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The characterisation of dissolved organic carbon and its influence on tri-halomethane formation potential

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**THE CHARACTERISATION OF
DISSOLVED ORGANIC CARBON AND ITS
INFLUENCE ON TRI-HALOMETHANE
FORMATION POTENTIAL**

A thesis submitted to the University of Bangor by:

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Abstract

Dissolved organic matter is best described as a mixture of soluble organic molecular structures derived principally from the degradation of plant matter by natural processes that occur in mineral and organic soils. The water industry currently utilise water catchments that are predominantly lowland or upland wetlands. These generally consist of bogs and fens that are currently under threat from changes in the global weather systems which pose a threat to the natural carbon cycling of these eco-systems.

Dissolved organic carbon (DOC) when disinfected during water processing reacts to form a series of disinfectant by products (DBPs) which have been reported to be detrimental to human health. With the threatened destabilisation of the water industry's water catchments, this thesis examines the character of the DOC being received into four lakes/reservoirs and attempts to establish what aspects or DOC characteristic relates to its reactivity when it is chlorinated with HOCl.

The series of samples were collected on a 30 day cycle for 24 months with every third month's samples being fractionated using DAX 8 and XAD 4 resins and an in-house designed and built instrument. The samples were characterised using a number of established literature methods and the tri-halomethane potential (THMFP) was determined by Gas Chromatography/Electron Capture Detection and Solid Phase Micro Extraction. Analysis of the samples highlighted differences in the DOC concentrations of the lakes over the seasons and the influences of dry, wet and cold periods upon the characteristics and concentration of the DOC. The results identified the lowland Cefni lake as the most responsive to environmental influences, but it had the lowest THMFP. The highest was exhibited by the upland Conwy lake DOC which also demonstrated the least response to external environmental influences. The dry/wet event investigated illustrated the differences between mineral and peat catchments, demonstrating rapid increases and decreases in DOC concentrations in the Mineral streams with relatively slower responses in the Peat stream. Although the upland Peat DOC peak concentration was higher than that of the upland Mineral, it was the upland Mineral that demonstrated the highest THMFP/mg of DOC.

Overall the results have indicated that the THMFP is independent of DOC concentration and that there is no single inference that controls their formation, but is a combination of a number of factors with ring substitution being the foremost influence.

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List of Abbreviations

AR	As Received
DBP	Disinfection by products
DOC	Dissolved organic carbon
ECD	Electron Capture Detector
EEM	Excitation Emission Matrices
FP	Finished Product
GC	Gas chromatography
HPLC	High Pressure Liquid Chromatography
MM	Molecular Mass
PARAFAC	Parallel Factor Analysis
RCR	Re-calculated Results
RSW	Raw Surface Water
THMFP	Tri-halomethane Formation Potential

Chapter 1

Introduction

Chapter 1. Introduction.

1.1 Chlorination of Raw Surface water

Water is one of the planet's most precious commodities and its capacity to sustain life is irrefutable, but it can also be the carrier of life threatening diseases. This was first highlighted by Dr John Snow when in 1854 he identified the Broad Street pump as the source of a cholera outbreak in London (Sack *et al.* 2004). The scientific community at the time believed that the transmission of cholera was by 'bad air'. John Snow in 1849 published an essay suggesting that the most probable route was *via* the mouth, but was ignored. The hypothesis was proved correct, when by the removal of the pump handle a sudden drop in new cholera cases were recorded.

Chlorine as a means of disinfecting the water supply was first suggested in the 1800s by William C. Cruikshank, but was met with resistance as people were seriously concerned about the health effects (Kleijnen *et al.* 2011). Later in the century the development of a chlorinated municipal water supply became more acceptable and Carl Rogers Darnall a Professor of Chemistry working for the United States Army Medical School in 1910 (Darnall C.R, 1911) began chlorinating water using sodium hypochlorite. Although chlorine is still one of the most effective water disinfectants and is still currently used by the water industry, it does form during the process a series of undesirable by-products when it comes into contact with dissolved organic carbon (DOC) in the water, which include; trihalomethanes (THMs), haloacetic acids (HAAs) and haloacetonitriles (HANs) (Rook, 1976; Oliver and Visser, 1980; Westerhoff *et al.* 2004).

Some of the major catchments that supply our water industry are upland and lowland wetlands, but the transfer of these waters to reservoirs and lakes includes the export of up to 20 % of stored terrestrial dissolved organic carbon (Fenner *et al.* 2007). The carbon fluxes which have been relatively balanced within these wetlands to date have recently become vulnerable to climatic effects. These, include higher temperatures, higher CO₂ atmospheric concentrations together with some hydrological changes. One impact of these associated changes will be the increased export of the DOC out of the catchments which will present the water industry with a challenge to keep the levels of the chlorinated by-

products in control and be aware of the climatic influences that may alter the trihalomethane formation potential (THMFP) of the organic compounds in the DOC.

1.2 Carbon Balance

The chemistry of organic compounds is based on carbon, the twelfth most abundant element in the Earth's crust (Greenwood & Earnshaw, 1993). Approximately 0.08% of the carbon is accounted for in the combined lithosphere, hydrosphere and atmosphere. The balance is held mainly in the form of carbonates as sedimentary rock. These reservoirs of carbon are effectively considered to be in equilibrium between their sources and sinks, so exist as global steady state systems (Killops and Killops, 1993). However since the beginning of the industrial revolution in the mid-1700s, there have been anthropogenic influences upon the natural flux between some carbon reservoirs. The most significant of these is the emission of CO₂ into the atmosphere from the burning of fossil fuels. The average atmospheric level for several thousand years prior to this period is believed to have been 280 ± 10ppm (Barnola *et al.*, 1999). Monitoring of atmospheric levels over the past 5 decades on the island of Manua Loa Hawaii has indicated a trend from 315.98 ppm in 1959 to 385.98 ppm in 2008. This represented an annual increase of 1.4 ppm for that period, but for 2010 the annual increase was 2.36 ± 0.09 ppm bringing the atmospheric concentration to 389.6ppm, which is approximately 39% above the pre-industrial revolution level (Carbon Dioxide Information Analysis Center, 2010).

One of the principal sinks of this atmospheric carbon dioxide occurs in northern hemisphere wetlands *via* plant photosynthesis and subsequent inorganic and organic transformations by microbial processes, resulting in the accumulation and locking of carbon within the wetland soils.

1.3 Wetlands

Wetlands cover approximately 4 to 6% of the earth's land surface (Maltby and Turner, 1986). They consist of areas of small lakes, floodplains and marshes. European wetland areas are primarily distributed in the cool boreal and sub-arctic areas of the northern hemisphere with an estimated area of about 3.5 x 10⁶ km² (Mitsch and Gosselink, 2000). The generic expression 'peat land' is used for any wetland that accumulates decaying plant matter in the form of peat. Gorham, (1991) stated that the largest tracts of peat land are found in Ireland, Scandinavia, Finland and Northern Russia. Within the British Isles,

smaller areas of peat lands are to be found and, although the areas are limited, they represent approximately 10% of the land mass (Dawson et al.2003). The characteristics of wetlands are complex but two principal systems, namely the ‘bog’ and the ‘fen’ can be classified by their hydrological processes and landscape features.

1.3.1 Bogs

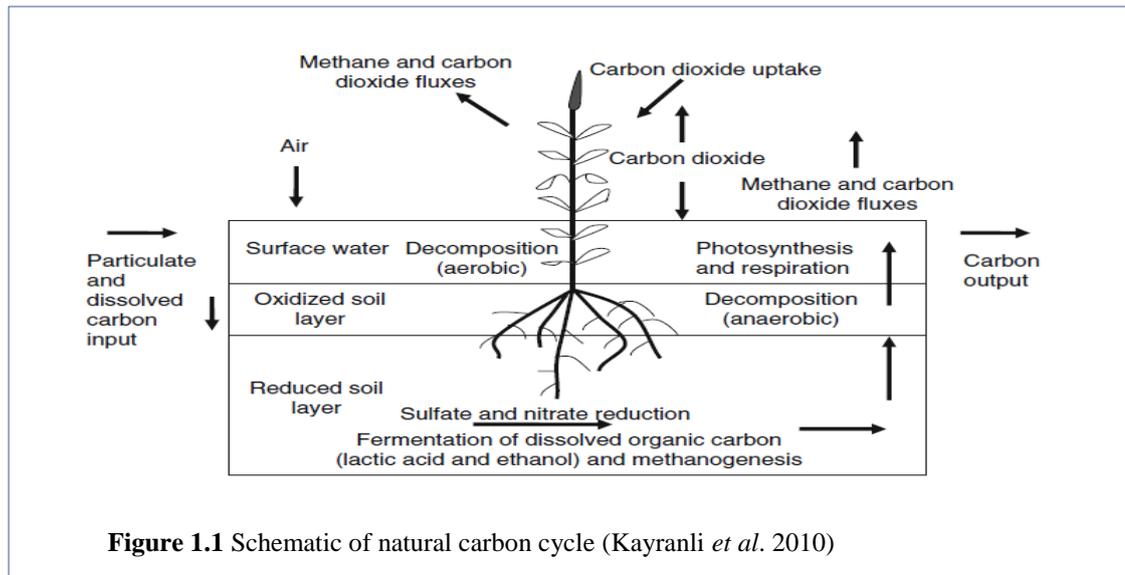
True bogs, or *ombrotrophic peatlands*, have developed peat layers which are level or higher than their surroundings and depend entirely on the atmosphere for water inputs and their nutrition. They are deficient in available plant nutrients and tend to be dominated by *sphagnums*. Bog water is generally acidic with a pH of 4.0 to 4.8 with acidity enhanced by the organic acids formed during slow decomposition of the vegetation (Warner and Rubec, 1997).

1.3.2 Fen

Unlike a bog, fens receive a large portion of their inputs from groundwater and surface run-offs of the surrounding watershed (Schlesinger, 1997). The surface water may flow through channels, forming pools and small lakes that are rich in dissolved minerals and can be described as *minerotrophic* (Avery, 1990). The vegetation in the richer fens is dominated by sedges and brown mosses, but in the poorer ones with lower levels of dissolved minerals, *Sphagnum* may be the dominant species which is said to have generated 10 – 15% of the current and historic, terrestrial carbon stock (Warner and Rubec, 1997; Fenner *et al.* 2004).

1.3.3 Wetlands as Carbon Reservoirs

Within this diverse system is stored a large proportion of the world’s carbon. Bridgham *et al.* (2008) estimated that peat land alone contains between 329 and 525 x 10¹⁵ Tg of C. Five main carbon reservoirs are contained within wetlands: plant biomass carbon, microbial biomass carbon, particulate organic carbon, dissolved organic carbon and the end product gases; carbon dioxide and methane. The accumulation and removal of this carbon is a continuum and the schematic (Fig 1.1) illustrates the basic carbon cycle of a functioning wetland.



Worrall *et al.* (2009) estimated a complete carbon budget for a peat covered catchment area of 11.4 km² in Northern England. The study included both fluvial and gaseous carbon fluxes together with the net ecosystem respiration of CO₂ and uptake of CO₂ by primary productivity. These workers calculated that over the 13 year period of data collection, the total carbon balance averaged a net sink of 56 g C/km²/yr. Extrapolation of these findings across all UK peat-land indicated that the carbon balance would be approximately -1.2 Tg C/yr (\pm 0.4 Pg C/yr).

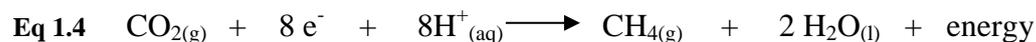
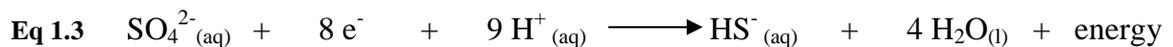
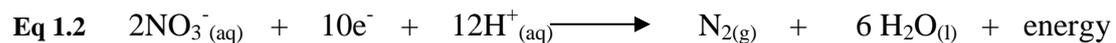
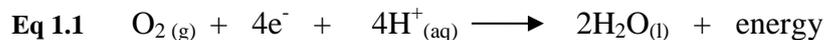
1.3.4 Decomposition.

Decomposition is the major process involved in the dissipation of energy stored in organic matter in wetlands. Both particulate and dissolved organic carbon (DOC) are subject to this process, part of which is *via* a number of physical-chemical steps such as grazing by zooplankton, photo-oxidation, lysis etc. Clymo, (1965) stated that the dominant decomposition within wetlands is orchestrated by microbial processes. The key microbial processes are respiration in the aerobic zone and sulphate, iron and nitrate reduction and fermentation in the anaerobic zone (Kayranli *et al.* 2010). The highest rates of organic matter decomposition are found in the near surface of the wetland where there is fresh litter and recently synthesised labile organic carbon for microbial respiration. The rapid reduction in available oxygen deeper within the anaerobic zone may result in the organic matter not being fully decomposed by the microbial processes. Hence the plant detritus and organic matter begins to accumulate as peat. Gorham (1991) estimated that the average accretion rate for boreal and sub-arctic peat-lands was 23g m²/year of carbon with

the accumulation of detritus/peat at 0.53 mm/year. Oxidation and reduction potentials (redox) are the major drivers of these processes.

1.3.5 Redox Potential.

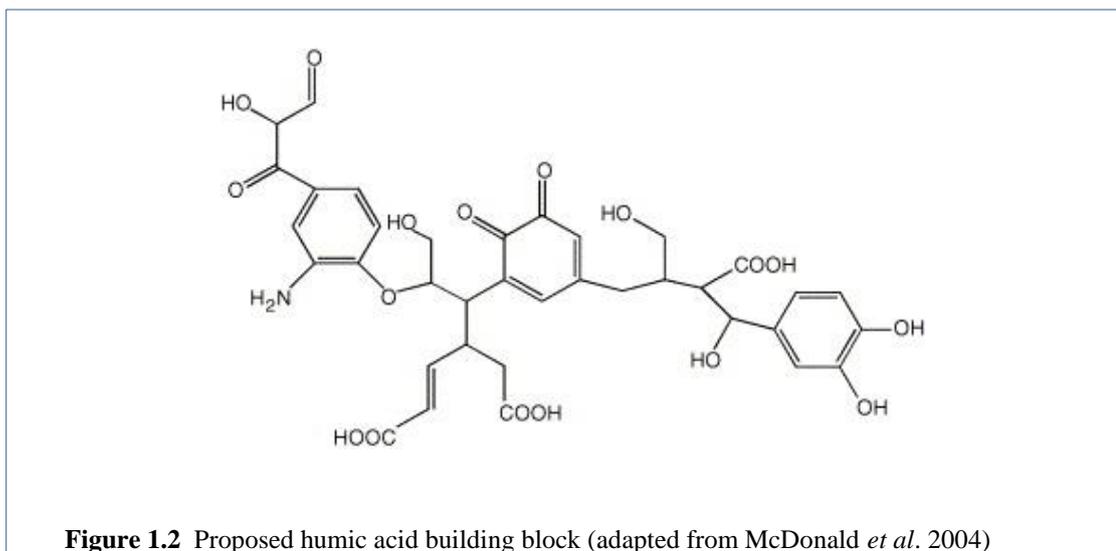
In wetland soils and peat reduction/oxidation (redox) processes are mediated by microbes (Mars *et al.* 1999) during respiration. That is, the microbe will use an electron transport system to remove electrons out of the cell and transfer them to an available electron acceptor, for example O₂, NO₃⁻ or SO₄²⁻, hence during the process the terminal electron acceptor is reduced and energy is generated (Lynch & Hobbie, 1988). With oxygen having limited solubility in water, aerobic conditions may give way to anaerobic conditions within millimetres of a saturated sediment surface. At the interface between the atmosphere and water layer where the concentration of dissolved oxygen is high and there are organic substrates available for microbial respiration, oxygen is the electron acceptor at a potential of about 400 mV (**Eq 1.1**). At lower redox potentials of 250 to -200mV, the electron acceptors may be NO₃⁻, SO₄²⁻ or CO₂ (**Eq 1.2, 1.3 and 1.4**) (Alwell *et al.* 2008). The reduction of CO₂ results in the production of the greenhouse gas methane.



1.4 Dissolved Organic Carbon

1.4.1 Character and structure

It is estimated that humic substances represent about 25% of the total organic carbon on earth with a substantial percentage of it held as DOC in lakes, waterways and the particulate interstitial sites of peat and soils (Robards *et al.* 1994).



Despite considerable efforts the exact composition of dissolved organic carbon (DOC) is not yet fully resolved due to its complex nature and the presence of mixtures of chemical species, although many published papers offer a compositional view of DOC (Rook, 1977; Jansen *et al.* 1996; McDonald *et al.* 2004; Samios *et al.* 2006;). Fig 2.1 shows a typical proposed building block of humic acid consisting of a number of different functional groups, but principally carbonyls, hydroxyls and carboxyls and amino groups. The influence of these functional groups on the aromatic ring activity can be pronounced and directional, from electron withdrawing properties by for example the (COOH and C=O), resulting in a lower rate of electrophilic substitution that is mainly directed to the *meta* position on the aromatic ring, to electron donating functional groups (OH,OR, NH₂ and OCOR) which are ring activators and *ortho* and *para* directors (Streitwieser and Heathcock, 1981).

The majority of the composition and structure of DOC results from the transformation of biomolecules by the metabolic processing of natural plant macromolecules (Peuravuori *et al.* 1999). Wetzel (1984) reported that DOC mass was decomposed by bacteria at a rate of 1-5% per day resulting in a growth of 25 – 50 % in bacterial biomass. The main DOC building blocks are believed to derive from cellulose, phenolic compounds (tannins and lignins) and water soluble compounds such as sugars, amino acids and aliphatic acids (McDonald *et al.* 2004). The resulting DOC is described as having two parts; a non-humic one which consists of known biomolecules such as lipids, carbohydrates, polysaccharides, amino acids, proteins, waxes and resins (Piccolo 2001), and a humic one which is defined as yellow to black heterogeneous organic substances that are refractory and of high

molecular weight $> 5,000$ Da (Macarthy *et al.* 1990). Thurman, (1985 b) described them as consisting of polyelectrolytic organic acids having a wide range of molecular sizes (2,000 – 5,000 and occasionally $\geq 100,000$ Da. Wershaw *et al.* (1990) subscribed to the existence of humic substances as pseudo-micellar structures which could not be represented by structural diagrams of functional groups with covalent bonds etc, but were similar to membranes and aggregates of plant degradation products such as fragments of lignin, tannins and lipids. The interiors of were made up of more hydrophobic parts with a freedom of motion similar to molecules suspended in a liquid. The outer surface of the aggregates are made up of more hydrophilic and polar groups with carboxylic acid functionalities.

Humic substances can be further subdivided into three components; humic acids, fulvic acids and humins (McDonald *et al.* 2004) Although the terminology is based on organic soil chemistry classifications, it has also been adopted for DOC of an aquatic nature. The humic acid components are described as the fraction that is not soluble at pH 2 or less, but increases in solubility with an increasing pH. Humic acids generally have a molecular weight range between 1,500 to 5,000 Da in stream water and between 50,000 to 500,000 in soils. (McDonald *et al.* 2004). The fulvic acid component is categorized as the fully soluble fraction from pH 1 to 14. Fulvic acid molecular weights range between 600 to 1000 Da in stream water and 1000 to 5000 Da in soils (McDonald *et al.* 2004). The last category of the humics are humins and these are considered to be insoluble in water at all pHs.

In the previous paragraph, the two divisions of the humic substances were assigned to a specific range of molecular weights. The humic acids are recognized as having larger molecular weights which precipitate readily when the pH is < 2 . This is partly thought to be due to aggregation of large polydisperse aromatic systems. These complex systems have a number of associated functional groups (Table 1.2) such as the phenolic hydroxyls, methoxyls and carboxylics which promote hydrogen bonding (Powell *et al.* 1992). Fulvic acids fractions have much lower molecular weights and are polar with more functional groups of an acidic nature; in particular carboxylics (McDonald *et al.* 2004). Both humic and fulvic acids exhibit hydrophilic properties in their natural state which is due principally to the strong hydrogen bonding between the molecular structures and the water molecules. However when the humic acid is isolated it can exhibit increased hydrophobic characteristics making it very difficult to re-dissolve without the application of ultra-sound and dissolution in a $\text{NaOH}_{(aq)}$ to increase the negative charges and reduce hydrogen bonding.

The major sources of humic and fulvic acids are decaying plant material under moist conditions (Jansen *et al.* 1996). The degradation products are believed to reflect fragments of molecular structures found in plants and microorganisms (Burdon, 2001). Hence, DOC from different environments is believed to have many common characteristics, although the abundance of some of the functional groups relative to each may vary (McDonald *et al.* 2004). Predominantly, DOC consists of a number of recurring functionally active structures (Table 1.1) that may determine the solubility, aggregation and trihalomethane formation potential (THMFP) of the DOC.

Table 1.1 Typical functional groups of Raw Surface Water DOC.		
R is the aliphatic backbone, Ar is a aromatic ring.		
Functional Group	Structure	Where found
Carboxylic acid	(Ar-) R- CO ₂ H	90% of all DOC
Phenolic OH	Ar - OH	Humic substances, phenols
Quinone	Ar = O	Humic substances (DOC)
Amine	(Ar-) R-CH ₂ - NH ₂	Proteins (DOC)
Amides	(Ar-) R=O (-NH-R)	Petides (DOC)
Ketone	(Ar-) R-C=O (-R)	Humic substances (DOC)
Aldehydes	(Ar-) R-C=O(-H)	Humic substances (DOC)

1.4.2 Changing Trends in DOC Concentrations.

Freeman *et al.* (2001) estimated that from (1988 -2000), stream and lake catchment DOC has increased by 65 % in the United Kingdom. There is also evidence of this increasing trend being displayed in both the rest of Europe and north America (Evans *et al.* 2006).

A common question is why are these increases occurring? A number of proposals have been advocated, principally based around global warming. Freeman *et al.* (2001) reported that the increase in DOC was associated with the rise in global temperatures over the past decades (+ 0.66° C from 1970 – 2000), leading to an increase in microbial activity and labile DOC. Laboratory simulations of temperature increases in peat matrices demonstrated that the extracellular enzyme *phenol oxidase* activity increased by 36 % for every 10° C rise in temperature. Clark *et al.* (2009) reported a decrease in DOC production during simulated drought and water table drawdown, which was ascribed to sulphur redox

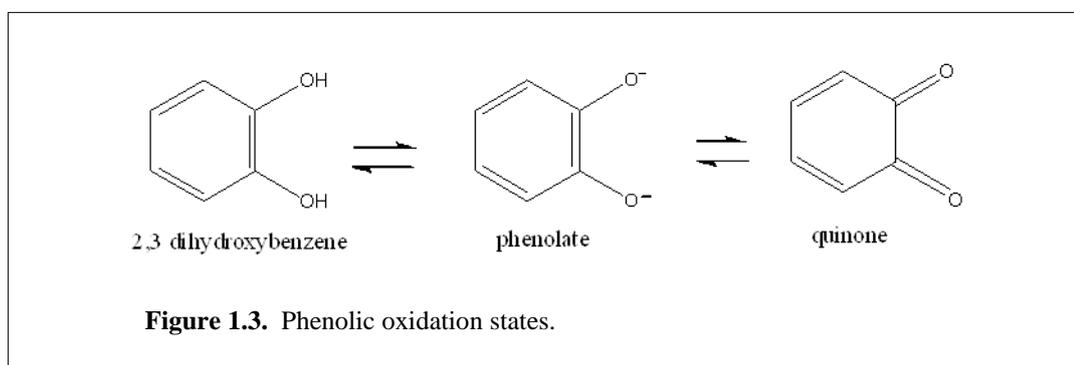
reactions decreasing the DOC solubility. Once the water table was restored DOC production increases were evident. Acidity has also been proposed as the controlling factor for the dissolution and export of DOC. Evans *et al.* (2012) demonstrated this by controlling the pH of separate sites over a period of 3 years (2009 – 2011) and reported an increase in DOC concentrations relative to an increase in pH and a decrease when the pH was lowered.

Table 1.2 Examples of UV-vis wavelength ratios and their application to DOC characteristics.			
Characteristic	Application	Wavelength ratio (nm)	Literature
Humification index	Degree of peat/soil organic matter decomposition	A_{465}/A_{665}	Kononova. (1966) Shirshova <i>et al.</i> (2006) McDonald <i>et al.</i> (2004)
Aromaticity	Measure of aromatic content in DOC	A_{250}/A_{365}	Peuravuori and Pihlaja (1997) McDonald <i>et al.</i> (2004)
Aromatic content of DOC/ water industry DOC indicator	Measure of aromatic/conjugated molecular systems in DOC	A_{254}	Edzwald <i>et al.</i> (1985)
Degree of aromatic ring activation	Prediction of the reactivity of aromatic moiety in chlorination reactions	A_{254}/A_{203}	Korshin <i>et al.</i> (1997)
Specific UV-vis absorbance (SUVA)	Provides an estimate of UV absorbing species and aromatic content	$A_{254}/\text{DOC (mg/L)}$	McDonald <i>et al.</i> (2004) Croue (2004) Hur <i>et al.</i> (2006)

1.4.3 Phenolic Compounds in DOC.

It has been reported that a major contributory group to dissolved organic carbons within the peat aquatic systems is derived from what is broadly termed the phenolics (Thurman, 1985). For phenolic compounds are a chemically diverse widespread group of hydrocarbons found in plants, decomposing matter and other biologically active systems (Ribereau-Gayon, 1972). They occur as both monomeric and polymeric forms and are

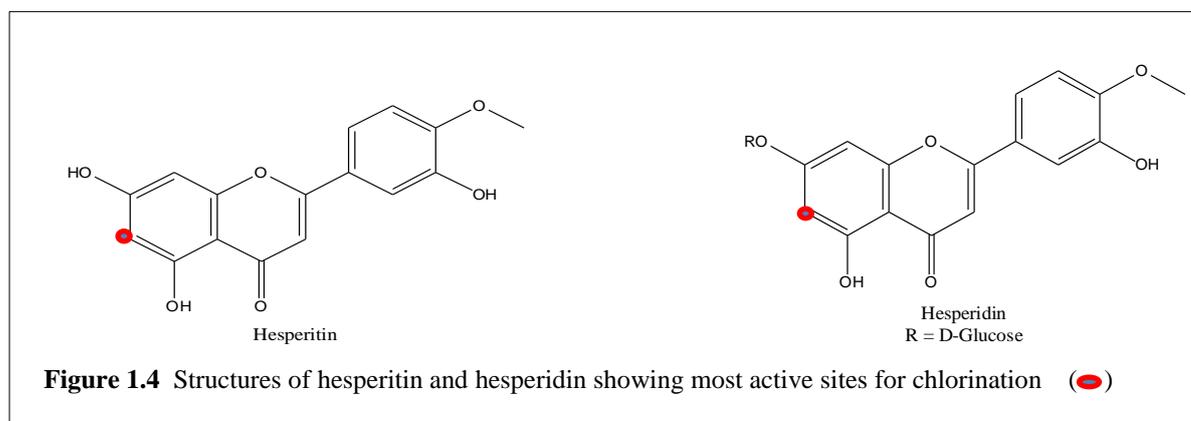
characterised by the presence of an aromatic ring bearing one or more hydroxyls (Fig 3). (Appel, 1993). Phenolics can participate in four major types of bonding, hydrophobic bonds where the aromatic ring of the phenolic attracts the hydrophobic regions of other compounds; for example aliphatic and aromatic side chains of amino acids (Oh *et al.* 1980). Phenolics can also form hydrogen bonds with other compounds at a pH below its pKa of ≈ 9.9 . Phenolics also have the capability of forming ionic bonds when it is deprotonated and is presented as a phenolate ion. The fourth type, is the covalent bond where the phenolics may polymerize by an oxidative process where radicals may be formed (Appel, 1993).



Microbial decomposers have been reported to be inhibited by the presence of phenolic compounds. For instance, Freeman *et al.* (2004) demonstrated this by monitoring the activity of peat hydrolase activities in peat slurries. The addition of the extracellular enzyme *phenol oxidase* to the slurries increased the activity of the hydrolases, β -glucosidase and phosphatase by a multiple of 6 and sulphatase by 18 %. Although effective in degrading the recalcitrant phenolic compounds, the *phenol oxidase* enzyme requires molecular oxygen for substrate oxidation (Rosenzweig and Sazinsky, 2006). Hence studies have reported that the long term increases in upland waters DOC concentrations after recent summer droughts may have been as a result of the aeration of the upper peat strata increasing the enzyme activity and solubilising the recalcitrant DOC (Worrall *et al.* 2004). This was initially suggested by Freeman *et al.* 2001 and was described as the ‘latch’ and is purported to be a mechanism that is currently near to a tipping point, which will be quickly unbalanced by an increase in changing temperatures and drought conditions.

Characterisation of DOC in the literature does not normally include phenolic content as part of the standard series of methods, but examination of model compounds that represent

THM precursors has demonstrated the importance of these. Rook, (1977) compared the reactivity of hesperitin and hesperidin when subjected to chlorination (Fig 1.4).



In that report, the yield of CHCl_3 from the chlorination of hesperitin was twice that of the hesperidin. This was considered to be due to the two free OH groups in the *meta* position of the hesperitin activating the position in between, whilst with one of the hesperidin OH groups subject to etherisation, hence reducing the chlorination activity of the site in between. Westerhoff *et al.* (2004) reported the same reactivity when comparing the reaction rates of a number of model compounds, such as maleic, benzoic acid and resorcinol. Resorcinol (1,3 dihydroxybenzene) with its two meta OH groups (electron donating), formed 0.1 moles of CHCl_3 /mole resorcinol over 40 minutes, but both maleic and benzoic acid, with the COOH electron withdrawing functional groups, demonstrated a much slower rate, only forming 0.001 moles CHCl_3 /mole benzoic and maleic acid over 40 minutes.

Phenolic compounds in DOC have been quantified by the standard Folin-Ciocalteu assay method described by Box (1981). In this assay samples containing one or more hydroxyl substituents bonded to aromatic rings are reacted at ambient temperature in the presence of Na_2CO_3 with Folin-Ciocalteu's reagent which contains both phosphomolybdic and phosphotungstic heteropoly acids. The reaction solutions have a general form of a central tetrahedral phosphate unit surrounded by octahedral molybdenum oxy acids. This structure can inter-change with tungsten. The DOC phenolates are oxidized by the oxidant, hence the heteropoly acid is reduced from a valence state of + 6 to mixture of + 6 and + 5. This results in the formation of a blue molybdenum-tungsten complex (Box, 1981).



Huang *et al.* 2005 and Everette *et al.* (2010) have reported that Folin-Ciocalteu reagent was significantly reactive towards other antioxidant compounds besides phenols and that the assay should be qualified as a total anti-oxidant capacity rather than total phenolics, but qualifies this statement by suggesting that it gives a rough approximation of total phenolic content.

1.4.4 Fractionation of DOC using Macroporous Resins.

Isolation of the DOC into specific fractions has been investigated by a number of researchers, using techniques such as precipitation, ultrafiltration, solvent extraction and freeze drying (Thurman and Malcolm, 1981). For a number of years a procedure using non-ionic macroporous sorbing resins such as the XAD 4 and DAX 8 has been utilised (Aiken *et al.* 1992). The DAX 8 resin is an hydrophilic acrylic ester with low ion exchange capacity (10^{-2} Mequiv g^{-1}), average pore size of 225 Å and surface area of 160 $m^2 g^{-1}$ and is reported to adsorb the large hydrophobic molecular structures from DOC (Malcolm and MacCarthy, 1992). The XAD 4 resin (a styrene-divinylbenzene copolymer) is highly aromatic and hydrophobic and possess no ion exchange capacity. The average pore size is much smaller at 40 Å and the surface area much larger at 725 $m^2 g^{-1}$. XAD resins have previously been reported for the successful separation/removal of fulvic and humic acids from water (Aiken *et al.*, 1979; Malcolm, 1992).

It has been reported that the resins in tandem (DAX 8 \rightarrow XAD 4) recover a large part of the DOC without contaminating the solutes with organic solvents or reagents (Malcolm and MacCarthy, 1992). When an acidified (pH 2) aqueous sample is passed through the DAX 8 resin, the hydrophobic acid fraction which is considered to be the fulvic/humic acids is sorbed by the resin. These large un-dissociated macromolecules are retained within the large pores (>25 nanometers) of the resin beads. Elution of these large retained molecules is achieved by simply passing 0.1M NaOH through the column which rapidly de-protonates the acidic moieties. The subsequent effluent is isolated and re-acidified to pH 2. The molecules described as the hydrophilic acids are not retained on the DAX 8 resin, but pass through to the XAD 4 resin where they are held. The resin is more hydrophobic than the more polar DAX 8 and has a large capacity for small uncharged solutes. The small pores of (*ca.* 5 nm) of the highly cross linked styrene divinylbenzene are uncharged and this favours the sorption of small hydrophilic acid molecules which have passed through the DAX 8 resin. These can then also be eluted by passing 0.1 M NaOH through the column (Malcolm and MacCarthy, 1992). The use of the XAD 4 resin is an alternative to the use of the weak anion exchange resin Duolite A-7 used by Leenheer and

Noyes (1984). The A-7 tended to concentrate inorganic anions as well as the organic ones during the process which then required other columns to isolate the inorganic ions. Further evidence of the DAX 8 and 4 system for fractionation was demonstrated when it was used in the HUMEX acid rain exercise in Norway where the important environmental project required accurate methods to determine slow changes in the organic aqueous solutes (Norwegian Institute for Water Research 1992).

The fractions recovered by the dual resin in tandem have been classified as per Table 3. (Leenheer, 1981 ; Aiken *et al.* 1992: Chow *et al.* 2003).

Table 1.3 Classification of fractions recovered from DAX 8 and XAD 4		
Resin	Eluent	Classification
DAX 8	NaOH (0.1 M)	Hydrophobic acid
DAX 8	HCl (0.1 M)	Hydrophobic base
XAD 4	NaOH (0.1 M)	Hydrophylic acid
XAD 4	HCl (0.1 M)	Hydrophylic base
DAX 8/XAD 4 (effluent)	None (not desorbed)	Neutral hydrophilic/hydrophobic

The organic compounds relative to the classifications (Table 1.3), that have been isolated from water have been described in the literature as listed below, but as (Marhaba *et al.* 2000) states, the makeup of the fraction components are dependent on the water condition and the watersheds;

- Hydrophobic acid - a soil fulvic acid/humic acids (Schnitzer, 1978; Malcolm and MacCarthy, 1992).
- Hydrophobic base – a humic substance containing amino acids, proteins, sugars and polysaccharides (Bruchet *et al.* 1990).
- Hydrophilic acid – an organic compound of the hydroxyl acid group (Leenheer, 1981).
- Hydrophilic base – a amphoteric proteinacious materials containing amino acids, amino sugars, peptides and proteins (Leenheer, 1981)
- Neutrals (Hydrophobic) – a mix of hydrocarbon and carbonyl compounds such as sugars (Leenheer, 1981),
- Neutrals (Hydrophilic) - an organic compound made up of polysaccharides (Tipson, 1968).

Peuravuori *et al.* (1997) noted that these fraction classifications are based on chemical isolation procedures rather than actual properties and quoted Frimmel and Shuman, (1990), who suggested that similarities in the samples derived from different origins posed the question, of whether this reflected the natural reality or artefacts due to the method of isolation. However, Peuravuori *et al.* (1998) suggested that the isolation of aquatic humic solutes using the XAD technique isolates fractions independent of molecular size and that they cannot be “accidental products of the isolation process”. Hence they can be classified as defined groups of entities of DOC.

A number of researchers have reported on the fractions recovered by the DAX 8 and XAD 4 resin technique. Marhaba *et al.* (2000) confirmed a mass balance tolerance of 10 – 15 % using the method whilst Croue *et al.* (1993) reported a mass tolerance of 8-12 %. The reported fraction distribution characteristics of the DOC were not similar for all sources, with the principal fraction being the hydrophobic acid of $\geq 40\%$ of DOC fractioned by Lu *et al.* (2009), Wei *et al.* (2008), Croue, (2004) but Lin *et al.* 2009 reported the hydrophilic acid as the major contributor (32 %) for one reservoir and the hydrophilic neutrals (39%) for another, with the hydrophobic acids of both averaging only 14 %. Barber *et al.* (2001) fractioned the DOC output of surface water passing through a series of treatment wetlands, and demonstrated the hydrophobic acid ranging from 33 – 46 % and hydrophobic bases ranging ≤ 1 %, with the hydrophobic neutrals exhibiting a 11 – 20 % DOC. Fractioning a Raw Surface Water before and after treatment, Croue (2004) reported a hydrophobic acid of 43 % before treatment. After treatment (concentration by reverse osmosis) the same fraction increased to 56 %. The hydrophilics also demonstrated an increase, 5 % before treatment and 8 % after. The concentrations of DOC in the pre-treated water was 8 mg/L and 1 mg/L in the treated.

1.4.5 Fourier Transform Infrared Spectroscopy of DOC.

Infrared spectroscopy has been used to determine the functional groups of the DOC molecular structures. Typically samples are freeze dried and to remove any moisture during storage and further dried at 60° C before analysis to reduce any contributory bands relative to water at 1640 cm^{-1} (Peuravuori *et al.* 2005; Santos *et al.* 2000). Samples tend to be measured as KBr pellets or using an attenuated total reflectance (ATR) attachment. The spectra reported in the literature have a number of common bands (Leenheer *et al.* 1987 ;

Nanny and Ratasuk, 2002; Santos *et al.* 2009; Chen *et al.* 2002). A broad strong band around 3400 cm^{-1} is generally attributed to OH stretching and aliphatic CH bands at the $2950 - 2850\text{ cm}^{-1}$ are reported by all in the literature. Strong bands have been reported in the $1734 - 1730\text{ cm}^{-1}$ region, attributed to non-conjugated C=O stretching of carboxyl groups, aldehydes and ketones ; $1437 - 1429\text{ cm}^{-1}$, deformation of aliphatic C-H stretch; $1093 - 1084\text{ cm}^{-1}$, C-O stretching of polysaccharides (Shirsikova *et al.* 2006). Santos *et al.* (2009) reported a band at 1580 cm^{-1} which was characteristic of a COO^- and a band at 1630 cm^{-1} attributed to C=C stretching of aromatic rings and to C=O stretching of conjugated carbonyl groups in ketones and quinones (Silverstein *et al.* 1991). An FT-IR analysis of a DOC recovered from the fresh water of Lake Savojarvi in Finland exhibited similar IR spectral bands to the above with bands attributed to C-C, C-OH, C-O-C and Si-O around 1054 cm^{-1} (Peuravuori *et al.* 2005).

1.4.6 Determination of Molecular Masses (MM) in DOC.

The most common methods for determining the molecular mass and size of molecules in DOC are ultra-filtration, electro-phoresis, osmometry, mass spectrometry and gel permeation chromatography or (size exclusion chromatography (SEC) (Abbt-Braun *et al.* 2004). The advantage of using SEC lies in the capability of being able to directly apply the samples onto the separation column. The technique can also be integrated with a series of in line methods such as ICP/MS, fluorescence or UV spectroscopy. Zhou *et al.* (1999) stated that the determination of the MM of humic substances is not a simple task as it represents a complex mixture of heterogeneous organic materials and a broad distribution of different structures. One of the factors that influence the determination of these MMs are the calibration standards. The following polymers have been tested; polydextranes (PDX), polyacrylates (PA) polymethacrylates (PMA) and polysaccharides, but these have not been successful as they did not represent the coiled structures of DOC (Peuravuori, 1997). For instance, Perminova *et al.* (1998) reported results using PDX, PA, PMA and poly(styrenesulfonic) acid sodium salt polymers (PSS) and that the accuracy of MM determination depended on the charge densities of the standards, i.e., the smaller the charge density, the higher the MM and *vice-versa*. It was established that the most representative standards of DOC character were the PSS polymers, for they demonstrated similar hydrodynamic radii, viscosity and acidity to humic material (Chin *et al.* 1994). The technique was further optimised by suppressing charge effects with a 0.1M solution of

NaCl. This effect induced the same coiled configuration in both the solutes and PSS standards (Leenheer and Croue, 2003; Peuravuori *et al.* 1997).

O'Loughlin *et al.* (1999) reported the effect of the UV-vis detector wavelength on MM determination. Because humic substances contain a range of chromophores which have a number of molar absorptivities (at any given wavelength) the MM detected may be biased by the wavelength set on the detector. For example, large MM may have an extinction coefficient different to the lower MMs, hence at the wavelength that detects the large MMs, the lower MMs may be relatively invisible and detection greatly reduced (low absorbance). This would lead to a distorted measure of the MMs distribution in the DOC. O'Loughlin and his co-workers reported that an increase in wavelength was accompanied by an increase in MM, for example one of the Lake Fryxell fulvic acids MM increased by 63% between 220 - 380 nm, but determined that the increases observed between 220 – 280 nm, which are the commonly used wavelengths for SEC were minimal.

Further evidence in support of using the wavelength range 220 - 280 nm for MM determinations in DOC was demonstrated by the following researchers; Chin *et al.* (2000), who reported the weight average (M_w) for Suwannee River humic acid of 3,703 and 2,243 Da for the fulvic acid at a 260 nm wavelength using PSS as calibration standards.

A sample of Suwannee River fulvic acid analysed by Zhou *et al.* (1999) exhibited an M_w of 1951 Da. Analysis of Suwannee River fulvic acid by Hur *et al.* 2006 at two separate wavelengths (224 and 254 nm) using the same SEC conditions and PSS standards demonstrated that the difference in apparent MM at the two wavelengths were negligible, i.e., 1,944 Da at 224 nm and 1,996 Da at 254 nm, hence confirming that any errors in MM relative to wavelength are minimal between 220 – 280 nm as suggested by O'Loughlin *et al.* (1999).

Seasonal and drought effects were suggested by Scott *et al.* (2001) to promote changes in MM distributions, with an increase from 8,000 – 10,000 Da in winter to 14,000 – 16,000 Da during a period of drought in a peat pool on Great Dunn Fell, Cumbria. After the drought, the average MM distribution was seen to drop to 6,000 – 8,000 Da. The SEC method has also been used to detect changes during water treatment processes. For instance, Korshin *et al.* (2009) analysed the changes in MM after coagulation with alum of a Raw Surface Water (Copi Hollow, New South Wales, Australia) and reported a drop in MM >1500 Da at low alum concentrations (40 mg/L).

1.4.7 Fluorescence Spectroscopy of DOC.

The application of fluorescence to the study of DOC is believed to be limited to a minor number of components (McDonald *et al.* 2004). The spectra are obtained by UV irradiation of the molecular structures in their lowest vibrational level of the ground electronic state. The molecules absorb the radiation and a transition to the second excited state takes place, after which some of the molecules can return to the ground state, by emitting some of the energy in the form of light, at a longer wavelength (Hollas, 1990). When the excitation and emission wavelengths are scanned simultaneously and the differences between them are kept constant the resulting spectrum is referred to as a synchronous spectrum (Goslan *et al.* 2004).

Coble, (1996) reported the fluorescence properties of DOC samples from two environments (sea and river water) and plotted the intensities as contours. An example of the results are shown in Table 1.4. Reasons for reporting them as humic ‘like’ was because of the overall similarity of the peaks but, minor differences existed in the peak positions which suggested differences in humic compositions between sample. To eliminate the visual identification of the maximum peak intensities, Stedmon et al (2003) suggested the use of a multivariate analysis to decompose the complex mixtures of fluorescence signals into individual fluorescing components. This approach was first proposed by Bro (1997) and suggested parallel factor analysis (PARAFAC). The analysis provides a means of collectively examining large sets of fluorescence excitation and emission data and identifies the most common fluorescing components in the group of samples.

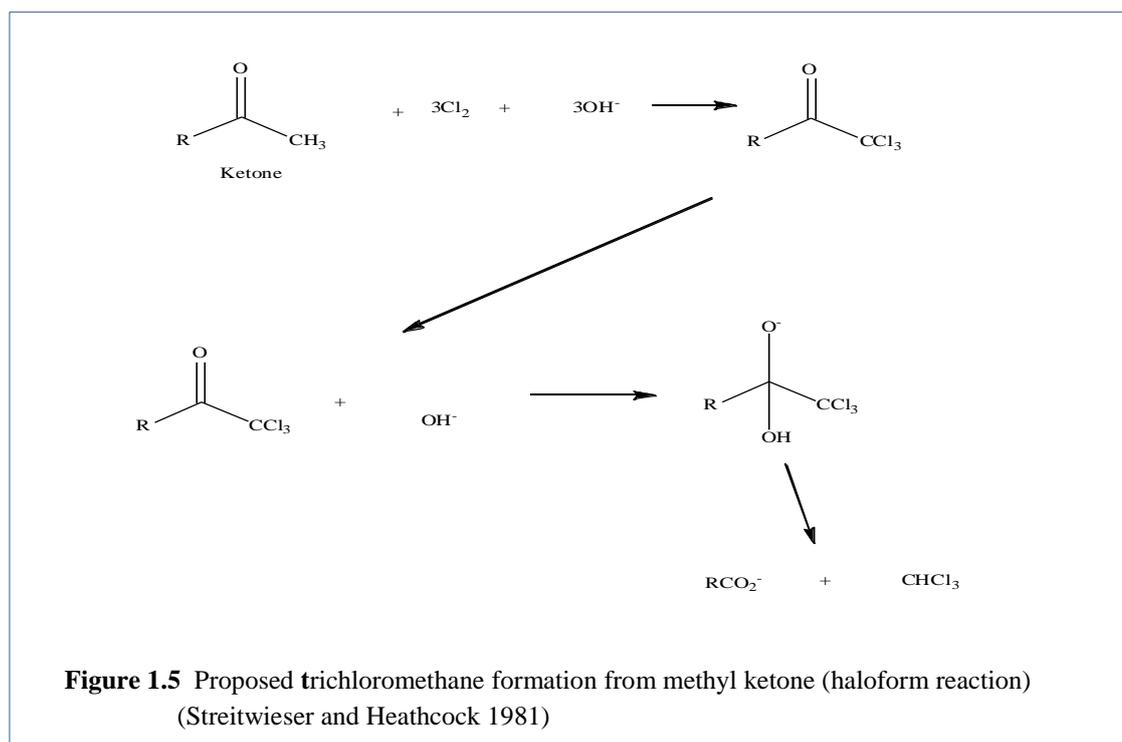
Peak	Ex(max)	Em (max)	Substance
B	275	310	Tyrosine like
T	275	340	Tryptophan like
A	260	380 - 460	Humic like
M	312	380 - 420	Marine humic like
C	350	420 - 480	Humic like

Due to the heterogeneous nature of DOC and the absence of fully validated reference standards, literature results are qualified with the word 'like' (Coble, 1996 ; Stedmon *et al.* 2003 ; Goslan *et al.* 2003; Fellman *et al.* 2009; Yamashita *et al.* 2011).

1.4.8 Tri-halomethane Formation Potential (THMFP) of DOC.

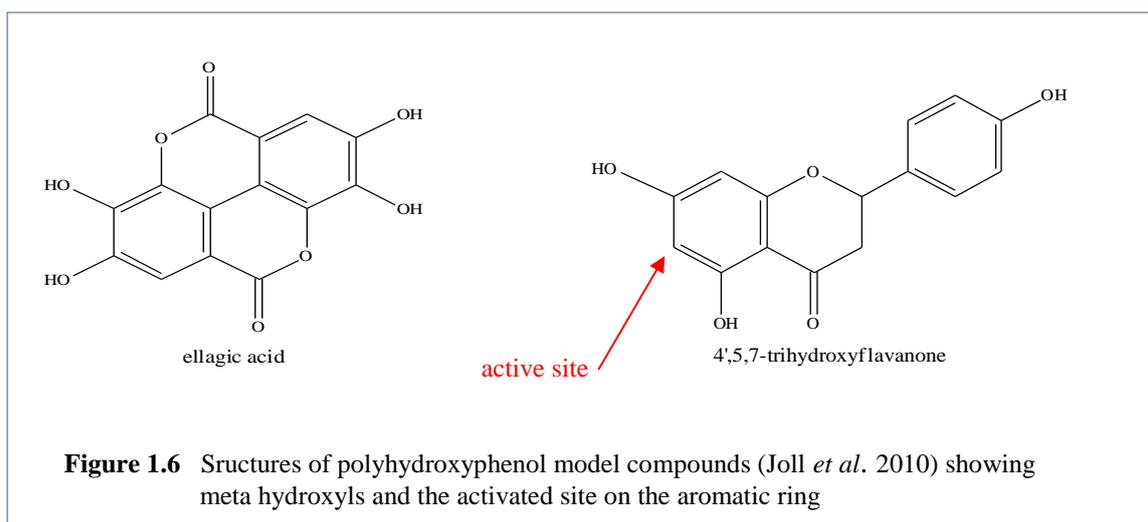
Chlorination of water can lead to the formation of organo-halides by the reaction of free chlorine with the organic matter (DOC) in the water (Sketchell *et al.* 1995). These by-products of the disinfection processes in water treatment works include a number of distinct compounds that can be broadly classified as the haloacetic acids (HAAs), haloacetonitriles and trihalomethanes (THMs) collectively known as disinfection by-products (DBPs). The best documented, and legislated for, are the THMs trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. DBP formation is considered to be a complex process that depends on a number of factors that include DOC concentration, chlorine dose, water temperature, reaction time and pH (Edzwald, 1987; Rodrigues *et al.* 2007). The two DOC reactants that have been suggested as the principal THM precursors are the humic and fulvic acids (Leenheer *et al.* 2001; Samios *et al.* 2007; Bond *et al.* 2009). Although the literature identify them and classify them for simplicity by the property of remaining in solution (fulvics) or falling out of solution (humics) at pH < 1; Rook, 1977 described them as being the same with the fulvic acids essentially being only smaller sized humic acid molecules. Samios *et al.* (2007) extends this by suggesting that humic acids have fewer acidic functional groups than fulvics but, the humic acids do demonstrate a more significant content of aromatic structures (benzene rings).

The formation of DBPs has been reported to take place *via* two routes; oxidation by the cleaving of carbon-carbon double bonds and substitution where a functional group is replaced by a halogen (Westerhoff *et al.* 2004; Ates *et al.* 2007). The reaction most quoted in the literature is the 'haloform reaction' (Blatchley *et al.* 2003). The usual reaction scheme is demonstrated using methyl ketone as a precursor. The initial step is the formation of an enol. Although the keto-enol equilibrium lies to the left, pH (acidic or basic) changes promote enolization (Fig 1.5) which is considered to be the rate limiting step. Rapid halogenation of the enol takes place with the methyl group being halogenated. The trihaloketone is then hydrolysed to yield the THM (CHCl₃).



A reaction of the haloform type is described by Samios *et al.* (2007) who reported rapid a reaction with HOCl at neutral pH of 3-ketoglutaric acid [COOHCH₂CH₂(CO)CH₂COOH] which has a molecular structure with two carboxylic groups next to a carbonyl and methyl group to yield trichloromethane. Chlorination of polyhydroxyphenol and 4',5,7-trihydroxyflavanone and ellagic acid compounds found free and/or combined in plants (Fig 1.6) was reported by Joll *et al.* (2010). The differences in reactivity were again highlighted and confirmed the influence the hydroxyl position (as described in 1.3.3) on the aromatic ring had on THM formation (Rook, 1977).

Another example of the hydroxyl groups *meta* (Fig 1.6) to each other was demonstrated by Joll *et al.* (2010) who reported a concentration of 2170 nM of CHCl₃ forming after 7 days incubation at pH 9 for (4',5,7-trihydroxyflavanone), but only 25 nM of CHCl₃ for the ellagic acid (Fig 1.6) under the same conditions. When the chlorination was repeated at pH 7, the formation of CHCl₃ was reduced to 1640 nM for the (4',5,7-trihydroxyflavanone) but remained virtually unchanged for the ellagic acid (26 nM). This experiment suggested the importance of both pH and *meta*-dioxygenated sites as the principal influences in THM formation.



Joll *et al.* (2010) also suggested possible THM formation reaction pathways of the terpenoids, retinol and β -carotene model compounds (Fig 1.7 shaded). These demonstrated an initial electrophilic addition of the HOCl to the double bond of the β -carotene of retinol. This was followed by a nucleophilic attack of water on the intermediate carbo-cation, and the internal attack by the hydroxide forming an epoxide intermediate. Water then reacts with the least hindered carbon of the epoxide and produces a vicinal diol which is cleaved in the reaction conditions to form β -ionone. The reaction could also form β -cyclocitral and trans- β -ionone, but β ionone and trans- β -ionone intermediates contain a methyl ketone structure. Hence Joll *et al.* argued that these are the most likely molecules to participate in an haloform reaction and produce THMs and a carboxylic acid, rather than the β -cyclocitral.

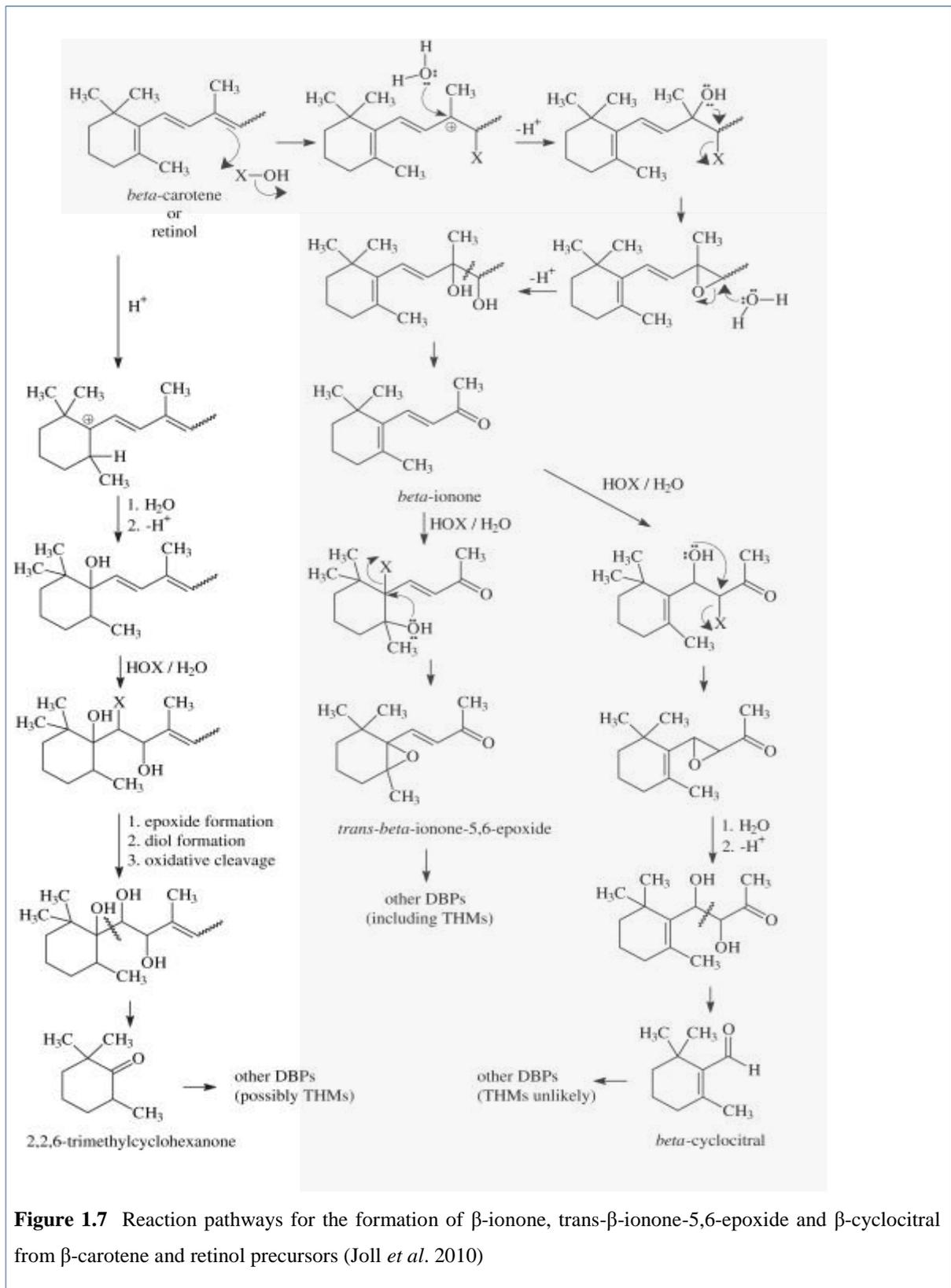


Figure 1.7 Reaction pathways for the formation of β -ionone, *trans*- β -ionone-5,6-epoxide and β -cyclocitral from β -carotene and retinol precursors (Joll *et al.* 2010)

The literature has reported the chlorination of fractioned Raw Surface Water with the majority of results concluding, that the hydrophobic fractions generated the highest concentrations of THMs (Xue *et al.* 2009; Yee *et al.* 2009; Buchanan *et al.* 2006; Chang *et*

al. 2001; Reckow *et al.* 1990) with CHCl_3 as the major species. Ates *et al.* (2007) reported the THM species formed by the fractionated samples of Raw Surface Water occur in the order of CHCl_3 (43.8 %), CHBrCl_2 (26.1 %), CHBr_2Cl (20.7 %) and CHBr_3 (9.4%).

The effect of pH and molecular mass (MM) has been documented. Adin *et al.* (1991) monitored the formation of THMs and demonstrated that a lower pH reduced THM formation and *vice versa*. This was as a result of the HOCl less dissociated at low pH (pKa: HOCl 7.5, K_a : 2.9×10^{-8}) and it was also suggested that the charge density on the surface of the DOC molecules was reduced. Hence solubility and aggregation of the DOC in the suspension was decreased. This may propagate aggregation and folding of the molecular structures resulting in the reduction of available chlorination sites. Conversely too high a pH will adversely affect THM formation as the HOCl becomes more dissociated and forms higher concentrations of OCl^- (Reckow *et al.* 1990). Solution pH effects on THM formation have also been reported to influence compound formation when a mixture of HOCl and KBr was used as the halogenating solution of the DOC. Initially between pH 4 – 6, CHCl_3 predominated, but as the pH increased the bromination products increased and chlorination products decreased (Italia *et al.* 1992). In other studies where HOCl was the only oxidant present, chlorinated products increased with increasing pH. The THM formation in this case was subject to the HOCl/HOBr ratio (1:1) where the HOBr (pKa : 8.7, K_a : 5×10^{-9}) dominated at the higher pH due to the HOCl oxidizing the Br^- to HOBr (Wong and Davidson, 1977).

Ishihashi *et al.* (1999) also demonstrated an increase in the formation of brominated products by varying the ratio of $[\text{NaOCl}]/[\text{NaOBr}]$, i.e., when the ratio was 0.04, CHCl_3 formation dominated, but when the ratio increased to 0.1, CHBr_3 predominated. The other THMs formed were in the order $\text{CHCl}_2\text{Br} > \text{CHClBr}_2 > \text{CHBr}_3$ and $\text{CHClBr}_2 > \text{CHCl}_2\text{Br} > \text{CHCl}_3$, respectively. These results suggest that the ratio $[\text{HOCl}/\text{HOBr}]$ may regulate the product distributions during the chlorination of Raw Surface Water if it has a natural high bromide concentration. The pH study relative to brominated and chlorinated THMs carried out by Ishihashi *et al.* (1999) reflected the results of Italia *et al.* (1992), with a relatively linear increase in CHCl_3 over a pH range of 4.9 – 10.7, but CHBr_3 increased between pH 6.8 – 9.9, after which it fell by 9 %.

A comparison of the THMFP of samples of Raw Surface Water and Treated Water MM ranges was reported by Wong *et al.* (2007). The highest reactivity was recorded for both waters by the MM range $< 1,000$ Da. Total THM formation was seen to decrease sharply from approximately $125 \mu\text{g}/\text{mg}$ DOC for MM < 1000 Da to $80 \mu\text{g}/\text{mg}$ DOC for MM $<$

3,000 Da. Between 3,000 – 100,000 Da the total THM formed over the range demonstrated less of a difference, with the < 10,000 Da forming $\approx 80 \mu\text{g}/\text{mg}$ DOC and the < 100,000 Da $\approx 75 \mu\text{g}/\text{mg}$ DOC. Over all the ranges the Raw Surface Water's THMFP was approximately $10 \mu\text{g}/\text{mg}$ DOC greater than the treated water.

A comparison of THM formation potentials demonstrated by both fulvic and humic acid MM distributions for both a stream and a lake was investigated by Oliver and Visser (1980). The results for the fulvic acid's potential to form CHCl_3 demonstrated the highest within the 5,000 – 30,000 Da range for both the stream and lake samples. The highest potential ($140 \mu\text{g}/\text{mg}$ DOC) in the stream samples was in the MM range (20,000 – 30,000 Da) but in the lake fulvic the highest formation ($> 140 \mu\text{g}/\text{mg}$ DOC) of CHCl_3 was within the 1,000 – 10,000 Da range. The highest CHCl_3 concentrations ($> 160 \mu\text{g}/\text{mg}$ DOC) formed by the humic acid were within the range from 10,000 – 30,000 Da, for both lake and stream samples, but evidence of formation was reported, although at a lower concentration ($< 100 \mu\text{g}/\text{mg}$ DOC) for the higher MMs (50,000 – 300,000 Da) with the lake humic demonstrating a greater variation in THMFP, i.e., 20 – $100 \mu\text{g}/\text{mg}$ DOC.

Conversely, Wei *et al.* (2008) demonstrated THMFP to be independent of predominant MM distribution. Fractionated DOC samples with a predominant MM distribution of (1,000 – 30,000 Da) were investigated for a series of monthly samples (April – October). The highest THMFP was recorded for the May samples and the lowest for October with the formation potential falling from the May high, of $130 \mu\text{g}/\text{mg}$ DOC to $70 \mu\text{g}/\text{mg}$ DOC in the October.

An interesting relationship was reported by Buchanan *et al.* (2006). They reported results of an experiment where DOC was photo-oxidized using $\text{UV}/\text{H}_2\text{O}_2$ and initially THMFP increased. This was presumed to be due to the breakdown of large conjugated compounds to molecules of a lower MM which were halogenated. However, over time THMFP dropped as the DOC was increasingly mineralised and CO_2 was released to the atmosphere by the photo oxidation process and the abundance of lower MMs reduced. This drop in THMFP may have been associated to the formation of carbonic acid and a decrease in the optimum pH for THM formation, but further irradiation led to an increase in the pH to 8 which did not demonstrate the subsequent expected increase in THMs. Although this method employing photo oxidation could be considered as a technique for removing THMFP precursors, Buchanan *et al.* (2006) reported an increase in nitrite formation from

the natural nitrate concentrations, hence introducing a mutagenic health risk (ADWG, 2004).

1.5 Guidelines and Regulation Limits of THMs in Drinking Water.

Concerns regarding the potential health effects of DBPs have prompted regulatory bodies to specify limits of exposure for a number of them (WHO, European Union Water Framework Directive, US Environmental Protection Agency and United Kingdom Drinking Water Inspectorate).

Although many benefits result from chlorination, the formation of halogenated organic compounds that include the trihalomethanes, haloaceticacids and haloacetonitriles are considered to pose a number of potential hazards to health. The majority are classified as carcinogenic/mutagenic and strict exposure limits are being established (Moseto *et al.* 2009).

Recent studies of birth defects attributed some obstructive urinary tract abnormalities and cardiac and ventricular septal defects to chlorination by-products (Tardiff *et al.* 2006). Daniel *et al.* (1986) reported laboratory-based experiments that concluded that the haloacetonitriles which include dibromoacetonitrile bromochloroacetonitrile and dichloroacetonitrile, are direct-acting genotoxins and are potent DNA strand breakers in human cells, mutagens in bacterial cells and as previously reported, by Bull *et al.* (1986) tumour initiators in mouse skin.

The UK water industry has established a threshold limit of the sum concentration of 100µg/L, for the four trihalomethanes; trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane (DWI 1998). Whilst the United States Environmental Protection Agency regulates the sum of the four THMs to a limit of 80µg/L, but also includes separately the sum of five haloacetic acids which include the monochloro-, monobromo-, dichloro-, dibromo- and trichloroacetic acid to a maximum limit of 60 µg/L (USEPA 1998). The current proposal by the European Union is that nine species of haloacetic acids should be considered and regulated to a maximum concentration of 80 µg/L (Cortvriend, 2008). A report by DEFRA (2008) to the Drinking Water Inspectorate stated that if England and Wales were subjected to the US disinfection by-product parameters, a high number of exceedances would be recorded using the current methods of disinfection and raw water sources. This thesis has shown that Raw Surface

Waters THMFP in the four lake/reservoirs in north Wales would not satisfy the set regulatory levels on a number of occasions.

1.6 Scope of the Project.

The initial objective of this study was to establish for four natural water sources the characteristics of their dissolved organic carbon (DOC) and the seasonal and other external influences that may promote DOC changes that might influence the potential for the formation of disinfectant by products (DBPs) from DOC during chlorination. The catchment areas of the sample sources were predominantly wetlands (lowland minerotrophic fen, ombrotrophic blanket bog and steep organic peaty rankers) with small areas of adjoining agricultural land. These represented a cross section of the majority of areas in north Wales where public drinking water reservoirs are situated.

The initial aims of the project were to monitor and characterise the DOC of four lake/reservoirs with the following catchment profiles over a twenty four month period;

- lowland minerotrophic fen/agricultural.
- upland oligotrophic blanket bog.
- steep upland thin peaty rankers/agricultural.
- upland thin peat soil/peat rankers with adjacent peat bog.

This has been carried out, utilising a series of analytical methods, that include the;

- determination of the DOC concentration in both Raw Surface Water and Pre-Chlorination Water (water having passed through the treatment processes but had not been chlorinated),
- UV spectroscopy, Fluorescence spectroscopy, Infrared spectroscopy, Size Exclusion Chromatography and Ion Chromatography.

Further characterisation has been carried out by fractioning the Raw Surface Waters using DAX 8 and XAD 4 macroporous resins. This was to determine any seasonal changes to the type and concentration (i.e., hydrophilic, hydrophobic, neutral) of molecular moieties being formed in the catchments. Each fraction was subjected to the same characterisation, chlorination and analysis methods as the Raw Surface Water and Pre-Chlorination water.

The study has also established the trihalomethane formation potential (THMFP) of both the Raw Surface Water, Pre-Chlorination and Fractioned Raw Surface Water DOC. This has

been carried out with the aim of identifying changes in reactivity relative to DOC characteristics influenced by seasonal changes, environmental events (e.g., dry/wet/cold periods) and catchment types.

1.7 Project objectives.

The objectives of the project were to identify and monitor the DOC concentration variations in upland and lowland environments. Noting any influences that contributed to a change in the characteristics and tri-halomethane formation potential (THMFP). This included characterization of the Raw Surface Waters, treatment works Pre-Chlorination waters and Finished Product, together with analysis to compare their THMFP when chlorinated by laboratory and treatment works methods. It was also proposed to examine SUVA, Aromaticity and the A_{253}/A_{203} ratio for correlations with the DOCs THMFP. With the advent of changes in weather patterns, it was intended to monitor and assess the impact of intense rain after a dry period on the DOC concentrations and THMFP of upland and lowland streams from peat and mineral sources.

1.8 Hypothesis

The DOC characteristics and THMFP are unique to individual catchments/lakes.

Chapter 2

Experimental Methods

Chapter 2 Experimental Methods

2.1 Method Overview

The experimental methods (2.1.4) within this chapter were used to characterize water samples collected from north Wales lakes and in water treatment works during the period of the project; October 2009 – October 2010. The methods and techniques were selected based upon in-house continual review and literature papers. For example, Leenheer and Croue (2003) and McDonald *et al.*, (2004) discussed methods for isolating and characterising dissolved organic matter (DOM) using a series of techniques that included size exclusion chromatography, followed by infrared, fluorescence and UV-visible spectroscopy along with an evaluation of XAD-type resins. The use of the DAX 8 and XAD 4 resins in this thesis for the fractionation of the samples was influenced by Aiken's (1979) paper on the comparison of XAD macroporous resins and Malcolm's (1992) paper on the quantitative evaluation of the DAX 8 and XAD 4 resins for DOC characterisation. Leenheer's (1981) calculation to determine the quantity of each resin required to fractionate 1L of water was used to establish the column parameters for of the fractionation system, with the technique for preparing the resins and carrying out the experiment developed from the paper by Aiken *et al.* (1992). The isolated fractions were identified using the same classification of Leenheer & Croue (2003 b) i.e., Hydrophobic acids/bases, Hydrophilic acids/bases and Neutrals/Transphilic Acids.

Poly(styrenesulphonic) acid sodium salt standards were selected as calibration standards for Size Exclusion Chromatography (SEC) as they have been reported to have hydrodynamic and chemical properties that reflect natural sample solutes (Peuravuori and Pihlaja, 1996). The Bio-Sep column used for SEC analysis had a size exclusion range of 1,000 to 300,000 Daltons with a quoted efficiency of 30,000 plates (Zhou *et al.*, 2000). The 'Cirrus' software of the Polymer Laboratories GPC-50 calculated the M_n which is the statistical average molecular weight of the polymer chains in the sample and M_w which is the molecular weight of the polymer chain contributing to the molecular weight average (Zhou *et al.* 2000). This chromatographic form of separation in SEC is based on the physical dimensions of the molecules, and the size separation takes place by repeated exchange of the solute molecules between the mobile phase (solvent) and the pores of the stationary phase containing stationary mobile phase. Molecules small enough enter the pores are repeatedly exchanged as they pass along the column whilst the larger molecules that are greater than the stationary phase pore size migrate along through the particles of

the stationary phase and are eluted first (USP 31. NF, 2008). Detection of the eluted molecular components of the DOM was by UV-vis detection at 254 nm, thus limiting the detection to chromophores absorbing at this wavelength. This was expected to be predominantly unsaturated [C=C] containing moieties with limited intensities and relatively lower responses at this wavelength for proteins, sugars and aliphatic acids (Leenheer & Croue, 2003).

The ultra-violet/visible (UV-vis) absorbance spectrum of each sample was collected over the range 700 to 200nm at 1nm/second increments. Although the collected spectra did not exhibit strong features, the absorbance at the 254 nm (used for the detection of unsaturated [C=C] molecular structures for the SEC method) has been reported to give an indication of the concentration of DOC in the sample (Weishaar *et al.*, 2003). The data generated was also used for the determination of Specific UV Absorbance (SUVA) and aromaticity. From the data recovered the wavelength ratio (253/203 nm) was calculated to investigate the suggestion by Korshin *et al.*, (1996), that a relationship between it and THM formation potential (see Chapter 7, 7.4)

Fourier transform infrared (FTIR) spectra were collected for a series of samples that had been freeze dried. They were prepared for analysis as KBr discs and interpretation of the spectra provided information on the vibrationally active functional groups present in the molecules. However, care had to be exercised during data analysis because of reported possible effects induced by the hygroscopic properties of KBr making it difficult to eliminate the interference of water bands in the spectra. (Macdonald *et al.* 2004).

Another technique used for sample characterisation was Fluorescence – related Excitation and Emission Spectroscopy (EEMS). The fluorescence spectra were obtained by scanning both the excitation (250 to 400nm) and emission wavelengths (280 to 500 nm) simultaneously with stepwise excitation increments of 5 nm. A 30 nm difference between the excitation and emission wavelength scans was maintained during data collection and the resulting spectra are hence described as synchronous spectra according to the literature (Goslan *et al.* 2004, Stedmon *et al.*, 2003). The major intensities and peak locations were identified and characterized using parallel factor analysis (PARAFAC) using MATLAB (Stedmon and Bro, 2008).

It is known that phenolic hydroxyl constituents are widely distributed in plants (Ribereau-Gayon, 1972). With the degradation of the plant material in soils and peat, it is reported that the mono and poly-phenolic molecules can undergo transformations to higher

molecular weight humic substances (Thoss *et.al.*, 2002) and can then be quantified in the laboratory using a colourimetric method. The method selected for this project was established by Box (1981) and the colour change was activated by the reduction of Folin-Ciocalteu reagent (acidic Mo, W) by the DOCs' phenolic components.

Quantitative analysis for the tri-halomethanes (THMs), CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3 was by GC using the technique of solid phase micro extraction (SPME) and detection by a ^{63}Ni Electron Capture Detector (ECD). The SPME fibre selection of carboxen-poly (dimethylsiloxane) CAR-PDMS, (75 μm film thickness) and choice of GC parameters was developed from investigations carried out by Sarrion *et al.*, (2000) and Pawliszyn (1997). Laboratory chlorination of the samples and their subsequent analysis at +7 days was adapted from the literature (Goslan, 2003), (EPA Method 501.2, 1979).

Cations and anion concentrations in the samples were analysed, using a dedicated Metrohm 850 ion chromatography instrument with dual columns capable of sequential analysis of the cations (0 – 50 mg/L) and anions (0 – 200 mg/L). Sample pH was determined with a calibrated double junction combination glass electrode and conductivity measurements were carried out using a pre - calibrated Primo 5 conductivity meter.

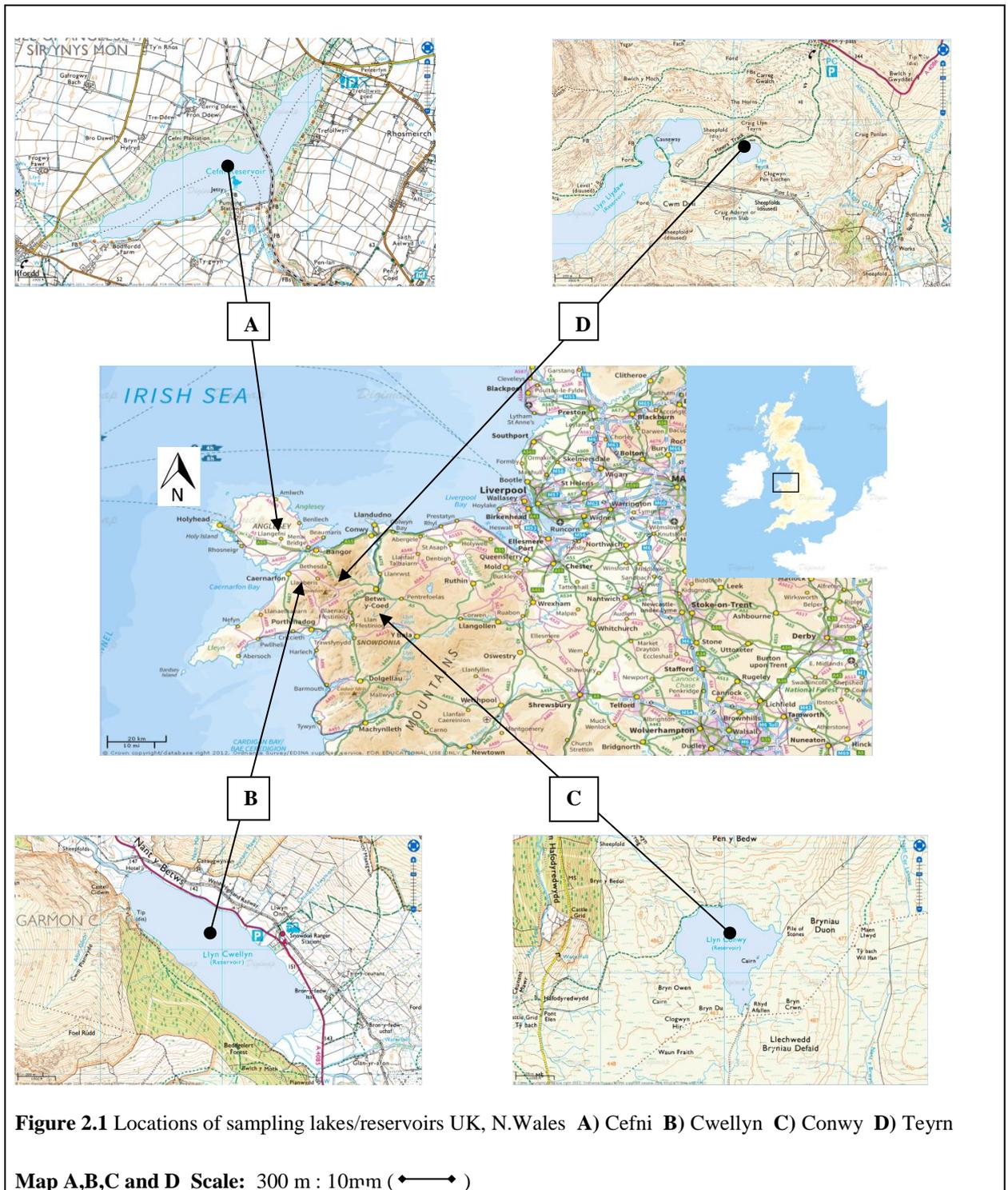
2.1.1 Sampling Sites

Four lakes/reservoirs in the north west of Wales were selected as water sampling sites for the project; Llyn Cefni, Cwellyn, Conwy and Teyrn. These were chosen because between them the four exhibit a broad spectrum of both topographical and geochemical characteristics that represent typical water resources available to the local water industry for production of potable water.

The Cefni reservoir (Figure 2.01 A) is located on the Isle of Anglesey, Wales at 53°16'16" N , 4°20'08"W. It has a maximum length of 2.3km, a surface area 0.86 km² and is 29 m above mean sea level. The reservoir, which has a capacity of 1.82 G L, was created in 1952 by damming the Erddreniog and Frogwy rivers (National Archives, 2011). The River Erddreiniog transports water approximately 5km from the Erddreiniog fen (Figure 2.03) and enters the reservoir to its eastern end. Erddreiniog fen is described as a mesotrophic fen overlying 4.5 metres of peat (Bragg, 1998). The fen covers an area of 467.19 hectares and occupies a shallow watershed valley which is fed by base rich (pH \geq 8) waters that emerge from an escarpment of limestone bedrock. The second source, the Frogwy river, enters the reservoir at its western end. Its catchment is a 6 km mixture of rich agricultural

land interspersed with small areas of wetland with a geology consisting of mudstone, sandstone and Coedana granite (British Geological Survey, 2011).

Llyn Cwellyn (Figure 2.01 B) is also used as reservoir and is located at the base of Snowdon within the Snowdonia National Park at coordinates 53°04'59''N 04°10'15''W. It has a surface area of 0.87 km², an average depth of 23m and is about 142 m above mean sea level (Hyder Consulting, 2007).



Llyn Cwellyn is considered to be an excellent example of a deep oligotrophic and slightly acidic lake formed during the ice age (Roberts, 1995). The lake's main watercourses are the Afon Gwyrfai with its source, Llyn y Gader situated at 191m above mean sea level and its tributary Afon Treweunydd which originates from a number of streams on the slopes of mount Snowdon. The catchments for the rivers are mainly upland, nutrient-poor thin peat soils and steep sided rocky slopes consisting mainly of the Nant Ffrancon sub-group; Devonian till and alluvium (Geological Map Data© NERC 2010).

The Conwy reservoir (Figure 2.01 C) lies at 452 m above mean sea level in a small basin at 52.99°N 3.80°W surrounded by a blanket bog with an area of up to 175 hectares referred to as the Migneint (Ellis and Tallis, 2001). The bog's average depth of peat is 1.2m with an underlying geology comprising of Ordovician mudstones with areas of basic and acidic volcanic tuffs (Billett *et al.*, 2010). The mean lake depth is 4 m with a water pH of 5.9.

The fourth lake, Teyrn (Figure 2.01 D) lies at about 384 m above sea level at 53° 4' 25''N 4° 1' 30'' W to the east of Snowdon. The catchment consists of steep slopes with a mixture of Ordovician acidic and basic volcanic rocks with localised igneous intrusions (British Geological Survey, 2011). Adjoining the lake to the south west, lies a small bog of equivalent area to the open water; average peat depth 1.2 m. The surrounding soils include organic peat soils, brown podzolic soil gleys and humic rankers. The lake water's mean annual pH is 6.1. To the south of the lake a small stream flows out to an artificial weir. This has been monitored as one of Environmental Change Network sites of fresh water lakes since 2000 (UK Environmental Change Network 2012).

2.1.2 Sampling Plan

The three reservoirs, Cefni, Cwellyn and Conwy supply their immediate surrounding areas with potable water. Each reservoir has a self-contained treatment works where the abstracted raw water is subjected to filtration, coagulation and chlorination before release (Dwr Cymru, United Utilities 2012). Cwellyn treatment works was fitted with a new dissolved air floatation (DAF) plant and new UV reactors during the winter of 2010/11 which were commissioned during the spring of 2011.

The raw water samples (1L) were recovered at monthly intervals from November 2009 to October 2011 at each treatment works *via* the fixed draw off taps on the raw water feed at each water treatment plant. Lake Teyrn samples were taken at the same reference point

(53°- 07'N, 4°- 03'W) one metre away from the water's edge by direct filling of the sample bottle at 10 cm below the water surface. A series of "finished" water samples (250 ml); i.e., water having been fully processed through the Cefni, Cwellyn and Conwy treatment works, were also collected from established draw off points at each water treatment works during the period December 2010 to October 2011. Additional samples (250ml) at the stage prior to chlorination were recovered from Cefni and Cwellyn treatment works from October 2010 to October 2011. Each sample taken were labelled accordingly with the reservoir / lake name and water sample classification; i.e., Raw Surface Water (water directly sampled from the lake or water treatment works raw water inlet), Pre-chlorination Water (water having been processed through the water treatment works but at a point before entering the chlorination contact tank) and Finished Water (water that was chlorinated and ready for distribution to the public water supply).

All water treatment works samples were collected after a 5 minute period of flushing to ensure a fresh sample of water was collected. Prior to sampling, all sample bottles were prepared and sterilised in the laboratory by washing with Decon 90 and Milli-Q water and finally de-ashing for 12 hours at 550°C in a muffle furnace. Transportation of all the samples to the laboratory was carried out under controlled conditions of light and temperature (sealed coolbox at 4° C).

2.2 Pre-Analysis Sample Treatment

2.2.1 Raw Water Samples

Within 4 hours of sampling, the Raw Surface Water samples (1L) were vacuum filtered *via* a 0.45 µm pre-washed (Milli Q water) Nylon membrane (Whatman) to remove particulate matter. The filtrate was then passed a second time through a 0.2 µm Nylon 66 membrane (Whatman) filter to minimize bacterial contamination. Conductivity and pH measurements (Methods 2.3.12, 2.3.13) of the raw waters were recorded prior to adjusting the pH to 2.8±0.2 with a few drops of HCl (4.0M). The acidified samples were sealed and stored under controlled conditions of darkness and temperature at 4°C.

2.2.2 Pre-Chlorination Water Samples.

The pH and conductivity of both Cefni and Cwellyn water treatment works Pre-chlorination water samples were measured prior to acidification to pH 2.8±0.2 with HCl

(4.0M). The samples were subjected to the same storage conditions as the Raw Surface Water samples.

2.2.3 Finished Water Samples.

The samples were not subjected to any further treatment before analysis. Conductivity and pH measurements (methods 2.1.4.11, 2.1.4.10) and analysis for tri-halomethanes by GC were carried out within 5 hours of sampling.

2.3 Analytical Methods.

2.3.1 Dissolved Organic Carbon (DOC).

An automated Thermalox 2000 (Analytical Sciences Ltd, Cambridge) was used to determine the concentration of dissolved carbon in the raw water and fractioned water samples. A 25 μL sample was introduced *via* a sample port into the reactor tube held at a temperature of $685 \pm 5^\circ\text{C}$ and containing a catalyst (5% platinum on a ceramic support). The reactor tube and catalyst were subjected to a continuous flow of oxygen (220 ml/minute) which promoted the thermal/catalytic oxidation of DOC to CO_2 (g). This was then passed into a cooling system held at a temperature of $2 \pm 1^\circ\text{C}$ and was identified and quantified by an infrared detector at $2640 \pm 20 \text{ cm}^{-1}$ (Analytical Sciences Ltd, 2009).

A series of DOC standards ranging from 0.1 to 30 mg/L were prepared with potassium hydrogen phthalate (Philip Harris) and the instrument was considered suitable for analysis when the calibration curve R^2 value was > 0.99 . System suitability was established by analysing three simultaneous sucrose samples (10 mg/L). The system was deemed suitable for analysis when the standard deviation of three consecutive results was $10.0 \leq 0.5$ mg/L.

Samples and check standards (every 5 samples) for analysis were prepared as per Section 2.2 and were transferred to 10 mL clean glass vials in the sampling tray of the instrument. The instrument was programmed to analyse each sample and check standard five times and calculate the final results were calculated from the mean of the five values subject to the collective suite of all the data having a co-variance of $< 3.0\%$.

2.3.2 Estimation of Total Phenolic Compounds

The analysis for phenolic compounds in the water samples was carried out using a modified version of the Folin-Ciocalteu colorimetric method described by Box (1983). In this assay, phosphomolybdic and phosphotungstic acids were reduced by any hydroxyl functional groups present within the DOC to form a molybdenum-tungsten blue complex. Absorbance at a fixed wavelength of 750nm and temperature of 22°C was measured using a Molecular Devices Spectramax M2 UV-VIS spectrometer. Samples were analysed *versus* a calibration curve ($R^2 > 0.99$) of phenol standards ranging from 0.0 to 3.0 mg/L. These were prepared making up a 1 L stock solution by dissolving 1.0 ± 0.2 g of phenol crystals (99% Analar: Lot121K1646 Sigma) and making up to 1L with Milli Q water (18 Ω). 5mL of the stock solution was transferred to a 1L volumetric flask and made up to the mark with Milli Q water (18 Ω) and identified as the working standard. From this solution, a series of calibration standards were prepared with a concentration range from 3.0, 2.0, 1.5, 1.0, 0.75, 0.5, 0.2, 0.0. mg/L. Both samples and standards were analysed using a clean 96-well Greiner micro-plate which was prepared for the analytical procedure by transferring 250 μ L of each phenol standard concentration (3.0, 2.0, 1.5, 1.0, 0.75, 0.5, 0.2, 0.0. mg/L) into separate, identified wells. 250 μ L of each sample was then transferred to separate, identified wells on the same micro-plate. Using a pipette, 12.5 μ L of Folin-Ciocalteu reagent (Lot 52K3671 Sigma) was added to all the standard and sample containing wells, followed by 37.5 μ L of 20% w/v sodium carbonate solution ($\geq 99.5\%$: Lot 058K0689 Sigma). The plate was then stored in the dark for 1.5 hours at a temperature of 22° C before transferring to the Spectramax M2 for analysis.

2.3.3 High Pressure Size Exclusion Chromatography (HPSEC)

Determination of the molecular weight distribution of DOC was carried out by High Pressure Size Exclusion Chromatography using a method developed and adapted from Zhou (2000). A Polymer Laboratories (Cirrus software, Varian, 2009) PL-GPC 50 instrument was used, employing a BioSep-SEC-s2000 (Phenomenex S/N 597004-6) column held at a temperature of 35°C for molecular weight separation. A 100 μ L sample loop delivered the sample to the column and the UV detector wavelength was fixed at 254 nm. Calibration standards were prepared using a range of poly(styrenesulfonic) acid sodium salt polymers (Fluka, SigmaAldrich); 4300 Da (Lot 001409884), 13000 Da (Lot 81608), 32000 Da (Lot 0001 409244), 77000 Da (Lot 81612), 155000 Da (Lot

0001391453), along with cyanobalamin (Vitamin B12) for the lowest molecular mass of 1340 Da (Lot LB68564).

A buffered (pH 6.8) mobile phase was prepared by dissolving 0.544 g (2. mmol) potassium dihydrogen phosphate (AnalaR, Lot 040M0126V), 0.913 g (2.0mM) dipotassium hydrogen phosphate (AnalaR, Lot A494776-430) and 11.690 g (0.1M) sodium chloride (SR grade Lot 2081) in 2.0L of Milli Q water ($> 18 \text{ m}\Omega$). The mobile phase was vacuum de-gassed before use.

The column was conditioned with mobile phase at a flow rate of 0.4 mL/minute for a period of 12 hours before increasing to the analysis flow rate of 1.3 mL/minute. A calibration curve (Appendix 2) was generated for determination of molecular mass (MM) distributions, using standards prepared as two separate solutions, with 3 molecular mass components as specified below dissolved in a 25ml volume of mobile phase :- Solution 1 (4,300, 32,000, 155,000 Da) Solution 2 (1,340, 13,000, 77,000 Da). Samples were injected *via* the 100 μ L sample loop onto the column and the resulting molecular mass distribution of each peak was determined using the 'Cirrus' program and the archived column calibration curve.

2.3.4 Ultraviolet Spectroscopy

Molecular absorption in the ultraviolet and visible region of the spectrum is dependent on the electronic structure of the molecule (Hobart *et al.* 1988). The energy absorbed by a molecule results in a transition of the electron generally from the highest occupied molecular orbital (HOMO) to a higher energy orbital referred to as an anti-bonding orbital usually designated with an asterisk (π^* or σ^*). The absorption data generated over the range of wavelengths provides information that can be assigned to known chromophores and the characteristics of the molecule can be determined (Silverstein *et al.* 1991).

A Hewlett Packard Lambda 35 was used to collect UV visible absorbance scan data. Raw Surface Water and fractioned Raw Surface Water samples were all analysed at pH 2.8 ± 0.2 *versus* Milli-Q water at pH 2.8 ± 0.2 and $> 18 \text{ m}\Omega$ conductance as a reference. The scan range was from 198 to 700 nm with a scan rate of 60 nm/minute and a slit width of 1 nm. The instrument was prepared for analysis by carrying out the auto-zero procedure using Milli-Q water in a 10mm quartz cuvette in the designated blanking sample holder. Each sample was analysed by transferring approximately 3 mL into a clean 10mm path length quartz cuvette which was placed in the designated sample holder of the instrument.

2.3.5 Fluorescence Spectroscopy

Excitation and Emission spectroscopy (EEMS) was conducted using a Spectramax M2 (Molecular Devices Ltd). The excitation wavelength range was run with stepped increases of 5 nm from 250 to 470 nm. The emission spectra were collected and included the equivalent 5.0 nm step increases over a range of 280 to 500 nm. During the scan a fixed synchronised differential of 30 nm was applied between the excitation and emission wavelengths (adapted the method described by Stedmon *et al.* 2003)

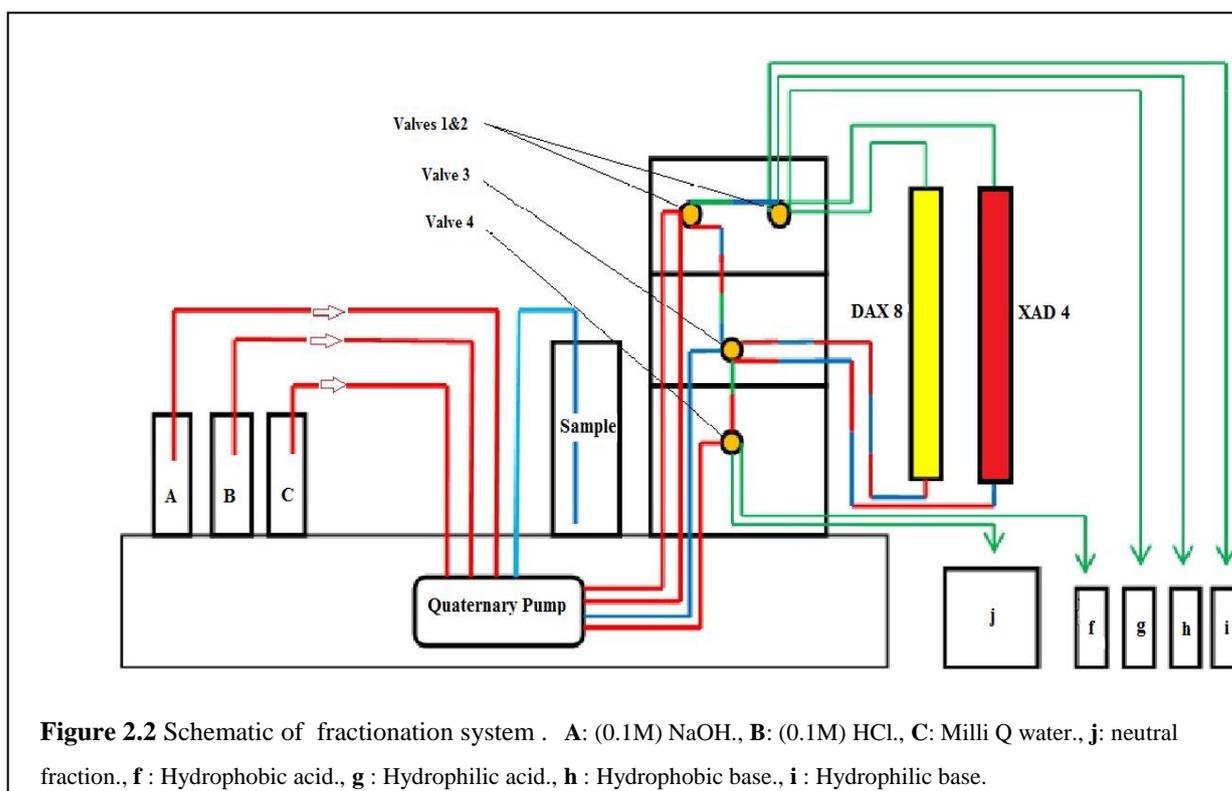
The DOC sample concentration was adjusted to 1 mg/L of carbon based on the sample's DOC concentration (determined using Method 2.1.4.1) and the pH to 2.8 ± 0.2 . To standardise the dimensions of the samples, 250 μ L of each was transferred into individual wells on the 96 well opaque micro-plate (Sterilin). A 250 μ L Milli-Q water (pH 2.8 ± 0.2 , > 18 m Ω conductance) blank was included in one well to correct for Raman effects (Hur *et al.* 2006). The data generated were subjected to Parallel Factor Analysis (Stedmon and Bro, 2008) and the subsequent results were considered relative to the literature (Bagoth *et al.* 2011).

2.3.6 Sample Fractionation

At 3 monthly intervals, all the raw water samples were subjected to fractionation using non-ionic macroporous resins. The method was developed and based on an earlier report by Aiken *et al.* (1992). One litre of filtered raw water (pH 2.8 ± 0.2) was passed at 1.0 mL/minute through two columns connected in series. The first column contained a Supelite DAX 8, acrylic ester resin with a pore size of 225 \AA and the second column, an Amberlite XAD 4 styrene - divinylbenzene resin with a pore size of 40 \AA . The 1L effluent collected after passage through both resins was labelled the 'Neutral fraction.' The columns were then separated and each was desorbed by eluting with 60mL of NaOH (0.1M, Analar, Lot 0031245670) followed by 40mL of Milli Q water. The fraction (100 mL total) collected off the DAX 8 was labelled 'Hydrophobic Acid' and that from the XAD 4 fraction, the 'Hydrophilic Acid.' The columns were further desorbed using 60mL HCl (0.1M, Analar, Lot 5612) followed by 40mL of Milli Q water. The recovered fractions were labelled 'Hydrophobic base' eluted from the DAX 8 and 'Hydrophilic base' eluted from the XAD 4. The Hydrophobic and Hydrophilic acids were acidified to $\text{pH } 2.8 \pm 0.2$ with HCl (4.0 M Analar, Lot 0031245670) and all the fractions were stored in darkness at 4°C.

The two resin columns were then reconnected in series (DAX 8 → XAD 4) and 100mL of Milli Q water was passed through them both at 1mL/minute. Regeneration was carried out by passing 50 mL of HCl (0.1M) at 1mL/minute.

Subsequent to this manually operated configuration, a fully automated system (Fig 2.02) was designed and built in-house (Appendix 1) for the project. This was capable of fractionating a 1L sample into the classified individual components mentioned above and complete a column wash and re-generation within an 8 hour period instead of > 24 hours by manual methods. The apparatus utilised an HPLC quaternary pump capable of being programmed to switch mobile phases and sample solutions automatically. The employment of four Rheodyne® valves to control the flow paths of the solutions and isolate the individual columns during desorption were also controlled by computer software. The operation of the automated system mirrored that of the method above using the same sample volume, desorption solution volumes, wash volumes and regeneration solution volumes. The fractionated samples collected were also treated in exactly the same manner as described above.



2.3.7 Chlorination of Water Samples

Chlorination of the Raw Surface Water and fractioned samples was carried out using a method adapted from Goslan (2003). The DOC sample concentration required for determining the tri-halomethane formation potential (THMFP) was 1 mg/L. This was calculated from the DOC surface water result acquired by Method 2.1.4.1. The volume of the surface water sample required to give a final concentration of DOC of 1mg/L was transferred to a measuring cylinder ($100 \pm 0.2\text{mL}$), then approximately 20mL of Milli Q water was added to the sample volume in the measuring cylinder. Following this, the diluted sample solution was transferred to a volumetric flask ($100 \pm 0.1\text{mL}$) with the measuring cylinder being washed into the volumetric flask with two further 20mL aliquots of Milli Q water. Phosphate buffer $2.0 \pm 0.1\text{mL}$ [range pH 6.8 ± 0.4] (potassium di-hydrogen phosphate, AnalaR Lot040M0126V, sodium hydroxide, AnalaR, Lot 0001427117) was pipetted into the flask and the contents were gently swirled. Using a positive displacement pipette, $\text{NaOCl}_{(\text{aq})}$ 0.5mL ($\equiv 5\text{mg Cl}_2/1\text{mg DOC}$, active Cl_2 : 6-14% , Lot 91820) was added and the solution was then made up to the mark.

The 100mL volume of chlorinated solution was then transferred to an amber glass bottle (100mL) and capped tightly. The bottle was then placed in a dark incubator for 7 days at 25°C. On the 7th day, any remaining unreacted chlorine in the sample was quenched with sodium sulphite (0.8M, AnalaR, Lot S7026 -250G) before analysis (see 2.1.4.8) for the tri-halomethanes, CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3 by GC-ECD as described below.

2.3.8 Analysis for Tri-halomethanes by Gas Chromatography

A Varian GC 450 using solid phase micro-extraction (SPME) and a ^{63}Ni Electron Capture Detector (ECD) was employed to analyse for the tri-halomethanes (THM) using a method developed from Sarrion *et al.* (2000). The following instrument parameters were applied during the analysis for the THMs. The column selected for the analysis was a Restek MX 1, 30m, 0.53mm id, phase: diphenyl/dimethyl polysiloxane with a carrier gas (N_2) flow of 10 mL/minute. The oven temperature gradient was programmed as follows; initial temperature: 35°C held for 9.0 minutes, increasing to 140°C at 10°C/ minute and held for 2 minutes, followed by an increase to 180°C at 10°C/minute, held for 3 minutes. The injection port was fitted with a straight glass liner; the temperature was 290°C with an applied split ratio of 35:1. The detector (ECD) temperature was maintained at 300°C with a make - up flow of 25 mL (N_2) and cell contact potential at -540 ± 100 mV (adjusted for

each analytical run) to establish maximum sensitivity and accommodate for any changes of the detector foil surfaces due to oxidation products over time.

Samples were prepared for analysis by GC-ECD by first transferring 400 μL sodium sulphite (0.8M) (to quench any remaining chlorine) into a clean dry measuring cylinder (25 ± 0.2 mL) using a pipette. A volume of chlorinated sample (14.6mL) was then added to the measuring cylinder to give a total volume of 15mL. This was then immediately transferred into the 20mL amber glass vial (Varian Analytical) and capped. The sample was then placed in the GC sample tray where it was incubated at a temperature of 40°C for a period of 10 minutes. A SPME fibre (CarboxenTM, polydimethylsiloxane, 75 μm , 23 gauge, Sigma-Aldrich) was then immersed to a depth of 5mm into the sample solution for 0.25 minutes before being desorbed within the injection port for 5 minutes at a temperature of 295°C . The desorbed sample was analysed for the selected THMs *versus* the method calibration curve which was generated by preparing a stock solution 20,000 $\mu\text{g/L}$ of chloroform, dichloromethane, dibromochloromethane and bromoform from a certified calibration mixture with a concentration of 2000 $\mu\text{g/mL}$ of each component (Sigma-Aldrich). A series of calibration solutions ranging from 5 to 100 $\mu\text{g/L}$ was prepared from this stock solution by diluting the calculated series of concentrations in Milli Q (18 Ω) water, followed by analysis as per the instruments parameters above. The data collected was used to generate individual calibration curves (Appendix 1) for each component. The resulting curves were attached to the programmed method and used for subsequent analysis. Before the samples were analysed, two blanks, one representing the matrix solution without NaOCl and the second, a matrix solution with NaOCl together with three check standards (5, 50 , 100 $\mu\text{g/L}$) were prepared and analysed to establish system suitability. If the blank chromatograms were clear of spurious peaks and the standard's results were $< 2\%$ CV (co-variance) relative to the theoretical concentration, system suitability was satisfied. The suites of samples were analysed in groups of ten followed by a check standard to establish system suitability. The final sample analysed was another blank to check for any carry-over.

2.3.9 Method Validation

A validation exercise (see Appendix 2) was carried out by determining the following validation requirements; the Linear Responses of a series of calibration standard concentrations for each of the four THMs; CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3 . This was determined by repeat analysis (x5) of a series of concentrations ranging from 0 – 100

$\mu\text{g/L}$. From the data recovered, the Galaxy[®] (Varian) software was used to calculate the R^2 values of the curves generated. Verification of accuracy and precision of the method was established by repeat analysis (x5) of the four THM components at the following concentrations; 2, 40, 60 and $80\mu\text{g/L}$ and the results were statistically analysed for the standard deviation (SD) and percentage residual standard deviation (%RSD). The limits of detection (LOD) and quantification (LOQ) were also established using the signal to noise ratio approach, i.e., LOD signal to noise ratio of 3:1, LOQ, signal to noise ratio of 10:1 (Chan *et al.* 2004).

2.3.10 Infrared Spectroscopy

Infrared spectra were measured using a Perkin Elmer Spectrum 100 FTIR spectrometer. Samples for analysis were initially freeze dried by dividing a litre of sample into four 250 mL aliquots. The samples were then transferred into 500mL round bottom flasks and attached to a dedicated freeze dryer (Modulyo 3) for 48 hours. The recovered DOC solid samples were then re-combined as one sample and stored in a desiccator for 48 hours before being prepared into KBr discs. The KBr (AnalaR) was pre-dried thoroughly before use (120°C). 0.4 ± 0.1 g was weighed onto a clean dry filter paper. With a pestle and mortar, the sample and KBr were ground to a fine powder and then formed into a 1mm transparent disc using a die under pressure (10 tonnes). The absence of a broad absorbance OH stretch at approximately $3000 - 3400\text{ cm}^{-1}$ for a blank disc indicated that the KBr was free from adsorbed water and suitable for use in the preparation of sample discs.

Sample spectra were collected from 450 to 4000 cm^{-1} with the band intensities reported as % transmittance.

2.3.11 pH Measurement

The pH of the samples was measured using a Metler Toledo, Expert Pro instrument and a Ag/AgCl double junction pH combination electrode. Daily calibration of the electrode was carried out using three pH calibration buffers of, pH 4.0 (Lot 039K0180 Sigma), 7.0 (Lot 060M1495 Sigma) and 9.0 (Lot 077J1567 Sigma).

2.3.12 Conductivity Measurements

Sample conductivity was measured using a Primo 5 instrument. The instrument was checked using a certified conductivity calibration solution of 1413 $\mu\text{S}/\text{cm}$ at 25°C (Lot H7031 Hannah). Varying temperatures generated drifts. These were corrected using the tabulated corrections on the standards certificate of analysis.

2.3.13 Ion Chromatography

Quantitative analysis of selected cations and anions was carried out on a Metrohm 850 Professional IC with an attached Metrohm 858 auto-sampler. Samples were prepared as per section 2.1.3.1. The columns employed for the analysis were a Metrosep A (150.0 x 4.0 mm ID) for anions and a Metrosep C4 (150.0 x 4.0 mm ID) column for cations. An isothermal oven temperature of 35.0 ± 2 °C was programmed for both columns together with an isocratic mobile phase flow rate of 0.9 mL/minute. The eluent for anion detection was an aqueous mixture of sodium carbonate (3.2 mM) and sodium bicarbonate (1.0 mM AnaLar) and the cation eluent was an aqueous mixture of nitric acid (1.7 mM) and 2,6-pyridinedicarboxylic acid (0.7 M). The ion suppressor unit was an MSM11 (Metrohm) and the re-generation solution was oxalic acid (0.1M), acetone and sulphuric acid (100mM) mixture in Milli Q water.

A calibration curve for each individual ion was carried out before every analysis, using a series of purchased external standards (Dionex 7 anion standard, product number 56933; (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-}) and Dionex 6 cation standard, product number 040187; (Li^+ , Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}) see 2.1.5 for concentrations. The acceptance criteria for continuing an analysis was a correlation coefficient of $R^2 > 0.99$ for each calibration curve generated. Samples (5mL), prepared as per 2.1.3.1 were transferred into 6ml clean dry headspace vials and placed in the auto-sampler carousel together with system suitability check standards every 10 samples. When the analysis for cations was complete the instrument mode automatically switched to anion analysis and the above procedure was repeated. Results are generated using the relevant calibration curves and instrument software.

2.4. Statistical Analysis

Statistical evaluation of the data generated (R^2 values) for the calibration curves which represented the linear dependence of the measured signal on the analytical value. This was determined using the dedicated instrument and software for the specific analysis. Establishing the precision and repeatability of the methods was determined from the standard deviation, calculated as the square root of the mean error squared and percent relative standard deviations (%RSD) or coefficient of variation which was expressed as percentage relative standard deviation. This was calculated by division of the standard deviation by the mean of the data ($\times 100$). Recoveries were calculated by division of the actual analytical result by the theoretical result and multiplying the product by 100.

2.5 Instrumentation

Instrumentation information, reagents and standards are to be found in Appendix 4

Chapter 3

Characterisation of Lakes/ Reservoir Raw Surface Water samples.

Chapter 3 Characterisation of Lakes/ Reservoir Raw Surface Water samples.

3.1 Introduction.

Dissolved organic carbon (DOC) is present in all natural water sources and its concentration and characteristics are considered to be dependent on number of factors that include catchment topography, microbial degradation, pH, ionic strength, hydraulics and various anthropological and environmental influences such as temperature, rainfall, industrial and agricultural effects. (Toberman *et al.* 2008; Evans *et al.* 2008; Sharp *et al.* 2006; Freeman *et al.* 2001; Wetzel, 1992; Gorham, 1991.) The aim of this chapter is to investigate the characteristics of the DOC in four selected north Wales lake waters (Fig 2.0.1). The recovered monthly lake water samples were analysed using the methods described in Chapter 2 with the intention of identifying and monitoring any relative changes in the Lowland Fen/Agricultural (Cefni), Upland/thin peat/glacial (Cwellyn), Upland/thin peat/shallow (Teyrn) and Upland/blanket bog (Conwy) DOC over a period from October 2009 – October 2011.

Monitoring of the receiving lake DOC concentrations and its varying characteristics identified the different catchments production potential of soluble carbon compounds, both seasonally and during different climatic episodes of the dry, cold and wet. For example, the analysis for DOC phenolic content and its association with inhibiting microbial activity allowed us to determine periods of low and high DOC export out of the catchments (Freeman *et al.* 2001) The UV-vis spectra, collected for each sample, were used to calculate the SUVA values. This value is considered to be a surrogate determinant of the concentration of aromatic molecular structures within the DOC. Further spectroscopic analysis was carried out using fluorescence spectroscopy to identify the presence of common DOC components, e.g., fulvic and humic like structures. A limited number of samples were freeze dried and analysed using infrared (IR) spectroscopy to identify any differences in the functional groups present and size exclusion spectroscopy (SEC) was used to compare, the molecular mass ranges (MM) present in the DOC during the period of the sampling exercise.

3.1.1 DOC Analysis

The samples were treated and prepared for analysis as per section 2.1.3 and DOC analysis was carried out on the prepared samples using Method 2.3.1.

The data show that Llyn Cefni (Fig 3.1) consistently generated a high level of DOC compared to the other sites and ranged from 5.5 ± 0.2 – 10.5 ± 0.3 mg/L. The 24 month study established that the DOC concentrations at the high end of the range occurred during each consecutive autumn (2009 – 2011) with the lower concentrations generally recorded in mid-winter (Table 3.1). Although in March 2010, Cefni's lowest recorded concentration over the 24 months of sampling was recorded (5.5 ± 0.2 mg/L). Following the March 2010 sample being taken and prior to the next sampling (April 14th 2010), a period of rainfall over 20 days occurred (132.6 mm) (Midas 2010) and the DOC concentration increased to 7.8 ± 0.2 mg/L, an increase of 2.3 mg/L.

Limited rainfall (< 10 mm) occurred after the April sample was taken with the total rainfall over the period January – July 2010 recorded at a seasonal low of 264 mm (ECBN 2011). The June and July 2010 DOC concentrations remained relatively similar at an average of 7.38 ± 0.2 mg/L. Preceding the August 2010 sample being taken, a 20 day period (14th July – 2nd August 2010) of rainfall occurred which yielded a 264 mm (ECBN 2011) precipitation total. The DOC level again demonstrated a step-change increase of 3.01 ± 0.1 mg/L up to levels of 7.39 ± 0.2 mg/L (July 2010) and 10.4 mg/L (August 2010). The equivalent period January – July 2011 was less dry (443mm, ECBN) and the averaged DOC (7.47 ± 0.2 mg/L) over the period was higher by 0.6 mg/L relative to the same period in 2010.

Lake /reservoir	Autumn (September – November) mean DOC mg/L	Mid -winter (December – February) averaged DOC mg/L
Cefni	10.0 ± 0.3	7.1 ± 0.2
Cwellyn	3.0 ± 0.1	2.0 ± 0.1
Conwy	5.0 ± 0.2	4.2 ± 0.1
Teyrn	2.2 ± 0.1	$1.4 \pm < 0.1$

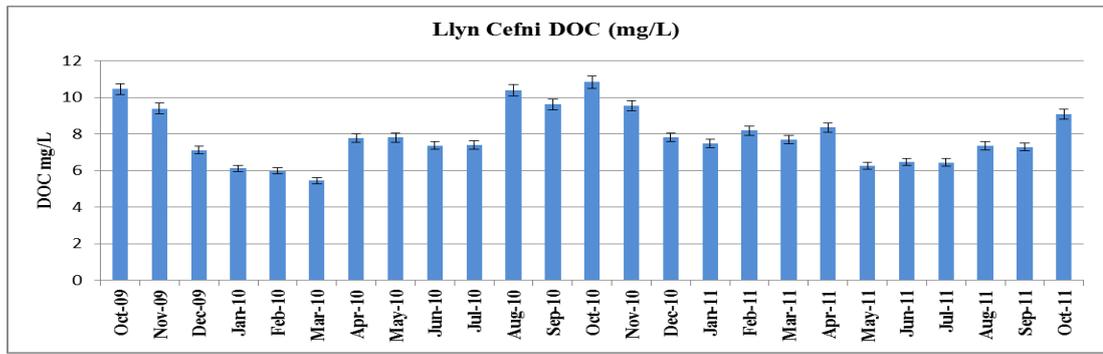


Figure 3.1 Llyn Cefni Raw Surface Water DOC concentrations (mg/L) October 2009 – October 2011.

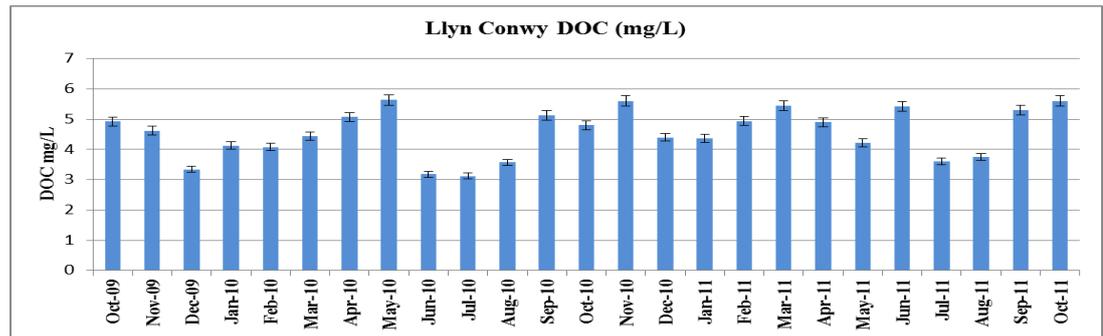


Figure 3.2 Llyn Conwy Raw Surface Water DOC concentrations (mg/L) October 2009 – October 2011.

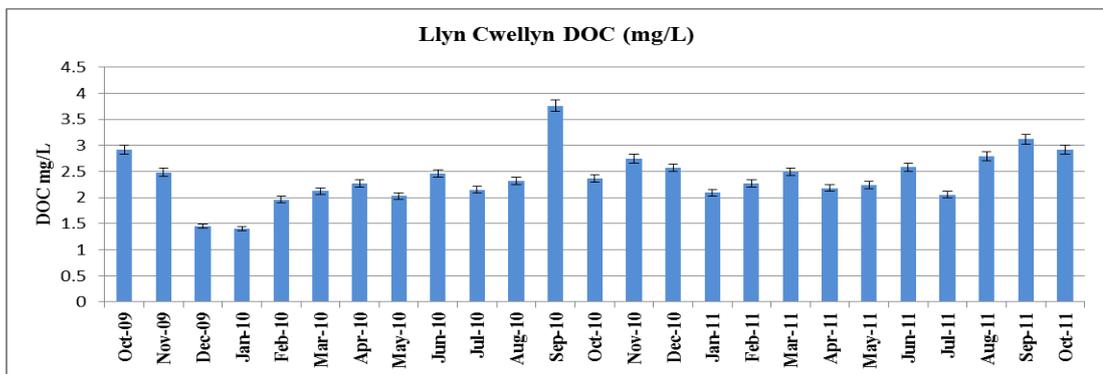


Figure 3.3 Llyn Cwellyn Raw Surface Water DOC concentrations (mg/L) October 2009 – October 2011.

