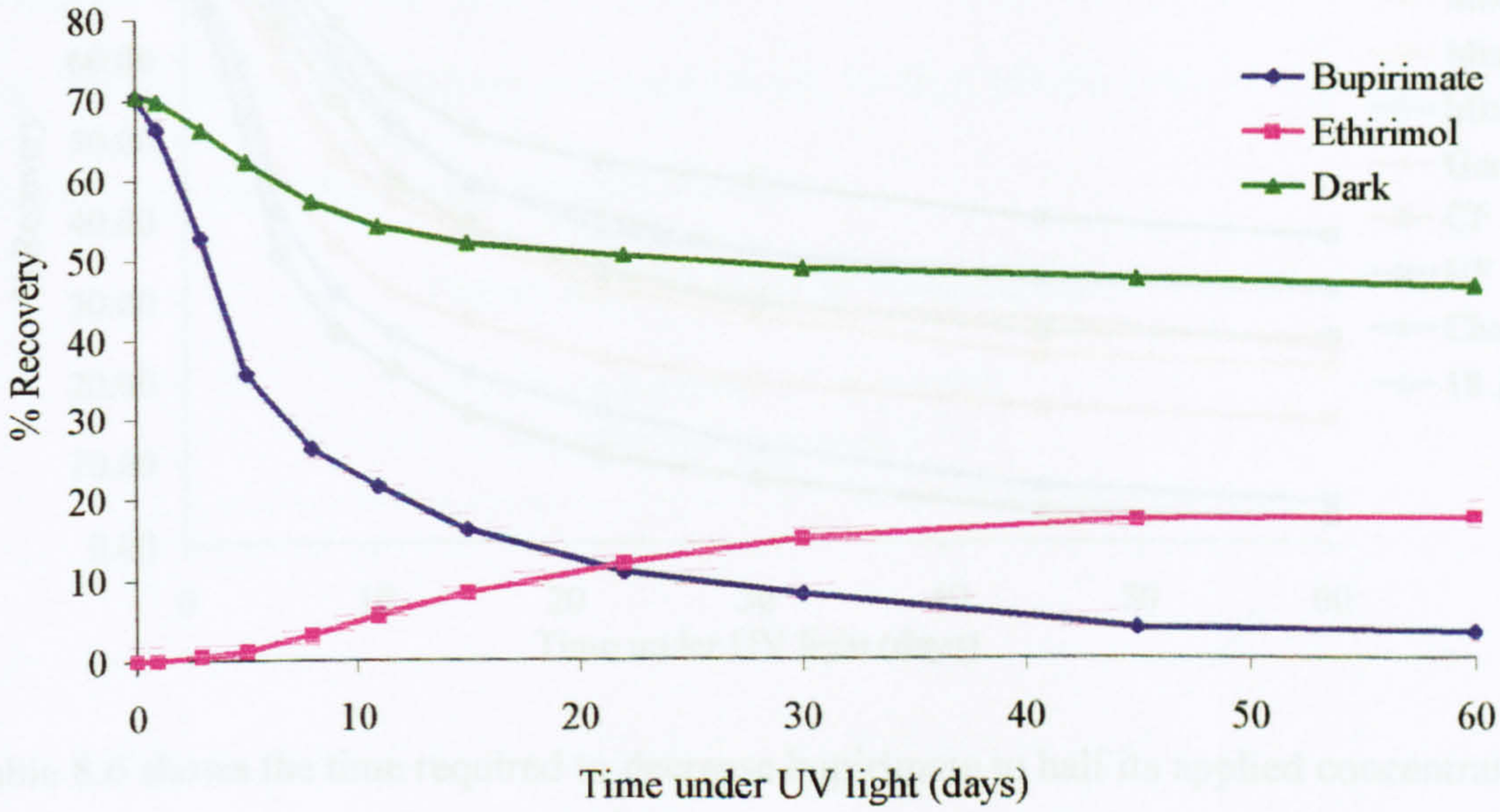
Figure 8.10 Disappearance of bupirimate on compost soil



Interpolation of figure 8.10 shows that after 4 days, less than half the applied concentration of bupirimate is extracted from compost. The solvent selection study

(chapter 9) shows that the decrease in bupirimate cannot be attributed to adsorption alone. This is also confirmed by the presence of ethirimol, the major degradation product of bupirimate (figure 8.11).[6]

Figure 8.11 Appearance of ethirimol



Appendix A5 shows similar data for the other eight soils included in the study. The results clearly show a correlation between the organic matter content of the soil and the degradation of bupirimate. The higher the organic matter content of the soil, the greater the photolysis of bupirimate. As in the case of PCP, degradation was different on each of the soils. Figure 8.12 compares the degradation of bupirimate on the various soil types. Appendix A6 shows a series of chromatograms for bupirimate photolysis on compost. After 3 days, the quantity of bupirimate has decreased slightly, and there is clear evidence of the presence of ethirimol. Two days later (day 5), the photolysis of bupirimate has significantly increased. This decrease in bupirimate with a corresponding increase in ethirimol continues until 22 day of UV exposure, when the loss in bupirimate is

statistically insignificant. The rate of increase of ethirimol has also slowed to a constant rate.

Figure 8.12 Photolysis of bupirimate on various soils

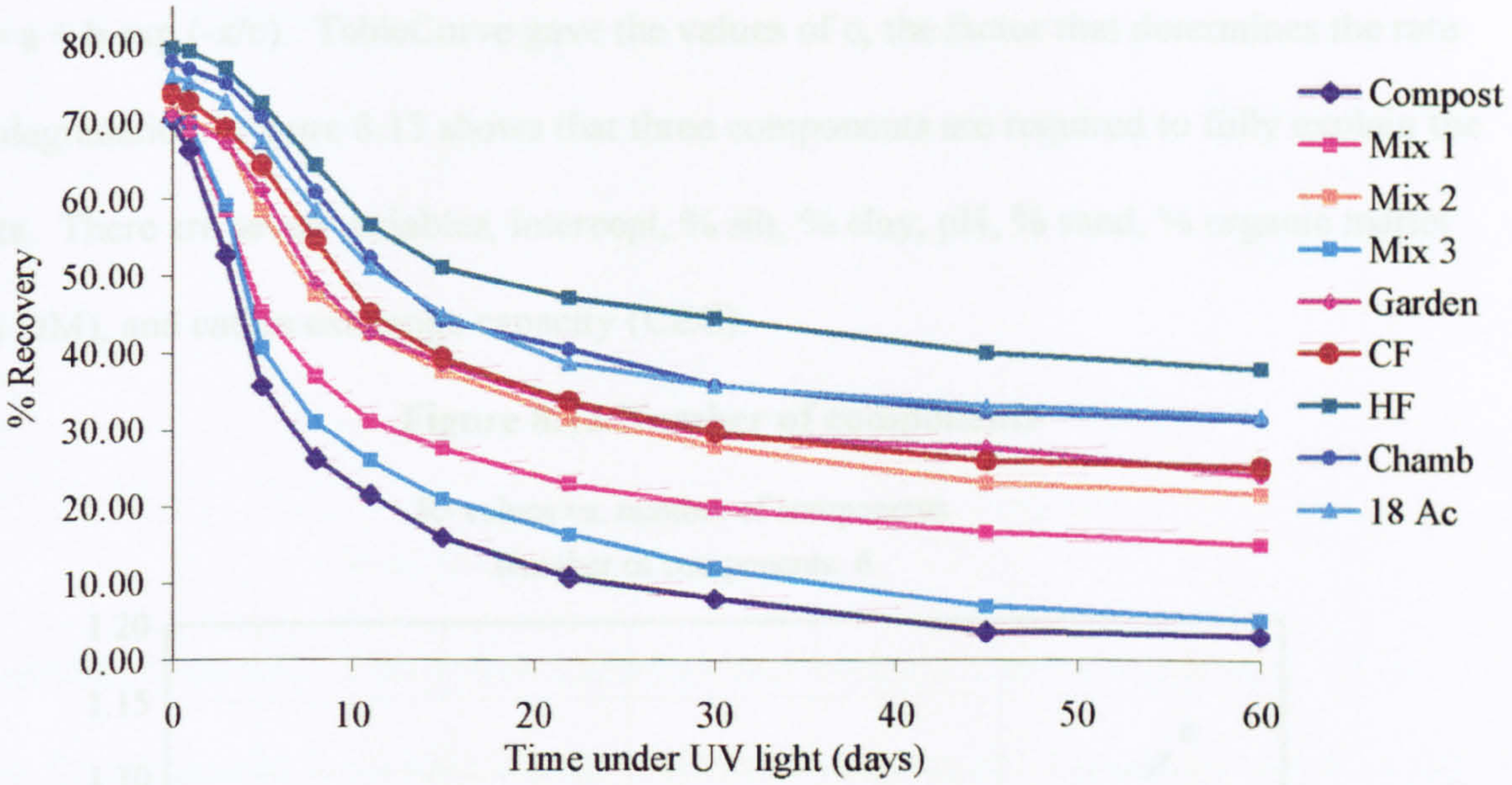


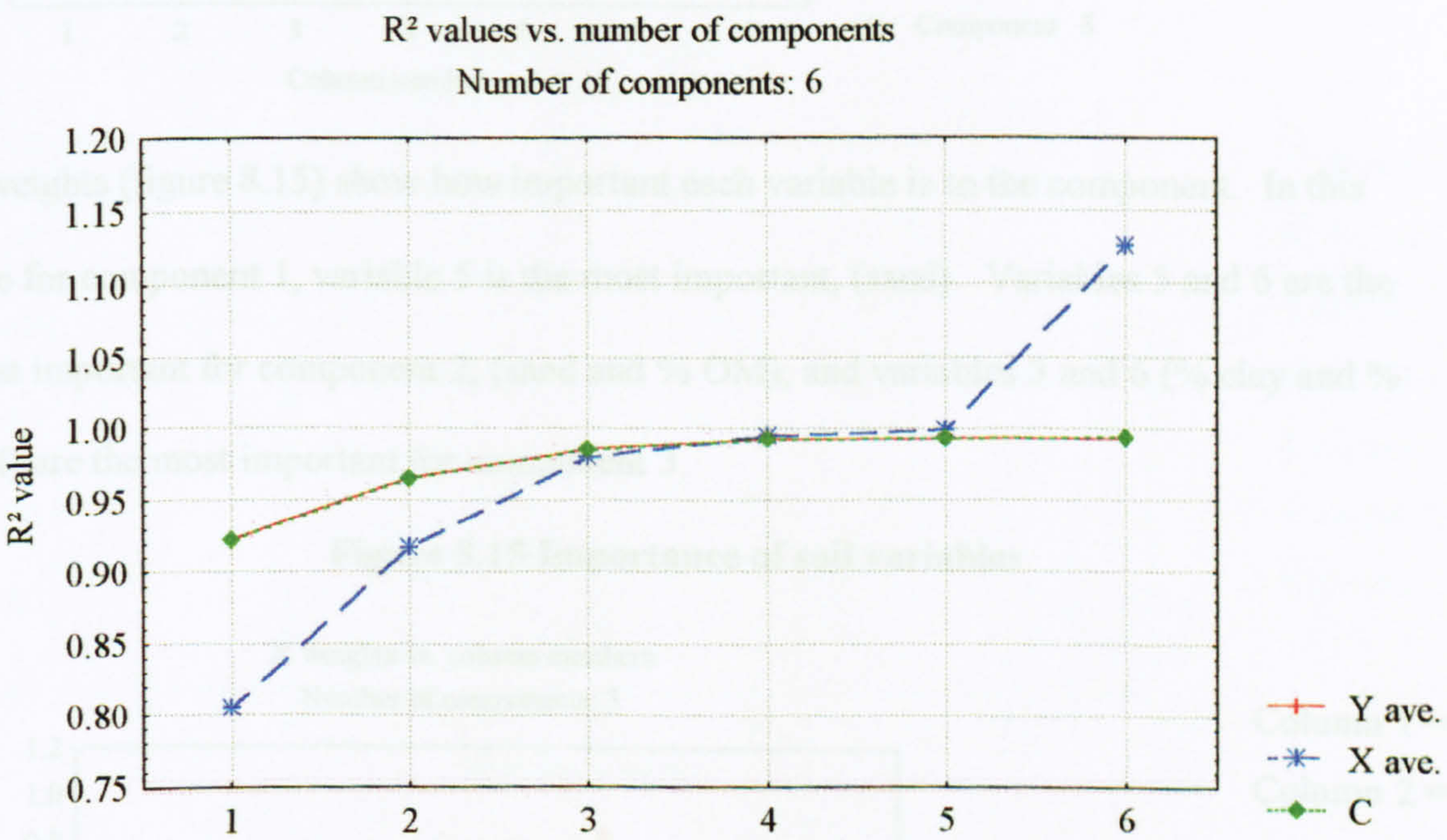
Table 8.6 shows the time required to decrease bupirimate to half its applied concentration on all nine soils.

