THE ISOLATION AND CHARACTERISATION OF DISSOLVED ORGANIC MATTER FROM FRESHWATER AND MARINE ENVIRONMENTS

BY

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LIST OF PUBLICATIONS AND PRESENTATIONS

Peer reviewed Publications

M. V. McCaul, D.Sutton, A. J. Simpson, A. Spence, D. J. McNally, B. W. Moran, A. Goel, B. O'Connor and B. P. Kelleher. Composition of Dissolved Organic Matter within a lacustrine environment. Environmental Chemistry 8(4) 146-154 2011.

Spence, A. J. Simpson, D. J. McNally, B. W. Moran, M. V. McCaul, B. O'Connor & B. P. Kelleher. The Degradation Characteristics of Microbial Biomass in Soil. Submitted to Geochimica et Cosmo Acta.

Woods GC, Simpson MI, Kelleher BP, McCaul M, Kingery WL, Simpson AJ. Online High-Performance Size Exclusion Chromatography-Nuclear Magnetic Resonance for the Characterization of Dissolved Organic Matter. Environmental Science And Technology, 44, 2, pp624-630. 2010

Poster Presentations

Sara Sandron; Ekaterina Nesterenko; Margaret McCaul; Brian Kelleher; Brett Paull, Application of multidimensional chromatography for the separation and identification of Dissolved Organic Matter in seawater and freshwater. Irish Separation Science Cluster Seminar, Dublin City University, Ireland, 2010

Sara Sandron; Ekaterina Nesterenko; Margaret McCaul; Brian Kelleher; Brett Paull, Application of two-dimensional chromatography (IC x RP) to the separation and identification of the components of Dissolved Organic Matter, 22nd International Ion Chromatography Symposium, Ohio, USA. 2010

Margaret McCaul, Andre Simpson, David Sutton, Brian P Kelleher. Watershed Influences on lacustrine dissolved organic matter. The 24th International Meeting on Organic Geochemistry 2009 in Bremen, Germany, Sep 6th - 11th, 2009.

Margaret McCaul, Andre Simpson, David Sutton, Brian P Kelleher. Chemical characteristics and viability of lacustrine dissolved organic matter. Poster Presentation GRC Organic Geochemistry, Holderness School, Holderness, NH, 2008

Oral Presentations

Margaret McCaul, Andre Simpson, David Sutton, Brian P Kelleher. Isolation and Characterisation of dissolved organic matter from freshwater. Irish Mass Spectrometry, Annual general meeting, Dublin 2010

'Environmental science has become too much a matter of dogma taught by 'professionals' in ivory towers as though it's all fact'

THESIS ABSTRACT

Freshwater and marine dissolved organic matter (DOM) is a complex mixture of chemical components that are central to many environmental processes, including carbon and nitrogen cycling. Due to its wide range of chemical-physical properties, the sampling and separation of its components is challenging, mainly due to co-elution and irreversible sorption issues on traditional chromatographic columns. For this reason, questions remain as to its chemical characteristics, sources and transformation mechanisms. Here, we employ novel passive sampling techniques for isolation coupled with advanced analytical techniques for the characterisation of DOM from marine and freshwater environments. The spatial and temporal variation of DOM composition was investigated in a number of freshwater bodies along with the development and application of novel passive samplers within marine environments. In Chapter 2 we employ 1- and 2-D nuclear magnetic resonance (NMR) spectroscopy to investigate the structural components of lacustrine DOM from Ireland, and how it varies within a lake system, as well as to assess potential sources. Major components found, such as carboxyl-rich alicyclic molecules (CRAM) are consistent with those recently identified in marine and freshwater DOM. Lignin-type markers and protein/peptides were identified and vary spatially. Phenylalanine was detected in lake areas influenced by agriculture, whereas it is not detectable where zebra mussels are prominent. The presence of peptidoglycan, lipoproteins, large polymeric carbohydrates and proteinaceous material supports the substantial contribution of material derived from microorganisms.

A major challenge in environmental chemistry at the moment is finding materials that can isolate all components of DOM from both fresh and marine water. In the freshwater studies of chapter 2 a cellulose sorbent was used to isolate the DOM from water. However, cellulose use is not possible in marine water studies as the Cl^- ions compete for binding sites affecting the sorption of DOM. Therefore activated carbon was investigated as a possible sorbent of DOM within marine environments in chapter 3. Activated carbon passive samplers showed an steady uptake of organic carbon over time, however NMR results were inconclusive as major DOM signals CRAM and MDLT were absent from ^1H spectra. Consequently, cation exchanged monmorillonite clays were investigated as sorbents and showed

very good potential, at least in the sorption of the aliphatic component of DOM. After a 28 deployment within marine and freshwater environments GCMS analysis identified a large aliphatic component sorbed to the clay. In addition sterols and sugars were also identified in the DOM matrix. To further investigate clay as an isolation medium for DOM it was included in a more in-depth study of water chemistry in the Shannon Pot, Co Cavan, Ireland (Chapter 4). The DOM composition and hydrochemistry within this karst aquifer was investigated using: 1. clay passive samplers followed by GCMS TMAH chemolysis, 2. NMR and 3. water quality indicators over a fifteen month period. This data was correlated with rainfall records. Phosphate and dissolved oxygen levels exceeded recommended concentrations at times of high precipitation indicating that fertiliser use is influencing the water chemistry at this important site. These events were also evident in DOM chemistry as an increase in biopolymers such as lignin was observed during the same periods of increased precipitation. Furthermore, evidence for anthropogenic influence in the surrounding landscape was found in the DOM including herbicides that may be more stable in the environment than previously thought. The results indicate than the DOM composition and its hydrochemistry is strongly influenced by the hydrological events such as rainfall.

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LIST OF ABBREVIATIONS

APS	Acylpolysaccarides
ACD	Advanced Chemistry Development
BSTFA	bis(trimethylsilyl)
Ca-Mt	Calcium Montmorillonite
CRAM	Carboxyl-Rich Alicyclic Molecules
COD	Chemical Oxygen Demand
D2O	Deuterium Oxide
DEAE	Diethylaminoethyl
DE	Diffusion Edited
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DO	Dissolved Oxygen
EI	Electron Impact
ESI-MS	Electrospray Ionisation Mass spectroscopy
FAME	Fatty Acid Methyl Esters
FT-ICR-MS	Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectrometry
HMQC	Hetronuclear Muluiple Quantum Coherence
HSQC	Hetronuclear Single Quantum Coherence
HDPE	High Density Polyethylene
HMW	High Molecular Weight
HPSEC	High Preformance Size Exclusion Chromatography
HS	Humic Substances
LO	Lake Ontario
LPS	Lipopolysaccharides
Li-Mt	Lithium Montmorillonite
LMW	Low Molecular Weight
MDLT	Material Derived from Linear Terpenoids
MWCO	Molecular Cut Off
Mont	Montmorillonite
NMR	Nuclear Magnetic Resonance
POC	Particulate Organic Carbon
POM	Particulate Organic Matter

PN	Peak Number
PG	Peptideglycon
PVDF	Poly Vinylindene Flouride
PUFA	Polyunsatturated Fatty Acids
PyGCMS	Pyrolysis Gas Chromatography Mass Spectrometry
Rt	Retention Time
Na-Mt	Soduim Montmorillonite
SOM	Soil Organic Matter
SPE	Soild Phase Extraction
TMAH	Tetramethylammonium Hydroxide
TPPI	Time Proportional Phase Incrimination
TOC	Totak Organic Carbon
TOCSY	Total Corellation Spectroscopy
UF	Ultra Filtration
UDOM	Ultra-filtrated Dissolved Organic Matter
UV	Utlra Violet
VFA	Volatile Fatty Acids
WISE	Water Information Systems for Europe
XRD	X Ray Diffraction

CHAPTER 1

INTRODUCTION

1.0 What is Dissolved Organic Matter?

Organic matter in natural waters exists as dissolved molecules, colloids and particulates and can be interconverted between these forms by precipitation, dissolution, sorption, desorption and aggregation (Perdue and Ritchie 2004). The dissolved fraction of organic matter can be divided into dissolved organic matter (DOM) and dissolved organic carbon (DOC). These two acronyms are often used interchangeably, however it is important to remember that there is a distinction between the two. DOM is incorporated into general discussions on dissolved organic matter whereas DOC is used when referring to specific results or carbon content. (McDonald et al. 2004, Hansell and Carlson 2002, Hiroshi Ogawa and Eiichiro Tanoue 2003). DOM in aquatic ecology is operationally defined as the dissolved phase of organic matter that can pass through a 0.45µm filter membrane, whereas DOC measurements can contain microscopic particulates <0.1 µm. However this is taken to be acceptable as particulates below 0.1µm do not tend to sink and are considered to be in the dissolved phase (Hansell and Carlson 2002).

1.1 Why is DOM significant?

DOM is one of the largest pools of carbon on Earth and plays an important role in the global carbon cycle. The amount of DOC in the ocean (~ 700 g x 10^{15} g) is approximately equal to the amount of CO_2 in the atmosphere (~ 750 x 10^{15} g) (Hansell, 2002) In one year the net oxidation of only 1% of the carbon bound in DOM would generate more CO_2 than that produced annually by fossil fuel combustion (Hansell and Carlson 2002). Significantly this oxidation can be brought about through the global warming phenomenon and subsequent rise in ocean temperatures.

In addition to DOM's contribution to the global carbon cycle, there are many other reasons for growing research into the characteristics of DOM in natural waters.

Traditionally DOM was considered to be resistant to microbial breakdown (refractory). However, it is now known that a portion of DOM can be utilized by the microbial community as an important energy and nutrient source (Aitkenhead-Peterson, McDowell and Neff 2002). Within many water bodies DOM can also effect light attenuation (both visible and ultraviolet light) and may limit the production of autotrophs. When present in high concentrations DOM shields aquatic organisms by absorbing harmful UV light (Blough and Del Vecchio 2002). The reactions of DOM with light may also reduce the oxygen levels in DOM rich waters, as the photochemical degradation of DOM consumes oxygen (Battin 1998, Morris et al. 1995, Moran and Zepp 1997). In addition, DOM can bind to trace metals affecting their speciation, solubility and mobility (Shiller 1997). DOM also affects aspects of water treatment which includes the formation of disinfection by-products such as trihalomethanes during disinfection (Wei et al. 2008, Rodrigues, Esteves da Silva and Antunes 2007) (Marhaba and Van 2000)).

Despite DOM's importance there is still much to be revealed regarding the composition of this complex environmental mixture and how its compounds vary between marine and freshwater environments. Globally, rivers transport approximately 0.25×10^{15} g of DOC annually into the ocean (Hedges, Keil and Benner 1997). These global discharges of riverine DOC are large enough to support the turnover of the entire pool of DOC in the ocean. However, the concentration of allochthonous derived DOC within the ocean does not correspond to these annual fluxes. Despite its refractory nature little is known about the quantities of allochthonous derived DOC that is rapidly degraded or persists in the ocean. The inference is that DOC is degraded, and modified in estuaries, on the contential margins or in the lower regions of the river itself (See section 1.6). In addition to transporting C in the form of DOC to the oceans, freshwater systems are active components of the global carbon cycle by storing terrestrial derived carbon in sediments and through the loss of CO_2 as emissions to the atmosphere. Cole et al (Cole et al. 2007) created a budget to highlight the role of freshwater systems in the global carbon cycle and analysis indicates that twice as much carbon is delivered from land to freshwater systems than is delivered to the ocean. They estimated that freshwater systems annually receive 1.9×10^{15} g C from terrestrial landscapes. Approximately 0.2×10^{15} g of C received by freshwater systems is buried in aquatic

sediments, 0.8×10^{15} g is returned to the atmosphere as gas exchange and 0.9×10^{15} g C is delivered to the ocean (See Figure 1.1).

Gas Exchange

1.9 x 10¹⁵ g C

Terrestrial Landscape

0.2 x 10¹⁵ g C

0.9 x 10¹⁵ g C
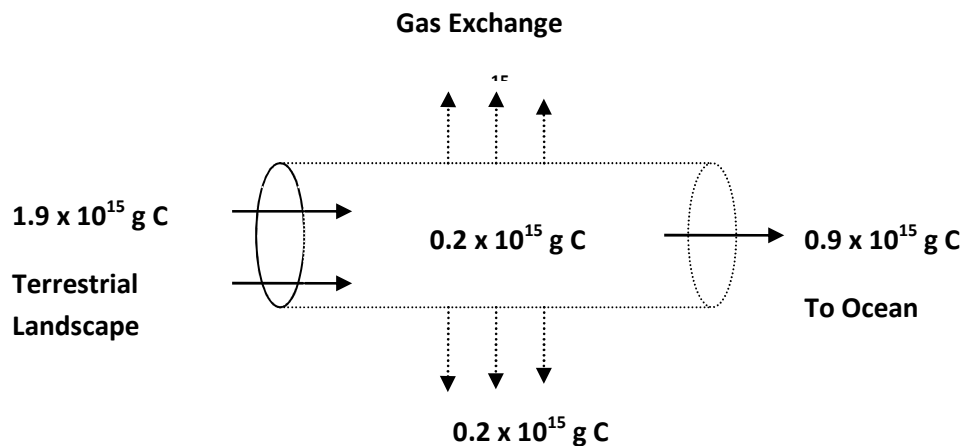
To Ocean

0.2 x 10¹⁵ g C

FIGURE 1.0 Schematic drawing of the role of freshwater systems in the global carbon balance (Adapted from Cole et al (Cole et al. 2007)

These results highlighted by Cole et al and others outline the "gaps" in the understanding of the flux's of DOC, and highlight the need for a further understanding of the composition and mechanisms transforming riverine DOC. This may also help explain its lower concentration within the marine environment (Hansell and Carlson 2002, Benner 2004).

1.2 DOM Composition

Despite the importance of DOM within natural waters knowledge of its molecular composition is far from complete. However, knowledge of the composition of DOM is increasing as a result of advances in isolalation and analytical techniques providing improved sensitivity for the characterisation of DOM.

Concentrations of DOC vary across different environments, for example concentrations vary within the ocean between 35-45 µg in deep oceans (>1000m) and 60-90 µg in surface waters (0 to 200m) (Hansell and Carlson 2002). Freshwater contains higher concentrations which vary from 0.5 to 50mg L^{-1} due mainly to the surrounding landscape. Amon et al (Amon and Benner 1994) isolated DOM from both oceanic and freshwater environments. DOM isolated from oceanic waters was

dominated (60-80%) by low molecular weight (LMW) material whereas DOM isolated from freshwaters was dominated (45-80%) by high molecular weight (HMW) material. This difference in molecular composition was suggested to be due to the origin of DOM isolated i.e. autochthonous or allochthonous. DOM within the ocean is mainly sourced from autochthonous sources and consists of several types of biochemical by-products such as carbohydrates (mono, oligo, and polysaccharides), amino acids, proteins and polypeptides, lipids and organic acids.

Freshwater DOM is predominantly influenced by terrestrial inputs. Terrestrial inputs contain more aromatic structures e.g. tannins and lignin's degradation products from plant materials. A major fraction of HMW DOM consists of humic substances. Humic substances (HS) are a large, operationally defined fraction of soil organic matter (SOM) and represent the largest pool of recalcitrant organic carbon in the terrestrial environment. It has traditionally been thought that HS consist of novel categories of cross-linked macromolecular structures that form a distinct class of chemical compounds (Stevenson 1994). However, advanced Nuclear Magnetic Resonance (NMR) has recently provided evidence that the vast majority of humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products and not a distinct chemical category as was traditionally thought (Kelleher et al 2003) . Furthermore, in another paper recently published (Woods et al 2009), the concept that extractable SOM is comprised mainly of humic materials was also challenged. The contribution of microbial biomass to SOM has been accepted to vary between 1-5% and is often associated with the labile, readily degradable component (Alef 1995). However, it was discovered that microbial presence far exceeds presently accepted values and large contributions of microbial peptide/protein are found in the HS fraction (50-69%). Considering the amounts of fresh cellular material in soil extracts, it would appear that contributions of micro-organisms in the terrestrial environment are seriously underestimated. As terrestrial inputs have such a large influence on the composition of DOM it is important to realise that their composition is still the subject of much debate but through the use of modern instrumentation we are beginning to see the actual make up of these complex mixtures.
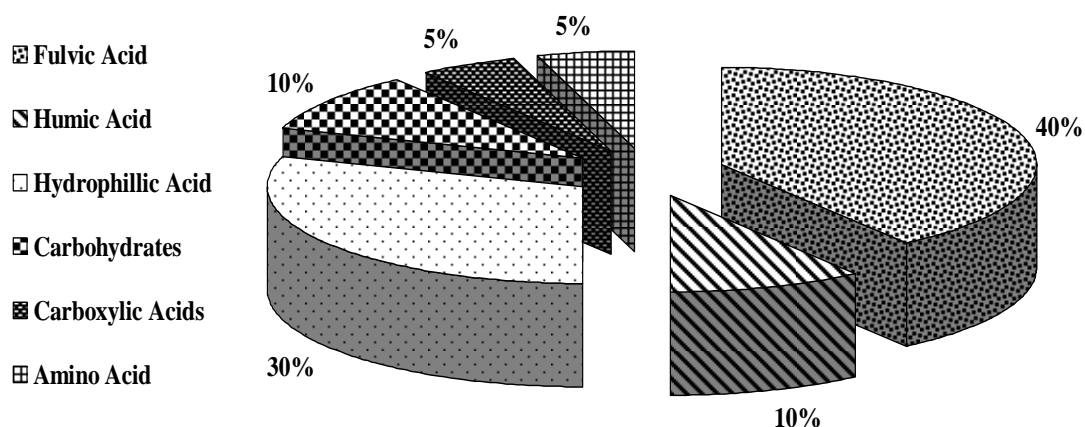
FIGURE 1.2 Major Classes of Dissolved Organic Carbon in freshwater (Adapted from Kaplan et al (Kaplan and Newbold 2002))

Although fresh and marine water DOM are considered to be two different carbon reservoirs due to their different cycling time, sources and fates, this only applies to the largest body of published literature in the area of humic substances (Hedges, Keil and Benner 1997). Humic substances only make up a small portion of the marine and freshwater DOM. The remaining portion of freshwater and marine DOM is still uncharacterised at a molecular or compound level. The uncharacterised portion may have cycling times, sources and fates different to humic substances. Repeta et al (Repeta et al. 2002) characterised high molecular weight (HMW; > 1kD) DOM in fresh and marine waters at a molecular level using spectroscopic techniques. Results indicated indistinguishable bulk and molecular properties between HMW DOM isolated from fresh and marine waters

Traditionally the chemical composition of DOM has been characterised by the analyses of concentrated DOM isolated from natural waters. A variety of isolation techniques have been used to isolate DOM from natural waters . However, variation in isolation techniques such as solid phase extraction and ultrafiltration may lead to compositional differences of DOM and therefore, may not be completely representative of DOM in its natural state and may be a limitation of some previous (Schumacher et al 2006, Schwede-Thomas et al 2005 and Hansell et al 2002). Direct

analysis of DOM in natural waters is therefore the more desirable as it can provide information on DOM in its natural state unaltered by isolation procedures. Spectroscopic methods such as fluorescence have been used to analyse unaltered DOM. However, resulting structural and compositional information can be limited. Nuclear magnetic resonance (NMR) is an emerging technique providing valuable information on the overall chemical composition of DOM. Lam et al (Lam and Simpson 2008) developed direct ^{1}H NMR spectroscopy of DOM in natural waters and NMR spectra were obtained directly from rivers, lakes, and oceans. To do this they employed water suppression techniques, facilitating the generation of NMR spectra of DOM directly from unaltered water samples without sample pre-treatment. This approach allowed detailed compositional information of the organic constituents of unaltered samples. The results are useful for the assessment of the impact that isolation, extraction and concentration techniques have on the composition of the sample. They reported that both river and lake samples produced natural abundance ^{1}H NMR spectra similar to spectra obtained from wetland water samples which were freeze-dried and re-dissolved prior to analysis. All spectra (river, lake and wetland) were recorded with identical parameters and therefore direct water analysis was identified as a possible way to elucidate compositional changes that may occur when isolation, preparation, and pre-concentration procedures are employed.

In a separate publication Lam et al.(Lam et al. 2007) investigated the major structural components of freshwater DOM using multidimensional NMR spectroscopy. DOM samples were isolated from Lake Ontario as using novel passive samplers. ^{1}H NMR data generated for DOM isolated from Lake Ontorio demonstrated major structural components which included aliphatics, carboxyl-rich alicyclic molecules (CRAM), carbohydrates and aromatics. Signals from larger macromolecular or aggregated species were emphasized by the use of diffusion editing, which retains only signals from aggregated or macromolecular species. Diffusion edited results of the ^{1}H NMR data produced a similar profile indicating that the structures present in the freshwater DOM are aggregates and/or macromolecular species. Further characterisation of chemical functionalities present in LO-DOM was achieved by employing 2D NMR. Heteronuclear multiple quantum coherence (HMQC) highlighted the presence of anomeric carbon from

carbohydrates, conjugated saturated aliphatics, aromatics, N-acetyl and/or O-acetyl, S-CH$_4$, aliphatics, CRAM, methylene from carbohydrates, and methine from carbohydrates. However, the methoxy group from lignin's which often is the most intense signal in soil organic matter is not present in LO-DOM. This significant result indicates that inputs from terrestrial origin are rapidly altered in Lake Ontario. Lam et al (Lam et al. 2007) showed that the DOM spectra obtained from their LO-DOM appears similar to spectra obtained from DOM isolated from the Pacific Ocean, described by Hertkorn et al.(Hertkorn et al. 2006). In this pioneering paper Hertkorn et al (Hertkorn et al. 2006) identified major components in marine DOM using 1-D and 2-D NMR in combination with Fourier transform ion cyclotron resonance mass spectroscopy (FT-ICR-MS). Hertkon et al (Hertkorn et al. 2006) described the three main components of marine DOM as carbohydrates, aliphatics, and CRAM. Employing the same method for integration and quantification of the ^{1}H NMR spectra, Lam et al found that 91% of the proton signals for LO-DOM comprise 17% carbohydrates, 12% aliphatics, and 62% CRAM (Lam et al. 2007). These results are similar to the quantities reported by Hertkorn et al (Hertkorn et al. 2006).

1.3 Freshwater DOM

DOM within freshwater systems (streams, rivers, lakes) influences many ecologically important processes such as light attenuation; it mediates the availability of dissolved nutrients and metals and plays significant roles in aquatic food webs. As such it is important to understand the processes that control its composition and concentration. An understanding of the factors that control the composition and concentration of DOC requires knowledge of the components of the landscapes that are responsible for its transport into freshwater systems, along with in-stream processes (Roulet and Moore 2006). The rate of introduction and concentration of DOM is influenced by the rate of production combined with the rate of sorption by mineral soils, and the flow path of water across the landscape and residence time within the system. DOM within a lake can be strongly influenced by differences in residence times. In lakes with short residence times in which the input rates of DOM is almost equal to the hydro-dynamically controlled output, DOM will be significantly influenced by physical process rather than biogeochemical processes (i.e. bacterial degradation) within the system. However, in lakes with water

residence times where the input and output of DOM are imbalanced, DOM is significantly influenced by bacterial degradation within the system (Mari et al. 2007).

1.3.1 Sources of Freshwater DOM

They are two major sources of DOM in freshwater, allochthonous and autochthonous. Allochthonous sources enter freshwater systems by the decomposition and subsequent leaching of organic litter from bogs, forests and wetlands. Allochthonous sources are to be the primary of terrestrial dissolved organic matter within freshwater systems. Most of these inputs enter the aquatic system through surface runoff passing through soil before entering surface water and results in a reduced portion entering into freshwater unaltered. However, the direct transfer of some organic material into a stream or lake is readily observed for example, when leaves fall into a stream or lake and DOM is leached. Hafner et al studied coarse woody debris as a source of DOM (Hafner, Groffman and Mitchell 2005). Over a one year period they studied litter leachate and coarse woody debris leachates in a lowland forest. Concentrations of DOC were found to be much higher in coarse woody debris leachates than in litter leachates (15nM and 1.6nM respectfully). In contrast O Connell et al on determining the rate of release of DOC leached from leaves, and woody debris in a forest in New South Wales Australia, reported that substantially more DOC was leached from leaves than from woody debris (O'Connell et al. 2000). Due to the refractory nature of terrestrially derived sources (allochthonous) it is commonly reported as being the main source of freshwater DOM (Wilson and Xenopoulos 2008). In contrast research by Bertilsson et al has indicated that autochthonous contributions to freshwater DOM are equal or if not more than allochthonous contributions (Bertilsson and Jones 2003b).

Autochthonous sources are produced within the freshwater system and are largely derived from algae and macrophytes. The algal source has been extensively researched (Bertilsson and Jones 2003a, Mannino and Harvey 2000a, Fogg 1971, Daniel et al. 2005, McKnight et al. 2001, Bade et al. 2004) as an autochthonous source of freshwater DOM due to the labile nature of molecules produced by algal and the large impact of these molecules on receiving waters (Farjalla et al. 2001,

Bertilsson and Jones Jr. 2003). Macrophytes which include, mosses, liverworts and vascular plants rooted or attached to the substrata of lakes, have received less attention as an autochthonous source of freshwater DOM. Early research in determining the source of DOM focused on the use of lipid biomarkers. Jaffe et al (Jaffé et al. 1995) investigated the lipid biogeochemistry of dissolved and particulate organic matter in a number of rivers in the Orinoco basin in South America to determine their origin. Aliphatic hydrocarbons, ketones, alcohols, triterpenoids and fatty acids were ubiquitous in all rivers. They reported that the distributions of the lipids identified were strongly influenced by phytoplankton composition and abundance as well as terrestrial inputs via soil runoff.
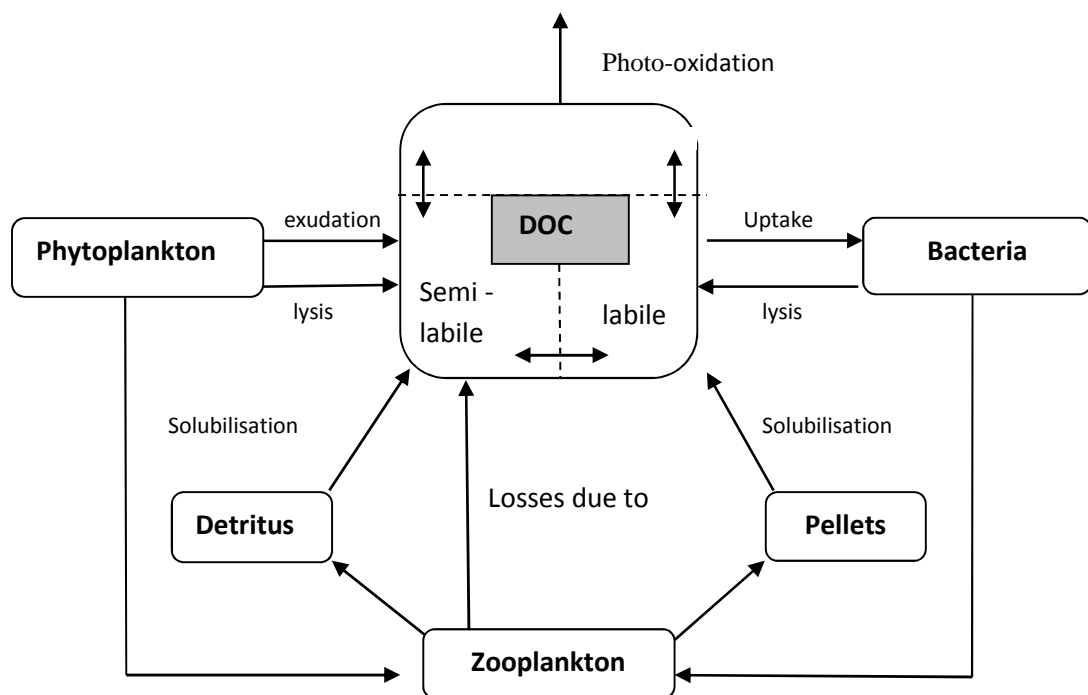
FIGURE 1.4 Schematic diagrams illustrating the four main production processes of autochthonous DOM (losses due to grazing, solubilisation, lysis, phytoplankton exudation). (Adapted from Hansell et al (Hansell and Carlson 2002))

Using chemical, spectroscopic, and isotopic analysis, Hood et al (Hood, Williams and McKnight 2005) investigated the source and composition of DOM during spatial and temporal changes in a mountain stream in Colorado. Samples were collected during a snowmelt season (May to September) at two sites along the stream, a low vegetated alpine site (downstream site) and a forested subalpine site

(upstream site). Concentrations of DOC varied during the snowmelt season at both sites. Concentrations were at their highest at both sites during early June, as the runoff from the snowmelt occurred and were at their lowest in September when the runoff reduced. However, concentrations were always 2-3 times greater at the downstream site (7.0 mg C l^{-1}) compared with the upstream alpine site (2.6 mg C l^{-1}). Isolated samples were fractionated into humic and non-humic fractions using XAD-8 Amberlite resins. Within the context of this paper (Hood, Williams and McKnight 2005) the humic fraction of DOC is referred to as humic acids while non-humic substances are referred to as transphillic acids. UV and fluorescence analysis indicated that the concentration of the fulvic acids decreased throughout the season which was consistent with the increase in humic substances derived from instream processes. Freeze-dried fulvic and transphillic samples from both sites were analysed for elemental and isotopic analysis. Samples from the alpine site were reported to have higher nitrogen content, depleted in ^{13}C, enriched in ^{15}N and had lower aromatic carbon content than samples isolated from the subalpine site. Overall this suite of chemical isotopic and spectroscopic analysis indicates that changes in DOM properties are the result of changes in the catchments source. This work highlights that isotopic and elemental analysis, when combined provide a useful tool in evaluating sources and transformations of DOM within freshwater systems.

Using an isotopic approach Helie et al (Hélie 2004) investigated the source of DOC and POC in the St Lawrence River, Canada from its source to its estuary. DOC samples and POC samples were collected on a bi-monthly basis from mid-1998 to mid-2003. They reported that DOC and POC had different origins, the POC samples analysed were dominated by terrestrial derived organic matter, while the DOC samples were found to be dominated by terrestrial derived organic matter with some evidence of aquatic POC influence in the summer. Additional ^{14}C activities of the sampled DOC were measured at the mouth of the St Lawrence River which enabled the research team to asses the age of DOC delivered to the estuary. Results show that the overall bulk of the DOC sampled is quite young indicating that DOC at the inlet to the estuary is derived from new organic matter from topsoil in the surrounding watershed (i.e. at the mouth of the river).

Bade et al (Bade et al. 2004) also used an 'isotopic approach' to investigate the sources and fates of DOM in lakes. Four whole-lake additions of ^{13}C-labeled DOM experiments were conducted to trace algal sources of carbon in lakes of differing trophic levels. Although DOC originally sourced from algal DOC is thought to be labile, Bade et al observed accumulation and persistence of ^{13}C labelled algal carbon in DOC analysed. By modelling isotopic results they were able to calculate the proportion of algal and terrestrial carbon within DOC, and estimate their fluxes to and from the DOC pool. The results conclude that algal was found to be the source of 20% of the DOC pool in two lightly coloured oligotrophic lakes. The same experiments were repeated the following year in one of these lakes under conditions of nutrient enrichment, and in a second humic lake. Within the nutrient enriched lake algae contributed to 40% of the DOC pool compared to 5% in the humic type lake. DOC from the above lakes was further analysed by elemental and spectroscopic techniques to confirm the levels of algal derived DOC. Results reported show increased levels of algal derived DOC in the nutrient enriched lake. Finally they compared the natural abundance measurements of δ^{13}C of DOC in 32 lakes to examine the contributions of both algal and terrestrial sources. Results revealed dual contributions of both terrestrial and algal sources to DOC.

1.3.2 Characterisation of Freshwater DOM

Understanding freshwater DOM at a molecular level is essential to further understand its role in the global carbon cycle as a major source of carbon flow to the ocean. Little is known about DOM at a molecular level. This deficit of information is mainly attributed to analytical difficulties arising from the complexity of DOM, high polarity and lack of non-invasive isolation and analytical techniques.

Gas-chromatography coupled with pyrolysis (PyGC/MS) (Schulten and Gleixner 1999, Lu et al. 2003), tetarmethylammonium hydroxide (TMAH) ((JANDL, SCHULTEN and LEINWEBER 2002)) and CuO degradation chemolysis (Benner and Opsahl 2001) have been used to chemically characterise freshwater DOM. Two of the techniques, PyGC/MS and chemolysis, are invasive and are selective in that only volatile compounds can be observed. Due to DOM's polarity and the destructive nature of the techniques only a small fraction of DOM may be

analysed using GC based methods. Advances in isolation and non-invasive analytical techniques such as (ESI-MS and NMR) provide greater insights into the molecular building blocks of DOM.

Kim et al (Kim et al. 200**3**) investigated the use of C_{18} solid phase disk extraction coupled with electrospray ionisation mass spectrometry (ESI-MS), for obtaining molecular level information of DOM from river water. The isolation technique can be achieved rapidly from acidified natural waters in remote sampling sites. They reported that UV-Vis absorbance and total organic carbon measurements show that over 60% of the DOM was recovered without the interference from salts (which can interfere in the ESI-MS process). NMR analysis indicated that the C_{18} isolated DOM had similar distributions of functional groups to the original DOM indicating that a large fraction of DOM was not altered by C_{18} solid phase disk extraction. Furthermore, results obtained from high resolution ESI-MS analysis of DOM at a molecular level revealed that a series of molecules differed by exact masses ($-H_2$, -0, or $-CH$) indicating a possible homologous series of structures.

Using Fluorescence, UV absorption, ^{13}C NMR spectroscopy and high performance size exclusion chromatography (HPSEC), Schwede-Thomas et al (Schwede-Thomas et al. 2005) compared the structural characteristics of DOM isolated by solid phase extraction (SPE), C-18 cartridges, ultrafiltration (UF), and XAD chromatography. Samples were isolated from the Suwannee River, Georgia, USA and McDonald's Branch, New Jersey USA which represents waters that are strongly influenced by terrestrial derived precursors. They reported that each method isolates a unique component of the DOM resulting in materials that differ in structure and reactivity. Samples isolated from Pony Lake Antarctica represented waters influenced by algal/microbial precursors. Slight variations between isolation methods were observed for terrestrial derived samples when analysed by UV and fluorescence spectroscopy. However, samples isolated from Pony Lake were reported to exhibit greater variability when analysed by these methods. ^{13}C NMR analysis showed structural differences between samples for all isolation techniques. HPSEC also revealed viability between samples isolated using C-18 that exhibited highest molecular weights. They therefore suggested that each method isolates

different fractions of DOM that can only be defined by a multi-analytical approach. These results highlight the need for new analytical approaches to analyse DOM in its natural state. Nuclear magnetic resonance (NMR) has proven to be very useful approach for determining the overall chemical composition of unaltered DOM (Lam and Simpson 2008). Electrospray-ionisation mass spectrometry (ESI-MS) is a relativity new technique for characterising polar organic compounds. ESI-MS has a low ionisation voltage which allows the characterisation of intact polar molecules (50 to 100,000 amu); and can therefore provide compound level information on mass, abundance and functional groups (50 to 100,000 amu). DOM compounds in water are generally polar, therefore ESI-MS has potential for the characterisation of a large fraction of unaltered DOM in freshwater

Seitzinger et al (Seitzinger et al. 2005) investigated the bioavailability and molecular level characterisation of DOM in two streams using ESI-MS. Despite the complexity of DOM, compositional similarities were reported with 70% of masses detected occurring in both streams. Seitzinger and his team carried out twelve day microbial decomposition experiments to determine which portion of DOM within the streamwater was biologically degraded. Results determined by ESI-MS analysis of both streams indicated that 40% of masses detected decreased in concentration after twelve days, <5% increased and 55% did not change. They reported that the compound-level microbial decomposition of DOM in the two streams suggests that microbial utilisation of DOM in freshwater is repeatable and therefore predictable.

Using different analytical methods Schumacher et al (Schumacher et al. 2006) studied the chemical composition and isotopic signature of ten aquatic DOM samples in five boreal forest catchments in Scandinavia. The DOM was isolated using reverse osmosis in spring and autumn. The isolated DOM samples were analysed by elemental analysis, FT-IR spectroscopy, solid state CP-MAS ^{13}C-NMR spectroscopy, synchrotron-based C-1s NEXFAS (Near edge X-ray absorption fine structure) spectroscopy and radiocarbon (^{14}C) analysis. There were no significant differences in DOM samples from spring and autumn, however minor variations in chemical compositions were observed between DOM samples isolated from the five catchments. Comparing, FT-IR, ^{13}C-NMR and synchrotron-based C-1s NEXFAS

with spectra obtained from fulvic and humic acid standards, Schumacher found that all samples contained less aromatic and phenolic carbon and were richer in O-alkyl carbon. All DOM samples were enriched with ^{14}C relative to a 1950 oxalic acid standard indicating that DOM contained significant amounts of organic compounds younger than 50 years old. δ^{13}C measurements ranged from -25% to -32% for all DOM samples indicating that the DOM isolated was of terrestrial rather than aquatic origin. This is as expected as C3 plants are predominant in the boreal catchments studied and have an average δ^{13}C value of -27% (range -22 to -32%) providing validation of the method used for future work in determining the origin of DOM within an freshwater system and how seasonal landscape and land use can influence the composition of aquatic DOM.