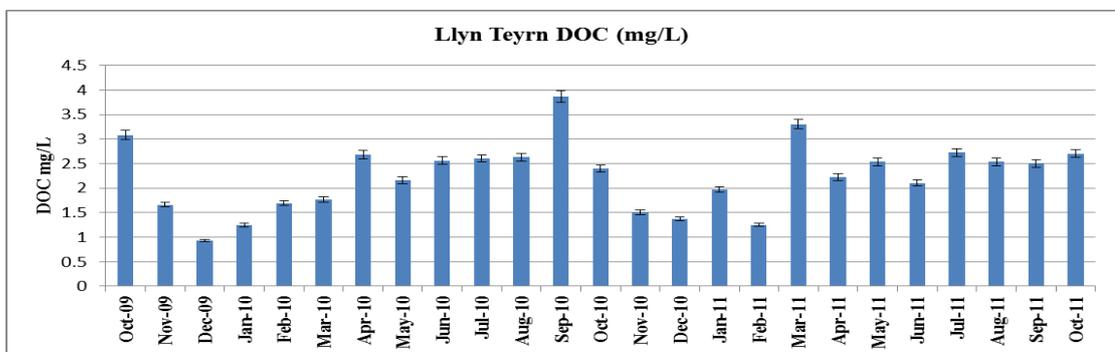


Figure 3.4 Llyn Teyrn Raw Surface Water DOC concentrations (mg/L) October 2009 – October 2011.

Conwy's DOC, mostly originates from its surrounding blanket bog and demonstrated a narrower range of concentrations over the period of sampling (2009 – 2011). The highest concentration recorded was 5.6 ± 0.2 mg/L in November 2010, with the lowest in July 2010 at 3.1 ± 0.1 mg/L. By comparison to the Cefni 24 month DOC concentration profile, the Conwy demonstrated a number of contrasting results. Unlike Cefni's fall in DOC concentration during the late winter months to early spring, Conwy's DOC concentrations for December 2010 and 2011 were 3.3 ± 0.1 and 4.4 ± 0.1 mg/L respectively, but increased and averaged 5.6 ± 0.1 mg/L for both early spring periods. The lowest DOC concentrations were recorded for both July's of 2010/11 and averaged 3.4 ± 0.1 mg/L. The step DOC increase observed for the Cefni in August 2010 after the rainfall (July 2010) was not as obvious in the Conwy, i.e., July (3.1 ± 0.1 mg/L) to August (3.6 ± 0.1 mg/L) which suggested that the blanket bog remained relatively unsaturated and the export water flow remained constant. When the rainfall increased during the autumns of 2010 and 2011 (September – October 2010, 395mm) and (September – October 2011, 486mm), the DOC concentration responded positively and the concentrations were seen to increase relative to the precipitation, 5.0 ± 0.1 mg/L (2010) and 5.4 ± 0.2 mg/L (2011).

The Cwellyn reservoir averaged DOC concentration for the 24 months was 2.5 ± 0.1 mg/L with the lowest recorded level of $1.4 \pm < 0.1$ mg/L noted in January 2010, compared to Cefni (6.1 ± 0.2 mg/L), Conwy (4.1 ± 0.1 mg/L) and Teyrn ($1.2 \pm < 0.1$ mg/L). Two step increases were seen in September 2010 and 2011. These coincided with increased rainfall for those months. In September 2010, the total rainfall was 288mm (ECBN 2011) and the DOC concentration increased from its August level of 2.3 ± 0.1 mg/L to 3.8 ± 0.1 mg/L. During September 2011, the DOC concentration was again above the 24 month average (2.4 ± 0.1 mg/L) at 4.6 ± 0.1 mg/L with a monthly rainfall of 312 mm (ECBN 2011). The changes in DOC concentrations seen in both Conwy and Cefni during the late winter to spring months were not as obvious in Cwellyn, but they did tend to be lower during December/January. This may have been due to the slow diffusion and mixing between the upper and lower water strata of the deep lake dampening subtle changes in the concentrations.

The Teyrn lake, which is 7.5 km from Cwellyn, had DOC concentration levels that ranged from a low of $1.0 \pm < 0.1$ mg/L to a high of 3.9 ± 0.1 mg/L which compared closely to the Cwellyn data ($1.4 \pm < 0.1$ – 3.8 ± 0.1 mg/L). Examination of Figs. 3.3 (Llyn Cwellyn) and 3.4 (Llyn Teyrn) showed the similar trends in DOC changes during the period January 2010 to September 2010, i.e., Teyrn ($1.0 \pm < 0.1$ mg/L) like Cwellyn had a low DOC

concentration followed by an increases over spring to late summer. In September 2010, Teyrn, like Cwellyn, demonstrated a step increase in DOC to 3.9 ± 0.1 mg/L which was the highest reading recorded for the 24 month sampling for this site. From October 2010 to February 2011 the DOC, unlike Cwellyn, reduced relatively steeply. Between February and March 2011, again a relatively sharp increase in DOC was recorded for Teyrn, from 1.3 mg/l in the February to 3.3 mg/L in March after which the DOC remained at an average of 2.5 mg/L with an SD of 0.23.

Fig 3.5 shows the DOC concentrations of the four sample sources, which demonstrated the different concentration levels of DOC observed over the 24 month sampling exercise.

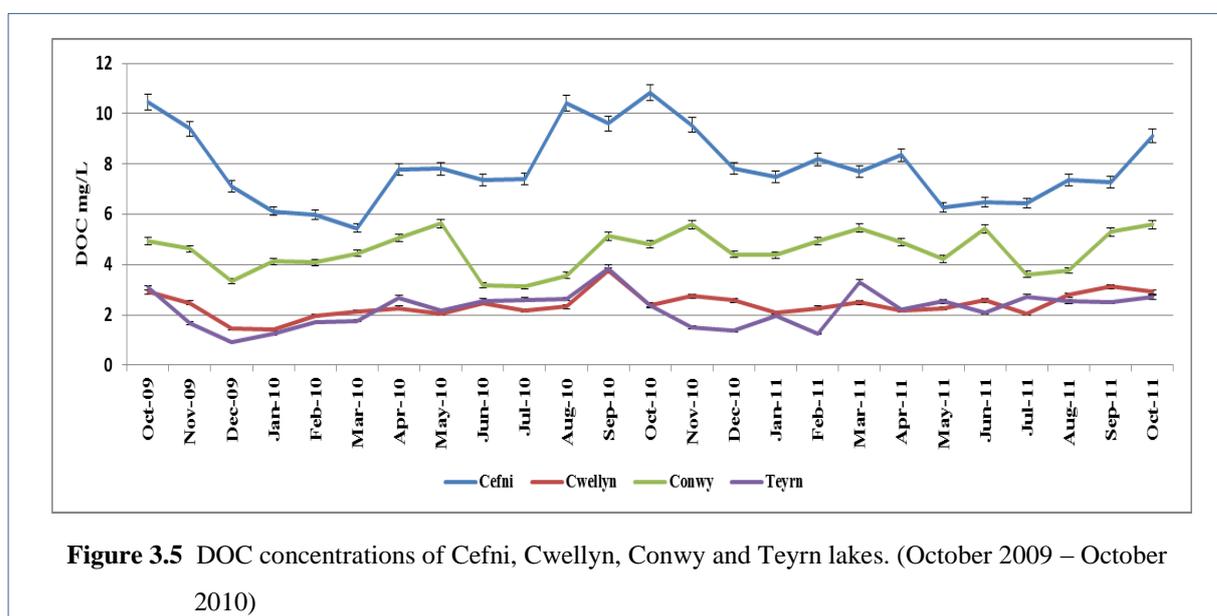


Table 3.2 showed that, overall Cefni’s DOC averaged 8.1 ± 1.5 mg/l whilst Conwy’s average was approximately half, at 4.5 ± 0.7 mg/L. Cwellyn and Teyrn tended to mirror each other with Cwellyn averaging over the sampling period 2.4 ± 0.5 mg/L and Teyrn 2.2 ± 0.7 mg /L.

Table 3.2 Mean DOC concentrations of lakes/reservoirs (October 2009 – October 2011)

Lake/Reservoir	Mean DOC (mg/L)	DOC range (mg/L)	Standard Deviation (L/mg-M)
Cefni (lowland fen)	8.1	5.5 – 10.5	1.5
Conwy (upland bog)	4.5	3.3 – 5.6	0.7
Cwellyn (upland)	2.4	1.5 – 4.6	0.5
Teyrn (upland)	2.2	0.9 – 3.3	0.7

The differences in the concentrations reflected the origin of the DOC. The Cefni with its combination of minerotrophic fen and agricultural catchment, both rich in nutrients and with a constant flow of water available to remove labile degraded DOC consistently presented the highest DOC levels. The Conwy catchment, an oligotrophic blanket bog with a low micro-organism activity level, limited by nutritional inputs exporting the DOC out of the deep peat regulated by diffusive hydraulic processes had half the DOC of Cefni. Teyrn and Cwellyn's catchment profile were relatively similar to each other, hence their DOC concentration (Fig 3.5) were mirrored. The relatively low concentration levels of the DOC may be attributed to the thin nutrient poor soils found on the steep catchment slopes and high rainfall introducing a dilution factor, hence limiting microbial activity and contact time during export of the DOC into the lakes, resulting in lower concentrations of soluble carbon molecular structures.

3.1.2 Determination of Phenolic Compounds in DOC

The concentration of phenolic compounds in the DOC of Llyn Cefni, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn were analysed using method (Estimation of Total Phenolic Compounds, 2.3.2) are shown in (Fig 3.6). Over the 24 months period of sampling Cwellyn and Teyrn demonstrated the lowest average concentrations of phenolics, Cwellyn averaged 0.56 ± 0.03 mg/L and Teyrn 0.57 ± 0.03 mg/L. Although Cefni (Fig 3.6) occasionally generated higher levels of phenolic compounds, both it and Conwy over the 24 months averaged similar levels at 1.08 ± 0.05 mg/L and 0.96 ± 0.05 mg/L respectively and both demonstrated statistically significant correlations (r 0.435, $p < 0.05$) between these two lakes for phenolic concentrations over the course of the study period.

Correlations between Cwellyn and Teyrn's relative phenolic concentrations were also significant (r 0.662, < 0.01), but when the group of four lakes were statistically analysed, differences were highlighted (Table 3.3). For example, Cefni showed a significant correlation with Conwy only, whilst Conwy correlated with both Cefni and Cwellyn. Cwellyn demonstrated a significant correlation with both Conwy and Teyrn, but Teyrn did not correlate with any other lake phenolic concentration other than Cwellyn.

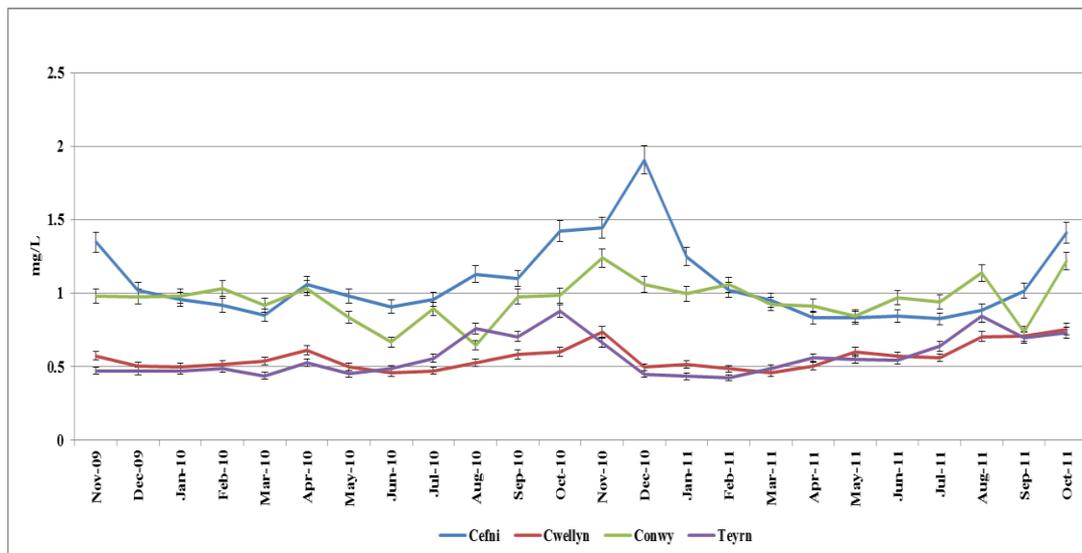


Figure 3.6 Phenolic concentrations of Cefni, Cwellyn, Conwy and Teyrn lake DOC (November 2009 – October 2011)

Table 3.3 Correlation coefficients of phenolic concentrations of each lake over the 24 month sampling period. (n 24)

Lake/Reservoir	Cefni (Pearson coefficient)	Cwellyn (Pearson coefficient)	Conwy (Pearson coefficient)	Teyrn (Pearson coefficient)
Cefni (Pearson coefficient)			0.435*	
Cwellyn (Pearson coefficient)			0.460*	0.662**
Conwy (Pearson coefficient)	0.435*	0.460*		
Teyrn (Pearson coefficient)		0.662**		

** Correlation is significant at the 0.01 level (2 tailed)

* Correlation is significant at the 0.05 level (2 tailed)

Using statistical analysis (Pearson's, two tailed correlation) of each lakes' DOC concentration *versus* phenolics concentration generated significant correlations. These supported the interpretation of results gathered from a number of sources and reported by Peacock *et al.* (2012). The results suggested that phenolic concentrations are representative of total DOC concentrations. Cwellyn demonstrated the significant correlation between DOC and phenolics of the group of lakes, with Conwy being the only lake to exhibit a non-significant correlation (Table 3.4).

Table 3.4 DOC <i>versus</i> phenolic correlation coefficients (<i>r</i>) for October 2009 – October 2011. (n = 24)		
Lake/Reservoir	<i>r</i>	<i>p</i>
Cefni	0.515*	< 0.05
Cwellyn	0.584**	< 0.01
Conwy	0.347	> 0.05
Teyrn	0.429*	< 0.05

Over the period 2009 to 2011, the highest phenolic concentrations for all of the lake samples were recorded between September 2010 and January 2011. Cefni with the lowland fen/agricultural catchment, demonstrated the greatest concentration increase of the four during this period increasing from 1.10 ± 0.05 mg/l in September to a high of 1.91mg/L in December 2010. This was followed by a gradual fall during the spring of 2011 to a level of concentration that averaged 0.86 ± 0.04 mg/L for the 2011 spring/summer months which was relatively comparable to the 2010 spring/summer months (0.94 ± 0.07 mg/L.)

Conwy's peak concentration occurred a month earlier (November 2010) than the Cefni's, increasing from the average spring/summer (2010) concentration of 0.85 mg/L (SD 0.14) to a peak of 1.24 ± 0.06 mg/L in November 2010. Again a drop during the spring months of 2011 was observed with an average for the spring/summer 2011 period 0.96 mg/L (SD 0.10). Teyrn and Cwellyn with the lowest phenolic concentrations of the series also reflected the same pattern of changing concentrations over the period, but Teyrn's highest concentration was a month earlier than both Conwy and Cwellyn, increasing from the spring/early summer (2010) average of 0.49 mg/L (SD 0.05) to 0.88 ± 0.04 mg/L. Although the phenolic concentration was seen to peak earlier, it also dropped back to its average spring/summer concentration faster (December 2010) and demonstrated an average concentration for the said period (2011) of 0.53 mg/L (SD 0.07). Cwellyn although reflecting a similar phenolic concentration profile to Teyrn, peaked as previously mentioned in November 2010 at a concentration of 0.74 ± 0.04 mg/L. This was the lowest phenolic peak concentration of the four lakes series. It was also noted that the maximum was only 0.2 mg/L greater than the spring/summer average, whilst the other upland lake's (Conwy, Teyrn) phenolics averaged a difference of 0.35 mg/L, but the greatest difference was exhibited by the Cefni (1.05 ± 0.05 mg/L).

When considering the phenolic data, it should be noted that the presence of phenolic compounds has been reported to inhibit biodegradation (Wetzel *et al.* 1992; Freeman *et al.*

2001). Fig 3.7 b shows the phenolic content in mg/L from October 2009 – October 2011. The analytical data clearly demonstrate that the highest phenolic content was in the Cefni lake due to its high Raw Surface Water DOC . However if the phenolic concentrations (mg/L) are presented as a percentage concentration (Fig 3.7 a) of the DOC, the results demonstrated that the phenolics concentrations relative to DOC are lowest for the Cefni and averaged 13.3% (Table 3.5) while the three upland lake phenolics relative to DOC concentration were > 20 %.

Lake/reservoir	% Phenolics/mg DOC	Average phenolics mg/L	Average DOC mg/L
Cefni	13.3	1.08 ± 0.05	8.1 ± 0.23
Cwellyn	24.4	0.56 ± 0.03	2.38 ± 0.07
Conwy	21.9	0.96 ± 0.05	4.54 ± 0.14
Teyrn	28.7	0.57 ± 0.03	2.24 ± 0.06

The generation of the labile DOC within catchment peat/soil is stated to be greatly influenced by phenolic compound concentrations and their relative oxidation/reduction state (Freeman *et al.* 1991) Oxidation of the phenolics is considered to be directly related to the activity of extracellular enzyme *phenol oxidase* (Freeman *et al.* 2004) The activity of the enzyme, is in turn subject to a series of three inter-related factors that include, the water table, molecular oxygen and temperature (Freeman *et al.*, 2001).

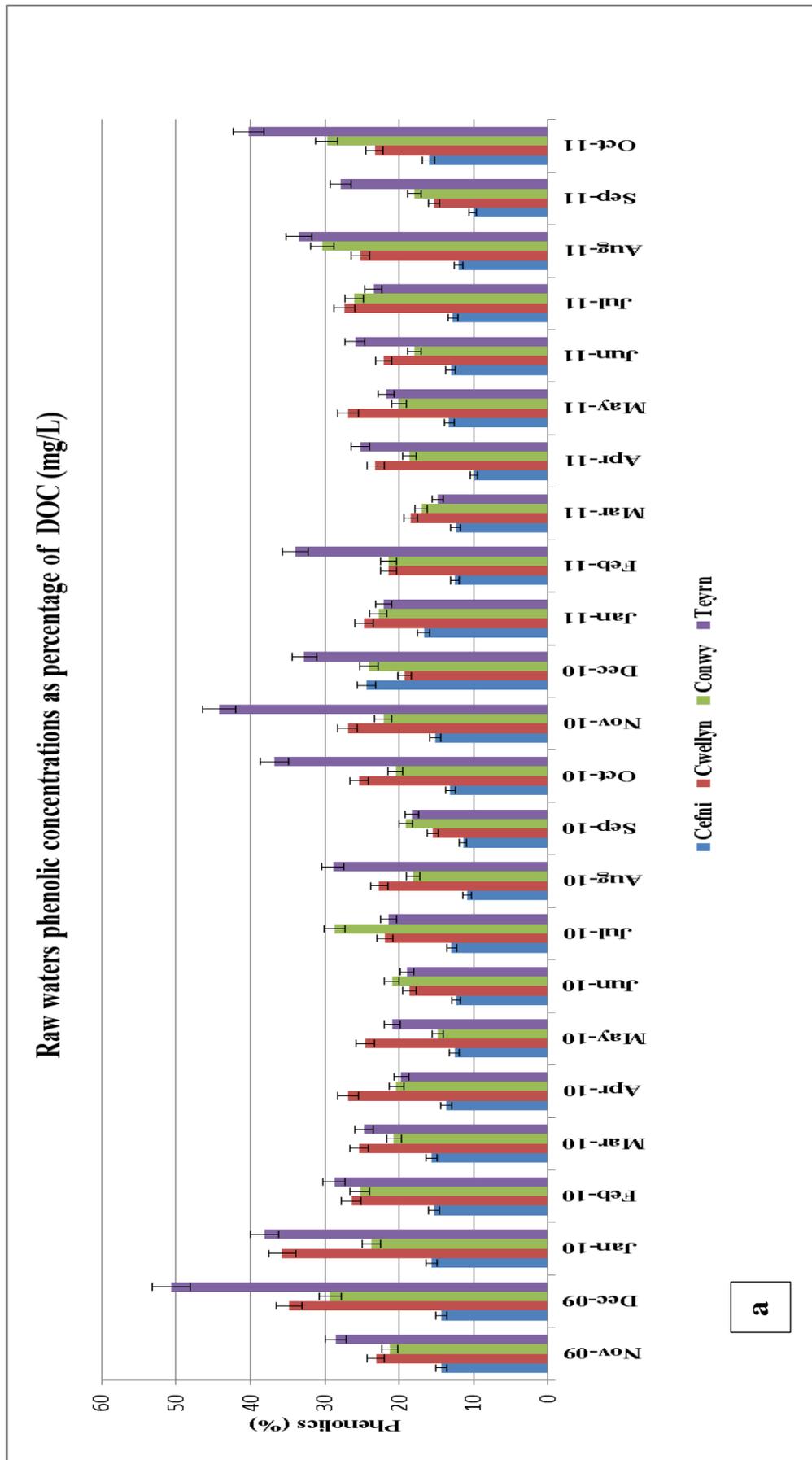


Figure 3.7 a) Cefni, Cwellyn, Conwy and Teyrn, phenolic concentrations shown as a percentage of DOC (mg/L).

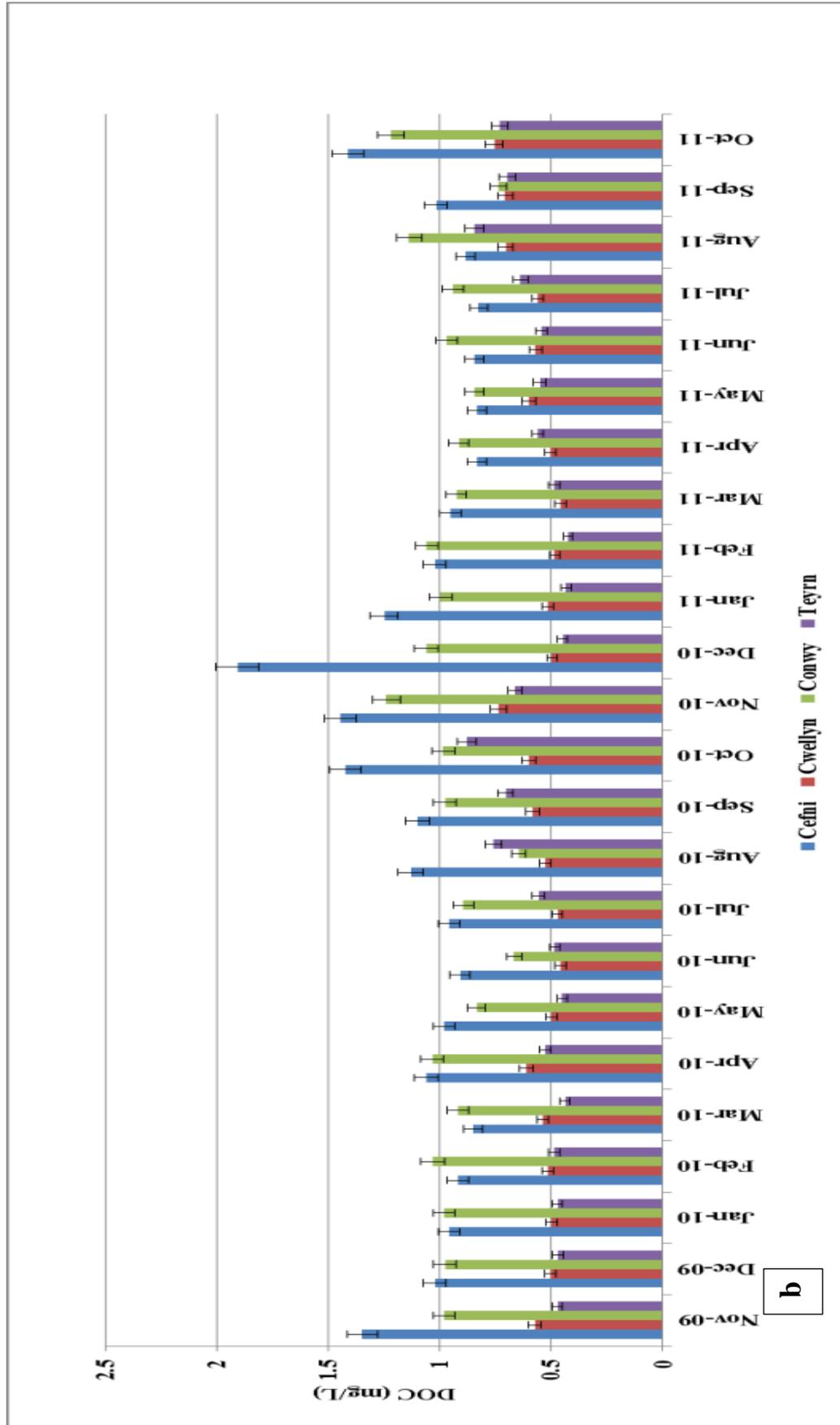


Figure 3.7 b) Cefni, Cwellyn, Conwy and Teyrn phenolic concentrations (mg/L).

With the phenolic compound concentrations appearing to follow a seasonal trend, i.e., the concentration levels increasing during late summer and peaking in autumn (Fig 3.6), this suggested that the three factors influenced and dominated the reactivity of the *oxidase* and the subsequent fall in phenolic oxidation, a) re-wetting of the catchment b) accompanying fall in available molecular oxygen and c) change in temperature (Fenner *et al.* 2005)

Linear regression analysis of the exported DOC concentration *versus* the phenolic concentrations in the DOC demonstrated significant relationships (Fig 3.8 a,b,c,d). As the phenolic content increased in the samples, the DOC concentrations decreased. This agrees with the relationship proposed by Freeman *et al.* (2001), that is, reduced phenolic compound concentrations within a catchment increases microbial activity and organic matter decomposition, subsequently resulting in an increase in DOC export. To further support these findings, the phenolic content of the DOC being exported from the Cefni catchment had the highest average DOC (8.1 mg/L) of the four catchments and the lowest percentage inclusion of phenolics (10 – 17%). Teyrn’s catchment, one of the lowest exporters of DOC (2.2 mg/L) had a higher relative phenolic content of (15 – 45%).

Fenner *et al.* (2005) reported that a relationship exists between microbial activity and temperature and phenolics. To explore this relative to these phenolic data, statistical analysis (Pearsons correlations) of phenolics concentrations in the DOC *versus* temperature was carried out (Table 3.7) using a 30, 60 and 90 day lags. The results for Cefni and Conwy each demonstrated increasing correlations with a negative value at the - 60 and - 90 day average, suggesting that higher temperatures (Fig 3.17) promoted greater phenolic oxidation, hence lower phenolic compound concentrations in the DOC. Although in the Conwy’s case, a decrease in phenolic concentration in the DOC did not result in an increase in DOC export. Cwellyn did not demonstrate any significant correlations. This may again be due to the catchment characteristics with the thin peaty soils being flushed regularly rather than having a slow diffusion.

Lake/Reservoir	(Month/Month)	r (2month MA)	r (3month MA)
Cefni	- 0.285, p > 0.05	- 0.408, p < 0.05	- 0.578, p < 0.01
Cwellyn	0.401, p > 0.05	0.252, p > 0.05	0.155 > 0.05
Conwy	- 0.276, p > 0.05	- 0.616, p < 0.01	- 0.622 < 0.01
Teyrn	0.668, p < 0.01	0.567, p < 0.01	0.412 > 0.05

Teyrn's correlation was significant for the first (-30 day) period, but the significance reduced sequentially when either 60 or 90 day lags was applied (Table 3.7). This suggested that a DOC equilibrium existed between the body of water and the adjacent small blanket peat bog, i.e., the adjacent bog and lake water were a continuum.

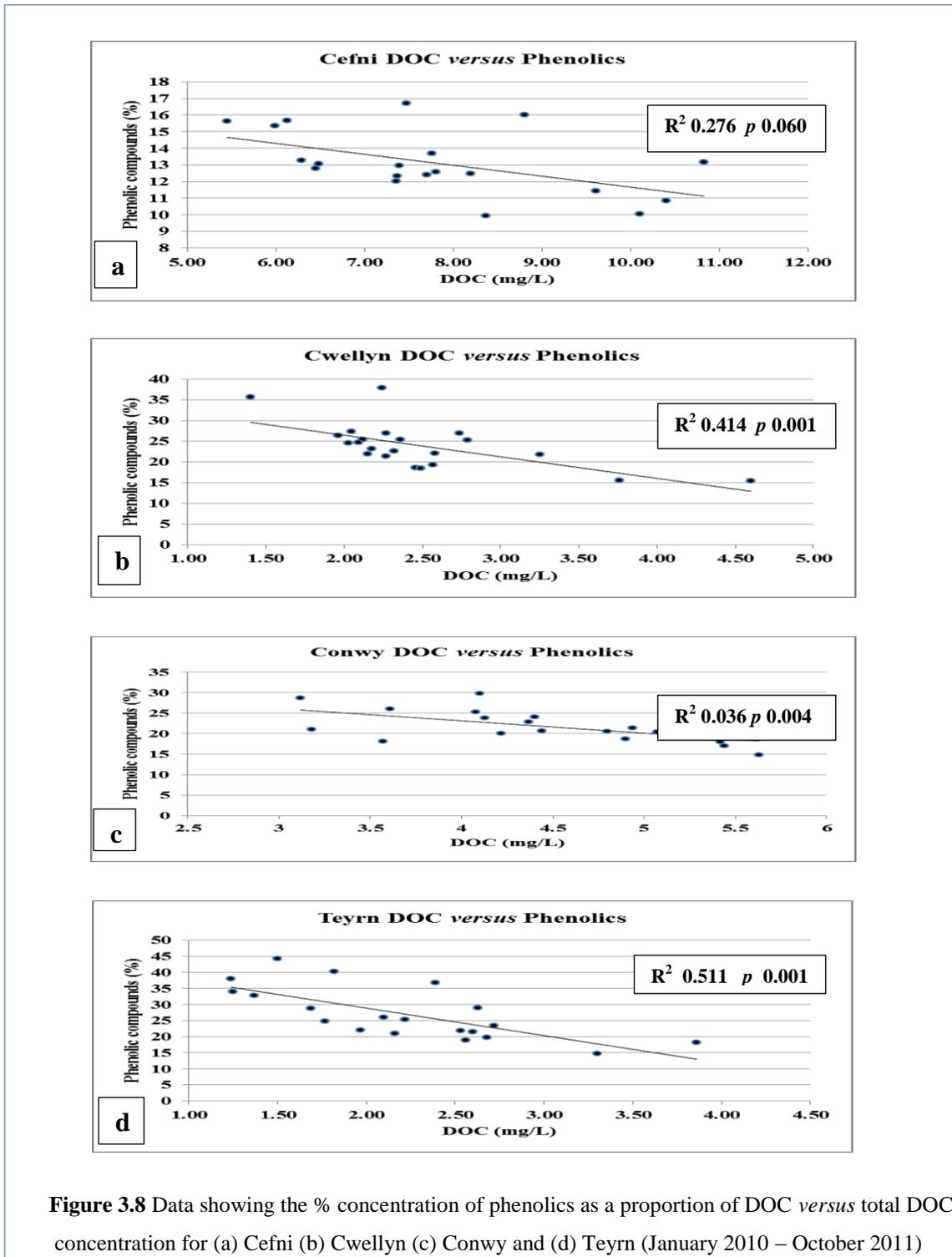


Figure 3.8 Data showing the % concentration of phenolics as a proportion of DOC versus total DOC concentration for (a) Cefni (b) Cwellyn (c) Conwy and (d) Teyrn (January 2010 – October 2011)

3.1.3 UV- visible Spectroscopy of DOC.

The UV spectra of the four lakes (Fig 3.7) during the 24 month sampling regime did not demonstrate any well resolved features, other than high absorption in the wavelength range 200 to 350 nm which is believed to be due to changes in absorbance related to DOC concentration. The absence of distinct features is believed to be due to the broad and overlapping absorption bands of a multiplicity of trace organic compounds typically found in DOC (Liu *et al.* 2010). In addition, it is reported that the wavelength range 200 - 230nm is affected by $n - \sigma^*$ transition of nitrate and bromide chromophores (Korshin *et al.*, 1997) in water. However, in fresh water, the majority of chromophores that have been reported to absorb in the 250 - 280 nm region are due to $\pi - \pi^*$ transitions associated with sp^2 hybridized carbon bonds, [C=C], [C=O] and aromatic structures (Westerhoff *et al.* 2004).

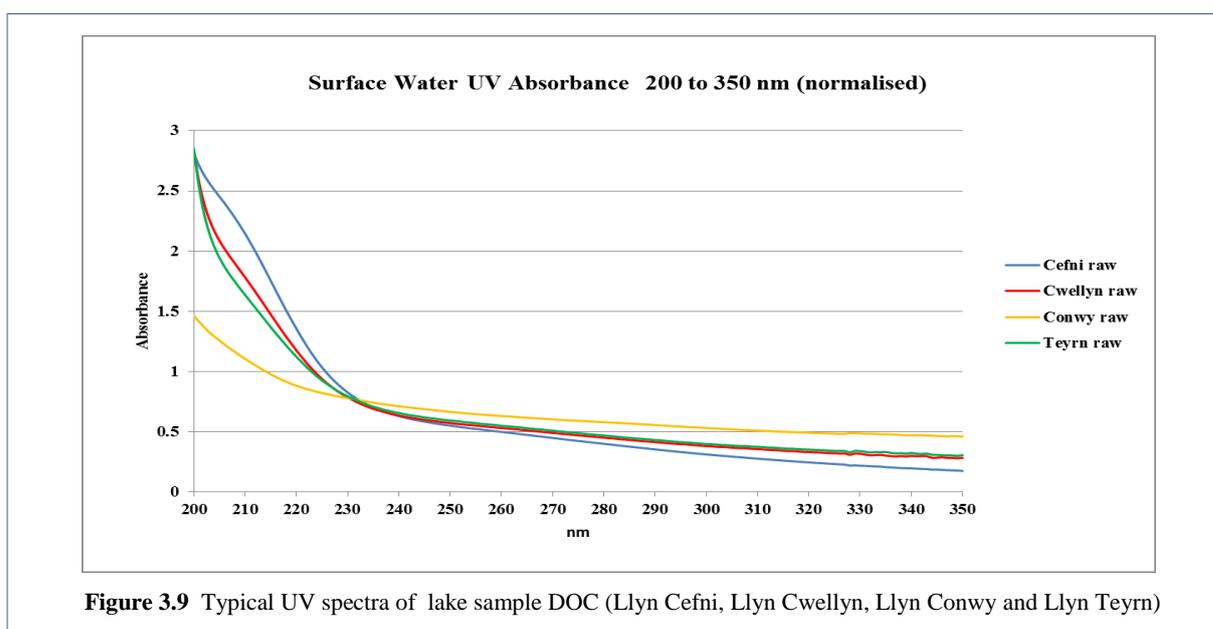


Figure 3.9 Typical UV spectra of lake sample DOC (Llyn Cefni, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn)

The wavelength 254 nm is referred to in the literature as the water treatment industry's method for monitoring the concentration of DOC (Edzwald *et al.* 1985). A plot of DOC concentration for each sample over the 24 month sampling period *versus* the UV-vis absorbance at 254nm (Fig 3.10) gave an R^2 value of 0.84 $p < 0.01$, thus confirming a linear relationship between these two parameters for all sites.

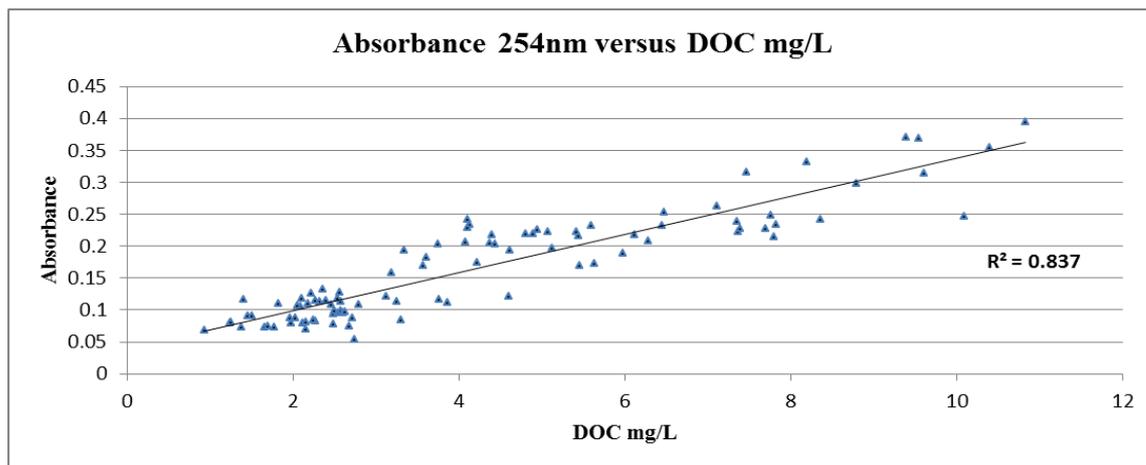


Figure 3.10 Absorbance at 254nm *versus* lake DOC concentration using data from Llyn Cefni, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn.

3.1.3.1 UV Spectroscopy (SUVA) of DOC

Specific Ultra Violet Absorbance (SUVA) values have been reported to be a measure of the aromatic compound content of DOC (Leenheer and Croue, 2003). Thus a high SUVA value (> 4) generally indicates DOC of high humic content with hydrophobic character (aromatic) and a lower value (< 4) has a more hydrophilic content with reduced aromatic character. SUVA is calculated by dividing the absorbance value at 254 nm of the sample by the DOC (mg/L) with the values reported in $\text{Lmg}^{-1}\text{m}^{-1}$ (EPA method 415.3, 2003).

Cefni SUVA ranged from 2.43 to 4.22 $\text{Lmg}^{-1}\text{m}^{-1}$ during the monitoring period with an overall average of 3.37 $\text{Lmg}^{-1}\text{m}^{-1}$. The highest SUVAs of the Cefni series were for January and February 2011 at 4.22 ± 0.08 and 4.06 ± 0.07 $\text{Lmg}^{-1}\text{m}^{-1}$, respectively. Cwellyn demonstrated higher SUVA values than Cefni, with an overall average of 4.33 ± 0.09 $\text{Lmg}^{-1}\text{m}^{-1}$. The highest values were recorded during the months of January 2010 at 8.40 ± 0.17 $\text{Lmg}^{-1}\text{m}^{-1}$ and 5.26 ± 0.11 $\text{Lmg}^{-1}\text{m}^{-1}$ for July 2011 (Fig 3.11). The intermediate months averaged 4.02 $\text{Lmg}^{-1}\text{m}^{-1}$, with a very low value of 2.00 ± 0.04 $\text{Lmg}^{-1}\text{m}^{-1}$ for November 2010. The Conwy SUVA values demonstrated higher values than Cwellyn, Cefni and Teyrn and averaged, 4.67 ± 0.09 $\text{Lmg}^{-1}\text{m}^{-1}$ for the 24 months. The highest SUVA values were recorded during the months of December 2010 and October 2011 at 5.83 ± 0.17 and 5.90 ± 0.12 $\text{Lmg}^{-1}\text{m}^{-1}$, respectively, with the lowest value (3.85 ± 0.08 $\text{Lmg}^{-1}\text{m}^{-1}$) observed in September 2010. SUVA values generated for Teyrn were the most

variable, 8 values out of the 24 were above 5.0 Lmg⁻¹m⁻¹ (average 6.1 ± 0.12 Lmg⁻¹m⁻¹). Two of the lowest values for the series of 24 were recorded for April 2010 at 2.68 ± 0.05 Lmg⁻¹m⁻¹ and March 2011 at 2.56 ± 0.05 Lmg⁻¹m⁻¹. Tabulated (Table 3.8) SUVA standard deviations (SD) illustrated that the Cefni showed the lowest deviation with Conwy second, both of which were below 1.0 Lmg⁻¹m⁻¹. The SD of Teyrn and Cwellyn were similar with both values greater than 1.00 Lmg⁻¹m⁻¹ and a 0.01 difference between the two.

Lake/Reservoir	Averaged SUVA values (Lmg ⁻¹ m ⁻¹)	Standard Deviation (Lmg ⁻¹ m ⁻¹)
Cefni	3.37 ± 0.07	0.44
Conwy	4.33 ± 0.09	0.70
Cwellyn	4.67 ± 0.09	1.30
Teyrn	4.60 ± 0.09	1.31

To explore and illustrate any correlations between SUVA values and the levels of aromatic content of the four lake's DOC the values from the sampling period (2009 – 2011) were statistically analysed using Pearsons correlation coefficient. From the statistical results, the three upland sources, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn demonstrated significant but, selective correlations, i.e., Llyn Teyrn correlated with both Conwy and Cwellyn, but Cwellyn did not with Conwy and *vice versa* (Table 3.9). The Cefni with its lower SUVA value, therefore exhibiting a less hydrophobic DOC, did not demonstrate any significant correlations with any of the upland lakes.

Lake/reservoir	Cefni (Pearsons coefficient)	Cwellyn (Pearsons coefficient)	Conwy (Pearsons coefficient)	Teyrn (Pearsons coefficient)
Cefni (Pearsons coefficient)				
Cwellyn (Pearsons coefficient)				0.418*
Conwy (Pearsons coefficient)				0.515**
Teyrn (Pearsons coefficient)		0.418*	0.515**	

** Correlation is significant at the 0.01 level (2 tailed)

* Correlation is significant at the 0.05 level (2 tailed)

Hence it can be stated that Llyn Teyrn's relatively high hydrophobic DOC content (SUVA 4.60) mirrored both Cwellyn (SUVA 4.67 Lmg⁻¹m⁻¹) and Conwy (SUVA 4.33 Lmg⁻¹m⁻¹)

over the period, but the SUVA values expressed a closer correlation with those of the Conwy's ($r = 0.515$ $p < 0.01$) than Cwellyn ($r = 0.418$ $p < 0.05$). Therefore, Teyrn and Conwy demonstrated similar patterns of hydrophobicity, while although Cwellyn exhibited a higher hydrophobicity it was independent of the Conwy's.

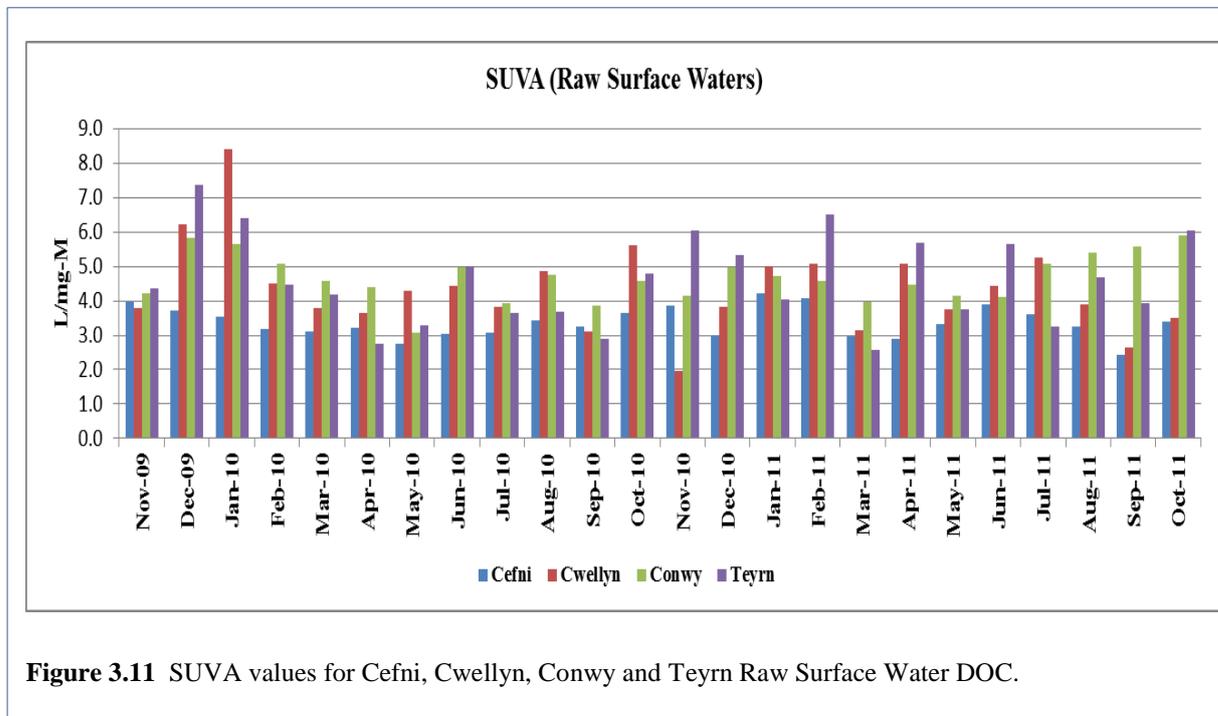


Figure 3.11 SUVA values for Cefni, Cwellyn, Conwy and Teyrn Raw Surface Water DOC.

3.1.4 Size Exclusion Chromatography (SEC) of DOC

Determination of the molecular mass (MM) distribution of the DOC of the four lakes over an 18 month period (January 2010 – July 2011) was carried out using Size Exclusion Chromatography (SEC) method 2.3.3. SEC is a method of fractionation that utilises the molecular dimensions of the DOC passing through the solid phase of the column. The retention time of the molecules on the column (BioSep SEC s2000) are directly related to molecular size and hence mass, with the largest having the shortest retention time etc. The adapted method uses a detection wavelength of 254nm, a buffered mobile phase and calibration is by a series of standards poly(styrenesulfonic acid sodium salt) (PSS) and Cyanocobalamin (B12) as per the method published by Zhou *et al.* (1999).

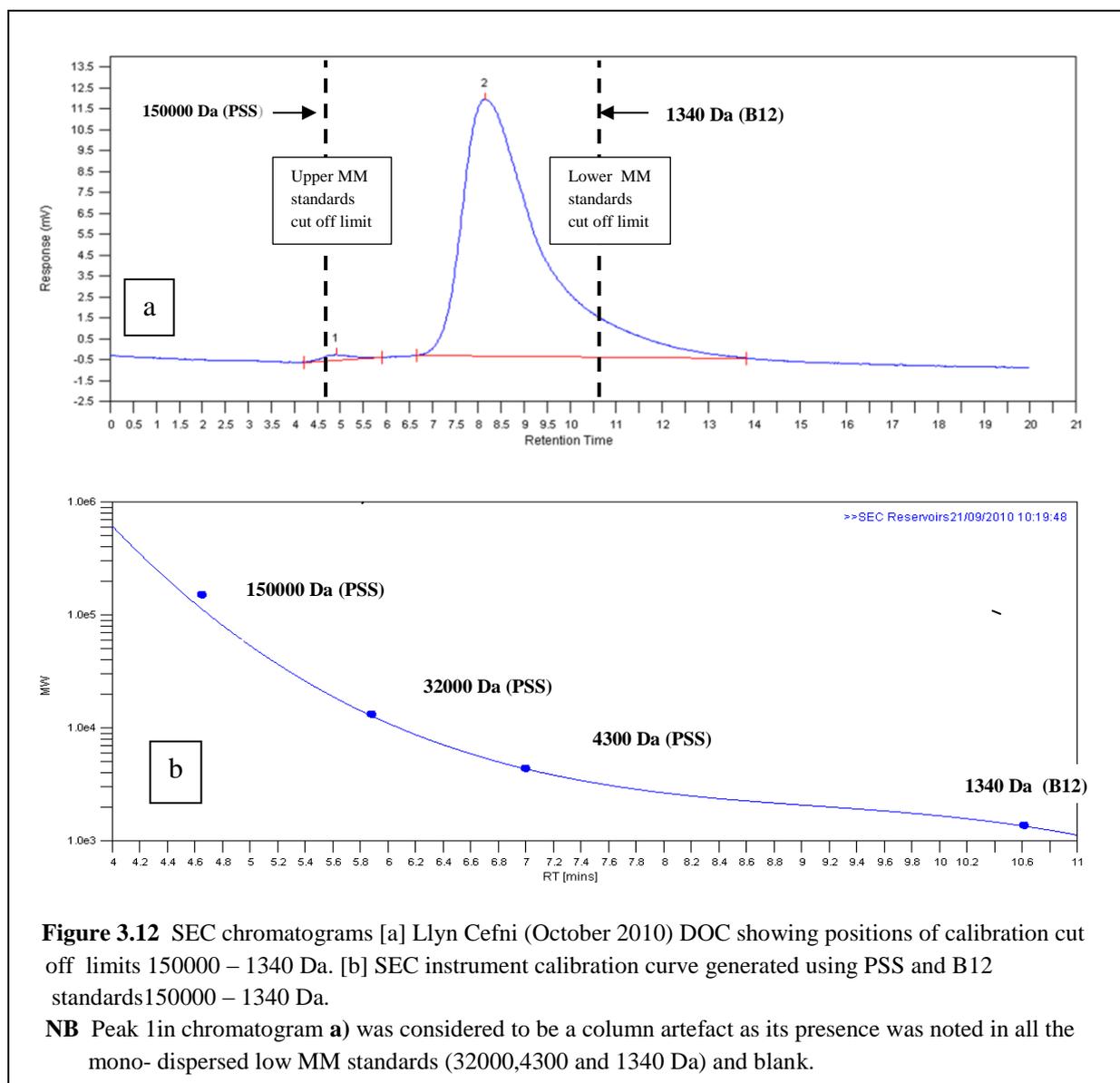


Figure 3.12 SEC chromatograms [a] Llyn Cefni (October 2010) DOC showing positions of calibration cut off limits 150000 – 1340 Da. [b] SEC instrument calibration curve generated using PSS and B12 standards 150000 – 1340 Da.

NB Peak 1 in chromatogram **a**) was considered to be a column artefact as its presence was noted in all the mono-dispersed low MM standards (32000, 4300 and 1340 Da) and blank.

PSS standards were selected for SEC analysis as they were considered to be monodispersed and representative of the coiled configuration of aquatic humic substances and were found to be similar to those of the Suwannee fulvic acid, although structural investigations reported the later as more branched with more cross linkages than PSS. (Chin *et al.* 1989; Peuravuori and Pihlaja.1997). It has been also reported that the use of, for example, globular standards can over predict the molecular masses in humic substances by a factor of 5 or more (Chin *et al.* 1994).

The elution time of the range of standards using the BioSep SEC s2000 column did not accommodate the peak elution of the sample, i.e., the tail of the sample peaks were not within the lower cut off point of the standard series (1340 Da at RT 10.6 minutes). Examination of all the samples demonstrated asymmetrical peaks with RTs between 7.5 and 8.5 minutes with peak tailing from approximately 9.5 minutes. A shorter complete

peak elution time could not be attained as an increase in mobile phase flow rate could not be accommodated by the stationary phase as the pressure approached its maximum recommended working limits (1,500 psi).

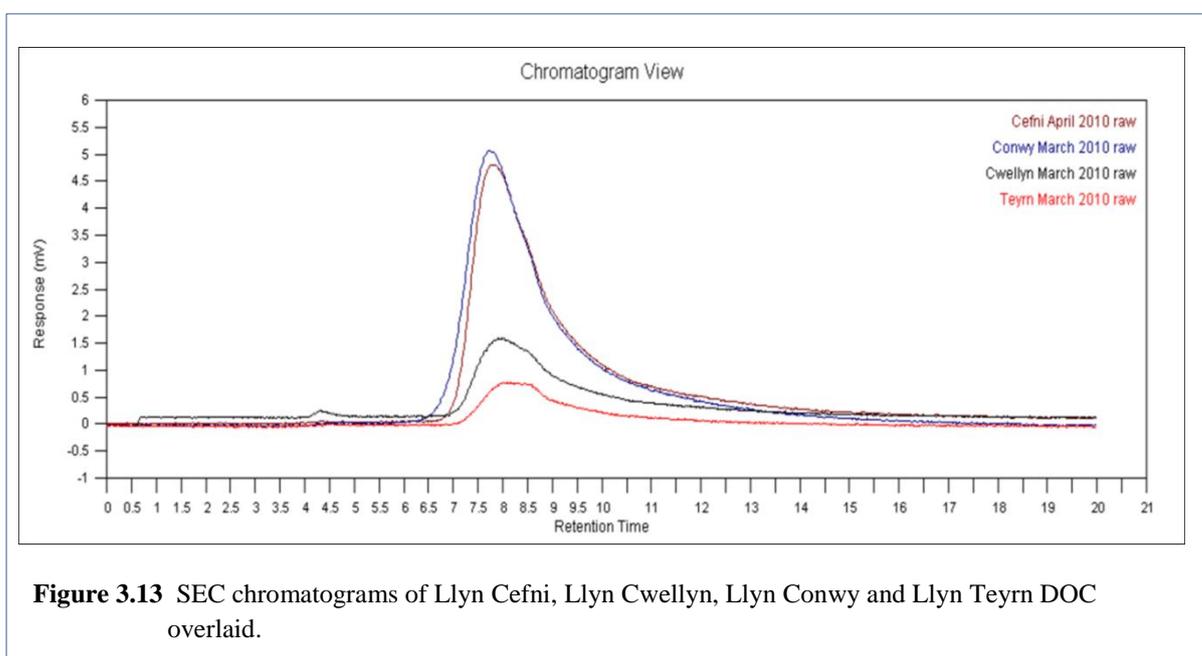
Analysis of the peak areas that were within the range did establish that the majority of the apparent molecular mass (MM) distributions of the DOC was < 5,000Da (RT; 7 – 12.1 minutes). The distribution of MMs within the DOC integrated sample peak areas were calculated by the ‘Cirrus’ software package of the Polymer Laboratories PL-GPC 50 instrument relative to pre-programmed selected ranges (Table 3.10). The ranges were inputted during the setting up of the method and the upper and lower MM represented a peak slice. The result relative to the peak slice range (Table 3.10) was reported as a percentage of the total area of the peak.

Table 3.10 Programmed peak slice MM ranges		
Upper MM (Da)	Lower MM (Da)	Retention time (RT ± 0.2 mins)
150000	100000	4.6 – 4.9
100000	50000	4.9 – 5.4
50000	40000	5.4 – 5.5
40000	30000	5.5 – 5.8
30000	20000	5.8 – 6.1
20000	10000	6.1 – 6.5
10000	5000	6.5 – 7.1
5000	1000	7.1 – 12.1
1000	10	> 12.1

The chromatogram (Figure 3.12 a) shows a typical peak eluted by the BioSep SEC s2000 (October 2010 Cefni surface water DOC). The highest MM (150000 Da) PSS standard and the lower (1350 Da) Cyanobalamin (B12) standard RT are indicated. Figure 3.12 b shows the non-linear calibration curve generated by the series of standards used (HPSEC 2.1.4.3) for analysis.

Figure 3.13 shows an overlay of the SEC chromatograms representing the MM distribution of the four lake DOCs. The peak shapes are relatively similar with a maximum response by each at 8.4 ± 0.2 minutes. The response (mV) values represented the intensities of each absorbing species at the 254nm wavelength. The differences in areas and peak heights represented the concentrations of aromatic/conjugated molecular structures present in each lakes DOC. With the samples being injected directly onto the column without concentration adjustments, the intensities are broadly considered to be representative of the

DOC concentrations also. Hence Cefni's intensity was the highest, closely followed by the Conwy. An interesting observation was that the SUVA values predicted a higher concentration of aromatic moieties in the Conwy (4.33 ± 0.09) than the Cefni (3.37 ± 0.07). Hence, although the Cefni has approximately double the Conwy DOC the peak heights are relatively close 5.2 mV and 4.9 mV respectively, thus demonstrating the higher concentration of 254 nm absorbing species present in the Conwy's DOC. Comparatively similar peak shapes confirmed the presence of similar ranges of MMs in both the Conwy and Cefni lake DOCs. Cwellyn and Teyrn peak shapes were less sharp and symmetric, with differing intensities although the DOC concentrations, SUVA values and UV-vis spectra demonstrated similarity.



The molecular weight distributions of the four lake DOCs (Llyn Cefni, Llyn Cwellyn, Llyn Conwy, Llyn Teyrn DOC) over a period from January 2010 to March 2011 were analysed within 48 hours after collection. The analysis was focussed on the peak areas between the upper and lower cut off limits shown in Fig 3.12 a.

The series of results reported in Figure 3.14 a,b,c,d demonstrate distinct patterns for the MM distributions. It was immediately apparent that the dominant MM in all the DOCs were within the ranges 5000 – 4000 Da and 4000 – 3000 Da for the period winter 2010 – winter 2011 with a couple of exceptions.

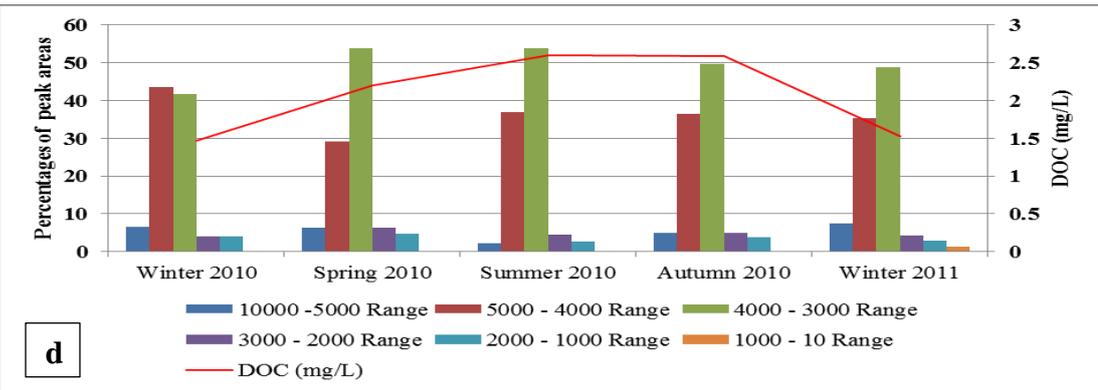
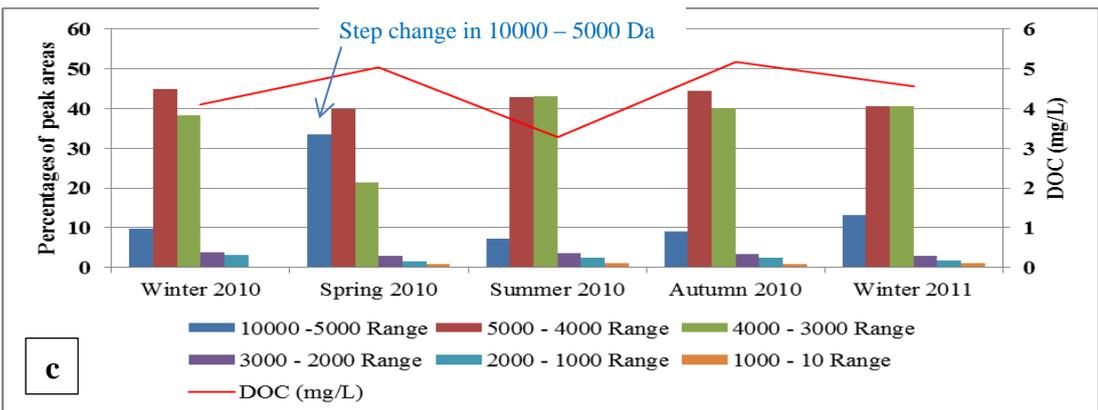
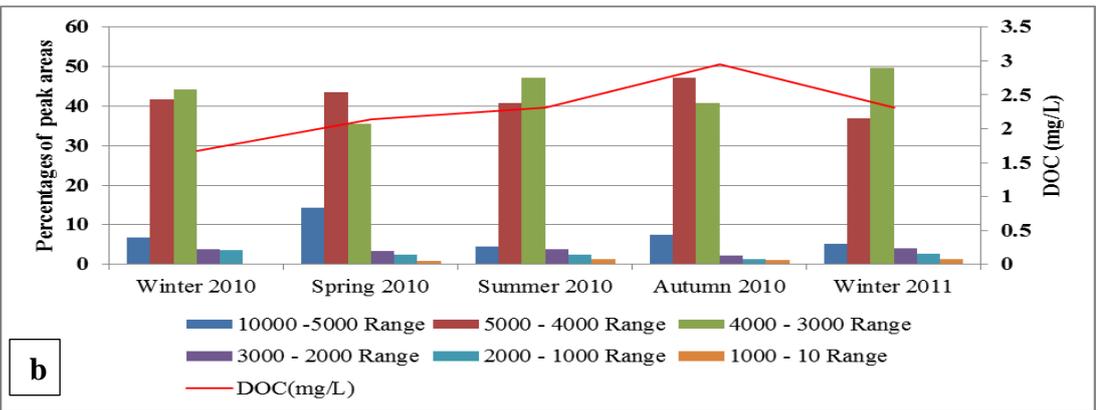
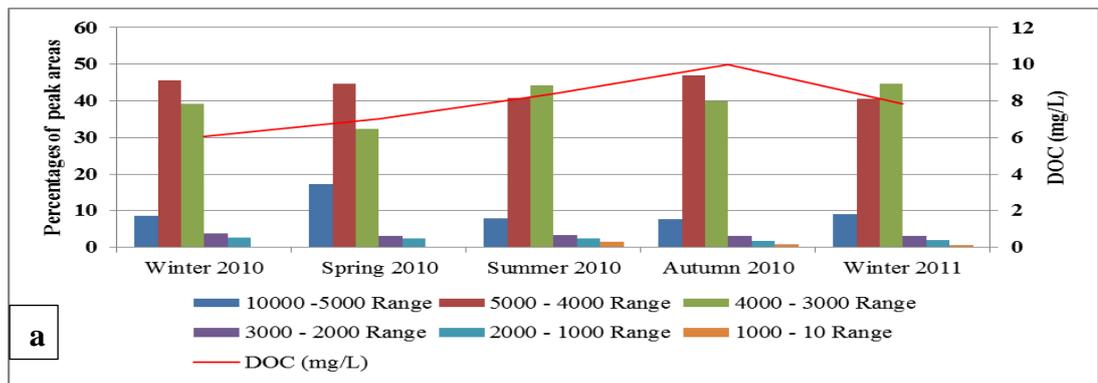


Figure 3.14 a) Cefni Raw Surface Water SEC b) Cwellyn Raw Surface Water SEC c) Conwy Raw Surface Water SEC d) Teyrn Raw Surface Water SEC.

Conwy exhibited a step change increase in the DOC material with MM in the range 10000 – 5000 Da from 10 % in the 2010 winter series to 33.2 % in the 2010 spring, whilst the 4000 to 3000 Da range reduced from a high of 38.1 % in the 2010 winter to 22.1% in the 2010 spring. It was also noted that the upland lake Teyrn demonstrated a change in the 4000 – 3000 range during the 2010 spring, from 41.1% in the 2010 winter to 53.0 % in spring 2010. This was accompanied by a reduction in the 5000 – 4000 Da range to 29.0 % from winter 2010 high of 42.9 %. During the dry period (June/July 2010) an increase was noted in the 4000 – 3000 Da range of the raw surface water DOC of all four lakes accompanied by a drop in the 10000 – 5000 Da ranges (Table 3.11).

Lake/ Reservoir	Average dry spring increase (%) 4000 – 3000 Da	Average dry spring reduction (%) 10000 – 5000 Da
Cefni	12.0	- 19.6
Cwellyn	12.0	- 10.1
Conwy	22.0	- 24.9
Teyrn	No change	- 5.6

The increase in rainfall post July 2010 (Fig 3.17) was associated with a reversal of the changes recorded in Table 3.11. A gradual increase in the 10000 – 5000 Da (Fig 3.15) and a decrease in the 4000 – 3000 Da (Fig 3.16) range between August 2010 - January 2011 for all four lake Raw Surface Water DOCs was observed. This suggested that during the dry period, when the DOC dwell time in the catchment increased, the macro molecular structures were being degraded by microbial activity to shorter chains with less MM. The increase in the longer chains with a higher MM related to a reduced retention of the DOC in the catchments, due to re-wet and increased water flow. Hence a rapid turnover of DOC leading to shorter microbial degradation and an accompanying increase in available DOC concentrations associated with the die off of the summer vegetation.

The DOC concentrations dropped during the winter period 2011 (Figs 3.14 a,b,c and d). This was also accompanied by an increase in the averaged 4000 – 3000 Da range in the Cefni, Cwellyn and Teyrn but not in the Conwy. Although the DOC concentration in the Conwy dropped, after the re-wet, it did not demonstrate large MM range perturbation. The 4000 – 3000 Da range demonstrated a steady percentage of 40 ± 2 % over the autumn and winter. This tends to suggest that the catchment processes were relatively stable, although there was a suggestion of a drop in microbial activity as the temperatures dropped (Fig

3.17) with the steady increase in the 10000 – 5000 Da range (Fig 3.15) and a decrease in the 4000 – 3000 Da range (Fig 3.16)

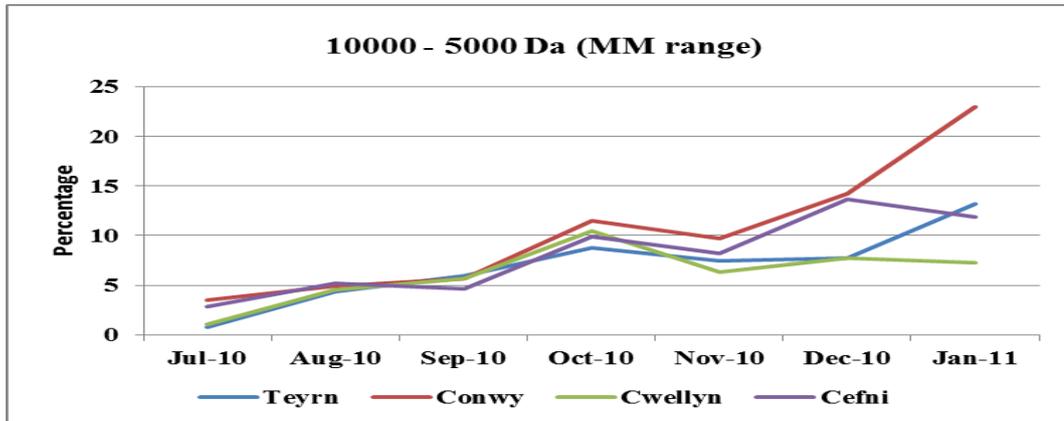


Figure 3.15 Increase in the 10000 – 5000 Da MM ranges of Llyn Cefni, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn after the 2010 dry spring.

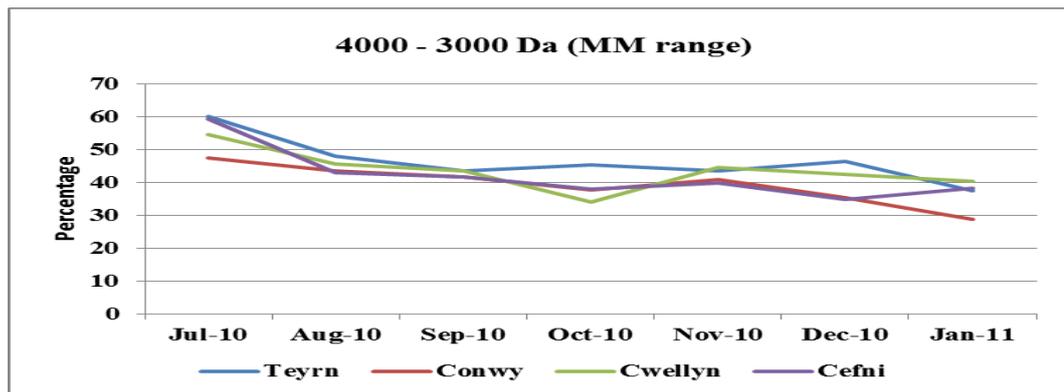


Figure 3.16 Decrease in the 4000 - 3000 Da MM ranges of Llyn Cefni, Llyn Cwellyn, Llyn Conwy and Llyn Teyrn after the 2010 dry spring.

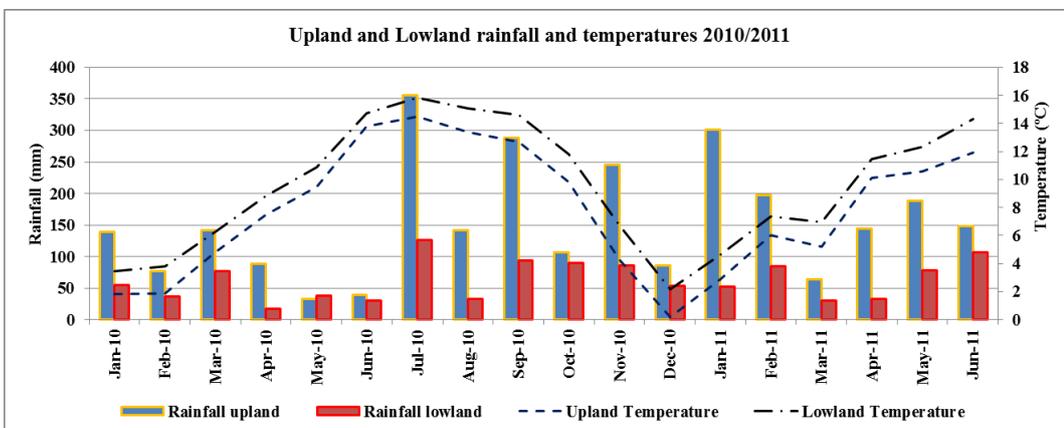


Figure 3.17 Upland and lowland rainfall and temperatures January 2010 – June 2011

To further investigate the character of the DOC using SEC, the poly-dispersity (\mathfrak{D}) of each lake's surface water DOC sample/month was calculated. Poly-dispersity values are used to characterize the 'dispersion of distribution' of MM in a given polymer. A poly - dispersity value of 1 indicates a 'uniform polymer' where the chain lengths and MM are consistent, but polymers having a poly-dispersity > 1 are considered to be non-uniform with variable MM making up the macromolecule (Stepto, IUPAC 2009).

Calculation of the poly-dispersity (\mathfrak{D}) for the surface water samples was determined using the "Cirrus" software calculation as shown in (Eq 3.1)

$$\mathfrak{D} = \frac{M_w}{M_n} \quad (\text{Eq 3.1})$$

The M_n (number average molecular weight) (Eq 3.2) is calculated by summing the (Number of molecules x Mass of each molecule) divided by the sum of the (number of molecules in the sample).

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \quad (\text{Eq 3.2})$$

Table 3.12 Example data for calculating M_n			
	N^o of molecules	Mass of each molecule (Da)	Total mass of each type of molecule
	2	2,000	4,000
	4	5,000	20,000
Total molecules	6	Total sample mass	24,000

$$M_n = 24,000 / 6 = \mathbf{4,000}$$

M_w (weight average molecular weight Eq 3.3) is calculated by: **1)** Calculating the weight fraction (W_i) of each molecule by dividing the total mass of each type of molecule ($N_i M_i$) with the summed mass of the molecules $\sum N_i M_i$ **2)** Multiplying the weight fraction by the total mass of each type of molecule ($N_i M_i$) to give ($W_i M_i$) and then summing all the results to give $\sum W_i M_i$) which is equal to M_w .

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \quad (\text{Eq 3.3})$$

where N_i is number of molecules and M_i is the MM. (Cirrus software, Varian 2009).

	Nº of molecules (N_i)	Mass of molecules (M_j)	Total mass of each type of molecule	Weight Fraction [W_i] ($N_i M_i / \sum N_i M_i$)	$[W_i M_i]$
	2	2,000	4,000	0.17	340
	4	5,000	20,000	0.83	4150
Total Molecules	6	Total sample mass	24,000	$\Sigma (W_i M_i)$	4490

$$M_w = \Sigma w_i M_i = 340 + 4150 = \mathbf{4490}$$

Polydispersity (\mathcal{D}) of example is calculated from (eq 3.1) = $4490/4000 = \mathbf{1.1}$

The polydispersity (\mathcal{D}) of the mono-dispersed PSS standards averaged 1.2 which indicates that the M_n and M_w values are relatively close. The results (Table 3.14) calculated using the above equations for the Raw Surface Water DOC of Cefni, Cwellyn, Conwy and Teyrn demonstrated a range from as low as 2.8 in the Cefni to a high of 10.8 in the Conwy. These are different to the \mathcal{D} values (c. 1.1) of the PSS standards which are mono-dispersed polymers.

Month	Cefni (Mw/Mn)	Cwellyn (Mw/Mn)	Conwy (Mw/Mn)	Teyrn (Mw/Mn)
Jan-10	5.9	7.6	9.5	8.9
Feb-10	7.4	7.1	8.6	8.8
Mar-10	10.7	9.9	10.8	4.9
Apr-10	2.8	3.2	4.2	4.6
May-10	6.9	4.7	6.1	5.1
Jun-10	6.9	4.9	7.3	5.3
Jul-10	5.3	3.8	3.8	2.6
Aug-10	4.9	5.2	5.8	5.3
Sep-10	3.6	4.8	4.5	4.5
Oct-10	4.1	4.0	6.3	5.4
Nov-10	3.8	3.3	5.7	6
Dec-10	5.2	5.9	5.8	7.2
Jan-11	6.4	5.6	5.2	6.4

Peuravuori *et al.* (1997) reported a range of polydispersity results between 2.7 and 9.1 for humic solutes in Raw Surface Water samples. These results indicated that the range of humic solutes in natural waters was wide and not homogeneous (Table 3.14).

Table 3.15 Correlation coefficients of poly-dispersity values

Lakes/Reservoirs	Cwellyn	Conwy	Teyrn	Cefni
Cefni	0.906**	0.907**	0.562*	
Cwellyn		0.889**	0.634**	0.906**
Conwy	0.889**		0.713**	0.907**
Teyrn	0.634**	0.713**		0.562*

** Significance at 0.01 level, * Significance at 0.05 level.

The highest \bar{D} value was in March 2010 for Cefni, Cwellyn and Conwy and was centred at 10.5, before dropping to an average of 3.4 in April 2010. Teyrn mirrored this pattern a month earlier and its highest \bar{D} value was in February 2010 (\bar{D} 8.8) before it dropped to a low of \bar{D} 4.9 in March 2010. The lower ratio values suggested that the weight average (M_w) of the molecular masses were not as diverse, (i.e., more homogenous). This may be as a result of an increase in microbial activity, cleaving polymers chains, thereby increasing the number of shorter chains present, hence reducing heterogeneity.

A series of low values (average \bar{D} 4.5) were again recorded for the period July to September 2010 with the lowest series of the group in July following the end of the very dry spring period. This tends to support the change in SEC data where evidence of an extended dwell time and microbial activity increased the 4000 – 3000 range, hence reducing heterogeneity. Statistical analysis (Pearsons two tailed correlation) demonstrated significant ($p < 0.01$) correlations (Table 3.15) between each of the lakes \bar{D} values with the exception of Teyrn which, although significant, presented weaker correlations with the other three sites. This might suggest possible differences in the rates of DOC processing at this site, possibly due to an environmental factor such as temperature and sunlight being efficiently collected simply by the nature of the lake's sheltered position on the south slopes of Mt Snowdon (Fig 2.1 D).

3.1.5 Fluorescence Spectroscopy of DOC

To further characterise the complex nature of the DOC and to try to further identify components in common in the four Raw Surface Water DOC samples over the 24 month sampling exercise, a fluorescence study was carried out using a Spectramax M2e instrument.

The synchronised fluorescence excitation and emission (EEMs) spectra of the samples were generated as per method (2.3.5). Figure 3.18 a,b,c and d show the results (January 2011) as 3-dimensional contour plots of EEMs emission spectra of the four Raw Surface Water DOCs after zeroing the instrument with Milli-Q water (18 M Ω .cm) to remove any interference (Hua *et al.*, 2010). The intensity axis for each plot ranges from 300 – 2800 R.U. (Raman Units). Plotting the start of the peak intensities from 300 removed noise from the surface plots and highlighted the major components contributing to the emission spectra.

The plots show different patterns of fluorescing components in each of the four lake DOCs January 2011 samples, but do demonstrate common excitation/emission species. The intense fluorescing component recorded at excitation/emission 255/490 nm in all four samples was also observed by Stedmon *et al.*, (2011) in their natural water samples and was classified as ‘broad humic like’. There was evidence of a fluorescing species between the emission wavelengths 300 – 500 nm, but ‘peak pick’ identification and comparison with the literature (Goslan *et al.*, 2004; Marhaba *et al.*, 1999; Her *et al.*, 2003) was difficult with the number of samples to consider.

Hence to analyse a large number of EEMs spectra 88 samples, (96 - 8 accidental loss) and establish the most common fluorescing components in the four surface water DOCs, a method was used that applied parallel factor analysis (PARAFAC) to the data based on a method of Stedmon *et al.* (2003). Using MATLAB software running N-way and DOMFluor toolboxes, the EEMs fluorescence spectra, which contained a large amount of three way data, i.e., (sample by excitation by emission), was used to generate matrices which were decomposed into trilinear components. From these, individual common fluorescent components were isolated as emission and excitation data and the results were plotted (Fig 3.22 a and b).). The maxima and minima of the spectra curve results were then compared to those reported in the literature.

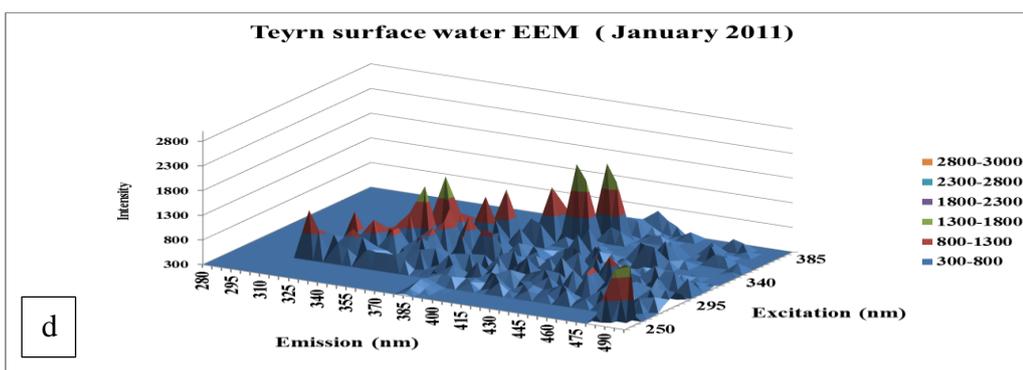
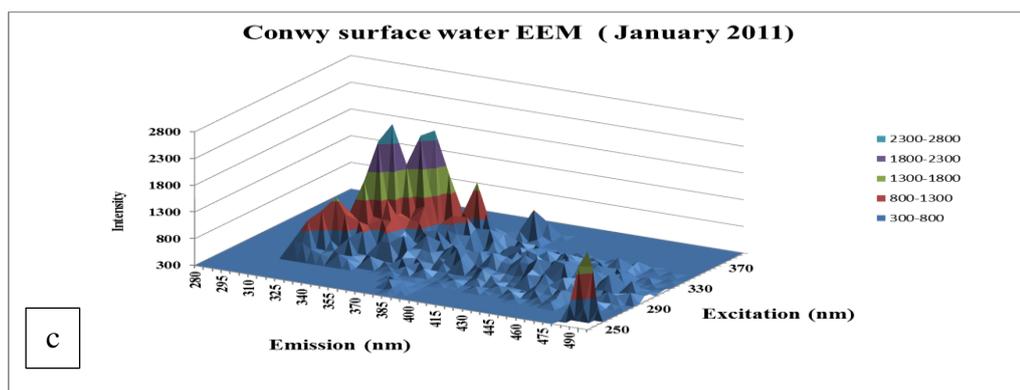
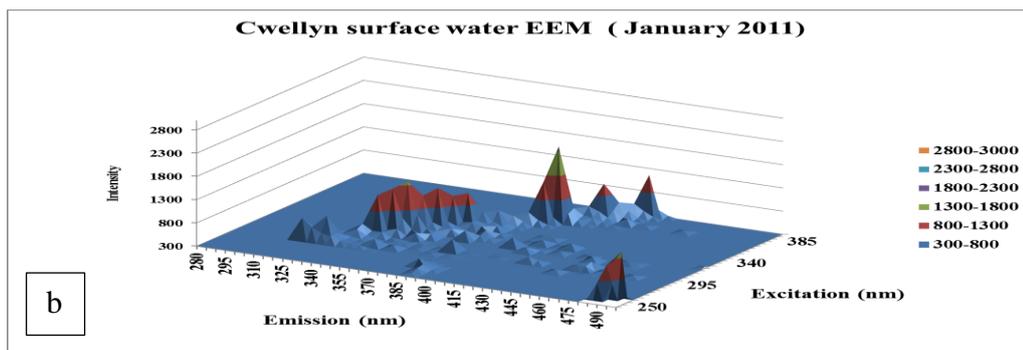
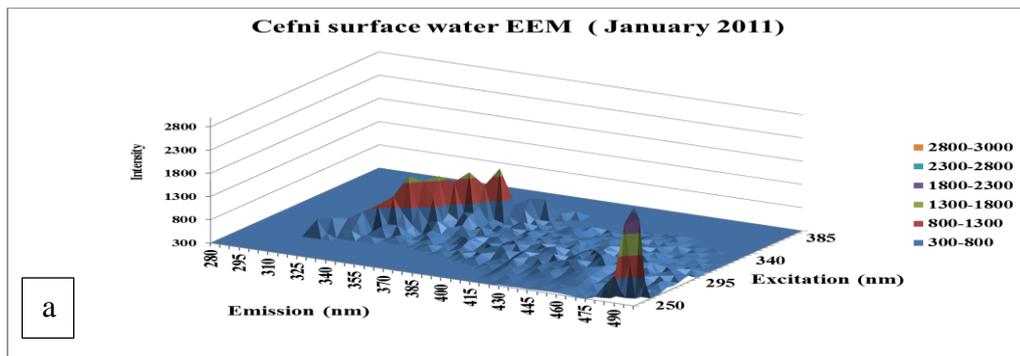
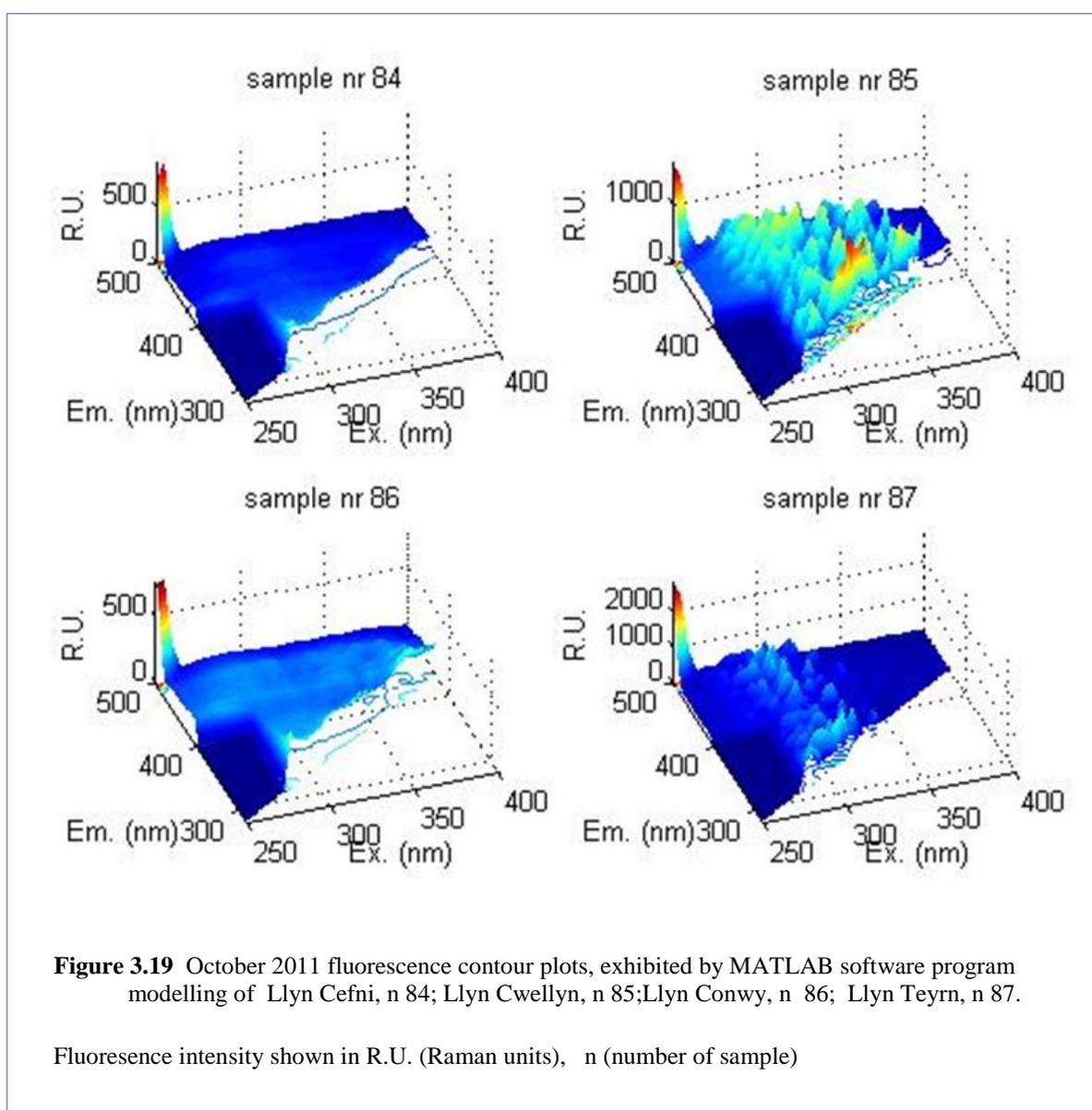


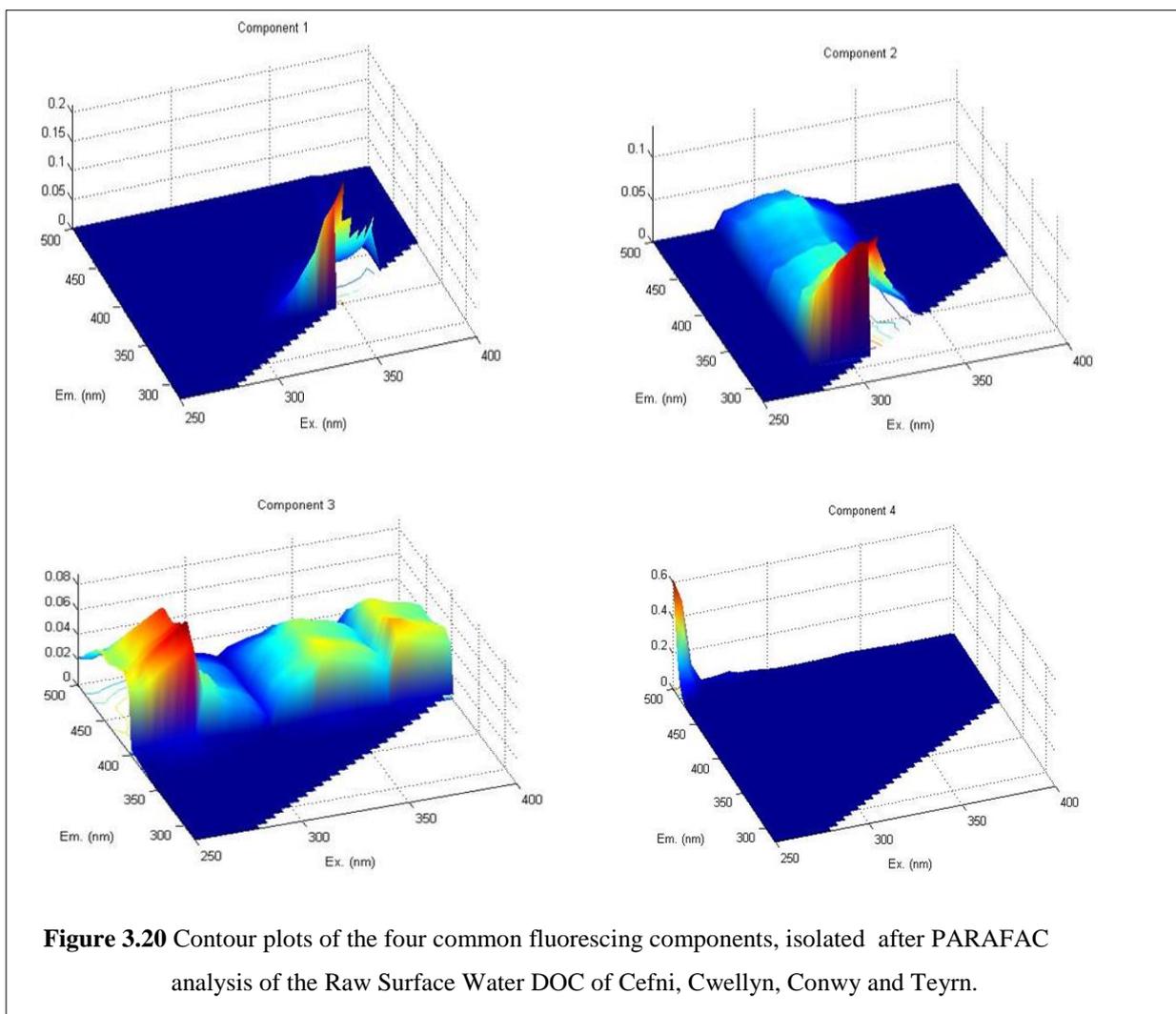
Figure 3.18 Fluorescence excitation and emission (EEM spectra) for January 2011 of Raw Surface Water from **a)** Llyn Cefni, **b)** Llyn Cwellyn, **c)** Llyn Conwy and **d)** Llyn Teyrn DOC . Excitation is from 250 – 390 nm and emission is from 280 – 490 nm.

The data were loaded into the toolbox and processed (Fig 3.19), initially to remove any wavelengths influenced by scatter (i.e., where emission wavelengths were less than excitation wavelengths) (Stedmon and Bro 2008). This was executed by the program and these data were replaced with zeros. Following this, the model was tested using the program until the best the fit of the sample data was established (Stedmon and Bro, 2008). This was executed by comparing the generated excitation and emission data from each sample as plotted curves. Any outlying data, for example specific data demonstrating negative fluorescence were removed. The program was iterated repeatedly with the data being re-evaluated for evidence of residual instrument noise and model fit.



Once the model fit was satisfactory, the principal fluorescing components (Fig 3.20) extracted from the data of the Raw Surface Waters were then isolated using the software

and the results of their excitation and emissions intensities were exported to a spreadsheet and plotted (Fig 3.22 a, b).



The emission components in the graph (Fig 3.20) represented the most common fluorescing species in the 88 sample set. Hua *et al.* (2010) suggested that direct comparisons with those reported in the literature is difficult since the positions of the fluorescence peaks are dependent on the type of DOC and the environment from which it was sampled. Hence the maximum excitation/emission intensities (Table 3.16) were tentatively identified using references from numerous literature sources.

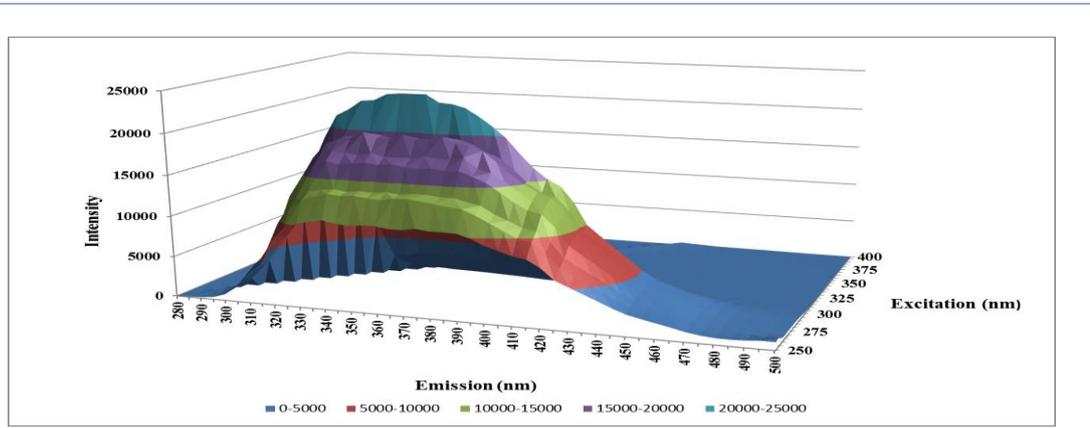


Figure 3.21 Fluorescence (excitation/emission spectrum) of tryptophan (1.0 ± 0.2 mg/L) in Milli Q water

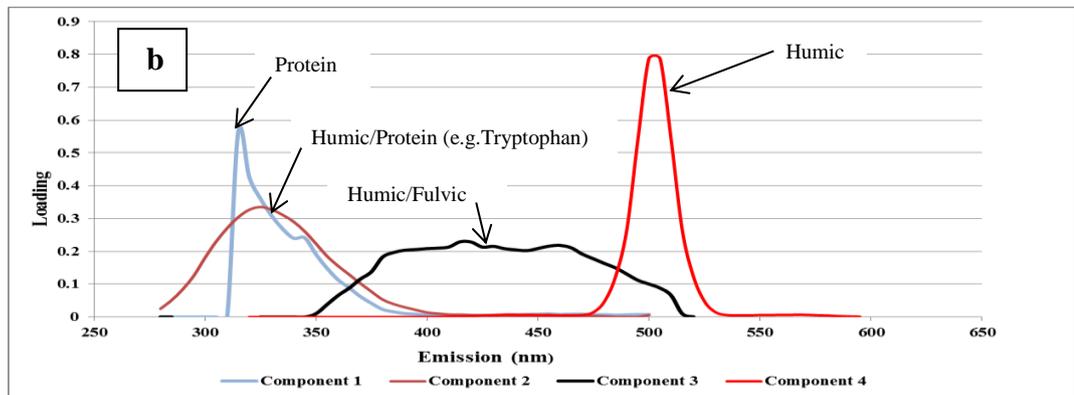
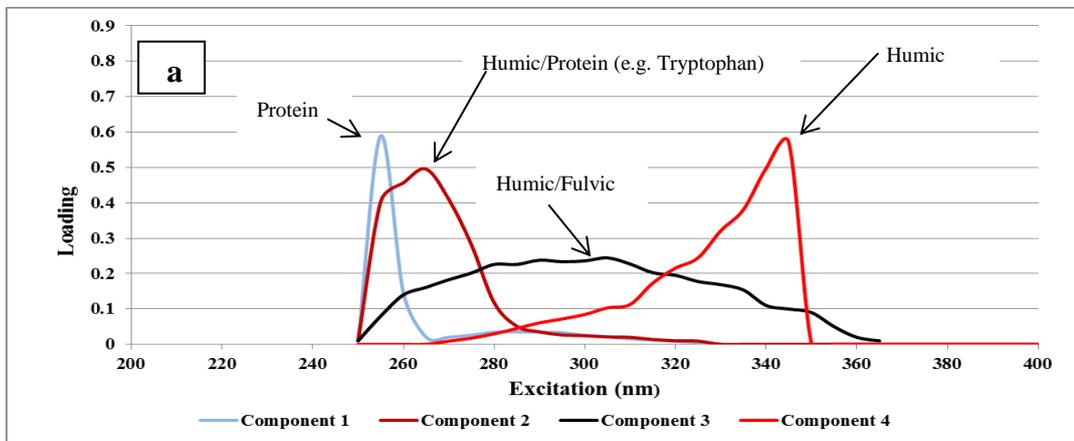


Figure 3.22 a, b, EEMs excitation and emission fluorescence components 1 - 4 of Raw Surface Waters DOC after PARAFAC modelling, of Cefni, Cwellyn, Conwy and Teyrn.

Absolute identifications could not be assigned as the reported excitation and emission peak descriptors and relative positions were qualified with the caveat that the results were ‘similar to’ or ‘like’ for example, humic like or similar to a protein.

Table 3.16 Positions of fluorescence maxima for excitation and emission spectra from results [Bracketed capitals refer to literature references]			
Component	Excitation (nm)	Emission (nm)	Identification
1	250 - 270	310 - 350	Protein like [A], [C] (Tryptophan)
2	250 - 280	300 - 400	Terrestrial humic [F], [H]. Protein like [D]
3	260 - 360	360 - 500	Humic like [E], [I], Fulvic like [I]
4	320 - 350	490 - 540	Humic like, high MM [C], [H]

([A] Coble, 1996; [B] Marhaba *et al.* 1999; [C] Hua *et al.* 2009; [D] Her *et al.* 2003; [E] Stedmon *et al.* 2003; [F] Ishii *et al.* 2012; [G] Bagoth *et al.* 2011; [H] Leenheer and Croue' 2003; [I] Goslan *et al.* 2004.).

Fig 3.21 shows the EEM of a tryptophan which was analysed as a reference standard to confirm reported literature results (Coble,1996; Goslan *et al.* 2004). The emission peak was centred at 340 - 360 nm with the excitation at 290 nm. These compare favourably with component 1 and 2 in Table 3.16.

3.1.6 Cation and Anion Analysis of Raw Surface Waters.

Different processes are proposed to be associated with changes in the export of DOC out of the catchments. For instance, the recent changes in atmospheric chemistry, mainly with the reduction in SO_4^{2-} concentrations and changes in marine depositions (Monteith *et al.* 2007; Evans *et al.* 2008; Hruska *et al.* 2009) has been identified as a key driver of rising DOC concentrations in N hemisphere freshwaters (Evans *et al.* 2012) To compare the ionic content of the four catchments and to try to identify possible influences they may have had on the DOC concentrations, an analysis for the cations Ca^{2+} , Mg^{2+} , K^+ , Na^+ NH_4^+ and the anions F^- , Br^- , NO_3^- , PO_4^{3-} and SO_4^{2-} was carried from November 2010 to October 2011 using Method 2.1.4.12.

The highest concentration of ions in the Raw Surface Water of all four lakes was demonstrated by the Cefni (Fig 3.23 A, A₁). It may be suggested that the influence of the limestone escarpments adjacent to the fen were responsible for the high concentration of Ca^{2+} ; it averaged 32 ± 2 mg/L over the twelve months. Cwellyn (Fig 3.23 B, B₁), Conwy (Fig 3.24 C, C₁) and Teyrn (Fig 3.24 D, D₁), although very much lower than Cefni, also had Ca^{2+} ions present as one of their dominant ionic species. Cwellyn demonstrated the

highest concentration within the group of three averaging 1.4 ± 0.3 mg/L. Conwy concentrations were the lowest, averaging 0.6 ± 0.1 mg/L with Teyrn not dissimilar at 0.7 ± 0.1 mg/L. The second most dominant ionic species of the Cefni was SO_4^{2-} with an average concentration of 13.7 ± 0.8 mg/L. In Cwellyn, Conwy and Teyrn, SO_4^{2-} was the principal species. The highest Ca^{2+} concentration was recorded for Cwellyn which averaged 2.6 ± 0.2 mg/L for the twelve months. Conwy and Teyrn were lower than Cwellyn but had similar concentration averages of 1.4 ± 0.1 and 1.5 ± 0.1 mg/L, respectively.

Nitrate (NO_3^-) concentrations were highest in the Cefni in the period from November 2010 to April 2011 averaging 6.0 ± 0.4 mg/L and dropped to low levels (average 0.60 ± 0.04 mg/L) from May to October 2011. The same pattern occurred in the Cwellyn samples but at lower concentrations and a smaller differential; November 2010 to April 2011 0.8 ± 0.1 mg/L and May to October 2011, 0.50 ± 0.03 mg/L. Teyrn and Conwy did not have the step changes observed in the other two sources, but Teyrn demonstrated a lowering of nitrates during the months of June to September (0.10 ± 0.01 mg/L). The twelve month average for Teyrn was 0.30 ± 0.02 mg/L and Conwy 0.50 ± 0.03 mg/L.

Magnesium (Mg^{2+}) and potassium (K^+) were present in all the four source water samples. Again in the Cefni samples these had a twelve month higher average concentration (Mg^{2+} 5.3 ± 0.2 mg/l; K^+ , 2.5 ± 0.1 mg/L) than the other three sites. Teyrn and Conwy both demonstrated similar average twelve month concentrations for both ions, with Mg^{2+} at 0.30 ± 0.02 mg/l and K^+ at 0.10 ± 0.01 mg/L. A drop in K^+ concentration during the summer months of June to September was observed for both Teyrn and Conwy to 0.04 ± 0.01 mg/L and 0.02 ± 0.01 mg/L, respectively. The concentration of the Mg^{2+} ion in Cwellyn (1.4 ± 0.2 mg/L) was approximately five times higher than Conwy and Teyrn, but the K^+ was only marginally higher at a twelve monthly average of 0.20 ± 0.02 mg/L.

The halogens (fluoride and bromide) were present in low concentrations in all four sampled sources (Figs 3.23 a₁, b₁; 3.24 c₁, d₁). Bromide ions were below the limit of detection ($5 \mu\text{g/L}$) for most months in the Conwy (Fig 3.25) and Teyrn (Fig 3.25) samples. In the Cefni (Fig 3.25) with the closest association to a maritime climate and limestone bedrock, concentration levels averaged $70 \pm 5 \mu\text{g/L}$ for the 12 months. Although maritime aerosols contributed (seawater Br^- concentrations $65 - 71$ mg/kg), limestone (Br^- 6 mg/kg) compared to other rock formations (Flury and Papritz, 1993), may be a major contributor in the Cefni. Cwellyn (Fig 3.25) was higher than the limit of detection for 5 months out of the twelve during early spring and mid-summer, with an average concentration of 14 ± 2

$\mu\text{g/L}$. Teyrn and Conwy Br^- levels were only above the limit of detection for one analysis for Teyrn ($12 \pm 2 \mu\text{g/L}$) during February 2011 and twice for Conwy during August and September 2011 (average $70 \pm 5 \mu\text{g/L}$)

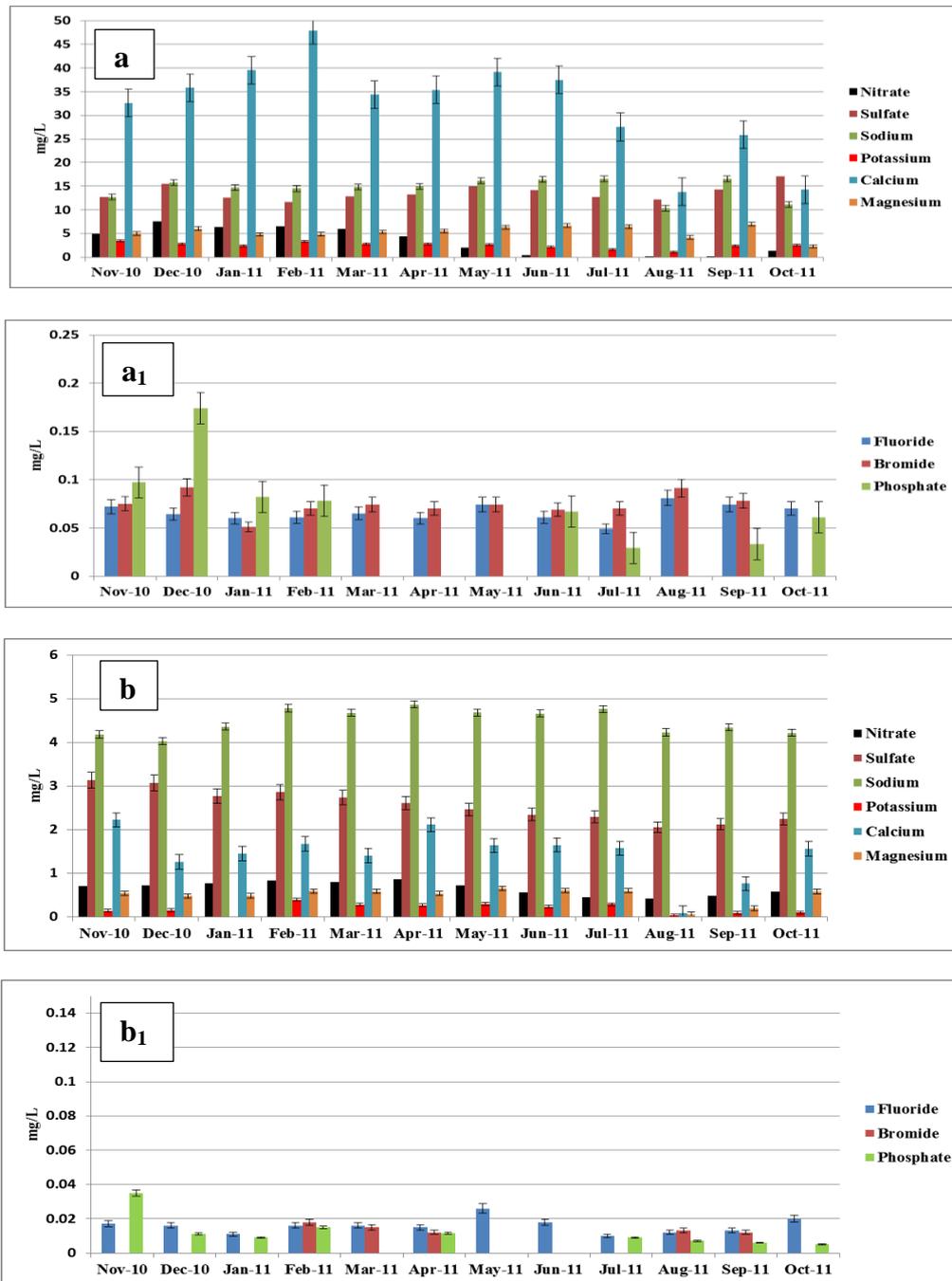


Figure 3.23 a) Cefni ionic concentrations for November 2010 to October 2011. **a₁**) Cefni, F^- Br^- PO_4^{3-} concentrations November 2010 to October 2011. **b**) Cwellyn ionic concentrations for November 2010 to October 2011, **b₁**) Cwellyn, F^- Br^- PO_4^{3-} concentrations for November 2010 to October 2011.

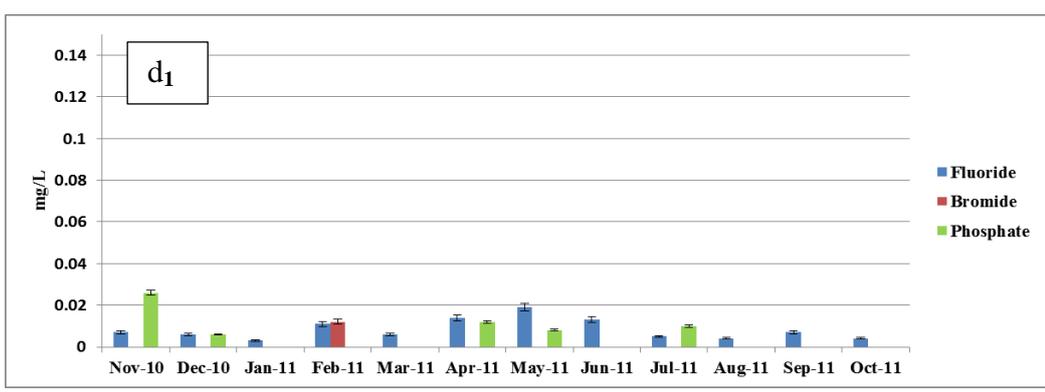
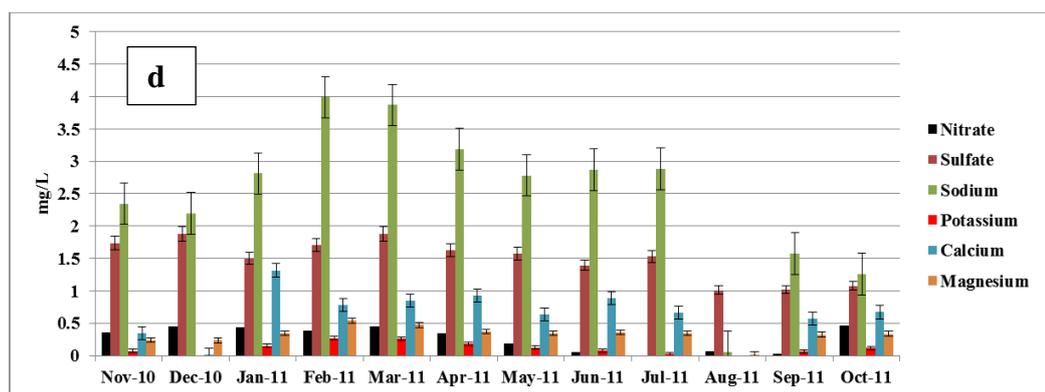
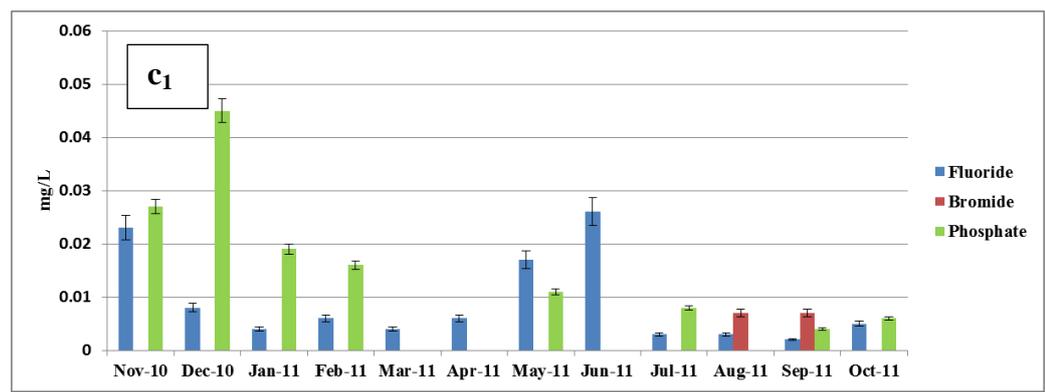
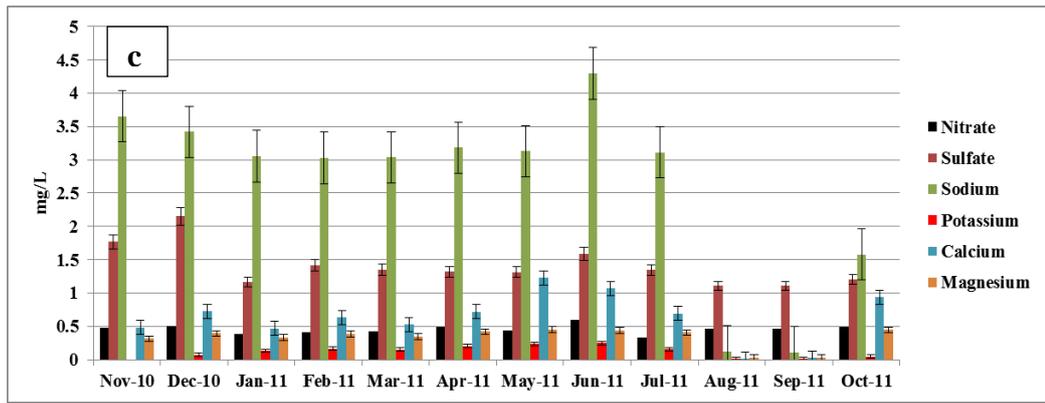
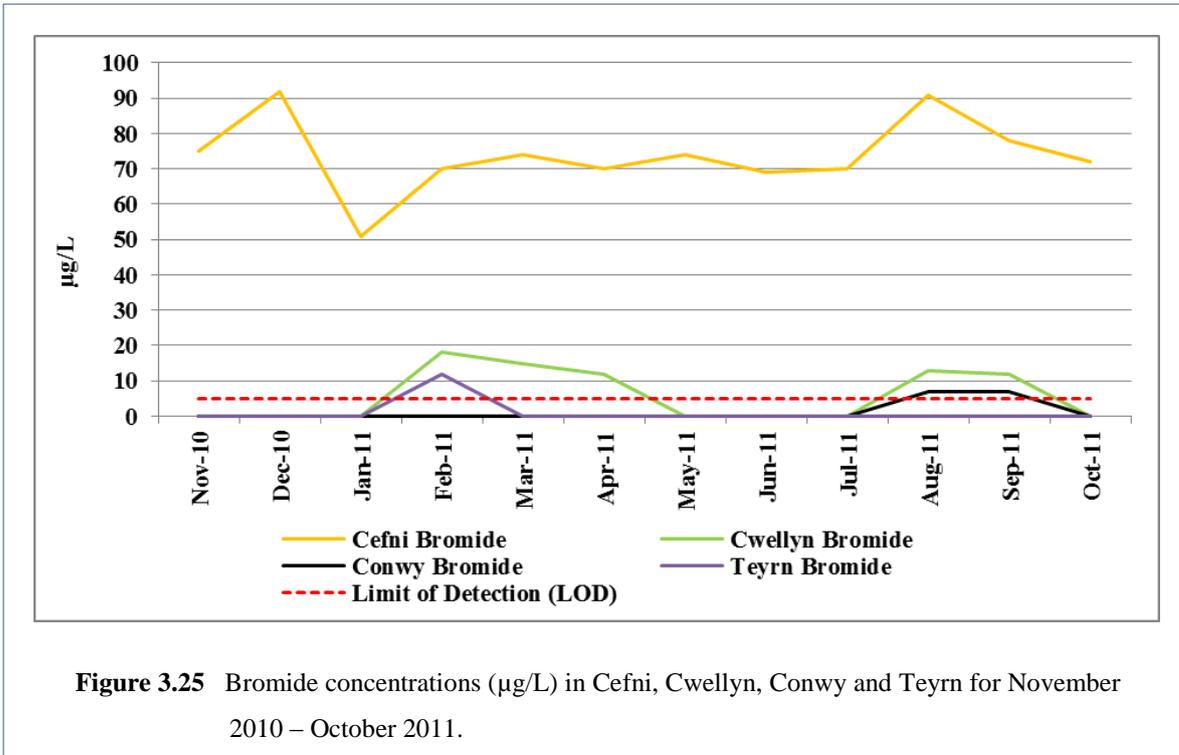


Figure 3.24 c) Conwy ionic concentrations for November 2010 to October 2011. c₁) Conwy, F⁻ Br⁻ PO³⁻ concentrations November 2010 to October 2011. d) Teyrn ionic concentrations for November 2010 to October 2011, d₁) Teyrn, F⁻ Br⁻ PO³⁻ concentrations for November 2010 to October 2011

Although analysis for chloride ions (Cl⁻) was carried out on a monthly basis during the 12 months these have not been included as part of the characterization suite as the use of HCl for preservation purposes obscured accuracy.



