THE APPLICATION OF EXCITATION-EMISSION FLUORESCENCE SPECTROPHOTOMETRY TO THE MONITORING OF DISSOLVED ORGANIC MATTER IN UPLAND CATCHMENTS IN THE UNITED KINGDOM.

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by

Lucy Bolton

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School of Geography, Politics and Sociology
University of Newcastle upon Tyne, U.K. NE1 7RU

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Declaration

I hereby certify that the work described in this thesis is my own, except where otherwise acknowledged, and has not been submitted previously for a degree at this or any other University

Lucy Bolton

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Abstract

This study details the investigation into the use of spectrophotometric methods, principally excitation emission fluorescence spectrophotometry, in the monitoring of dissolved organic matter (DOM) in upland catchments. A protocol for the storage and analysis of DOM solutions was designed. To minimise deterioration immediate analysis was recommended. Long term storage, by freezing, resulted in significant and unpredictable alteration of the spectrophotometric properties. A post analytical correction was applied to overcome concentration related interferences. Solutions were analysed at natural pH, with consideration of the influence this property has on the spectrophotometric properties of DOM. Two study areas: the Coalburn Experimental Catchment (Northumberland) and the Loch Assynt area (Sutherland) were monitored. Spatial assessment of surface waters indicated that the distribution of DOM spectrophotometric properties was related to the influence of inorganic material in soils. This was observed as DOM in runoff from peat dominated areas, compared to non-peat, the former DOM having greater aromaticity or higher molecular weight. Distinct DOM spectrophotometric properties were observed in rainwater and throughfall and DOM from fresh and partially degraded spruce needles had a unique spectrophotometric signal. The two study areas exhibited limited variations in DOM properties, when compared to DOM from a wider range of sources. The mean estimated export DOC of from the Coalburn Experimental Catchment was 22.00 $gm^{-2}a^{-1}$ but the rate varied through the year. DOM spectrophotometric properties in both study areas varied seasonally exhibiting production and flushing periods with changes in catchment conditions. Discharge relationships indicated DOM sources in peat dominated area, however, these sources are only important when hydrologically active. A mild aqueous extraction method, to obtain dissolved organic mater from peat, was designed. This method obtained DOM, which reflected the distribution of spectrophotometric properties in related surface water. The method was applied to peat profiles from both study areas and the spectrophotometric properties of the DOM indicated relatively homogenous peat derived DOM. Peat DOM depth variations were observed and in some cases related to the presence of litter and inorganic layers. There was a broad spectrophotometric change with depth indicating increased aromaticity or molecular weight.

Chapter 1.

Introduction and Literature Review

This thesis presents the study of dissolved organic matter (DOM) using spectrophotometric techniques. The use of such methods, especially excitation emission matrix (EEM) fluorescence spectrophotometry, in the study of DOM has become widespread in the last 10 years. These studies have not concentrated in detail on DOM in upland areas at high resolution. The following chapters describe the spectrophotometric examination and characterisation of DOM composition, sources and processes from two such areas. The importance of DOM in upland catchments is two fold, firstly the negative impact it's presence and composition has upon drinking water quality and secondly on habitats. With future predictions of climate change these aspects become more important as estimates of DOC in rivers indicate an increase in exports. It is therefore essential to be able establish accurate concentrations and compositions of DOM. There are many methods for this, however each has drawbacks, in addition to benefits.

DOM is a complex aquatic component and thus requires extensive isolation and sample preparation prior to analysis. This study applies a method, fluorescence spectrophotometry, which does not require isolation and maintains the natural associations by analysis bulk samples. Also it is quick, easy and cheap method, when previously been applied to DOM studies (Baker, 2001). This is the first detailed use of these analytical techniques for the detailed examination of DOM in upland areas and also the first detailed examination of DOM in a forested peat catchment. The study aims both to utilise spectrophotometric properties to investigate DOM composition, but also to consider flow paths and DOM sources.

The aims of this study are presented in each chapter with respect to the specific aspects of the research presented therein. The aims relate to the investigation into spatial and temporal variations in aquatic and peat DOM in locations in the UK: the Coalburn Experimental Catchment (Northumberland) and the Loch Assynt area (Sutherland). Variations in DOM were monitored using EEM spectrophotometry and UV-visible absorbance analytical methods and a further aim of the study is to assess

these methods. Utilising such methods provides the ability to analyse DOM *in situ* incorporating the multiple interactions with other aquatic components.

The following chapter presents an overview of the literature regarding the current understanding and importance of DOM in the environment and the methods employed in the monitoring of it. A summary of the use of spectrophotometric methods in the analysis of DOM is also detailed as are the field areas monitored.

1.1 Dissolved organic matter

Dissolved organic matter is ubiquitous in soil and aquatic ecosystems. In aquatic environments natural organic matter (NOM) ranges in concentration from 0.5 mgL^{-1} DOC in alpine streams to 100 mgL^{-1} in wetland draining streams (Spitzy and Leenheer, 1991; Frimmel, 1998). An operational classification is applied to NOM, between particulate organic matter (retained on 0.45μm filter) and dissolved organic matter (DOM) (Aiken *et al.*, 1985; Spitzy and Leenheer, 1991). Organic carbon occurs bound into organic molecules and the terms DOM and DOC, are used interchangeably in the literature (Eatherall *et al.*, 1998). In aquatic environments, NOM is composed of carbon compounds and related nitrogen or phosphorus compounds (Spitzy and Leenheer, 1991).

In addition to naturally derived organic matter there are many classes of anthropogenically derived DOM in aquatic systems. These components are derived from specific sources such as agriculture or sewage, or can be present as pollutants such as pesticides, petroleum products and industrial effluents (Manahan, 1994). This work is concerned with NOM and DOM is used to denote dissolved NOM in both soil and aquatic environments. DOC is used to indicate the concentration of dissolved organic carbon.

Riverine DOM is composed of a variety of substances, which vary in time and space. Approximately 25% of DOM is fully characterised; this comprises amino acids, nucleic acids, carbohydrates, hydrocarbons, fatty acids and phenolic compounds (Spitzy and Leenheer, 1991; Thomas, 1997) the rest being composed of humic substances (HS). Estimates of the amount of HS in aquatic DOM are in the region of 40-60% (Senesi, 1993) and 50-70% (Thurman, 1985). In soil systems HS are closely

associated with non-humic components and inorganic material involving multiple interactions and aggregations (MacCarthy, 2001).

1.1.1 Humic substances

Humic substances (HS) are natural, complex, macromolecular substances that form from the breakdown of plant and animal debris and are ubiquitous in soil, sediments and water (Thurman, 1985; MacCarthy, 2001). HS are the main components of soil organic matter (~80%) but due to the complexities of formation processes and composition they are not completely described (Hayes and Clapp, 2001; MacCarthy, 2001).

Aiken *et al*. (1985) defined HS as:
"*A general category of naturally occurring heterogeneous organic substances that can generally be characterised as being yellow to black in colour, of high molecular weight, and refractory*".

This definition is still considered to be valid, however the refractory nature may only exist in protected environments, (Hayes, 1998) and the term "high molecular weight" is not always applicable (Hayes, 1997). MacCarthy (2001) has proposed a more recent definition relating to the basic principles of HS. This addresses questions about the nature of the composition and formation of HS:

"Humic substances comprise an extraordinarily complex, amorphous mixture of highly heterogeneous, chemically reactive yet refractory molecules, produced during early diagenesis in the decay of biomatter and formed ubiquitously in the environment via processes involving chemical reaction of species randomly chosen from a pool of diverse molecules and through random chemical alteration of precursor molecules."

The traditional view of humification involves the products of the biodegradation of plant and animal material in polymerisation and condensation reactions leading to a range of high molecular weight material. Many transformations during decomposition and humification have been identified, including the loss of polysaccharides and phenolic moieties, modification of lignin structures and enrichment of recalcitrant non-lignin aromatic structures (Zech *et al*., 1997).

As reviewed by Hayes (1998) HS consist of aromatic rings with substitution by hydroxyl, methoxyl and aliphatic hydrocarbon groups, some of which link the aromatic structures, in conjunction with ester functionalities. There are a number of theories regarding the molecular nature of HS, as reviewed by Hayes and Clapp (2001). HS are thought to exist as a pseudo molecular structure of associations of smaller molecular species, as large macromolecules or as micelle associations.

Aqueous solubility is used to operationally segregate HS into commonly used fractions, as defined by Aiken *et al*. (1985). The “humic acid” (HA) fraction is not soluble in acid solutions (pH 1 in soil chemistry and pH 2 in aquatic chemistry), but is at higher pH, the “fulvic acid” (FA) fraction is soluble at all pH conditions and “humin” is entirely insoluble.

The amount and composition of riverine HS is controlled by catchment soils (Hayes and Clapp, 2001) and is primarily considered to be derived from here, however, compositional differences have been observed. Malcolm (1990) found distinct differences between HS derived from soil, stream and marine environments. FA in streams were found to be intermediate between highly aromatic soil and more aliphatic marine FA. HA in soil was found to be more aromatic but similar to stream HA, both of which were more aromatic than marine HA. Stream HA was more phenolic compared to soil and marine HA. Soil HS have been found to contain less amino acids and sugars compared to aquatic derived HS (Hayes, 1998).

There is a continuum of composition in HS. HA are moderately aliphatic, highly aromatic (25-45%) and contain more phenolic and methoxy moieties compared to simple FA, which are highly aliphatic and moderately aromatic and more highly oxidsed (Malcolm, 1993; Ma *et al.*, 2001). The molecular weight of HS varies from lower values in aquatic derived HA (2000-5000 Da) to higher levels in soil derived HA (greater than $1x10^6$Da) (Aiken *et al*., 1985). FA have lower average molecular weights of 500-2000Da (Senesi, 1993), are smaller, more polar and more highly charged in comparison to HA, which suggests a more linear rather than coiled structure (Hayes, 1998).

The complex nature and polydispersity of HS, causes practical difficulties in characterising composition and establishing molecular structures (Krasner *et al.*, 1996). Hayes (1998) summarised the current information on the molecular structures and composition of HS, however at the current level of analytical ability no precise

structure can be proposed. Analysis can allow chemical characterisation, which enables predictions of functionality and an understanding of many of the interactions between DOM and other environmental components (Hayes, 1998; Frimmel, 1998).

Thurman (1985) defined aquatic HS as:
"coloured polyelectrolytic acids isolated from water by sorption onto XAD resins at pH 2".

A commonly used resin is XAD-8, which is a non-ionic and macroporous (pore size 25 μm), methyl methacrylate ester resin. The use of resins to isolate humic substances from aquatic samples was developed by Leenheer (1981) and Thurman and Malcolm (1981) and has been widely used since. In this procedure less polar fractions of DOM, including HA and FA, are sorbed onto a resin at low pH, desorbed with NaOH and HA is then precipitated at low pH.

Definitions of aquatic HS that are based on isolation and fractionation techniques have been considered to be artificial (Huatala *et al.*, 2000), as there is no chemical division between humic and non-humic substances (Peuravuori and Pihlaja, 1998b). This technique has, however, been used by many authors (Malcolm, 1990; Malcolm, 1993; Ma *et al*., 2001) and has been adopted by International Humic Substances Society (IHSS) to produce standard and reference aquatic HA and FA (Averett *et al*., 1994; Leenheer *et al*., 1994; Ma *et al*., 2001).

1.2 Aquatic DOM

Aquatic DOM originates either allochthonously, from outside the water environment or autochthonously from within the water environment (Spitzy and Leenheer, 1991; Hope *et al.*, 1994). A paucity of data has been identified in the determination of the mechanistic processes that govern DOM variations in these environments (Eatherall *et al.*, 1998).

1.2.1 Allochthonous DOM

The source, abundance, characteristics and variability of naturally derived DOM in rivers have been studied by a number of authors. These studies show that allochthonous sources are dominant over autochthonous sources in the majority of

river environments (Malcolm, 1985). The primary source of allochthonous DOM is material flushed from catchment soils and vegetation. Additionally, inputs from wind blown material, direct precipitation and leaf fall (Stockley *et al*., 1998) account for a small proportion of allochthonous aquatic DOM.

A number of factors have been identified that influence the composition and concentration of allochthonous DOM in aquatic systems. These include soil type, catchment physiography, precipitation, vegetation, peat and wetland cover, flow path of water through different soil horizons and other soil processes (Hope *et al*., 1997a; Aitkenhead *et al*., 1999). Organic carbon storage in soils has been found to explain 91% of the variance in the annual flux of DOC concentration in 17 British rivers (Hope *et al*., 1997a) and spatial changes in DOC concentration are primarily controlled by the composition and abundance of soil water inputs from different soil types (Dawson *et al.,* 2001). In Britain peat cover has been found to be the most important and useful factor in predicting annual DOC concentration export variations (Hope *et al*., 1997b; Aitkenhead *et al*., 1999).

There are two recognised sources of DOM in soils. Firstly, mature organic matter more or less humified (Zsolnay *et al*., 1999). As plant material decomposes at different rates soil organic matter is present in different states of transformation and degradation (Hayes, 1997). Secondly, fresh organic material from, for example cell lysis or rhizoexudation, which is not well humified and is not strictly classed as HS (Zsolnay *et al*., 1999). Fresh, less degraded matter is found in litter layers at the surface of soils. DOC concentrations are highest in the interstitial waters of organic-rich upper horizons declining lower down with less vegetation derived inputs (Boyer *et al*., 1997). In forested catchments fresh leaf litter has been identified as an important source of DOM as runoff from this layer has higher DOC concentrations than older litter and soils (Hongve, 1999). The leaching of fresh deciduous litter has been suggested as the controlling factor of DOC concentration seasonality in forested areas (Hongve, 1999).

Humification and production of soil DOM is microbially driven, and is thus dependent on the temperature and moisture of the soil and is ultimately climatically controlled (Zech *et al.,* 1997; Tipping *et al.,* 1999). Tipping *et al.* (1999) found that warming and drying can accelerate leachable DOM production in soils. DOM mineralization, due to heightened biodegradation of lower molecular weight fractions by biological activity is greater in pore waters with less acidic conditions (Kaiser *et al.,* 2002).

Soil type can also affect DOC concentration flux, as mineral soil horizons can act as a buffer removing DOM abiotically by adsorption as it passes through the soil. Soil interactions can remove DOM entirely or attenuate and delay the flush from the soil to the stream (McDowell and Likens, 1988). David *et al*. (1992) and Tipping *et al*. (1999) found that stream DOC concentrations and fluxes, from organic horizons in soils of upland catchments, are controlled by mineral soil adsorption of DOM. Research has shown that flow paths through the soil, antecedent conditions and soil compositions affect the composition and concentration of DOM entering streams (Hope *et al*., 1997a). The composition of DOM has been observed to be influenced by soil type, as inorganic interactions preferentially retain higher molecular weight and more aromatic components (Zhou *et al.,* 2001).

Other allochthonous inputs to surface water environments such as dry and wet deposition are low in DOC concentration (McDowell and Likens, 1988) as are ground water inputs (Fraser *et al.,* 2001). Throughfall and DOM from washing and leaching of leaves have a higher DOC concentration compared to precipitation, and may have a characteristic DOM signature (McDowell and Likens, 1988; Katsuyama and Ohte, 2002).

The yellow to dark brown/black appearance of water in rivers and streams is caused by the absorbance of light at certain wavelengths by dissolved substances (Huatala *et al*., 2000). In unpolluted waters this is derived from the presence of DOM. Functional groups that are responsible for colour in DOM are suggested to be conjugated double bonds, keto-enol groups and quinones (Gjessing *et al*., 1998). The principle colour causing materials are considered to be HS, specifically HA, which being more aromatic in composition can absorb more light (Mitchell, 1990; Huatala *et al*., 2000). Similar processes are thought to control the production of water colour and aquatic DOM (Mitchell and McDonald, 1995). Coloured streams have DOC concentrations in the range 3-50 mgL^{-1} compared to uncoloured water <2-8 mgL^{-1} (Malcolm, 1993). Similarly, water colour levels have been correlated with a number of catchment factors such as peat coverage (Mitchell and McDonald, 1995; Watts *et al.*, 2001)

Variations in land use and vegetation cover have been identified to influence the DOM fluxes in river water. Due to soil disturbance, agriculture and forestry have been observed to increase DOC concentration flux. This is observed in lowland areas,

which usually exhibit low DOC concentration levels (Aitkenhead *et al.,* 1999) and in upland forested areas when compared to natural moorland (Grieve and Marsden, 2001). Burning, gripping (ditching), afforestation and deforestation in peat areas are thought to be likely causes of increased water colour, due to drying of the peat, water table modifications and changing of flow patterns (Mitchell and McDonald, 1992; Watts *et al*., 2001).

Urbanisation has also been identified in altering DOC concentration fluxes (Westerhoff and Anning, 2000). Long term intensive land use has been observed to alter the composition of DOM, strong peat decomposition has resulted in increasing aromatic structures within water soluble FA (Kalbitz *et al.,* 1999).

1.2.2 Autochthonous DOM

The DOM produced by autochthonous processes is derived from polymerisation and degradation of existing DOM, release from living and dead organisms, and microbial syntheses within the water body (Thomas, 1997). These inputs are considered to be less important than allochthonous DOM and the net effects are not completely understood (Mitchell, 1990; Eatherall *et al.*, 1998; Lara *et al.*, 1998). Autochthonous DOM can become dominant if, for example, water bodies are ice-covered (Tao, 1998). As stream order increases, autochthonous DOM inputs from primary production and transportation from upstream become more important, when compared to headwaters. Spatial variations in soil inputs and soil types are, however, the primary control on stream DOM (Dawson *et al*., 2001).

Allochthonous and autochthonous riverine DOM have been recognised to exhibit different compositions and processes. Aquatic FA derived from allochthonous soil and litter sources generally has a higher aromatic carbon content, compared to autochthonous microbially derived FA (Malcolm, 1990; McKnight *et al*., 1994).

1.2.3 Seasonal patterns in DOM

In rivers DOC concentration is on the whole positively correlated with discharge, however hysteresis and seasonal variations complicate this relationship (Kullberg *et al.,* 1993, Frank *et al.,* 2000). Many authors have observed annual cycles in aquatic DOM concentrations and compositions. As riverine DOM is predominantly

allochthonous these cycles reflect the properties of soil and are strongly influenced by regional vegetation and climate (Lobbes *et al*., 2000).

Variations in DOC concentration, and to some extent DOM composition, are commonly related to changing flow paths through catchment soils. During low flow conditions it is thought that subsurface flow is through DOM poor soil horizons and as the water table rises DOM is flushed from upper organic rich horizons (Easthouse *et al*., 1992; Boyer *et al*., 1997). This is corroborated by DOM composition, Ivarsson and Jansson (1994) found that during summer and autumn flushing episodes DOM is less decomposed and is derived from soil surface and litter material and that base flow DOM is more decomposed and is derived from deeper soil sources. Similarly, Easthouse *et al*. (1992) used DOM hydrophobic acid and hydrophilic acid content in conjunction with inorganic components to trace runoff pathways. In comparison to soil water, base flow was found to have the characteristics of deep water and during peak flow DOM was similar to upper soil horizon derived DOM.

Maurice *et al.* (2002) observed in a small freshwater wetland that in periods of low flow, when ground water discharge to the stream was dominant, stream water DOM had low aromaticity, (weight averaged) molecular weight and DOC concentration. The opposite was observed when soil pore water was dominant during high flow. The difference was related to preferential adsorption to mineral surfaces in lower soil horizons of components of higher molecular weight and aromaticity.

Summer and autumn maxima in DOC concentration are observed in many river and lake systems, where flow is continuous throughout the year (for example Scott *et al*., 1998; Eatherall *et al*., 2000; Fenner *et al*., 2001). The main export of organic carbon occurs during autumn and winter months (Tipping *et al.,* 1997). Water colour in upland UK catchments exhibits seasonal variations that mirror DOC concentration with an autumn maximum (Mitchell and McDonald, 1992). After the autumnal maximum colour levels have been observed to decline to a winter low level, then rise during summer (Pattinson, 1994).

DOC concentration maxima in streams and rivers have been related to the production of soil DOM and subsequent flushing. Scott *et al.* (1998) found that seasonal variations in DOC concentrations, in a UK upland peat system, were consistent with the production of dissolvable organic carbon during the dry and warm, summer months when soil microbial activity is high. As soil moisture is recharged,

after a dry summer, DOC concentration in soil solution increases and when the system is flushed, by rainfall events, the flux in DOM from the soil to aquatic environments increases (Kullberg *et al*., 1993). Flushing can occur for extended periods of time until the soil DOC concentration store is depleted and the river DOC concentration falls to a winter/spring low level.

It has been observed that higher colour values are influenced by drought periods (Mitchell and McDonald, 1992). From a long-term record Naden and McDonald (1989) identified that the highest colour levels during autumn related both to soil moisture deficits in both the previous summer and the summer months immediately prior to the event. Butcher *et al.* (1992) and Watts *et al.* (2001) identified this pattern in a number of upland UK catchments, after the droughts of 1975/1976 and 1995. A low colour level was observed during these dry summers, with high autumn values occurring two years after the drought, when soil moisture had recovered. Eatherall *et al.* (2000) observed a similar pattern in DOC concentration. During autumn 1996 DOC concentration was much higher than previously seen, suggesting that it was produced during the dry summer of 1995 and flushed a year later after saturation of the soil.

Scott *et al*. (1998) and Scott *et al*. (2001) found that after the drought of 1995 the DOM in an upland peat system changed molecular composition with a suggested drop in aromatic carbon content, an increase in carboxyl group content and an increase in molecular weight before and after dry periods. These differences were suggested to be due to the oxygenation of normally anoxic peat, during dry periods, changing the DOM processing in these layers. The authors also suggest that DOM with these characteristics (low aromaticity) may preferentially be flushed after dry periods due to its greater solubility.

Mitchell and McDonald (1992) experimentally reproduced the prolonged drying of Winter Hill Peat to establish the relationship of water colour to soil moisture. They found that peat surface drying was possibly the cause of the greatest proportion of water colour. This was related to the enhanced production of coloured DOM by oxidation and microbial activity in soil pore spaces. This process and the controlling factors mirror the processes of soil organic matter formation described by Tipping *et al.* (1999). Year to year variations in DOC concentration and water colour are considered to be closely related to the response of soil microbial action and

production of DOM and to rainfall event timing, frequency and intensity (Scott *et al.*, 1998).

In catchments which are snow covered and experience low flow during winter months spring snowmelt events coincide with DOC concentration maxima even though snowmelt itself has a relatively low DOC concentration (Boyer *et al.*, 1996; Boyer *et al*., 1997). Boyer *et al*. (1996) proposed a simple model for DOC concentration during spring floods, where an initial rise in DOC concentration with rising flow is related to activation of new DOM sources in higher soil horizons. As these sources become depleted in DOM a gradual fall in DOC concentration occurs. The flow paths activated during spring floods may be unique to this type of event and thus, in such environments are important in the export of DOM (Bishop and Pettersson, 1996).

Superimposed on to annual cycles are short-term peaks of DOM concentration during storm events (Tipping *et al.,* 1997; Frank *et al.*, 2000). DOC concentrations during storm events have been explained by changing flow paths, in a similar manner to the spring flush DOC concentration maxima. During base flow subsoil is the main water runoff source. The contribution by upper soil layers and riparian areas increases during the storm and is dominant during later stages (Hinton *et al*., 1998; Frank *et al*., 2000). During storm events water colour has been observed to increase with an increase in discharge and peak colour levels occurring two hours after peak discharge (Pattinson, 1994).

1.3 The environmental importance of DOM

As DOM is environmentally ubiquitous it plays many important roles in natural ecosystems. In soil and sediments DOM greatly affects the stabilisation of colloids and aggregates (Kalbitz *et al*., 2000). This is critical in soils for maintaining the physical quality and to prevent soil degradation.

DOM can play an extensive and diverse role in aquatic ecosystems as both a beneficial and harmful component. For example, DOM is recognised as a key source of energy in stream ecosystems (Wetzel, 1992) but can limit biological activity by absorbing light at the same wavelengths as chlorophyll (Markager and Vincent, 2000). DOM is also known to play a role in the protection of freshwater ecosystems from UV radiation, by absorbance at such wavelengths (Schindler *et al*., 1997). Similarly, HS may buffer against acidification, but can also add to acidity in surface

waters (Kullberg *et al*., 1993). In aquatic ecosystems where nitrogen is limited but HS are abundant degradation by UV light and the consequent release of ammonia has an important implication for the availability of nitrogen (Bushaw *et al*., 1996).

HS interact with metals, radionuclides and nutrients and can alter the transport, reactivity and behaviour of these materials (Senesi, 1993). These interactions can have important environmental influences upon such components, making them more bioavailable or sequestering them. This may reduce both toxicity (for example, toxic metals) and biological benefits (for example, micronutrients) (Belzile *et al*., 1997). An example of this is aluminium, which during weathering of aluminosilicate rocks can be mobilized by DOM, but can also be complexed with it, resulting in reduced toxicity (Smith and Kramer, 1998). Interactions with metal ions can also affect the DOM itself, for example the tendency to aggregate (Tipping *et al*., 1988).

Organic chemicals such as pesticides similarly interact with HS influencing the fate of these in the aquatic environment. Hydrophobic pollutants can bind with the hydrophobic regions of DOM (Benson and Long, 1991). The organic chemical may be completely or temporarily immobilized when bound to the HS (Senesi, 1993), thus affecting transport and bioavailibiltiy. Due to the complex nature of HS the mechanisms of such interactions are poorly understood (Senesi, 1993; Chin *et al*., 1994; Bryan *et al*., 1998).

1.3.1 The influence of DOM on drinking water quality

DOM derived from non-anthropogenic sources is not directly toxic in drinking water supplies; however, related water quality parameters are regulated. True water colour occurs when dissolved constituents of the water absorb light within the region of visible wavelengths (380-760 nm). Water colour is not considered to be harmful to health and the World Health Organisation has not proposed health based guidelines regarding water colour (W.H.O., 1996). There are aesthetic reasons for the regulation of colour in drinking water and water producers must comply with the EC maximum of about 20 Hazen units (1.5 absorbance units m^{-1} measured at 400 nm) (Mitchell, 1990).

During water treatment DOM may react with disinfectant chlorine to form a range of compounds such as trihalomethanes (THMs), haloacetic acids and many other halogenated disinfection by-products (DBPs) (Singer, 1999). The aromatic content of

the precursor DOM has been linked with the production of such by-products, indicating that HS have a role in the formation of these molecules (Singer, 1999). Variations in DOM of the raw water can result in variations in the production of DBPs. Due to the complexities of the reactivities and composition of DOM many of the processes and end products of DBP formation are poorly understood (Li *et al.,* 2000). It has, however, been identified that the most reactive hydrophobic and aromatic fractions of DOM contribute to the potential formation of DBP in drinking water (Kitis *et al*., 2002).

Some DBP have been shown to have carcinogenic effects upon lab animals and epidemiological studies have indicated that the consumption of them in drinking water is related to cancer of the urinary and digestive tracts (Singer, 1999). These studies have shown inconclusive outcomes, however the cause for concern has resulted in the regulation of disinfection by-products. Drinking water supplies in the UK are required to have a THM level below 100 μgL^{-1} (Drinking Water Inspectorate, 1999). Alternative water disinfection techniques are available, to reduce the amounts of harmful DBPs produced.

The best option in preventing DOM related drinking water concerns is the prediction and prevention of DOM rich source waters entering drinking water supply. In the UK over 70% of the potable water supply is derived from upland areas, the major source of water colour (Watts *et al*., 2001) thus catchment management strategies have been devised to reduce the amount of coloured water reaching water treatment systems (Mitchell and McDonald, 1995).

1.3.2 The influence of climate change on DOM

Local climate changes in response to global change may influence DOM and water colour production, due to changes in temperature. Similarly, effects on flushing and transport processes due to changes in rainfall intensity and patterns may occur. As noted by Watts *et al*. (2001) in the southern Pennines (UK), future predictions of climate change involving more frequent warm and dry summers will result in a greater production of DOM and coloured water runoff, as higher temperatures and lower water tables results in the drying of peat, and led to increased rates of DOM production (Evans *et al*., 1999). The extent and regularity of such summers will have

a direct effect upon the recovery of catchments after DOM and water colour events and the associated benefits and problems of high or low DOC concentration levels.

DOC concentration has been recognised to respond to climate change. Freeman *et al*. (2001) have observed that over 12 years there was a 65% increase in the DOC concentration of freshwater derived from upland areas in the UK. The authors suggest that this is a response to increasing temperatures, stimulating the export of DOM from peat areas. With global warming, therefore, the export of DOM from the large carbon store in peat-lands will increase in extent, compared to the normal slow turnover rates.

In contrast to this, it has been observed in Canadian lake environments that over 20 years of rising temperatures DOC concentration showed a decrease of 15-25% (Schindler *et al*., 1997). The authors related this to reduced inputs of DOM due to lower rainfall levels and less runoff. This has resulted in a potentially harmful increase in UV light penetration in the lake ecosystems. Additionally, it indicates that although warmer and drier conditions may increase DOM production in soils under such circumstances it will remain there until sufficient rainfall followed by flushing occurs.

1.4 The characterisation of DOM

To understand how aquatic DOM interacts in environmental systems it is important to establish composition and variability (Hayes, 1998). To conduct accurate chemical analyses on the structures and composition of aquatic DOM it is necessary to use pure substances, which requires extraction and isolation from other aquatic solutes (Hayes, 1998). Some methods of analysis can use bulk DOM, which has undergone no processing, and additionally some research aims require this to ensure natural responses from the analysed material.

1.4.1 Extraction and concentration methods

Aiken (1985) reviewed the major methods possible to concentrate aquatic DOM and recommended the use of a resin based solid phase extraction technique. The predominant resins used in such techniques are non-ionic macroporous resins (Peuravuori and Pihlaja, 1998a and 1998b) and the extraction method involves

eluting water samples through a column of resin to concentrate hydrophobic solutes, including the humic components (Leenheer, 1981; Thurman and Malcolm, 1981). Resin-based methods can extract 80-85% of aquatic DOC concentration (Malcolm, 1993).

The resin extraction methods are difficult, time consuming and can require a large volume of sample at low DOC concentrations (Pettersson *et al.*, 1994). Acid precipitation and resin interactions can cause unavoidable structural and compositional alterations of the DOM and loss of certain fractions (Green and Blough, 1994; Peuravuori and Pihlaja 1998a and 1998b). Scott *et al*. (2001) suggested that such extraction procedures are selective, to the extent that variations observed in bulk DOM were not seen in the fractionated material. These factors present problems with this widely employed method, however the technique has been used to produce a series of aquatic and soil derived references and standards by the International Humic Substances Society (Averett *et al*., 1994).

Isolation techniques which are based on physical extraction methods, such as reverse osmosis (RO), which can concentrate up to 98.5% DOM (Clair *et al.*, 1991; Sun *et al.*, 1995; Gjessing *et al.*, 1998). Serkiz and Perdue (1990) developed a portable RO system, which has been used in a number of studies of aquatic DOM (Sun *et al.*, 1995; Crum *et al.*, 1996; Anderson *et al.*, 2000). Gjessing *et al.* (1999) suggested that, even though there is a selective loss of low molecular weight DOM during extraction, RO should be used by aquatic DOM researchers to promote international co-operation and analytical consistency. The major benefit of RO is the speed at which large volumes of water can be processed, in comparison to for example a low-pressure, low temperature evaporation technique (Aiken, 1985). Gjessing *et al.* (1999) compared an evaporation technique with RO and found that evaporation was a more efficient extraction, with less loss of DOM, but required a long processing time.

1.4.2 Analysis methods

When concentration and fractionation has been performed there are a variety of analytical techniques that can be employed to determine composition, structure and concentration of DOM. A number of these methods can be performed on bulk waters with no pre-treatment.

The most commonly used analytical methods in DOM studies are summarised in Table 1.1, with representative references. NMR techniques are considered to be very powerful tools and possibly the most useful in the elucidation of HS composition (Hayes, 1998). The technique however, may, suffer from quantitative errors, but is generally considered to be qualitatively comparable across different samples (Sihombing *et al*., 1996).

Aquatic HS are commonly considered to be derived primarily from soil humic substances, however different analytical methodologies have produced data that both confirms and contradicts this theory. Malcolm (1990) used NMR techniques to show that stream humic extracts are distinct from their respective fractions in soils. Hedges (1990) and Lu *et al*. (2000) used various analytical methods, including elemental analysis, atomic ratios and NMR to determine the sources of aquatic HS, and identified that they were derived from the surrounding soils. This discrepancy may be attributable to varying extraction and analysis methods and shows that it is important to select the correct analytical technique, to gain comparable accurate data.

Krasner *et al.* (1996), Hayes (1998), Gjessing *et al.* (1998) and Frimmel (1998) have reviewed the major analytical techniques that are available in the characterisation of bulk and extracted aquatic DOM. Extensive analyses of extracted DOM may require more purified material than it is possible to obtain from some DOM poor sources (Pettersson *et al.*, 1994; Frimmel, 1998) thus emphasis in method development has often been placed on soil rather than aquatic organic extracts (Anderson *et al.*, 1990). Recently however, there have been interdisciplinary studies dedicated to the characterisation of aquatic DOM, such as the "NOM-typing project" (Gjessing *et al.*, 1999), which aims to develop a method to classify DOM using a multi-method approach. Assessment of the current literature suggests that no one single method of analysis or process of isolation and fractionation is considered to be the best and a combination of different techniques is required for comprehensive DOM characterisation.

Method/Technique	Typical Uses	Example references
Nuclear magnetic resonance (NMR) spectroscopic techniques	Elucidation of structure including quantification of various functional groups	Sihombing *et al*. (1996); Belzile *et al.* (1997); Monteil-Rivera *et al*. (2000); van Heemst *et al*. (2000); Ma *et al*. (2001)
Pyrolysis techniques	Characterisation, functionality	Sihombing *et al*. (1996); van Heemst *et al*. (2000)
FTIR/ NIR	Characterisation, structure and functionality	Gressel *et al*. (1995); Belzile *et al.* (1997); Christy and Egeberg (2000)
UV-vis absorbance	DOC concentration and molecular composition, THM formation potential, water colour	Krasner *et al*. (1996); Belzile *et al.* (1997); Huatala *et al*. (2000)
Fluorescence spectrophotometry	Metal/pesticide interactions, composition, source and molecular characteristics	Gjessing *et al*. (1998); Mounier *et al.* (1999)
Stable isotopes/ radio isotopes	Source and age	Clapp and Hayes (1999); van Heemst *et al*. (2000)
Immobilized metal ion affinity chromatography	Separation based on affinity for metal ions	Wu *et al*. (2002)
X-ray photo electron spectroscopy	Structural information	Monteil-Rivera *et al.* (2000)
Capillary isoelectric focusing	Structural information	Schmitt *et al.* (1997)
High performance liquid chromatography	Amino acid composition	Thomas and Eaton (1996)
Potentiometric titration	Proton dissociation behaviour, acidic functional group analysis	Patterson *et al.* (1992)
Electron spin resonance	Organic radical content	Peuravuori and Pihlaja (1998b), Scott *et al*. (1998); Chen *et al*. (2002)
Elemental analysis	Nature and origin. Elemental concentrations, atomic ratios (C, N, H, O, S, P)	Belzile *et al.* (1997)
Field flow-flow fractionation	Molecular size fractionation, molecular mass and diffusion coefficients determination	Zanardi-Lambardo *et al*. (2002)
Ultra-centrifugation, High-pressure size exclusion chromatography, Gel electrophoresis	Molecular size/weight determination and distributions	Perminova *et al*. (1998); Everett *et al.* (1999); Pelekani *et al.* (1999)

Table 1.1
Analysis methods commonly applied in studies of soil and aquatic DOM.

1.5 Spectrophotometric analysis of DOM

A number of analytical techniques mentioned in Table 1.1 can be applied to water samples with no pre-treatment or extraction, these methods may not provide the molecular detail of other analyses, but providing information on DOM in its natural state is often preferable, or necessary (Krasner *et al.*, 1996). This study concentrates on the use of two such methods, those of UV-visible absorbance and fluorescence spectrophotometry. These methods can be applied to the characterisation of DOM samples and extracts, as certain constituents of DOM respond to irradiation by UV and visible wavelengths of energy.

1.5.1 The use of UV-visible absorbance spectrophotometry in the analysis of DOM

Aquatic DOM strongly absorbs energy in the UV-visible (UV-vis) wavelength range, and this has led to the use of UV-vis absorbance spectrophotometry as a method to determine composition and concentration of DOM (Korshin *et al.*, 1997). Electrons in certain functional groups, termed chromophores, are promoted in energy level, upon absorption of light energy by a molecule. Different chromophores absorb energy at different wavelengths, thus variations in composition can be inferred. Absorption of energy in the UV range is due to π electrons and reflects the presence of aromatic, carboxylic and carbonylic groups and their conjugates (Abbt-Braun and Frimmel, 1999). The π electrons are those involved in π bonding in double and triple bonds. As discussed in Section 1.2.1 visible wavelength absorption is due to keto-enol systems and quinones (Gjessing *et al.*, 1998). Additionally, the amount of absorbance increases proportionally with the concentration of chromophores (Kemp, 1991).

DOM contains many chromophoric moieties, which makes interpretation of the UV-vis absorbance spectra difficult and the resolution of specific chromophores within the spectra impossible (Korshin *et al.*, 1997). Typical UV-vis absorbance spectra of DOM, in both isolated and raw states, exhibit featureless trends of decreasing absorbance with increasing wavelength (Kalbitz *et al.*, 1999). This lack of overall resolution has led to the measurement of UV-vis absorbance at single wavelengths or wavelength ratios to determine specific compositional variations in DOM (Huatala *et al.*, 2000) as summarised in Table 1.2.

Wavelength (nm)	Property	Example reference
250nm/365nm	Aromaticity and molecular size	Peuravouri and Pihlaja (1997)
203nm/253nm	Functionality	Korshin *et al*. (1997)
254nm/436nm 270nm/350nm 465nm/665nm	Aromaticity (Humification)	Gjessing *et al*. (1998); Trubetskoj *et al*. (1999)
272nm and 280nm	Aromaticity and molecular weight	Triana *et al*. (1990); Chin *et al*. (1994); Kalbitz *et al*. (1999)
340nm	Aromaticity	Scott *et al*. (2001)
254nm and 272nm	DBP formation	Banks and Wilson (2002); Korshin *et al*. (2002)
260nm to 280nm	Hydrophobic and aromatic content	Dilling and Kaiser (2002)
254nm/400nm	Aromaticity and humification	Abbt-Braun and Frimmel (1999); Vogt *et al*. (2001)
254nm/365nm	Molecular weight	Anderson *et* al. (2000); Anderson and Gjessing (2002)
265nm/465nm	Aromaticity	Chen *et al*. (2002)

Table 1.2
The wavelengths at which UV-vis absorbance is measured, in studies of soil and aquatic DOM.

The value of 465nm/665nm has been used in many studies of DOM; especially soil derived matter, however, this parameter has no single accepted interpretation (Clapp and Hayes, 1999). The ratio has been used as a measure of humification (Trubetskoj *et al*., 1999), molecular weight and aromaticity (Chen *et al.,* 1977). Gressel *et al*. (1995) suggested that the ratio is indicative of molecular structure, and not humification and Howard *et al*. (1998) correlated increased ratios with more highly oxidised HA and suggested that a decrease in ratio corresponds with an increase in the degree of condensation.

A ratio of the absorbance at 203nm, at which bezenoid compounds usually absorb light, and 253nm, which is attributed to a charge transfer transition was used by Korshin *et al*. (1997) to indicate a change in the functionality of aromatic systems. This ratio was used by Kumke *et al*. (2001) to investigate HS response to hydrolysis. The authors related an increase in the ratio to an increase in the degree of effective functionality.

As different functional groups are responsible for absorbance at different wavelengths Abbt-Braun and Frimmel (1999) used the value of 254nm/436nm as an indicator of the proportion of UV to visible light absorbing functional groups. Gjessing *et al*. (1998) used a number of such short wavelength/ long wavelength absorbance ratios to estimate aromaticity. Low ratio values corresponded with increased aromatic carbon content measured by NMR.

The use of absorbance to estimate the aromaticity of DOM has been widely used, as it has been observed that the aromatic content of DOM, measured by other means, is proportional to the absorbance (Triana *et al*. 1990; Chin *et al*. 1994). Specific absorbance at 280nm (absorbance/DOC concentration) has been used as such a measure of the aromatic nature of DOM (for example Maurice *et al*., 2002) as in this wavelength region π to π^* electron transitions occur. Other single wavelengths have been used to determine aromaticity for example, absorbance at 340nm (per unit organic carbon) was found to correlate with the aromaticity of DOM (Aiken, 1997) and this was used by Scott *et al.* (2001) to monitor peat DOM.

The relationship of UV-vis absorbance to DOC concentration in natural waters has been utilised in an attempt to develop a quick and easy analytical technique to determine DOC concentrations. In the water treatment industry absorbance at 245nm is measured to monitor DOC concentration (Dobbs *et al.*, 1972) and in natural systems a number of wavelengths have been used; 250nm (DeHaan *et al.*, 1982), 360nm (Grieve, 1985), 330nm (Moore, 1985) and 340nm (Tipping *et al.*, 1988). These studies find a good prediction of DOC concentration by absorbance; however, Edwards and Cresser (1987) found that regression equations linking organic carbon concentration and absorbance might not be reliable, due to natural fluctuations in organic carbon. This problem was further examined by Dilling and Kaiser (2002) who measured the absorbance (260nm) of hydrophobic fractions of aquatic DOM, which contain the majority of the aromatic moieties of DOM. The authors found that absorbance was directly proportional to the concentration of the hydrophobic fraction and suggested that absorbance may be used as a measure of the hydrophobic content. This, however, also suggests that using absorbance, as a measure of DOC concentration is only valid if the DOM in question has a constant aromatic content, because the absorbance of DOM is strongly dependant on the aromatic nature.

The relationship between water colour and DOM has led to colour being used as a simple proxy for DOM concentration (Huatala *et al.*, 2000). Water colour is often

determined by comparison to a standard solution of hexachloroplatinate and cobalt ions in hydrochloric acid (Pt-Co solution) developed by Hazen in 1892. $1mgL^{-1}$ Pt is equal to one Hazen Unit and these standards can be directly, visually, compared to natural waters, as either a Pt-Co solution or in the form of coloured glass filters (Crowther and Evans, 1981).

The visual method however has been described as "not very precise" (Peuravouri and Pihlaja, 1997; Huatala *et al.,* 2000) and of lower precision than required by EU water quality analysis methods (Hongve and Åkesson, 1996). Consequently instrumental techniques have been developed which measure absorbance at different visible wavelengths, as summarised in Table 1.3. The usefulness of Pt-Co solutions as colour standards has been questioned and tannic acid has been proposed as an alternative (Cuthbert and Giorgio, 1992).

Wavelength (nm)	**Reference**
400nm	Mitchell and McDonald (1992)
440nm	Cuthbert and Giorgio (1992)
410nm	Hongve and Åkesson (1996)
Various	Crowther and Evans (1981)
400nm or465 nm	Huatala *et al*. (2000)
436nm (525nm, 620nm)	EN ISO 7887:1994
430nm	Gjessing *et al*. (1999)
462nm	Malcolm (1990)
456 nm	Bennet and Drikas (1993)

Table 1.3
Absorbance wavelengths commonly used in the instrumental analysis of water colour. Methods either quote colour as absorbance units, or standardised to Hazen units ($mgPtL^{-1}$).

1.5.2 Fluorescence spectrophotometry

Fluorescence occurs, in certain types of molecular species, when absorption of light energy from an external source results in the emission of light. A simple energy diagram explains these processes; Figure 1.1. Excitation occurs when a fluorescent species absorbs (**1**) a photon and electrons are promoted (excited) from ground state to higher vibrational energy levels. Excitation is followed by a transition to ground state from the first excited singlet state, with the emission of a photon, usually in the ultraviolet to visible range of the spectrum, shown as fluorescence (**3**) (Senesi, 1990).

Phosphorescence processes occur on the scale of seconds, due to a change in electron spin to a triplet state, whereas fluorescence happens effectively immediately on the scale of nanoseconds (Bashford and Harris, 1987). Inter-system crossing (**4**) and decay by phosphorescence (**5**), shown in Figure 1.1, results in the photon being emitted later compared to fluorescence emission.

A molecule that can exhibit fluorescence (fluorophore) exists in the excited state for a finite period (~1-10x10^{-9} seconds) and during this interval undergoes multiple possible interactions with the molecular environment, conformational changes and energy loss. The term fluorophore is usually used to denote the fluorescing component of a molecule. As the fluorescence of DOM is due to a mixture of such components it is used in this study as a term to represent all molecular components involved in fluorescence. Fluorescence intensity is proportional to the number of fluorophores in the solution (Senesi, 1990).

The wavelengths of fluorescence emission are longer (red shifted) and of lower energy than excitation or absorbance wavelengths. This energy difference is termed the Stoke's Shift and, as shown in Figure 1.1, is due to the loss of energy via non-radiative emission, such as collisional deactivation (**2**) and intersystem crossing (**4**). The measure of the relative extent to which these processes occur is the quantum yield or quantum efficiency of fluorescence, Q.

Q = F/A ***(Equation 1.1)***

Where A is the number of photons absorbed (**1** in Figure 1.1) and F is number of fluorescence photons emitted (**3** in Figure 1.1). It represents the proportion of fluorophores that are excited and then contribute to fluorescence emission, thus the probability that a molecule will fluoresce (Schulman and Scharma, 1999). Q depends upon the rate of fluorescence emission compared to the rates of non-radiative emissions and is also related to molecular structure (Senesi, 1990). In practice, Q is usually determined by comparison of the fluorescence emission of the species of interest to a standard that has a known Q. In DOM studies this standard is usually quinine sulphate in sulphuric acid (Ferrari *et al*., 1996).

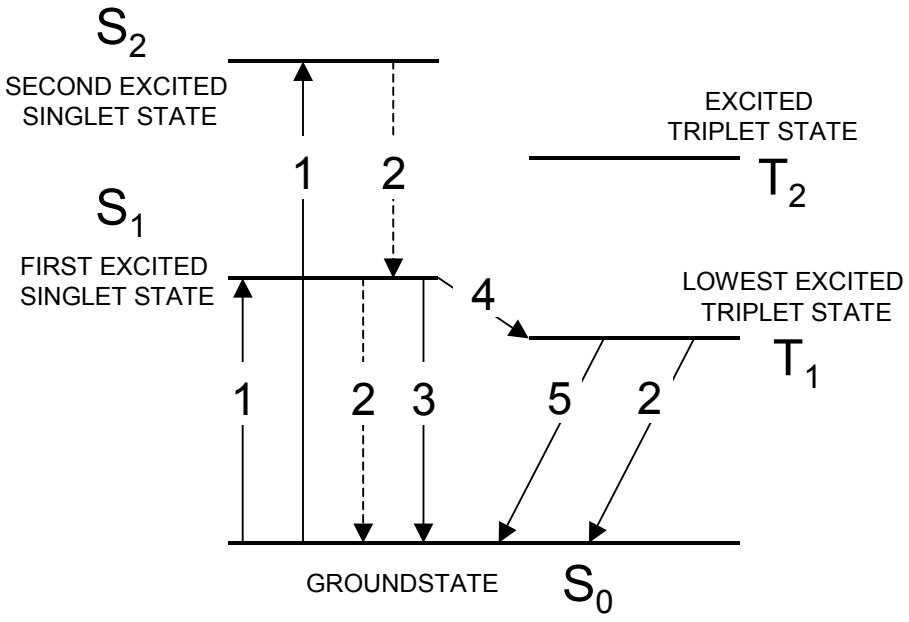


Figure 1.1
Energy transfer diagram (Jablonskii diagram) showing the photoprocesses in a typical photoactive molecule (Olmstead and Gray, 1997). ***1*** *= absorption;* ***2****= collisional deactivation and internal conversion;* ***3****= fluorescence;* ***4****= intersystem crossing;* ***5****= phosphorescence. Dashed lines represent non-radiative processes.*

Senesi (1990) described in detail the structural effects that control fluorescence processes, in relation to HS. Briefly, molecules with π bond systems, aromatic molecules and highly unsaturated aliphatic molecules fluoresce efficiently. The greater the extent of the π bond systems the lower the energy between groundstate and the first excited state, thus the longer the wavelengths of fluorescence. The presence of substituent groups, such as carbonyl, hydroxide, alkoxide and amino groups also shift fluorescence to longer wavelengths. The presence of metals in organic compounds decreases Q, due to enhanced intersystem crossing.

1.5.3 Fluorescence spectrophotometric analysis of DOM

When DOM is stimulated by excitation the resulting fluorescence occurs due to molecules containing the structures detailed above, therefore, the fluorescence processes undergone by DOM are dominated by HS and aromatic amino acids (Coble, 1996). Fluorescence spectra represent the signal from only a small fraction of the total DOM and are derived from many fluorescing molecules and associations of molecules. The molecular complexity of DOM is reflected in the fluorescence emission, which generates a broad featureless spectrum, and makes interpretation of spectra, in terms of composition and structural components, impossible (Senesi, 1990). Fluorescence spectrophotometry has, however, been used in numerous studies of DOM. Careful choice of excitation and emission wavelengths can allow the monitoring of changes in DOM composition (Vodacek, 1992) and DOC concentrations (Smart *et al*., 1976)

This technique can only provide a broad characterisation of DOM, based on the known fluorescence responses to molecular composition and structure changes, especially in comparison to more specific techniques, such as NMR methods. Fluorescence spectrophotometric analyses have a range of benefits specific to the analysis of DOM. These include simplicity, low cost, rapid analysis time, small sample volume, no necessity for pre-treatment or isolation (Kalbitz and Geyer, 2001) and the ability to analyse at natural and low concentrations (Patterson *et al.,* 1992, Frund *et al.*, 1994, Coble, 1996).

Early uses of the methods concentrated on “single-scan” techniques, which investigated excitation and emission spectra. Emission spectra are generated by measuring fluorescence emission over a range of wavelengths, at a constant excitation wavelength. Excitation spectra are obtained by the measurement of fluorescence at one emission wavelength while varying the excitation wavelength. In DOM samples a broad peak characterises emission spectra, the excitation spectra exhibit more resolved peaks and shoulders (Senesi *et al.,* 1991). The broad shape of the emission spectra indicates the presence of more than one fluorophore. The maximum fluorescence intensity of HS has been determined to occur at excitation wavelengths from 350nm to 360nm and the emission wavelengths between 420nm and 480nm (Webber, 1988).

In addition to basic excitation and emission single spectra fluorescence analysis synchronous scan fluorescence (SSF) has been used in DOM analysis. These spectra are obtained by varying both emission and excitation wavelength while maintaining a constant difference between, thus fluorescence intensity is measured as both a function of emission and excitation wavelength (Patterson *et al.*, 1992). In DOM studies such spectra are more resolved than emission or excitation spectra (Miano and Senesi, 1992) and can often separate overlapping fluorescence bands (Cabaniss, 1991) but interpretation of such SSF spectra remain difficult.

1.5.3.1 Fluorescence spectrophotometric analysis of humic substances

To identify the causes of observed variations in the fluorescence signatures of DOM, comparisons have been made to both HA and FA extracts (Senesi *et al.,* 1989; Senesi *et al.,* 1991) and simple compounds representing structural components (Senesi, 1990; Cronan *et al.*, 1992; Matthews *et al.,* 1996; Kumke *et al*., 2001).

Senesi *et al*. (1991) determined from analysis of HA and FA from different sources that relative fluorescence intensity and maximum emission wavelengths varied according to the origin and nature of the DOM. The authors, thus, determined that fluorescence can be used as a diagnostic criteria to distinguish HA and FA of different sources and to differentiate between the two. The authors described the fluorescence observed in the following manner:

"The long wavelengths and low fluorescence intensities….. mainly ascribed to the presence of linearly-condensed aromatic ring and other unsaturated bond systems, capable of a high degree of conjugation and bearing electron-withdrawing substituents such as carbonyl and carboxyl groups, and their high molecular weight units.
The short wavelengths and high intensities measured for main fluorescence peaks …….. are associated with the presence of simple structural components of low molecular weight, low degree of aromatic polycondensation, low levels of conjugate chromophores, and bearing of electron-donating substituents such as hydroxyls, methoxyls and amino groups."

This description and classification has formed the basis for numerous subsequent fluorescence spectrophotometric studies of DOM. Fluorescence emission intensity peaks with long wavelengths are postulated to be caused by fluorophores of HA-like

substances (Miano and Senesi, 1992; Mobed *et al*., 1996) and peaks with short wavelengths are attributed to FA-like fluorophores (Senesi *et al.,* 1989; Senesi *et al.,* 1991; Barancíková *et al*., 1997).

It has been observed that the analysis of smaller molecular mass fractions of DOM results in higher fluorescence emission intensities and shorter emission wavelengths, in comparison to larger mass fractions (Miano and Alberts, 1999; Wu and Tanoue, 2001b; Wu *et al*., 2002). Senesi (1990) related this to the greater proximity of chromophores in higher molecular weight DOM and an increased probability of internal quenching occurring such as collisional deactivation (**2** Figure 1.1). Additionally, increased rigidity in molecules was related to increase in fluorescence intensity, due to a reduction in internal conversions (Senesi, 1990).

1.5.3.2 Environmental influences on the spectrophotometric properties of DOM

DOM fluorescence is highly sensitive to changes in the environmental conditions of the sample. These conditions were reviewed by Senesi (1990) and include temperature, pH, metal ions, solvent interactions and other solutes. These factors can influence the processes involved in fluorescence or the structure of the fluorophores, which in turn can affect the environmental conditions resulting in complex interrelations.

In the literature regarding measurement of DOM properties by different fluorescence spectrophotometric techniques, the concentration of DOC often varies. This ranges from solutions of HS at 100mgL^{-1} (Senesi *et al*., 1991) to natural solutions of 2.00 mgL^{-1} DOC (Baker and Genty, 1999). Under constant conditions fluorescence intensity is directly proportional to the concentration of fluorophores in the solution, which corresponds to DOC concentration. It has been observed that with increasing concentrations of DOM solutions this relationship becomes non-linear over similar ranges absorbance exhibits a consistent linear relationship with concentration (Senesi, 1990; Yang and Zhang, 1995; Matthews *et al*., 1996; Mobed *et al.*, 1996).

At high solute concentrations chromophores and fluorophores interfere with the normal process of excitation and emission. This results in suppression of

fluorescence intensity (Bashford and Harris, 1987), and is described as inner-filter effects (IFE).

The processes occurring at high concentrations are summarised in Figure 1.2. The chromophore at **a** absorbs light at the wavelength of excitation of the fluorophores present, preventing this energy from reaching the fluorophore at **X**. This fluorophore is not excited at this wavelength and does not contribute to emission energy (primary IFE). Similarly emission energy from fluorophore **X** is absorbed by the chromophore at **b**, preventing this light from leaving the cuvette and being detected instrumentally (secondary IFE). The fluorophore at **Y**, however, is positioned closer to the edge of the cuvette and does not experience these interferences (MacDonald *et al.*, 1997).

This example describes a simple solution containing only one fluorophore and one chromophore; solutions of DOM contain a more complex composition and may exhibit many inner-filtering interactions. With increasing DOC concentration, and thus increasing absorbance more of these interactions can occur. As HS are thought to have a low quantum efficiency (Green and Blough, 1994) the non-fluorescing chromophores dominate in IFEs (Matthews *et al.*, 1996).

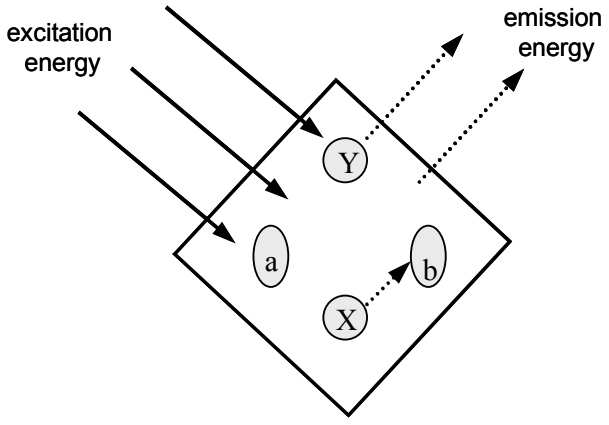


Figure 1.2
A simplified example of inner filter effects within an analysis cuvette (adapted from MacDonald et al., 1997).

As absorbance spectra of DOM show maximum absorbance at shorter wavelengths, IFE is encountered at these excitation and emission wavelengths at lower concentrations compared to longer fluorescence wavelengths (Mobed *et al.*, 1996).

This has led to the following observation by Mobed *et al*., (1996):

"If absorbance correction were ignored shifts in peak maxima with increasing concentration would be erroneously attributed to actual changes in the fluorescence spectral features of the humic substances instead of to the inner filter effects"

This indicates that consideration of and correction for IFE is vital in the analysis of DOM solutions that contain any absorbing components. In EEM studies both primary and secondary IFE must be considered (Ohno, 2002).

IFE can be reduced by viewing the fluorescence closer to the surface of the cell, reducing the path length and the potential for absorbance, dilution, standard additions, measurement at a long wavelength or application of a correction factor and the use of a triangular analysis cell (Senesi, 1990; McKnight *et al*., 2001; Chen *et al*., 2003). Dilution has been suggested as an easy method to reduce IFE (Senesi, 1990) and it has been recommended to keep absorbance below 0.05cm^{-1} at the excitation wavelength as good analytical practice (Bashford and Harris, 1987).

For DOM solutions absorbance levels of 0.5 cm^{-1} at A_{250nm} (Stewart and Wetzel, 1981) and less than 0.1 cm^{-1} (Zsolnay *et al.*, 1999; Cox *et al.*, 2000) have been suggested to avoid IFE. Alternatively maintaining a sufficiently low concentration may be used to reduce the effects of IFE. Kalbitz and Geyer (2001) found that between 10 mgL^{-1} and 3 mgL^{-1} DOC is a suitable analytical range for FA fluorescence analysis. A linear relationship of concentration to fluorescence intensity, indicating no IFE, has been found in the wider range of 2.5 to 25 $mgCL^{-1}$ in soil FA (Lombardi and Jardim, 1999). Dilution and low DOC concentration may reduce primary IFE but correction for secondary IFE may require other corrections (Ohno, 2002).

Techniques for the correction of IFE have been derived and applied to DOM analysis. Zimmerman *et al*. (1999) reviewed such correction procedures in relation to the fluorescence quenching of anthropogenic DOM by HS. McKnight *et al.* (2001) applied such a correction factor, to natural water samples and extracted FA solutions, where

the absorbance of excitation (A_{ex}) and emission (A_{em}) light is determined as follows, in Equation 1.2:

$$A_{ex} = \varepsilon c b$$

(Equation 1.2)

Where ε is specific absorptivity, c is DOC concentration and b is path length of analysis, assumed to be 0.5cm for both excitation and emission light. A_{em} is calculated in the same manner. A_{total} is the sum of A_{ex} and A_{em} and the correction, Equation 1.3, was applied to every point in the EEM.

$$I_o = \frac{I}{10^{-A_{total}}}$$

(Equation 1.3)

Where I_o is fluorescence intensity with IFE removed and I is the detected fluorescence intensity. Ohno (2002) applied a similar correction (Equation 1.4) in the investigation of humification indices, however in Equation 1.4 there is no necessity for prior knowledge of DOC concentration.

$$I_o = \frac{I}{10^{-b(A_{ex}+A_{em})}}$$

(Equation 1.4)

In this equation the absorbance of the solution at the excitation and emission wavelengths are used for A_{ex} and A_{em}. Path length is again assumed to be 0.5 cm. Following the application of this correction to soil DOM extracts a solution absorbance of 0.3 cm^{-1} was found to be the upper limit of absorbance that fluorescence derived humification index can be analysed without correction being required (Ohno, 2002). This correction does not take into account the effects of aggregation or configuration changes, which are known to change with concentration (Kalbitz and Geyer, 2001) and to date no method of correction for these phenomena have been published.

Some published work has ignored this phenomenon and the fluorescence variations yielded require reassessment in light of this. These studies include those involving natural samples, when fluorescence has been measured on raw water with high natural absorbance (Thoss *et al.*, 2000; Newson *et al.*, 2001) or extracted DOM solutions where high concentrations are used (Senesi, 1990).

Newson *et al.* (2001) used the ratio of the intensity of peaks to monitor DOM temporally and spatially. This ratio increased with increasing DOC (mgL^{-1}), suggesting that attenuation of shorter wavelength fluorescence may be occurring at higher concentrations. This response to concentration may be a compositional change as Kalbitz and Geyer (2001) found that a similar humification index, calculated from emission ratios, had a linear relationship with DOC concentration after Equation 1.4 had been applied.

In comparison to Senesi (1990) and Senesi *et al.* (1991) Yang and Zhang, (1995) found that HS cannot be compositionally fingerprinted by fluorescence spectrophotometry, and HA and FA from different sources are similar when measured at low concentrations. Other work at low absorbance and DOC concentration, such as that on marine waters (for example, Coble 1996) and cave waters (Baker and Genty, 1999) have yielded information on the variations of DOM in time and space. This indicates that even at low concentrations fluorescence spectrophotometry is a useful qualitative analysis technique.

Spectrophotometric properties of DOM are known to be highly sensitive to changes in solution pH (Senesi, 1990). A variety of responses to such changes have been observed in the analysis of both extracted HS and raw DOM samples. The majority of investigations resulted in an increase of fluorescence intensity with increasing pH, however some also observed decreases. Westerhoff *et al.* (2001) observed a 30 to 40% increase of fluorescence intensity in response to an increase in pH from 3 to 7. This corresponded to a 25% increase in absorbance (at A_{200nm} to A_{350nm}) in Suwannee River FA. Other observed fluorescence intensity increases depend on the source of the DOM and the observed pH range. For example, Yang *et al.* (1994) observed a 10% increase over pH 4.0 to 5.5 in pine litter extracts and Huatala *et al.* (2000) an increase of 19% over pH 4.4 to 7.0 in fresh water extracts.

Patel-Sorrentino *et al.* (2002) similarly observed an increase in fluorescence intensity with increasing pH over the range of 1 to 10-11, with a decrease at pH 12 in DOM

extracted from river water. This pattern of increase in intensity with increasing pH and then a decline at high pH has been observed by a number of authors. The pH at which fluorescence intensity maxima occur varies. For example, Cabaniss (1991) observed an increase in intensity to pH 2 to 5 than a decrease at higher pH levels, Smart *et al*. (1976) observed a maximum at pH 5-6 and decline to pH 13.

This response to pH has been observed at different fluorescence wavelengths, for example, Patel-Sorrentino *et al.* (2002). Shorter wavelength fluorescence, in comparison to longer wavelength fluorescence, exhibited a greater increase in intensity with increasing pH. This difference led Patel-Sorrentino *et al.* (2002) to caution against the use of fluorescence intensity ratios, as descriptions of DOM when solutions are measured at different pH levels. Differences in fluorescence intensity response to changes in pH vary due to the wavelengths observed, and thus to the characteristics of the fluorophores and DOM composition.

Spectral shifts are also observed in response to changing pH. Vodacek (1992) and Mobed *et al*. (1996) observed a red shift, in fluorescence intensity maxima, with increasing pH at long wavelengths (EXλ=~390nm) and a similar red shift at shorter wavelengths (EXλ=320nm), in soil derived HS. In aquatic derived DOM, the shorter wavelength fluorescence peak was observed to blue shift, with increasing pH (Cassasas *et al*., 1995; Mobed *et al*., 1996). Other authors have observed no wavelength change with pH (Tam and Sposito, 1993; Patel-Sorrentino *et al.,* 2002). The wide range of responses to pH reflects the complex nature and heterogeneous composition of DOM and may be additionally influenced by different analytical conditions and sample preparation.

A number of compositional reasons behind the described responses to pH change in DOM fluorescence have been discussed. Firstly, it is thought that the effects of pH are related to the presence of various acidic functional groups in the DOM (Miano and Senesi, 1992). The spectral red shift observed by Mobed *et al*. (1996) was related to phenolic groups, which have been observed to exhibit such a shift with increasing pH. Deprotonation of acidic /electron donating functional groups is related to increases in fluorescence intensity with increasing pH and blue shifts in wavelength (Senesi, 1990; Casassas *et al.,* 1995). Cabaniss (1991) noted that the fluorescence intensity of many phenols is quenched by deprotonation at high pH, which may explain the decrease of fluorescence intensity at such levels.

Spectral shifts in emission wavelengths and changes in fluorescence intensity have been related to disruption of hydrogen bonds and conformational changes in the macromolecular configuration of HS (Senesi, 1990; Pullin and Cabaniss, 1995). At high pH the macromolecule has a linear structure, and at low pH these structures contract, to form coiled pseudomicelles (Ghosh and Schnitzer, 1980; von Wandruska *et al.*, 1998) and DOM exhibits a different structure at different pH levels. It has been suggested that at low pH fluorophores may be situated within the coiled structure and are masked by non-fluorescent components and accordingly do not contribute to the fluorescence intensity (Patel-Sorrentino *et al.,* 2002). This may be used to explain both the fluorescence intensity and wavelength changes with pH, as within different pH ranges the composition and quantity of contributing fluorophores will vary. The intramolecular coiling of HS and the formation of pseudomicelles has been directly related to molecular composition (von Wandruska *et al.*, 1998)

A final explanation for pH responses involves DOM and metal ion interactions. The following points, however, indicate that metal ion interactions are not the major influence on fluorescence responses to pH change. At low pH most metal-DOM complexes will be disassociated thus fluorescence intensity quenching from metals will be reduced (McKnight *et al*., 2001). Observed data, discussed above, shows that at low pH fluorescence intensity is generally lower. Additionally, it has been suggested that the concentration of such quenching metals in fresh waters is not great enough to explain the responses to pH (Patel-Sorrentino *et al.,* 2002). The response of fluorescence intensity to pH is also observed in solutions of DOM extracts that, through the processes of extraction and fractionation, have had the metal content removed (for example Mobed *et al*., 1996).

Due to such pH effects on the fluorescence characteristics of DOM many studies use solutions adjusted to constant values. This value varies widely, for example, pH=7.8 (Matthews *et al*., 1996), pH=5 (Mounier *et al.,* 1999) and pH=2 (Zsolnay *et al*., 1999), additionally a number of studies have monitored DOM at natural pH levels (for example, Yan *et al.*, 2000; Baker 2002c). When comparing data between studies pH must be taken into account.

The influences on DOM of metal ions and other environmental agents have been studied using the phenomenon of quenching, which alters the intensity of DOM fluorescence due to influences on excited state energy processes. Examples of this

are interactions of HS with metal ions (Senesi, 1993) and pesticides (Fang *et al.,* 1998). The use of fluorescence spectrophotometric techniques in the analysis of the interactions of DOM and contaminants is recognised as a highly useful method, as the spectra reflect the energy levels of the electronic state of DOM that governs the reactions with other environmental constituents (Frimmel and Abbt-Braun, 1999). Additionally, as the technique requires no pre-concentration or separation it may only minimally disturb the equilibria that exist between the constituents in natural systems (Kumke, *et al*., 1999). The technique has been used in conjunction with most of the techniques detailed in Table 1.1 and in international DOM characterisation projects (Gjessing *et al*., 1998; Frimmel and Abbt-Braun, 1999).

1.5.3.3 Fluorescence spectrophotometric analysis of amino acids

In DOM proteinaceous material also exhibits fluorescence, derived from the presence of aromatic amino acids (Coble, 1996). There are three such amino acids: - phenylalanine; tryptophan and tyrosine and the details of fluorescence are described in Table 1.4.

Of the three amino acids phenylalanine has the lowest Q and the weakest fluorescence, as it consists of only a benzene ring and a methylene group. Fluorescence due to phenylalanine can be observed only in the absence of both tyrosine and tryptophan a combination not observed in the literature of DOM composition. The fluorescence of amino acids illustrates the effect of molecular structure upon quantum efficiency. Phenylalanine exhibits low relative fluorescence intensity, but the addition of a hydroxyl group, to form tyrosine, increases this 20 times and an indole ring to form tryptophan increases it by 200 times (Lacowicz, 1999).

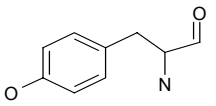
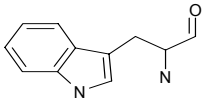
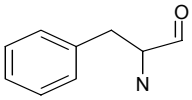
Amino Acid		Wavelengths of maximum fluorescence Excitation (nm)	Emission (nm)	Q	Abundance in DOM
Tyrosine		230 280	302 302	0.14	0.75*
Tryptophan		230 280	350 350	0.20	0.54*
Phenylalanine		260	282	0.04	1.08*

Table 1.4
*The properties of fluorescent amino acids (Lacowicz, 1999). Q= quantum efficiency. *Molar percent of amino acids in stream water from Wu and Tanoue (2001a).*

In aquatic DOM protein-like fluorescence has been correlated with tryptophan concentration as it dominates over tyrosine even though it is present in lower concentrations (Table 1.4). This is due to the higher Q of tryptophan and the quenching of tyrosine fluorescence due to energy transfer effects. Tyrosine is observed in the fluorescence signature of DOM when it is highly concentrated, such as sewage impacted waters (Wu and Tanoue, 2001a) and waters of high productivity (Determann *et al*., 1998).

It should be noted that isolated HS and DOM produced by the fractionation methods detailed in Section 1.4.1 do not exhibit any fluorescence derived from amino acids as the techniques do not retain proteinaceous material. Bulk analyses of raw water samples and DOM extracts that do not fractionate protein indicates that fluorescence spectrophotometry can be a simple and powerful method to monitor protein material in natural and waste water systems (Baker, 2001). Additionally, it presents a method to specifically monitor the concentration of tryptophan and proteins it is bound to without the need for traditional lengthy chemical and chromatographic analytical techniques (Wu and Tanoue, 2001a).

1.5.4 The use of single scan fluorescence spectrophotometry in the analysis of DOM

SSF and single scan spectra analyses have been used, often in conjunction, to investigate the influence of different environmental conditions on fluorescence characteristics of DOM extracts, such as alkaline hydrolysis (Kumke *et al*, 2001) pH (Miano and Senesi, 1992; Pullin and Cabaniss, 1995), concentration (Yang and Zhang, 1995), photo-oxidation (Vodacek, 1992), metal ion (Senesi, 1990; Cabaniss, 1992) and herbicide (Miano *et al.*, 1992) interactions and chlorination (Korshin *et al.*, 1999). In natural systems these methods have been used to monitor variations of DOM during river and lake or ocean water mixing (Ferrari *et al.*, 1996; Pullin and Cabaniss, 1997; Esteves *et al.*, 1999), degradation of pine litter (Tam and Sposito, 1993), metal complexation in pore waters (Nagao and Nakashima, 1992) and the effects of agricultural soil degradation (Kalbitz *et al*., 1999).

1.5.4.1 Qualitative fluorescence indices

Fluorescence spectrophotometry has been used in a number of ways to characterise the composition, concentration and source of DOM, primarily based on the interpretation and definition of Senesi (1990). The following section describes a number of these techniques.

The humification of DOM has been investigated by a number of authors, by quantifying the amount of red shift of emission spectra, which for this purpose is equated with increasing aromaticity. Kalbitz *et al.* (1999) used SSF to calculate the fluorescence intensity ratios of emission at 400nm/360nm and 470nm/360nm as a measure of the degree of polycondenstion and humification. Increasing values indicating an increase in both. By comparison to other analytical techniques the ratios were confirmed to increase with increasing aromatic content. This technique has been used to examine DOM change with land use in FA and original aqueous DOM solutions (Kalbitz *et al.,* 1999; Kalbitz *et al.,* 2000). Comparison of this index in original aqueous DOM samples to fractionated FA indicated that both types of DOM gave comparable humification ratio data, indicating that this technique could be used without the lengthy processing and extraction of FA (Kalbitz *et al*., 2000).

Zsolnay *et al.* (1999) used Equation 1.5 to calculate a humification index (*HIX*) using a fluorescence emission spectra (excitation of 254nm) measuring emission between 300nm and 345nm and between 435nm and 480nm. This follows the same principle as Kalbitz *et al.* (1999) of a ratio of fluorescence intensity at long wavelength to short wavelength. This method, however, specifically measures the ratio of fresh water soluble DOM (short wavelength) to more humified material (longer wavelength).

$$HIX = \frac{\sum_{W1=435}^{480} I_{W1}}{\sum_{W1=300}^{345} I_{W1}}$$

Equation 1.5

Equation 1.5 is taken from Cox *et al*. (2000), where $W1$ is the wavelength and I_{W1} is the fluorescence intensity at this wavelength. The authors used the index in the monitoring of soil amendments. Zsolnay *et al.* (1999) used Equation 1.5 to compare fresh DOM from cell lysis, aqueous soil DOM and a soil FA. This study was mirrored by Ohno (2002), who looked at the influence of concentration on *HIX* and analysed corn residue, as a source of fresh DOM material. These studies found that *HIX* increased from fresh DOM, to aqueous soil DOM, to soil FA, indicating a decrease in proteinaceous fluorescence and an increase in humification. With consideration of concentration the method was suggested to be a suitable tool to measure humification (Ohno, 2002).

As the index in Equation 1.5 measures HS fluorescence intensity (excitation = 254nm emission = 435-480nm) and tryptophan related fluorescence intensity (excitation = 254nm emission = 300-345nm) other considerations must be made in the interpretations of these limited examples. As the process of extraction and fractionation of FA can alter or entirely remove protein components (Sihombing *et al*., 1996) in comparison to milder aqueous extractions of soil DOM where protein would be obtained with the HS (Erich and Trusty, 1997). Extraction with resins, as used by Ohno (2002) to derive soil FA, is used specifically to remove non-humic molecules from soil HS (Hayes, 1998), thus, it would be expected that no short wavelength fluorescence would be seen in the FA and a high *HIX* would be obtained. This indicates that on the basis of the examples explored this index may be more

sensitive to the processing of the DOM rather than the humification degree. These humification indices reflect those discussed in Section 1.5.1 derived from absorbance ratios at different wavelengths (Gjessing *et al*., 1998).

McKnight *et al*. (2001) developed a fluorescence index to determine the source of aquatic FA and whole water samples. Fluorescence spectra from terrestrially derived FA were found to have longer peak emission wavelengths than microbially derived FA. The authors used the ratio of fluorescence emission intensity at 450nm to 500nm (excitation 370nm) to determine source and autochthonously derived DOM was found to have a higher ratio value compared to allochthonous DOM.

A number of authors have applied this index such as Westerhoff and Anning (2000) and Fraser *et al.* (2001). Donahue *et al*. (1998) used this index to identify autochthonous DOM in acidified lakes, and inferred that an increase in the proportion of this form of DOM was generated by chemical and physical changes to allochthonous DOM, rather than an increase in biological activity.

Huatala *et al*. (2000) used simultaneous measurement of absorbance and fluorescence intensity to estimate "total humus content" C_{tot} of water samples and derived Equation 1.6

$$C_{total} = C_{HA} + C_{FA} = 110A + 0.18I$$

Equation 1.6

Where C_{HA} and C_{FA} are the HA and FA type humus content (mgL^{-1}), A is absorbance at 465nm and I is the fluorescence intensity at excitation 450nm and emission 350nm.

1.5.5 The use of excitation emission fluorescence spectrophotometry in the analysis of DOM

Three dimensional excitation-emission matrix spectrophotometry (also known as total fluorescence spectrophotometry) has been used in recent years in the analysis of DOM. This method allows emission and excitation wavelength to be scanned simultaneously, producing geometric hyper-surfaces (excitation-emission matrix) defined by excitation and emission wavelengths and fluorescence intensity (Yang *et*

al., 1994; Coble, 1996; Mobed *et al.,* 1996). An excitation-emission matrix (EEM) is composed of individual excitation and emission spectra, thus combining all the information that can be derived from multiple single scan excitation or emission analyses (Figure 1.3). In these studies fluorescence intensity maxima are identified within the EEM independently of excitation or emission wavelength, unlike in single scan spectra.

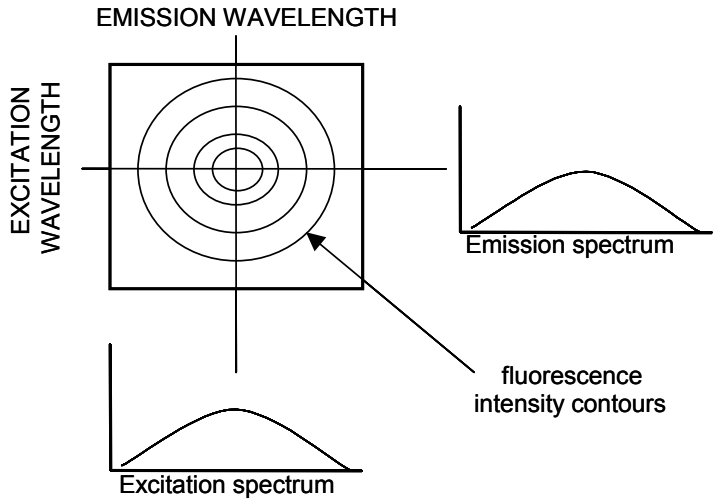


Figure 1.3.
A schematic representation of an excitation emission matrix (EEM).

EEM fluorescence variations have been used as the primary analytical technique in a number of studies, with much of the original work predominantly investigating the nature, distribution and sources of marine DOM (for example, Mopper and Schultz, 1993; Coble, 1996; Mayer *et al*., 1999). Terrestrial aquatic systems have also been studied, such as cave waters (Baker and Genty, 1999), ground water (Baker and Lamont-Black, 2001) and river and stream waters (Mounier *et al.*, 1999; Yan *et al* 2000; Wu and Tanoue 2001a; Baker 2002c) to characterise and monitor the composition of DOM. The method has been used to look at sources and changes in DOM extracted from a number of diverse sources, such as coral exoskeletons (Matthews *et al.*, 1996); aqueous extracts of pine litter (Yang *et al.*, 1994); soil organic layers (Erich and Trusty, 1997) and IHSS references and standards (Mobed

et al., 1996). In a similar manner to single scan analyses EEM has also been used in DOM metal interaction studies (Smith and Kramer, 1998; Sharpless and McGown, 1999; Elkins and Nelson, 2001; Wu and Tanoue, 2001a).

A compilation of the average positions of fluorescence maxima identified, in EEM studies of DOM is shown in Figure 1.4. The distribution of these peaks which are derived from the analysis of materials from a wide variety of sources and under differing analytical conditions shows that DOM fluorescence has consistently similar excitation and emission wavelength ranges. It should be noted, however, that the data shown in Figure 1.4 were derived in most cases using different experimental conditions, such as condition of the analyte, wavelength range, concentration and machine specification, all of which must be considered when comparing such data (Kalbitz and Geyer, 2001; McKnight *et al.,* 2001). Due to these differing conditions and the strong influences they have on fluorescence intensities the variations observed in this parameter are not discussed.

The data in Figure 1.4 were taken from the following:

Alberts *et al*. (1998); Alberts *et al*. (2002); Aoyama *et al*. (1999); Baker (2001); Baker (2002a); Baker (2002b); Baker (2002c); Baker and Genty (1999); Baker and Lamont-Black (2001); Blaser *et al*. (1999); Boehme and Coble (2000); Caseldine *et al*. (2000); Coble (1996); Coble *et al*. (1990); Coble *et al*. (1993); Coble *et al*. (1998); Del Castillo *et al*. (1999); Dettermann *et al*. (1996); Dettermann *et al*. (1998); Elkins and Nelson (2001); Erich and Trusty (1997); Esparza-Soto and Westerhoff (2001); Frund *et al*. (1994); Gjessing *et al*. (1998); Goldberg and Weiner (1994); Hemmingsen and McGown (1997); Katsuyama and Ohte (2002); Klapper *et al*. (2002); Komada *et al*. (2002); LeCoupannec *et al*. (2000); Lochmuller and Saevedra (1986); Marhaba (2000); Marhaba and Pu (2000); Matthews *et al*. (1996); Mayer *et al*. (1999); McKnight *et al*. (2001); Mobed *et al*. (1996); Mopper and Schultz (1993); Mounier *et al*. (1999); Newson *et al*. (2001); Parlanti *et al*. (2000); Patel-Sorrentino *et al*. (2002); Persson and Wedborg (2001); Sharpless and McGown (1999); Smith and Kramer (1998); Vogt *et al*. (2002); Westerhoff *et al*. (2001); Wolfe *et al*. (2002); Wu and Tanoue (2002a); Wu and Tanoue (2002b); Xiaying (2000); Yan *et al*. (2000); Yang *et al*. (1994).

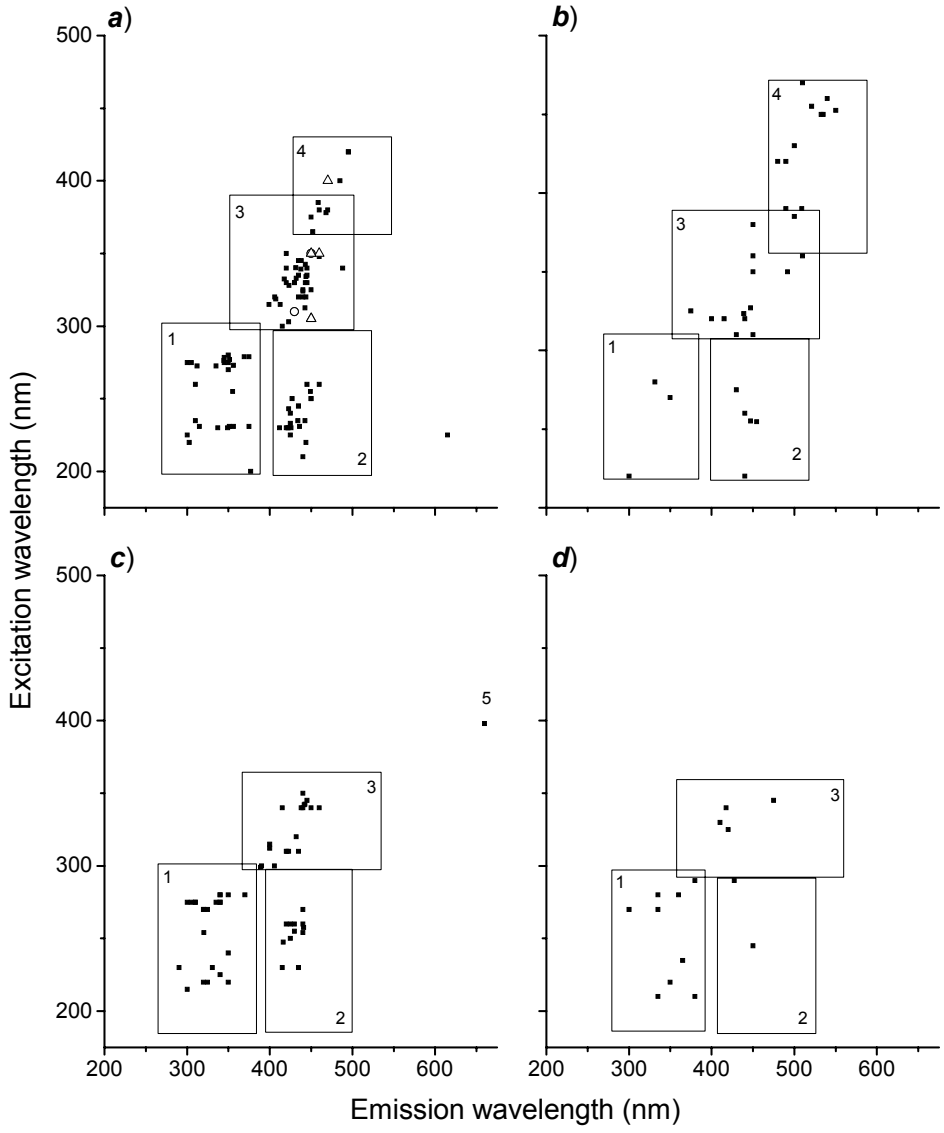


Figure 1.4
The average positions of maximum fluorescence intensity identified in EEM fluorescence spectrophotometry of DOM. ***a)*** *river/lake water* ***b)*** *soil/litter* ***c)*** *marine/estuarine* ***d)*** *wastewater.* (■) *all references* (○) *fulvic acid* (△) *humic acid from Mobed et al., (1996) boxes indicate the range of values observed. Numbers refer to DOM fractions identified in the text 1=protein-like 2,3 and 4= humic-like.*

The two major divisions of fluorescent DOM are easily identifiable from EEM analysis studies, as indicated on Figure 1.4. Firstly, protein-like fluorescence is observed in the wavelength regions detailed in Table 1.4 (region **1** in Figure 1.4). Tyrosine and tryptophan have been identified in marine water (Coble, 1996) stream water (Wu and Tanoue, 2001a) and soil DOM (Erich and Trusty, 1997). Fluorescence in the excitation wavelength ranges 250-280nm/200-240nm and emission wavelength range 300-360nm is often attributed simply to protein-like fluorescence, with no differentiation between amino acid (for example, Baker and Genty, 1999; Yan *et al*., 2000). Tryptophan emission wavelength has been recorded in the range 320-350nm (Determann *et al.*, 1996) and tyrosine in the range 300-320nm (Parlanti *et al.*, 2000) thus differentiation of the two in natural systems can be problematic.

From Figure 1.4 b it can be seen that soil derived DOM has a lower proportion of instances that identify fluorescence in region **1**, compared to aquatic sources. This however may not indicate a lower content of proteinaceous material, but may reflect the wavelength ranges examined, or the processing of the soil DOM and fractionation including removal of non-humic substances.

In the EEM fluorescence of DOM the remaining fluorescence peaks identified in Figure 1.4 are attributed to HS. Two fluorophores assigned to this source were identified in river and marine water by Coble (1996): - UV-humic fluorescence (region **2**) and visible-humic fluorescence (region **3**), each excited in the corresponding wavelength ranges. Fluorescence intensity peaks ascribed to these two major divisions have been recognised in DOM from a wide variety of sources and is observed in both raw samples and extracted DOM as shown in Figure 1.4 a, b and c. In raw river water region **3** has been further divided into two different peaks, identified at excitation ~340nm and ~380nm (Xiaying, 2000; Newson *et al*, 2001).

A basic interpretation of the fluorophores responsible for these peaks are the presence of simple aquatic phenolics, such as hydroxy-substituted benzoic acid and cinnamic acid derivatives, or simple aromatic fluorophores at shorter wavelengths and at longer wavelengths highly conjugated aromatic compounds such as coumarins and xanthones (Yang *et al.*, 1994; Blaser *et al.*, 1999). Alberts *et al.* (2002) suggested that the fluorescence intensity maxima in region **3** (Figure 1.4) are derived from the presence of “simple oxygenated aromatic components of the structural material of plants”.

As shown in Figure 1.4 a and b fluorescence peaks have been identified at longer wavelengths (region **4**) of both excitation and emission than the visible-humic fluorescence peak (region **3**). These peaks are predominantly observed in soil derived DOM (Lochmuller and Saevedra, 1986; Mobed *et al.,* 1996; Sharpless and McGown, 1999; Aoyama *et al.*, 1999), however, such peaks are also observed in DOM from aquatic sources (Blaser *et al.*, 1999).

An individual component of DOM that can be identified in EEM analysis is chlorophyll (point **5** Figure 1.4 c). This has only been recognised once in the literature (Coble *et al.*, 1998) associated with biological productivity in upwelling ocean water.

It has been suggested that fluorescence at different wavelengths is derived from the same fluorophore, as in the case of the two fluorescence maxima identified for tyrosine and tryptophan (Erich and Trusty 1997). Coble (1996), however, concluded that the behaviour of two fluorophores in region **2** and **3**, under different conditions, indicated that an additional fluorophore was contributing to the UV peak. Patel-Sorrentino *et al.* (2002) similarly found that this like peak was comparatively more sensitive to pH changes. Various authors have given different identifications to the fluorescence peaks in the three HS DOM fluorescence regions, especially assigning them to HA and FA.

In river water the fluorescence peaks in region **3** (Figure 1.4 a), at excitation 360-370 have been interpreted as being more HA-like and the peaks at excitation ~340nm to be more FA-like (Newson *et al.*, 2001; Baker, 2002c). Mounier *et al*. (1999) identified, again in river water, fluorescence in region **2** to be more FA like and in region **3** to be more HA like. Both of these interpretations are derived from the description of HS fluorescence characteristics of Senesi (1990) (Section 1.5.3.1).

Fluorescence peaks exhibited by EEM analysis of IHSS aquatic HA and FA are shown on Figure 1.4 a. HA fluoresces at similar wavelengths to FA, however exhibits an additional peak at long wavelengths, in region **4** (Mobed *et al.*, 1996). Mobed *et al.* (1996) did not monitor fluorescence at region **2** wavelengths, thus, the use of this data to assign HA and FA fluorescence wavelengths is biased toward long wavelengths. As there is a continuum in DOM composition from FA to HA the three commonly identified wavelength regions may mirror this continuum. From region **2** a more FA derived fluorescence to region **4** more HA derived. This difficulty in

differentiating fluorescence peaks and the operational definition of HS in terms of HA and FA suggests that a description of DOM in terms of its fluorescence characteristics would be more suitable in these types of study. This description may take the form of a ratio of intensity at different wavelengths (Mounier *et al*., 1998; Newson *et al.*, 2001).

From this overview of EEM fluorescence spectrophotometric studies of DOM there is a recognisable range of fluorescence wavelengths that relate to the source of the DOM. Marine and waste water DOM exhibits fluorescence in shorter wavelength regions compared to aquatic sources, which in turn are shorter in comparison to soil DOM (Figure 1.4 a, b and c). This reflects the different processing and source of DOM in each environment. As increasing fluorescence wavelengths is in part attributed to increasing aromaticity of the fluorophores this continuum mirrors the compositional differences observed by Malcolm (1990).

The comparison of the fluorescence signal between quite similar DOM may reveal how EEM fluorescence spectrophotometry can identify more subtle differences. For example Yang *et al.* (1994) analysed DOM from leaf litter that did not yield a fluorescence peak at the long wavelengths observed in soil DOM (Aoyama *et al*., 1999). This may be due to compositional differences which result in longer wavelengths of fluorescence in more humified soil DOM compared to fresher litter DOM as is expected by the breakdown of plant material and the formation of HS (Zech *et al*., 1992).

EEM fluorescence spectrophotometry benefits from the advantages of single scan analyses but yields a greater amount of data. Resulting from this it is becoming a common method of rapid DOM analysis. Although the technique does not provide specific compositional and structural data it can differentiate in terms of source and broad compositional variations.

1.6 Extraction of Soil DOM for spectrophotometric analysis

A variety of methods have been published and used in the extraction of organic matter from soils, as reviewed by Kögel-Knabner (2000) and Clapp and Hayes (2001). The purpose behind such an extraction method, for example, which soil organic fraction is of interest and what analytical techniques are to be employed, governs the method used. These methods can often involve treatments that can alter the natural state of DOM and the resulting organic matter extracts have been described as artefacts, rather than DOM components that reflect the state as it is present in the soil (Hayes and Clapp, 2001). As spectrophotometric properties of DOM are sensitive to many environmental and compositional factors extraction processes potentially result in alteration of the spectrophotometric signatures. For example, in the commonly used methods to isolate HS (Howard *et al*., 1998), extraction with a basic solution is followed precipitation with acid to separate HA and FA fractions. As discussed in Section 1.5.3.2 fluorescence signatures are highly sensitive to pH changes. This method is susceptible to such alterations of the natural fluorescence properties.

Most methods of soil DOM extraction involve fractionation stages to obtain different classes of material, separated on the basis of hydrophobic character, size or charge density. Fractionation includes resin absorption methods and electrophoretic techniques, as reviewed by Hayes and Clapp (2001). Fractionation can disrupt the associations of different DOM fractions and other inorganic components of the soil matrix, however a suitably “mild” method will result in DOM of natural compositions (Hayes and Clapp, 2001).

Other processes involved in soil extraction, such as drying of the soil sample, can alter the soil OM properties. Zsolnay *et al*. (1999) found that fluorescence emission of oven dried soil DOM was blue shifted, with a greater proportion of fluorescence in the protein-like region, compared to field moist samples. This was attributed to biomass lysis during drying.