1.3.3 Land use and landscape influences on Freshwater DOM

Landscape and land use can influence concentrations and chemical compositions of DOM by influencing hydrological connections between land and receiving waters. The landscape of a particular watershed consists of an array on different land use and land cover that involves a multitude of plant and animal species. Water and its dissolved and suspended load follows a number of stochastic pathways through these zones as it moves across the landscape from uplands to receiving water (i.e. lakes, rivers and oceans). Uptake, transformation and release of dissolved and suspended matter occurs within the water as it travels across the landscape is altered due to path-specific material dynamics (Peterson et al 2002). Boyer et al (Boyer et al. 1997) investigated how variations, such as lateral versus vertical, occur in flow paths through a landscape (See figure 1.5).
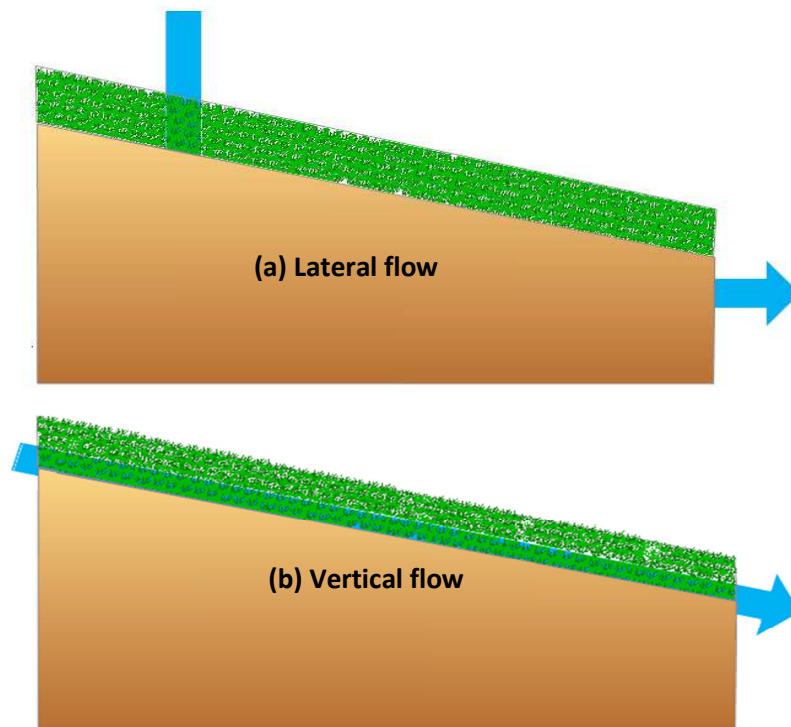
FIGURE 1.5 Conceptual models of (a) lateral and (b) vertical flow paths across a landscape.

This variation of lateral versus vertical flow can control differences in dissolved organic carbon. This paper reports that these flow path variations can result in large changes in the concentration of DOC delivered to surface waters over short time intervals altering the aquatic chemistry of the receiving water. Bogland, forest, grasslands and soil are the principle sources of DOC within the landscape (see figure 1.6). Deforestation, intensive agriculture, increased human activity and presence of riparian zones within the landscape can directly or indirectly influence the concentration and composition of DOM.

Gregel et al (Gergel, Turner and Kratz 1999) quantified the extent of landscape influence on DOC concentrations in a series of Wisconsin lakes and rivers. The selected area was influenced by a large proportion of wetlands in the watershed. Using a series of chemical analysis, spatial data and statistical analysis Gergel et al (Gergel, Turner and Kratz 1999) measured the proportion of wetlands in the watershed at different distances from the lake, to quantify the extent of the landscape influence on DOC concentrations in lakes and rivers.
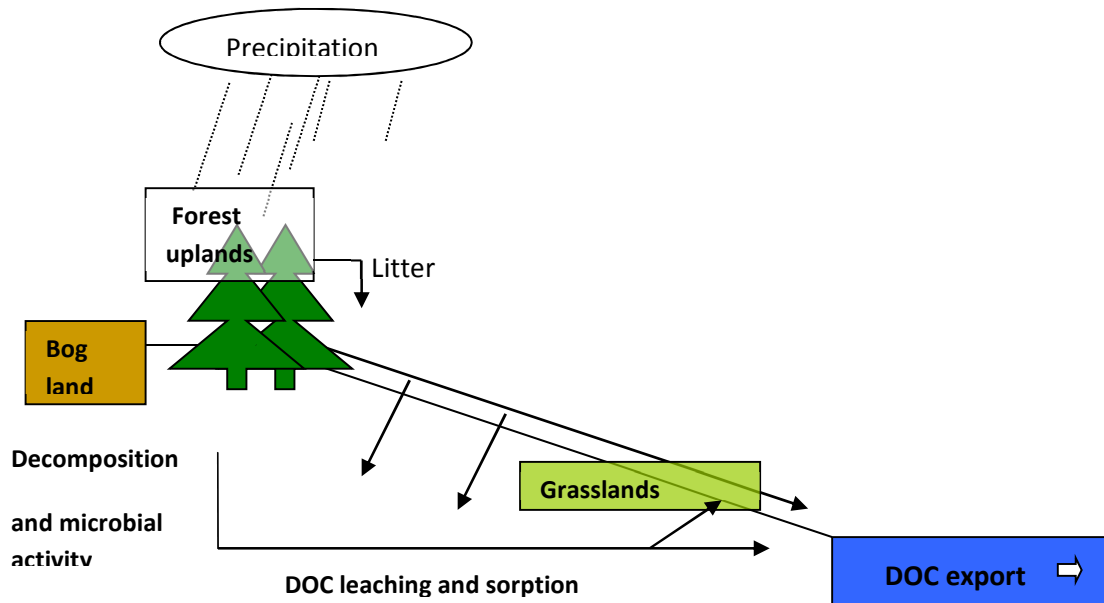
FIGURE 1.6 Pathway of DOC through the landscape (Adapted from Roulet et al (ROULET and MOORE 2006))

The wetlands were sampled at increasing distances from the rivers and lakes until the total watershed area was reached. The proportion of wetlands measured in near-shore riparian zones (25-100m) revealed r^2 values which varied between 1-3%. However, for rivers the amount of wetlands measured in the total watershed explained more about the variability in DOC concentrations than wetlands measured in near-shore riparian zones. In addition, the amount of wetlands in the watershed also had seasonal influences on DOC concentrations in rivers with greater variance observed in autumn than in spring in agreement with Wilson et al (Wilson and Xenopoulos 2008). Although Gregel and others have identified the proportion of wetlands within a watershed as a major predictor of DOC concentrations in lakes and rivers, Wilson et al (Wilson and Xenopoulos 2008) identified several other landscape characteristics with equal or greater predictive strength than that of wetlands. Wilson et al (Wilson and Xenopoulos 2008) studied DOC concentrations in 32 streams with varying agricultural land-use intensities within their catchments in south central Ontario. Streams in this region were identified as better predicators of DOC concentrations than the proportion of wetlands in the watershed. Poorly drained soils accounted for up to 67% of the variation in DOC concentration observed, which was 2.6 times greater than that of wetlands. Wilson et al (Wilson

and Xenopoulos 2008) also reported agricultural land-use did not strongly influence the concentration of DOC within the area. However, alterations in land use (e.g. urbanisation and deforestation) alter concentration of DOC entering the streams within the study.

Frost et al (Frost et al. 2006) also investigated the influence of contrasting landscape characteristics on DOM concentrations and their physicochemistry in a large heterogeneous watershed. DOC concentrations varied from 4 to 35mg C L $^{-1}$ among the 60 steams studied from the Ontonagon River watershed in northern Michigan, USA. Variations in DOC concentrations between each stream correlates to differences in watershed area, watershed slope and type of land cover within the stream watershed (e.g. lakes, total wetlands, emergent wetlands, and lowland conifers forests). In particular the percentage of the watershed area found around lakes was found to be an important predictor of DOM concentration. DOM physicochemical properties, molar absorptivity (280nm) and average molecular weight, varied amongst streams. Streams with high DOM concentrations also had higher molecular weight components and molar absorbtivity at 280nm than streams with low DOM concentrations. However, less variation was observed in DOM physicochemical properties than in DOM concentrations. Results indicate that the type of landscape within a watershed has a greater influence on the concentration of DOM within a stream than its physiochemical properties. This stronger correlation between landscape and DOM concentration indicates that in-stream processes (DOM loading, degradation, and dilution) rapidly alter the structure but not the concentration of DOM transported within the watershed. It was concluded that the DOM concentration and physicochemical properties are influenced by many biogeochemical processes. These processes, which vary across large watersheds, control DOM input, transportation and degradation ultimately changing the composition of DOM.

Findlay et al (Findlay et al. 2001) studied a set of near stream flow paths in pasture, native forest and exotic pine plantations in New Zealand to determine their effect on DOC. They reported that the quantity and bioavailability of DOC varied in flow paths (groundwater, surface and subsurface flow) with different land uses.

Finlay et al (Findlay et al. 2001) investigated the flow paths of pasture sites, groundwater seep and two flow paths from these seeps to a stream, one across the soil surface (surface flow) and the second between the underlying bedrock and the lower boundaries of the soil (subsurface flow) They concluded that higher concentrations of amino acid were observed in subsurface flow than groundwater samples. Amino acids constituted 17% of groundwater DOC compared to 5% in pasture subsurface waters. They also showed how different riparian zones can influence DOC by altering exposure to UV radiation through shading. Photolytic effects on DOC concentrations were not significant following a 2hr exposure of water from all flow paths; however a significant decrease (37%) in fluorescence characteristics was observed in pasture subsurface waters in comparison to waters from the native forest catchments which experienced little or no change in fluorescence. In contrast bacterial growth and activity was higher in native catchment waters exposed to sunlight, whereas there was no light effect on bacterial growth from pasture subsurface waters.

Larson and Frost et al (Larson et al. 2007) examined the ability of upstream lakes to alter DOM quantity and the absorbance of UV radiation in streams. They assessed 15 streams with upstream lakes and 17 streams without upstream lakes located in northern Michigan. Results reported showed a significant difference between streams with or without lakes. The concentration, UVB absorbance and molar absorbivity of DOC in streams with lakes were significantly lower than in streams with no lakes. This reduction in concentration which results in a reduced ability to absorb UV-light may be due to water residence time in upstream lakes. It is postulated that this increase in water residence time could increase opportunities for DOM processing (i.e. bacterial degradation) resulting in the observed effect. They also reported that upland lakes within the sample area had the ability to act as a sink for allochthonous DOM.

Using a series of chemical, biological, and isotopic tracers, Bernardes et al (Bernardes et al. 2004) investigated the forms and composition of particulate (fine and coarse) and ultra-filtrated dissolved organic matter (UDOM) as an indicator of land use changes. Samples were taken from five sites along Ji -Parana River and

eight sites in six of its tributaries at the southern limits of the Amazon lowlands. To test for pasture derived signals in riverine organic matter, C_4 signals obtained from UDOM sampled were compared to C_4 signals from leaf and pasture soils. The coarse particulate matter and UDOM fraction were identified as being derived from fresh leaves in lowland forest. The fine fraction was identified as being mostly influenced by a mineral soil phase. The coarse fraction was least degraded in contrast to the UDOM fraction which was reported to be the most degraded of the three fractions (fine, coarse and UDOM). Compositional differences were also observed among the 3 fractions along the Ji -Parana River. The UDOM fractions contained higher $\delta^{13}C$ values than fine the coarse POC when the surrounding landscape was covered by pasture soils. This indicates that the higher the pasture area the greater the possibility that the C_4 derived organic matter signal will be detected in the UDOM due to its faster cycling time. It was proposed in this work that when assessing the land use effect on organic matter composition (within the Ji -Parana River basin), UDOM cycling times must be taken into account as replacement of forests by C_4 pastures has taken place in the last 30 to 40 years and has already altered the composition of riverine organic matter.

1.4 Marine DOM

The majority of DOM within the ocean is produced within the surface of the ocean and thus is considered "fresh" DOM. This DOM is considered to be the bioavailable fraction of DOM in the ocean and a large portion of this bioavailable fraction is rapidly consumed by zooplankton grazing, microbial exudation and cell lysis (see figure 1.3 section 1.2). Due to its rapid turnover times this bioavailable fraction of DOM does not accumulate rapidly in the ocean and is therefore found in low concentrations. Conversely, deep ocean DOM is considered very resistant to microbial degradation (refractory). Although all sources of DOM within the deep ocean have not yet been identified, the hypothesis is that it may be sourced from the residue of microbial processes and sedimentary release. Marine DOM can have an average ^{14}C age of 6000 B.P., which is approximately 7-8 times the oceanic cycling time (Ogawa and Tanoue 2003).

1.4.1. Source of Marine DOM

 The concentration, composition and bioavailability of DOM within the ocean is variable depending on its source. Benner et al (Benner and Kaiser 2003) investigated the abundance of amino sugars in Ultra-filtered DOM, POM and in a variety of marine organisms. Analysis was performed on samples isolated from a number of ocean basins at various depths. Amino sugars, glucosamine and galactosamine were abundant in all samples, and accounted for 2.5% of the carbon present. Nitrogen accounted for 7.1% of N in UDOM isolated from surface waters within the oceans sampled. Glucosamine and glactosamine were also present in all organisms analysed. Due to their many potential sources the exact source could not be determined in this study. However, low concentrations of chitin, a structural polymer of glucosamine produced by a large variety of marine organisms, indicate that they are not a primary source of UDOM and POM. In addition, muramic acid, an amino acid only found in peptidoglycan, was found in all UDOM samples in low concentrations but was relatively abundant in POM, indicating that bacterial detritus were a major component in marine POM and a minor component of UDOM. Kawasaki et al (Kawasaki and Benner 2006) investigated the heterotrophic bacterial growth and chemical composition of DOM produced by bacteria from marine and freshwater environments. Bacterial growth was monitored during experiments using natural bacterial assemblages collected from aquatic ecosystems with artificial media containing glucose as the sole carbon source. Glucose was consumed rapidly and directly produced refractory DOM growth in all experiments. The percentage yields of DOM produced from bacteria ranged from 14% to 31%. These results are in agreement with Ogawa et al (Ogawa et al. 2001) who also investigated the production of DOM by bacteria. Using glucose or glutamate as the sole C sources, they found that natural assemblages of marine bacteria rapidly consumed labile compounds (glucose, glutamate) and produced refractory DOM that persisted for more than a year. Percentage yields of DOM produced from glucose and glutamate incubations ranged from 22 to 35%. Ogawa et al (Ogawa et al. 2001) concluded that of the bacterial derived DOM only 10 to 15% was identified as hydrolysable amino acids and sugars typical of the concentrations found in marine DOM (Tanoue et al. 1995, McCarthy, Hedges and Benner 1998).

Wetz et al. (Wetz and Wheeler 2007) studied the release of DOM from five marine diatom species. Release rates of DOC varied from each species during different growth stages. All species showed release rates significantly higher in transition or exponential growth than during stationary growth. On average *C. decipiens* released 21% of its fixed C as DOC, whereas the remaining four diatoms released 5% of fixed C as DOC. Wetz also noted that DOM produced by some diatom species with benthic life history adhere to filters and are measured as POM which can pose problems when measuring "true" DOM. Wetz et al (Wetz et al 2007) therefore reported results for Chaetocerous, a species with no benthic life history, and thus concluded these results to be accurate. These observations highlight the complexity in determining the source and fate of DOM within the marine environment. Wakeham et al. (Wakeham, Pease and Benner 2003) investigated hydroxy fatty acids in UDOM from the equatorial Pacific Ocean, Gulf of Mexico and North Sea to evaluate potential inputs from bacterial membrane. Several samples contained β-hyroxy acids that range from C_{10}-C_{18} dominated by β-12:0, β-14:0 and β-16:0. This indicates that hydroxyl fatty acids in marine DOM could derive from membrane lipopolysaccarides (LPS) of gram negative bacteria.

1.4.2 Characterisation of marine DOM

Aluwihare et al.(Aluwihare, Repeta and Chen 1997) investigated the chemical characterisation of macromolecular DOC in surface seawater. Macromolecular DOC was recovered from several sites in the Atlantic and Pacific Ocean by ultrafiltration and dialysis. Proton NMR of ultrafiltered dissolved organic matter (UDOM) displayed similar spectra for all samples analysed. The abundance of major biochemical's; carbohydrates, acetates and lipids in (UDOM) was relatively consistent in all samples (see table 1.5.2.1), and had an average carbohydrate/acetate/lipid ratio of 8/1/1. These results suggest that chemical composition of DOM in oceanic surface waters exhibits little variation between ocean basins.

Sample	Average DOC conc. (μM)	Carbon (relative %)		
		Carbohydrates	Acetate	Lipid
Atlantic Ocean	104	79	10	10
Pacific Ocean	Not Reported	82	11	8
Average composition		80	10	9

TABLE 1.1 DOC concentration and relative abundance of biochemical's in UDOM obtained from several sampling points in the Atlantic and Pacific Oceans adapted from (Aluwihare, Repeta and Chen 1997)

Monosaccharide analysis of hydrolysed UDOM from surface and deep water samples also yielded similar compositions. These observations indicate that a significant fraction of DOC in seawater consists of a group of closely related oligosaccharides, formed by direct biosynthesis, which may persist for long periods of time. Using laboratory culture experiments Aluwihare et al.(Aluwihare, Repeta and Chen 1997) also found that macromolecular DOC produced during decomposition of algae exudates had similar compositions and linkage patterns to the oligosaccharide portion of UDOM. However, Aluwihare emphasised the need for further analysis to fully assess algal production of DOC, and its accumulation in seawater.

In a later paper using NMR spectroscopy and molecular level analysis, Aluwihare et al.(Aluwihare and Repeta 1999) compared the chemical characteristics of extra cellular HMW DOM produced by marine algae to HMW DOM isolated from seawater. Three species of marine phytoplankton 1. *Thassiosira weissflogii,* 2. *Emilliana huxleyi and* 3.*Phaeocystis sp.*, were grown in nutrient enriched seawater that was pre-filtered to remove natural HMW DOM. All species were found to produce DOM rich in polysaccharides. Species 1 and 2 produced polysaccharides

similar to acyl hetero polysaccharides (APS), a major constituent of naturally occurring HMW DOM in seawater. They showed that different polysaccharides within these exudates showed different rates of degradation. The polysaccharides produced that were similar to APS exhibited slower degradation rates relative to other polysaccharides. Their results are in agreement with Carlson et al.(Carlson, Ducklow and Hansel 1998) and indicate that APS within the surface water can be present due to direct algal contribution, and their accumulation within seawater may be due to its inherent resistance to microbial degradation.

Hertkon et al.(Hertkorn et al. 2006) characterised refractory carboxyl rich molecules (CRAM) in marine ultrafiltered dissolved organic matter (UDOM) by employing NMR. CRAM are distributed throughout the water column and were reported to be the most abundant identified components if deep ocean DOM. CRAM is defined by a complex mixture of carboxylated and fused alicyclic structures with a carboxyl-C: aliphatic-C ratio ranging from 1:2 to 1:7. CRAM are expected to constitute a strong ligand for metal binding, and multiple coordination across cations could promote aggregation due to marine gel formation with DOM, affecting its reactivity and bioavailability of nutrients and trace metals. Molecular formulae acquired from FTCIR mass spectra data indicated that the CRAM isolated by Hertkon et al. was derived from biomolecules with structural similarities to sterols and hopanoids.

Using Electrospray ionisation Fourier transform ion cyclotron resonance mass spectroscopy (ESI FT-ICR-MS), Koch et al (Koch et al 2005) characterised and compared molecular formulae of algal derived DOM from the Weddell Sea in Antarctica and Terrigenous DOM from saline pore water of a tropical mangrove area in Northern Brazil. DOM was isolated from both marine and terrigenous waters using solid phase extraction. Several thousand molecular formulas were identified in the mass range of 300 – 600Da. These molecular formulas were reproduced in elemental ratio plots. Mangrove derived DOM displayed a higher number of unique elemental compositions exclusive to mangrove DOM compared to marine DOM. However, approximately one third of molecular formulas present in marine DOM were also present in mangrove derived DOM. These apparent similarities indicate

that independent to the origin of organic matter, degradation processes ultimately lead to similar structural components. Koch et al also found that there was no significant difference in elemental composition between samples from the surface and deep water DOM. In a later paper Koch, B.P. et al (Koch et al 2005) combined reversed phase liquid chromatography with FT-ICR-MS to achieve a more detailed structural characterisation of marine dissolved organic matter. With the addition of reverse phase chromatography they observed enhanced separation when: 1. The aqueous phase does not degrade over time and is 100% buffer free- water, 2. Resolution of water soluble compounds is improved when water eluent is adjusted to pH 7 and 3. Separation of polar compounds is improved when initial flow rate was maintained at a low level. Differences in peak distributions were observed between both samples analysed. A number of peaks detected in DOM obtained from sea ice were absent from samples obtained from Antarctic and Wendell deep water. Samples were separated into four fractions and analysed by FT-ICR-MS to assess the quality of separation at a molecular level. A large group of molecular formula obtained were identified in specific fractions. Differences in molecular formula observed between DOM isolated may be due to the origin of the samples. Sea Ice is considered to have a relatively fresh source of DOM as it hosts a multitude of different organisms, whereas Antarctic and Wendell deep waters exhibit old organic matter signatures. Koch et al (Koch et al 2005) also reported that differences in molecular formulae specific to each fraction may have potential as biomarkers for DOM, highlighting the need for further target orientated analytical techniques to determine the origin and source of DOM.

1.5 DOM in Estuaries

Rivers are the main conduits for the transport of DOM and POC from land to sea. Globally rivers transport an estimated 0.9gt C of DOM and POC annually (Cauwet 2002a). This flux is comprised primarily of degraded vegetation that enters the aquatic ecosystem through leaching and physical incorporation. The amount of DOM delivered to oceans is sufficient to support its turnover and enough POC to account for all the organic carbon being buried in marine sediments. However, the concentration of terrestrial derived DOM within the ocean does not correspond to the continental fluxes (Hedges, Keil and Benner 1997, Schlünz and Schneider 2000). Despite its refractory nature the hypothesis is that a large fraction of terrestrial

derived DOM is degraded upon mixing with seawater (Cauwet 2002b). Physical-chemical processes such as flocculation and photooxidation within the estuarine mixing zone can alter the structures and quantities of both DOC and POC of terrestrial derived DOM (Benner. 2004 and Cauwet 2002). Estuaries and deltas therefore provide an invaluable insight into the fate of terrestrial organic carbon as it enters the sea. Lignins, which are unique to vascular plants, are recalcitrant in nature have proven to be unique biochemical tracers employed to asses the fate of terrestrial organic matter in marine areas (Benner 2004),(Mannino and Harvey 2000b, Spencer, Aiken, Wickland, Striegl and Hernes. 2007, Engelhaupt and Bianchi 2001)

Louchouran et al (Louchouarn, Lucotte and Farella 1999) studied a series of sediment samples taken along a land to sea transect along the St Lawrence system, using a combination of molecular (lignin) and isotopic signatures of sedimentary organic matter. They observed a decreasing gradient in terrigenous organic matter. Assessing all data available on lignin derived oxidation products from riverine, shelf and deep basin sediments, along with riverine and marine suspended particles, they estimated a global mass balance. This mass balance suggests that almost half the annual riverine flux is degraded, the other half accumulating within shelf and slope sediments. They concluded that lignin does not behave predictably in the marine environment but supports some organic matter degradation.

These findings were further supported by Gordon and Goni et al (Gordon and Goń i 2004) on their study of the Mississippi and Atchafalaya river margins. They examined sediment samples across the Mississippi and Atchafalaya river margins by using; 1.molecular biomarkers (lignin phenols) 2.elemental 3.isotopic, and 4.mineral surface area analysis. From this study they were able to assess the composition and magnitude of the organic matter deposited from riverine discharges. They found that the concentration of lignin decreased from 61 % on the inner shelf (water depth <10m) to 37% on the outer shelf (water depth 10m-200m) and finally 2% on the outer shelf. The initial deposition of POC, primarily composed of plant debris, explains this dramatic decrease of lignin content as they are the main source of lignin. The application of lipid biomarkers can further differentiate sources of organic matter. Saliot et al (Saliot et al. 1991) investigated lipid biomarkers in POC

from the Danube delta to the North-eastern Black Sea. Over a series of nine sampling stations, Saliot compared concentrations of different lipid biomarkers prominent in TOC from the river mouth (station 1) to the amount found in open seawaters (station 9). He recorded a decrease in all vascular plant biomarkers along the 9 sampling stations. Sterols such as *n*-alkanols, and *n*-fatty acids showed a decrease five times greater than that of *n*-alkanes (4%). Saliots findings indicate that n-alkanes are the most recalcitrant within estuarine systems, making them valuable extractable lipid biomarkers for tracing the fate of TOC as it enters the ocean.

Abril et al (Abril et al. 2002) assessed the behaviour of dissolved organic carbon in nine contrasting European estuaries. In addition to terrestrial organic carbon, Abril assessed the contribution of organic matter from domestic, agricultural and industrial wastes. Results obtained suggest that the oxidation of POC within the estuaries was significantly influenced by that residence time, liability increasing with increased pollution levels, and the source. This is in agreement with Saliot et al's (SALIOT et al. 1991) findings who found that DOC concentrations usually decreased with increased salinity, possibly due to flocculation. Sholkovitz et al (Sholkovitz and Gieakiz 1971) demonstrated that up to 11% of DOC derived from rivers rapidly flocculated with increased salinity. Assessing all POC data enabled Abril et al (Abril et al. 2002) to estimate the loss of contential POC. Results reported showed a decrease in POC within all estuaries. They concluded that the majority of POC was mineralised in all estuaries. However, the rate that this mineralisation occurred was influenced by three factors: 1.The origin of the POC (i.e. anthropogenic or allochthonous) 2. The residence time of particles within the estuarine zone and 3. Lability, which increased with increasing pollution levels. However, they emphasised the need for parameters such as long-term bioassays, isotopic tracers and/or elemental composition of DOC to further assess the sources and sinks of DOC in estuaries.

Middleburg et al (Middelburg and Herman 2007) examined the same nine contrasting European estuaries using elemental and isotopic composition to examine the distribution of total organic matter. Their findings suggested that estuaries with low residence time showed evidence of low POC concentrations with variable

organic carbon contents and had consistent δ^{15}N values and C/N ratios. Tidal estuaries showed evidence of high concentrations of suspended matter with consistent organic matter contents and C/N values.

Using a multi-tracer approach Mc Callister et al (McCallister et al. 2006) used lipid biomarkers, elemental ratios, and stable carbon isotopes (δ^{13}C and δ^{15}N) to assess the source and distribution of dissolved and particulate organic matter along the York River, Virginia USA. Isotopic signatures for HMW (High molecular Weight) DOM in freshwater were different to POM in freshwater and signatures found in HMW DOM with increasing salinities. To further investigate source contributions Mc Callister et al used lipid biomarkers. Contributions of polyunsaturated fatty acids (PUFA) to HMW DOM were nominal in contrast to that of POM. POM showed characteristic contributions from labile compounds indicative to phytoplankton/zooplankton. With FA in HMW DOM reflecting bacterial and vascular plant signatures. The difference in composition of HMW DOM and POM indicate the variable degrees of susceptibility of terrigenous POM and HMW DOM to physico-chemical and biogeochemical breakdown. These degrees of susceptibility may ultimately regulate the persistence of TOM within an estuary.

Despite the extent of research carried out on DOM, the chemical nature, variability and transformations are still poorly understood. Progress in studying the chemical composition of DOM in both fresh and marine environments has been hindered by its complexity and lack of suitable techniques for isolating and concentrating DOM for subsequent characterisation. The research presented here focuses on; 1) the optimisation and development of novel passive sampling techniques for the concentration of DOM, 2) the development and optimisation of analytical techniques to study the complex mixtures in DOM and 3) The characterisation and dynamics of marine and freshwater DOM.

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CHAPTER 2

COMPOSITION OF DISSOLVED ORGANIC MATTER WITHIN A LACUSTRINE ENVIRONMENT

2.0 INTRODUCTION

DOM from fresh to marine water is not only important in the global carbon cycle but also plays an important role in the enhanced solubility, bioavailability and fate of chemical contaminants and their global transport. Despite this importance, there is still much to learn about the chemical composition of freshwater DOM and how chemical constituents vary worldwide, and between freshwater and marine environments. Landscape and land use within a watershed can influence the concentrations and chemical composition of DOM (Frost, et al. 2006). Watershed properties such as riparian zone (type and condition), geology and the vegetation contained within a watershed can affect the structure and function of aquatic ecosystems and DOM by influencing the hydrological connections between lands and receiving waters (Gergel, Turner and Kratz 1999).

This study was undertaken to establish spatial and temporal variations in the major structural components of DOM at 13 sampling points throughout Lough Derg, the largest lake in the Shannon catchment. Variations in DOM as a result of the watershed influence, variations with season, landscape and land use were monitored over a period of 8 months. Water quality parameters at each sampling site

(temperature, pH, conductivity, D.O., COD, and TOC, orthophosphate, F⁻, Cl⁻, $\underline{NO_3}^-$ and SO_4^-) were also monitored during the sampling period. This data will be used to further our knowledge of the structures that comprise DOM and assess the relationship between water quality and composition and concentration of DOM. Isolation of DOM from Lough Derg was performed using a passive sampler system at thirteen sampling points around the lake, from July to September 2007 and January to March 2008. 1D H and 2D Heteronuclear Single Quantum Coherence (HSQC) NMR was then employed to assess the chemical characteristics of DOM throughout the lake system. In recent work, one and two dimensional solution-state NMR spectroscopy was employed to show that major structural components of lake freshwater include carboxyl-rich alicyclic molecules (CRAM), heteropolysaccharides, and aromatic compounds (Lam, et al. 2007). These components were first reported and are consistent with those identified in marine DOM (Hertkorn and Kettrup 2005). Furthermore, it has been tentatively suggested that CRAM may be derived from cyclic terpenoids (Lam, et al. 2007). However, it is not clear whether these precursors are of terrestrial or aquatic origin or whether transformations proceed via biological and/ or photochemical processes. These components were first reported, and are consistent with those identified, in marine DOM. Furthermore, it has been tentatively suggested that CRAM may be derived from cyclic terpenoids. However, it is not clear whether these precursors are of terrestrial or aquatic origin or whether transformations proceed viabiological and photochemical processes. Traditional methods of DOM isolation require large sample volumes to overcome the low concentration in natural waters or are laborious and time consuming. Sampling is often carried out over just 1 or 2 days, which is unlikely to be long enough to provide a representative sample of the area. The samplers employed in this study were deployed over a 4-week period and provide a more representative material that is less susceptible to specific daily fluxes. Another advantage of using passive samplers of this kind is that filtration is not required, reducing the possibility of contamination and loss of material.

2.1 MATERIALS AND METHODS

2.1.1 Sampling Site

The River Shannon is the largest catchment within Britain and Ireland draining a land area of approximately 18,000km^2. Lough Derg (Fig 2.2.1.1), which is the third largest lake in Ireland, is located at the southern end of the Shannon River. Lough Derg covers an area of 120 km^2, has a mean depth of 7.6m, a maximum depth of 36m and a residence time of 0.15years. The lake extends from Ballina (Sample Site 9) in the south to Portumna (Sample site 1) in the north with a distance along its south-north axis of 35km. It has a mean width of 5 km and a maximum width of 14.5 km across the Scariff to Youghal bay transect. The greater part of the lake lies on carboniferous limestone but the narrow southern section is underlain by Silurian. The majority of the southern portion of the lake is enclosed by hills on either side; the Arra Mountains to the east, and Aughty Mountains to the west. The northern part of the lake is bordered by relatively flat agricultural land. The catchment is not notably industrialised. Land use within the catchment is approximately 73% grass based agriculture with the remaining 27% divided between forest, tillage and peat harvesting. Peat harvesting is evident on western shores of the lake between Rinbarra point and Rossmore Pier (Sample Site 4). Rossmore is an area with little boat or human activity and is located at the mouth of the Woodford River. The Woodford River originates in the Slieve Aughty Mountains; the river begins at a high elevation of coniferous forests. A mixture of high shrub-land, grasslands, and bog make up the middle and lower section of the watershed.

There are 12 towns located on the watershed which discharge treated effluent into the lakes tributaries. The largest town within the catchment is Nenagh in Co Tipperary with a population of approximately 7000. Nenagh town sited on the river Nenagh enters Lough Derg at Dromineer 9km to the North West. Located on the eastern side of the
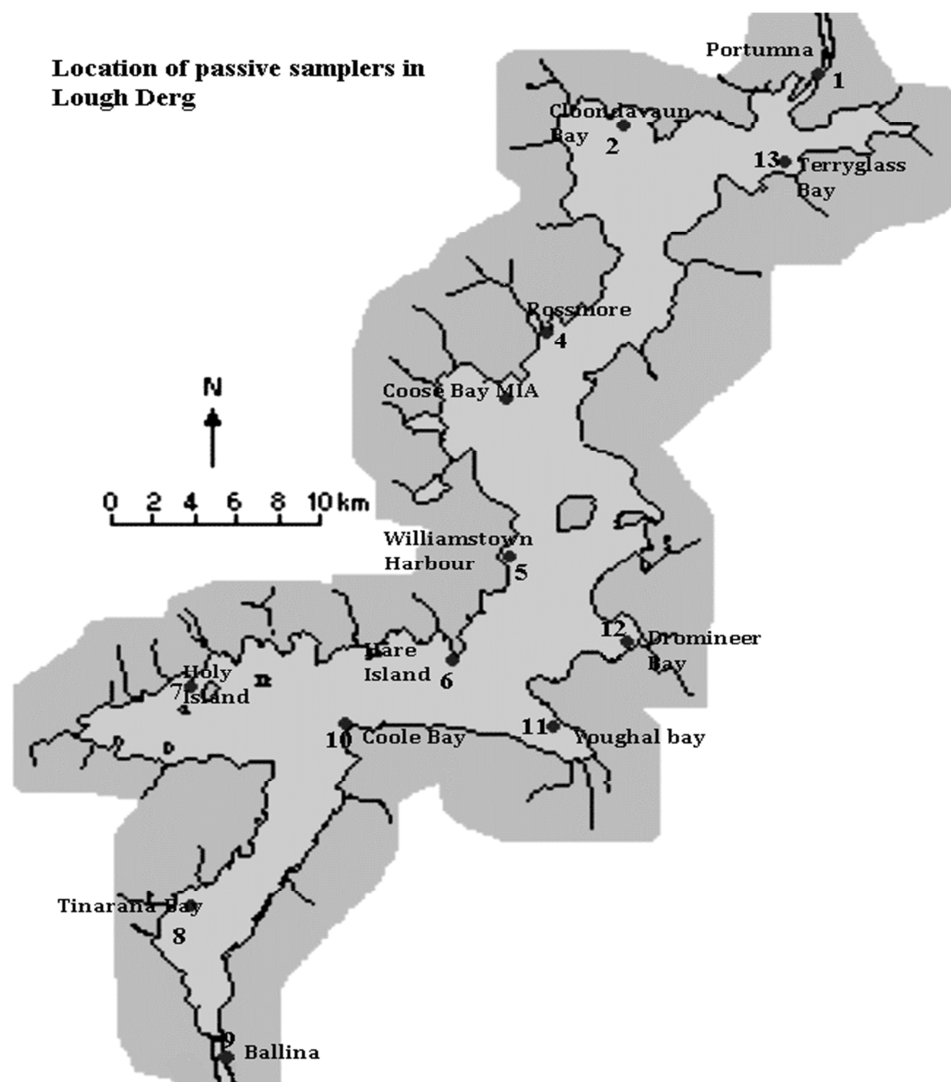
FIGURE 2.1 Map of Lough Derg showing the location of each sampling site

Lake, Dromineer is significantly influenced by the Nenagh River which originates in the Silvermine Mountains in Co. Tipperary and undertakes a 28 mile journey before entering Lough Derg at Dromineer. Most of the land that the river passes through is utilized for agriculture and raising livestock. Dromineer also accommodates a sizable public marina which sees a significant increase in activity from March to August.

In the early 1990's Lough Dreg was classed as being strongly eutrophic. This was due to an increase in the level of planktonic algae (dominated by blue green algae). Since 1997 there as been a decrease in the levels of euthrophiction within the lake, placing Lough Derg in the mesothrophic category (Moriarty and International Association of Theoretical and Applied Limnology 1998) . This reduction in eutrophication coincides with increased measures to reduce phosphorus from point

source discharges and land disposals of agricultural wastes. However, despite these improvements some areas of the lake still experience high algae blooms within the summer months. The decrease in euthrophication has also coincided with an invasion by Zebra Mussels (Dreissena polymorpha pallas) (Minchin, Maguire and Rosell 2003). Zebra Mussels filtering activities often result in the reduction of phytoplankton. While the presence of Zebra Mussels appears to have improved the water quality, in the long-term these invasive species may threaten the ecology of the lake (Lucy, Sullivan and Minchin 2005). During the sampling period from August to September Zebra Mussels were evident at highest concentrations on the eastern side of Lough Derg at Coole.

2.1.2 Sample Collection

Thirteen sampling sites around Lough Derg were chosen to represent areas influenced by different aspects of the surrounding landscape. At each sampling site passive samplers (containing six membranes (see section 2.2.3) were placed and suspended (using fishing line) approximately 100 cm below the surface of the water. Samples were removed from the lake after 28 days, returned to the laboratory and extracted using the procedure outlined in section 2.2.3.2. At each sampling point a series of composite samples were collected in 500ml polypropylene sampling bottles on the date the passive samplers were deployed. The samples were immediately placed in an ice cooler and brought to the laboratory for further analysis. Temperature, pH and conductivity were measured on-site at each sampling point using EUTECH waterproof hand-held meters. Dissolved Oxygen measurements were carried out on site using a Schott Handi-lab hand-held DO probes.
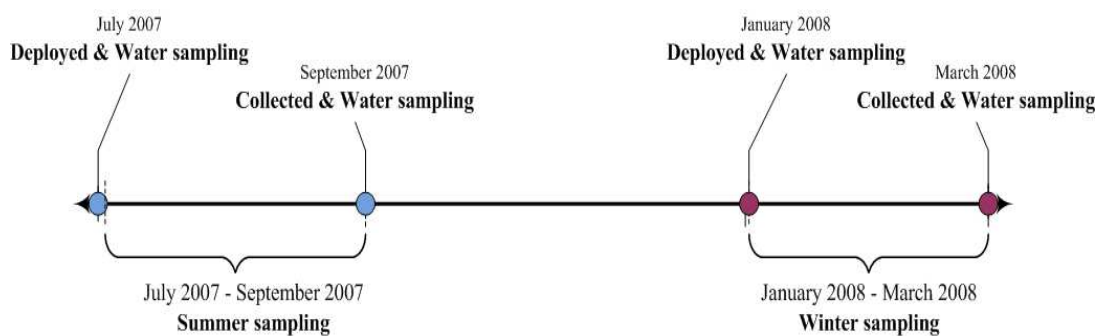
FIGURE 2.2 Timeline of summer and winter sampling periods.

2.1.3. Isolation of DOM

DOM samples were isolated at each sampling site using priority passive samplers described in this section.