The sodium (Na⁺) concentration, which is predominantly impacted upon the catchments relative location to a marine environment, was high in the Cefni and samples varied from a low of 11.1 ± 0.8 mg/L to a high of 16.6 ± 1.2 mg/L and averaged 14.5 ± 1.0 mg/L over the 12 months. The high levels were recorded during the months of May to September with lows predominantly in the late winter to early spring. From the graph (Fig 3.23 a), the Na⁺ concentration of the Cefni was less than the Ca²⁺, but the other sample sources, demonstrated the opposite trend with Na⁺ being the ion with the highest concentration. Although much lower than the Cefni, Cwellyn averaged 4.6 ± 0.3 mg/L from November 2010 to 0.24 mg/L in July 2011 after which an increase to 2.4 ± 0.2 mg/L in October was recorded. Conwy also demonstrated a similar pattern, with a high average concentration of 3.3 ± 0.2 mg/L followed by a drop in August 2011 to 0.12 ± 0.01 mg/L then a concentration increase to 1.6 ± 0.1 mg/L in October. Teyrn displayed a slightly different pattern of concentrations (Fig 3.24 D). Between November 2010 and January 2011, the Na⁺ ion concentration averaged 2.4 ± 0.2 mg/L. This then increased to a 3 month average of 3.5 ± 0.2 mg/L before reducing to 2.8 ± 0.2 mg/L for a further 3 months to July 2011.

The August concentration decreased to a low of 0.057 mg/L after which an increase to 1.5 ± 0.1 mg/L was recorded for September and October 2011.

Increased SO_4^{2-} concentrations have been associated with the suppression of DOC release (Clark *et al.* 2005) in soils so, conversely, a decrease in SO_4^{2-} would hence promote an increase in DOC release. These ion concentration changes would also have an impact on the ionic strength of the Raw Surface Water solutions, which have been reported to have an influence on the release of DOC, for instance, a decline in ionic strength was accompanied by a three-fold increase in DOC (Hruska *et al.* 2009).

Our exploration of the relationship between the ionic concentration and DOC concentration, although for only a short period, compared to the 14 years of monitoring by Hruska *et al.* Statistical analysis (Pearson correlation and linear regression) of each individual lake's SO_4^{2-} versus DOC concentration demonstrated no significant correlations. Total ionic concentrations versus DOC of each lake did not demonstrate any significance either.

3.1.7 pH and Conductivity of Raw Surface Water

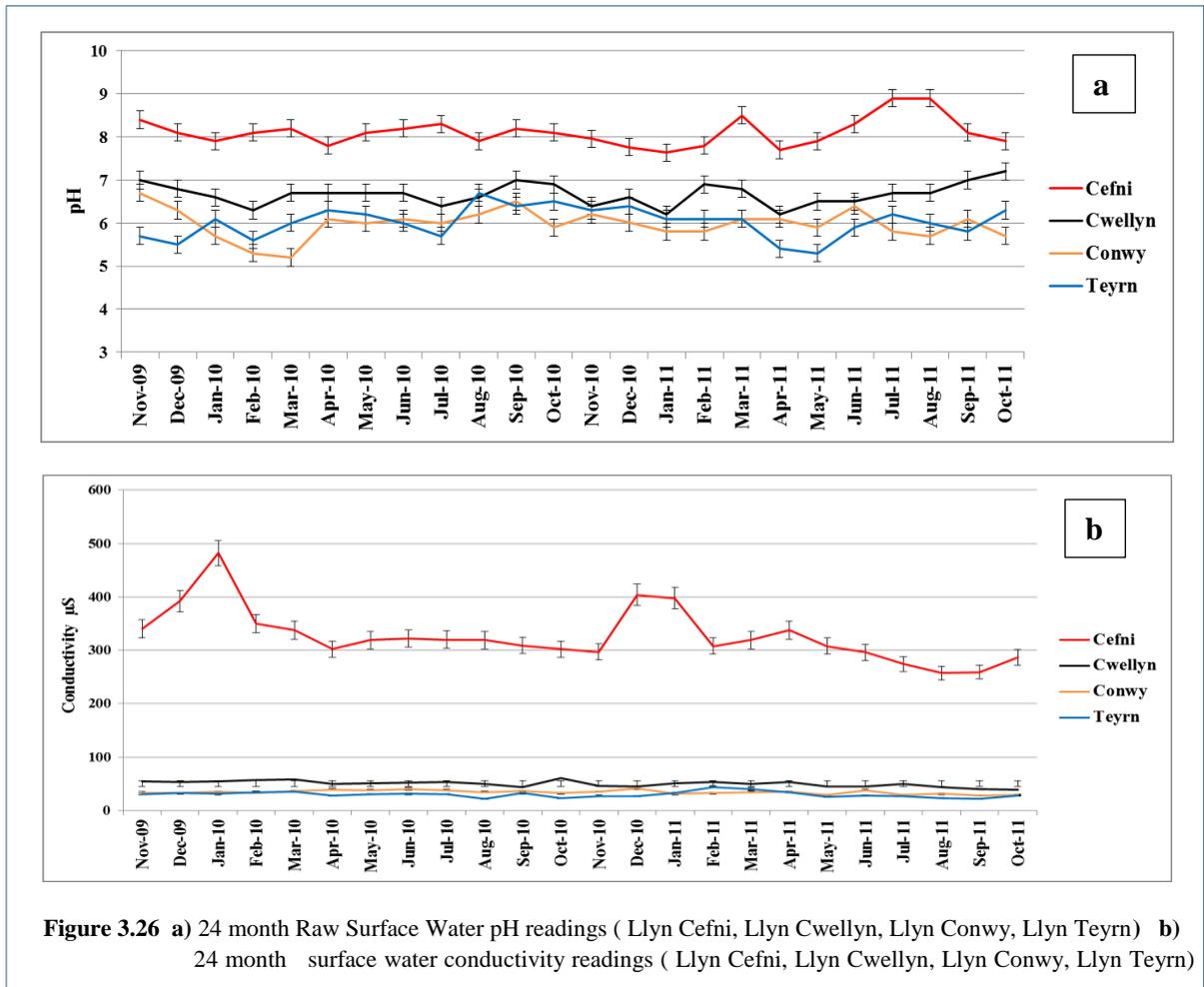
The pH readings (Fig 3.26 a) of the surface waters were measured in the laboratory. Three of the Raw Surface Waters, (namely Cwellyn, Conwy and Teyrn) were slightly acidic with respective 24 month averages of 6.7, 6.0 and 6.0 pH units. Cefni described as a calcareous fen catchment recorded an average pH of 8.1 (SD 0.3). The high concentrations of Ca^{2+} (32 ± 2 mg/L, 3.1.6) suggest that erosion by natural processes (Eq 3.4) of the limestone escarpments and bedrock to the east of the fen catchment were influencing the pH (Eq 3.4 and 3.5) (Wissmar *et al.* 1997).



The pH values over the period of sampling demonstrated fluctuations in all four lake Raw Surface Waters of ± 0.3 pH units. Cefni's alkaline water tended to average pH 8.1 ± 0.2 for the 24 months, with the lowest pH recorded at 7.6 ± 0.2 . The highest recorded pH (8.9 ± 0.2) for Cefni was during July and August 2011. At this time excavation work was being

conducted in the Erddreiniog fen, near the base of the eastern limestone outcrops. Here large tracts of peat 4.5 hectares were removed and the limestone marl was exposed to the atmosphere and direct rainfall runoff (Leonard *et al.* 2013).

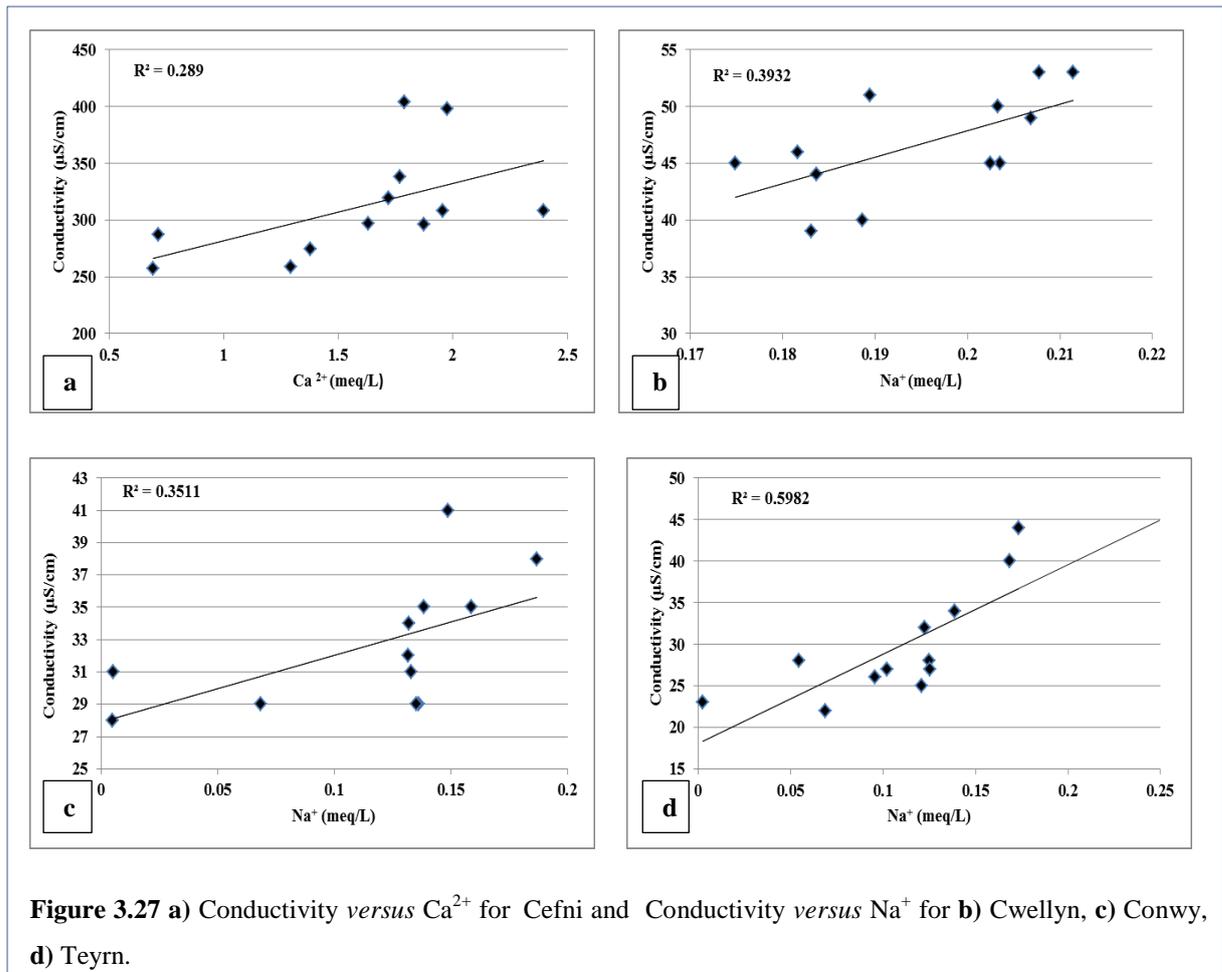
Cwellyn demonstrated the second highest average pH over the 24 months (6.7, SD 0.3). Insignificant decreases were observed during the summer period but in late summer 2011 an increasing trend was recorded from pH 6.2 ± 0.2 in August to 7.2 ± 0.2 in October.



Conwy and Teyrn demonstrated month to month fluctuations with a small trend upwards during the autumn and winter months of September to March 2010/11, Teyrn (6.3, SD 0.2; March 2011) and Conwy (6.0, SD 0.3; March 2011). The March to September for both years were insignificantly lower, at an average pH (6.1, SD 0.2) and (5.9, SD 0.3) respectively for Teyrn and Conwy.

The conductivity (Fig 3.26 b) of each Raw Surface Water sample was measured after filtering through a 0.2 µM membrane filter. The Cefni demonstrated the highest

conductivity of the samples, averaging for the 24 month period $326.7 \pm 10 \mu\text{S}/\text{cm}$, which was expected with the sample waters having higher ionic concentrations (Section 3.1.7, Fig 3.26 b). Plotting the conductivity *versus* the cationic charge (meq/L) demonstrated that in the Cefni Raw Surface Water the major cation was the Ca^{2+} . Although Mg^{2+} was relatively high in the three upland lakes, it was the Na^+ (Fig 3.27 b,c and d) that demonstrated a more linear correlation with conductivity.



The conductivity for the period November 2009 to February 2010 for Cefni (3.26 b) was high with a $(482 \pm 10 \mu\text{S}/\text{cm})$ spike in January 2010. A similar perturbation was repeated in the November 2010 – February 2011 with two spikes; the first peaked at $404 \pm 10 \mu\text{S}/\text{cm}$ in December and the second at $398 \pm 10 \mu\text{S}/\text{cm}$ in January 2011. These tend to suggest that there was an increase in one of the ionic species during these periods, but no evidence was found in the group of cations and anions analysed for. The readings for the periods between the isolated increases were relatively stable at an average of $314 \pm 10 \mu\text{S}/\text{cm}$, with the exception of a decline in conductivity from $308 \pm 10 \mu\text{S}/\text{cm}$ in May 2011

to $259 \pm 10 \mu\text{S/cm}$ in October 2011. Again this was accompanied by a drop in Ca^{2+} concentrations (Fig 3.23, A). The step change increases recorded in the Cefni were not repeated by the three other surface waters samples; Cwellyn, Conwy and Teyrn. These sites displayed much lower overall conductivity readings for the 24 month period. Of the group of three Cwellyn was the highest, averaging $49.8 \pm 10 \mu\text{S}$ with Conwy second at $34.1 \pm 10 \mu\text{S/cm}$ and Teyrn the lowest at $29.5 \pm 10 \mu\text{S/cm}$. These variations were reflected also by the Na^+ ion concentrations. The conductivity (3.26 b) levels for all of these three were relatively stable with Cwellyn varying between 57 and 45 $\mu\text{S/cm}$, Conwy between 38 and 30 $\mu\text{S/cm}$ and Teyrn 25 and $32 \pm 10 \mu\text{S/cm}$.

3.1.8 Infrared Spectroscopy of Freeze Dried Raw Surface Water DOC Samples.

The Cefni dried DOC residual consisted of large, pale mottled brown flakes, whilst Conwy's was darker brown with more uniform fine particles (Figs 3.31 a,b). The infrared spectra (Fig 3.28A; 3.28B) were collected as per method 2.3.10 and demonstrated similar characteristic bands to the Nordic Humic and Fulvic reference samples (Figs 3.29, 3.30).

Broad O-H stretches were prominent in both Cefni and Conwy surface water DOC with Cefni being slightly broader than the much sharper Conwy. This suggested that the Cefni DOC had intermolecular hydrogen bonding while the Conwy had more intramolecular bonding. There was also evidence of 'free hydroxyl' groups in the Conwy at 3546 cm^{-1} and an amine stretch at 3246 cm^{-1} . The asymmetrical and symmetrical stretching of aliphatic (C-H) are recorded in both DOC spectra at 2926 and 2860 cm^{-1} respectively with a scissoring band at 1451 cm^{-1} but this may have been due to cyclic hydrocarbons, for example cyclohexane absorbs at 1452 cm^{-1} (Silverstein *et al.*, 1991)

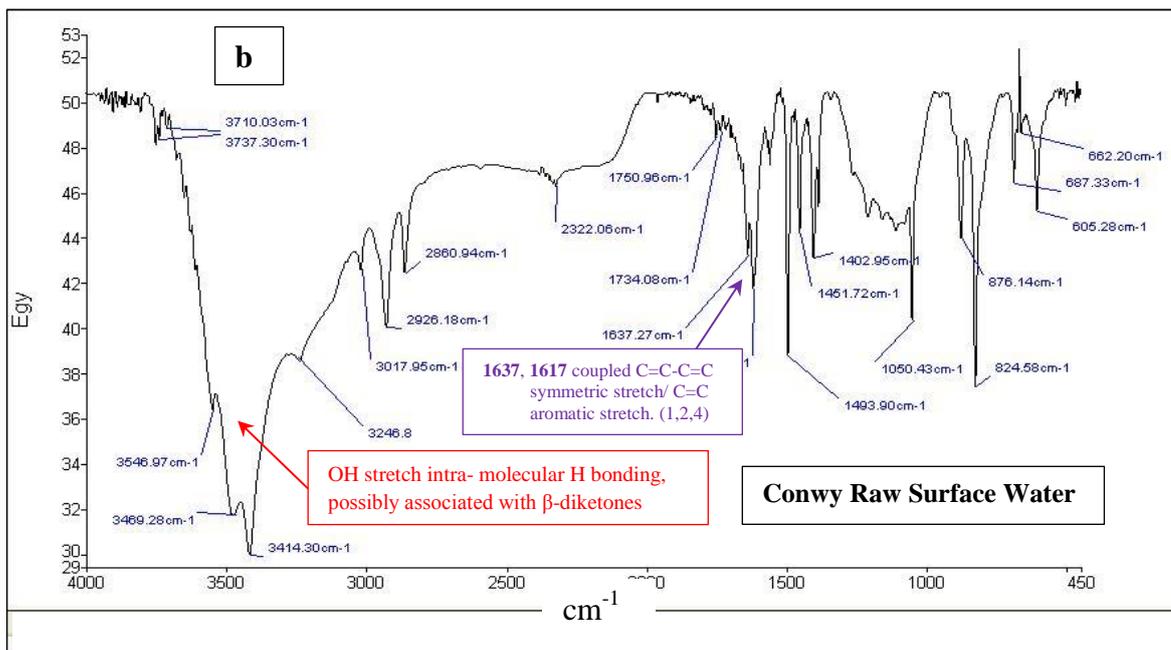
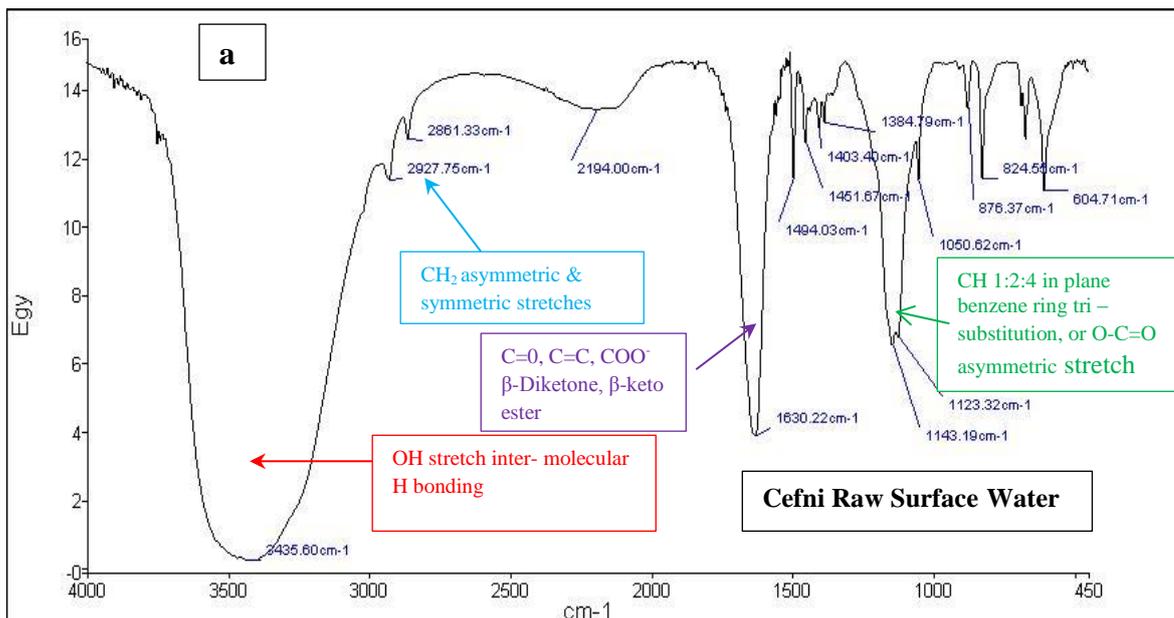


Figure 3.28 Infrared spectra (KBr disc) of **a)** of Llyn Cefni (October 2011) freeze dried Raw Surface Water DOC, **b)** Llyn Conwy (October 2011) freeze dried Raw Surface Water DOC

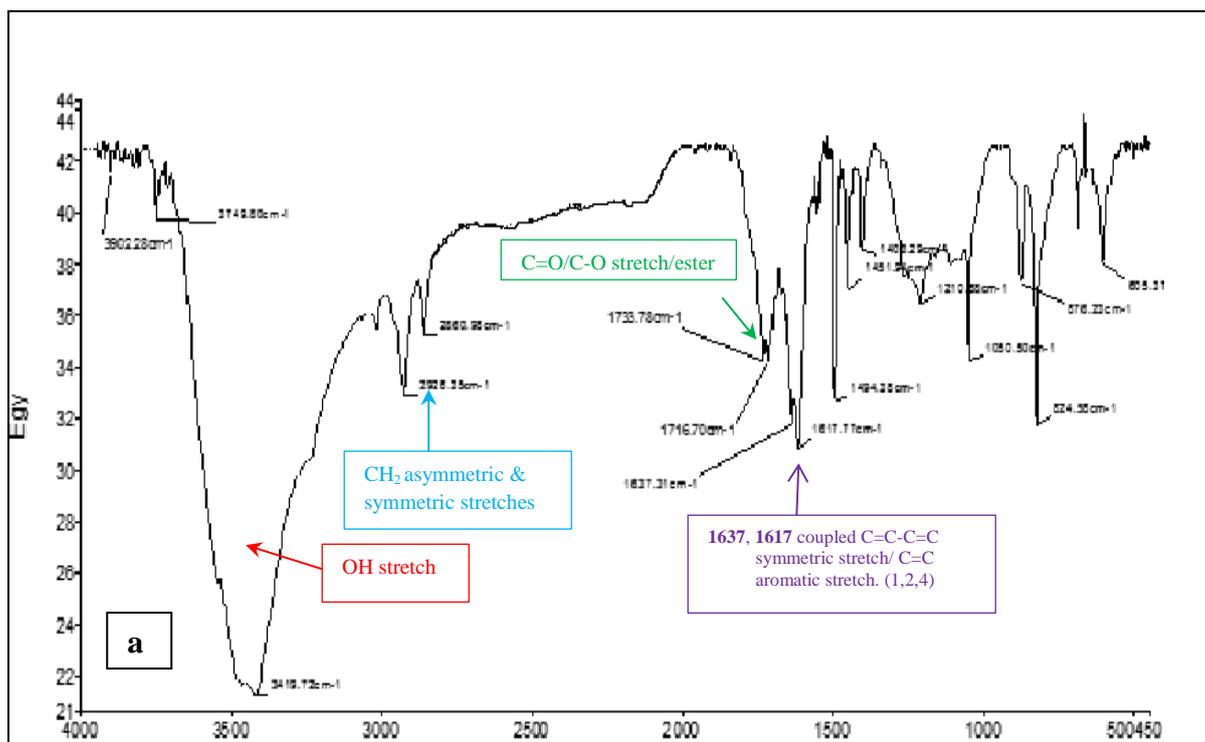


Figure 3.29 a) Infra-red spectra (KBr) of Nordic fulvic acid (1R 105 F reference standard).

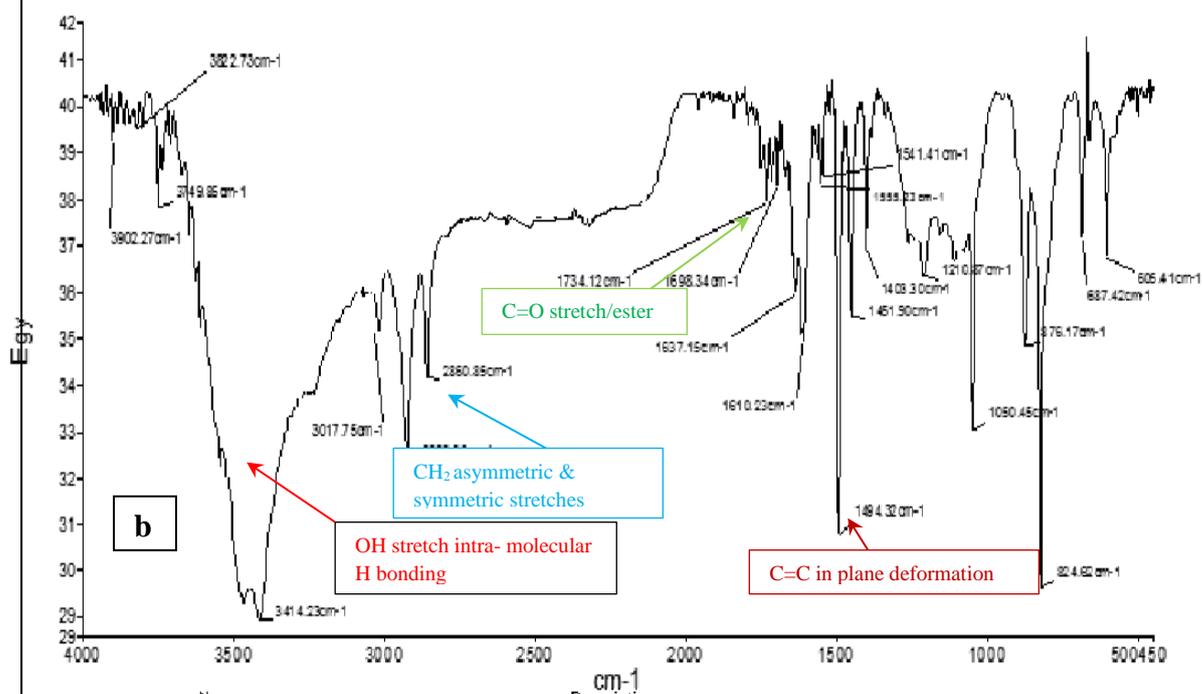


Figure 3.30 b) Infra-red spectra (KBr) of Nordic humic acid (1R 105 H reference standard)

Table 3.17 FTIR bands of Cefni and Conwy freeze dried surface water DOC		
Bands Assignments	Cefni Raw Surface Water DOC (cm⁻¹)	Conwy Raw Surface Water DOC (cm⁻¹)
OH stretching (phenol, alcohol, carboxyl, amine, Si) [s]	<p>3435 OH broad, intermolecular H-bonded. [m broad] (2)</p> <p>604 out of plane OH bend [m] (2)</p> <p>2900 -2000 b NH₃⁺ stretch [b](2)</p> <p>1630 C=C ring stretch, C=O associated with conjugated system, amide. [s] (1,2,4)</p> <p>1630 β Diketones/ester, in enolic form intramolecular H bonded [s] (2) (8)</p> <p>1630 asymmetrical stretch COO⁻ associated with inorganic ions) [ms]</p>	<p>3546 free OH [sharp] (2,5)</p> <p>3469 - 3414.3 OH polymeric association [v] (5)</p> <p>3414 band suggests intramolecular H-bonding extended band to 2000 (overtone combinations) [s] (2) and β diketones (5)</p> <p>3000 – 2000 broad NH₃⁺ stretch [w](2)</p> <p>3246 suggests N-H stretch [w] (2).</p> <p>2322 phthalic acid like (3)</p> <p>604 out of plane OH bend [m](2)</p>
CH ₂ stretching methylene groups	<p>2927 symmetric CH₂, [v] (2,4)</p> <p>2861 CH₂ asymmetric. [v]</p> <p>1451 CH₂ out of plane deformation [s]</p> <p>1143 suggests CH 1:2:4 in plane benzene ring tri – substitution or ester asymmetric stretch [s broad](2)</p>	<p>2926 symmetric CH₂, [v] (2,4)</p> <p>2860 CH₂ asymmetric [v]</p> <p>1451 CH₂ out of plane Deformation [s]</p>
Conjugated C=C, aromatic C=C, carboxylate ion (COO ⁻), N-H stretch?	<p>1494 C=C in plane deformation [s] (2,5)</p> <p>1403 symmetrical stretch COO⁻ [w]</p> <p>1384 carboxylate ion binding to silicate [w] (7)</p> <p>1050 CO stretching alcohols Ethers [s] (6)</p>	<p>1750,1734 C=O/ C-O ester stretch [v]</p> <p>1637, 1617 coupled C=C-C=C symmetric stretch/ C=C aromatic stretch. [v w] (1,2,4)</p> <p>1637 β-Diketone or β-keto ester in enolic form, H bonded [s] (2) (8)</p> <p>1637 asymmetrical stretch COO⁻ [s] associated with inorganic ion (3)</p> <p>1558 C=C-C=C asymmetric stretch C=C aromatic asymmetric stretch. [w] (2)</p> <p>1493 C=C in plane deformation [s] (2,5)</p> <p>1403 symmetrical stretch COO⁻ [w]</p> <p>1384 carboxylate ion binding to silicate [w] (7)</p> <p>1050 CO stretching alcohols ethers [s].</p>

[S = strong m = medium w = weak v = variable]

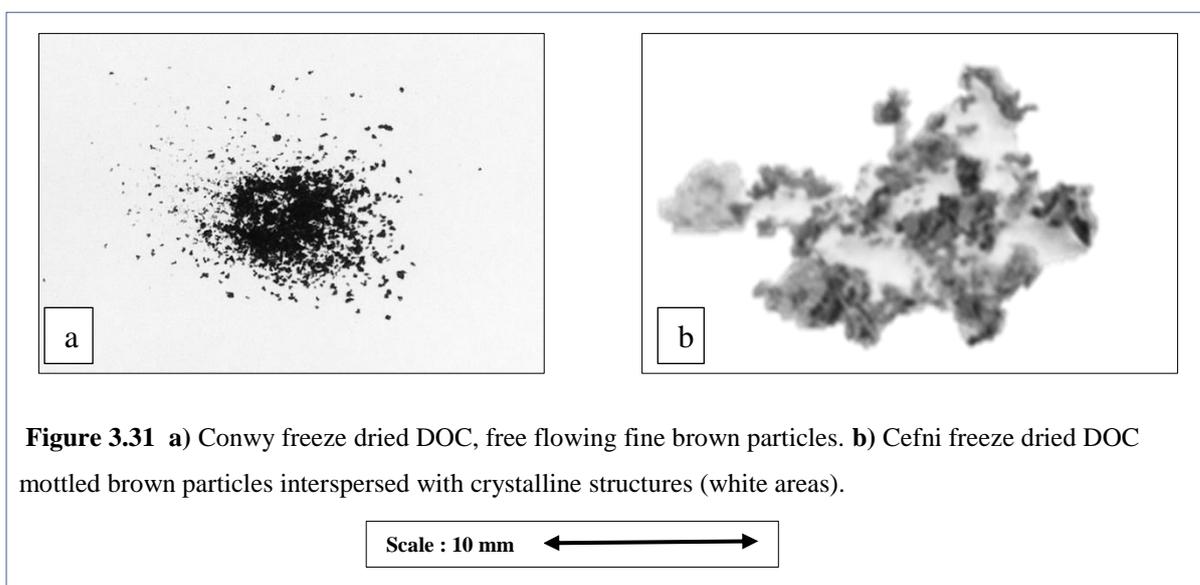
- | | | |
|-------------------------------------|------------------------------------|--------------------------------|
| 1) Stevenson and Goh (1974) | 4) Chen <i>et al.</i> (2002) | 7) Marley <i>et al.</i> (1992) |
| 2) Silverstein <i>et al.</i> (1991) | 5) Cross and Jones. (1969) | 8) Hesse <i>et al.</i> (1997) |
| 3) Elkins <i>et al.</i> (2001) | 6) Peuravuori <i>et al.</i> (2005) | |

A shoulder indicating aromatic C-H stretches was recorded at 3019 cm^{-1} (Marley *et al.* 1992) for both spectra. A very broad band was present in both the Cefni and Conwy ($3000 - 2000\text{ cm}^{-1}$) which is characteristic of N-H multiple combination and overtone bands of $[\text{R-NH}_3^+]$ possibly representative of primary amino acids. Evidence of a carbonyl group stretch was absent in both spectra at the usual frequency, i.e., textbook frequency of *ca.* $1750 - 1715\text{ cm}^{-1}$, (Silverstein *et al.*, 1991). The strong relatively broad stretching vibration recorded for the Cefni DOC at 1630 cm^{-1} may be attributed to four vibrations, a) the aromatic C=C stretch conjugated with an alkene. b) a carbonyl stretch with an associated conjugated system, hence lowering the frequency, β -diketone c) the association of a cation such as Na^+ or Ca^{2+} with a carboxylate anion (asymmetric stretch) thus forming a salt. d) an NH_3^+ or amide stretch, possibly hydrogen bonding (primary or secondary). Although the four may be considered to contribute, the position and strength of the absorbance tends to favour the COO^- as the principal contributing structure, i.e., strong asymmetrical C=O stretch at 1630 cm^{-1} coupled with a weaker symmetrical stretch 1494 cm^{-1} (Elkins and Nelson, 2001). The absorbance in this region for the Conwy appears as a doublet $1637, 1617\text{ cm}^{-1}$ which may be due to Fermi resonance (Silverstein *et al.* 1991), but again suggests similar band contributions as those for Cefni but with less intensity.

The Nordic fulvic and humic acid spectra and the Conwy and Cefni DOC spectra demonstrated a number of similar characteristics. The OH and CH_2 and aromatic CH stretches at 3700 to 2000 cm^{-1} were present in all four spectra (Figs 3.28a,b; 3.29; 3.30). Differences were recorded between the Cefni and both of the Nordic spectra in the 2000 to 1400 cm^{-1} region. The Nordic fulvic exhibited a C=O stretch doublet at $1733\text{-}1716\text{ cm}^{-1}$ as a result of a coupled asymmetric/symmetric stretching (Silverstein *et al.*, 1991; Leenheer *et al.* 1987). They were absent in both the Cefni and Conwy's October 2011 DOC, but the doublet previously mentioned at $1637\text{-}1617\text{ cm}^{-1}$ in the Conwy spectrum was also present in both Nordic sample spectra (Leenheer *et al.* 1987). The intense sharp band at 1493 cm^{-1} which may be assigned to the C=C aromatic ring stretch or a C-O symmetric stretch of a carboxylate, was present in the Conwy and Cefni together with the two Nordic samples.

Section 3.1.6 (cation and anions) indicated relatively high concentrations of anions that may have presented IR absorption frequencies. The principal ones recorded by IC were the NO_3^- , SO_4^{2-} . Although, CO_3^{2-} was present in the Raw Surface Water samples, it was

removed by acidification with HCl (3 M) and sparge (N_2). Examination of the spectra in the absorption band regions for the remaining ions (SO_4^{2-} , $1130 - 1080\text{ cm}^{-1}$) and (NO_3^- , $1410 - 1340\text{ cm}^{-1}$) (Cross and Jones, 1969) indicated bands that may have been associated with SO_4^{2-} stretches in both the Cefni (1403 cm^{-1}) and Conwy (1402 cm^{-1}) spectra. A second series of bands possibly associated with SO_4^{2-} were recorded between $687 - 604\text{ cm}^{-1}$ in both. Cefni and Conwy spectra also exhibited an absorption frequency at 1050 cm^{-1} which correlated with the NO_3^- reported stretches (Silverstein *et al.* 1991), these were also accompanied by bands at *ca.* 824 cm^{-1} which were within the frequencies reported by Cross and Jones, 1969.



3.2 Discussion.

The average DOC concentrations for the upland lakes (Cwellyn, Conwy and Teyrn) were lower than the lowland Cefni reservoir. This difference was ascribed to the nutritionally rich fen/agricultural catchment of the Cefni having a high carbon loading as a result of a large turnover of plant growth and decay compared to the nutritionally poorer upland catchments with reduced vegetation (Wetzel, 1992).

Three of the four sources, Cefni, Cwellyn and Teyrn were seen to be affected by the seasonal changes in rainfall, temperature and related microbial activity. Although the coldest periods (December 2009 through to February 2010) demonstrated DOC concentration declined in all of the four lakes, this was followed, with the exception of the Conwy, by a steady increase peaking in late summer with the early autumn flush of carbon

when higher rainfall occurred and the water table increased. The Conwy reservoir's dissimilar response to rainfall suggested that the dynamics of the surrounding peat blanket bog were different and less responsive to environmental changes. That is, it is likely that the bog's water table did not fall as much as the other sample catchments during the dryer summer period and remained relatively saturated. June to August 2010 saw comparative stability in the DOC concentrations in the upland lakes. This suggested that the dry period, from March to July 2010 when the monthly rainfall averaged 84.6mm (normal average 288mm), may have introduced a period of stability where the export of DOC out of the catchments was not subjected to irregular flushing. This low rainfall period was followed by a 330.8mm precipitation during July/August 2010 which was associated with an upward trend in the upland and lowland lake DOC from September 2010 (Figs 3.1 – 3.4).

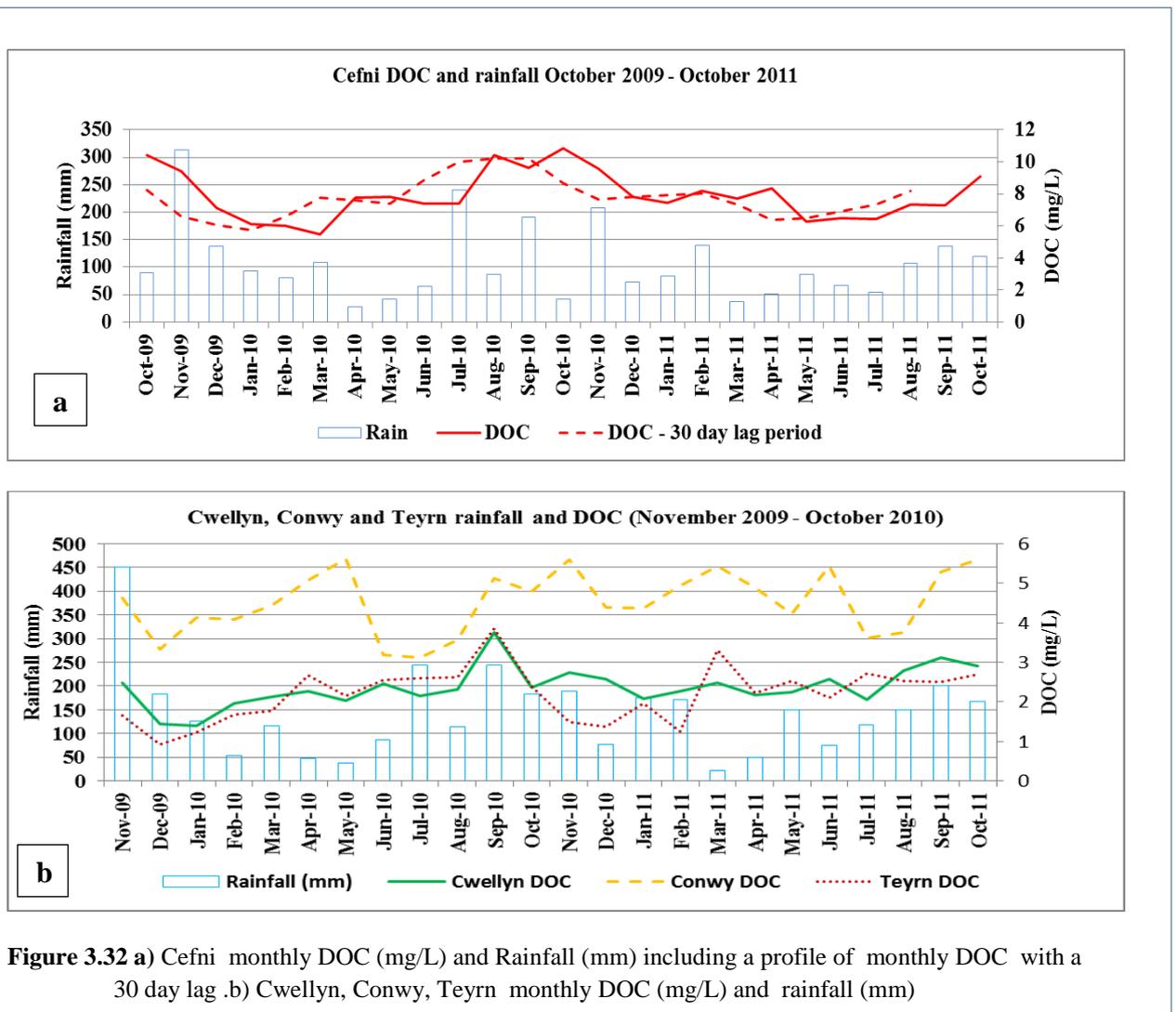
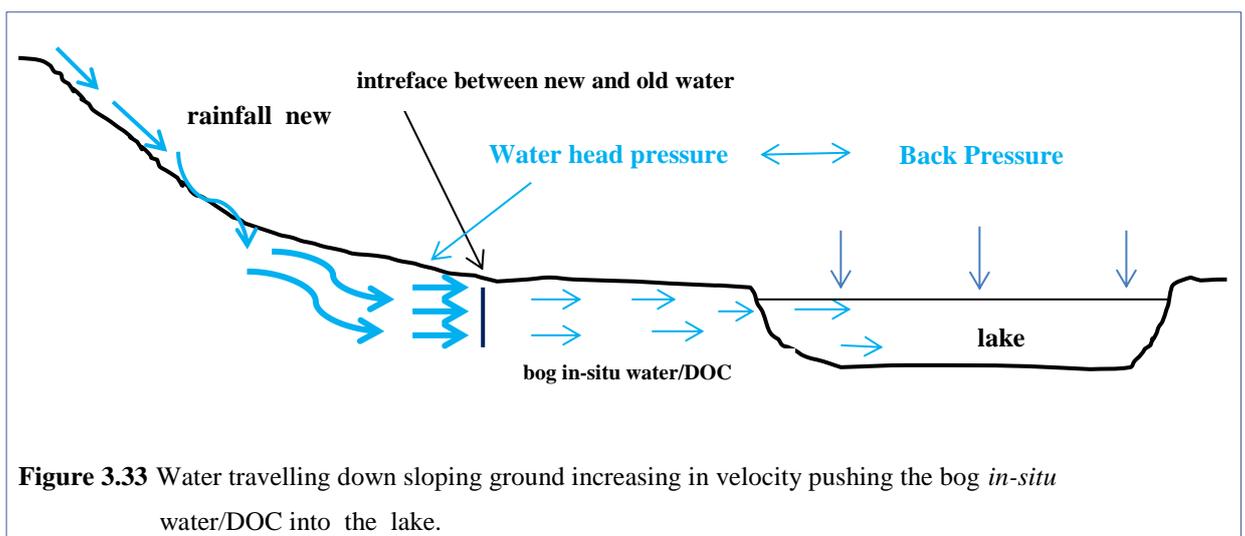


Figure 3.32 a) Cefni monthly DOC (mg/L) and Rainfall (mm) including a profile of monthly DOC with a 30 day lag .b) Cwellyn, Conwy, Teyrn monthly DOC (mg/L) and rainfall (mm)

Fig 3.32a shows the DOC concentration trend of the Cefni reservoir changing with rainfall. Application of statistical analysis (Pearson correlation) proved the link was not significant (r 0.343, $p > 0.05$). A further exercise carried out with the DOC concentration calculated using a 30 and 60 day moving average relative to the monthly rainfall also failed to generate a significantly improved Pearson correlation. Application of the immediate, 30 and 60 DOC moving averages to rainfall for the three upland lakes did not yield any significant correlations for Cwellyn and Teyrn either, but Conwy did demonstrate a significant correlation (r 0.553, $p < 0.05$) between the DOC and rainfall without any lag period. This positive result can possibly be ascribed to the Conwy lake's hydraulics. As mentioned above when the dry period was being discussed, the Conwy exhibited a steady state which could be best described as an equilibrium between catchment and lake. When the rainfall returned; with it being surrounded by mostly saturated peat soils on gentle slopes, the rainfall (new water) flowed rapidly *via* the upper dry soil/peat more open and textured layers (Fig 3.33).



The surge of new water would have gained momentum and started to collect on the periphery of the denser peat of the bog. This would temporarily have increased the water table and form a header volume and a pressure pulse, hence the new water would have pushed the residual older water within the peat into the lake to bring the system into equilibrium. This effect was previously described by Zaslavsky and Sinai (1981) as the hydraulic conductivity processes of a sloping catchment being analogous to a 'thatched roof' and the push of new water as the 'piston effect' (Hewlett and Hibbert, 1967). This hydraulic effect would have been absent in the three other lakes as the topography was

different. Teyrn with steep slopes and a very short peat/soil apron surrounding it would have been limited to a form of thatched roof/through-flow effect with the physical gradient of the catchment reducing the sub-surface contact time (Dawson *et al.* 2011). This is described as ‘overland flow’ and the water is believed to travel as quasi laminar sheets or minor rivulets into the lake (Ward and Robinson, 1990). This form of water transfer would have been evident in the Cwellyn after a dry period and the absence of correlations for Cwellyn may have been due to a number of factors, for example the reservoirs volume (18 Mm³) and depth (25m) would have influenced residence times and dilutions. Hence a response to rapid change in DOC concentrations being exported out of the catchment would have a limited impact on the *in-situ* lake DOC. Although the hydraulic and topographical properties have been considered as the principal contributors towards poor correlations, they may not have been observed simply due to temporal influences, where the flush of DOC was missed due to the fixed sampling timetable. This is addressed in Chapter 8.

Although it was ambient rather than soil temperatures that were recorded, the temperatures when correlated with the DOC suggested that there was evidence of a relationship. Fenner *et al.* (2005) demonstrated that a temperature-related mechanism described as microbial/enzyme activity was conducive to generating labile DOC. For example, an increase in temperature by 4° C during spring generated a > 100% increase in DOC. Worrall *et al.* (2003) suggested that the major DOC generation takes place close to the top of the water table. Therefore, if Worrall’s hypothesis is valid for these sites, the ambient temperature *versus* DOC rather than the deep soil structure temperature, should exhibit a correlation.

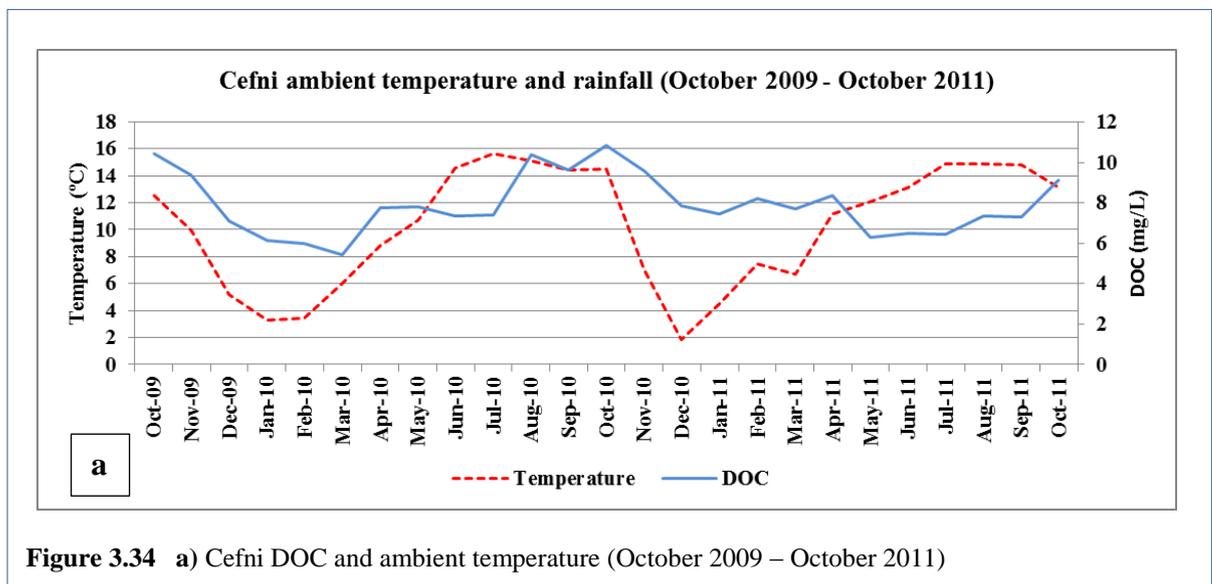


Figure 3.34 a) Cefni DOC and ambient temperature (October 2009 – October 2011)

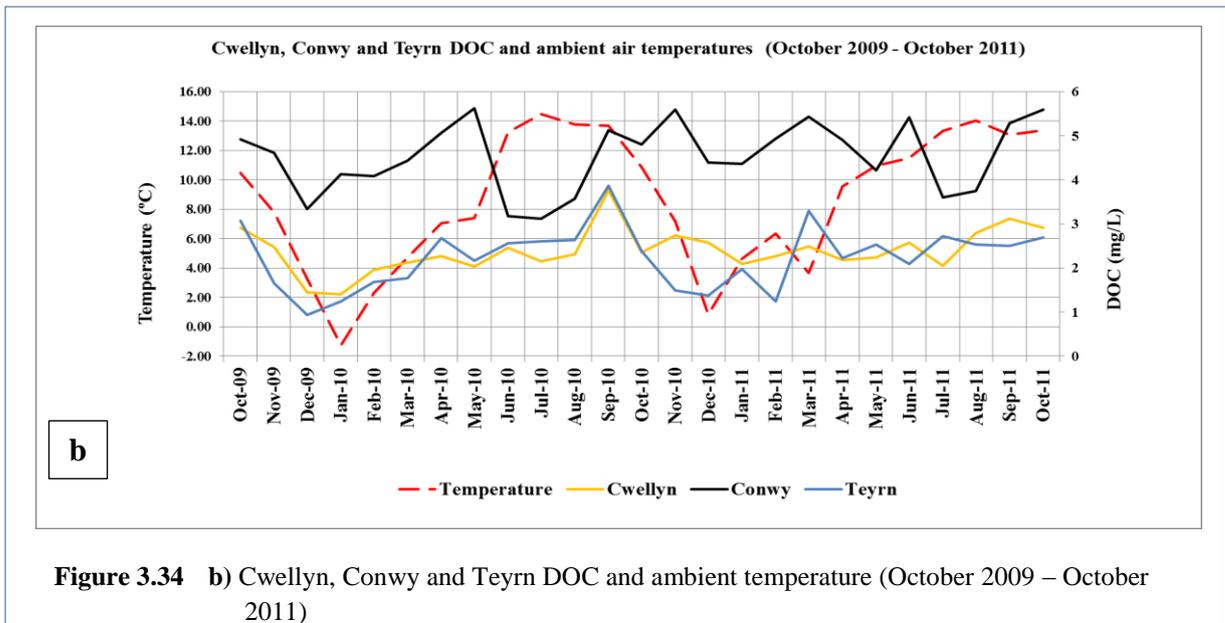


Figure 3.34 b) Cwellyn, Conwy and Teyrn DOC and ambient temperature (October 2009 – October 2011)

To explore this further, the data for temperature and DOC concentrations were statistically analysed. (Figs 3.34 a and b). Visually there appears to be some correlation between the changes in temperatures which is reflected by the DOC concentrations, with the exception of the Conwy. Statistical evaluation (Pearson’s correlations) of the temperature and DOC concentration data on the day of sampling (no lag) exhibited the following results (Table 3.18).

Table 3.18 Correlation coefficients of Cefni, Cwellyn, Conwy and Teyrn DOC concentration <i>versus</i> temperature for, No lag (NL), 30 day and 60 day lag intervals.		
Lake/Reservoir	Lag period (days)	Correlation coefficients (r)
Llyn Cefni	NL	0.472*
	30	0.675**
	60	0.651**
Llyn Cwellyn	NL	0.490*
	30	0.525*
	60	0.262
Llyn Conwy	NL	0.231
	30	0.172
	60	0.131
Llyn Teyrn	NL	0.640**
	30	0.539*
	60	0.257

** Correlation is significant at the 0.01 level (2 tailed)

* Correlation is significant at the 0.05 level (2 tailed)

Re-analysis of the temperature *versus* DOC data using mean temperature data blocks of 30 and 60 day intervals before each DOC recorded data point was noted to improve the correlations coefficients and significance for Cwellyn but, particularly for Cefni. However, Teyrn's correlation dropped from (r 0.640 p < 0.01) to (r 0.539 p < 0.05) for the 30 day data and, at 60 days it was insignificant. This tended to suggest that the direct proximity of the catchment and higher rain flow transfer rate into the Teyrn promoted a rapid transit of DOC into the lake with less of a time lag than for Cwellyn or Cefni. It was also noted that significance was improved in the Cefni correlations at the 30 to 60 day lag, from < 0.05 to < 0.01. By comparison, Conwy DOC concentration changes relative to temperature did not demonstrate any significant correlations when statistically analysed using any combination of antecedence. This again suggested that conditions for DOC generation within the blanket bog were stable, with the very limited seasonal changes in water drawdown, thus restricting the oxic and anoxic interface to a relatively fixed position (Freeman *et al.* 2001).

Whilst the data show that different concentrations of DOC were being exported out of the catchments, the concentration of actual phenolic compounds within it also varied, with Cefni demonstrating the lowest concentrations per mg of DOC. This was highlighted by presenting the phenolic content as a percentage of the DOC concentration. This suggested that as the phenolic content increased in the DOC samples, the DOC concentration being exported decreased (Figs 3.8 a,b,c and d). The resulting data, expressed as linear regressions graphs agree with the findings of Freeman *et al.* (2001) who demonstrated, that reducing the phenolic compounds concentrations, (which are considered to inhibit microbial activity) within a catchment, indirectly results in an increase in DOC export. The lowest concentrations of phenolics recorded during the late springs to late summers also supported Freeman's proposal that water drawdown resulting in higher O₂ infiltration of the upper peat layers also promoted greater enzyme activity, hence increased phenolic compound oxidation leading to increased microbial carbon processing. Fenner's temperature gradient experiment (Fenner *et al.* 2005) also confirmed that a relationship existed between temperature and optimum microbial activity; that is, it was suggested that the microbial populations within the peat not only respond to changes in temperature but also may have a unique range of temperatures within which they have optimised their reactivity. Hence it may be suggested that the reason for the increased phenolic concentrations (Figs 3.6; 3.7 b) being staggered may have been due to temperatures changes going beyond the catchment specific enzymes optimal range.

The UV absorbance spectra profiles ranging from 200 to 700nm reflected those reported in the literature (Moore, 1985; Korshin *et al.* 1996; Chen *et al.* 2002). Each spectrum had high absorption at 200nm dropping to a low at *ca.* 350nm. A plot of the absorbance at 254nm for all the reservoir/lake DOC *versus* the measured DOC concentrations satisfied a linear regression curve generating an R^2 value of 0.837, hence confirming a relatively positive relationship between the DOC concentration and DOC aromatic/conjugated system content arising from C=C bonds in the DOC which absorb at 254 nm. Reported SUVA values > 4 are considered to be an indicator of the presence of hydrophobic aromatic compounds with values < 3 , indicative of hydrophilic material (Matilainen *et al.* 2011). Conwy, Cwellyn and Teyrn exhibited average levels > 4 with Conwy the highest at 4.67, whilst Cefni (which generated the highest average DOC) showing an average SUVA value of 3.37 suggesting that the Cefni DOC had less of a hydrophobic content, and relatively less aromatic character than the upland lakes.

Analysis of the DOC using size exclusion chromatography (SEC) highlighted that the dominant ranges centred from 5000 – 3000 Da for the Cefni, Cwellyn, Conwy and Teyrn for the majority of the time. Examination of the data recovered at the zenith of the dry period during July 2010 exhibited a range bias towards the lower MM ranges in all the of the lake DOC as the re-wet began. Figs 3.14 a,b,c and d which show the MM ranges as percentages of the peaks, highlighted two dominant molecular mass ranges, the 5,000 - 4,000 Da and 4,000 - 3,000Da. There was little evidence of high molecular masses above 10,000 Da, suggesting that they were either absent or that they absorbed outside the single wavelength (254nm); hence non-chromaphoric DOC was missed, as suggested by Peuravuori and Pihlaja, (1997) and Rosario – Ortiz *et al.* (2007).

Figs 3.35 a,b,c,d show the MM distributions from January 2010 to December 2010 over the MM range 10000 – 1000 Da. The red line of each graph represents the MM distribution of the dry July 2010 DOC. The MM distribution range appears to be biased towards the 4000 – 3000 Da range (highlighted) and demonstrated $> 50\%$ of the overall distribution, with the exception of the Conwy DOC (Fig 3.35 c). Again this complements the suggestion that Conwy was less affected by what are termed ‘events’, (i.e., high rainfall or severe cold periods) which impose sudden shifts to the catchments equilibrium.

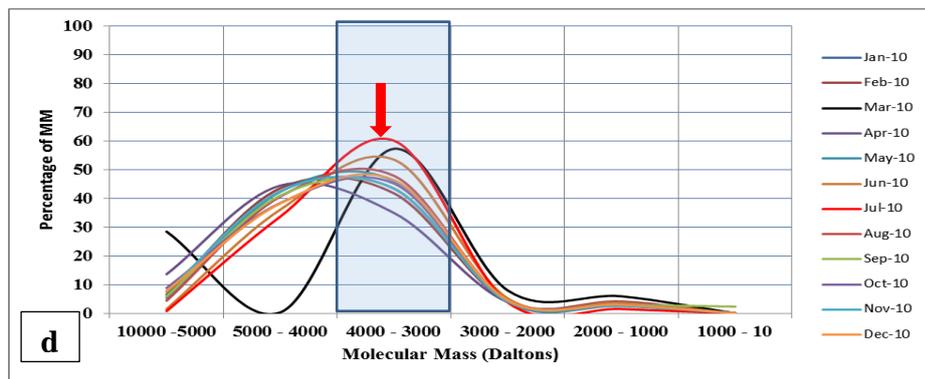
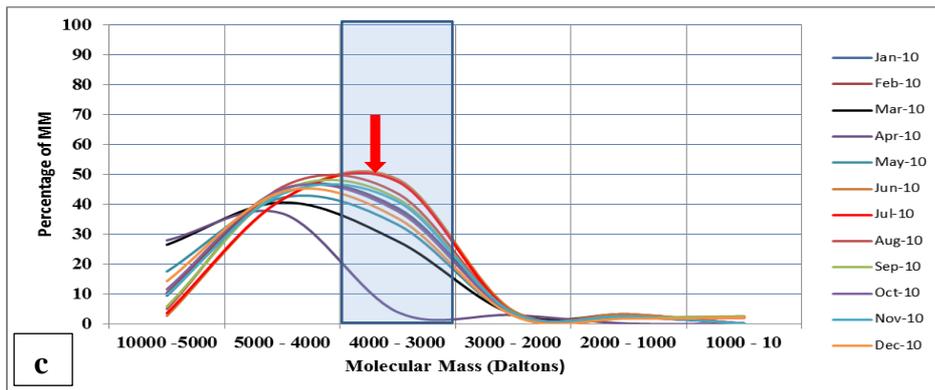
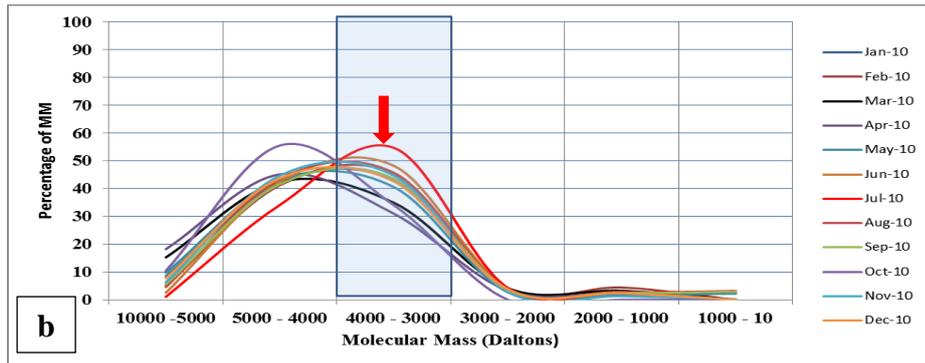
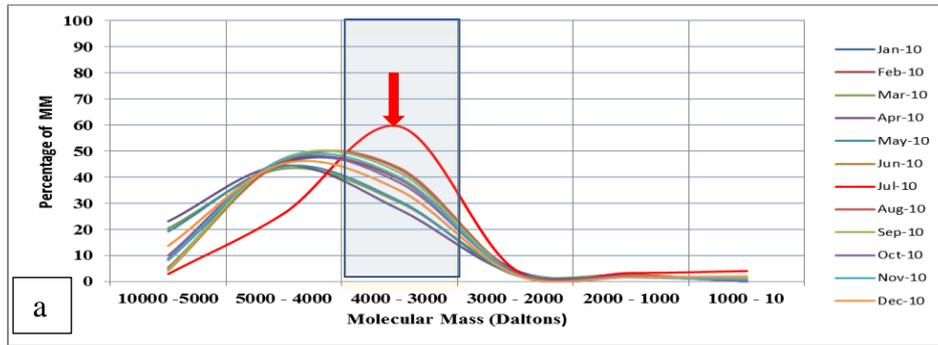


Figure 3.35 Percentage of MM *versus* range distributions for **a)** Cefni **b)** Cwellyn **c)** Conwy **d)** Teyrn. The red arrows indicate the increases recorded for July 2010 in the 4,000 to 3,000 Da range (shaded) in all four lake DOCs.

The increase in the lower MM range of the Cefni, Cwellyn and Teyrn suggested a longer DOC residence time in the catchments, thereby subjecting that DOC to prolonged microbial degradation and possibly photolysis in the lake itself (Rosario-Ortiz *et al.* 2007; Wetzel *et al.* 1995).

From the twelve month data (Fig 3.35 a) the Cefni distributions appeared to be relatively stable and repeatable but, in the upland lakes, differences were noted in March and October. Teyrn's March 2010 range (4000 – 3000 Da) demonstrated a similar response to that recorded in July 2010. This increase, again may have been due to longer residence, thereby increased polymer chain cleavage by microbial activity and degradation *via* photolysis. As the lake is shallow and the catchment is surrounded by steep slopes to the north and open to the south (Fig 2.1 D); hence higher ambient spring temperatures. Conwy, Cwellyn and Teyrn demonstrated percentage increases in the 5,000 – 4,000 Da for October 2010 (Figs 3.35 b,c and d). Again Conwy did not show the same increase (38%) compared to that of Cwellyn and Teyrn's, 55 and 45 % respectively. Reasons for these perturbations are not clear, but the upland lake DOC appears to correspond with the spring increase in plant/microbial activity and the late summer's relative abundance of degrading vegetation. Unlike the upland lakes, where vegetation growth is limited by low nutrients, cool temperatures and low sunlight the catchment of the Cefni is relatively rich in vegetation and therefore, the availability of degradation products is a continuum and hence not a limiting factor. Therefore, the upland lakes with the exception of Conwy demonstrate positive responses to environmental/seasonal changes.

Fluorescence spectroscopic analysis using PARAFAC analysis of the four surface water samples over the period of 24 months (n = 88) identified four common components in the DOC namely, **a**) protein like, (e.g tryptophan) **b**) terrestrial like humic, protein like (tryptophan) **c**) humic like, fulvic like and **d**) humic like. Assignment of the components to the wavelength excitation and emission pairs were made using comparisons from the literature and although the Stedmon *et al.* (2003) PARAFAC analysis method was utilised satisfactorily and common components (1,2,3 and 4) were isolated, their identification and assignment was tentative rather than absolute. This was due to the fact that the excitation and emission wavelengths and assigned descriptive identifications reported in the literature were also proposed by those authors (Section 3.1.5, [A, B, C, D, E, G, H, I]) rather than positively assigned. Reasons for this can be accounted for by the complexity of the chemical composition of the DOC structures influencing the transition energy states (electronic, vibrational and rotational) (Hollas, 1990) of the electrons. These can be

influenced by local conditions, different catchments and instrument configuration (Coble, 1996 ; Stedmon and Bro, 2008).

Cation and anion analysis of the samples over the period of 12 months (November 2010 – October 2011) highlighted the differences in the geology and anthropogenic influences of the catchments. The Cefni lake is a classic combination of two separate inputs; the western inlet has a distinct agricultural catchment and the eastern inlet sources from a large area of fen with limestone escarpments to the east of it and further agricultural areas to its west (Sampling Sites 2.1.1). Analysis of the Cefni Raw Surface Water demonstrated the highest inference of inorganic cations and anions of all the four lakes analysed. The high concentrations of calcium and magnesium ions (Ca^{2+} : 31.9, Mg^{2+} : 5.3) suggested chemical weathering of the limestone, whilst the high nitrates (average 3.6 ± 0.2 mg/L) especially during November to April may have resulted from agricultural outputs and run offs from fertilizers and animal manure spreading. In support of this, the three upland sampling sources did not have a high nitrate and remained below 1 mg/L, (Conwy 0.40 ± 0.05 mg/L, Teyrn 0.29 ± 0.06 mg/L). Cwellyn demonstrated the highest of the three (0.60 ± 0.04 mg/L), which suggested a more anthropogenic influence on its catchment. This may have been due to its southwest inlet passing through an agricultural area and a small hamlet, where each household had its self-contained sewage treatment plant with the effluent being removed *via* ground soakaways.

The drop in nitrate concentration (e.g., Cefni 4.9 mg/L spring 2011 to 0.5 mg/L summer 2011) recorded during the summer months in all four, but much smaller ($\approx 40\%$ less) in the upland lakes (av. 0.7 mg/L to 0.4 mg/L) could be associated with the growing season and high uptake by the vegetation. The upland lake catchments for Conwy and Teyrn would be classified as oligotrophic/ombrotrophic, obtaining their nutrients principally from rainfall and geological sources (Steinmann and Shotyk, 1997). The October 2011 analysis for NO_3^- concentration demonstrated an increase in all of the lake samples, hence suggesting a return of NO_3^- back into the peat/soil as the summer vegetation degraded.

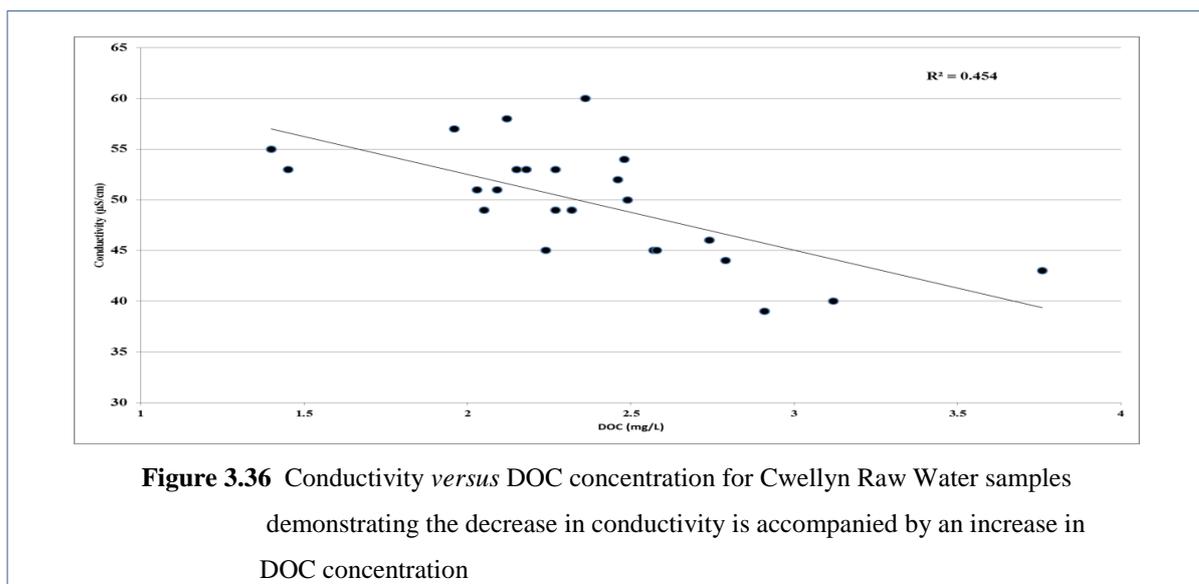
Although the sodium concentration varied in Cwellyn, Conwy and Teyrn, it did not increase above an average of 4.6 mg/L, but Cefni again demonstrated a high average concentration of > 14.0 mg/L. This is probably due to its island location, i.e., strong marine inferences (Gorham *et al.* 1985) and again other contributory factors such as agricultural run-offs such as dairy farm slurries, manures etc. (Li-Xiam *et al.* 2007). Bromide ions usually associated with a marine environment (70 mg/L in seawater, Lowery and Cavell 1962) were found to be present at trace level concentrations in the Cefni water, averaging

70 $\mu\text{g/L}$ (lower by a factor of 10^3) whilst the upland lakes were lower at a concentration of less than 10 $\mu\text{g/L}$. The Cefni catchment, although influenced by its marine environment also has a secondary source, in the form of limestone bedrock and escarpment where up to 6 mg/kg of Br^- may be available (Flury and Papritz, 1993). Although the concentration levels of Br^- are well within the safe levels recommended of 0.4mg/kg bodyweight (WHO 2009), bromide is readily oxidised and hydrolyzed during the Cl_2 disinfection of Raw Surface Water to both free Br_2 and HOBr . These species can react with the DOC to form brominated compounds (Chapter 5 section 5.6) that are considered to be carcinogenic and are subject to strict control (Chapter 1)

The origins of the sulphate levels in the environment could be attributed to a number of sulphur sources; metal sulphides, geothermal activity, marine, biological processes (est 300×10^6 tonnes pa) (Greenwood and Earnshaw, 1993) and atmospheric deposition (acid rain) which has been in decline since the 1980s (Evans *et al.* 2006) and which continues to decline as coal fired power stations are de-commissioned. Research into the changing concentrations of the sulphate anion, pH and its relationship to the export of DOC out of peatlands has been investigated (Clark *et al.* 2005, Evans *et al.* 2008). Cefni's average sulphate concentration (13.7 ± 0.8 mg/L) was approximately seven times higher than that of the three upland lake averaged levels (1.8 ± 0.1 mg/L). The four lakes demonstrated significant correlations (r 0.884, $p < 0.01$) between their pH and SO_4^{2-} concentrations. The high levels of SO_4^{2-} in the Cefni may be attributed to a series of sulphur/sulphate sources in the area, such as the poly-metallic mine (Parys Mountain, Anglesey) within 5 miles and the marine influence. The lower SO_4^{2-} concentrations recorded in the upland lakes was typical, as the majority of sulphur deposition is dependent on the geology and rainfall (Steinmann and Shotyk, 1997). The bedrock geology of the upland lake catchments consisting of principally, Ordovician igneous rock formations with low levels of sulphur. Hence the release and solubility are relatively low (Scheider, 1978).

A relationship has also been reported to exist between ionic strength, pH and the solubility of DOC, i.e., DOC solubility/release is inhibited by high concentrations of ions and an acidic pH (Evans *et al.* 2006; Steinmann and Shotyk. 1996; Tipping and Woof. 1990). In this thesis, it has been demonstrated (Figs 3.27 a,b,c and d) that conductivity and ionic charge concentration of the principal cations present in the Raw Surface Water had a significant relationship. Hence the conductivity of the samples, because of this linear relationship represented the ionic strength. This, as previously mentioned, may have influenced the export of DOC. Therefore plots of conductivity *versus* DOC should

demonstrate a linear relationship if the hypothesis is correct. Figure 3.36 shows the 24 month data for conductivity *versus* DOC concentration for the upland lake Cwellyn at average pH 6.7 ± 0.2 . A significant inverse linear relationship (R^2 0.454), was established between the conductivity (ionic strength) and DOC concentration. That is, the DOC increased with falling conductivity (ionic strength), which satisfied the proposed hypothesis.



Cefni's Raw Surface Waters had the highest conductivity of the four lakes, hence the highest ionic strength, but the pH was basic (8.1 ± 0.2). Thus the DOC was deprotonated and carried a negative surface charge which promoted solubilisation of the DOC. Unlike the Cwellyn where a relationship between conductivity and the DOC export was not supported (R^2 0.06). Conwy and Teyrn's samples averaged pH 6 ± 0.2 and although slightly acidic, exhibited insignificant correlations for conductivity *versus* DOC concentrations, (0.04 and 0.01) respectively. These results suggest that the pH which influences the dissociation of the DOC acidic functional groups (Maurer *et al.* 2010) of the Raw Surface Water is a critical component that influences DOC export.

This can be rationalised using the pK_a of an acid and its ability to dissociate. That is, the lower the pK_a the higher the dissociation and the stronger the acid and *vice versa* (Sykes, 1986). Hence if the dissociation constant (pK_a value) of DOC is considered, the pH of the solution/solvent acts in effect as a chemical switch, i.e.,



The dissociation of the humic and fulvic acids which are the major constituents of DOC, as previously mentioned, are therefore influenced by pH. A pH of < 2 has been reported to induce agglomeration (Janos, 2003) of humic acid molecules, hence indicating that dissociation is suppressed by the increase in H^+ and is therefore less than the DOCs actual pKa value. Hence polarisation of the water molecules surrounding the DOCs molecular structures is reduced, resulting in the limitation of the water molecules to form a solvation envelope around the DOC molecules. With the pH of the Cefni waters being basic, the DOC in effect would be dissociated ($pH > pKa$) and would exhibit a negative delocalised charge density, which would be conducive to increasing its solubility. Hence it is suggested that the export of DOC from the fen would be favourable and greatly influenced by this property rather than changes in ionic strength. Although it is suggested that pH may be considered a driving mechanism, correlation between DOC and pH for the Cefni surface waters was not established ($r\ 0.189, p > 0.05$). This was probably due to the water with a pH averaging 8.1 ± 0.2 (SD: 0.3) continually de-protonating the acidic DOC moieties and hence, subtle pH changes did not influence the dynamics of the system. If we considered the upland sources, Cwellyn's pH was closest to neutral (pH 7) averaging 6.7 ± 0.2 (SD: 0.3), a weak but significant correlation was noted ($r\ 0.426, p < .05$). This suggests that the pKa value is relatively close to the pH value with small changes in the pH influencing the solubility by altering the solubility/charge density properties of the DOC. Conwy and Teyrn both with an averaged pH of 6 ± 0.2 appear to have suppressed de-protonation, which in turn would have impacted DOC solubility. Again like the Cefni, but with an opposite effect, the DOC remained relatively protonated, i.e., the pKa value was greater than the subtle pH changes. Hence pH did not influence solubility. This was confirmed by the insignificant correlations for pH *versus* DOC (Conwy: $r\ 0.169\ p > 0.05$) (Teyrn: $r\ 0.246\ p > .05$).

Infrared spectroscopic analysis of the Cefni and Conwy freeze dried samples of Raw Surface Water DOC exhibited functional group bands that were also present in the Nordic Fulvic and Humic acid reference samples (IR 105 F, IR 105H). Cefni's OH stretch was broad ($432\ cm^{-1}$; peak half width) compared to the Conwy's indicating strong inter-molecular hydrogen bonding, whilst Conwy, although broad it did exhibit a sharper band ($243\ cm^{-1}$; peak half width) and did tend to indicate some intra-molecular hydrogen bonding. The broad OH band of the Cefni suggested that its inter-molecular bonding may have consisted of several molecular structures participating in the H-bonding (polymeric) whilst the sharper intra molecular H-bonding of the Conwy OH stretch suggested dimeric participation for instance as occurs in β -diketones (Cross and Jones, 1969). Evidence of a

C≡N stretch was present in both the Conwy and Cefni at 2322 and 2194 cm^{-1} although they were weak, but may indicate the presence of protein type structures. The strong stretch at 1630 cm^{-1} in the Cefni spectrum (Fig 3.23 A) was difficult to assign, although the stretch was present in the Conwy and Nordic samples, they were less strong and appeared as two signals. References (Silverstein *et al.* 1991) indicated that this band could be assigned to an ester or a carbonyl attached to a conjugated system, both of which would lower the usual carbonyl (1750 -1715 cm^{-1}) frequency. It could also have related to an absorption belonging to an overlap carbonyl amide band of a primary, secondary or tertiary amide in the solid state. (Silverstein *et al.* 1991) but the expected out of plane N-H wagging broad band from 800 to 600 cm^{-1} was absent. The Conwy and Nordic humic (Figs 3.28 B, 3.30 B) spectra were similar in all aspects with each band labelled being present in both. The doublet at 1637 and 1610 cm^{-1} suggested a carboxylate, possibly associated with an inorganic cation such as Ca^{2+} (Elkins *et al.* 2001) or a quinone in conjugation with alkenes (Shirshova *et al.* 2006). Evidence of stretching frequencies for both SO_4^{2-} and NO_3^- were observed in all the spectra, including the Nordic humic and fulvic acids. A series of absorbances were recorded in the Conwy at 3710 and 3737 cm^{-1} representing Si-OH stretches together with Si-O bends between 830 - 1100 cm^{-1} (Silverstein *et al.* 1991) although this region could also be assigned to the SO_4^{2-} and NO_3^- . The presence of Si structures in the Conwy may have resulted from the weathering of natural rocks and sediments by acidic pH water. In aqueous solution it exist as (H_4SiO_4) , but can also be present in a polymeric form in conjunction with the acid (Wonisch *et al.* 2008).

3.3 Conclusions.

This chapter has explored, the changes within lake DOC and indirectly that of the catchments and their characteristics. The data have established that:

- The lowland minerotrophic fen/agricultural catchment exported on a continual basis the highest concentration of DOC.
- The upland oligotrophic/ombrotrophic blanket bog was the second highest exporter with both Cwellyn and Teyrn the other upland catchments with steep rocky, thin peat/soil catchments demonstrating the lowest DOC exported concentrations.
- Conwy's DOC concentration changes correlated with rainfall but not with temperature. Cefni, Cwellyn and Teyrn DOC concentrations demonstrated correlations with ambient temperature, but not with rainfall.

- The SEC results highlighted that the DOCs dominant MM ranges were between 5,000 -3,000 Da with only 8 -11 % present in the 10,000 – 5,000 Da range.
- Phenolic concentration demonstrated a controlling influence on DOC solubility, with a $\pm 1\%$ variation in concentration prompting a $\pm 0.5\%$ change DOC concentration.
- From the fluorescence spectroscopy exercise, the molecular structures detected in the SEC ranges consisted of mainly humic substances, although the identifications were not conclusive as the published data is not comprehensive.
- The IR spectra of both samples had bands representing functional groups that were common to both the Nordic humic and fulvic acid (reference material). Hence it can be concluded that although there was a common series of functional groups within the DOCs molecular structures, the number of bands and overlap made it difficult to positively identify all the functional groups by IR spectroscopy.
- The Cefni fen/agricultural catchment had the highest ion concentrations with Ca^{2+} being the dominant cation due to the limestone escarpment to the east of the catchment. The anthropogenic and marine inputs were believed to be responsible for the high concentrations of Na^+ and SO_4^{2-} .
- The three upland lakes most dominant ions was Na^+ and SO_4^{2-} , influenced by both the marine environment and anthropogenic inputs *via* rainfall.
- NO_3^- concentrations were higher in the Cefni owing to possible anthropogenic (agriculture) inputs rather than predominantly atmospheric ones in the three upland lakes.
- The halogens F - and Br – concentrations were best described to be at trace levels in the three upland lakes, but the Cefni demonstrated concentrations above the LOD. These contributions were considered to be of a marine source the limestone escarpment and bedrock.
- The pH of the Cefni water was basic > 7 suggesting a buffering capacity by the dominant limestone in the fen catchment. Whilst the pH of the upland lakes were < 7 owing to the acidic nature of the geology and the influence of the DOC acidic groups.
- Conductivity in the lowland fen catchment reflected the high ionic concentrations of the samples. The three upland lakes demonstrated a lower conductivity, reflecting the incidence of low ionic concentrations.

Chapter 4

Characterisation of Water Treatment Works Pre-Chlorination Water Samples.

Chapter 4 Characterisation of Water Treatment Works Pre-Chlorination Water Samples.

4.1 Introduction

Water samples were collected on a monthly basis from two of the treatment plants, Cefni and Cwellyn, for a 12 month period from October 2010 to October 2011. The two plants serve two different water sources; Cwellyn a deep oligotrophic glacial lake fed by an upland catchment and the Cefni a man-made reservoir fed by a lowland fen and a nutrient rich agricultural catchment. Recent refurbishment and updating of the Cwellyn Dissolved Air Floatation (DAF) plant took place during the sampling regime (Spring 2011) together with the replacement of UV reactors that have been incorporated at the end of the treatment as a secondary disinfection process for removal of pathogens (e.g., *Cryptosporidium*).

The water treatment processes for both treatment plants are similar (Figs 4.1 and 4.2), but differ in the pH adjustment of the Raw Surface Water on intake. For the Cefni (pH \approx 8) H₂SO₄ is added to reduce the pH to \leq 7 and at Cwellyn (pH \approx 6.7) is elevated using Na₂CO₃ to elevate the pH to \leq 7 (if required) to aid the removal of manganese on the filters (Dwr Cymru. Welsh Water, 2009).

Samples for characterisation and laboratory chlorination were taken at points [A] indicated on Fig 4.1 and 4.2 after processing through the water treatment that laboratory filtration was excluded (2.3.8).

The aim of the sample characterisation and chlorination was to compare the effects of the water treatment process *versus* the partially (laboratory filtered 0.2 μ m) treated Raw Surface Water. The comparative study would highlight any impacts on; the DOC content, MM distributions, SUVA, fluorescing components and ionic concentrations that may aid in to begin to identifying and isolating molecular structures that are considered to be THMFP precursors. Such precursors are thought to include typical plant degradation products, for example, resorcinol, catechols, aromatic amino acids and various phenolic compounds (Rook, 1977; Westerhoff *et al.* 2004).

Characterisation of the samples was carried out using the range of methods described in Chapter 2 and the determination of their THMFP using Method 2.3.8

4.1.1 Cwellyn Water Treatment.

Raw water is abstracted from the Cwellyn lake *via* an intake (depth 4.5 m) connected to an 800 mm diameter pipe that feeds the works directly. At the water treatment works, the Raw Surface Water is monitored for pH, temperature, conductivity and turbidity before passing through four rapid pressure filters arranged in parallel to remove insoluble debris. The data recovered from the monitoring process is used to adjust the dosage parameters for; chlorine, carbon dioxide and orthophosphoric acid prior to entering the Cl₂ contact tank (Fig 4.1).

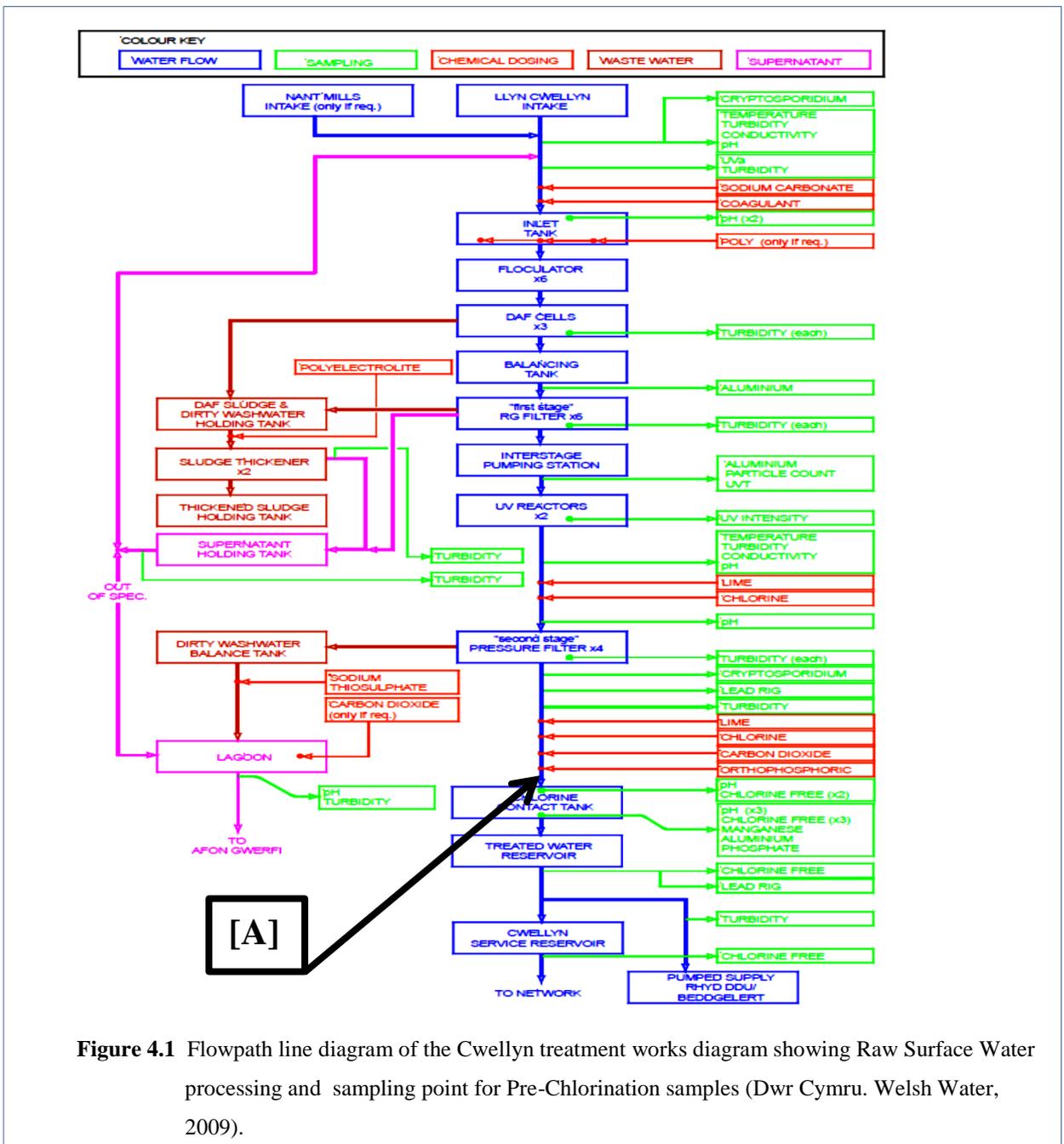
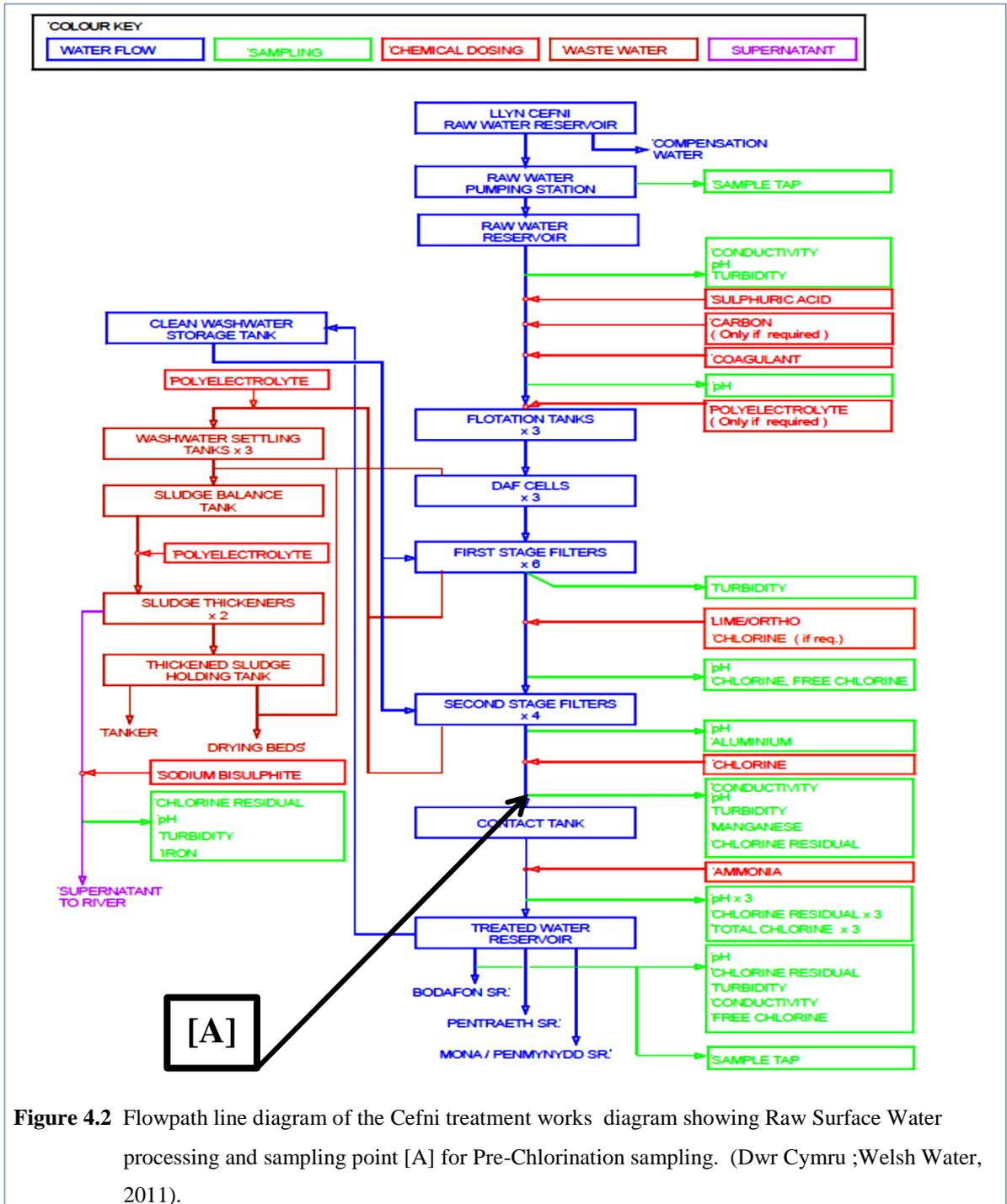


Figure 4.1 Flowpath line diagram of the Cwellyn treatment works diagram showing Raw Surface Water processing and sampling point for Pre-Chlorination samples (Dwr Cymru, Welsh Water, 2009).



4.1.2 Cefni Water Treatment.

The raw surface water is pumped from the reservoir to an elevated holding tank from which it is gravity fed into the treatment plant (Fig 4.2). The water is monitored for pH, conductivity, and turbidity. Prior to the addition of flocculant $[Al_2(SO_4)_{3(aq)}]$ the pH is adjusted as required with H_2SO_4 to below pH 8, especially during the algal blooms which propagate an increase in the pH. Flocculation is carried out in three units where the mixture

is stirred gently with a continuous up-flow of micro-air bubbles to buoy and suspend the floating sludge blanket. The clarified water is then passed through a series of rapid gravity filters after which it flows to the chlorine contact tank (Fig 4.2).

4.2 Coagulation and Flocculation Process Fundamentals.

The process of removing suspended particulates in Raw Surface Water is carried out by coagulation and flocculation. The existence of a surface charge on these particulates keep them suspended and relatively stabilized (Franceschi *et al.* 2002). Three mechanisms have been reported to exist during coagulation, charge neutralization, sweeping and bridging (Li *et al.* 2006). Charge neutralization is carried out to destabilize the particulates by adding a hydrolysable metal salt, for example, $[Al_2(SO_4)_3(aq)]$. These hydrolyse to form cationic species, which are adsorbed on the surface of the negatively charged suspended particulates, hence reducing the charge and introducing disruption of the suspension and encouraging micro-flocs to form, as the particulates start to collide. Gentle mixing of the solution increases the collision of the neutralized particulates and the micro-flocs, resulting in larger flocs being formed which can be removed by sedimentation or can be subjected to a system where micro bubbles of air buoy up the floc to the surface where it forms a floating blanket.

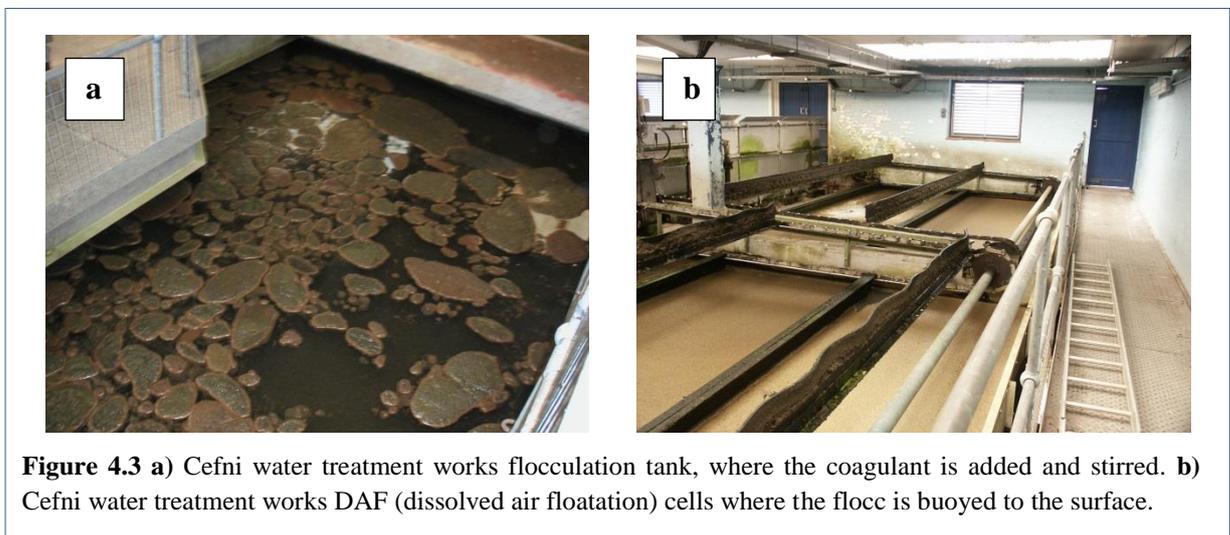


Figure 4.3 a) Cefni water treatment works flocculation tank, where the coagulant is added and stirred. b) Cefni water treatment works DAF (dissolved air floatation) cells where the floc is buoyed to the surface.

The two other mechanisms mentioned above for removing suspended particulates are referred to as Sweeping and Bridging. Sweep flocculation is initiated by adding high concentrations of an amorphous metal hydroxide to the water where the precipitate as it falls through the water column captures the suspended particles. Simple metal salts are not used for what is described as bridging flocculation. This mechanism utilises polymeric

flocculants. These destabilize the particulates by the absorption of segments of the polymer chain onto their surface, thus forming a series of linkages which grow into larger flocs, which then fall out of suspension.

4.3 Characterisation

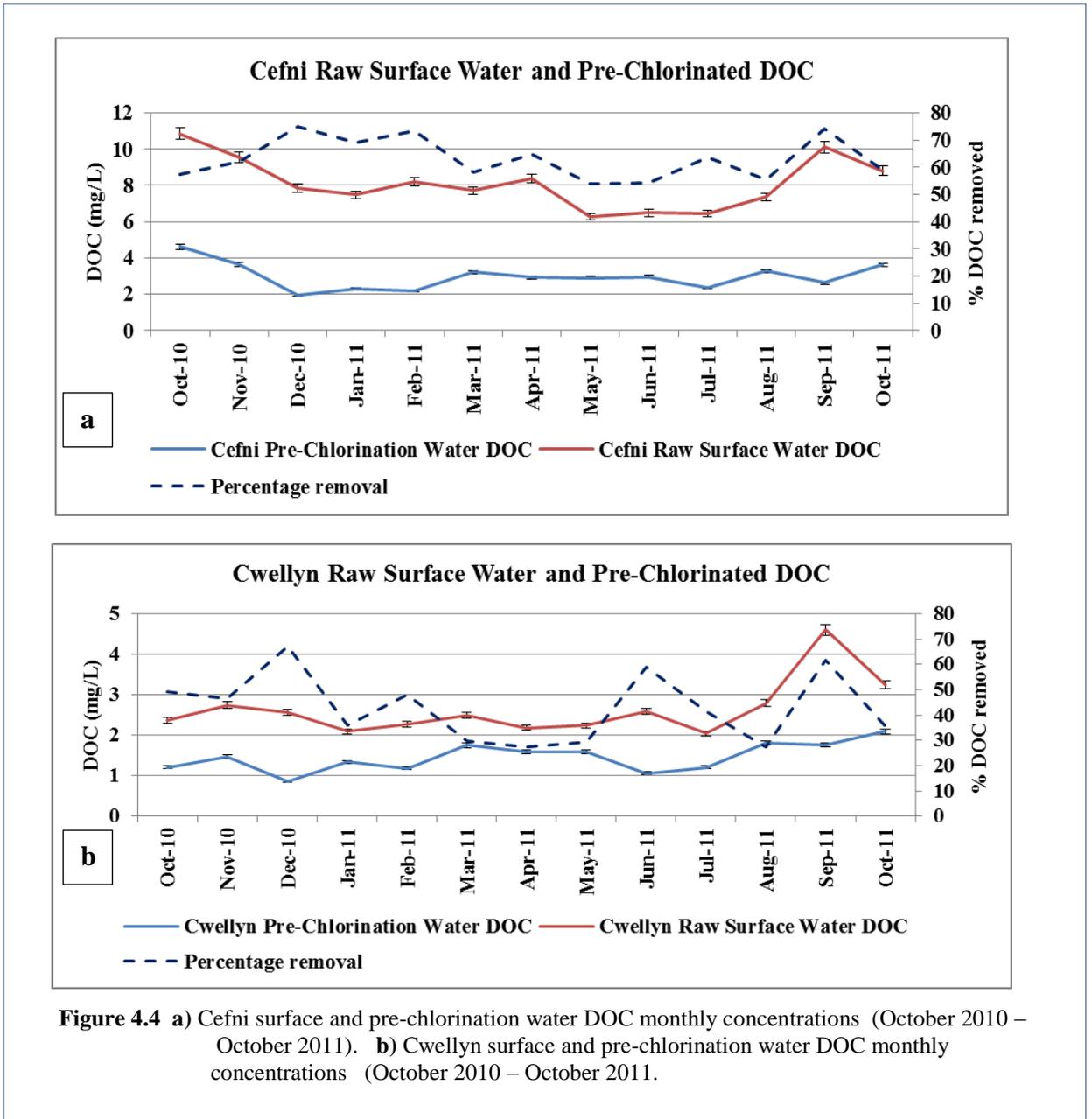
To characterise the Cefni and Cwellyn's DOC in the pre-Chlorination samples for the period October 2010 – October 2011, they were subjected to a selected series of analyses as described in Chapter 2.

4.3.1 DOC Analysis of Pre-Chlorination Samples.

The samples were prepared for analysis and adjusted to $\text{pH } 2.8 \pm 0.2$ with HCl (4.0 M) as per the Raw Surface Water (Method 2.1.4.1).

As discussed in Chapter 3, the Cefni's mean Raw Surface Water DOC (Fig 3.1) for the October 2010 to October 2011 period was 8.1 ± 1.4 mg/L. After processing through the treatment works, the Pre-Chlorinated water DOC averaged 3.0 ± 0.1 mg/L (Fig 4.4 a), equating to a mean removal of approximately 5 mg/L or 63 % of DOC. Cwellyn's average Raw Surface Water DOC for the same period (Fig 3.3) was 2.6 ± 0.7 mg/L. Following processing at the treatment works a drop of 42 % was recorded, which represented the removal of 1.1 mg/L of DOC (Fig 4.4 b). The difference between the two works' capability of removing DOC was on average 21 %. This may have resulted from differences in composition and character of the DOC and suspended particulates. For example, the SUVA values suggested that the Cefni was less aromatic than the Cwellyn's DOC, hence surface charge would be different, with the humics (high aromatic content) having more surface charge than the fulvic (lower aromatic content). This would have an effect on the neutralising capacity of the coagulant (Sharp *et al.* 2006).

Both Cefni and Cwellyn's residual DOC in the Pre-Chlorination water samples demonstrated their lowest concentration in December 2010 (Cefni 1.96 ± 0.06 mg/L, Cwellyn 0.85 ± 0.02 mg/L) although the concentration in both of the Raw Surface Waters before passing through the water treatment process were relatively high, i.e., Cefni Raw Surface Water: 7.83 ± 0.2 mg/L, Cwellyn Raw Surface Water: 2.57 ± 0.08 mg/L). In the March of 2011, Pre-Chlorination DOC concentration levels for both increased (Cefni 3.2 mg/L, Cwellyn 1.8 mg/L).



The coagulation and flocculation processes which removes the suspended charged particulates reflected the changes in the DOC surface charge characteristics of both Cwellyn and Cefni over the months. Although the March 2011 Cefni Raw Surface Water DOC concentration only increased by 6.3% above the December 2010 level, the Pre-Chlorination residual DOC level increased by 38.7%. Cwellyn demonstrated an actual decrease in March 2011 of 3 % below the December 2010 level to that of the Raw Surface Water DOC, but the Pre-Chlorination DOC increased by 51.4%. This tends to suggest that the DOC charge character had changed and did not respond in the same way as the December 2010 DOC to processing. A drop of $23.5 \pm 1\%$ in the Pre-Chlorination DOC

was recorded for both reservoirs in June/ July 2011 (Cefni 2.35 mg/L, Cwellyn 1.2 mg/L) suggesting both DOCs demonstrated similar charge characteristics and were being efficiently removed. The Raw Surface Water DOC increased in September 2011 for both lakes as the autumn flush of the catchments became evident, but the Pre-Chlorination DOC remained relatively low and the percentage removal by processing was Cefni 74.1 % and Cwellyn 61.7 %. This would suggest that again a large concentration of the DOCs charge characteristics were being effectively neutralised by the coagulant and removed with the floc.

4.3.2 Determination of Phenolic Compounds in Pre-Chlorination Samples

The DOC remaining after removal by the filtration and flocculation/coagulation of the Raw Surface Water in the treatment works was analysed for the presence of phenolic compounds by the Folin Ciocalteu's modified method described in section 2.3.2. The analytical results for both Cefni and Cwellyn's Pre-Chlorination samples (October 2010 – October 2011) are presented together with the Raw Surface Water phenolic results for the same 12 month period in Fig 4.5, with the mean data including the standard deviations (SD) tabulated in Table 4.1.

Lake/Reservoir	Mean Raw Surface Water DOC (mg/L)	Mean Raw Surface Water DOC phenolics (mg/L)	Mean Pre-Chlorination DOC (mg/L)	Mean Pre-Chlorination DOC phenolics (mg/L)
Cefni	8.1 (SD 1.4)	1.1 (SD 0.34)	3.0 (0.7)	1.1 (SD 0.33)
Cwellyn	2.6 (SD 0.7)	0.59 (SD 0.10)	1.5 (0.4)	0.46 (SD 0.14)

From the results (Fig 4.5) it was evident that the phenolic concentrations were not affected by the water treatment process and remained at similar concentration levels to those of the Raw Surface Water, although at times higher. On average over the twelve month period (Table 4.1) they remained the same for the Cefni and demonstrated a slight drop in the Cwellyn as phenolics, presented in the format mg/L.

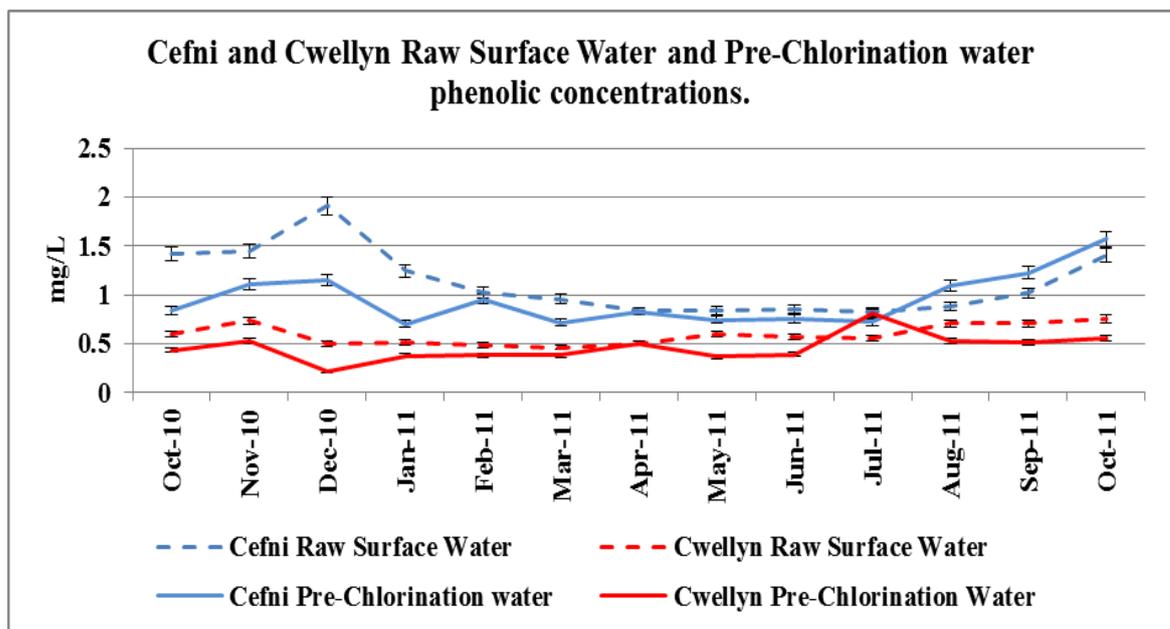


Figure 4.5 Cefni and Cwellyn Pre-Chlorination Water phenolic samples (mg/L) and Raw Surface Water phenolics samples (mg/L).

In Fig 4.6 a,b the phenolics data for the Raw Surface Water and Pre-Chlorination water for both Cefni and Cwellyn is shown as a percentage of the total DOC concentration (Fig 4.6 a,b). The results demonstrate that in the Pre-Chlorination DOC phenolics were present at a higher percentage than in the Raw Surface Water phenolic samples. For example, the Cefni Raw Surface Water % phenolics, averaged 13.9 %, while the Pre-Chlorination samples averaged 37.2 % (difference 23.3 %) and ranged from 20 – 60 % over the 12 month period. The Cwellyn exhibited a higher phenolic/mg DOC in the Raw Surface Water (23.0 %) than the Cefni but demonstrated after treatment a Pre-Chlorination percentage of 32.7 %. These data demonstrate that both treatment plants removed proportionally more of the non-phenolic compounds during flocculation and filtration, leaving the majority of the phenolic compounds still suspended. This suggested that the surface charge of the phenolic DOC was negligible or absent and was not matched by the cationic hydrolysed coagulant charge, hence suggesting that complexation/precipitation of the phenols was in-effective. This may have been due to the phenolic groups high dissociation constants which range from (pKa 9.9 – 13.95) depending on their ring position and other associated functional groups present (Guan *et al.* 2006). With aluminium based coagulents, optimum coagulation occurs at pH 5 - 6 (Sharp *et al.* 2006), hence ionisation of the phenolics with the high pKa values would not take place and they would not participate in the coagulation process.

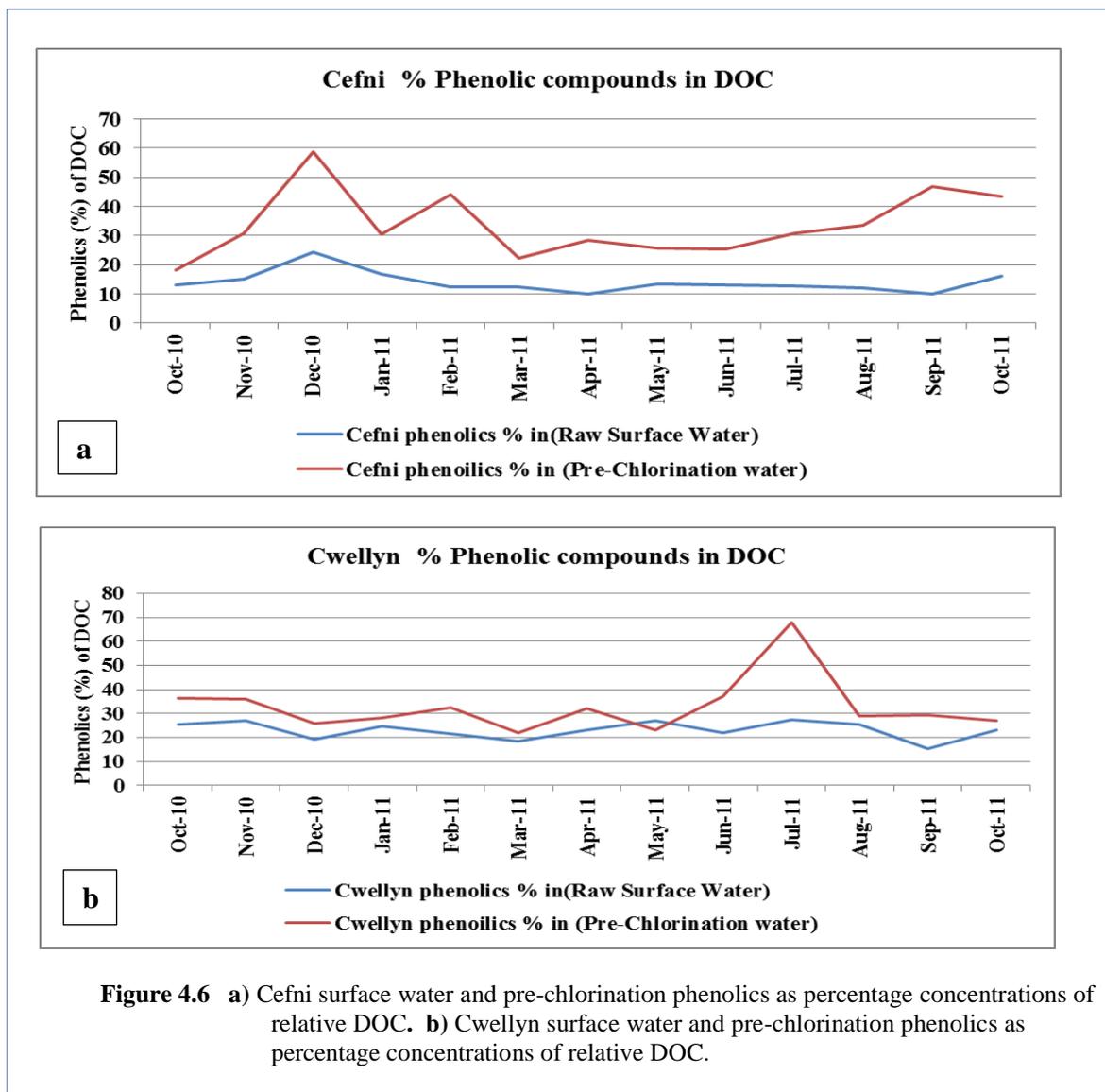


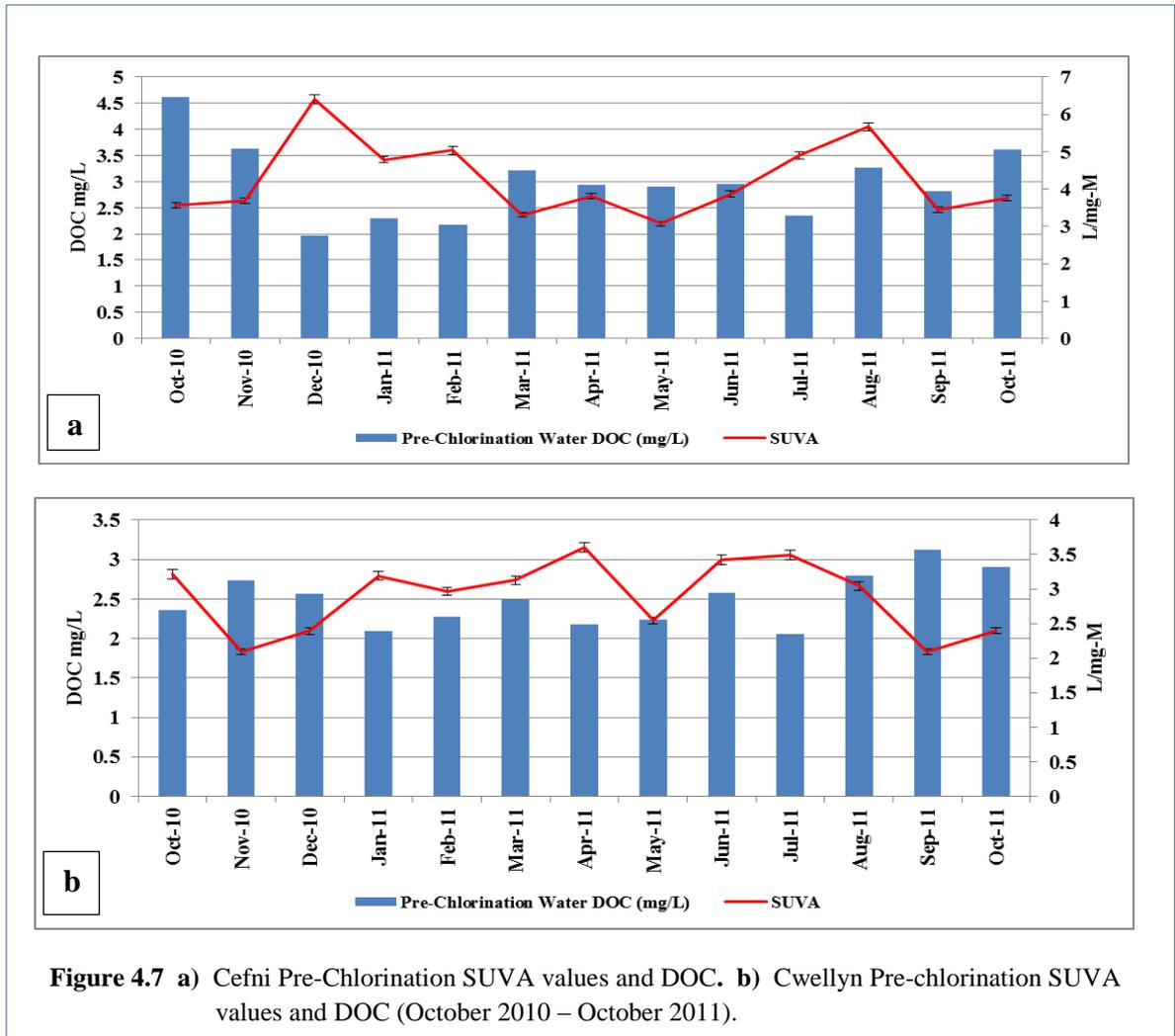
Figure 4.6 a) Cefni surface water and pre-chlorination phenolics as percentage concentrations of relative DOC. b) Cwellyn surface water and pre-chlorination phenolics as percentage concentrations of relative DOC.