Table 8.6 Measured half lives for bupirimate on various soils

Soil	Time required to reduced concentration by 50% (days)
Hyde Farm	12.5
Chamberlain	12.5
18 Acres	12
Chalgrove Farm	9.5
Garden	8
Mix 2	7.25
Mix 1	4.25
Mix 3	4
Compost	3.25

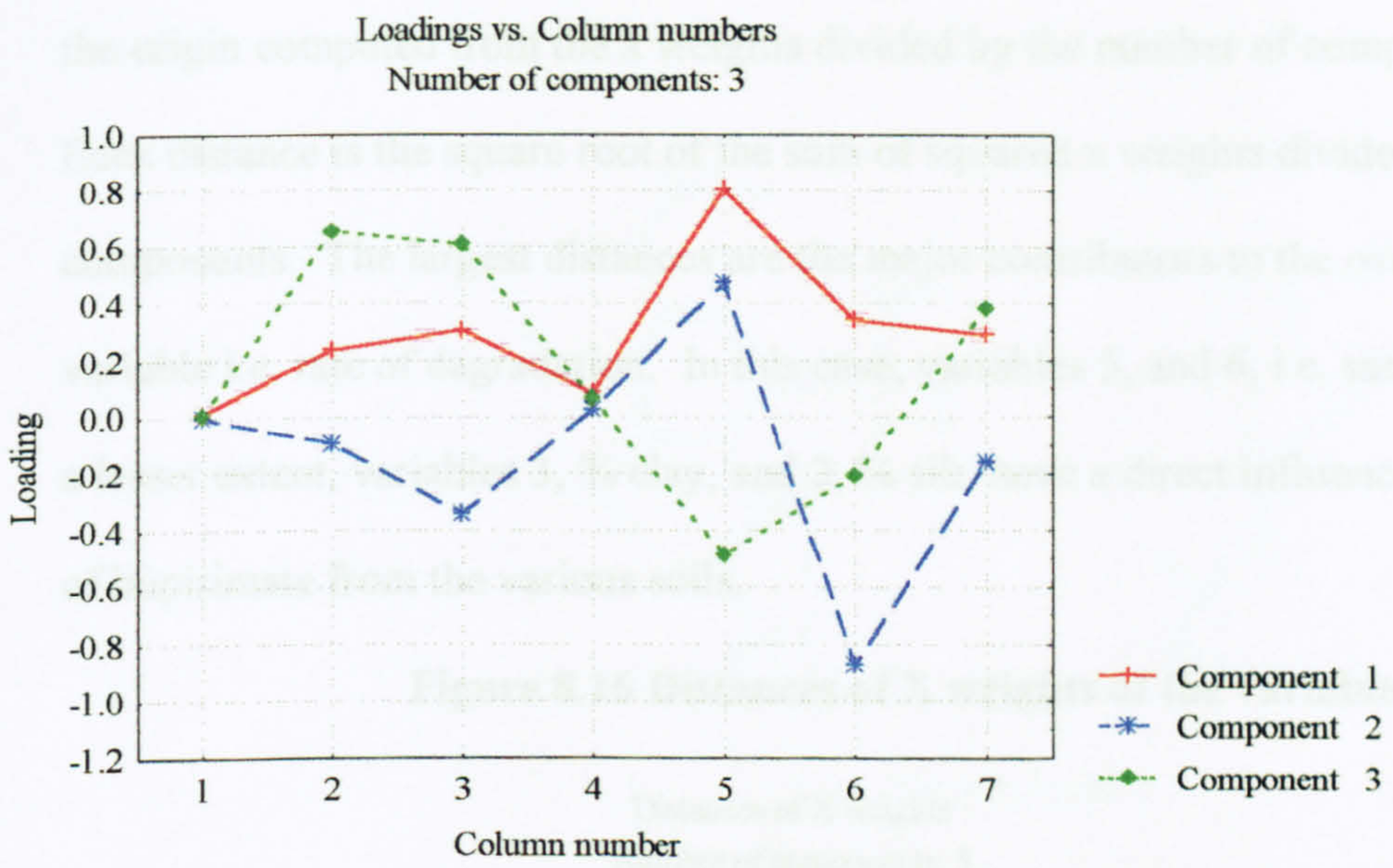
It is clear from the combined data that soil type has an influence on the degradation of bupirimate to ethirimol. To determine which soil parameters are important in the degradation of bupirimate, PLS was also performed on the data, using the same equation, $y = a + b \exp(-x/c)$. TableCurve gave the values of c, the factor that determines the rate of degradation. Figure 8.13 shows that three components are required to fully explain the data. There are seven variables, intercept, % silt, % clay, pH, % sand, % organic matter (% OM), and cation exchange capacity (CEC).

Figure 8.13 Number of components



The loadings for the analysis (figure 8.14) show that columns 4 and 6 (i.e. pH and % OM) are the most influential variables that define component 1, columns 5 and 6 (i.e. % sand and % OM) define component 2, and columns 2 and 5 (i.e. % silt and % sand) define component 3.

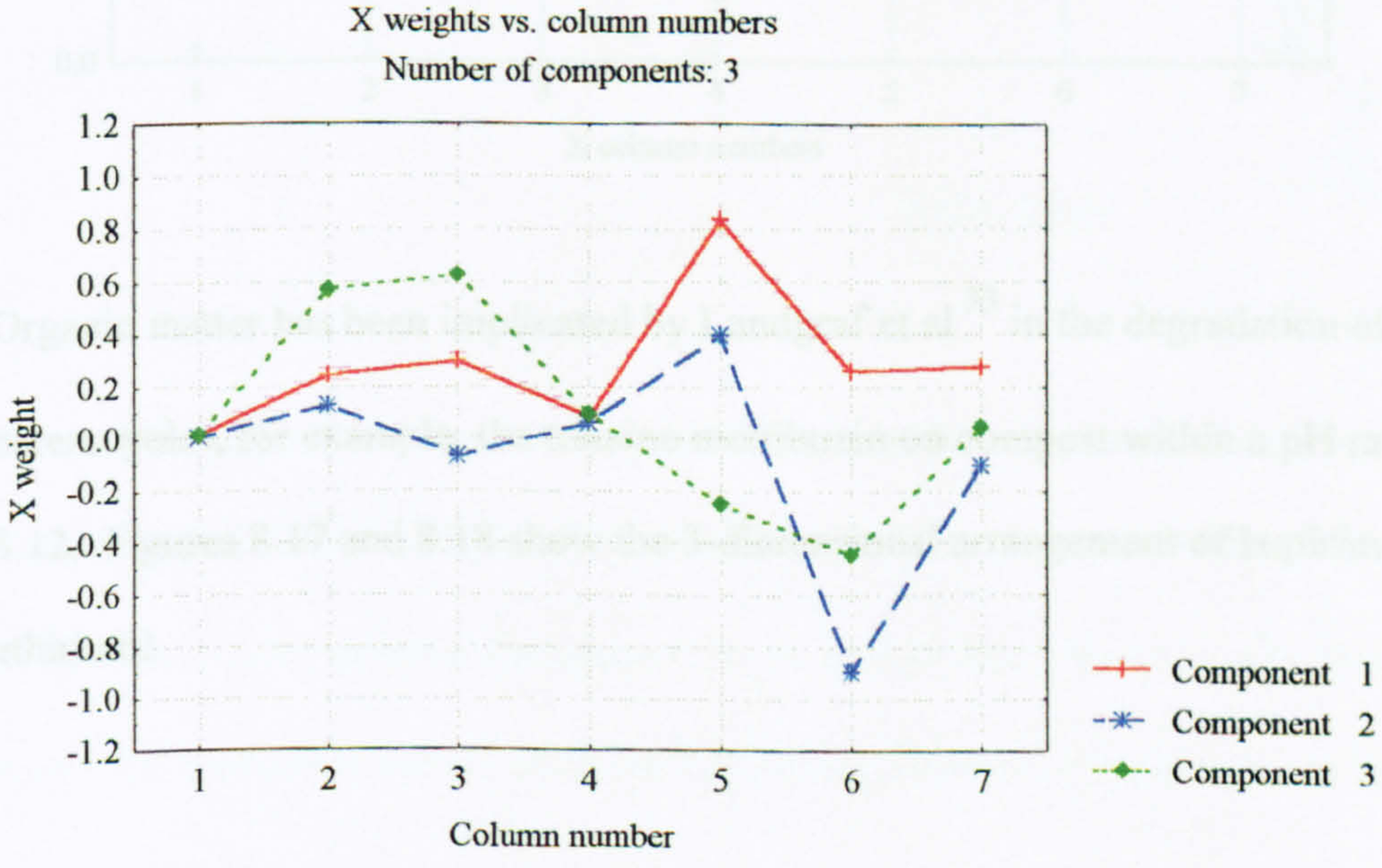
Figure 8.14 Loadings for two components



Column 1 = Intercept
Column 2 = % Silt
Column 3 = % Clay
Column 4 = pH
Column 5 = % Sand
Column 6 = % OM
Column 7 = CEC

X weights (figure 8.15) show how important each variable is to the component. In this case for component 1, variable 5 is the most important, (sand). Variables 5 and 6 are the most important for component 2, (sand and % OM), and variables 3 and 6 (% clay and % OM) are the most important for component 3.

