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Climate change and dissolved organic carbon : impacts on drinking water supplies

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CLIMATE CHANGE AND DISSOLVED ORGANIC CARBON: IMPACTS ON DRINKING WATER SUPPLIES

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Abstract

The presence of natural dissolved organic carbon (DOC) in drinking water supplies is undesirable due to its reaction with chlorine during water treatment and the resulting formation of disinfection by-products (DBPs) (Rook, 1976). Some of these compounds, principally trihalomethanes (THMs), have demonstrated carcinogenic properties and can potentially cause damage to the reproductive system (Bull, *et al.* 1995; Nieuwenhuijsen, *et al.* 2000). Concentrations of DOC in freshwater rivers and lakes in the UK have increased by 91% during the last 15 years (Evans, *et al.* 2005), with the rise attributed to the destabilisation of peatland soils through climate change, and the release of some of the vast carbon store of 455 Pg they have accumulated over the last few thousand years (Gorham, 1991). If the increase continues, it will create a serious challenge to water companies due to the need to minimise the formation of harmful DBP compounds. This project investigated the role of peatlands in the formation of THMs at selected reservoirs in north Wales. Water draining from Cors Erddreiniog fen into Cefni reservoir was found to be the most important source of DOC, comprising 33-57% of all the inputs to the lake on an annual basis; however, it was also demonstrated that sunlight can strongly degrade the DOC entering the lake from this source, thereby reducing its THM-forming potential. Rapid growth of algae during the summer months contributes up to 45% of all the DOC inputs, although the lower molecular weight of the carbon compounds ensures that algae do not generate THMs as readily as terrestrially-derived DOC during water treatment. At Marchlyn Bach and Llugwy reservoirs, the DOC concentration of the lake water was lower, due to both the bog soils within the catchment releasing less DOC than the fen, and the much lower autochthonous production. The impact of rising temperatures on peat soil was to increase the leachability of DOC during spring for bogs and summer for fens, the latter being important for the Cefni as this is when THM concentrations are highest. Exposure to elevated concentrations of atmospheric ozone reduced the porewater DOC concentration of fen peat by as much as 55%, suggesting that through its effects on the carbon allocation within the above ground vegetation, elevated ozone could partially offset the predicted increase in DOC leaching from fen ecosystems through enhanced warming and CO_2 concentrations.

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List of abbreviations

AOT40	Accumulate dose (of ozone) over 40 ppb
BrTHMs	Brominated trihalomethanes
CEC	Cation Exchange Capacity
CFCs	Chlorofluorocarbons
CIN	‘Cefni inflow North’
CIS	‘Cefni inflow South’
DBPs	Disinfection by-products
DIC	Dissolved Inorganic Carbon
dicq	2,3-dihydroindole-5,6-quinone-2-carboxylate
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ECP	Extracellular Products
EOM	Extracellular Organic Material
HAAs	Haloacetic Acids
HI	Humification Index
HMW	High Molecular Weight
HPLC	High Pressure Liquid Chromatography
HPSEC	High Pressure Size Exclusion Chromatography
IC	Inorganic Carbon
IMW	Intermediate Molecular Weight
IPCC	Intergovernmental Panel on Climate Change
L-DOPA	L-dihydroxyphenylalanine
LMW	Low Molecular Weight
MCL	Maximum Contaminant Level
MUF	Methylumbelliferone
MW	Molecular Weight
NMR	Nuclear Magnetic Resonance
NOM	Natural Organic Matter
NOx	Nitrogen Oxides
PAR	Photosynthetically Active Radiation
POC	Particulate Organic Matter
SOM	Soil Organic Matter
SUVA	Specific Ultraviolet Absorbance
THMs	Trihalomethanes
THMFP	Trihalomethane Formation Potential
TOC	Total Organic Carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VOCs	Volatile Organic Compounds
WHO	World Health Organisation

Chapter 1

Introduction

1.1 General introduction

The availability of clean drinking water is one of the most important influences on the quality of human health around the world. Developed countries spend billions of dollars on water treatment processes to ensure a safe drinking water supply, whilst those living in developing countries are not so fortunate and are often exposed to water that is unfit for consumption (van Leeuwen, 2000). The majority of treatment costs are for the removal of natural dissolved organic matter (DOM) and the addition of a disinfectant to remove microorganisms. Chlorine is the most frequently used type of disinfectant, it has been employed for over a century and the health benefits it has provided have been unprecedented (Bull, *et al.* 1995). It has even been stated that chlorine has saved more lives than any other chemical (Craun, *et al.* 1994). A vast number of harmful bacterial, viral, protozoan and parasitic diseases can exist in natural, untreated sources of water, which can easily be transmitted to humans and animals upon consumption. However, there are billions of people throughout the world whose only source of water is contaminated. In these often poor, isolated regions of the world, where untreated well and lake water are used for drinking and bathing, deaths from infectious diseases are extremely high. Diarrhoea caused by these water-borne diseases accounts for more deaths world-wide than cancer and AIDS put together (Craun, *et al.* 1994). Consequently, the issue of water quality, especially in the third world, is one of the greatest environmental challenges facing mankind in the 21st century.

The health benefits of chlorination cannot de disputed, but the discovery that potentially harmful by-products (disinfection by-products – DBPs) can be generated in water following the addition of chlorine has raised much concern in the water industry (Rook, 1976). The production of these by-products centres on the presence of dissolved organic carbon (DOC) in the water that enters the treatment plant; when chlorine is added it reacts with this organic material to form a class of compounds known as organo-halides (Sketchell, *et al.* 1995). The most commonly formed group of organo-halides are trihalomethanes (THMs) (Peterson, *et al.* 1993). They are a major cause for concern, because some tests have shown them to be carcinogenic to laboratory animals (Bull, *et al.* 1995).

Although the link between organic matter, chlorination and THMs was recognised 30 years ago, there is still much to be understood about the exact nature of the THM forming reactions and what specific precursor compounds are involved (Pomes, *et al.* 1999; Nikolaou & Lekkas, 2001; Uyguner, *et al.* 2004). However, it is understood that the dominant source of carbon to most lakes is allochthonous, i.e. produced outside the lake, and that peatlands are the most important soil type influencing this external input (Dillon & Molot, 1997). Concern is growing that climate change is leading to the increased export of carbon from peatland ecosystems, as DOC concentrations in many northern hemisphere freshwater systems have risen substantially over the last few years (e.g. Worrall, et al. 2004*b*; Evans, *et al.* 2005). Without the costly upgrading of treatment processes, this could lead to an increased formation of THMs and a deterioration in the quality of final drinking water.

1.2 Disinfection by-products (DBPs) and trihalomethanes (THMs)

1.2.1 Overview

Disinfection of water with chlorine has dramatically lowered the occurrence of water-borne diseases in all developed countries and saved millions of lives. One hundred years ago, typhoid and cholera epidemics were common throughout the developed world (USEPA, 1998), but now occurrences of such diseases have virtually ceased (Richardson, 2003). However, concern has developed over the formation of products that are potentially harmful to human health when water containing organic matter (DOM or DOC) is chlorinated. DOC itself is considered harmless, but the substitution of hydrogen ions in DOC by chlorine can lead to the formation of hundreds of halogenated by-products (DBPs), some of which are known to be carcinogenic (Jimenez, *et al.* 1993; Palacios, *et al.* 2000). They are considered to be the greatest health threat to consumers of treated water. A U.S. Environmental Protection Agency (EPA) survey discovered that DBPs are present in virtually all chlorinated water supplies (Capece, 1998).

It is important to put into perspective that DBPs are usually only present in trace amounts in chlorinated drinking water (approximately tens to hundreds of micrograms per litre) and the health risk they pose, in comparison to the harmful pathogens that are killed by chlorine, are small. However, because exposure to these trace compounds is over such a long period of time, governments in the developed world have deemed the health risk of DBPs great enough to require water companies to take action to reduce their level in drinking water supplies.

1.2.2 Trihalomethanes

Trihalomethanes (THMs) are the most common type of DBP that forms when chlorine reacts with aqueous organic matter (Sketchell, *et al.* 1995). There are four compounds within this class and they are similar in structure to methane but have three hydrogen atoms substituted with chlorine or bromine. The most common type of THM formed is chloroform ($CHCl_3$), the structure of which is shown in *figure 1.01*. Bromide is incorporated into the reaction when it is present in the raw water supply. This leads to the formation of the three other THM compounds; Bromodichloromethane ($CHCl_2Br$), Dibromochloromethane ($CHClBr_2$) and Bromoform ($CHBr_3$), which have a similar structure to chloroform except for the presence of one or more bromide ions. The formation of THMs containing bromide (brominated THMs) during water treatment is usually much lower than the production of chloroform.

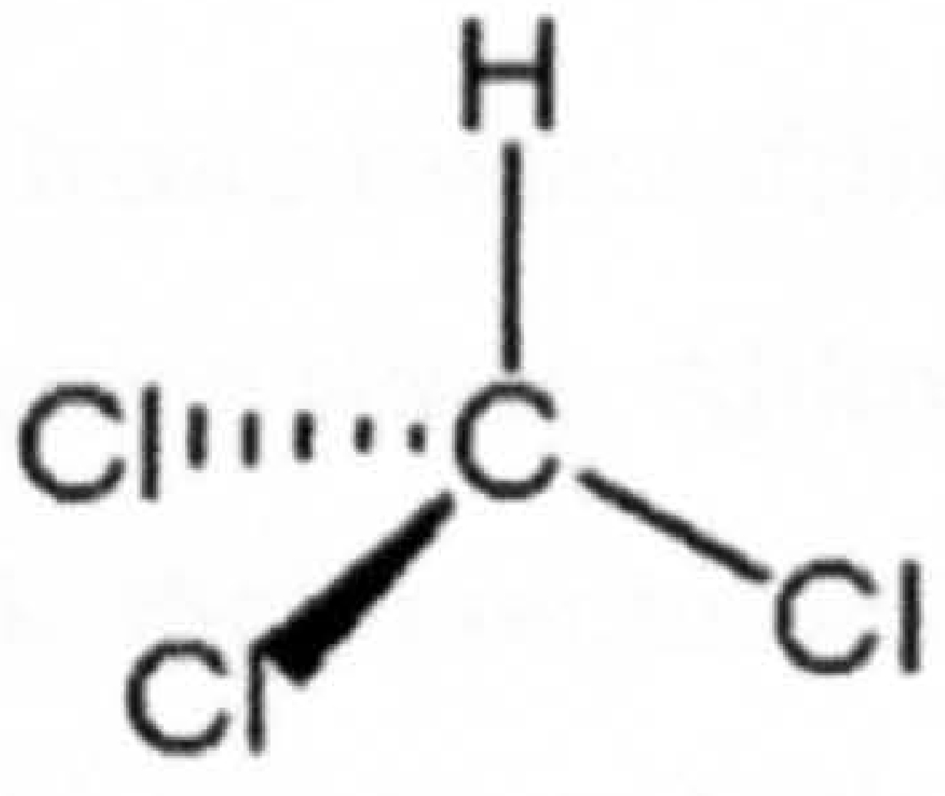

Figure 1.01 – The chemical structure of chloroform

THMs belong to a class of compounds called organohalides, which are naturally occurring and structurally diverse (Gribble, 2003). They are produced naturally by bacteria, plants, fungi, lichen and insects, whilst volcanoes and forest fires also contribute large quantities. Chloroform is the most abundant organohalide in the environment; an estimated 4 million tonnes are produced by natural sources annually, dwarfing the 10,000 tonnes released anthropogenically.

The formation of THMs during chlorination was first discovered in the 1970s but despite extensive research into the problem since then, the specific DOC precursor compounds involved remain largely unknown. However, the larger molecular weight/size compounds, such as humic acids, are generally thought to be most reactive to chlorine (Oliver & Visser, 1980; Uyguner, *et al.* 2004). The problem is alleviated somewhat by the fact that these types of compounds are more amenable to removal during water treatment processes (Collins, *et al.* 1986; Alarcon-Herrera, *et al.* 1994; Goslan, *et al.* 2002), but the incomplete removal of organic matter at most treatment works ensures THMs are a serious problem.

A variety of methods have been developed for measuring DBPs in water, as summarised by Cho, *et al.* (2003). They include direct aqueous injection, liquid-liquid extraction, static and dynamic headspace techniques (including purge and trap) and solid-phase microextraction. There are a number of advantages and disadvantages to all the methods; for example, the direct aqueous injection procedure is simple and quick, but suffers from column instability, and the liquid-liquid extraction method is sensitive, but contamination from the solvent can easily occur. The most reliable methods seem to be those that employ gas chromatography.

1.2.3 The health risk posed by DBPs

The risk to human health of DBPs in treated water differs from the short-term biological hazard that exists from drinking untreated water. Although most studies have shown that DBPs pose only a low-magnitude risk, their ubiquitous presence in public water supplies ensures that they are an issue of great importance to the long term health of humans (Singer, 2006). The continuous intake of these trace compounds through drinking water, showering (Nazir & Khan, 2006), bathing (Xu, *et al.* 2002) and even the use of public swimming baths (Chu & Nieuwenhuijsen, 2006) can result in a high degree of exposure to these compounds over the course of a person's life.

DBPs are a significant concern for the water industry as they have been linked to an increased risk of cancer (Peterson, *et al.* 1993; Bull, *et al.* 1995; Singer, 1999). The first major epidemiologic study on the health effects of trihalomethanes was conducted in 1974 when chloroform was first identified in chlorinated water supplies (IARC, 1991). In that and numerous epidemiologic studies since, strong links have been found between colon, rectum and bladder cancer and waters which contain high levels of THMs. Long-term carcinogenic tests on rats have also led scientists to conclude that THMs, and also haloacetic acids (the second main type of DBP), have a carcinogenic effect. Consequently, the International Agency for Research on Cancer has classified chloroform as a 2B carcinogen: possibly carcinogenic to humans (Chu & Nieuwenhuijsen, 2002). In rodent experiments comparing the different types of THM compounds, exposure to the brominated THMs has lead to a greater development of tumours than chloroform (Nobukawa & Sanukida, 2001; Villanueva, *et al.* 2003). Several DBP compounds regularly found in chlorinated drinking water supplies have also demonstrated reproductive and developmental toxicity in laboratory animals at high doses (Nieuwenhuijsen, *et al.* 2000). The most adverse effects were reductions in body weight and survival of offspring. A study in the USA found that women who drank 5 or more glasses of cold water per day that contained more than 75 $\mu g\ l^{-1}$ THMs had a miscarriage rate of 15.7%, compared with a rate of 9.5% for women with a low THM exposure (Capece, 1998). However, other studies have yielded contrasting results, with one large epidemiological study using laboratory animals finding no reduction in birth weight, despite administering a high dose of THMs (Dodds, *et al.* 1999).

New work has emphasised the problem of DBPs (Richardson, 2003; Richardson & Ternes, 2005). Specifically, there are concerns over adverse reproductive and developmental effects recently observed in human populations, concerns that the types of cancer observed in laboratory animals (for regulated DBPs) do not correlate with the cancers observed in human populations (indicating that other DBPs may be important), and concerns that other routes besides ingestion (i.e. inhalation and dermal adsorption) are also significant sources of DBP exposure. In addition, new types of DBPs are being discovered for which little toxicological information exists.

Current levels of DBPs in drinking water (tens to hundreds of $\mu g\ l^{-1}$) are thought to pose a sufficient carcinogenic risk for their levels to need regulation (Waller, *et al.* 1998). For example, in the USA, the maximum contaminant level (MCL) for THMs in drinking water is 80 $\mu g\ l^{-1}$ (Yoon, *et al.* 2003). MCLs in other countries vary, ranging from 100 $\mu g\ l^{-1}$ in most, including the UK (DWI, 1998), to just 10-15$\mu g\ l^{-1}$ in Denmark.

1.2.4 Other DBP compounds

Chlorinated drinking water contains a complex cocktail of DBPs, each with their own chemical and toxicological properties (Villanueva, *et al.* 2003). Whilst THMs dominate, and are often used as a surrogate for other DBPs, an understanding of the other by-products is necessary.

After THMs, the non-volatile haloacetic acids (HAAs) are the most prevalent DBP, occurring generally at about half the concentration of THMs in final drinking water (Nieuwenhuijsen, *et al.* 2000). Their formation is favoured over THMs the lower the pH of the water (Bull, *et al.* 1995). Like THMs, studies have linked HAAs with an increased risk of cancer (Sérodes, *et al.* 2003). Experiments on rats have demonstrated a potential link with some reproductive problems too; specifically testicular damage, disruption of spermatogenesis and reduced foetal weight and survival (Nieuwenhuijsen, *et al.* 2000). Other studies have suggested an increase in damage to the cardiovascular, digestive and urino-genital systems.

Most other DBPs occur at concentrations below 1 $\mu g\ l^{-1}$ (Nieuwenhuijsen, *et al.* 2000) and the commonest are bromate, chlorite, haloketones and haloacetonitriles (Yoon, *et al.* 2003). Bromate forms when ozone is used as the disinfectant and it reacts with naturally occurring bromide in the source water. It is currently regulated to a MCL of 10 $\mu g\ l^{-1}$. Chlorite is formed when chlorine dioxide is used as the disinfectant and is regulated to a level of 1 $\mu g\ l^{-1}$ (Siddiqui, *et al.* 1997).

1.2.5 Potential solutions to the DBP problem

There are 2 main ways in which the formation of DBPs can be moderated; a reduction in the concentration of organic material in the raw water, and the use of a disinfectant other than chlorine. It has always been a necessity for water treatment works to ensure the efficient removal of organic matter, in order to improve the aesthetics, taste and odour of drinking water (Sketchell, *et al.* 1995), but the added problem of DBP formation gives this greater emphasis (Black, *et al.* 1996). DOC also adds to the problem of bacterial re-growth in the distribution system (Lehtola, *et al.* 2001). A number of treatment options are available to reduce the concentration of organic matter, such as enhanced coagulation, oxidation/biodegradation, photocatalysis, magnetic ion exchange resin, adsorption, nanofiltration, etc., the merits of which are discussed by Balch (2005). Although a reduction in the precursor DOC concentration would certainly result in a final water containing less THMs, it can alter the relative proportion of the four individual compounds (Sketchell, *et al.* 1995). Treatment techniques that lower the levels of DOC without affecting bromide (i.e. increase the bromide:DOC ratio) have been implicated in a shift from chlorinated to brominated THM compounds (Sinha, *et al.* 1997). This is of particular concern, because, as previously discussed, there is a greater theoretical risk to humans from the brominated THMs.

The other main method for reducing the concentration of DBPs in drinking water is by using alternative disinfectants to chlorine; however, there is still much debate about how effective this technique is. Chloramine is the most widely used alternative to chlorine; its main advantage being that it produces a lower concentration of THMs (and HAAs). In a study by Peterson, *et al.* (1993), THM concentrations in drinking water were 14 times lower when using chloramination rather than chlorination. However, chloramine is a much less potent disinfectant, and therefore requires longer contact times to eliminate pathogens (Bull, *et al.* 1995; Peterson, *et al.* 1993). Although the elimination of THMs and HAAs is a huge advantage, other by-products are produced, namely cyanogen chloride and N-organochloramines, and even less is known about the health implications of these compounds (Bull, *et al.* 1995). Chloramination also results in a final water which contains more nitrifying bacteria (Siddiqui, *et al.* 1997).

Another popular alternative to chlorine is ozone and unlike chloramine, it is a very effective disinfectant (Bull, *et al.* 1995). It has a number of drawbacks, however, including the fact that it cannot be used as the residual disinfectant in the distribution system; therefore chlorine or chloramine would have to be added. Ozonation also leads to the production of other DBPs, such as bromate, bromoform, dibromacetic acid, peroxides, aldehydes and ketoacids (Bull, *et al.* 1995; Siddiqui, *et al.* 1997). Studies have shown that some of these compounds are mutagenic and carcinogenic. Ozone also has a tendency to increase the level of biodegradable organic carbon in the water, leading to an enhanced growth of biofilms in the distribution pipes. This can cause a variety of problems, including corrosion of the pipe and worsening of the taste, colour and odour of the drinking water (Siddiqui, *et al.* 1997). Chlorine dioxide is the other main disinfectant. Like ozone, it is an excellent disinfectant, but produces by-products which have not been studied extensively, namely chlorate and chlorite, although it does offer a significant reduction in THMs and HAAs (Bull, *et al.* 1995). Ozone and chlorine dioxide are also unfavourable disinfectants because they are relatively expensive compared to chlorine.

1.3 Organic matter in freshwaters

1.3.1 Overview

Natural organic matter (NOM), which is central to the formation of DBPs, is a constituent of all freshwater ecosystems. It consists of a mixture of plant and animal products in various stages of decomposition, of substances synthesised biologically and/or chemically from the breakdown products, and microorganisms and small animals and their decomposing remains (Schnitzer & Khan, 1972). The individual components of organic matter are all at various stages of cycling, and most exist in a decompositional state. Carbon is the key constituent of NOM and in freshwaters is present mostly in the dissolved form (Wetzel, 1975), i.e. below 0.45 µm (Thurman, 1985). Organic matter in freshwaters is therefore usually referred to as dissolved organic carbon (DOC), although a significant portion of material below 0.45 µm in size can be colloidal in nature (Chow, *et al.* 2005*b*). To describe the carbon cycle simply, organic carbon originates from the fixing of atmospheric CO_2 by plant photosynthesis, which leads to the formation of complex carbon compounds such as lignin and cellulose. Eventually, most of this carbon is returned to the atmosphere when dead plant material is decomposed in soil ecosystems. An important intermediate step in this process is the leaching of DOC from soils to freshwater lakes and streams; it is the dominant type of dissolved substance entering these systems and a vital component of the global carbon cycle (Schindler, *et al.* 1997). Freshwaters with a high concentration of DOC will often be stained brown due to the presence of aquatic humic substances.

1.3.2 The structure and composition of DOC compounds

The number of carbon compounds comprising this dissolved fraction is limitless and its structural complexity remains poorly resolved. This is despite copious amounts of research focussed on characterising DOM using analytical tools such as UV-visible absorbance, HPLC-SEC (High Pressure Liquid Chromatography-Size Exclusion Chromatography), fluorescence, NMR (Nuclear Magnetic Resonance), XAD fractionation, ultrafiltration, etc. (Her, *et al.* 2003; Uyguner, *et al.* 2004; Chow, *et al.* 2005*a*). One of the most basic methods of separating DOC compounds is into non-humic and humic substances, which are defined overleaf:

Non-humic compounds – This polar fraction is comprised mostly of decomposing animal and plant residues, products of their decomposition and products of re-synthesis in bacterial cells (Kononova, 1966). They include carbohydrates, proteins, peptides, fats, resins and other low molecular weight compounds (Schnitzer & Khan, 1972). They are uncoloured, labile compounds which are easily broken down by microorganisms and consequently exhibit rapid flux rates in lakes.

Humic compounds – These are non-polar, amorphous, brown/black coloured, hydrophobic, acidic compounds and are the more stable, recalcitrant, larger components of organic matter. They may remain unchanged in the environment for thousands of years (Vaughan & Malcolm, 1985) and form most of the organic matter of soils and waters. Humic substances are by far the most abundant organic molecules on Earth; their carbon contents are estimated to be two or three times higher than those in living matter (Davies & Ghabbour, 1998), whilst they are thought to comprise 50-75% of DOC in freshwaters (Hope, *et al.* 1994). All humic compounds are formed largely as a result of the microbial metabolism of plant and animal material, with the products formed being relatively resistant to microbial degradation in freshwaters (Fukushima, *et al.* 1996). They are able to complex with metal ions and hydrous oxides and can interact with clay minerals and organic chemicals, including toxic pollutants (Schnitzer & Khan, 1972). Therefore, the presence of humic substances in soils and waters, together with inorganic and organic compounds and the properties of the products formed, are of great importance in understanding environmental pollution.

1.3.3 Allochthonous vs. autochthonous sources

The concentration and characteristics of reservoir DOC depends on the degree of in-lake production (autochthonous sources) and the influx from the lake's catchment (allochthonous sources). The origin of DOC from allochthonous sources is chiefly from the decay of dead vegetation and the leaching of this organic matter in various stages of decomposition. This input represents a major source of energy and material for freshwaters, although by the time DOC reaches lake systems via inflowing streams its composition can become enriched in refractory compounds due to microbial transformation in the transporting waters (Wetzel, 1975). Other allochthonous sources include the exudation of material from plant roots, the erosion of topsoil by rain or wind, and input via rainfall, which generally has a DOC concentration of 1 mg l^{-1} (Wetzel, 2001). Autochthonous DOC is mainly composed of photosynthetic inputs of the littoral (lake shore) and pelagic (open water) flora through secretion and autolysis of cellular contents, excretions by zooplankton and higher animals, and bacterial decomposition of organic matter, with subsequent DOC release (Fukushima, *et al.* 1996). This decomposition leads to a build-up of DOC in the lake's hypolimnion (Parks & Baker, 1997). The chemical characteristics of DOC vary markedly between sources. In general, DOC from terrestrial sources is nitrogen poor, optically dense, highly coloured, rich in aromatic structures and has a high molecular weight. In contrast, autochthonous DOC is nitrogen-rich, relatively transparent and low in aromaticity and molecular weight (Curtis & Schindler, 1997; Gergel, 1999).

The contribution from each source varies both spatially and temporally between aquatic systems, however, the predominant origin of DOC to most freshwaters, especially in the northern temperate regions, is allochthonous (Wetzel, 2001). For example, Schindler (1971) observed a correlation between lake water colour and the ratio of terrestrial drainage area to lake surface area, a finding confirmed in many other studies. However, the contribution from each source changes seasonally; in late spring, summer and early autumn, autochthonous production is much greater than during the winter because increased sunlight and warmth allows for a much higher rate of photosynthesis and primary production by algae and vegetation. The contribution from allochthonous sources varies seasonally too and tends to peak during the autumn, as vegetation within the catchment starts to decompose as the weather turns cooler and wetter. In upland regions in winter, where snow is the dominant form of precipitation, there is a build-up of DOC which can be washed out of the soil during spring snowmelt (Parks & Baker, 1997). Such differences will have an influence on the formation of THMs.

1.3.4 Functions and losses of DOC from reservoirs

As summarised by Worrall, *et al.* (2002), DOC performs several functions in a lake, including acting as an energy and nutrient source, assisting in the transport of metals, and buffering pH. The DOC concentration can be the main factor controlling light penetration (Perez-Fuentetaja, *et al.* 1999) and the chromophoric functional groups are important for blocking out UV light and minimising the exposure of aquatic life to harmful UV-B radiation (Brooks, *et al.* 2005). DOC can influence the availability of some forms of phosphorus to phytoplankton and alter sedimentation rates (Gergel, 1999). It has also been observed to affect the thermocline depth (Perez-Fuentetaja, *et al.* 1999). There are a number of pathways by which DOC is lost in a reservoir. These are generally divided into biological and chemical:

Biological losses

- Oxidation and respiration – The continuous biotic oxidation of DOC compounds such as lignin by microbes and invertebrates generates carbohydrates, amino acids and phenolic compounds (Degens, 1982). When completely broken down, this can represent a sizeable export of carbon as CO_2 to the atmosphere.
- Immobilization – Biota, especially biofilms, can convert DOC to particulate organic carbon (POC) and back again, at rates ranging from 0.1 – 1.1 g^{-2} h^{-1} (Kuserk, *et al.* 1984; McDowell, 1985). However, the presence of recalcitrant high molecular weight compounds may inhibit this process (Freeman, *et al.* 1990).

Chemical losses

- Adsorption – DOC can be removed from the water column in large amounts due to adsorption by iron and aluminium oxides (McDowell, 1985).
- Photochemical oxidation – Exposure to UV radiation can break down recalcitrant fractions of DOC into inorganic compounds, such as carbon dioxide, and labile low molecular weight DOC (Parks & Baker, 1997) which may act as a carbon source for bacterial growth (Wetzel, *et al.* 1995).
- Sedimentation – DOC loss to the lake bed is also a major sink.

Non-living autochthonous DOC is more labile and decomposition of these carbon compounds is much more rapid (Steinberg & Muenster, 1985). They therefore only contribute a small proportion of freshwater DOC, especially in the cool, temperate regions of the world where most freshwater lakes exist.

1.4 The role of wetland ecosystems

1.4.1 Overview

Despite their straightforward sounding name, wetlands are extremely difficult ecosystems to define. This is because the depth of water fluctuates over time, their range in size is vast, they occupy many different geographical locations (from inland to the coast) and the level of human influence varies greatly between each one (Mitsch & Gosselink, 2000). They are also often temporally transitional, between ecosystems that are at a greater or lesser state of succession. Consequently, many definitions of wetlands exist, but the most widely used is that which was established at the international *Ramsar Convention* in 1971:

> Areas of marsh, fen, peatland, or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish, or salt including areas of marine water, the depth of which at low tide does not exceed 6 metres.
>
> (Groombridge, 1992)

The above definition incorporates all of the variable aspects of wetlands, but the dominant characteristic of all wetlands is the persistently high water-table and the effect this has on biogeochemical reactions. As a result, wetlands possess soils with very unique properties and are inhabited by specialised flora and fauna. From the unproductive, upland, oligotrophic bogs of the UK, to the diverse, tropical swamps of South America, wetlands contain some of the most unique and important features of all of the world's ecosystems, despite occupying just 4-6% of the earth's land surface (Maltby & Turner, 1983; Matthews & Fung, 1987). They are valuable as sources, sinks and transformers of a multitude of chemical, biological and genetic materials (Mitsch & Gosselink, 2000). For example, Braekke (1981) observed that 62% of dissolved materials exported from a Norwegian watershed were chemically changed by passing through wetlands, even though the wetlands occupied just 24% of the catchment. Their ecological niche between more terrestrial and more aquatic environments often leads to a high biodiversity, as wetlands can provide a home for species from both extremes (Mitsch & Gosselink, 2000).

SOME PARTS
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1.4.2 Peatlands

Peatlands are freshwater wetlands found extensively in the boreal zone of the world, where cool, humid conditions prevail and precipitation is moderate to high (*figure 1.02*). Estimates of the world's surface area occupied by peatlands are in the region of 400 million ha (Gorham, 1991; Immirzi, *et al.* 1992), approximately half of the world's total of wetland ecosystems. Like all wetlands, the dominant feature of peatlands is the presence of a high water-table, with estimates of soil water content being 88-97% (Heathwaite, *et al.* 1993). The waterlogged conditions and resulting lack of oxygen create a situation where decomposition processes fail to keep pace with the production of organic matter (Mitsch & Gosselink, 2000). Consequently, peat soil is dark and fibrous, with a dry weight of over 65% organic matter (Clymo, 1983). Peatlands are generally classified as soils with a surface layer of peat greater than 30-40cm (Glaser, 1987), but they can contain peat up to a depth of several metres.

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There are 2 contrasting types of peatland; bogs and fens, as defined below:
Bogs – An ombrotrophic (entirely rain-fed), oligotrophic (nutrient-poor), acidic peatland, with no significant inflow or outflow of surface water or groundwater, which supports acidophilic (acid-loving) vegetation, particularly mosses.
Fens – A minerotrophic, meso- or eutrophic open peatland system that generally receives some drainage from surrounding mineral soils and is often covered by grasses, sedges or reeds. (Mitsch & Gosselink, 2000).

A true bog will only form when the soils become isolated from surface water inputs and the influx of water from precipitation exceeds the efflux by evapotranspiration. This dramatically lowers the nutrient content of the soil and consequently the potential for organic matter decomposition (Mitsch & Gosselink, 2000). The resulting accumulation of organic compounds lowers the soil's pH (usually <4.5), which is enhanced further by the strong cation exchange capacity of the *sphagnum* moss that dominates the vegetation of bog ecosystems (Clymo, 1963). Fens receive a significant supply of nutrients from groundwater sources, ensuring a pH which is less acidic than bog soils (rarely <4.5) and a greater nutrient content. This in turn allows for faster rates of decomposition within the soil, and vegetation which is much more productive (Farrish & Grigal, 1988). The vegetation associated with peatlands is often highly adapted to the unfavourable conditions of the soil. One of the most notable adaptations to the low nutrient availability in bog soils is displayed by the carnivorous plants (e.g. *Dionaea muscipula*), which obtain nourishment by trapping and digesting insects (Moore & Bellamy, 1974).

1.4.3 Biogeochemistry in peatlands

Figure 1.03 presents a summary of the major biogeochemical processes occurring in a typical bog. It shows that the input of nutrients is solely from rainfall, that *sphagnum* moss takes up nutrients such as ammonium and potassium and releases pH lowering hydrogen ions, that the decomposition of organic matter releases acidic compounds and that processes such as sulphate reduction by microorganisms occur in the deep anaerobic layers of peat.

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The waterlogging of soil instantly alters chemical and microbiological processes, influencing nutrient cycling and leading to the accumulation of toxins (Pulford & Tabatabai, 1987). At the surface of bogs and fens there is usually a thinly oxidised layer of soil, where air is incorporated into the pores. This creates a steep gradient in redox potential (Eh) within the soil profile; from +700 mV at the surface to -400 mV in the deeper layers. As the redox potential and oxygen level decrease with increasing soil depth, alternative terminal electron acceptors are utilized by anaerobic microorganisms during respiration. These may be organic compounds, or more commonly, inorganic compounds such as NO_3^-, MnO_2, $Fe(OH)_3$ and SO_4^{2-} (*figure 1.04*).

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The thin, aerobic surface layer plays a vital role in many biogeochemical reactions within peatlands. Nutrient cycling is one such process, whereby nutrients that might not be available in the bulk of the oxygen-free soil are transformed into different types and transported into the deeper anaerobic zones (Gambrell & Patrick, 1978).

1.4.4 The carbon cycle in peatlands

Except for the thin layer at the surface, waterlogged peat soils are strongly anaerobic. Perhaps the most important aspect of this is the impact on the carbon cycle, which is slowed down greatly in peatlands compared to more aerated soils (Gorham, 1991). The growth of vegetation and uptake of carbon is a slow process, as photosynthesis is hampered by the poor quality soil and its lack of nutrients. However, the decomposition of organic matter, principally dead plant material, is even slower due to the anaerobic, acidic conditions. Moore & Bellamy (1974) showed that the numbers of carbon cycling microorganisms in peatlands are much fewer than in an aerobic soil. This has led to the continuous build-up of vast amounts of carbon in the soil profile, carbon that was originally derived from the atmosphere by photosynthetic CO_2 assimilation (Gore, 1983). The result is that peatlands are net carbon sinks and, despite occupying only 3% of the world's terrestrial surface, contain an estimated 455 gigatonnes of carbon, the equivalent of 60% of the atmospheric carbon dioxide pool (Gorham, 1991; Oechel, *et al.* 1993), or one third of the total soil carbon pool (Eswaran, *et al.* 1995). Rates of carbon accumulation by peatlands on a global scale are estimated to be between 0.05-0.11 Pg/yr, at a rate of 0.5 mm/yr (Armentano & Menges, 1986; Silvola, 1986). Considering that the total annual quantity of CO_2 released from soils around the world during decomposition is 0.2-0.5 Pg/yr (Wild, 1993), this peatland accumulation is substantial. The build up of carbon is principally from decaying plant material and is therefore mainly high molecular weight compounds, such as phenolic and cellulose material. Organic matter decomposition occurs mostly to DOC, rather than CO_2, so the porewater of peatlands can be DOC-rich, with concentrations generally in the region of 20-60 mg l^{-1}. Peatland soils boast these characteristics and therefore exhibit high DOC effluxes (Grieve, 1990), with estimates typically around 1-50 g m^{-2} yr^{-1} (Blodau, 2002).

1.4.5 Peatland organic matter decomposition and hydrolase enzymes

Soil enzymes are the biological catalysts of organic matter decomposition (Dick & Tabatabai, 1993). Extracellular *hydrolase enzymes* are the dominant decomposers of organic matter and they are produced by microorganisms (Burns, 1978). The low oxygen content, poor nutrient status and acidic pH of peatlands, especially bogs, ensures that these enzymes have a low rate of activity, resulting in minimal organic matter decomposition (Gammelgaard, *et al.* 1992; Kang & Freeman, 1999). However, experiments have shown that this suite of enzymes increase in activity in more aerated peat, despite their physiologically lack of a requirement for bimolecular oxygen (Lee, *et al.* 1999). For example, during an experiment to assess the effect of water-table drawdown on hydrolase enzyme activities, a water-table drop of 5.2cm significantly increased the activity of β-D-glucosidase, phosphatase and sulphatase by up to 70% (Freeman, *et al.* 1996). Subsequent studies on carbon cycling in peat linked the observed response in hydrolase enzyme activities to a group of carbon compounds and an enzymic 'latch' mechanism (Freeman, *et al.* 2001*a*), discussed below.

1.4.6 Phenolic compounds and phenol oxidase

Phenolics are present in all soils and are a suite of compounds which have a high molecular weight, consisting of an aromatic ring with branching hydroxyl groups (Ostle, 1994). They are derived from the microbial breakdown of lignin and cellulose compounds from decaying vegetation (Dickinson, 1983). Phenol oxidase is a microbially-produced, natural soil enzyme which is one of only a few capable of degrading phenolic compounds (Sinsabaugh, *et al.* 1991; Pind, *et al.* 1994; McLatchey & Reddy, 1998). Phenol oxidase is severely inhibited in anaerobic peat soils as it has an absolute requirement for oxygen (McLatchey & Reddy, 1998; Freeman, *et al.* 2001*a*). Consequently, high concentrations of phenolic compounds are known to exist in peat water (Hartley & Whitehead, 1985). Studies have shown that these phenolic compounds possess inhibitory characteristics towards hydrolase enzymes (Wetzel, 1992). This implies that phenol oxidase plays a critical role in the rates of organic matter decomposition occurring in peatlands, or rather, its lack of activity in the predominantly anaerobic peat soil helps explain why enzyme activities, microbial respiration and organic matter decomposition are so low in peatlands. The lack of oxygen suppresses phenol oxidase activity, allowing phenolics to build up, inhibiting hydrolase enzymes and impairing rates of decomposition. Phenol oxidase therefore acts as a 'latch', preventing the release of a vast terrestrial store of carbon (Freeman, *et al.* 2001*a*; Freeman, *et al.* 2004*a*).

1.4.7 The role of peatlands in the freshwater DOC cycle

As previously stated, allochthonous inputs tend to dominate as the source of DOC in most freshwater lakes. Often the most influential determinant on the size of this allochthonous input are the peatlands that occupy the lake's catchment (Curtis & Schindler, 1997; Curtis, 1998). The persistently saturated soils of peatlands means there is always potential for leaching large quantities of DOC and export rates are readily described by a simple linear function based on the percentage of peat coverage in the catchment (Dillon & Molot, 1997). Consequently, they play a hugely influential role in the export of organic matter to lakes which serve as drinking water sources.

A number of studies have demonstrated the influence peatlands have on the carbon content of freshwaters. McDowell & Likens (1988) showed that within upland ecosystems, a greater quantity of DOC per unit area is exported from sites with thicker soils. Aitkenhead, *et al.* (1999) observed that Scottish rivers with the highest DOC concentration have extensive peatlands in their catchments. Urban, *et al.* (1989) reported that the largest single variable controlling the DOC yield from a catchment is the proportion of the area occupied by peatlands. In Scotland, annual fluxes of DOC in two rivers showed strong positive correlations with hill peat in the catchment area (Hope, *et al.* 1997). Strong correlations have also been reported for lake-water DOC and the abundance of wetlands within the watershed (Gergel, *et al.* 1999; Dillon & Molot, 1997). Wilson *et al.* (1981) demonstrated that the humic acid composition of an upland lake in north Wales was largely determined by the input of terrestrial humic substances from the surrounding blanket bog. A study by Sachse, *et al.* (2001) of a fen within the catchment of a dystrophic lake reported higher concentrations of DOC and humic acids in the section of the lake closest to the fen.

The role of peatlands in influencing the DOC concentration of freshwaters is therefore critical, particularly in the temperate regions of the northern hemisphere where most peatlands exist. They therefore have an important detrimental impact on the quality of drinking water sources and raise the potential for THM formation, particularly as the DOC they leach is often of a high molecular weight and therefore strongly reactive with chlorine (Uyguner, *et al.* 2004). Previous studies have shown that peatlands leach significant quantities of THM precursors into freshwaters (Chow, *et al.* 2003; Fleck, *et al.* 2004). A schematic for this peatland-reservoir-drinking water carbon flux is illustrated in *figure 1.05* and demonstrates that peatlands input large quantities of DBP-forming carbon compounds into drinking water reservoirs.

The role of peatlands in the freshwater carbon cycle is particularly important in the UK, as they are the single largest terrestrial store of carbon (Cannell, *et al.* 1999) and dominate the headwaters of many major water supply catchments (Evans, *et al.* 1999). The release of DOC from peatland soils is considered a two stage process; the production of a soil organic carbon pool through primary production and decomposition processes and the subsequent efflux of this carbon through the drainage network (Freeman, *et al.* 2002). This process is largely dependent on the local hydrological flow regime (Worrall, *et al.* 2002). The majority of DOC production from peatlands occurs during the summer, due to high rates of decomposition (Tegen & Dorr, 1996). However, the low rainfall during summer tends to lead to a build up of DOC which is then washed out during the heavy rainfall that is characteristic of the autumn season (Scott, *et al.* 1998). These seasonal variations have a very important effect on the concentration of disinfection by-product (DBP) forming compounds (DBP precursors) in reservoir waters and consequently the concentrations of DBPs in drinking waters (Goslan, *et al.* 2002; Whitaker, *et al.* 2003). Long-term data of DOC concentrations in Alaw lake, on the island of Anglesey in north Wales show the typical seasonal variation experienced in most lakes in the northern hemisphere (*figure A.01,* appendix A1). The highest concentrations for most years were recorded near the beginning of October, when rainfall and the flux of allochthonous DOC is highest.

1.5 The problem of climate change

1.5.1 The changing nature of organic matter in freshwaters

Concerns are growing within the water industry that concentrations of DOC are increasing in many of the upland, freshwater lakes that are used as sources of drinking water in the northern hemisphere. There have been many studies publishing such findings, with increases recorded in the UK (Robson & Neal, 1996; Freeman, *et al.* 2001*b*; Worrall, *et al.* 2003*a*; Evans, *et al.* 2005), central Europe (Hejzlar, *et al.* 2003; Korth, *et al.* 2004), Scandinavia (Hongve, *et al.* 2004) and the USA (Schindler, *et al.* 1997). In the UK, the concentration of freshwater DOC has increased by 91% in the last 15 years (Evans, *et al.* 2005) (*figure 1.06*).

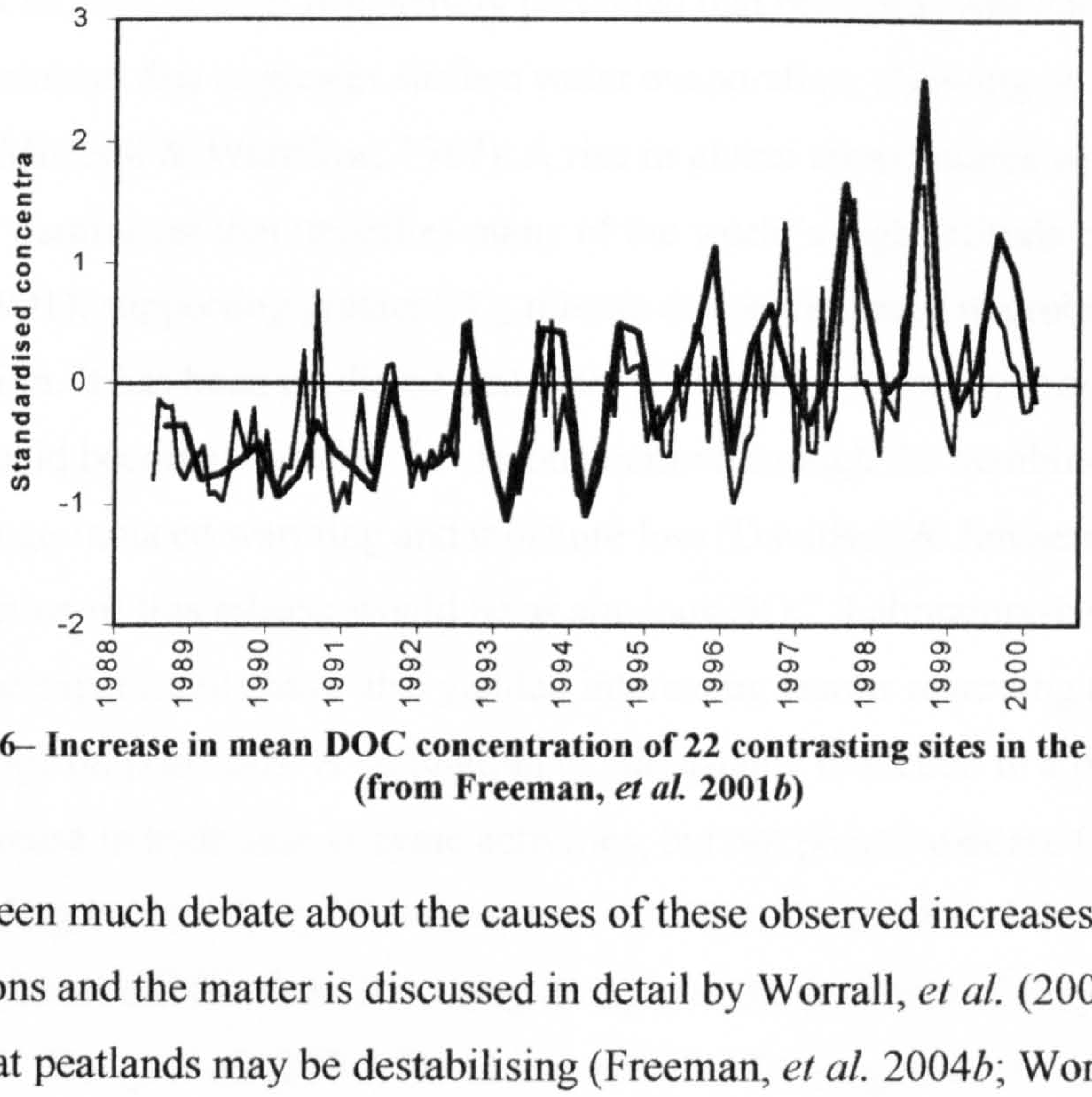

Figure 1.06– Increase in mean DOC concentration of 22 contrasting sites in the UK since 1988 (from Freeman, *et al.* 2001*b*)

There has been much debate about the causes of these observed increases in DOC concentrations and the matter is discussed in detail by Worrall, *et al.* (2004*b*). There are concerns that peatlands may be destabilising (Freeman, *et al.* 2004*b*; Worrall & Burt, 2005) and slowly beginning to release some of the vast store of carbon they contain. With freshwater DOC concentrations being related to the degree of storage and release of carbon in peatland catchment soils, there must be a coherent driving force causing these increases. This was thought to be increasing temperature (Freeman, *et al.* 2001*b*), but this has since been ruled out as the sole cause. Elevated CO_2, hydrological changes, drought, continuing nitrate deposition and decreasing sulphate deposition have all been put forward as potential causes, but there is still little consensus on what might be the most likely reason (Freeman, *et al.* 2004*b*; Worrall, *et al.* 2004*a*; Evans, *et al.* 2005; Findlay, 2005; Clark, *et al.* 2006; Worrall, *et al.* 2006*a*).

1.5.2 Peatlands and future climate change

The main effects of future climate change are likely to be elevated temperatures, atmospheric carbon dioxide levels and an increased occurrence of drought and storm events (Fowler & Hennessy, 1995). For example, the continually increasing anthropogenic release of greenhouse gases is expected to increase the average global surface temperature of the Earth by between 1.47°C and 5.87°C by 2100 (IPCC, 2001). A number of studies have investigated the potential effects of such changes on peatlands with respect to DOC flux. It is generally predicted that peatlands will become drier if the world gets warmer due to greater surface water evaporation, allowing increased aeration of the soil (Mitchell & Warrilow, 1987). A rise in global temperatures would also melt some of the permafrost that underlies many of the world's high-latitude peatlands (Gorham, 1991), supporting greater CO_2 release due to increased microbial decomposition. It has been predicted that 100 Pg of carbon normally locked up inside peatlands could become available for decomposition through the combined effects of climate change-induced warming and moisture loss (Davidson & Janssens, 2006), and a large proportion of this release would be as aqueous DOC. Laboratory-based manipulative experiments have also yielded interesting results regarding the effects of climate change on peatlands. A simulation of water-table reduction in a peat soil lead to a strong increase in hydrolase enzyme activities, but not phenol oxidase (Freeman, *et al.* 1996); a finding backed up by Williams, *et al.* (2000), who attributed the lack of an increase in phenol oxidase activity during drought to the presence of enzyme inhibitors and a low pH. Kang, *et al.* (2001) found that elevated CO_2 significantly increased pore-water DOC in peat cores. The effect of a combination of drought and increased heavy rainfall was investigated by Fenner, *et al.* (2001) in a Welsh peatland. It was found that the lowering and raising of the water-table dramatically increased DOC concentrations leaching from these soils. Chow, *et al.* (2003) also observed that this process released large amounts of THM precursors from peat soils.

It is not just CO_2 that is on the rise in the atmosphere. Concentrations of ozone have more than doubled during the past century; typical surface measurements today are 30-40 ppb, compared with 10-15 ppb during the pre-industrial era (Volz & Kley, 1988). Ozone is regarded as the most potent phytotoxic regional scale air pollutant (Andersen, 2003). Analysis of the single-species response database has indicated that the highest number of potentially-ozone sensitive communities are found in grasslands, heathlands and mire, bogs and fens (Mills, *et al. in press*), but these predictions have yet to be tested on whole ecosystems and the impacts on below ground carbon cycling have not be investigated at all.

Worrall & Burt (2005) have modelled potential future DOC release from UK peatlands with increasing temperature and decreasing water-table depth. The results suggest that peat bogs will become a net source of carbon within 25 years, unlocking some of the vast store of 455 Pg that has built up over thousands of years. This is a significant concern for the water industry and may mean that through the increased leaching of DOC into freshwaters, some treatment works that draw water from reservoirs with peat-dominated catchments become inoperable.

1.6 Overall summary

Since its introduction early in the twentieth century, the process of water chlorination has saved millions of lives and the benefits to mankind cannot be understated. However, the recent discovery of the formation of potentially harmful disinfection by-products (DBPs) in chlorinated drinking water, generated from the reaction between chlorine and natural organic matter, has shed a negative light on the process. The main group of disinfection by-products (DBPs) are trihalomethanes (THMs), which have been studied extensively since their discovery in treated drinking water in 1976. As a result, much literature is available on their nature, formation and health impacts, although there is still much to be learnt. Epidemiological research has suggested that there is a possible increase in the risk of cancer upon ingestion of THMs via drinking water, especially cancers of the rectum, colon and bladder (Bull, *et al.* 1995). Studies have also linked THMs to reproductive and developmental problems (Nieuwenhuijsen, *et al.* 2000). Other DBPs, such as haloacetic acids, haloketones and bromate have also demonstrated negative health impacts during experiments on laboratory animals, and new DBP compounds are being discovered for which little is known about their potential health impacts (Richardson, 2003).

The solution to the DBP problem is not simple. The use of disinfectants other than chlorine, such as chloramine and ozone, reduces THM and HAA concentrations but also produces alternative DBPs, are less effective at disinfecting water and are relatively expensive. The most reliable method would seem to be to try and remove as much organic matter as possible prior to disinfection. Whilst this would also improve the taste, odour and colour of the water, it is not a cheap process.

An additional worry for the water industry is that the concentration of organic matter compounds, the precursors to DBP formation, seem to be increasing in the freshwaters that are common in the northern, temperate zone of the northern hemisphere and which are used as drinking water sources. Freshwater DOC concentrations have increased by 91% in the last 15 years at 22 contrasting sites within the UK (Evans, *et al.* 2005) and peat bogs may become net sources of carbon in 25 years (Worrall & Burt, 2005). This places an even greater emphasis on improving the efficiency of organic matter removal.

1.7 Aims of the project

The project will focus on 3 reservoirs in north-west Wales. The Cefni is characterised by high raw water DOC and final water THM levels and a series of experiments will attempt to identify the most important sources of THM-forming DOC to the lake. The wetland soils surrounding the lake may be responsible for a large allochthonous loading of DOC to the lake, but autochthonous sources of carbon are expected to play an important role in THM formation. The characteristics of the Cefni reservoir with respect to THM formation will be compared to those at Marchlyn Bach and Llugwy, which contain only low levels of DOC but typify the type of environment predicted to experience rising freshwater DOC concentrations through the effects of climate change (Freeman, *et al.* 2001*b*; Worrall & Burt, 2005).

Rising temperature is one of the certainties of climate change, and its effect on the carbon leachability of peatland soils during heavy rainfall events will be investigated. Storm conditions wash large concentrations of terrestrially-derived carbon into freshwaters (Royer & David, 2005) and the effect of warming on the stability of peatland soils during periods of heavy rainfall would provide an interesting new aspect concerning the influence of warming on terrestrial carbon stocks.

Ultraviolet light is a key influence on the quantity and characteristics of DOC in freshwaters lakes and therefore the potential formation of THMs following water treatment. Its influence on the flux of terrestrially-derived DOC to the Cefni and Llugwy lakes will therefore be investigated. It is expected that UV light will reduce the molecular weight of DOC compounds, making them less likely to form THMs.

It is anticipated that rising concentrations of atmospheric CO_2 will increase the flux of DOC from peatlands (Freeman, *et al.* 2004*b*), but one aspect of climate change that hasn't been considered is rising levels of ozone. The effect of elevated ozone concentrations on DOC release from peat cores will therefore be investigated.

Chapter 2

A field survey of factors affecting the formation of trihalomethanes at three contrasting reservoirs in north Wales, UK

2.1 Reservoir characteristics

2.1.1 Materials and methods

2.1.1.1 Field sites

Three contrasting reservoirs in north-west Wales were chosen for the survey; the Cefni (UK grid ref. SH4450077100), Marchlyn Bach (SH60376302) and Llugwy (SH69266241). The Cefni (*figures 2.01 & 2.02*) is a man-made, shallow (max. 4m depth), lowland lake on the island of Anglesey. This site was chosen because of the consistently high DOC concentration of the lake water and the resulting high concentration of final water THMs formed at the treatment works. Compliance for THMs at this site has historically been poor (Balch, 2005). The red circle in *figure 2.02* shows a large area of foam which can be typical of high DOC freshwaters.

Figure 2.01 – Cefni lake

Figure 2.02 – Cefni lake dam and outflow

Marchlyn Bach (*figure 2.03*) and Llugwy (*figure 2.04*) reservoirs are deep, oligotrophic, upland lakes within the Snowdonia mountain range. The Llugwy is approximately twice the size of Marchlyn Bach and, although their DOC concentrations are relatively low, they were chosen for analysis because they typify the type of environment prone to increased DOC exports in response to climate change (Freeman, *et al.* 2001*b*; Worrall & Burt, 2005). Both lakes feed a single water treatment works, at Mynydd Llandegai, which supplies drinking water to the cities of Bangor and Caernarfon and the surrounding regions of north-west Wales.

Figure 2.03 – Marchlyn Bach reservoir

Figure 2.04 – Llugwy reservoir

2.1.1.2 Sampling regime

Samples were taken at monthly intervals from February 2003 to March 2004. Lake water samples for all three reservoirs were taken from the raw water taps at the Cefni and Mynydd Llandegai water treatment works, rather than directly at the lakes themselves. This was to ensure that the water sampled represented as closely as possible the water entering each treatment works immediately prior to treatment. The raw water tap was allowed to run for approximately 5 minutes to ensure the collection of fresh water and samples were collected in pre-ashed (550°C) 20 ml glass vials (5 per reservoir). For the analysis of dissolved carbon gases, 12 ml of raw water was drawn into a 20 ml syringe, which was capped and sealed. All samples were carefully transported back to the laboratory, where they were stored in a 4°C refrigerator. pH measurements and the extraction of dissolved gases (see later for method) were carried out immediately and all water samples were filtered through sterilised 0.45 µm membranes (Whatman, Kent, UK) within 24 hours to remove particulate organic carbon (POC) and minimize bacterial degradation (Fenner, 2002).

2.1.1.3 Experimental analyses

Lake water dissolved organic carbon analyses

- Dissolved organic carbon (DOC) concentrations were measured with a Shimadzu TOC-5000 using a carrier gas of high purity air at a flow rate of 150 ml min^{-1} and an injection volume of 26 µl. A one-point calibration was performed, using 100 mg l^{-1} of potassium hydrogen phthalate (total organic carbon – TOC) and 100 mg l^{-1} of sodium carbonate/sodium hydrogen carbonate solution (inorganic carbon – IC). DOC concentrations were calculated by subtracting IC from TOC. Analysis of TOC and IC standard solutions at 10 mg l^{-1} intervals demonstrated that the machine was linear from 0 up to at least 200 mg l^{-1}, with R^2 values >0.9.
- Phenolic compounds were assayed using a modified version of the spectrophotometric method developed by Box (1983). Within 300 µl microplate wells, replicates of 250 µl of sample, 37.5 µl of Na_2CO_3 solution (200 mg l^{-1}) and 12.5 µl of Folin-Ciocalteau phenol reagent were dispensed and mixed thoroughly. A standard curve was prepared by adding the same chemicals to 0-5 mg l^{-1} phenol solution. After two hours of incubation at room temperature, the colour change of the reactants was measured at 750nm using the absorption option of a BMG Fluostar Galaxy fluorimeter. Samples out of range of the standard curve were re-measured using a standard curve with a higher upper concentration.
- Characterisation of the molecular size of DOC was employed using high pressure size exclusion chromatography (HPSEC). The smaller compounds are eluted later due to greater permeation on the porous matrix. Spectra were obtained on a Cecil 1100 series High Performance Liquid Chromatograph (HPLC) with a gel filtration column (PL-GFC 8µm, 300Å, 300 mm x 7.5 mm inner diameter; Polymer Laboratories, Shropshire) and a Cecil 1200 variable wavelength monitor detecting at 254nm. The flow rate was 1 ml min^{-1} and the loop size 120 µl. Tris (Tris hydroxymethane aminomethane) hydrochloric acid (0.01M, pH 7.5) was used as the eluent, following the recipe of Dawson, *et al.* (1986). The system was calibrated using polystyrenesulfonic acid compounds (Polysciences, Inc., USA) with molecular weights (MW) of 4,600, 8,000, 18,000 and 35,000 and salicyclic acid (MW 138) and

acetone (MW 58), as described in Zhou, *et al.* (2000). These compounds were preferred for column calibration because proteins tend to overestimate the molecular weight of humic substances (Chin, *et al.* 1994). For the purposes of integration, the spectra were divided into three size fractions; high molecular weight (HMW), comprising carbon compounds between 10,000-100,000 daltons; intermediate molecular weight (IMW), 1,000-10,000 daltons; and low molecular weight (LMW), 100-1,000 daltons.

- UV-visible absorbance has many useful applications for characterising aquatic organic matter, as summarised by Hautala, *et al.* (2000). Absorbencies at 254, 465 and 665nm were measured on a Camspec M330 UV-visible spectrophotometer, using a 1cm quartz cell zeroed to a blank of deionised water. Absorbance at 254nm (UV-254) is closely related to aromaticity (and molecular weight) of organic compounds (Korshin, *et al.* 1997*a*) and is often used as a surrogate measurement for DOC in the water industry (Edzwald, *et al.* 1985; Korshin, *et al.* 1997*b*). SUVA (L(mg m)) was calculated as the ratio of UV absorbance at 254nm (m^{-1}) to DOC (mg l^{-1}); the higher the value the more aromatic and higher molecular weight the DOC compounds (Volk, *et al.* 2002) and the higher the potential to form THMs (Kitis, *et al.* 2002). The ratio of absorbencies at 465nm and 665nm, known as the E4:E6 ratio or Humification Index, is inversely proportional to the aromatic composition and molecular weight of the carbon compounds (Kononova, 1966; Chen, *et al.* 1977). The smaller the value the lower the degree of decomposition that the soil organic matter has undergone.

Hydrochemical analysis

- A Dionex DX-120 Ion Chromatograph was used to measure the concentration of anions (chloride, bromide, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, magnesium and calcium). Anions were measured using an IonPac AS4A anion analytical column and an eluent of 1.7 mM Na_2HCO_3/1.8 mM Na_2CO_3. Cations were measured using an IonPac CS12 4mm cation analytical column and an eluent of 31 mM H_2SO_4. Both columns were calibrated using standard Dionex solutions and a flow rate of 1 ml min^{-1} was used.

Lake water dissolved gas analysis

- Dissolved methane and carbon dioxide were extracted from the lake water using a similar headspace equilibrium method described by Hope, *et al.* (1995) and Dawson, *et al.* (2002). To the 20 ml syringes, which contained 12 ml of lake water, 6 ml of inert nitrogen gas was added and the syringes capped. They were then vigorously shaken for 2 minutes to equilibrate the headspace with the lake carbon gas concentrations. The headspace was analysed on an Ai Cambridge model 92 gas chromatograph, with a Porapak QS column at 350°C and N_2 carrier gas at a flow rate of 30 ml min^{-1}. The GC includes a flame ionization detector incorporating a CO_2 to CH_4 catalytic converter and an electron capture detector.

Treated water DOC and final water trihalomethane (THM) concentrations

The concentrations of treated water DOC (following coagulation and filtration) and final water THMs (following chlorination) at the Cefni and Mynydd Llandegai treatment works were made available by Welsh Water once every week.

2.1.1.4 Statistical analysis

Outliers within the raw data sets were removed using the method of Davis & Goldsmith (1972). *Figures 2.05-2.11 and 2.13* show data for one replicate and *figures 2.12 and 2.14-2.23* show mean monthly values for 5 replicates (n=5), with error bars indicating ± standard error of the mean. Significant differences over time (with months as the factor) were determined for all analyses using General Linear ANOVA, although evidence of seasonality was only discussed based on qualitative observations. Pearson correlation was performed to test for significant correlations between lake analyses (*tables 2.01 & 2.02*). Statistical analyses were performed using SPSS version 9.0 (SPSS, Inc.) and Graphpad InStat version 3.05 (Graphpad Software, San Diego, California, USA).

2.1.2 Results

2.1.2.1 Final drinking water analyses

The concentration of trihalomethanes at the Cefni (*figure 2.05*) showed a strong seasonal trend, with the highest concentrations recorded during the months of May, June and July and the lowest during the winter months. The average concentration of THMs at Mynydd Llandegai (*figure 2.06*) (33.6 µg l^{-1}) was lower than at the Cefni (45.5 µg l^{-1}) and did not exhibit an obvious change with season. The proportion of brominated THMs (*figure 2.07*) averaged 54% at the Cefni, compared to just 12% for Mynydd Llandegai. *Figure 2.08* shows that the DOC concentration of the water following coagulation and filtration correlated significantly with the formation of THMs at the Cefni treatment works ($p<0.001$).

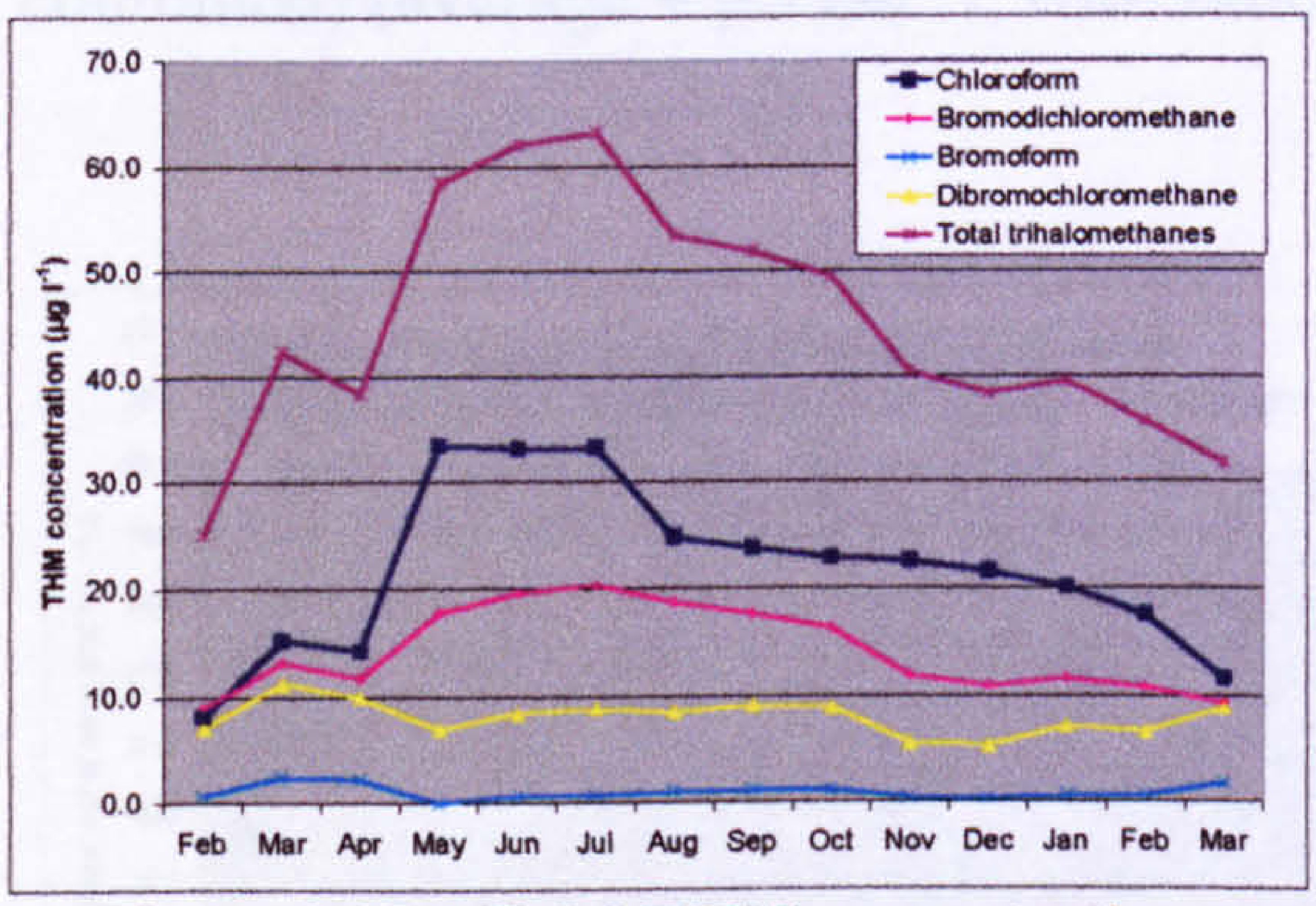

Figure 2.05 – Cefni THM concentrations

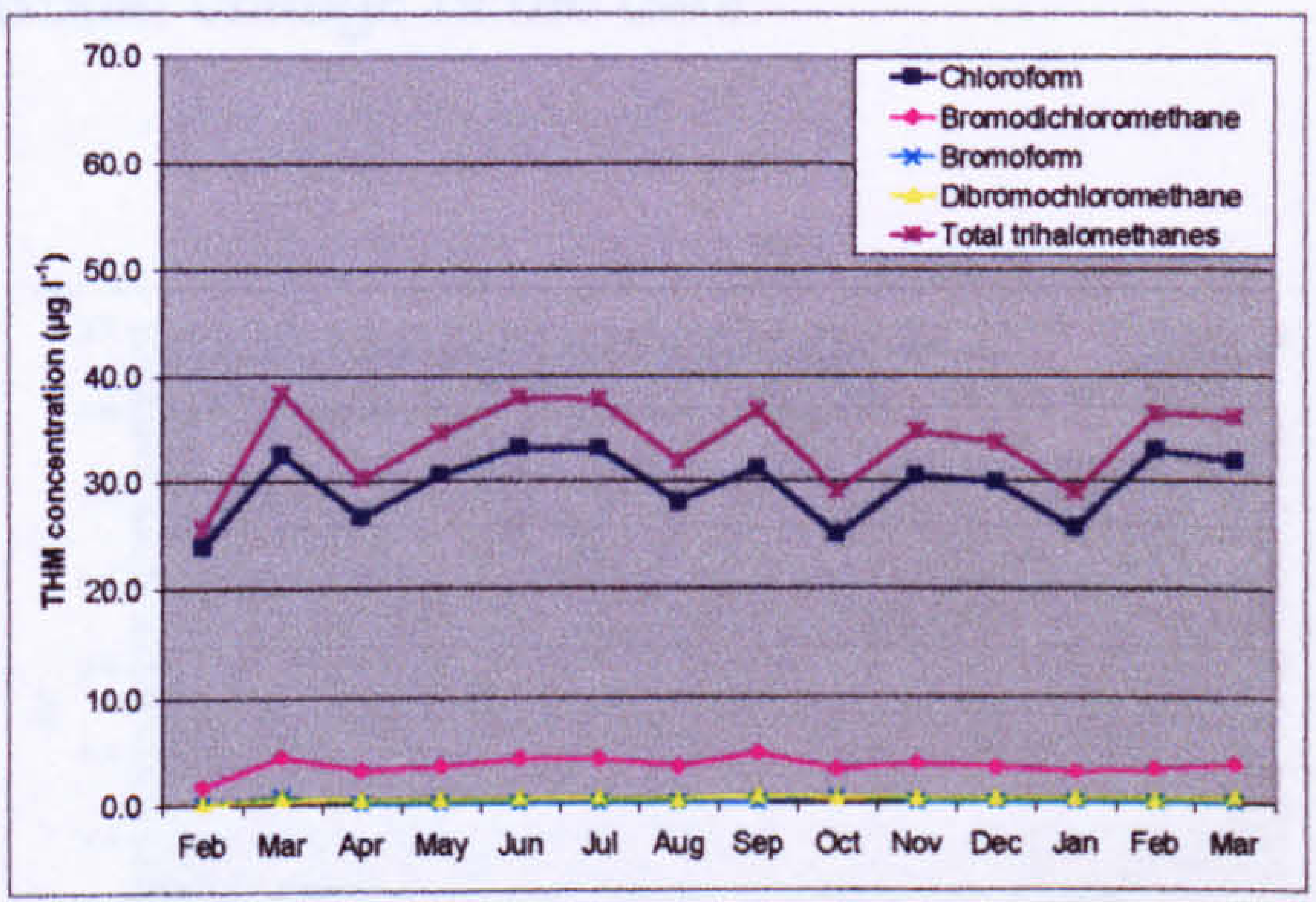

Figure 2.06 – Mynydd Llandegai THM concentrations

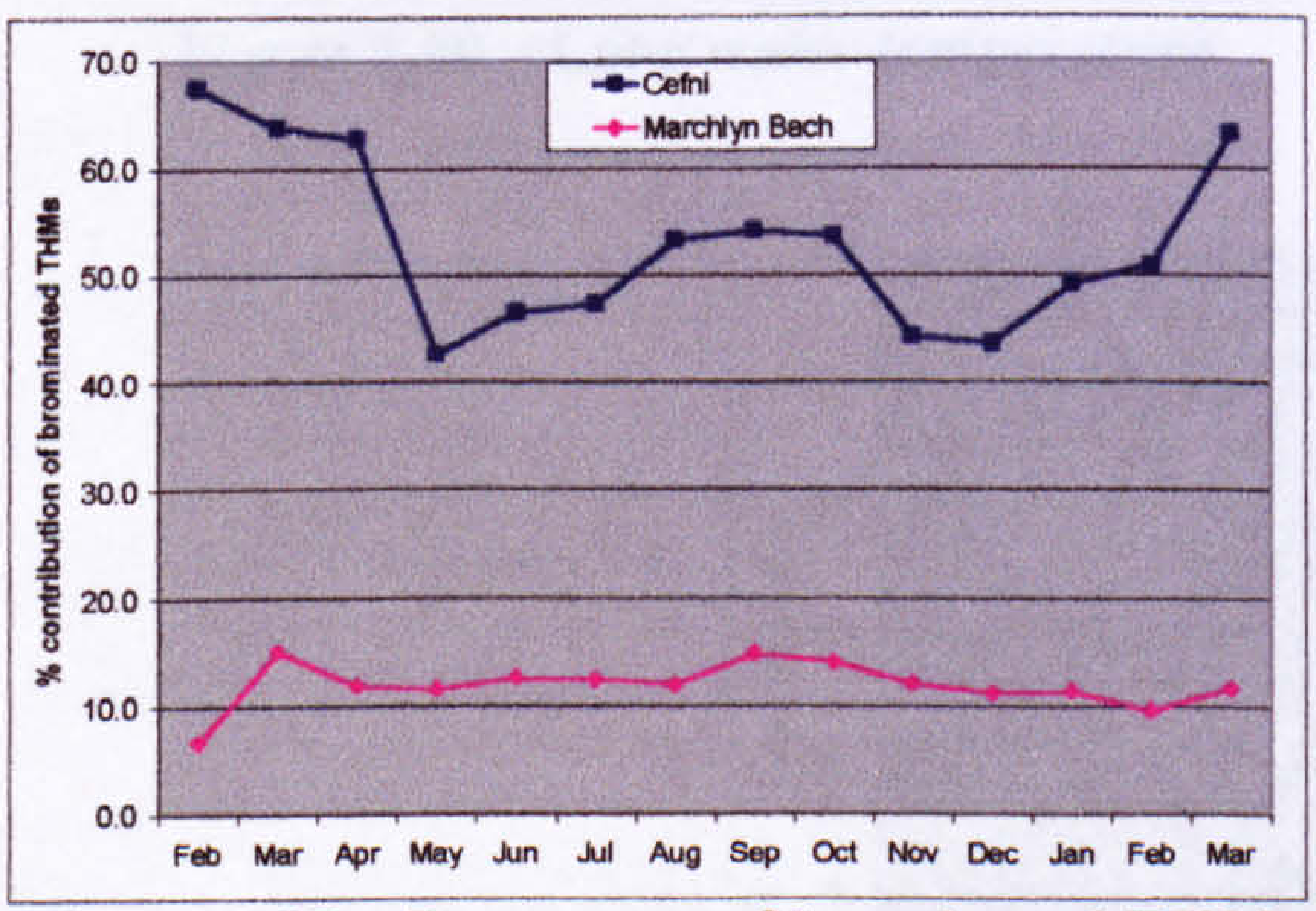

Figure 2.07 – Percentage of brominated THMs at Cefni and Mynydd Llandegai

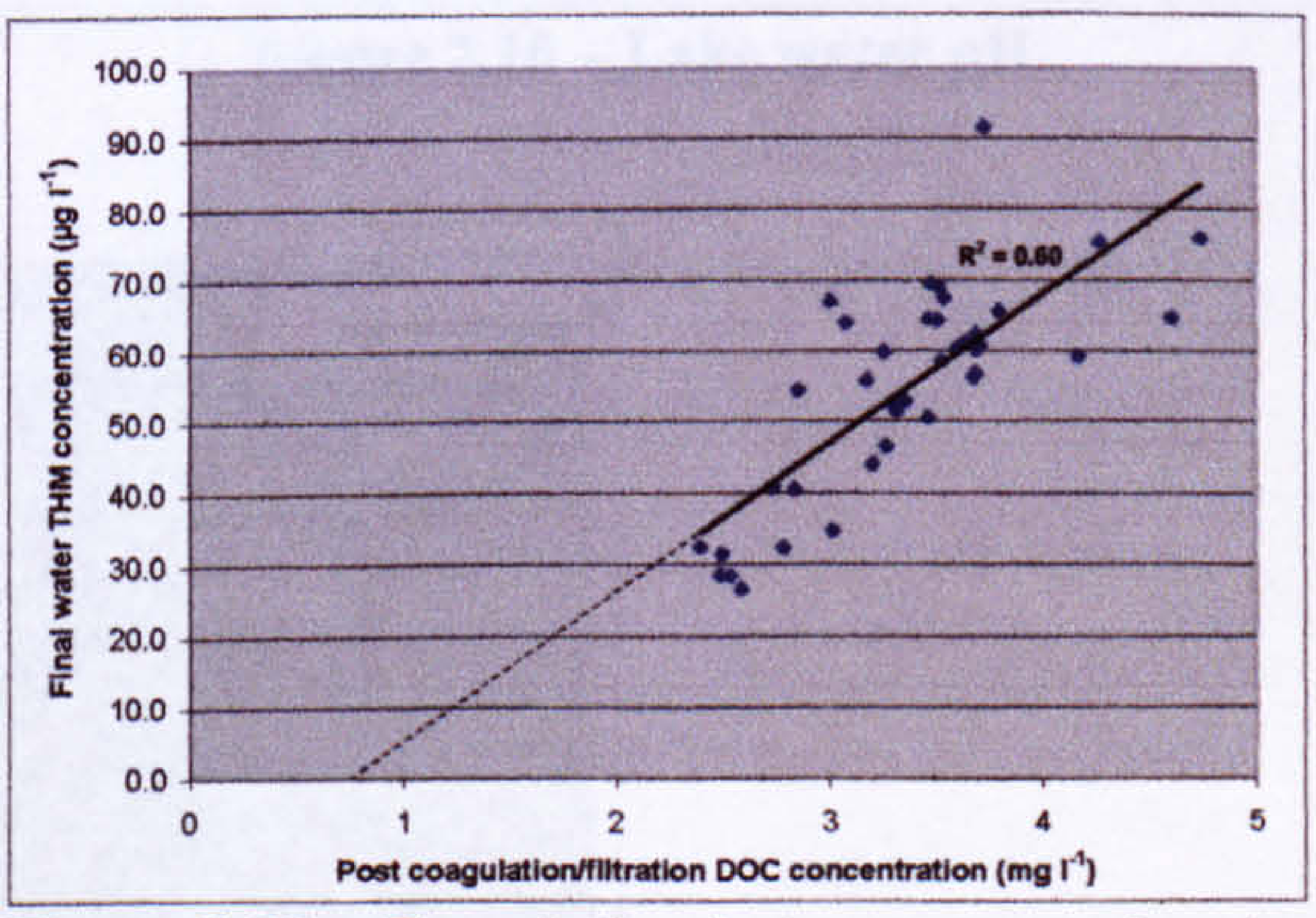

Figure 2.08 – Correlation between treated water DOC and final water THM concentrations at the Cefni (data provided by Welsh Water)

Raw water characteristics

2.1.2.2 Physicochemical analyses

Water temperature (*figure 2.09*) showed strong seasonal variation, being highest in August or September 2003 and lowest in January 2004 for all three lakes. The Cefni was usually the warmest, especially during the summer when 20°C was reached, 5°C greater than the two upland lakes. The water temperature at Marchlyn Bach varied similarly to the Llugwy, but during the summer months was 3-4°C warmer.

Lake water pH (*figure 2.10*) was consistently greater at the Cefni, averaging 7.1, with little seasonal variation. The average pH of the Llugwy (5.1) was slightly lower than Marchlyn Bach (5.3), with little seasonal change for either.

Lake water conductivity (*figure 2.11*) was approximately 29-fold higher at the Cefni (average 245 $\mu S\ cm^{-1}$) than at Mynydd Llandegai (Marchlyn Bach and Llugwy combined) (average 9 $\mu S\ cm^{-1}$), with little seasonal change in the data.

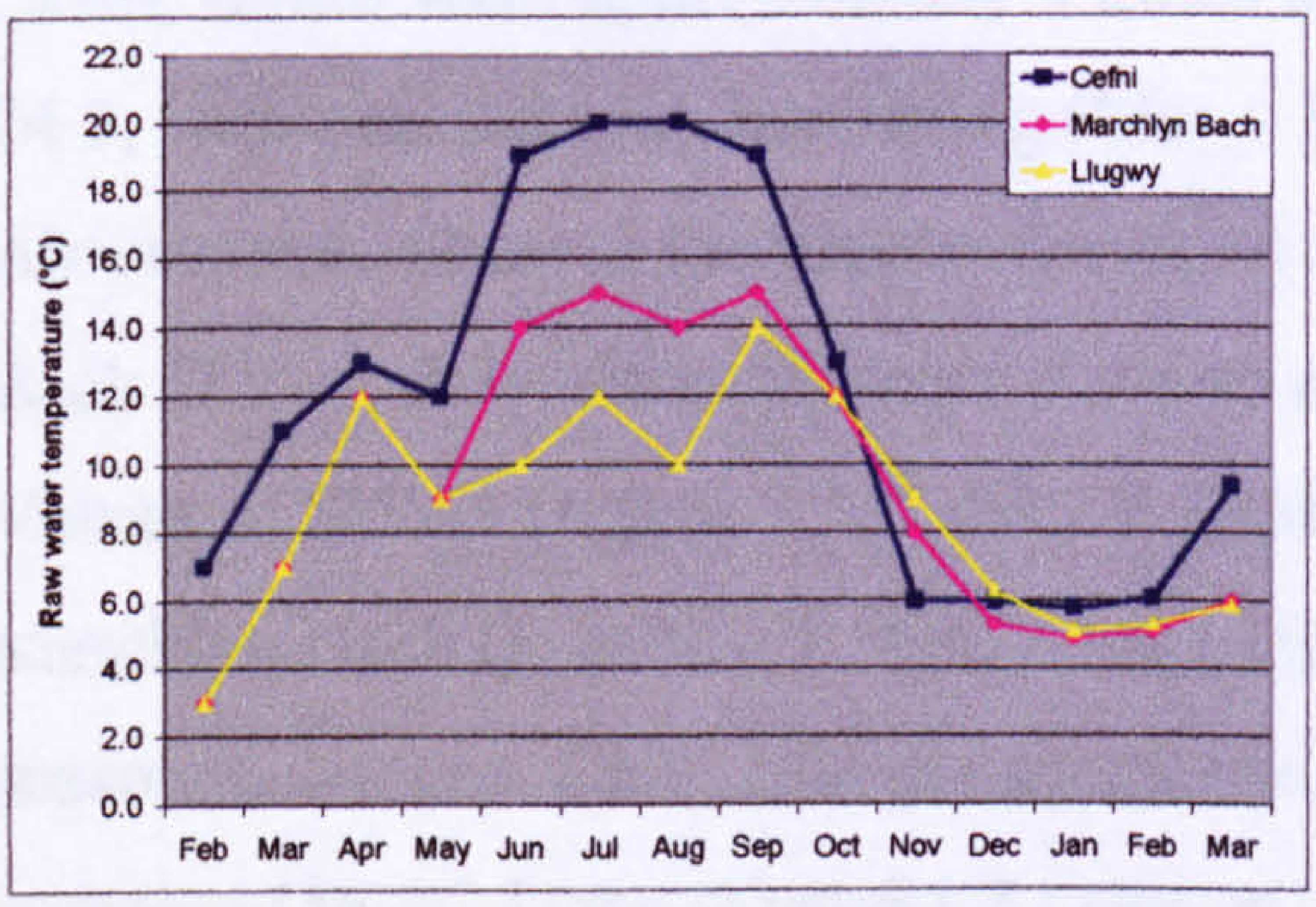

Figure 2.09 – Lake water temperature

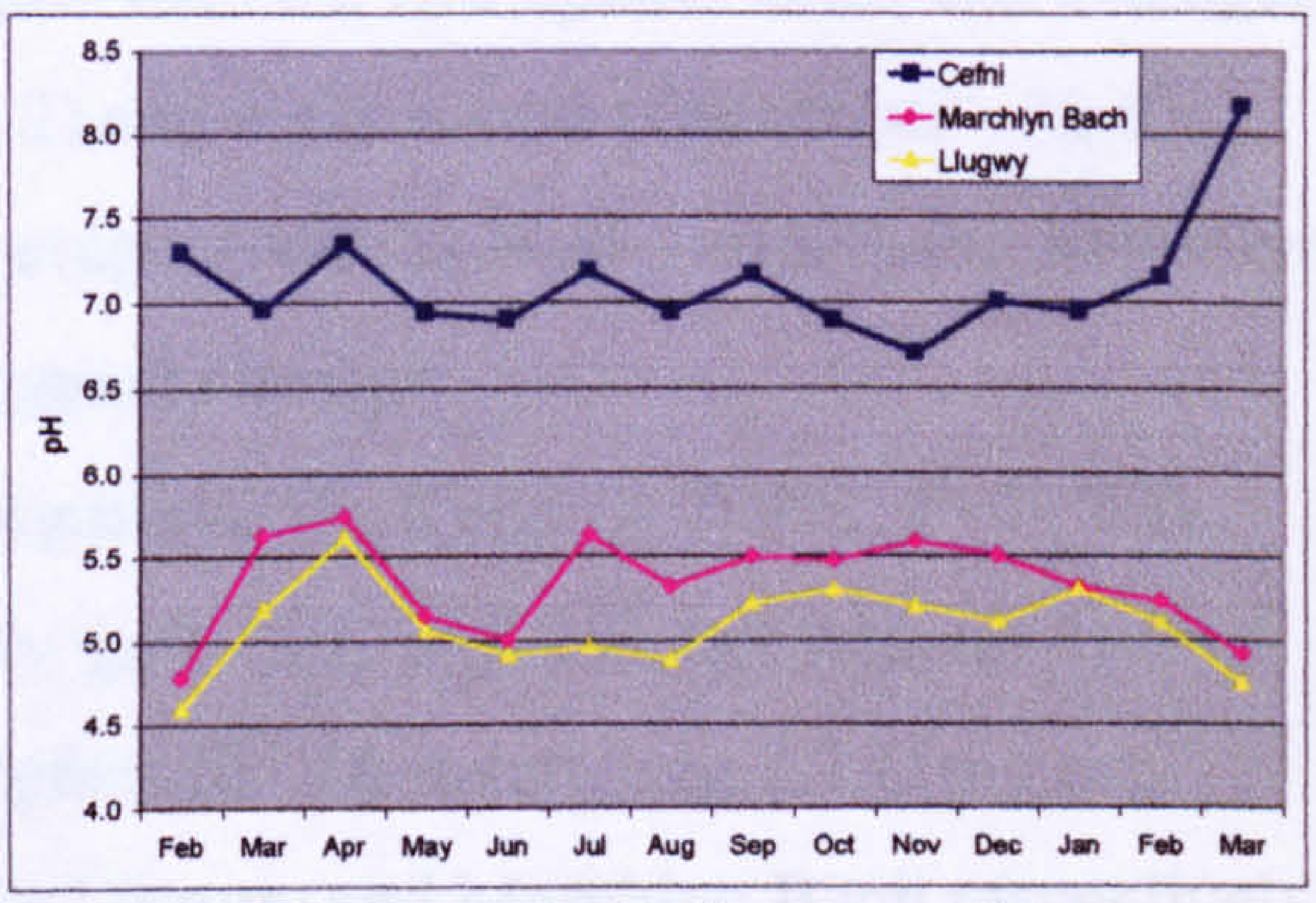

Figure 2.10 – Lake water pH

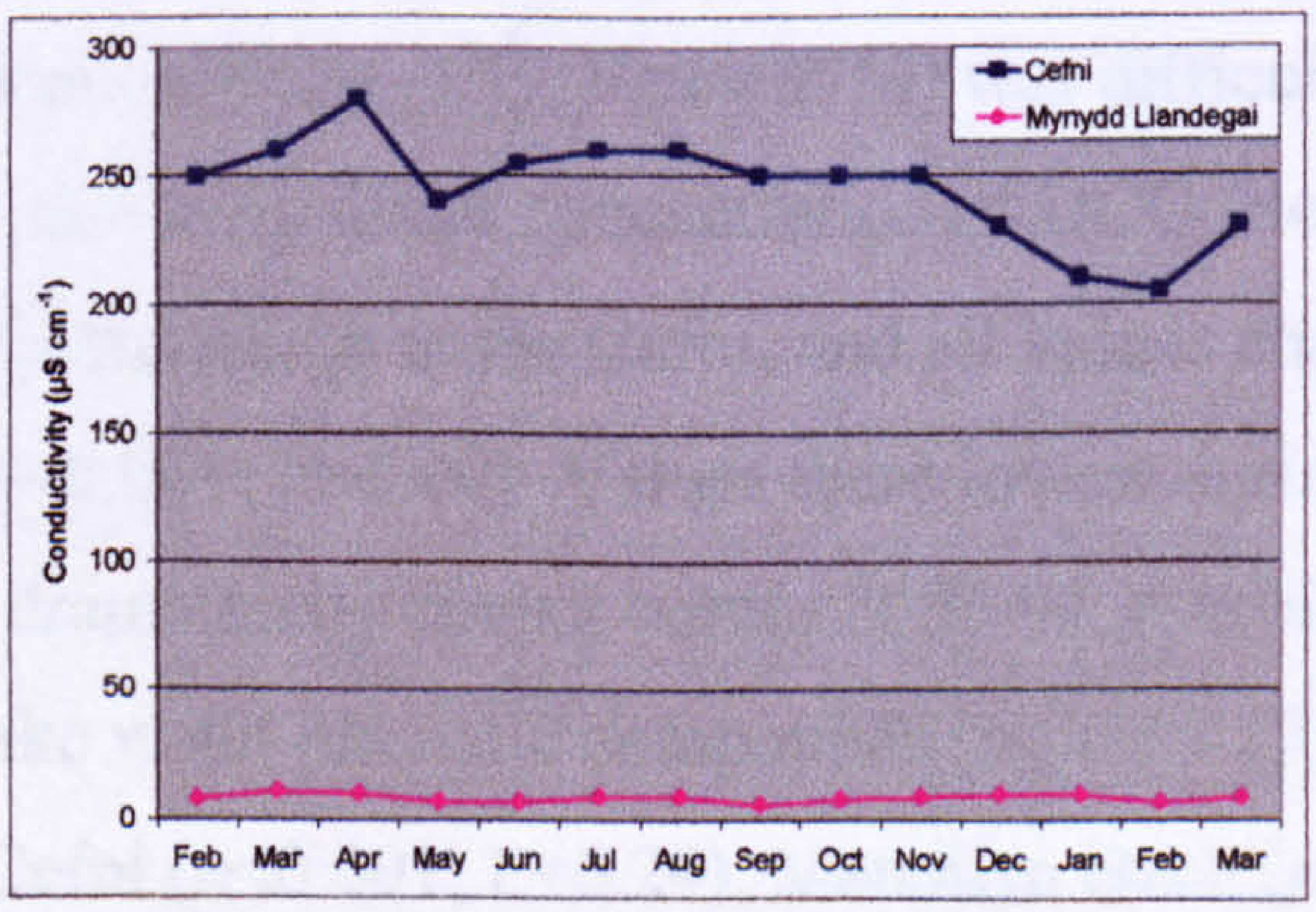

Figure 2.11 – Lake water conductivity

2.1.2.3 Dissolved organic carbon analyses

The concentration of DOC (*figure 2.12*) varied significantly over time for all three lakes; Cefni (p<0.001; F=7.52), Marchlyn Bach (p<0.001; F=3.62) and Llugwy (p<0.05; F=2.21), although there was only minor visual evidence of seasonal changes in the data set. The concentration of DOC at the Cefni averaged 9.3 mg l^{-1}, with the highest value in August 2003 (12.3 mg l^{-1}) and the lowest in February 2003 (6.7 mg l^{-1}). DOC concentrations seemed consistently high during late autumn and winter 2003-04, when an average of almost 10 mg l^{-1} was recorded from November-February. DOC was higher at the Llugwy than the Marchlyn Bach during 12 out of 14 months of the study and averaged 3.2 mg l^{-1}, with 2.8 mg l^{-1} for Marchlyn Bach. There was little effect of season at either lake. *Figure 2.13* shows that the raw water DOC concentration of Cefni reservoir correlated significantly with the treated water concentration at the treatment works (p<0.001).

Values of UV-254 absorbance (*figure 2.14*) varied for all lakes; Cefni (p<0.001; F=281.02), Marchlyn Bach (p<0.001; F=5.02) and Llugwy (p<0.05; F=6.41). For the Cefni, values were approximately 4 times higher than the two upland lakes and averaged 24.3, with peaks during late spring (May - 30.2) and early winter (December - 32.8). Absorbance values at the Llugwy were, on average, twice as high compared to Marchlyn Bach (7.2 vs. 3.3), although there were no seasonal changes.

Values of SUVA (*figure 2.15*) also changed significantly; Cefni (p<0.001; F=10.62), Marchlyn Bach (p<0.001; F=6.45) and Llugwy (p<0.001; F=8.26), but with no distinct seasonal changes. Cefni lake possessed the highest SUVA, averaging 2.7 L(mg m), compared to 2.3 L(mg m) and 1.3 L(mg m) for Llugwy and Marchlyn Bach respectively. SUVA values for the Llugwy were consistently higher than Marchlyn Bach.

Analysis of the Humification Index (HI) (*figure 2.16*) was difficult with Marchlyn Bach and Llugwy lake water due to its dilute concentration of DOC. However, the DOC concentration was sufficiently high at the Cefni, and HI values changed significantly throughout the study (p<0.001; F=5.00). Values were lowest during the summer (e.g. August - 1.5) and rose dramatically during winter 2003-04, reaching 7.7 in December.

The concentration of lake water phenolic compounds (*figure 2.17*) varied significantly throughout the study; Cefni (p<0.001; F=6.24), Marchlyn Bach (p<0.001; F=28.87) and

Llugwy ($p<0.001$; F=6.18). Concentrations were similar between all three reservoirs, although the Cefni usually possessed higher values and had a 14-month average twice that of the two upland lakes. The concentration was greater during the autumn and winter (e.g. December – 2.8 mg l^{-1}) than spring and summer (e.g. July - 0.5 mg l^{-1}). The average phenolics concentration was almost identical between Marchlyn Bach and Llugwy (approximately 0.62 mg l^{-1}).

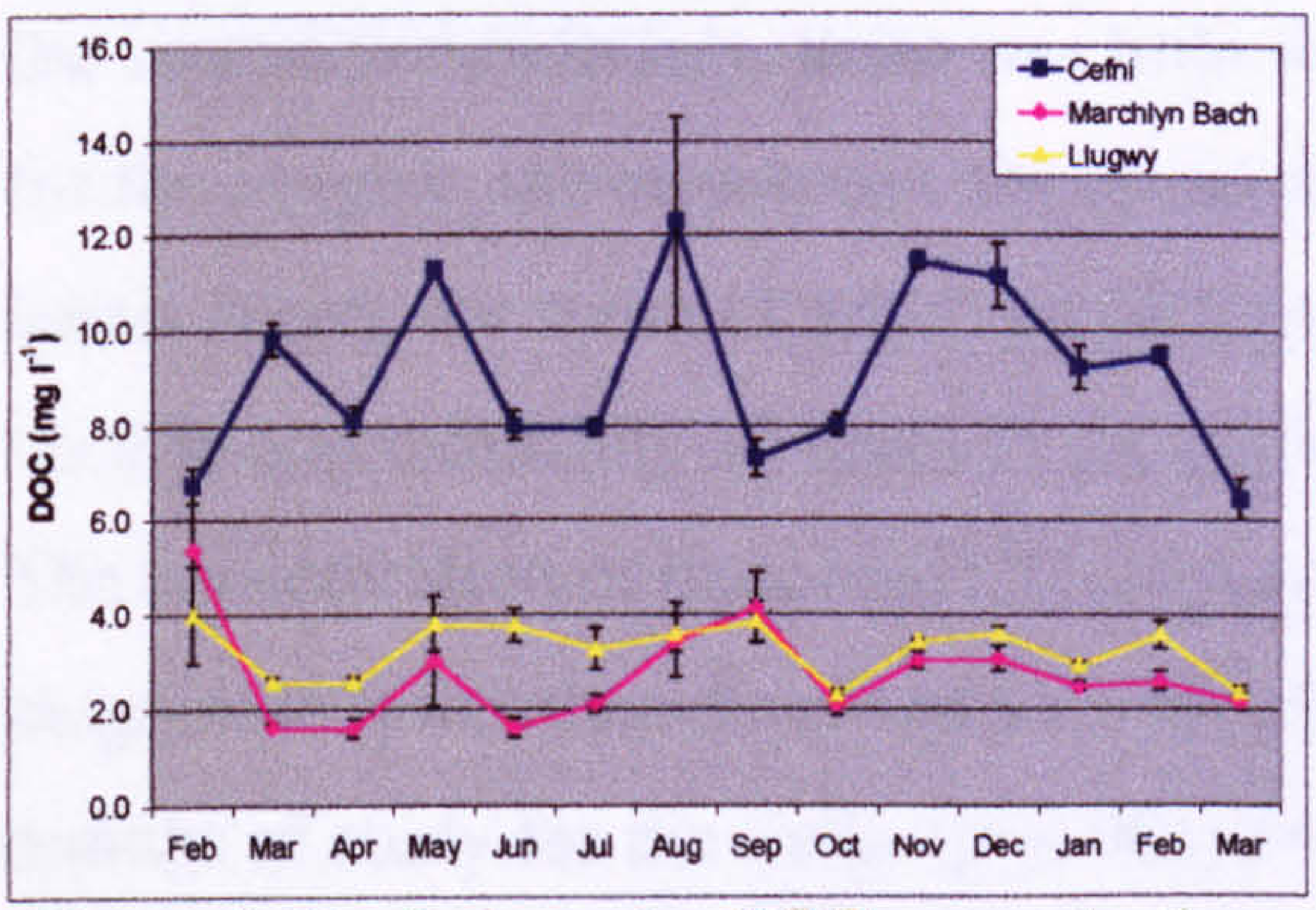

Figure 2.12 – Lake water DOC concentrations

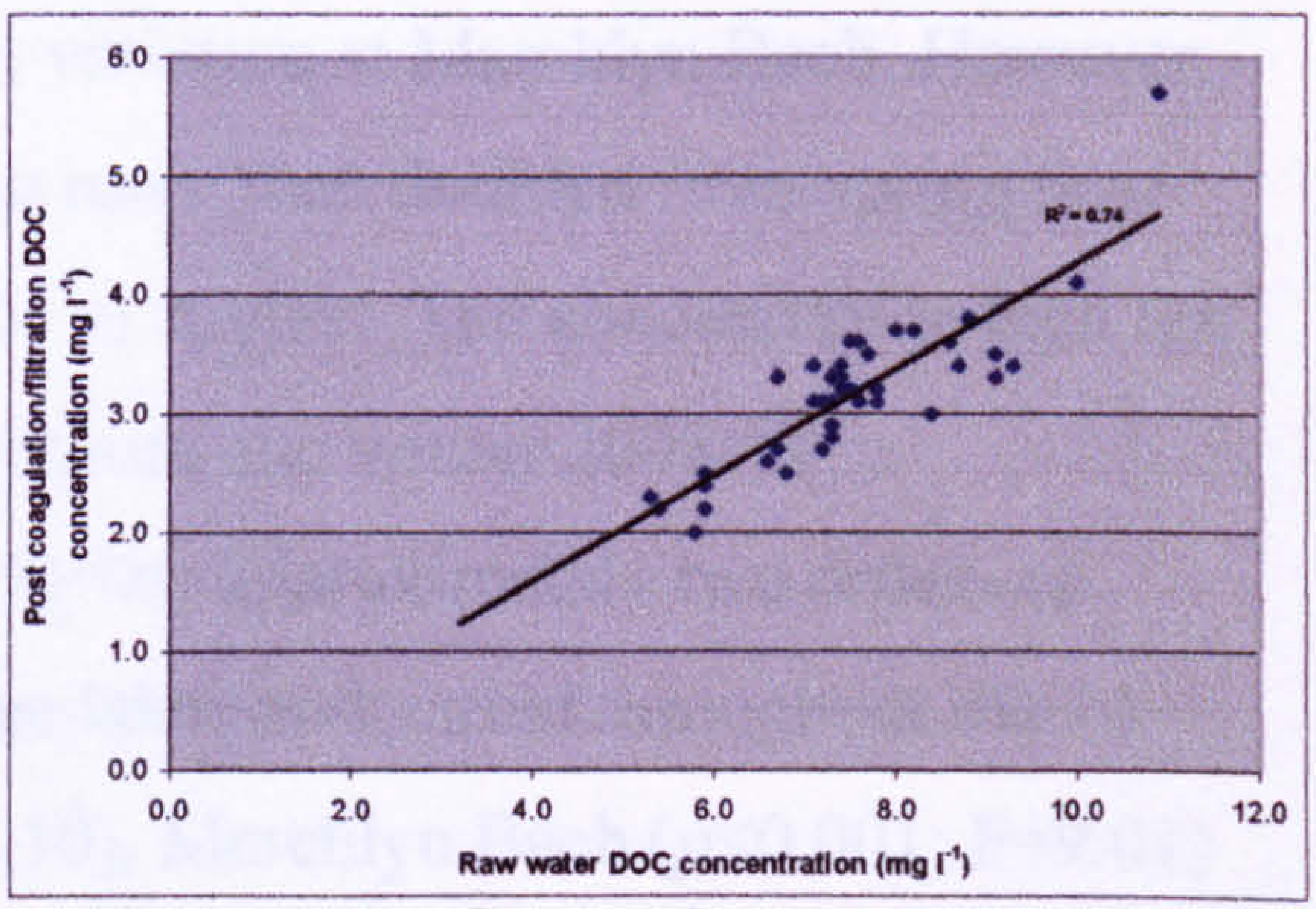

Figure 2.13 – Correlation between raw and treated water DOC concentrations at the Cefni (data provided by Welsh Water)

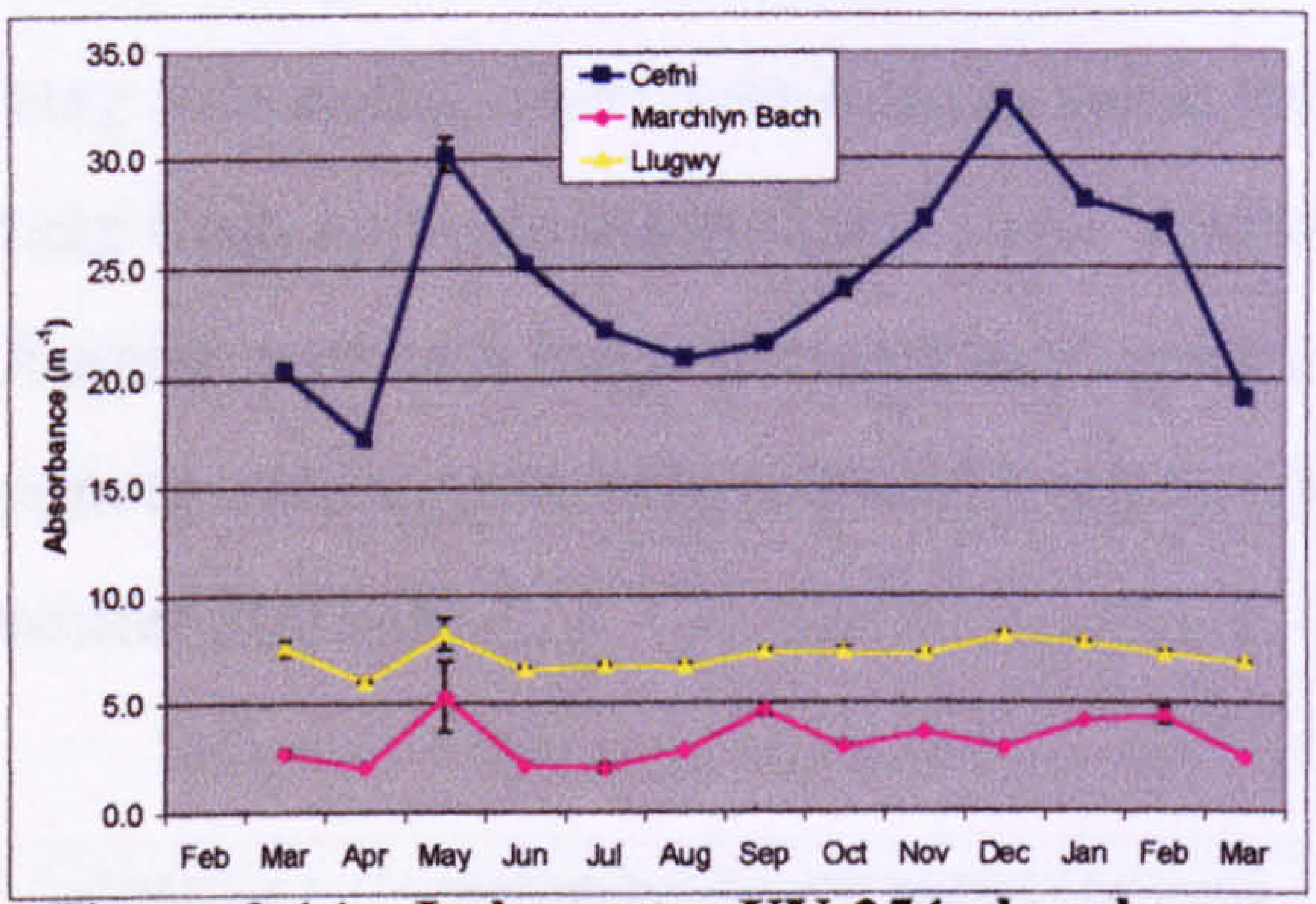

Figure 2.14 – Lake water UV-254 absorbance

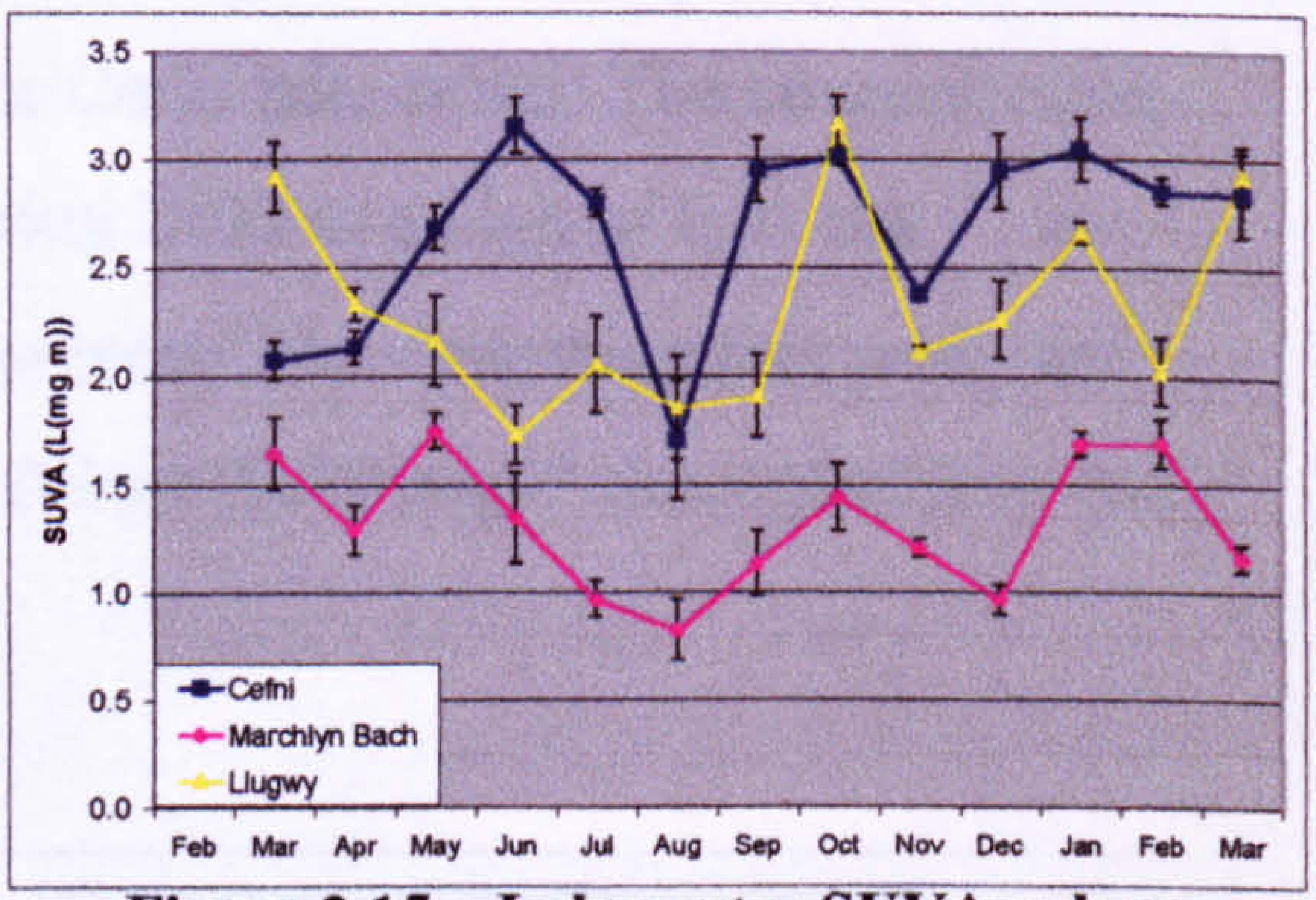

Figure 2.15 – Lake water SUVA values

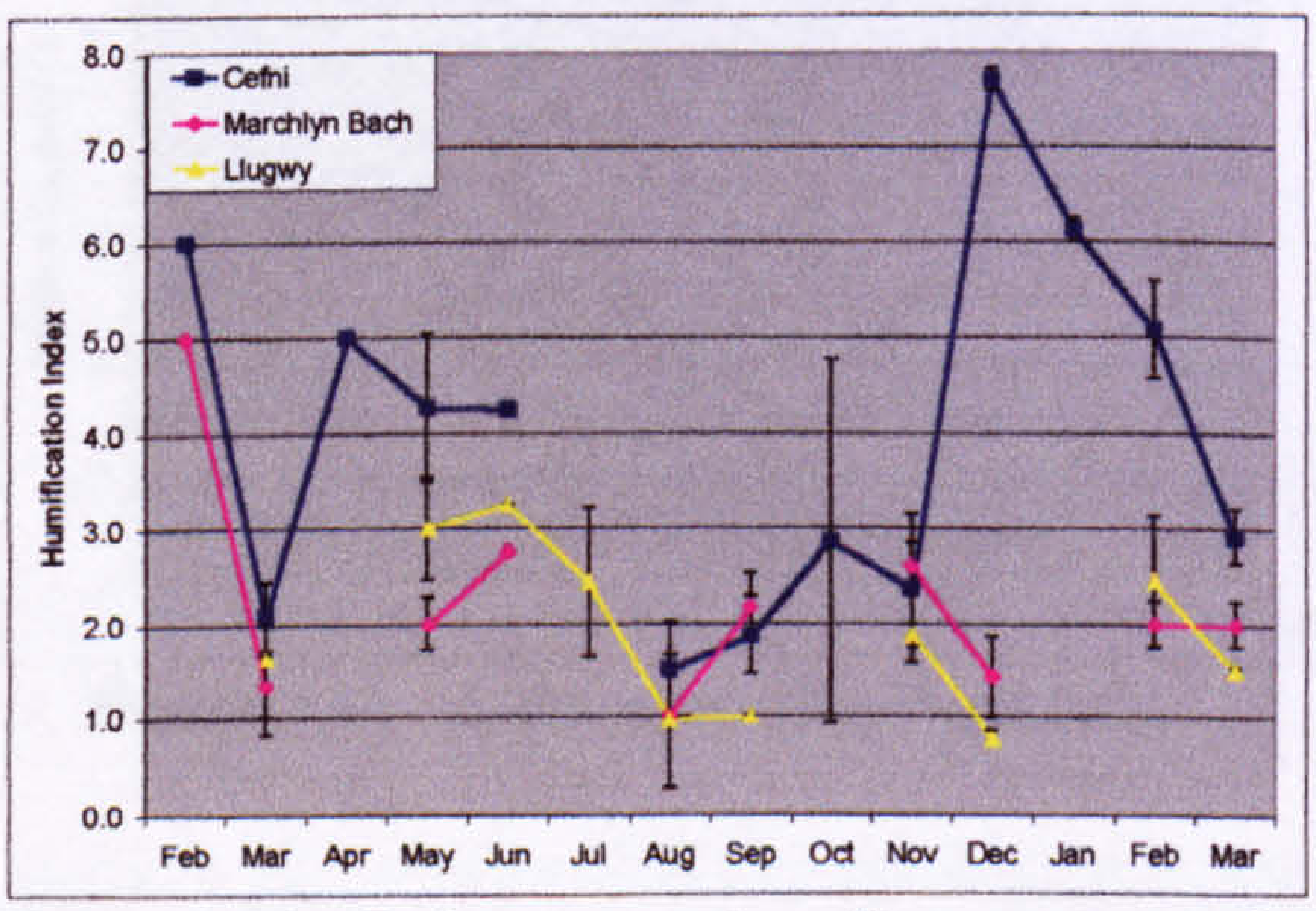

Figure 2.16 – Lake water phenolics concentrations

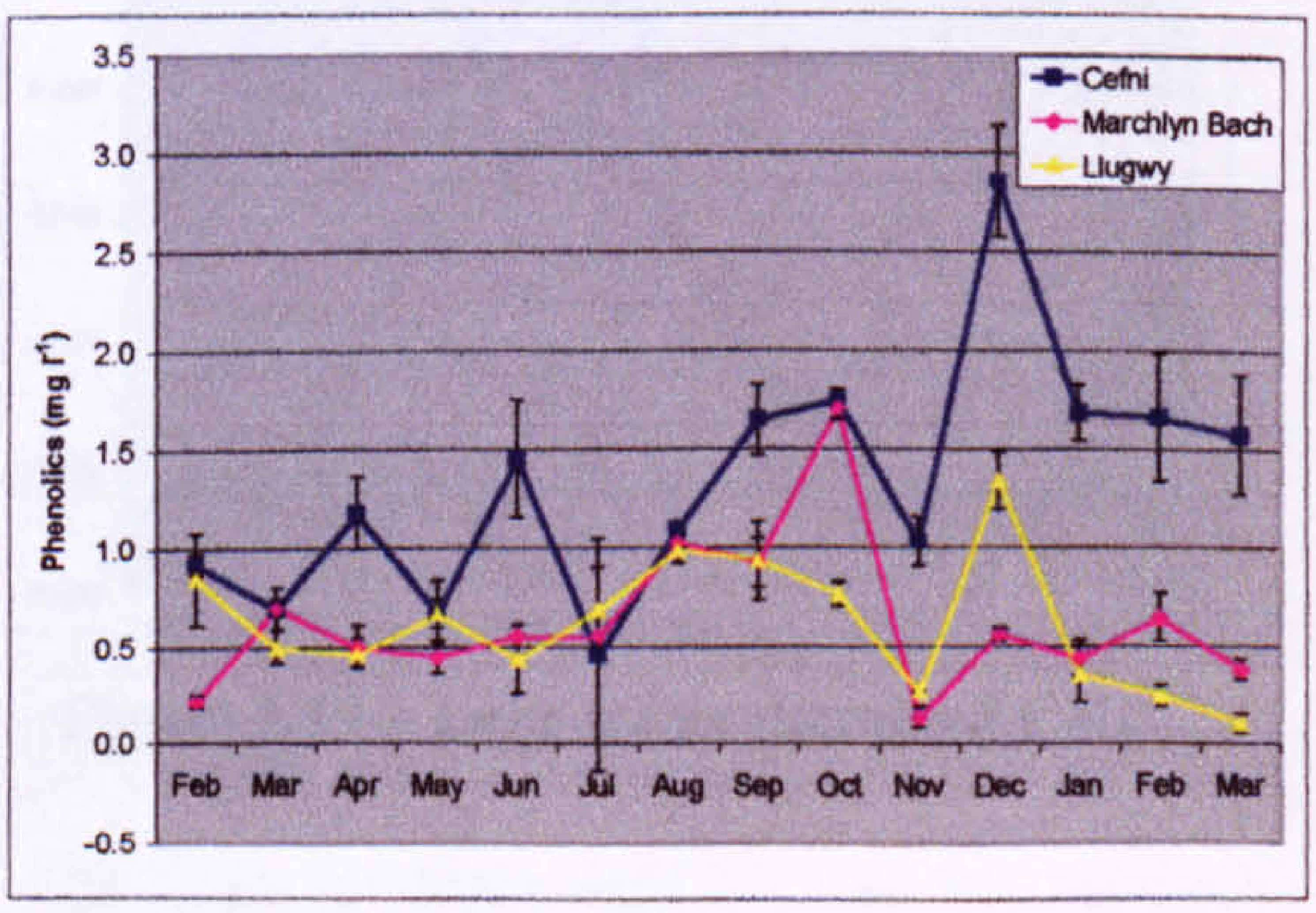

Figure 2.17 - Lake water Humification Index

2.1.2.4 Dissolved lake carbon gases

The concentration of dissolved CO_2 (*figure 2.18*) showed pronounced seasonal changes and varied significantly for all three lakes; Cefni ($p<0.001$; F=48.19), Marchlyn Bach ($p<0.001$; F=12.13) and Llugwy ($p<0.001$; F=68.66). Values at the Cefni ranged from a dip in the summertime (13.2 µmol l^{-1} in August) to an autumn/winter time peak (65.7 µmol l^{-1} in December 2003). Despite their general similarity, concentrations of dissolved CO_2 were much different between Marchlyn Bach and Llugwy. Apart from a large dip in the concentration in July, there was little season variation at Marchlyn Bach. However, for the Llugwy, the dissolved CO_2 concentration more than doubled from spring time levels during the summer, reaching 64.9 µmol l^{-1} in August. The concentration then fell back to approximately 30 µmol l^{-1} during late autumn and winter 2003-04.

The concentration of dissolved CH_4 (*figure 2.19*) was approximately two orders of magnitude lower than dissolved CO_2 for all three lakes and varied throughout the 14 months of study for the Cefni ($p<0.001$; F=206.10), Marchlyn Bach ($p<0.001$; F=9.01) and Llugwy ($p<0.001$; F=4.69). Whilst the concentration of dissolved CH_4 was consistently low for Marchlyn Bach and Llugwy (average of 0.001 mg l^{-1}), and did not vary seasonally, there was a remarkable trend for Cefni lake water. The concentration rose from approximately 0.002 mg l^{-1} during spring 2003 to a high of 0.01 mg l^{-1} in August, part of a large summer time peak in dissolved CH_4. Following this peak, the concentration gradually reduced back to a level below 0.002 mg l^{-1} during autumn and winter 2003-04.