4.3.3 Specific UV-vis. Spectroscopy (SUVA) of Pre-Chlorination Samples

Specific Ultra Violet Absorbance (SUVA) values, a DOC aromatic concentration indicator (Rodriguez and Nunez, 2009), were determined for the series of Cefni and Cwellyn Pre-Chlorination samples using the method of calculation reported in section 3.1.4.

The average SUVA value for the Cefni was $4.4 \pm 0.1 \text{ Lmg}^{-1}\text{m}^{-1}$ for the 12 months. Within this series the highest value was in December 2010 at $6.4 \pm 0.1 \text{ Lmg}^{-1}\text{m}^{-1}$. This trended downward after the December peak to a relatively stable period from March to June 2011 averaging $3.0 \text{ Lmg}^{-1}\text{m}^{-1}$. It then increased and peaked in August at $5.7 \pm 0.1 \text{ Lmg}^{-1}\text{m}^{-1}$, before declining again in October 2011 ($3.8 \text{ Lmg}^{-1}\text{m}^{-1}$) to a level similar to that of October 2010 ($3.6 \text{ Lmg}^{-1}\text{m}^{-1}$). Cwellyn's highest and lowest SUVA values were recorded in April

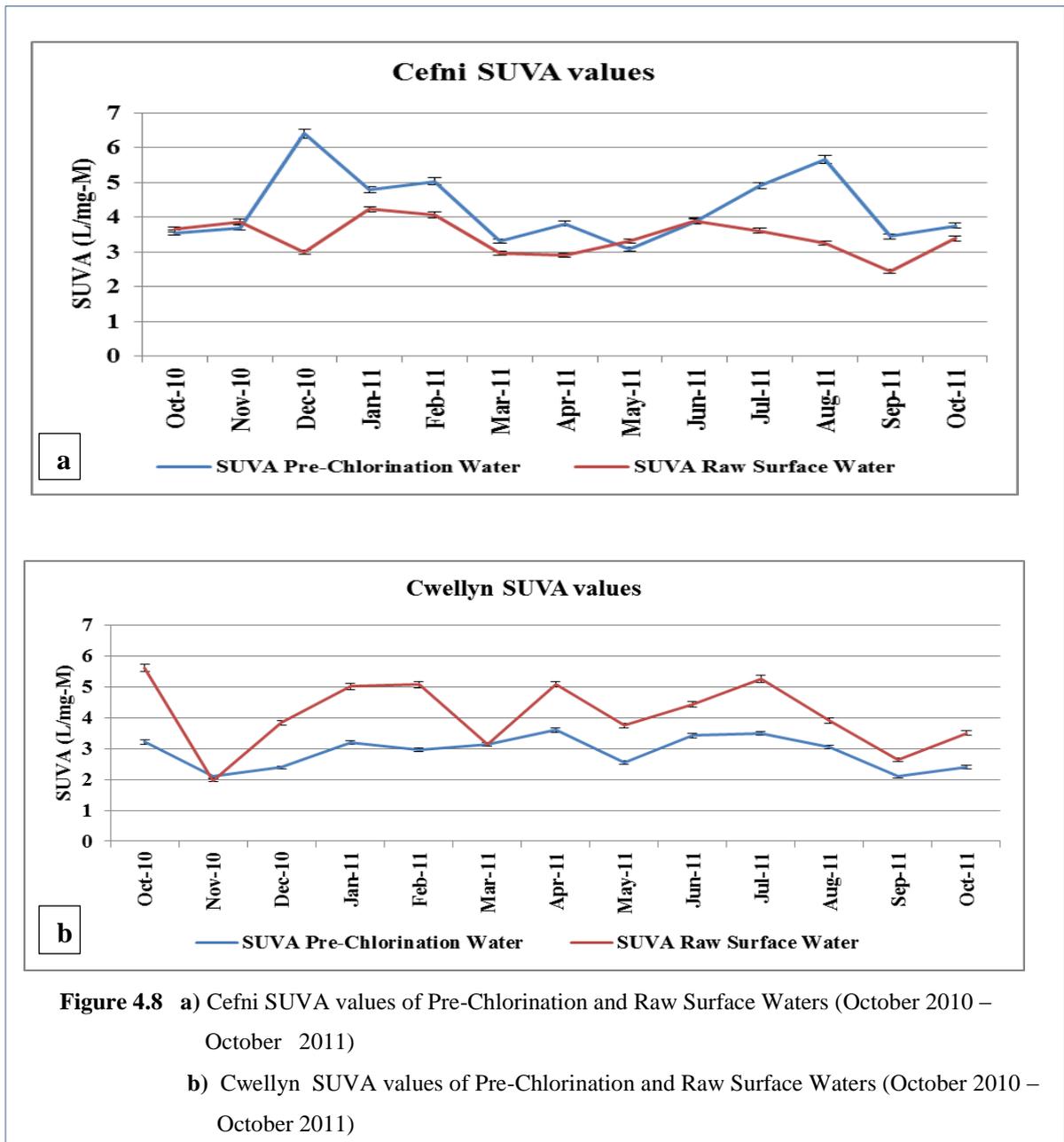
and September 2011 at $\text{Lmg}^{-1}\text{m}^{-1}$ and $2.1 \text{Lmg}^{-1}\text{m}^{-1}$ respectively with the average over the 12 month period at $2.9 \text{Lmg}^{-1}\text{m}^{-1}$.



Unlike the Cefni, the Cwellyn SUVA values were higher during the months January to July 2011 and from July onwards the value fell to below $2.4 \text{Lmg}^{-1}\text{m}^{-1}$. In contrast to the Cefni results, the October 2011 value ($2.4 \text{Lmg}^{-1}\text{m}^{-1}$) did not correspond to the October 2010 value of $3.2 \text{Lmg}^{-1}\text{m}^{-1}$. This difference also corresponded with the Raw Surface Waters (Fig 4.8 b) of the Cwellyn with the October 2011 SUVA value at $3.3 \text{Lmg}^{-1}\text{m}^{-1}$ and the October 2010 at $5.6 \pm 0.1 \text{Lmg}^{-1}\text{m}^{-1}$. This demonstrated a drop for the October 2010 and 2011 of 42.9 % and a 27.3 % respectively in the SUVA values. This suggested that the DOC characteristics had altered or the commissioning of Cwellyn’s updated plant (DAF cell upgrade 2011) had improved the flocculation process.

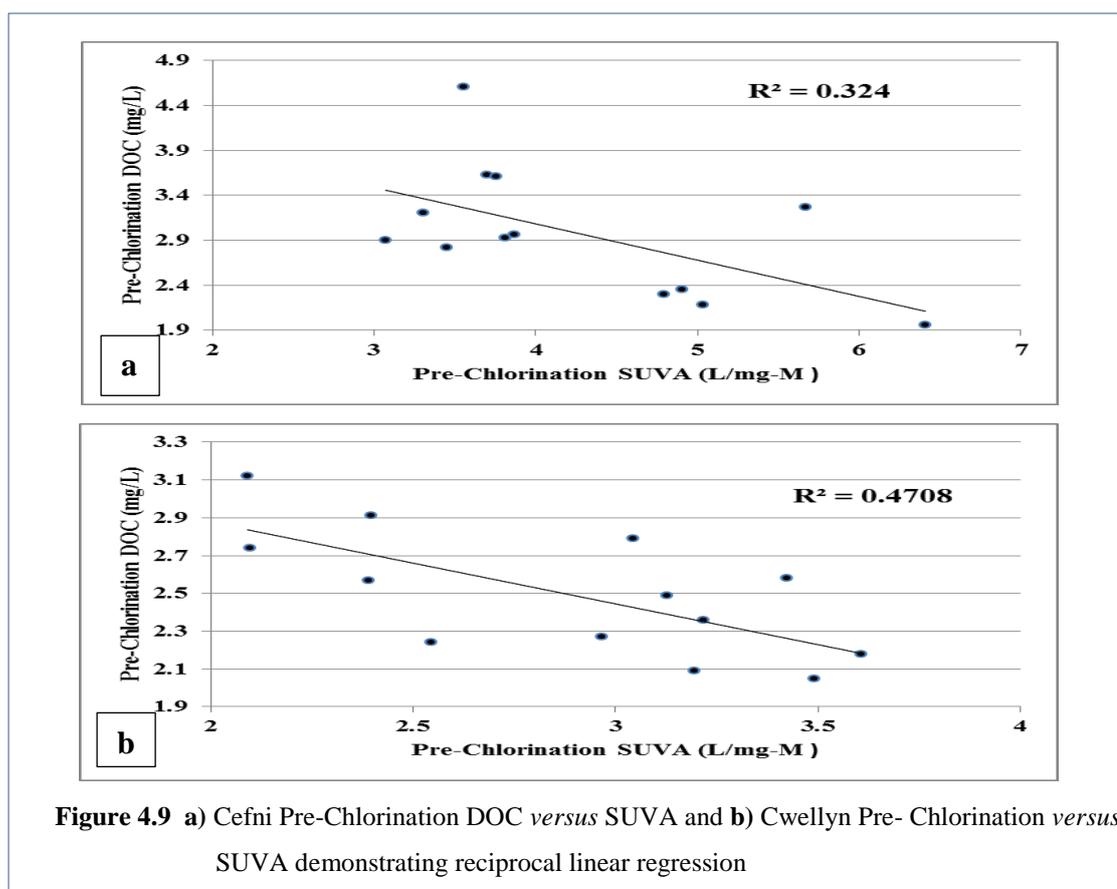
Fig 4.7 a and b show the Pre-Chlorination DOC and SUVA values. With the SUVA values normalised and representing the value/mg of DOC, a trend was demonstrated that

suggested that the SUVA values tended to counter the DOC concentrations in both of the sample series, i.e., a number of high DOC concentration values corresponded with low SUVA and *vice-versa*. Statistical analysis (Pearson correlations) demonstrated that this was so, and the reciprocal relationships were significant, Cefni Pre-Chlorination ($r = -0.569$, $p < 0.05$) and Cwellyn Pre-Chlorination ($r = -0.686$, $p < 0.05$). The linear regression curves (Fig 4.9 a and b) demonstrated a similar response to the data with R^2 values for Cefni and Cwellyn exhibiting 0.324 and 0.471 respectively.



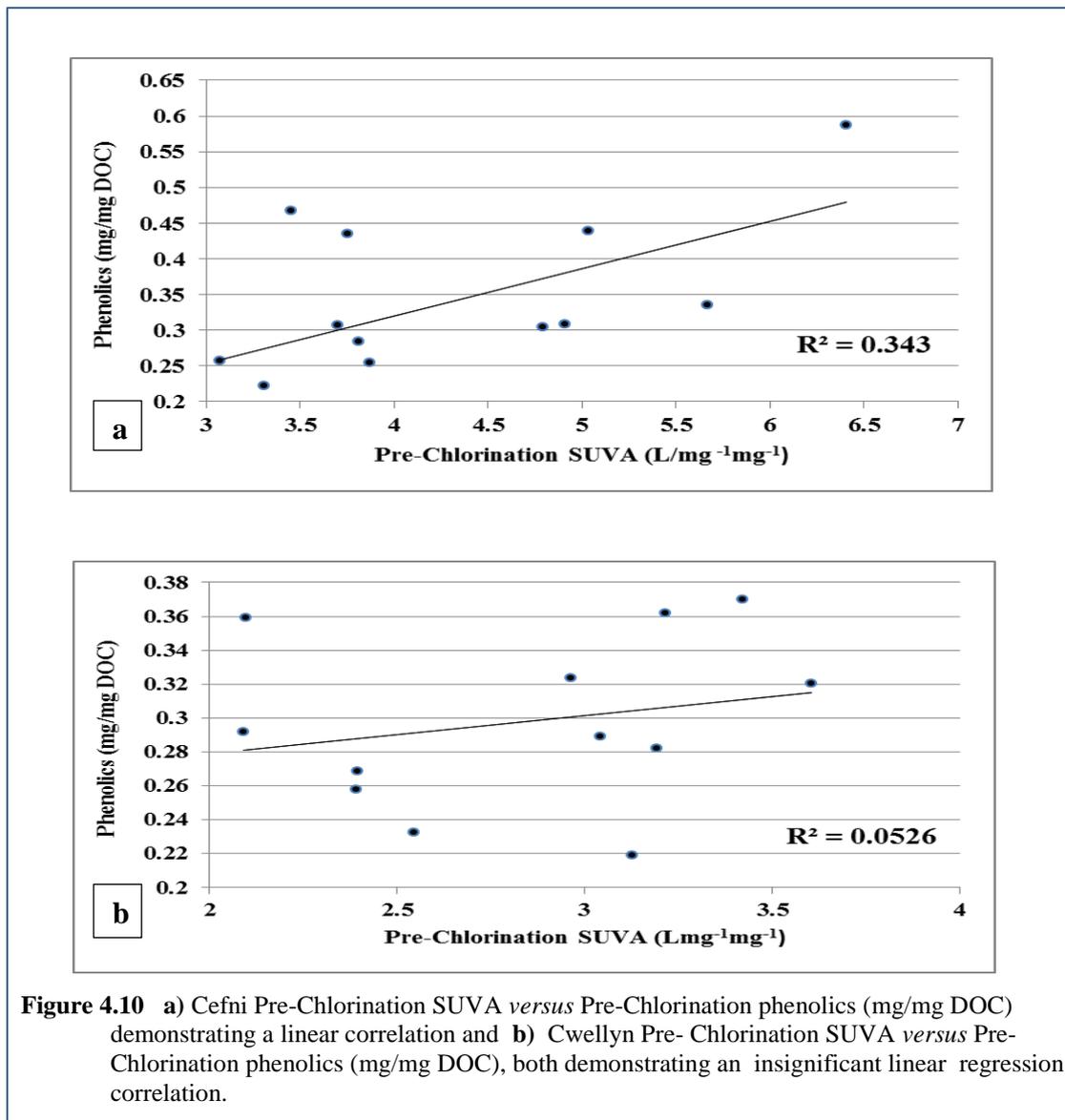
A comparison of the SUVA values for the Pre-Chlorination and Raw Surface Water DOCs are shown in Fig 4.8 (a,b). The graphs demonstrated that the SUVA values of Cefni and

Cwellyn differed, with the Cefni having a higher average value for the Pre-Chlorination samples (4.2 L/mg-M, SD 1.0) compared to the Raw Surface Water samples (3.4 L/mg-M, SD 0.5), while Cwellyn’s SUVA values were the reverse, with the averaged Pre-Chlorination samples (2.9 L/mg-M, SD 0.5) lower and the Raw Surface Water samples (4.1 L/mg-M, SD 1.1) higher. This suggested that the Cefni treatment works on average removed marginally higher concentrations of the aliphatic or non-absorbing DOC while Cwellyn’s treatment removed the more aromatic DOC. This again may relate to the flocculation process of phenolics discussed in (Section 4.3.2). The Cefni aromatic structures may be predominantly phenolic, hence are not removed effectively by coagulation. As the SUVA values are normalised values, the decrease in the DOC concentration (the divisor in the SUVA calculation) increases the value above that of the Raw Surface Water values, therefore it could be suggested that the actual concentration of the aromatics are biased towards phenolics and may exhibit a minimal concentration change in the Cefni between the Raw and Pre-Chlorinated Waters.



Examination (Fig 4.10 a and b) of the Pre-Chlorination SUVA values versus the Pre-Chlorination phenolic concentrations (mg/mg DOC) did not exhibit a significant correlation for the Cwellyn data (R^2 0.053), but the Cefni did exhibit a weak linear

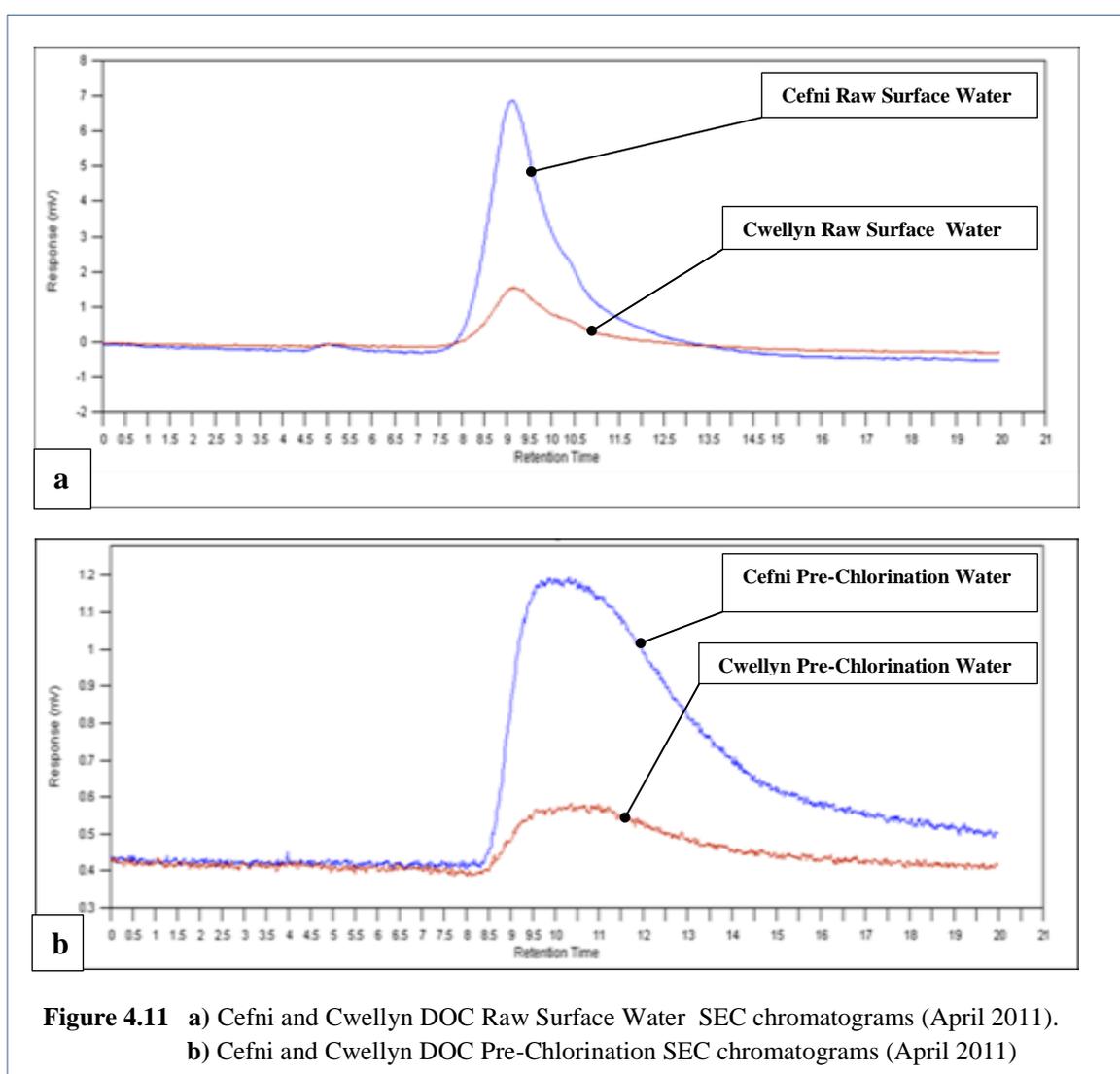
correlation (R^2 0.343). This again, suggests that the concentration of molecular structures relative to the DOC concentration had a consistent phenolic characteristic.



The data also suggested (Fig 4.10 b) that the Cwellyn, although demonstrating higher SUVA values than the Cefni, unlike the Cefni, a relatively linear relationship between the SUVA and phenolics was absent which suggests that although aromatic in character its phenolic content inconsistent.

4.3.4 Size Exclusion Chromatography (SEC) of Pre-Chlorination Samples.

The total peak areas (Fig 4.11 a) for the Raw Surface Water DOC concentrations for Cefni and Cwellyn averaged 1009.1 ± 50 and 191.7 ± 9 mV.s, respectively. After processing at the treatment works (Fig 4.11 b), the Pre-Chlorination sample for Cwellyn demonstrated a peak area of 59.4 ± 3 mV.s and the Cefni, a peak area of 171.9 ± 8 mV.s. The results before and after treatment demonstrated that the Cefni Raw Surface Water DOC (determined by SEC peak area) was reduced by approximately 82.9% and Cwellyn's by 69.0%. This suggested that the treatment process (April 2011) did not remove 31 % of Cwellyn's and 17.1 % of Cefni's molecular compounds that absorbed at the 254 nm wavelength normally associated with aromatic/conjugated systems.



These differences calculated using the peak areas were also mirrored by the percentage differences taken between the Raw Surface Water and Pre-Chlorination Water SUVA values (Figs 4.8 a,b) of Cwellyn (31 %) and Cefni (23.7 %) for April 2011. Although the Cefni demonstrated a difference of approximately 6.6 % between the two, this may be accounted for because the peak area being calculated by the SEC instrument was within the limits of the standards (Fig 3.12 a)

The monthly Pre-Chlorination samples collected from both the Cefni and Cwellyn sampling points annotated [A] (Figs 4.1 and 4.2) were analysed as per (Method 2.3.3). The results (Fig 4.12 (a and b) show the specific ranges of molecular masses (MM) as percentages of the overall MM distribution for each sample.

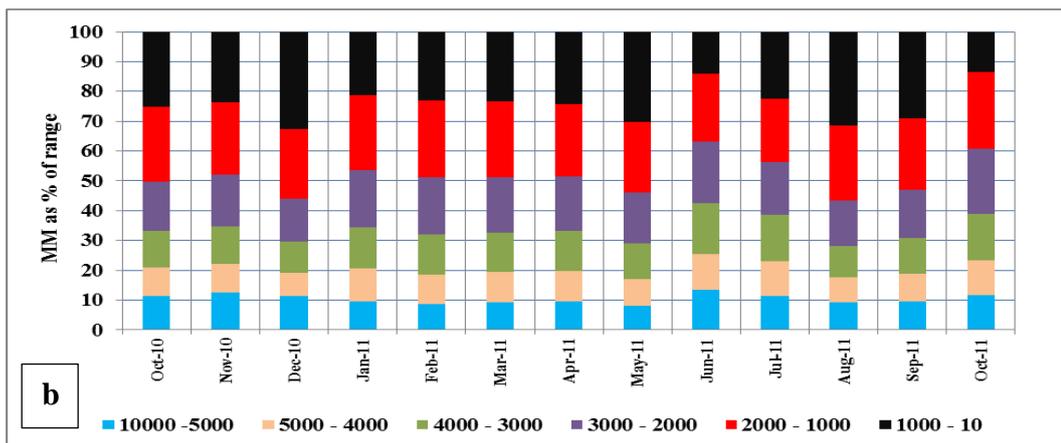
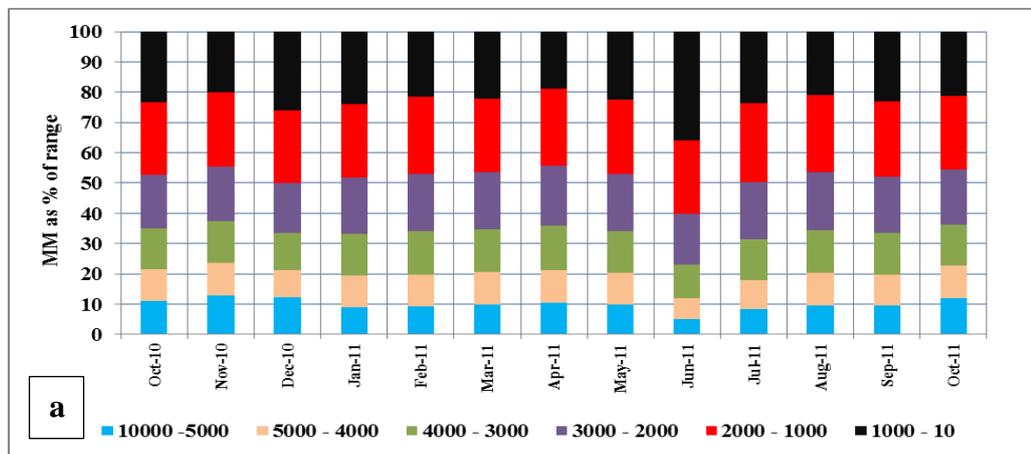


Figure 4.12 a) Cefni Pre-Chlorination SEC, showing MM distribution ranges (%) for each sample collected October 2010 – October 2011.
b) Cwellyn pre-chlorination SEC, showing MM distribution ranges (%) for each sample collected October 2010 – October 2011.

Both Cefni and Cwellyn demonstrated similar distributions with two of the ranges 2000 - 1000 Da and the 1000 - 10 Da dominating approximately 50% of all the sample distributions, with the other 50% consisting of four ranges representing 10000 - 2000 Da. The Raw Surface Water distributions (Chapter 3, 3.1.4) for both the Cefni and Cwellyn's lake DOCs were dominated by the 5000 - 3000 Da distribution range, with the MM range 5000 – 4000 Da representing 29.4% for the Cefni and 24.5% for Cwellyn and the 4000 – 3000 Da range consisted of 55.3 % and 59.6 % for the Cefni and Cwellyn, respectively. This demonstrated that the Raw Surface Water MM distributions, after passing through the treatment works had been altered by the flocculation and coagulation process, easily removing the larger macro molecules. Both Cefni and Cwellyn's Raw Surface Water MM distribution range percentages were very similar in both the 5000 – 4000 and 4000 – 3000 Da. Post treatment, the Pre-Chlorination MMs of both ranges for both of the treatment works exhibited a drop in the MM range % to give similar final % distribution values (Table 4.2).

Table 4.2 Molecular mass range averaged percentage changes after Cefni and Cwellyn treatment works.

Lake/Reservoir	Raw Surface Water 5000 – 4000 Da (%)	Raw Surface Water 4000 – 3000 Da (%)	Pre-chlorination Water 5000 – 4000 Da (%)	Pre-chlorination Water 4000 – 3000 Da (%)
Cefni	29.4	55.3	10.1	13.5
Cwellyn	24.5	59.6	9.9	13.2

The (Đ) polydispersity, (representative of polymer homogeneity) of the DOC polymeric structures within the MM distributions were determined for the Pre-Chlorinated DOC using the 'Cirrus' software (section 3.1.5) utilising (Eq 3.1) and the results are tabulated (Table 4.3).

Table 4.3 Polydispersity of Pre-Chlorination Water samples of Cwellyn and Cefni with the Raw Surface Water results in brackets

Month	Cefni (Mn/Mw)	Cwellyn (Mn/Mw)
Oct-10	4.3 (4.1)	4.4 (4.0)
Nov-10	4.7(3.8)	3.8 (3.3)
Dec-10	6.2(5.2)	6.0(5.9)
Jan-11	7.1(6.4)	5.2(5.6)
Feb-11	3.7(3.6)	4.4(5.0)
Mar-11	3.2(2.4)	4.5(3.2)
Apr-11	3.9(2.7)	4.8(3.4)

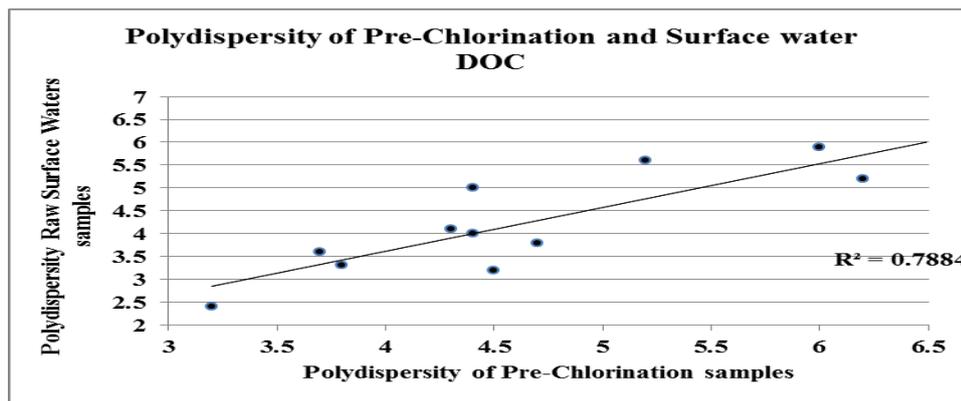


Figure 4.13 Correlation between Raw Surface Water and Pre - Chlorination water for Cefni and Cwellyn

A significant linear relationship ($R^2, 0.7884$) was established between the polydispersity of the Raw Surface and Pre-Chlorination water DOC (Fig 4.13), suggesting that although the DOC was quantitatively reduced the remaining DOC proffered a similar molecular multimodal distribution. This tended to suggest that the mixture of polymer chain lengths and accompanying MM were both relatively similar in both the Raw Surface Water and the Pre-Chlorination Water samples of Cefni and Cwellyn, although they may have exhibited different flocculation charge characteristics, thus reducing their removal during processing through the treatment plant. The gradient in the graph suggests that the \bar{D} changes over the period October 2010 to April 2011. The same data presented in (Fig 4.14) demonstrates that the \bar{D} increases in both Cefni and Cwellyn DOC for the Raw Surface Water and Pre-Chlorination Water from November 2010 to January 2011.

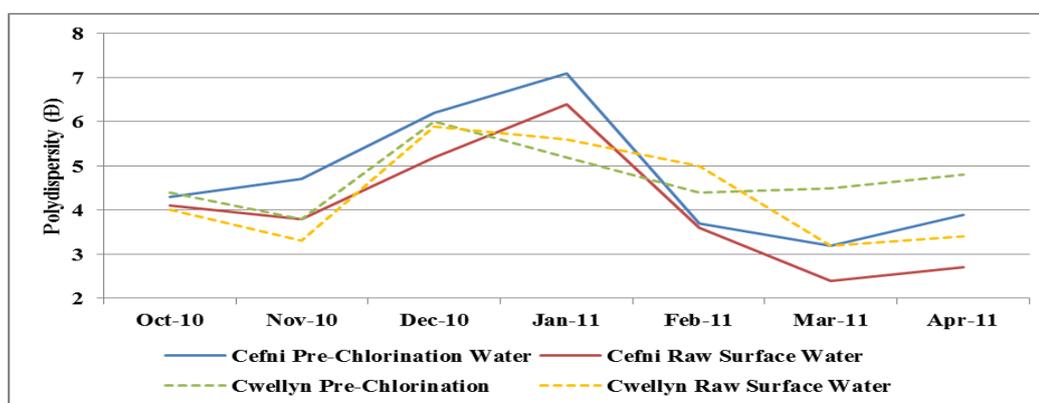
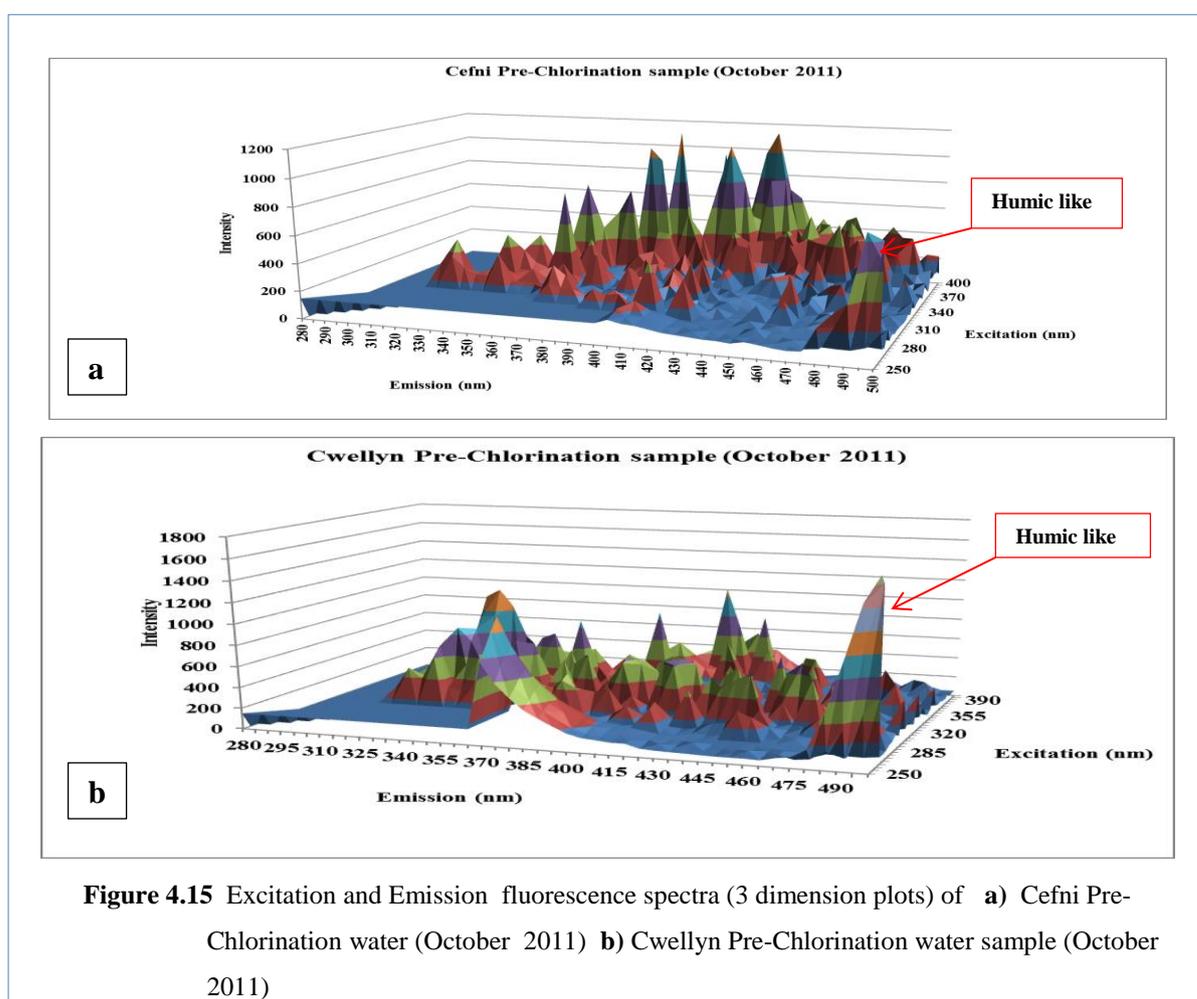


Figure 4.14 Cefni and Cwellyn \bar{D} values for both Raw Surface Water and Pre-Chlorination DOC (October 2010 – April 2011)

This suggests that it may be a response to reduced microbe activity during the colder mid-winter period (Fig 3.17), resulting in a reduction of polymer chain breakdown to an increased number of relatively uniform masses.

4.3.5 Fluorescence Spectroscopy of the Pre-Chlorination Samples

Determination of the principal fluorescence components of the Cefni and Cwellyn pre-chlorination samples was carried out using method 2.3.5 (Chapter 2), and PARAFAC analysis was applied to the data using MATLAB software and the DOMFluor toolbox to determine the principal fluorescing components in the Pre-Chlorination water samples.



The three surface plots (Fig 4.15 a,b) show the raw excitation and emission data before PARAFAC analysis. Raman scatter was corrected for, by subtracting the Milli-Q water spectrum from each sample spectrum (Hua *et al.*, 2010). As in the Raw Surface Water

spectra (Fig 3.18 a,b,c,d), they both showed an intense fluorescing emission at 475-490 nm which was assigned as ‘broad humic like’ (Stedmon *et al.*, 2011). The 22 samples were then loaded into the MATLAB program and each of the spectra was explored for outliers, negative and scatter peaks as described in Chapter 3, 3.1.5.

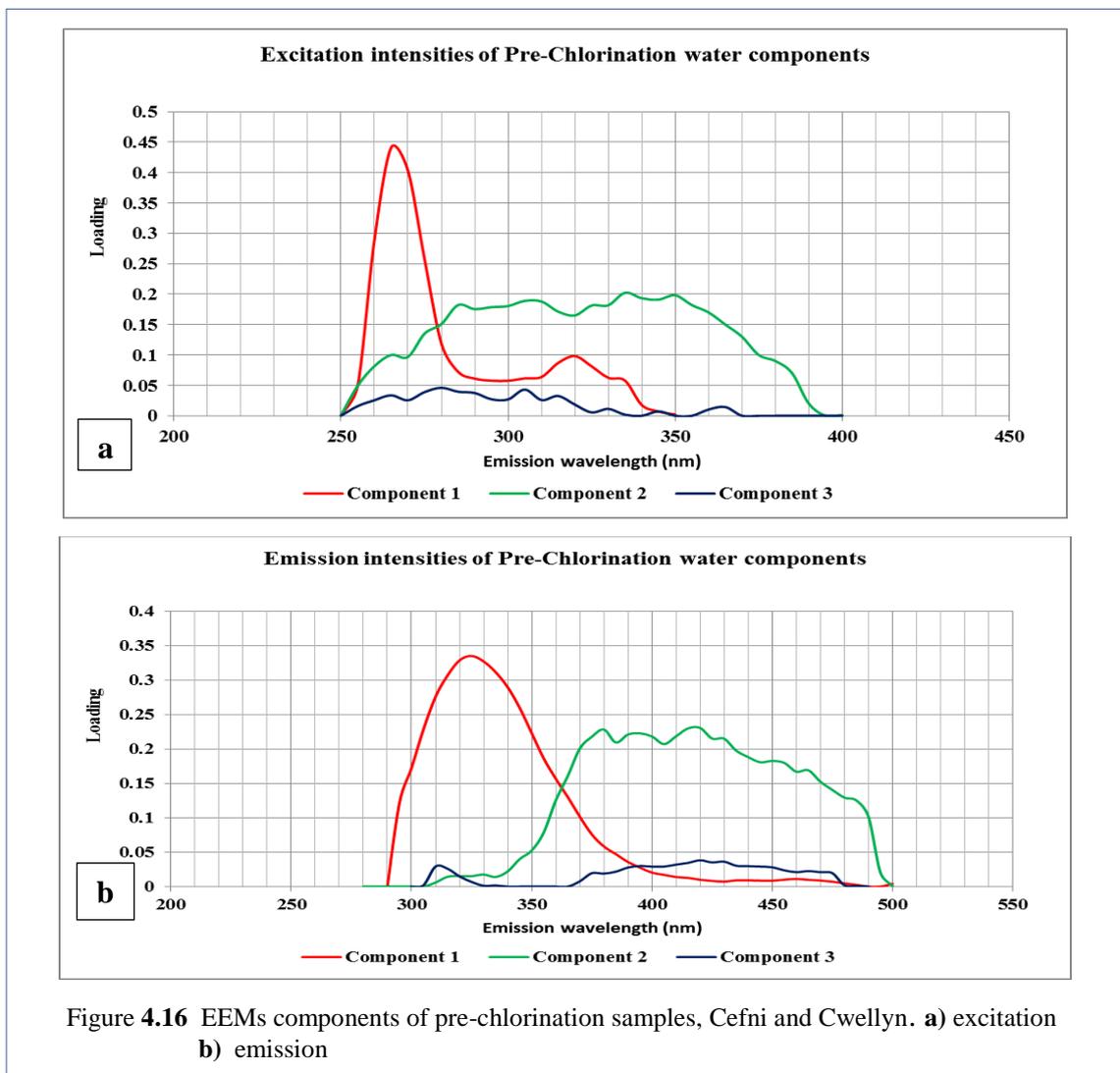


Figure 4.16 EEMs components of pre-chlorination samples, Cefni and Cwellyn. a) excitation
b) emission

The results generated a model that identified the three most common components with the highest fluorescing intensity values. The results were exported out of the program into an Excel file and the data is presented in graphical form to show the relative excitation/emission intensities (Fig 4.16, a and b). Identification (Table 4.4) of the 3 peak emission intensities was carried out using the available information published in the literature.

Table 4.4 Fluorescence excitation/emission maxima and potential identification, suggested from references.

Component	Peak Pair (Ex/Em) nm	Identification
1	250 - 280/300 - 400	Terrestrial Humic like [F], [H], [D]
2	250- 400/ 350 - 500	Humic like, [E], [I], fulvic like [I].
3	250 - 350/370 - 470	Humic like, protein like [A], [C], [H]

([A] Coble, 1996; [B] Marhaba *et al.* 1999; [C] Hua *et al.* 2009; [D] Her *et al.* 2003; [E] Stedmon *et al.* 2003; [F] Ishii *et al.* 2012; [G] Bagoth *et al.* 2011; [H] Leenheer and Croue' 2003; [I] Goslan *et al.* 2004.).

4.3.6 Cation and Anion Analysis of Pre-Chlorination Samples.

The ion concentration profiles of the two Pre-Chlorination samples (Figs 4.13 a, b) demonstrated high sulphate (SO_4^{2-}) concentrations for both. The mean concentration for the Cefni (60.7 mg/L, SD 14.57) was approximately six times that of Cwellyn (10.6 mg/L, SD 0.54). The Raw Surface Water samples for Cefni and Cwellyn averaged (13.65 mg/L, SD 1.57) and (2.55 mg/L, SD 0.36) respectively. The SO_4^{2-} concentration difference between the Raw Surface Water and Pre-Chlorination water represented a percentage increase for both Cefni and Cwellyn of 77.6 % and 75.9 % respectively. When considering the ion concentrations, it should be noted that both Cwellyn and Cefni use $[\text{Al}_2(\text{SO}_4)_{3\text{aq}}]$ as a flocculation reagent, so it is suggested that the higher residual concentrations of the SO_4^{2-} ion was a carry-over from the treatment process. Cefni's pre-chlorination water also exhibited higher Ca^{2+} average concentrations (48.8 mg/L, SD 16.3) compared to the Raw Surface Water (31.97 mg/L, SD 10.1). This increased concentration also suggested that it may have resulted from the treatment process from the lime addition, pH adjustment Cefni, (Fig 4.2). Cwellyn's Ca^{2+} levels (1.5 ± 0.08 mg/L) remained similar to the raw water (1.4 ± 0.07 mg/L) although there is facility to add lime. The dominant cation of the Cwellyn samples was Na^+ and the concentration relative to the averaged Raw Surface Water more than doubled, from 4.5 mg/L (SD 0.28) to 10.6 mg/L (SD 1.4). This again suggested that this was as a result of the treatment process, as Na_2CO_3 is used for pH correction (Flowpath 4.1). The Cefni although having a higher average Na^+ than Cwellyn did not show an increase after processing and remained at a level of 14.2 mg/L (SD 4.6) , 0.3 mg/L lower than the original Raw Surface Water concentration (14.5 mg/L).

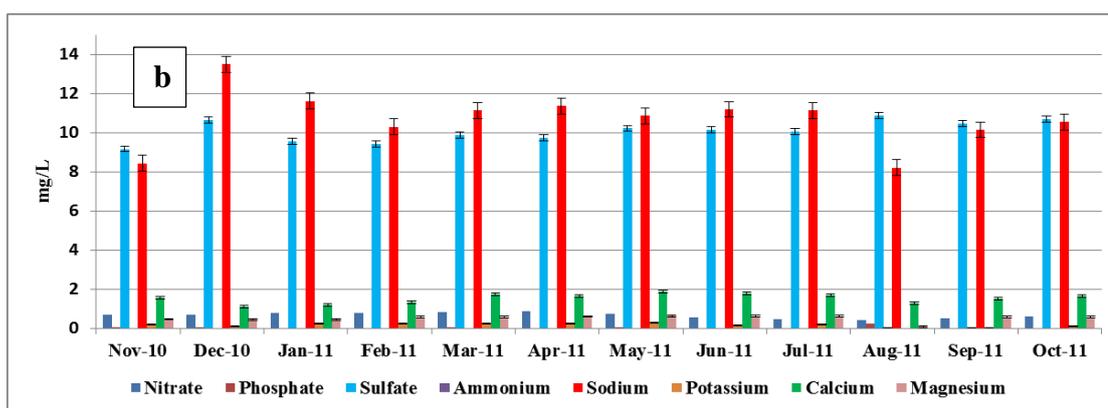
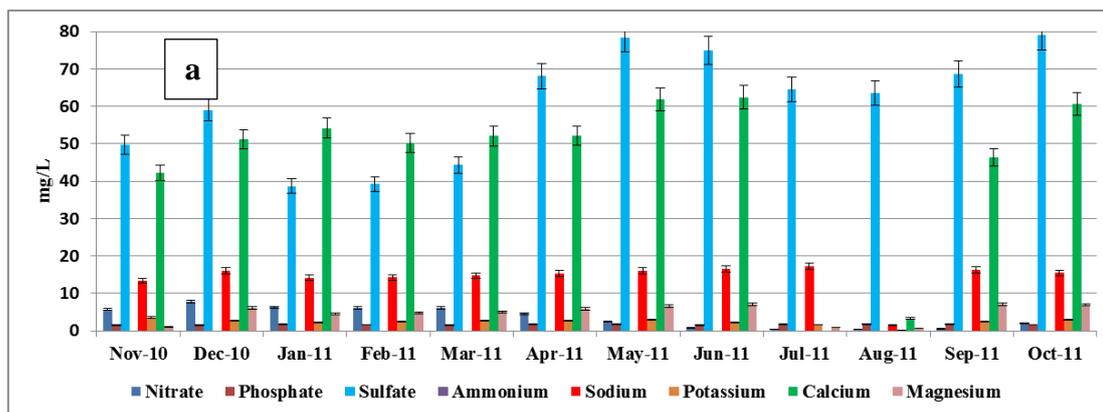


Figure 4.17 Cation and anion concentrations (mg/L) for **a**) Cefni pre-chlorination ion concentrations (mg/L) **b**) Cwellyn pre-chlorination ion concentrations (mg/L)

The results (Table 4.5) of the other cation and anion concentrations before and after treatment showed much smaller changes with the exception of the phosphate concentrations for both the Cefni and Cwellyn which increased from an average of 0.34 and 0.03 mg/L respectively before treatment to 1.62 ± 0.08 mg/L and 0.07 ± 0.004 mg/L, respectively after treatment. This change, again suggested a treatment input from the H_3PO_4 which was added during the process to reduce plumbo-solvency of lead pipes in the distribution system (Water Treatment Works Operators Manual, 1999). The addition of 0.5 mg/L of orthophosphoric acid to water with a $pH < 7$ prevents the lead found in old pipework and soldered joints being released, by forming an insoluble coating of $Pb_3(PO_4)_2$ (Water and Wastewater Treatment, 1999). From the results (Table 4.5) the Cwellyn concentration of 0.07 mg/L (70 μ g/L) after treatment does not suggest that the increase was as a result of addition, therefore it can only be considered as contamination of the sample by an unspecified artefact.

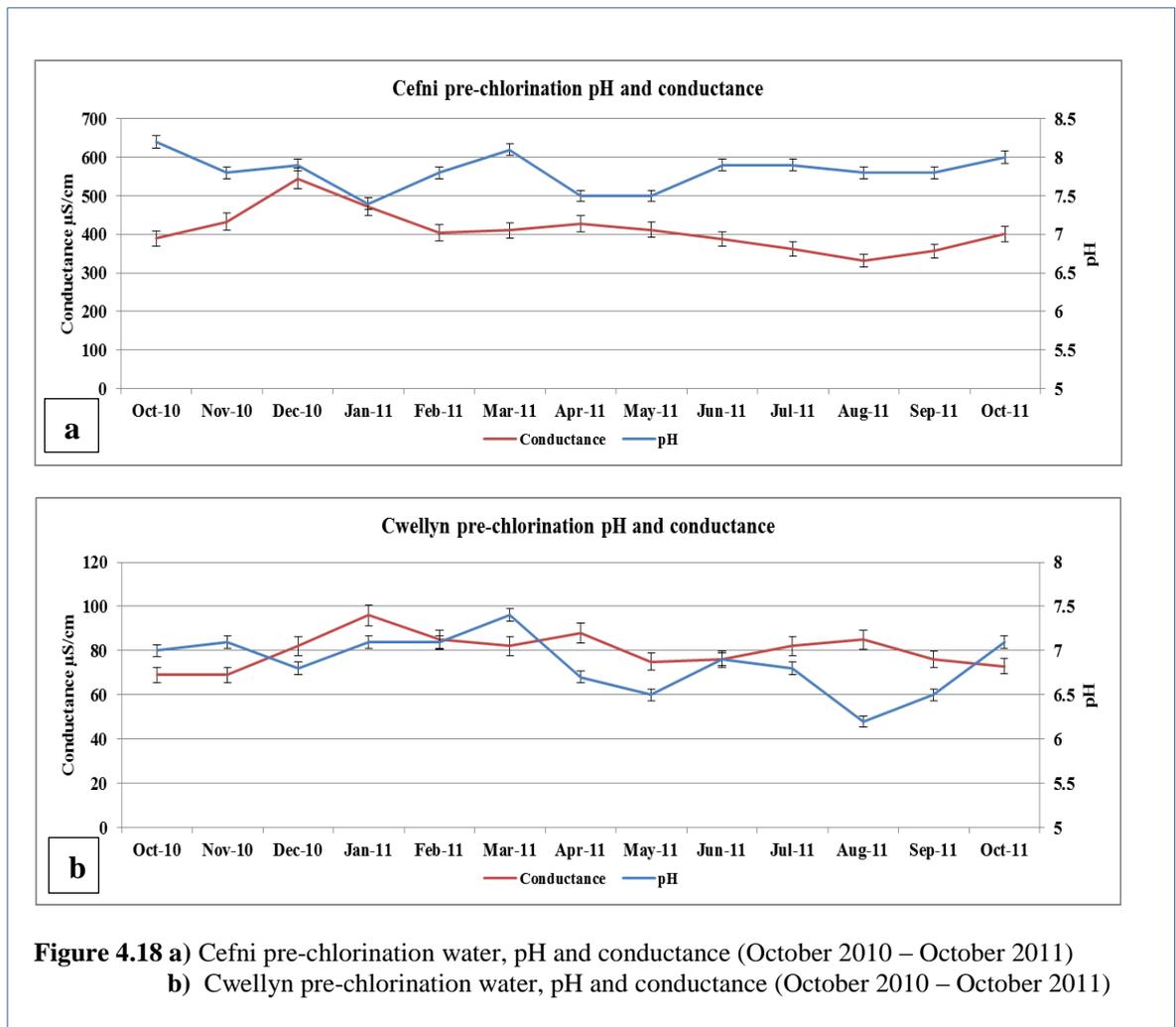
Table 4.5 Comparison of average ion concentrations in pre-chlorination and surface water Cefni and Cwellyn samples.			
Lake/Reservoir	Cation/Anion	Raw Surface Water (average mg/L)	Pre-Chlorinated Water (average mg/L)
Cefni	Fluoride	0.07	0.05
	Bromide	0.07	0.07
	Nitrate	3.61	3.59
	Phosphate	0.34	1.62
	Potassium	2.48	2.38
	Magnesium	5.34	4.71
Cwellyn	Fluoride	0.02	0.01
	Bromide	0.01	0.02
	Nitrate	0.65	0.66
	Phosphate	0.03	0.07
	Potassium	0.20	0.18
	Magnesium	0.48	0.51

4.3.7 pH and Conductivity of Pre-Chlorination samples.

The pH and conductivity (Figs 4.11 a,b) were measured for both Pre-Chlorination samples within 3 hours of collection. The Cefni's average pH for the period was 7.8 ± 0.2 (SD 0.2), which was relatively similar to the Raw Surface Water ($\text{pH } 8.11 \pm 0.2$, SD 0.4) over the comparable months. Cwellyn also demonstrated little change in the pH; the Pre-Chlorination samples averaged 6.8 ± 0.2 (SD 0.3) whilst the comparable Raw Surface Waters averaged $\text{pH } 6.7 \pm 0.2$ (SD 0.3).

Conductivity measurements, after treatment with coagulants $[\text{Al}_2(\text{SO}_4)_3]$ and other reagents including H_3PO_4 , CaCO_3 and Na_2CO_3 , demonstrated an increase compared to the Raw Surface Water for both sites. The Cefni's average conductivity increased from, 312 $\mu\text{S/cm}$ for the Raw Surface Water samples to an average of $410.2 \pm 10 \mu\text{S/cm}$ for the Pre-Chlorination water samples. Cwellyn's conductivity for the comparable samples were $46.7 \pm 1 \mu\text{S/cm}$ for the Raw Surface Water and $82.2 \pm 2 \mu\text{S/cm}$ for the Pre-Chlorination samples. This increase represented a 24% and a 43 % increase in Cefni and Cwellyn's conductivity after treatment. The overall percentage increase in the measured ion concentrations between the Raw Surface Water and Pre-Chlorination was 47 % and 58 % for Cefni and Cwellyn respectively. Although Cefni (Fig 4.14 a) had the highest average conductivity, it also demonstrated a large increase in December 2010 to $545 \pm 11 \mu\text{S/cm}$ (average $410.2 \pm 10 \mu\text{S/cm}$) after which it declined to its lowest in August 2011 (332 ± 7

$\mu\text{S}/\text{cm}$). This was accompanied by an increase in both the SO_4^{2-} ($+ 9.2 \pm 0.6 \text{ mg}/\text{L}$) and Ca^{2+} ($+ 8.9 \pm 0.5 \text{ mg}/\text{L}$) ion concentrations. A step increase was also recorded over a two month period November 2010 to January 2011 for the Cwellyn's conductance which represented an increase from 82 to 96 $\mu\text{S}/\text{cm}$, but no increase in SO_4^{2-} , Ca^{2+} or any other ions in the suite analysed for was observed. This suggests that another ion species may have been responsible for the small increase. Cwellyn's conductivity profile demonstrated very little fluctuation during early to late spring of 2011 and after January's 2011 high conductivity it dropped to $83 \pm 1.7 \mu\text{S}/\text{cm}$ in March and continued at a relatively similar level from April to October 2011.

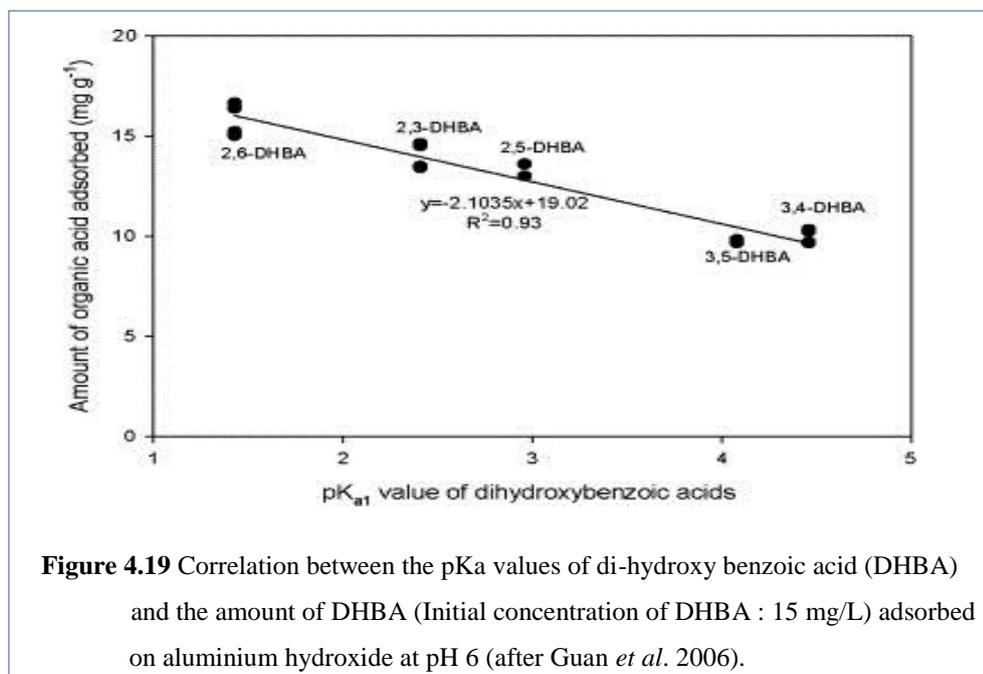


4.4 Discussion

The Pre-Chlorination sample analytical profile compared to the Raw Surface water demonstrated that the treatment processes', filtration, flocculation and pH adjustments altered a number of the Raw Surface Waters characteristics. Both treatment works had a similar scheme of treatment (Figs 4.1 and 4.2), but with two initial differences, ie., the Cefni with a basic pH is dosed with H₂SO₄ and the Cwellyn with a water feedstock that is neutral to acidic has Na₂CO₃ added as required. The coagulant [Al₂(SO₄)_{3aq}] is used by both treatment works with the flocculation process aided by stirring. The flocculated particulates are buoyed to the surface by micro air bubbles forming a sludge blanket, with the clarified water being removed *via* a series of baffles. The water is then passed through a series of filters to remove any coagulant carry over and suspended solids. Again in Cwellyn a further step is introduced, where the filtered water is UV irradiated to ensure pathogens are destroyed. The next stage in the process for both treatment works is, chlorination, where the water is dosed with chlorine in the contact tank. The chlorinated finished water product is then transferred to a holding reservoir before distribution.

The key changes observed during processing, over the process is the drop in DOC concentration. Both Cwellyn and Cefni demonstrated DOC removal of up to 57.7 and 62.9 %, respectively. Seasonal trends were reflected in the results and significant correlations between Raw Surface Water and Pre-Chlorination samples for DOC was evident with Cefni (r 0.678, $p < 0.05$) generating a stronger Pearsons coefficient than Cwellyn's (r 0.426, $p < 0.05$). This may have been due to a number of factors such as lower concentrations of more easily coagulated DOC present in the Cwellyn, or possibly the commissioning of the treatment works upgrade during of 2010 – 2011 which included a series of new flocculation tanks. Hence optimum coagulation process had not been fully refined during this sampling period. Although the overall DOC in both Cefni and Cwellyn pre-Chlorination samples suites dropped, the phenolic content of the DOC concentration remaining after treatment was higher by 23.3 % in the Cefni and 9.7% in the Cwellyn, suggesting that the treatment processes did not effectively remove OH substituted molecular structures. This may have been due to the range of pKa values associated with the phenolics, as their dissociation are greatly influenced by pH, other substituents and their position (Sykes, 1991). For example, Guan *et al.* (2006) reported pKa values for the hydroxyls of a series of di-hydroxy benzoic acid (DHBA) structures, ranging from 9.9 to 13.95 compared to the DHBA carboxyl functional group pKa values ranging from 1.43 – 4.08 (Fig 4.19). In the study, Guan *et al.* (2006) identified the involvement of the phenolic

groups with the Al^{3+} complexation and demonstrated that OH groups *ortho* to each other, or to a carboxylic, tended to increase the HPOA complexation with Al^{3+} as the phenolic groups were deprotonated. Deprotonation increased with increasing pH, hence cogulation and flocculation of molecular structures with high OH substitution would be subject to the constraints of process pH.



Hence it could be suggested that the majority of the Cefni's DOC phenolics may be structurally different to that of the Cwellyn's and/or that the pH adjustments carried out before addition of the coagulant may have suppressed dissociation or even protonated the groups. This would have had an effect on the subsequent removal efficiency, which is considered to be optimal at pH 5-6 for aluminium coagulants (Amirtharajah and O'Melia, 1990).

SUVA which is considered an indicator of the aromaticity of DOC, again demonstrated changes in the Pre-Chlorination samples relative to the Raw Surface Water SUVA results. Figs 4.5 a,b demonstrated that the relative aromaticity of the Cefni increased after treatment and reduced in the Cwellyn samples. Again as discussed above this may be ascribed to the DOC characteristics influencing the removal efficiencies. For example, the absorbance values for Cefni Pre-Chlorination and Raw Surface Water samples at 254 nm differed by 0.01 A.U, but the DOC concentration in the Pre-Chlorination sample was lower. Hence the SUVA value would show an increase above the value of the Raw Surface Water SUVA value, demonstrating an increase in the overall relative aromaticity of the

sample. This suggests that although DOC concentrations are lowered by the process, the relative aromaticity is increased by the flocculation due to the selectivity of the removal process in the Cefni. This did not happen for the Cwellyn; the fall in DOC concentration in the Pre-Chlorination samples was also accompanied by a fall in the DOC aromaticity according to the calculated SUVA values.

An overall change in the MM distribution could be seen after the surface water had been passed through the process (Figs 4.12 a,b). Analysis of the SEC curves demonstrated the reduction of the two principal ranges in the surface water DOC, namely the 5,000 – 4,000 and 4,000 – 3,000 Da from a relative contribution of > 80% to < 24 %. The major ranges within the DOC after processing were the 2,000 – 1,000 and 1,000 – 10 Da (50%), which the treatment process was less effective at removing (Wong *et al.*, 2007). The polydispersity of the samples demonstrated significant correlations with the Raw Surface Water polydispersity (Fig 4.13) indicating that the process modified DOC retaining similar polymeric dimensions within the MM distributions left. This tends to suggest that the polydispersity of each range is similar and remains a heterogeneous mixture of MMs.

The PARAFAC analysis result for the Pre-Chlorination water exhibited only 3 fluorescing components compared to the 4 fluorescing components in the Raw Surface Water. The absent component was classified in the Raw Surface Water DOC as humic like with a large MM. This may have been associated with the SEC distributions (Figs 4.12 a and b) that demonstrated a reduction in the 5,000 to 2,000 Da range and an increase in the < 2,000 Da in the Pre-Chlorination samples (3.14 a,b,c and d). Although PARAFAC analysis reduced a considerable volume of work when analysing large data sets, the literature appears to be limited in its interpretation, with a number excitation and emission ranges ascribed to ‘humic like’. This together with the broad range and overlaps of the excitation and emission wavelengths limit its value as an analytical tool.

Differences were also recorded in the ion concentrations of the pre-chlorination samples compared to the Raw Surface Water. In the Cefni, SO_4^{2-} replaced the Ca^{2+} in the surface water as the dominant species with Cwellyn both increasing the Na^+ and SO_4^{2-} concentrations in its samples after processing. These changes reflected additions made during the process, such as the flocculation reagent ($\text{Al}_2(\text{SO}_4)_3$), the addition of H_2SO_4 and NaHSO_4 and Na_2CO_3 for pH adjustment. As discussed in 4.3.6, the increase in PO_4^{3-} was due to the reagent source. H_3PO_4 which is added to the Raw Surface Water (Figs 4.1, 4.2) during processing as an inclusion to reduce dissolution of Pb from the household and

distribution system's lead pipework. An interesting reference was made by Werner *et al.* (2007) of another source of phosphates from ruptured algal cells releasing their accumulated ions. Although this may not be the principal source of PO_4^{3-} in the Pre-Chlorination water, evidence of an increase from June – September 2011 in the Cefni may suggest a contribution from its algal blooms. The late spring PO_4^{3-} concentration increased from 1.5 ± 0.1 mg/L to an average of 1.7 ± 0.1 mg/L through June to September, after which it fell back to an average of 1.5 ± 0.1 mg/L. This was not observed in the Cwellyn which is considered to be low in algal growth during the summer months.

Although the pH going into the chlorine contact tank was adjusted as close as possible to neutral (pH 7) the Cefni did demonstrate alkalinity (pH 7.8 ± 0.2) at that point which would accordingly promote an increase in trihalomethane formation potential (Rodrigues *et al.*, 2006). Cwellyn's pH was seen to be relatively neutral tending to slightly acidic (pH 6.8 ± 0.2) which was not dissimilar to the Raw Surface Water pH. These also are considered to impact the flocculation process which as mentioned above is optimised at pH 5 - 6 for aluminium salt coagulants.

4.5 Conclusions

This chapter has investigated the Raw Surface Water after it has been processed in the Cefni and Cwellyn water treatment plants.

- The results established that > 50 % of the DOC was removed by filtration and flocculation, but the residual Cefni DOC after passing through the process had higher concentrations of phenolic type molecular systems per mg of DOC remaining compared to the Raw Surface Water.
- Cwellyn samples also demonstrated a percentage increase in its phenolics post cogulation.
- Cefni's Pre-chlorination SUVA values were higher than its Raw Surface Water, but Cwellyn's Pre-Chlorination values were lower than its Raw Surface Water SUVA values.
- The treatment process increased the concentration of some ion species, namely Ca^{2+} and SO_4^{2-} and PO_4^{3-} for the Cefni and principally Na^+ for Cwellyn. These increases resulted from the reagents used in the treatment process.

- Treatment of the Raw Surface Water using filtration and the coagulant $[\text{Al}_2(\text{SO}_4)_3]$ reduced the MM range 5000 – 3000 Da of the Raw Surface Water.

Chapter 5

**Tri-halomethane Formation Potential (THMFP)
of the Raw Surface Water and Cefni and Cwellyn
Pre-Chlorination Dissolved Organic Carbon
(DOC) samples.**

Chapter 5 Tri-halomethane Formation Potential (THMFP) of the Raw Surface Water and Cefni and Cwellyn Pre-Chlorination Dissolved Organic Carbon (DOC) samples.

5.1 Introduction

Although a number of other halogenated by-products are formed during this process (Goslan *et al.* 2009), the ones regulated in the United Kingdom to a maximum total concentration at the consumer's tap of $100\mu\text{g/L}$ are trichloromethane; CHCl_3 , dichlorobromomethane; CHBrCl_2 , dibromochloromethane; CHBr_2Cl and tribromomethane; CHBr_3 (DWI, 1998). This chapter investigates the THM formation potential of DOC in Raw Surface Water in its natural state prior to any form of treatment with a view to identifying any common associations that may predict changes or influence THM formation potential.

THMFP of the four Raw Surface Water sources, Cefni, Cwellyn, Conwy and Teyrn collected between October 2009 and October 2011 was carried out on a regular monthly basis, with the exception of the samples October 2009 to June 2010. Their THMFP analysis using the Varian GC (Gas Chromatography) 450 was delayed due to commissioning and validation issues which were satisfactorily completed by June 2010.

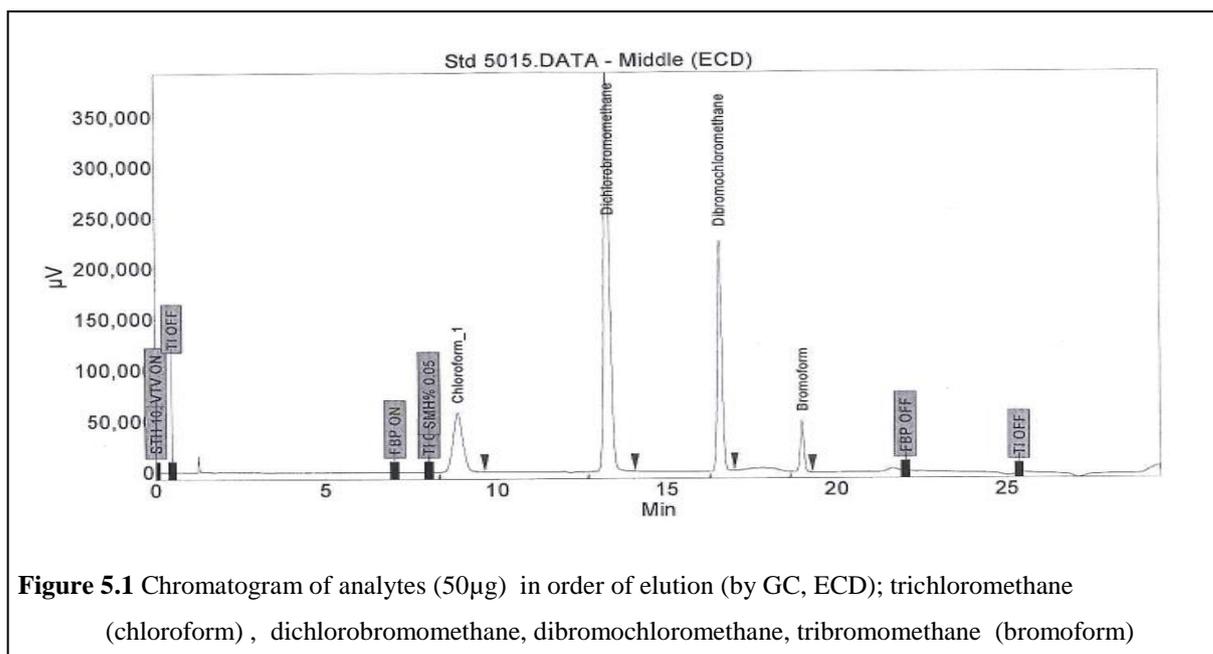


Fig 5.1 shows a typical chromatogram of the target THM analytes each at a concentration of $50 \pm 1.5 \mu\text{g/L}$ with their respective retention times (RT); CHCl_3 (8.84 mins), CHBrCl_2 (13.27 mins), CHBr_2Cl (16.55 mins), CHBr_3 (18.99mins).

5.2 Results of Raw Surface Water Dissolved Organic Carbon (DOC) Tri-halomethane Formation Potential (THMFP) .

The presentation of the THMFP analytical results for the period November 2009 to October 2011 for all the four lakes set out in the text, begins with the tri-halomethane (THM) that has the shortest retention time (RT) CHCl_3 followed by CHBrCl_2 , CHBr_2Cl and CHBr_3 .

5.2.1. Trichloromethane Formation Potential of Cefni, Cwellyn, Conwy and Teyrn (DOC: 1mg/L).

The concentration of trichloromethanes in the four lake sources sampled over the 24 month period exhibited a number of variations (Fig 5.2). Conwy demonstrated the highest concentrations throughout the series with two exceptions, March 2010 and June 2011 when the Cefni's concentration was higher than Conwy by $12 \pm 0.4 \mu\text{g/mg DOC}$ and $20 \pm 0.6 \mu\text{g/mg DOC}$ respectively.

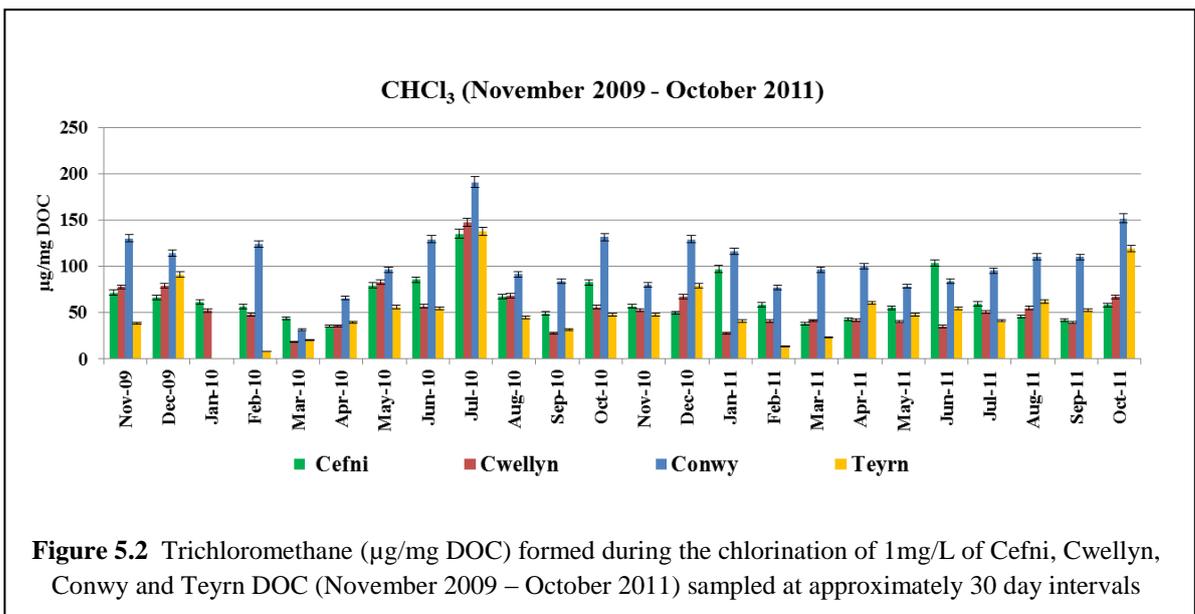


Figure 5.2 Trichloromethane ($\mu\text{g/mg DOC}$) formed during the chlorination of 1mg/L of Cefni, Cwellyn, Conwy and Teyrn DOC (November 2009 – October 2011) sampled at approximately 30 day intervals

The highest formation potential of CHCl_3 for the 24 months in all four lakes was recorded in July 2010. Conwy DOC demonstrated the highest at $190 \pm 5.7 \mu\text{g}/\text{mg DOC}$ followed by Cwellyn, Teyrn and Cefni (147 ± 4.4 , 138 ± 4.1 , $135 \pm 4.1 \mu\text{g}/\text{mg DOC}$) respectively. The lowest concentration of CHCl_3 was recorded for March 2010, when each of the four lakes formed $< 50 \mu\text{g}/\text{mg DOC}$, this was again observed in March 2011 with the exception of Conwy which formed $96 \mu\text{g}/\text{mg DOC}$ of CHCl_3 . Although seasonal trends could not be determined from the results, the formation potential of CHCl_3 demonstrated significant correlations.

Table 5.1 Correlations of CHCl_3 formation potentials of Cefni, Cwellyn, Conwy and Teyrn (November 2009 – October 2011)

Lake	Cefni	Cwellyn	Conwy	Teyrn
Cefni		0.595**	0.477*	0.436*
Cwellyn	0.595**		0.634**	0.697**
Conwy	0.477*	0.634**		0.729**
Teyrn	0.436*	0.697**	0.729**	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

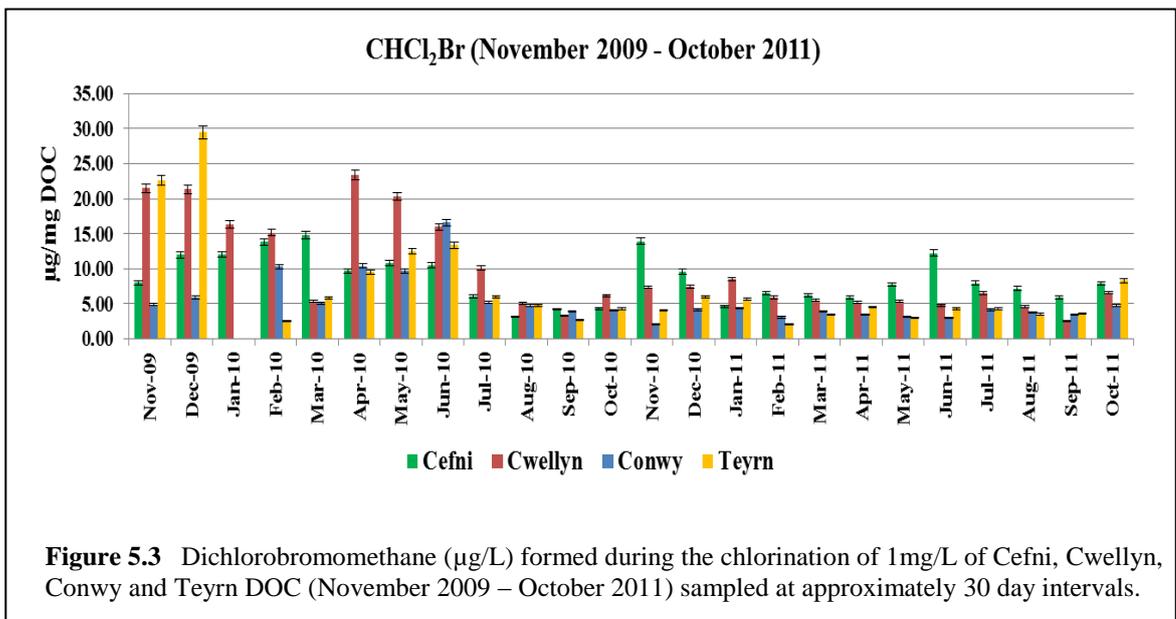
From the correlation results (Table 5.1), the upland lake DOC, Cwellyn, Conwy and Teyrn demonstrated significant correlations at the < 0.01 level, while Cefni, although the results correlated with the upland ones, they exhibited a lower significance < 0.05 with Teyrn and Conwy.

5.2.2 Dichlorobromomethane Formation Potential of Cefni, Cwellyn, Conwy and Teyrn (DOC: 1 mg/L).

Dichlorobromomethane (CHCl_2Br) was the second highest concentrated component (Fig 5.3) of the four THMs analysed for during the laboratory chlorination/mg DOC of the lake/reservoir Raw Surface Waters.

The generation of CHCl_2Br was subject to Br^- being available in either the sample source or the HOCl during the chlorination step. Although Conwy DOC demonstrated a high formation potential for CHCl_3 the potential for forming CHCl_2Br was lower, with the highest concentration recorded ($16.58 \pm 0.5 \mu\text{g}/\text{mg DOC}$) for the June 2010 sample with no comparable increase to that observed in the July 2010 sample for CHCl_3 .

Concentrations remained below $5 \mu\text{g}/\text{mg DOC}$ for the remainder of the Conwy series. For the period November 2010 to October 2011, Cefni demonstrated the highest concentrations and averaged, $7.9 \pm 0.2 \mu\text{g}/\text{mg DOC}$ of the suite.



Both Cwellyn and Teyrn averaged $\leq 5.0 \pm 0.1 \mu\text{g/mg DOC}$ for the suite of samples from July 2010 to October 2011 with Cwellyn demonstrating the highest standard deviation (SD) of $\pm 3 \mu\text{g/mg DOC}$, with Teyrn concentrations showing less deviation at $\pm 1.5 \mu\text{g/mg DOC}$.

For the sampling period November 2009 to June 2010 the concentrations of CHCl_2Br in all lake samples were higher, especially in Cwellyn and Teyrn. Teyrn exhibited concentrations during the two months of 2009 that averaged $26.0 \pm 0.8 \mu\text{g/mg DOC}$. For the same two months, Cwellyn also demonstrated high concentrations of CHCl_2Br that averaged $21.34 \pm 0.6 \mu\text{g/mg DOC}$. These high concentrations were also recorded for the other brominated species (CHBr_2Cl , CHBr_3) and having considered possible contamination from other sources, (such as the reagents and glassware and SPME fibres) we concluded that it may have been propagated by a change in the DOC during the period storage before analysis. An experiment to establish this was carried out by comparing the THMFP of a series of new and aged samples (see Section 5.3) with the highest concentration of the series being recorded in April 2010 at $23.31 \pm 0.7 \mu\text{g/mg DOC}$.

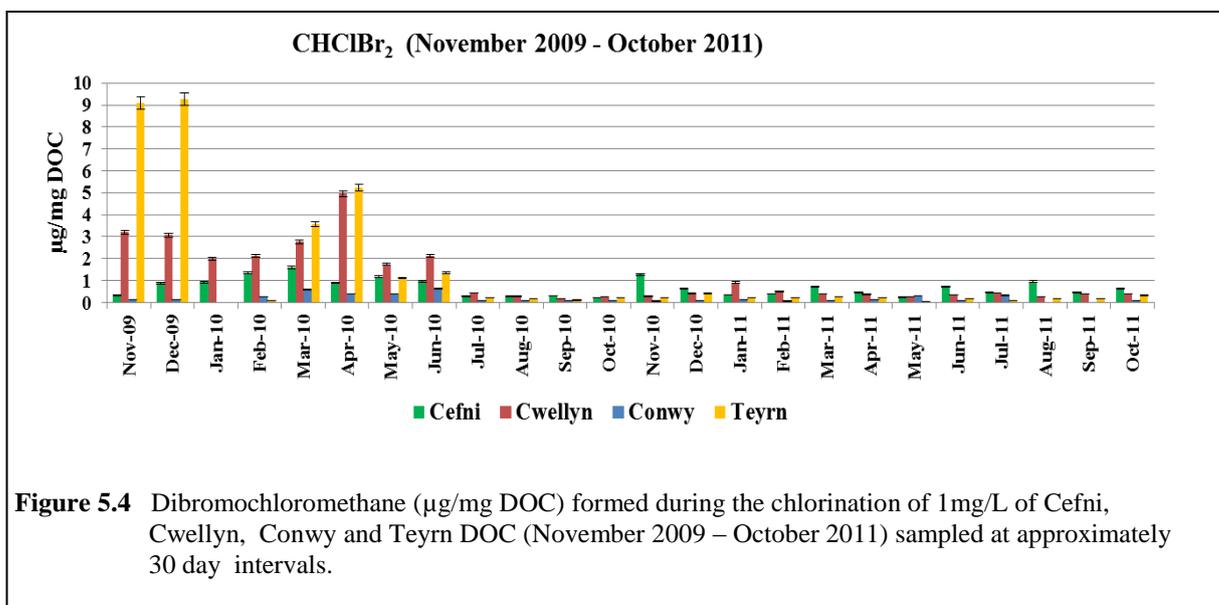
Lake	Cefni	Cwellyn	Conwy	Teyrn
Cefni		0.167	0.613*	0.084
Cwellyn	0.167		0.354	0.552*
Conwy	0.613*	0.354		0.596*
Teyrn	0.084	0.552*	0.596*	

* Correlation is significant at the 0.05 level.

Statistical analysis of the results (Table 5.2) from July 2010 to October 2011 demonstrate that Cefni had a significant correlation with the Conwy formation potential, but not with the other upland lake DOC. Teyrn exhibited significant correlation with both Cwellyn and Conwy, but Cwellyn and Conwy, although there was a relationship ($r = 0.354$) it was > 0.05 .

5.2.3 Dibromochloromethane Formation Potential of Cefni, Cwellyn, Conwy and Teyrn (DOC: 1 mg/L).

Again the results for the CHClBr_2 component of the THMs (Fig 5.4) showed both Cwellyn and Teyrn as having the highest formation concentrations/mg of DOC during November to December 2009 although both Cefni and, to a lesser extent, Conwy demonstrated relatively low concentrations for the same months. The high concentrations of CHClBr_2 continued but at a reduced level up to June 2010. This response to chlorination and an increase in brominated products had been observed in the formation of CHBrCl_2 and has been addressed in Section 5.3. Teyrn exhibited the highest concentrations ($9.2 \pm 0.3 \mu\text{g}/\text{mg DOC}$) of all the lakes during Nov/Dec 2009 and again in April 2010, both Teyrn and Cwellyn recorded high concentrations, with Cwellyn demonstrating its highest for the series ($5.2 \pm 0.2 \mu\text{g}/\text{mg DOC}$) Conwy had comparatively low levels of CHBr_2Cl throughout the 24 months, averaging $0.2 \pm 0.01 \mu\text{g}/\text{mg DOC}$.



The Cefni's CHClBr_2 formation potential over the 24 months averaged $0.7 \pm 0.02 \mu\text{g}/\text{mg DOC}$, but again as with all the brominated products, during the period Nov 2009 to June 2010 it expressed higher concentration that averaged $1.0 \pm 0.03 \mu\text{g}/\text{mg DOC}$, with the

highest concentration during March 2010 ($1.59 \pm 0.05 \mu\text{g}/\text{mg DOC}$). Higher than average concentrations for Cefni were again recorded in the months of November 2010, March and August 2011 at 1.27 ± 0.04 , 0.73 ± 0.02 and $0.95 \pm 0.03 \mu\text{g}/\text{mg DOC}$, respectively. Considering the higher of bromide concentrations in the Cefni samples (average $70 \pm 5 \mu\text{g}/\text{L}$) compared to the upland lakes (average $<10 \pm 0.7 \mu\text{g}/\text{L}$), the results suggest that DOC structures are an important controlling factor during chlorination.

Table 5.3 Correlations of CHClBr_2 formation potentials of Cefni, Cwellyn, Conwy and Teyrn (July 2010 – October 2011)

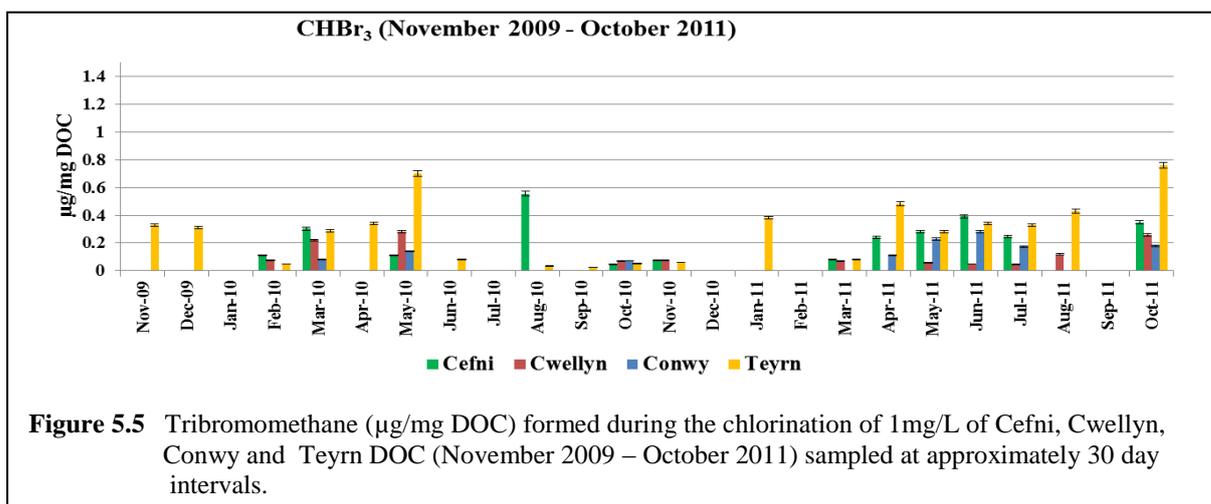
Lake	Cefni	Cwellyn	Conwy	Teyrn
Cefni		- 0.139	- 0.336	0.292
Cwellyn	- 0.139		0.081	0.280
Conwy	- 0.336	0.081		- 0.442
Teyrn	0.292	0.280	0.442	

There were no significant correlations between the CHClBr_2 formation potentials of the lakes, although Teyrn exhibited a correlation with the three others, but all were $p > 0.05$. This suggests that the formations were more random than the previous brominated analyte (CHCl_2Br).

5.2.4 Tribromomethane Formation Potential of Cefni, Cwellyn, Conwy and Teyrn (DOC: 1 mg/L).

The formation of CHBr_3 within the quartet of THMs, again relied on the presence of the bromide concentrations within the samples (see Section 5.6, effects of bromide on THMFP). The majority of the results were below the limit of quantification LOQ ($1.8 \mu\text{g}/\text{L}$) and limit of detection LOD ($0.54 \mu\text{g}/\text{L}$) parameters of the method. Hence they could not be considered to be of analytical value other than as indicators of the presence of CHBr_3 in the samples. For a number of months (January, July, December 2010 and September 2011) Figure 5.5 showed that CHBr_3 was below the LOD.

There were also a number of months when only one or two lake samples demonstrated any inference of CHBr_3 formation. Evidence of CHBr_3 was seen more consistently over the period from March 2011 to August 2011 when all the four lakes showed data close to the LOD, but a complete absence of CHBr_3 was recorded for September 2011.



The final set of results generated for October demonstrated a CHBr₃ presence in all four samples with Teyrn’s at 0.76 $\mu\text{g}/\text{mg}$ DOC being the highest, although still below the LOQ, with Cefni Cwellyn and Conwy having even lower concentrations at 0.35, 0.26 and 0.18 $\mu\text{g}/\text{mg}$ DOC respectively. Hence these are tentative results, that could not be confirmed.

A statistical analysis of the months where CHBr₃ was detected, although at very low concentrations, is shown in (Table 5.4). Teyrn demonstrated significant correlations with both Conwy and Cwellyn, but not with the Cefni.

Table 5.4 Correlations of CHBr₃ formation potentials of Cefni, Cwellyn, Conwy and Teyrn (July 2010 – October 2011)

Lake	Cefni	Cwellyn	Conwy	Teyrn
Cefni		- 0.138	0.334	0.160
Cwellyn	- 0.138		0.160	0.650*
Conwy	0.334	0.160		0.616*
Teyrn	0.160	0.650*	0.616*	

* Correlation is significant at the 0.05 level.

5.2.5 Tri-halomethane Formation Potential of Cefni, Cwellyn, Conwy and Teyrn samples, re – calculated to represent ‘as received’ DOC.

Using the analytical results (AR) in sections 5.2.1 – 5.2.4 that were reported as $\mu\text{g}/\text{mg}$ DOC, the results were re-calculated to express the THMFP as if the samples had not been diluted. The purpose was to illustrate the THMFP of each DOC source before any form of water treatment other than the laboratory removal of suspended solids was applied. The calculation was carried out by multiplying the Raw Surface Water DOC concentration

(mg/L) by the THMFP results ($\mu\text{g}/\text{mg}$ DOC). The re-calculated results (RCR) are reported as ($\mu\text{g}/\text{L}$).

The results (Figs 5.6 a,b,c and d) show that the Cefni had the highest concentrations of CHCl_3 for 15 out of the 24 months, whilst for the (AR) results (Fig 5.2) the Conwy demonstrated the highest, for 21 out of the 24 months.

The highest concentration of CHCl_3 recorded for the (RCR) 24 month series was for the Cefni in July 2010 at $999.12 \pm 30 \mu\text{g}/\text{L}$, compared to the uncorrected (AR) concentration of $147.01 \pm 4.4 \mu\text{g}/\text{mg}$ DOC. Conwy due to its lower actual DOC concentration exhibited an RCR value of $596.33 \pm 18 \mu\text{g}/\text{L}$ in contrast to its AR value of $190.61 \pm 5.7 \mu\text{g}/\text{mg}$ DOC. Teyrn and Cwellyn also demonstrated a potential for high concentrations of CHCl_3 during July 2010 exhibiting RCR values of 316.11 ± 9.5 and $358.01 \pm 10.7 \mu\text{g}/\text{L}$, respectively. The overall averages for the time series of both the diluted samples and the undiluted Raw Surface Water samples for the THM CHCl_3 are presented in Table 5.5.

Table 5.5 Mean concentrations of CHCl_3 in Cefni, Cwellyn, Conwy and Teyrn shown as concentration/mg DOC and corrected for actual surface water DOC concentrations in mg/L as sampled. NB DOC (mg/L) is a calculated concentration.				
Lake/Reservoir	DOC (mg/L) AR diluted concentration	Mean (AR) CHCl_3/mg DOC. ($\mu\text{g}/\text{mg}$)	DOC (mg/L) un-diluted concentration	RCR mean CHCl_3 ($\mu\text{g}/\text{L}$)
Cefni	1.0 ± 0.1	64.1 ± 1.2	8.0 ± 0.2	505.0 ± 10.1
Cwellyn	1.0 ± 0.1	54.5 ± 1.1	2.4 ± 0.1	129.6 ± 2.6
Conwy	1.0 ± 0.1	100.7 ± 2.0	4.5 ± 0.1	434.8 ± 8.7
Teyrn	1.0 ± 0.1	50.5 ± 1.0	2.2 ± 0.1	109.1 ± 2.2

Applying the concentration re-calculation to the CHCl_2Br results using the actual Raw Surface Water DOC concentrations demonstrated the potential to form the product (Fig 5.6 b). With its high DOC (average 8.0 mg/L) Cefni would have generated in November 2010 a RCR concentration of $133.13 \pm 4.0 \mu\text{g}/\text{L}$ and a low in August 2010 ($32.95 \pm 1.0 \mu\text{g}/\text{L}$). Re-calculation of the CHCl_2Br concentrations in the upland lakes, Cwellyn, Conwy and Teyrn, demonstrated that the Conwy was potentially the highest generator of the three with a concentration in May 2010 of $54.61 \pm 1.6 \mu\text{g}/\text{L}$.

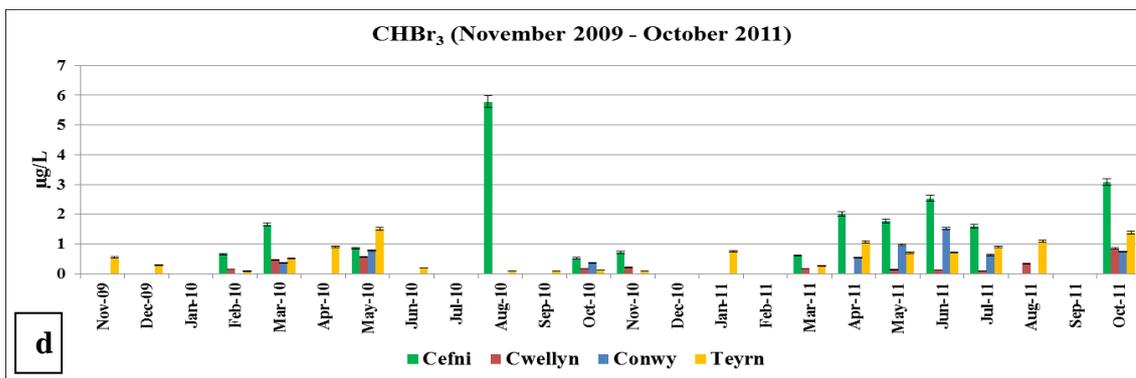
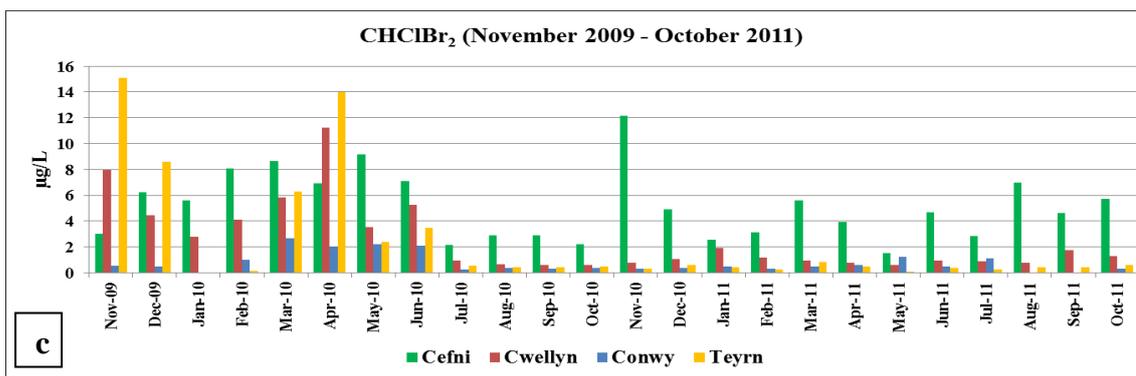
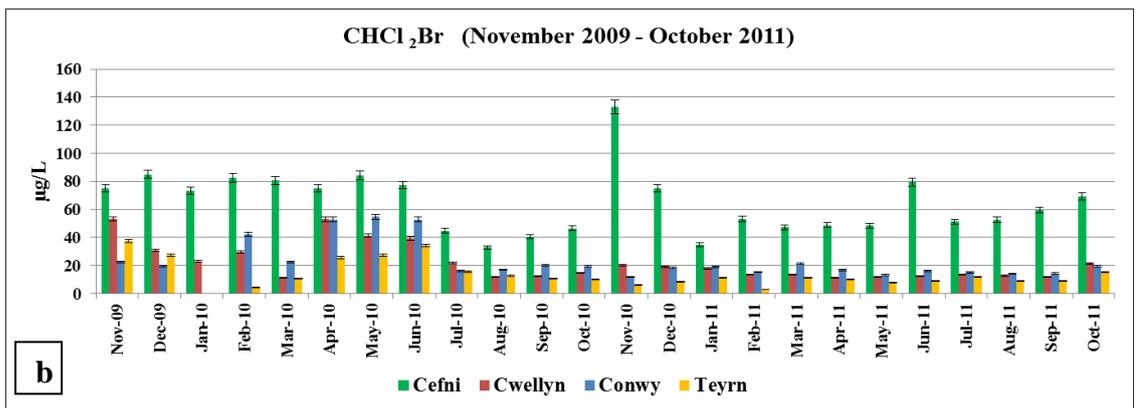
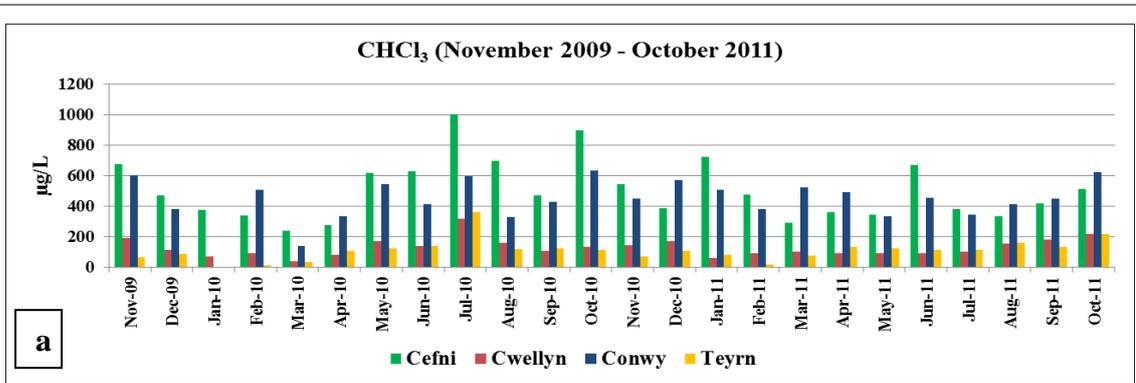


Figure 5.6 THMFP of Cefni, Cwellyn, Conwy and Teyrn re-calculated using the ‘as received’ Surface Raw Water DOC concentration results multiplied by the THMFP ($\mu\text{g}/\text{mg}$ DOC) of the diluted Raw Surface Water ($1\text{mg}/\text{L}$ DOC). **a)** CHCl_3 , **b)** CHCl_2Br , **c)** CHClBr_2 , **d)** CHBr_3 .

Cwellyn and Teyrn having lower DOC concentrations (< 3mg/L) demonstrated relatively low levels of re-calculated (RCR) CHCl₂Br, Cwellyn averaged from July 2010 to October 2011, 14.9 µg/L (SD 3.8) and Teyrn 9.9 µg/L (SD 3.2).

The tabulated means over the 24 month sampling period (Table 5.6) exhibited the THMFP of the DOCs relative to the THM CHCl₂Br before and after RCR. Cwellyn demonstrated the greatest (AR) before the high concentration of brominated analytes were not included in the calculation of the mean. A recalculation of the AR means, with the results for November 2009 to June 2010 excluded showed the Cefni (7.1 ± 0.1 µg/mg DOC) with the highest potential for forming CHCl₂Br with Conwy the lowest.

Table 5.6 Mean concentrations of CHCl ₂ Br in Cefni, Cwellyn, Conwy and Teyrn shown as concentration/mg DOC and corrected for actual surface water DOC concentrations in mg/L as sampled. The values in brackets represent the FP from July 2010 – October 2011, i.e. possible distortion of mean by the high concentration of brominated analytes November 2009 – June 2010. NB DOC (mg/L) is a calculated concentration.				
Lake/Reservoir	DOC (mg/L) AR diluted concentration	Mean (AR) CHCl ₂ Br/mg DOC. (µg/mg)	DOC (mg/L) un-diluted concentration	RCR mean CHCl ₂ Br (µg/L)
Cefni	1.0 ± 0.1	8.5 ± 0.2 (7.1)	8.0 ± 0.2 (8.3)	64.6 ± 1.3 (57.3)
Cwellyn	1.0 ± 0.1	9.7 ± 0.2 (5.9)	2.4 ± 0.1 (2.7)	21.7 ± 0.4 (14.9)
Conwy	1.0 ± 0.1	5.2 ± 0.1 (3.8)	4.5 ± 0.1 (4.5)	22.2 ± 0.4 (16.6)
Teyrn	1.0 ± 0.1	6.9 ± 0.1 (4.4)	2.2 ± 0.1 (2.3)	13.6 ± 0.3 (9.9)

The RCR mean results including the range between November 2009 and October 2011 show the Cefni with the highest formation potential (FP) for CHCl₂Br followed by Conwy, Cwellyn and Teyrn respectively. This sequence of FP concentrations is repeated when the high concentration of brominated analytes (November 2009 – June 2010) is not included in the calculation.

The RCR for the CHClBr₂ are shown (Fig 5.6 c). Although the results are theoretical and based on experimental data they do highlight how the individual Raw Surface Water total DOCs may have reacted to halogenation by chlorine and bromine. Teyrn with its relatively low DOC concentration (average 2.2 ± 0.07 mg/L), demonstrated the highest concentration of CHClBr₂ in November 2009 at 15.1 ± 0.5 µg/L, although this may have been a characteristic not normally exhibited by the sample, but may be as a consequence of a

storage influence (see Section 5.3) as previously mentioned. During April 2010 - October 2011 Cefni's concentrations of CHClBr_2 were recorded as the highest of all the four lakes. The November 2010 spike recorded for the Cefni for the RCR of CHCl_2Br data (Fig 5.6 b) was also seen in (Fig 5.6 c), for the CHClBr_2 suite which had a concentration of $12.2 \pm 0.4 \mu\text{g/L}$. The series for Cwellyn Conwy and Teyrn from June 2010 - October 2011 remained relatively low at $< 2.0 \mu\text{g/L}$, with Cwellyn's RCR increasing to 0.92, 1.8 and 1.3 $\mu\text{g/L}$ respectively for June, September and October 2011.

The means of the results for both the AR and RCR (Table 5.7) again demonstrated the potential reactivity and formation of CHClBr_2 over the 24 month period of sampling, with the mean (high concentrations of CHClBr_2 from (November 2009 – June 2010 removed) of the July 2010 – October 2011 shown bracketed. Cwellyn and Teyrn generated higher concentrations of $\text{CHClBr}_2/\text{mg DOC}$ than both the DOCs of Cefni and Conwy, but when corrected for the actual DOC concentration within the lakes, Cefni had the highest FP.

Table 5.7 Mean concentrations of CHClBr_2 in Cefni, Cwellyn, Conwy and Teyrn shown as concentration/mg DOC and corrected for actual surface water DOC concentrations in mg/L as sampled. The values in brackets represent the FP from July 2010 – October 2011, i.e. representative of the mean values, without the high concentration of brominated analytes recorded for November 2009 – June 2010, which may bias the mean values. NB DOC (mg/L) is a calculated concentration.				
Lake/Reservoir	DOC (mg/L) AR diluted concentration	Mean (AR) $\text{CHClBr}_2/\text{mg DOC}$. ($\mu\text{g}/\text{mg}$)	DOC (mg/L) un-diluted concentration	RCR mean CHClBr_2 ($\mu\text{g}/\text{L}$)
Cefni	1.0 ± 0.1	0.68 ± 0.01 (0.14)	8.0 ± 0.2 (8.3)	5.20 ± 0.10 (4.3)
Cwellyn	1.0 ± 0.1	1.16 ± 0.02 (0.05)	2.4 ± 0.1 (2.7)	2.50 ± 0.05 (1.0)
Conwy	1.0 ± 0.1	0.17 ± 0.01 (0.07)	4.5 ± 0.1 (4.5)	0.70 ± 0.01 (0.4)
Teyrn	1.0 ± 0.1	1.36 ± 0.03 (0.20)	2.2 ± 0.1 (2.3)	2.40 ± 0.30 (0.4)

Very low concentrations of CHBr_3 were recorded for all lake DOCs in the AR, with a number below the LOD ($0.54 \mu\text{g/L}$) and LOQ ($1.8 \mu\text{g/L}$). Re-calculation (RCR) of the analytical results (AR) results are shown in (Fig 5.6 d) and the mean results are tabulated in Table 5.8. The RCR means compared to the AR mean results and although they cannot be considered reliable (as we did not test the linearity of the DOC concentration relative to

chlorination) they do present a pattern that has been observed through-out the series; that is, with Cefni demonstrating the highest DOC concentration, it tended to dominate the highest concentrations when the ARs were corrected to the actual DOC concentration of the Raw Surface Water DOC. After Cefni, Teyrn tended to have the highest concentration of CHBr₃ which was twice that of the Conwy CHBr₃ concentration and over three times Cwellyn's concentration.

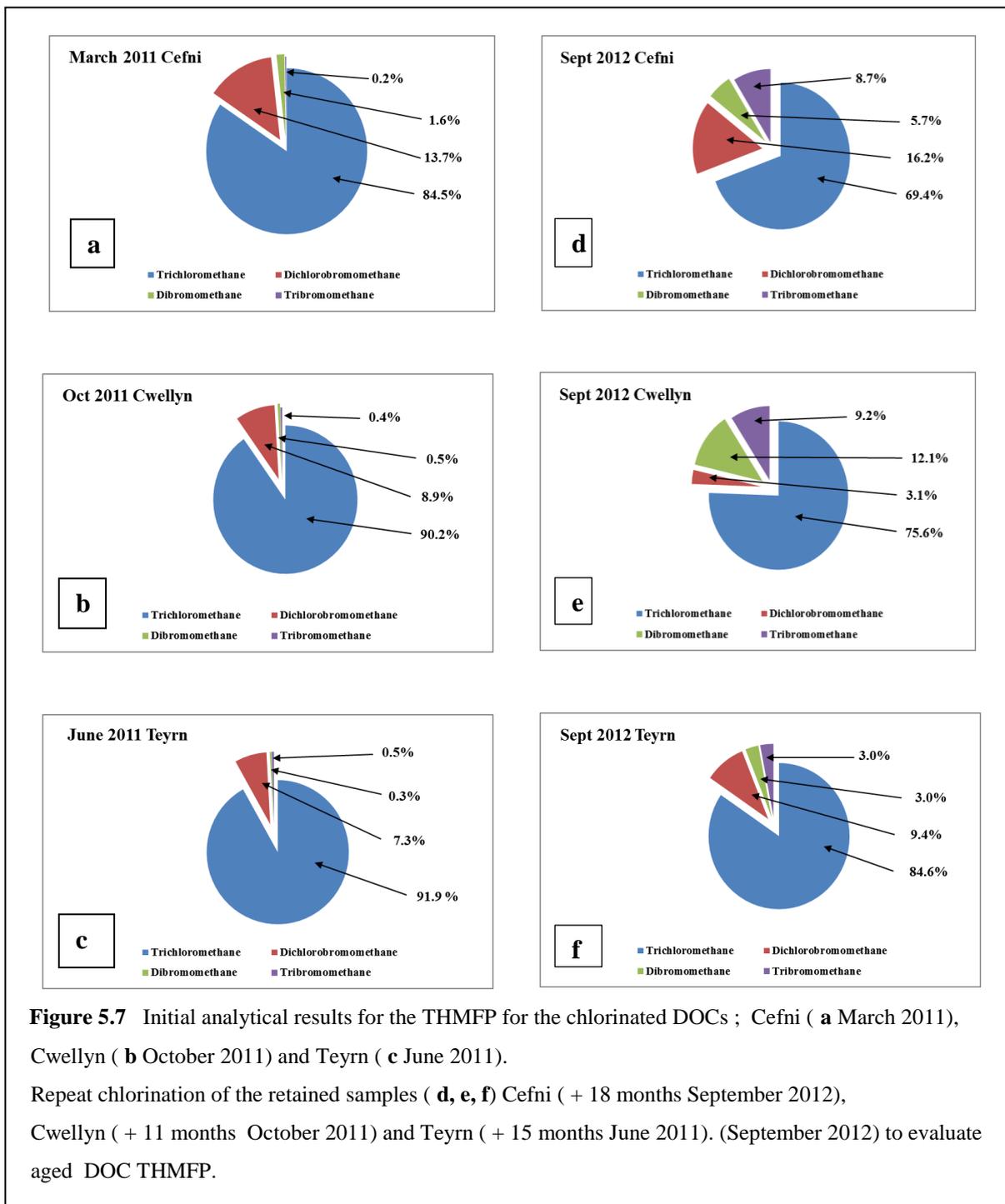
Table 5.8 Mean concentrations of CHBr ₃ in Cefni, Cwellyn, Conwy and Teyrn shown as concentration/mg DOC and corrected for actual surface water DOC concentrations in mg/L as sampled. The values in brackets represent the FP from July 2010 – October 2011, i.e. representative of the mean values, without the high concentration of brominated analytes recorded for November 2009 – June 2010, which may bias the mean values. NB DOC (mg/L) is a calculated concentration.				
Lake/Reservoir	DOC (mg/L) AR diluted concentration	Mean (AR) CHBr₃/mg DOC. (µg/mg)	DOC (mg/L) un-diluted concentration	RCR mean CHBr₃ (µg/L)
Cefni	1.0 ± 0.1	0.14 ± 0.01 (0.11)	8.0 ± 0.2 (8.3)	0.91 ± 0.10 (1.16)
Cwellyn	1.0 ± 0.1	0.05 ± 0.02 (0.06)	2.4 ± 0.1 (2.7)	0.14 ± 0.05 (0.13)
Conwy	1.0 ± 0.1	0.07 ± 0.01 (0.05)	4.5 ± 0.1 (4.5)	0.25 ± 0.01 (0.30)
Teyrn	1.0 ± 0.1	0.20 ± 0.03 (0.22)	2.2 ± 0.1 (2.3)	0.47 ± 0.30 (0.46)

5.3 Chlorination of Laboratory Stored Raw Surface Water DOC.

During the initial stages of the project samples were required to be stored at pH 2.8 in darkness at 4°C for periods ranging from 1 to 8 months (November 2009 – June 2010) before being chlorinated and analysed due to GC commissioning. The results for this delayed analysis appeared to present some anomalies in the results when they were compared to the samples that were analysed within a week of being received into the laboratory and chlorinated using Method 2.1.4.7. The series analysed for THMFP that had been stored for a period of time demonstrated a high influence of brominated analytes compared to the samples that had not been held in storage, although conditions of analysis and reagents of both sample sets were exactly the same, the only difference was the holding period in the laboratory storage. Hence this experiment was undertaken to examine

the influence of timed storage on the THMFP of a series of retained samples. The samples used, were original samples that were re- analysed at points ranging from 11 – 18 months after their initial sampling.

The samples were originally chlorinated and analysed during the month of collection, i.e., Cefni (March 2011), Cwellyn (October 2011) and Teyrn (June 2011). An un-chlorinated aliquot of the each surface water samples were stored at a pH 2.8 and 4° C in dark conditions. They were chlorinated and analysed in September 2012 as per Method 2.1.4.8.



The results established differences between the initial and aged samples (Fig 5.7) and an increase in the brominated species were recorded by all three samples. For example, Cefni which exhibited a FP of 84.5% (45.3 µg/mg DOC) for CHCl₃ for its initial analysis (March 2011) of the total THMs, demonstrated a drop to 69.4% (37.6 µg/mg DOC) when the sample was chlorinated and re-analysed 18 months later. The brominated analytes CHCl₂Br, CHClBr₂ and CHBr₃ initial analysis demonstrated a % total THM concentration of 13.7 % (6.1 µg/mg DOC) , 1.6% (0.7 µg/mg DOC) and 0.2 % (0.1 µg/mg DOC), respectively. For the re-chlorinated and re-analysed sample at 18 months the concentration THMFP % had increased to 16.2 % (10.6 µg/mg DOC) for CHCl₂Br, 5.7% (3.7 µg/mg DOC) for CHClBr₂ and 8.7% (5.7 µg/mg DOC) for CHBr₃. Table 5.9 shows the increases and decreases in the THMFP relative to the initial analysis.

Lake DOC	% Increase/Decrease CHCl₃	% Increase/Decrease CHCl₂Br	% Increase/Decrease CHClBr₂	% Increase/Decrease CHBr₃	Storage time (months)
Cefni	- 15.1	3	4.1	8.5	18
Cwellyn	- 14.6	5.8	11.6	8.8	11
Teyrn	- 7.3	2.1	2.7	2.5	15

Although, the results demonstrated a positive influence of storage time on the THMFP, the results had to be deemed in-conclusive. Reasons for this was limited by not having an adequate number of retained samples to fully evaluate the implied influence of stored aged samples. Hence, although the results were limited they suggest that the THM formation during the period November 2009 to June 2010 may have been influenced by their sample storage conditions and the original results should be treated with caution.

5.4 THMFP of Cefni and Cwellyn Pre-Chlorination DOC samples

To compare differences between Raw Surface Water, subjected to water treatment works coagulation, flocculation and filtration etc, and Raw Surface Water that had limited treatment (filtered 0.2 μm) in the laboratory, the Pre-Chlorination samples collected (Chapter 4) at the point before being chlorinated in the two water treatment works Cefni and Cwellyn were adjusted after analysis for DOC concentration (Method 2.3.1) to represent a DOC concentration of 1mg/L. The samples were chlorinated and analysed for THMFP as per Methods 2.3.7 and 2.3.8. The results are reported as μg of THM per 1mg of DOC and similarly to the results presented in (Section 5.2.5) they are re-calculated to represent the actual DOC concentration of the treated water being chlorinated in the treatment works. Samples of Finished Product (i.e. chlorinated at the treatment works and ready for distribution) were taken at the same time as the Pre-Chlorination and Raw Surface Water. These samples were analysed for THMs, as received within 4 hours of recovery by Method 2.3.8. Hence the results are reported in $\mu\text{g/L}$.

5.4.1 Results of the Total Tri-halomethanes Formation Potential of Pre-Chlorination DOC of Cefni and Cwellyn.

The results shown in Fig. 5.8 for both Pre-Chlorination samples demonstrate a similar pattern for the majority of both the Cefni and Cwellyn samples. The highest THM analyte formed in both suites was CHCl_3 . For both lakes the highest concentrations of total THMs was recorded for both Cefni and Cwellyn at 82 and 85 $\mu\text{g/mg}$ DOC for Cefni and Cwellyn respectively. Of this total Cefni's CHCl_3 was 79.9 ± 2 $\mu\text{g/mg}$ DOC and Cwellyn's 66.5 ± 2 $\mu\text{g/mg}$ DOC. These represented 97 % and 78 % of the total THMs formed during January 2011. Following this, the FP of CHCl_3 in both sample series dropped and was at its lowest in August 2011, Cefni (6.8 ± 0.2 $\mu\text{g/mg}$ DOC) and Cwellyn (15.1 ± 0.5 $\mu\text{g/mg}$ DOC), which represented 53 % and 88 % of the respective total THMs. The relatively low CHCl_3 concentrations recorded from July - August 2011 were seen to increase in October 2011. Cefni demonstrated an 8 fold increase in concentration to 55.3 ± 2 $\mu\text{g/mg}$ DOC, while the Cwellyn doubled to 31.1 ± 0.9 $\mu\text{g/mg}$ DOC. These represented a CHCl_3 inference of total THMs of 75 % and 83 % respectively.