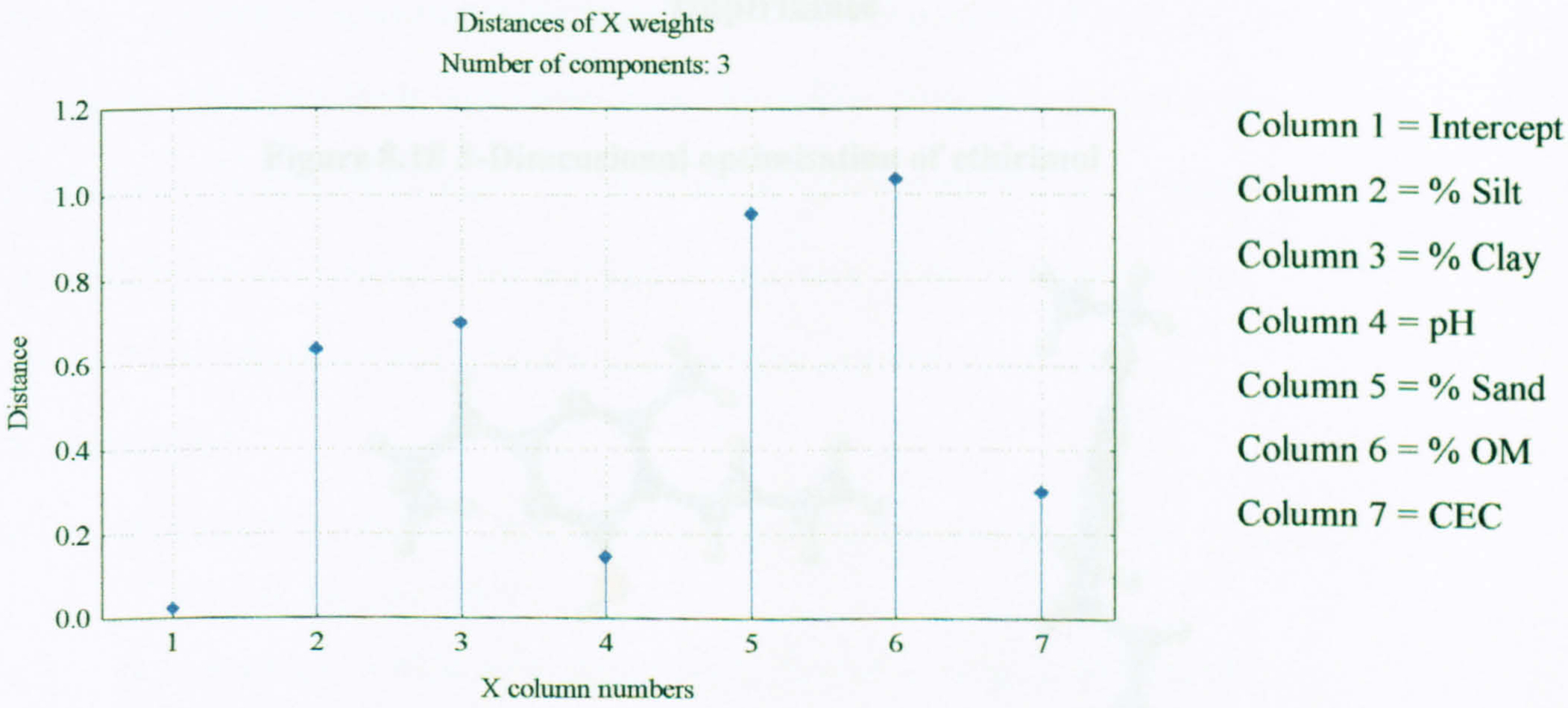
Figure 8.15 Importance of soil variables



Column 1 = Intercept
Column 2 = % Silt
Column 3 = % Clay
Column 4 = pH
Column 5 = % Sand
Column 6 = % OM
Column 7 = CEC

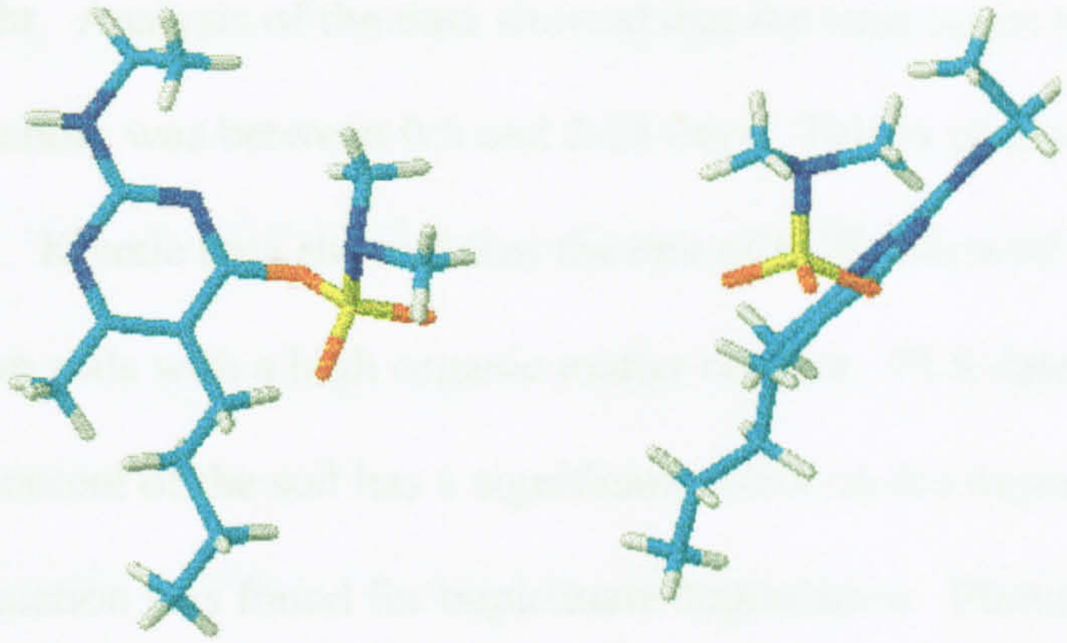
The distances of x weights (figure 8.16), is the Euclidean distances of the variables from the origin computed from the x weights divided by the number of components i.e. 3. Each distance is the square root of the sum of squared x weights divided by the number of components. The largest distances are the major contributors to the overall conceptual variable i.e. rate of degradation. In this case, variables 5, and 6, i.e. sand and OM, and to a lesser extent, variables 3, % clay, and 2, % silt, have a direct influence on the extraction of bupirimate from the various soils.

Figure 8.16 Distances of X weights of the variables



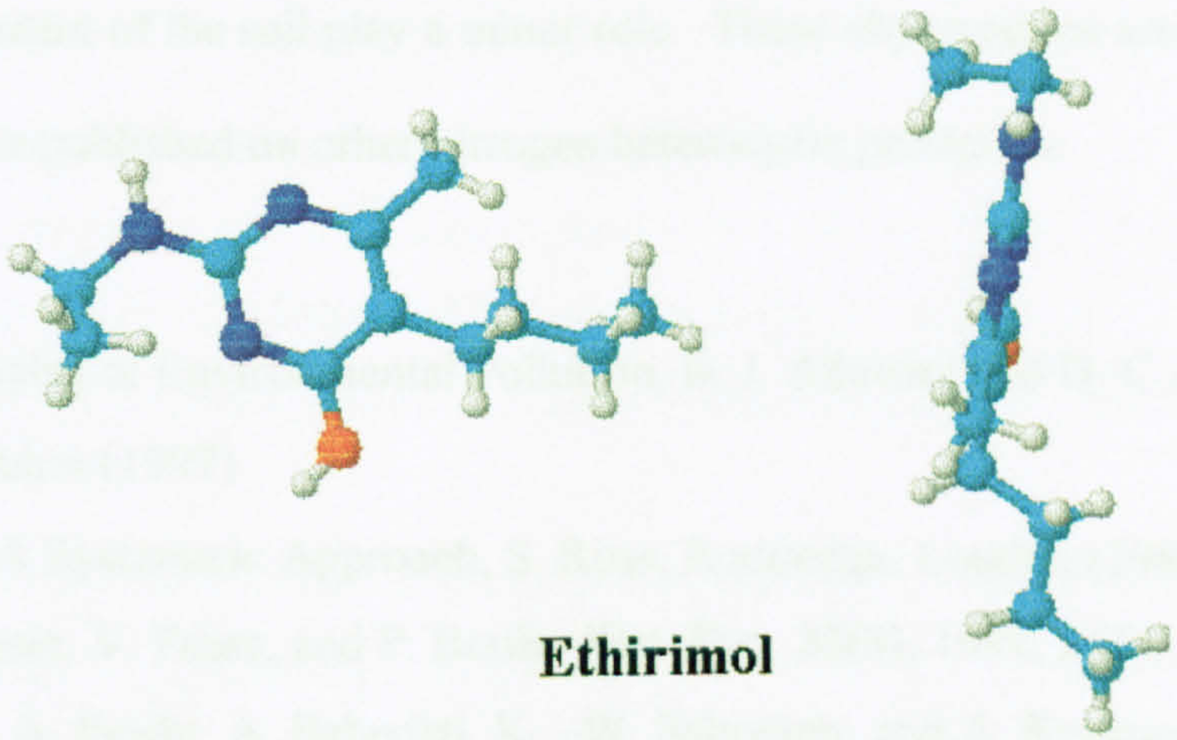
Organic matter has been implicated by Landgraf et al.[33] in the degradation of nitrogen heterocycles, for example, the triazine metribuzin on compost within a pH range of 5.8 – 8.12. Figures 8.17 and 8.18 show the 3-dimensional arrangement of bupirimate and ethirimol.

Figure 8.17 3-Dimensional arrangement of bupirimate



Bupirimate

Figure 8.18 3-Dimensional optimisation of ethirimol



Ethirimol

As can be seen both molecules are relatively planar and hence could be incorporated into the clay mineral structure. Small particle size is often associated with a high surface area and hence a large potential for adsorption, as well as degradation.[34] The results of PLS show that both these factors contribute to the degradation of bupirimate on soil.

8.3 Summary.

Both PCP and bupirimate have been successfully degraded on various soil types in the presence of ultraviolet light. Analysis of the data showed that the time taken to reduce PCP to half its applied quantity was between 0.5 and 2.25 days. This is consistent with previously published data. Kinetic data showed that the rate of PCP followed first order kinetics, and was fastest on soils with a high organic matter content. PLS determined that sand and organic matter content of the soil has a significant effect on the degradation rate of PCP. An analogous situation was found for bupirimate degradation. Photolysis of bupirimate reduced the concentration to half its initial concentration between 3.25 and 12.5 days. This value is also soil composition dependent. PLS determined that two major factors influenced the degree of degradation, sand and organic matter content, and that the silt and clay content of the soil play a minor role. These observations are consistent with literature published on other nitrogen heterocycle pesticides.

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Chapter 9

Conclusions and Furture Work

Conclusions and future work.

9.0 Conclusions

9.0.1 PFE optimisation.

Using three classes of molecules, the PFE extraction parameters were optimised. The only significant extraction parameter was temperature of extraction in the bupirimate and ethirimol study. Pressure was not deemed to have any effect in the study. The extraction of bupirimate and ethirimol showed that PFE was superior in both the quantity extracted and the precision when compared to sonication and shake flask extraction techniques. PFE was found comparable to Soxhlet extraction. Sonication and shake flask extractions were not used in subsequent investigations. The initial studies on bupirimate and ethirimol were performed using spiked inert matrix, hydromatrix. Application of the optimised parameters to the extraction of these molecules from spiked soil showed that the soil matrix influenced the recovery of the molecules. An investigation into the extraction solvent also showed that certain solvents gave better extraction recoveries than others did. An analogous situation was found when pentachlorophenol was extracted from a certified reference material (CRM 524), and spiked aged soils. Both solvent and soil matrix have a significant effect on the recovery of the extraction. A third class of pesticides were also investigated, the organochlorine pesticides DDT, and its degradation products, DDD and DDE. The extraction of this natively contaminated soil showed that ASE did not degrade any of the analytes of interest, and highlighted the need for a method of solvent prediction.