There has been limited previous work on the examination of spectrophotometric properties of DOM in peat. An example of this work represents the problems associated with DOM extraction. Caseldine *et al*. (2000) examined DOM extracted using NaOH, in comparison to humification data measured by the transmisivity of the DOM solutions. These extracts were obtained by boiling in NaOH and the method

was found to result in “considerable breakdown” of the organic material and the resulting EEMs showed no long wavelength (peak B) fluorescence. This suggests that the extracts do not reflect the original composition of the DOM but are a product of the procedure. This extraction is essentially the same as used by Kumke *et al.* (2001) who employed alkali hydrolysis to specifically separate DOM into smaller constitutes, which also resulted in a loss of long wavelength fluorescence.

1.7 Field Areas

Sampling of DOM was performed in two areas in the UK: The Coalburn Experimental Catchment and the Loch Assynt area (Figure 1.5). These areas were chosen as both encompass peat dominated areas and areas of mineral soil. Each site has contrasting vegetation and land use and provide opportunities to investigate DOM in relation to these factors. In addition to these areas samples were taken from water bodies throughout the UK. This provided an opportunity to examine DOM from differing sources, with relation to soil type and land use.

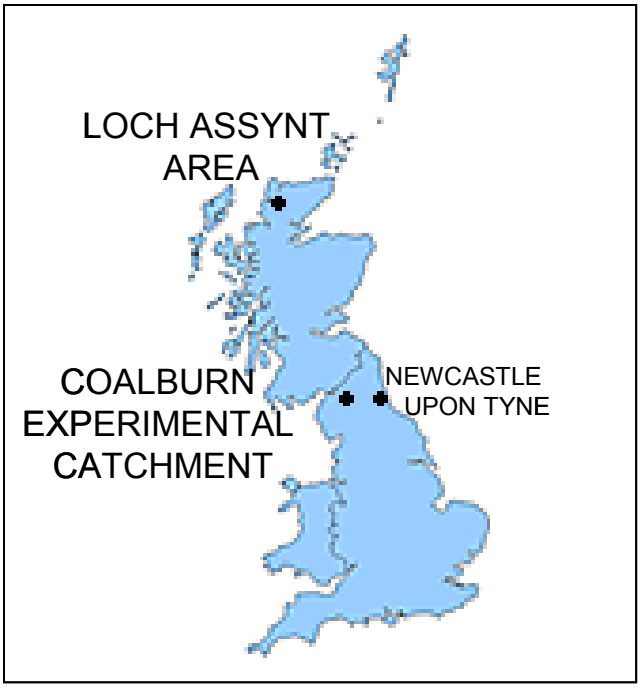


Figure 1.5
Map of Great Britain, showing the location of the field areas in this study.

1.7.1 The Coalburn Experimental Catchment

This area is located within Kielder Forest in an upland area of peat land that has been largely forested for commercial exploitation. The Coalburn catchment is typical of many upland catchments, having original waterlogged soils and, thus, requiring extensive pre-plantation ground drainage to allow tree establishment. This practice is widespread through Northern Europe (Robinson *et al*., 1998) where, on blanket peat over 45cm deep, there is an estimated 190 000 ha of forestry (Byrne and Farrel, 1997).

The effects of forestry on peat areas have been recognised to impact on hydrology, ecology, surface water quality and carbon cycling. For example, forested areas have been recognised to have runoff of greater DOC concentration and water colour compared to unforested areas (Grieve, 1990; Mitchell and McDonald 1992). In peat land areas this can result in very highly coloured waters and enhance DOM export in rivers of naturally high concentrations, as typified by the Coalburn Catchment and surrounding area. DOM export increases present water quality concerns of water colour and disinfection by-product formation in drinking water supplies. Broader concerns come with the increasing emphasis on the export of organic carbon from peat lands with changing climate conditions in relation to global climate change.

In 1966, the Coalburn Experimental Catchment was established and the extensive research at this site provides a long term background to this study and the use of data from existing monitoring equipment, installed by different agencies. Additionally, the physiography of the catchment allows studies of two sub-catchments, within the Coalburn catchment as a whole. The examination of DOM properties and fluxes in the Coalburn catchment incorporates the study of the spectrophotometric properties of DOM from highly coloured river waters and peat DOM sources.

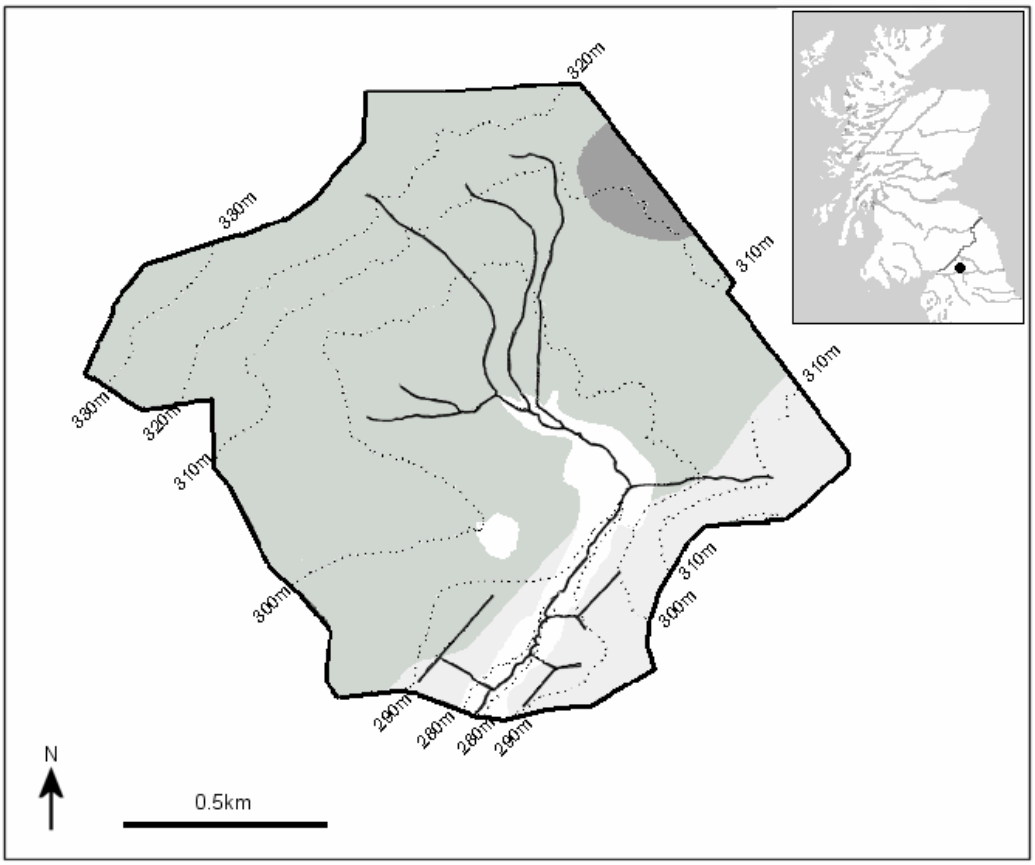
The Coalburn is a headwater tributary of the River Irthing, within Kielder Forest located approximately 40km to the northeast of Carlisle (Cumbria). The Coalburn Experimental Catchment (Figure 1.6) is a 1.5km^2 upland area with an altitude varying from 270m (AOD) to 330m (AOD), 275.3m at the catchment outfall. There is a main channel gradient of 25m km^{-1}.

Annual mean precipitation is approximately 1350mm (mean 1967-1996), which is distributed relatively evenly throughout the year and snowfall is usual most years.

Forest interception losses were measured at ~21 to 27% of the gross rainfall (1994-1998). Mean stream flow at the catchment outfall is 0.046 m^3s^{-1}. The maximum recorded flow value was 6.00 m^3s^{-1} and zero flow is observed during dry periods (Robinson *et al*., 1998).

The geology of the catchment consists of Lower Carboniferous sediments (Upper Border Group) covered by locally derived glacial/fluvioglacial boulder clay, of a thickness up to 5m. Above this is a surface layer of blanket peat generally 0.6 to 3m deep. As shown in Figure 1.6 approximately 75% of the catchment is covered by peat bog. The remaining 25% of the area, in the southeast of the catchment, has steeper slopes (>5°), and is covered by peaty gley soils (Robinson *et al*., 1998).

The catchment has been monitored since 1966 to investigate the hydrological impacts of the local forestry activities: peat drainage and tree planting, through to future felling. Prior to forestry the catchment was used for rough grazing and vegetation consisted of *Molinia* grassland and peat bog species, such as *Eriophium* spp., *Sphagnum* spp., *Juncus* spp. and *Plantago* spp. The area was ploughed and drained in 1972 and, following a year for the improvement of soil conditions, Sitka spruce (*Picea sitchensis*) and some Lodgepole pine (*Pinus contorta*) were planted in spring 1973. Approximately 90% of the catchment was planted (Figure 1.6). Boundary ditches were dug prior to plantation to define the exact area of the catchment.



Winter Hill soil series

Longmoss soil series

Wilcocks 1 soil series

Major unplanted areas

Figure 1.6
The Coalburn Experimental Catchment, showing location, soil types, topography and main surface water channels. Catchment outfall: national grid reference NY693777; 55:05:39N 2:28:40W. (Adapted from Robinson et al., 1998).

The drainage system, constructed to provide drier and more aerated soils for tree growth, increased the natural drainage density of the catchment by approximately sixty times, to 200km km^{-2}. The artificial drainage network consists of ditches (plough-furrows) spaced at about 4.5m, which are intercepted by deeper drains or allowed to run directly into the natural streams. Vegetation growth, litter accumulation and sedimentation have resulted in the infilling of the majority of these ditches that are currently 0.4 to 0.5m deep.

At the end of 1992 60% of the forest in the catchment had reached canopy closure stage and by the end of 1996 the canopy had closed and trees grown to approximately 10m tall (Robinson *et al*., 1998). The understory currently consists of *Sphagnum* spp. and some *Molinia,* with a spruce needle layer along tree rows (Hind, 1992).

Robinson (1998) summarised the implications of the hydrological effects of forestry in the catchment. The effects observed are due to artificial drain network and are manifested in increased water yield after planting and an increase in peak flows. Both of these factors have now been reduced, after tree growth and the infilling of the drainage system, however an increase in low flows has been observed that is thought to be effectively permanent during the period of forestry.

1.7.1.1 Water chemistry in the Coalburn Experimental Catchment.

The Environment Agency has performed water quality monitoring in the catchment since 1992. As part of this monitoring and other research projects there is a comprehensive record of the temporal variations in the water chemistry of the main channel. For example, the water has been monitored in particular with respect to acidification (Mounsey, 1999) and the processes relating to canopy closure (Hind, 1992). These studies have mostly concentrated on inorganic water chemistry; however, Mounsey (1999) monitored DOC concentration and water colour over the period 1993 to 1997.

The spatial variability of precipitation, surface and soil water has been monitored in Coalburn Experimental Catchment in terms of the two different pedological areas shown on Figure 1.6. These comprise, to the west, raw oligofibrous peats (Long Moss and Winter Hill series) and to the east cambic stagnohumic gleys (Wilcocks 1

series) (Robinson *et al.*, 1998). The eastern area “peaty-gley sub-catchment” has a lower mean soil moisture content compared to the western “peat sub-catchment”. “V-notch” weirs have been installed on drainage ditches on each sub-catchment for the monitoring of hydrology and geochemistry.

A selection of water quality data from the two sub-catchments and the main channel is detailed in Table 1.5 and has been summarised as follows:

“… to the eastern side of the main stream, waters are characterised by high values of pH, conductivity and concentration of sodium and calcium; there is no discolouration of these waters. The western-side waters are the converse of this…”
From Robinson *et al.* (1998).

These variations have been directly related to the sub-catchment soil properties. Robinson *et al*. (1998) suggested that the high pH and corresponding high calcium concentrations in the peaty-gley sub-catchment, as detailed in Table 1.5, are derived from the calcareous boulder clay beneath the shallow surface peat. Similarly, the increased colour from the peat sub-catchment reflects the higher organic content in soils of this area.

The broad classifications do not reflect the full variability of water quality in the catchment as a whole. For example, Mounsey (1999) identified periods when high pH levels were observed in peat sub-catchment waters. Additionally, even though the peat sub-catchment dominates in spatial extent the different characteristics of runoff from both sub-catchments influence the water characteristics of the main channel at the catchment outfall. As detailed in Table 1.5 the main channel mean characteristics of calcium concentration and pH exhibit an intermediate value between each sub-catchment. Newson *et al.* (2001) also recognised this in DOC concentration, although this is not reflected in Table 1.5. As the peaty-gley area is located nearer to the catchment outfall, it has been recognised that in the early part of a rainfall event water is displaced from here, thus, influencing outfall geochemistry, possibly acting as a buffer to pH in the main channel (Mounsey, 1999).

	Stem flow	Throughfall	Rainwater	Coalburn (main channel)			Sub-catchments	
				Mean	Low flow	High flow	Peat	Peaty -gley
pH	4.2 [b]	4.8 [b]	5.4 [a] (4.4-7.5)	4.8 [a] (3.6-7.9)	7.3 [a]	4.5 [a]	3.9 [b]	6.8 [b]
Conductivity (μScm^{-1})	320 [b]	160 [b]	40.0 [c] (27-78)	75.9 [c] (49-216)	*na*	*na*	83 [b]	120 [b]
Water colour (Hazen)	*na*	*na*	4.87 [c] (0.5-20)	124.9 [c] (50-199)	141.6 [c]	104 [c]	*na*	*na*
DOC (mgL^{-1})	*na*	*na*	3.3 [a] (1.0-23.6)	18.2 [a] (7.4-30.2)	11.7 [a]	15.9 [a]	27.5[d]	19.6[d]
Calcium (mgL^{-1})	*na*	*na*	1.1 [a] (0.1-8.5)	7.4 [a] (1.8-33.1)	27.6 [a]	2.4 [a]	3.9 [a]	12.8 [a]
Sodium (mgL^{-1})	*na*	*na*	2.4 [a] (0.1-10.7)	4.6 [a] (1.6-7.6)	4.8 [a]	4.0 [a]	4.8 [b]	5.1 [b]

Table 1.5
Selected Geochemistry of Surface Water from the Coalburn Catchment.
Adapted from Robinson et al. (1998) sampled [a]02/03/92 to 17/12/96 and [b]11/88 to 07/92; [c]Mounsey and Newson (1994) sampled 02/03/92-12/02/95 and [d]Newson et al. (2001) sampled 01/09/98-01/09/99
na =data not available; all values are means; ranges are given in brackets.

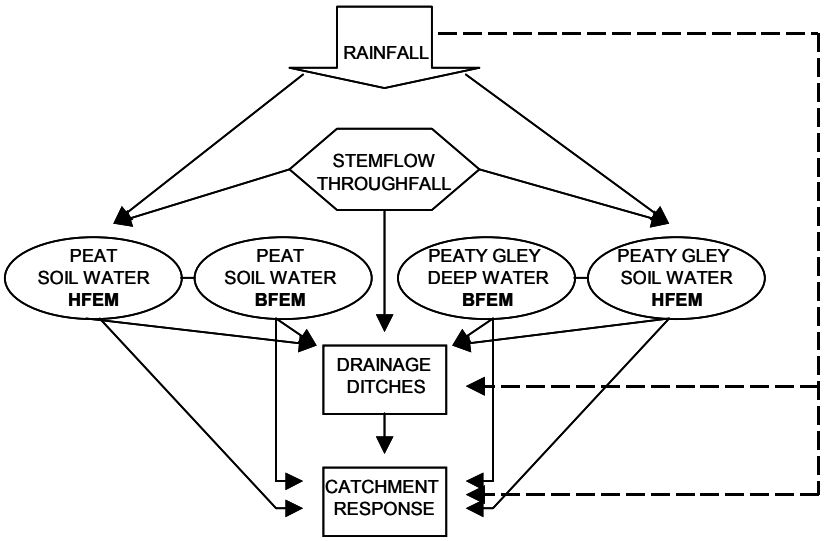


Figure 1.7
Hydrological runoff sourcing model of the Coalburn Experimental Catchment, from Mounsey (1999, page 242). HFEM= high flow end-member BFEM = base flow end-member

From investigation of water quality Mounsey (1999) devised a hydrological model of the Coalburn Experimental Catchment, to establish the flow paths associated with acidification during hydrological events. This model is reproduced in Figure 1.7 and it provisionally identifies runoff sources in the catchment. It indicates a change in source, between low and high flow conditions in the main channel, at the catchment outfall. These changes can be used to explain the variations in stream water chemistry during different flow conditions, as shown in Table 1.5.

The main points identified in this model are as follows: -

On entering the catchment soils precipitation, partitioned into stem flow and through fall in the canopy (trees and grass), becomes modified, taking on a chemical composition dependent on residence time, flow paths and soil type.

During base flow conditions the water in the main channel and ditches is derived from inputs of 'deep water' derived from lower soil levels, resulting in the well buffered (high pH) stream water composition as shown in Table 1.5. Due to the hydraulic conductivity of the soils precluding 'piston-flow' (Newson *et al.*, 2001) it is suggested that this input is transferred via seepage and slow travel along preferential pathways in the peaty gley sub-catchment.

Main channel water is derived from soil water sources during rainfall events, transported via near surface through flow and surface flow through drainage systems. This can result in the high flow stream water composition as shown in Table 1.5, typically low pH and low calcium concentration. Soil water levels have been observed to have a rapid response to rainfall and once this flow has reached the drainage network can rapidly be transferred to the main channel (Robinson *et al.*, 1998). The extensive artificial drainage system can store pooled water between rainfall events. The chemical characteristics of this water contribute to the early chemical signal in subsequent events. These ditches are now largely overgrown, but have been recognised to still be important hydrologically and to have a significant effect on catchment hydrochemistry (Robinson *et al.*, 1994; Newson *et al.*, 2001).

Precipitation, stemflow and throughfall may pass directly to the ditches and the main channel, if the catchment is saturated. This results in rapid dilution and modification of the high flow water characteristics in the main channel by water with compositions

as summarised in Table 1.5. For example, a dilution in the DOC and calcium concentration would occur if a significant precipitation input were introduced to the main channel or sub-catchment waters.

Throughfall accounts for 97% and stemflow 3% of net precipitation (Hind, 1992). No differences in chemical composition in throughfall and stemflow have been observed between the two sub-catchment areas, it is after interaction with soils that modification and differentiation occurs (Robinson *et al*., 1998). As shown in Table 1.5 stemflow and throughfall have typically lower pH and higher conductivity than rainfall, indicating that compositional modification occurs during passage through the canopy.

1.7.1.2 Previous studies of DOM in the Coalburn Experimental Catchment

As discussed above DOC concentration and water colour have been routinely measured in previous studies of the catchment water quality. In addition to spatial variability, DOC concentration and colour also exhibit the typical seasonal variations observed in many rivers (Section 1.2.1). This consists of low levels in winter and spring, rising to a maximum during the end of the summer/autumn (Mounsey, 1999). Additionally, it was noted that over the period 1993-1997 colour levels and DOC concentration increased, possibly indicating a long term increase. This pattern has been recognised by other authors in the UK and related to climate variations and recovery after drought years, which are known to generate high levels of colour (Watts *et al.*, 2001).

As discussed in Section 1.2 it has been recognised that DOM is somewhat derived from soil water, thus displaying a positive relationship with flow (Hope *et al*., 1994). Mounsey (1999) did not observe such an association, relating this to the strong seasonal trends masking short term variations. Additionally, the importance of peat as a DOM rich source during low flow conditions was noted and, thus, the maintenance of relatively high DOC concentrations during such conditions.

United Utilities plc (formerly North West Water Ltd) have studied the Coalburn Experimental Catchment in relation to disinfection by-product formation and investigated the precursor materials in upland raw water. A seasonal pattern was identified with highest concentration of trihalomethanes formed (on experimental

chlorination) during autumn and early winter and the lowest between January and March. Additionally, an increase in total trihalomethane formation was observed on the rising limb of storm hydrographs (Robinson *et al.*, 1998).

Newson *et al.* (2001) utilised the fluorescence spectrophotometric properties of DOM to examine the pathway model shown in Figure 1.7. The authors found that in the Coalburn Experimental Catchment the main channel water and the two sub-catchments could be differentiated in terms of fluorescence intensity peak wavelengths and, specifically, that peak $A_{EM\lambda}$ was significantly different at each sampling point. As discussed in Section 1.5.3.2 the influence of IFEs on waters with high concentrations of DOC, and the corresponding high levels of absorbance, requires that fluorescence intensity data undergo post analytical corrections. The authors did not employ this procedure, thus, the interpretation of annual variations in the fluorescence intensity signatures and the separation of runoff sources by season require re-evaluation. For example, the authors found an increase in fluorescence intensity during summer in the peaty-gley sub-catchment water but not in the peat sub-catchment. Through consideration of absorbance or colour, known to be highest during this period (Mounsey, 1999), the lack of a summer peak in intensity is potentially due to high IFEs and the suppression of fluorescence intensity.

The authors identified the need for further work to explore the variations in DOM fluorescence spectrophotometric properties in the Coalburn Experimental Catchment, both spatially and temporally and to evaluate the methods use in monitoring runoff pathways. These suggestions comprise some of the aims of this study.

1.7.2 The Loch Assynt area and River Traligill catchment

The Loch Assynt area (Sutherland, N.W. Scotland) represents a natural aquatic system that has undergone little anthropogenic alteration to the peat and water resources. The area provides an entirely natural end member, without the influence of forestry in the study of aquatic and peat DOM. Additionally, in comparison to the Coalburn Experimental Catchment there is a much wider range of water colour observed in the area, ranging from highly coloured water associated with upland peat areas, to low coloured river and loch waters in areas of mineral soils and bedrock. The catchment of the River Traligill provides an example of this variation, including areas of distinct geology and both peat and mineral soils.

In such areas DOM is important both in terms of drinking water quality, the majority of Scottish drinking water is derived from such upland areas, and in relation to aquatic ecosystems. DOM can limit UV light penetration in large water bodies, metal transport and bioavailabiltiy. In the Loch Assynt area these factors are both important to natural ecosystems and commercial fisheries.

The geology of the Loch Assynt area consists of Lewisian Complex gneiss unconformably overlain by Torridonian sandstones, which, in turn are unconformably overlain by a Cambrian succession of quartzites. The upper strata consist of carbonates of the Cambro-Ordovician Durness group. The area is cut by a series of horizontally–directed thrusts related to the Moine Thrust, resulting in a complex structural geology. The thrusts are the main control on groundwater movement in the area (Smart *et al.*, 1986).

Soils of the area consist of varying depths of blanket peat and localised mineral soils, which overlie a variety of glacial and fluvioglacial deposits and bedrock. Altitude ranges from ~50 m AOD at the edge of Loch Assynt to 998 m AOD (Ben More Assynt). Annual rainfall is >1200 mm based on 1961 to 1991 averages (measured at Stornoway). The climate, as described by Charman *et al.* (2001), is oceanic and the area experiences an average of 250 to 270 rain days and 4 to 6 snow days annually. The study area comprises part of the Inchnadamph National Nature Reserve and is predominantly wild.

The catchment of the River Traligill has an extent of ~21 km^2 in the area to the east of Inchnadamph (Latitude 58°08´N Longitude 4°55´W) (Figure 1.8). The catchment consists of tributaries draining areas of different geological and geomorphological character. Streams flowing from the north drain areas with steep slopes of exposed bedrock and thin peat, the solid geology consisting of Lewisian gneiss and Cambro-Ordovician quartzite.

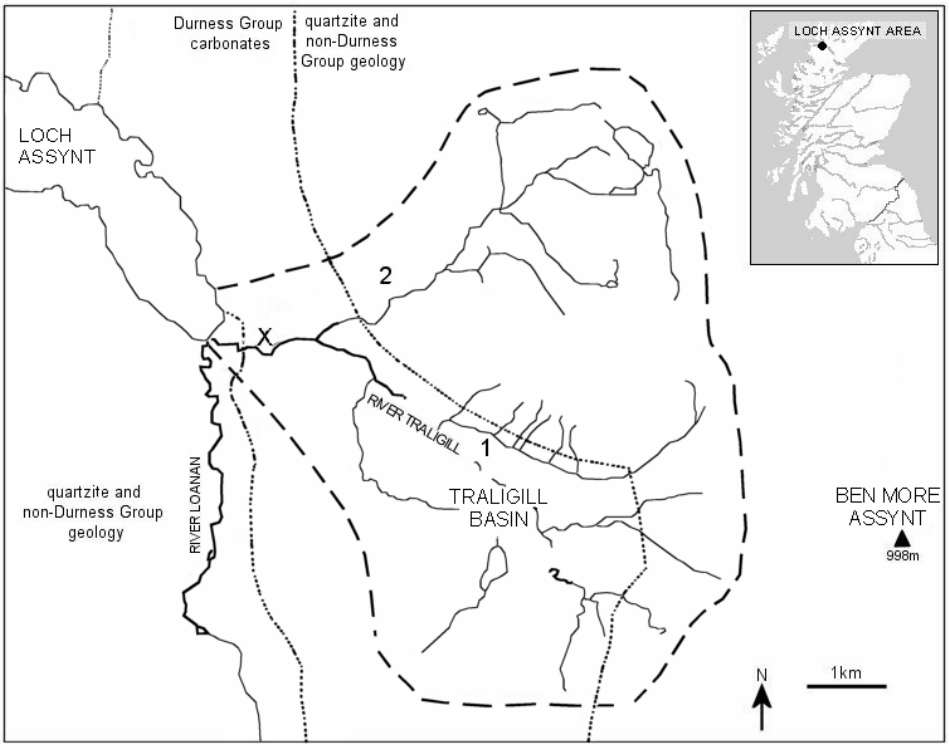


Figure 1.8
The Loch Assynt Area showing the River Traligill Catchment and geological boundaries. (**X**) *River Traligill sampling point, national grid reference NC 252218; 58:08:59N 4:58:16W.* ***dashed line*** *Estimated extent of River Traligill catchment* ***dotted line*** *Geological boundary* ***1,2*** *peat core sampling points*

In the southern area of the upper catchment of the River Traligill streams drain a peat dominated area, the Traligill Basin (NC 290200 AOD ~300 m). This consists of blanket mire overlying glacial till which in turn overlies Cambro-Ordovician Durness Group carbonates. This area of the catchment is characterised by intermittent streams, fed by runoff from the Basin, which only flow during wet periods. Due to the permeability of the underlying strata there is no input of groundwater to the peat and consequently it can dry out during prolonged dry periods. Dwarf shrubs and discontinuous *Sphagnum* cover (Charman *et al.,* 2001) are the dominant vegetation in the Traligill Basin.

Down slope of the Traligill Basin, in the middle section of the catchment, the area consists of Durness Group carbonate bedrock exposures, mineral soils and localised peat. The surface streams draining quartzite and peat sink at the contact with the

underlying carbonate geology. This results in a series of sinks and resurgences through the middle section of the catchment. In the lower section of the catchment, downstream of the Lower Traligill resurgence (NC 26732123), the channel has permanent surface flow to the confluence with Loch Assynt (NC 251219; AOD 70m).

1.8 Thesis structure

In Chapter 2 a discussion of the analytical conditions used in the study is presented. This includes sample treatment and preservation, an assessment of possible interferences and a method to obtain DOM from peat. Chapter 3 and 4 describe the spectrophotometric properties of aquatic DOM from the Coalburn Experimental Catchment. The former detailing spatial variations in surface, soil, throughfall and precipitation DOM and the latter the changes observed over time. A comparison to the observations made regarding DOM from the Coalburn Experimental Catchment is presented in Chapter 5 and 6, by discussion of the DOM spectrophotometric properties from the Loch Assynt area, together with a comparison to DOM from a wider area (Chapter 7).

The analysis of DOM derived from peat profiles is presented in Chapter 8 and the temporal, spatial and depth variations discussed. Chapter 9 concludes the study and suggestions are made for future work.

Chapter 2.

Method Development

2.1 Introduction

The following chapter will discuss the analytical methods, sample storage and post analytical considerations in the examination of the spectrophotometric properties of DOM. The interpretations of previous analyses of DOM are summarised. A wide variety of analytical conditions have been used in the analysis of DOM by fluorescence spectrophotometry. There are no standard methods of analysis, in terms of solution properties, machine conditions and sample preservation. This chapter addresses some of these points, prior to the large-scale analysis of DOM. Specifically, the influence of solution concentration and pH on spectrophotometric properties of DOM is assessed and recommendations regarding sample storage are made.

The extraction of DOM from soil and peat is often considered to involve harsh chemical and physical treatments (Hayes and Clapp, 2001). These treatments can alter the physiochemical characteristics of DOM, however, these alterations have not been quantified or consistently monitored in terms of spectrophotometric properties. The following chapter describes a “mild” aqueous dissolution method of peat DOM extraction. This method produces sufficient material for analysis and naturally analogous DOM for the application to field samples.

2.1.2 Aims

The chapter aims are to define the analytical conditions to be used in this study. This will comprise the following:

- Reproducibility
- DOC concentration influence on fluorescence spectrophotometry
- pH influence on fluorescence spectrophotometry
- Sample storage and stability

- A method to obtain DOM from peat

From this method development recommendations for analysis methods and procedures will be made.

2.2 Analytical conditions

The following section details the analytical methodology used throughout the study in the analysis of DOM.

2.2.1 Excitation emission fluorescence spectrophotometric analysis

Fluorescence was measured using a Perkin-Elmer luminescence spectrometer LS-50B. The machine derives excitation from a pulsed xenon discharge lamp, with pulse power of 20 kW and pulse width at half peak height of <10 msec and produces fluorescence using a Monk-Gillieson type monochromator (excitation range 200-800nm; emission range 200-900nm) and detects using a grated photomultiplier. Samples were analysed in a 10mm far UV silica cell and at a constant temperature of 22 ± 2°C (Newson *et al*., 2001).

Validation was performed daily using a sealed water cell containing distilled water to ensure performance within the ranges specified in Table 2.1. The signal to noise was measured using the Raman band of water with excitation at 350 nm and 10 nm excitation and emission band pass over 10 minutes analysis time.

	Minimum	**Maximum**
Raman Signal to noise ratio	500:1	
Raman Peak Wavelength (nm)	392	402
Rayleigh Scatter wavelength 1 (nm)	348.5	351.5
Rayleigh Scatter wavelength 2 (nm)	548.5	551.5

Table 2.1
Validation parameters of the LS-50B Perkin-Elmer luminescence spectrometer

Sealed water cell blank scans were run every 10-15 samples to test machine stability using the Raman peak of water, at excitation 350nm and emission 340nm-420nm.

Raman emission intensity, at 390nm averaged 20.69 ± 2.43 intensity units (n=245) (December, 1999 to April, 2002). Fluorescence emission intensities were standardised to this peak (Baker, 2002c).

It has been observed by, Kalbitz and Geyer (2001), that differing performance, reproducibility and accuracy can be obtained by using different spectrophotometric equipment. All comparisons to fluorescence data obtained using different types of machine must therefore be made cautiously. This is true for the same spectrophotometer model as the operational parameters, such as slit widths, may be different. Throughout this study all machine conditions were kept constant.

All samples were scanned in the following wavelength regions: excitation 200nm to 500nm at 5nm steps and emission 200nm to 600nm at 0.5nm steps. Analysis was performed and excitation emission matrices produced using Perkin-Elmer FL WinLab software. All samples were filtered prior to analysis using Whatman GF/C glass microfibre filter papers pre-ashed at 400°C.

2.2.2 Interpretation of fluorescence excitation emission matrices

A fluorescence excitation emission matrix (EEM) is a two-dimensional contour plot that displays fluorescence intensities as a function of a range of both excitation and emission wavelengths. Figure 2.1 presents a schematic EEM derived from the analysis of river water and shows the fluorescence centres identified in such samples. Within EEMs each contour represents points of iso-fluorescence intensity.

Distinct areas of fluorescence intensity maxima have been attributed by several authors to different components of DOM, derived from different compositional features. The fluorescence in the areas indicated on Figure 2.1, as A, B, E and F have been are related to “humic-like” substances, as discussed in Section 1.5.5 (Figure 1.3). The fluorescence maxima represented by C and D are indicative of protein-like substances.

Excitation wavelength (peak $X_{EX\lambda}$), emission wavelength (peak $X_{EM\lambda}$) were recorded at points of maximum fluorescence intensity (peak X_{Fint}) for peak A, B and C in all analyses. Specific fluorescence intensity, peak XS_{Fint}, was determined as a ratio of peak X_{Fint}/DOC mgL^{-1}. In a small number of samples peak B and C were not

identifiable. Peak D, attributable to tyrosine-derived fluorescence was monitored when present in the EEM. The position within the EEM of this peak coincides with the Raman line of water, as shown on Figure 2.1, which may interfere with fluorescence.

The scatter features shown in Figure 2.1 were ubiquitous in all EEMs. Rayleigh scattering occurs when an electron re-emits a photon at the same energy as the excitation photon, thus EXλ=EMλ. Secondary Rayleigh scattering occurs where 2EXλ=EMλ. These lines occur as diagonal features of very high fluorescence intensity across the EEM. The Raman effect is related to Rayleigh scattering, and is caused by vibrational energy being subtracted from or added to the excitation photon, which is responsible for the Rayleigh scattering (Senesi, 1990). The Raman ridge is dominant in dilute samples, as DOM concentration increases this features becomes less obvious.

A maximum fluorescence intensity centre is ubiquitous in DOM derived EEMs in the UV excitation regions E and F. The area of high fluorescence intensity F (EXλ = 220 $\pm$ 20nm) includes short wavelengths maxima attributed to tyrosine and tryptophan fluorescence. This area often contains multiple maxima, which overlap secondary scatter features, resulting in problematic identification of individual fluorescence peaks. Additionally, at low wavelengths (<250nm) lamp performance degrades, resulting in greater errors in fluorescence intensity (Mayer *et al*., 1999). With consideration of this fluorescence maxima in this area were not routinely recorded in all samples. Other interferences incurred during these analyses include possible contribution from peak A and E to peak C emission, due to the broad spectral slope extending into this region.

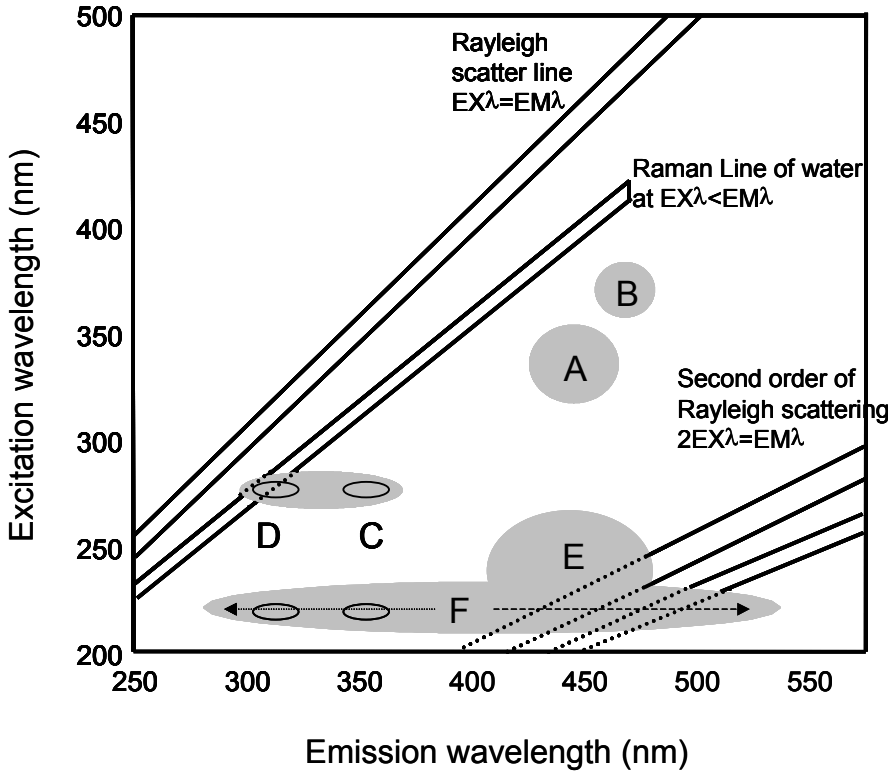


Figure 2.1
Schematic representation of a typical EEM, showing the major fluorescence intensity centres and scatter features.

2.2.3 Ultraviolet-visible absorbance

Ultraviolet-visible absorbance (UV-vis) was measured using a WPA Lightwave UV-visible Diode-array spectrophotometer (S2000), with a single beam diode array using Rowland Circle optics with a flat field corrected concave grating and pulsed deuterium and pulsed tungsten sources.

Absorbance (A_{xnm}) spectra were obtained between A_{200nm} and A_{700nm} and individual absorbance values were recorded at A_{254nm}, A_{272nm}, A_{340nm}, A_{365nm}, A_{410nm}, A_{465nm} and A_{665nm}. Samples were analysed in 10mm far UV silica cell and were blanked against distilled water. Samples were diluted with distilled water of zero absorbance if the measured absorbance exceeded the analytical range (1.999 cm^{-1}).

Absorbance ratios were calculated as follows: A_{254nm}/A_{365nm}, A_{465nm}/A_{665nm}, A_{254nm}/A_{410nm}, specific UV absorbance SUV_{254nm} (A_{254nm}/DOC mgL^{-1}) and specific visible

absorbance $Svis_{410nm}$ (A_{410nm}/DOC mgL^{-1}). Molar absorptivity (ε), absorbance normalized to moles of carbon, ($moleCL^{-1}cm^{-1}$) at A_{272nm} was calculated as an estimate of aromaticity.

2.2.3.1 Water colour

Water colour was determined by conversion of visible absorbance, A_{410nm}, to Hazen units ($mgL^{-1}Pt$) following the method of Hongve and Åkesson (1996). Conversion was performed using a dilution series of a stock solution of 500 mgL^{-1} Pt units (1.245g of potassium (IV) hexachloroplatinate and 1g Cobalt (II) chloride hexahydrate in 100ml HCl, 900ml water) as detailed in EN-ISO 7887:1994.

2.2.4 pH, conductivity and TOC

The pH and conductivity of all water samples was measured using a Myron L Company model 6P ultrameter. Modification of pH for method development experiments was performed by the addition of dilute NaOH or HCl and pH measurement using Jenway bench pH meter, calibrated daily. Samples were analysed for TOC using a Shimadzu 5000 TOC analyser.

2.2.5 Reproducibility

The reproducibility values of the major spectrophotometric characteristics of river water are detailed in Table 2.2 calculated from triplicate analysis of river water samples. When the distribution of data, for example in the of the means of two populations, is below the levels in Table 2.2 the difference observed may be explained by the reproducibility of the technique

a)

	Excitation wavelength (nm)	Emission wavelength (nm)	Fluorescence intensity
Peak A	5	7	3.6%
Peak B	6	8	3.8%
Peak C	10	10	9.7%

b)

A_{254nm}	A_{272nm}	A_{340nm}	A_{365nm}	A_{410nm}	A_{465nm}	A_{665nm}
5.0%	4.8%	5.8%	6.3%	10%	10%	27%

Table 2.2.
The reproducibility of spectrophotometric parameters of river water DOM, from triplicate analyses. (n=150) ***a)*** *fluorescence spectrophotometric properties.* ***b)*** *UV-vis absorbance properties*

2.2.6 Statistical analysis

Correlation coefficients were calculated using the Spearman's rho method and significant differences were calculated using independent sample t-tests, throughout the study. All statistical analyses were performed using SPSS (v 11).

2.2.7 Interpretation of spectrophotometric properties of DOM

A summary of the interpretations placed upon spectrophotometric properties of DOM in published studies is presented in Table 2.3. The interpretations of DOM spectrophotometric properties made in this study are based upon these previously described properties and upon the basic principles of spectrophotometry, as summarised in Section 1.5. Due to the difference in measurement methods between studies comparisons of absolute figures cannot be always made and the interpretation made are only comparative.

Spectrophotometric properties	Interpretation	references
Excitation and emission wavelengths	▪ Red shift in peak $B_{EM\lambda}$ increase in aromaticity (measured by NMR) ▪ Blue shift in emission wavelengths is a reduction in conjugation/aromaticity and the presence of hydroxy/metohoxy groups	Senesi, 1990; Senesi *et al.,* 1991; McKnight *et al.* 2001
Fluorescence intensity peak A, B, E and F	Humic substance concentrations	Coble, 1996
Fluorescence intensity Peak C and D	Amino acid/protein concentrations	Coble, 1996
Absorbance	DOM concentrations	Tipping *et al.,* 1988; Dilling and Kaiser, 2002
Peak A_{Fint}/peak B_{Fint}	Proportion of fulvic to humic acid	Newson *et al*., 2001
Specific fluorescence intensity Peak AS_{Fint} (peak A_{Fint}/DOC mgL^{-1})	Increase with lower molecular weight	Wu and Tanoue, 2001
Specific absorbance SUV_{254nm} (A_{254nm}/DOC mgL^{-1}) $Svis_{410nm}$ (A_{410nm}/DOC mgL^{-1})	Increase with increased aromaticity	Chin *et al*., 1994; Maurice *et al*., 2002
Molar absorptivity (ε) ($moleCL^{-1}cm^{-1}$)	Increase with increased aromaticity	Maurice *et al*., 2001
A_{465nm}/A_{665nm}	Aromaticity (Humification)	Gjessing *et al*., 1998;Trubetskoj *et al*., 1999
A_{254nm}/A_{365nm}	Increase with decreased aromaticity and/or molecular weight	Peuravuori and Pihlaja, 1997;Chen *et al*., 2002
A_{254nm}/A_{410nm}	▪ Increase with decreased aromaticity and/or molecular weight. ▪ Values up to 10 in DOM fractions of >50,000 and above 10 values sizes smaller than this. ▪ High values indicate the presence poorly degraded organic material, e.g. carbohydrate rich plant matter.	Vogt *et al.,* 2001; Anderson *et al.* 2000
peak A_{Fint}/A_{340nm} (fluorescence intensity efficiency)	Increase with lower molecular weight/smaller mass fractions	Wu and Tanoue, 2001; Miano and Alberts, 1999

Table 2.3
Summary and interpretation of the spectrophotometric properties analysed in this study.

2.3 Determination of the environmental influences on the spectrophotometric properties of DOM

Spectrophotometric properties of DOM vary due to compositional differences; thus, the method can be applied to DOM characterisation. The environmental conditions of the sample in the analytical solution also influence the spectrophotometric signal. These conditions include pH, temperature, concentration, solvent and solute interactions, as discussed in Section 1.5.3.2. Senesi (1990) reviewed the full range of such relationships.

In this study environmental conditions, such as temperature and solvent remained constant throughout all analyses. The interactions with other solutes, such as metal ions, was not investigated, or corrected for. Depending on the metal ion such components can enhance or reduce fluorescence intensity and blue or red shift excitation and emission wavelengths (Elkins and Nelson, 2001). As the purpose of this study is to examine the fluorescence of DOM *in situ*, in natural systems such interactions with metals, or other components are considered to be integral features of the natural fluorescence signal.

Both the pH and concentration of DOM solutions vary greatly in analytical studies involving spectrophotometric techniques. A consistent response to variations in these conditions have been observed in DOM solutions from different sources and subjected to different treatments (Mobed *et al*., 1996). Assessment of the variations in natural fluorescence signal due to fluctuations in these two parameters was required to determine the influence on the range of DOM solutions seen in this study. The following section discusses the influence that pH and DOC concentration has on river water samples and the considerations that must be given to them, in the study as a whole.

2.3.1 The correction of inner filter effects

The following section will identify a suitable and practical method to remove the influence of IFE (concentration related interference) in the spectrophotometric analysis of river water. The method is required to not undermine the benefits of

fluorescence spectrophotometry as a rapid, cheap, easy technique that monitors DOM in its natural state.

There are a number of methods that can be applied to reduce or remove the effects of absorbance, as in Section 1.5.3.2; the simplest of these is dilution or application of a correction. The use of both of these techniques was examined to determine a suitable method of correction for IFE in this study.

It has also been suggested that measurement of fluorescence emission at long excitation wavelengths (for example, 370nm; McKnight *et al*., 2001) will minimise IFE. This is due to the comparatively lower influence of IFE at such wavelengths. This method has not been considered, as a large amount of information would be lost from the EEM if this technique were employed. In addition to this, even at longer wavelengths IFE occurs in solutions of high absorbance, as illustrated below.

Dilution of a solution results in a weakening of IFE with a reduction in absorbance and it has been used as a method to remove the problem (Cox *et al*., 2000), by creation of a solution that has a linear relationship of absorbance to fluorescence intensity. Both dilution to a level of absorbance at which no IFEs occur and to a constant level of absorbance and thus a constant level of IFE have been used in previous studies (Ohno, 2002). Constant concentration of DOC has also been used, however this does not directly address the cause of IFEs. Due to compositional variations in DOM, solutions of the same concentration can exhibit different absorbance levels, and different IFEs.

To assess the use of dilution in removing IFEs from a range of DOM, 15 river water samples (D1-D15) from different sources were sequentially diluted, with distilled water (absorbance = 0), to absorbance <0.1cm^{-1} at A_{340nm} and analysed as detailed in Section 2.2. Details of these samples are recorded in Appendix 1a. The original samples and diluted solutions were not treated in any other manner.

The effects of increasing absorbance on fluorescence intensity can be seen in Figure 2.2. At absorbance of greater than ~0.25cm^{-1} the relationship with peak A_{Fint} becomes non linear as IFE suppression occurs. This level varies between samples from ~0.2 to 0.45cm^{-1}. When plotted against DOC concentration similar trends are also observed.

IFE occurs at lower absorbance levels at shorter excitation and emission wavelengths, thus longer wavelength peaks exhibit intensity attenuation at higher absorbance. This is illustrated by comparison of Figure 2.2a to 2.2b where only in a number of samples is the influence of IFE on peak B_{Fint} seen. In all of the dilutions performed that absorbance had a positive relationship with solution strength.

All dilutions shown in Figure 2.2 exhibit different relationships of fluorescence intensity to absorbance, reflecting the natural variations in raw DOM spectrophotometric properties. The estimated absorbance level at which the linear relationship to fluorescence intensity ends does not correlate with any properties of the original undiluted sample, such fluorescence peak intensity, wavelength, absorbance or source (95% confidence level). Due to these variations it would be impossible to design a broad correction for IFEs, in multiple samples based on the dilution curve of a different sample and using the undiluted characteristics. Therefore, it is required that each individual sample is diluted, for example to a low absorbance level at which IFE is not thought to occur.

To examine the application of this method to the analysis of river water samples a set of 31 samples from Coalburn Weir (Chapter 3) were diluted to an absorbance level of $0.05cm^{-1}$ $\pm 0.002cm^{-1}$ at A_{340nm} (peak $A_{EX\lambda}$ of all of the samples). This level has been suggested to be a suitable level for analysis with no IFE (Bashford and Harris, 1987). On dilution of the samples peak A_{Fint} exhibited a mean decrease of 70.10% (s.d. 1.96), peak B_{Fint} of 77.22% (s.d. 1.61) and A_{340nm} of 91.15% (s.d. 0.59). This disparity indicates the suppression in fluorescence intensity, at the natural concentration levels of the samples, compared to absorbance. The potential use of such a method is discussed further below.

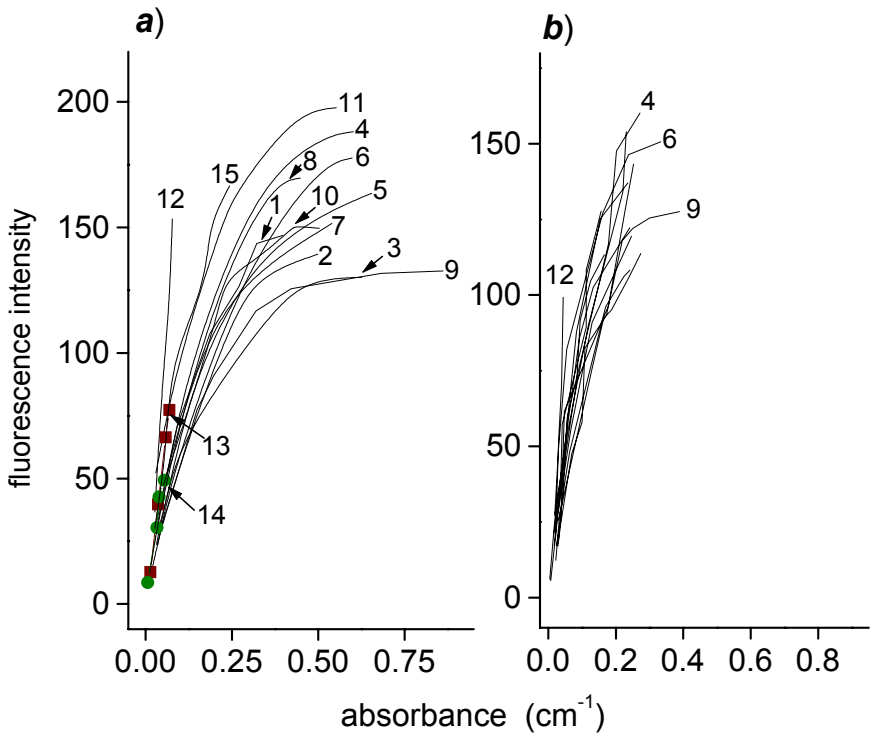


Figure 2.2
The relationships of absorbance (at EXλ) to fluorescence intensity on dilution of river water. ***a)*** *peak* A_{Fint} ***b)*** *peak* B_{Fint} *Samples 13 and 14 and numbering are excluded from 2.3b. For sample details see Appendix 1a.*

Correction method	Peak A_{Fint}	Peak B_{Fint}
Equation 1.4	+120.29% (s.d. 10.59)	+49.97% (s.d. 9.69)
Extrapolation of diluted data	+239.44% (s.d. 33.86)	+158.31% (s.d. 22.59)
Application of Equation 1.4 to samples diluted to constant absorbance	+7.07% (s.d. 0.72)	+5.07% (s.d. 1.28)

Table 2.4
Summary of the changes in fluorescence intensity observed on application of IFE correction methods.

Equation 1.4, presented by Ohno (2002), was applied to the data set of 31 water samples from Coalburn Weir. The resultant amount of fluorescence intensity change is summarised in Table 2.4. From these values the higher absorbance and thus greater influence of IFE at shorter wavelengths can be recognised in the difference between peak A_{Fint} and peak B_{Fint}. The fluorescence intensity change observed when the original sample fluorescence intensity was adjusted using data from dilution is also summarised on Table 2.4. This adjustment to remove IFE was performed by multiplication of the diluted fluorescence intensity by the amount of dilution.

After application of Equation 1.4 to the fluorescence intensity of the samples diluted to constant absorbance both peak A_{Fint} and increased (Table 2.4). This indicates that IFE is greatly reduced by dilution, however not entirely removed, if the equation is correct. To obtain a percentage increase of fluorescence intensity that is within the reproducibility of this technique (Table 2.2) it would be required to dilute solutions to absorbance $A_{ex} + A_{em} = 0.031$ for peak A and $B_{ex} + B_{em} = 0.033$ for peak B. At such low levels of absorbance fluorescence intensity approaches the lower limit of detection of the LS50 B and fluorescence peaks are hard to identify in the background fluorescence of water, which can exhibit fluorescence intensity up to approximately 10 intensity units.

Determination of fluorescence intensity without IFE through dilution appears to overestimate the amount of suppression compared to correcting using Equation 1.4, as shown in Table 2.4. This is possibly due to complex modifications in the degree of association and to configuration rearrangements, which have been recognised to occur due to changes in concentration in DOM solutions (Senesi, 1990; Tam and Sposito, 1993). These changes are likely to be related to the composition of the DOM, which can vary with source and may explain the different dilution trends in Figure 2.2. Due to the heterogeneous nature of DOM such alterations are difficult to quantify or predict. Application of Equation 1.4 may also result in an overestimation of IFE as it assumes that primary and secondary IFEs are equal, secondary processes are not as important as primary (Bashford and Harris, 1987).

As shown on Figure 2.2 peak A_{Fint} and peak B_{Fint} respond differently to changes in absorbance. This results in a consistent response of peak B_{Fint}/peak A_{Fint} to absorbance, as shown in Figure 2.3. Changes in this ratio seen in river waters may reflect concentration or absorbance variations rather than DOM composition or changes in the proportion of the fluorophores responsible for peak A_{Fint} and peak B_{Fint}. After application of Equation 1.4 the ratio value shows no change with changing absorbance. This suggests the necessity for IFE correction when ratios of fluorescence intensity at different wavelengths are being used as a qualitative measure of DOM.

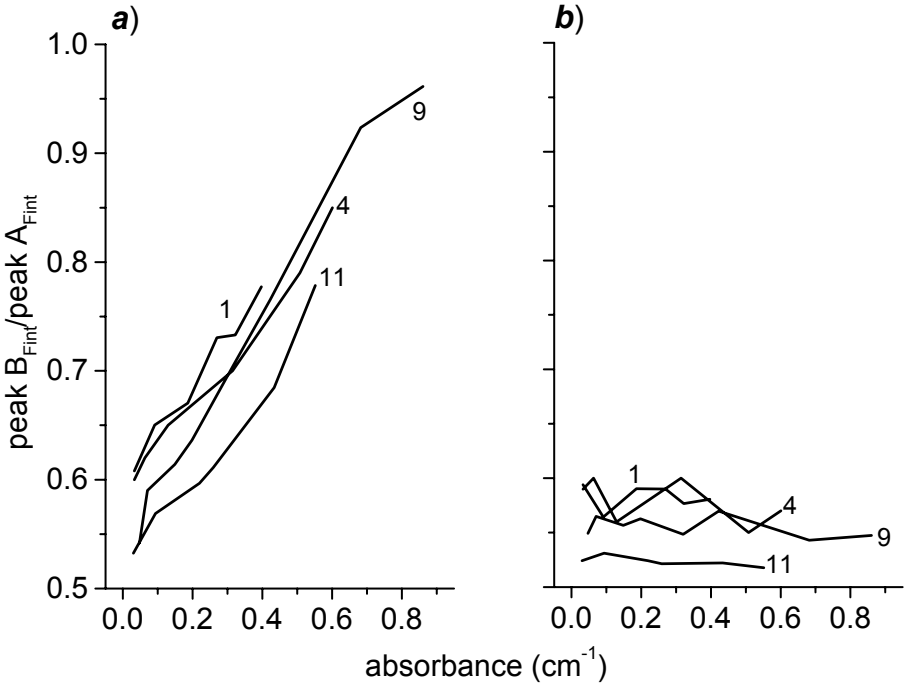


Figure 2.3
*The response of peak B_{Fint}/peak A_{Fint} to changes in absorbance (at peak $A_{EM\lambda}$) in a number of representative samples **a)** uncorrected intensity data **b)** intensity data corrected using Equation 1.4. For sample details see Appendix 1a.*

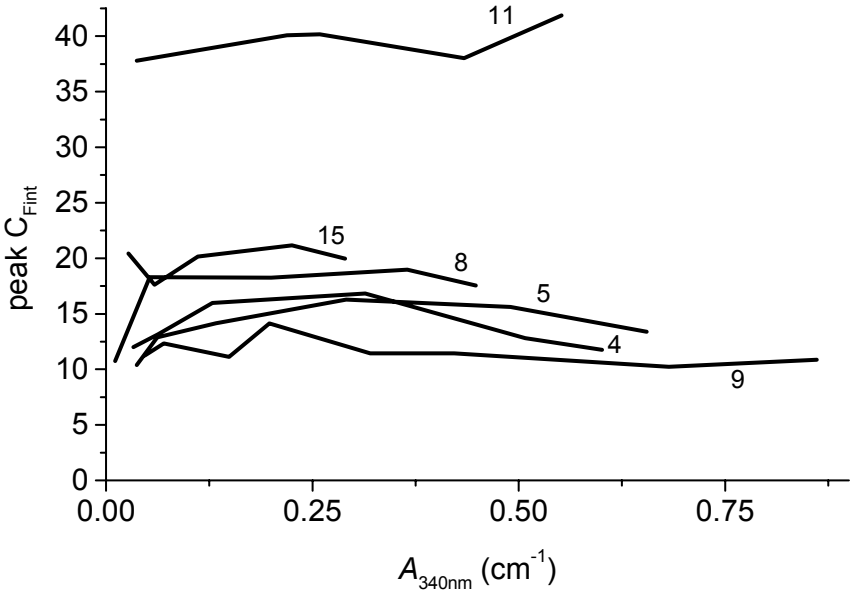


Figure 2.4
Dilutions of river waters showing the relationships of absorbance to peak C_{Fint}. Representative samples are shown. For sample details see Appendix 1a.

Peak C_{FInt}, as shown in Figure 2.4, did not respond to dilution in the same manner as peak A_{FInt} and peak B_{FInt} and was independent of absorbance in the observed range. This is due to the high quantum efficiency of the fluorophores that contribute to peak C_{FInt}, primarily the amino acid tryptophan. These molecules exhibit a greater proportion of fluorescence emission intensity of the energy absorbed by the chromophores compared to HS-like substances (Mayer *et al.*, 1999). Alteration of absorbance has little effect on the peak C_{FInt}, additionally; it confirms that the majority of the absorbance in DOM solutions is derived from humic like material.

Excitation and emission wavelengths similarly do not show any relationship with absorbance, outside the reproducibility of the technique. Wavelengths changed up to ±5nm on dilution. It can be assumed therefore that any changes of wavelengths or peak C_{FInt} with absorbance or DOC concentration in data sets are compositional variations, not IFE artefacts. A similar result was obtained by Mobed *et al.* (1996), who found that concentration had little effect on the spectral characteristics of HA and FA.

2.3.1.1 Recommendations for the correction of inner filter effects

On dilution different samples exhibit varying absorbance levels below which peak A_{FInt} and peak B_{FInt} have linear relationships with absorbance. Dilution, as a method to remove IFEs would require each sample to be diluted to very low (for example, $<0.2cm^{-1}$ A_{340nm}) absorbance levels. To confirm that this dilution has removed IFE and the relationship between absorbance and intensity is linear, a dilution series has to be made for each individual sample. This is time consuming in terms of both preparation and analysis and additionally, would require a sufficiently large sample. This procedure would eliminate some of the benefits of the technique, fast processing times and small sample requirement. As dilution relationships reflect the variations in the DOM, and possible alterations are incurred due to dilution, analyses of diluted samples result in spectrophotometric properties of DOM that are no longer in the natural state. The different relationships observed in the dilution series may provide information on the characterisation of DOM, if the complex interactions that occur during dilution are understood and quantified.

It is recommended for the analysis of DOM in this study, and in other work, that data from all analyses have Equation 1.4 applied to fluorescence intensities. This will

ensure identical treatment of data from all samples, and provide fluorescence intensities that are potentially comparable to other published work (for example Kalbitz and Geyer, 2001). Examination of uncorrected fluorescence intensity data is also suggested to compare to other studies that have not corrected for IFE.

2.3.2 Determination of the influence of pH on the spectrophotometric properties of DOM

To establish how natural variation in river water pH may influence DOM properties a number of pH manipulations of such samples were performed. Modification of pH was performed; on samples number F1 to F28, detailed in Appendix 1b, by addition of dilute HCl or NaOH. The buffers were used to replicate the treatments performed in various other studies and as there was no intrinsic fluorescence derived from them. Details of the samples used are in Appendix 1b. The observed response to the increase in pH in summarised in Table 2.5, Figures 2.5 to 2.7, for four representative samples (F4, F11, F13, F18). These examples show the range of trends observed in all samples examined and represent riverine DOM from different sources.

Spectrophotometric properties	Response to increase in pH (2-10)
Peak C variables	No response
Peak $A_{EX\lambda}$ and peak $B_{EX\lambda}$	No response
Peak $A_{EM\lambda}$	No consistent response or variation outside the reproducibility of the method.
Peak $B_{EM\lambda}$	A significant (95% confidence level) red shift was observed in all samples, over a different pH range and magnitude for each sample, (approximate range 4 to 8), summarised in Table 2.6. A number of samples this shift exceeded the reproducibility of the method
Peak A_{Fint}	Increase, to a maximum at variable pH, decrease at higher pH, mean difference between minimum and maximum 15.75%(s.d. 5.38)
peak B_{Fint}	Increase, mean difference between minimum and maximum 41.82%(s.d. 7.43)
peak B_{Fint}/peak A_{Fint}	Increase, some samples exhibited a constant level below pH~7
A_{340nm}	Increase, mean difference between minimum and maximum 17.79% (s.d. 3.45)

Table 2.5
Summary of the changes in spectrophotometric properties observed on modification of solution pH (range pH 2 to 10).

An overall significant (95% confidence level) red shift in peak $B_{EM\lambda}$ with increasing pH was observed in all samples over varying pH ranges summarised in Table 2.5. The maximum wavelength shifts observed exceeded variable reproducibility (Table 2.2) and indicate a molecular response. This red shift is similar to those seen by Mobed *et al.* (1996) in a fluorescence intensity peak with similar excitation and emission wavelengths. As discussed in Section 1.5.3.2 was related to changes in phenolic functional groups. The contrasting response in peak $A_{EM\lambda}$ and peak $B_{EM\lambda}$ to pH change suggests a different composition between the fluorophores.

The specific functional groups responsible for the different responses are, however, unclear. As discussed in Section 1.5.3.1 fluorescence at shorter wavelengths (peak A) is attributed to the presence of simple structural components with electron donating substituents and long wavelength (peak B) to more conjugated structures with electron withdrawing groups (Senesi *et al*., 1991). The response known to occur due to changes in pH in electron withdrawing groups is the opposite of that observed for peak $B_{EM\lambda}$. Due to changes in the stabilisation of the excited state of such groups wavelengths of emission are red shifted on protonation (Schulman and Scharma, 1999). The opposite, a blue shift is observed for electron donating substituents. This indicates that firstly it is difficult to predict pH response in compounds of unknown structure (Sensei, 1990).

As discussed in Section 1.5.3.2 fluorescence intensity of DOM is known to increase with increasing pH and then to decline at higher pH levels. This pattern is seen, with a small amount of decline at high pH, in Figure 2.6 and summarised in Table 2.6, for peak A_{Fint}. The response of peak B_{Fint} to pH changes, however, exhibited an overall increase, as summarised in Table 2.5 and shown on Figure 2.6.

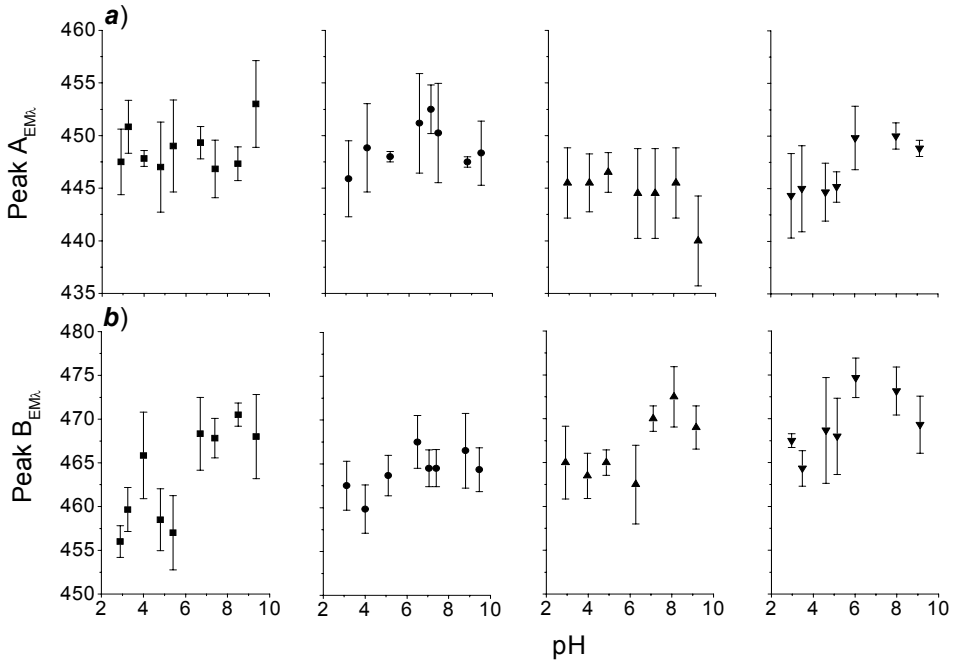


Figure 2.5
The relationships of emission wavelength to changes in solution pH. ***a)*** *peak* $A_{EM\lambda}$ ***b)*** *peak* $B_{EM\lambda}$ (■) *F18,* (●) *F11,* (▲) *F4;* (▼) *F13 For sample details see Appendix 1b.*

	F18	F11	F4	F13
Mean before (nm) (s.d.)	459.4 (3.86)	462.0 (1.97)	464.4 (1.23)	467.1 (1.92)
Mean after (nm) (s.d.)	468.7 (1.24)	465.5 (1.45)	470.8 (2.47)	472.4 (2.75)
Difference (nm)	9.3	3.5	6.4	5.3
pH range	5.4→6.7	5.11→6.5	6.27→7.11	5.15→6.04
pH of maximum peak A_{Fint}	~8	~8	~6	4

Table 2.6
Details of the spectral red shift observed in peak $B_{EM\lambda}$ *and pH of maximum peak* AF_{int} *on modification of the pH of the solution and the pH range at which it occurs. For sample details see Appendix 1b.*

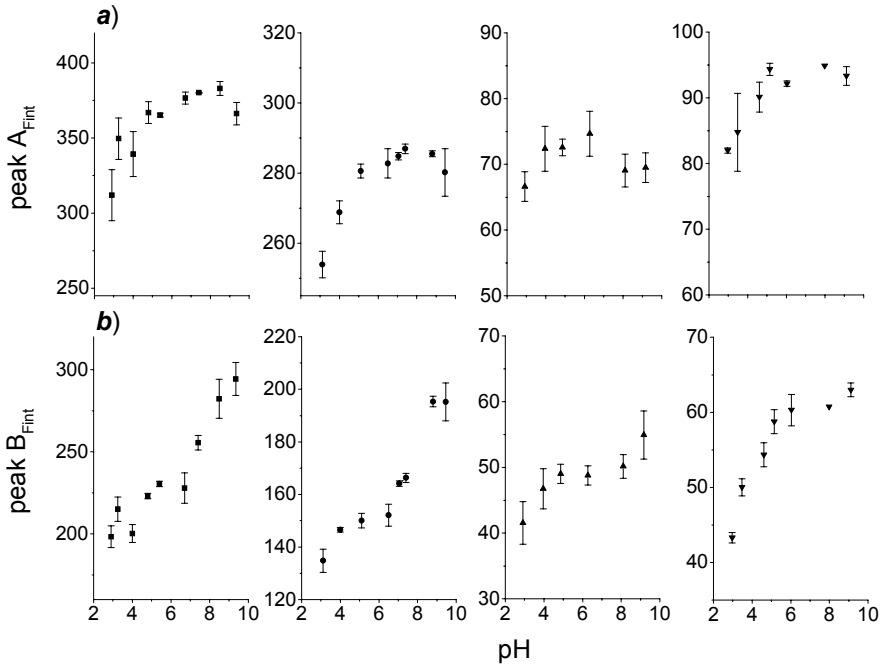


Figure 2.6
The relationships of ***a)*** *peak* A_{Fint} ***b)*** *peak* B_{Fint} *to changes in solution pH.* (■) *F18,* (●) *F11,* (▲) *F4;* (▼) *F13. For sample details see Appendix 1b.*

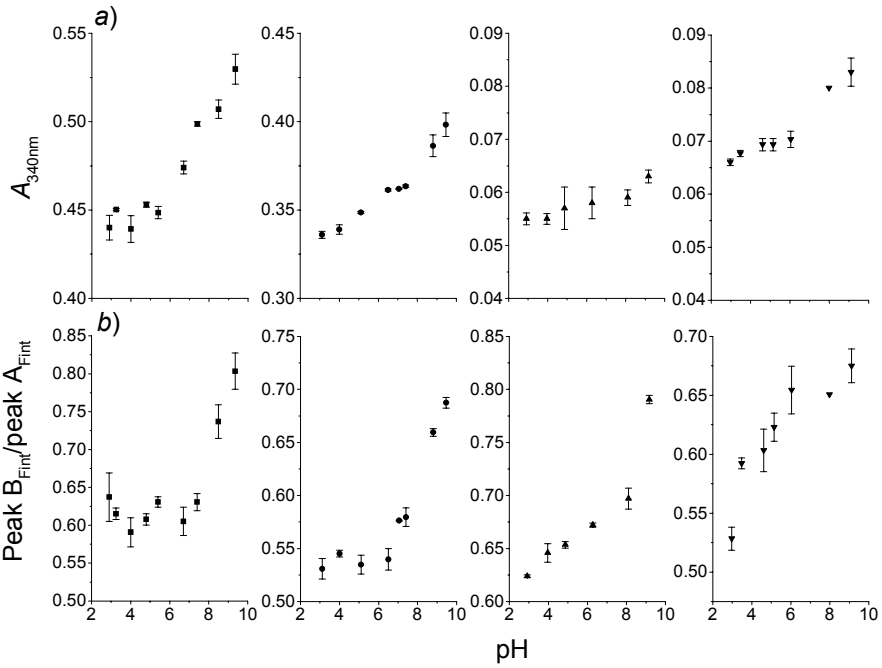


Figure 2.7
The relationships of ***a)*** A_{340nm} ***b)*** *peak* B_{Fint} */peak* A_{Fint} *to changes in solution pH.* (■) *F18,* (●) *F11,* (▲) *F4;* (▼) *F13. For sample details see Appendix 1b.*

Absorbance has been previously observed to increase with increasing pH (Anderson *et al*., 2000); Figure 2.7a shows such a relationship in the response of A_{340nm} to pH. The difference in response to pH between A_{340nm} and fluorescence intensity suggests differing composition of chromophores and fluorophores. The amount of change due to pH modification was similar for A_{340nm} and peak A_{Fint} and the trend observed in Figure 2.7a was similar to peak B_{Fint}. This may indicate that the absorbing components are chromophores that have compositional components in common with both of the fluorophores observed. The difference in response of fluorescence intensity at different wavelengths is demonstrated in Figure 2.7b. As with the different response to pH in peak $A_{EM\lambda}$ and peak $B_{EM\lambda}$ the different response in intensity reflects the differing composition of fluorophores responsible for each peak.

This study confirms observation made by Patel-Sorrentino *et al.* (2002) who observed a different response to pH at different wavelengths. Fluorescence at shorter wavelengths (peak E) was found to be more sensitive to pH than at longer wavelengths (peak A).

A_{340nm}, peak A_{Fint} and peak B_{Fint} show a greater percentage increase, with increasing pH, if the original sample had higher values of these parameters. As both fluorescence intensity and absorbance suggests that the response to pH is not only compositionally controlled, but also influenced by the DOC concentration of the original solution.

The influence of pH must be considered in the interpretation of spectrophotometric parameters of DOM, especially if samples with a wide range of pH are being examined. Modification of all samples to the same pH is not recommended. As illustrated by a limited number of samples, the DOM from 28 river waters exhibit responses to pH, for example the increase in peak B_{Fint} with increasing pH ranged from 32.1% to 74.8%. Thus, changing the solution pH may result in varying responses between DOM solutions. To avoid such alterations and maintain the natural signal of the DOM samples analysis at natural pH is required.

2.3.4 The implications of DOC concentration and solution pH to the spectrophotometric properties of DOM

1. Application of Equation 1.4, to remove the effects of IFE, is vital for comparable data, which reflect the natural signal of the DOM.

2. Spectrophotometric properties are sensitive to pH change and the change varies with concentration and the wavelength observed. It is recommended to analyse at field pH, as the modification to constant pH will result in spectrophotometric changes. These changes are consistent; however vary in extent between samples.

2.4 DOM storage and stability

After sampling DOM in solutions, such as river or marine waters, can degrade over time. Fluorescence characteristics may be altered during this period by evaporation, photodegradation, volatilization, microbial activity and container interactions (Yan *et al.*, 2000). Photodegradation processes can be minimised by storage of the sample in the dark. Low temperature storage and secure bottle seals can reduce evaporative loss. Sample container interactions may vary with different container composition, both glass and plastic have been used in fluorescence studies and rigorous cleaning of the bottles may reduce this effect.

Refrigeration is commonly used for short-term storage of DOM solutions and natural samples (Ferrari *et al*., 1996), however room temperature has also been used (Yan *et al*, 2000). For longer term storage and archiving freezing is used (Mayer *et al*., 1999). The stability of fluorescence characteristics has been noted by a number of authors. Coble (1996) found that fluorescence intensity of solutions of concentrated marine DOM analysed after three months frozen and two weeks refrigerated varied by 8%. No effects due to frozen storage were observed in EEM characteristics of peat DOM extracts (Caseldine *et al*., 2000). Yan *et al.* (2000) found that river water analysed after storage at room temperature for 43 days exhibited fluorescence characteristics within the experimental error when compared to analysis 24 hours after sampling.

A complete storage and preservation method for river water in fluorescence studies has not been designed. To determine the best conditions of river water sample storage for fluorescence analysis a number of tests were made on DOM solutions stored and preserved in differing manners. River water from different sources were analysed to assess stability during refrigeration, acidification and freeze defrost processes.

Acidification is widely used as a method of sample preservation of natural waters for the analysis of metals it is also recommended to preserve samples for total organic carbon analysis, and thus has been used in the preservation of samples prior to fluorescence analysis. As discussed in Section 2.3.3 modification of pH alters the spectrophotometric properties of DOM, however investigation in to the stability of such solutions at low pH is made, to provide a comparison to other literature in which this has been performed.

2.4.1. The assessment of conditions and containers for storage of DOM samples

To examine the behaviour of spectrophotometric properties during storage and determine what, if any, degradation takes place two river water samples were analysed. Two different samples were observed to monitor the comparative stability:

Sample 1, Coalburn Weir (09/12/1999) ; **Sample 2**, Peaty-gley Weir (13/01/2000)

The locations of the samples are discussed in Chapter 3. Both samples were filtered and analysed prior to and periodically during storage, as detailed in Section 2.2. Amber glass bottles, ashed at 400°C and plastic bottles, soaked in 10% HCl and rinsed with distilled water were used to examine sample-container interactions.

The samples were kept under the following conditions:

1. Sample 1 and 2 stored in both container types for 64 days, in the dark, at ~5°C. The samples were monitored until the solution had been exhausted.
2. Sample 1 stored in both container types in the dark at room temperature
3. Sample 1 and 2 stored in plastic containers, in the dark, at ~5°C after acidification to pH =2± 0.05 with dilute HCl

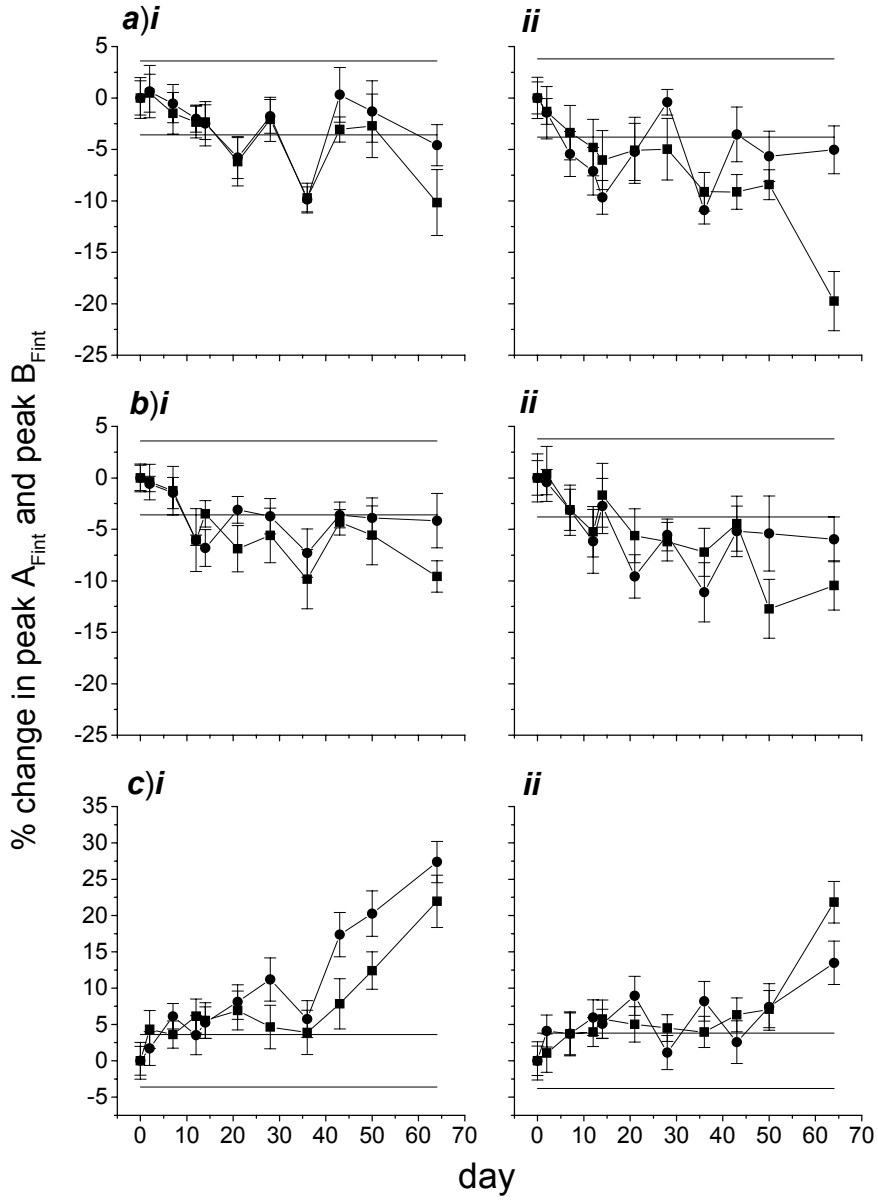


Figure 2.8
Changes in fluorescence intensities of river water DOM with time, stored in (■) *glass bottle* (●) *plastic bottle*
a) Sample 1 i *peak* A_{Fint} ***ii*** *peak* B_{Fint} *at ~5°C.*
b) Sample 2 i *peak* A_{Fint} ***ii*** *peak* B_{Fint} *at ~5°C.*
c) Sample 1 i *peak* A_{Fint} ***ii*** *peak* B_{Fint} *at room temperature.*
—— *analytical reproducibility*

2.4.2.1 Storage temperature

The results of the storage of two river water samples in glass and plastic bottles under refrigeration are shown in Figure 2.8. Peak A_{FInt} and peak B_{FInt}, for both samples in both container types, show similar fluctuations with an overall decrease of ~ 10%. There is an extreme decrease of 20% of peak B_{FInt} in sample 1, stored in glass, after 64 days. After 5 to 20 days the intensity loss is greater than the analytical errors, however, after longer periods the fluorescence intensity change is within errors for example sample 1, peak A_{FInt} after 40 days. This indicates the unstable nature of DOM fluorescence during storage.

There is a statistically significant relationship in the variation over time of peak A_{FInt} in both samples, when stored in plastic bottles (Spearman's rho=0.633; 95% confidence level). The fluctuations in peak A_{FInt} when stored in glass and peak B_{FInt}, stored in both types, show no statistically significant correlation (95% confidence level) between each sample. This indicates that, in this case, the two samples behave differently on prolonged storage and that as samples age it may not be possible to predict the change in fluorescence character from one sample to another. The mechanisms that would contribute to such loss in fluorescence intensity are unclear and may stem from degradation of the fluorophores. Additionally, within one sample properties at different wavelengths behave differently.

To illustrate this, the change in peak B_{FInt}/peak A_{FInt} over storage time is shown in Figure 2.9. At two points sample 2 has a lower peak B_{FInt}/peak A_{FInt} than sample 1, inverting the relationship of the original fresh samples. If this ratio were used as a measure of the characteristics of DOM (Newson *et al*., 2001) the changes during storage may result in data interpretations opposite to those given to the original samples.

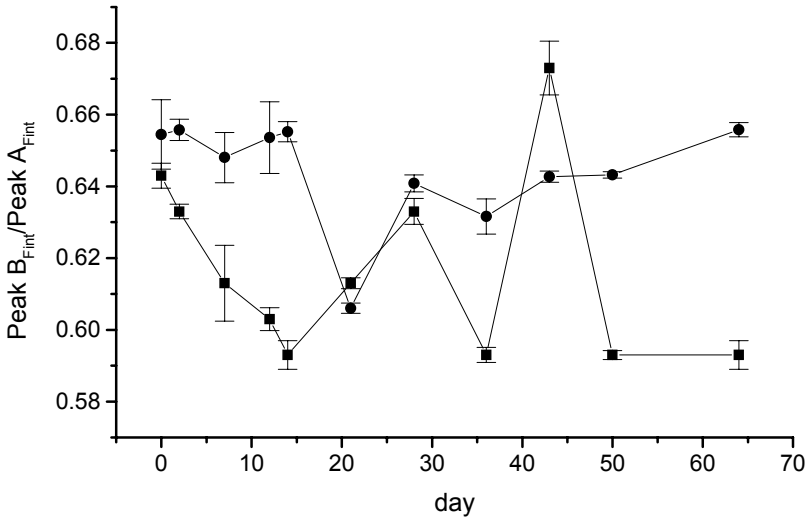


Figure 2.9
Changes in peak A_{Fint}/ peak B_{Fint} fluorescence intensities of river water DOM with time stored at ~5° C in plastic containers. (■) *sample 1* (●) *sample 2.*

The samples stored at room temperature (Figure 2.8) showed an increase in peak A_{Fint} and peak B_{Fint} that exceeded normal reproducibility after 2 days and 7 days for plastic and glass storage respectively. The maximum fluorescence intensity increase was 27%. This suggests an accelerated degradation in warmer conditions, possibly due to evaporation, or microbial activity. The former process explains the increase in fluorescence intensity, which would occur with progressive concentration of the solution.

The excitation and emission wavelengths of the peak A and peak B did not vary outside the range of the normal reproducibility of river water samples, during storage.

2.4.2.2 Sample containers

The fluorescence intensity relationships between each sample, stored in glass bottles and plastic bottles (refrigerated) are given in Table 2.7. For both sample 1 and 2 over the full 64 day experimental period peak A_{Fint} shows a statistically significant (95% confidence level) correlation in the pattern of change between samples, stored in glass and plastic. Peak B_{Fint} only exhibited such a relationship for sample 2.

This was calculated for different periods of storage and it was found that up to and during the first 14 days both samples exhibited significant correlations between sub samples stored in glass and plastic bottles (Table 2.7). This indicates that over such a storage period the fluorescence intensity of samples stored in glass and plastic behave in a similar manner.

		Peak A_{Fint}	Peak B_{Fint}
Sample 1	0-64 days	*rho*=0.87 99%	*rho*=0.20 ns
	0-14 days	*rho*=0.90 95%	*rho*=0.99 99%
Sample 2	0-64 days	*rho*=0.73 95%	*rho*=0.67 95%
	0-14 days	*rho*=0.90 95%	*rho*=0.98 99%

Table 2.7
The correlations of the change in fluorescence intensity between river water DOM stored in glass and plastic containers. (Spearman rho correlation coefficient and confidence level; ns= not significant)

2.4.2.3 Acidification of river water samples

Immediately upon acidification fluorescence intensity, for both fluorescence peaks in both river water samples, decreased by 20 to 22%. The fluorescence intensity remained at 18.64%±8.4 below the original intensity over 30 day storage. During this period wavelengths did not vary outside normal ranges. Acidification, in addition to altering the spectrophotometric properties of DOM, as discussed in Section 2.3.3 has been recognised to cause potential problems in DOC analysis, such as loss of analyte, by precipitation (Malcolm, 1993)

2.4.3 DOM sample storage and preservation by freezing

To assess the use of sample freezing as a storage method in this study 35 river water samples from a range of sources, detailed in Appendix 1b, were routinely analysed and immediately frozen, in plastic bottles for up to 1 year. The samples were entirely defrosted and re-analysed. The changes in spectrophotometric properties of DOM samples after freezing storage and complete defrosting are summarised in Table 2.8 and Figures 2.10 to 2.13. Upon freeze and defrost the amount and direction 9increase and decrease) of spectrophotometric properties varied significantly between and within samples.

Spectrophotometric properties	Changes observed after freeze and defrost
Excitation and emission wavelengths of peaks A, B and C	Mean changes were within analytical errors, individual samples exhibited up to ±20nm shift. The greatest proportion of wavelength change was a blue shift for all wavelengths, except peak $C_{EM\lambda}$. Both direction and magnitude of wavelength change varied.
Peak A_{Fint}, peak B_{Fint} and peak C_{Fint}	80% of the samples exhibited a change in fluorescence intensity greater than the analytical reproducibility, both as increases and decreases. Max change peak A_{Fint} -38.24%; peak B_{Fint} -40.58%; peak C_{Fint} +52.02&%
Peak B_{Fint}/peak A_{Fint}	Range from -7.89% change to +38.81% change
Peak C_{Fint}/peak A_{Fint}	Range from -13.01% change to +98.37% change
Absorbance	The majority of samples show a decrease in A_{340nm} and 77% of the samples exhibited a change outside the analytical reproducibility.
Peak AS_{Fint}	Range from –35.08% change to +30.66% change
SUV_{254nm}	Range from –34.44% change to +7.03% change

Table 2.8
Summary of the changes of spectrophotometric properties with freeze and defrost

A greater change in peak C_{Fint} was observed compared to peak A_{Fint} or peak B_{Fint}, as indicated in Figure 2.11c and Table 2.8. This possibly relates to the stability of the fluorophores that contribute to this fluorescence and indicates that the proteinaceous fraction of fluorescent DOM is less stable in response to freeze defrost in comparison to the humic-like fraction.

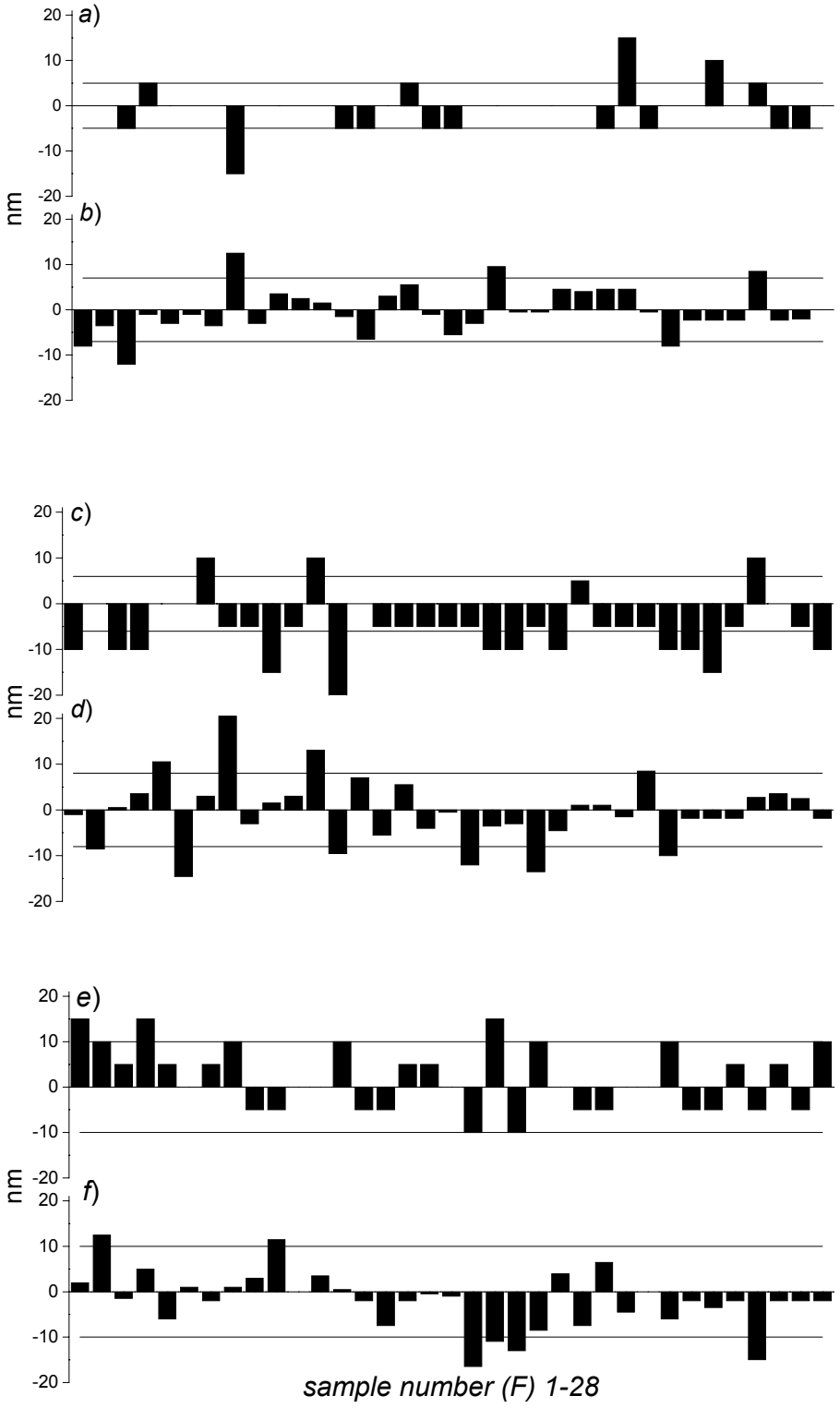


Figure 2.10
Spectral shifts after freeze defrost, change in ***a)*** *peak* $A_{EX\lambda}$ ***b)*** *peak* $A_{EM\lambda}$ ***c)*** *peak* $B_{EX\lambda}$ ***d)*** *peak* $B_{EM\lambda}$ ***e)*** *peak* $C_{EX\lambda}$ ***f)*** *peak* $C_{EM\lambda}$ *⁻-------analytical reproducibility. For sample details see Appendix 1b.*

It is important to recognise changes in fluorescence intensity ratios if such values are being used as a qualitative measure of DOM. In some cases there was little change from the original signal, however, as expected from the range of responses in fluorescence intensity shown in Figure 2.11, this was not consistently the case. An extreme example of this is sample F28 which exhibited an increase in peak C_{Fint}/peak A_{Fint} of ~100%, effectively doubling the apparent proportion of peak C (tryptophan-protein) content. This was due to both a decrease in peak A_{Fint} and an increase in peak $C_{Fint.}$ The changes in fluorescence intensities caused by freezing and thawing could potentially led to erroneous interpretation of the fluorescence signal. As observed for fluorescence wavelengths the changes in fluorescence intensities and fluorescence intensity ratios did not correlate with any of the original properties of the samples (95% confidence level).