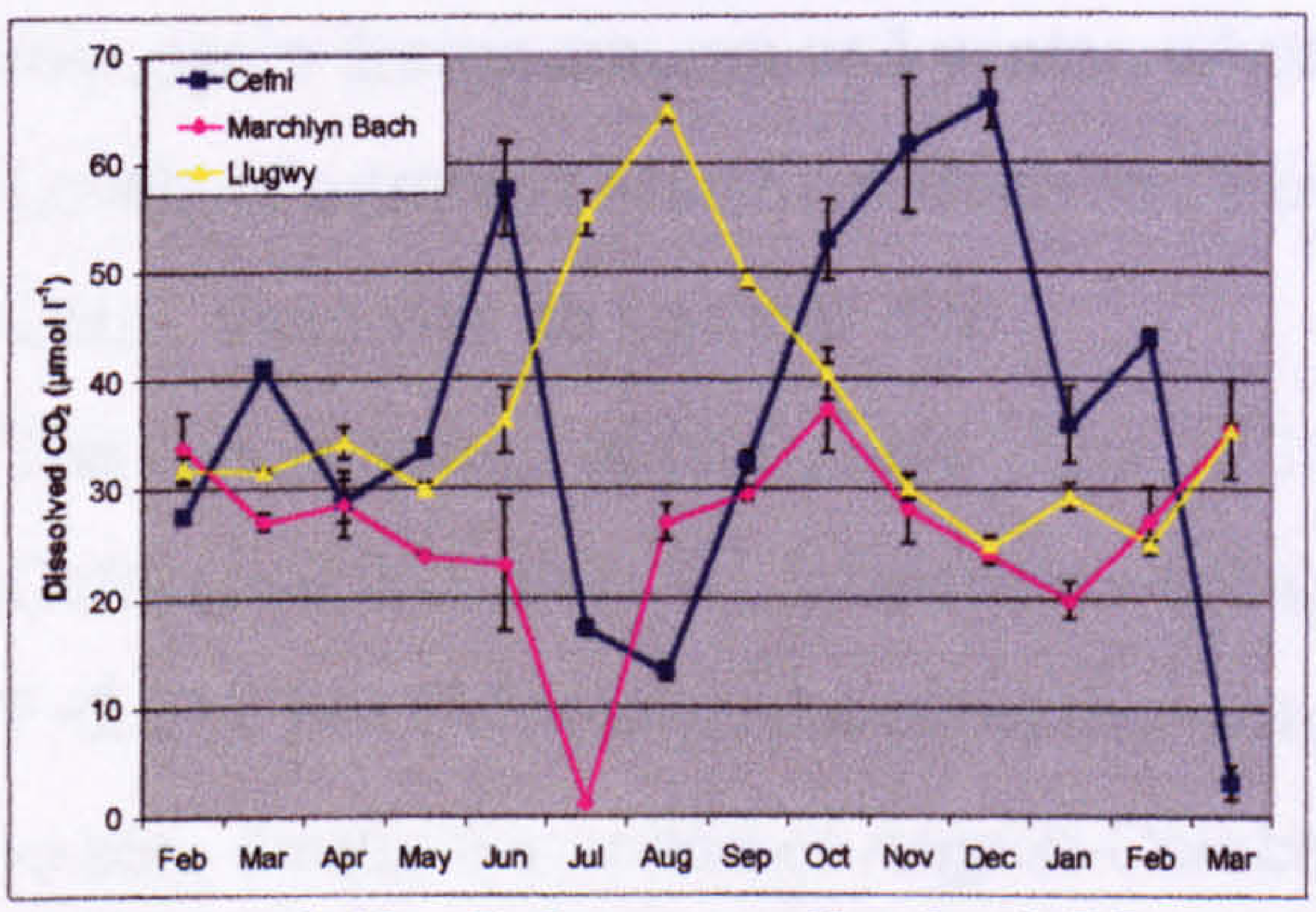

Figure 2.18 – Lake water dissolved CO_2

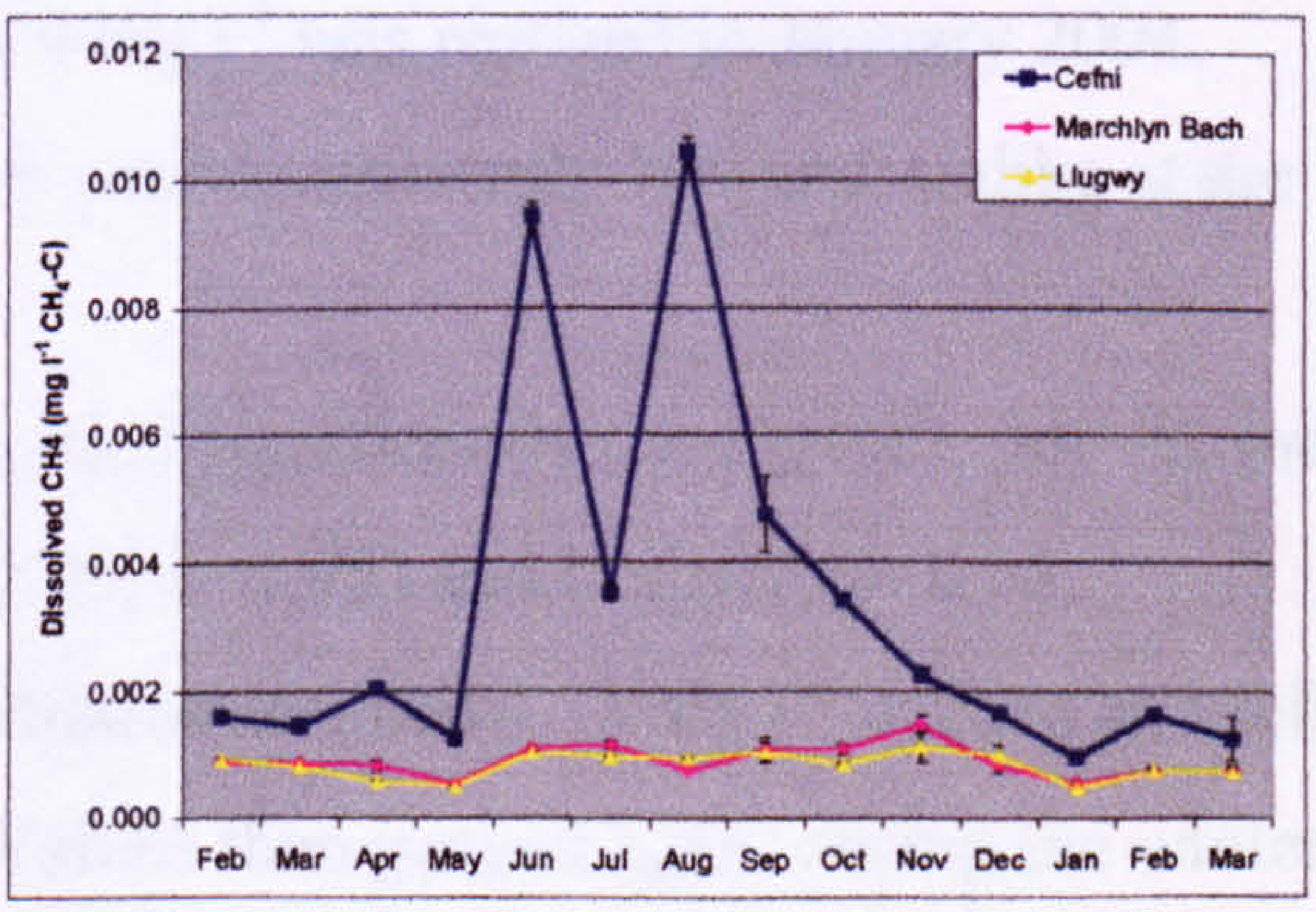

Figure 2.19 – Lake water dissolved CH_4

2.1.2.5 Ion analyses

The concentration of chloride (*figure 2.20*) in the lake water varied significantly throughout the year for the Cefni ($p<0.001$; F=239.72), Marchlyn Bach ($p<0.001$; F=270.53) and Llugwy lakes ($p<0.001$; F=207.36). Apart from a dip in the concentration in September for all three lakes, there was little seasonal variation in the data. The concentration of chloride was almost identical between Marchlyn Bach and Llugwy for all the months of sampling and, on average, approximately 5 times lower than values for the Cefni. This difference in the average concentration between Cefni and the two upland lakes, and the general trend in the data set, was matched by other ions, such as sulphate, sodium, potassium, magnesium and calcium (data not shown).

Bromide concentrations (*figure 2.21*) varied significantly throughout the period of study for the Cefni ($p<0.001$; F=5.04), Marchlyn Bach ($p<0.001$; F=5.34) and Llugwy ($p<0.01$; F=2.63). Values were low for the two upland lakes, averaging 0.017 and 0.011 mg l^{-1} for Marchlyn Bach and Llugwy respectively and there was no effect of season. Concentrations in Cefni lake were consistently higher and averaged 0.16 mg l^{-1}, ten times the level of the two upland lakes. During autumn and winter bromide concentrations peaked, reaching 0.4 mg l^{-1} in December and February, compared to an average of approximately 0.1 mg l^{-1} during spring and summer.

The concentration of nitrate (*figure 2.22*) varied throughout the course of the study for the Cefni ($p<0.001$; F=457.84), Marchlyn Bach ($p<0.001$; F=101.00) and Llugwy ($p<0.001$; F=30.58). The seasonal change for the Cefni was remarkable, with the concentration falling from 9.3 mg l^{-1} in February 2003 to almost zero in August. It then rose again during autumn and winter, when 11.9 mg l^{-1} was reached in January 2004. Levels of nitrate at Marchlyn Bach and Llugwy were consistently low and, unlike at the Cefni, there was no summer dip.

The concentration of phosphate (*figure 2.23*) varied significantly during the study for the Cefni ($p<0.001$; F=6.54), Marchlyn Bach ($p<0.05$; F=1.95) and Llugwy ($p<0.05$; F=2.21). For Cefni lake, the phosphate concentration reached 0.13 mg l^{-1} in June and fell to zero during the period of August-October. Values then peaked again during the winter of 2003-2004. Concentrations were, on average, lower for the two upland lakes but the number of months when none was detected was less than for the Cefni.

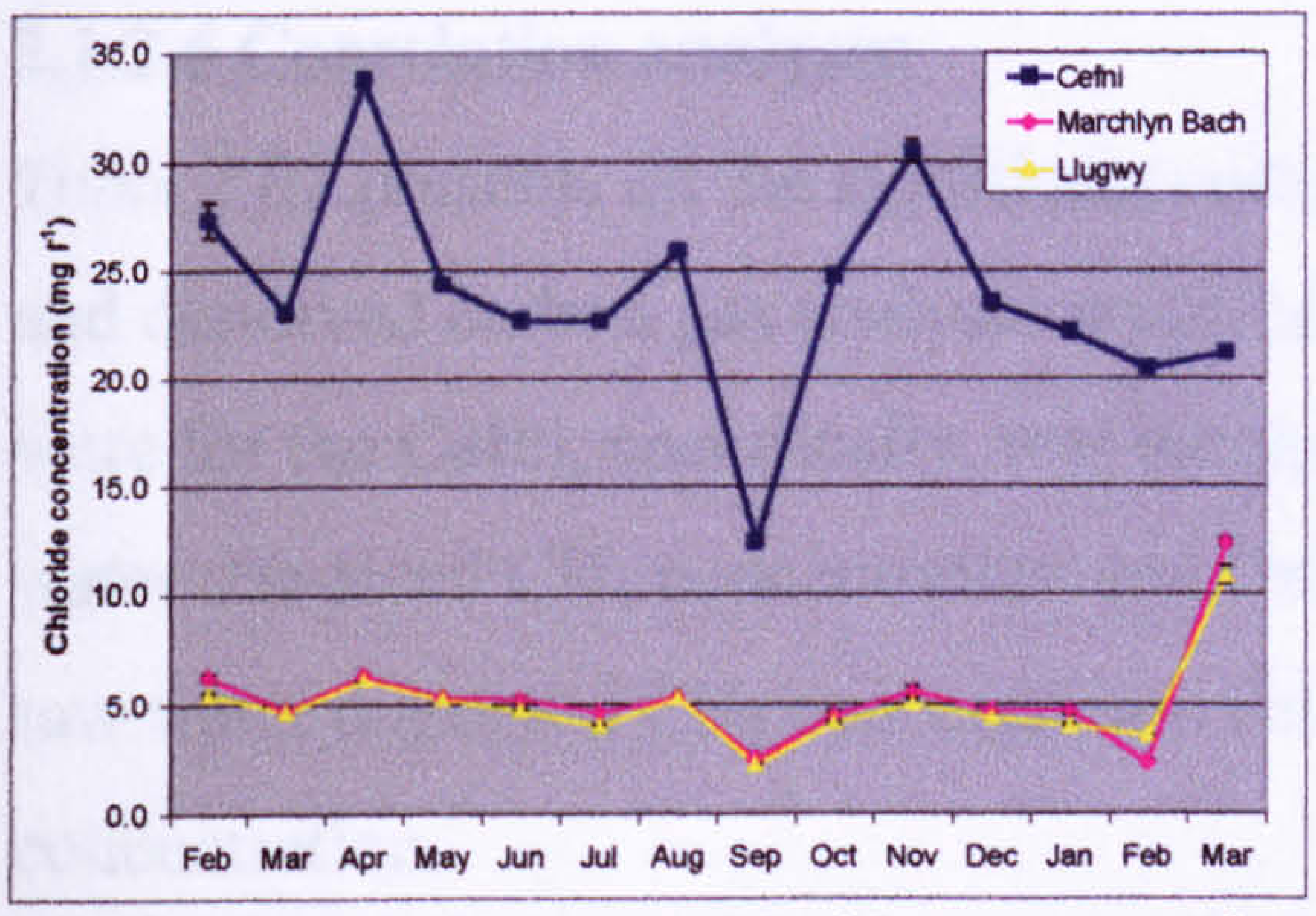

Figure 2.20 – Lake water chloride concentrations

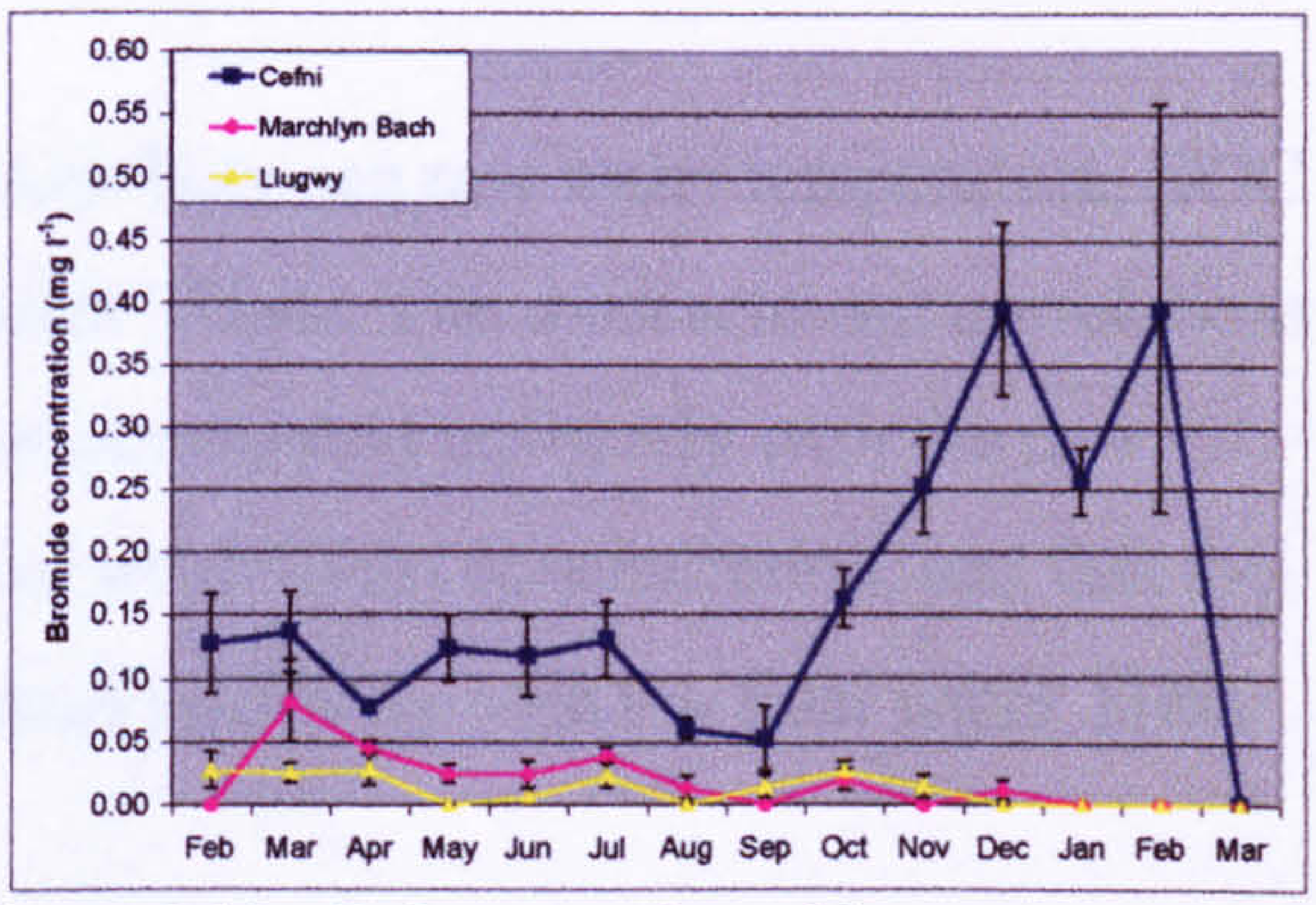

Figure 2.21 – Lake water bromide concentrations

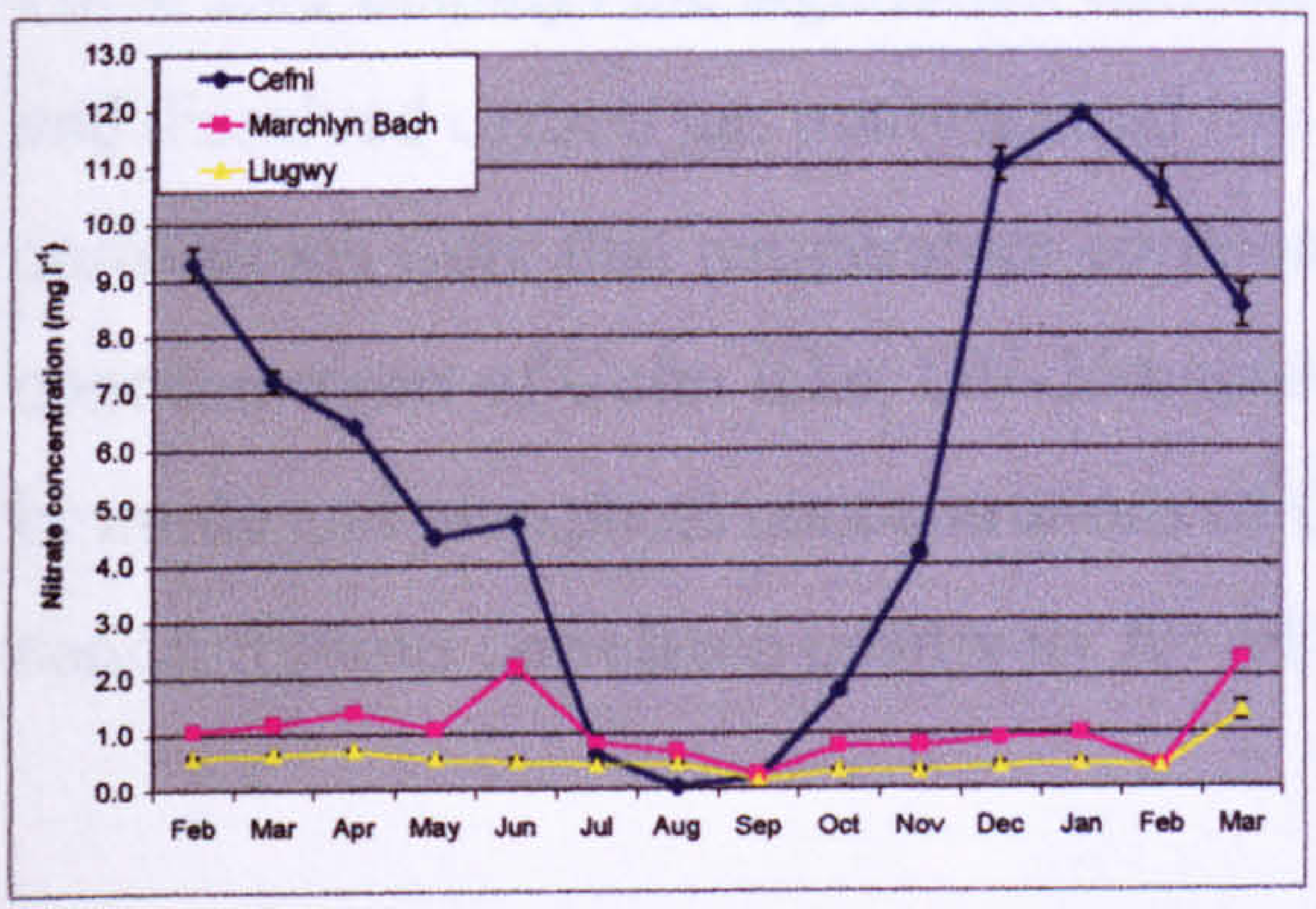

Figure 2.22 – Lake water nitrate concentrations

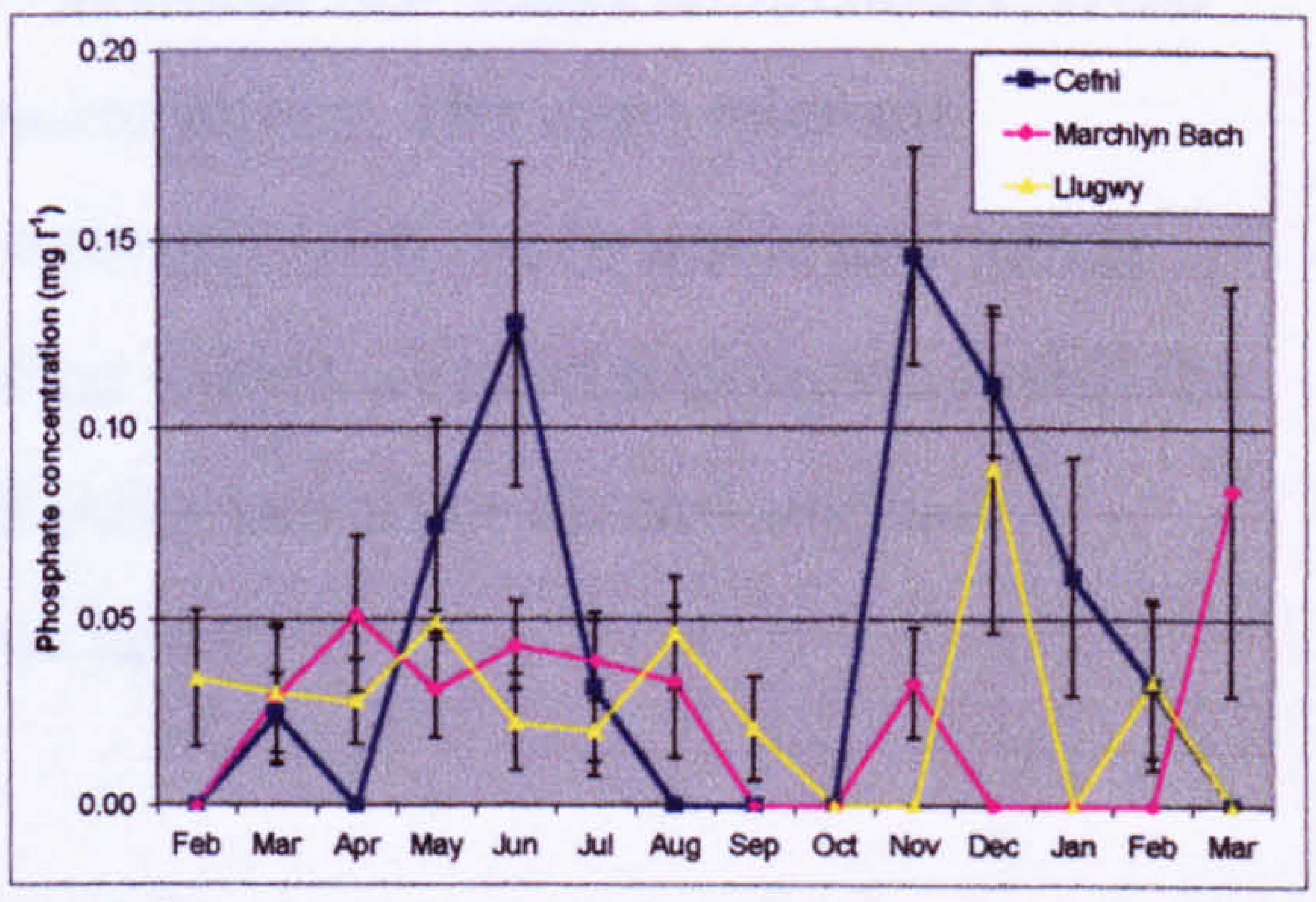

Figure 2.23 – Lake water phosphate concentrations

2.1.2.6 Correlation analyses

Table 2.01 presents all the significant correlations between raw water temperature, DOC and dissolved carbon gas analyses and final water THMs. The most relevant correlations were for the Cefni; specifically, that temperature correlated positively with the raw water dissolved CH_4 concentration and the final water THM concentration, and that the raw water dissolved CH_4 concentration correlated positively with the final water THM concentration.

Table 2.02 displays the significant correlations between raw water temperature, DOC and dissolved carbon gas analyses and ion concentrations. The most relevant correlations were that temperature correlated inversely with the bromide and nitrate concentrations of Cefni lake, UV-254 absorbance values correlated positively with the bromide and phosphate concentrations of Cefni lake and chloride and sulphate concentrations correlated positively for all three lakes.

	DOC	UV-254	SUVA	Phenolics	Dissolved CO_2	Dissolved CH_4	THMs
Temperature					L (0.60*)	C (0.76**)	C (0.80**)
DOC		M (0.65*)	L (-0.89***)				
UV-254					C (0.64*)		
SUVA							
Phenolics							
Dissolved CO_2							
Dissolved CH_4							C (0.59*)

Table 2.01 – Pearson correlation between lake temperature, dissolved organic and gaseous carbon analyses and final water THMs (n=70) (* p<0.05, ** p<0.01, *** p<0.001) (C-Cefni, M-Marchlyn Bach, L-Llugwy)

	Cl^-	Br^-	NO_3^-	PO_4^{2-}	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
Temperature		C (-0.63*)	C (-0.84***)						
DOC		M (-0.57*)							
UV-254		C (0.76**)		C (0.71**)					
CO_2		C (0.68**)		C (0.71**)			L (0.57**)		
CH_4			C (-0.61*)			M (-0.66*) L (-0.70**)			
Cl^-			M (0.77**) L (0.95***)	M (0.77**)	C (0.70*) M (0.88***) L (0.90***)				
Br^-			C (0.55*)						
NO_3^-				M (0.74**)	M (0.77**) L (0.96***)				
PO_4^{2-}					M (0.75**)				
Na^+							M (0.75**)	C (0.75**)	
Mg^{2+}									M (0.93***) L (0.89***)

Table 2.02 – Pearson correlation between lake temperature, dissolved organic and gaseous carbon analyses and ion concentrations (n=70) (* p<0.05, ** p<0.01, *** p<0.001) (C-Cefni, M-Marchlyn Bach, L-Llugwy)

2.1.3 Discussion

The concentration of DOC within Cefni lake was approximately 3 times higher than in Marchlyn Bach and Llugwy (*figure 2.12*), with the concentration in the two upland lakes being lower than is typically recorded in lakes with bog-dominated catchments (Wetzel, 2001). Despite the employment of four carbon-removal stages at the Cefni (coagulation, DAF cells and 2 rapid gravity filters), the concentration of DOC in the water following these treatment processes correlated positively with the raw water concentration (*figure 2.13*) and the formation of trihalomethanes following chlorination (*figure 2.08*), indicating that the higher the concentration of organic carbon in the lake itself, the more carbon that passes through the treatment works and is available to form THMs. However, not all DOC present in the treated water is chlorinated as shown by the trendline in *figure 2.08*. When traced back towards the axes, it does not go through the origin, indicating that there are organic compounds that pass through the treatment works that do not form THMs. Due to the greater amount of organic matter in Cefni lake, significantly higher concentrations of THMs were formed at this treatment works compared to at Mynydd Llandegai ($p<0.001$). Concentrations of THMs in the final water from the Cefni also correlated positively with the raw water temperature, as the chlorination of organic matter is a temperature-dependent reaction (Knocke, *et al.* 1986) The concentration of bromide in lake water is crucial to the formation and speciation of THMs (Myllykangas, *et al.* 2003) and, at the Cefni, bromide values were consistently higher than at Marchlyn Bach and Llugwy and exhibited a large rise during autumn and winter. The principle source of bromide in freshwater lakes is from the sea (Duce, *et al.* 1965; Weinberg, *et al.* 2002) and concentrations are known to be much higher in lowland surface waters than upland environments (Whitaker, *et al.* 2003). The rise during autumn and winter at the Cefni most likely reflects the much higher rate of precipitation, highly marine-influenced, which is typical of these seasons. However, bromide levels in the Cefni also correlated with the lake's UV-254 absorbance, so it may be that the source of terrestrial carbon to the Cefni was also a significant source of bromide. The observation that bromide correlated with colour for 135 Swedish lakes and rivers by Asplund & Grimvall (1991) suggests this could be true. At the Marchlyn Bach and Llugwy, concentrations of lake water bromide were much lower, due to the reduced marine influence. The dominant trihalomethane compound recorded at each treatment works each month was always chloroform, an observation recorded in many other studies (Vansteenderen, *et al.* 1991, Jack, *et al.* 2002; Whitaker, *et al.* 2003). However, the influence of bromide ions in the raw water on THM formation is demonstrated well in this study. The formation of the three bromine-containing THMs was, on average, 5-fold greater at the Cefni compared to Mynydd Llandegai, where approximately 88% of THMs occurred as chloroform. However, the higher bromide concentrations in Cefni lake water during autumn and winter did not increase the proportion of brominated THMs in the final water.

It was unusual that there was a lack of a strong seasonal effect on DOC concentrations in the three reservoirs, as values in northern, temperate freshwater lakes are usually highest in late summer and autumn and lowest in late winter and early spring due to the allochthonous input of carbon (Goslan, *et al.* 2002; Whitaker, *et al.* 2003) (see also *figure A01*, appendix A1). The lack of seasonality in this data set may have been caused, in part, by the exceptionally dry weather experienced during 2003 (*figure A02*, appendix A2). Within Wales, 10 out of the 12 months experienced below average rainfall, whilst August, September and October were exceptionally dry, averaging just 23, 49 and 65% of normal rainfall levels respectively. The year as a whole was the 7th driest out of the last 90 (*figure A03*, appendix A2). Schindler, *et al.* (1997) and Hongve, *et al.* (2004) found that during drier years there any generally lower inputs of DOC to lakes than during wetter years and therefore lower DOC concentrations in lakes. Whilst low rainfall will be the main reason for this reduced input, it could also be caused by a lower water-table in peatland soils stimulating organic matter decomposition to an end-point of CO_2, reducing the formation of DOC intermediates (Scott, *et al.* 1998). Whitaker, *et al.* (2003) recorded reduced THM levels during 1995 for 3 water companies in the UK and related this to a severe drought and the subsequent reduction in runoff and input of terrestrial organic matter to reservoirs. In this study, trihalomethane levels throughout the year were much lower than during previous years, suggesting a similar trend.

Whilst there was a lack of any seasonality in the DOC data set, the same cannot be said of UV-254 absorbance values at the Cefni. As would be expected, the highest value was recorded in December as part of a late autumn/winter peak. This is probably due to a combination of heavy rainfall (*figure A04*, appendix A2), high inputs of leaf litter and decaying plant matter, and low temperatures; the latter reduces the degradation and utilisation of carbon compounds by heterotrophic organisms. The large rise in the Humification Index for the Cefni in December reflects this input of 'new', non-decomposed carbon. There was also a large rise in UV-254 in May; there are two possible explanations for this. During spring, the lake water temperature increases, as does the supply of nutrients to the lake due to the use of agricultural fertilisers within the catchment (refer to section 3.1). This combination of factors may stimulate algal production, increasing the size of the autochthonous carbon pool. However, algal DOC is generally low molecular weight and has a weak absorbance at 254 nm (Nguyen, *et al.* 2005). Also, the peak does not continue throughout the summer, when algal productivity is typically greatest. A more likely explanation is, again, high rainfall and the resulting increase in the input of terrestrial organic carbon. This process has been demonstrated previously for wetland dominated catchments (Hinton, *et al.* 1997; Inamdar & Mitchell, 2006). In 2003, May had six days of rainfall above 10 mm, as many as November and December and the same number as the whole of the February to April period (*figure A04*, appendix A2); it therefore seems likely that rainfall is the reason for the high UV-254 value in May. The summertime dip in UV-254 absorbance values probably reflects the increased presence of low molecular weight, UV-transparent, algal DOC.

One of the most interesting findings to emerge from the survey was that, at the Cefni, raw water dissolved methane correlated positively and significantly with the formation of trihalomethanes. The concentration of CH_4 was highest in summer at the Cefni, with low concentrations recorded throughout the sampling period for Marchlyn Bach and Llugwy. Dissolved methane can originate from several sources; input from inflowing streams, diffusion from littoral and lake-bed sediments and the internal anaerobic decomposition of organic matter through methanogenesis (Smith, *et al.* 1993; Jones & Mulholland, 1998; Murase, *et al.* 2003). Temperature correlated with dissolved methane concentrations at the Cefni throughout the study and is one of the most important factors controlling these processes (Murase, *et al.* 2003). The high concentrations of CH_4 recorded during the summer at the Cefni most likely represent the greater breakdown of organic matter by methanogenesis within the lake water and its sediments during these warm months, as has been observed in previous studies (Utsumi, *et al.* 1998; Casper, *et al.* 2000; Kuznetzova & Dzyuban, 2005). Despite the strong structural and compositional similarity between methane (CH_4) and THMs (e.g. $CHCl_3$), there appear to have been no studies to elucidate whether dissolved methane in lake water could form THMs during drinking water treatment.

Another interesting observation was the contrast in nitrate, phosphate and dissolved CO_2 concentrations between Cefni lake and the two upland lakes, and the implications this has for algal productivity and the quality of the lakes from a drinking water perspective. Cefni lake is known to experience algal blooms during the summer months (Malthus & George, 1997) and the graph of nitrate concentrations (*figure 2.22*) provides evidence of a eutrophic state of the Cefni during summer. During winter time, the concentration was typically 10-12 mg l^{-1}; this was much higher than the two upland lakes and is probably due to the influence of groundwater and leaching of nitrate from agricultural land within the catchment of the Cefni. During the summer month of August, the concentration fell to a level close to zero; a typical finding of freshwater eutrophic lakes (Stewart, *et al.* 1982) and is due to the biological demand outstripping the supply as the lake water temperature increases (Heathwaite, 1993). The fact that nitrate concentrations fell to this level during the summer suggests that the lake is nitrate-limited for algal growth; according to Wetzel (2001), this is a sign of extreme eutrophy. From a drinking water quality perspective, the nitrate concentration is well below the limit of 50 mg l^{-1} defined by the World Health Organisation (WHO, 2004), but the formation of algal blooms represents a serious treatment cost to the water industry and reduces the efficiency of organic matter removal.

Phosphate is the other key nutrient for algal growth; concentrations in the two upland lakes were within the range typical of uncontaminated surface lakes, whilst the Cefni sometimes exceeded this range (Wetzel, 2001). Phosphate is the most limiting nutrient in freshwaters to algal growth (Schindler, *et al.* 1977) and concentrations fell to below the limit of detection from Autumn to October, probably due to stimulated algal productivity and phosphate uptake in the warmer temperatures. The peak in June may have been caused by increased fertiliser use and it's leaching into the reservoir (see section 3.1), whilst the peak in November was most likely due to both an increase in rainfall (Moss, *et al.* 2005), the cooling of the lake and a reduction in algal productivity. Evidence of algal productivity can also be found in the trends for raw water dissolved CO_2 concentrations. Dissolved CO_2 within a lake can be derived from a number of sources; externally, from inflowing terrestrial surface runoff (esp. from organic rich soils) and groundwater, both of which are often supersaturated in CO_2, and internally, from the photo-oxidation and biological decomposition of organic matter and carbon-cycling processes within the lake bed sediment (Granéli, *et al.* 1996; Hope, *et al.* 2001; Dawson, *et al.* 2002; Jonsson, *et al.* 2003; Worrall & Lancaster, 2005). CO_2 can be removed from the lake water by algal photosynthesis and exchange with the atmosphere. A survey of 1835 freshwater lakes worldwide revealed that 87% were supersaturated with CO_2 with respect to the atmosphere, indicating that lakes play an important role in the global carbon cycle (Cole, *et al.* 1994). At the Cefni, the dissolved CO_2 concentration was lowest in summer; this is likely to be due to the uptake of CO_2 during photosynthesis by algae and other aquatic plants, namely species of macrophytes, phytoplankton and periphyton (Berman-Frank, *et al.* 1995; Barth & Veizer, 1999). During the winter time, the dissolved CO_2 concentration increased; this can probably be attributed to the lowering of the water temperature and the subsequent reduction in photosynthetic activity within the lake, and also the increased terrestrially-derived input of DOC and dissolved CO_2 which is typical of this time of the year.

The trends of dissolved CO_2 at the two upland bog lakes were very different to the Cefni because, at both locations, the algal and microbial productivity is assumed to be low and the lake is surrounded by peat soils. In soils of this type, the vertical movement of carbon gases up through the soil profile and into the atmosphere is restricted and much slower than the lateral movement into the draining freshwaters (Hope, *et al.* 2001). Consequently, the greatest concentrations of dissolved CO_2 have been recorded in streams that flow through the more peaty areas of their catchment (Dawson, *et al.* 2002).

At the Lluwgy, there was a strong seasonal change in dissolved CO_2, with much greater values measured during summer than winter. This may be related to processes occurring within the catchment soils.

In comparing the two upland lakes, it is interesting that the values of raw water SUVA, which reflect the aromaticity of the DOC and generally correlate with molecular weight and THM formation (Uyguner, *et al.* 2004), were almost twice as high for the Llugwy compared to Marchlyn Bach. Coupled with the higher DOC values recorded, this implies that the lake water from the Llugwy not only possesses more organic matter, but that it is more aromatic and consequently more likely to form THMs. At the Mynydd Llandegai treatment works, the raw water is drawn from both of these upland lakes with a usual blend ratio of 3:1 in favour of the Llugwy (pers. comm. Quinn, 2003). A change in this ratio to allow for more water to be drawn from Marchlyn Bach would lead to a reduction in THMs formed at this treatment works. Currently, final water THM levels at this water treatment works are well below the limit of 100 $\mu g\ l^{-1}$, but if legislation becomes stricter as it has done recently in the USA (USEPA, 1998) and if freshwater DOC concentrations increase with climate change, as has been predicted (Freeman, *et al.* 2001*b*; Worrall & Burt, 2005), this could be a cheap, viable option to reducing the final water concentration of THMs.

The use of measurements of UV absorbance at 254nm for characterising the organic matter content of lake and river water is common (Karanfil, *et al.* 2002); both in addition to (Nguyen, *et al.* 2002; White, *et al.* 2003) and instead of DOC (Uyguner, *et al.* 2004). However, in this study a positive correlation between DOC and UV-254 was only recorded for Marchlyn Bach lake ($R^2=0.65, p<0.05$). Although including the data from all three lakes together resulted in a very strong and highly significant positive correlation ($R^2=0.89, p<0.01$), the lack of correlations for individual lakes implies that measurements of UV-254 absorbance as a proxy for DOC concentrations cannot always be relied upon. This can be attributed to UV-254 measurements not detecting some low molecular weight carbon compounds that do not absorb UV light.

2.2 Catchment soil characteristics

2.2.1 Materials and methods

2.2.1.1 Field sites

The lowland Cefni and upland Marchlyn Bach and Llugwy lakes differed in the types of soils immediately bordering the lake. Following the artificial creation of Cefni lake, a marshy wetland (*figure 2.24*) formed at the edges of the lake on all sides, especially where trees were also felled. Beyond this wetland, the catchment soils are mostly farmland. Marchlyn Bach and Llugwy are surrounded completely by acidic, ombrotrophic, carbon-rich *Sphagnum*-dominated bog soils (*figures 2.25 & 2.26*).

Figure 2.24 – Marshy wetland soils surrounding Cefni lake

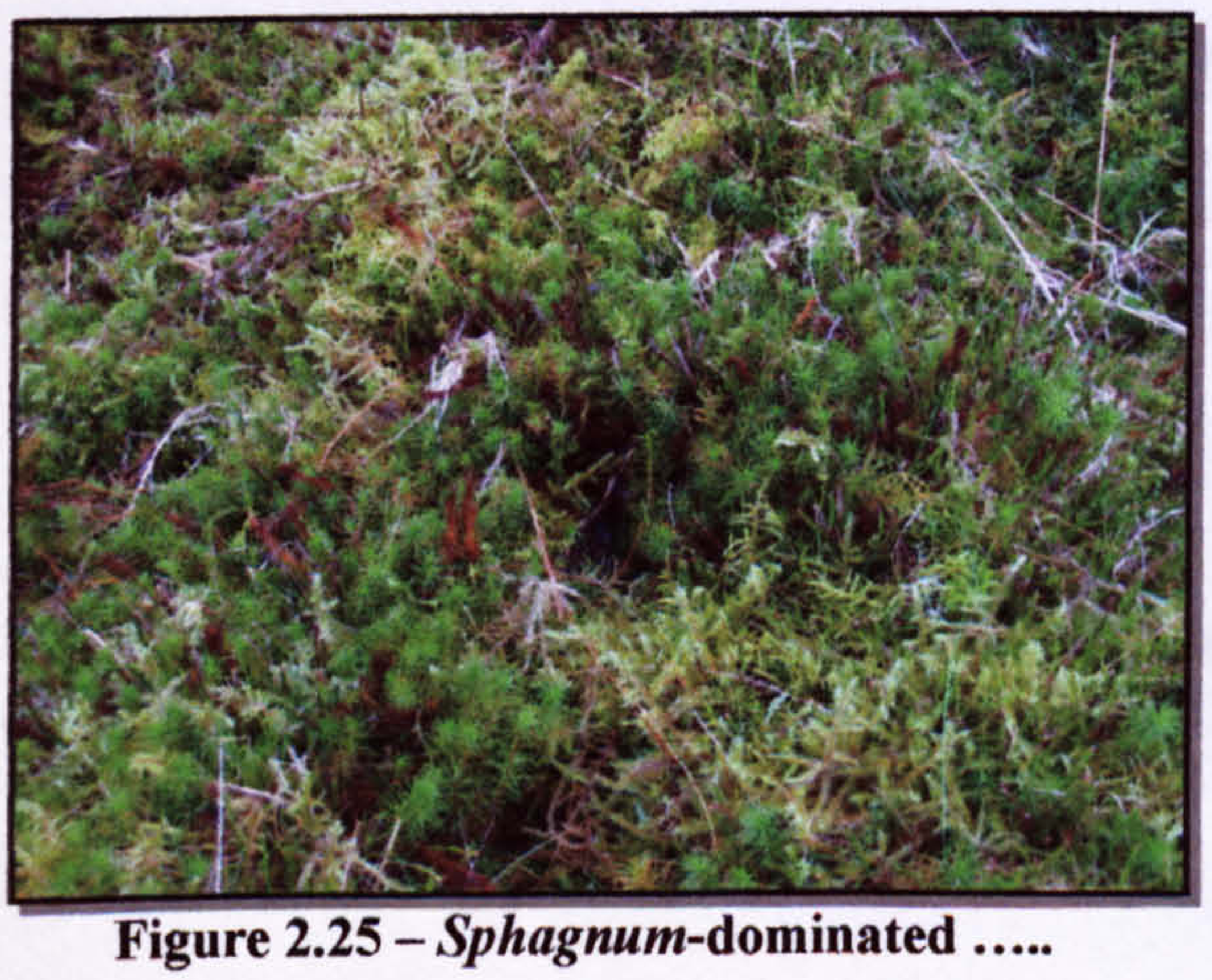

Figure 2.25 – *Sphagnum*-dominated .....

Figure 2.26 - ...organic rich bog catchment soils

2.2.1.2 Sampling regime

Similar to the sampling of the reservoir waters, soil samples at the Cefni and Marchlyn Bach were taken at monthly intervals from February 2003 to March 2004, with the survey extended to the Lluwgy soils in June 2003. In April 2003, an additional sample of peat was taken at Marchlyn Bach two days after the main sampling was undertaken, after rainfall had ended a small drought.

A representative area of wetland soil within the catchment of the three lakes was chosen to evaluate the soil catchment properties. Soil water samples (approx. 5 x 20 ml) were extracted at a depth of 10 cm using Plastipak syringes and Tygon auto analyzer transmission tubing following the design described by Freeman, *et al.* (1994). The water was transferred to 20 ml glass vials. For the analysis of carbon leachability and soil enzyme activities, one block of soil per site (approx. 12 cm x 8 cm) was carefully extracted using a sharp knife and transported back to the laboratory in plastic bags to prevent water-loss. Soil temperature at a depth of 10 cm, water-table depth and the general weather conditions at the time of sampling were also recorded.

All samples were transported back to the laboratory, where they were filtered and stored as described in section 2.1 (p31).

2.2.1.3 Experimental analyses

For details of analyses of DOC and phenolic compound concentrations, apparent molecular weight, UV/visible absorbencies and ion concentrations, please refer to section 2.1 (p32-33).

Soil enzyme activities and microbial respiration analyses

The following analyses conducted on the peat matrix itself were performed at field temperature in a Gallenkamp cooled incubator. They were performed on the sample of soil collected from the three field sites.

- Soil bulk density (dry weight) and gravimetric water content were determined by drying replicates of 1 cm^3 of peat at 105°C for 24 hours in a Carbolite muffle furnace and calculating the weight loss.

- The activities of three extracellular hydrolase enzymes (β-D-glucosidase, sulphatase and phosphatase) were assayed using a modified version of the methods developed by Freeman, *et al.* (1995) and Kang (1999). MUF-free acid (4-methylumbelliferone) and MUF enzyme substrates (400 μM MUF-β-D-glucoside, 400 μM MUF-sulphate and 200 μM MUF-phosphate) were dissolved in methyl cellosolve (ethylene glycol monomethyl ether) and diluted in deionised water. A standard curve was prepared by placing six 1 cm^3 cubes of peat in separate stomacher bags and adding 7ml of milli-q water. They were then homogenised for 60 seconds (Seward Colworth model 400) and 1.5 ml of solution from each centrifuged at 7,200 g for 5 minutes. Of the supernatant, 250 μl was transferred from each vial to six separate wells on a microplate. Fifty microlitres of varying concentrations of MUF free acid solution were added to the supernatant to create a concentration curve of 0-100 μM, from which the enzyme activity was determined. For the enzyme assay, 7 ml of enzyme substrate was added to 1 cm^3 cubes of peat and the bags incubated at field temperature for 60 minutes (45 minutes for phosphatase). Five replicates were performed for each soil type. When the time had elapsed, the reactant was centrifuged and 300 μl of supernatant transferred to the wells of a black fluorescent microplate. The fluorescence of the standard curve and enzyme wells was measured at 450 nm emission and 330 nm excitation, with a slit setting of 2.5, on a BMG Fluostar Galaxy fluorimeter. Values of enzyme activity were automatically calculated from the standard curve by the software program. Enzyme activities were expressed as MUF produced (nmoles g^{-1} min^{-1}), correcting for the dry weight of the soil.
- Phenol oxidase was assayed using a modified version of the method developed by Pind, *et al.* (1994). Twenty cubes of peat (1 cm^3) per site were placed in separate stomacher bags, 9 ml of deionised water was added and the contents homogenised for 60 seconds. Of this homogenate, 300 μl was transferred to centrifuge vials and 450 μl of deionised water and 750 μl of 10 mM L-DOPA (dihydroxyphenylalanine) solution were then added, and the reaction allowed to continue for 1 minute in ten of the vials and 9 minutes in the other ten vials. All vials were then centrifuged at 7,200 g for 5 minutes. Of the supernatant, 300 μl was transferred to wells of a clear

microplate, and the absorbance read at 460nm. The activity of phenol oxidase was calculated using a formula encompassing Beers Law, the difference in the absorbance values between 1 and 9 minutes, the value of molar absorptivity (3.7×10^4) and the dry weight of the peat. Phenol oxidase activity was expressed in μmol dicq g^{-1} min^{-1}.

- Microbially-derived soil gases (respirometric technique) were collected in the lab by placing 1 cm^3 of peat soil from a depth of 10 cm into a darkened bottle of known volume which was sealed tightly with a screw-fit cap containing a Suba-Seal septum. For each site, 5 bottles were used, as well as 5 'blank' bottles. Five millilitres of gas sample was withdrawn from each bottle after 90 minutes of incubation at field temperature, with the gases being drawn through the septum into a 10 ml gas-tight syringe. The gas samples were analysed on the GC, as described on page 34. The respirometric method measures those gases derived principally from microbial processes within the soil matrix.

Soil leachability/water-extractable analyses

- The total quantity of water-extractable (leachable) carbon in each of the three soils was assessed by placing 1 cm^3 of peat in a stomacher bag, adding 9 ml of milli-q water, homogenising and centrifuging 1.5 ml of homogenate at 10,000 rpm for 5 minutes. The supernatant was then filtered through a 0.45μm filter and DOC and phenolics concentrations were determined.

2.2.1.4 Statistical analysis

Please refer to section 2.1 (p34) for details of the types of statistical analyses performed. *Figures 2.27-2.29 and 2.36-2.37* show data for one replicate and *figures 2.30-2.35 and 2.38-2.49* show mean monthly values for 5 replicates, with error bars indicating ± standard error of the mean. Pearson correlation was performed to test for significant correlations between soil analyses (*tables 2.03-2.04*), and in testing for correlations between soil and lake analyses (*tables 2.05-2.06*).

2.2.2 Results

During the late summer of 2003 a major drought was experienced in the UK, which unfortunately meant that soil porewater was unable to be sampled in August and September at the Cefni and Marchlyn Bach sites. However, there was sufficient water at the Llugwy site to enable sampling during these months.

2.2.2.1 Physicochemical analyses

Soil temperature (*figure 2.27*) showed strong seasonal variation, being highest in August 2003 for all three sites (15-16°C) and lowest in December 2003 (2-4°C). The Cefni soils were usually slightly warmer than the bog soils. At Marchlyn Bach, the extra sample collected at Marchlyn Bach in April 2003 showed that the soil temperature dropped by approximately 4°C following a heavy rainfall event which marked the end of a small drought. The soil moisture content (*figure 2.28*) did not show a seasonal trend, but was always higher for the two bog soils (averaging 85-90%) than for Cefni soils (average of 65%). Values of soil pH (*figure 2.29*) at the Cefni tended to decrease from spring to autumn (6.3 in March 2003 to 4.9 in November 2003) and then rose once again during winter (5.8 in March 2004). pH values from the bog soils were consistently more acidic. At Marchlyn Bach, the pH averaged 3.7 throughout the year, but increased to 5.6 during a small drought in April 2003. Following heavy rainfall, the pH reduced back down to 3.3. At the Llugwy, the pH averaged 4.2, but exhibited a seasonal trend; highest during the summer and lowest during the winter.

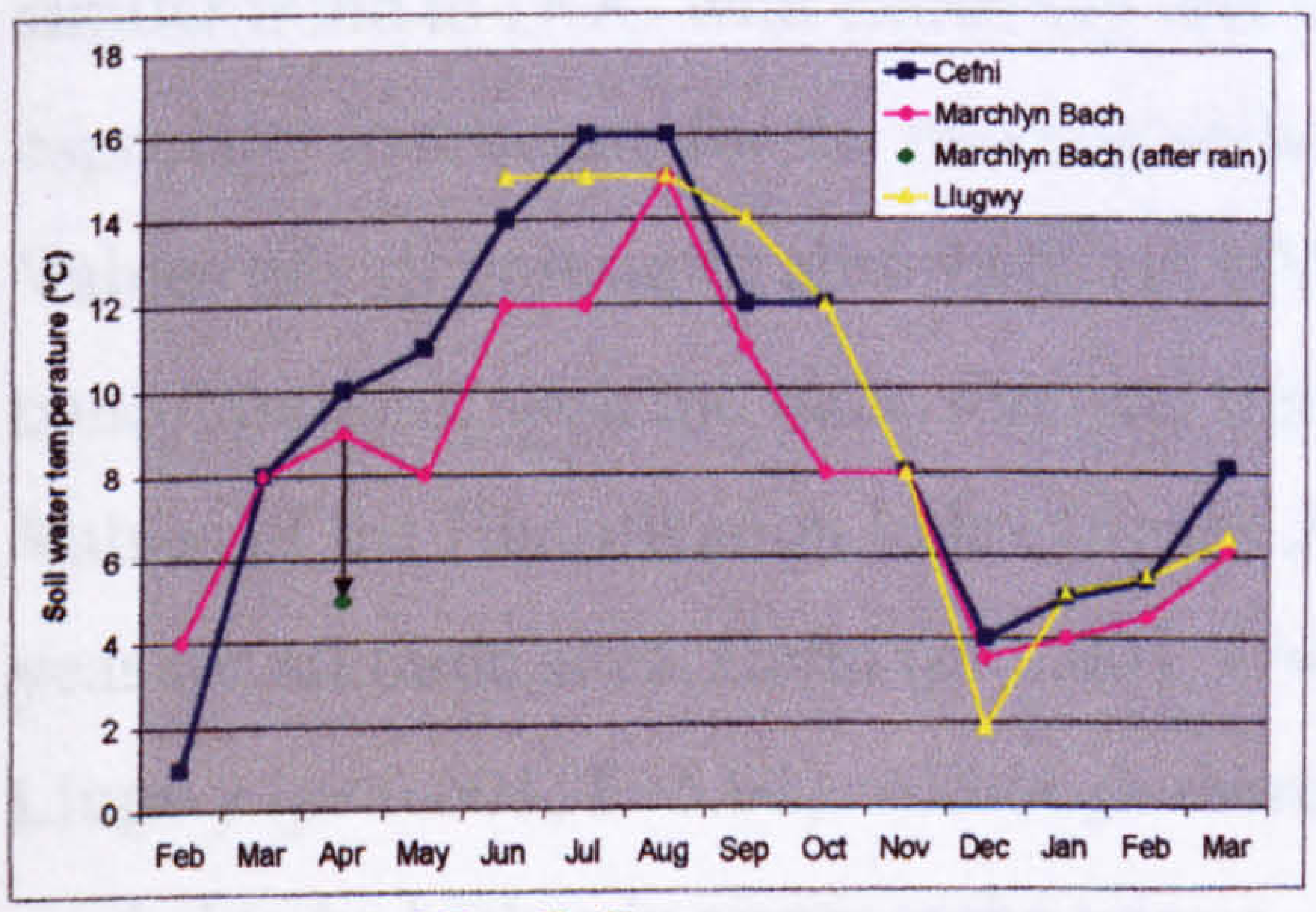

Figure 2.27 – Soil water temperature

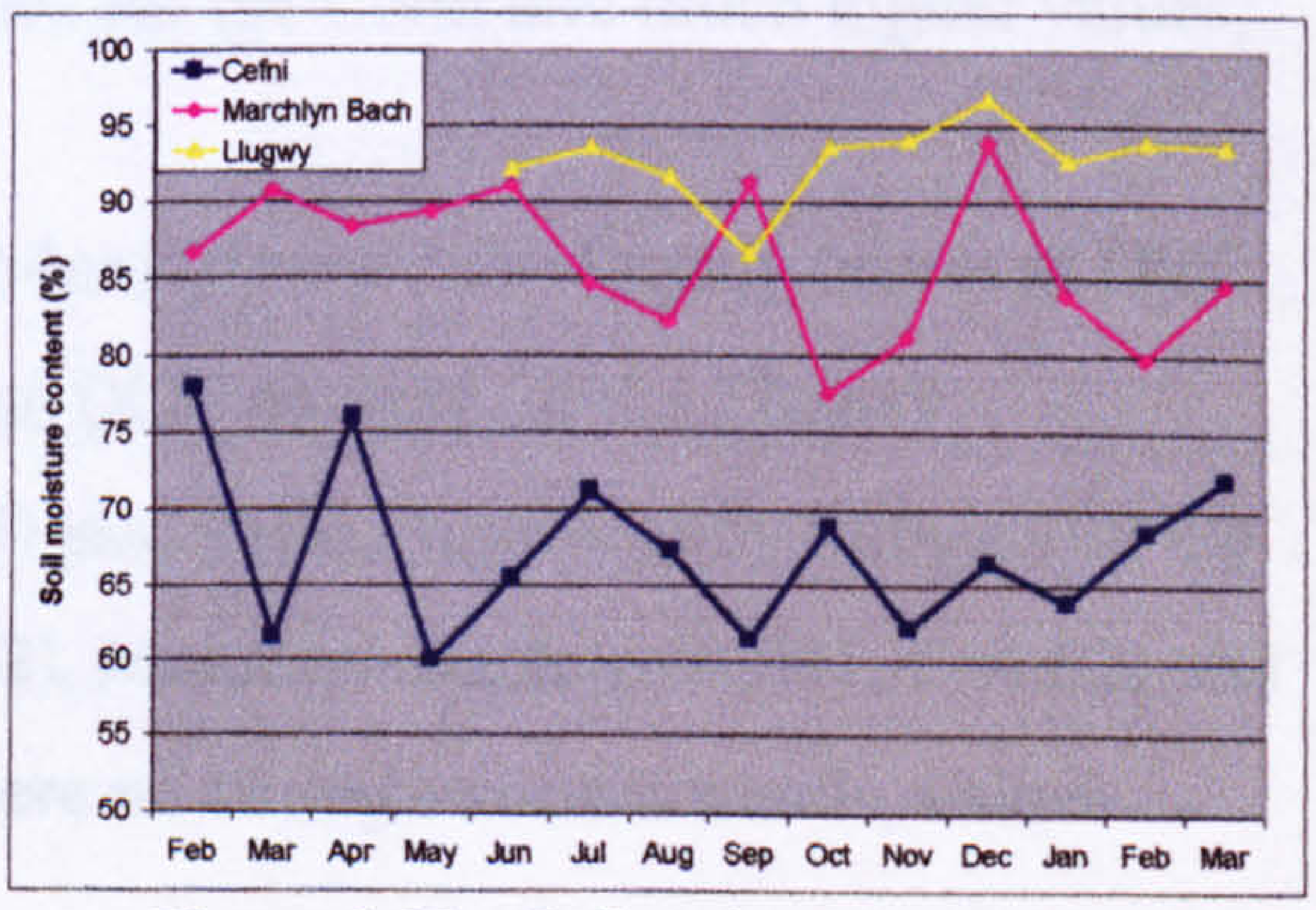

Figure 2.28 – Soil moisture content

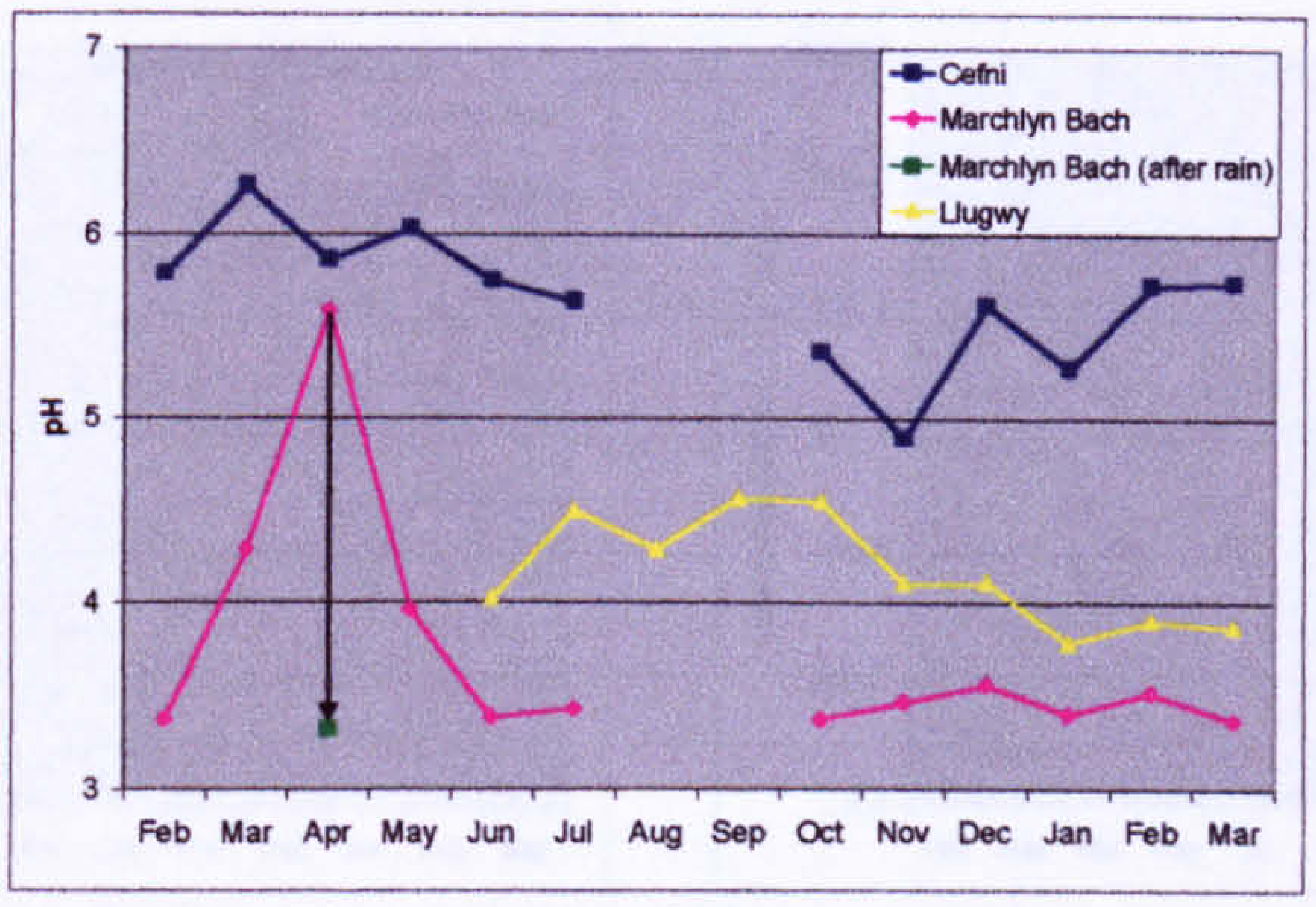

Figure 2.29 – Soil water pH

2.2.2.2 Dissolved organic carbon analyses

General linear ANOVA showed that the soil porewater DOC concentration (*figure 2.30*) varied significantly over the 14 month sampling period at the Cefni (p<0.001; F=37.36), Marchlyn Bach (p<0.001; F=4.23) and Llugwy (p<0.001; F=22.02). Only the Llugwy exhibited a strong seasonal trend, with values being highest during summer (33.9 mg l^{-1} for August 2003) and lowest during winter (3.8 mg l^{-1} for March 2004). DOC leachability (*figure 2.31*) was extremely low for the Cefni, averaging just 14.7 mg l^{-1} g^{-1}. Much higher concentrations of leachable DOC were recorded for the two bog soils, with both exhibiting a strong leachable potential during the autumn.

The concentration of porewater phenolics (*figure 2.32*) varied significantly throughout the year for Cefni (p<0.001; F=4.62), Marchlyn Bach (p<0.001; F=4.62) and Llugwy (p<0.001; F=10.06) soils. Again only Llugwy exhibited a strong seasonal response, with phenolics concentrations being highest in summer (5.4 mg l^{-1} in June 2003) and lowest in winter (1.1 mg l^{-1} in January). Leachable phenolics (*figure 2.33*) exhibited a very similar trend to DOC with extremely low values for the Cefni and much higher values, especially in autumn, for the two bog soils.

Values of soil porewater absorbance at UV-254nm (*figure 2.34*) largely mirrored DOC concentrations, with the same seasonal trend as DOC evident for the Llugwy.

Values of the Humification Index (*figure 2.35*) also varied significantly throughout the year for all three sites; Cefni (p<0.001; F=4.62), Marchlyn Bach (p<0.001; F=4.62) and Llugwy (p<0.001; F=5.01). Although there were no strong seasonal trends, values tended to be higher in summer the winter.

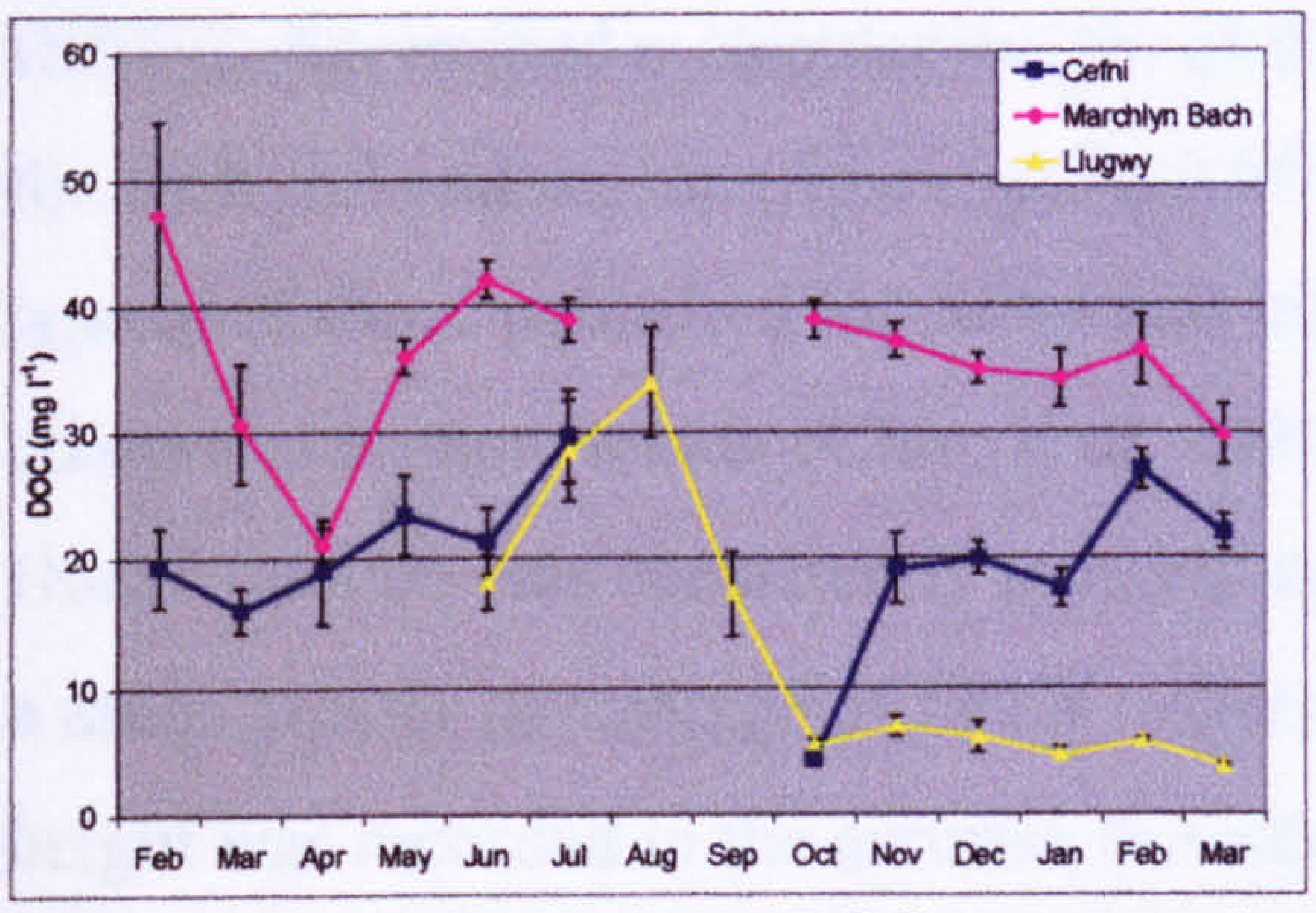

Figure 2.30 – Soil porewater DOC concentrations

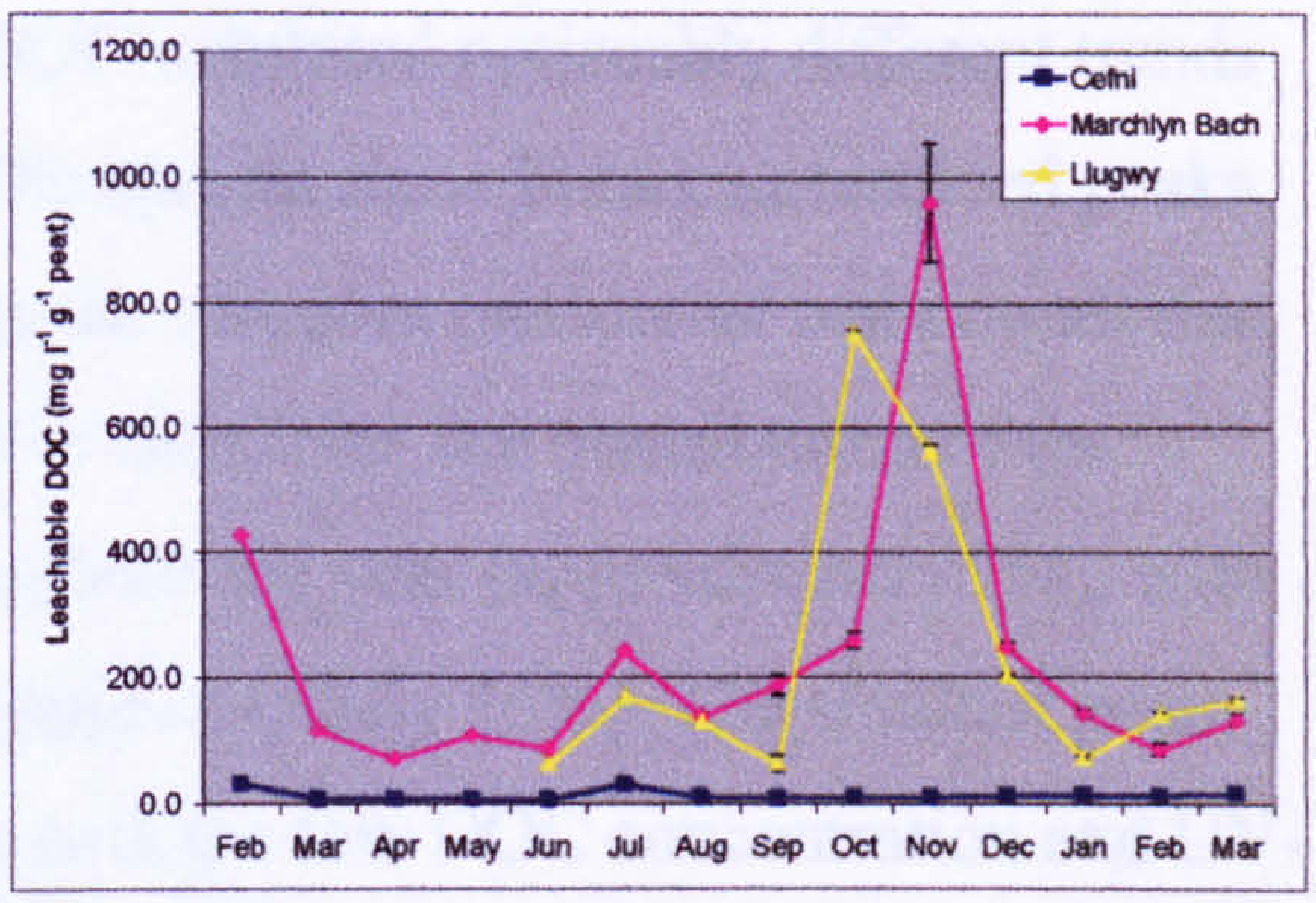

Figure 2.31 – Soil leachable DOC concentrations

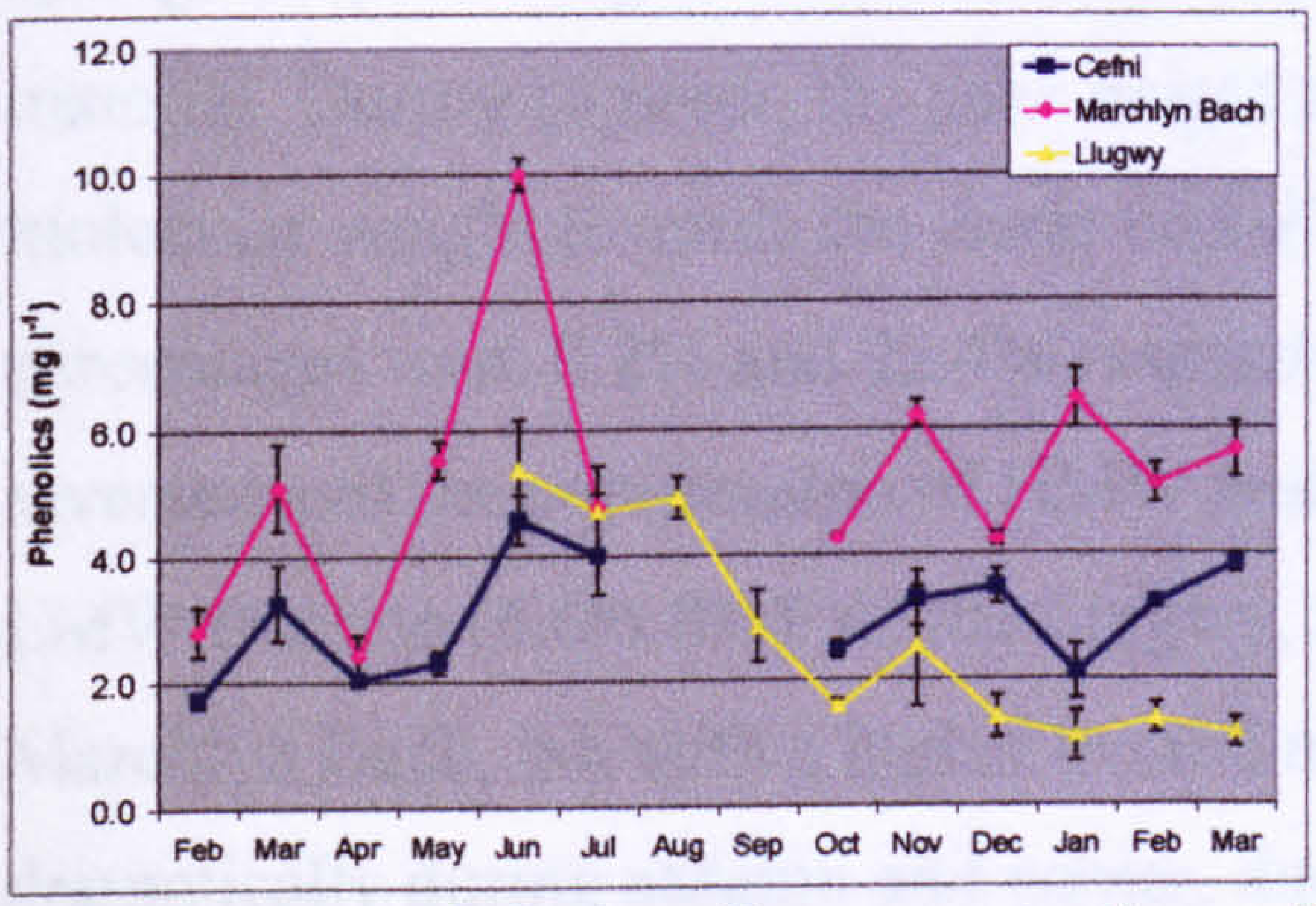

Figure 2.32 – Soil phenolics concentrations

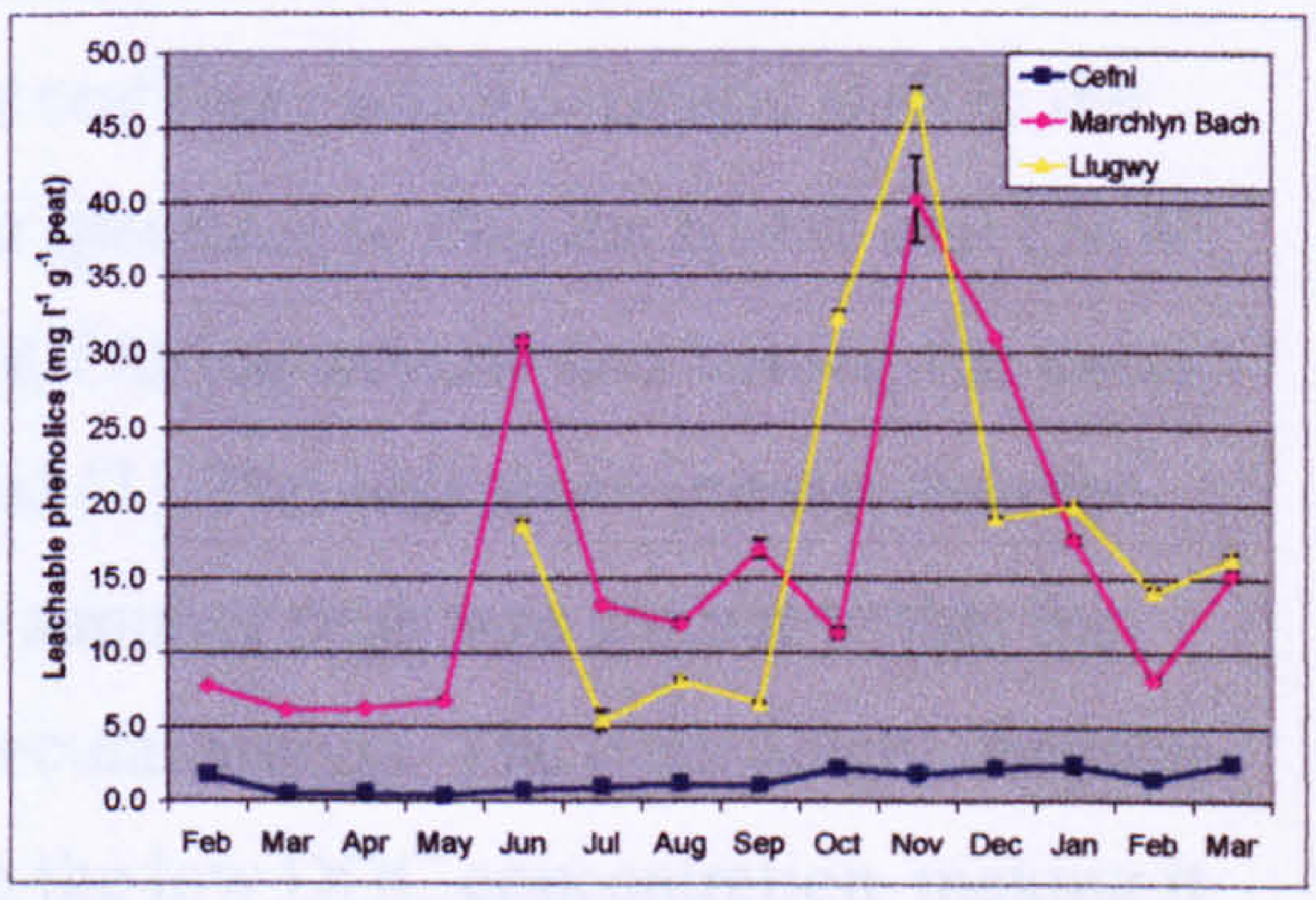

Figure 2.33 – Soil leachable phenolics concentrations

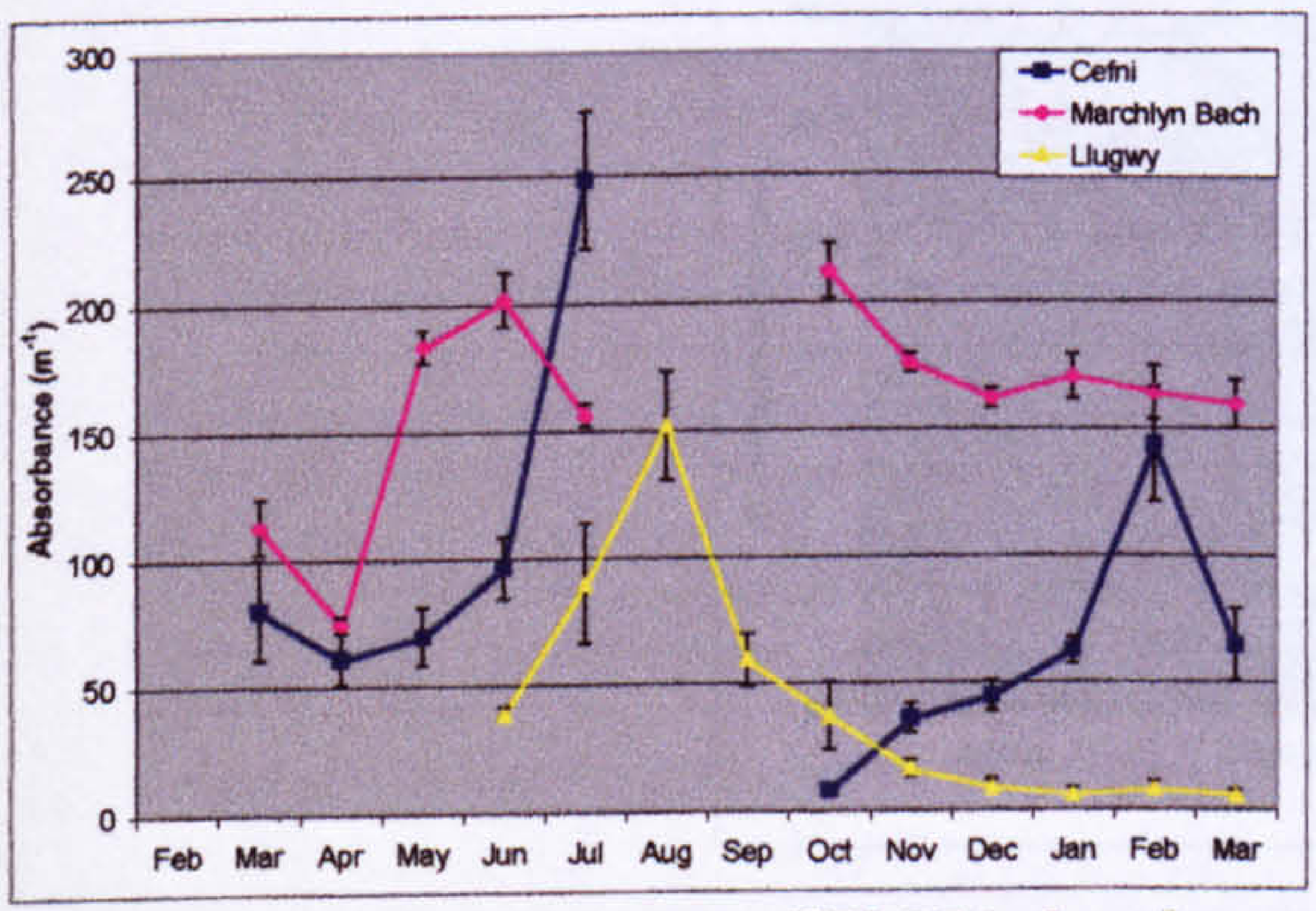

Figure 2.34 – Soil porewaterUV-254 absorbance

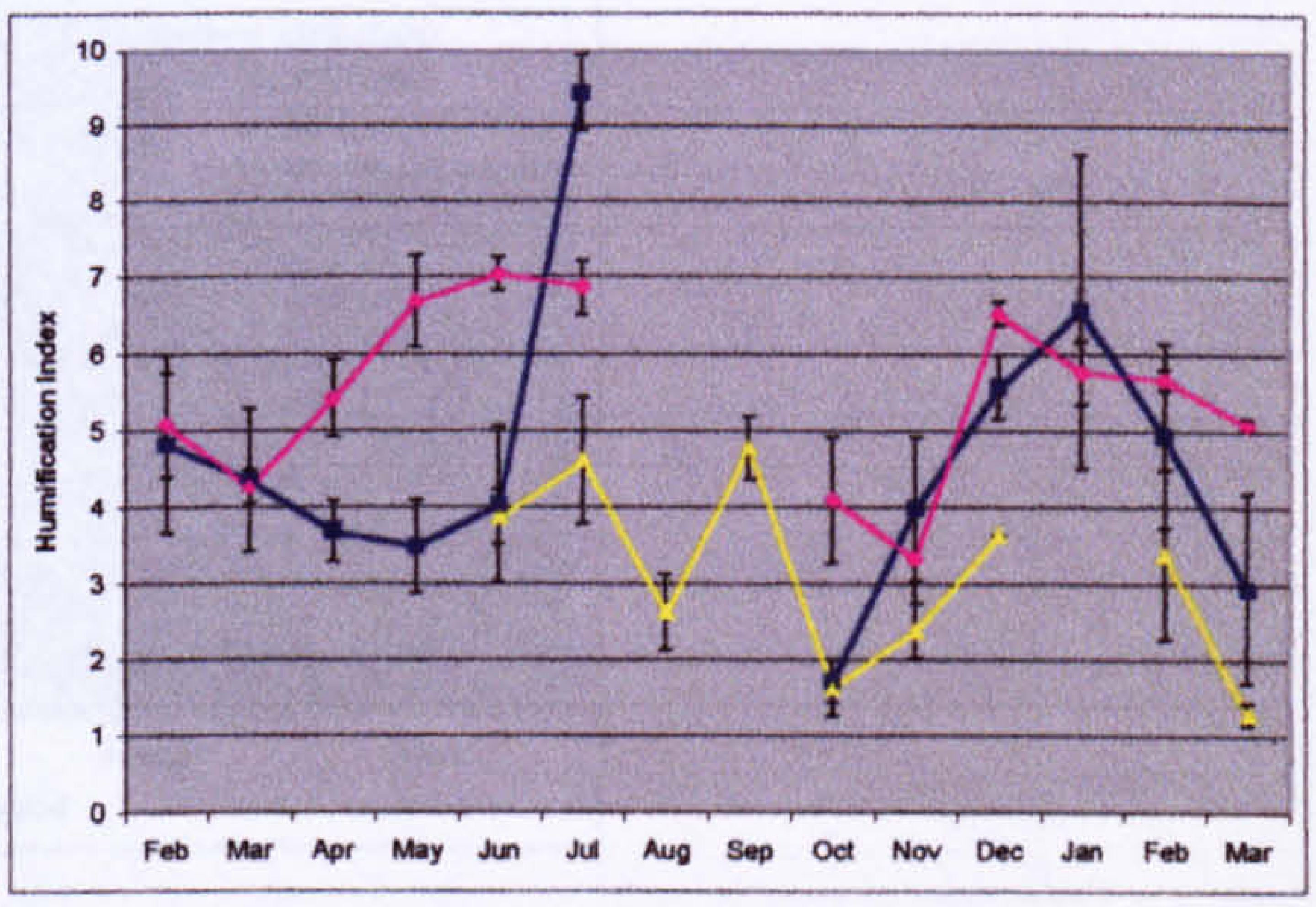

Figure 2.35 – Soil porewater Humification Index

HPSEC-determined molecular weight of the DOC exhibited noticeably different trends for each soil and season (*figures 2.36-2.37*). The spectra show broad, unresolved peaks typical of those published for NOM and reflect the complex mixture of compounds that comprises organic matter (Chin, *et al.* 1994). For the Cefni, the contribution of the HMW fraction was consistently minimal throughout the year (approximately 2%), with a much greater percentage of LMW material (approximately 15%). The smallest peak height was recorded in the autumn; this ties in with the low DOC concentration and UV-254 absorbance values. For Marchlyn Bach, the peaks were consistently higher than the Cefni, due to its greater DOC concentration and there was more of a seasonal effect. In spring, the peak height was smallest and there was a minimal contribution of HMW material. During summer, the peak height rose and there was a dramatic shift in the molecular weight towards the lower end of the spectrum so that the HMW and LMW percentages were 0.2% and 22.4% respectively. During autumn and winter, the trend reversed and the contribution of HMW material (21.2%) was much greater than the LMW fraction (6.6/6.8%). At the Llugwy, the summer peak was similar to that for Marchlyn Bach, but with a higher overall molecular weight. The peak height dropped dramatically during autumn and winter, due to the low DOC concentration, making it difficult to assess the contribution of each molecular weight fraction.

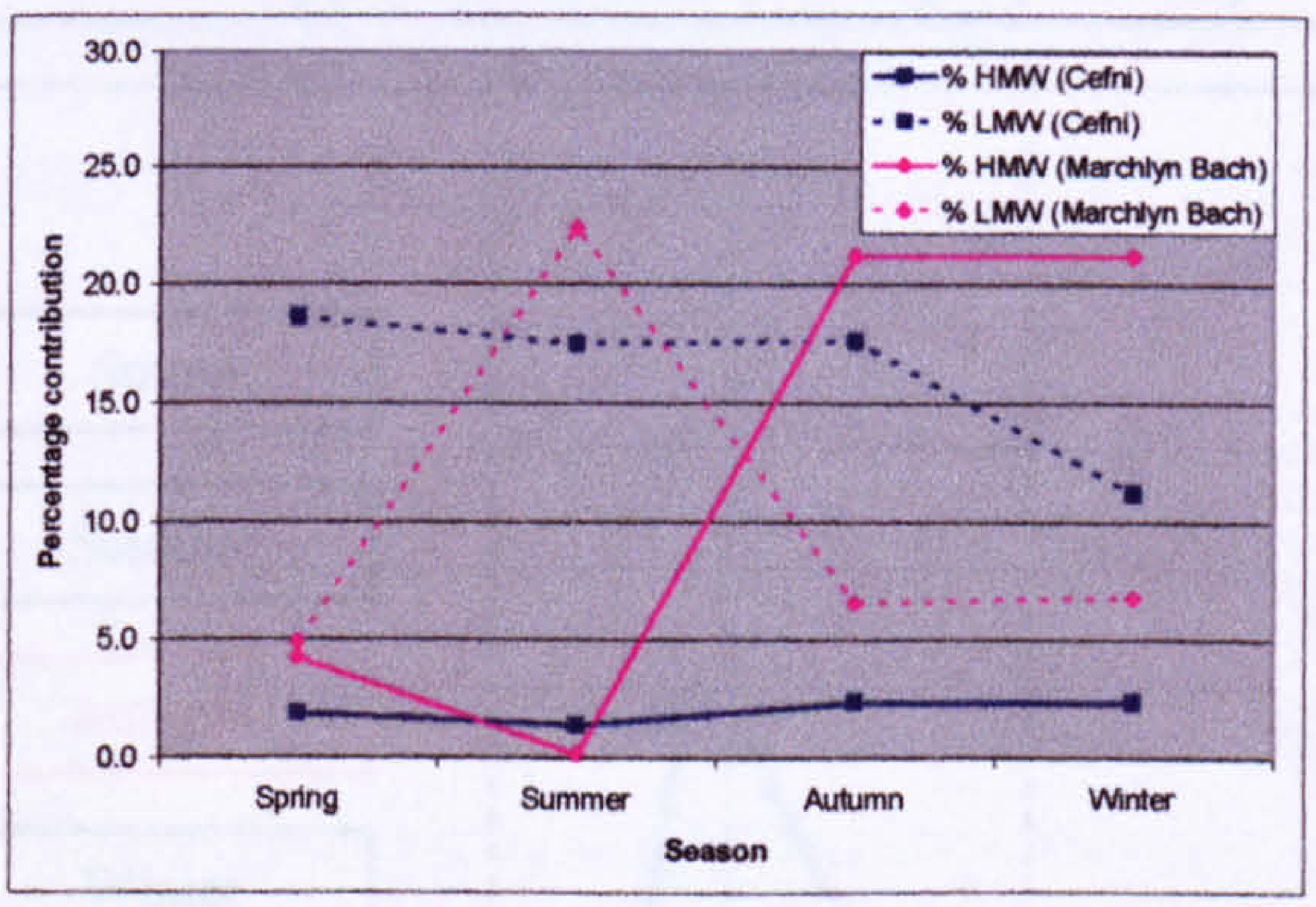

Figure 2.36 – Percentage abundance of the HMW and LMW fraction of DOC for Cefni and Marchlyn Bach soil porewaters

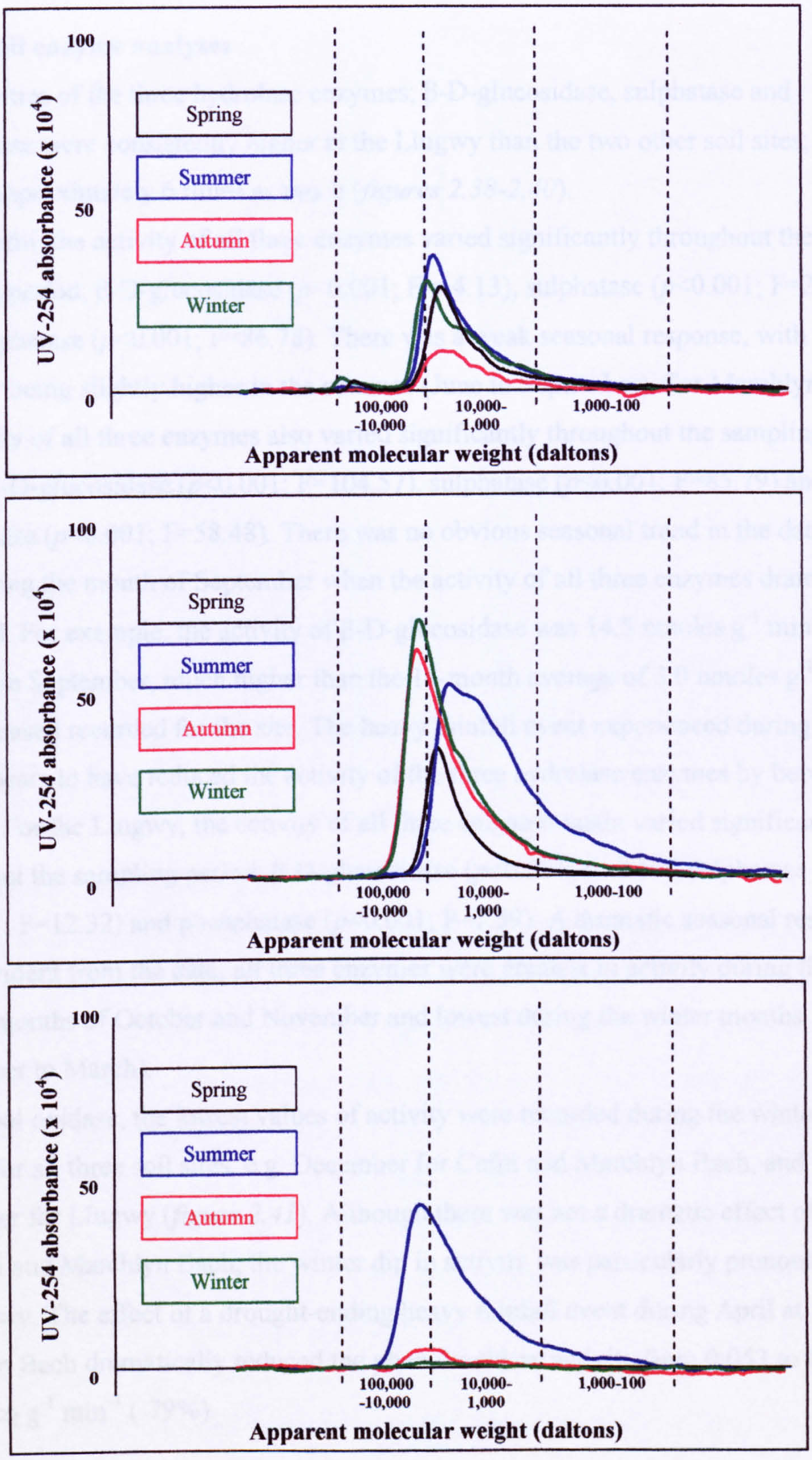

Figure 2.37 – HPSEC-determined molecular weight spectra for Cefni (top), Marchlyn Bach (middle) and Llugwy (bottom) soil porewaters.

2.2.2.3 Soil enzyme analyses

The activities of the three hydrolase enzymes; β-D-glucosidase, sulphatase and phosphatase were consistently higher at the Llugwy than the two other soil sites; on average approximately 6 times as much (*figures 2.38-2.40*).

At the Cefni, the activity of all three enzymes varied significantly throughout the sampling period; β-D-glucosidase (p<0.001; F=64.13), sulphatase (p<0.001; F=26.82) and phosphatase (p<0.001; F=86.78). There was a weak seasonal response, with values generally being slightly higher in the summer (June to September). For Marchlyn Bach, the activity of all three enzymes also varied significantly throughout the sampling period; β-D-glucosidase (p<0.001; F=104.57), sulphatase (p<0.001; F=85.79) and phosphatase (p<0.001; F=58.48). There was no obvious seasonal trend in the data, apart from during the month of September when the activity of all three enzymes dramatically increased. For example, the activity of β-D-glucosidase was 14.5 nmoles g^{-1} min^{-1} MUF released in September, much higher than the 14-month average of 3.0 nmoles g^{-1} min^{-1} MUF released recorded for the site. The heavy rainfall event experienced during April 2003 appears to have reduced the activity of the three hydrolase enzymes by between 58-87%. For the Llugwy, the activity of all three enzymes again varied significantly throughout the sampling period; β-D-glucosidase (p<0.001; F=5.13), sulphatase (p<0.001; F=12.32) and phosphatase (p<0.001; F=7.59). A dramatic seasonal response seems evident from the data; all three enzymes were greatest in activity during the autumn months of October and November and lowest during the winter months (December to March).

For phenol oxidase, the lowest values of activity were recorded during the winter months for all three soil sites, e.g. December for Cefni and Marchlyn Bach, and November for Llugwy (*figure 2.41*). Although there was not a dramatic effect of season for Cefni and Marchlyn Bach, the winter dip in activity was particularly pronounced at the Llugwy. The effect of a drought-ending heavy rainfall event during April at Marchlyn Bach dramatically reduced the phenol oxidase activity from 0.053 to 0.011 μmol dicq g^{-1} min^{-1} (-79%).

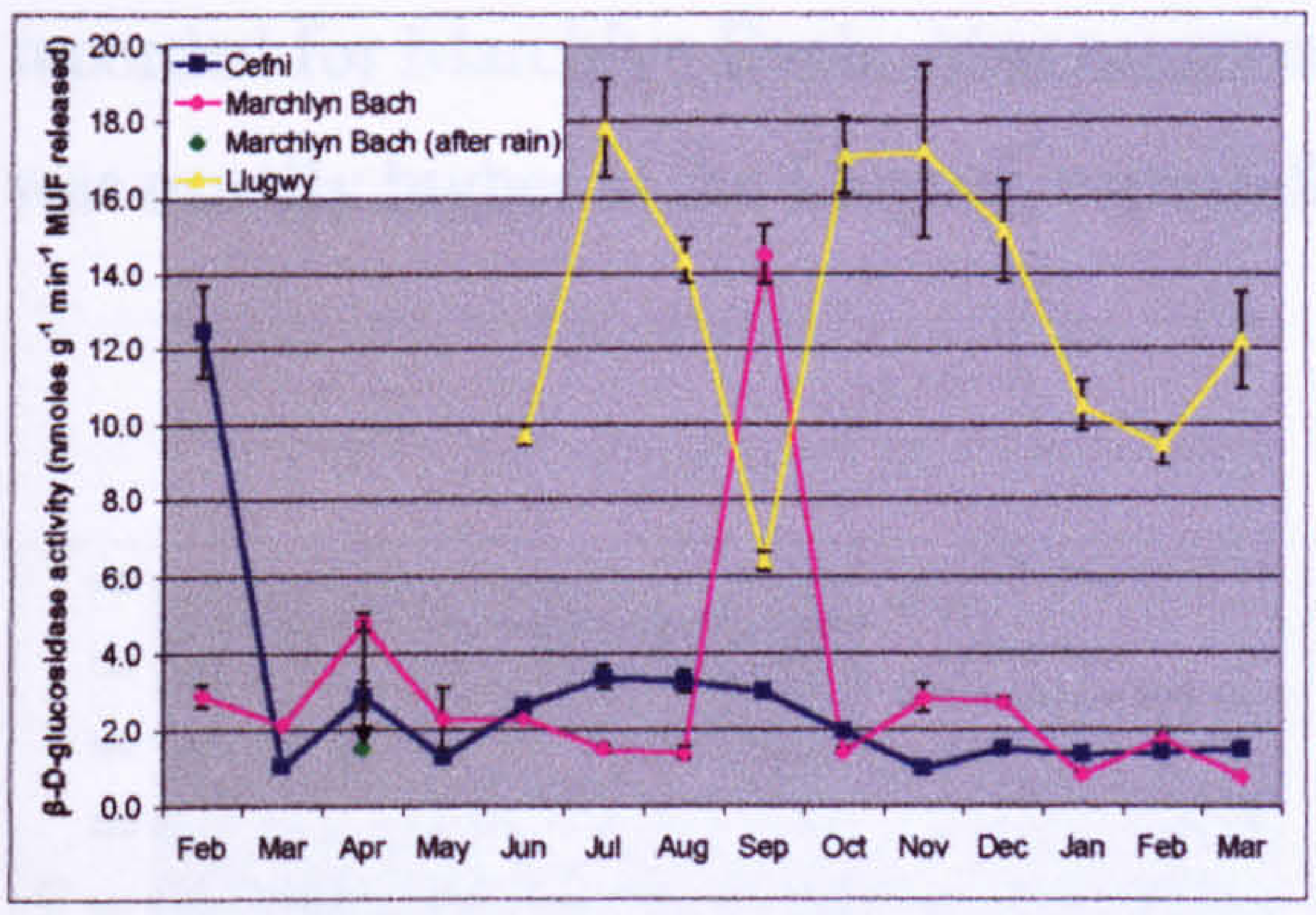

Figure 2.38 - β-D-glucosidase activity

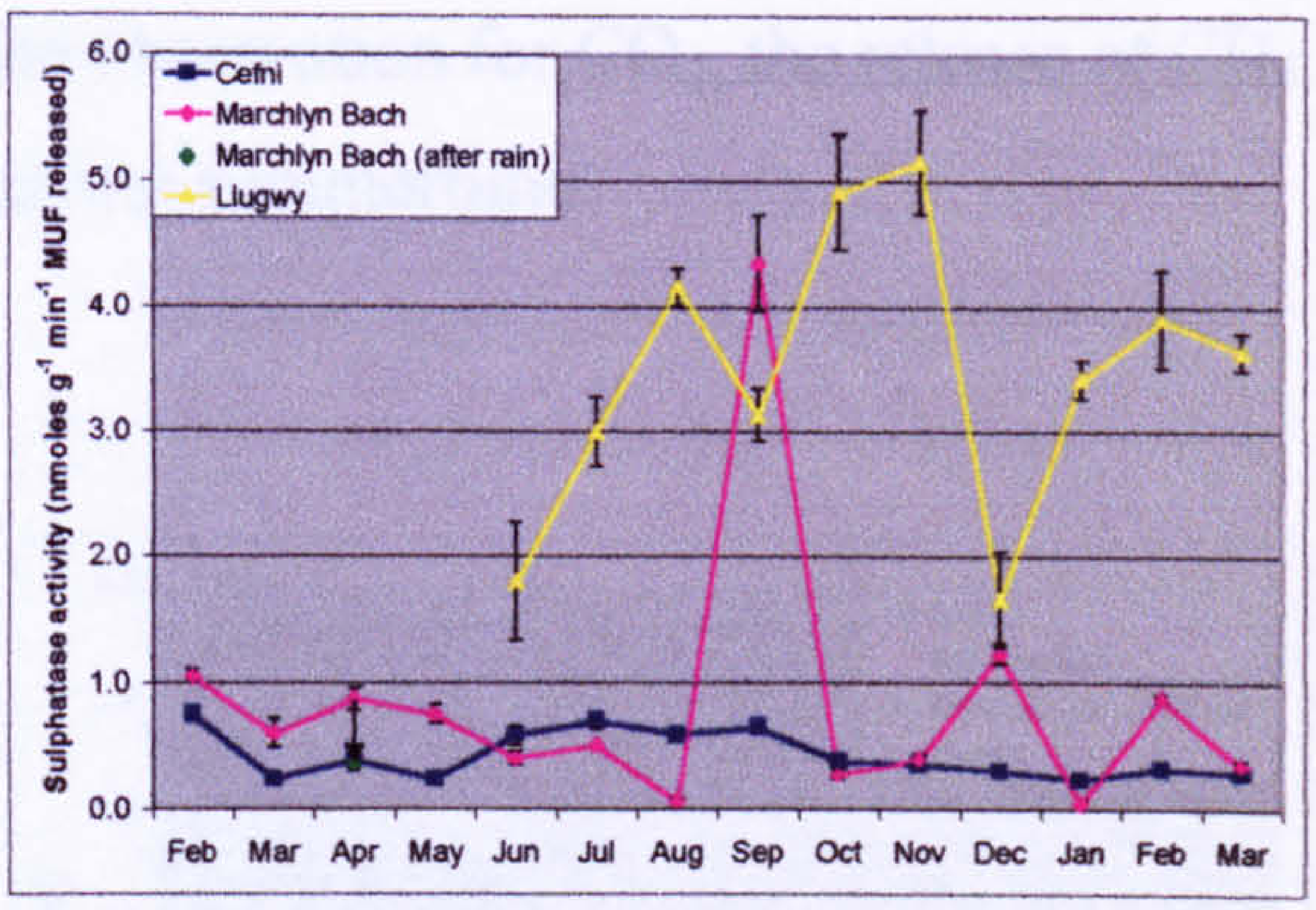

Figure 2.39 – Sulphatase activity

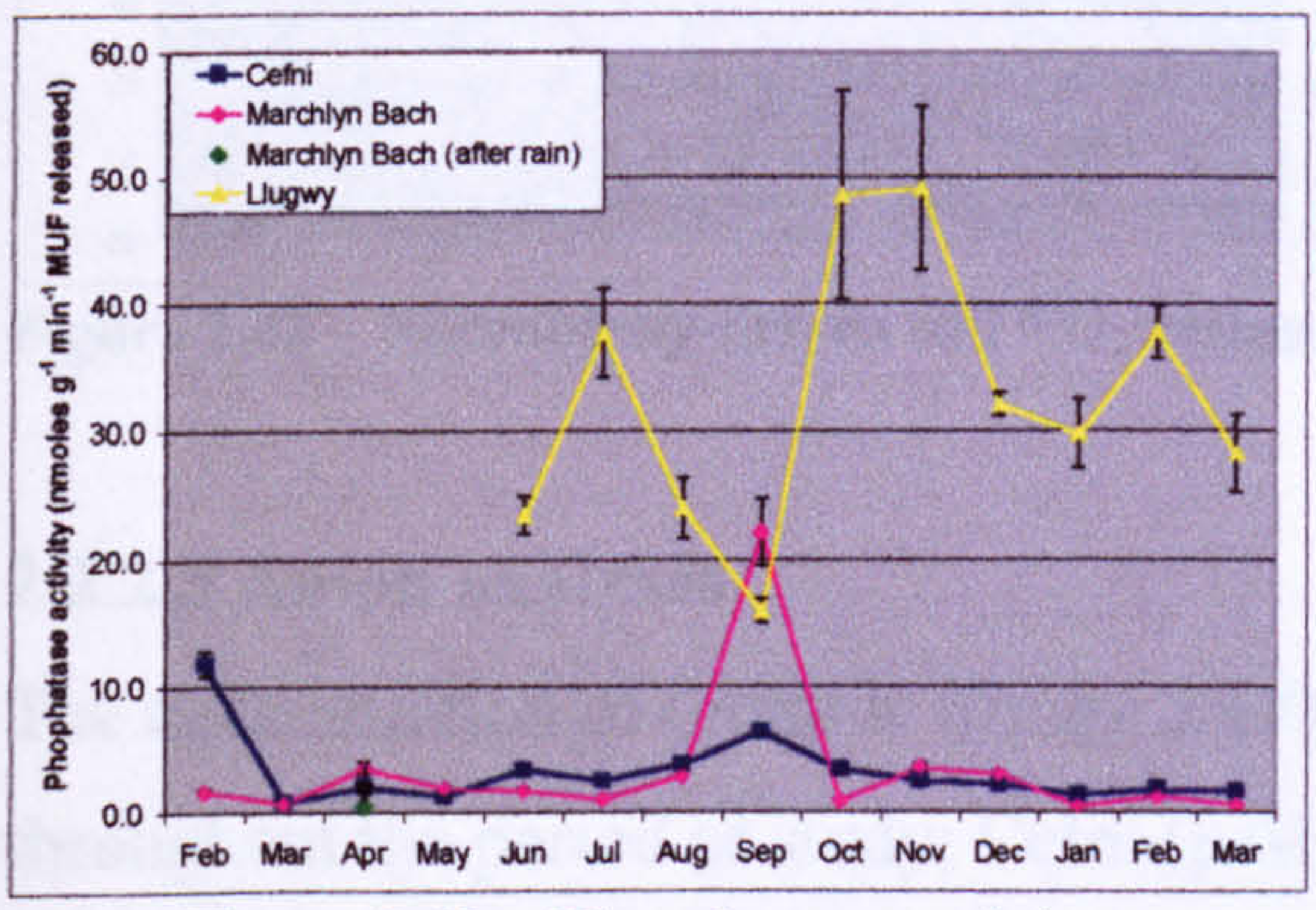

Figure 2.40 – Phosphatase activity

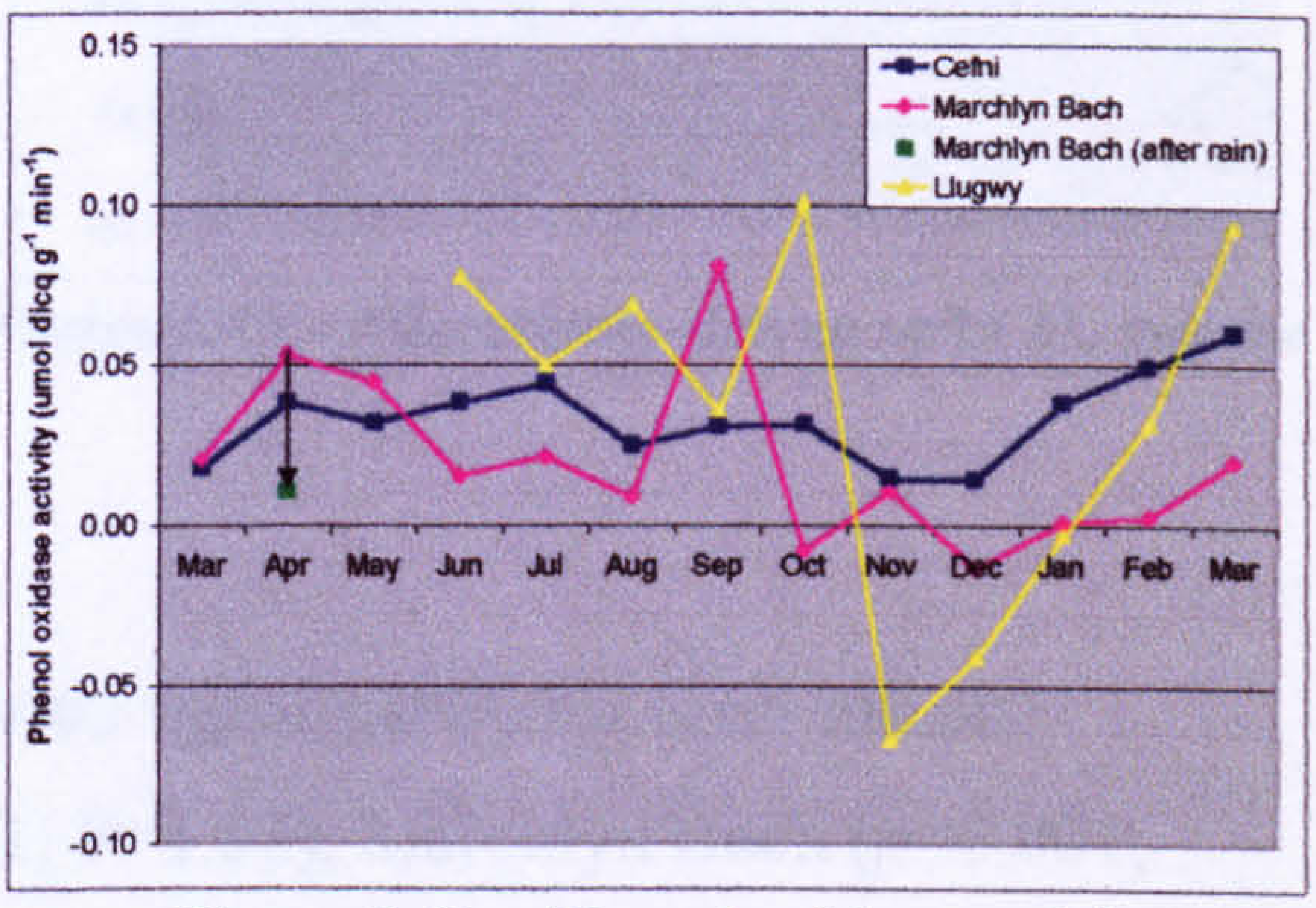

Figure 2.41 – Phenol oxidase activity

2.2.2.4 Microbial respiration analysis

Values of microbially-driven CO_2 release (*figure 2.42*) were similar for the three sites and varied significantly throughout the sampling period; Cefni ($p<0.001$; F=21.45), Marchlyn Bach ($p<0.001$; F=14.59) and Llugwy ($p<0.001$; F=23.07). All the sites appear to show comparable seasonal trends, with the highest values recorded during the spring and the lowest during the winter of 2003. For example, for the Cefni, the highest value recorded was 90.3 $\mu g\ g^{-1}\ h^{-1}$ in April and the lowest was 5.7 $\mu g\ g^{-1}\ h^{-1}$ in December 2003. The soils of the Llugwy consistently had a higher rate of microbial CO_2 release, especially during the summer. The heavy rainfall event at Marchlyn Bach reduced the rate of microbial respiration by 63%.

As with CO_2, microbially-driven CH_4 release (*figure 2.43*) varied over the course of sampling for all 3 sites; Cefni ($p<0.001$; F=9.96), Marchlyn Bach ($p<0.001$; F=4.86) and Llugwy ($p<0.01$; F=3.54), although the effect of seasonality was less clear. The highest values were during spring for the Cefni and summer for the Llugwy, with little change recorded for Marchlyn Bach. Also mirroring the observation for CO_2, the release of CH_4 was usually higher at the Llugwy, especially during summertime.

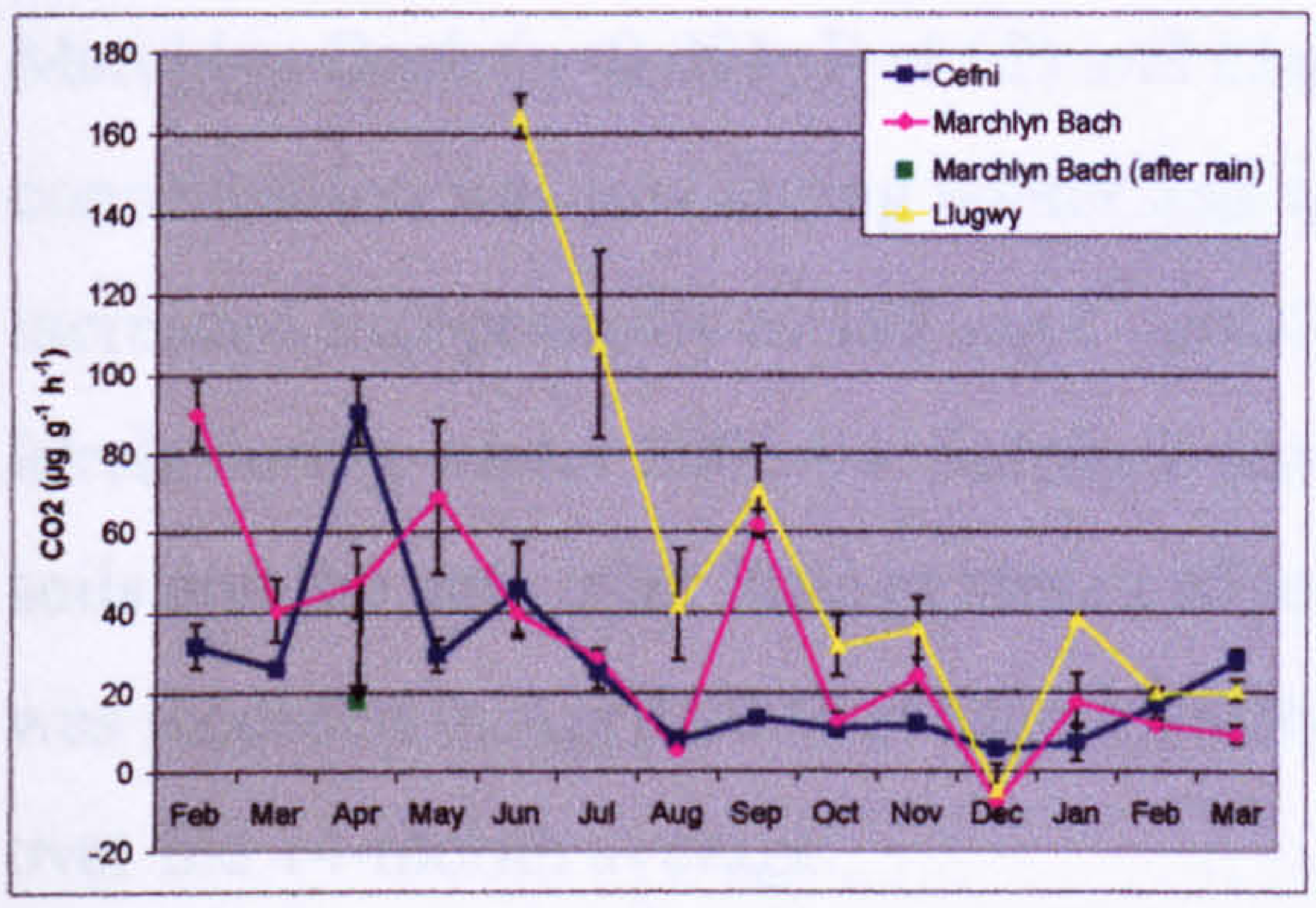

Figure 2.42 – Microbially-driven soil CO_2 release

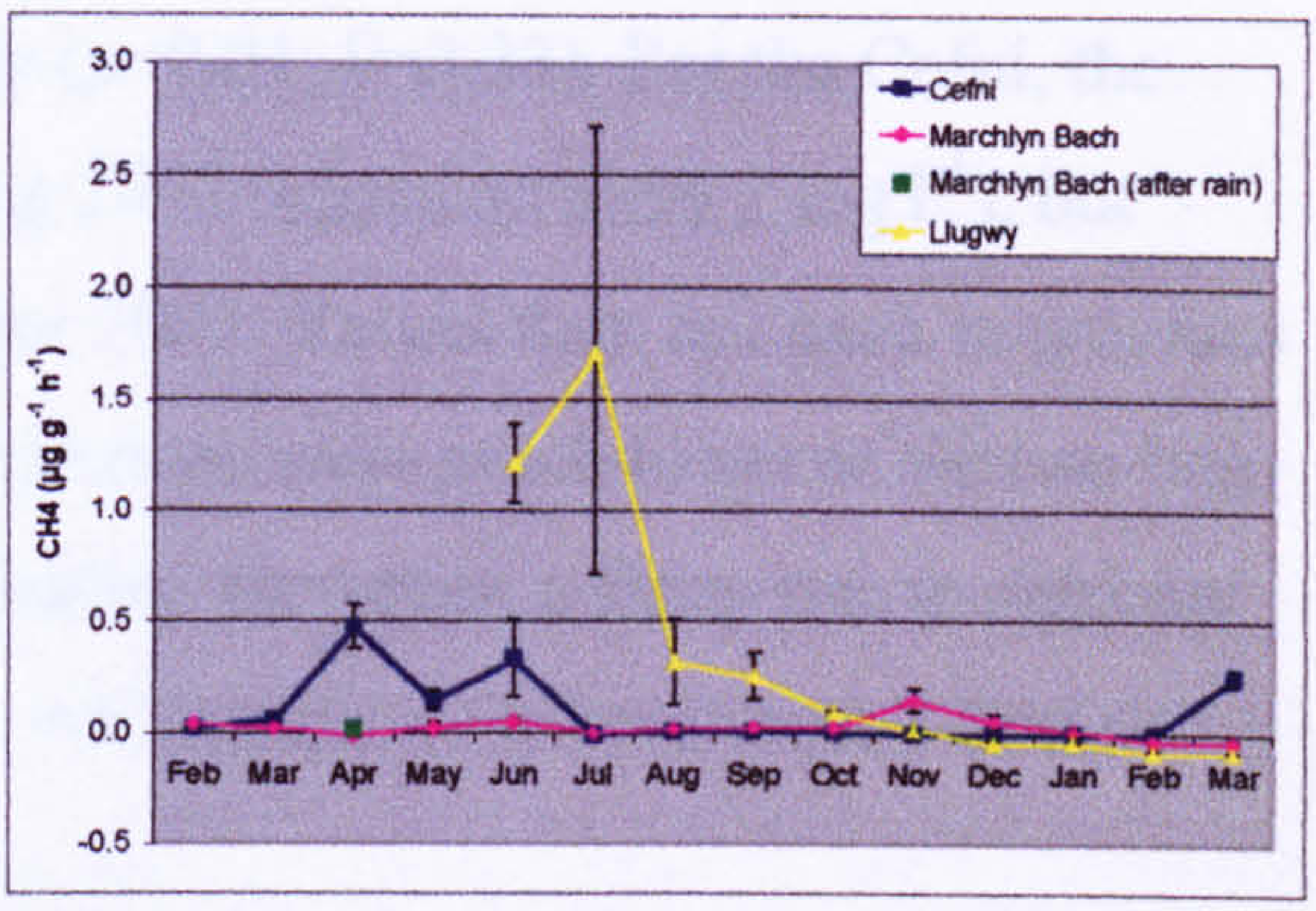

Figure 2.43 – Microbially-driven soil CH_4 release

2.2.2.5 Anion analyses

The concentration of bromide (*figure 2.44*) varied significantly for each soil site throughout the period of study; Cefni ($p<0.001$; F=4.62), Marchlyn Bach ($p<0.001$; F=4.62) and Llugwy ($p<0.01$; F=3.62). The concentrations at Marchlyn Bach and Llugwy were consistently low (averaging 0.03 mg l^{-1}) and did not exhibit much of a change with season. However, the concentrations at the Cefni were much higher, averaging 0.23 mg l^{-1} over the 14-month sampling period, with the highest concentrations recorded during spring 2003.