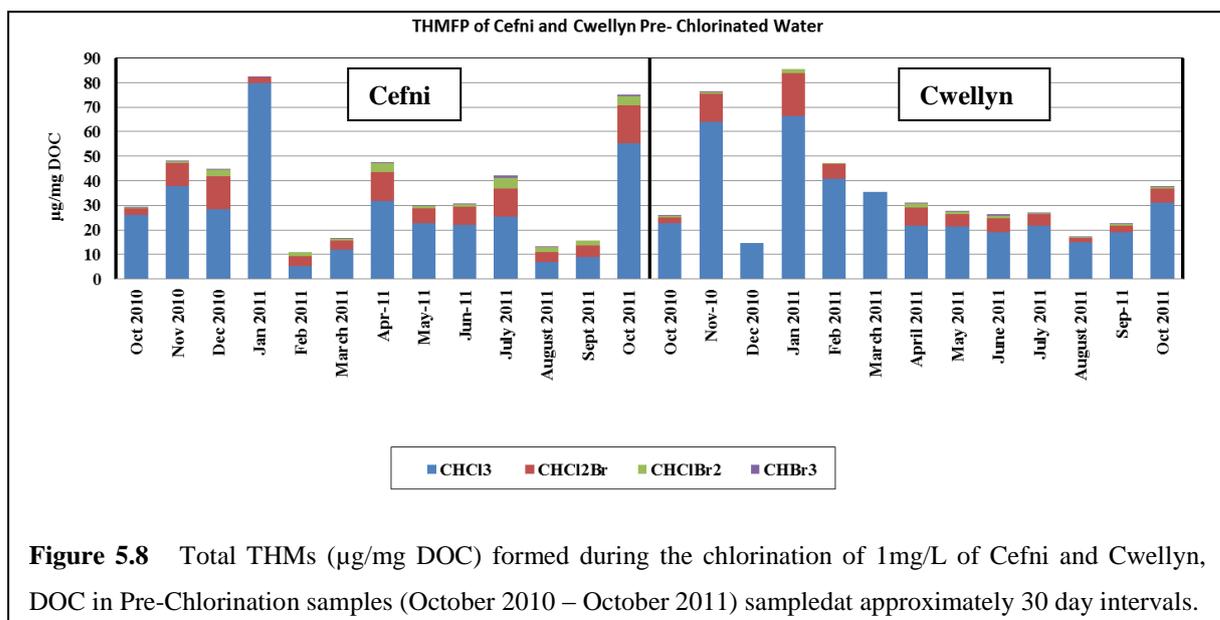


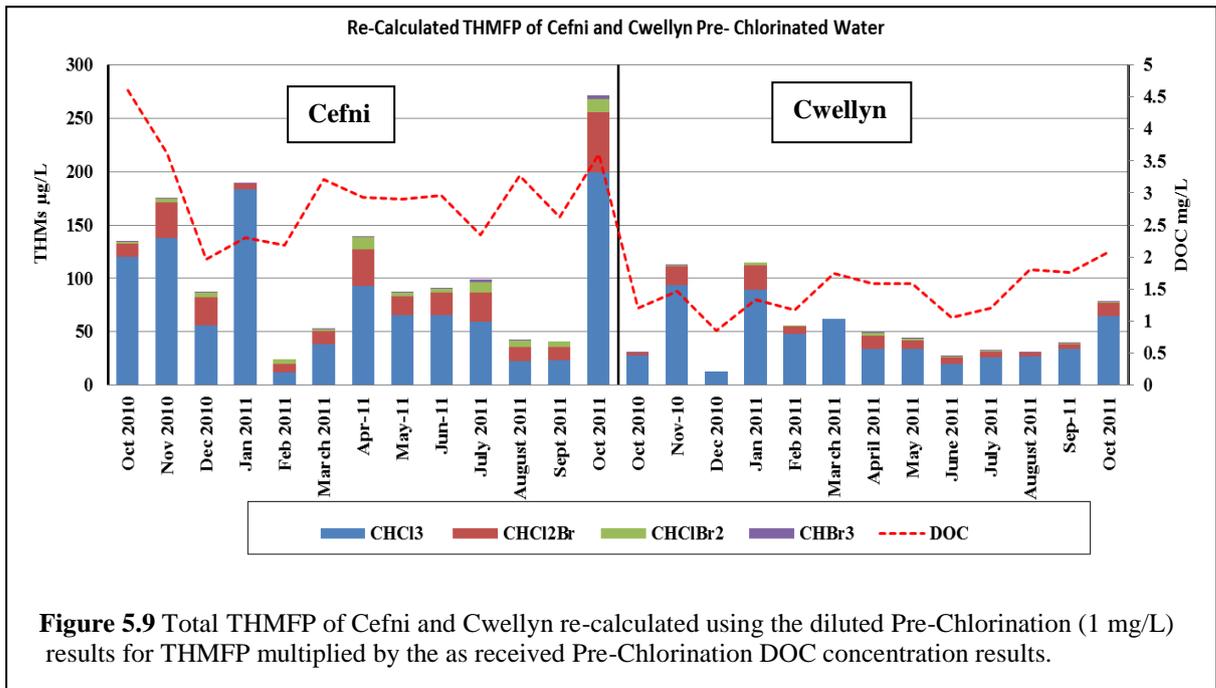
Figure 5.8 Total THMs ($\mu\text{g}/\text{mg}$ DOC) formed during the chlorination of 1mg/L of Cefni and Cwellyn, DOC in Pre-Chlorination samples (October 2010 – October 2011) sampled at approximately 30 day intervals.

Concentration of CHBrCl_2 (Fig 5.8) were much lower in both Cefni and Cwellyn than CHCl_3 , with the highest recorded in Cwellyn in January 2011 ($17.3 \pm 0.5 \mu\text{g}/\text{mg}$ DOC), which represented 20 % of the total THMs, but in both December 2010 and March 2011 evidence of CHBrCl_2 was absent in the Cwellyn samples. Over-all the average CHBrCl_2 percentage inclusion for Cwellyn over the months the samples were analysed was 16 %. Unlike Cwellyn, Cefni demonstrated a concentration of CHBrCl_2 in all the samples and tended to mirror the CHCl_3 result profile, but at a much reduced concentration. They ranged from a low in January 2011 ($2.3 \pm 0.1 \mu\text{g}/\text{mg}$ DOC) to a high in October 2011 ($15.5 \pm 0.5 \mu\text{g}/\text{mg}$ DOC) and for the period sampled (October 2010 – October 2011) CHCl_2Br represented an average of 20 % of the total THMs.

For October 2010, July 2011 and August 2011 the results for the concentration of CHClBr_2 in the Pre-Chlorination samples of Cwellyn were below the LOQ ($0.5 \mu\text{g}/\text{L}$) whilst the Cefni demonstrated the same for two of the samples (October 2010 and January 2011). Cwellyn's CHClBr_2 concentrations throughout the Pre-Chlorination series were $< 2 \mu\text{g}/\text{mg}$ DOC with the highest in April 2011 at $1.73 \pm 0.05 \mu\text{g}/\text{mg}$ DOC and the lowest in August 2011. Cefni exhibited relatively high concentrations for 4 of the 12 months, December 2010, April 2011, July 2011 and October 2011 at 2.48 ± 0.1 , 3.75 ± 0.1 , 4.14 ± 0.1 and $3.54 \pm 0.1 \mu\text{g}/\text{mg}$ DOC respectively. The May and June 2011 samples demonstrated similar THMFP for both Cefni and Cwellyn and averaged for the 4 results $0.97 \mu\text{g}/\text{mg}$ DOC with an SD of 0.08. The over all averages for both Cefni and Cwellyn for the concentration of CHBr_2Cl presented as a percentage of total THMs was 5% and 2 % respectively.

Both Cefni and Cwellyn Pre-Chlorination sample results for CHBr_3 were below the LOQ ($1.8 \mu\text{g/L}$) with only 3 of all the series satisfied the LOD ($0.54 \mu\text{g/L}$), namely Cefni July 2011 ($1.01 \pm 0.03 \mu\text{g/mg DOC}$), Cefni October 2011 ($0.86 \pm 0.03 \mu\text{g/mg DOC}$) and Cwellyn June 2011 ($0.66 \pm 0.02 \mu\text{g/mg DOC}$). Although the results were not analytically valid, Cwellyn had a higher concentration of CHBr_3 on six out of the twelve samples, but from November 2010 to April 2011 a concentration for CHBr_3 was not detected. Cefni also exhibited two months when a concentration was not detected, February 2011 and September 2011.

Fig 5.9 represents the theoretical THMFP of the actual Pre-Chlorination DOC concentration passing into the Cl_2 contact tank. The total THMFP concentrations were calculated by multiplying the THMFP results of the diluted Pre-Chlorination DOC (1mg/L) by the Pre-Chlorination ‘as sampled’ DOC concentrations determined by Method 2.3.1.



The re-calculated THMFP for Cwellyn only altered the CHCl_3 concentration by a low margin, as the initial DOC concentrations were already close to 1 mg/L for the Pre-Chlorination samples. For example, the difference between the Cwellyn chlorinated (1mg/L) samples and the re-calculated average was $16.1 \mu\text{g/L}$, representing a difference of 30.3% . Cefni with its higher DOC concentrations in the treated waters expressed an average difference of $72.6 \mu\text{g/L}$, representing a 65.8% increase. For example the January 2011 uncorrected CHCl_3 FP was $79.9 \pm 2.4 \mu\text{g/L}$ and the re-calculated was 183.8 ± 5.5

µg/L. Substantial differences were also seen in the brominated analytes especially the CHCl_2Br of the Cefni which demonstrated an average increase of 65.7%. This in µg/L of CHCl_2Br represented an increase from 7.4 ± 0.1 µg/L to 21.6 ± 0.4 µg/L.

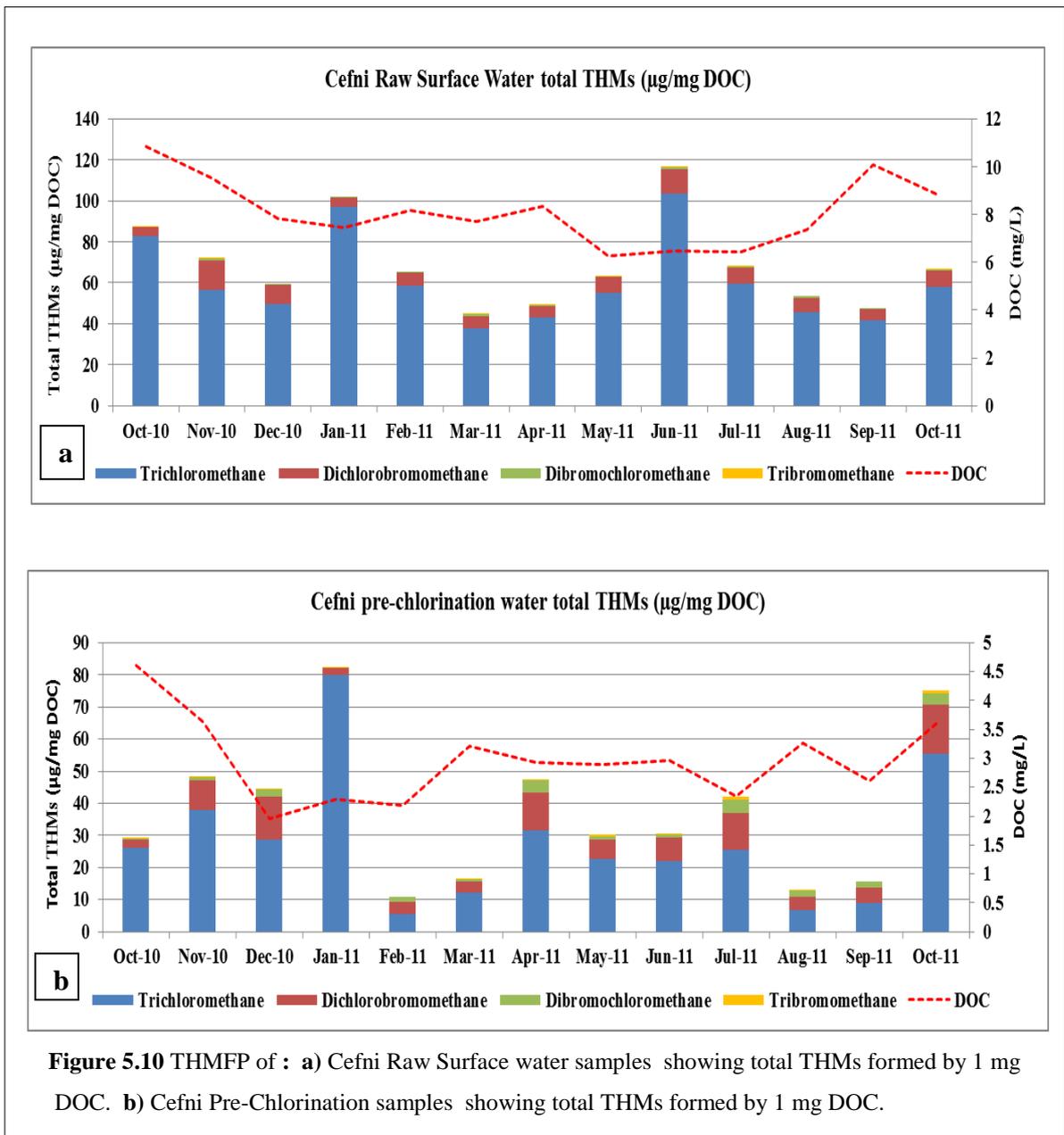
An interesting feature of the re-calculated THMFP is the high concentration recorded in all the analytes in October 2011. Although the diluted (1 mg/L) formed total THMs of 75 ± 1.5 µg/L, when corrected to represent the actual THMFP of the undiluted Pre-Chlorination water, the THMs was 271.5 ± 5.4 µg/L with a DOC concentration of 3.6 ± 0.1 mg/L. Reviewing the results in the months previous to this, for example, the Cefni November 2010 and January 2011, although the DOC concentrations were 3.6 and 2.3 mg/L respectively the total THMFPs did not reflect the changes, that is, for the November 2010 sample it was 174.6 ± 3.5 µg/L and the January 2011 sample 189.1 ± 3.8 µg/L.

The re-calculated values for both CHClBr_2 and CHBr_3 demonstrated low concentrations of both analytes in Cwellyn. The values for CHBr_3 below the limit of quantification (LOQ 1.8 µg/L) The values for CHClBr_2 averaged for the period 0.98 ± 0.02 µg/L, but demonstrated three high values during January (2.10 ± 0.04 µg/L), April (2.70 ± 0.05 µg/L) and October 2011 (1.40 ± 0.03 µg/L). The Cefni FP concentrations for CHClBr_2 averaged five times higher than Cwellyn's at 5.0 ± 0.1 µg/L. Cefni again demonstrated above average concentrations during April, July and October 2011 at 10.98 ± 0.2 µg/L, 9.7 ± 0.2 and 12.7 ± 0.3 µg/L respectively. These higher values were also associated with increases in the other THMs, which suggested changes in the DOC that increased the response to chlorination and bromination, as the DOC remained at a lower concentration than when the FP of brominated products was also low. For example, Cefni March 2011, DOC 3.21 mg/L : (CHClBr_2 2.18 µg/L); April 2011, DOC 2.93 mg/L (CHClBr_2 10.9 µg/L).

5.5 Pre-Chlorination THMFP results compared to Raw Surface Water THMFP and Finished Product.

In (Figs.5.10 a,b and 5.11 c,d), the Raw Surface Water and Pre-Chlorination water THMFP for Cefni and Cwellyn were compared. Differences in the THM formation concentrations were pronounced especially between the two Cefni suites with the Raw Surface Water forming a higher concentration of THMs per mg of DOC for each of the samples, but with the exception of October 2011 samples where the converse occurred. The total THMFP for the Pre-Chlorination water was 75.2 ± 1.5 µg/mg DOC with the Raw

Surface Water concentration lower at $58.0 \pm 1.2 \mu\text{g}/\text{mg}$ DOC. This exception was also recorded in the Cwellyn samples in January 2011, where the Pre-Chlorination water total THMs were $114.5 \pm 3.4 \mu\text{g}/\text{mg}$ DOC with the Raw Surface Water forming a low total THMs of $37.3 \pm 1.1 \mu\text{g}/\text{mg}$ DOC. Reasons for these differences were not obvious as there was no observable differences in measurements of DOC character such as MM distributions, phenolics or SUVA. The principal THM formed in all the sample suites was CHCl_3 , but in the Pre-Chlorination samples of both Cefni and Cwellyn an increase in the brominated THMs, CHBr_3 and CHBr_2Cl was recorded (Fig 5.10 b and 5.11,d).



This increase in brominated analytes was most apparent in the Cefni and was not conducive with the ion results, as the Pre-Chlorination samples exhibited a decrease of $1.00 \pm 0.06 \mu\text{g/L}$ in Br^- concentration (Raw Surface Water Br^- , average 74 ± 4 ; Pre-Chlorination water Br^- , average $73 \pm 4 \mu\text{g/L}$) from October 2010 – October 2011. This again suggested a change in the DOCs character to one that favours bromination rather than chlorination, although the average FP of CHCl_2Br in both Cwellyn and Cefni Pre-Chlorination and Raw Surface water remained relatively similar, with the Cefni average FP in The Raw Surface Water at $7.7 \pm 0.2 \mu\text{g/mg DOC}$ and Pre-Chlorination $7.4 \pm 0.2 \mu\text{g/mg DOC}$ and Cwellyn, The Raw Surface Water at $5.9 \pm 0.1 \mu\text{g/mg DOC}$ and Pre-Chlorination $5.6 \pm 0.1 \mu\text{g/mg DOC}$.

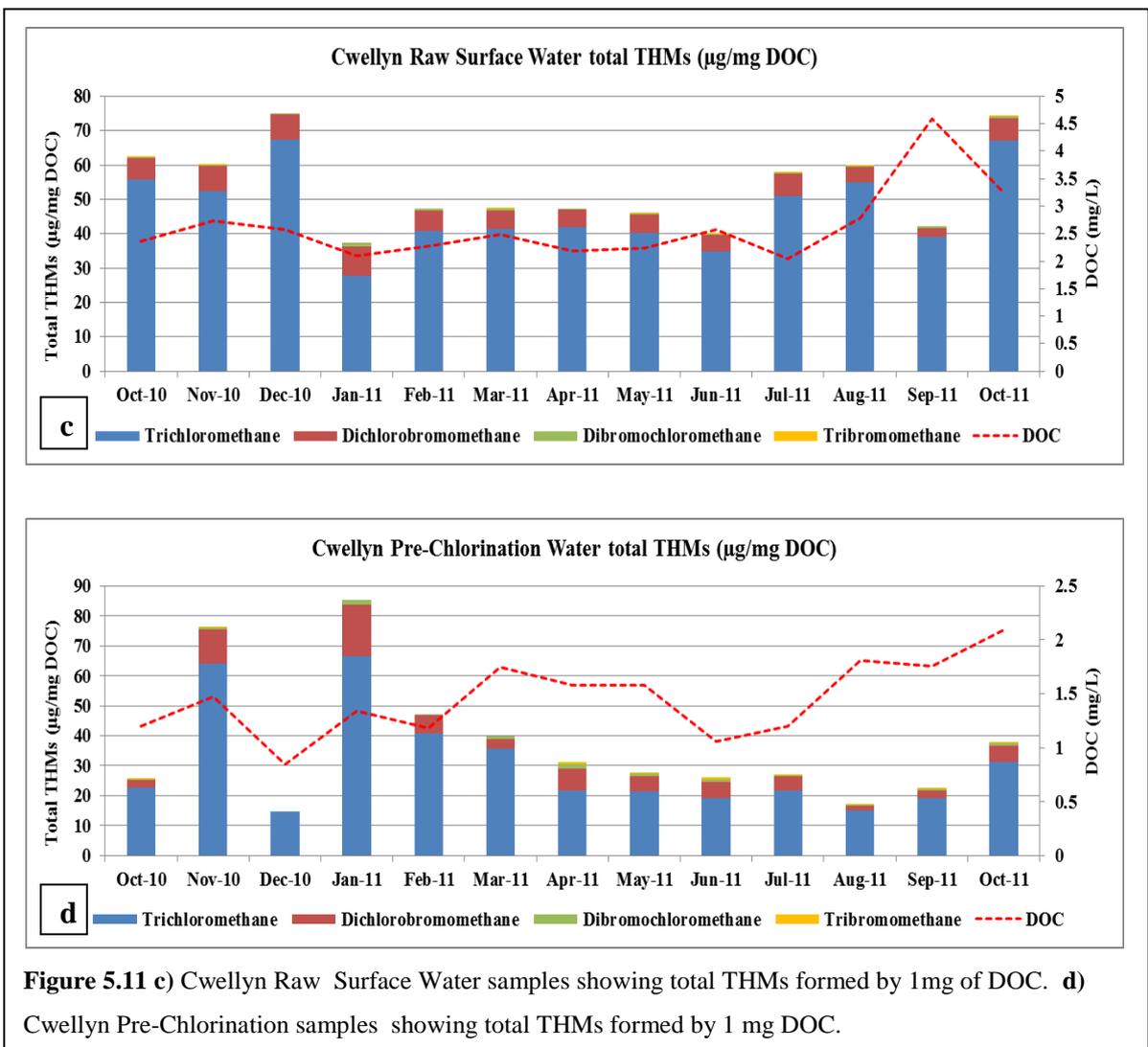
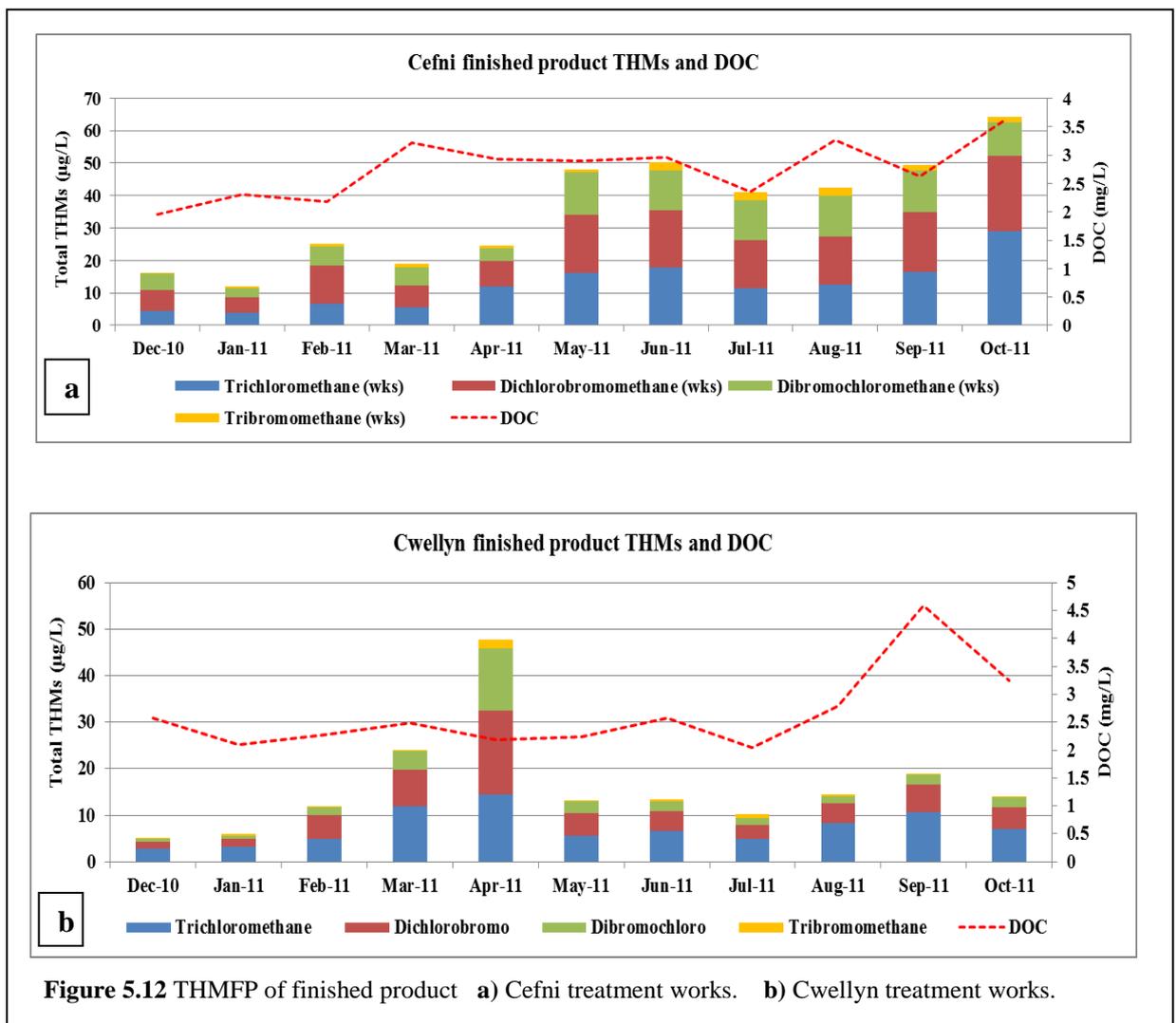


Figure 5.11 c) Cwellyn Raw Surface Water samples showing total THMs formed by 1mg of DOC. d) Cwellyn Pre-Chlorination samples showing total THMs formed by 1 mg DOC.

During the period (December 2010 – October 2011) Finished Product samples collected from the sampling point after the Cl_2 contact tank were also monitored. They were collected and quenched (Na_2SO_4) at the point of sampling at the same time as the Pre-

Chlorination samples and were analysed for both DOC (Method 2.3.1) and THMFP products as per the Method (2.3.8) within 3 hours of sampling. This study was conducted to establish the concentrations of THMs that were being distributed for consumption *via* the distribution network.

The results were reported as $\mu\text{g/L}$ which complimented those reported for the theoretical THMFP calculated for the un-diluted Pre-Chlorination samples hence a like-for-like comparison. The Finished Product demonstrated a lower concentration of total THMs which may have resulted from the short period in the contact tank and lower reaction temperatures compared to the 7 day incubation of the Pre-Chlorination samples. Evidence of a relative decrease in CHCl_3 formation and an increase in the brominated THMs (CHCl_2Br , CHBr_2Cl and CHBr_3) for both Cefni and Cwellyn was recorded (Figs 5.12 a, b) for the Finished Product, (although below the $100 \mu\text{g/L}$ legislated limit DWI 1998) compared to the Pre-Chlorination samples.



A number of quantifiable concentrations of CHBr_3 (Table 5.5) were recorded together with a higher inference of the other two brominated THMs (CHBrCl_2 , CHBr_2Cl). A substantial total THM concentration change in them for Cefni was recorded for the period May – October 2011, from an average of 13.14 $\mu\text{g/L}$ (SD 4.2) during the spring, to an average of 32.06 $\mu\text{g/L}$, (SD 2.01) for May – October with CHCl_3 averaging 17.2 $\mu\text{g/L}$ (SD 6.3). A higher concentration of total brominated molecules in the Cwellyn was only recorded during the month of March 2011 ($12.22 \pm 0.7 \mu\text{g/L}$) and April 2011 when the highest of the sample series concentration was recorded ($33.47 \pm 1.0 \mu\text{g/L}$).

A comparison of the THMFPs of the Pre-Chlorination analytes to the Finished Product analytes are shown in (Table 5.10) as percentages of each total THMFP.

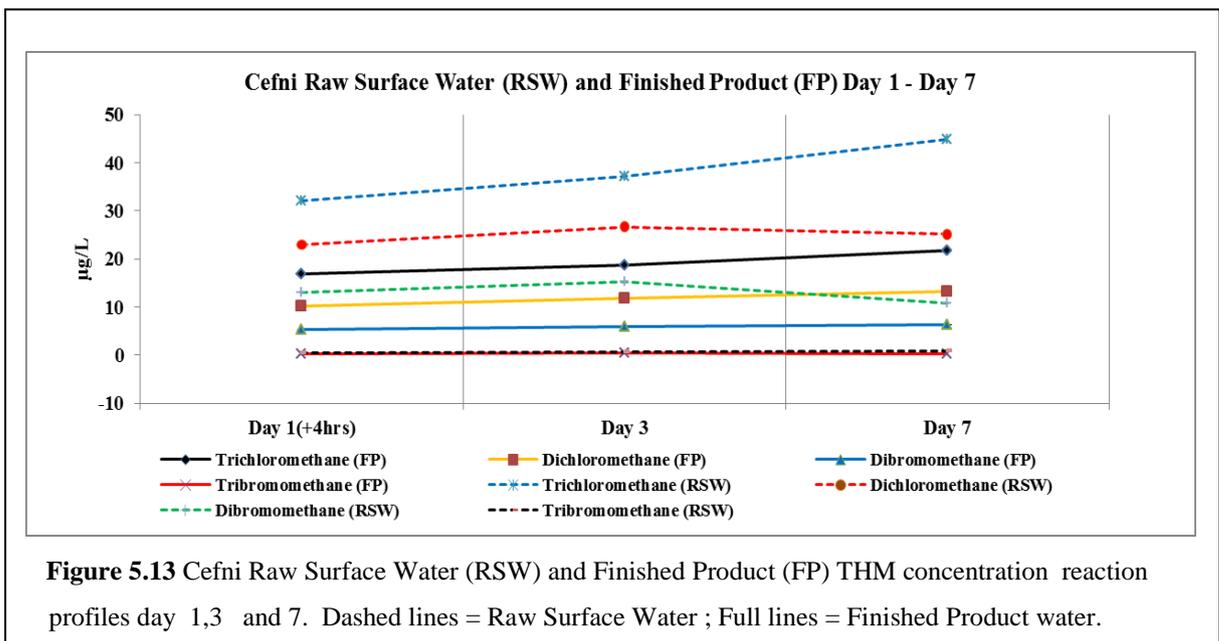
Table 5.10 THMFP of Cefni and Cwellyn Finished product analysed on day 1 and cefni and Cwellyn Pre-Chlorination sample analysed at day +7. The analytes are shown as percentages of the total THMs

	Cefni Pre-Chlorination	Cefni Finished Product	Cwellyn Pre-Chlorination	Cwellyn Finished Product
Total THMs $\mu\text{g/L}$	102.2	35.7	50.5	16.3
CHCl_3 (%)	72.8	34.7	81.4	44.8
CHCl_2Br (%)	20.8	36.7	15.6	34.4
CHClBr_2 (%)	5.5	24.7	2.4	17.7
CHBr_3 (%)	0.9	3.9	0.6	3.1

The results clearly show the different concentration of analytes formed between the water treatment works chlorination and laboratory chlorination. The major difference was the predominant formation of CHCl_3 in both Cefni and Cwellyn's laboratory chlorinated samples. This may have been ascribed to the different conditions employed during chlorination, that is; contact time and temperature (lab: 7days at 25°C , treatment works: ambient temperature with an average 30 minute hold in the contact tank during chlorination).

With the Finished Product being chlorinated at ambient temperature and analysed on the day collected and the laboratory chlorinated Raw Surface Water being analysed after being incubated at a fixed temperature $25 \pm 2^\circ\text{C}$ for 7 days, an experiment was conducted to study any observable differences between the Finished Product and Raw Surface Water THMFP with both sets of samples subjected to the same incubation period (7days) and temperature (25°C) conditions.

The exercise was carried out by sampling the Raw Surface Water and Cefni Finished Product water on the same day and time in August 2012. Following the standard THMFP analysis as per Method 2.3.8, the samples were analysed for THMs over a period of 7 days with analysis being carried out on day 1, day 3 and day 7. The results were plotted (Fig 5.13) and the concentrations at the points; day 1, 3 and 7 were recorded for each THM. From the data (Table 5.11) an increase in each THM was recorded from day 1 – 3, although the Raw Surface water and Finished Product demonstrated different rates of formation (Fig 5.13). It could be clearly seen that the THMFP (Table 5.11), of the Finished Product DOC was greater over a longer time period for all four of the THMs. After day 3 the formation potential of CHCl_3 formation increased in both sample suites, with the Finished Product demonstrating the highest rate of formation. Formation of the brominated products CHBrCl_2 and CHBr_2Cl after day 3 slowed for the Finished Product and the Raw Surface Water formation became negative, thus indicating a rapid fall in brominated product formation compared to a continuing increase in CHCl_3 .



The concentration of CHBr_3 was low for both sample sets over the 7 day exercise but tended to show a drop in concentration in the Finished Product from day 3 and a negligible increase in the Raw Surface Water (Table 5.11).

Table 5.11 Changes in THMFP over a 7 day period for Cefni Finished Water and Raw Surface Water.			
Samples	THM	Increase/decrease (µg/L) day 1 to 3	Increase/decrease (µg/L) day 3 to 7
Finished Product water	CHCl ₃	+ 6.17	+ 10.7
	CHBrCl ₂	+ 5.69	+ 4.76
	CHBr ₂ Cl	+ 2.08	+ 1.11
	CHBr ₃	+ 0.85	+ 0.25
Raw Surface water	CHCl ₃	+ 5.2	+ 7.6
	CHBrCl ₂	+ 3.7	- 1.6
	CHBr ₂ Cl	+ 2.2	- 4.5
	CHBr ₃	+ 0.17	+ 0.26

This elevation of brominated components in the Finished Product and Pre-Chlorination water tended to agree with results reported by Westerhoff *et al*, (2004). They reported high concentrations of brominated products for water that had been subjected to alum coagulation, with brominated products increasing relative to the alum [KAl(SO₄)₂. 12 H₂O] concentration used, this suggested that coagulation preferentially removed organic material capable of forming haloforms in the presence of chlorine, plus the ratio of Br⁻ to DOC may have changed due to the bromides not being removed by the coagulant.

5.6 Effect of Bromide on THMFP

Exposure of the DOC of each lake sample over a period of 7 days in darkness at 25 °C to excess chlorine generated data that suggested differences in their THMFP. The principal product formed during chlorination of DOC in all four lakes was CHCl₃. Although the Conwy's (mean 4.5 mg/L) catchment DOC concentration was lower than the Cefni's (mean 8.0 mg/L), it had the highest THMFP for CHCl₃ per mg of DOC (average 100.7 µg/mg DOC, Section 5.2.1) of all four lakes.

The brominated THMs, (CHBrCl₂, CHBr₂Cl and CHBr₃) were present in low concentrations for all four of the lake chlorinated DOCs. The principal brominated THM (CHBrCl₂) averaged 9.7 and 8.5 µg/ mg DOC for Cwellyn and Cefni, respectively. Teyrn averaged 6.9 µg/mg DOC and the Conwy generated the lowest concentration at 5.2 µg/mg DOC. These differences in the literature have been attributed to the available bromide (Nikolaou, 2004; Platikanov, 2010) present in, for example; the Raw Surface Water samples and catchments. To explore this, the moles of bromide available to partake in the reactions and the final molar concentration of Br⁻ in the brominated THMs formed were

computed and compared for some of the results in this thesis to establish whether an excess of Br^- remained after the 7 day incubation in the presence of excess chlorine.

Cefni, Cwellyn and Teyrn are close to the north Wales western coastline with its prevailing westerly wind, hence they have a greater exposure to a marine environment (see Fig 2.01), whilst the Conwy bog catchment is further inland and is isolated from the marine impact.

Although Cefni surface water had the highest Br^- concentration (Table 5.12) which averaged 70 $\mu\text{g/L}$ (equivalent to 8.76×10^{-7} moles), the averaged summed brominated THM species (9.18 $\mu\text{g/mg DOC}$) represented a molar concentration of 5.7×10^{-8} moles of Br^- . This suggested that not all the Br^- was utilised in the formation of the three brominated THMs generated by the Cefni DOC. Cwellyn samples also demonstrated this with 1.87×10^{-7} moles of Br^- available in the Raw Surface Water, but only 7.0×10^{-8} moles were accounted for in the averaged summed brominated THM species formed. Conwy's summed brominated THMs averaged 5.37 $\mu\text{g/mg DOC}$ (Tables 5.6 – 5.8) representing 3.2×10^{-8} moles of Br^- which was less than the molar concentration of Br^- (8.7×10^{-8} moles) available in the Raw Surface Water.

To establish during the 24 month exercise that sufficient free chlorine remained after the 7 day incubation of the samples, an iodometric titration were carried out and the results averaged 3.1×10^{-4} moles of free Cl_2 remaining at the point of quenching. Sketchell *et al.* (1995) stated that brominated THMs were formed when the chlorine in the form of hypochlorous acid/hypochlorite ion (HOCl/OCl^-) oxidized the bromide in the water to hypobromous acid/hypobromite ion (HOBr/OBr^-). As an adequate chlorine concentration was available for oxidation of the bromide ions to free bromine / hypobromous acid, it suggests that another mechanism may affect the formation of brominated products.

Although the results above indicate that not all the available Br^- was consumed by the analyte concentrations recorded, it must be noted that other brominated products may have been present which may have accounted for the excess moles.

Table 5.12 Molar concentrations of Br⁻ in the Raw Surface Water samples of the Cefni and Cwellyn and the summed brominated THM molar concentrations after laboratory chlorination.

Lake/Reservoir	Moles Br ⁻ (Raw Surface Water)	Moles Br ⁻ utilised in THM formation.
Cefni	8.8×10^{-7}	5.7×10^{-8}
Cwellyn	1.8×10^{-7}	7.0×10^{-8}

An interesting feature of the THM reactions highlighted that, as brominated THMs increased in concentration the CHCl₃ reduced. Hence when the ratio of these two analytes was computed for the Cefni samples and the results were presented (Fig. 5.14), the ratio demonstrated a linear relationship with the CHCl₃ concentrations (R^2 0.5012). Hence as the ratio decreased the CHCl₃ also decreased and *vice versa*.

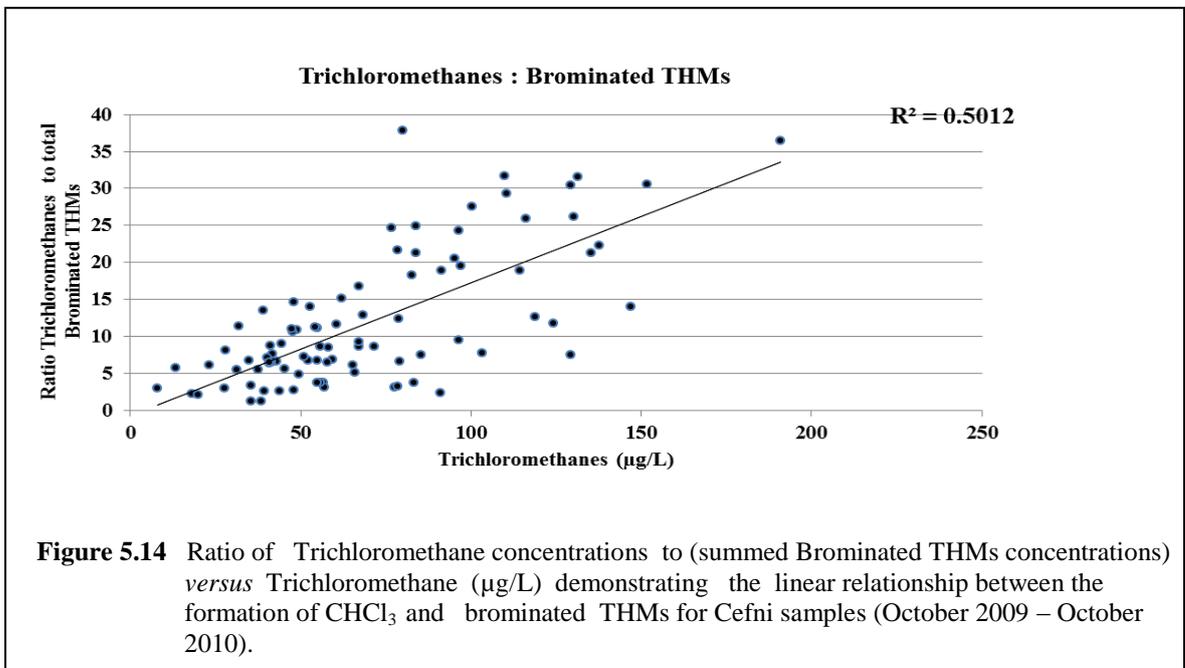


Figure 5.14 Ratio of Trichloromethane concentrations to (summed Brominated THMs concentrations) versus Trichloromethane (µg/L) demonstrating the linear relationship between the formation of CHCl₃ and brominated THMs for Cefni samples (October 2009 – October 2010).

5.7 THMFP *versus* DOC, SUVA, Phenolics and Aromaticity.

Exploration and evaluation of the THMFP of DOC from the four lakes was carried out using the statistics package (SPSS. v 16) to determine Pearson's correlation coefficients. The four (THMs, CHCl₃, CHBrCl₂, CHBr₂Cl and CHBr₃) were summed for each sample and tested for any significant correlations relative to the; DOC, SUVA, phenolics and aromaticity (Chapter 3). The following results were generated:-

- a) DOC concentrations *versus* summed THMFP concentrations did not show significant correlations for Cefni, Cwellyn, Conwy or Teyrn.
- b) Only the Cefni's summed THMs demonstrated a weak significant correlation with SUVA (Table 5.6).
- c) Conwy's summed THMs correlated weakly (Table 5.6) with aromaticity (E₂/E₃).

The results (Table 5.13) for SUVA reflected the findings of Weishaar *et al*, (2003) , Qunshan *et al*. (2008) and Fram *et al*. (1999). They concluded that SUVA was a weak universal indicator for the formation of THMs. Their studies tended to suggest that THMs were also formed by non- aromatic molecular structures.

Lake/Reservoir (THMs)	SUVA	Aromaticity	Phenolics	DOC
Cefni	0.436*	-	-	-
Cwellyn	-	-	-	-
Conwy	-	0.297*	-	-
Teyrn	-	-	-	-

* Correlation is significant at the 0.05 level.

In Figure 5.15 a,b,c and d the summed THMs for each lake together with the DOC concentrations are shown for the period of sampling. Although there are no significant correlations between the characterization surrogates (Table 5.13) and THMs, correlations of the summed THMFP between the individual lake samples are significant (Table 5.14).

Lake/Reservoir	Cefni THMs	Cwellyn THMs	Conwy THMs	Teyrn THMs
Cefni THMs	-	-	0.443*	-
Cwellyn THMs	-	-	0.592**	0.660**
Conwy THMs	0.443*	0.592**	-	0.617**
Teyrn THMs	-	0.660**	0.617**	-

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level.

The Cefni correlated weakly with only the Conwy, but the three upland lake THMFP showed significant correlations with each other. These results tend to suggest that the respective upland DOC being exported out of the unique catchments may have had relatively common molecular structures that presented active sites for chlorination compared to the Cefni. The Cefni result inferred that the DOC being exported out of its catchment was different to both Cwellyn and Teyrn's but had a reactivity profile that was relatively similar to the Conwy's THMFP DOC.

During the months March – June 2010 which represented a dry period (Chapter 3, Fig 3.17), it was noted that the THMFP increased in all the lakes (Fig 5.15 a,b,c and d) with the Conwy demonstrating a month by month increase. In July 2010, a universal increase in THMFP was recorded for all four lakes although the rainfall before and during the day of sampling was approximately 4.4 mm (lowland) and 12.2mm (upland) with the DOC concentrations not being affected significantly. The increases in the July 2010 total THMs (as percentages) over the June 2010 concentrations are shown (Table 5.15). A steep fall in total THMs was recorded for the August 2010 samples at all four sites, with Teyrn and Cwellyn demonstrating the greatest drops at 65.6 and 53.3 % respectively.

Lake/Reservoir	% increase in THMFP from June- July 2010	% decrease in THMFP from July -August 2010
Cefni	31.5	49.8
Cwellyn	52.4	53.3
Conwy	25.3	51.0
Teyrn	51.7	65.6

This variation in THMFP concentrations suggested that the DOC being received into the lakes may have been influenced by the dry periods, i.e., longer residence time in the catchments and increasing spring temperatures (ranging 6 – 14° C) suitable for increased microbial activity. Hence, during dry conditions the catchment released increasingly higher concentrations of molecular structures susceptible to chlorination (July 2010). Once the catchment water tables had returned to normal, the residence of DOC was reduced and so presumably was the microbial degradation and it was exported in a form less suitably modified for chlorination (August 2010).

Total THMs in (Fig 5.15) are reported in µg/mg of DOC and DOC concentrations are shown as mg/L. The relationship between the amount of DOC being exported and chlorination susceptible DOC appears to be random and demonstrated insignificant correlation coefficients. This suggested that, although varying concentrations of DOC are exported from the catchments, the THMFP was dependent on a property of the DOCs which may be a modification/degradation by microbial sources, or the absence of it.

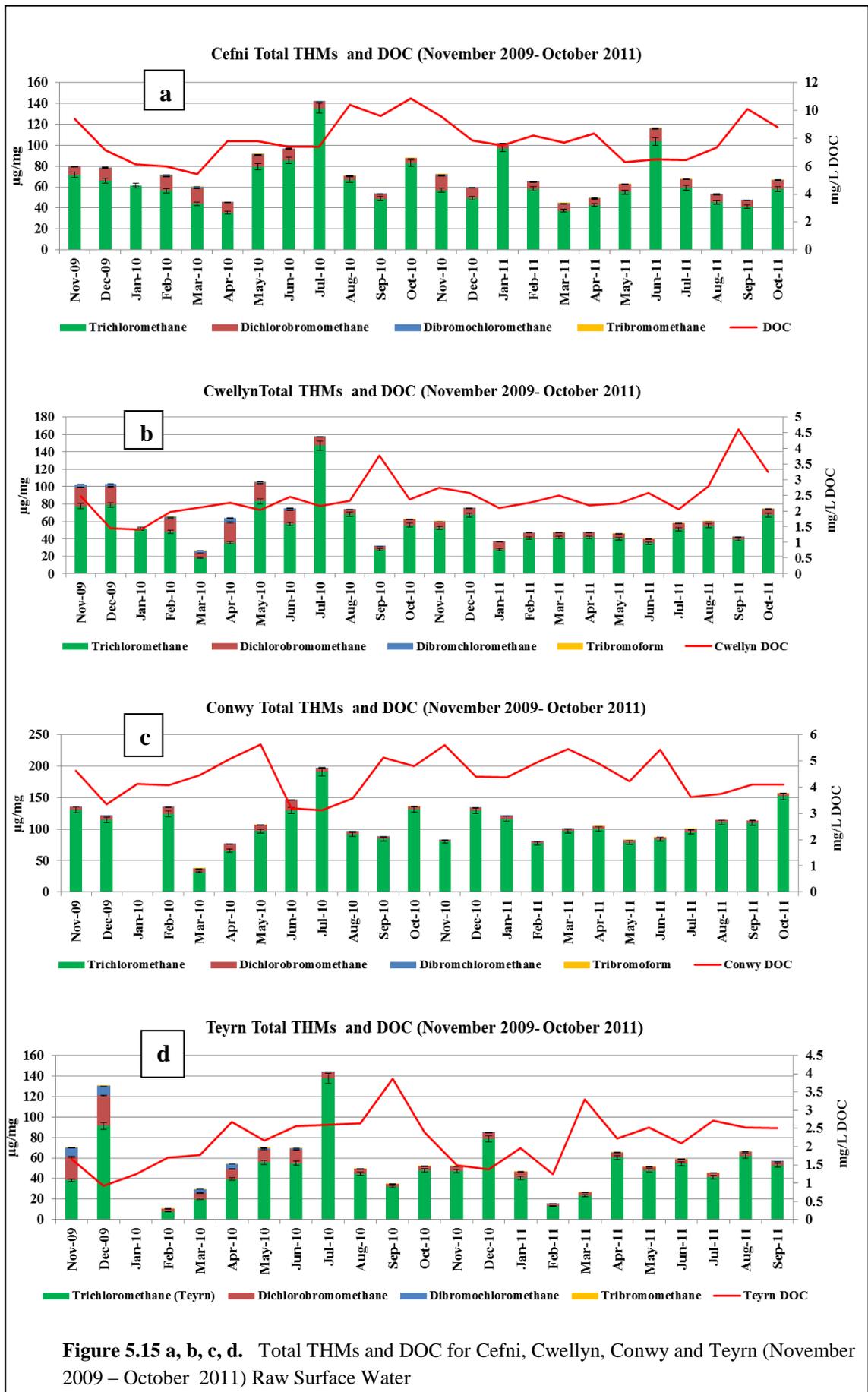
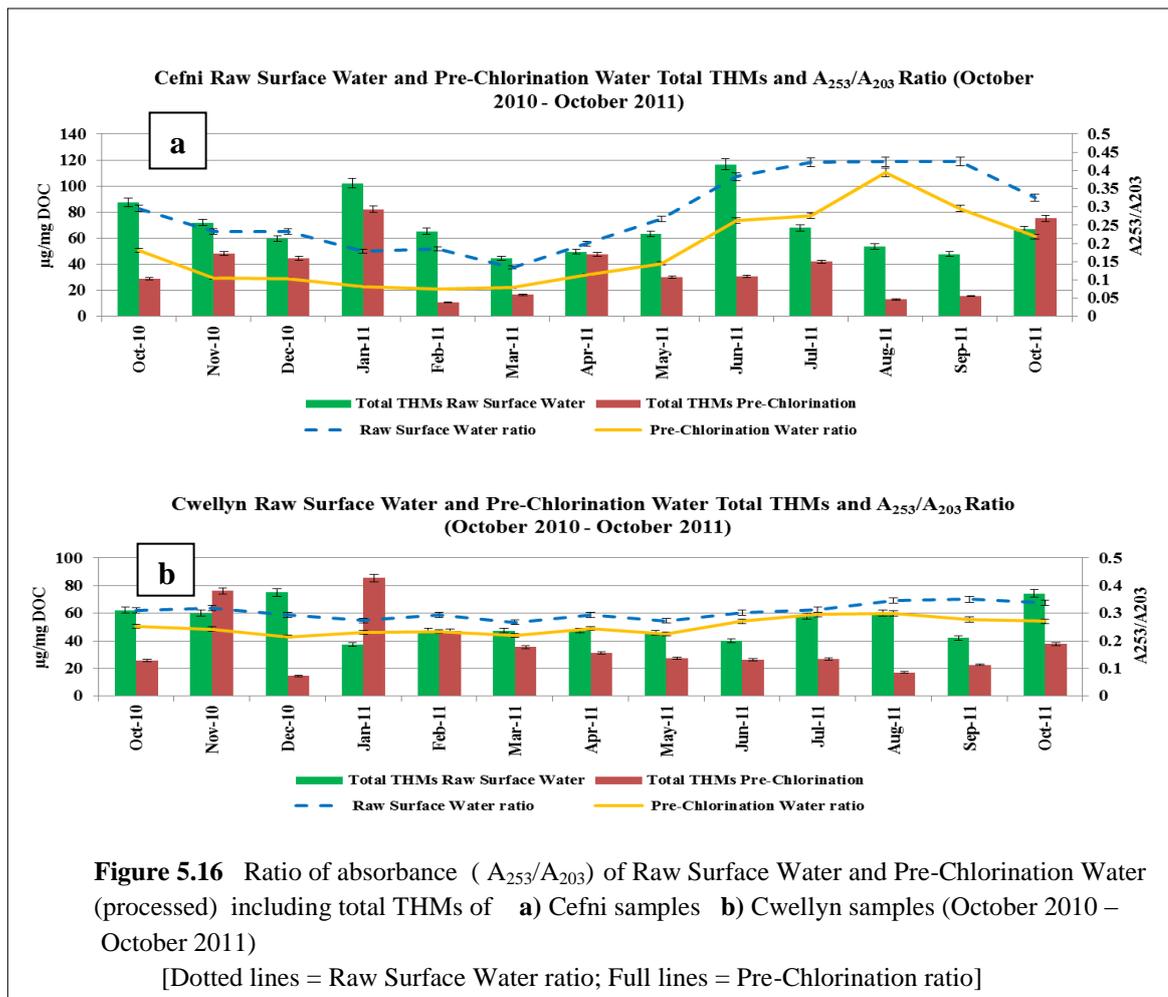


Figure 5.15 a, b, c, d. Total THMs and DOC for Cefni, Cwellyn, Conwy and Teyrn (November 2009 – October 2011) Raw Surface Water

5.8 Absorbance Ratio (A_{253}/A_{203}) DOC functional group relationship and THMFP in Pre-Chlorination and Raw Surface Water DOC.

Korshin *et al.* (1997) suggested that a relationship was evident between THMFP and the 253nm/203nm absorbance ratio. The product of the ratio was considered to be related to aromatic ring substitution. For example, a value of 0.027 was typical of an un-substituted aromatic ring and a ratio between 0.25 and 0.35 was characteristic of the catechols and related phenolics. Ratios of 0.4 and above were typical of carbonyl, carboxyl or ester substitution. Korshin *et al.* demonstrated a linear relationship between the ratio and the THMFP of post coagulation DOC. These workers also reported a drop in the ratio after the selective removal of the DOC and its accompanying functional groups during the coagulation process, suggesting a removal of DOC with functional groups conducive to THMFP, i.e., carboxyl, carbonyl and esters (Korshin *et al.* 1997)



We explored this relationship using data generated from Cefni and Cwellyn's Raw Surface Water and Pre-Chlorination Water samples. Differences were noted between both

lake DOC (A_{253}/A_{203}) ratios. The Raw Surface Water and Pre-Chlorination sample ratios (Fig 5.16 a, b) for Cefni demonstrating a larger difference between each other, compared to Cwellyn's. This suggested that significantly more aromatics substituted with carbonyl, carboxyls and esters were removed by coagulation than during the treatment of the Cwellyn Raw Surface Water.

Significant correlations were not evident between the Raw Surface Water ratios and THMFP or the Pre-Chlorination sample ratios and their THMFP. When the Cefni's ratios dropped during the winter/ early spring period to < 0.1 and increased during the late spring/summer months to > 0.35 they were not accompanied by equivalent changes in total THMs. Although this suggested a changing DOC chromophore content as Korshin *et al.* postulated, it did not directly influence changes in THM formation.

Application of the (A_{253}/A_{203}) ratio to Cefni, Cwellyn, Conwy and Teyrn Raw Surface Water over the 24 month period (Fig 5.17 a,b,c and d) indicated subtle differences in the DOC molecular substitution. The Cefni ratio (Fig 5.17 a) demonstrated the greatest seasonal fluctuation, with a ratio below 0.25 during the coldest periods, late winter early spring. Two peaks occurred in mid-summer (July 2010, June 2011) which were greater than 0.4, indicating carboxyl and ester substituted aromatics. These peaks were seen to be associated with an increase in total THMFP. The Cwellyn ratio (Fig 5.17 b) did not exhibit the same fluctuations as the Cefni, but in March 2010 the ratio went below 0.25 which suggested that the DOC aromatics were substituted with hydroxyls. During this low ratio phase the total THMs concentrations dropped, but in September 2010 the ratio was > 0.33 and the THMFP was equally as low. Although in July 2010 when there was a substantial THMFP increase at the end of the dry period of the previous three months, the ratio was approaching < 0.33 . Korshin *et al.* (1997) proposed that the ratio 0.25 – 0.35 represented hydroxyl substitution, for example, catechols and phenolic pure compounds. The effect of the ring positions of these hydroxyls are suggested to influence the THMFP (Chapter 1: Rook 1977; Westerhoff *et al.* 2004).

Conwy (Fig 5.17 c) demonstrated a ratio that was above 0.4 for the majority of the time suggesting that the DOC was highly substituted with carbonyls, esters and carboxyl groups (Korshin *et al.*, 1997).

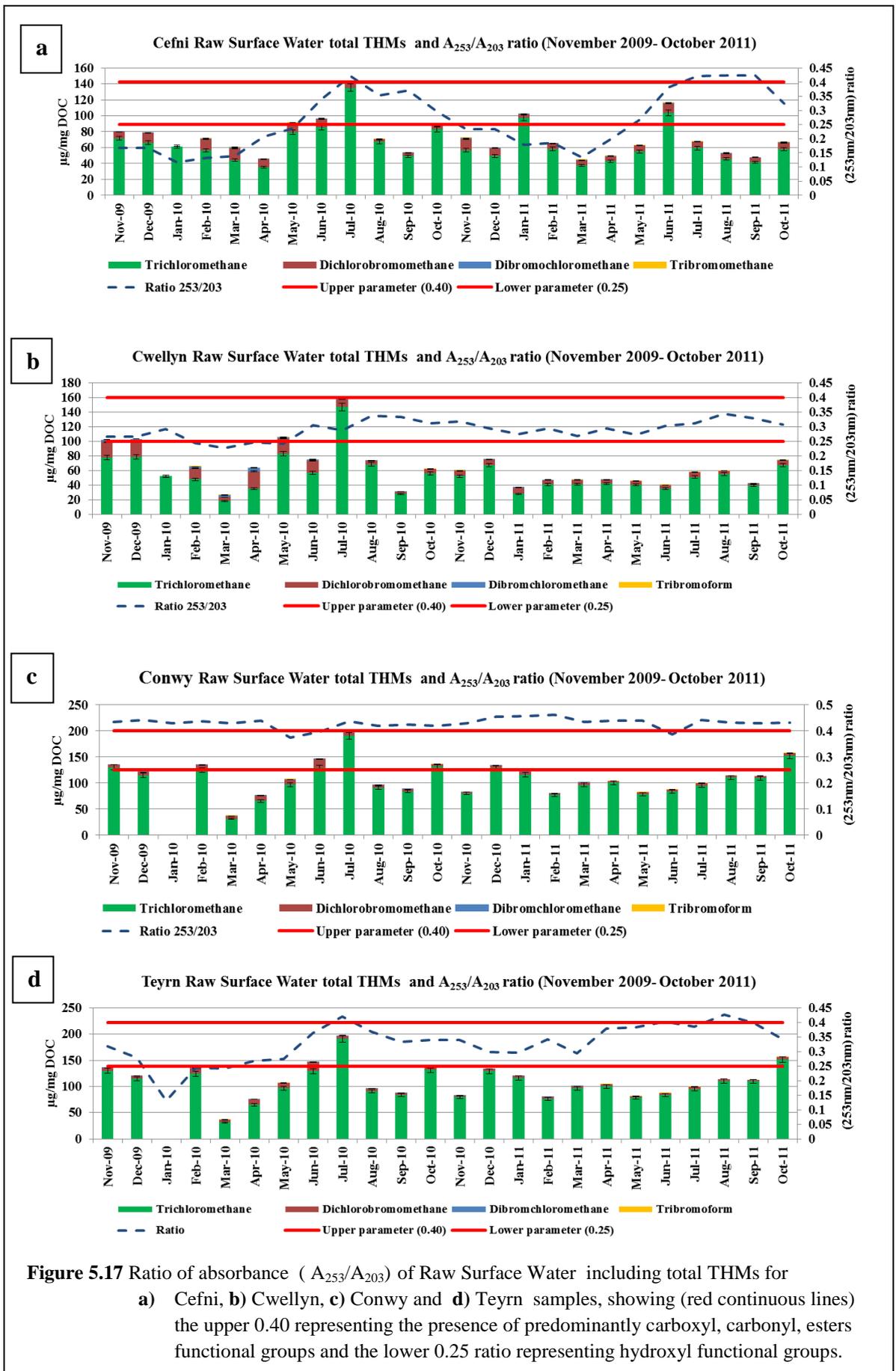


Figure 5.17 Ratio of absorbance (A_{253}/A_{203}) of Raw Surface Water including total THMs for a) Cefni, b) Cwellyn, c) Conwy and d) Teyrn samples, showing (red continuous lines) the upper 0.40 representing the presence of predominantly carboxyl, carbonyl, esters functional groups and the lower 0.25 ratio representing hydroxyl functional groups.

Compared to the other three lake DOCs the Conwy generated on average, the highest concentrations of THMs/mg DOC throughout the project. This agrees with the literature that the afore mentioned functional groups especially, esters and their participation in the haloform reaction and suggested subsequent THM formation (Rook, 1977; Reckhow and Singer, 1990).

The July 2010 increase in THMFPs that were recorded for each individual lake sample was reflected in an increase in each of their ratios, with the exception of Cwellyn. Finally Teyrn's ratio (Fig. 5.17 d) demonstrated a similarity to Cefni's, but was more constrained and fluctuated within the 0.25 and 0.4 parameters. The July 2010 THMFP spike recorded for all the lakes, was accompanied by an increase in the ratio to above 0.4. The ratio again peaked above 0.4 during the August of 2011, but a relative increase in THMs was small.

5.9 Comparison of Raw Surface Water and Pre-Chlorination DOC Molecular Mass (MM) ranges to THMFP.

Figure 5.18 (a,b, c and d) show the monthly molecular mass (MM) ranges in percentage form for each category of MM, from January 2010 to July 2011 for the four lakes. The line graph represents the total THMs generated by the Cefni, Cwellyn, Conwy and Teyrn Raw Surface Water DOC during the period January 2010 – October 2011. THM concentrations for Teyrn and Conwy (January 2010) are not shown because an insufficient sample (accidental breakage of sample bottle) was available for chlorination (Figs 5.18 c,d).

Statistical analysis of the individual lake molecular mass (MM) ranges *versus* μg THM per mg DOC did not generate significant correlations ($p > 0.05$). The highest concentration (July 2010) of total THMs recorded during the 24 month survey did not coincide with any major changes in the lake Raw Surface water MM range distributions. The 4000-3000 Da was the dominant range in the Cefni, Cwellyn and Teyrn for the month (Figs 5.18 a, b and d) averaging 58% for all three which represented an approximate increase of 10 % from the June 2010 figure. Conwy also demonstrated an increase in THMs for July 2010, but did not show any significant change in its MM distribution that accompanied the THM increase, i.e., June 2010; 4000 – 3000 Da range 48.4%; July 2010; 47.5 %.

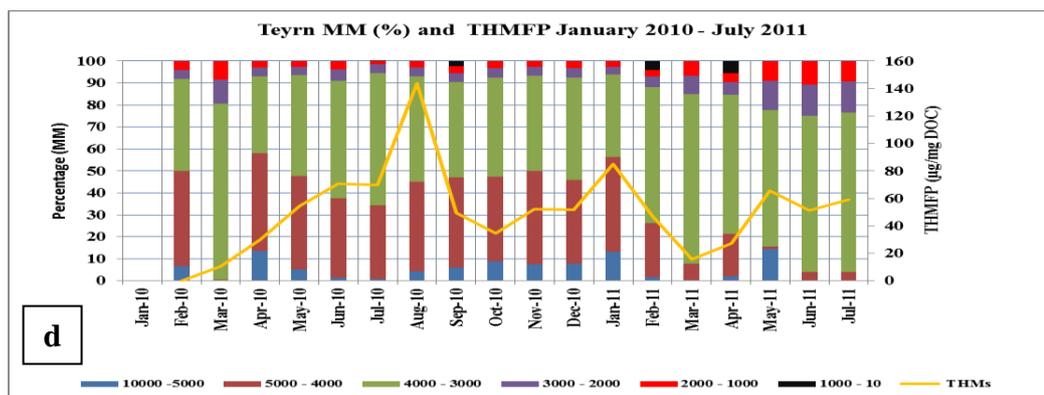
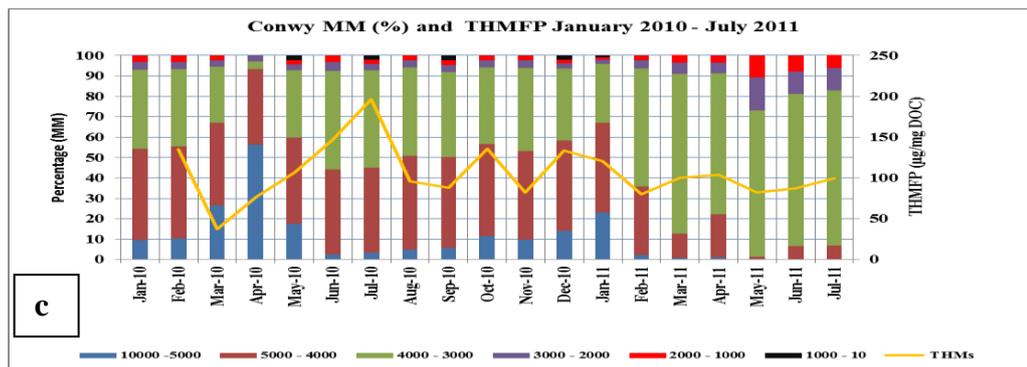
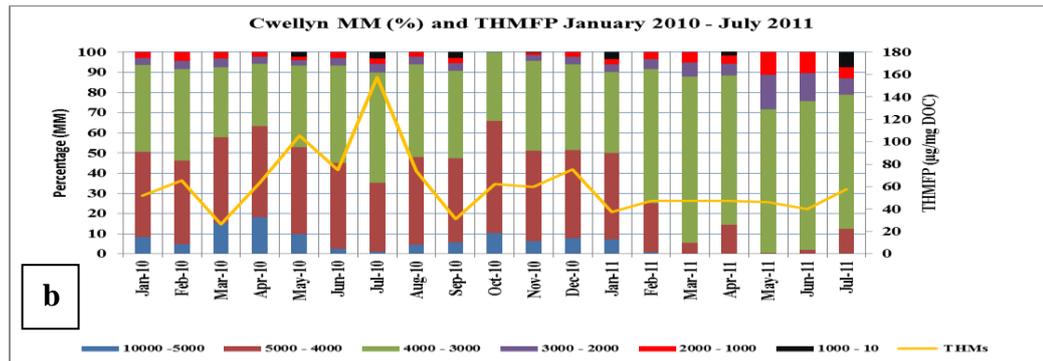
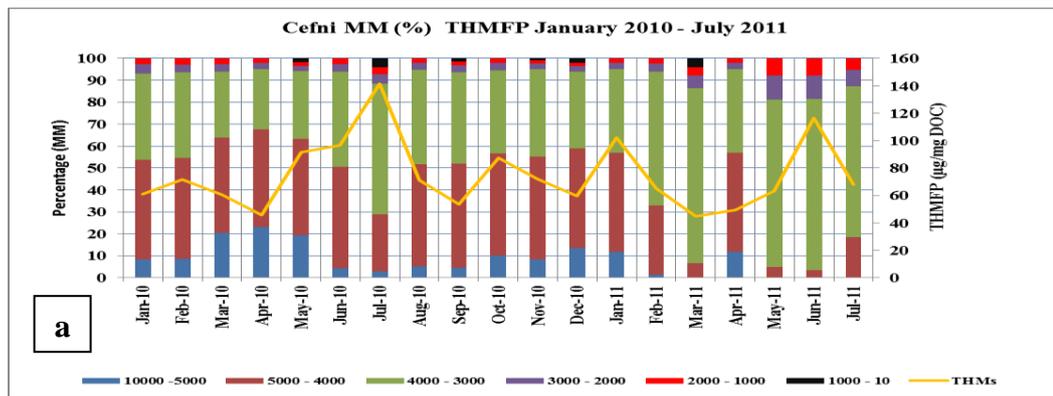


Figure 5.18 Molecular mass ranges (%) and THMFDP ($\mu\text{g}/\text{mg}$ DOC) for each lake Raw Surface water sample series (January 2010 – October 2011), exhibiting the THM formation potential relative to MM. a) Cefni DOC(MM) and THMFDP b) Cwellyn DOC (MM) and THMFDP. c) Conwy DOC (MM) and THMFDP . d) Teyrn DOC (MM) and THMFDP.

The removal/reduction of the higher MM ranges (5000-4000, 4000-3000 Da) by filtration, coagulation and flocculation of the Raw Surface Water DOC by the water treatment processes was apparent in the Pre-Chlorination water DOC after analysis by SEC (Fig 5.19 a, b). The two lower ranges (2000-1000, 1000-10 Da) now dominated the monthly series, and averaged 50% of the overall DOC distribution (Fig 5.19 a, b).

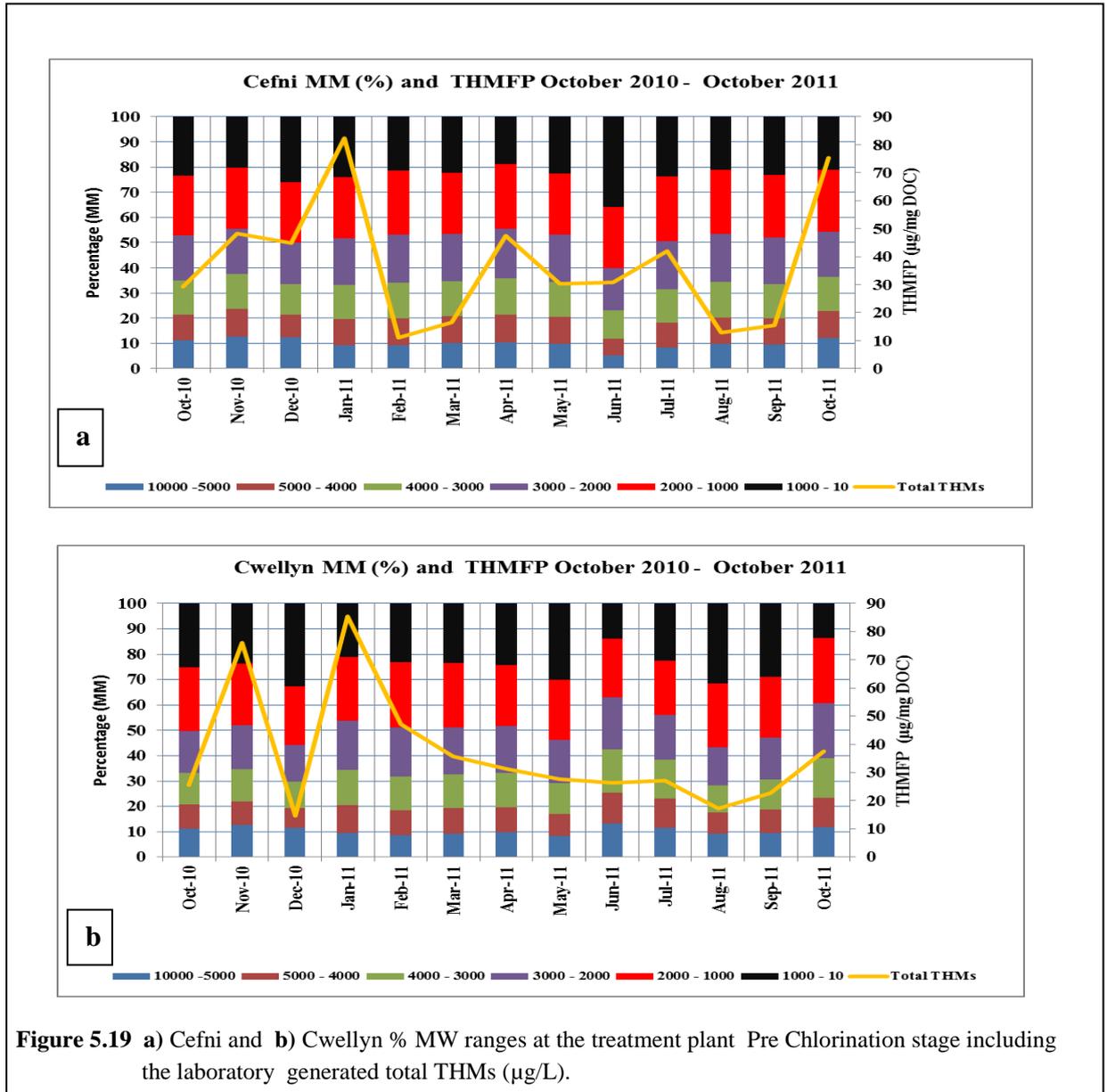


Figure 5.19 a) Cefni and b) Cwellyn % MW ranges at the treatment plant Pre Chlorination stage including the laboratory generated total THMs (µg/L).

Both Cefni and Cwellyn Pre-Chlorination total THMs demonstrated their highest concentrations in January 2011, but no change in the MM distribution ranges of both samples was noted. Drops in total THMFP recorded over the months February – August 2011 did not coincide with any obvious changes in the MM distributions either. Statistical

analysis of the different ranges *versus* THMFP for all the results, again did not exhibit significant correlations ($p > 0.05$).

5.10 Discussion

Of the 4 THMs, CHCl_3 was the major component formed by the DOC of all the four lake surface waters sampled during the 24 month period. The three other components CHBrCl_2 , CHBr_2Cl and CHBr_3 were present in successively lower concentrations. Of the four lakes sampled, the Conwy surface water DOC which originated from a blanket peat bog was found to form the highest concentrations of CHCl_3/mg of DOC throughout the sampling period. The exception to this was in March 2010 when all the lake DOCs demonstrated some of the lowest THM concentrations (Fig 5.15 a,b,c,d) of the whole series. There was no obvious reason for Conwy's and the other lakes fall during this month as all the characterising data used in this exercise did not demonstrate a particular step change. A contributing factor may have been the cold period experienced during the 2010 early spring (Chapter 3; Fig 3.17) which may have affected peat degradation and formation of less active aromatic THM precursors.

The second highest generator of CHCl_3 per mg DOC during the sampling period was the Cefni which has a large nutrient rich fen in its catchment. Both the Cefni and Conwy were the only two Raw Surface Water DOCs which demonstrated a tentative correlation between THMFP, Aromaticity and SUVA (Conwy, $r\ 0.30\ p < 0.05$, Aromaticity) and (Cefni, $r\ 0.44\ p < 0.05$, SUVA) hence suggesting a weak relationship with THM formation and the aromatic DOC content. There is no evidence in the data set that the exceptionally high THM concentrations recorded in July 2010 and the low concentrations measured in March 2010 were caused by changes in DOC quality, which would be indicated by analyses of SUVA, Aromaticity for any of the other lake samples. This reflected a common throughout this time series analysis which supports the literature's suggestion that the DOC of Raw Surface Waters consisted of a contribution of organic compounds of which not all were THM precursors (Mosteo *et al.* 2009; Engelage and Stringfellow, 2009; Gehr *et al.* 1993). For example, for every 1mg of DOC in the Cefni, an average 6.8 μg of carbon is utilised to form the total THMs/mg DOC, expressed as a percentage, this represents 0.7 % of the DOC. Conwy which had the highest total average THMFP/mg DOC (106.1 μg) converted 10.5 μg of carbon per 1 mg of DOC, i.e., 1.1 %.

The formation of brominated THMs were relatively low for the 24 month sample series. Although it was the Cefni Raw Surface Water samples that exhibited the highest bromide ion concentrations (0.07mg/L), it was Cwellyn that formed the highest brominated THMs overall. This was as a result of it and Teyrn in particular exhibiting higher concentrations of brominated THMs than the Conwy and Cefni for the period November 2009 to June 2010. This was reciprocated by lower concentrations of CHCl₃ for the same months, which agreed with Sketchell *et al.* (1995) and Italia *et al.* (1991), that an inverse relationship exists between trichloromethane and brominated THM species. The linear correlation (R^2 0.50) of the ratio of (CHCl₃: Brominated species) *versus* CHCl₃ (Fig 5.14) for the sampling period supported these observations. Table 5.16 demonstrates the drop observed in the brominated species relative to the increase in CHCl₃ during July 2010 compared to the 24 month averages. This may as, suggested by Westerhoff *et al.* (2004) been due to the DOC aromatic ring bonds being cleaved by the chlorine, with the bromine demonstrating substitution into the ring rather than cleavage, hence a reduction in brominated halomethanes.

Lake/Reservoir	% Total Brominated THMs July 2010	% CHCl ₃ July 2010	% Averaged Brominated THMs for 24 months	% Averaged CHCl ₃ for 24 months
Cefni	4.5	95.5	12.9	87.1
Conwy	2.7	97.3	5.3	94.7
Cwellyn	6.6	93.4	15.9	84.1
Teyrn	4.3	95.7	14.4	85.6

Westerhoff *et al.* (2004) carried out experiments on the reaction kinetics of aqueous bromine and chlorine with DOC and model compounds (recorcinol, benzoic acid, 3,5-dimethoxy benzoic acid and phenol). They suggested that the bromine and chlorine reactions were competitive during the chlorination process and that in the absence of HOCl, the HOBr reaction rate with DOC was 10 times faster. In Raw Surface Water, bromine is usually in the reduced form (Br⁻). Hence when the Raw Surface Water is chlorinated using chlorine or NaOCl the HOCl formed in the aqueous state demonstrates a redox potential of ($E^0 = + 1.630V$). This oxidizes the Br⁻ to HOBr ($E^0 = + 1.331V$) (Eqn 5.1) which then becomes a competitor for active sites on the DOC molecules.



Although the formation of HOBr is dependent on HOCl. Sketchel *et al.*(1995) suggested that their reactivity with DOC is not competitive but independent, with the formation of brominated THMs being biased to substitution reactions due to the higher electron density of the Br⁻ stabilizing the carbocation for electron release (Sykes, 1986). The chlorine a stronger oxidant is believed to target electron rich sites which includes the cleaving of double bonds.

Ishihashi *et al.* (1999) established that HOCl was also significant in the formation of brominated THM precursors. They reacted the DOC with a known concentration of NaOBr and measured the formation of CHBr₃. These workers repeated the experiment, but included NaOCl, which resulted in the formation of 7 times the initial experiment's concentration of CHBr₃ being formed.

Westerhoff *et al.* (2004) demonstrated that the halogen consumption and THM yields by organic compounds representative of some of the DOC moieties such as resorcinol (1,3-dihydroxybenzene) and benzoic acid were affected by the degree of aromaticity and the presence of electron donators (hydroxyls) and electron withdrawing functional groups (carboxyls) (see Chapter 7, Sec 7.5). Korshin *et al.*, (1996) also proposed that THMFP was related to the ratio (A₂₅₃/A₂₀₃) which was indicative of aromatic ring substitution. The results in this study of the actual total THM concentrations and the ratio did not generate significant correlations, but the THM concentrations did demonstrate changes relative to the product of the ratio. Conwy which had a ratio > 0.4 for the majority of the period had the highest levels of THMs (averaging > 106 µg/mg DOC), whilst Teyrn and Cwellyn whose ratio tended to remain between the upper and lower parameters (0.25 - 0.40) generated much lower THM concentrations (average 59.0 and 64.7 µg/mg DOC respectively). Cefni's ratio product demonstrated that the DOC's aromatic substituents changed, with mainly hydroxyls during the winter period, but peaked above 0.4 ratio line in summer. Cefni averaged total THMs of 72.9 µg per mg of DOC over the 24 month period, with the highest ratio (0.42) occurring in July 2010 together with a high THMFP of 141.5 µg/mg DOC. A second high ratio was recorded in June 2011, accompanied with a THMFP of, 116.7 µg/mg DOC. Although the ratio was indicative of the changing absorption characteristics of the aromatic ring when subjected to substitution it could not be used as an indicator THMFP.

The surface water MM ranges of all the four lakes were dominated by the 5000-4000 and 4000-3000 Da. A reduction of this range was exhibited after being processed (pre-Chlorination samples) through both the Cwellyn and Cefni treatment works. This left the lower ranges (2000-1000, 1000-10 Da) as the dominant MW distributions and laboratory chlorination of these samples demonstrated a reduction of 45.8 % for the Cefni and 31.9 % for Cwellyn THMFP in comparison to the surface water samples. Chang *et al.*, (2001) reported that low MM distributions generated higher SUVA values and higher THMFP. The data presented here agreed with their initial findings as regards to higher SUVA values (Table 5.17) but the data for THMFP contradicted their findings by demonstrating lower THMs / mg DOC after treatment..

Sample	THMFP ($\mu\text{g}/\text{mg}$ DOC) Raw Surface Waters	SUVA ($\text{L}/\text{mg m}^{-1}$)	THMFP ($\mu\text{g}/\text{mg}$ DOC) Pre- Chlorination	SUVA ($\text{L}/\text{mg m}^{-1}$)
Cefni	68.9	3.4	37.4	4.2
Cwellyn	53.6	4.1	36.5	5.2

When we considered THMFP of the lake DOCs relative to each other over the 24 month period, the results highlighted significant correlations (Table 5.14). Cefni the lowland fen catchment demonstrated a weak correlation ($r = 0.443$, $p < 0.05$) with the Conwy (upland blanket bog catchment) indicating that both catchments were exporting DOC with relatively similar active sites susceptible to chlorination.

Cwellyn and Teyrn the other upland sources did not demonstrate any correlation with the Cefni, but did exhibit significant correlations with the Conwy and each other (Table 5.11). This tends to confirm similarities in the THMFP of the upland site DOCs.

Lakes/ Reservoirs	Correlation coefficient (r)
Cefni/Conwy	0.443, $p < 0.05$
Cwellyn/Conwy	0.592, $p < 0.01$
Cwellyn/ Teyrn	0.660, $p < 0.01$
Conwy/ Teyrn	0.617, $p < 0.01$

5.11 Conclusions

Similar to the findings reported in the literature, the DOC of the Cefni, Cwellyn, Conwy and Teyrn form four THMs in the concentration sequence $\text{CHCl}_3 > \text{CHBrCl}_2 > \text{CHBr}_2\text{Cl} > \text{CHBr}_3$.

- The Cefni catchments exported the highest concentrations of DOC, but it was the Conwy that had the highest THMFP /mg DOC. Its THMFP for CHCl_3 was 36 % higher than the Cefni and approximately 50 % more than both Cwellyn and Teyrn.
- The formation potential of brominated THMs was low. Of the brominated THMs CHBrCl_2 demonstrated the highest formation concentration by all of the four lake DOCs.
- The formation of CHBr_3 was sporadic and close to the LOD.
- Cefni and Cwellyn's Finished Product and Pre-Chlorination THMFP results demonstrated increases in brominated products suggesting either contamination by the flocculation/filtering reagents, or secondly the DOC' associated with Br^- in the natural state was not removed during the flocculation/ filtration operation.
- The increase could also be an effect of the coagulant itself, as Westerhoff *et al.* (2004) indicated, that an increase in brominated products over chlorinated products may be recorded with increasing coagulant concentration.
- THMFP was found to be very high for all the lake Raw Surface Water DOCs after the 2010 dry period.
- Stored samples, present different THM formation potential.
- There were no correlations between THMs, SUVA, aromaticity and phenolics.
- The (A_{253}/A_{203}) ratio for the Conwy demonstrated the optimum ratio for THM formation.
- A reduction in the MM ranges 5,000 – 3,000 Da with an increase in the 2,000 – < 1,000 Da range was noted after coagulation and flocculation.
- THMFP was reduced in the Pre-Chlorination and Finished Product samples.

Chapter 6

Characterisation of Lake/ Reservoir Raw Surface Water Fractions.

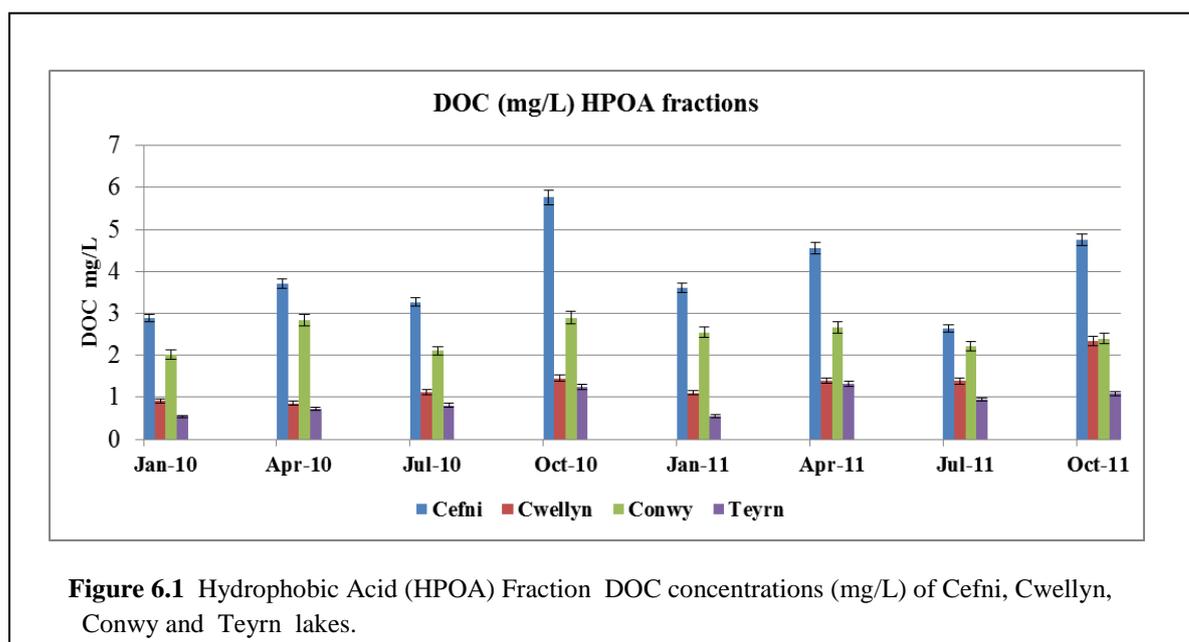
Chapter 6 Characterisation of Lake/ Reservoir Raw Surface Water Fractions.

6.1 Introduction

Fractionation of the four Raw Surface Waters was carried out every 3 months by the method described in Chapter 2 (2.3.6) using non-ionic DAX 8 and XAD 4 resins in tandem. The samples were prepared and stored before being fractionated using the standard procedure previously described for Raw Surface Waters. The methods described in Chapter 2 were used to characterise the fractions recovered.

6.1.1 DOC Analysis

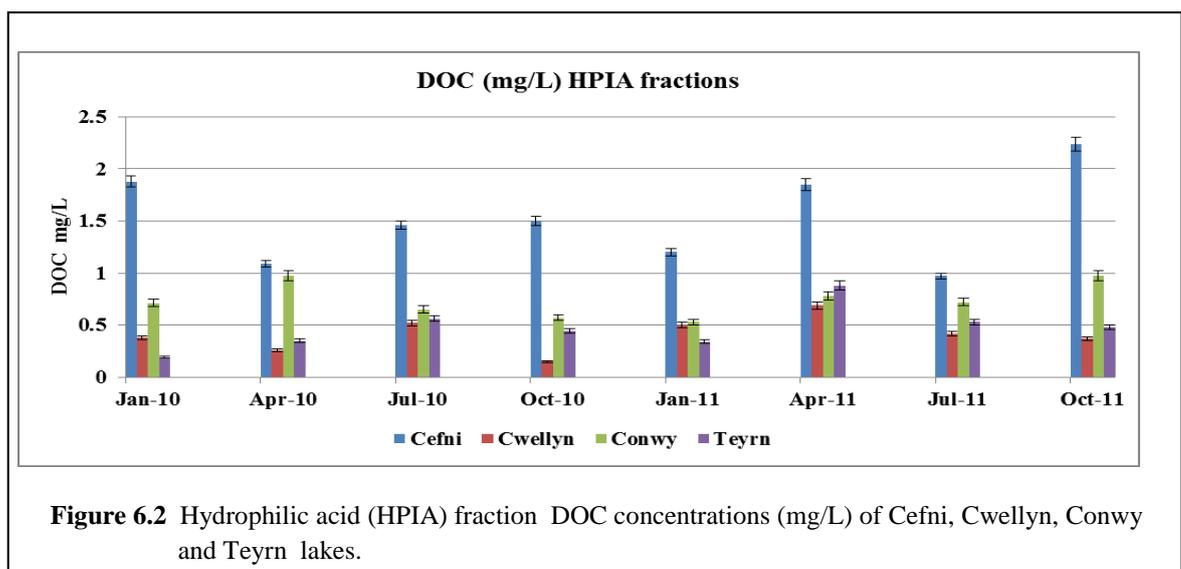
The recovered HPOA, HPIA, HPOB and HPIB fractions (100 ml) were diluted to 1L with Milli Q (18M Ω) water. The recovered effluent after passing the 1L Raw Surface Water sample through both resins was identified as the Neutral fraction. Unlike the above fractions, its volume was not diluted. All the fractions were adjusted to pH 2.8 and DOC analysis was carried out on the fractionated samples using Method 2.3.1.



The hydrophobic acid (HPOA) demonstrated (Fig 6.1) the highest concentration of DOC of all fractions of the series. Of the 4 sites, the Cefni exhibited the highest HPOA DOC throughout, ranging from a high of 5.77mg/L during October 2010 to a low of 2.64 mg/L during July 2011. The highest concentration levels coincided with the autumnal flush when

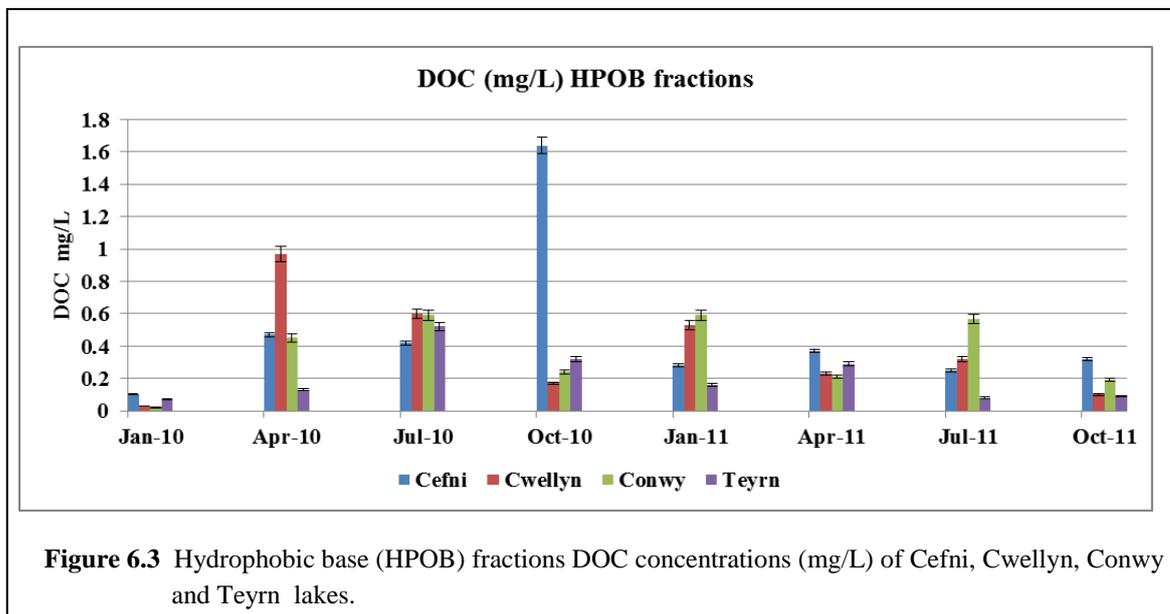
rainfall generally increased during the months of October 2010/11, DOCs (5.77 and 4.75 mg/L) respectively. The second highest series of HPOA DOC concentrations were noted for the Conwy fractions, but at a much lower range; 2.02mg/L in January 2010 to the highest of 2.90mg/L during October 2010. Lower DOC levels of the HPOA fractions were exhibited by Cwellyn and Teyrn. Evidence of the autumn flush was present in the Cwellyn series, with two relatively high concentrations being recorded for the months of October 2010/11 at 1.45 and 2.34mg/L respectively. Teyrn DOC concentration for the HPOA fraction demonstrated the lowest average level (0.91mg/L) of all the lakes. The lowest Teyrn HPOA levels were recorded for January 2010 and 2011 at 0.54 and 0.55 mg/L, respectively with the highest again in the autumn flush during the Octobers of 2010/11 (average 1.2mg/L).

Cefni also dominated the highest concentration levels (Fig 6.2) of the hydrophilic acid fractions (HPIA) but their levels compared to the HPOA's were much lower, averaging 1.52 mg/l for the overall series with the uppermost recorded in October 2011 at 2.24mg/L.



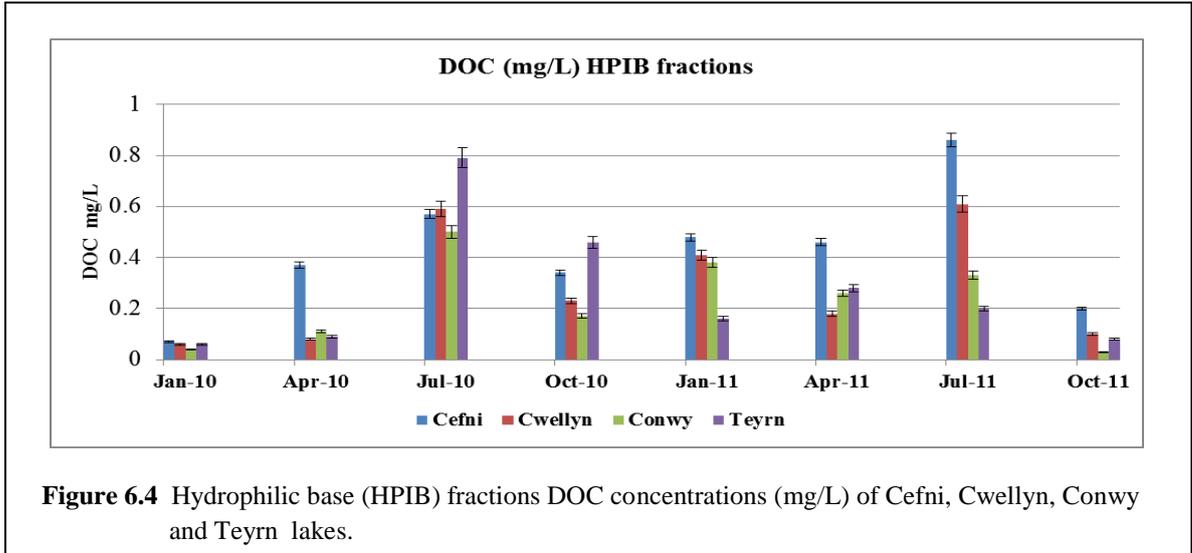
Whilst the HPOA fractions, for the four lakes all demonstrated distinct concentration differences, the HPIA fractions could be grouped into the Cefni and then the three upland lakes Cwellyn, Conwy and Teyrn. Of the upland sites Conwy had the highest concentration average of 0.74mg/L. Teyrn which had an overall (average 0.47 mg/L), exhibited during April 2011 the highest concentration of all three at 0.88mg/L compared to both Conwy's (0.78mg/L) and Cwellyn's (0.69mg/L). Teyrn consistently had a higher HPIA DOC than Cwellyn (Fig 6.2) with October 2010 showing the greatest difference between them of 0.15mg/L.

The hydrophobic base fractions (HPOB) exhibited very low DOC concentrations (Fig 6.3) with two exceptions. Cefni, during October 2010, demonstrated the highest level of the series at 1.64mg/L and Cwellyn in April 2010 showed 0.97mg/L.

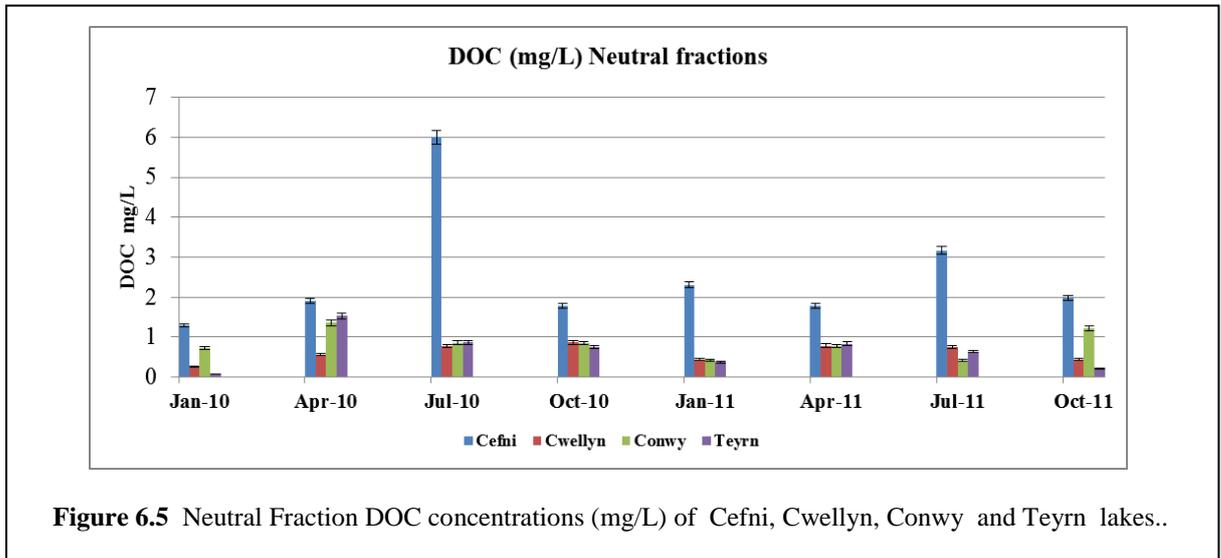


Otherwise the DOC levels of the HPOB fractions for all four of the lakes remained below 0.6mg/L during the sampling period. Cefni exhibited lower concentrations than Conwy and Cwellyn during July 2010, January 2011 and July 2011. Cwellyn and Conwy appeared to exhibit a similar trend in HPOB DOC concentrations for most of the period with an 0.02 mg/L average difference between each other. An exception occurred during the month of April 2010 when Cwellyn demonstrated an increase and the average difference between it and Conwy was 0.52 mg/L. This was also recorded for July 2011 when Conwy average concentration increased above that of Cwellyn's by 0.25mg/L.

The hydrophilic base fraction (HPIB) DOC concentrations (Fig 6.4) were all < 0.9mg/L. Cefni demonstrated the highest average concentration over the sampling period at 0.4 mg/L, whilst the three upland lakes averaged 0.2 mg/L. Teyrn exhibited a higher concentration than the other three sites during July 2010 (0.79mg/L) and again in October 2010, but at a much lower level (0.46mg/L). During July 2011 both Cefni and Cwellyn recorded the highest (HPIB) concentrations (Cefni 0.86mg/L and Cwellyn 0.61mg/L). January 2010 and October 2011 exhibited the lowest levels of DOC for all the four lake HPIB fractions, two of the lowest (0.1mg/L) were for Cwellyn and Conwy.



The neutral fractions consisted of DOC molecules that were not adsorbed on to either the DAX 8 or XAD 4 resins. This fraction demonstrated distinctively higher concentrations (Fig 6.5) in the Cefni samples for the majority of the sampling period, compared to those of the upland sites.



The average concentration over the sampling period was 2.5mg/L for the Cefni whilst Cwellyn, Conwy and Teyrn exhibited averages of 0.6, 0.8 and 0.7 mg/L respectively. The July 2010 and 2011 DOC concentrations for the Cefni were 6.0 mg/L and 3.17 mg/L respectively, whilst Cwellyn Conwy and Teyrn only averaged 0.8 mg/L and 0.6mg/L for the same months.

6.1.2 Determination of Phenolic Compounds in Raw Surface Water Fractions.

The analysis for phenolics in the surface water fractions was carried out, but the results were considered to unreliable due to large discrepancies between the mass balances (e.g Cefni Raw Surface Water: 1.1mg/L, Cefni total fractioned Raw Surface Water 1.9 mg/L) of the fractioned phenolic results and the Raw Surface Water phenolic results. Validation of the method required investigation and further research before the results could be deemed valid (See Appendix 3).

6.1.3 UV Spectroscopy of Raw Surface Water Fractions.

The UV spectra of the fractioned samples were relatively featureless and presented a similar profile to those of the Raw Surface Water spectra, with the exception that the HPOA and Neutrals exhibited higher intensity values. Figures 6.8 a,b were typical of the spectra generated by the fractions, with the highest absorbance's occurring in the 200 to 350 nm spectral region associated with $\pi - \pi^*$ transitions (Silverstein *et al.* 1997).

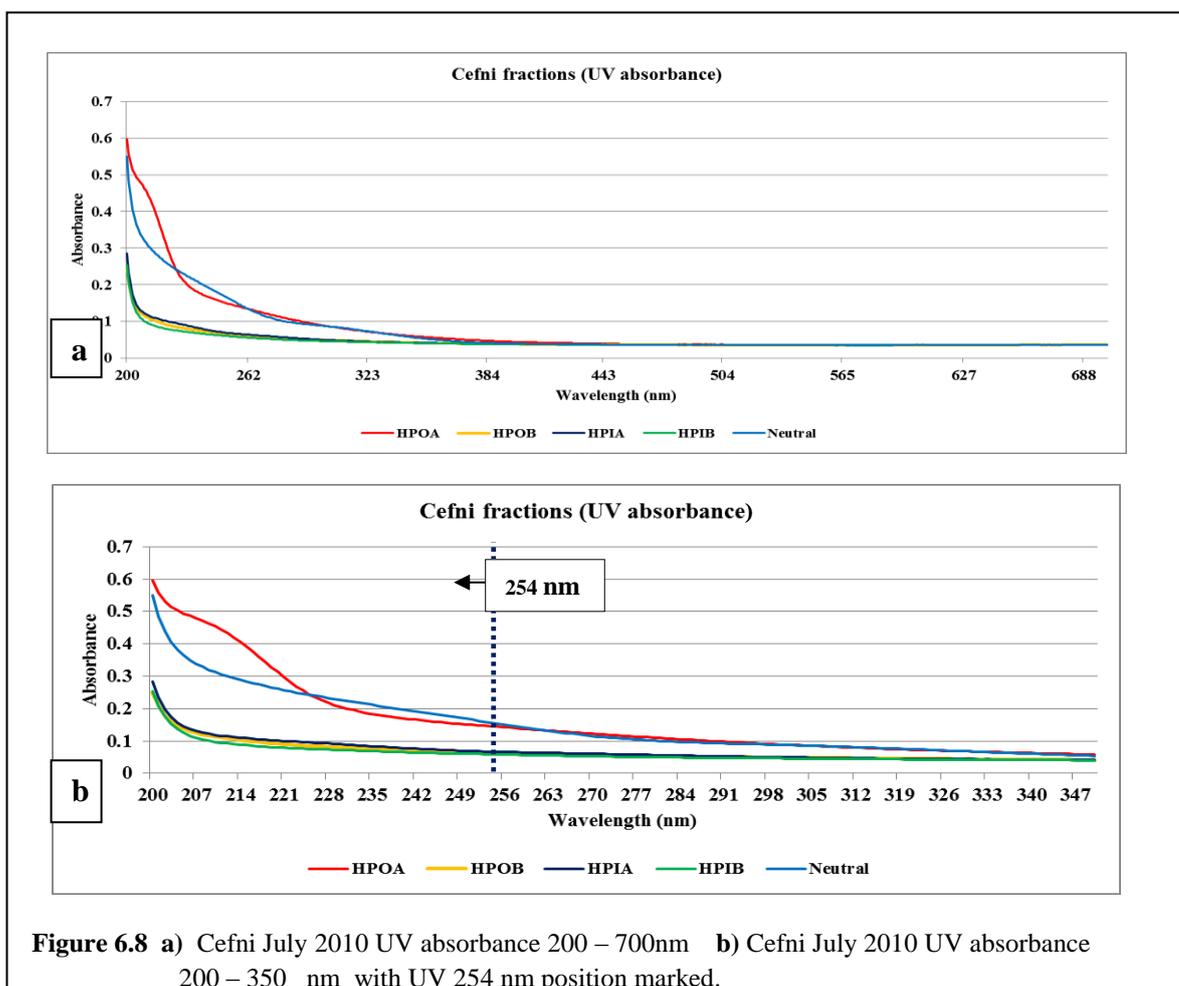
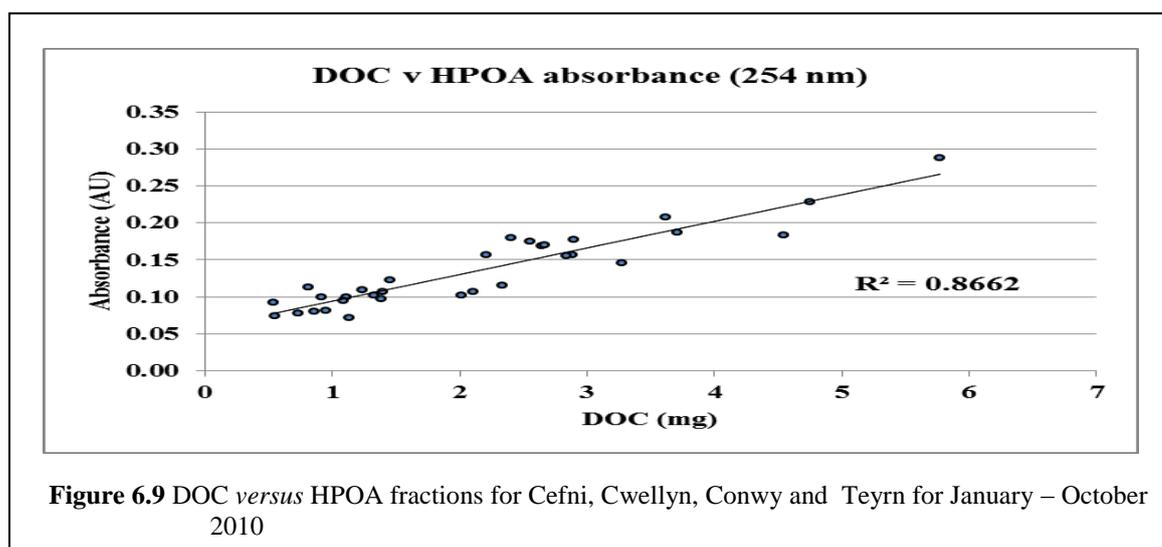


Figure 6.8 a) Cefni July 2010 UV absorbance 200 – 700nm b) Cefni July 2010 UV absorbance 200 – 350 nm with UV 254 nm position marked.

The most intense absorbance in the HPOA and Neutral fractions appeared at *ca.* 214 nm although there were no well defined peaks present. This was as a result of the numbers and heterogeneity of different types of molecules in the DOC and the resulting coalescence of bands. The broad absorption intensities suggest aromatics with a number of possible substitutions, for example, an unsubstituted benzene ring would exhibit an absorbance at 254 nm but when substituted with an OH it absorbs at 210.5 nm (hypsochromic shift). If the OH was oxidized to a phenolate anion, a bathochromic shift occurs and it absorbs at 235 nm (Silverstein *et al.*, 1997). In addition *o*-catechols absorb at 214 nm, but if the catechol was present as the *o*-catecholate anion, again a bathochromic shift to 236.5 nm would be observed (Silverstein *et al.*, 1997). Thus absorbance in the near UV (200 – 380 nm) is greatly influenced by the changing oxidation states, structured substitution and ionic state of the DOC

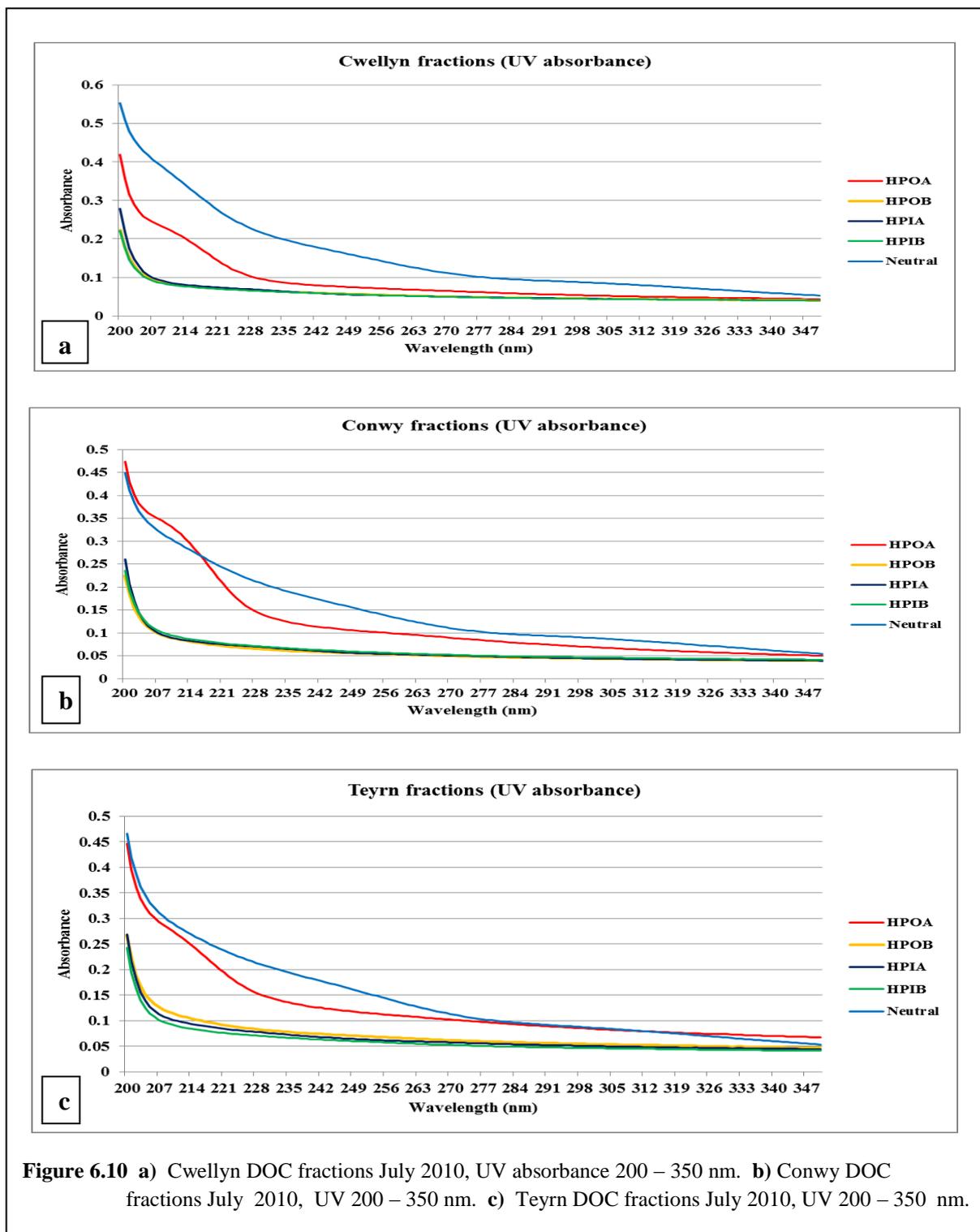
The four lake DOC concentrations *versus* the HPOA absorbances at 254 nm gave an R^2 value of 0.8662 (Fig 6.9), but the four other fractions (HPOB, HPIB, HPIA and Neutrals) *versus* DOC demonstrated poor linear correlations ($R^2 < 0.2$).



This tends to suggest that the aromatic moieties predominated the HPOA fraction as suggested in the literature (Aiken *et al.* 1992 ; Croue *et al.* 2004), but these data were less consistent in the other fractions.

Figs 6.10 a,b and c show the spectra of Cwellyn, Conwy and Teyrn fractions for July 2010, and the highest absorbance, like the Cefni, was demonstrated by the HPOA and Neutrals. Although again there were no distinct peaks observed for the overall series, the

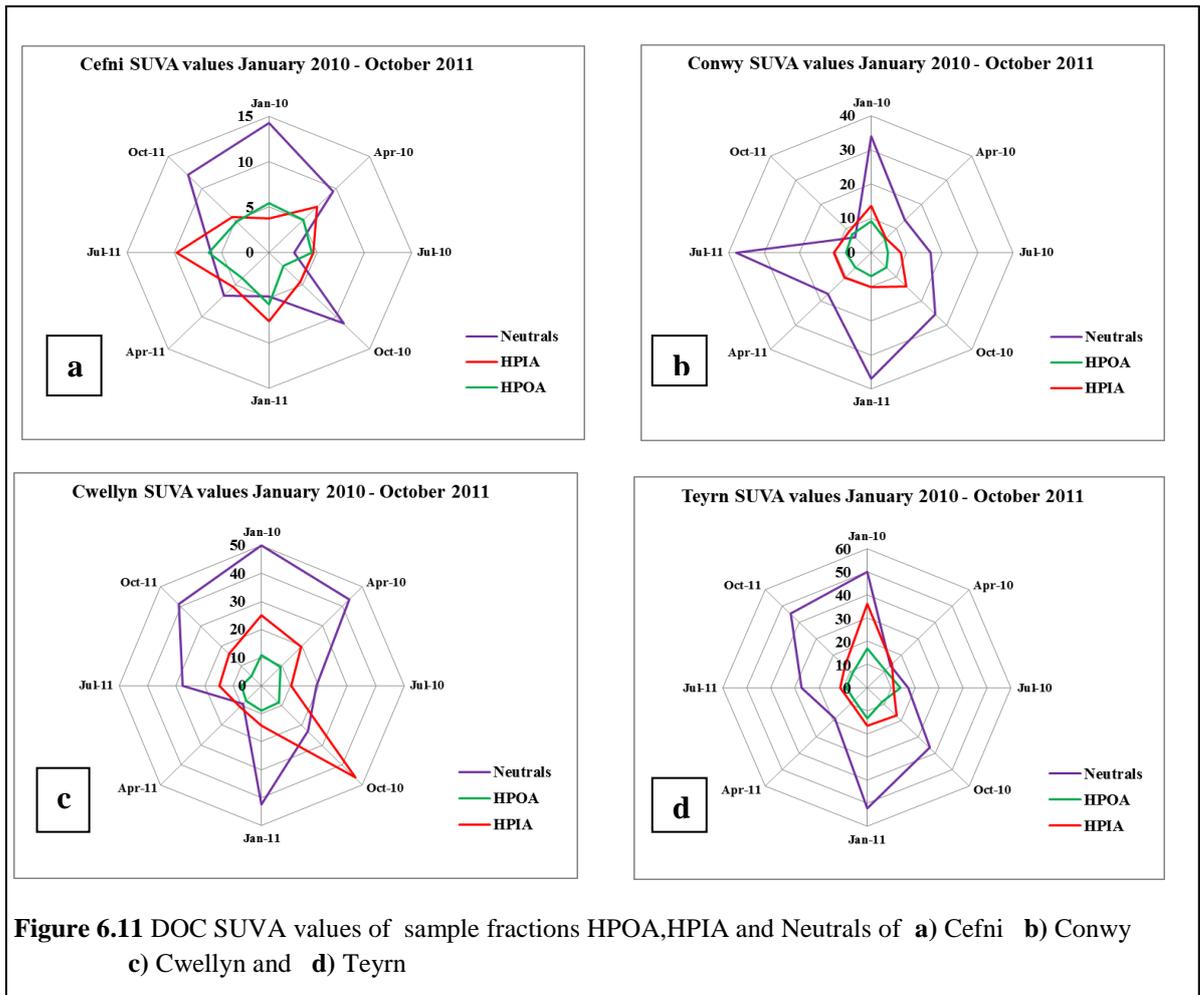
Neutrals of the upland DOC demonstrated the highest absorbance intensities for the majority of the fractionated series. This was not consistent in the Cefni lowland DOC as the Neutral and HPOAs' tended to randomly exchange from sample series to sample series.