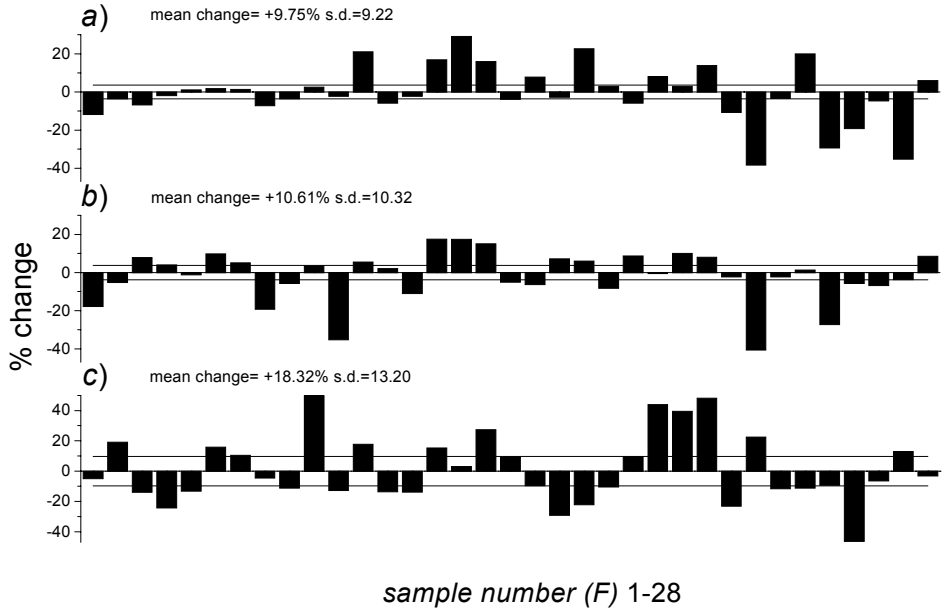


Figure 2.11
Changes in fluorescence intensities after freeze defrost ***a)*** *peak* A_{Fint} ***b)*** *peak* B_{Fint} ***c)*** *peak* C_{Fint}*. ------- analytical reproducibility For sample details see Appendix 1b.*

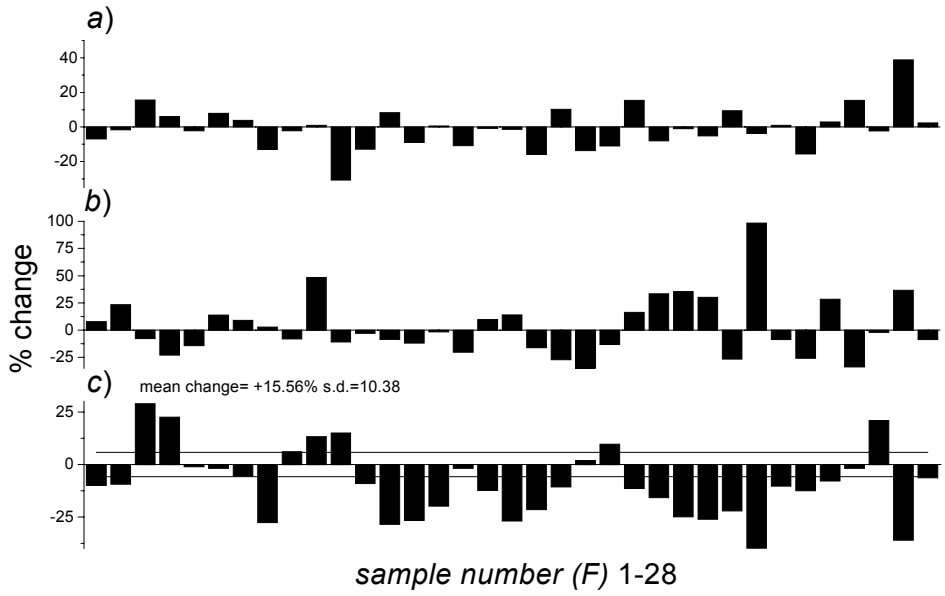


Figure 2.12
Changes in spectrophotometric properties after freeze defrost ***a)*** *peak* B_{Fint} */peak* A_{Fint} ***b)*** *peak* C_{Fint} */peak* A_{Fint} ***c)*** A_{340nm}*. --------analytical reproducibility. For sample details see Appendix 1b.*

Not all samples exhibited the same magnitude of change in absorbance at different wavelengths. For example Table 2.9 details the change in absorbance in sample F4. In this example A_{254nm}/A_{410nm} changed by +85.60% and A_{254nm}/A_{365nm} changed by -21.12%. This again presents problems when using such ratios in examining compositional differences in DOM. This pattern is not typical of those observed and is used as an illustration of the variations in response to freeze defrosts in this data set.

	A_{254nm}	A_{272nm}	A_{340nm}	A_{365nm}	A_{410nm}	A_{465nm}
Change due to freeze defrost	+2.54%	+5.43%	+22.55%	+30.00%	–44.75%	–77.78%

Table 2.9
Percentage changes in absorbance at different wavelengths after freeze defrost in sample F4. For sample details see Appendix 1b.

Sample F28 showed a ~40% loss in A_{340nm} this, coupled with a loss in peak A_{Fint} and peak B_{Fint}, suggests an overall loss of DOC concentration in the sample, as changes in both variables are closely related to concentration. To examine this a number of defrosted samples were analysed for DOC concentration. As shown in Figure 2.13

DOC decreases by 4.87% for sample F28. This reduction in concentration cannot explain the greater decrease in absorbance and fluorescence intensity. Similarly, sample F23 exhibited a 7.24% increase in DOC concentration, but a corresponding decrease in both A_{340nm} and peak A_{Fint}.

In all the samples looked at, neither a change in $A_{340nm,}$ peak A_{Fint} or peak B_{Fint} correlated with change in DOC concentration (95% confidence level). Before freezing peak A_{Fint} and peak B_{Fint} correlated significantly with DOC (Spearman's rho =0.654 rho=0.539 95% confidence level) and a similar relationship was seen for A_{340nm} (Spearman's rho = 0.921 99% confidence level). After defrosting these relationships did not exist. These examples suggest a compositional or physical change, such as disaggregation, rather than concentration related spectrophotometric response to freeze defrost processes, but that these processes also alter DOC concentration.

Additionally, as shown in Figure 2.13 b and c individual samples show different responses in peak AS_{Fint} and SUV_{254nm} values, indicating that after freeze defrost DOM has a lower absoptivity (per mg organic carbon L^{-1}) and more fluorescent (per mg organic carbon L^{-1}). As with the other examined properties this was not consistent, For example, sample F23, which showed an increase in DOC concentrations also shows a decrease peak A_{SFint} and SUV_{254nm}, indicating that the proportion of fluorescent and absorbant DOM in this sample has decreased.

This experiment has only examined a limited number of DOM samples and has revealed a variety of combinations of responses to freezing and defrosting. This includes varying amounts of both increase and decrease in fluorescence intensity and absorbance, at different wavelengths within the same sample.

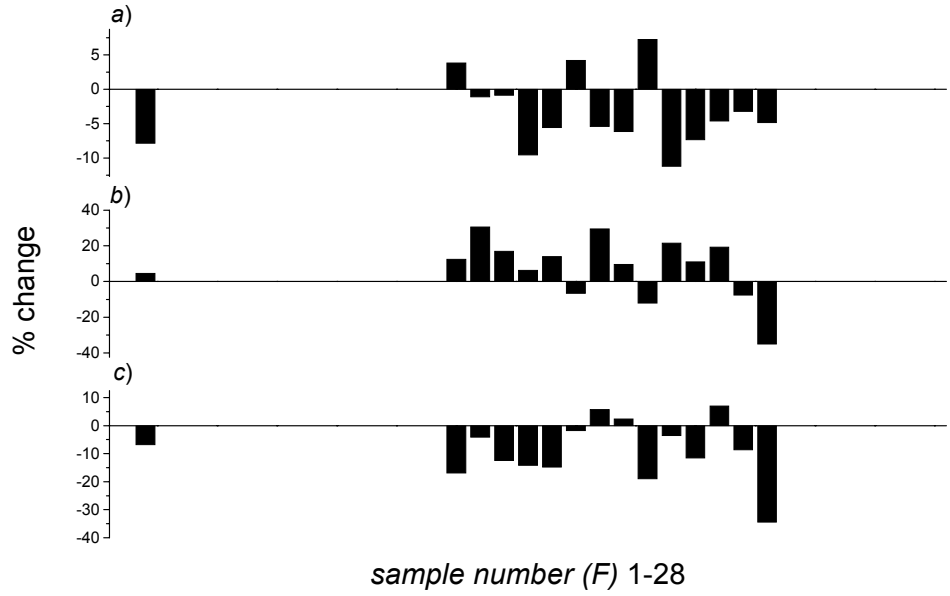


Figure 2.13
Changes in after ***a)*** *DOC (mgL^{-1})* ***b)*** *peak AS_{Fint}* ***c)*** *SUV_{254nm} freeze defrost, no bar represents missing data. For sample details see Appendix 1b.*

The amount of influence freeze and defrost has upon samples in real data sets can be made by the comparison of a number of samples examined in this experiment to data discussed in Chapter 3. A summary of these comparisons is made in Table 2.10.

Sample number	Variable	Change after freeze defrost	Range in the whole data set [1]	% of the total data range[2]
F19	peak B_{Fint}/peak A_{Fint}	0.102 (0.648 → 0.546)	0.692 →0.480	48%
F21	peak B_{Fint}/peak A_{Fint}	0.077 (0.571 → 0.494)	0.512 → 0.705	40%
F23	peak B_{Fint}/peak A_{Fint}	0.092 (0.599 → 0.691)	0.490 → 0.718	42%
F28	peak A_{Fint}	100.99 (280.29 → 179.30)	202.27 → 369.01	67%
	peak B_{Fint}	76.216 (187.81 → 111.60)	135.41 →217.95	96%

Table 2.10
Summary of the comparison of the change in spectrophotometric properties of selected samples after freeze defrost, to the range of data observed from the sample source. [1]range of the data from all analyses from this source of DOM [2]percentage of the range of the data from this source that the changes after freeze and defrost represent.

The examples in Table 2.10 indicate that the changes observed in spectrophotometric properties after freeze storage and defrosting were not only different in each case, but occurred to an extent that may seriously alter the distribution of data within a set of samples from the same source. Overall relationships were not lost by freeze thaw; for example a strong positive correlation of peak A_{Fint} and peak B_{Fint} with absorbance. In the data this process may not affect broad relationships, however, subtle variations maybe masked.

As there was no correlation of original sample properties with the amount of change in any of those properties or the signal of the sample after freeze defrost, it is concluded that knowledge of the original properties cannot be used to determine the amount of change that will occur if this method is used as a preservation technique. A small set of DOM samples have been monitored and a proportion of these show significant change in spectrophotometric properties due to this process. If defrosted samples are solely analysed, or examined in combination with fresh material the potential results of these changes must be taken in to consideration.

On defrosting insoluble black particulate matter was observed in a number of samples. This material was removed by filtration and spectrophotometric properties were not altered outside normal reproducibility by this filtration step. The decrease in absorbance, fluorescence intensity and DOC concentration of certain samples may be explained by this precipitate, due to the loss of original DOM that has been rendered insoluble by freeze defrost. Not all samples exhibited such losses in combination with precipitation. These precipitates have been previously observed by Malcolm (1993) who recommended that freezing of samples for preservation is undesirable due to loss of DOM, in most samples, by flocculation on thawing.

Other workers have observed little or no change in defrosted samples. A number of samples discussed above show small changes in certain properties that can be accounted for by analytical reproducibility. It must be noted that no sample exhibited a signal after freeze defrost that was the same as the natural signal. Similarly, no sample exhibited all changes within reproducibility for all parameters.

There are no published investigations into how freeze defrost may affect DOM or integral components of DOM. von Wandruska *et al*. (1998) used unspecified crude freeze-thaw cycles to separate out three fractions of soil HA solutions. These fractions did not result in size or functional group separations, but were found to

show distinct structural differences. It is these differences that may result in the response to freeze defrost discussed above.

2.4.4. Summary and recommendations regarding DOM storage and preservation

Storage and preservation of river water samples for spectrophotometric analysis have been examined. The following points summarise the recommended procedures to be used throughout this study.

- Spectrophotometric properties of river water, in particular fluorescence intensities, change over time during storage.
- The change during storage under refrigeration is similar between samples and fluorescence intensities at different wavelengths; however, with increasing time these trends diverge.
- Analysis is recommended as soon as possible after sampling to obtain a signal, within the analytical reproducibility of the technique. Data obtained after 5 days storage may not reflect the natural DOM signal, having degraded to values outside the reproducibility ranges.
- Storage should be under refrigerated conditions in suitably cleaned containers.
- Storage at room temperature is not recommended
- Plastic and glass containers can be used, and data is comparable between samples stored in either type, over the recommended 5 days storage period.
- Acidification is not recommended as a preservation method
- After defrosting and reanalysis of water samples alterations of fluorescence has been observed, this varies between samples in an inconsistent manner and cannot be predicted from original spectrophotometric characteristics.
- Analysis of defrosted samples must be undertaken with caution, as the spectrophotometric data obtained may not reflect the natural state of the DOM.
- Long term storage is problematic and may not be achieved without spectrophotometric modifications.
- To ensure that the spectrophotometric properties of undegraded and unaltered DOM in its natural state are obtained it is recommended that only fresh samples be analysed.

2.5 The extraction of DOM from peat for spectrophotometric analysis

Extraction of DOM from soil and peat commonly uses harsh chemical and physical methods, as reviewed in Section 1.6. The following section discusses a method of mild extraction of soil DOM that is designed to investigate variations in spectrophotometric signatures. The method is applied in Chapter 8 to examine variations in peat DOM profiles.

The method to extract peat DOM was required firstly, to attempt to establish links between the bulk fluorescence spectrophotometric properties of soil DOM with the properties of the catchment river water DOM at different periods within the annual DOM flux cycle. Secondly the method was developed to identify, both inter and intra catchment variations in soil DOM fluorescence spectrophotometric properties and characterize differences with depth in the soil column. To achieve these objectives the DOM derived from soil must retain its original spectrophotometric characteristics.

Catchment soils are recognised to be the major control on the amount and composition of riverine DOM, especially HS (Hayes and Clapp, 2001). It has been suggested, by Malcolm (1990) that in most streams HS are distinctly different in composition from their respective fractions in soils, as discussed in Section 1.1.1. Malcolm (1990) also noted that in peat areas in Great Britain stream waters retain an organic fingerprint of their peat soil origin. Similarly, Easthouse *et al*. (1992) observed that soil solution DOM gave a relatively good estimate of river DOM composition and content, in a small headwater catchment and this has also been recognised in swamp environments (Sihombing *et al*., 1996). Although differences between soil and riverine DOM properties are expected, it is assumed that there is a component of soil DOM in the aquatic environment and that links between the two can be observed, especially in peat dominated catchments (Maurice *et al*., 2002).

In the linking of DOM from soil sources to that in riverine settings a method to obtain soil DOM that represents the processes of flushing by rainwater would be ideal. A method that extracts all readily water soluble DOM and associated soil components with little solvent interactions, physical or chemical alterations is required to crudely mimic the hydrological flushing of the soils. As the purpose of the study, as a whole, is to examine the character of riverine DOM in its natural state, with minimal perturbation a method of mild extraction of bulk peat DOM was developed to ensure that the DOM analysed reflects as closely as possible the natural state.

To determine a satisfactory method of extraction of DOM from peat using a method based on Patterson *et al.* (1992) a simple aqueous dissolution was used. Various parameters - time of extraction, peat to water ratio and pH of the solvent were assessed and the best method determined. Additionally, the relationship of the spectrophotometric signature of such extracted DOM and the signature from related aquatic DOM were compared, to determine the applicability of the method to real situations. As the method is designed for application to field moist material it results in a composite solution of both soluble matter in the peat matrix and any interstitial water present.

A method such as this was preferred over direct sampling of soil water, to obtain a bulk signal from all DOM that can potentially be flushed from the soil. Previous water sampling from soil water in peat areas has yielded relatively low concentrations of DOC, for example 7.6 mgL^{-1} (Hinton *et al*., 1998) and 2.8-5.5 mgL^{-1} (Easthouse *et al*., 1992), from a wide depth range, in the soil column. Concentrations such as these may result in relatively low fluorescence intensities, especially if a higher depth resolution was sampled.

2.5.2 Method development

The following section describes the different parameters examined to develop the optimum method of simple dissolution to extract peat DOM. The requirements of the technique are, principally, that sufficient fluorescent and absorbant DOM is extracted, which can be detected using the methods detailed in Section 2.2 and that the signal obtained from the peat DOM has similar properties to DOM naturally derived from peat.

A test core (55cm) of peat was taken from the Coalburn Catchment, (28/09/00) (Chapter 3) and was divided into 5cm segments down the length, each segment was stored in foil in airtight containers at 5°C. Triplicate sub-samples, from each segment, of peat were dissolved in non-fluorescent distilled water and extracted under different conditions, in pre-cleaned plastic bottles (as used in Section 2.4), at room temperature. Two additional cores were taken on the same day to determine the reproducibility of data from triplicate extracts of each 5cm section. DOM was obtained by filtration of the solution through Whatman GF/C glass micro fibre filter

papers, pre-ashed at 400°C. Field moist peat was used to minimise any potential alteration from drying the material, and to ensure that all DOM in pore spaces was included in the extract. The solutions obtained were analysed as in Section 2.2. Table 2.11 summarises the conditions used.

Extract Condition	Purpose	Method
Peat to water ratio	Identify the minimum amount of peat that could be successfully extracted to obtain a sufficient yield for analysis	Triplicate extraction of 0.5; 1.0; 2.0; 3.0 and 4.0g wet weight peat in 50ml distilled water for 1hr
Time of extraction	Identify the amount of time required to aqueous extract a sufficient yield for analysis	Triplicate extraction of 1g wet weight peat in 50ml distilled water between 1, and 1800 minutes (30hr) at room temperature
pH of distilled water	Determine the optimum pH of the solvent to obtain a sufficient yield for analysis	Triplicate extraction of 1g wet weight peat in 50ml with pH of distilled water 2 to 10, for 2 hours

Table 2.11
The variations in the experimental conditions in the development of a peat DOM extraction technique.

The EEMs and absorbance spectra derived from the extraction of peat DOM using the simple aqueous dissolution method described resembled those observed in river water analysis. All of the features described on Figure 2.1 were present on the EEM, within the wavelength regions indicated. Fluorescence intensities and absorbance levels were lower than those seen in river waters from the same area. The similarities between the DOM extracts and natural DOM solutions indicate that, the solutions contain similar components, with comparable spectrophotometric properties. As this was found to be the case the method was fully investigated.

2.5.3.1 Reproducibility of data from peat DOM extracts

The errors observed in the analyses of triplicate sub samples from 5cm depth sections of the three test cores (Table 2.12) were found to be higher than those observed in triplicate analyses of river water samples (Table 2.2). The natural variability of peat at such a depth resolution accounts for this. The reproducibility

indicates that triplicate extractions can provide useful data on the spectrophotometric variability of the peat. The low level of reproducibility of A_{665nm} is representative of the overall low absorbance of the DOM, which in many analyses was below zero at >480nm.

a)

	Excitation wavelength (nm)	Emission wavelength (nm)	Fluorescence intensity
Peak A	±7.5nm	±9.0nm	±7.2%
Peak B	±8.0nm	±9.0nm	±7.8%
Peak C	±10.0nm	±15.0nm	±12.7%

b)

A_{254nm}	A_{272nm}	A_{340nm}	A_{365nm}	A_{410nm}	A_{465nm}	A_{665nm}
6.4%	5.8%	5.9%	7.7%	12.1%	12.4%	29%

Table 2.12
The reproducibility of spectrophotometric parameters of extracted DOM from triplicate extractions of peat samples from the same 5cm depth range within a core. (n=31) ***a)*** *fluorescence spectrophotometric properties* ***b)*** *UV-vis absorbance properties*

2.5.3.2 The influence of parameter variations on peat DOM extracts.

Table 2.13 and Figures 2.14 to 2.16 summarise the spectrophotometric properties observed under differing peat DOM extraction conditions.

As shown in Figure 2.14a the response to an increase in the proportion of peat in the extract mixture indicates that spectrophotometric are controlled by DOC concentration rather than compositional variations due to preferential extraction of different DOM fractions. It is indicated by these results that to obtain sufficiently high fluorescence intensities and absorbance for analytical detection a solution of >1g peat $50ml^{-1}$ is required. At approximately >1.5g peat $50ml^{-1}$ clogging of filter paper occurred and resulted in low extract yields and variable extraction times.

The spectrophotometric properties of time-varied extracts, shown Figure 2.14b, suggest that when this parameter is varied the response is derived from the amount of DOM extracted. The increase in fluorescence intensity and absorbance after 1440 minutes may possibly be due to desorption of inorganically associated DOM or microbial activity releasing DOM. These processes governing the release of DOM after an extended period of dissolution do not represent the hydrological flushing of

peat as desired by this experiment. 120 minutes is recommended as an extraction period, in combination with 1g of wet weight sample in 50ml of water. This will result in a sufficient signal in both fluorescence intensity and absorbance and allows analysis to be performed within one day, thus maintaining constant conditions.

Extraction parameter	Spectrophotometric property	Response
Peat water ratio	Peak A_{Fint}, peak B_{Fint} and absorbance	Linear increase with increasing peat volume
	Peak wavelengths, peak B_{Fint}/ peak A_{Fint}, and peak C variables	None
Time of extraction	Peak A_{Fint}, peak B_{Fint} and absorbance	Rapid increase over 1-120 minutes, peak at 1800 minutes
pH of solvent	Peak wavelength, peak B_{Fint}/ peak A_{Fint} and peak C variables	None
	Peak A_{Fint}, peak B_{Fint} and absorbance	Linear increase with increasing pH
	Peak wavelength, peak B_{Fint}/ peak A_{Fint} and peak C variables	None

Table 2.13
Summary of the response of spectrophotometric properties of extracted DOM on varying peat extraction parameters

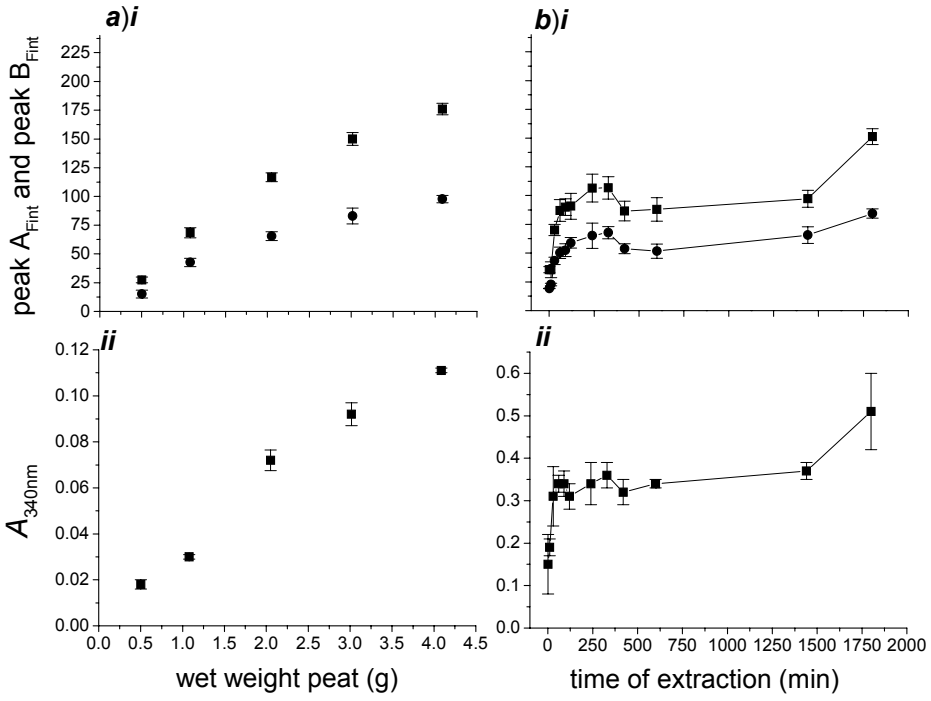


Figure 2.14
*The response of spectrophotometric properties to **a)** peat: water volume and **b)** time of extraction **i** (■) peak A_{Fint} and (●) peak B_{Fint} **ii** A_{340nm}*

As discussed in Section 2.3 solution pH is an important consideration in the examination of the spectrophotometric properties of DOM. The pH of the extracts was constant with all of the varying parameters at 4 ± 0.5. This indicates that the response seen in Figure 2.15 was related to the pH of the solvent in the extraction rather than reflecting a change in solution pH. An increase in the release of DOM from soil at high pH has been observed in other work (Shen, 1999).

A pH in the region of natural rainwater, for example in the Coalburn Catchment where the mean has been recorded at pH=5.4 (range = 4.4 to 7.4) (Robinson *et al*., 1998) results in sufficient signal in both fluorescence intensity and absorbance, thus a natural pH level may satisfactorily be used. Natural rainwater would provide a better solvent than distilled water in mimicking natural flushing processes. Collection of rainwater uncontaminated by fluorescent material in sufficient quantities was not possible. A pH of 6±0.5 was selected for this extraction. This will result in a range of intensity and absorbance within analytical errors and avoids extreme pH changes, which may result in alteration of the natural state of the DOM such as dissociation of DOM.

2.5.4 Proposed peat dissolved organic matter extraction technique

From the above investigations a method to extract DOM from peat has been devised. The method produced is summarised as follows: -

- 1g field moist peat
- Dilution to 50 ml distilled water (pH=6±0.5)
- 2 hours (at room temperature; shake twice)
- Filter supernatant (GFC pre-ashed)

Analysis

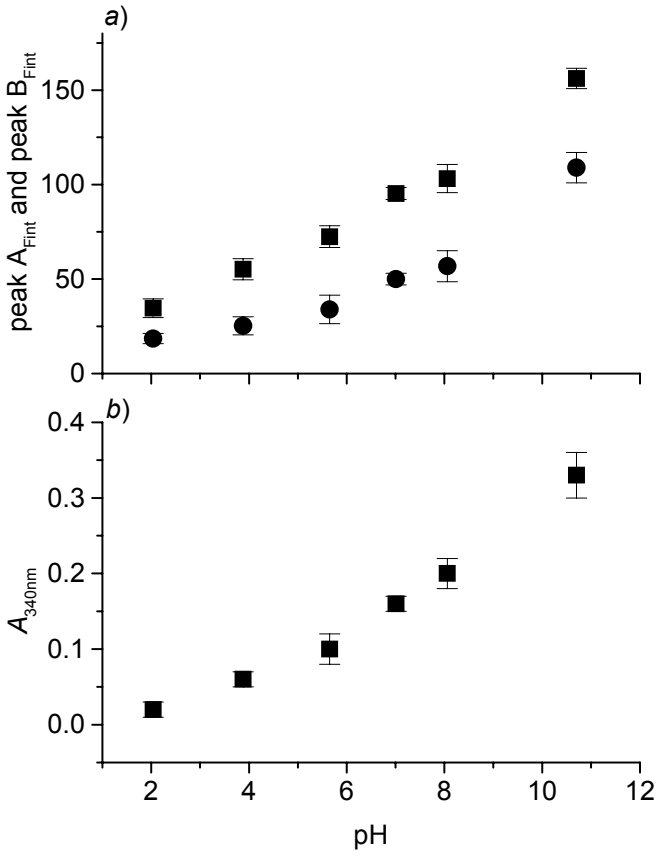


Figure 2.15
*The response of **a)** fluorescence intensity* (■) *peak A_{Fint} and* (●) *peak B_{Fint} and **b)** A_{340nm} to changes in pH of the solvent during peat DOM extraction.*

2.5.5 Comparison of the spectrophotometric properties of peat derived DOM to aquatic DOM

As mentioned in Section 1.6 the products of soil DOM extraction are sometimes considered as artefacts of the extraction and fractionation procedure, having little or no relevance to natural soil condition or soil processes. To establish if the spectrophotometric properties of experimentally extracted DOM, using the above method, were related to natural DOM and to that displaced from soils to river waters, a set of natural analogues were examined. Paired samples of peat and water were taken from standing pools of water (<3m^2) (pp1 to pp8) directly on exposed peat at eight sites in the Loch Assynt area (Chapter 5). These pools are fed only from precipitation and soil water. Triplicate samples of peat were extracted using the

method outlined in Section 2.5.4. The resulting DOM extracts and water samples were analysed using the methods in Section 2.1.

Similar water bodies, in a UK upland peat system, have been used previously as a sampling source of DOM directly derived from the underlying peat, thus, using the pool water as natural soil water source (Scott *et al*., 1998).

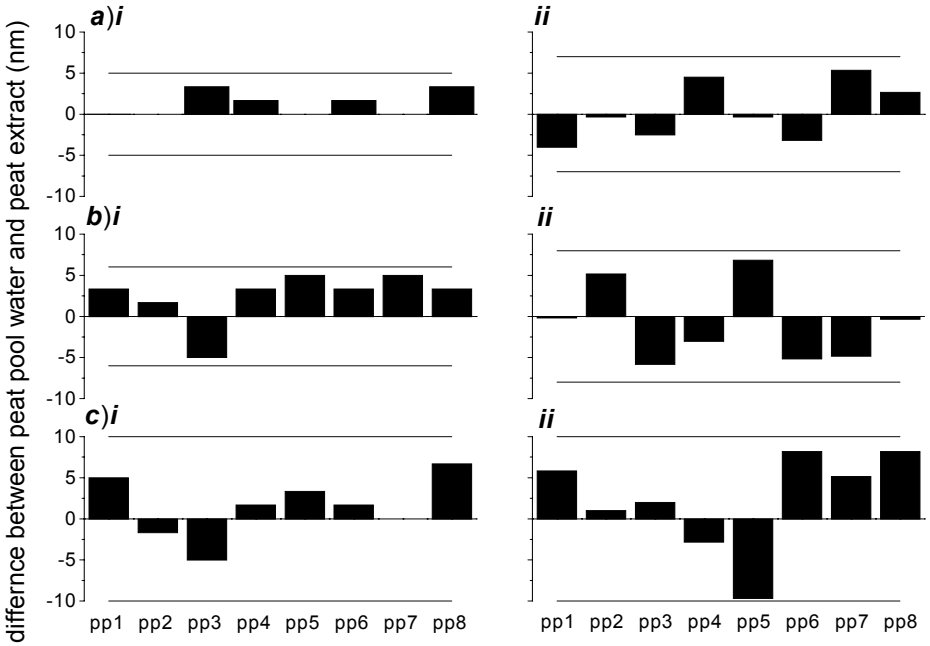
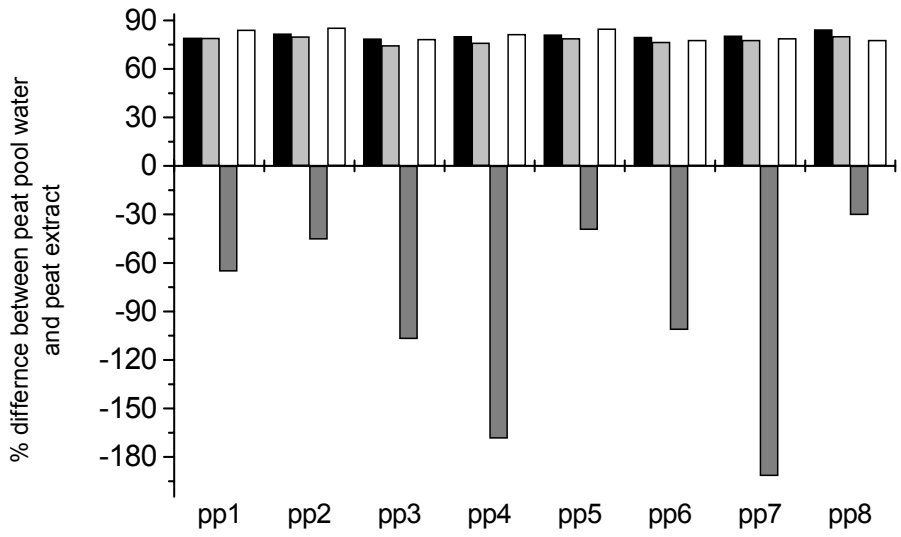


Figure 2.16
Differences in fluorescence wavelengths between extracted DOM and peat pool DOM. Positive values represent longer wavelengths in the peat pool water. ***a)i*** *peak* $A_{EX\lambda}$ ***ii*** *peak* $A_{EM\lambda}$ ***b)i*** *peak* $B_{EX\lambda}$ ***ii*** *peak* $B_{EM\lambda}$ ***c)i*** *peak* $C_{EX\lambda}$ ***ii*** *peak* $C_{EM\lambda}$
--------- analytical reproducibility (river water). For sample details see Chapter 5.

Figure 2.16 compares excitation and emission wavelengths of peak A, B and C in DOM from extracts and peat pool waters. Although most of the comparisons indicate different wavelengths for each peak, none of these differences are outside the reproducibility errors for river water analyses (Table 2.2). The differences in wavelengths did not correlate to any of the properties of either the DOM extracts or the peat pool waters.

a)



b)	**Peak A_{Fint}**	**Peak B_{Fint}**	**Peak C_{Fint}**	***A_{340nm}***
Mean difference between pool water and DOM extract (s.d.)	80.72% (1.76)	77.90% (1.98)	-93.54% (60.42)	81.13% (3.32)

Figure 2.17
Differences in fluorescence intensity and absorbance between extracted DOM and peat pool DOM, positive values indicate a greater proportion in the peat pool water. ***a)*** **black** *peak A_{Fint}* **light grey** *peak B_{Fint}* **dark grey** *peak C_{Fint}* **white** *A_{340nm}.* ***b)*** *mean differences in each parameter. For sample details see Chapter 5.*

In the comparison of DOM extracts to peat pool water peak A_{Fint}, peak B_{Fint} and absorbance (A_{340nm}), as shown in Figure 2.17 were approximately 80% higher in the peat pool water samples. This offset was relatively constant in the eight cases examined and suggests that the experimental extraction produces DOM of consistent properties, in relation to the naturally derived DOM.

The offset indicates that experimental extraction results in a lower concentration of DOC, compared to natural processes. This would arise due to the differences between natural and experimental DOM extraction, such as time scale, volumes of water to peat, microbial activity and drying of the peat (Mitchell and McDonald, 1992).

There were a number of observed differences in the spectrophotometric properties between extracted DOM and peat pool water that may be related compositional differences. Peak B_{Fint}/peak A_{Fint} was higher in the peat DOM extracts (12.46% s.d. 1.85). This may be related to processes undergone by the DOM whilst held in the peat pool, for example photo-degradation, which has been recognised to cause the preferential decay of fluorescence at different wavelengths (Coble *et al.,* 1998) or rapid biodegradation before entering the aquatic environment (Blaser *et al*., 1999).

Previous studies of variously extracted soil DOM commonly show greater fluorescence intensity at longer wavelengths in comparison to aquatic DOM (Figure 1.4) (Senesi *et al.,* 1991; Mobed *et al.,* 1996). This red shift may be a function of the extraction method. As it was observed in the mildly extracted DOM in this study (increase in peak B_{Fint}/peak A_{Fint}) it suggests that soil derived DOM is preferentially composed of fluorophores that contribute to longer wavelength fluorescence, when compared to aquatic DOM. This reflects the compositional differences noted by Malcolm (1990) who observed a greater aromaticity in soil HS when compared to stream HS. A shift to longer fluorescence wavelengths is associated with an increasing content of aromatic nuclei in DOM (Senesi *et al*., 1989; Miano and Senesi, 1992), which suggests that peat DOM is more aromatic than peat pool water DOM.

Kalbitz *et al*. (2000) observed a similar relationship in peat topsoil and surface water using synchronous fluorescence spectra. Here a humification ratio of long to short wavelength fluorescence was higher in the extracted peat DOM compared to the surface water at the same sites. Similarly, aromatic content, determined using UV absorbance and FTIR spectra was found to be higher in the peat DOM. Examination of directly related soil and aquatic DOM by EEM fluorescence spectrophotometry has not been previously performed and further investigation is required for a more comprehensive interpretation. When comparing peat DOM spectrophotometric properties extracted using this method to riverine DOM it is important to consider the compositional differences observed in the peat pool example.

A component that is inconsistent between extracted and pool water DOM is peak C_{Fint}. As shown on Figure 2.17 this is significantly enriched in the extracts, by 30% to ~190% (99% confidence level). As in the case of the discrepancy seen in peak B_{Fint}/peak A_{Fint} this offset maybe due either to a differing composition of the peat DOM and peat pool water DOM, modification of the DOM in the water body or the extraction process.

Malcolm (1990) observed an approximately ten times higher amino acid content in soil HS compared to stream HS and Thomas (1997) reviewed amino acid compositions in aquatic settings and noted higher concentrations in soil pore waters. The greater proteinaceous fluorescence in the extracts may simply reflect a higher concentration in soil derived DOM. This may also reflect the quantum yield of the tryptophan present, which if located within the proteins is less fluorescent, compared to a location on the outside of such molecules (Mayer *et al.,* 1999). The process of extraction may disrupt the protein molecules and render more tryptophan able to contribute to the fluorescence of the proteins. Zsolnay *et al.* (1999) observed an increase in protein-like fluorescence due to drying of soil material, which was related to cell lysis. Similar physical disruption of fresh cellular material by sampling and extraction processes may result in a higher proportion of fluorescent tryptophan, thus a higher peak C_{FInt} in comparison to naturally derived DOM.

2.5.6 Summary of the proposed peat DOM extraction technique

In the comparison of extracted peat DOM to related aquatic DOM there is little shift in fluorescence wavelengths, and none outside errors. Fluorescence intensity at peak A_{FInt}, peak B_{FInt} and absorbance are depleted in the extracted DOM in comparison to aquatic DOM. This depletion is constant and as the technique is not intended to be a quantitative investigation of soil DOC concentration it indicates that the method is suitable for comparisons of spectrophotometric properties. The compositional differences between peat DOM extracts and the related aquatic DOM can be seen in peak B_{FInt}/peak A_{FInt}, which is consistently higher in the extracted material, this may be a feature of the extraction or reflect real greater aromaticity in peat DOM. Similarly, peak C_{FInt} is enriched in the extracts and the scale of this difference suggests that it is unlikely that the signal in the peat DOM is related to that observed in the riverine systems.

The peat pool systems investigated may not wholly represent the spectrophotometric properties of DOM flushed from soils to rivers, as processes occurring over greater transportation times and distances will influence the final signature. These processes include retention by adsorption to inorganic material and microbial processing during dry periods (Scott *et al*., 1998; Tipping *et al*., 1999). The evidence that all DOM extracts behave in the same manner in respect to directly related aquatic DOM suggests that the method can be used to compare soil and river DOM if the above

considerations are made. Additionally, disparities between peat DOM and potentially related riverine DOM may provide information regarding the influences on DOM by such transportation processes.

There are a number of potential problems in extracting peat DOM by the proposed method, in addition to the bias in properties when related to aquatic DOM. The method only dissolves the DOM that could potentially be released from the soil and be present in river water however it does not take into account residence times, flow paths or other processes that may influence DOM during the transport from sampling point to the river. Only broad relationships between soil DOM and river DOM may be drawn. Additionally, as the peat cores were arbitrarily divided into 5cm depth segments the resolution of spectrophotometric variations will only reflect this resolution and will not indicate any smaller scale changes.

The method has only been applied to high organic content peat. Other soil types, with a lower DOC yield, and greater inorganic content may not be suitable for this technique, as fluorescence signal of the extracts may be low.

This section has outlined the method to obtain DOM from peat material that will be applied in the wider study. The conditions under which this is to be performed have been assessed and recommendations made. The relation of the extracted DOM to related aquatic DOM has been evaluated and although a number of differences between the spectrophotometric signals from the two sources have been identified with consideration of these factors and other limitations this technique is consistent and reproducible and will provide information on DOM that is present in aquatic systems.

2.6 Summary and conclusions

This chapter has outlined the analytical methods used throughout this study and recommendations for the treatment of samples and data have been made. These recommendations are as follows:

- Post analysis corrections are applied to fluorescence intensities to remove the effects of IFE at high concentrations and absorbance. Experimental dilution shows that peak A and peak B contribute to sample absorbance to a greater extent than peak C.

- All samples are analysed at natural pH. The experimental modification pf solution pH shows the presence of different fluorophores and that chromophores have compositional components that behave in the same manner as fluorophores.
- Samples are to be analysed in a fresh state, with minimum storage time, and freezing is to be avoided. Both of these recommendations stem from the changes observed in spectrophotometric properties over time.

A method of obtaining easily soluble DOM from peat has been described. It was found that DOM obtained using this method reflects the variations in spectrophotometric properties seen in related aquatic DOM.

Chapter 3.

Spatial Variations in the Spectrophotometric Properties of Dissolved Organic Matter in the Coalburn Experimental Catchment

3.1 Introduction

To investigate the variations in spectrophotometric properties of DOM two upland areas in the UK were monitored. The following chapters present the results of these studies, describing spatial and temporal variations in aquatic DOM and water extractable peat DOM and characterisation using spectrophotometric techniques. The application of EEM fluorescence spectrophotometry to such studies is assessed.

The following chapter will discuss the comprehensive examination of the spectrophotometric properties of DOM from the Coalburn Experimental Catchment (Northumberland, England). Details of the field area are presented in Section 1.7.1.

3.2 General aims of the study of the Coalburn Experimental Catchment

The broad aims of the study of DOM are summarised below. More specific aims are detailed in Section 3.4.1 and 4.1.1.

- To examine the spatial variations in the spectrophotometric properties of aquatic DOM, and to relate these variations to the influences of vegetation, soil type and hydrology.
- To monitor temporal variations in the spectrophotometric properties of aquatic DOM in the Coalburn, to examine seasonal variations in relation to temperature and rainfall.
- To apply the spatial variations in aquatic DOM properties to time series data and assess the use of this method to determine sources and flow paths of DOM.
- To characterise DOM using EEM fluorescence spectrophotometry, both spatially and temporally and to assess the analytical method in such applications.

3.3 Sampling and monitoring programme in the Coalburn Experimental Catchment

3.3.1 Sampling point identification and locations

The abbreviations used in this study to denote samples from each of the points are detailed in Table 3.1. The locations of the sampling points within the catchment are shown on Figure 3.1.

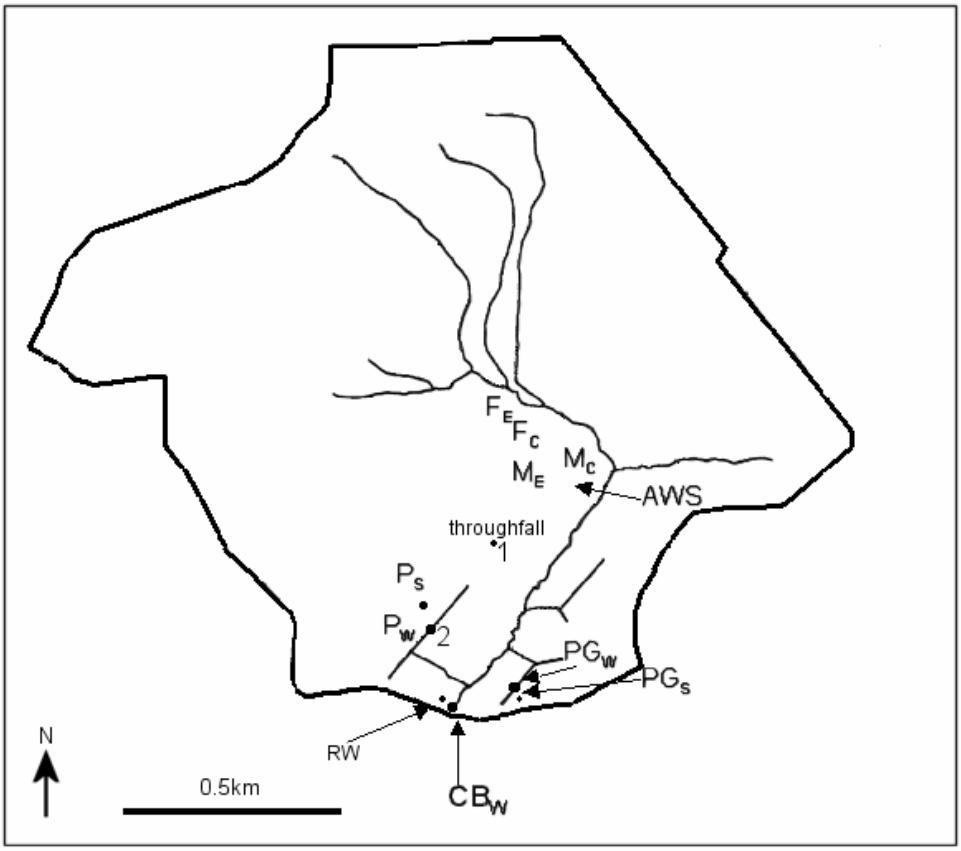


Figure 3.1
Sampling locations in the Coalburn Experimental Catchment. 1, 2 peat core sampling sites CB_w =main channel P_w = peat sub-catchment surface sample P_s = peat sub-catchment soil water sample PG_w = peaty-gley sub-catchment surface sample PG_s= peaty-gley sub-catchment soil water sample.

Location/Description	Abbreviation	No. samples
Main channel	CB_{weir}	62/320

(manual/autosampler)		
Peat sub-catchment weir	P_{weir}	31
Peaty-gley sub-catchment weir	PG_{weir}	28
Rainwater	RW	19
Throughfall and stemflow	Throughfall	9
Moorland-experimental*	M_E	19
Moorland-control*	M_C	7
Forest-experimental*	F_E	19
Forest-control*	F_C	19
Peat sub-catchment dipwell water	P_{soil}	9
Peaty-gley sub-catchment dipwell water	PG_{soil}	10

Table 3.1
*Details of the abbreviations of samples sites used in the text. *paired micro-catchment ditches*

3.3.2 Automatic measurements

An automatic weather station was used to measured rainfall, which was recorded with a 0.2mm tipping bucket rain gauge, and mean daily temperature. Data were supplied by the Environment Agency. Stream flow from the catchment main channel was recorded on a fifteen minute basis using a compound, broad-crested weir (with low flow V notch section). The Environment Agency is responsible for the validation and archiving of this data and a full description of the validation and conversions used are given in Mounsey (1999).

3.3.3 Sampling of water

Water sampling was performed from January 2000 to January 2002. All samples were filtered with Whatman GF/C glass microfibre filter papers, pre-ashed at 400°C and analysed using the method detailed in Section 2.2. Analysis techniques replicate those used by Newson *et al*. (2001). During March to August 2001 the site was not accessible due to the Foot and Mouth Disease outbreak and subsequent closure of access routes, thus, no data was available for this period.

3.3.3.1 Coalburn main channel sampling

Water samples were regularly taken (approximately weekly) from the main channel at a point upstream of the weir (CB_{weir} Figure 3.1). High resolution sampling of the main channel was performed between 02/01/01 to 20/02/01 and 01/08/01 to 21/10/01 at 8 hour intervals using a Rock and Taylor auto-sampler. Bottles were cleaned by soaking in 10% HCl and thorough rinsing with non-fluorescent distilled water. Due to the nature of the equipment each bottle had to be reused. Initial checks revealed that if thoroughly cleaned there was no potential for cross contamination from previous contents. Sample stability was also addressed as samples were collected at approximately 14 day intervals. Duplicate samples taken at the beginning of each auto-sampler run, one of which was analysed immediately and the other left for 14 days in the auto-sampler duplicated well, not exceeding analytical errors detailed in Section 2.2.5. This time period is longer than that recommended in Section 2.4 and as seen in stability monitoring may have undergone degradation; thus, larger errors in spectrophotometric properties are potentially incurred with samples taken in this manner.

3.3.3.2 Sub-catchment sampling

Water samples were taken at v-notch weirs from ditches draining each sub-catchment, located on Figure 3.1. Both of these ditches are located at the edge of the forested area and intercept flow from ditches draining from closed canopy forest. The sampling points replicate sites sampled by Mounsey (1999) and by Newson *et al.* (2001). Soil water samples were taken from a dipwell on each sub-catchment. These were part of two transects of dipwells monitored for soil water depths, approximately bi-monthly as part of the long term study of the site. Both transects were situated under closed canopy.

A further four ditches were sampled representing micro-catchments, all located in the peat sub-catchment. The locations of these ditches are given on Figure 3.1. One pair of micro-catchments drain established forest (F_C & F_E) and the other pair drain moorland (M_C & M_E). Two of the ditches were deepened to their original depth of 0.9m, as experimental systems, one forest (F_E) and one moorland (M_E) micro-catchment; the others were left as controls. This was originally performed to investigate the effect of remedial drainage treatment on the generation of extreme flows by comparison of excavated and partially infilled ditches. Sampling was performed adjacent to v-notch weirs installed on all the ditches. Table 3.2 describes the state of the ditches during the study period.

	Design	Filling	Canopy
Forest experimental (F_E)	Trapezoidal	Bare peat and spruce needles	None
Forest control (F_C)	Parallel	Sphagnum and sedge	50% cover
Moorland experimental (M_E)	Trapezoidal	Bare peat	None
Moorland control (M_C)	Parallel	Sphagnum filled	70% cover

Table 3.2
Description of the condition of the micro-catchments drainage ditches during sampling program.

3.3.3.3 Rainwater; throughfall and stemflow sampling

Bulk deposition was sampled as a composite of rainwater, cloud mist, snow and dry deposition. Due to the sampler used the bulk of this was rainwater and these samples are discussed as such. Collection of rainwater was from a ground level collector, located in an unplanted area adjacent to the main channel weir, and was made bi-monthly if sufficient was present. Samples were a composite of precipitation since the previous sampling date. The collector consisted of a plastic funnel and bottle designed to limit avian contamination (Mounsey, 1999).

Analysis of duplicate samples, collected in a pre-cleaned (10% HCl soak and rinse with distilled water) glass collector, indicated that there were no significant differences in the mean spectrophotometric properties (95% confidence level) between this and the plastic sampler. Interferences from the collector were negligible.

Due to the nature of the collector potential interferences to the natural signal of precipitation, during sample collection by, for example, evaporation, microbial activity and particulate matter falling into the sampler were assessed. Investigation of rainwater sampled over short periods, with minimal opportunity for such interferences showed similar spectrophotometric characteristics in comparison to routine samples taken as discussed above. This suggests that the rainwater properties observed were not dominated by such contaminations or interferences.

Both throughfall and stemflow were sampled in combination from the runoff from interception sheets (Figure 3.1) from beneath closed canopy Sitka Spruce. For ease this composite is termed throughfall.

3.4 Spatial variations in DOM in the Coalburn Experimental Catchment

The following section presents and discusses the results of the analyses of the water sampled, from all of the sites shown on Figure 3.1, and compares each source to identify the spatial variations in DOM in the Coalburn Experimental Catchment during the study period. The focus of this chapter is to summarise this information and to identify significant spectrophotometric characteristics using both EEM fluorescence and absorbance spectrophotometric properties.

As discussed in Section 1.7 the physical structure of the catchment and hydrological flow pathways have been observed to control stream water chemistry, each sub-catchment having a distinct geochemical signal. Runoff from both sub-catchments has been identified to significantly influence the water quality at the catchment outfall. Surface ditch water and soil dipwell water from each area was assessed to investigate spectrophotometric character of water from different sources for the investigation of the flow paths of DOM within the catchment discussed in Section 1.7. The spectrophotometric properties of precipitation, throughfall and spruce needles are also discussed to identify the spectrophotometric properties of DOM inputs to the catchment.

3.4.1 Aims of the study of spatial variations in DOM in the Coalburn Experimental Catchment

The following aims are related to the spatial variations investigated in the Coalburn Experimental Catchment

- To identify the comparative spectrophotometric character of DOM throughout the catchment from each component of the flow paths described in Figure 1.7.
- To investigate the DOM properties from contrasting ditches within the peat sub-catchment, comparing the influence of micro-catchment vegetation and ditch infill condition
- To characterise the spectrophotometric properties of precipitation

- To characterise the spectrophotometric properties of throughfall and investigate the input of DOM to the catchment from vegetation and litter interactions.
- To establish a basis from which the temporal dynamics of spectrophotometric properties can be assessed.

3.5 Spatial variations in surface water of the Coalburn Experimental Catchment

3.5.1 Spatial variability in pH and conductivity

Both pH and conductivity data, presented in Figure 3.2, observed in this study were comparable to that seen in previous work (Table 1.5), having similar ranges and means, and replicating the broad spatial differentiation of the catchment (Robinson *et al*, 1998). As shown in Figure 3.2 there was a significantly higher mean pH in PG_{weir} (5.84 s.d. 0.55) compared to CB_{weir} (4.76 s.d. 0.73) and all peat sub-catchment derived waters (99% confidence level). As expected from previous observations PG_{weir} had the highest surface water pH (7.30) in the catchment, due to buffering by the inorganic component in the soil (Robinson *et al*., 1998). CB_{weir} exhibited a significantly higher mean pH in comparison to all peat sub-catchment derived waters (4.15 s.d. 0.78) and this suggests that inputs from both sub-catchments can be recognised in the water chemistry at the catchment outfall, during this study. The four monitored ditches in the peat sub-catchment had statistically indistinguishable mean pH values (95% confidence levels).

Mounsey (1999) recognised that water of high pH buffers the water of CB_{weir}, especially at low flow. The observations of pH in the main channel were made during a range of flow conditions (0.00 to 1.28 m^3s^{-1}, mean=0.039 m^3s^{-1}). pH exhibited a significantly negative relationship with discharge (99% confidence level) with the lowest observed pH values occurring during higher flow conditions. Runoff from surface ditches and soil water of the relatively smaller area of peaty-gley sub-catchment therefore has an important influence on the chemistry of the main channel.

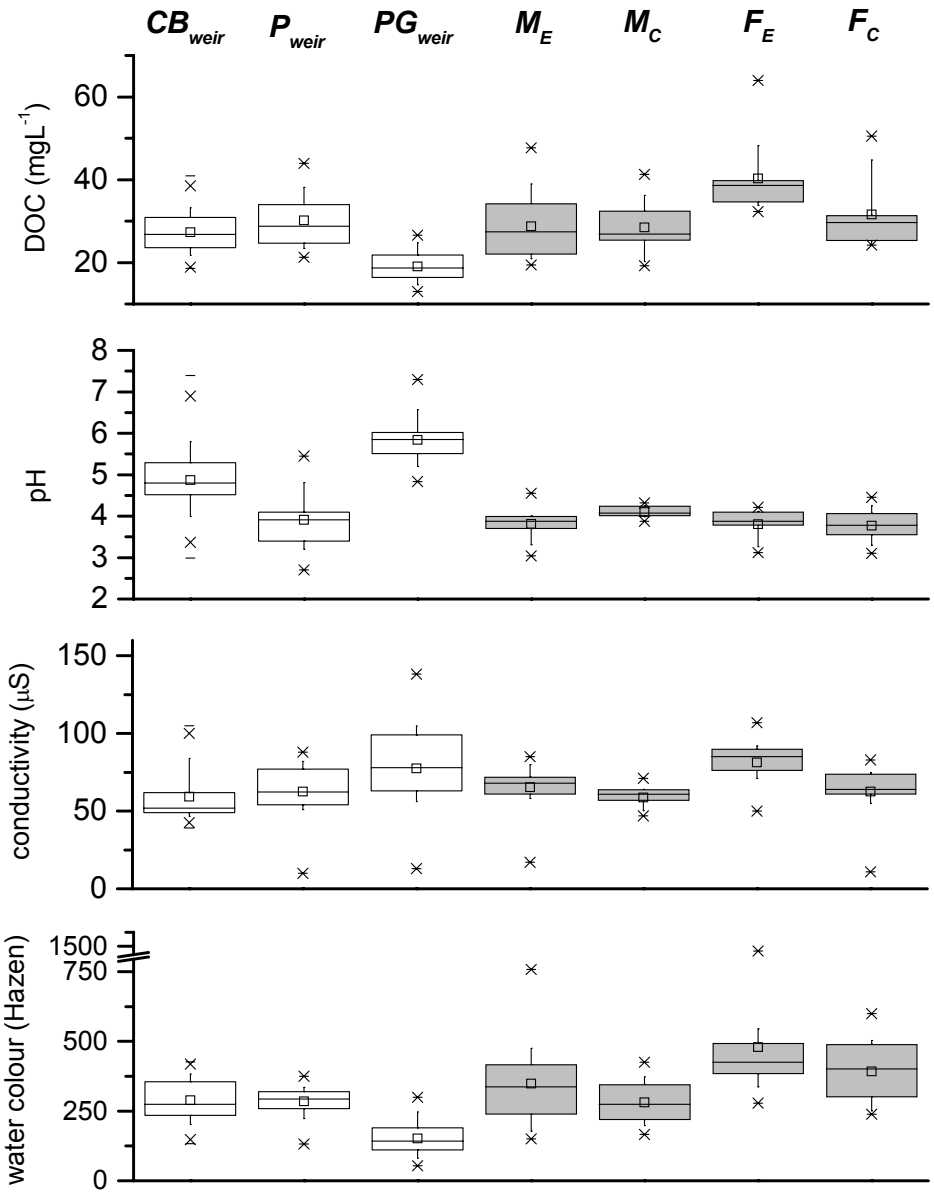


Figure 3.2
Box plots of DOC concentration (mgL^{-1}); pH; conductivity (μS) and water colour (Hazen) in surface water from the Coalburn Experimental Catchment.
Key: The square symbol in the box denotes the mean of the column of data. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values; error bars denote the 5th and 95th percentile values; two symbols below the 5th percentile error bar denote the 0th and 1st percentile values; the two symbols above the 95th percentile error bar denote the 99th and 100th percentiles.

Mean conductivity exhibited the patterns seen previously in the catchment, however, there were no significant differences between CB_{weir}; peat sub-catchment derived surface waters and PG_{weir} (95% confidence level), the latter exhibiting the highest mean. High conductivity levels of PG_{weir} can be attributed to the comparatively high concentrations of solutes (Table 1.5) and relates to the inorganic nature of the soil in this area of the catchment. Conductivity data throughout this study was lower compared to the values in Table 1.5, at duplicated sampling sites. This may indicate a response to different climatic conditions during the respective monitoring periods, however; similarly it may indicate a difference in sampling frequencies and analytical methods.

3.5.2 Spatial variability in DOC concentration and water colour in surface water

In this study samples from CB_{weir} were found to have a higher mean DOC concentration (27.02 mgL^{-1}) than data from Newson *et al.* (2001) 24.3 mgL^{-1} and Robinson *et al.* (1998) 18.2 mgL^{-1}; this may be due to different analytical and sampling procedures. Mounsey (1999) noted an increase in DOC concentration over time (1994 -1997). A continuation of this overall trend may be reflected in this study. It has been recognised that measurement of total organic carbon in aquatic samples is poorly reproduced using different analytical methods (Koprivnjak *et al*., 1995) such as those employed in this and previous studies based in the Coalburn Experimental Catchment. Thus direct comparison of DOC concentration values are not made. The difference in DOC concentration appears to be consistent over the catchment as both P_{weir} and PG_{weir} mean DOC concentrations are slightly higher than those shown in Table 1.5.

Although there is a discrepancy in absolute values the data from this study replicates that observed previously and replicates the broad description of the catchment made by Robinson *et al.* (1998). Mean DOC concentration was 32.93% and water colour was 48.43% significantly higher in P_{weir} and CB_{weir} in comparison to PG_{weir} (95% significance level). P_{weir} had a higher mean value of DOC concentration (30.15 mgL^{-1}) compared to CB_{weir} however this was not significant.

In a similar manner to DOC concentration data it was not possible to compare absolute water colour values in this and previous work due to differences in

measurement techniques. In previous studies of the catchment the specific method of colour measurement has not been detailed and variations in the technique, such as the wavelength of absorbance used, can result in wide differences in Hazen value calculated. Water colour replicates the spatial variations of that previously observed in the general catchment description. In samples from all sources DOC concentration and water colour correlated positively (95% confidence level Spearman's Rho 0.655 to 0.979) and 69.4% of the variations in water colour could be explained by DOC concentration. This indicates that water colouration in the catchment is related to DOC concentration, as discussed in Section 1.2.1, and is primarily derived from DOM.

In the peat sub-catchment ditches mean DOC concentrations and water colour levels were the highest in ditch F_E, (40.27 mgL^{-1} s.d. 9.44 and 479.74 mgL^{-1} s.d. 239.68) significantly higher than ditch $M_{E,}$ M_C and P_{weir} (95% confidence level). Ditch M_C had the lowest mean DOC concentration values of the peat sub-catchment surface water (28.45 mgL^{-1}), as shown on Figure 3.2. F_E exhibited the highest DOC concentration seen within the catchment (max=63.97 mgL^{-1}); such elevated levels of DOC concentration have not been previously reported in the Coalburn catchment. Similar values however have been identified in peat land environments; using the same analytical method (Fraser *et al*., 2001) and higher DOC concentration has been reported in peat lands that have undergone cutting and disturbance (Glatzel *et al.,* 2003).

From these limited examples it appears that a greater proportion of planted area in the micro-catchment of the ditch enhances DOC concentration in the ditch water, a finding previously observed in other upland environments, on a larger scale (Grieve and Marsden, 2001). Water from the four sampled ditches all exhibited higher mean DOC concentrations, compared to PG_{weir} (99% confidence level) and in the case of ditches F_E and F_C higher than CB_{weir} (99% confidence level).

The variations in water colour values recorded in Figure 3.2 closely correspond to DOC concentration distribution. The calculation of colour/DOC concentration (Table 3.3) indicates the proportion of coloured DOM in each water source. CB_{weir} and waters derived from the peat sub-catchment had significantly more coloured DOM compared to peaty-gley sub-catchment derived DOM (99% confidence level). This shows that the peat sub-catchment exports runoff with greater colouration compared

to the peaty-gley sub-catchment and with a higher proportion of coloured components in the DOM.

	Mean and standard deviation colour/DOC
$\mathbf{CB_{weir}}$	10.746 (1.387)
$\mathbf{P_{weir}}$	9.952 (1.246)
$\mathbf{PG_{weir}}$	8.397 (2.547)
$\mathbf{M_E}$	10.818 (3.745)
$\mathbf{M_C}$	9.518 (0.786)
$\mathbf{F_E}$	12.350 (3.695)
$\mathbf{F_C}$	11.469 (1.965)

Table 3.3
Summary of water colour/DOC in surface water in the Coalburn Experimental Catchment. Standard deviations are shown in brackets

3.5.3 Spatial variations in the fluorescence properties of DOM in the Coalburn Experimental Catchment

3.5.3.1 Excitation emission matrices

Analyses of all water samples from the catchment exhibited the features seen in EEMs discussed in Section 2.2 comprising peak A, B and C. Peak E and fluorescence maxima in region F, at excitation wavelengths <300nm, (Figure 2.1) were observed throughout the samples, however neither was consistently monitored due to the errors discussed in Section 2.2.2. Peak D was not observed in any of the samples.

The excitation and emission wavelengths of fluorescence intensity maxima within EEMs throughout the catchment are presented in Figure 3.3, together with the mean values of EXλ and EMλ of each identified peak. From examination of this data it can be seen that there are consistent locations of fluorescence intensity peaks within the EEMs. One exception to this was observed, in the analysis of a PG_{weir} sample, which resulted in a blue shift of peak $A_{EM\lambda}$ to 408.88nm, in comparison to the mean shown on Figure 3.3 (peak $A_{EM\lambda}$ 441.86nm). Peak B and peak C were absent. An additional peak (EXλ = 280$\pm$0nm and EMλ = 409.25nm) of lower fluorescence intensity than peak A was identified, which was unrelated to any of the typical peaks observed in DOM analysis (Figure 2.1).

The peaks exhibited near identical emission wavelengths, which never exceeded 1.5nm in replicate analyses, suggesting that both peaks were related to the presence of the same fluorophores. The configuration of the fluorescence intensity peaks within this EEM resembled that observed in non-DOM analyses, such as single compound solutions of for example, quinine sulphate. The EEM exhibited rounded maxima and definite peaks within single scan excitation and emission spectra, in comparison to the poorly defined peaks seen in typical DOM analyses. Compounds which have fluorescence maxima identified in the regions in question include salicylic acid, 3-hydroxycinnamic acid and variously substituted coumarins, all of which have been suggested as possible contributors to the fluorescent signature of DOM (Senesi, 1990), however none of these compounds replicate the distinctive fluorescence characteristics observed in this sample. An identification of the fluorophore responsible for this EEM was beyond the scope current study and requires further investigation into simple organic molecules present, by isolation and analysis of these components.

The positions of peaks identified in this unique EEM are included in Figure 3.3. As this EEM was not identified in any other analysis the fluorophores present and the distinct DOM composition responsible may be attributed to the specific catchment conditions during sampling. During this period (May 2000) there were relatively dry, low flow conditions and PG_{weir} was stagnant with algal and microbial growth apparent. This EEM pattern was not seen in the catchment in DOM sampled during other low flow conditions, and is unlikely to represent a different signal derived from deeper water sources and flow paths, that have been identified to predominate under such conditions. It is suggested that the fluorescence signature is directly due to the biological activity within the water modifying the typical spectrophotometric signal. However, in other stagnant ditches sampled that exhibited some algal growth, this signal was not observed.

As this distribution of peaks within EEMS appears to only exist during specific conditions it influences the overall signal of the catchment DOM little. This is due firstly to the limited time during which this DOM was observed to occur. Secondly, this was due to the negligible flow during this period resulting in DOM remaining in the ditch at PG_{weir} and not being transferred to the main channel. Upon increasing flow conditions this EEM disappeared and the typical signal was observed.

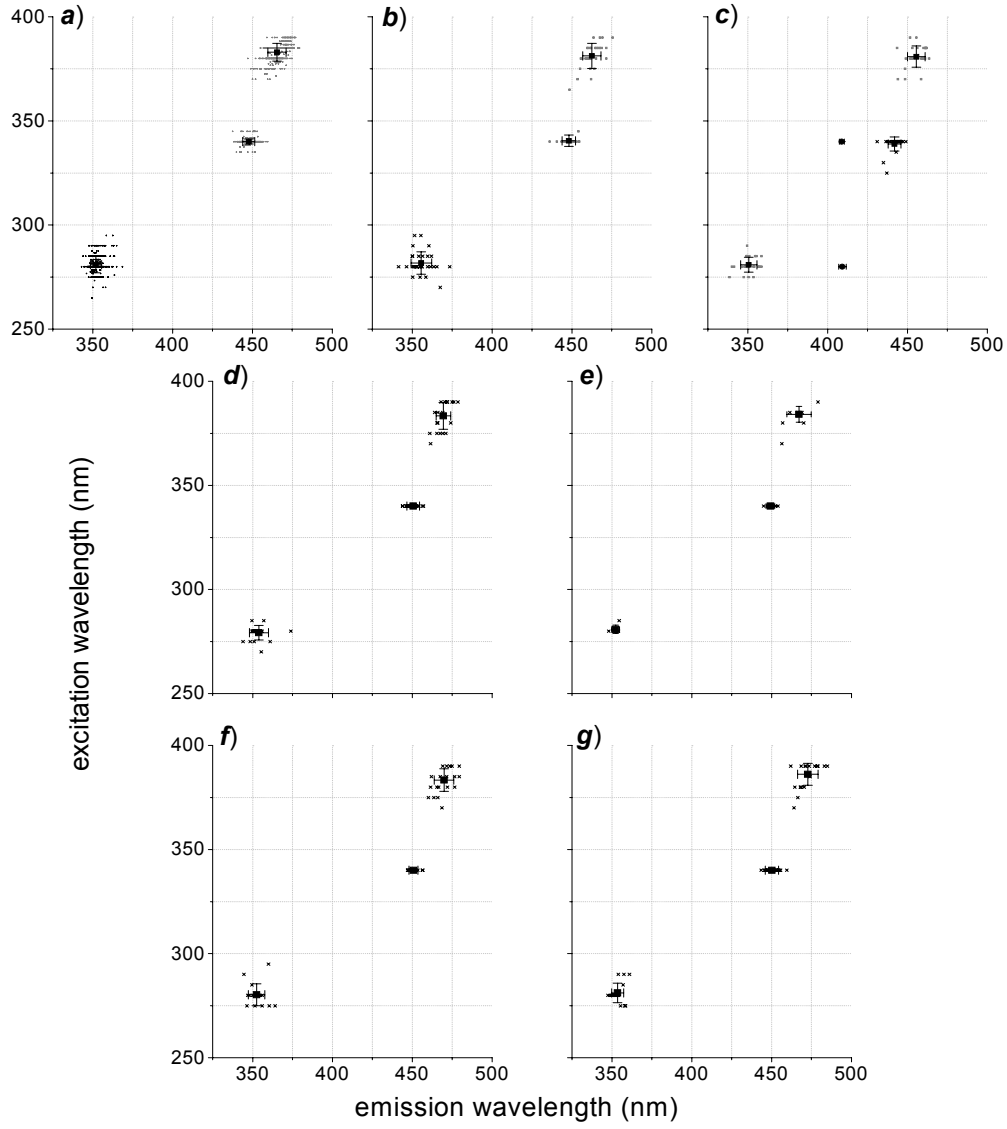


Figure 3.3
The positions, within EEMs, of all the fluorescence intensity maxima, identified in surface water from the Coalburn Experimental Catchment (x) *all data* (■) *mean.* ***a)*** *CB_{weir}* ***b)*** *P_{weir}* ***c)*** *PG_{weir}* (●) *peaks identified from May 2000* ***d)*** *M_E* ***e)*** *M_C* ***f)*** *F_E* ***g)*** *F_C*

3.5.3.2 Excitation and emission wavelengths of fluorescence intensity peaks

The consistent position of fluorescence intensity peaks within EEMs is shown on Figure 3.3. The range of observed wavelengths was limited, in both the data set as a whole and in each individual sample source, as summarised in Table 3.4. The standard deviations about the means did not exceed the reproducibility of the method as quoted in Table 2.2.

		EXλ mean (nm)	EMλ mean (nm)
All data	peak A	340.056 (1.491)	447.772 (4.070)
	peak B	382.790 (4.800)	465.297 (6.416)
	peak C	280.937 (4.044)	352.278 (4.132)
CB_{weir}	peak A	340.100 (1.173)	447.737 (3.692)
	peak B	382.978 (4.302)	465.445 (5.685)
	peak C	281.010 (3.917)	352.008 (3.517)
P_{weir}	peak A	340.484 (2.694)	448.081 (4.211)
	peak B	381.290 (5.051)	465.435 (5.734)
	peak C	281.774 (5.408)	355.684 (6.508)
PG_{weir}	peak A	338.929 (3.431)	441.857 (3.986)
	peak B	380.893 (5.101)	455.571 (5.515)
	peak C	280.893 (3.614)	350.643 (5.115)
M_E	peak A	340.000 (0.000)	450.158 (4.123)
	peak B	382.632 (5.946)	469.026 (4.789)
	peak C	278.947 (3.566)	353.368 (6.220)
M_C	peak A	340.000 (0.000)	449.450 (2.598)
	peak B	381.500 (5.297)	467.050 (6.990)
	peak C	280.500 (1.581)	351.550 (2.351)
F_E	peak A	340.263 (1.147)	450.763 (2.725)
	peak B	382.632 (6.094)	469.763 (5.992)
	peak C	280.263 (5.130)	352.605 (5.054)
F_C	peak A	340.000 (0.000)	450.421 (4.217)
	peak B	385.263 (5.341)	472.211 (6.501)
	peak C	281.053 (4.588)	353.526 (3.627)

Table 3.4
Summary of the mean fluorescence peak wavelengths in the Coalburn Experimental Catchment. Standard deviations are given in brackets

In the comparisons of peak $A_{EM\lambda}$ the mean differences between sample sources were less that the reproducibility, with the exception of the comparison of PG_{weir} to F_E, a difference of 8.906nm. This relationship was observed in the comparison of PG_{weir} and all other sources in peak $B_{EM\lambda}$, the maximum difference being with F_C (16.639nm). These differences were statistically significant (99% confidence level). With the exception of the short wavelengths observed in PG_{weir} DOM the data

showed no further differences between sources. This indicates the different physical conditions of peat sub-catchment ditches did not influence this excitation and emission wavelengths.

No significant relationships were seen between fluorescence peak wavelengths and conductivity or pH. Within the data set as a whole a weak negative correlation was observed between pH and peak $B_{EM\lambda}$ (Spearman's rho = -0.194 99% confidence level). Experimentally a red shift in emission wavelengths was observed with increasing pH, however in this data set the natural gradients in geochemistry dominate over the relationships observed in Chapter 2.

All wavelengths in the data set as a whole were independent from changes in DOC concentration, except peak $A_{EM\lambda}$ and peak $B_{EM\lambda}$, which had weak positive correlations with DOC concentration (99% level Spearman's Rho). This represents the DOC concentration and wavelengths observed in peat sub-catchment water compared to the peaty-gley sub-catchment. The differences in emission wavelength between peat and peaty-gley sub-catchment derived DOM indicates a difference in composition of DOM in waters of high and low DOC concentration such as those described by Senesi *et al*. (1991).

3.5.3.3 Peak fluorescence intensities and fluorescence intensity ratios

Fluorescence intensity and fluorescence intensity ratio data is presented in Figure 3.4 and Figure 3.5 and in Table 3.5. The results of t-tests indicating significant differences between the mean values of fluorescence intensity variables in different sources are summarised in Table 3.6.

A comparison was made between fluorescence intensity maxima that had not been corrected for IFEs to compare the current study to Newson *et al*. (2001). As discussed in Section 1.5.3, it is essential to consider IFEs in the examination of fluorescence intensities of solutions with high DOM concentrations and high absorbance levels. The previous investigations into fluorescence spectrophotometry of DOM at Coalburn (Newson *et al*., 2001) as in the case of a number of other studies exclusively used fluorescence intensity data without such a correction.

To illustrate the importance of IFEs Figure 3.4 presents peak A_{Fint} data from CB_{weir}, P_{weir} and PG_{weir} before and after application of the correction discussed in Section 2.3.1.1. The data from PG_{weir} shows less suppression of fluorescence intensity prior to correction compared to CB_{weir} and $P_{weir.}$ After correction, as shown in Figure 3.4 mean peak B_{Fint}/peak A_{Fint} was significantly higher in P_{weir} and PG_{weir} compared to CB_{weir} and in PG_{weir} compared to P_{weir} (95% confidence level). As discussed in Section 1.7.1 Newson *et al*. (2001) monitored the same sites and observed that mean peak A_{Fint} was higher in PG_{weir} compared CB_{weir} and P_{weir} and that peak B_{Fint}/peak A_{Fint} was higher in CB_{weir} and P_{weir} compared to PG_{weir}. Indicating the data discussed by the authors is highly influenced by IFEs.

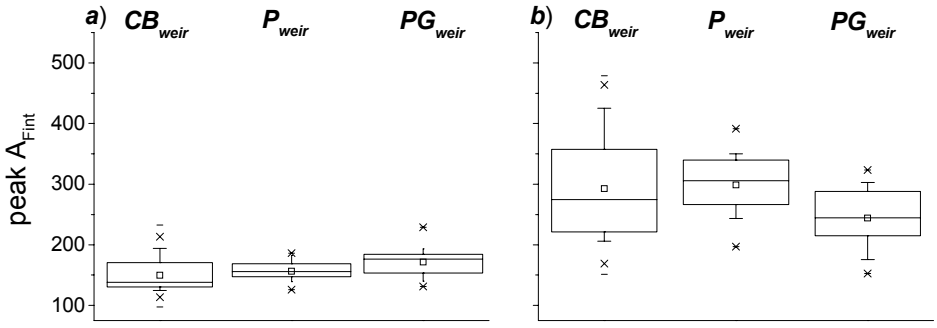


Figure 3.4
Box plots of Peak A_{Fint} ***a)*** *without correction for IFE* ***b)*** *with correction for IFE in surface water from Coalburn Experimental Catchment. For key to box plots see Figure 3.2*

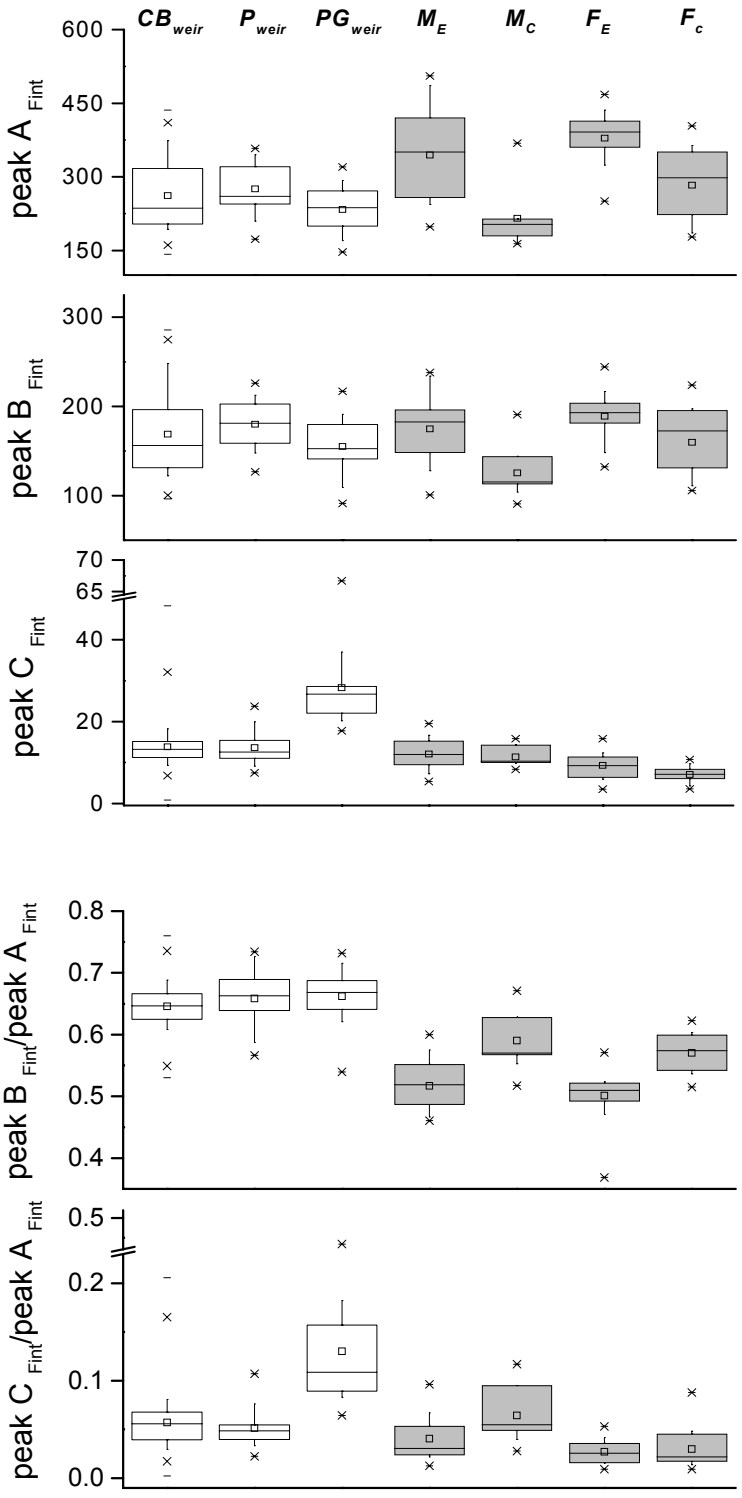


Figure 3.5
Box plots of peak A_{Fint}; peak B_{Fint}; peak C_{Fint}; peak B_{Fint} /peak A_{Fint}; peak C_{Fint} /peak A_{Fint} in surface water from the Coalburn Experimental Catchment. For key to box plots see Figure 3.2.

	peak A_{Fint}	peak B_{Fint}	peak C_{Fint}	peak B_{Fint}/ peak A_{Fint}	peak C_{Fint}/ peak A_{Fint}
CB_{weir}	292.339 (84.531)	168.827(46.239)	13.693 (4.288)	0.581 (0.032)	0.052 (0.023)
P_{weir}	298.910 (50.535)	179.911(28.240)	13.662 (4.201)	0.605 (0.044)	0.048 (0.020)
PG_{weir}	244.334 (50.537)	154.950 (33.995)	28.315 (9.928)	0.634 (0.040)	0.125 (0.070)
M_E	344.362 (100.782)	174.805 (40.947)	12.056 (4.053)	0.517 (0.043)	0.041 (0.022)
M_C	255.553 (88.566)	135.723 (32.191)	11.185 (3.050)	0.551 (0.079)	0.064 (0.029)
F_E	378.482 (54.799)	188.993 (27.955)	9.312 (3.406)	0.501 (0.041)	0.027 (0.012)
F_C	282.824 (974.675)	159.647 (36.314)	7.078 (2.216)	0.570 (0.031)	0.030 (0.019)

Table 3.5

Summary of mean peak A_{Fint}; peak B_{Fint}; peak C_{Fint}; peak B_{Fint} /peak A_{Fint}; peak C_{Fint} /peak A_{Fint} in surface water from the Coalburn Experimental Catchment. Standard deviation is given in brackets.

		P_{weir}	PG_{weir}	M_E	M_C	F_E	F_C
peak A_{Fint}	CB_{weir}	ns	4.508	ns	ns	6.478	ns
	P_{weir}		4.071	ns	ns	5.130	ns
	PG_{weir}			3.988	ns	8.440	ns
	M_E				3.154	ns	3.654
	M_C					4.004	ns
	F_E						4.052
peak B_{Fint}	CB_{weir}	ns	1.998	ns	ns	2.949	ns
	P_{weir}		2.997	3.872	ns	3.187	ns
	PG_{weir}			ns	ns	6.716	ns
	M_E				3.245	ns	ns
	M_C					4.427	ns
	F_E						2.791
peak C_{Fint}	CB_{weir}	ns	7.741	ns	ns	5.397	11.938
	P_{weir}		7.512	ns	ns	4.488	7.235
	PG_{weir}			7.768	8.120	9.350	10.925
	M_E				ns	2.259	4.696
	M_C					ns	3.700
	F_E						2.395
peak B_{Fint}/ peak A_{Fint}	CB_{weir}	3.751	6.553	6.372	ns	8.348	ns
	P_{weir}		2.574	6.859	ns	8.343	ns
	PG_{weir}			9.214	3.136	10.797	6.958
	M_E				3.145	ns	4.354
	M_C					ns	ns
	F_E						5.828
peak C_{Fint}/ peak A_{Fint}	CB_{weir}	ns	5.446	ns	ns	8.166	4.684
	P_{weir}		5.562	ns	ns	4.005	3.043
	PG_{weir}			5.875	3.814	7.172	6.727
	M_E				ns	2.313	ns
	M_C					3.824	3.299
	F_E						ns

Table 3.6

Summary of the results of t-tests, comparing significant differences in mean peak A_{Fint}; peak B_{Fint}; peak C_{Fint}; peak B_{Fint} /peak A_{Fint}; peak C_{Fint} /peak A_{Fint} in surface water from the Coalburn Experimental Catchment. T values; higher values indicate greater differences in means, ns = not significant; all significant differences are at 95% confidence level

As shown in Figure 3.5 and Table 3.6 mean peak A_{Fint} and peak B_{Fint} were highest in F_E (378.482 and 188.993 respectively), significantly so in comparison to all other sources except M_E (344.362 and 174.808 respectively). Both experimental ditches exhibited significantly higher mean peak A_{Fint} and peak B_{Fint} compared to control ditches. The highest individual value of peak A_{Fint} was observed in M_E (505.789) and highest peak B_{Fint} in CB_{weir} (285.570) the lowest of peak A_{Fint} was seen in PG_{weir} (151.208) and peak B_{Fint} in M_C (90.752).

The maximum value of peak B_{Fint}/peak A_{Fint} (0.718) was observed in PG_{weir}, DOM from this source also had the highest mean peak B_{Fint}/peak A_{Fint} (0.634 s.d. 0.040). This mean value was significantly higher than all other sources (Table 3.6). P_{weir} exhibited the highest mean in peat derived DOM (0.581), including CB_{weir} (0.605), both of these sources exhibited significantly higher and than experimental ditches (95% confidence level) (Table 3.6) Mean peak B_{Fint}/peak A_{Fint} was significantly higher in both F_C and M_C compared to M_E and $F_{E,}$ which had the lowest value in the catchment (0.369).

Peak C and peak C_{Fint} /peak A_{Fint} were highest in PG_{weir} (28.315 and 0.125 respectively) (Table 3.6). DOM from this source also exhibited the maximum values of peak C_{Fint} (66.691) and peak C_{Fint} /peak A_{Fint} (0.419); minimum values were observed in F_E (3.12 and 0.009 respectively). This distribution resulted in significantly higher mean values in PG_{weir} DOM compared to other sources (Table 3.6).

Throughout the catchment peak B_{Fint} and peak A_{Fint} strongly correlated (Spearman's rho 0.964 99% confidence level) and both of these values had a negative correlation with peak C_{Fint} (99% confidence level). This negative relationship replicates the increased peak B_{Fint} and peak A_{Fint} in peat sub-catchment DOM compared to increased peak C_{Fint} in PG_{weir}.

Peak B_{Fint}/peak A_{Fint} was calculated as a possible measure of humification. This technique is based upon the observed increase in the number of highly substituted aromatic nuclei aromaticity and conjugated unsaturated systems (Senesi *et al.,* 1991) in DOM with increasing wavelength. Other indices using this assumption have been applied to DOM spectrophotometric analyses (for example Kalbitz *et al.,* 1999; Zsolnay *et al.,* 1999, McKnight *et al*., 2001). High values of such ratios have been associated with increased humification and decomposition of DOM (Kalbitz *et al.,* 1999).

In the current study the interpretation of this ratio indicates that peaty-gley derived DOM had higher values of peak B_{Fint}/peak A_{Fint} has more aromatic or humified DOM compared to peat waters. This is contrary to the relationship seen in specific absorbance and emission wavelengths, and that discussed by Newson *et al*. (2001). The relationship of estimated aromaticity with this ratio was a significant, weakly negative one (99% confidence level) replicating the lower aromaticity seen in the peaty-gley sub-catchment waters. This suggests that in the data set as a whole peak B_{Fint}/peak A_{Fint} may not represent a measure of aromatic content and thus humification.

The index developed by McKnight *et al.* (1999) as discussed in Section 1.5.4 was applied to a number of samples to further investigate the results of peak B_{Fint}/peak A_{Fint} analyses. The McKnight *et al.* (1999) index ratios the fluorescence intensity at EMλ = 450nm and 500nm, at a constant EXλ = 370nm. The authors calculated 450nm/500nm and higher values were attributed to less humified DOM, with an autochthonous source. Data obtained in this study was examined using this method and values of 450nm/500nm calculated. This analysis resulted in a significantly higher mean value of the ratio in PG_{weir} DOM compared to both P_{weir} and CB_{weir} (95% confidence level). These values correlated negatively with estimated aromaticity (Spearman's rho = 0.786 99% confidence level) and specific absorbance. Interpretation of this data results in DOM of lower aromaticity and less humified than from peaty-gley sub-catchment.

The difference between the two ratios may be due to the wavelengths of fluorescence intensity that are being considered. In the McKnight *et al.* (1999) index fluorescence intensity is measured at differing emission wavelengths, however peak B_{Fint}/peak A_{Fint} is measured at different excitation wavelengths (~375nm/340nm). In this ratio emission wavelengths are relatively close (~465nm/450nm) and the values calculated may not identify the shift of emission wavelength that is known to occur with changes in aromaticity and humification.

The observed changes in peak B_{Fint}/peak A_{Fint} may be explained with reference to the influence of pH on DOM discussed in Chapter 2. Experimental data in Section 2.3.2 indicated that in aquatic DOM the two fluorescence intensity peaks were controlled in different manners by pH and that the ratio increased with increasing pH over different ranges for different samples. In the overall relationship of spatial data in the

catchment there is a weak positive correlation of pH and peak B_{FInt}/peak A_{FInt} suggesting that the spectrophotometric properties of DOM in catchment may vary in response to the pH of the water. The complete interpretation of this ratio requires isolation and specific analytical identification of peak A and B fluorophores. The ratio is further explored in later chapters.

3.5.3.4 The relationship of fluorescence intensity to DOC concentration

To investigate fluorescence intensity normalized to DOC concentration peak AS_{FInt} (peak A_{FInt} /DOC mgL^{-1}) was calculated. This revealed, as shown on Figure 3.6, that PG_{weir} DOM was significantly more fluorescent per mgL^{-1} OC (13.229 s.d. 2.181) than both CB_{weir} (10.317 s.d. 1.922) and P_{weir} (10.637 s.d. 1.799) (95% confidence level). M_C and F_C exhibited the lowest mean values of peak AS_{FInt} (8.527 s.d. 1.074 and 8.507 s.d. 1.709) compared to other peat derived DOM, as shown in Figure 3.6, significantly so in comparison to CB_{weir}, P_{weir} and M_E (mean = 11.410 s.d. 2.547) (95% confidence level).

The overall relationship of peak A_{FInt} with DOC concentration is shown in Figure 3.7 and indicates the strong positive correlation (Spearman's rho = 0.721 99% confidence level). Peak B_{FInt} exhibited a similar positive correlation with DOC concentration (Spearman's rho = 0.639 99% confidence level). This demonstrates that there is a strong concentration component to fluorescence intensity; however, the presence of a positive intercept on the DOC concentration axis of the linear regression line indicates that there is a non-fluorescent component of the DOM ranging from approximately 5 -15mgL^{-1} between each sample group.

Large errors and relatively low R^2 values were incurred in the linear regression of fluorescence intensity and DOC concentration suggesting that if this technique were employed as a method to determine DOC concentration inaccuracies would occur. This is also suggested by the percentage variation in fluorescence intensity that is explained by DOC concentration (Table 3.7). Additionally the examination of the DOC concentration relationship with fluorescence intensity in waters from each sample source resulted in different linear regression equations. This suggests that a calibration of fluorescence intensity to DOC concentration such as that in Figure 3.7 may not be applicable to DOM from different sources within one catchment.

In the data set as a whole there was a negative correlation between peak C_{Fint} and DOC concentration (95% confidence level), as shown on Figure 3.8 This showed the small contribution in comparison to peak A_{Fint} and peak B_{Fint} of peak C_{Fint} derived fluorophores to the total DOC concentration. The DOC concentration gradient in the catchment is shown in Figure 3.8, peaty-gley derived water has a low DOC concentration and high peak C_{Fint}.

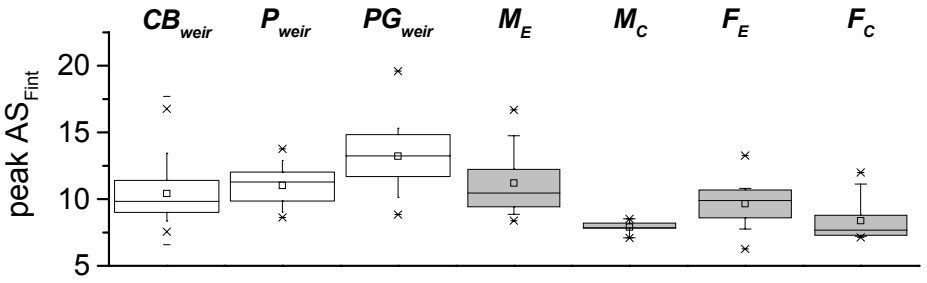


Figure 3.6
Box plots of peak AS_{Fint} in surface water from the Coalburn Experimental Catchment. For key to box plots see Figure 3.2

	Peak A_{Fint}		Peak B_{Fint}	
CB_{weir}	49.3%	DOC=15.427+A_{Fint}*0.045	44.7%	DOC=15.324+B_{Fint}*0.045
P_{weir}	32.3%	DOC=5.928+A_{Fint}*0.077	29.4%	DOC=6.816+B_{Fint}*0.077
PG_{weir}	50.6%	DOC=5.551+A_{Fint}*0.054	45.8%	DOC=7.110+B_{Fint}*0.054
F_C	58.0%	DOC=9.487+A_{Fint}*0.084	53.6%	DOC=7.608+B_{Fint}*0.084
F_E	25.8%	DOC=23.335+A_{Fint}*0.038	25.2%	DOC=22.501+B_{Fint}*0.038
M_C	90.9%	DOC=8.208+A_{Fint}*0.082	80.5%	DOC=-2.289+B_{Fint}*0.082
M_E	43.5%	DOC=11.371+A_{Fint}*0.055	39.5%	DOC=8.366+B_{Fint}*0.055

Table 3.7
The results of linear regression of fluorescence intensity against DOC concentration in surface water from the Coalburn Experimental Catchment, showing the percentage variation explained by DOC concentration and the equation of the linear regression.