Nitrate levels (*figure 2.45*) varied significantly throughout the 14 months of sampling for the Cefni ($p<0.001$; F=4.62) and Marchlyn Bach ($p<0.001$; F=4.62), but not for the Llugwy ($p>0.05$). The lowest values were recorded during winter for all three sites, especially for the Cefni, where the concentrations during spring 2003 were typically 3 times those in winter 2003. A large peak in the nitrate concentration of Marchlyn Bach porewater was observed in April 2003, which equated to a 7-fold increase over the 14-month average.

Like nitrate, concentrations of phosphate (*figure 2.46*) varied significantly throughout the study for Cefni ($p<0.001$; F=4.62) and Marchlyn Bach ($p<0.001$; F=4.62), but not for the Llugwy ($p<0.05$). No strong seasonality is obvious from the data set, although the concentration of phosphate did consistently decrease at Marchlyn Bach from winter to summer 2003.

Concentrations of porewater sulphate (*figure 2.47*) were remarkably different between the three sites and varied significantly for all three sites; Cefni (p<0.001; F=4.62), Marchlyn Bach (p<0.001; F=4.62) and Llugwy (p<0.01; F=3.33). For the Cefni, the concentration was low during winter and spring 2003 (approximately 2 mg l^{-1}), but increased exceptionally to 192 mg l^{-1} in October 2003. Values then fell back to previous levels during winter 2003-04. Sulphate concentrations were much lower at the two bog soils and the data offer little evidence of seasonality. However, a sharp rise in sulphate was recorded in April 2003 at Marchlyn Bach, which equated to an almost 7-fold rise over the 14-month average.

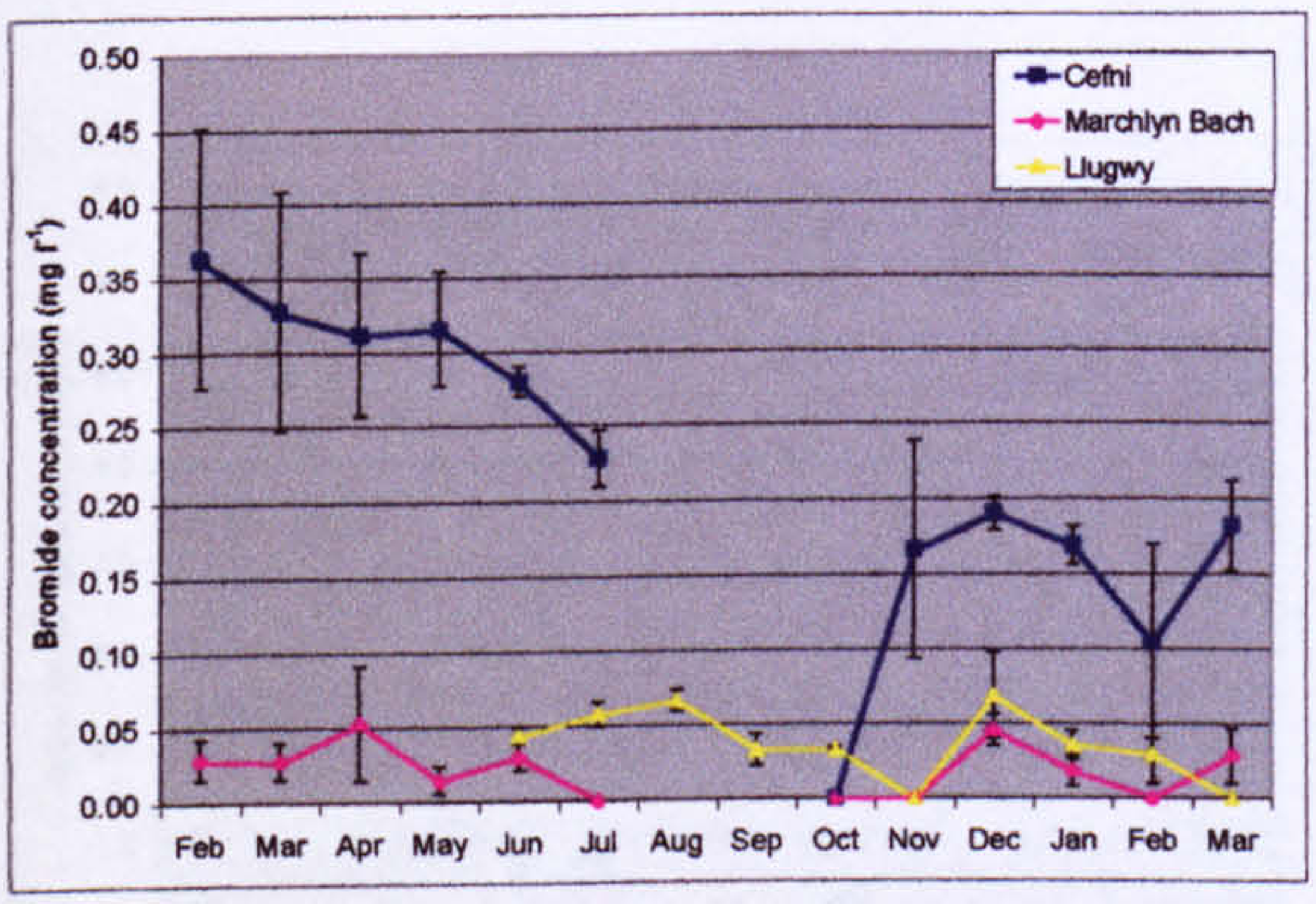

Figure 2.44 – Porewater bromide concentrations

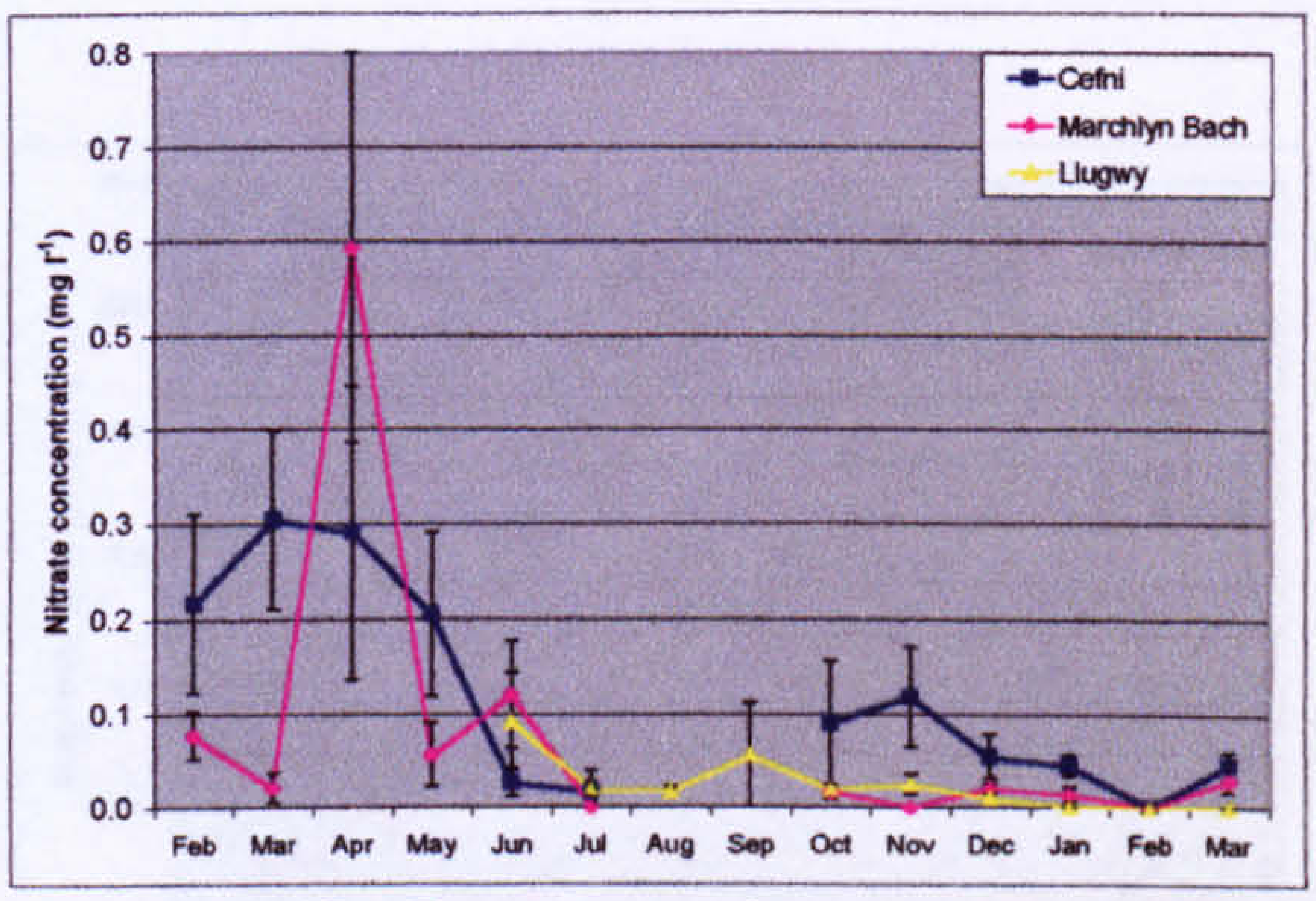

Figure 2.45 – Porewater nitrate concentrations

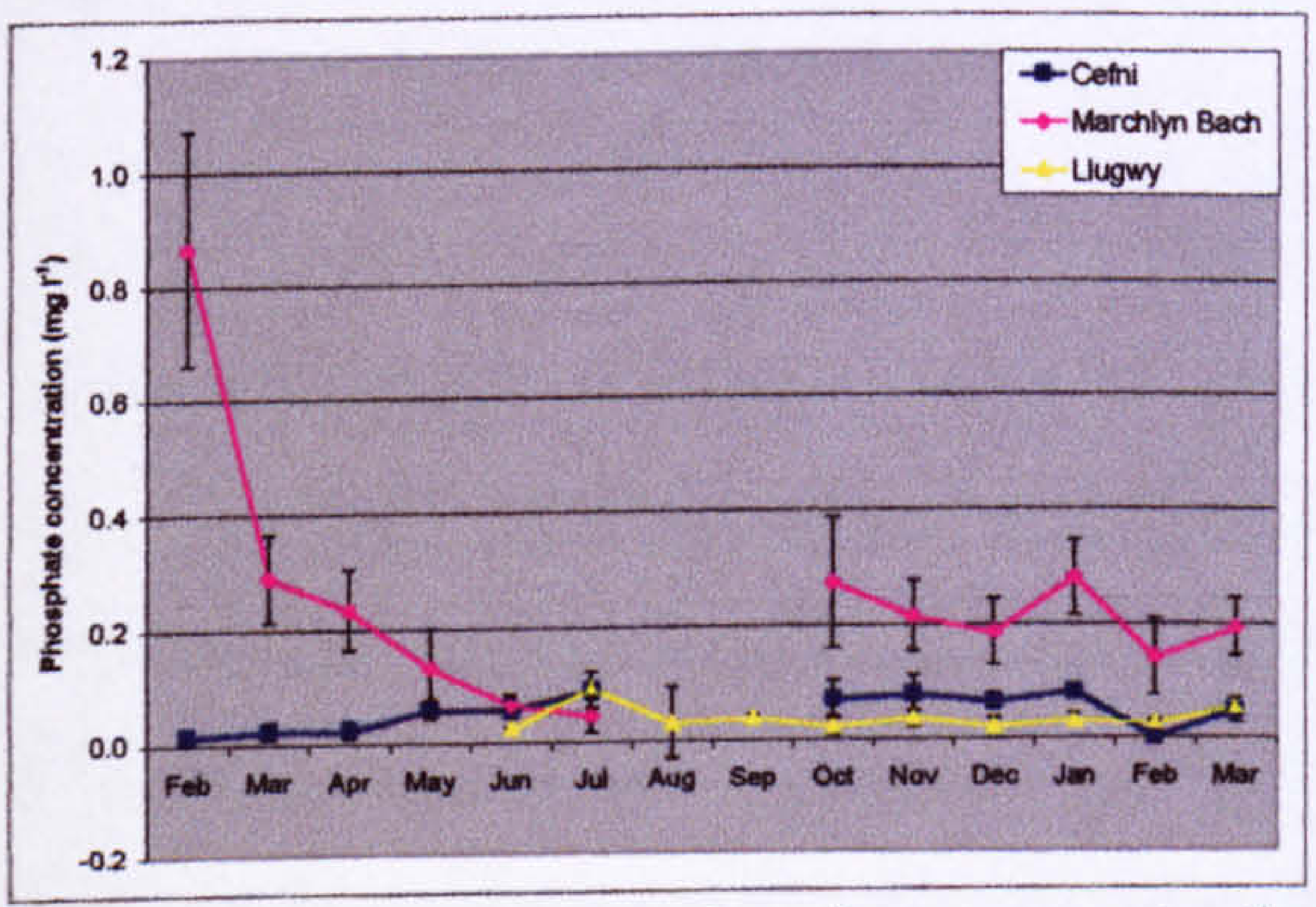

Figure 2.46 – Porewater phosphate concentrations

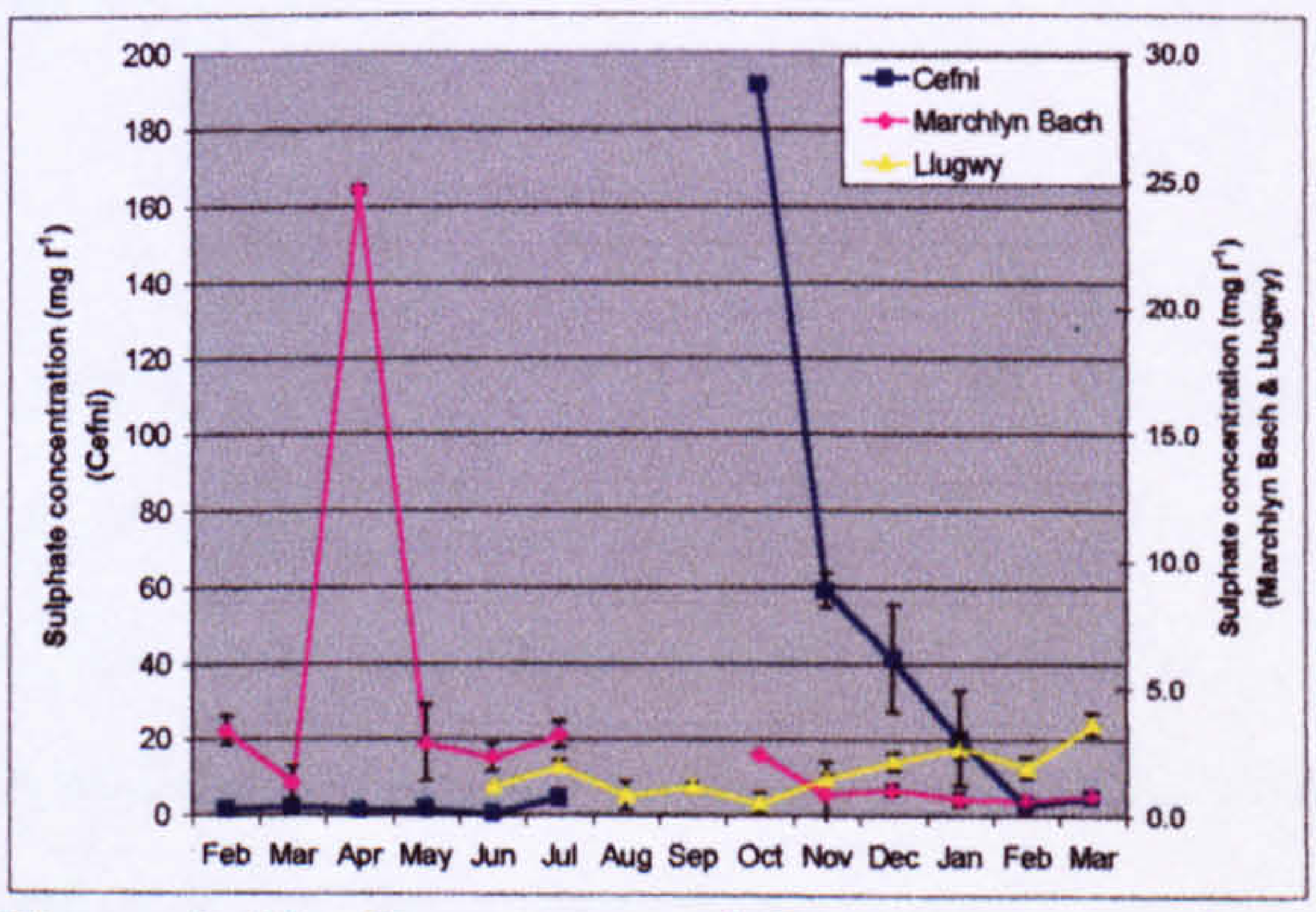

Figure 2.47 – Porewater sulphate concentrations

2.2.2.6 Cation analyses

The concentration of potassium (*figure 2.48*) varied throughout the period of study for all three sites; Cefni ($p<0.001$; F=4.62), Marchlyn Bach ($p<0.001$; F=4.62) and Llugwy ($p<0.001$; F=7.17). Concentrations were lower for the two bog soils; Marchlyn Bach did not show seasonal changes but concentrations were higher during summer than winter 2003 for the Llugwy. Conversely, potassium levels at the Cefni seemed to dip during the summer, with the highest concentration recorded in March 2003 (6.6 mg l^{-1}).

The concentration of magnesium (*figure 2.49*) also varied significantly for all three sites throughout the study; Cefni ($p<0.001$; F=4.62), Marchlyn Bach ($p<0.001$; F=4.62) and Llugwy ($p<0.001$; F=8.16). The concentration was consistently much higher at the Cefni than the two bog soils and highest during spring 2003 (approximately 11 mg l^{-1}) than the rest of the year (average of 7 mg l^{-1}). Values of magnesium at the bogs were on average 8 times lower than at the Cefni, approximately 1 mg l^{-1}.

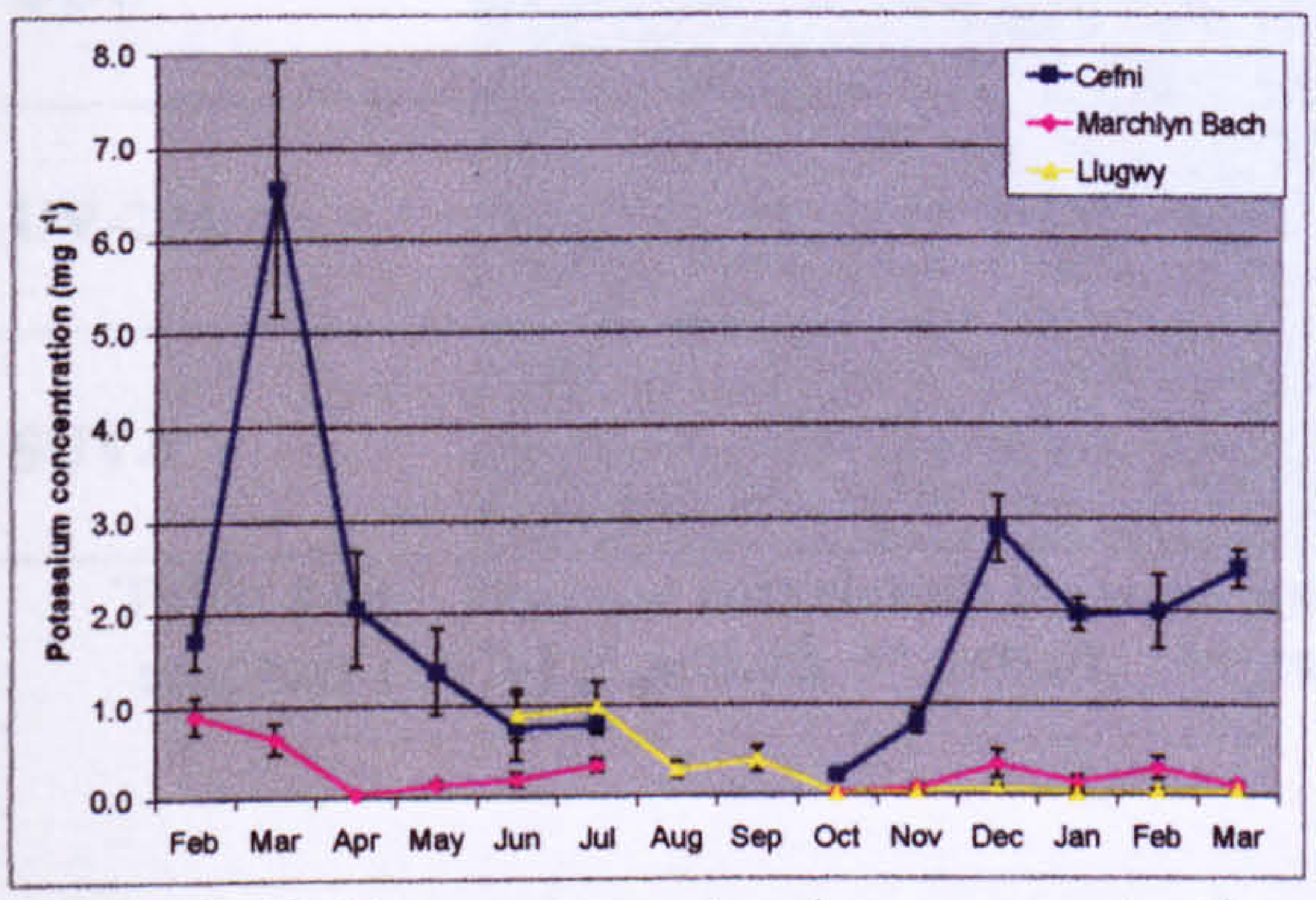

Figure 2.48–Porewater potassium concentrations

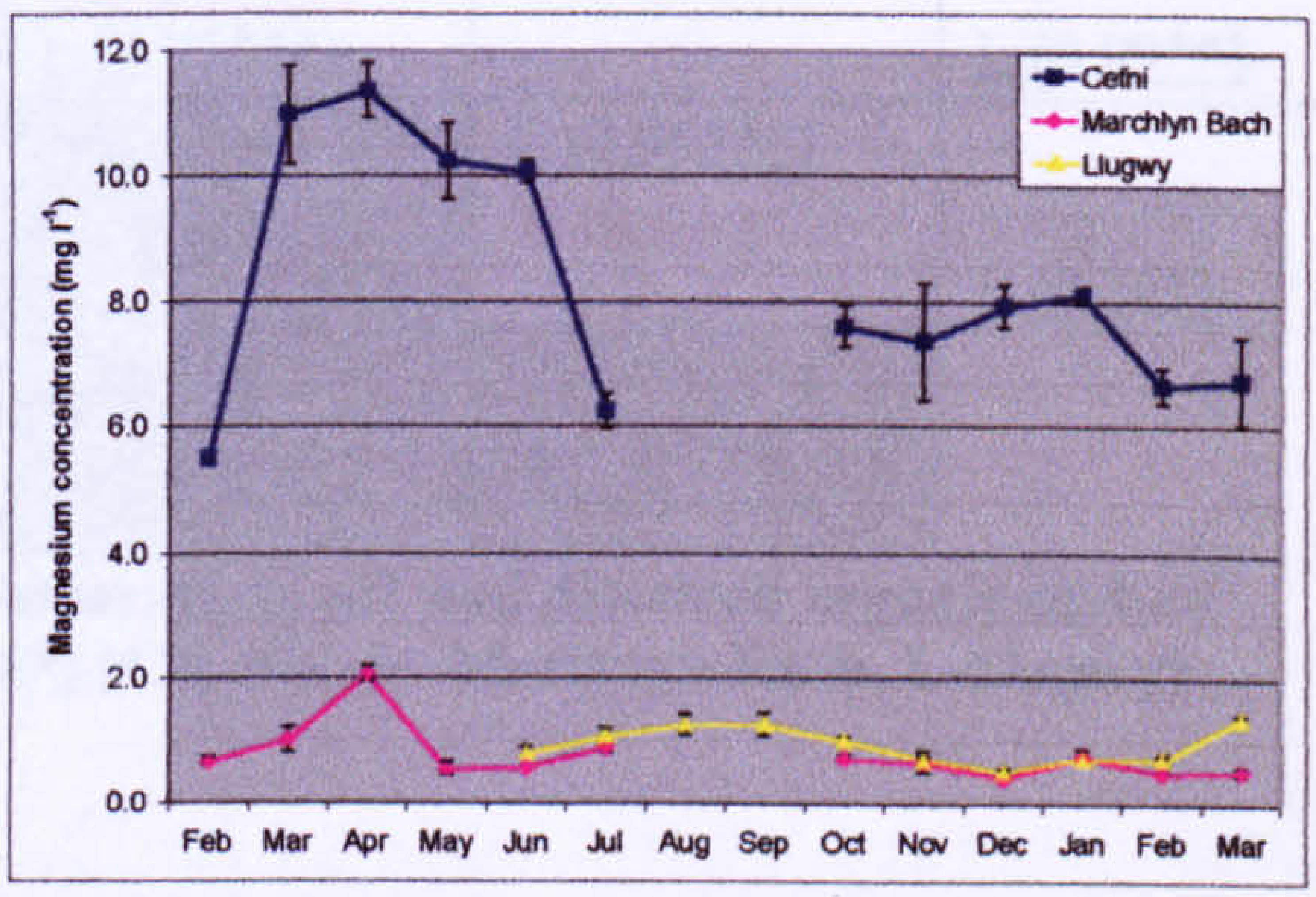

Figure 2.49–Porewater magnesium concentrations

2.2.2.7 Correlation analyses

Table 2.03 shows the significant correlations between soil temperature, pH and dissolved organic carbon analyses for all three sites. The most relevant observations are that DOC correlated positively with UV-254 for all three sites, temperature correlated positively with DOC, UV-254 and phenolics for the Llugwy and pH correlated negatively with DOC, UV-254 and SUVA of Marchlyn Bach porewater.

	pH	**DOC**	**UV-254**	**SUVA**	**Phenolics**
Temperature	L (0.67*)	L (0.79**)	L (0.76*)		L (0.84 **)
pH		M (-0.77**)	M (-0.85**)	M (-0.70*) L (0.79**)	
DOC			C (0.77**) M (0.88***) L (0.95***)	C (0.63*)	L (0.89**)
UV-254				C (0.96***) M (0.83 **)	L (0.76*)
SUVA					

Table 2.03 – Pearson correlation between soil temperature, pH and dissolved organic carbon analyses (n=70) (* *p*<0.05, ** *p*<0.01, * *p*<0.001) (C-Cefni, M-Marchlyn Bach, L-Llugwy)**

Table 2.04 displays the significant correlations between various soil parameters, enzyme activities and microbial release of carbon gases. The key observations are that temperature influenced the microbial release of CO_2 and CH_4 from Llugwy soils, the three hydrolase enzymes generally correlated positively with each other for each site and phenol oxidase correlated with the hydrolase enzymes at Marchlyn Bach.

	β-D-glucosidase	Sulphatase	Phosphatase	Phenol Oxidase	CO_2 resp.	CH_4 resp.
Temperature					L (0.74*)	L (0.70*)
pH	MB (0.74**)			MB (0.72*)		
% water content	C (0.64*) L (0.63*)					
DOC						L (0.68*)
Phenolics					L (0.80**)	L (0.81**)
β-D-glucosidase		C (0.70**) MB (0.96***)	C (0.92***) MB (0.98***) L (0.74*)	MB (0.78**)		
Sulphatase			C (0.76**) MB (0.95***)	MB (0.70**)		
Phosphatase				MB (0.72**)		
Phenol Oxidase					MB(0.85***)	
CO_2 resp.						C (0.89***) L (0.85**)

Table 2.04 – Pearson correlation between soil parameters, enzyme activities and microbial carbon gas release (n=70) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) (C-Cefni, M-Marchlyn Bach, L-Llugwy)

Tables 2.05 & 2.06 show the significant correlations between all the measured soil analyses with all of the lake analyses. Soil parameters are displayed in the left column, lake parameters on the top row. The most relevant correlations were that the soil DOC concentration positively correlated with the lake's DOC concentration at Marchlyn Bach, whilst soil DOC, UV-254 and phenolics correlated positively with lake water dissolved CO_2 concentration at the Llugwy (*table 2.05*); the pH of Marchlyn Bach and Llugwy soils positively correlated with the bromide concentration of their respective lakes and the activity of phenol oxidase and the three hydrolase enzymes within Marchlyn Bach soils correlated positively with the lake's magnesium and calcium concentration (*table 2.06*).

SOIL \ LAKE	pH	DOC	UV-254	SUVA	Phenolics	Dissolved CO_2	Dissolved CH_4
pH						L (0.66*)	
DOC		M (0.60*)	L (-0.64*)			L (0.89***)	
UV-254						L (0.95***)	
SUVA	M (-0.68*)						
Phenolics			L (-0.75*)	L (-0.69*)		L (0.69*)	C (0.59*)
Phenol Oxidase	C (0.73**)	C (-0.69**)	L (-0.64*)			C (-0.59*)	
β-D-glucosidase							
Sulphatase							C (0.55*)
Phosphatase							
CO_2 resp.			L (-0.65*)				
CH_4 resp.							M (0.64*)
Cl^-							
Br^-					L (0.79**)		
NO_3^-				C (-0.85***)			
PO_4^{2-}		M (0.78**)					
SO_4^2							
Na^+				C (-0.71*)			
NH_4^+		M (0.59*)		C (-0.71*)			
K^+		M (0.59*)					
Mg^{2+}						L (0.73*)	
Ca^{2+}			L (-0.69*)			L (0.95***)	

Table 2.05 – Pearson correlation between soil (left column) and lake (top row) parameters (n=140) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) (C-Cefni, M-Marchlyn Bach, L-Llugwy)

LAKE SOIL	Cl^-	Br^-	NO_3^-	PO_4^{2-}	SO_4^{2-}	K+	Mg^{2+}	Ca^{2+}	THMs
pH		M (0.59*) L (0.78**)							
DOC					C (-0.63*)	L (0.89***)			
UV-254						L (0.80**)			
SUVA		M (-0.71*) L (0.69*)							
Phenolics						L(0.74*)			
Phenol Oxidase							M (0.67*)	M (0.75**)	
β-D-glucosidase						C (-0.60*)	M (0.88***) L (-0.75*)	C (0.58*) M (0.91***)	
Sulphatase						C (-0.62*)	M (0.91***)	M (0.88***)	
Phosphatase						C (-0.56*)	M (0.91***)	M (0.93***)	
CO_2									
CH_4						L (0.75*)			
Cl^-	C (0.65*)				C (0.67*)				
Br^-				L (0.73*)					
NO_3^-	C (0.61*)								
PO_4^{2-}						L (0.72*)			M (-0.74**)
SO_4^{2-}			L (0.74*)		C (0.69*) L (0.71*)				
Na^+							L (0.69*)		
NH_4^+								L (0.73*)	M (-0.58*)
K^+						L (0.72*)		M (-0.59*)	
Mg^{2+}									
Ca^{2+}		M (0.61*)				L (0.85**)			

Table 2.06 – Pearson correlation between soil (left column) and lake (top row) parameters (n=140) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) (C-Cefni, M-Marchlyn Bach, L-Llugwy)

2.2.3 Discussion

The most important observation of the survey is that the soils bordering Cefni lake are unlikely to be a significant source of organic matter to the lake and are therefore not contributing to the high THM levels at this water treatment works. The concentration of porewater and leachable DOC, phenolics and the percentage of HMW DOC were all lower than would be expected for a typical wetland and could not explain the high concentrations of DOC in Cefni lake that were recorded throughout the year. In addition, the activity of the hydrolase enzymes was lowest at the Cefni, despite the soils higher pH, high ion concentrations and lower water content. This inactivity may reflect the consistently low concentration of DOC leachability of the Cefni soils; the low potential to leach carbon suggests that there is little organic matter available as a substrate within the soil matrix for the enzymes to break down, and consequently little leachable DOC. However, it is important to point out that it was assumed that the site of soil sampling was representative of the majority of soils bordering the lake. This was based on a thorough visual observation, but a more detailed study would be needed to confirm this. The situation at the two upland lakes was very different, with the soils being high in DOC and phenolics, especially in the leachable form. HPSEC-determined molecular weight spectra for Marchlyn Bach, in particular, were typical of the seasonal variation you would expect for a peatland (Li, *et al.* 2003). The spring peak was smallest, reflecting the low DOC concentration measured in April. The summer peak saw a substantial enrichment in low molecular weight material; this reflects the enhanced decomposition of organic matter that would occur in the warm summer season. During autumn and winter the peak shifted markedly towards the high molecular weight end, which can be attributed to increased vegetation death and reduced organic matter decomposition that occurs during these cooler, wetter months. Despite this evidence of organic rich catchment soils, the DOC concentrations of Marchlyn Bach and Llugwy lakes were low and the soil DOC only correlated with the lake DOC for Marchlyn Bach. This implies that the soils do not necessarily contribute substantial amounts of DOC to the lake, or perhaps that heavy rainfall, which is typical of upland catchments, and the large lake volume, dilutes the DOC influx substantially.

There was an especially strong potential for leaching DOC during the autumn. This is most likely due to changes in the organic matter quality in the acrotelm of the peat. As summer ends, vegetation death occurs rapidly, inputting large quantities of decaying plant material into the soil's surface layers. Due to the dominant *Sphagnum* vegetation in bogs, a large proportion of this material will be phenolic in nature (Wetzel, 1992), accounting for the similarly large increase in potentially leachable phenolics observed. This build up of organic matter therefore created a soil with a large reserve of potentially leachable carbon, able to be flushed out of the peat matrix; this was also observed by Worrall, *et al.* (2002). This process will have been exacerbated during 2003 because the severe drought experienced from August to mid-October will have increased decomposition and the production of water-soluble carbon with the peat matrix, whilst the associated low soil through-flow will have allowed for greater accumulation of these compounds (Scott, *et al.* 1998).

Similar to the lake water observations, concentrations of porewater DOC did not vary on a seasonal basis at the Cefni and Marchlyn Bach as would perhaps be expected. There was no rise in porewater or leachable DOC at the Cefni during the 2003-04 winter despite the rise in UV-254 absorbance and humification index values observed for the lake water; this again implies that these soils did not influence the organic matter content of the lake. At the Llugwy, however, DOC concentrations were much higher during the summer than the winter. There was also a strong positive correlation with phenolic compounds. Similar findings were observed by Kaiser, *et al.* (2001) and were attributed to greater microbial and enzyme activities during the summer months, when temperatures are higher and the soil may be drier. In this study, the peak in DOC can also be linked to enzyme activities and temperature. As would be expected, the soil temperature was highest in the summer and correlated with the DOC and phenolics concentrations. The warmer temperatures would have increased the rate of microbial respiration (Freeman, *et al.* 1996; Bonnett, 2004), as demonstrated by the positive correlation between the two parameters. Although a significant correlation was not recorded with temperature or the microbial release of carbon gases, phenol oxidase activity was higher during the summer and could be responsible for the higher DOC concentrations. The increase in DOC may also be explained by an increase in *Sphagnum* plant metabolism that would occur during the summer months; *Sphagnum* species have recently been shown to contribute rapidly and significantly to the below-ground DOC pool of peatlands (Fenner, *et al.* 2004). The impact of increased spring/summer vegetation growth can also be seen indirectly in the values of porewater pH at the Llugwy, which were higher during the summer and correlated with temperature. Similar results were observed by Conyers, *et al.* (1997) and Bonnett (2004). The rise in pH may also be partly responsible for the increase in porewater DOC, as DOC is known to be more soluble at a higher pH (Evans, *et al.* 2006).

One of the important findings of the reservoir survey was that the DOC concentration and SUVA values (and hence quantity of THM precursors) of Llugwy lake water were consistently higher than Marchlyn Bach. This may partly be due to the larger peaty catchment size, but can also be explained by observed differences in the soil properties. In the Llugwy soils, the activity of phenol oxidase was approximately twice that in Marchlyn Bach, consequently the observed concentration of phenolic compounds, known inhibitors of hydrolase enzymes (Freeman, *et al.* 1990; Wetzel, 1992) was half as high. This may partially explain the activities of the three hydrolase enzymes in the Llugwy being approximately six times as high as those recorded in Marchlyn Bach soil. However, the phenolics concentrations were similar between the soils during summertime and there was still a large difference in the hydrolase activities, so phenolics alone cannot explain the difference. There were also only minor differences in soil temperature and ion concentrations, so these factors are insufficient to account for the observations. It is well known that pH is one of the reasons why the decomposition of organic matter in peatlands is so low (Clymo, 1983), and Kang & Freeman (1999) showed that increasing the pH can increase the mineralisation of organic matter. The pH at the Llugwy was consistently higher than at Marchlyn Bach and probably at least partially accounted for the difference in enzyme activities. The greater activity of the hydrolase enzymes within the Llugwy soils suggests a much greater rate of organic matter decomposition. This was confirmed by values of the Humification Index, which, for the Llugwy, were almost half that for Marchlyn Bach, indicating the Llugwy possessed the more degraded DOC.

This data collectively suggests that at the Llugwy there was a consistently greater rate of organic matter decomposition and therefore a greater release of DOC into the lake. Also of interest was the observation that lake water dissolved CO_2 concentrations correlated positively with soil porewater DOC concentrations, both peaking in the summertime. As peat is a significant source of dissolved CO_2 to freshwaters (Dawson, *et al.* 2002), this correlation suggests a concurrent release of carbon into the lake in both the solid and gaseous forms from the organic rich soils.

Interesting data was also recorded during and at the end of the two periods of drought. During the April 2003 drought at Marchlyn Bach, the pH rose from 3.5 to 5.5. Clark, *et al.* (2006) simulated drought in peatland cores and observed an increase in SO_4^{2-} ions, due to the oxidation of sulphides (Ponnamperuma, 1972), a resulting increase in acidity and a reduction in porewater DOC. A notable reduction in DOC and a large rise in porewater sulphate (+1323%) were also observed in this study during the drought, so the large rise in pH is somewhat unusual. An important source of acidity in bogs is the build up of organic acids resulting from the incomplete decomposition of organic matter (McKnight, *et al.* 1985). It may have been that during the drought the soil became sufficiently aerobic to stimulate microbial and enzymic decomposition of these acidic compounds, a process that would release cations into the soil solution and raise the pH (Gorham, *et al.* 1984). However, apart from magnesium, there were no peaks in cation concentrations at Marchlyn Bach during the April drought. Collectively, this suggests that the relationships between drought, sulphate and acidity are not as clear cut as the data of Clark, *et al.* (2006) suggests. The contrary findings of drought and DOC interactions reported by Freeman, *et al.* (2004) and Tipping, *et al.* (1999), and DOC and acidity published by Schindler, *et al.* (1992) and Strand, *et al.* (2002) highlight the difficulties in attaining a consistent theory. An increase in porewater nitrate was also observed for Marchlyn Bach bog during the April drought; this has been measured in previous studies (Freeman, *et al.* 1993) and is due to the increased mineralisation of nitrogen-containing organic compounds resulting from water-table drawdown (Williams & Wheatley, 1988; Bayley, *et al.* 2005). The rainfall event that ended the April drought at Marchlyn Bach had an effect on all the analyses undertaken. Soil temperature lowered appreciably (4°C reduction), pH reduced from 5.5 to 3.5, the activity of the soil enzymes fell by 58-87% and microbial respiration dropped by 63%. This shows that the moisture content of soils plays a critical role in regulating processes occurring with the soil. The severe summer drought which peaked in September 2003 led to a considerable increase in the activity of all three hydrolase enzymes and phenol oxidase at Marchlyn Bach. This is not surprising; the lowering of the water-table that occurred would have increased the aeration of the peat (Worrall, *et al.* 2002), raising the concentration of bimolecular oxygen and stimulating the activity of phenol oxidase (Pind, *et al.* 1994). This would have reduced the concentration of inhibitory phenolics (Freeman, *et al.* 2001*b*), leading to the observed increase in hydrolase enzyme activities. Unfortunately, the lack of porewater due to extensive water-table drawdown during this summer drought meant that a comparison of porewater pH and sulphate levels with those recorded during the April drought was not possible.

As with the lake water, the ion concentrations of the soils highlight the contrasting hydrological influences at the upland and lowland sites, with values for the Cefni generally much higher. This can be attributed to the greater influence of the sea and groundwater. The two major sea ions are sodium and chloride, with calcium, potassium, magnesium and sulphate also present in high concentrations (Pilson, 1998). All of these ions were present in greater concentrations within the Cefni soils and lake and can be partially attributed to the site's greater proximity to the sea. These significant inputs of base cations explain the greater pH and conductivity in both the soil and lake waters when compared to the bog sites, whose pH is much lower due to the presence of acidic organic acids within and leaching from the peat soils (Kortelainen & Saukkonen, 1995), and the lesser marine influence. Of all the measured ions, bromide is the most important in this study because of its effects on THM speciation. The higher concentrations in the Cefni soil porewater compared to the bog soils again represents the greater marine influence of the lowland site; a one-off measurement of the anion levels of rainfall incident at the Cefni revealed a bromide concentration of 0.277 mg l^{-1} (data not shown), similar in magnitude to that recorded in Cefni soils and lake water. Bromide can be strongly retained in soils with a high concentration of organic matter (Vinogradov, 1959; Biester, *et al.* 2004); the soils surrounding the Cefni are therefore not likely to represent a buffer zone where bromide ions are retained, due to their low concentration of organic material. Instead bromide ions are free to leach directly into the lake, which has consequences for THM formation. The lack of a concurrent autumn and winter bromide peak in the Cefni soils that matched the trend for the lake water would also seem to suggest the soils were not a significant source of bromide. The high values of bromide in Cefni lake during autumn and winter (*figure 2.21*) probably reflect the greater amount of rainfall at this time of the year. The situation concerning bromide at the upland bog catchments is opposite to that at the Cefni, with a lesser marine influence and input of bromide ions, but the soils have a much higher composition of organic matter. Interestingly, concentrations of bromide within the two upland lakes correlated positively with the catchment soil's pH. This suggests that raising the pH of the bog soil can release bromide, probably due to enhanced organic matter decomposition and the mineralisation of bromide-binding organic compounds. The significant correlation between soil pH and the key carbon cycling enzymes β-D-glucosidase and phenol oxidase at Marchlyn Bach would seem to suggest this is viable.

The concentration of total DOC determined by the combustion method of the Shimadzu TOC-5000 correlated strongly and positively with spectrophotometric absorption at 254nm for all three soils. The use of UV-254 absorbance is rare for soil waters but these correlations show that it can be used as a reliable, time-saving surrogate measurement for DOC concentrations.

The lack of strong, significant or consistent correlations between the basic soil parameters (pH, temperature, etc.), soil enzymes and microbial respiration show the difficulty in assessing soil processes over a range of different soil types. For example, whereas temperature correlated with the microbial release of CO_2 and CH_4 (as might be expected) within soils from the Llugwy, the same finding was not observed for Marchlyn Bach, despite being a similar upland bog. This is testimony to the vast number of environmental variables that can influence soil processes; for examples, the activity of hydrolase enzymes can be affected by pH, temperature, water content, oxygen availability, presence of inhibitory phenolics and iron, ion concentrations, microbial abundance, etc. (Brock & Bregman, 1989; Freeman, *et al.* 1990; Sinsabaugh, *et al.* 1991; Freeman, *et al.* 1995; Freeman, *et al.* 2001*b*), all of which will vary between sites.

2.2.4 Conclusions

A number of interesting observations and conclusions can be drawn from the data presented in chapter 2:

a) The sampled wetland soils immediately bordering Cefni lake appeared to leach only very low levels of DOC and phenolics, and have very low leachable carbon potentials, suggesting they may not be important sources of DOC to the lake,
b) Significant correlations between raw and treated water DOC and final water THM concentrations at the Cefni indicate that the more organic matter in the lake, the greater the formation of THMs at the treatment works,
c) Dissolved methane in Cefni lake correlated with the final water concentration of THMs at the treatment works,
d) The much greater formation of brominated THMs at the Cefni treatment works compared to Mynydd Llandegai would seem to be due to the greater concentration of bromide in the lake itself,
e) Temperature correlated with final water THM concentrations at the Cefni,
f) Cefni lake appears to be eutrophic during the summer. Input of nutrients, presumably from the farmland which surrounds all sides of the lake, coupled with high summer temperatures and high DOC concentrations, support the growth of algal blooms,
g) Despite their apparent similarity, the Llugwy contains more total DOC and more THM-forming DOC than Marchlyn Bach. This appears to be due to the greater rates of organic matter decomposition within the Llugwy catchment soils and the resulting extra release of DOC.
h) Drought can increase soil temperature, pH, and nitrate and sulphate concentrations, whilst stimulating soil enzyme activities. This can lead to an increased build up of DOC compounds in the soil but a reduced leaching of carbon due to a lower soil through flow. Rainfall has the opposite effect and washes large concentrations of organic carbon out of the soil profile.

i) The strong influence of ground- and seawater at the lowland Cefni dramatically increases the concentration of ions such as chloride and sodium; levels at the upland sites were much lower.
j) Absorbance at 254nm is a reliable, cost effective alternative for measuring carbon concentrations in soil water due to its strong, positive correlation with DOC.

Based on these conclusions, a number of follow-up experiments are required:

a) Is there a dominant source of DOC to Cefni lake that can explain the lakes high DOC concentration?
b) Can dissolved methane in water form THMs upon chlorination?
c) How does the THM formation potential of algal DOC contrast to terrestrially-derived DOC?
d) Is there a source of bromide to Cefni lake in addition to rainfall?

Chapter 3

Identifying the most important sources of terrestrial and non-terrestrial THM precursors to Cefni lake

3.1 Terrestrial THM precursors

3.1.1 Introduction

There are likely to be a number of sources of dissolved organic carbon to Cefni lake, due to both the large number of inflowing streams, which will supply terrestrially-derived organic matter, and the high lake water temperature and ion concentrations, which aid the autochthonous production of DOC. The latter will be addressed in section 3.2, whilst this section will deal with terrestrial sources of DOC. Analysis of DOC concentrations and characteristics, presented in chapter 2, demonstrated that the sampled wetland soils surrounding Cefni lake seem to release too little DOC to be a major allochthonous source of organic matter. The aim of this chapter was therefore to determine if there was a dominant point source of terrestrial DOC and THM precursors. A series of measurements were undertaken on soils located at selected points within the catchment of the Cefni, in addition to the major inflowing streams. Dissolved organic carbon compounds are poorly defined and structurally very complex (Thurman, 1985). However, when derived from a certain source they can retain the structural characteristics from where they originated (Lu, *et al.* 2004). For example, Wilson, *et al.* (1980) identified a high percentage of aromatic compounds within the DOC composition of an upland lake in north Wales and attributed it to leaching of carbon from bog peatlands within the lake's catchment.

One of the best methods currently available for the analysis of DOC structures is NMR (Nuclear Magnetic Resonance) spectroscopy. For an experiment such as this, ^{1}H-NMR is most useful, as it offers quick resolution of the major structural groups of NOM with a limited sample weight (Grasso, *et al.* 1990; Cook, 2004). It targets protons bonded to carbon atoms and can therefore be used as an indirect method for determining the structure of carbon compounds. ^{13}C-NMR, which is often used for quantitative determination of soil organic carbon compound structures, is unpractical for the characterisation of lake water DOC because the large amount of sample needed (due to low natural abundance of ^{13}C) requires the collection of hundreds of litres of lake water. ^{1}H-NMR has been used with great success for characterising soil humic acids (Lentz, *et* *al.* 1977), marine humic acids (Hatcher, *et al.* 1980), soil and freshwater stream humic substances (Malcolm, 1990), and for assessing changes in DOC quality during drinking water treatment (Kim & Yu, 2005). The ability of ^{1}H-NMR to ascertain the percentage of compounds that are aromatic is useful because it is these types of compounds that are most reactive with chlorine (Liang & Singer, 2003). It is often stated that ^{1}H-NMR is best used for 'fingerprinting' freshwater organic matter from particular sources (Fujita, *et al.* 1996; Lu, *et al.* 2004) and for estimating the relative abundance of functional groups (Cardoza, *et al.* 2004), rather than identifying specific compounds, and this is how it will be used in this investigation.

In order to identify potentially important sources of organic carbon to Cefni lake, soils at seven locations from within 100 metres of the lake were sampled, in addition to Cors Erddreiniog fen located 5 km to the north of the lake. The five main inflowing streams were also sampled, as well as raw water from Cefni lake itself.

3.1.2 Materials and methods

3.1.2.1 Experimental design, site descriptions and sample collection procedures

The sampling sites for soils and inflowing streams around the Cefni are shown in *figures 3.01 & 3.02*. Most of the soils are typical of arable farmland soils within the UK, being dominated by grassland vegetation and having a poor soil structure due to the action of ploughing. They could therefore probably be classified as mollisols (Wild, 1993), although a more thorough investigation would be needed to confirm this. The two soils not falling within this category are those from 'Cors Erddreiniog', which is a typical calcareous fen and is dominated by *Schoenus nigricans*, *Juncus subnodulosus* and *Carex rostrata* species, and 'Frogwy', which is a marsh-like wetland similar to that surrounding Cefni lake. The sites for soil extraction were chosen for their proximity to the streams flowing into the Cefni and after a visual determination of the extent of their representation of the local soils. At each site, five 8 x 12 cm blocks of soil were extracted with a sharp knife and transported back to the laboratory in plastic bags to prevent water-loss.

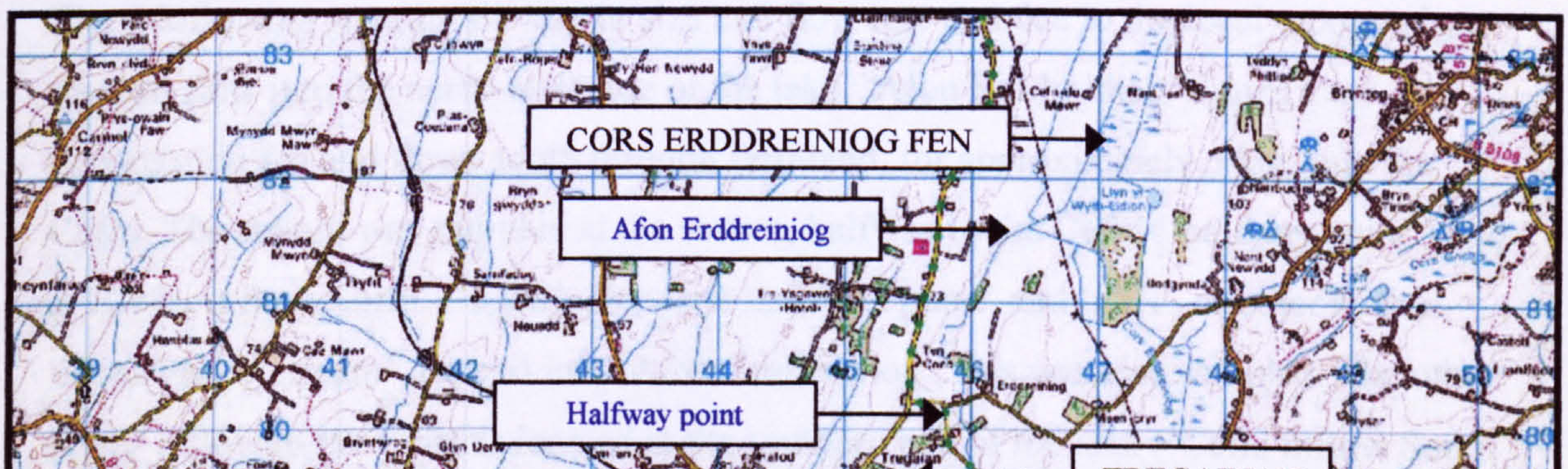

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Figure 3.01

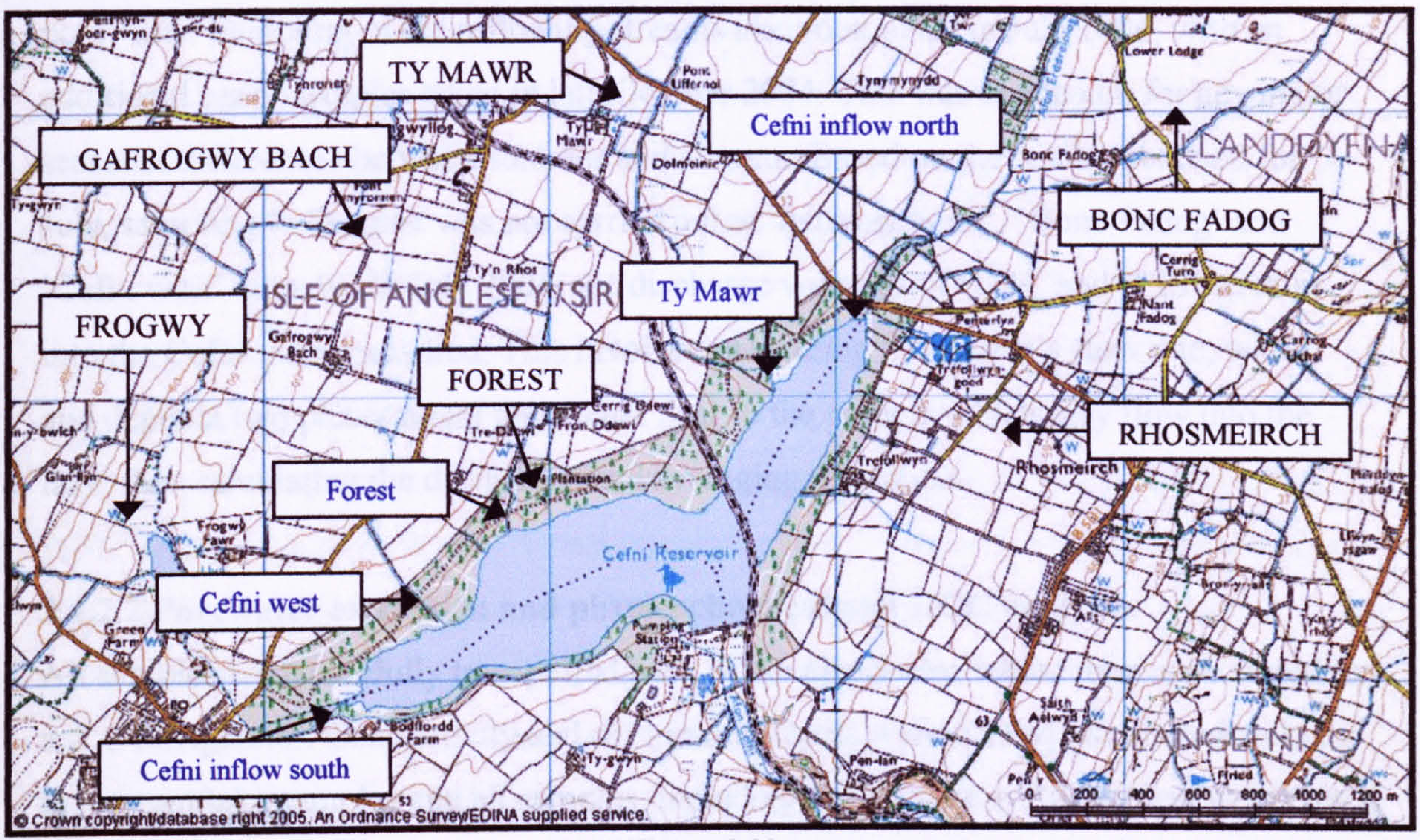

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Figure 3.02

Figures 3.01 & 3.02 – Sampling sites within the catchment of the Cefni reservoir showing sites where soil (black, upper case letters) and stream water samples (blue, lower case letters) were taken.

The 5 inflowing streams all vary in size and flow rate and due to the local topography, they all flow into the north-west side of the lake. 'Afon Erddreiniog' drains Cors Erddreiniog fen and flows south through farmland, for approximately 5 km, into the Cefni. The stream was sampled at the source, halfway to the Cefni ('halfway point') and at 'Cefni inflow North' ('CIN'). Between 'halfway point' and 'CIN', another stream, 'Bonc Fadog stream', flowed into 'Afon Erddreiniog'; this was also sampled. The other major inflow to the Cefni is located at the southern end of the lake ('Cefni inflow South' – 'CIS') and flows through mainly farmland. The other sampled streams, named 'Ty Mawr', 'Forest' and 'Cefni west' for the purposes of this study, are minor in comparison and have a much lower discharge.

Water from each stream was collected in five pre-ashed (550 °C) 20 ml glass vials, with an additional 1 litre sample collected in an amber glass bottle for ^{1}H-NMR analysis.

Cefni lake water was collected from the raw water tap at the treatment works.

The sampling regime was carried out once for the soils, in early July 2004, and during a period of normal rainfall, thereby ensuring the soils were sufficiently moist to extract porewater. Sampling of the inflowing streams also took place in July 2004, with an additional set of samples taken in late October 2004. This was to account for any major seasonal differences between summer and autumn. Based on the results obtained for July, sampling in October was not carried out at 'halfway point', 'Bonc Fadog' and 'Cefni west' streams. In July 2004, the discharge volumes of 'CIN' and 'CIS' streams into the Cefni were measured. This involved measuring the stream's flow rate, width and depth at two places along the stream near to the point at which they flow into the lake, then calculating the discharges and averaging the values.

3.1.2.2 Porewater extraction and physicochemical and DOC analyses

All samples were carefully transported back to the laboratory, where they were stored in a 4°C refrigerator. Conductivity and pH measurements were carried out immediately for the stream/lake samples and all samples, including those in the 1 litre bottles, were then filtered through sterilised 0.45 μm membranes within 24 hours.

For the soils, porewater was extracted using the centrifugation technique described by Reynolds (1984). For each block of soil, three bespoke centrifuge tubes were filled with approximately 50 cm^3 of soil from a depth of 10 cm. The tubes were then centrifuged at 10,000 g for 20 minutes in a Beckmann J2-21 centrifuge. This process yielded between 10-20 ml of soil water per tube, depending on the moisture content of the soil. These samples were then analysed for pH and conductivity and filtered, as described above. For each of the five replicate blocks, 20 ml of the porewater was combined into a composite of 100 ml for ^{1}H-NMR analysis.

Analyses of DOC and phenolic compound concentrations, UV-254 absorbance values, HPSEC-determined molecular weight and ion concentrations were then carried out on all soil and lake water samples (please refer to chapter 2 for methods). In addition, UV spectrophotometer absorbance readings were also made at 250 and 365 nm. The ratio of values at these wavelengths, known as the E2:E3 ratio, has been demonstrated to be *inversely* proportional to the size and molecular weight of DOC compounds (Osburn, *et al.* 2001).

3.1.2.3 ^{1}H-NMR analysis

^{1}H-NMR analysis was undertaken in October only for the stream samples as the presence of autochthonous DOC during July would have made it difficult to distinguish the terrestrial source of DOC to Cefni lake. Data from Welsh Water showed that chlorophyll *a* levels were very low in the Cefni during October 2004 (*figure A05*, Appendix A3); therefore the input of autochthonous DOC would also have been low. One hundred millilitres of soil water and 1 litre of lake water were individually freeze-dried to remove the water molecules and to extract the dissolved materials, including DOC. This involved decanting the samples into round-bottomed, sterilised glass flasks and, whilst continuously rotating the flask, rapidly freezing the samples in liquid nitrogen. This created a 'shell' of frozen water on the inside of the flask and maximised the surface area, thereby reducing the length of time for the sublimation process. The extracted powder was placed into a 2 ml centrifuge tube and dissolved in 0.6 ml NaOD (0.54 ml D_2O + 0.06 ml 5 M NaOH), similar to the methods of Gillam & Wilson (1985) and Ma, *et al.* (2001). The tubes were placed on an IKA KS501 orbital shaker for 4 hours to ensure that the sample was completely dissolved and then centrifuged at 7,200 g to remove any particulates that may interfere with the NMR process (Ma, *et al.* 2001). The supernatant was then transferred to a 5 mm NMR tube and analysed on a Bruker 500 MHz superconducting NMR spectrophotometer. Each sample was run for 512 scans to ensure a sufficiently high signal:noise ratio, each with an acquisition time of 3.17 seconds and using a sweep width of 10330 Hz, a 30° pulse and a 2 second pulse delay. During data processing, a line broadening of 0.3 Hz was applied and integration was performed with WinNMR, version 6.1 software, using the D_2O signal at 4.9 ppm as a reference.

3.1.2.4 Trihalomethane Formation Potential (THMFP)

The formation of trihalomethanes was measured using adapted versions of procedure 5710 in 'Standard Methods for the Treatment and Examination of Water and Wastewater' (American Public Health Association, 1992) and the methods given in Goslan (2003). For the purposes of this investigation, the THMFP was determined for 'Afon Erddreiniog', 'CIN' and 'CIS' samples.

All glassware for THM analysis had previously been thoroughly cleaned using phosphate-free detergent and soaked overnight. It was then rinsed with tap water and then three times with milli-q water and allowed to dry. All reagents were prepared in advance using the purest quality chemicals commercially available and milli-q water with a resistance of 18.2 MΩ.

The chlorine dosing solution (1000 mg l^{-1} Cl_2) was prepared following the titrametric determination of the volume of sodium hypochlorite solution required. A phosphate buffer solution was prepared using potassium dihydrogen phosphate and sodium hydroxide, the use of which eliminates concerns over pH differences between samples. The samples were chlorinated based on their DOC concentration; 5 mg Cl_2 l^{-1} per 1 mg l^{-1} DOC, where 0.125 ml of chlorine dosing solution equals 5 mg Cl_2 l^{-1}. Therefore, a sample of 10 mg l^{-1} DOC requires 1.25 ml of chlorine dosing solution. The appropriate volume of chlorine dosing solution was transferred to a 25 ml amber vial with a PTFE-lined screw cap and 0.5 ml of phosphate buffer added. The vial was completely filled with sample and capped to ensure there was no headspace, with replicates of five used per stream. The samples were stored for 7 days at 25°C in a darkened incubator.

The samples were analysed using a headspace technique. Following the 7 day chlorination procedure, 10 ml of sample from each vial was discarded and the vials recapped. Each vial was analysed separately and the procedure involved submerging the vial in a Grant Instruments W14 water bath at 90°C for 10 minutes; this converted the THMs to a gaseous form in the newly created headspace. After heating, the vial was left to stand for 1 minute to equilibrate. Using a Hamilton 2.5 ml gas-tight syringe, 2.0 ml of headspace was drawn from the headspace and injected into the 500 µl sample loop of a Hewlett Packard 5890 gas chromatograph. The GC uses a flame ionisation detector and a 100% dimethyl polysiloxane fused silica capillary column (Restek), with dimensions of 30 m x 0.53 mm i.d. and a film thickness of 7.0 µm. The carrier gas was helium, the injector temperature was 200°C and the detector temperature was 300°C. The temperature programme used was 60°C for 2 minutes and a 3°C/minute rise to 84°C and each analysis ran for 10 minutes. The chromatograms were processed using a Hewlett Packard Controller 7673A and a Varian Star 800 module interface.

Calibration was performed using standards prepared in the laboratory. Solutions of varying known concentrations of the four THM compounds were prepared from stock solutions of chloroform, bromodichloromethane, dibromochloromethane and bromoform (Aldrich, >98% purity). The solutions were prepared in ultra pure water (Elga Option 4) which had been boiled down to half its volume and allowed to cool. This was performed to increase the purity of the water and remove any THMs that may have been present. Calibration was performed once a month and the samples were prepared when needed as they were not suitable for storage. Each time the GC was used a blank and standard were analysed at the beginning of the day to check for machine reliability and calibration performed again if the standard was more than 10% out.

3.1.2.5 Statistical analyses

To enable comparison of the THM yields from different sources of organic matter, and with data from following chapters, THM values were normalised to the DOC concentration (to give THM yields in micrograms of THMs per milligram of DOC). *Figures 3.05-3.08 and 3.13-3.15* show data for one replicate and *figures 3.03-3.04, 3.09-3.12 and 3.16-3.18* show mean values for 5 replicates (n=5), with error bars indicating ± standard error of the mean. Significant differences between mean values were tested for using General Linear ANOVA analysis and the Tukey-Kramer multiple comparisons post-hoc test. Pearson correlation coefficient was performed to test for significant correlations between the various analyses for soil and stream/lake water samples separately. Statistical analyses were performed using SPSS version 9.0 (SPSS, Inc.) and Graphpad InStat version 3.05 (Graphpad Software, San Diego, California, USA).

3.1.3 Results

3.1.3.1 Catchment soils analyses

3.1.3.1.1 Dissolved Organic Carbon analyses

The concentration of DOC (*figure 3.03*) at 'Cors Erddreiniog' fen averaged 95.9 mg l^{-1} and was significantly higher than any of the other soils ($p<0.001$). The concentrations of DOC for the other 7 soils averaged approximately three times less than the fen and were all similar in magnitude, ranging from 13.3 mg l^{-1} for 'Frogwy' to 31.8 mg l^{-1} for 'Rhosmeirch'. Values of UV-254 absorbance correlated with DOC concentrations significantly ($R^2=0.96, p<0.001$).

SUVA values (*figure 3.04*) were generally similar for each of the 8 soils and averaged approximately 2.0 L(mg m). The highest value was for 'Forest' (3.2 L(mg m)), which was significantly higher than most of the other soils ($p<0.05$-0.001), and the lowest was for 'Rhosmeirch' (1.2 L(mg m)), which was significantly lower than 3 of the other soils ($p<0.05$-0.001). For the 8 soils, the concentration of phenolic compounds correlated positively with DOC ($R^2=0.93, p<0.001$) and UV-254 absorbance ($R^2=0.83, p<0.05$). The value at 'Cors Erddreiniog' fen (4.7 mg l^{-1}) was significantly higher and the value at 'Frogwy' (0.3 mg l^{-1}) significantly lower than any of the other soils ($p<0.001$).

Molecular weight spectra, displayed in *figure 3.05*, show that, as expected, the soil with the highest DOC concentration, 'Cors Erddreiniog', also had the greatest peak height. *Figure 3.06* shows the contribution of each of the three molecular weight fractions. The soil at 'Gafrogwy Bach' had the highest %HMW fraction, 'Cors Erddreiniog' fen the highest %IMW fraction and 'Bonc Fadog' the highest %LMW fraction. 'Cors Erddreiniog' fen also had the lowest %LMW fraction of all the soils.

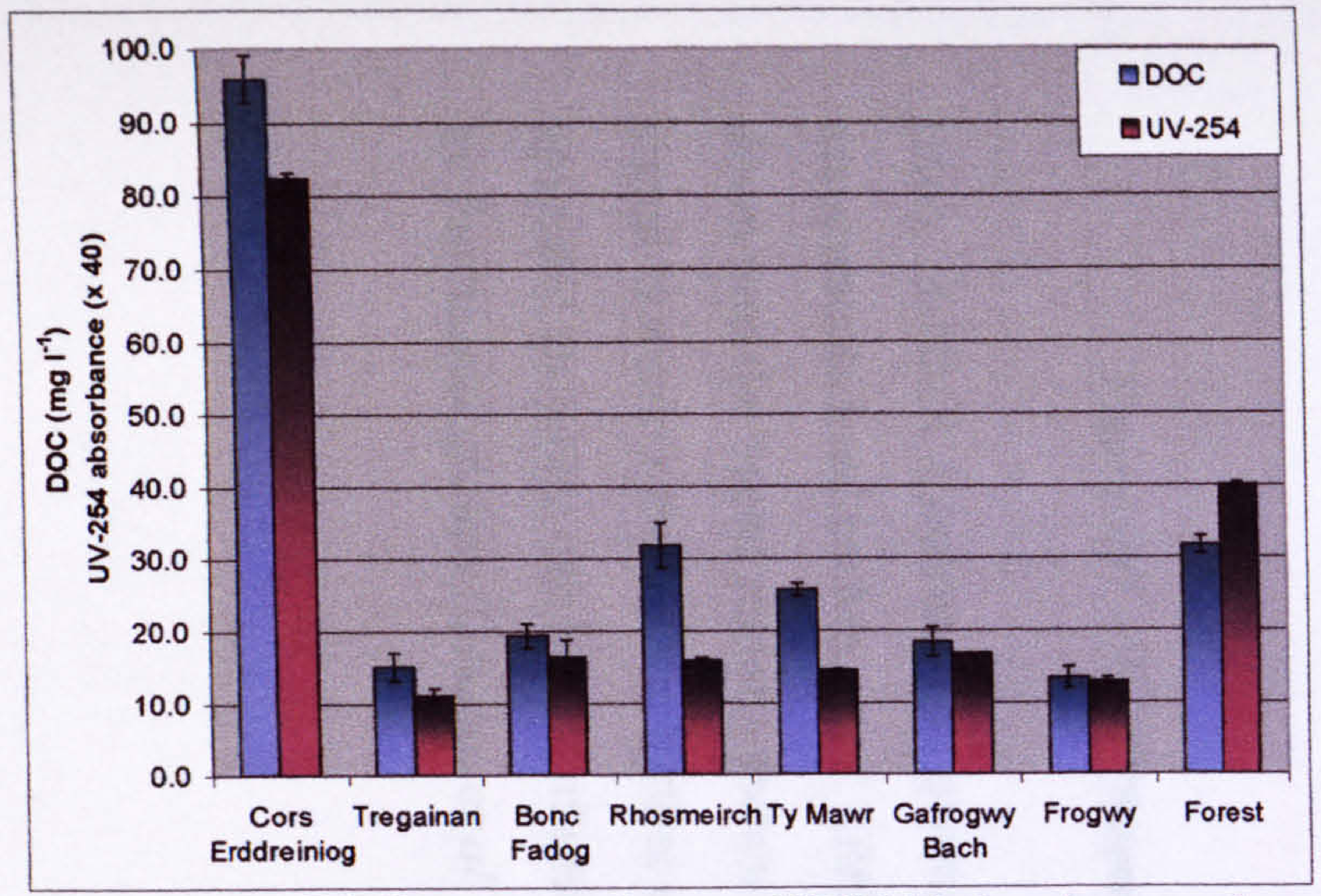

Figure 3.03 – DOC concentrations and UV-254 absorbance values of porewater from each of the 8 soils.

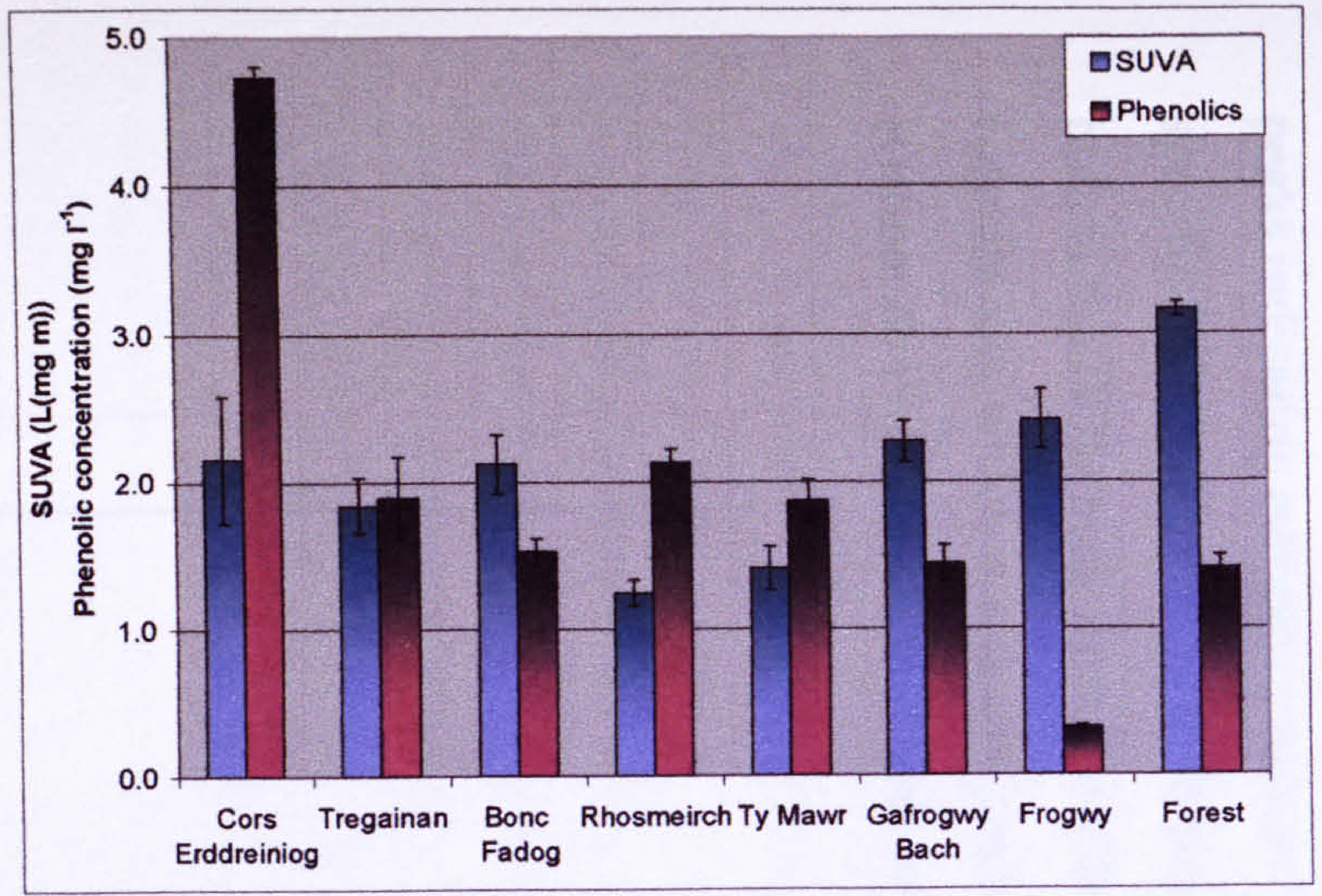

Figure 3.04 – SUVA values and phenolic compound concentrations of porewater from each of the 8 soils.

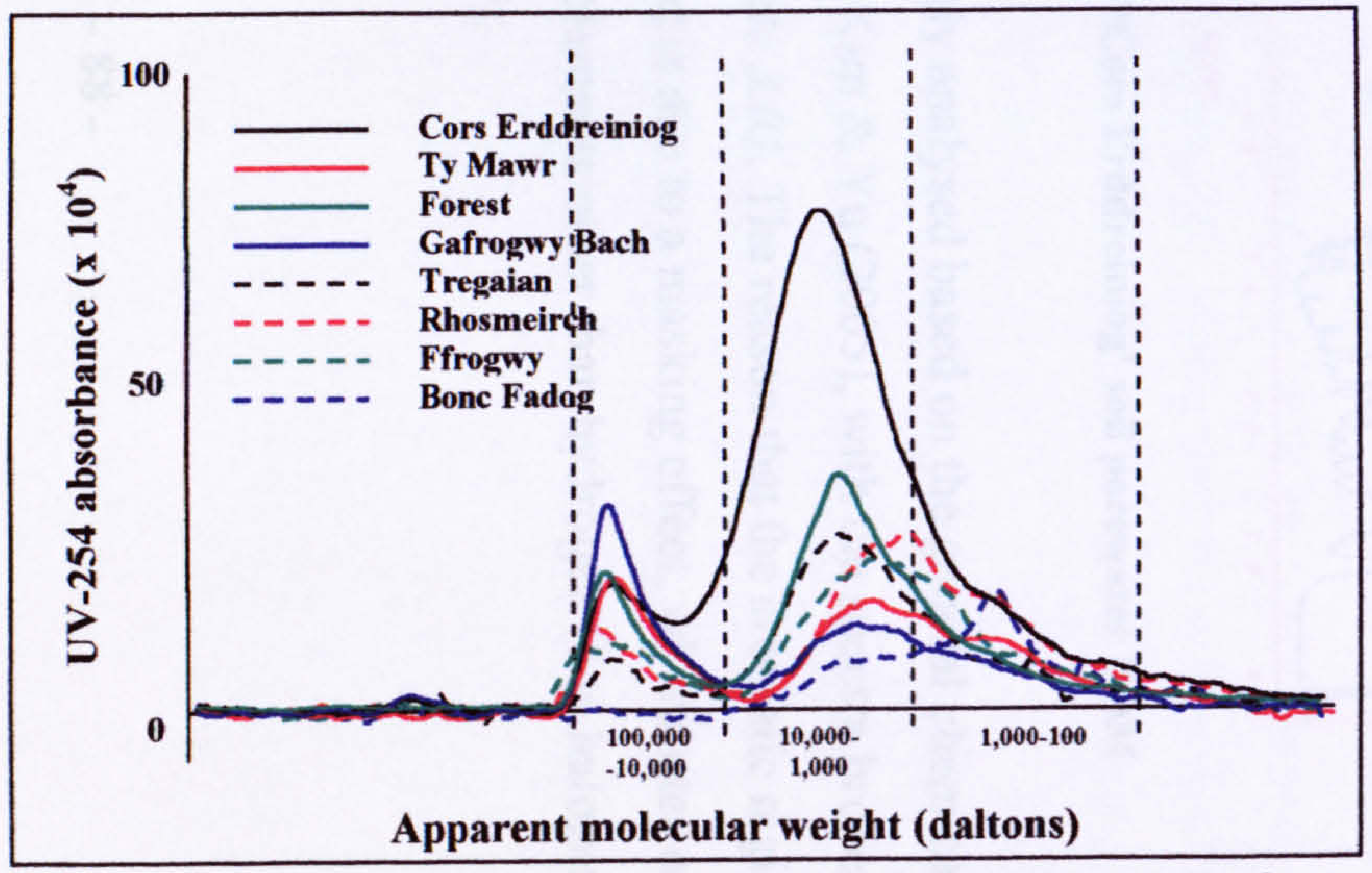

Figure 3.05 – HPSEC-determined molecular weight spectra of porewater from each of the 8 soils.

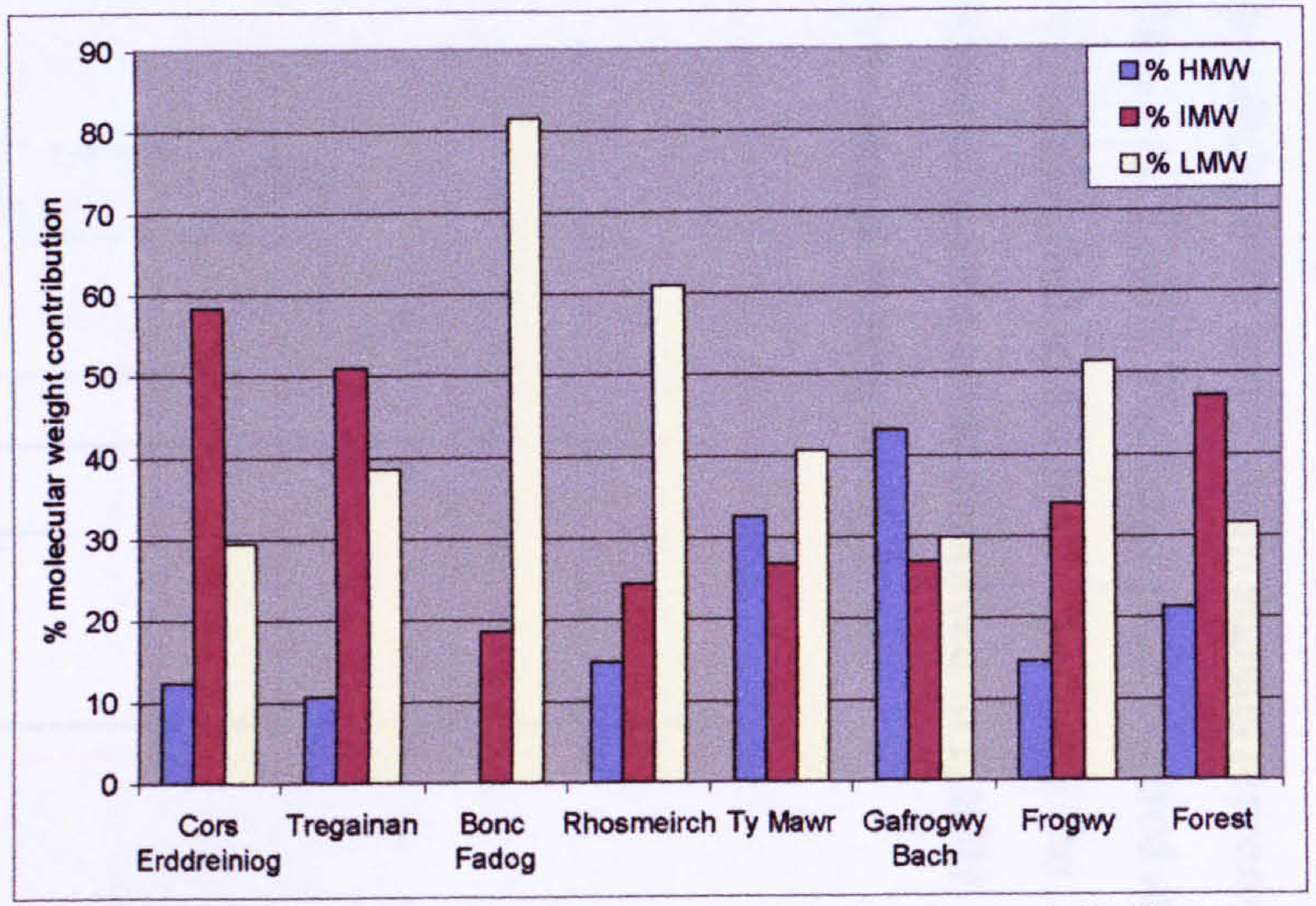

Figure 3.06 – The contribution of each molecular weight fraction to the porewater DOC pool of each of the 8 soils.

3.1.3.1.2 ^{1}H-NMR spectroscopy

The spectra shown in *figure 3.07* is of 'Cors Erddreiniog' soil porewater DOM and is typical of the type of spectra obtained for DOM, being dominated by broad unresolved peaks indicating complex mixtures of compounds, with some sharp signals representing specific functional groups or structures (Ma, *et al.* 2001). The large peak centred at 4.9 ppm is the HOD peak from the solvent D_2O, whilst that at 8.4 ppm represents the formate ion, which is a decomposition product of humic acids dissolved in NaOH (Gillam & Wilson, 1985). These peaks were ignored during integration.

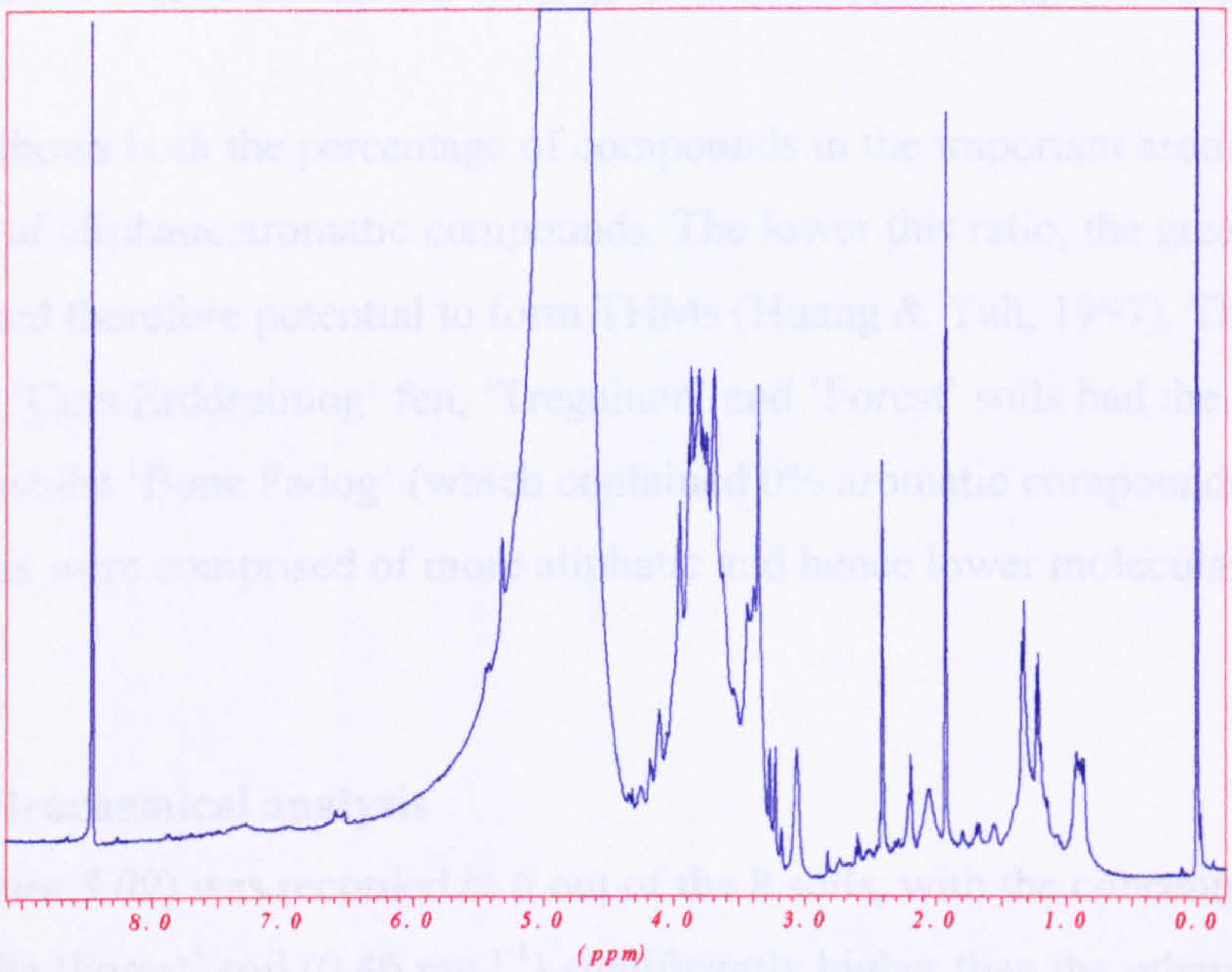

Figure 3.07 – ^{1}H-NMR spectra of 'Cors Erddreiniog' soil porewater DOM

Each ^{1}H-NMR spectrum was quantitatively analysed based on the general chemical shift assignments used by Ma, *et al.* (2001) & Kim & Yu (2005), with the spectra broken down into the broad regions shown in *table 3.01*. The reason that the aromatic region signal is weaker than those further upfield is due to a masking effect, where sites on the aromatic structure can be substituted by elements other than hydrogen (e.g. halogens), or by functional groups (Grasso, *et al.* 1990).

Chemical shift region (ppm)	Type of compounds found in this region
I (0.0 – 1.6)	Aliphatic methyl and methylene
II (1.6 – 3.2)	Protons of the methyl and methylene groups α to aromatic rings, protons on carbons in α position to carbonyl, carboxylic acid, ester, or amino acid
III (3.2 – 4.3)	Protons on carbon or hydroxyl, ester and ether, and protons on methyl, methylene and methyne carbons directly bonded to oxygen and nitrogen
IV (6.0 – 8.5)	Aromatic protons including quinines, phenols, oxygen containing hetero-aromatics
P_{Al}/P_{Ar} ratio	Ratio of aliphatic to aromatic protons (regions I & II/region IV)

Table 3.01 – ^{1}H-NMR chemical shift regions and the type of compounds detected

Figure 3.08 shows both the percentage of compounds in the important aromatic region, and the ratio of aliphatic:aromatic compounds. The lower this ratio, the greater the aromaticity and therefore potential to form THMs (Huang & Yah, 1997). The data suggests that 'Cors Erddreiniog' fen, 'Tregainen' and 'Forest' soils had the greatest aromaticity, whilst 'Bonc Fadog' (which contained 0% aromatic compounds) and 'Frogwy' soils were comprised of more aliphatic and hence lower molecular weight material.

3.1.3.1.3 Hydrochemical analysis

Bromide (*figure 3.09*) was recorded in 6 out of the 8 soils, with the concentration recorded in the 'Forest' soil (0.46 mg l^{-1}) significantly higher than the others (p<0.01-0.001). The bromide concentration correlated positively and significantly with the percentage of aromatic compounds (R^2=0.84, p<0.01).

The concentration of nitrate (*figure 3.10*) was lowest and almost below the limit of detection for 'Cors Erddreiniog' fen and below 20 mg l^{-1} for all of the soils apart from at 'Bonc Fadog', which had a concentration significantly much higher (325.6 mg l^{-1}, p<0.001). The nitrate concentration correlated positively and significantly with the HPSEC-determined percentage of LMW DOC (R^2=0.81, p<0.05) and the ^{1}H-NMR-determined aliphatic:aromatic ratio.

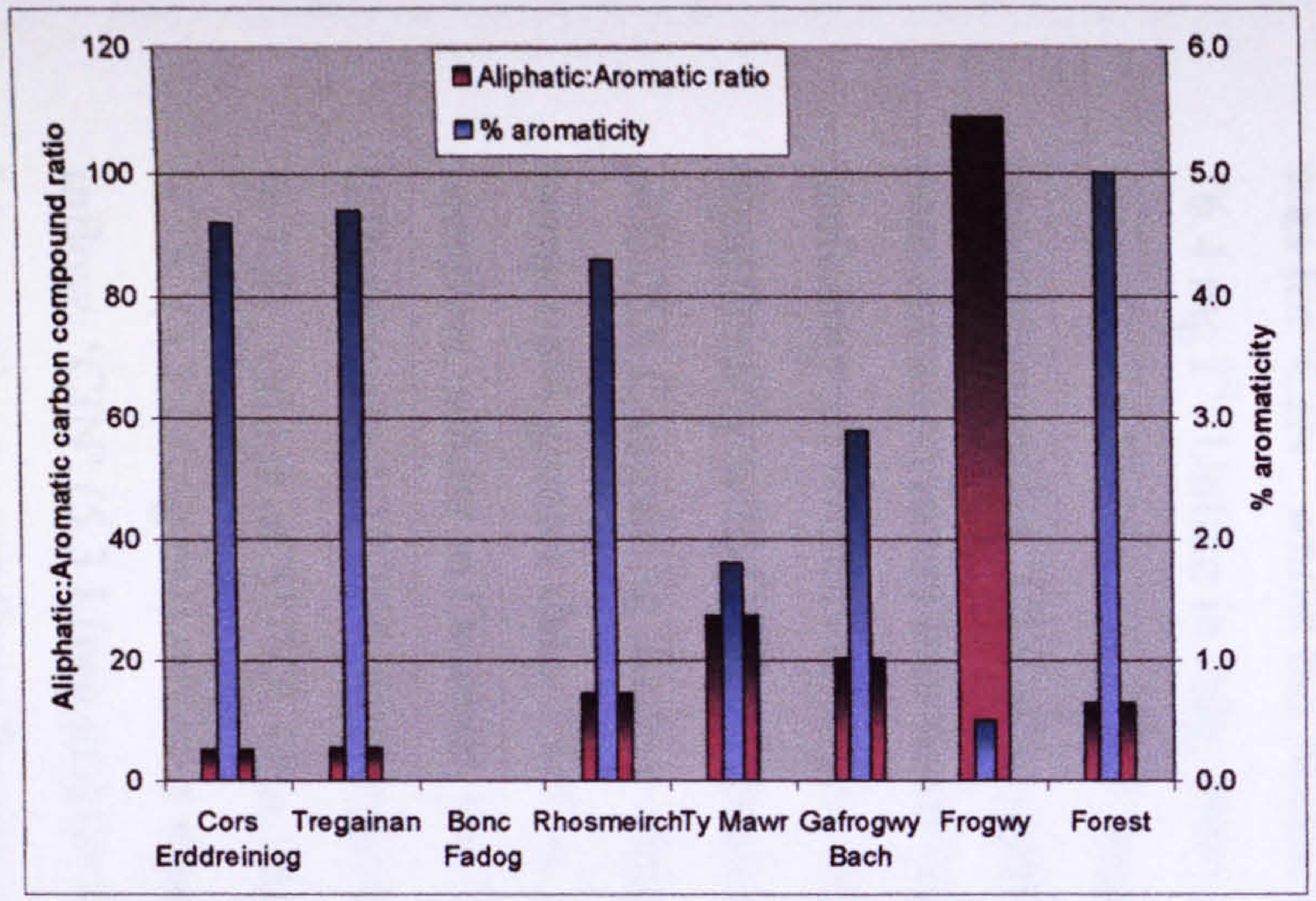

Figure 3.08 - Percentage aromaticity and aliphatic:aromatic carbon compound ratio of the 8 sampled soils

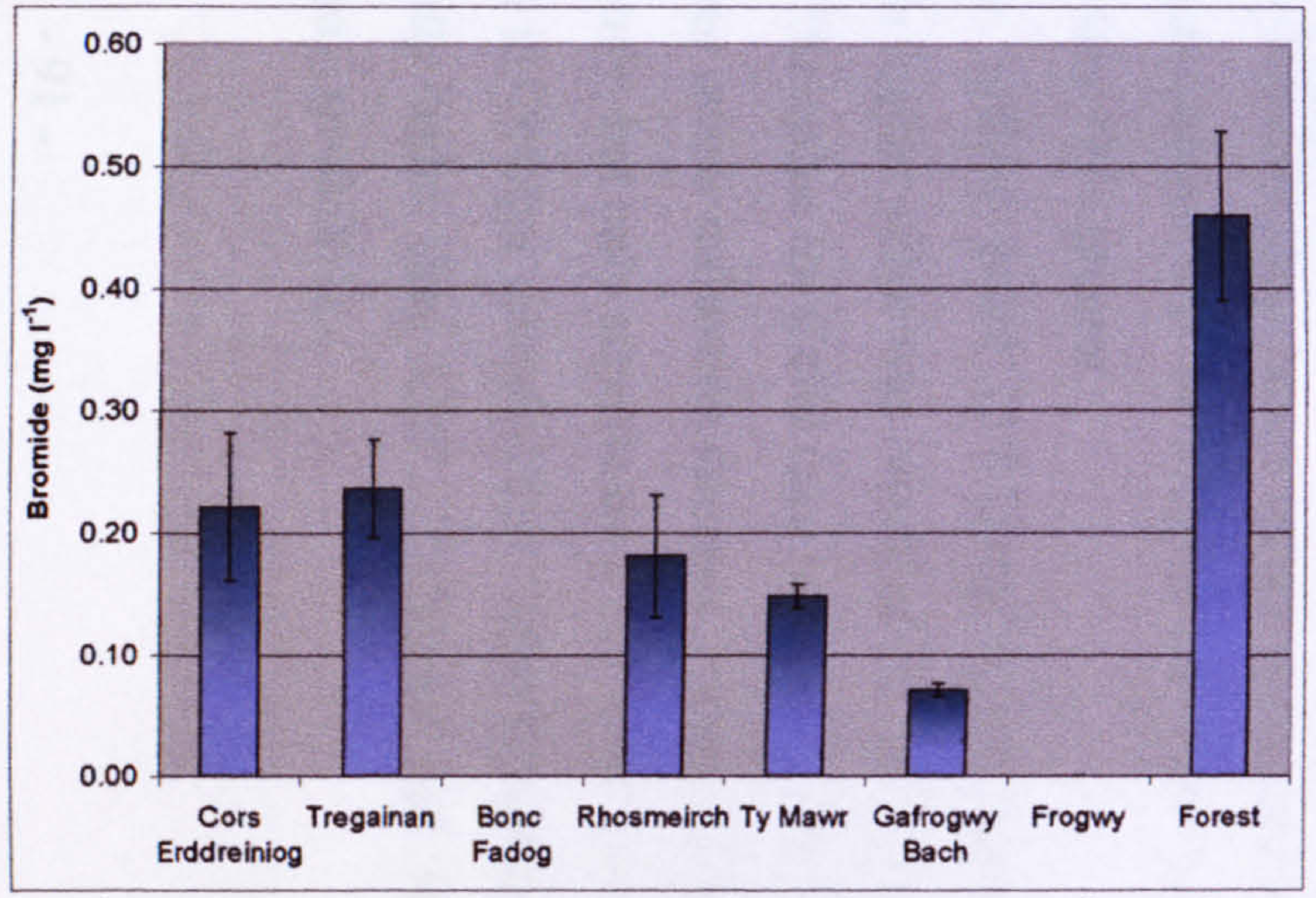

Figure 3.09 – Bromide concentrations of porewater from the 8 sampled soils

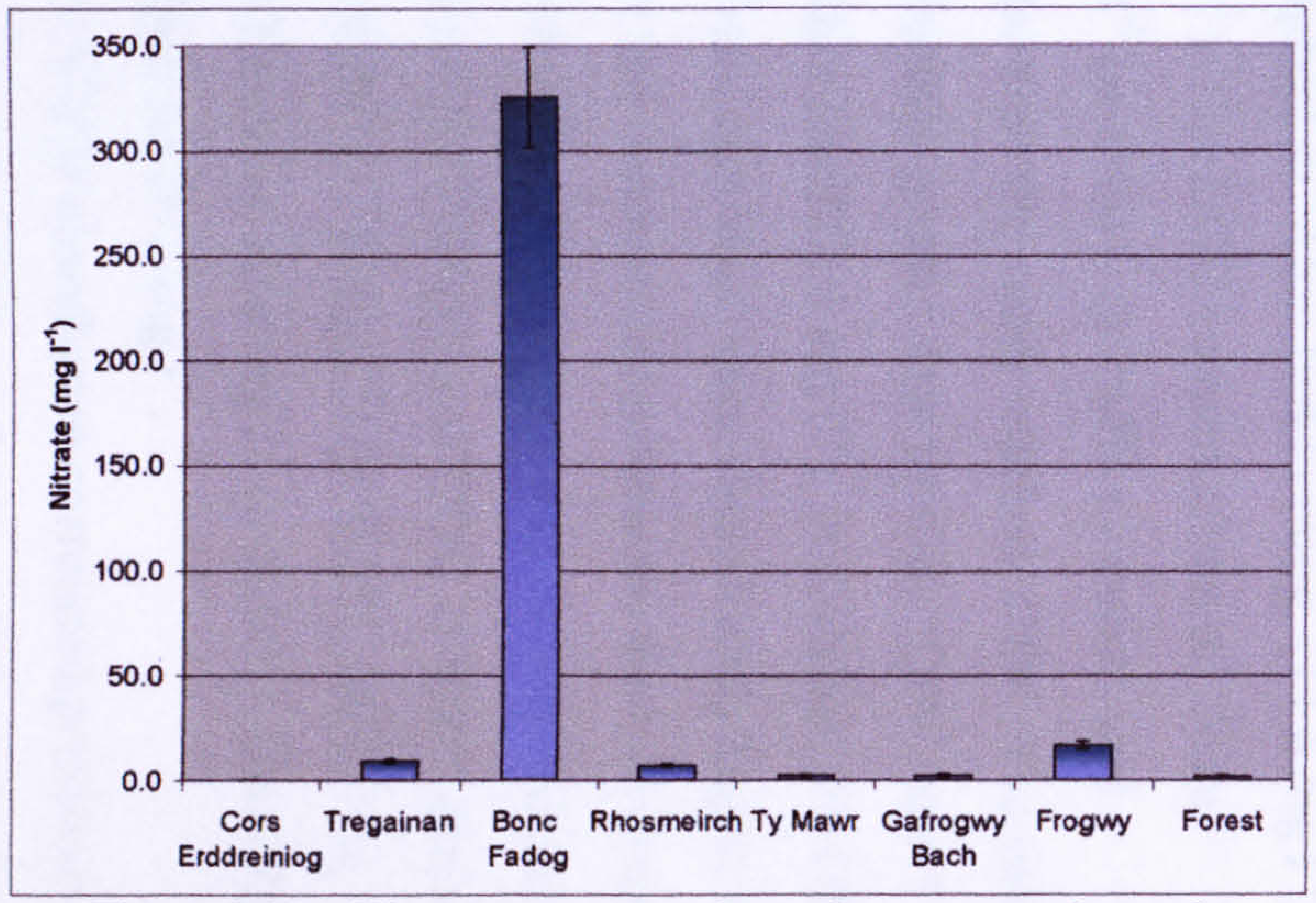

Figure 3.10 – Nitrate concentrations of porewater from the 8 sampled soils

3.1.3.2 Catchment stream analyses

3.1.3.2.1 Dissolved organic carbon analyses

The concentration of DOC in July was highest in 'Afon Erddreiniog' (11.6 mg l^{-1}) and lowest in 'Bonc Fadog stream' (3.8 mg l^{-1}). The DOC concentration of 'Afon Erddreiniog' fell significantly to 5.8 mg l^{-1} ($p<0.001$) at 'halfway point', and significantly to 4.0 mg l^{-1} ($p<0.001$) by the time the water reached 'CIN'. The value at 'CIS', the other major inflow, was significantly greater than that for 'CIN', at 6.7 mg l^{-1} ($p<0.001$), with a similar value in Cefni lake itself (6.1 mg l^{-1}). In October, the DOC concentrations of all the streams, as well as Cefni lake, were significantly higher than in July ($p<0.001$). The greatest difference in the concentration between the two months was for 'CIN', whilst the stream with the highest DOC concentration was 'Forest', at 16.4 mg l^{-1}. Unlike in July, there was not a significant loss of DOC as water drained from 'Afon Erddreiniog' (15.2mg l^{-1}) to 'CIN' (14.6 mg l^{-1}) ($p>0.05$), whilst the concentration for 'CIS' was significantly lower, at 12.6 mg l^{-1} ($p<0.001$). The DOC concentration in the lake itself was also high, at 16.3 mg l^{-1}.

Including all streams together, values of UV-254 absorbance correlated positively and significantly with DOC concentrations in July ($R_2=0.89, p<0.01$) and October ($R_2=0.87$, $p<0.05$). Comparing the two major inflows, despite the DOC concentration for 'CIS' being 68% greater than 'CIN' in July, the UV-254 absorbance values were almost identical, whilst in October, the DOC concentration was 14% higher for 'CIN' but the difference in UV-254 absorbance values was 33% greater.

In July, values of SUVA were highest for 'halfway point' (5.8 L(mg m)) and lowest for 'CIS' (3.1 L(mg m)) whilst a significantly higher value was recorded for the other major inflow, 'CIN' (5.3 L(mg m)) ($p<0.001$). The value for Cefni lake itself was also low (3.8 L(mg m)), as were those for the other three minor inflowing streams. Values of SUVA in October were generally lower than in July, but only significantly for 'CIN' ($p<0.01$). Comparison of the values for the streams in October revealed that 'Afon Erddreiniog' and 'CIN' (3.9 L(mg m)) were significantly higher than 'CIS', 'Ty Mawr' and 'Forest' streams and the lake itself (all 3.3 L(mg m)) ($p<0.001$).

Values of the E2:E3 ratio, inversely proportional to molecular weight, varied insignificantly for the stream waters in July and averaged 5.2, but a significantly higher value of 6.9 was recorded for Cefni lake ($p<0.001$). Compared to the July data, the values for October were significantly lower for 'Afon Erddreiniog' and 'CIN' ($p<0.05$-0.001) only. Comparison of the 6 streams sampled in October revealed that values were significantly lower for 'Afon Erddreiniog' and 'CIN' compared to 'CIS', 'Ty Mawr' and 'Forest' ($p<0.001$), whilst the high value recorded in July for Cefni lake reduced significantly ($p<0.001$) to a level comparable with the stream waters.
The spectra shown in *figure 3.13* display the molecular weight distributions for the 6 stream/lake waters in October, with the peak heights relating closely to the DOC concentrations and UV-254 absorbance values. Separation of the peaks into the three molecular weight fractions (*figure 3.14*) revealed no detection of the HMW fraction in July for any of the samples, whilst the percentage of LMW material was lowest for 'Afon Erddreiniog' (35%) and 'halfway point' (33%) and highest for Cefni lake (51%). In October, HMW material was detected for most of the samples, whilst the percentage of LMW material was lower than in July. The percentage of the HMW fraction was greatest for Cefni lake (7.8%), whilst 'CIN' (4.6%) contained a greater quantity than 'CIS' (3.8%).

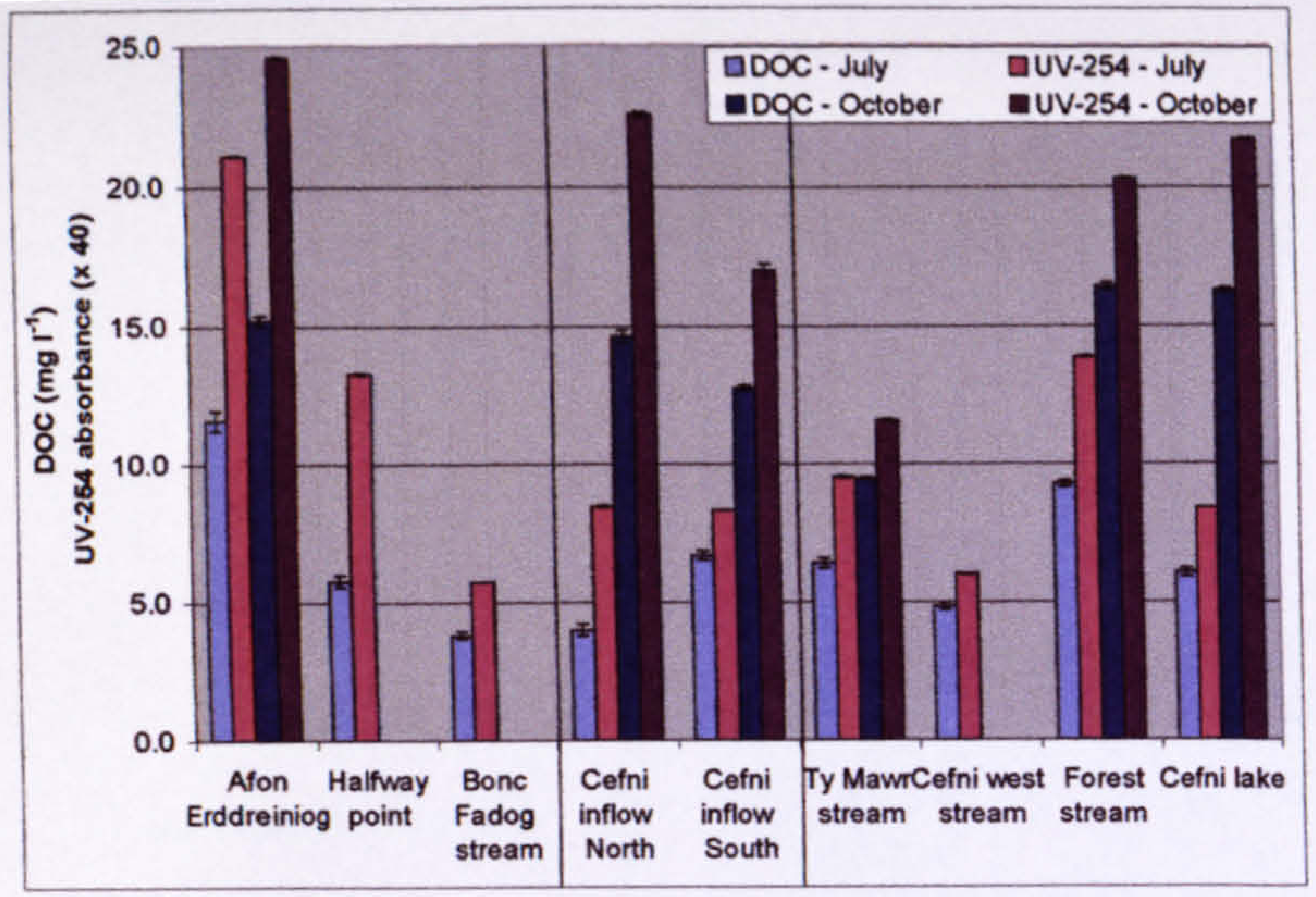

Figure 3.11 – DOC concentrations and UV-254 absorbance values for the stream/lake samples collected during July and October

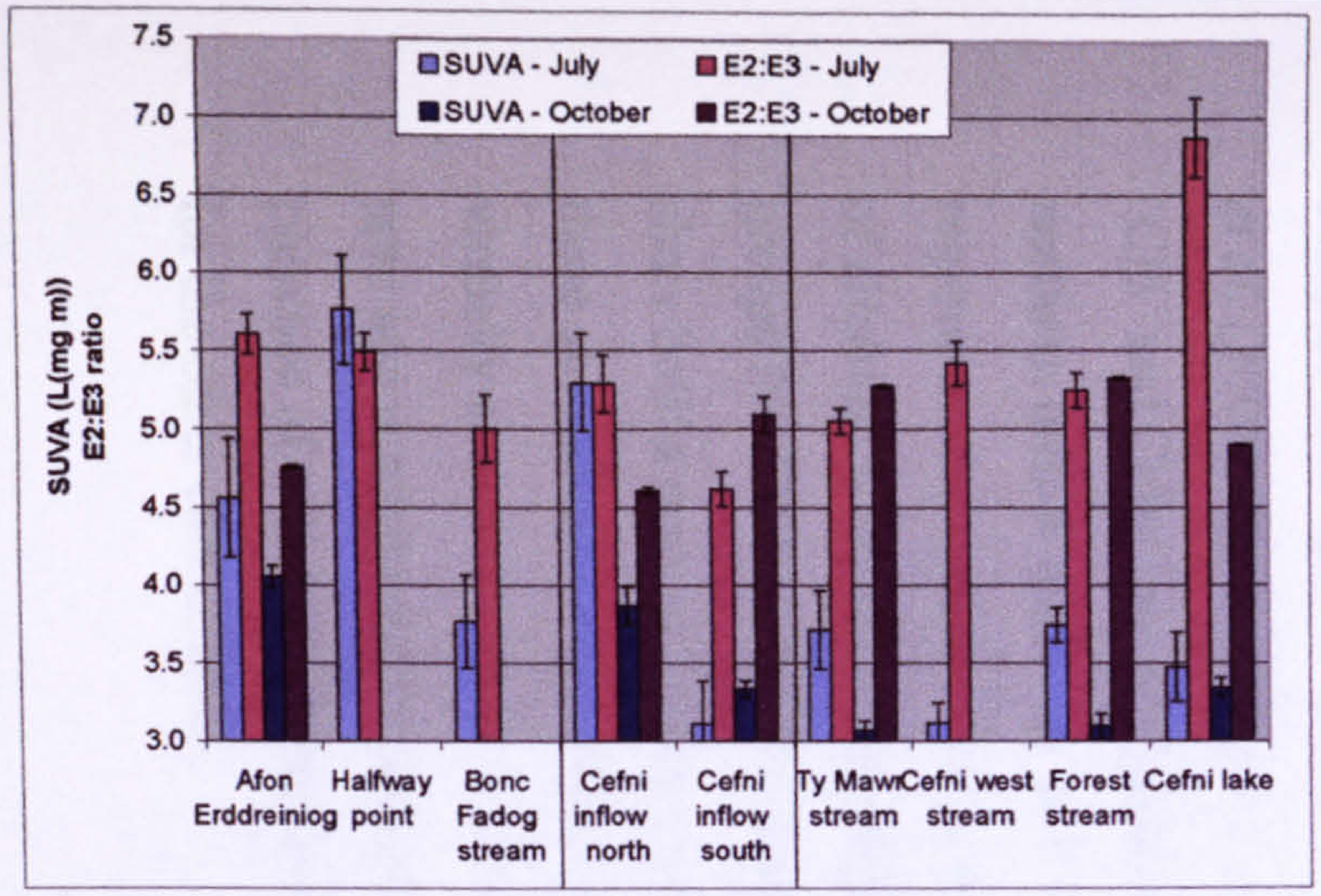

Figure 3.12 – SUVA and E2:E3 values for the stream/lake samples collected during July and October

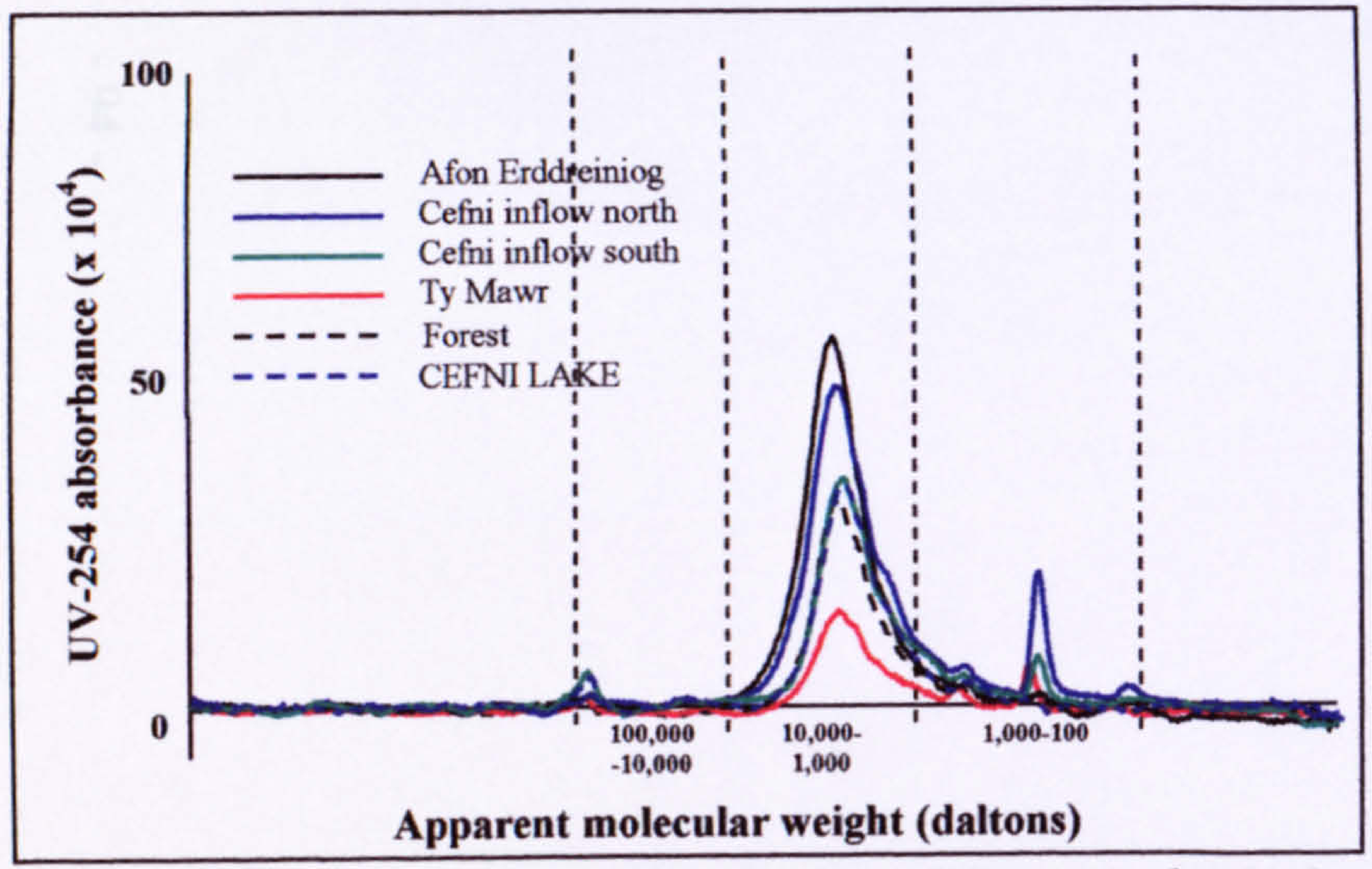

Figure 3.13 – HPSEC-determined molecular weight spectra for the 6 stream/lake samples collected during October

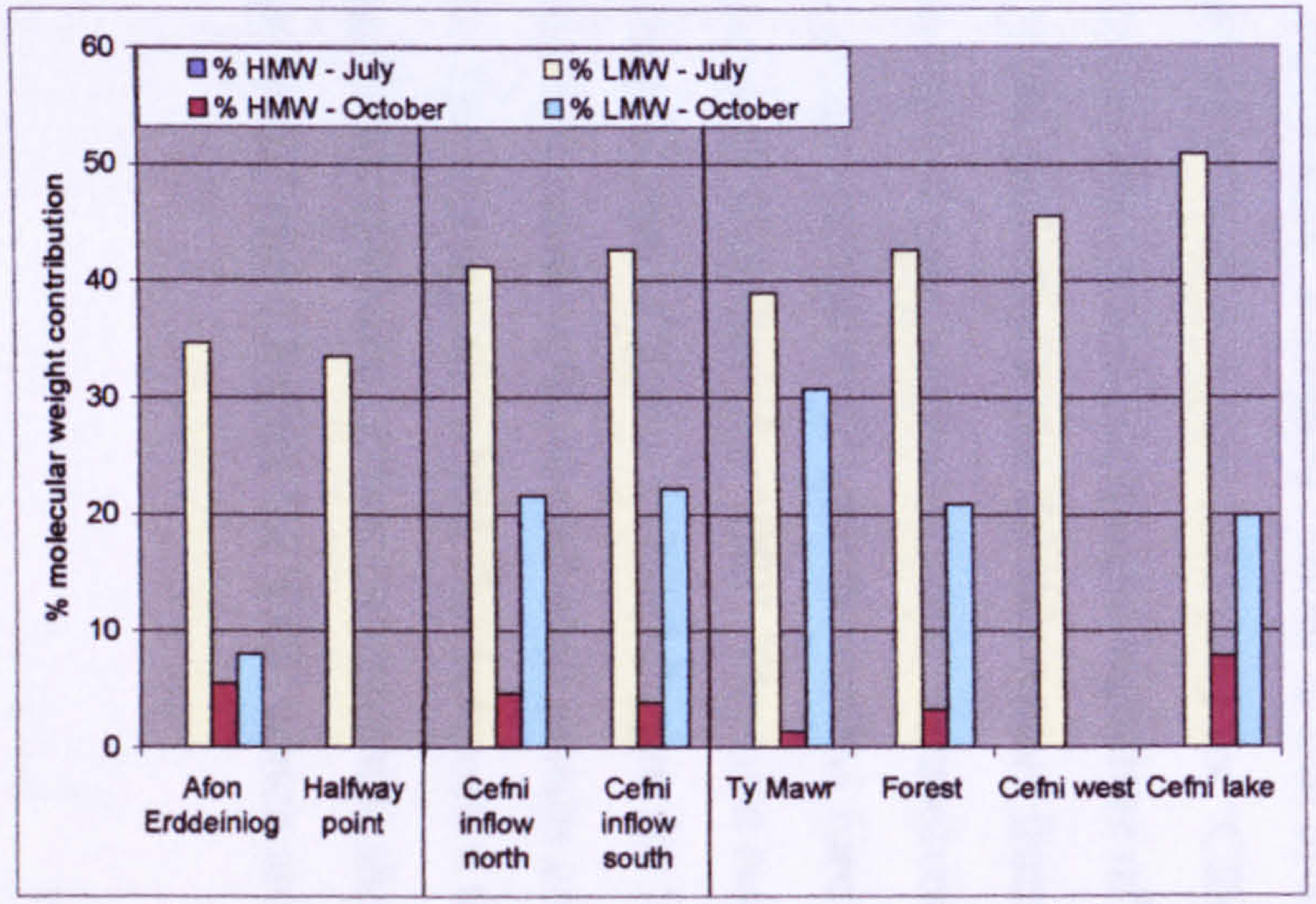

Figure 3.14 – Percentage values of the 3 molecular weight fractions for the 6 main stream/lake samples collected during October

3.1.3.2.2 ^{1}H-NMR analysis

Using ^{1}H-NMR spectroscopy, an attempt was made to 'fingerprint' the terrestrially-derived carbon compounds entering Cefni lake via the two major inflowing streams and compare their spectra to that of the lake water itself; a method similar to that used by Lu, *et al.* (2004). The raw spectra for October are shown in *figure 3.15 (a-c)* for 'CIN', 'CIS' and Cefni lake and seem to suggest that the carbon compound signature of the northern inflow match up best with that in the lake. Evidence for this comes from four regions on the spectra. The first, between 0.5-1.5 ppm, shows a broad, unresolved peak of aliphatic structures with three distinct peaks indicative of specific methyl functional groups for 'CIN' and Cefni lake; only one peak is evident for 'CIS'. The next two areas show both a peak at 2.3 ppm and a very large series of peaks at 3.0-3.2 ppm for 'CIS'; these are indicative of protons next to aromatic, carboxylic or carbonyl groups and are virtually absent for 'CIN' and Cefni lake. The last region, which lies between 6.0-8.0 ppm and is attributed to protons associated with aromatic carbon compounds, shows the presence of two distinct functional groups at 6.2 and 6.8 ppm for 'CIS' which are absent for the other two samples.