6.1.4 UV Spectroscopy (SUVA) of Raw Surface Water DOC Fractions

SUVA values which are considered to be a numerical indicator of the aromatic content (i.e., high values are indicative of high DOC aromatic content and *vice versa*). These were calculated out using the formula (EPA/600/R-09/122, 2009) :

$$(F. 1) \text{ SUVA (L/mg C m)} = [\text{Abs (nm)} / \text{DOC (mg/L)}] * 100$$

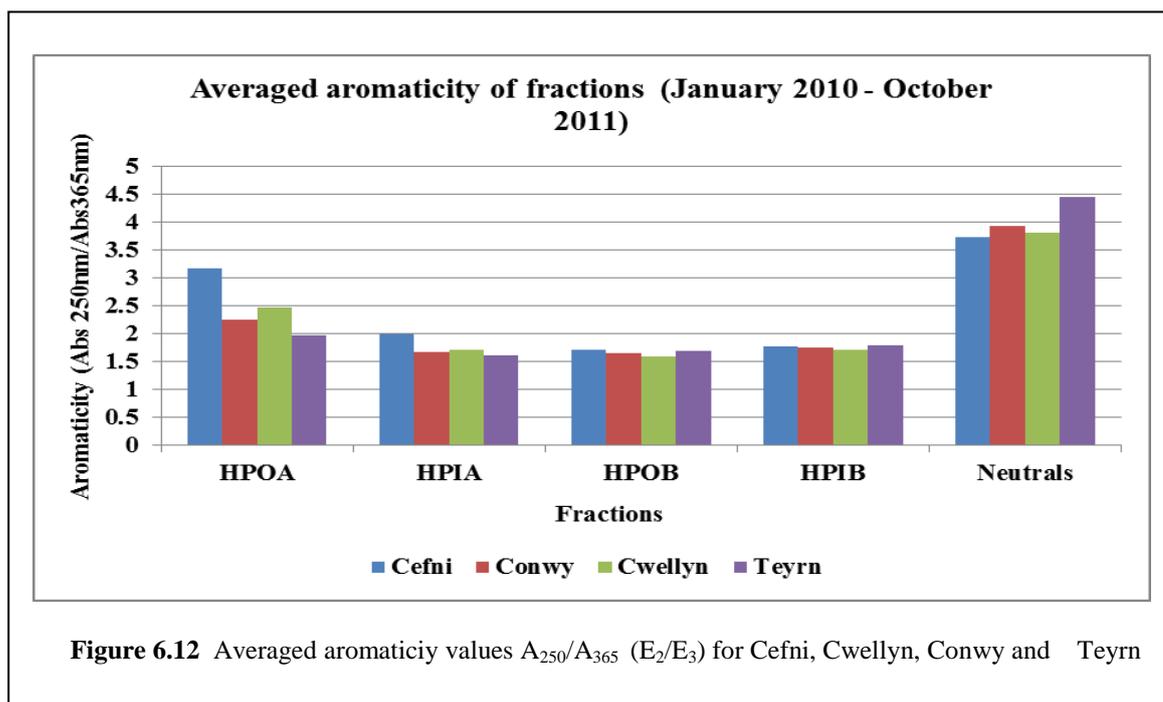


The data is presented as radar plots (Fig 6.11 a,b,c and d) to display the variation in values recorded for the SUVA calculations within the same suite of results. Although the value ranges changed from fraction to fraction within the suite, the pattern from lake to lake DOC SUVA values were similar. The SUVA for the HPOA demonstrated the lowest series of values (average 5 L/mg m⁻¹) for the fraction series of each lake DOC. This was similar to those reported in the literature (Edzwald and Tobiasson, 1999), but the other values for the Neutrals, HPIA, HPOB and HPIB varied from > 10 to > 50 L/mg m⁻¹, which did not reflect any reported literature values (Chow *et al.* 2003). The HPIAs' series

generated higher values than the HPOA for all fractions with the values for the Neutral fractions exceeding both, but with some limited exceptions. For example, both July 2010 and 2011 the Cefni fractions generated values of less than 5.0 L/mg m⁻¹. The lowest for the three upland lakes were for Cwellyn April 2011 (9.4 L/mg m⁻¹), Conwy October 2011 (6.3 L/mg m⁻¹) and Teyrn April 2010 (13.6 L/mg m⁻¹). Evaluation of the data sets highlighted that when the concentration of the DOC was < 1 mg/L, and the absorbance values approached < 0.1 A.U the resulting SUVA values increased greatly.

6.1.5 UV Spectroscopy (Aromaticity, A_{250}/A_{365}) of Raw Surface Water DOC Fractions.

The aromaticity ratio values were calculated by dividing the 250 nm absorbance value by the 365 nm absorbance value defined as (E_2/E_3).



Peuravuori and Pihlaja (1996) and Chin *et al.* (1994) suggested that the greater the ratio value, the less aromatic moieties were present and their associated average molecular masses were also considered to be lower. The averaged results (Fig 6.12) show that although the DOC was from 4 different sources, each fraction reflected similar aromaticity values. Hence the lowest values for all four lake DOCs were demonstrated by the HPIA

(1.74), HPIB (1.75) and HPOB (1.67) fraction ranges, thus proposing a higher aromatic content than the averaged Neutrals (3.98) and HPOA (2.46). Reviewing the individual results, the Cefni exhibited slightly higher averaged values for all of the four fractions (HPOA, HPIA, HPIB and HPOB). The Cefni's Neutral fraction, demonstrated the lowest aromaticity value of the averaged lake group, thus indicating a higher aromatic content. This was also observed in the other lake fractions with Teyrn demonstrating the highest, hence it had the lowest aromatic content.

6.1.6 Size Exclusion Chromatography (SEC) of Fractioned Raw Surface Water DOC.

SEC analysis was carried out using Method 2.3.3 (Chapter 2).

The suite of fractions collected for each individual lake surface water DOC were analysed at pH 6.8 after being diluted from 100 ml to 1L with Milli Q water (18M Ω). The final dilution represented the concentration of the fraction (e.g. HPOA) that was present in the Raw Surface Water DOC. Each series of chromatogram peak overlays (Figs 6.13 a,b,c,d) represent the Raw Surface Water sample and its fraction traces. The Raw Surface Water fraction peak distributions appear to reflect those of the Raw Surface Water samples, but with reduced intensities. Subtle changes in retention times (RT) between the Raw Surface Water peaks and the fraction peaks were noted. The HPOA had an RT of approximately 9.0 minutes indicating a MM with a higher overall distribution average than that of the Raw Surface Waters (RT 9.3). The remaining fractions appeared to have RTs \geq 9.3 minutes which tended to indicate lower MMs distribution ranges. There were also differences recorded for the start and end of peak base times which indicated that some of the MM ranges were absent or at very low concentrations. This is demonstrated for example, in the Teyrn sample (6.13 d), where the HPOB, HPIA, HPIB and Neutrals peaks start at \approx RT 8.8 and end at 10.7 minutes which represents a range of 5000 – 1000 Da. The peaks shapes for all four of the lake fractions were asymmetrical with a tendency to tail rather than front. This tendency was also present in the Raw Surface Water peaks thus limiting the absolute determination of the molecular mass distribution, as the cut off of the standard curve was at RT 10.7 minutes which represented the calibrant (MM 1350 Da).

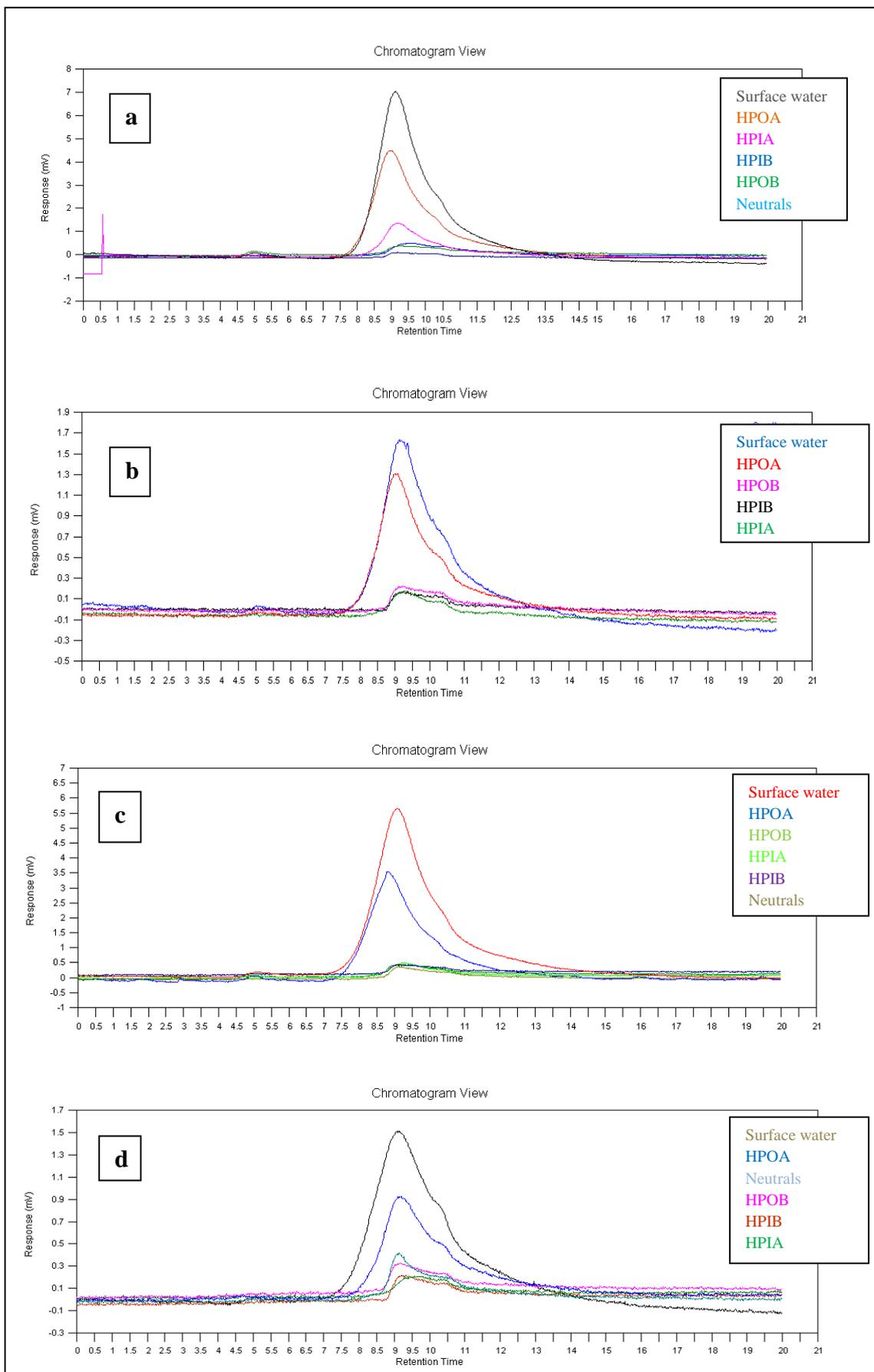


Figure 6. 13 SEC chromatograms showing fraction MM distributions for **a)** Cefni, **b)** Cwellyn, **c)** Conwy **d)** Teyrn

Table 6.1 Pearson's correlation coefficients of the suites of fractions January 2010 – January 2011, demonstrating similarities in the range distributions from lake to lake DOC.

<i>HPOA fractions</i>				
Lake/reservoir	Cefni	Cwellyn	Conwy	Teyrn
Cefni		0.966**	0.955**	0.925**
Cwellyn	0.966**		0.946**	0.984**
Conwy	0.955**	0.946**		0.939**
Teyrn	0.925**	0.984**	0.939**	
<i>HPOB fractions</i>				
Cefni		0.786**	0.874**	0.820**
Cwellyn	0.786**		0.788**	0.823**
Conwy	0.874**	0.788**		0.951**
Teyrn	0.820**	0.823**	0.951**	
<i>HPIA fractions</i>				
Cefni		0.775**	0.972**	0.966**
Cwellyn	0.775**		0.714**	0.691**
Conwy	0.972**	0.714**		0.962**
Teyrn	0.966**	0.696**	0.962**	
<i>HPIB fractions</i>				
Cefni		0.706**	0.874**	0.820**
Cwellyn	0.706**		0.788**	0.823**
Conwy	0.874**	0.788**		0.951**
Teyrn	0.820**	0.823**	0.951**	
<i>Neutral fractions</i>				
Cefni		0.421*	0.883**	0.543**
Cwellyn	0.421*		0.694**	0.706**
Conwy	0.883**	0.694**		0.881**
Teyrn	0.543**	0.706**	0.881**	

** Significance at 0.01 level, * Significance at 0.05 level

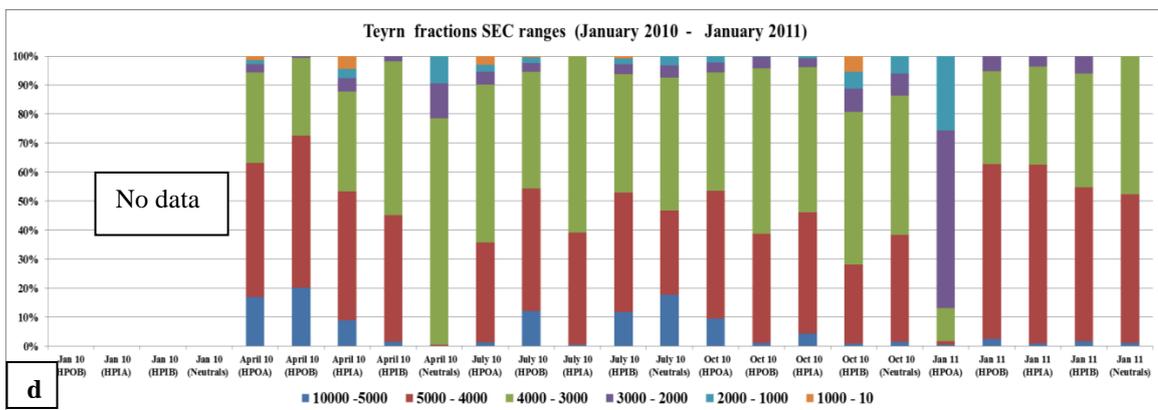
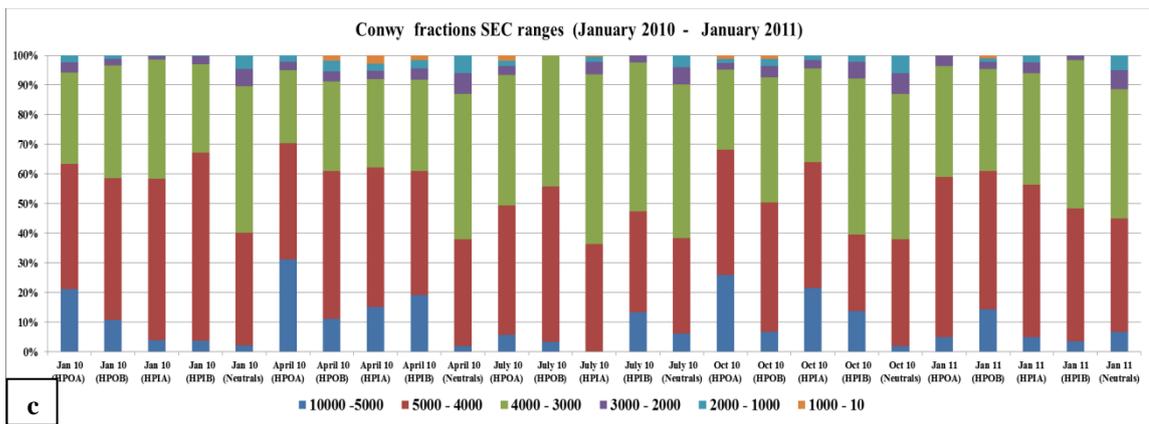
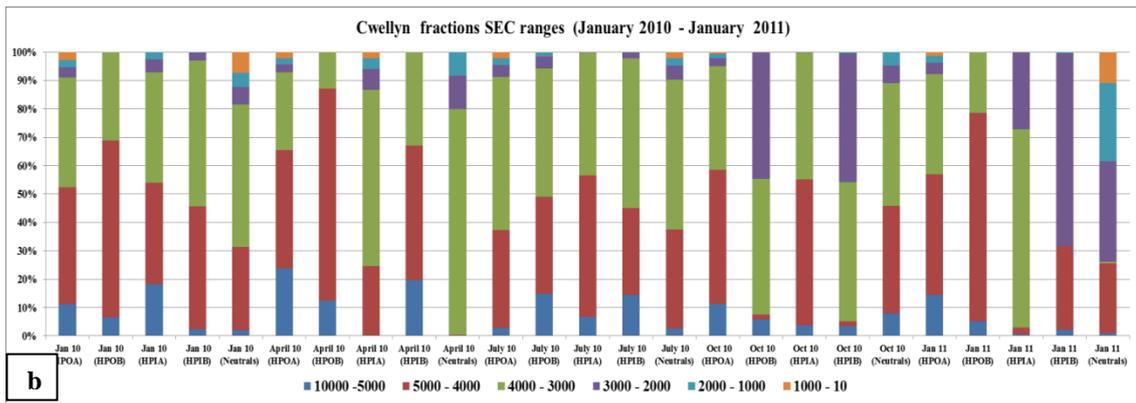
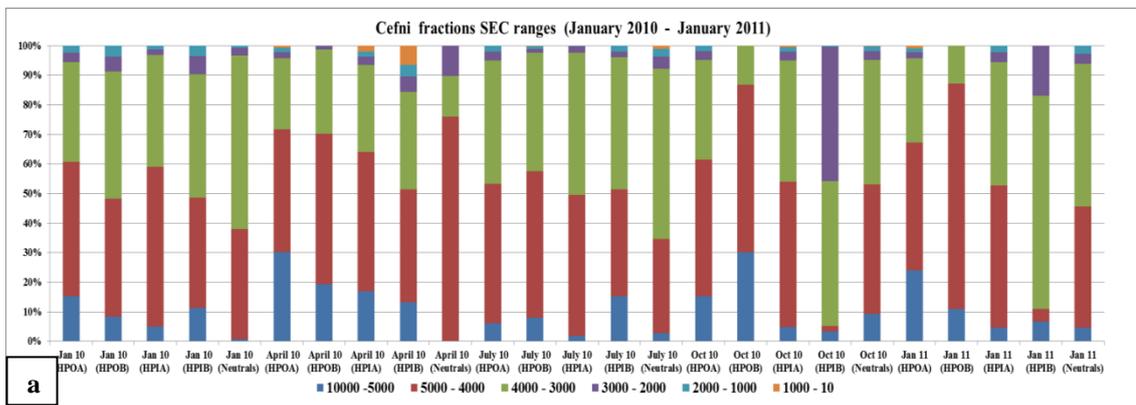


Figure 6.14 SEC MM ranges shown as percentages of peak distributions for a) Cefni b) Cwellyn c) Conwy d) Teyrn for the period January 2010 – January 2011.

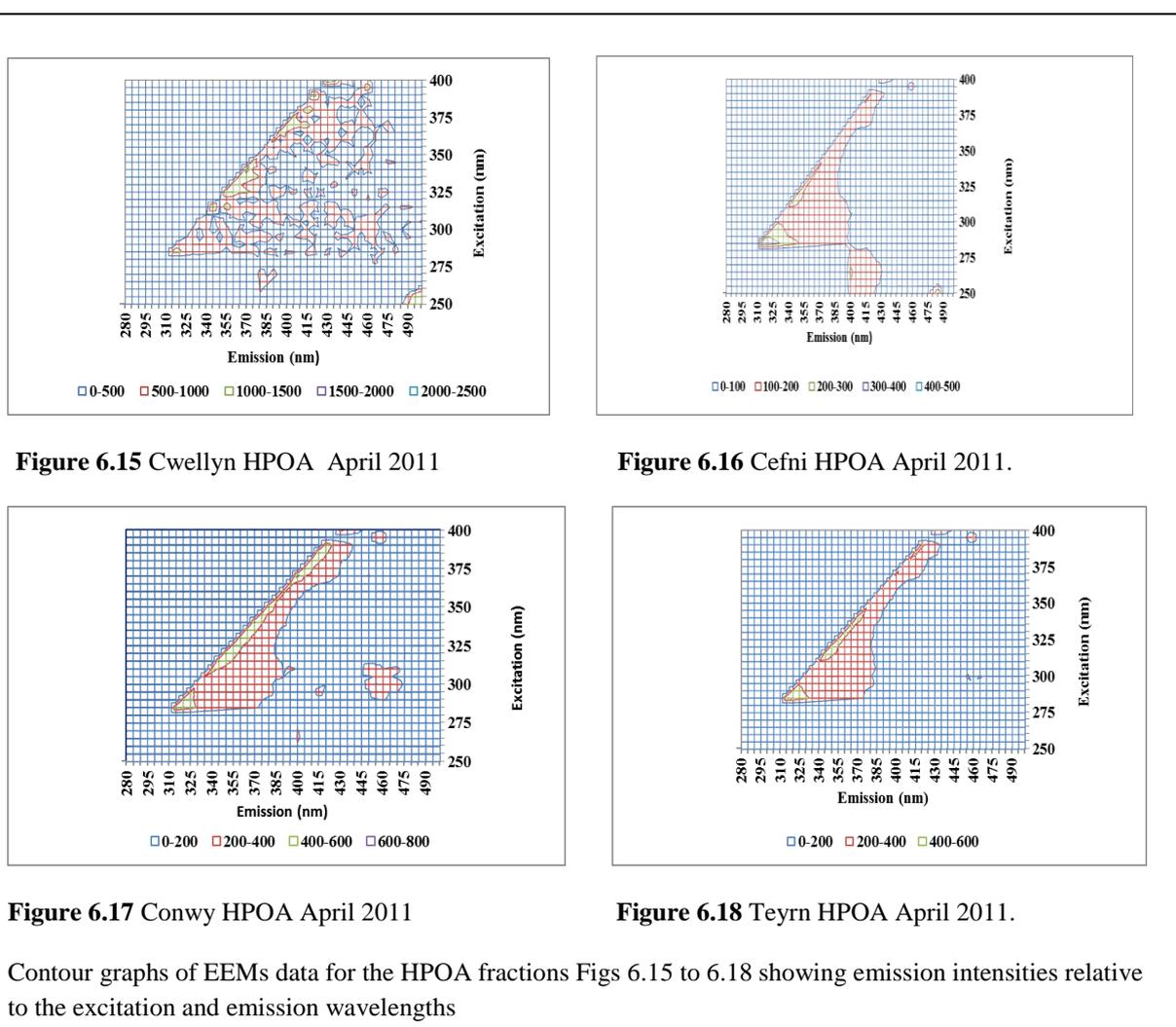
To overcome this, the area of the peak within the limits of the highest and lowest standards were normalised to 100%. This enabled the characterisation of the sample peaks within the upper and lower limits of the standards.

The results are shown in a similar format (Figs 6.14 a,b,c and d) to those of the surface water DOC (Chapter 3 Figs 3.14 a,b,c,d), i.e., peak area slices relative to the MM transformed into percentages. Like the Raw Surface water the dominant MM were the distributions 5000 – 4000 and 4000 – 3000 Da in each fraction. A subtle difference was observed in the majority of the Neutral fractions, where there was an increase in 4000 – 3000 Da distribution compared to the 5000 - 4000 Da ranges by approximately 1.5 : 1. Both Teyrn and Cwellyn April 2010 Neutrals demonstrate significant increases in the 4000 – 3000 Da > 75 % with < 1 % of the 5000 – 4000 Da range, but for the same month and fraction for the Cefni, the 5000 – 4000 Da range was dominant at > 75.5%. Seasonal influences were not observed in the MM ranges of the fractions, although the relatively low molecular mass 3000 -2000 Da range increased in the Cefni, Cwellyn and Teyrn from October 2010 onwards, suggesting a release of DOC that had been degraded over a longer period or by higher microbial activity usually associated with the higher temperatures of summer, being flushed out of the catchments by the increasing autumnal rainfall.

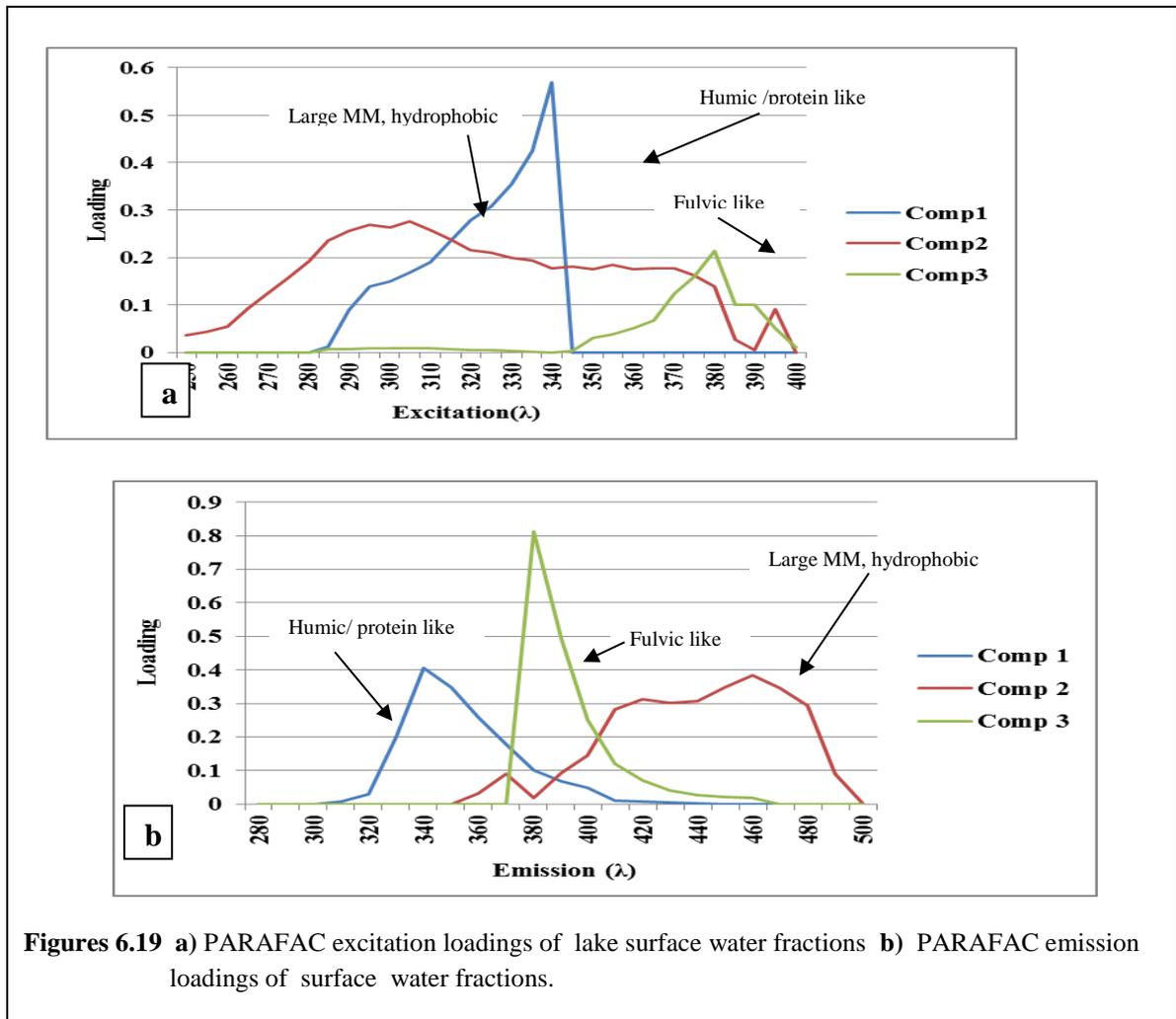
A statistical evaluation of the relationship between the fractions was established using Pearson correlation coefficients. Table 6.1 shows the results of correlations exercise, with the HPOA suites demonstrating the most significant relationships. The Neutrals demonstrated weaker correlations, especially for the Cefni with Cwellyn and Teyrn, hence highlighting the MM range differences and their Neutral hydrophilic properties. Whilst Conwy's Neutrals demonstrated a strong correlation with the Cefni Neutral DOC, suggesting that the hydrophilic molecular structures within the samples had similar MM distributions. The results established the selectivity of the DAX 8 and XAD 4 relative to the hydrophobic, hydrophilic property, i.e., the MM ranges observed at 254 nm were regularly distributed throughout the fractions indicated that resin column desorption was selective.

6.1.7 Fluorescence Spectroscopy of Raw Surface Water DOC Fractions.

Method 2.1.4.5 was employed to collect synchronous fluorescence spectra of the fractionated Raw Surface Waters of the four lakes. The data generated was subjected to parallel factor analysis (PARAFAC) using MATLAB and the N-Way Toolbox (Stedmon *et al.* 2008). A typical series of contour plots (Figs 6.15 – 6.18) demonstrate and highlight different fluorescing components within the HPOA fraction for the April 2011 series of fractions before PARAFAC analysis as this series represented temperatures and precipitation that were relatively stable and did not exhibit extremes (Fig 3.17).



The results after treatment of the collected spectra (n=74) of all the fractions using the PARAFAC model isolated 3 common fluorescing components (Figs 6.19 a,b) within the fractions of the 4 lakes.



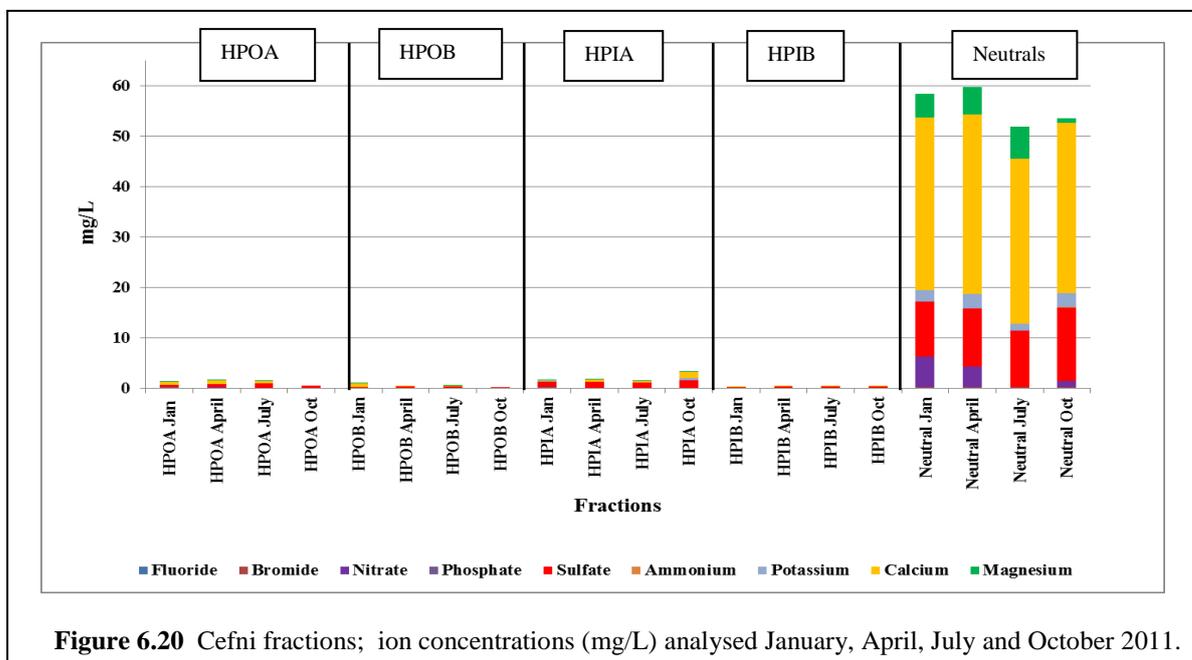
Figures 6.19 a) PARAFAC excitation loadings of lake surface water fractions b) PARAFAC emission loadings of surface water fractions.

The components were tentatively identified using references from literature sources (Marhaba *et al.*, 1999; Goslan *et al.*, 2003; Her *et al.*, 2003; Stedmon *et al.*, 2003; Ishii *et al.*, 2012) and fluorescing components in the Raw Surface Water were also observed in the fractions, but with one missing which may have not been resolved because of its low concentration, or it could be included with component 2, which may relate to component 4 in the Raw Surface Water.

Component	Peak (Ex/Em)	Identification
1	280-340/310- 400(350)	Humic like/protein
2	250-390/380-500(420)	Large MM, hydrophobic
3	350-400/370-420(390)	Fulvic like

6.1.8 Cation and Anion Analysis of Raw Surface Water Fractions.

Analysis of the lake fractions was carried out for selected cationic and anionic species every three months from January - October 2011 by Method 2.3.13.



The results (Figs 6.20) of the Cefni fractions demonstrated that the highest concentrations of ions were predominantly in the Neutral fractions, with Ca^{2+} (Table 6.3) as the major ion concentration (average 34.1 ± 2 mg/L). Evidence of high concentrations of SO_4^{2-} (average 12.1 ± 1.0 mg/L) and Mg^{2+} were also recorded (4.4 ± 0.3 mg/L), with a drop in October 2011 in the Mg^{2+} and an increase in SO_4^{2-} .

Fraction (2011)	F^- (mg/L)	Br^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	NH_4^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)
Neutral January	0.06	0.04	6.11	ND	10.95	ND	2.32	34.22	4.74
Neutral April	0.06	0.06	4.15	ND	11.58	ND	2.81	35.60	5.54
Neutral July	0.06	0.06	0.80	ND	11.23	ND	1.47	32.65	6.50
Neutral October	0.07	0.05	1.28	0.02	14.58	0.07	2.77	33.91	0.83

ND: Not Detected

NO_3^- was present in both the January and April 2011 Cefni Neutral fractions, but was relatively low (0.80 ± 0.05 mg/L) in the July 2011 Neutral fraction, which may have been as a response to plant uptake during the growing season. Concentrations of PO_4^{3-} were not detected in the three Neutrals of January, April or July 2011, but 0.02 mg/L was recorded in the October Neutral. The halides F^- and Br^- were present in all the Neutral fractions and averaged 60 and 50 $\mu\text{g/L}$ respectively.

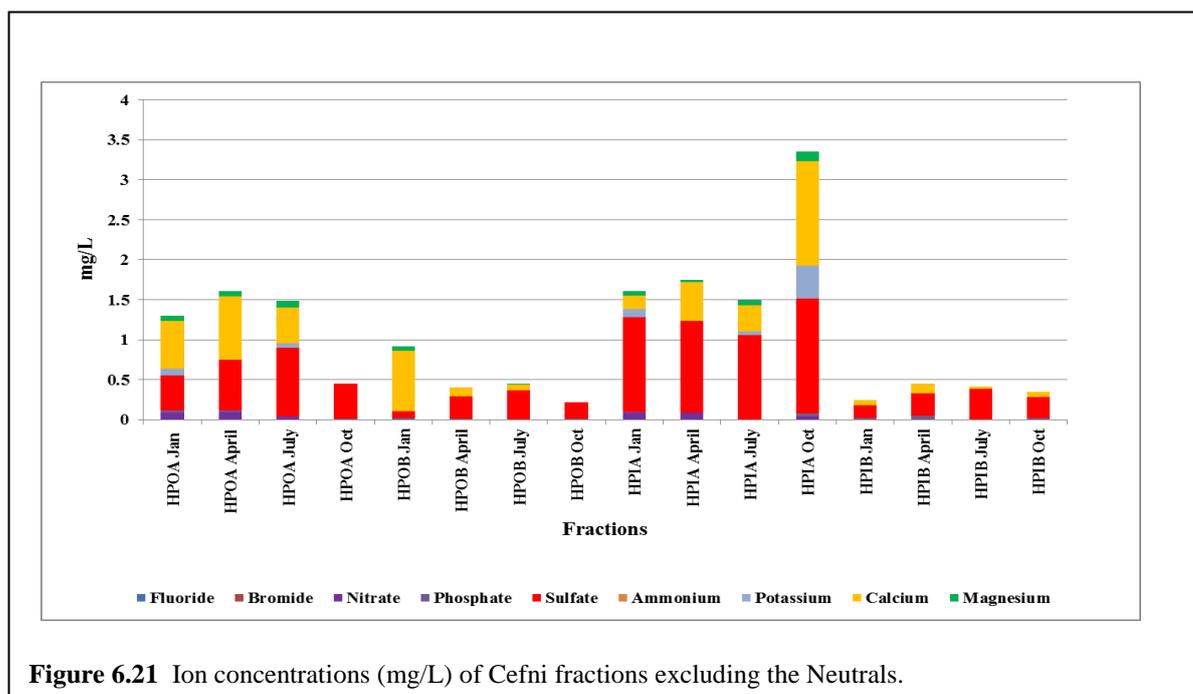


Figure 6.21 is the same data as Fig 6.20, but with the Neutral fractions excluded. The majority of these fractions were dominated by the presence of both SO_4^{2-} and Ca^{2+} ions but at much reduced concentrations averaging 0.6 and 0.4 mg/L respectively. Concentrations of the other ions within the fractions were relatively low with K^+ being present at levels ranging from a low of 0.056 to 0.40 mg/L in selective fractions such as the HPOA and HPIA during the months of January, July and October 2011. The halogen ions F^- and Br^- were below the level of detection (LOD 0.005 mg/L) and limit of quantitation LOQ (0.02 mg/L) in these fractions.

The Cwellyn fractions (Fig 6.22) demonstrated a similar trend to the Cefni, with the highest ion concentrations being exhibited in the Neutral fractions. Concentrations of Ca^{2+} and SO_4^{2-} were dominant, averaging 1.7 ± 0.1 and 1.9 ± 0.1 mg/L respectively.

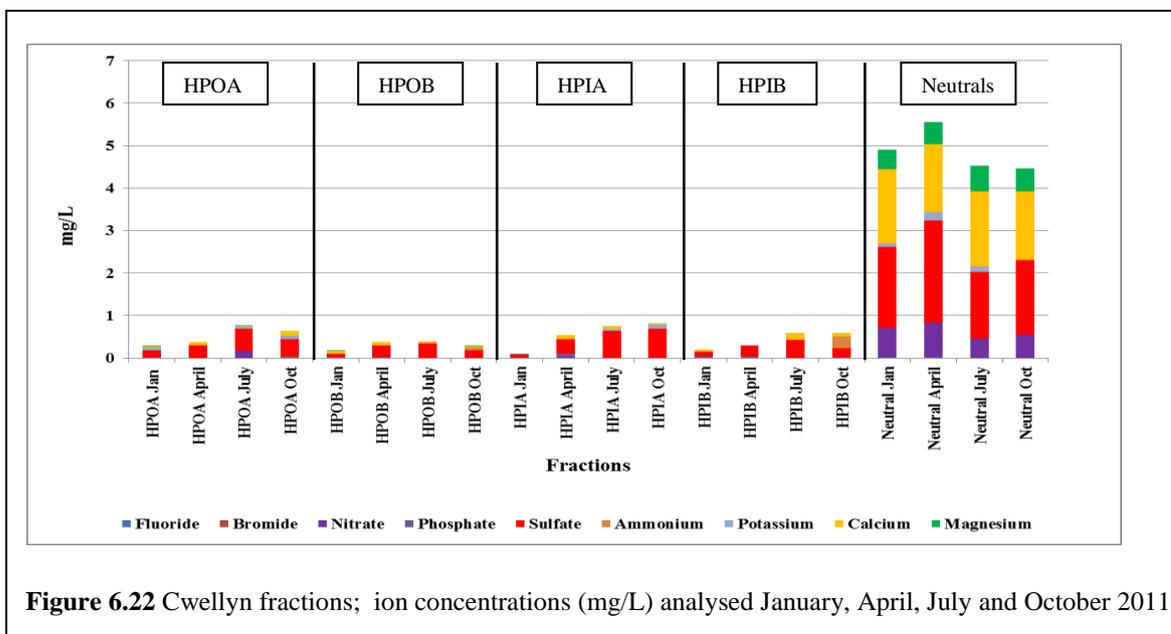


Table 6.4 Cwellyn Neutral fraction ion concentrations

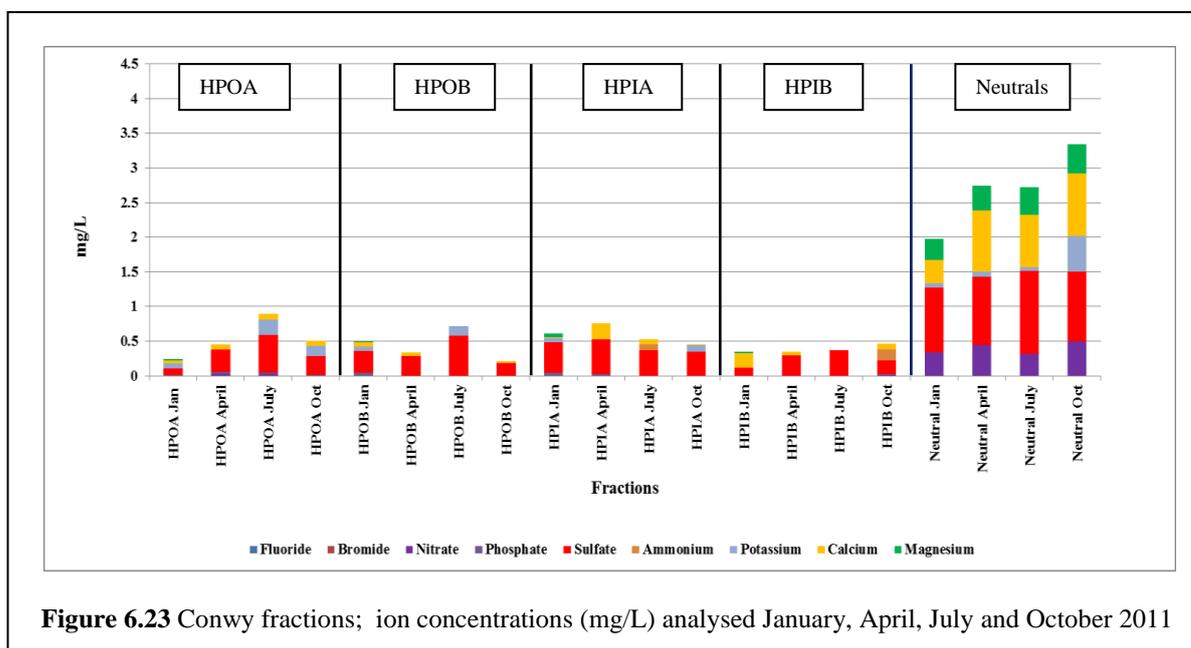
Fraction (2011)	F ⁻ (mg/L)	Br ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NH ₄ ⁺ (mg/L)	K ₊ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)
Neutral January	0.01	ND	0.69	ND	1.90	ND	0.10	1.74	0.45
Neutral April	0.01	ND	0.82	ND	2.40	ND	0.20	1.61	0.51
Neutral July	0.01	ND	0.45	ND	1.56	ND	0.13	1.77	0.61
Neutral October	0.01	ND	0.53	ND	1.76	ND	ND	1.63	0.54

ND: Not Detected

No Br⁻, NH₄⁺ or PO₄³⁻ were detected in the Neutral fraction for this site (Table 6.4). The analysis highlighted the presence of NO₃⁻ at an average level of 0.62 mg/L and also Mg²⁺ at a similar average of 0.53 ± 0.03 mg/L.

The four other fractions of the Cwellyn series of ion concentrations when showed concentrations that were < 1 mg/L. The dominant ions that were present in all fractions of each monthly set were, Ca²⁺ (0.06 mg/L) and SO₄²⁻ (0.31 mg/L). The halogens F⁻ and Br⁻ were not detected with one exception; Br⁻ (0.05 mg/L) in the HPOA fraction in October 2011. The concentrations of PO₄³⁻ averaged 0.02 mg/L for the fractioned sample series, but in July 2011 HPOA, HPOB, HPIA and HPIB fractions no PO₄³⁻ was detected suggesting possible uptake by the growing vegetation. Sporadic detection of concentration levels around the limits of detection were recorded for NH₄⁺, NO₃⁻ and Mg²⁺.

The analysis of ion concentrations in the Conwy fractioned samples again demonstrated a similar profile to those of both Cefni and Cwellyn, with the highest concentrations being found in the Neutral fractions (Figure 6.23).



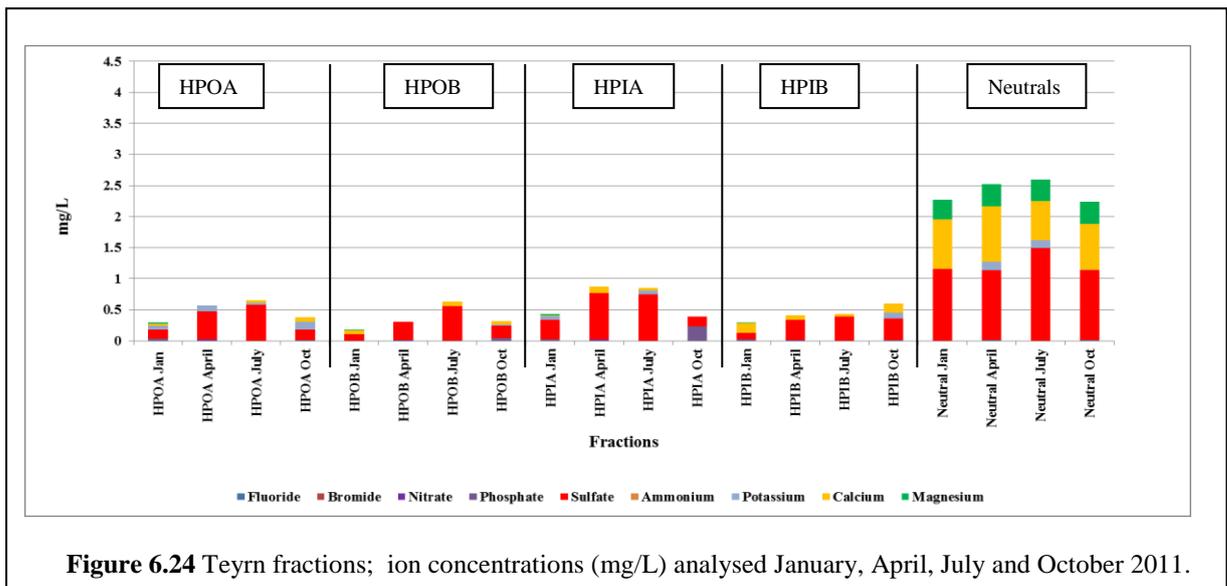
Tabulating the results of the Neutral fractions (Table 6.5) shows that, although at lower concentrations than Cefni and Cwellyn, the dominant ion species were SO_4^{2-} (average 1.03 mg/L) and Ca^{2+} at 0.71 mg/L. Concentration levels of both Mg^{2+} and NO_3^- were relatively similar at respective averages of 0.37 and 0.40 mg/L.

Fraction (2011)	F^- (mg/L)	Br^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	NH_4^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)
Neutral January	0.002	ND	0.34	ND	0.93	ND	0.07	0.33	0.31
Neutral April	0.005	ND	0.44	ND	1.00	ND	0.07	0.88	0.36
Neutral July	ND	ND	0.32	ND	1.20	ND	0.06	0.75	0.40
Neutral October	0.001	ND	0.50	ND	1.01	ND	0.51	0.90	0.42

ND: Not Detected

The ions Br^- , NH_4^+ and PO_4^{3-} were not detected, but the halide F^- concentration was recorded at levels close to the limit of detection for three out of the four months. The

remaining fractions HPOA, HPOB, HPIA and HPIB demonstrated ion concentrations of < 1.0 mg/L with a similar distribution of concentrations to both Cefni and Cwellyn. Thus SO_4^{2-} and Ca^{2+} ions predominate at average concentrations of 0.33 and 0.08 mg/L respectively, which were relatively similar to Cwellyn's. Although PO_4^{3-} was not detected in the Neutral fractions, it was present at very low concentrations in the majority of the remaining ones, averaging 0.017 mg/L but was absent in the April and July 2011, HPOB, HPIA and HPIB fractions. The halides F^- and Br^- were only detected in the Neutral fractions and at very low concentrations.



Teyrn (Fig 6.24) also presented a similar ion concentration profile to those of the fractioned samples of Cefni, Cwellyn and Conwy. The SO_4^{2-} (average 1.2 mg/L) and Ca^{2+} (average 0.8 mg/L). were at relative similar levels to the Conwy Neutrals together with Mg^{2+} averaging 0.3 mg/L (Table 6.6).

Fraction (2011)	F^- (mg/L)	Br^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	NH_4^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)
Neutral January	0.003	ND	ND	ND	1.15	ND	ND	0.80	0.32
Neutral April	0.008	ND	ND	ND	1.13	ND	0.14	0.89	0.36
Neutral July	0.004	ND	ND	ND	1.50	ND	0.12	0.64	0.34
Neutral October	0.007	ND	ND	0.007	1.12	0.007	ND	0.74	0.35

ND: Not Detected

Very low concentrations of F^- (average 0.005 mg/L) were recorded, but neither Br^- or NO_3^- were detected. Both NH_4^+ , PO_4^{3-} were only detected in the October 2011 Neutrals at low concentrations (0.007 mg/L). The remaining fractions exhibited the presence of both SO_4^{2-} and Ca^{2+} at 0.35 and 0.07 mg/L respectively. There was no evidence of the presence of the halogens F^- and Br^- or the NH_4^+ ion. The April 2011 fractions HPOA, HPOB, HPIA and HPIB exhibited a NO_3^- concentration that averaged 0.02 mg/L. Nitrate was not detected in the January, July or October 2011 fractions

6.1.9 Infrared Spectroscopy of Freeze Dried Raw Surface Water Fractioned Samples.

Cefni and Conwy (October 2011) Raw Surface Water was freeze dried for the IR spectra experiment carried out in (Chapter 3, Section 3.1.8). To compare the DOC of the Raw Surface Water to one that had been fractioned, a further 1L of each of the same Raw Surface Water (October 2011) samples were fractioned as per Method (2.3.6). The recovered fractions were adjusted with HCl (3.0 M) to pH 2.8 and each aliquot was freeze dried with an adapted Modulyo freezer unit. The resulting dried samples were prepared as KBr discs (100:1) with the infra-red spectra collected using a FT-IR Perkin Elmer Spectrum 100 instrument.

Figures 6.25 a, b show the spectra of the HPOA fraction for Cefni and Conwy. This fraction is considered to be relatively highly hydrophobic and have the highest aromatic content of the fractions and the principal THM precursor (Aiken *et al.* 1992 ; Mash *et al.* 2004) Inspection of the spectra identified OH stretch differences. Conwy's was sharper than that of the Cefni's, suggesting differences in H bonding, i.e., Cefni had a broad stretch usually associated with intermolecular hydrogen bonding and Conwy's sharper OH stretch which suggested less intermolecular and more intramolecular hydrogen bonding as previously seen in the Raw Surface Water freeze dried spectra (Figs 3.23 B).

There was evidence of a broad band extending from 3000 – 2000 cm^{-1} in both spectra (Fig 6.25 a,b) that suggested an associated N-H stretch of an amino acid (Silverstein *et al.*, 1991). In Fig 6.25 b Conwy, two bands were recorded at 3478 and 3414 cm^{-1} which could be ascribed to an $[-NH_2$ or $=NH]$ (Hesse *et al.* 1997; Ratpukdi *et al.* 2009).

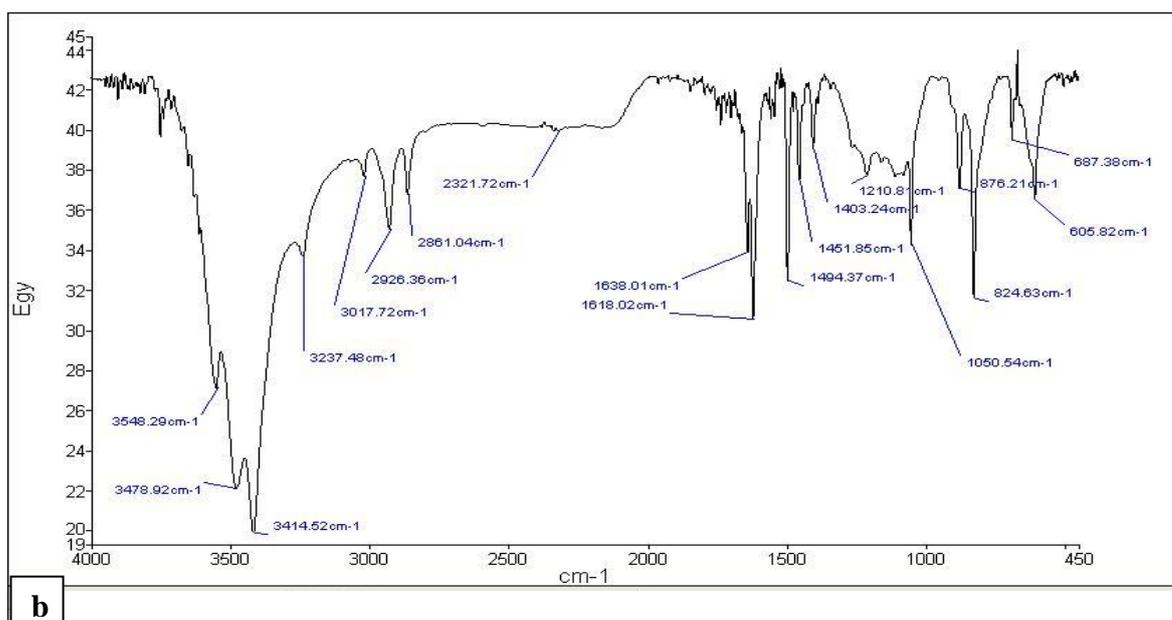
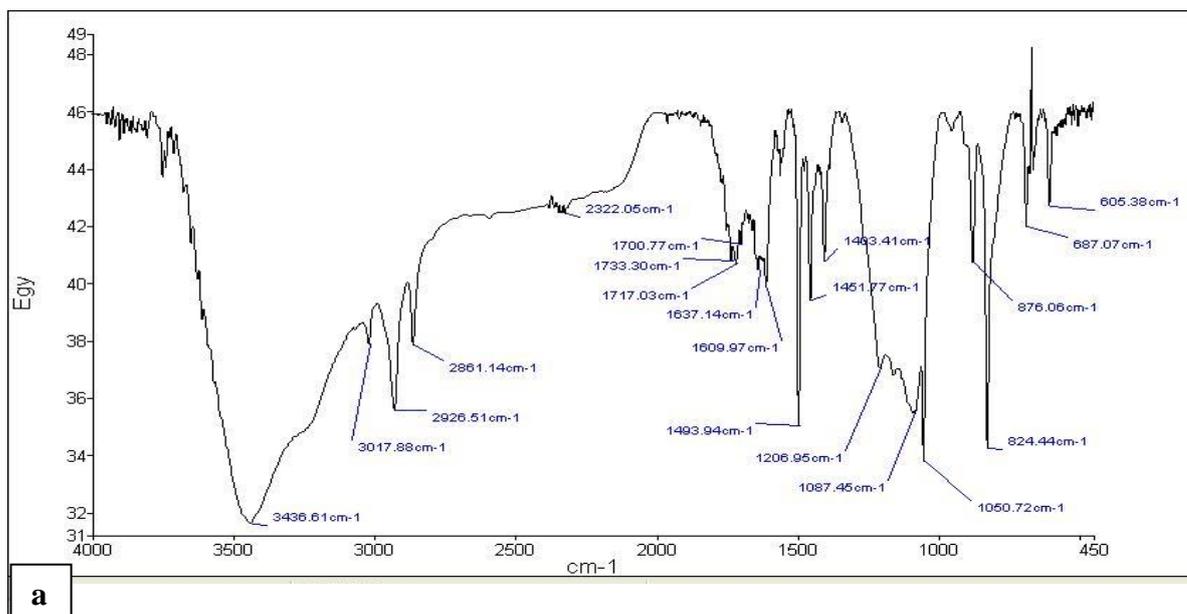
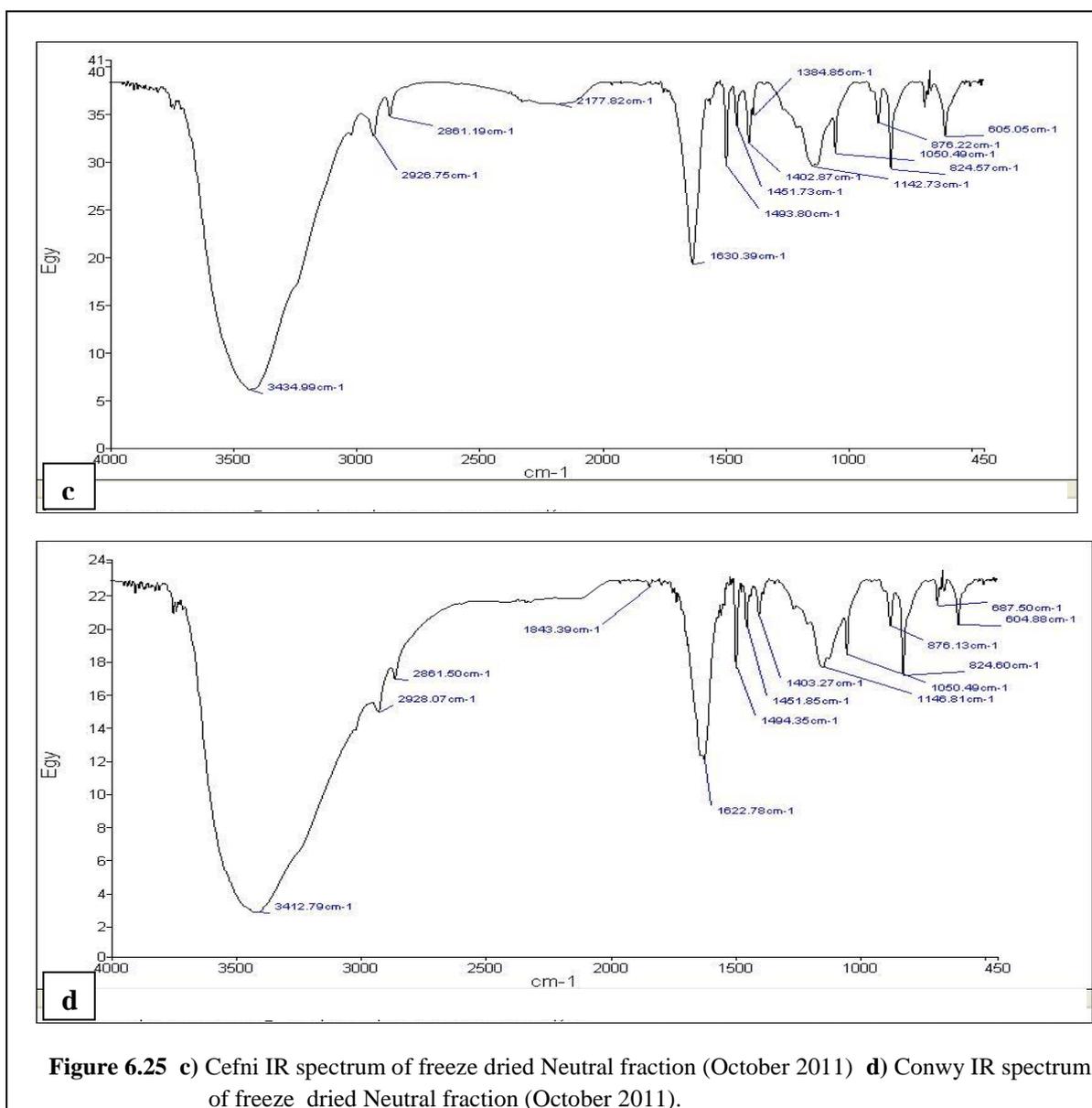


Figure 6.25 a Cefni IR spectrum of HPOA freeze dried fraction (October 2011) **b** Conwy IR spectrum of freeze dried HPOA fraction (October 2011)

The absence of these two in the Cefni HPOA (Fig 6.25 a) spectra may have been as a result of being hidden by the large OH stretch. A feature of both spectra (6.25 a,b) is the band in both at $\approx 3650 \text{ cm}^{-1}$ which may be assigned to a free OH. A feature of the Cefni spectrum (Fig 6.25 a), was the group of bands in the $1730 - 1600 \text{ cm}^{-1}$ region which reflected that of the Nordic fulvic acid (Fig 3.2). These were considered to be

representative of carbonyl functional groups that included aldehydes, ketones or esters with intramolecular hydrogen bonds (Hesse *et al.* 1997; Leenheer *et al.*, 1987; Peuravuori *et al.* 2005). The Conwy HPOA demonstrated a sharp doublet at 1630 and 1618 cm^{-1} which may have been attributed to OCOR (β -keto esters in the enol form with hydrogen bonding), *ortho* enolic OH, C=C aromatic stretching or COO^- asymmetric stretching (Hesse *et al.*1997).



Figs 6.25 c,d show the Neutral fractions of Cefni and Conwy. The OH band region in both demonstrated broad stretches relative to strong intermolecular hydrogen bonding associated, for example with polyphenolic structures. The bands at 3478 and 3414 cm^{-1} were not observed, possibly due to the large OH signal. The (C-H) aliphatic stretches were present

at 2926 - 2860 cm^{-1} but were less prominent due to the broad OH band. A strong absorption was recorded by both in the 1620 – 1630 cm^{-1} region which may be attributed to aromatic structures possibly conjugated to C=O (Chen *et al.*, 2002) or (β -keto esters in the enol form with hydrogen bonding or β -Diketones in the enolic form stabilized by resonance and the absorbance displaced by the intramolecular hydrogen bonding Silverstein *et al.*, 1991), *ortho* enolic OH (Cross and Jones, 1969), C=C aromatic stretching or COO^- asymmetric stretching (Hesse *et al.* 1997).

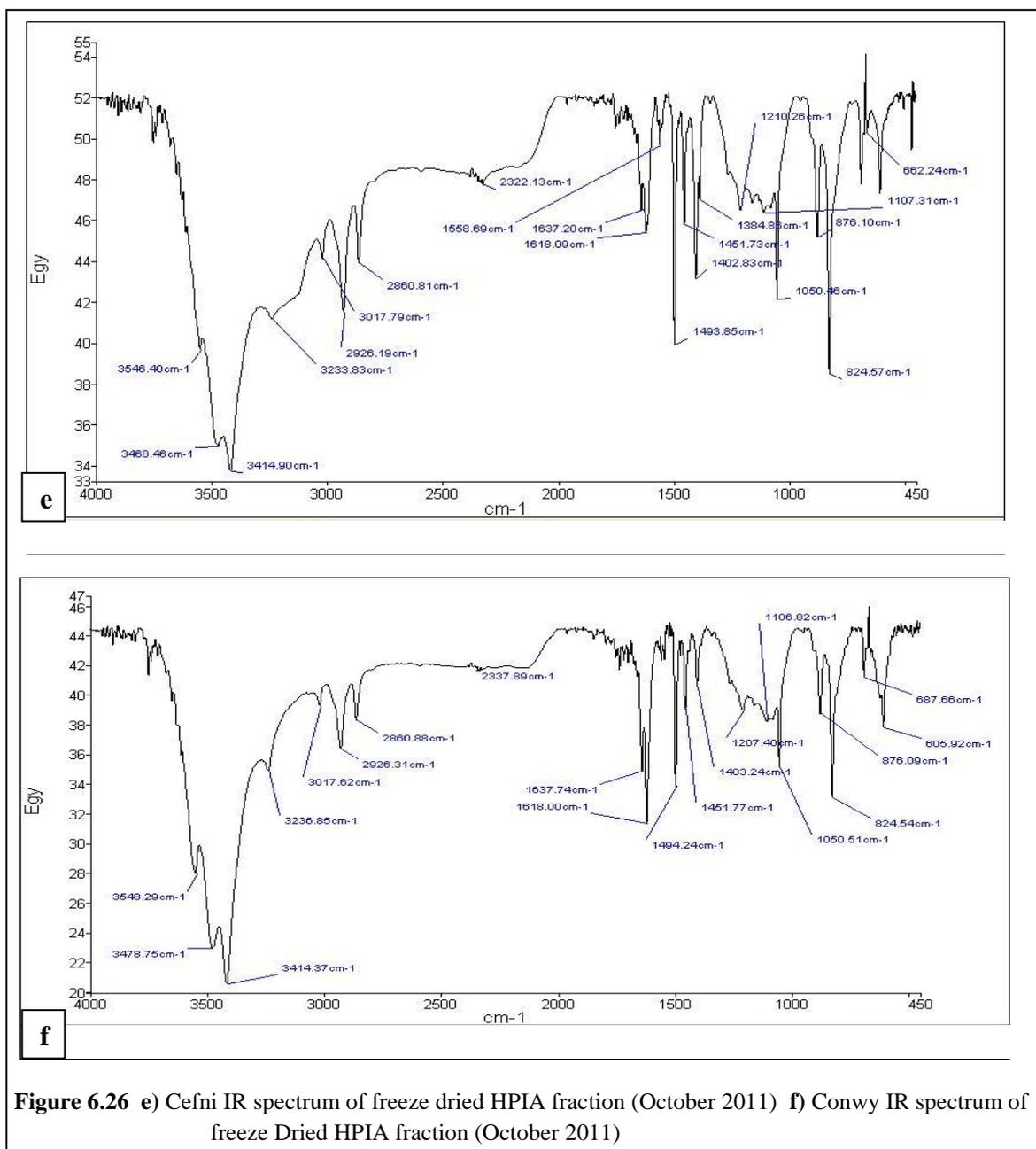
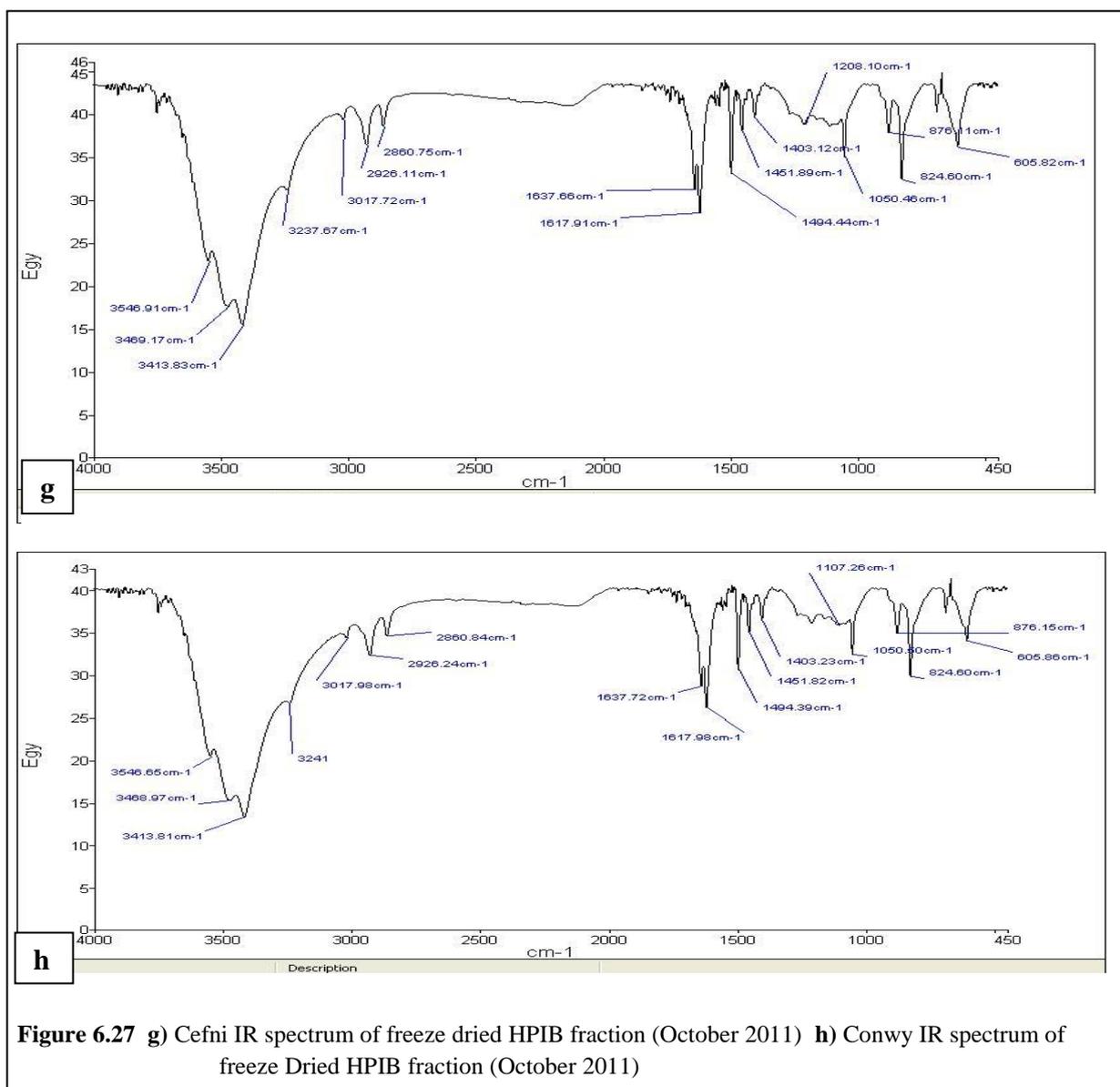


Figure 6.26 e) Cefni IR spectrum of freeze dried HPIA fraction (October 2011) f) Conwy IR spectrum of freeze Dried HPIA fraction (October 2011)

Both spectra of the HPIA fractions (Figs 6.26 e,f) demonstrated similar absorbance bands, with the OH band stretch showing less H bonding than the neutrals. The broad band from 3000 – 2000 cm^{-1} observed in the HPOA spectra (Figs 6.25 a,b) was also present in the HPIA spectra, it may be tentatively ascribed to the inference of the N-H stretch of amino acids. The suggested primary amine stretching bands at 3478 and 3414 cm^{-1} were also present in both spectra. The HPIA spectra of Cefni and Conwy when compared relative to the Nordic humic acid (Fig 3.26 b) demonstrated similar band features with the aliphatic stretches (3017 – 2860 cm^{-1}) present and the doublet at 1637-1610 cm^{-1} suggesting aromatic/conjugated C=O systems, *ortho* enolic OH C-CO-O (β -keto esters in the enol form with hydrogen bonding), C=C aromatic stretching or COO^- asymmetric stretching (Hesse *et al.*1997).
 or an N-H bend (1610 cm^{-1}).



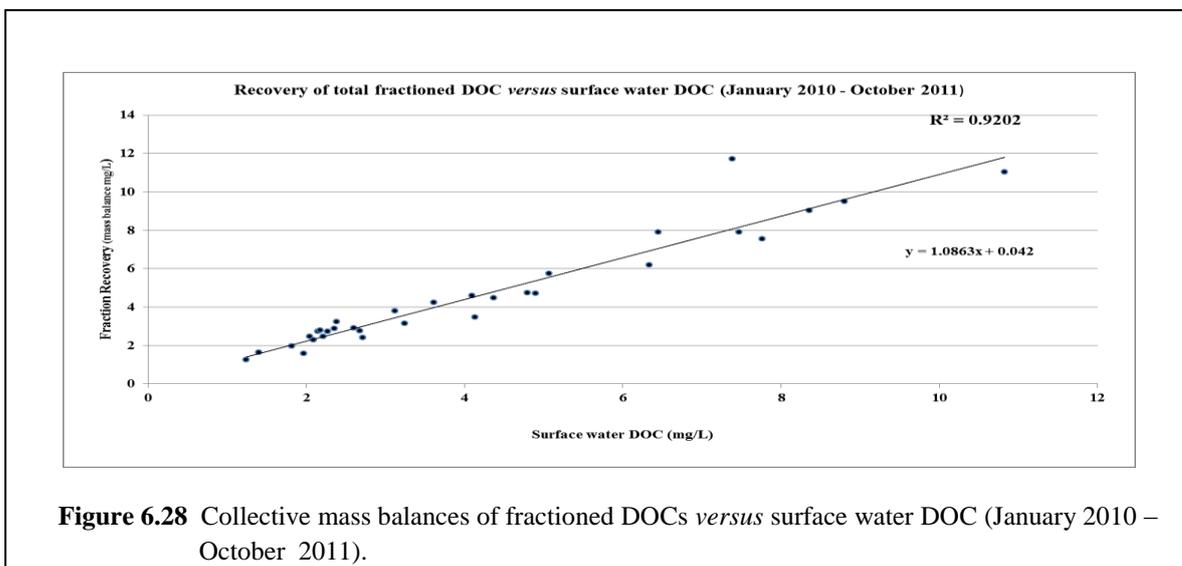
The spectra (Fig 6.27 g,h) are of the HPIB which were desorbed off the XAD 4 column with HCl (0.1M). Both spectra appeared to be mirror images of each other, this was also observed in the two Neutral spectra (Figs 6.25 c,d) and it is interesting to note that these fractions were adsorbed and desorbed at the same pH.

The region below 1500 cm^{-1} in all the spectra demonstrated similar bands with strong sharp absorbance at 1494 cm^{-1} which suggests C=C ring stretches or C-H asymmetric bending. The bands at 1050 cm^{-1} as suggested by Chen *et al.*, (2002) may be the C-O stretching and O-H deformation of the COOH groups. The $870 - 824\text{ cm}^{-1}$ band recorded in all the spectra suggested substitution patterns of the benzene ring, e.g., C-H out of plane bending of a 1,2,4 – substituted aromatic ring (Streitwieser and Heathcock (1981). Shirshova *et al.*, (2006) also suggested that these bands can also relate to C-O stretching of polysaccharides.

6.2 Discussion.

6.2.1 Raw Surface Water Fractioned DOC.

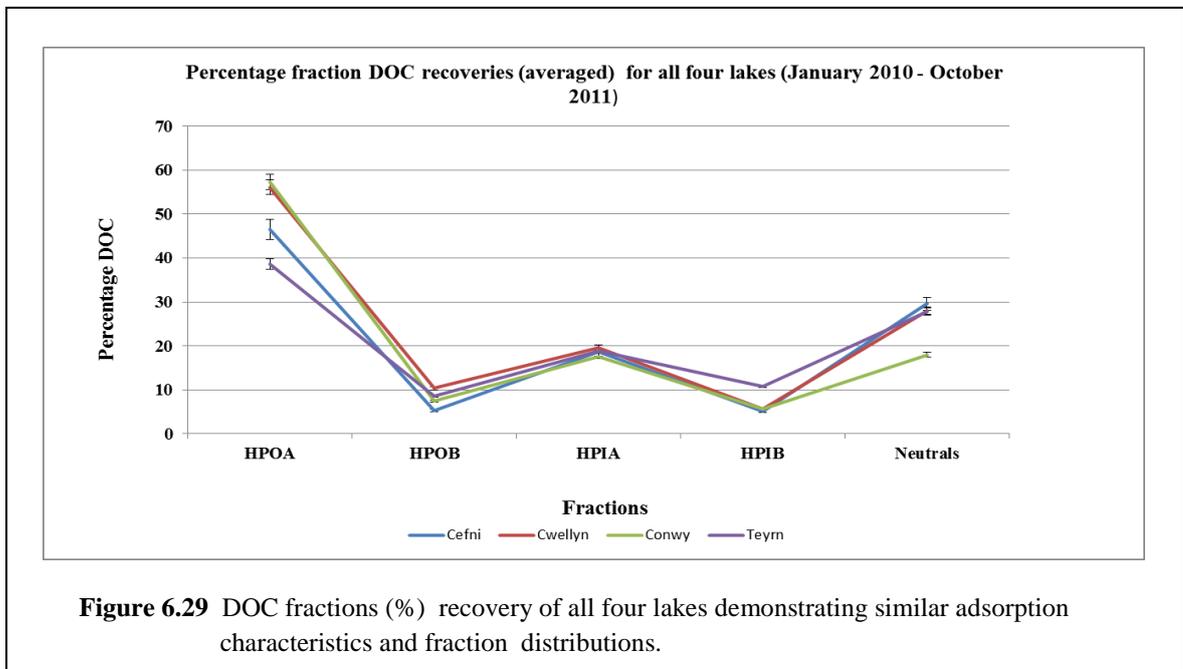
The lake Raw Surface Waters DOC when fractionated as the HPOA, HPIA, HPOB, HPIB and Neutrals, presented a mass balance recovery, (i.e., the summed individual masses of DOC in the fractions of Raw Surface Water compared to the initial Raw Surface Water sample DOC mass) ranging between 84.3% and 127.9 %. A plot of the surface raw waters DOC (mg/L) *versus* the collective mass of fractioned DOC (mg/L) generated an $R^2 = 0.9697$ (Fig 6.28) which demonstrated that the fraction mass balances and recoveries were relatively stable over the range of concentrations presented.



The recovery percentage (Fig 6.29) of each fraction relative to the individual lakes over the period compared well with that found in the literature (Malcolm *et al.* 1992; Aiken *et al.* 1992 ; Qunshan *et al.* 2008). Although the DOC fraction concentrations were seen to vary and change over the sampling period, significant correlations (Table 6.7) were found to exist between the Raw Surface Water DOC samples and individual fraction DOC concentration. This indicated that although the Raw Surface Water DOC concentrations varied within the data set the distribution pattern of each fraction concentration, although variable demonstrated a repeatable pattern within each set.

Table 6.7 Fractions vs Raw Surface Water DOC showing the significant relationship between the original DOC concentration and the individual fraction DOC concentrations.

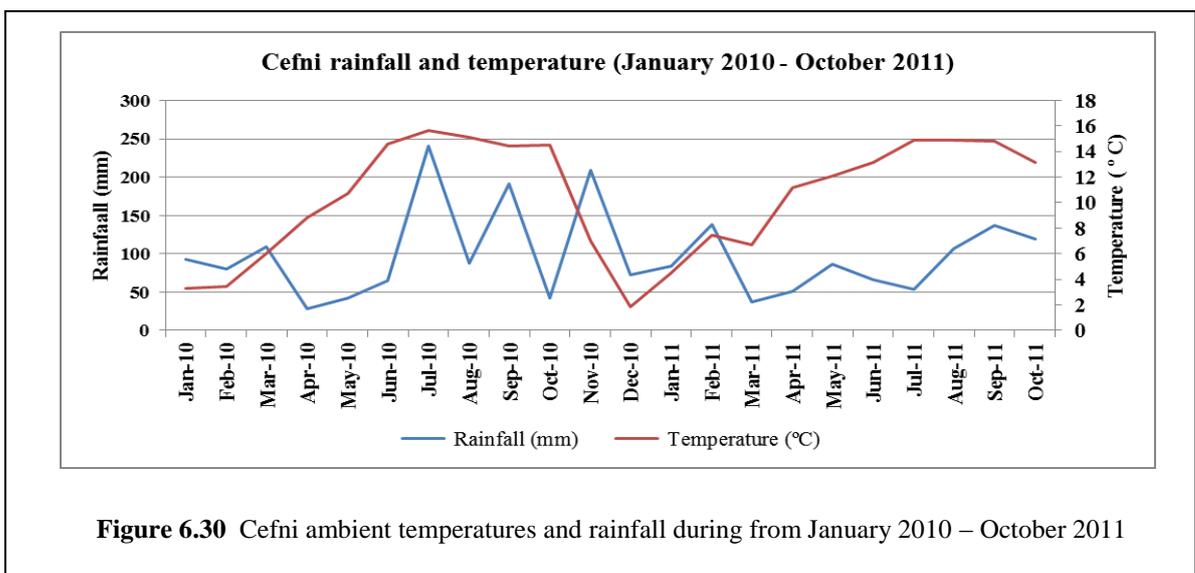
Fraction	<i>r</i>	<i>p</i>
HPOA	0.911	< 0.01
HPIA	0.851	< 0.01
HPOB	0.475	< 0.01
HPIB	0.399	< 0.05
Neutral	0.792	< 0.01



The concentrations for example of HPOA and HPIA found in the DOC of each lake may be attributed to a number of influences. Mash *et al.* (2004) suggested that the HPOA, HPIA

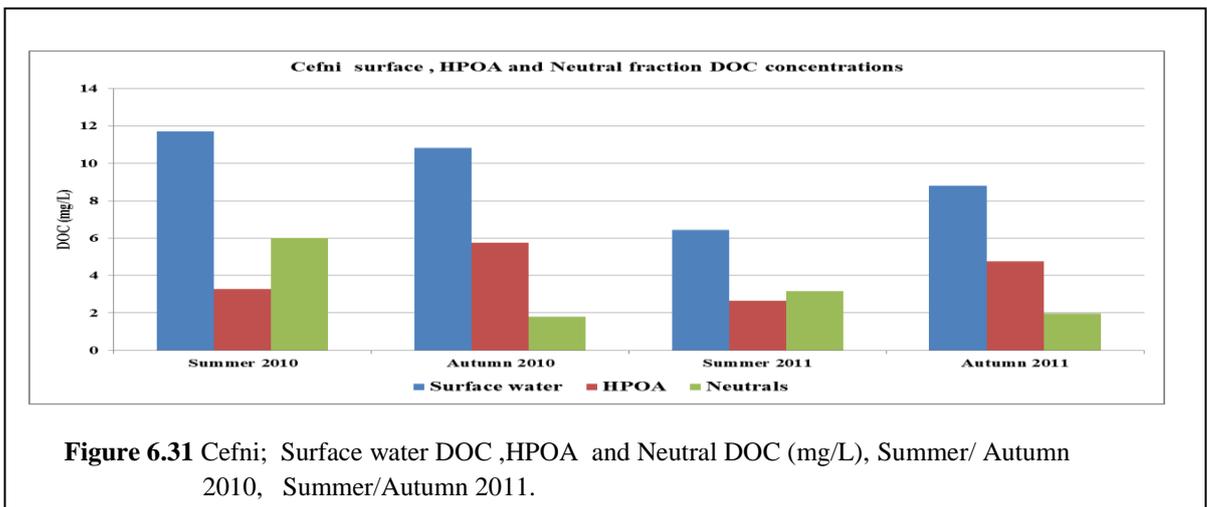
concentrations present in the receiving lake environments are subject to their original production source, i.e., allochthonous (DOC sourced from outside catchment/lake) or autochthonous (DOC source within catchment/lake). Barber *et al.* (2001) demonstrated this in two experimental treatment wetlands in the United States. He identified high concentrations of autochthonous DOC being generated in the experimental wetland with semi-stagnant conditions and high algal growth. Fractioning and analysing the surface water samples exiting from the source resulted in low HPOA DOC (i.e., Hydrophobic/aromatics) and high Neutral DOC (less aromatic/algal source). This was reversed when the water was passed through the plant dominated treatment wetland. Analysis of the fractionated samples from this surface water demonstrated high HPOA DOC (increased hydrophobic/aromatic structures) and a low Neutral DOC (decrease in algal DOC) concentration.

Although the system described by Barber *et al.* (2001) and co-workers was subject to artificial controls, it may be suggested that similar inferences were exhibited by the Cefni lake. This can be demonstrated if the two lakes Cefni and Cwellyn are considered. For example, the warmer summer months (Figure 6.30) of 2010/11 were preceded by unique dry springs. This resulted in the Cefni having a lower hydraulic loading, longer water residence time, high evapo-transpiration with algal blooms developing in the early summers of 2010 and 2011. These conditions may have promoted autochthonous DOC in the lake. Following early summer, increased rainfall and decreased temperatures towards autumn would promote an increase in the water flows and remove the algal blooms within the lake. These conditions would have established predominantly allochthonous DOC within the lake.

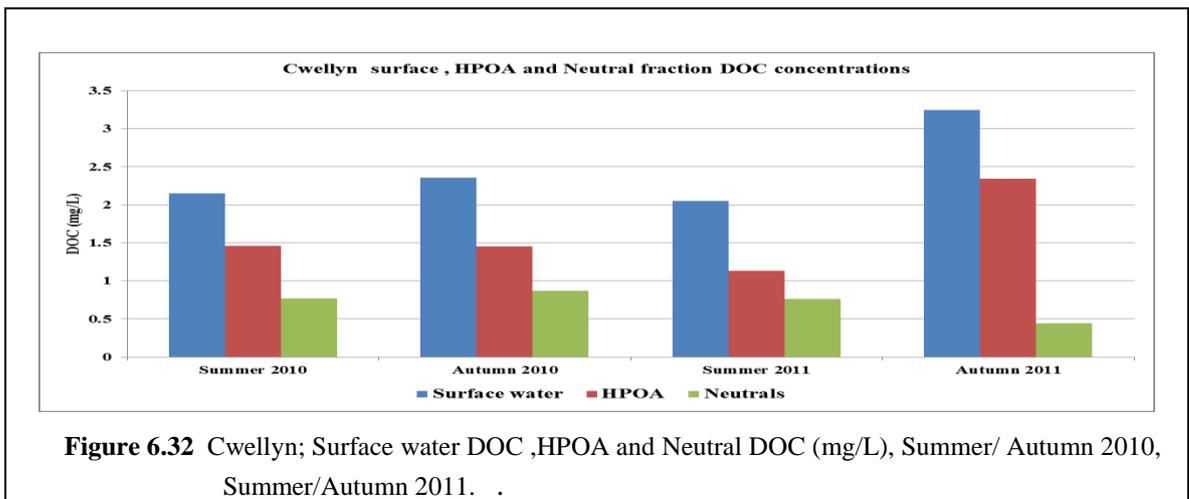


The results of the surface water DOC and HPOA and neutral fractions collected during the four seasons are shown in Figure 6.31. From these it could be seen that the HPOA and

Neutral fractions concentration levels exchanged during the autumn/summer periods and *vice versa*. The results show lower HPOA and a higher Neutral DOC concentrations during the two July's (2010/11) and a reversal of this during both the cooler October 2010/11 months. These results tend to support the findings of Barber *et al.* (2001) and Mash *et al.* (2004) that autochthonous and allochthonous DOCs are different, i.e., allochthonous DOC inputs are generally have a higher aromatic content and autochthonous DOC is less aromatic.



Considering the upland lake Cwellyn, this is a deep glacial lake (Chapter 2; 2.01 b) with continual water movement, shorter residence times and no evidence of algal blooms formed during 2010 and 2011. Hence the DOC would be classified allochthonous. The results show (Fig 6.32) that the HPOA and Neutral DOC fractions unlike the Cefni, did not demonstrate any exchanges during the seasonal temperature and rainfall variations.



The highest concentrations of the HPIA fraction were present in the Cefni (1.5 mg/L) samples with the other lake samples averaging below 1.0 mg/L. The overall HPIA results demonstrated a significant correlation ($r = 0.851$, $p < 0.01$) with the DOC concentrations. Aiken *et al.* (1992) stated that the HPIA fraction contained poly-functional organic acids and aliphatic acids with five or fewer carbons. Relative to the Raw Surface Water, DOC concentrations in the HPIA fraction accounted for $< 20\%$ (Fig 6.29) with all four of the lakes demonstrating a standard deviation of 0.8% between their collective means. The HPOB and HPIB fraction of the DOC exhibited concentrations with no fixed discernible pattern between lakes or the sampling periods and accounted for $\leq 10\%$ of the surface water DOC (Fig 6.29).

The fraction that was not retained on any of the macroporous resins classified as the Neutral and according to Leenheer, (1981) and Buchanan *et al.* (2006) has been reported to consist of hydrophilic organic species such as simple polysaccharides and two to three carbon alcohols and ketones. The concentration of this Cefni DOC Neutral fraction was the highest of all of the four lakes with the exception of July and October 2010 when Teyrn exhibited the highest concentration [Teyrn residence time may have increased due to low flow $< 0.1 \text{ m}^3/\text{s}$ in the dry period compared to an average of $0.2 \text{ m}^3/\text{s}$ (ECN data centre, 2012)], suggesting an increase in autochthonous DOC. All four lakes demonstrated the highest concentration of Neutrals in July 2010. This coincided with the preceding dry warm periods (Fig 6.30), hence suggesting a possible increase in for example a carbohydrate exudates (Thurman, 1985) by algae. The low concentrations ($\leq 1.5 \text{ mg/L}$) of the three upland lakes were relatively consistent over the sampling period. Their lowest concentrations were during January 2010/11 when very low temperatures (Figure 6.33) were experienced in the areas, thus suggesting inhibition of microbial activity (Fenner *et al.*, 2005) especially in the upper peat/soil layers.

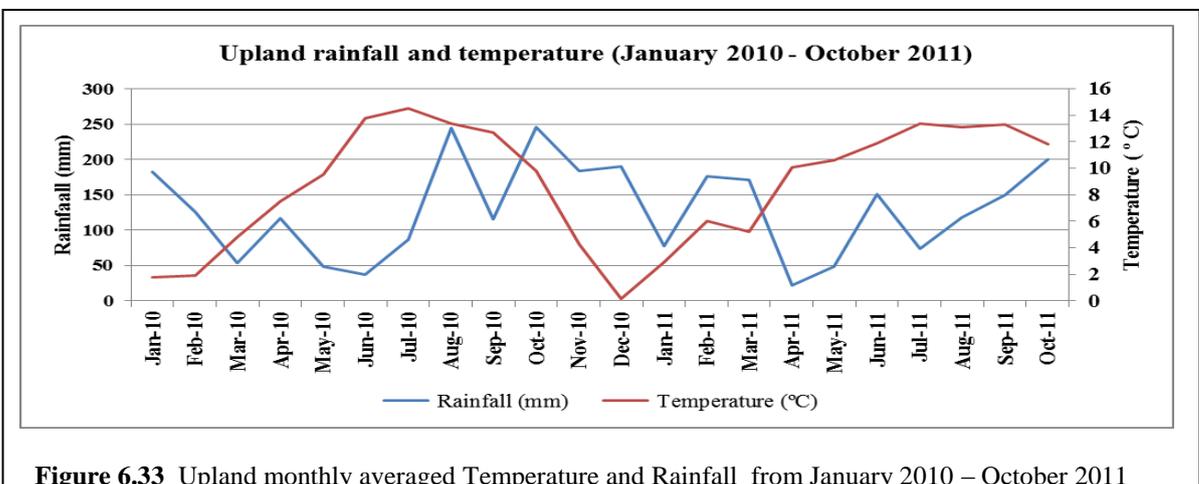
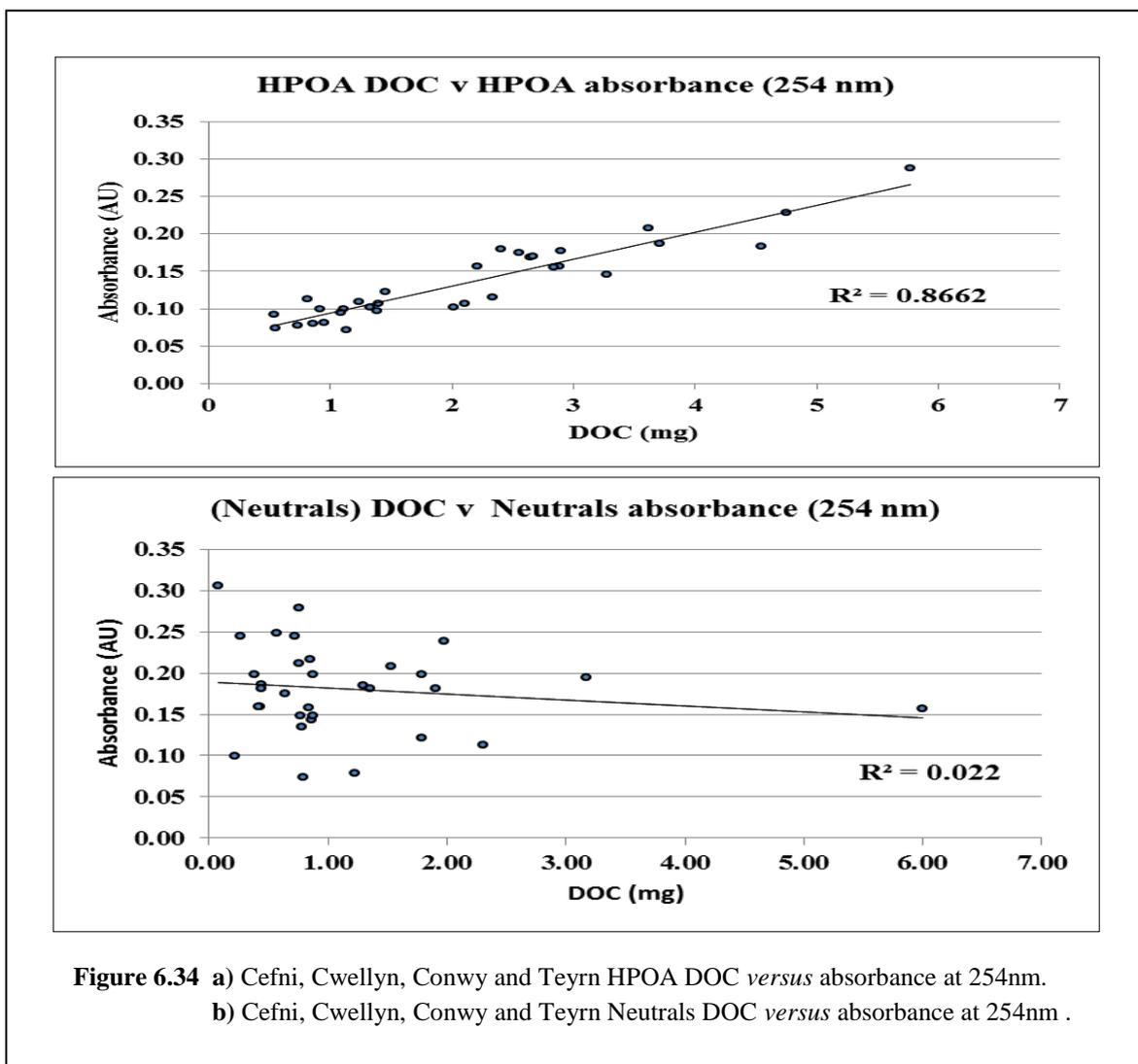


Figure 6.33 Upland monthly averaged Temperature and Rainfall from January 2010 – October 2011

6.2.2 UV-visible Spectra of Raw Surface Water DOC Fractions.

The UV-vis absorbance profiles were similar to those of the Raw Surface Water DOC and were relatively featureless because the spectra did not exhibit unique and distinguishable chromophores due to being low concentrations of DOC and many species contributing by a multiplicity of overlapping transitions.



A plot of the DOC concentrations of all the HPOA fractions versus the absorbance wavelength of 254nm demonstrated (Fig 6.34 a) a significant correlation (R^2 0.866). The linear regression plot of the Neutral fraction DOC versus its absorbance at 254 nm (6.34b) demonstrated an insignificant correlation (R^2 0.022). These results support a linear relationship between the [C=C] species absorbing at 254 nm in the HPOA fractions and DOC concentrations, but the Neutrals DOC did not exhibit this linear relationship. This suggested that the Neutral DOC fraction consisted of compounds proposed by Leenheer

and Croue (2003) that did not have a high incidence of unsaturated [C=C] bonds, i.e., proteins, sugars and aliphatic acids that did not readily absorb at 254 nm.

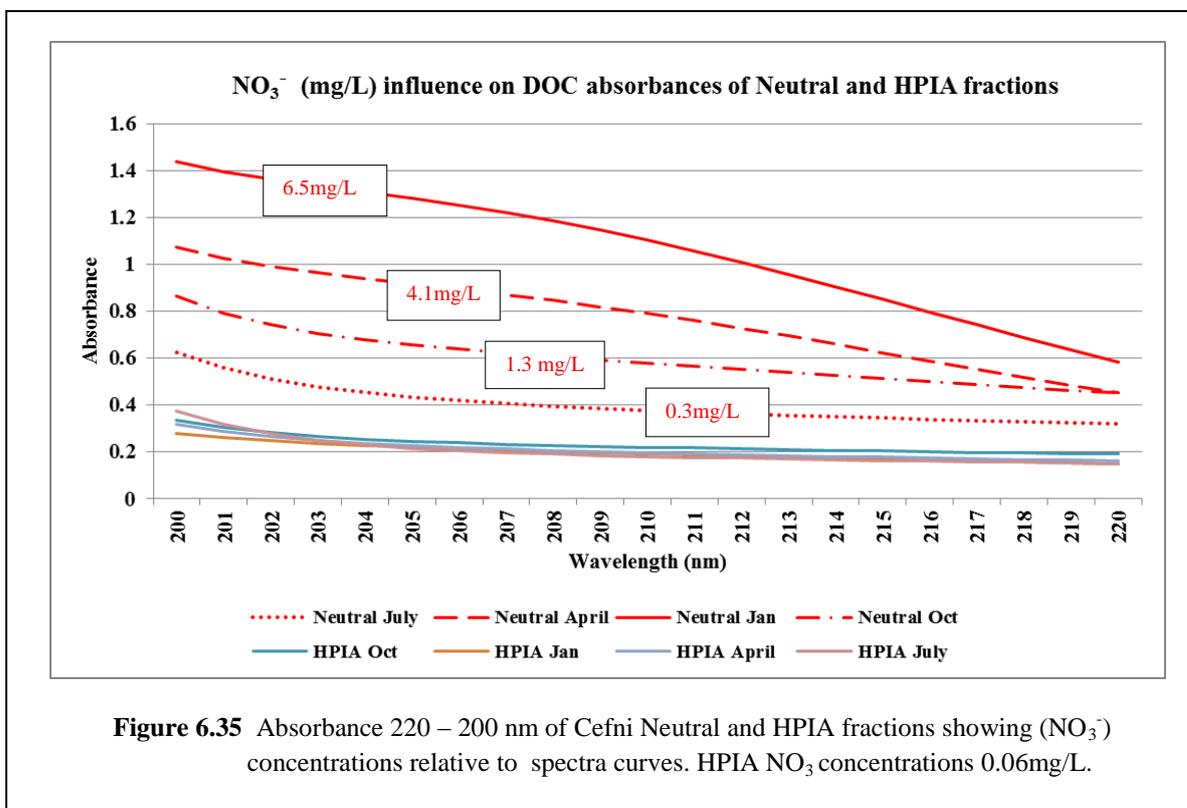
Both the HPIB and HPOB also demonstrated insignificant correlations ($R^2 = 0.218$) and ($R^2 = 0.118$) respectively between the DOC concentrations and UV absorbance at 254 nm, but the HPIA fraction presented a significant correlation ($R^2 = 0.584$) thus indicating the presence of 254 nm absorbing species which increased/decreased relative to the DOC concentration. This was in contrast to that proposed by Dilling and Kaiser (2002) who stated that the HPOA fraction contained the majority of aromatic moieties.

6.2.3 Influence of Inorganic Anions on UV-vis Spectra.

Examination of the UV spectra showed that beyond the maximum absorbance intensities at 200nm absorption decreased rapidly with the increasing wavelength. Figures 6.8 a,b show typical examples of UV spectra for the individual fractions for the four lakes for July 2010 and October 2011. The highest intensities were recorded by the HPOA and Neutrals between 384 and 200nm. In the region 300 to 250 nm, small shoulders were observed in both the HPOA and Neutrals which suggested $\pi - \pi^*$ electron transitions associated with unsaturated systems (Peuravuori and Pihlaja, 1997). Her *et al.* (2008) stated that NO_3^- and SO_4^{2-} demonstrated an absorbance at these shorter wavelengths. The two fractions that exhibited the highest concentrations of these two inorganic anions were the Neutrals and HPIA (6.1.8). Both indicated high SO_4^{2-} concentrations, but as previously noted (Section 6.1.7) the Neutrals exhibited the highest NO_3^- levels.

To explore this statement by Her and co-workers, the NO_3^- ion concentrations relative to absorbance changes in the Neutral and HPIA fractions were investigated over a series of months (Fig 6.35). The results demonstrated the contributory influences by the NO_3^- ions on the UV-vis absorbance between wavelengths 220 – 200 nm. Absorbance relative to NO_3^- concentrations increased as the wavelength reduced from 0.04 AU/mg at 220 nm to 0.13 AU at 207 nm. The very low concentration of the NO_3^- ions (average 0.06 mg/L) and high SO_4^{2-} (average 0.33mg/L) in the HPIA were accompanied by relatively low absorbance values which suggested that absorbance was influenced by the presence of NO_3^- but not by SO_4^{2-} . Spiking the HPIA (0.06mg/L) with 0.5 mg of NaNO_3 to give a solution of 0.42mg/L (NO_3^-) increased the absorbance at 207 nm from 0.2 to 0.42 AU. As the absorbance influence of the NO_3^- is within the 225 – 200 nm range (Hioki and

McLaren, 2008) it does not influence the aromaticity determinations, but may impact the functional group ratio calculation (A_{253}/A_{203}) reported by Korshin *et al.* (1997), if NO_3^- concentrations are high.



6.2.4 Aromaticity of Fractions.

The DOC aromaticity ratio (E_2/E_3) (6.1.5) proposed by Peuravuori and Pihlaja (1996) indicates that the higher the ratio quotient value the lower the aromatic content and averaged molecular mass and *vice versa*. An overall ratio (E_2/E_3) that averaged 3.98 was computed for the Neutral fractions and a lower overall average was recorded for the HPOA fractions at 2.5, which suggested that the HPOA was of a higher aromaticity and would have a bias towards higher molecular mass (MM) ranges, than the Neutral fractions. Relating the two averages (E_2/E_3 and MM ranges) to the SEC distribution the molecular mass distributions agreed with that which was proposed; for example, the Neutrals had a relatively low aromatic content (aromaticity: 3.98), and the MM ranges with the highest percentage (57.8 %) was in the lower 4000 to 2000 Da range. The HPOA with the lower aromaticity ratio (2.46), which suggested a high aromatic content than the Neutrals was also accompanied by a higher MM range, i.e., a percentage (78.7 %) in the 5000 to 3000

Da. The results confirm the observations reported in the literature, of lower aromatics and smaller molecules in Neutral fractions compared to the hydrophobic acids and the relationship proposed by Peuravuori and Pihlaja that the aromaticity ratio and MM were seen to relate.

Edzwald and Tobiason (1999) stated that SUVA values $> 4 \text{ L/mg m}^{-1}$ were an indication of a mainly hydrophobic material, considered to be high in aromatic content, whilst a SUVA $< 3 \text{ L/mg m}^{-1}$ was mainly hydrophilic. The SUVA values generated by the fractions were all $> 4 \text{ L/mg m}^{-1}$ (Section 6.1.4) indicating that they had a relatively high aromatic content. Although the only series that demonstrated what may be regarded as normal values (i.e., $< 10 \text{ L/mg m}^{-1}$) were the HPOA. To establish if the SUVA and aromaticity ratio (E_2/E_3) values correlated, a statistical exercise was carried out. Although both are cited to be guides to the aromatic content, the results (Table 6.8) did not establish any significant correlations for the fraction HPOA, or when all the fraction SUVAs and aromaticities were statistically analysed.

Sample	Fraction	<i>r</i>	<i>p</i>
Cefni	HPOA	-0.673	> 0.05
Conwy	HPOA	0.261	> 0.05
Cwellyn	HPOA	-0.459	> 0.05
Teyrn	HPOA	-0.654	> 0.05
Cefni	All fractions	-0.173	> 0.05
Conwy	All fractions	- 0.052	> 0.05
Cwellyn	All fractions	0.028	> 0.05
Teyrn	All fractions	0.206	> 0.05

6.2.5 Size Exclusion Chromatography (SEC) of Raw Surface Water DOC Fractions.

The SEC chromatograms in Section 6.1.6 (Figure 6.13) show the overlaid molecular distribution peaks of the nominated fractions. The dominant peak of the fractioned series was the HPOA representing 40 – 60% of the DOC while the HPIA and Neutrals represented 14 – 28%. This relates well to the fraction isolations reported in the literature (Thurman and Malcolm 1979; Aiken *et al.*, 1993; Korshin *et al.* 1997; Quansham *et al.* 2008b). Collating all the lakes fraction result (Table 6.9) from January 2010 to April 2011, the MM ranges as individual sets demonstrated standard deviations (SD) $< 18\%$.

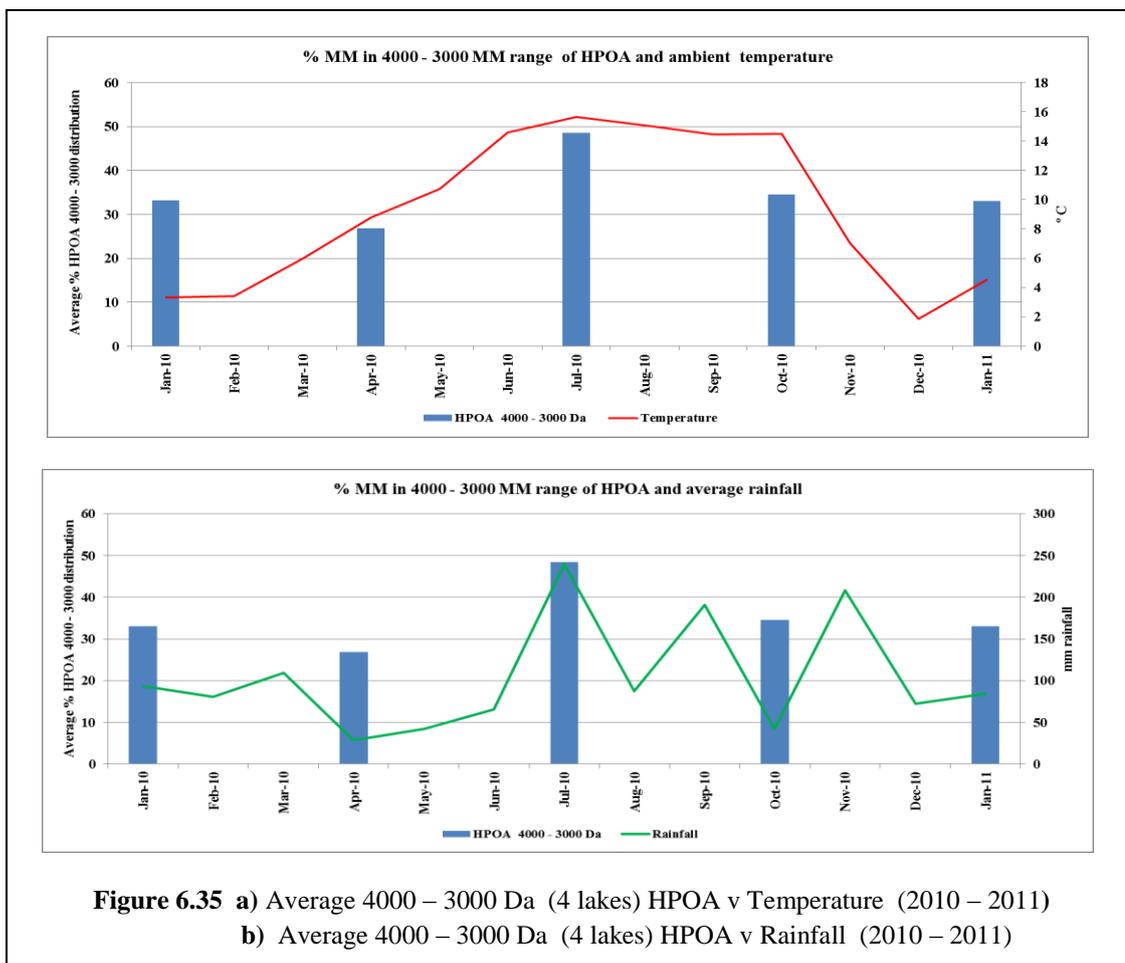
Table 6.9 Mean % distributions and standard deviations of individual fractions for combined data from all the 4 lakes						
MM distribution (%)						
	10000 - 5000	5000 - 4000	4000 - 3000	3000 - 2000	2000 - 1000	1000 - 10
Fraction						
HPOA (mean %)	15.2	42.5	36.2	3.1	2.0	1.1
SD	9.6	4.1	9.5	0.6	0.4	1.1
HPIA (mean %)	7.5	44.3	43.3	2.7	1.4	0.8
SD	7.1	8.0	10.6	2.0	1.2	1.4
HPIB (mean%)	9.0	31.8	41.7	15.0	1.5	1.0
SD	6.8	17.6	13.3	15.5	1.7	2.0
HPOB (mean%)	11.4	46.4	36.1	4.9	1.6	0.3
SD	7.4	15.9	12.3	11.1	1.3	0.5
Neutrals (mean %)	3.8	33.3	51.3	6.6	4.3	0.8
SD	4.8	17.3	15.2	2.9	2.6	2.0

The changes in the molecular mass distributions within the samples over the period appeared to be remarkably constrained with the majority of the MM within the two ranges 5000 – 4000 and 4000 – 3000 Da, but there were subtle variations, influenced possibly by a series of inter-related factors that included rainfall, temperature and microbial activity.

As the HPOA fraction represented the highest percentage inclusion in the Raw Surface Water DOC the results for the four lake HPOA DOC' with respect to temperature and rainfall were plotted (Fig 6.35 a,b). These two parameters were chosen as they have been reported to indirectly influence microbial degradation of the DOC and its subsequent export out of the catchments (Fenner *et al.* 2005b; Chow *et al.* 2003).

The range 4000-3000 Da was chosen as it demonstrated the largest percentage changes in the overall series. Hence it could be considered a surrogate for the environmental factors, temperature and rainfall and indirectly microbial activity. For example, during the month of July 2010 when low rainfall had been documented for the previous months, a change in the HPOA 4000 – 3000 Da range was recorded, with an increase from an average of 26.8% recorded in April 2010 to 48.5% in the July 2010, which was the zenith of the dry period. The increasing temperature during the preceding months and the reduced export of DOC due to the fall in rainfall may have allowed an accumulation of DOC to occur, thus subjecting it to prolonged microbial degradation and the consequential reduction in MM. Although the increase in air temperature may not have been reflected to a great extent in the mean soil temperature (Clark *et al.*, 2005) the rapid lowering of the water table, thereby

increasing available O₂ within the upper peat layers would have been expected to increase microbial aerobic activity (Freeman *et al.* 2001).



Related variations in the MM of exported DOC were observed by Scott *et al.*, (2001), who noted changes in MM during wet and dry periods, although they reported a bias towards higher MM averages after dry conditions unlike this projects results. However they did acknowledge that specific influences within contrasting catchment environments propagated the final DOC makeup.

6.2.6 Fluorescence of Raw Surface Water DOC Fractions

Examination of the fluorescence results after PARAFAC analysis generated 3 common components within the DOC, classified in the literature simply as protein like, humic like and fulvic like (Marhaba *et al.* 1999b, Goslan *et al.* 2003, Her *et al.* 2003, Stedmon *et al.* 2003b, Ishii *et al.* 2012). These data were derived from the fluorescence spectra of all the pooled fractions (n=74) as the separate unique classifications were not sufficient in number

to be modelled successfully. Although various components were identified in the exercise, the model presented a number of uncertainties as a number of weak fluorophores were evident in the majority of the samples and could not be distinguished from the noise levels very easily. The fluorophores identified did relate to those observed in the Raw Surface Water, but they could not be assigned to individual fractions due to the design of the experiment, i.e., all the fractions were pooled together for analysis.

6.2.7 Cations and Anions of Raw Surface Water DOC Fractions

The DAX 8 and XAD 4 resins do not have an affinity for inorganic ions and accordingly have been used to fraction a wide range of water, from saline to fresh without effect on the distribution coefficients (Aiken *et al.* 1992). This was demonstrated by the analytical results of the ionic content of the fractions collected. The major concentrations of both cations and anions were present in all the Neutral fractions. The major ion concentrations in the fraction were Ca^{2+} , Mg^{2+} , NO_3^- and SO_4^{2-} (Tables 6.3 – 6.6) with trace levels of K^+ , Br^- and F^- . The ion concentrations of the other fractions, i.e., the resin desorbed, demonstrated that only the HPOA and HPIA tended to retain a number of the ions at trace levels (< 1.4 mg/L). These fractions represented the highest percentage of DOC, and the retention of inorganic ions within these fractions suggests that the macro-molecules may capture the ions within the DOC aggregations, by the formation of a complex/ clathrate (Srivathsa 2004) or simply a surface charge interaction rather than an ion/resin interface reaction although the DAX 8 resin has some polarity (Sigma Aldrich., 2012).

6.2.8 Infra-red Spectra of Raw Surface Water DOC Fractions.

Spectra were only collected for freeze dried fraction samples of the Cefni and Conwy. This was due to the limitations of the Modulyo freeze dryer and the time available. The spectra of the fractions demonstrated similarities to those of the Raw Surface Water (Figs 3.23 A,B). The broad OH stretch was present in the Cefni HPOA fraction but appeared sharper than that recorded for the Raw Surface Water. A distinct change was seen in the 1600 – 1800 cm^{-1} region of the Cefni's HPOA compared to the Raw Surface Water. That is, the single broad stretch at 1630 cm^{-1} which may be assigned various structures such as C=C aromatic stretching or aromatic structures conjugated to C=O or C-CO-O=C (β -keto

esters in the enol form with hydrogen bonding) or β -Diketones in the enolic form, stabilized by resonance and intramolecular hydrogen bonding was replaced with a series of absorbance peaks that suggested a change in for example hydrogen bonding and resonance which may move the absorption to higher or lower frequencies. Unsaturation in the conjugation associated with the functional groups may also have an effect on the absorption frequencies. Hence it could be suggested that the fractioning process had an effect, but the Conwy HPOA spectrum in this region did not change, although it was subjected to the same desorption method as the Cefni, hence it could be counter argued, that the fraction isolation process did not influence the desorbed product. An interesting feature of the spectra was noted in the Neutrals, both the Conwy and Cefni demonstrated broad OH stretches which inferred that intermolecular hydrogen bonding was present. To establish that this was not a hygroscopic feature related to the use of KBr, the KBr and samples were dried a number of times and the analysis repeated with no discernible effect between the spectra.