2.1.3.1 Passive Sampler Construction

The passive sampler consists of; 1. DEAE cellulose, a selective resin that absorbs negatively charged species at neutral pH, 2. A poly-vinylidene fluoride (PVDF) porous membrane, with a molecular cutoff (MWCO) of 1000 kDa, and 3. A high density polyethylene (HDPE) nagelene bottles with pre-drilled holes (constructed in-house). Prior to use, DEAE cellulose was pre-cleaned using a cycle of acid, base, and distilled water washings. The cleaning regime consisted of 0.1M hydrochloric acid, 0.1 M sodium hydroxide, and distilled water washings. A minimum of 10 full acid-water-base cycles were preformed followed by a minimum of a 100 rinses with distilled water and finally freeze-dried. Cleaned DEAE-cellulose (250mg) was slurry packed with distilled water into 7 cm length, 24mm width PVDF porous membranes, which were presoaked in 0.1% sodium azide for 48hrs. Packed membranes were then placed in the constructed HDPE casings to form the passive samplers.

2.1.3.2 Extraction of DOM

The PVDF membrane was cut and resin removed to extract the sorbed DOM from the passive sampler. The resin was then placed in a 250ml Teflon centrifuge tubes and extracted with 40ml of 0.1M sodium hydroxide. The tubes were centrifuged at 1000g for 15 min to pellet the resin, and the supernatant was decanted. The resin was then re-suspended in 40ml 0.1M sodium hydroxide, and the previous steps were repeated 4 times or until the solution was clear to ensure complete extraction of the
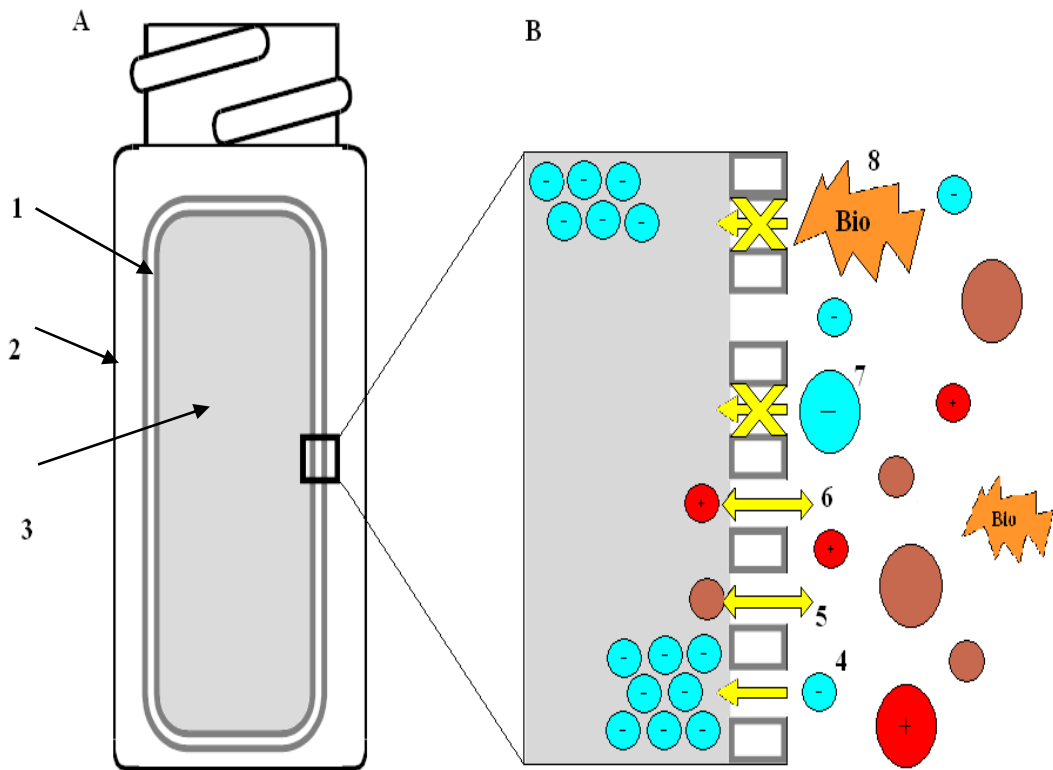
FIGURE 2.3 *(A) Diagrammed schematic showing the components of the passive sampler. (1)1,000 kDa Molecular Weight Cut Off (MWCO) Poly (vinylidene fluoride) (PVDF) membrane. (2) Porous high density polyethylene (HDPE) casing to house sampler unit (designed in house) to prevent large organisms (fish etc.) and debris from compromising the membrane. 3) Resin for DOM sorption. (**B**) Expanded region showing the resin/membrane/water interface. 4) dissolved negatively charged DOM enters the membrane and are sorbed onto the resin and concentrated many fold, (5 and 6) dissolved neutral or positively charged species (for example, metals in the case of positively charged) can enter the membrane but are not retained (note the vast majority of DOM is negatively charged), (7 and 8) large species including particulate organic matter and biological species cannot enter the membrane. The use of the membrane removes the need for filtering. (*Adapted from Lam et al (Lam and Simpson 2006)).

2.1.4 NMR analysus

Each sample (100 mg) was re-esuspended in 1 mL of deuterium oxide (D_2O) and titrated to pH 13.1 using NaOD (40% by weight) to ensure complete solubility. Samples were analyzed using a Bruker Avance 500 MHz NMR spectrometer equipped with a 1H-BB-13C 5 mm, triple resonance broadband inverse probe. 1-D solution state 1H NMR experiments were performed with 256 scans, a recycle delay of 3 s, 32768 time domain points, and an acquisition time of 1.6 s. Solvent suppression was achieved by pre-saturation utilizing relaxation gradients and echoes ((Simpson and Brown 2005)). Spectra were apodized through multiplication with an exponential decay corresponding to 1 Hz line broadening, and a zero filling factor of 2. Diffusion-edited experiments were performed using a bipolar pulse longitudinal encode-decode sequence ((Wu, Chen and Johnson 1995)). Scans (1024) were collected using a 2.5 ms, 49 gauss/cm, sine-shaped gradient pulse, a diffusion time of 100 ms, 8192 time domain points, 410 ms acquisition time, and a sample temperature of 298 K. Spectra were apodized through multiplication with an exponential decay corresponding to 10 Hz line broadening and zero filling factor of 2.

Total Correlation Spectroscopy (TOCSY) spectra were obtained in the phase sensitive mode, using time proportional phase incrimination (TPPI). These 2-D NMR experiments were carried out using 128 scans with 128 time domain points in the F1 dimension and 2048 time domain points in the F2 dimension. A mixing time of 60 ms was used with a relaxation delay of 1 s. Processing of both dimensions used a sine-squared function with a /2 phase shift and a zero-filling factor of 2.

Heteronuclear Multiple Quantum Coherence (HMQC) spectra were obtained in phase sensitive mode using Echo/Antiecho gradient selection. The HMQC experiments were carried out using 256 scans with 128 time domain points in the F1 dimension and 1024 time domain points in the F2 dimension. A relaxation delay of 1 s and 1J 1H-13C of 145 Hz were used. In processing the F2 dimension it was multiplied by an exponential function corresponding to a 15 Hz line broadening. The F1 dimension was processed using a sine-squared function with a /2 phase shift and a zero-filling factor of 2.

Spectral predictions were carried out using Advanced Chemistry Development's ACD/SpecManager and ACD/2D NMR Predictor using Neural Network Prediction algorithms (version 10.02). Parameters used for prediction including line shape, spectral resolution, sweep width, and spectrometer frequency were set to match those of the real datasets as closely as possible.

2.1.5 Growth and degradation of soil microbial biomass

Soil microbes were cultivated according to a modified version of the protocol described by Janssen et al (Janssen, et al. 2002). 1 g of soil was added to 100-ml aliquots of sterile distilled water and dispersed with a magnetic stirrer. One-millilitre aliquots of soil suspension were added to 9-ml portions of dilute nutrient broth (DNB), containing gL^{-1}: Lab-Lemco' Powder 1.0; Yeast Extract 2.0; Peptone 5.0; and Sodium Chloride 5.0, at a concentration of 0.08 g per litre of distilled water (Oxoid Ltd., Hampshire, England). Diluted soil suspensions were mixed by vortexing at approximately 150 rpm for 10 s, and used to prepare serial dilutions containing 10^{-2} to 10^{-4} g of soil suspension. One hundred-microlitre aliquots of each dilution series was plated on duplicate LB agar plates containing 0.5% dripstone, 0.25% yeast extract, 0.1% D-glucose, 0.25% NaCl and 1.5% agar. Serially inoculated LB plates were incubated at RT for 2 days and all isolated colonies were selected from the 10^{-4} dilution of the soil and used to inoculate 3.0 ml of LB broth. Cultures were incubated at RT for 48 h.

The degradation experiment was conducted according to a modified version of the protocol described by Kelleher et al. (Kelleher, Simpson and Simpson 2006). The experimental design attempted to mimic *in situ* conditions and enable the collection of transformed and leached organic matter for further analysis. Glass funnels with borosilicate sintered discs, with a porosity grade of 4 were submerged until flush with soil in a clay pot. The soil used was a native light clay-loam soil taken from fields surrounding Lough Derg. The cavity beneath the sintered disc was filled with the native soil and secured with glass wool and 0.4 g of the soil microbial biomass evenly distributed on the surface of the sintered disc. This set up enables

microbes in the soil to access the microbial biomass. The biomass was sprinkled with water every second day to mimic rain and the runoff was collected in a vial attached to the end of the funnel. Moisture levels were kept constant throughout the experiment. Runoff and microbial biomass were collected at 6 and 14 weeks post degradation.

2.1.6 Water Quality Analysis

Samples were contained in 500ml heavy duty polypropylene bottles provided with hermetic-locking caps. Bottles and caps were cleaned by soaking in 10% HCl, and rinsed with deionised water, drained, wrapped in polyethylene bags and stored until required. Composite samples were collected at each sampling point at a depth of 30cm below the surface of the water. Prior to sample collection, sampling containers were rinsed twice with the water to be sampled. Temperature, pH, conductivity and dissolved oxygen measurements were performed in situ at each sample site. Duplicate samples were taken from each sampling site. One of these was acidified to pH 2 by addition of H_2SO_4 and used for the determination of total organic carbon (TOC). The second was kept at its natural pH and used for determination of fluoride, chloride nitrate, sulphate, orthophosphate and chemical oxygen demand (COD). Samples were immediately transported to the laboratory and stored at 4°C until their analysis, which was accomplished within two days. Physico-chemical parameters have been determined by following standard methods of analysis (Greenberg, Clesceri and Eaton 1992).

2.1.6.1 Physical-chemical parameters.

2.1.6.1.1 TOC analysis

TOC was measured directly from 10ml water samples using the HACH direct method (HACH method 10129 (Manual 1998). To remove inorganic carbon a representative 10ml portion of the sample was placed in a 30ml beaker. H_2SO_4 was added to reduce the pH to 2. The acidified sample was then allowed to stir for 10 minutes. Using a funnel, one persulfate powder pillow was added to each acid digestion vial. 3ml of treated sample was added to the vial and mixed. Indicator

ampules were rinsed with distilled water, wiped with a soft lint free wipe cloth. One ampule was lowered into each acid digestion vial when the score mark of the amplule was level with the top of the digestion vial, the top of the amplule was snapped off and the ampule was allowed to drop into the acid digestion vial. Each vial was capped tightly and placed in a COD reactor for 2hrs at 103-105 °C. The vials were then removed from the reactor and allowed to cool for 1 hour prior to analysis. During digestion the organic carbon in the sample is digested by the persulphate and acid to form carbon dioxide. The carbon dioxide diffuses from the outside vial into the inner ampule containing a pH indicator reagent. The absorption of the carbon dioxide forms carbonic acid, which alters the pH of the indicator reagent thus changing the colour. The intensity of the colour change is indicative of the amount of carbon within the sample. A blank using distilled water and an internal reference standard of 10mg/l C was used in place of the sample to measure the degree of contamination within the method and to validate the accuracy of the analytical method. The concentration of C mg/l was then estimated from the absorbance of the sample measured at 600nm using a DR2000 spectrophotometer.

2.1.6.1.2 COD analysis

COD analysis was measured directly from 2mls of the water sampled using the reactor digest method (HACH method 8000 (Manual 1998). Holding the digestion vials at a 45-degree angle 2mls of the sample was added using a clean volumetric pipette. Each vial was tightly capped, rinsed with distilled water and wiped with a soft lint free cloth. The vials were inverted several times to mix and placed in a DRB200 reactor for two hours at 150°C. The vials were allowed to cool for 20 minutes, inverted several times, placed in a test-tube rack and allowed to cool to room temperature. During the 2hr reaction time the sample is heated with sulphuric acid and potassium dichromate (a strong oxidising agent). Oxidizable organic compounds within the sample react, thus reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). A blank using distilled water and an internal reference standard of 100 mg/l COD was used in place of the sample to measure the degree of contamination within the method and to validate the accuracy of the analytical method. The concentration of COD mg/l was then estimated from the absorbance of the sample measured at 350 nm using a DR2000 spectrophotometer. The results in

mg/l COD are defined as the milligrams of O_2 consumed per litre of sample under the conditions of the method.

2.1.6.1.3 Orthophosphate

Orthophosphate was directly measured from 25 ml of the water sampled using the Phosver 3 (Ascorbic acid) method (HACH method 8178 (Manual 1998). A 25ml sample cell was filled with 25ml of the sample to be analysed. One Phosver 3 powder pillow was added to each sample cell the cell was capped and shaken vigorously for 60 secs. The sample was then left to settle for two minute to allow for the reaction to take place. During that time orthophosphate within the sample reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduced the complex, giving an intense molybdenum colour proportional to the concentration of orthophosphate in the sample.

A blank using distilled water and an internal reference standard of 2.0mg/l phosphate was used in place of the sample to measure the degree of contamination within the method and to validate the accuracy of the analytical method. The concentration of orthophosphate was then estimated from the absorbance of the sample measured at 890 nm using a DR2000 spectrophotometer.

2.1.6.1.4 Fluoride, Chloride, Nitrate, and Sulphate analysis

Fluoride, Chloride, Nitrate and Sulphate analysis was performed using a Dionex 4500i ion chromatograph fitted with an electrolytic conductivity detector. Sample and eluant (3.5mM sodium carbonate and 1.0mM sodium bicarbonate) were lead via a Dionex AG10 anion guard through a Dionex AG10 anion column, through a Dionex GDM-2 pump. The IC system was automated in terms of sample introduction by means of a Dionex ASM-11 sample changer and data processing using Dionex A1450 software.

To improve the accuracy of the results, a blank sample of ultra pure water was prepared in the same way as each sample and analyzed under identical conditions. The blank results were subtracted from the results of the samples to improve accuracy. For calibration proposes duplicate injections of four different levels of standards (2.5mg/l, 5mg/l, 10mg/l, and 20mg/l) containing each individual ion were carried out. Correlation coefficients of 0.995 or better were obtained.

2.2. RESULTS AND DISCUSSION

2.2.1 Watershed influences on water quality

Isolated DOM yields from each site are tabulated and divided into both winter and summer sampling periods. Water quality results are divided into each physico-chemical parameter i.e. temperature, pH, Conductivity, D.O., COD, and TOC, orthophosphate, F^-, Cl^-, NO_3^- and SO_4^-. Spatial and temporal variations for each parameter at each sampling site are tabulated and presented graphically and collated in excel.

2.2.2. Isolated DOM Yields

Table 2.1 and Figure 2.4 compare yields collected from various sampling sites during both summer and winter sampling periods. Total yields were isolated on six passive samplers over a 28 day period at each site during both summer and winter sampling period. Variations in the yields observed can be a result of numerous influences. The dramatic increase in the yield obtained at Williamstown Harbour is likely to be a result of a considerable amount of anions dissolved alongside the organic compounds at the sampling site indicated by the increase in the conductivity level during the same sampling period (see Figure).

TABLE 2.1 Comparison of Isolated DOM yields obtained with the passive samplers from each sample site

Sampling site	Summer (mg)	Winter (mg)
Portumna	56.2	109.4
Cloondavaun Bay	176.2	59.7
Rossmore	48.0	124.7
Williamstown Harbour	409.2	45.6
Hare Island	109.0	n/a *
Holy Island	96.7	87.4
Tinnarana	89.5	n/a *
Ballina	147.3	112.5
Coole Bay	86.0	76.0
Youghal Bay	134.0	105.4
Dromineer Bay	69.5	115.3
Terryglass Bay	66.2	n/a *

* Yields were not available due to loss of passive samplers at the site

Negative ions mainly Cl$^-$ within the water body will compete for binding sites on the DEAE-cellulose and slow down or prevent the sorption of DOM components (Lam, et al. 2007). High concentrations of SO_4^- reduce the solubility of OM by reducing pH and increasing ionic strength (Kalbitz, et al. 2000). Anion concentrations is just one of the factors that can affect the uptake of DOM by the passive samplers in the environment, water dynamics amongst others play an important key role.
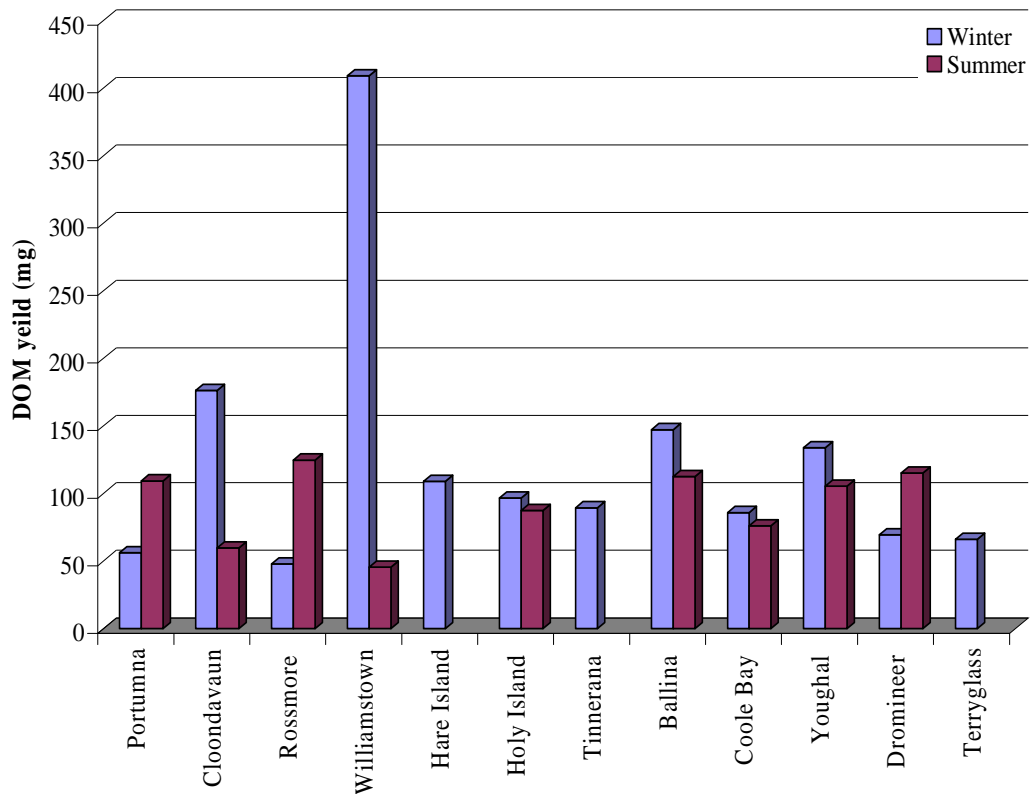
FIGURE 2.4 Spatial and temporal variability of DOM yields isolated using passive samplers at each sample site

Spatial and temporal variations in DOM yields observed at Portumna, Rossmore and Dromineer are likely the result of seasonal shifts in hydrological pathways within the watershed and across the landscape into receiving waters (Evans, Monteith and Cooper 2005). Hydrological pathways are controlled by environmental parameters that include temperature, geology, soil type and rainfall intensity. Each sampling point is fed by a river draining considerable catchments of varying land use. Increased rainfall during the winter sampling period may increase the concentration of DOM within receiving lake and rivers as it diverts the hydrological flow path from deeper mineral soil horizons to flow over shallow organic rich soil horizons (Erlandsson, et al. 2008). Therefore each river may transport increased fluxes of DOM to each sampling site during periods of increased rainfall (Evans, Monteith and Cooper 2005). Higher COD mg/l, TOC mg/l and low conductivity observed at Rossmore during the winter sampling also indicate high organic load at the site, influenced by the Woodford River which drains large areas of forest and peat lands.

Temperature can also play a role in influencing the yield obtained by passive samplers within a water body. Results obtained indicate that increased temperatures observed during the summer sampling period could increase DOM production through increased organic matter decomposition (Clark, et al. 2005).

2.2.3. Physical-chemical parameters

The influence of season on the physical-chemical parameters of water was investigated by sampling during the summer and winter of 2007. Water sampling was conducted in tandem with the DOM passive sampler deployment at 13 different sampling points. In this way, spatial and temporal influences on water quality and composition of DOM could be established. A temporal comparison at Hare Island and Coose Bay is not available due to limited access to both sites during winter sampling periods.

2.2.3.1. Temperature

The temperature values show a seasonal variation that fluctuated from 7.88 ˚C (winter $\bar{x}$), ($\sigma = 0.65$) to 18.65 ˚C (summer $\bar{x}$), ($\sigma = 0.91$) for the entire research project. Seasonal variations in temperature were consistent between each sampling point with an average drop in temperature of 10.82 ˚C. Spatial variations in temperature were observed at Ballina. Temperatures recorded at Ballina during both summer and winter sampling periods were 2 ˚C lower than the majority of the other sampling sites. This decrease in temperature at Ballina can be associated with the increased depth and flow of water at the sampling point.
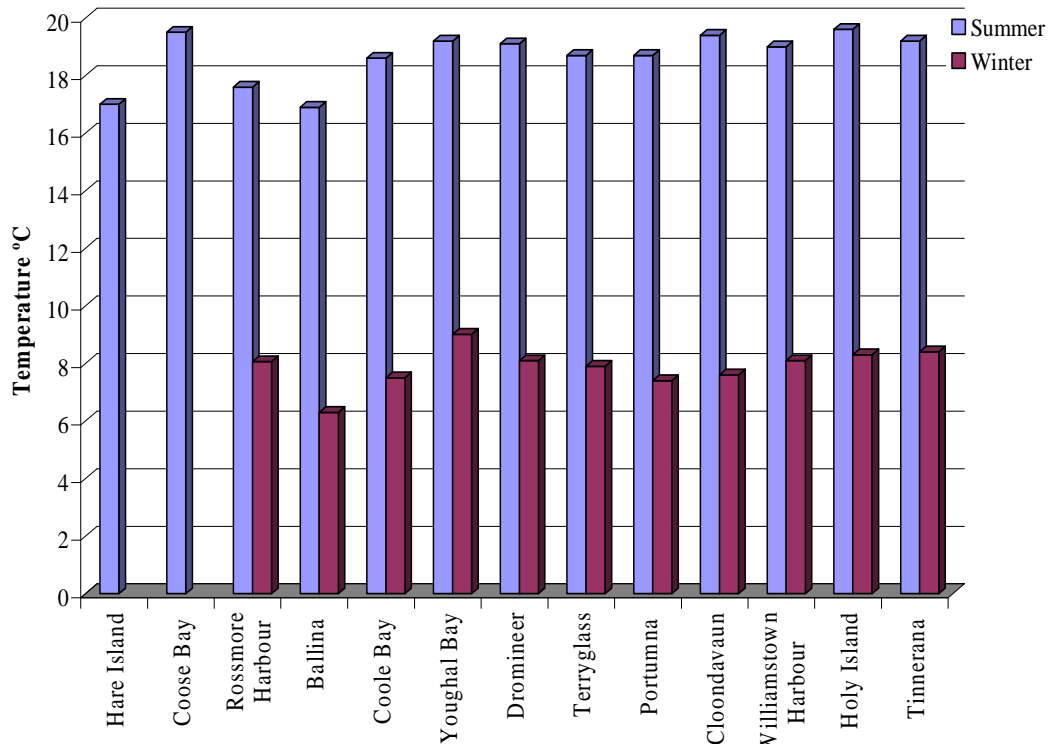
FIGURE 2.5 Spatial and temporal variability of temperature concentrations at each sampling site studied within Lough Derg.

2.2.3.2. pH

Temporal and spatial variations in pH within the lake ranged from 6.30 to 8.80, slightly acidic to alkaline for the entire research project. The majority of the samples sites were slightly alkaline ranging from 7.15 to 8.80 (summer $\bar{x} = 7.94$), (σ 0.50) (winter $\bar{x} = 7.63$), ($\sigma = 0.61$). The variation in pH range between each sampling point rarely exceeded one pH unit affirming the good buffer capacity of the lake. A variation in the pH above one pH unit was observed at Ballina with a difference of 1.48 pH units which can be attributed to the depth and flow of water at the sampling point.
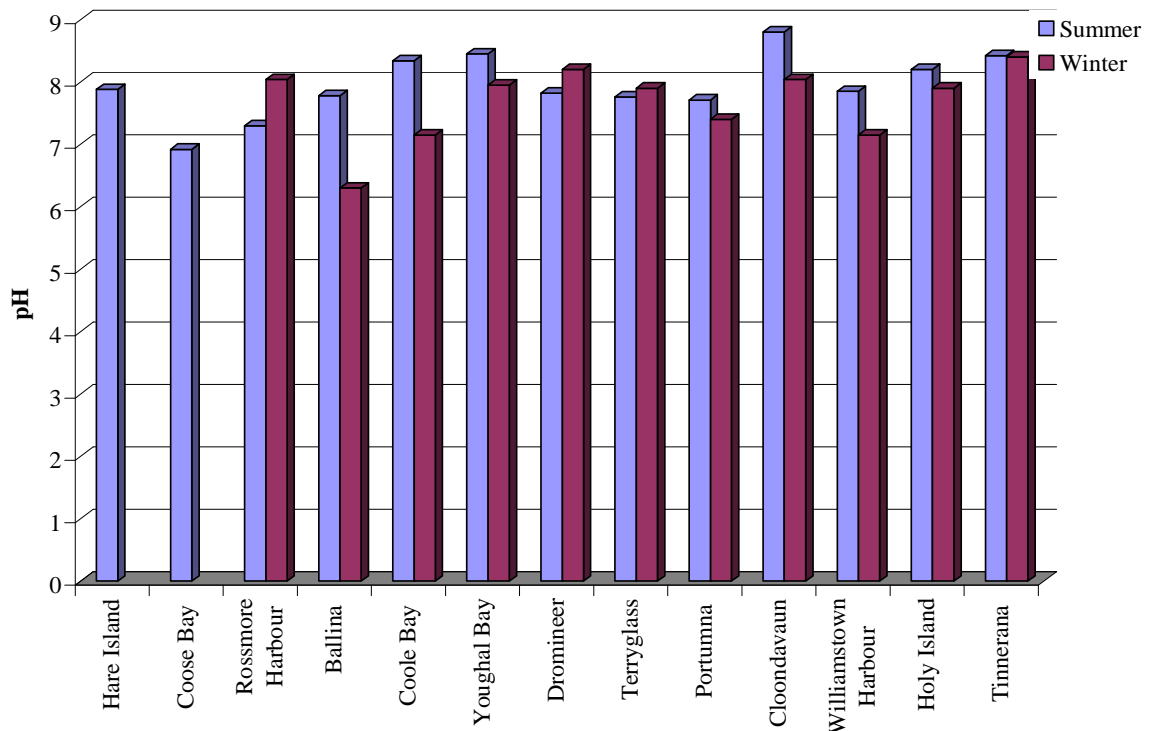
FIGURE 2.6 Spatial and temporal variability of pH in particular sampling sites studied in Lough Derg.

2.2.3.3 Conductivity

Conductivity is the measure of the ability of water to conduct an electrical current. Conductivity will increase as dissolved minerals accumulate within a water body. As a result, conductivity is an indirect measure of the number of ions in solution. Conductivity of lakes is affected primarily by the geology of its surrounding watershed. Significant spatial variations were observed during summer with conductivity fluctuating between each sampling point and ranging from 476μScm to 1340 μScm with an average of 727.6 μScm for the entire project. Lower conductivity was observed during winter at almost all sampling sites (176 μScm to 610 μScm, $\bar{x}$ = 472.6 μScm), the exception was at Cloondavaun and conductivity was higher in winter than summer by 17 μScm. Seasonal variations at each sampling point maybe associated with water levels within the lake. During the summer sampling period water levels within the lake are lower than levels during winter due to reduced rainfall and evaporation. The increased conductivity levels during the summer period indicate that evaporation possibly concentrates the number of ions in solution (Kadlec 1982).
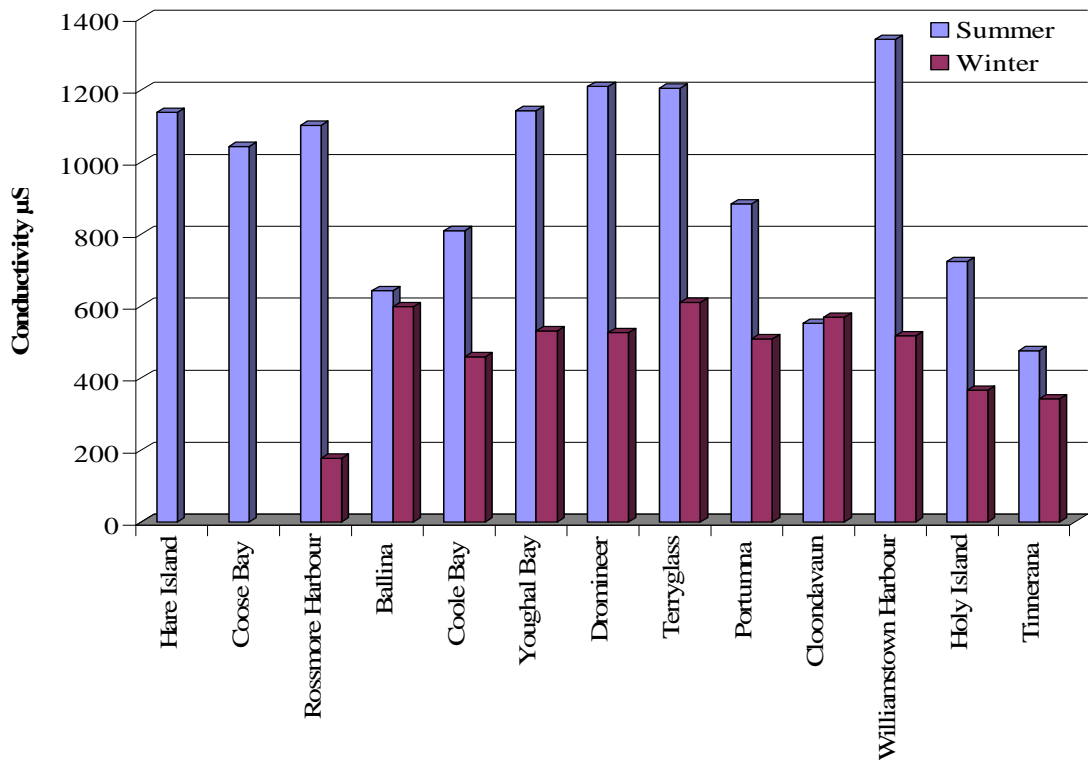
Figure 2.7 Spatial and temporal variability of conductivity in particular sampling sites studied in Lough Derg.

During the summer sampling period maximum conductivity levels were observed at Williamstown harbour. High conductivity levels correspond to high DOM yields recorded at Williamstown during the same sampling period. High yields and high conductivity levels observed are likely due to high chloride levels at the sample site.

2.2.3.4. Dissolved Oxygen

The dissolved oxygen (DO) measurement is used to measure the amount of oxygen dissolved within a lake. EPA guidelines suggest that lake water should have an optimum value of 100%, anything above this value is said to be super-saturated. Dissolved oxygen measurements taken during the summer exceeded that value by greater than 10% (i.e. 110% DO) at four sampling locations (Rossmore harbour, Coole Bay, Youghal Bay). This increase in dissolved oxygen can be associated with the occurrence of algal blooms during sampling the summer sampling period.

Super-saturation (over 100% DO saturation) can occur when there is a large algal bloom. During the daylight, when the algae are photosynthesizing, they can produce oxygen so rapidly that it cannot escape into the atmosphere, thus leading to short-term saturation levels of greater than 100%. During the same sampling period the lowest DO levels were observed at Portumna (77.1%). This level was significantly lower than the % DO observed at the same sampling point during the winter sampling period.
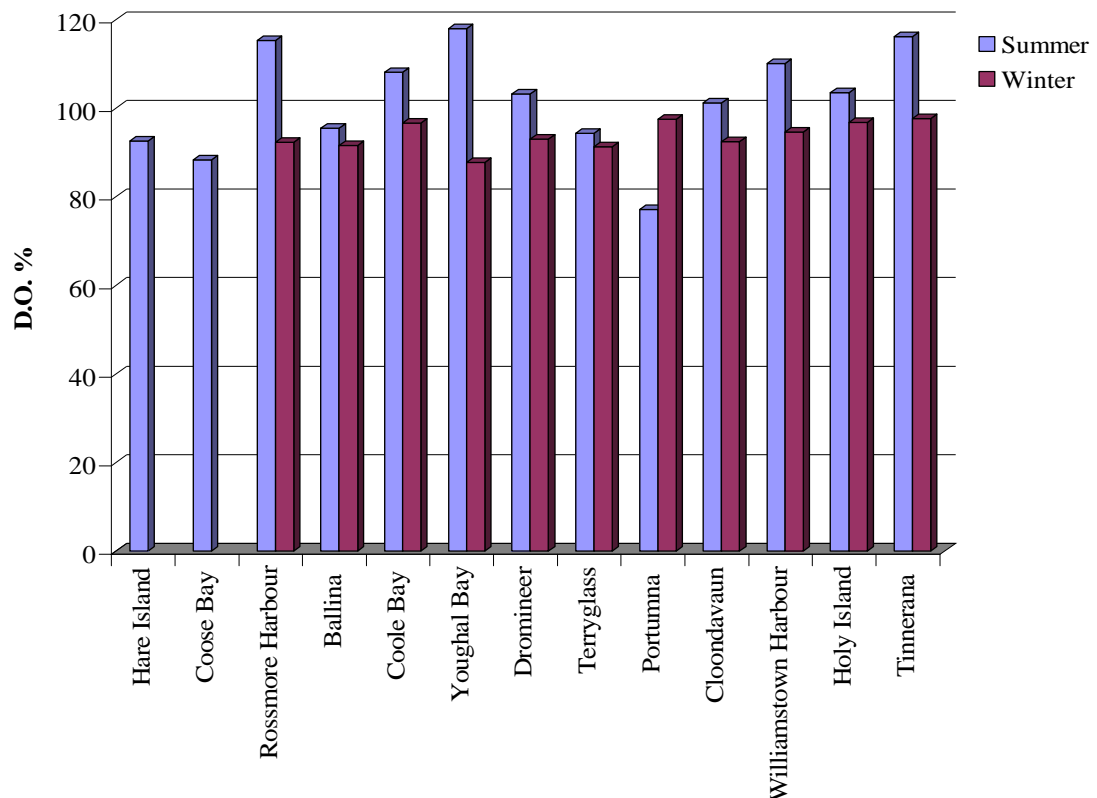
FIGURE 2.8 Spatial and temporal variability of the percentage of dissolved oxygen in particular sampling site studied in Lough Derg.

2.2.3.5. TOC Analysis

Temporal variations in TOC concentrations within the lake were evident. Concentrations during summer (at all sampling sites) ranged from between 10.22mg/l C and 22.76 mg/C with an arithmetic mean of 16.90mg/C. The range of TOC concentrations during the winter sampling was between 3.30 mg/l C and 10.66 mg/l C with an arithmetic mean of 5.59 mg/l C. Maximum concentrations in summer were observed at Terryglass which also showed the largest range in

concentration between summer and winter with a difference of 16.56 mg/l C. Minimum concentrations of 10.22 mg/l and the narrowest range of 6.92 mg/l were observed at Tinnerana. These results suggest that seasonal changes rather than spatial differences are the main factor to influence TOC concentrations within the Lake.
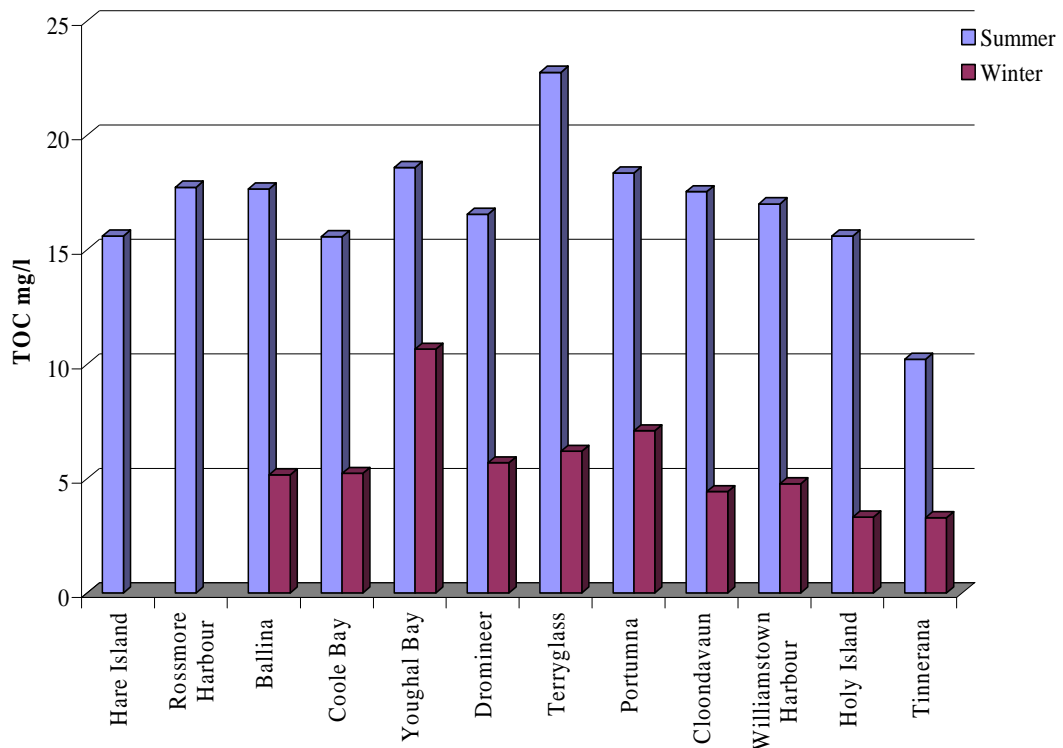
FIGURE 2.9 Spatial and temporal variability of TOC concentrations in particular sampling site studied in Lough Derg.