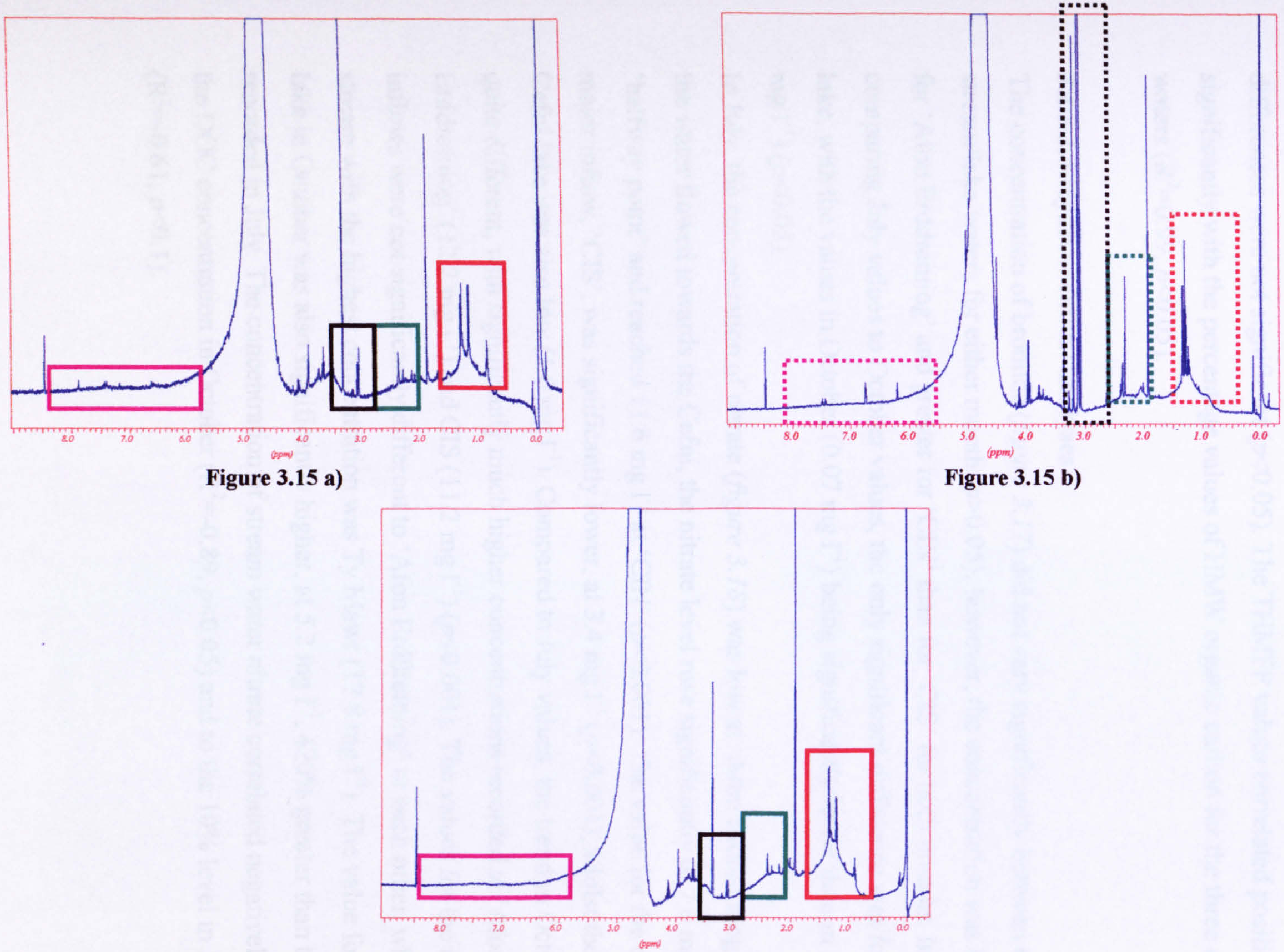

Figure 3.15 a)

Figure 3.15 b)

Figure 3.15 c)

Figure 3.15 – ^{1}H-NMR spectra of a) Cefni inflow North, b) Cefni inflow South and c) Cefni lake water for samples collected during October

3.1.3.2.3 Trihalomethane formation potential

The trihalomethane formation potential (*figure 3.16*) of the three stream samples tested was, on average, 54% greater in October compared to July and higher for 'Afon Erddreiniog' than the two inflowing streams for both months. The THMFP was 23% and 30% higher for 'CIN' than 'CIS' in July and October respectively, however, the differences were not significant ($p<0.05$). The THMFP values correlated positively and significantly with the percentage values of HMW organic carbon for the three stream waters ($R^2=0.99, p<0.05$).

3.1.3.2.4 Hydrochemical analyses

The concentration of bromide (*figure 3.17*) did not vary significantly between the 9 stream/lake waters for either month ($p>0.05$); however, the concentration was highest for 'Afon Erddreiniog' and greater for 'CIN' than for 'CIS' for both months. In comparing July values to October values, the only significant difference was for Cefni lake, with the values in October (0.07 mg l^{-1}) being significantly lower than in July (0.16 mg l^{-1}) ($p<0.05$).

In July, the concentration of nitrate (*figure 3.18*) was low at 'Afon Erddreiniog', but as the water flowed towards the Cefni, the nitrate level rose significantly to 7.6 mg l^{-1} at 'halfway point' and reached 11.6 mg l^{-1} at 'CIN' ($p<0.001$). The value for the other major inflow, 'CIS', was significantly lower, at 3.4 mg l^{-1} ($p<0.001$), whilst the value for Cefni lake was also low (1.0 mg l^{-1}). Compared to July values, the trend in October was quite different, with significantly much higher concentrations recorded at 'Afon Erddreiniog' (12.2 mg l^{-1}) and CIS (11.2 mg l^{-1}) ($p<0.001$). The values for the two major inflows were not significantly different to 'Afon Erddreiniog' or each other, whilst the stream with the highest concentration was Ty Mawr (17.8 mg l^{-1}). The value for Cefni lake in October was also significantly higher, at 5.2 mg l^{-1}, 420% greater than that recorded in July. The concentration of stream water nitrate correlated negatively with the DOC concentration in October ($R^2=-0.89, p<0.05$) and to the 10% level in July ($R^2=-0.61, p<0.1$).

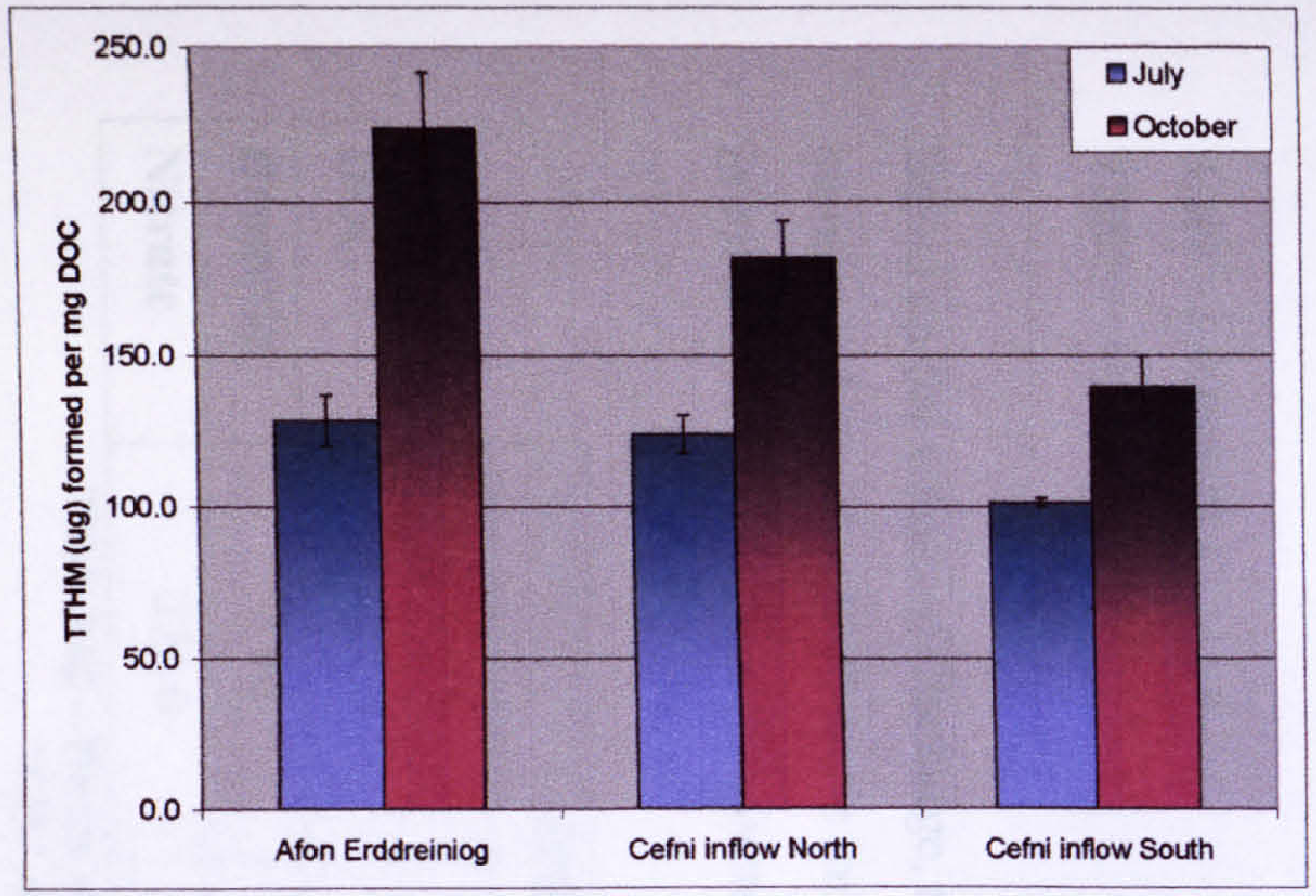

Figure 3.16 – THMFP of the 3 main stream samples collected during July and October

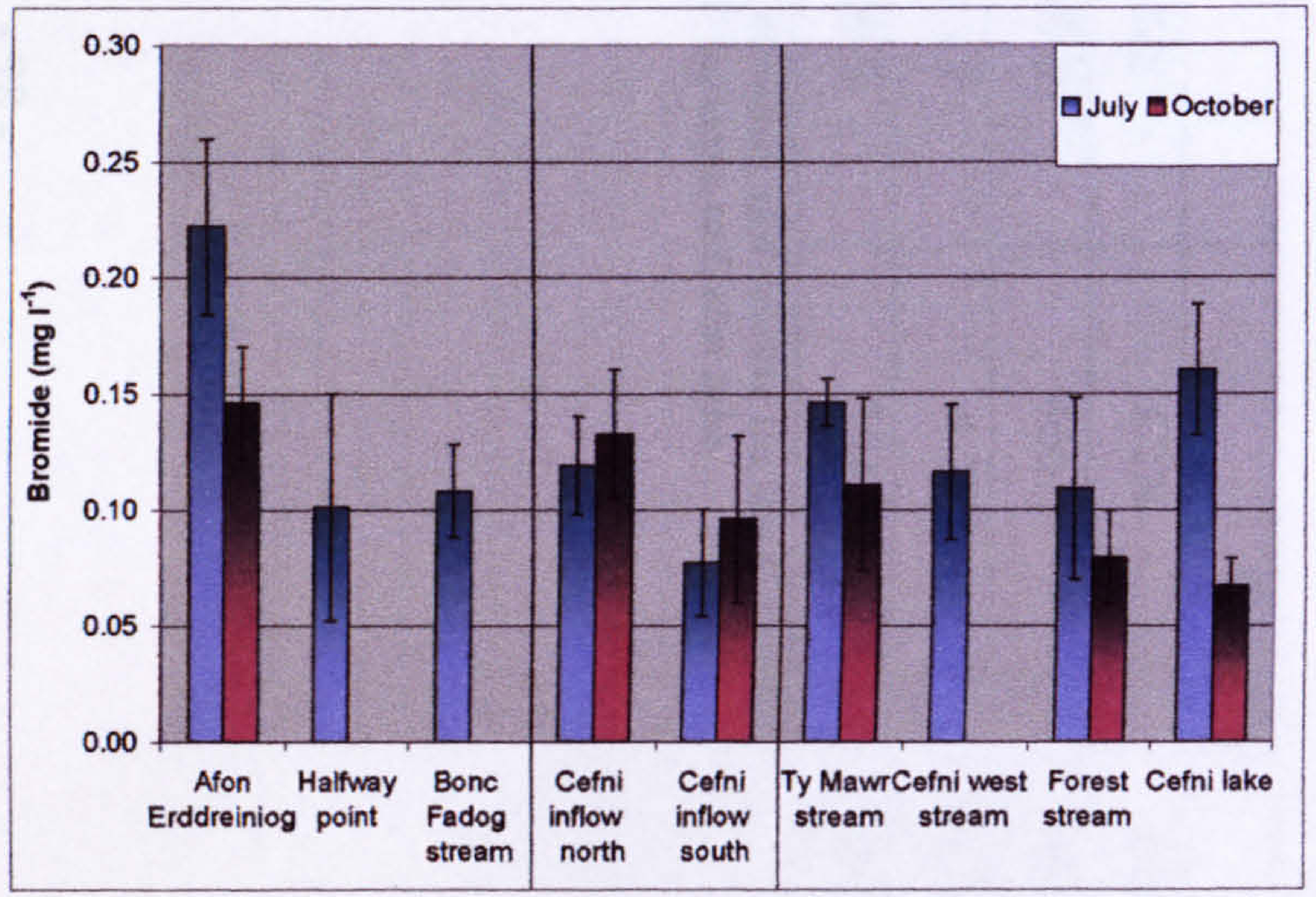

Figure 3.17 – Bromide concentrations of the stream/lake samples collected during July and October

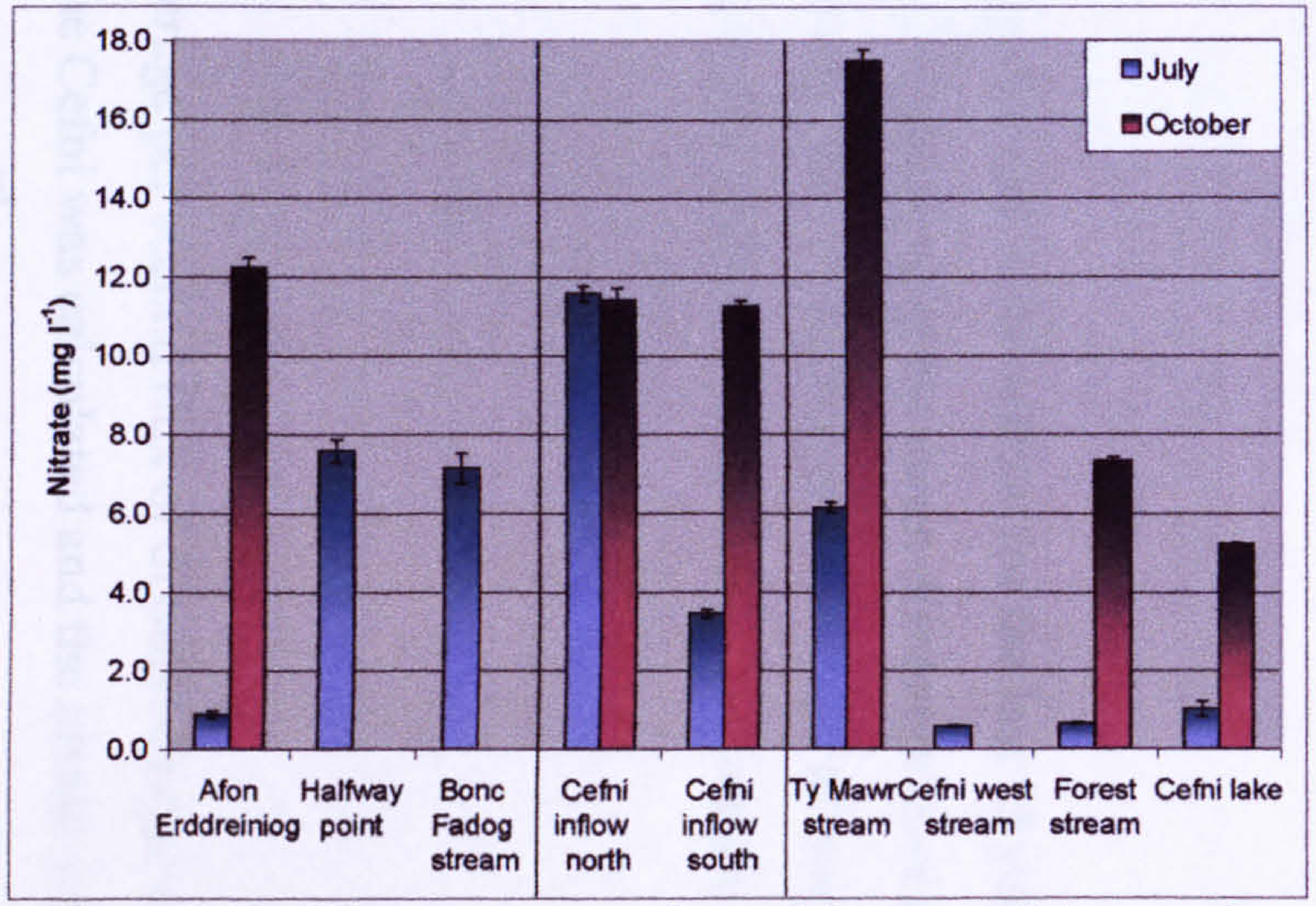

Figure 3.18 – Nitrate concentrations of the stream/lake samples collected during July and October

3.1.3.2.5 Major inflow discharges and fluxes of dissolved materials

The calculated average discharge volumes for 'CIN' and 'CIS' streams were:

'CIN': 31.9 l s^{-1}
'CIS': 14.5 l s^{-1}.

Data provided by the Environment Agency for the 'CIS' inflow for the last 15 years is displayed in *figure A06*, of appendix A3 and shows that the mean discharge over that time period has been 17.8 l s^{-1}. Therefore the rates of discharge were close to average. These near average figures would lead to a total influx of water from each stream to the Cefni per year of:

'CIN': 1,005,998,400 l yr^{-1}.
'CIS': 457,272,000 l yr^{-1}.

Based on the values of discharge, the average per second flux of dissolved organic carbon and dissolved inorganic ions to the Cefni was calculated and the results presented in *table 3.02*. The values are given in mg $second^{-1}$.

	July		**October**	
	CIN	**CIS**	**CIN**	**CIS**
DOC	127.6	97.2	465.7	182.7
Bromide	3.80	1.12	4.21	1.39
Nitrate	370.0	49.3	364.3	163.0

Table 3.02 – Fluxes of DOC, bromide and nitrate (in mg s^{-1}) from 'CIN' and 'CIS' streams to Cefni lake

An additional input of water to the Cefni is direct rainfall. To work out the volume contribution of water to the Cefni from this source, the following calculation was employed:

Area of Cefni = 710,000 m^2

Average annual rainfall at Cefni = 1,060 mm = 1.06 m (data provided by Welsh Water)

Total volume of water falling directly on lake per year = 710,000 x 1.06 = 752,600 m^3

752,600 x 1000 = 752,600,000 l yr^{-1}.

3.1.3.2.6 Comparison of yearly fluxes of DOC from 'CIN', 'CIS' and rainfall

Figure 3.11 shows that the DOC concentration of 'CIN' was approximately 4 and 14 mg l^{-1} and for 'CIS' approximately 7 and 13 mg l^{-1} for samples collected in July and October respectively, and these values probably represent the two extremes of concentrations that would be recorded at throughout the year. If it is therefore assumed that the average annual DOC concentration of 'CIN' is 9 mg l^{-1} and 'CIS' is 10 mg l^{-1}, and the typical concentration of rainwater 1 mg l^{-1} (Wetzel, 2001), the annual flux of DOC from the two major inflowing streams and rainfall can be calculated:

'CIN': 1,005,998,400 l yr^{-1} x 9 mg l^{-1} = <u>9,053,986 g DOC</u>

'CIS': 457,272,000 l yr^{-1} x 10 mg l^{-1} = <u>4,572,720 g DOC</u>

Rainfall: 752,600,000 l yr^{-1} x 1 mg l^{-1} = <u>752,600 g DOC</u>

3.1.4 Discussion

The data collected in this study implies that the most important source of terrestrially-derived THM precursors to Cefni lake is from the stream that drains 'Cors Erddreiniog' fen and flows south into the northern edge of the lake. DOC concentrations and SUVA, percentage HMW and THMFP values, coupled with the discharge rates of the two major inflowing streams, all point to 'CIN' supplying the greater quantity of DOC and, importantly, THM-forming DOC. As reported by Kang, *et al.* (2002) for a similar fen site (Cors Goch) elsewhere on the island of Anglesey, 'Cors Erddreiniog' fen possesses an organic-rich soil, with the DOC concentration being three times as high as any of the other soils surveyed. Consequently, 'Afon Erddreiniog' has a high DOC concentration for a freshwater stream (Wetzel, 2001). With the 'Cors Erddreiniog' fen ecosystem having an elevation above sea-level 35 metres higher than Cefni lake, the topography is established for a natural flow of drainage water rich in THM-forming compounds from the fen into the lake.

The concentration of DOC in 'Cors Erddreiniog' fen was also higher than that recorded for the bog soils in section 2.2 (*figure 2.30*, p57); a similar observation was recorded by Kang, *et al.* (2002). Whilst the fen soil's higher DOC concentration compared to the other 7 catchment soils is due to the high water-table and resulting inhibition of organic matter decomposition, the higher concentration of DOC compared to the bog soils is due to the fen's greater nutrient content and pH, which would allow for greater enzymic production of water-soluble DOC compounds (Kang & Freeman, 1999).

In addition to a high concentration of DOC, structural analyses of the fen peat porewater DOC suggests a greater composition of THM precursor compounds than the other 7 soils. It boasted the lowest %LMW value and the lowest ratio of aliphatic to aromatic compounds, indicating that the soil was composed of mostly medium to high molecular weight compounds with a high degree of aromaticity. This was also indicated by the concentration of phenolic compounds, which were, on average, more than twice as high as any of the other soils. It is known that such structural attributes exist with the carbon compounds that are most reactive with chlorine (Huang & Yeh, 1997; White, *et al.* 2003; Uyguner, *et al.* 2004).

The leaching of significant concentrations of THM-forming organic matter from the fen into 'Afon Erddreiniog' was obvious by the brown staining of the water and confirmed by the high DOC values for both July and October. Out of the two major inflows, 'CIN' supplied the greater quantity of organic matter due in part to its discharge rate, which was twice as high as 'CIS'. Although the DOC concentration of 'CIS' was almost twice as high as 'CIN' in July, the greater discharge of 'CIN' meant it had a higher flux of DOC to Cefni lake. The difference in the flux between the two streams was even greater in October due to the DOC concentration being higher for 'CIN'. Compositional analysis revealed that not only was the flux of total DOC greater for 'CIN', the 'CIN' inflow contributed DOC which had a higher SUVA and molecular weight due to its peatland source. This explains the greater THMFP values observed for 'CIN' and suggests that it inputs more THM-forming compounds than 'CIS'; this has important consequences for the compliance of THMs at the Cefni and the production of safe drinking water.

In addition to the quantitative carbon data obtained for the two major inflowing streams, a visual comparison of their structural characteristics obtained by ^{1}H-NMR seems to confirm that 'CIN' is the more important in influencing the DOC concentration and composition of Cefni lake. The highlighted regions on *figure 3.15* show differences in the functional group peaks and hence DOC composition between 'CIS' and Cefni lake; differences that were largely absent when comparing the 'CIN' signature with that of the lake. The absence of the large peaks at 2.3 ppm and 3.0-3.2 ppm on the Cefni lake spectra that were present for 'CIS' are particularly obvious, indicating that these functional groups either became broken down upon entering the lake, or, perhaps more likely, they became less identifiable in the signature of Cefni lake due to dilution because of the lower discharge volume of 'CIS'. 'Fingerprinting' using ^{1}H-NMR spectroscopy to distinguish sources of DOM in a similar way has been used successfully by Fujita, *et al.* (1996) and Lu, *et al.* (2004).

Comparison of the stream samples in July with those in October reveal the effect of seasonal influences, which have implications for the quality of drinking water. DOC concentrations in October were generally higher than in July, e.g. 'Afon Erddreiniog' had a 31% higher DOC concentration in October. This can probably be attributed to the higher rate of precipitation (that is typical during autumn) causing a greater leaching of DOC from Cors Erddreiniog fen. It was the 'CIN' samples that exhibited the greatest difference between the July and October values. The DOC concentration was 265% higher in October and was just 4% lower than the value at the 'Afon Erddreiniog' source, but in July was 66% lower, indicating that much more carbon was lost from the stream water as it travelled from 'Afon Erddreiniog' to 'CIN' in the summer. The input of DOC represents a major energy source to aquatic microbes in freshwater lakes (Hessen, 1992) and the greater rate of reduction observed during summer as water travelled from 'Afon Erddreiniog' to 'CIN' can be partially attributed to the higher rate of microbial mineralisation of organic matter that would take place in the warmer water. The other major DOC-reducing process that occurs within streams is photodegradation, i.e. the removal of organic matter by sunlight. This breaks down UV-absorbing organic material into lower molecular weight compounds and inorganic carbon, such as CO_2 (Parks & Baker, 1997). It has been shown that photodegradation may be more important in removing DOC from freshwaters than microbial processing (Köhler, *et al.* 2002) and with the sun higher in the sky and ground level UV radiation much higher during July than October, this likely represents an important DOC removal process. It is therefore realistic to assume that 'Cors Erddreiniog' fen supplies a continuously large concentration of DOC to 'Afon Erddreiniog', but during summer the concentration of DOC in the stream is strongly reduced by solar degradation and microbial metabolism so that there is a much reduced input of DOC to the Cefni from this inflow by the time the stream reaches 'CIN'. The cooler temperatures experienced during autumn and winter, and the lower level of UV radiation, ensures that DOC concentrations reaching the Cefni from 'Afon Erddreiniog' remain high. The photodegradation of organic compounds may also be the reason that no HMW fraction of DOC was detected by HPSEC in any of the streams in July, as high molecular weight compounds are known to be reduced in molecular weight/size by natural sunlight (Wetzel, *et al.* 1995). However, it may also be due to a reduced leaching of HMW DOC from the soils within the catchments of the streams during summer, due to organic matter decomposition in the soils being higher in the warmer temperatures experienced in July (Kaiser, *et al.* 2001).

Given the impact that photodegradation has on the DOC content and quality within a stream, it was perhaps unexpected to record a significantly higher SUVA value for 'CIN' in July compared to October, especially given that the value for 'Afon Erddreiniog' in July was significantly lower than 'CIN'. This implies that as water travelled from 'Afon Erddreiniog' to 'CIN' the carbon compounds became comprised of a greater proportion of aromatic groups. The most viable explanation for this is a reduction in the abundance of labile compounds due to microbial and biofilm metabolism (Freeman, *et al.* 1990; Lock & Ford, 1985). DOC utilised as an energy source by microbes and biofilms in freshwaters represents a major loss pathway, for example Hynes, *et al.* (1974) recorded a 75% loss of newly added DOC in a 30 metre stretch of river. It has been demonstrated that the uptake of labile DOC compounds can leave behind those that are more recalcitrant, increasing their proportion in the lake water (Wetzel & Manny, 1972; Kaplan, *et al.* 1980; Sabater, *et al.* 1993). This would explain the higher SUVA value recorded for 'CIN' compared to 'Afon Erddreiniog', as the recalcitrant fraction of DOC is known to be more aromatic than the labile fraction (Kiem, *et al.* 2000). The fact that an increase was only recorded in July is probably because the higher temperatures could support greater microbial metabolism than in October. In previous photodegradation experiments, it has been shown that the aromatic compounds can also be recalcitrant to sunlight, due to their structural stability (Allard, *et al.* 1994); this may also explain the rise in SUVA.

Seasonal differences in the data were also evident in the samples taken from Cefni lake. In July, the E2:E3 ratio was significantly higher than in any of the inflowing streams, indicating a lower overall molecular weight; this was confirmed by HPSEC analysis which showed that the greatest percentage of LMW material in July was for Cefni lake. Such a difference was not present in the samples collected in October, with the E2:E3 ratio and %LMW values being comparable between the inflowing streams and Cefni lake. The concentration of nitrate also differed between the two sampling months, with much higher concentrations recorded in October. These nitrate concentrations were also comparable to those recorded for the corresponding months in the reservoir survey in section 2.1 (*figure 2.22*, p41). This DOC molecular weight and nitrate data again highlights the autochthonous production of DOC that occurs during the warm, summer months in Cefni lake (Malthus & George, 1997). Being derived principally from algae and plankton, such DOC compounds are typically transparent and low in molecular weight (Curtis & Schindler, 1997) and explain the significantly higher E2:E3 ratio of the Cefni lake water in July. In addition, the very low concentration of nitrate in the lake, despite the higher concentrations in the major inflowing streams, also reflects this extensive growth of algae (see sections 2.1 & 3.2). The greater nitrate concentration in October in Cefni lake can be attributed to the lower temperatures and lesser amount of sunlight, hence a reduced growth of algae within the lake.

The stream named 'Bonc Fadog' flows into 'Afon Erddreiniog' and therefore indirectly into the Cefni reservoir, but relative to the water at 'halfway point', which immediately precedes the point at which the stream flows into 'Afon Erddreiniog', 'Bonc Fadog' stream is low in DOC and SUVA, and therefore dilutes the DOC concentration and aromaticity of the water flowing into the Cefni. Analysis of the farmland soil surrounding 'Bonc Fadog stream' revealed that it had the highest LMW fraction and was the only soil to contain below detectable amounts of HMW or aromatic material, which partly explains the low DOC concentration of this stream. Whilst the contribution of DOC from 'Bonc Fadog' soil and stream appears to be low, the same cannot be said of nitrate. The soil possessed an extremely high nitrate concentration of 325 mg l^{-1}, which indicates a strong use of fertilisers. In addition, in July a progressive and huge increase of 1208% in nitrate was recorded as water flowed from 'Afon Erddreiniog' to 'CIN'. This suggests that the farmland soils to the north of the Cefni are contributing a large concentration of nitrate to Cefni lake and this nitrate has an important influence on the growth of undesirable algal blooms during the summer months. Strong positive correlations between the nitrate content of stream water and the proportion of agricultural land within the catchment has been previously reported (Woli, *et al.* 2002). Higher concentrations of nitrate were recorded in October in the inflowing streams, which are probably due to a reduced uptake of nitrate by plants and microbes in the catchment soils, increasing the amount available for leaching into the streams (Chapman, *et al.* 2001). The concentrations of nitrate recorded for 'CIS' and 'Ty Mawr' streams in October were much higher than in July. This probably reflects the fact that fertiliser is applied to agricultural land at different times of the year, indicating that the importance of nitrate sources varies on a temporal basis. A positive correlation was reported of both the percentage of low molecular weight DOC and the aliphatic:aromatic ratio with the nitrate concentration of the soil, and a negative correlation between stream water nitrate and DOC concentrations, and these correlations are likely to be related. The consumption of nitrate through microbial denitrification, a process which converts NO_3^- to N_2 and N_2O gas, is greater in organic soils compared to mineral soils (Davidsson & Ståhl, 2000) and is also influenced by the soil's water content (Chapman, *et al.* 2001). Consequently, wetland soils have a strong denitrification potential and often contain little free nitrate; this explains the values of nitrate being lowest for 'Cors Erddreiniog' fen. The higher concentration of nitrate in the soils with a higher percentage of lower molecular weight material most likely reflects a greater rate of organic matter decomposition and mineralisation (perhaps due to a more neutral pH and/or lower water content), processes that would degrade organic matter into lower molecular weight material and release nitrate from organic compounds. The negative correlation between DOC and nitrate concentrations recorded in the stream waters could also be due to decomposition/mineralisation in the catchment soils; those soils experiencing higher rates would produce more nitrate but less DOC, with the breakdown of organic matter proceeding to an end-point of CO_2, rather than producing DOC intermediates. Therefore rain water washing through soils with higher rates of decomposition are likely to contain more nitrate and less DOC. Nitrate ions are not strongly absorbed to soil particles due to their negative charge, and are therefore free to leach out of the soil profile if they are not taken up by plants or microbes (Wild, 1993). In section 2.1 it was stated that the unknown source of terrestrial organic matter to Cefni lake may also be a source of bromide. In this chapter it has been proven that 'Cors Erddreiniog' fen is an important source of DOC, and the hydrochemical data suggests it may be an important source of bromide. Of all the stream/lake waters, the concentration of bromide in Afon Erddreiniog was greatest during both months, indicting that the fen leached bromide in addition to DOC. Similar studies have shown that peatlands can positively influence the bromide concentration of freshwater streams (Asplund & Grimvall, 1991). Although the concentration of 'Afon Erddreiniog' was not significantly higher, nor was the 'CIN' inflow compared to the 'CIS' inflow, the higher discharge rates of 'CIN' equate to this inflow having a 200-240% greater contribution of bromide than 'CIS'. Although this would seem to suggest that the fen may be an important source of bromide, precipitation is also likely to be a significant source due to the site's close proximity to the sea (see page 45, section 2.1 and page 73, section 2.2).

The other three inflowing streams to Cefni lake, 'Ty Mawr', 'Cefni west' and 'Forest', all had DOC concentrations that were comparable to those from 'CIN' and 'CIS' but because they were much smaller and consequently had a much lower discharge rate their contribution of organic carbon to Cefni lake is much less important.

The discharge value of 14.5 $l\ s^{-1}$ measured for the 'CIS' inflow during this study was similar to the mean value of 17.8 $l\ s^{-1}$ for the last 15 years provided by the Environment Agency. The rate of discharge was measured during July and when referring to *figure A07*, in Appendix A3, it is apparent that the value of 14.5 $l\ s^{-1}$ is much higher than is usually experienced in July. This may reflect the above average rainfall that was experienced from April to July (see *figure A08*, Appendix A3), or that sampling was undertaken after a period of rainfall. In any case, it can be assumed that the discharge measurements reported here are for near average flow conditions. Although no data is available for the 'CIN' stream from the Environment Agency, it must be assumed that the discharge value of 31.9 $l\ s^{-1}$ is also close to the mean annual average for this stream too. Compared to the contribution of DOC from 'CIN' and 'CIS', the influx of DOC from rainfall is approximately 18 times lower. Therefore, the potential role of DOC from direct rainfall to the formation of THMs at the Cefni would appear to be minimal, although the impact of rainfall on raising THM levels through the washing of high concentrations of dissolved material from catchment soils into Cefni lake cannot be disputed. It is important to point out that storms, or heavy rainfall events, are the primary mechanism of DOC export from catchments to freshwaters because they result in both high DOC concentrations and a greater rate of discharge (Manny & Wetzel, 1973). Most allochthonous DOC therefore enters freshwaters during periods of high water flow (Royer & David, 2005), which is due mainly to the increased flushing of DOC from terrestrial carbon storage sites. Therefore, storms greatly affect DOC release from peatlands and will represent the periods of greatest DOC flux from Cors Erddreiniog fen into Cefni lake.

3.1.5 Conclusions

The data gathered in this study suggest that the most important flux of terrestrially-derived THM precursors to Cefni lake is from the stream entering the lake at 'CIN', due to both its DOC concentration and quality and higher discharge rate. Being derived from a peatland source, the stream is high in DOC and this DOC is more aromatic than that from other terrestrial sources. The water from this source is therefore more likely to contribute THM precursors to the Cefni than that from the other major inflow, 'CIS'. It appears that 'CIN' contributes twice as much DOC to Cefni lake than 'CIS'. If it is assumed that the other minor inflows ('Ty Mawr', 'Forest' and 'Cefni west') contribute 10% of all terrestrially-derived stream DOC to the lake, then the figures for 'CIN' and 'CIS' would be 60% and 30% respectively.

Important influences on the contribution of terrestrial organic carbon via inflowing streams are natural removal processes such as solar photolysis and microbial metabolism. The influence of these processes on the carbon concentrations and quality of the 'CIN' inflow were particularly obvious in July and appear to lessen the concentration of DOC entering the lake from Cors Erddreiniog.

Hydrochemical data also seems to imply that the 'CIN' inflow is an important contributor of bromide and nitrate, the former originating from the fen and the latter from the farmlands which the stream passes through on its journey to the Cefni. However, other sources of these ions are also important, such as nitrate draining from farmlands into 'CIS' and the input of bromide from precipitation.

3.2 Non-terrestrial THM precursors

3.2.1 Introduction

It was demonstrated in chapter 2 that a peak in dissolved methane concentrations occurred in Cefni lake during the summer of 2003 (*figure 2.19*, p39) and that concentrations correlated with the formation of trihalomethanes (THMs). There was also indirect evidence of high algal productivity during the summer months, when THM formation was greatest. Thus, there is a need to determine the potential contribution of dissolved methane and algal DOC to the formation of THMs at the Cefni reservoir.

Methane (CH_4) in lake water is typically supersaturated compared to atmospheric concentrations. Lakes are therefore usually sources of methane to the atmosphere (Jones & Mulholland, 1998; Hope, *et al.* 2001). The methane concentration of a lake changes seasonally and depends on both the trophic status of the lake and the balance between methane production and consumption (Murase, *et al.* 2003, 2005). In eutrophic lakes, the principal origin of methane is methanogenesis through the anaerobic decomposition of organic matter by bacteria in anoxic portions of the lake (St. Louis, *et al.* 2000). Other sources include diffusion from the anoxic layers of lake-bed sediments, groundwater and inflowing stream/river contributions and external inputs from catchment soils (Jones & Mulholland, 1998; Murase, *et al.* 2003; Kuznetsova & Dzyuban, 2005). Temperature is one of the most important factors controlling the development of anoxia and occurrence of methanogenic activity in eutrophic lakes and lake sediments; for example, Murase, *et al.* (2005) attributed high methanogenesis in littoral sediments to high summer temperatures. The flux of allochthonous dissolved methane to lakes depends on processes occurring within the catchment soils, principally the extent of organic matter storage, the soil's hydraulic conductivity and the availability of carbon dioxide and other terminal electron acceptors in determining the extent of methanogenesis (Jones & Mulholland, 1998; Mitsch & Gosselink, 2000). Peatland soils are usually the greatest external source of dissolved methane to freshwaters, because the dominantly anaerobic nature of the soils leads to high rates of methanogenesis. In addition, most gaseous carbon is transported laterally (into freshwaters), rather than vertically (into the atmosphere), from peat soils (Hope, *et al.* 2001). Consequently, the highest concentrations of dissolved methane in freshwater streams are usually found in areas draining peatlands. For example, for a site in north-east Scotland, Hope, *et al.* (2001) observed peaks in dissolved CH_4 (and CO_2) as streams passed through areas of peaty soils.

Within a lake, methane is lost by evasion to the atmosphere, which is controlled by temperature and turbulence (Jones & Mulholland, 1998), and oxidation by methanotrophs, which occurs mostly in the presence of oxygen, but can take place anaerobically (Hanson & Hanson, 1996; Utsumi, *et al.* 1998). Oxidation is considered the primary mechanism of loss in all lake types (Utsumi, *et al.* 1998; Wetzel, 2001). Concentrations of dissolved methane in temperate freshwaters are typically in the region of 5-10 $\mu g\ l^{-1}$ (Jones & Mulholland, 1998), but can rise to 60 $\mu g\ l^{-1}$ when passing through peaty areas (Dawson, *et al.* 2002). The concentration of dissolved methane is therefore approximately three orders of magnitude lower than the typical concentration of dissolved organic carbon (DOC) found in temperate freshwaters.

There appear to have been no published studies that have attempted to assess the formation of THMs from the dissolved methane contained within raw water used for drinking water supplies. This is somewhat unusual, given the ubiquitous presence of dissolved methane in most temperate streams, rivers and lakes, and the structural similarity of methane and the four THM compounds. An attempt was therefore made to manipulate the dissolved methane concentration of pure distilled water and determine its potential to form THMs through chlorination. It was hoped that, from this, the potential contribution of THMs from dissolved methane at environmentally significant levels during water treatment could be established.

The presence of algae in reservoirs used as sources of drinking water is undesirable as it increases the DOC concentration of the water, impairs the water's taste and odour, blocks water treatment filters and contributes to the formation of disinfection by-products (Palmer, 1977; Plummer & Edzwald, 2001; Nguyen, *et al.* 2005). If inefficiently removed during water treatment processes, some species of 'blue-green' algae have also been shown to lead to illnesses such as gastroenteritis in humans (Palmer, 1977). Most treatment works benefit from extracting water from reservoirs where the algal productivity is low, but in some lowland areas this is not possible. In extreme cases, rising costs associated with removing algae from highly productive lakes used as sources of drinking water have led to the treatment works being decommissioned. High costs are generated because sophisticated treatment processes such as dissolved air flotation (DAF) (Gehr, *et al.* 1993) are needed to remove algae and prevent the clogging of filters, which are usually employed later in the treatment process. Even enhanced coagulation, the most efficient carbon-removal process in widespread use, cannot effectively remove algal DOC (Cheng & Chi, 2003). It has been stated that high levels of algae in raw waters can reduce the time between filter washes from an average of 100 hours to just 10 hours (Palmer, 1977).

A number of studies have determined the potential of algae-derived DOC to form THMs following chlorination. The results are reasonably consistent and suggest that algal DOC is an important source of THM precursors, but at lower levels than terrestrially-derived DOC. This is thought to be due to the lower aromaticity and molecular weight of algal DOC (Nguyen, *et al.* 2005). Algae can contribute DOC to freshwaters in two forms; actual cell biomass or by the release of extracellular products (ECP)/organic material (EOM). It is thought that the contribution from ECP to total DOC concentrations in freshwater lakes is greater than that from biomass (Widrig, *et al.* 1996). It is also less easy to remove during treatment (Wachter & Andelman, 1984; Amy, *et al.* 1992; Plummer & Edzwald, 2001). The production of THMs depends on factors such as algal species and growth phase (Plummer & Edzwald, 2001), although published data is somewhat inconsistent. For example, Plummer & Edzwald (2001) and Nguyen, *et al.* (2005) reported opposing findings of the reactivity of 'green' and 'blue-green' type algae, suggesting that each individual species possess their own degree of chlorine reactivity.

Oliver & Shindler (1980) measured a mean THMFP of 5.0 μg per mg DOC for seven species of algae, compared to a value of 17 μg per mg DOC for an aquatic fulvic acid. Yields of THMs per mg of algal DOC in other studies have typically yielded figures of between 20-50 μg, with those that have compared the THMFP values with a terrestrially-derived organic carbon compound usually finding that the algal DOC had a 2-3 times lower reactivity (Oliver, 1983; Wacther & Andelmann, 1984; Plummer & Edzwald, 2001; Nguyen, *et al.* 2005). Contrasting results have been published for the THMFP of biomass and ECP-type DOC. Wacther & Andelman (1984) recorded similar values of THMFP for biomass and ECP type DOC, but Plummer & Edzwald (2001) measured 2-3 times as much THMs following the chlorination of the biomass of a species of the diatom *Cyclotella*, compared to its ECP.

Although algae-derived DOC is less likely to produce THMs than that originating from terrestrial sources, due to its lower composition of chlorine-reactive structures, the rapid growth of large blooms of algae and the resulting high input of autochthonous DOC that can occur in eutrophic freshwaters lakes imply that algae can be an important source of THM precursors during the summer. However, previous studies have shown that the formation of THMs from algal DOC is strongly influenced by the species and growth stage of the algae, therefore the production of THMs from algal DOC is likely to be highly variable between sites and on a temporal basis. Therefore an attempt was made to assess the potential of algae in the Cefni reservoir to form THMs on a number of occasions during the summer of 2005 and to compare the values obtained with those of terrestrially-derived DOC entering the lake by the inflowing streams.

3.2.2 Materials and methods

3.2.2.1 Dissolved methane experiment

3.2.2.1.1 Saturation of Milli-Q water with dissolved methane

According to Walsh & McLaughlan (1999), the maximum possible concentration of dissolved methane in water at room temperature is 22 mg l^{-1}. Their technique involved the use of Milli-Q water that had been degassed completely using vacuum sonication and then bubbling pure methane through the water in a sealed vessel. In this experiment, CH_4-free Milli-Q water (at 25°C) was also used but it was not degassed and a simpler method of saturation was employed. Although the elimination of the degassing phase meant that the water would not be truly 'saturated' with methane, it was deemed sufficient for the purposes of this experiment. A 60 ml syringe was filled to the 40 ml mark with CH_4-free Milli-Q water and then 20 ml of pure methane (99.995%, Cryoservice, UK) was added and the syringe capped. This gave a 2:1 water to headspace ratio. The syringe was then shook vigorously for a set number of minutes, with separate syringes being used with shaking times of 0, 1, 2, 3, 4 and 5 minutes. To determine the methane concentration of the Milli-Q water following shaking, the simple headspace extraction method of Kling, *et al.* (1991) was employed. The headspace was removed and replaced with 20 ml of pure nitrogen (BOC gases, UK) and the syringe capped and shook for 2 minutes. The methane concentration of the headspace was then analysed on a gas chromatograph (see page 34, section 2.1 for instrument details).

3.2.2.1.2 Chlorination of water with varying dissolved methane concentrations

The saturation of non-degassed Milli-Q water yielded a dissolved methane concentration of 7.3 mg l^{-1} CH_4-C. A range of dissolved methane concentrations were tested for their potential to form THMs following chlorination of the water sample, following dilution of the methane-saturated stock solution. They were 0.02, 0.04, 0.06, 0.08, 0.1 and 1.0 mg l^{-1} CH_4-C, with the 7.3 mg l^{-1} CH_4-C solution also tested for THM formation. For details of the chlorination and analytical details of the THMFP determination, please refer to page 83. For each 25 ml vial, 0.5 ml of chlorine dosing solution was used, irrespective of the dissolved methane concentration.

3.2.2.1.3 Dissolved methane concentrations of natural waters

Figure 2.19, page 39 of section 2.1 shows seasonal changes in the dissolved methane concentration of Cefni lake water, with the highest concentration being recorded in the summer. Consequently, the dissolved methane concentration of natural waters within the catchment of the Cefni was tested in July 2005 to determine the potential contribution to THM formation from the dissolved CH_4 if the sample was chlorinated. Based on the data presented in section 3.1, that Cors Erddreiniog fen was the most important source of terrestrially-derived carbon to Cefni lake, the sampling sites chosen were Cors Erddreiniog fen, Afon Erddreiniog, Cefni inflow North ('CIN'), Cefni lake and, to test whether dissolved methane is removed during the treatment processes employed at the Cefni reservoir, post-filtration (i.e. after all the carbon removal stages) and Cefni final (i.e. after chlorination). At each site, 40 ml of water was taken into a 60 ml syringe, which was capped, and 5 syringes were used for each site. Soil water was obtained from Cors Erddreiniog fen using the method described on page 52 of section 2.2. The syringes were transported back to the laboratory, where the headspace extraction method detailed on the previous page was performed to determine the methane concentrations.

3.2.2.1.4 Calculations and statistical tests

Dissolved methane concentrations were converted to mg l^{-1} CH_4-C using the method of Kling, *et al.* (1991), which corrected for stream temperature and site elevation. THM values were normalised to the DOC concentration, as described on page 85. *Figure 3.19* shows data for one replicate and *figures 3.20-3.22* show data for 5 replicates, with error bars indicating ± standard error of the mean. One-way ANOVA and the Tukey-Kramer multiple comparisons post-hoc test were used to test for significant differences between the chloroform yields obtained from the varying dissolved methane concentrations of the Milli-Q water and between the samples tested for their natural dissolved methane concentrations. This was preformed using GraphPad InStat version 3.06 (GraphPad Software, San Diego, California, USA).

3.2.2.2 Algae experiment

3.2.2.2.1 Collection and incubation of natural algal samples

During the spring, summer and autumn of 2005, algal productivity was high in Cefni lake, particularly during September and October. A sample was collected at the lake on 6 occasions during this period; specifically 17th May, 21st July, 27th August, 11th September, 1st October and 4th October. At each visit, a 1 litre sample of lake water was collected in a clean, clear bottle from the same place each time and transported back to the laboratory, where the sample was incubated to promote algal growth. This involved transferring the sample to a pre-autoclaved 10 litre clear Pyrex glass vessel and adding 4 litres of deionised water. To increase growth of the algae, Conway growth medium (composition detailed in *table 3.03*) and a vitamin/silicate nutrition solution were added at 1 ml l^{-1} and 0.1 ml l^{-1} respectively. Air, which had been passed through a 0.2 µm filter to remove particulates, was bubbled through the solution to increase aeration and improve mixing. The vessel was placed in front of a continuous light bank, whilst the temperature of the solution was maintained at room temperature (24-25°C).

Constituent	Amount
EDTA disodium salt	90.0g
H_3BO_3 crystals	67.2g
$NaNO_3$	200.0g
$NaH_2PO_4.H_2O$	40g
$MnCl_2.4H_2O$	0.72g
$FeCl_3$ (anhydrous)	2.6g
Distilled water	2.0 litres
Trace metal solution	2.0 ml

Table 3.03 – Composition of Conway medium, used to stimulate the growth of freshwater algae.

3.2.2.2.2 Sample treatment and analytical details

After 10 days of growth, a 1 litre sample of the algal culture was removed and filtered through a 0.45 µm cellulose nitrate filter at high vacuum. Although this would remove most of the algal cells, it is a necessary procedure to remove the particulate organic carbon that would interfere with the chlorination process and to enable a direct comparison of the THMFP of algal DOC with that of terrestrial DOC. The filtrate would therefore be composed of mostly ECP and some degraded cell components. As the contribution of ECP to total DOC in lakes is greater than that from biomass (Widring, *et al.* 1996) and it is less easily removed during water treatment (Plummer & Edzwald, 2001), this method was deemed appropriate. The solution was stored in a 1 litre amber glass bottle in an incubator set at 4°C until the undertaking of dissolved organic carbon analyses. DOC concentration, UV-254 absorbance, SUVA, HPSEC molecular weight and THMFP analyses were determined on all six of the algal bloom filtrate solutions. The THMFP method was kept the same as that performed on the stream/lake water DOC in section 3.1 to enable direct comparison between the THMFP of allochthonous and autochthonous DOC.

3.2.2.2.3 Data analysis regarding THM formation at the Cefni

Raw water chlorophyll *a*, final water DOC and THM concentrations and rainfall levels from the Cefni water treatment works was examined to ascertain the influence of algal DOC on the formation of THMs. Average daily rainfall totals were calculated for the period between each THM measurement. These data were provided by the water company.

3.2.2.2.4 Statistical analyses

As for the dissolved methane data, THM values were normalised to the DOC concentration. *Figures 3.24, 3.25 and 3.27* show data for one replicate and *figures 3.25-3.26* show data for 5 replicates, with error bars indicating ± standard error of the mean. Significant differences in SUVA and THMFP values between the six algal cultures, and compared to the data for the inflowing streams to the Cefni from section 3.1, were determined using one-way ANOVA and the Tukey-Kramer multiple comparisons post-hoc test. Pearson correlation was used to test for correlations between raw water chlorophyll *a* and final water DOC with final water THM concentrations. Statistical analyses were preformed using SPSS version 9.0 (SPSS, Inc.) and GraphPad InStat version 3.06 (GraphPad Software, San Diego, California, USA).

3.2.3 Results

3.2.3.1 Dissolved methane experiment

Figure 3.19 shows the effect of saturating pure, CH_4-free Milli-Q water with pure methane gas using an increasing number of minutes of shaking. After 1 minute of shaking, the methane concentration of the Milli-Q water had reached 6.6 mg l^{-1}. Shaking for 3 minutes increased the methane content by 8% to 7.3 mg l^{-1}. An overall shaking of 5 minutes increased the concentration by just a further 1%, by which point methane saturation appears to have occurred. Based on this data, a shaking time of 3 minutes was used to saturate Milli-Q with pure methane.

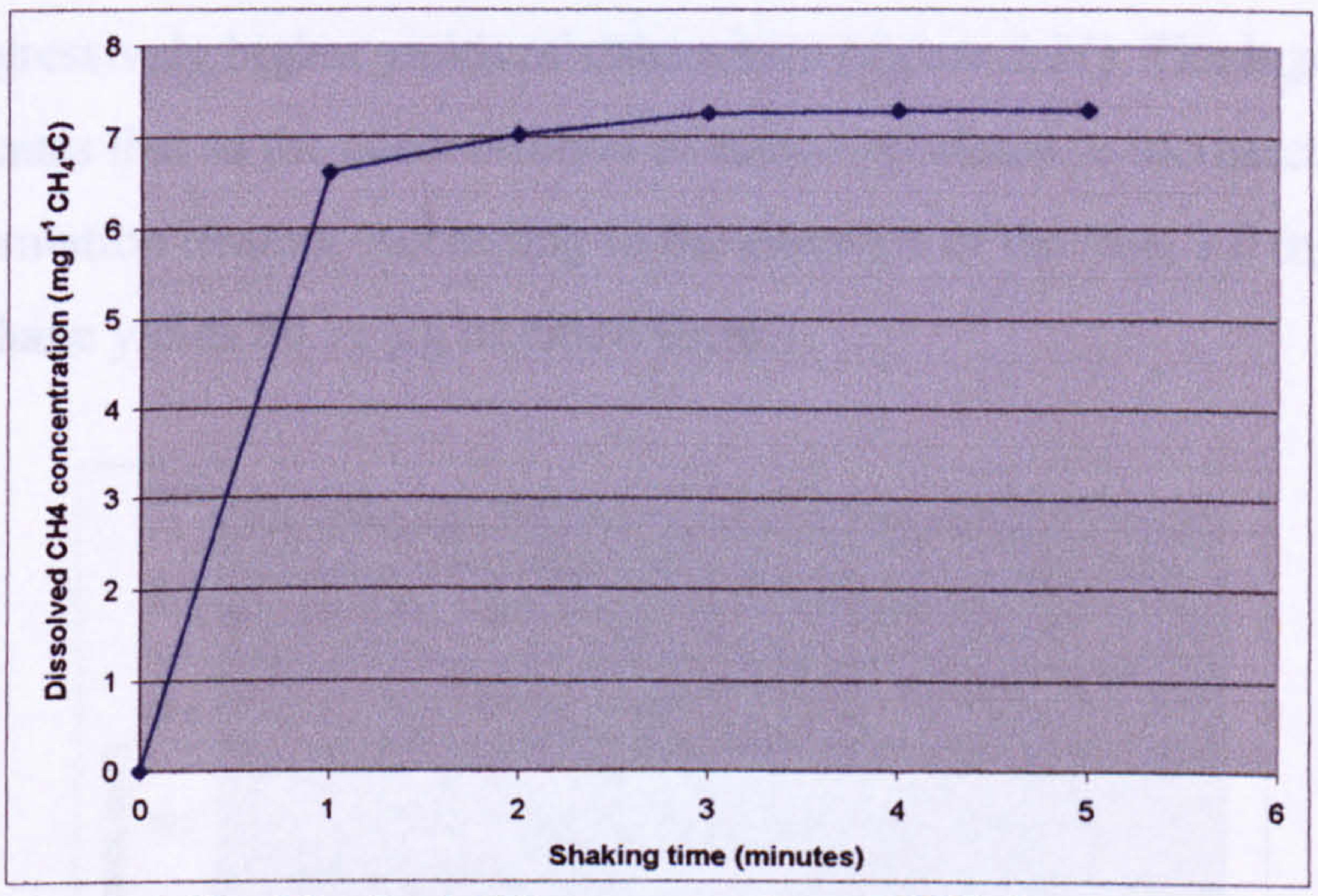

Figure 3.19 – Dissolved CH_4 concentration of Milli-Q water using a headspace extraction method involving increasing lengths of shaking

The chlorination of methane-saturated Milli-Q water yielded a chloroform concentration significantly higher than that for pure, CH_4-free Milli-Q (*figure 3.20*), with the value being 924% greater (p<0.001). Due to the absence of bromide in the pure Milli-Q water, chloroform was the only THM generated.

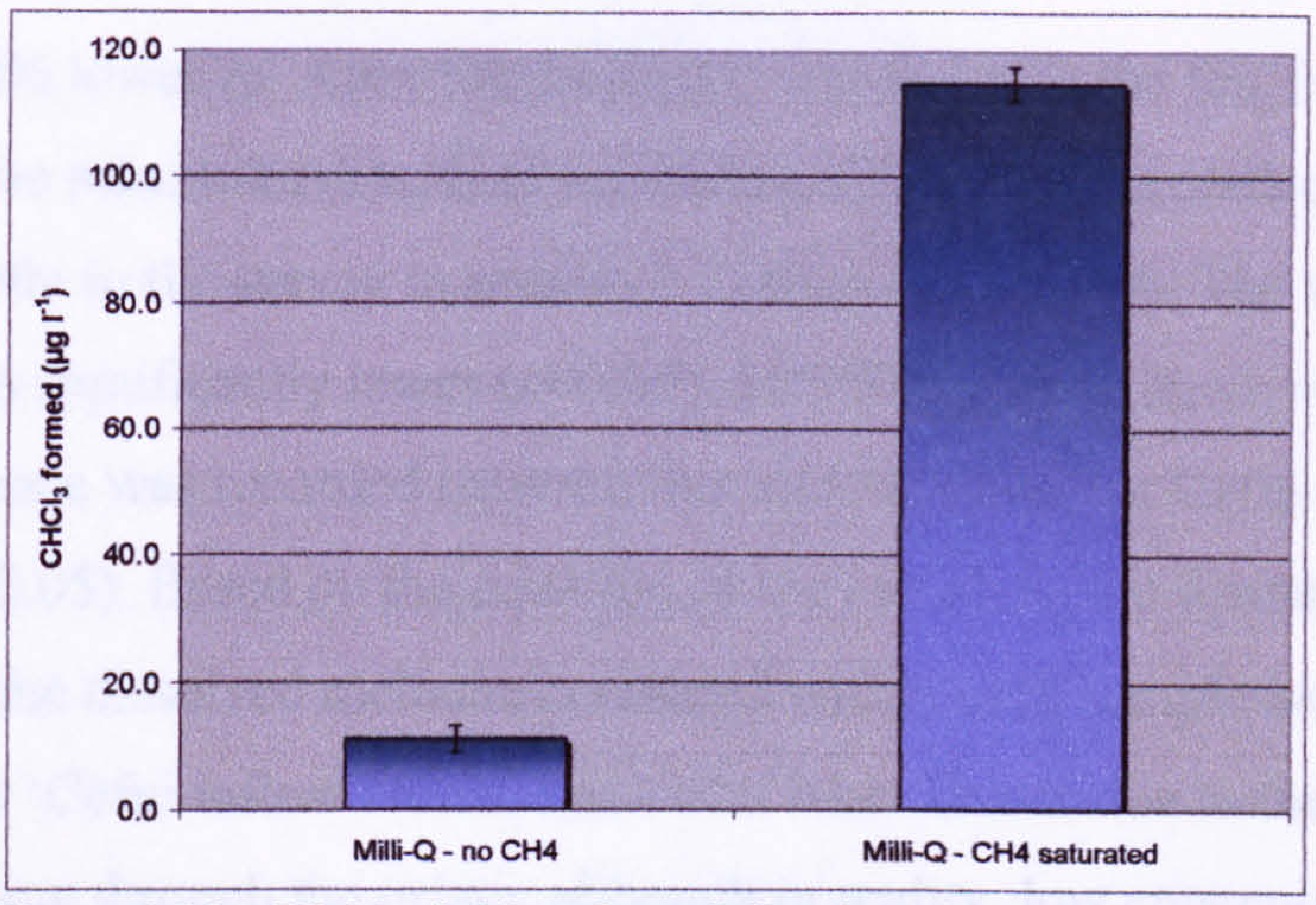

Figure 3.20 – Yield of chloroform following chlorination of pure, CH_4-free and CH_4-saturated Milli-Q water

The chlorination of Milli-Q water with increasing concentrations of dissolved methane generated progressively higher yields of chloroform (*figure 3.21*). The logarithmic trendline indicates that as the concentration of dissolved methane increases the yield of chloroform formation lessens. According to the equation of the line, 1.0 mg l^{-1} of dissolved methane yields 50.31 µg of chloroform.

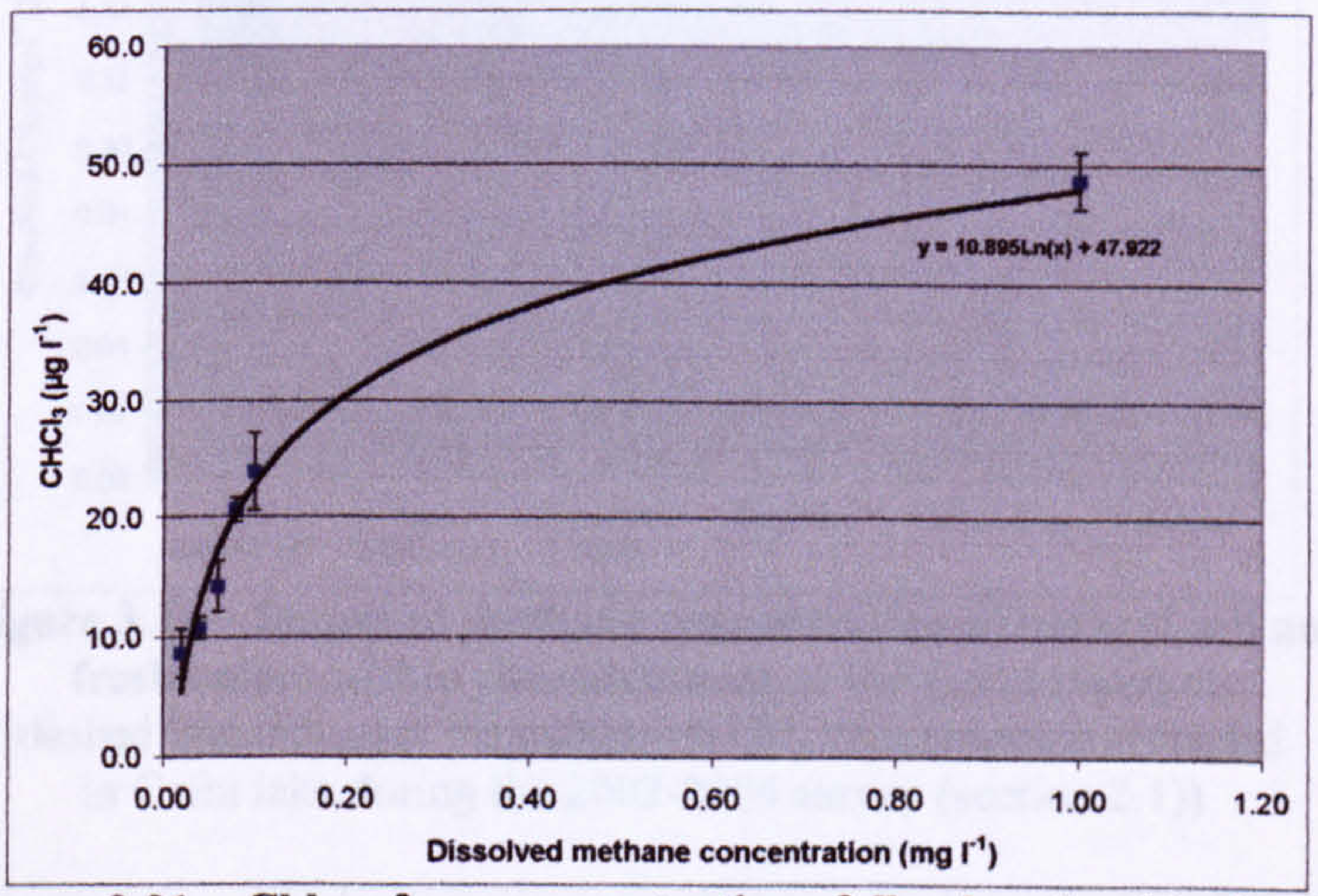

Figure 3.21 – Chloroform concentrations following the chlorination of solutions of Milli-Q water with a range of dissolved methane concentrations

The dissolved methane concentration of natural waters was determined (*figure 3.22)* and, based on this, the amount of chloroform that would be generated if all the methane in the sample was chlorinated (*table 3.04*). The porewater of the 'Cors Erddreiniog' fen soil was enriched in dissolved methane compared to the freshwater samples, having a methane concentration of 0.199 mg l^{-1}. The concentration of dissolved methane was almost exactly 10% lower in 'Afon Erddreiniog', which drains the fen, but was almost twice as high as the maximum dissolved methane concentration recorded during the summer at the Cefni in the survey in section 2.1 (*figure 2.19*, p39). The value at 'Cefni inflow North' was significantly lower ($p<0.05$), at 0.003 mg l^{-1} CH_4-C, but no significant difference was recorded between this and the value for Cefni lake (0.0017 mg l^{-1} CH_4-C; $p>0.05$). Based on the equation of the trendline, the formation of chloroform from the dissolved methane contained within each sample would yield a negative value for 'Cefni inflow North' and Cefni lake. This is due to the logarithmic trendline not passing through the origin, although in reality, low concentrations of methane would probably yield undetectable concentrations of chloroform. The treatment processes employed at the Cefni seemed to effectively remove the dissolved methane, with values below the limit of detection being recorded for both the post-filtration and final water stages.

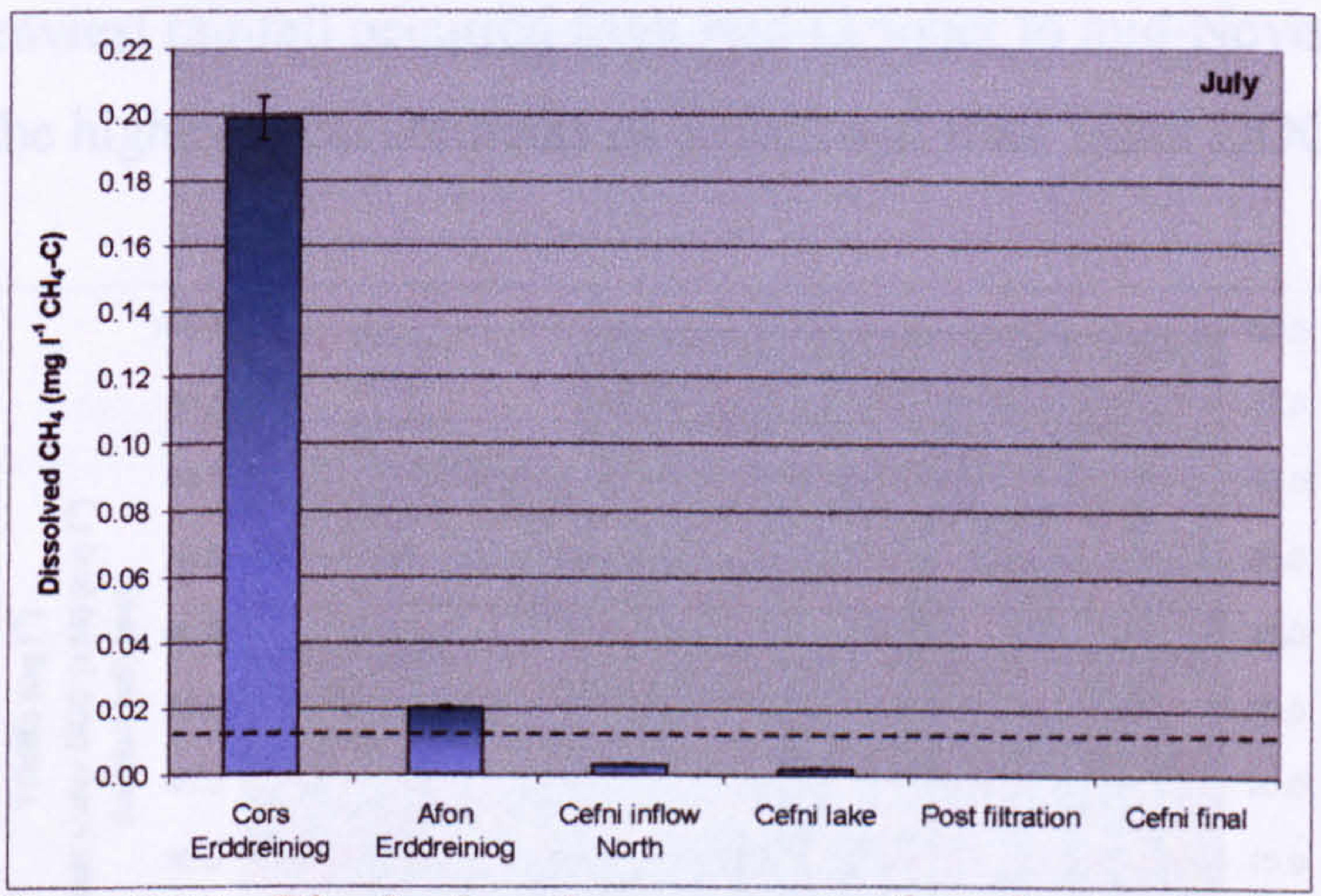

Figure 3.22 – Dissolved methane concentration of natural soil and freshwaters within the catchment of the Cefni reservoir
(dashed line indicates the maximum CH_4 concentration recorded in Cefni lake during the 2003-2004 survey (section 2.1))

Sample name	Dissolved CH_4 concentration (mg l^{-1})	Chloroform yield if chlorinated (µg)
Cors Erddreiniog fen	0.1990	30.33
Afon Erddreiniog	0.0202	5.51
Cefni inflow North	0.0030	-15.37
Cefni lake	0.0017	-21.56
Post-filtration	below detection limit	N/A
Cefni final	below detection limit	N/A

Table 3.04 – Potential yields of chloroform from the dissolved methane present in natural samples following chlorination

3.2.3.2 Algae experiment

Measurements of chlorophyll *a* concentration, an indicator of algal productivity, show that a dip in late spring/early summer (lowest - 3.9 µg l^{-1}) was followed by a progressive rise to a peak during late summer/early autumn, when a concentration of 45.2 µg l^{-1} was reached on 15th September. *Figure 3.23* shows this increase in raw water chlorophyll *a*, alongside measurements of final water DOC and THMs and rainfall levels. The black arrows show the times when samples were taken from Cefni lake. Although the concentration of final water DOC and THMs increased steadily during the summer, there was no obvious large increase in concentration at the same time as the September-October peak in chlorophyll *a* concentrations. As indicated by the red box on the graph, the concentration of THMs actually fell during the peak in chlorophyll *a*, and increased again once the chlorophyll levels reduced. Likewise, the smaller peaks in chlorophyll *a* at the beginning of April and August did not impact on final water THM concentrations. The period of heaviest rainfall occurred from mid-October to mid-November, which coincided with the highest measurements of THMs and final water DOC.

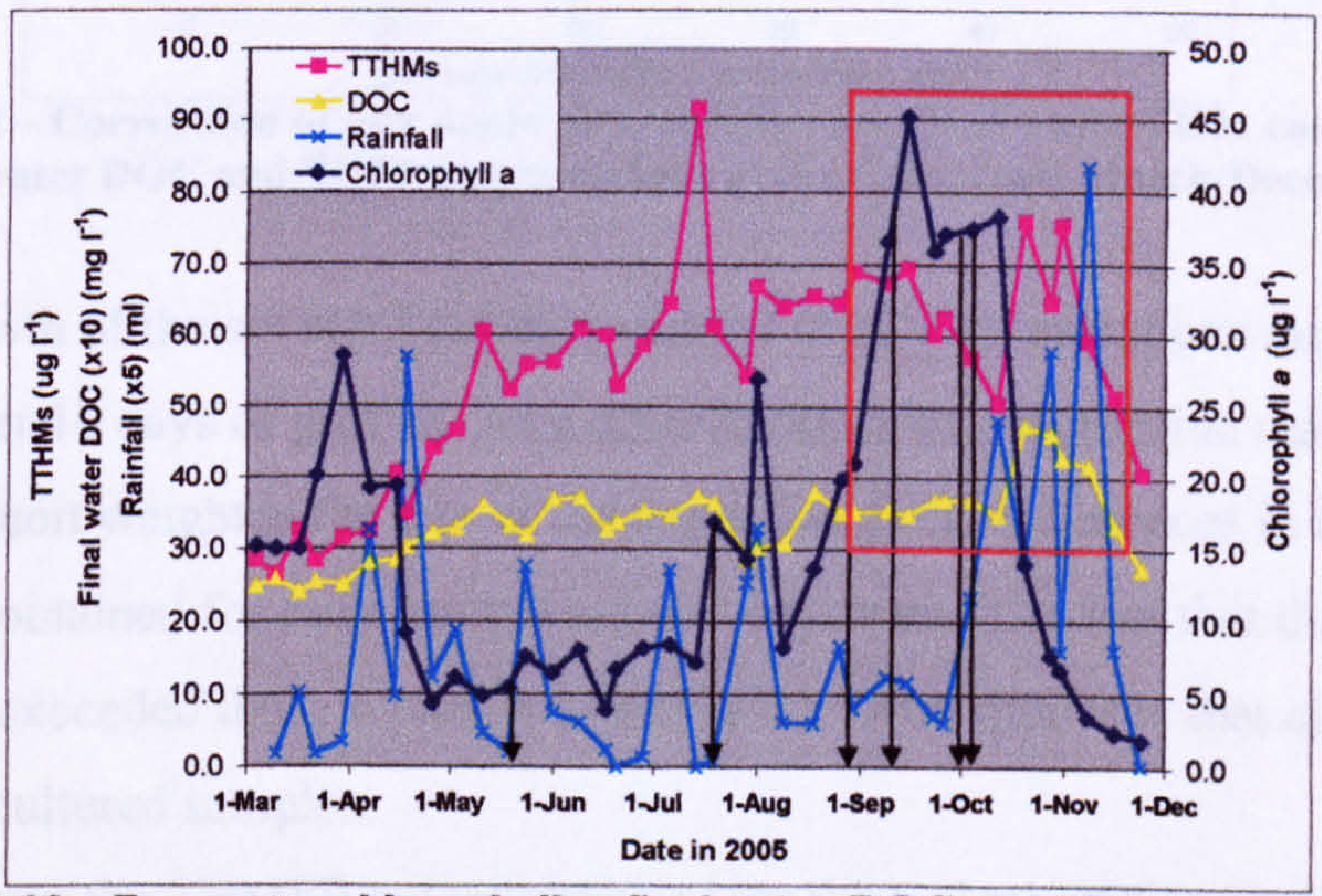

Figure 3.23 – Raw water chlorophyll *a*, final water DOC and THM concentrations and rainfall levels for the Cefni water treatment works from March-December 2005

Based on a carbon:chlorophyll a ratio of 100, which was used to estimate the concentration of algal DOC in Dutch eutrophic lakes by Van Der Vlugt & Klapwijk (1990), the contribution by algae to total DOC concentrations in Cefni lake during 2005 would have ranged from approximately 0.5-4.5 mg l^{-1} (5-45% of total DOC within the lake, assuming an average DOC concentration of 10 mg l^{-1}, based on data in *figure 2.12*, page 38).

Figure 3.24 shows the correlation between raw water chlorophyll *a* and final water THM concentrations. The R^2 value of just 0.0014 seems to suggest that periods of high lake chlorophyll *a* values did not result in elevated THMs during 2005. This is despite the positive significant correlations between raw water and treated water DOC (*figure 2.13,* page 38) and treated water DOC and final water THMs (*figure 2.08*, page 35) measured at the Cefni treatment works during 2003.

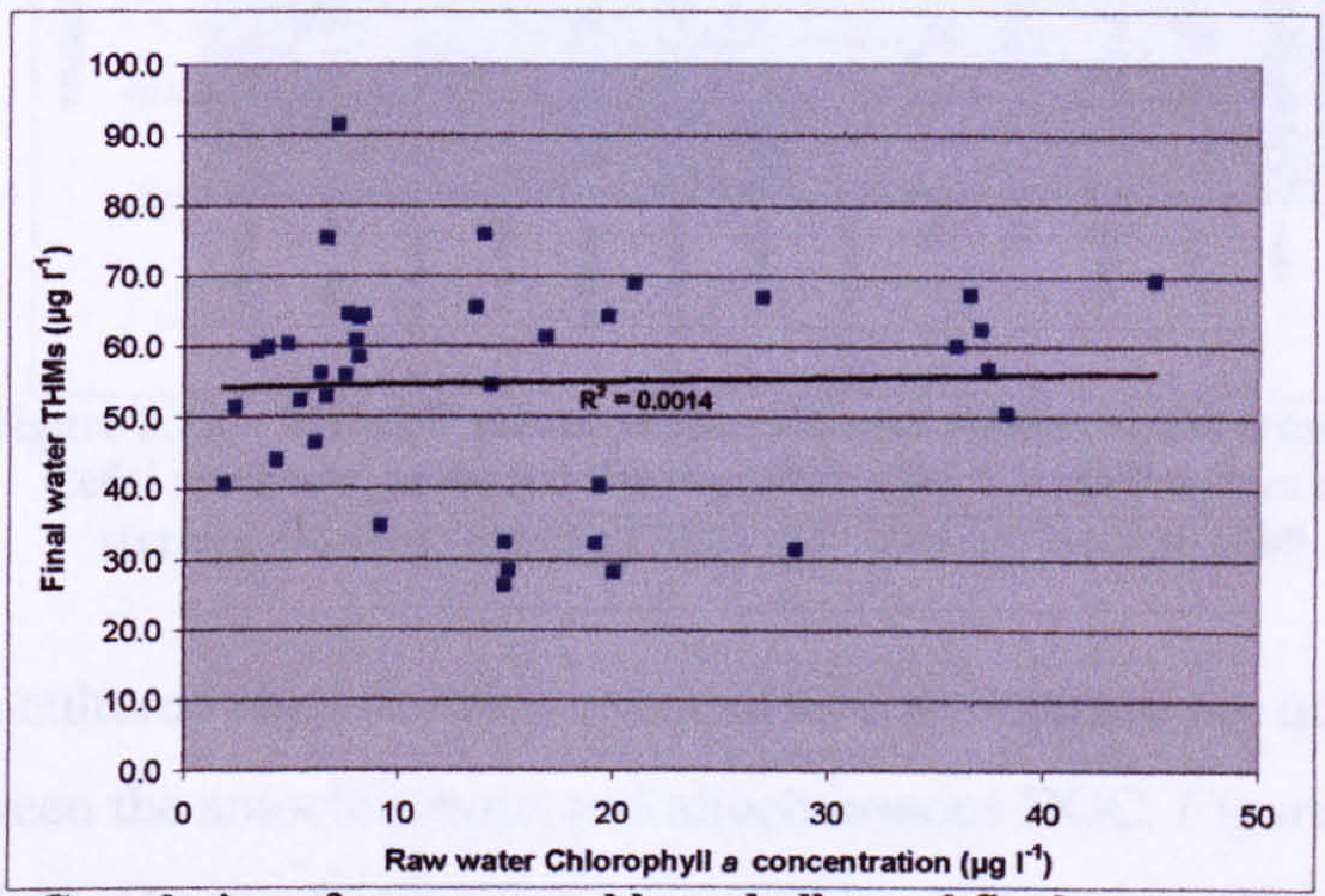

Figure 3.24 – Correlation of raw water chlorophyll *a* and final water THM concentrations and final water DOC and THM concentrations at the Cefni from March-December 2005

Successful growth of the six algal cultures yielded DOC concentrations ranging from 20 to 37 mg l^{-1} after 10 days of growth. As a determination of the chlorine reactivity of the algal DOC per unit weight is the aim of the experiment, the differences in DOC concentrations obtained for each sample are not important. The fact that the DOC concentrations exceeded those typically found at the Cefni indicates that algal DOC dominated the cultured samples.

Figure 3.25 shows the THMFP values obtained for the 6 algal DOC samples and, to enable comparison between autochthonous and allochthonous sources of DOC, the THMFP values of the inflowing streams to the Cefni in October 2005. The values for the 1st and 4th October samples were approximately twice as high as the mean value and significantly different to the other 4 cultures ($p<0.01$). The mean algal THMFP value was significantly lower than the mean inflowing stream THMFP values (72% lower, $p<0.01$). The THMFP of the 'Afon Erddreiniog' DOC was significantly higher than all six algal THMFP values ($p<0.01$-0.001), whilst 'CIN', 'CIS' and 'Forest' stream THMFP values were all significantly higher than the 17th May-11th September and mean algal THMFP values ($p<0.05$-0.001).

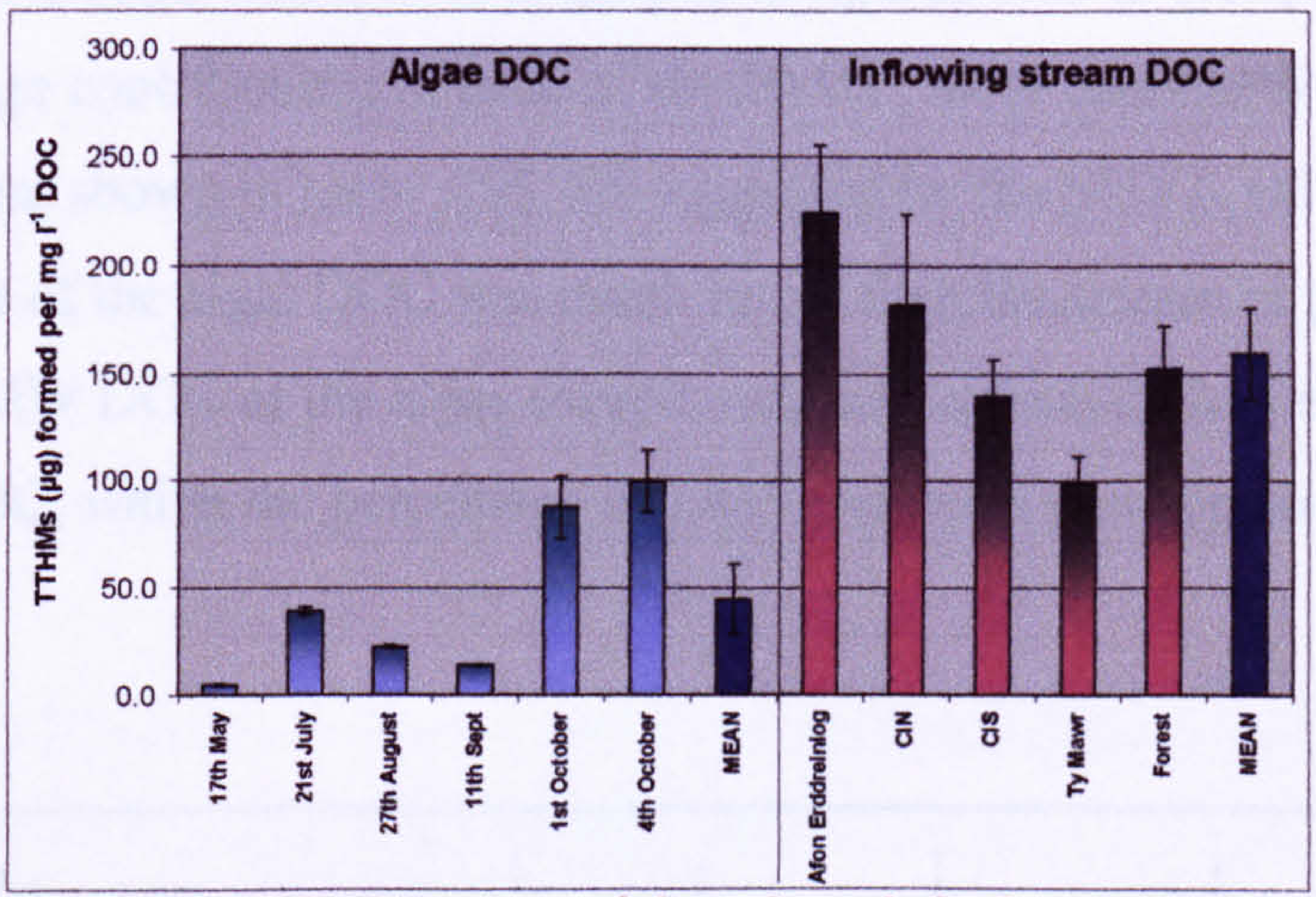

Figure 3.25 – THMFP values of the cultured algal samples from the Cefni reservoir, collected during 2005, plus THMFP values of streams flowing into the Cefni, collected in October 2005.

The DOC of the cultured algal samples was analysed to compare the qualitative differences between the autochthonous and allochthonous DOC. *Figure 3.26* shows the SUVA values of the algal and inflowing stream DOC. Values were significantly lower for the algal DOC than the terrestrial DOC, with the mean value of the six algal samples being just 15% of the mean value of the inflowing stream samples ($p<0.001$). None of the algal samples were significantly different to one other ($p>0.05$), but they were all significantly lower than the five inflowing stream SUVA values ($p<0.001$).

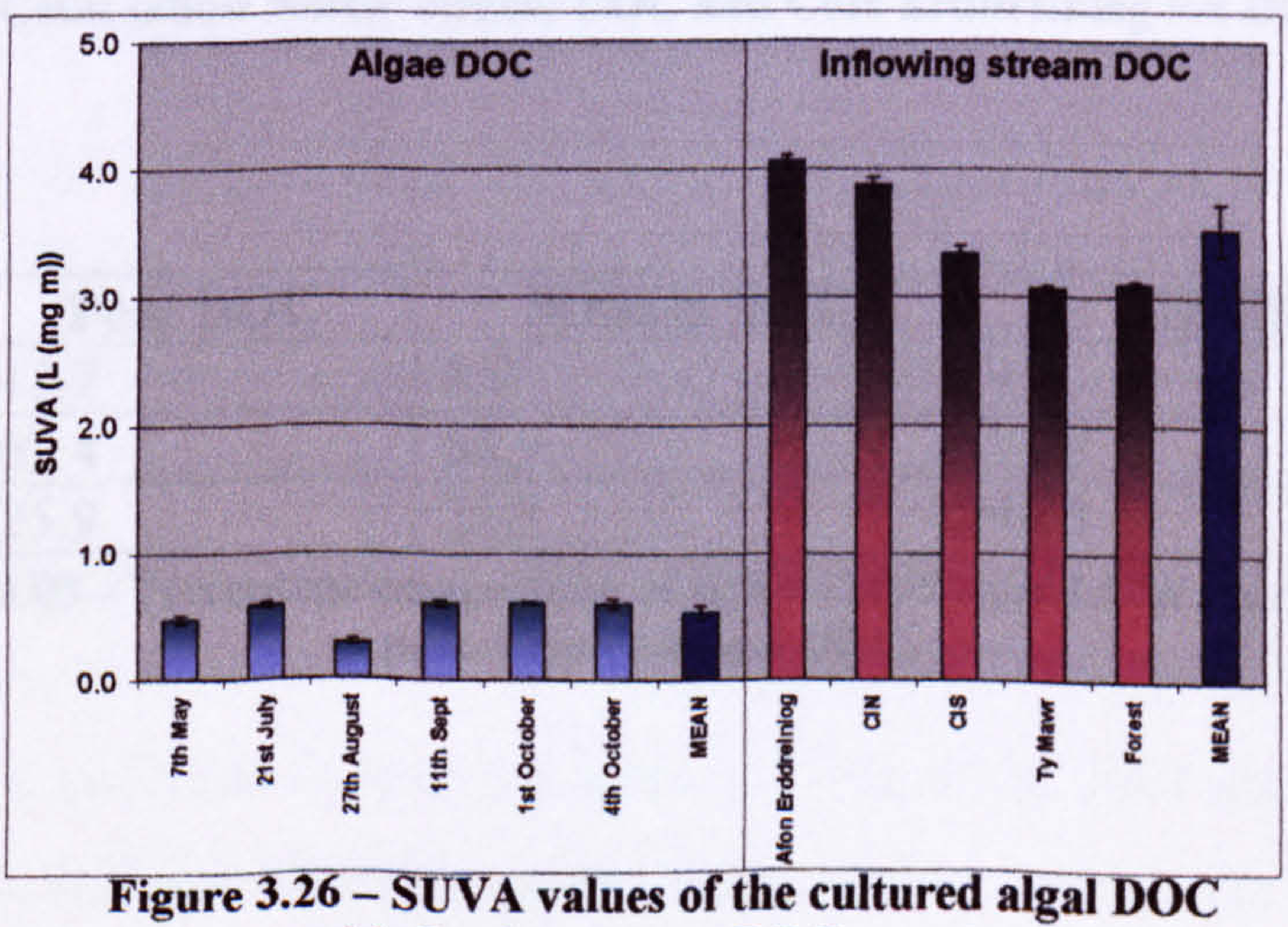

Figure 3.26 – SUVA values of the cultured algal DOC and inflowing stream DOC samples

Molecular weight data of the algal DOC was also determined by HPSEC and the spectra are shown in *figure 3.27*. The algal DOC spectra, which is a combination of the six cultured algae samples, is shown alongside the October 2005 'Cors Erddreiniog' peat and 'CIN' stream spectra which were displayed in *figures 3.05 & 3.13*, on pages 87 & 93. The percentage contributions of each of the HMW, IMW and LMW fractions to the total DOC pool are shown in *table 3.05*. As suggested by the SUVA values, the molecular weight of the algal DOC was much lower than the stream or peat DOC. The abundance of HMW DOC of the algae sample was approximately half the value of the lake and peat DOC, whilst the percentage of LMW material was approximately twice as much.

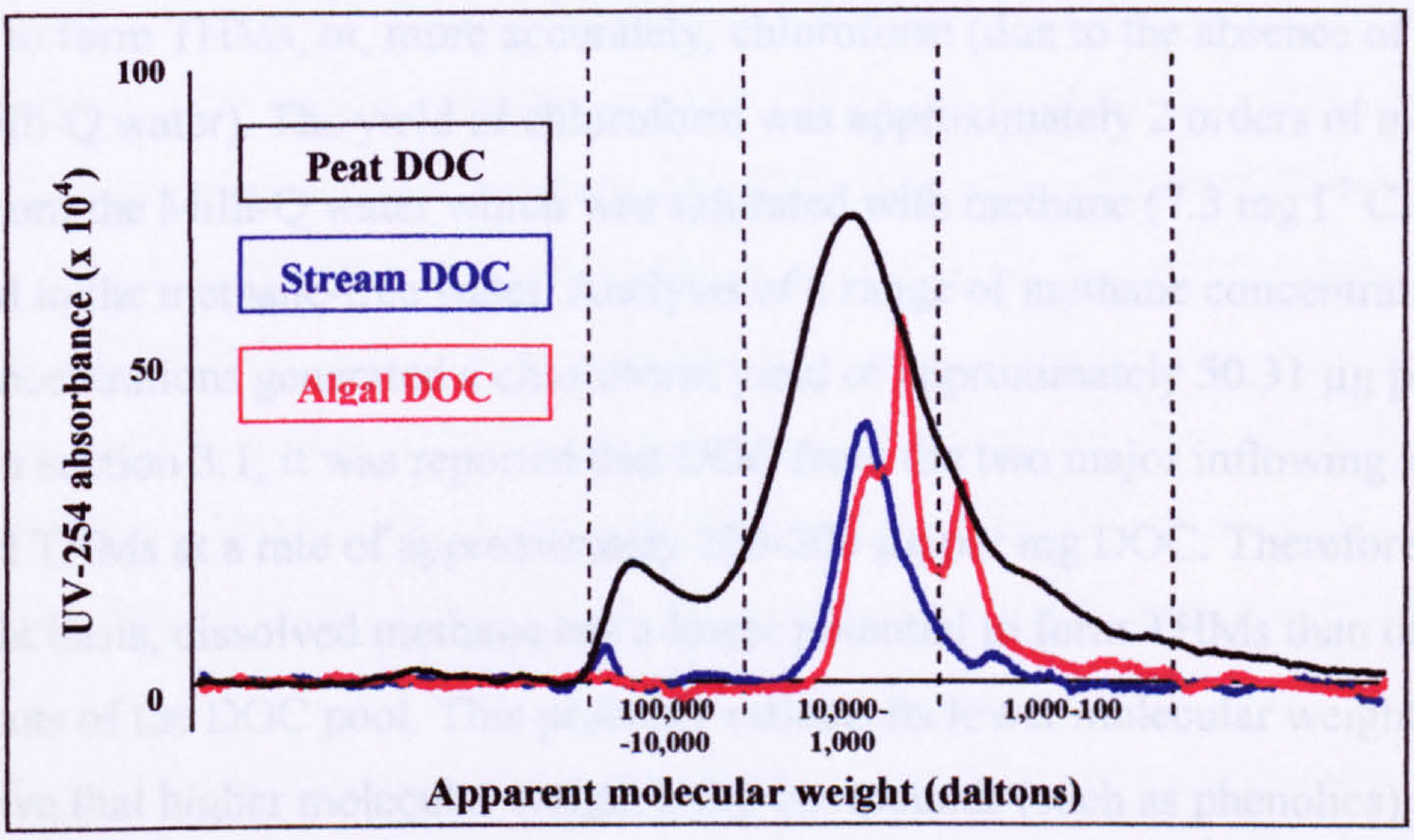

Figure 3.27 – HPSEC-determined molecular weight spectra of algal DOC, alongside 'Cefni inflow North' stream DOC and Cors Erddreiniog fen DOC.

	Peat DOC	Stream DOC	Algal DOC
HMW	11.7	8.9	4.8
IMW	62.4	68.9	51.7
LMW	25.9	22.2	43.5

Table 3.05 – Percentage composition of HMW, IMW and LMW fractions of peat, lake and algal DOC.

3.2.4 Discussion

3.2.4.1 Dissolved methane experiment

The results of this study show that dissolved methane can form chloroform following the addition of chlorine. Whilst methane in freshwaters can be considered a component of the DOC reservoir, since it is dissolved, organic and carbon-based, its gaseous nature ensures it is a unique component of the organic carbon cycle. The observation that THMs can be generated following the chlorination of methane is therefore an interesting new angle.

The chlorination of methane-saturated Milli-Q water demonstrated the potential of methane to form THMs, or, more accurately, chloroform (due to the absence of bromide in the Milli-Q water). The yield of chloroform was approximately 2 orders of magnitude greater from the Milli-Q water which was saturated with methane (7.3 mg l^{-1} CH_4-C) compared to the methane-free water. Analysis of a range of methane concentrations at lower concentrations generated a chloroform yield of approximately 50.31 μg per mg CH_4-C. In section 3.1, it was reported that DOC from the two major inflowing streams generated THMs at a rate of approximately 100-200 μg per mg DOC. Therefore, on a per weight basis, dissolved methane has a lower potential to form THMs than other components of the DOC pool. This probably reflects its lower molecular weight and it is well known that higher molecular weight DOC compounds (such as phenolics) are generally more reactive with chlorine than those with a low molecular weight (Uyguner, *et al.* 2004).

Despite the potential of dissolved methane to form THMs, an analysis of the typical concentrations of methane in the freshwaters associated with the Cefni reservoir reveal that dissolved methane is unlikely to contribute to the formation of THMs at the treatment works. In the survey described in chapter 2, it was shown that in Cefni lake itself, the concentration of dissolved methane peaked at approximately 0.01 mg l^{-1} during the summer months of 2003. The measurement taken in July 2005 shows that the concentration was much lower, at approximately a fifth of the 2003 value. However, as for the 2003 data, the concentration may have been higher during other times in the summer. Whilst a significant source of this dissolved methane is likely to have been from methanogenesis through the anaerobic breakdown of organic matter (Jones & Mulholland, 1998; Murase, *et al.* 2005), a potentially important source seems to be from the fen. Peatlands are known to possess high concentrations of dissolved methane compared to other soils due to the lack of oxygen and the resulting occurrence of methanogenesis. They play an important role in the global methane budget, contributing 5-10% of all the methane released to the Earth's atmosphere (Whalen, 2005). The concentration of dissolved methane in the porewater of Cors Erddreiniog fen (0.2 mg l^{-1}) was comparable to that recorded by Hope, *et al.* (2001) and Beckmann, *et al.* (2004) for a range of peat soil types collected from northern Europe.

This high concentration in the fen porewater explains the relatively high concentration in 'Afon Erddreiniog' (0.02 mg l^{-1}), the stream draining the fen. This value is higher than is typically recorded for a freshwater river (Scranton & McShane, 1991), but comparable to that recorded for a stream draining a peatland in Scotland (Hope, *et al.* 2001). As would be expected, the concentration in the stream was much lower than in the fen, as methane is lost by a number of pathways once it leaches out of the soil. A major mechanism is oxidation by methanotrophic bacteria (Utsumi, *et al.* 1998), a process which will be quite rapid, because the dissolved methane enters an aerobic environment after being generated in the oxygen-free soil. Most lakes and streams possess methane at a concentration which is supersaturated (Jones & Mulholland, 1998); this is especially so for peatland-draining streams. A net flux of methane to the atmosphere therefore occurs and partly explains why the loss of methane was so high. Another reason why the methane concentration of 'Afon Erddreiniog' was so much lower than the fen porewater is because of dilution. The concentration of dissolved methane dropped significantly again (almost 90%) as the water travelled from the source, at the fen, to Cefni lake, at 'CIN'. Evasion and oxidation would again account for the majority of this loss, the latter of which varies seasonally and would have been high at the time of sampling due to the warmer temperatures leading to greater methanotrophic bacterial activity. Unlike in lakes, evasion is thought to be the main pathway of loss in rivers and streams (Wetzel, 2001).

It is apparent from the data collected that the concentration of dissolved methane in Cefni lake and its major inflowing stream is very low; it is approximately three orders of magnitude lower than the total concentration of DOC. It therefore comprises a very minor component of the aquatic DOC pool. Based on the yield of 50 micrograms of $CHCl_3$ generated per milligram of methane from *figure 3.21*, the chlorination of untreated solutions of water from the three freshwater bodies ('Afon Erddreiniog', 'CIN' and 'Cefni lake') would generate extremely small concentrations of $CHCl_3$ from the dissolved methane they contain.

Further evidence of the unimportance of dissolved methane as a THM precursor was found when analysing the dissolved methane concentration of the treated water. At the Cefni, raw water is subject to coagulation, dissolved air flotation and two sets of rapid gravity filters, all of which efficiently remove a large percentage of the DOC contained within the water. As shown in *figure 3.22*, these processes also seem to remove the dissolved methane to a level below the limit of detection. As a consequence, there will be very little methane available for chlorination following treatment of the water at the Cefni.

It can therefore be concluded that the positive correlation between raw water dissolved methane and final water THMs observed at the Cefni during 2003-2004 (described in section 2.1) was not caused by the direct influence of CH_4 on the formation of THMs. Instead, the correlation is likely to have been due to the influence of temperature on the processes that affect the concentration of both of these compounds. The highest concentration of both methane and THMs were recorded in the summer months, when the lake temperature was highest. Temperature is known to strongly influence the activity of methanogenesis in the lake water and its sediments (Murase, *et al.* 2005; Kuznetsova & Dzyuban, 2005), explaining the higher concentrations recorded in June and August. In addition, the reaction of chlorine with organic matter to produce THMs is strongly temperature-dependent (Knocke, *et al.* 1986; Sérodes, *et al.* 2003).

3.2.4.2 Algae experiment

Following the successful laboratory stimulation of algal growth in the samples collected from the Cefni, chlorination of the dominantly algal-derived DOC led to the formation of THMs (*figure 3.25*). The yield of THMs was variable, ranging from 5 μg per mg DOC for the May sample to 99 μg per mg DOC for the 4th October sample, giving a mean value of approximately 44 μg per mg DOC. This mean value is similar to that obtained by Oliver (1983) for a species of the 'blue-green' alga *Anabaena*, but almost twice that recorded for a species of the 'green' alga *Scenedesmus*. Similarly, Plummer & Edzwald (2001) recorded a similar yield of THMs as the mean value for this study for a diatom, but half the value for a species of 'green' alga. However, Wacther & Andelmann (1984) measured a similar value to this study for the ECP of a 'green' alga. Nguyen, *et al.* (2005) recorded a mean value of 43 μg per mg DOC for the average THMFP of 4 freshwater algal cultures, almost identical to the mean value measured in this study. Algae-derived DOC therefore contributes to the formation of THMs at the Cefni, an observation that conforms to previous experiments on the THM-forming potential of autochthonous DOC. However, as *figure 3.25* indicates, the algal DOC is significantly less likely to form THMs than that entering Cefni lake via the major inflowing streams, whose DOC originates from mainly terrestrial sources. The mean THMFP of the inflowing stream DOC was over three times higher than the mean algal THMFP, whilst the value for 'CIN', demonstrated in section 3.1 to be the most important point source of terrestrial DOC, was over four times greater. Similar differences have been recorded in previous studies, with Oliver & Shindler (1980), Oliver (1983) and Nguyen, *et al.* (2005) recording 2-4 times more THMs produced from a standard, terrestrially-derived fulvic or humic acid isolate than DOC derived from algal cultures.

The differences can be attributed to the contrasting molecular weights of the opposing sources; DOC originating from allochthonous sources is generally more aromatic and has a higher molecular weight (Curtis & Schindler, 1997; Gergel, 1999). Such attributes give organic compounds a greater affinity for chlorine, so they are more likely to form THMs (Oliver & Visser, 1980; Uyguner, *et al.* 2004). Evidence of low aromaticity and molecular weight of the algal DOC was observed in this study, from analyses of SUVA and HPSEC molecular weight distribution. The SUVA value of the six algal cultures was consistently low and averaged just 0.5 L(mg m), whereas the SUVA of the inflowing stream DOC was approximately 7 times greater, at 3.5 L(mg m). In addition, the algal DOC had approximately half the percentage of HMW material, and twice the percentage of LMW compounds. This data conforms to previous studies that have demonstrated a lower molecular weight of algal biomass and ECP DOC than terrestrially derived DOC, such as humic and fulvic acids. For example, Nguyen, *et al.* (2005) recorded a similar SUVA trend to that reported here, with a mean value of 0.7 L(mg m) for three algae cultures contrasting with a value of 4.1 L(mg m) observed for a fulvic acid standard. Lower aromaticity of the algal cultures compared to the fulvic acid was also measured using ^{13}C-NMR by Nguyen, *et al.* (2005), who commented that the low aromaticity explains the low THMFP of the algal DOC. It can therefore be concluded that, in this study, the much lower molecular weight and aromaticity of the six algae cultures compared the five inflowing streams is sufficient to explain its significantly lower potential to form THMs.

The spring to autumn period of 2005 saw high algal productivity in Cefni lake. Concentrations of chlorophyll *a* peaked at 29 and 45 $\mu g\ l^{-1}$ in spring and autumn respectively, but were lower during the summer, with the June-August period averaging almost half the concentration of the September-November maximum (*figure 3.23*). Data provided by the water company of chlorophyll *a* concentrations from 2004-2006 also show a dip in summer concentrations during 2006 (*figure A05*, Appendix A03). Despite the high temperatures and availability of light during summer, algal growth can decline sharply following a spring bloom due to a reduction in nutrient levels in the photic zone of the lake (Wetzel, 2001); the chlorophyll *a* concentrations suggest this occurred in the Cefni during 2005 (and 2006). The data of nitrate and phosphate concentrations for the 2003 survey in section 2.1 (*figures 2.22 & 2.23*, p41) suggest that the Cefni does become limited of the most important nutrients for algal growth during the summer. As shown by the red box in *figure 3.23*, at the peak of chlorophyll *a* concentrations, a reduction in THMs was observed, with a subsequent rise in THMs occurring at the same time that chlorophyll *a* concentrations rapidly declined. In addition, there was no correlation between either of the two variables throughout the March-November period. This suggests that algal blooms do not necessarily result in an increase in the formation of THMs. However, as demonstrated by Jack, *et al.* (2002), the senescent phase of algal growth can contribute THM-forming DOC to reservoirs. It may be possible that the death of algae and release of DOC led to a rise in the formation of THMs. However, the most likely reason for the increase in THMs from 12th October is rainfall, with average daily totals being much higher from 4th October -16th November than during the spring or summer period. Heavy rainfall often washes large concentration of organic matter from catchment soils into lakes, either directly or via inflowing streams, and is known to strongly influence the DOC concentrations of reservoirs and affect the formation of THMs (Goslan, *et al.* 2002; Whitaker, *et al.* 2003). The peak in final water DOC concentrations that occurred at the same time as THMs suggests that the heavy rainfall during October and November did increase the DOC concentration of the reservoir. The lack of a positive correlation between chlorophyll *a* and THM concentrations in this study may be because there were other factors influencing the formation of THMs at the same time that the highest chlorophyll levels were observed. The main factor is likely to have been temperature, which has a strong influence on both algal growth and the formation of THMs. Oliver & Shindler (1980) also observed no correlation between chlorophyll *a* and THM formation from seven algal cultures, attributing it to the chlorophyll *a* pigment not reacting with chlorine (Oliver & Shindler, 1980). The measurement of chlorophyll *a* as an indicator of algal biomass and productivity may therefore not be the most reliable method in studies of the THMFP of algal DOC.

The significantly higher THMFP of the 1st and 4th October algal samples cannot be explained by differences in molecular weight and/or aromaticity, since SUVA values and HPSEC spectra were almost identical for each of the six cultures. The only possibility is that differences in algal species composition could account for the higher values for the October cultures. Different algal species have shown contrasting potentials to form THMs upon chlorination, although the published data is somewhat contradictory. Oliver (1983) and Plummer & Edzwald (2001) demonstrated that the 'green' type alga are less reactive with chlorine than the 'blue-greens' and diatoms, however, Nguyen, *et al.* (2005) reported the opposite trend. Data provided by the water company of algal speciation in the lake at the time of sampling show that the types and cell numbers of each species differed markedly between sampling visits (*table A01*, Appendix A03). For example, the 27th May sample was dominated by species of the 'green' alga *Scenedesmus*, and the 27th August sample by species of the 'blue-green' alga *Anabaena*. The dominant species for the October samples was the 'green' alga *Ulothrix*, and it may be that this species is more reactive with chlorine than others. However, there have been no published studies on the reactivity of this species of algae, so it can only be speculated that it may yield more THMs upon chlorination than other species of algae.

3.2.5 Conclusions

The data presented in this study shows for the first time that the chlorination of dissolved methane, an important component of the global organic carbon cycle, can generate THMs when dissolved in water. However, the concentration of dissolved methane in natural freshwaters is usually at such a low concentration that it should not be considered as a significant precursor for THMs in freshwater reservoirs. This is certainly the case at the Cefni reservoir, where even at its most concentrated (during the summer), dissolved methane comprised approximately 0.1% of the total DOC pool. Coupled with the efficient removal of dissolved methane during water treatment, this suggests that methane can be discounted as an important THM-forming fraction of DOC.

It was also demonstrated in this study that DOC derived from algal sources at the Cefni can lead to the formation of THMs. Although no correlation was observed between raw water chlorophyll *a* measurements and the formation of THMs following water treatment, probably due to the influence of temperature in affecting both variables, the chlorination of algal cultures led to the formation of THMs. However, the reactivity of the algal DOC was just 28% of the mean value of the terrestrially-derived DOC entering the lake via the inflowing streams, which can be attributed to the much lower molecular weight of the algal DOC. In terms of its concentration, the algal DOC comprised 5-45% of the total DOC concentration of the lake during 2005. It is therefore calculated that the contribution of DOC derived from algae to the total THMFP of the DOC within Cefni lake was 1.4-12.6% during the year 2005. This calculation assumes that DOC in Cefni lake is derived solely from algae and the inflowing streams, the latter of which would make up 87.4-98.6% of the lake's THMFP values, with the contribution from the fen-derived 'CIN' inflow only ranging from 52.5-59.2%. However, these values are likely to be slightly overestimated, as DOC will also enter Cefni lake via a number of other routes, including upwelling from the lake bed and rainfall, both directly falling onto the lake surface and that which falls within the catchment and washes DOC from the soils into the lake.

Chapter 4

The effects of rising temperature on the quantity and quality of dissolved organic carbon leaching from peatlands and the implications for the formation of trihalomethanes

4.1 Introduction

The continually increasing anthropogenic release of greenhouse gases is expected to increase the average global surface temperature of the Earth by between 1.47°C and 5.87°C by 2100 (IPCC, 2001). Temperature is a key influence on the rates of many biological processes in natural systems and the potential effects of warming on the global stocks of soil organic matter (SOM) are of considerable importance due to the potential feedbacks to climate change (Schlesinger & Andrews, 2000). Disagreements exist, however, concerning the implications of global warming for the storage of carbon in soils and the topic is reviewed in great detail by Davidson & Janssens (2006). If warming accelerates the decomposition of organic matter, a positive feedback would occur, but if warming increases the input of plant-derived carbon below-ground at a faster rate than increasing decomposition, a negative feedback would result. It is generally agreed that rising temperatures alone will increase rates of decomposition in soils, but it is how other factors, such as changes in moisture content, interact to affect decomposition that remains a relative unknown (Davidson, *et al.* 2000).

The issue is complex for a number of reasons, for example, the extreme heterogeneity of organic carbon compound characteristics makes attempting to generate a general model of warming-induced effects on soils an extremely challenging task. Soils contain a vast mixture of thousands of different carbon compounds, plants produce a wide range of carbon exudates and the decomposition of dead plant matter generates a suite of organic compounds entirely different from the parent material. All have contrasting kinetic properties and levels of recalcitrance (Thornley & Cannell, 2001; Davidson & Janssens, 2006), although even measurements of the temperature sensitivity of different pools of carbon have yielded entirely contrasting results, with Knorr, *et al.* (2005) reporting greater sensitivity of the recalcitrant compared to the labile pool, Liski, *et al.* (1999) recording the opposite and Fang, *et al.* (2005) observing comparable sensitivities. Other climate change impacts have the potential to override the effects of increased temperature, making it difficult to make general assumptions about the effects of warming alone. For example, it has been shown that through increasing gross primary productivity and the exudation of carbon compounds below-ground in a forested ecosystem, the length of the growth season has more of an impact on soil decomposition processes than temperature (Janssens, *et al.* 2001).

Due to the complexity of the issue, it is not surprising that contrasting results have been obtained for experiments measuring the effects of warming on soil carbon stocks. For example, Jarvis & Linder (2000) found that after 5 years, there was no effect of warming on soil CO_2 efflux from boreal forest plots in Sweden, but Fang & Moncrieff (2001) recorded a significant increase in CO_2 efflux in the soils of a sitka spruce site in Scotland under increased temperature.

The rate of increase in global temperatures is expected to be greatest at the high latitudes of the northern hemisphere (Billings, 1987), and recent measurements in western Canada, the European Russian Arctic, Greenland and Scandinavia suggests this is already occurring (Chapin III, *et al.* 2005). It is these regions of the world where the majority of the Earth's peatlands exist (Gorham, 1991, Mitsch & Gosselink, 2000), suggesting that they may be particularly prone to rising global temperatures. Due to the anaerobic conditions within peat soils, decomposition proceeds extremely slowly (Gore, 1983). This has led to the build-up of vast stores of carbon within the world's peatlands over the last few thousand years, so that peatlands have an estimated organic carbon content of 455 gigatonnes, the equivalent of 60% of the atmospheric carbon dioxide pool (Gorham, 1991; Oechel, *et al.* 1993), or one third of the total soil carbon pool (Eswaran, *et al.* 1995). They therefore play a critical role in the carbon cycle and the potential effects of rising temperatures on peatlands have very important global implications.

The importance of temperature in controlling rates of enzyme and microbial activities, the cycling of carbon and the release of nutrients in peat soils has been demonstrated in numerous studies (Ross, 1985; Koerselman, *et al.* 1993; Updegraff, *et al.* 1998; Scanlon & Moore, 2000; Chow, *et al.* 2006). As a general rule, as temperature increases, the rate of microbial and enzymic decomposition of organic matter in peat increases (Tate, 1987; Kang & Freeman, 1999; Freeman, *et al.* 2001*a*); a process that releases carbon into the porewater and eventually out of the soil as DOC (Blodau, 2002). Consequently, concentrations of dissolved organic carbon leaching from peatlands into freshwater rivers and lakes are usually highest in summer and autumn (Visser, 1984; Grieve, 1990). Experiments on the response of decomposition in peatland soils to rising temperatures have yielded contrasting results. Hogg, *et al.* (1992), Tipping, *et al.* (1999) and Silvola, *et al.* (1996) found no effect of temperature alone on rates of CO_2 efflux, but values did increase when water-table drawdown occurred in conjunction. This was attributed to the incorporation of oxygen into the soil profile. Likewise, Chow, *et al.* (2006) found a significant stimulation of CO_2 efflux following warming in association with water-table drawdown, but no effect on DOC release from peat. However, Dioumaeva, *et al.* (2002) recorded an exponential increase in CO_2 efflux with increasing temperature alone for a boreal forested peatland and Domisch, *et al.* (2006) recorded higher rates of decomposition of pine needles in peat cores with increasing temperature. The results of Minkinnen & Laine (1998) offer an added complexity, with the study observing that warming and a small water-table draw down actually increased carbon accumulation in an ombrotrophic bog, due to increased primary production and input of carbon.

Despite the uncertainties that exist in predictions of the impacts of rising temperatures on soil carbon stocks, it is generally agreed that net carbon loss from peatlands will occur in a warmer world due to a combination of rising temperatures and lower water-tables and it has been suggested that 100 Pg of carbon normally locked up inside peatlands could become available for decomposition (Davidson & Janssens, 2006). Based on the calculation that anthropogenic carbon emissions are increasing the CO_2 content of the atmosphere by 3 Pg/year (IPCC, 2001), a CO_2 release of this magnitude would be equivalent to approximately 33 years of current anthropogenic emissions. A recent study of soils in England and Wales has revealed that carbon is currently being lost at a rate of 0.6% yr^{-1}, with the greatest losses being for those soils with the highest carbon contents (e.g. peatlands) (Bellamy, *et al.* 2005). This loss of soil carbon may be at least partly to blame for the 91% increase in freshwater DOC concentrations that have been recorded in the UK during the last 15 years (Evans, *et al.* 2005). Hence, there are concerns that peatlands may already be destabilising and it has been predicted that they could be a net source of carbon within the next 25 years (Worrall & Burt, 2005).

Although the specific causes of this increase are still subject to much debate, it is likely that rising temperatures are at least partially to blame (Freeman, *et al.* 2004*b*; Worrall, *et al.* 2004*b*; Evans, *et al.* 2005; Clark, *et al.* 2006).

Temperature-manipulation experiments carried out previously in this laboratory have used temperature-gradient bars (Dowrick, 1998; Ross, 2004; Fenner, *et al.* 2005*b*). This is a custom-built piece of equipment measuring 1.15m in length with an aluminium, heat-conducting base. At one end of the bar is an electronic heat source and at the other, a heat sink. When in operation, a temperature gradient is created along the length of the aluminium bar, the magnitude of which can be altered by changing the voltage of the heat source and/or sink. Measurements of greenhouse gas exchange, enzyme and microbial activities and DOC concentrations during previous thermal-gradient bar experiments have demonstrated that peat soils respond dramatically to rising temperatures. Dowrick (1998), for example, observed a linear increase in carbon dioxide emissions with increasing incubation temperature of both bog and fen peat, a consequence of the higher microbial activity occurring in the warmer peat soil.

These previous studies subjected peat to a temperature range of 2-20°C. The purpose of this study was different, because the aim was to assess the effect of *predicted* future climate change-induced warming on the DOC release from peat during heavy rainfall, as it is under storm conditions that most allochthonous carbon enters freshwaters (Royer & David, 2005). The experiment also focussed on changes in the potential of the leaching DOC to form THMs. Therefore, peat was incubated from 2-8°C above the temperature recorded at the time of peat collection for each season, hence potential future peatland DOC release was determined under a range of global temperature projections for the year 2100.

4.2 Materials and methods

4.2.1 Soil collection, preparation and incubation

Peat samples were collected from Marchlyn Bach ombrotrophic bog and Cors Erddreiniog minerotrophic fen (locations described in chapters 2 & 3). Similar to the seasonal sampling regime used by Fenner, *et al.* (2005*b*), peat collection was undertaken during January, May, August and November 2005 to represent natural winter, spring, summer and autumn conditions respectively. At each site, two 20 cm deep sections of peat soil, complete with overlying vegetation and measuring approximately 15 cm x 15 cm, were extracted using a sharp knife and a spade. The temperature of the soil at a depth of 10 cm was recorded at 5 places within an approximate 3 m^2 area around the site of peat collection and the mean value calculated in order to record the peat collection temperature (*table 4.01*). The peat samples were then transported back to the laboratory carefully in plastic bags, which minimised water loss (Dowrick, 1998).

	Bog	*Fen*
Winter	1.7	2.3
Spring	7.0	10.1
Summer	12.4	15.0
Autumn	7.5	4.1

Table 4.01 – Mean temperature of the peat soil at the time of sample collection for each of the four seasons

In a room maintained at a constant 10°C, the soil samples were prepared for incubation following the method of Dowrick (1998). This involved removing the top layer of vegetation and roots down to a depth of approximately 5 cm and extracting peat soil between depths of 5-15 cm. Once a sufficient quantity had been collected, it was homogenised by hand in a bucket for approximately 10 minutes in order to minimise spatial heterogeneity and any stones or large roots were removed. The peat was then placed on a temperature-gradient bar, with a layer of thin polythene plastic placed below and above the peat in order to reduce evaporative water losses (*figure 4.01*). The peat was built up to a depth of 3 cm and was covered in a layer of aluminium foil to prevent exposure of the peat soil to light that would be absent in the natural environment. Finally, a glass lid was placed on top to provide thermal insulation. Two thermal-gradient bars were employed simultaneously during each season, one each for the fen and bog soils. The peat was incubated in the thermal-gradient bars for two weeks. The temperature of each bar was maintained at 1-2 °C below the collection temperature at the cold end and 7-8 °C above at the warm end (therefore an 8-10°C thermal gradient).

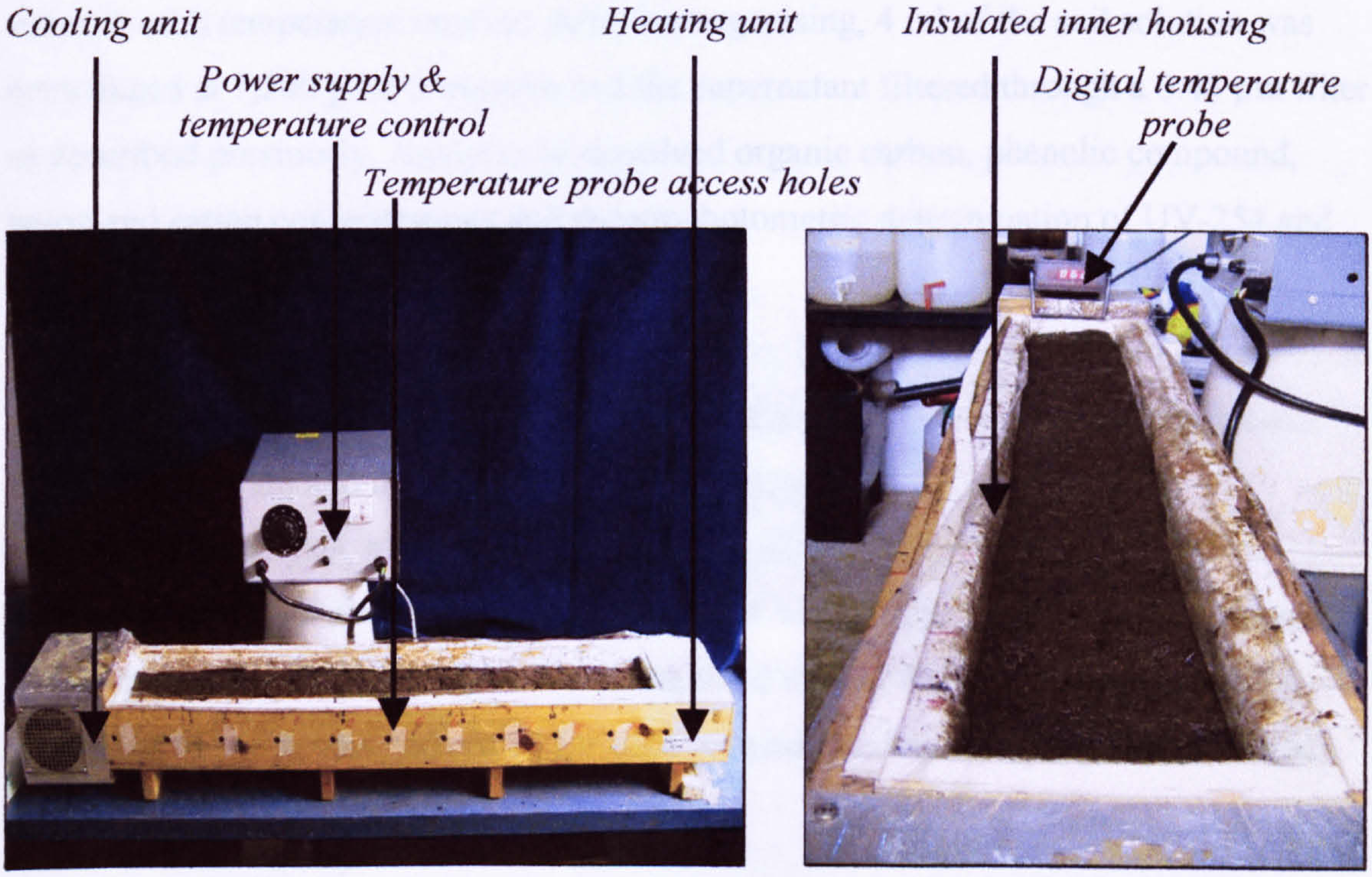

Figure 4.01 – The thermal-gradient bar apparatus custom-built for incubating peat soil at below and above ambient temperatures.