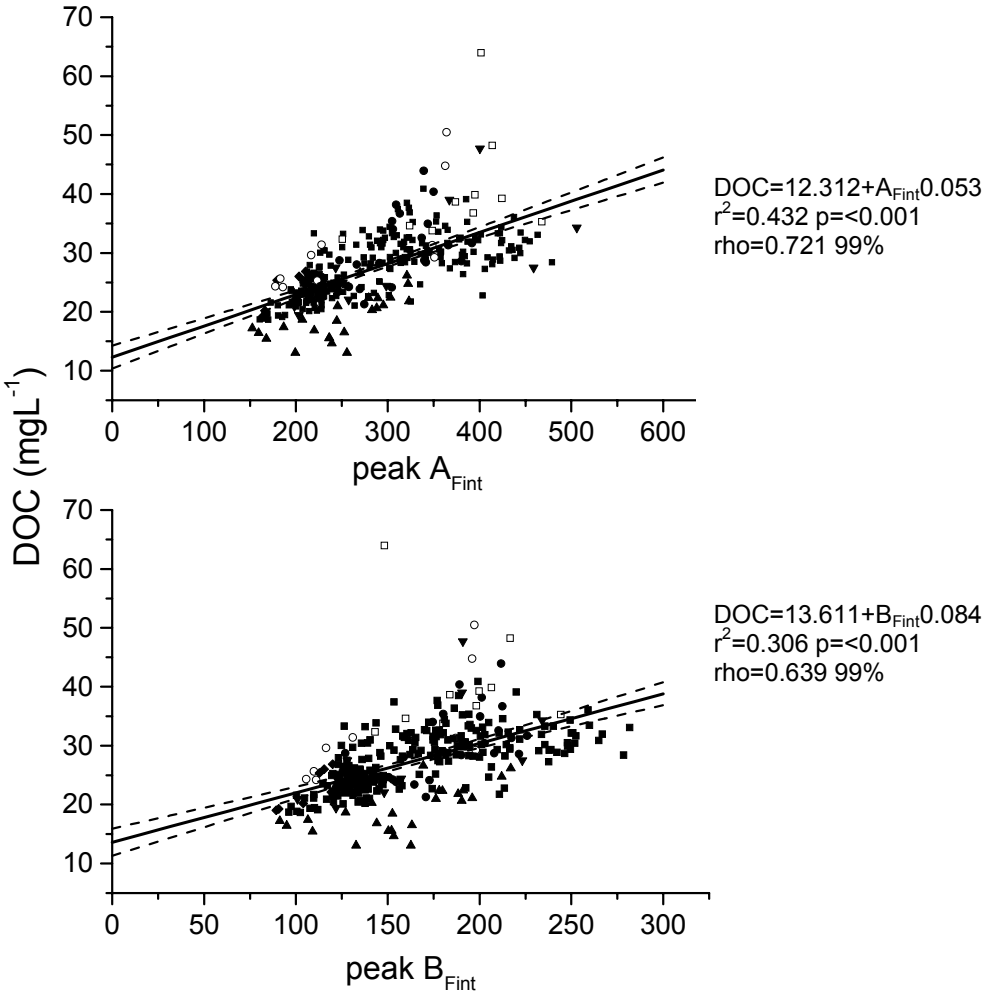


Figure 3.7
The relationship of peak A_{Fint} and peak B_{Fint} to DOC concentration in surface water from the Coalburn Experimental Catchment. (■) CB_{weir} (●) P_{weir} (▲) PG_{weir} (▼) M_E (♦) M_C (□) F_E (○) F_C (——) *linear regression* (- - - -) *95% confidence level; equations refers to combined data from all sources*

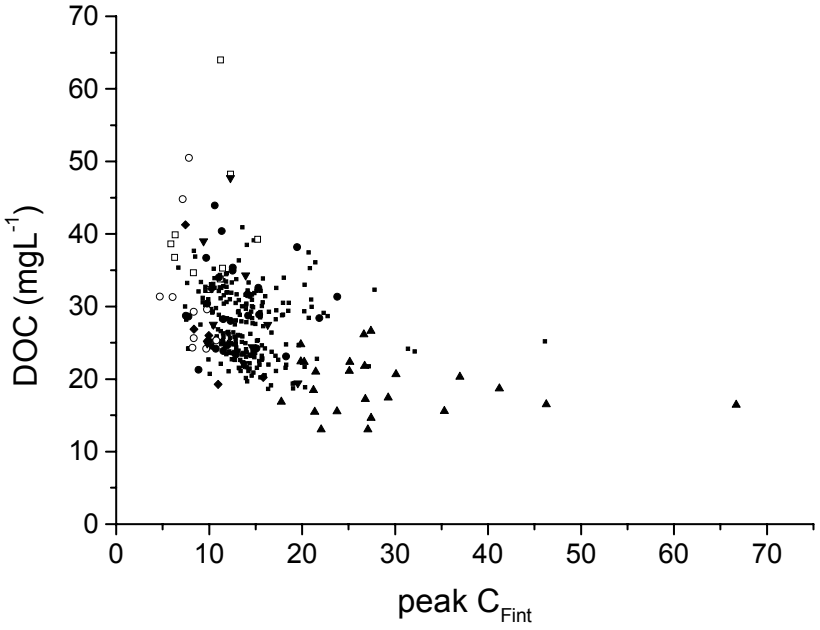


Figure 3.8
The relationship of peak C_{Fint} to DOC concentration in surface water from the Coalburn Experimental Catchment (■) CB_{weir} (●) P_{weir} (▲) PG_{weir} (▼) M_E (♦) M_C (□) F_E (○) F_C

3.5.4 Spatial variations in the UV-visible absorbance properties of DOM in the Coalburn Experimental Catchment

DOM from the Coalburn Experimental Catchment exhibited typical absorbance spectra, comparable to that recognised in DOM analyses from many sources (Section 1.5.1). As all measured individual wavelengths correlated positively in the data set as a whole and in each individual data set (95% confidence level) a single wavelength, A_{340nm}, is presented on Figure 3.9. This represents the distributions within and between data from each sample source. The spectra reproduced in Figure 3.10 show featureless curves of decreasing absorbance with increasing wavelengths. These spectra were observed in all analyses throughout the catchment.

PG_{weir} exhibited the lowest absorbance values (A_{340nm} = 0.082) and the lowest mean values (0.232 s.d. 0.086), as shown in Figure 3.9. Mean absorbance from CB_{weir} was indistinguishable from P_{weir} at wavelengths longer than ~A_{300nm}, however, at A_{254nm} and A_{272nm} P_{weir} was significantly higher than CB_{weir} (95% confidence level). CB_{weir}

additionally exhibited a wider range of values. Maximum absorbance values at all measured wavelengths were observed in F_E (A_{340nm} = 1.588). Forest micro catchment had significantly higher mean absorbance compared to other peat sub-catchment derived DOM (95% confidence level).

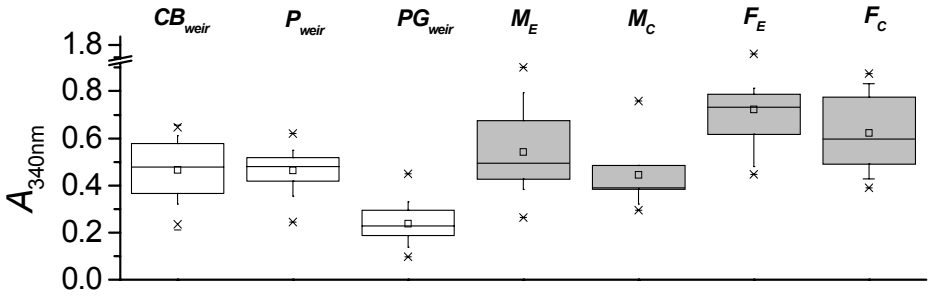


Figure 3.9
Box plots of A_{340nm} in surface water from the Coalburn Experimental Catchment. For key to box plots see Figure 3.2

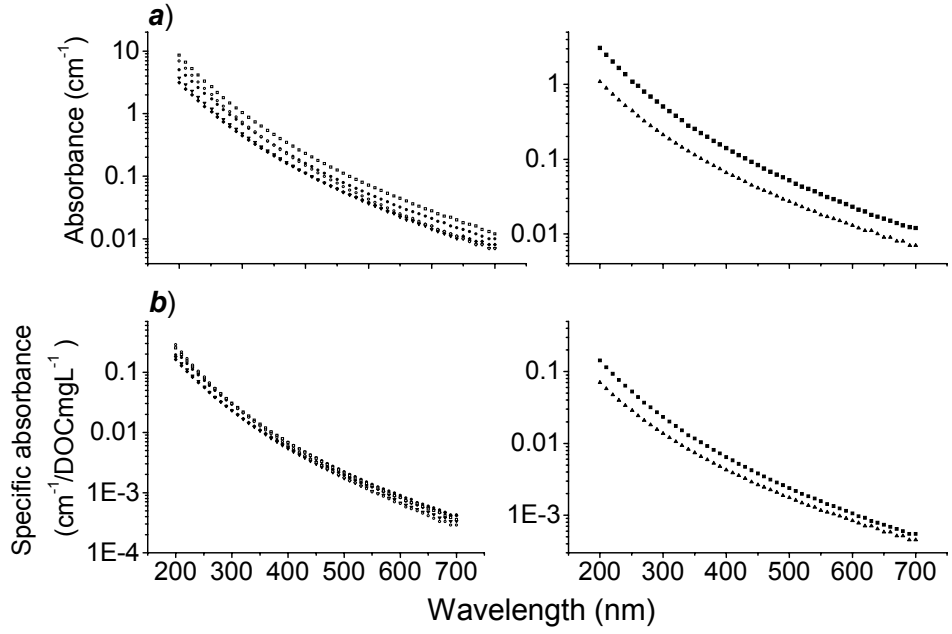


Figure 3.10
Typical absorbance spectra in surface water from the Coalburn Experimental Catchment. ***a)*** *absorbance (cm^{-1})* ***b)*** *specific absorbance ($cm^{-1}/DOCmgL^{-1}$) (■) CB_{weir} (●) P_{weir} (▲) PG_{weir} (▼) M_E (♦) M_C (□) F_E (○) F_C (all sampled on 20/02/01).*

3.5.4.1 The relationship of UV-visible absorbance to DOC concentration

Absorbance at all wavelengths correlated strongly positively with DOC concentration, in all sample sets and the data set as a whole, as shown on Figure 3.11. This indicates that there was a strong component of concentration in the absorbance signal. This was not seen at wavelengths >A_{500nm}. Within each sample group the correlation was strongly positive with Spearman's rho >0.73 (99% confidence level).

The linear regression relationships between DOC concentration and absorbance are summarised in Table 3.8. These relationships indicate that absorbance is explained by variations in DOC concentration to a greater extent than fluorescence intensity in the data set as a whole (Table 3.7). This, however, varies between each group. For example, in data from M_C DOC concentration explained variations in peak A and peak B to a greater extent than at all absorbance wavelengths. Additionally, the relationship of absorbance and DOC concentration varied between the wavelengths observed in DOM from the same sample source. For example CB_{weir} exhibits the greatest relationship of absorbance to DOC concentration at A_{340nm} however, this occurs at A_{272nm} in DOM from P_{weir}.

The maximum variation in absorbance explained by DOC concentration in DOM from M_E was 54%, however this was up to 95% in DOM from F_E. In addition, to the DOC concentration of the solution the aromatic and the content of hydrophobic material of the DOM can influence the absorbance of DOM (Dilling and Kaiser, 2002). The varying relationship presented in Table 3.8 may show a spatial variation in DOM composition.

	A_{254nm}		A_{272nm}	
CB_{weir}	41.23%	DOC=14.24+A_{254nm}*10.13	44.94%	DOC=12.29+A_{272nm}*13.70
P_{weir}	70.05%	DOC=18.17+ A_{254nm}*6.69	72.85%	DOC=13.75+A_{272nm}*12.02
PG_{weir}	69.01%	DOC=13.76+A_{254nm}*6.66	67.19%	DOC=12.93+A_{272nm}*9.68
F_C	84.95%	DOC= 8.94+A_{254nm}*13.97	87.21%	DOC= 6.09+A_{272nm}*18.84
F_E	76.63%	DOC=-3.78+A_{254nm}*26.87	81.16%	DOC=-2.32+A_{272nm}*28.68
M_C	61.49%	DOC=13.07+A_{254nm}*13.09	69.13%	DOC=13.71+A_{272nm}*14.69
M_E	54.53%	DOC= 6.20+A_{254nm}*16.43	52.47%	DOC= 5.98+A_{272nm}*19.16
	A_{340nm}		A_{365nm}	
CB_{weir}	60.75%	DOC=10.81+A_{340nm}*36.31	56.40%	DOC=11.62+A_{365nm}*51.09
P_{weir}	70.22%	DOC= 6.11+A_{340nm}*49.36	65.99%	DOC=3.29+A_{365nm}*84.36
PG_{weir}	59.29%	DOC=10.46+A_{340nm}*35.25	57.64%	DOC=11.40+A_{365nm}*47.44
F_C	91.22%	DOC=11.75+A_{340nm}*33.63	92.79%	DOC=10.41+A_{365nm}*55.67
F_E	88.94%	DOC=-1.46+A_{340nm}*66.09	82.77%	DOC=-3.50+A_{365nm}*110.22
M_C	53.24%	DOC=18.08+A_{340nm}*29.15	57.56%	DOC=20.15+A_{365nm}*38.48
M_E	29.10%	DOC= 6.34+A_{340nm}*44.01	30.81%	DOC= 7.60+A_{365nm}*63.36
	A_{410nm}		A_{465nm}	
CB_{weir}	37.76%	DOC=10.18+A_{410nm}*113.02	2.28%	DOC=10.70+A_{465nm}*230.26
P_{weir}	67.65%	DOC=-4.63+A_{410nm}*224.99	11.52%	DOC=14.14+A_{465nm}*218.18
PG_{weir}	46.74%	DOC=11.687+A_{410nm}*87.56	0.01%	DOC=12.99+A_{465nm}*157.30
F_C	95.07%	DOC=14.850+A_{410nm}*86.67	89.79%	DOC=19.18+A_{465nm}*125.77
F_E	69.26%	DOC=-0.395+A_{410nm}*206.80	26.26%	DOC=-11.59+A_{465nm}*694.86
M_C	34.06%	DOC=25.52+A_{410nm}*54.96	11.59%	DOC=26.55+A_{465nm}*100.44
M_E	20.03%	DOC= 8.834+A_{410nm}*121.79	3.08%	DOC= 6.47+A_{465nm}*308.29

Table 3.8
The results of linear regression of absorbance against DOC concentration in surface water from the Coalburn Experimental Catchment showing the percentage variation explained by DOC concentration and the equation of the linear regression.

	SUV_{254nm}	$Svis_{410nm}$	Estimated aromaticity
CB_{weir}	0.047 (0.008)	0.005 (0.000)	476.767 (70.574)
P_{weir}	0.056 (0.015)	0.005 (0.000)	533.446 (100.606)
PG_{weir}	0.041 (0.016)	0.004 (0.001)	390.275 (133.357)
M_E	0.049 (0.010)	0.005 (0.002)	502.880 (90.871)
M_C	0.045 (0.004)	0.005 (0.000)	478.138 (48.686)
F_E	0.049 (0.008)	0.005 (0.001)	532.948 (68.642)
F_C	0.051 (0.007)	0.005 (0.001)	510.414 (82.598)
All peat sub-catchment	0.048 (0.009)	0.006 (0.001)	485.362 (75.983)

Table 3.9
Summary of mean SUV_{254nm}, $Svis_{410nm}$ and estimated aromaticity in the Coalburn Experimental Catchment standard deviations are given in brackets.

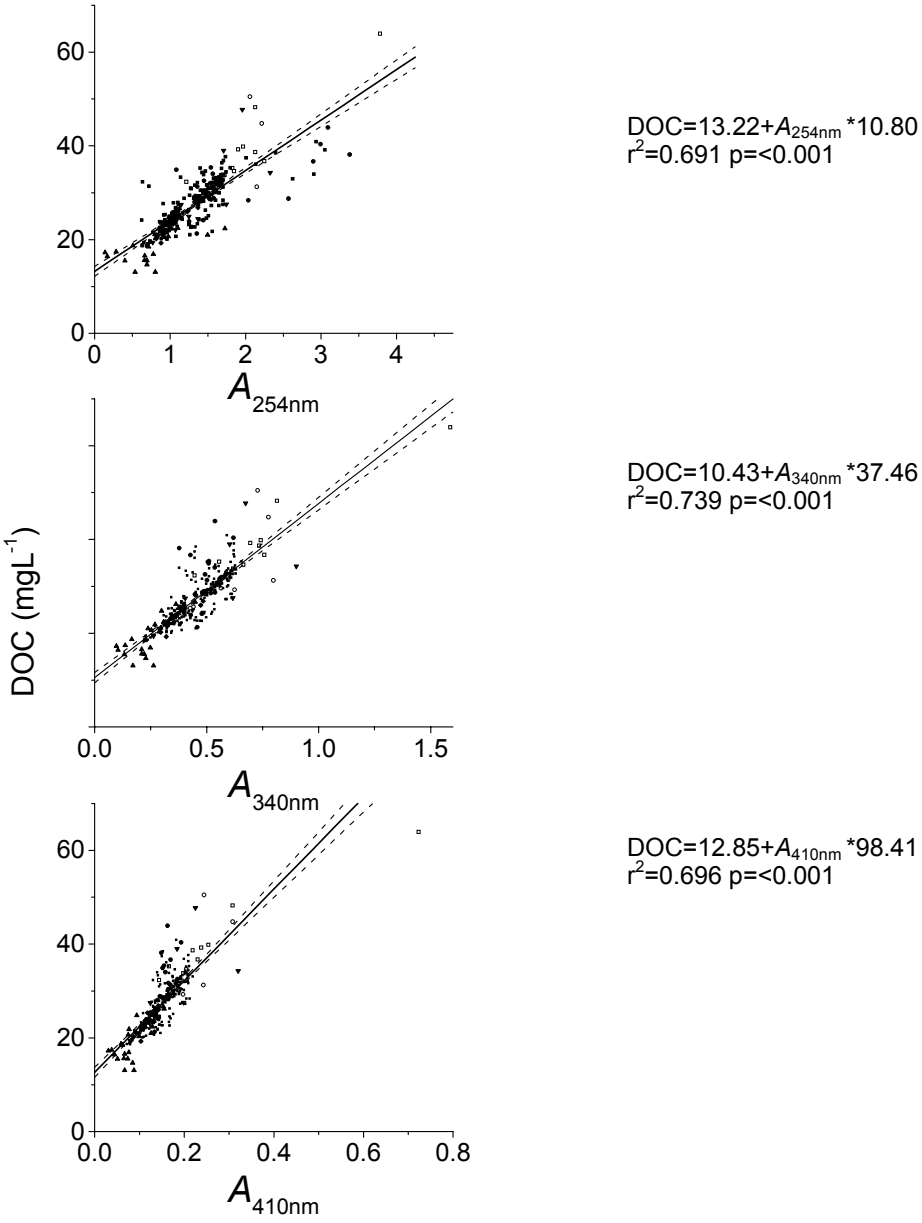


Figure 3.11
The relationship of A_{254nm}; A_{340nm} and A_{410nm} to DOC concentration in surface water from the Coalburn Experimental Catchment. (■) CB_{weir} (●) P_{weir} (▲) PG_{weir} (▼) M_E (♦) M_C (□) F_E (○) F_C (——) *linear regression* (- - - -) *95% confidence level equations refers to combined data from all sources*

The lowest values of SUV_{254nm} (0.008), $Svis_{410nm}$ (0.002) and estimated aromaticity (80.840) (Section 2.2) were observed in data form PG_{weir}. DOM from this source also exhibited the lowest mean values of these variables (Table 3.9). DOM from throughout the peat sub-catchment catchment had consistent values of SUV_{254nm}, $Svis_{410nm}$ and estimated aromaticity, as shown in Table 3.9. DOM from this source exhibited a 16% variation in these variables, compared to 35% variation of DOM from PG_{weir}. Throughout the catchment surface water specific absorbance exhibited no significant spatial differences in mean values, as shown in Table 3.9.

3.5.4.2 The relationship of UV-visible absorbance to fluorescence intensity

As discussed above both peak A_{Fint}, peak B_{Fint} and absorbance exhibited a positive relationship to DOC concentration. Figure 3.12 shows the relationships of peak A_{Fint} to absorbance measured at different wavelengths. This indicates a similar relationship throughout the absorbance spectrum and spatially in the Coalburn Experimental Catchment. A distinct grouping of data points from PG_{weir} can be observed on Figure 3.12 to the left of the regression line. This data indicates different fluorescence intensity to absorbance relationship in the DOM from this source.

The proportion of chromophores in the DOM that on absorbance results in the emission of energy is represented by peak A_{Fint}/A_{340nm}, shown in Figure 3.13. In the comparison of this parameter PG_{weir} DOM had a significantly higher mean (188.942 s.d. 207.891) than the peat sub-catchment derived DOM, including CB_{weir} (95% confidence level). Within the peat sub-catchment derived DOM peak A_{Fint}/A_{340nm} showed no significant differences in mean values (Figure 3.13).

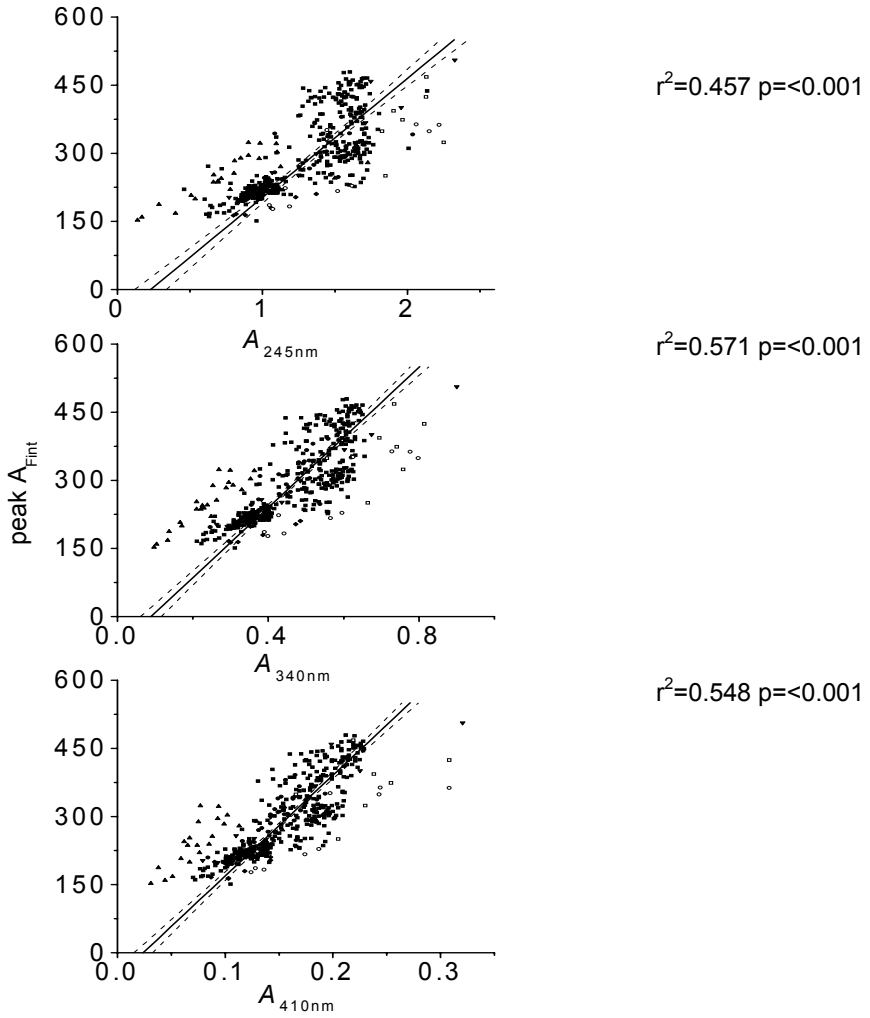


Figure 3.12
The relationship of A_{254nm}; A_{340nm} and A_{410nm} to peak A_{Fint} in surface water DOM from the Coalburn Experimental Catchment. (■) *CB_{weir}* (●) *P_{weir}* (▲) *PG_{weir}* (▼) *M_E* (♦) *M_C* (□) *F_E* (○) *F_C* (——) *linear regression* (- - - -) *95% confidence level*

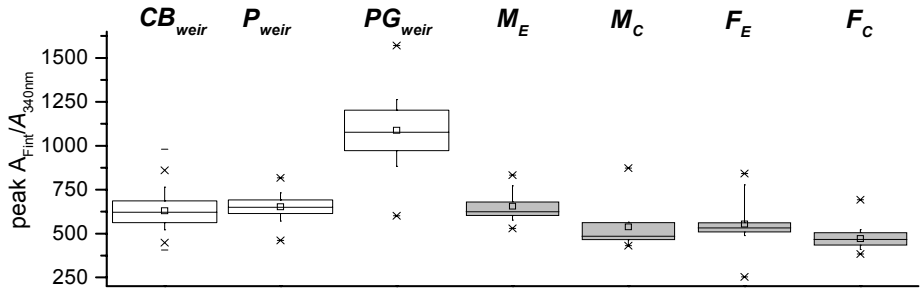


Figure 3.13
Box plots of peak A_{Fint} /A_{340nm} in surface water DOM from the Coalburn Experimental Catchment. For key to box plots see Figure 3.2

3.5.4.5 Absorbance ratios (A_{465nm}/A_{665nm}, A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm})

As discussed in Section 1.5.1 ratios of absorbance values at different wavelengths correlate with certain properties of DOM. In a number of cases these relationships have been used to apply absorbance ratios as proxies for DOM compositional variations. The ratios shown in Figure 3.14 have been calculated to identify spatial differences within the catchment and to establish compositional differentiations, in conjunction with fluorescence properties.

A_{465nm}/A_{665nm} varied little between surface water DOM and no significant differences were observed in the mean values of CB_{weir}, P_{weir}, M_E and F_E (95% confidence level). M_C (mean 13.982 s.d. 7.035) and F_C (mean 10.122 s.d. 4.611) exhibited significantly higher values than all other sources and PG_{weir} showed a significantly lower mean value (mean 5.263 s.d. 3.585) compared to all other sources (95% confidence level).

A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} measure ratios of short and long wavelengths and exhibit the same spatial patterns. As shown in Figure 3.14 the means of both A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} were significantly higher in P_{weir} and PG_{weir} compared to CB_{weir} and all other peat sub-catchment derived DOM. P_{weir} also had significantly higher means when compared to PG_{weir} (95% confidence level). This is largely accounted for by a number of high values in P_{weir}, which were the highest observed (maximum A_{254nm}/A_{410nm} 22.685 and A_{254nm}/A_{365nm} 10.795). If these extreme figures, sampled under dry and low flow conditions, discussed in Chapter 4, are removed the PG_{weir} has significantly higher mean values of A_{254nm}/A_{410nm} (mean 9.093 s.d. 1.472) and A_{254nm}/A_{365nm} (mean 4.902 s.d. 1.016) compared to peat sub-catchment DOM (A_{254nm}/A_{410nm} mean 7.537 s.d. 1.430; A_{254nm}/A_{365nm} mean 3.204 s.d. 0.625) (95% confidence level).

The three absorbance ratios detailed in Figure 3.14 did not correlate with DOC concentration, suggesting that the variations observed are related more to compositional differences in DOM, however these appear spatially limited in the examples investigated.

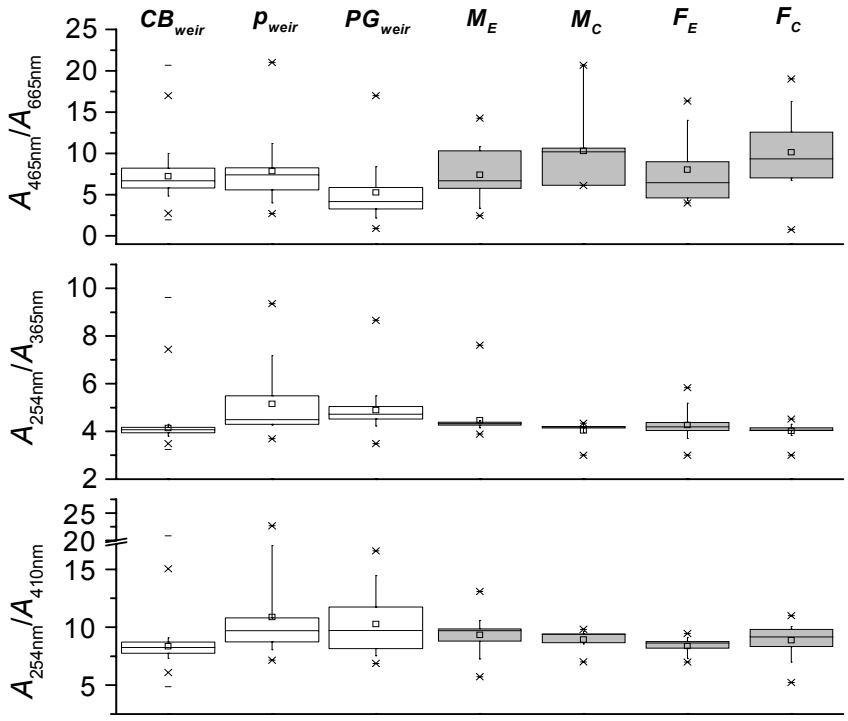


Figure 3.14
Box plots of A_{465nm}/A_{665nm}; A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} in surface water DOM from the Coalburn Experimental Catchment. For key to box plots see Figure 3.2

3.5.5 Summary of the spatial variations in spectrophotometric properties of surface water DOM in the Coalburn Experimental Catchment

The spatial variations in aquatic DOM properties in the Coalburn Experimental Catchment are presented in the previous section. From the examination of this data it can be seen that DOM exhibits a range of spectrophotometric properties in the catchment. The following points summarise these variations.

1. Fluorescence intensity peak wavelengths were constant. Spatial variations were greater than reproducibility only in the comparison of PG_{weir} and F_E (peak $A_{EM\lambda}$) and PG_{weir} and F_C (peak $B_{EM\lambda}$).

2. DOC concentration has a strong positive correlation with absorbance and fluorescence intensity. Absorbance and fluorescence intensity also have a strong positive correlation with each other.

3. The overall catchment pattern of spectrophotometric properties shows DOM of peat type and peaty-gley type with CB_{weir} generally closer to P_{weir}. The variations seen spatially reproduce the DOC concentration gradient and the geochemical definition of Robinson *et al.* (1998), delineating a difference between sub-catchments. The percentage difference in spectrophotometric properties between peaty-gley sub-catchment and peat sub-catchment DOM is summarised in Table 3.10. It can be seen that peaty-gley sub-catchment DOM has a lower DOC concentration and from peak $A_{Fint}/A_{340nm,}$ specific absorbance and absorbance ratios that peaty-gley sub-catchment has lower aromaticity and molecular weight DOM based on the interpretations discussed in Section 2.2.

Spectrophotometric property	Percentage difference
DOC concentration (mgL^{-1})	32.533
A_{340nm}	52.121
Peak A_{Fint}	17.762
Peak B_{Fint}	8.587
SUV_{254nm}	15.192
$Svis_{410nm}$	21.812
Estimated aromaticity	19.591
Peak AS_{Fint}	-29.035
Peak C_{Fint}	-115.549
Peak C_{Fint} / peak A_{Fint}	-152.561
Peak B_{Fint} / peak A_{Fint}	-10.027
Peak A_{Fint} / A_{340nm}	-75.797
A_{254nm}/A_{410nm}	-11.426
A_{254nm}/A_{365nm}	-12.712

Table 3.10
Summary of the percentage difference of spectrophotometric properties between PG_{weir} and peat sub-catchment DOM (including CB_{weir}). A negative value indicates higher means in PG_{weir} and positive higher in peat-sub catchment DOM.

4. The ditches sampled from the four micro catchments in the peat sub-catchment displayed spectrophotometric properties similar to P_{weir} suggesting a relatively homogeneous signal from peat sub-catchment derived DOM. A number of spectrophotometric properties indicate variations within the DOM from the peat sub-catchment. In the comparison between the four ditches a number of significant variations were observed. These are summarised below and expressed as percentage differences.

- **F_E higher than all other peat DOM**

DOC concentration	45.226%
A_{340nm}	51.926%
Peak A_{Fint}	28.854%
Peak B_{Fint}	12.031%

- **M_E higher than M_C**

Peak A_{Fint}, peak B_{Fint}, DOC concentration and A_{340nm}	mean 15.898%

- **F_E higher than F_C**

Peak A_{Fint}, peak B_{Fint}, DOC concentration and A_{340nm}	mean 19.024%

- **M_C and F_C higher than M_E and F_E**

Peak B_{Fint} / peak A_{Fint}	11.207%

- **M_E and F_E higher than M_C and F_C**

Peak AS_{Fint}	21.266%

These differences relate principally to DOC concentration and indicate that water in experimental ditches, which have undergone excavation, has a higher concentration of DOM in comparison to ditches that have been allowed to infill.

3.6 Spatial variations in soil water DOM in the Coalburn Experimental Catchment

The following section presents and discusses the data from soil water sampled from dipwells located on each sub-catchment within the Coalburn Experimental Catchment. The samples represent bulk DOM from the total of the soil depth and are used for a broad comparison to the surface water characteristics and for a comparison between each sub-catchment. Further examination of peat derived DOM is presented in Chapter 8. The properties observed in soil waters sampled from the Coalburn Experimental Catchment are summarised in Table 3.11 and 3.12 relating to the peaty-gley sub-catchment and the peat sub-catchment respectively. Appendix 2 summarises the results of t-test used to statistically compare the means of the spectrophotometric properties of DOM from each soil.

From the data presented in Table 3.12 and Figure 3.2 it can be observed that PG_{weir} and PG_{soil} have the highest pH in the catchment due to buffering by the inorganic component in the soil (99% confidence level). A similar pattern of conductivity to that observed in surface water was seen in soil water, P_{soil} had a lower mean conductivity compared to PG_{soil}, but the difference was not statistically significant (95%

confidence level). Both soil waters had higher conductivity means compared to corresponding surface waters. High conductivity levels of PG_{soil} and PG_{weir} can be attributed to the comparatively high concentrations of solutes and indicates the inorganic nature of the soil in this area of the catchment.

Mean DOC concentration and water colour values from soil waters were statistically indistinguishable from the corresponding surface water means. The means of DOC concentration and water colour in P_{soil} were statistically indistinguishable from all peat sub-catchment derived water and CB_{weir} (95% confidence level).

DOC concentration in surface waters has been related to the organic content of the sub-catchment soils (Newson *et al.,* 2001). The wetter conditions in the peat sub-catchment as discussed in Section 1.7.1 may enhance the release of DOM. Under anaerobic conditions decomposition results in the production of a greater proportion of water soluble metabolites compared to under aerobic conditions (Kalbitz *et al*., 1997). Furthermore, comparatively depleted levels of DOC concentration in the peat-gley sub-catchment may also involve increased sorption of organic matter by soil inorganic components, which can restrict movement of larger molecular weight macromolecular components (Zhou *et al*., 2001; Maurice *et al*., 2002). Adsorption of DOM under aerobic conditions is additionally suggested to be greater than under anaerobic conditions (Kaiser and Zech, 1997). This may enhance such abiotic processes in the peaty-gley sub-catchment soils resulting in preferential retention within the soil matrix compared to the peat sub-catchment.

	Mean	Std. Dev.	Min.	Max.
DOC (mgL^{-1})	18.93	7.16	11.32	32.49
Water Colour (Hazen)	161.58	128.65	18.86	407.56
pH	5.39	0.84	4.24	6.41
Conductivity (μS)	99.23	29.47	65.00	170.00
Peak A_{EX} (nm)	339.50	1.58	335.00	340.00
Peak A_{EM} (nm)	433.65	11.52	420.00	449.50
Peak B_{EX} (nm)	384.50	4.97	380.00	390.00
Peak B_{EM} (nm)	457.40	6.26	447.50	467.50
Peak C_{EX} (nm)	279.00	3.16	275.00	285.00
Peak C_{EM} (nm)	353.55	4.64	349.00	364.00
Peak A_{Fint}	195.68	62.85	96.12	327.24
Peak B_{Fint}	115.42	26.00	74.18	153.63
Peak C_{Fint}	38.73	9.75	25.97	57.98
Peak B_{Fint}/Peak A_{Fint}	0.612	0.109	0.470	0.889
Peak C_{Fint}/Peak A_{Fint}	0.223	0.108	0.079	0.471
Peak AS_{Fint}	10.20	3.14	6.53	15.49
Peak BS_{Fint}	6.34	1.92	4.05	9.71
$A_{340nm}cm^{-1}$	0.2055	0.1158	0.0710	0.3950
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	0.0263	0.0042	0.0207	0.0342
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	0.0036	0.0018	0.0011	0.0065
ε A_{272nm} ($L(moleC)^{-1}cm^{-1}$)	261.32	51.75	163.32	342.46
Peak A_{Fint}/A_{340nm}	1116.11	400.42	537.52	1680.77
A_{465nm}/A_{665nm}	3.68	1.52	2.34	6.50
A_{254nm}/A_{365nm}	4.06	1.23	2.41	6.13
A_{254nm}/A_{410nm}	9.02	5.05	3.79	20.42

Table 3.11
Properties of DOM from PG_{soil} (n=15)

	Mean	Std. Dev.	Min.	Max.
DOC (mgL^{-1})	28.48	4.53	20.58	35.25
Water Colour (Hazen)	328.60	116.66	228.91	599.95
pH	3.56	0.51	2.94	4.46
Conductivity (μS)	109.33	34.38	62.00	155.00
Peak A_{EX} (nm)	340.56	1.67	340.00	345.00
Peak A_{EM} (nm)	444.83	3.69	437.50	449.50
Peak B_{EX} (nm)	380.00	2.50	375.00	385.00
Peak B_{EM} (nm)	459.33	3.00	455.00	465.50
Peak C_{EX} (nm)	279.44	3.91	275.00	285.00
Peak C_{EM} (nm)	352.22	4.15	347.50	360.00
Peak A_{Fint}	325.94	85.77	222.94	474.42
Peak B_{Fint}	200.17	48.86	137.86	276.05
Peak C_{Fint}	15.36	5.72	6.53	23.58
Peak B_{Fint}/Peak A_{Fint}	0.617	0.024	0.576	0.651
Peak C_{Fint}/Peak A_{Fint}	0.050	0.021	0.014	0.079
Peak AS_{Fint}	10.93	1.76	7.34	13.46
Peak BS_{Fint}	6.72	0.97	4.66	7.83
$A_{340nm}cm^{-1}$	0.4940	0.1521	0.3200	0.8530
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	0.0510	0.0084	0.0436	0.0698
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	0.0060	0.0012	0.0048	0.0087
ε A_{272nm} (moleC $L^{-1}cm^{-1}$)	515.84	79.97	444.90	696.17
Peak A_{Fint}/A_{340nm}	681.31	168.12	420.64	1030.99
A_{465nm}/A_{665nm}	5.27	2.22	2.73	8.22
A_{254nm}/A_{365nm}	4.17	0.57	3.03	4.78
A_{254nm}/A_{410nm}	8.47	1.41	7.08	10.52

Table 3.12
Properties of DOM from P_{soil} (n=15)

3.6.1 Spectrophotometric properties of soil DOM in the Coalburn Experimental Catchment

The EEMs obtained from analysis of soil waters resulted in typical distributions of peaks shown in Figure 3.15. Peaks A, B and C were consistently observed. Peak E and fluorescence maxima F were also seen, but not monitored, due to the interferences discussed in Section 2.2. Neither peak D nor any additional peaks were observed.

In the comparison of all excitation and emission wavelengths of fluorescence intensity maxima, with the exception of peak $A_{EM\lambda}$, in soil waters to the corresponding surface waters PG_{soil} and P_{soil} were statistically indistinguishable from PG_{weir} and P_{weir} (95% confidence level). This was also the result in the comparison of PG_{soil} and P_{soil}. Mean peak $A_{EM\lambda}$ was significantly shorter in PG_{soil} when compared to PG_{weir} (8.207nm), P_{weir} (14.403nm), CB_{weir} (14.087nm) and P_{soil} (11.183nm) (99% confidence level). These differences are all greater than the reproducibility of the method (Table 2.2).

Peak $A_{EM\lambda}$ in PG_{soil} had a wide range of values, greater than the analytical reproducibility of the variable, which suggests that this property is sensitive to changes in this source that is not seen in water from other sources. PG_{soil} also exhibits the lowest mean value of this parameter compared to all other surface derived samples. This may be due to interactions with the inorganic component, such as sorption of the peaty-gley soil that do not occur in relation to the DOM in the peat sub-catchment.

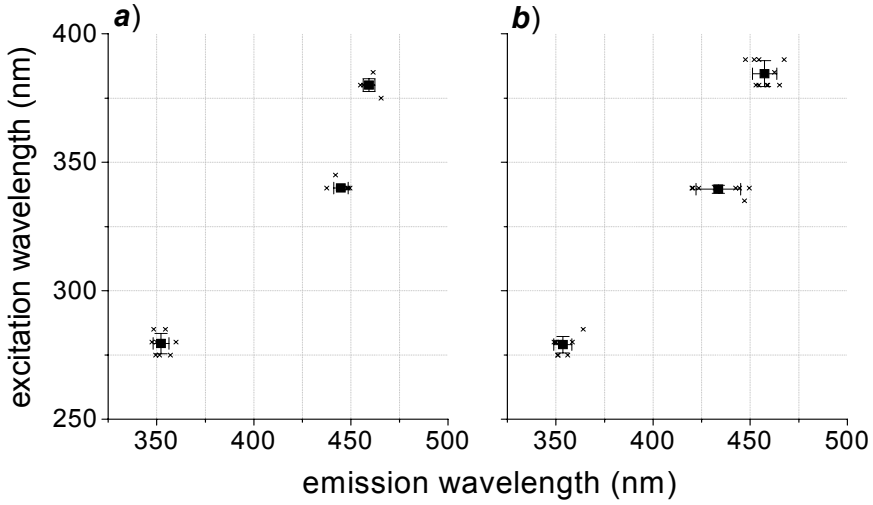


Figure 3.15
The positions, within EEMs, of all the fluorescence intensity maxima, identified in soil water DOM from the Coalburn Experimental Catchment. (x) *all data* (■) *mean* ***a)*** P_{soil} ***b)*** PG_{soil}

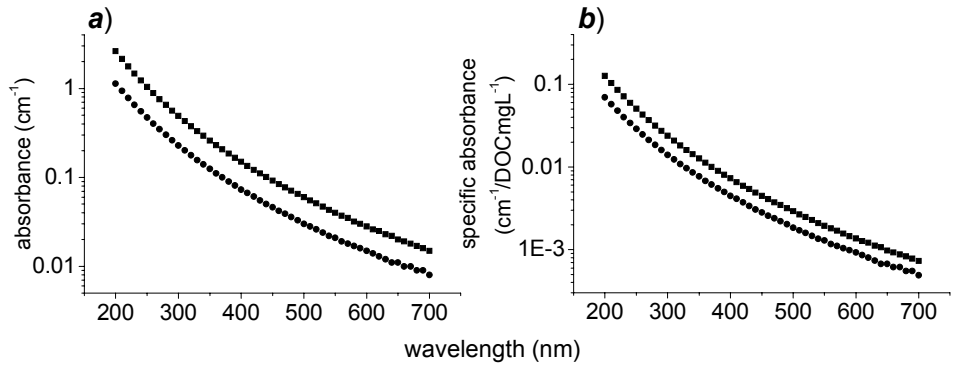


Figure 3.16
Typical absorbance spectra in soil water DOM from the Coalburn Experimental Catchment. ***a)*** *absorbance* (cm^{-1}) ***b)*** *specific absorbance* ($cm^{-1}/DOCmgL^{-1}$) (■) P_{soil} (●) PG_{soil} *(sampled on 20/02/01).*

In the comparison of mean fluorescence intensities both peak A_{Fint} and peak B_{Fint} were significantly higher in P_{soil} compared to PG_{soil} (99% confidence level). This was also observed in mean absorbance measured at all wavelengths and replicates the patterns seen in surface waters and the strong positive correlation of both variables with each other and with DOC concentration. In relation to all surface waters, including PG_{weir}, PG_{soil} exhibited significantly lower mean peak A_{Fint} and peak B_{Fint} and higher peak C_{Fint} and peak C_{Fint}/peak A_{Fint} (95% confidence level). P_{soil} was statistically indistinguishable from all peat sub-catchment surface waters in mean fluorescence intensities and peak C_{Fint}/peak A_{Fint} however peak B_{Fint}/peak A_{Fint} was significantly lower in the four monitored ditches (95% confidence level).

Soil derived DOM exhibited typical featureless absorbance spectra observed throughout surface water analysis, as shown on Figure 3.16, and at all measured wavelengths soil water mean absorbance was statistically indistinguishable from the corresponding surface waters (95% confidence level). Mean P_{soil} absorbance was also indistinguishable from all peat sub-catchment and CB_{weir} waters (95% confidence level). PG_{soil} exhibited a wider range of absorbance values compared to PG_{weir} having both higher and lower values.

The positive relationship of fluorescence intensity and absorbance to DOC concentration in soil water DOM replicated that seen in surface waters. In contrast to surface waters, however, both peak A_{Fint} and peak B_{Fint} were not significantly correlated with DOC concentration. In both soil DOM data sets absorbance was strongly positively correlated with DOC concentration (99% confidence level). Both peak A_{Fint} and peak B_{Fint} increased with increasing absorbance but this relationship was only significant in PG_{soil} at absorbance $<A_{340nm}$ (95% confidence level) and not significant at any wavelengths in P_{soil}. At all measured wavelengths >90% of the variation in absorbance was explained by DOC concentration in PG_{soil}, this was >70% for P_{soil}. In comparison to surface waters these relationships indicate a stronger relationship of absorbance to DOC in soil derived DOM. No relationship was observed between DOC concentration and peak C_{Fint} or fluorescence peak wavelengths.

Mean SUV_{254nm}, $Svis_{410nm}$ and estimated aromaticity were significantly lower in PG_{soil} compared to all peat sub-catchment derived DOM and compared to PG_{weir} in SUV_{254nm} and estimated aromaticity (95% confidence level). P_{soil} was significantly

higher compared to PG_{soil} and indistinguishable from peat sub-catchment waters and CB_{weir} in these variables (95% confidence level).

Peak AS_{Fint} was indistinguishable between the soil DOM samples (95% confidence level), however PG_{soil} exhibited a greater range of values. Mean peak AS_{Fint} was significantly higher in PG_{weir} compared to PG_{soil} and statistically indistinguishable between all peat sub-catchment derived DOM and P_{soil} (95% confidence level).

Peak A_{Fint}/A_{340nm} in PG_{soil} was significantly higher, compared to P_{soil} and all other peat sub-catchment derived DOM (99% confidence level). Both soil waters were statistically indistinguishable from the corresponding surface waters. PG_{soil} and P_{soil} were not significantly different in any of the measured absorbance ratios. Similarly, the soil DOM means were not statistically distinguishable from the corresponding surface samples in these variables. P_{soil} was not significantly different to P_{weir} or PG_{weir}. In comparison to all peat sub-catchment derived surface DOM, PG_{soil} had lower mean A_{465nm}/A_{665nm} (95% confidence level).

3.6.2 Summary of soil DOM in the Coalburn Experimental Catchment

Soil derived DOM exhibited spectrophotometric properties of similar character to surface water. P_{soil} DOM exhibited statistically indistinguishable spectrophotometric properties from P_{weir}, more differences were observed between PG_{soil} and PG_{weir}. These differences are summarised in Table 3.13. The differences between P_{soil} and PG_{soil} correspond to those seen in surface waters in each sub-catchment and indicate a link between the two pools of DOM. A further examination of the link between surface water and soil derived DOM is presented in Chapter 8.

In comparison to peat sub-catchment waters, peaty-gley DOM manifests a character, which can be interpreted as having a lower molecular weight and aromaticity (Section 2.2). The preferential retention by inorganic material in PG_{soil} of DOM with higher molecular weight and/or higher aromatic content can be observed in the spectrophotometric properties. The variations between PG_{soil} and PG_{weir} suggest that there are inputs of DOM into PG_{weir} from sources other than soil water, or than soil water DOM is altered on transport to the ditch; this is discussed further in Section 3.9.

	P_{soil} compared to PG_{soil}	PG_{soil} compared to PG_{weir}
DOC concentration	33.533	-0.263
Peak $A_{EM\lambda}$	8.207	-11.180
Peak A_{Fint}	39.964	-24.862
Peak B_{Fint}	42.340	-34.253
A_{340nm}	58.405	-13.104
Peak C_{Fint}	-152.192	26.893
Peak C_{Fint}/peak A_{Fint}	-343.879	43.973
Estimated aromaticity	49.341	-49.530
SUV_{254nm}	48.506	-54.391
$Svis_{410nm}$	39.630	-19.463
Peak AS_{Fint}	*ns*	-29.745
Peak A_{Fint}/A_{340nm}	-63.818	*ns*

Table 3.13
Summary of the percentage differences in spectrophotometric properties of DOM between P_{soil} and PG_{soil} (positive values are higher in the former) and between PG_{soil} and PG_{weir} (positive values are higher in the former).

3.7 DOM in rainwater in the Coalburn Experimental Catchment

The following section describes the properties of DOM in rainwater in comparison to surface and soil DOM and the overall rainwater spectrophotometric signal is identified and discussed. There have been no previously published details of the spectrophotometric properties of DOM in precipitation. This is due to the relatively low levels of DOC concentration in rainwater, typically 1 to 10mgL^{-1}.

Sampling of rainwater was performed as described in Section 3.4.3.3 and analysed as detailed in Section 2.2 in tandem with the surface water sampling program. Within this study bulk deposition was analysed, which includes rainwater, cloud mist deposition and snow fall. Cloud mist is estimated to contribute an input of ~50-90mm yr^{-1} (Robinson *et al*., 1998) in comparison to 1350mm yr^{-1} rainwater and as no samples were taken during snowfall the bulk of the sample was rainwater and is referred to as such.

	Mean	Std. Dev.	Min.	Max.
DOC (mgL^{-1})	1.94	0.66	1.80	3.41
Water Colour (Hazen)	*n/a*	*n/a*	*n/a*	*n/a*
pH	5.49	0.54	4.82	6.47
Conductivity (μS)	31.47	16.31	13.00	67.00
Peak A_{EX} (nm)	333.42	7.4634	320.00	340.00
Peak A_{EM} (nm)	410.02	5.73	402.50	421.00
Peak B_{EX} (nm)	*n/a*	*n/a*	*n/a*	*n/a*
Peak B_{EM} (nm)	*n/a*	*n/a*	*n/a*	*n/a*
Peak C_{EX} (nm)	277.81	4.46	270.00	290.00
Peak C_{EM} (nm)	346.53	9.72	332.50	369.50
Peak A_{Fint}	23.10	15.54	7.27	59.66
Peak B_{Fint}	*n/a*	*n/a*	*n/a*	*n/a*
Peak C_{Fint}	24.52	8.82	11.39	37.59
Peak B_{Fint}/Peak A_{Fint}	*n/a*	*n/a*	*n/a*	*n/a*
Peak C_{Fint}/Peak A_{Fint}	0.966	0.500	0.407	2.093
Peak AS_{Fint}	11.99	7.52	3.15	24.39
Peak BS_{Fint}	*n/a*	*n/a*	*n/a*	*n/a*
A_{340nm} **cm^{-1}**	0.0164	0.0163	0.010	0.070
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	0.0226	0.0106	0.0047	0.0438
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	*n/a*	*n/a*	*n/a*	*n/a*
ε A_{272nm} (moleC $L^{-1}cm^{-1}$)	212.47	112.55	41.02	433.84
Peak A_{Fint}/A_{340nm}	1930.58	340.96	1121.54	2535.00
A_{465nm}/A_{665nm}	*n/a*	*n/a*	*n/a*	*n/a*
A_{254nm}/A_{365nm}	*n/a*	*n/a*	*n/a*	*n/a*
A_{254nm}/A_{410nm}	*n/a*	*n/a*	*n/a*	*n/a*

Table 3.14
The properties of precipitation in the Coalburn Experimental Catchment.
Peak B_{Fint} and absorbance > A_{340nm} were below detection levels in rainwater (n=19)

3.7.1 General properties of rainwater

The general properties of rainwater previously identified in the Coalburn Experimental Catchment are summarised in comparison to surface water data, in Table 1.5. From assessment of the chemical composition of wet deposition it was described as slightly acidic with a chemical composition of moderate pollution (Mounsey, 1999). Mounsey (1999) further explored the characterisation of wet deposition and two distinct chemical composition signatures were recognised. Firstly, a marine signature derived from southerly and westerly winds and secondly a terrestrial signature from easterly winds. The former had typically lower pH, higher DOC concentration and water colour compared to the latter. The DOC concentration and water colour in rainwater was recognised to correlate negatively with rain volume, indicating a dilution relationship and 13-22% of the DOC exported from the Coalburn Experimental Catchment was estimated to derive from precipitation sources. The derivation of the bulk of the DOC in precipitation was, however, determined to be from rainfall, due to the comparatively lower volumes of cloud mist. The latter precipitation source was found to be enriched in both DOC concentration and water colour.

The properties of the rainwater examined in this study are detailed in Table 3.14. The mean value of pH compares closely to that previously observed. Mean, minimum and maximum conductivity values however, were slightly lower in this study over the same range, this may relate to analytical differences. Similarly, DOC concentration was lower in the current data compared to that presented in Table 1.5 and that described by Mounsey (1999). Principally, the maximum values previously seen are higher than those observed in this study. This may also be due to differing analytical conditions, thus the data cannot be compared between studies. The difference is exemplified by water colour, which was not detected in the rainwater analysed in the current study, as absorbance at wavelengths longer than $A_{340m,}$ was not detectable.

The mean value of DOC concentration in rainwater in this study is significantly lower compared to all data from surface and soil derived water (99% confidence level). DOC concentration compares well to values observed in other studies, for example, 1.2mgL^{-1} (Soulsby, 1995), 55.1μM carbon L^{-1} (6.61 mgL^{-1}) (Neal *et al*., 2001) and 2.8mgL^{-1} (Fraser *et al*., 2001) where contribution from precipitation is thought to be negligible to the overall DOC export from the catchments studied.

The variation in DOC concentration in rainwater exhibited no seasonal or volumetric relationships, indicating a relatively stable annual input of DOM. This may be due to the composite nature of the sampling, which may have effectively smoothed any seasonal variations.

3.7.2 Spectrophotometric properties of DOM in rainwater

Analyses of rainwater produced distinctive and consistent EEMs. The positions of maximum fluorescence intensity identified are shown on Figure 3.17. Rainwater analyses revealed the presence of peaks in the wavelength ranges ascribed to both peak A and peak C fluorescence in surface water, however, peak B was not present. Fluorescence intensity measured at typical peak B wavelengths (EXλ= 370nm EMλ= 460nm) was at background levels. No additional peaks to those identified in the analyses of DOM from the catchment, such as peak D, were identified in rainwater. Fluorescence maxima were observed in the areas related to peak E and F. Peak Aexλ and peak Aemλ were both significantly shorter in rainwater compared to mean surface water DOM. These differences were greater than the reproducibility of the method; peak $A_{EX\lambda}$ 6.633nm and peak $A_{EM\lambda}$ 37.427nm.

In rainwater EEMs peak $C_{EM\lambda}$ exhibited a wide range of values (332.5nm to 369.5nm), compared to surface water sources. Longer peak $C_{EM\lambda}$ may be due to low peak A fluorescence intensity and a reduction of the spectral overlap of the two peaks that may reduce peak C fluorescence intensity at longer emission wavelengths.

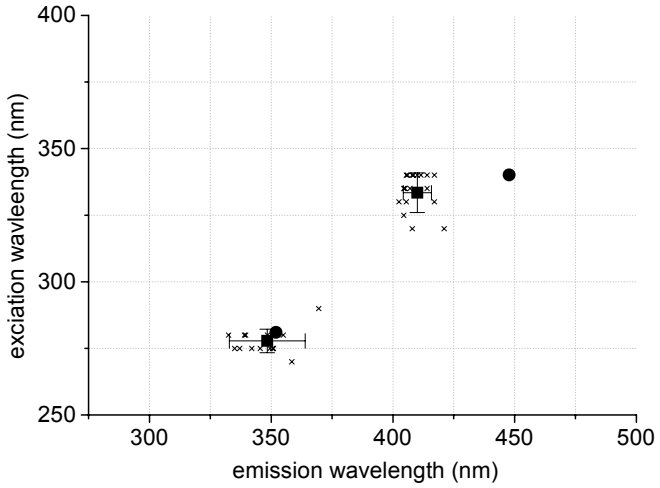


Figure 3.17
The positions, within EEM, of all the fluorescence intensity maxima, identified in rainwater from the Coalburn Experimental Catchment (x) *all data* (■) *mean* (●) *mean* CB_{weir} *wavelengths*

The broad and featureless typical absorbance spectra observed in DOM, of decreasing absorbance with increasing wavelength in the UV–visible range, were exhibited by rainwater. Absorbance values and specific absorptivity were significantly lower at all measured wavelengths compared to surface and soil waters. At measured wavelengths longer than A_{340nm} no absorbance was detected. This type of spectrum, with very low visible wavelength absorbance has been related by Chen *et al*. (2002) to a greater influence and/or abundance of ketonic C=O functional groups compared to aromatic C=C functional groups (Chen *et al*., 2002). In comparison to surface and soil water DOM this suggests that rainwater DOM is less aromatic. This is further indicated by the significantly low mean estimated aromaticity and blue shifted emission wavelengths in rainwater analyses compared to all surface waters.

Rainwater exhibited a significantly lower (10 to 20 times) mean level of peak A_{Fint} and absorbance compared to surface and soil waters (99% confidence level), replicating the low DOC concentration levels seen. DOM from rainwater exhibited a significantly higher mean peak C_{Fint} value compared to all surface waters except PG_{weir} (95% confidence level). Mean peak AS_{Fint} was statistically indistinguishable in rainwater compared to surface water of all sources in the catchment (95% confidence level). This suggests that the fluorescence efficiency of the DOM may be similar, however the contrasting fluorescence intensity peak wavelengths indicated that the overall composition was different, mirroring that observed in absorbance data. No

relationship was observed between fluorescence intensity and absorbance at all wavelengths. This resulted in a wide range of peak A_{Fint}/A_{340nm} values the mean of which was significantly higher than surface water (99% confidence level). This suggests that peak A fluorescence is derived from lower molecular weight DOM compared to surface waters (Wu and Tanoue, 2001). This corresponds to the blue shift of peak A wavelengths and absence of peak B fluorescence. A shift has been related to the presence of simple molecules of low molecular weight and aromaticity (Senesi *et al*., 1991).

Rainwater DOM exhibited a wide range of fluorescence intensity and absorbance variables in comparison to the ranges seen in surface water DOM indicating a more variable DOM composition or source. The controls on these variations and the source of DOM are unclear. As the potential for contamination was monitored and minimised it is thought that the DOM analysed is from natural processes and sources within the precipitation cycle. No relationships between spectrophotometric properties and the volume of rainfall or the seasonal sampling conditions were observed.

To summarise the spectrophotometric properties of rainwater, in comparison to surface and soil water, it exhibits a range of different properties. Firstly, DOC concentration is low, as is absorbance and fluorescence intensity, however neither correlates with DOC concentration. This low fluorescence intensity and absorbance levels is also manifested in no detectable signal of absorbance at long wavelengths and the absence of peak B fluorescence. Mean values of both peak C_{Fint} and peak A_{Fint}/A_{340nm} were high in rainwater DOM whereas specific absorbance and estimated aromaticity was low. Due to the non-specific nature of the analytical techniques employed in this study the composition of DOM in rainwater cannot be determined. Overall, the data shows that the fluorophores responsible for peak A are different to surface water DOM, having a lower aromatic content or molecular weight.

3.8 DOM in throughfall/stemflow in the Coalburn Experimental Catchment

The following section will describe the characteristics observed in throughfall (a composite of stemflow and throughfall) sampled from beneath Sitka Spruce on the peat sub-catchment of the Coalburn Experimental Catchment. The monitoring and analysis performed on surface samples was replicated in samples taken from the

runoff of interception sheets beneath closed canopy forest. To expand this DOM monitoring investigation into DOM related to spruce needles of varying decomposition was performed.

The model of flow paths in the catchment discussed in Section 1.7 includes the amendment of rainwater character by interaction with the forest and further alteration within soils. As a source of DOM to surface environments precipitation is not considered as important as soils due to the low DOC concentrations. On interaction with vegetation, however, the DOC concentration may rise significantly. The DOM derived from such canopy interactions is discussed below and compared to the rainwater and surface water DOM signal identified in the catchment. The spectrophotometric signal of throughfall DOM properties is identified to establish if there is a significant input to surface water and influence on the quality of water exported from the catchment, from this source.

As discussed in Section 1.7.1 throughfall is enriched in most solutes in comparison to rainwater (Table 1.5) due to the flushing of accumulated material in the canopy. In a comprehensive study of the inorganic composition of throughfall in the Coalburn Experimental Catchment Hind (1992) observed no spatial variations and no overall correlation of throughfall volume to ion concentrations. The concentration of ions in throughfall/stemflow was related to the presence of solutes in the canopy, on needles and branches, existing as stable water droplets or deposited by evaporation. Light rainfall and cloud mist are thought to be important as sources of solute deposition in the canopy. In the Coalburn Experimental Catchment cloud mist deposition never exceeds forest storage capacity, thus solutes deposited in the canopy from this process remain until flushing by the next rainfall event (Robinson *et al*., 1998). Although cloud mist accounts for only 5% of the annual precipitation volume it is enriched in all solutes, in comparison to rainwater including DOC concentration, indicating that cloud mist deposition within the canopy may be a significant source of DOM in throughfall. Occult deposition has been recognised to further contribute to solute deposition within the canopy (Soulsby, 1995). In the Coalburn Experimental Catchment occult deposition in lower branches has been observed to occur during periods of low rainfall and high soil water evaporation rates (Hind, 1992). Due to the configuration of the sampler potential inputs from occult deposition were minimised.

As previous investigation of throughfall composition revealed little spatial variation in chemical composition the characteristics observed in this study are assumed to be

applicable throughout the forested area of the Coalburn Experimental Catchment. The amount of interception loss and therefore the volume of throughfall and stemflow within the catchment have been observed to vary. For example, there is greater interception loss from taller trees compared to shorter and the amount of interception loss is expected to increase as the trees age (Robinson *et al.,* 1998). The processes involved in throughfall generation and composition have been related to tree species, age and spacing and to antecedent climatic conditions and rainfall intensity (Soulsby *et al*., 2002).

The following section details the properties of throughfall in the Coalburn Experimental Catchment and compares it to both rainwater and to soil and surface waters DOM properties within the catchment. Throughfall showed no variations related to changes in the amount of preceding rainfall, the moisture deficits calculated for the catchment or to any seasonal variations. This suggests that the DOM generated within the catchment was relatively constant in relation to the wide temporal variations seen in surface water DOM properties (Chapter 4).

3.8.1 DOM properties of throughfall

The distribution of pH, conductivity, DOC concentration and water colour data from the analysis of throughfall is presented in Table 3.15. The mean values of both pH and conductivity compare closely to those previously observed, at the same location, by Robinson *et al*. (1998) (Table 1.5).

In comparison to rainwater throughfall exhibits statistically indistinguishable mean pH and significantly higher conductivity (95% confidence level). It has been recognised that throughfall becomes enriched in most solutes, during precipitation passage through the canopy (Soulsby, 1995) resulting in enhanced conductivity. In this study the enrichment is also apparent in DOC concentration (99% confidence level). Throughfall additionally exhibited colouration in comparison to the uncoloured rainwater. Throughfall exhibited, in comparison to surface and soil water from the peat sub-catchment, a significantly higher mean pH. It was statistically indistinguishable from CB_{weir}, PG_{weir} and PG_{soil} mean values (95% confidence level). Mean conductivity was similar to that seen in all surface and soil waters.

	Mean	Std. Dev.	Min.	Max.
DOC (mgL^{-1})	12.34	3.65	8.81	19.22
Water Colour (Hazen)	104.80	38.39	60.08	171.98
pH	5.04	0.60	4.28	5.92
Conductivity (μS)	75.39	35.20	20.00	120.50
Peak A_{EX} (nm)	338.33	3.54	330.00	340.00
Peak A_{EM} (nm)	441.22	3.99	435.50	447.50
Peak B_{EX} (nm)	383.33	6.12	370.00	390.00
Peak B_{EM} (nm)	464.61	4.03	459.50	472.50
Peak C_{EX} (nm)	278.33	4.33	270.00	285.00
Peak C_{EM} (nm)	350.89	2.26	348.00	354.50
Peak A_{Fint}	213.08	67.22	103.29	302.47
Peak B_{Fint}	118.66	43.02	49.04	168.91
Peak C_{Fint}	39.97	12.55	29.65	66.81
Peak B_{Fint}/Peak A_{Fint}	0.548	0.049	0.475	0.606
Peak C_{Fint}/Peak A_{Fint}	0.225	0.167	0.101	0.647
Peak AS_{Fint}	17.12	3.18	10.94	20.30
Peak BS_{Fint}	9.35	2.24	5.20	12.12
A_{340nm} (cm^{-1})	0.2064	0.0897	0.0960	0.3810
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	0.0594	0.0168	0.0308	0.0826
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	0.0044	0.0005	0.0037	0.0052
ε A_{272nm} ($moleC\ L^{-1}cm^{-1}$)	602.32	160.43	317.80	794.76
Peak A_{Fint}/A_{340nm}	1076.35	142.22	793.89	1308.32
A_{465nm}/A_{665nm}	3.94	1.97	1.87	7.60
A_{254nm}/A_{365nm}	5.93	0.90	4.34	7.56
A_{254nm}/A_{410nm}	13.16	3.32	7.28	17.64

Table 3.15
Properties of throughfall DOM

Mean DOC concentration was significantly higher in all surface and soil waters in comparison to throughfall, indicating the importance of litter and soil processes as a source of DOM in the catchment. The maximum DOC concentration values observed in throughfall equate to the minimum seen in both PG_{weir} and PG_{soil}. Water colour was similarly higher in peat sub-catchment derived water including CB_{weir}, compared to throughfall; however, the mean values observed in peaty-gley derived waters were statistically indistinguishable from throughfall mean value (95% confidence level). These points suggest that the input from throughfall to the runoff from this sub-catchment may contribute significantly to water colour and enhance the DOC concentration. This may be important during winter periods, when DOM production is low within soil and litter.

In throughfall both fluorescence intensity and absorbance exhibited positive relationships with DOC concentration. Using linear regression 75.7% of the variation in peak A_{Fint} and 66.4% of peak B_{Fint} was explained by DOC concentration. In comparison to fluorescence intensity absorbance had a stronger relationship to DOC concentration, as was observed in surface waters. 90.8%, 92.6% and 96.4% of the variations in A_{254nm}, A_{340nm} and A_{410nm} respectively were explained by DOC concentration. The distribution of DOC concentration in throughfall is replicated by peak A_{Fint}, peak B_{Fint} and absorbance, which were significantly lower than peat sub-catchment derived DOM, as shown in Table 3.16 and higher than rainwater DOM.

Analyses of throughfall revealed EEMs similar to the typical pattern observed in surface and soil water throughout the catchment. Peaks A, B and C were identified throughout, the mean positions, of which are shown on Figure 3.18. The mean positions of a number of surface water fluorescence intensity peaks are included as a comparison. Peak E and area F were consistently observed, however not monitored. No other fluoresce intensity peaks, including peak D, were noted in the EEMs.

In comparison to rainwater analyses mean Peak $A_{EM\lambda}$ was significantly longer (31.196nm) in throughfall (99% confidence level) $A_{EX\lambda}$ and peak C wavelengths were also longer in throughfall, however not significantly so. Peak B was observed throughout throughfall data in contrast to rainwater in which no longer wavelength fluorescence was observed. Both mean peak A_{Fint} and peak C_{Fint} were significantly higher in throughfall compared to rainwater; however, peak C_{Fint}/peak A_{Fint} was significantly higher in rainwater (99% confidence level). This indicates that the DOM

signal and spectrophotometric properties of precipitation is significantly modified by canopy interactions.

EEMs from the analysis of throughfall have the same overall appearance, as surface and soil water. In comparison to peat sub-catchment DOM differences in mean wavelengths did not exceed the method reproducibility. A difference greater than the reproducibility was, however, observed in the comparison of throughfall to PG_{weir} DOM. peak $B_{EM\lambda}$ was 9.040nm longer in throughfall.

	Peat sub-catchment	Peaty-gley sub-catchment
Peak A_{Fint} peak B_{Fint}	-29.326	ns
Peak C_{Fint}	203.325	*ns*
Peak AS_{Fint}	66.747	29.441
Peak A_{Fint}/A_{340nm}	73.442	ns
A_{254nm}/A_{365nm} A_{254nm}/A_{410nm}	46.082	35.356
SUV_{254nm} $Svis_{410nm}$	*ns*	24.001

Table 3.16
Summary of the percentage differences between the mean spectrophotometric properties of throughfall DOM and surface water DOM; negative values indicate higher in the latter ns= not significant.

Throughfall had a significantly higher mean peak AS_{Fint} compared to all other sampling sites (99% confidence level) with the exception of rainwater. Throughfall exhibited a higher mean peak AS_{Fint} compared to rainwater but not significantly so due to the wide range of values in rainwater.

Absorbance and specific absorbance spectra, shown in Figure 3.19, in throughfall exhibited the typical decrease with increasing wavelengths seen in DOM. The distributions of absorbance ratios in throughfall are shown in Table 3.16. Throughfall had mean values for both A_{254nm}/A_{365nm} and $A_{254nm}/A_{410nm,}$ which were significantly higher than all other sources. High values of absorbance ratios are related to comparatively lower aromaticity and/or molecular weight (Peuravuori and Pihlaja, 1997, Chen *et al*., 2002). This does not correspond to the SUV_{254nm} and estimated aromaticity seen in throughfall DOM, which were indistinguishable to peat sub-catchment waters and significantly higher compared to CB_{weir} and peaty-gley sub-catchment waters. This suggests a similar or higher aromaticity, in relation to the total carbon (Abbt-Braun and Frimmel, 1999).

The overall spectrophotometric properties of throughfall DOM may be due to influences on that are not significant in surface and soil waters. For example, high values of A_{254nm}/A_{365nm} have been related to lower molecular size (Peuravuori and Pihlaja, 1997) and a relative abundance of carbohydrate components (Chen *et al*., 2002). It has been identified, in different tree species, that 50% of the DOM in throughfall is composed of carbohydrates (Guggenberger and Zech, 1994). Specific absorbance however suggests relatively more aromatic DOM.

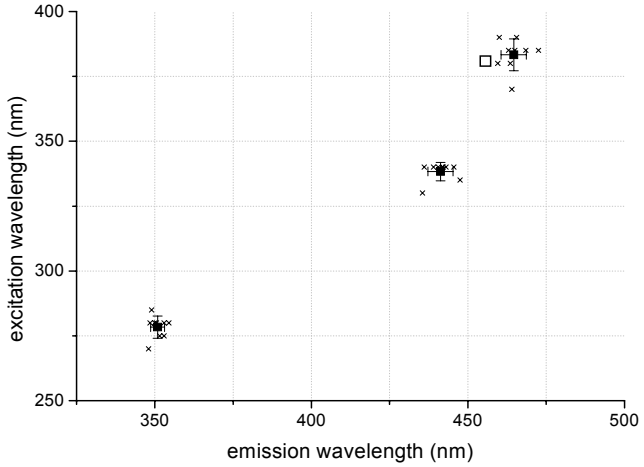


Figure 3.18
The positions, within EEMs of the fluorescence intensity maxima identified in throughfall from the Coalburn Experimental Catchment (x) *all data* (■) *mean values* (□) *mean peak B in* PG_{weir}

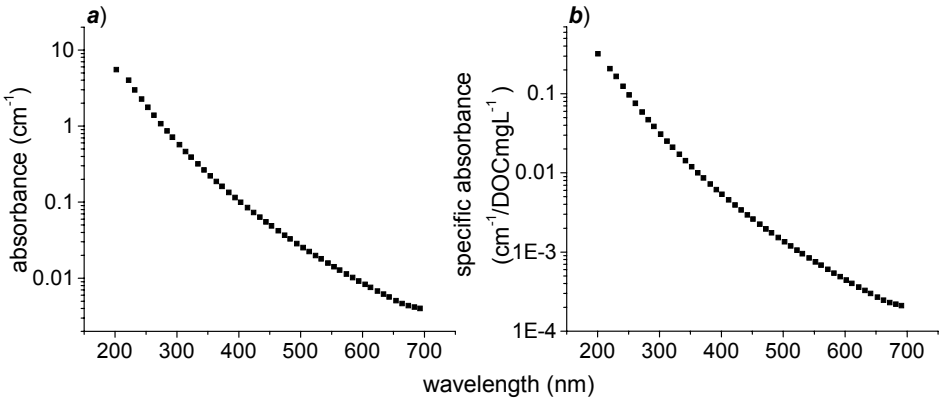


Figure 3.19
Typical absorbance spectra in throughfall from the Coalburn Experimental Catchment. ***a)*** *absorbance* (cm^{-1}) ***b)*** *specific absorbance* ($cm^{-1}/DOCmgL^{-1}$) *(sampled on 16/01/02).*

3.8.4 Spruce needle DOM

The identified differences between throughfall and surface water DOM may be indicative of the signal of fresh DOM leached from deposits within the canopy and from branch, needle and stem interactions. The similarities of EEMs to those commonly observed in "humic-like" DOM (Figure 1.3) suggests that there are similar fluorophores present and that DOM from various sources has a homogeneous spectrophotometric signal. The following section details an investigation into the spectrophotometric properties of DOM associated with spruce needles and relates this to the signal seen in DOM throughout the catchment and specifically to throughfall. To examine the spectrophotometric properties of the DOM potentially derived during interactions with forest canopy and with spruce needles of a varying degree of degradation a number of simple extractions were performed.

Sampling was performed on 16/01/02 at site 1, shown on Figure 3.1, adjacent to the throughfall sampling site. Triplicate samples of fresh needles, attached to the stalk were sampled, from a branch at approximately 2 m height. Partially degraded needles (50% green) and needles of greater degradation (100% senescent brown) were sampled in triplicate, from the surface of the litter. The surfaces of the fresh needle samples were washed with non-fluorescent distilled water; 1g of needles of varying degradation were shaken in 50 ml distilled water and the resulting solution filtered, as described in Section 2.5. Experimental conditions, as discussed in relation to peat DOM extraction were maintained at constant temperature and pH of solution. The resulting solutions were analysed as discussed in Section 2.2.

DOM obtained from partially degraded needles and washes from fresh needles were found to have similar spectrophotometric properties, both differing in the same manner and to the same extent from the more degraded needles. This was manifested as distinct EEMs and absorbance spectra, which differed from all other DOM analysed in this study.

Fresh and partially degraded needles exhibited EEMs containing the following fluorescence intensity maxima and peaks that are summarised Figure 3.20:

- A highly fluorescent peak EXλ=250$\pm$5nm EMλ=309$\pm$3nm, termed peak **X**.
- A secondary diffuse peak EXλ=~200nm EMλ=309$\pm$7nm. This peak appeared to be related to peak X, as both peaks exhibited the same emission wavelengths on

replicate analysis and dilution of the solution. These peaks appeared close to the wavelength ranges where peak C and peak D are commonly observed and similarly, two intensity peaks were observed, as is recognised in the analysis of pure tyrosine and tryptophan (Table 1.4). An identification of a modified peak C or peak D was not made as peak X exhibited significantly shifted wavelengths and a different shape to amino acid maxima seen in DOM analyses. Peak X exhibited the shape and configuration typical of those observed in EEMs from the analysis of solutions with single compounds present.

- A diffuse peak of lower fluorescent intensity compared to peak X and multiple maxima, at wavelengths that approximated to peak A EXλ=310$\pm$5nm EMλ=417.5nm $\pm$4.7nm, termed peak **A′**. In comparison to peak A in surface waters peak A′ was significantly blue shifted in both excitation and emission wavelengths. In comparison to mean peak A seen in rainwater (Figure 3.17) peak A′ excitation wavelength was blue shifted, however, emission wavelength was significantly longer (99% confidence level).

The positions of the peaks observed are shown on Figure 3.20, the mean positions of throughfall intensity peaks are included for comparison. Figure 3.20 is a composite image of EEMs at different concentrations as peak X was not observed with peak A′ at the same concentrations. In solutions at relatively high absorbance; A_{254nm} >~0.05nm, the fluorescence intensity of peak X was above the level of detection. Peak A′ however was present in the EEMs of solutions at these concentrations. On dilution to a concentration resulting in the resolution peak X fluorescence peak A′ was not present, and fluorescence intensity at such wavelengths was at background levels.

Degraded litter DOM solutions exhibited EEMs closer in appearance to riverine DOM analyses with similar wavelength of fluorescence intensity maxima, as shown on Figure 3.3. The highly fluorescent peak X was not observed in the degraded litter analyses. Figure 3.20 shows peak $A_{EM\lambda}$ and peak $B_{EM\lambda}$ significantly shorter than all surface and soil waters and throughfall, however peak C and E wavelengths were not significantly different to surface and soil waters. In comparison to rainwater DOM from degraded litter exhibited significantly longer mean peak $A_{EM\lambda}$ (99% confidence level).

Absorbance spectra of DOM from both partially degraded needles and fresh needle washes did not show the typical decrease in absorbance with increasing wavelength of DOM, but exhibited a shoulder at A_{250nm} ±1.5nm, as shown in Figure 3.25. This shoulder was observed at all strengths of solution and exhibited a linear relationship with concentration. Absorbance at >A_{400nm} approached zero. Degraded litter DOM did not show this shoulder and exhibited the typical DOM absorbance spectrum. Absorbance at such wavelengths has been related to the presence of aromatic material, such as phenol (Senesi *et al.*, 1999).

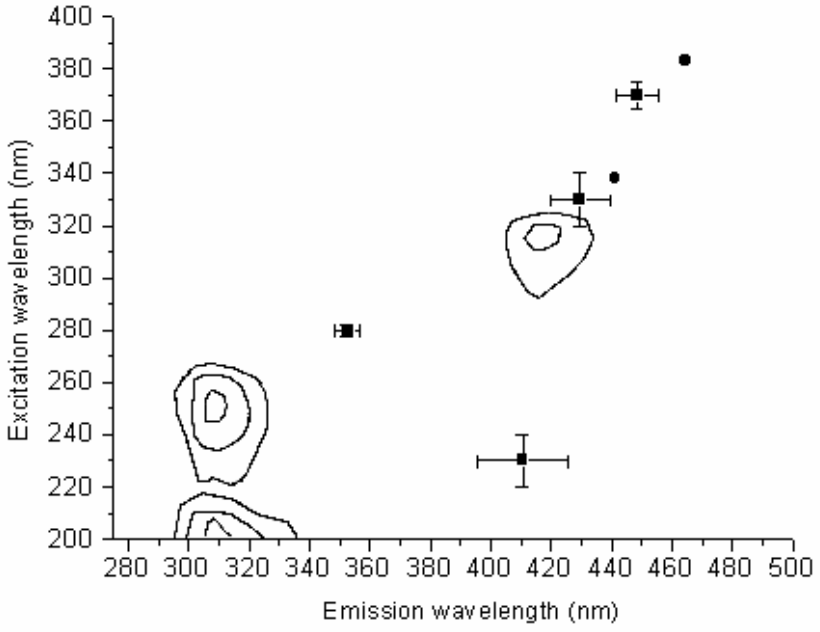


Figure 3.20
Composite EEM showing the relative positions of fluorescence intensity maxima from fresh and partially degraded spruce needle related DOM; contours indicate areas of equal fluorescence intensity. (■) *mean positions of fluorescence intensity peaks in degraded litter DOM* (●) *peak A and peak B in throughfall*

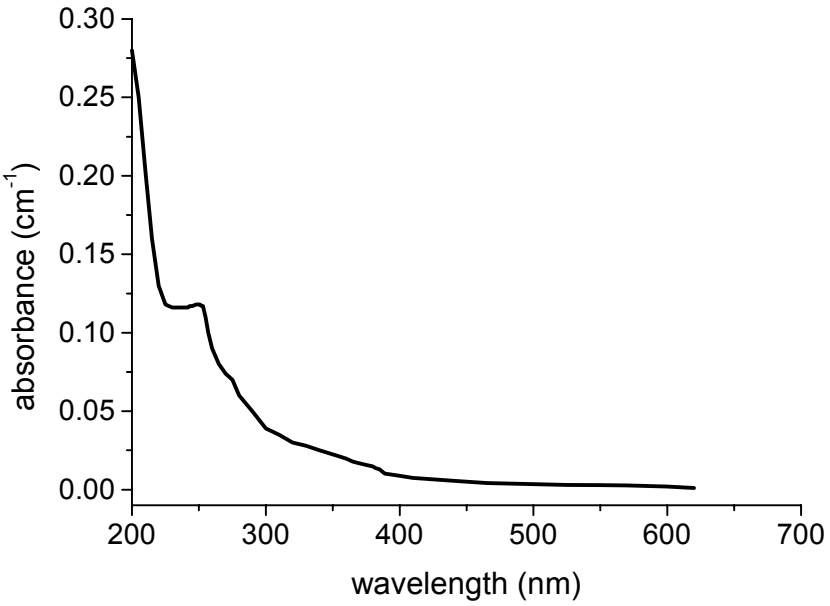


Figure 3.21
Absorbance spectrum of fresh and partially degraded spruce needle related DOM

Fluorescence intensity and absorbance ratios did not vary in any of the needle DOM solutions, with changing dilution of the analytical solution, suggesting that the variations observed were compositional rather than a concentration effect. Fluorescence ratios were measured for the degraded needle DOM solutions and mean peak B_{Fint}/peak A_{Fint} (0.615 s.d. 0.041) was not significantly different to surface and soil water but was significantly higher than throughfall (95% confidence level). Mean peak C_{Fint}/peak A_{Fint} (0.455 s.d. 0.024) was significantly higher than throughfall and all other surface waters and lower than rainwater (95% confidence level).

The ratio of fluorescence intensity to absorbance, at peak excitation wavelength, in fresh and partially degraded needle DOM indicated that peak X (1738.80 s.d 904.73) compared closely to peak A' (Table 3.17). This indicated that although peak X exhibited a high level of fluorescence intensity both peaks had similar fluorescence efficiency per unit absorbance. DOM from degraded needles however exhibited a significantly higher peak A_{Fint}/A_{340nm} (95% confidence level) compared to fresh and partly degraded needles (Table 3.17). Mean peak A_{Fint}/A_{340nm} in all needle DOM solutions was significantly higher than surface and soil waters and throughfall DOM (99% confidence level).

Absorbance ratios were determined for all needle solutions and replicate the spectrum presented in Figure 3.21 having significantly high values of A_{254nm}/A_{365nm}, and in the case of degraded needle DOM, A_{254nm}/A_{410nm} compared to surface and soil water and to throughfall DOM. Degraded needles also showed significantly higher mean values of A_{254nm}/A_{365nm} compared to fresh needles (99% confidence level). These relationships are replicated in some of the data discussed in Chapter 8 regarding the properties of needle litter material in a peat column sampled from this site, which shows high absorbance ratios and peak A_{Fint}/A_{340nm}.

	A_{254nm}/A_{365nm}	A_{465nm}/A_{665nm}	A_{254nm}/A_{410nm}	Peak A_{Fint}/A_{340nm}
Fresh and partially degraded needles	6.97 (0.52)	n/a	n/a	1785.58 (322.29)
Degraded needles	19.75 (0.87)	0.40 (0.75)	31.60 (2.48)	6585.54 (724.52)

Table 3.17
Details of spruce needle related DOM mean absorbance ratios, standard deviations are given in brackets

Coniferous litter degrades by the action of micro organisms and the removal of labile components resulting in the accumulation of recalcitrant material. Due to the different decomposition rates of various litter components the composition changes over time (Coûteaux *et al*., 1998). These changes may explain the differences between the fresh and partly degraded needle DOM and the more degraded needle DOM signal. The distinct signal in fresh needle DOM spectrophotometric properties may derive from such labile components that are readily lost or altered upon decomposition. Coniferous needles comprise primary components such as lignin and cellulose and secondary components such as terpenoids, monoterpenes and phenolics. The latter two components have been recognised to be water soluble and to decrease in concentration from fresh green litter upon decomposition (Kainulainen and Holopainen, 2002). These compounds are also potential fluorophores, having aromatic ring components; however further characterisation of the observed DOM by other techniques, such as NMR, is required to identify the fluorophores present.

3.8.5 Discussion of throughfall and spruce needle DOM spectrophotometric properties

A number of the characteristics of throughfall DOM spectrophotometric properties observed in comparison to surface and soil DOM were exhibited in spruce needle derived DOM. This includes high values of absorbance ratios, peak A_{Fint}/A_{340nm} and peak C_{Fint}/peak A_{Fint}. The distinct spectrophotometric properties observed in fresh needle DOM are not observed in either throughfall or surface water in the catchment, which suggests that this signal does not naturally contribute to throughfall. The extraction method used may have released DOM that is not naturally released from the needles in this form.

Throughfall exhibits spectrophotometric properties that closely compare to the more degraded needle DOM analysed. This may result from interactions of throughfall with needle material on the interception sheets during sampling. The differences in DOM character observed between needle derived DOM and throughfall (absorbance ratios, peak B_{Fint}/peak A_{Fint} and fluorescence wavelengths) suggest that this is not the only source of DOM in throughfall.

The input from precipitation may affect throughfall composition, however, the low mean DOC concentration in rainwater suggests that this influence may be limited. Cloud mist deposition may represent a more DOM rich source and further work is required to investigate the spectrophotometric properties of other forms of wet deposition. Similarly, occult deposition, although minimised in this study, may contribute to the signal of throughfall. Both cloud mist deposition and occult deposition require further examination of the DOM spectrophotometric properties to fully understand the processes that contribute to throughfall DOM.

3.9 Summary of the spatial variations in DOM in the Coalburn experimental catchment

The most obvious spatial variation in DOM properties identified within the catchment is the DOC concentration in waters from the peat sub-catchment and CB_{weir} compared to the peaty-gley. This is higher in the former waters and is derived from the greater extent of organic rich soils in the peat sub-catchment and replicates previous studies. Evidence from DOM replicates the spatial division observed in

inorganic geochemistry, pH and conductivity and can be observed in DOC concentration, fluorescence intensity (peak A and peak B), absorbance and water colour. The highest DOC concentration, fluorescence intensity and absorbance were seen in ditches draining predominantly forested area micro-catchments; those with a mix of open moor and forest have slightly lower values. Further to this the four micro-catchments observed indicated enhanced DOC concentration in ditches that have been experimentally cleared.

CB_{weir} exhibited spectrophotometric properties that were closer to peat sub-catchment waters and although the water sampled at the catchment outfall is a composite from both sub-catchments it was apparent that the peat sub-catchment was dominant in the spectrophotometric signal. The differences observed between each sub-catchment will be applied to the temporal observations of CB_{weir} to establish if different sources dominate under different flow or seasonal conditions (Chapter 4).

The control of DOC concentration on the differentiation of DOM properties such as emission wavelength and peak C_{Fint}, resulted in this being the major factor when applying statistical classification methods to the properties discussed here. Spatially, the waters of the Coalburn Experimental Catchment are defined adequately using DOC concentration. In tandem within the spatial DOC concentration gradient a gradient is also seen in spectrophotometric properties, such as peak A_{Fint}/A_{340nm} that do not relate to concentration. This indicates a spatial variation in DOM composition. From the interpretation of DOM spectrophotometric properties discussed in Section 2.2 DOM from the peaty-gley sub-catchment, both surface and soil water derived, smaller molecular size and less aromatic DOM when compared to peat sub-catchment DOM. This was observed in specific absorbance, emission wavelength and peak A_{Fint}/A_{340nm}. As discussed in relation to soil DOM spectrophotometric properties this differentiation may relate to the stabilisation of aromatic and/or higher molecular weight DOM in the inorganic components of the peaty-gley sub-catchment soil in comparison to peat sub-catchment (Zhou *et al*., 2001; Maurice *et al*., 2002) resulting in an effective fractionation of the surface water DOM.

Although peat sub-catchment DOM exhibited little variation in spectrophotometric properties a number of differences were observed. Peak AS_{Fint} was at the same level in excavated ditches, P_{weir} and P_{soil}, and depressed in control ditches. This suggests that the input from soil waters to the ditches may be modified or retarded in the infilled ditches. From the examination of DOM in spruce litter, which comprises a part

of the fill of the ditches, this material does not appear to modify DOM spectrophotometric properties in control ditches. Relatively enhanced peak A_{Fint}/A_{340nm} would be expected if this were a significant source of DOM in the control ditches.

Experimentally cleared ditches exhibit high values of DOC concentration related variables in relation to both control ditches and to P_{weir} and $CB_{weir.}$ It is concluded that this is due to the exposure of bare peat and removal of vegetation. Bare peat faces within and adjacent to the ditches are more susceptible to drying, oxidation and other DOM production processes compared to vegetated areas. This contributes to the generation of more DOM during dry periods, over a greater surface area. The removal of vegetation during ditch clearing resulted in a greater proportion of precipitation reaching the ditch and surrounding area, compared to control ditches, thus allowing the DOM produced within in surface peat layers to be exported. The large extent of this drainage network acts as both a rapid transport network increasing hydrological connectivity and a pool for the storage of Dom under low flow conditions.

Further study is required to explore the variability of spectrophotometric properties of DOM in forestry ditches, using a greater variety of ditch physical conditions. From this limited study it is apparent that the state of the ditch influences the DOM exported from the micro-catchment, and to some extent the quality of it.

Peak C_{Fint} and peak C_{Fint}/peak A_{Fint} were higher in peaty-gley sub-catchment waters in comparison to peat sub-catchment. This may derive from a significantly greater proportion of protein-like DOM in the former resulting in greater fluorescence from tryptophan components. The source of this material is unclear, it has been recognised in river waters impacted by anthropogenic inputs that enhanced peak C_{Fint} is related to for example sewage inputs and farm wastes (Baker, 2002a and b) and in marine waters from phytoplankton (Mayer *et al*., 1999).

In the Coalburn Experimental Catchment the high values of peak C_{Fint}/peak A_{Fint} were observed in peaty gley soil, throughfall and the highest in rainwater. Litter derived DOM also exhibited elevated peak C_{Fint}/peak A_{Fint} values in comparison to peat sub-catchment DOM. This may indicate that DOM from litter, precipitation and throughfall DOM was combined with that from PG_{soil} to give a comparatively higher peak C_{Fint}/peak A_{Fint} and peak C_{Fint} in PG_{weir}. In the peat sub-catchment DOM from peat

derived soil water dominates over litter derived DOM, in the observed spectrophotometric properties.

The intrinsic spectrophotometric properties of the DOM, however, may control peak C_{Fint} distribution. Energy transfer can occur when the emission energy from peak C (340-360nm) is reabsorbed by peak A, or other non-fluorescent chromophores. The relatively high specific absorbance and DOC concentration of peat sub-catchment waters suggests that this may occur preferentially in this DOM compared to peaty-gley sub-catchment DOM. Resulting in suppressed peak C_{Fint} in the former. The highest levels of peak C_{Fint} were seen in waters with the lowest DOC concentration and specific absorbance. This is complicated by the high specific absorptivity seen in throughfall data and suggests that a source related to the vegetation does influence peak C_{Fint}. As no peak C_{Fint} was observed in fresh needles DOM this may be derived from the more degraded litter.

3.10 Conclusions

This chapter has presented spatial characterisation of DOM in the Coalburn Experimental Catchment. Spectrophotometric properties of DOM from each component of the flow paths within the catchment were described and the aims were achieved with the following conclusions:

- *To identify the comparative spectrophotometric character of DOM throughout the catchment from each component of the flow paths described in Figure 1.7.*

DOM in the main channel is similar to the peat sub-catchment DOM. In comparison to this DOM peaty-gley sub-catchment DOM had a different spectrophotometric character that can be concluded as relatively lower molecular weight and aromaticity. This difference is related to the interaction of runoff from the peaty-gley sub-catchment with inorganic components in the soil.

Both surface and soil water exhibited the same distribution of spectrophotometric properties in catchment showing that soil derived DOM is the main influence upon surface water DOM composition in this catchment.

- *To investigate the DOM properties from contrasting ditches within the peat sub-catchment, comparing the influence of micro-catchment vegetation and ditch infill condition*

Within the peat sub-catchment ditches with different infilling exhibited similar DOM spectrophotometric properties. It was observed that soil water DOM may be transferred preferentially to experimentally cleared ditches in comparison to overgrown ditches. The ditch infill did not appear to influence the DOM spectrophotometric properties however; the extensive ditch drainage has changed the water regime within the catchment generating conduits for the transport and storage of DOM.

- *To characterise the spectrophotometric properties of precipitation*

Precipitation exhibited fluorescence properties, although DOC concentration was low. Rainwater DOM has low aromaticity and molecular weight characteristics, in comparison to surface water DOM. These properties were modified during passage through the canopy and DOC concentration significantly enhanced.

- *To characterise the spectrophotometric properties of throughfall and investigate the input of DOM to the catchment from vegetation and litter interactions*

Throughfall exhibited characteristics, in comparison to surface water DOM, of lower molecular weight. Similar DOM spectrophotometric properties were observed in degraded spruce needle DOM. DOM from fresh and partially degraded DOM, however, exhibited specific unique spectrophotometric properties, possibly relating to the presence of specific compounds. This signal does not contribute to the overall spectrophotometric characteristics of surface water DOM in the catchment due to modification, dilution and degradation.