2.2.3.6 COD analysis

A seasonal comparison of COD levels was not available due to analytical limitations during the winter sampling period. The mean COD for the summer sampling period was 33.4 mg/l (ranging from 13- 47mg/l). Lowest COD values were recorded at both Ballina and Tinnerana (13 and 14 mg/l respectively). Highest COD values were recorded at Dromineer, Williamstown Harbour and Youghal Bay. High values recorded at Dromineer and Williamstown Harbour may be attributed to an increase in human activity at each sampling point during the sampling period as both accommodate large in-land marinas. The increased human activity at both sites can

increase the organic load in the area and therefore increase the level of COD. High COD values recorded at Youghal Bay coincide with a large algal bloom accumulation in the area during the sampling period. Low COD values recorded at Ballina can be associated with the depth and flow of water at the sampling point.
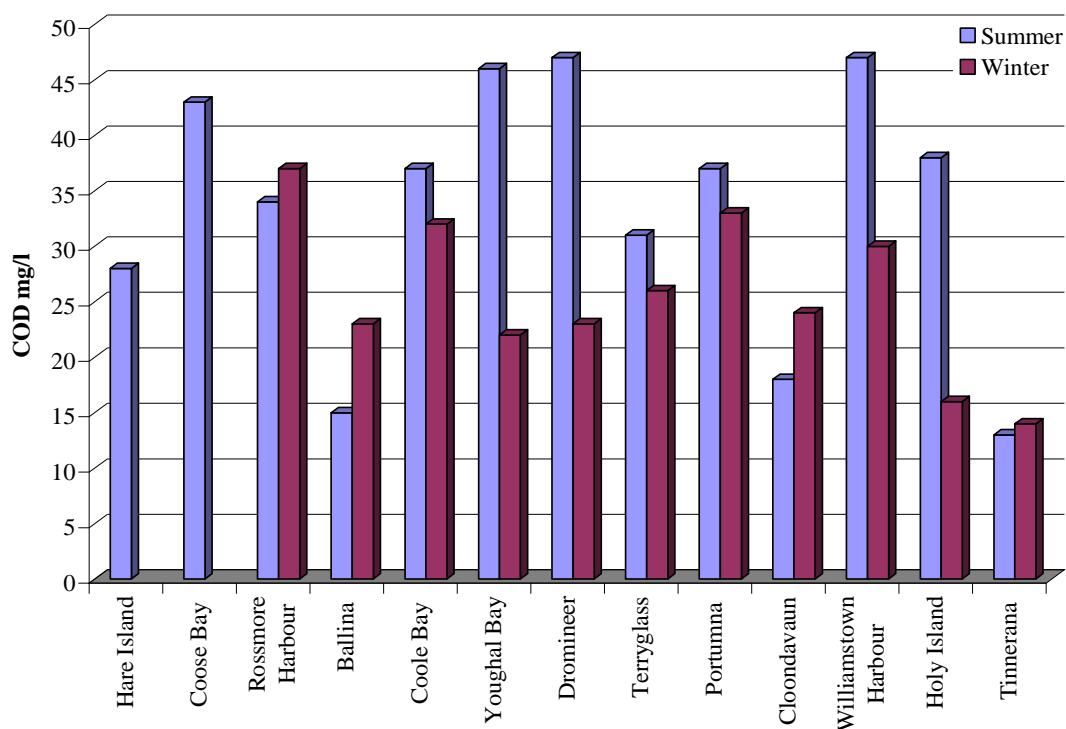
FIGURE 2.10 Spatial and Temporal variability of the percentage of C.O.D in Lough Derg during winter sampling period**.**

2.2.3.7. Fluoride, Chloride, Nitrate, and Sulphate

Fluoride, chloride, nitrate and sulphate are naturally present in the environment but could also originate from waste-water discharge or runoff from agricultural land containing soil amendments such as fertilisers and lime. No seasonal comparison can be made as anion analysis was only carried out during the winter sampling period. During this period, small spatial variations were observed, in F^-, Cl^-, $\underline{NO_3}^-$ and SO_4^- concentrations.
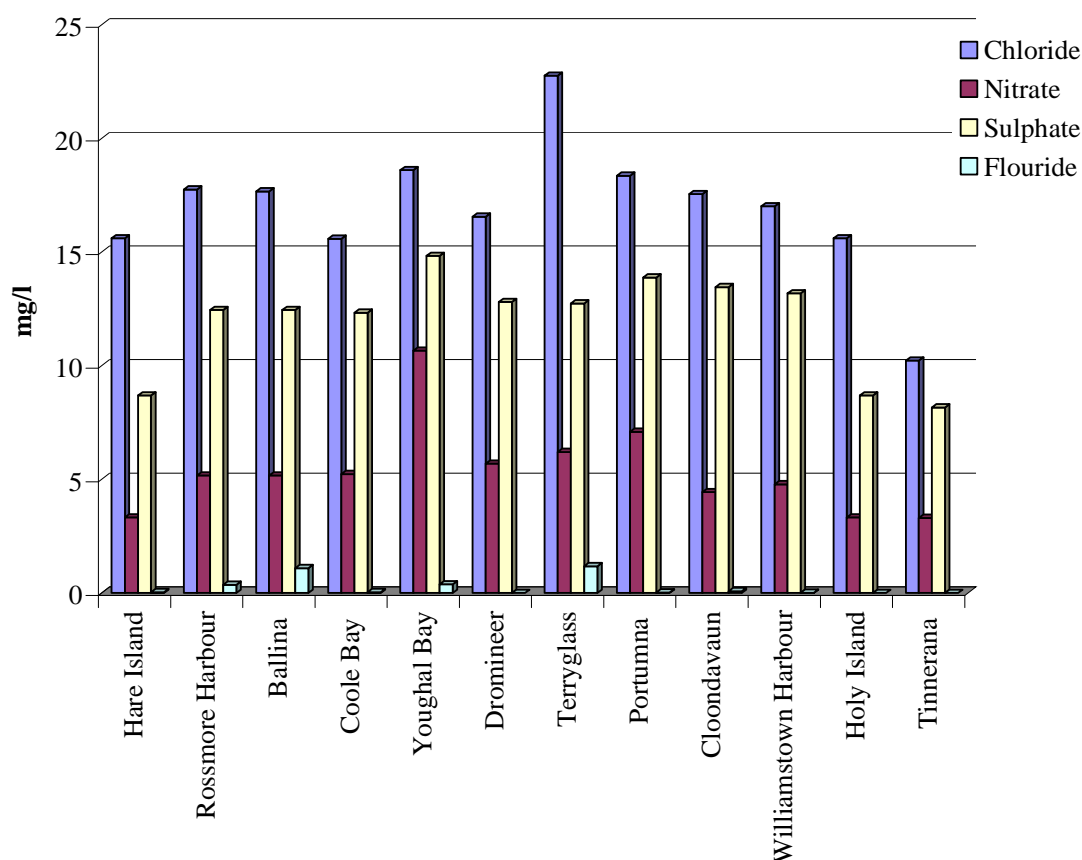
FIGURE 2.11 Spatial variability of anions in particular sampling sites studied in Lough Derg during summer sampling period.

The F^- concentrations were between < 0.1 and 1.17 mg/l throughout the sampling period, with the majority of the samples containing <0.1 mg/l F^- with few exceptions. Rossmore harbour, Ballina, Youghal Bay and Terryglass contained F^- concentrations of 0.36 mg/l, 1.09mg/l, 0.38mg/l and 1.17mg/l respectfully. Of the samples analysed none exceeded the maximum admissible concentration (MAC) of 1.5mg/l. Elevated fluoride levels at both Ballina and Terryglass can be attributed to discharge from urban developments at each site. Chloride concentrations ranged from 10.22 to 22.76 mg/l none exceeding the MAC for fluoride in drinking water 250mg/l Cl^-. Highest concentrations were recorded from samples taken at Youghal bay (22.52 mg/l Cl^-) and Terryglass (22.76 mg/l Cl^-) with lowest concentrations recorded from samples taken from Tinnerana (3.3mg/l Cl^-). Nitrate concentrations within the lake ranged from 1.46mg/l at Rossmore Harbour to 8.49 mg/l at Youghal Bay. Higher concentrations of NO_3^- at Youghal can be attributed to runoff from the surrounding landscape of intense agricultural exploitation. The mean concentration

of NO_3^- for the entire sampling period was 4.46 mg/l below the MAC of 50mg/l NO_3^-. The SO_4^{-2} concentrations ranged from 5.68mg/l at Rossmore harbour to 13.69mg/l at Williamstown Harbour and are below the MAC of 250mg/l SO_4^{-2}.

Results obtained indicate that physical-chemical parameters and yield of DOM isolated vary both spatially and temporally within the Lough Derg. Correlation between physical-chemical parameters and the quantity of DOM were observed at numerous sampling points most noticeably at Williamstown, Terryglass, Portumna, and Dromineer. High conductivity, COD and TOC, dissolved oxygen, temperature and pH were found to influence to varying degrees the quantity of DOM isolated at each sampling site. These variances in yield may in part be due to the watershed influences which alter the water chemistry within the lake.

2.2.4 NMR analysis of Isolated DOM

In order to assess the watershed influences on the quality of DOM, NMR analysis was carried out on samples isolated from each sampling site. Figure 2.12 compares the conventional ^{1}H (Figure 2.12 a) and diffusion edited (Figure 2.12 b) NMR spectra for the Ballina DOM sample and also shows the diffusion edited ^{1}H spectrum of the Coole Bay sample area of lake (Fig. 2.12 c). General assignments, consistent with those reported in the literature are; aliphatics, including material derived from linear terpanoids; (1), carboxyl-rich alicyclic molecules (CRAM) (see also Figure 2.12 A); (2), a mixture of carbohydrates and amino acids; (3) and aromatics, including resonances from amino acid side chains; (4)(Hertkorn, et al. 2006)(Lam, et al. 2007) . More specific assignments refer to (i); CH_3, likely including resonances from aliphatic species and methylated amino acid side-chain residues in peptides/protein, (ii); consistent with a side chain residue also seen in the ^{1}H NMR spectrum for bovine serum albumin, (iii); aliphatic methylene $(CH_2)n$, (iv); aliphatic methylene units β to an acid or ester i.e. R_2-OCO-CH_2-R_1 (v); contributions from both N-acetyl group in peptidoglycans and other units in branched lipids waxes, (vi); anomeric protons in

carbohydrate. Si indicates a natural silicate species and not TMS (a commonly used NMR reference standard) (Simpson, et al. 2007).
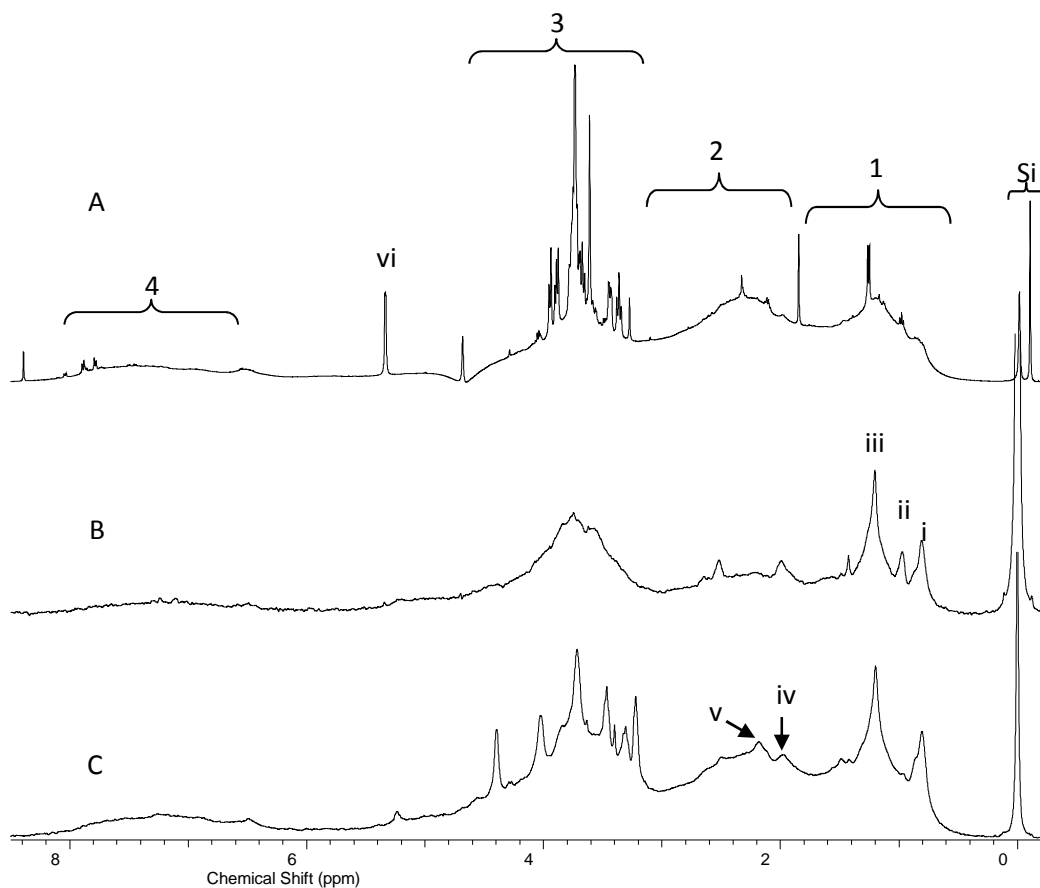
FIGURE 2.12 ^{1}H NMR spectra for (A) Ballina DOM sample, (B) diffusion edited 1H spectrum of Ballina DOM sample, (C) diffusion edited 1H spectrum of Coole Bay sample area of lake.

Figure 2.12 1a displays sharp peaks especially in the carbohydrate region (region 3). Sharper lines observed in NMR are often characteristic of smaller structures, and this may indicate the breakdown of the carbohydrates from large polymeric structures into smaller fragments. To test this hypothesis we applied diffusion edited experiments to the Ballina sample. In diffusion edited NMR experiments small molecules are essentially gated from the final spectrum but signals from macromolecules which display little translational diffusion are not gated

and appear in the spectrum (Wu, Chen and Johnson 1995). Any similarities between the 1-D ^{1}H and diffusion edited spectra would suggest that those components are macromolecular and/or rigid and exhibit little, if any translational diffusion. However, for the Ballina DOM sample (Fig. 2.12b); many signals of small molecules are greatly attenuated in the diffusion edited spectrum. Aliphatic chains dominate the diffusion edited spectrum indicating they are preserved in rigid domains, whereas the relative intensity of the carbohydrate signals have declined suggesting a larger contribution from smaller units that have some translation diffusion. A characteristic resonance for CH_3 in methylated amino acid side-chain residues (signal i.) is now easily distinguishable in the diffusion edited NMR suggesting the presence of protein/peptide. Furthermore, the resonance at ~1 ppm (signal ii.) can again be attributed to protein/peptide as this peak is also present in the ^{1}H NMR spectrum of bovine serum albumin (Simpson, et al. 2007). Complimentary evidence for protein/peptide presence is provided by the emergence of alpha protons from amino acids in Figure 2.14 Proteinaceous compounds are viewed as labile in the environment (Fuhrman 1990) and their survival and occurrence has been explained through protection mechanisms such as encapsulation and formation of microbially resistant complexes with carbohydrates and lignin (Hedges, et al. 2000, Tanoue, et al. 1995, Ogawa, et al. 2001).

Lam et al. (Lam, et al. 2007)detected very weak protein/peptide contributions in Lake Ontario DOM and it is thought that protein/peptide is only present in DOM as a minor constituent. However, the spectra generated in this study show that the protein/peptide contribution varies between DOM from different sources and that it may be a significant component of freshwater DOM. It is estimated that plants often contain only 1–5% protein by weight and that protein structures are known to degrade rapidly in a soil environment (Park, Hettiarachchy and Were 2000, Herman, Wolt and Halliday 2002). It therefore seems unlikely that the preservation of plant-derived peptide/protein structures can completely account for the contributions of proteins and peptides in DOM. One possibility is that a significant portion of peptide/protein in DOM arises from the cells of dead and living microbes. Alternatively, microbially resistant ligno-protein may also account for some of the protein present (Waksman 1932). Figure 2.13 highlights crosspeaks that represent lignin derived methoxy carbons and protons, often the most intense signal in soil

organic matter (Simpson 2001). Methoxy crosspeaks are clearly present in all of the lake samples (overlapped with carbohydrate crosspeaks), especially Hare Island and Dromineer. Lignin is a strong indicator of plant inputs and may be an indication of the age of DOM and/or the influence of surrounding environment. Proteins originating from microbial cells may be encapsulated by lignin making them less susceptible to degradation.
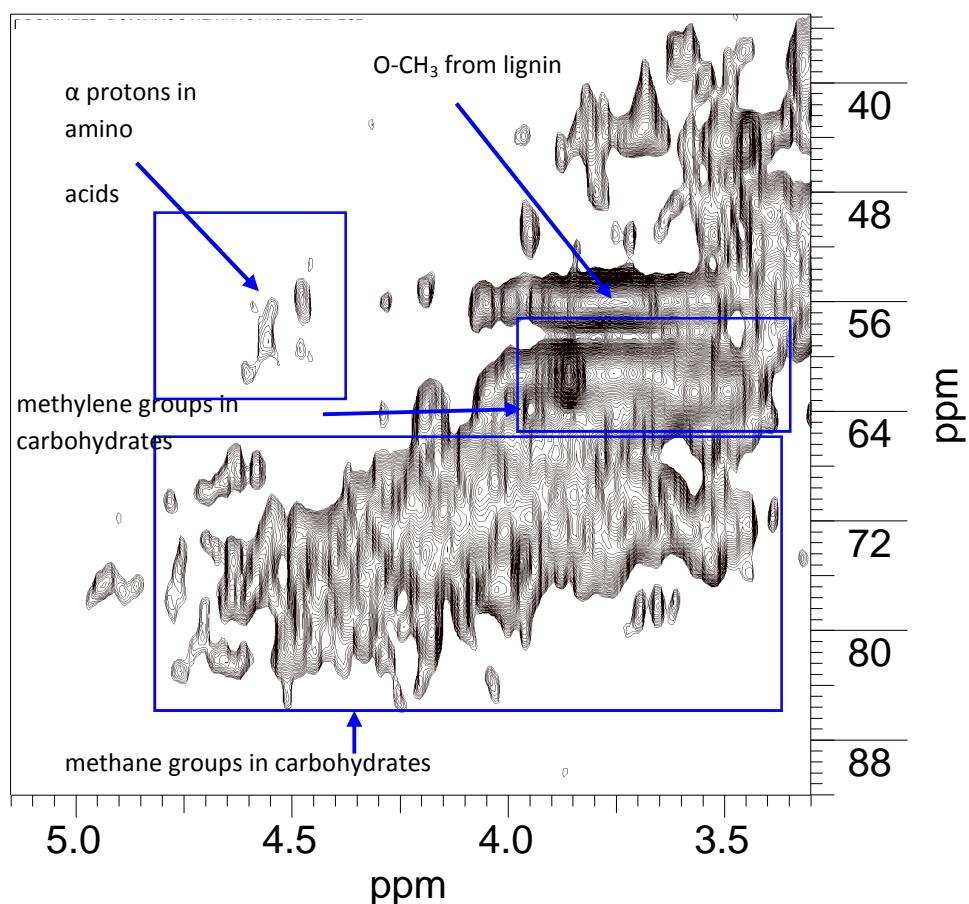
Figure 2.13 Zoom region of DOM HSQC (Dromineer).

All Lough Derg DOM samples contain a contribution from carbohydrates that are not removed during diffusion editing (Figure 2.12 b and 2.12 c) indicating that there is a polymeric carbohydrate component present that could potentially be associated with the cell walls of micro-organisms (Lam, et al. 2007). Signal (iii) (aliphatic methylene $(CH_2)_n$) and (v) (aliphatic methylene units β to an acid or ester) in Figure 2.12 b and 2. c are consistent with aliphatic structures. The aliphatic methylene $(CH_2)_n$ peak is dominant indicating the presence of stable waxes and lipids (Deshmukh, Simpson and Hatcher 2003). Waxes and cutins derived from

plants have been identified in abundance in humic extracts (Simpson, et al. 2006), and are likely to be preserved due to their cross-linked structure and hydrophobicity (Simpson, et al. 2007). Figure 2.12c shows the DE ^{1}H NMR spectrum for the Coole Bay DOM sample and the presence of signal (v) indicates that some of the lipids present are associated with peptide, thus supporting the presence of lipoprotein in the sample. Lipoprotein is a key component of bacterial cells, is structurally diverse, and is released during bacterial growth (Moore, et al. 1998). The exact structure of the lipoprotein cannot be determined, but it is likely that a portion of this material may be from soil or aquatic microbes. Microbial contributions are also supported by the presence of signal (iv) in Figure 2.12c. This can be assigned to peptidoglycan that comprises up to 90% by weight of grampositive bacteria and is the key structural component in all microbial cell walls. It is important to note that peptidoglycan does contain small peptide linkers in its structure but it is not possible to accurately quantify contributions of peptide in the form of peptidoglycan in the various samples due to spectral overlap. The presence of peptidoglycan in DOM is logical as it is resistant (as microbe cell walls) to many chemical and biological processes and has been found to be present in the most refractory components of soil organic matter (Simpson, et al. 2007).

The potential contribution of surrounding soil microbial biomass to DOM was studied by conducting a complimentary laboratory experiment that monitored the degradation of soil microbial biomass over time. Degradation occurred over 14 weeks allowing NMR experiments to be conducted on degraded soil microbial biomass residue and leachate. Figure 2.14 compares the DE ^{1}H NMR spectrum of the 14 week leachate from degraded soil microbial biomass (A), to the DE ^{1}H NMR spectrum of the "Dromineer" DOM sample from Lough Derg (B). Characteristic resonances such as CH_3 in methylated amino acid side-chain residues (signal i.) and aliphatic methylene $(CH_2)_n$ (signal ii.) that are present in the Dromineer DOM (Figure 3B) are also present in the microbial leachate. These signals also persist in degraded plant matter and therefore it is not possible to say that they originate solely from soil microbial biomass (Kelleher and Simpson 2006). However, peptidoglycan (PG, Figure 2.15 A and B), is present in both the DOM and the soil microbial biomass leachate and this is confirmed in the HMQC spectra in Figure 2.15
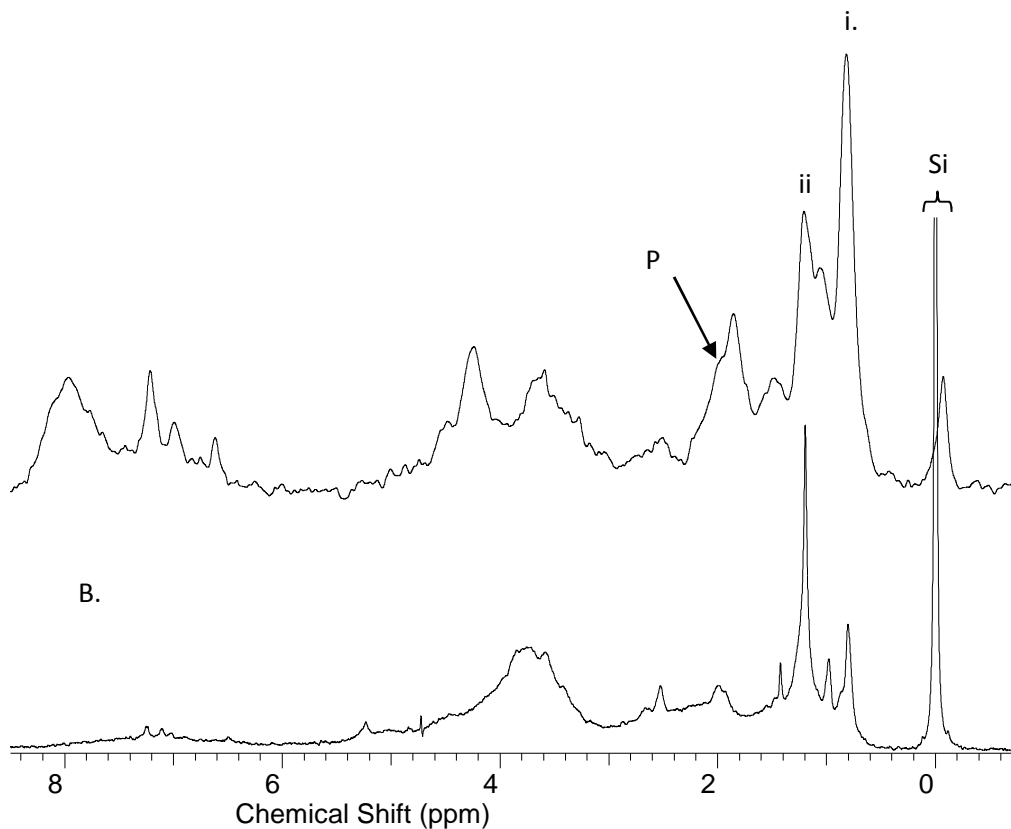
FIGURE 2.14 (A) DE ^{1}H NMR of 14 week leachate from degraded soil microbial biomass, and (B), the DE ^{1}H NMR of the "Dromineer" DOM sample from Lough Derg. Specific assignments are: (i); CH_3, likely including resonances from aliphatic species and methylated amino acid side-chain residues in peptides/protein, (ii); aliphatic methylene $(CH_2)n$, (PG); peptidoglycan (Simpson et al 2007 ES and T humin) and Si indicates a natural silicate species and not TMS (a commonly used NMR reference standard).

This would suggest that complex biomaterials such as peptidoglycan from the cell walls of soil micro-organisms can persist in the water environment and that it is possible that the peptidoglycan we see in DOM is originally derived from microbes in soil. Interestingly, natural silicate species (Si) that are present in DOM samples are also present in the microbial leachate spectrum. Carbon sequestration in the oceans is known to be coupled with the global cycle of silicon (Treguer, et al. 1995, Ragueneau, et al. 2000). Rivers provide the conduit for 5 Tmol of silicon per year into the oceans which is 80% of the total annual flux (Treguer, et al. 1995,

Conley 2002). The remaining 20% comes from dust and submarine hydrothermal sources. It is thought that the ultimate source of continental silicon flux into the oceans is the weathering processes in terrestrial biogeosystems (Birkeland 1997)(Van Breemen and Buurman 2002). However, silicon dynamics in terrestrial biogeosystems cannot be understood solely by mineral weathering (Sommer, et al. 2006). The organic (crosspeaks also appear in the HMQC spectra) silicate species in the soil microbial leachate would suggest that soil micro-organisms accumulate their own stable silicon pools and may play a larger role in silicon cycling than presently thought
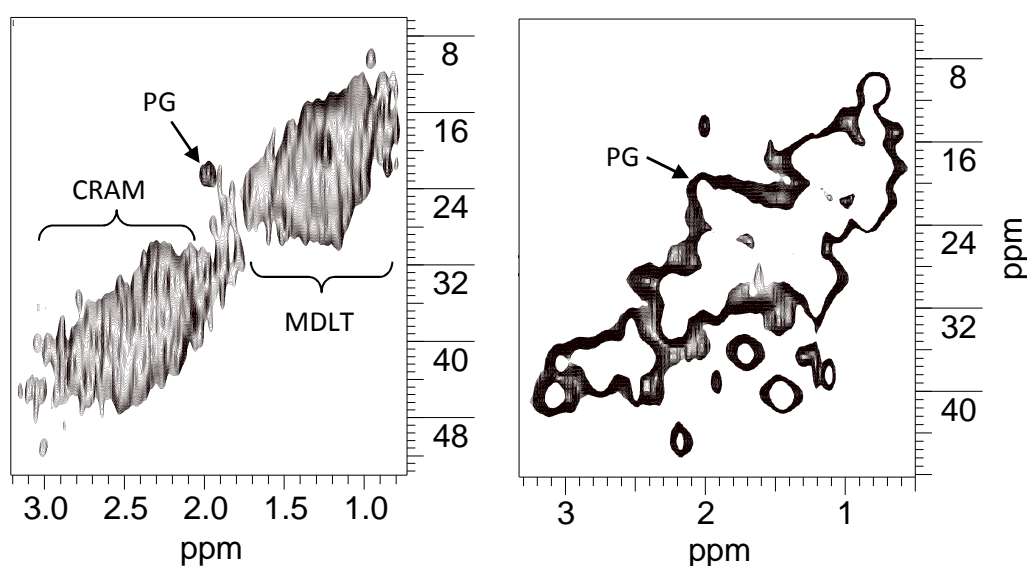
Figure 2.15 HMQC of the expanded aliphatic region of A. Dromineer and B. 14 week leachate. Abbreviations are: CRAM; carboxyl-rich alicyclic molecules, PG; peptidoglycan and MDLT; material derived from linear terpenoids.

Figure 2.16 displays the ^{1}H NMR spectra for the aromatic region of two sample sites in L. Derg (Coole Bay and Dromineer). The samples display generally similar profiles and ratios of major chemical constituents. However, strong resonances that can be assigned to phenylalanine in the Dromineer spectrum (and to a lesser extent Portumna and Williamstown) do not appear to be present in the Coole Bay sample (Simpson, et al. 2007). Phenylalanine is the most commonly found aromatic amino acid in proteins and enzymes, is invariably present in any animal tissue and is also synthesised by common pathways in phytoplankton and bacteria.
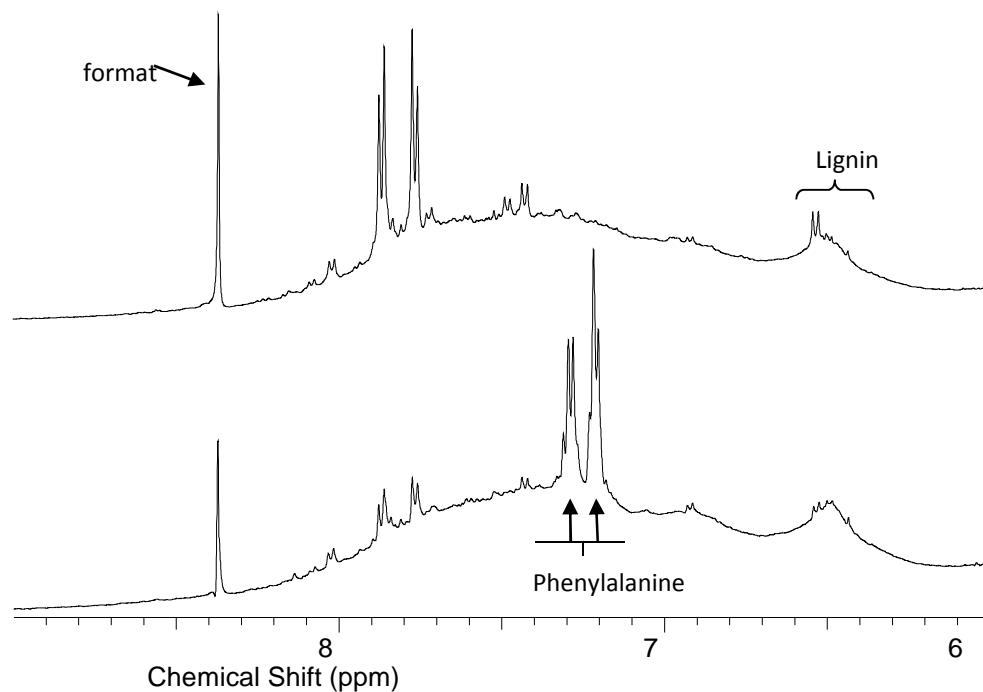
FIGURE 2.16 ^{1}H NMR spectra for (A) Coole and (B) Dromineer samples.

It is considered an easily degraded hydrolysable amino acid (Yamashita and Tanoue 2003) and therefore its elevated presence in some samples is interesting. Phenylalanine has been associated with increased NH_4^+ concentrations in water (Jones, Dennison and Stewart 1996) which in turn is a product and indicator of the presence of nitrogenous organic wastes. Dromineer is strongly influenced by the Nenagh River which passes through land that is utilized for agriculture and raising livestock and it also accommodates a sizable public marina. Higher phenylalanine concentrations may therefore be an indicator of elevated organic wastes from agriculture and industry. Interestingly, there appears to be little phenylalanine present in the Coole Bay sample which is south of the Dromineer sampling site. Two possible reasons for this are: 1. the site is secluded, surrounded by forestry and not fed or influenced directly by a river and 2. during the sampling period from August to September, Zebra Mussels (*Dreissena polymorpha*) were evident at

highest concentrations on the eastern side of Lough Derg at Coole Bay. The filtering activities of zebra mussels have been shown to have a large ecosystem-level influence on nitrogen cycling (Mellina, Rasmussen and Mills 1995, Arnott and Vanni 1996, Conroy, et al. 2005) and organic nitrogen concentrations decrease in water columns in microcosms with live zebra mussels (Bykova, et al. 2006). It is therefore possible that the filtering activities of Zebra mussels results in a recycling of larger organic nitrogen compounds such as phenylalanine. The presence of formate in both samples suggests a pathway of organic carbon degradation mainly reported for anoxic marine sediments (Hansell and Carlson 2002) and indicates that anoxic breakdown by various micro-organisms is taking place in the lake. Formate and other volatile fatty acids (VFA) are products of hydrolysis and anaerobic fermentation (Mopper and Kieber 1991). The anomeric section of the Ballina DOM HSQC spectrum is shown in Figure 6 and again confirms the strong resonance in the anomeric CH region that is seen in Figure 2.3.2.1 A. Assignments are consistent with those of degrading celluloses and xyloses as reported by (Matulova, et al. 2008) and their presence is likely due to the degradation of plant material.

Given the influence of terrestrial organic matter on marine DOM and the similarity of the structures of both, it is difficult to assess the source of DOM and whether it is aquatic or terrestrial in origin. The findings in this study point to a strong terrestrial input of recalcitrant material that can be influenced by land management and human activities. The traditionally held view that soil microbial biomass is often associated with the labile, readily degradable component has recently been challenged and it has been shown that larger contributions of microbial peptide/protein are found in the HS fraction than previously thought. Considering the amounts of microbial material in soil extracts and the results of this study, it is likely that soil and aquatic microbial biomass has a major influence on the structures in DOM.

3.0 CONCLUSION

Given the influence of terrestrial organic matter on marine DOM and the similarity in the structures of both, it is challenging to assess the source of DOM and whether it

is aquatic or terrestrial in origin. The findings here suggest a strong terrestrial input of recalcitrant material. Land management and human activities are important factors influencing the spatial distribution of DOM within the lacustrine environment. Differences observed in the yields, ^{1}H and HMQC spectra of DOM isolated indicate that watershed influences both the chemical composition and quantity of DOM within Lough Derg. Yields varied both temporally and spatially between each sampling point. The most notable variance in quantity being at Williamstowns Harbour. High yields observed at this site were likely due to the presence of inorganic salts at the site indicated by the high conductivity levels recorded at the same site during the same sample period. The input of plant material is confirmed by the presence of lignin-type signatures, whereas the influence of microbial biomass from either terrestrial or aquatic sources is highlighted by resonances for peptidoglycan and protein. The presence of phenylanine at varying quantities at a number of sample points which indicates influence of agricultural waste on the watershed, which is evident at Domineer due presence of phenylanine, high TOC and COD values at the site. Further to this the absence of phenylanine and low TOC values coincided with the high population of zebra mussels at Coole bay. Soil microbes may also contribute to silicon cycling through stable organo-silicon structures within the cells. The study also confirms the presence of CRAM in DOM from an Irish lake, which suggests that it may be globally ubiquitous.

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CHAPTER 3

PASSIVE SAMPLER FOR THE ISOLATION OF DISSOLVED ORGANIC MATTER FROM MARINE ENVIRONMENTS

3.0 INTRODUCTION

Oceanic DOM represents one of the most important reservoirs of organic carbon within the ocean. The amount of DOC in the ocean ($700x\ 10^{15}g$) is comparable to the amount of CO_2 within the atmosphere ($750 \times 10^{15}g$) (Benner 2004). Net oxidation of just 1% of this marine DOC would generate more CO_2 than that produced annually by fossil fuel combustion (Hansell and Carlson 2002a). In addition to its importance in the global carbon cycle, DOM within the ocean can alter the spectral properties of seawater and the surface properties of minerals. It effects metal bioavailability and toxicity and plays a vital role in the cycling of bioactive elements (Benner 2004).

Despite its importance in global and oceanic processes, many of DOM's chemical and structural components have yet to be elucidated due to current isolation and analytical limitations. Furthermore, it is estimated that erosion-induced deposition of terrestrial organic carbon (TOC) into coastal water and sediments is between 0.4-0.6 Gt/year (Lal, R. *Science*, 2004. 304, 1623-1626. The fate of this huge carbon deposit and mechanisms controlling biodegradation remain uncertain due to the challenges posed by the need to sample in both fresh and ocean water. In order to compare fresh water to marine water DOM similar sampling approaches need to be taken and this has provided a challenge to natural organic matter scientists. The development of isolation and concentration techniques to recover DOM from marine environments in its natural form can be problematic due to high salt concentrations compared with the low concentrations of DOM. A wide variety of DOM isolation and concentration techniques have been used in marine environments. Currently the most commonly used are solid-phase extraction and ultrafiltration that recovers approximately 30% of marine DOM. In solid-phase extraction DOM is adsorbed onto a single non-ionic macroporous sorbent (XAD-2 or XAD-8). DOM isolated using this technique undergoes very large changes in pH as

water samples are acidified to pH 2 before extraction. This fluctuation in pH may alter the structure and composition of the isolated material. Tangential-Flow Ultrafiltration relies on forcing the water sample through 1000-Da cut-off filters (Hansell and Carlson 2002b). These techniques rely on grab/active sampling techniques requiring large sample volumes to be taken at any one location for the duration of the sampling period. These approaches can lead to costly, time consuming, labour intensive onsite practices. The passive sampling approach avoids some of these problems due to its low cost and need for maintenance, unattended operation and its independence from power sources (Kot, Zabiegała and Namieśnik 2000, Seethapathy, Gorecki and Li 2008) The passive sampling approach provides a means to collectively filter, isolate and concentrate DOM in the field for the duration of the sampling period (Lam and Simpson 2006) . Lam et al (Lam and Simpson 2006) successfully developed a passive sampler to economically and effectively isolate and concentrate DOM from freshwater (see chapter 2). However, the sampler developed was found to be unsuitable for saltwater environments. DEAE cellulose is the sorbent used in these passive samplers and is an ion exchange based resin which adsorbs negatively charged DOM at neutral pH. Therefore in a saltwater environment, the high concentration of Cl^- ions will compete for binding sites on the resin. Activated carbon and montmorillonite clay matrix has been shown to be a successful sorbent in the removal of DOM from salt water environments at neutral pH (Newcombe, Drikas and Hayes 1997). Here we investigate the activated carbon and cation exchanged montmorillonite as potential sorbents in isolation of DOM from both saltwater and freshwater environments. We have designed an experiment that will allow a complete assessment of activated carbon as an efficient DOM sampler.