9.0.2 Solvent selection model.

The initial experimentation showed that a robust and quantitative method of solvent selection was required. Investigation into this area of extraction showed that solvent

selection models were not readily available, or easy to understand. A method based on the Hildebrand solubility parameter was developed. The basis of the solubility parameter is that molecules with similar solubility parameters form mixtures. Hence, a method of calculating the solubility parameter for common environmental analytes and extraction solvents was required. Fedor's reported a method of predicting the solubility parameter using a group addition method, where the molecule of interest is broken down into its constituents, and each group is given a value for the influence of hydrogen bonding, dispersion and polarity. Comparison of literature values and calculated values showed excellent correlation ($R^2 = 0.92$). Individual parameters representing the magnitude of hydrogen bonding, polarity and dispersion were calculated for the analytes of interest, bupirimate, ethirimol, PCP, DDT, DDD and DDE. A ternary plot was used to visually represent the positions of the analytes in relation to the different extraction solvents investigated. The predicted optimum extraction solvent for DDT, DDD and DDE was dichloromethane, and for bupirimate, ethirimol and PCP, a mixture of acetontrile:dichloromethane 1:1. PFE extraction of these molecules from spiked soil (bupirimate and ethirimol), natively contaminated soil (DDT, DDD and DDE) and a certified reference material (PCP) confirmed the predicted solvent was the optimum for the extraction. The model was applied to examples of extraction of analytes from real (not spiked) soil in the literature. There was good agreement between the predicted solvent and the experimental optimum.

9.0.3 Photolysis of PCP on soil.

PCP was spiked to nine different soils and subjected to UV light. Samples were removed and extracted at different time intervals. Six replicates were extracted per time interval.

Other chlorinated compounds were identified, indicating photolysis was occurring and the reduction in PCP extraction was not simply a result of adsorption to the soil matrix. The rate of PCP degradation followed first order kinetics, with rate constants from - $8.69x10^{-6}$ s^{-1} and - $2.00x10^{-6}$ s^{-1}. The time required for reducing the amount of PCP to half its applied quantity varied between 12 hours and 3 days. These data implied that soil composition had a direct effect on PCP degradation. Further investigation into the effect of soil composition showed that due to the high degree of correlation between the soil variables, multilinear regression would not give an accurate overview of the effect of soil variables. Hence, partial least squares regression was performed to determine the soil variables that influenced the rate of degradation. Partial least squares multiple regression determined that the quantity of sand and organic matter had a significant effect on the rate of degradation. Percentage clay was also implicated, but to a lesser extent.

9.0.4 Bupirimate photolysis on soil.

Bupirimate was spiked to nine different soils and subjected to UV light. Samples were removed and extracted at different time intervals. Six replicates were extracted per time interval. The presence of ethirimol, the major degradation product of bupirimate confirmed that photolysis was occurring and the reduction for bupirimate extracted was not simply a result of adsorption to the soil matrix. The time required to reduce the amount of Bupirimate to half its applied value ranged from 3.25 days on Compost soil, to 12.5 days on both Chamberlain and Hyde Farm soil. As in the case of PCP, the highly correlated soil variables precluded the use of multiple linear regression to elucidate the significant soil parameters. Partial least squares multiple regression determined that the

quantity of sand and organic matter had a direct effect on the rate of bupirimate photolysis. Percentage silt and percentage clay also played a minor role on the rate of Bupirimate degradation.

11.2 Future work

This work has shown that ASE is capable of extracting a wide range of analytes from soil, quickly and efficiently. It has also produced a robust method for the prediction of the optimum extraction solvent. Two molecules have been successfully degraded under UV light and their degradation products have been identified and quantified. Future work arising from these studies could include;

- Investigation of the kinetics of bupirimate degradation.
- Investigation of mechanism for PCP and bupirimate photolysis.
- Incorporation of the effect of the matrix into the solvent selection model
- Application of the model to matrices other than soil – spiked and aged samples, as well as literature examples.

Appendices

Appendix A1. Calculation of parameters for methanol

Dispersion contribution, δ_d

Equation 7.4 $\quad \delta_d = \dfrac{(\Sigma F_d)}{V}$

Total F_d (table 7.1)	= 630	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
divide by V (table 7.1)	= 630	$\dfrac{J^{1/2}\ cm^{3/2}\ \cancel{mol}^{-1}}{cm^3\ \cancel{mol}^{-1}}$
	= 43.5	
	= 14.48	$J^{1/2}\ cm^{-3/2}$
	= 14.48	$(J\ cm^{-3})^{1/2}$
	= 14.48	$(MPa)^{1/2}$

Polarity contribution, δ_p

Equation 7.5 $\quad \delta_p = \dfrac{(\Sigma F_p)}{V}$

Total (table 7.1)	= 500	$J^{1/2}\ cm^{2}\ mol^{-1}$
divide by V	= 500	$\dfrac{J^{1/2}\ cm^{2}\ \cancel{mol}^{-1}}{cm^3\ \cancel{mol}^{-1}}$
	= 43.5	
	= 11.49	$J^{1/2}\ cm^{-3/2}$
	= 11.49	$(J\ cm^{-3})^{1/2}$
	= 11.49	$(MPa)^{1/2}$

Hydrogen bonding contribution, δ_h

Equation 7.7. $\quad \delta_h = \left(\dfrac{\Sigma U_h}{V}\right)^{1/2}$

Total (table 7.1)	= 20000	$J^{1/2}\ mol^{-1}$
divide by V (table 7.1)	= 20000	$\dfrac{J^{1/2}\ \cancel{mol}^{-1}}{cm^3\ \cancel{mol}^{-1}}$
	43.5	
	= 459.77	$J^{1/2}\ cm^{-3/2}$
take square root	= 21.44	$(J\ cm^{-3})^{1/2}$
	= 21.44	$(MPa)^{1/2}$

Appendix A2. Calculation of parameters for DDT

Dispersion contribution, δ_d

Equation 7.4 $$\delta_d = \frac{(_z\Sigma^z F_d)}{V}$$

Total F_d (table 7.2)	= 4800	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
divide by V (table 7.2)	= 4800	$\frac{J^{1/2}\ cm^{3/2}\ \cancel{mol}^{+}}{cm^3\ \cancel{mol}^{+}}$
	= 204.6	$cm^3\ \cancel{mol}^{+}$
	= 23.46	$J^{1/2}\ cm^{-3/2}$
	= 23.46	$(J\ cm^{-3})^{1/2}$
	= 23.46	$(MPa)^{1/2}$

Polarity contribution, δ_p

Equation 7.6 $$\delta_p = \frac{(_z\Sigma^z Fp^2)^{1/2}}{V}$$

Square each	= 48400	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
Group contribution	= 1210000	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
	= 2722500	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
Add up squared contributions	= 3980900	$J\ cm^3\ mol^{-2}$
Take square root	= 1995	$J^{1/2}\ cm^{3/2}\ mol^{-1}$
Divide by V	= 1995	$\frac{J^{1/2}\ cm^{3/2}\ \cancel{mol}^{+}}{cm^3\ \cancel{mol}^{+}}$
	= 204.6	$cm^3\ \cancel{mol}^{+}$
	= 9.75	$J^{1/2}\ cm^{-3/2}$
	= 9.75	$(J\ cm^{-3})^{1/2}$
	= 9.75	$(MPa)^{1/2}$

Hydrogen bonding contribution, δ_h

Equation 7.7 $$\delta_h = \frac{(_z\Sigma^z U_h)}{V}^{1/2}$$

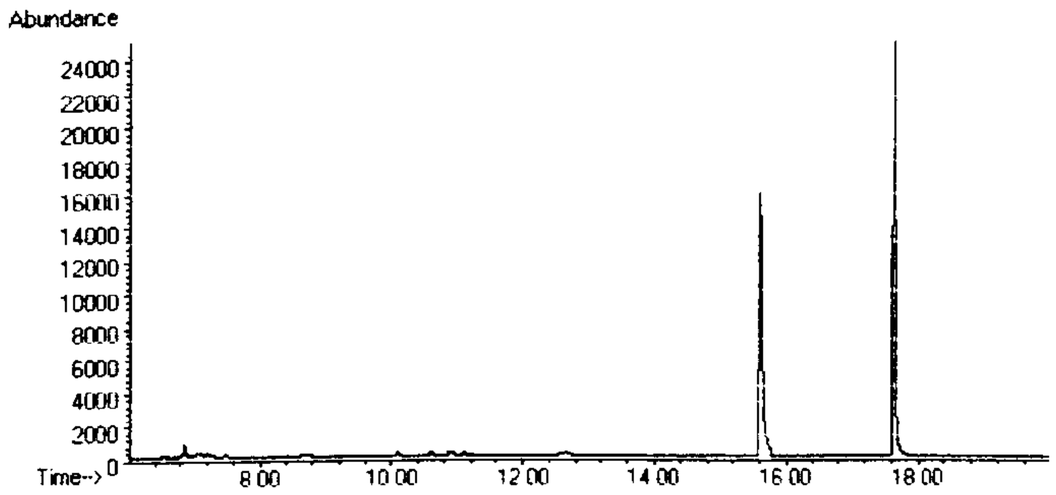
Total (table 7.2)	= 2000	$J^{1/2}\ mol^{-1}$
divide by V (table 7.2)	= 2000	$\frac{J^{1/2}\ \cancel{mol}^{+}}{cm^3\ \cancel{mol}^{+}}$
	= 204.6	$cm^3\ \cancel{mol}^{+}$
	= 9.75	$J^{1/2}\ cm^{-3/2}$
take square root	= 3.13	$(J\ cm^{-3})^{1/2}$
	= 3.13	$(MPa)^{1/2}$

Appendix A3

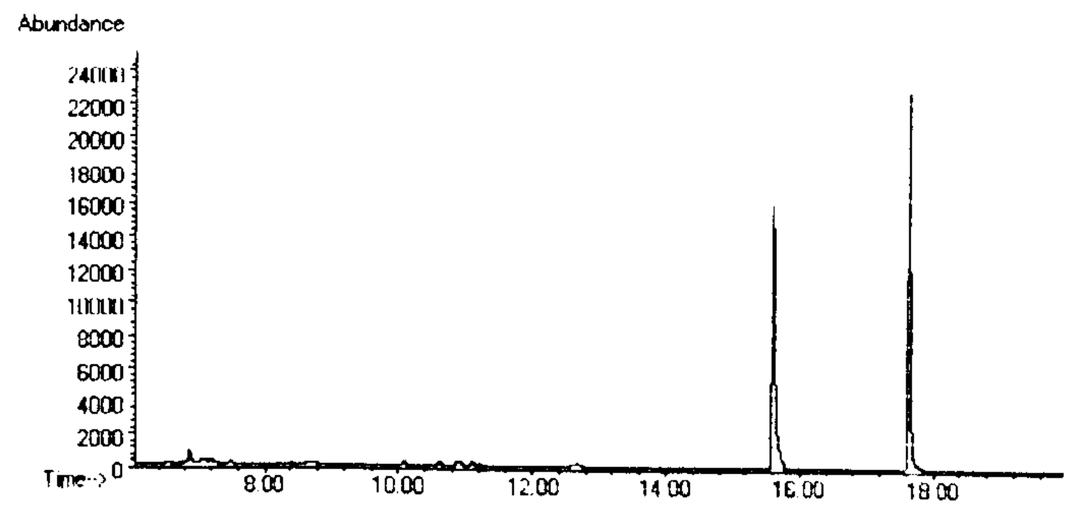
Mean µg / g (%RSD), 100 % recovery = 20 µg / g