The broad stretches seen from $3000 - 2000 \text{ cm}^{-1}$ also suggested an amino acid, i.e., NH_3^+ stretching band $3100 - 2600 \text{ cm}^{-1}$ with an associated asymmetric bending band at 1610 cm^{-1} (Silverstein *et al.* 1991), the absence of an expected prominent overtone band assigned to the asymmetrical bending vibration and torsional oscillation at 2020 cm^{-1} of the NH_3^+ was not present, hence it may be that the extended stretch was most likely generated by coupling of the (O-H, C-O) vibrational modes. But the absence of contributions by N-H groups cannot be ruled out, as evidence of work carried out by Croue (2004) on colloidal DOC assigned the 1050 cm^{-1} band, (which was present in all the fractions analysed during this project) was considered to be indicative of the presence of N-acetylglucosamine. Croue, stated that this was formed from the oxidation of carbohydrates with amino groups from bacterial cell wall structures.

Although the fractioned DOC exhibited similar bands the results did confirm that the resins remained selective and did not modify the fractions, That is, subtle differences in the molecular structures were retained e.g., the HPOA fractions demonstrated different hydrogen bonding in both Conwy and Cefni OH stretch bands. The bands in the 1600 cm^{-1} region of the HPOA samples also showed differences although those recorded for both Neutral samples of the OH stretch (3500 cm^{-1}) and the 1600 cm^{-1} generated similar spectra, which may suggest that the desorption step using NaOH (0.1M) for the hydrophobic acids (HPOA) may disrupt the intra-molecular hydrogen bonding of the molecular structures that absorb in the 1600 cm^{-1} region, but not so, in the Neutral fraction which is not

subjected to desorption reagents, hence molecular structure may not be disrupted. The broad stretch noted in both Cefni and Conwy spectra suggests a β -Diketone, although usually in tautomeric form, this broad stretch indicates that it is in the enol form and is displaced from the normal conjugated ketone absorption of $\approx 1715 \text{ cm}^{-1}$ by intramolecular hydrogen bonding. Silverstein *et al.* (1991) also propose that the enolic O-H stretching absorption is also seen as a hallow band at $3000 - 2700 \text{ cm}^{-1}$.

6.3 Conclusions

This chapter has presented the characterisation results of the fractioned raw surface water DOC of the four lakes of the project. The main conclusions that can be drawn are;

- The concentrations of the DOC when fractioned were generally distributed in the trend : HPOA>Neutrals>HPIA> HPIB and HPOB representing from 38 – 58%, 18 - 28%, 17 – 19% and $\leq 10\%$ respectively of the surface water DOC.
- The Cefni and Cwellyn HPOA contained the highest percentage of the DOC, whilst Teyrn demonstrated the lowest average.
- Phenolic concentration results were outside accepted analytical limits recovery limits (80 – 120 %).
- UV-vis spectra demonstrated that the HPOA fractions had the highest absorbance at 254 nm, hence suggesting that this fraction had the highest concentrations of aromatic molecular structures.
- A linear relationship existed between absorbance at 254 nm and the DOC concentrations of the HPOA fractions for all the lakes sampled.
- SUVA values (section 6.1.4) which were calculated using the UV-vis spectra did not correspond to any published values for the fractioned DOC.
- Calculation of the aromaticity ratio (E_2/E_3) values (Section 6.1.5) using the same UV-vis data, indicated that the aromatic concentration was again highest in the HPOA and lowest in the Neutral fraction.
- The SUVA values were higher than those recorded for the Raw Surface Water DOC, with the exception of the HPOA fraction.
- Size exclusion chromatography (SEC) demonstrated (Fig 6.14) that the distribution of MM at a detector wavelength of 254 nm were relatively similar for all the fractions. The major distribution ranges were between 5000 – 3000 Da. The relationship between the individual suites of lake fractions demonstrated significant

correlations, thus concluding that the DOC being exported from the catchments exhibited similar MM distributions.

- A response between temperature and HPOA MM distribution was noted, with an increase in the lower MMs during higher temperatures.
- The fluorescence results established the presence of three major fluorescing components in the fractioned samples, which was one less than those identified in the Raw Surface Water samples.
- Low concentrations of cations and anions associated with the DOC were found in the fractions, the majority passed through both resin columns, confirming that the columns did not carry any charges and were relatively neutral.
- Major differences were not found in the IR spectra of the fractions. Band variations were recorded for the OH stretches, i.e., hydrogen bonding was strong in the Cefni fractions HPOA and Neutrals, but reduced in the HPIA. Conwy's hydrogen bonding suggested intramolecular bonding, rather than the inter-molecular hydrogen bonding suggested by the Cefni spectra. The spectra collected, suggested heterogeneous molecular structures, with functional groups of the type: COOH, COO⁻, CH_n, N-H, C_nH_n, OH, (β-keto esters or β-Diketones) both in the enol form stabilized by resonance and hydrogen bonding.

The data collected by fractioning the Raw Surface Water suggests that the DOC is made up of a series of molecular compounds with different physical and chemical properties which are capable of being isolated.

Chapter 7

Tri-halomethane Formation Potential (THMFP) of Fractioned Raw Surface Water DOC.

Chapter 7 Tri-halomethane Formation Potential (THMFP) of Fractioned Raw Surface Water DOC.

7.1 Introduction

Chapter 5 presented the results of the chlorination of the Raw Surface Water DOC. In this chapter the results of the fractioned samples of the Raw Surface water characterised in Chapter 6 are reported. The exercise was intended to determine each fraction's trihalomethane formation potential (THMFP) and establish any common property that promoted trihalomethane (THM) formation. The differences in the THMFP of each fraction for each lake will be investigated and reported on to identify differences promoted by the catchments characteristic and location. The series of fractions recovered (Chapter 6) were chlorinated at a ratio of 5:1 (5mg Cl₂: 1mg DOC). Residual chlorine after incubation was analysed for by iodometric titration. To determine chlorine demand, the chlorinated samples were analysed by GC- ECD using solid phase micro extraction (SPME) technique (Method 2.3.8, Chapter 2). Each THM component was quantified by external standard calibration curves (Appendix 2).

7.2 THMFP results of Cefni, Cwellyn, Conwy and Teyrn DOC fractions.

The results are reported as µg/mg DOC, this as previously noted in Chapter 5 is to demonstrate the standardised THMFP of the DOC rather than the formation potential of the concentration of the DOC in the sample. Where results are reported relative to a complete suite, this represents in effect (µg/5 mg DOC). In Section 7.2.5 we present the individual lake DOC fraction results in the form of stacked columns to make easy comparisons of the THMFP each fraction.

7.2.1 Trichloromethane (CHCl₃) Formation Potential of Cefni, Cwellyn, Conwy and Teyrn Fractioned Raw Surface Water DOC.

Fig 7.1 shows results for the CHCl₃ THMFP of the individual suites of fractions of the Raw Surface Water samples for each lake over the period January 2010 – October 2011. The results show that each fraction within a suite has a different THMFP. To establish the

concentrations of CHCl_3 formed by each complete suite over the period (January 2010 – October 2011) of the exercise, the THMFP of each individual lake suite were summed to give a total CHCl_3 / mg DOC concentration. The results established that Cwellyn, Teyrn and Conwy generated the highest concentrations: 2215, 2245 and 2169 μg of CHCl_3 respectively and Cefni the lowland fen catchment formed the lowest concentration total at 1589 μg of CHCl_3 . This highlighted a difference between the upland lake DOC THMFPs of the fractions and that of the Cefni's.

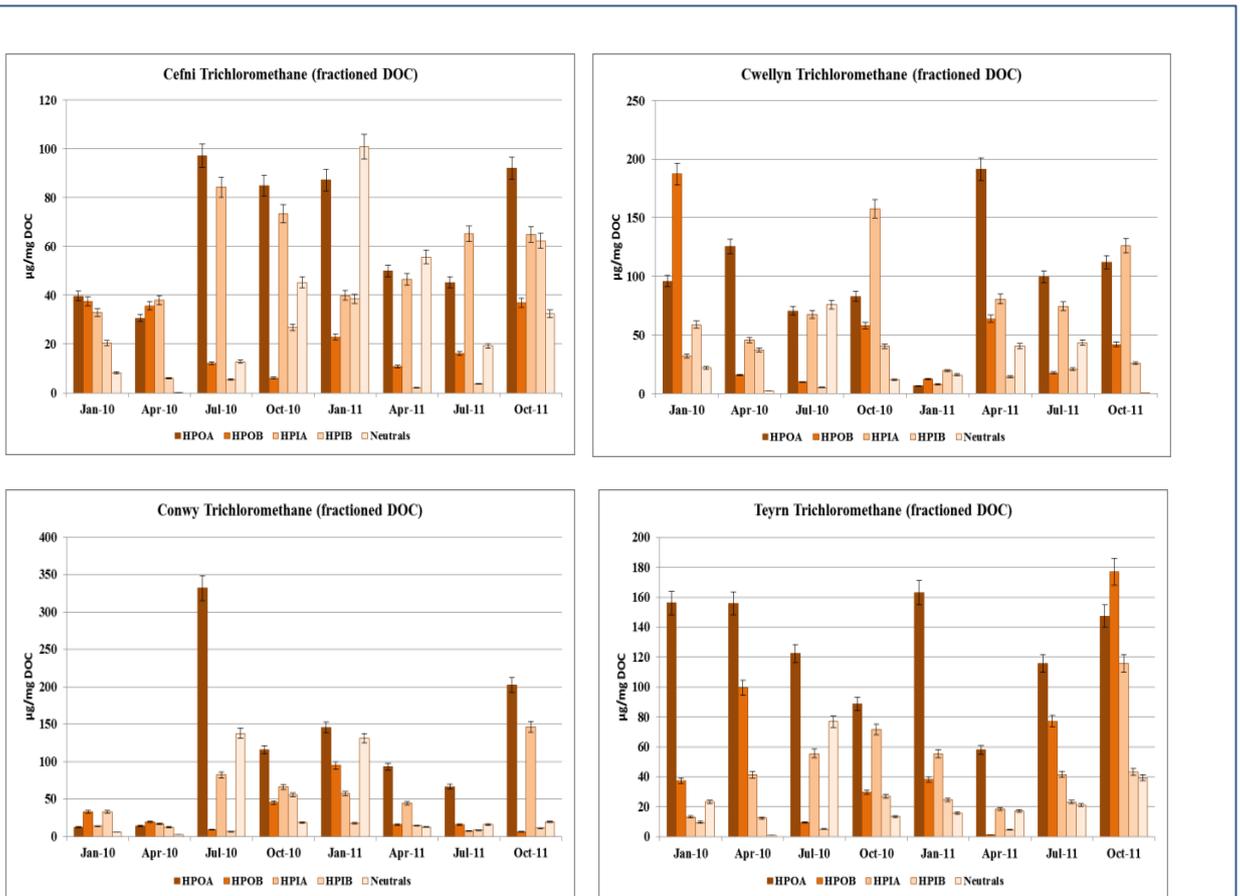


Figure 7.1 Trichloromethane formation potential presented as $\mu\text{g}/\text{mg DOC}$ of the hydrophobic, hydrophilic acids, bases and neutrals of the fractioned Cefni, Cwellyn, Conwy and Teyrn DOC. (January 2010 – October 2011)

This difference in upland and lowland THMFP of the fractions was again illustrated when the July 2010 results at the end of the dry period were considered and a general increase was recorded for all four THMFPs. Cefni although exporting the highest concentration of DOC out of the catchment ($7.4 \pm 0.2 \text{ mg/L}$) formed $211 \pm 4 \mu\text{g}$ CHCl_3 for the suite of fractions with $97 \mu\text{g}/\text{mg DOC}$ ascribed to the HPOA fraction, while Conwy, Cwellyn and Teyrn with their lower Raw Water DOC concentrations formed 568 ± 11 , 228 ± 4 and $269 \pm 5 \mu\text{g}$ CHCl_3 respectively for their suite of fractions. The HPOA fraction of the upland

lakes for the July 2010 samples contributed CHCl_3 concentrations of ; Conwy 331 ± 6 $\mu\text{g}/\text{mg}$ DOC, Cwellyn 70 ± 1 $\mu\text{g}/\text{mg}$ DOC and Teyrn 122 ± 2 $\mu\text{g}/\text{mg}$ DOC to their suites of fractions for the month. The Neutral fractions also exhibited different THMFPs during the same month, with Cefni again forming the lowest CHCl_3 concentration (13 ± 0.3 $\mu\text{g}/\text{mg}$ DOC) of the four and Conwy, Cwellyn and Teyrn forming 137 ± 3 , 75 ± 2 and 76 ± 2 $\mu\text{g}/\text{mg}$ DOC respectively.

During January 2011 cold conditions were experienced (Fig 3.1.7) which may have affected the THMFP. Conwy formed the highest CHCl_3 (448 ± 9 μg CHCl_3) concentration, followed by Teyrn (296 ± 6 μg CHCl_3), demonstrating both as relatively similar to the July 2010 THMFPs, but Cwellyn exhibited a much lowered formation potential for the suite and only formed 62 μg of CHCl_3 . Cefni did not exhibit this extreme change and for the cumulative suite of concentrations formed a total of 289 ± 6 μg CHCl_3 . The interesting feature about the January 2011 THMFP was that the Conwy and Teyrn's fraction with the highest CHCl_3 formation potential (FP) was the HPOA, but Cwellyn and Cefni's was the Neutral fraction. This was demonstrated by Cwellyn during the dry July 2010 month also.

To show the mean concentration of CHCl_3 formed by each fraction for each of the lakes over the period January 2010 to October 2011, the results in $\mu\text{g}/\text{mg}$ DOC have been presented as percentages (Table 7.1). The results bracketed show the same results, with those for January and April 2010 removed to accommodate for possible FP errors by DOC changes due to storage (reported in Chapter 5, Section 5.3)

From the tabulated results (Table 7.1) the highest % concentration of CHCl_3 was generated by the HPOA fraction of each lake DOC, with Conwy and Teyrn's demonstrating the highest and relatively similar FP. The HPIA had the second highest FP, with both Cefni and Cwellyn showing similar FP and Conwy and Teyrn, except that Teyrn is approximately 2 % less than Conwy.

Table 7.1 Percentage of CHCl_3 formed/fraction for eight fractioned samples of Cefni, Cwellyn Conwy and Teyrn. Bracketed results are calculated without the January and April 2010 results included.				
Fraction	Cefni (%)	Cwellyn (%)	Conwy (%)	Teyrn (%)
HPOA	33.1 (34.05)	35.4 (35.30)	45.4 (47.79)	44.8 (40.98)
HPOB	11.2 (7.81)	18.3 (12.75)	11.2 (9.41)	20.9 (19.61)
HPIA	27.9 (27.91)	26.8 (32.26)	20.1 (20.23)	18.4 (21.10)
HPIB	10.4 (10.35)	10.0 (7.90)	7.4 (5.74)	6.6 (7.49)
Neutrals	17.4 (19.87)	9.5 (11.73)	15.9 (16.82)	9.3 (10.81)

For the HPOB fraction the Cefni and Conwy demonstrated the similar FP at 11.2 %, whilst Cwellyn and Teyrn displayed a higher percentage FP of 8.3 and 20.9 % respectively. Although Cwellyn's pattern of fraction THMFP was comparable to both Cefni and Conwy it demonstrated a 7.1% higher formation for its HPOB fraction. Differences were also exhibited by the Neutral fraction THMFP, both Teyrn and Cwellyn were similar $\approx 9\%$, but Cefni and Conwy's formation potentials were much higher at 17.4 and 15.9% respectively. Statistical analysis to determine any significant correlations between the same fractions of the different lake DOCs exhibited only three. The HPOA of both Cefni and Conwy ($r\ 0.866, p < 0.01$), the HPIA for Conwy and Teyrn ($r\ 0.858, p < 0.01$) and for Cefni and Teyrn HPIB ($r\ 0.843, p < 0.01$). No correlations were demonstrated between any lake DOC for the Neutrals or HPOB.

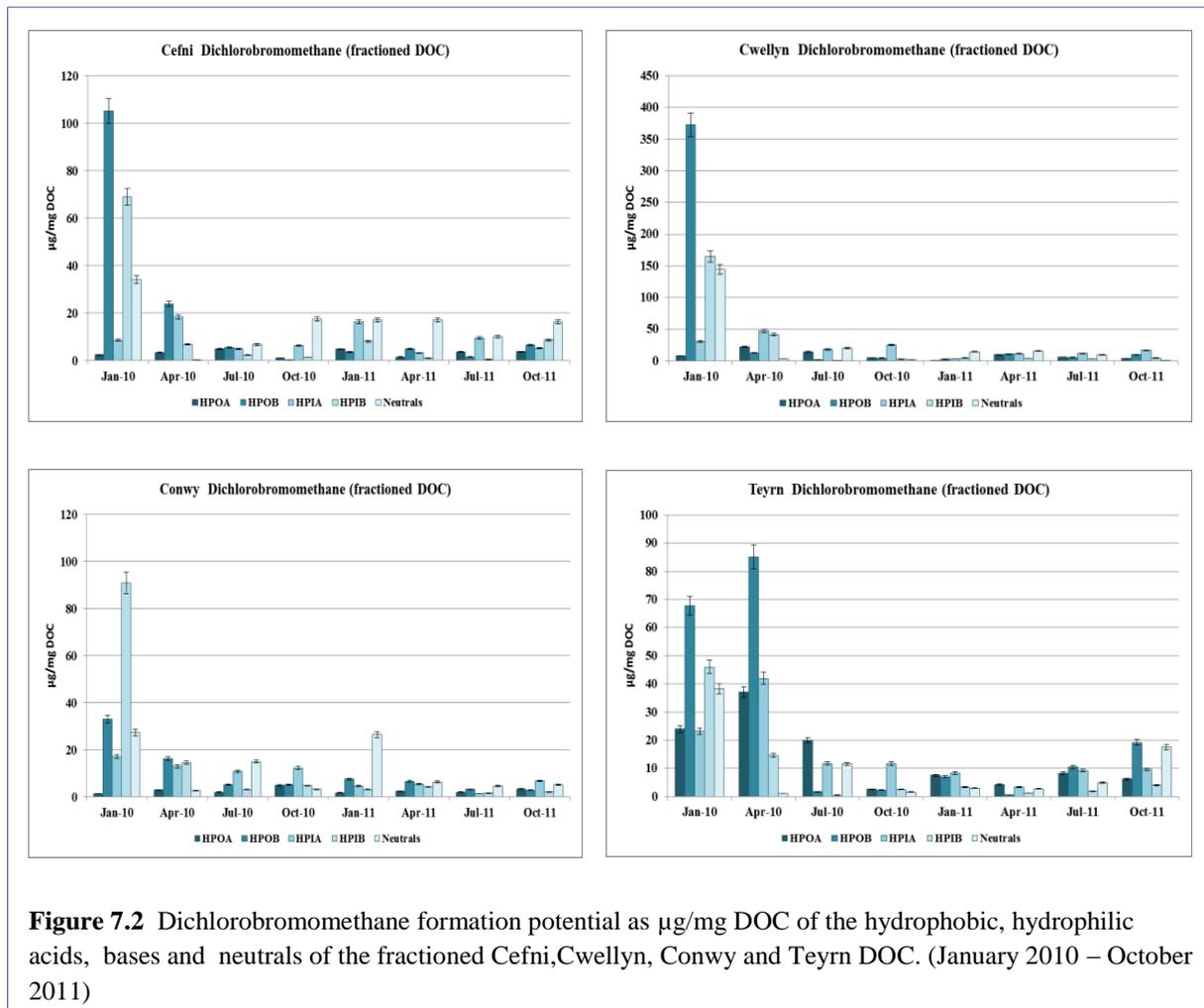
The quarterly data collected over the period January 2010 to October 2011 generated 8 complete fractioned suites, four for 2010 and an equivalent temporal series of 4 for 2011. The results were statistically analysed to explore for any correlations between the CHCl_3 FP for equivalent months and lakes, for example, all lake fractions April 2010 *versus* all lake fractions April 2011. From the results the October 2010 and October 2011 suites demonstrated significant correlations ($r\ 0.862, p\ 0.01$) with the exception of Teyrn which did not show any significant correlations in its FP for all months considered.

7.2.2 Dichlorobromomethane (CHCl_2Br) Formation Potential of Cefni, Cwellyn, Conwy and Teyrn Fractioned Raw Surface Water DOC.

The results (Fig 7.2) suggested that the storage of the Raw Surface Water as discussed in Chapter 5 (section 5.3), had an effect on the formation potential (FP) of the brominated products. This was evident in the FP results for January 2010 in all of the four lakes and was seen to be much reduced in the April 2010 fractioned suites, although Teyrn demonstrated an exception with its April 2010 HPOB fraction forming the highest concentration of CHCl_2Br ($85.2 \pm 3\ \mu\text{g}/\text{mg}\ \text{DOC}$) of all of its fractioned sample series (January 2010 – October 2011).

Excluding the January and April 2010 results, unlike CHCl_3 the major formation of CHCl_2Br was by the fractions other than the HPOA. Over the period of sampling the two principal fractions that generated CHCl_2Br were the HPIA and Neutrals for three of the lake samples. Again Teyrn did not follow the trend of Cefni, Cwellyn and Conwy, it demonstrated relatively similar FP averages for CHCl_2Br by HPOA ($8.1\ \mu\text{g}/\text{mg}\ \text{DOC}$),

HPOB (6.9 $\mu\text{g}/\text{mg}$ DOC), HPIA (9.0 $\mu\text{g}/\text{mg}$ DOC) and Neutrals (6.9 $\mu\text{g}/\text{mg}$ DOC) with HPIB averaging the lowest FP at an average of 2.3 $\mu\text{g}/\text{mg}$ DOC.



The lake DOC that had the highest FP for CHCl_2Br when fractioned was the Cwellyn with the other three ordered as Teyrn > Cefni > Conwy. In Table 7.2 the percentage formation potentials of the fractions are shown for CHCl_2Br . The bracketed figures were calculated without the January and April 2010 samples to eliminate distortions due to the increased formation of brominated products.

Table 7.2 Percentage of CHBrCl_2 formed/fraction for eight fractioned samples of Cefni, Cwellyn Conwy and Teyrn. Bracketed results are calculated without the January and April 2010 results included.

Fraction	Cefni (%)	Cwellyn (%)	Conwy (%)	Teyrn (%)
HPOA	5.3 (9.99)	6.4 (16.44)	5.2 (9.72)	19.1 (24.52)
HPOB	32.7 (11.50)	38.6 (14.42)	20.6 (18.13)	33.6 (20.76)
HPIA	15.5 (23.35)	15.1 (35.91)	18.4 (24.61)	20.6 (27.12)
HPIB	20.9 (11.14)	20.7 (7.91)	32.2 (11.13)	12.8 (6.83)
Neutrals	25.6 (44.01)	19.2 (25.32)	23.6 (36.41)	13.9 (20.77)

From these data that include the whole series (January 2010 – October 2011) the highest formation potential was by the HPOB and HPIB and Neutrals with the lowest by the HPOA and HPIA fractions. When the percentages were recalculated without the January and April 2010 results the FP were altered. The highest FP was demonstrated by the HPIA and Neutrals (with the exception of Teyrn as previously noted) and the HPOB and HPIB FP was reduced. These results tend to suggest that the hydrophobic and hydrophilic base DOC molecular structures were the most influenced by the extended storage.

Statistical analysis of the FPs did not demonstrate any significant correlations between fractions or lakes for both the complete series (January 2010 – October 2011) and the series with January and April 2010 removed.

7.2.3 Dibromochloromethane (CHBr₂Cl) Formation Potential of Cefni, Cwellyn, Conwy and Teyrn Fractioned Raw Surface Water DOC.

Analysis of the THMFP potential of the fractions for CHBr₂Cl demonstrated that, similar to the results for CHCl₂Br, the concentrations were higher in all the lake fractioned DOC for the January and April 2010 samples. Figure 7.3 shows the distribution of the concentrations within each suite of fractions. It was evident that the formation potential for CHBr₂Cl was lowest for the HPOA fraction in all the lake fractioned samples (Table 7.4).

Table 7.3 Percentage of CHBr ₂ Cl formed/fraction for the fractioned samples of Cefni, Cwellyn Conwy and Teyrn. Bracketed results are calculated without the January and April 2010 results included.				
Fraction	Cefni (%)	Cwellyn (%)	Conwy (%)	Teyrn (%)
HPOA	1.5 (3.5)	2.1 (12.0)	1.4 (4.9)	9.6 (12.7)
HPOB	22.6 (9.5)	26.3 (14.6)	10.8 (17.0)	30.1 (10.2)
HPIA	11.6 (12.1)	12.0 (28.0)	9.3 (15.4)	19.7 (13.6)
HPIB	9.4 (7.2)	23.3 (8.1)	34.5 (18.9)	19.4 (8.4)
Neutrals	54.8 (67.9)	36.4 (37.4)	43.9 (43.8)	21.1 (55.2)

The highest concentration of CHBr₂Cl was formed by the DOCs' Neutral fraction in all of the four lakes. This was also reflected in the results with both January and April 2010 removed to provide a series without the high concentration of brominated species that were considered to be generated by DOC after a period of storage. Before removal the results indicated that the HPOB and HPIB fractions exhibited the major FPs with the exception of Conwy (10.8 %). The three others showed a potential contribution of over 20%.

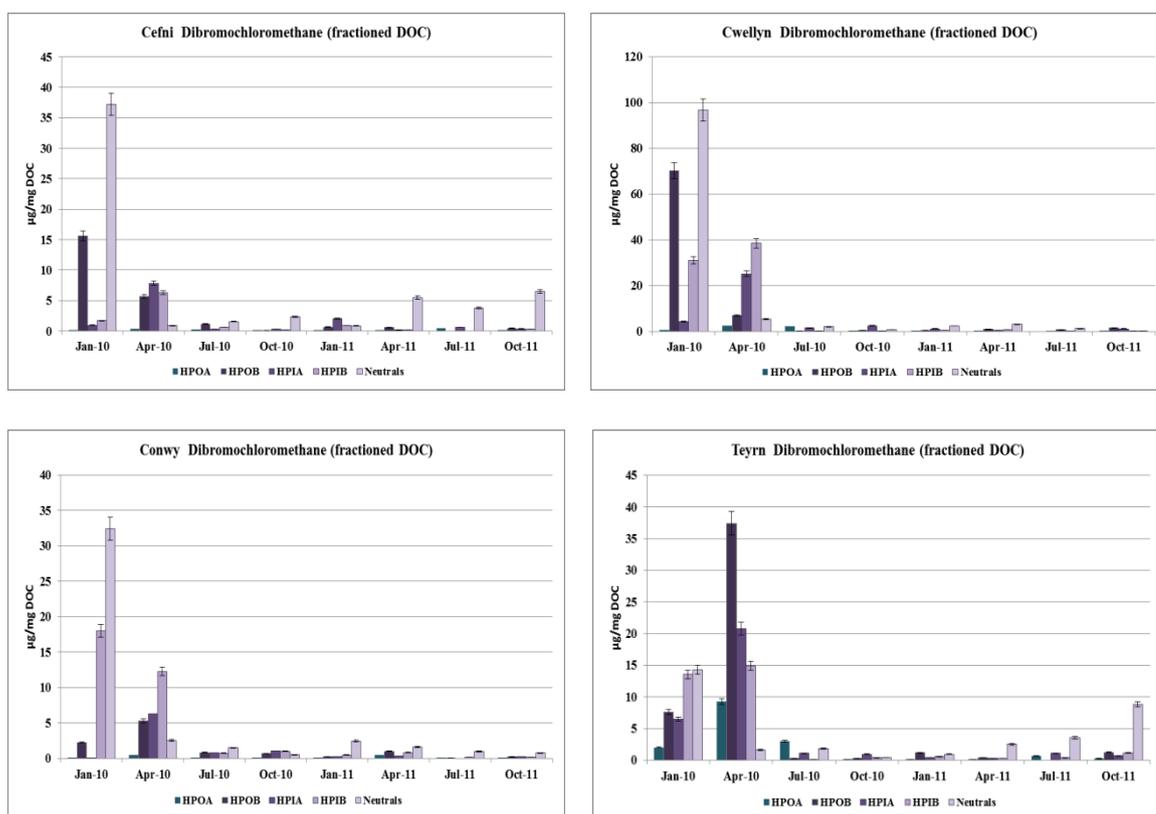


Figure 7.3 Dibromochloromethane formation potential as $\mu\text{g}/\text{mg}$ DOC of the hydrophobic, hydrophilic acids, bases and neutrals of the fractioned Cefni, Cwellyn, Conwy and Teyrn DOC (January 2010 – October 2011).

When the January-April results were not included, a change in FP was observed, the Neutral fraction remained as the CHBr_2Cl principal FP, together with the HPIA fraction. The three others reduced their HPOB FPs from an average of 26.3 % to an average of 11.4% whilst the HPOB fraction of the Conwy increased its FP of CHClBr_3 from 10.8 % to 17 %. A similar change was also observed in the HPIB FP, i.e., the FP percentage was reduced in all the fractions when the January and April 2010 contributions were excluded (Table 7.4). This again suggested that the HPOB and HPIB of the Raw Surface Water DOC was modified in the aged DOC.

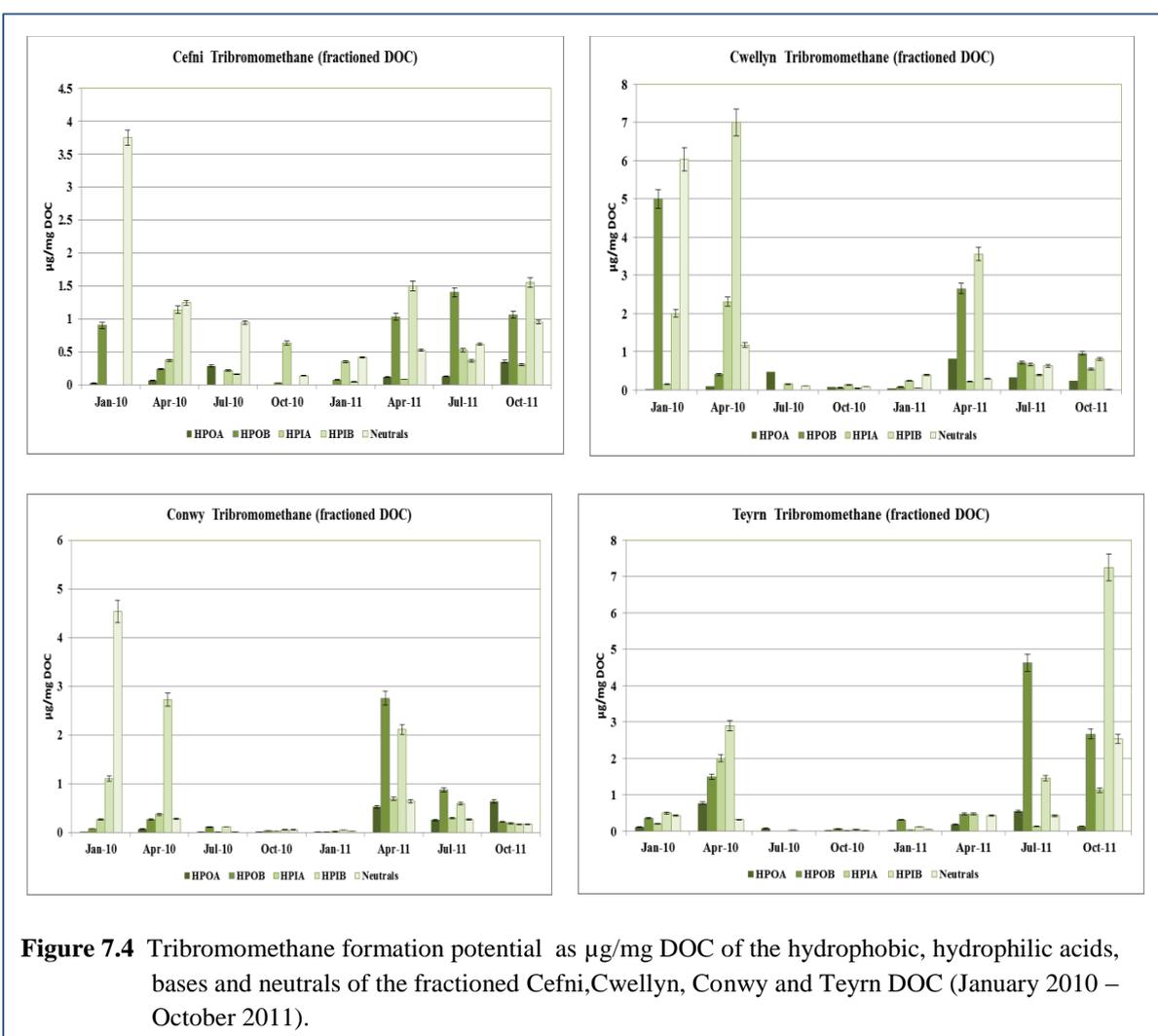
7.2.4 Tribromomethane (CHBr_3) Formation Potential of Cefni, Cwellyn, Conwy and Teyrn Fractioned Raw Surface Water DOC.

Many of the results were close to the limit of detection (LOD $0.54\mu\text{g}/\text{L}$) and limit of quantitation (LOQ $1.8\mu\text{g}/\text{L}$) for CHBr_3 . Hence these data can be considered to give qualitative rather than quantitative results. Table 7.4 presents the complete series and suites

of fractions, again with a second series (bracketed) of which the results for January and April 2010 have been removed.

Table 7.4 Percentage of CHBr_3 formed/fraction for eight fractionated samples of Cefni, Cwellyn Conwy and Teyrn. Bracketed results are calculated without the January and April 2010 results included.

Fraction	Cefni (%)	Cwellyn (%)	Conwy (%)	Teyrn (%)
HPOA	4.5 (6.4)	5.4 (13.4)	7.4 (13.3)	5.8 (4.2)
HPOB	22.0 (26.1)	25.3 (30.2)	21.1 (36.6)	30.9 (35.0)
HPIA	11.5 (15.3)	11.3 (13.2)	9.0 (11.2)	12.3 (7.6)
HPIB	22.1 (26.2)	35.5 (32.8)	33.5 (28.2)	38.1 (38.3)
Neutrals	39.9 (26.0)	22.4 (10.4)	28.9 (10.7)	13.0 (14.9)



Comparatively high concentrations of CHBr_3 were recorded for the months of January and April 2010 especially in Cwellyn where collectively the fractions generated an average of $12.1 \pm 0.4 \mu\text{g CHBr}_3$ compared to the other three samples Cefni ($3.9 \pm 0.1 \mu\text{g CHBr}_3$), Conwy ($4.9 \pm 0.1 \mu\text{g CHBr}_3$) and Teyrn ($4.6 \pm 0.1 \mu\text{g CHBr}_3$). There was a discernible

pattern to the formation potential of the four fractioned DOC', i.e., the period from July 2010 to April 2011 demonstrated a lower formation potential for CHBr_3 in all the fractions, after which increases were recorded. In October 2011, Teyrn increased its potential from a total of $7.2 \pm 0.2 \mu\text{g CHBr}_3$ for the combined suite of fractions in July 2011 to $13.7 \pm 0.4 \mu\text{g CHBr}_3$, while the other three sites demonstrated smaller changes.

Table 7.5 shows that the HPOA had the lowest formation potential for CHBr_3 in all four fractioned DOC samples with HPOB, HPIB and Neutrals having the highest in line with the other brominated THMs. With the January and April 2010 results not included, the Cefni demonstrated that the formation potential of HPOB, HPIB and Neutrals were similar at $26.1 \pm 0.1 \%$. The highest CHBr_3 formation fractions were HPOB and HPIB with their Neutrals having an equivalent potential to HPOA.

7.2.5 Review of THMFP of the DOC fractions.

In Figures 7.5 to 7.8 (a,b) the results of the formation potentials of each fraction relative to CHCl_3 , CHCl_2Br and CHClBr_2 are represented by the stacked columns. Each column consists of the concentration of the THM formed by the fraction that is specific to each lake DOC.

In Figure 7.5 the results of the CHCl_3 formation potential (FP) relative to the suite of DOC fractions show that overall, the highest concentrations were generated by the HPOA fraction. The individual concentration contributions to each of the columns showed a number of variations. For example, the July 2010 HPOA fractions collectively showed an overall summed concentration of $> 600 \mu\text{g CHCl}_3$, but the principal contributor was the Conwy fraction ($332.0 \pm 10 \mu\text{g}/\text{mg DOC}$), whilst Cefni, Cwellyn and Teyrn contributed 97.1, 70.5 and 122.3 $\mu\text{g}/\text{mg DOC}$ respectively. In April 2011 the highest CHCl_3 FP by the individual contributors was Cwellyn followed by Conwy Cefni and Teyrn. The random changes in FP also occurred in the other fractions and suggested that although the CHCl_3 FP was biased principally to the HPOA (hydrophobic properties) and HPIA (hydrophilic properties) fractions, the THMFP of the other molecular structure classifications were favourable to chlorination in the Raw Surface Water DOC and were subject to constant change.

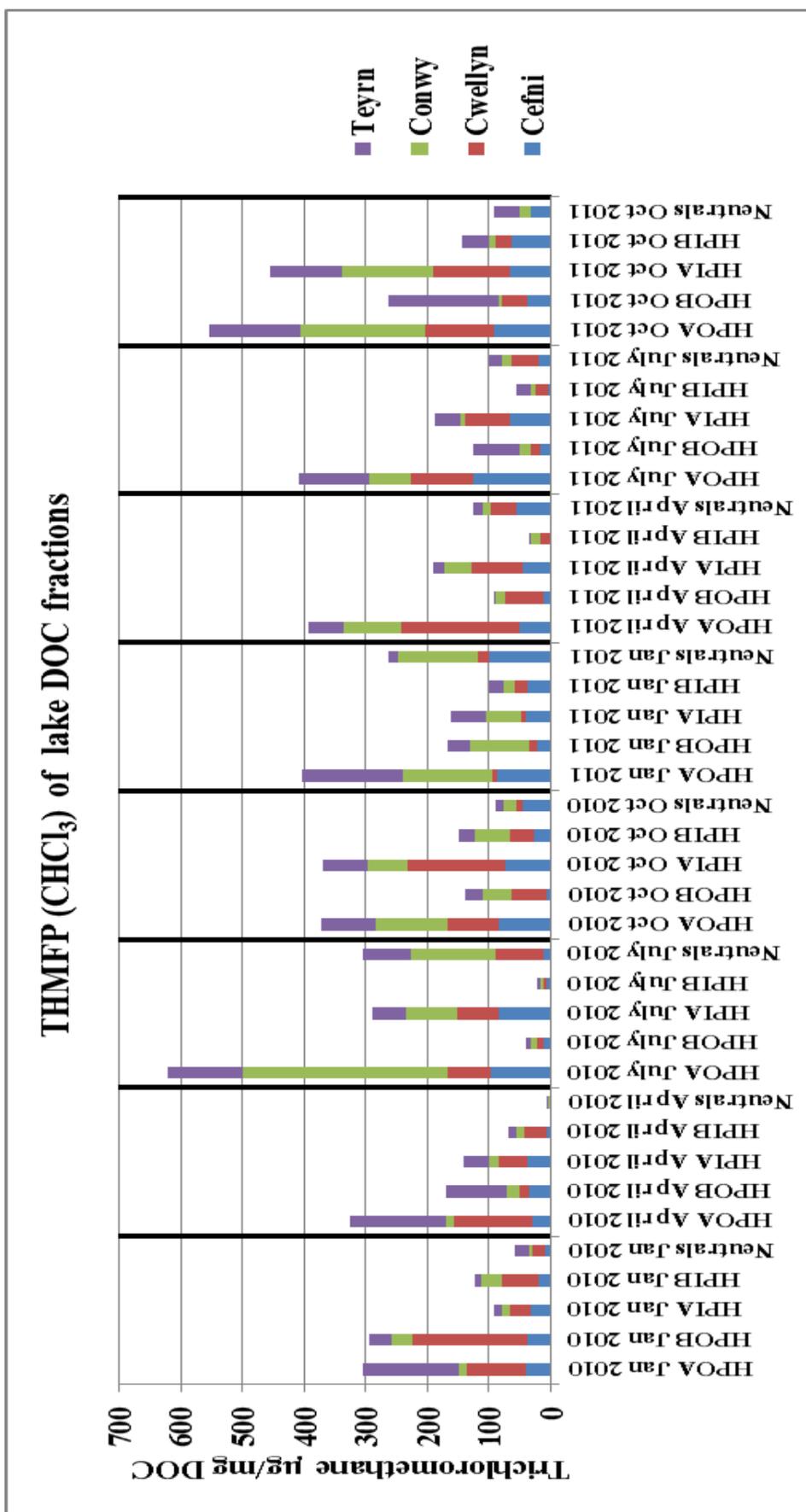


Figure 7.5 THMFP (CHCl₃) for fractionated samples (HPOA, HPOB, HPIA, HPIB and Neutrals) for the four lake sites (Teyrn, Cwellyn, Conwy and Cefni) from January 2010 to October 2011.

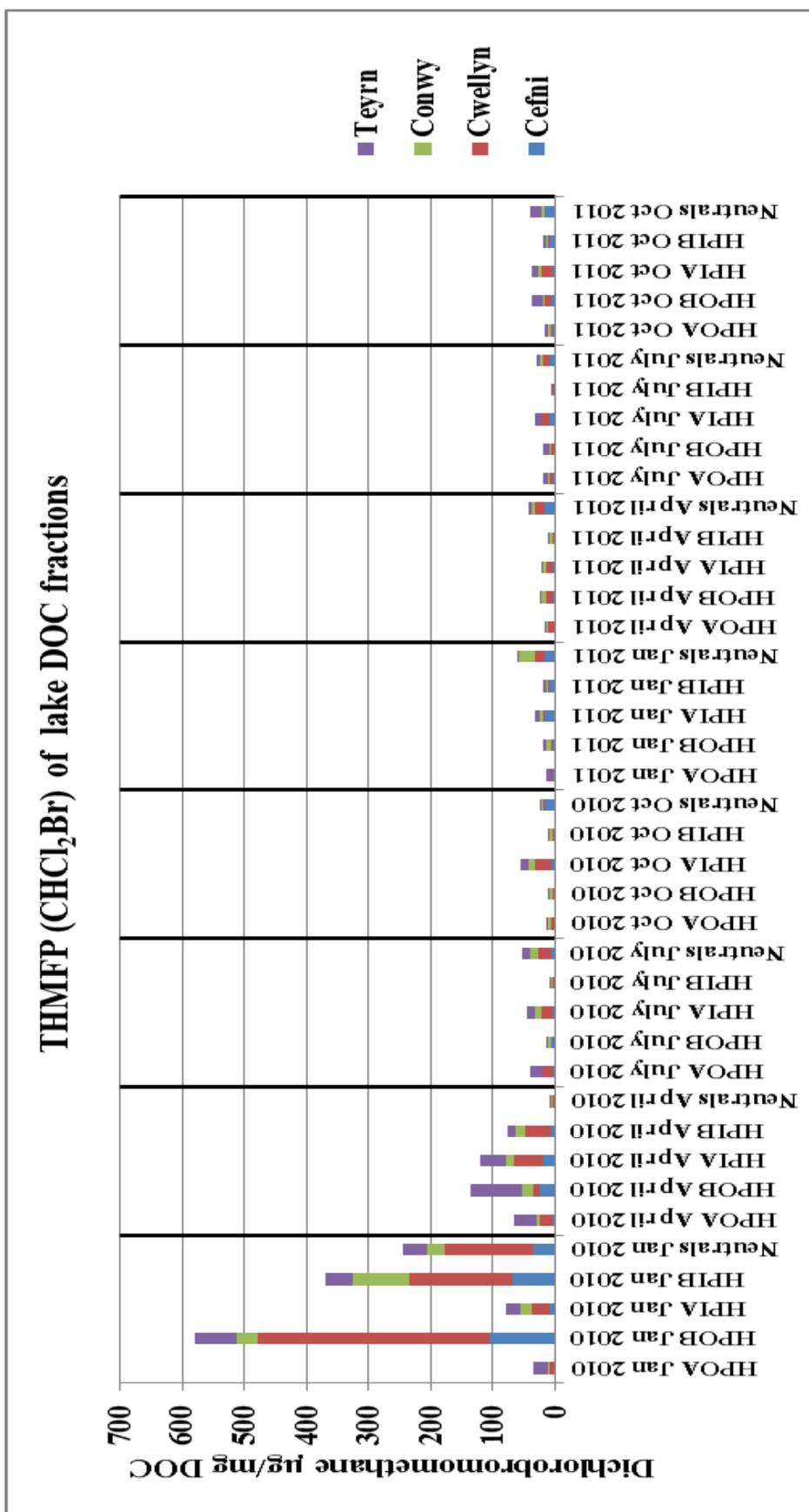


Figure 7.6 THMFP (CHCl₂Br) for fractioned samples (HPOA, HPOB, HPIA, HPIB and Neutrals) for the four lake sites (Teyrn, Cwellyn, Conwy and Cefni) from January 2010 to October 2011.

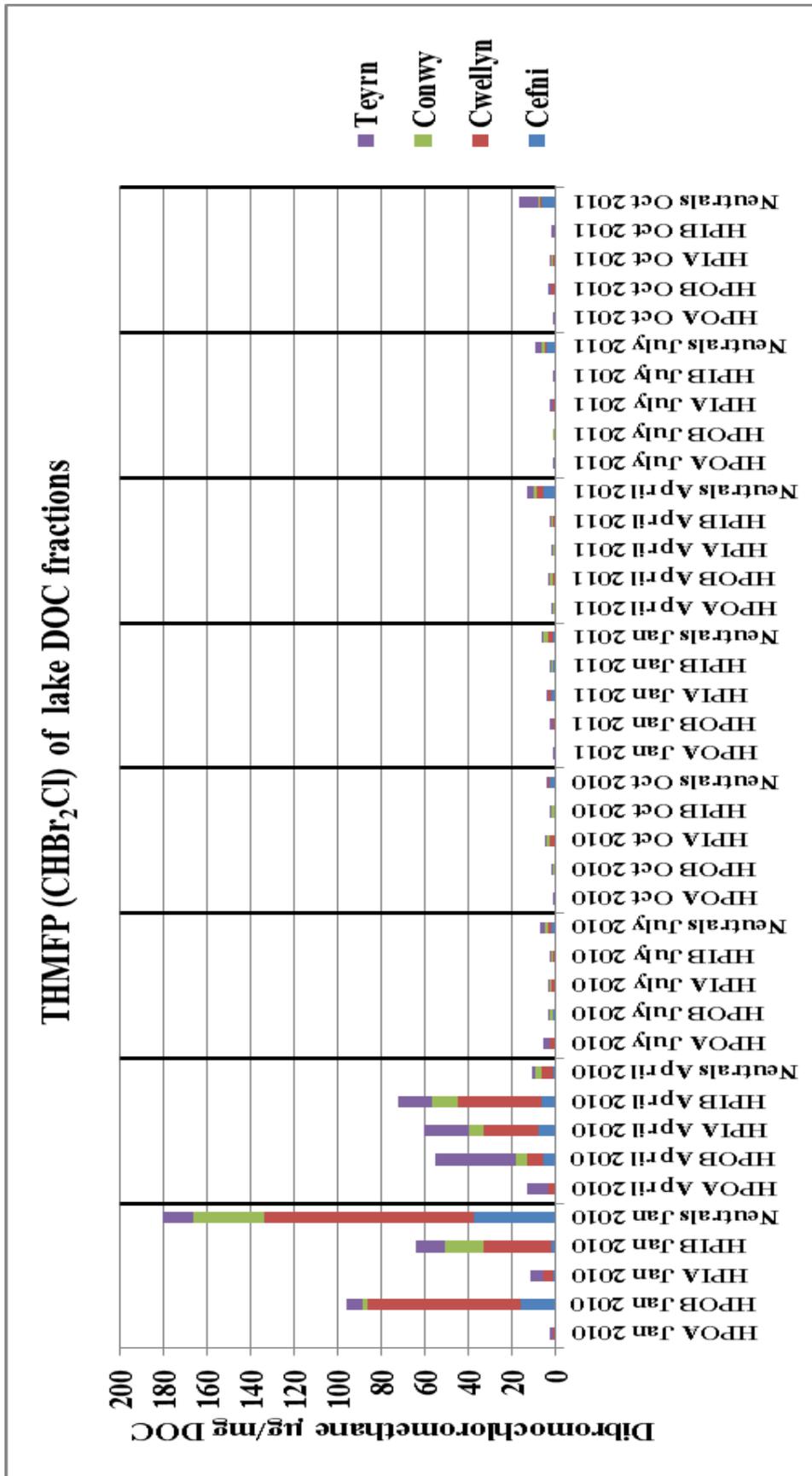


Figure 7.7 THMFP (CHBr₂Cl) for fractionated samples (HPOA, HPOB, HPIA, HPIB and Neutrals) for the four lake sites (Teyrn, Cwellyn, Conwy and Cefni) from January 2010 to October 2011.

An interesting feature of the Conwy and Cefni CHCl_3 FP fraction series was relative to the step change discussed in Chapter 5, Section 5.3. That is, for both January and April 2010 there was an increase in brominated products in all the fractions of the lakes, this was considered to relate to the DOC molecular structure being modified during the storage period, hence making it more susceptible to a reaction with bromine rather than chlorine. For example, the Conwy FP of CHCl_3 by the HPOA fraction averaged $159.1 \pm 5 \mu\text{g}/\text{mg}$ DOC for the series from July 2010 to October 2011 but for the January/April 2010 analysis the FP was much reduced and averaged $13.2 \pm 0.4 \mu\text{g}/\text{mg}$ DOC. This trend was also evident in the Cefni FP, the CHCl_3 HPOA formation potential averaged $35.2 \pm 0.7 \mu\text{g}/\text{mg}$ DOC for January and April 2010, but for the rest of the sampling period (July 2010 – October 2011), it like the Conwy, demonstrated an increased FP and averaged $76.1 \pm 1.5 \mu\text{g}/\text{mg}$ DOC.

Step changes in FP were more obvious in the brominated fractions (Figure 7.6, 7.7). These showed distinct differences in the FP between the DOC that was fractioned immediately (July 2010 – October 2011) and the series that were stored (January to April 2010). Cwellyn's HPOB, HPIB and Neutrals fractions demonstrated the highest FP for both CHCl_2Br and CHBr_2Cl (combined average $936 \pm 19 \mu\text{g}$) compared to the other lakes during this period (Cefni, Conwy and Teyrn (combined average $250 \pm 5 \mu\text{g}$) for the January 2010 fractioned suites).

The analytical data, when reproduced without the January and April 2010 results for both THMs, CHCl_2Br and CHBr_2Cl (Fig 7.8 a and b) demonstrated reduced concentration of the brominated species for the fractioned DOCs. For example, the series from July 2010 to October 2011 for both Cwellyn's Neutrals and HPOB FP of brominated products were reduced, averaging 6.6 and $1.5 \mu\text{g}/\text{mg}$ DOC respectively. The CHCl_3 FP demonstrating an average concentration for the same fractions of 33.5 ± 0.7 and $30.9 \pm 0.6 \mu\text{g}/\text{mg}$ DOC respectively. When the overall THMFP was considered for both brominated and chlorinated products the highest concentrations were generated by the HPIA and Neutral fractions for the brominated products with the higher concentrations of CHCl_3 being formed by the HPOA.

Although Conwy and Teyrn, like Cwellyn had low natural Br^- , relatively high total concentrations from July 2010 to October 2010 of brominated THMs were found, (Conwy; average $32.5 \pm 0.7 \mu\text{g}$) and (Teyrn; $42.5 \pm 0.9 \mu\text{g}$). Cefni with its relatively high natural Br^- (average 0.04 mg/L) concentration did not demonstrate as high a FP for both CHCl_2Br and CHBr_2Cl as expected, with a combined average of $40.7 \pm 0.8 \mu\text{g}$, which was less than that of Teyrn's.

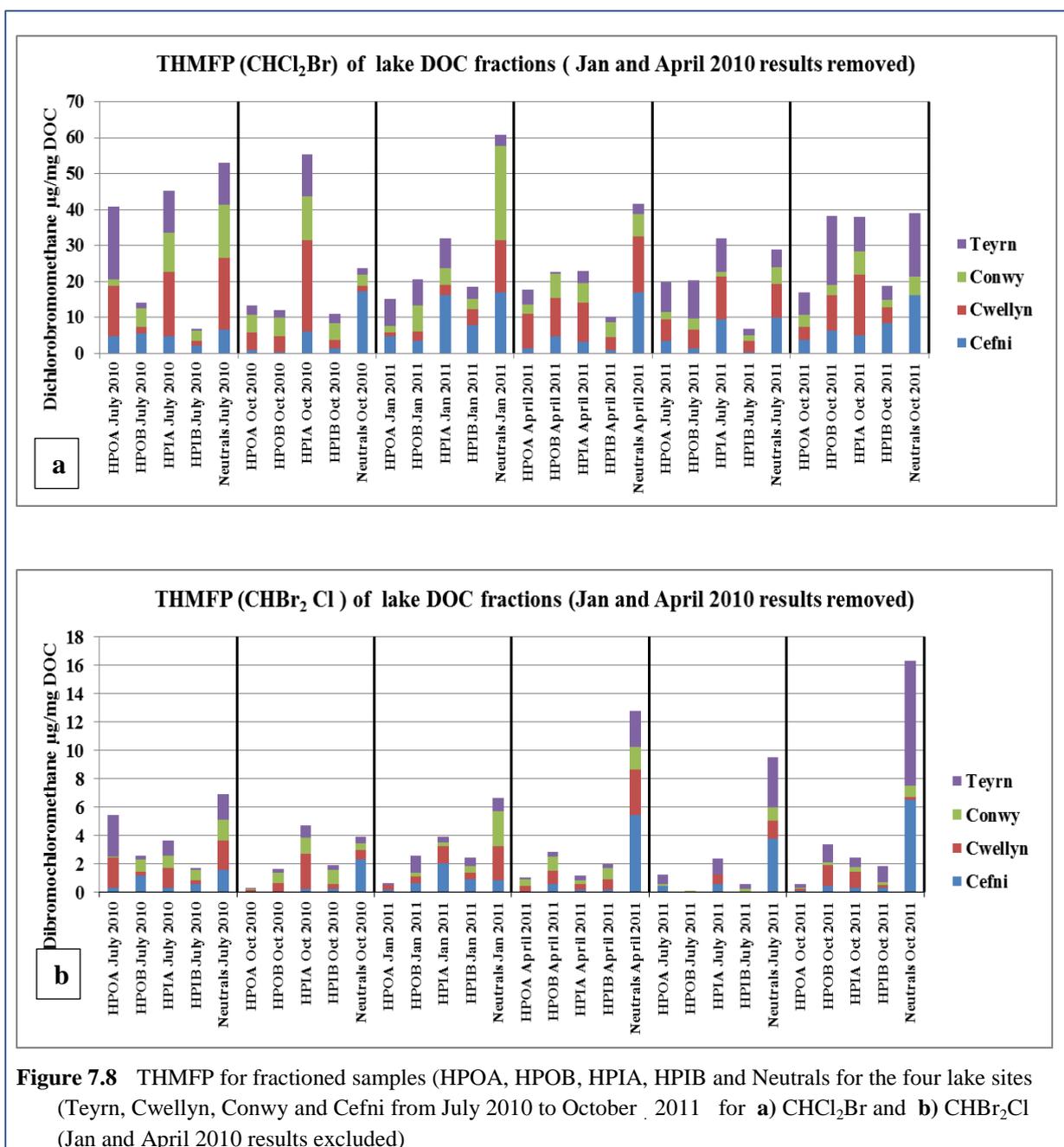


Figure 7.8 THMFP for fractioned samples (HPOA, HPOB, HPIA, HPIB and Neutrals for the four lake sites (Teyrn, Cwellyn, Conwy and Cefni from July 2010 to October 2011 for a) CHCl₂Br and b) CHBr₂Cl (Jan and April 2010 results excluded)

7.3 Fractioned DOC THMFP correlations with SUVA, Aromaticity and Phenolics

The results of the correlation analysis of aromaticity, SUVA and phenolics *versus* the THMFP of the fractioned DOC are presented in Tables 7.5 to 7.7. Aromaticity as previously stated in Chapter 6 is the product of the ratio of absorbance 250 : 365 nm. The product relates qualitatively to the concentration of aromatic moieties in the DOC; i.e., the

higher the value the lower the aromatics present (Peuravuori and Pihlaja (1996). The Aromaticity of the fractions ranged from a low of 1.3 to 6.1, with the greatest range occurring in the Neutral fraction (1.6 – 6.1) whilst the smallest range was for the HPOB fraction at 1.37 to 2.10.

The results (Table 7.5) demonstrate only three significant correlations between the THMFP and Aromaticity values of the fractions. Relatively weak correlations were recorded for HPOA trichloromethane (CHCl₃) and dichlorobromomethane (CHCl₂Br) with a similar weak correlation coefficient for HPIB's THMFP of trichloromethane (CHCl₃).

Table 7.5 Pearsons correlations (*r* values) of THMFP concentrations *versus* to aromaticity ratio (E₂/E₃) of Raw Surface Water fractions.

Fraction	Trichloromethane	Dichlorobromomethane	Dibromochloromethane	Tribromomethane
HPOA	0.399 <i>p</i> < 0.05	0.421 <i>p</i> < 0.05	0.302 > 0.05	0.04 > 0.05
HPOB	0.107 <i>p</i> > 0.05	0.249 > 0.05	0.352 > 0.05	0.07 > 0.05
HPIA	0.220 <i>p</i> > 0.05	0.207 > 0.05	- 0.238 > 0.05	0.03 > 0.05
HPIB	0.427 <i>p</i> < 0.05	- 0.256 > 0.05	- 0.221 > 0.05	- 0.01 > 0.05
Neutrals	0.249 <i>p</i> > 0.05	- 0.006 > 0.05	- 0.042 > 0.05	- 0.01 > 0.05

SUVA is also considered to be an indicator of the aromatic content of the DOC (Chapter 6, 6.1.4), hence the higher the SUVA value the greater the aromatic content (Croue, 2004). The degree of electron rich aromatic content has been reported to be one of the important controlling properties of the formation of chlorinated disinfection by-products (Reckhow *et al.*, 1990) For the fractions the SUVA values ranged from < 5 to > 500 L/mg m⁻¹. Although the calculated values did not correspond with the literature, the relationship between THMFP and SUVA data were analysed (Table 7.6). The results demonstrated positive correlations for the HPOA fractions for all the THM components, with the exception of CHBr₃. A correlation also existed for the SUVA value and the THM CHCl₂Br.

Table 7.6 Pearsons correlations (*r* values) of THMFP concentrations *versus* SUVA values (L/mg m⁻¹) of Raw Surface Water fractions.

Fraction	Trichloromethane	Dichlorobromomethane	Dibromochloromethane	Tribromomethane
HPOA	0.387 < 0.05	0.621 < 0.01	0.400 < 0.05	0.01 > 0.05
HPIA	- 0.173 > 0.05	0.668 < 0.01	0.318 > 0.05	0.01 > 0.05
Neutrals	- 0.083 > 0.05	- 0.098 > 0.05	0.034 > 0.05	0.01 > 0.05

Comparing the statistical results of both the reported indicators of the aromatic content of DOC, Aromaticity and SUVA demonstrated a correlation between the HPOAs aromatic

content and its CHCl_3 formation potential. Another significant correlation was recorded between the formation potential of CHCl_2Br and the HPOA aromatic content by both the Aromaticity and SUVA values. Other common relationships for correlations between fraction formation potentials and aromaticity and SUVA values were not found.

Exploration of the THMFP of the fractioned DOC relative to phenolics only generated two significant correlations (Table 7.7) for CHBrCl_2 , CHBr_2Cl for the HPOB fraction while all the other fractions and components did not demonstrate any significant correlations.

Fraction	Trichloromethane	Dichlorobromomethane	Dibromochloromethane	Tribromomethane
HPOA	0.034 > 0.05	- 0.240 > 0.05	- 0.280 > 0.05	- 0.328 > 0.05
HPOB	0.244 > 0.05	0.509 < 0.01	0.431 < 0.05	0.275 > 0.05
HPIA	0.058 > 0.05	- 0.190 > 0.05	- 0.268 > 0.05	- 0.324 > 0.05
HPIB	0.048 > 0.05	0.131 > 0.05	- 0.266 > 0.05	- 0.324 > 0.05
Neutrals	- 0.144 > 0.05	- 0.193 > 0.05	- 0.155 > 0.05	- 0.090 > 0.05

7.4 Absorbance ratio ($A_{253\text{nm}}/A_{203\text{nm}}$) of Fractioned DOC.

Evidence of the relationship between substituted aromatic functional groups and THMFP suggested by Korshin *et al.* (1996), was again explored for the fractioned THM results. The ratio was calculated for the HPOA fractions which are considered to be composed of predominantly aromatic moieties (Croue, 2004). The calculated ratios ($A_{253\text{nm}}/A_{203\text{nm}}$) together with the HPOA THM total concentrations for each lake, are shown in Figure 7.9. In July 2010 when the dry period ended, a drop in the ratio ($A_{253\text{nm}}/A_{203\text{nm}}$) for each of the DOC lake samples was recorded. The drop which approached the 0.25 ratio lower parameter was assigned by Korshin to denote greater OH substitution, e.g., catechols and other related phenolic compounds. The fall in the ratio occurred once in the Cefni and Conwy HPOA series, but in Cwellyn and Teyrn a second ratio drop was again recorded in July 2011. Although in the Teyrn the drop was only just below 0.4 whilst in Cwellyn it fell to approximately 0.3. Apart from the July 2010 dip both Cefni and Conwy ratios remained above 0.4, suggesting that the aromatics were highly substituted with carbonyls, esters and carboxylic groups (Korshin *et al.* 1996).

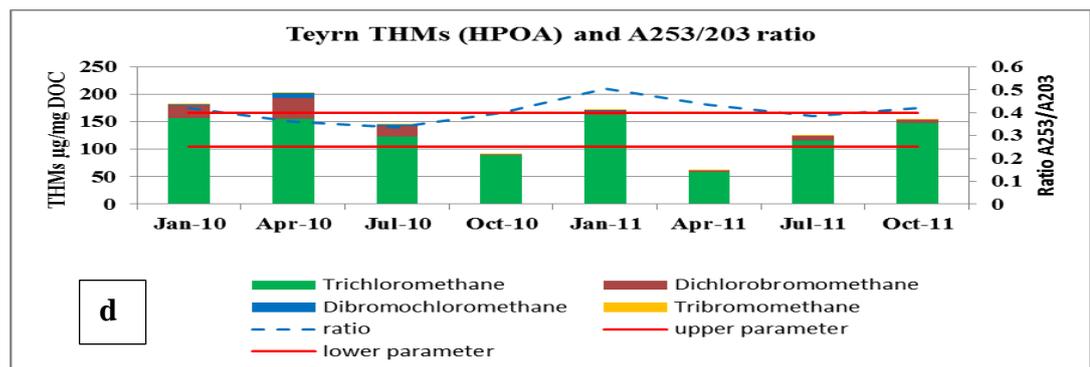
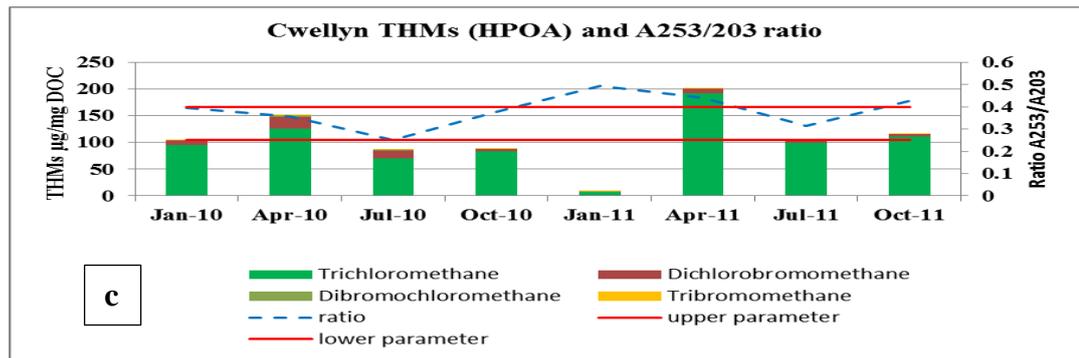
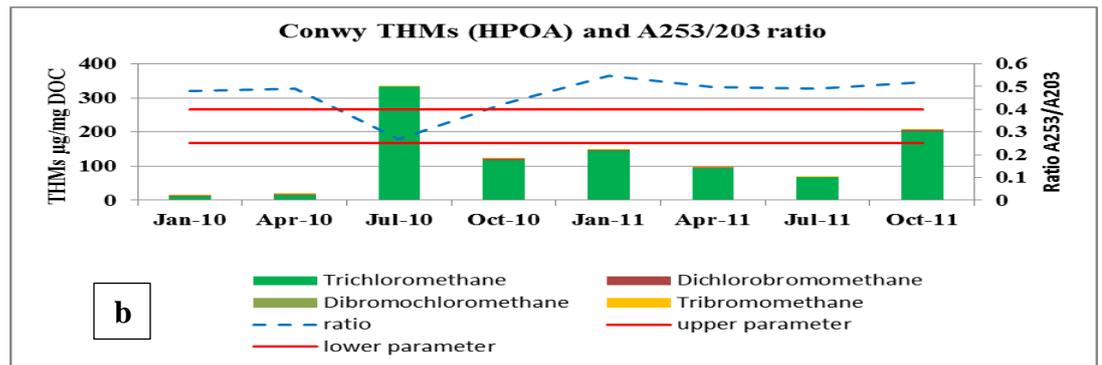
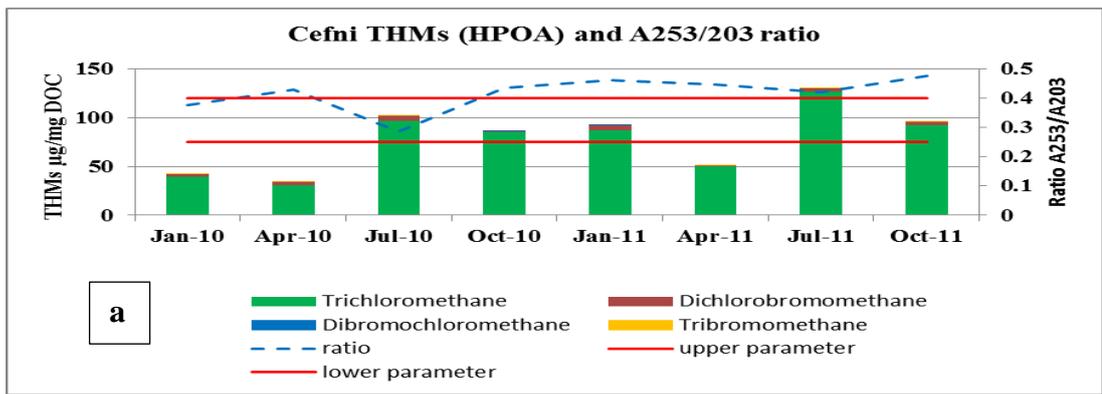


Figure 7.9 a) Cefni, b) Conwy c) Cwellyn and d) Teyrn cumulative THMs formed by the chlorination of DOC fraction HPOA, showing the ratio product of A_{253}/A_{203} relative to the upper and lower parameters 0.25 and 0.40 suggested to represent substituted benzene (Korshin *et al.*, 1996).

It has been reported that the reactivity of the aromatic ring may be directly influenced by these substituted functional groups which can be grouped into two classes, i.e., ortho, para directing and activating; OH, OR and OCOR (hydroxyl, alkoxy and ester) and meta directing and de-activating COOH, COOR, CHO and COR (carboxylic acids, esters, aldehydes and ketones) (Streitwieser and Heathcock 1981).

For example, Rook (1977) demonstrated the influence of two hydroxyl substituents in the meta position during the chlorination of hesperetin and hesperidin which were considered as model molecular structures present in the fulvic acids. The hesperetin formed twice the concentration of CHCl_3 compared to hesperidin (see Section 7.1.4). This was due to the two hydroxyls activating the carbon between them, whereas the hesperidin which had one of its hydroxyls etherized, hence de-activating the centre carbon.

Evidence of a statistically significant relationship between the total THMs of the HPOA results could not be established (R^2 0.009). Although a drop in the ratio indicated an increase in hydroxyl substitution that was accompanied by an increase in THM formation in July 2010 in both Cefni and Conwy, the opposite was recorded for Cwellyn and Teyrn, i.e., a drop in THM formation was recorded in association with a drop in the ratio. It could be suggested that although the ratio value indicated hydroxyl substitution, the UV-vis spectrum may not have been adequately sensitive to differentiate between substitution positions on the aromatic ring. Hence Cwellyn and Teyrn's aromatic ring hydroxyl ring substitutions may have been in deactivating positions.

Examination of the ratio (A_{253}/A_{203}) profiles of the Neutral fractions Figure 7.10 present the same similarities of those of the HPOA. The upland lakes Cwellyn, Conwy and Teyrn ratios demonstrated a dip that was indicative of a greater substitution by hydroxyls during the July 2010 samples. The Cefni's ratio demonstrated a similar profile to that of the ratio exhibited by the Raw Surface Water plot (Figure 5.17a). Although both ratios mirrored each other, the formation of the collective fractioned and surface water THMs for the Cefni did not. In (Figure 7.10 a) the concentration for the July 2010 total THMs in the Neutrals were $21.94 \pm 1 \mu\text{g}/\text{mg DOC}$, but the surface water demonstrated a collective THM concentration of $141.6 \pm 4 \mu\text{g}/\text{mg DOC}$ (Chapter 5, 5.15a). Both ratios were similar, 0.39 and 0.42 respectively which was as previously stated indicative of esters and carboxylic substituents. This tended to suggest that the Neutral fraction which was isolated during fractionation was less heterogeneous than the Raw Surface water and had a limited activated aromatic content which included the more hydrophilic structures.

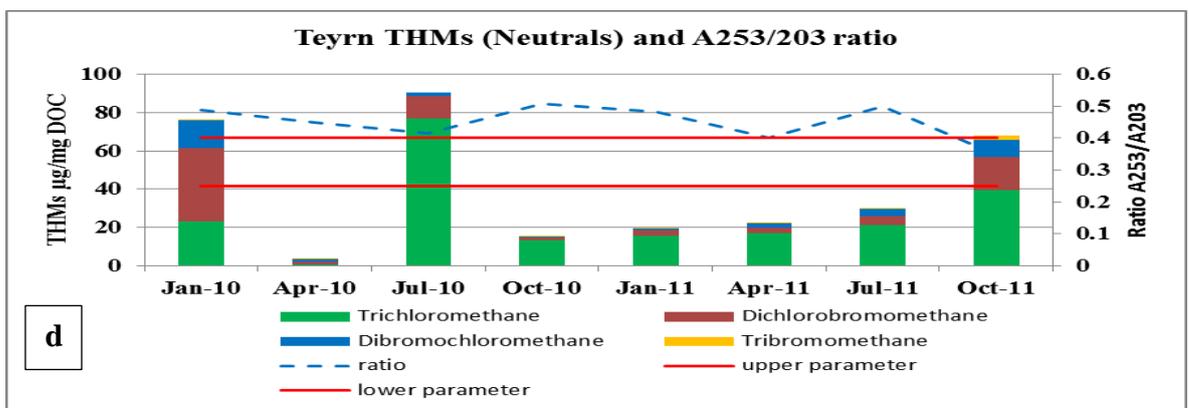
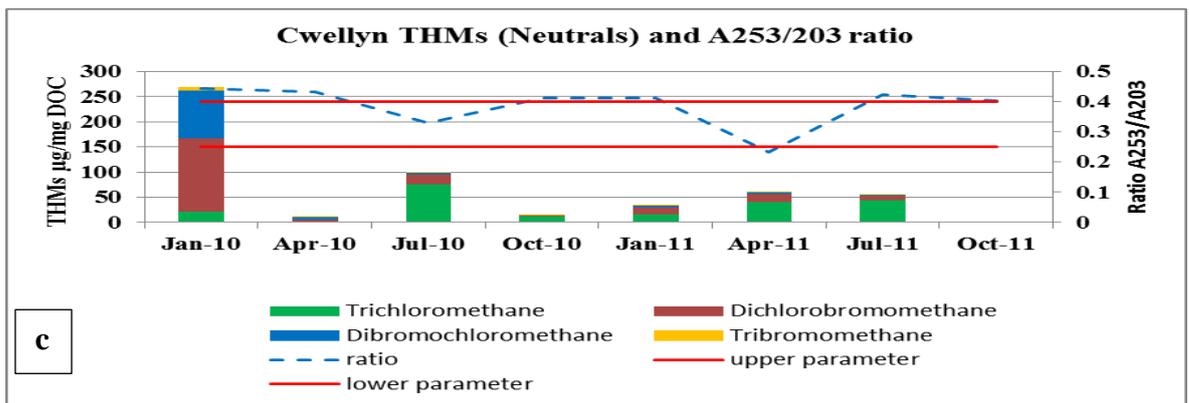
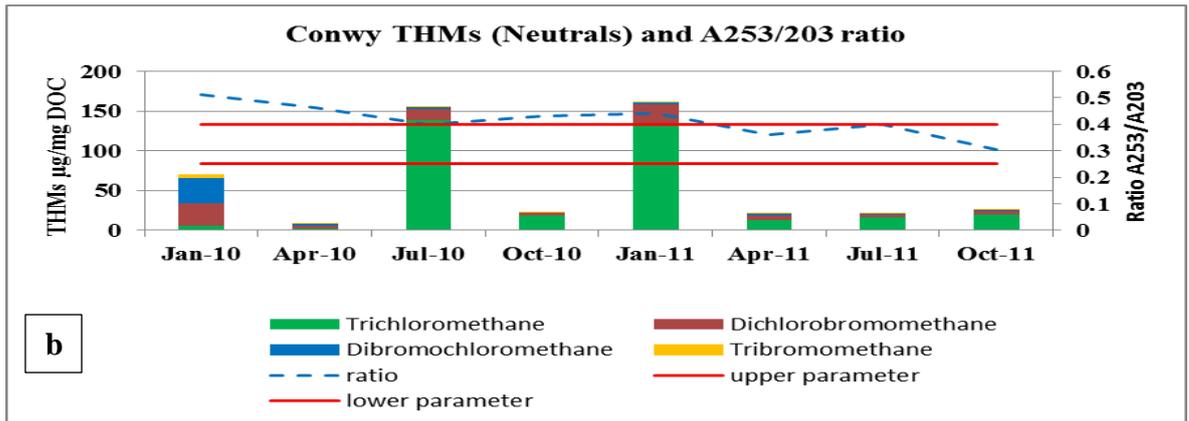
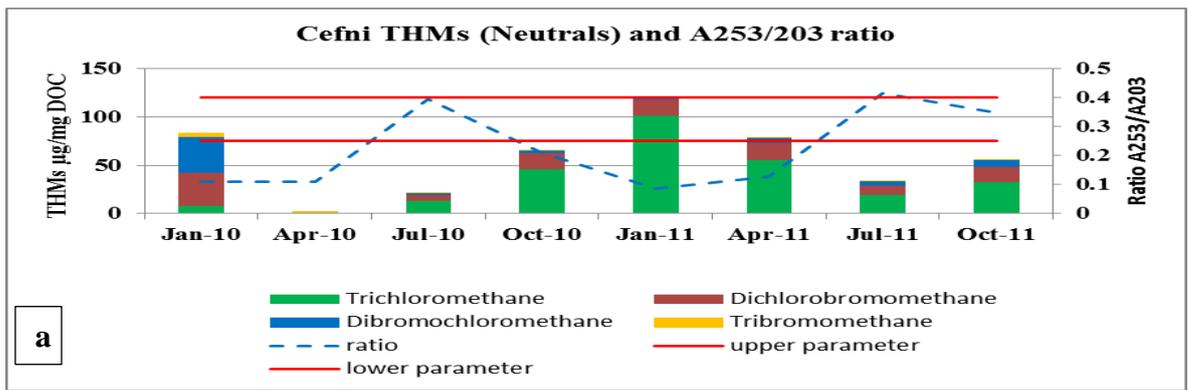


Figure 7.10 a) Cefni, b) Conwy c) Cwellyn and d) Teyrn cumulative THMs formed by the chlorination of DOC Neutrals fraction, showing the ratio product of (A_{253}/A_{203}) relative to the upper and lower parameters 0.25 and 0.40 suggested to represent substituted benzene (Korshin et al., 1996).

In January 2011 the Neutrals Cefni ratio was low (< 0.25) and this correlated with a high cumulative THM concentration (Jan 2011, ratio 0.1, THMs $119.0 \pm 3.5 \mu\text{g}/\text{mg DOC}$). The three upland lakes did not demonstrate the extreme variations in the ratio's that were recorded in the Cefni Neutrals, but the Cwellyn and Teyrn, like the Cefni exhibited increases in cumulative THMs when the ratio dropped. Conwy's Neutral DOC demonstrated the opposite of this with the THMs high ($154.1 \pm 3 \mu\text{g}/\text{mg DOC}$) with the ratio > 0.4 and tending to drop when the ratio reduced, but there was no consistency, for the ratio was > 0.04 for April 2010 and October 2010 and total THMs were 8.3 ± 0.2 and $22.8 \pm 0.5 \mu\text{g}/\text{mg DOC}$.

The HPOB fractions (Fig 7.11) A_{253}/A_{203} ratio for all the four lake DOC' was centered about the 0.4 upper parameter indicating a high level of ester/ carboxyl ring substituents. In the group a spike was seen in the January 2011 ratio which complemented an increase in the Conwy's cumulative THMs, but to a lesser extent in the Cefni and Teyrn results. The Cwellyn did the opposite, the increase corresponded with a drop in cumulative THMs.

The HPIA fraction (A_{253}/A_{203}) ratio results (Fig 7.12) were again closely related to the 0.4 upper parameter, but when compared to the HPOB (Fig 7.11) the ratio spike recorded for January 2011 was less distinct and on average lower for Cefni (HPOB 0.44, HPIA 0.39) and Conwy (HPOB 0.49, HPIA 0.46), but for Cwellyn and Teyrn they were relatively similar for HPOB. Again with the exception of Cwellyn there was no distinct response to the ratio changes. Cwellyn's upward spike in the ratio for January 2011, recorded a drop in cumulative THMs from the October 2010 high of $185.6 \pm 5.6 \mu\text{g}/\text{mg DOC}$ to $12.3 \pm 0.4 \mu\text{g}/\text{mg DOC}$. Although the Conwy HPIA had a total THMFP of $9.1 \pm 0.3 \mu\text{g}/\text{mg DOC}$ for July 2011 the ratio remained above the 0.4 ratio parameter (Fig 7.12) at 0.46 while in October 2011 the collective THMFP was $153.9 \pm 4.6 \mu\text{g}/\text{mg DOC}$ and the ratio was recorded slightly higher than the July 2011 one at 0.48.

Figure 7.13 shows the four graphed results of the A_{253}/A_{203} ratio of each HPIB fraction, of DOC over the sampling period plus the cumulative THMFP concentrations. Again the ratio products were in the 0.4 region and above. In January 2011 the ratio spiked upwards in all four samples, but relative movements in the THMFP did not correspondingly change. The feature that was very obvious was the generation of CHCl_2Br in all the January and April 2010 HPIB fractions due to the proposed storage effect. The ratio for the two months, were relatively high for all four HPIB samples, hence suggesting that ester/carboxylic functional groups were present.

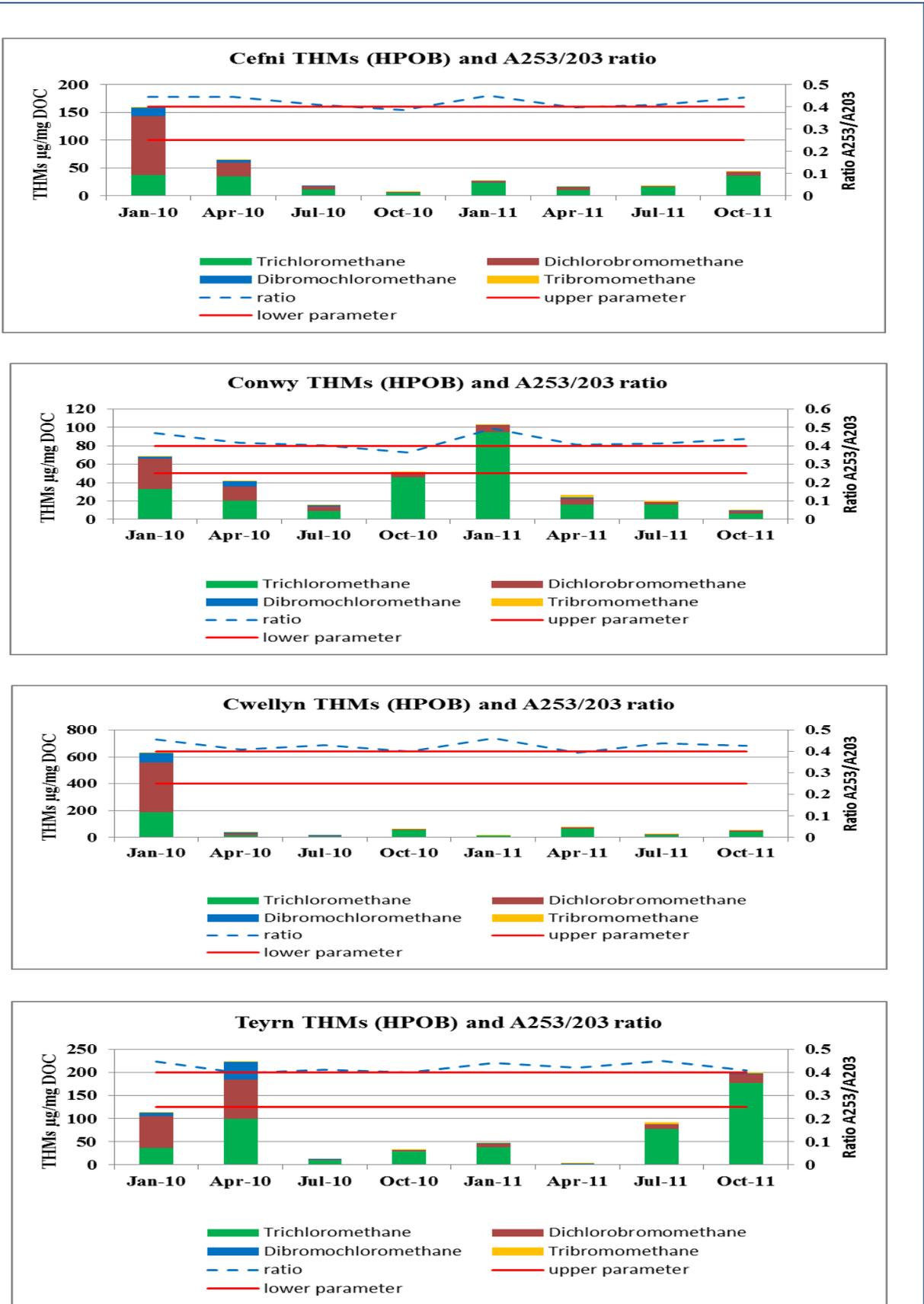


Figure 7.11 Cefni, Cwellyn, Conwy and Teyrn cumulative THMs formed by the chlorination of DOC fraction HPOB, showing the ratio product of A_{253}/A_{203} relative to the upper and lower parameters 0.25 and 0.40 suggested to represent substituted benzene (Korshin *et al.* 1996)

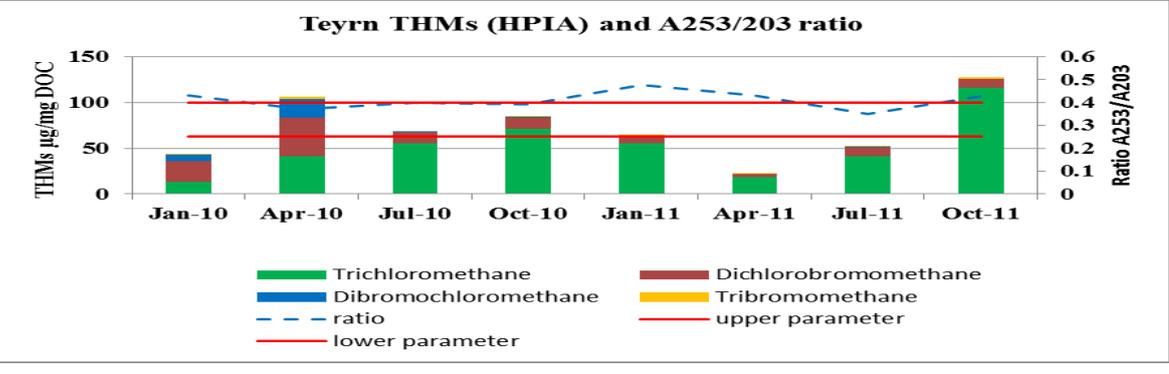
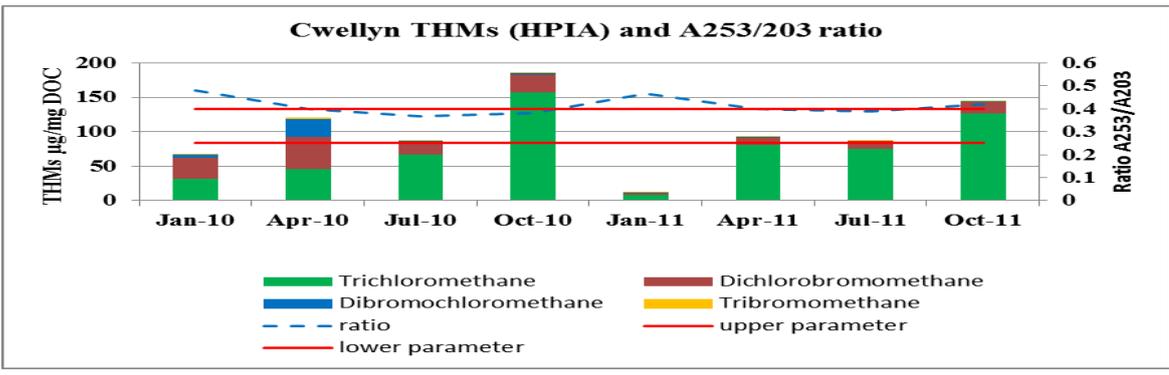
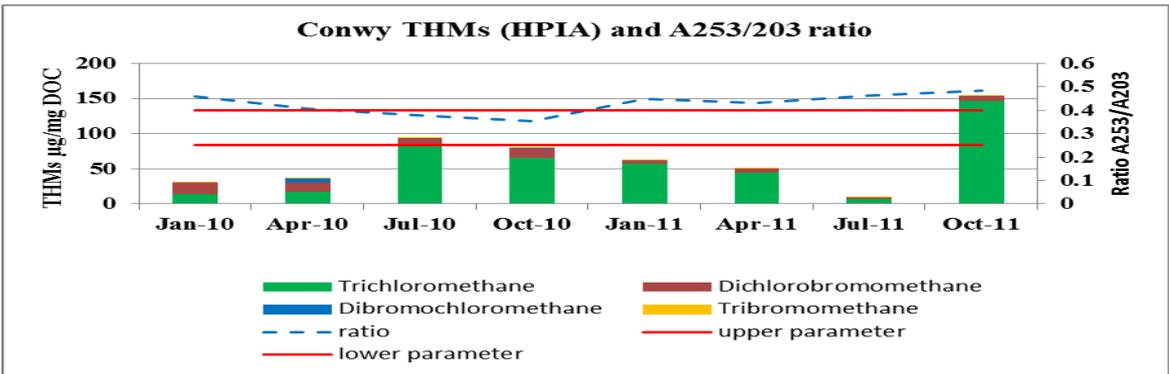
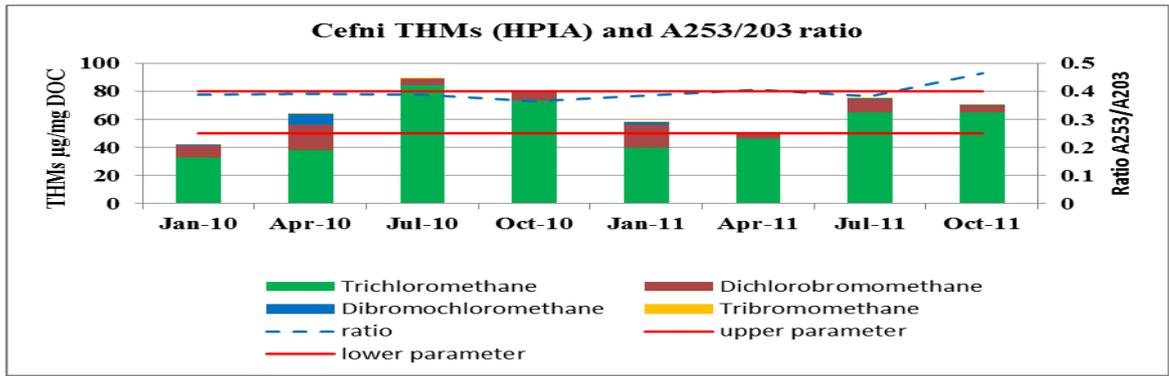


Figure 7.12 Cefni, Cwellyn, Conwy and Teyrn cumulative THMs formed by the chlorination of DOC fraction HPIA, showing the ratio product of A_{253}/A_{203} relative to the upper and lower parameters 0.25 and 0.40 suggested to represent substituted benzene (Korshin *et al.* 1996).

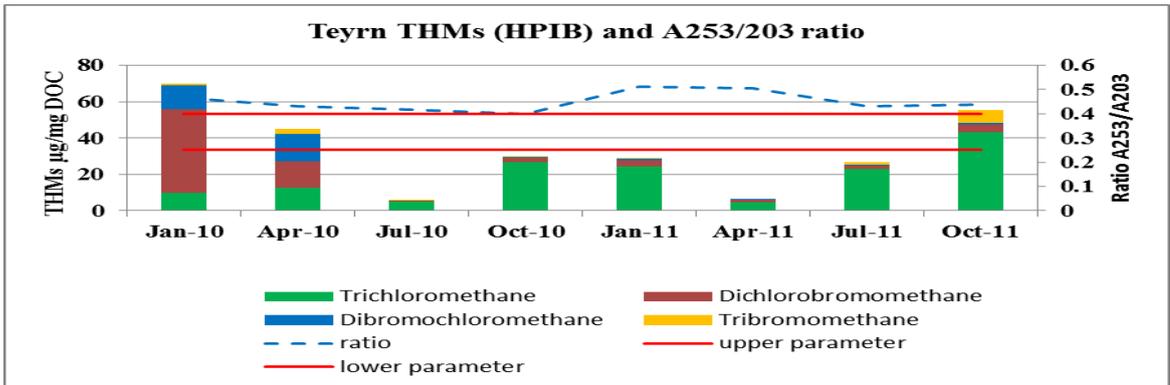
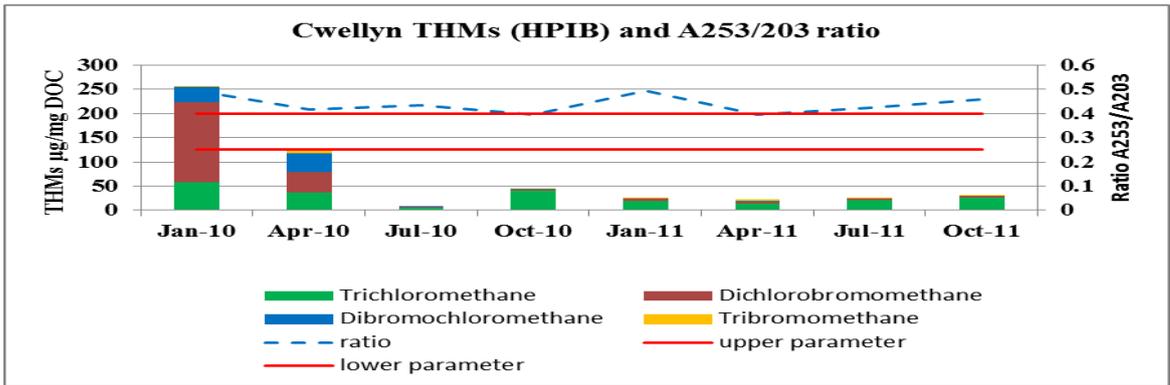
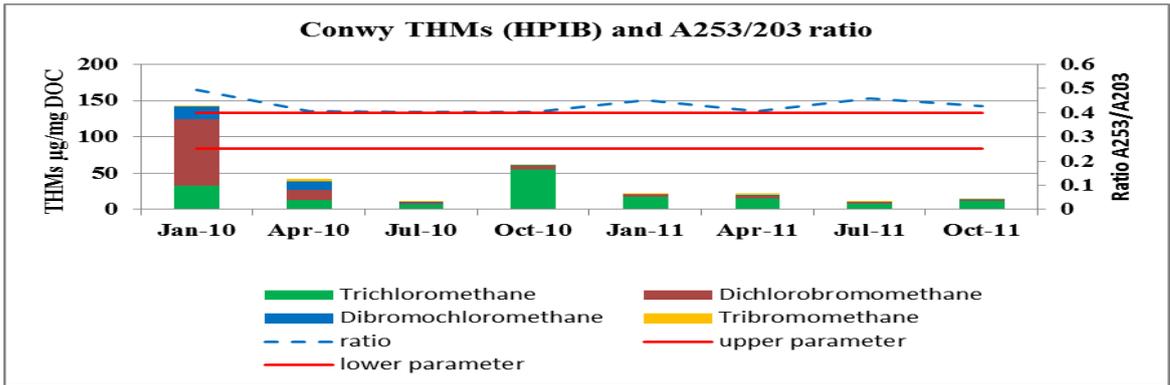
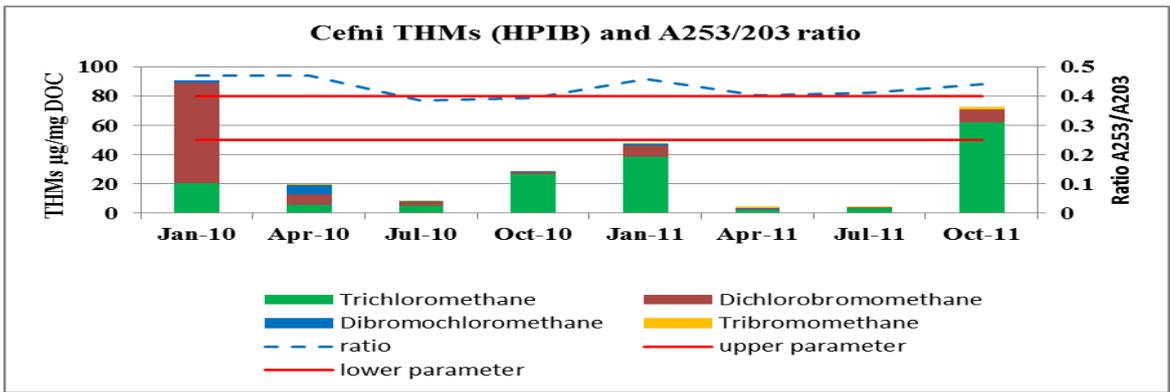


Figure 7.13 Cefni, Cwellyn, Conwy and Teyrn cumulative THMs formed by the chlorination of DOC fraction HPOA, showing the ratio product of A_{253}/A_{203} relative to the upper and lower parameters 0.25 and 0.40 suggested to represent substituted benzene (Korshin *et al.* 1996).

With the suggestion that the ratio represented aromatic ring functional group substituents, statistical analysis was carried out to determine if any lake to lake DOC (A_{253}/A_{203}) ratio correlations could be established to demonstrate that the aromatics being exported out of each of the catchments into the lakes had common properties. From these data the Cefni ratio did not demonstrate any significant correlations (Table 7.8) with any of the three upland lakes, but the Conwy, Cwellyn and Teyrn exhibited significant correlations with each other, with Conwy and Cwellyn having the most significant (r 0.608, $p < 0.01$).

DOC fraction source	Cefni	Cwellyn	Conwy	Teyrn
Cefni correlation (Pearson)	1	0.296	0.011	-0.094
Cwellyn correlation (Pearson)	0.296	1	0.608**	0.448**
Conwy correlation (Pearson)	0.011	0.608**	1	0.462**
Teyrn correlation (Pearson)	-0.094	0.448**	0.462**	1

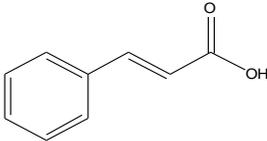
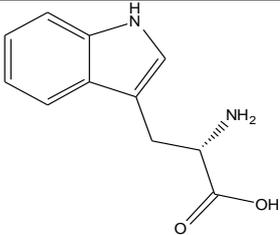
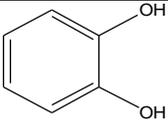
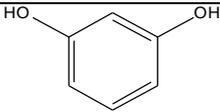
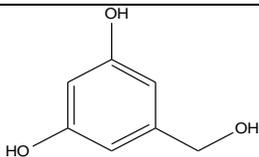
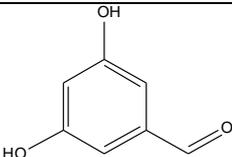
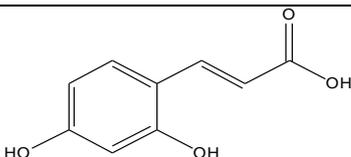
** . Correlation is significant at the 0.01 level (2-tailed).

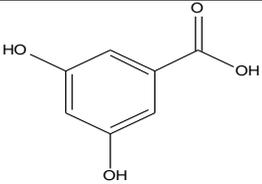
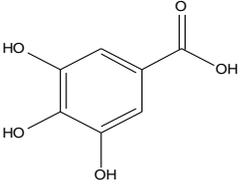
This suggested that the upland lake DOC was represented by similar aromatic substitutions with the changes from the high ratio (≥ 0.4) representing carbonyls, esters and carboxylates to the lower ratio (≤ 0.25) representing greater hydroxyl substitution were relatively synchronised. Although the Cefni demonstrated large changes in the ratio, especially in the HPOA and Neutral fractions together with the absence of correlations with any of the other lakes suggested that the processing of DOC molecular structures within the fen/agricultural catchment were unique and isolated from the upland processes

7.5 Model compounds

To illustrate the effect of substituted functional groups on the aromatic ring's susceptibility to chlorination, a series of reactions were carried out by dissolving each compound listed (Table 7.9) in Milli Q water, adjusting the DOC concentration to represent 1.0 ± 0.15 mg/L and chlorinating and analysing for THMs as per methods 2.3.7 and 2.3.8. The range of compounds were deemed to be potential THM precursors and considered to be representative of some surface water DOC molecular structures (Rook. 1977; Westerhoff *et al.* 2004; Bond *et al.* 2009).

Table 7.9 A series of model compounds chlorinated to establish functional group influence on THM formation over a 7 day period. Each chlorinated compound was analysed for the formation of CHCl_3 at + 2, + 96 and + 168 hours after chlorination

Compound	+ 2 hours ($\mu\text{g/L}$ of CHCl_3)	+ 96 hours ($\mu\text{g/L}$ of CHCl_3)	+168 hours ($\mu\text{g/L}$ of CHCl_3)
 Cinnamic acid	None detected	10	12
 tryptophan	< 1	21	20
 1,2 dihydroxybenzene	1	13	6
 3,5 dihydroxybenzene (resorcinol)	1025	1319	1218
 3,5 dihydroxybenzyl alcohol	743	711	751
 3,5 dihydroxybenzaldehyde	118	91	180
 2,4 dihydroxycinnamic acid	719	829	833

 <p>3,5 dihydroxybenzoic acid</p>	669	514	476
 <p>3,4,5 trihydroxybenzoic acid (gallic acid)</p>	Non detected	< 1	< 1

Hypochlorous acid's (HOCl) structure and bond polarization ($\text{Cl}^{\delta+} - \text{OH}^{\delta-}$) demonstrates three types of reaction; a) oxidation reactions b) addition reactions to unsaturated bonds and c) electrophilic substitution reactions at nucleophilic sites (Deborde and von Gunten, 2008).

The compounds selected for the exercise, included a series of functional groups in different positions on the benzene ring to investigate and identify their influence on the reactivity of the ring during chlorination.

The effect of the OH groups on the benzene ring was demonstrated by a number of the model compounds. When the OHs were on adjacent carbons (1,2 dihydroxy benzene) the formation of CHCl_3 was poor (averaged 7 $\mu\text{g/L}$ over 7 days), but if the positions of the two hydroxyls were separated by one carbon as in 3,5 dihydroxy benzene, the rate of reaction demonstrated a CHCl_3 FP of 1025 $\mu\text{g/L}$ over 2 hours.

When the two OH were *meta* to COOH, CHO and CHOH reactivity was reduced by the electron withdrawing effect of the functional groups. For two OHs *meta* to COOH as in 3,5 dihydroxy benzoic acid the rate of reaction for CHCl_3 formation was lower at 669 $\mu\text{g/L}$ over 2 hours, replacing the COOH with the CHOH group, the rate over 2 hours was faster at 743 $\mu\text{g/L}$. With the dihydroxyls *meta* to a CHO the CHCl_3 formation was very much reduced (2 hours : 118 $\mu\text{g/L}$). The inclusion of a third OH between the two *meta* OHs as in gallic acid (3,4,5 trihydroxybenzoic acid) deactivated the aromatic ring and the CHCl_3 yield was less than 1 $\mu\text{g/L}$ over the 7 day incubation. The reactivity of cinnamic acid and tryptophan were also examined over a 7 day period and the results demonstrated low yields of CHCl_3 over the 7 days (average 10 $\mu\text{g/L}$). The same experiment was carried out with a 2,4 dihydroxycinnamic acid and the formation of CHCl_3 over 7 days averaged 794 $\mu\text{g/L}$ (719 $\mu\text{g/L}$ over 2 hours) . Hence, indicating the pronounced activation of the aromatic ring at C3 by the two OHs.

7.6 THMFP and Molecular Mass (MM)

Chang *et al.* (2001) and Oliver and Visser, (1980) reported a relationship between molecular masses (MM) had THMFP. Oliver and Viser concluded that the major CHCl_3 precursors were low MM ($< 30,000$ Da) fulvic acid fractions, whilst Chang *et al.* reported that the highest THMFP was by the (< 1000 Da) MM and was found to decrease with increasing MM.

Exploration of the molecular mass (MM) distribution data of the fractions HPOA, HPOB, HPIA, HPIB and Neutrals relative to the THM components CHCl_3 , CHCl_2Br , CHBr_2Cl and CHBr_3 did not demonstrate any significant correlations. Figure 7.15 demonstrated the typical relationship of the percentage molecular mass present (2 – 1 kDa) of the HPOA relative to the concentration of the THM component CHCl_3 for each month sampled. This range and THM (CHCl_3) were chosen as it represented the lowest MM distribution within the apparent MM standards and also the principal THM formed during chlorination.

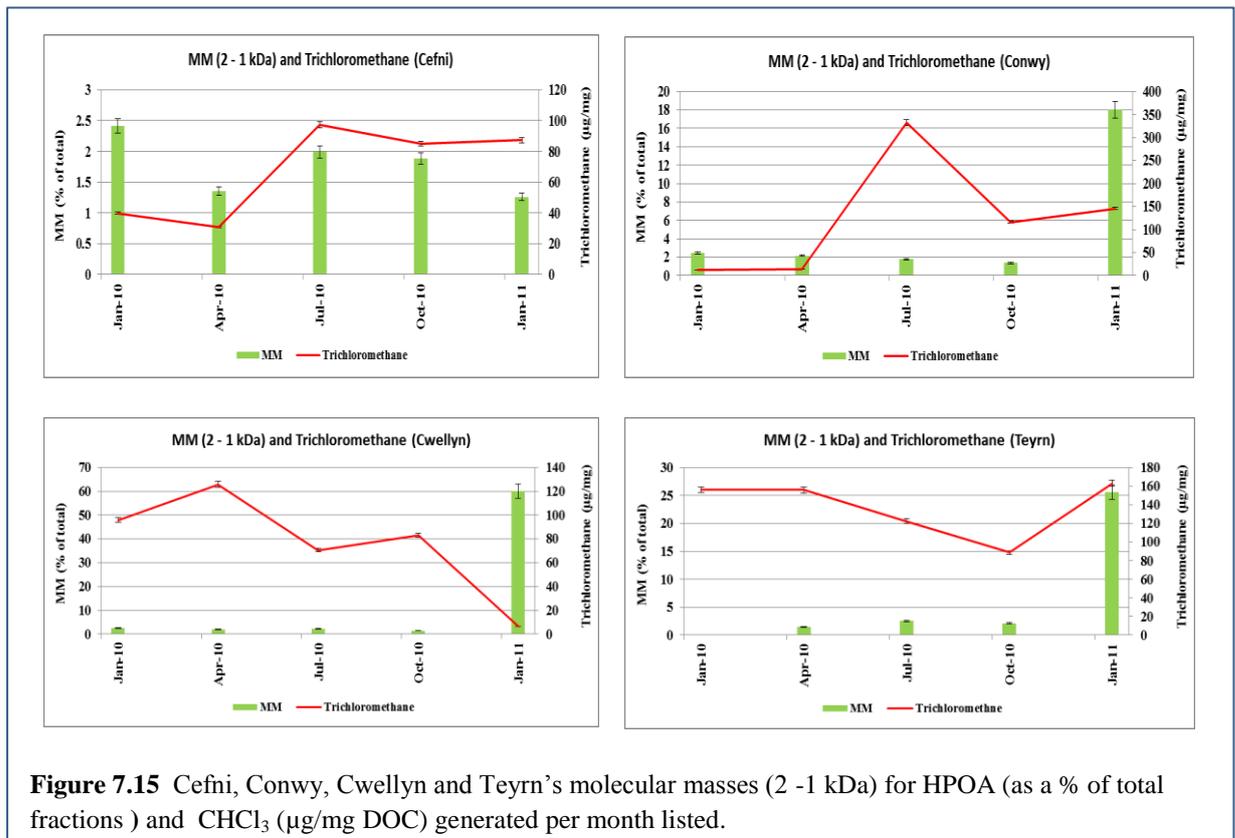


Figure 7.15 Cefni, Conwy, Cwellyn and Teyrn's molecular masses (2 -1 kDa) for HPOA (as a % of total fractions) and CHCl_3 ($\mu\text{g}/\text{mg}$ DOC) generated per month listed.

Although there appeared to be a relationship between the changes in the percentage MM during April 2010 to October 2010 and the concentration of CHCl_3 for the Cefni, this was not apparent for the Conwy, Cwellyn and Teyrn results.

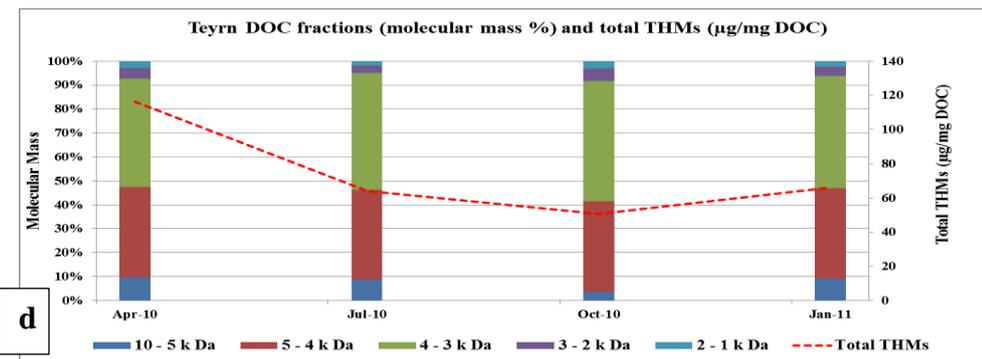
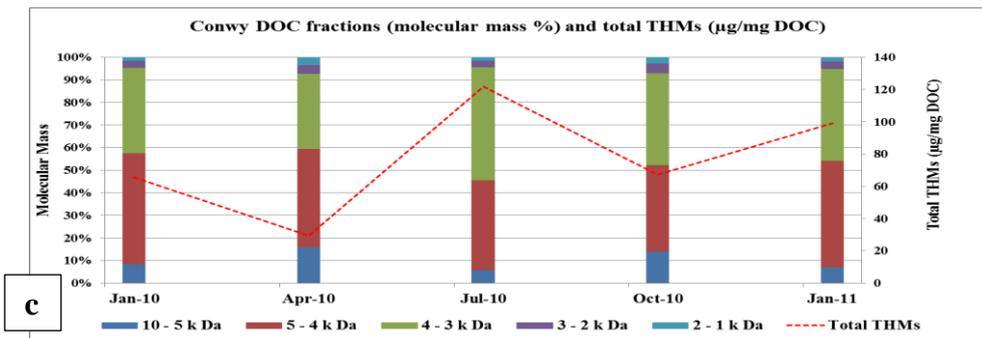
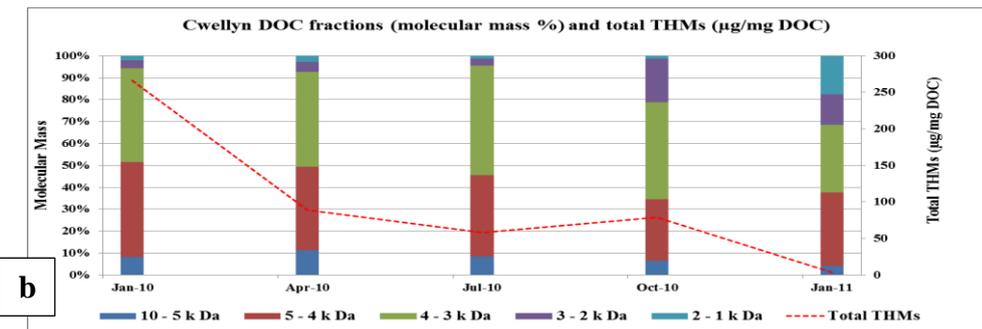
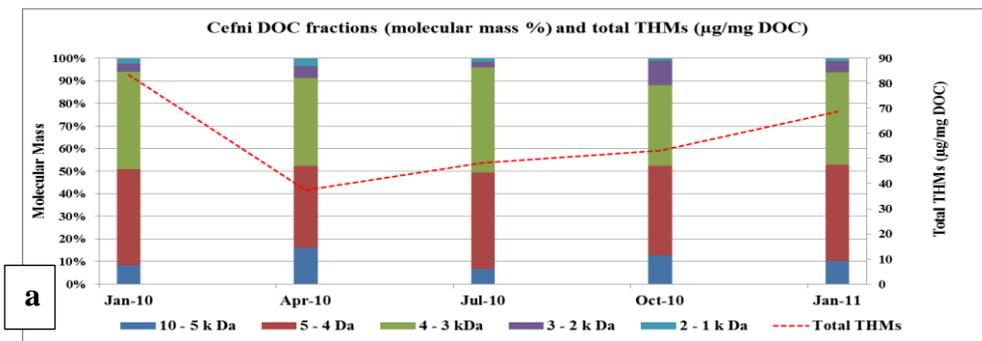


Figure 7.17 a) Cefni, b) Cwellyn, c) Conwy and d) Teyrn representing the MM ranges (%) for each complete fraction set (HPOA, HPOB, HPIB, HPOB and Neutrals) per month of the Raw Surface water with total THMs for each suite of fractionated DOC.

Examples of this relationship were observed in the January 2011 series. The Cefni concentration of CHCl_3 increased and the percentage MM decreased relative to each other, but the Conwy's MM percentage increased and so did the CHCl_3 concentration. The Cwellyn's HPOA molecular mass percentage for January 2011 relative to the October 2010 level was higher, but the CHCl_3 concentration dropped from, 82.8 ± 2.5 to 6.5 ± 0.2 $\mu\text{g}/\text{mg}$ DOC. Teyrn demonstrated the opposite response, with both the MM percentage increasing and the CHCl_3 concentration increasing in January 2011 (MM 25.9 %, CHCl_3 63.1 ± 1.3 $\mu\text{g}/\text{mg}$ DOC) relative to October 2010 (MM 2.2 %, CHCl_3 53.7 ± 1.1 $\mu\text{g}/\text{mg}$ DOC)

In Figure 7.17 a,b,c and d the molecular mass of the individual fraction ranges have been summed and each has been illustrated as a percentage of the complete DOC suite. The red broken line represents the total THM concentrations for all the DOC fractions together. A clear distinct statistical and visual relationship could not be determined between any of the ranges and summed THMs. This was also found in the Raw Surface Water THM and MM ranges (Chap 5, 5.1.7).

7.7 Discussion

The formation of THMs has been reported to be first order in respect to soluble DOC and that their production can be attributed to the humic acid fraction of the DOC rather than the fulvic acid (Babcock and Singer, 1979).

The analytical procedure termed the 'DOC fractionation analysis' was developed by Leenheer and reported in the literature in 1981. The procedure fractionated and classified the DOC into a series based on their resin adsorption characteristics which defined the physio-chemical properties of the fractions, although Peuravuori *et al.* (1998) initially suggested that the humic water solutes, although from different sources may be altered in such a way by the method of fractionation that their properties may be artificially generated. (Peuravuori *et al.* 2005) later reported that base-catalyzed ester hydrolysis that may take place during isolation of the HPOA and HPIA fractions was very limited and hence, did not affect THMFP.

Each fraction within the complete suite representing a lake fractioned Raw Surface Water samples demonstrated a unique THMFP. For example, (Table 7.10) the HPOA fraction exhibited a high THMFP in the Cefni, Conwy and Teyrn for CHCl_3 , while the Cwellyn's principal formation of CHCl_3 was less defined, with the highest THMFP by the HPOA for

50 % of the samples and the other 50 % by the remaining hydrophilics and neutral fractions during the period January 2010 to October 2011.

Conwy and Teyrn's principal THM generators were the hydrophobic fractions (HPOA and HPOB). Tables 7.2 and 7.3 exhibited the averaged results (January 2010 – October 2011) for the brominated components CHCl_2Br and CHBr_2Cl expressed as the percentages generated by each fraction. From the tabulated results it can be clearly seen that the HPOA fraction generate the lowest concentrations of these components with the exception of Teyrn, which had a different THMFP profile to the other three lakes. That is, the formation potential (FP) of HPOA, HPOB and HPIA were the highest for CHBrCl_2 , but for Cefni Conwy and Cwellyn the highest FP were demonstrated by the HPOB, HPIB and Neutrals. This was again noted for the FP of CHBr_2Cl with all five (HPOA, HPOB, HPIA, HPIB and Neutrals) DOC fractions from the Teyrn but with a much lower FP observed by its HPOA fraction. The highest concentrations of CHBr_2Cl were generated by the Neutral fractions in the other three lakes with HPOB having the second highest CHBr_2Cl FP for Cefni and Cwellyn. The second highest FP CHBr_2Cl for Conwy was recorded by the HPIB which in turn had the lowest FP for the Cefni. These results do not support the suggestion in the literature (Peuravuori *et al.* 1998; Aiken and Malcolm. 1986) that DOC molecular structures were regularised and altered during the isolation process. If this was so, with the chlorination concentration of DOC being 1 mg/L, the THMFP results for each fraction would have been regular and similar, hence they are considered to agree with Peuravuori *et al.* (2005), that changes, (e.g., hydrolysis of esters etc) are negligible during fractionation of DOC .