3.1 MATERIALS AND METHODS

3.1.1 Preparation of various monmorillonite complex's for use as sorbent materials in marine and freshwater passive samplers

Monmorillonite (STx-1b (250 grm/unit) Ca-rich Montmorillonite White, Gonzales County, Texas, USA) was purchased from the clay minerals society and used without further purification. Approximately 34g of the source clay was exchanged with sodium (18.8023g) and Lithium (15.051g).

3.1.1.1 Preparation of sodium montmorillonite

Sodium-montmorillonite (Na-Mt) was prepared from Calcium-montmorillonite (Ca-Mt) by placing 18.8023g Ca-Mt, 1.2051g Na_2CO_3 and 200ml DI H_2O into a conical flask and then incubating this in a bath at 80^0C with magnetic stirring for a period of 3hrs. During mixing several drops of HCl were added to remove carbonate ions (CO_3^-). The mixture was then centrifuged at 6000rpm for 10mins a number of times. The supernatant containing salts was decanted and fresh DI H_2O added and mixed vigorously before centrifuging again. The resulting Na-Mt was then dried at 105^0C overnight, after which the dry clay was ground up and sieved through 200µm sieve. The sieved clay was then transferred to a glass jar and stored under vacuum in a desiccator. The final yield of Na-Mt was calculated to be 10.0844g

3.1.1.2 Preparation of Lithium montmorillonite

Lithium-montmorillonite (Li-Mt) was also prepared from Ca-Mt in a similar fashion except using $200cm^3$ 1M LiCl and 15.0031g Ca-Mt (with small volume of DI H_2O added to create slurry). The slurry was added to the 1M LiCl with magnetic stirring and no temperature setting. The reaction was left for 48hrs. Centrifugation was carried until the resulting supernatant yielded a negative (clear instead of white precipitate) result for Cl^- using the 0.1M $AgNO_3$ test. The final weight for the Li-Mt clay was 6.7660g.

3.1.2 Passive Sampler Construction

Passive samplers were constructed as follows

3.1.2.1 Construction of activated carbon passive samplers

The passive sampler (figure 3.2) consists of 1 g of Activated Carbon (sorbent), contained within a Polyvinylidene Fluoride PVDF membrane, 0.22 µm, all contained within a 125ml Nagelene high density polyethylene (HDPE) screw top containers with pre-drilled holes. After drilling, any sharp splinters remaining on the inside of the container were filled to prevent damage to the membrane. The activated carbon is placed into the PVDF membrane (8.5cm length, 3cm width) and heat sealed. This length and width of membrane allow activated carbon mobility within the sealed membrane. Six sealed membranes are placed in the pre-drilled HDPE protective casing to form the passive samplers.
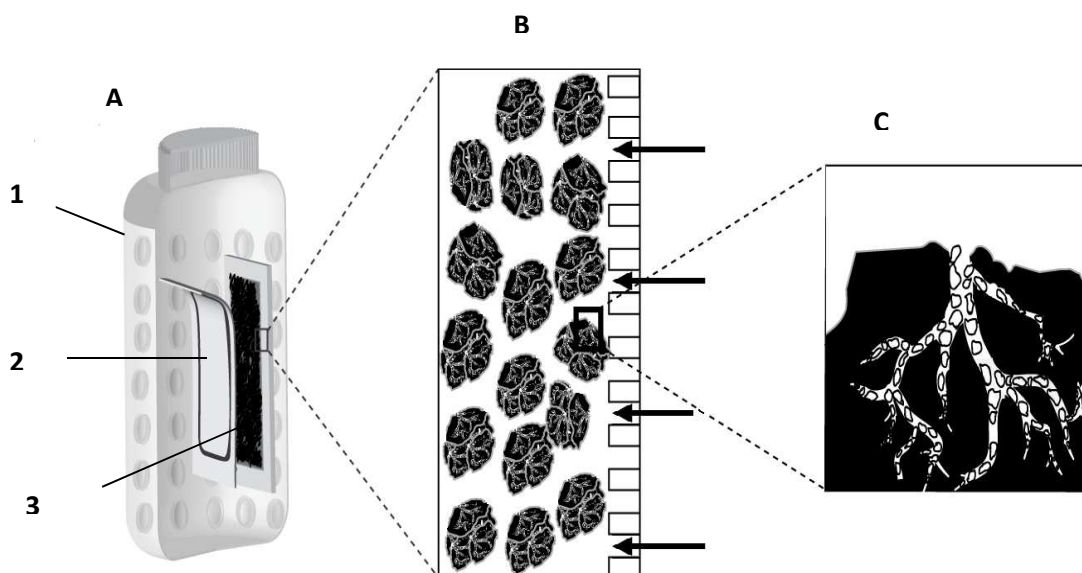
FIGURE 3.1 (A) Schematic showing the components of the passive sampler for saltwater environments. (1) Porous HDPE casing to house sampler unit to prevent large organisms such as fish and debris compromising the membrane. (2) Polyvinylidene Fluoride PVDF membrane, 0.22 µm. (3) Activated Carbon. (B) Expanded region of the activated carbon/membrane/water interface. Dissolved organic matter enters the membrane and is adsorbed on to the activated charcoal. Large species such as particulate organic matter and large biological species cannot

enter the membrane. The use of the membrane removes the need for filtering. (C) Expanded region of the surface of an activated carbon particle.

3.1.2.2 Construction of clay passive samplers

1 g of each montmorillonite was contained in 0.22µm polyvinylidene flouride PVDF membrane (3cm x 7cm) and contained within a 125ml Nagelene high density polyethylene (HDPE) screw top containers with pre-drilled holes as described in section 3.3.2.1

3.1.3 Sampler Deployment within a marine environment

Activated carbon studies took place over 56 day period. A total of 60 passive samplers were deployed (20 at each site) at 3 sites 15km apart. Prior to deployment geophysical properties such as depth, wave exposure and surrounding geological influences were determined. Clay sorption studies were carried out over a 28 day period at sample site 2 (see figure 3.3)
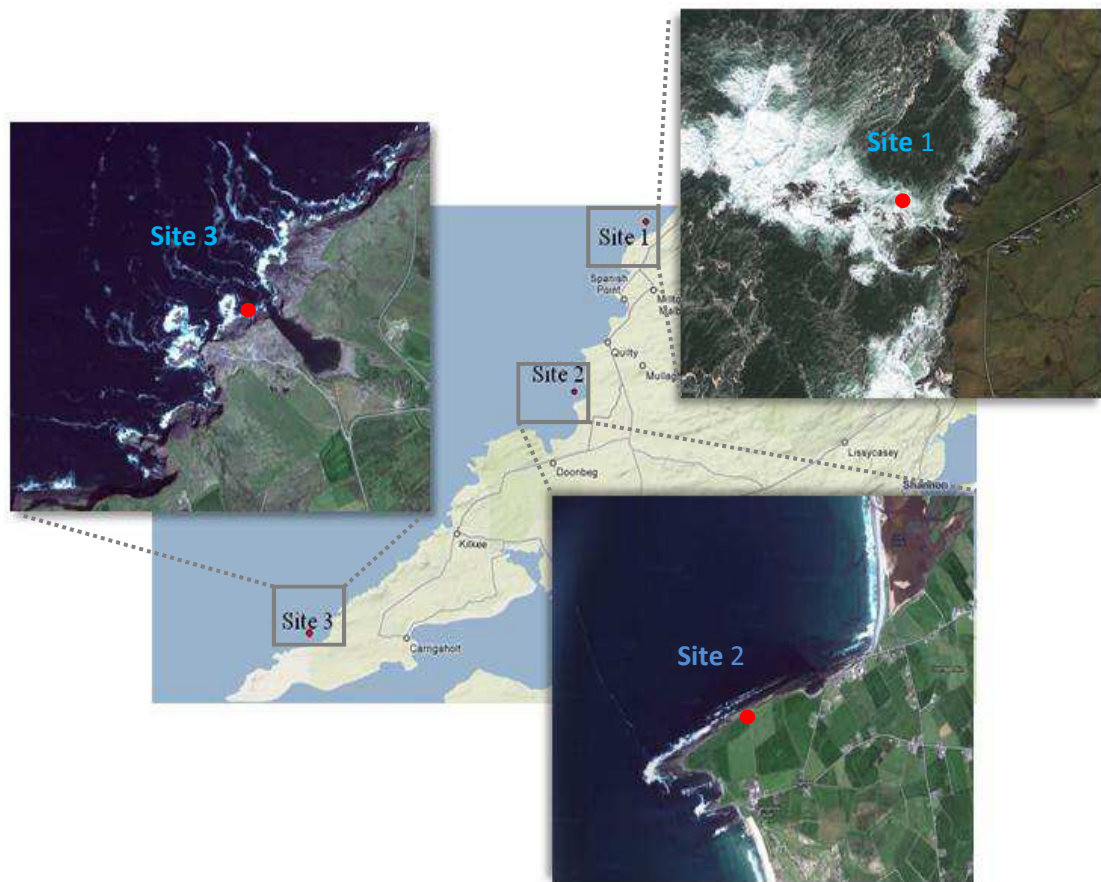
FIGURE 3.3 Map of the west Clare Coastline, Ireland, showing each sampling location and the approximate location of each passive sampler.

Field studies for activated carbon samplers were carried out at three separate sampling locations (figure 3.3) in the Atlantic sea off the west coast line Ireland. Prior to deployment at each sampling location passive samplers were attached in triplicate to a weighted suspension line using a 2mm braided polyethylene braided rope. The suspension line used to keep the samplers in a vertical position (see figure 3.2) consisted of a folding anchor attached to the bottom of the line and a coloured buoy fixed to the top. At each site samplers were deployed below the surface of the water and at a fixed distance from the sea bed to ensure samplers were submerged below the water level at all times. Three activated carbon passive samplers were retrieved from each site after days 1, 3, 7, 14, 28, 52. The samplers were then placed in a sample bottle filled with the water from the sampling site to avoid the receiving phase (activated carbon) from drying out.

Buoy

Water level

Suspension line

2mm braided polyethylene
braided rope

2mm braided polyethylene braided rope

125ml Nagelene HDPE containers with pre-drilled holes

3.7kg folding anchor
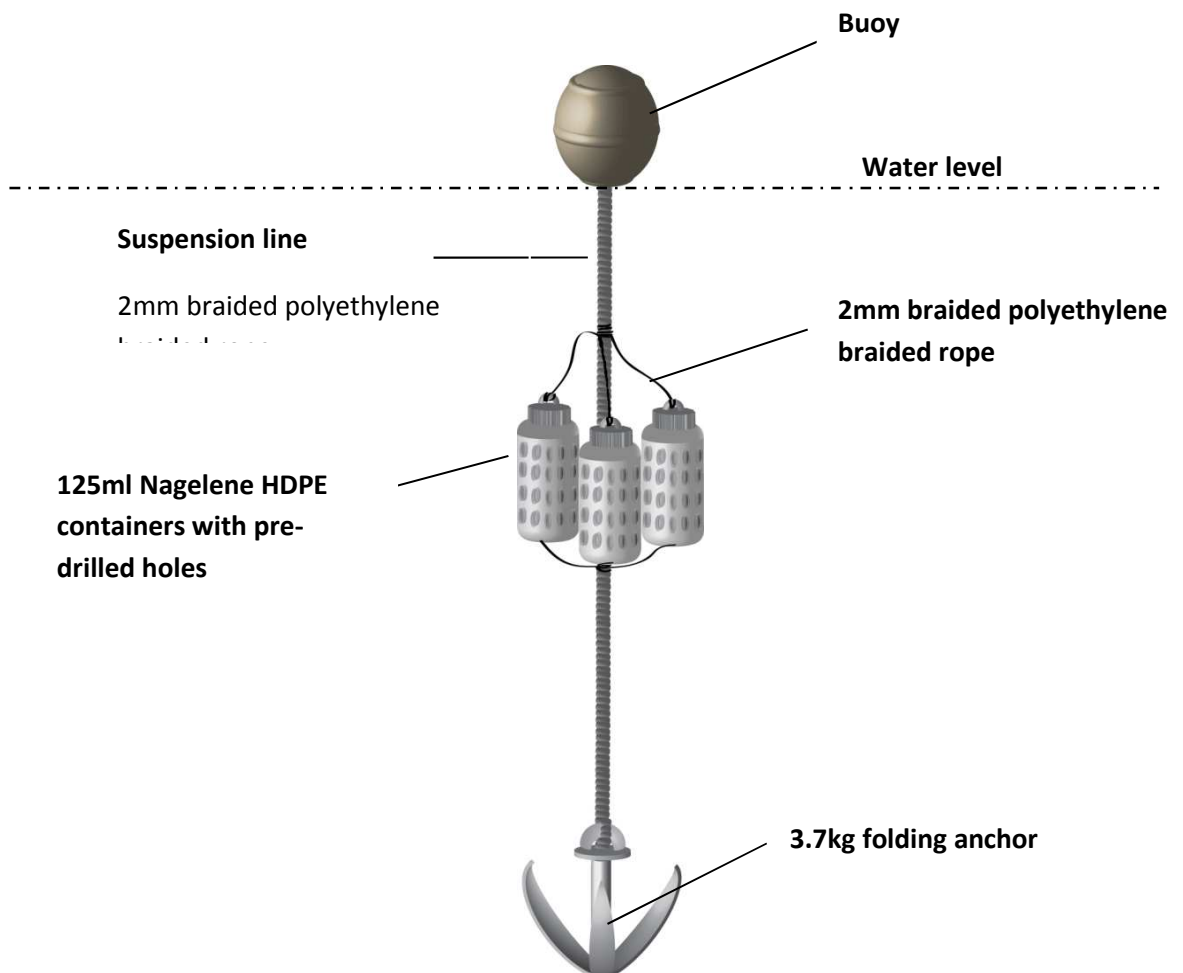
FIGURE 3.4 Diagram illustrating the components necessary for the deployment of the passive samplers

Three composite sea water samples (5 L) were collected from each site. Water samples were collected in 5 L amber (HDPE) bottles. All samples were refrigerated during transport to the laboratory and stored at 4ºC until analysis. The pH of the water at each site was measured *in situ* using a EUTECH waterproof hand-held meters.

3.1.4 Deployment of clay passive samplers in a Freshwater environment

Clay passive samplers described in section were deployed for a 28 day period at the mouth of the Ardcloony River on the River Shannon south of Lough Derg. The surrounding catchment is largely dominated by grass based agriculture.
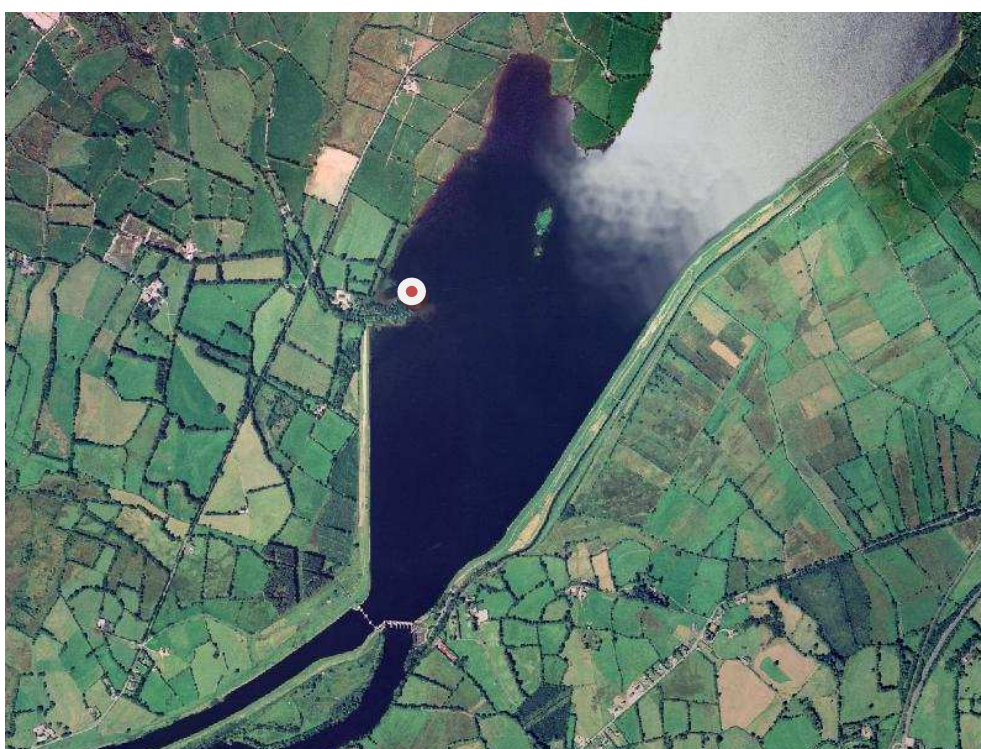
FIGURE 3.5 Picture showing the approximate location of the fresh water clay passive samplers at Ardcloony, Co. Clare.

3.1.5 Sample Recovery

Extraction of adsorbed DOM from the passive samplers was performed by cutting the sealed PVDF membrane and removing the activated carbon. The activated carbon was then placed in clean 250ml Teflon centrifuge tubes and extracted using 40mls of 0.5 M sodium hydroxide. The tubes were then sonicated for three hours and passed through a 0.22 µm PVDF membrane using vacuum filtration. An aliquot of the resulting solution was isolated for TOC analysis while the the remaining sample

was ion exchanged using Amberjet 1200H Plus resin adjusted to pH 6 and freeze-dried. The resulting material was weighed and stored at -22°C for further analysis.

3.1.6 Sample Extraction

3.1.6.1 Extraction of DOM from activated carbon passive samplers

Extraction of the bound DOM was performed by cutting and removing the activated carbon from the PVDF membrane using a Teflon spatula. The activated carbon was then placed in a 250ml centrifuge tubes and extracted using 40mls of 0.5M sodium hydroxide. The tubes were sonicated for 3hrs and centrifuged at 10000g to pellet the activated carbon. The supernatant was passed through a 0.22µm PVDF filter membrane using Buchner filtration to remove activated carbon. The extracted DOM was then ion- exchanged using Amberjet 1200H plus resin and freeze-dried. Before freeze drying an aliquot of each sample was removed for TOC analysis.

3.1.6.2 NMR analysus

Each sample (100 mg) was re-esuspended in 1 mL of deuterium oxide (D_2O) and titrated to pH 13.1 using NaOD (40% by weight) to ensure complete solubility. Samples were analyzed using a Bruker Avance 500 MHz NMR spectrometer equipped with a 1H-BB-13C 5 mm, triple resonance broadband inverse probe. 1-D solution state 1H NMR experiments were performed with 256 scans, a recycle delay of 3 s, 32768 time domain points, and an acquisition time of 1.6 s. Solvent suppression was achieved by pre-saturation utilizing relaxation gradients and echoes (Simpson and Brown 2005). Spectra were apodized through multiplication with an exponential decay corresponding to 1 Hz line broadening, and a zero filling factor of 2. Diffusion-edited experiments were performed using a bipolar pulse longitudinal encode-decode sequence (Wu, Chen and Johnson 1995). Scans (1024) were collected using a 2.5 ms, 49 gauss/cm, sine-shaped gradient pulse, a diffusion time of 100 ms, 8192 time domain points, 410 ms acquisition time, and a sample temperature of 298 K. Spectra were apodized through multiplication with an exponential decay corresponding to 10 Hz line broadening and zero filling factor of 2. Total Correlation Spectroscopy (TOCSY) spectra were obtained in the phase sensitive mode, using time proportional phase incrimination (TPPI). These 2-D NMR experiments were carried out using 128 scans with 128 time domain points in the F1 dimension and 2048 time domain points in the F2 dimension. A mixing time

of 60 ms was used with a relaxation delay of 1 s. Processing of both dimensions used a sine-squared function with a /2 phase shift and a zero-filling factor of 2.Heteronuclear Multiple Quantum Coherence (HMQC) spectra were obtained in phase sensitive mode using Echo/Antiecho gradient selection. The HMQC experiments were carried out using 256 scans with 128 time domain points in the F1 dimension and 1024 time domain points in the F2 dimension. A relaxation delay of 1 s and 1J 1H-13C of 145 Hz were used. In processing the F2 dimension it was multiplied by an exponential function corresponding to a 15 Hz line broadening. The F1 dimension was processed using a sine-squared function with a /2 phase shift and a zero-filling factor of 2. Spectral predictions were carried out using Advanced Chemistry Development's ACD/SpecManager and ACD/2D NMR Predictor using Neural Network Prediction algorithms (version 10.02). Parameters used for prediction including line shape, spectral resolution, sweep width, and spectrometer frequency were set to match those of the real datasets as closely as possible.

3.1.6.3 Extraction of DOM from clay passive samplers

Extraction of DOM bound to clay passive samplers was performed by cutting and removing the clay from activated carbon from the PVDF membrane using a Teflon spatula. The clay was then placed in a 250ml centrifuge tubes and extracted as in section 3.2.5.3.

3.1.6.4 XRD analysis of marine clays

For powder XRD analysis the clay samples were placed evenly into a circular hollow aluminum holder. XRD analysis was carried out on a Phillips X'Pert diffractometer using Cu$K\alpha$ radiation. The samples were measured in the step–scan mode with steps of 0.04 [∘2θ] and a counting time of 1 s. To negate the influence of interlayer expansion due to water molecules, XRD analysis was carried out after the clays were dried at 110°C.

3.1.6.5 Ultra sonic assisted extraction of clay isolates

Isolated DOM fractions (200-250mg) were sonicated twice for 15 min each time with 2.5mls of methanol, then dichloromethane: methanol (1:1; v/v), followed by dichloromethane. The combined extracts were centrifuged and the supernatant was

decanted. The combined extracts were concentrated by rotary evaporation and completely dried in a 2 ml vial under a steady stream of nitrogen gas. The solvent extracts were redissolved in 500µl dichloromethane: methanol (1:1; v/v). Aliquots (approx 100µl) qof each extract were dried under a stream of nitrogen. The total extract is a mixture of non-polar (e.g. saturated hydrocarbons) and polar compounds (e.g. acids and alcohols). Carboxylic acids and alcohols are very polar and do not elute well on most GC-MS capillary columns, therefore aliquots of the total extracts were converted to their trimethyl silyl (TMS) derivatives by derivatives by reaction with 90µl N, O-bis-(trimethylsilyl) triflouroroacetamide (BSTFA) and 10µl pyridine. The vial was placed in an oven at 70°C for 3 hours. The vial was allowed to cool and reduced to complete dryness; 100µl of hexane containing 100ppm of the external standard cholestane was added to re-dissolve the analytes. The derivatisation of carboxylic acid yields TMS esters and alcohols yields TMS ethers. Pyridine is added as a catalyst to complete the reaction.

3.1.6.6 GC-MS analysis of clay isolates

The GC-MS analysis of the derivatised extracts was performed on an Agilent model 6890N GC coupled with to an Agilent Model 5973N quadrupole mass selective detector (MSD). The helium carrier gas was set at a flow rate of 1ml/min. Separation was achieved on an Agilent HP 5MS fused silica column (30m × 0.25mm i.d., 0.25 µm film thickness). The GC operating conditions were as follows; temperature hold at 65°C for 1min, a temperature rise from 65°C to 300°C at 6°C /min with a final isothermal hold at 300°C for 20min. 1µl of each sample was injected using an Agilent 7683 auto sampler the MSD was operated as for 4.3.3.1.

3.2 RESULTS AND DISCUSSION

3.2.1 Sampling Considerations

Although the potential advantages for use of the passive samplers described for the isolation and concentration of DOM from saltwater environments are numerous, it is important to discuss the potential drawbacks associated with the deployment of these samplers within the marine environment. The selection of an appropriate sampling point is vital for the survival of the passive samplers within the marine environment. For near shore sampling, the sampling point should have safe, easy access for the sampler deployment and collection and should be chosen at low tide to ensure that

the sampler is submerged at all times. The suspension line should be measured and marked prior to deployment so that the sampler can be set to the desired depth.
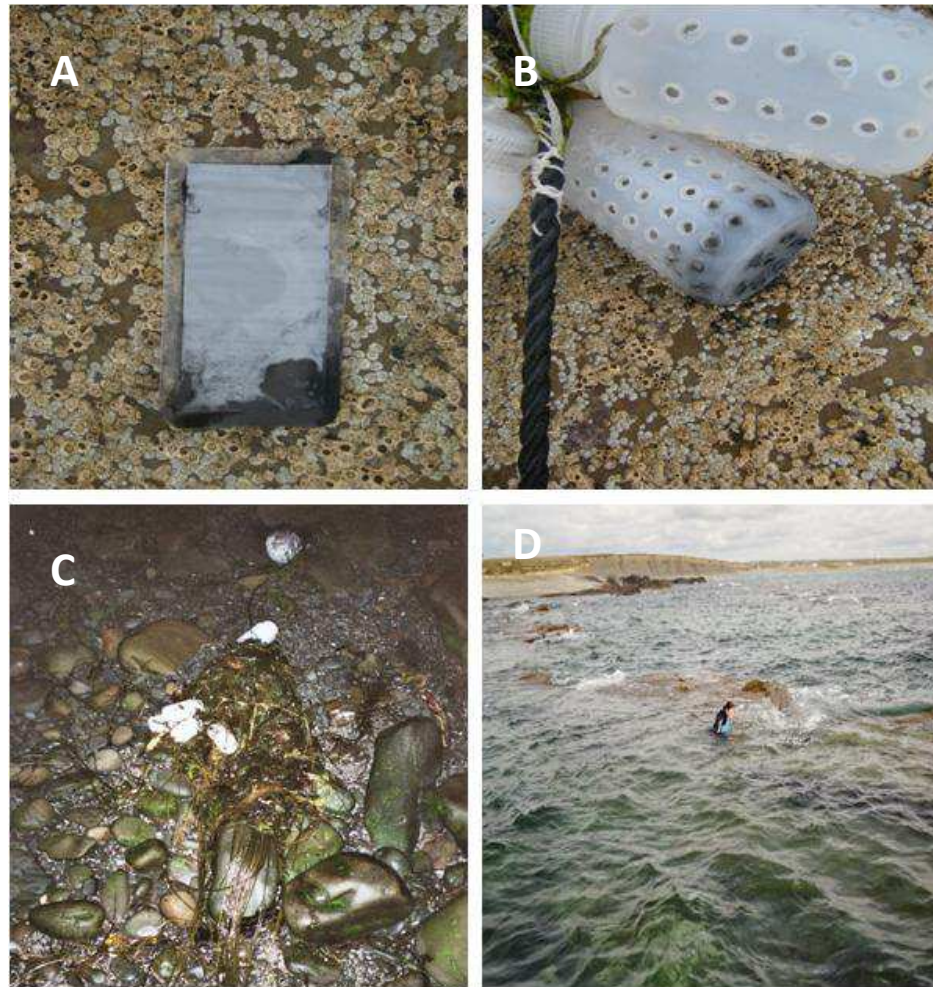
FIGURE 3.6 A, B, C, and D illustrating problems associated sampling in a marine

During the initial sampling period some of the samplers were torn (Figure 3.6 A and B) and lost (Figure 3.6 D) due to the vigorous hydrodynamic conditions of the marine environment. Reducing the size of the heat seal on the membranes from 3mm to less than 2mm reduced tearing and subsequent loss of activated carbon from the membrane. Excessive fouling (Figure 3.6 C) of the samplers occurred at sampling site one during high tide, this caused the samplers to become dislodged from its anchor and the samplers were washed inland. These samplers were disregarded and the area was re-sampled. It must be noted that the presence of a buoy is critical for identification purposes of the sampler; however it can also draw attention to the sampler increasing the threat of vandalism.

3.2.2 Sampling yields and TOC analysis for activated carbon studies

Table 1 summarises the yields isolated using from passive samplers at each sampling point. The yields are expressed as per individual sampler. Samplers were deployed in triplicate each containing 1g of activated carbon. Variations in yields are evident between each site which can be attributed to the difference in water chemistry at each site. Highest yields were isolated after samplers were deployed for 3 days at all three sites nearly twice that, was isolated after 14 days. The decrease in yield after seven days indicates desorption of the DOM from the activated carbon within the passive sampler. The capacity of the activated carbon to adsorb organic matter in saline solutions has been speculated by many researchers. Bjelopavlic et al (Bjelopavlic, Newcombe and Hayes 1999) found that increased salt contents increases the absorptive capacity of adsorbents for organic molecules. It is therefore possible to suggest that after day 3 the activated carbon within the passive sampler had reached its adsorption capacity.

Time (Days)	Site 1 Yield (mg)	Site 2 Yield (mg)	Site 3 Yield (mg)
1	246.567	195.033	137.200
3	276.900	509.267	199.833
7	265.333	209.778	181.100
14	217.200	120.067	96.000
28	58.467	182.733	100.067
56	45.666	201.733	75.367

* Samplers were deployed in triplicate each containing 1g of activated Carbon. Yields are expressed per individual sampler

Due to the interference of salts yields alone cannot be used to assess the amount of DOM adsorbed by the activated carbon. To further assess the ability of the passive sampler to adsorb DOM from the ocean the amount of TOC within each sample was determined. Figures below illustrate the uptake of TOC on passive samplers from each site. Error bars indicate the standard deviation between triplicate samplers. The uptake of TOC at sample site one and two increased overtime, however at sample site two the amount of TOC decreases after 30 days which can be attributed to loss of activated carbon from the sampler at the site. This increase of TOC over time and

decrease in yield obtained suggests that salt is initially adsorbed initially adsorbed but is desorbed over time whereas the adsorption of DOM increases overtime
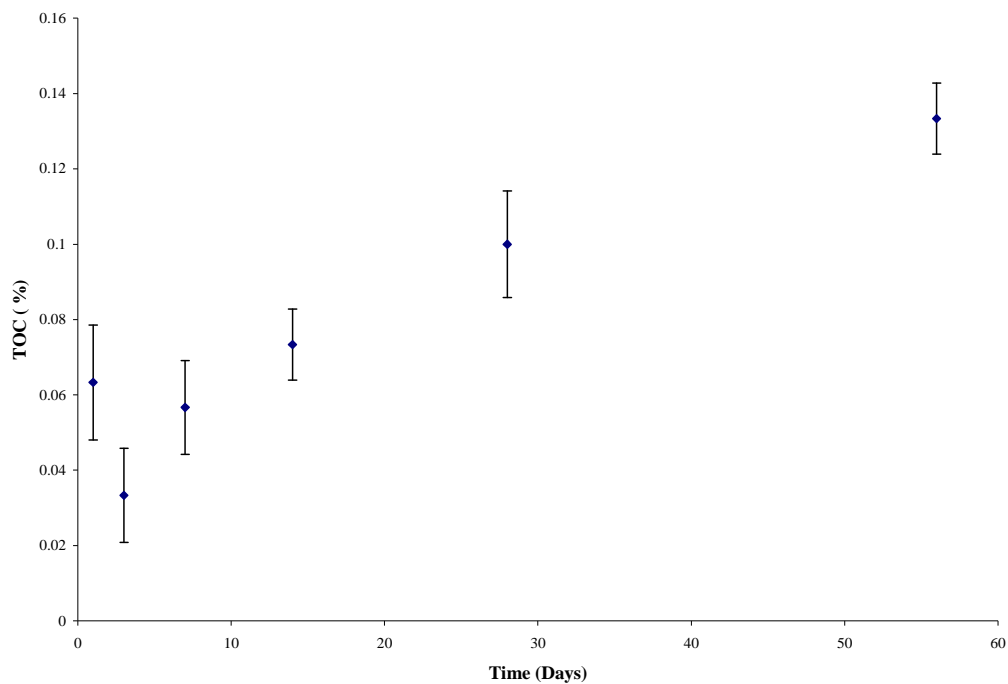
FIGURE 3.6 Illustrates the uptake of TOC on passive samplers at sample site one (figure 3.3)
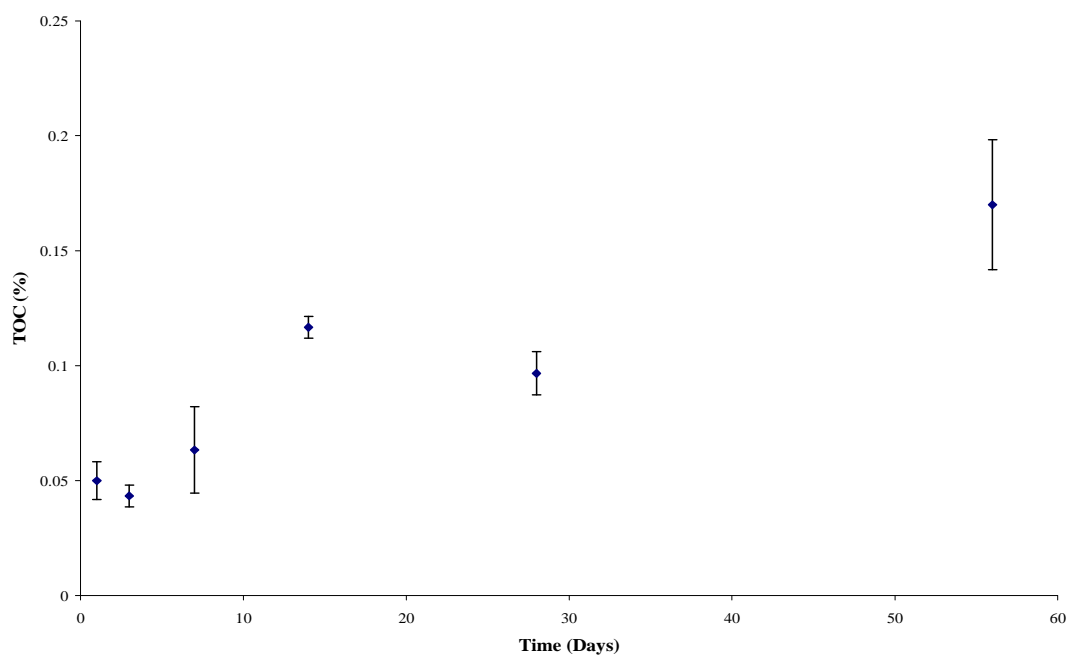
FIGURE 3.7 Illustrates the uptake of TOC on passive samplers at sample site two (figure 3.3)
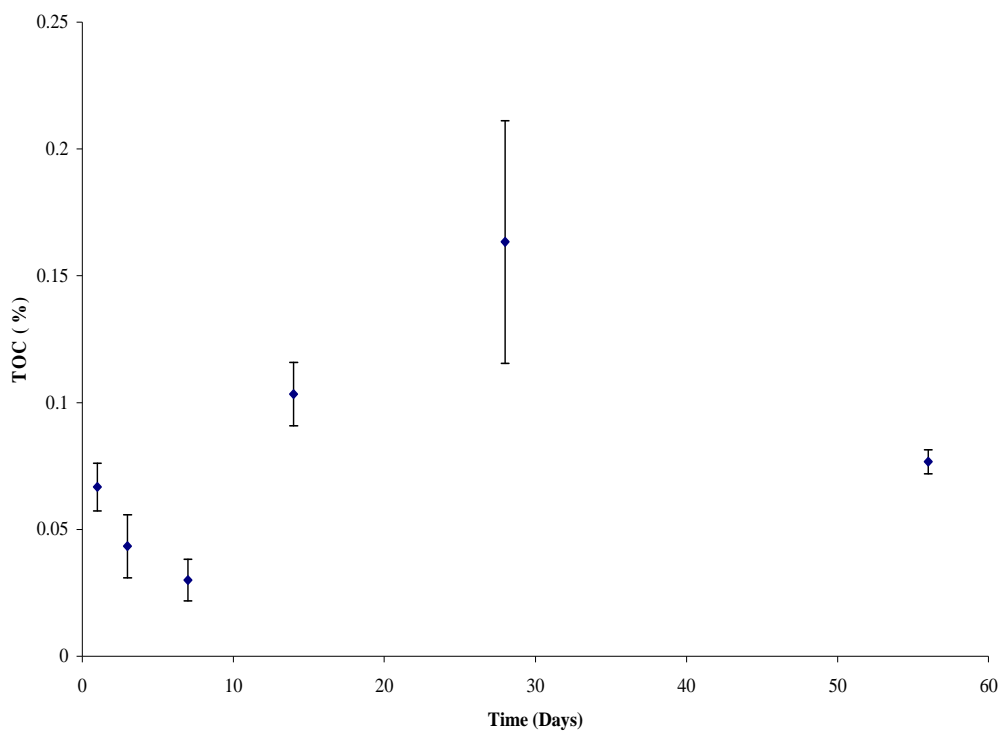
FIGURE 3.8 Illustrates the uptake of TOC on passive samplers at sample site three.

3.2.3 NMR analysis on activated carbon sorbents

The results from NMR analysis of the activated carbon DOM were disappointing in that the spectra are not similar to any previous work on freshwater or marine DOM. Potential components of DOM are present as seen in Figure 4.8. There is a broad protein background (designated "P" in the spectrum, Figure 4.9) and evidence of the recent degradation of organic matter (formic, lactic and acetic acid) but the signature resonances for DOM that depict CRAM and MDLT are absent (McCaul et al). The presence of glycerol is also unusual and some of the samples turned blue in colour when dissolved in NMR solvent (D_2O). For these reasons it was decided not to proceed any further with this part of the project but it would be interesting to investigate what happened in future work.