Time (days)	Compost	Mix 1	Mix 2	Mix 3	Garden	Chalgrove Farm	Hyde Farm	Chamberlain	18 Acres
0	16.07 (2.8)	17.04 (2.1)	18.31 (3.1)	16.83 (3.1)	17.79 (3.3)	16.46 (3.6)	17.65 (2.6)	18.84 (4.0)	17.58 (2.9)
0.04	14.49 (3.1)	16.25 (2.2)	15.07 (3.2)	15.16 (3.2)	16.99 (3.2)	16.39 (4.5)	15.65 (3.6)	16.51 (4.3)	17.10 (3.5)
0.25	12.23 (3.0)	13.93 (2.6)	13.68 (3.3)	13.56 (4.1)	15.86 (4.1)	14.07 (3.2)	14.31 (3.3)	15.60 (3.0)	14.51 (3.3)
0.5	9.85 (3.2)	12.15 (3.1)	13.04 (4.2)	12.00 (3.7)	14.50 (2.7)	11.33 (4.5)	12.76 (3.2)	14.76 (3.7)	12.62 (4.2)
0.75	8.08 (3.2)	11.10 (4.3)	12.76 (4.4)	10.76 (3.5)	13.34 (3.1)	9.87 (4.2)	11.75 (4.4)	14.25 (3.2)	11.45 (4.4)
1	7.18 (2.9)	10.50 (4.4)	12.29 (4.1)	9.73 (2.9)	12.49 (2.8)	9.05 (3.8)	10.51 (4.5)	13.65 (3.6)	10.41 (3.9)
3	2.5 (3.6)	5.90 (4.1)	8.30 (3.9)	4.80 (4.4)	8.67 (2.9)	5.02 (3.7)	6.53 (2.9)	9.56 (3.7)	6.50 (3.1)
5	1.20 (3.0)	3.53 (4.9)	5.11 (3.7)	3.16 (4.6)	6.05 (3.6)	2.95 (4.7)	4.33 (2.7)	6.26 (2.6)	4.13 (4.3)
8	0.70 (3.8)	2.52 (3.7)	3.21 (3.3)	2.24 (3.8)	3.70 (4.2)	2.09 (5.4)	2.61 (3.2)	3.96 (4.4)	2.53 (4.6)
11	0.43 (4.3)	1.75 (3.6)	2.41 (4.2)	1.7 (3.9)	2.66 (4.5)	1.24 (5.1)	2.01 (3.0)	3.52 (4.6)	1.83 (4.4)
15	0.27 (4.5)	1.42 (3.6)	2.00 (4.5)	1.36 (3.6)	1.74 (4.7)	0.80 (4.7)	1.55 (4.8)	3.15 (3.8)	1.28 (3.9)
22	0.00	1.32 (4.2)	1.88 (4.4)	1.22 94.1)	1.66 (3.7)	0.50 (5.2)	1.48 (4.6)	3.07 (3.2)	1.18 (5.2)
30	0.00	1.25 (4.0)	1.84 (4.9)	1.15 (4.0)	1.59 (4.9)	0.00	1.43 (3.4)	3.00 (4.1)	1.00 (4.9)

Appendix A4



Compost day 0



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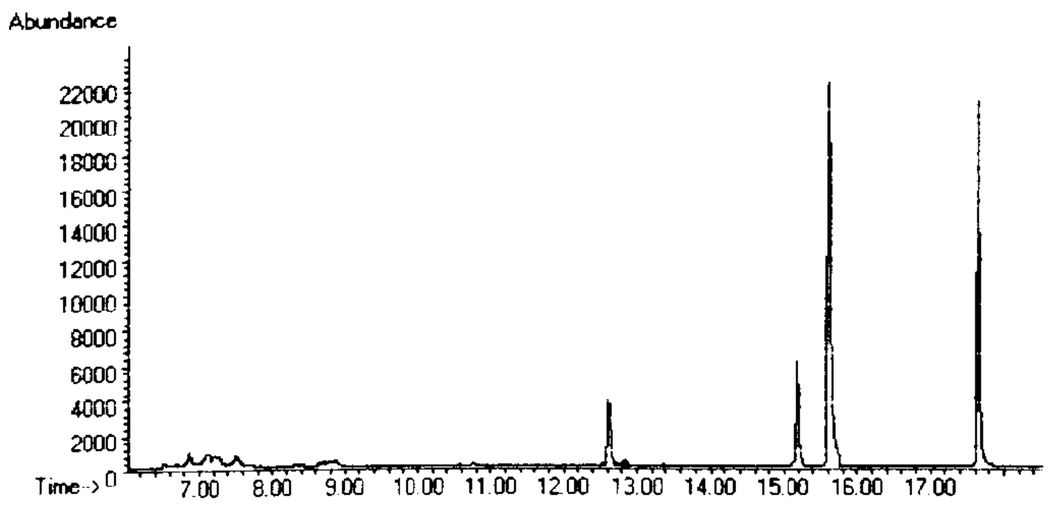

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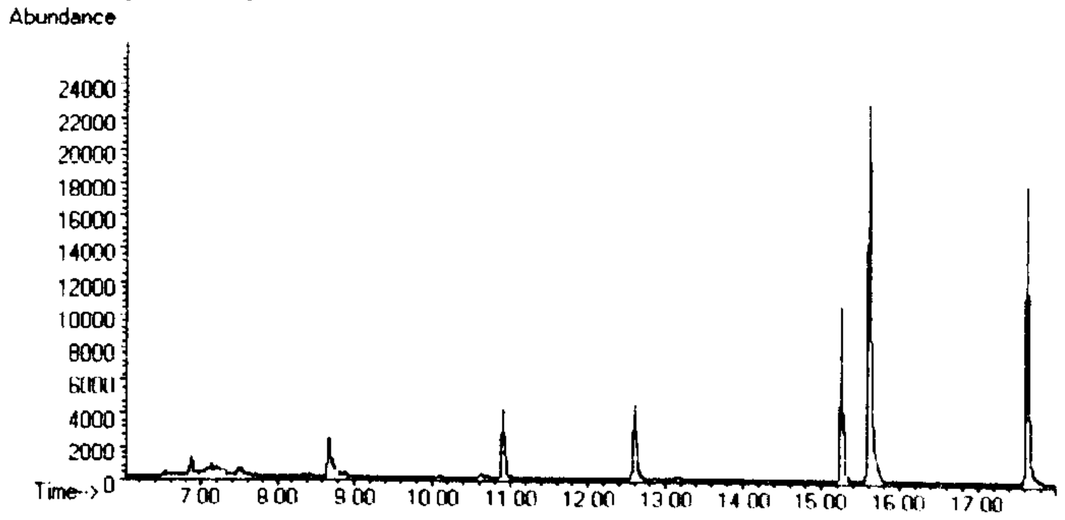

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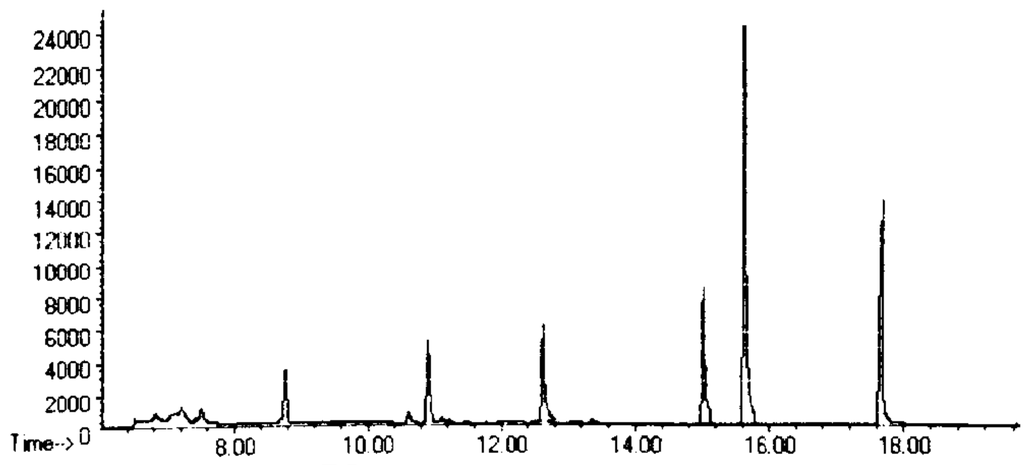