- *To establish a basis from which the temporal dynamics of spectrophotometric properties can be assessed.*

The observations made in this chapter are further discussed in relation to temporal DOM patterns, in Chapter 4, and examination of peat derived DOM, in Chapter 8.

Chapter 4.

Temporal Patterns of Dissolved Organic Matter in the Coalburn Experimental Catchment

4.1 Introduction

The following chapter will discuss temporal variations in DOM properties sampled from the locations in the Coalburn Experimental Catchment. Firstly, seasonal variations in CB_{weir}, PG_{weir}, P_{weir} and other peat sub-catchment waters will be discussed. Secondly, a high resolution study during two periods of monitoring of CB_{weir} is detailed. The differences between and during these periods are discussed and high resolution variations in DOM spectrophotometric properties are assessed. This presents the first high resolution fluorescence investigation of DOM in such a catchment.

Previous studies in the catchment have revealed a distinct seasonal pattern of DOC concentration and water colour, which exhibited maximum values during late summer/autumn and low values in winter (Mounsey, 1999). In other peat areas similar patterns are observed and this is related to the export of DOM that is produced by soil microbial activity and oxidation during warm and dry periods by subsequent rainfall and displacement to streams (Mitchell and McDonald, 1992; Scott *et al*., 1998).

As concluded in Chapter 3 DOM from precipitation, throughfall and litter was determined to have a minimal effect of surface water DOM. The variations observed in surface waters are therefore thought to reflect processes within the soils of the catchment. Due to gaps in sampling resulting in a relatively low resolution data set of PG_{soil} and P_{soil} no seasonal variations could be seen, with the exception of higher DOC concentration during summer compared to winter.

In Chapter 3 spectrophotometric properties were identified that defined the DOM from different sources within the catchment, these were DOC concentration, peak AS_{Fint} peak A_{Fint}/A_{340nm}, SUV_{254nm}, absorbance ratios and peak B_{Fint} / peak A_{Fint}. These values are discussed and presented in this chapter. Absorbance and peak A_{Fint} were

found to be closely related to DOC concentration in Chapter 3 however divergences in the temporal characteristics were observed and are discussed. Other spectrophotometric properties did not exhibit any temporal variations.

4.1.1 Aims of the study of temporal patterns in DOM in the Coalburn Experimental Catchment

- To identify seasonal differences in DOM spectrophotometric properties
- To examine the response of DOM to changes in rainfall and discharge, over on both an annual cycle and during individual events to relate these variations to catchment conditions, discharge, flow paths and sources, using the spatial characteristics discussed in Chapter 3.
- To estimate the DOC export from the catchment.

4.2 Conditions in the Coalburn Experimental Catchment during sampling

Water sampling was performed from January 2000 to February 2002 at approximately bi monthly intervals, with more regular sampling from CB_{weir}. No sampling was possible from February 2001 to August 2001. During low flow conditions PG_{weir} was completely dry and sampling was not performed during these periods (July to August 2000).

The catchment conditions recorded during the sampling program are detailed in Figure 4.1. Rainfall was relatively evenly distributed throughout the year. Periods of significantly low rainfall were recorded during May, June and July 2000, and September and January 2001. Temperature showed broad annual cycles of winter lows from approximately October to May (mean daily temperature = 4.48°C $\pm$ 2.64). Higher mean daily temperatures were recorded in spring and summer (mean daily temperature = 12.04°C $\pm$ 2.47). Temperature maxima were recorded in May, June and August 2000 and in June, July and August 2001.

Rainfall and temperature data were used to calculate monthly hydrologically effective precipitation (Figure 4.1). This was calculated using the method of Thornthwaite (Shaw, 1994), which is used in this study to provide a general indication of the periods of relatively wet and dry conditions in the catchment. Details of the

calculation are in Appendix 3. The driest conditions were determined to occur during May and June 2000 and August 2001, when no precipitation was hydrologically effective. In both years monitored there was hydrologically effective precipitation after September and throughout the winter, however, this was not of a constant amount.

Discharge was measured at fifteen minute intervals at the catchment outfall and the mean daily discharge is shown on Figure 4.1. Over the study period this was typified by mean discharge of 0.0503 m^3s^{-1} (s.d. 0.0993). The highest discharge was recorded during 11/01/00, with a maximum level of 1.754 m^3s^{-1}. A number of periods of zero discharge were recorded during May and July 2000 and August 2001.

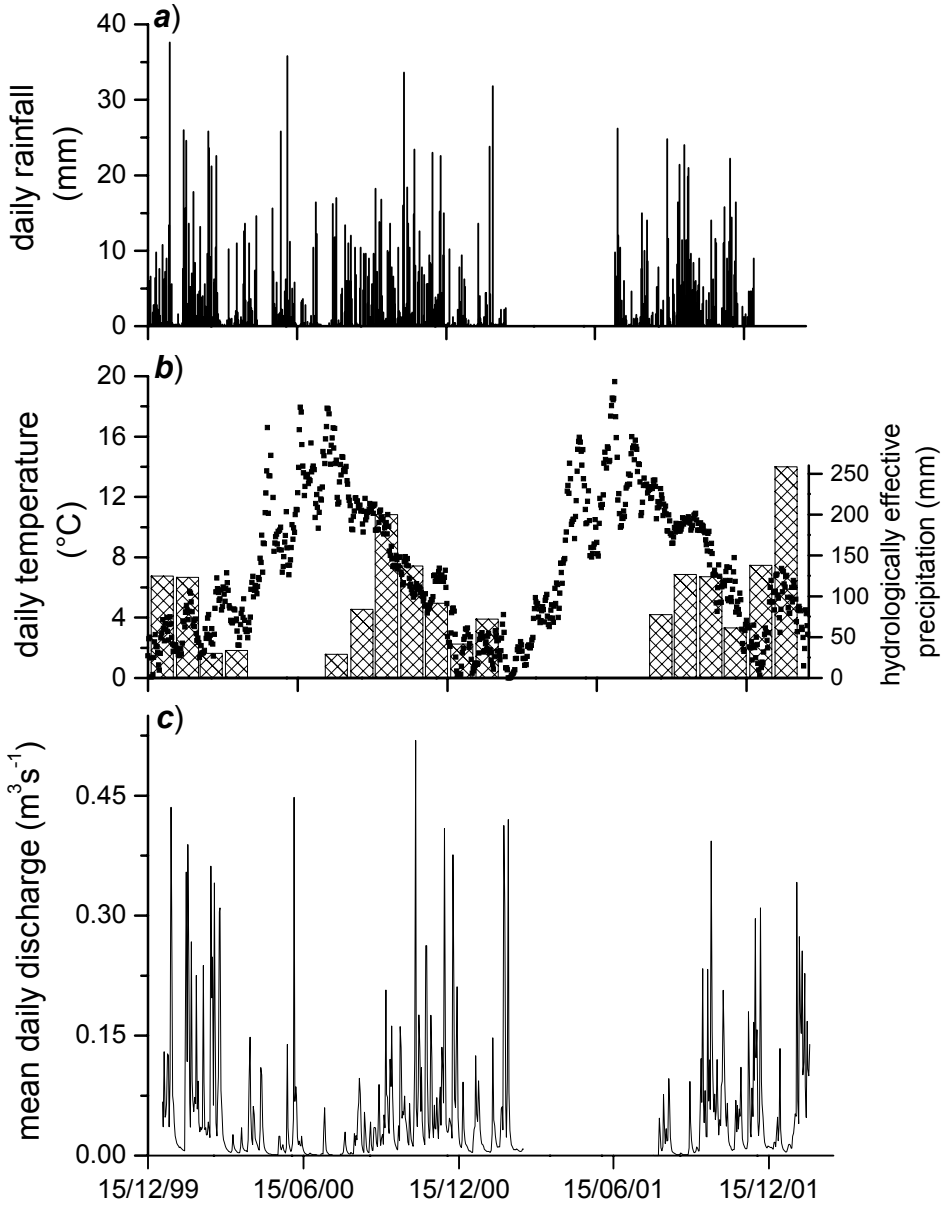


Figure 4.1
Conditions in the Coalburn Experimental Catchment during the study period ***a)*** *total daily rainfall (mm)* ***b)*** (■) *mean daily temperature* (°C) (bars) *hydrologically effective precipitation* (mm) *(calculated using Thornthwaite equation, Appendix 3)* ***c)*** *mean daily discharge* (m^3s^{-1}), *at the catchment outfall. Data was collected and supplied by the Environment Agency. No rainfall or discharge data were available from 27/02/01 to 01/07/01.*

4.3 Temporal patterns of DOM in the Coalburn Experimental Catchment during January 2000 to January 2002

Mean pH was highest, in $CB_{weir,}$ during March to August 2000 (mean = 5.312 s.d.= 0.881) and was lowest (mean = 4.512 s.d. = 0.912) during higher flow periods of September to November 2000). The high levels were close to the mean of PG_{weir} (mean = 5.582 s.d.= 0.545) and the low values to the mean of peat sub-catchment waters (mean = 3.915 s.d.= 0.639). This distribution results in a negative correlation of pH to discharge (Spearman's rho 99% confidence level). This relationship agrees with that observed by Mounsey (1999) where it was suggested that during low flow there is a source, other than the peat derived water, of a well buffered pH, probably from a deep source more typical of peaty-gley sub-catchment water.

The pattern of DOC concentration over time is shown in Figure 4.2 and summarised in Table 4.1 for CB_{weir}. As discussed in Chapter 3 water colour, absorbance and peak A_{Fint} and peak B_{Fint} correlated highly with DOC concentration and further to this these variables replicated the temporal trends in DOC concentration, as summarised in Table 4.1. CB_{weir} P_{weir} and PG_{weir} showed a similar DOC concentration trend. The annual pattern of DOC concentration is one high concentration during the summer and autumn periods related to DOM production and mobilisation and lower DOC concentration during winter periods. These periods are summarised in Table 4.1. As shown in Figure 4.2 highest DOC concentration (>30 mgL^{-1} in CB_{weir}) occurred from June to October 2000.

Peat sub-catchment ditches also exhibited a similar overall trend in DOC concentration to CB_{weir}. A significant peak, during July 2000 (mean = 35.27 mgL^{-1}) was seen in all four ditches but not observed at the other sample sites. This is related to the low flow and rainfall conditions (Figure 4.1) during this period when DOM was accumulating in the ditches; however, it was not being exported to the main channel.

	DOC (mgL^{-1})	**Peak A_{Fint}**	**Peak B_{Fint}**	***A*$_{340nm}$**
Aug. to Oct. 2000	34.135(2.873)	315.524(28.665)	181.276(19.034)	0.598(0.048)
Aug. and Sept. 2001	31.308(1.112)	382.540(65.030)	218.698(32.448)	0.566(0.045)
Nov. to Feb. 2000-2001	25.304(1.981)	218.913(28.022)	135.109(14.679)	0.402(0.071)

Nov. to Feb. 2001-2002	23.418(1.415)	268.950(21.772)	155.745(13.180)	0.438(0.045)

Table 4.1
Summary of DOC concentration related variables in CB_{weir} (standard deviations given in brackets)

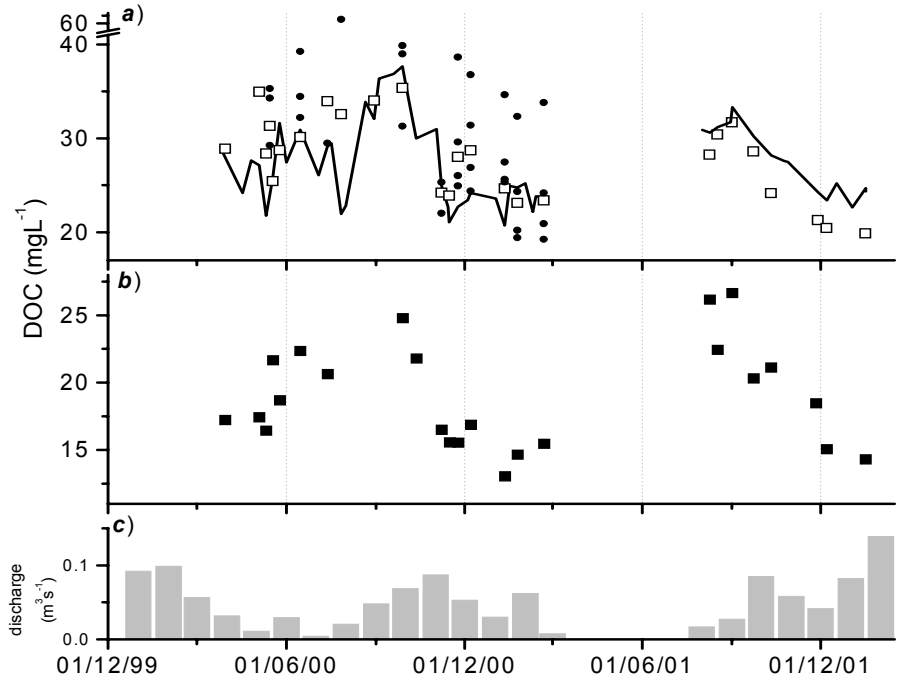


Figure 4.2
Time series of DOC concentration (mgL^{-1}) in the Coalburn Experimental Catchment a) (——) CB_{weir} (□) P_{weir} (●) peat sub-catchment ditches b) PG_{weir} c) Mean monthly discharge at the catchment outfall (m^3s^{-1}).

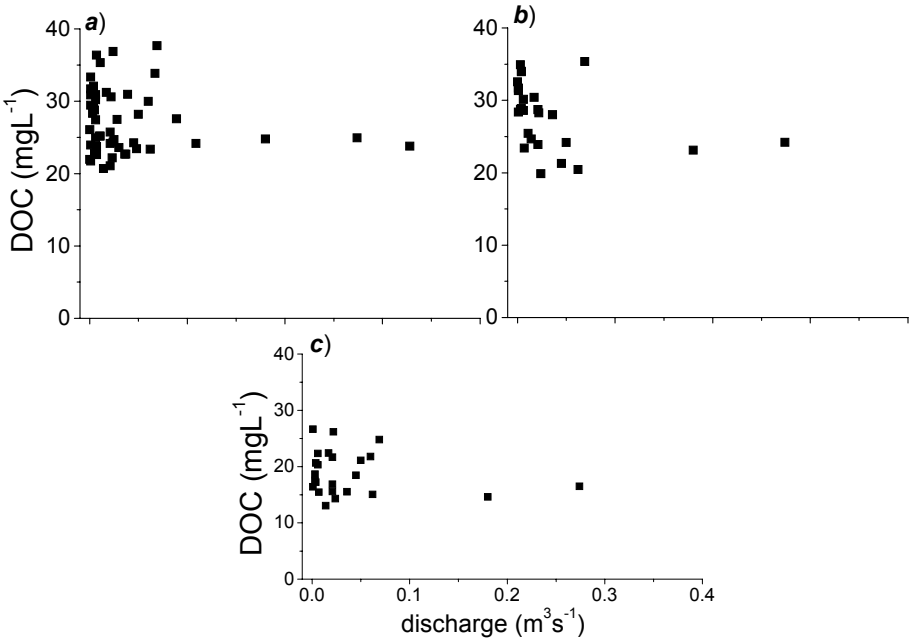


Figure 4.3
The relationship of DOC concentration (mgL^{-1}) to discharge at the catchment outfall (m^3s^{-1}) ***a)*** *CB_{weir}* ***b)*** *P_{weir} and* ***c)*** *PG_{weir}*

The relationship of DOC concentration to discharge at the main channel is shown in Figure 4.3. Both P_{weir} and CB_{weir} exhibited a significantly negative correlation of DOC concentration with discharge from the main channel (Spearman's rho 95% confidence level). A wide range of DOC concentration was observed at low flow (21.75 to 36.36 mgL^{-1} at <0.01 m^3s^{-1}).

The temporal variations in peak AS_{Fint} are presented in Figure 4.4. CB_{weir} expressed high values (>12.5) during May to July and November 2000 and in September 2001. A mean value of 9.62 (s.d. =1.13) was observed during winter 2000. In comparison to this, a mean of 13.81 (s.d. = 2.88) was observed in September 2001 to February 2002. Peak AS_{Fint} in P_{weir} exhibited maxima during May and November 2000 and in September 2001 (>11.23), in a similar pattern to CB_{weir}; however, a peak was not observed in July 2000. The peat sub-catchment ditches showed levels of >13.15 peak AS_{Fint} in May to July 2000.

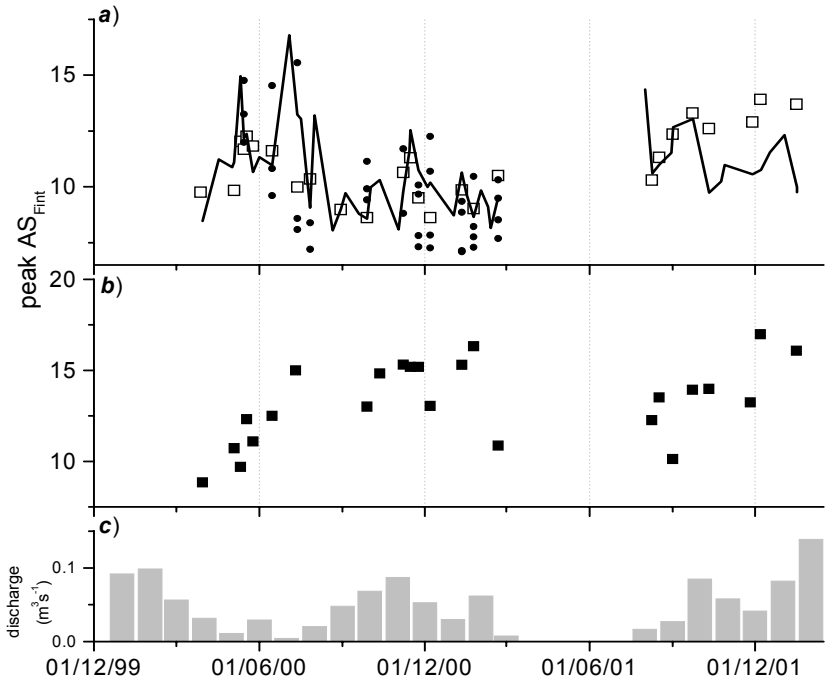


Figure 4.4
Time series of peak AS_{Fint} in the Coalburn Experimental Catchment ***a)*** (——) CB_{weir} (□) P_{weir} (●) *peat sub-catchment ditches* ***b)*** PG_{weir}. ***c)*** *Mean monthly discharge at the catchment outfall (m^3s^{-1}).*

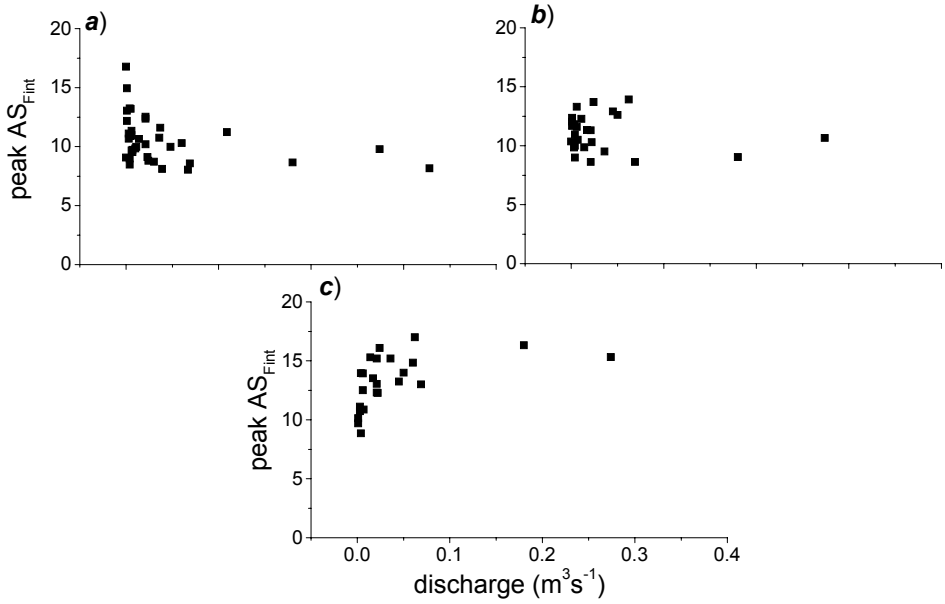


Figure 4.5
The relationship of peak AS_{Fint} to discharge at the catchment outfall (m^3s^{-1}) ***a)*** CB_{weir}, ***b)*** P_{weir} *and* ***c)*** PG_{weir}

As shown in Figure 4.5 PG_{weir} exhibited a significant positive correlation between peak AS_{Fint} and discharge (Spearman's rho= 0.733 99% confidence level). A significantly negative relationship of these variables was observed in CB_{weir} (Spearman's rho= -0.507 99% confidence level).

As presented in Figure 4.6 maxima of peak A_{Fint}/A_{340nm} > 800 in CB_{weir} occurred in May, July and November 2000. P_{weir} showed a similar pattern to CB_{weir}, however, no peak in July 2000 was observed. PG_{weir} exhibited values of peak A_{Fint}/A_{340nm} of >1250 during March to May 2000 and >1100 during November 2000 and February 2001. Peat sub-catchment ditches had a mean peak A_{Fint}/A_{340nm} of 558 (s.d.= 126) individual ditches replicated the P_{weir} trend.

In CB_{weir} specific absorbance (SUV_{254nm}), as shown in Figure 4.7 manifested significantly high values of > 0.09 in mid May 2000 and mid June to July 2000, this was also seen in the P_{weir} and PG_{weir}. The peat sub-catchment ditches exhibited a constant mean throughout the study period. The time series of A_{254nm}/A_{410nm} as presented in Figure 4.8 in the peat sub-catchment ditches, CB_{weir} and P_{weir} DOM exhibited A_{254nm}/A_{410nm} values of >14 and >15 respectively during May to June 2000. PG_{weir} showed a peak value in June 2000 of >14. Values of A_{254nm}/A_{365nm} in CB_{weir} and peat sub-catchment waters exhibited the same temporal pattern as A_{254nm}/A_{410nm}. In PG_{weir} the trend of A_{254nm}/A_{410nm} was replicated in A_{254nm}/A_{365nm} with an additional peak during May 2000.

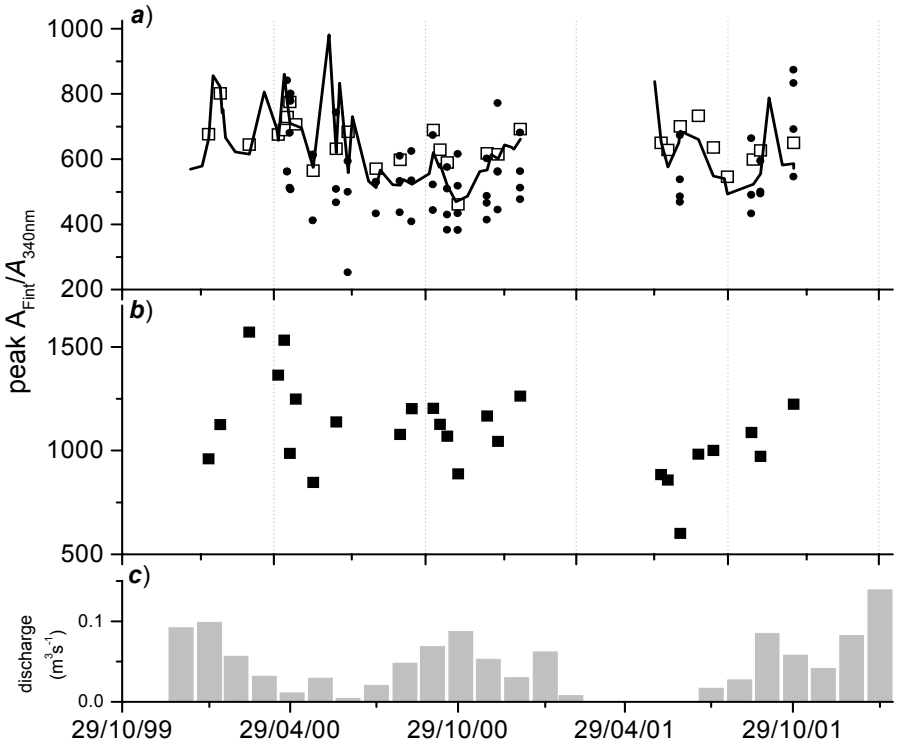


Figure 4.6

Time series of peak A_{Fint}/A_{340nm} in the Coalburn Experimental Catchment ***a)*** (——) *CB_{weir}* (□) *P_{weir}* (●) *peat sub-catchment ditches* ***b)*** *PG_{weir}.* ***c)*** *Mean monthly discharge at the catchment outfall (m^3s^{-1}).*

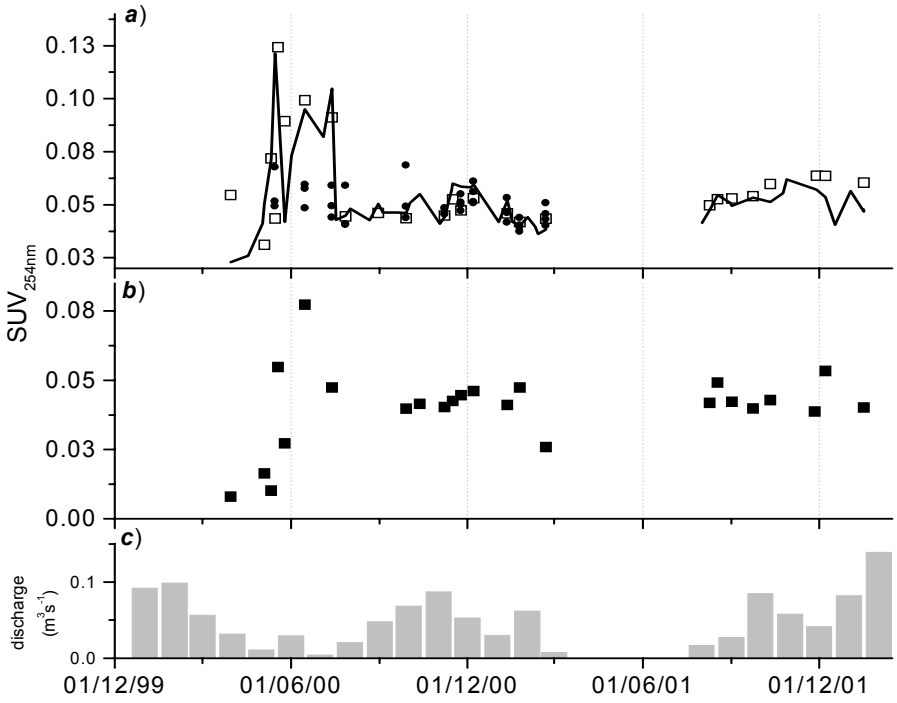


Figure 4.7

Time series of SUV_{254nm} in the Coalburn Experimental Catchment ***a)*** (——) CB_{weir} (□) P_{weir} (●) *peat sub-catchment ditches* ***b)*** PG_{weir} ***c)*** *Mean monthly discharge at the catchment outfall (m^3s^{-1}).*

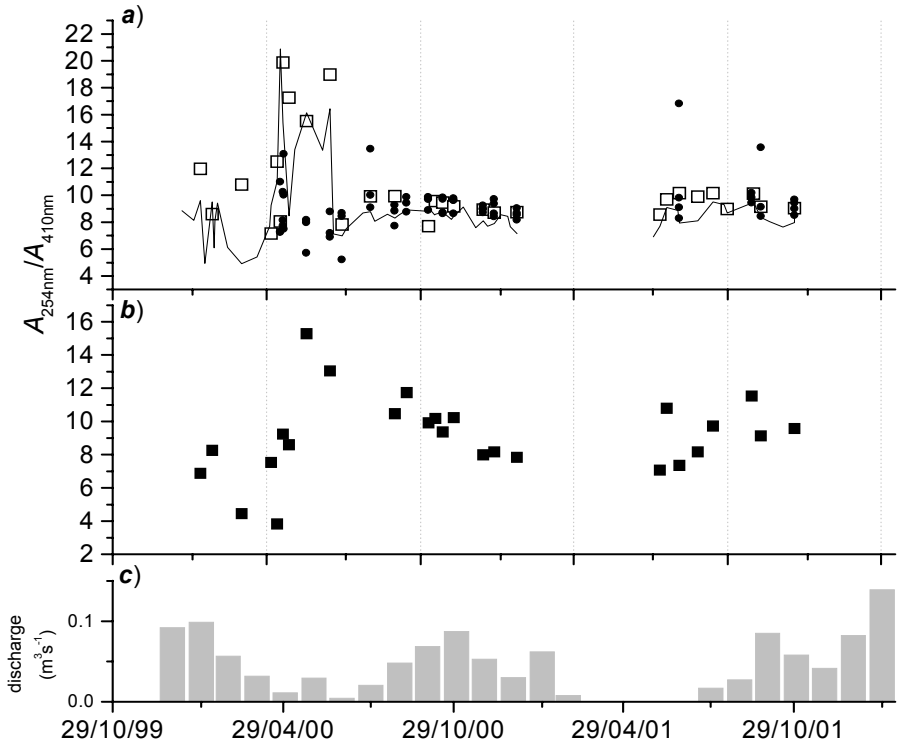


Figure 4.8
Time series of A_{254nm}/A_{410nm} in the Coalburn Experimental Catchment ***a)*** (——) CB_{weir} (□) P_{weir} (●) *peat sub-catchment ditches* ***b)*** PG_{weir} ***c)*** *Mean monthly discharge at the catchment outfall (m^3s^{-1}).*

"Deeper water sources", as described by Mounsey (1999) have been recognised in the inorganic geochemistry of ditch water during low flow periods. Specific fluorescence intensity and peak A_{Fint}/A_{340nm} exhibited highest values during relatively dry periods, indicating DOM of this character was derived from such sources. The relative spectrophotometric characteristics of the DOM observed under these conditions indicate that the DOM source is the peaty-gley sub-catchment, as DOM from this area exhibits high peak A_{Fint}/A_{340nm} and peak AS_{Fint} as discussed in Chapter 3. Flow paths may be preferential within this sub-catchment through pathways resulting from slumping/cracking (Mounsey, 1999). The values of peak A_{Fint}/A_{340nm} in CB_{weir} under low flow conditions do not reach the levels seen in peaty-gley sub-catchment DOM, indicating multiple sources or processing of DOM. The DOM observed at the catchment outfall is not entirely derived from the peaty-gley sub-catchment during low flow.

In data from PG_{weir} there was a significant negative relationship of SUV_{254nm} to peak A_{Fint}/A_{340nm} that was also seen in CB_{weir}. This suggests that DOM exists on a gradient from lower molecular weight and less aromatic DOM to more aromatic larger DOM, based on the interpretations of these variables discussed in Section 2.2.7. This reflects a transition from DOM that has interacted with inorganic material in the soils, resulting in the retention of a significant fraction, to DOM derived from primarily organic soil and litter horizons.

4.3.1 The properties of DOM in the Coalburn Experimental Catchment during May to August 2000

The period of May to August (2000) exhibited a range of spectrophotometric properties in the catchment that were distinct from other periods of the time series. The following section considers the variations in DOM during this period in greater detail. Table 4.2 and 4.3 summarise the spectrophotometric properties of DOM and the condition in the catchment at this time. The highest DOC concentration ($63.97 mgL^{-1}$) seen in the catchment was observed at this time in F_E. During this period the unique EEM recorded in PG_{weir} (Section 3.5.3.1) was observed.

DOM source	Variable	Mean (standard deviation)
CB_{weir}	DOC concentration	31.849 (6.039)
	Peak AS_{Fint}	10.303 (2.099)
	SUV_{254nm}	0.058 (0.015)
	Peak A_{Fint}/A_{340nm}	696.671 (125.389)
	A_{254nm}/A_{410nm}	11.660 (4.242)
PG_{weir}	DOC concentration	19.827 (2.356)
	Peak AS_{Fint}	11.803 (1.445)
	SUV_{254nm}	0.041 (0.026)
	Peak A_{Fint}/A_{340nm}	1157.207 (241.677)
	A_{254nm}/A_{410nm}	9.534 (0.024)
Peat sub-catchment	A_{254nm}/A_{410nm}	10.293 (4.157)
	SUV_{254nm}	0.058 (0.016)

Table 4.2
Summary of the properties of DOM during May to August 2000

Rainfall records mean daily rainfall	
28/04/00 to 15/05/00	0.0mm
10/07/00 to 28/07/00	0.35mm ± 0.207
03/06/00	rainfall event (35.8mm)
Mean daily temperatures	
minimum in April	4.58 °C ± 1.57
maximum in July	14.16 °C ± 2.23
effective precipitation for May, June and July	zero
Recorded discharge at the main channel outfall	
04/05/00 to 17/05/00 and 16/06/00 to 02/08/00	zero
08/07/00 to 13/07/00	max. discharge 0.060m^3s^{-1}
04/06/00	max. discharge 0.447m^3s^{-1}

Table 4.3
Summary of the catchment conditions during May to August 2000

In the peat sub-catchment mean peak A_{Fint}/A_{340nm}, peak AS_{Fint} and SUV_{254nm} were significantly lower and DOC concentration higher than the main channel (95% confidence level). DOC concentration was higher, compared to winter levels but not as high as the autumn level in the main channel and PG_{weir}.

High DOC concentration (25/05/00 31.63mgL^{-1}) (Figure 4.2) in CB_{weir} occurred synchronously with a period of rainfall. Prior to this the peat sub-catchment ditch water exhibited higher DOC concentration (34.27). This temporal pattern suggests that DOM produced in early May, under warm dry conditions was then transferred to the main channel during increased precipitation. The transfer of DOM declined during dry conditions in June and as the catchment wetted up in August and September a major period of DOM displacement occurred with high DOC concentration exported from the main channel.

In CB_{weir} DOC concentration remained lower than in peat sub-catchment waters and during June when DOC concentration declined in the main channel, peat sub-catchment runoff DOC concentration did not. This suggests that DOM was being produced within the peat sub-catchment throughout this period and was being stored in the ditch system. Robinson *et al*. (1998) discussed the soil water levels recorded in the catchment annually concluding that there was a constant direction in the water table gradient throughout the year, resulting in seepage from drain sides. This mechanism may account for the displacement of peat derived DOM into ditch water throughout this dry period. DOM accumulated within the ditch network until catchment conditions resulted in sufficient flow for displacement of the high DOC concentration water to the main channel. In addition to this PG_{weir} exhibited relatively

high DOC concentration during this period, suggesting that DOM was being produced and stored in ditches throughout the catchment.

The temporal patterns of spectrophotometric properties during this period were variable in the catchment, having a number of significantly high values, as summarised in Table 4.2. Peak AS_{Fint} and peak A_{Fint}/A_{340nm} manifested similar trends in CB_{weir} exhibiting two peaks higher than levels in peat sub-catchment DOM. These peaks occurred during periods of precipitation but no discharge increase and may be derived from peaty-gley sub-catchment waters that were comparatively enhanced in peak A_{Fint}/A_{340nm} (Section 3.5.4.2). A slight increase in rainfall during this period may have resulted in the transport of readily mobile DOM with this spectrophotometric signal from the peaty-gley sub-catchment influencing the DOM in the main channel. Previously, it has been observed that due to the location, topography and soil of this area the typical geochemical signal of peaty-gley sub-catchment runoff can be recognised early in rainfall events, and that lower levels of precipitation may displace water from here, compared to the peat sub-catchment (Mounsey, 1999).

Specific absorbance and estimated aromaticity during this period were higher in CB_{weir}, PG_{weir} and P_{weir} compared to other periods in the year, as summarised in Table 4.2. The values observed in these sources were similar, however the four peat sub-catchment micro-catchment ditches did not exhibit enhanced levels, with values lower than other surface waters. Specific absorbance values decreased, in CB_{weir} and P_{weir}, with the onset of increased flow conditions. This suggests an input of forestry ditch derived DOM, with increased flow. This is further suggested by the decrease in peak A_{Fint}/A_{340nm} and peak AS_{Fint} in CB_{weir} with increased flow. During the period of increases in flow DOM properties in the main channel take on the character that is similar to forestry ditch DOM.

The observed temporal variations in spectrophotometric properties during spring-autumn 2000 exhibit a complex pattern. This pattern relates to the sources of DOM and flow paths. From the examination of spectrophotometric properties during this period of relatively dry conditions the methods used are useful in identifying flow paths and sources of DOM in the catchment.

4.3.2 Summary of the temporal patterns in DOM in the Coalburn Experimental Catchment from January 2000 to January 2002

This section has discussed the temporal changes in DOM spectrophotometric properties in the Coalburn Experimental Catchment. It is difficult to interpret the entire data set due to sampling gaps. High DOC concentration occurs during the "autumn flush" when DOM produced during previous drier periods is displaced to the main channel.

It is apparent that the DOM signal is related closely to catchment conditions during 2000. A period of dry conditions (May-August) exhibited variations in properties and distinct characteristics suggesting that the DOM in the main channel was derived from different sources in the catchment.

DOM in the main channel water switched between low flow peaty-gley sub-catchment/deep water sources and high flow peat sub-catchment derived DOM as flow patterns changed. A switch from the former to the latter can be seen between high levels of DOC concentration in peat ditches during the DOM production period and high levels in the main channel during flushing. This indicates that the DOC concentration increase during the autumn flush and the changes in DOM spectrophotometric properties are due to DOM derived from peat sub-catchment which is transported via the ditch network. This is observed as a fall in peak A_{Fint}/A_{340nm} and SUV_{254nm} in the main channel to levels similar to the means in the peat sub-catchment monitored ditches. The rapid preferential transport of DOM from the peaty-gley sub-catchment was also observed when rainfall occurred, without an increase in discharge.

4.4 DOM patterns during rainfall events in the Coalburn Experimental Catchment

The following section describes the spectrophotometric analysis of water samples that were taken at eight hourly intervals from CB_{weir} during two periods of the study. Firstly, samples were taken from January to March 2001 and secondly during August and September 2001. These periods represent distinct stages in the annual DOM cycle in upland catchments: - "winter" and "late summer/autumn". As discussed in Section 1.2.3 during winter months DOC concentration exhibits low levels after depletion of the DOM produced in soils in the previous summer, by flushing events, as the catchment wets up during the autumn. This cycle is identified in the temporal patterns observed in the Coalburn Experimental Catchment and the high resolution periods cover distinct periods within the DOC concentration.

4.4.1 Catchment conditions during rainfall event sampling

Rainfall, temperature and discharge from the catchment outfall, covering the periods 01/01/01 to 20/02/01 and 01/08/01 to 23/09/01, are shown on Figure 4.9 and summarised in Table 4.4.

Winter	**01/01/01 to 20/02/01**		
Daily rainfall	Mean 2.50mm ± 5.89	Maximum 10/02/01 (31.8mm)	Rainfall events 06/02/01 and 23/01/01
Mean temperature	1.92°C ± 0.97		
Discharge from CB_{weir}	Mean 0.052m^3s^{-1} ± 0.114	Peak flow 06/02/01 and 11/02/01 >1m^3s^{-1}	Discharge events 24/01/01, 4/01/01 and 07/01/01; snowmelt 10/02/01
Summer/autumn	**01/08/01 to 23/09/01**		
Daily rainfall	Mean 4.60mm ± 5.98	Rainfall events 12-13/09/01, 18/08/01 and 12/08/01 (> 10mm daily rainfall total).	
Mean temperature	11.21°C ± 1.50		
Discharge from CB_{weir}	Mean 0.014m^3s^{-1} ± 0.026	Peak flow 13/09/01 0.209 m^3s^{-1}	Discharge events 08/08/01, 13/08/01 and 19/08/01 > 0.07m^3s^{-1}

Table 4.4
Summary of the catchment conditions during rainfall event sampling

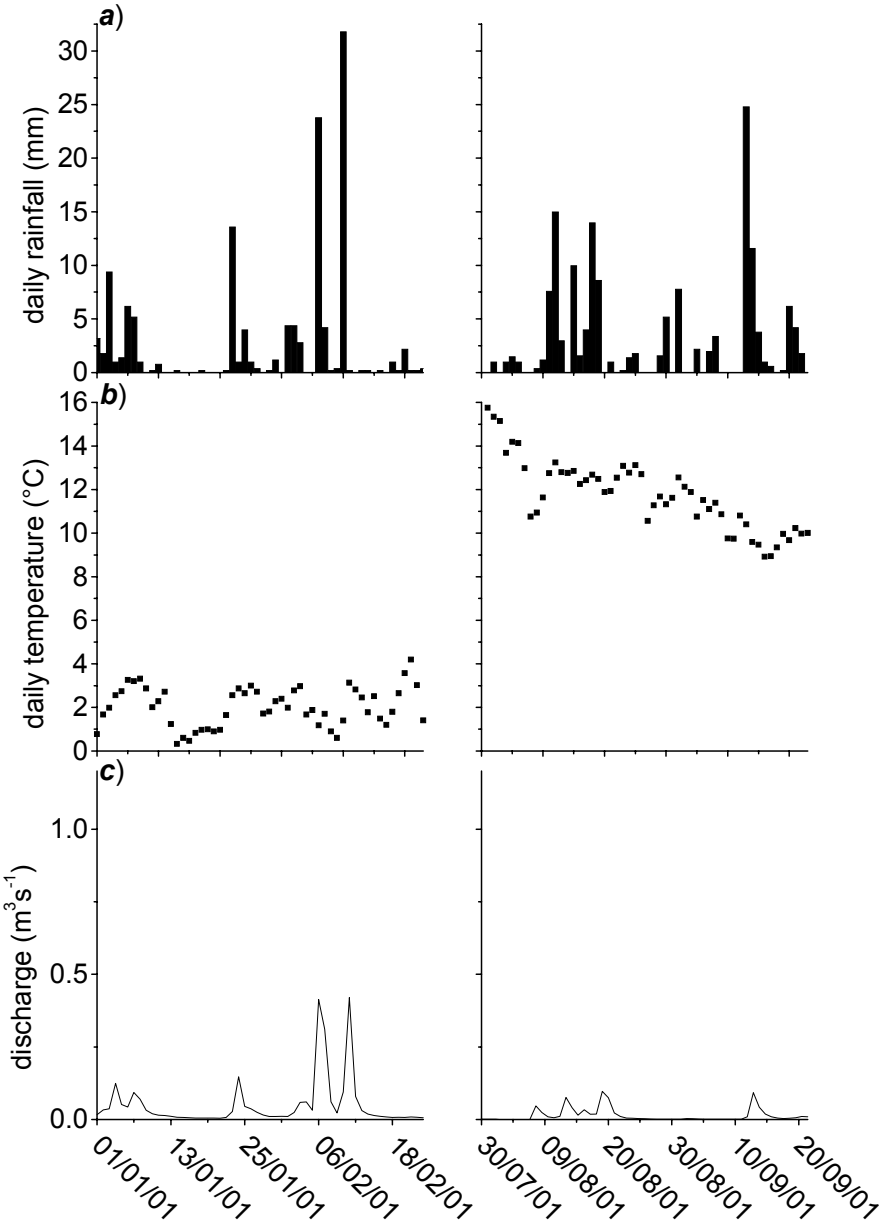


Figure 4.9
Conditions in the Coalburn Experimental Catchment during high resolution sampling periods winter 2001 summer/autumn 2001 ***a)*** *total daily rainfall (mm)* ***b)*** *mean daily temperature (°C)* ***c)*** *discharge from the main channel at 15 minute intervals (m^3s^{-1}). Data was collected and supplied by the Environment Agency.*

4.4.2 Comparison of winter and summer/autumn DOM characteristics

The distribution of DOM properties in each sampling period are summarised in the following section in Table 4.5, this data is presented in Appendix 4. The seasonal difference in DOC concentration related can be attributed to the autumn flux of DOM from soils into surface waters. Manifested as the flush of soluble DOM produced and stored in soils and litter during summer. In the Coalburn Experimental Catchment this is also related to displacement of DOM from the ditch system. Winter periods exhibit lower DOC concentrations in surface waters, as DOC concentration declines when the pool of soluble and mobile DOM has been depleted.

The differences in spectrophotometric properties also indicate that DOM sampled during summer/autumn was of a more aromatic composition in comparison to winter sampled DOM. Absorbance and fluorescence ratios a greater variance was observed in the summer/autumn data set compared to winter, this suggests that DOM during this period was more variable, related to the greater variability of catchment conditions.

Spectrophotometric property	Comparison of high resolution sampling period data
Peak $A_{EX\lambda,}$ peak $B_{EX\lambda}$, peak $A_{EM\lambda}$, peak $B_{EM\lambda}$, peak C variables, peak A_{Fint}/A_{340nm} A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	No significant differences in mean values outside reproducibility
DOC concentration, peak A_{Fint}, peak B_{Fint}, water colour and absorbance (at all measured wavelengths)	Summer/autumn exhibited significantly higher means compared to winter (99% confidence level); mean difference 34.74%
Mean peak AS_{Fint}, estimated aromaticity; SUV_{254nm}, $Svis_{410nm}$ and A_{465nm}/A_{665nm}	Summer/autumn exhibited significantly higher means compared to winter (99% confidence level); mean difference 16.67%

Table 4.5
Summary of the differences in spectrophotometric properties in DOM sampled during high resolution monitoring of CB_{weir}.

4.4.3 The relationship of fluorescence intensity and absorbance to DOC concentration during rainfall events

As discussed in Section 3.6 DOM in the catchment exhibits a positive correlation, of varying strength, of peak A_{Fint}, peak B_{Fint} and absorbance to DOC concentration. In both of the high resolution data sets both peak fluorescence intensities and absorbance correlated positively with DOC (99% confidence level). As shown in Figure 4.10 and summarised in Table 4.6 the relationship of fluorescence intensity

and DOC concentration differed between each data set. The distribution of these variables was similar between each data set, for DOC concentration and A_{340nm}, as shown in Table 4.7. Peak A_{Fint} however exhibited a greater range of values and variance in summer/autumn compared to winter. Resulting from this difference DOC concentration explains more of the variations in the absorbance and fluorescence intensity data in the winter data set compared to summer/autumn, when using linear regression. Variations in absorbance are explained to a greater extent by DOC concentration compared to fluorescence intensity. Additionally, in the summer/autumn data set the relationship of peak A_{Fint} to A_{340nm} is weak with 12.3% of variation in absorbance explained by fluorescence intensity compared to winter in which 65.6% was explained.

It has been suggested that absorbance and fluorescence intensity could be used as a proxy for DOC concentrations. It is apparent that not only as discussed in Section 3.6 does this relationship vary spatially but also over time. Averaged calibrations may be not applicable to DOM sampled over different periods of time. Differences in DOC concentration-spectrophotometric property relationships are explained by compositional changes in DOM chromophores and fluorophores and the proportion of non-spectrophotometric DOM.

	Winter	Summer/autumn
A_{254nm}	62.90%	40.10%
A_{272nm}	62.50%	38.30%
A_{340nm}	62.50%	48.30%
A_{365nm}	62.30%	48.40%
A_{410nm}	64.60%	45.00%
A_{465nm}	62.30%	40.30%
A_{665nm}	27.20%	7.70%
Peak A_{Fint}	29.20%	1.70%
Peak B_{Fint}	21.20%	3.20%

Table 4.6
The results of linear regression of peak A_{Fint}, peak B_{Fint} and absorbance against DOC concentration showing the percentage variation explained by DOC concentration sampled from CB_{weir} at high resolution during winter and summer/autumn, 2001

		Winter	Summer/autumn
DOC concentration	Range	14.66	12.50
	Variance	5.580	6.240
A_{340nm}	Range	0.199	0.250
	Variance	0.002	0.002
Peak A_{Fint}	Range	90.572	291.703
	Variance	252.184	4356.271

Table 4.7
Summary of the distribution of DOC concentration, A_{340nm} and peak A_{Fint} in DOM sampled during high resolution monitoring of CB_{weir}.

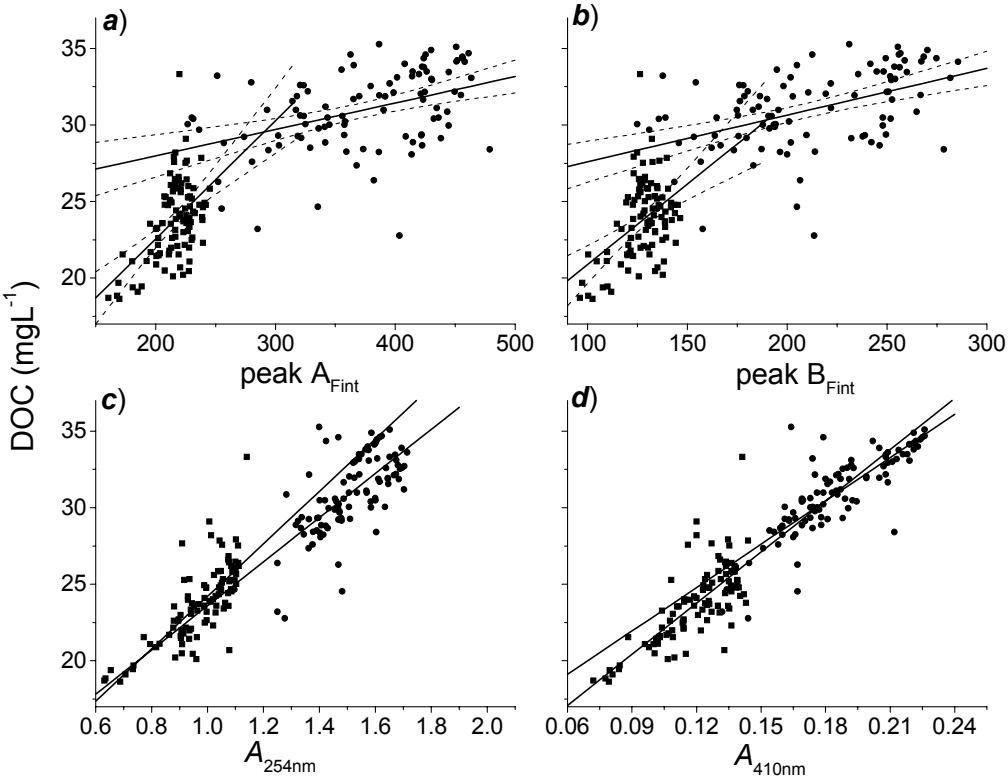


Figure 4.10
The relationship of peak A_{Fint} peak B_{Fint} A_{254nm} and A_{410nm} to DOC concentration from CB_{weir} sampled at high resolution during (■) winter and (●) summer/autumn, 2001 ***a)*** *peak A_{Fint}* ***b)*** *peak B_{Fint}* ***c)*** *A_{254nm}* ***d)*** *A_{410nm} (——) linear regression (- - - -) 95% confidence level (not shown on* ***c)*** *and* ***d)****)*

4.4.4 Rainfall events during winter (January / February 2001)

During the eight hourly sampling of CB_{weir} from 11/01/01 to 20/02/01 DOC concentration exhibited little variation over time with a constant mean level. As can be seen in Figure 4.11, during the major discharge events (06/02/01 and 10/02/01) and periods of increased rainfall and snowmelt DOC concentration decreased at peak discharge. The dilutions of DOC concentration is due to influxes of low DOC concentration event water; rainwater and/or snow melt, and to runoff being sourced primarily from low DOC concentration sources. DOM is generally depleted during this period in all surface waters, indicating that throughout this period there were low levels of DOM in the catchment, and little reaching the catchment out fall, in comparison to other periods in the year (Figure 4.2).

Absorbance, peak A_{Fint} and peak B_{Fint} exhibited the same responses as DOC concentration to changes in discharge (Figure 4.11 b and c). The discharge events of 06/02/01 and 10/02/01 resulted in the lowest values of these properties (A_{340nm}=0.211; peak A_{Fint}=160.52; peak B_{Fint}= 97.1). This accounts for a decrease of 25% in DOC concentration and fluorescence intensity and 50% in absorbance). DOC concentration, absorbance, peak A_{Fint} and peak B_{Fint} all increased on the falling limb of the hydrographs.

As shown in Figure 4.12 SUV_{254nm} had a constant value over time (0.041 $\pm$ 0.003), however, it exhibited a decrease coinciding with peak discharge during 06/02/01 and 10/02/01 of ~3%, which was not significant. Specific fluorescence intensity exhibited a variable level, with a number of peaks values however these were not significant and did not relate to other variables or to the catchment conditions.

The patterns over time in absorbance ratios are shown in Figure 4.12. A_{465nm}/A_{665nm} did not exhibit any significant trends over time. Both A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm} exhibited significant variations. There was a decrease to a minimum (A_{254nm}/A_{410nm} =7.24 A_{254nm}/A_{365nm} =3.75) on 22/01/01 (19:30). From this minimum level values rapidly increased over the period 22/01/01 19:30 to 24/01/01 by 21% in A_{254nm}/A_{410nm} and 11% in A_{254nm}/A_{365nm} to a maximum value on 24/01/01 (11:45). This coincided with significantly higher rainfall and discharge conditions. A_{254nm}/A_{365nm} as shown on Figure 4.12 exhibited a significant peak of 4.44 during 06/02/01 and 07/02/01, which coincided with the peak in discharge and high rainfall. Both ratios exhibited a

significantly positive correlation with discharge, over this period of sampling (99% confidence level). The peak in discharge on 10/02/01 did not coincide with a peak in absorbance ratio values. As this discharge peak had a high snowmelt component the corresponding lack of change in DOM properties may result from the discharge increase being due to an input of snow with negligible DOM content (DOC =0.00 mgL^{-1}).

In the time series fluorescence intensity peak wavelengths related to peak A and C showed no variations over time or with changing catchment conditions. Peak C_{Fint} also showed no variation over this period. Peak $B_{EX\lambda}$ however exhibited a mean blue shift of 7.4nm from 24/01/01 to 25/01/01, a change greater than reproducibility of the method ($\pm$6nm). As shown in Figure 4.11 there were red shifts in both peak $B_{EX\lambda}$ and peak $B_{EM\lambda}$ on 08/02/01 (11:45) occurring between the peaks in discharge and coinciding with peaks in absorbance, peak A_{Fint} and peak B_{Fint} at low flow. These are significant shifts of 15nm in peak $B_{EX\lambda}$ and 16.5nm in peak $B_{EM\lambda}$, on the falling limb of the hydrograph.

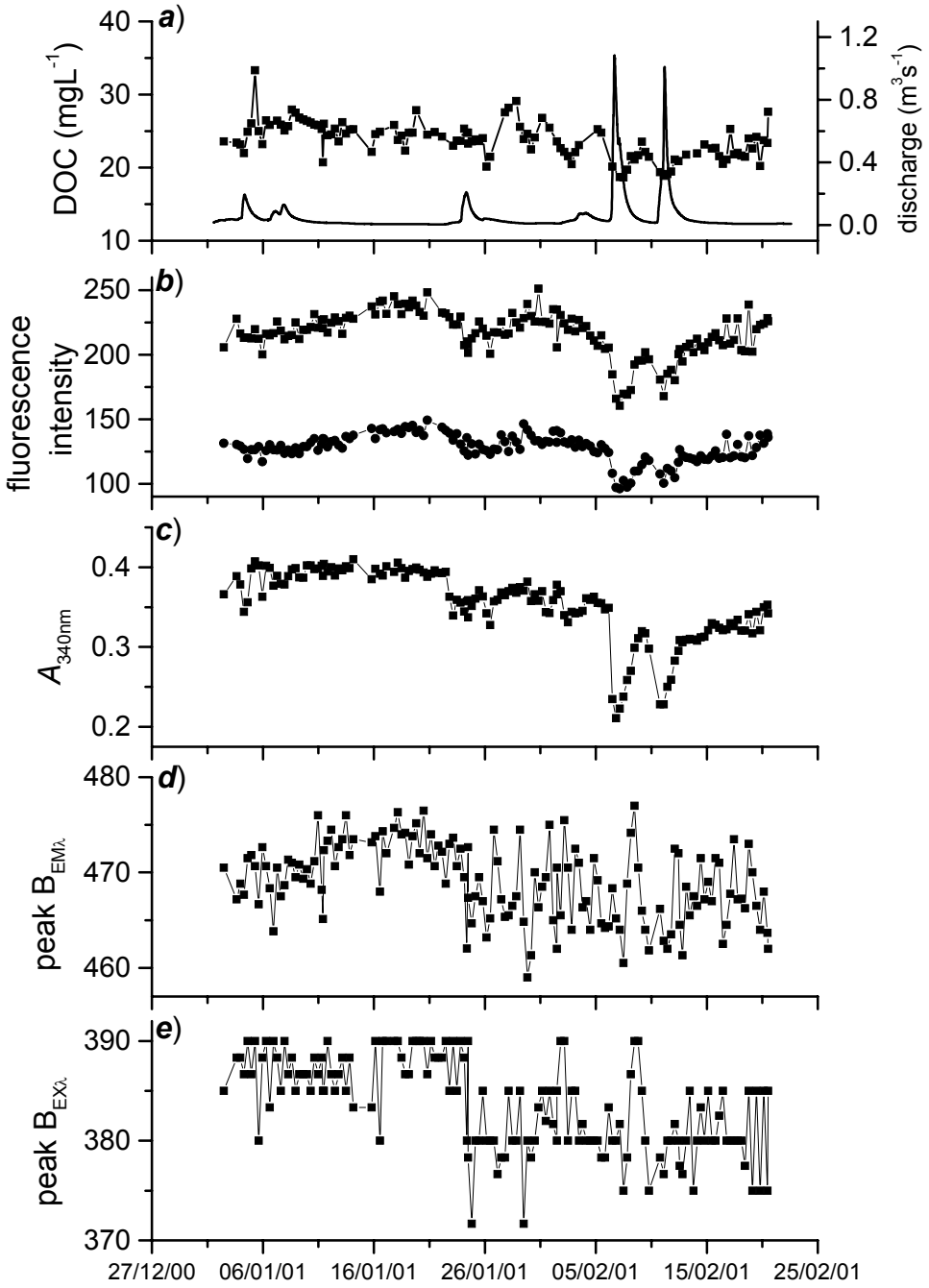


Figure 4.11
Time series from winter high resolution sampling of CB_{weir} ***a)*** *DOC concentration (mgL^{-1}) and discharge (m^3s^{-1})* ***b)*** (■) *peak A_{Fint}* (●) *peak B_{Fint}* ***c)*** *A_{340nm}* ***d)*** *peak $B_{EM\lambda}$* ***e)*** *peak $B_{EX\lambda}$*

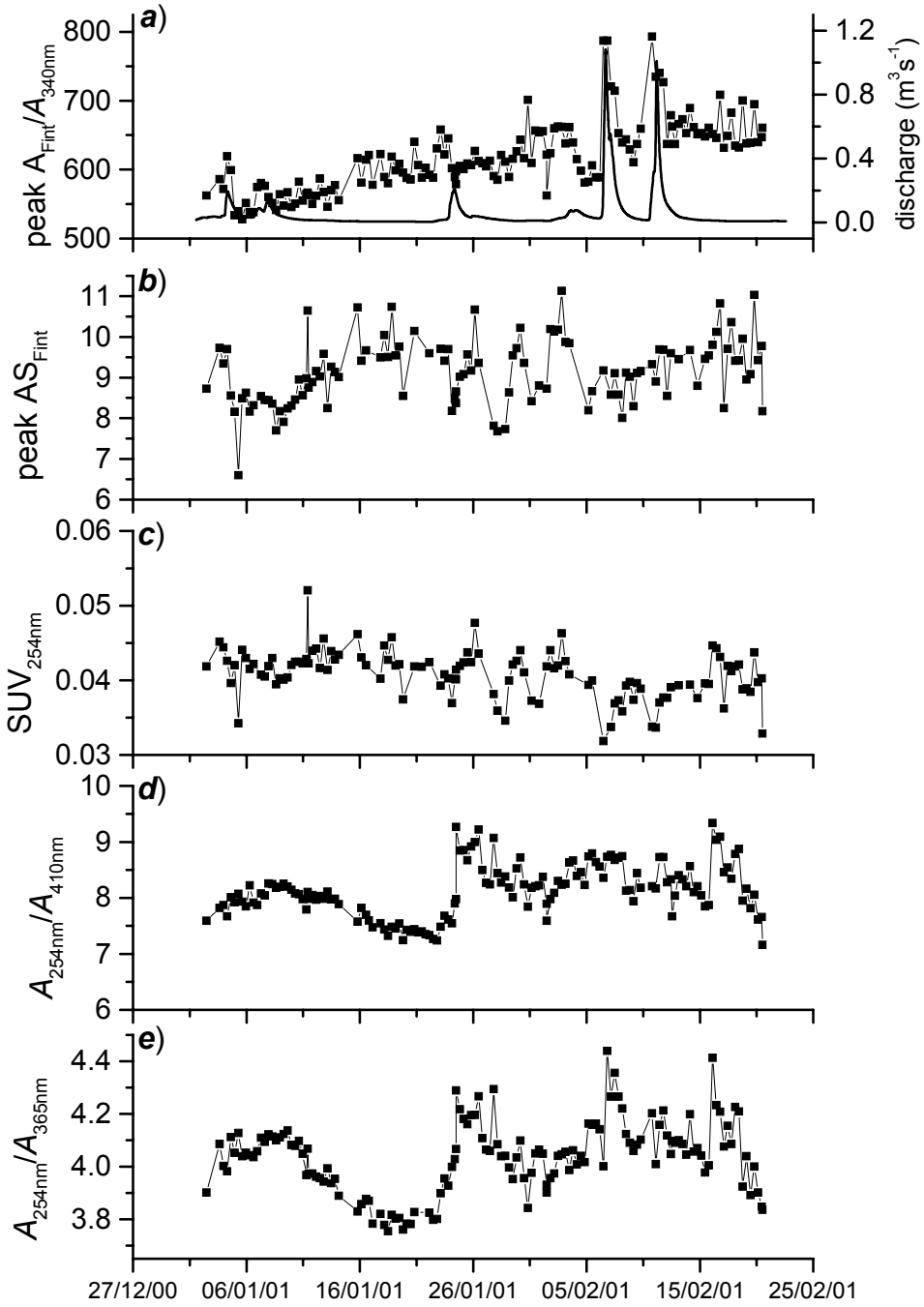


Figure 4.12
Time series from winter high resolution sampling of CB_{weir} ***a)*** *peak A_{Fint}/A_{340nm} and discharge (m^3s^{-1})* ***b)*** *peak AS_{Fint}* ***c)*** *SUV_{254nm}* ***d)*** *A_{254nm}/A_{410nm}* ***e)*** *A_{254nm}/A_{365nm}*

As presented in Figure 4.12 peak A_{Fint}/A_{340nm} significant peak values of ~790 coincided with the peaks in discharge on both 06/02/01 and 10/02/01. The maxima in peak A_{Fint}/A_{340nm} occurred prior to the peak in discharge, on the rising limb of the hydrograph. Between the discharge events levels dropped by ~22%.

From the examination of flow relationships in this data set during the event of 06/02/01 there was a significant increase of peak A_{Fint}/A_{340nm} with changing discharge. This relationship exhibited hysteresis, as shown in Figure 4.13, with peak A_{Fint}/A_{340nm} preceding flow and changes occurring rapidly on the rising limb. The same overall pattern was observed during the event of 10/02/01. At this time mean peak A_{Fint}/A_{340nm} was 641 (s.d. 44) in P_{weir} and 1156 (s.d. 109) in PG_{weir} and from the examination of the spatial differences in the variable (Figure 4.6) this relationship can be interpreted as a change in the source of the DOM. As rainfall and discharge increases peak A_{Fint}/A_{340nm} also increases as DOM is preferentially transported from peaty-gley sub-catchment. This is followed by an influx of DOM from peat sub-catchment as DOM sources in this area are activated. From the distribution of DOM spectrophotometric properties the shift in peak B wavelengths and the increasing DOC concentration, during this event, also indicates a source of DOM the peat sub-catchment after peak flow.

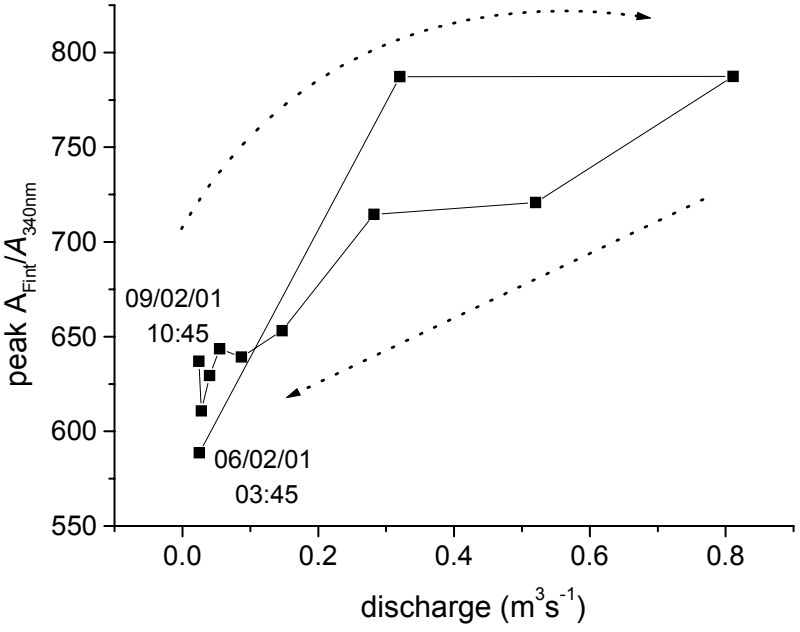


Figure 4.13
The relationship of peak A_{Fint} /A_{340nm} in CB_{weir} to flow, during the rainfall event of 06/02/01.

4.4.5 Rainfall events during summer (August / September 2001)

DOC concentration exhibited a constant mean level over this period (31.12±2.56), having a similar range and variance in the data as the winter sampling period. In the time series shown on Figure 4.14 two responses to hydrological conditions related to the peaks in discharges can be observed. A decrease of 19% on 08/08/01 and 12.5% on 13/08/01 coincided with an increase in discharge. This was not observed in the discharge event of 19/08/01, when no response was apparent. The first two discharge events (08/08/01 and 13/08/01) were dilution responses, due to an input of low DOC concentration event water such as rainwater or from the peaty-gley sub-catchment. Subsequent to this, the response to rainfall and increased discharge did not affect the DOC concentration.

It is unclear, due to lack of prior data, if the peak in DOC concentration, seen in Figure 4.2, during September (2001) was the major or the only peak in DOC concentration and if it represented the "autumn flush" of DOM. It was, however the final flush of DOM before DOC concentration declined to lower winter average level. The DOC concentration peak identified in Figure 4.2 lasted for a number of days 19/08/01 to beginning of 12/09/01 (mean DOC concentration = 32.39 s.d = 1.53). DOC concentration significantly fell by 35% with the next discharge event.

		Response of peak A_{Fint}
1	*01/01/01 → 07/08/01 06:30*	Decrease in values (01/08/01 to 03/08/01) (mean = 406.03 s.d. = 34.18)
	07/08/01 → 08/08/01 14:30	Rapid decrease, coinciding with increased rainfall and discharge
2	*08/08/01 14:30 → 21/08/01 5:40*	Period of varying intensity, having a low value at peak discharge (not significant 95% confidence level) (mean = 299.87 s.d. = 41.77)
	21/08/01 05:40 → ~26/09/01 21:40	Rapid increase in values
3	*26/08/01 → 12/09/01 23:00*	Relatively constant and overall high values (mean = 430.47 s.d. = 28.19)

Table 4.8
Summary of the changes over time of peak A_{Fint} in CB_{weir} during high resolution sampling, summer/autumn 2001.

Peak A_{Fint} and peak B_{Fint} exhibited somewhat similar patterns to DOC concentration however the two related variables diverged, as shown in Table 4.6. Fluorescence intensity exhibited three periods of different mean values, summarised in for peak A_{Fint}. The mean values of peak A_{Fint} and peak B_{Fint} in period 1 and 3 were significantly higher than period 2 and period 3 was higher than period 1 (95% confidence level). Specific fluorescence intensity exhibited the same trend as peak A_{Fint} and peak B_{Fint}.

The pattern over time observed in absorbance is shown in Figure 4.14 as A_{340nm}. The trend in A_{340nm} was similar to DOC concentration, however, exhibited slightly different variations. There was a rapid decrease to a minimum coinciding with the decrease seen in peak A_{Fint} Table 4.8 07/08/01 to 08/08/01 of 23.7%, the minimum in A_{340nm} (0.405) occurred at approximately maximum discharge on 08/08/01.

As shown in Figure 4.14 there was a significant decrease in A_{340nm} values, which occurred at the same time as the discharge event on 19/08/01 of 24.95% (95% confidence level). Lowest absorbance (A_{340nm} 0.505 and 0.494) corresponded to points both prior to and immediately after peak discharge and at peak discharge there was an increase to 0.574 in absorbance. This event resulted in both a dilution and flushing in relation to DOM absorbance. As rainfall increased dilution of the ambient signal at CB_{weir} by low absorbance event water occurred, as discharge peaked and rainfall totals declined, water with higher absorbance was then transported to CB_{weir}. After peak discharge further dilution was observed, as rainfall briefly increased, followed by a rapid rebound to pre-event levels.

This cycle may be explained by comparison to the catchment runoff model of Mounsey (1999) and the observations made in relation to pH. The initial dilution by low absorbance water may be derived from rainfall input direct to the main stream and possibly to a greater extent by peaty-gley sub-catchment water being displaced to the catchment outfall. As the drainage ditches and surface peat layers are flushed water of higher absorbance was displaced and during peak flow this was the dominant signal. The second dilution event however, may represent the input of water to CB_{weir} that has had little interaction with the peat, possibly transported in infilled ditches or as surface flow, which as discussed in Section 3.6 exhibited overall lower absorbance compared to ditches with bare peat faces. As flow decreases absorbance returns to pre-event levels.

The pattern observed in absorbance was not seen in DOC concentration or fluorescence intensity indicating a variation in DOM composition relating directly to absorbance. SUV_{254nm} and $Svis_{410nm}$ exhibited noisy trends, with no significant variations. This may reflect the smaller degree of definition of these variables between sources within the catchment. As discussed in Section 3.6.4 the DOM from peat sub-catchment and peaty-gley sub-catchment have similar specific absorbance compared to the greater difference in absorbance. During this period waters from both sub-catchments and the main channel had similar specific absorbance values (CB_{weir}=0.05 s.d. 0.006; P_{weir}=0.051 s.d. 0.003; PG_{weir}=0.049 s.d. 0.005). The difference in absorbance between DOM in peaty-gley sub-catchment waters (A_{340nm} ~0.44) and peat sub-catchment waters (A_{340nm} ~0.6 to 0.7) was significant at this time. This suggests that a dilution by the former may occur during the increased discharge on 19/08/01, however, is only recognised in the DOM absorbance signal.

The variations in pH over this event were dominated by a decrease in pH at peak flow; this was typical of the responses observed by Mounsey (1999) and indicates the inputs of low pH peat sub-catchment waters at peak discharge. There was a slight increase in pH coinciding with the second decrease in absorbance, although not conclusive this may suggest the influence of a pulse of water form peaty-gley sub-catchment contributing to both the absorbance and pH signals.

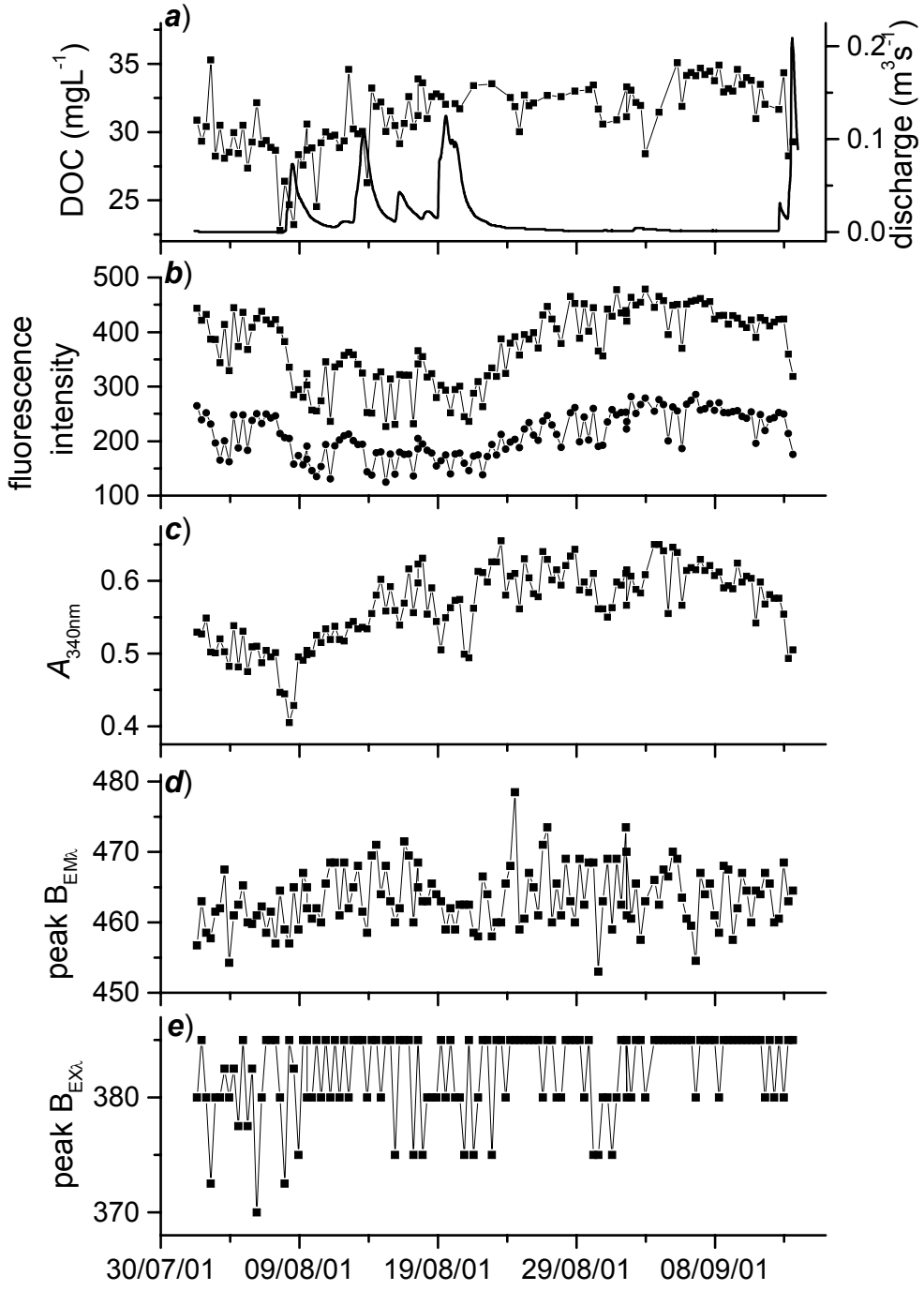


Figure 4.14
Time series from summer/autumn high resolution sampling of CBweir ***a)*** *DOC concentration (mgL^{-1}) and discharge (m^3s^{-1})* ***b)*** (■) *peak* A_{Fint} (●) *peak* B_{Fint} ***c)*** A_{340nm} ***d)*** *peak* $B_{EM\lambda}$ ***e)*** *peak* $B_{EX\lambda}$

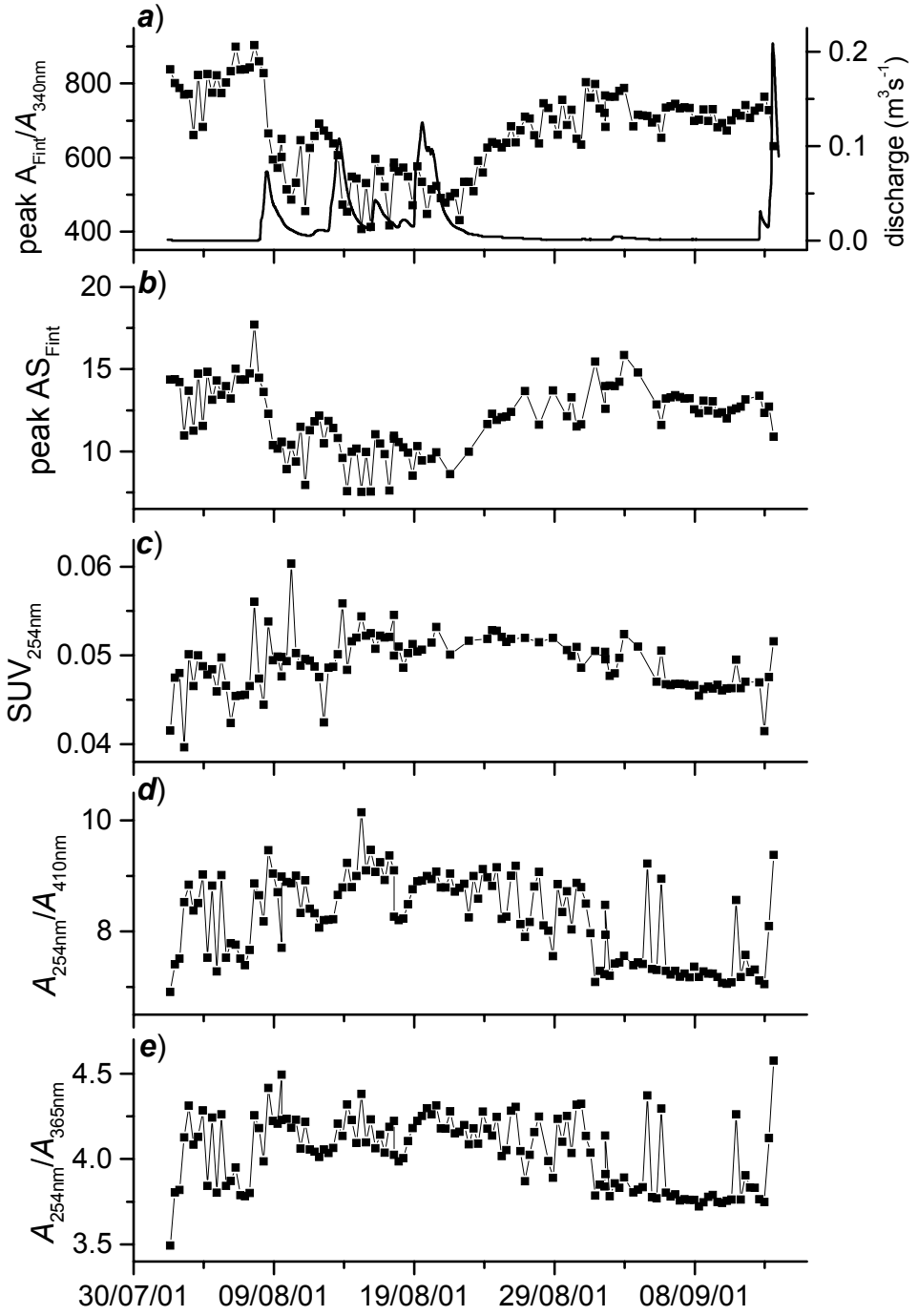


Figure 4.15

Time series from summer/autumn high resolution sampling of CB_{weir} ***a)*** *peak A_{Fint} /A_{340nm} and discharge (m^3s^{-1})* ***b)*** *peak AS_{Fint}* ***c)*** *SUV_{254nm}* ***d)*** *A_{254nm}/A_{410nm}* ***e)*** *A_{254nm}/A_{365nm}*

As shown in Figure 4.15 peak A_{Fint}/A_{340nm} had high levels (mean = 801.97 s.d. = 64.82) before the first discharge event, on 08/08/01. These values corresponded to the levels observed in PG_{weir} (sample 01/08/01 CB_{weir} = 838 PG_{weir} = 883) suggesting that during this low flow DOM was derived from this sub-catchment. During the first discharge event levels of peak A_{Fint}/A_{340nm} declined to a significantly lower value from 08/08/01 to 22/08/01 (mean = 542.31 s.d. = 73.14; 99% confidence level). Within this period levels increased between the first and second discharge events (12/08/01) by 22% and then rapidly fell as rainfall and discharge increased. After 22/08/01 peak A_{Fint}/A_{340nm} rapidly increased to a higher level that was significantly lower than the level seen prior to 08/08/01 (mean =704.31 s.d. = 49.83; 99% confidence level).

The relationship of peak A_{Fint}/A_{340nm} to discharge, with particular reference to 09/08/01 and 13/08/01, is shown on Figure 4.14 and exhibits clockwise hysteresis. During both of these events peak A_{Fint}/A_{340nm} decreased and post-event levels are lower than pre-event levels. Between the events levels increased but a further increase in flow rapidly depressed peak A_{Fint}/A_{340nm} by ~30%. From the differentiation of high peak A_{Fint}/A_{340nm} in peaty-gley sub-catchment waters and low in peat sub-catchment waters discussed in Section 3.6 this trend identifies the partitioning of DOM sources. The low levels observed during higher flow periods represent inputs of DOM from peat sub-catchment sources. Over the events observed the progressive lowering of peak A_{Fint}/A_{340nm} may indicate that runoff from peat sub-catchment sources is increasing in importance as a source of DOM as catchment conditions change. Such changes include increased flow in forestry ditches, which are often stagnant during early summer.

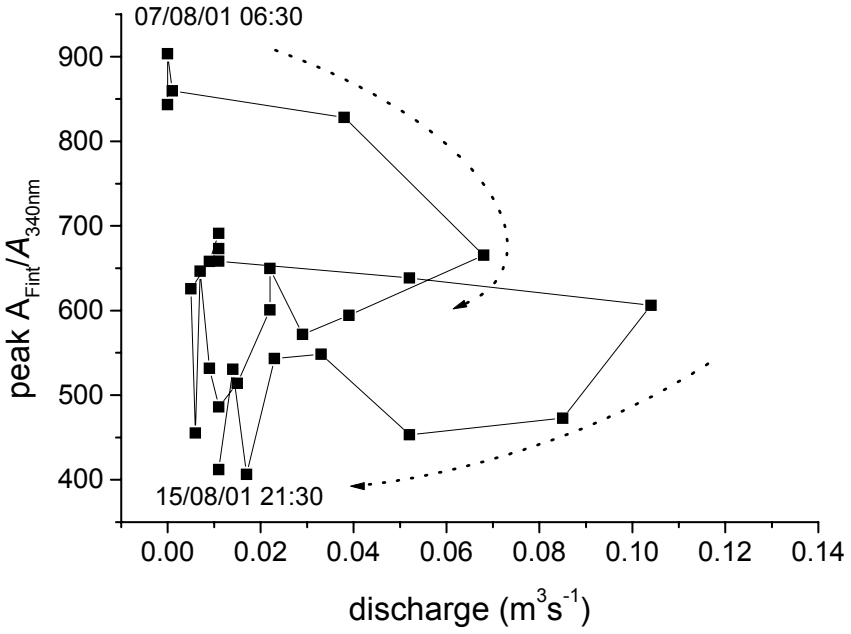


Figure 4.16
The relationship of peak A_{Fint} /A_{340nm} in CB_{weir} to flow, during the rainfall events of 09/08/01 and 13/080/1

As shown in Figure 4.15 prior to peak discharge and coinciding with maximum rainfall there was a decrease of 12% and 9% in A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm} respectively. This was seen for the three main peaks of discharge and the differences were significant between high and low values in A_{254nm}/A_{410nm} (95% confidence level). This indicates that rainfall activated a source of low absorbance ratio DOM prior to peak discharge. During this period there was a relatively lower mean for both A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm} in PG_{weir} (7.33) compared to P_{weir} (9.90) and peat sub-catchment ditches indicating that a similar pattern is observed to that seen in absorbance, peaty-gley sub-catchment DOM is transferred to the main channel with an increase in rainfall.

Fluorescence intensity peak wavelengths showed little variations, as shown in Figure 4.15 for peak $B_{EX\lambda}$ and peak $B_{EM\lambda}$. There were no obvious relationships to other variables or the catchment conditions, such as discharge. Peak C values also showed little variation over this period.

4.4.6 Summary of rainfall event DOM monitoring

From the examination of high resolution DOM variations the principal fluctuations were observed to occur synchronously with changes in rainfall amounts and discharge. The trends observed during both the winter and summer/autumn periods reveal temporal DOM variations related to changes in DOM source between the peat and peaty-gley sub-catchments.

During the winter period this was manifest as a dilution of DOC during high flow, due to an influx of peaty-gley sub-catchment derived DOM or precipitation to the main channel. Between the observed discharge events DOC concentration increased due to waters from peat sub-catchment reaching the catchment outfall. This is most clearly expressed in the temporal variations of peak A_{Fint}/A_{340nm} as this variable defines the DOM from each sub catchment well. Peak B wavelengths also show this pattern. The discharge event of 10/01/01 included a snowmelt component which in some studies snowmelt has been observed to result in an increase in DOC concentration in surface waters (Sakamoto *et al*., 1999) due to flushing of DOM from surface soil layers. In this study snowmelt results in the dilution of surface waters. Surface flow paths of snowmelt being relatively depleted in DOM by previous flushing during the period of maximum DOC concentration. Due to the forested nature of the catchment snowmelt may also not significantly displace high DOC concentration water from the ditch network into the main channel. Runoff and input to CB_{weir} from the peaty-gley sub-catchment may be preferential during snowmelt due to the proximity to the catchment outfall and the steeper slopes of this area.

The summer/autumn period also exhibited a differentiation of DOM relating to changes in the source between the two sub catchments. This was demonstrated in the trends of absorbance and peak A_{Fint}/A_{340nm}. Prior to the onset of rainfall on 14/08/08 peak A_{Fint}/A_{340nm} was similar in the main channel to peaty-gley sub-catchment DOM, however over time this is reduced, suggesting an increasing input form the peat sub-catchment. Within individual discharge events a change between DOM from the two sub catchments can be identified. With increasing flow the main channel receives inputs from peat sub-catchment resulting in increased absorbance and decreased peak A_{Fint}/A_{340nm}. After peak flow this is reversed and DOM is derived from the peaty-gley sub-catchment or other DOM depleted sources. Absorbance specifically indicates the switching of sources with increased rainfall, exhibiting inputs

of event or peaty-gley sub-catchment water, followed by peat sub-catchment water as discharge increases.

The first observed discharge event exhibited a decrease in DOC concentration indicating that high DOC concentration peat sub-catchment sources were not activated in this event. Only after sufficient rainfall occurred is DOM exported from here. A source of DOM is the forestry ditch network and, as discussed in Section 4.3.3 the accumulation of DOM can occur here even during low flow. This DOM is rapidly transferred from the ditch network to the main channel during rainfall. During the observed period sufficient rainfall did not occur until 14/08/01 to facilitate this transport route of DOM, all monitored ditches did not exhibit flow until this date. Thus, the initial discharge event was one of DOC dilution, whereas later this became flushing, observed in absorbance trends, as DOM was transported from the ditches.

The spectrophotometric properties of DOM in the main channel during rainfall events show a change in composition with source. This was discussed in Chapter 3 and is observed as a change from low molecular weight/aromaticity when peaty-gley sub-catchment inputs are dominant to high molecular weight/aromaticity when DOM is derived from the peat sub-catchment.

The long term temporal trends shown in Figure 4.2 indicate that there was a peak in DOC concentration values during September 2001. From the examination of the high resolution data set this peak was not as abrupt as it appears in Figure 4.2. Over the high resolution sampling period there was an overall increase in DOC concentration, however no individual peaks were seen. This indicates the care that is required in the examination the long term data sets with sampling at relatively low resolution, and the necessity of examination of such data in combination with the catchment conditions at the time of sampling.

4.5 Rates of DOC export from the Coalburn Experimental Catchment

DOC flux was calculated using Equation 4.1. This calculation is “Method 2” described by Walling and Webb (1981) and as applied to DOC fluxes by Hope *et al*. (1997b).

$$Load = K\sum_{n}^{i=1}\left[C_i Q_i / n\right] \qquad \textbf{Equation 4.1}$$

Where

K = conversion factor to take account of the period of record

C_i = instantaneous concentration measurement (mgL^{-1})

Q_i = instantaneous discharge measurement at the time of sampling (Ls^{-1})

n = number of samples

The calculated annual flux of DOC from the Coalburn Experimental Catchment is detailed in Table 4.9. The figures calculated from all the data obtained during the study are elevated in comparison to observations from other peat dominated areas, as shown in Table 4.10. Absolute comparisons cannot be made between these studies, due to differing estimation methodologies and possible responses to long term climate fluctuations. This study compares more closely to the higher values seen in peatlands including forested areas (Moore, 1989) which exhibit a greater DOC export in comparison to unforested peatlands.

Due to the gaps in sampling the total annual flux may be biased, thus figures for winter/spring and summer/autumn periods are detailed in Table 4.9. These estimates of annual flux and rates of export include data from different years during the study. From the calculated values it can be seen that overall there was a greater export of DOC from the catchment during winter and spring periods. This differentiation was also seen in the comparison of fluxes calculated from high resolution sampling data only.

In comparison to winter periods, during summer and autumn the catchment exhibited an overall mean higher DOC concentration. The difference in export rates and amounts during these periods, however, is due to the low measurable discharge at the catchment outfall during summer/autumn.

During winter and spring ~54% of the DOC export occurred during relatively high discharge conditions (>$0.1m^3s^{-1}$) whereas during the summer this accounted for ~24%. This suggests that although there was a higher level of DOC concentration in the catchment surface water during summer and autumn (Figure 4.2) it was not entirely exported during this time due to relatively low flow and that during winter periods high flow conditions account for the majority of DOC export. This has been recognised in other studies, where storm events have been observed to be important in the export of DOC from catchments (Hinton *et al*., 1997). During the winter high resolution sampling period an increase in discharge coincided with a dilution of DOC

concentration, however, the increased flow during this period results in a relatively high rate and amount of DOC export, shown in Table 4.9.

	Estimated annual export of DOC (g DOC m^2yr^{-1})	Rate of DOC flux (g DOC ms^{-1})
Total sampling period	22.00 ± 5.67	
Winter/spring	29.64 ± 3.07	1.400 ± 1.12
Summer/autumn	11.10 ± 1.00	0.528 ± 0.04
"Autumn flush"	17.67 ± 1.24	0.841 ± 0.01
Winter high flow	105.16 ± 1.55	5.002 ± 0.01

Table 4.9
Estimated annual export of DOC and the rate of DOC flux from the Coalburn Experimental Catchment, calculated from all the data available and data from selected periods of the study, using Equation 4.1.

DOC export (g DOC $m^{-2}yr^{-1}$)	Study area	Reference
8.4	Thoreau's Bog (USA)	McKnight *et al*. (1985)
8-21	forested catchments (NZ)	Moore (1989)
3-4	peatlands (USA/Canada)	Urban *et al*. (1989)
20	northern peatland	Gorham (1995)
7-15	upland peat (UK)	Scott *et al*. (1998)
8.3	ombrotrophoic bog (Canada)	Fraser *et al*. (2001)
2.8	forested catchment (USA)	McDowell and Likens (1988)
8.4	moorland (UK)	Grieve (1984)
2.5	forested catchment (USA)	David *et al*. (1992)
1.85/1.08/0.84	forested/grassland/headwater (Switzerland)	Frank *et al*. (2000)
2.88	forested catchment (China)	Tao (1998)

Table 4.10
Summary of DOC exports from forested and wetland catchments.

The period recognised to exhibit the greatest DOC concentration, as discussed above occurs during the autumn, when catchment conditions result in sufficient flow to displace DOM produced during the previous drier conditions. This flush period was identified by calculation of the rate of DOC flux using Equation 4.1 to monthly periods throughout the study. August and September in both 2000 and 2001 resulted in the highest rate of DOC flux, in a monthly period. As shown in Table 4.9 the "autumn flush" exhibits a high rate and relatively large export of DOC. Using the current method of estimating DOC flux winter high flow periods exhibited a greater rate and amount of DOC flux, in comparison to the "autumn flush". The winter high flow

periods, however, had a relatively limited temporal extent. Overall the major periods of DOC export were autumn-winter.

4.6 Chapter 4 Conclusions

In this chapter the temporal variations in DOM in the Coalburn Experimental Catchment have been presented and discussed to achieve the aims stated.

- *To identify seasonal differences in DOM spectrophotometric properties*

Seasonal differences in DOM manifested as a period of DOM export during autumn and DOM production and storage during spring/summer. The DOM in the main channel was closely related to catchment conditions and transfer from specific areas of the catchment. The observation that these changes can be recognised in DOM spectrophotometric properties indicates that this analytical technique has potential as a tracer in flow path studies in such areas.