3.2.3 X-ray diffraction analysis of marine clays

On comparison of XRD patterns generated before the use of the montmorillonite clay as a passive sampler and after show that the basal spacing representing montmorillonite, originally at approx. 7.2 [∘2θ] (this peak shifts slightly depending on the cation exchanged) had moved downwards to an average of 6.5 [∘2θ]. This is interesting as it may mean that intercalation of DOM components is occurring in the interlamellar spacings of the clay. Further work will be required to prove this but if it is the case then it would be interesting to know what species are preferentially intercalated as they will afforded most protection from degradation over time. A suitable technique to analyse the organic material that is intercalated would be High Resolution Magic Angle Spinning NMR (HR-MAS-NMR) that allows the analysis of solid materials by adding a solvent to the analyte (in this case the clay-organo complex) and after swelling, the organic components become NMR observable (Keifer et al., 1996). The challenge here however would be to initially extract all the DOM attached to the outer surfaces so as not to mix this component with the intercalated material.
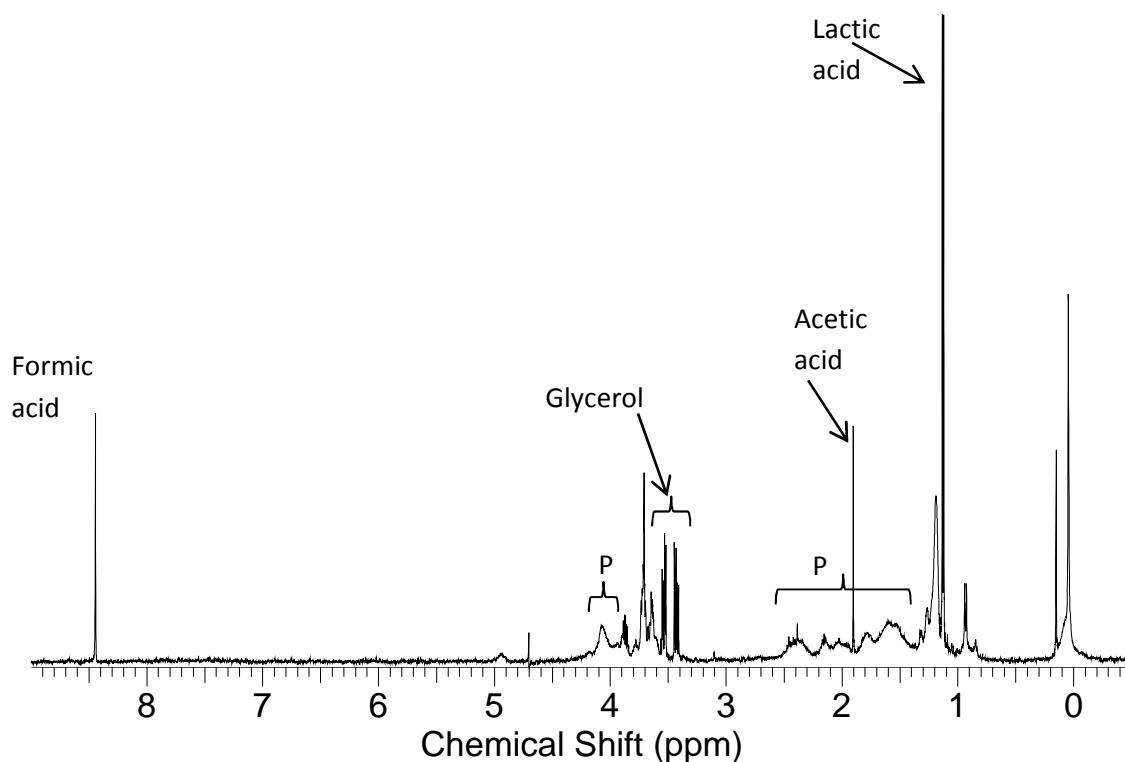
FIGURE 3.9 ^{1}H NMR spectrum for activated carbon at site 2. "P" represents areas on the spectrum that can be attributed to protein.

3.2.5 Montmorillonite as a possible sorbent for DOM in marine and freshwater environments.

The aim of this study was to evaluate the use of cation (Li and Na) exchanged montmorillonite as a possible sorbent of DOM in situ within marine and freshwater environments. Clay and mineral surfaces are known to stabilize and preserve natural organic matter in nature. For example, Mayer, 1994 demonstrated a strong correlation between organic carbon concentration and mineral surface area in continental shelf sediments (Mayer 1994). Intimate relationships have been found to exist between soil organic matter and clay surfaces (Baldock and Nelson 2000)(Chorover and Amistadi 2001) and organic matter conformation at the solid-aqueous interface has been proposed to govern pollutant sorption (Simpson 2001). Furthermore, these crystalline surfaces are likely to have played several important roles in life's geochemical origins (Hazen and Sverjensky 2010). Understanding the mechanisms of sorption and preservation is therefore necessary for improving the fundamental understanding of soil, sediment and water biogeochemistry and environmental reactivity. Most studies on mineral-organic matter interactions in an aqueous environment focus on one solute in water with at most a single electrolyte at room conditions. These experiments are essential to obtain baseline information. However, these studies do not replicate environmental complexities, including salts in seawater, competition among organic species, and numerous competing mineral phases and surfaces, all present over a range of temperature, pressure, pH, and solute concentrations (Hazen and Sverjensky 2010). The use of clay as a passive sorbent is, to our knowledge, the first time that clay has been used to isolate and characterise DOM from natural environments. From the mass spectroscopy results the clay appeared to attract at least as much DOM as more traditional techniques, including the simple filtration and freeze drying of water. Further work is required to validate and quantify its use as a passive sampler but these initial results show that there is great potential for its use. As an exercise to investigate what organic species preferentially sorbs to a clay mineral in the environment, this also has been an interesting study. Aliphatic species are known to preferentially sorb to clay surfaces (Simpson, et al. 2006) and this is what has occurred here. However, we have also seen sugars sorb onto the surface and the mechanisms of how this occurs would be an interesting subject for further study.

Solvent extraction followed by GCMS analysis showed the presence of organic matter in all clays after interaction with marine and freshwaters. GCMS investigations revealed the sorption of fatty acid methyl esters (TMS), alkanes, alkenes, sugars and sterols (see table 3.2). X-ray diffraction analysis showed that intercalation between clay platelets was also taking place. This would suggest that the interaction of the montmorillonite and DOM also involves a change in the structure and properties of the clay itself (Kelleher, et al. 2004). The identity of the intercalating species is unknown but it would be very interesting to identify them in future work as they are likely to be well protected against chemical and biological degradation interatctions (Majzik and Tombácz 2007). These results contradict the findings of Drouin et al (Drouin, et al. 2005) who found that organic molecules sorbed onto montmorillonite could not be removed using traditional solvents such as those employed in this study. Overlaid GCMS chromatograms for freshwater Li montmorillonite and Na montmorillonite are shown in 3.10 with marine Li montorillonite and marine Na monmorillonite shown in Figure 3.11. Table 4.2 shows the accompanying peak identifications for each chromatogram; the 'tick' in each column indicates the presence of that compound in that chromatogram. Li montmorillonite in both fresh and marine waters yielded chromatograms with a greater peak distribution and higher abundance than that obtained from fresh water and marine Na. One possible explanation for this variation could be the possible aggregation of the Na montmotillonite within the passive sampler 'pocket' affecting its adsorption properties (Yoko, et al. 2009).
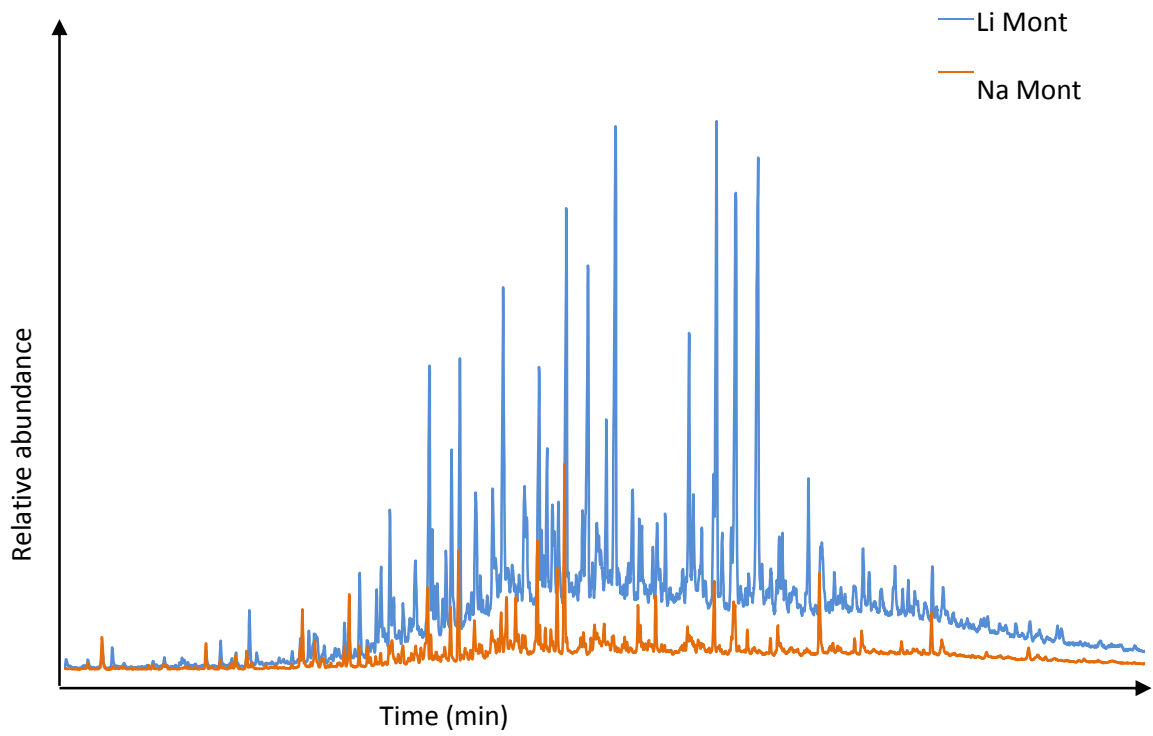
FIGURE 3.10 GC chromatograms (TIC) for DOM isolated onto lithium and sodium exchanged clays within a fresh water environment.
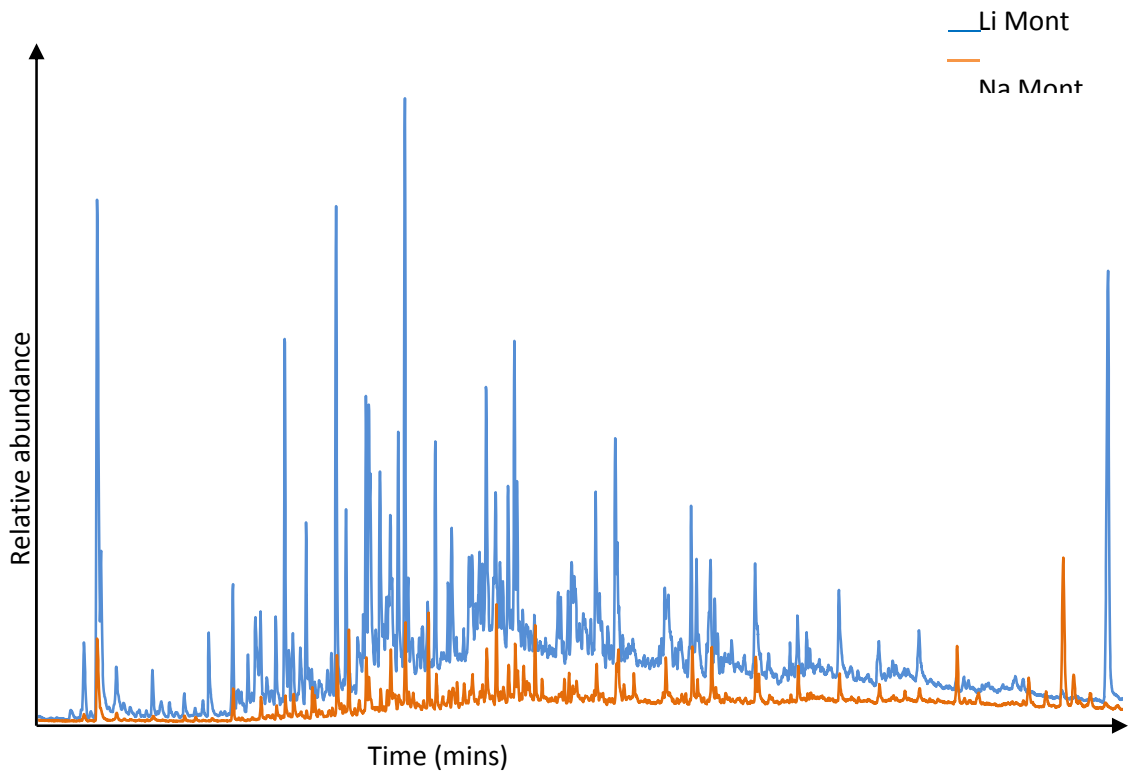
FIGURE 3.11 GC chromatograms (TIC) for DOM isolated onto lithium and sodium exchanged clays within a marine environment.

In total 45 compounds were identified with alkanes dominanting the compound classes in both fresh and marine water isolates.

n-Alkanes isolated using Li montmorillonite (mont) passive samples ranged from C_{13} to C_{30} with no odd over even predominance. Branched alkanes 7 and 8 methylpentadecane were identified in marine Li mont and are found in lagoonal algal mats (Hansell and Carlson 2002b). This alkane distribution suggests influences from marine macrophytes including maerl, kelp, and filamentous red and green algae (Wild et al. 2009). Na mont n-alkane distributions were bimodial, the first mode ranged from C_{13} to C_{19} with an odd over even predominance with C_{15} and C_{19} being the most abundant. The second mode consists of C_{23} to C_{28} with an even over odd dominance suggesting microalgal influences. However, it could be argued that the molecular n-alkanes were derived from organic matter of terrestrial origin (Aichner, Herzschuh and Wilkes 2007). Odd long chain alkanes from C_{25}, to C_{31} are particular abundant in higher plant waxes (Frazier, et al. 2003). These alkanes were not abundant in marine isolates, and during the sampling period red and green algal along with kelp were particularly abundant in the seawater at the sampling site (site 2). The presence of these macrophytes may account for the alkane distributions identified. Alkanes isolated from fresh waters using both Li and Na montmorillonite ranged from C_{14} to C_{26} with a strong even over odd predominance and indicate contributions from terrestrial plants (Aichner, Herzschuh and Wilkes 2007). Fatty acid TMS profiles are similar for both freshwater clays ranging C_{15} to C_{19} are indicative of algal distributions. Octadecanoic fatty acid methyl ester is also indicative of algal contribution and was found in all clay isolates.

TABLE 3.2 List of elucidated compounds isolated from marine and freshwater environments using Li and Na montmorillonite

Compound	LiFresh	NaFresh	LiMarine	NaMarine
Alkanes				
Tridecane			✓	
Tetradecane	✓		✓	
Pentadecane			✓	✓
Heptadecane			✓	
Octadecane			✓	
Nonadecane			✓	✓
Eicosane	✓	✓	✓	✓
Heneicosane	✓		✓	
Docoane	✓	✓		
Tricosane				✓
Tetracosane	✓	✓	✓	✓
Pentacosane		✓		✓
Hexacosane	✓		✓	
Heptacosane	✓		✓	✓
Octacosane			✓	✓
Nonacosane	✓		✓	
Tricontane			✓	
Methylated Alkanes				
7 methylheptadecane			✓	
8 methylheptadecane			✓	
10 Nonadecane				
2-methyl-7-nonadecane	✓		✓	
FAME				
Tetradecanoic Acid, trimethylsilyl ester	✓	✓		
Heaxdecanoic Acid, trimethylsilyl ester	✓	✓		
Octadecanoic Acid, methyl ester	✓	✓	✓	✓
cis-9-Octadecenoic acid, trimethylsilyl ester		✓		

Sugars				
Sucrose	✓	✓		
Sterol				
Cholesteral trimetyl ester	✓	✓	✓	✓
Stigma sterol trimetyl ester				✓
Beta Sitosterol	✓			✓
N containing				
Dodecanenitrile	✓			
Tetradecanenitrile	✓			
hexadecanitrile	✓		✓	
Phenol				
1,2-Dimethyl-3-(4-methylphenylsulphonamido)-indole	✓			
Phenol 2,4 bis(1,1 di methyl ethyl)			✓	
Diterpene				
Cembrane	✓			
Ketone				
8 Pentadecone	✓			✓

Cholesterol was found in both freshwater and marine clays, its origin within dissolved organic matter can be difficult to identify as cholesterol is almost ubiquitous in nature, and is found in most animal's macrophytes, and phytoplankton (Hassett and Anderson 1979). The clay passive sampler's location during this study can give us further insight into the origin of cholesterol. Freshwater passive samplers were located at Ardcloony (see figure) on the Shannon River south of Lough Derg. The surrounding landscape is predominantly grass based agriculture suggesting that the cholesterol isolated from freshwater originated from animal influences. Marine passive samplers were located at sample site 2 (figure 3.2) large red and green algae

mats were observed during the sampling period suggesting algal as the main source of cholesterol during the sampling period. Stigma sterol and beta sitsterol are indicative of terrestrial plant influences but can also be found in algae. The presence of sitosterol in Na mont marine and the alkane distributions observed suggest that that marine macrophytes are the main influence on the composition of DOM at sample site 2 (figure 3.2).

3.4 CONCLUSIONS

Initial TOC results indicated that activated carbon may be a potential sorbent for DOM within a marine environment. Results suggested that organic carbon sorption onto activated carbon increased over time. However ^{1}H NMR spectra did not include DOM signatures such as CRAM and MDLT which are ubiquitous in DOM originating from marine and freshwaters (Simpson, et al. 2006, Hertkorn and Kettrup 2005). These results suggest that although organic carbon may have sorbed onto activated carbon it did so in small amounts due to possible interferences from Cl^{-} ions. Alternatively, DOM sorption was successful but we were unable to extract it all despite the traditional conditions employed. If harsher conditions were used it is likely that the structural integrity of the DOM would be compromised and therefore an alternative sorbent was studied. Cation exchanged montmorillonite clay was then used in the passive sampler as a possible sorbent for DOM. Due to the laborious nature of the sampling protocol involved in the activated carbon study it was decided for initial studies to use the sampling protocol employed in chapter 2. Mass spectroscopy results showed that montomorillonite clay appeared to be successful in isolating at least a fraction of DOM from fresh and marine environments. Further work is required to validate and quantify its use as a passive sampler but these initial results show that there is great potential for its use in fresh and marine water environments. As an exercise to investigate what organic species preferentially sorbs to a clay mineral in the environment, this also has been an interesting exercise. Aliphatic species are known to preferentially sorb to clay surfaces (Simpson et al, 2006) and GCMS results show that this is happening here. However, it is important to highlight that the type of extractions that were used and the GCMS analysis employed will detect predominantly the aliphatic component (plus sugars, amino acids and sterols). This means that without NMR analysis at this point it is difficult to say what else, if anything was sorbed to the clay surface (e.g. CRAM and MDLT). Alkane distributions observed in both clays (Li and Na exchanged) suggest that

DOM composition is very much dependant on its surroundings. Distributions observed for marine clays suggest that the most likely source of DOM is the macrophytes observed at the sampling site during the sampling period and distributions observed from freshwater clay show a strong terrestrial influence. In addition to alkanes here we have also seen sugars and sterols sorb onto the surface and why and how this occurs would be an interesting subject for further study. Although successful in isolating a portion of DOM from both fresh and marine environments a majority of the peaks were unresolved and almost impossible to identify using NIST and Wiley libraries along with comparisons to relevant literature. To further assess the potential for such passive samplers described in this chapter other appropriate extraction and derivatisation techniques must be employed. The use of TMAH chemolysis has been previously used as a derivatisation technique for DOM. In the next chapter we l employ these techniques along with improved sampling techniques to further assess the potential of these passive samplers. Although the passive samplers described in this show great potential it is important to discuss the drawbacks associated with the sampler that may affect or alter the isolation of DOM. Firstly within the marine environment biological growth was observed on the surface of the membrane after three weeks. The presence of this growth could block pores on the membrane and/or the biological species could contribute exudates that are sorbed onto the clay effecting the overall interpretation. Therefore pre-treatment of the membrane prior to deployment maybe necessary for future studies. Secondly the aggregation of the clay within the sampling pocket may affect the sorption of organic molecules. In-laboratory studies on aggregation and sorption of DOM must therefore be carried out.

3.4 References

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CHAPTER 4

HYDROGEOCHEMISTRY OF THE SHANNON POT, CO. CAVAN

4.0 INTRODUCTION

The Shannon river basin, the largest river basin in the British Isles drains approximately 16,000 km^2 over the 280 km of the rivers course(Moriarty, International Association of Theoretical and Applied Limnology and Congress 1998). Originating in County Cavan the Shannon flows in a southerly direction to enter the Atlantic Ocean via a 113 km estuary below Limerick city. The bedrock geology of the upper region of the Shannon is highly karstified with pure bedded limestone and is of the Carboniferous age, approximately 359.2 ± 2.5 Mya. The principle industry along the course of the river is agriculture, primarily dairy and livestock farming with little heavy industry. The Shannon is named after Sionnan, who was the granddaughter of Manannan Mac Lir, or God of the Sea of ancient Irish mythology. Legend has it that she came to this spot to eat the fruit of the forbidden, or the Tree of Knowledge, which was planted by the Druids. As she began to eat it, the waters of the pool sprang up and overwhelmed her, drawing her down into it to flow out later across the land, thus the River Shannon sprung up (Gunn 1982).

The traditional source of the River Shannon is the Shannon Pot, a karst aquifer situated in the Cuilcagh Mountains, Co. Cavan. The 'pot' is a naturally fed fluctuating pool, the Shannon Pot has been explored to 14.2meters where it emerges from a 2m fissure. Water tracing experiments carried out by Gunn et al. (Gunn 1982) suggests that that the Shannon Pot drains an immediate area of about 12.8km² of which, about 60% is underlain limestone. High flow conditions during periods of heavy rainfall, suggest that the Pot may have a substantially larger catchment area than previously outlined by Gunn et al. (Gunn 1996). Gunn (Gunn 2007) further reported two sinks, 10 to 11km east of the rising, to be hydro-logically connected, thus increasing the catchment area. Of this, the immediate catchment area is largely

grass based agriculture, with forestry and peat harvesting also present to a lesser extent. The Shannon Pot's perimeter is overhung by lichen covered trees. Large detritus matter from the surrounding vegetation is a constant associate of the Shannon Pot. The hydrochemistry of the 'Pot' is strongly influenced by precipitation events which reduce residence times of water in the karst and increase runoff from surrounding catchment. In this chapter the major physcio-chemical constituents of groundwater within the Shannon Pot are presented with correlation to influences by anthropogenic and natural constituents. The natural quality of groundwater varies greatly as groundwater flows from its recharge area (elevated topography) and its discharge area (aquifer). The groundwater chemistry can change as it passes through soils, subsoils or rocks with different mineralogy. The 'Shannon Pot' study reported in this chapter was conducted over a fifteen month period, in tandem with other studies conducted along the course of the river which have been reported elsewhere (McCaul, et al. 2011).

Over the fifteen month period samples were analysed for conductivity, temperature, pH Dissolved Oxygen (DO) as well as macro nutrients (nitrate, phosphate). Chemical Oxygen Demand was carried out to assess potential pollution as a result of anthropogenic activities, which are seasonal in the area. Such farming activities have an influence upon the water quality and are exacerbated by the recorded weather conditions, namely rainfall. Macro nutrient minerals of calcium, magnesium, potassium, sodium, chloride and sulphate were also traced over the period. Heavy metals cadmium, chromium, lead and mercury were determined to investigating their potential presence within the aquifer. Rainfall for the period is also reported and employed to rationalise propositions relating to variations in parameters. In previous chapters (Chapter 2) we have shown nuclear magnetic ressonance (NMR) as a successful tool in identifying the major structural components of fresh water DOM using diethylaminoethyl (DEAE) cellulose passive samplers to isolate and concentrate DOM. In this chapter the chemical nature and structural composition of the DOM isolated using different techniques were elucidated with an offline TMAH process, followed by GCMS analysis. The goals of this study were:

1. To look at temporal variability of DOM over the 15 month study period.
2. To compare and contrast the traditional DOM isolation technique of filtration

(Perdue and Ritchie 2003) against our employ of clay as a sorbent to isolate DOM within its natural environment.

3. To determinate the hydro chemical functioning of the 'pot' over a 14 month period and to assess both anthropogenic and natural influences on the water quality and the composition of DOM within the Shannon Pot.
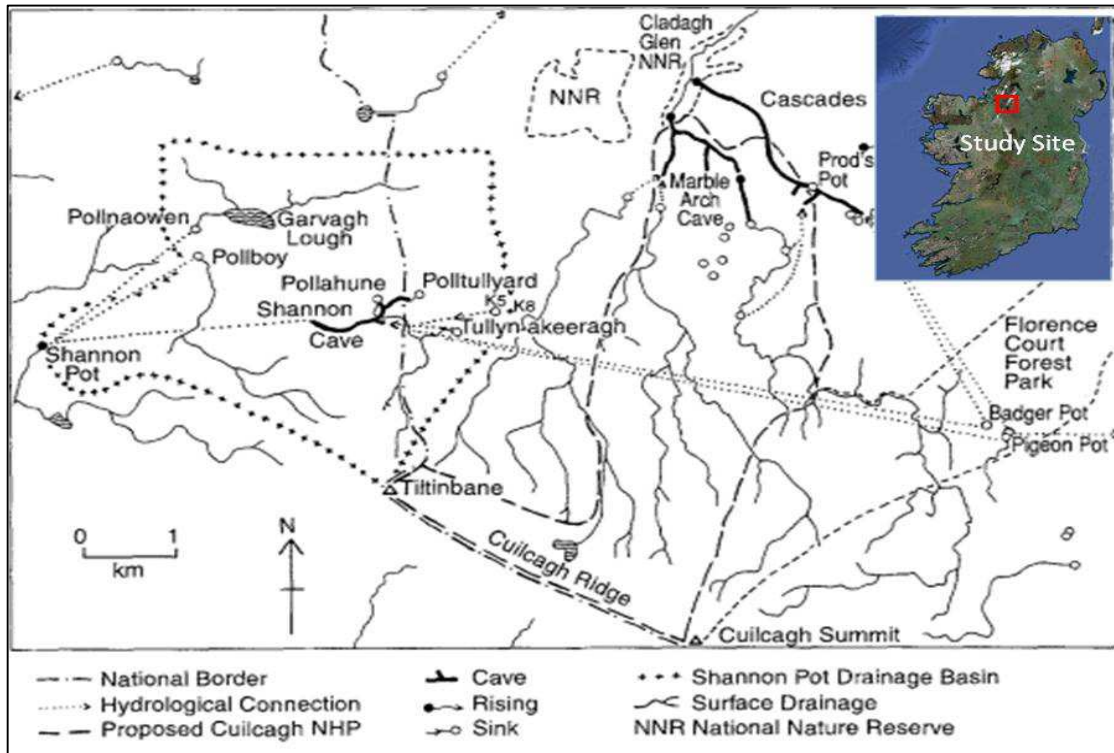
FIGURE 4.1 Sampling location adapted from Gunn et al. (Gunn 1996)

We believe this study to be novel and the most detailed hydro geochemical study of the Shannon Pot to date and that of a karst aquifer over a fifteen month period. The work presented identifies and confirms temporal, geological and seasonal variation with listed chemical constituents, which indicate the influencing factors on the water quality of this aquifer and links such results to rainfall and influencing agricultural practices which are seasonally dependant.

4.1 EXPERIMENTAL

In this section the experimental techniques of the study are outlined.

4.1.1 Sampling

The Shannon Pot (Lag na Sionna) is the recognised source of the River Shannon (Section 2.1). It is located on the western slopes of the Cuilcagh Mountains Co. Cavan. The spring is one of the most famous in Ireland; The Shannon Pot's fame can
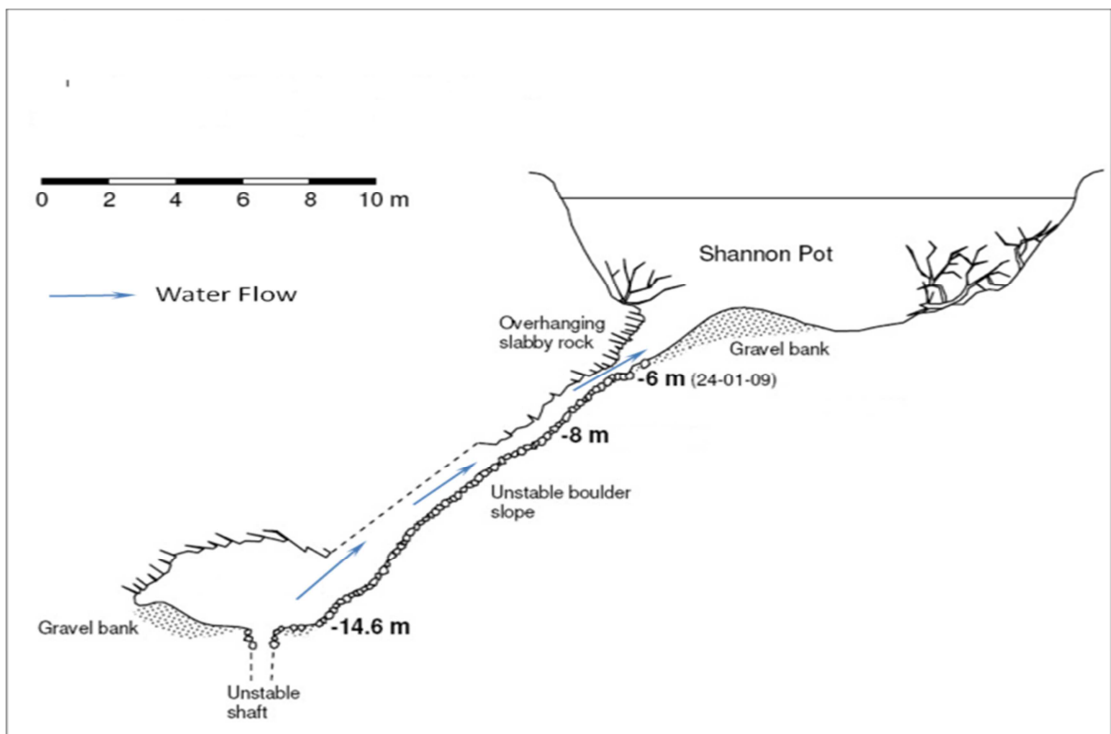
FIGURE 4.2 Picture of the Shannon Pot explored to depth of 14.6m by Boycott et al.(Boycott, et al. 2008)

be traced back to the very early times of the legendary Finn MacCool and the Fianna, the great warriors of Irish mythology. A picture of the Shannon Pot is shown in Figure 4.2 showing the out let for the aquifer which is the opening of the river Shannon. Rainfall was recorded over the course of the sampling period at the Met Eireann weather observation station, Ballyhaise Co. Cavan (Location: 54.051°N; 7.31°; W 67M above mean sea level). DOM was isolated using passives samplers containing DEAE cellulose (see chapter 2), clay matrices and filtration.

Water samples were collected on the date of deployment and collection of passive samplers. In addition on sampling dates, general weather conditions and

observations of the sampling site were recorded. Initial test of pH, conductivity, temperature, alkalinity, colour and dissolved oxygen content were carried out on site using standard instrumentation. The sample container employed for all samples collected were 500ml heavy duty polypropylene bottles provided with hermetic-locking caps. Bottles and caps were cleaned by soaking in 10% HCl, and rinsed with deionised water, drained, wrapped in polyethylene bags and stored until required. Composite samples were collected at each sampling point at a depth of 30cm below the surface the surface of the water. Prior to sample collection, sampling containers were rinsed twice with the water to be sampled.

FIGURE 4.3 Picture of the Shannon Pot illustrating the outflow to the Shannon River

Temperature, pH, conductivity and dissolved oxygen measurements were performed in situ at each sample site. Duplicate samples were taken on each sampling occasion. One duplicate was acidified to pH 2 by addition of H_2SO_4. The second duplicate was kept at its natural pH and used for determination of fluoride, chloride nitrate, sulphate, orthophosphate and chemical oxygen demand (COD). Samples were immediately transported to the laboratory and stored at 4°C until their analysis, which was accomplished within two days. Physico-chemical parameters have been determined by following standard methods of analysis (APHA-AWWA-WPCF, 1985; AOAC (1992).

Table 4.1 Sampling period for the Shannon Pot, Co. Cavan

Sampling Period	Month	Sampling Period	Month
1	October	9	June
2	November	10	July
3	December	11	August
4	January	12	September
5	February	13	October
6	March	14	November
7	April	15	December
8	May		

4.1.2 Isolation of DOM within the Shannon Pot

Isolation techniques and sorbent material used are outlined in this section.

4.1.2.1 Isolation of DOM using Clay Matrices

As well as DEAE cellulose, some forms of clay have been shown to be successful in the absorption of DOM from freshwater environments (Meier, et al. 1999). Montmorillanite is one such clay. Various cationic forms (Na, Li) were prepared to establish each clays sorbtion affinities for DOM. Depending on the element cationically exchanged in the clay, there is a resultant size variation between the clay's layers, where intercalation of organic matter may occur (Jiang and Cooper 2003).

4.1.2.2.1 Sodium Montmorillonite

Sodium-montmorillonite (Na-Mt) was prepared from calcium-montmorillonite (Ca-Mt) by placing 18.8023g of Ca-Mt and 1.2051g of Na_2CO_3 and 200ml Di H_2O into a conical flask and placing in a water bath stirring at 80°C for a period of 3hrs. During mixing several drops of HCL were added to remove carbonate ions (CO_3^-). The resulting solution was centrifuged at 6000rpm for 10mins, the supernatant was decanted and the resulting clay slurry was washed with DiH_2O until all carbonate

ions were removed (Cases, et al. 1992). The clay exchanged with Na ion was placed in an oven for 24hrs at 105°C. The clay was ground in a pestle and mortar and passed through a 200µm sieve. The sodium mont-morillonite was stored under vacuum until required for further analysis.

4.1.2.2.2 Lithium Montmorillonite

Lithium-montmorillonite (Li-Mt) was prepared by placing 15.0031g of Ca^{2+} montmorillonate in a beaker and exchanging with a 1.0M solution of LiCl for 24hrs. The Li exchanged form was washed with distilled water until free of chloride ions, dried in a oven at 60°C and ground to pass through a 0.22mm sieve. The Li-exchanged form was headed for 24 hours at 110°C to evoke partial fixation of the Li^+ on the layers (Komadel, Hrobarikova and Koppelhuber-Bitschnau 2002).

4.1.2.2 Isolation of DOM using filtration and reconstitution in laboratory

Water samples were collected on date of deployment and collection of passive samplers from September 2010 until January 2012. Thirty liters of water was collected three 10l PTFE sampling containers approximately 8 to 12 inches *beneath the surface of the* water. On arrival to the university laboratories, the 30l of water were vacuum filtered through 0.22um PVDF porous membrane (9mm). The filtrate was frozen; freeze dried and stored at -80 until further analysis. This further analysis yielded two sets of data: the initial filtrate was analysed directly according to the technique outlined in section (4.3). The remaining filtrate was employed in different study.

This procedure sought to investigate the absorption of natural DOM onto a Ca montmorillanite under laboratory conditions. This was carried out by reconstituting the filtrate in 10l of deionised water. Passive samplers as used in the environment were deployed within the 10l and remained in situ for 28 days. The samples were kept at 16°C and in the absence of light to prevent thermal and photo decomposition. After 28 days the water was filter freeze-dried and analysed by GCMS. The laboratory passive samplers were removed, centrifuged at 6500 rpm and filtered through a 0.22 µm PVDF filter membrane. The filter and the filtrate were frozen, freeze-dried and stored at -80°C for subsequent analysis.

4.2 Solvent extraction

DOM components were extracted using both soxhlet and ultrasonic assisted extraction.

4.2.1 Soxhlet Extraction of DOM for GC analysis

All DOM isolated using the four techniques DOM (200-250mg) were soxhlet extracted at 80°C for 28 hours using dichloromethane/acetone (9:1) (v/v). This technique suspended the DOM fraction in a suitable solvent for GCMS analysis. The solvent extracts were reduced to approximately 2mls under a steady stream of nitrogen gas. The resulting extracts were methylated by adding 50ml of 25% (w/w) aqueous TMAH solution, to the volume reduced extract. The resulting mixture was treated in an ultrasonic bath for 15 min at 50°C.

4.2.2 GC-MS analysis of soxhlet extracts

Separation of derivatised compounds was performed on an Agilent model 6890N GC coupled with to an Agilent Model 5973N quadrupole mass selective detector (MSD). Derivatised extracts (1µl) were injected for each GC-MS run. The injector temperature was set at 280°C with a splitless injection. The helium carrier gas was set at a flow rate of 1ml/min. Separation was achieved on an Agilent HP 5MS fused silica column (30m × 0.25mm i.d., 0.25 µm film thickness). The GC operating conditions were as follows: temperature hold at 150°C hold at 1 min, increased from 150°C to 280°C at a rate of 5°C with a final isothermal hold for 30 min. The mass spectrometer was operated in the electron impact mode (EI), at 70eV ionisation energy and scanned from 50 to 650 Da. Data was acquired and processed with Agilent Chemstation G1701DA software. Individual compounds were identified by mass spectra literature NIST and Wiley MS data libraries.

4.2.3 Quality Control

All glassware was cleaned by boiling in detergent (Decon 90), rinsing 6 times with DiH$_2$O, placing in a muffled furnace at 450°C for 3 hours and finally rinsing twice with acetone, methanol, and dichloromethane immediately before use. Prior to injection the instrument was tuned and calibrated according to the instruments specifications. To ensure tuning and calibration were complete a 'spiked' sample containing a known concentration of cholestane was injected and its concentration verified. If the concentration was found to be greater or less than the known concentration the instrument was recalibrated. Blank samples containing no detectable compounds were injected before each sample injection, to ensure there was no column contamination or carryover between sample injections. Method blanks were performed for all extractions to check for contamination during the extraction process.

Table 4.2 Outlines each sample analysed by GCMS and subsequent sample references that are used throughout in the remainder of this chapter

Sample Reference	Sample Description	No. of samples*
WinterFilt	30 litres of on collection and deployment of passive samplers during winter periods.	14
SummerFilt	30 litres of samples of water were filtered at collection and deployment of passive samplers during summer periods.	14
WinterClay	Clay Passive Samplers deployed in situ during winter periods	39
SummerClay	Clay Passive Samplers deployed in situ during summer periods	41
WinterLabClay	Clay Passive Samplers deployed in winter DOM suspensions within the laboratory	14**
SummerLabClay	Clay Passive Samplers deployed in winter DOM suspensions within the laboratory	14**

* Indicates the number of samples taken for the entire sampling period (15 months). ** Winter and summer suspensions were composite filtered samples, taken for each season.