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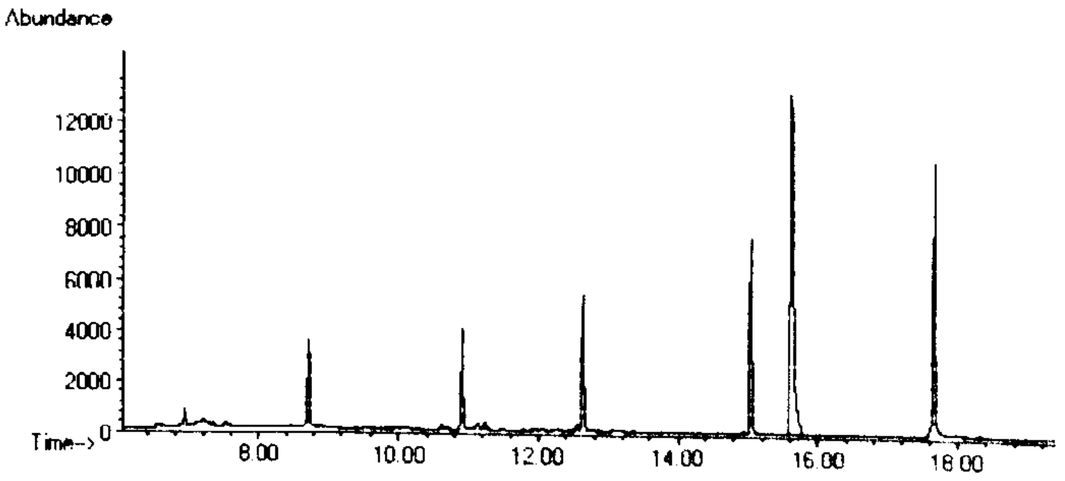
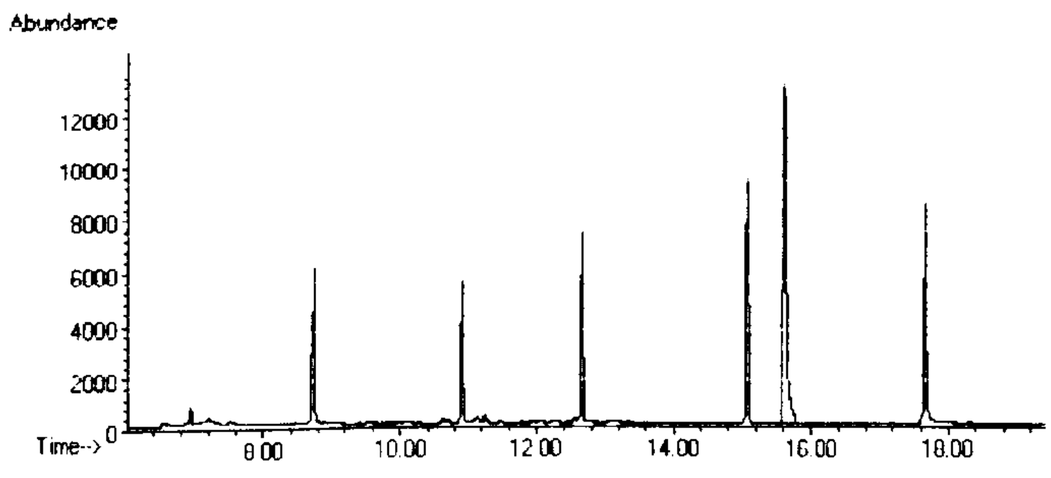
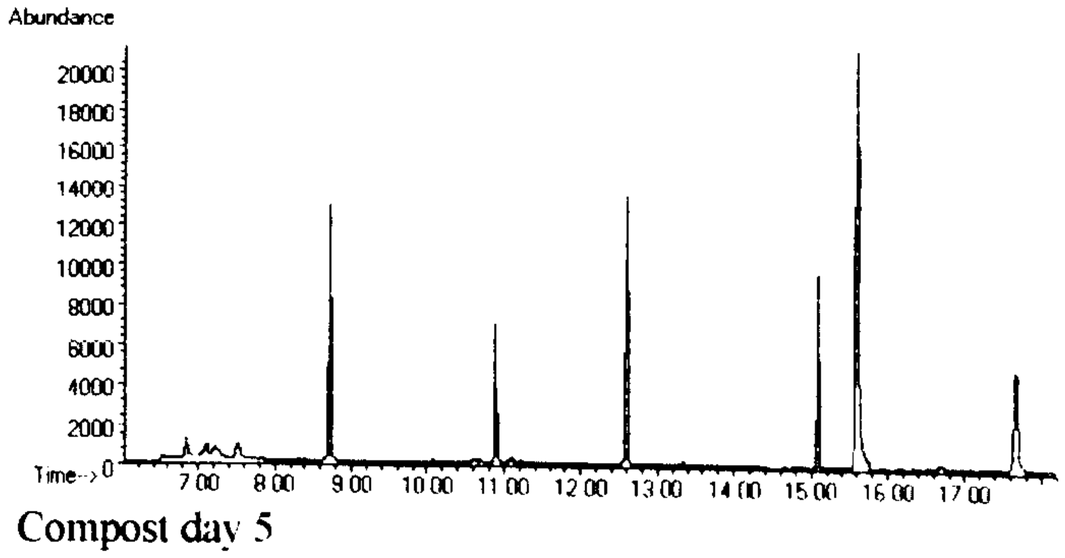


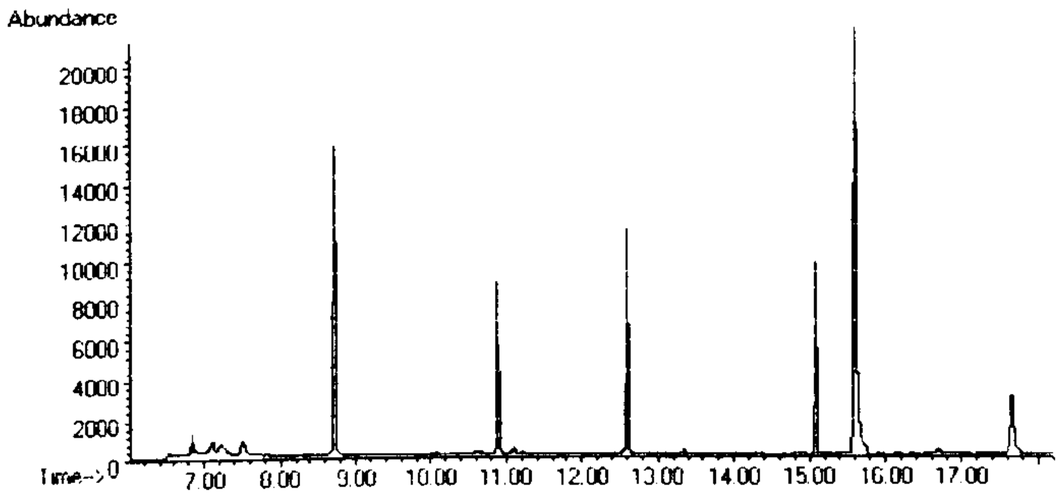
Compost day 1



Compost Day 3



Compost day 5



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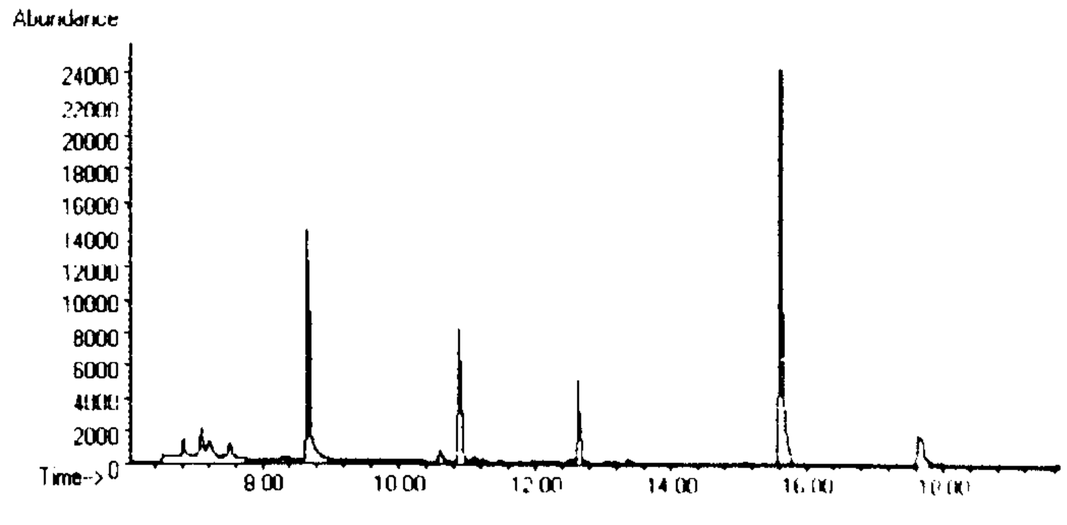
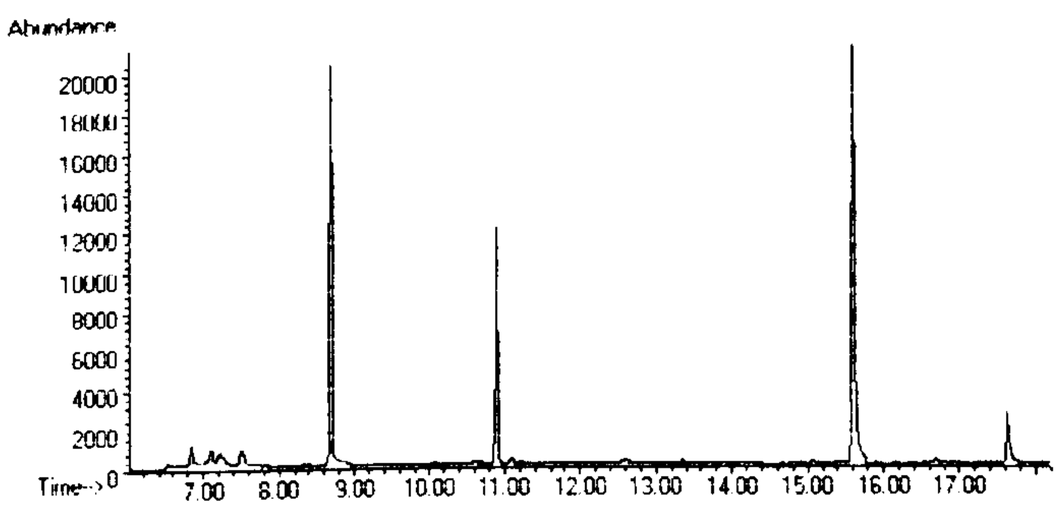
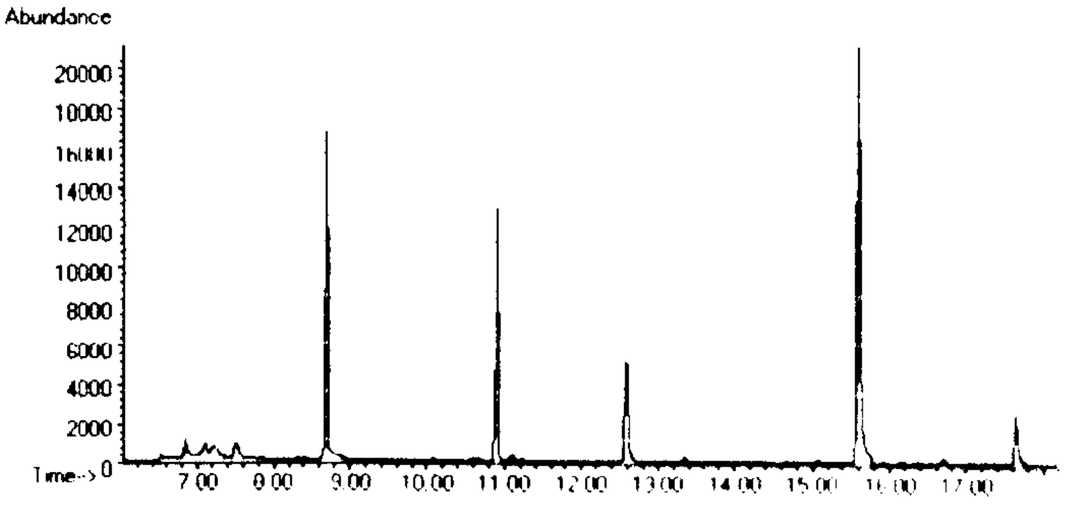