- *To examine the response of DOM to changes in rainfall and discharge, over on both an annual cycle and during individual events to relate these variations to catchment conditions, discharge, flow paths and sources, using the spatial characteristics discussed in Chapter 3.*

It can be concluded that both qualitatively and quantitatively DOM export is controlled by the influence of precipitation upon different areas of the catchment. Two periods can be identified which DOM exhibited different responses to rainfall:
1. During spring-autumn (approx May-September) when the catchment is under low flow conditions the rapid preferential transport of DOM from the peaty-gley sub-catchment to the main channel occurs during low magnitude rainfall and at the onset of rainfall events, on the rising limb of the hydrograph. At peak flow DOM sourced from the peat sub-catchment become dominant. This source of DOM is the forestry ditch network, where DOM accumulates during low flow and is flushed only when catchment conditions become sufficiently wet for hydrological connectivity in the ditch network to activate flow here.

2. During winter (approx September to April) DOM in the main channel is derived from the peat sub-catchment. During rainfall events DOM is preferentially transported from the peaty-gley sub-catchment. Snowmelt does not result in DOM export.

- *To estimate the DOC export from the catchment.*

It was estimated that the total annual export of DOC was 22.00 g DOC m^2yr^{-1} a value at the high end of the range observed in previous work. This value varied throughout the year and DOC export was greatest during autumn. Export rates were highest during winter high flow conditions, however these periods were limited in extent.

Chapter 5

Spectrophotometric Properties of Aquatic Dissolved Organic Matter in the Loch Assynt Area

5.1 Introduction

The following chapter will discuss spatial variations in spectrophotometric properties of DOM in the Loch Assynt, area using spectrophotometric techniques to establish how DOM. DOM sampled from streams and standing water throughout the area will be compared to evaluate the source controls on spectrophotometric properties. These controls and the overall character of the DOM will be compared to the DOM from the Coalburn Experimental Catchment, discussed in Chapter 3. In Chapter 6 temporal fluxes in DOM will be assessed using a time series from River Traligill. An examination of peat derived DOM is made in Chapter 8 from profiles sampled in three locations in the area.

5.1.1 The aims of the spatial monitoring of DOM in the Loch Assynt area

- To characterise using spectrophotometric techniques DOM in the Loch Assynt area and to compare these characteristics to DOM from the Coalburn Experimental Catchment.
- To compare river and stream water DOM spectrophotometric properties to DOM from peat pools and from loch water, to investigate variations across this area and to identify the mechanisms that influence this pattern, such as soil, flow paths and autochthonous processing.
- To compare river and stream water draining two different lithologies. This comparison aims to establish if DOM in runoff from these areas is different, as detected using the current methods.

5.2. Water sampling in the Loch Assynt area

The temporal variations and characteristics of DOM were examined through sampling of the River Traligill (April 2000 to March 2002) and spatial variations by sampling of a variety of water bodies throughout the area. Details of the locations and dates of sampling are presented in Appendix 5.

Water samples from the River Traligill were routinely taken and flow was gauged at Inchnadamph (NC 25152175) (Figure 1.8), from April 2000 to March 2002. Periods of high intensity sampling were performed during April and September 2000, May and September 2001 and March 2002. During September 2001 sampling was performed at 1.5 hourly intervals. Additional water samples were taken throughout the River Traligill catchment and the wider Loch Assynt area from streams and lochs, of a range of sizes, and pooled water.

All water samples were taken, stored and analysed using the parameters discussed in Section 2.2. In addition to these analyses selected water samples were acidified to pH 2 and analysed for calcium concentration using ICP (Unicam 701 ICP-OES).

5.2.1 Spatial grouping of samples

For the purpose of relating spectrophotometric properties to source and processing the samples were divided into four groups, related to the aquatic setting and visually observed comparative water colour. The groups represent the range of local biogeochemical influences on DOM composition and a wide range of previously observed water colour. The division was as follows: -

Group 1. Rivers and streams draining carbonate bedrock dominant catchments**.** Very low water colour to uncoloured water.
Group 2. Rivers and streams draining quartzite dominant and non-carbonate bedrock catchments. Very low to moderate water colour. Groups 1 and 2 represent streams draining the two dominant lithologies in the area, above which soils consist of peat and mineral soils of varying thickness and extent. Quartzite draining group 2 streams are predominantly surface draining, whereas group 1 streams undergo varying amounts of flow in underground conduits and waters from both groups are more or less influenced by inorganic interactions.
Group 3. Small pools of standing water directly on peat surfaces of moderate to high water colour. Water in such pools is entirely derived from precipitation interactions with the peat, especially in the Traligill Basin where it is recognised that there is no

groundwater input to the peat (Charman *et al.*, 2001). It provides direct information on the characteristics of unaltered source DOM in areas of peat cover.

Group 4. Lochs and lochans of very low to low water colour. DOM in lake water undergoes distinct processing and characteristics may relate to these or to the properties of the inflowing DOM. Processes that may be recognisable in spectrophotometric properties include changes in composition and molecular size via microbial action, photodegradation and flocculation and also production of autochthonous DOM by phytoplankton (Schindler *et al*., 1997).

5.3 Spatial variations in surface water in the Loch Assynt area

To establish if any seasonal trends were present the data were divided into three groups, sampled during April 2000, September 2000 and May 2001. There were no significant differences (95% confidence level) in all the analysed variables between samples taken during each period. As samples were not consistently taken from replicated locations during the different sampling periods, the spatial variability in DOM accounted for a greater proportion of the differences observed compared to temporal variability.

The non-spectrophotometric characteristics of the samples from the Loch Assynt area are summarised in Figure 5.1. Group 3 exhibited the lowest mean pH, which was significantly lower than the other groups. Conductivity was highest in group 3, the but means from all groups were statistically indistinguishable (95% confidence level). The highest mean DOC concentration and water colour was observed in group 3 samples (208.3 mg Pt L^{-1}). These mean concentrations were significantly higher, compared to the other sample groups, which were statistically indistinguishable (99% confidence level). Colour correlated positively with DOC concentration in groups 1, 2 and 4 (Spearman's rho 95% confidence level).

A gradient of DOC concentration and water colour from high concentrations in group 3 to group 2 and low concentrations in groups 1 and 4 can be seen in Figure 5.1. This reflects the influence of peat on the control of DOM in the area. Water in contact with peat (for example, peat pools) exhibited high DOC concentration due to greater direct dissolution of organic matter. The rivers sampled in the area are fed by such peat derived waters. The low DOC concentration and increased pH indicate significant inputs of water from other sources, such as groundwater, and modification or dilution of the peat derived geochemistry.

Loch and lochan water (group 4) had a mean DOC concentration of 4.4mgL^{-1}. This data included lochans situated in peat dominated areas, where the loch water was on average 67.2% reduced in DOC concentration compared to the inflowing streams. These water bodies had a mean DOC concentration of 10.2mgL^{-1}in comparison to larger lochs located in mineral soil and or bedrock dominated areas, which had a mean of 2.0mgL^{-1}. These mean concentrations were significantly different (95% confidence level). Thus, the lochs situated in more upland areas have a significant input from high DOC concentration peat derived runoff.

In lakes the balance of DOC concentration is related to the inputs from the catchment and biological production and removal from the system by export, sedimentation, microbial and photochemical mineralization (Reche and Pace, 2002). In-lake processes of photodegradation and photobleaching have been recognised to remove the coloured fraction of DOM more rapidly in comparison to uncoloured. These processes were not recognised, as the ratio of water colour to DOC concentration is constant across the sample groups.

From the fluorescence spectrophotometric analysis of all DOM from the Loch Assynt area the EEMs obtained compared closely to the typical results discussed in Section 1.5. Peaks A, B and C were identified throughout. High fluorescence intensity, at excitation wavelengths of <250 nm relating to peak E and F, was also present. Peak D was not observed. No other fluorescence intensity peaks were identified. The means and ranges of wavelengths of the monitored fluorescence intensity maxima are summarised in Figure 5.2.

Peaks A and B exhibited maximum fluorescence within the regions identified in previous work. The standard deviation about the mean of data from each group and in the data set as a whole of both wavelengths for each peak did not exceed the analytical error (Section 2.2). The difference in emission wavelengths between sample groups was, on average 5nm, which is lower than the reproducibility of the method (Section 2.2).

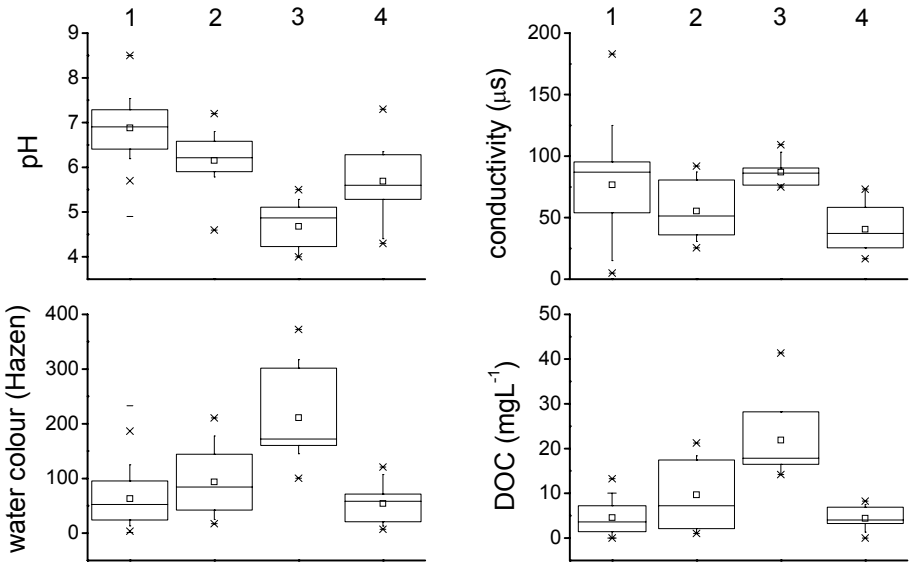


Figure 5.1
Box plots of DOC concentration (mgL^{-1}), pH, conductivity (μS) and water colour (Hazen) in surface water in each sample group from the Loch Assynt Area. For key to box plots seen Figure 3.2.

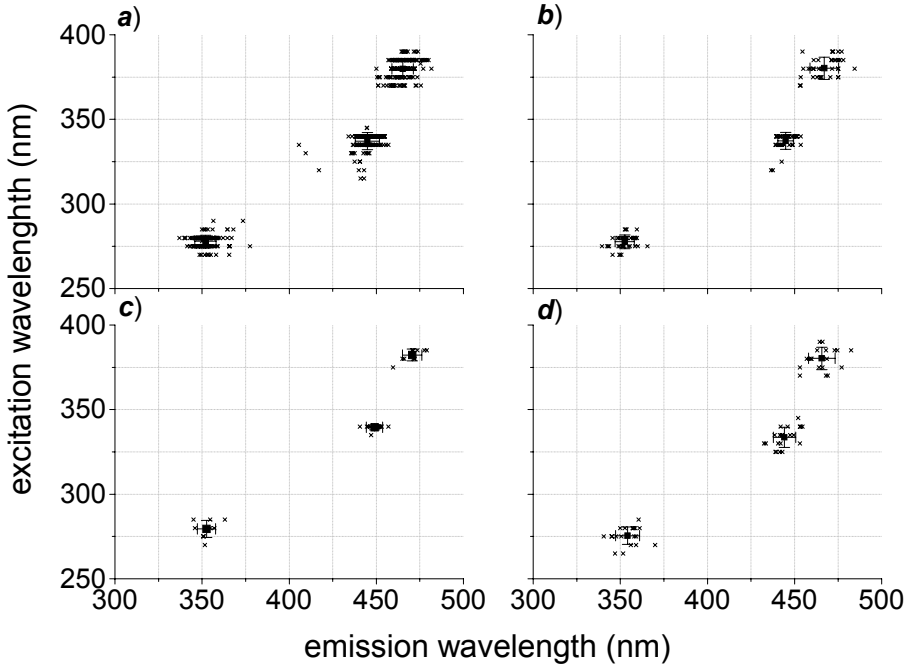


Figure 5.2
The positions, within EEMs, of all the fluorescence intensity maxima, identified in surface water from Loch Assynt area (+) *all data* (■) *mean values* ***a)*** *group 1* ***b)*** *group 2* ***c)*** *group 3* ***d)*** *group 4*

In a number of samples peak A had short excitation wavelengths <325 nm and emission wavelengths <425 nm, a shift of 12nm and 20xnm from the mean.

The three samples identified to have peak $A_{EM\lambda}$ of <425 nm were sampled from similar streams, within one area on the same day. The conditions during this sampling period included periods of snowmelt and the short wavelengths may represent inputs from snow, which exhibited no peak A-like fluorescence, or from sources activated during snowmelt. During this period, however, other sampled streams that drain similar locations, with respect to soil and altitude did not exhibit such short wavelengths, indicating the complexities in the spatial variation in DOM spectrophotometric properties.

The samples with blue shifted emission wavelength also exhibited the highest measured calcium concentrations in the study (mean = 31.20 mgL^{-1} s.d. = 0.576). Calcium exhibited significantly higher mean concentrations in group 1 (mean = 10.29 mgL^{-1} s.d. = 6.72) compared to the other groups, which were statistically indistinguishable (95% confidence level). The calcium concentration decreased from group (mean = 4.03 mgL^{-1} s.d. = 4.75) to group 4 (mean = 3.11 mgL^{-1} s.d. = 2.72) with the lowest mean in group 3 (mean = 2.24 mgL^{-1} s.d. = 0.95).

Romkens and Dolfing (1998) showed that calcium preferentially flocculated higher molecular weight DOM suggesting that longer wavelength fluorophores may be removed from solution or retained in the calcium rich soils. This was discussed by Baker and Genty (1999), in relation to groundwater in the Traligill catchment. The authors observed calcium concentrations of 24-40 mgL^{-1} and peak $A_{EX\lambda}$ 306.1 $\pm$ 4.7nm and peak $A_{EM\lambda}$ 414.6 $\pm$ 3.3nm and a negative relationship between calcium concentration and wavelength. The groundwater calcium concentrations to emission wavelength relationships are replicated in the blue shifted emission samples of this study. These relationships indicate that the inorganic components of soil and water can act to alter DOM spectrophotometric properties. The blue shifted samples did not exhibit different aromaticity to other samples, indicating the emission wavelength shift is more sensitive measure of the influence of calcium ions on DOM.

The distribution of fluorescence intensities, fluorescence intensity ratios and absorbance, represented by A_{340nm} are summarised in Figure 5.4. The same

relationships were observed in absorbance at all measured wavelengths. Group 3 DOM exhibited mean peak A_{Fint} of 60% peak B_{Fint} of 33% and A_{340nm} of 68% in comparison to the means of the other three groups (99% confidence level). Significant differences in the means of peak A_{Fint}, peak B_{Fint} and A_{340nm} can be summarised as follows: group 3> group 2> group 1= group 4 (95% confidence level).

Typical absorbance and specific absorbance spectra of DOM from each of the four groups of samples from Loch Assynt area are shown in Figure 5.5. The spectra show featureless curves, resembling those previously reported and shown in Figure 3.10 from the Coalburn Experimental Catchment. Absorbance, in a number of samples was low and approached the minimum detection limit at long wavelengths. This occurred at approximately $>A_{500nm}$ and in the example shown in Figure 5.5a no absorbance was measured for the group 1 sample at longer than A_{605nm}. Specific absorbance (mg DOC L^{-1} cm^{-1}) spectra as shown in Figure 5.5b exhibited statistically indistinguishable means in all groups throughout the spectra.

Mean peak B_{Fint}/peak A_{Fint} as shown in Figure 5.4 was highest in group 1, significantly so when compared to the other sample groups (99% confidence level). This mean, however, was primarily derived from results of analyses of River Traligill samples, which had peak B_{Fint} mean of 0.684. When data from River Traligill is discounted the means of peak B_{Fint}/peak A_{Fint} are statistically indistinguishable between group 1 and group 2. Mean peak C_{Fint} was highest in group 4 (23.29 s.d. 9.79) and both this and the mean in group 3 (21.15 s.d. 5.48) were significantly higher compared to groups 1 and 2 (95% confidence level). Mean peak C_{Fint}/peak A_{Fint} was highest in group 4 significantly so, in comparison to group 2 and group 3 (95% confidence level).

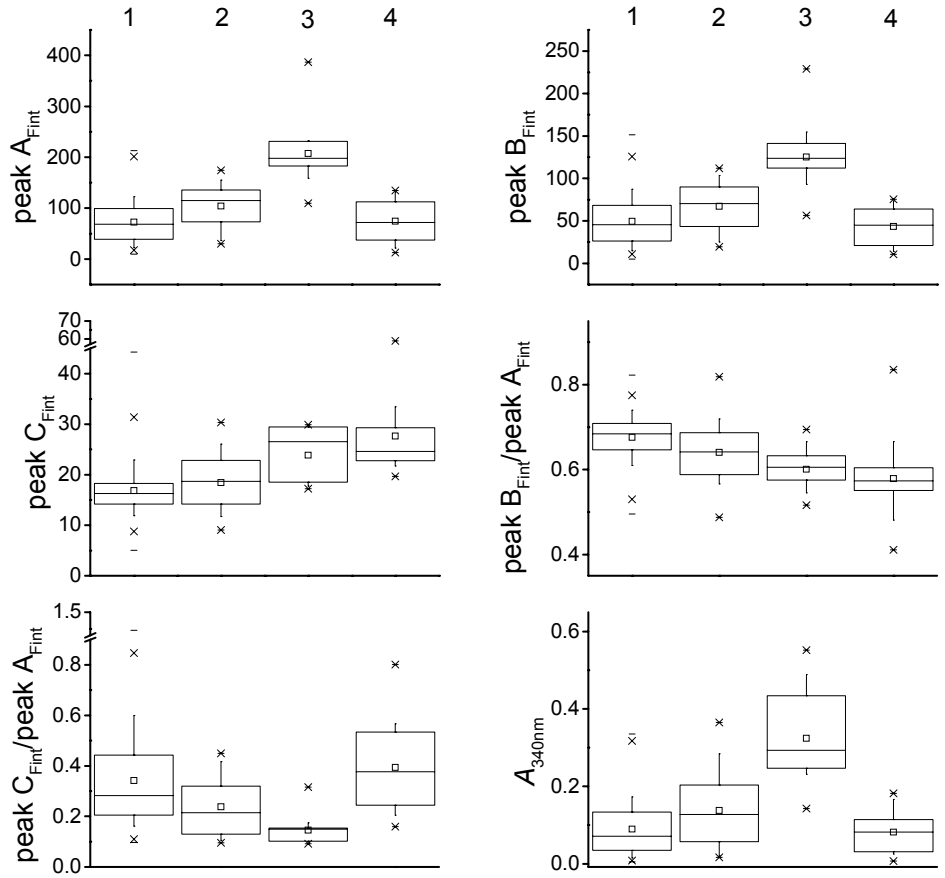


Figure 5.3
Box plots of peak A_{Fint}, peak B_{Fint}, peak C_{Fint}, peak B_{Fint} /peak A_{Fint}, peak C_{Fint} /peak A_{Fint} and A_{340nm} in surface water in each sample group from the Loch Assynt Area. For key to box plots see Figure 3.2.

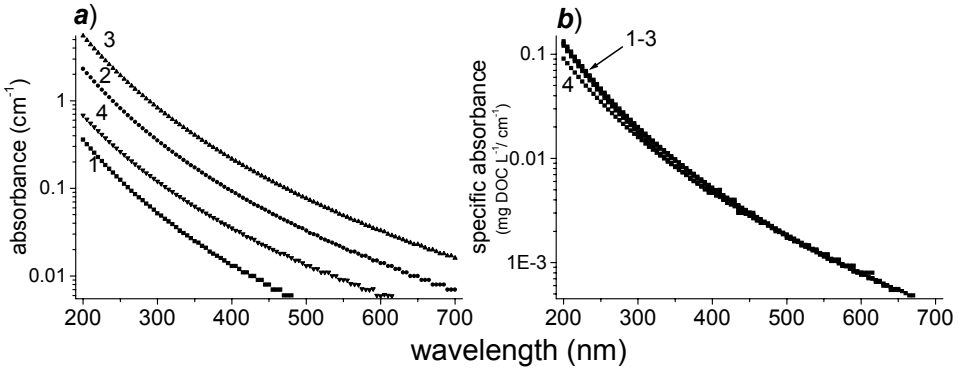


Figure 5.4
Typical absorbance spectra in surface water from the Loch Assynt area ***a)*** *absorbance (cm^{-1})* ***b)*** *specific absorbance (mg DOC L^{-1}/cm^{-1})*

Peak A_{Fint}, peak B_{Fint} and absorbance exhibit significant positive correlations with DOC concentration (99% confidence level). In the data set as a whole and in all individual groups except group 3 both peak A_{Fint} and peak B_{Fint} correlated positively (Spearman's rho 99% confidence level) with all absorbance wavelength measurements. This indicates the control that DOC concentration has over both absorbance and fluorescence intensity. As presented in Table 5.1 the amount of variation in the fluorescence and absorbance data that was explained by DOC concentration varies between each sample group. For example, the variations in group 4 fluorescence intensity data is only explained ~30-40% by DOC concentration compared to up to 79% in the other groups. This suggests that within loch water there may be a significant component of non-fluorescent DOM.

In relation to absorbance data the amount of variation explained by DOC concentration varies with sample source and wavelength observed. For example group 3 samples are better explained at A_{254nm} and group 4 at A_{410nm}. This may indicate the depletion of aromatic UV absorbing chromophores in loch water by photo-degradation (Donahue *et al*., 1998). Group 2 samples had the strongest relationship of fluorescence intensity and absorbance to DOC concentration. Overall absorbance had a stronger relationship with DOC concentration compared to fluorescence intensity, replicating the relationships observed in the Coalburn Experimental Catchment.

	Peak A_{Fint}		**Peak B_{Fint}**	
group 1	55.2%	DOC=-1.000+A_{Fint}*0.095	56.0%	DOC=-0.591+B_{Fint}*0.129
group 2	67.5%	DOC=-4.893+A_{Fint}*0.145	79.7%	DOC=-6.133+B_{Fint}*0.233
group 3	63.3%	DOC=-3.295+A_{Fint}*0.109	65.6%	DOC=-3.712+B_{Fint}*0.183
group 4	37.9%	DOC= 1.161+A_{Fint}*0.049	32.9%	DOC= 1.599+B_{Fint}*0.074
	A_{254nm}		***A_{340nm}***	
group 1	52.5%	DOC= 0.520+A_{254nm}*20.967	59.3%	DOC= 0.452+A_{340nm}*60.181
group 2	96.4%	DOC=-1.717+A_{254nm}*28.244	96.8%	DOC=-0.597+A_{340nm}*71.454
group 3	70.2%	DOC=-1.417+A_{254nm}*24.670	60.9%	DOC= 0.948+A_{340nm}*58.582
group 4	31.7%	DOC= 2.139+A_{254nm}* 9.302	57.1%	DOC=-0.357+A_{340nm}*60.670
	A_{410nm}			
group 1	61.2%	DOC=-0.134+A_{410nm}*165.785		
group 2	93.6%	DOC=-1.865+A_{410nm}*222.178		
group 3	54.3%	DOC= 4.218+A_{410nm}*147.938		
group 4	73.4%	DOC=-0.665+A_{410nm}*175.353		

Table 5.1
The results of linear regression of fluorescence intensity and absorbance against DOC concentration in surface water from the Loch Assynt area showing the percentage variation explained by DOC concentration and the equation of the linear regression.

Mean peak AS_{Fint}, specific absorbance and estimated aromaticity calculated from molar absorptivity ($moleCL^{-1}cm^{-1}$) at A_{272nm} were statistically indistinguishable between each sample group (95% confidence level), as presented in Figure 5.6 When samples of very low DOC concentration (<1.5mgL^{-1}) were discounted mean SUV_{254nm}, absorbance and estimated aromaticity was significantly higher in group 3 (0.055 s.d. 0.014) than group 1, 2 and 4, (0.038, 0.047, 0.045) however, not significantly so (95% confidence level). Removal of these low DOC concentration data points did not alter the distribution of specific fluorescence intensity data.

As shown in Figure 5.7 mean peak A_{Fint}/A_{340nm} was significantly lower in group 3 (676.49 s.d. 177.41), compared to groups 1, 2 and 4 (95% confidence level) (1125.30 s.d. 905.91). Group 2 had the highest mean, however groups 1, 2 and 4 were statistically indistinguishable. A_{465nm}/A_{665nm} was only consistently measured in groups 2 and 3 as absorbance at long wavelengths approached zero in groups 1 and 4. The available data indicated no significant differences in this variable (95% confidence level).

DOM from group 3 had significantly higher A_{254nm}/A_{410nm} (8.27 s.d. 1.34) compared to groups 1 and 2 (95% confidence level) (6.92 s.d. 2.18 and 6.98 s.d. 2.00). Group 4 samples had similar mean A_{254nm}/A_{410nm} compared to group 3 and showed a greater range in values of 46%. Mean A_{254nm}/A_{365nm} was statistically indistinguishable between the sample groups (95% confidence level).

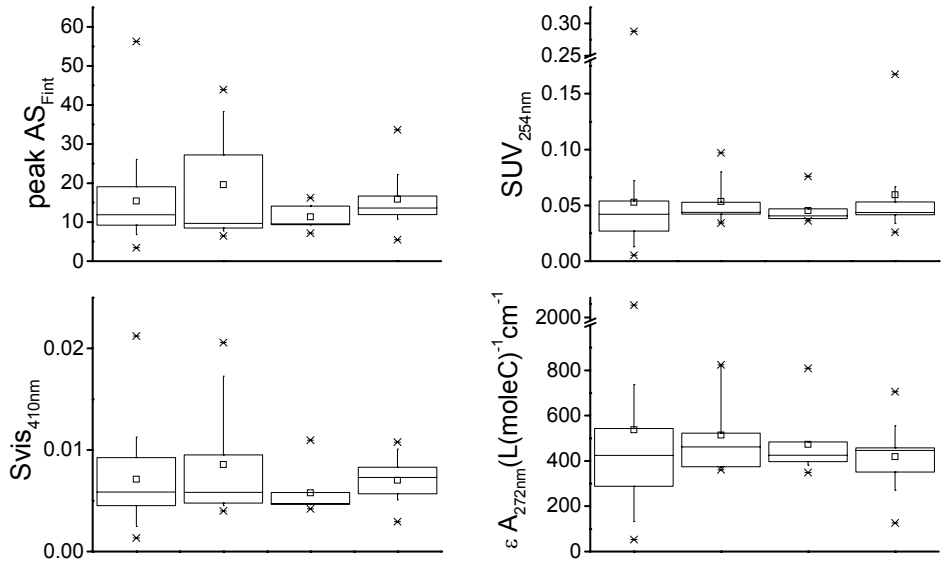


Figure 5.5
Box plots of peak AS_{Fint} SUV_{254nm}, $Svis_{410nm}$ and aromaticity estimated from molar absorptivity ($molCL^{-1}cm^{-1}$) at A_{272nm} in surface water from the Loch Assynt area. For key to box plots seen Figure 3.2.

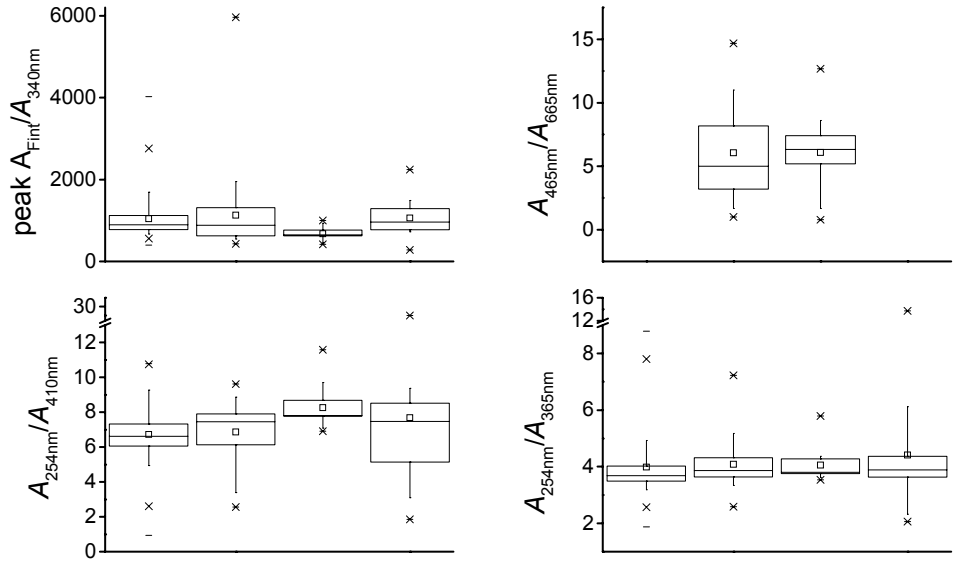


Figure 5.6
Box plots of peak A_{Fint} /A_{340nm} A_{465nm}/A_{665nm} A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm} in surface water from the Loch Assynt area. For key to box plots seen Figure 3.2.

To investigate the spatial variations on a smaller scale within the Loch Assynt area the catchment of the River Traligill was examined. The main channel, where surface flow occurred, and the major tributaries were sampled. Monitoring was performed on 19/05/01.

Samples taken from the River Traligill between the confluence with Loch Assynt to the Lower Traligill resurgence (Figure 1.8) showed no significant variations in spectrophotometric properties. Tributaries draining from the north and south areas of the catchment exhibited similar spectrophotometric properties to the main channel. Tributary water had a 27% higher DOC concentration and concentration related variables, compared to the main channel (95% confidence level).

Surface waters draining the Traligill Basin area had significantly 60% higher DOC concentration compared to River Traligill main channel. There were no other significant differences in spectrophotometric properties (95% confidence level). This represents a gradient of DOC concentration down stream in the catchment; however, this gradient is not mirrored by compositional differences, such as that observed between stream and peat pool data. This suggests that peat pool type DOM becomes modified if it is transferred to streams. This DOM character, may only relate to DOM formed by leaching from the peat and modification with the standing water. DOM flushed from the acrotelm to streams may not have this character.

5.3.1 Discussion of the spatial variations in spectrophotometric properties of DOM in Loch Assynt area

The spatial assessment of DOM in the Loch Assynt area reveals a source of DOM in upland areas. A continuum in DOC concentration was observed from upland to lowland surface waters. This was related both to the proximity of organic rich soils in the former and the flow paths and processes in the latter.

DOM in peat pools had a specific character. Peak C_{Fint} and absorbance ratios show, in comparison, to stream and loch water the presence of poorly degraded DOM rich in carbohydrates and protein (Section 2.2). This can be derived from biological activity and autochthonous DOM production or breakdown of plant matter (Spitzy and Leenheer, 1991; Zsolnay *et al.*, 1999). The absorbance ratios from peat DOM from these pools was low (mean 4.29 s.d. 1.25)(Section 2.5) indicating that when

transferred from peat to water modification of DOM occurs. In further comparison to other surface waters peat pools exhibited higher molecular weight DOM This distribution can be interpreted with reference to soil type and the retention of DOM with a higher molecular weight in inorganic soils (Section 3.6). Reduced aromaticity or molecular weight with interactions with inorganic matter is exhibited in the relationship of emission wavelengths and calcium.

Overall, loch water DOM was relatively similar to stream waters. The spectrophotometric signal of entirely autochthonously produced DOM, identified in McKnight *et al.* (2001) was not observed in loch water DOM, for example, in emission wavelengths. This may suggest that the DOM spectrophotometric properties in the loch water monitored are due to a combination of the properties of the terrestrial inputs and further modification by in-lake processes. Loch water was however found to contain a significant component of non-fluorescent DOM.

The comparison of surface water draining different lithologies indicated that quartzite streams had elevated DOC concentration compared to limestone, however this may be related to the dominance of peat above the former. There appears to be no control on DOM properties by underlying bedrock and associated groundwater sources and processes.

The examination of spatial variations as discussed above poorly defines the DOM from each sample source, however, samples of varying DOC concentration exhibited specific characteristics. When broader spatial variations were examined higher DOC concentration and absorbance was noted in samples from group 1, 2 and 4 that were associated with the peat covered areas of the study area. To identify these broader spatial variations in spectrophotometric properties samples were ranked according to DOC concentration. This was performed using A_{340nm} as a proxy for DOC concentration. Absorbance and DOC concentration are highly correlated in the data set and although the variation in absorbance is not completely explained by DOC concentration a proxy was used, as DOC concentration data was not available for all of the samples.

The 25th, 50th and 75th percentiles were used to rank samples into the ranges shown on Table 5.2. Samples from groups 1,2 and 4 were included in each ranked group. As shown in Table 5.2 group 3 was only represented at greater than the 50th percentile level. Spectrophotometric properties observed to significantly vary

between ranked groups are summarised in Table 5.3. Other variables showed no significant patterns.

The examination of Loch Assynt area data when ranked, based on DOC concentration indicates a range of lower molecular weight, simpler, less aromatic and less conjugated DOM at low DOC concentration compared to more aromatic higher molecular weight DOM at high concentrations. The relative spectrophotometric characterisation of DOM reflects a broad spatial relationship to soil. Lower DOC concentration derives from soils with less organic content and the retention of specific fractions of DOM in inorganic material resulting in lower DOC concentration and related compositional variations.

			Percentage of samples in each absorbance ranked group			
Ranked group	Percentile	A_{340nm} range	Group1	Group 2	Group 3	Group 4
(1)	0-25th	0.0000-0.0368	26.99	21.95	0.00	28.57
(2)	25th-50th	0.0368-0.0875	29.45	14.63	0.00	23.81
(3)	50th-75th	0.0875-0.1533	24.54	26.83	9.09	33.33
(4)	75th-100th	0.1533-0.5520	19.02	36.59	90.91	14.29

Table 5.2
Details of the division of samples from the Loch Assynt area when ranked according to A_{340nm}.

Peak C_{Fint}/peak A_{Fint}	Mean decrease with increased ranked group (**1**) 0.545 (s.d. 0.186) (**4**) 0.153 (s.d. 0.041)
Peak A_{Fint}/A_{340nm}	Mean decrease with increased ranked group (**1**) 1586.998 (s.d. 606.249) (**4**) 663.298 (s.d. 120.612)
Peak A_{EM}	+7.59nm shift with increased from ranked group (**1**) to (**4**)
Peak AS_{Fint}	Mean in ranked group (**4**) 10.268 (s.d. 2.741) lower than ranked group (**1**) 18.23639 (s.d. 14.249) and (**2**) 19.489 (s.d. 12.616)
A_{254nm}/A_{410nm}	Mean in ranked group (**1**) 5.829 (s.d. 2.897) lower than ranked group (**2**) 7.476 (s.d. 2.374) (**3**) 7.424 (s.d. 1.815) and (**4**) 7.326 (s.d. 1.174)

Table 5.3
Summary of significant variations in DOM spectrophotometric properties from the Loch Assynt area when ranked according to A_{340nm}. All trends 95% confidence level.

5.4 Comparison of the DOM from the Loch Assynt area to DOM from the Coalburn Experimental Catchment

The following section will discuss, identify and summarise the differences and similarities of the surface water DOM spectrophotometric properties in the Loch Assynt area and the Coalburn Experimental Catchment discussed in Chapter 3. This comparison will be used to establish if the spectrophotometric techniques applied can differentiate between DOM from distinct areas, with different soil type, vegetation and flow paths, and if these methods can provide evidence for compositional differences.

Overall this comparison indicates that the Loch Assynt area peat pool DOM has similar spectrophotometric properties to the Coalburn Experimental Catchment peat sub-catchment derived DOM. PG_{weir} DOM has a number of characteristics that are closer to stream and loch water from the Loch Assynt area. These similarities are seen in fluorescence intensity, absorbance, peak wavelengths, specific absorbance and absorbance ratios. The significant differences observed in spectrophotometric properties from the two study areas are summarised in Table 5.4

Absorbance ratios that increase with decreasing molecular size fraction (Peuravuori and Pihlaja, 1997) were relatively low in the Loch Assynt area stream and loch DOM compared to DOM from Coalburn Experimental Catchment. The high values of A_{254nm}/A_{410nm} in the Coalburn Experimental Catchment observed appear to be derived from a small proportion of the data from peat derived waters. Of the non-peat derived DOM samples 82.6% had A_{254nm}/A_{410nm} of lower than 10 and in peat derived DOM this was 93.1%. 10 was chosen as a cut off as it was found by Anderson *et al.* (2000) that values up to 10 were identified in DOM fractions of >50,000 Da in molecular size and above 10 values represented sizes smaller than this. Overall, peat derived DOM appears to have relatively higher molecular weight, compared to non-peat DOM, however, there is a wide range of values that indicates this property is variable.

When the relationships of both absorbance and fluorescence intensity to DOC concentration are examined in a combination of all the data from Loch Assynt area and Coalburn Experimental Catchment a greater proportion of the variation can be explained than when examined in individual data sets (Table 5.5). From examination

of the literature a number of similar relationships of absorbance and DOC concentration have been reported. For example, Tipping *et al*. (1988) 57% and 76% (A_{340nm}); Vogt *et al.* (2001) 88% (A_{400nm}); Reche and Pace (2002); 74% (A_{440nm}) and Worral *et al*. (2002) 80% (A_{400}). These values compare well to data in this study.

From the examination of the relationships detailed in Table 3.7, 3.8 and 4.6 the amount of variation in the absorbance and fluorescence intensity data that was explained by DOC concentration from both areas was similar. A wide variation from different sources within each area was observed. On the whole there was a closer relationship of absorbance to DOC concentration than fluorescence intensity to DOC concentration in both data sets. This was not always the case in each sample site.

DOC concentration, absorbance, peak A_{Fint}, peak B_{Fint} and water colour	CB_{weir} and peat sub-catchment > Loch Assynt area streams and lochs by ~76.3% CB_{weir} and peat sub-catchment > Assynt peat pools by ~28.2%
Peak $A_{EM\lambda}$ and peak $A_{EM\lambda}$	Assynt peat pools > PG_{weir} by 9.5nm and 15nm
Peak A_{Fint}/A_{340nm}	Loch Assynt area streams and lochs >CB_{weir} and peat sub-catchment by 39.5%
Peak B_{Fint}/peak A_{Fint}	Loch Assynt area streams and lochs >CB_{weir}, peat sub-catchment and PG_{weir} by 14.9%
Peak C_{Fint}	Loch Assynt area streams and lochs >CB_{weir} and peat sub-catchment by 29.2% PG_{weir} > Assynt peat pools by 48.3%
A_{254nm}/A_{410nm}	CB_{weir} and peat sub-catchment > Loch Assynt area streams and lochs by ~20.1%
Peak AS_{Fint}	Loch Assynt area streams and lochs >CB_{weir} and peat sub-catchment by 35.5%
$Svis_{410nm}$	Loch Assynt area streams and lochs >CB_{weir} and peat sub-catchment by 35.3%

Table 5.4
Summary of the significant differences between DOM spectrophotometric properties from Loch Assynt area and Coalburn Experimental Catchment. All relationships 95% confidence level.

		Variation explained by DOC concentration
peak A_{Fint}	DOC=3.34+peak A_{Fint}*0.08	75.2%
peak B_{Fint}	DOC=2.84+peak B_{Fint}*0.15	69.4%
***A*$_{254nm}$**	DOC=6.18+A_{254nm}*15.47	81.2%
***A*$_{340nm}$**	DOC=4.25+A_{340nm}*49.72	86.7%
***A*$_{410nm}$**	DOC=5.33+A_{410nm}*140.41	81.6%

Table 5.5
Linear relationships of DOC concentration to fluorescence intensity and absorbance in data from Loch Assynt area and Coalburn Experimental Catchment combined.

From spectrophotometric properties a distinction can be made between low DOC concentration waters of lower molecular weight dominated DOM versus higher DOC concentration and molecular weight DOM. The first category of DOM includes Loch Assynt area streams and loch water and $PG_{weir.}$ The second other DOM sampled in the Coalburn Experimental Catchment and peat pools in the Loch Assynt area.

5.4.1 Discriminant analysis of the spatial variations in DOM

Using the parameters discussed above that are significantly different with DOM source further statistical analysis was performed on the data from the Loch Assynt area and the Coalburn Experimental Catchment. Discriminant analysis has been found to be useful in the examination of spectrophotometric data from river water. Baker (2002c) found the technique differentiated between DOM from individual tributaries. The method allocates an individual (a water sample), on the basis of its properties (x), to one of n groups or populations (sample source). The variables selected for the discrimant analysis are shown in Table 5.6. DOC concentration related variables were not included as discriminant analyses performed with these resulted in the data being entirely discriminated by this variable. This indicates the strong relationship of DOC concentration to source.

The results of the discriminant analysis are shown in Table 5.6, 5.7 and 5.8 and Figure 5.8. Figure 5.8 presents a plot of the first two discriminant functions, which, as shown in Table 5.7 explained 97.9% of the variance in the data set. Function 1 explained 85.3% of this variance and, as shown in Table 5.6, peak B_{Fint}/peak A_{Fint} and peak A_{Fint}/A_{340nm} exhibited the highest correlations with this function. Function 2 explains a further 12.6% of the variance and A_{254nm}/A_{410nm} exhibited the highest correlations with this function. Peak $A_{EM\lambda}$ was negatively correlated in both functions and SUV_{254nm} exhibited little correlation with either. The latter is not differentiated between DOM source and it exhibits greater correlations with higher numbered

functions, as shown in Table 5.7. These functions account for a small proportion of the variance in the data set.

As shown in Figure 5.8 Loch Assynt stream water and PG_{weir} have the highest scores in the first function, due to values of peak B_{Fint}/peak A_{Fint} in the former and peak A_{Fint}/A_{340nm} in the latter. These two sample groups are discriminated in function 2 due to the comparatively higher levels of A_{254nm}/A_{410nm} in PG_{weir} DOM. The greatest discrimination in function 1 can be seen between PG_{weir} and Loch Assynt streams compared to peat sub-catchment ditches, due to the long mean peak $A_{EM\lambda}$ in this DOM.

Within function 1 there is a sequence, from low DOC concentration (<20 mgL^{-1}) at positive scores to high (>20 mgL^{-1}) at negative scores, which is represented by the difference between PG_{weir} and peat sub-catchment ditches. Within this gradient CB_{weir} Loch Assynt area peat pools and P_{weir} all plot close to zero in this function, suggesting that the sample sources cannot be differentiated using these parameters. In addition to this Loch Assynt area loch water plots close to CB_{weir} and has wide range of scores overlapping the distribution of Coalburn Experimental Catchment waters, suggesting that although there is an apparent DOC concentration gradient along function 1 lower DOC concentration waters have a similar position to higher. This suggests that the differences in DOM characteristics are not entirely controlled by DOC concentration.

P_{weir} had positive scores in function 2 due to the levels of A_{254nm}/A_{410nm} in this data set. CB_{weir} could not be discriminated from peat sub-catchment waters and a large proportion of CB_{weir} samples were predicted as belonging to these sources (Table 5.6), which indicates the close relationship of DOM in CB_{weir} to the peat sub-catchment. The close plots of Loch Assynt stream and PG_{weir} reflect the influences of inorganic interactions in the immobilization of DOM and DOM fractions in soils on DOM from these sources.

As shown in Table 5.6 the prediction of group membership calculated from this analysis is correct for 54.39% of the samples, however, this varies between each group. For example, PG_{weir} and peat sub-catchment ditches were identified in the majority of cases correctly. P_{weir} and Loch Assynt loch and peat pool DOM, however, were identified correctly in less than half the samples. This can be seen in Figure 5.8 where group centroids plot close together.

From the application of discriminant analysis it is suggested that the parameters used have a limited use in the discrimination between DOM from different sources. Furthermore the discrimination that has been identified is related to DOC concentration differences. The scores for function 1 correlate significantly negatively (99% confidence level) with sample DOC concentration, absorbance and peak A_{Fint}. This reflects the wide range of DOC concentrations observed and the identification of distinct spectrophotometric properties at high and low concentrations.

The spectrophotometric properties of loch water DOM plot closely to peat derived DOM in comparison to stream derived DOM on Figure 5.8. This suggests that the DOM sampled was distinct in comparison to the overall nature of that inflowing to the lochs. This is not, as suggested, due to photo-degradation, or biological activity fractionating the DOM. If this were the overriding process governing DOM spectrophotometric properties in loch water a character of overall lower absorbance, especially at longer wavelengths would be expected (Donahue *et al*., 1998). This is not seen. The positions of the respective DOM sources shown on Figure 5.8 suggest that the DOM properties result from the observed abundance of peat sediment within the lochs of the area (Boomer, 2003, personal communication) and the derivation of loch water DOM from this

	Function				
	1	2	3	4	5
A_{254nm}/A_{410nm}	-0.111	0.739	0.455	0.456	-0.163
Peak B_{Fint}/peak A_{Fint}	0.624	-0.102	0.719	-0.239	0.163
Peak A_{Fint}/A_{340nm}	0.601	0.243	-0.666	0.316	0.191
SUV_{254nm}	0.035	-0.098	0.349	0.817	-0.447
Peak $A_{EM\lambda}$	-0.293	-0.155	0.349	0.322	0.815

Table 5.6
The correlations of discriminating variables to canonical discriminant functions. source.

Function	% of Variance	Cumulative %
1	85.3	85.3
2	12.6	97.9
3	1.3	99.3
4	0.6	99.9
5	0.1	100.0

Table 5.7
The variance in the dataset explained by the first five canonical discriminant functions of the discriminant analysis of Loch Assynt and Coalburn Experimental Catchment data.

	Cases correctly assigned by discriminant analysis
CB_{weir}	51.79%
P_{weir}	42.30%
Coalburn peat ditches	70.27%
PG_{weir}	80.00%
Loch Assynt lochs and peat pools	38.46%
Loch Assynt streams	64.70%
total	54.39%

Table 5.8
The percentage of samples correctly classified into the sample group by discriminant analysis

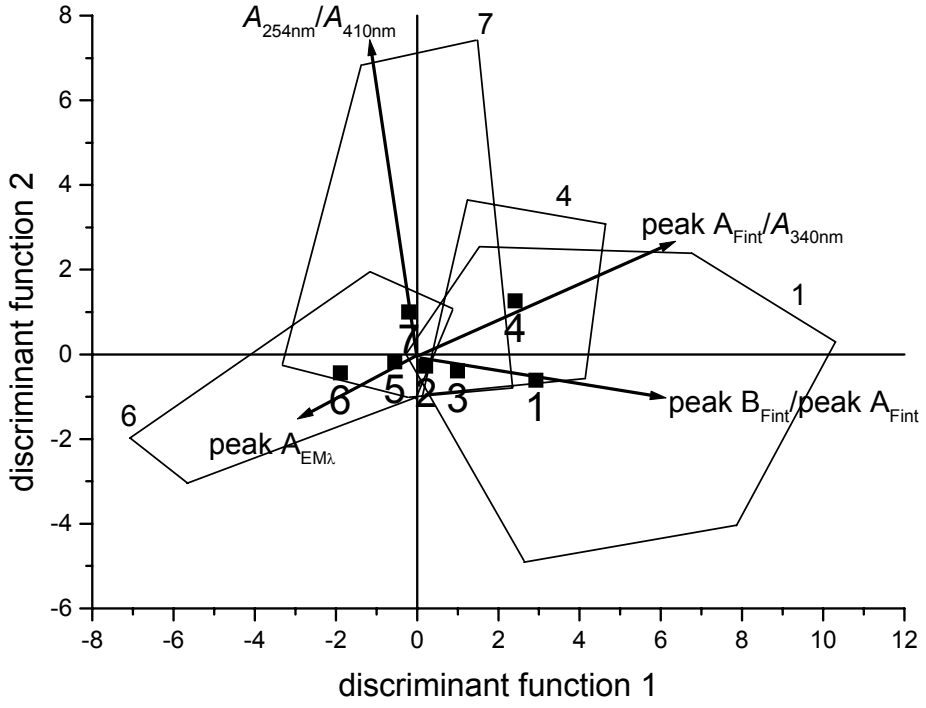


Figure 5.7
Discriminant analysis of data from Loch Assynt area and Coalburn Experimental Catchment: scatter plot of the first two discriminant functions.
Data points indicate the group centroids of each data set. 1=Loch Assynt streams 2= Loch Assynt peat pool 3= Loch Assynt loch water 4= PG_{weir} 5= CB_{weir} 6= peat sub-catchment ditches 7= P_{weir}. Arrows represent the direction in which discriminant variables increase. Enclosed areas represent the spread of data point for Loch Assynt streams, PG_{weir}, peat sub-catchment ditches and P_{weir}. Data points for the other data sets plot within these areas.

5.4.2 Summary of the spatial variations in DOM from the Coalburn Experimental Catchment and the Loch Assynt area

From the comparison of the Loch Assynt area and the Coalburn Experimental Catchment it can be recognised that DOM from peat pools in the former area and that related to peat sub-catchment waters in the latter area were similar in comparison to non peat sourced DOM. These samples, from PG_{weir}, lochs and streams in the Loch Assynt area had overall similar spectrophotometric properties. These patterns were recognised statistically by discriminant analysis. The identification of DOM properties using these techniques in these examples appears limited, as there are a wide range of values and only small differences between DOM from different sources. A molecular weight difference may be observed ranging from a prevalence of smaller DOM in non-peaty derived waters to higher in waters from peat dominated sources. This is related to inorganic interactions that retard the movement of larger molecular material and aggregates by sorption (Zhou *et al*., 2001).

High values in certain parameters, for example A_{254nm}/A_{410nm} in P_{weir} and short wavelengths in Loch Assynt area stream waters, skew the data sets. These extreme values represent instances of specific conditions and indicate the natural variations observed in DOM spectrophotometric properties. This suggests that the measured properties are highly sensitive to the conditions at the time of sampling. Broad spatial variations are observed, however, seasonal and climate differences may require consideration in the interpretation of DOM spectrophotometric properties.

5.5 Conclusions

The aims of this section were to use spectrophotometric properties to analyse DOM from an area in northwest Scotland. Samples were designated according to the source and the methods were used to identify differences between DOM from each setting. The conclusions made from this chapter are further considered in Chapter 6 relation to temporal changes in DOM in the River Traligill.

- *To characterise using spectrophotometric techniques DOM in the Loch Assynt area and to compare these characteristics to DOM from the Coalburn Experimental Catchment.*

This study provides an indication of the spectrophotometric character of DOM on a wider scale than the Coalburn Experimental Catchment and with differing vegetation cover and influence from both peat and mineral soils. In the comparison of DOM from the Loch Assynt area and the Coalburn Experimental Catchment it can be concluded that DOM from peat areas is similar in composition in both study areas, as is DOM in non-peat areas.

- *To compare river and stream water DOM spectrophotometric properties to DOM from peat pools and from loch water, to investigate variations across this area and to identify and suggest the mechanisms that influence this pattern, such as soil, flow paths and autochthonous processing.*

The DOM source and flow paths can be identified as a control upon the spectrophotometric properties and composition of surface water DOM. The influence of inorganic soil components on DOM and the retention of high molecular weight and aromatic material is the principle factor that spatially differentiates DOM. Association with peat-dominated areas also controls DOM concentration, during transport from such source areas both dilution and modification of DOM occurs, indicating that flow paths strongly influence DOM.

A source of DOM is observed specifically in peat pool derived DOM. This source is poorly degraded plant material and/or autochthonous production or modification of DOM within the peat pool. This DOM is not observed in other parts of the catchment, as it is either not exported from the peat pools, or is altered or diluted upon transport again showing the importance of flow paths upon DOM.

The factors controlling on loch water DOM not were found to be photodegradation or other processes within the water body. Loch water DOM was sourced from both inflowing streams and peat rich sediment in the lochs. A significant component of DOM in loch water was non-fluorescent.

- *To compare river and stream water draining two different lithologies. This comparison aims to establish if DOM in runoff from these areas is different, as* detected using the current methods.

Surface water in areas of peat cover is enriched in DOM, which has a more aromatic composition. This is in comparison to DOM depleted surface water in non-peat areas. The influence of underlying lithology upon surface water DOM cannot be separated from the influence of soil type.

Chapter 6

Temporal Patterns in Dissolved Organic Matter in the Loch Assynt Area

6.1 Introduction

The following chapter will discuss the variations in spectrophotometric properties of DOM in the River Traligill over time during sampling from April 2000 to March 2002. The general distribution of spectrophotometric properties in the river water and broad patterns over time will be discussed. From the observations in spatial data discussed in Section 5.3 possible flow paths and DOM sources are examined. The data will be compared to that observed in the Coalburn Experimental Catchment as discussed in Chapter 4 to investigate the temporal patterns observed in different rivers.

The catchment of the River Traligill includes areas of distinct geology and both peat and mineral soils as described in Section 1.7.2 and shown in Figure 1.8. Sampling and flow measurement was performed at Inchnadamph (NC 25152175). High resolution sampling was performed during April and September 2000, May and September 2001 and March 2002. Sampling was performed at 1.5 hourly intervals during September 2001.

Samples taken intermittently from the River Traligill were not consistently stored as recommended in Section 2.4, due to conditions during transit. The errors relating to sample storage may have been incurred in the analyses of these samples. In the statistical analyses of the data discussed in the following chapter these samples are not considered and are included in the discussion of temporal DOM patterns as a background and indicator of long term variations only.

6.1.1 Aims

- To characterise the spectrophotometric properties of DOM from the River Traligill
- To identify temporal patterns in DOM in the River Traligill and relate to DOM seen in the wider Loch Assynt area to suggest sources and flow paths of DOM in the River Traligill
- To compare the temporal patterns observed in this area to those observed in Coalburn Experimental Catchment, described in Chapter 4.

6.2 The spectrophotometric properties of DOM in the River Traligill

The properties of water sampled from River Traligill are shown in Table 6.1. Overall there was little variation in relation to other riverine DOM sampled throughout the Loch Assynt area. In the examination of the general water quality properties water colour showed a range of values that correlated positively with DOC concentration (Spearman's Rho=0.685 99% confidence level). In the River Traligill a number of samples were of very low colour, 19% of the samples were below the EU limit for colour in drinking water, $20mg^{-1}$ Pt/Co scale (Schedule 5 Form B 1998 EU Drinking Water Directive 98/83/EC). This indicates that although a proportion of the Traligill catchment is peat land and a DOM rich source with water of high colouration (Figure 5.1), water sources, flow paths or processes that generate low coloured water or remove coloured material contribute to the signal at the sampling point.

	Mean	Std. Dev.	Min.	Max.
Calcium (mgL-1	10.026	3.168	3.220	19.250
DOC (mgL^{-1})	4.972	3.886	0.000	13.258
Water Colour (Hazen)	60.692	41.384	3.174	168.056
pH	6.943	0.555	5.750	8.500
Conductivity (μS)	93.387	23.083	14.000	183.000
Peak $A_{EX\lambda}$ (nm)	337.355	5.025	315.000	345.000
Peak $A_{EM\lambda}$ (nm)	445.449	5.079	435.500	457.000
Peak $B_{EX\lambda}$ (nm)	379.768	5.716	370.000	390.000
Peak $B_{EM\lambda}$ (nm)	465.228	5.696	450.500	480.000
Peak $C_{EX\lambda}$ (nm)	278.138	3.625	270.000	290.000
Peak $C_{EM\lambda}$ (nm)	351.924	6.294	337.000	377.500
Peak A_{FInt}	70.768	35.810	17.850	145.700
Peak B_{FInt}	48.893	25.777	11.600	105.220
Peak C_{FInt}	16.777	4.444	6.694	41.759
Peak B_{FInt}/Peak A_{FInt}	0.684	0.047	0.565	0.822
Peak C_{FInt}/Peak A_{FInt}	0.340	0.195	0.137	1.228
Peak AS_{FInt}	15.462	12.099	3.459	56.288
Peak BS_{FInt}	10.431	8.101	2.017	36.652
***A*$_{340nm}$ (cm^{-1})**	0.086	0.056	0.006	0.195
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	0.055	0.062	0.005	0.288
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	0.007	0.005	0.001	0.021
ε A_{272nm} ($moleC\ L^{-1}cm^{-1}$)	568.284	625.827	53.341	2927.273
Peak A_{FInt}/*A*$_{340nm}$	1006.663	403.685	526.968	3193.333
***A*$_{465nm}$/*A*$_{665nm}$**	*n/a*	*n/a*	*n/a*	*n/a*
***A*$_{254nm}$/*A*$_{365nm}$**	4.007	1.028	2.583	8.065
***A*$_{254nm}$/*A*$_{410nm}$**	7.050	2.045	2.818	14.778

Table 6.1
Summary of data from the River Traligill. A_{465nm}/A_{665nm} was not measured due to low absorbance at $>A_{500nm}$

Calcium concentrations were monitored in the River Traligill, to examine if changes in this parameter related to the spectrophotometric properties of DOM. This parameter did not significantly correlate with any properties of the DOM. Increased calcium concentrations in stream waters are often related to a groundwater input compared increased DOC concentration which are related soil inputs (Neal *et al.,* 2001). In this data set there was no significant relationship between calcium and DOC concentration.

Excitation emission matrices in all the River Traligill analyses were closely comparable to those discussed in Section 2.2. The same peaks observed in samples from around the Loch Assynt area were identified in the River Traligill waters. Peak A dominated the fluorescence characteristics and samples consistently exhibited this maximum. Peak B and peak C were also ubiquitous, however, no fluorescence maxima related to peak D or any other unclassified peaks were observed. Fluorescence intensity maxima were observed in the regions attributed to peak E and F. Due to the reasons discussed in Section 2.2 these maxima were not monitored.

For peak A, peak B and peak C the standard deviation about the mean wavelengths were within the reproducibility (Table 6.1) of the method indicating that the distribution of peaks could be explained by variation within the analytical method. Six measurements of peak $A_{EM\lambda}$ exhibited comparatively shorter excitation wavelengths, of 315 to 325nm.

Table 6.1 shows the range of fluorescence intensities observed in the River Traligill. Peak A_{Fint} was consistently higher than peak B_{Fint}, as indicated by peak B_{Fint}/peak A_{Fint}. The intensities of the two peaks correlated highly, indicating the close relationship between the fluorophores (99% confidence level). The DOC concentration influence on fluorescence intensity can be seen in peak A_{Fint} and peak B_{Fint} both of which correlated with DOC concentration (99% confidence level Spearman's Rho 0.639 peak A_{Fint} and 0.629 peak B_{Fint}). 49.9% and 50.6% of the variation in peak A_{Fint} and peak B_{Fint} data respectively was explained by DOC concentration in this data set. Peak C_{Fint} did not correlate with DOC (95% confidence level).

River Traligill DOM exhibited featureless absorbance spectra similar to those observed previously in riverine DOM. Single absorbance measurements correlated positively with each other and this correlation was also observed with peak A_{Fint}, peak

B_{FInt} and DOC concentration. On average 42.5% (±16.17) of the variations in absorbance measured at different wavelengths was explained by DOC concentration, the maximum being A_{410nm}. 95.2% of the variations in peak A_{FInt} were explained by A_{340nm}. These figures are within the ranges discussed in Section 3.5 for data from Coalburn Experimental Catchment and the Loch Assynt area.

The ranges SUV_{254nm}, $Svis_{410nm}$, A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} are shown in Table 6.1. A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} did not correlate significantly with either fluorescence intensity or DOC concentration, indicating that these ratios relate to compositional rather than concentration changes in DOM. Peak A_{FInt}/A_{340nm} exhibited a wide range of values and was found to be negatively related to peak A and B wavelengths suggesting that DOM with greater fluorescence efficiency exhibited lower molecular weight (Section 2.2). Specific absorbance and estimated aromaticity did not significantly correlate with any other variables (95% confidence level).

6.3 Temporal patterns in DOM in the River Traligill

The River Traligill was monitored between April 2000 and March 2002; including five periods of high intensity sampling. Long term sampling was performed at approximately monthly intervals. Each individual sample set provides a detailed record of DOM fluctuations over short time periods superimposed on the long term record. Additionally these sampling periods can be generally grouped into autumnal (September 2001 and 2000) and winter/spring (April 2000, May 2001 and March 2002), periods.

Spectrophotometric data, with a high R^2 value when linearly regressed with time, was detrended to remove temporal autocorrelations. This data comprised the following variables :-

- DOC concentration, absorbance and specific fluoresce intensity (May and September 2001)
- Specific absorbance (September 2000 and May 2001)
- Fluorescence intensity (September 2001)

The remaining data, including the long term record exhibited a stationary relationship and did not require detrending.

6.3.1 Catchment conditions during sampling

The discharge observed in the River Traligill during the study period is shown in Figure 6.1. The long term trend shows periods of high flow from September to November 2000, June to September 2001 and in January and February 2001. Lowest flow periods occurred during December 2000 and May 2001. As the measurement was discontinuous this data can only provide an indication of the discharge of the River Traligill.

High resolution sampling periods provide more detailed information on the discharge patterns of the River Traligill. As is expected in this area (Soulsby *et al.,* 2002) observed discharge is highest during September 2000 and 2001 (max=4.1m^3s^{-1}) compared to the winter/spring sampling periods. Average discharge in September 2001 was significantly higher than September 2000 (99% confidence level) and during May 2001 there was significantly lower average discharge than during the other sampling periods (99% confidence level). Within individual sampling periods the River Traligill showed little variation in discharge as total rainfall, the major control of surface water in the catchment varied little. During September 2000 discharge initially increased then decreased in response to rainfall prior to the observation period. Daily cycles of snowmelt occurred during sampling in April 2000 and accounts for the peaks in discharge during this period.

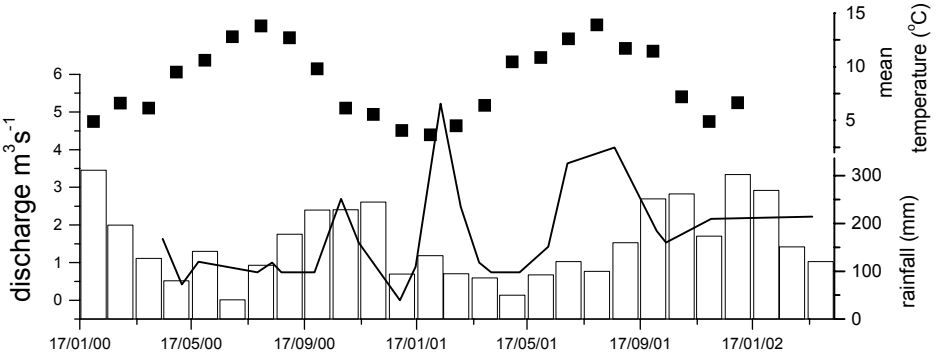


Figure 6.1
Conditions in the Traligill catchment during the study period. (■) Mean monthly temp (bar) total monthly rainfall converted from measurement at Stornoway (Stornoway rainfall (mm) x 1.7407), (——) measured discharge in the River Traligill.

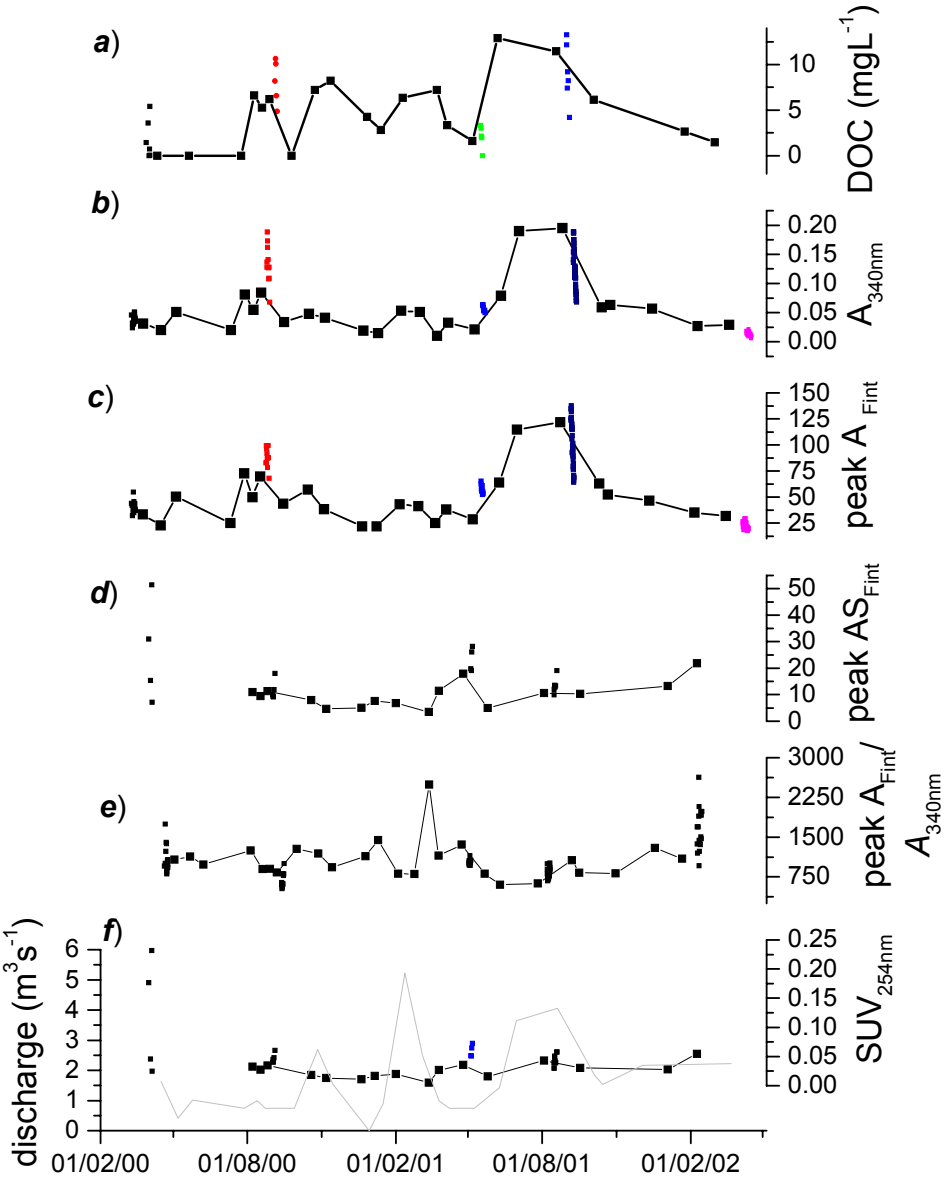


Figure 6.2
Time series of a) DOC (mgL^{-1}) b) A_{340nm} c) peak A_{Fint} d) peak AS_{Fint} e) peak A_{Fint}/A_{340nm} f) SUV_{254nm} and (——) discharge (m^3s^{-1}) in the River Traligill

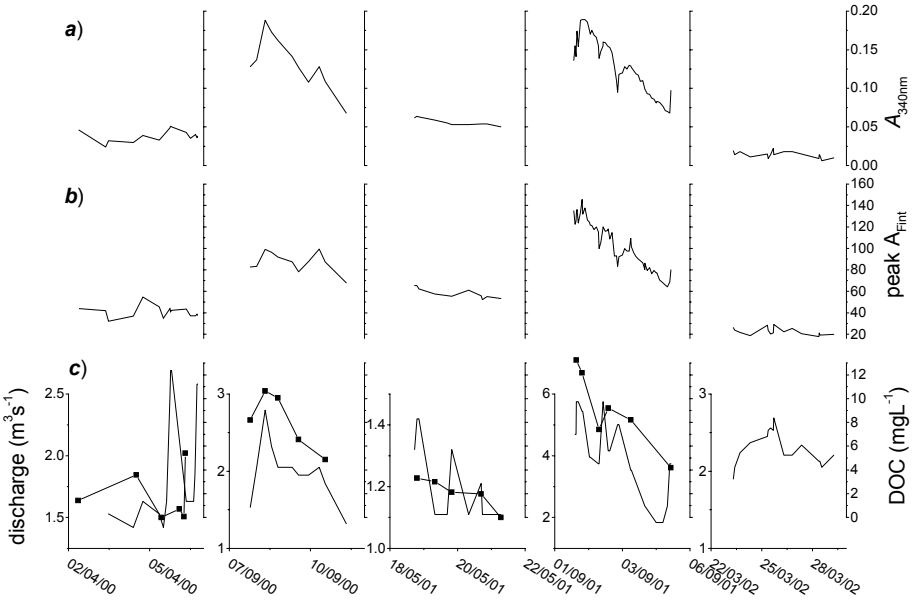


Figure 6.3
*Time series of **a)** A_{340nm} **b)** peak A_{Fint} **c)** DOC (mgL^{-1}) and (——) discharge (m^3s^{-1}) during high resolution sampling of the River Traligill.*

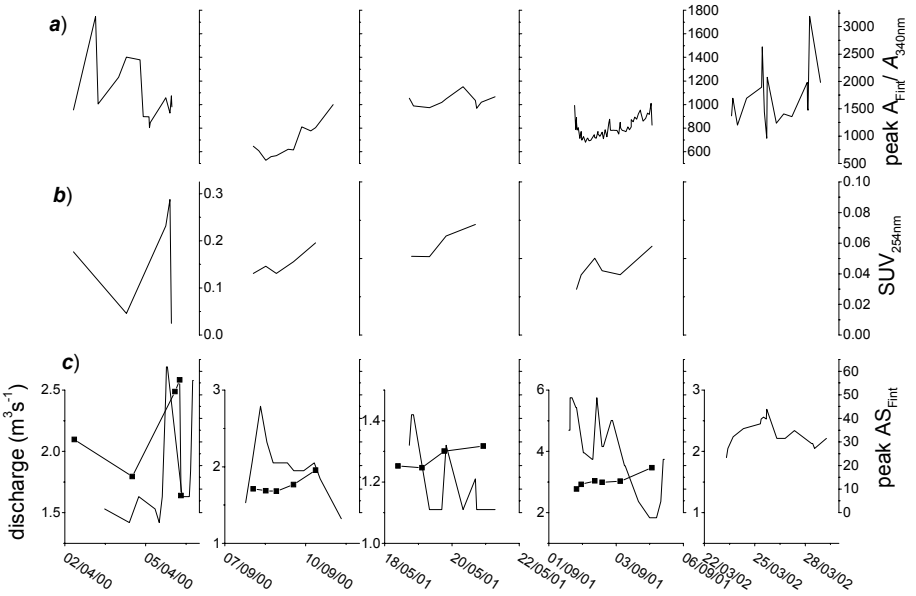


Figure 6.4
*Time series of **a)** peak A_{Fint} /A_{340nm} **b)** SUV_{254nm} **c)** peak AS_{Fint} and (——) discharge (m^3s^{-1}) during high resolution sampling of the River Traligill*

6.3.2 Temporal patterns in the spectrophotometric properties of DOM in the River Traligill

The time series of selected data are shown in Figures 6.2 to 6.4. Conductivity and pH both showed no seasonal variation and small ranges during high resolution periods, the means of which were statistically indistinguishable. The calcium concentration exhibited no variation over time and did not correlate with discharge during any of the high resolution monitoring periods, except during April 2000 (Spearman's Rho – 0.842; 99% confidence level). This relationship is a dilution effect caused by the influx of snowmelt water, which exhibited low calcium concentrations ($0.5 \pm 0.07 mgL^{-1}$). During this period 54% of the variation in calcium concentration could be explained by changes in discharge.

No relationship with discharge, and therefore snowmelt, was observed during April 2000 in DOC concentration and water colour data. Snow exhibited zero detectable DOC concentration and water colour. This is in contrast to the snowmelt influenced DOC concentration pattern seen in the Coalburn Experimental Catchment (Section 4.4.5) where an influx of snowmelt significantly lowered DOC concentration. As shown in Figure 6.2 the levels of DOC concentration seen in the River Traligill were also comparatively low at this time, thus a dilution signal would have had minimal effect on the river water signal.

DOC concentration and water colour showed the same temporal patterns; both exhibited maximum concentrations during August to November 2000 and June to September 2001 ($max=11.2 mgL^{-1}$). These correspond to the summer/autumn maxima in organic matter concentration that has been identified in other rivers (Section 1.2). Both peak A_{Fint} and peak B_{Fint} showed the same temporal pattern. High levels were observed in July to September 2000 and June to September 2001 (max peak A_{Fint} =126.4). Low levels were observed during the winter months and the lowest in March 2002 (min peak A_{Fint} =17.4). The significant differences in mean values of spectrophotometric properties form each high resolution sampling period are summarised in Table 6.2.

DOC concentration, peak A_{Fint} and peak B_{Fint} and absorbance	September 2000 and 2001 > other sampling periods March 2002 < other sampling periods
Peak B_{Fint}/peak A_{Fint}	September 2000 and 2001 > other sampling periods
Peak $B_{EX\lambda}$	April 2000 to September 2000 shift of +11.68nm
Peak C_{Fint}	March 2002 < other sampling periods
Peak AS_{Fint}	September 2000 and 2001 < May 2001
Peak A_{Fint}/A_{340nm}	September 2000 and 2001 < other sampling periods
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	April 2000 > other sampling periods

Table 6.2
Summary of the significant variations in DOM spectrophotometric properties in the River Traligill during periods of sampling (all relationships 95% confidence level).

During April 2000 DOM exhibited short excitation wavelengths ($B_{EM\lambda}$=442.5) high A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} (5.81 and 9.46) and peak A_{Fint}/A_{340nm} (1089.403) and during May 2001 DOM exhibited high peak AS_{Fint} (23.24) and peak A_{Fint}/A_{340nm} (1030.92). This combination is interpreted as a DOM of lower molecular weight /aromaticity in comparison to DOM observed during September 2000 and 2001.

DOC concentration correlated positively with discharge in the entire data set (Spearman's rho 0.490; 99% confidence level) as shown in Figure 6.5 and during September 2001 (Spearman's rho 0.81; 95% confidence level). This has been observed in other rivers (for example Kullberg *et al*., 1993; Hope *et al*., 1994) and in the Traligill catchment is probably due to increased input from runoff from the peat areas during high flow conditions. As discussed in Chapter 5 there is significantly higher DOC concentration and water colour in waters from such areas. Runoff from the Traligill Basin is intermittent and can cease during dry conditions. The combination of increased production of DOM within the peat during warmer dry conditions in summer, (Scott *et al*., 1998) and the release by increased rainfall during late summer/autumn, results in the monitored data exhibiting such a relationship. This relationship may not occur during parts of the hydrological year that were not sampled. The positive relationships of discharge to absorbance and fluorescence intensity, shown in Figure 6.5, appears to relate to broad seasonal variations, when higher flow results in the greatest export of DOM.

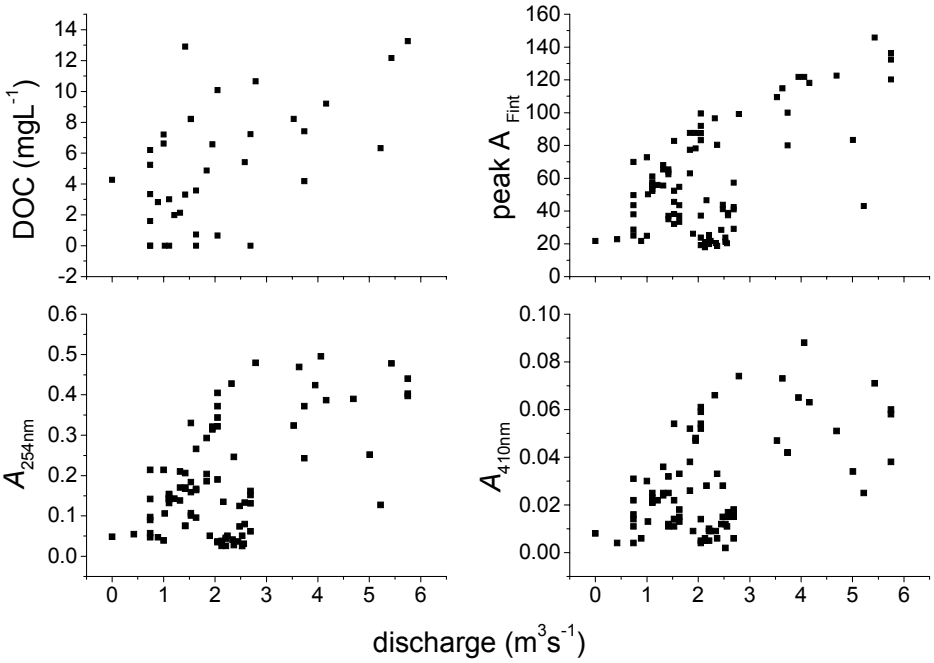


Figure 6.5
The relationship of DOC (mgL^{-1}), peak A_{Fint}, peak B_{Fint} and absorbance to discharge in the River Traligill

In the data set as a whole discharge correlates positively with peak A_{Fint} and peak B_{Fint} (99% confidence level). In the individual high resolution data this was only reproduced in September 2000 (Spearman's rho peak A_{Fint} 0.742; peak B_{Fint} 0.821 99% confidence level). Examination of the data suggests that during September 2001 peak A_{Fint} and discharge exhibited the same pattern, both decreasing over time, however, when this temporal effect was removed the two variables did not correlate significantly. The 1.5 hour resolution sampling during September 2001 showed 3 peaks in fluorescence intensity that occurred after peaks in discharge, on the falling limb of the hydrograph. Each successive discharge peak had a correspondingly lower fluorescence intensity maximum, which may suggest that with successive flushing fluorescence intensity was depleted, representing depletion in DOM. This was also observed in absorbance data.

The discharge relationships shown in Figure 6.5 are quite weak. The amount of variation explained by discharge in each is as follows:

DOC concentration 30%; peak A_{Fint} 36%; peak B_{Fint} 37%; A_{254nm} 30%; A_{410nm} 26%.

These values compare closely to other quoted for the DOC concentration – discharge relationship. For example, 38.5% (Grieve, 1984) and less than 30% (Tipping *et al*., 1988). Weak correlations have been related to seasonal effects and hysteresis.

The relationships shown on Figure 6.7 differ to the discharge relationships observed in the Coalburn Experimental Catchment. In that data set less than 10% of the variation in DOC concentration was explained by discharge. As discussed in Section 4.4 DOC concentration, absorbance and fluorescence intensity had an overall negative relationship with discharge, exhibiting high values at both high and low flow. In comparison to the River Traligill the Coalburn has a significant input of higher DOC concentration waters during all flow conditions.

The observed changes in DOM spectrophotometric properties over time exhibit a change in composition that can be interpreted as a change in DOM source, as shown by DOC concentration. A significant negative relationship exists between peak A_{Fint}/A_{340nm} and discharge. This variable was significantly lower in DOM from peat pools in the Traligill Basin area thus demonstrating high flow condition DOM source from this area.

6.4 Summary of temporal patterns in DOM in the Loch Assynt area and comparison to the Coalburn Experimental Catchment

The overall variations in spectrophotometric properties reveal distinct differences in DOM in the River Traligill during different times of the year. The major division, which can be recognised in enhanced levels of DOC concentration, absorbance and fluorescence intensity during summer and autumn. This is recognised in other such systems (Scott *et al.,* 1998; Tipping *et al.,* 1999) and reflects the export of DOM produced under dry warm conditions when catchment becomes wet enough for net export. The DOM exported at this time also has longer fluorescence intensity wavelengths and low peak A_{Fint}/A_{340nm}, indicating a flush of higher molecular weight DOM. This flush relates to the displacement of DOM previously associated with soil inorganic material that has become solubilised by increased rainfall (Scott *et al.,* 1998). DOM with these characteristics is observed in the peat dominated area of the Traligill Basin and changes in spectrophotometric properties indicate export of DOM

from this area with increased rainfall and discharge. This temporal pattern was also identified in the Coalburn experimental catchment, where DOM source in forestry ditches become important upon increased rainfall and discharge. However, unlike the Coalburn experimental catchment DOM is only exported from the peat areas under high flow conditions. Under low flow conditions DOM in the River Traligill is derived from areas with varying soil types and the fractionation and retardation of DOM with relatively high molecular weight and aromaticity (Zhou *et al*., 2001) in these soils influences DOM spectrophotometric signal.

6.5 Conclusions

This chapter has presented and discussed the temporal patterns observed in spectrophotometric properties of DOM in the River Traligill, Assynt.

- *To characterise the spectrophotometric properties of DOM from the River Traligill*

The River Traligill exhibited typical DOM spectrophotometric properties of group (1) discussed in Chapter 5.

- *To identify temporal patterns in DOM in the River Traligill and relate to DOM seen in the wider Loch Assynt area to suggest sources and flow paths of DOM in the River Traligill*

From the examination of temporal patterns in DOM a number of conclusions relating to flow paths and sources in the River Traligill catchment can be made.

1. During autumn DOM is exported from the peat associated areas of the Traligill Basin, such as standing peat pools. This results in enhanced DOC concentration in comparison to other periods of the year - the “autumn flush”.

2. The activation of peat associated DOM sources during the autumn flush results in export of more aromatic and higher molecular weight DOM, in comparison to other periods of the year. Such DOM has two possible sources. Firstly, peat associated surface and pore water DOM flushed to the main channel unaltered when the upland peat areas are hydrologically active. Secondly, the export of DOM otherwise retained in mineral soils. As increased DOM is transported through the catchment the sorbing capacity of the soils may become saturated allowing DOM to pass to surface water and be transported downstream.

3. Under low flow conditions the sources of DOM in the River Traligill are controlled by the mineral soils and DOM-inorganic interactions controlling both DOC concentration and DOM composition.

- *To compare the temporal patterns observed in this area to those observed in the Coalburn Experimental Catchment, described in Chapter 4.*

The Traligill exhibits a different DOM-flow relationship to the Coalburn Experimental Catchment. In the latter under all flow conditions DOM is derived from peat areas and only under specific conditions is the mineral soil important (Chapter 4). This distinction indicates the importance of the influence of soil type on the temporal patterns of DOM spectrophotometric characteristics in surface water. Similarities exist in annual DOC concentration cycle, both rivers being typified by an autumn flush of DOM.

Chapter 7

The Wider Context of the Spectrophotometric Properties of Dissolved Organic Matter

7.1 Introduction

In Chapters 3-6 DOM from two areas was discussed and found to exhibit similar spectrophotometric properties. DOM from both areas was divided by source into peat-derived and non peat-derived. Each type of DOM from both areas was similar. These comparisons may stem from both equivalent soil type and DOM sources and DOM flow paths and processing. A number of questions are raised in the interpretations of these data sets regarding the variations and relationships in variables. Further assessment of the distributions and relationships of these spectrophotometric properties is, therefore, required to fully interpret the causes of such variations. The following section will address these points by reference to DOM sampled from surface water from wider source areas, and by comparison to other studies of DOM.

To further examine spatial DOM spectrophotometric properties sample from a wide range of sources were analysed. All samples were analysed using the methods detailed in Table 2.3 and stored as discussed in Section 2.2. These samples provide the potential to both expand the spectrophotometric characterisation of DOM and to evaluate further the analytical technique. Samples were taken from rivers draining a range of soil types and land uses. In addition to this urban rivers and urban impacted rivers were included. These have been found to have a distinct spectrophotometric character, related to inputs of sewage and farm wastes (Baker, 2001) typified by protein concentrations resulting in enhanced peak C_{Fint}.

The samples considered in this chapter were divided into the groups (types) representing different DOM sources:

Type (1) rivers draining predominantly peat areas including forested peat areas

Type (2) rivers draining from non-peat areas

Type (3) urban rivers and rivers with inputs of sewage/farm waste DOM

The source, dates and classification of the samples used in this chapter are detailed in Appendix 6.

7.1.1 Aims

- To characterise DOM from surface water influenced by different sources and processes and to place the DOM from Coalburn Experimental Catchment and Loch Assynt area into a context of DOM from a variety of source areas.

- To evaluate the analytical method using the following variables:
  - Emission wavelengths, excitation wavelengths and peak A_{Fint}/A_{340nm}
  - UV-visible absorbance ratios
  - The relationship of peak C_{Fint} to DOC concentration
  - Specific absorbance and estimated aromaticity

7.2 Emission wavelengths, excitation wavelengths and peak A_{Fint}/A_{340nm}

DOM fluorescence intensity maxima emission wavelengths are generally interpreted as shorter values indicate simpler molecules of lower molecular weight and of lower aromaticity (Senesi *et al*., 1991). Variations in excitation wavelengths have a similar interpretation. With the exception of a limited number of instances excitation and emission wavelengths, throughout this study, exhibited variations that could be explained by reproducibility of the method.

In other studies such shifts have been observed on a wider scale (for example McKnight *et al*., 2001). Other data analysed under the same analytical conditions indicate that emission wavelength can vary to a greater extent than the data presented in this study. Baker (2002c) found a mean difference of up to 12.9nm in tributaries within one catchment. McKnight *et al*. (2001) found that a peak $B_{EM\lambda}$ red shift of 5nm was the equivalent of a 5-7% increase in aromaticity, measured by NMR.

The excitation and emission wavelengths of DOM in samples taken from a range of surface water sources are shown in Table 7.1. A number of significant patterns can be observed, firstly a distinct significant shift in peak A and B wavelengths from short in type (3) DOM to longer in type (1) DOM, with type (2) DOM as an intermediate (99% confidence level). The shift in peak $A_{EM\lambda}$ from type (1) to type (3) DOM is on average 21.03nm, which is significant and has a greater magnitude compared to the reproducibility of the method. The shift between type (2) and type (3) DOM (15nm) was also greater than the analytical reproducibility.

It can also be seen in Table 7.1 that the range and variance in the data sets was greater in type (3) DOM than both (1) and (2) DOM. This shows that, peat derived DOM has a relatively limited range of excitation and emission wavelengths, compared to that derived from non-peat sources. Influences other than DOM composition may affect wavelengths, such as pH and metal ion interactions, as discussed in Section 1.5.3. These interactions were not observed, as both pH and conductivity did not correlate with wavelength (95% confidence level).

The influence that molecular weight has on emission wavelength in this data set can be seen in Figure 7.1, which shows the significant relationship of peak $A_{EM\lambda}$ to peak A_{Fint}/A_{340nm}. Higher values of peak A_{Fint}/A_{340nm} are observed to occur in fractions of DOM of lower molecular weight (Miano and Alberts, 1999; Wu *et al*., 2002). This variable was found to be useful in the differentiation of DOM spectrophotometric properties in both spatial and temporal settings. Figure 5.10 shows the limited range of values observed in peat derived DOM compared to other sources. The red shifted emission wavelength and low values of peak A_{Fint}/A_{340nm} indicate higher molecular weight in peat derived DOM.

	type(1)				type(2)				type(3)			
	n	Mean	±	Variance	*n*	Mean	±	Variance	*n*	Mean	±	Variance
Peak $A_{EX\lambda}$	578	340.14	1.49	2.23	431	336.53	6.23	38.81	128	334.72	7.10	50.51
Peak $A_{EM\lambda}$	577	447.90	3.97	15.81	431	441.76	9.21	84.99	128	426.81	11.44	130.97
Peak $B_{EX\lambda}$	578	382.88	4.89	23.94	429	379.94	5.98	35.83	120	380.41	6.30	39.74
Peak $B_{EM\lambda}$	578	465.62	6.67	44.61	429	463.11	7.85	61.76	120	460.62	10.08	101.79
Peak $C_{EX\lambda}$	567	280.80	4.20	17.67	430	278.53	3.93	15.50	128	279.21	3.57	12.77
Peak $C_{EM\lambda}$	567	352.67	4.50	20.25	430	352.95	6.39	40.90	128	351.00	6.41	41.20

Table 7.1
Summary of fluorescence intensity maxima wavelengths

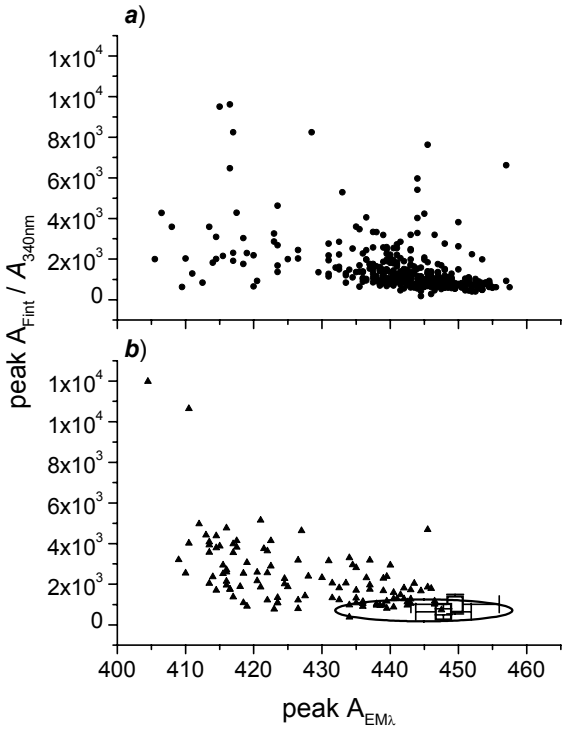


Figure 7.1
The relationship of peak A_{Fint}/A_{340nm} to peak $A_{EM\lambda}$ in surface water in the large scale monitoring of DOM ***a)*** *type (2) DOM* ***b)*** (▲) *type (3) DOM* (□) mean data from the *Coalburn Experimental Catchment and Loch Assynt area. Enclosed area type (1) DOM*

7.3 UV-visible absorbance ratios: A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}

Ratios of absorbance measured at UV and visible wavelengths have previously been successfully applied to the study of DOM and higher values have been related to lower molecular weight and an increase aromaticity (Peuravuori and Pihlaja, 1997; Huatala *et al.*, 2000). In previous chapters the values of A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} exhibited significant spatial variations, the distributions, however, were

principally controlled by a number of high values. These values were related to conditions specific to the period of sampling.

Figure 7.2 shows the distribution of these ratios in DOM from the large dataset. In data from different sources mean absorbance ratios were not significantly different (95% confidence level). The distributions indicate in each DOM type a number of samples with high values. These samples did not have any other defining spectrophotometric characteristics. This indicates that the limited differences of absorbance ratios in DOM from the Coalburn Experimental Catchment and Loch Assynt area are observed in DOM from more diverse sources and that the ratios have a limited use in the differentiation of DOM. Chen *et al.* (2002) identified that DOM rich in carbohydrates exhibits a higher A_{254nm}/A_{365nm} compared to more aromatic DOM. DOM with high absorbance ratio values may reflect such a compositional differences, which is specific to the conditions it was sampled under.

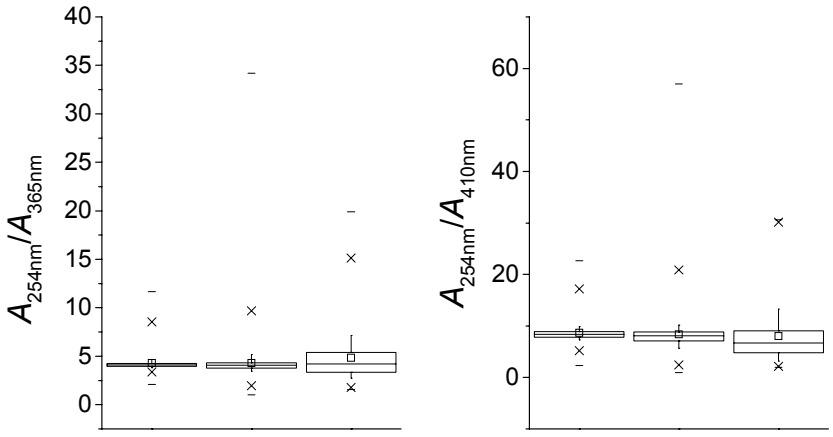


Figure 7.2
Box plots of ***a)*** *A_{254nm}/A_{365nm} and* ***b)*** *A_{254nm}/A_{410nm} in surface water in the large scale monitoring of DOM. For key to box plots see Figure 3.4.*

7.4 The relationship of peak C_{Fint} to DOC concentration, peak A_{Fint} and UV-visible absorbance

DOM from type (3) rivers exhibited significantly higher mean peak C_{Fint} (67.27 s.d. 52.61) compared to DOM from more natural sources (type (1) = 54.96 and type (2) = 23.19). A number of the rivers sampled had inputs from farm wastes and sewage; these exhibited the highest peak C_{Fint} levels >100. The relationships of peak C_{Fint} to peak A_{Fint} and absorbance are summarised in Figure 7.3. In type (3) DOM peak C_{Fint}

showed little variation with absorbance compared to the negative relationship in type (1) DOM. In contrast to this both type (3) and (2) DOM had a positive relationship of peak C_{Fint} to peak A_{Fint}, compared to no relationship in type (1) DOM (95% confidence level). This indicates that although the input of highly proteinaceous material may control peak C_{Fint} in certain settings, rivers with entirely natural DOM both peak C_{Fint} and peak A_{Fint} can be relatively enhanced.