4.2.3.1 Quantification

Components were quantified using the external method of quantification. A cholestane standard with a concentration of 1000ppm was diluted to 100ppm by a 10:1 dilution with hexane. Prior to injection 100ul of the 100ppm standard was added to each sample.

4.2.4   Physico-chemical parameters.

4.2.4.1 COD analysis

COD analysis was measured directly from 2mls of the water sampled using the reactor digest method (HACH method 8000). Holding the digestion vials at a 45-degree angle, 2mls of the sample was added using a clean volumetric pipette. Each vial was tightly capped, rinsed with distilled water and wiped with a soft lint free cloth. The vials were inverted several times to mix and placed in a DRB200 reactor for two hours @ 150°C. The vials were allowed to cool for 20 minutes, inverted several times and allowed to cool to room temperature. During the 2hr reaction time the sample was heated with sulphuric acid and potassium dichromate. A blank using distilled water and an internal reference standard of 100 mg/l COD was used in place of the sample, to measure the degree of contamination within the method and to validate the accuracy of the analytical method. The concentration of COD mg/l was then estimated from the absorbance of the sample measured at 350 nm using a DR2000 spectrophotometer. The results in mg/l COD are defined as the milligrams of O_2 consumed per litre of sample under the conditions of the method.

4.2.4.2 Orthophosphate

Orthophosphate was directly measured from 25 ml of the water sampled using the Phosver 3 (Ascorbic acid) method (HACH method 8178). A 25ml sample cell was filled with 25ml of the sample to be analysed. One phosver 3 powder pillow was added to each sample cell the cell was capped and shaken vigorously for 60 seconds. The sample was then left to settle for two minutes to allow the reaction to take place. During that time orthophosphate within the sample reacts with molybdate in an acid medium, to produce a phosphomolybdate complex. Ascorbic acid then reduced the complex, giving an intense molybdenum colour proportional to the concentration of orthophosphate in the sample.

A blank using distilled water and an internal reference standard of 2.0mg/l phosphate was used in place of the sample to measure the degree of contamination

within the method and to validate the accuracy of the analytical method. The concentration of orthophosphate was then estimated from the absorbance of the sample measured at 890 nm using a DR2000 spectrophotometer.

4.2.4.3 Fluoride, Chloride, Nitrate, and Sulphate analysis

Fluoride Chloride Nitrate and Sulphate analysis was performed using a Dionex 4500i ion chromatograph fitted with an electrolytic conductivity detector. Sample and eluant (3.5mM sodium carbonate and 1.0mM sodium bicarbonate) were lead via a Dionex AG10 anion guard through a Dionex AG10 anion column, through a Dionex GDM-2 pump. The IC system was automated in terms of sample introduction by means of a Dionex ASM-11 sample changer and data processing using Dionex A1450 software.

To improve the accuracy of the results, a blank sample of ultra pure water was prepared in the same way as each sample and analysed under the same identical conditions. The blank results were subtracted from the results of the samples to improve accuracy of the results. For calibration proposes, duplicate injections of four different levels of standards (2.5mg/l, 5mg/l, 10mg/l, and 20mg/l) containing each individual ion were carried out.

4.2.4.4 Metals Analysis

Acidified samples were analysed for the range of metals understudy using AAS (Varian SpectrAA50). Calibration was carried out using standard solutions, and the instrument was adjusted to the associated wavelength for each metal being investigated. Statistical analysis of data was performed using Origin 6.0 and Microsoft Excel Analysis ToolPak. Linear regression was used to correlate AAS and each data set checked for potential outliers. Any measurement where the ratio of the calculated residual of the regression model and the standard error was larger than 2, was considered to be an outlier.

4.3 RESULTS AND DISCUSSION

4.3.1 Hydrogeochemical analysis

This section outlines the result for the hydro geochemical properties recorded over the 15 month period. The major chemical components of groundwater within the

Shannon Pot are examined to assess influences by anthropogenic and natural constituents and in turn their influence on dissolved organic matter. The natural quality of groundwater varies greatly as groundwater flows from its recharge area (elevated topography) and its discharge area (aquifer). The groundwater chemistry can change as it passes through soils, subsoils or rocks with different mineralogy. The Shannon Pot is a karstified aquifer situated in the Culilagh Mountains composed of limestone bedrock and dominated by limestone subsoil the surrounding area is dominated by light agriculture.
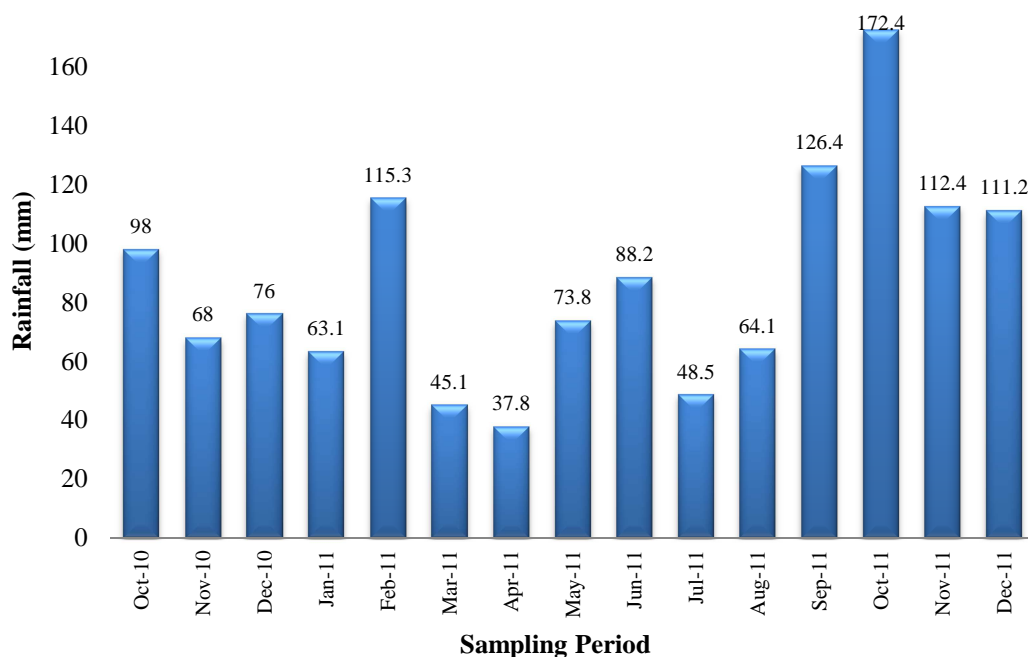
FIGURE 4.4 Rainfall recorded during each sampling period at the weather observing station, Ballyhaise, Co. Cavan

The data presented here was collected over a 14 month period. All comparisons are made with threshold values outlined by European Environment Agency WISE (Water Information Systems for Europe) (Marra and Carlei 2011).

4.3.1.1 Rainfall for the sampling Period

The rainfall values obtained from Met Eireann were recorded at Ballyhaise Automatic Weather Station (54.051°N 7.31° W), Ballyhaise Co. Cavan. These values are used as an indicator of rainfall levels at the Shannon Pot, Co. Cavan. Rainfall data for Cavan during the sampling period are presented in figure 4.4. The

average rainfall for the sampling period was 84.9mm. Rainfall during October was substantially higher than any other monthly average and in excess of the monthly average (67.5mm) recorded for October in 2010.

4.3.1.2 Nitrate levels recorded during the study period
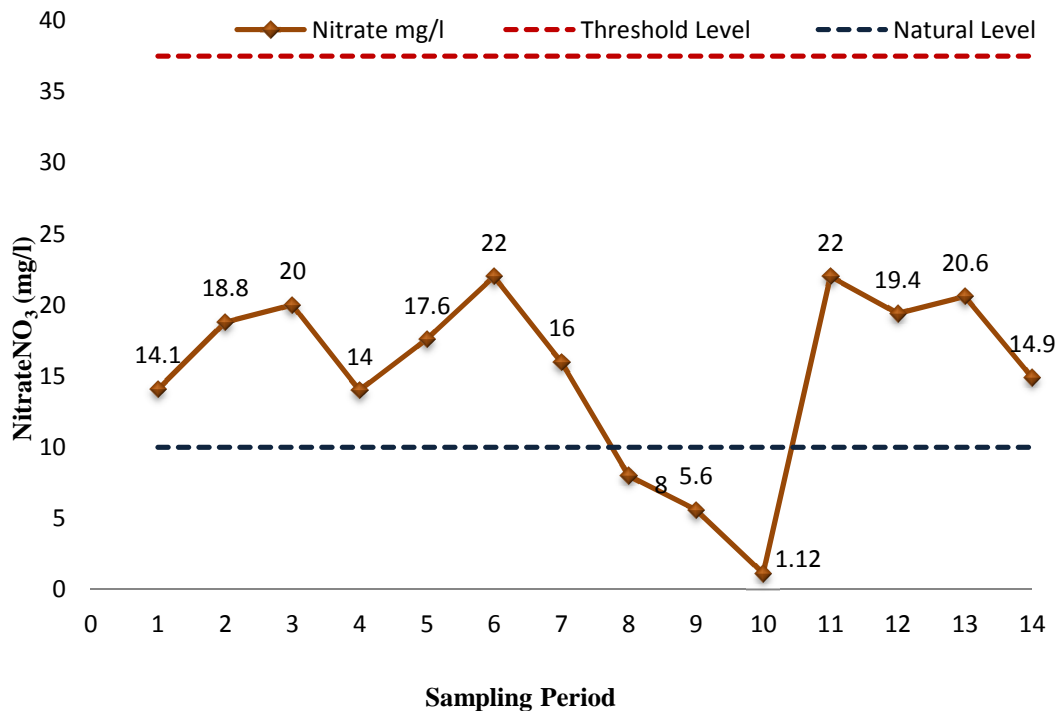
FIGURE 4.5 Nitrate levels recorded in ground water samples from the Shannon Pot
A threshold level of 37.5mg/l for nitrate set by the European Environment Agency WISE (Water Information Systems for Europe). Nitrate is found naturally in groundwaters at concentrations lower than 10mg/l NO_3. Concentrations greater than 10mg/l NO3 indicate influences from organic anthropogenic sources such as slurry and agricultural runoff or inorganic sources such as spreading of artificial fertiliser. Samples were collected in triplicate on deployment and collection of passive samplers. The mean concentrations recorded during each sampling period are shown in Figure 4.5. A total of 84 samples were analysed for nitrates with a mean concentration for the entire sampling period being 17.0mg/l NO_3. Concentrations greater than 37.5mg/l NO_3 were not recorded, 10 sampling periods recorded values that exceeded the natural concentrations of nitrate, with the highest concentration (22mg/l NO_3) recorded during April and September. Natural concentrations were not

exceeded during June (8.6mg/l NO$_3$), July (5.6mg/l NO$_3$) and August (1.12mg/l NO$_3$). In general nitrate concentrations observed were above the natural concentration of nitrates in groundwater, light agriculture surrounds the Shannon Pot and increase in rainfall (met eireann) which increases runoff into the pot are probable causes for these elevated concentrations.

4.4.1.3 Phosphate levels recorded during the study period
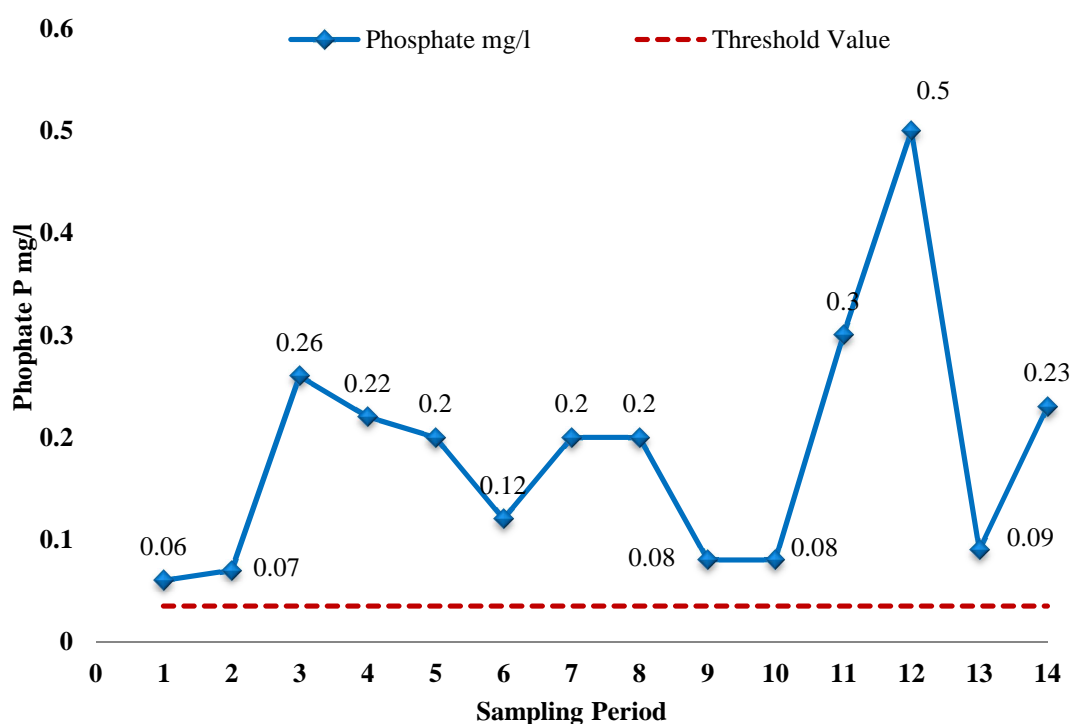
FIGURE 4.6 Phosphate levels recorded during the entire sampling period

Phosphate in groundwater can act as a nutrient pathway for receiving waters (Takatert, Sanchez-Pérez and Trémolières 1999) contributing to eutrophication within rivers and lakes. The Shannon pot is particularly vulnerable as it is a karst aquifer with shallow soils and subsoil's. Phosphate levels within the Shannon Pot for the period October 2010 to December 2011 are presented in figure 4.6. The mean concentration of phosphate in the Shannon pot for the entire sampling period was 0.172mg/l far exceeding the threshold level 0.035mg/l set by WISE (Marra and Carlei 2011). All sampling periods exceeded the threshold value with the most significant increase during September and October. This significant rise in phosphate levels during October corresponds with an increase in rainfall during the same

period. Kilroy et al.(Kilroy and Coxon 2005) examined phosphorous concentrations in 8 karstic springs in the west of Ireland. All eight springs showed an increase in phosphate concentration after the first autumnal rainfall recorded in September. This temporal change in concentration may reflect the change in soil moisture content from deficit to soil moisture surplus. This increase in soil moisture can attribute to the release of loosely bound phosphate within the soil, accumulated during the summer following inorganic and organic fertiliser manipulations (McBeath, et al. 2012). In addition agricultural point sources of pollution can attribute to an increase in phosphate levels. During adverse weather conditions it was noted that cattle and sheep congregated under the vegetation surrounding the Shannon pot, which can lead to severe localised pollution and increased soil erosion feeding the Shannon pot.

4.3.1.4 pH, conductivity, alkalinity, and colour levels recorded for the study period

Table 3.1 shows the pH, conductivity, alkalinity and colour for the extended sampling period. The pH values ranged from 7.10 to 8.24 with a mean of 7.67 for the entire sampling period. The water within the Shannon pot is slightly alkaline and falls between the threshold values for pH levels set by WISE (Marra and Carlei 2011). Conductivity (mS) ranged from 108.3mS to 268.1mS with a mean of 295.7mS. Conductivity values above the threshold values (187.5 mS) were observed during October. This coincides with an increase of nitrates and phosphates (figure 4.6) and a rise in rainfall (figure 4.4) during the same sampling period. Conversely D.O. levels reduced during the same period.

TABLE 4.2 pH, conductivity, alkalinity, and colour in groundwater samples from the Shannon Pot

Sampling Period	pH	Conductivity (mS)	Alkalinity ($CaCO_3$) mg/l	Colour (Hazen)
October-10	8.24	165.0	73	70
November-10	8.10	164.8	74	70

December-10	7.67	149.2	64	40
January-11	7.24	166.4	65	55
February-11	7.38	152.8	70	60
March-11	7.10	108.3	68	70
April-11	8.04	130.2	60	45
May-11	7.38	156.7	70	70
June-11	7.40	120.5	68	75
July-11	7.98	138.9	74	80
August-11	7.48	166.4	70	65
Sepember-11	7.94	158.6	68	70
October-11	8.11	268.1	62	80
November-11	7.38	178.5	70	65
December-11	7.56	154.6	62	70
Mean	7.67	295.7	68.28	65.35
Min	7.10	108.3	60.00	40.00
Max	8.24	268.1	74.00	80.00

4.3.1.5 Chemical oxygen demand and Dissolved Oxygen levels recorded for the study period

This decrease in D.O. (48.2) contradicts findings by Karakoc et al. (Karakoç, Ünlü Erkoç and Katircioglu 2003). Karakoc et al found that D.O. levels increased during periods of recharge as a result of precipitation, at high flow rates the water table rises causing intermixing (aeration) and reduced residence times within the aquifer. However, this apparent decrease in DO observed in October coincides with an increase in COD levels during the same period. Capraro et al. (Capraro, et al. 2011) also observered a similar increase in COD levels as a response to increased precipitation in karst aquifers within the Vento Region Italy. This increase in COD levels can be attributed to the direct injection of agricultural runoff with limited filtration by soil and rock interfaces. The decrease in DO levels during the same period may be attributed to this influx of agricultural runoff, and detritus from surrounding vegetation increasing microbial activity at the time of sampling, along with an surge of 'old aquifer' water that has been displaced (Wong, Mahler and Musgrove 2011). Reduction in DO levels was also observed during April, a period of low precipitation levels (see figure 4.3), indicating a reduction in residence time

within the aquifer as a result of low precipitation. The reduction in flow decrease mixing increasing microbial activity. DO levels recorded during all other sampling periods were slightly above or close to saturation for all sampling period which is optimum to support aquatic life (Breitburg 2012). This is essential for the region as a small trout stream emerges from the 'pot'.
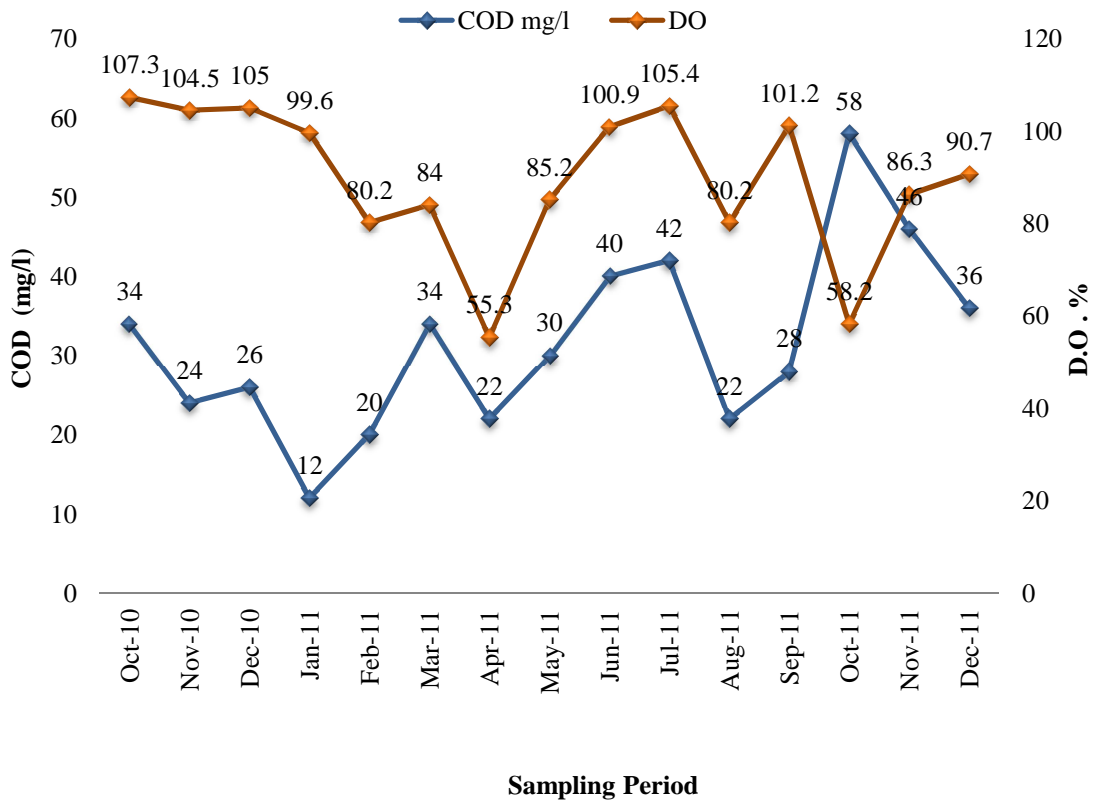
FIGURE 4.7 COD and DO measurements recorded for the entire sampling period

4.3.1.6 Major cations and anions

The variations in the major dissolved ions found in groundwater are presented in table. Major anions chloride and sulphate, and major cations sodium, calcium, magnesium and potassium were recorded.

TABLE 4.3 Major Cations and Anions measured in samples taken from the Shannon Pot

Sampling Period	Calcium (mg/l)	Magnesium (mg/l)	Potassium (mg/l)	Sodium (mg/l)	Chloride (mg/l)	Sulphate (mg/l)
Oct-10	28.6	1.72	0.48	5.12	42.7	34.9
Nov-10	29.7	1.78	0.46	5.31	36.1	42.7
Dec-10	23.7	1.48	0.4	6.32	20.6	38.1
Jan-11	28	1.58	0.44	5.24	18.9	40.4
Feb-11	30.4	1.7	0.46	5.83	35	38.2
Mar-11	26.2	1.74	0.38	5.26	39.4	40.1
Apr-11	28.3	1.64	0.42	5.84	28.1	42.4
May-11	22.04	1.72	0.4	5.04	16.4	30.2
Jun-11	24.6	1.5	0.38	5.6	34	36
Jul-11	28.4	1.76	0.42	5.11	32.4	40.3
Aug-11	29	1.69	0.46	5.56	20.8	39.8
Sep-11	24.5	1.72	0.39	5.29	25.5	40
Oct-11	48.4	2.52	0.43	5.52	32.5	35.9
Nov-11	32.4	1.52	0.48	6.00	26.5	38
Dec-11	24.8	1.48	0.32	5.18	42.7	34.9
Mean	27.4	1.6	0.42	5.48	29.2	38.3
Max	38	1.78	0.48	6.32	42.7	42.7
Min	22.0	1.48	0.32	5.04	16.4	30.2

 Concentrations of the major cations and ion fluctuated throughout the sampling period but did not exceed threshold values set by WISE (Capraro, et al. 2011). However, levels of calcium and magnesium concentrations were lower than expected for sampling area which may be attributed to pH levels for sampling period being slightly basic, as dissolution of ions requires the pH to be slightly acidic. The levels of calcium within the aquifer increased significantly during October and November this difference in chemical behaviour could be interpreted as follows: Rinck-Pfeiffer et al. (Rinck-Pfeiffer, et al. 2000)explained that an increase in calcium levels during periods of increased rainfall is attributed to flushing of water with long residence times from the phreatic zone (see figure 4.3). The stagnant water is forced out by the fresh recharge through the fissure causing a piston like process (Rinck-Pfeiffer, et al.

2000). Magnesium, Potassium, Sodium, Chloride and Sulphate showed no significant increase in concentrations during the same sampling period.

Minor ions such as cadmium, chromium, copper, manganese, lead, and mercury were also determined for each sampling period. Levels were found to be below limit of detection for the instrument which was <0.01mg/l. Iron was detected for each sampling period all levels were < 0.08mg/l which was below average levels recorded in aquifers (Singh, et al. 2010) which was found to be 0.12mg/l. Hydrochemical analysis of the Shannon pot provides an insight into the major hydrogeochemical functioning of the 'pot' and it's response to precipitation events.

4.3.2 NMR analysis of DOM isolated from the Shannon Pot.

Recent investigations of DOM that employ NMR spectroscopy show that it shares many structural similarities despite its source, i.e. marine, freshwater or where in the world it is from (McCaul, et al. 2011, McCaul, et al. 2011, Hertkorn, et al. 2006,

Lam, et al. 2007). These major structural components are also present in the DOM isolated from the Shannon Pot as is evident in Figure 4.8.
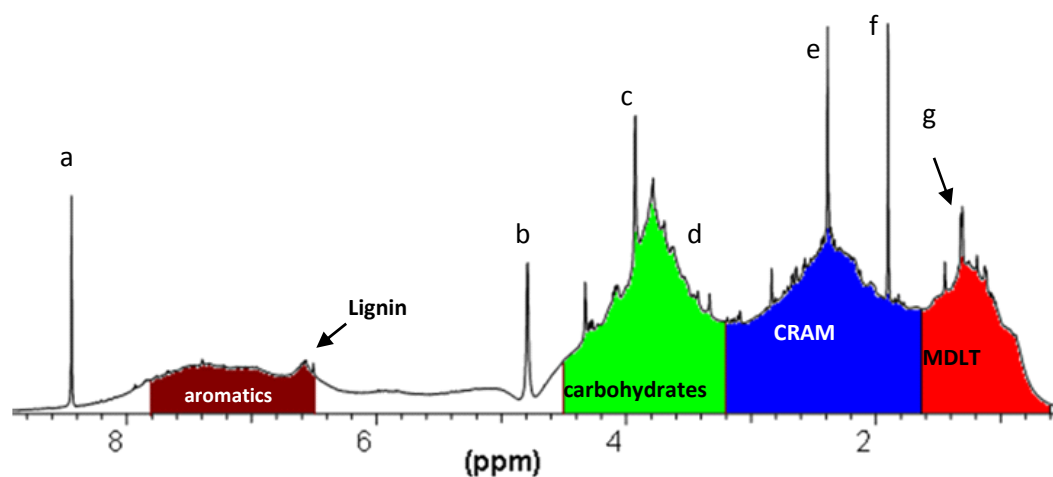
FIGURE 4.8 1D ^{1}H NMR spectrum of the Shannon Pot DOM. Colours relate to the different structural groupings in DOM. Specific assignments are as follows: (a) formic acid, (b) residual water, (c) glycolic acid, (d) methanol, (e) succinic acid, (f) acetic acid, and (g) lactic acid.

General assignments, consistent with those reported are: (Red), material derived from linear terpanoids (MDLT), a region dominated by aliphatics; (Blue), carboxyl-rich alicyclic molecules (CRAM) or the region characterized by groups such as ester, amide, methyl ketones, and carboxylic acids; (Green), carbohydrates and amino acids and (Brown) aromatics, including resonances from amino acid (AA) side chains (lignin methoxyl also resonates under this region) (Hertkorn, et al. 2006, Lam, et al. 2007). More specific assignments of simple molecules from a 1D spectrum of DOM by Woods et al, 2011(Woods, et al. 2009) allows us to identify the same carboxyl and alpha hydroxy acids in the Shannon Pot spectrum (see figure 4.8). The source of low molecular weight acids in DOM may vary. For example, succinic acid is created as a byproduct of the fermentation of sugar. It and other low molecular weight aliphatic acids are also transient components of plant and animal tissues (Rowe 1989) . Lactic acid is an end product of plant metabolism that generally accumulates in cell vacuoles(Hertkorn and Kettrup 2005). A source of acetic acid may through a reaction of OH• radicals with organic matter (Goldstone et

al, 2002) or the hydrolytic degradation of hemicelluloses by hemicellulases that can also produce monomeric saccharides (Pérez, et al. 2002).
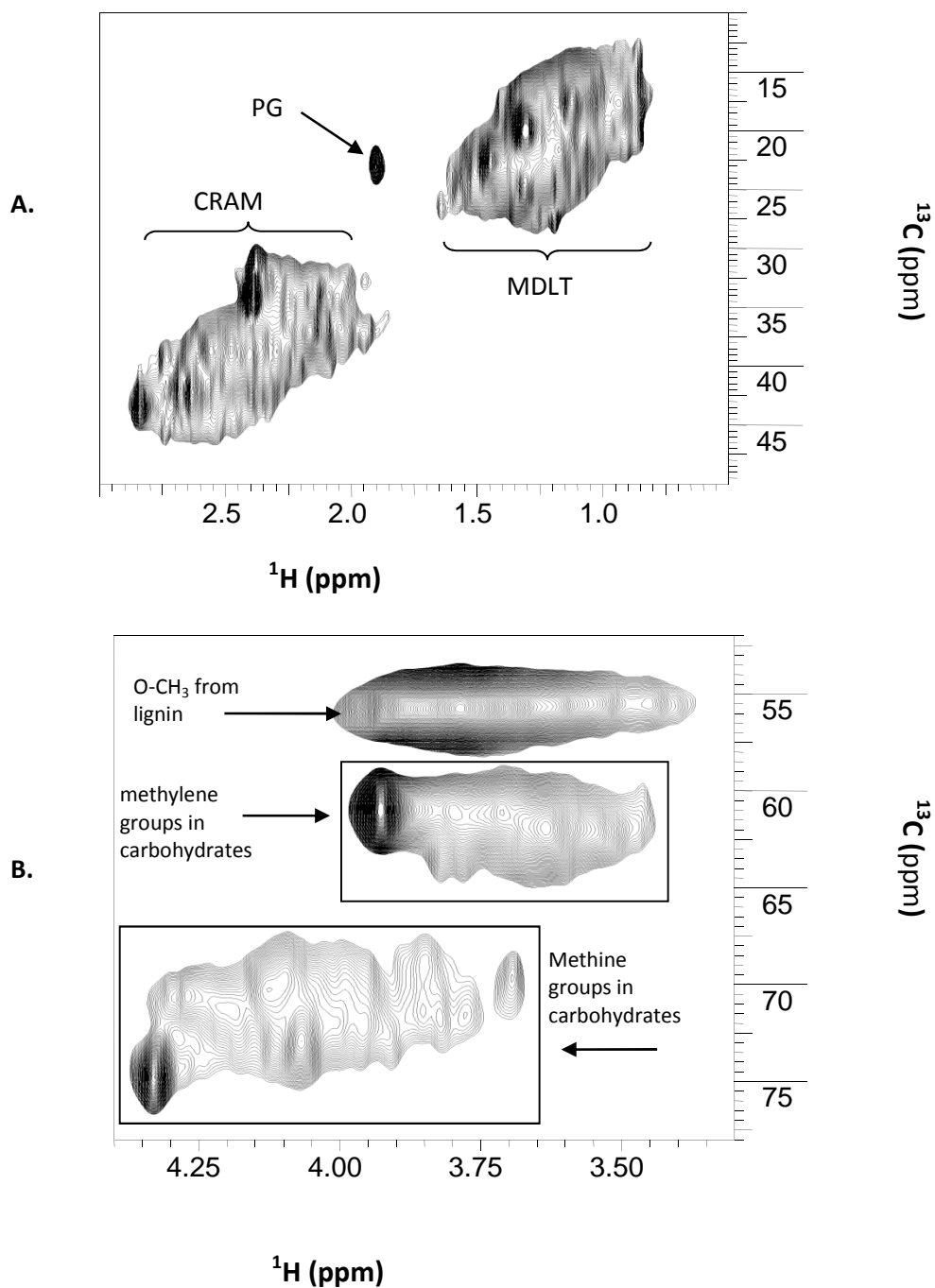
FIGURE 4.9 HMQC of expanded carbohydrate (A) and aliphatic region (B) of the Shannon Pot DOM. Abbreviations: CRAM, carboxyl-rich alicyclic molecules; PG, peptidoglycan; MDLT, material derived from linear terpenoids.

However, Brinkmann et al.(Brinkmann, et al. 2003) proposed that the production of acetic, and succinic acids was the result of a combination of photochemical and biochemical degradation reactions of plant material (Brinkmann, et al. 2003).

The presence of methanol is not surprising as it and other oxygenated volatile organic carbon compounds are known components of DOM (Dixon, Beale and Nightingale 2011). Methanol is also the second most abundant organic gas in the atmosphere after methane (Jacob, et al. 2005). Despite the fact that methanol is biogeochemically active and plays a significant role in atmospheric chemistry large uncertainties exist as to its origins. Dixon and co-workers recently provided evidence that the atmosphere is not a major source of methanol and suggested that it is predominantly produced by sunlight driven decomposition of organic matter (Dixon, Beale and Nightingale 2011).

Again, there is a characteristic peak from lignin at ~6.5 ppm in figure 4.8 (Woods, et al. 2009)(McCaul, et al. 2011).This peak cannot be assigned with certainty from the ^{1}H NMR spectra alone but 2D experiments (such as HMQC NMR spectroscopy) provides ^{1}H-^{13}C bond correlations which help resolve overlapping signals from ^{1}H NMR data (Simpson 2001) and thus with some degree of confidence we can assign this peak as lignin. Figure 4.9 shows the HMQC NMR spectrum of the Shannon Pot DOM. The presence of lignin-type material is confirmed by the intense methoxy signal seen in the HMQC data (Fig. 4.9). Lignin is a strong indicator of terrestrial plant inputs and may be an indication of the age of DOM and/or the influence of the surrounding environment. Microbial contributions to the DOM are supported by the presence of the *N*-acetyl functional group from peptidoglycan that is prominent in the HMQC NMR spectrum (Figure 4.9). Peptidoglycan is a polymer that consists of sugars and amino acids that forms a layer outside the plasma membrane of bacteria. It has been used to estimate bacterial concentrations (Benner and Opsahl 2001, Simpson, et al. 2004) and can be protected from microbial degradation after cell death by copolymerization reactions and transformation, substantially adding to the refractory nitrogen pool (Benner and Kaiser 2003). Strong resonances for CRAM and MDLT are also clear in Figure 4.9 B.

4.3.3 Analysis of DOM isolated from the Shannon Pot

Presented in this section are the qualitative analysis of DOM isolated by the four main techniques employed as described in section 4.2and displayed in table 4.2 All

results refer to analysis over a temporal variation over a consecutive 15 month period for the Shannon Pot.
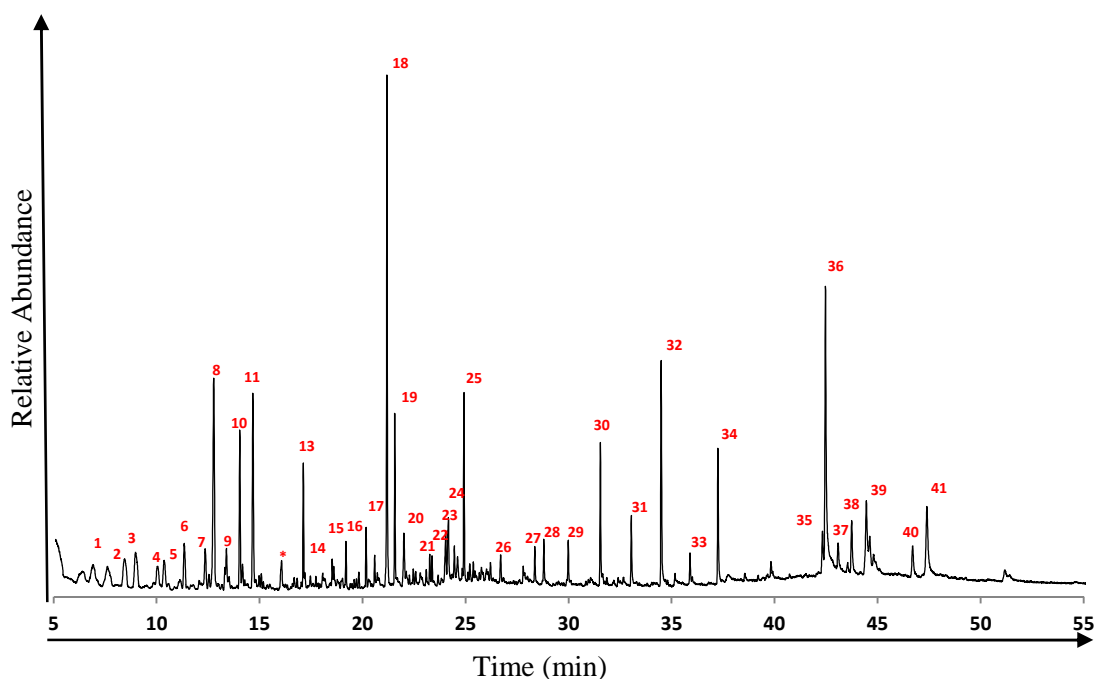
FIGURE 4.10 TMAH chemolysis GC-MS chromatograms (TIC) for summer clay DOM. Peak indications are given in table 4. "*" corresponds to a series of siloxanes seen in winter filtered and winter clay samples.