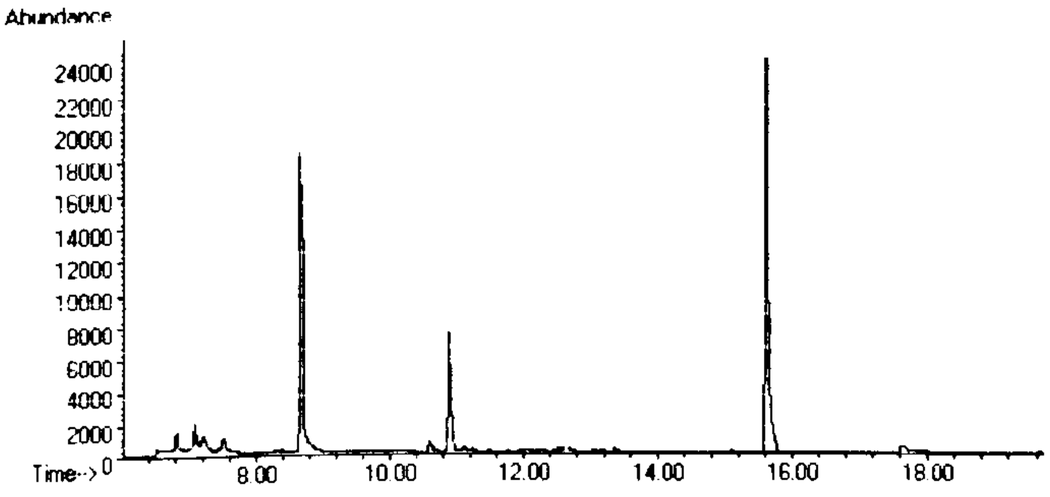
Compost day 11



Compost day 15



Compost day 22



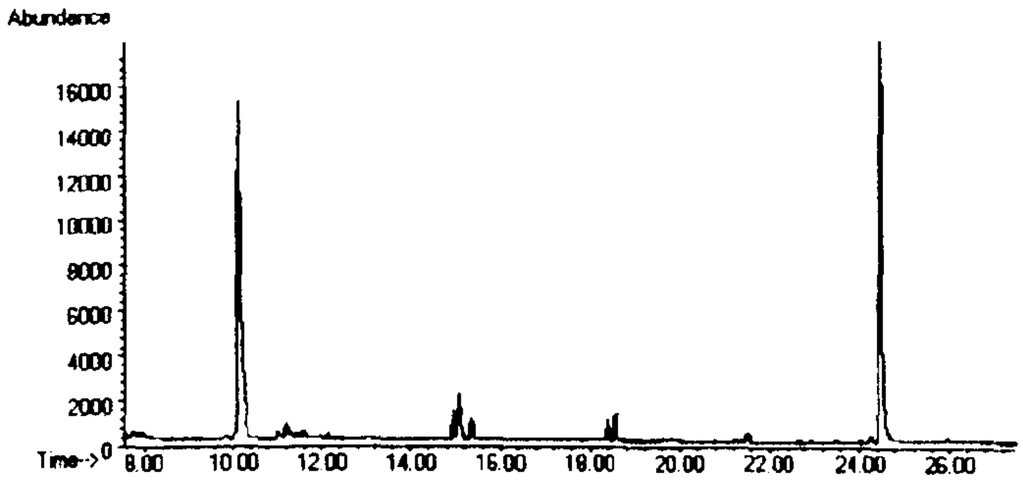
Compost day 30

Appendix A5

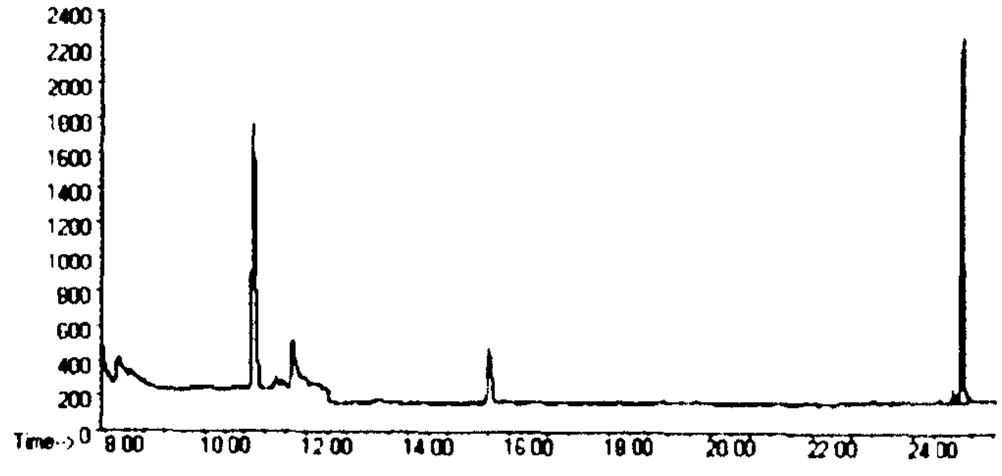
Mean µg / g (%RSD), 100 % recovery = 20 µg / g

Time (days)	Compost	Mix 1	Mix 2	Mix 3	Garden	Chalgrove Farm	Hyde Farm	Chamberlain	18 Acres
0	17.59 (3.3)	17.68 (3.3)	17.78 (3.3)	17.62 (3.3)	17.74 (4.3)	18.42 (4.4)	19.89 (3.3)	19.49 (4.2)	18.98 (3.2)
1	16.61 (4.3)	17.24 (3.4)	17.6 (4.2)	17.48 (4.2)	17.51 (3.3)	18.18 (3.2)	19.79 (4.1)	19.21 (3.7)	18.77 (2.1)
3	13.19 (2.8)	14.63 (2.7)	16.95 (3.9)	14.79 (3.8)	16.80 (3.7)	17.29 (3.1)	19.21 (2.5)	18.75 (3.2)	18.15 (2.5)
5	8.94 (3.1)	11.32 (3.4)	14.60 (4.5)	10.17 (3.5)	15.29 (3.6)	16.08 (2.3)	18.10 (2.8)	17.64 (4.2)	16.84 (3.0)
8	6.56 (4.8)	9.23 (2.6)	11.88 (2.7)	7.77 (4.2)	12.27 (4.7)	13.62 (2.6)	16.08 (3.1)	15.23 (4.0)	14.63 (3.1)
11	5.40 (3.3)	7.79 (4.1)	10.59 (3.8)	6.50 (2.7)	10.63 (4.3)	11.26 (3.6)	14.13 (4.2)	13.09 (3.2)	12.79 (4.0)
15	4.01 (3.7)	6.90 (4.3)	9.39 (4.6)	5.26 (2.9)	9.65 (4.1)	9.83 (4.4)	12.76 (4.1)	11.05 (3.5)	11.23 (3.2)
22	2.71 (5.0)	5.75 (4.1)	7.86 (3.2)	4.11 (4.0)	8.33 (3.8)	8.40 (4.8)	11.78 (3.8)	10.12 93.6)	9.66 (3.7)
30	2.00 (5.2)	5.00 (5.2)	6.95 (3.5)	3.00 (4.5)	7.28 (3.3)	7.42 (3.7)	11.13 (3.4)	8.97 (4.7)	8.94 (4.2)
45	0.93 (4.7)	4.2 (3.9)	5.78 (2.5)	1.76 (4.7)	6.91 (4.2)	6.51 (3.2)	10.00 (3.7)	8.07 (4.3)	8.29 (4.1)
60	0.80 (4.4)	3.81 (3.7)	5.45 (5.1)	1.33 (5.5)	6.08 (4.8)	6.32 (5.1)	9.49 (4.9)	7.85 (5.2)	8.00 (5.1)