4.2.2 Soil carbon and hydrochemical analyses

Rather than using the centrifugation technique of Reynolds (1984) that was employed by Dowrick (1998) and Fenner, *et al.* (2005*b*) in previous temperature-bar experiments, the water-extractable (leachable) method for soil porewater collection was used. This technique accounts for carbon compounds washed out of the soil profile during heavy rainfall events, and rainfall is known to strongly influence the flux of DOC from peat soils (Scott, *et al.* 1998). The knowledge that heavy rainfall washes large concentrations of DOC into freshwater lakes (Forsberg, 1992; Hillman, *et al.* 2004), especially the THM-forming, HMW fraction (e.g. phenolics) (Fenner, *et al.* 2005*a*), coupled with the

prediction that heavy rainfall or storm events in the UK may increase in frequency in the future (Rowntree, *et al.* 1993) means this different method for obtaining soil water would provide an interesting new angle compared to previous, similar experiments.
After the two week incubation period, peat was extracted at five 2°C intervals along the 10°C thermal gradient. This involved extracting five 1 cm^3 cubes of peat, placing them in separate stomacher bags, adding 9 ml of milli-q water and homogenising for 1 minute using a Seward Stomacher 80 (see section 3.1 for more details). This was repeated five times at each temperature interval. After homogenising, 4 ml of the soil solution was centrifuged at 7,200 g for 5 minutes and the supernatant filtered through a 0.45 μm filter as described previously. Analyses of dissolved organic carbon, phenolic compound, anion and cation concentrations and spectrophotometric determination of UV-254 and E2:E3 values were then undertaken.

The remaining 4-5 ml of solution in the stomacher bags was combined into one for each of the five replicates and centrifuged and filtered as described above. These solutions were kept separate and their THMFP determined (see p83 for method).

For ^{1}H-NMR analysis, a further ten 1 cm^3 cubes of peat were extracted at each temperature interval and the above procedures of water addition, homogenisation and filtration performed. The samples were combined into 90 ml of soil solution and ^{1}H-NMR spectroscopy was performed for each temperature interval (see p83 for method).

4.2.3 Statistical analyses

For each type of analysis, a linear trendline was displayed in the graphs (*figures 4.02-4.17*) for each season's 5 point dataset, along with the equation of the line. The data in all of the figures are for the mean values of 5 replicates, with error bars indicating ± standard error of the mean. The trends for each season for each analysis were tested for significance against a straight line with an equation of zero, and then against each other season to determine differences in trends throughout the year, using linear regression analysis, described by Fowler, *et al.* (1998). Additionally, correlations between all determinants were performed using Pearson correlation coefficient and the significant correlations detailed in *tables 4.02 & 4.03*. Statistical analyses were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego California USA) and SPSS version 9.0 (SPSS, Inc.).

4.3 Results

4.3.1 Bog peat soils

4.3.1.1 Dissolved organic carbon analyses

Only DOC and phenolic compound concentrations were determined for the winter bog peat, due to the results of previous studies. Freeman, *et al.* (2001*a*) and Fenner, *et al.* (2005*b*), using the centrifugation method, and Ross (2004), using the leachable method, all failed to elicit a positive response for dissolved organic carbon and phenolic concentrations, enzyme activities and the final production of CO_2 when winter peat was incubated under temperatures up to 15°C above the ambient. As the identification of increases in THMFP values under increasing soil temperature is the aim of this experiment, and a decrease in DOC and phenolic compound concentrations was recorded for winter peat, further analyses, such as THMFP were not undertaken.

For spring peat, the THMFP increased at a rate of 0.67 mg l^{-1} $°C^{-1}$, for summer peat decreased at a rate of 0.15 mg l^{-1} $°C^{-1}$ and for autumn peat remained almost completely unchanged with increasing temperature (*figure 4.02*). Linear regression analysis showed that only the trend for summer was significant and it was significantly different to the spring trend only ($p<0.05$).

Although bromide was not detected for any of the three seasons, the presence of brominated THMs (BrTHMs) (*figure 4.03*) suggests that 'free' bromide must have been present at concentrations below the detection limit of the ion chromatograph. For the spring peat, BrTHMs increased by 0.24%/°C, for summer peat fell by 0.05%/°C and for autumn peat decreased by 0.23%/°C, although only the decreasing trend for autumn was significant.

Temperature-induced changes in DOC concentrations followed a similar pattern to those of the trihalomethane potential of the solution leaching from the peat (*figure 4.04*). Following incubation, winter, summer and autumn peat DOC concentrations reduced as the temperature rose, but they increased dramatically for spring peat. Winter peat responded least to the temperature increase, with the DOC concentration reducing at a rate of 1.04 mg l^{-1} $°C^{-1}$. Summer and autumn peat reacted almost identically to the warmer conditions, reducing at a rate of 1.94 and 1.97 mg l^{-1} $°C^{-1}$. For the spring peat, an increase of 3.46 mg l^{-1} $°C^{-1}$ was recorded under rising temperatures. According to linear regression analysis, the decreasing trends for winter, summer and spring were significantly different, but not the spring increase. However, the response for spring was significantly different to summer and autumn ($p<0.05$). Changes in the DOC concentration correlated positively and significantly with THMFP for spring ($R^2=0.90$, $p<0.05$) and summer ($R^2=0.92, p<0.05$).

Values of absorbance at 254nm (data not shown) correlated significantly with DOC for all seasons with increasing temperature (spring - $R^2=0.94, p<0.05$; summer – $R^2=0.99$, $p<0.01$; autumn – $R^2=0.98, p<0.01$) (*table 4.02*). UV-254 also correlated positively and significantly with percentage BrTHM values for spring ($R^2=0.94, p<0.05$).

Concentrations of phenolic compounds varied in a similar manner to DOC with increasing incubation temperature (*figure 4.05*), with the greatest change in concentration occurring for spring peat. Concentrations fell 0.10, 0.18 and 0.22 mg l^{-1} $°C^{-1}$ for summer, winter and autumn peat respectively, but for spring peat rose by 1.03 mg l^{-1} $°C^{-1}$. Like DOC, only the decreasing trends for winter, summer and spring were significant ($p<0.05$). The change in phenolics concentrations correlated positively and significantly with DOC for summer ($R^2=0.97, p<0.01$) and autumn ($R^2=0.95, p<0.05$), with the THMFP for summer ($R^2=0.89, p<0.05$) and with the formation of BrTHMs in spring ($R^2=0.96, p<0.01$).

Changes in E2:E3 ratio values with rising temperature followed a similar but *inverse* trend to those of the DOC concentrations for the 3 seasons (*figure 4.06*). Both summer and autumn peat exhibited an increasing trend; 0.02/°C for summer peat and 0.06/°C for autumn peat, with the spring peat reducing at a rate of 0.03/°C. Linear regression analysis demonstrated that only the summer rise was significant, although the spring decrease was significantly different to the summer and autumn trends ($p<0.05$). Values of the E2:E3 ratio correlated negatively and significantly with values of DOC for spring and summer (both $R^2=-0.93, p<0.05$) and negatively with THMFP values ($R^2=-0.93$, $p<0.05$) and BrTHM formation ($R^2=-0.96, p<0.01$) for spring peat.

Changes in the proportion of aromatic compounds within the DOC pool mirrored the trends for phenolic compounds (*figure 4.07*). For spring peat, the percentage composition of aromatic compounds increased sharply, by 1.04%/°C, but for summer and autumn peat reduced by 0.09% and 0.44%/°C respectively. However, none of the trends were significant or significantly different from one another, although the percentage of aromatic compounds did correlate with the formation of BrTHMs in spring ($R^2=0.97$, $p<0.01$).

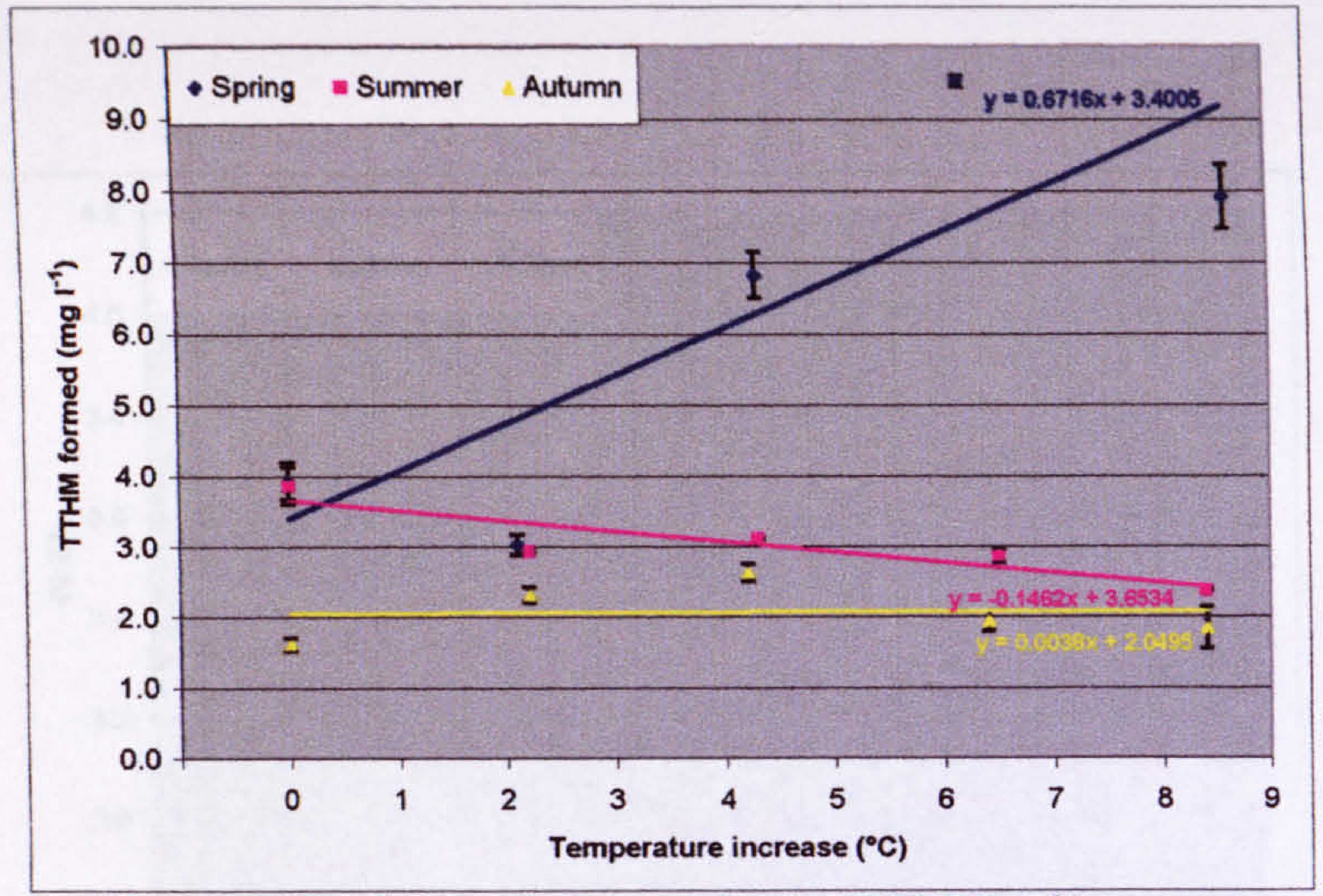

Figure 4.02 – Seasonal changes in the THMFP of bog peat leachable DOC with increasing soil temperature

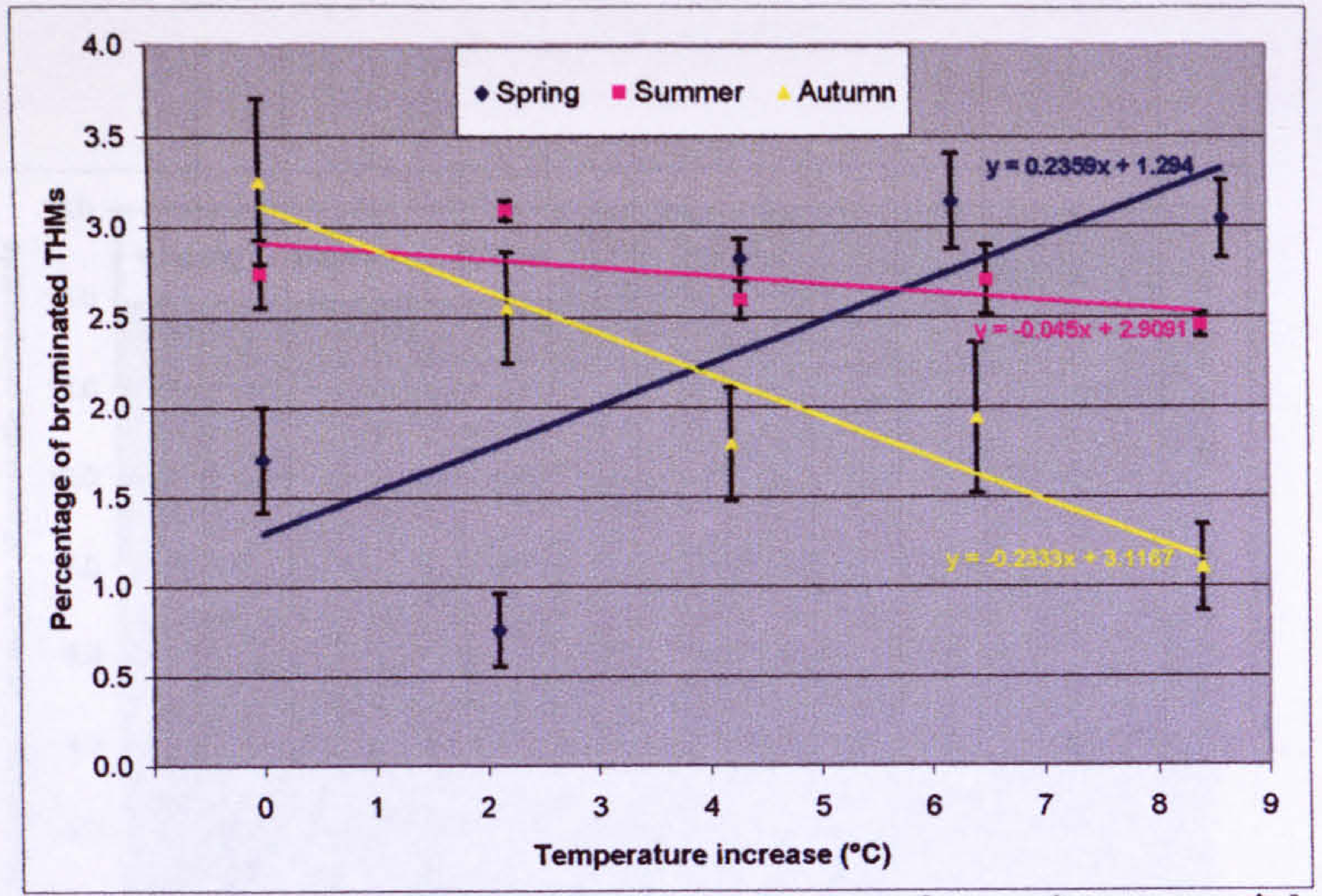

Figure 4.03 – Seasonal changes in the percentage formation potential of BrTHMs of bog peat DOC with increasing soil temperature

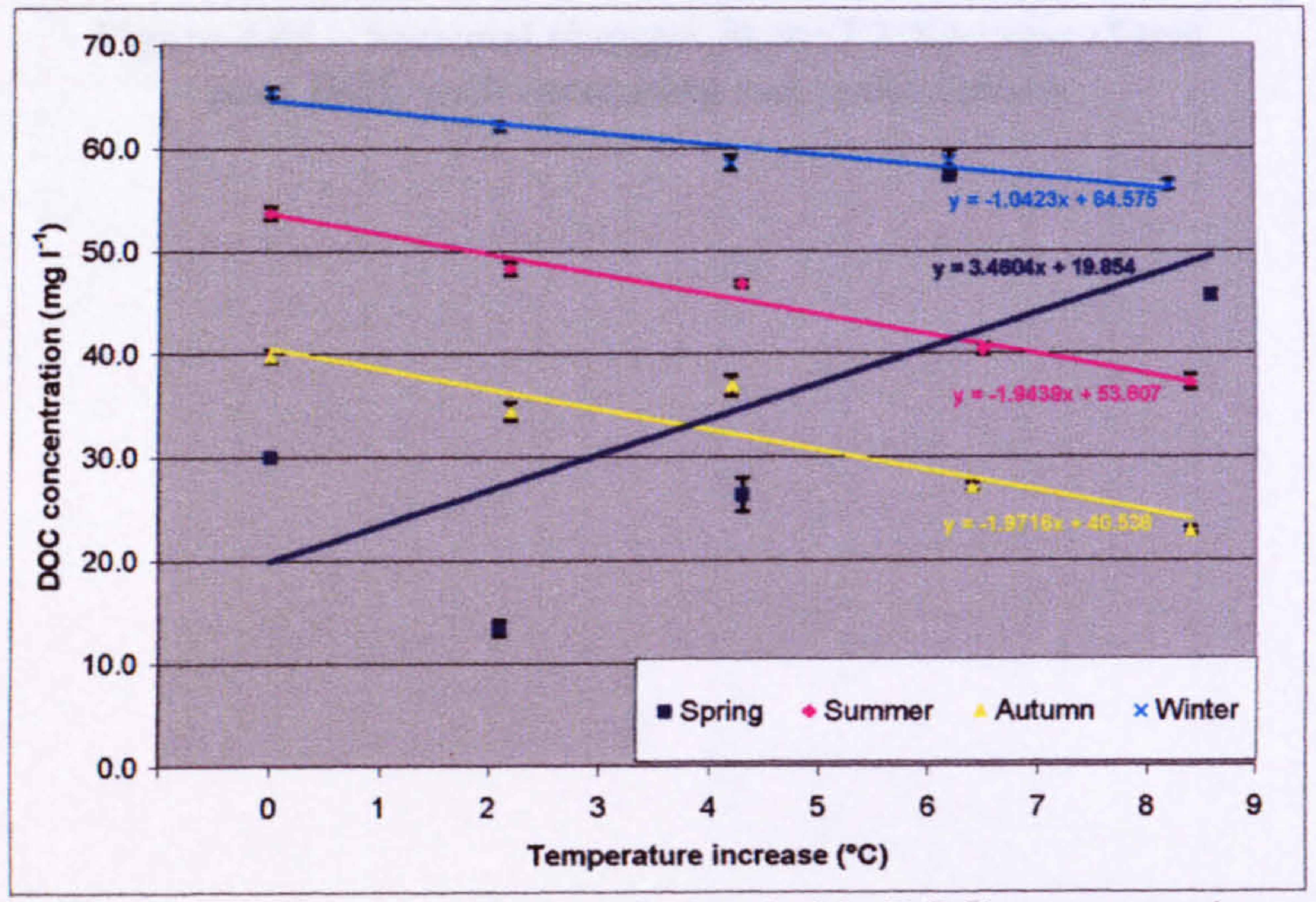

Figure 4.04 – Seasonal changes in leachable DOC concentrations of bog peat DOC with increasing soil temperature

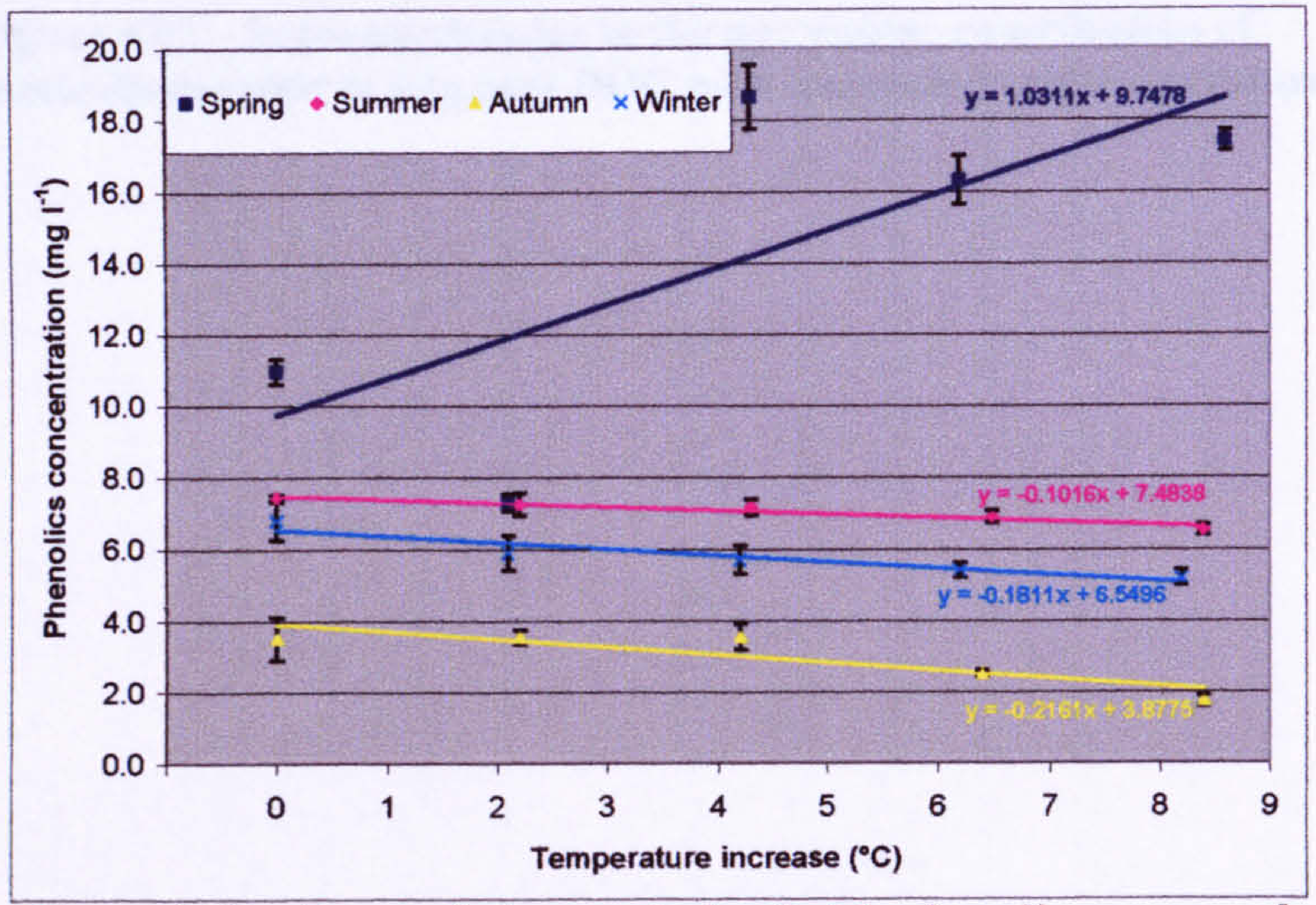

Figure 4.05 – Seasonal changes in leachable phenolic compound concentrations of bog peat with increasing soil temperature

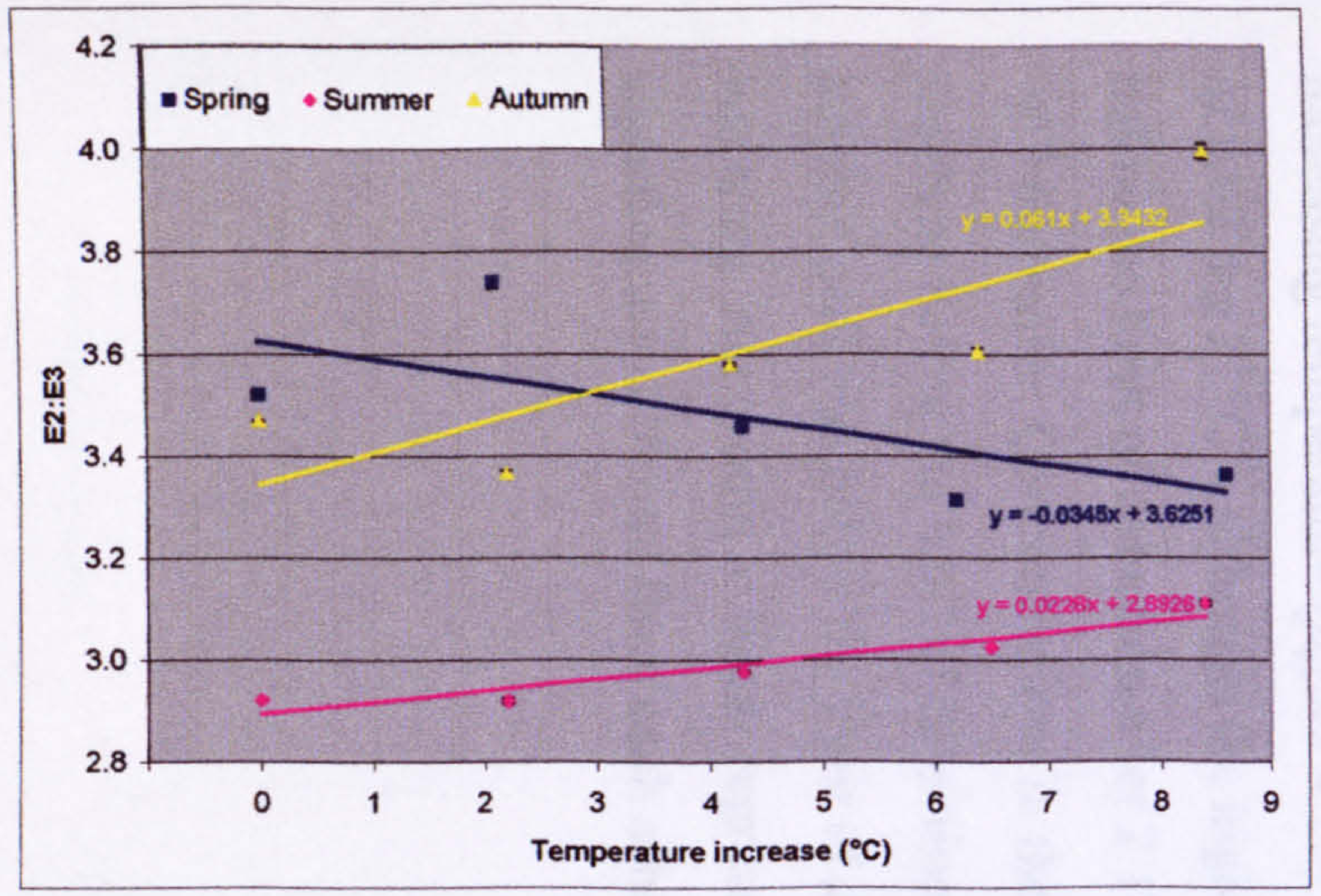

Figure 4.06 – Seasonal changes in the E2:E3 ratio of bog peat DOC with increasing soil temperature

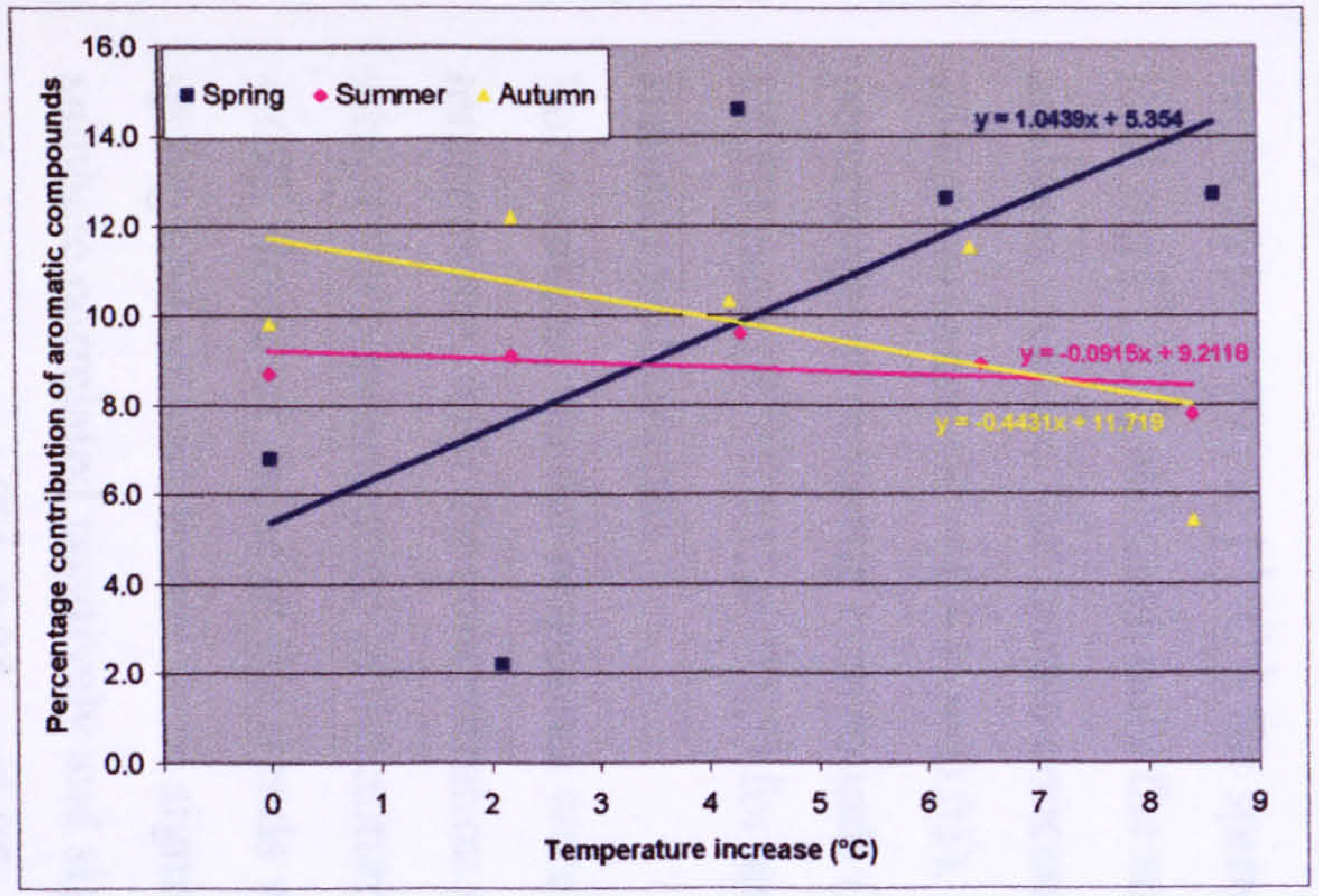

Figure 4.07 – Seasonal changes in the percentage contribution of aromatic compounds to bog peat DOC with increasing soil temperature

4.3.1.2 Hydrochemical analyses

Concentrations of some ions also fluctuated as the peat temperature increased, especially nitrate, sodium and ammonium. For nitrate, the concentration reduced as the temperature increased for each season (*figure 4.08*). For summer peat, the concentration reduced by 0.09 mg l^{-1} °C^{-1}, for spring peat by 0.05 mg l^{-1} °C^{-1} and for autumn peat by 0.02 mg l^{-1} °C^{-1}, although only the summer trend was significant (p>0.05). Correlation analyses revealed that nitrate concentrations correlated negatively with values of UV-254 absorbance (R^2=-0.91, p<0.05), phenolic concentrations (R^2=-0.98, p<0.01) and the percentage of aromatic compounds (R^2=-0.97, p<0.01) and positively with values of the E2:E3 ratio (R^2=0.91, p<0.05) for spring peat, but no correlations were recorded for summer and autumn.

For sulphate, similar responses were recorded for spring and summer under increased temperature, with the concentration reducing by 0.17 and 0.15 mg l^{-1} °C^{-1} respectively, whilst the concentration in the autumn peat increased slightly (0.01 mg l^{-1} °C^{-1}) (*figure 4.09*). However, none of the trends were significant, although the difference between the spring and autumn trends were significantly different (p<0.05). The concentration of sulphate correlated positively and significantly with nitrate for spring (R^2=0.93, p<0.05) and summer peat (R^2=0.94, p<0.05).

Concentrations of ammonium exhibited dramatically different seasonal responses with increasing temperature (*figure 4.10*). The concentration in spring peat increased at a rate of 0.02 mg l^{-1} °C^{-1}, although the high value at the collection temperature decreased to below the limit of detection after 2.1°C of warming and increased at a rate of 0.25 mg l^{-1} °C^{-1} thereafter. Concentrations in the summer peat reduced at a rate of 0.12 mg l^{-1} °C^{-1}, reaching below the limit of detection after 8.4°C of warming. The concentration in the autumn peat was below the limit of detection at all temperatures, even after substantial warming. According to linear regression, none of these trends were significant or significantly different from each other (p>0.05).

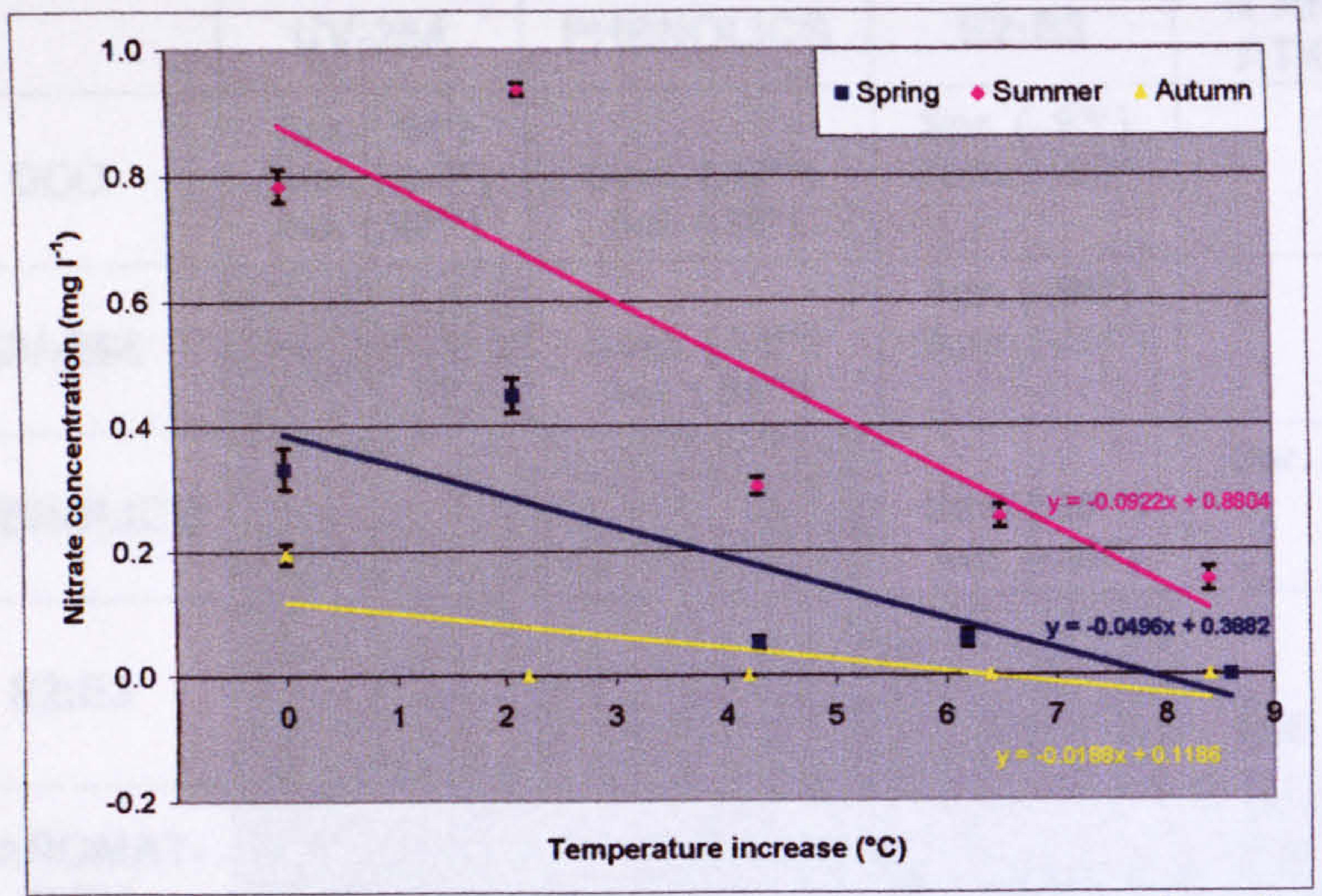

Figure 4.08 – Seasonal changes in the nitrate concentration of bog peat with increasing soil temperature

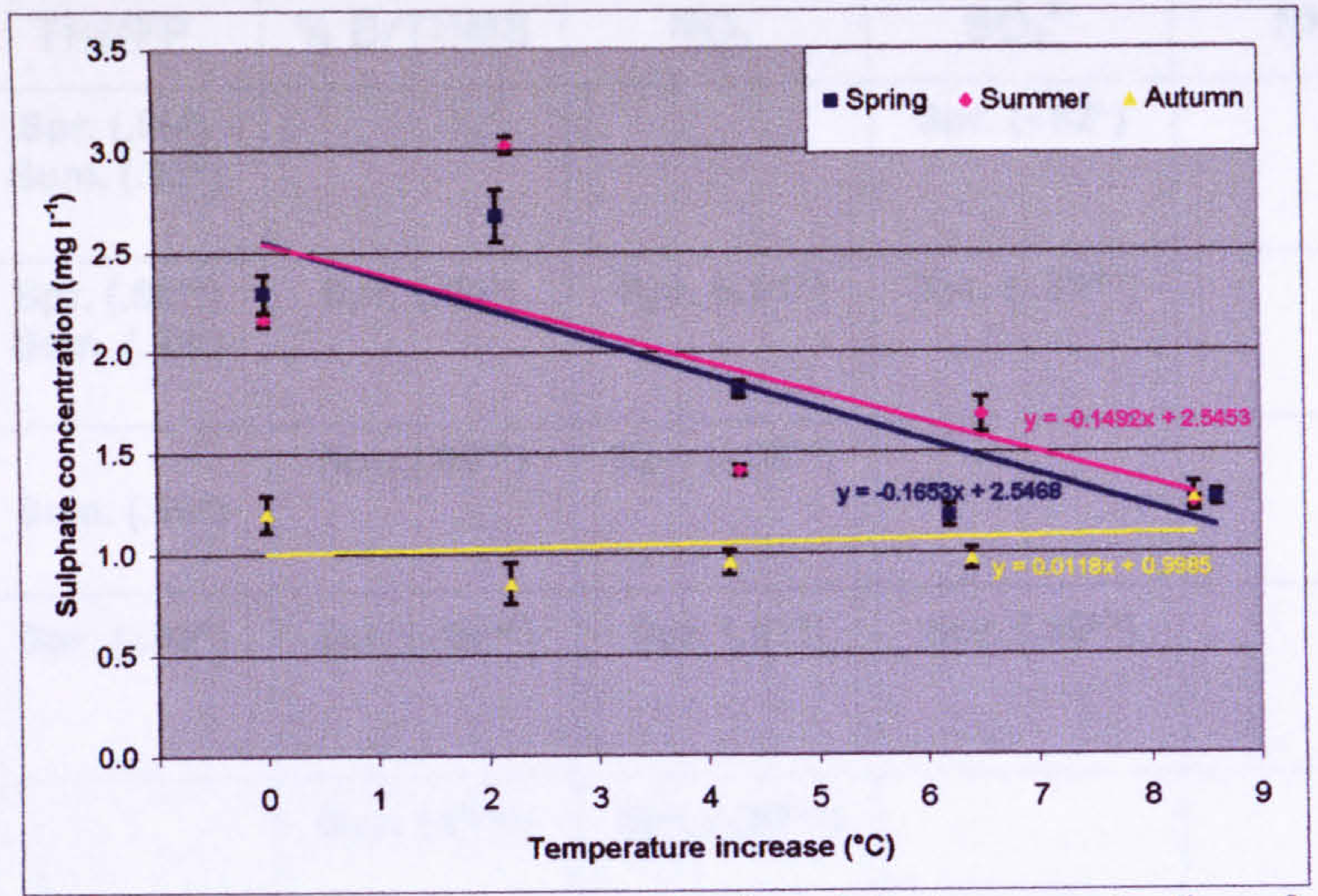

Figure 4.09 – Seasonal changes in the sulphate concentration of bog peat with increasing soil temperature

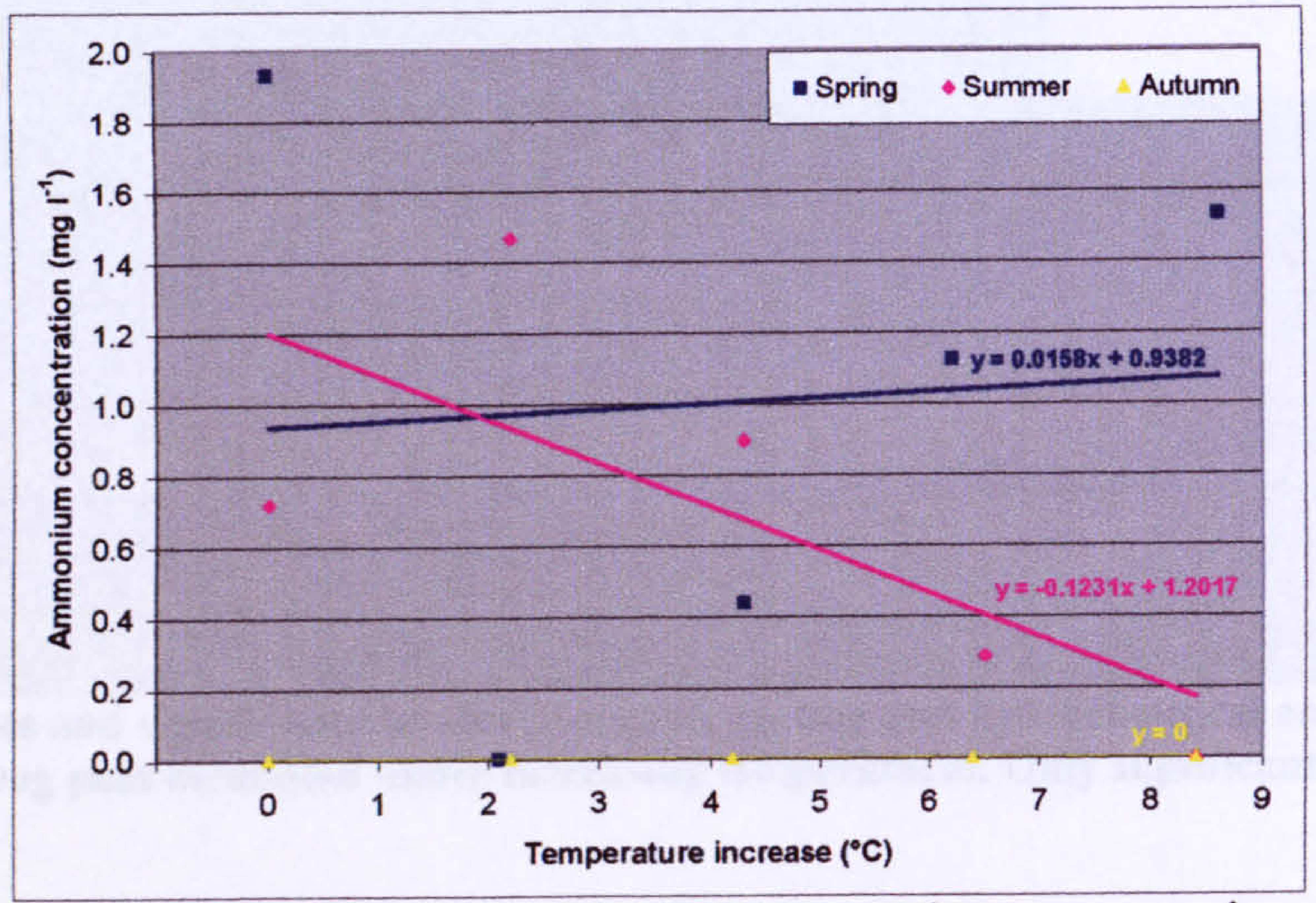

Figure 4.10 – Seasonal changes in the ammonium concentration of bog peat with increasing soil temperature

	UV-254	PHENOLICS	E2:E3	% AROM-ATICITY	THMFP	% BrTHMS	NO_3^-	SO_4^{2-}	NH_4^+
DOC	Spr. (.94*) Sum. (.99**) Aut. (.98**)	Sum. (.97**) Aut. (.95*)	Spr. (-.93*) Sum. (-.93*)		Spr. (.90*) Sum. (.92*)			Spr. (-.92*)	
UV-254		Sum. (.98**) Aut. (.98**)	Spr. (-.96*) Sum. (-.94*)		Spr. (.98**) Sum. (.92*)	Spr. (.94*)	Spr. (-.91*)	Spr. (-.99**)	
PHENOLICS			Sum (.98**) Aut. (-.90*)	Spr. (.99**)	Sum. (.89*)	Spr. (.96**)	Spr. (-.98**)		
E2:E3				Aut. (-.91*)	Spr. (-.93*)	Spr. (-.96**)	Spr. (.91*)	Spr. (.96**)	
% AROMAT-ICITY						Spr. (.97**)	Spr. (-.97**)		
THMFP						Spr. (.95*)	Spr. (-.92*)	Spr. (-.98**)	
% BrTHMS							Spr. (-.98**)	Spr. (-.98*) Sum. (.96**)	
NO_3^-								Spr. (.93*) Sum. (.94*)	
SO_4^{2-}									

Table 4.02 – Pearson correlation values and significance levels for organic carbon and hydrochemical analyses for individual spring, summer and autumn seasons for bog peat incubated under increasing temperatures. Only significant correlations are shown.

4.3.2 Fen

4.3.2.1 Dissolved organic carbon analyses

Values of THMFP demonstrated contrasting trends with each season under increasing temperature (*figure 4.11*). Values of THMFP increased with warming for summer (0.18 mg l^{-1} °C^{-1}) and autumn (0.06 mg l^{-1} °C^{-1}) peat, but decreased for winter (0.09 mg l^{-1} °C^{-1}) and spring (0.001 mg l^{-1} °C^{-1}) peat. The winter decrease and summer increase trends were significant, with the winter decrease being significantly different to the trends of the other three seasons (p<0.05). Only minor variations in the proportions of BrTHMs were recorded with increasing incubation temperature (0.03-0.07% change), with none of the trends being significant (*figure 4.12*).

The concentrations of dissolved organic carbon exhibited similar responses to THMFP with increasing temperature. Winter and autumn peat both exhibited a reduction in DOC concentrations, of 0.66 and 0.55 mg l^{-1} °C^{-1} respectively. Autumn peat showed a small rise, 0.24 mg l^{-1} °C^{-1}, whilst summer peat demonstrated a large rise of 1.16 mg l^{-1} °C^{-1}. Linear regression revealed that, like DOC, the winter and summer trends were significant. Additionally, the rising trend for summer peat was significantly different to those of the other three seasons (p<0.05). DOC concentrations correlated significantly with DOC only in winter (R^2=0.89, p<0.05). Values of UV-254 absorbance (data not shown) correlated significantly with DOC concentrations for winter (R^2=0.91, p<0.05), spring (R^2=0.90, p<0.05) and autumn peat (R^2=0.78, p<0.001), but not for summer peat (*table 4.03*).

The concentration of phenolic compounds varied in a similar manner to DOC concentrations with increasing temperature for each season (*figure 4.14*). Summer peat was the only season to demonstrate a rise in phenolic compounds, at a rate of 0.15 mg l^{-1} °C^{-1}. Concentrations in winter, spring and autumn peat reduced by 0.25, 0.15 and 0.02 mg l^{-1} °C^{-1} respectively. Similarly to THMFP and DOC values, the summer and winter trends were significant, whilst the summer rise was significantly different to the trends for autumn and winter (but not spring). The concentration of phenolic compounds correlated positively with DOC (R^2=0.89, p<0.05) and THMFP values (R^2=0.99, p<0.001) for winter peat and with the percentage of BrTHMs for summer peat (R^2=0.99, p<0.01).

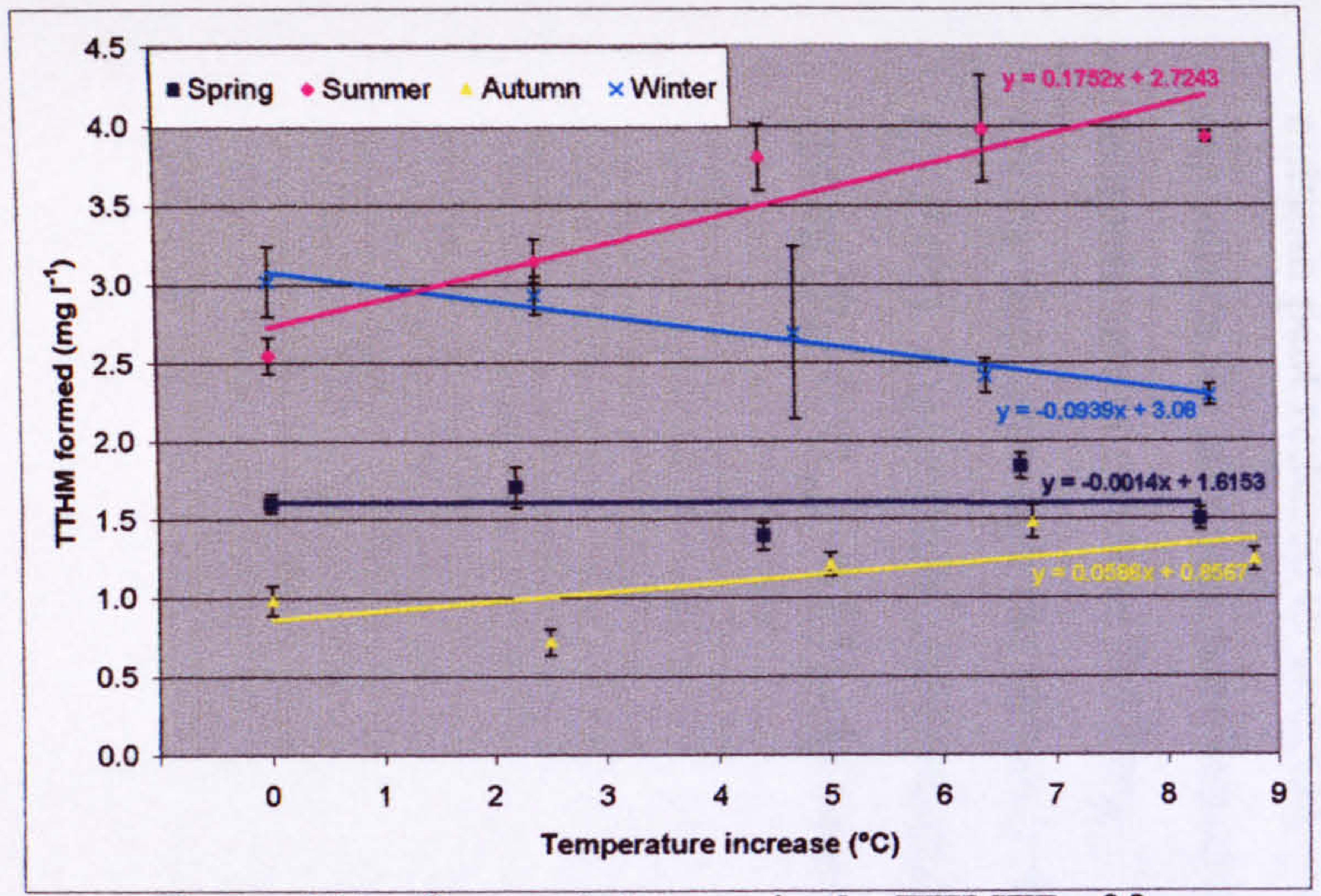

Figure 4.11 – Seasonal changes in the THMFP of fen peat DOC with increasing soil temperature

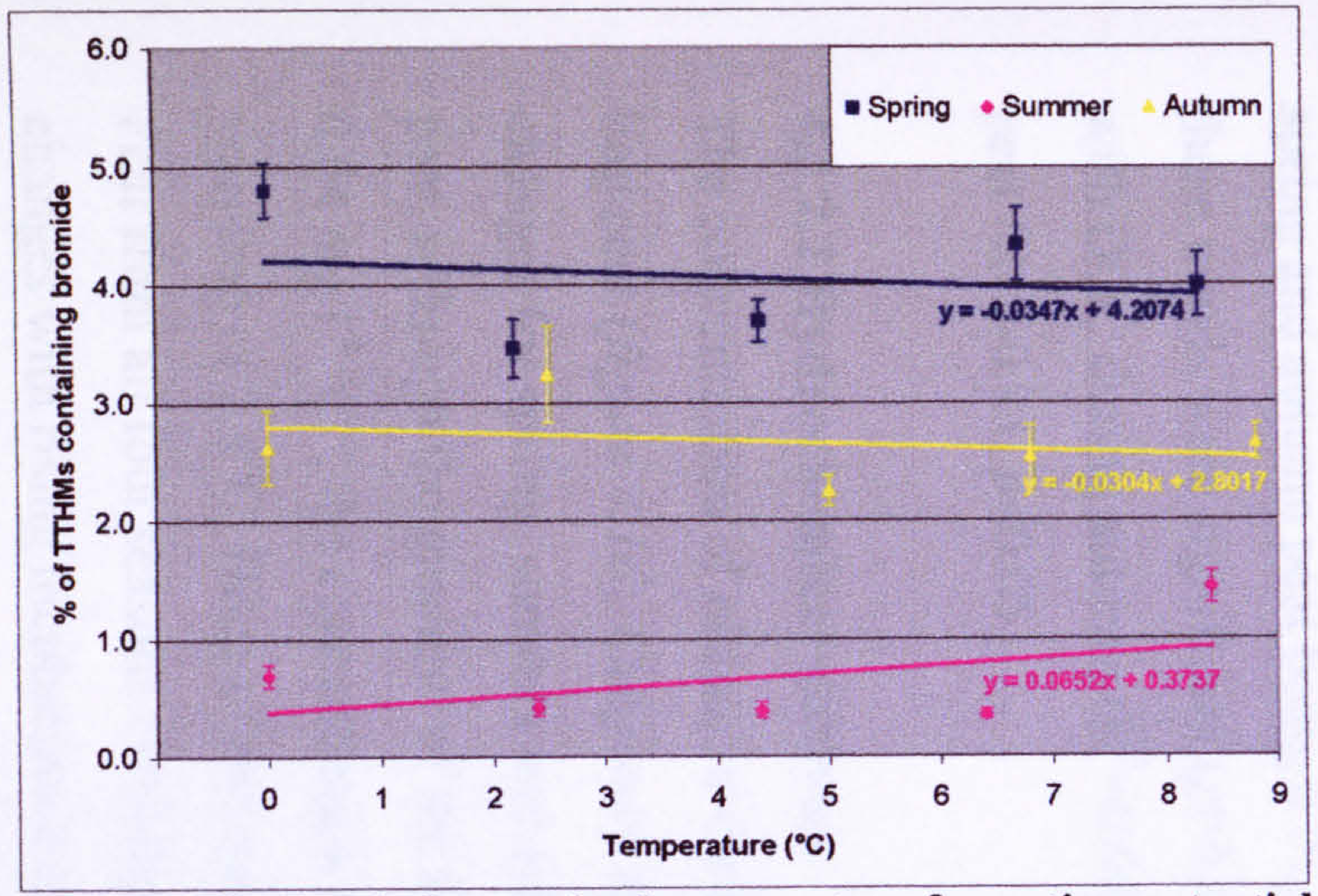

Figure 4.12 – Seasonal changes in the percentage formation potential of brominated THMs of fen peat DOC with increasing soil temperature

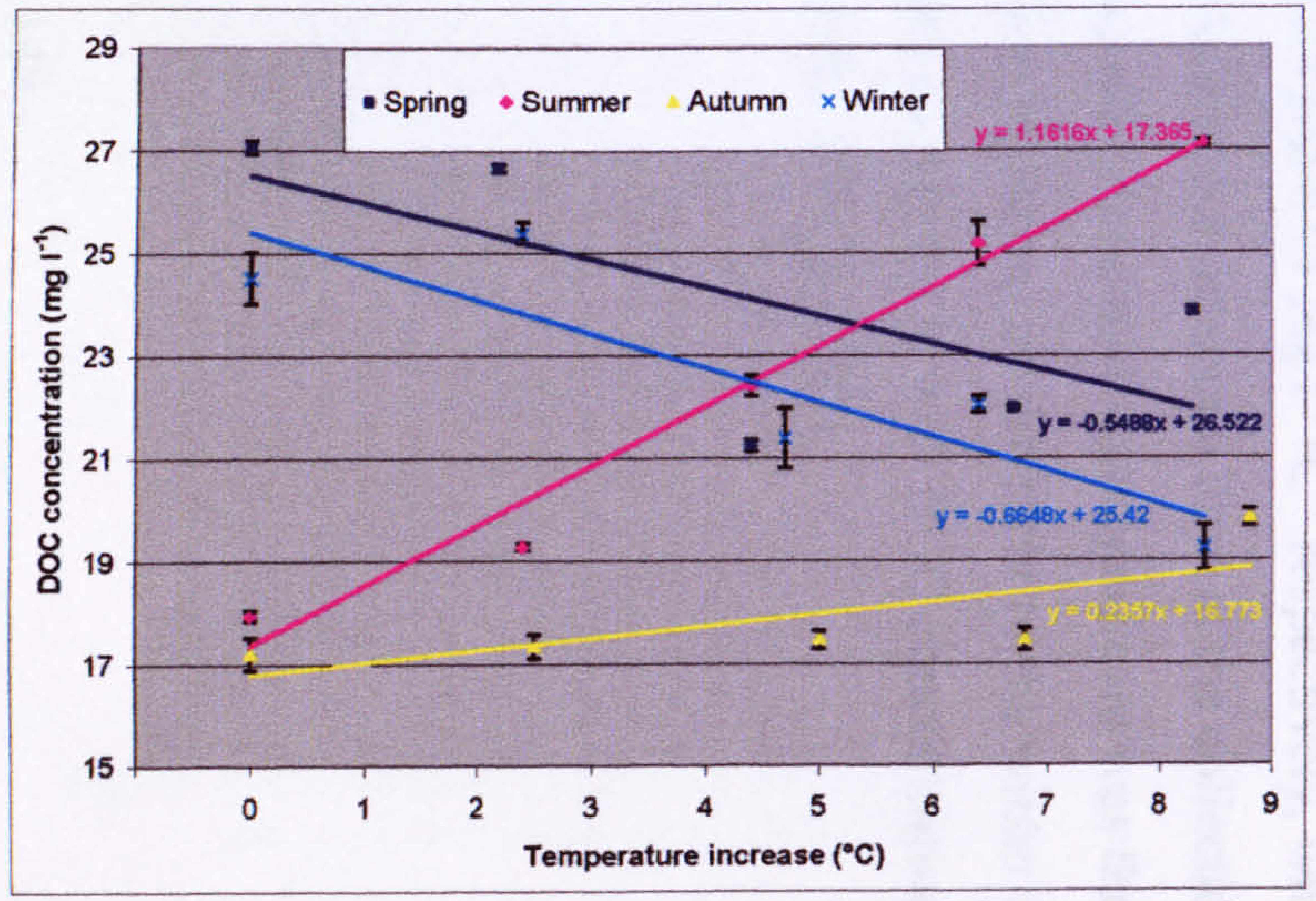

Figure 4.13 – Seasonal changes in leachable DOC concentrations of fen peat with increasing soil temperature

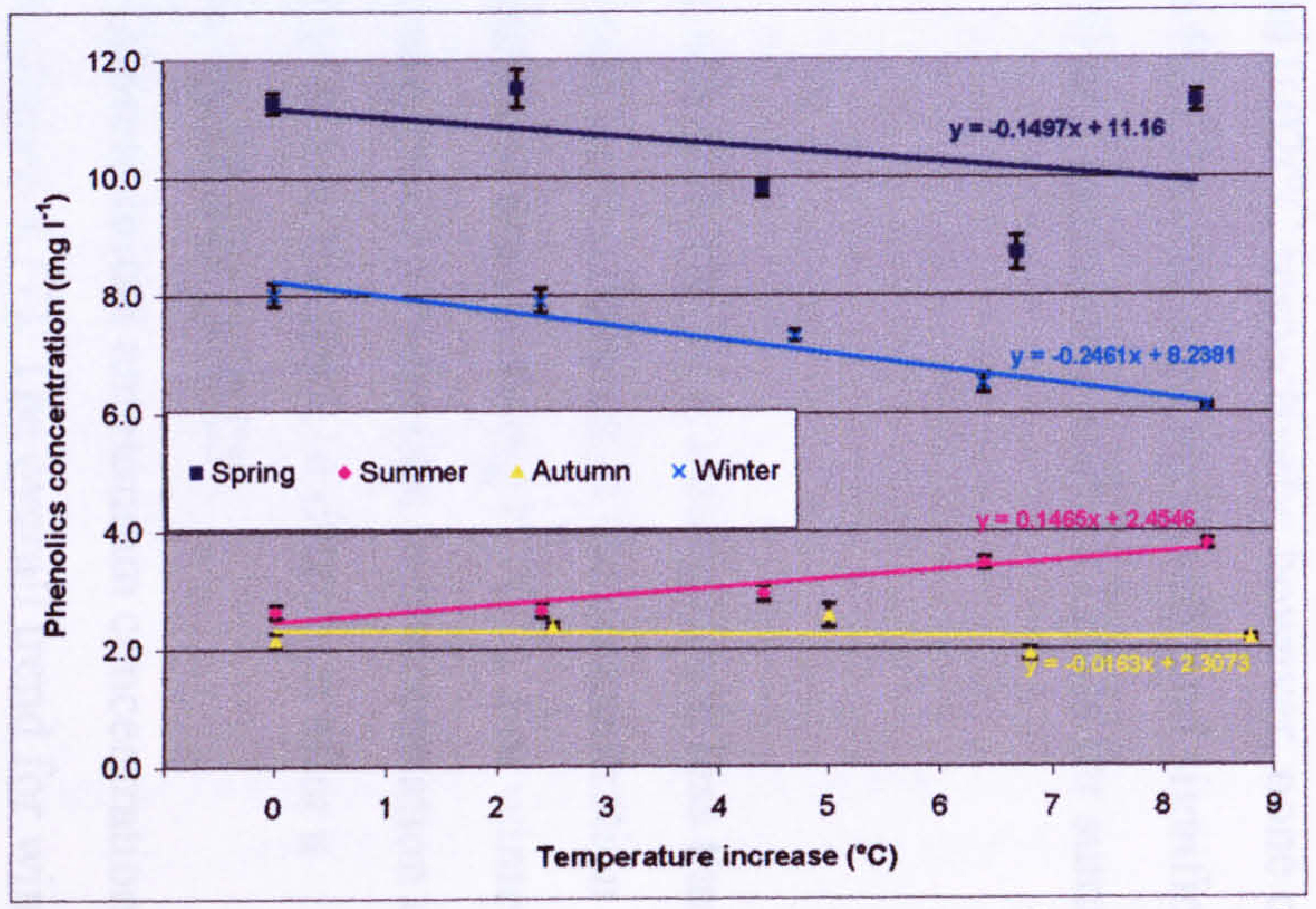

Figure 4.14 – Seasonal changes in leachable phenolic compound concentrations of fen peat with increasing soil temperature

Warming induced an inverse response in values of the E2:E3 ratio to that of phenolics, with a reduction for summer peat and a rise for the other three seasons (*figure 4.15*). The rate of reduction for summer peat was 0.01/°C, with the rates of increase for winter, spring and autumn peat being 0.01, 0.01 and 0.02/°C respectively; however, none of these trends were significant (p>0.05). The E2:E3 correlated positively and significantly with DOC during autumn (R^2=0.98, p<0.05) and negatively with BrTHMs for summer peat (R^2=-0.93, p<0.05).

4.3.2.2 Hydrochemical analyses

The concentration of nitrate in the fen peat was affected by warming much less than the bog peat (*figure 4.16*). Only very minor trends were exhibited in the concentration changes for spring, summer and autumn peat (less than 0.00 mg l^{-1} °C^{-1}). For winter peat, although the gradient of the trendline suggested a reduction in concentration of 0.04 mg l^{-1} °C^{-1}, the concentration reduced to below the limit of detection after a warming of 2.4°C. None of the trends were significant (p>0.05).

Peat from all four seasons exhibited very different initial ammonium concentrations and changes with rising incubation temperature (*figure 4.17*). The overall trend for winter peat was a reduction in concentration at a rate of 0.18 mg l^{-1} °C^{-1}, although the concentration reached below detectable limits after a warming of 4.7°C. Both spring and autumn peat reduced in concentration by 0.07 and 0.01 mg l^{-1} °C^{-1} respectively, whilst the values for summer peat increased from below the detection limit at the collection temperature at a rate of 0.12 mg l^{-1} °C^{-1}. The only trend that was significant was the spring rise, although the summer increase was significantly different to the winter fall (p<0.05). Ammonium concentrations correlated positively with DOC concentrations for spring (R_2=0.91, p<0.05) and summer peat (R_2=0.94, p<0.05).

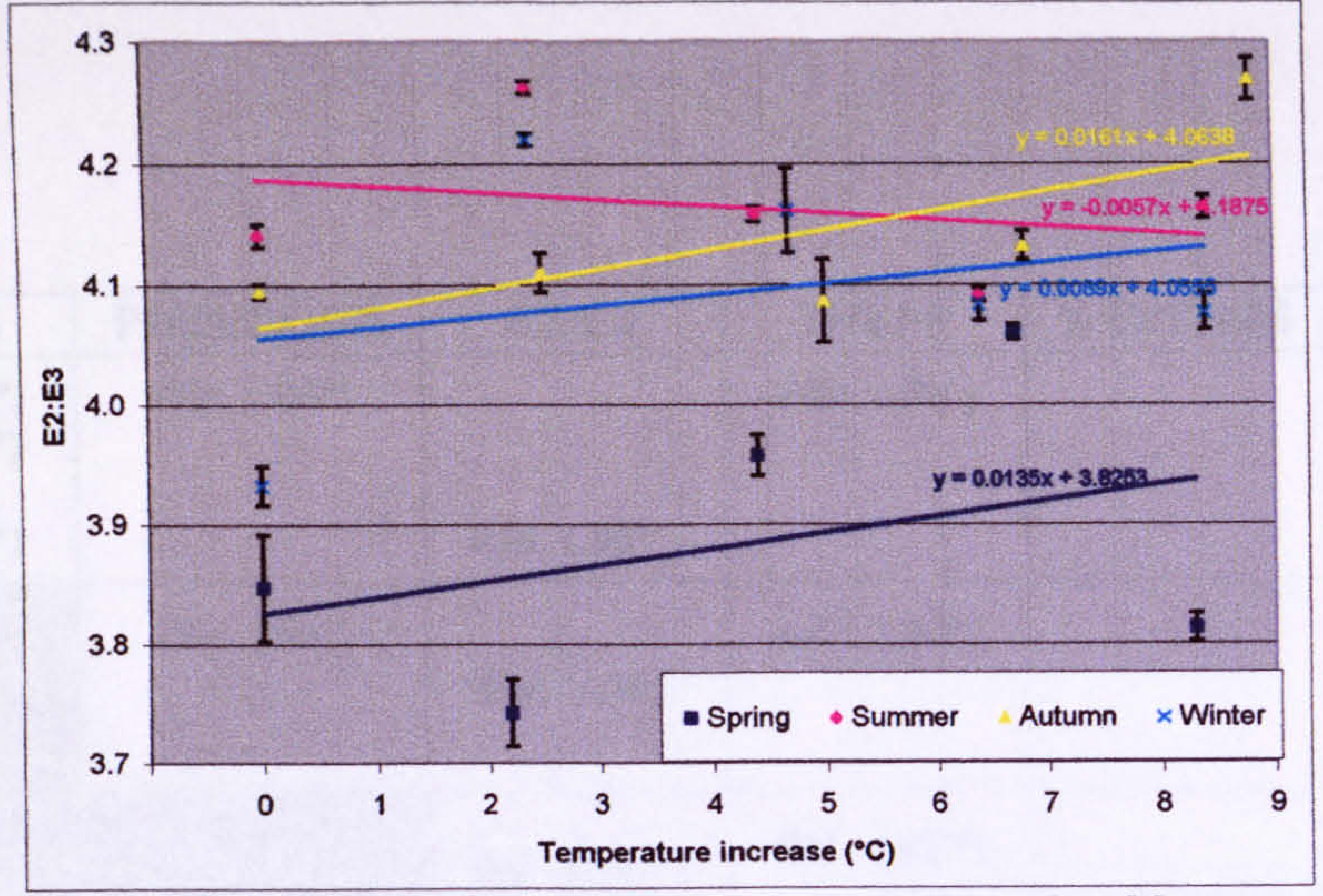

Figure 4.15 – Seasonal changes in E2:E3 ratio of fen peat DOC with increasing soil temperature

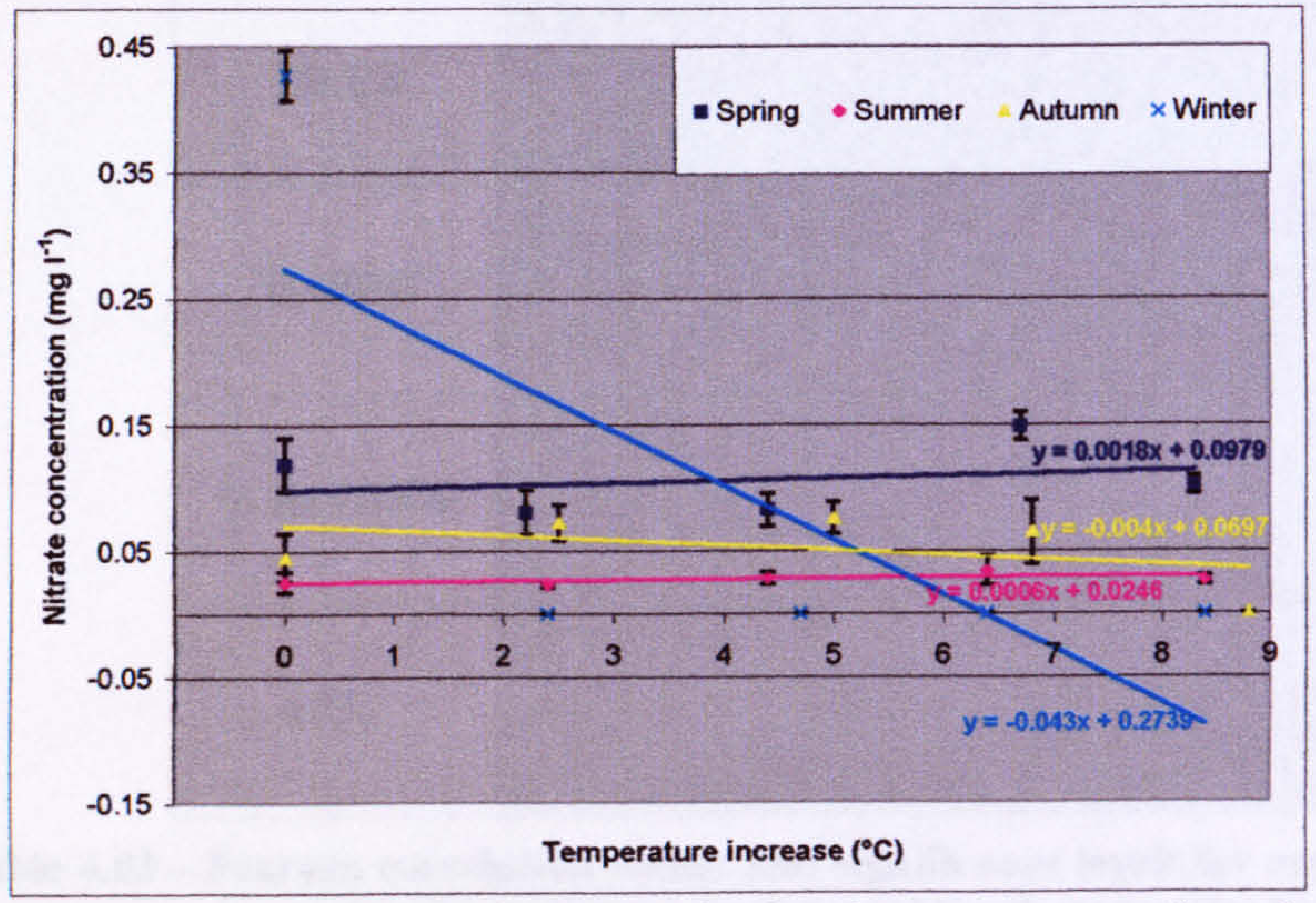

Figure 4.16 – Seasonal changes in the nitrate concentration of fen peat with increasing soil temperature

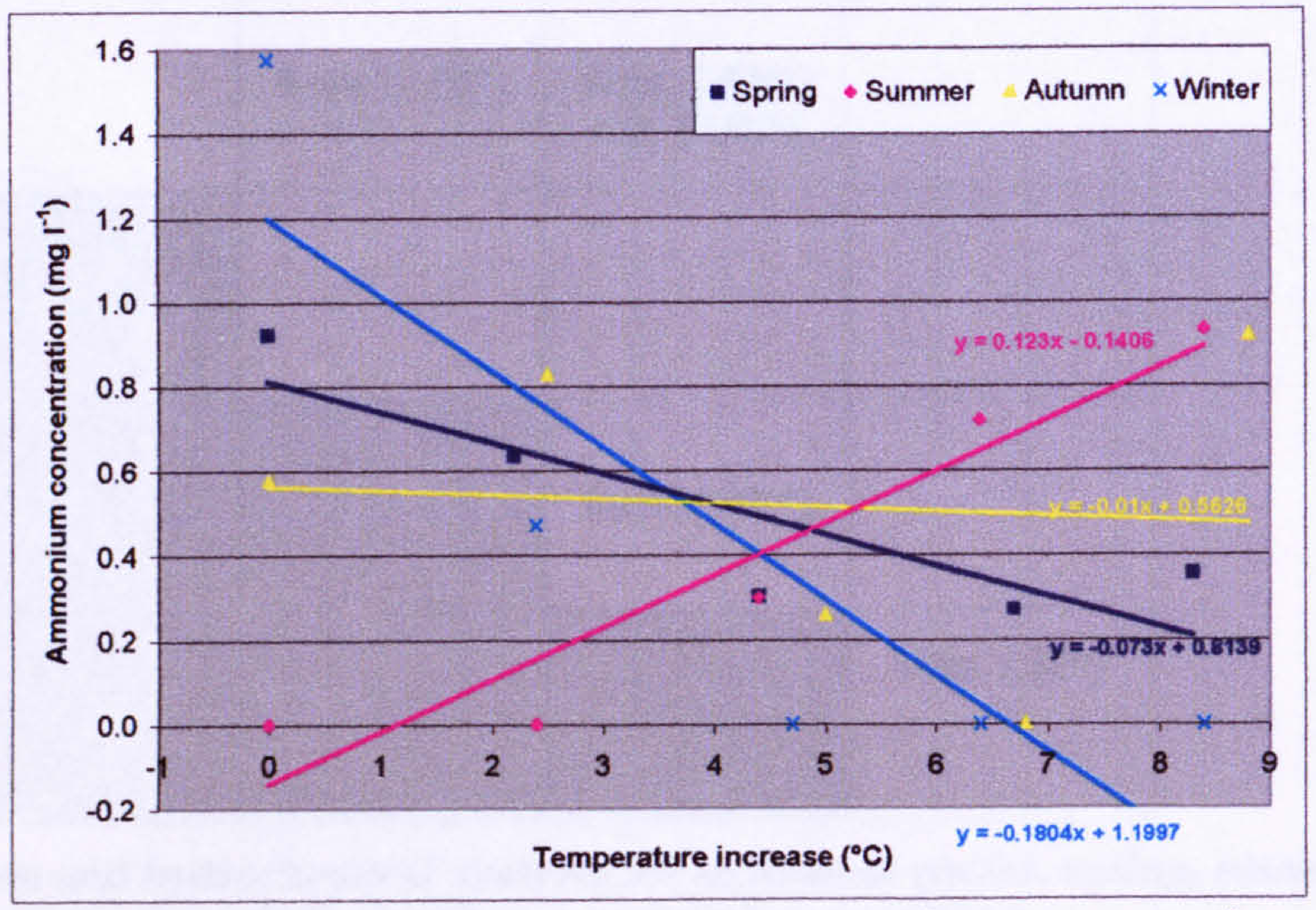

Figure 4.17 – Seasonal changes in the ammonium concentration of fen peat with increasing soil temperature

	UV-254	PHENOLICS	E2:E3	THMFP	% BrTHMS	NO_3^-	NH_4^+
DOC	Win. (.91*) Spr. (.90*) Aut. (.89*)	Win. (.89*)	Aut. (.98**)	Win. (.89*)		Aut. (-.89*)	Spr. (.91*) Sum (.94*)
UV-254		Win. (.91*)	Spr. (-.90*)	Win. (.90*)			
PHENOLICS			Spr. (-.97**) Sum. (-.95*)	Win. (.99**)	Sum. (.99**)	Sum. (.99**)	
E2:E3					Sum. (-.93*)	Sum. (.96**) Aut. (-.89*)	
THMFP							
% BrTHMS						Sum. (.98**)	
NO_3^-							Win. (.95*)

Table 4.03 – Pearson correlation values and significance levels for organic carbon and hydrochemical analyses for individual winter, spring, summer and autumn seasons for fen peat incubated under increasing temperatures. Only significant correlations are shown.

4.4 Discussion

4.4.1 Bog observations

The general trend of potentially-leachable DOC concentration changes with increasing soil temperature were a gradual, but significant, decrease for winter, summer and autumn peat, and a large, but insignificant rise for the spring peat. The significant decreasing trends are most likely to have been due to the increasing temperatures enhancing microbial and enzymic decomposition, resulting in a greater production of CO_2 rather than DOC. Although the spring trend was insignificant, perhaps due to the high degree of variance of the data points around the trendline rather than the lack of a response, the fact that a significant decrease was not recorded for this season, and that the trend was significantly different to the summer and autumn decreases, suggests the peat may possess unique characteristics during springtime. The data suggests that if peat in springtime is warmed sufficiently, one or more temperature-constrained processes may be stimulated, potentially mobilising large concentrations of carbon which then become available for release during heavy rainfall events. The potential mechanisms behind this opposing spring trend are discussed below.

During winter time, cold temperatures inhibit the growth of *Sphagnum* moss (Moore, 1989), and reduce soil microbe and enzyme activities (Fenner, *et al.* 2005*b*). This severely impairs the decomposition of the large accumulation of senescent and dead vegetation that occurs from the end of the warm summer season (Blodau, 2002). Whilst some of this poorly decayed material would be washed out of the soil, as illustrated by the winter peat having the largest leachable DOC concentration at the collection temperature, a large quantity of non-water-soluble carbon compounds would remain in the soil. It may be that as winter turns to spring and the photosynthesis and productivity of the overlying *Sphagnum* increases (Lindholm, 1990; Moore, *et al.* 2002), a 'priming' of the soil microbial populations occurs (Lynch & Whipps, 1990). It has previously been shown that photosynthetically-derived root exudates from *Sphagnum* can be an important source of carbon within the DOC pool of ombrotrophic bog systems (Fenner, *et al.* 2004). As *Sphagnum* productivity increases at the beginning of the spring growth season, the increase in root carbon and nutrient exudation would stimulate the activity of soil microbial populations (Cheng & Coleman, 1990; McLatchey & Reddy, 1998; Hamilton & Frank, 2001), and, along with rising temperatures, would contribute to an increase in the release and activity of extracellular enzymes (Burns, 1978). This enzyme stimulation, especially of the key soil enzyme phenol oxidase, which has been shown to be higher in activity during spring compared to winter (Fenner, *et al.* 2005*b*), would allow for increased cleaving of water-soluble carbon compounds from the peat matrix, ultimately resulting in their leaching out of the soil during heavy rainfall.

If this was a viable mechanism, it may be expected that the concentration of leachable DOC at the collection temperature would have been higher during spring. However, concentrations at the collection temperature (i.e. not having been exposed to increased temperature) were lowest for the spring peat. This implies that the decomposition of winter accumulated carbon is temperature constrained, or, perhaps more accurately, an increase in temperature such as that which the spring peat was subject to in this experiment, in combination with an increased influx of labile low molecular weight root exudates and nutrients, can dramatically stimulate microbial and enzymic activities to degrade some of the winter accumulation of dead plant-derived organic material. During spring, the microbes are emerging from a long, cold season of relative inactivity and the total biomass is lower (Bardgett, *et al.* 1997); a rise in temperatures would be expected to induce a substantial stimulation of their activity. There is evidence of this; the study by Fenner, *et al.* (2005*b*) observed that the activity of the key cellulose degrading enzyme β-D-glucosidase increased with rising temperatures fastest in the spring season.

The difference in the temperature-induced trend for spring peat between leachable and porewater DOC recorded by Fenner, *et al.* (2005*b*) is interesting and is probably due to the difference in the procedures involved for both of the techniques. The leachable technique is a simulation of heavy rainfall and involves the physical removal of some of the carbon compounds from the peat matrix into solution via the action of water molecules, whereas the porewater technique extracts only DOC already in solution. Moore & Dalva (2001) showed that when peat comes into contact with water of a low

DOC concentration, such as rainwater or the deionised water used in this study, carbon compounds are released into solution and this mechanism probably accounts for some of the difference. Hagedorn, *et al.* (2004) reviewed the differences in DOC quantity and quality using the porewater and leachable methods. They reported that the leachable method yields significantly more DOC; this was observed during the 2003 survey (*figures 2.30 & 2.31*, p57) and is to be expected given the physical action involved. They also stated that the leachable solution contained significantly more 'new' carbon than the porewater solution. Additionally, Hill & Cardaci (2004) noted that leachable carbon is particularly susceptible to decomposition. These are interesting findings, as they show that the mechanism proposed above for the rise in spring peat DOC is viable, because the decomposition of the winter carbon accumulation during spring will generate new carbon because it is derived from recently dead vegetation. The fact that a rise in DOC was only observed for leachable and not porewater solutions indicates that the physical action of water movement through the soil is needed to remove most of this carbon into solution, and this mechanism is dramatically temperature-constrained, despite the increased influx of nutrients and labile carbon associated with *Sphagnum* growth during spring.

Although also not significant trends, the analyses of E2:E3 ratios, aromatic percentages and especially phenolic concentrations reveal evidence of increasing molecular weight with rising temperature for the spring peat. As for DOC, the lack of a significant decrease, which was recorded for all three other seasons, and the significant differences for phenolics concentrations between the summer and autumn decreases compared to the spring increase, suggests that spring peat possesses unique characteristics and is particularly responsive to increased temperature. The rise in DOC and phenolics for spring were comparable, a finding matched by Freeman, *et al.* (2001*b*). In addition, DOC concentrations correlated negatively and significantly with values of the E2:E3 ratio, which is inversely proportional to molecular weight (Osburn, *et al.* 2001). This selective leaching of high molecular weight carbon during spring again seems to support the idea that warming led to an increased mobilisation of winter accumulated carbon from the peat matrix. This accumulation would have input mostly *Sphagnum*-derived high molecular weight material, such as humic acids, into the soil (Clymo, 1983; Aerts,

et al. 1999). As the conditions improved during spring, allowing for greater decomposition, the dead vegetation will have become more susceptible to leaching into solution.
Given the inhibitory effect of phenolic compounds on enzymic activities (Wetzel, 1992; Pind, *et al.* 1994; Freeman, *et al.* 1996; Freeman, *et al.* 2001*a*), it may seem unusual that phenolic compounds could increase simultaneously to the proposed increase in enzyme activities discussed previously. However, as described by Fenner, *et al.* (2005*b*), if the conditions are right, phenolic compounds can be cleaved from the peat matrix by the action of soil enzymes faster than they can be decomposed by the microbial community. This is an important process and must have occurred in the spring peat to explain the observed rise in DOC.

Ion data for springtime also adds to the theory of an increased mobilisation of organic matter, with losses, albeit insignificant, of nitrate and sulphate concentrations and an increase in ammonium with rising temperature. Under the anaerobic conditions found within the peat used in this experiment, microbes utilize oxidized soil ions, such as nitrate and sulphate, as electron acceptors for respiration (Pulford & Tabatabai, 1988). The decrease of nitrate with increasing temperature shows that microbial respiration must have been stimulated; measurements of soil CO_2 release by Fenner, *et al.* (2005*b*) confirm this effect. The reduction of nitrate to below the limit of detection demonstrates that the ombrotrophic bog sampled was, as would be expected, nutrient limited. It has been demonstrated in previous studies that temperature can indirectly influence the sulphate concentration of peat by enhancing evapotranspiration and drying (Eimers, *et al.* 2003), however, in this study, such a process cannot account for the loss of sulphate because no significant differences were measured in the water content of the peat along the temperature-gradient bar (data not shown). The loss of sulphate is, like nitrate, probably due to the stimulation of enzymic processes, as sulphate is also used as an electron acceptor during microbial respiration. The loss of sulphate may offer another explanation of why DOC concentrations increased, as a reduction in sulphate would cause an increase in pH and therefore an increase in DOC solubility (Evans, et al. 2006). However, the lack of an increase in DOC concentrations for the summer peat, for which a decrease in sulphate concentration was recorded with increasing temperature, suggests this chemical process is not as important as the biological mechanisms explained above. The positive correlation for nitrate and sulphate with the E2:E3 ratio indicates that as nitrate and sulphate were consumed, the molecular weight of the leachable DOC increased, adding to the growing evidence that there was a temperature-induced mobilisation of the peat matrix. The fact that sulphate did not get utilised to levels below the limit of detection, unlike nitrate, is that it is an energetically less favourable electron acceptor (Lucassen, *et al.* 2005). The increase in ammonium is also probably linked to decomposition, as it is sourced principally from the mineralisation of N-containing organic matter (Bayley, *et al.* 2005). *Sphagnum* has been shown to be an important source of nitrogen to peatland ecosystems (Bragazza & Limpens, 2004); the enhancement in DOC concentration for the spring peat that is thought to be due to the decomposition of dead vegetation would therefore release previously organically-bound nitrogen, possibly explaining the rise in ammonium concentrations.

Perhaps the most important finding of this study was that there was a large increase in the THMFP of the solution leaching from the spring peat with increasing temperature. Although the rise was again an insignificant trend, the fact that it was significantly different to the summer decrease suggests there are important differences between the soil characteristics throughout the year. The increase in the THMFP values can be attributed to the increase in the DOC concentration and molecular weight. For every °C increase in temperature, values of THMFP increased by 670 micrograms of THMs. Relative to the increase in DOC concentration, there was a slightly greater rate of increase in the THMFP of the leachate (approximately 2.5-fold increase for DOC, 3-fold increase for THMFP over the full temperature range), indicating the greater mobilisation of THM-forming over non-THM-forming DOC. The THMFP correlated positively with DOC and UV-254 absorbance and negatively with values of E2:E3, signifying that it was the higher molecular weight fraction of DOC that contributed most to THM formation.

The percentage formation of BrTHMs correlated significantly with UV-254, phenolic and percentage aromaticity values, and negatively with the E2:E3 ratio. These correlations exist because of the strong binding of bromide with the aromatic compounds of soil organic matter (Biester, *et al.* 2004). With the increased mobilisation of high molecular weight (and therefore aromatic) carbon with increasing temperature for the spring peat, there will therefore have been a concurrent increase in the release of bromide-containing organic compounds. During the THMFP reaction, the break up of these aromatic compounds by chlorine will have released bromide, which would have then become incorporated into the carbon-chlorine THM-forming reaction. This explains the increase in the percentage of BrTHMs that occurred for the spring peat following warming and strengthens the previously discussed argument that the spring DOC rise was due to a mobilisation of the carbon matrix.

The decrease in DOC concentrations and molecular weight for the other seasons is simpler to explain and is based upon the fact that enzymes work more efficiently in warmer temperatures; therefore the decomposition of organic matter will occur more quickly and slightly less inefficiently in the peat studied here. For examples, Fenner, *et al.* (2005*b*) observed an increase in β-D-glucosidase and phenol oxidase activities following a rise in temperature for summer and autumn peat.

During the summer and autumn seasons, the temperature, *Sphagnum* productivity, input of labile carbon, and microbe and enzyme activities are greater, so decomposition rates are faster than at other times of the year (Kang & Freeman, 1999; Scott, *et al.* 2001; Moore *et al.* 2002). The winter build-up of relatively labile portions of dead vegetation has been reduced, so that the peat matrix is composed of a greater proportion of older plant material which is harder to decompose (Freeman, *et al.* 2001*a*). The increase in temperature of the peat soil may therefore have brought about a reduction in DOC and the overall molecular weight because the enzymic rate of decomposition may have been greater than the cleaving of non-water soluble carbon compounds from the peat matrix. This was also suggested by the nitrate concentration, which significantly decreased in the summer peat with increasing temperature, for reasons discussed previously. The concentration of sulphate also reduced with increasing peat temperature for summer, implying enhanced decomposition, but remained unchanged for autumn peat, suggesting sulphate-reduction did not increase with rising temperature, although these trends were insgnificant. The concentration of ammonium exhibited very contrasting relationships;

none was detected in the autumn peat, whilst concentrations decreased for the summer peat (as opposed to the increase recorded for spring peat). This possibly suggests that, for the summer peat, the higher temperatures stimulated nitrosomonas (ammonium-oxidising) bacteria faster than the ammonium-releasing mineralisation of N-containing organic compounds. Nitrosomonas bacteria have previously been shown to be highly responsive to temperature (Groeneweg, *et al.* 1994). The fall during summer may also be because the reserve of easily-decomposable *sphagnum*-derived organic compounds had become depleted.

As a consequence of the small but significant reduction in DOC and molecular weight, the THMFP of the leachable solutions reduced significantly for the summer peat, with no change recorded for the autumn peat. The lack of significant increases for the BrTHMs in response to rising temperatures, and the lack of significant correlations with measurements of organic matter, shows that the mobilisation of aromatic or high molecular weight carbon compounds from the peat matrix, and therefore the release of bromide, is not as prevalent when summer and autumn peat are subject to warming. This mobilisation effect would appear to make the spring bog peat unique.

4.4.2 Fen observations

The main temperature-induced effect for the fen peat was that a significant increase in leachable DOC was recorded for only the summer peat as the incubation temperature rose, and this increased the THMFP of the leachate solution. The rate of increase in DOC concentrations and THMFP values was approximately equal, indicating mobilisation of similar quantities of THM-forming and non-THM-forming DOC. There was no significant effect of warming on spring DOC concentrations and the responses of autumn and winter peat were minor. Unfortunately, no other published studies exist on the seasonal responses of fen peat soil to warming. Compared to the bog peat, the responses of the fen peat to warming were generally less dramatic. Although they are both anaerobic, peat-accumulating ecosystems, fens differ from bogs in a number of ways. The most important is their source of freshwater, with fens (especially Cors Erddreiniog) benefiting from nutrient-rich groundwater and bogs (especially Marchlyn Bach) receiving only nutrient-poor rainwater (Mitsch & Gosselink, 2000). This contrast in nutrient status means the pH of fens is usually higher (Gorham, 1967) and enzyme activities (Kang & Freeman, 1999) and microbial respiration (Verhoeven, *et al.* 1994; Kang, *et al.* 2002) are greater, creating a more stable environment which is more suitable for organic matter decomposition (Malmer, 1986). This creates a soil whose DOC is of a lower overall molecular weight (Kang, *et al.* 2002; Brooks, 2005). The high phenolic concentration of *sphagnum* moss also increases the molecular weight of bog organic matter (Verhoeven & Liefveld, 1997). These biogeochemical contrasts may explain the differences in the temperature response for the fen peat, and also the disparity in the seasonal data recorded at the collection temperature. The concentration of DOC, phenolic compounds and THMFP values were, on average, higher for the bog peat at the collection temperature, whilst the E2:E3 ratio was lower. Although levels of nitrate and ammonium were similar, there was overall a greater nutrient concentration in the fen peat (data not shown).

The significant rise in DOC for the summer peat may be due, in part, to an abundance of nutrients, which will be higher in summer due to greater plant productivity (Warembourg & Paul, 1977). In fens, especially Cors Erddreiniog which was used during this study, nutrients are not generally limiting (Mitsch & Gosselink, 2000). This was observed for nitrate and sulphate (data not shown) concentrations, which remained constant as the summer peat temperature increased. This suggests that during summertime, the increase in temperature led to an increase in the enzyme-induced mobilisation of organic matter from the peat matrix, which then became available for leaching. Although no significant, the trend of ammonium concentrations may also imply this; concentrations correlated with DOC changes, suggesting the increased mineralisation of nitrogen-containing organic compounds from the peat matrix (Bayley, *et al.* 2005).

The reason why this rise occurred for summer peat DOC concentrations and not for any of the other seasons may be that the temperature the peat was exposed to was greater than is typically experienced throughout the year for only this season. For example, spring peat was warmed from 10-18 °C and summer peat from 15-23 °C, therefore the summer peat was incubated at temperatures (at the upper end of the scale especially) which are not experienced in soils during current summers, but which they may experience in the future. According to Fenner, *et al.* (2005*b*), the dominant type of microorganisms in peat are psychrophiles, which have an optimal growth temperature below 20°C, and mesophiles, which have an optimal growth between 20-50°C. Pietikainen, *et al.* (2005) and Metje & Frenzel (2005) both illustrated that microbial growth rates in peaty soils were optimal at around 25°C; the rise for the summer peat may therefore be due to a stimulation of the mesophilic microbes and this would have only occurred for the summer peat. The rise may also be due to the combination of these high temperatures in association with labile root exudates, the influx of which are generally higher in the summer months (Warembourg & Paul, 1977). Interestingly, at the collection temperature the concentration of leachable DOC and phenolics was low compared to winter and spring. This can probably be attributed to the higher rates of enzymic activities during the summer and the greater breakdown of DOC, resulting in less that can be leached out of the soil (Kang & Freeman, 1999).

DOC molecular weight changes for summer also seem to support the principle of enhanced peat mobilisation for the summer peat, with a significant increase in phenolic compound concentrations recorded with rising temperature. This is only possible if organic matter is cleaved from the peat matrix, as decomposition processes, which are stimulated by rising temperatures, would result in a decrease of DOC molecular weight. The concurrent increase in both DOC concentrations and molecular weight as the soil temperature rose resulted in a leachable solution with a significantly greater THMFP. The rate of increase (180 micrograms of THMs per °C temperature increase) was much lower than the increase recorded for the bog soils; this reflects the lesser temperature-induced increases in DOC concentrations and molecular weight for the fen leachate.

The only similarity between the data for bog and fen peat was for the winter, which exhibited similar minor, but significant DOC and phenolic compound losses. It may be that the lack of labile root exudates input during this cold season may be to blame for the lack of an increase in leachable DOC concentrations (resulting from the decomposition of the winter-accumulated organic matter) on a similar magnitude to that observed for the spring bog peat. The data for nitrate concentrations from the winter fen peat suggests that exhaustion of key nutrient supplies may also be a cause. After just a 2.4°C temperature increase, the nitrate concentration reduced to below the limit of detection, while phosphate was not detected at all (data not shown). These are key nutrients controlling the cycling of organic matter in soils (Cleveland, *et al.* 2006) and their absence in winter peat would seem to suggest that decomposition was consequently impaired. A rise in temperature would therefore have little effect.

The data for spring and autumn peat did not show significant temperature-mediated biogeochemical changes, but these seemed to be a seasonality effect. At the collection temperature, DOC and phenolic compound concentrations for spring peat were similar to winter, whilst autumn values were similar to summer. This can be attributed to the degree of organic matter cycling occurring during these seasons. During autumn, enzyme activities and the decomposition of organic matter are greater and are also high during the summer (Kang & Freeman, 1999). This ensures there is less leachable carbon available to be flushed out of the soil profile during rainfall. Increasing incubation temperature of both spring and autumn peat therefore had minor effects on carbon quantity and quality. The lack of an effect on nitrate, ammonium and sulphate concentrations (data not shown) would seem to illustrate that temperature had little effect on the microbial populations in the peat and that rising temperatures in spring and autumn will not result in increased mobilisation of the peat matrix. This may imply that during these seasons, microbial and enzyme activities are operating at optimal conditions, although obviously constrained by the lack of oxygen in the soil pores. The contrast in the response of spring fen peat to warming compared to bog peat is particularly stark. The reason for this may again be traced back to the difference in the quality of the organic matter between fens and bogs. With fens having a lower pH, more nutrients, greater enzyme activities and microbial respiration, and being composed of lower molecular weight, less inhibitory DOC, there is less potential for a large build up of dead vegetation, such as during wintertime, which can then be flushed out during heavy rainfall. The lower overall DOC values at the collection temperature for the fen peat would seem to suggest this.

The percentage contribution of brominated THMs did also not exhibit a significant change with temperature for any of the seasons. This may suggest that bromide ions are not bound as strongly to organic matter within fens compared to bogs, possibly due to their lower content of aromatic carbon (Kang, *et al.* 2002; Brooks, 2005). Brominated percentages instead exhibited a greater seasonal fluctuation, with values being lowest for the summer peat and highest during autumn and spring. This may simply reflect patterns of rainfall, which during 2005 were higher in spring and autumn compared to summer (*figure A08*, Appendix A3). Rainfall has been shown to be an important source of bromide to Anglesey, due to its close proximity to the sea (see p45 & 73 of chapter 2).

4.5 Conclusions

These data suggest that increasing the temperature of peat soil to levels that may be expected under a warmer climate may significantly increase the mobilisation and leaching of high molecular weight carbon from fen peat during summertime. For the bog peat, warming is likely to significantly increase decomposition processes within the soil in winter, summer and spring, but may lead to an increased leaching of organic carbon during spring. The extra carbon leaching from fens during summer and bogs during spring includes THM-forming compounds, so there are implications for freshwaters and the quality of drinking water. The most likely explanation for the increase is that warming led to an increased mobilisation of the peat matrix, especially as phenolic and other high molecular weight compounds also increased in leachability. For the bog peat, the increase may be due to the 'priming' of soil microbial populations through the increase in labile carbon and nutrient exudates that occurs during spring and the resulting increase in soil enzymes causing a cleaving of carbon from the peat matrix. This process also seemed to mobilise organically-bound bromide ions, as the percentage of brominated THMs also increased with temperature. For the fen peat, the summer increase may have been due to the increase of soil temperatures to levels sufficiently high to stimulate mesophilic bacteria and the resulting increase in enzyme activities in the high pH, nutrient rich soil cleaving organic carbon from the peat matrix.

Chapter 5

Short-term impacts of elevated concentrations of atmospheric ozone on peatland carbon cycling

5.1 Introduction

Ozone gas (O_3) is a natural constituent of the troposphere, originating from its transfer from the stratosphere and photochemical reactions between nitrogen oxides (NOx) and volatile organic compounds (VOCs). Concentrations have more than doubled during the past century; typical surface measurements today are 30-40 ppb, compared with 10-15 ppb during the pre-industrial era (Volz & Kley, 1988). The annual rate of increase during the last three decades has been approximately 0.5-2% (Vingarzan, 2004), or 0.1 ppb per year (NEGTAP, 2001). The dominant factor behind the increasing background concentration is the global increase in ozone precursor emissions associated with the extraction and use of fossil fuels, which has greatly elevated the concentrations of VOCs and NOx in the atmosphere (Finlayson-Pitts & Pitts, 1997). In the last decade, a clear signal has also emerged that although background concentrations are rising, peak ozone concentrations in the UK have declined by about 30% as European controls on precursor emissions have taken effect (NEGTAP, 2001). A similar pattern is emerging in the rest of Europe and North America (Oltmans, *et al.* 1998). This changing profile is expected to continue, with IPCC modelling data indicating that mean surface ozone concentrations are predicted to continue to rise throughout the 21st century, possibly to levels as high as 70 ppb (Vingarzan, 2004). For example, predictions for 8 sites in the UK indicate increases in annual mean concentrations of 13% by 2030, 29% by 2060 and 55% by 2100 (Coyle & Fowler, 2003).

Numerous studies have shown that tropospheric ozone can have a number of detrimental impacts on vegetation functioning, and it is regarded as the most potent phytotoxic regional scale air pollutant (Krupa & Kickert, 1989; Runeckles & Krupa, 1994; Fuhrer, *et al.* 1997; Andersen, 2003; Ashmore, 2005). The primary impacts of ozone occur in the leaves, as ozone is taken up by the plant's open stomata. Some studies have reported decreased photosynthesis and reproductive functioning in a range of plants after ozone exposure (Krupa & Kickert, 1989; Black, *et al.* 2000) and others have recorded reduced leaf conductance, leaf area and carbon assimilation, reduced water use efficiency and flowering, and loss of stomatal control (Runeckles & Krupa, 1994; Andersen, 2003). Ozone can alter leaf chemistry, and increases in specific leaf weight have been observed, due to a rise in phenolic and lignin compound concentrations within the leaf to aid defence and repair processes against ozone (Kim, *et al.* 1998; Saleem, *et al.* 2001). Ozone has been identified as a contributing factor in the long-term decline of forest growth in areas of high exposure (Peterson, *et al.* 1995). It is thought that the impacts of ozone worldwide today are considerable, and with background concentrations likely to increase during this century, it is envisaged that ozone may seriously impair food production and ecosystem function in the future (Ashmore, 2005).

For economic and geographical reasons, more studies have been conducted on the impacts of ozone on crop and tree species than on natural or semi-natural vegetation. So far, it has only been possible to derive dose-response functions for 80 species of natural vegetation exposed singularly (Hayes, *et al. in press*), with very few dose-response functions available for intact communities. Analysis of the single-species response database has indicated that the highest number of potentially-ozone sensitive communities are found in grasslands, heathlands, bogs and fens (*Mills, et al. in press*), but these predictions have yet to be tested on whole ecosystems. Thus, despite their unique role in the global carbon cycle (Gorham, 1991), relatively few studies have addressed the effects of O_3 on peatland ecosystems.

The vegetation of peatlands is dominated by *Sphagnum* moss, whose one-cell-thick leaves make it particularly susceptible to ozone uptake (Niemi, *et al.* 2002; Rinnan, *et al.* 2003). It has been mentioned previously that plants growing in moist habitats are likely to be more susceptible to ozone than those existing in drier soils (Fanzaring, 2000).

Of the few studies that have been conducted on the responses of peatland vegetation to ozone there have been a few significant responses. Potter, *et al.* (1996) measured significantly lower net CO_2 assimilation, photosynthetic efficiency and an increased leakage of potassium from *Sphagnum recurvum.* Widespread signs of visible injury, reduced photosynthetic rate and overall growth in a large number of fen species exposed to ozone suggest that these ecosystems may be more vulnerable to ozone than other, more intensively studied types of semi-natural vegetation (Power & Ashmore, 2002). The most extensive studies into the impacts of ozone on peatland functioning have been carried out recently by Niemi/Rinnan and co-workers in Finland. Findings from the first study included a short-term significant increase in dark ecosystem respiration of ozone-fumigated *Sphagnum* species, a slight insignificant increase in photosynthesis but no responses in overall CO_2 exchange (Niemi, *et al.* 2002). A significant stimulation in methane production was observed at the 100 ppb level compared to the control; this may have resulted from increased primary production. Rinnan, *et al.* (2003) observed that net CO_2 exchange was reduced by ozone for both *Sphagnum* and *Eriophorum* dominated peatland microcosms. After an initial stimulatory effect, it was concluded that high ozone doses decrease the sink strength of peatlands. Significant increases in dark ecosystem respiration indicate a stimulation of plant or microbial respiration under elevated ozone, whilst CH_4 efflux was again highest under the greatest O_3 concentration for the *Eriophorum* species. Although initial findings suggested that *Sphagnum* dominated peatlands are less sensitive to rising ozone concentrations than most higher plants (Niemi, 2003), later work by the same author demonstrated that *Sphagnum* species have differing levels of ozone tolerance, with *Sphagnum recurvum*, the most sensitive, displaying similar symptoms of chloroplast structural changes as higher plants (Rinnan & Holopainen, 2004).

The limited numbers of investigations that have been conducted on peatland responses to atmospheric ozone have indicated that future concentrations have the potential to upset current CO_2 and CH_4 fluxes, with implications for atmospheric greenhouse gas concentrations (Rinnan, *et al.* 2003). However, there is limited understanding of how such disturbances might impact on soil organisms within the rhizosphere and, consequently, how they would influence the soil carbon cycle (Andersen, 2003; Ashmore, 2005). Due to the highly reactive nature of the ozone molecule, plant roots are not directly exposed to ozone (Blum & Tingey, 1977), but there is potential for an upset in the normal carbon flux to and from soils because ozone reduces carbon acquisition and alters carbon partitioning within the overlying vegetation (Cooley & Manning, 1987). A number of studies have reported reduced allocation of carbon below ground for many difference vegetation types (McCool & Menge, 1983; Edwards, 1991; Coleman, *et al.* 1995; Yoshida, *et al.* 2001), but there are also a few results to the contrary (Duckmanton & Widden, 1994; Nouchi, *et al.* 1995; McCrady & Andersen, 2000). Similarly, there are mixed results of the resulting impact on root respiration (Hofstra, *et* *al.* 1981; Edwards, 1991; Nouchi, *et al.* 1995; Scagel & Andersen, 1997). However, the general consensus is that exposure to ozone will increase the carbon demand and sink strength in leaves, to aid in defence and repair processes, leading to a reduced allocation of carbon below ground (Andersen, 2003). A reduction in this flux would be expected to lead to reduced root growth and there are numerous studies confirming this (Cooley & Manning, 1987; Andersen & Scagel, 1997; Grulke, *et al.* 1998), sometimes even before any impacts above-ground were observed (Mortensen, 1998; Wonisch, 1999). A review of the impacts of ozone on shoot and root growth amongst a variety of different species found greater inhibition of roots for 17 out of the 20 studies listed (Cooley & Manning, 1987).

A reduction in the quantity of root exudates has the potential to suppress microbial activity and therefore the rate of decomposition of soil organic matter. This is suggested by Kim, *et al.* (1998), who observed reduced rates of decomposition within a forest soil due to ozone-induced changes in substrate quality and soil microbial activity. A recent long term study into the impacts of ozone on extracellular soil enzymes within a forest soil yielded interesting results, with a significant 25% reduction in the carbon cycling enzyme 1,4-β-glucosidase (Chung, *et al.* 2006).

Although there is a degree of disparity in the results obtained for assessing below-ground responses, most likely due to differences in the species and developmental stage of the vegetation analysed, variations in soil types and contrasting experimental methods for ozone exposure, there is sufficient evidence to suggest that atmospheric ozone has the potential to seriously impact on above and below ground carbon cycling. The aim of this study was therefore to determine the potential effects of ozone on the below ground carbon cycling in carbon-rich peatlands and to infer what these alterations might mean for the quantity and characteristics of carbon leaching from peatlands into freshwater systems.

5.2 Materials and methods

5.2.1 Site descriptions and peat microcosm collection

Twenty peat microcosms were collected from each of two contrasting peatlands representative of those in Wales; Cors Goch fen (UK National Grid Ref. SH 501 813), on the island of Anglesey, and an acidic bog within Snowdonia's Nant Francon valley (UK National Grid Ref. SH 641 616). Cors Goch is a typical calcareous, minerotrophic fen, dominated by *Festuca rubra* and *Juncus acutiflorus* vegetation (Kang, *et al.* 1998). The peatland within the Nant Ffrancon valley is a typical acidic bog and is dominated by *Sphagnum recurvum* moss and similar species. The cores were collected in PVC drainpipes measuring 10 cm diameter x 40 cm depth. Extraction of the peat involved cutting into the soil with a sharp knife around the base of the drainpipe and pushing the tube into the soil slowly to minimise disturbance. Surface vegetation was kept intact and as uniform as possible between the cores. Five replicate cores were collected for each of the four treatments used during the experiment. Once collected, the cores were kept in a cold room and the bottoms capped using plastic containers and a silicone sealant. This ensured there was no soil-water admixture from one sample to another. The cores were then placed at random into plastic boxes (eight boxes containing five cores each) which were filled with deionised water. Holes drilled into the side of each drainpipe ensured the water-table was maintained to a level at, or just below, the soil surface (*figure 5.01*).

Figure 5.01 – Peatland microcosms placed inside plastic boxes ready for ozone fumigation

5.2.2 Ozone exposure regime

The forty cores were then incubated at different ozone concentrations for six weeks during the summer of 2003 at the CEH Solardome climate change research facility at Abergwyngregyn, Bangor, north Wales in four hemispherical solardomes (height 2.1m, diameter 3.1m) ventilated with computer-controlled ozone concentrations (*figure 5.02*).

Figure 5.02 - CEH solar dome facility located at Abergwyngregyn, near Bangor.

The solardomes provided two air changes per minute of charcoal-filtered air injected with ozone to simulate current and 2050 rural ozone profiles (*table 5.01).* In addition, low local pollution events (+5 ppb over background) were employed in domes 1 and 4 and high local pollution events (+ 55ppb) in domes 2 and 3 during the daytime for four days during the week. The cores were watered by an automatic irrigation system that maintained the water-table depth, and were maintained at near-ambient temperature, humidity and field radiation conditions inside the solardomes.

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The actual concentrations employed during the experiment were measured continuously and used to calculate the AOT40, which is the cumulative exposure of ozone during each daylight hour above a critical level of 40ppb, expressed in ppb h^{-1}. Critical values of 3000 ppb h^{-1} over a 3 month growing season have been proposed for semi-natural vegetation (Fuhrer, *et al.* 1997). The AOT40 values for the four treatments are shown in *table 5.02.*

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5.2.3 Sampling procedures

The full suite of analytical measurements employed throughout the experiment was also performed one week after the cores were collected, and prior to their placement inside the domes. Once the cores were placed inside the domes and the ozone switched on, routine sampling was undertaken after 0, 6, 20 and 41 days of exposure. Peat porewater sampling was performed using a device consisting of a length of plastic Tygon auto analyser transmission tubing (20 cm long, 3 mm diameter) attached to a sawn-off syringe tip inserted into the soil profile to a depth of 12 cm (Freeman, *et al.* 1994). The tip was packed with glass wool to prevent the uptake of large soil particles that cause blockages in the tubing. These devices were inserted into the soil cores one week before the first sampling date. During sampling, 20 cm^3 syringes were attached to the end of the tube and 3 ml of soil porewater was extracted and discarded to prevent the sampling of water that may have collected in the tube. Twenty millilitres of porewater from all 40 cores was then taken up through the tubing, filtered through 0.2 μm syringe filters (Whatman, UK), decanted into separate pre-ashed (550°C) glass vials and stored at 4°C until analysis. To prevent post-sampling aeration of the soil, the tubing was capped with a spare plastic syringe. The soil temperature of each core was measured using a digital thermometer.

5.2.4 Soil carbon quantity and quality and hydrochemical analyses

Please see chapter 2 for methodical details of total DOC and phenolic compound concentrations, UV-visible absorbencies, HPSEC molecular weight and cation concentrations.

5.2.5 Statistical analyses

Data for dissolved organic carbon characteristics (*figures 5.03-5.07*) and ion concentrations (*figures 5.11-5.14*) were standardised (e.g. DOC_{std}) following the method of Clark, *et al.* (2006). The mean measured value (e.g. DOC concentration) at day 0 for each core was subtracted from the values for days 0, 6, 20 and 41, so that, for example, the DOC_{std} at day 0 was 0 mg l^{-1}. This was deemed necessary because the measured values for all analyses on day 0 differed and standardisation was the most effective way of ensuring comparable data between cores and treatments. *Figures 5.03-5.07 and 5.11-5.14* show mean data for 5 replicates (n=5), with error bars indicating ± standard error of the mean and *figures 5.08-5.10* show data for one replicate.

One-way ANOVA with Tukey-Kramer post-hoc test were used to test for significant differences in replicated analyses between the four different ozone fumigation treatments at days 6, 20 and 41. However, it is acknowledged that three-way ANOVA should have been used to test for significant differences using time, treatment and peatland type as the factors. Statistical analyses were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, California, USA).

5.3 Results

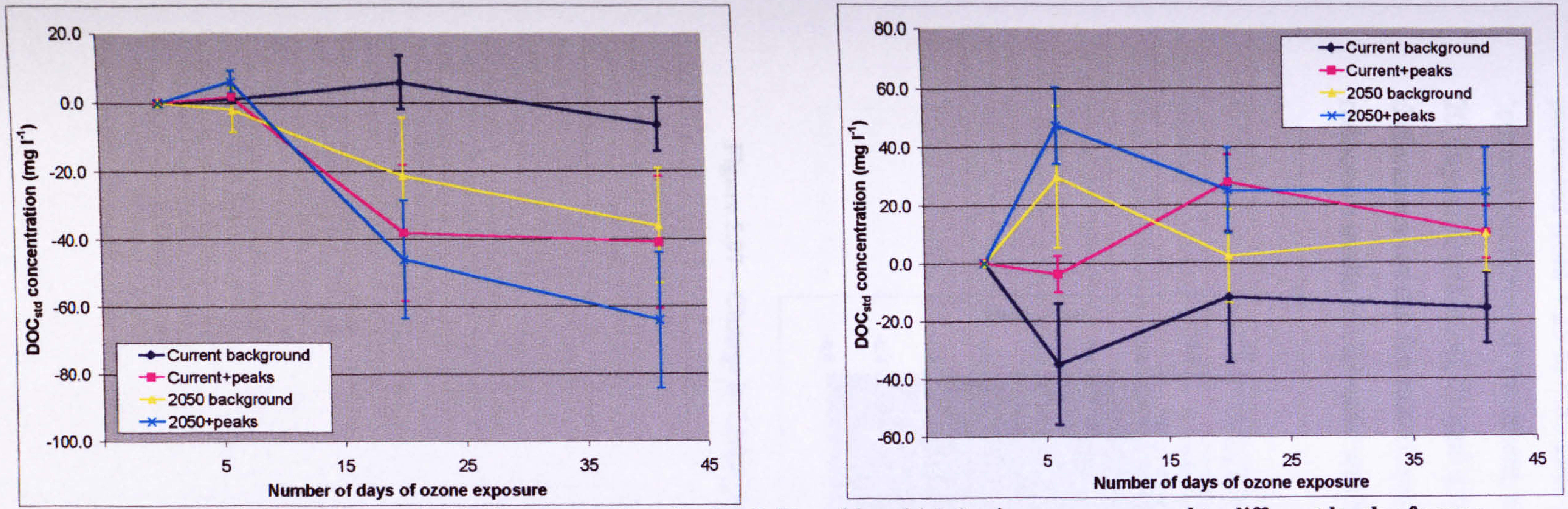
5.3.1 Soil carbon observations

Figures 5.03 & 5.04 show that ozone fumigation led to markedly different responses in the size of the dissolved organic carbon pool in the fen and bog microcosms. For the fen, DOC within the 'current background' treatment fell slightly, from 55.1 mg l^{-1} on day 0 to 48.8 mg l^{-1} after 41 days of exposure, whereas DOC concentrations dropped over 55% from 114.7 mg l^{-1} to 50.7 mg l^{-1} for the '2050+peaks' treatment over the same time period. The difference in these changes was significant at $p<0.05$. Concentrations of DOC fell approximately 40 mg l^{-1} (equating to a 40% loss) within the two other treatments.

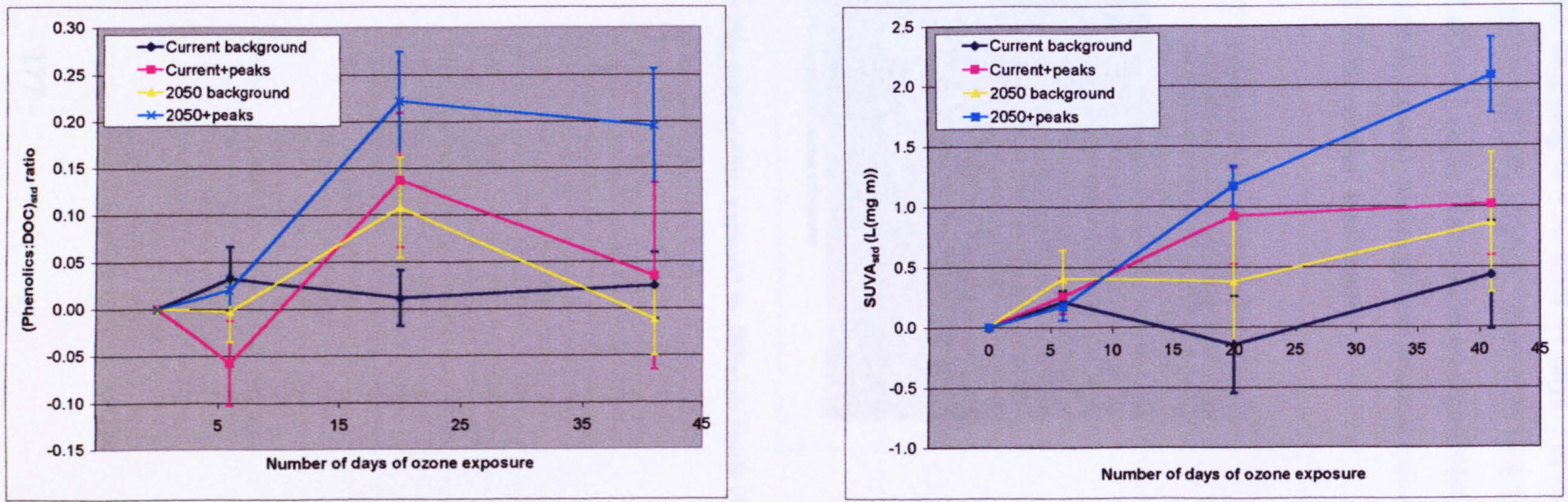
The concentrations of DOC within the bog microcosms incubated in the 'current background' treatment decreased sharply after just 6 days of exposure, falling from 111.7 to 76.8 mg l^{-1} (-31%). Large increases were recorded for the two 2050 treatments; 81.4 to 110.9 mg l^{-1} (+37%) for the '2050 background' treatment and 64.1 to 111.6 mg l^{-1} (+75%) for the '2050+peaks' treatment. The difference in this DOC change after 6 days between the 'current background' and '2050+peaks' treatments was significant ($p<0.05$). Although the divergence in the change in DOC concentrations between the three elevated ozone treatments and the 'current background' was not as strong after 20 and 41 days, there was a net increase in DOC for the three elevated treatments (+12-19%) and a net decrease (-14%) for the 'current background' treatment after the 41 days of ozone exposure.