4.3.3.1 Results for DOM isolated using filtration and Clay passive samplers

In total we tentatively identified 69 different compound peaks in the chromatograms elucidated from DOM isolated from the Shannon Pot using clay passive samplers and the filtration procedures on site. Sixty compounds were identified from winter DOM isolated using passive samplers with clay as a sorbent material, while 50 compounds were isolated using ultra-filtration procedures for the same sampling period. Summer sampling yielded only 19 compounds using both techniques. Nine compounds identified were common in both summer and winter isolates and found using both isolation techniques. Figures 4.10 and 4.11 show the total ion chromatograms for DOM obtained from the Shannon Pot. All compounds were identified using NIST and WILEY libraries along with relevant published literature. Table 4.4 shows a complete set of elucidated chromatograms of DOM within the Shannon Pot which employed two isolation techniques and are reported using seasonal variations of winter and summer. Derivatisation using TMAH yielded

products which on identification were catergorised according to their recognised potential biomolecular precursors. Potential precursors yielded from the TMAH treatment of isolated DOM include fatty acid methyl esters, alkanes, aldehydes, ketones, terpenoids and lignins.   Table 4.4? consists of 5 columns, the first column contains the identified compound list, the second column contains the molecular formula for each TMAH derived product identified, the next 4 columns relate to each DOM sample analysed. Each sample column is split into two. The "tick" indicates if the compound was identified in that sample and the number adjacent is the peak identification number for each compound identified in the subsequent chromatogram e.g. Figure 4.1 . The chemolysis product distribution observed in this study is similar to that obtained by Fraizer et al. (Frazier, Kaplan and Hatcher 2005a) and Jose et al. (del Rio and Hatcher 1996) with good correlations to their distributions. Noticeably the resultant chromatograms for the summer and winter sampling periods were quite different from each other. Considerably more compounds were detected and identified for the winter sampling period. Such a quantity and variation in compounds has not been previously reported in the literature. A conclusion for this observation could be related to several factors including the extensive nature of the sampling program, the techniques employed in deriving a sample suitable for GCMS analysis and the advances in identification software for such studies. However the hydromorpholghy of the waters investigated will significantly contribute to the results. The following discuss each group of compounds isolated in turn comparing them to the literature.
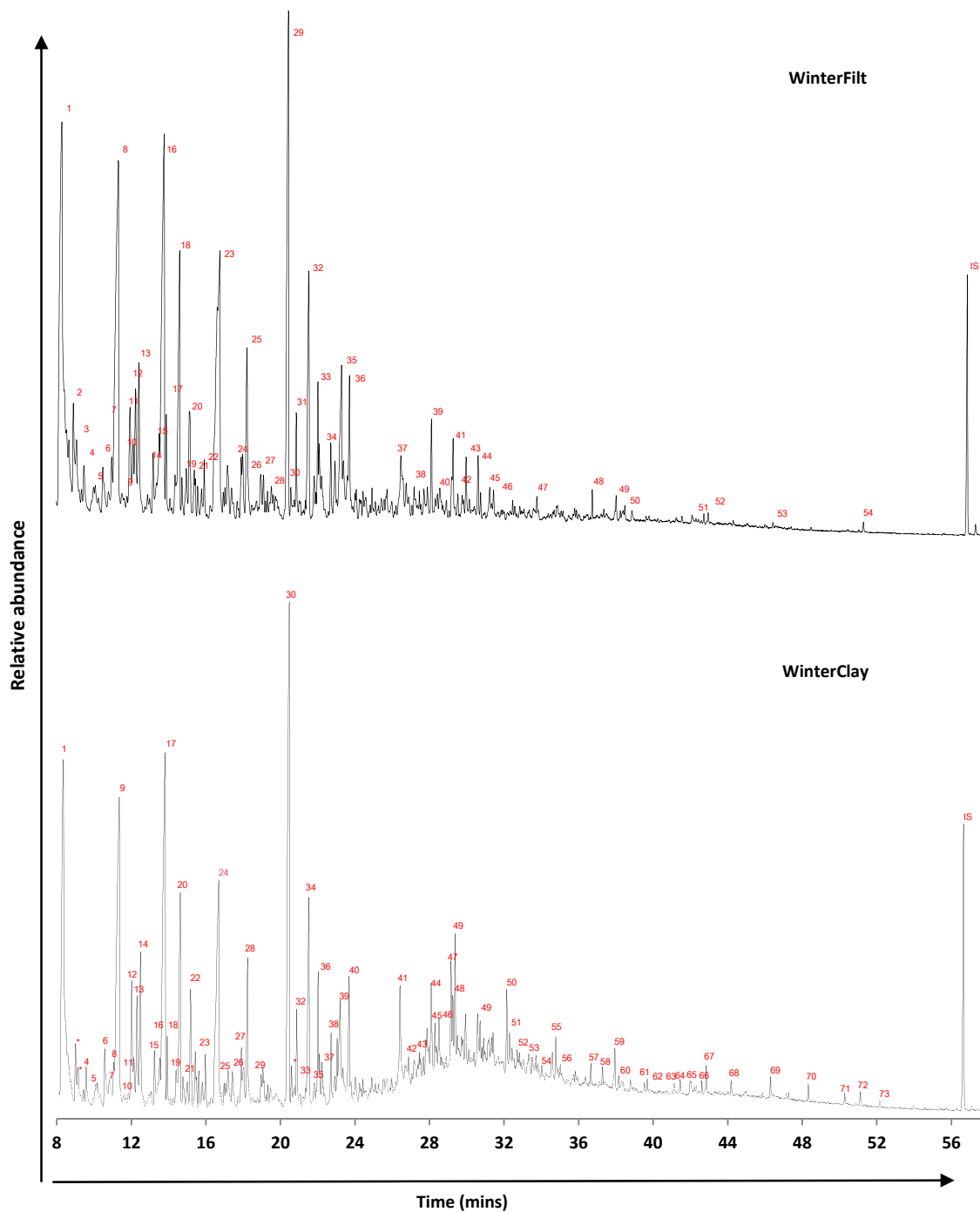
FIGURE 4.11 TMAH chemolysis GC-MS chromatograms (TIC) for winter filtered DOM. Peak indications are given in table 4. . "*" corresponds to a series of siloxanes seen in winter filtered and winter clay samples.

TABLE 4.4 Peak Identifications for Winter Clay, Winterfilt, Winter lab clay and Summer Clay samples. (Table is continued on subsequent pages)

Compound Name	MF	WinterClay		WinterFilt		Winterlabclay		SummerClay	
		Y	PN	Y	PN	Y	PN	Y	PN
Alkanes									
Pentadecane	$C_{15}H_{32}$	✓	41						
Hexadecane	$C_{16}H_{34}$	✓	48						
Heptadecane	$C_{17}H_{36}$	✓	50						
Pristane	$C_{19}H_{40}$	✓	51						
Octadecane	$C_{18}H_{38}$	✓	55						
Phytane	$C_{20}H_{42}$	✓	56						
10-Nonadecane	$C_{19}H_{38}O$	✓	57						
Nonadecane	$C_{19}H_{40}$	✓	58						
10 Nonadecane	$C_{19}H_{38}O$	✓	64	✓	48	✓	48		
Eicosane	$C_{20}H_{42}$	✓	62						
Heneicosane	$C_{21}H_{44}$	✓	65						
Docosane	$C_{22}H_{46}$	✓	68					✓	26
Tricosane	$C_{23}H_{48}$	✓	70	✓	53	✓	53		
Tetracosane	$C_{24}H_{50}$	✓	69						
Pentacosane	$C_{25}H_{52}$	✓	71						
Hexacosane	$C_{26}H_{54}$	✓	74						
Methylated Alkanes									
3-Penten-2-one, 4-methyl	$C_6H_{10}O$	✓	24						
2-Pentanol, 2,3-dimethyl	$C_7H_{16}O$	✓	25						
Pentadecane, 2 methyl	$C_{16}H_{34}$	✓	45						
Pentadecane, 3 methyl	$C_{16}H_{34}$	✓	46						

Compound Name	MF		ClayWinter		FiltWinter		LabClayWinter		Summerfilt	
			Y	PN	Y	PN	Y	PN	Y	PN
Hexadecane, 3 methyl	$C_{17}H_{36}$		✓	54						
Fatty Acids										
Butanoic acid	$C_4H_6O_4$				✓	9	✓	9		
Dodecanoic acid	$C_{12}H_{24}O_2$				✓	43	✓	43		
Fatty Acid Methyl Esters										
Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	10:0								3
Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	12:0	✓	43	✓	38	✓	38	✓	8
Tetradecanoic acid methyl ester	$C_{15}H_{30}O_2$	14:0	✓	53					✓	13
Tetradecanoic 12-methyl, methyl ester	$C_{16}H_{32}O_2$	*iso* 15:0			✓	47	✓	47	✓	15
Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	15:0							✓	16
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	16:0	✓	59	✓	49	✓	49	✓	18
9-Octadecanoic acid (Z), methyl ester	$C_{19}H_{36}O_2$	18:1							✓	24
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	18:0	✓	66	✓	52	✓	52	✓	25
Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	19:0							✓	9
Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	20:0							✓	27
Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	21:0							✓	29
Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	22:0							✓	30
Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	23:0							✓	31
Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	24:0							✓	32
Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	25:0							✓	33

Compound Name	MF	ClayWinter		FiltWinter		LabClayWinter		Summerfilt	
		Y	PN	Y	PN	Y	PN	Y	PN
Alkene									
Tetradecene	$C_{14}H_{28}$	✓	38						
1-Hexadecene	$C_{16}H_{32}$	✓	47						
1-Octadecene	$C_{18}H_{26}$							✓	15
Docosene	$C_{22}H_{44}$	✓	61						
Docosene	$C_{22}H_{44}$	✓	72						
Hexacosene	$C_{26}H_{52}$	✓	73						
Aldehydes and Ketones									
ethanone, 1-phenol, (acetophenone), phenyl, methyl ketone	C_8H_8O	✓	10	✓	9	✓	9		
4-hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$	✓	13						
Phorone (2,5-Heptadien-4-one, 2,6-dimethyl-)	$C_9H_{14}O$	✓	15	✓	14	✓	14		
Umbelliferone	$C_9H_6O_2$							✓	11
Isophorone (2-cyclohexen-1-one, 3,5,5 trimethyl)	$C_9H_{14}O$			✓	16	✓	16		
2-Acetyl-3-methylthiophene	C_7H_8OS	✓	16	✓	15	✓	15		
Cis-2,7, Dimethyl-4-octene	$C_{10}H_{20}O_2$	✓	19	✓	18	✓	18		
3-Penten-2-one, 4 methyl-	$C_6H_{10}O$	✓	24	✓	23	✓	23		
2-Pentanol,2,3-dimethyl	$C_7H_{16}O$	✓	25						
Isojamone	$C_{11}H_{18}O$	✓	32	✓	30	✓	30		
p-flourobutyrophenone	$C_{10}H_{11}FO$	✓	33						
2-propanone, 1-(3,5,5 trimethyl-2-cyclohexane-1-ylidene) (Z)	$C_{12}H_{18}O$	✓	37	✓	33	✓	33		
2,6,6, trimethyl 2 cyclohexene 1,4 dione	$C_9H_{12}O_2$	✓	40						

Compound Name	MF	ClayWinter		FiltWinter		LabClayWinter		Summerfilt	
		Y	PN	Y	PN	Y	PN	Y	PN
Terpenes									
Artemisia ketone	$C_{10}H_{16}O$	✓	20	✓	19	✓	19		
Pipertone	$C_{10}H_{14}O$	✓	34	✓	31	✓	31	✓	3
Megastimatrienone	$C_{13}H_{18}O$	✓	49	✓	44	✓	44		
Manool	$C_{20}H_{34}O$	✓	63						
Artemisinin	$C_{15}H_{18}O_4$			✓	51	✓	51		
Terpenoids									
4,8,12,16-Teteramethylheptadecan-4-oldie	$C_{21}H_{40}O_2$							✓	28
Carbohydrates									
2 cyclohexen-1-one, 3 methyl	$C_7H_{10}O$	✓	9	✓	8	✓	8	✓	11
2,3,5-trimethylfuran	$C_7H_{10}O$	✓	11	✓	10	✓	10		
Dimethyl maleate	$C_6H_8O_4$	✓	12						
2 Butanol-5-methyl furan	$C_9H_{12}O_2$	✓	23	✓	22	✓	22		
2-cyclopentene-1-carboxylic acid, 1-methl-methyl ester	$C_8H_{12}O_2$	✓	27						
trans-2-(-1-hydroxycyclohexyl)furan	$C_{10}H_{14}O_2$	✓	36	✓	32	✓	32		
Sterols									
Stigmast-5-en-ol, 3 beta	$C_{29}H_{50}$							✓	35
Ergosta-5,7,22-trien-3β-ol	$C_{28}H_{44}O$							✓	36
5-Alpha-stigmast-3-one	$C_{29}H_{48}O$							✓	37
Stigmasta-3-5-diene-7-one	$C_{29}H_{46}O$							✓	38

Compound Name	MF	ClayWinter		FiltWinter		LabClayWinter		Summerfilt	
		Y	PN	Y	PN	Y	PN	Y	PN
Stigmastanol	$C_{29}H_{52}O$							✓	39
Stigmasterol	$C_{29}H_{48}O$							✓	40
Lignin									
Methoxy benzene	C_7H_8O							✓	1
1-(4-hydroxy-3-methoxphenyl)ethanone	$C_9H_{10}O_3$	✓	29	✓	26	✓	26		
1-Methoxy-4-(1-methylethnyl)benzene	$C_{10}H_{12}O$	✓	26	✓	24	✓	24		
3,4 dimethoxy benzaldehyde	$C_9H_{10}O_3$	✓	30	✓	28	✓	28		
3,4,5-trimethoxybenzoic acid methyl ester	$C_{11}H_{14}O_2$	✓	39	✓	36	✓	36	✓	4
Phenol									
Phenol methoxy acetate	$C_9H_{12}O_3$							✓	5
Phenol,2,6,-bis(1-dimethylethyl)-	$C_{14}H_{22}O$	✓	42						
3,5-Diisopropylphenol	$C_{12}H_{18}O_2$			✓	48	✓	48		
Possible pesticides									
Carbamic acid, (2-phenylethyl)-ethyl ester	$C_{11}H_{15}NO_2$	✓	44	✓	39	✓	39		
Uncertain Origin									
Tert-butyl alcohol	$C_4H_{10}O$	✓	1	✓	1	✓	1		
3-Furancarboxylic acid, 2-mrthyl-methyl ester	$C_7H_8O_3$	✓	5	✓	4	✓	4		
Methyl 1-cyclohexane-1-carboxylate	$C_{18}H_{12}O_2$	✓	6	✓	5	✓	5		
1,1,3-Trimethoxy Propane	$C_8H_{18}O$	✓	7						
Acetamide, N-methyl	C_3H_7NO	✓	8	✓	7	✓	7		
Acetamide, N-(2,4-dimethylphenyl)	$C_{10}H_{13}NO$			✓	35	✓	35		

Compound Name	MF	ClayWinter		FiltWinter		LabClayWinter		Summerfilt	
		Y	PN	Y	PN	Y	PN	Y	PN
Benzene, 1-(chloromethyl)-3-fluoro-	C_7H_6ClF	✓	18	✓	17	✓	17		
1-(2-furyl)cyclohexan-1-ol	$C_{10}H_{41}O_2$	✓	22	✓	21	✓	21		
Methyl-3-(3,5 diterbutyl-4-hydroxy phenyl)	$C_{18}H_{28}O_3$							✓	19
2-dichloromethyl-5,6-dihydro-2H-pyran	$C_7H_{10}Cl_2O$	✓	28	✓	25	✓	25		
2,6,6, trimethyl 2 cyclohexene 1,4 dione	$C_9H_{12}O_2$	✓	40						
2,6-Dimethyl-6-nitro-2-hepten-4-one	$C_9H_{15}NO_3$			✓	23	✓	23		
E-15 Heptadecanol	$C_{17}H_{32}O$	✓	60						
1-(1-Hydroxybutyl)-2,5-dimethoxybenzene	$C_{12}H_{18}O_3$			✓	43	✓	43		
Methyl ester of 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-propionic acid	$C_{18}H_{28}O_3$			✓	50	✓	50		

4.3.3.1.1 Alkanes

Alkanes derived from 'clay winter' samples were the most abundant class of compounds identified and ranged in carbon length from C_{15} to C_{26}. The distribution of alkanes for these winter samples did not display any odd or even preference for carbon number. However, a relatively low molecular weight distribution of the alkanes detected in the clay during the winter sampling period are centred around low carbon numbers with C_{17} compounds being the most abundant . Frazier et al. (Frazier, Kaplan and Hatcher 2005a) among others (Schulten and Schnitzer 1997, Saiz-Jimenez and Hermosin 1999) have proposed that this lower end molecular weight distribution pattern for isolated alkanes strongly suggests microbial/algal origins as opposed to higher plant sources such as plant waxes such as epicuticular waxes (Frazier, Kaplan and Hatcher 2005a)(Otto and Simpson 2007). The presence of pristane and phytane (C_{19} & C_{20} range) is indicative of algal sources of DOM. The proposed origin of these alkanes is from the transformations of 'phytol' the isoprenoid side-chain of chlorophyll during TMAH chemolysis as reported by Rontani and Meyers. (Rontani and Volkman 2003)(Meyers and Ishiwatari 1993). Conversely alkanes were smaller component in the summer clay with little or no abundance. Notably for the filtering techniques, summer filtered and winter filtered DOM only yielded classifications of n-heneicosane and n-docosane.

4.3.3.1.2 Fatty Acid Methyl Esters (FAME)

Fatty Acid Methyl Esters (FAME) are the most dominant TMAH chemolysis products observed in the chromatogram of the 'summer filtered' and 'summer clay' isolates. The series of methyl esters identified ranged from C_{12} to C_{25}, the methyl ester of hexadecanoic acid (16:0) was the most abundant compound followed by those of octadecanoic (18:0) acid and tetradecanoic (14:0) acid. Even numbered fatty acids were more prevalent than odd numbered has previously been reported by Jandl, Schulten and Lienweber (JANDL, SCHULTEN and LEINWEBER 2002). Burdige et al also reported evidence for FAME content in DOM derivied from a wide range of living material such as algae (C_{16} to C_{20}) and terrestrial vascular plants (C_{22} to C_{26}) (Burdige 2006). Furthermore the FAME distributions during summer sampling periods are very closely supported by Burdige et al.(Burdige 2006) and Jandl et al. (Jandl, et al. 2005) work. A series of mid-longchain fatty acid methyl

esters, including those corresponding fatty acid methyl esters identified in this study (docosanoic acid, methyl ester, tricosanoic acid methyl ester and pentacosanoic acid methyl ester) have been reported elsewhere ((Jandl, et al. 2005)(Čechlovská, et al. 2009)) . These authors suggested that the prevalence of even-carbon-numbered long-chain fatty acids is due to inputs of plant biopolymers such as cutin, cutan, suberin, and suberan and there evidence for this as shown in Table 4.4 by the isolation of such compounds. In addition the presence of iso C_{15} and C_{17} fatty acids suggests inputs arising from bacterial membranes (Kwon, Shon and Cho 2009). Rontani et al. (Rontani and Volkman 2005) and others suggest that the occurrence of these odd numbered fatty acids in DOM may be as a result of influences from soil sulphate SO_4 reducing bacrteria (*desulfobulbus* and *desulfovibrio)*. This is also supported by Li et al. (Li, et al. 2011) who studied the fatty acid distributions in dissolved and particulate matter from cave water in Hesang Cave, Central China. In our study, TMAH chemolysis did not release short chain fatty acids (up to 8 carbon atoms) in any of the isolated DOM. The absence of short chain fatty acids maybe due to these fatty acids being bound to lignin and other compounds within the DOM matrix (Pulchan, Helleur and Abrajano 2003). However FAME released from chemolysis of 'winter clay' DOM and 'winter filtered' DOM only released short mid chain fatty acids (C_{12} to C_{18}). This distribution of mid chain even numbered FAME is most likely to be as a consequence of contributions from algal sources (Haas and Wild 2010).

4.3.3.1.3 Sterols

Sterols were identified in 'summer clay' DOM samples but were absent from 'winter clay' DOM samples. Sterols observed in summer isolates are indicative of plant sources with some input from fungi and animal inputs.This would be expected given the period of growth experienced during spring/summer months and subsequent influence on the water body. Among the sterols indentified, β-sitosterol and stigmasterol are widely distributed among the plant kingdom and are the most common sterols in waxes and higher plants (Santos, Carreira and Knoppers 2008). Stigmasta-3-5-dien-7-one and sitosterene found in this study and are thought to be degradations products of β-sitosterol and stigmasterol and are therefore plant derived a fact borne out on several occasions in the litereature (Frazier, Kaplan and Hatcher 2005a) (Frazier, Kaplan and Hatcher 2005b) (Santos, Carreira and Knoppers 2008). The most abundant sterol identified during summer sampling periods was ergosterol,

which has been previously identified as a biomolecular precursor for fungal contributions in freshwater DOM (Santos, Carreira and Knoppers 2008). This high abundance of ergosterol suggests significant contributions from fungi during summer sampling periods.

4.3.3.1.4 Lignins

Lignin is sometimes an important component of DOM and in other studies lignin related materials have been released using TMAH/thermochemolysis procedures(del Rio and Hatcher 1996, Sleighter and Hatcher 2008, Frazier, et al. 2003)(Pulchan, Helleur and Abrajano 2003). Table 4.1 shows lignin-derived compounds released using TMAH derivatisation techniques in the *absence* of pyrolysis. Both 'winter clay' and 'summer clay' isolates yielded products that have previously been used to identify lignin precursors and their parent material within the DOM pool (Frazier, Kaplan and Hatcher 2005a). The lignin products observed were syringl (S), guaiacyl (G), or p-hydroxyphenyl (P) groups and from the lignin distributions observed in this study of the Shannon system in chapter 2 it would appear that that lignins are spatial and seasonally dependent.(Spencer, et al. 2008)

Three compounds (1-(4-hydroxy-3-methoxyphenyl) ethanone (G), 3,4 dimethoxy benzaldehyde (G), 3,4,5-trimethoxybenzoic acid methyl ester (G)) were observed only in winter isolates and indicate the presence of lignin that originates predominantly from gymnosperms (conifers). Plantations of which exist throughout the Shannon Pot catchment. Methoxy benzene (P) was observerd in summer isolates, and had been found to be a derivative of p-hydroxylphenol originating from grass also originating from grasses an Guaiacyl derivative 3,4,5-trimethoxybenzoic acid methyl ester was indentified in both summer and winter sampling periods(Pérez, et al. 2002). Although the TMAH lignin derived products indentified in this study are likely to be specific to lignin sources, Frazier et al.(Frazier, Kaplan and Hatcher 2005a) found that not all lignin products were specific to lignin sources and can be derivative from other biopolymers such as carbohydrates and tannins. The presence of lignin derived by-products in winter and absence in summer as evident in table 4.4 and may be attributed to rainfall events (those being higher in winter) (see figure 4.4). The ubsequent changes to residence time of the DOM within the 'Pot' and the ultimate changes in hydro-geochemical parameters are most easily explained by

these differences in rainfall. A similar conclusion was proposed by Ward et al.(Ward, Richey and Keil) who observed a high correlation between river charge and discharge rates and the subsequent changes in the nature and character of dissolved organic carbon of the water body as a result of rainy periods. Ward et al. (Ward, Richey and Keil) found that dissolved lignin increased by 240% with an increase in river discharge. This increase in lignin's concentration was as a direct result of rainfall events mobilising lignin from soils from surrounding landscape (figure 4.4). Jaques et al. (Jaques and Pinto 1997) also found a decrease in lignin levels during summer sampling periods as flow rates decreased. The study suggested that plant debris released lignin after the second phase of the decomposition process, which occurred during the third week after the debris sank to the river bed. This increased residence time of the plant debris and slow degradation is a likely reason for lower concentrations of lignin derived TMAH by products from summer DOM isolates.

4.3.3.1.5 Terpenes and Terpenoids

Monoterpenes (piperitone) and diterpenes (manool) were identified in 'winter clay' samples. Both terpenes which are indicative of higher plants, most likely originated from forestry plantations located on the southern slope of the Cuilcagh Mountains north of the Shannon Pot. This plantation is dominated by Sitka Spruce and Logpole Pine trees (Smith, et al. 2006). Kanerva et al. (Kanerva, et al. 2008) found manool to be the most abundant diterpene in pine needles. Artemisinin was detected in winter filtered DOM samples, artemisia ketone was detected in both winter filtered DOM and winter clay samples. The presence of these terpenes may suggest terrestrial sources from the vascular plant family artemisia (Yang, Zhu and Yu 2012, Brown 2010). The Artemisia family of plants contain about 200 species of plant including those evident around the perimeter of the Shannon Pot and surrounding landscape. The terpenoid 4,8,12,16-tetramethylheptadecan-4-oldie was also identified during the winter sampling period. 4,8,12,16-teteramethylheptadecan-4-oldie has previously been described as an oxidation degradation by-product of α tocopherol (Adsul, et al. 2009)), α tococpherol's have been reported to be likely sources of pristine (Baldock and Nelson 2000) originating from higher plants and can accumulate as resistant remnants.

4.3.3.1.6 Polysaccharides

Carbohydrates are ubiquitous in nature and are major components of the most common biopolymers such as cellulose and lignin. TMAH chemolysis of plants, soil organic matter, and DOM results in complex chemolytic products, which include a wide variety of polysaccharide containing compounds encompassing a wide range of molecular weights(Page, et al. 2001)(Leenheer and Croue 2003). Cellulose is the most analysed polysaccharide using the TMAH chemolysis outlined in section 4. Its chemolysis products have been found to consist mainly of levoglusan along with furans, furanones and pyranones. Here we identified a variety of furans (2,3,5-trimethylfuran, 2 Butanol-5-methyl furan, trans-2-(-1-hydroxycyclohexyl)furan) from the summer clay and filtered DOM samples. These furans have been identified by Mannino and Harvey in DOM samples isolated from both fresh and marine environments and are indicative of cellulose contributions (Mannino and Harvey 2000, Minor, et al. 2001). Carbohydrate TMAH derived 2-cyclopentene-1-carboxylic acid 1-methl-methyl was also present in winter DOM isolates. Dimethyl maleate was also indentified in summer isolates and is indicative of contributions from lichens which are evident in trees surrounding the Shannon Pot (Crow, et al.).

4.3.3.1.7 Aldehydes and ketones

Several TMAH derived products identified as aldehydes and ketones contributed to DOM isolated in this study. Considerable variations in peak identifications were observed between winter and summer, in total 8 potential bimolecular precursors were identified in winter sampling periods compared to one identified in summer isolates. The ketone umbrelliforone was unique to summer isolates, and originates from the plant family *apiaceac* which are ubiquitous in nature and dominant during summer periods (Stace 2010). Umbrellifone has been previously identified as a photochemical and microbial degradation lignin by product (Skórczewski, Mudryk and Kukliński 1999). During the summer sampling periods there may have been increased degradation of plant material from plant debris present in the Shannon Pot due to increased residence time therefore increasing exposure to sunlight. This was also supported by Faeth et al. (Faeth, et al. 2006) who showed that photochemical degradation of DOM increased by 80% after exposure to sunlight.

Degradation products were also present in winter DOM isolates. The 4-hydroxy-4-methyl-2-pentanone compound was tentatively identified in 'winter clays' and is likely to originate from grass as it has been previously identified as a precursor for the grass family Poaceae (Faeth, et al. 2006). The algal contributions of phorone and 4-methyl-3-penten-2-one were also present in winter isolates (see table 4.), The suggested origin of these compounds is as a result of algal degradation exudates. Both compounds were evident in 'early' winter samples while summer algae decompose too rapidly for detection . However, on a technical note it should be reported that 4-hyroxy-4-methyl-pentanone and phorone had previously been identified as contamination from impurities in acetone. Given the attention to 'method blanks' or 'controls' employed in this study it is with confidence that we report their presence here in the study as a contribution from sampled DOM and not a cross contamination from acetone solvents employed in processing.

4.3.3.1.8 Anthropogenic compounds

In addition to the naturally occurring compounds isolated, *anthropogenic compounds were also found in DOM isolates. One group of compounds identified were phthalates. They were presence in the form of* 1, 2 benzene dicarboxyl acid methyl *in all samples.* Isolation and extraction procedures are a common source of these phthalate methyl esters (Bazes, et al. 2009). However, here phthalate esters were not present in the method blanks carried out indicating that the entered the sample during storage from leaching of PTFE containers. A second group of compounds most likely pesticides were identified in all DOM isolates. These are evident by the presence of two TMAH derivatives (1) 3-methyl 2 cyclohexen-1-one and (2) Carbamic acid. The presence of 3-methyl 2 cyclohexen-1-one (MCH) in DOM is an anti-aggregation pheromone. The compound MCH has successfully been used to control bark beetle from attacking susceptible host tress such as Sitka spruce, Norwegian spruce and Logpole pine trees ((Smith, et al. 2006, Bilby 1984, Ross, Daterman and Munson 2004)). While the bark beetle is endemic in the US and mainland Europe it is not present in Ireland. However, MCH is still used in Irish forestries as a precaution against the threat of this invasive species. Carbamic acid, (2-phenylethyl)-ethyl ester was also identified in all isolates and is a possible TMAH

by-product from n-methyl carbamate insecticide (Gruber and Munn 1998), which are also used in forestries to prevent bark beetle infestation in pine trees (Hastings, et al. 2001). Gruber et al. (Gruber and Munn 1998) also found that these compounds are not persistent in nature and therefore the pesticides must have entered the system at the time during sampling periods. However they are found in all isolates that suggests that in this chemical form, they do survive in nature and can be found in DOM.

4.3.3.1.9 TMAH products of uncertain origin

As a matter of course for these types of studies many of the TMAH chemolysis products detected by the GCMS analysis are of uncertain origin within DOM sampled from aqueous environments (Frazier, Kaplan and Hatcher 2005a). In many cases bio-molecules that are unidentified from their TMAH derivatives are most likely derived from more than one precursor. An examples of the deratitives of such biomoleules are acetamide, tert-butyl alcohol and methyl-3-(3,5 diterbutyl-4-hyroxy phenyl) which are in evidence in our samples. Predominantly the products are general by-products of TMAH reactions with simple organic matter present in samples (Frazier, Kaplan and Hatcher 2005a). These products are not source specific and are ubiquitous in origin as a result of the reaction of organic matter with TMAH. In previous studies these compounds which yield definitive GCMS chromatogram traces have been used as a 'fingerprinting' tool in the identification of DOM as coming from a specific geography of aquatic environment, in essence a forensic tool. . We can thus propose that this section of the chromatogram be used as a fingerprint tool for the Shannon Pot and possibly for karst aquifers in similar regions

4.3.4 Laboratory investigation of the adsorption of filter isolated DOM onto clay passive samplers

In this study, laboratory control passive sampling methods were investigated using actual DOM isolated in-situ at the Shannon Pot. The Filtered DOM was reconstituted in an aqueous environment under laboratory conditions. The DOM was filtered from 30 liters of water at the Shannon Pot and was reconstituted in 10 litres of DI water as described in section 4.1. Clay passive samplers were suspended

in the reconstituted DOM water and the resultant clay processed as per environmental samples (see Section 4.1) Chromatograms obtained from filtered DOM (from the Shannon Pot) showed little or no difference to DOM isolated in the laboratory clay passive samplers study. These results indicate that the DOM isolated by clay passive samplers are the same as those isolated using the filtration technique. It is also notable that the in-situ clay passive samplers which were in the Shannon Pot for periods of 28 days at a time did have differences to that of the filtered processed DOM from the Shannon Pot. Twice as many compounds were recovered using clay passive samplers and for the first time addresses any issues that may exist when choosing one techniques over the other within DOM sampling and analysis. This is an important result as it validates all previous studies which were conducted using the filtration technique as a method for the isolation of DOM. However the results also show that clay passive samplers isolate more DOM components than direct filtration . Significantly the clay passive sampler by its nature will allow a better study of the dynamic nature of an aquatic environment over a sustained period. In contrast the onsite filtration technique only allows a snap shot of the chemistries of an aquatic environment on the day of sampling. It is significant that the flux of chemistries within the Shannon Pot were such that in this situation passive sampling yields more information as to the nature and changes in DOM over the 28 day sampling period.

4.4 CONCLUSION

The influence of seasonal changes and land use practices along with an evaluation of isolation techniques revealed natural and anthropogenic differences in water chemistry and composition of DOM from the Shannon Pot. Different land use and local climate are important in controlling the hydrologic processing of the Shannon Pot. Rainfall events are the major driving mechanism for the changes in hydrochemistry and composition of DOM. For example biopolymers such as lignin increased in concentration as a response to rainfall events. This increase may be attributed to the flushing of vascular plant inputs from watershed soil and/or flushing of detritus from the underground cave system. Conversely during dry periods in this study the terrestrial inputs from the surrounding catchment appear to be reduced. During these events results indicate that the DOM is derived from in-situ events such as algal input and photo-degradation of detrital matter due to increased exposure to

sunlight and residence time. Anthropogenic influences were also evident with forestry being the main anthropogenic activity observed.

The hydro chemical sensitivity to hydrological changes was evident as nitrate, phosphate, DO and COD values responded to variations in precipitation. Increases in nitrates, phosphates and COD may be the result of direct injection of agricultural runoff with little soil and rock interaction. The decrease in DO levels during the same period may be attributed to this influx of agricultural runoff, and detritus from surrounding vegetation increasing microbial activity at the time of sampling. Additionally, surges of old aquifer water may also decease oxygen concentrations. Variations in DOM composition due to the materials employed for passive sampling were also observed. Montmorillonite passive samplers yielded similar compounds in both in house and in-situ investigations versus filtration techniques. This highlights the potential of such passive samplers for isolating the identifiable fraction DOM within the environment and shows promising alternative to traditional filtration techniques for temporal studies. Although the interface between terrestrial and aquatic systems is intricate and the resultant DOM pool complex our study provides a small insight into the identifiable pool of DOM, and links it to land-use and seasonal changes within the watershed. Unidentifiable portions of the subsequent DOM GCMS chromatograms provide finger print data bases for the Shannon Pot and possibly geographically similar Karst aquifiers.

4.5 References

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CHAPTER 5

CONCLUSIONS AND FUTURE CONSIDERATIONS

5.0 CONCLUSIONS AND FUTURE CONSIDERATIONS

Isolation and characterisation of DOM from freshwater and marine waters was performed using novel sampling techniques and advanced analytical techniques. In chapter 2 we employed DEAE cellulose passive samplers coupled with 1- and 2-D nuclear magnetic resonance (NMR) to investigate the structural components and sources of DOM and how it varies within a lake system. Major components found, such as carboxyl-rich alicyclic molecules (CRAM) are consistent with those recently identified in marine and freshwater DOM. Lignin-type markers and protein/peptides were identified and vary spatially. Phenylalanine was detected in lake areas influenced by agriculture, whereas it is not detectable where zebra mussels are prominent. The presence of peptidoglycan, lipoproteins, large polymeric carbohydrates and proteinaceous material supports the substantial contribution of material derived from microorganisms. Evidence is provided that peptidoglycan and silicate species may in part originate from soil microbes.

Although the isolation and subsequent characterisation of DOM within the lake system proved successful it is much more challenging within a salt water environment where the Cl$^-$ ions will compete for binding site on the DEAE cellulose. Therefore in Chapter 3 we proposed the use of activated carbon and cation exchanged montmorillonite as a possible sorbents within passive samplers for use in saline conditions. Activated carbon studies showed potential as a possible sorbent for marine DOM as initial TOC analysis showed an increase in organic carbon over time at two sampling points. However ^{1}H NMR analysis was inconclusive in that the spectra are not similar to any previous work on freshwater or marine DOM. Protein and evidence from recent degradation of organic matter (formic, lactic and acetic acid) were detected but the signature resonances for DOM such as CRAM and

MDLT are absent. These results indicate that only trace amounts of DOM were sorbed onto the activated carbon and salt may have played a role in inhibiting the sorption of DOM. This investigation was not continued and cation exchanged montmorillonite was used within the passive sample as a sorbent for DOM. Calcium montmorillonite was cation exchanged with Na and Li, the resultant clays were placed in passive samplers and deployed in both freshwater and marine waters for a 28 day period. GC/MS investigations, performed on clays, revealed the sorption fatty acids, alkanes, sugars and sterols to all clays, with alkanes being the most abundant compound class identified in all isolates. Alkane distributions identified indicated that DOM within the marine environment sampled was strongly influenced by macrophytes which were evident in large quantities at the time of sampling. Alkane and fatty acid distributions identified in freshwater were indicative of terrestrial plant origins. These initial results indicate the potential for clay as a possible sorbent for the identifiable fraction of DOM within freshwater and marine environments. Although initial results obtained by solvent extraction followed by N,O-bis(trimethylsilyl) BSTFA derivatisation yielded chromatograms with considerable peak distributions the majority of peaks co-eluted and were unidentifiable using NIST and Wiley libraries along with comparisons to relative literature. With this in mind in chapter 4 we further investigated the use of clay as a possible sorbent for organic matter using alternative extraction and derivatisation techniques. Soxhlet extraction followed by off-line chemolysis with tetramethylammonium hydroxide (TMAH) was employed to investigate the composition and temporal variations of DOM within the Shannon Pot, Co. Cavan. In addition a comparison was made between the clay sorbents and traditional filtration procedures.

Chemolysis TMAH products produced were similar to that found by Frazier et a (Frazier 2003). Winter DOM isolates yielded considerably more compounds than that observered in summer isolates. A direct correlation was observed between rainfall events and increased lignin distributions along with an elevation in nitrate and phosphate concentrations. Conversely a decrease in compound distribution was observed during summer sampling periods. These results indicate that DOM composition and hydrology within the Shannon Pot is strongly influenced by hydrological events and the geology in it's surrounding area. Results also highlight the use of clay sorbent passive samplers as an alternative to traditional isolation methods such as filtration. Both laboratory tests and field tests showed that clay

passive samplers were more successful in isolating a wider range of biomolecular compounds associated with DOM in comparison to filtration techniques. Although isolation of DOM by the clay passive samplers described in this study along with TMAH chemolysis and GCMS procedures provides us with an insight into the structural composition of DOM, further investigations are required to fully assess its potential. Some suggestions for further consideration are as follows:

TMAH chemolysis GCMS analysis of clay DOM herein was effective in qualitatively investigating the lignin, carbohydrate and lipid signatures of freshwater DOM, quantitative assessment was not carried out but is recommend as to fully assess its potential as a sorbent for DOM in freshwater.

If the sources of TMAH products of uncertain origin could be identified they could combine with TMAH products of 'known' origin to provide a larger picture of the composition of DOM within natural waters.

The comparison of TMAH chemolysis GCMS data and ^{13}C with ^{1}H NMR would provide a more accurate picture of the DOM isolated using clay passive samplers. The power of NMR is that it can give a general overview of all components present in DOM. Even though we may not be able to identify them, we know they are present and could compare this to DOM extracted from clay to ascertain what chemical classes, if any, have been excluded in the clay sorption process. In-situ time related field studies of clay passive samplers coupled with chemolysis GCMS and ^{13}C with ^{1}H NMR carried out over a two month period to evaluate the sorption of DOM over time in both fresh and marine water. Clay passive samplers had been placed in our marine sampling point for subsequent TMAH chemolysis GCMS but these samplers were lost at sea!. A repeat of this study is essential to fully assess clay as a possible sorbent in passive samplers. The development of a passive sampler to sorb DOM from both marine and freshwater is necessary if we are to answer the question of what happens to DOM as it transits from freshwater to marine waters. The potential of clay as a sorbent for DOM in passive samplers is described in thesis. The use of these samplers in marine and freshwater environments coupled with analytical techniques such as TMAH GC-MS and NMR analysis could provide an accurate and more comprehensive picture of the sources and fate of DOM in natural waters.