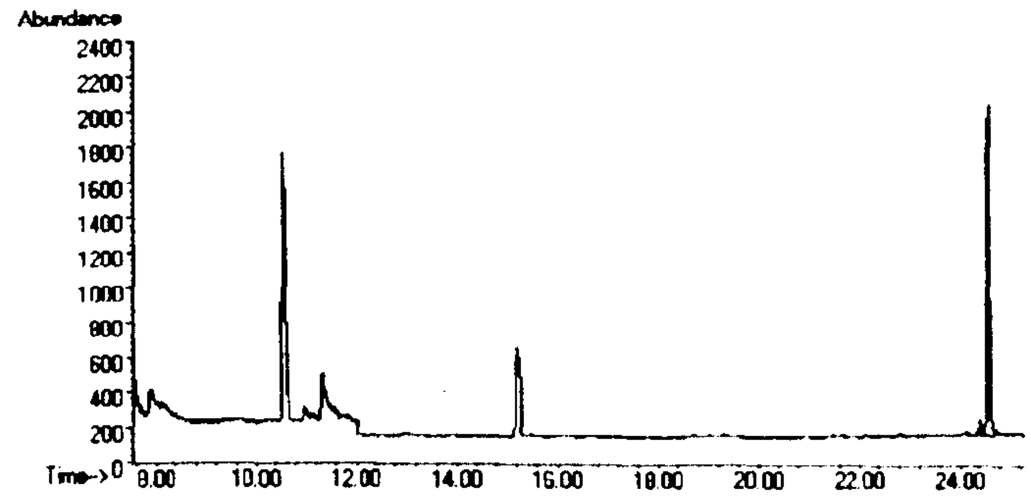
Appendix A6



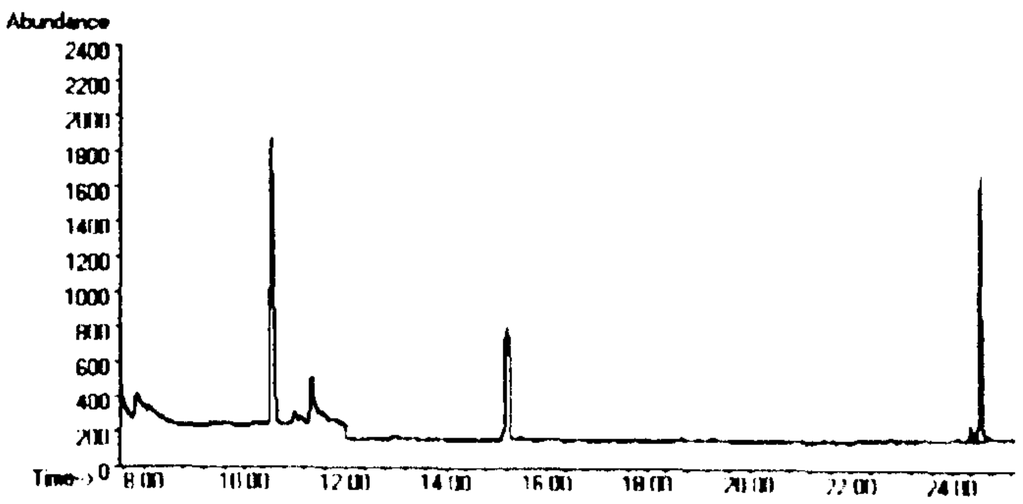
Day 1



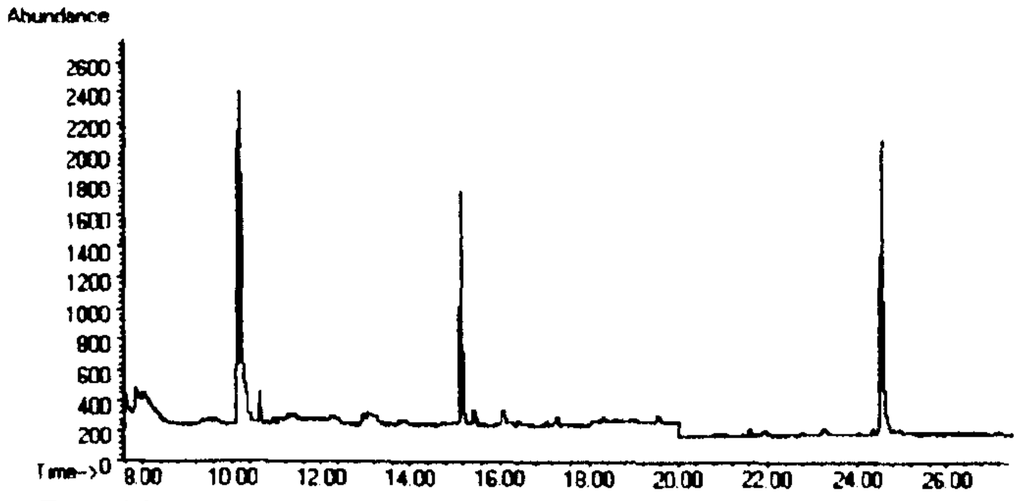
Day 3



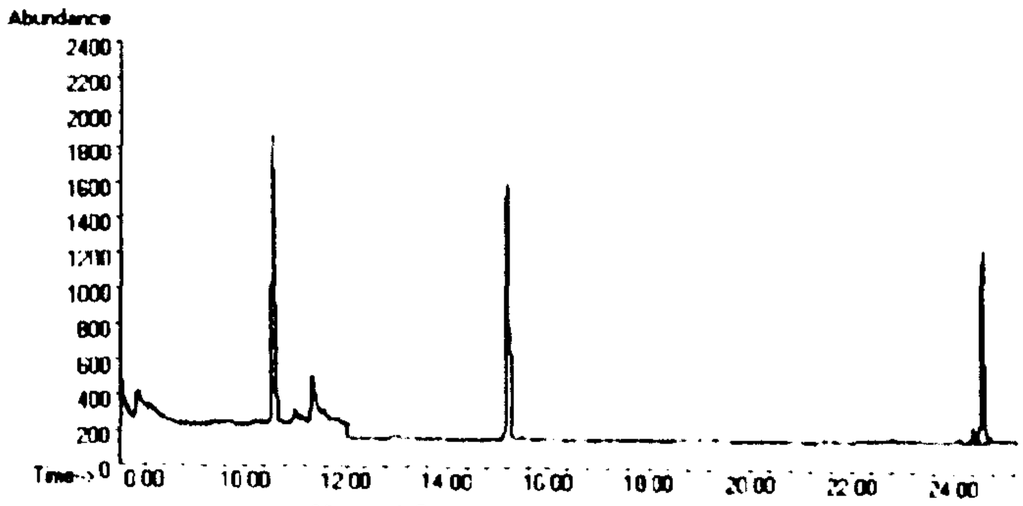
Day 5



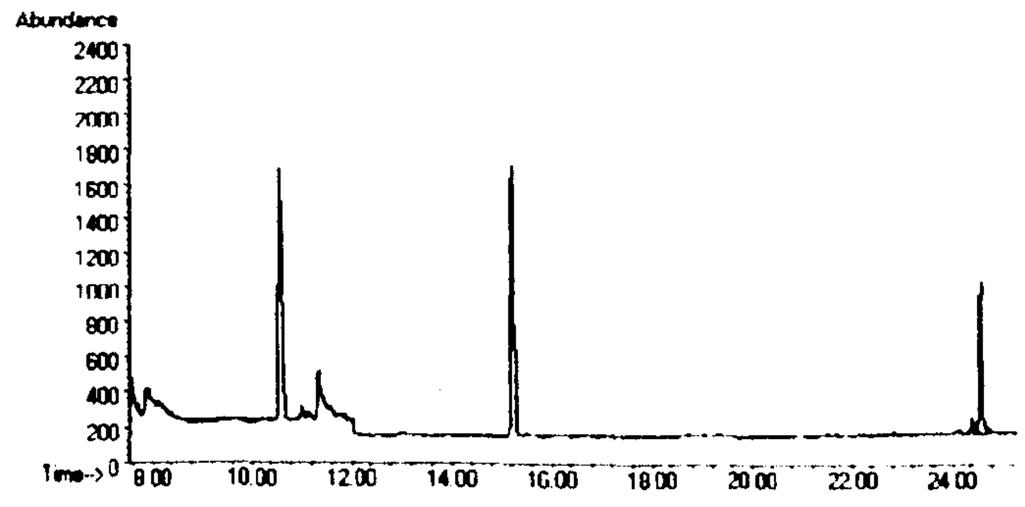
Day 8



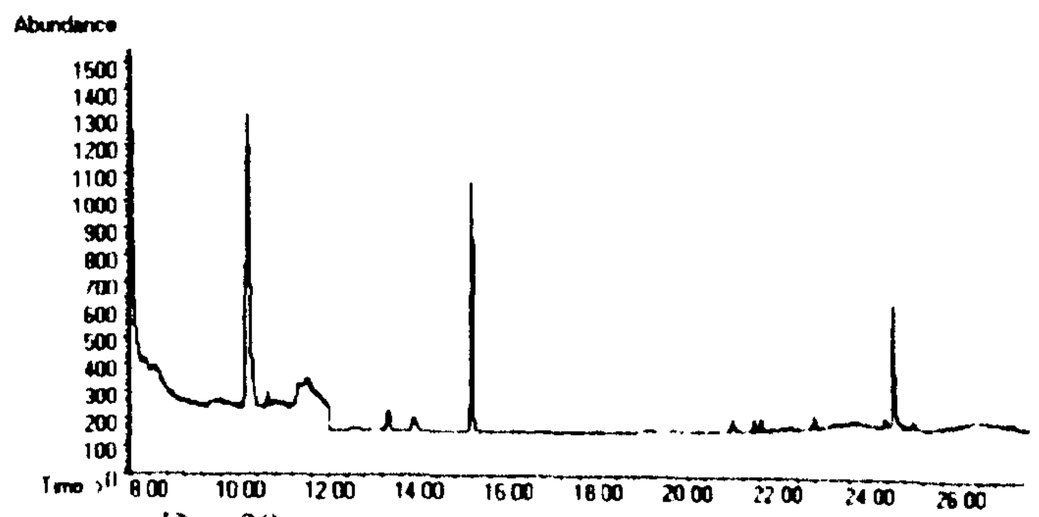
Day 11



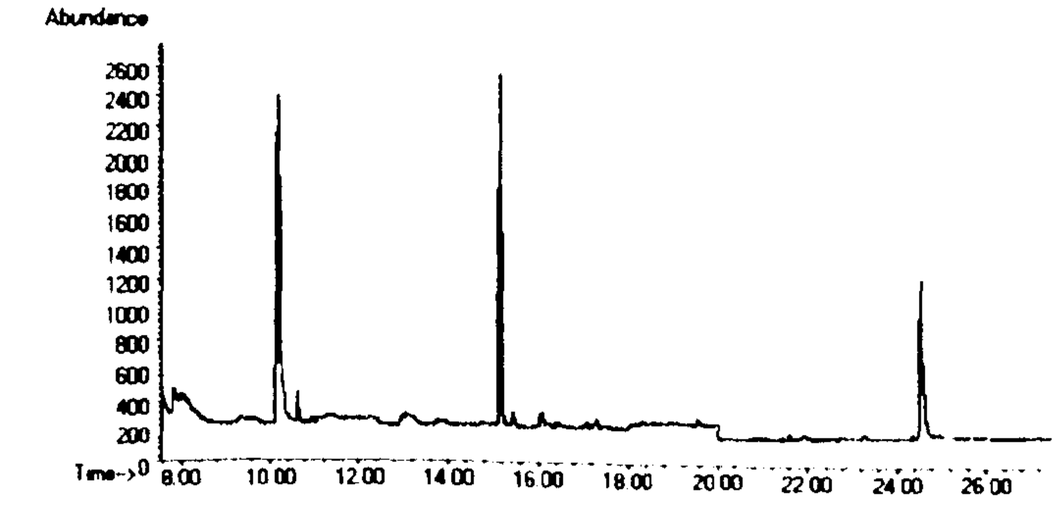
Day 15



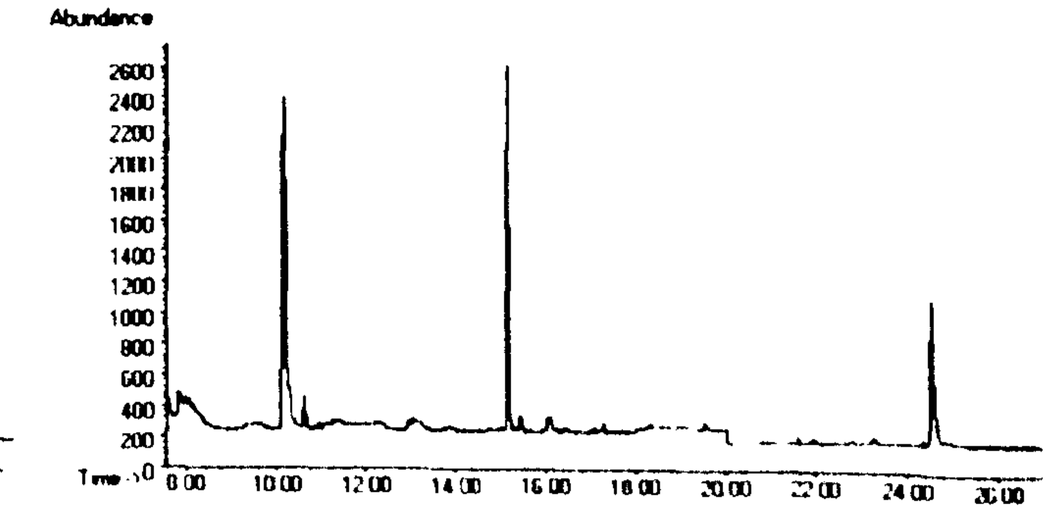
Day 22



Day 30



Day 45



Day 60

Appendix A7 Publications

"Microwave-assisted solvent extraction", J. R. Dean, L. J. Fitzpatrick and C. Heslop, in "Extraction Methods in Organic Analysis", A. Handley (Ed), Sheffield Academic Press, Sheffield (1999) Chapter 7, pp.166-193.

"Accelerated solvent extraction of pentachlorophenol from industrially relevant matrices", L. J. Fitzpatrick, J. R. Dean, M. H. I. Comber, K. Harradine, K. P. Evans and S. Pearson, J. Chromatogr., 873 (2000) 287-291.

"Extraction of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and its metabolites (DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane]from aged, contaminated soil", L. J. Fitzpatrick, J. R. Dean, M. H. I. Comber, K. Harradine, K. P. Evans and S. Pearson, J. Chromatogr., *in press*.

"Pesticides defined by matrix", J. R. Dean and L. J. Fitzpatrick, in "Handbook of Analytical Separations", R.M. Smith (Series Editor). Volume 7, "Environmental Analysis", W. Kleibohmer, (Ed), Elsevier Science, Amsterdam *(due 2001)*.

"Extraction solvent selection in environmental analysis", L. J. Fitzpatrick and J. R. Dean, Analyst, *submitted*.

"Environmental Applications of Pressurised Fluid Extraction", L. J. Fitzpatrick, O. Zuloaga, N. Etxebarria, and J. R. Dean, Reviews in Analytical Chemistry, *in press*.

"Pressurised Fluid Extraction of PAH's from Soil: Influence of Soil Type", O. Zuloaga, L. J. Fitzpatrick, N. Etxebarria, and J. R. Dean, *submitted*.

"Photochemical Degradation of Organic Pollutants on Soil", L. J. Fitzpatrick, and J. R. Dean, *in preparation*.