Unlike DOC, concentrations of porewater phenolic compounds within the fen microcosms remained largely unchanged following incubation in each of the four treatments. With the decrease in DOC recorded for the elevated ozone treatments, the proportion of phenolic compounds comprising the DOC pool of the cores in these treatments therefore increased. After 20 days of exposure, values of the mean phenolic:DOC ratio increased from 0.149 to 0.370, a rise of 0.22, for the '2050+peaks' treatment, with only a slight increase of 0.01 for the same time period within the 'current background' treatment. The difference between the change in the phenolics:DOC ratio was significant after day 20 between these two treatments ($P<0.05$), but only significant to the 10% level after 41 days. No significant differences or trends were observed for the bog microcosms for the phenolics:DOC ratio.

SUVA values of the porewater DOC increased under all treatments for the fen microcosms (*figure 5.06*) and were significantly higher after 20 and 41 days of ozone exposure for the '2050+peaks' treatment relative to the 'current background' ($P<0.05$). Values increased from 1.62 L(mg m) on day 0 to 3.70 L(mg m) (+2.08 L(mg m) rise) after 41 days of exposure in the '2050+peaks' treatment. Values for the 'current background' treatment increased from 2.94 to 3.36 L(mg m) (+0.42 L(mg m)) for the same time period. For the two other treatments, there was an overall increase in the SUVA values when compared to the 'current background' treatment, especially for the 'current+peaks' treatment, but the difference was not significant ($p>0.05$). There was no trend in SUVA values for the bog microcosms.



Figures 5.03 & 5.04 – Change in DOC concentrations for fen (left) and bog (right) microcosms exposed to different levels of ozone



Figures 5.05 & 5.06 – Change in phenolics:DOC ratios (left) and SUVA values (right) for fen microcosms exposed to different levels of ozone

The E2:E3 ratio of the bog DOC (*figure 5.07*) was significantly higher under the '2050+peaks' ozone treatment compared to the 'current background' after 20 days of fumigation ($p<0.05$), but not significantly higher after 41 days ($p>0.05$). Values increased from 4.25 to 4.80 after 20 days, dropping to 4.55 after 41 days. For the 'current background' treatment, values increased only slightly, from 4.25 to 4.38 after 20 days of incubation, dropping to 4.27 after 41 days. There were no significant differences for the fen microcosms (data not shown), although the greatest overall reduction in the E2:E3 ratio was for the '2050+peaks' treatment.

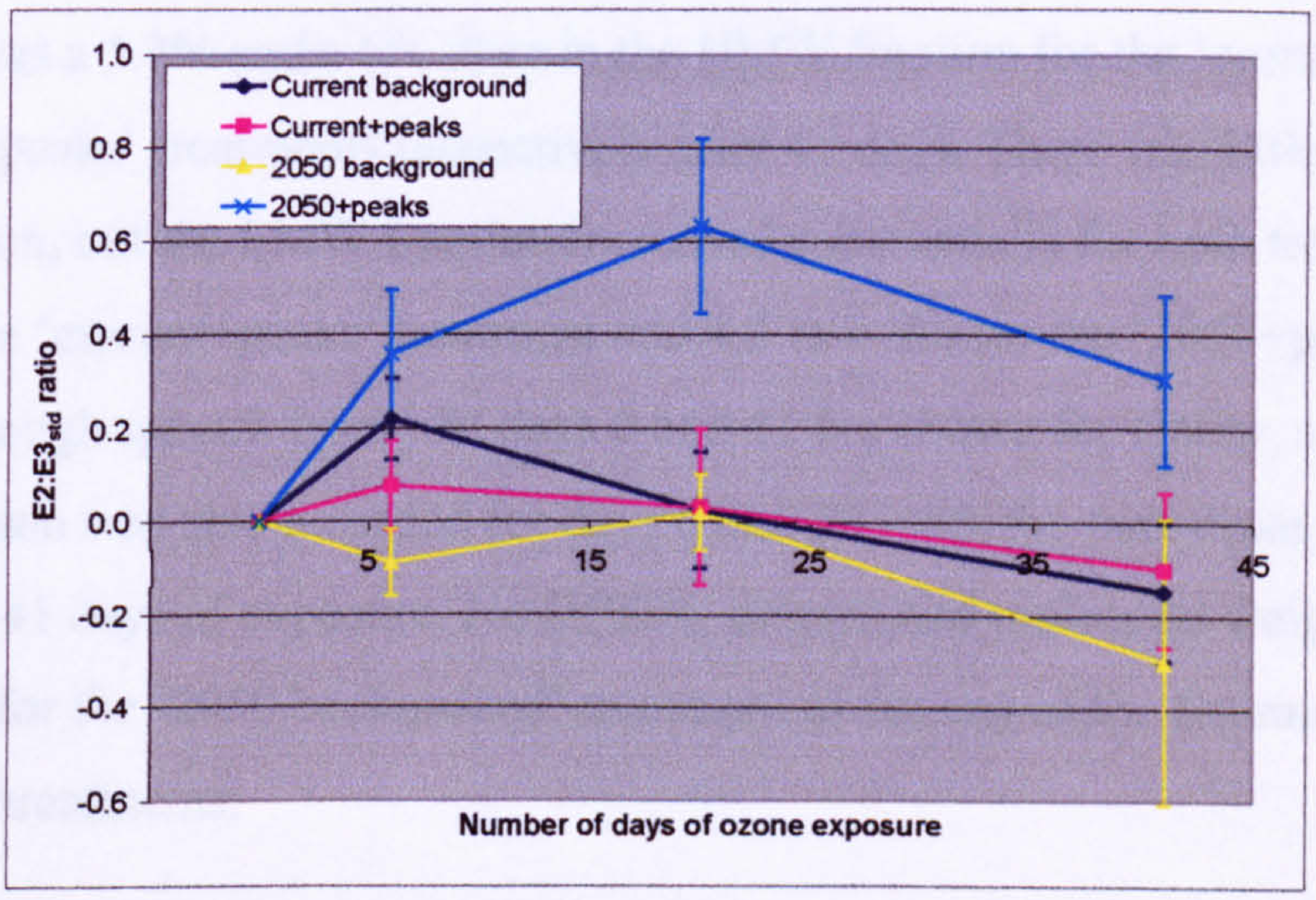


Figure 5.07 – Change in E2:E3 ratios for bog microcosms exposed to different levels of ozone

HPSEC spectra of the dissolved organic carbon from the bog microcosms for three of the treatments on day 0 and 41 of ozone exposure are shown in *figures 5.08 – 5.10* and the percentage contribution from the HMW, IMW and LMW fractions to the total DOC pool are shown in *table 5.03.*

The composition of the DOC within the 'current background' treatment changed very little over the period of ozone exposure and the HMW and LMW fractions remained virtually unchanged. There was a small rise in the amount of IMW material, but this only represented an approximately 2% increase. A much more pronounced change in the molecular weight distribution was observed for the treatments incorporating peaks in ozone. There was a 5.2% and a 6% drop in the HMW fraction for the 'current+peaks' and the '2050+peaks' treatments respectively after 41 days. There was little change in the IMW fraction, but the LMW fraction increased substantially for both treatments; 6.7 to 14.6% for the 'current+peaks' treatment and 4.3 to 9.9% for the '2050+peaks' treatment. Although spectra from just days 0 and 41 are shown for clarity, a reduction in the HMW fraction was also recorded for days 6 and 20, with the maximum reduction occurring after 41 days of exposure. No HPSEC determined molecular weight changes were observed for the '2050 background' treatment or for any of the fen microcosms within the four treatments.

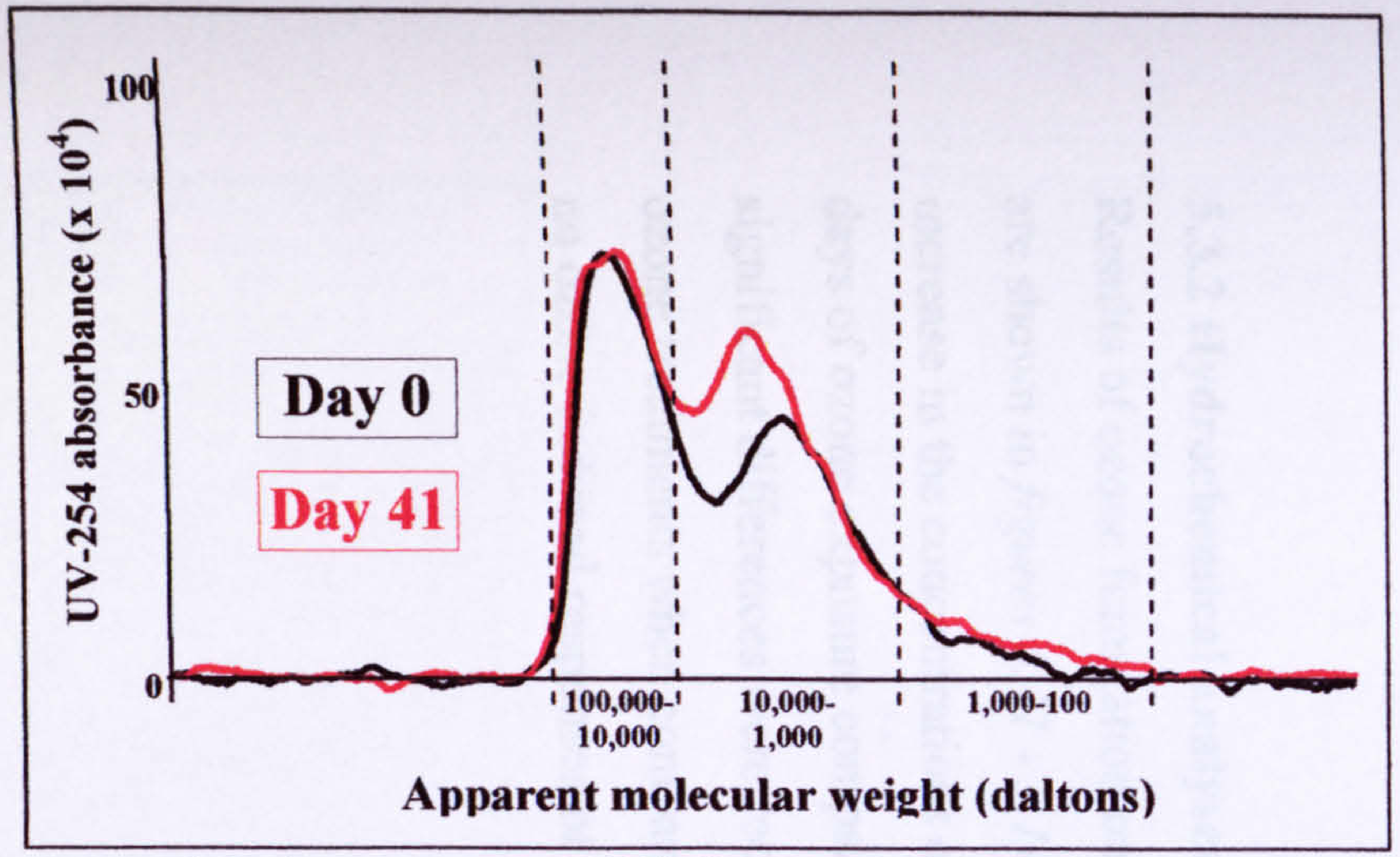


Figure 5.08 - HPSEC chromatograph for bog porewater DOC after 0 and 41 days of exposure ('current background' treatment)

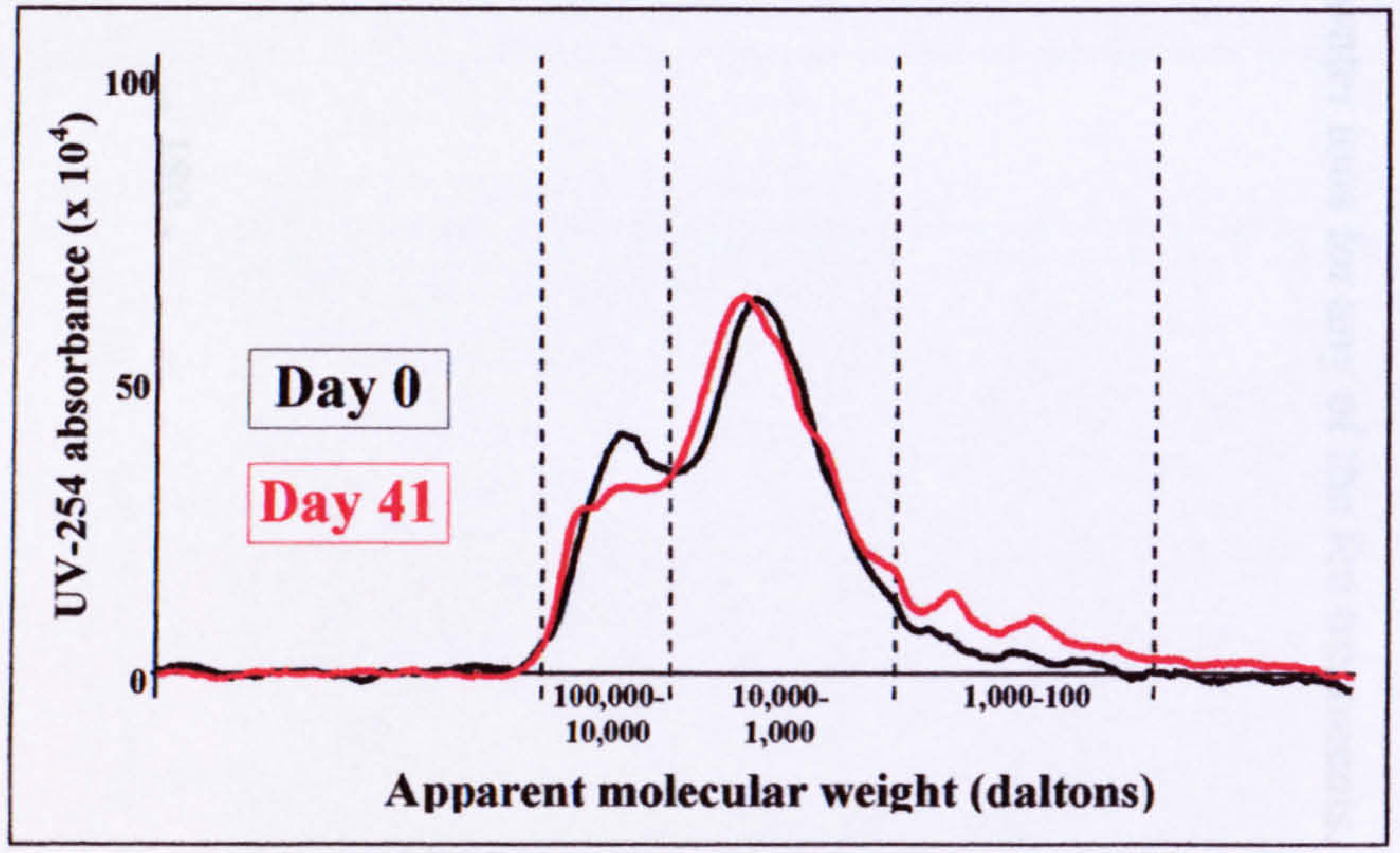


Figure 5.09 - HPSEC chromatograph for bog porewater DOC after 0 and 41 days of exposure ('current+peaks' treatment)

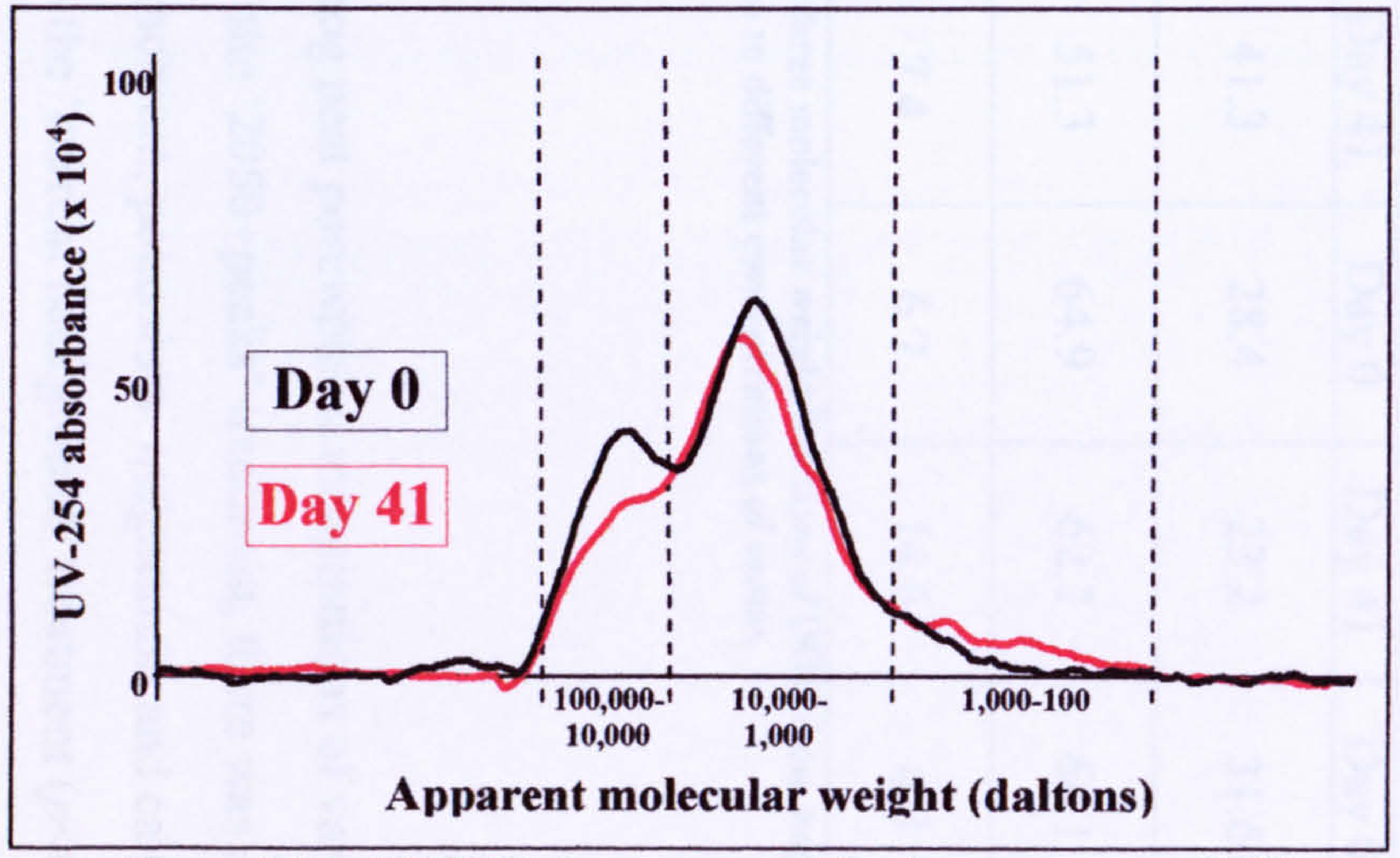


Figure 5.10 - HPSEC chromatograph for bog porewater DOC after 0 and 41 days of exposure ('2050+peaks' treatment)

DOC molecular weight fraction (daltons)	**Treatment** ***'Current background'***		***'Current+peaks'***		***'2050+peaks'***	
	Day 0	Day 41	Day 0	Day 41	Day 0	Day 41
100,000-10,000 (high)	42.2	41.3	28.4	23.2	31.6	25.6
10,000-1,000 (intermediate)	49.2	51.3	64.9	62.2	64.1	64.5
1,000-100 (low)	8.6	7.4	6.7	14.6	4.3	9.9

Table 5.03 – Percentage composition of the three molecular weight fractions of DOC from bog microcosms following exposure to different concentrations of ozone.

5.3.2 Hydrochemical analyses

Results of ozone fumigation on the bog peat porewater concentrations of various cations are shown in *figures 5.11 - 5.14*. For the '2050+peaks' treatment, there was a significant increase in the concentrations of ammonium, potassium, magnesium and calcium after 6 days of ozone exposure compared to the 'current background' treatment ($p<0.05$). No significant differences were recorded beyond day 6, or for any of the other elevated ozone treatments when compared to the 'current background' treatment. There were also no ozone-induced responses of porewater ions for any of the fen treatments.

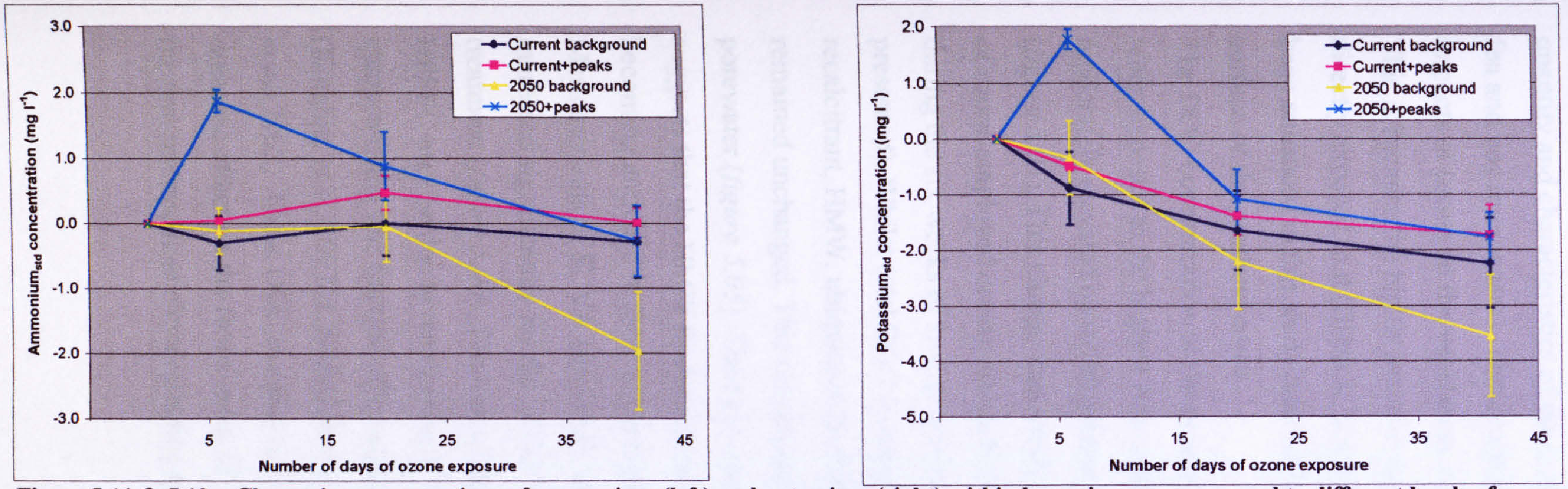


Figure 5.11 & 5.12 – Change in concentrations of ammonium (left) and potassium (right) within bog microcosms exposed to different levels of ozone

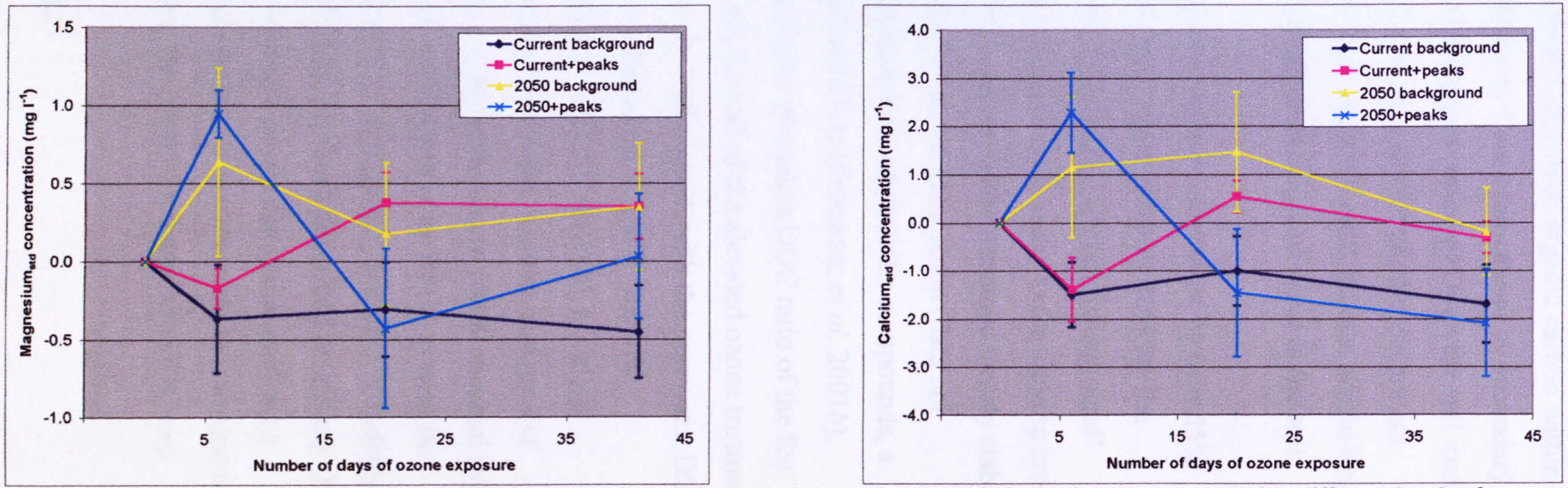


Figure 5.13 & 5.14 – Change in concentrations of magnesium (left) and calcium (right) within bog microcosms exposed to different levels of ozone

5.4 Discussion

Exposure to elevated levels of atmospheric ozone induced a significant change in the quantity and characteristics of the pool of porewater dissolved organic carbon within the fen and bog microcosms. The alterations are likely to have been caused by secondary impacts of ozone on the vegetation, rather than the direct action of O_3 on the soil itself. This is because the highly reactive nature of ozone ensures that plant roots are not directly exposed to it (Blum & Tingey, 1977). Such an attenuation process would have been enhanced in this study because of the continuous presence of water at the soil surface of the peatland cores.

The DOC concentration of the porewater from the fen cores decreased by over 55% when exposed to the highest concentration of ozone, approximately 40% for the moderately elevated ozone treatments, and only 11% for the 'current background' (*figure 5.03*). This change was initiated upon exposure of the cores to the varying levels of ozone employed during the study; their DOC concentrations remained largely stable during the six weeks of storage prior to the onset of ozone fumigation (data not presented). Whilst total DOC declined, the concentration of phenolic compounds, a recalcitrant, HMW, ubiquitous fraction of peatland DOC (Freeman, *et al.* 2001*b*), remained unchanged. This resulted in a much higher phenolics:DOC ratio of the fen porewater (*figure 5.05*). Their persistence in the fen soil of the elevated ozone treatments suggests that the HMW fraction remained largely undisturbed, with the porewater DOC becoming enriched in these compounds due to a reduction in LMW material. Measurements of SUVA also imply an enrichment in HMW material, as values increased significantly for the '2050+peaks' compared to the 'current background' treatment (*figure 5.06*). The lack of a detection of this reduction in LMW material using HPSEC may be due to the drawback of using UV detection in an HPLC system for characterising this fraction of compounds, as they do not typically absorb UV radiation (Korshin, *et al.* 1997*b*). Such a limitation of HPSEC has been described previously (Her, *et al.* 2003). These DOC quantity and quality changes imply that ozone induced a dramatic effect on the functioning of the fen ecosystem. It is interesting to note that for the two intermediate elevated ozone treatments, the greatest reduction in DOC and increase in both phenolics:DOC and SUVA occurred for the 'current+peaks' treatment, which had a higher AOT40 value than the '2050 background' treatment.

Similar findings of changes in qualitative soil DOC characteristics have been observed after exposing wheat and sorghum species to elevated ozone (Islam, *et al.* 1999) and it is likely that the observed changes occurred as a result of an alteration to one or two fundamental processes. The most likely mechanism is that there was a significant reduction in the allocation of carbon below ground. It is well documented that exposure to ozone can have serious implications on the flux of photosynthetically-produced carbon normally transported from the leaves to the roots, leading to suppressed root growth and, ultimately, a reduction in the exudation of C compounds into the rhizosphere (Edwards, 1991; Yoshida, *et al.*, 2001; Andersen, 2003). Although the flux was not directly measured, as has been carried out in other studies (McCrady & Andersen, 2000), the fall in dissolved organic carbon concentrations under elevated ozone was so dramatic that a reduction in root exudates must at least partly be responsible for the change. Root exudates can account for up to 40% of all organic inputs to soil and are principally comprised of LMW sugars, organic and amino acids (Fox & Comerford, 1990; Whipps, 1990; Chen, *et al.* 2004). The large reduction in porewater LMW material for the fen cores exposed to the elevated ozone levels suggests that an inhibition in the transport of photosynthetically produced carbon to the roots was a viable cause. This response is likely to have been due to increased respiratory demands for the maintenance and repair of leaf tissues damaged by O_3, as has been reported elsewhere (Edwards, 1991).

Such a disturbance in this flux of LMW material to the soil matrix would have knock-on effects for the enzyme-mediated cycling of organic matter within the soil, a process already constrained within peatland soils (Freeman, *et al.* 2001*a*). The reduction in LMW compounds and essential nutrients entering the soil via plant roots would reduce the metabolism of soil microbes within the rhizosphere, as it is their primary food source (Cheng & Coleman, 1990; Edwards, 1991; Kraffczyk, *et al.* 1984). As microbes are an important source of extracellular enzymes (McLatchey & Reddy, 1998), this would reduce the abundance of enzymes within the soil matrix and impair rates of organic matter decomposition. Another consequence of a reduction in the supply of LMW root exudates may be the direct inhibition of soil enzymes due to the relative enrichment of the soil porewater in recalcitrant phenolic compounds. It is well known that phenolic compounds are strong inhibitors of the main suite of enzymes responsible for degrading organic matter in soil; the hydrolase enzymes (Wetzel, 1992; Freeman, *et al.* 1996; Freeman, *et al.* 2001*a*). It has also been postulated in previous studies that the phenolics:DOC ratio, and the subsequent competition between substrate DOC (LMW) and phenolic DOC (HMW), is an important variable influencing the inhibition of enzymes (Freeman, *et al.* 1990; Kang, *et al.* 2002). It is therefore likely that within the soil matrix of the fen microcosms incubated in the elevated ozone treatments the increase in the phenolics:DOC ratio led to a suppression of the organic matter degrading enzymes, a process which would have reduced the porewater DOC concentration of the soil even further. Suppression of a suite of soil enzymes has been recorded recently under elevated ozone conditions (Chung, *et al.* 2006), through an inhibition of plant production and microbial metabolism.

The potential upset in the normal plant-soil carbon cycle that the DOC data from this study suggests warrants a much more intensive study of the soil enzyme and microbial activities of a minerotrophic fen soil exposed to elevated ozone.

One of the remarkable aspects of the loss of dissolved organic carbon within the porewater of the fen microcosms was the speed at which the change occurred, with a 48% reduction in porewater DOC occurring after just 20 days of exposure for the '2050+peaks' ozone treatment. Similar rapid below-ground responses have also been recorded in other studies, with Gorissen, *et al.* (1991) reporting a 19% reduction in the flux of carbon from shoots to roots and a 34% reduction in soil/root respiration of Douglas fir after just 28 days.

It has been mentioned in previous ozone studies that impacts on below-ground functioning may occur even earlier than changes in above-ground processes (Hofstra, *et al.* 1981; Kasurinen, *et al.* 2004). The findings of Power & Ashmore (2002) and Rinnan & Holopainen (2004) of greater ozone sensitivity amongst fen species than other semi-natural vegetation types seem to hold true for this study. This is thought to be due to the high growth rates, stomatal conductance and leaf area ratio of these species, characteristics that are synonymous with high ozone sensitivity.

For the bog microcosms there was a different, and much less clear, response in the dissolved organic carbon quantities and characteristics. After just 6 days of exposure there was a significant divergence in the porewater DOC concentrations, with a sharp increase for the '2050+peaks' treatment and a sharp decrease for the 'current background' treatment (*figure 5.04*). Although the divergence was not as pronounced and there were no significant differences after day six, there was an overall increase in DOC for the three elevated ozone treatments. Additional data on the characteristics of the porewater DOC suggests that there was an opposite effect to that which occurred in the fen microcosms. From measurements of the E2:E3 ratio, there was a significant shift in the overall molecular weight of the DOC to a lower value for the '2050+peaks' treatment, with little change recorded for the other treatments (*figure 5.07*). These observations are strongly backed up by HPSEC analysis, with a notable drop in the HMW fraction and a corresponding increase in the composition of LMW material (*figures 5.08–5.10; table 5.03*).

This data implies that under the highest ozone concentration, the decomposition of recalcitrant HMW organic carbon compounds into LMW material increased after just 6 days. Such a process is strongly constrained within peatlands (Freeman, *et al.* 2001*a*), so it is interesting that a stimulation of this process seemed to occur.

The significant increase in the DOC concentration after day 6 for the bog porewater exposed to the highest concentration of ozone was matched by significant increases in the concentrations of the cations ammonium, potassium, magnesium and calcium (*figures 5.11 – 5.14*). Like DOC, the increase was not sustained beyond 6 days. This adds weight to the theory that there may have been a stimulation of organic matter decomposition, a process which releases ions into the porewater solution (Mitsch & Gosselink, 2000; Charman, 2002). The increase in ion concentration may also be due to the effect of ozone on the *Sphagnum* vegetation. Peat and *Sphagnum* moss are known to possess a strong cation exchange capacity (CEC), where cations such as potassium and magnesium are taken up by the plants and replaced by H^+ ions. This is one of the reasons why decomposition within bogs is so low, as cations are required by the microorganisms that break down organic matter (Thomas & Pearce, 2004). It may have been that the highest ozone treatment induced a stress response in the *Sphagnum* vegetation which reduced its CEC, allowing for greater cation concentrations in the normally nutrient-poor peat porewater and therefore increased microbial organic matter decomposition. Niemi, *et al.* (2002) proposed a similar mechanism of CEC reduction in *Sphagnum* microcosms, but there is little additional evidence to back this up, and more intensive studies are needed to investigate this potential stress response.

It is widely known that ozone molecules can break down large, HMW DOC and its use in the removal of organic matter and as a disinfectant within the water industry has been discussed (Siddiqui, *et al.* 1997; Melin & Ødegaard, 2000; Galapete, *et al.* 2001; Fahmi, *et al.* 2003). Although this is a reaction that would have occurred in the peat water at the very surface of the cores, as reported by Niemi, *et al.* (2002), it would not be sufficient enough to explain the reduction in the HMW fraction that we observed for the bog cores under the highest ozone treatment, because of the inability of ozone to penetrate below the surface of soil.

Previous studies of ozone effects on *Sphagnum* moss are limited in both quantity and their investigation of below-ground functions. Studies by Potter, *et al.* (1996) and Rinnan & Holopainen (2004) suggest that *Sphagnum recurvum*, the species that dominated our bog microcosms, is particularly sensitive to ozone but it is not known how the changes in the above-ground vegetation functioning that they recorded would impact on carbon cycling below ground. The data presented here implies that there was a small but significant initial stimulation in the decomposition of organic matter within the soil, and the most likely explanation for this was an increase in microbial activity resulting from the significant rise in nutrient concentrations.

5.5 Conclusions

The results of this study suggest that exposure to levels of ozone predicted for the middle of this century had a dramatic impact on the quantity and quality of the organic carbon pool within the peatland soils investigated. The most serious impact was for the fen microcosms, where a remarkable reduction in the porewater DOC concentrations, but increase in molecular weight, was recorded for the three elevated ozone treatments. It is postulated that there may have been a double stress mechanism occurring in the fen microcosms that led to this change. Similar to findings from other studies (Yoshida, *et al.* 2001; Andersen, 2003), there may have been a decreased allocation of low molecular weight photosynthetically-derived carbon to the root system and ultimately the soil, lessening the DOC concentration. This reduction in the flux of LMW carbon would have reduced the microbial activity and hence enzyme production within the soil, impairing the rate of organic matter decomposition, further reducing the DOC concentration. This process is initiated through ozone-induced changes in the vegetation characteristics and morphology and previous studies have shown that typical fen vegetation is particularly sensitive to ozone (Power & Ashmore, 2002; Rinnan & Holopainen, 2004).

There was a much less dramatic impact on the soil carbon store of the bog microcosms. Although not significant, there was a net increase in DOC for the three elevated ozone treatments and a net decrease for the 'current background' over the 41 days of ozone exposure. After just six days, significant stimulations in DOC and four cation concentrations was observed for the highest ozone treatment. The most likely reason for this was that the overlying *Sphagnum recurvum* vegetation, previously shown to be particularly sensitive to ozone (Potter, *et al.* 1996; Rinnan & Holopainen, 2004), may have lost some of its ability to retain cations upon exposure to elevated ozone, releasing them into the nutrient-poor soil and stimulating microbial decomposition of the some of the HMW fraction of DOC that is prevalent in bog soils. More intensive studies are required to investigate such a possible stress response.

Taken together, the data suggest that fen-like ecosystems, which are dominated by higher more ozone-sensitive plants (Rinnan & Holopainen, 2004) are much more likely to exhibit below-ground alterations in their carbon cycling than more stable, less ozone-sensitive *Sphagnum* dominated bog ecosystems. Future increases in the concentration of tropospheric ozone are therefore likely to lead to a reduction in the DOC leaching from fen soils into freshwaters, as the sensitive vegetation responds by locking up more carbon within the plant itself. Predicting the effects of ozone on bog soils is much less obvious, although it is clear from this, and other studies, that ozone does not have the potential to unlock the vast stores of carbon housed within this soil type in the same way that elevated CO_2 might (Freeman, *et al.* 2004*b*).

It is important to consider that all the significant changes recorded in this study were for the microcosms exposed to an increased background concentration incorporating episodic pollution events. Since ozone precursors are transported long distances, including globally, and with concentrations being highest in remote areas away from local sources of other pollutants that can react with ozone to reduce concentrations, even some of the world's remotely located peatlands (especially bogs) occurring at high latitudes are potentially at risk if ozone concentrations continue to rise as predicted. Effects on lowland fen ecosystems, such as those described here, are also likely when these ecosystems are located downwind of conurbations and away from local pollution sources.

Chapter 6

The effects of solar radiation on the characteristics of dissolved organic carbon compounds in peatlands and freshwater lakes

6.1 Introduction

Ultraviolet light (UV) is a highly energised, invisible part of the solar radiation spectrum, which is often divided into four bands; vacuum UV (100-280nm), UV-C (200-280nm), UV-B (280-320nm) and UV-A (320-400nm). The protective ozone layer within the Earth's upper stratosphere filters out the vacuum UV, UV-C and the majority of the UV-B band, with longer wavelength UV-A able to pass through the atmosphere without being absorbed. Even though UV-B radiation comprises <1% of all the sun's energy reaching the Earth's surface, its short wavelength and high quantity of energy per photon make it particularly damaging to plant and animal life. It is thought that 3 billion years ago, the earliest forms of life on Earth were restricted to aquatic environments until a sufficiently thick layer of protective ozone built up to block out UV-B and allow evolution onto terrestrial environments, a process which took 200 million years (Schlesinger, 1991).

Ever since the discovery of a large hole in the protective layer of ozone within the Earth's stratosphere above Antarctica, concern has grown about rising levels of harmful ultraviolet light reaching the earth's surface (Molina & Rowland, 1974; Farham, *et al.* 1985). The cause has been directly linked to anthropogenic emissions of chlorofluorocarbons (CFCs), leading to a reaction which depletes the ozone layer (Crutzen, 1992). Since 1974, an average 0.5% per year reduction in the Earth's ozone layer has been recorded (Rozema, *et al.* 2005), which has lead to an approximate 5% increase in the global average UV-B radiation reaching ground level (Madronich, *et al.* 1998). Worryingly, the increase in UV-B radiation has been much greater at the mid-latitudes of the northern hemisphere (Stolarski, *et al.* 1992) and, in the United Kingdom, an 11-13% rise in ground level UV-B radiation was recorded between 1980 and 1997 (*figure 6.01*) (EEA, 2000). It has been predicted that by 2010-2020, relative to 1979-92 levels, there could be a 60-90% increase in springtime UV radiation reaching the Earth's surface at a latitude of 60°N (Taalas, *et al.* 2000).

The 1987 Montreal Protocol Treaty and its subsequent amendments have banned the use of CFC products in over 150 countries and the rate of ozone depletion is expected to eventually lessen. Although a full recovery of the ozone layer is expected by 2050-2060, there is little evidence of this yet (Weatherhead & Andersen, 2006), with the 2003 hole above the Antarctic being the second largest ever recorded (WMO, 2003). Since 1996, a hole in the ozone layer over the Arctic ice cap has also been detected every spring due to the greenhouse effect (Rex, *et al.* 2004).

The impacts of increasing levels of ultraviolet light (specifically UV-B) on the Earth's biota is a relatively new and rapidly expanding area of study and most experiments have been conducted on plant species. Plants typically react to UV-B stress by changing the chemical composition of their leaves (Rozema, *et al.* 1997), although responses are dependent upon variables such as species (Cybulski & Peterjohn, 1999) and season (Hunt & McNeil, 1999). Similar to ozone exposure responses, increasing the abundance of UV-B filtering phenolic and flavonoid compounds is a typical defence mechanism (Paul & Gwynn-Jones, 2003; Selas, 2006). It is also thought that UV-B can dramatically affect carbon allocation within the plant and alter the quantity and quality of root exudates, with repercussions for the soil microbial and fungal species composition and abundance (Johnson, *et al.* 2002; Avery, *et al.* 2003). Changes in soil microbial and fungal community diversity and respiration have also been detected under enhanced UV-B (Newsham, *et al.* 1997).

One aspect of research into solar radiation that has received a great amount of attention is its impacts on lake biogeochemistry and ecosystem functioning. Visible light (herein referred to as photosynthetically active radiation (PAR), 400-700nm), UV-A and UV-B radiation are all able to penetrate to some extent into the water column of lakes; a process largely dependent on the lake's DOC concentration and absorptivity (Morris, *et al.* 1995). Whilst the life-supporting properties of PAR are obvious, UV has a number of detrimental impacts. The shortwave UV-B band in particular has been shown to have negative impacts on a wide range of aquatic organisms, ranging from plankton to fish (Williamson, *et al.* 1996*a*) and the most sensitive can display symptoms of nucleic acid damage and inhibition of important physiological processes (Chatila, *et al.* 2001). Such impacts of UV-B have been reported to reduce numbers and survival rates of invertebrates (Kiffney, *et al.* 1997; McNamara & Hill, 1999) and rates of photosynthesis and growth of algal species (Watkins, *et al.* 2001; Xenopoulos, *et al.* 2000).

There are also a host of UV-induced effects on the biogeochemical properties of lakes and, ultimately, these changes can have a bearing on its entire ecosystem (Vinebrooke & Leavitt, 1998; Tank, *et al.* 2003). One of the principal ways in which UV affects lake properties is through the degradation of aqueous humic substances and other DOC compounds, a process which is an integral component of carbon cycling in freshwaters lakes (Morris & Hargreaves, 1997). DOC is a ubiquitous component of freshwater ecosystems (Thurman, 1985), and its ability to attenuate solar radiation within the upper portions of lakes helps to reduce the penetration of harmful UV-B radiation (Wetzel, 1975; Morris, *et al.* 1995; Williamson, 1995; Danilov & Ekelund, 2001; Brooks, *et al.* 2005). Even a small decrease in the DOC concentration of a lake can dramatically reduce the efficiency of UV attenuation (Williamson, *et al.* 1996*b*).

The absorption of UV light occurs at sensitive sites on organic molecules called chromophores (Zepp, 1988), which are mostly conjugated double bonds usually found on the aromatic rings and phenolic functional groups of humic substances (Thurman, 1985). Most carbon compounds in northern hemisphere freshwater lakes are terrestrial, and often peatland, in origin, with high aromaticity (Urban, *et al.* 1989; Malcolm, 1990; Dillon & Molot, 1997) and are consequently rich in these chromophoric structures (Vähätalo, *et al.* 1999). Phenolic compounds, for example, comprise a large proportion of the DOC content of peatland-dominated freshwater ecosystems (Wetzel, 1992) and Chen, *et al.* (1978) demonstrated the complete removal of phenolics after irradiating a sample of fulvic acid with UV. Conversely, autochthonous, photosynthetically-produced DOC, a generally less dominant fraction in northern hemisphere freshwaters, has a lower average molecular weight, contains fewer chromophores and is less photo-labile (Thomas & Lara, 1995; Bertilsson & Tranvik, 2000).

The absorption of solar radiation induces a photo-oxidation reaction which can completely degrade the organic matter into inorganic compounds, such as carbon monoxide, carbon dioxide and dissolved inorganic carbon (DIC), or partly degrade it into lower molecular weight/size UV-transparent organic molecules (Gjessing & Gjerdahl, 1970; Strome & Miller, 1978; de Haan & de Boor, 1991; Salonen & Vähätalo, 1994; Zepp, *et al.* 1995; Zepp, *et al.* 1998; Waiser & Robarts, 2004). Solar radiation is known to appreciably reduce the concentration of DOC in some natural systems (Stewart & Wetzel, 1981; Miller & Zepp, 1995), with one study recording a 40% loss of DOC in just one year in the upper 10m of a lake (Hoingé, *et al.* 1989). Photochemical DIC production has been shown to be within one order of magnitude of plankton respiration in the upper 2.5m of a series of lakes with a range of humic contents (Granéli, 1996). DIC production from a humic lake has been observed at levels several times greater than from a clear lake, indicating that photolysis of terrestrially-derived DOC is a significant source of DIC (Lindell, *et al.* 2000).

The photochemical degradation of DOC is often called 'photobleaching', due to the loss of colour following UV exposure. Most DOC compounds are reduced in molecular size and weight rather than being completely removed (Wetzel, *et al.* 1995). Due in part to its allochthonous source and high aromaticity, molecular weight and size, DOM resident in northern hemisphere temperate lakes is often unsuitable for use as a carbon source for microbes and algae. However, the lower molecular weight products formed by the photo-degradation of organic matter are usually significantly more biological active than the parent DOM from which they were formed (Granéli, *et al.* 1998). Wetzel, *et al.* (1995) observed rapid formation of LMW fatty acids, such as acetic and citric acid, upon exposure of plant leachate DOM to natural solar radiation. Radio-labelling of these products traced their fate to bacterial metabolism, with bacterial productivity increasing 35% in just 5 hours of exposure. Following 24 hours of exposure to simulated solar radiation, Brinkmann, *et al.* (2003*a*) recorded a rise in the percentage contribution of low molecular weight (LMW) organic products comprising the total DOC pool from 0.31% to 6.4%, dramatically increasing the amount of biologically available carbon substrates. A review of numerous studies showed that photo-bleaching of DOM increased bacterial activity 1.5- to 6-times greater than DOM not exposed to solar radiation (Moran & Zepp, 1997). It is possible that the photo-formation of LMW products can somewhat offset the direct negative impacts of harmful UV-B radiation on bacterial populations (Zepp, *et al.* 1998).

In addition to DIC and LMW photoproducts, the break-up of DOC compounds can release nutrients such as nitrogen and phosphorus which can become available for biological uptake. The release of ammonium by the action of UV-A and UV-B radiation is particularly pronounced, with production rates in the order of 0.1-0.4 $\mu M\ h^{-1}$ recorded for a boreal freshwater lake (Bushaw, *et al.* 1996). Nitrite release from DOM has also been observed (Kieber, *et al.* 1999). Photochemical phosphate release from DOM has been shown as an important source for bacterial growth in humic lakes (Vähätalo, *et al.* 2003). It is thought that the photochemical release of nitrogenous compounds and phosphate may be key processes in stimulating microbial activity in freshwaters.

Depending on the concentration and quality of DOC, the depth to which shortwave UV-B can reach in lake water can be just a few centimetres to 10 metres (Morris, *et al.* 1995). Considering that the average depth of lakes worldwide is less than 10 metres (Wetzel, 1990), harmful levels of UV-B can reach significant depths. It has been reported that the ability of UV-B to degrade DOC compounds is two orders of magnitude higher than UV-A, and three to four orders of magnitude more than PAR (Reche, *et al.* 2000). However, growing evidence suggests that PAR and UV-A are more important than previously thought in terms of photobleaching within the overall water column. Due to its longer wavelength and lower energy, UV-A can pass through the water column about 2.5 times further than UV-B, whilst PAR penetration can be 10 times as much (Williamson, *et al.* 1996*a*). So although UV-B radiation possesses much more energy per photon (Granéli, *et al.* 1998), and consequently has a higher photobleaching coefficient, its faster rate of attenuation in lake water means the degradative properties of UV-B are usually restricted to the surface layers (Vähätalo, *et al.* 2000). On the other hand, although longer wavelength UV-A and PAR are less efficient at degrading DOC, their ability to penetrate to greater depths within lake water allows for absorption by a larger number of carbon compounds, so they account for a high proportion of total lake DOC photobleaching.

Measurements of lake DOC characteristics over a period of a year or more have shown the dramatic seasonal effect of sunlight. The typical finding is of less aromatic, chromophoric, photo-reactive DOC in the water column during summer compared to winter months (Clair & Sayer, 1997; Lindell, *et al.* 2000). Morris & Hargreaves (1997) observed that the depth to which 10% of ground level solar radiation at a wavelength of 320 nm reached within the water column of a lake in the USA increased from 1.8 m in early spring to 7.5 m in midsummer, coinciding with the height of the summer solstice.

The continuous action of solar radiation in reducing the molecular weight of DOC compounds can have both beneficial and detrimental effects on the quality of drinking water reservoirs. The input of peatland-derived HMW, highly coloured DOC to freshwater lakes within the northern hemisphere temperate zone can create problems for drinking water treatment, but it is this type of DOC, rich in aromatic structures, that is highly responsive to solar radiation photolysis. Therefore the action of sunlight can serve to reduce the potential of lake water to form disinfection by-products.

Whilst this would ultimately improve the chemical quality of final drinking water, the photo-conversion of HMW to LMW carbon compounds, coupled with the potential release of nitrogen and phosphorus, would reduce the water's biological quality. DOM of lower average molecular weight is less easily removed by conventional water treatment processes such as coagulation and filtration (Alarcon-Herrera, *et al.* 1994;

Goslan, *et al.* 2002), therefore the selective photo-degradation of HMW DOC would result in final water containing a greater quantity of LMW material. Such compounds are known to be important carbon sources for undesirable microbes such as nitrifying bacteria that can flourish in water distribution pipes (Huck, 1990; Ribas, *et al.* 1997). The temperate latitudes of the northern hemisphere have seen the greatest rise in ground-level UV-B radiation over the past few decades and, if this rise continues as expected in the short-term, there is potential for increased rates of photobleaching and degradation of DOM in many freshwater ecosystems in the decades that lie ahead. The consequences of such an increase on biogeochemical aspects of freshwaters lakes are discussed by Zepp, *et al.* (1998) and the effects will have important consequences for freshwater carbon cycling.

In section 3.1, it was suggested that the action of sunlight reduced the total flux of DOC reaching Cefni lake from Cors Erddreiniog fen, as a much greater loss of DOC was observed in July compared to October. The purpose of this experiment was to test whether sunlight could be partly the cause of the 66% reduction in DOC that was recorded in July. With the Cefni known to experience algal blooms during the summer (Malthus & George, 1997; also section 3.2) the break-up of fen-derived DOC compounds by ultraviolet radiation could potentially be a significant source of inorganic nitrate and phosphate to the lake. This experiment also aimed to assess the contrasting reactions of the fen and bog DOC to UV, which are known to be structurally diverse (Brooks, 2005).

6.2 Materials and methods

6.2.1 Experimental design, site descriptions and sample collection procedures

Pilot experiment

A small pilot experiment was conducted at the beginning of the study to identify a suitable length of time for which the main experiment should run for. At Cors Erddreiniog fen (location described in chapter 3, p80), 900 ml of peat porewater (high DOC, high molecular weight) was collected by extracting a large cube of peat and squeezing out the porewater by hand into a bucket, which was covered in foil to prevent exposure to light. Once transported back to the laboratory, the sample was filtered through 0.45 μm 47 mm cellulose nitrate filters (Whatman, UK), to isolate the dissolved organic carbon, and stored at 4 °C. Fifty millilitres of sample was then decanted into 16 separate 60 ml Whirl-Pak ® polyethylene bags (M-Tech Diagnostics, Ltd., UK). Whirl-Pak bags have been used successfully in previous studies requiring a UV-transparent plastic (Smith, *et al.* 1992; Holm-Hansen & Helbing, 1993; Noble & Fuhrman, 1997). Eight of the 16 bags were used as controls and wrapped in foil to prevent exposure to solar radiation. The 16 bags were sealed and placed flat on the roof of the Memorial Building (UK National Grid Ref. SH 578 720, latitude 53° 15') for 2 weeks during June 2005, where sunlight was incident for up to 12 hours every day. When lying flat the height of the bags was just 5 cm, so it is assumed that all of the sample in the non-control bags would have been exposed to the full range of the solar radiation spectrum incident at ground level. The weather conditions during the period of exposure were mostly sunny with occasional cloud cover, so levels of UV light were medium to high. On days 0, 2, 4, 6, 8, 10, 12 and 14 one bag was removed from the control and exposed treatments and analysed for DOC concentration, UV absorbencies and HPSEC apparent molecular weight distribution. For details of these analytical procedures, please refer to chapter 2, pages 32-33.

Main experiment

Based on the results obtained at the end of the pilot experiment, the experiment was run for 14 days, beginning on 1st August. Conditions during these 2 weeks were mostly sunny and cloud-free and the sun was still high in the sky. Six different sites were chosen for sample collection based on their differing DOC characteristics and 'age' with respect to UV exposure. Cors Erddreiniog fen porewater was selected because it represents an important source of HMW fen DOC to Cefni lake (discussed in section 3.1), and it has not been exposed to sunlight. 'Afon Erddreiniog' drains the fen, hence its high DOC concentration (*figure 3.11*, p93). As a freshwater source of DOC, it is therefore relatively new, but will have been exposed to some solar radiation. By the time water draining from the fen reaches Cefni lake (inflow North), it has travelled approximately 5 miles, therefore any carbon present will have been exposed to much more solar radiation. The sample taken from Cefni lake was extracted from the surface, the site of most photochemistry within a lake (Granéli, *et al.* 1996), so the organic carbon compounds will be relatively 'old' with respect to solar radiation exposure. These four sampling sites represent the 'fen' DOC and are abbreviated herein as 'CEF' (Cors Erddreiniog fen), 'AE' (Afon Erddreiniog), 'CIN' (Cefni Inflow North) and 'CL' (Cefni lake). These sites can be seen on the map in *figure 3.01*, page 81, of section 3.1.

Two sites were also sampled at the Llugwy catchment to represent the 'bog' DOC; the peatland itself and the lake surface water (*figure 6.02*). It has been shown previously that the organic carbon characteristics within the Anglesey (fen) and Snowdonia (bog) sampling sites are quite different (Brooks, 2005), therefore it is expected that they will react differently upon exposure to sunlight.

Figure 6.02 - Llugwy bog and lake sampling sites, located within the Snowdonia mountain range

At the fen and bog peatlands, 2 litres of soil water was extracted as described previously (p197). For each of the four lake/stream water samples, 10 litres was decanted into large plastic containers. All samples were carefully transported back to the laboratory and filtered and stored as described previously.

6.2.2 Treatment regimes

There were four treatment regimes used during the experiment; dark (control), PAR (700-400nm), 'PAR+UVA' (700-320nm) and 'PAR+UVA+UVB' (700-280nm). The treatments consisted of bespoke plastic chambers covered in different types of filters. The control treatment was covered in three layers of black Neutral Density filter 211 (Lee Filters, UK), which excluded all radiation below 700nm. The 'PAR' treatment used a UV filter sheet (UV Filter 226, Lee Filters, UK) which is only able to transmit radiation above 400 nm. The 'PAR+UVA' treatment employed a Melinex layer of plastic sheeting (Melinex 400, PSG Group Ltd, UK), which filtered out all radiation below 320nm. The 'PAR+UVA+UVB' treatment was covered in a sheet of Plexiglas 2458 (Amari Plastics PLC) which can transmit all radiation above 280 nm. The transmission of the four types of plastic is shown in *figure 6.03*. The chambers (*figures 6.04 & 6.05*) were raised off the ground slightly to ensure a constant passage of air beneath them, which prevented heat build-up and maintained similar temperatures between the treatments. This was checked regularly using an Oregon Scientific RAR621 wireless thermometer, with a sensor under each chamber. Levels of UV-B radiation at the experimental site were monitored using an International Light IL1700 research radiometer equipped with a SED240/ACT3/W UV detector (Able Instruments & Controls, Ltd.). The bandwidth of the detector employed was 185-310 nm with a peak response at 274 nm. It could therefore not measure UV-A radiation so was only used to give rough estimates of ground-level UV light. PAR levels were measured using a Skye SKP200 (Skye Instruments Ltd.). During the main experiment, and on a cloudless day, ultraviolet and visible light were measured at midday at the experimental site in the open air and under all four chambers.

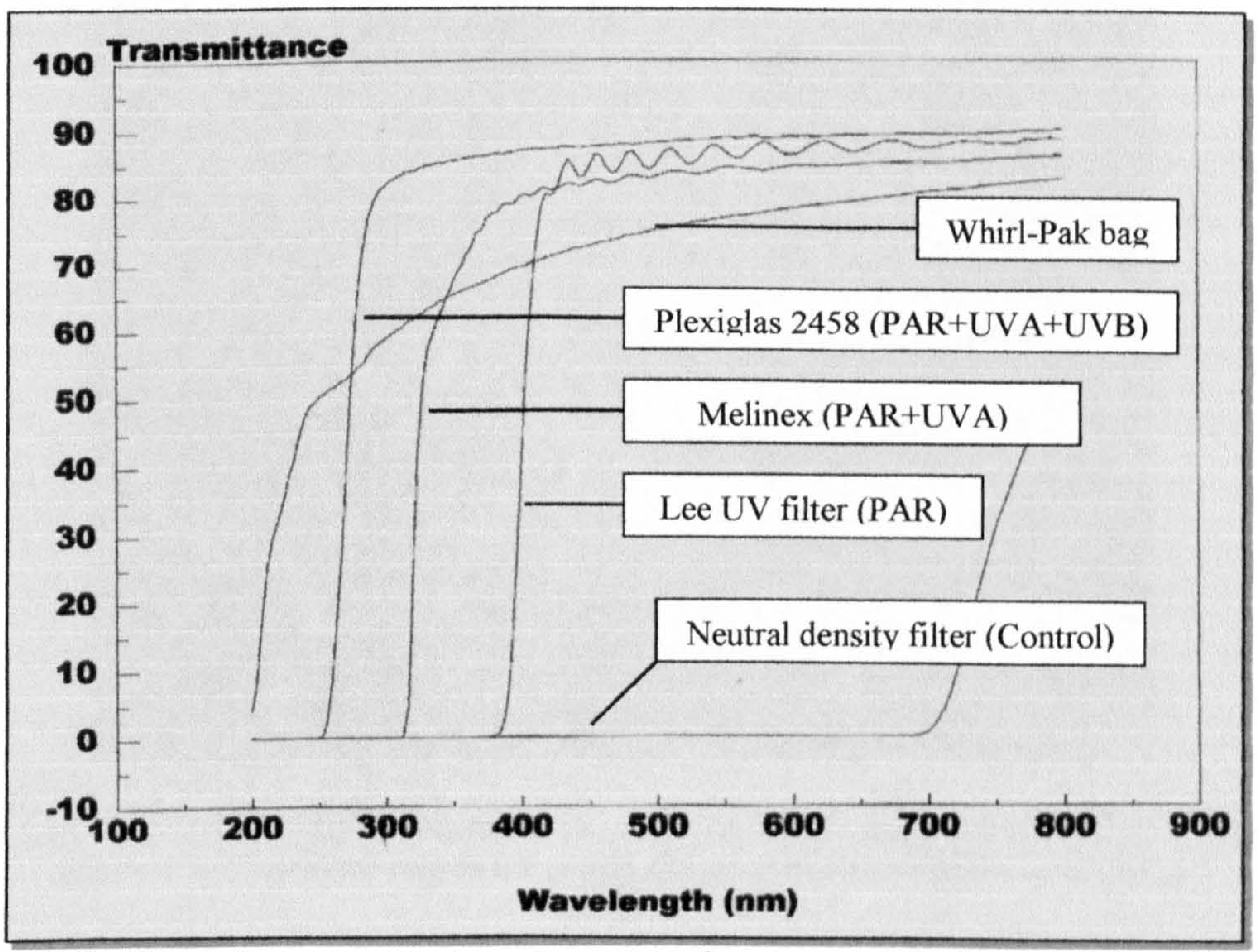


Figure 6.03 – Percentage transmission values of the four types of plastic material used for the different treatments, plus the Whirl-Pak plastic used for holding the samples.

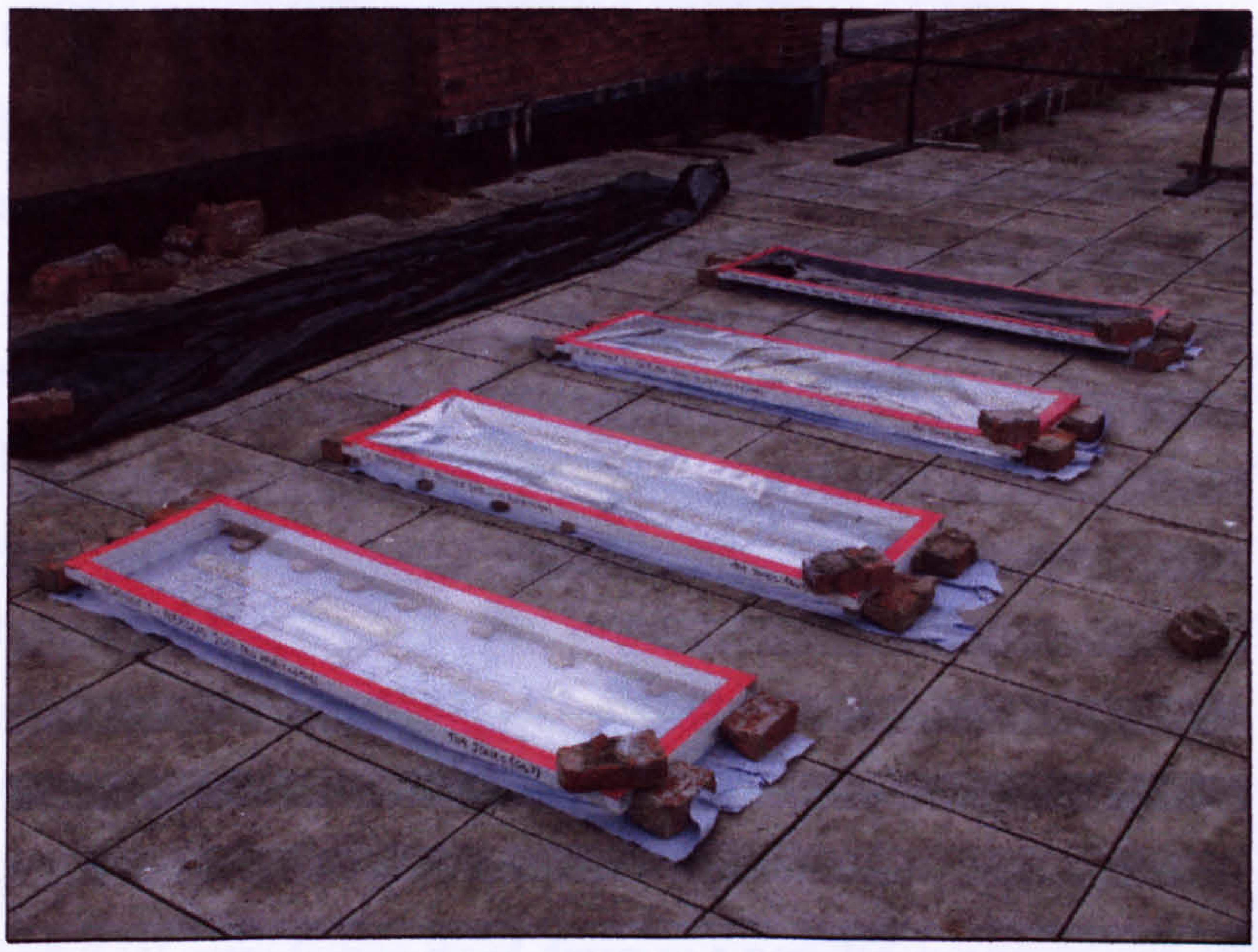


Figure 6.04 - The four treatment chambers, situated on the roof of the Memorial building

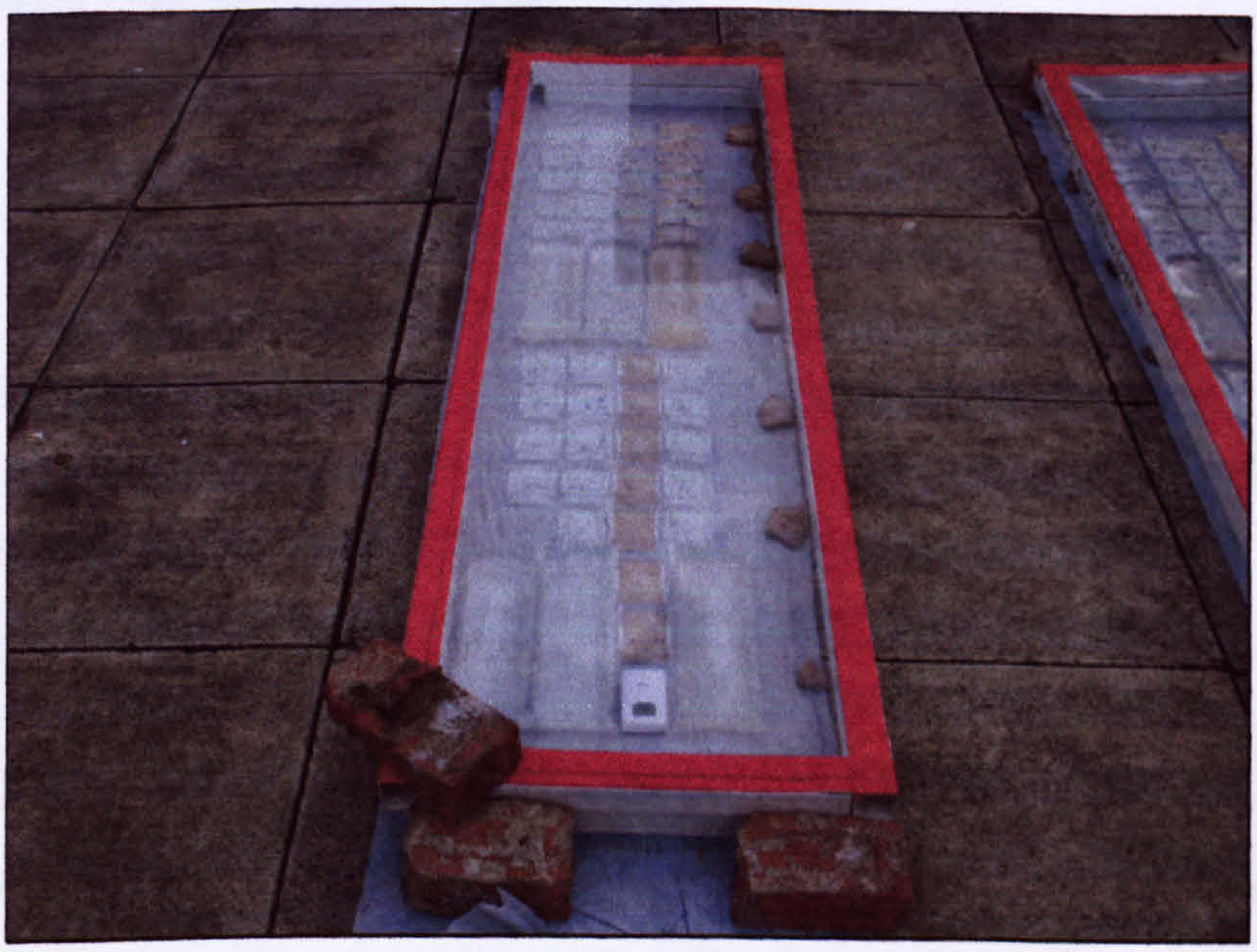


Figure 6.05 – 'PAR+UVA+UVB' treatment chamber, showing sample-filled Whirl-Pak bags and wireless thermometer sensor for continuous remote temperature monitoring

6.2.3 Exposure protocol

For each of the four treatments and six sample types, 5 Whirl-Pak bags were filled with 50 ml of sample, as described on page 197. In addition, extra bags for 'AE', 'CL' and 'LB' samples were filled so that ^{1}H-NMR analysis could be performed at the end of the experiment. This involved completely filling four 1 litre Whirl-Pak bags for the 2 stream samples, and eight extra 50 ml (4 x 100 ml) bags for the 'LB' sample. All of the bags, totalling 128 x 50 ml and 8 x 1 litre, were divided up between the four treatments and exposed to natural sunlight.

After 14 days of exposure, the bags were moved from the roof back to the laboratory, where they were decanted into 50 ml or 1 litre amber glass bottles and stored at 4 °C. Samples contained within the 50 ml bottles were tested for DOC concentration, UV absorbencies, HPSEC molecular weight and ion concentrations. The extra samples for 'AE', 'CL' and 'LB' were freeze-dried down to a powder, which was analysed using ^{1}H NMR spectroscopy. For details of all of these methods, please refer to chapters 2 and 3.

6.2.4 Graphical representation and statistical analyses

Main experiment

The graphs of DOC quantity and quality (*figures 6.10-6.13*) show the percentage change of the mean values (n=5) for each treatment compared to the control, whilst those of anion concentrations (*figures 6.17-6.19*) show the actual change, with error bars indicating ± standard error of the mean. One-way ANOVA and the Tukey-Kramer multiple comparisons post-hoc test was used for each analysis type to compare the mean values of each treatment. Significant differences between the three 'light' treatments and the control are indicated on each graph by * ($p<0.05$), ** ($p<0.01$) or *** ($p<0.001$). *Tables 6.03-6.06 & 6.09-6.11* display significant differences between the PAR and the two UV treatments for DOC characteristics and anion concentrations to indicate the individual effect of the UV-A and UV-B band. Numbers indicate the p values, whilst 'ns' signifies no significant difference was found. Statistical analyses were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, California, USA). It is acknowledged that the data gathered would have been suitable for testing for differences between sample types and wavelength treatments using two-way ANOVA.

6.3 Results

6.3.1 Pilot experiment

6.3.1.1 Solar UV and visible light measurements

Figures A09 & A10 of Appendix A4 show that UV and visible light levels at midday varied throughout the experiment and correlated positively, whilst ground-level UV correlated negatively with percentage cloud cover.

6.3.1.2 Dissolved organic carbon characteristics

Solar radiation progressively reduced the colour of Cors Erddreiniog porewater in the exposed bags with increasing exposure time (*figure 6.06*).

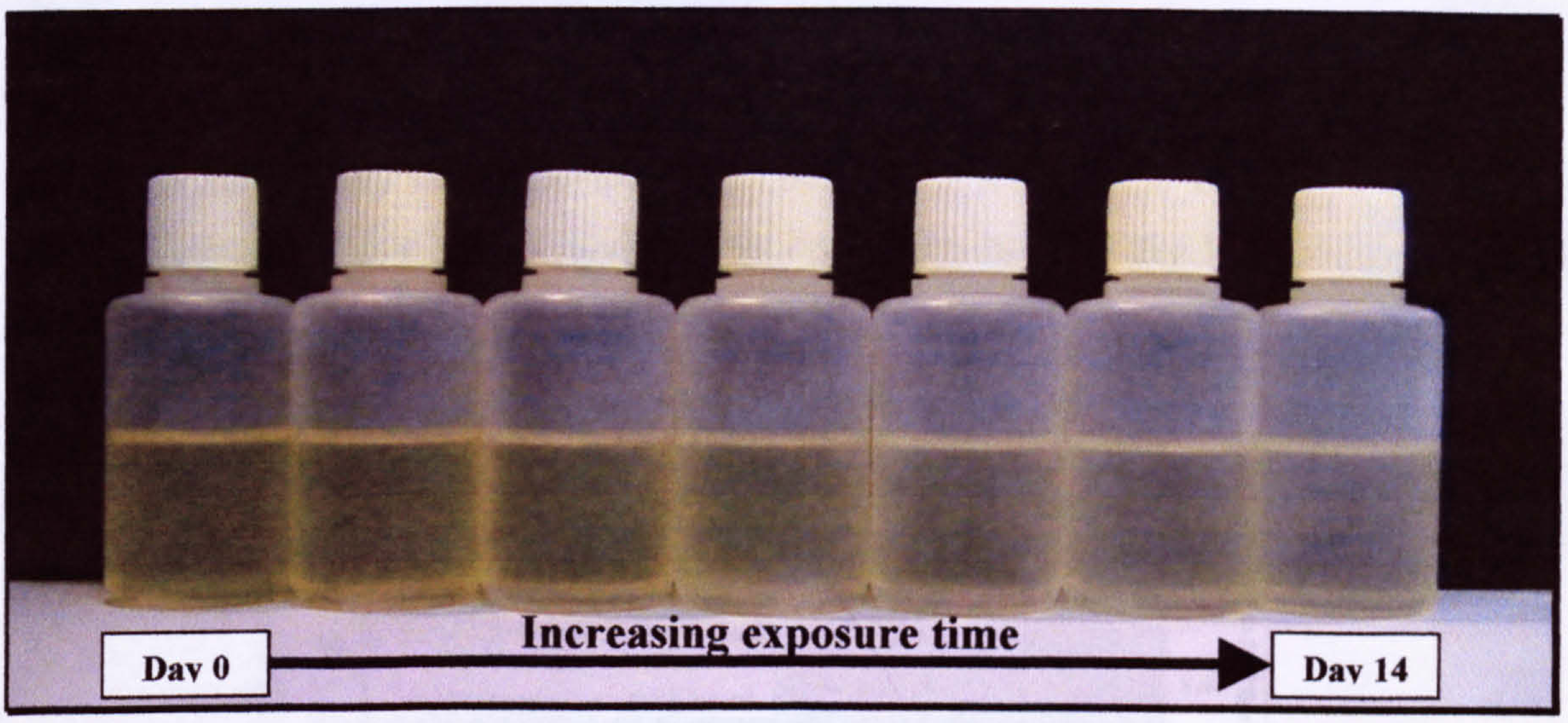


Figure 6.06 - Soil porewater solutions from Cors Erddreiniog fen exposed to solar radiation from 0 to 14 days.

Figures 6.07 and 6.08 show the main results obtained during this pilot experiment. For the exposed bags, DOC values decreased from 52.1 mg l^{-1} on day 0 to 35.0 mg l^{-1} on day 14, equating to a 32.8% loss. DOC concentrations in the control bags dropped just 5.4%. Values of UV-254 absorbance for the exposed samples fell from 1.64 to 0.84 after 14 days, translating to a 48.8% loss. For the control samples, the value increased by 2.2%.

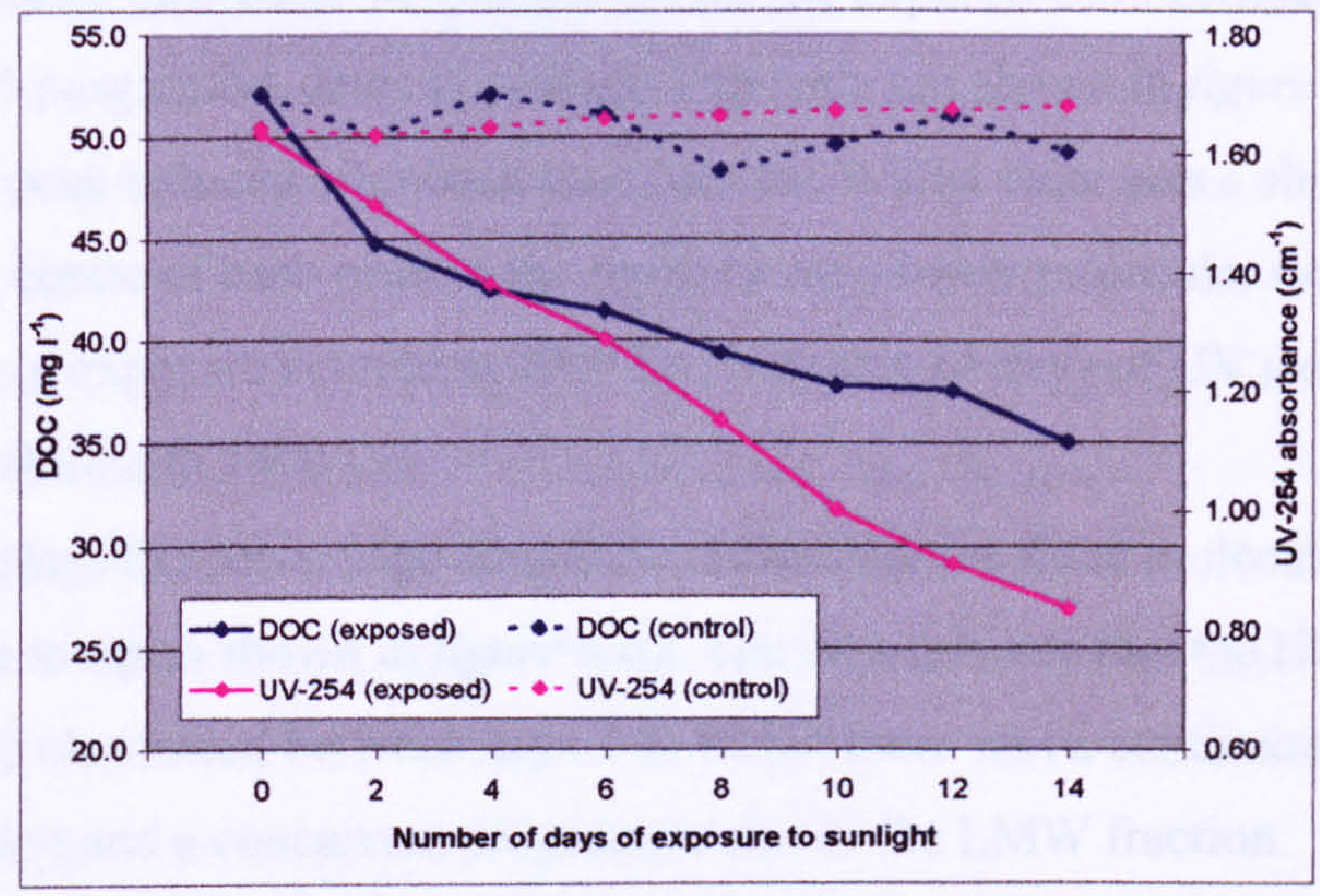


Figure 6.07 - DOC concentration and UV-254 values for samples of Cors Erddreiniog fen porewater covered and exposed to sunlight for a period of 14 days

Values of E2:E3, inversely proportional to molecular weight, increased continuously throughout the 14 days for the exposed samples. The ratio rose from 5.5 to 10.9, an increase of 98.3%, but increased by only 1.3% for the control samples. Despite a small rise after day 2, SUVA values, proportional to aromaticity, for the exposed samples decreased consistently with exposure time, falling from 3.14 to 2.39 L(mg m) (-23.8%). For the control samples, values increased by 8.0% from day 0 to day 14.

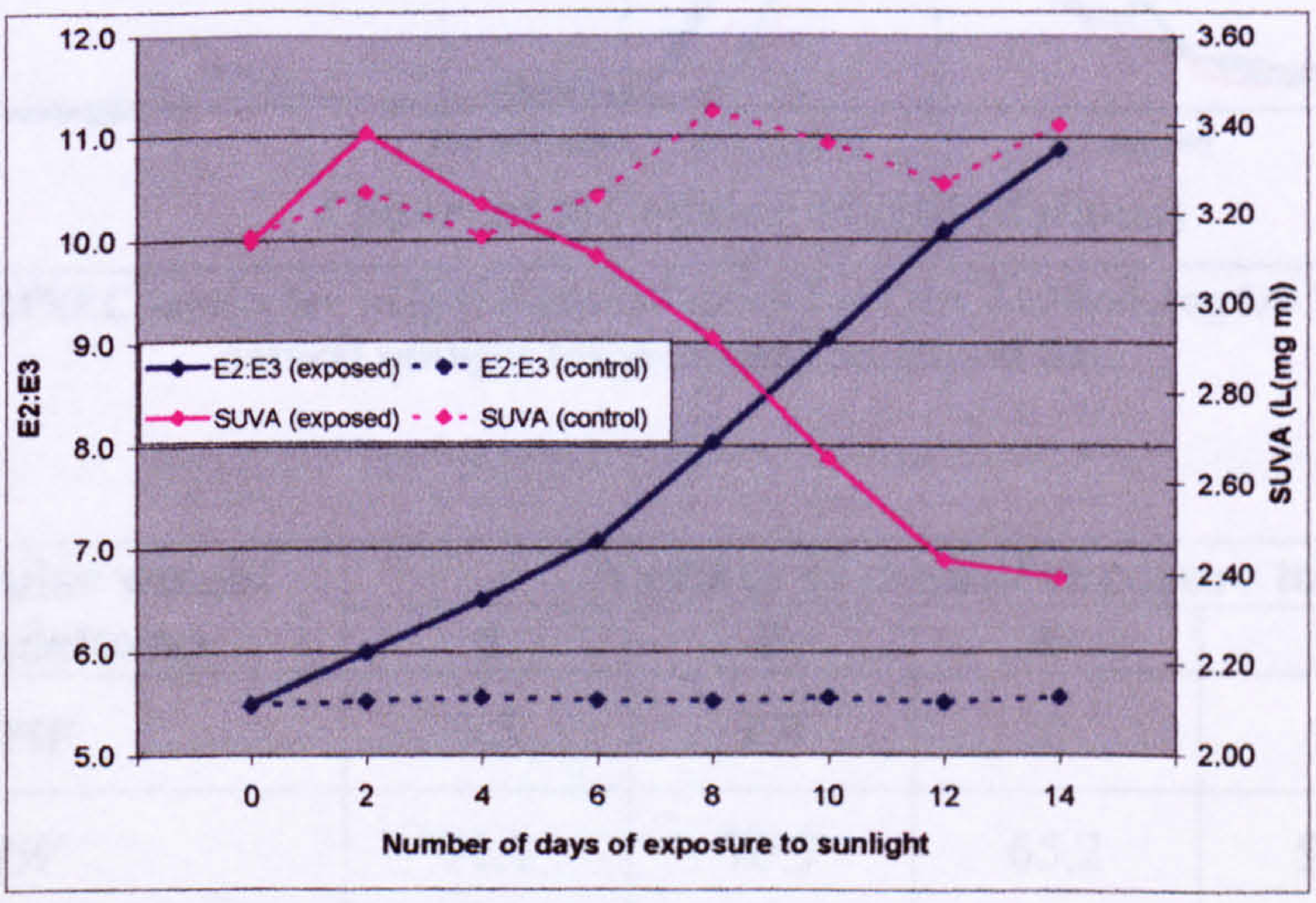


Figure 6.08 - E2:E3 and SUVA values for samples of Cors Erddreiniog fen porewater covered and exposed to sunlight for a period of 14 days

HPSEC determined molecular weight spectra for the exposed Cors Erddreiniog fen porewater for 5 progressive dates of sunlight exposure are shown in *figure 6.09*. The height of each peak reduced after each time interval, whilst there was a shift in the position of the centre of each peak to the right, i.e. to a lower molecular weight. The peak for day 0 of exposure centred at 2000 Da, but after 14 days of UV sunlight exposure, had shifted to 1400 Da.

Table 6.01 displays the percentage abundance values for the three molecular weight fractions of the samples shown in *figure 6.09*. The data indicate that the HMW fraction was completely eliminated between days 2-6, whilst there was a continuous decrease in the IMW fraction and a concurrent progressive rise in the LMW fraction.

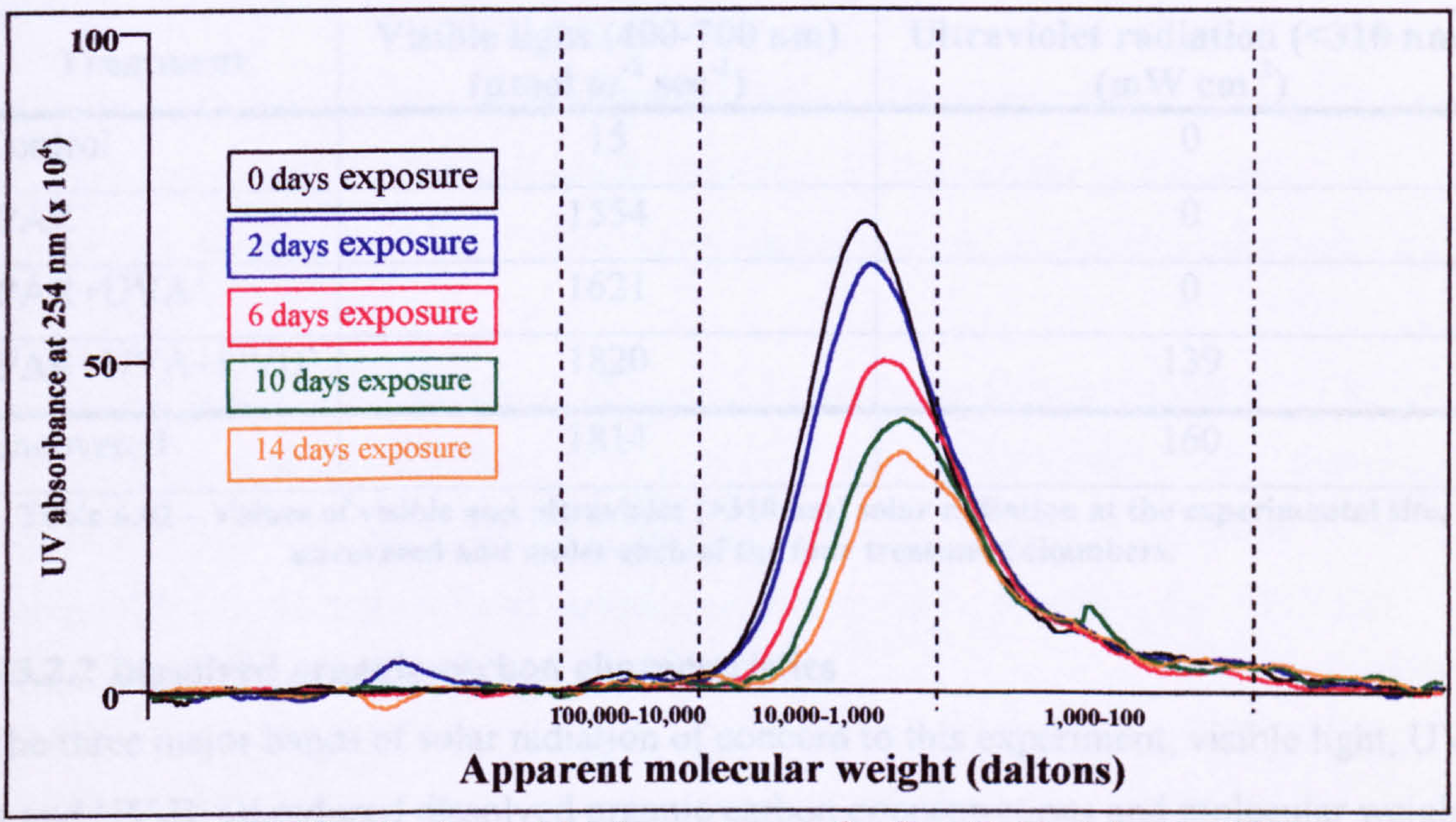


Figure 6.09 – HPSEC molecular weight chromatogram for Cors Erddreiniog fen DOC exposed to natural sunlight for increasing lengths of time

DOC molecular weight fraction (daltons)	Number of days of exposure to sunlight				
	0	2	6	10	14
HMW	3.3	1.4	0	0	0
IMW	71.1	70.5	65.2	59.0	53.8
LMW	25.6	28.1	34.8	41.0	46.2

Table 6.01 – Percentage abundance of the three molecular weight fractions for Cors Erddreiniog fen DOC exposed to natural sunlight for increasing lengths of time

6.3.2 Main experiment

6.3.2.1 Solar UV and visible light measurements

Measurements of incident solar radiation in the open air and under all four treatments are shown in *table 6.02.* Ultraviolet radiation greater than 310 nm was only detected in the 'PAR+UVA+UVB' treatment, at a level 87% of that compared to outside the chamber. Visible light (PAR) transmission was 100%, 89% and 86% for the 'PAR+UVA+UVB', 'PAR+UVA' and 'PAR' treatments respectively. Levels of visible light recorded under the control treatment were negligible, just 0.8% of that recorded outside the chamber.

Treatment	Visible light (400-700 nm) (μmol m^{-2} sec^{-1})	Ultraviolet radiation (<310 nm) (mW cm^{-2})
Control	15	0
'PAR'	1554	0
'PAR+UVA'	1621	0
'PAR+UVA+UVB'	1820	139
Uncovered	1814	160

Table 6.02 – Values of visible and ultraviolet (>310 nm) solar radiation at the experimental site, uncovered and under each of the four treatment chambers.

6.3.2.2 Dissolved organic carbon characteristics

The three major bands of solar radiation of concern to this experiment, visible light, UV-A and UV-B, all reduced dissolved organic carbon concentrations and molecular weight to a varying degree.

Figure 6.10 shows the percentage reduction in DOC concentrations for the three 'light' treatments compared to the control. Visible light (PAR) significantly reduced the DOC concentration of 'AE' and 'CL' (p<0.05), but it was the combined 'PAR+ UVA' treatment that had the greatest effect, significantly reducing the amount of DOC in all but the 'CIN' sample (p<0.05-0.001) (e.g. 41.9 to 33.2 mg l^{-1} for LB). Statistical analysis (*table 6.03*) shows that the UV-A only band significantly reduced DOC for the two peatland samples (p<0.001), whilst the UV-B band only had an impact on the Llugwy bog DOC concentration (p<0.01). Comparison of the fen stream sample types shows that the greatest loss of DOC upon exposure to solar radiation was 'AE' (-30%), followed by 'CIN' (-21%) and 'CL' (-10%). Sunlight exposure caused a much smaller loss of DOC for the fen peatland DOC (-13%) than the bog peatland DOC (-26%), whilst 'LL' was hardly affected (-6%).

Values of the percentage change in UV adsorption at 254 nm are shown in *figure 6.11*. PAR significantly reduced the UV-254 adsorption for all samples apart from Llugwy lake (p<0.001), with the greatest change occurring for 'AE' (-17%) and the lowest for Cefni lake (-4%). It was the UV-A band that had the greatest effect, with *table 6.04* showing that the effect was significant for all samples (p<0.001). The lowest response was for Cefni lake (-23%), with the highest responses recorded for 'AE', 'CIN' and 'LL' samples (-45 to -47%). The UV-B band reduced total adsorption values slightly more for all samples, although the individual affect of this band was much less than that of UV-A. Although UV-B significantly reduced UV-254 adsorption for the 4 stream/lake samples (p<0.001), there was no additional response for Llugwy bog (p>0.05).

The total loss of adsorption at UV-254 was 2-5 times greater than the loss of DOC for all samples. For example, PAR, UV-A and UV-B induced losses of UV-254 absorbance 27%, 103% and 78% greater than that of DOC for 'AE'. The effect was more pronounced for the two peatland samples, especially for PAR (299% for 'CEF' and 454% for 'LB').

Percentage changes in the E2:E3 ratio are shown in *figure 6.12* for each of the six sampling sites. PAR significantly increased the ratio for 'AE' (+48%), 'CIN' (+53%), 'CL' (+9%) and 'LB' (+9%) samples (p<0.05-0.001), and in combination with UVA for all samples. The greatest increase for the "PAR+UVA" treatment was for 'AE' and 'CIN' (+124%), whilst the smallest was for 'LB' (+16%). UV-A alone increased the E2:E3 ratio significantly for all but the two peatland sites, whilst UV-B alone had little additional effect. Solar radiation had much more of an effect on the fen peatland DOC than the bog, increasing the ratio by 56% compared to 12% for the bog.

Concentrations of dissolved inorganic carbon (DIC) are shown in *figure 6.13* and show marked differences in the impacts of solar radiation between the fen and bog sites. PAR significantly reduced the DIC concentration of the 'CEF' (53.0 to 43.3 mg l^{-1}) and 'AE' samples (49.6 to 40.6 mg l^{-1}) ($p<0.001$) by about 25% but had no effect on 'CIN' and 'CL'. The UV-A and UV-B treatments had little additional effect, although the UV-B band did reduce DIC concentrations slightly more and significantly for the 'CEF' and 'CIN' samples ($p<0.05$). The trend for the bog was opposite, with PAR in particular dramatically and significantly increasing the DIC concentrations by up to 50% for both 'LB' (0.78 to 1.15 mg l^{-1}) and 'LL' (0.88 to 1.32 mg l^{-1}) ($p<0.001$). UV-A increased the amount of DIC for 'LB' a further 35% (+0.29 mg l^{-1}) ($p<0.01$), although there was no effect of UV-B radiation on either of the bog samples.

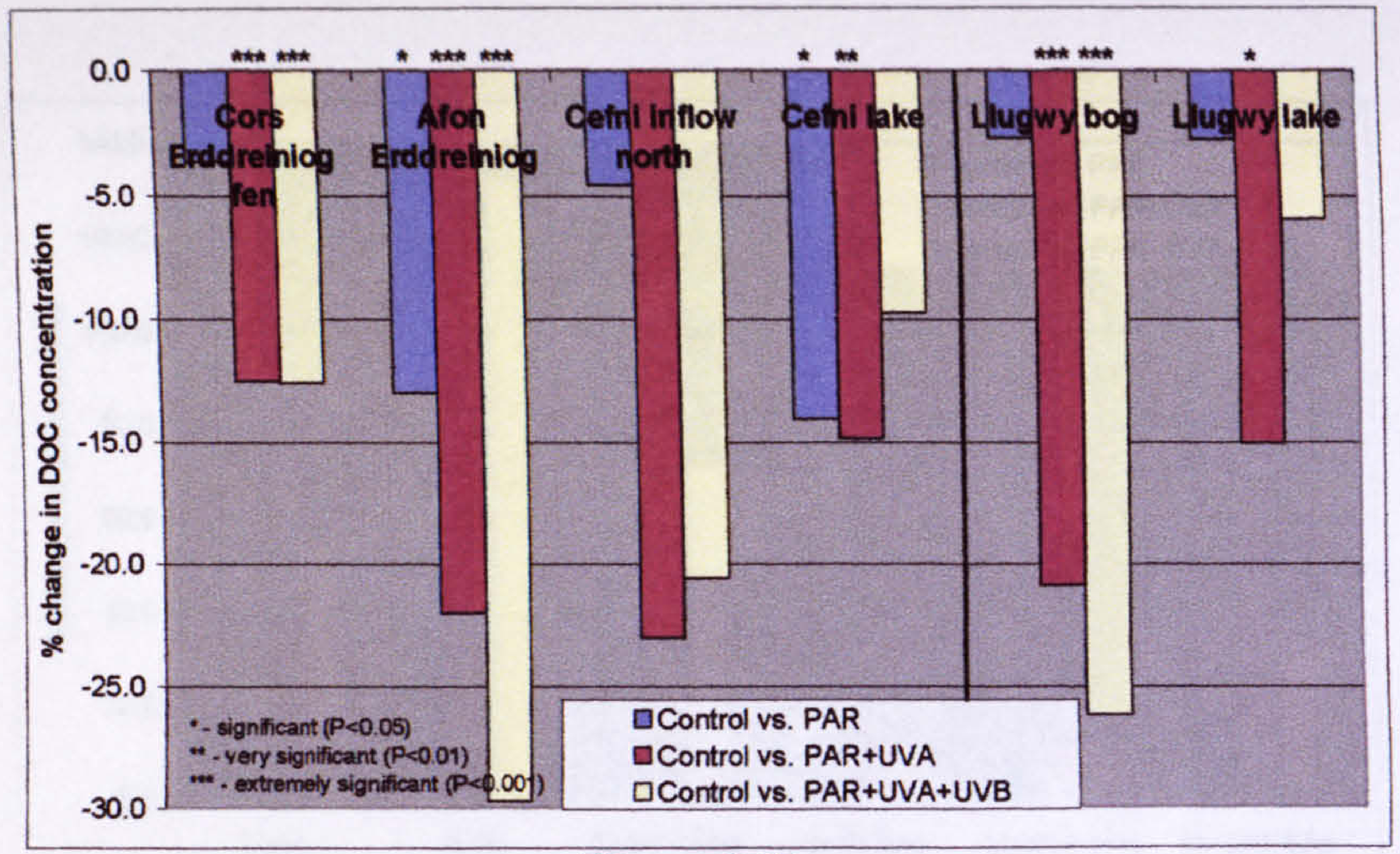


Figure 6.10

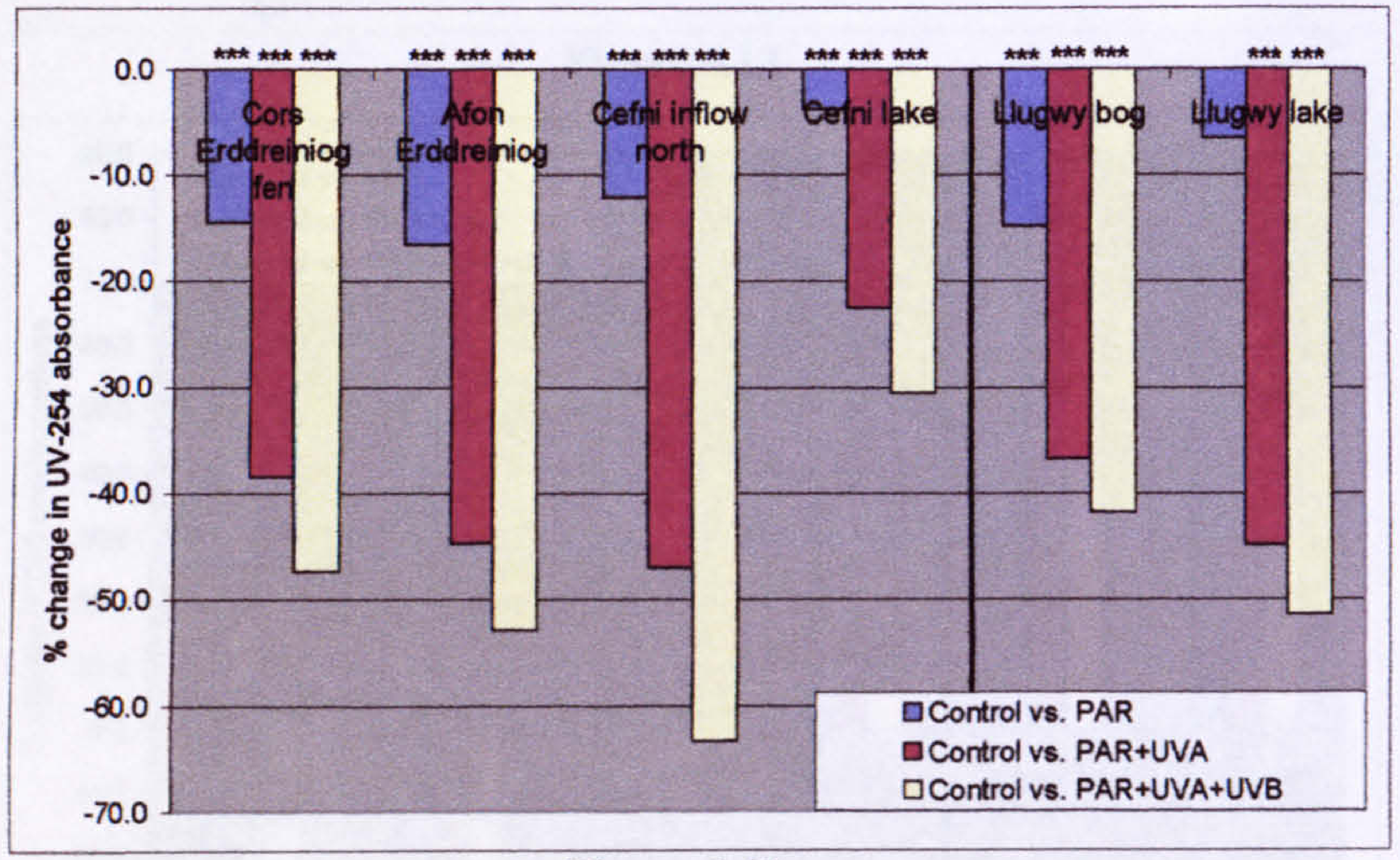


Figure 6.11

Figures 6.10 & 6.11 - Percentage changes in DOC concentrations &UV-254 absorbance values of samples exposed to solar radiation

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Cors E fen	<0.001	ns
Cors E stream	ns	ns
Cefni inflow North	ns	ns
Cefni lake	ns	ns
Llugwy bog	<0.001	<0.01
Llugwy lake	ns	ns

Table 6.03

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Cors E fen	<0.001	<0.01
Cors E stream	<0.001	<0.001
Cefni inflow North	<0.001	<0.001
Cefni lake	<0.001	<0.001
Llugwy bog	<0.001	<0.01
Llugwy lake	<0.001	ns

Table 6.04

Tables 6.03 & 6.04 – One-way ANOVA comparison of the changes in DOC concentrations and UV-254 absorbance values of samples exposed to UV radiation. Column 1 - UV-A only, column 2 - UV-B only.

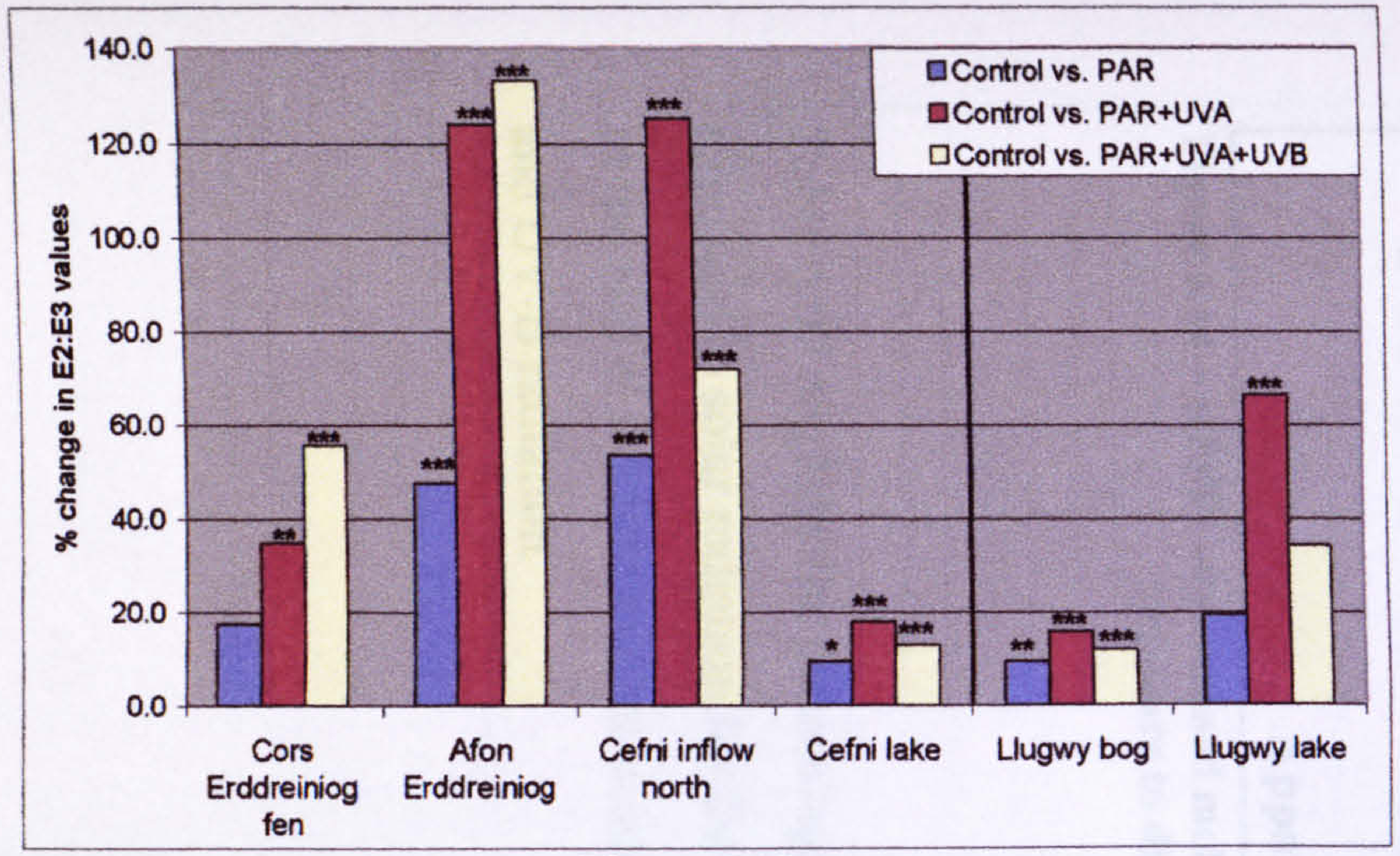


Figure 6.12

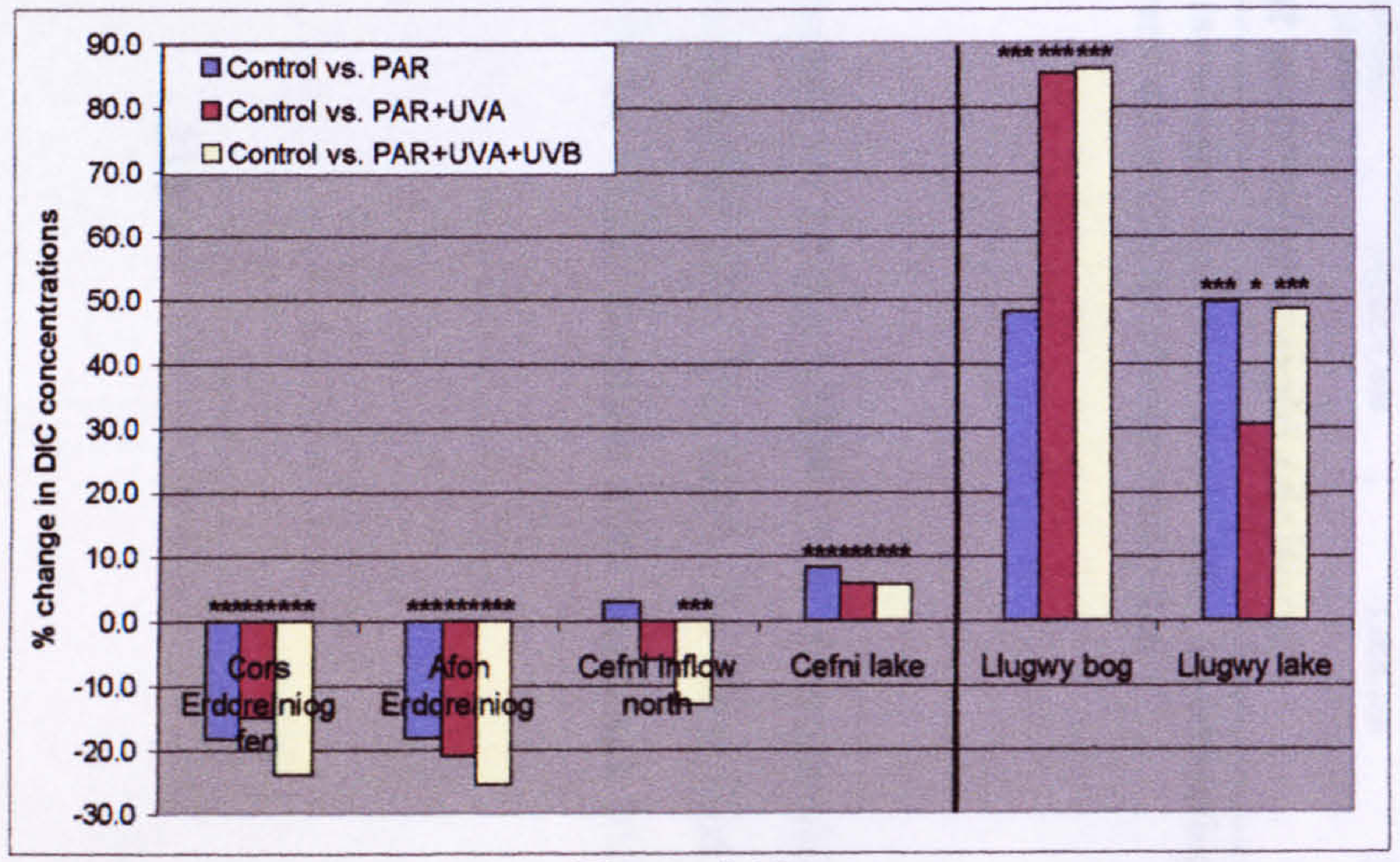


Figure 6.13

Figures 6.12 & 6.13 - Percentage changes in E2:E3 values and DIC concentrations of samples exposed to solar radiation

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Cors E fen	ns	ns
Cors E stream	<0.001	ns
Cefni inflow North	<0.001	<0.001
Cefni lake	<0.05	ns
Llugwy bog	ns	ns
Llugwy lake	<0.01	ns

Table 6.05

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Cors E fen	ns	<0.05
Cors E stream	ns	ns
Cefni inflow North	<0.01	<0.05
Cefni lake	<0.01	ns
Llugwy bog	<0.01	ns
Llugwy lake	ns	ns

Table 6.06

Tables 6.05 & 6.06 – One-way ANOVA comparison of the changes in E2:E3 values and DIC concentrations of samples exposed to UV radiation. Column 1 – UV-A only, column 2 – UV-B only.

The molecular weight distribution of the DOC was assessed for all samples and, as an example, a chromatogram is shown below of changes in the molecular weight distribution of DOC from 'Afon Erddreiniog' (*figure 6.14*). The trend is typical of that recorded for all six sample types, and signifies that exposure to a greater portion of the solar radiation spectrum decreased the DOC concentration, as indicated by the smaller peak heights, and reduced the molecular weight, as shown by the slight shift to the right of the centre of each peak.

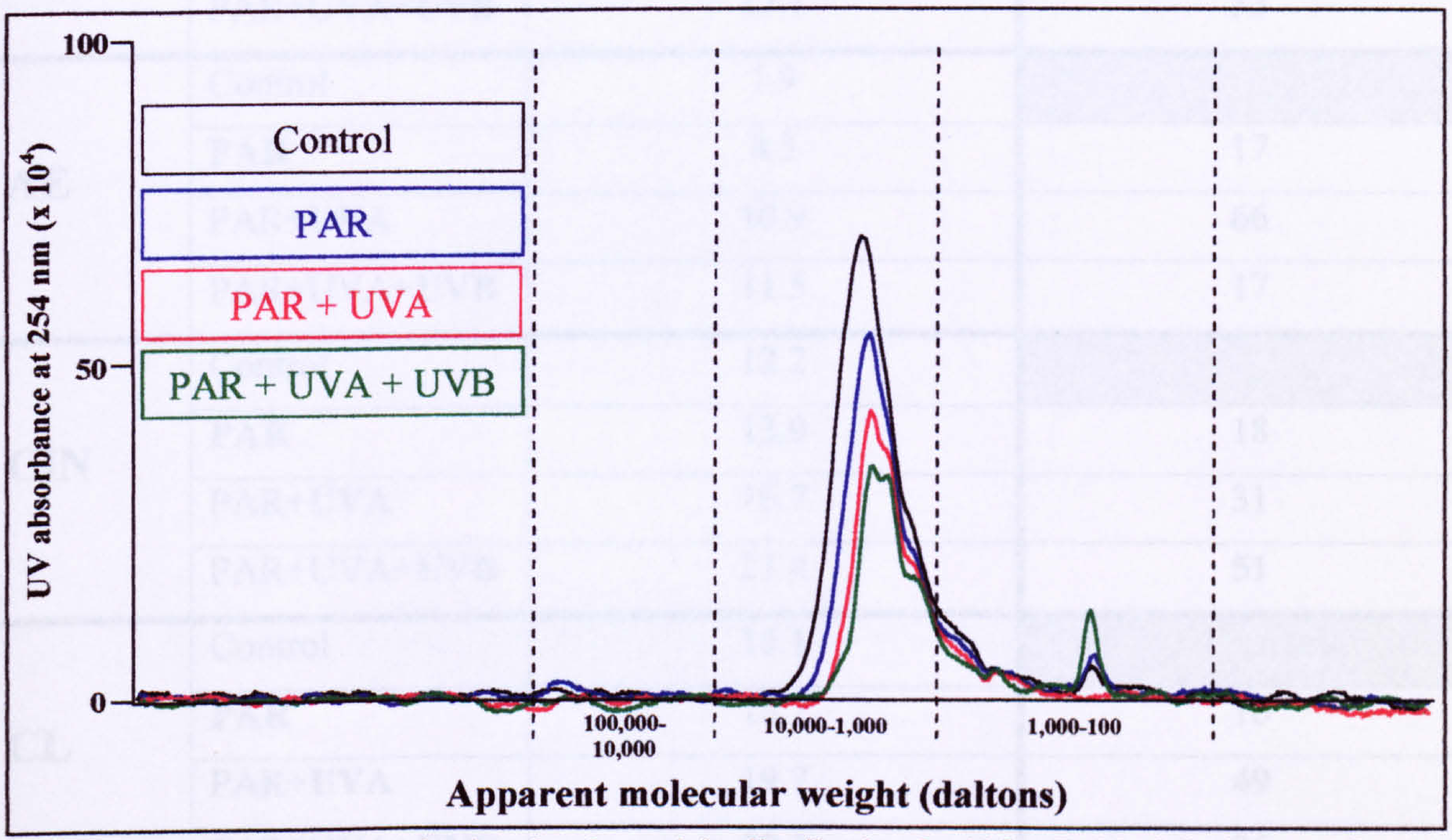


Figure 6.14 – HPSEC-determined molecular weight distribution for 'Afon Erddreiniog' water after exposure to different wavelengths of solar radiation

Table 6.07 displays the percentage abundance of the **LMW** DOC fraction following exposure to solar radiation for the five sampled sites. For each site there was a decrease in the HMW fraction and an increase in the LMW fraction after exposure to PAR, UV-A and UV-B radiation.

Sample site	Treatment	Actual percentage abundance of LMW compounds (100-1,000 Daltons)	Percentage contribution of PAR, UVA or UVB to the total increase in LMW abundance
CEF	Control	9.5	
	PAR	11.5	26
	PAR+**UVA**	14.6	**41**
	PAR+UVA+**UVB**	17.1	33
AE	Control	7.9	
	PAR	8.5	17
	PAR+**UVA**	10.9	**66**
	PAR+UVA+**UVB**	11.5	17
CIN	Control	12.2	
	PAR	13.9	18
	PAR+**UVA**	16.7	31
	PAR+UVA+**UVB**	21.4	**51**
CL	Control	15.1	
	PAR	16.2	16
	PAR+**UVA**	19.7	**49**
	PAR+UVA+**UVB**	22.2	35
LB	Control	6.5	
	PAR	10.1	**60**
	PAR+**UVA**	12.2	35
	PAR+UVA+**UVB**	12.5	5

Table 6.07 – Percentage abundance of the LMW DOC fraction following exposure of five different samples to varying wavelengths of solar radiation.

6.3.2.3 ^{1}H-NMR spectra of DOC

Figures 6.15-6.16 display the ^{1}H-NMR spectra of 'Afon Erddreiniog' DOC incubated under the control and 'PAR+UVA+UVB' treatments. Spectra for 'CL' and 'LB' are not shown, but showed similar trends to those of 'AE'.

The most obvious alteration to the composition of the DOC caused by sunlight exposure evident from the spectra is a reduction in the size of the broad aromatic region (region IV), highlighted by the red box. The reduction in the size of this aromatic region was accompanied by an increased number of small, sharp, well-defined peaks.

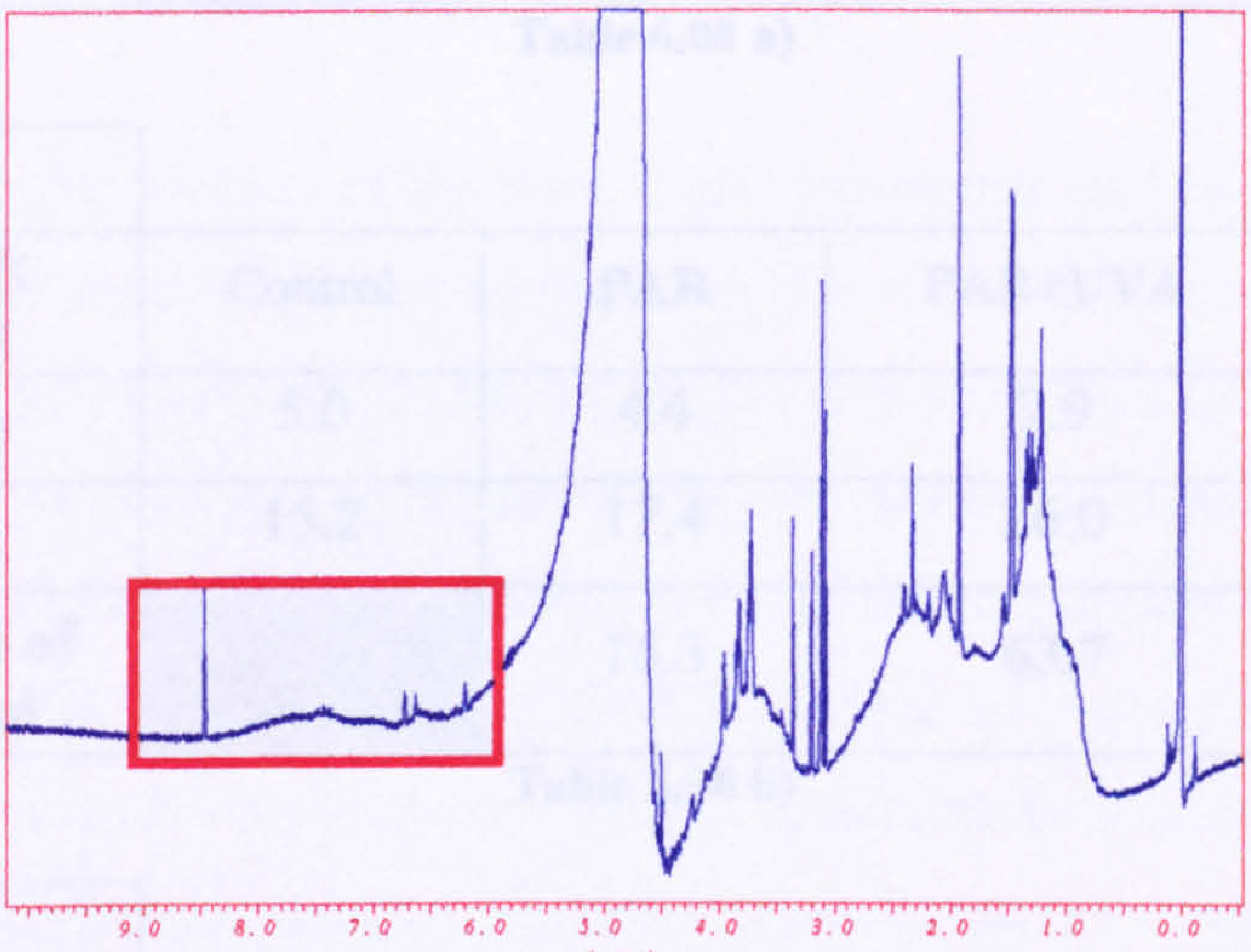


Figure 6.15 - ^{1}H-NMR spectra of 'Afon Erddreiniog' DOC (control treatment)

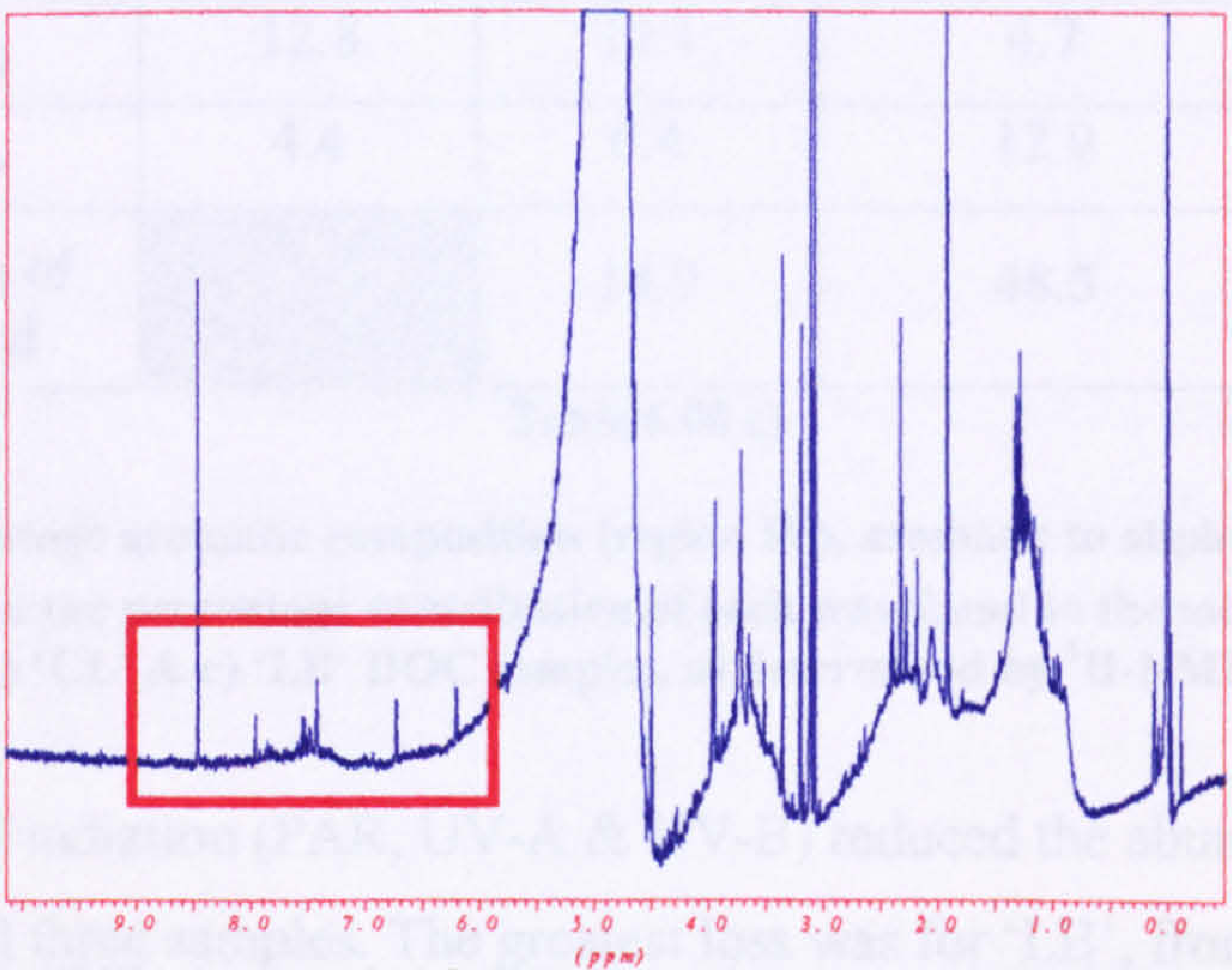


Figure 6.16 - ^{1}H-NMR spectra of 'Afon Erddreiniog' DOC ('PAR+UVA+UVB' treatment)

Tables 6.08 a-c, below, show the change in the composition of the DOC for 'AE', 'CL' & 'LB' samples exposed to solar radiation under the four different treatments.