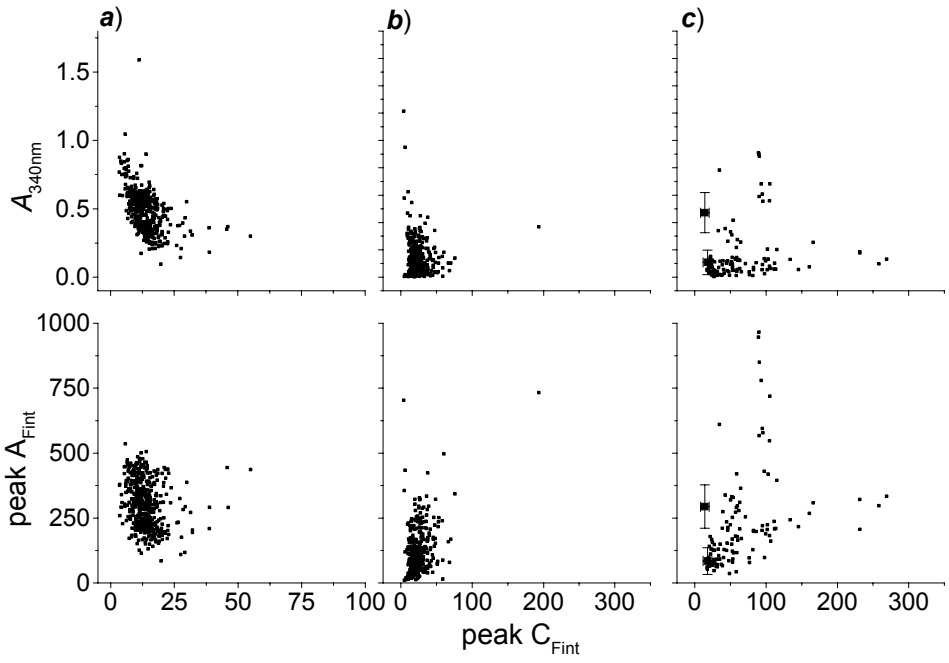


Figure 7.3
*The relationship of peak C_{Fint} to A_{340nm} and peak A_{Fint} in surface water in the large scale monitoring of DOM **a)** type (1) DOM **b)** type (2) DOM **c)** type (3) DOM*
(▪) *mean data from the Coalburn Experimental Catchment and Loch Assynt area*

The positive relationships of peak C_{Fint} to peak A_{Fint} may stem from an increased fluorescence emission intensity from peak C resulting in increased excitation of peak A and, thus, emission of this fluorophore. The relationship, however, indicates that in DOM of a varied spectral character than that seen in the Coalburn Experimental Catchment and Loch Assynt area peak C_{Fint} is not controlled by DOC concentration, absorbance or peak A_{Fint}. More work is required to further establish if in river water DOM peak C_{Fint} is directly proportional to the concentration or fluorescence efficiency of proteinaceous material present.

7.5 Specific absorbance and estimated aromaticity of DOM

It has been previously reported that absorbance of DOM correlates well with aromatic content (for example Croué *et al*., 1999). Measurements such as these have been used as a proxy for DOM aromaticity, in this study it was calculated from molar absorptivity ($molCL^{-1}cm^{-1}$) at A_{272nm}.

A summary of the published values of SUV_{254nm} is presented in Table 7.2. The source of the DOM and the analytical conditions of this data varies. Within this data it can be seen that surface waters compare closely, having a higher value than wastewaters and groundwater. The mean value of SUV_{254nm} in surface water data from the two study areas, indicated on Figure 7.4 are similar to the values shown in Table 7.2. In the data in this study only a limited differentiation of specific absorbance between DOM from different sources could be identified, and this may derive from the relatively constant values observed from DOM of different areas as shown in Table 7.2 resulting from a homogenous aromatic content, or the limited sensitivity of the method.

To further investigate variations in specific absorbance a wider range of values were considered. In the large data set a limited amount of specific absorbance data was available. This showed no significant difference between the SUV_{254nm} mean values of type (1) DOM (0.048 s.d. 0.009) and type (2) DOM (0.046 and 0.038) (99% confidence level). Data from river water DOM sampled in the River Tyne catchment (Appendix 6b) was assessed for specific absorbance properties, as this data set ranged from coloured upland peat associated DOM (type (1)) to urban derived DOM (type (3)). The range of SUV_{254nm} (0.0107 to 0.0528) observed reflects the data presented in Table 7.2; low values were observed in urban rivers and higher values in upland sources.

Reference	Source	Specific absorbance ($mgL\ DOC^{-1}cm^{-1}$)
Reckhow and Singer (1984)	River and stream FA	0.035
	River and stream HA	0.054
Gjessing *et al*. (1998)	Lake water	0.0489
	Groundwater	0.040
Westerhoff *et al*. (1998)	Lake water	0.0097
	Lake water	0.0467
	Brown water	0.0436
Abbt-braun and Frimmel (1999)	Soil seepage	0.0316
	Groundwater	0.0292
	Waste water	0.0144
Westerhoff and Anning (2000)	Waste water	0.012
	Suwannee river FA	0.044
	Brown coal	0.0418
Baker (2001)	River water	0.031-0.058
Vogt *et al*. (2001)	Surface water	0.05
Vogt *et al*. (2002)	Lake water	0.034-0.059
Muller and Frimmel (2002)	Bog water	0.0055
	Waste water	0.0008

Table 7.2
Published values of SUV_{254nm} from DOM analyses.

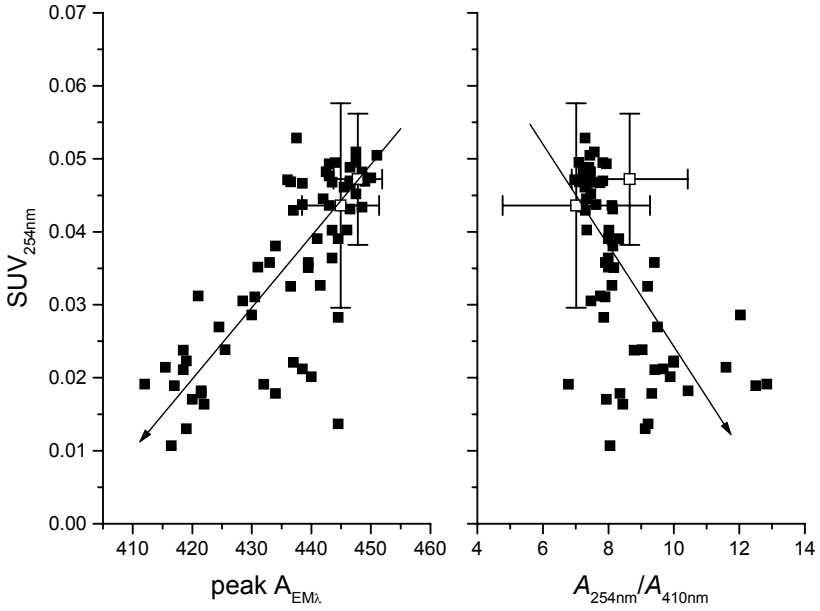


Figure 7.4
The relationship of SUV_{254nm} to peak $A_{EM\lambda}$ and A_{254nm}/A_{410nm} in data from the River Tyne catchment and (□) mean data from the Coalburn Experimental Catchment and Loch Assynt area. Arrows indicate the transition from type (1) to type (3) DOM

In this data set the relationship of other variables to specific absorbance also represents patterns not observed previously in this study. As shown in Figure 7.4 peak $A_{EM\lambda}$ and A_{254nm}/A_{410nm} had significant relationships with specific absorbance (Spearman's rho 0.731 and -0.781 respectively 99% confidence level). This indicates that as defined by Senesi *et al*. (1991) peak $A_{EM\lambda}$ has a positive relationship with aromatic content. A significant red shift of 39nm is observed between DOM of specific absorbance 0.031 to 0.62. Similarly, higher A_{254nm}/A_{410nm} values (>10) represent DOM of specific absorbance (<0.022).

The ratio of peak B_{Fint}/peak A_{Fint} has been proposed as a possible index for DOM variation (Newson *et al*., 2001), specifically as a proxy for humification. As discussed in Section 3.9 this value did not exhibit the same patterns as other aromaticity estimates. The reason for this was suggested to be due to the two fluorescence intensity peaks being measured at similar emission wavelengths and different excitation wavelengths and that the latter is less sensitive to DOM variations. As shown in Figure 7.4 peak $A_{EM\lambda}$ is positively correlated with SUV_{254nm} a similar relationship was observed for peak $A_{EX\lambda}$ (Spearman's rho 0.456 99% confidence level), however, the amount of variation explained by SUV_{254nm} at each wavelengths varied. Using linear regression to estimate this 19% of peak $A_{EX\lambda}$ and 60% of peak $A_{EM\lambda}$ variations were explained by SUV_{254nm}. This discrepancy related to a wide range of SUV_{254nm} observed at longer peak $A_{EX\lambda}$ and indicates that excitation wavelength is less sensitive to DOM variations compared to emission.

7.6 Summary of the comparison of the spectrophotometric properties of DOM from various sources

This section has discussed the comparison of a number of spectrophotometric properties in DOM of the Coalburn Experimental Catchment and Loch Assynt area to DOM from other sources. In the principle two study areas, both of which are peat influenced, it was observed that DOM spectrophotometric properties were able to identify spatial and temporal differences in DOM. Aquatic DOM derived from peat dominated areas in the Coalburn Experimental Catchment and Loch Assynt area exhibited similar spectrophotometric properties although the two areas are distinct in morphology and land use. The distinct DOM signatures of runoff from peat areas and from areas of more inorganic soils could be observed in the River Traligill and the

Coalburn, depending on the catchment conditions. The dominance of peat and the influence of ditching in the Coalburn Experimental Catchment resulted in a signal similar to this DOM under all flow conditions; however, an input from the peaty-gley sub-catchment was observed under changing flow conditions. In the Loch Assynt area the River Traligill exhibited an input from peat dominated runoff under higher flow conditions. Under lower flow conditions the DOM exhibited a signal typical of the non-peat areas indicating runoff from these areas. These variations indicate the methods used can be applied to monitor changing flow paths. When samples taken from a wide range of sources are divided on the basis of source and influences on DOM the relative spectrophotometric properties of DOM can be summarised and interpreted as detailed in Table 7.3. In a wider context the spectrophotometric properties of aquatic DOM from peat areas exhibited a limited range.

Peak C_{Fint} of type (3) DOM indicates a source of DOM possibly derived from external proteinaceous material. Both types (2) and (3) exhibited DOM characteristics, possibly resulting from interactions with inorganic soils that was not an influence on peat derived DOM. Such interactions may retard higher molecular weight and more aromatic DOM within soils, thus changing the character of aquatic DOM (Zhou *et al*., 2001), and resulting in an overall lower DOC concentration in surface waters.

	Type (1) Peat derived DOM	**Type (2) Non-peat derived DOM**	**Type (3) Urban rivers**
Summary	High DOC concentration and specific absorbance, long peak $A_{EM\lambda}$, low peak C_{Fint}, A_{254nm}/A_{410nm} and low peak A_{Fint}/A_{340nm}	Range of peak $A_{EM\lambda}$ and peak A_{Fint}/A_{340nm}, low A_{254nm}/A_{410nm}, high peak C_{Fint}, medium-low DOC concentration	Short peak $A_{EM\lambda}$, low specific absorbance, high A_{254nm}/A_{410nm}, peak A_{Fint}/A_{340nm}.and peak C_{Fint}, low DOC concentration.
Interpretation	High aromaticity and molecular weight	Intermediate to low molecular weight relatively low aromaticity	Intermediate to low molecular weight, low aromaticity, presence of external DOM sources

Table 7.3
Summary of the relative spectrophotometric properties of DOM from different sources.

7.7 Conclusions

The previous chapter has examined the spectrophotometric properties of DOM from a range of sources. The conclusions reached fulfilled the aims as follows:

- *To characterise DOM from surface water influenced by different sources and processes and to place the DOM from Coalburn Experimental Catchment and Loch Assynt area into a context of DOM from a variety of source areas.*

Peat derived DOM, including that from Coalburn Experimental Catchment and the Loch Assynt area, is more aromatic in comparison to DOM from non-peat dominated and urban areas. DOM from the latter sources is also of a lower molecular weight and more proteinaceous. The former DOM is more homogenous than the latter. It is concluded that this variability is due to the down stream location of this DOM and the numerous modification, interactions and secondary sources that can influence DOM in these areas. Peat derived DOM is concluded to be a source area of DOM and therefore has a limited potential for alterations to occur. DOM from the two main study areas was similar in spectrophotometric properties in comparison to DOM sampled from other places in the UK, with the same soil characteristics.

- *To evaluate the analytical method using spectroscopic variables*

The spectrophotometric variables: emission wavelengths, excitation wavelengths and peak A_{Fint}/A_{340nm}, UV-visible absorbance ratios, peak C_{Fint}, specific absorbance and estimated aromaticity examined in this chapter show a greater variability than that observed in Chapters 3-6. All of the monitored variables utilised were found to be useful in differentiating DOM on this scale. It is recommended that these spectrophotometric properties be used in further studies of aquatic DOM.

Chapter 8.

The Spectrophotometric Properties of Peat Dissolved Organic Matter

8.1 Introduction

The following chapter applies the method of obtaining DOM from peat proposed in Section 2.5 to peat sampled from the Coalburn Experimental Catchment and the Loch Assynt area. Within both of the study areas the association of surface water with peat has an influence upon the spectrophotometric properties identified in aquatic DOM. The difference in extent and state of the peat may influence the differences in surface water DOM observed between each site. In comparison to P_{soil} DOM spectrophotometric properties discussed in Section 3.6 the examination of peat profiles will provide stratified DOM-depth relationships. The measured spectrophotometric properties that were identified in Chapter 7 to be useful in the characterisation of DOM (peak C_{Fint}, A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm} and peak A_{Fint} $/A_{340nm)}$ are assessed together with absorbance and fluorescence intensity

The annual cycles of DOC concentration in river waters are commonly related to the processes occurring within catchments soils. In peat systems this cycle is closely related to the seasonal changes in water table, moisture content and temperature. In turn, these conditions control the physical and biological processes of DOM formation and export (Mitchell and McDonald, 1992; Worral *et al*, 2002). Changes in the composition, the aromatic and hydrophobic nature of surface water DOM, have also been related to processes within peat (Scott *et al*., 1998). In peat areas it is thought that such processes control the flux of DOM rather than the influence of sorptive processes in mineral soil horizons (Worral *et al*., 2002).

There has been limited previous analysis of peat-derived DOM using the spectrophotometric techniques employed in this study and no previous work has related such properties in peat to those in surface waters. Pore waters and peat associated waters have been analysed in relation to peat degradation and restoration using fluorescence humification indices (Kalbitz *et al.,* 1999; Glatzel *et al*., 2003).

Cocozza *et al*. (2003) characterized the properties of DOM from a peat profile using spectrophotometric analysis of pore waters. Peaks in excitation and emission spectra exhibited increases in fluorescence intensity and a red shift in wavelengths with increasing depth. The authors identified a transition zone in fluorescence properties and related this to the acrotelm-catotelm division. Describing an upper zone of transformation processes with heterogeneous DOM, and a lower zone of more homogeneous DOM of simple highly degraded aromatic humic material that accumulates in the saturated portion of the peat profile. Newson *et al*. (2001) examined the emission wavelengths of aqueous extracts of dried peat and also observed a red shift of emission wavelengths (~30nm) with depth occurring at the junction of the acrotelm-catotelm shift. A maximum was observed at the transition and this horizon was thought to be the level at which lateral flow is likely to occur.

8.1.2 Aims

- To extend of the evaluation of EEM fluorescence spectrophotometry as an analytical technique to peat DOM, by comparison to surface water DOM.

- To compare the peat DOM from both the Coalburn Experimental Catchment and Loch Assynt area and to identify temporal or spatial patterns.

- To identify the spectrophotometric properties of DOM from peat within individual profiles, identify changes in DOM with depth and to relate such depth variations to surface water sources of DOM.

8.2 Peat DOM from the Coalburn Experimental Catchment

The following section will discuss the variations in peat DOM extracts from two sites, with the Coalburn Experimental Catchment. One site in open peat and one under closed canopy forest were investigated to determine the broad influence that forestation has on the spectrophotometric properties of DOM. Due to the problems associated with sampling and the lack of structure within the peat comparisons are made on broads units characterised by physical properties. The sampling was performed from September 2001 to January 2002 a period encompassing changing moisture conditions in the catchment and distinct DOM properties in surface water between the autumn DOC concentration flush and winter low concentration levels.

8.2.1 Sampling locations, pH and moisture measurements

Peat profiles were sampled from the two locations, shown on Figure 3.1. The obtained cores were separated in to 5cm portions, and stored in airtight conditions at ~5°C. DOM was obtained in triplicate using the method outlined in Section 2.5 and the resulting solutions were analysed as described in Section 2.2. Peat moisture content was measured by drying ~10g of field moist peat (105°C) to a constant weight (±0.005g). The pH of the extraction solutions were measured prior to the 2 hour period of dissolution. Peat physical characteristics were described and assigned a humification level according to the scheme of von Post (Appendix 7).

Site one was located under closed canopy tree cover ~1.3m from the closest ditch. The surface covering of spruce litter was sampled at the surface of each profile. Site two was located in an area of open ground ~4.7m from the tree line and ditch upstream of P_{weir}. Vegetation at this site consisted of *Molinia*.

Location	Name	Sampling Date
Site 1	CB 01/09/01 (1)	**01/09/01**
	CB 14/10/01 (1)	**24/10/01**
	CB 28/11/01 (1)	**28/11/01**
	CB 16/01/02 (1)	**16/01/02**
Site 2	CB 01/09/01 (2)	**01/09/01**
	CB 11/10/01 (2)	**11/10/01**
	CB 28/11/01 (2)	**28/11/01**
	CB 16/01/02 (2)	**16/01/02**

Table 8.1
Peat core sampling details from the Coalburn Experimental Catchment

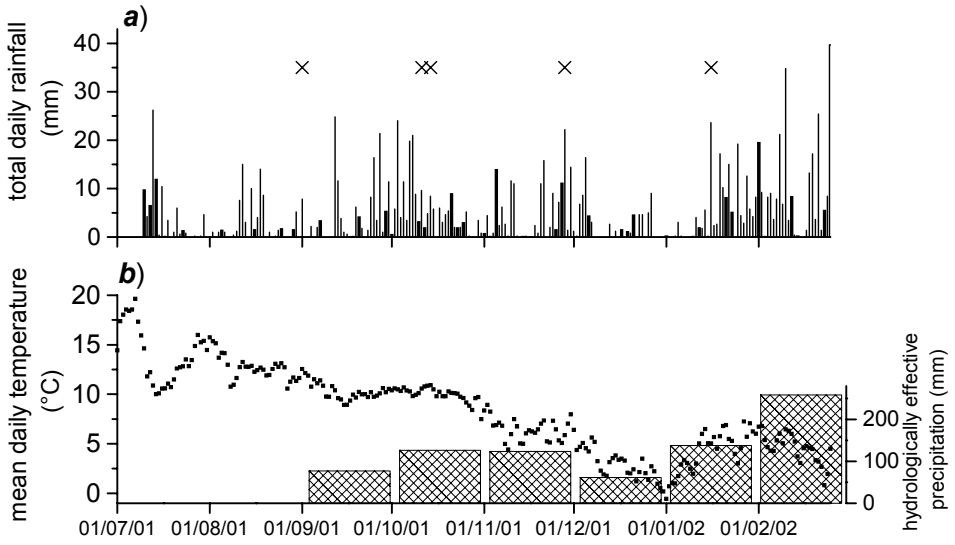


Figure 8.1
Conditions in the Coalburn Experimental Catchment during peat DOM monitoring ***a)*** *total daily rainfall (mm)* (X) *peat sampling date* ***b)*** (■) *mean daily temperature* (°C) *and* (bars) *hydrologically effective precipitation* (mm) *(calculated using Thornthwaite equation, Appendix 3). Data was collected and supplied by the Environment Agency.*

8.2.2 Conditions in the Coalburn Experimental Catchment during peat sampling

Peat sampling was performed on the dates shown on Table 8.1 through autumn/winter 2001/2002. As shown in Figure 8.1 the first samples were taken at the beginning of the period of wetting-up of the catchment, following dry conditions (hydrologically effective precipitation = 0mm). Antecedent conditions were not entirely dry and a period of rainfall occurred 10-15 days prior to sampling on 01/09/01. During this period observed flows within the catchment and discharge at the outfall were low, as shown in Figure 4.1.

Cores were sampled on 24/10/01 at site (1) and 11/10/01 at site (2). Prior to both of these dates there was relatively high rainfall, and an increasing level of hydrologically effective precipitation. Sampling in November was performed following, in comparison to the October sampling dates, a relatively dry period, with a similar level

of hydrologically effective precipitation. The final peat samples were taken in January 2002, following the dry conditions observed in December and early January.

8.2.3 Characteristics of peat in the Coalburn Experimental Catchment

As the peat examined was strutureless and no defining markers were found to link each profile and remove the problems of compaction and distortion during sampling. Absolute comparisons of peat at the same depth cannot be made. Comparisons between cores are made on broader depth units of similar vegetation content, degradation, moisture and appearance.

8.2.3.1 Coalburn Experimental Catchment site (1)

The physical description of soil sampled from site (1), situated under closed canopy forest on the peat sub-catchment, is detailed in Table 8.2. The depth of peat was relatively shallow, in comparison to other areas in the catchment (Rayner, 1997) and consisted of a layer of fresh vegetation above approximately 25cm of organic rich peat. Below this level, in unit 3, there was an increasing content of inorganic sand and clay, which became entirely inorganic below approximately 27cm depth.

In all of the profiles from this site the moisture content (% loss on drying), as shown in Figure 8.2, decreased down the core and was strongly negatively correlated with depth (Spearman's rho = -0.709 to -0.989 99% confidence level). It exhibited significantly higher means in unit 1 and 2 compared to lower units and in unit 3 compared to unit 4 (95% confidence level). The opposite relationship was seen in pH having a positive correlation with depth (Spearman's rho = 0.790 to 0.847 99% confidence level). All profiles had a significantly higher mean pH in unit 4 (5.1) compared to the other units (95% confidence level).

The moisture and pH variations reflect the physical composition of the material. Mounsey (1999) discussed the influence of organic acids and inorganic buffering in controlling pH, in the catchment, and this is reflected in these examples. Inorganic material significantly buffers pH at depth.

Mean values of both pH and moisture content were statistically indistinguishable between all site 1 samples, showing no significant changes over time. This suggests

that measured soil moisture at this site has little relation to the changing rainfall and temperature in the catchment shown on Figure 8.1. The influence of the forest by interception may smooth the soil moisture response as measured by this method. The recognition of the limited usefulness of this measure of soil moisture to estimate ambient conditions has been commented upon in previous studies of the catchment (Hind, 1992).

	Description	von Post scale
1	Undegraded to degraded spruce needles and woody material	H1
2	Increasing degradation of peat with depth; some fresh plant material	H1-H5
3	Transition from organic to inorganic material increasing sand and clay with depth	-
4	More inorganic than organic material	-
5	Entirely inorganic	-

Table 8.2
Description of sampled material from site (1) in the Coalburn Experimental Catchment. Details of von Post classification in Appendix 7.

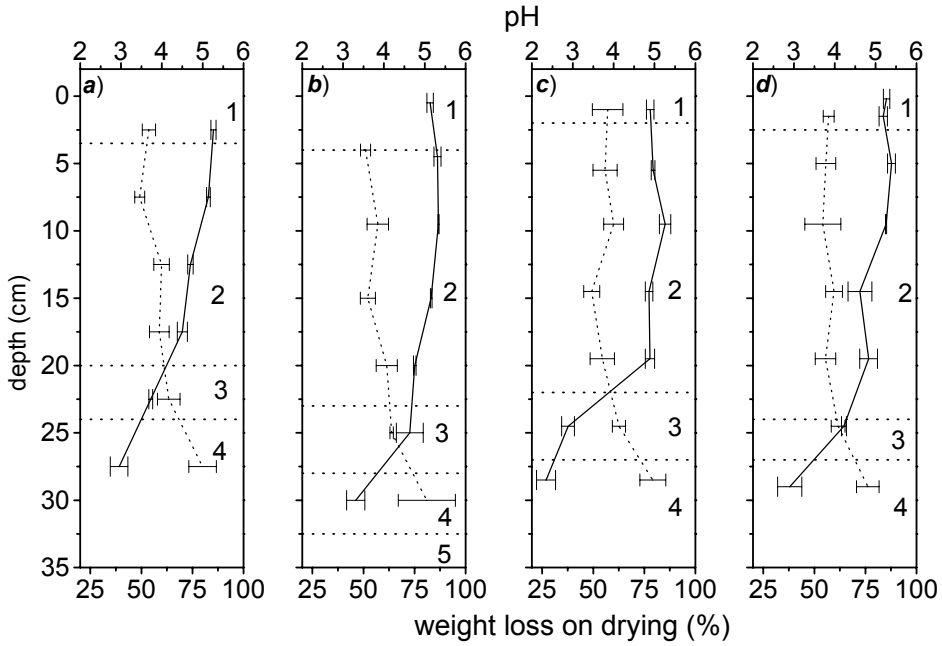


Figure 8.2
Details of peat cores from site (1) in the Coalburn Experimental Catchment showing moisture content (percentage weight loss on drying) (——) *and* (- - - -) *pH.*
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1) Numbers refer to units described in Table 8.2*

8.2.3.2 Coalburn Experimental Catchment site (2)

The characteristics of the sampled soil at site (2) are detailed in Table 8.3. The peat was structureless and homogenous throughout, showing gradual increased decomposition of vegetation with depth. The boundaries between each unit in cores from site (2) are represented on Figure 8.3 as distinct levels; however, these transitions were more diffuse occurring over up to 10cm of peat. The dotted line on Figure 8.3 represents a mean depth of change. Hind (1992) described peat from a similar site approximately 300m from the current sampling site. The A_1 horizon extended to depths of 1-2cm, the A_2 horizon to 14cm, below which was the B horizon to depths of 150cm. The difference between this profile composition and the current study may reflect spatial variations in peat over small area, or varying sampling technique.

The relationships of moisture content with depth, as shown in Figure 8.3, were different to those observed at site (1). Significantly drier peat was observed in unit 2 (84.2%). The variations seen in CB 01/09/01 (2) and CB 16/01/02 (2) showed an overall increase in moisture content with depth and a significantly higher mean value in unit 3 compared to unit 2 (95% confidence level). CB11/10/01 (2) and CB28/11/01 (2) showed significantly higher mean values in unit 1 and the top of unit 2 decreasing to minima in the bottom half of unit 2, and increasing in unit 3 (95% confidence level).

Moisture content was consistently significantly higher (99% confidence level) at site (2) compared to site (1) on the same sampling day. Lower soil water content was recognised under forest, in comparison to unplanted peat, within the catchment, by Robinson *et al*. (1998). Monitoring has shown that at an unplanted peat site, comparable to site (2), the water table water was within 50cm of the ground surface throughout the year, however, in a planted area this was seen for only 20% of the recorded period (1990-1993). At 20cm depth the unplanted site was saturated for over 50% of the monitored time, this was only seen in the forested site for 5% of the time. This disparity was solely attributed to vegetation and represents the higher total water use of the forest as combined losses from interception and transpiration.

	Description	von Post scale
1	Undecomposed vegetation, *Molinia* roots; little peat	H1
2	Decreasing proportion of plant material and increasing decomposition with depth; homogeneous	H2-H5
3	Decomposed peat; small amounts of plant matter, decomposing wood fragments; homogeneous	H6-H8

Table 8.3
Description of sampled material from site (2) in the Coalburn Experimental Catchment. Details of von Post classification in Appendix 7.

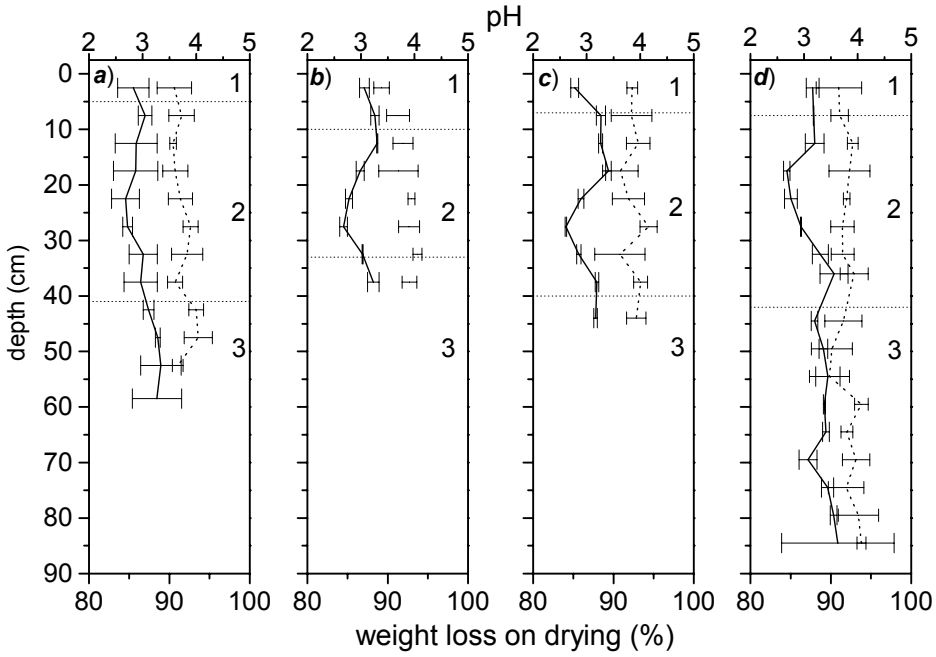


Figure 8.3
Details of peat cores from site (2) Coalburn Experimental Catchment showing moisture content (percentage weight loss on drying) (——) *and* (- - - -) *pH*
a) *CB 01/09/01(2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01(2)* ***d)*** *CB 16/01/02 (2) Numbers refer to units described in Table 8.3*

There were no significant differences in mean pH values in all cores at site (2) and no consistent changes with depth. Hind (1992) recognised that, in peat from a similar site in the catchment, pH showed an increase with depth. This was not seen in the current work, possibly due to different analytical techniques, or reflecting the different seasonal or spatial variations. The mean values of peat pH were not significantly

different between each monitored site, with the exception of significantly higher pH levels at site (1) with depth, due to the influence of buffering.

8.2.4 Spectrophotometric characteristics of peat DOM in the Coalburn Experimental Catchment

The following section describes the significant trends in spectrophotometric properties in each core. Full graphical representation of this data is presented in Figure 8.4 to Figure 8.15, at the end of this section.

In the analyses of peat DOM a number of general relationships were consistently seen throughout all peat extracts. Firstly, peak A_{Fint} and peak B_{Fint} correlated positively with each other and showed similar trends with depth in all cores. This reflected the same relationship seen throughout the data from surface and soil waters in the catchment. Secondly, there were strong positive correlations between absorbance measured at different wavelengths, however, the strength of this correlation decreased with increasing wavelength of analysis. Absorbance at $>A_{500nm}$ approached the lower limits of detection and was not recorded.

Throughout all the analyses absorbance exhibited the typical featureless DOM spectra of decreasing absorbance with increasing wavelength, as discussed in Section 1.5.1. EEMs from peat DOM had similar features to that seen in river water, in some cases peak D was noted (Figure 2.1).

The excitation and emission wavelengths of peak A, B and C were found to be consistent throughout the depths of all analysed cores. This resulted in no significant variations with depth or differences within or between the two sampling sites. Additionally, within each core the variations in wavelengths were found to be within the reproducibility of the extraction and analysis technique as discussed in Section 2.5. The spectrophotometric properties of DOM from each profile are summarised in Table 8.4 and 8.5.

Core CB 01/09/01 (1)	
Peak B_{FInt} and peak B_{FInt}/ peak A_{FInt}	Maximum at the top of unit 2
Peak C_{FInt} and peak C_{FInt}/ peak A_{FInt}	Decrease with depth
Absorbance	Increase in unit 3 and 4
Peak A_{FInt} /A_{340nm}	Decrease in unit 3 and 4
A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm}	Negative correlation with depth (Spearman's rho = -0.860 and -0.947)
Core CB 24/10/01 (1)	
Peak A_{FInt}, peak B_{FInt}, peak B_{FInt}/ peak A_{FInt} and peak C_{FInt}/ peak A_{FInt}	Maxima in the top half of unit 2
Absorbance	Maximum in the top half of unit 2 and a significant maximum in unit 4
A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm}	Maximum values in unit 1. Values of A_{254nm}/A_{410nm} were exceptionally high (mean = 20.70 s.d. 1.57)
Core CB 28/11/01 (1)	
Peak A_{FInt} and peak B_{FInt}	Increase from unit 1 to the middle of unit 2
Peak B_{FInt}/ peak A_{FInt}	Minimum in unit 1
Peak C_{FInt} and peak C_{FInt}/peak A_{FInt}	Maximum in unit 1
Absorbance	Maximum at the top of unit 2 and an increase with depth in unit 3 and 4
Peak A_{FInt} /A_{340nm}	Minimum at the top of unit 2 and low values in units 3 and 4
A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm}	Negative correlation with depth
Core CB 16/01/02 (1)	
Peak A_{FInt} peak B_{FInt} and peak B_{FInt}/ peak A_{FInt}	Maximum at the top of unit 2
Peak C_{FInt} and peak C_{FInt}/peak A_{FInt}	Maximum in unit 1
Absorbance	Maximum in unit 4
Peak A_{FInt} /A_{340nm}	Minimum in unit 4
A_{254nm}/A_{410nm} and A_{254nm}/A_{365nm}	Negative correlation with depth

Table 8.4
Summary of the significant relationships of the spectrophotometric properties of peat derived DOM with depth in peat cores from Coalburn Experimental Catchment ***Site (1)****. All significant relationships at 95% confidence level. For values refer to Figures 8.4 to 8.15.*

Core CB 01/09/01 (2)	
Peak A_{Fint} and peak B_{Fint}	Negative correlation with depth (Spearman's rho –0.699 and –0.763)
Peak B_{Fint} /peak A_{Fint}	Negative correlation with depth (Spearman's rho –0.791)
Absorbance	Negative correlation with depth (Spearman's rho= -0.384 to -0.631)
Peak C_{Fint}/peak A_{Fint}	Positive correlation with depth (Spearman's rho = 0.700).
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	Negative correlation with depth (Spearman's rho =-0.723 and -0.720)
Peak A_{Fint} /A_{340nm}	Minimum in unit 2 and unit 3
Core CB11/10/01 (2)	
Peak A_{Fint} and peak B_{Fint} Peak C_{Fint}	Minimum in the middle of unit 2. Peak A_{Fint} and peak B_{Fint} maximum ~25-30cm
Absorbance	Maximum 30-35cm
Peak A_{Fint} /A_{340nm}	Maximum centre unit 2
A_{254nm}/A_{365nm}	Decrease with depth
A_{254nm}/A_{410nm}	Decrease with depth, maximum at the base of unit 2
Core CB28/11/01 (2)	
Peak A_{Fint} and peak B_{Fint}	Negative correlation with depth (Spearman's rho = -0.843 and -0.857
Absorbance	Negative correlation with depth
Peak C_{Fint}	Positive correlation with depth (Spearman's rho = 0.766). Maximum in the middle of unit 2
Peak A_{Fint} /A_{340nm}	Decrease through unit 2
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	Negative correlation with depth
Core CB16/01/02 (2)	
Peak A_{Fint} and peak B_{Fint}	Negative correlation with depth (Spearman's rho= -0.600 and -0.744)
Peak B_{Fint}/peak A_{Fint}	Negative correlation with depth
Peak C_{Fint}/peak A_{Fint}	Positive correlation with depth (Spearman's rho = 0.874)
Absorbance,	Positive correlation with depth
Peak A_{Fint} /A_{340nm}	Increase with depth
A_{254nm}/A_{410nm}	Negative correlation with depth

Table 8.5
Summary of the significant relationships of the spectrophotometric properties of peat derived DOM with depth in peat cores from Coalburn Experimental Catchment ***Site (2)****. All significant relationships at 95% confidence level. For values refer to Figures 8.4 to 8.15.*

8.2.5 Depth relationships of the spectrophotometric characteristics of peat in the Coalburn Experimental Catchment

In profiles from both sites absorbance ratios decreased with depth. In litter layers (site (1)) the values observed were higher than those seen in surface water in this area, but were similar to that seen in degraded spruce needles (Section 3.8.4). The values rapidly decreased with depth, indicating that the DOM spectrophotometric properties are altered with degradation and spruce needle DOM is lost. Spruce needle DOM also exhibited high peak A_{Fint}/A_{340nm} (6585, see Table 3.17). Such levels were not observed in peat DOM even in litter layers, however peak A_{Fint}/A_{340nm} was significantly higher in units 1 and 2 at site (1) (minimum =1315) than surface (95% confidence level). Profiles from site (2) were also significantly higher in peak A_{Fint}/A_{340nm} (minimum= 798) than peat sub-catchment surface DOM.

At the base of profiles from site (1) in inorganic material peak A_{Fint}/A_{340nm} significantly decreases (<1100; 99% confidence level). DOM of relatively higher molecular weight is observed, this may be a function of the extraction method, which is preferentially releasing DOM sorbed onto the inorganic matrix.

In peat DOM from site (2) peak A_{Fint} and peak B_{Fint} exhibited a decrease with depth. Mean absorbance also decreases with depth, the strength of the relationship depending on the wavelength measured. As discussed in Section 3.5 throughout the Coalburn Experimental Catchment both intensity and absorbance are positively correlated with DOC concentration and it can be assumed that a similar relationship exists in the peat DOM spectrophotometric properties. This indicates that there is a decrease in either the extractability of DOM, or the concentration of DOM with depth. Similar trends have been observed with depth in peat and soil water DOM (Fraser *et al*., 2001) and have been related to microbial processing and sorption to inorganic material.

In peat DOM from site (1) peak A_{Fint} and peak B_{Fint} showed no overall differences in value between each peat core, but exhibited a consistent mean peak at the top of unit 2. Peak B_{Fint}/peak A_{Fint} values mirror this trend, as does absorbance. Absorbance also showed a high level in unit 3 and unit 4 (core CB 01/09/0 (1) and 28/11/01 (1)).

Mounsey (1999) suggested that lower levels of the catchment soils are active under lower flow conditions. As discussed in Chapter 4 the relationship of absorbance and fluorescence intensity to discharge at CB_{weir} is overall one of dilution, with the highest values occurring at low flow. Under low flow conditions a deep source of DOM is assumed to be active, if the model in Figure 1.7 is applied. The decrease in fluorescence intensity and absorbance with depth at site (2) does not correspond with this and may suggest that under low flow conditions DOM may be derived from seepage from ditch faces rather than lateral movement from lower levels of the peat. This exemplifies the complex nature of the DOM sources within the catchment.

The values of peak C_{Fint} in site (1) and (2) show little variation with depth, except in core CB 28/11/01 (1) and 16/01/02 (1), which exhibit high values in unit 1. A similar high mean value is observed in core CB 16/01/02 (1), in peak D_{Fint} ($EX\lambda$=220 $EM\lambda$=300nm). This peak is not commonly observed in DOM analyses, and it was not observed in any other samples from the catchment. Lower intensity levels below the litter layer suggest that both of the amino acid fluorophores are rapidly processed within the soil, as plant material is degraded.

A number of significant differences in mean values from the whole of the cores can be seen in all of the data: A_{254nm}/A_{365nm}; A_{254nm}/A_{410nm}; peak B_{Fint} /peak A_{Fint} and peak A_{Fint} /A_{340nm} were higher in site (1). Peak A_{Fint}; peak B_{Fint}; peak C_{Fint} were higher in site (2). Absorbance was only significantly different at A_{272nm} having higher means in site (2) at all depths. The differences observed between forested and unforested DOM spectrophotometric properties indicates a less aromatic and/or lower molecular weight DOM with greater depth variations in site (1) compared to site (2) which exhibits a greater abundance of extractable DOM.

8.2.6 Seasonal patterns in the spectrophotometric characteristics of peat DOM in the Coalburn Experimental Catchment

In the peat profile data from site (1) there were no significant changes identified over time. The changes over time in peat DOM at site (2) were dominated by a significant increase in peak A_{Fint}, peak B_{Fint} and absorbance (of 13.7%) in units 1 and 2 over the sampling period. Unit 3 was not included in this comparison to remove the bias due to extended depth in core CB16/01/02 (2). In comparison to the trend seen in P_{weir} adjacent to site (2) a similar relationship was observed in both specific fluorescence

intensity and absorbance, which increased over the sampling period. Although no absolute correlations were seen between peat DOM and the surface water DOM signals the same seasonal changes can be recognised. Additionally, peak A_{Fint}/A_{340nm} showed a similar increase over the sampling period of site (2) peat DOM and P_{weir}.

In all of the profiles there were no correlations found between the rainfall and temperature observed prior to and during each sampling date and the spectrophotometric characteristics of the peat DOM. Together with the limited changes over time this, suggests that this method of investigation is not sensitive enough to recognise such changes in DOM properties, or alternatively the peat within the catchment is relatively stable through out such a period. The period in question may not adequately reflect the cycles observed in the catchment peat and further work is required to continue this study, with observations made during spring and summer periods.

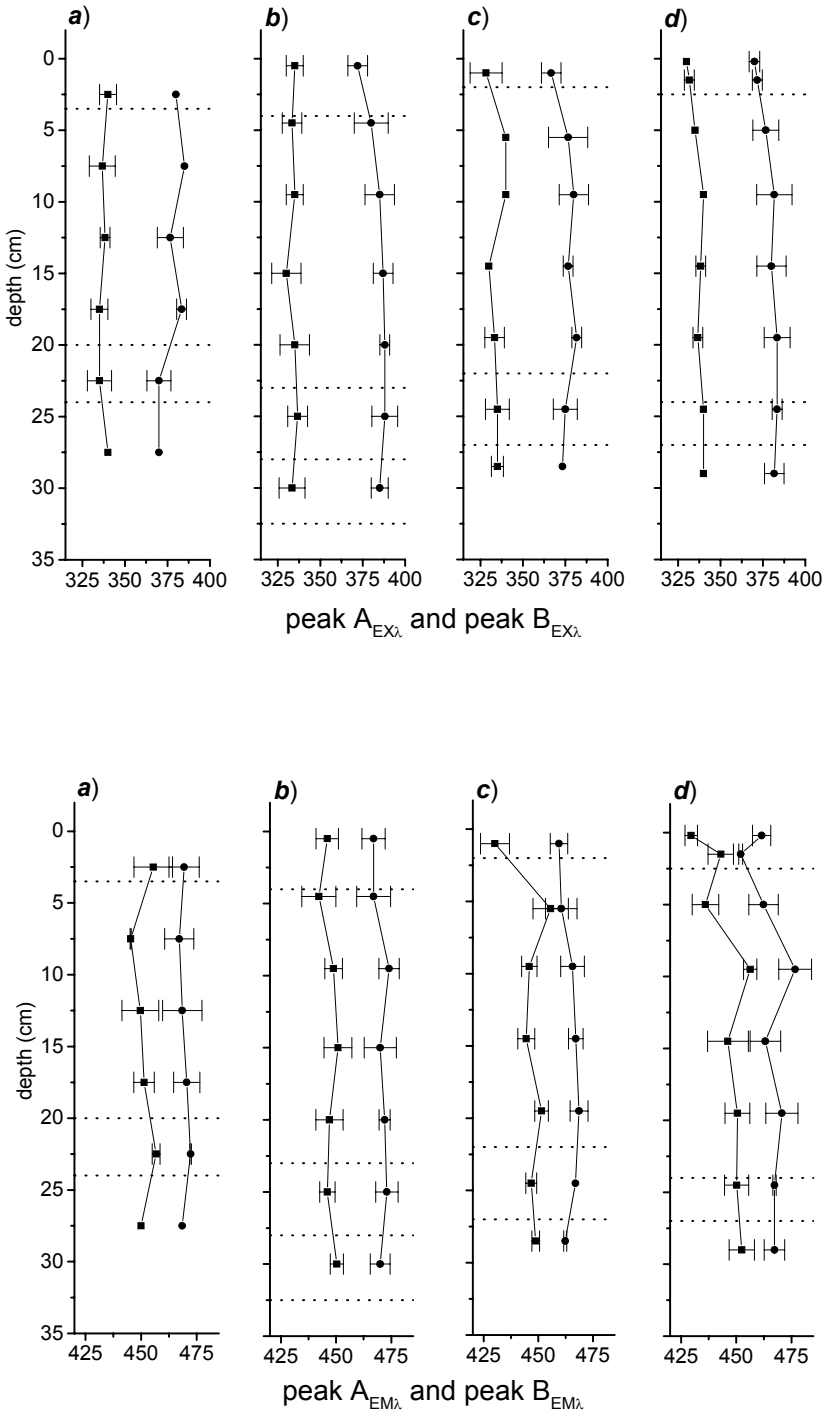


Figure 8.4
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: ■ peak* $A_{EX\lambda}$ *● peak* $B_{EX\lambda}$ ***Bottom****: ■ peak* $A_{EM\lambda}$ *and ● peak* $B_{EM\lambda}$
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

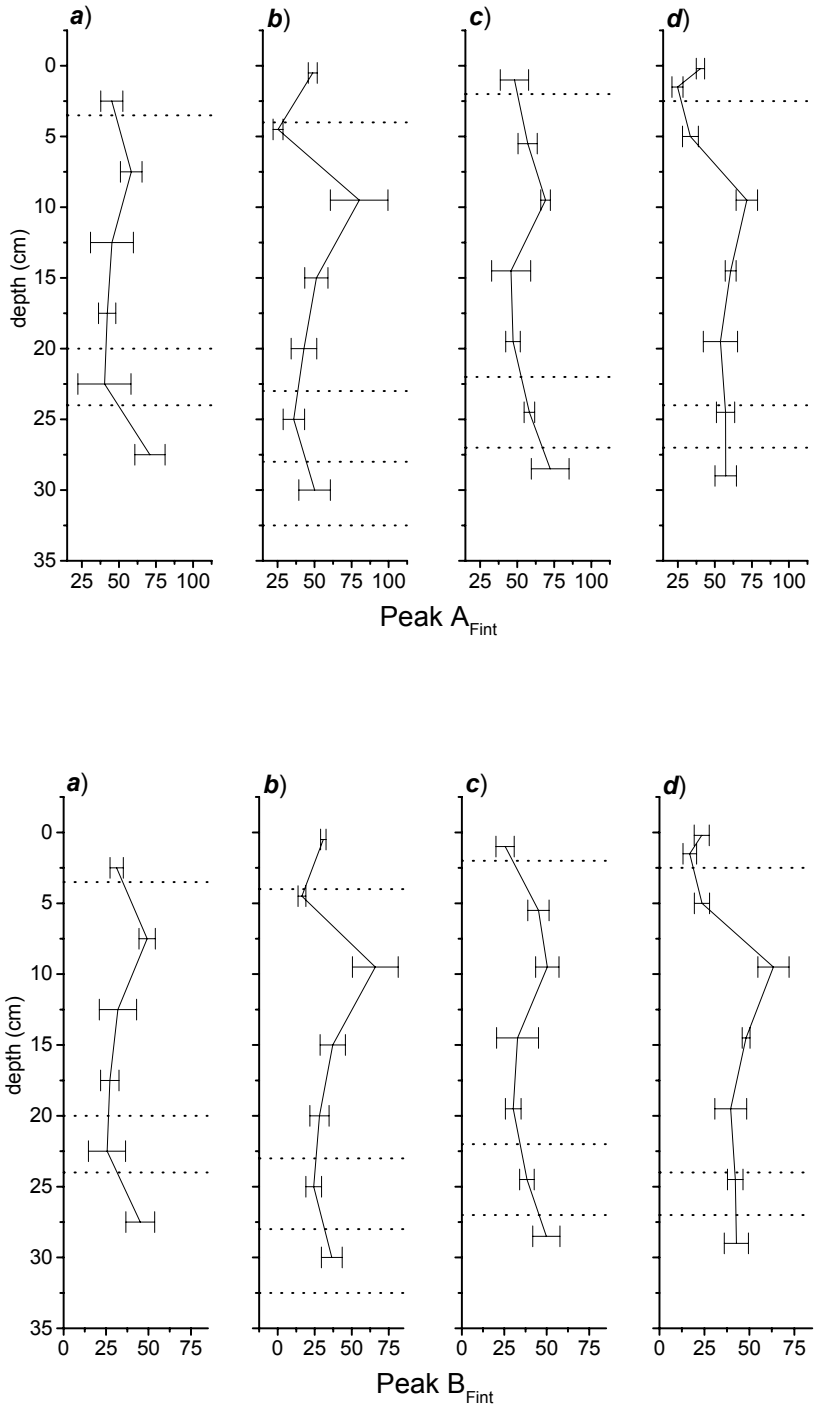


Figure 8.5
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* A_{Fint} ***Bottom****: peak* B_{Fint}
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

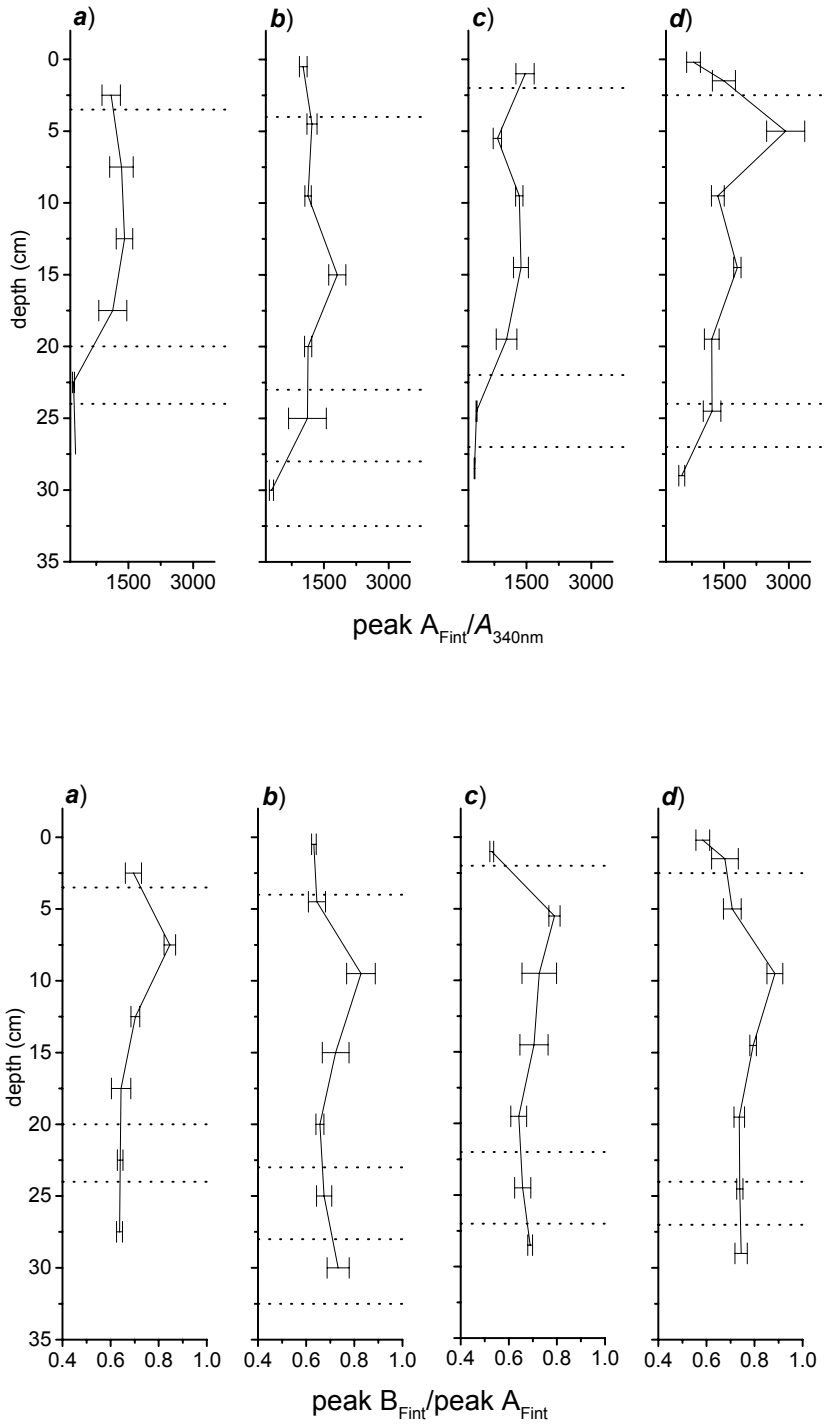


Figure 8.6
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* A_{Fint}/A_{340nm} ***Bottom****: peak* B_{Fint}/peak A_{Fint}
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

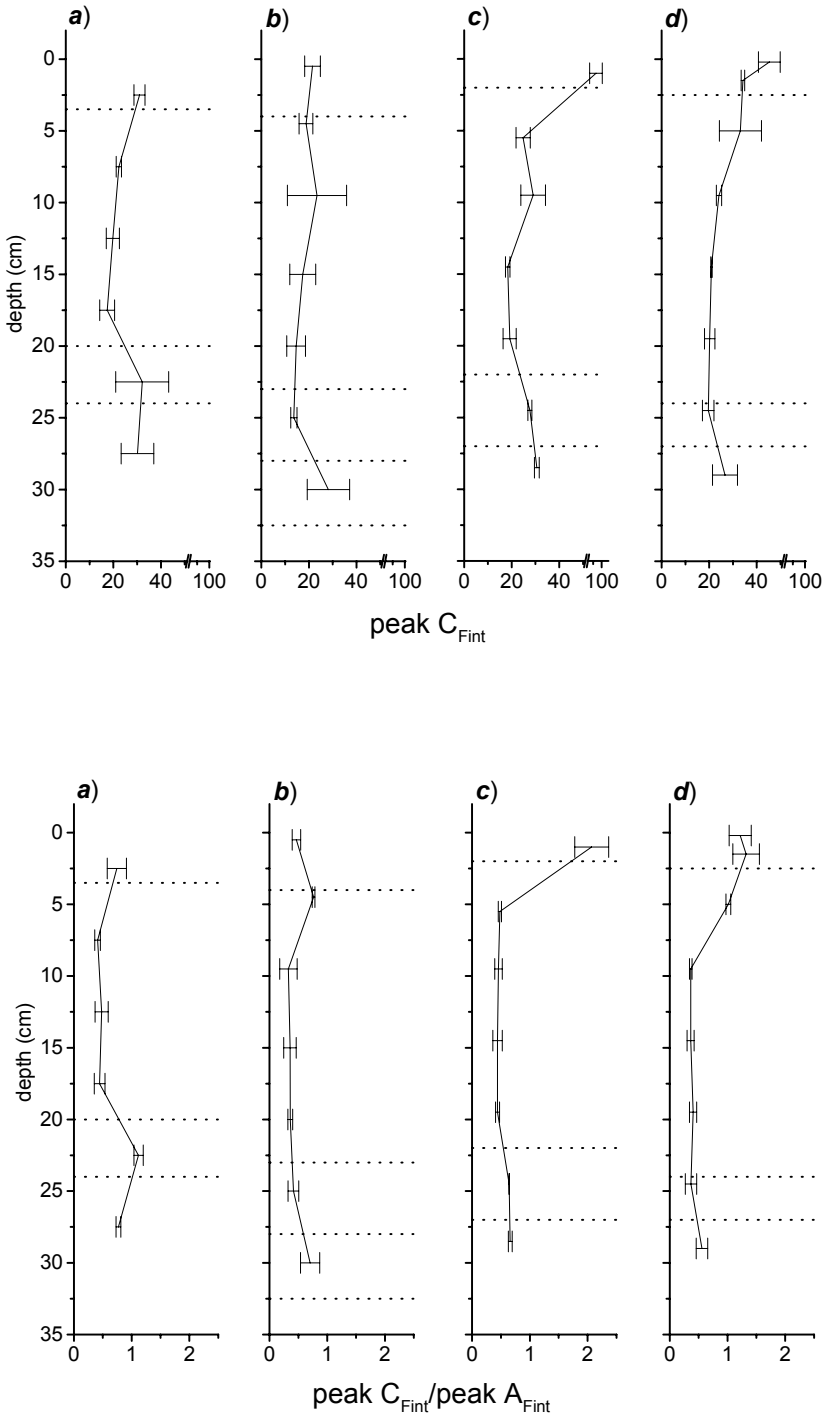


Figure 8.7
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* C_{Fint} ***Bottom****: peak* C_{Fint}*/peak* A_{Fint}
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

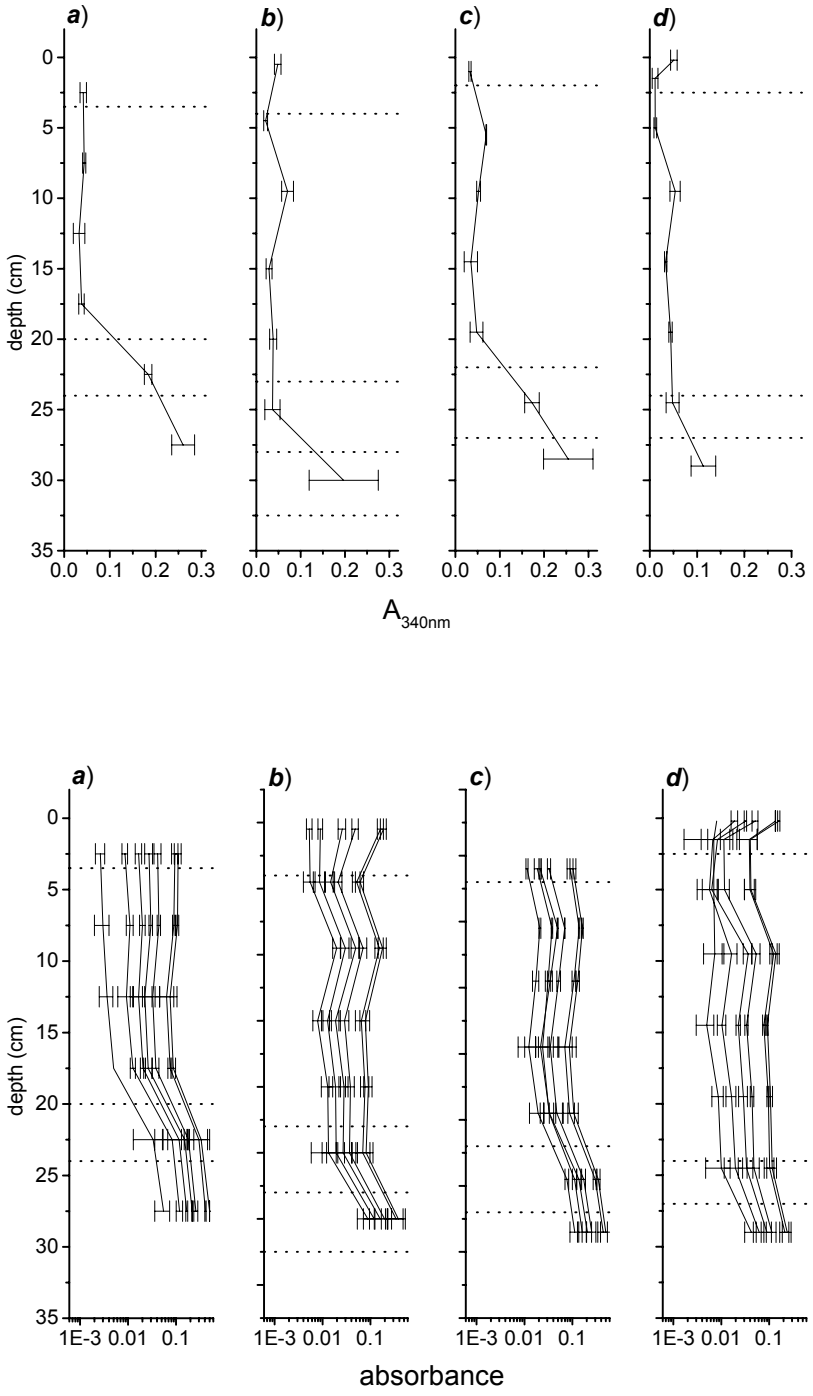


Figure 8.8
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: A_{340nm}* ***Bottom****: absorbance (cm^{-1})*
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

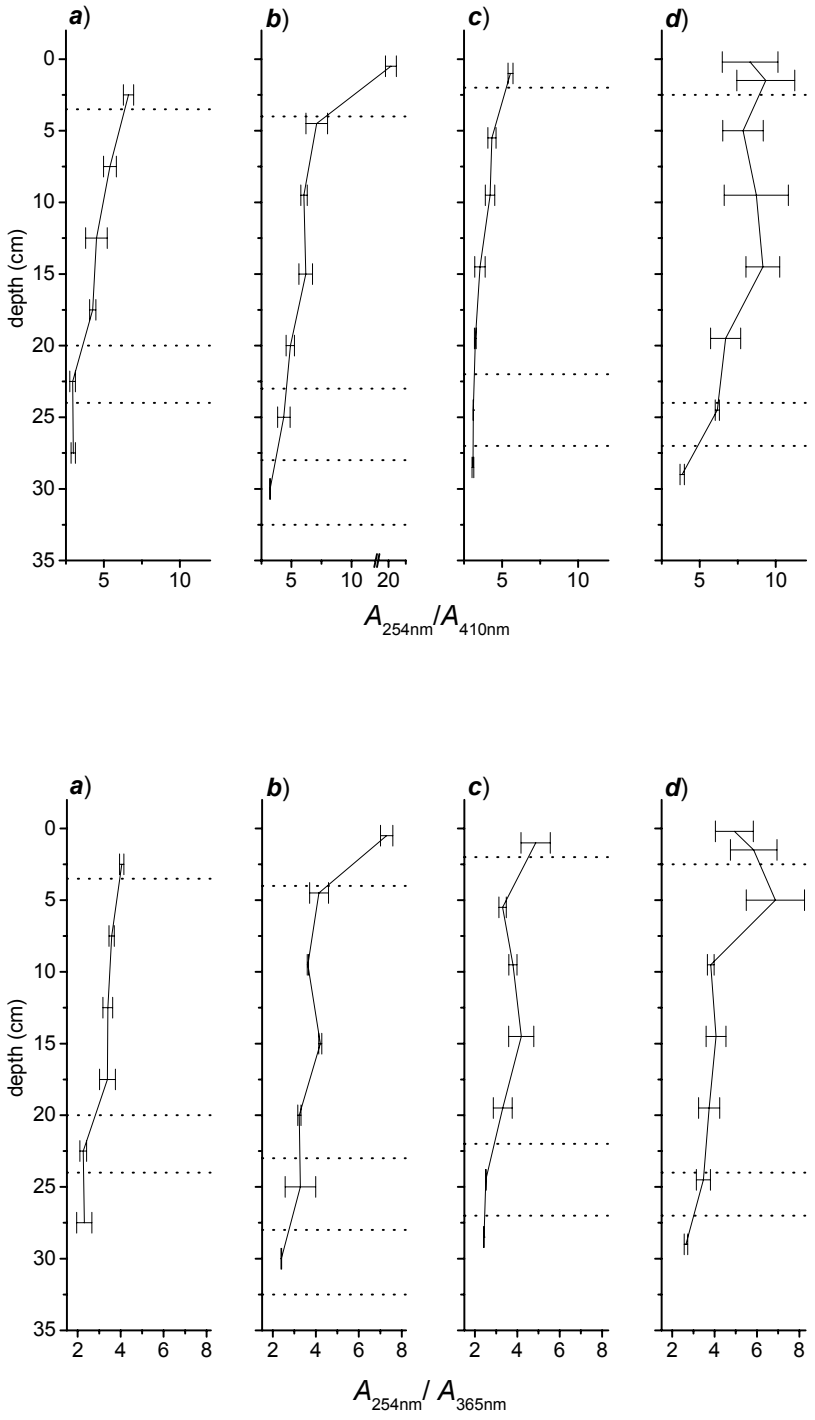


Figure 8.9
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: A_{254nm}/A_{410nm}* ***Bottom****: A_{254nm}/A_{365nm}*
a) *CB 01/09/01 (1)* ***b)*** *CB 24/10/01 (1)* ***c)*** *CB 28/11/01 (1)* ***d)*** *CB 16/01/02 (1)*

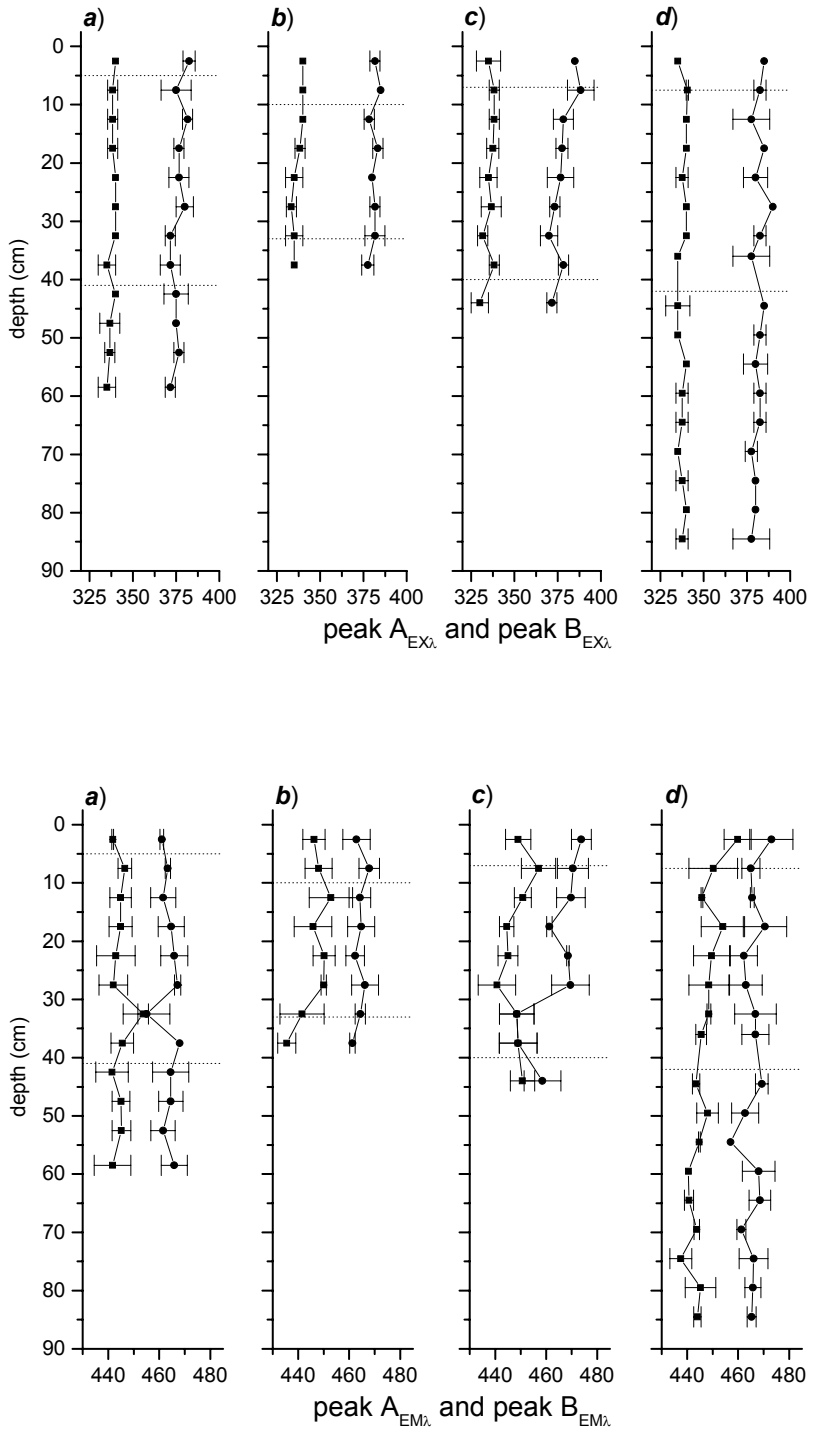


Figure 8.10
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: ■ peak* $A_{EX\lambda}$ *● peak* $B_{EX\lambda}$ ***Bottom****: ■ peak* $A_{EM\lambda}$ *and ● peak* $B_{EM\lambda}$
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

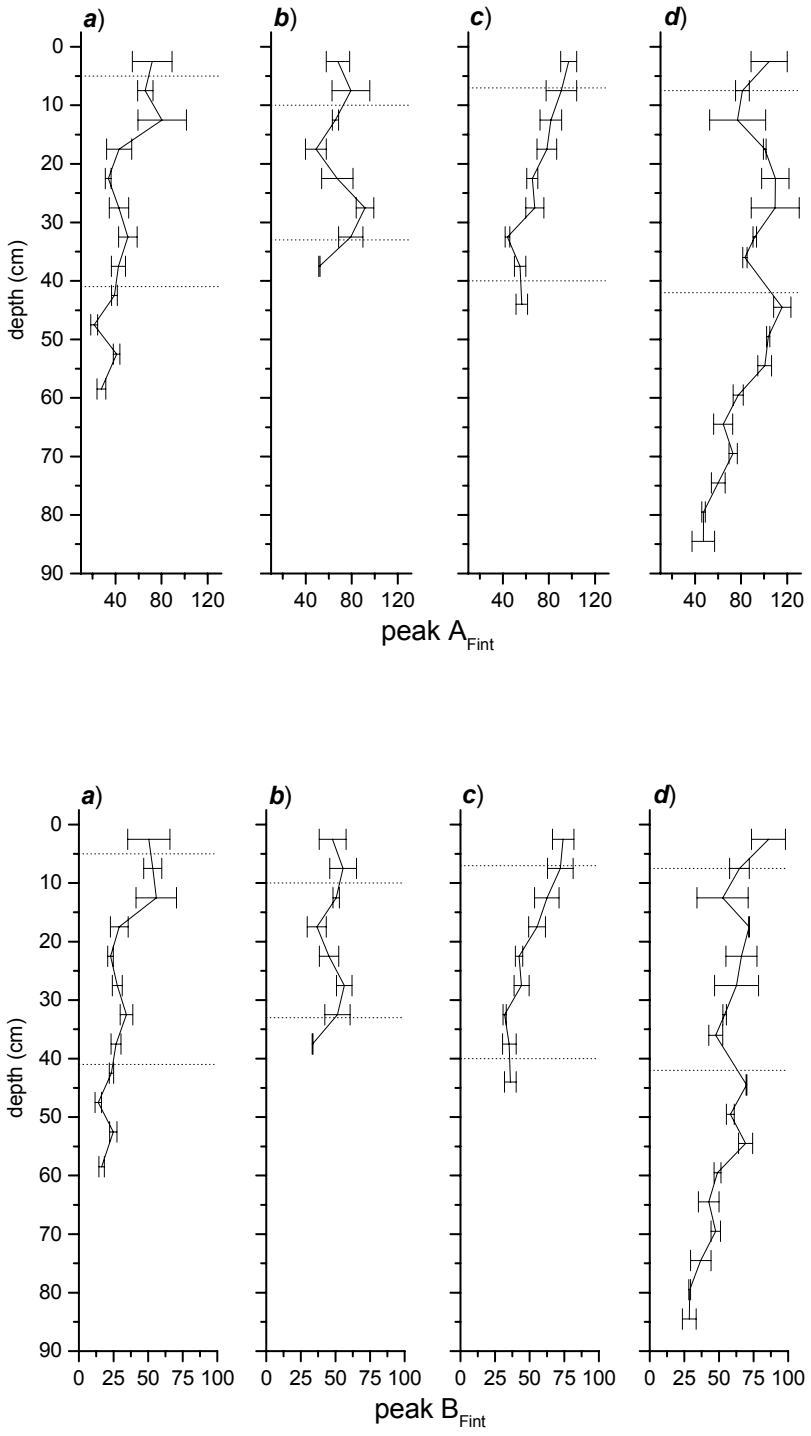


Figure 8.11
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* A_{Fint} ***Bottom****: peak* B_{Fint}
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

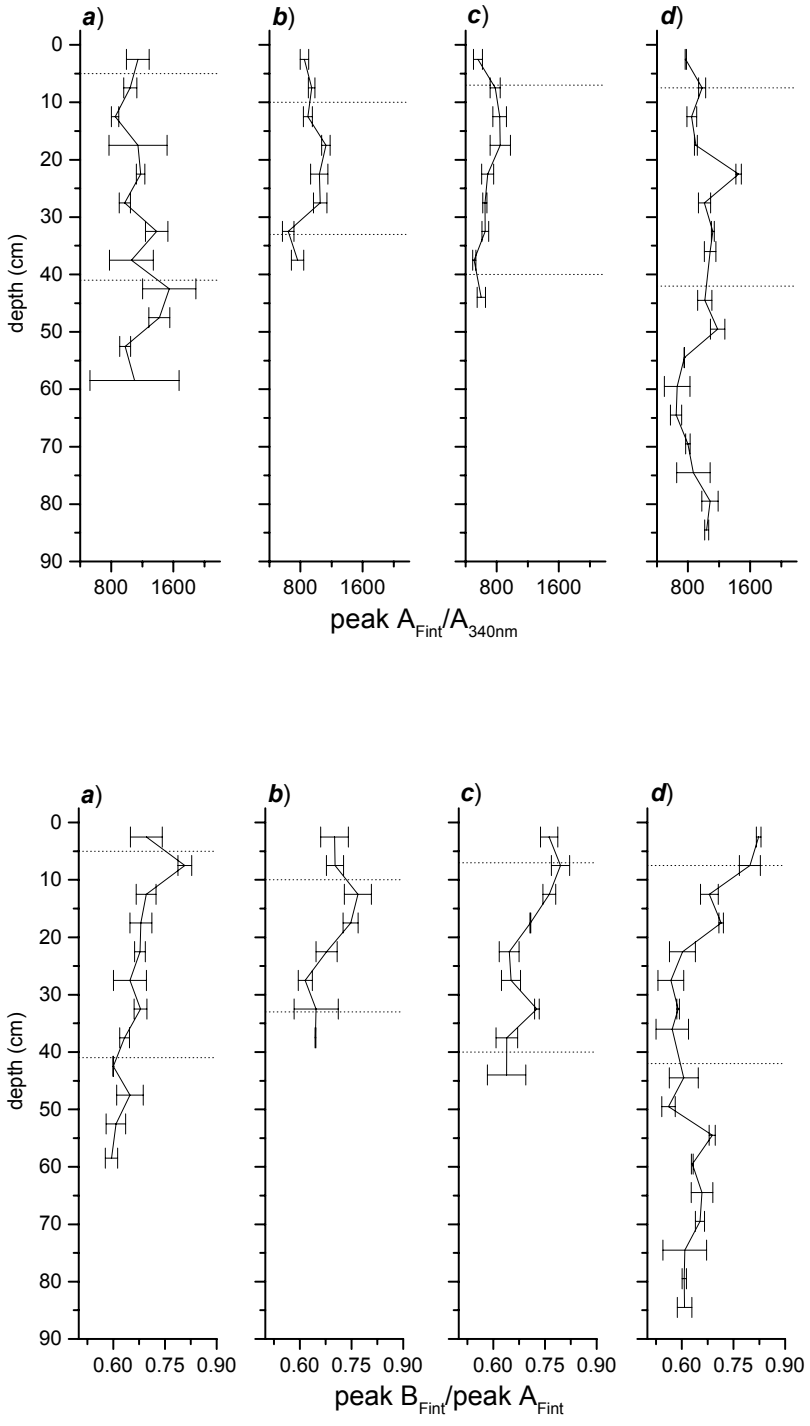


Figure 8.12
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* A_{Fint}/A_{340nm} ***Bottom****: peak* $B_{Fint}/peak\ A_{Fint}$
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

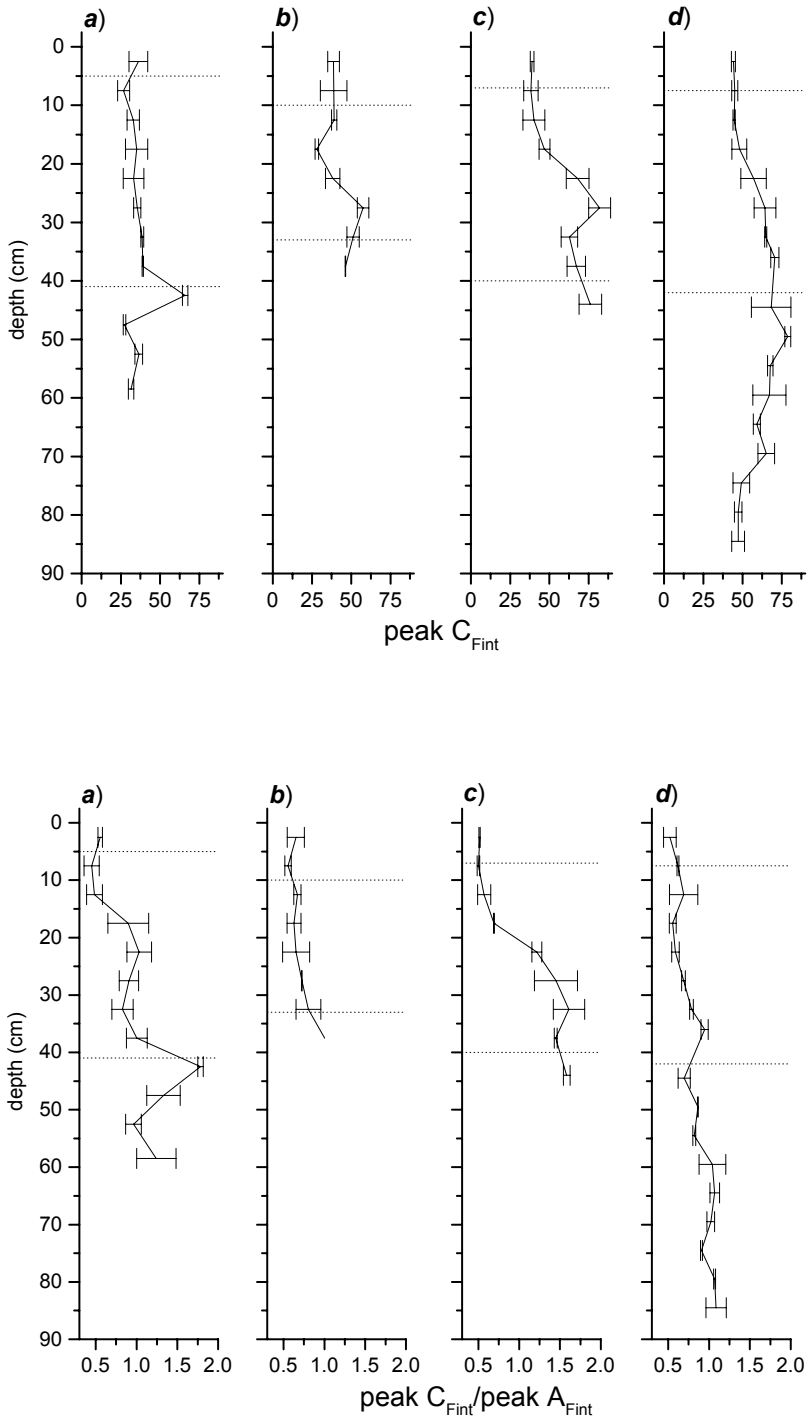


Figure 8.13
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: peak* C_{Fint} ***Bottom****: peak* C_{Fint}*/peak* A_{Fint}
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

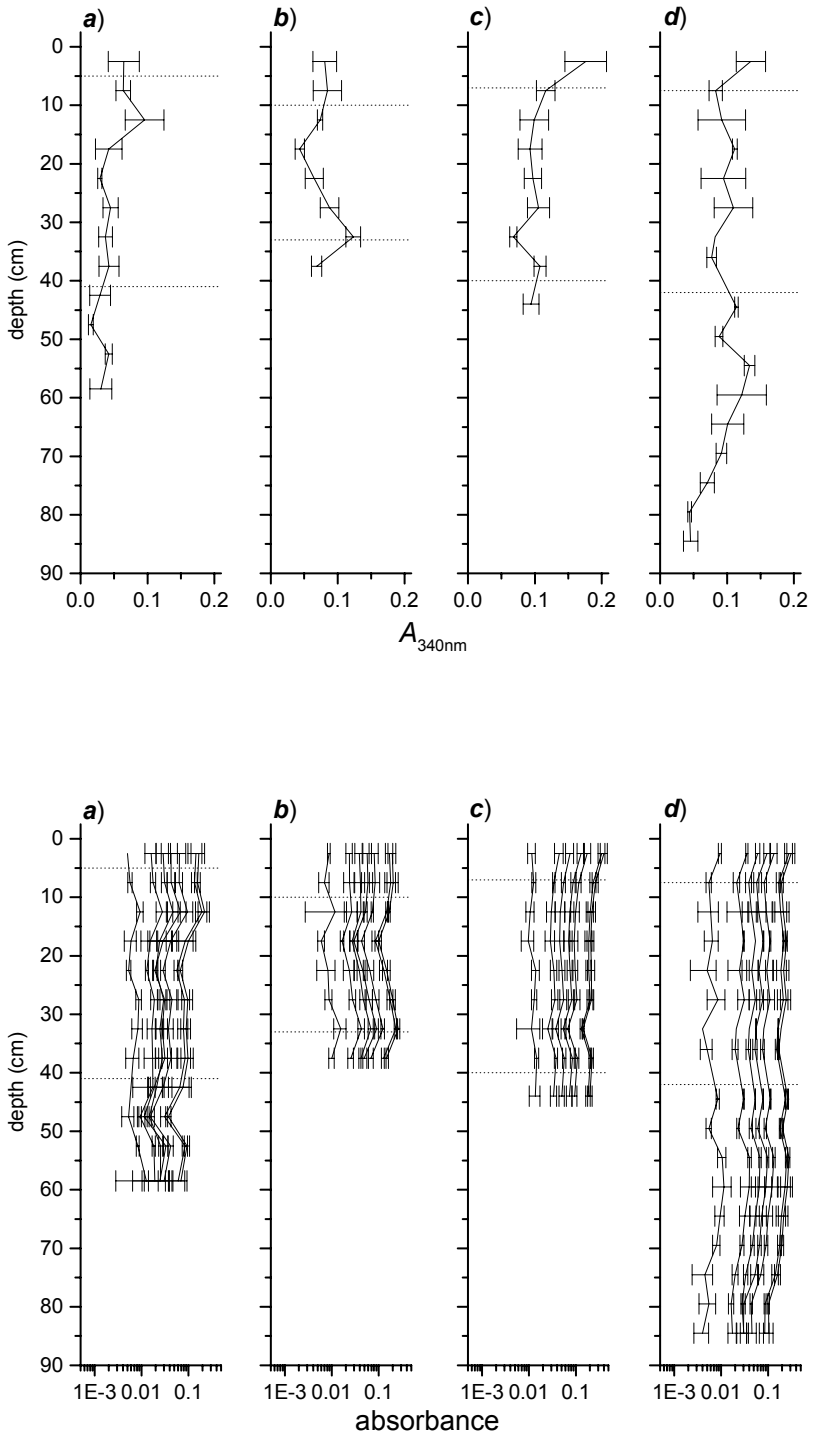


Figure 8.14
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****: A_{340nm}* ***Bottom****: absorbance (cm^{-1})*
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

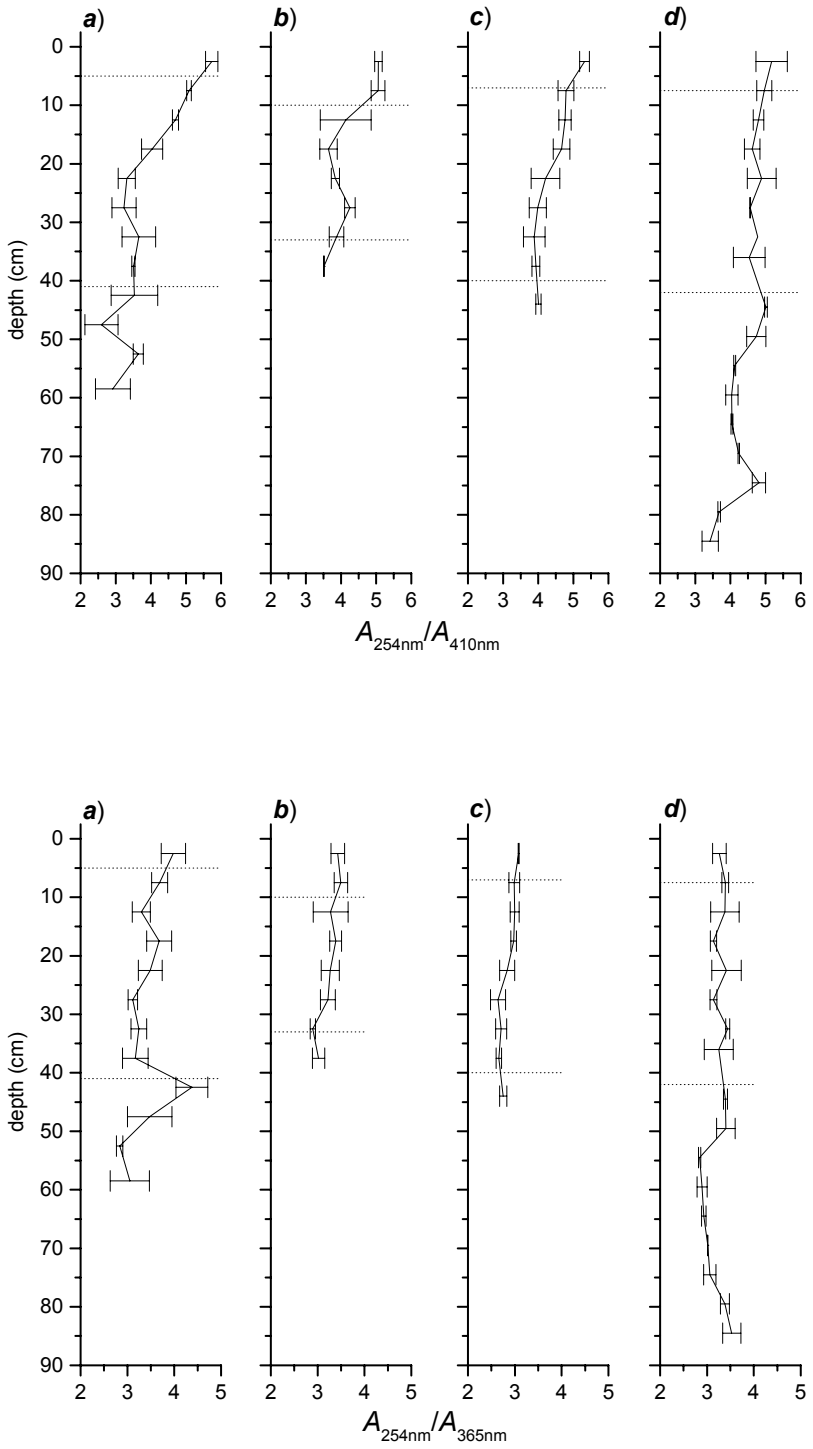


Figure 8.15
Spectrophotometric properties of peat DOM in Coalburn Experimental Catchment ***Top****:* A_{254nm}/A_{410nm} ***Bottom****:* A_{254nm}/A_{365nm}
a) *CB 01/09/01 (2)* ***b)*** *CB 11/10/01 (2)* ***c)*** *CB 28/11/01 (2)* ***d)*** *CB 16/01/02 (2)*

8.3 Peat DOM from the Loch Assynt area

The following section will describe the spectrophotometric properties of extractable DOM from peat in the Loch Assynt area. The spatial variations in peat from three sites, during spring and autumn, will be examined to establish any significant differences in the DOM obtained from peat over space and time in this area. These variations will be compared to the identified spatial and temporal surface water DOM spectrophotometric properties discussed in Chapter 5 and 6. The characteristics of peat DOM from the Loch Assynt area will be compared to that discussed in Section 8.2 from the Coalburn Experimental Catchment.

To compare seasonal and spatial variations in the spectrophotometric properties of peat DOM cores were taken during May and September 2001 from two different localities. In contrast to the temporal variations discussed in relation to peat DOM from the Coalburn Experimental Catchment the study of the Loch Assynt area examines broad seasonal variations, during distinct periods of the annual cycle in DOM export. A typical DOC concentration cycle as discussed in Section 6.4 was recognised in the River Traligill catchment. May 2001 sampling represents the spring period when water had a low DOC concentration and water colour compared to September 2001, which was during the summer/autumn flush of DOM.

Three locations were selected to sample peat, two in contrasting locations within the Traligill catchment. The first location (site 1) was within the upper Traligill catchment in an area of comparatively low DOC concentration runoff (Chapter 5; group 1). The second location (site 2) was, in comparison, situated in an area with typically higher DOC concentration runoff (Chapter 5; group 2) and the third site (3) was located in an area of generally higher DOC concentration runoff than both site 1 and 2.

8.3.1 Sampling locations

Three locations were sampled, to investigate the spatial variability or similarity of peat in the region. Site 1 was located in the Upper Traligill above cave Uamh an Tartair, replicating the location used by (NC 276205) close to the site of UAM 4 of Charman *et al.,* (2001). Site 2 is located in the lower reaches of the Traligill catchment (NC 265223) in the valley of a tributary (Allt Poll an Droighinn) and is situated above non-Durness geology. These sites are located on Figure 1.8. The third site was located

outside the Traligill catchment (NC 230273) approximately 5km NNE of Inchnadamph on the eastern slope of Quinag, above non-Durness geology. All sites were at an altitude of approximately 250m.

Peat cores were sampled on 22/05/01 at site 1 and 2 and 23/05/01 at site 3, replicates at site 1 and 2 were sampled on 03/09/01. The cores are named as follows relating to sampling date and location: - AS 21/05/01 (1); AS 21/05/01 (2); AS 22/05/01 (3); AS 03/09/01 (1); AS 03/09/01 (2).

The samples were taken as described in Section 2.5 and analysed using the procedure outlined in Section 2.2. Moisture content and pH were measured as detailed in Section 8.2.1.

8.3.2 Conditions in the Loch Assynt area during peat sampling

The temperature and rainfall prior to and during peat sampling in the Assynt region are detailed in Figure 8.16 with calculations of moisture excess using the Thornthwaite method. Prior to sampling in May 2001 the area experienced a drier period, (mean daily rainfall = 1.77mm) during the previous month, this resulted in a negative hydrologically effective precipitation value for the month of May. In contrast there was positive value prior to the September sampling date when the catchment began to wet up after the summer dry period. Immediately prior to the September sampling date the first major rainfall event occurred (30/08/01 33.08mm).