AE				
Chemical shift region (ppm)	Control	**PAR**	PAR+**UVA**	PAR+UVA+**UVB**
IV (6.0 – 8.5)	6.3	6.1	5.0	4.1
P_{Al}/P_{Ar} ratio	12.0	12.6	15.8	18.9
% contribution of each waveband		8.7	**46.4**	44.9

Table 6.08 a)

CL				
Chemical shift region (ppm)	Control	**PAR**	PAR+**UVA**	PAR+UVA+**UVB**
IV (6.0 – 8.5)	5.0	4.4	2.9	2.7
P_{Al}/P_{Ar} ratio	15.2	17.4	26.0	28.7
% contribution of each waveband		16.3	**63.7**	20

Table 6.08 b)

LB				
Chemical shift region (ppm)	Control	**PAR**	PAR+**UVA**	PAR+UVA+**UVB**
IV (6.0 – 8.5)	12.8	10.1	4.7	2.8
P_{Al}/P_{Ar} ratio	4.4	6.4	12.9	17.8
% contribution of each waveband		14.9	**48.5**	36.6

Table 6.08 c)

Tables 6.08 - Percentage aromatic composition (region IV), aromatic to aliphatic carbon compound ratios (P_{Al}/P_{Ar}) and the percentage contribution of each waveband to the total increase in P_{Al}/P_{Ar} of a) 'AE', b) 'CL' & c) 'LB' DOC samples, as determined by ^{1}H-NMR spectroscopy.

All three bands of radiation (PAR, UV-A & UV-B) reduced the abundance of aromatic compounds for all three samples. The greatest loss was for 'LB', from 12.8% for the control to 2.8% for the 'PAR+UVA+UVB' treatment. The progressive increase in the

P_{Al}/P_{Ar} ratio for each sample indicates a conversion of aromatic to aliphatic compounds. Again, the most dramatic change occurred for the 'LB' sample.

Out of the three wavelength bands, UV-A appeared to have the greatest effect on the samples, contributing 46%, 64% and 49% to the total increase in the P_{Al}/P_{Ar} for 'AE', 'CL' and 'LB' respectively. UV-B had the next greatest contribution, averaging approximately 30%, with PAR having the least overall effect (average 12%).

6.2.2.4 Hydrochemical characteristics

Exposure of the samples to UV radiation led to some notable impacts on dissolved anion concentrations.

Figure 6.17 shows the impacts of the three 'light' treatments on Llugwy bog and lake phosphate concentrations. Exposure to PAR and UV-A had no effect, but UV-B increased phosphate concentrations from 0 for the control to 0.11-0.13 mg l^{-1}. No impact of solar radiation exposure on phosphate was found for the fen samples, with concentrations below the limit of detection recorded for each treatment.

Figure 6.18 shows the change in bromide concentrations of the two bog samples. The concentration of bromide was below the limit of detection for the control and PAR treatments for both the 'LB' and 'LL' samples. Exposure to UV-A increased the concentration of bromide up to 0.06 mg l^{-1} for the 'LB' sample, with no additional effect of UV-B radiation. UV-A had no effect on the 'LL' sample, but UV-B increased the bromide concentration to 0.01 mg l^{-1}. Like phosphate, there was no detectable increase for the fen samples.

Figure 6.19 displays the sulphate concentration of the four fen samples. No effects were observed on the three freshwater samples, but there was a significant effect observed for 'CEF'. PAR significantly increased the sulphate concentration by 1.9 mg l^{-1}, whilst exposure to UV-B increased the sulphate concentration by 19.1 mg l^{-1}, from 42.9 to 62.1 mg l^{-1}, a large rise of 45%.

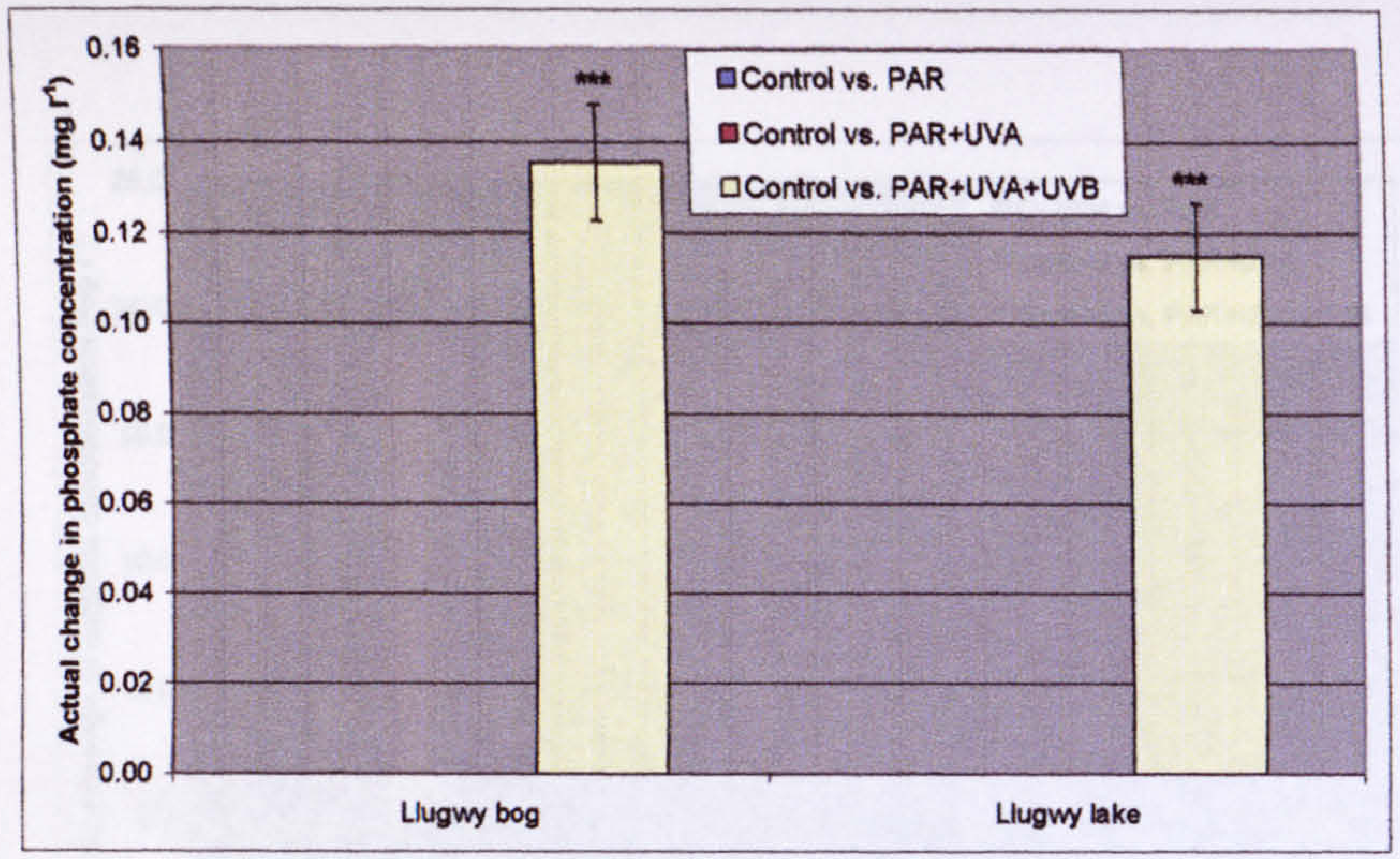


Figure 6.17

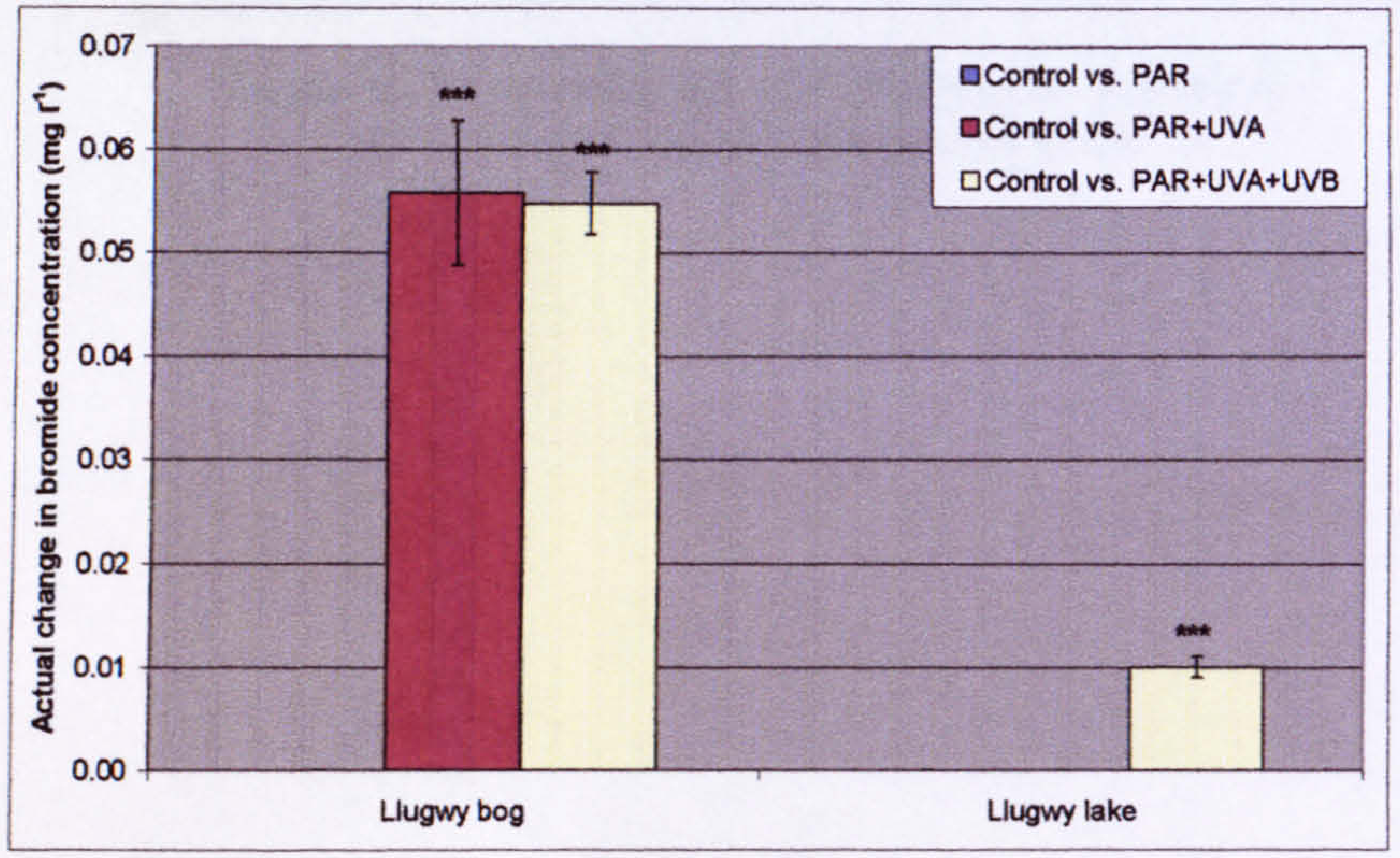


Figure 6.18

Figure 6.17 & 6.18 - Actual changes in phosphate & bromide concentrations of bog samples exposed to solar radiation

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Llugwy bog	ns	<0.001
Llugwy lake	ns	<0.001

Table 6.09

	'PAR' vs. 'PAR+UVA'	'PAR+UVA' vs. 'PAR+UVA+UVB'
Llugwy bog	<0.001	ns
Llugwy lake	ns	<0.001

Table 6.10

Table 6.09 & 6.10 – One-way ANOVA comparison of the changes in phosphate & bromide concentrations of bog samples exposed to UV radiation. Column 1 - UV-A only, column 2 - UV-B only.

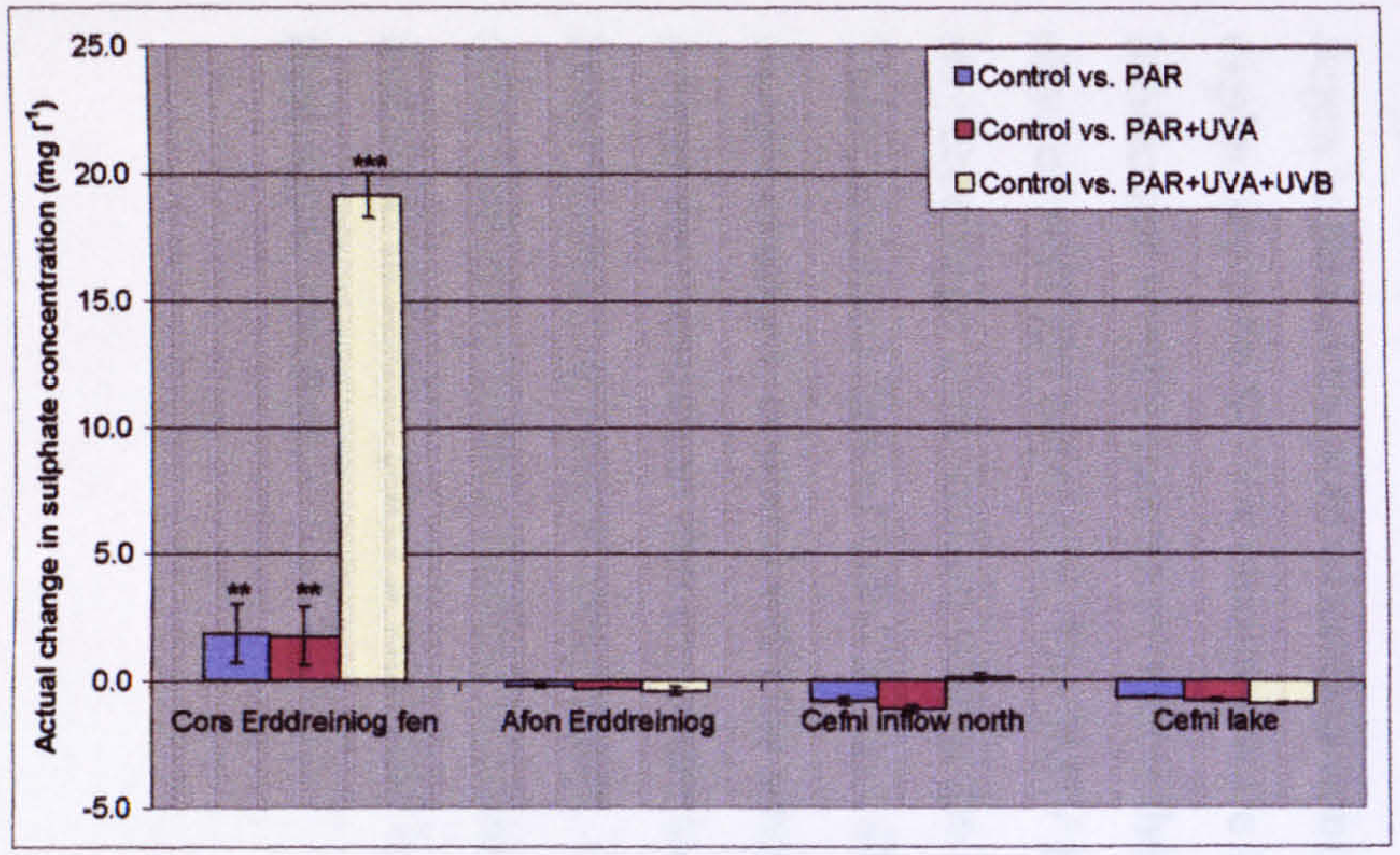


Figure 6.19 - Actual changes in sulphate concentrations of fen samples exposed to solar radiation

	‘PAR’ vs. ‘PAR+UVA’	‘PAR+UVA’ vs. ‘PAR+UVA+UVB’
Cors E fen	ns	<0.001
Cors E stream	ns	ns
Cefni inflow North	ns	ns
Cefni lake	ns	ns

Table 6.11 – One-way ANOVA comparison of the changes in sulphate concentrations of fen samples exposed to UV radiation. Column 1 - UV-A only, column 2 - UV-B only

6.4 Discussion

6.4.1 Pilot experiment

Continuous exposure of Cors Erddreiniog fen porewater to solar radiation reduced the value of UV-254 absorption to a greater extent than the total loss of DOC. Such a finding is common amongst the literature of similar studies, and can be explained by the conversion of high molecular weight, aromatic humic compounds, whose spectral absorption reaches a maximum at around 254nm (Korshin, *et al.* 1997*a*), to lower molecular weight, UV-transparent organic compounds. For example, Allard, *et al.* (1994) recorded a 50% reduction in the DOC concentration of a lake water fulvic acid after 12 hours, but a 95% decrease in UV-254.

The 98% rise in the E2:E3 ratio after 14 days of exposure indicates a dramatic reduction in the molecular weight of the DOC compounds. Increases of a similar magnitude are common in the literature; De Hann (1993), for example, measured a 66% increase in the E2:E3 value of lake water exposed to natural sunlight for 6 days, a similar rate of increase as measured in this pilot study. SUVA values for the DOC compounds did not decrease to the same magnitude as the increase in the E2:E3 ratio; this may have been because SUVA incorporates values of total carbon, which includes low molecular weight compounds that do not absorb at 250 and 365 nm. Solar radiation would not degrade this extra pool of carbon compounds that only SUVA can detect, possibly explaining why SUVA changes were lower than those for E2:E3.

Molecular weight spectra revealed the HMW fraction was entirely degraded after 2-6 days of sunlight exposure. The IMW fraction was also reduced by solar radiation, with its percentage contribution within the DOC pool falling from 71% to 54% after 14 days. This combined data of UV-254 and SUVA losses and E2:E3 and HPSEC molecular weight changes, coupled with the smaller magnitude loss of total DOC concentration, is a clear demonstration that exposure to solar radiation selectively degrades higher into lower molecular weight compounds, which then have a much reduced ability to absorb solar radiation. Such a finding is commonplace amongst the literature and emphasises that solar radiation plays a vital role in the cycling of allochthonous carbon inputs to freshwater systems.

6.4.2 Main experiment

Measurements of visible and ultraviolet light passing through the four different types of filters (*table 6.02*) revealed their efficiency in blocking out the required wavelengths of solar radiation. The three 'light' treatments were able to transmit visible light efficiently (>86%). Only a negligible level of visible light was able to pass through the neutral density control filter, equating to a 0.8% transmission rate. Plexiglas 2458 plastic used in the 'PAR+UVA+UVB' treatment transmitted approximately 85-90% of radiation in the UV-B range. This corresponded very closely to the actual level of UV-B recorded within this treatment under natural conditions, which was 87% of the level measured outside the chamber

Figures 6.10-6.15 show that solar radiation had a dramatic impact on all the analyses of dissolved organic carbon characteristics performed during this experiment. In terms of the DOC concentration, solar radiation had the greatest effect on the 'AE' sample, with a 30% reduction observed. It is not surprising that out of the four stream/lake waters studied 'AE' exhibited the greatest rate of photo-degradation; the stream drains from 'CEF' and it's DOC will be relatively 'new' with respect to sunlight exposure. DOC losses from 'CIN' and 'CL' were approximately 21% and 10% respectively, demonstrating their progressively older solar exposure 'age'. Stream water reaching 'CIN' from 'AE' has travelled several miles, during which time some photo-degradation will have occurred, explaining the smaller DOC loss observed. The lake-water extracted from the surface of the Cefni will have been resident in the lake for a relatively long period of time; hence its DOC would have received much more solar degradation. This finding confirms that discussed in section 3.1, that the action of UV light serves to reduce the concentration of DOC draining from Cors Erddreiniog fen into Cefni lake during the summer.

Comparing the two peatland samples, DOC loss from the bog was twice as high as that from the fen. The reason for this may be that 'LB' DOC possesses a much lower overall molecular weight; it's E2:E3 value being 3.55, compared to 6.42 for 'CEF'. Of the two peatland samples, it is therefore likely to contain the highest composition of chromophoric structures. The sample exhibiting the smallest loss of DOC was 'LL', with just a 6% reduction in concentration recorded. This may be due to the fact that, like at the Cefni, the sample was extracted at the surface during the summer, therefore it will have already been exposed to solar radiation.

The losses of DOC reported are within the typical range observed for studies such as these. For example, Molot & Dillon (1997) measured a mean DOC loss of 32% following an 11 day natural sunlight exposure of stream water draining extensive peatlands but a much lower reduction in DOC that was extracted from the lake that they flowed into (similar to 'AE' vs. 'CL' in this study).

Analysis of concentration changes alone would be insufficient for assessing the reactivity of carbon compounds to sunlight, as a large proportion are converted to smaller compounds with different characteristics and degrees of photochemical and biological lability. Absorbance at 254 nm (UV-254) is an analytical technique that selectively detects the more coloured, aromatic components of the DOC (Korshin, *et al.* 1997*a*). It is not surprising that for all 6 samples, rates of absorption loss were greater than total DOC losses, typically by about 200-300% (*figure 6.11*). As for DOC, the smallest loss of absorbance was for the 'older' sample from 'CL', with the 'younger' 'AE' and 'CIN' samples reacting greatest. It is interesting to observe that when comparing the two peatland samples, the loss of absorbance was slightly higher for 'CEF' than 'LB', despite the latter demonstrating twice the DOC loss. Indeed, the loss of absorbance was only 60% higher than total DOC loss for 'LB', but 276% higher for 'CEF'. This suggests that despite both samples being highly coloured porewater from anaerobic peatland systems, their DOC composition is different, a finding also reported by Brooks, (2005). There was generally a greater difference between the losses of DOC absorbance and concentration for the two peatland samples than the four freshwater samples. This is to be expected and is testimony to the darker colour and higher proportion of UV-absorbing chromophores that is typical of high molecular weight soil carbon compounds.

The change in the E2:E3 ratio of the 6 samples offers an insight into the impact of solar radiation on the molecular weight of the DOC compounds. Similarly to DOC, the greatest increase (therefore decrease in molecular weight) was observed for 'AE' (+133%), with lower values for 'CIN' (+72%) and 'CL' (+13%). This again highlights the progressively older age of these three samples, with the freshly leached DOC found in 'AE' containing more UV absorbing structures not yet degraded by sunlight exposure. It is surprising that there was very little change in the E2:E3 value for 'LB' (+12%), however, it is almost identical to the +10% change in the ratio that was observed by Osburn, *et al.* (2001) following exposure of a highly coloured bog porewater to sunlight for 7 days. For the fen samples, the E2:E3 increase for 'CEF' was less than half of that for 'AE', suggesting that, despite being highly coloured samples which have never been exposed to sunlight, the two peatland porewaters are not as responsive to sunlight as the DOC that leaches from them into freshwater streams and rivers. The E2:E3 value for Llugwy lake DOC decreased by more than twice that of 'CL'. This can probably be attributed to the proximity of the peatland DOC source to each lake. Unlike 'CL', 'LL' is surrounded by steeply sloping mountain sides with thick, bog soils and there is a continuous leaching of carbon directly into the reservoir. 'LL' therefore receives peatland-sourced carbon that has not already been exposed to sunlight, whereas DOC leaching from 'CEF' via 'AE' to 'CL' has, as previously stated, already undergone a certain degree of photo-degradation.

The HPSEC determined apparent molecular weight data clearly demonstrate the ability of solar radiation to selectively degrade high molecular weight carbon compounds. This is shown visually in *figure 6.14* by the greater reduction in the left-hand side of the main peak. Similar HPSEC trends have been published in the literature, for example, Brinkmann, *et al.* (2003*b*) published almost identically shaped DOC curves to those in *figure 6.14* after artificially irradiating bog lake DOM. Allard, *et al.* (1994) demonstrated the progressive disappearance of the broad peak of an isolated fulvic acid and the emergence of a sharper peak at a later retention time following 28 hours of artificial sunlight exposure, whilst Frimmel (1998) observed a similar response for lake water DOM using an HPLC system comparable to that used here. In this study, the percentage values of the LMW fraction under the control treatment again demonstrate both the natural sunlight exposure 'age' of the freshwater fen samples, with progressively higher percentages in order from 'AE' < 'CIN' < 'CL', and the observation that the bog peatland DOC is of higher average molecular weight than the fen DOC, due to its lower LMW value. All of the 5 samples in this study contained DOC which was mostly in the IMW fraction (10,000-100,000 Da) and although it still dominated following exposure to solar radiation, there was a much greater proportion of LMW material. Comparison of the two peatland samples confirms the results obtained by the E2:E3 ratio of a greater reduction in molecular weight for 'CEF', with the LMW abundance increasing 7.6% for the 'PAR+UVA+UVB' treatment compared to the control, and a 6% increase for 'LB'.

One major drawback of the results obtained using our HPLC system is that the UV detector cannot identify LMW as efficiently as IMW and HMW compounds; this has been discussed in chapter 5, page 182 and by Her, *et al.* (2003). The use of a UV detector is especially restrictive in this experiment because the samples have, of course, been degraded by ultraviolet radiation and some of these will have become undetectable by UV absorbance. Ideally, an HPLC system employing a DOC detector would be used for this study and the advantage of this over UV-254 detection in studies of solar radiation was discussed by Brinkmann, *et al.* (2003*b*).

The three samples whose DOC was analysed by ^{1}H-NMR spectroscopy revealed the presence of large, broad, indefinable peaks that are typical for DOM. Following exposure to sunlight there was a reduction in the percentage composition of aromatic compounds, i.e. those that absorb solar radiation more efficiently, for all three samples. The loss of aromaticity generally resulted in an increased proportion of aliphatic compounds and suggests that solar radiation can induce the break-up of benzene rings and double bonds into open chain carbon compounds; a degradation process highlighted in other studies (Waiser & Robarts, 2000 & 2004; Brinkmann, *et al.* 2003*b*). As pictured by the increased number of small, sharp peaks between 6.0-8.5 ppm in *figure 6.16*, the break-up of the broad, featureless aromatic region also resulted in the formation of smaller, specific compounds that, to some extent, still retained their aromatic structure. Unsurprisingly, the greatest reduction in aromaticity and a threefold increase in the P_{Al}/P_{Ar} ratio was observed for LB, which had a much higher aromatic composition to begin with.

Taken together, the data for DOC, UV-254, E2:E3, HPSEC molecular weight and ^{1}H-NMR spectroscopy demonstrate the ability of sunlight to degrade organic matter, especially those compounds of higher molecular weight. The most surprising finding of the experiment was that losses of DOC concentration and absorbance characteristics were generally lower for the two peatland porewaters compared to the freshwaters which they drain into ('AE' & 'LL'). In addition, out of the two peatland samples there was generally less of a reduction in DOC characteristics, especially the E2:E3 ratio, for the bog porewater. This is despite the higher initial DOC concentration, colour, aromaticity, UV absorbance and molecular weight of the porewaters, especially from the bog, and therefore the greater abundance of chromophoric, light-absorbing functional groups. Although these characteristics would be expected to make carbon compounds more photo-labile, it may be that their aromaticity and molecular weight and size were high enough to ensure they were partially recalcitrant to ultraviolet radiation. Such reasoning has been implied in previous studies; for example Allard, *et al.* (1994) recorded a 5-fold slower rate of reduction in the DOC concentration and UV absorbance of a humic acid compared to a fulvic acid during ultraviolet irradiation and attributed it to the humic acid's greater proportion of aromatic structures and therefore it's higher stability. Another reason may be that due to the high initial DOC concentration of the peatland samples (25.6 mg l^{-1} for 'CEF', 41.9 mg l^{-1} for 'LB') there may have been high attenuation and dissipation of the UV-B band, resulting in a lower percentage of the carbon compounds being exposed to the higher energy UV-B in the porewater than the freshwater samples (Wetzel, *et al.* 1995). Another reason may be due to the greater acidity of the porewaters (pH 4.0 for 'LB' vs. 5.0 for 'LL', and 5.5 for 'CEF' vs. 6.5-7.0 for 'AE', 'CIN' and 'CL'). For a fulvic acid, Chen, *et al.* (1978) reported a decreasing rate of DOC absorbance loss with increasing acidity. As postulated by Reche, *et al.* (1999), the reason acidity may increase photo-recalcitrance is that it promotes the condensation of humic acids into larger colloids which may reduce the access of sunlight to chromophoric functional groups. This would also explain the lower losses of DOC absorbance observed for 'LB' compared to 'CEF'.

The reduction in DIC concentrations for 'CEF', 'AE' and 'CIN' samples following sunlight exposure are somewhat unusual, given the vast number of studies reporting DIC photo-production from DOC breakdown (e.g. de Haan, & de Boor, 1991; Salonen & Vähätalo, 1994; Granéli, *et al.* 1996; Lindell, *et al.* 2000). This reduction can perhaps be attributed to the high initial DIC concentrations of these samples (40-50mg l^{-1} for the control treatment). The detection of photochemically produced DIC that is normally in the order of a few tenths to a few milligrams per litre against a background concentration this high would be difficult, a drawback discussed by Granéli, *et al.* (1996). The high initial DIC concentration for the fen samples is assumed to be due to high biological production, the influx of marine-influenced rainwater and calcium carbonate originating from the limestone bedrock. The decrease in DIC concentrations for the fen samples may have been that during the 14 days of sunlight exposure some calcium carbonate precipitated out. Although no visible precipitation was obvious inside the bags at the end of the experiment, for 'CEF' and 'AE' there was a slightly lower calcium concentration of 35 mg l^{-1} for the 'PAR+UVA+UVB' treatment compared to 40 mg l^{-1} for the control (data not shown). It may be that exposure to ultraviolet light can cause a slight increase in the precipitation of calcium carbonate, although there are no studies to confirm this and it requires further investigation. The reduction in DIC may also have been due to degassing of dissolved CO_2.
Large increases in DIC concentrations were recorded for the two bog samples; 86% for 'LB' and 46% for 'LL', therefore complete mineralization of some DOC to DIC occurred. Having said that, the DIC increase was, on average, less than 10% of the total reduction in DOC (e.g. 11mg l^{-1} DOC loss, 0.66 mg l^{-1} DIC increase for 'LB'). This suggests that, despite resulting in a large percentage increase in the total amount of DIC in the bog samples, the photo-formation of DIC from the breakdown of carbon compounds was a relatively unimportant process for the bog samples.

Comparison of the individual contribution of each waveband to total photo-degradation reveals that UV-A generally had the greatest effect. For example, by analysing the size of each of the bars in *figure 6.10* for DOC concentrations, PAR, UV-A and UV-B individually reduced DOC by an average of 6.7%, 11.7% and 0% for the six samples, with similar trends for UV-254 absorbance and E2:E3. This is despite the familiar concept that UV-B radiation has the highest energy per photon and photobleaching coefficient (Moran & Zepp, 1997; Reche, *et al.* 2000). Statistical analysis of the contribution of UV-A and UV-B radiation to DOC degradation (*tables 6.03-6.06*) shows that UV-B alone significantly impacted on fewer samples than UV-A. It is interesting that UV-B alone only significantly reduced DOC concentrations for Llugwy bog. This ties in with the suggestion that the highly aromatic bog porewater is more recalcitrant to solar radiation than the other samples used in this study, and that exposure to solar radiation with the highest energy (UV-B) is needed to break some of these bonds. This concept would also explain why the band with the lowest quantity of energy per photon, PAR, had the least effect on 'LB' out of all the samples.

The general lack of an effect for the UV-B band for the freshwater samples can perhaps be traced back to the UV transmission values of the Whirl-Pak bags used for holding the samples during sunlight exposure (*figure 6.03*). Although the Plexiglas material used for the 'PAR+UVA+UVB' treatment could let through approximately 90% of UV-B radiation, the Whirl-Pak bags were not so efficient, transmitting only 60%. It may be assumed from this that the samples exposed under this treatment received less of the radiation they should have done compared to the UV-A and PAR treatments. Future experiments of this kind should look into choosing a storage material with a better rate of transmission in the UV-B range. Quartz glass would be ideal, but it would have been too expensive for this study.

Despite this, the fact that UV-A radiation had the most significant impact on DOC characteristics falls in line with the growing number of studies that have reported this. Granéli, *et al.* (1996), for example, observed a better correlation between production of DIC and loss of DOM fluorescence with UV-A than UV-B for humic lake waters. The reason why UV-A may be more important is that, although it has a weaker photobleaching coefficient, it comprises 95% of all UV radiation reaching the Earth's surface (i.e. a greater weighted quantum yield) as well as being able to penetrate to much greater depths within lakes. Based on these factors, a study on a temperate freshwater lake by Molot & Dillon (1997) concluded that UV-A was responsible for 60% of the total DOC photolysis, with a similar value of 68% recorded by Vähätalo, *et*

al. (2000). Reche, *et al.* (2000) and Vähätalo, *et al.* (2002) both concluded that in the humic lakes that dominate the northern hemisphere freshwater ecosystems, UV-B may be of minor significance in terms of total water column photo-degradation.
The role of PAR as an important contributor to freshwater DOC photolysis was also highlighted in this study. It significantly reduced values of DOC concentration, UV-254 absorbance and E2:E3 for 11 out of a possible 18 samples/analyses. Although it's contribution was generally not as high as UV-A, the HPSEC and ^{1}H-NMR data indicate that PAR accounted for, on average, 20% of the DOC structural changes detected by these methods. There is also growing evidence that PAR plays an important role in freshwater photolysis. Reche, *et al.* (1999) stated that, like in this study, PAR in tandem with UV-A had the greatest photobleaching effect on lake water. The ability of PAR to penetrate to much greater depths within lakes than radiation in the UV region ensures that it is not just the ultraviolet component of sunlight that is involved in photoreactions in freshwaters.

In addition to the conversion of organic carbon compounds to DIC and LMW photo-products, the break-up of DOC released a number of ions into the sample solutions. However, the break-up DOC compounds derived from the fen did not lead to a significant release of inorganic nitrogen and phosphate, therefore UV light does not contribute to the formation of algal blooms at the Cefni through nutrient release. Phosphate release was observed for the bog porewater and lake water samples, with exposure to the total spectrum of solar radiation (PAR+UVA+UVB) increasing phosphate concentrations from below the limit of detection for the control to approximately 0.12 mg l^{-1} (*figure 6.17*). It is interesting that this values falls within the range of concentrations for eutrophic lakes (0.016 – 0.386 mg l^{-1}) (Wetzel, 2001). The fact that the lake water increase of PO_3^{4-} was almost identical to that of the soil implies that the source of phosphate-bound humics to the lake was the continuous leaching from the peatland within the lake's catchment. The observation that no phosphate was detected for the PAR and UV-A treatments indicates that the higher energy of UV-B is required to break the bonds between phosphate ions and dissolved carbon compounds. The lack of a concurrent increase in PO_3^{4-} for the fen samples may be explained by the role of iron in the phosphate bonding. Although iron was not detected in the freshwater lakes or streams, total iron was measured in the 'LB' control porewater samples at a concentration of 0.36 mg l^{-1} (data not shown), similar to that reported by Freeman, *et al.* (1993) for the surface of a Welsh bog, whereas none was detected in 'CEF'. As iron in association with dissolved humic compounds can strongly bind phosphate (Koenings & Hooper, 1976; Francko & Heath, 1979), which can then be broken by UV light to release the PO_3^{4-} ions (Francko & Heath, 1982), the presence of iron in the bog porewater, and the probable leaching of 'Fe-DOC-PO_3^{4-}' complexes into Llugwy lake, may explain why UV-induced phosphate release was only observed for the bog samples and not the iron-free fen-fed freshwaters. Despite their collection in mid-summer, the time of the year when photolysis is greatest, no phosphate was detected in the 'LL' control samples. This is probably due to the immediate utilisation of the phosphate ions, as it is often present in concentrations low enough to limit microbial and algal growth in lake waters (Schindler, 1977). Based on the observation of Vähätalo, *et al.* (2003) of increased bacterial growth following photo-production of phosphate within lake water, this seems like a plausible explanation.

In addition to the release of phosphate, concentrations of bromide also increased for the bog soil and lake samples from below the limit of detection in the control treatments following exposure to ultraviolet light. As discussed in chapter 2 (p73) and chapter 4 (p157), bromide can be retained in quite large concentrations in soils with a high content of organic matter, such as peatlands (Vinogradov, 1959), largely bound to organic compounds (Biester, *et al.* 2004). High concentrations of bromide have been observed leaching from a naturally drained peatland following artificial rewetting, with the mechanism of release attributed to the biological breakdown of organic matter (Hughes, *et al.* 1996). A similar mechanism, albeit abiotic, is likely to be responsible for the bromide release that occurred in this study. For the 'LB' porewater, it was the action of the lower energy UV-A radiation only that was responsible for the bromide release, indicating that the binding of Br^- ions with carbon compounds is not as strong as for PO_3^{4-}. The photo-release of bromide occurring within 'LL' was much lower than for the peatlands, probably due to the retention of some bromide with the catchment soil itself, and only occurred with exposure to UV-B. Similarly to phosphate, it was unusual that no bromide was detected in the 'LL' control samples, however, data from section 2.1 (*figure 2.21*, p41) show that bromide concentrations in 'LL' averaged 0.011 mg l^{-1} over the 15 months of sampling, the same concentration detected for the UV-B exposed samples. This data suggests that UV-B break-up of DOC in the surface waters of bog-fed lakes can be an important source of bromide. This has important implications for the quality of final drinking water, as the presence of bromide will increase the formation of more harmful brominated THMs. It was unusual that no bromide release was detected in the fen samples and suggests that bromide may be leached out of the fen profile rather than being retained by the organic matter. The observation of high bromide concentrations in 'Afon Erddreiniog' in section 3.1 (*figure 3.17*, p97) suggests this may be the case.

Whilst there was no detectable release of bromide and phosphate for any of the fen samples, there was an effect of sunlight exposure on sulphate concentrations for the fen porewater. The action of UV-B led to the release of almost 20 mg l^{-1} extra sulphate ions, equating to a 45% increase. With no effect of sunlight on sulphate concentrations for the bog samples, the data suggests that sulphate is strongly bound to organic material within fens, and in bogs is either not bound to dissolved carbon compounds or, more likely, due to the higher recalcitrance of bog DOC, is bound too strongly to be affected by ultraviolet radiation. The lack of an increase in sulphate concentrations for 'AE' may be due to the retention of sulphate in the fen itself, or that there had already been a rapid photolytic release of SO_2^{4-} for the samples before they were collected. The photo-production of sulphate has been recorded previously; Chen, *et al.* (1978) observed the formation of inorganic sulphate following the artificial irradiation of a fulvic acid solution.

6.5 Overall conclusions

The data gathered in this experiment demonstrate that solar radiation is a vital component of the carbon cycle of freshwater ecosystems. Although the experiment was conducted under ideal natural conditions (e.g. in direct sunlight for 14 days), the results demonstrate that as water drained from Cors Erddreiniog fen into Cefni lake (AE ▶CIN ▶ CL), the DOC compounds progressively became less reactive to solar radiation. As these samples were collected in midsummer, it highlights the fact that they have already undergone a certain amount of natural photo-degradation. The results suggest that solar radiation must at least be partially responsible for the loss of DOC observed during July in section 3.1.

The conversion of DOC from higher into lower molecular weight carbon compounds, as evidenced by the E2:E3 values, HPSEC molecular weight curves, ^{1}H-NMR spectra and the greater reduction in UV-254 absorbance over DOC concentration, has important implications for freshwater bacterial and algal growth. Whilst a reduction in the overall molecular weight of DOC residing in reservoirs would ultimately reduce the quantity of disinfection by-product precursors and therefore the concentration of THMs in final drinking water, the enrichment in low molecular weight compounds would reduce the efficiency of organic matter removal at the water treatment works and lead to an increased carbon source for harmful bacteria in the distribution system following water treatment.

The action of UV-B radiation in releasing bound phosphate ions in the surface of lakes with bog catchment soils is potentially very environmentally significant. Phosphate is often the limiting nutrient in freshwaters and a photo-release of the magnitude recorded could dramatically stimulate algal growth. Although algae have been shown to be a less potent THM precursor than allochthonous DOC inputs (section 3.2), it's presence in raw water supplies is known to reduce the efficiency of water treatment processes. The lack of a photo-release of inorganic nitrogen and phosphate from the fen-derived DOC suggests that UV radiation does not elevate the levels of the two most important algal nutrients at the Cefni reservoir.

As well as phosphate release negatively affecting the biological quality of lake water, the measured release of bromide reduces its chemical quality and suggests that, in some lakes, sunlight can be an important indirect source of bromide. Bromide incorporation during the formation of THMs and other DBPs can lead to the enrichment of more harmful brominated species in the final drinking water.

The results of this study also demonstrate the importance of PAR and UV-A radiation in causing the break-up of DOC compounds. Although these bands of radiation have a lower photobleaching coefficient than UV- B, they represent a much greater proportion of total solar energy within a lake's water column. More often than not, UV-A was the dominant degradative waveband, which agrees with the increasing number of studies that are reporting this.

Chapter 7

Final discussion

7.1 The 'current' scenario

7.1.1 Historical perspective

In the world's developed countries, widespread access to clean drinking water has been one of the greatest achievements of the twentieth century. Water-born diseases, such as cholera and typhoid, which killed many thousands of people only a hundred years ago, have been virtually eradicated in first-world countries due to the efficient treatment of drinking water sources and the elimination of harmful pathogens by chlorination (Pontius, 2003). However, while a biological hazard has been virtually eradicated, a new chemical one has emerged. The reaction of chlorine with organic matter to produce a suite of disinfection by-products (DBPs) is a serious side-effect of disinfection, as some of these compounds have been shown to be carcinogenic and to cause reproductive damage in rodent bioassay tests (Dunnick & Melnick, 1993; Bull, *et al.* 1995; Dodds, *et al.* 2004). After the removal of harmful pathogens, the intake of DBP compounds represents the greatest threat to the health of consumers of chlorinated drinking water. Although water treatment techniques can remove DBP precursors reasonably efficiently, positive and significant correlations between raw and treated water DOC at the Cefni ($R^2=0.74, p<0.001$; *figure 2.13*, p38) and final water DOC and THM concentrations at the Cefni reservoir ($R^2=0.60, p<0.001$; *figure 2.08*, p35) demonstrates that the greater the content of organic matter in the reservoir, the more there is in the water at the chlorination stage and the greater the formation of THMs. The concentration, characteristics and seasonal variability of organic matter in reservoirs is therefore crucial to understanding the formation of THMs during water treatment.

7.1.2 Sources of organic matter to Cefni lake

Due to the high raw water DOC (*figure 2.12*, p38) and final water THM concentrations (*figure 2.05*, p35), the Cefni reservoir, in north Wales, was the main focus of the studies presented in this thesis. A multiplicity of sources of carbon to the lake were analysed and their role in the formation of THMs investigated. *Figure 7.01* shows the relative contribution of DOC to the Cefni from each of the sources studied and the role they play in the formation of THMs. Data from section 2.2 shows that the wetland soils surrounding the lake contribute only minor amounts of DOC (*figure 2.30*, p57) and this DOC contains a very low percentage of HMW material (*figure 2.36*, p58). Instead, the most important source of terrestrially-derived DOC to the Cefni appears to be from Cors Erddreiniog fen (section 3.1), the character of which is higher in molecular weight than any of the other catchment soils (*figures 3.03 & 3.06*, p87; *figure 3.08*, p90) and would therefore be expected to contain the greatest concentration of THM precursors. Of all the streams draining these soils and flowing into the Cefni, Afon Erddreiniog had the greatest concentration of both total and HMW DOC (*figures 3.11-3.14*, p93), which is testament to the influence of the fen. A similar finding was reported by Sachse, *et al.* (2001), who measured high concentrations of total DOC and HMW DOC in the portion of a lake closest to an adjacent fen. It is also in agreement with numerous studies that have demonstrated the role of carbon-rich soils in increasing the DOC concentration of freshwaters lakes (e.g. Urban, *et al.* 1989; Aitkenhead, *et al.* 1999; Gergel, *et al.* 1999; Elder, *et al.* 2000).

Nuclear Magnetic Resonance (NMR) is one of the most powerful tools for assessing the structure of NOM (Peuravuori, 2005) and is the method of choice for rapid characterization of dilute NOM samples, such as lake water (Cook, 2004). It has been used to trace the source of nitrogen-bound organics in groundwater (Lu, *et al.* 2004) and the results of this study suggest that, by 'fingerprinting', it could also be used to identify sources of DOM to lakes, especially in cases where there are contrasting sources (e.g. peatland vs. farmland in this study). Using ^{1}H-NMR to trace the DOC compounds entering Cefni lake from the two major inflows confirms the greater influence of the northern (fen) inflow (*figure 3.15*, p95). Of great importance to Welsh Water is that the DOC leaching into the lake via the fen inflow contains more THM precursors than the southern inflow (*figure 3.16*, p97), an observation which can be linked to the differences in molecular weight characteristics and aromaticity (e.g. SUVA - *figure 3.12*, p93). The fact that the average drainage volume of the northern inflow is approximately twice as high as the southern inflow (and much higher than the other inflowing streams) accentuates the role of the fen inflow in determining the characteristics of the DOC in Cefni lake. It was calculated that the inflow from the fen contributes 33-57% of all the

DOC within the lake on an annual basis, and, based on analyses of its reactivity, contributes 53-59% of the total THM formation potential of the DOC within Cefni lake. Another important source of DOC is autochthonous production within the lake itself (Fukushima, *et al.* 1996). The growth of algae in source waters is highly undesirable due to its negative effects on the taste and odour of the water (Nguyen, *et al.* 2005) and at the Cefni water treatment works, a special dissolved air flotation (DAF) treatment stage has been employed specifically to remove algae. The contribution of algae-derived DOC to Cefni lake was estimated at between 5-45% during 2005, therefore during times of high algal growth, there can be a significant contribution of DOC. Indirect evidence of high algal productivity was observed in the 2003 survey of the Cefni reservoir, when, from winter to summer, nitrate concentrations fell approximately 99.7% (*figure 2.22*, p41), phosphate concentrations reduced to below the limit of detection (*figure 2.23*, p41) and there was a notable dip in the concentration of dissolved CO_2 (*figure 2.18*, p39). Although the production of THMs from the cultured algal DOC was significantly less than the terrestrial stream DOC (*figure 3.25*, p121), and the overall contribution of algal DOC to the total THMFP of the lake was just 1.4-12.6%, the removal of algal DOC is much less efficient than the removal of terrestrial DOC (due to its lower molecular weight/size) (Cheng & Chi, 2003). Therefore the algae:terrestrial DOC ratio of the chlorinated water will be much higher than the algae:terrestrial DOC ratio of the raw water. Although the DAF treatment stage will remove most of the algal biomass, it is likely that most of the algal ECP will pass through (Plummer & Edzwald, 2001). The fact that the algal chlorination experiment was performed on mostly algal ECP highlights the fact the algal DOC is likely to be a significant precursor for THMs at the Cefni.

Another source of carbon to Cefni lake is dissolved methane, which is greater in the summer due to higher temperatures (Murase, *et al.* 2005). Section 2.1 showed that dissolved methane in the lake correlated with the concentration of THMs in the final water and section 3.2 demonstrated that methane can form chloroform following chlorination. This is an important finding, as Schindler, *et al.* (1992) stated that methane is often not considered as part of the DOC pool. It is actually the lowest molecular weight DOC compounds that exists and the observation that it can form THMs following chlorination exemplifies the fact that it should be considered as a component of DOC. Almost identical THM formation potentials were recorded for the dissolved methane and algal DOC (50 and 44 micrograms per milligram respectively), suggesting that algal DOC also has a *very* low molecular weight. However, dissolved methane is present at such low concentrations in natural systems that it should not be considered an important THM precursor by water companies. Algae, on the other hand, are much more abundant in the Cefni and other eutrophic lakes and the DOC it produces is much less efficiently removed during water treatment. Coupled with the fact that algae can impact on the taste and odour of source waters ensures that it poses a much greater threat to the quality of drinking water than dissolved methane.

The final source of DOC to Cefni lake considered in this study was rainfall. Its low DOC concentration (typically just 1 mg l^{-1} (Wetzel, 2001)) and lower contribution by volume of water (section 3.1, p99) ensures that its direct influence on THM formation is less important than the two major inflowing streams. However, the identification of highly absorbing and fluorescing organic carbon compounds in rainwater by Kieber, *et al.* (2006) suggests that rainwater is composed of compounds that can contribute to the formation of THMs.

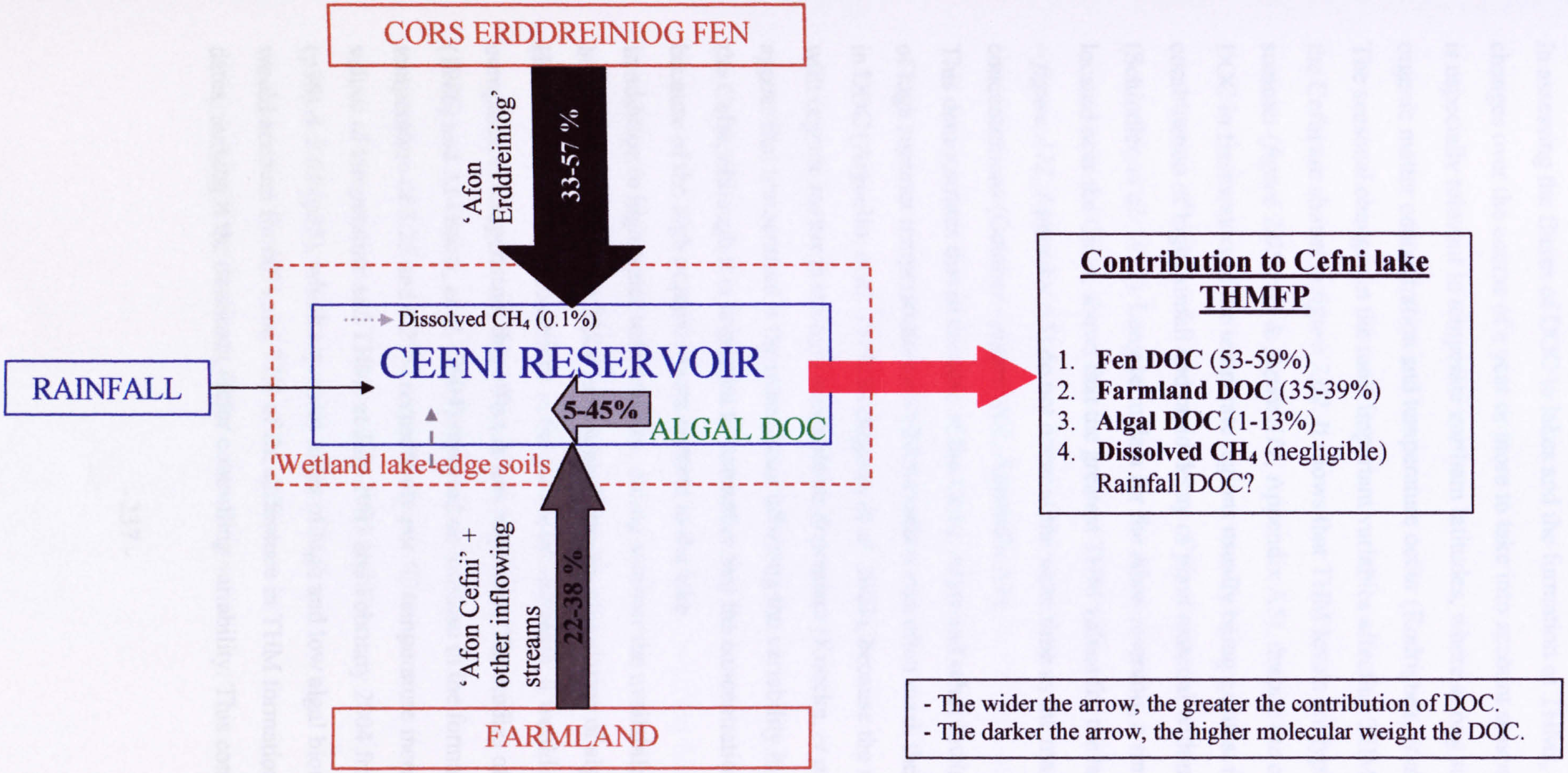


Figure 7.01 – Sources of DOC to Cefni lake and their importance in the formation of THMs

7.1.3 The effects of seasonality

In assessing the fluxes of DOC to lakes and the formation of THMs, one must observe changes over the course of a year or more to take into account seasonal variations. This is especially relevant in temperate northern latitudes, where strong seasonal changes in organic matter concentration and temperature occur (Rodriguez, *et al.* 2004).

The seasonal changes in the most important variables affecting THM concentrations at the Cefni are shown in *figure 7.02.* It shows that THM levels are typically highest in the summer (*figure 2.05*, p35 & *figure A11*, Appendix A5), despite the concentrations of DOC in freshwaters of the temperate regions usually being greatest in the autumn due a combination of high rainfall and rapid decay of plant material within the catchment (Schindler, *et al.* 1992). Long-term data for the Alaw reservoir, a similar lowland lake located near the Cefni, shows that the greatest THM values for the last 10 years (August – *figure A12*, Appendix A5) do not occur at the same time as the maximum DOC concentrations (October – *figure A01*, Appendix A1).

This demonstrates that in the case of the Cefni, Alaw and other lowland lakes, the effect of high summer temperatures on THM formation can often mask the seasonal variations in DOC (Arguello, *et al.* 1979; Rodriguez, *et al.* 2003), because the reaction of chlorine with organic matter is strongly temperature dependent (Knocke, *et al.* 1986). It would appear that temperature is the main factor affecting the variability in THM formation at the Cefni, although it is important to remember that the concentration of THMs is high because of the high organic matter content in the lake.

In addition to high water temperatures, during summer the availability of sunlight is also high and will impact on THM formation through a stimulation of algal biomass. However, based on calculations in the published literature, it would appear that compared to temperature, this effect is less important. The studies of Knocke, *et al.* (1986) and Al-Omari, *et al.* (2004) reported an increase in the formation of THMs with temperature of 3.25 and 4.23% respectively per °C temperature increase. Taking the values of temperature and THMs at July 2003 and February 2004 from *figures 2.09* (p36) & *2.05* (p35), which represent times of high and low algal biomass, temperature would account for 60% and 77% of the difference in THM formation between these two dates, making it the dominant factor controlling variability. This conclusion is backed up by Golfinopoulos & Arhonditsis (2002), who stated that temperature caused more of the THM variability than other seasonal factors such as light intensity and photoperiod.

One of the most important external factors that affects the characteristics of DOC within a lake is ultraviolet radiation (Williamson, 1995) and with levels in the summer being almost 10 times higher than in the winter in the UK (*figure A13*, Appendix A5), the seasonal variability is extremely high. DOC compounds are broken down by sunlight and the importance of this process in affecting the terrestrial DOC being supplied to the Cefni from the fen inflow was demonstrated in chapters 3 and 6. Both of these separate studies reported an approximate two thirds loss of DOC from Afon Erddreiniog, where water drains from the fen, to the point at which the stream enters the Cefni, during the summers of 2004 and 2005. In chapter 3, a 66% loss of DOC was recorded in July, when conditions were warm and sunny (*figure 3.11*, p93), whilst in chapter 6, exposure of water collected from these same sites to sunlight for 14 days resulted in a loss of DOC for 'Cefni inflow North' that was 66.7% lower than the loss of DOC for Afon Erddreiniog. A comparison of the natural loss in October, when conditions were cool and cloudy, revealed just a 4% loss of DOC (*figure 3.11*, p93). This demonstrates that during the summer, and at times when the levels of sunlight are high, the DOC leaching out of the fen is strongly degraded by sunlight. Due to the high molecular weight of the fen DOC, which was discussed previously, solar degradation would be expected to reduce the DOC concentration of the fen inflow more than any of the other sources of DOC shown in *figure 7.01*. However, it is important to point out that in addition to UV, DOC will also be lost from the stream through microbial metabolism, adsorption, precipitation and by dilution from other smaller streams, direct rainfall and groundwater (Kaplan, *et al.* 1980).

The light blue arrows on *figure 7.02* show the average influx of rainfall for the four seasons based on long term data from the Met Office (*figure A14*, Appendix A5). Although the direct contribution of DOC from rainfall is minimal (*section 3.1*, p99), rainfall is the medium through which the large concentrations of decaying organic carbon enters the lake from the catchment soils (e.g. Scott, *et al.* 1998). The fact that rainfall is usually highest in autumn means that the flux of allochthonous DOC and THM precursors is usually greatest in this season too (e.g. *figure A01*, Appendix A1).

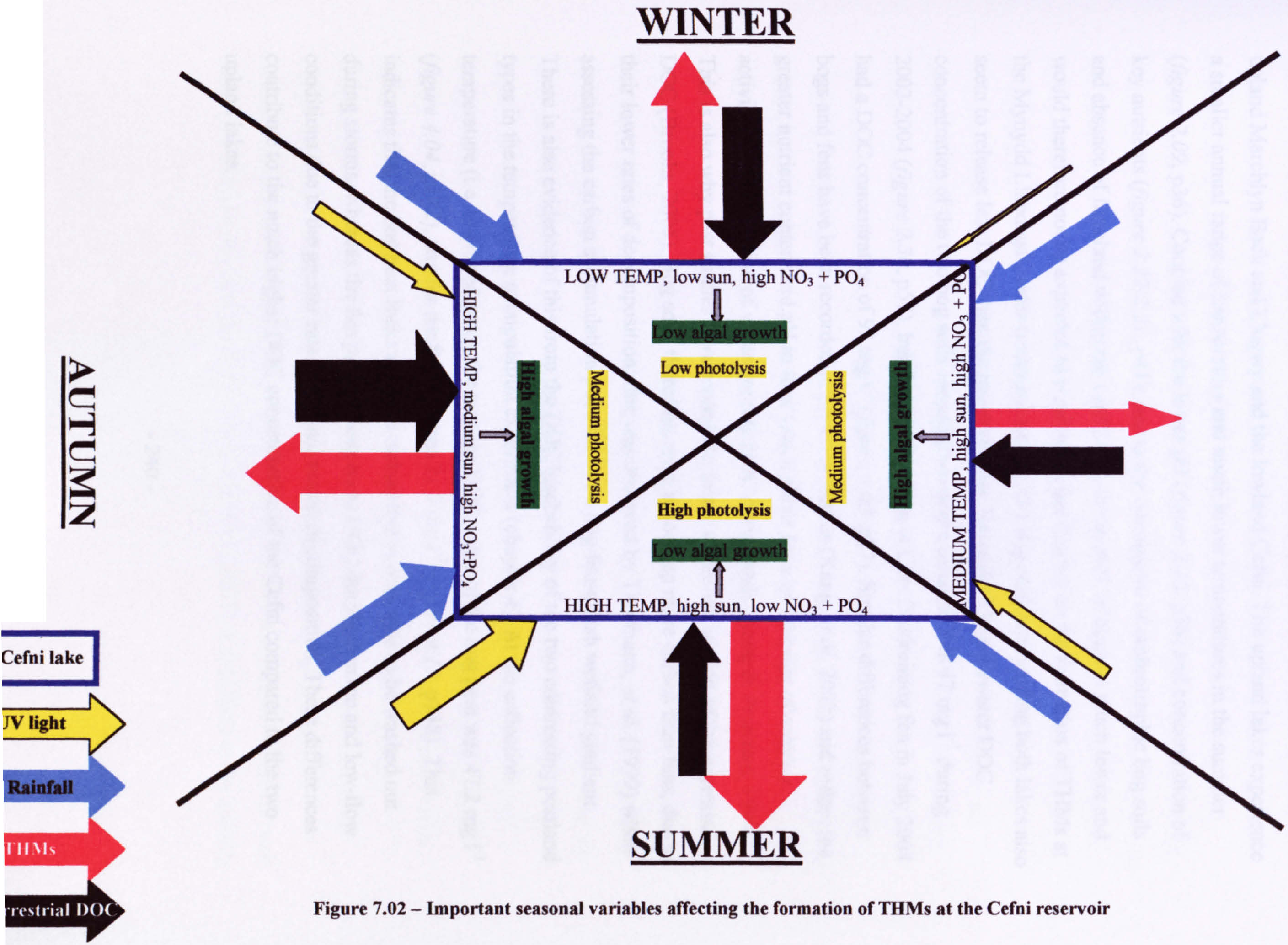


Figure 7.02 – Important seasonal variables affecting the formation of THMs at the Cefni reservoir

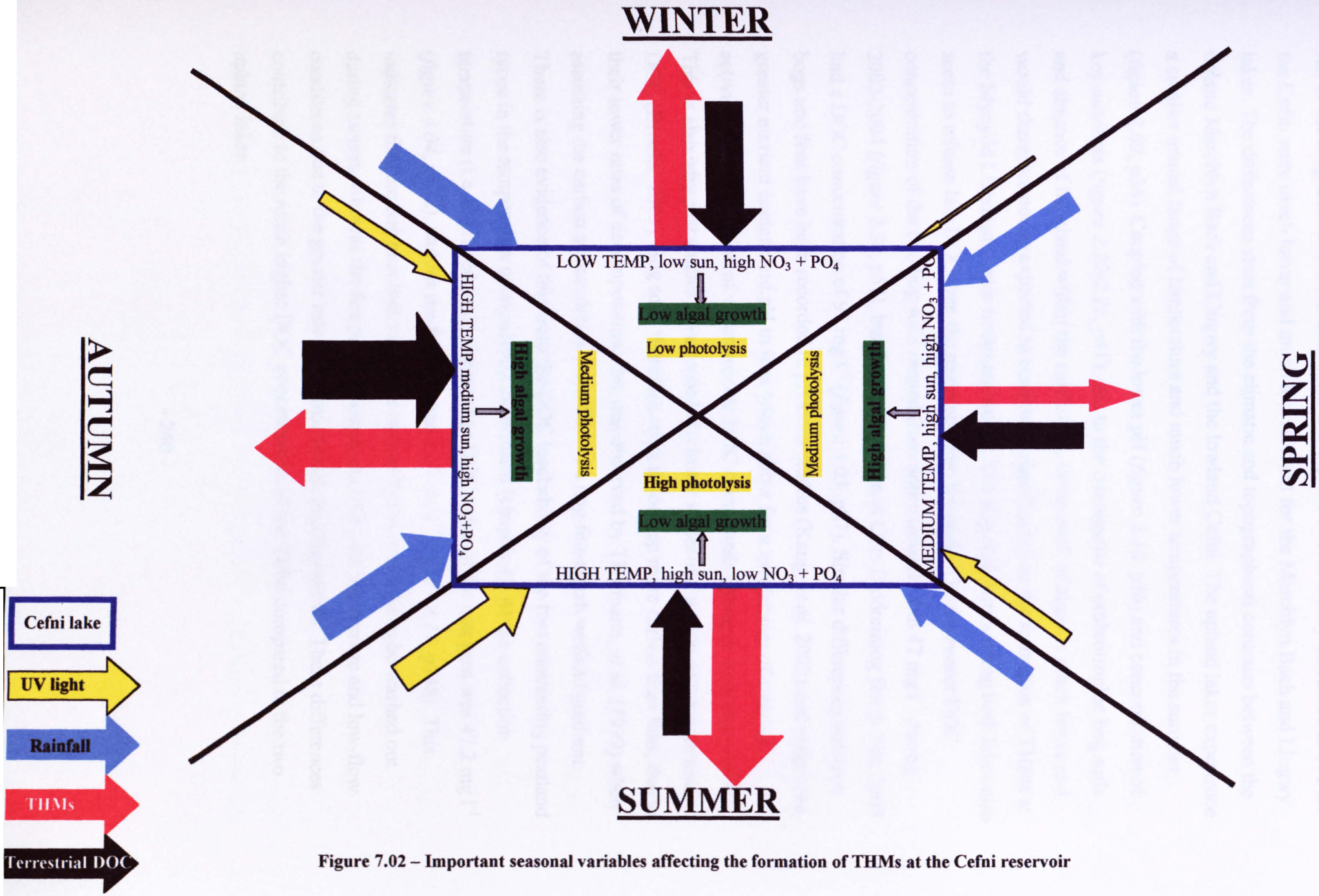


Figure 7.02 – Important seasonal variables affecting the formation of THMs at the Cefni reservoir

7.1.4 Upland catchment contrasts

All of the biogeochemical characteristics and aspects of seasonality discussed above for the Cefni were much lower and much less relevant for the Marchlyn Bach and Llugwy lakes. The differences stem from the climatic and topographical contrasts between the upland Marchlyn Bach and Llugwy and the lowland Cefni. The upland lakes experience a smaller annual range of temperature and much lower temperatures in the summer (*figure 2.09*, p36). Coupled with the lower pH (*figure 2.10*, p36) and concentration of key nutrients (*figure 2.22-2.23*, p41), due to the dominance of ombrotrophic bog soils and absence of farmland within the catchment, the growth of algae is much lower and would therefore not be expected to contribute significantly to the formation of THMs at the Mynydd Llandegai water treatment works. The bog soils surrounding both lakes also seem to release less DOC than the minerotrophic fen soils. The porewater DOC concentration of the two bog soils ranged from approximately 5 to 47 mg l^{-1} during 2003-2004 (*figure 2.30*, p57), but the sample taken at Cors Erddreiniog fen in July 2004 had a DOC concentration of 95 mg l^{-1} (*figure 3.03*, p87). Similar differences between bogs and fens have been recorded in previous studies (Kang, *et al.* 2002) and reflect the greater nutrient content and pH in fens which allow for a greater rate of enzyme activities and production of water-soluble DOC compounds (Kang & Freeman, 1999). This is also why past studies have recorded a lower molecular weight of fen porewater DOC (Brooks, 2005). Bog soils therefore seem to lock up more carbon than fens, due to their lower rates of decomposition; this was observed by Thormann, *et al.* (1999) when assessing the carbon accumulation potentials of a bog-fen-marsh wetland gradient. There is also evidence of this from the DOC leachability of the two contrasting peatland types in the temperature manipulation experiment (chapter 4). At the collection temperature (i.e. unaltered peat), the average DOC value of the bog peat was 47.2 mg l^{-1} (*figure 4.04*, p142), and for the fen peat was 21.7 mg l^{-1} (*figure 4.13*, p148). This indicates that the bog peat locks up more carbon that is only able to be washed out during storms, whereas the fen peat releases more DOC during average and low-flow conditions due to the greater rate of organic matter decomposition. These differences contribute to the much higher DOC concentration of the Cefni compared to the two upland lakes.

7.2 The 'future' scenario - the potential impacts of climate change

'Climate change' is a broad term which in today's world is synonymous with the anthropogenically-induced alterations to the world's climate that are likely to occur in the next hundred years. Often, the phrase 'global warming' is used to describe such a process, but it is becoming increasingly unfashionable because the impacts of rising atmospheric carbon dioxide concentrations will induce more than just warming. Whilst elevated CO_2 and temperature will have dramatic impacts on the global carbon cycle and the flux of organic matter to freshwaters (Freeman, *et al.* 2004*b*; Davidson & Janssens, 2006), other factors such as changes in discharge (Tranvik & Jansson, 2002; Worrall, *et al.* 2003*a*), precipitation patterns (Tipping, *et al.* 1999), drought (Freeman, *et al.* 1993; Worrall & Burt, 2004), anthropogenic pollution and land use (Siepak, 1999), etc. all have the potential to alter the fluxes of carbon. With peatlands often being the primary source of carbon to freshwaters in the northern, temperate zone (Urban, *et al.* 1989; Aitkenhead, *et al.* 1999), the potential effects of climate change on these systems are crucial to predicting the likely changes in the DOC concentrations of sources of drinking water. *Figure 7.03* shows some of the implications of climate change on major processes which affect the flux of DOC from peatlands to freshwaters.

7.2.1 Potential impacts of elevated atmospheric ozone

One factor that has received almost no attention in predictions of future fluxes of carbon from peatlands is the effect of rising atmospheric ozone. This is despite ozone being the most potent phytotoxic regional scale air pollutant (Krupa & Kickert, 1989) and any impacts on vegetation could have knock-on effects for the soil carbon store through changes in net primary productivity (Cao & Woodward, 1998; Minkkinen & Laine, 1998). The results of chapter 5 show that exposure to elevated concentrations of ozone led to a dramatic 40-55% reduction in the concentration of DOC leaching from a minerotrophic fen peatland, with the remaining carbon shifting to a higher molecular weight, implying an impairment in the flux of photosynthetically-produced root exudates and a reduction in enzymic carbon cycling. With concentrations of background ozone predicted to rise 29% by 2060 (Coyle & Fowler, 2003), a reduction in the quantity of DOC leaching from fen systems may occur. This is the first study to record a depletion of organic carbon in a soil in response to elevated ozone. However, rising ozone concentrations are just one of a number of changes likely to occur under the general climate change scenario, so it is important to consider the results of chapter 5 alongside predictions for altered DOC fluxes from peatlands under other climate change predictions. For example, atmospheric CO_2 concentrations have been predicted to increase 60% by 2050 (Kattenburg, *et al.* 1995) and numerous recent experiments have shown that elevated CO_2 can partially offset some of the negative impacts of ozone, such as reduced photosynthesis, plant growth, etc. (Andersen, 2003; Holmes, *et al.* 2003; Loya, *et al.* 2003). Very few studies have addressed the impact of more than two variables on factors that can potentially upset the current organic carbon cycle. This is because the number of possible combinations and long time periods over which they operate make it difficult to assess future changes through controlled experiments alone (Ollinger, *et al.* 2002).

7.2.2 Potential impacts of increased global temperatures

Elevated global temperature is one of the certainties of climate change and although doubts exist over whether it will have a positive or a negative feedback on overall soil carbon stocks because of the potential stimulation of primary production (Davidson & Janssens, 2006), it is generally accepted that peatlands will sequester less carbon in a warmer world (Freeman, *et al.* 2001*b*). The temperature manipulation experiment in chapter 4 demonstrated that under conditions of elevated temperature, significantly more DOC and THM precursors leached out of fen soils in summer during heavy rainfall events. The data also suggests that greater concentrations of DOC may leach from bog soils during spring. These increases were linked to an increased mobilisation of the peat carbon matrix. The potential increase in THM-forming DOC washing out of bog soils during heavy rainfall in spring should not be a major concern for water treatment works which source water from upland lakes surrounded by bog peat soils, as spring is usually the time of year when DOC release from peat soils into freshwaters is lowest (Worrall, *et al.* 2006*b*). For example, in the survey described in chapter 2, the lowest concentrations of DOC in Marchlyn Bach lake were recorded in March and April 2003.

The potential increase in DOC leaching from fen peat in the summertime is a concern, however, for the treatment of drinking water at the Cefni reservoir. In chapter 2 it was explained that a significant autochthonous algal production of DOC occurs during the summer in the Cefni reservoir and in chapter 3 that this algal DOC can be important precursors for the formation of THMs at the Cefni. The potential increase in DOC leaching from fens may therefore represent a further deterioration in water quality during the summer under warmer conditions, as some of this extra carbon will leach directly into the Cefni reservoir. Coupled with the high temperatures that are typical of the Cefni during the summer, this could lead to problems with THM compliance.

7.2.3 Potential impacts of increased levels of UV radiation

In addition to elevated carbon dioxide, ozone, temperature and altered rainfall patterns, changes in exposure to UV radiation are likely to have important consequences for global biogeochemical cycles (Zepp, *et al.* 1998). Recent work suggests that although CFC emissions have dramatically lowered, ozone depletion may continue for some time due to the greenhouse effect, where solar energy becomes trapped near the Earth's surface, lowering the temperature of the upper stratosphere where the ozone layer exists (Shindell, *et al.* 1998). Models of future ozone depletion with rising greenhouse gas emissions have demonstrated that the greatest loss of ozone over the Arctic is expected to be between 2010 and 2019, similar in magnitude to the losses experienced over the Antarctic in the early 1990s (Shindell, *et al.* 1998). With ground-level UV radiation expected to increase over the coming decades as a result, there is potential for an enhancement in the impacts of UV radiation on the global carbon cycle. It was shown in section 3.1 that as water derived from Cors Erddreiniog fen flowed south into the Cefni reservoir, its DOC concentration reduced by 66% in July (*figure 3.11*, p93). Chapter 6 proved that the photodegrading action of UV light could have been responsible for much of this reduction, although microbial and biofilm metabolism will also reduce the concentration of DOC. With the quality of the organic carbon leaching from Cors Erddreiniog fen being relatively enriched in HMW and IMW material (*figures 3.05*, p87 *& 3.14*, p93), the chromophoric carbon content is likely to be higher than organic material originating from other sources of terrestrial carbon (Vähätalo, *et al.* 1999), explaining the high loss of carbon recorded in both chapters. Future increases in UV radiation may therefore offset to a certain degree increases in carbon leaching into Cefni lake from Cors Erddreiniog fen due to climate change (e.g. chapter 4). However, with only a 4% loss of DOC recorded in October 2004 (*figure 3.11*, p93), at a time of the year when solar activity is almost 3 times lower than during the summer maximum (*figure A13*, Appendix A5), the extent to which increased UV radiation could reduce DOC fluxes to lakes at the time of the year when they are greatest would appear minimal. Also, with the majority of allochthonous carbon entering lakes during storms (Royer & David, 2005), when solar activity would be minimal due to cloud cover, enhanced UV would have little impact on the fluxes of DOC to lakes on an annual basis. It has also been shown that tropospheric ozone, predicted to increase during this century (Coyle & Fowler, 2003), will partially offset some of the future predicted increases in ground-level UV radiation (Bruhl & Crutzen, 1989).

In addition to reducing the DOC concentrations of Llugwy bog and lake water, exposure to sunlight also released organically-bound phosphate and bromide into the lake water (*figures 6.17 & 6.18*, p216). In the case of phosphate, the release is due to the action of UV light in breaking up iron-DOC-phosphate complexes (Francko & Heath, 1982) and for bromide is due to the break up of bromide-absorbing aromatic bonds (Biester, *et al.* 2004). Increases in UV radiation could therefore lead to increased autochthonous production in lakes fed by bog soils and increase the percentage of brominated THMs at water treatment works that extract from these reservoirs; both would reduce the quality of the drinking water.

7.3 Overall conclusions

With the Cefni and upland (Marchlyn Bach and Llugwy) lakes being fed by fen and bog peatlands respectively, there is potential for climate change-induced alterations to the fluxes of DOC to these lakes, which will have consequences for the quality of drinking water. If the rising concentrations of DOC in freshwater rivers and lakes that have been observed in many regions of the northern hemisphere over the last few decades are to continue (Robson & Neal, 1996; Schindler, *et al.* 1997; Freeman, *et al.* 2001*b*; Hejzlar, *et al.* 2003; Worrall, *et al.* 2003*a*; Hongve, *et al.* 2004; Korth, *et al.* 2004; Evans, *et al.* 2005), it is likely to pose a serious challenge to water companies to reduce DBP concentrations and maintain clean drinking water in an affordable manner. Most studies suggest that continually rising temperatures, levels of atmospheric carbon dioxide, occurrence of drought and storms, etc. will increase the flux of DOC from peatlands, although making accurate predictions of future fluxes is difficult due to the complexity of the global carbon cycle and the number of processes influencing it. The results obtained in this study of a reduction in DOC leaching from fens under elevated ozone add another complication.

Water companies that exist in the temperate zone of the northern hemisphere, where the greatest concentration of both peatlands and freshwater lakes are found, are likely to observe the greatest deterioration in water quality, as the organic, carbon-rich peat soils that have been sequestering carbon for thousands for years begin to release some of their vast store. The problem may be particularly pronounced in Wales, due to the mountainous topography and abundance of freshwater lakes and peatlands. The reservoirs Marchlyn Bach and Llugwy are typical of the type that exist in mountainous regions of the northern hemisphere and the observation that peatlands may become net sources of carbon within the next 25 years are likely to result in a serious deterioration in the quality of water in lakes such as these (Worrall & Burt, 2005). Those water treatment works that currently draw water from lakes with high DOC concentrations and whose catchments contain peatlands may therefore need to invest heavily in order to improve organic matter removal in the future. The Cefni reservoir is also likely to experience rising DOC concentrations in the future, via the stream flowing to the lake from Cors Erddreiniog fen. An additional problem at the Cefni concerns the lake's eutrophic status and the high algal productivity during the summer. Given that the problem of eutrophication is expected to worsen with climate change in lakes that already experience eutrophic conditions (Malmaeus, *et al.* 2006), algal productivity in the Cefni would be expected to increase in a warmer world, causing a further deterioration in water quality. Given the high concentrations of raw water DOC that already exists in the Cefni, the potential for a breach in THM compliance in the future is more likely.

The intake of THM compounds via drinking water has been linked to the development of cancer (Bull, *et al.* 1995), and cancer is the second biggest killer in the developed world (WHO, 2006). Although smoking and unhealthy lifestyles are generally accepted as having the greatest contribution to increasing rates of cancer (Ames, *et al.* 1995), with chlorinated drinking water having existed for over a hundred years, carcinogenic disinfection by-products could be responsible for a significant proportion of cancers in the developed world. Coupled with the uncertainty in knowing exactly what level of exposure to DBPs is truly 'unsafe', and the emerging evidence of a new generation of DBP compounds (Richardson & Ternes, 2005; Krasner, *et al. in press*), for which little toxicological information exists, the need for water companies to minimise the formation of these compounds in drinking water should therefore be high. However, it is of the utmost importance that water companies do not compromise the safety of drinking water by reducing the efficiency of pathogen removal (e.g. by reducing the chlorine dose) in an attempt to lessen the formation of disinfection by-products. A recent outbreak of cholera in the American continents highlights the significance of this (Glass, *et al.* 1992).

In addition to reducing DBP concentrations in drinking water, water companies are likely to face challenges in the supply of water too. It is expected that the per capita demand for water will increase in the UK in the coming decades (Thomsen, 1990), along with an increased occurrence of droughts (Arnell, 1992). This will place a huge demand on water supply in the UK, especially in the south-east of England, where the driest conditions and the greatest concentration of people exist.

Legislation governing concentrations of THMs and other disinfection by-products in final water is likely to become stricter in the future, and coupled with potential increases in the concentration of DOC in freshwaters, the need for Welsh Water to take steps to lessen the risk of failing water quality tests at the Cefni is imperative. Given the data on DOC flux measurements and reactivity for the inflowing streams, the most obvious action to take would be to divert 'Afon Erddreiniog', the stream that flows from Cors Erddreiniog, away from the lake, due to the inflow's high concentration of THM-forming DOC. However, such a measure would need careful planning, particularly concerning the local hydrology, as this stream also contributes the greatest point-source volume of water to the lake and, in times of drought, the loss of water volume in the lake may seriously undermine water quality and lead to restrictions on water usage for the customers that the treatment works serves.

Another way in which Welsh Water could lower the DOC concentration of Cefni lake and ultimately reduce the formation of THMs is to work with local farmers to minimise the leaching of nitrogen and phosphorus-containing fertilisers from the catchment soils into the lake. Nitrate and phosphate are the key nutrients for influencing the growth of algae, which have been shown to make a small, but significant contribution of DOC to Cefni lake.

7.4 Potential future work

Algal productivity at the Cefni

It was demonstrated that at the Cefni reservoir, algae are an important source of THM precursors. However, it is not known how much DOC in the Cefni reservoir is actually derived from algae and how much leaches into the lake from terrestrial sources. This is critical to understanding the role of organic matter in the formation of THMs at this reservoir. An assessment of the contribution of algal DOC could be achieved by performing whole-lake inorganic ^{13}C additions, to strongly label autochthonous primary production, according to the method of Kritzberg, *et al.* (2004). However, due to the size of the Cefni, such an experiment would not be economically viable; therefore it may have to be performed on a series of lake mesocosms. If autochthonous sources contribute most of the DOC, then the water company can work with local farmers to reduce the nutrient loading from the catchment to the lake, which would decrease the productivity of algae and reduce the formation of THMs. If allochthonous sources dominate, then management of Cors Erddreiniog fen based on predictions of future DOC fluxes under climate change scenarios would become a priority.

It would also be sensible to assess the reactivity of species of Ulothrix algae, which may have been responsible for the greater THMFP of the 1st and 4th October algal cultures (*figure 3.25*, p121).

Presence of THM precursors in rainfall

Although rainfall contributes less total DOC than other sources to the Cefni reservoir, a determination of the characteristics of the DOC it contains and its potential to form THMs is essential to improving the understanding of THM precursor fluxes to reservoirs. With algal productivity and the catchment soils contributing less DOC at the two upland lakes compared to the Cefni, the role of rainfall in the carbon budget of upland lakes is likely to be much more important. The observation that highly absorbing and fluorescing organic carbon compounds exist in rainwater (Kieber, *et al.* 2006) suggests that it does contain THM precursors.

Seasonal changes in the reactivity of DOC

The effect of seasonal changes in the reactivity of organic matter at the Cefni was masked by the high lake water temperatures experienced during the summer and the effect this had on THM formation. It would be sensible for a future experiment to assess the reactivity of the DOC throughout the year using the constant temperature THMFP method, rather than just obtaining actual THM values for final water from the water company. This would allow for determination of the influence of the DOC characteristics alone to the formation of THMs at the Cefni.

Source of phosphate

Phosphate is a key nutrient for algal growth and is often the most limiting in freshwater lakes. Section 2.1 showed that phosphate is reduced to levels below the limit of detection in Cefni lake during times of high algal growth, but section 3.1 failed to identify a point-source of phosphate (it was not measured in any of the inflowing streams in either July or October). Sources of phosphate include precipitation and atmospheric deposition, groundwater and surface drainage, the latter of which is the greatest contributor and depends on the amount of phosphorus in soils, the local topography and land use (Wetzel, 2001). Given the high algal productivity of the Cefni during the late spring to early autumn period, identification of the source of phosphate is essential.

Interactive effects of elevated atmospheric ozone and carbon dioxide

Given the large reduction in porewater DOC in the fen microcosms observed under elevated ozone, a greater understanding is needed of the possible interactive effects of elevated ozone and carbon dioxide, along with variables such as increased water-table drawdown, temperature and UV light, on the flux of DOC from peatlands. With elevated temperature and CO_2 expected to increase the amount of carbon leaching from peatlands in the future (Freeman, *et al.* 2001*b* & 2004*b*) experiments assessing interactive effects on peatland carbon fluxes involving elevated ozone now seem essential to increase our understanding of the flux of DOC to freshwaters in a changed climate.

Appendices

Appendix A1

(for chapter 1)

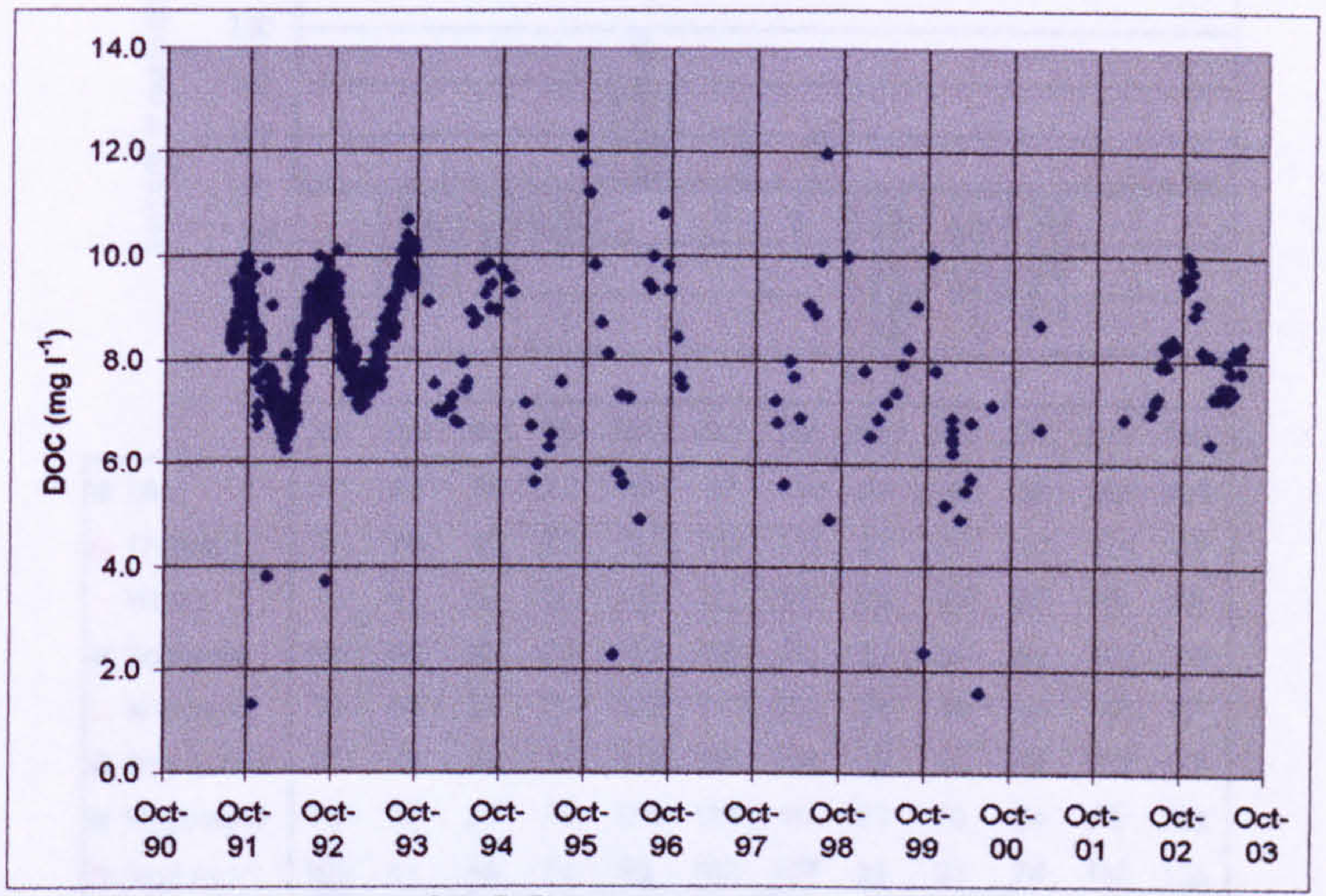
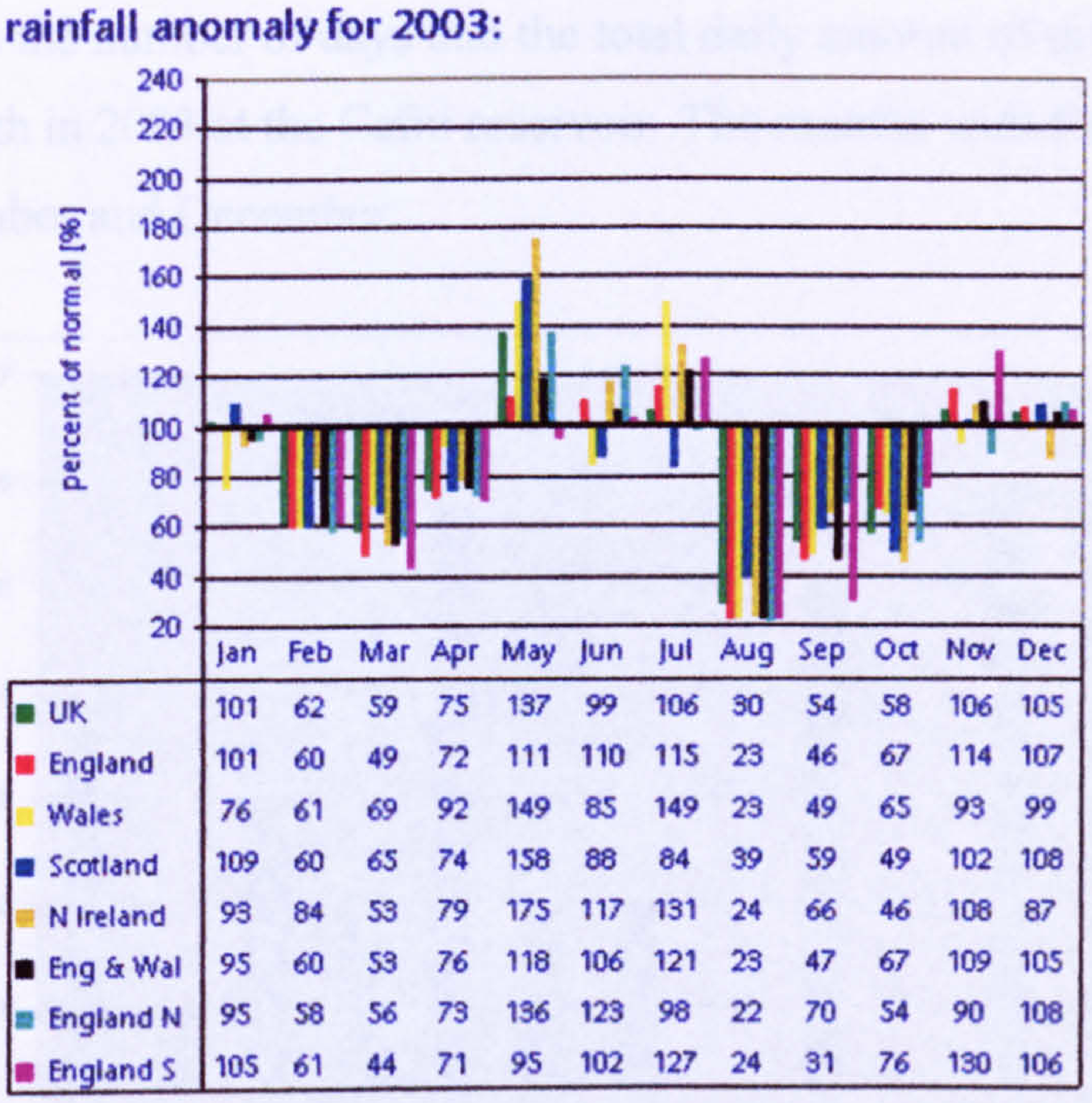


Figure A01 – Long-term data of DOC concentrations at Alaw reservoir, north Wales. Data provided by Welsh Water.

Appendix A2

(for chapter 2)

Figure A02 shows the monthly rainfall received in all parts of the UK for the year 2003, based on the percentage difference from the average. Values in Wales were below average for 10 out of the 12 months, with August and September being exceptionally dry.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
🟩 UK	101	62	59	75	137	99	106	30	54	58	106	105
🟥 England	101	60	49	72	111	110	115	23	46	67	114	107
🟨 Wales	76	61	69	92	149	85	149	23	49	65	93	99
🟦 Scotland	109	60	65	74	158	88	84	39	59	49	102	108
🟧 N Ireland	93	84	53	79	175	117	131	24	66	46	108	87
⬛ Eng & Wal	95	60	53	76	118	106	121	23	47	67	109	105
▨ England N	95	58	56	73	136	123	98	22	70	54	90	108
🟪 England S	105	61	44	71	95	102	127	24	31	76	130	106

Figure A02 – Rainfall values for the UK for 2003 as monthly percentage differences from the long-term average (Met Office, 2004).

Figure A03 shows the total annual rainfall received in the UK from 1915-2005. The year 2003 was the 7th driest in that period.

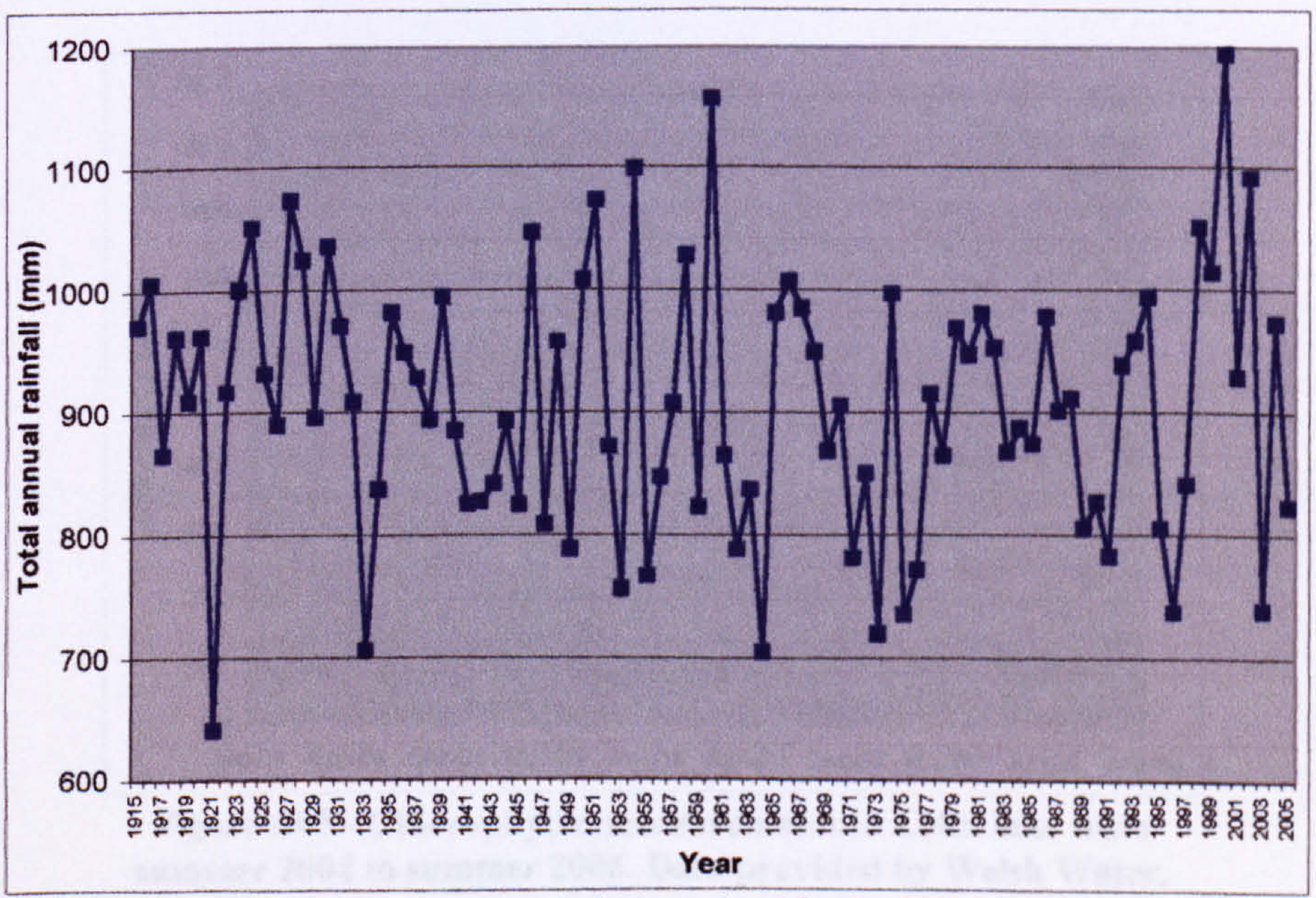


Figure A03 – Total annual rainfall received in the UK from 1915-2005 (data provided by the Met Office).

Figure A04 shows the number of days that the total daily amount of rainfall exceeded 10 mm for each month in 2003 at the Cefni reservoir. The months with the highest values were May, November and December.

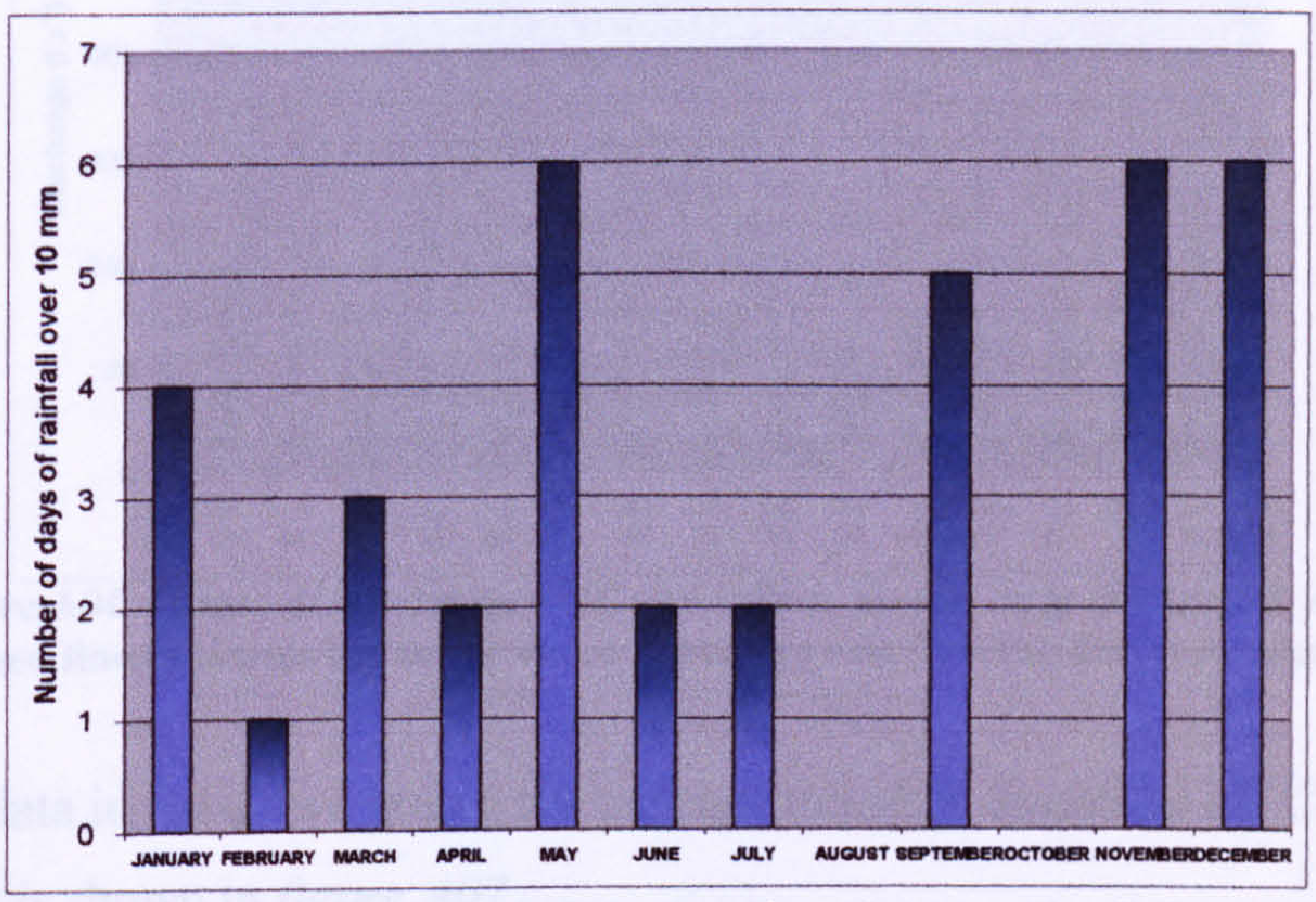


Figure A04 – Number of days of rainfall exceeding 10 mm at the Cefni reservoir for each month during 2003. Data provided by Welsh Water.

Appendix A3

(for chapter 3)

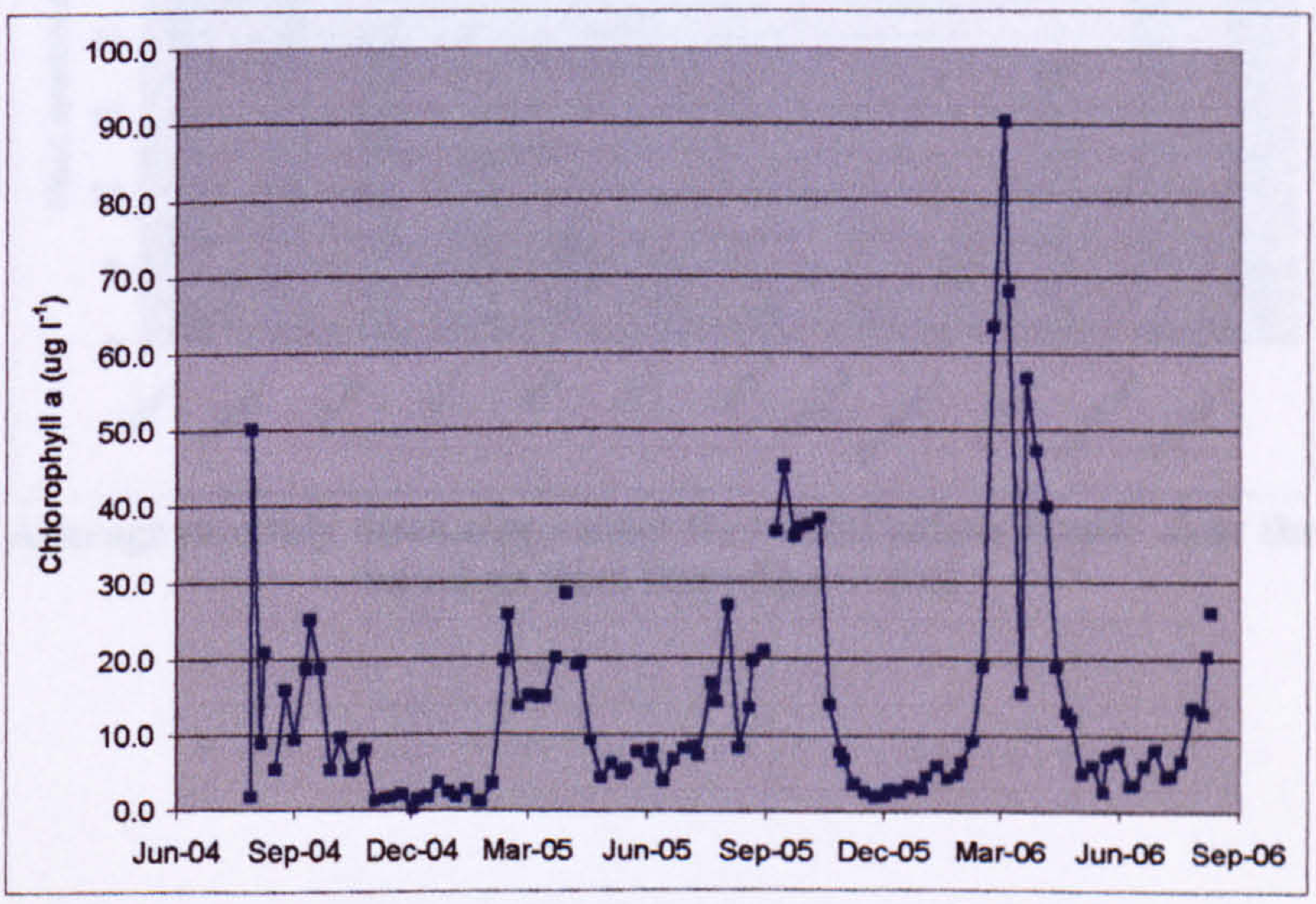


Figure A05 - Chlorophyll *a* measurements in Cefni lake from summer 2004 to summer 2006. Data provided by Welsh Water.

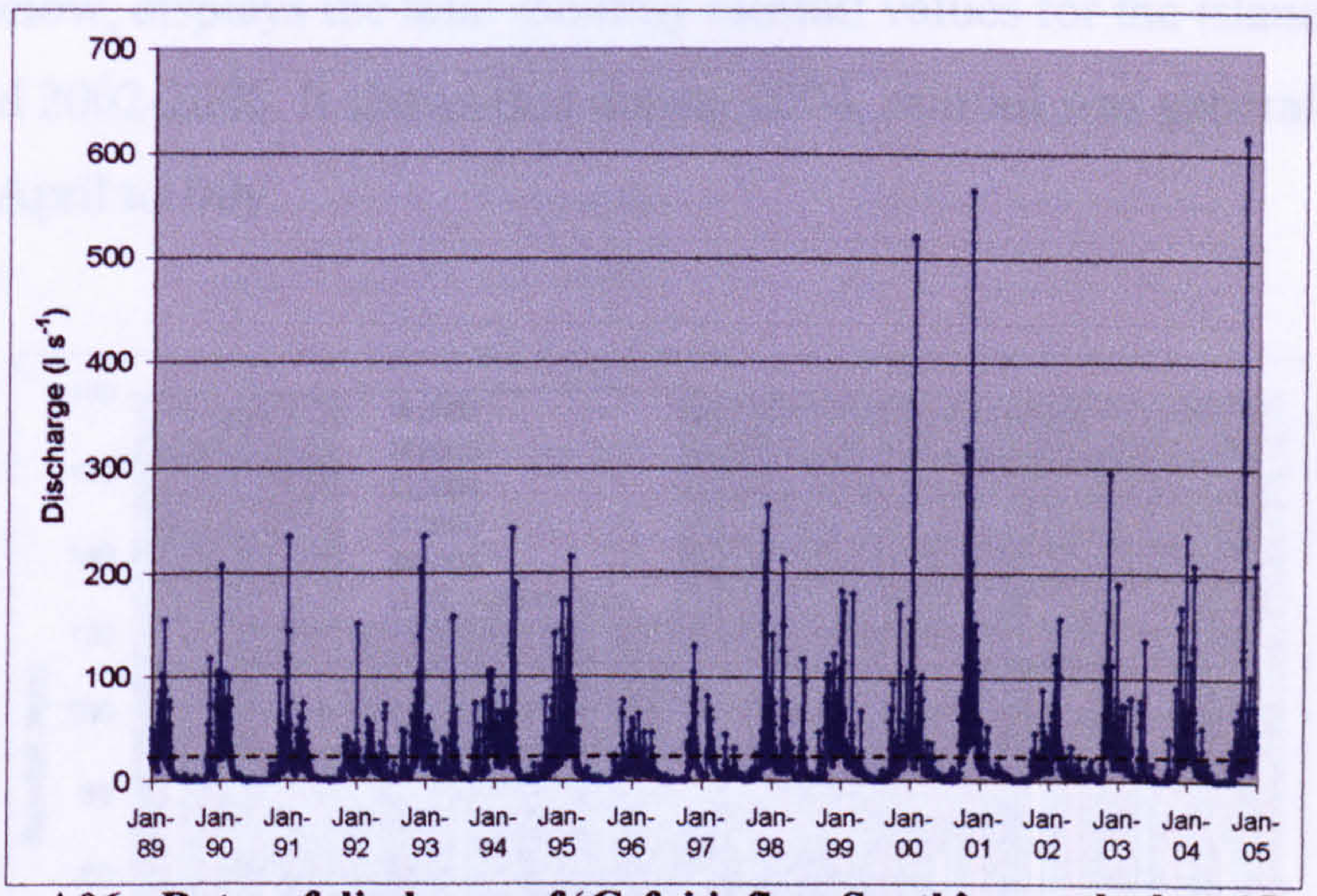


Figure A06 - Rates of discharge of 'Cefni inflow South' over the last 15 years. The dashed line indicates the mean value. Data provided by the Environment Agency.

Based on the data in the above graph, the average monthly discharge of 'CIS' was calculated and is shown in *figure A07*.

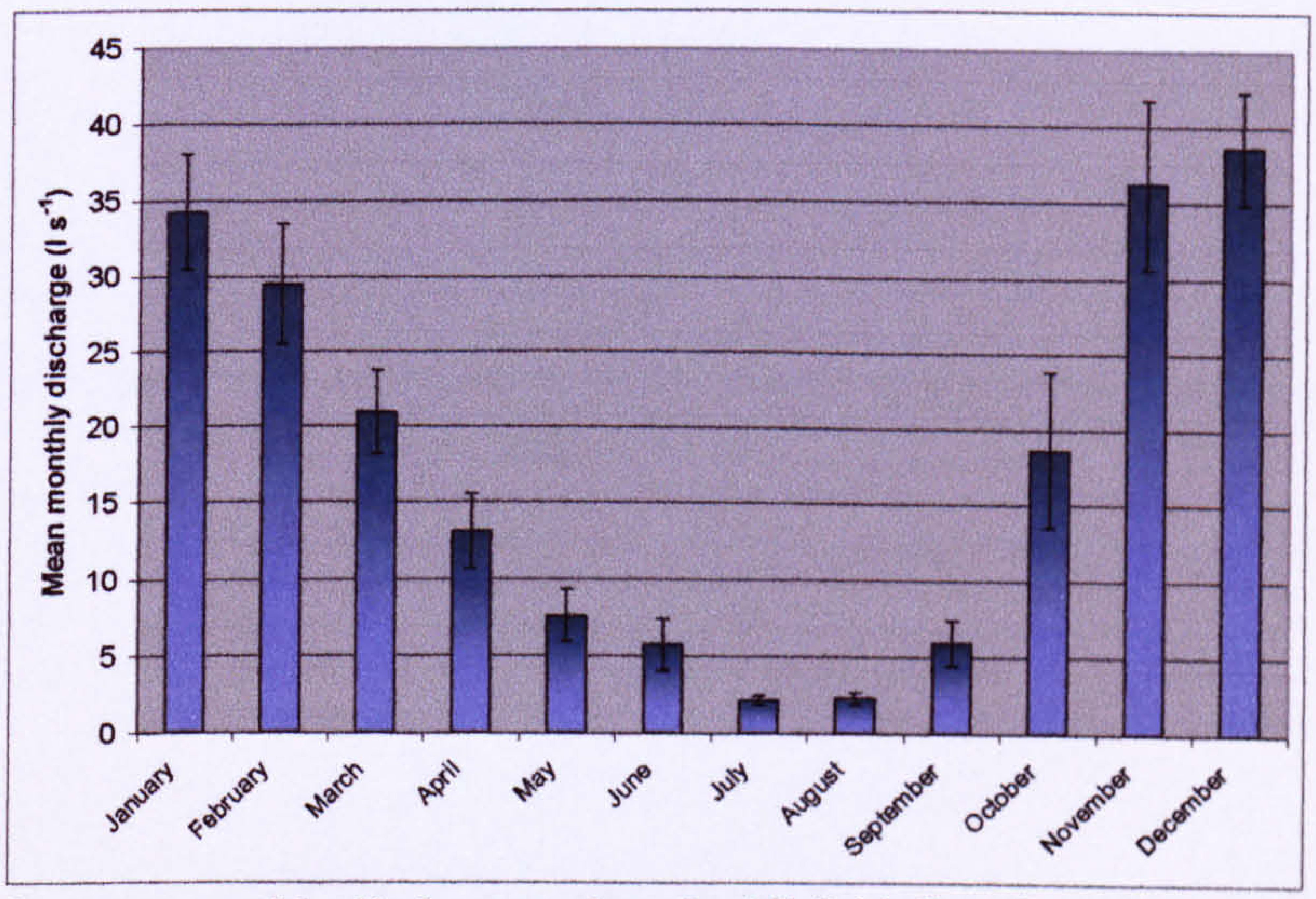


Figure A07 – Average monthly discharge values for 'Cefni inflow South' over the last 15 years, based on data from *figure A06*.

Figure A08, below, displays the total monthly rainfall values for the island of Anglesey over the period 2002-2006. It shows that during 2004, rainfall was generally above average from April to July.

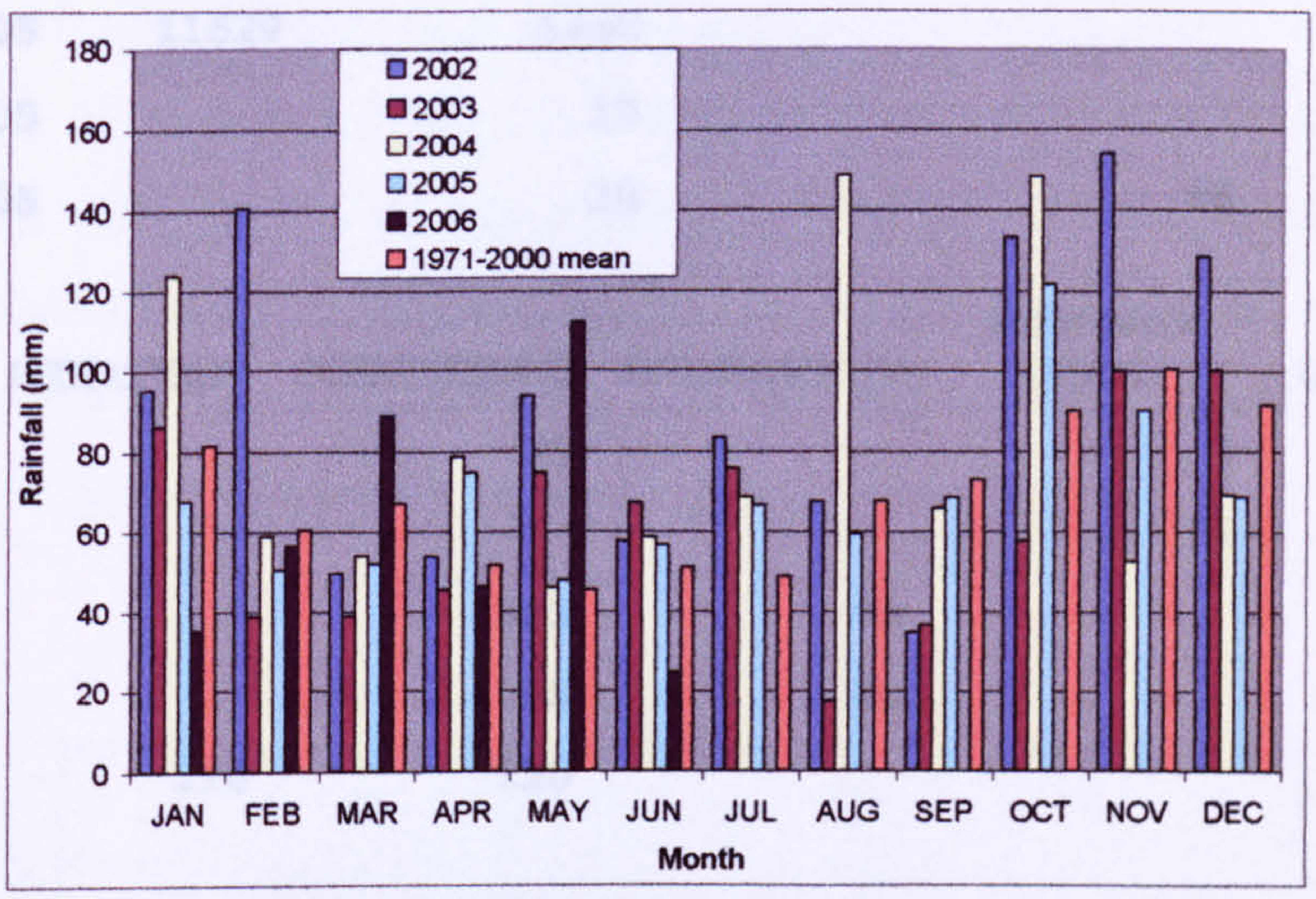


Figure A08 – Monthly totals of rainfall for 2002-2006, together with the 1971-2000 average. Data provided by the Met Office.

	ANABENA	ASTERIONELLA	CHLORELLA	DINOBRYON	MICROCYSTIS	PEDIASTRUM	SCENEDESMUS	STAURASTRUM	STEPHANO-DISCUS	ULOTHRIX	CRYPTOMONAS	UROGLENA	MOUGEOTIA	RHODOMONAS
4-Oct-05		1069	122				75		9	3376	47			56
9-Sep-05	253	206							75	141	19			
25-Aug-05	11629	1466					351	49			49	15636	293	
21-Jul-05		19					38	28						
16-May-05		38		66	28	216	150				47			

Table A01 – Speciation of algae in Cefni lake at the time of collection of lake water for the culturing of algae. Data provided by Welsh Water.

Appendix A4

(for chapter 6)

Measurements of solar UV and visible radiation made at midday during each day of the pilot experiment are shown in *figure A09* and correlate strongly (R^2=0.96), as expected. Percentage cloud cover had a dramatic impact on ground-level UV (R^2=-0.88), reducing values from about 200 mW m^{-2} under cloudless skies to 50 mW m^{-2} during complete cloud cover (*figure A10*). Levels of visible light fell by similar amounts to UV with increasing cloud cover (2000 to 50 µmol m^{-2} sec^{-1}).

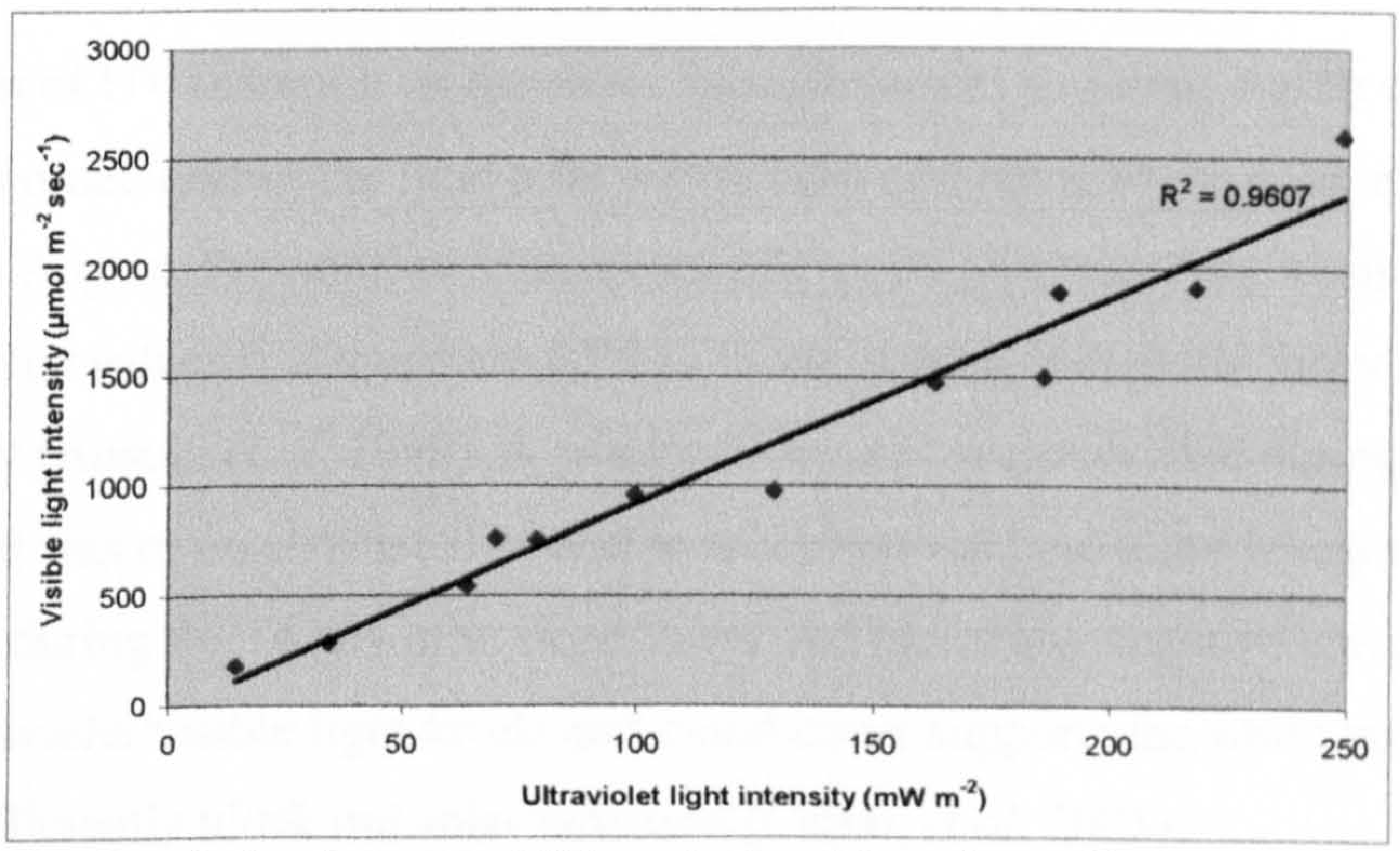


Figure A09 - Correlation between visible and ultraviolet solar radiation recorded at near ground- level during 12 days in June 2005.

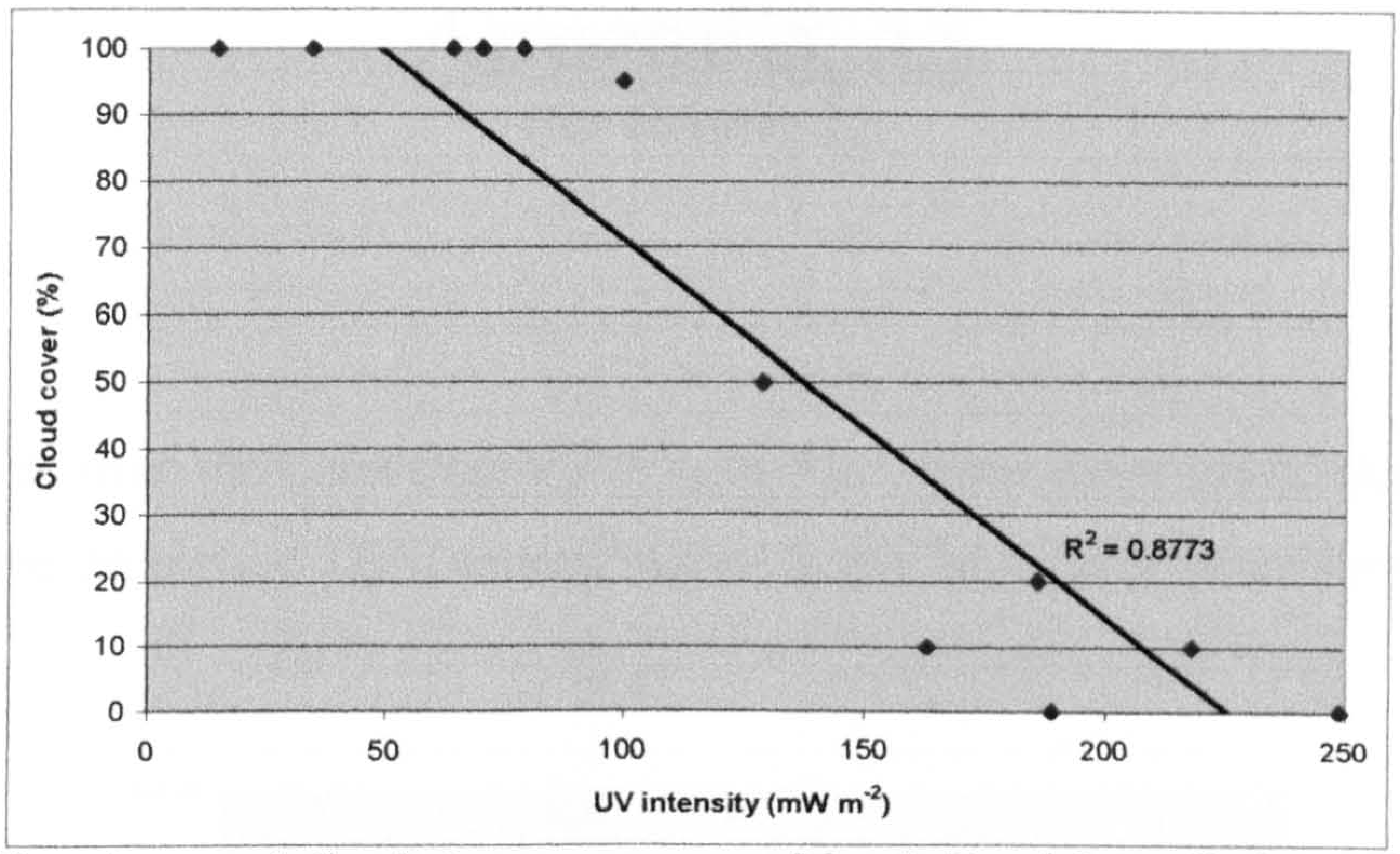


Figure A10 - Negative correlation between near ground-level ultraviolet radiation and cloud cover during 12 days in June 2005.

Higher values of UV radiation on the roof of the Memorial Building during clear, sunny days were recorded during the June pilot experiment compared to the main experiment, performed in August. Previous measurements of ground level ultraviolet light in the UK have shown that values in August are 80% of those in June, due to the lower angle of the sun in the sky (Austin, *et al.* 1999). A similar difference was recorded during this experiment. It was of no surprise that visible and ultraviolet radiation levels correlated so positively during the 14 day pilot experiment and the strong negative correlation between ultraviolet/visible light levels and cloud cover supports the well known fact that clouds can efficiently block out solar radiation (Calbo, *et al.* 2005).

Appendix A5

(for chapter 7)

Long-term data from the Cefni (*figure A11*) and Alaw water treatment works (*figure A12*) show that the highest THM concentrations in the final water occur during the summer.

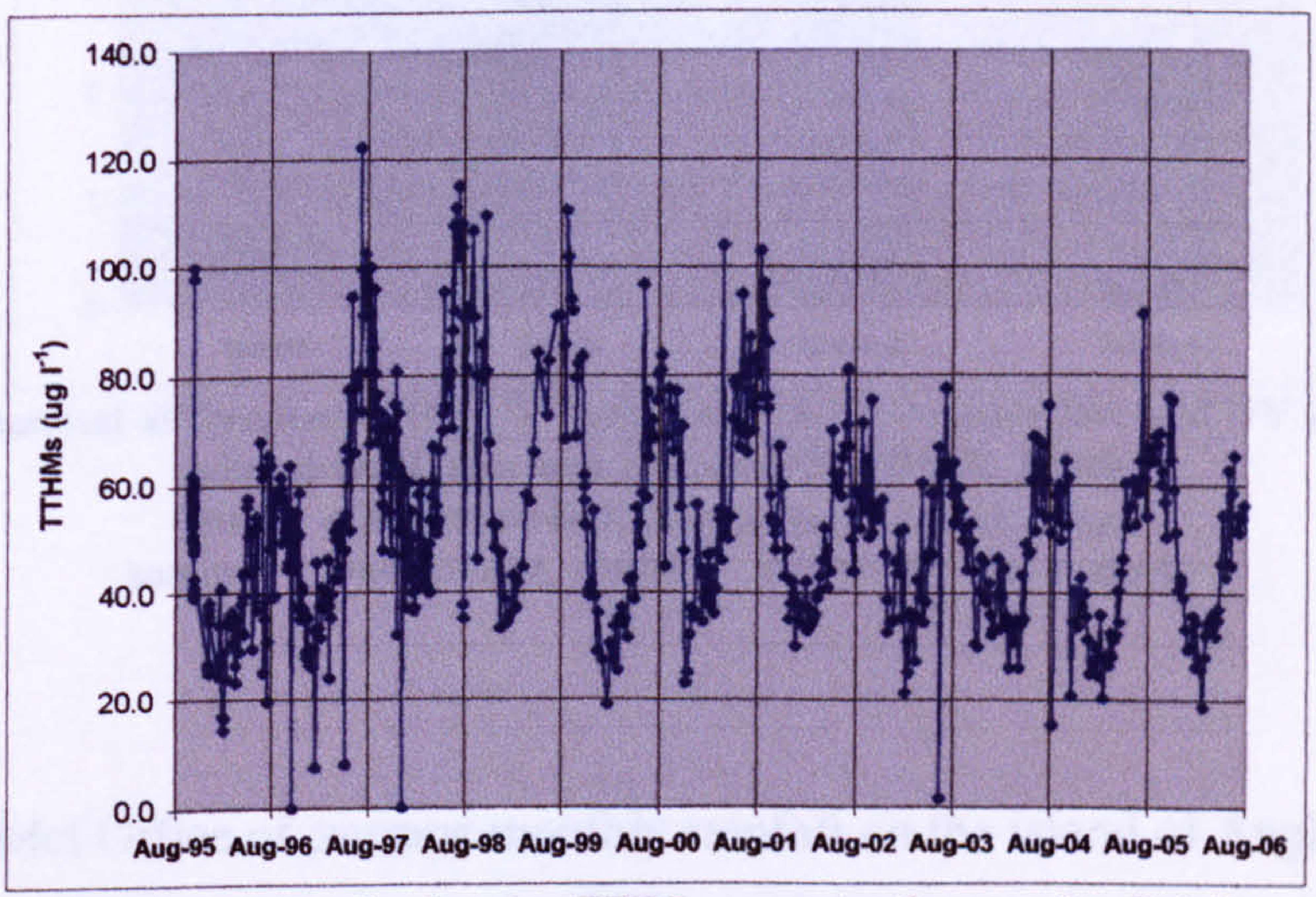


Figure A11 – Long-term final water THM concentrations at the Cefni reservoir. Data provided by Welsh Water.

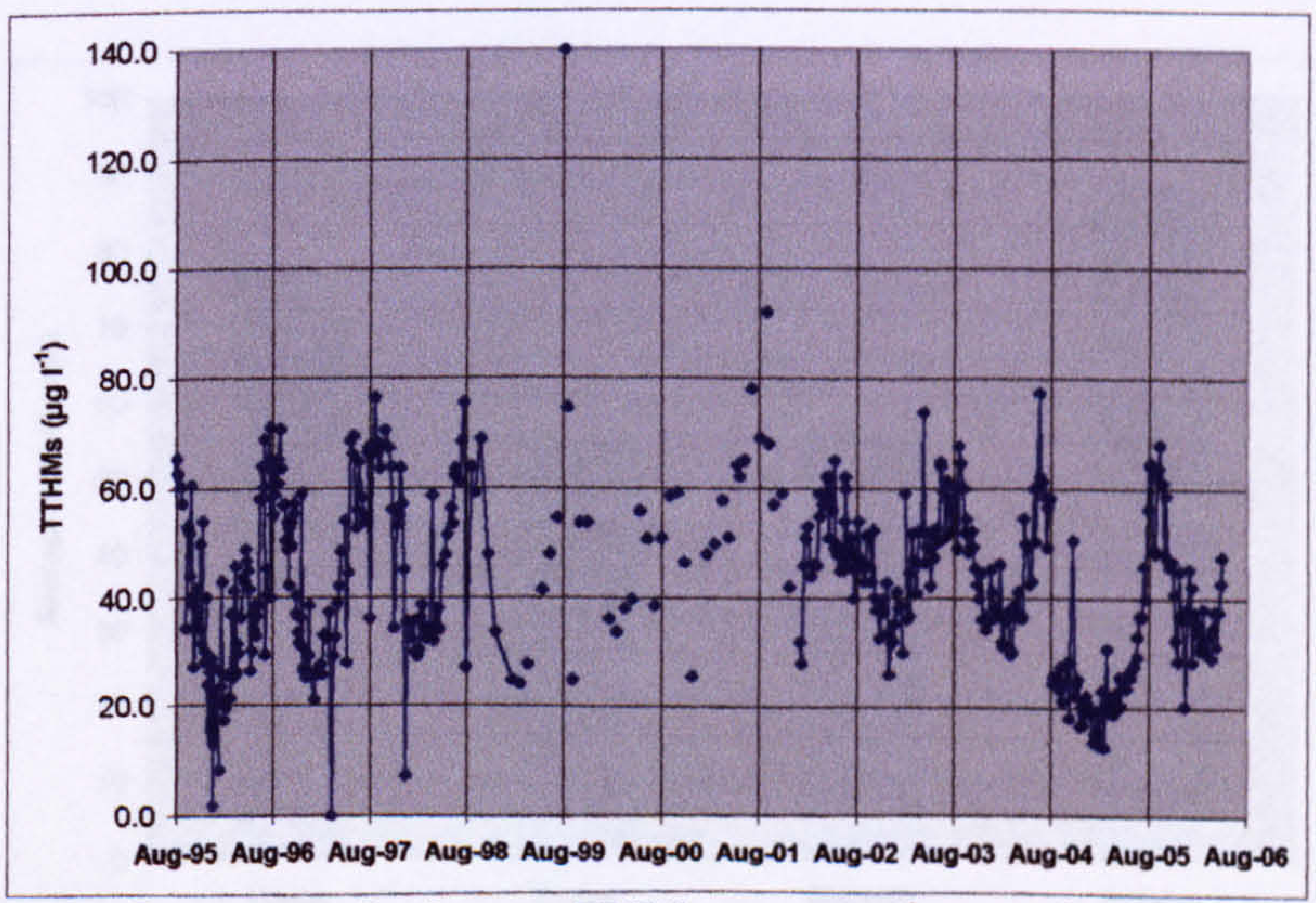


Figure A12 – Long-term final water THM concentrations at the Alaw reservoir. Data provided by Welsh Water.

Ultraviolet light has an important influence on the global freshwater carbon cycle, and *figure A13* shows that levels of UV light are understandably greatest in the summertime.

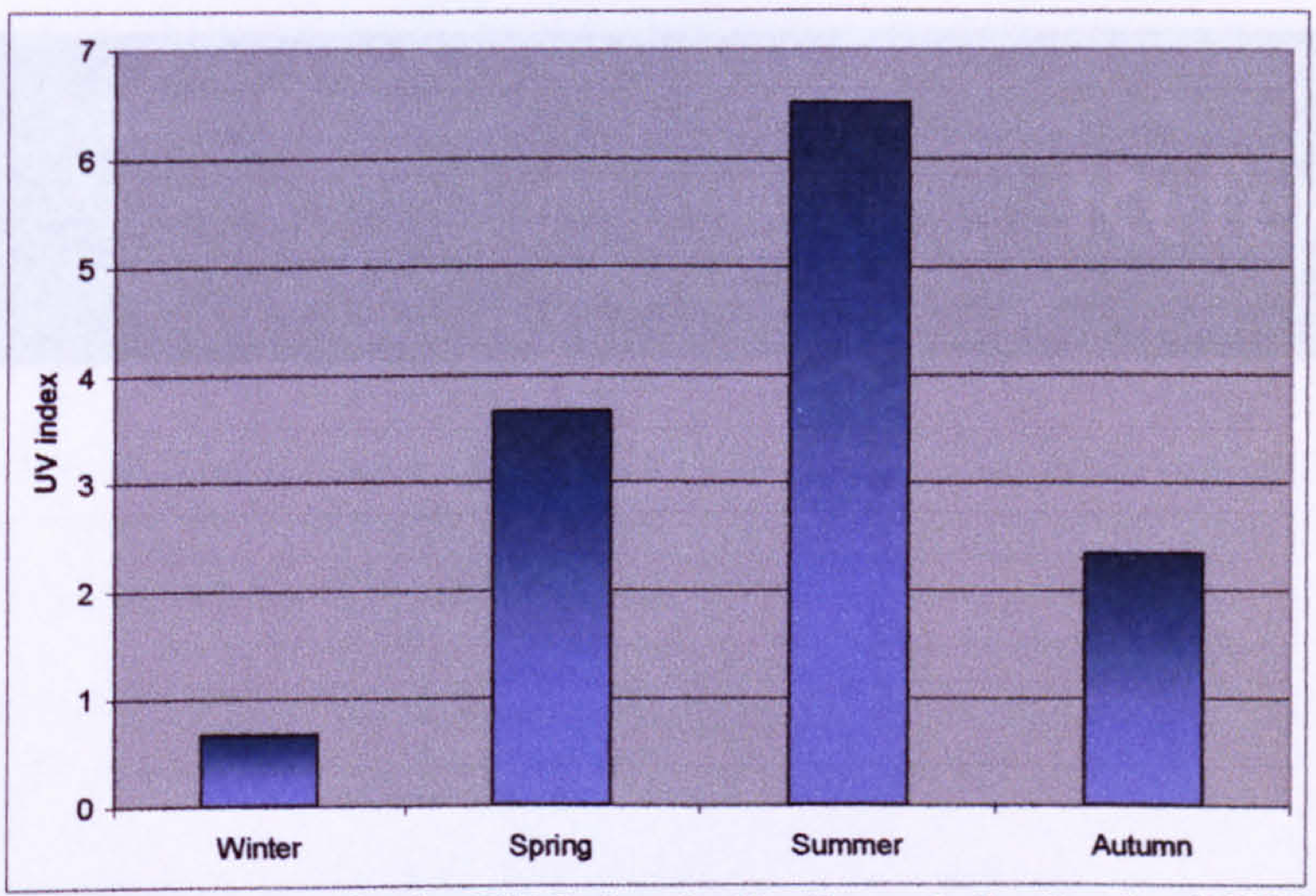


Figure A13 – Seasonal differences in the UV Index, indicating relative levels of UV light incident at ground-level, at a latitude of 56°N (NRPB, 2003)
(winter – December-February, spring – March-May,
summer – June-August, autumn – September-November)

Data from the Met Office of average monthly rainfall on the island of Anglesey for the period 1971-2000 (*figure A14*) shows that more rainfall is experienced in Autumn, with approximately 11% less falling in winter and 38% less falling in spring and summer.

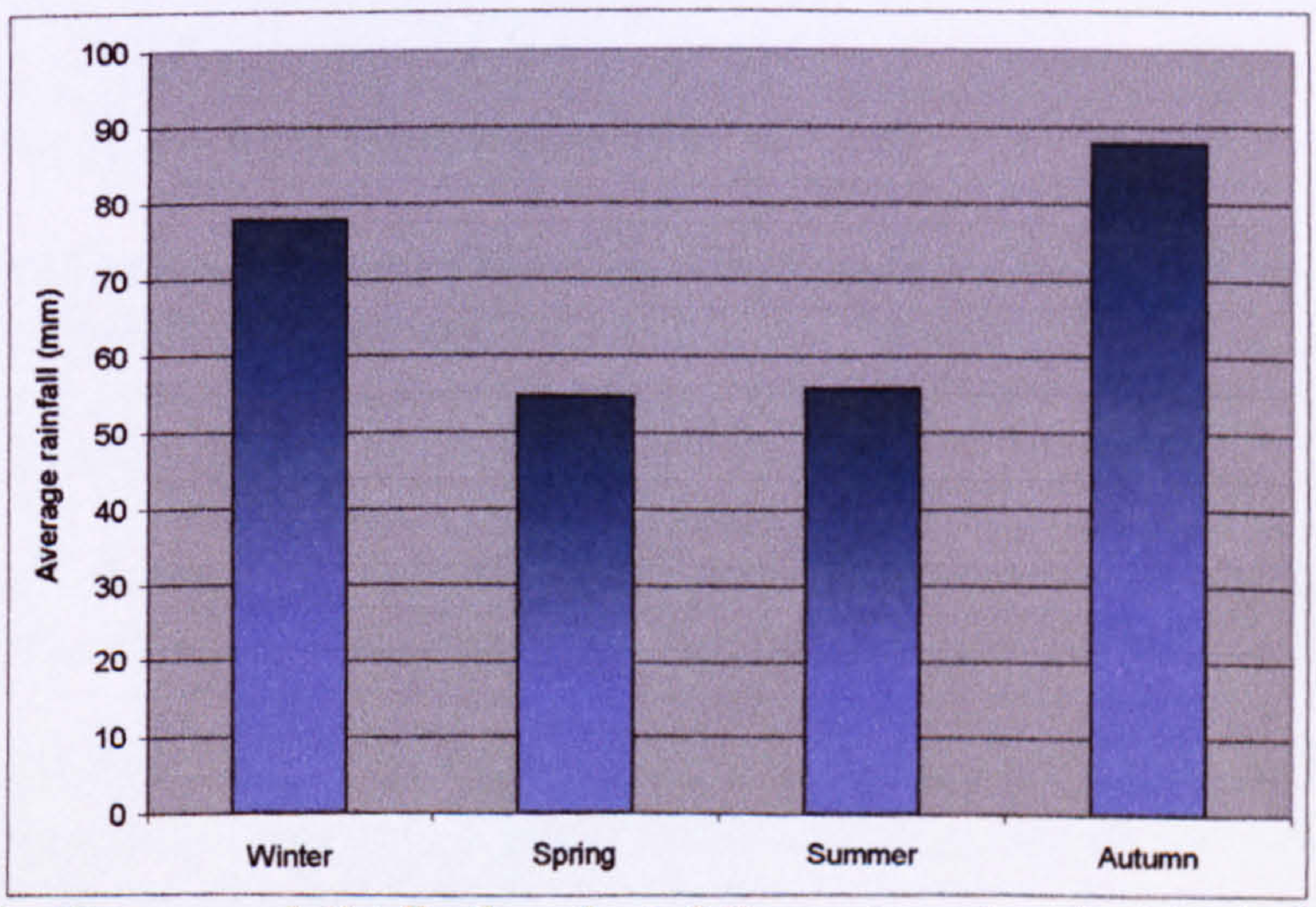


Figure A14 – The seasonal distribution of rainfall experienced on the island of Anglesey. Data provided by the Met Office and is based on measurements between 1971-2000.

References

Aerts, R., Verhoeven, J.T.A. & Whigham, D.F. (1999) *Plant-mediated controls on nutrient cycling in temperate fens and bogs.* Ecology, **80**: 2170-2181.

Aitkenhead, J.A., Hope, D. & Billet, M.F. (1999) *The relationship between dissolved organic carbon in stream water and soil organic carbon pools at different spatial scales.* Hydrological Processes, **13**: 1289-1302.

Al-Omari, A., Fayyad, M. & Qadar, A.A. (2004) *Modeling trihalomethane formation for Jabel Amman water supply in Jordan.* Environmental Modeling and Assessment, **9**: 245-252.

Alarcon Herrera, M.T., Bewtra, J.K. & Biswas, N. (1994) *Seasonal variation in humic substances and their reduction through water treatment processes.* Canadian Journal of Civil Engineering, **21**: 173-179.

Allard, B., Borén, H., Pettersson, C. & Zhang, G. (1994) *Degradation of humic substances by UV irradiation.* Environment International, **20**: 97-101.

American Public Health Association/American Water Works Association/Water Environment Federation (1992) *Standard methods for the examination of water and wastewater, 18th Edition.* APHA/AWWA/WEF, Washington DC, USA.

Ames, B.N., Gold, L.S. & Willett, W.C. (1995) *The causes and prevention of cancer.* Proceedings of the National Academy of Sciences of the United States of America, **92**: 5258-5265.

Amy, G.L., Sierka, J., Bedessem, D.P. & Tan, L. (1992) *Molecular size distribution of dissolved organic matter.* Journal of the American Water Works Association, **84**: 67-75.

Andersen, C.P. & Scagel, C.F. (1997) *Nutrient availability alters belowground respiration of ozone-exposed ponderosa pine.* Tree Physiology, **17**: 377-387.

Andersen, C.P. (2003). *Source-sink balance and carbon allocation below ground in plants exposed to ozone.* New Phytologist, **157**: 213-228.

Arguello, M.D., Chriswell, C.D., Fritz, J.S., Kissinger, L.D., Lee, K.W., Richard, J.J. & Svec, H.J. (1979) *Trihalomethanes in water – report on occurrence, seasonal-variation in concentrations, and precursors of trihalomethanes.* Journal of the American Water Works Association, **71**: 504-508.

Armentano, T.V. & Menges, E.S. (1986) *Patterns of change in the carbon balance of organic-soil wetlands of the temperate zone.* Journal of Ecology, **74**: 755-774.

Arnell, N.W. (1992) *Impacts of climatic change in river flow regimes in the UK.* Journal of Institution of Water and Environmental Management, **6**: 432-442.

Ashmore, M.R. (2005) *Assessing the future global impacts of ozone on vegetation.* Plant, Cell and Environment, **28**: 949-964.

Asplund, G. & Grimvall, A. (1991) *Organohalogens in nature.* Environmental Science and Technology, **25**: 1346-1350.

Austin, J., Driscoll, C.M.H., Farmer, S.F.G. & Molyneux, M.J. (1999) *Late spring ultraviolet levels over the United Kingdom and the link to ozone.* Annales Geophysicae-Atmospheres Hydrospheres and Space Sciences, **17**: 1199-1209.

Avery, L.M., Smith, R.I.L. & West, H.M. (2003) *Response of rhizosphere microbial communities associated with Antarctic hairgrass (Deschampsia antartica) to UV radiation.* Polar Biology, **26**: 525-529.

Balch, M. (2005) *The use of enhanced coagulation at a lowland WTW to improve THM compliance.* MSc thesis, School of Water Sciences, Cranfield University.

Bardgett, R.D., Leemans, D.K., Cook, R. & Hobbs, P.J. (1997) *Seasonality of the soil biota of grazed and ungrazed hill grasslands.* Soil Biology & Biochemistry, **29**: 1285-1294.

Barth, J.A.C. & Veizer, J. (1999) *Carbon cycle in St. Lawrence aquatic ecosystems at Cornwall (Ontario), Canada: seasonal and spatial variations.* Chemical Geology, **159**: 107-128.

Bertilson, S. & Tranvik, L.J. (2000) *Photochemical transformations of dissolved organic matter in lakes.* Limnology and Oceanography, **45**: 753-762.

Bayley, S.E., Thormann, M.N. & Szumigalski, A.R. (2005) *Nitrogen mineralization and decomposition in western boreal bog and fen peat.* Ecoscience, **12**: 455-465.

Beckmann, M., Sheppard, S.K. & Lloyd, D. (2004) *Mass spectrometric monitoring of gas dynamics in peat monoliths: effects of temperature and diurnal cycles on emissions.* Atmospheric Environment, **38**: 6907-6913.

Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M. & Kirk, G.J.D. (2005) *Carbon losses from all soils across England and Wales 1978-2003.* Nature, **437**: 245-248.

Berman-Frank, I., Kaplan, A., Zohary, T. & Dubinsky, Z. (1995) *Carbonic Anhydrase Activity in the Bloom Forming Dinoflagellate Peridinium Gatunense,* Journal of Phycology, **31**: 906-913.

Biester, H., Keppler, F., Putschew, A., Martinez-Cortizas, A. & Petri, M. (2004) *Halogen retention, organohalogens, and the role of organic matter decomposition on halogen enrichment in two Chilean peat bogs.* Environmental Science and Technology, **38**: 1984-1991.

Billings, W.D. (1987) *Carbon balance of Alaska tundra and taiga ecosystems: past, present and future.* Quaternary Science Reviews, **6**: 165-177.

Black, B.D., Harrington, G.W. & Singer, P.C. (1996) *Reducing cancer risks by improving organic carbon removal.* Journal of the American Water Works Association, **88**: 40-52.

Black, V.J., Black, C.R., Roberts, J.A. & Stewart, C.A. (2000) *Impact of ozone on the reproductive development of plants.* New Phytologist, **147**: 421-447.

Blodau, C. (2002) *Carbon cycling in peatlands – A review of processes and controls.* Environment Reviews, **10**: 111-134.

Blum, U. & Tingey, D.T. (1977) *A study of the potential ways in which ozone could reduce root growth and modulation of soybean.* Atmospheric Environment, **11**: 737-739.

Bonnett, S. (2004) *Biogeochemical implications of plant-soil interactions in wetland ecosystems.* PhD thesis, Biological Sciences, University of Wales, Bangor.

Box, J.D. (1983) *Investigation of the Folin-Ciocalteau Phenol reagent for the determination of polyphenolic substances in natural waters.* Water Research, **17**: 249-261.

Braekke, F.H. (1981) *Hydrochemistry of high altitude catchments in southern Norway. 3, dynamics in water flow and release-fixation of sulphate, nitrate and hydronium.* Norsk Institut for Skogforskning, **36**: 1-21.

Bragazza, L. & Limpens, J. (2004) *Dissolved organic nitrogen dominates in European bogs under increasing N deposition.* Global Biogeochemical Cycles, **18**: GB4018.

Brinkmann, T., Hörsch, P., Sartorius, D. & Frimmel, F.H. (2003*a*) *Photoformation of low-molecular-weight organic acids from brown water dissolved organic matter.* Environmental Science and Technology, **37**: 4190-4198.

Brinkmann, T., Sartorius, D. & Frimmel, F.H. (2003*b*) *Photobleaching of humic rich dissolved organic matter.* Aquatic Science, **65**: 415-424.

Brock, T.C.M. & Bregman, R. (1989) *Periodicity in growth, productivity, nutrient content and decomposition of Sphagnum recurvum var. mucronatum in a fen woodland.* Oecologia, **80**: 44-52.

Brooks, E. (2005) *Assessing the potential of terrestrially derived dissolved organic carbon to form carcinogenic trihalomethanes during water treatment.* MSc thesis. Department of Chemistry, University of Wales, Bangor.

Brooks, P.D., O'Reilly, C.M., Diamond, S.A., Campbell, D.H., Knapp, R., Bradford, D., Corn, P.S., Hossack, B. & Tonnessen, K. (2005) *Spatial and temporal variability in the amount and source of dissolved organic carbon: implications for ultraviolet exposure in amphibian habitats.* Ecosystems, **8**: 478-487.

Bruhl, C. & Crutzen, P.J. (1989) *On the disproportionate role of tropospheric ozone as a filter against solar UV-B radiation.* Geophysical Research Letters, **16**: 703-706.

Bull, R.J., Birnbaum, L.S., Cantor, K.P., Rose, J.B., Butterworth, B.B., Pegram, R. & Tuomisto, J. (1995) *SYMPOSIUM OVERVIEW – Water Chlorination: Essential Process or Cancer Hazard?* Fundamental and Applied Toxicology, **28**: 155-166.

Burns, R.G. (1978) *Soil Enzymes.* Academic Press, London, UK.

Bushaw, K.L., Zepp, R.G., Tarr, M.A., Schulz-Jander, D., Bourbonniere, R.A., Hodson, R.E., Miller, W.L., Bronk, D.A. & Moran, M.A. (1996) *Photochemical release of biologically available nitrogen from aquatic dissolved organic matter.* Nature, **381**: 404-407.

Buzby, K.M. & Perry, S.A. (2000) *Modelling the potential effects of climate change on leaf pack processing in central Appalachian streams.* Canadian Journal of Fisheries and Aquatic Sciences, **57**: 1773-1783.

Calbo, J., Pages, D. & Gonzales, J.A. (2005) *Empirical studies of cloud effects on UV radiation: A review.* Reviews of Geophysics, **43**: Art No. RG2002.

Cannell, M.G.R., Milne, R., Hargreaves, K.J., Brown, T.A.W., Cruickshank, M.M., Bradley, R.I., Spencer, T., Hope, D., Billett, M.F., Adger, W.N. & Subak, S. (1999) *National inventories of terrestrial carbon sources and sinks: The UK experience.* Climatic Change, **42**: 505-530.

Cao, M. & Woodward, I. (1998) *Net primary and ecosystem production and carbon stocks of terrestrial ecosystems and their responses to climate change.* Global Change Biology, **4**: 185-198.

Capece, J. (1998) *Trihalomethanes and Our Water Supply.* Available from http://www.southerndatastream.com/thm/. Last accessed 19-09-2006.

Cardoza, L.A., Korir, A.K., Otto, W.H., Wurrey, C.J. & Larive, C.K. (2004) *Applications of NMR spectroscopy in environmental science.* Progress in Nuclear Magnetic Resonance Spectroscopy, **45**: 209-238.

Casper, P., Maberly, S.C., Hall, G.H. & Finlay, B.J. (2000) *Fluxes of methane and carbon dioxide from a small productive lake to the atmosphere.* Biogeochemistry, **49**: 1-19.

Chapin III, F.S., Sturm, M., Serreze, M.C., *et al.* (2005) *Role of land-surface changes in Arctic summer warming.* Science, **310**: 657-660.

Chapman, P.J., Edwards, A.C. & Cresser, M.S. (2001) *The nitrogen composition of streams in upland Scotland: some regional and seasonal differences.* The Science of the Total Environment, **265**: 65-83.

Charman, D. (2002) *Peatlands and Environmental Change.* John Wiley & Sons, Ltd.

Chatila, K., Demers, S., Mostajir, B., Gosselin, M., Chanut, J-P., Monfort, P. & Bird, D. (2001) *The responses of a natural bacterioplankton community to different levels of ultraviolet-B radiation: a food web perspective.* Microbial Ecology, **41**: 56-68.

Chen, Y., Senesi, N. & Schnitzer, M. (1977) *Information provided on humic substances by E4/E6 ratios.* Soil Science Society of America Journal, **41**: 352-358.

Chen, Y., Khan, S.U. & Schnitzer, M. (1978) *Ultraviolet irradiation of dilute fulvic acid solutions.* Soil Science Society of American Journal, **42**: 292-296.

Chen, C.R., Condron, L.M., Davis, M.R. & Sherlock, R.R. (2004) *Effects of plant species on microbial biomass phosphorus and phosphatase activity in a range of grassland soils.* Biology and Fertility of Soils, **40**: 313-322.

Cheng, W. & Chi, F. (2003) *Influence of eutrophication on the coagulation efficiency in reservoir water.* Chemosphere, **53**: 773-778.

Cheng, W. & Coleman, D.C. (1990) *Effect of living roots on soil organic matter decomposition.* Soil, Biology & Biochemistry, **22**: 781-787.

Chin, Y., Aiken, G. & O'Loughlin, E. (1994) *Molecular weight polydispersity, and spectroscopic properties of aquatic humic substances.* Environmental Science and Technology, **28**: 1853-1858.

Cho, D-H., Kong, S-H. & Oh, S-G. (2003) *Analysis of trihalomethanes in drinking water using headspace-SPME technique with gas chromatography.* Water Research, **37**: 402-408.

Chow, A.T., Tanji, K.K. & Gao, S. (2003) *Production of dissolved organic carbon (DOC) and trihalomethane (THM) precursor from peat soils.* Water Research, **37**: 4475-4485.

Chow, A.T., Gao, S. & Dahlgren, R.A. (2005*a*) *Physical and chemical fractionation of dissolved organic matter and trihalomethane precursors: a review.* Journal of Water Supply Research and Technology – AQUA, **54**: 475-507.

Chow, A.T., Guo, F., Gao, S., Breuer, R. & Dahlgren, R.A. (2005*b*) *Filter pore size selection for characterizing dissolved organic carbon and trihalomethane precursors from soils.* Water Research, 39: 1255-1264.

Chow, A.T., Tanji, K.K., Gao, S. & Dahlgren, R.A. (2006) *Temperature, water content and wet-dry cycle effects on DOC production and carbon mineralization in agricultural peat soils.* Soil Biology & Biochemistry, 38: 477-488.

Chu, H. & Nieuwenhuijsen, M.J. (2002) *Distribution and determinants of trihalomethane concentrations in indoor swimming pools.* Occupational Environmental Medicine, 59: 243-247.

Chung, H., Zak, D.R. & Lilleskov, E.A. (2006) *Fungal community composition and metabolism under elevated CO_2 and O_3.* Oecologia, 147: 143-154.

Clair, T.A. & Sayer, B.G. (1997) *Environmental variability in the reactivity of freshwater dissolved organic carbon to UV-B.* Biogeochemistry, 36: 89-97.

Clark, J.M., Chapman, P.J., Heathwaite, A.L. & Adamson, J.K. (2006) *Suppression of dissolved organic carbon by sulfate induced acidification during simulated droughts.* Environmental Science and Technology, 40: 1776-1783.

Cleveland, C.C., Read, S.C. & Townsend, A.R. (2006) *Nutrient regulation of organic matter decomposition in a tropical rain forest.* Ecology, 87: 492-503.

Clymo, R.S. (1963) *Ion exchange in Sphagnum and its relation to bog ecology.* Annals of Botany (London). New series, 27: 309-324.

Clymo, R.S. (1983) *Peat.* In: Goodawll, D. W. (Ed.) Ecosystems of the World, vol. 4A, Mires: Swamp, Bog, Fen and Moor. Elseview, Amsterdam, pp. 159-224.

Cole, J.J., Caraco, N.F., Kling, G.W. & Kratz, T.K. (1994) *Carbon-dioxide supersaturation in the surface waters of lakes.* Science, 265: 1568-1570.

Coleman, M.D., Dickson, R.E., Isebrands, J.G. & Karnosky, D.F. (1995) *Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone.* Tree Physiology, 15: 593-604.

Collins, M.R., Amy, G.L., Steelink, C. (1986) *Molecular Weight Distribution, Carboxylic Acidity, and Humic Substances Content of Aquatic Organic Matter: Implications for Removal during Water Treatment.* Environmental Science & Technology, 20: 1028-1032.

Cook, R.L. (2004) *Coupling NMR to NOM.* Analytical and Bioanalytical Chemistry, 378: 1484-1503.

Cooley, D.R. & Manning, W.J. (1987) *The impact of ozone on assimilate partitioning in plants: a review.* Environmental Pollution, 47: 95-113.

Conyers, M.K., Uren, N.C. & Helyar, K.R., Poile, G.J. & Cullis, B.R. (1997) *Temporal variation in soil acidity.* Australian Journal of Soil Research, 35: 1115-1129.

Coyle, M. & Fowler, D. (2003) *New Directions: Implications of increasing tropospheric background ozone concentrations for vegetation.* Atmospheric Environment, 37: 153-154.

Craun, G.F., Bull, R.J., Clark, R.M., Doull, J., Grabow, W., Marsh, G.M., Ojun, D.A., Regli, S., Sobsey, M.D. & Symons, J.M. (1994) *Balancing chemical and microbial risks of drinking water disinfection. I. Benefits and potential risks.* Journal of Water Supply and Research Technology-AQUA, 43: 192-199.

Crutzen, P.J. (1992) *Ultraviolet on the increase.* Nature, 356: 104-105.

Curtis, P.J. & Schindler, D.W. (1997) *Hydrologic control of dissolved organic matter in low-order Precambrian Shield lakes.* Biochemistry, 36: 125-138.

Curtis, P.J. (1998) *Climatic and hydrologic control of DOM concentration and quality in lakes.* Ecological Studies, 133: 93-105.

Cybulski, W.J. & Peterjohn, W.T. (1999) *Effects of ambient UV-B radiation on the above-ground biomass of seven temperate-zone plant species.* Plant Ecology, 145: 175-181.

Danilov, R.A. & Ekelund, N.G.A. (2001) *Effects of solar radiation, humic substances and nutrients on phytoplankton biomass and distribution in lake Solumsjö, Sweden.* Hydrobiologia, 444: 203-212.

Davidson, E.A., Trumbore, S.E. & Amundson, R. (2000) *Biogeochemistry: Soil warming and organic carbon content.* Nature, 408: 789-790.

Davidson, E.A. & Janssens, I.A. (2006) *Temperature sensitivity of soil carbon decomposition and feedbacks to climate change.* Nature, 440: 165-173.

Davidsson, T.E. & Ståhl, M. (2000) *The influence of organic carbon on nitrogen transformations in five wetland soils.* Soil Science Society of America Journal, 64: 1129-1136.

Davies, G. & Ghabbour, E.A. (eds.) (1998) *Humic substances: structures, properties and uses.* Royal Society of Cambridge.

Davis, O.L. & Goldsmith, P.L. (eds.) (1972) *Statistical methods in research and production.* Oliver & Boyd.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H. & Jones, K.M. (1986) *Data for biochemical research (3^{rd} ed.).* Clarendon Press.

Dawson, J.J.C., Billett, M.F., Neal, C. & Hill, S. (2002) *A comparison of particulate, dissolved and gaseous carbon in two contrasting upland streams in the UK.* Journal of Hydrology, **257**: 226-246.

De Haan, H. & De Boor, T. (1991) *UV-degradation of aquatic humic substances.* Finnish Humus News, **3**: 177-182.

De Hann, H. (1993) *Solar UV-light penetration and photodegradation of humic substances in peaty lake water.* Limnology and Oceanography, **38**: 1072-1076.

Degens, E.T. (1982) *Transport of carbon and minerals in major world rivers, part 1.* Proceedings of a workshop arranged by scientific committee on problems of the environment (SCOPE) and the United Nations Environment Programme (UNEP). Hamburg University, Hamburg, Germany.

Dick, W.A. & Tabatabai, M.A. (1993) *Significance and potential uses of soil enzymes.* In: Soil Microbial Ecology: Applications in Agricultural and Environmental Management (Metting BJ, ed), pp. 95–127. Marcel Dekker Inc., New York.

Dickinson, C.H. (1983) *Microorganisms in Peatlands.* In: Gore, A.J.P. (Ed.), Ecosystems of the World 4A. Elsevier, Amsterdam, pp. 225–243.

Dillon, P.J. & Molot, L.A. (1997) *Dissolved organic and inorganic carbon mass balances in central Ontario lakes.* Biogeochemistry, **36:** 29-42.

Dioumaeva, I., Trumbore, S., Schuur, E.A.G., Goulden, M.L., Litvak, M. & Hirsch, A.I. (2002) *Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon.* Journal of Geophysical Research – Atmospheres, **108**: Art. No. 8222.

Dodds, L., King, W., Woolcott, C. & Pole, J. (1999) *Trihalomethanes in public drinking water supplies and adverse outcomes.* Epidemiology, **10**: 233-237.

Dodds, L., King, W., Allen, A.C., Armson, B.A., Fell, D.B. & Nimrod, C. (2004) *Trihalomethanes in public water supplies and the risk of stillbirth.* Epidemiology, **15**: 179-186.

Domisch, T., Finer, L., Laine, J. & Laiho, R. (2006) *Decomposition and nitrogen dynamics of litter in peat soils from two climatic regions under different temperature regimes.* European Journal of Soil Biology, **42**: 74-81.

Dowrick, D. (1998) *Laboratory studies of biogeochemical processes in wetlands subject to simulated climate change.* PhD thesis. School of Biological Sciences, University of Wales, Bangor.

Drinking Water Inspectorate – DWI (1998) *New Drinking Water Regulations in the UK.*

Duce, R.A., Winchester, J.W. & Van Nahl, T.W. (1965) *Iodine, bromine and chlorine in the Hawaiian marine atmosphere.* Journal of Geophysical Research, **70**: 1775.

Duckmanton, L & Widden, P. (1994) *Effect of ozone on the development of vesicular-arbuscular mycorrhizae in sugar maple saplings.* Mycologia, **86**: 181-186.

Dunnick, J.K. & Melnick, R.L. (1993) *Assessment of the carcinogenic potential of chlorinated water – experimental studies of chlorine, chloramine, and trihalomethanes.* Journal of the National Cancer Institute, **85**: 817-822.

Edwards, N. (1991) *Root and soil respiration responses to ozone in Pinus taeda L. seedlings.* New Phytologist, **118**: 315-321.

Edzwald, J.K., Becker, W.C. & Wattier, K.L. (1985) *Surrogate parameters for monitoring organic matter and THM precursors.* Journal of the American Water Works Association, **77**: 122-132.

EEA (European Environment Agency) (2000) *EEA – Reports – Environmental Signals 2000 – 9. Stratospheric Ozone Depletion.* Available at http://reports.eea.eu.int/signals-2000/en/page010.html Last accessed 25-09-2006.

Eimers, M.C., Dillon, P.J., Schiff, S.L. & Jeffries, D.S. (2003) *The effects of drying and re-wetting and increased temperature on sulphate release from upland and wetland material.* Soil Biology & Biochemistry, **35**: 1663-1673.

Elder, J.F., Rybicki, N.B., Carter, V. & Weintraub, V. (2000) *Sources and yields of dissolved carbon in northern Wisconsin stream catchments with differing amounts of peatland.* Wetlands, **20**: 113-125.

Eswaran, H., Van den Berg, E., Reich, P. & Kimble, J. (1995) *Global soil carbon resources.* In: Soil and global change. Lal, R., *et al.* (eds.), pp. 27-45. Boca Raton: Lewis Publishers.

Evans, M.G., Burt, T.P., Holden, J. & Adamson, J.K. (1999) *Runoff generation and water table fluctuations in blanket peat: evidence from UK data spanning the dry summer of 1995.* Journal of Hydrology, **221**: 141-160.

Evans, C.D., Monteith, D.T. & Cooper D.M. (2005) *Long-term increases in surface water dissolved organic carbon: observations, possible causes and environmental impacts.* Environmental Pollution, **137**: 55-71.

Evans, C.D., Chapman, P.J., Clark, J.M., Monteith, D.T. & Cresser, M.S. (2006) *Alternate explanations for rising dissolved organic carbon export from organic soils.* Global Change Biology, **12**: 2044-2053.

Fahmi, Nishijima, W. & Okada, M. (2003) *Characterization of organic matter in ozonation and biological treatment.* Journal of Water Supply Research and Technology-AQUA, **52**: 291-297.

Fang, C. & Moncrieff, J.B. (2001) *The dependence of soil CO_2 efflux on temperature.* Soil Biology & Biochemistry, **33**: 155-165.

Fang, C.M., Smith, P., Moncrieff, J.B. & Smith, J.U. (2005) *Similar response of labile and resistant soil organic matter pools to changes in temperature.* Nature, **433**: 57-59.

Fanzaring, J., Tonneijck, A.E.G., Kooijman, A.W.N. & Dueck, Th.A. (2000) *Growth responses to ozone in plant species from wetlands.* Environmental and Experimental Botany, 44: 39-48.

Farham, J.C., Gardiner, B.G. & Shanklin, J.D. (1985) *Large losses of total ozone in Antarctica reveal seasonal ClOx/NOx interactions.* Nature, **315**: 207-210.

Farrish, K.W. & Grigal, D.F. (1988) *Decomposition in an ombrotrophic bog and a mineratrophic fen in Minnesota.* Soil Science, **145**: 353-358.

Fenner, N., Freeman, C., Hughes, S. & Reynolds, B. (2001) *Molecular weight spectra of dissolved organic carbon in a rewetted Welsh peatland and possible implications for water quality.* Soil Use and Management, **17**: 106-112.

Fenner, N. (2002) *The effects of climate change on dissolved organic carbon release from peatlands.* PhD thesis. School of Biological Sciences, University of Wales, Bangor.

Fenner, N., Ostle, N., Freeman, C., Sleep, D. & Reynolds, B. (2004) *Peatland carbon efflux partitioning reveals that Sphagnum photosynthate contributes to the total DOC pool.* Plant and Soil, **259**: 345-354.

Fenner, N., Freeman, C. & Reynolds, B. (2005*a*) *Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling.* Soil Biology & Biochemistry, **37**: 1277-1287.

Fenner, N., Freeman, C. & Reynolds, B. (2005*b*) *Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for the global carbon cycle and soil enzyme methodologies.* Soil Biology & Biochemistry, **37**: 1814-1821.

Findlay, S.E.G. (2005) *Increased carbon transport in the Hudson River: unexpected consequence of nitrogen deposition?* Frontiers in Ecology and the Environment, **3**: 133-137.

Finlayson-Pitts, B.J. & Pitts, J.N. (1997) *Tropospheric air pollution: Ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles.* Science, **276**: 1045-1052.

Fleck, J.A., Bossio, D.A. & Fujii, R. (2004) *Dissolved organic carbon and disinfection by-product precursor release from managed peat soils.* Journal of Environmental Quality, **33**: 465-475.

Forsberg, C. (1992) *Will an increased greenhouse impact in Fennoscandia give rise to more humic and coloured lakes.* Hydrobiologia, **229**: 51-58.

Fowler, A.M. & Hennessy, K.J. (1995) *Potential impacts of global warming on the frequency and magnitude of heavy precipitation.* Natural Hazards, **11**: 283-303.

Fowler, J., Cohen, L. & Jarvis, P. (1998) *Practical statistics for field biology.* John Wiley & Sons.

Fox, T.R. & Comerford, N.B. (1990) *Low-molecular-weight organic acids in selected forest soils of the Southeastern USA.* Soil Science Society of America Journal, **54**: 1139-1144.

Francko, D.A. & Heath, R.T. (1979) *Functionally distinct classes of complex phosphorus compounds in lake water.* Limnology and Oceanography, **24**: 463-473.

Francko, D.A. & Heath, R.T. (1982) *UV-sensitive complex phosphorus: Association with dissolved humic material and iron in a bog lake.* Limnology and Oceanography, **27**: 564-569.

Freeman, C., Lock, M.A., Marxsen, J. & Jones, S.E. (1990) *Inhibitory effects of high molecular weight dissolved organic matter upon metabolic processes in biofilms from contrasting rivers and streams.* Freshwater Biology, **24**: 159-166.

Freeman, C., Lock, M.A. & Reynolds, B. (1993) *Impacts of climatic change on peatland hydrochemistry; a laboratory-based experiment.* Chemistry and Ecology, **8**: 49-59.

Freeman, C., Hudson, J., Lock, M.A., Reynolds, B. & Swanson, C. (1994) *A possible role of sulphate in the suppression of wetland methane fluxes following drought.* Soil Biology & Biochemistry, **26**: 1439-1442.

Freeman, C., Liska, G., Ostle, N.J., Jones, S.E. & Lock, M.A. (1995) *The use of fluorogenic substrates for measuring enzyme activity in peatlands.* Plant and Soil, **175**: 147-152.

Freeman, C., Liska, G., Ostle, N.J., Lock, M.A., Reynolds, B. & Hudson, J. (1996) *Microbial activity and enzymic decomposition processes following peatland water table drawdown.* Plant and Soil, **180**: 121-127.

Freeman, C., Ostle, N.J. & Kang, H. (2001*a*) An enzymic 'latch' on a global carbon store. *Nature*, 409: 149.

Freeman C., Evans, C.D., Monteith D.T., Reynolds, B. & Fenner N. (2001*b*) *Export of organic carbon from peat soils.* Nature, **412**: 785.

Freeman C., Evans, C.D., Monteith D.T., Reynolds, B. & Fenner N. (2002) *Climate change – terrestrial export of carbon - reply.* Nature, **415**: 862.

Freeman, C., Ostle, N.J., Fenner, N. & Kang, H. (2004*a*) *A regulatory role for phenol oxidase during decomposition in peatlands.* Soil Biology & Biochemistry, **36**: 1663-1667.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S. & Hudson, J. (2004*b*) *Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels.* Nature, **430**: 195-198.

Frimmel, F.H. (1998) *Impact of light on the properties of aquatic natural organic matter.* Environment International, **24**: 559-571.

Fuhrer, J., Skarby, L. & Ashmore, M.R. (1997) *Critical levels for ozone effects on vegetation in Europe.* Environmental Pollution, **97**: 91-106.

Fujita, Y., Ding, W.H. & Reinhard, M. (1996) *Identification of wastewater dissolved organic carbon characteristics in reclaimed wastewater and recharged groundwater.* Water Environment Research, **68**: 867-876.

Fukushima, T., Park, J., Imai, A. & Matsushige K. (1996) *Dissolved organic carbon in a eutrophic lake; dynamics, biodegradability and origin.* Aquatic Sciences, **28**: 139-157.

Galapate, R.P., Baes, A.U., Ito, K., Iwase, K. & Okada, M. (1999) *Trihalomethane formation potential prediction using some chemical functional groups and bulk parameters.* Water Research, **33**: 2555-2560.

Galapete, R.P., Baes, A.U. & Okada M. (2001) *Transformation of dissolved organic matter during ozonation: Effects on trihalomethane formation potential.* Water Research, **35**: 2201-2206.

Gambrell, R.P. & Patrick, W.H. (1978) *Chemical and microbiological properties of anaerobic soils and sediments.* In Plant Life in Anaerobic Environments. D. D. Hook and R. M. M. Crawford (eds.). Ann Arbor Scientific Publishing Company, pp. 375-423.

Gammelgaard, A., Freeman, C. & Lock, M.A. (1992) *The measurement of electron transport system activity in sphagnum-derived peat.* Soil Biological Biochemistry, **25**: 147-152.

Gehr, R., Swartz, C. & Offringa, G. (1993) *Removal of trihalomethane precursors from eutrophic water by dissolved air flotation.* Water Research, **27**: 41-49.

Gergel, S.E., Turner, M.G. & Kratz, T.K. (1999) *Dissolved organic carbon as an indicator of the scale or watershed influence on lakes and rivers.* Ecological Applications, **9**: 1377-1390.

Gillam, A.H. & Wilson, M.A. (1985) *Pyrolysis-GC-MS and NMR studies of dissolved seawater humic substances and isolates of a marine diatom.* Organic Geochemistry, **8**: 15-25.

Gjessing, E.T. & Gjerdahl, T. (1970) *Influence of ultraviolet radiation on aquatic humus.* Vatten, **2**: 144-145.

Glaser, P. (1987) *The development of streamlined bog islands in the continental interior of north America.* Arctic and Alpine Research, **19**: 402-413.

Glass, R.I., Libel, M., Brandling-Bennet, A.D. (1992) *Epidemic cholera in the Americas.* Science, **256**: 1524-1525.

Golfinopoulos, S.K. & Arhonditsis, G.B. (2002) *Multiple regression models: A methodology for evaluating trihalomethane concentrations in drinking water from raw water characteristics.* Chemosphere, **47**: 1007-1018.

Gore, A.J.P. (ed.) (1983) *Ecosystems of the World 4A. Mires: swamp, bog, fen and moor.* Elsevier Science Publishing Company, Inc.

Gorham, E. (1967) *Some chemical aspects of wetland ecology.* Technical Memorandum 90, Committee on Geotechnical Research, National Research Council of Canada, pp. 2-38.

Gorham, E., Eisenreich, S.J., Ford, J., & Santelmann, M.V. (1984) *The chemistry of bog waters.* In W. Strum, ed. Chemical processes in lakes. John Wiley & Sons, New York, pp. 339-363.

Gorham, E. (1991) *Northern peatlands; role in the carbon cycle and probable responses to climatic warming.* Ecological Applications, **1**: 182-195.

Gorissen, A., Joosten, N.N. & Jansen, A.E. (1991) *Effects of ozone and ammonium sulphate on carbon partitioning to mycorrhizal roots of juvenile Douglas fir.* New Phytologist, **119**: 243-250.

Goslan, E.H., Fearing, D.A., Banks, J., Wilson, D., Hills, P., Campbell, A.T. & Parsons, S.A. (2002) *Seasonal variations in the disinfection by-product precursor profile of a reservoir water.* Journal of Water Supply: Research and Technology – AQUA, **51**: 475-482.

Goslan, E.H. (2003) *Natural organic matter character and reactivity: Assessing seasonal variation in a moorland water.* Eng. D. Thesis. School of Water Sciences, Cranfield University.

Granéli, W., Lindell, M. & Tranvik, L. (1996) *Photo-oxidative production of dissolved inorganic carbon in lakes of different humic content.* Limnology and Oceanography, **41**: 698-706.

Granéli, W., Lindell, M., De Faria, B.M. & Esteves, F.D. (1998) *Photoproduction of dissolved inorganic carbon in temperate and tropical lakes – dependence on wavelength band and dissolved organic carbon concentrations.* Biogeochemistry, **43**: 175-195.

Grasso, D., Chin, Y. & Weber, W.J. (1990) *Structural and behavioral characteristics of a commercial humic acid and natural dissolved aquatic organic matter.* Chemosphere, **21**: 1181-1197.

Gribble, G. (2003) *Organohalides, naturally.* Education in Chemistry, March 2003, 40-41, 45.

Grieve, I.C. (1990) *Seasonal, hydrological and land management factors controlling dissolved organic carbon concentrations in the loch fleet catchments, southwest Scotland.* Hydrological Processes, **4**: 231-239.

Groeneweg, J., Sellner, B. & Tapp, W. (1994) *Ammonia oxidation in nitrosomonas at NH_3 concentrations near Km: effects of pH and temperature.* Water Research, **28**: 2561-2566.

Groombridge, B. (ed.) (1992) *Global Biodiversity: Status of the Earth's living resources.* Chapman & Hall.

Grulke, N.E., Andersen, C.P., Fenn., M.E. & Miller P.R. (1998) *Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California.* Environmental Pollution, **103**: 63-73.

Hagedorn, F., Saurer, M. & Blaser, P. (2004) *A ^{13}C tracer study to identify the origin of dissolved organic carbon in forested mineral soils.* European Journal of Soil Science, **55**: 91-100.

Hamilton, E.W. & Frank, D.A. (2001) *Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass.* Ecology, **82**: 2397-2402.

Hanson, R.S. & Hanson, T.E. (1996) *Methanotrophic bacteria.* Microbial Review, **60**: 439-471.

Hartley, R.D. & Whitehead, D.C. (1985) *Phenolic acids in soils and their influence on plant growth and soil microbial processes.* In: Vaughan, D. & Malcolm, R. E. (1985) Soil Organic Matter and Biological Activity, chpt 3, pp 77-100. Martinus Nijhoff/Dr. W. Junk Publishers.

Hatcher, P.G., Rowan, R. & Mattingly, M.A. (1980) *^{1}H and ^{13}C NMR of marine humic acids.* Organic Geochemistry, 2: 77-85.

Hautala, K., Peuravuori, J. & Pihlaja, K. (2000) *Measurement of aquatic humus content by spectroscopic analyses.* Water Research, 34: 246-258.

Hayes, F., Jones, M.L.M., Mills, G. & Ashmore, M. *Meta-Analysis of the relative sensitivity of semi-natural vegetation to ozone.* Environmental Pollution, in press.

Heathwaite, A.L. (1993) *Nitrogen cycling in surface waters and lakes. In: Nitrate: Processes, patterns and management.* John Wiley & Sons.

Heathwaite, A.L., Eggelsmann, R., Göttlich, Kh. & Kaule, G. (1993) *Ecohydrology, mire drainage and mire conservation.* In: Mires: Process, Exploitation and Conservation (ed. by A. L. Heathwaite and Kh. Göttlich), pp. 417-484. John Wiley & Sons, Ltd., Chichester.

Hejzlar, J., Dubrovsky, M., Buchtele, J. & Ruzicka, M. (2003) *The apparent and potential effects of climate change in the inferred concentration of dissolved organic matter in a temperate stream (the Malse River, South Bohemia).* The Science of the Total Environment, **310**: 143-152.

Her, N., Amy, G., McKnight, D., Sohn, J. & Yoon, Y. (2003) *Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC and fluorescence detection.* Water Research, 37: 4295-4303.

Hessen, D.O. (1992) *Dissolved organic carbon in a humic lake: effects on bacterial production and respiration.* Hydrobiologia, 229: 115-123.

Hill, A.R. & Cardaci, M. (2004) *Denitrification and organic carbon availability in riparian wetland soils and subsurface sediments.* Soil Science Society of America Journal, 68: 320-325.

Hillman, G.R., Feng, J.C., Feng, C.C. & Wang, Y.H. (2004) *Effects of catchment characteristics and disturbances on storage and export of dissolved organic carbon in a boreal headwater stream.* Canadian Journal of Fisheries and Aquatic Sciences, 61: 1447-1460.

Hinton, M.J., Schiff, S.L. & English M.C. (1997) *The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments.* Biogeochemistry, 36: 67-88.

Hofstra, G., Ali, A. Wukasch, R.T. & Fletcher, R.A. (1981) *The rapid inhibition of root respiration after exposure of bean (Phaseolus vulgaris L.) plants to ozone.* Atmospheric Environment, **15**: 483-487.

Hogg, E.H., Lieffers, V.J. & Wein, R.W. (1992) *Potential carbon losses from peat profiles: Effects of temperature, drought cycles, and fire.* Ecological Applications, **2**, 298-306.

Hoingé, J., Faust, B.C., Haag, W.R., Scully, F.E. & Zepp, R.G. (1989) *Aquatic humic substances as sources and sinks of photochemically produced transient reactants.* In: Suffet, I. H. & MacCarthy, P. (Eds.) Aquatic humic substances. Influence on fate and treatment of pollutants. Advances in Chemistry Series 219. Washington: American Chemical Society; 1989: 333-362.

Holm-Hansen, O. & Helbling, E.W. (1993) *Polyethylene bags and solar ultraviolet radiation.* Science, **259**: 534-535.

Holmes, W.E., Zak, D.R., Pregitzer, K.S. & King, J.S. (2003) *Soil nitrogen transformations under Populus tremuloides, Betula papyrifera and Acer saccharum following 3 years exposure to elevated CO_2 and O_3.* Global Change Biology, **9**, 1743-1750.

Hope, D., Billett, M.F. & Cresser, M.S. (1994) *A review of the export of carbon in river water – fluxes and processes.* Environmental Pollution, **84**: 301-324.

Hope, D., Dawson, J.J.C., Cresser, M.S. & Billett, M.F. (1995) *A method for measuring free CO2 in upland streamwater using headspace analysis.* Journal of Hydrology, **166**: 1-14.

Hope D., Billett M.F. & Cresser, M.S. (1997) *Exports of organic carbon in two river systems in NE Scotland.* Journal of hydrology, **193**: 61-82.

Hope, D., Palmer, S.M., Billett, M.F. & Dawson, J.J.C. (2001) *Carbon dioxide and methane evasion from a temperate peatland stream.* Limnology and Oceanography, **46**: 847-857.

Hongve, D., Riise, G. & Kristiansen, J.F. (2004) *Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water – a result of increased precipitation?* Aquatic Sciences, 66: 231-238.

Huang, W.J. & Yeh, H.H. (1997) *The effect of organic characteristics and bromide on disinfection by-products formation by chlorination.* Journal of Environmental Science and Health, Part A – Environmental Science and Engineering & Toxic and Hazardous Substance Control, **32**: 2311-2336.

Huck, P.M. (1990) *Measurement of biodegradable organic-matter and bacterial-growth potential in drinking-water.* Journal of the American Water Works Association, **82**: 78-86.

Hughes, S., Reynolds, B. & Hudson, J. (1996) *Release of bromide following rewetting of a naturally drained acid gully mire.* Soil Use and Management, **12**: 62-66.

Hunt, J.E. & McNeil, D.L. (1999) *The influence of present-day levels of ultraviolet-B radiation on seedlings of two southern hemisphere temperate tree species.* Plant Ecology, **143**: 39-50.

Hynes, H.B.N., Kaushik, N.K., Lock, M.A., Lush, D.L., Stocker, Z.S.J., Wallace, R.R. & Williams, D.D. (1974) *Benthos and allochthonous organic-matter in streams.* Journal of the Fisheries Research Board of Canada, **31**: 545-553.

Immirzi, C.P., Maltby, E. & Clymo, R.S. (1992) *The global status of peatlands and their role in carbon cycling.* A report for Friends of the Earth by the Wetland Ecosystems Research Group, Dept of Geography, University of Exeter, pp. 1-145. FoE, London.

Inamdar, S.P. & Mitchell, M.J. (2006) *Hydrologic and topologic controls on storm-event exports of dissolved organic carbon (DOC) and nitrate across catchment scales.* Water Resources Research, **42**: Art. No. W03421.

IARC (International Agency for Research on Cancer) (1991) *IARC monographs on the evaluation of carcinogenic risks to humans. Chlorinated drinking water; chlorination by-products; some other halogenated compounds; cobalt and cobalt compounds*, vol. 52. Lyon, IARC.

IPCC (Intergovernmental Panel on Climate Change) (2001) *Climate Change: the Scientific Basis.* Cambridge University Press, Cambridge, 2001.

Islam, K.R., Mulchi, C.L. & Ali, A.A. (1999) *Tropospheric carbon dioxide or ozone enrichments and moisture effects on soil organic carbon quality.* Journal of Environmental Quality, **28**: 1629-1636.

Jack, J., Sellers, T. & Bukaveckas, P.A. (2002) *Algal production and trihalomethane formation potential: an experimental assessment and inter-river comparison.* Canadian Journal of Fisheries & Aquatic Sciences, **59**: 1482-1491.

Janssens, I.A., Lankreijer, H., Matteucci, G., et al. (2001) *Productivity overshadows temperature in determining soil and ecosystem respiration across European forests.* Global Change Biology, **7**: 269-278.

Jardine, P.M., Wilson, G.V., McCarthy, J.F.M., Luxmoore, R.J., Taylor, D.L. & Zelazny, L.W. (1990) *Hydrogeochemical processes controlling the transport of dissolved organic carbon through a forested hillslope.* Journal of Contaminant Hydrology, **6**: 3-19.

Jarvis, P.G. & Linder, S. (2000) *Constraints to growth of boreal forests.* Nature, **405**: 904-905.

Jimenez, M.C.S., Pedraza, D.A. & Silverio, J.M. (1993) *Reaction kinetics of humic acid with sodium hypochlorite.* Water Research, **27**: 815-820.

Johnson, D., Campbell, C.D., Lee, J.A., Callaghan, T.V. & Gwynn-Jones, D. (2002) *Arctic microorganisms respond more to elevated UV-B radiation than CO_2.* Nature, **416**: 82-83.

Jones, B.J. & Mulholland, P.J. (1998) *Methane input and evasion in a hardwood forest stream: Effects of subsurface flow from shallow and deep pathways.* Limnology and Oceanography, **43**: 1243-1250.

Jonsson, A., Karlsson, J. & Jansson, M. (2003) *Sources of carbon dioxide supersaturation in Clearwater and humic lakes in northern Sweden.* Ecosystems, **6**: 224-235.

Kaiser, K., Guggenberger, G., Haumaier, L. & Zech, W. (2001) *Seasonal changes in the chemical composition of dissolved organic matter in organic forest floor leachates of old-growth Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) stand in northeatern Bavaria, Germany.* Biogeochemistry, **55**: 103-143.

Kang, H., Freeman, C. & Lock, M.A. (1998) *Trace gas emissions from a north Wales fen – role of hydrochemistry and soil enzyme activity.* Water, Air and Soil Pollution, **105**: 107-116.

Kang, H. (1999) *The significance of enzyme activities in wetland biogeochemistry.* PhD thesis. School of Biological Sciences, University of Wales, Bangor, United Kingdom.

Kang, H. & Freeman, C. (1999) *Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors.* Soil Biology and Biochemistry, **31**: 449-454.

Kang, H.J., Freeman, C. & Ashendon, T.W. (2001) *Effects of elevated CO_2 on fen peat biogeochemistry.* Science of the Total Environment, **279**: 45-50.

Kang, H., Freeman, C. & Kim, S. (2002) *Variations of DOC and phenolics in porewater of peatlands.* Korean Journal of Limnology, **35**: 306-311.

Kaplan, L.A., Larson, R.A. & Bott, T.L. (1980) *Patterns of dissolved organic carbon transport.* Limnology & Oceanography, **25**: 1034-1043.

Karanfil, T., Schlautman, M. & Erdogan, I. (2002) *Survey of DOC and UV measurement practices with implications for SUVA determination.* Journal of the American Water Works Association, 94: 68-80.

Kasurinen, A., Kokko-Gonzales, P., Riikonen, J., Vapaavuori, E. & Holopainen, T. (2004) Soil CO_2 efflux of two silver birch clones exposed to elevated CO_2 and O_3 levels during three growing seasons. Global Change Biology, 10: 1654-1665.

Kattenburg, A., Giorgi, F., Grassl, H., Meehl, G.A., Mitchell, J.F.B., Stouffer, R.J., Tokioka, T., Weaver, A.J., Wigley, T.M.L. (1995) *Climate Models - Projections of Future Climate. In* Climate Change 1995: The Science of Climate Change, Working Group I (1996). International Governmental Panel on Climate Change, Cambridge University Press.

Kieber, R.J., Li, A. & Seaton, P.J. (1999) *Production of nitrite from the photodegradation of dissolved organic matter in natural waters.* Environmental Science and Technology, 33: 993-998.

Kieber, R.J., Whitehead, R.F., Reid, S.N., Willey, J.D. & Seaton, P.J. (2006) *Chromophoric dissolved organic matter (CDOM) in rainwater, southeastern North Carolina, USA.* Journal of Atmospheric Chemistry, 54: 21-41.

Kiem, R., Knicker, H., Körschens, M. & Kögel-Knabner, I. (2000) *Refractory organic carbon in C-depleted arable soils, as studied by 13C NMR spectroscopy and carbohydrate analysis.* Organic Geochemistry, 31: 655-668.

Kiffney, P.M., Clements, W.H. & Cady, T.A. (1997) *Influence of ultraviolet radiation on the colonization dynamics of a Rocky mountain stream benthic community.* Journal of the North American Benthological Society, 16: 520-530.

Kim, J.S., Chappelka, A.H. & Miller-Goodman, M.S. (1998) *Decomposition of blackberry and broomsedge bluestem as influenced by ozone.* Journal of Environmental Quality, 27: 953-960.

Kim, H. & Yu, M. (2005) *Characterization of natural organic matter in conventional water treatment processes for selection of treatment processes focused on DBPs control.* Water Research, 39: 4779-4789.

Kitis, M., Karanfil, T., Wigton, A. & Kilduff, J.E. (2002) *Probing reactivity of dissolved organic matter for disinfection by-product formation using XAD-8 resin absorption and ultrafiltration fractionation.* Water Research, 36: 3834-3848.

Kling, G.W., Kipphut, G.W. & Miller, M.C. (1991) *Arctic lakes and streams as gas conduits to the atmosphere – implications for tundra budgets.* Science, 251: 298-301.

Koenings, J.P. & Hooper, F.F. (1976) *The influence of colloidal organic matter on iron-phosphorus cycling in an acid bog lake.* Limnology and Oceanography, **21**: 684-696.

Koerselman, W., Van Kerkhoven, M.B. & Verhoeven, J.T.A. (1993) *Release of inorganic N, P and K in peat soils; effects of temperature, water chemistry and water level.* Biogeochemistry, **20**: 63-81.

Köhler, S., Buffam, I., Jonsson, A. & Bishop, K. (2002) *Photochemical and microbial processing of stream and soil water dissolved organic matter in a boreal forested catchment in northern Sweden.* Aquatic Sciences, **64**: 269-281.

Kononova, M.M. (1966) *Soil Organic Matter.* Pergamon Press.

Korshin, G.V., Li C-W. & Benjamin, M.M. (1997*a*) *The decrease of UV absorbance as an indicator of TOX formation.* Water Research, **31**: 946-949.

Korshin, G.V., Li, C.W. & Benjamin, M.M. (1997*b*) *Monitoring the properties of natural organic matter through UV spectroscopy: A consistent theory.* Water Research, **31**: 1787-1795.

Kortelainen, P. & Saukkonen,S. (1995) *Organic vs minerogenic acidity in headwater streams in Finland.* Water, Air and Soil Pollution, **85**: 559-564.

Korth, A., Fiebiger, C., Bornmann, K. & Schmidt, W. (2004) *NOM increase in drinking water reservoirs – relevance for drinking water production.* Water Science and Technology: Water Supply, **4**: 55-60.

Knocke, W.R., West, S., Hoehn, R.C. (1986) *Effects of low-temperature on the removal or trihalomethane precursors by coagulation.* Journal of the American Water Works Association, **78**: 189-195.

Knorr, W., Prentice, I.C., House, J.I. & Holland, E.A. (2005) *Long-term sensitivity of soil carbon turnover to warming.* Nature, **433**: 298-301.

Kraffczyk, I., Trolldenier, G. & Beringer, H. (1984) *Soluble root exudates of maize: influence of potassium supply and rhizosphere microorganisms.* Soil Biology and Biochemistry, **16**: 315-322.

Krasner, S.W., Weinberg, H.S., Richardson, S.D., Pastor, S.J., Chinn, R., Sclimenti, M.J., Onstad, G.D. & Thruston, A.D. *Occurrence of a new generation of disinfection byproducts.* Environmental Science and Technology, in press.

Kritzberg, E.S., Cole, J.J., Pace, M.L. & Granéli, W. & Bade, D.L. (2004) *Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake 13C addition experiments.* Limnology & Oceanography, **49**: 588-596.

Krupa, S.V. & Kickert, R.N. (1989) *The greenhouse effect: impacts of ultraviolet-B (UV-B), carbon dioxide (CO_2) and ozone (O_3) on vegetation.* Environmental Pollution, 61, 263-293.

Kuserk, F.T., Kaplan, L.A. & Bott, T.L. (1984) *In situ measurements of dissolved organic carbon flux in a rural stream.* Canadian Journal of Fisheries and Aquatic Sciences, 41: 964-973.

Kuznetzova, I.A. & Dzyuban,, A.N. (2005) *Microbial processes of methane transformation in the shallow-water zone of the Rybinsk reservoir.* Microbiology, 74: 744-745.

Lee, S.S., Shin, K.J., Kim, W.Y., Ha, J.K. & Han, I.K. (1999) *The rumen ecosystem: as a fountain source of noble enzymes-review.* Asian-Australasian Journal of Animal Sciences, 12: 988-1001.

Lehtola, M.J., Miettinen, I.T., Vartiainen, T., Myllykangas, T. & Martikainen, P.J. (2001) *Microbially available organic carbon, phosphorus, and microbial growth in ozonated drinking water.* Water Research, 35: 1635-1640.

Lentz, H., Lüdermann, H.D. & Ziechmann, W. (1977) *Proton resonance spectra of humic acids from the solum of a Podzol.* Geoderma, 18: 325-328.

Li, L., Huang, W.L., Peng, P., Sheng, G.Y. & Fu, J.M. (2003) *Chemical and molecular heterogeneity of humic acids repetitively extracted from a peat.* Soil Science Society of America Journal, 67: 740-746.

Liang, L. & Singer, P.C. (2003) *Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water.* Environmental Science and Technology, 37: 2920-2928.

Lindell, M.J., Graneli, H. & Bertilsson, S. (2000) *Seasonal photoreactivity of dissolved organic matter from lakes with contrasting humic content.* Canadian Journal of Fisheries and Aquatic Sciences, 57: 875-885.

Lindholm, T. (1990) *Growth dynamics of the peat moss Sphagnum fuscum on hummocks on a raised bog in southern Finland.* Annales Botanici Fennici, 27: 67–78.

Liski, J., Ilvesniemi, H., Makela, A. & Westman, C.J. (1999) *CO_2 emissions from soil in response to climatic warming are overestimated – The decomposition of old soil organic matter is tolerant of temperature.* Ambio, 28: 171-174.

Lloyd, A. (2004) *The effects of tropospheric ozone on carbon cycling in peatlands.* MSc Ecology thesis. School of Biological Sciences, University of Wales, Bangor, United Kingdom.

Lock, M.A. & Ford, T.E. (1985) *Microcalorimetric approach to determining relationships between energy supply and metabolism in river epilithon.* Applied and Environmental Microbiology, **49**: 408-412.

Loya, W.M., Pregitzer, K.S., Karberg, N.J., King, J.S. & Glardina, C.P. (2003) *Reduction of soil carbon formation by tropospheric ozone under increased carbon dioxide levels.* Nature, **425**: 705-707.

Lu, J., Chang, A.C. & Wu, L. (2004) *Distinguishing sources of groundwater nitrate by ^{1}H NMR of dissolved organic matter.* Environmental Pollution, **132**: 365-374.

Lucassen, E.C.H.E.T., Smolders, A.J.P., Lamers, L.P.M. & Roelofs, J.G.M. (2005) *Water table fluctuations and groundwater supply are important in preventing phosphate-eutrophication in sulphate-rich fens: Consequences for wetland restoration.* Plant and Soil, **269**: 109-115.

Lynch, J. & Whipps, J.M. (1990) *Substrate flow in the rhizosphere.* Plant and Soil, **129**: 1-10.

McCool, P.M. & Menge, J.A. (1983) *Influence of ozone on carbon partitioning in tomato – potential role of carbon flow in regulation of the mycorrhizal symbiosis under conditions of stress.* New Phytologist, **94**: 241-247.

McCrady, J.K. & Andersen, C.P. (2000) *The effect of ozone on below-ground carbon allocation in wheat.* Environmental Pollution, **107**: 465-472.

McDowell, W.H. (1985) *Kinetics and mechanisms of dissolved organic carbon retention in a headwater stream.* Biogeochemistry, **1**: 329-352.

McDowell, W.H. & Likens, G.E. (1988) *Origin, composition and flux of dissolved organic carbon in the Hubbard Brook Valley.* Ecological Monographs, **58**: 177-195.

McKnight, D., Thurman, E., Wershaw, R. & Hemond, H. (1985) *Biogeochemistry of aquatic humic substances in Thoreau Bog, Concord, Mass.* Ecology, **66**: 1339-1352.

McLatchey, G.P. & Reddy, K.R. (1998) *Regulation of organic matter decomposition and nutrient release in a wetland soil.* Journal of Environmental Quality, **27**: 1268-1274.

McNamara, A.E. & Hill, W.R. (1999) *Effects of UV-B dose and irradiance: comparison among grazers.* Journal of the North American Benthological Society, **18**: 370-380.

Ma, H., Allen, H.E. & Yin, Y. (2001) *Characterization of isolated fractions of dissolved organic matter from natural waters and a wastewater effluent.* Water Research, **35**: 985-996.

Madronich, S., McKenzie, R.L., Björn, L.O. & Caldwell, M.M. (1998) *Changes in biologically active ultraviolet radiation reaching the Earth's surface.* Journal of Photochemistry and Photobiology B: Biology, **46**: 5-19.

Malcolm, R.L. (1990) *The uniqueness of humic substances in each of soil, stream and marine environments.* Analytica Chimica Acta, **232**: 19-30.

Malmaeus, J.M., Blenckner, T., Markensten, H. & Persson, I. (2006) *Lake phosphorus dynamics and climate warming: A mechanistic model approach.* Ecological Applications, **190**: 1-14.

Malmer, N. (1986) *Vegetational gradients in relation to environmental-conditions in northwestern European mires.* Canadian Journal of Botany, **64**: 375-383.

Maltby, E. & Turner, R.E. (1983) *Wetlands of the world.* Geographic Magazine, **55**: 12-17.

Maltby, E. & Immirzi, P. (1993) *Carbon dynamics in peatlands and other wetland soils regional and global perspectives.* Chemosphere, **27**: 999-1023.

Malthus, T.J. & George, D.G. (1997) *Airbourne remote sensing of macrophytes in Cefni reservoir, Anglesey, UK.* Aquatic Botany, **58**: 317-332.

Manny, B.A. & Wetzel, R.G. (1973) *Diurnal changes in dissolved organic and inorganic carbon and nitrogen in a hardwater stream.* Freshwater Biology, **3**: 31-43.

Matthews, E. & Fung, I. (1987) *Methane emissions from natural wetlands: global distribution, area, and environmental characteristics of sources.* Global Biogeochemical Cycles, **1**: 61-86.

Melin, E.S. & Ødegaard, H. (2000) *The effect of biofilter loading rate on the removal of organic ozonation by-products.* Water Research, **34**: 4464-4476.

Metje, M. & Frenzel, P. (2005) *Effect of temperature on anaerobic ethanol oxidation and methanogensis in acidic peat from a northern wetland.* Applied and Environmental Microbiology, **71**: 8191-8200.

Met Office (2004) *Met Office: 2003 dry spell.* Available from:
http://www.metoffice.com/climate/uk/interesting/2003dryspell.html
Last accessed 20-09-2006.

Miller, W.L. & Zepp, R.G. (1995) *Photochemical production of dissolved inorganic carbon from terrestrial organic matter – significance to the oceanic organic-carbon cycle.* Geophysical Research Letters, **22**: 417-420.

Mills, G., Hayes, F., Jones, M.L.M. & Cinderby, S. *Identifying ozone-sensitive communities of (semi-) natural vegetation suitable for mapping exceedance of critical levels.* Environmental Pollution, in press.

Minkinnen, K. & Laine, J. (1998) *Long-term effect of forest drainage on the peat carbon stores of pine mires in Finland.* Canadian Journal of Forest Research, **28**: 1267-1275.

Mitchell, J.F.B. & Warrilow, D.A. (1987) *Summer dryness in northern mid-latitudes due to increased CO_2.* Nature, **330**: 238-240.

Mitsch, W.J. & Gosselink, J.G. (2000) *Wetlands (3rd ed.).* Van Nostrand Reinhold.

Molina, M.J. & Rowland, F.S. (1974) *Stratospheric sink for chlorofluoromethanes – chlorine atomic catalysed destruction of ozone.* Nature, **249**: 810-812.

Molot, L.A. & Dillon, P.J. (1997) *Photolytic regulation of dissolved organic carbon in northern lakes.* Global Biogeochemical Cycles, **11**: 357-365.

Moore, P.D. & Bellamy, D.J. (1974) *Peatlands.* Elek Science, London, UK, pp. 84-117.

Moore, T.R. (1989) *Growth and net production of Sphagnum at 5 fen sites, subarctic eastern Canada.* Canadian Journal of Botany, **67**: 1203-1207.

Moore, T.R. & Dalva, M. (2001) *Some controls on the release of dissolved organic carbon by plant tissues and soils.* Soil Science, **166**: 38-47.

Moore, T.R., Bubier, J.L., Frolking, S.E., Lafleur, P.M. & Roulet, N.T. (2002) *Plant biomass and production and CO2 exchange in an ombrotrophic bog.* Journal of Ecology, **90**: 25-36.

Moran, M.A. & Zepp, R.G. (1997) *Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter.* Limnology and Oceanography, **42**: 1307-1316.

Morris, D.P., Zagarese, H., Williamson, C.E., Balseiro, E.G., Hargreaves, B.R., Modenutti, B., Moeller, R. & Queimalinos, C. (1995) *The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon.* Limnology and Oceanography, **40**: 1381-1391.

Morris, D.P. & Hargreaves, B.R. (1997) *The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau.* Limnology and Oceanography, **42**: 239-249.

Mortensen, L.M. (1998) *Growth responses of seedlings of six Betula pubescens Ehrh. Provenances to six ozone exposure regimes.* Scandinavian Journal of Forest Research, **13**: 189-196.

Moss, B., Barker, T., Stephen, D., Williams, A.E., Balayla, D.J., Beklioglu, M. & Carvalho, L. (2005) *Consequences of reduced nutrient loading on a lake system in a lowland catchment: deviations from the norm?* Freshwater Biology, **50**: 1687-1705.

Murase, J., Sakai, Y., Sugimoto, A., Okubo, K. & Sakamoto, M. (2003) *Sources of dissolved methane in Lake Biwa. Sources of dissolved methane in Lake Biwa.* Limnology, **4**: 91-99.

Murase, J., Sakai, Y., Kametani, A. & Sugimoto, A. (2005) *Dynamics of methane in mesotrophic Lake Biwa, Japan.* Ecological Research, **20**: 377-385.

Myllykangas, T., Nissinen, T.K., Mäki-Paakanen, J., Hirvonen, A. & Vartiainen, T. (2003) *Bromide affecting drinking water mutagenicity.* Chemosphere, **53**: 745-756.

Nazir, M. & Khan, F.I. (2006) *Human health-risk modeling for various exposure routes of trihalomethanes (THMs) in potable water supply.* Environmental Modelling & Software, **21**: 1416-1429.

NEGTAP (2001) *Transboundary air pollution: Acidification, eutrophication and ground-level ozone in the UK.* Report of the National Expert Group on Transboundary Air Pollution. Department of Environment Food and Rural Affairs, London. Available at: http://www.nbu.ac.uk/negtap/finalreport.htm. Last accessed 22-09-2006.

Newsham, K.K., McLeod, A.R., Roberts, J.D., Greenslade, P.D. & Emmett, B.A. (1997) *Direct effects of elevated UV-B radiation on the decomposition of Quercus robur leaf litter.* Oikos, **79**: 592-602.

Nguyen, M., Baker, L.A. & Westerhoff, P. (2002) *DOC and DBP precursors in western US watersheds and reservoirs.* Journal of the American Water Works Association, **94**: 98-112.

Nguyen, M.L., Westerhoff, P., Baker, L., Hu, Q., Esparza-Soto, M. & Sommerfield, M. (2005) *Characteristics and reactivity of algae-produced dissolved organic carbon.* Journal of Environmental Engineering-ASCE, **131**: 1574-1582.

Niemi, R., Martikainen, P.J., Silvola, J. & Holopainen, T. (2002) *Ozone effects on Sphagnum mosses, carbon dioxide exchange and methane emission in boreal peatland microcosms.* The Science of the Total Environment, **289**: 1-12.

Niemi, R. (2003) *Peatland vegetation and carbon gas dynamics under increasing UV-B radiation and ozone concentrations.* PhD thesis. Faculty of Natural and Environmental Sciences, University of Kuopio, Finland.

Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J. & Elliot, P. (2000) *Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review.* Occupational Environmental Medicine, **57**: 73-85.

Nikolaou, A.D., Kostopoulou, M.N. & Lekkas, T.D. (1999) *Organic by-products of drinking water chlorination.* Global Nest: the International Journal, **1**: 143-156.

Nikolaou, A.D. & Lekkas, T.D. (2001) *The role of natural organic matter during formation of chlorination by-products: A review.* Acta Hydrochimica Et Hydrobiologica, **29**: 63-77.

Noble, R.T. & Fuhrman, J.A. (1997) *Virus decay and its causes in coastal water.* Applied and Environmental Microbiology, **63**: 77-83.

Nobukawa, T. & Sanukida, S. (2001) *Effect of bromide ions on genotoxicity of halogenated by-products from chlorination of humic acid water.* Water Research, **35**: 4293-4298.

Nouchi, I., Ito, O., Harazono, Y. & Kouchi, H. (1995) *Acceleration of ^{13}C-labelled photosynthate partitioning from leaves to panicles in rice plants exposed to chronic ozone at the reproductive stage.* Environmental Pollution, **88**: 253-260.

NRPB (2003) *Solar radiation measurements at the network of six sites in the UK, January-December, 2002.* National Radiological Protection Board, report NRPB-W37.

Oechel, W.C., Hastings, S.J., Vourlitis, G., Jenkins, M., Riechers, G. & Grulke, N. (1993) *Recent change of Arctic tundra ecosystems from a net carbon-dioxide sink to a source.* Nature, **361**: 520-523.

Oliver, B.G. & Shindler, D.B. (1980) *Trihalomethanes from the chlorination of aquatic algae.* Environmental Science and Technology, **14**: 1502-1505.

Oliver, B.G. & Visser, S.A. (1980) *Chloroform production from the chlorination of aquatic humic material: the effect of molecular weight, environment and season.* Water Research, **14**: 1137-1141.

Oliver, B.G. (1983) *Dihaloacetonitriles in drinking water: Algae and fulvic acid as precursors.* Environmental Science and Technology, **17**: 80-83.

Ollinger, S.V., Aber, J.D., Reich, P.B. & Freuder, R.J. (2002) *Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO_2 and land use history on the carbon dynamics of northern hardwood forests.* Global Change Biology, **8**: 545-562.

Oltmans, S.J., Lefohn, A.S., Scheel, H.E., Harris, J.M., Levy, H., Galbally, I.E., Brunke, E.G., Meyer, C.P., Lathrop, J.A., Johnson, B.J., Shadwick, D.S., Cuevas, E., Schmidlin, F.J., Tarasick, D.W., Claude, H., Kerr, J.B., Uchino, O. & Mohnen, V. (1998) *Trends of ozone in the troposphere.* Geophysical Research Letters, **25**: 139-142.

Osburn, C.L., Morris, D.P., Thorn, K.A. & Moeller, R.E. (2001) *Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation.* Biogeochemistry, **54**: 251-278.

Ostle, N.J. (1994) *Regulation of enzyme activities in peatlands.* MSc thesis. School of Biological Sciences, University of Wales, Bangor

Palacios, M., Pampillon, J.F. & Rodriguez, M.E. (2000) *Organochlorinated drinking waters and current compliance with quality standards throughout the European Union.* Water Research, **34**: 1002-1016.

Palmer, C.M. (1977) *Algae and water pollution.* Cincinnati Municipal Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency.

Parks, S.J. & Baker, L.A. (1997) *Sources and transport of organic carbon in an Arizona river-reservoir system.* Water Research, **31**: 1751-1759.

Paul, N.D. & Gwynn-Jones, D. (2003) *Ecological roles of solar UV radiation: towards an integrated approach.* Trends in Ecology & Evolution, **18**: 48-55.

Perez-Fuentetaja, A., Dillon, P.J., Yan, N.D. & McQueen, D.J. (1999) *Significance of dissolved organic carbon in the prediction of thermocline depth in small Canadian Shield lakes.* Aquatic Ecology, **33**: 127-133.

Peterson, H.G., Milos J.P., Spink, D.R., Hrudey, S.E. & Sketchell J. (1993) *Trihalomethanes in finished drinking water in relation to dissolved organic carbon and treatment processes for Alberta surface waters.* Environmental Technology, **14**: 877-884.

Peterson, D.L., Silsbee, D.G., Poth, M., Arbaugh, M.J. & Biles, F.E. (1995) *Growth responses of bigcone Douglas fir to long-term ozone exposure in Southern California.* Journal of Air Pollution Management Association, **45**: 36-45.

Peuravuori, J. (2005) *NMR spectroscopy study of freshwater humic material in light of supramolecular assembly.* Environmental Science and Technology, **39**: 5541-5549.

Pietikainen, J., Pettersson, M. & Baath, E. (2005) *Comparison of temperature effects on soil respiration and bacterial and fungal growth rates.* Fems Microbiology Ecology, **52**: 49-58.

Pilson, M.E.Q. (1998) *An Introduction to the Chemistry of the Sea.* Prentice-Hall, Inc.

Pind, A., Freeman, C. & Lock, M.A. (1994) *Enzymic degradation of phenolic materials in peatlands – measurement of phenol oxidase activity.* Plant and Soil, **159**: 227-231.

Plummer, J.D. & Edzwald, J.K. (2001) *Effect of ozone on algae as precursors for trihalomethane and haloacetic acid production.* Environmental Science and Technology, **35**: 3661-3668.

Pomes, M.L., Green, W.R., Thurman, E.M., Orem, W.H. & Lerch, H.E. (1999) *DBP formation potential of aquatic humic substances.* Journal of the American Water Works Association, **91**: 103-115.

Ponnamperuma, F.M. (1972) The chemistry of submerged soils. Advances in Agronomy, **24**: 29-96.

Pontius, F.W. (2003) *Drinking water regulation and health.* John Wiley & Sons.

Potter, L., Foot, J.P., Caporn, S.J.M. & Lee, J.A. (1996) *Responses of four Sphagnum species to acute ozone fumigation.* Journal of Bryology, **19**: 19-32.

Power, S.A. & Ashmore, M.R. (2002) *Responses of fen and fen-meadow communities to ozone.* New Phytologist, **156**: 339-408.

Pulford, I.D. & Tabatabai, M.A. (1987) *Effect of waterlogging on enzyme activities in soils.* Soil Biology and Biochemistry, **20**: 215-219.

Reche, I., Pace, M.L. & Cole, J.J. (1999) *Relationship of trophic and chemical conditions to photobleaching of dissolved organic matter in lake ecosystems.* Biogeochemistry, **44**: 259-280.

Reche, I., Pace, M.L. & Cole, J.J. (2000) *Modeled effects of dissolved organic carbon and solar spectra on photobleaching in lake ecosystems.* Ecosystems, **3**: 419-432.

Rex, M., Salawitch, R.J., von der Gathen, P., Harris N.R.P., Chipperfield, M.P. & Naujokat, B. (2004) *Arctic ozone loss and climate change.* Geophysical Research Letters, **31**: L04116.

Reynolds, B. (1984) *A simple method for the extraction of soil solution by high speed centrifugation.* Plant and Soil, **78**: 437-440.

Ribas, F., Frías, J., Huguet, J.M. & Lucena, F. (1997) *Efficiency of various water treatment processes in the removal of biodegradable and refractory organic matter.* Water Research, **31**: 639-649.

Richardson, S.D. (2003) *Disinfection by-products and other emerging contaminants in drinking water.* Trends in Analytical Chemistry, **22**: 666-684.

Richardson, S.D. & Ternes, T.A. (2005) *Water analysis: emerging contaminants and current issues.* Analytical Chemistry, **77**: 3807-3838.

Rinnan, R., Impio, M., Silvola, J., Holopainen, T. & Martikainen, P.J. (2003) *Carbon dioxide and methane fluxes in boreal peatland microcosms with different vegetation cover – effects of ozone or ultraviolet-B exposure.* Oecologia, **137**: 475-483.

Rinnan, R. & Holopainen, T. (2004) *Ozone effects on the ultrastructure of peatland plants: Sphagnum mosses, Vaccinium oxycoccus, Andromeda polifolia and Eriophorum vaginatum.* Annals of Botany, **94**: 623-634.

Robson, A.J. & Neal, C. (1996) *Water quality trends at an upland site in Wales, UK, 1983-1993.* Hydrological Processes, **10**: 183-203.

Rodriguez, M.J., Vinette, Y., Serodes, J.B. & Bouchard, C. (2003) *Trihalomethanes in drinking water of greater Quebec region (Canada): Occurrence, variations and modelling.* Environmental Monitoring and Assessment, **89**: 69-93.

Rodriguez, M.J., Serodes, J.B. & Levallois, P. (2004) *Behaviour of trihalomethanes and haloacetic acids in a drinking water distribution system.* Water Research, **38**: 4367-4382.

Rook, J.J. (1976) *Haloforms in drinking-water.* Journal of the American Water Works Association, **68**: 168-172.

Ross, S.M. (1985) *Field and incubation temperature effects on mobilization of nitrogen, phosphorus and potassium in peat.* Soil Biology and Biochemistry, **17**: 479-482.

Ross, A. (2004) *The influence of global warming on DOC release.* BSc thesis. School of Biological Sciences, University of Wales, Bangor.

Rowntree, P.R., Murphy, J.M. & Mitchell, J.F.B. (1993) *Climatic-change and future rainfall predictions.* Journal of the Institution of Water and Environmental Management, **7**: 464-470.

Royer, T.V. & David, M.B. (2005) *Export of dissolved organic carbon from agricultural streams in Illinois, USA.* Aquatic Sciences, **67**: 465-471.

Rozema, J., van de Staaij, J., Björn, L.O. & Caldwell, M. (1997) *UV-B an environmental factor in plant life: stress and regulation.* Trends in Ecological Evolution, **12**: 22-28.

Rozema, J., Boelen, P. & Blokker, P. (2005) *Depletion of stratospheric ozone over the Antarctic and Arctic: Responses of plants of polar terrestrial ecosystems to enhanced UV-B, an overview.* Environmental Pollution, **137**: 428-442.

Runeckles, V.C. & Krupa, S.V. (1994) *The impact of UV-B radiation and ozone on terrestrial vegetation.* Environmental Pollution, **83**: 191-213.

Sabater, F., Meyer, J.L. & Edwards, R.T. (1993) *Longitudinal patterns of dissolved organic-carbon concentration and suspended bacterial density along a blackwater river.* Biogeochemistry, **21**: 73-93.

Sachse, A., Babenzien, D., Ginzel, G., Gelbrecht, J. & Steinberg, C.E.W. (2001) *Characterization of dissolved organic carbon (DOC) in a dystrophic lake and an adjacent fen.* Biogeochemistry, **54**: 279-296.

Saleem, A., Lopnonen, J., Pihlaja, K & Oksanen, E. (2001) *Effects of long-term open-field ozone exposure on leaf phenolics of European Silver Birch.* Journal of Chemical Ecology, **27**: 1049-1062.

Salonen, K. & Vähätalo, A. (1994) *Photochemical mineralisation of dissolved organic matter in Lake Skjervatjern.* Environment International, **20**: 307-312.

Scagel, C.F. & Andersen, C.P. (1997) *Seasonal changes in root and soil respiration of ozone-exposed ponderosa pine (Pinus ponderosa) grown in different substrates.* New Phytologist, **136**: 627-643.

Scanlon, D. & Moore, T. (2000) *Carbon dioxide production from peatland soil profiles: The influence of temperature, oxic/anoxic conditions and substrate.* Soil Science, **165**: 153-160.

Schindler, D.W. (1971) *An hypothesis to explain differences and similarities among lakes in the Experimental Lakes Area, north-western Ontario.* J. Fish Res. Bd Can., **28**: 295-301

Schindler., D.W. (1977) *Evolution of phosphorus limitation in lakes.* Science, **195**: 260-262.

Schindler, D.W., Bayley, S.E., Curtis, P.J., Parker, B.R., Stainton, M.P. & Kelly C.A. (1992) *Natural and man-caused factors affecting the abundance and cycling of dissolved-organic substances in Precambrian shield lakes.* Hydrobiologia, **229**: 1-21.

Schindler, D.W., Curtis, P.J., Bayley, S.E., Parker, B.R., Beaty, K.G. & Stainton, M.P. (1997) *Climate-induced changes in the dissolved organic carbon budgets of boreal lakes.* Biogeochemistry, **36**: 9-28.

Schlesinger, W.H. (1991) *Biogeochemistry: an analysis of global change.* Academic Press, Inc.

Schlesinger, W.H. & Andrews, J.A. (2000) *Soil respiration and the global carbon cycle.* Biogeochemistry, **48**: 7-20.

Schnitzer, M. & Khan, S.U. (1972) *Humic substances in the environment.* Marcel Dekker, Inc.

Scott, M.J., Jones, M.N., Woof, C. & Tipping E. (1998) *Concentrations and fluxes of dissolved organic carbon in drainage water from an upland peat system.* Environment International, **24**: 537-546.

Scott, M.J., Jones, M.N., Woof, C., Simon, B. & Tipping, E. (2001) *The molecular properties of humic substances isolated from a UK upland peat system.* Environment International, **27**: 449-462.

Scranton, M.I. & McShane, K. (1991) *Methane fluxes in the southern North Sea – the role of European rivers.* Continental Shelf Research, **11**: 37-52.

Selas, V. (2006) *UV-B induced plant stress as a possible cause of ten-year hare cycles.* Population Ecology, **48**: 71-77.

Sérodes, J-B., Rodriguez, M.J., Li, H. & Bouchard, C. (2003) *Occurrence of THMs and HAAs in experimental chlorinated waters of the Quebec City Area (Canada).* Chemosphere, **31**: 253-263.

Shafer, S.R. (1988*) Influence of ozone and stimulated acidic rain on microorganisms in the rhizosphere of Sorgham.* Environmental Pollution, **51**, 131-152.

Shindell, D.T., Rind, D. & Lonergan, P. (1998) *Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentrations.* Nature, **392**: 589-592.

Siddiqui, M.S., Amy, G.L. & Murphy, B.D. (1997) *Ozone enhanced removal of natural organic matter from drinking water sources.* Water Resources, **31**: 3098-3106.

Siepak, J. (1999) *Total organic carbon (TOC) as a sum parameter of water pollution in selected Polish rivers (Vistula, Odra, and Warta).* Acta Hydrochimica et Hydrobiologia, **27**: 282-285.

Silvola, J. (1986) *Carbon-dioxide dynamics in mires reclaimed for forestry in eastern Finland.* Annales Botanici Fennici, **23**: 59-67.

Silvola, J., Alm, J., Ahlholm, U., Nykänen, H. & Martikainen, P.J. (1996) *CO_2 fluxes from peat in boreal mires under varying temperature and moisture conditions.* Journal of Ecology, **84**: 219-228.

Singer, P.C. (1999) *Humic substances as precursors for potentially harmful disinfection by-products.* Water Science and Technology, **40**: 25-30.

Singer, P.C. (2006) *Disinfection byproducts in drinking water: Additional science and policy considerations in the pursuit of public health protection.* National Water Research Institute, The 2006 Clarke Lecture. Available from: http://www.nwri-usa.org/uploads/2006%20Clarke%20lecture.pdf. Last accessed 19-09-2006.

Sinha, S., Amy, G.L. & Sohn, J. (1997) *Reactivity of NOM fractions in forming chlorinated DBPs.* Presented at the AWWA Annual Conference, Atlanta, GA, June 1997.

Sinsabaugh, R.L., Antibus, R.K. & Linkens, A.E. (1991) *An enzymic approach to the analysis of microbial activity during plant litter.* Agricultural Ecosystems & Environments, **34**: 43-54.

Sketchell, J., Peterson, H.G., & Christofi, N. (1995). *Disinfection by-product formation after biologically assisted GAC treatment of water supplies with different bromide and DOC content.* Water Research, **29**: 2635-2642.

Smith, R.C., Prezelin, B.B., *et al.* (1992) *Ozone depletion – ultraviolet-radiation and phytoplankton biology in Antarctic waters.* Science, **255**: 952-959.

Smith, R.L.,, Miller, L.G. & Howes, B.L. (1993) *The geochemistry of methane in lake Fryxell, an amictic, permanently ice-covered, Antarctic lake.* Biogeochemistry, **16**: 43-61.

St. Louis, V.L., Kelly, C.A., Duchemin, E., Rudd, J.W.M. & Rosenberg, D.M. (2000) *Reservoir surfaces as sources of greenhouse gases to the atmosphere: A global estimate.* Bioscience, **50**: 766-775.

Steinberg, C. & Muenster, U. (1985) *Geochemistry and ecological role of humic substances in lakewater.* In: Aiken, G. R., McKnight, D. M., Wershaw, R. L. & McCarthy, P. (eds.) Humic substances in soil, sediment and water. John Wiley & Sons, New York, USA.

Stewart, A.J. & Wetzel, R.G. (1981) *Dissolved humic materials: photodegredation, sediment effects, and reactivity with phosphate and calcium carbonate precipitation.* Archiv Fur Hydrobiologie, **92**: 265-286.

Stewart, W.D.P., Preston, T., Peterson, H.G. & Christofi, N. (1982) *Nitrogen cycling in eutrophic freshwaters.* Philosophical Transactions of the Royal Society of London, series B, 296, 491-509.

Stolarski, R., Bojkov, R., Bishop, L., Zerefos, C., Staehelin, J. & Zawodny, J. (1992) *Measured trends in stratospheric ozone.* Science, **256**: 342-349.

Strand, L.T., Abrahamsen, G. & Stuanes, A.O. (2002) *Leaching from organic matter-rich soils by rain of different qualities: I. Concentrations.* Journal of Environmental Quality, **31**: 547-556.

Strome, D.J. & Miller, M.C. (1978) *Photolytic changes in dissolved humic substances.* Internationale Vereinigung für Theoretische und Angewandte Limnologie, Verhandlungen, **20**: 1248–1254.

Taalas, P., Kaurola, J., Kylling, A., Shindell, D., Sausen, R., Dameris, M., Grewe, V. & Herman, J. (2000) *The impact of greenhouse gases and halogenated species on future solar UV radiation doses.* Geophysical Research Letters, **27**: 1127-1130.

Tank, S.E., Schindler, D.W. & Arts, M.T. (2003) *Direct and indirect effects of UV radiation on bethic communities: epilithic food quality and invertebrate growth in four montane lakes.* OIKOS, **103**: 651-667.

Tate, R.L. (1987) *Soil organic matter: biological and ecological effects.* New York: Wiley, Inc.

Tegen, I. & Dorr, H. (1996) *Mobilisation of cesium in organic rich soils: correlation with the production of dissolved organic carbon.* Water, Air, Soil Pollution, **88**: 133-144.

Thomas, D.N. & Lara, R.J. (1995) *Photodegradation of algal derived dissolved organic carbon.* Marine Ecology-Progress Series, **116**: 309-310.

Thomas, P.A. & Pearce, D.M.E. (2004) *Role of cation exchange in preventing the decay of anoxic deep bog peat.* Soil Biology & Biochemistry, **36**: 23-32.

Thomsen, R. (1990) *Effect of climate variability and change in groundwater in Europe – keynote paper presented at the conference on climate and water, Helsinki, 11-15 September, 1989.* Nordic Hydrology, **21**: 185-194.

Thormann, M.N., Szumigalski, A.R. & Bayley, S.E. (1999) *Aboveground peat and carbon accumulation potentials along a bog-fen-marsh wetland gradient in southern boreal Alberta, Canada.* Wetlands, **19**: 305-317.

Thornley, J.H.M. & Cannell, M.G.R. (2001) *Soil carbon storage carbon response to temperature: an hypothesis.* Annals of Botany, **87**: 591-598.

Thurman, E M. (1985) *Organic Geochemistry of Natural Waters.* Nijhoff/Junk, Dordrecht.

Tipping, E., Woof, C., Rigg, E., Harrison, A.F., Ineson, P., Taylor, K., Benham, D., Poskitt, J., Rowland, A.P., Bol, R. & Harkness, D.D. (1999) *Climatic influences on the leaching of dissolved organic matter from upland UK moorland soils, investigated by a field manipulation experiment.* Environment International, **25**: 83-95.

Tranvik, L.J. & Jansson, M. (2002) *Climate change – Terrestrial export of organic carbon.* Nature, **415**: 861-862.

Updegraff, K., Bridgham, S.D., Pastor, J. & Weishampel, P. (1998) *Hysteresis in the temperature response of carbon dioxide and methane production in peat soils.* Biogeochemistry, **43**: 253-272.

Urban, N.R., Bayley, S.E. & Eisenreich, S.J. (1989) *Export of dissolved organic carbon and acidity from peatlands.* Water Resources Research, **25**: 1619-1628.

USEPA (1998) *National Primary Drinking Water Regulations: Disinfection and Disinfection Byproducts; Final Rule.* Federal Register, **63**: 69390-69476.

Utsumi, M., Nojiri, Y., Nakamura, T., Nozawa, T., Otsuki, A., & Seki, H. (1998) *Oxidation of dissolved methane in a eutrophic, shallow lake: Lake Kasumigaura, Japan.* Limnology and Oceanography, **43**: 471-480.

Uyguner, C.S., Hellriegel, C., Otto, W. & Larive, C.K. (2004) *Characterization of humic substances: Implications for trihalomethane formation.* Analytical and Bioanalytical Chemistry, **378**: 1579-1586.

Vähätalo, A.V., Salonen, K., Salkinoja-Salonen, M.S. & Hatakka, A. (1999) *Photochemical mineralization of synthetic lignin in lake water indicates enhanced turnover of aromatic organic matter under solar radiation.* Biodegradation, **10**: 415-420.

Vähätalo, A.V., Salkinoja-Salonen, M.S., Salonen, K., & Taalas, P. (2000) *Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake.* Limnology and Oceanography, **45**: 664-676.

Vähätalo, A.V., Salonen, K., Saski, E. & Salkinoja-Salonen, M.S. (2002) *Bleaching of color of kraft pulp mill effluents and natural organic matter in lakes.* Canadian Journal of Fisheries and Aquatic Sciences, **59**: 808-818.

Vähätalo, A.V., Salonen, K., Münster, U., Järvinen, M. & Wetzel, R.G. (2003) *Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake.* Archiv. Fur Hydrobiologia, **156**: 287-314.

van der Vlugt, J.C. & Klapwijk, S.P. (1990) *The Reeuwijk Lakes: A five years water quality study in a eutrophic ecosystem.* Aquatic Ecology, **24**: 57-67.

van Leeuwen, F.X.R. (2000) *Safe drinking water: the toxicologist's approach.* Food and Chemical Toxicology, **38**: S51-S58.

Vansteenderen, R.A., Pieterse, M.J. & Bourne, D. (1991) *THM formation in potable waters with reference to related variables and health data-bases.* Water SA, **17**: 269-272.

Vaughan, D. & Malclom, R.E. (eds.) (1985) *Soil organic matter and biological activity.* Martinus Nijhoff/Dr. W. Junk Publishers.

Verhoeven, J.T.A., Whigham, D.F., van Kerkhoven, M., O'Neill, J. & Maltby, E. (1994) *Comparative study of nutrient-related processes in geographically separated wetlands: Toward a science base for functional assessment procedures.* In W. J. Mitsch, ed. Global Wetlands: Old World and New. Elsevier, Amsterdam, pp. 91-106.

Verhoeven, J.T.A. & Liefveld, W.M. (1997) *The ecological significance of organochemical compounds in Sphagnum.* Acta Botanica Neerlandica, **46**: 117-130.

Villanueva, C.M., Kogevinas, M. & Grimalt, J.O. (2003) *Haloacetic acids and trihalomethanes in finished drinking waters from heterogeneous sources.* Water Research, **37**: 953-958.

Vinebrook, R.D. & Leavitt, P.R. (1998) *Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web.* Limnology and Oceanography, **43**: 1065-1081.

Vingarzan, R. (2004) *A review of surface ozone background levels and trends.* Atmospheric Environment, **38**: 3431-3442.

Vinogradov, A.P. (1959) *The geochemistry of rare and dispersed chemical elements in soils, 2nd ed.* Consultants Bureau, New York.

Visser, S.A. (1984) *Seasonal changes in the concentration and colour of humic substances in some aquatic environments.* Freshwater Biology, **14**: 79-88.

Volk, C., Wood, L., Johnson, B., Robinson, J., Zhu, H.W. & Kaplan, L. (2002) *Monitoring dissolved organic carbon in surface and drinking waters.* Journal of Environmental Monitoring, **4**: 43-47.

Volz, A. & Kley, D. (1988) *Evaluation of the Montsouris series of ozone measurements made in the nineteenth century.* Nature, **332**: 240-242.

Wachter, J.K. & Andelman, J.B. (1984) *Organohalide formation on chlorination of algal extracellular products.* Environmental Science and Technology, **18**: 811-817.

Waiser, M.J. & Robarts, R.D. (2000) *Changes in composition and reactivity of allochthonous DOM in a prairie saline lake.* Limnology and Oceanography, **45**: 763-774.

Waiser, M.J. & Robarts, R.D. (2004) *Photodegradation of DOC in a shallow prairie wetland: evidence from seasonal changes in DOC optical properties and chemical characteristics.* Biogeochemistry, 69: 263-284.

Waller, K., Swan, S.H., DeLorenze, G. & Hopkins, B. (1998) *Trihalomethanes in drinking water and spontaneous abortion.* Epidemiology, 9: 134-140.

Walsh, K.P. & McLaughlan, R.G. (1999) *Bubble extraction of dissolved gases from groundwater samples.* Water, Air, and Soil Pollution, **115**: 525-534.

Warembourg, F.R. & Paul, E.A. (1977) *Seasonal transfers of assimilated ^{14}C in grassland: plant production and turnover, soil and plant respiration.* Soil Biology & Biochemistry, 9: 295-301.

Watkins, E.M., Schindler, D.W. & Turner, M.A. (2001) *Effects of solar ultraviolet radiation on epilithic metabolism, and nutrient and community composition in clear-water boreal lake.* Canadian Journal of Fisheries and Aquatic Sciences, **58**: 2059-2070.

Watts, C.D., Naden, P.S., Machell, J. & Banks, J. (2001) *Long term variation in water colour from Yorkshire catchments.* The Science of the Total Environment, **278**: 57-72.

Weatherhead, E.C. & Andersen, S.B. (2006) *The search for signs of recovery of the ozone layer.* Nature, **441**: 39-45.

Weinberg, H.S., Krasner, S.W., Richardson, S.D. & Thruston, A.D. (2002) *The occurrence of disinfection by-products (DBPs) of health concern in drinking water: results of a nationwide DBP occurrence study.* United States Environmental Protection Agency, Athens, GA. Available at http://www.epa.gov/athens/publications/EPA_600_R02_068.pdf. Last accessed 20-09-2006.

Wetzel, R.G. & Manny, B.A. (1972) *Decomposition of dissolved organic carbon and nitrogen compounds from leaves in an experimental hard-water.* Limnology & Oceanography, **17**: 927-931.

Wetzel, R.G. (1975) *Limnology.* W. B. Saunders Company.

Wetzel, R.G. (1990) *Land-water interfaces: metabolic and limnological regulators.* Verhandlungen. Internationale Vereinigung fur Theoretische und Angewandte Limnologie, **24**: 6-24.

Wetzel, R.G. (1992) *Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems.* Hydrobiologia, **229**: 181-198.

Wetzel, R.G., Hatcher, P.G. & Bianchi, T.S. (1995) *Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism.* Limnology and Oceanography, **40**: 1369-1380.

Wetzel, R.G. (2001) *Limnology: Lake and river ecosystems, 3rd ed.* Academic Press.

Whalen, S.C. (2005) *Biogeochemistry of methane exchange between natural wetlands and the atmosphere.* Environmental Engineering Science, **22**: 73-94.

Whipps, J.M. (1990) *Carbon Economy.* In: Lynch, J. M. (Ed.) *The Rhizosphere.* John Wiley, West Sussex, UK, pp. 59-90.

Whitaker, H., Nieuwenhuijsen, M.J., Best, N., Fawell, J., Gowers, A. & Elliot P. (2003) *Description of trihalomethane levels in three UK water supplies.* Journal of Exposure Analysis and Environmental Epidemiology, **13**: 17-23.

White, R.E. (1997) *Principles and Practice of Soil Science* (3rd ed.) Blackwell Science.

White, D.M., Garland, D.S., Narr, J. & Woolard, C.R. (2003) *Natural organic matter and DBP formation potential in Alaskan water supplies.* Water Research, **37**: 939-947.

WHO (World Health Organisation) (2004) *Guidelines for drinking water quality.* 3rd ed, Volume 1, Recommendations. Geneva: World Health Organisation.

WHO (World Health Organisation) (2006) *Cancer: diet and physical activity's impact.* Available from:
http://www.who.int/dietphysicalactivity/publications/facts/cancer/en/
Last accessed 25-09-2006.

Widrig, D.L., Gray, K.A. & McAuliffe, K.S. (1996) *Removal of algal-derived organic material by preozonation and coagulation: Monitoring changes in organic quality by pyrolysis-GC-MS.* Water Research, **30**: 2621-2632.

Wild, A. (1993) *Soils and the Environment: An introduction.* Cambridge University Press.

Williams, B.K. & Wheatley, R.E. (1988) *Nitrogen mineralization and water-table height in oligotrophic deep peat.* Biology and Fertility of Soils, **6**: 141-147.

Williams C.J., Shingara, E.A. & Yavitt, J.B. (2000) *Phenol oxidase activity in peatlands in New York state: Response to summer drought and peat type.* Wetlands, **20**: 416-421.

Williamson, C.E. (1995) *What role does UV-B radiation play in freshwater ecosystems?* Limnology and Oceanography, **40**: 386-392.

Williamson, C.E., Stemberger, R.S., Morris, D.P., Frost, T.M. & Paulsen, S.G. (1996*a*) *Ultraviolet radiation in North American lakes: attenuation estimates from DOC measurements and implications for plankton communities.* Limnology and Oceanography, **41**: 1024-1034.

Williamson, C.E. (1996*b*) *Effects of UV-radiation on freshwater ecosystems.* Journal of Environmental Studies, **51**: 245-256.

Wilson, M.A., Barron, P.F. & Gillam, A.H. (1981) *The structure of freshwater humic substances as revealed by ^{13}C-NMR spectroscopy.* Geochimica et Cosmochimica Acta, **45**: 1743-1750.

Woli, K.P., Nagumo, T. & Hatano, R. (2002) *Evaluating impact of land use and N budgets on stream water quality in Hokkaido, Japan.* Nutrient Cycling in Agroecosystems, **63**: 175-184.

Wonisch, A., Muller, M., Tausz, M., Soja, G. & Grill D. (1999) *Simultaneous analyses of chromosomes in root meristems and of the biochemical status of needle tissues of three different clones of Norway spruce trees challenged with moderate ozone levels.* European Journal of Forest Pathology, **29**: 281-294.

WMO (World Meteorological Organisation) (2003) *Scientific assessment of ozone depletion.* Global ozone research and monitoring project. Report 47, pp. 498.

Worrall, F., Burt, T.P., Jaeban, R.Y., Warburton, J. & Shedden, R. (2002) *Release of dissolved organic carbon from upland peat.* Hydrological Processes, **16**: 3487-3504.

Worrall, F., Burt, T. & Shedden, R. (2003*a*) *Long term records of riverine dissolved organic matter.* Biogeochemistry, **64**: 165-178.

Worrall, F., Reed, M., Warbuton, J. & Burt, T. (2003*b*) *Carbon budget for a British upland peat catchment.* The Science of the Total Environment, **312**: 133-146.

Worrall, F., Burt, T. & Adamson, J. (2004*a*) *Can climate change explain increases in DOC flux from upland peat catchments?* Science of the Total Environment, **326**: 95-112.

Worrall, F., Harriman R., Evans, C.D., Watts, C.D., Adamson, J., Neal, C., Tipping, E., Burt, T., Grieve, I., Montieth, D., Naden, P.S., Nisbet, T., Reynolds, B. & Stevens, P. (2004*b*) *Trends in dissolved organic carbon in UK rivers and lakes.* Biogeochemistry, **70**: 369-402.

Worrall, F. & Burt, T. (2004) *Time series analysis of long-term river dissolved organic carbon records.* Hydrological Processes, **18**: 893-911.

Worrall, F. & Burt, T. (2005) *Predicting the future DOC flux from upland peat catchments.* Journal of Hydrology, **300**: 126-139.

Worrall, F. & Lancaster, A. (2005) *The release of CO_2 from riverwaters – the contribution of excess CO_2 from groundwater.* Biogeochemistry, **76**: 299-317.

Worrall, F., Burt, T.P. & Adamson, J.K. (2006*a*) *Trends in drought frequency – The fate of DOC export from British peatlands.* Climatic Change, **76**: 339-359.

Worrall, F., Burt, T.P. & Adamson, J.K. (2006*b*) *The rate of and controls upon DOC loss in a peat catchment.* Journal of Hydrology, **321**: 339-359.

Xenopoulos, M.A., Prairie, Y.T. & Bird, D.F. (2000) *Influence of ultraviolet-B radiation, stratospheric ozone variability, and thermal stratification on the phytoplankton biomass dynamics in a mesohumic lake.* Canadian Journal of Fisheries and Aquatic Sciences, **57**: 600-609.

Xu, X., Mariano, T.M., Laskin, J.D. & Weisel, C.P. (2002) *Percutaneous absorption of trihalomethanes, haloacetic acids, and haloketones.* Toxicology and Applied Pharmacology, **184**: 19-26.

Yoon, J., Choi, Y., Cho, S. & Lee, D. (2003) *Low trihalomethane formation in Korean drinking water.* The Science of the Total Environment, **302**: 157-166.

Yoshida, L.C., Gamon, J.A. & Andersen, C.P. (2001) *Differences in above- and below- ground responses to ozone between two populations of a perennial grass.* Plant and Soil, **223**: 203-211.

Zepp, R.G. (1988) Environmental photoprocesses involving natural organic matter. In: Frimmel, F. H. & Christman, R. F. (Eds) *Humic substances and their role in the environment* (pp 193-214). J. Wiley & Sons.

Zepp, R.G., Callaghan, T.V. & Erickson, D.J. (1995) *Effects of increased solar ultraviolet radiation on biogeochemical cycles.* Ambio, **24**: 181-187.

Zepp, R.G., Callaghan, T.V. & Erickson, D.J. (1998) *Effects of enhanced solar ultraviolet radiation on biogeochemical cycles.* Journal of Photochemistry and Photobiology B: Biology, **46**: 69-82.

Zhou, Q., Cabaniss, S.E. & Maurice, P.A. (2000) *Considerations in the use of high-pressure size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances.* Water Research, **34**: 3505-3514.