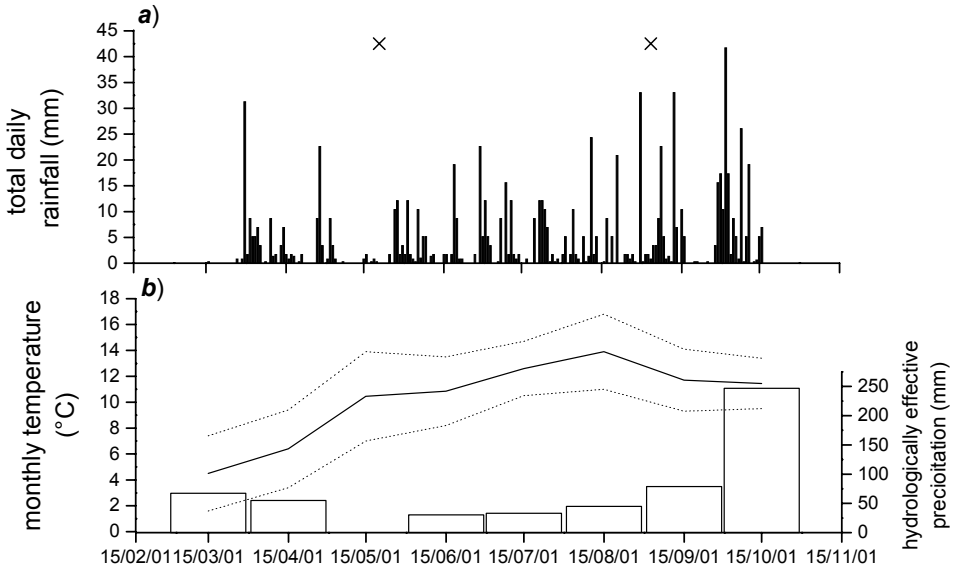


Figure 8.16
Conditions in the Loch Assynt area during peat DOM monitoring ***a)*** *total daily rainfall (mm)* (**X**) *peat sampling date* ***b)*** (——) *mean monthly temperature* (- - -) temperature max and min (°C) (bars) *hydrologically effective precipitation (mm) (calculated using Thornthwaite equation, Appendix 3) (source Met Office).*

8.3.3 Characteristics of peat from the Loch Assynt area

The peat sampled from site 1 and 2 showed no physical differences between each sampling date and in all cases the profiles examined exhibited homogeneous peat with no visible structure. The profiles were divided into units of similar appearance and humification, measured on the von Post scale (Appendix 7), for comparison of DOM spectrophotometric data. The details of these units are recorded in Table 8.6 for each site. The transition depths between each unit shown in Figure 8.17 were not definite boundaries and represent the averages of the gradual change with depth.

Profiles from site 2 and 3 were comparatively similar to each other, in relation to site 1. The latter peat exhibited a lighter colour and less humification compared to site 2 and 3. A different loss on drying (moisture content) relationship with depth was also observed between the sites.

The moisture content profile (Figure 8.17) was replicated in both site 1 and 2 during both sampling dates. Peat from site 1 exhibited higher moisture content in unit 2 compared to above and below. This was in contrast to site 2 which had lower moisture content in unit 2, compared to peat above and below. The profiles from this site showed maximum values over the transition from unit 2 to 3. In peat from site 3 the moisture content was constant with a low mean at the top of unit 2 this core showed the highest overall mean moisture content. Although as shown in Figure 8.16 there were differing moisture regimes at each sampling date there was, however, no consistent or significant difference in peat sampled on each date in either variation with depth or absolute values. The mean values of pH of did not significantly vary with depth, or between sampling sites.

a)

	Description	**von Post scale**
1	Undecomposed vegetation dominant	H1
2	Increasing decomposition, decreasing plant material and proportion of peat with depth	H1-H4
3	Little plant material, degraded peat dominant of darker colour than above, degraded wood	H3-H6

b)

	Description	**von Post scale**
1	Undecomposed vegetation dominant	H1
2	Increasing decomposition, decreasing plant material with depth	H1-H6
3	No plant material highly decomposed peat homogeneous greater moisture compared to above	H6-H9

c)

	Description	**von Post scale**
1	Undecomposed vegetation dominant	H1
2	Increasing decomposition, decreasing plant material with depth, increased moisture with depth	H1-H6
3	No plant material highly decomposed peat of darker colour than above homogeneous	H6-H8

Table 8.6
*Description of sampled material from Loch Assynt Area **a)** site (1) **b)** site (2) **c)** site (3) Details of von Post classification in Appendix 7.*

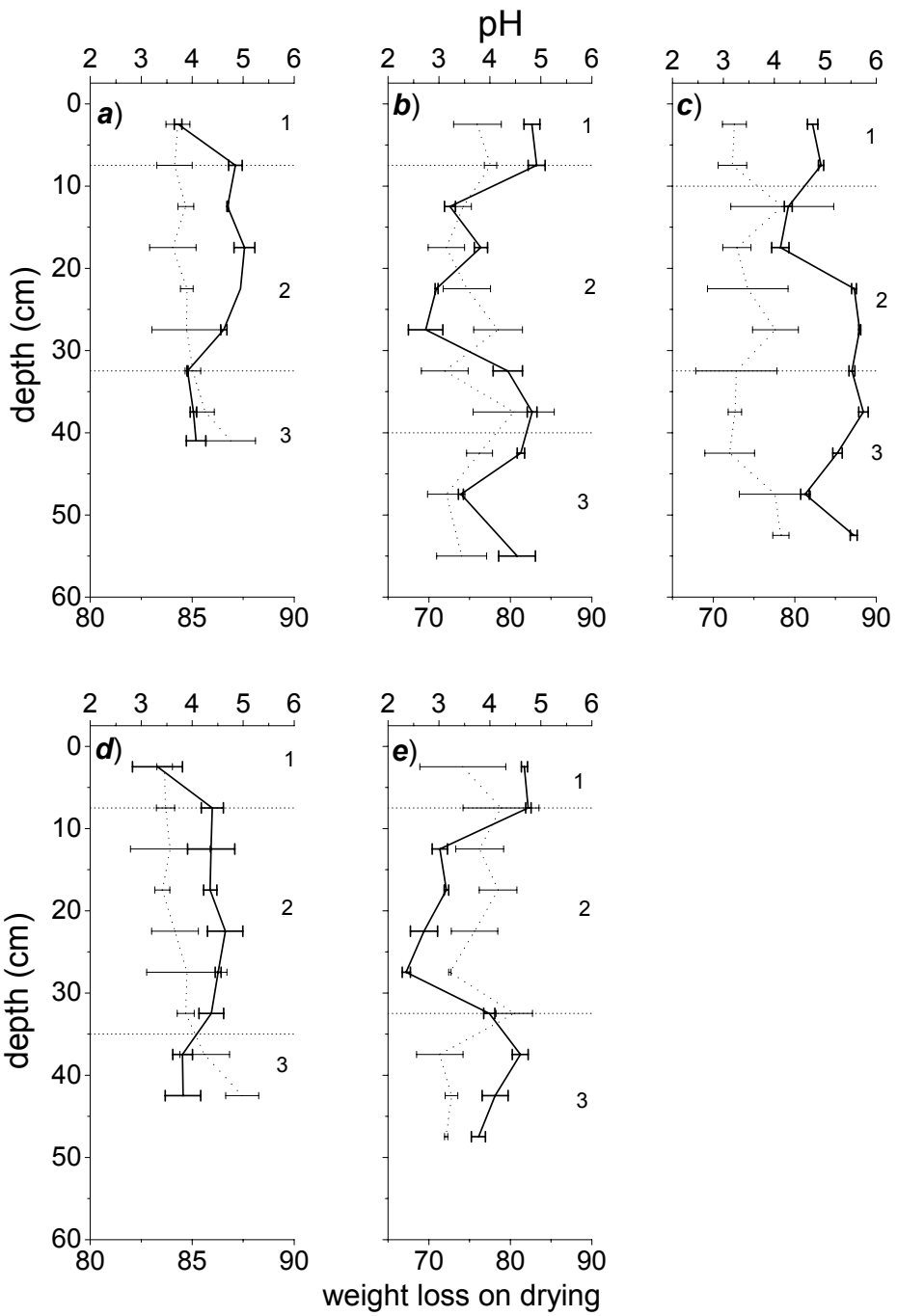


Figure 8.17
Details of peat cores from Loch Assynt area, showing moisture content (percentage weight loss on drying) (——) and (- - - -) pH. ***a)*** *AS 21/05/01 (1),* ***b)*** *AS 21/05/01 (2),* ***c)*** *AS 22/05/01 (3),* ***d)*** *AS 03/09/01 (1),* ***e)*** *AS 03/09/01 (2)*
Numbers refer to units described in Table 8.4

8.3.4 Spectrophotometric characteristics of peat DOM in the Loch Assynt area

In all analyses EEMs resembled those discussed in Section 2.2 of typical DOM samples. Fluorescence intensity peaks A, B and C were identified throughout the profiles, as was peak E, however, for reasons discussed in Section 2.2 this was not monitored. No other fluorescence intensity peaks, including peak D, were observed within the EEMs. Absorbance exhibited the typical DOM spectra of decreasing absorbance with increasing wavelength. The measured absorbance at all wavelengths was low, in comparison to surface waters and in a number of cases below detection levels. This primarily occurred at absorbance wavelengths greater than A_{340nm}. Due to this lack of data at long wavelengths and the increased measurement incurred error at low values the ratio of A_{465nm}/A_{665nm} was not calculated. Within each profile no correlations were found with moisture content or pH and spectrophotometric properties. Graphical presentations of the profiles are detailed in Figure 8.18 to 8.23, at the end of this section.

8.3.5 Depth relationships of the spectrophotometric characteristics of peat DOM from the Loch Assynt area

Depth relationships on peat DOM spectrophotometric properties are summarised in Table 8.7. An increase in peak A_{Fint}, peak B_{Fint} and absorbance with depth was consistent in all profiles and possibly related to the amount of available DOM for extraction. The greatest component of this increase in fluorescence intensity and absorbance was over the top ~20-35cm in site (2) and (3) profiles suggesting an accumulation of DOM over this depth.

The gradual blue shift in peak $B_{EM\lambda}$ (17nm) in AS 03/09/01 (1) and AS 03/09/01 (2) suggests a decrease in aromaticity/molecular weight. The wavelength shift in these profiles does not correspond to the pattern of peak A_{Fint}/A_{340nm} both of which decreased with depth, suggesting an increase in molecular weight of the DOM. This can be interpreted as an accumulation of low molecular weight/high aromaticity DOM at the surface.

Core AS 21/05/01 (1)	
Peak $C_{EM\lambda}$	352 to 333.5nm shift with depth
Peak A_{Fint}, peak B_{Fint} and absorbance	Positive correlation with depth
Peak A_{Fint}/A_{340nm}	Maximum at top (7967.8±4613.74, highest value from Assynt DOM) decrease in unit 3
Core AS 21/0/501 (2)	
Peak $C_{EM\lambda}$	348.5 to 333nm shift with depth
Peak A_{Fint}, peak B_{Fint} and absorbance	Positive correlation with depth
Peak A_{Fint}/A_{340nm}	Increase in unit 2 and 3; decrease across transition of 2-3
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	Negative correlation with depth
Core AS 22/05/01 (3)	
Peak A_{Fint} and peak B_{Fint} and absorbance	Increase to unit 2-3 transition
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	Negative correlation with depth
Core AS 03/09/01 (1)	
Peak $B_{EM\lambda}$	467±4.24 to 455±3.4nm shift with depth
Peak A_{Fint} and peak B_{Fint}	Positive correlation with depth
Peak A_{Fint}/A_{340nm}	Maximum unit 1-2 transition
Core AS 03/09/01 (2)	
Peak $B_{EM\lambda}$	472±4.95 to 455±4.73nm shift with depth
Peak A_{Fint}, peak B_{Fint} and absorbance	Positive correlation with depth
Peak A_{Fint}/A_{340nm}	Decrease in unit 1 and 2
A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}	Negative correlation with depth

Table 8.7
Summary of the significant relationships of the spectrophotometric properties of peat derived DOM with depth in peat cores from the Loch Assynt area. All significant relationships at 95% confidence level. For values refer to Figures 8.18 to 8.23.

8.3.6 Spatial variations in the spectrophotometric characteristics of peat DOM from the Loch Assynt area

The spectrophotometric properties of peat DOM from site (2) and site (3) were closer, in comparison to site (1). A significant gradient in fluorescence peak intensity and absorbance, can be observed from site (1) to site (3), with site (2) as an intermediate. The opposite relationship is significantly observed in A_{254nm}/A_{365nm} and A_{254nm}/A_{410nm}, site (3) having lower values than site (2) and (1). These differences between locations are observed in peat DOM from profiles as a whole and in units of similar von Post scale humification. The remaining spectrophotometric properties do not vary between peat profiles from each location.

From the interpretation placed on the absorbance ratios this suggests that site (3) peat DOM has a more aromatic composition and higher molecular weight and has a greater store of readily soluble DOM. The compositional differentiation is not further evidenced in fluorescence intensity peak wavelengths or peak A_{Fint}/A_{340nm} values.

8.3.7 Seasonal patterns in the spectrophotometric characteristics of peat DOM from the Loch Assynt area

Surface water samples taken from rivers adjacent to peat sampling points 1 and 2 show clear seasonal variations in DOM. During May 2001 there was low DOC concentration and water colour compared to September 2001 when DOC concentration and water colour were significantly higher and sampling took place during a period of DOM export. The values are summarised in Table 8.8 from site (1) and (2).

	Site	
	1	2
Peak $B_{EM\lambda}$(nm)	-11.910nm	-8.547nm
Peak $C_{EM\lambda}$ (nm)	12.322nm	8.013nm
A_{340nm} (%)	23.838	69.183
A_{254nm}/A_{410nm} (%)	42.925	5.800
Peak A_{Fint}/A_{340nm} (%)	-44.814	-12.461
Peak A_{Fint}, peak B_{Fint}(%)	29.269	47.821

Table 8.8
Summary of the mean seasonal difference between peat profiles in the Loch Assynt area. Negative values indicate higher values during May.

The differences in peak A_{Fint}/A_{340nm} A_{254nm}/A_{410nm} peak $B_{EM\lambda}$ suggest that the DOM sampled during spring had a relatively higher aromatic content in relation to autumn, however, peak A_{Fint}/A_{340nm} suggests that this DOM is of a lower molecular weight. These seasonal variations are replicated in surface waters. River waters sampled in the localities of each peat sampling site exhibited higher peak A_{Fint}, peak B_{Fint}, absorbance lower peak A_{Fint}/A_{340nm} and higher A_{254nm}/A_{410nm} during autumn compared to spring. The accumulation of low molecular weight/high aromaticity DOM at the

surface in the cores from September 2001 suggest that this DOM is being exported in the catchment surface water at his time

The River Traligill DOM exhibits the seasonal differences identified in peat DOM from sites (1) and (2) further indicating the link between peat DOM and aquatic DOM. Specifically, River Traligill sampled during spring 2001 had significantly higher mean values of peak A_{Fint}/A_{340nm} and peak $B_{EM\lambda}$. In comparison absorbance at all wavelengths peak A_{Fint}, peak B_{Fint} and DOC concentration were higher in autumn 2001. Absorbance ratios and peak $C_{EM\lambda}$ did not significantly differ (95% confidence level). Although the surface water variations in spectrophotometric properties relate to the preferential inorganic interactions and retention of DOM (Section 5.4) seasonal variations in peat can also be identified using spectrophotometric properties

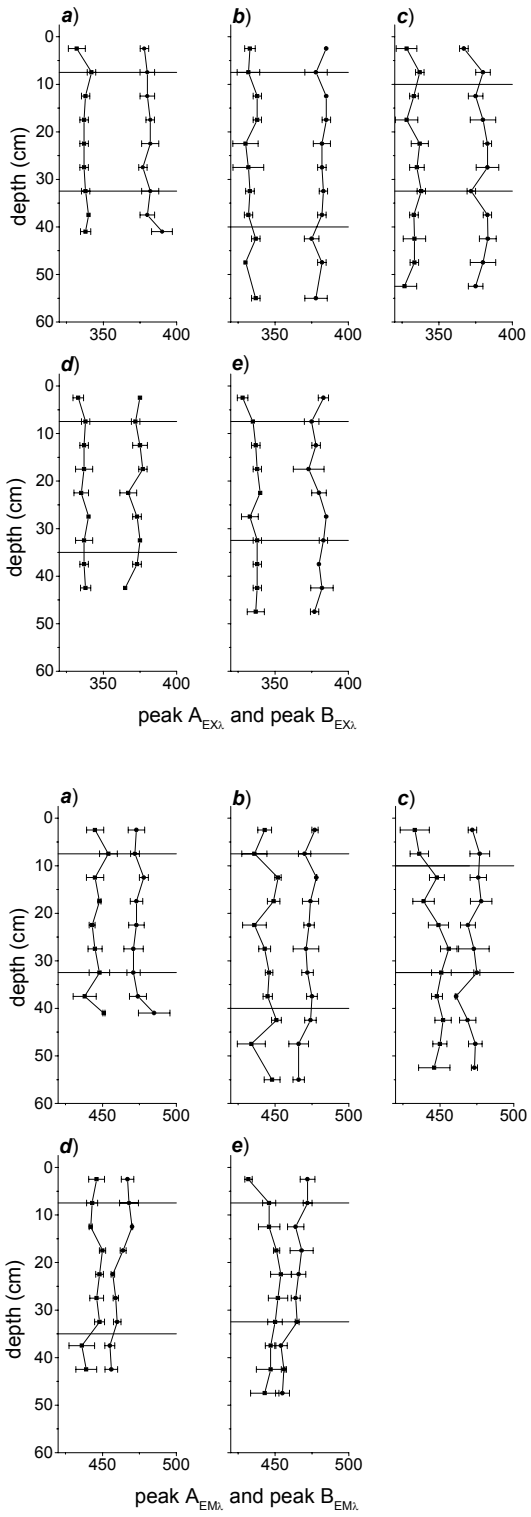


Figure 8.18
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: ■ peak $A_{EX\lambda}$ ● peak $B_{EX\lambda}$ **Bottom**: ■ peak $A_{EM\lambda}$ and ● peak $B_{EM\lambda}$ **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

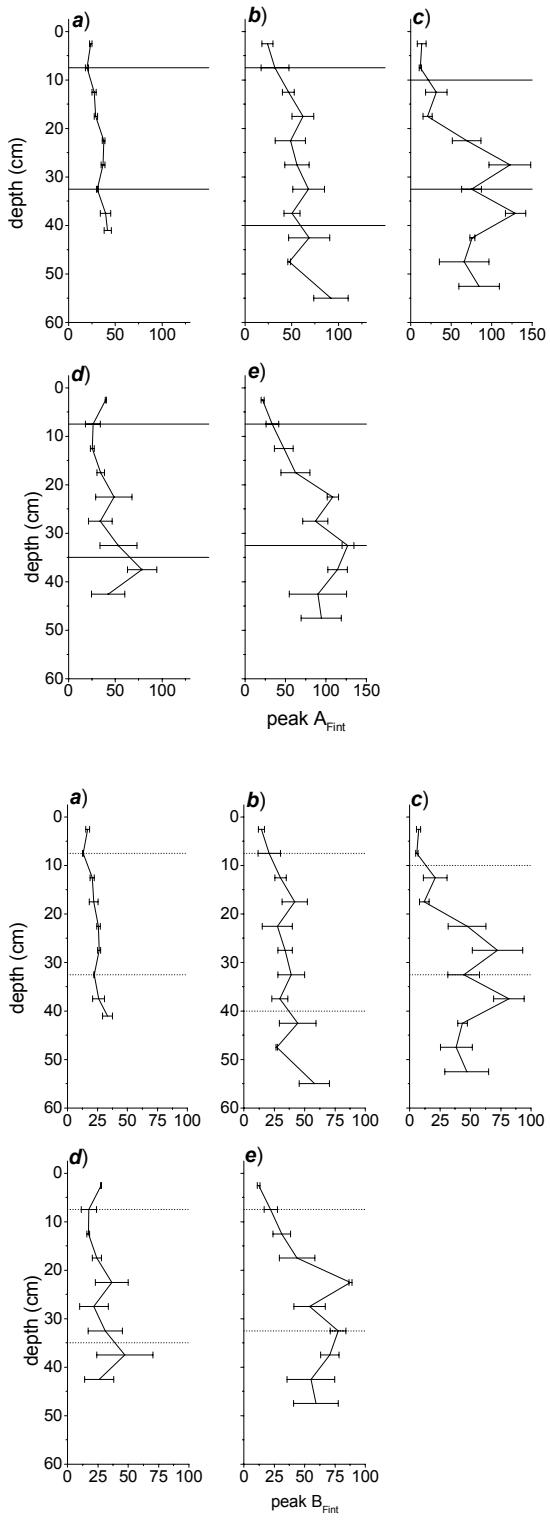


Figure 8.19
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: peak A_{Fint} **Bottom**: peak B_{Fint} **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

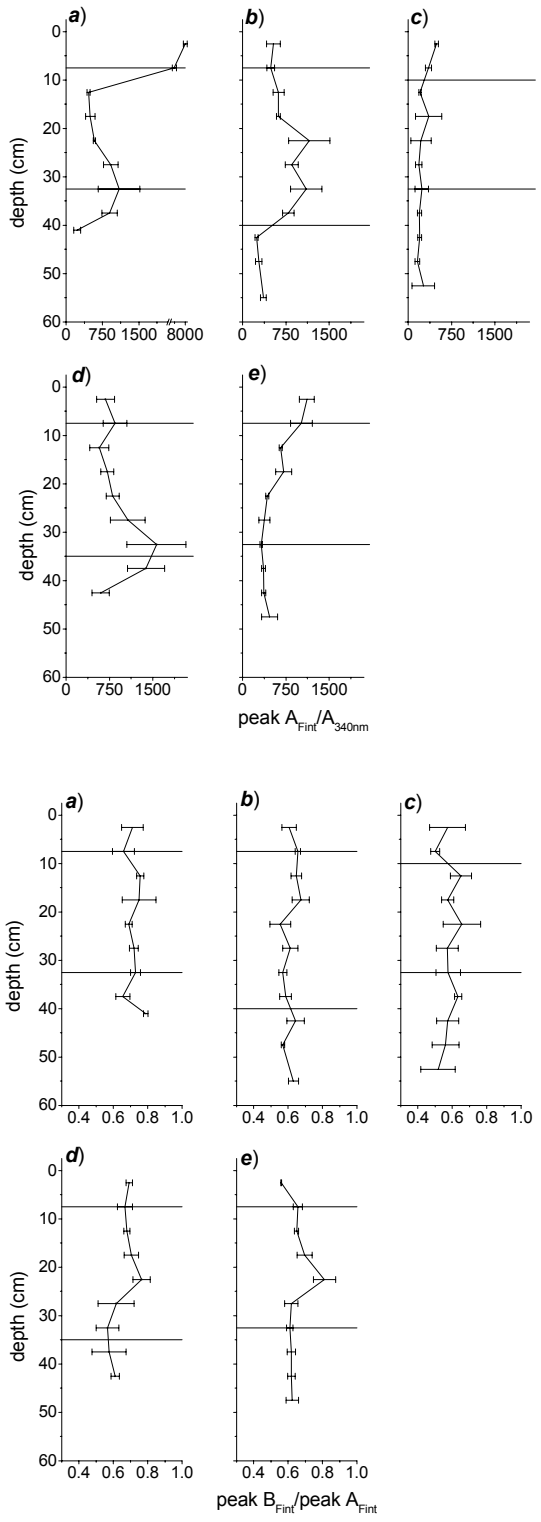


Figure 8.20
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: peak A_{Fint}/A_{340nm} **Bottom**: peak B_{Fint}/peak A_{Fint} **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

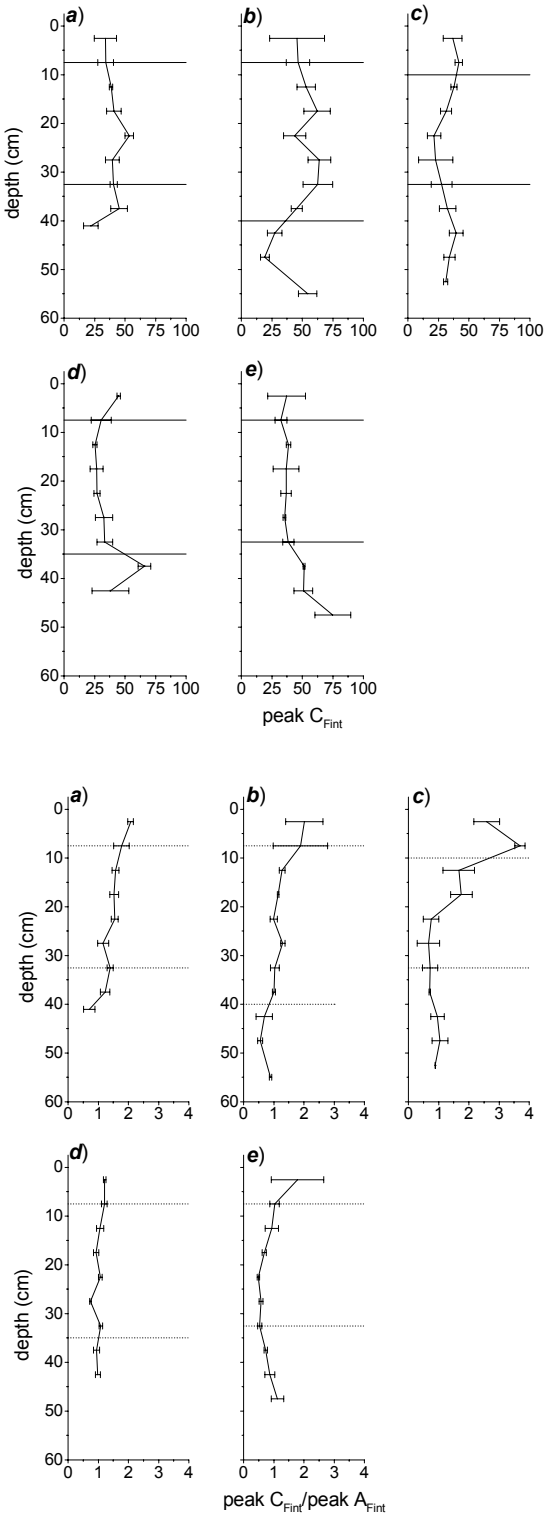


Figure 8.21
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: peak C_{Fint} **Bottom**: peak C_{Fint}/peak A_{Fint} **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

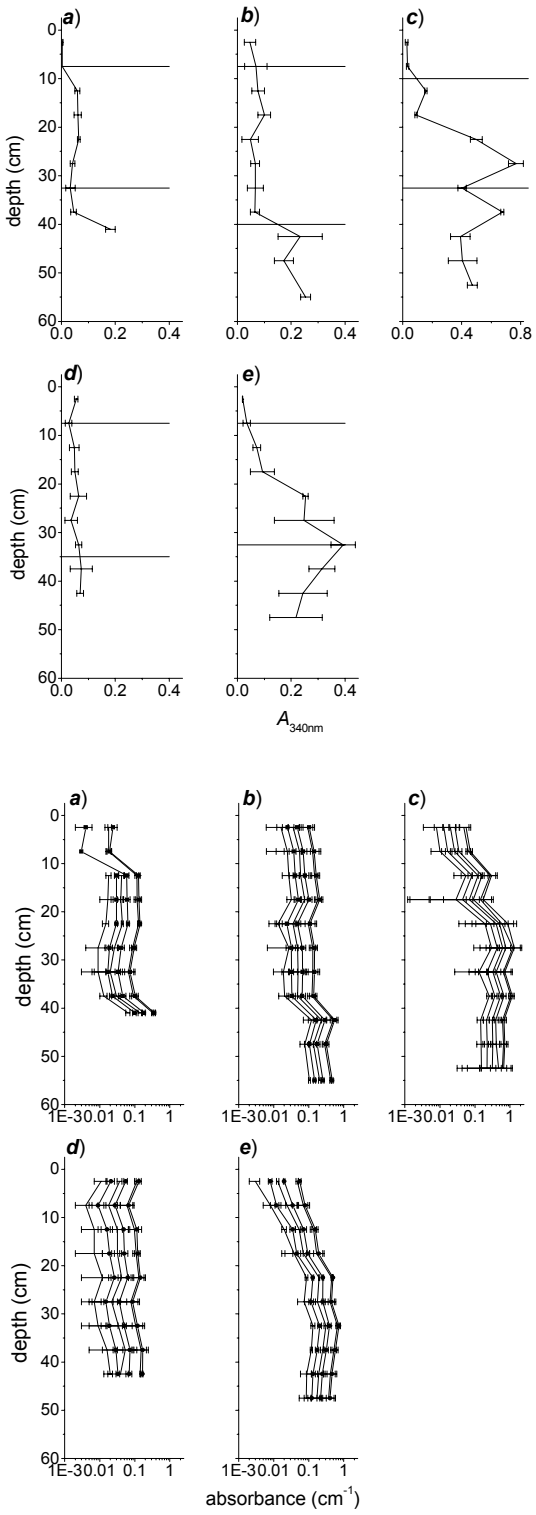


Figure 8.22
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: A_{340nm} **Bottom**: absorbance (cm^{-1}) **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

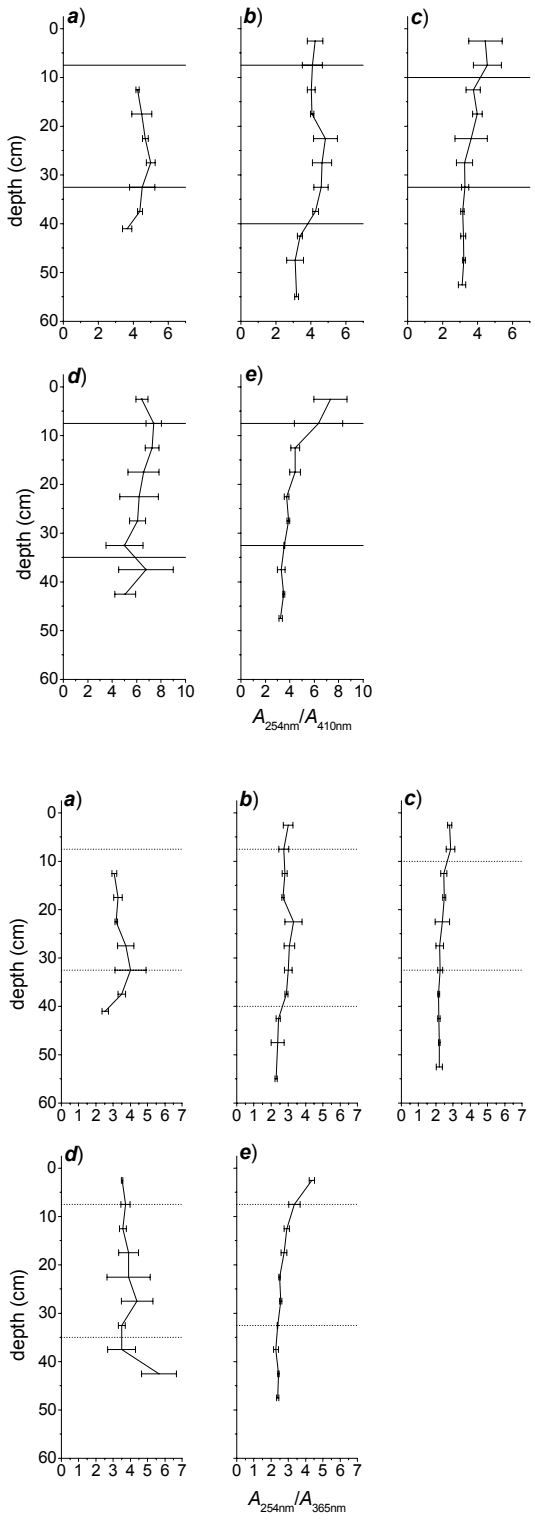


Figure 8.23
Spectrophotometric properties of peat DOM in the Loch Assynt Area
***Top**: A_{254nm}/A_{410nm} **Bottom**: A_{254nm}/A_{365nm} **a)** AS 21/05/01 (1) **b)** AS 21/05/01 (2) **c)** AS 22/05/01 (3) **d)** AS 03/09/01 (1) **e)** AS 03/09/01 (2).*

8.4 Comparisons of the spectrophotometric properties of peat derived and surface water DOM

Surface water DOM from the two study areas DOM had similar spectrophotometric properties, especially when compared to DOM derived from other sources (Section 7.6). These similarities were also observed in absolute values and in the depth trends of the peat profiles. For example, a decrease in A_{254nm}/A_{410nm} with depth was seen in profiles from both areas. A number of differences were observed, none of which indicated an overall difference in the DOM from the two study areas. Differences in DOM in the two monitored sites in the Coalburn Experimental Catchment related to the presence of litter and inorganic layers in site (1) profiles.

As the method of extraction was non-quantitative the amount of soluble organic matter present in the peat was not established. Absorbance was used as a rough proxy for the amount of DOM extracted. Peat from Loch Assynt area sites (2) and (3) exhibited the highest absorbance values, compared to all Coalburn Experimental Catchment and other Loch Assynt area peat derived DOM. An increase in absorbance may reflect the ease in which DOM is dissolved from the peat or the abundance in which is present. Easily soluble DOM has low hydrophobicicty and low aromaticity (Scott *et al*., 1998; Zhou *et al*., 2001). This is not reflected by absorbance ratios or emission wavelengths that relate to estimated aromaticity in surface water DOM (Figure 7.4).

In the data from all of the peat derived DOM high values of A_{254nm}/A_{410nm} (o5) and A_{254nm}/A_{365nm} (o3.8) were recorded only at the lower limit of the range of absorbance values observed (A_{340nm} <1.105). Lower values of the absorbance ratios, A_{254nm}/A_{410nm} (<5) and A_{254nm}/A_{365nm} (<3.8), were observed throughout the range of DOM absorbance. This suggests that the spectrophotometric signature of peat derived DOM with a smaller proportion of extractable DOM (low absorbance), is of lower aromaticity (high absorbance ratio). Peak A_{Fint}/A_{340nm} was highest in Coalburn Experimental Catchment site (1) peat DOM, discounting the DOM associated with the lower inorganic layers. This indicates a relatively lower molecular weight DOM and this DOM may in part be derived from the input of DOM from litter layers, which as discussed in Section 3.8 exhibited high peak A_{Fint}/A_{340nm} values.

In the entire record of peat profiles examined peak C_{Fint} exhibited a significantly higher level than surface waters in peat areas (62%). This indicates a greater proportion of protein related fluorophores in the peat derived DOM. This was also observed in river waters from sources impacted by sewage and farm waste runoff DOM, discussed in Section 7.4. This relationship was related to an external source of DOM with a high peak C_{Fint}. The variability in peak $C_{EM\lambda}$ in certain profiles suggests a different conformation of tryptophan compared to surface waters. The emission wavelengths can relate to the position of fluorophores within proteins and peptides (Mayer *et al*., 1999).

A further indication of the increased protein-derived fluorophores in peat DOM is the presence of peak D in a number of profiles. This fluorescence intensity peak is derived from the amino acid tyrosine and is not commonly observed in riverine DOM due to low fluorescence efficiency (Figure 1.3, Table 1.4). This is the only instance of such a peak in this study. Peak D has been recognised in DOM EEMs and related to the presence of animal wastes (Baker, 2002c) and high productivity waters (Dettermann *et al.*, 1998). For tyrosine fluorescence intensity to be observed in EEMs the concentration must be relatively high as it is usually not observed due to low fluorescence efficiency. The value of peak C_{Fint} /peak A_{Fint} (mean = 0.926) observed in peat derived DOM is at the maximum equivalent to that observed in rivers impacted by sewage (Baker, 2001).

DOM fractions with enhanced protein derived fluorescence have been related to "fresh" material that has not yet been but is susceptible to degradation (Zsolnay *et al*., 1999; Wu and Tanoue, 2001a). The observed protein fluorescence indicates that DOM analysed is similarly "fresh" and the extractive process releases DOM of this type. The enhanced peak C_{Fint} in litter layers (for example core CB 28/11/01 (1)) further suggests the signal is derived less degraded DOM of plant origin.

In comparison to related surface water peat derived DOM exhibits enhanced peak C_{Fint} and peak C_{Fint} /peak A_{Fint}. This was noted in Section 2.5.5 in relation to peat pool surface water and peat derived DOM, indicating a difference between directly related DOM. This suggests either an extractive bias or that the protein-derived DOM spectrophotometric properties are modified or diluted during the natural removal from peat to surface waters. In addition to change in DOM on transfer from peat to surface water dilution of the peak C_{Fint} by the increased proportion of other fluorophores may also occur. This would result in the lower peak C_{Fint} and peak C_{Fint}/ peak A_{Fint}

observed in peat sub-catchment DOM in the Coalburn Experimental Catchment. In areas with a greater proportion of inorganic soils, such as the peaty-gley sub-catchment, this dilution effect may not occur due to lower overall DOC concentration levels.

From the comparison of peat derived DOM properties to surface runoff in these areas in addition to the enhanced peak C_{Fint}, absorbance ratios are lower and peak A_{Fint}/A_{340nm} is higher. If the interpretations (Table 2.2) of the latter parameters are applied the DOM observed in the peat profiles are relatively more aromatic with a lower molecular weight, compared to surface waters. Extraction bias may control this difference, however, this mirrors the observations relating to HS differences between soil and surface waters of Malcolm (1990), discussed in Section 1.1.1.

Within the layers of each profile DOM properties varied and peak A_{Fint}/A_{340nm} had a value similar to surface waters at depths of, approximately, 30-60 cm in Coalburn Experimental Catchment site (2) profiles. This suggests the association of DOM from this depth to surface waters. The signal from peat derived DOM at these depths does not, however, exhibit similar absorbance ratio values to surface water. Using the spectrophotometric signal of peat profiles assigning a surface water source of DOM has not been possible. The difference between surface water DOM and peat derived DOM are also observed in the comparison of P_{soil} to peat derived DOM. This further shows that the DOM observed in the peat profiles is not of the same spectrophotometric composition as that which is transferred from the peat to the surface waters of the catchment.

8.5 Conclusions

This chapter has presented the results of the extraction, by a mild method, of peat DOM from the Coalburn Experimental Catchment and Loch Assynt area. From the examination of spectrophotometric properties of peat derived DOM a number of conclusions can be made to achieve the aims.

To extend of the evaluation of EEM fluorescence spectrophotometry as an analytical technique to peat DOM, by comparison to surface water DOM.

The method developed in Chapter 2 is suitable for the extraction of easily soluble DOM in sufficient amounts for spectrophotometric analysis and of a character similar

to aquatic DOM. Peat derived DOM has the same spectrophotometric properties as surface water, having a distribution of fluorescence intensity in EEMs and absorbance spectra of the same shape.

To compare the peat DOM from both the Coalburn Experimental Catchment and Loch Assynt area and to identify temporal or spatial patterns.

There was limited differentiation of peat DOM spectrophotometric properties from inter and intra catchment comparisons, thus the peat DOM extracted and analysed was homogeneous.

In the Loch Assynt area DOM the spatial and temporal distribution of peat DOM was related to that observed in surface water DOM, leading to the conclusion that in this area the composition of DOM exported has a significant relationship to the peat at the time of export. DOM of high aromaticity/low molecular weight is observed to accumulate in the surface of peat during autumn, in this area.

To identify the spectrophotometric properties of DOM from peat within individual profiles, identify changes in DOM with depth and to relate such depth variations to surface water sources of DOM.

Comparison of peat derived DOM to surface water indicates a number of parameters with overall similar spectrophotometric properties. A more detailed assessment indicates differences, which are related to modification or dilutions of the DOM during transport from peat to surface water. Spectrophotometric properties suggest that the peat derived DOM is more aromatic with a lower molecular weight when compared to surface waters.

In the Coalburn Experimental Catchment peat DOM was spatially defined by litter and inorganic layers, in site (1) profiles. DOM derived from pine litter layers has a composition indicative of poorly degraded DOM. From the spatial examination of Coalburn Experimental Catchment peat it can be concluded that although different vegetation and land management are present at both sites, peat derived DOM is not influenced by these processes.

Within the profiles the physical characteristics of the peat changed with depth and an increase in the aromaticity and/or molecular weight of DOM was observed. This

indicates an enrichment of recalcitrant non-lignin aromatic structures, related to increased humification (Zech *et al*., 1997) and can also be concluded from the visual assessment of humification. In the Coalburn Experimental Catchment site (1) profiles this compositional change is related to the release of sorbed DOM associated with inorganic material. In profiles from both areas the variables measured do not provide an overall consistent DOM relationship with depth, thus the homogeneous spatial nature of peat derived DOM is also observed with depth.

The lack of overall obvious stratification in spectrophotometric signal renders the distinction of acrotelm and catotelm difficult, although this has been performed in previous studies (Section 8.1). This may result from a number of methodological problems such as depth sampling resolution and extraction procedure; however, it may also indicate that the easily extracted DOM in these profiles has a relatively consistent composition. Due to this limited depth stratification within peat profiles the identification of sources of surface water DOM under different flow conditions is difficult.

Chapter 9.

Conclusions and Further work

9.1 Summary and Conclusions

Spectrophotometric techniques were applied to DOM from two main study areas – the Coalburn Experimental Catchment and the Loch Assynt area and to DOM from a wider range of sources. The methods applied were assessed and a recommended analytical method presented. These DOM analysis methods comprised EEM fluorescence and UV-visible absorbance spectrophotometry. DOM was sampled over a spatial and temporal scale to determine the variations in these properties over such ranges. Peat derived DOM was extracted using a mild method of dissolution to examine depth, seasonal and temporal variations in spectrophotometric properties of such DOM in relation to surface waters.

From the application of spectrophotometric techniques to the analysis of DOM from upland areas it is apparent that the method provides useful information regarding spatial and temporal variations. These variations reflect the overall distinct molecular characteristic of DOM derived from and influenced by different processes. Such processes reflect DOM sources. The signal derived from runoff from areas with a greater inorganic soil component was distinct to that from peat derived soils. The former exhibiting a signal related to lower molecular weight DOM. Additionally, the response of DOM spectrophotometric properties to the catchment conditions reflected a change in hydrological pathways, DOM sources and molecular properties.

In the primary study areas DOM had an overall relatively homogeneous spectrophotometric signal, in comparison to DOM from wider source areas. A number of the spectrophotometric properties monitored had limited use in differentiating DOM. Analysis of peat derived DOM indicated a relatively homogeneous spectrophotometric signal.

9.1.1 Sample storage and treatment

A sample storage protocol, consisting of immediate analysis and the use of suitably cleaned glass or plastic containers was found to give reproducible data. Refrigeration, freezing and defrosting were found to have a significant effect upon DOM spectrophotometric properties. Storage altered the signal of different DOM samples in a varying and inconsistent manner that was not related to properties of the fresh sample.

Varying the pH of the sample solution resulted in changes in both fluorescence and absorbance properties of the DOM. The extent of these changes and the pH range at which they were observed varied between samples. It is therefore recommended that samples be analysed at natural pH, to ensure an unaltered spectrophotometric signal was recorded, but that the pH of the solutions be considered when interpretation of this data was made.

The DOC concentration and absorbance of DOM solutions were observed to have a significant influence upon the fluorescence characteristics, due to IFEs. It was therefore required to employ a method to reduce such interferences. Dilution was discounted, and to entirely remove IFEs a post analytical correction was applied. This was found to result in data that retained the original spectrophotometric properties without the interferences due to absorbance.

9.1.2 Spatial variability in DOM spectrophotometric properties

In DOM from the Coalburn Experimental Catchment and Loch Assynt area DOC concentration was related to fluorescence intensity, absorbance and water colour: all variables exhibiting the same spatial patterns. Higher levels of DOC concentration were found in surface water of peat dominated areas.

In the study areas surface water DOM was found to have a number of spatial spectrophotometric variations and these were used to discriminate the sample sources statistically. The discrimination defined DOM spatially into water with a high DOC concentration, and DOM of a relatively higher molecular weight and aromaticity in comparison to lower DOC concentration waters.

The influence of the inorganic components of soils within each area was proposed to control the spatial definition of DOM in surface waters. Low DOC concentration waters were observed in areas with a greater proportion of inorganic soils compared to peat. Runoff from peat areas exhibited a consistently higher DOC concentration. Retention of DOM and, in particular, fractions with higher molecular weight and aromatic content have been observed in such soils (Zhou *et al*., 2001). This process reflects the DOM signal observed in low DOC concentration surface waters, indicating the preferential retention of DOM within soils. The primary control on DOM spectrophotometric spatial variations was determined to be soil interactions. In the Loch Assynt area, however, the spectrophotometric properties observed in loch water suggested that although this DOM was sampled in mixed soil areas the peat sediment in the loch had a significant impact on the quality of the DOM present.

In the Coalburn Experimental Catchment the main channel DOM exhibited spectrophotometric properties closer to those observed in the peat sub-catchment DOM compared to the peaty-gley sub-catchment DOM. DOM sampled from across the peat sub-catchment was homogeneous. A number of variations in DOM within this sub-catchment were observed. Forestry ditches in the Coalburn Experimental Catchment exhibited differing DOM properties relating to ditch infill. The ditches with exposed surfaces contribute to a greater proportion of DOC export compared to infilled ditches.

Undegraded DOM was observed in peat pools and under specific dry and low flow conditions in the Coalburn Experimental Catchment surface water. Such DOM is derived from leaching from vegetation, such as spruce litter in ditches under low flow conditions. Under similar dry and low flow catchment conditions DOM from PG_{weir} exhibited a unique EEM, suggesting a different source of DOM during such conditions.

Further spatial examination of DOM in the Coalburn Experimental Catchment was made by sampling of throughfall, precipitation and spruce litter derived DOM. These samples manifested specific spectrophotometric properties that were distinct from DOM in surface waters. Degraded spruce litter and throughfall both contained DOM of a low molecular weight material. Undegraded spruce needles exhibited a specific and unique EEM, due to specific compounds related to such material. This specific DOM was not recognised in any other samples suggesting that it was modified as the

needle degraded. It is concluded that this pool of DOM does not contribute to the DOM spectrophotometric properties observed in the catchment.

Precipitation had low DOC concentration and DOM of comparatively low aromaticity and/or molecular weight. This indicated that the bulk of the DOM exported from the catchment was generated by interaction with vegetation and soils. Throughfall had a relatively higher DOC concentration and may be a significant input to the catchment DOM. Soil water from each sub-catchment had similar spectrophotometric properties to and the same spatial distributions as surface water DOM. Thus it is concluded that DOM spectropohotometric properties of surface water are primarily controlled by soil water and soil DOM interactions.

It can be concluded that in the Coalburn Experimental Catchment surface water DOM is derived from soils. Throughfall and needle DOM are a source of DOM under specific conditions and in the low DOC concentration areas, however precipitation is not a source of DOM.

9.1.3 Temporal variability in DOM spectrophotometric properties

Seasonal DOM patterns exhibited export in the summer and autumn compared to spring and winter. This was observed in both study areas and describes a cycle of production and export of DOM. The DOM exported during the summer/autumn period was of higher molecular weight.

In the Coalburn Experimental Catchment spectrophotometric techniques were able to distinguish between runoff from each sub-catchment in the main channel. The most useful parameter in distinguishing source and flow path influences on the main channel DOM was peak A_{Fint}/A_{340nm}. This parameter was found to be useful due to the distinction observed between runoff from both sub-catchments, in the same manner, changes in DOC concentration also provided a method to recognise inputs from different sources of DOM.

During May-August (2000) in the Coalburn Experimental Catchment the main channel exhibited specific DOM spectrophotometric properties. During this period, under low flow, DOM in the main channel was sourced from the peaty-gley sub-catchment. Rainfall preferentially displaced DOM from here. As conditions changed and precipitation increased DOM from the peat sub-catchment dominated the main

channel. A large amount of DOM was stored in the peat sub-catchment forestry ditches during this period due to low hydrological connectivity. The ditches not only act as a store of DOM but also are a rapid transport route to the main channel, as hydrological conditions change.

A range of responses to changes in rainfall and discharge was seen in DOM spectrophotometric properties in both study areas. In the Coalburn Experimental Catchment an increase in discharge resulted in an overall dilution of the low flow signal and a decrease in DOC concentration.

- During winter sampling this dilution was related to inputs of low DOC snowmelt or runoff from DOC depleted sources.
- During summer/autumn a dilution was related to inputs of peaty-gley sub-catchment runoff or precipitation inputs.
  - As the catchment wetted up, following the previous summer, no dilution response to rainfall was observed due to the constant inputs of Dom fro peat sub-catchment ditches.
  - The switch between the two responses suggests that export form the peat sub-catchment only occurred after sufficient rainfall to displace DOM from soils to ditches to the main channel.

In the Coalburn Experimental Catchment the summer DOM production and autumn DOM export cycle was observed, the major control upon DOM export was rainfall amounts.

In the River Traligill DOC concentration had a positive relationship with flow. At high flow and during the “autumn flush” DOM with characteristics that reflected the spectrophotometric properties of DOM from peat areas of the catchment was observed. From the comparison of the two study areas rainfall is the controlling factor in triggering DOM export elaboration upon this relation will prove useful in the prediction of potential water quality issues relating to DOM.

The difference in DOC concentration-discharge relationships in the two study areas reflects the greater variability of soils in the River Traligill catchment compared to the dominant peat of the Coalburn Experimental Catchment. In the latter area high DOC concentration runoff from peat areas is observed at high and low flow, however, in the former area higher DOC concentration runoff occurs only when peat sources are activated. Upon activation of peat sources of more aromatic DOM the DOM exported

from both of the study areas becomes more likely to form disinfection by-products, compared to other periods of flow.

In the Coalburn Experimental Catchment it was estimated that the total annual export of DOC was 22.00 g DOC m^2yr^{-1}. DOC concentration in the Coalburn main channel was highest during autumn periods; however, export was estimated to be greatest during winter, under high flow conditions.

9.1.4 Spectrophotometric properties of peat-derived DOM

A method that obtains peat DOM in a mild manner and in sufficient quantities for spectrophotometric analysis was designed. This consists of dissolution in distilled water. The resultant solutions exhibited EEMs and DOM spectrophotometric properties that resembled surface waters. This indicted that the DOM obtained was possibly related to that naturally transported from peat to surface waters. A number of spectrophotometric properties, however, suggest that the peat derived DOM is relatively more aromatic with a lower molecular weight when compared to surface waters.

A number of peat profiles were examined from the Coalburn Experimental Catchment and Loch Assynt area to examine spatial, depth and temporal variations in peat derived DOM spectrophotometric properties. The observed profiles had limited differences in spectrophotometric properties indicating that peat derived DOM was relatively homogeneous. The limited temporal changes reflected changes observed in surface waters over the same period. Limited spatial variations in peat derived DOM corresponds to the lack of spatial variation in the surface waters of peat dominated areas.

In a number of peat profiles a possible increase in the aromaticity and/or molecular weight with depth of the DOM was observed, this agreed with visual observation of the physical properties of the peat and may indicate an enrichment of recalcitrant non-lignin aromatic structures, related to increased humification (Zech *et al*., 1997). In the Coalburn Experimental Catchment site (1) profiles this compositional change appear to related to the release of sorbed DOM associated with inorganic material.

9.1.5 The wider context of DOM spectrophotometric properties

From the comparison of the spectrophotometric properties of Coalburn Experimental Catchment and Loch Assynt area derived DOM to DOM from other sources it was found that EEMs were similar in all analyses. Aquatic DOM derived from peat dominated areas in the Coalburn Experimental Catchment and Loch Assynt area exhibited similar spectrophotometric properties although the two areas are distinct in morphology and land use, indicating the homogeneous nature of peat derived DOM. An overall continuum of DOM spectrophotometric properties was observed from peat derived DOM to DOM derived from urban influenced catchments with inputs of DOM from sources, such as sewage and farm wastes. The former DOM exhibited high DOC concentrations and spectrophotometric properties of more aromatic and higher molecular weight DOM. DOM from the latter sources exhibited the opposite characteristics with a significant presence of protein. A range of intermediate spectrophotometric properties was observed in DOM derived from non-peat dominated areas influenced by inorganic soils. Thus is can be concluded that DOM is highly influence by land use and soil type and that spectrophotometric methods of analysis are capable of identifying DOM from different sources.

Observations of distinct DOM spectrophotometric properties from different sources indicate the potential for the use of spectrophotometric methods in characterising such DOM. Peak $A_{EM\lambda}$ and A_{254nm}/A_{410nm} exhibited relationships with specific absorbance and estimated aromaticity in DOM from a range of sources not seen in the two main study areas. This indicates that these variables both respond to changes in the aromatic nature of the DOM on a wider scale and are useful in the characterisation of DOM. This also presents the use of spectrophotometric methods to monitor the potential of forming disinfection by-products in areas other than upland catchments.

9.2 Future work

The following section presents a discussion of how further work can be used to expand on the current study.

Further laboratory experiments involving freezing and defrosting of DOM solutions

This process resulted in a change in the spectrophotometric properties due to such processes. Further analysis of DOM subjected to such processes and the analysis of particulate matter precipitated on defrosting is required to determine the mechanisms, which alter DOM under processed in such a manner.

Further observation of temporal and spatial variations in DOM within the Coalburn Experimental Catchment

This is required to clarify and number of patterns observed in this study. Firstly, temporal monitoring of DOM spectrophotometric properties of surface water DOM, at weekly resolution, is required to assess the reproducibility of the annual and seasonal variations observed in this study. Higher resolution sampling of runoff from each sub-catchment is required to fully understand the sources and flow paths of DOM in the catchment during rainfall events. The rapid response of runoff and discharge to rainfall in the catchment was not fully monitored in the eight hourly resolution sampling therefore higher resolution is suggested. Monitoring of DOM properties such as peak A_{Fint}/A_{340nm} in tandem with pH and other water quality parameters that have been previously observed to define water sources would establish a more detailed model of runoff in the catchment.

Extended monitoring of other DOM sources will assist in the further understanding of seasonal patterns of DOM production and export. This includes the sampling of a full annual record of soil water DOM and sampling of peat profiles from a wider spatial temporal range

From the limited study of forestry ditches it was apparent that the state of the ditch influences the DOM exported. Further monitoring of a range of such ditches in the catchment would expand this picture and identify how the varying state of the ditch influences spectrophotometric properties. This study would also encompass the observation of DOM export timing and investigate the prediction of periods of DOM export in relation to water quality issues. DOM spectrophotometric properties of both cloud mist deposition and occult deposition require further examination to fully understand the processes that contribute to throughfall. Similarly the DOM derived from stemflow alone requires assessment.

Expansion of the assessment of the techniques used to a wider area with different influences on DOM sources and processes.

This suggestion stems from the observations that DOM appears to have a relatively limited range of properties in the two study areas in question. To fully utilise the methods of DOM characterisation in this study application to areas on a larger scale, or with a greater range of physiographic properties may be required. For example, sampling from both surface and soil DOM encompassing a soil transect from peats through gleys to brown earths. This would establish the influence of changing soils on DOM spectrophotometric properties such as molecular weight indicated by peak A_{Fint}/A_{340nm}.

Assessment of the influence of forestry practices on DOM

Forestry was found to have a possible influence on DOM spectrophotometric properties and impact on DOM and DOC concentration transport through the Coalburn Experimental Catchment. To further understand the influence ditching and forestry has upon surface water DOM a duplicate study is suggested monitoring an unforested area, such as a location in the Border Mires complex close to Coalburn Experimental Catchment.

Calibration of DOM spectrophotometric properties by compositional analysis using other methods

The tentative compositional variations interpreted from the spectrophotometric properties of DOM in this study are derived from comparison to the trends observed in the data to previously published information. This information, however, was obtained from DOM different from sources and in different states using different analytical techniques. To fully establish the molecular derivation of variations in DOM spectrophotometric properties the DOM from the two study areas requires further analysis by more specific techniques. This will increase the potential application of the method especially with the respect of predicting disinfection bi-product formation in drinking water. In particular the relationship of peak A_{Fint}/A_{340nm} to molecular weight require further definition, as this parameter was useful in the discrimination of DOM.

The distribution of peak C_{Fint} in DOM was suggested, in some cases, to be related to the presence of proteinaceous fluorophores. Further characterisation of the amino acid composition of DOM is required to identify if such DOM components are responsible for this fluorescence and if this or energy transfers between fluorophores controls peak C_{Fint}.

To perform such detailed characterisations requires a large amount of sample. Using the data obtained in the spatial and temporal monitoring of DOM surface water with DOM of a different quality can be predicted. Samples can be taken of distinct DOM spectrophotometric properties accordingly. DOM characterisation can also be employed to further understand the fluorophores present in EEMs from specific sources, such as spruce litter and throughfall or that sampled under specific catchment conditions.

In situ DOM monitoring

Spectrophotometric analysis is a rapid and reproducible method with the possibility of automation and *in situ* measurement. DOM spectrophotometric properties, such as peak $A_{Fint}/A_{340nm,}$ A_{254nm}/A_{410nm} and peak $A_{EM\lambda}$, have been observed to have a significant relationship to specific absorbance, which is used a proxy for trihalomethane formation potential. Specific absorbance requires the analysis of DOC concentration, whereas using spectrophotometric properties would be possible on-line with out this analytical procedure. Further definition of the compositional changes related to DOM spectrophotometric properties would indicate if such on-line monitoring would be useful regarding this and other DOM related water quality issues, such as metal transport and light penetration.

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Appendix 1. Details of the samples used in Chapter 2

1.a Samples used in dilution experiment

D1	[1]CB_{weir}	19/01/01
D2	[1]CB_{weir}	09/08/01
D3	[1]CB_{weir}	28/10/01
D4	[1]CB_{weir}	27/08/01
D5	[1]CB_{weir}	23/08/01
D6	[1]CB_{weir}	22/08/01
D7	[1]P_{weir}	01/09/01
D8	[1]P_{weir}	09/08/01
D9	[1]F_C	01/09/01
D10	[1]F_E	20/02/01
D11	[2]peat pool	19/05/01
D12	[1]PG_{weir}	20/02/01
D13	[3]Agill Beck (Lofthouse Moor)	16/04/01
D14	[2]River Traligill	20/05/01
D15	[3]Chirdon Burn (NY 73458475)	11/04/01

1.b Samples used in freeze-defrost and pH modification experiments

F1	[2]River Traligill	08/09/00
F2	[2]River Traligill	08/09/00
F3	[2]River Traligill	08/09/00
F4*	[3]River Taw (Devon)	03/04/01
F5	[1]M_E	12/10/00
F6	[1]F_C	12/10/00
F7	[1]F_E	12/10/00
F8	[3]River Blythe (NZ 190776)	01/05/00
F9	[3]Glenridding Valley Stream (NX 355157)	02/06/00
F10	[3]Fold Sike (NY 834293)	08/01/01
F11*	[3]Chirdon Burn (NY 73458475)	11/04/01
F12	[3]Shooter's Clough (SK 005747)	15/08/00
F13*	[3]Agill Beck (Lofthouse Moor)	16/04/01
F14	[3]River Coquet (NT 956035)	17/02/01
F15	[1]CB_{weir}	30/03/00
F16	[1]CB_{weir}	30/08/00
F17	[1]CB_{weir}	16/01/01
F18*	[1]CB_{weir}	24/01/01

F19	[1]CB_{weir}	11/05/00
F20	[1]P_{weir}	30/03/00
F21	[1]P_{weir}	30/08/00
F22	[1]P_{weir}	11/05/00
F23	[1]PG_{weir}	30/03/00
F24	[1]PG_{weir}	15/11/00
F25	[1]PG_{weir}	20/02/01
F26	[1]PG_{weir}	11/05/00
F27	[3]Howan Burn (NY 705768)	30/03/00
F28	[3]Howan Burn (NY 705768)	25/05/00
F29	[3]Rookhope Burn (NZ 915425)	09/05/00
F30	[3]Rookhope Burn (NZ 915425)	13/06/00
F31	[3]River Teign (Chagford, Devon)	18/04/00
F32	[3]River Exe (Exeter)	20/04/00
F33	[3]Wash Leat (Chagford, Devon)	23/04/00
F34	[3]Gruntley Beck (NY 826104)	11/05/00
F35	[3]Howgill Sike (NY 826104)	11/05/00

Key: refer to [1]Chapter 3 [2]Chapter 5 [3]Appendix 4 for sampling information *Samples represented on Figure 2.6, 2.7 and 2.8, showing the response to the modification of pH

Appendix 2

T-test comparison of P_{soil} and PG_{soil}

	t-value	% Confidence level
DOC (mgL^{-1})	-3.235	95
Water Colour (Hazen)	-2.952	99
pH	5.358	99
Conductivity (μS)	-0.650	ns
Peak A_{EX} (nm)	-1.416	ns
Peak A_{EM} (nm)	-2.781	ns
Peak B_{EX} (nm)	2.446	ns
Peak B_{EM} (nm)	-0.842	ns
Peak C_{EX} (nm)	-0.274	ns
Peak C_{EM} (nm)	0.654	ns

Peak A_{Fint}	-3.805	99
Peak B_{Fint}	-4.792	99
Peak C_{Fint}	6.273	99
Peak B_{Fint}/Peak A_{Fint}	-0.133	ns
Peak C_{Fint}/Peak A_{Fint}	4.717	99
Peak AS_{Fint}	-0.585	ns
A_{340nm} **cm^{-1}**	-4.683	99
SUV_{254nm} ($mgCL^{-1}cm^{-1}$)	-7.848	99
$Svis_{410nm}$ ($mgCL^{-1}cm^{-1}$)	-3.157	99
ε A_{272nm} $(L(moleC)^{-1}cm^{-1})$	-7.885	99
Peak A_{Fint}/A_{340nm}	3.020	99
A_{465nm}/A_{665nm}	-1.618	ns
A_{254nm}/A_{365nm}	-0.248	ns
A_{254nm}/A_{410nm}	0.312	ns

The results of t-tests comparing the mean spectrophotometric properties of P_{soil} and PG_{soil}. Positive values indicate a higher mean in latter.

Appendix 3. Calculation of Hydrologically Effective Precipitation

The following method was used to calculate monthly hydrologically effective precipitation (PE_m), following the method of Thornthwaite (Shaw, 1994, page 249).

$$PE_m = 16N_m \left(\frac{10\overline{T}_m}{I} \right)^a mm$$

m = months 1,2,3…12

N_m = adjustment factor related to hours of daylight

T_m = monthly mean temperature °C

$a = 6.7\text{x}10^{-7} I^3 - 7.7\text{x}10^{-5} I^2 + 1.8\text{x}10^{-2} I + 0.49$

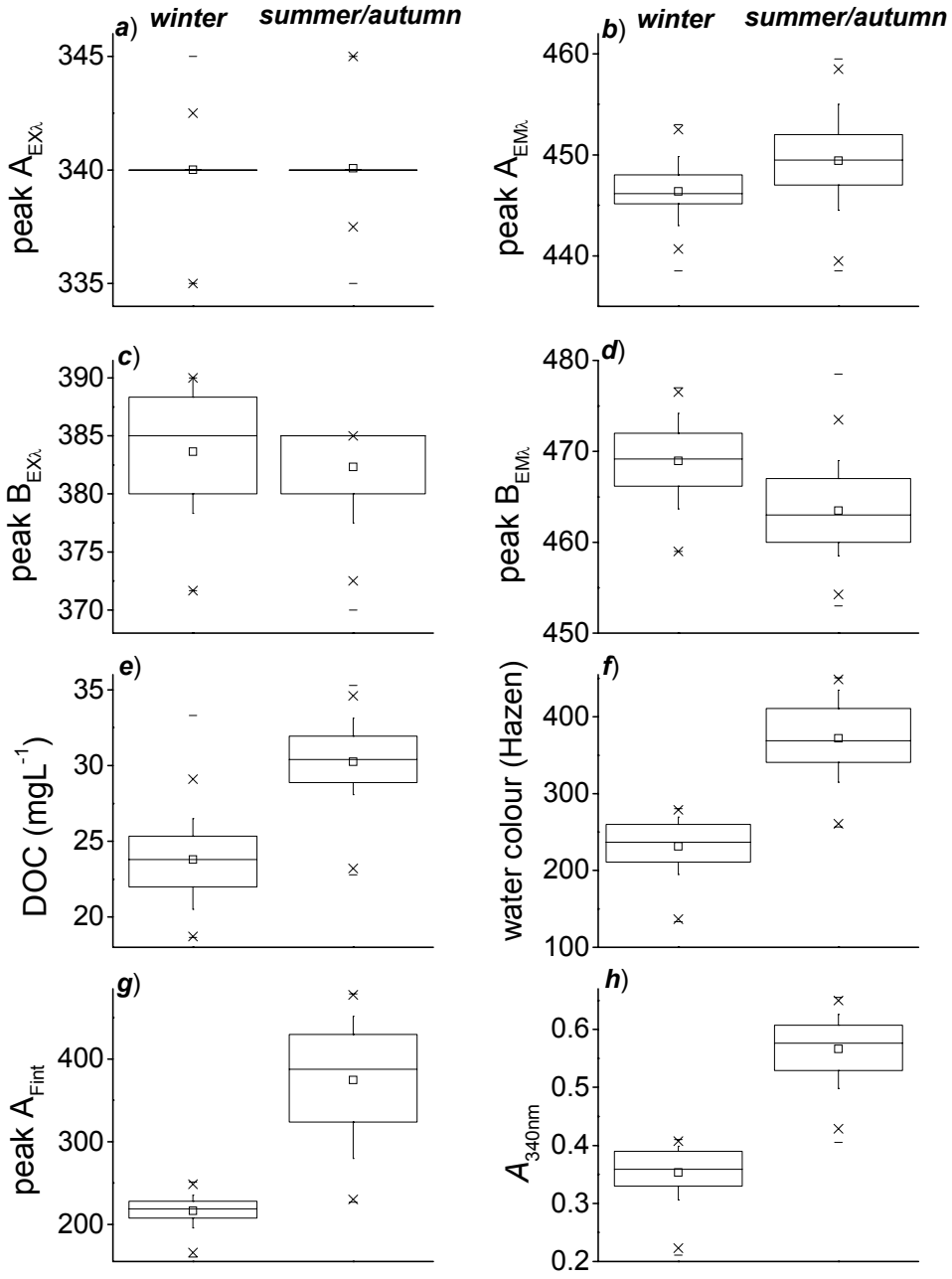
I = heat index given by

$$I = \sum im = \sum \left(\frac{\overline{T}_m}{5} \right)^{1.5}$$

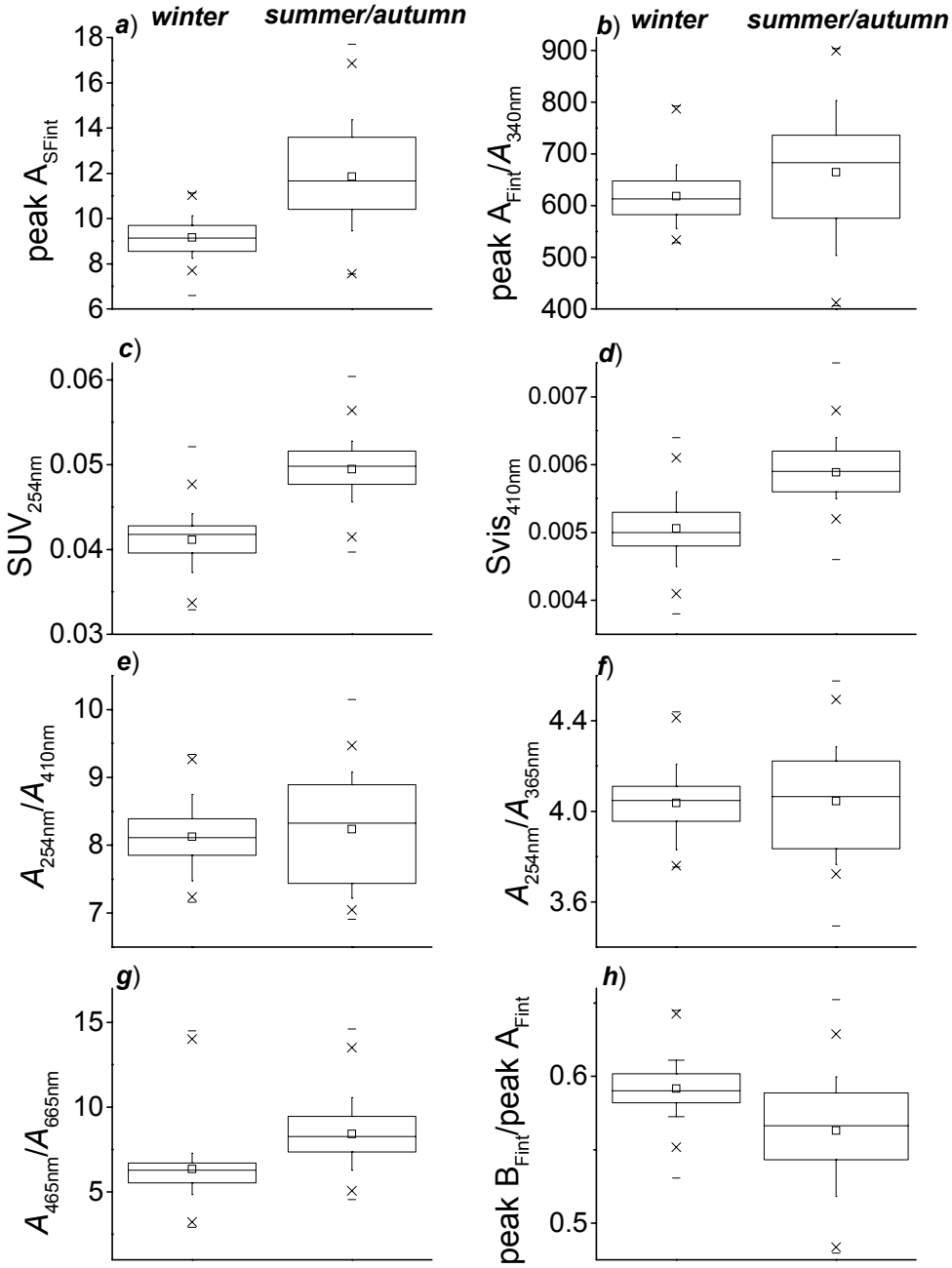
mm = total monthly rainfall (mm)

Appendix 4

Graphical presentation of the distribution of spectrophotometric properties in CB_{weir} during high resolution sampling.



Box plots of DOM characteristics in water samples from CB_{weir} sampled at high resolution during winter and summer/autumn, 2001 **a)** peak $A_{EX\lambda}$ **b)** peak $A_{EM\lambda}$ **c)** peak $B_{EX\lambda}$ **d)** peak $B_{EM\lambda}$ **e)** DOC concentration (mgL^{-1}) **f)** water colour (Hazen) **g)** peak A_{Fint} **h)** A_{340nm}. For key to box plots see Figure 3.2.



Box plots of DOM characteristics in water samples from CB_{weir} sampled at high resolution during winter and summer/autumn, 2001 **a)** peak AS_{Fint} **b)** peak A_{Fint}/A_{340nm} **c)** SUV_{254nm} **d)** $Svis_{410nm}$ **e)** A_{254nm}/A_{410nm} **f)** A_{254nm}/A_{365nm} **g)** A_{465nm}/A_{665nm} **h)** peak B_{Fint} /peak $A_{Fint.}$ For key to box plots see Figure 3.2.

Appendix 5. Details of Water Samples from the Assynt Region

Location	Grid Ref. (NC)	Sampling date	
Allt a' Chalda Mór	245235	05/04/00	1
Inflow to Loch Assynt, Calda House	245235	05/04/00	1
Inflow to Loch Assynt	248228	05/04/00	1
Inflow to Loch Assynt (by A837)	242238	05/04/00	1
Small stream, inflow to Loch Assynt	238241	05/04/00	1
Allt Poll an Droighinn	260220	02/04/00	1
River Traligill (footbridge)	272210	02/04/00	1
Stream (Glenbain Hole)	265217	02/03/00	1
Small stream (by track)	263218	02/03/00	1
Stream (near plantation)	271212	02/03/00	1
River Traligill (footbridge	272210	02/03/00	1
Frozen pool (Allt à Bhealaich)	282200	02/03/00	1
Allt a' Chalda Mór	245235	08/09/00 13:20	1
Allt Poll an Droighinn	260220	09/09/00 09:10	1
Outflow of Loch Mhaolach-coire	276197	09/09/00	1
Stream (near plantation)	271212	09/09/00 13:30	1
Allt Poll an Droighinn	260220	19/05/01	1
Very small stream, Glenbain	263218	19/05/01	1
River Traligill (footbridge)	272210	19/05/01	1
Allt à Bhealaich	282200	19/05/01	1
Tributary of Outflow of Loch Mhaolach-coire	276197	19/05/01	1
Outflow of Loch Mhaolach-coire	276197	19/05/01	1
Stream (Creagan Breaca)	275198	19/05/01	1
Tributary of Allt Poll an Droighinn	264224	19/05/01	1
Tributary of Allt Sgiathaig (by car park)	234275	20/05/01	1
Inflow to Loch Assynt, Ardvreck Castle	241288	20/05/01	1
River Traligill (footbridge)	272210	02/09/01	1
Allt Sgiathaig	235245	05/04/00	2
Inflow to Loch Assynt	226246	05/04/00	2
Small stream, inflow to Loch Assynt	229246	05/04/00	2
Small stream, inflow to Loch Assynt	227246	05/04/00	2
Inflow to Loch Assynt	226246	05/04/00	2
Alltan Leacach	235185	05/04/00	2

Alltan Beithe	233194	05/04/00	2
Allt Cuil Fraoich	237189	05/04/00	2
River Loanan, Stronchrubie	244192	05/04/00	2
Tributary of River Loanan (Sròn Crùbaidh)	248199	05/04/00	2
River (near Eas a Chùal Aluinn)	274277	04/04/00	2
Stream (near Loch Bealach a' Bhùirich)	268278	04/04/00	2
River Loanan, inflow to Loch Assynt	246218	07/09/00 19:15	2
River Inver, Little Assynt	155251	08/09/00 09:30	2
Inflow to Loch Assynt, Rubha an Doire Cuillinn	207259	08/09/00 10:15	2
Allt na Doire Cuillinn (by A837)	207258	08/09/00 10:30	2
Lochan Feòir outfall	228248	08/09/00 10:45	2
Allt Sgiathaig	235254	08/09/00 11:00	2
Allt Sgiathaig	233254	08/09/00 11:20	2
Lochan Feòir inflow	227252	08/09/00 11:30	2
Tributary of Allt Sgiathaig	232252	08/09/00 12:20	2
Outfall of Loch na Gainmhich	243294	08/09/00 12:40	2
Inflow to Lochan an Duibhe	221255	08/09/00 17:00	2
Inflow to Loch na Gainmhich	244288	09/09/00 15:40	2
Allt Mhic Mhurchaidh Ghèir	248159	09/09/00 17:15	2
Drainage ditch	247159	09/09/00	2
River Loanan, Loch Awe outfall	249161	09/09/00 19:20	2
Allt na Beinne Gairbhe	204244	10/09/00 11:15	2
Inflow to loch Assynt, Torr an Eileinn	201246	10/09/00 11:30	2
Tributary of Allt Poll an Droighinn	266225	19/05/01	2
River Inver, Blàr nam Fear Mora	143253	19/05/01	2
River Loanan, Loch Awe outfall	249161	20/05/01	2
Inflow to Lochan an Ais	188903	20/05/01	2
River Canaird, Strath Canaird	146014	20/05/01	2
Main inflow to Loch na Gainmhich (by A894)	240288	20/05/01	2
Inflow to Loch na Gainmhich (by A894)	240288	20/05/01	2
Unapool Burn	235308	20/05/01	2
Allt Sgiathaig	232274	20/05/01	2
Tributary of Allt Sgiathaig	229274	20/05/01	2
Allt na Doire Cuillinn (by A837)	207258	20/05/01	2
Allt Poll an Droighinn	260220	02/09/01	2
Peat pool (pp1)	226246	05/04/00	3
Peat pool (by A837) (pp2)	236243	05/04/00	3
Peat pool (pp3)	235270	04/04/00	3
Peat pool (near Allt à Bhealaich) (pp4)	281203	19/05/01	3
Peat pool (pp5)	282200	19/05/01	3

Peat pool (pp6)	277200	19/05/01	3
Peat pool, Loch na Gainmhich (pp7)	242289	20/05/01	3
Peat pool (pp8)	282201	20/05/01	3
Loch Assynt	236240	05/04/00	4
Loch Assynt	227246	05/04/00	4
Lochan, Cnoc an Droighinn	275240	04/04/00	4
Loch Fleodach Coire	275247	04/04/00	4
Lochan, Cnoc an Droighinn	273244	04/04/00	4
Lochan, Glas Bheinn	268265	04/04/00	4
Loch Assynt, Inchnadamph Church	248221	07/09/00 19:05	4
Loch Assynt, Rubha an Doire Cuillinn	207258	08/09/00 10:15	4
Loch Leitir Easidh	175265	08/09/00 09:50	4
Lochan Feòir	230252	08/09/00 11:30	4
Loch na Gainmhich	243289	08/09/00 12:30	4
Loch Assynt, Ardvreck Castle	240238	08/09/00 13:05	4
Lochan an Duibhe	221255	08/09/00 16:45	4
Loch Mhaolach-coire	276196	09/09/00	4
Loch na Gruagaich	245159	09/09/00 17:30	4
Loch Awe	247157	09/09/00 17:45	4
Loch Mhaolach-coire	276196	19/05/01	4
Loch nan Eun	109238	19/05/01 19:25	4
Small loch (Druim na h-Uamha Móire)	227275	20/05/01	4
Loch Assynt, Ardvreck Castle	240238	20/05/01	4
Loch Mhaolach-coire	276196	02/09/01	4

1= streams and rivers draining carbonates
2=streams and rivers draining non-carbonate
3=peat pools
4=lochs and lochans

Appendix 6. Details of samples used in Chapter 7
a. All samples analysed

Sampling location	Dates	No. samples	Category
Peat drains, Upper Wharfedale (SE845815)	01/08/00 to 02/10/01	18	1
Stream, Birdoswald Mire (NY615665)	15/05/00	1	1
Stream, Spadeadam Mire (NY665712)	15/05/00; 13/07/00; 27/07/00	3	1
Felecia Moss Mire (NY721775); ditch	26/02/00	2	
pool;	26/02/00	1	1
standing water;	26/02/00	3	
stream	15/05/00	1	
Yellow Mire (NY690773); standing water	26/02/00	3	1
Muckle Samuel's Mire (NY679790); stream;	27/02/00	3	
ditch;	27/02/00	2	1
standing water;	27/02/00	1	
Coom Rigg Mire (NY690795); standing water;	27/02/00	4	1
stream	27/02/00	3	
Whickhope Nick Mire (NY673815); ditch;	27/02/00	3	1
standing water	27/02/00	1	
Howan Burn (NY705768)	19/01/00-16/01/02	21	1
Peat pool, Goyt Valley (SJ995771)	15/08/00	1	1
Shooter's Clough, Goyt Valley (SK005747)	15/08/00	1	2
Shooter's Clough Goyt Valley (SK006748)	15/08/00	1	2
River Dove, Mill Dale (SJ140548)	16/08/00	1	2
Stream, Dove Dale (SJ143292)	16/08/00	1	2
River Manifold, Hulme End (SJ102590)	17/08/00	1	2
Burbage Brook, Foxhouse (SE255795) main stream and tributaries	03/09/00	3	2
River Irthing, Churnsike Bridge (NY662766) to Newby Bridge (NY476522)	11/01/00; 04/05/00	9	2
Stream (NY693768)	11/01/00	1	2
Butter Burn (NY677743)	11/01/00	1	2
Lawrence Burn (NY686776)	11/01/00	1	2
Churn Sike (NY763773)	11/01/00	1	2
Linen Sike (NY683735)	11/01/00	1	2
Caw Burn (NY749690)	22/07/00	1	2
Knag Burn (NY791689)	22/07/00	1	2
Streams, Hadrian's Wall area (NY782701; NY771702; NY751695)	22/07/00	4	2
Chirdon Burn (NY73458475)	11/04/01	1	2
River Rede (Northumberland)	25/11/00; 24/11/01	22	2

River Eden (NY782043; NY684205; NY761133; NY701176)	13/01/00; 04/05/00; 11/05/00; 15/05/00	6	2
Scandal Beck (NY750110; NY783028; NY722045)	13/01/00; 04/05/00; 11/05/00	4	2
Hilton Beck (NY755155)	13/01/00	1	2
Borrowdale Beck (NY832157)	13/01/00	1	2
Foss Gill (NY754113)	13/01/00	1	2
Helm Beck (NY748145)	13/01/00	1	2
Swindale Beck (NY824188; NY774135)	13/01/00; 04/05/00; 11/05/00	3	2
Augill Beck NY833157	11/05/00	3	2
Argill Beck (NY868130; NY825128; NY849147)	11/05/00; 13/01/00	3	2
Sticegill Beck (NY855117)	11/05/00	1	2
Pottersike (NY875087)	11/05/00	1	2
River Belah (NY824120; NY794120)	11/05/00; 13/01/00	3	2
Gruntley Beck (NY826104)	11/05/00	1	2
Howgill Sike, Coldkeld (NY826104)	11/05/00	1	2
Stream, Red Gate Farm (NY812110)	11/05/00	1	2
Tarn Sike (NY743028)	11/05/00	1	2
Artlegarth Beck (NY722045)	11/05/00	1	2
Hoff Beck (NY675175)	15/05/00	1	2
High Cup Beck (NY684234)	15/05/00	1	2
Jed Water (NT652204)	18/03/00	1	2
Hen Poo (NT785561)	20/03/00	1	2
Oxcleugh Burn (NT237204)	21/03/00	1	2
Yarrow water (NT238204)	21/03/00	1	2
Stream, Melrose Abbey (NT548341)	18/03/00	1	2
Whituir Lake (NT500274)	20/03/00	1	2
River Tweed, Kelso (NT728336)	20/03/00	1	2
How Beck, Wharfedale (SE064592)	06/05/00	1	2
Fir Beck, Wharfedale (SE060593)	06/05/00	1	2
River Wharfe (SE058593; SD884802)	06/05/00; 10/07/00	1	2
Beck, Littondale (SD904739)	06/05/00	1	2
Beck, Ribblesdale (SD787749)	06/05/00	1	2
River Skirfare (SD880763)	06/05/00	1	2
Rookhope Burn (NZ 915425)	09/05/00; 13/06/00; 13/07/00; 10/08/00; 17/10/00; 15/11/00; 13/12/00; 24/01/01	10	2
Red Tarn Beck (NY357167)	02/06/00	1	2
Red Tarn (NY350154)	02/06/00	1	2
Grisedale Beck (NY375153; NY359138)	04/06/00	2	2
Nethermostove Beck (NY361144)	04/06/00	1	2
Stream, Place Fell (NY403157)	03/06/00	1	2
Stream, Glenridding Valley (NY355157)	02/06/00	1	2

Stream, Place Fell (NY418190)	03/06/00	1	2
Stream, Ullswater (NY396183)	03/06/00	1	2
Stream, Grisedale (NY358137)	04/06/00	1	2
Baybridge Burn, Blanchland (NY965504)	12/06/00	1	2
River Swale (SE022987)	08/07/00	1	2
Bleaberry Gill Beck, Swaledale (SD993009)	09/07/00	1	2
Annaside Beck, Swaledale (SD949071)	09/07/00	1	2
Lad Gill Beck, Swaledale (SD885045)	09/07/00	1	2
Thwaite Beck, Swaledale (SD893982)	09/07/00	1	2
Barney Beck, Swaledale (SE008998)	09/07/00	1	2
Small stream,Goyt Valley (SK014764	15/08/00	1	2
Mill Clough, Goyt Valley (SK008783)	15/08/00	1	2
Serpentine Reservoir; Knypersley, Staffs.	24/12/00	1	2
Knypersley Stream Staffs.	24/12/00	1	2
River Trent; Knypersley, Staffs.	24/12/00	1	2
Barton Spring, Beds.	26/12/00	1	2
Fold Sike (NY834293)	08/01/01	1	2
River Tees, Holmwath (NY835293)	08/01/01	1	2
Tinklers Sike (NY816284)	08/01/01	1	2
River Tees, Cauldron Snout (NY815286)	08/01/01	1	2
Red Sike (NY817296)	08/01/01	1	2
Pegham Sike (NY817296)	08/01/01	1	2
Sand Sike (NY845315)	08/01/01	1	2
River Nent, Cumbria	09/01/01	3	2
Haweswater Silverdale, Lancs.	10/01/01	1	2
River Kent Silverdale, Lancs.	10/01/01	1	2
Reigh Burn, Thropton	17/02/01	1	2
River Coquet, Northumberland	17/02/01; 14/04/01; 16/12/00	9	2/3
Streams, Blanchland (NY957500; NY959498; NY962500; (NY965502)	12/06/00	4	2/3
Blossom Hill Farm, Hexham U/S- outfall-D/S farm waste runoff	19/08/00; 21/08/00; 05/09/00	15	2/3
Tyne Valley; Haltwistle Burn, Melkridge Burn, Bardon Mill Burn, Settlingstone Burn, River South Tyne, Forstones, Brockhole Burn	14/02/01	6	2
River Tyne, Hexham	05/09/00		3
Devil's Brook (Dorset)	09/08/00	10	3
Briardene Burn, Tyne and Wear	25/11/00	1	3
Wallsend Burn, Tyne and Wear	25/11/00	1	3
Seaton Burn, Tyne and Wear	25/11/00	1	3
Todd Burn, Tyne and Wear	16/12/00	1	3
River Lea, Luton	26/12/00	1	3

Stanley Burn, Wylam	27/12/00	1	3
River Tyne, Park Burn	20/12/00; 27/12/00; 04/01/01	3	3
River Derwent, Blanchland (NY9835131)	12/06/00	1	3
River Wansbeck, Mitford (NZ148857)	26/03/00	1	3
River Font, Mitford (NZ172862)	26/03/00	1	3
Ouse Burn, Newcastle	02/05/00	2	3
River Blyth, Belasis Bridge (NZ190776)	01/05/00; 10/06/00	2	3
River Pont, Ponteland	01/05/00	1	3
Catraw Burn, Stanington (NZ213790)	10/06/00	1	3
River Tyne, Corbridge (NY980647)	12/06/00	1	3
Aydon Beck, Corbridge (NY980647)	12/06/00	1	3
River Severn, Shrewsbury	17/03/00	1	3
River Ouse, York	25/03/00; 01/05/00	2	3
River Colne, Huddersfield	25/03/00	1	3
River Skerne, Darlington (NZ285135)	17/05/00; 13/06/00	2	3
River Tees, Darlington (NZ273133)	17/05/00	1	3
Woodham Burn, Newton Aycliffe (NZ262246)	17/05/00	1	3
Corner Beck, Newton Aycliffe (NZ263246)	17/05/00	1	3
Cong Burn, Chester le Street (NZ277515)	17/05/00	2	3
River Wear (NZ280518) D/S STW	17/05/00	1	3
Lumley Burn (NZ284514)	17/05/00	1	3
River Wear (NZ284500)	17/05/00	1	3
South Burn (NZ274498)	17/05/00	2	3
Kyo Burn (River Team) Causey Arch U/S-outfall – D/S STW	19/05/00	4	3
Bogbins Burn, Causey	19/05/00; 02/11/00	2	3
River Tees, Croft on Tees (NZ290099)	13/06/00	1	3
River Skerne, (NZ290099) D/S STW	13/06/00	1	3
Arnside Tower Rising, Silverdale, Lancs.	10/01/01	1	3
Black Dyke, Silverdale, Lancs.	10/01/01	3	3
River Aire, Leeds	22/02/01	1	3
Agill Beck, Lofthouse Moor	16/04/01	1	3
Dowcy Sike, Lofthouse Moor	16/04/01	1	3
Streams, Isle of Skye.	21/07/01	5	2
River Exe, Exeter	18/04/00-22/04/00; 27/06/00	5	3
River Exe, Exebridge	02/10/00	1	3
Taddiford Brook, Exeter	19/04/00	1	3
River Teign, Chagford	18/04/00; 21/04/00; 26/06/00; 02/10/00; 03/04/01; 18/04/00; 28/08/01	8	3
Natterdon Brook, Chagford	18/04/00	1	3
Wash Leat, Chagford	23/04/00; 28/08/01	2	3
Meldon Stream, Devon	02/10/00; 03/04/01	2	3

South Zeal Brook, Devon	03/04/01	1	3
River Taw, Devon	03/04/01	1	3
East Dart, Devon	28/08/01	1	3
Walla Brook, Devon	28/08/01	1	3
Team Valley; Rowletch Burn, Hellhole Wood stream, Home Farm, Beamish Burn, Causey Burn, Houghwell Burn, Coltspool Burn; Coltspool Bridge	02/11/00	11	3

Key : STW sewage treatment works, D/S downstream U/S upstream.

Category 1= rivers draining predominantly peat areas

2= rivers draining from non-peat areas

3= urban rivers and rivers with inputs of sewage/farm waste DOM

b. Samples from the River Tyne catchment

Location	Grid ref	Location	Grid ref
Allen (Allenbanks)	NY8010064800	North Tyne (Kielder)	NY6320092800
Beamish Burn	NZ2050054700	Otter Burn (Otterburn)	NY8860094200
Black Burn (D/S STW)	NY6590058700	Ouse Burn	NZ2140069900
Black Burn (Intack)	NY7070043600	Ouse Burn	NZ2410069500
Bolts Burn	NY9580049700	Ouse Burn (Woolsington)	NZ2000070000
Chirdon Burn (Tarset)	NY7830085100	Park Burn (Park Village)	NY6850062000
Derwent (Allensford)	NZ0850050400	Pont Burn (Road Bridge)	NZ1470056200
Derwent (Ruffside Hall)	NY9850051500	Rede (Cottonshopefoot)	NT7780001200
Derwent (Clockburn Drift)	NZ1860060400	Rede (Otterburn)	NY8880092700
Derwent (Eddys Bridge)	NZ0380050800	Rede (Redesmouth)	NY8630082400
Derwent (Shotley Bridge)	NZ0910052700	Sills Burn (A68 Road)	NY8280092200
Don (Mount Pleasant)	NZ3450060800	South Tyne	NY9100065900
Derwent (U/S Bolts Burn)	NY9560049800	South Tyne (Alston)	NY7160046200
Devils Water (Dilston Hall)	NY9750063600	South Tyne (Eals)	NY6820055400
Don (Jarrow Cemetery)	NZ3310064500	South Tyne (Haltwhistle)	NY7050063700
Derwent (Lintzford Bridge)	NZ1470057000	South Tyne	NY7460041300
East Allen (Huntwell)	NY8510047700	Stocksfield Burn	NZ0540061300
Elsdon Burn	NY9340092800	Swinburn (Barrasford)	NY9200073100
Elsdon Burn (Road Bridge)	NY9110092100	Tarset Burn (Tarset)	NY7780085900
Erring Burn (Chollerton)	NY9310071600	Team	NZ2450060600
Gunnerton Burn (Burnmouth)	NY8980074500	Team	NZ2460055000
Hareshaw Burn (Bellingham)	NY8400083500	Tipalt Burn	NY6880063600
Horsleyhope Burn	NZ0640047300	Tyne (Bywell)	NZ0520062000
Houghwell Burn	NZ1890053700	Tyne (Crew Hall)	NY79606470
Lewisburn (Kielder)	NY6460090400	Tyne (Hexham)	NY9410064600
March Burn (Dipton House)	NY9950060800	Tyne (Ovingham)	NZ0860063600
Nent (Alston)	NY7170046700	Tyne (Wylam Bridge)	NZ1190064600
Newbrough Burn	NY8720067900	Wallish Walls Burn	NZ0750050500
North Tyne (Barrasford Intake)	NY9200073200	Warks Burn (Wark)	NY8620076600
North Tyne (Chollerford)	NY9180070500	West Allen	NY8030046700
North Tyne (Tarset)	NY7760086200	Wharnley Burn	NZ0750050100
North Tyne (Wark)	NY8630077000	Whittle Burn (Ovingham)	NZ0840063700

(sampled 01/06/02 and 01/08/02)

Appendix 7. Von Post Scale of Humification

Scale	Peat Characteristics
H1	Completely undecomposed peat; only clear water can be squeezed from peat
H2	Almost undecomposed; mud free peat; water squeezed from peat is almost clear and colourless
H3	Very little decomposition; very slightly muddy peat; water squeezed from peat is muddy; no peat passes through fingers when squeezed; residue retains structure of peat
H4	Poorly decomposed; somewhat muddy peat; water squeezed from peat is muddy; residue is muddy but it shows structure of peat
H5	Somewhat decomposed; muddy; growth structure discernible but indistinct; when squeezed some peat passes through fingers but most muddy water passes through fingers; compressed residue is muddy
H6	Somewhat decomposed; muddy; growth structure indistinct; less than one-third of peat passes through fingers when squeezed; residue very muddy
H7	Well decomposed; very muddy, growth structure indistinct; about one-half of peat passes through fingers when squeezed; exuded liquid has a "pudding-like" consistency
H8	Well decomposed; growth structure very indistinct; about two-thirds of peat passes through fingers when squeezed; residue consists mainly of roots and resistant fibres
H9	Almost completely decomposed; peat is mud-like; almost no growth structure can be seen; almost all of peat passes through the fingers when squeezed
H10	Completely decomposed; no discernible growth structure; entire peat mass passes

(Damman and French, 1987)