An interesting feature of this series of brominated fractioned samples were of the changes in the fraction's THMFP after being subjected to a delay in chlorination and analysis as mentioned in Chapter 5 (section 5.2). If the THMFP results for CHBrCl_2 relative to the January and April 2010 data are removed, the principal FP fraction switched from the HPOB to the HPIA fraction (Table 7.2) for the three upland lake DOC' (Cwellyn, Conwy and Teyrn) and to the Neutral fraction for the lowland Cefni DOC. It was also noted that the Neutral and HPOA formations of CHBrCl_2 at all four sites increased, with an accompanying reduction in the HPOB and HPIB formation potential. Again re-calculation, with the January and April 2010 data absent for the fractions with the THMFP for CHBr_2Cl also demonstrated changes (Table 7.3). The Neutral fraction increased its THMFP for the Cefni, Cwellyn and Teyrn fractions but remained similar for the Conwy. Further to the discussion about resin effects and their possible regularization of molecular structure by reaction with the desorption reagents, it was interesting to note that the

changes recorded in the Raw Surface Water THMFP were also reflected in the THMFP of the suites of fractions. Hence the surface water DOC was not modified when fractioned, but retained the molecular structures promoting the formation of brominated THMs in the January, April 2010 fraction suites.

Aromaticity and SUVA have previously been reported to be the principal indicators of aromatic moieties in the DOC (Leenheer and Croue, 2003), a correlation exercise carried out on the SUVA and Aromaticity data generated by the fractions showed a significant relationship between the two for the HPOA fraction ($r = 0.626$, $p < 0.01$) and a weaker one for the Neutral fractions ($r = 0.381$, $p < 0.05$). Although researchers report significant correlations for THM formation with SUVA and Aromaticity (Lu *et al.* 2009; Kim and Yu. 2007; Reckhow *et al.* 1990) for samples of treated lake water (coagulation/filtration) and a natural river and lake water, the data in this thesis does not support their findings. The significant correlation coefficients reported in Tables 7.4 and 7.5 were poor, with only 3 correlations of significance out of twenty results for the THMFP *versus* Aromaticity. For SUVA *versus* THMFP only 4 out of 12 results demonstrated correlations. Our results tend to agree with Ates *et al.* (2007) and Weishaar *et al.* (2003) that SUVA identifies a general characteristic of a pool of molecules and that the compositional characteristics that demonstrate reactivity towards chlorine are not directly related to the SUVA value. Weishaar (2003) suggested that there was also present, heterogeneous material such as β -ketones, pyroles (chlorophyll) and proteins that do not absorb at UV 254 nm which can act as THM precursors.

The ratio, A_{253}/A_{203} is again one of the proposed spectroscopic methods that is considered to be a surrogate for THM formation (Kim and Yu, 2007). They suggested that it may be a better indicator than the SUVA which depends on one wavelength rather than two. The Raw Surface Water fractions having been isolated by resin desorption rather than ion exchange were relatively low in inorganic interference (Aiken *et al.* 1992) at the shorter wavelengths (200 – 230 nm). The inclusion of inorganics in the sample are believed to introduce errors in the ratio (Matilainen *et al.* 2011), but with only trace levels of inorganics in the fractions analysed, their consideration as discussed in Chapter 6 (6.2.4) could be ignored for all the fractions with the exception of the Neutrals. These demonstrated the highest concentrations of inorganics, and the NO_3^- concentrations appeared to increase the absorbance in the 200 - 230 nm region.

The investigation of the ratio, highlighted the variations in the aromatic substitutions of the fractioned sample DOCs. The HPOA which has been reported to have the highest concentration of aromatics (Croue, 2004) demonstrated the largest variation in the

(A_{253}/A_{203}) ratio. Of particular interest was the dip recorded during July 2010 following a dry spell. Here the (A_{253}/A_{203}) ratio approached 0.25 in all of the four lake HPOAs. This was indicative of ring substitution being predominantly by the OH functional group (Korshin *et al.* 1996). This dip supported the Scott *et al.* (2001) proposal that, after a dry period, there can be lower carboxyl groups in humic substances due to re-wetting and the oxygenated peat becoming gradually anoxic. For Cefni and Conwy the July 2010 total concentration of THMs (Fig 7.9) increased relative to that of April 2010, but Cwellyn and Teyrn did the opposite and decreased in July 2010, from the April 2010 level. It could be proposed that the reason for these differences was due to the catchment profiles. For example, both Cefni and Conwy with deep areas of peat of an anoxic nature were subjected to water table drops together with an accompanying oxygen influx, which would promote an increase in microbial activity (Freeman *et al.* 2001; Fenner *et al.* 2005). Cwellyn and Teyrn, alternatively, having relatively thin peat layers on steep slopes as their principal catchments would have experienced larger areas of desiccated peat with little microbial activity. The first rainfall after the dry period could have passed rapidly through this, i.e., ‘the thatched roof effect’ described by Zaslarsky and Sinai (1981). Thus upon arriving in the lake incoming water would have had a diluting effect but, more importantly, a lower incidence of flushed microbially modified DOC. The other extreme weather event observed during the sampling period was in January 2011 when below average temperatures were experienced in all the four catchments. The (A_{253}/A_{203}) ratio for the HPOA for the January 2011 series increased in all four samples to above the 0.4 parameter which has been reported to be associated with esters and carboxylic functional groups (Korshin *et al.* 1996). The Cwellyn collective THMs were seen to drop compared to Cefni, Conwy and Teyrn’s. This could not be explained relative to its ratio, phenolics or aromaticity values as they did not demonstrate any obvious changes compared to the other lakes, but the THMFP suggested that the Cwellyn DOC exhibited different properties to that of the other lakes. The exercise using model compounds (Sec 7.1.4) demonstrated the importance of the position of the functional groups on the rings and their capability to activate or de-activate. Hence it is suggested that the Cwellyn’s DOC microbial processes had been limited during the cold event, resulting in the DOC degradation not generating molecular structures with active sites.

Evidence of changes in the molecular mass (MM) distributions were apparent in the Cwellyn’s fractionated series for January 2011. Both the MM range 3000 – 2000 and 2000 - 1000 Da increased by 13 and 17% respectively. This suggested that the 3000 – 1000 Da range of molecular structures may have limited THMFP, this observation was also reported

by (Kim *et al.* (2007)). The change exhibited by Cwellyn's MM for January 2011 was not recorded in the other three lakes and their dominant major MM range remained centred on 5000 - 3000 Da, neither was a drop in accumulated THMs recorded. The small step change in the distribution ranges of Cwellyn's MM, was demonstrated by an increase of \approx 20% in the 10000 – 5000 Da range. This could not be explained, other than it may be as a result of the impact of low temperatures, ice and snow on the microbial community. Chow *et al.* (2003) demonstrated the biotic effects on the THMFP when the micro-organism environment is artificially changed in the laboratory. Although Chow's experiment did not reflect the temperatures recorded for the catchments (Fig 3.15) these workers reported that the THMFP responded for both anaerobic and aerobic samples when water content and temperatures were varied over an eight week period. With Cwellyn's catchment having thin peaty soil the oxidized upper peat layer may have been subjected to freezing, hence reducing water flow, microbe activity and DOC export, altering the DOC concentration and the mixing dynamics in the deep lake.

7.8 Conclusions

- The principal THM component formed is CHCl_3 with HPOA as the major fraction responsible, but other fractions also exhibited a THMFP for CHCl_3 on occasions. For example, the Neutrals for the Cefni and Cwellyn, with the HPOB fraction for Conwy, Teyrn and Cwellyn
- The highest formation of CHCl_2Br on average ($> 30\%$) was by the HPOB fraction of Cefni, Cwellyn and Teyrn, but the Conwy HPOB averaged ($< 30\%$) FP.
- The Neutrals also demonstrated a high average CHCl_2Br FP, with $> 20\%$ formation. Teyrn's Neutrals were the exception and had an average FP of $< 20\%$.

When the January and April 2010 results (their FP was considered to be influenced by the period of storage) were not included in the survey of fraction FPs, the principal FP fraction for CHCl_2Br exchanged and were as follows:

- The HPIA fraction demonstrated the highest FP average for CHCl_2Br for Cwellyn and Teyrn.
- Cefni and Conwy's averaged FP for CHCl_2Br ($> 30\%$) was by the Neutrals.

The HPIA, HPOB, HPIB and principally the Neutral fractions demonstrated the highest FP for CHBr_2Cl for all the fractioned lake samples. Removal of the January and April 2010 results again exchanged the fraction FP results:

- The Cefni and Teyrn's Neutral fraction FP for CHBr_2Cl increased.
- Cwellyn and Conwy's Neutral fraction FP for CHBr_2Cl remained unchanged but the HPIA fraction FP increased.

The conclusion that can be drawn from the above results is that all the fractions react to halogenation, but chlorination reactions are more directed to the HPOA fraction, which are considered to have a higher aromatic content. The bromination reactions are biased to the more hydrophilic Neutrals, although the hydrophobic bases also react positively in the extended stored samples, tending to suggest that more nucleophilic sites are available for the polarizable HOBr.

- The fractioned samples also confirmed that the upland lake DOCs have greater overall FP compared to the lowland lake, with a difference of approximately 30%.
- Aromaticity and SUVA values demonstrated correlations with a limited number of fraction FP.
- Both SUVA and Aromaticity (E_2/E_3) correlated with the HPOA relative to CHCl_3 and CHCl_2Br ,
- The HPIA SUVA exhibited correlations with the THMs CHBr_2Cl and CHCl_2Br .
- The ratio A_{253}/A_{203} to determine the aromatic ring substitution suggested a relationship with the THMFP of the fractions, but did not express statistical correlations. The overall results for this ratio (A_{253}/A_{203}) as a technique for determining THMFP is inconclusive.
- Model compounds confirmed that the positions of the OH substitutions were and their electron donating effect influenced the formation of THMs. The electron withdrawing groups COOH and CHO were found to deactivate the aromatic rings relative to THM formation.
- Molecular mass distributions of the fractions relative to their THMFP did not demonstrate any correlations with THM formation.

Chapter 8

Conwy and Cefni Catchments, Rainfall Event.

Chapter 8 Conwy and Cefni Catchments, Rainfall Event.

8.1 Introduction.

When catchments are subjected to an extreme weather event such as extended dry periods followed by an intense period of rainfall, changes have been reported in the concentrations and properties of the DOC being exported (Clark *et al.* 2007; Zielinski *et al.* 2009). These are believed to impact the quality and hence the THMFP of the water entering the reservoirs (Worrall *et al.* 2008).

8.2 Site Locations and Monitoring

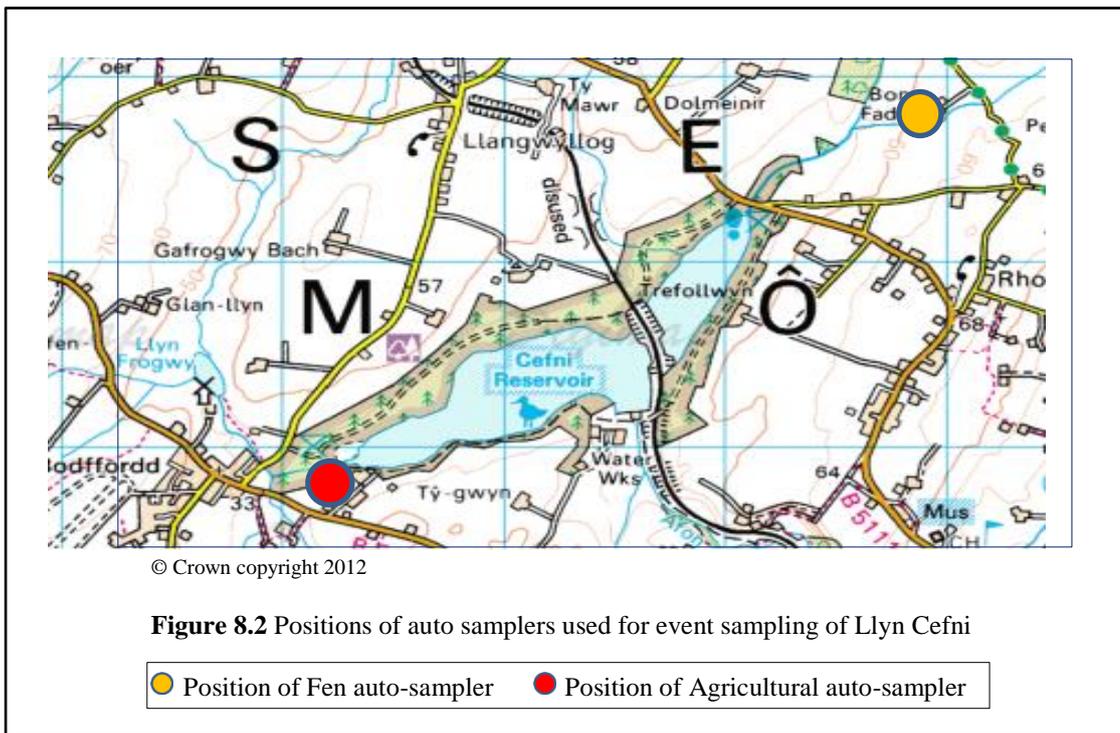
In Chapter 3 changes were observed in the DOC concentrations for August 2010, these were associated with the end of a relatively dry period spanning from the end of March 2010 to the beginning of July 2010. The DOC samples resulting from this rainfall increase on the Cefni, Cwellyn, Conwy and Teyrn catchments were recovered from the lakes during the routine monthly sampling. The samples collected did not accurately reflect a true flushing of the catchment due to the receiving lake's dilution effects and fixed sampling routine. Hence a planned monitoring of a rainfall event (intense period of rain) took place in May 2011 after another relatively long period of dry weather.

The sampling was carried out using four auto-samplers that could be programmed to collect 0.5 L of sample at 3.0 hourly intervals (Fig 8.1). The samplers were positioned at two stream inlet points for the Cefni and two streams, Nant y Brwyn and an unnamed one (upland mineral stream) at grid reference SH 76381 45870 for the Conwy catchment. These two streams were chosen because the Nant y Brwyn's (SH 79191 45565) source represented water run-offs of the Conwy blanket bog and the upland mineral stream (SH 76381 45870) was considered as a typical representative upland mineral stream that flowed into lakes such as Cwellyn and Teyrn. The two sampling units assigned to the Cefni were positioned to collect a series of a) fen inflow and b) agricultural inflow. The ones assigned to the Conwy were positioned to collect; a) upland peat flow b) mineral flow. Flow rates (m^3/s) of the lowland agricultural and upland peat streams were monitored by (CEH and DE)

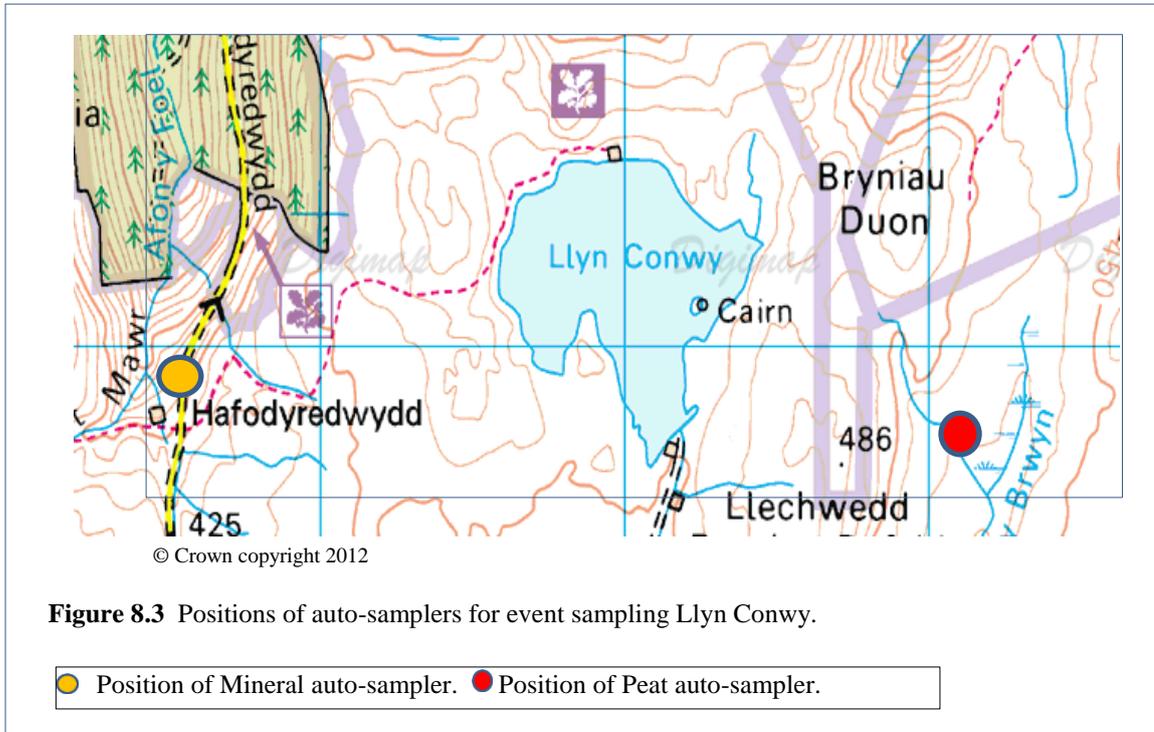


The monitoring was carried out for 69 hours during a period of rainfall between the 6th and 9th May 2011. Unfortunately the auto-sampler for the Cefni Fen catchment was damaged during sampling and these samples were lost.

8.2.1 Auto-sampler Locations



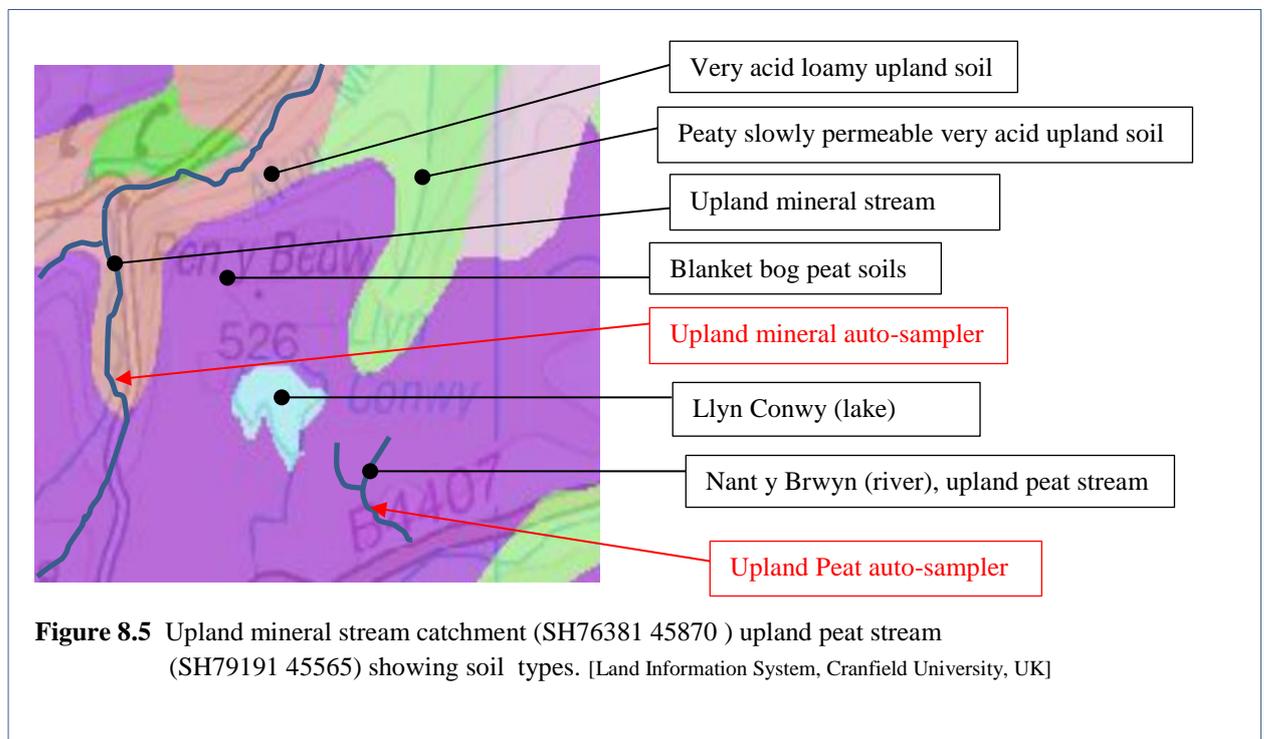
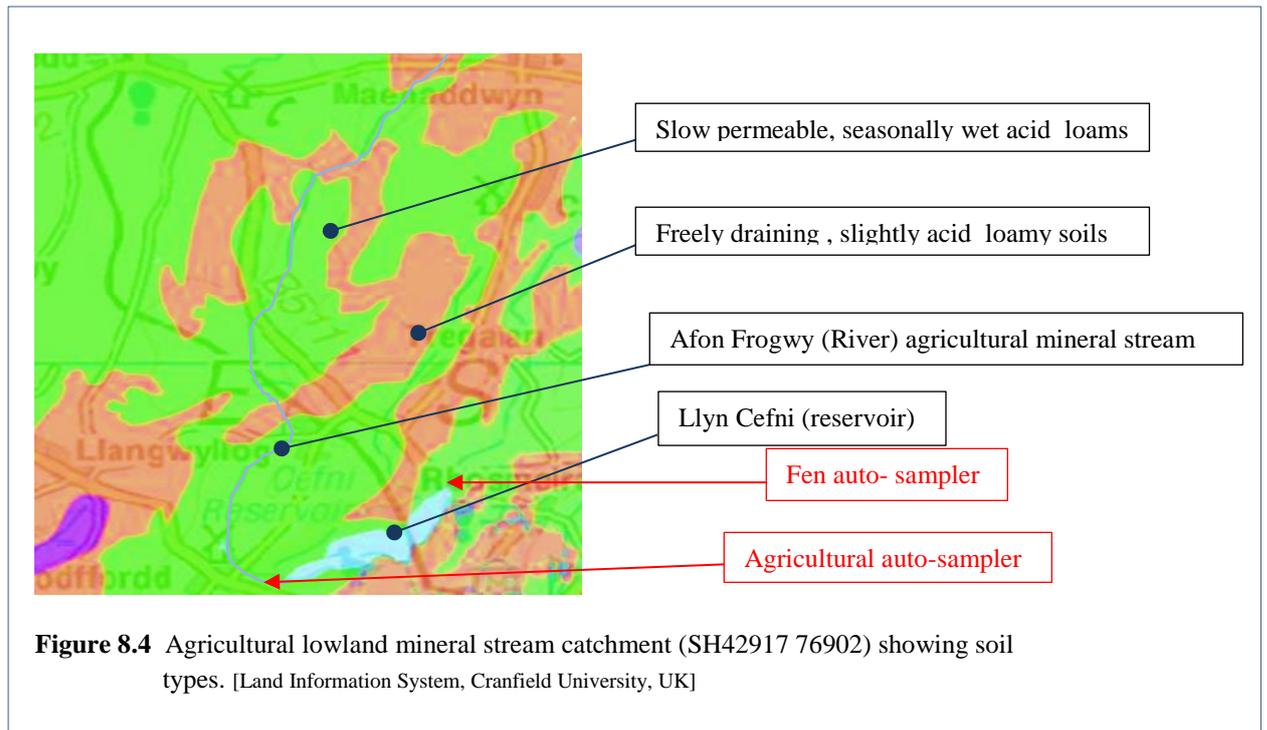
The Cefni Fen stream catchment sampler was placed at grid reference SH 46162 78832 and the Cefni Agricultural mineral stream catchment sampler was at grid reference SH 42917 76902. (see Fig 2.01 A)



The Conwy upland Mineral catchment sampler was placed at grid reference SH 76381 45870 and the Conwy Peat catchment sampler at SH 79191 45565. (see Fig 2.01 C).

8.2.2 Catchment Soil Types.

The soil types in the catchment areas chosen for monitoring varied from mineral loam to organic peat soils. This range of soil types was most prominent in the Cefni catchment, where to the west, Afon Frogwy enters the reservoir having passed through an area of a mixture of wet acid mineral coarse loamy soils (Avery, 1990) with anthropogenic inputs from agricultural processes that provide both inorganic and organic materials in the form of land treatments (fertilizer/liming etc) and animal waste products. At the east end of the Cefni reservoir, lies the Afon Erddreinig with a catchment consisting principally of a calcareous deep semi-fibrous peat (Avery, 1990). The upland Conwy samplers were placed in two regions, the Nant y Brwyn peat stream with the catchment consisting of organic peaty rankers and oligotrophic blanket peat (Avery, 1990). The soils of the Conwy mineral stream catchment consist of a range of soils from of acidic upland loamy to acidic peat.



8.2.3 Rainfall and Stream Flow results.

Over the monitoring period of 69 hours (h) during the 6th - 9th May 2011 the stream flow rates and cumulative rainfall data was collected (Figs 8.6 a,b) for the upland peat and

lowland agricultural stream (CEH and EW, 2011). The results show that the agricultural catchment (Fig 8.6 a) received the maximum rainfall (21.8 mm) between 03.00 h on the 7th May 2011 and 06.00 h on the 8th May 2011 after which only a further 2.8mm fell (Fig 8.6 a).

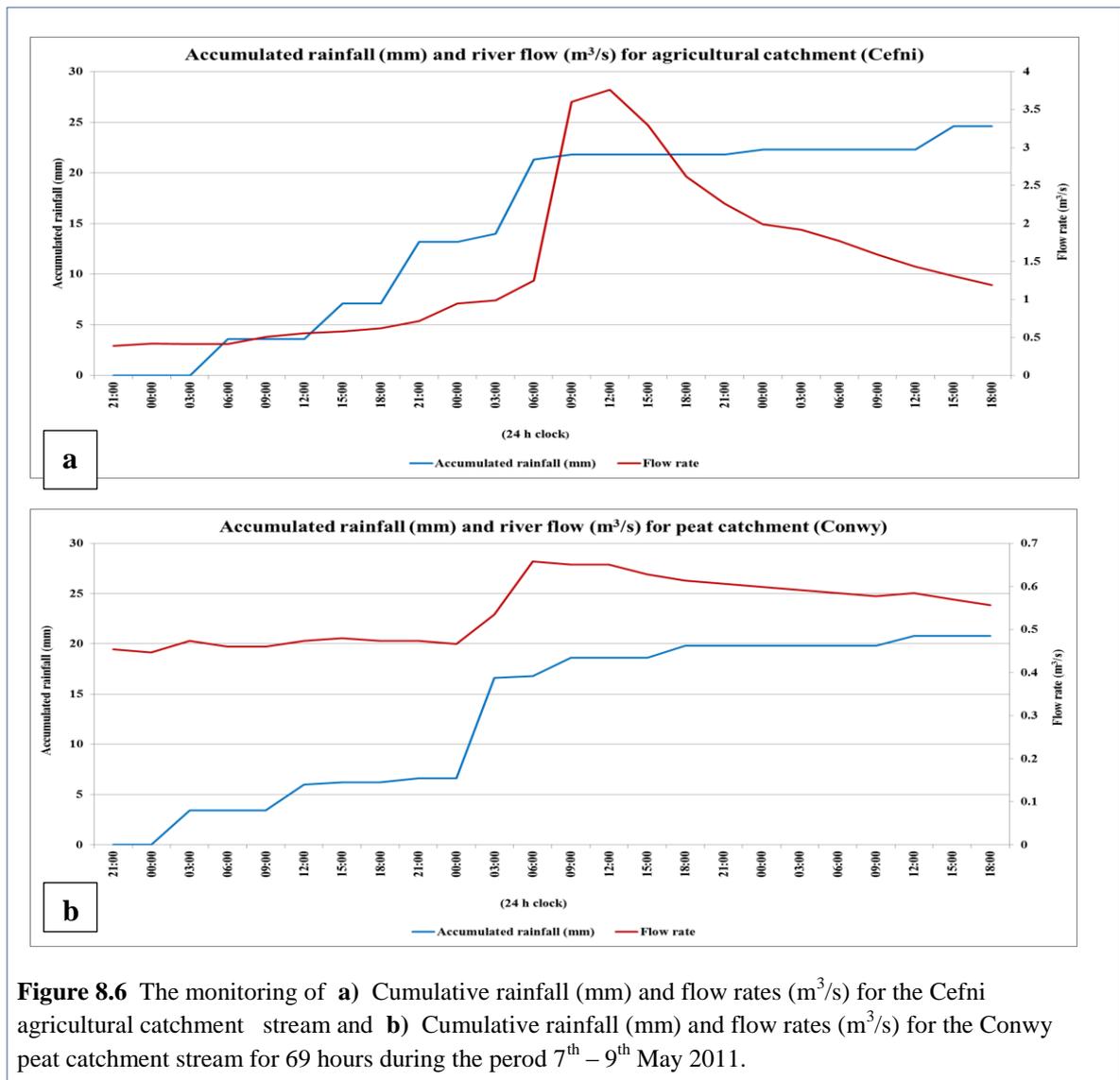
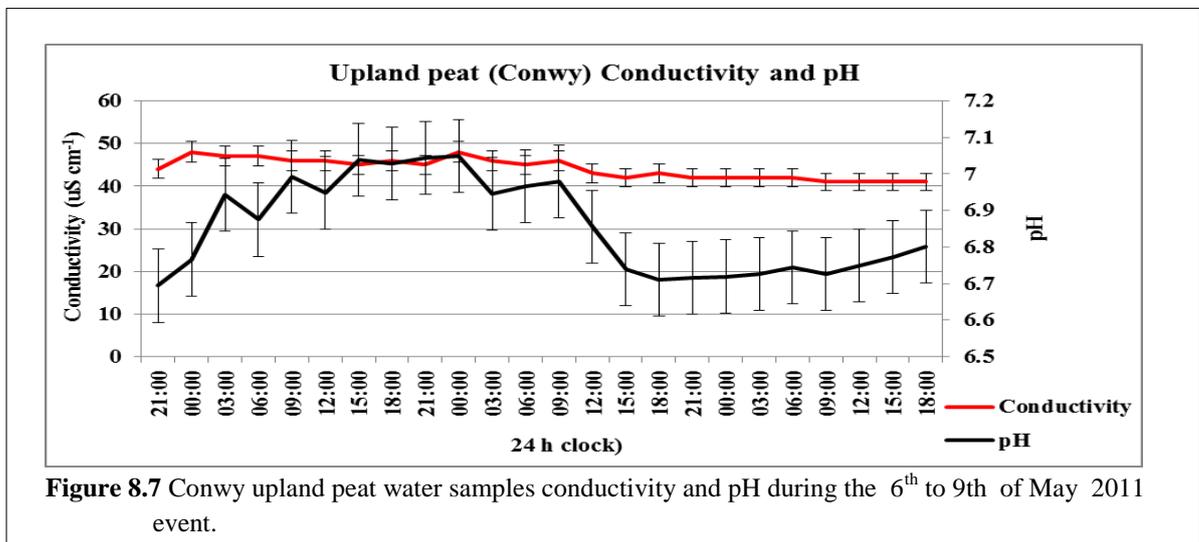


Figure 8.6 shows a similar profile with the maximum cumulative precipitation (18.6 mm) occurring between 00.00 h (7th May 2011) and 09.00 h (8th May 2011) with a further 2.2mm of rain falling during the following 33 hours. The flow rates for both streams were measured at a rate of m³/second and the results of the intense rainfall accumulation is seen in both graphs with sharp increases recorded in the flows at or after the maximum precipitation.

8.3 Results and DOC Characterisation.

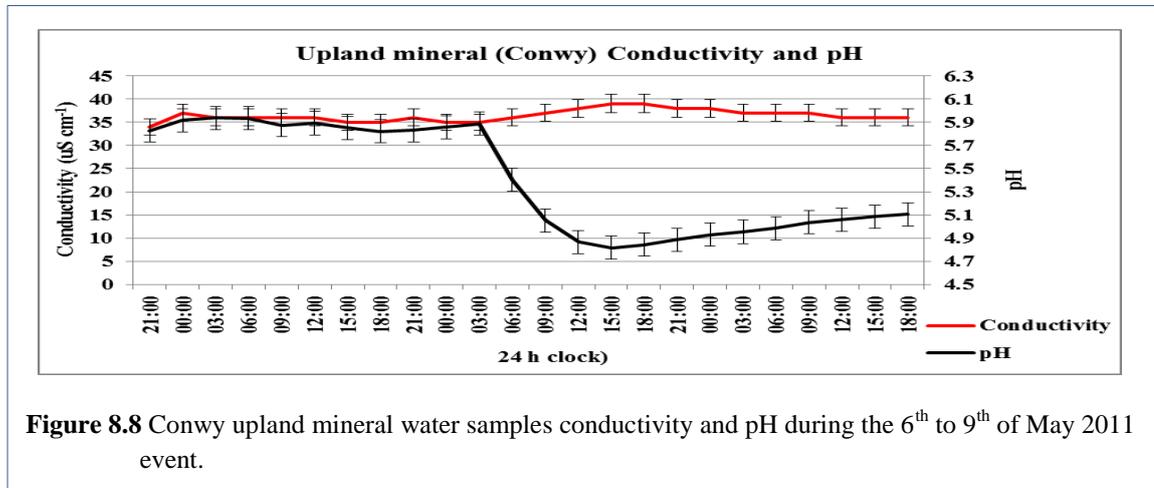
8.3.1 Conwy Peat and Mineral Stream Water Conductivity and pH.

The results for the pH and Conductivity profiles for both sets of sample series were different both in profile and values. The pH of the sample series classified as ‘upland peat’ (Fig 8.7) was initially at 6.7 ± 0.2 , at 21.00hrs on the 6th May 2011 after which it increased and plateaued at approximately 15.00hrs on the 7th May 2011 at $\text{pH } 7.0 \pm 0.2$ (cumulative rainfall 6 mm). The ‘Mineral’ (Fig 8.8) series during this comparable period did not change and remained at an average $\text{pH } 5.9 \pm 0.2$ (cumulative rainfall 13 mm). The sample represented as ‘Peat’ demonstrated a pH drop from 21.00hrs on the 7th May 2011 and continued to drop over 18 hours to $\text{pH } 6.7 \pm 0.2$ (cumulative rainfall 20 mm). The pH measurements for the equivalent ‘Mineral’ samples remained relatively stable, for over a period of 3 hours between 00.00 to 03.00 hours on the 8th May after which, a rapid drop was recorded to $\text{pH } 4.9 \pm 0.2$ (cumulative rainfall 28 mm). Both the ‘Peat’ and ‘Mineral’ sample series pH reading were seen to increase steadily and at the end of the exercise at 18.00hrs on the 9th May 2011, the ‘Peat’ sample water was at $\text{pH } 6.8 \pm 0.2$ and the ‘Mineral’ was $\text{pH } 5.1 \pm 0.2$.



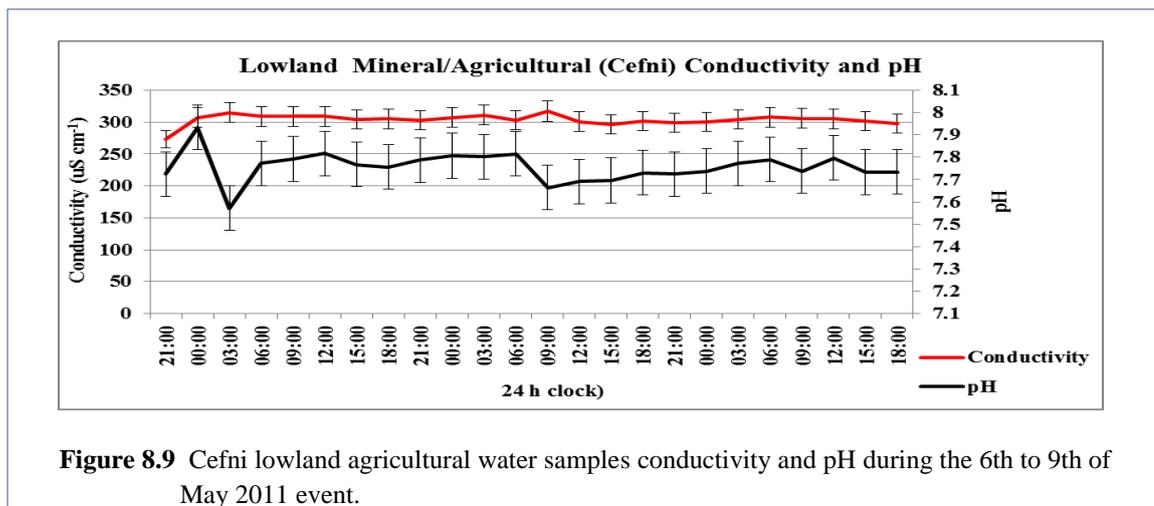
The conductivity of both samples during the exercise did not exhibit large fluctuations. The ‘Peat’ water conductivity demonstrated a decline over the period from its initial level of $44 \pm$ to $41 \mu\text{S/cm}$ at 1800 hrs on the 9th may 2011. Over the same timescale the ‘Mineral’ water sample’s conductivity remained the same at $36.5 \mu\text{S/cm}$ with a standard deviation of 1.4. Although there was an overall limited change in the ‘Mineral’ samples, a perturbation

was recorded when the (pH 4.8 ± 0.2) was at its lowest (Fig 7.4) the conductivity did demonstrate its highest reading (39 μS) for the period 12.00 -1800hrs on 8th May 2011.



8.3.2 Cefni Mineral/Agricultural Stream Water Conductance and pH.

Figure 8.9 shows the results for the pH and conductivity of the lowland Mineral agricultural catchment of Llyn Cefni. These data do not exhibit large changes in either conductance or pH (7.8 ± 0.2) with an SD 0.07. There was evidence of a small drop in pH, from 7.8 to 7.6 during the increase in river flow rate, i.e., $1.25 - 3.6 \text{ m}^3/\text{s}$ (0600 – 0900hrs) on the 8th May 2011.



The conductivity for this series of samples also exhibited a similar profile to the pH, with an average of $303.9 \mu\text{S}/\text{cm}$ and an SD of 8.2 which was similar to the conductivity average recorded in the Raw Surface Water of the Cefni reservoir samples (326.7 ± 10

$\mu\text{S}/\text{cm}$) . A small spike occurred during the same time period as pH change (06.00 – 09.00hrs) with the conductivity increasing from 303 to 311 μS which then reduced to 301 μS by 12.00hrs.

8.3.3 Event DOC Concentrations.

The literature report changes in the DOC concentration with increasing rainfall and run off through the soil profiles (Worrall *et al.* 2008). To determine any DOC concentrations changes relative to the event the samples were analysed using a Thermalox 2000 and method 2.3.1 (Chapter 2).

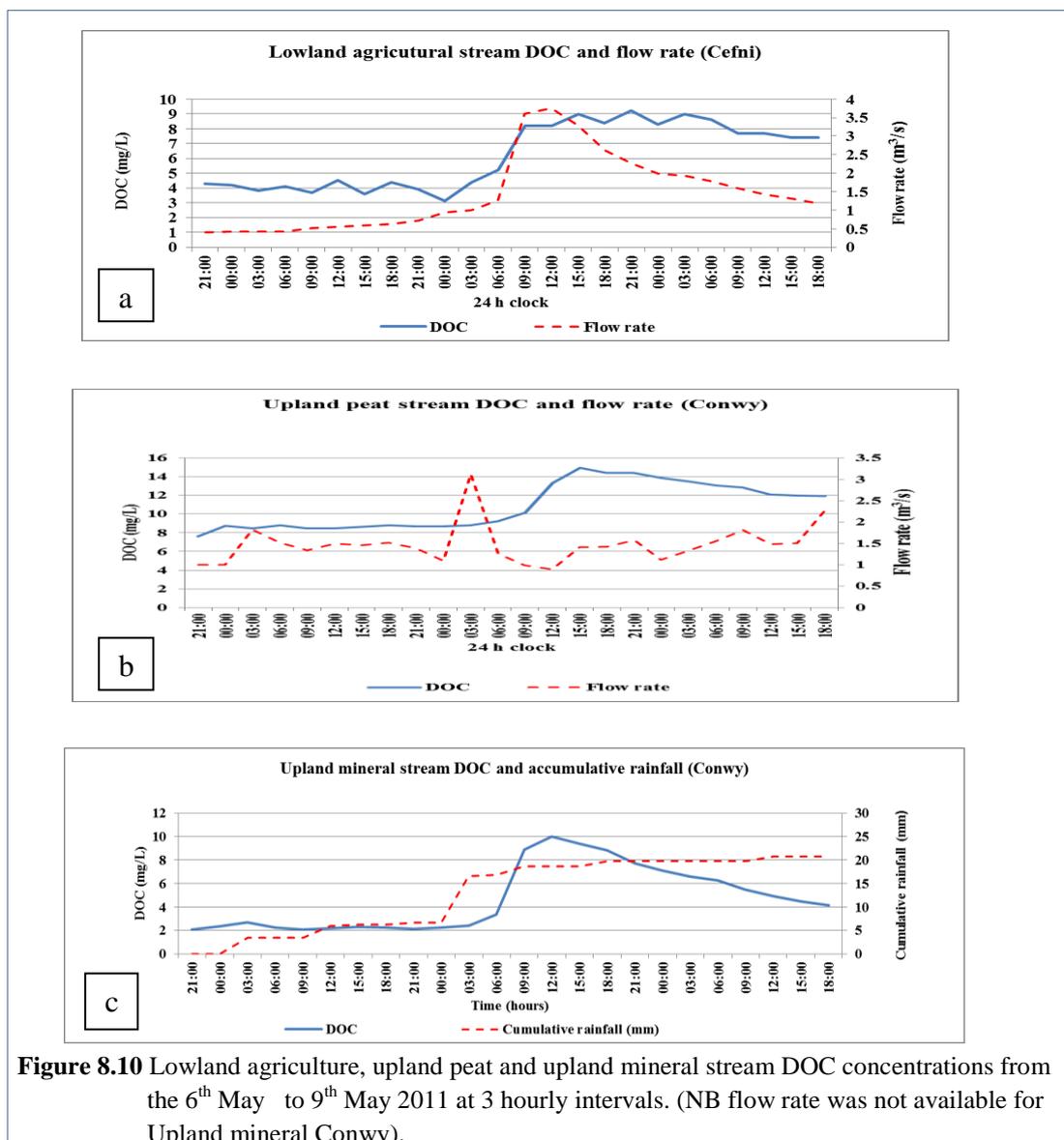
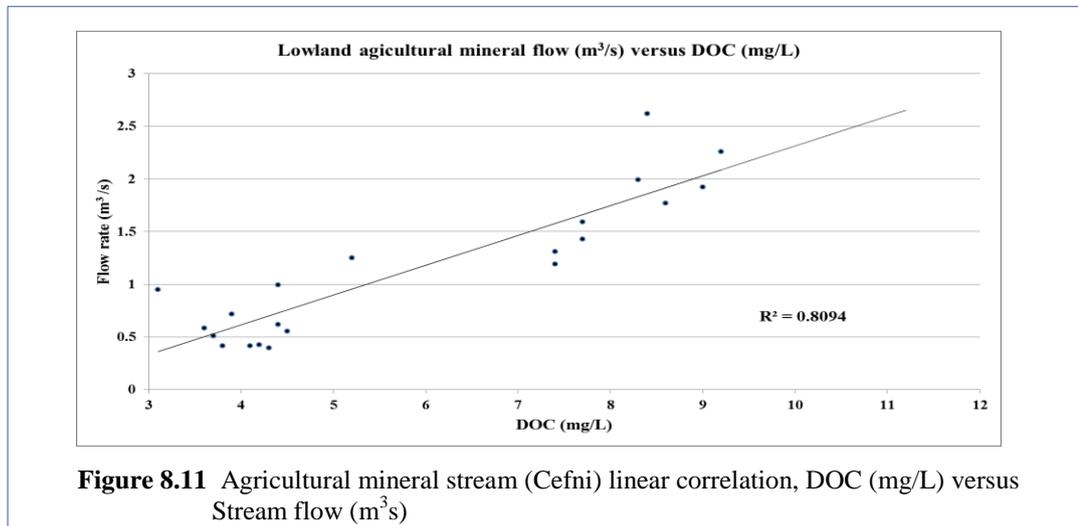


Figure 8.10 Lowland agriculture, upland peat and upland mineral stream DOC concentrations from the 6th May to 9th May 2011 at 3 hourly intervals. (NB flow rate was not available for Upland mineral Conwy).

The results for DOC concentration reported in Figs 8.10 show that all three streams experienced an increase in DOC concentrations during the storm event. The DOC

concentrations were relatively stable for the first 24 hours, but as the rainfall accumulated and the flow rates in the streams increased the DOC concentrations also began to change. The lowland Mineral agricultural DOC concentration tended to increase as the flow rate increased and demonstrated a significant correlation (Pearson $r = 0.815$ $p < 0.01$) and a linear correlation (Fig 8.11) $R^2 = 0.809$.



Although a similar response was observed in the upland peat stream, the flow rate peaked sharply at 0300 hours on the 8th May before reducing back to an average of 1.5 m³/s. However, visual inspection of the data (Fig 8.10 b) shows a clear correlation but with a time lag of 10 hours between the peak of the stream flow and peak DOC concentration was recorded, with the start of the DOC increase beginning at 09.00hours on the 8th May 2011 and reaching its maximum (14.9 ± 0.4 mg/L) at 15.00hrs (Table 8.1). For the upland mineral stream a steep upward trend in the DOC concentration was recorded at 06.00 hrs (8th May 2011) with the maximum concentration (9.9 ± 0.3 mg/L) recorded for the 12.00hrs sample at the highest 19 mm cumulative rainfall with the DOC concentration falling at a rate of ≈ 0.2 mg/L/hour.

Table 8.1 Lowland mineral, upland peat and upland mineral stream water , showing the DOC concentrations before, during and after the rainfall event(6 th May – 9 th May 2011).			
Stream	Pre-storm (21.00 hrs, 6th May 2011) DOC (mg/L)	Storm (8th May 2011) DOC (mg/L)	Post-storm (18.00hrs, 9th May 2011) DOC (mg/L)
Lowland mineral (Cefni)	4.3	9.2 (15.00hrs)	7.7
Upland peat (Conwy)	7.6	14.9 (15.00hrs)	11.9
Upland mineral (Conwy)	2.1	9.9 (12.00hrs)	4.1

8.3.4 Phenolic Analysis of Event DOC.

The phenolic content of the DOC was analysed for, using the Folin-Ciocateau colormetric method described by Box (1983) as detailed in Chapter 2 (2.3.2).

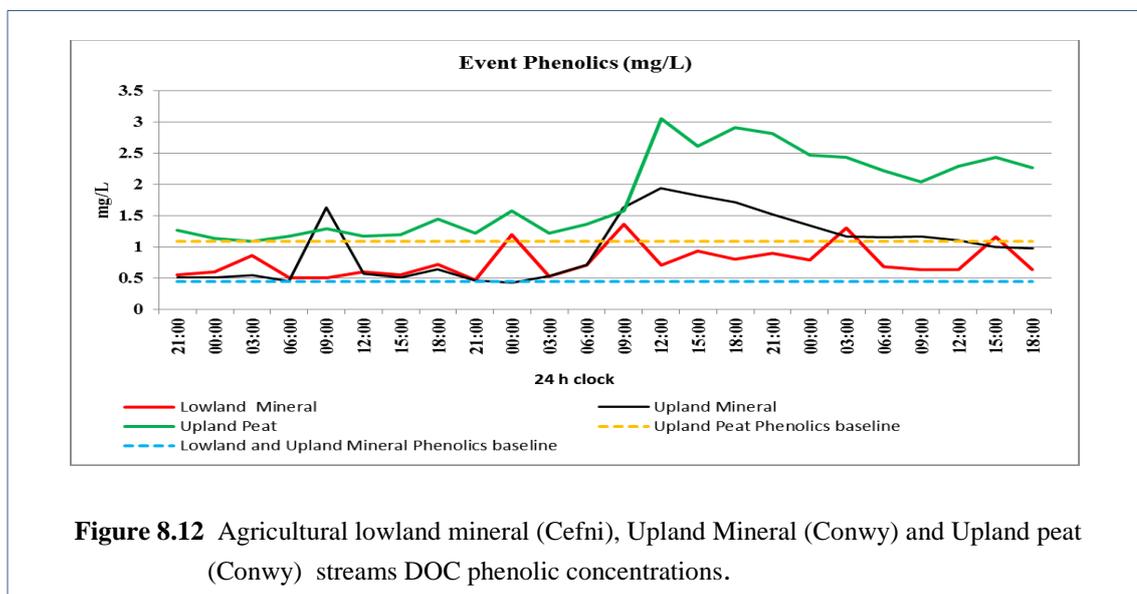


Figure 8.12 shows the phenolic concentrations during the event period. Both the upland stream samples demonstrated a change in phenolic concentration coinciding with the increased DOC concentration and exhibited significant correlations, but with a reduced significance for the lowland mineral stream (Table 8.2) which at its maximum at 09.00 h on the 8th May was approximately 0.85 ± 0.04 mg/L above the baseline concentration of 0.45 mg/L, representing a phenolic concentration of 0.14 mg/mg DOC during the event peak, compared to ≈ 0.11 mg/mg DOC at the pre-event baseline concentration.

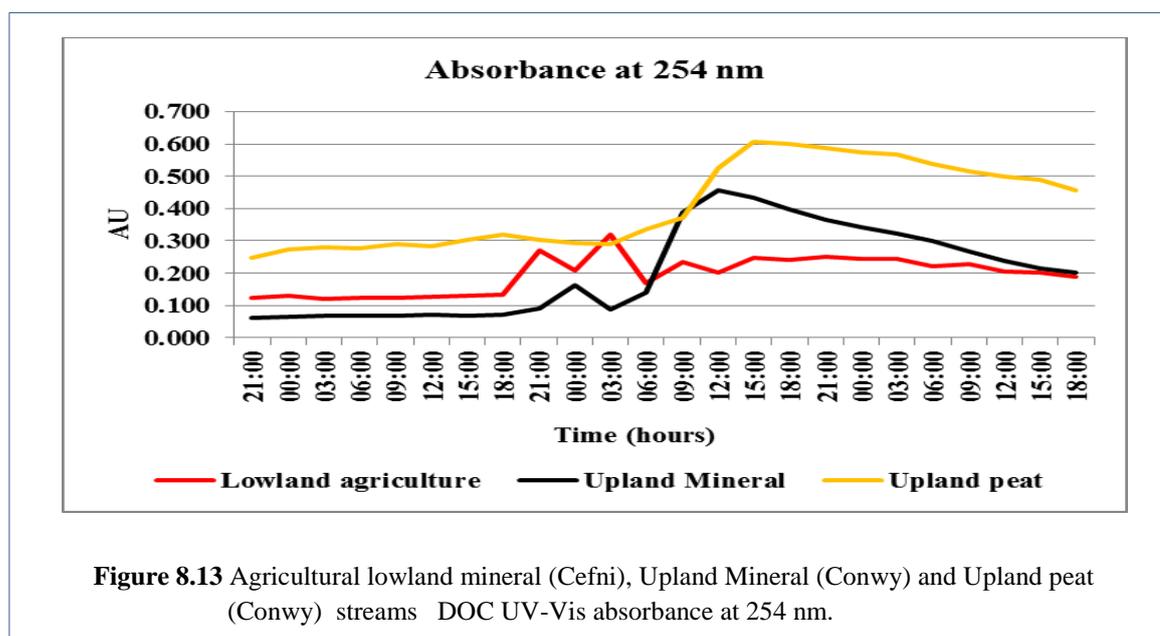
Stream	Correlation coefficients (DOC <i>v</i> Phenolics)
Lowland agricultural (Cefni)	0.441 < 0.05
Upland peat (Conwy)	0.949 < 0.01
Upland mineral (Conwy)	0.881 < 0.01

Phenolic concentrations (Table 8.3) were highest in the upland peat stream. At the peak of the event DOC concentration the phenolic concentration represented 0.22 mg/mg of DOC, compared to 0.13 mg/mg DOC before the event. The upland mineral exhibited a phenolics concentration of 0.19 mg/mg DOC at the event peak DOC concentration, but demonstrated higher phenolic concentrations (0.23 mg/mg DOC) for the pre-event period.

Table 8.3 Lowland agricultural, upland peat and upland mineral stream phenolic concentrations (6th May – 9th May 2011).			
Stream	Phenolics (mg/L)		
	Pre-event (6 th May 2011)	Event (8 th May 2011)	Post-event (9 th May 2011)
Lowland agricultural (Cefni)	0.5 (21.00 h)	1.4 (09.00hrs)	0.6 (18.00 h)
Upland peat (Conwy)	1.1 (21.00 h)	3.1 (12.00hrs)	2.3 (18.00 h)
Upland mineral (Conwy)	0.4 (21.00 h)	1.9 (12.00hrs)	0.9 (18.00 h)

8.3.5 UV 254nm of Event DOC

UV- visible spectra were collected using a Hewlett Packard Lambda 35 spectrometer with a 1 cm quartz cuvette. Absorbances' at (λ 254 nm) have been reported to give a qualitative indication of the aromatic molecular structures present in the DOC of the stream samples. The results (Fig 8.13) for the three stream samples over the exercise period demonstrated a similar graphical profile to the DOC (Fig 8.10) and phenolic concentrations (Fig 8.12).



Statistical analysis yielded significant correlations for DOC *versus* 254nm (Table 8.4). The most significant were demonstrated by both the upland streams, with correlation coefficients close to 1. The lowland agricultural stream was weaker but still significant with a $p < 0.01$. Consideration of the phenolics *versus* absorbance 254 nm correlations were significant for the two upland streams suggesting that the aromatic content of the DOC was highly substituted with hydroxyl functional groups. The agricultural mineral stream did not demonstrate a correlation (Table 8.4) between the phenolic concentration

and absorbance at 254 nm, thus suggesting intermittent substitution of the aromatic/conjugated systems with hydroxyl functional groups

Table 8.4 Correlation coefficients of 254 nm *versus* DOC concentrations and 254nm *versus* phenolics for Agricultural lowland mineral (Cefni), Upland Mineral (Conwy) and Upland peat (Conwy) streams

Stream	Correlation coefficients (254 nm ν DOC)	Correlation coefficients (254 nm ν Phenolics)
Lowland agricultural (Cefni)	0.566 < 0.01	0.300 > 0.05
Upland peat (Conwy)	0.995 < 0.01	0.948 < 0.01
Upland mineral (Conwy)	0.985 < 0.01	0.860 < 0.01

8.3.6 SUVA and Aromaticity of Event DOC

The SUVA and Aromaticity values were calculated for the three streams. The significance of the values as previously mentioned, are considered to be indicators of the presence of aromatic structures in the DOC with the low SUVA value (< 3) indicating a hydrophilic low aromatic concentration in the DOC, whilst a highly aromatic hydrophobic DOC has been reported to demonstrate a SUVA value > 4 (Ates *et al.* 2007). The product of the (250nm/365nm), i.e., the Aromaticity ratio, which is also considered to be a measure of DOC Aromaticity, with low values suggesting high aromatic content and *vice versa*. Figure 8.14 a) and b) show the results of both the SUVA and Aromaticity values in the stream DOCs. A change in both SUVA values was clearly shown for the lowland Mineral agriculture and upland Mineral DOC. The increase (3 \rightarrow 7 L/mg m⁻¹) began at approximately 18.00 h on the 7th May with a reversal occurring (7 \rightarrow 3 L/mg m⁻¹) in the upland mineral at 00.00 hours, 3 hours later at 03.00 hours the agricultural Mineral lowland followed suit. The upland Peat stream did not show any noticeable change in either SUVA or Aromaticity values over the exercise period, but the SUVA increased slowly from 03.00hrs on the 8th May 2011 from 3.3 to 4.2 L/mg m⁻¹ on the 9th May 2011. Correlation of the SUVA and Aromaticity *versus* changing DOC concentrations were statistically significant for the lowland agricultural Mineral and upland Mineral streams, indicating that the aromatic content responded relative to the changing stream DOC concentrations. The agricultural Mineral stream (Table 8.5), exhibited a less clear but negative correlation ($r = -0.528$ $p < 0.01$). It indicated that as the DOC increased/decreased the aromatic content reciprocated. Unlike the mineral streams, there were no obvious changes demonstrated in the (8.14 a,b) of upland Peat stream SUVA and Aromaticity, but the correlations was strongly significant and positive with SUVA (Table 8.5).

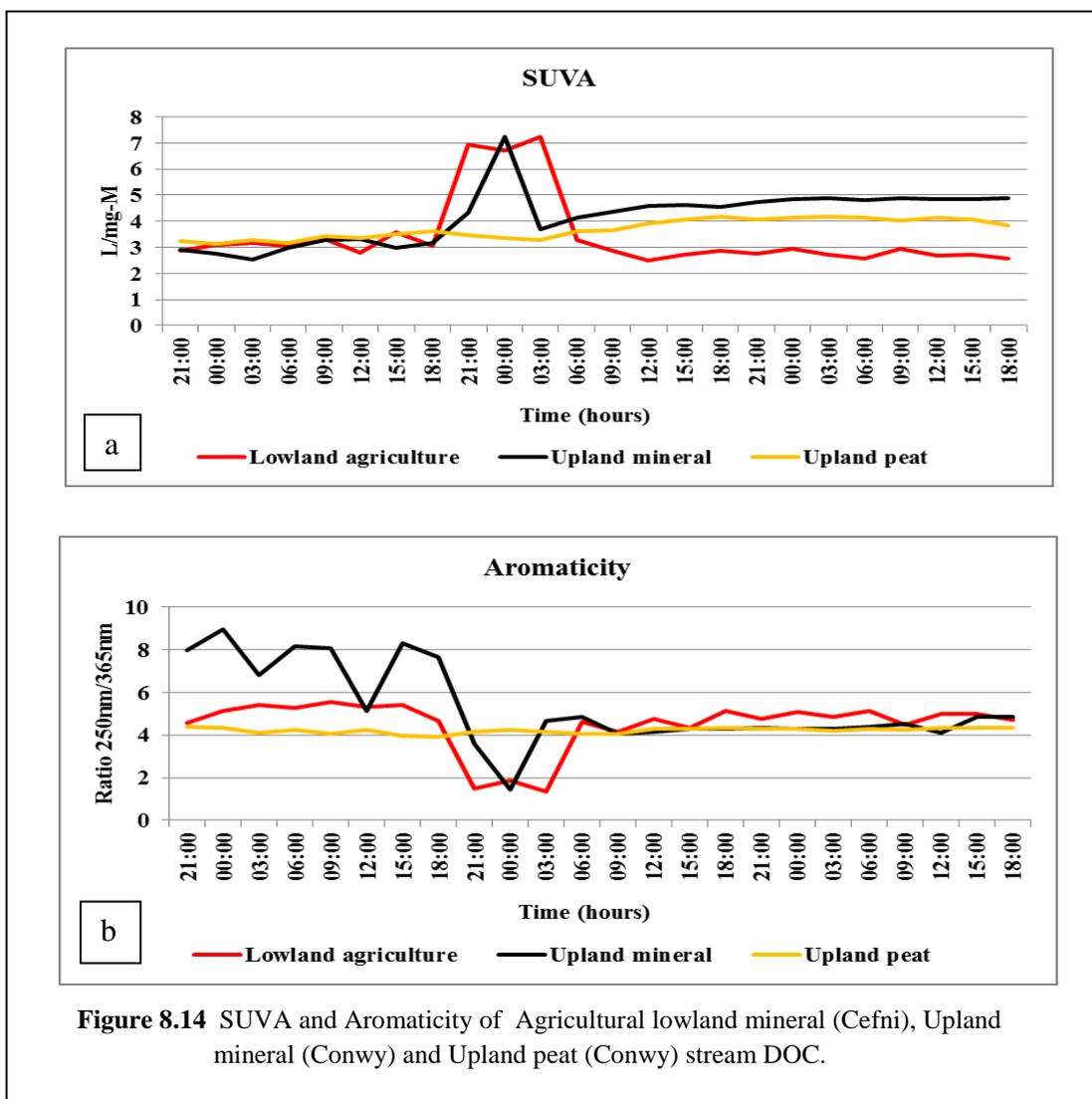


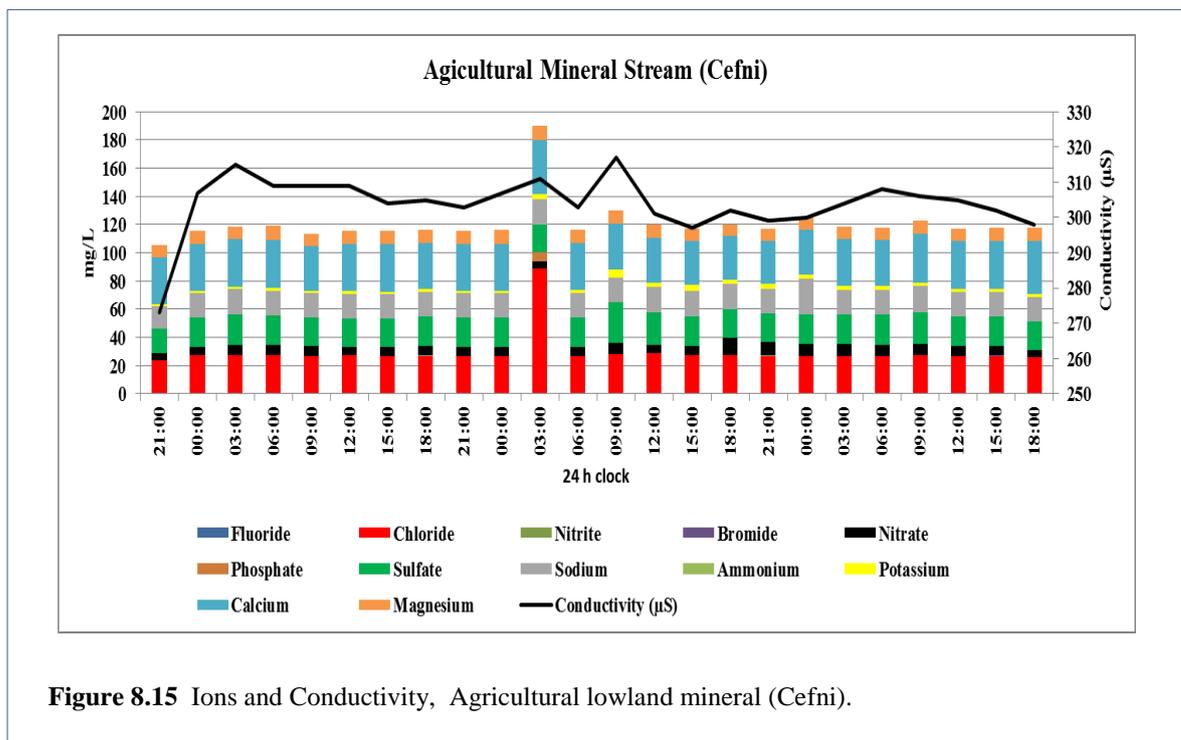
Table 8.5 SUVA and DOC correlations in Agricultural lowland mineral (Cefni), Upland mineral (Conwy) and Upland peat (Conwy) stream DOC.

Stream	Correlation coefficients (SUVA ν DOC)	Correlation coefficients (Aromaticity ν DOC)
Lowland agricultural (Cefni)	- 0.528 < 0.01	0.262 > 0.05
Upland peat (Conwy)	0.924 < 0.01	0.479 < 0.05
Upland mineral (Conwy)	0.422 < 0.05	- 0.491 < 0.05

8.3.7 Cations and Anions in the Event Stream Waters

The agricultural Mineral stream demonstrated the highest concentration of cations and anions of the three streams that were monitored during the event exercise. The predominant cation was Ca^{2+} with an average concentration of 33.3 mg/L (SD 1.8). Na^+

and Mg^{2+} were also present with relatively high concentrations, at 17.6 and 9.2 mg/L (SDs 1.7 and 0.5), respectively.



The principal anion present which was also the ion species with the highest concentration in the sample suite was Cl^- , averaging 29.4 mg/L with an SD of 12.7. The second highest anion concentration was SO_4^{2-} (21.2 mg/L, SD of 1.90). The SO_4^{2-} concentrations demonstrated significant correlations with conductivity and stream flow of the lowland agricultural Mineral stream (Table 8.6). The NO_3^- concentrations in the stream also remained high throughout the event (average 7.0 mg/L, SD 1.6), with a spike recorded (12.3 mg/L) at 1800hrs on the 8th May 2011. This coincided with a drop in flow rate from 3.29 m³/s (15.00 hours) to 2.62 m³/s (18.00hours). The concentration then reduced over the period to 5.0 mg/L for the last sample at 18.00hours on the 9th May 2011.

The most significant correlations (Table 8.6) were demonstrated for K^+ , stream flow (r 0.829) and DOC concentrations (r 0.648) which tends to suggest agricultural run-off after spring fertilizer and manure spreading.

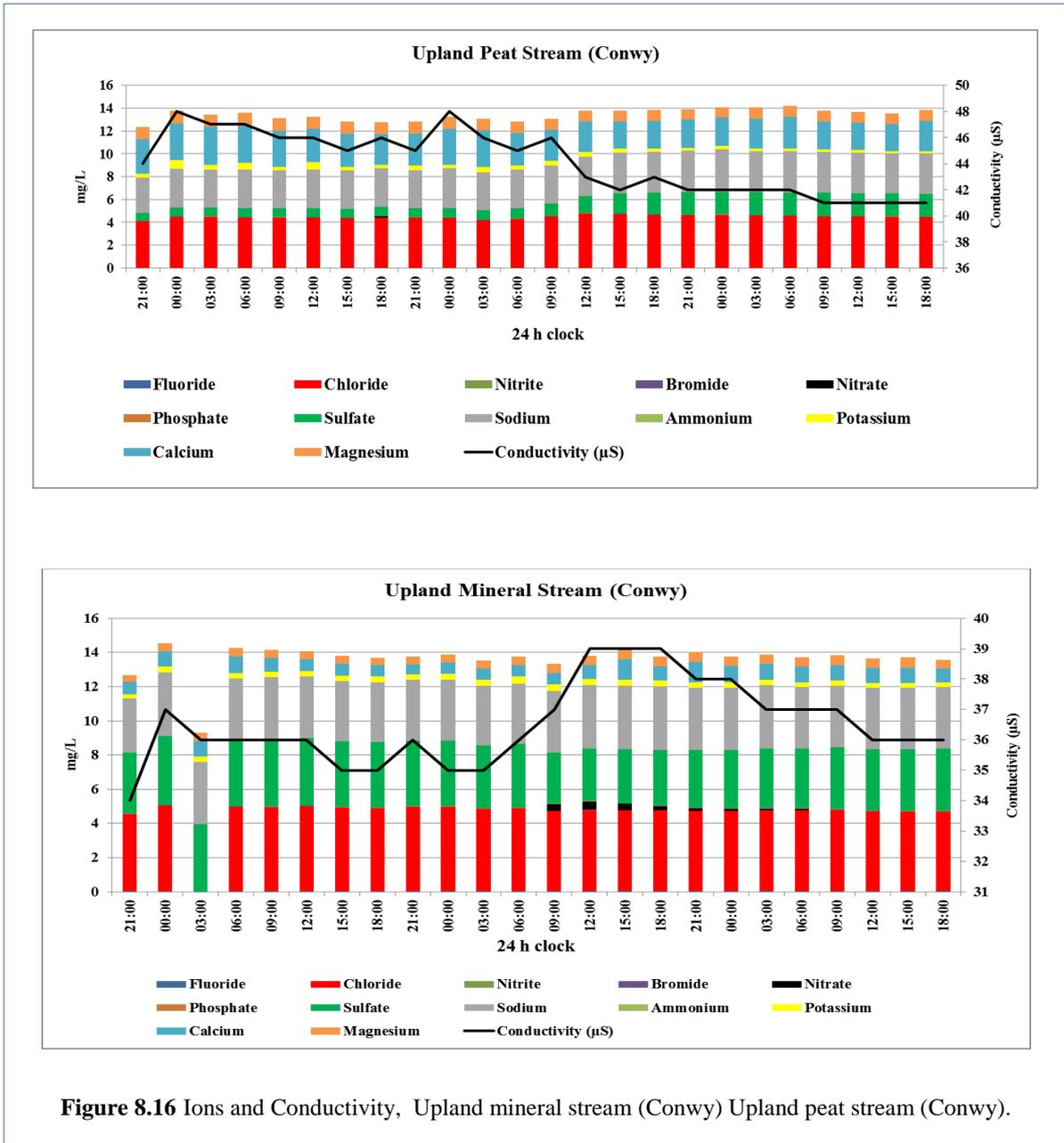
Stream	Ion	Correlation (DOC)	Correlation (StreamFlow)	Correlation (Conductivity)
Agricultural lowland (Cefni)	Magnesium	- 0.561**	- 0.506*	
	Sodium			
	Calcium		- 0.419*	
	Potassium	0.648*	0.829**	
	Sulphate		0.563**	0.564**
	Chloride			
	Bromide			
	Nitrate	0.515*	0.414*	

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

The upland Mineral and upland Peat streams exhibited lower ionic concentrations to those of the agricultural Mineral stream. The lowland agricultural Mineral stream's principal cation was Ca^{2+} , but it was Na^+ for the upland peat and mineral streams with an average concentration of 3.4 (SD 0.14) and 3.6 mg/L (SD 0.12) respectively. The Ca^{2+} ion had a relatively low concentration with an average of 0.8mg/L (SD 0.2) in the upland Mineral stream, but the peat stream average was 3.5 times higher at 2.8 mg/L (SD 0.3). The analysis also highlighted differences in Mg^{2+} concentrations, with the peat stream demonstrating an average 1.0 mg/L compared to 0.5 mg/L by the upland Mineral. Both upland streams had the same dominant anion, Cl^- with similar concentrations, the upland peat (4.5 mg /L, SD 0.16) and the upland Mineral (4.8 mg/L, SD 0.13). The second highest ion concentration in both was SO_4^{2-} , averaging 3.6 mg/L (SD 0.29) in the upland mineral and 1.3 mg/L (SD 0.58) in the upland peat. The SO_4^{2-} remained at a relatively similar concentration during the event in the Mineral stream, but increased in the upland Peat stream from a low of 0.53 mg/L to 2.0 mg/L at the end of the event monitoring, with the step change occurring 6 hours after the highest flow rate at 03.00 hours (8th May 2011). The NO_3^- anion which had a high average concentration in the lowland agricultural stream (6.9 mg/L) was not detected on a continual basis in the upland Peat stream, but did exhibit detectable traces towards the end of the event 0.017 - 0.007 to mg/L during the hours 00.00 – 06.00 hours on the 9th May 2011 which accompanied a small drop in the flow rate (0.59 – 0.58 m³/s). In the upland Mineral stream, again a concentration was not detected for the whole event period, but evidence of a detectable concentration was recorded at 09.00 hours (0.39 mg/L) on the 8th May 2011, which peaked at a concentration of 0.48 mg/L at 12.00 hours and then dropped over a period of 21 hours to a concentration of 0.013 mg/L at

09.00 hours on the 9th May 2011, after which it was not detected. The halogens F⁻ and Br⁻ exhibited traces (mean 0.02 mg/L) for the upland Mineral stream and (mean 0.01 mg/L) for the Peat stream.



The correlation coefficients of the upland peat and mineral ion concentration data relative to DOC, conductivity and stream flow (Table 8.7) show significant positive and negative values. The upland Peat stream's suite of ions with exception of NO₃⁻ correlated with the DOC, demonstrating a significant positive correlations (<0.01) with Na⁺, Cl⁻ and SO₄²⁻ with Mg²⁺, Ca²⁺, K⁺ and Br⁻ demonstrating significant negative correlations.

The upland Mineral stream ions Mg^{2+} , Na^+ , Ca^{2+} and NO_3^- exhibited significant positive correlations with conductivity, but SO_4^{2-} showed a significant negative correlation. Statistical

Statistical analysis of the upland Peat stream exhibited significant negative correlations between the concentrations of the Na^+ , Cl^- and SO_4^{2-} ions and conductivity, but a positive series of correlations with Mg^{2+} , Ca^{2+} and K^+ .

An interesting observation of the correlation coefficients is that the majority of the significant ones are between the upland Peat stream ions and DOC (> 0.8). Both the positive and negative are related to the DOC concentration. The positive coefficients suggest a close association and entrapment within the molecular structures of DOC rather than as suspended electrolytes in the water body which is suggested by the negative coefficients.

Table 8.7 Correlation of ions with DOC, conductivity and stream flow in Upland peat stream (Conwy) and Upland mineral stream (Conwy)

Stream	Ion	Correlation with (DOC)	Correlation with (StreamFlow)	Correlation with (Conductivity)
Upland peat stream (Conwy)	Magnesium	- 0.856**		0.860**
	Sodium	0.872**		- 0.651**
	Calcium	- 0.808**		0.850**
	Potassium	- 0.542**		0.723**
	Sulphate	0.922**		- 0.913**
	Chloride	0.832**		- 0.465*
	Bromide	- 0.905**		
	Nitrate			
Upland mineral stream (Conwy)	Magnesium	0.776**		0.739**
	Sodium	0.530**		0.763**
	Calcium	0.522**		0.610**
	Potassium			
	Sulphate	- 0.939**		- 0.683**
	Chloride	- 0.480*		
	Bromide			
	Nitrate	0.871**	- 0.762*	0.680*

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

8.3.8 THMFP of Event Streams.

Laboratory chlorination and analysis of the samples was carried out as per methods (Chapter 2, sections 2.3.7 and 2.3.8). Three samples from each stream were selected for THMFP analysis. The first sample of each stream before the event, the second was the highest concentration of DOC recorded in each stream during the event and the third; the last sample from each stream after the event. The total THMFP results are presented in graphical form (Figure 8.17 a,b and c) and show both actual DOC concentrations and SUVA values for each sample.

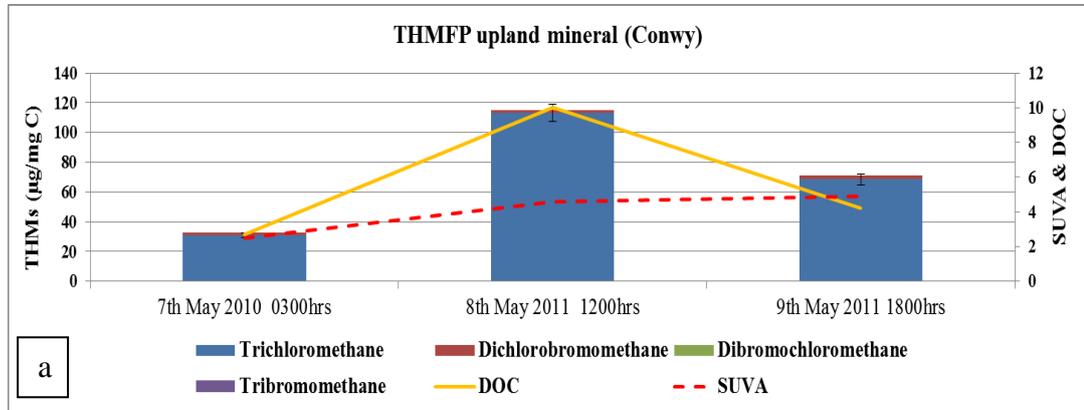
The results, were dominated by the CHCl_3 component with relatively low concentrations of brominated components being formed by the upland Mineral and Peat streams, which had mean Br^- stream sample concentrations of $19 \mu\text{g/L}$. The highest SUVA value for the lowland agricultural stream was recorded for the 03.00 hours sample on the 7th May 2011 (3.3 L/mg m^{-1}) with a DOC of 4.4 mg/L . This was approximately half the 2100 hrs (8th May 2011) DOC concentration (9.3 mg/L). The total THM concentration generated was $56.7 \pm 1.7 \mu\text{g/mg DOC}$. The highest total THMFP potential for the lowland agricultural Mineral stream was $63.4 \pm 1.9 \mu\text{g/mg DOC}$ at 2100hrs (8th May 2011) of which $15.3 \pm 0.3 \mu\text{g/mg DOC}$ were brominated products (mean stream sample Br^- : $85 \mu\text{g/L}$). This coincided with the highest concentration of DOC ($9.2 \pm 0.3 \text{ mg/L}$) but was also accompanied by a low SUVA (2.7 L/mg m^{-1}).

The upland Mineral stream THMFP demonstrated its highest total THM concentration ($115.4 \pm 3.5 \mu\text{g/mg DOC}$) at 12.00 hours (8th May 2011) when the DOC concentration was at the event maximum of $9.9 \pm 0.3 \text{ mg/L}$ with a SUVA value of 4.6 L/mg m^{-1} . On the previous day at 03.00 hours (7th May 2011) the actual DOC (2.7 mg/L) and SUVA (2.5 L/mg m^{-1}) were relatively low and the collective THMs were 8.5 times lower at $32.8 \mu\text{g/mg DOC}$.

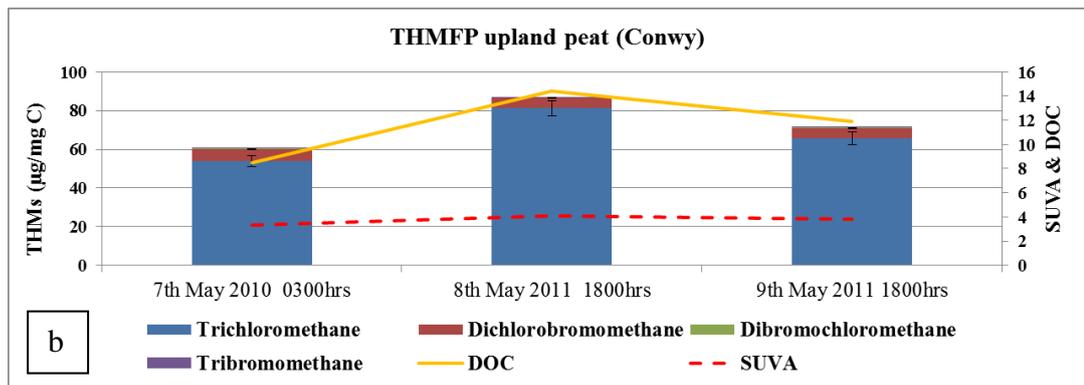
The upland Peat stream's collective THMFP demonstrated its highest concentration ($87.2 \pm 2.6 \mu\text{g/mg DOC}$) at 18.00 hours (8th May 2011) when the DOC concentration was $14.4 \pm 0.4 \text{ mg/L}$ (SUVA 4.1 L/mg m^{-1}). At the point of minimum DOC concentration ($8.8 \pm 0.3 \text{ mg/L}$, 03.00 hours, 7th May 2011), the total THMs were $60.8 \pm 1.8 \mu\text{g/mg DOC}$ (SUVA 3.3 L/mg m^{-1}).

Statistical analysis demonstrated significant correlations between DOC and THMs ($r = 0.837$, $p < 0.01$), but insignificant correlations between SUVA values and collective total THMs ($p > 0.05$), suggesting as previously mentioned in Chapter 5, Section 5.7, that

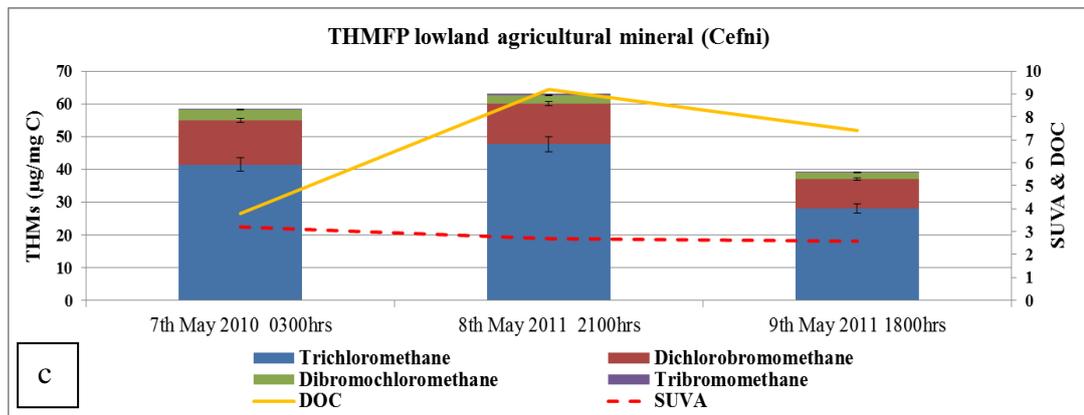
SUVA was a weak indicator of THMFP (Weishaar *et al.*, 2003; Qunshan *et al.* 2008 and Fram *et al.* 1999). Significant correlations between DOC and THMs ($r = 0.837, p < 0.01$).



a) Conwy upland mineral stream demonstrated the highest THMFP during the event

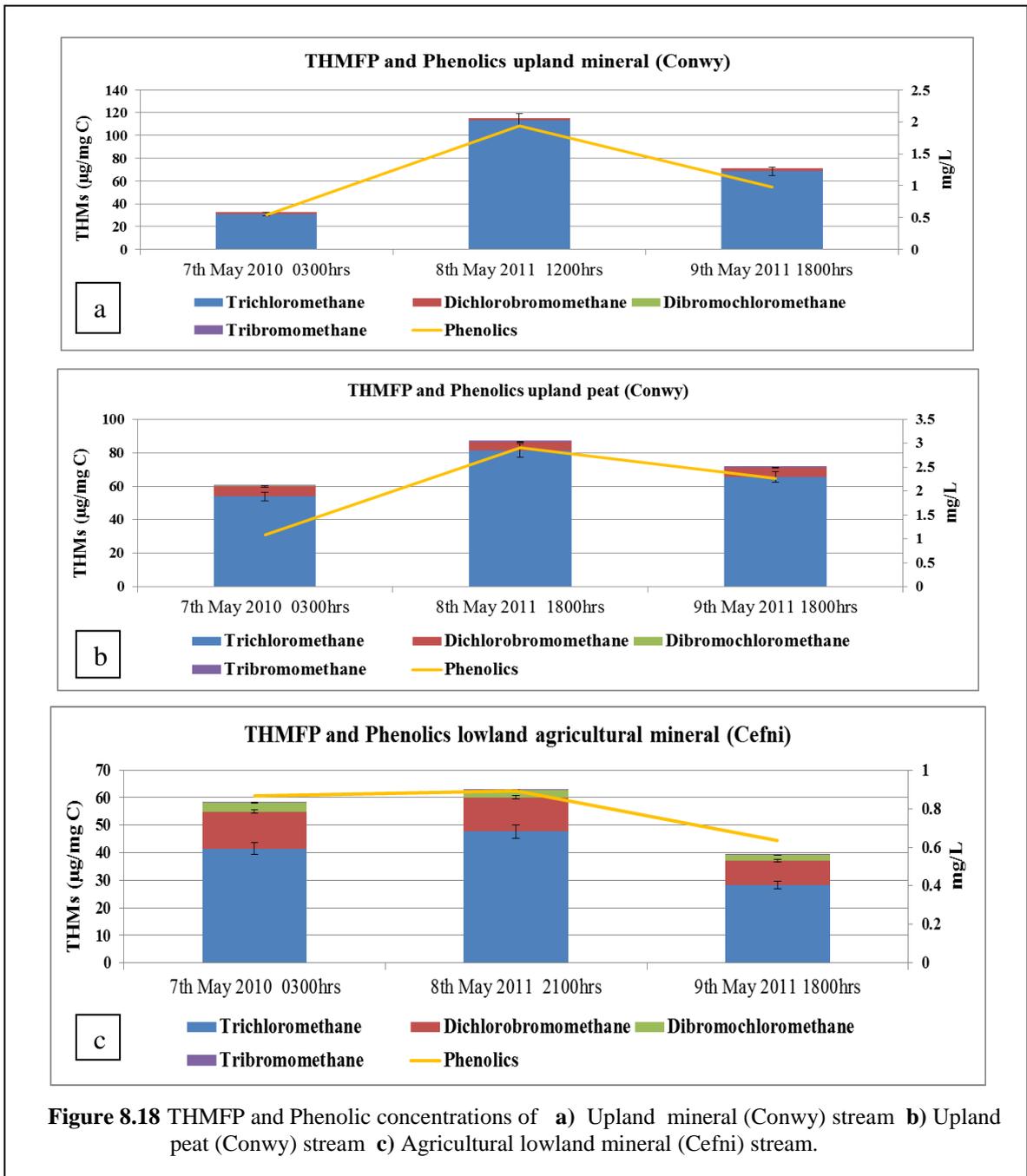


b) Conwy upland peat stream demonstrated the second highest THMFP during the event



c) Cefni lowland agricultural mineral demonstrated the lowest THMFP during the event.

Figure 8.17 THMFP, DOC and SUVA of a) Upland mineral stream (Conwy) b) Upland peat stream (Conwy) c) Agricultural lowland mineral stream (Cefni)



The results of the collective THMFP (Fig 8.18 a,b and c) with the phenolic concentrations over-laid displayed a relationship between the concentration of THMs and phenolics. Statistical analysis of the concentration of phenolics in mg/L demonstrated significant correlations (r 0.854, p < 0.01). To explore the relationship between phenolics and THMFP further, the concentration of phenolics per mg of DOC was calculated. Statistical analysis demonstrated insignificant correlations (p > 0.05) and the results demonstrated that the THMFP did not reflect relative increases or decreases in the phenolic concentrations as mg/mg DOC (Table 8.8).

Table 8.8 The concentration of phenolics (mg/mg DOC) relative to the THMFP at the time shown of the upland peat stream (Conwy) and upland (Conwy) and lowland (Cefni) mineral streams (May 7th – May 9th 2011)

Stream	Date (May 2011)	Time (24 hour clock)	DOC (mg/L)	Phenolics (mg/mg DOC)	Total THMs (µg/mg DOC)
Lowland mineral	7	03.00	4.3	0.129	58.6
Upland mineral	7	03.00	2.1	0.220	32.8
Upland peat	7	03.00	7.6	0.130	60.8
Lowland mineral	8	21.00	8.2	0.136	63.0
Upland mineral	8	21.00	9.9	0.194	115.4
Upland peat	8	21.00	13.3	0.174	87.2
Lowland mineral	9	18.00	7.4	0.092	39.4
Upland mineral	9	18.00	4.2	0.224	71.3
Upland peat	9	18.00	11.9	0.203	71.8

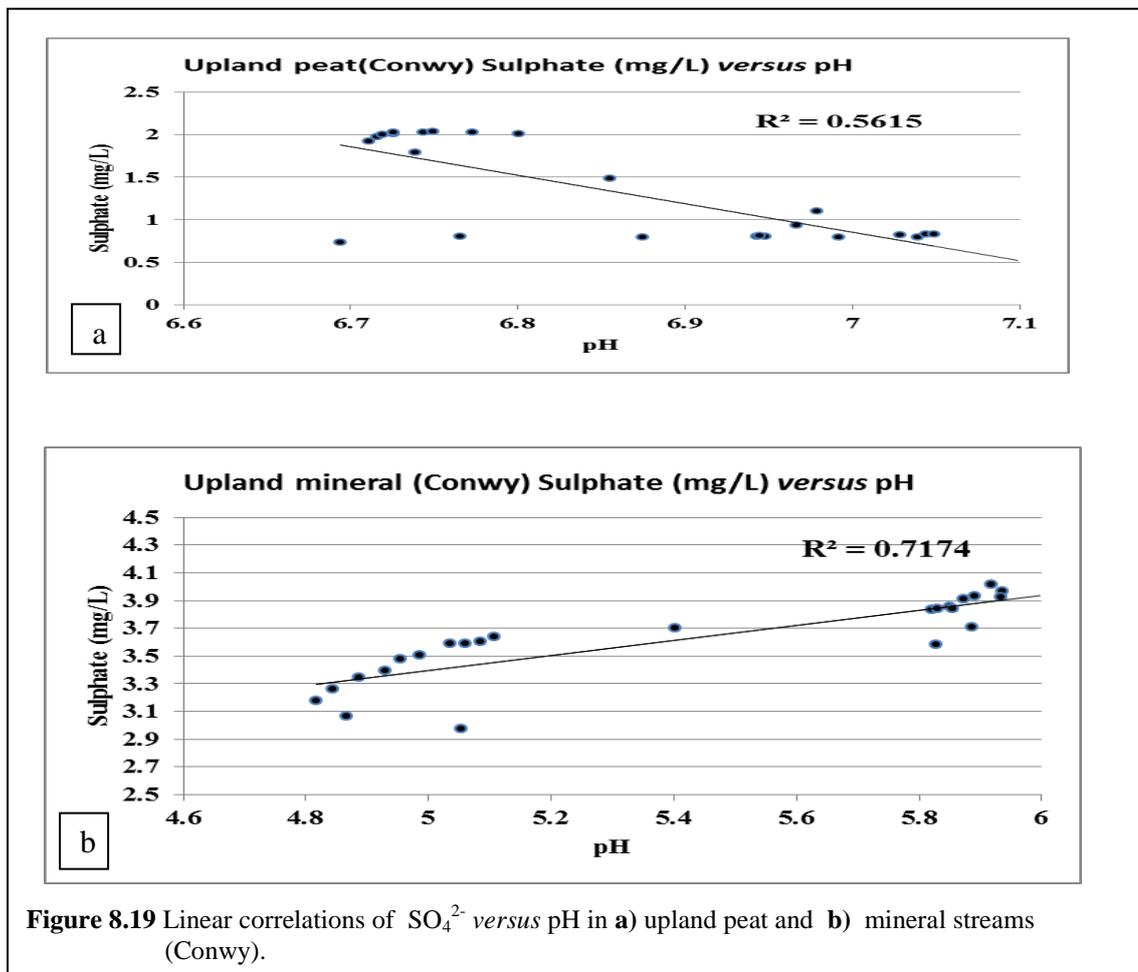
8.4 Discussion.

The total rainfall for March and April 2011 for the Cefni agricultural stream was 61.8 mm (BADC 2012) preceding the rainfall event. During the event, 24.6 mm of rain fell over the 69 hours of monitoring, with 21.8 mm falling within the first 24 hours.

The upland catchments, peat and mineral streams received 20.8 mm of rainfall over the 69 hours of monitoring exercise, of which 16.8 mm or the total rainfall fell within the first 24 hours. The effects of these intense precipitations demonstrated an effect on the flows, DOC, pH, ionic content and conductivity of the streams. The stream flows (Figure 8.6 a and b) show that although the precipitation began at approximately 0300hrs on the 7th May 2011 the upland and lowland streams achieved their maximum flows at different times. The agricultural mineral stream peaked at 1200hrs on the 8th May 2011 whilst the upland peat stream flow peaked much earlier at 0600hrs on the 8th. The differences were most likely to have been influenced by the hydrological status of the catchment topography. Thus the relatively level agricultural catchment of the Cefni composed of field enclosures with ditching and a mixture of free draining and slow draining permeable acid soils. These soil properties, during rainfall may have developed a water gradient in the vertical direction which would saturate the upper soil strata. This would then be followed by lateral hydraulic surface conductivity towards the water course (Ward and Robinson, 1989). By comparison the upland peat catchment consisted of a less dense fibrous organic peat on the gradients, thus inducing a faster water flow rate which may have been contained in the upper surface layers, analogous to a thatched roof (Zaslusky and Sinai, 1981). With parts of the blanket bog being poorly drained (Avery, 1990) water flowing off the gradients may have continued as an overland flow (Tetzlaff *et al*, 2009).

The rainfall event following a dry period, has to be considered as a mixing of rainfall with what may be termed ‘old water’, that is water that was in the catchment/ streams before the rainfall. This old water represented the catchment water table discharge into the streams. Hence this ‘old water’ had had a long residence time to evolve its solute composition (Worrall *et al.*, 2002) and the ‘new water’ which was the rainfall would also have had its own chemical signature.

The pH and conductivity of the streams demonstrated changes and influences most likely to have been induced by the event. For instance, the upland acidic and lowland mineral streams exhibited pH drops during the highest flows. Clark *et al.* (2005) and Evans *et al.* (2006) suggested that pH changes in catchments are related to the dominant anion (SO_4^{2-}), i.e., Clark (2005) stated, that SO_4^{2-} concentrations $> 2.5 - 5 \text{ mg/L}$ control the pH and lead to pH falls in acidic peats during droughts and SO_4^{2-} concentrations of less than 2.5 mg/L in soil solutions, the acidity is controlled by organic acids.



In Figure 8.19 the suggestion by Clark *et al.* (2005) was demonstrated by the Upland Peat stream ($R^2 = 0.5615$). The pH increased with the fall in SO_4^{2-} concentration. The converse

was observed in the Upland Mineral, the pH increased with the increase in SO_4^{2-} concentration (R^2 0.7174) demonstrating that although SO_4^{2-} is an acid anion its counter ions were not protons in this case. The Lowland Agricultural Mineral stream pH (av 7.8) did not exhibit a directional change during the event and demonstrated a weak linear correlation (R^2 0.1111) with SO_4^{2-} concentrations (21.24 mg/L).

The pH falls in the two upland streams during the highest flow may have been related to the increase in DOC and its organic acid moieties. Steinman and Shoty, 1996 reported that acid producing reactions occurred in the upper layers (i.e., dissociation of organic acids and H_2CO_3), whilst deeper down the soil profile, acid consuming reactions (biological SO_4^{2-} and NO_3^-) take place and Holden and Burt, (2003) also reported typical water movement during an event taking place principally in the top 10 cm soil layer. The relative negative correlation coefficients (upland peat; $r = 0.708$, $p < 0.01$ and upland mineral; $r = 0.906$, $p < 0.01$) exhibited by the DOC *versus* pH, suggests that the water flow is through the top acidic layer, hence the drop in pH as the DOC concentration increased.

The lowland agricultural mineral stream pH averaged 7.8 with a very small SD of 0.07, although the DOC increased over the event from 4.3 mg/L to 9.2 mg/L, the pH remained relatively stable. This tends to suggest a possible buffering capacity existed within the system that suppressed proton release (Worrall *et al.*, 2008).

The conductivity of the Agricultural Mineral indicated a high ionic content (Fig 8.9). With the exception of SO_4^{2-} ($r = 0.564$, $p < 0.01$), correlations (Table 8.6) did not demonstrate a direct relationship with any specific ion. Both upland streams did demonstrate significant positive and negative correlation coefficients between a number of ions and the DOC concentration (Table 8.7) which suggested a direct relationship, with the positive correlations suggesting adsorption by the DOC matrix and the negative correlations suggesting non-adsorbed or desorbed ions which were suspended in the water body. This supports what Freeman *et al.* (1993) who suggested that the DOC matrix traps ions that have been generated during respiration processes and the aerobically degraded re-wetted DOC matrix releases the ions.

Both the Upland and Lowland Agricultural Mineral streams exhibited NO_3^- inferences during the event. The lowland agricultural (av 6.98 mg/L) demonstrated the highest and constant concentration throughout. The largest most probable contributor was dominated by agricultural runoff. The Upland Mineral stream NO_3^- concentration demonstrated an association with both the DOC, stream flow and conductivity (Table 8.7) with a peak in concentration (0.48 mg/L) coinciding with that of the stream flow. The stream source can

only be assumed, due to its isolation from possible anthropogenic terrestrial sources to have been derived from atmospheric inputs, microbial activity and plant processes.

The SUVA and aromaticity values, calculated using UV-vis spectra absorbance again demonstrated relative fluctuations to DOC during the event (Fig 8.14) in the upland and lowland agricultural mineral streams. The normalised SUVA spikes suggested a change in the relative aromatic content of the DOC during the highest flow rate. This indicated that the physical flushing effect of the new water, washed out a higher concentration of the hydrophobic aromatic molecules into the two mineral streams. The upland peat SUVA and aromaticity values did not respond to the higher flow rates but a gradual increase over the event period was recorded for the UV-vis 254 nm λ absorbance value, i.e., 0.247 to 0.458 AU, rather than sudden sharp increases that was observed in the 254 nm λ during the DOC flush for the upland and lowland mineral (0.139 to 0.456 ; 0.135 to 0.319 AU) respectively. The SUVA correlation of the upland peat stream relative to its DOC was significant ($r = 0.924, p < 0.01$). It suggested that the catchment DOC was homogenous and consisted of a proportional concentration of aromatic molecules. The phenolic content also reflected the SUVA results and generated a significant correlation with the upland peat and upland mineral stream DOC (Table 8.2).

The Upland Mineral stream sample exhibited the highest THMFP for the set of stream samples chlorinated from each catchment. The total THMs generated consisted of 93.9 % CHCl_3 and 6.1% CHBrCl_2 which suggests that Br^- (15 $\mu\text{g/L}$) reactions may have been limited by the concentration of Br^- in the source waters.

Both the total THMs formed by the upland Peat and the lowland agricultural Mineral DOC especially, generated varying concentrations of all four THMs. The lowland agricultural Mineral DOC generated the highest averaged concentrations of them both, e.g., CHCl_3 (75.7%, 47.7 $\mu\text{g/mg DOC}$), CHBrCl_2 (19.7%, 12.4 $\mu\text{g/mg DOC}$), CHBr_2Cl (4.1%, 2.6 $\mu\text{g/mg DOC}$) and CHBr_3 (0.5%, 0.3 $\mu\text{g/mg DOC}$), thus demonstrating that an increase in the concentration of Br^- (80 $\mu\text{g/L}$) influenced the specific THMs formed.

The upland Peat stream DOC with a Br^- concentration of 14 $\mu\text{g/L}$ which was 1 $\mu\text{g/L}$ less than the upland Mineral stream had a THMFP with the following distribution CHCl_3 (90.6 %, 81.2 $\mu\text{g/mg DOC}$), CHBrCl_2 (8.6 % 5.3 $\mu\text{g/mg DOC}$), CHBr_2Cl (0.2%, 0.2 $\mu\text{g/mg DOC}$) and CHBr_3 (0.6%, 0.5 $\mu\text{g/mg DOC}$). This suggested that, although the Br^- concentration is a controlling factor in the formation of brominated products it is not necessarily the limiting factor as THMFP may also depend on DOC molecular properties

All the highest concentrations of THMs/mg of DOC appear to have coincided with the highest flow rate. Considering that the THMFP is determined relative to a mg of DOC, therefore it is divorced from the actual overall change in DOC concentration. It is suggested that the DOC with high concentrations of THM precursors were increasing *in-situ* during the dry period and were released during the flush. The post event samples demonstrated the exhaustion of these THMFP precursors as the formation per mg of DOC dropped noticeably. The two mineral streams, Upland and Lowland falling by 37.9 and 37.8% respectively, but the upland Peat recorded less of a drop (17.7%). This tended to suggest that the removal of the residual pool of THM precursors was subject to the dynamics of the flow. This was exhibited by the flow characteristic of the upland peat (Fig 8.10) which was more dampened than those of the mineral stream, i.e., upland peat flow rate change over the event 0.2 m³/s; lowland agricultural Mineral flow rate change 3.4 m³/s.

8.5 Conclusions

The event described in this chapter demonstrated that a short dry episode during the spring period can result in the increased export of DOC out of both mineral and peat dominated catchments with the concentrations closely related to flow rates. The impact of the mixing of the 'new water' (rainfall average pH 5.5 – 6.5) and 'old water' (ground water) appears to have had a minimal influence on the pH of the upland Peat and lowland Mineral streams.

The upland Mineral pH did demonstrate a lowering (pH 5.9 → 4.8), this tended to suggest that the increase in H⁺ concentrations were due to DOC organic acids concentrations from the acidic upper layer of the catchment peat/mineral soils (Fig 8.5) being washed out and dissociating. Dilution/flush effects were recorded in the ionic content of the streams, these were demonstrated by negative and positive correlations (Tables 8.6, 8.7). A tentative conclusion could be proposed that the positive correlations with the DOC concentrations suggested possible aggregation and a capability to complex certain ions (Alvarez-Puebla and Garrido, 2005). The negative correlations implied a disassociated relationship with the DOC and the change in ionic concentration was subject to the flow rate removing the free residual ion content within the interstitial sites of the peat or soil structures.

The increase and decrease in SO₄²⁻ concentrations relative to pH changes proposed by Clark *et al*, (2005), (i.e., SO₄²⁻ concentrations > 2.5 – 5 mg/L control the pH) were not confirmed, although a pH increase was noted (Fig 8.19) when the SO₄²⁻ concentrations

dropped in the upland Peat stream, but was seen to increase with the increasing concentration of SO_4^{2-} in the upland Mineral stream concentration

The SUVA values which are considered to indicate DOC aromatic content could not be assigned as a THMFP surrogate which tended to confirm a number of reports in the literature, that SUVA is a weak indicator of THMFP Weishaar *et al.*, (2003) , Qunshan *et al.* (2008) and Fram *et al.* (1999).

Phenolic concentrations did not demonstrate a relationship to THMFP (Fig 8.18) although a significant correlation (r 0.854, $p < 0.01$) exhibited when analysed using the mg/L concentration *versus* THMFP. Statistical re-analysis using the actual concentration of phenolics per mg of DOC demonstrated insignificant correlations, suggesting that although having overall phenolic concentrations determined, only the activated OH substituted aromatics participate in THMFP.

The export of DOC is limited during dry periods and the concentration of THMFP molecular structures remain *in-situ* and increase relative to microbial activity. The re-wetting hydraulics of predominantly high mineral soils were recorded to flush the THMFP precursors out over a short period, whilst the peat dominated catchment THMFP precursors concentrations remained higher over a longer period indicating a diffusion mechanism within the peat structures. Comparing the actual export of DOC from the catchments, the lowland Mineral stream DOC at normal flow rates ($0.5 \text{ m}^3/\text{s}$) removed 7.2 Kg/h and at the height of the event was removing 105.8 Kg/h. The upland Peat stream at its normal flow rate of $0.45 \text{ m}^3/\text{s}$ would have removed 12.3 Kg/h with an increase to 34.9 Kg/h when the flow was at its highest. The theoretical THMFP of these DOC loadings at their highest during the event would represent a total THMs/hour of 6.7 Kg/h for the Mineral stream and 3.0 Kg/h for the upland Peat stream. This represented a THMFP percentage of 6.3 % for the lowland Mineral and 8.6 % for the upland Peat streams, compared to 5.9 % for the lowland Mineral and 6.1 % for the upland Peat streams THMFP at normal flow.

Chapter 9

Conclusions.

Chapter 9 Conclusions.

9.1 Introduction.

Each of the previous chapters have been dedicated to an aspect relating to the characterisation and THMFP of the four lakes (Cefni, Cwellyn, Conwy and Teyrn) selected for the project. This chapter summarises the results and findings presented throughout the thesis identifies specific aspects of the DOC character of each lake that may influence THMFP and draws conclusions from the work.

9.2 Overview

This project has investigated the influences of DOC concentrations, and characteristics and on THMFP of the water in the lakes/reservoir catchments described below:

- a) Cefni : minerotrophic fen/agricultural catchment. (lowland)
- b) Conwy: ombrotrophic blanket bog catchment. (upland).
- c) Cwellyn: mixture of thin peat rankers /soil/agricultural steep slopes catchment (upland).
- d) Teyrn: thin peat soils/steep slopes shallow/adjacent bog catchment (upland).

Having sampled the four lakes monthly over a period of 24 months the data collected has highlighted the importance and role of uncontrolled environmental factors on the type and concentration of DOC in the lakes. The drought/re-wet event sampled in (May 2011) with demonstrated how rapid changes associated with a dry warm period followed by an intense pulse of rainfall altered the equilibrium of the catchment streams. A similar dry/wet event, during the late spring/early summer period of 2010 was also captured during the monthly sampling regime.

In conjunction with the collection of the monthly Raw Surface Water samples, treated water samples (Pre-Chlorination) which had been subject to filtration and coagulation within the treatment plants were also collected over a twelve month period. These samples were collected for comparison of the THMFPs between treated and untreated water and demonstrated that coagulation efficiencies could vary relative to the seasons, suggesting molecular structural/charge differences in the DOC.

Surprisingly, the overall THMFP of the actual DOC samples collected over the 24 months only represented 0.7 to 1.1 % of each mg of DOC, which by weight/weight was 7 to 11 μg per 1000 μg of DOC. The presence of the very low concentrations of active DOC,

effectively generated a difficulty in associating bulk characterising properties such as SUVA, Phenolics, Aromaticity, MM and the ratio A_{253}/A_{203} with the THMFP. Hence to utilise them as THMFP surrogates requires care.

The order of mean Raw Surface Water DOC concentrations in the 4 lakes over the period of sampling October 2009 – October 2011 were as follows: Cefni > Conwy > Cwellyn > Teyrn (Chapter 3, section 3.1.1). The differences between lakes can mainly be attributed to the topography, soil type and extent of anthropogenic influence within the catchments, but it was also demonstrated from the monthly sampling that DOC concentrations responded positively to two external influences, rainfall and temperature (Chapter 3, Table 3.8) which complemented the work of Fenner *et al.* 2005; Worrall *et al.* 2003 and Wetzel *et al.* 1992. The three lakes Cefni, Cwellyn and Teyrn DOC exhibited significant correlations with temperature. The Conwy lake with its blanket bog demonstrated an insignificant response to temperature, but evidence of a destabilisation of the steady state was exhibited between its DOC concentration and rainfall (r 0.553, $p < 0.05$) (Table 3.18). This we concluded, resulted from the physical proximity of the lake to the surrounding bog. With the water table being maintained at a steady state, and any oxic/anoxic interface changes were minimal and restrained (Clark *et al.* 2009; Freeman *et al.* 2001).

DOC concentrations increased during the autumn in the four lakes. This change is mostly due to the vegetation death within the catchments and the subsequent release of degradation products and DOC during the increasing autumn rainfall. This was described by Scott. (2001) and Dawson *et al.* (2008) as the autumn flush. The dry period during the spring of 2010 also highlighted the influence of reduced rainfall and the subsequent water table drawdown which would be accompanied by an increasing influx of O_2 into the upper peat layers. Along with increasing late spring temperatures, this would promote increased microbial activity and the consequential increase in degradation products (Freeman *et al.* 2004). The samples taken after the late July rains demonstrated an increase of approximately (+ 29%) in the Cefni DOC concentration during August, but only a small increase was initially recorded in the upland lakes, e.g., Conwy (+14%) but it increased to (+39 %) in September 2010. This was probably due to initial re-saturation of the peat upper surface layers. Evidence of increases in DOC concentrations after a dry period was also recorded during the event monitoring (May 2011). Rapid increases were recorded with the flow (Figs 8.10) in the lowland mineral stream, but a time lag of 10 hours was evident for the peat stream. This we concluded was related to flow dynamics through the different mineral soil/peat structures and depths Dawson *et al.* (2010); Clark *et al.* (2007); Zaslausk

and Sinai, (1981) and a slower diffusion rate of DOC out of the peats immobile water as reported by Worrall et al. (2008). The data demonstrated rapid responses in some of the DOC documented characteristics. For example, SUVA and Aromaticity values in the lowland and upland agricultural/mineral demonstrated obvious step change increases, but did not do so in the upland peat stream. This we conclude was due to the rapid passage of new water through the organic rich surface layers of the mineral soils, as described by Horberger *et al.* (1994). The flush of new water was also seen to influence the pH in both upland streams, with the upland peat stream demonstrating a small increase in pH (6.7 to 7) at peak flow before falling back to a background average of pH (6.7). The opposite occurred for the upland mineral, with a drop in pH during the peak flow (pH 5.9 to 4.8). The lowland agricultural/mineral did not demonstrate any obvious variation in its pH. Similarity between the phenolic concentrations changes relative to DOC was also seen in the event and monthly samples, i.e an increase during high rainfall.

This relationship between phenolics and DOC demonstrated increases during the autumn flush and correlations were significant (Chapter 3, Table 3.4) with again the exception of the Conwy lake which demonstrated an insignificant correlation (r 0.347, p >0.05). Although phenolic compounds are a major part of DOC and increased rainfall will inevitably flush the less microbially processed (reduced form) out of the catchments, an interesting relationship between phenolics and the DOC concentration was established. We established that as the percentage of phenolic compounds per mg of DOC increased the DOC concentrations in the lake Raw Surface Water decreased and *vice versa* (Chapter 3, Fig 3.8). This restraining influence by phenolic compounds on the lability of DOC was suggested by Freeman *et al.* (2001) and labelled the 'latch'. In our data this was further highlighted by the low percentage of phenolics (mean 13% per mg DOC) in the Cefni compared to the upland lakes (> 20% per mg DOC). Hence it could be concluded that the phenolic concentrations that are part of the overall DOC makeup are one of the principal factors influencing its mobility and THMFP. Other factors considered to promote solubility of DOC, i.e., lowering ionic strength (Evans *et al.* 2006, Tipping and Woof 1990) did not exhibit any clear relationships in our data. Cefni with the highest ionic strength and a relatively high stable conductivity did not correlate with either the high DOC concentrations or the concentration changes recorded within the sampling period, but Cwellyn with a pH close to neutral (Chapter 3, Section 3.1.7) was influenced by the changes in ionic strength. The decrease in conductivity was accompanied with an increase in DOC concentration, hence it suggested that the pH in combination with ionic strength may be another controlling factor in DOC transportation out of catchments. The

monitoring of the MM distributions by SEC at 254 nm did not establish large obvious changes relative to, for example the dry wet periods of 2010, but there was an increase in the larger MM distribution from late summer (Fig 3.15) which could be assigned to the reduction of microbial activity with temperature. With the reduction in activity an expected drop in phenol oxidation would be expected. This was recorded and the phenolic concentrations increased in all four lakes in the late autumn (Fig 3.6) by 100% in the Cefni and approximately 25 % in the upland lakes. Although the model compound data confirmed the reactivity of phenolic compounds with HOCl there were ring activation and de-activation properties associated with the hydroxyls. The reactivity of the aromatic ring was highly dependent on the positions of the hydroxyls, with di-hydroxyls *meta* to each other being the most favourable, with major ring de-activators, the carboxyl groups, demonstrating large reductions in reactivity. This was also reported by Westerhoff *et al.* (2004), Bond *et al.* (2009) and Rook, (1977). These reactivity differences could be directly attributed to the higher THMFP of the upland DOC compared to the lowland. A comparison of the relative infrared spectra of the freeze dried Raw Surface Water DOCs furthered this argument as the upland and lowland DOCs exhibited two obvious molecular H-bonding differences. Although difficult to assign specific functional groups, Cefni tended to suggest a higher inference of carboxylics with its broad OH stretches and broad stretch at 1630 cm^{-1} . Hence, suggesting an increased de-activation of the phenolic substituted rings. There was also evidence of a more hydrophilic DOC in the Cefni compared to the Conwy and the two other upland lakes, with a much lower absorbance at 254 nm (Fig 3.9), this was further validated by a mean SUVA of $3.4\text{ Lmg}^{-1}\text{m}^{-1}$ for Cefni and an average of $5.2\text{ Lmg}^{-1}\text{m}^{-1}$ for the upland lakes, with Conwy demonstrating a particularly high value of $5.9\text{ Lmg}^{-1}\text{m}^{-1}$. Comparable infrared spectra (Figs 6.25) to that of the Cefni Raw Surface Water were collected for both Neutral fractions of the Cefni and Conwy. Hence it would be expected that the THMFP of both would be relatively similar and lower. This was confirmed with the Cefni and Conwy's Neutral fraction demonstrating a CHCl_3 formation potential of 32.4 and 19.4 $\mu\text{g} / \text{mg DOC}$, respectively compared to that of the HPOA fraction (Chapter 6, Figs 6.25), which is considered to be the more hydrophobic and is usually reported as the principal THM precursor (Xue *et al.* (2009). The infrared spectrum of both Cefni and Conwy HPOA samples showed an increase in intra-molecular hydrogen bonding, although less for the Cefni. The resulting CHCl_3 formation potential for both were the Cefni: 91.97 and Conwy: 202.6 $\mu\text{g} / \text{mg DOC}$. SUVA in effect supported these results, with a higher value for the HPOA and lower for the Neutrals, although there were no significant correlations for SUVA and THMFP found

in the overall sample data. This absence of significant correlation between THMFP and SUVA was also reported by White *et al.* (2003), but not for the data reported by Kristiana *et al.* (2009) although their observations included disinfectant by products other than THMs.

Further evidence of a higher inference of carboxylic functional groups in the Cefni DOC was suggested by the increase in phenolics (+ 23.3 %) after coagulation. This, as has been discussed in the main body of the thesis may have been due to the easier removal of the poly-hydroxy aromatic molecular moieties with high levels of carboxylic substitution, hence they can be easily deprotonated (pKa, *ca.* 4) to form charge moieties, resulting in suspension disruption by the coagulant and the resulting floc being effectively removed (Franceschi *et al.* 2002). The data associated with the SEC of the Pre-Chlorination samples indicated a removal of the larger MM ranges, with the majority of the remaining range (50%) with a mean MM of 2,000 to 1,000 Da, but significant increases in THMs reported by Chang *et al.* (2001) and Wong *et al.* (2007) for low MMs were not recorded. Although a reduction in both DOC concentrations, MMs and THMFP was established by the treated water (Pre-Chlorination) and the (Finished Water) the THM formed were of the same order, i.e., $\text{CHCl}_3 > \text{CHCl}_2\text{Br} > \text{CHBr}_2\text{Cl} > \text{CHBr}_3$, the distribution of the species changed, with an increase in the brominated compounds recorded in both the laboratory and works chlorinated samples in both monitored sites, Cefni and Cwellyn. The Cefni Finished Water brominated compounds increased by + 53 % and Cwellyn's by + 42 % above that of the Raw Surface Water laboratory chlorinations. The laboratory chlorinations of the works filtered and treated samples (Pre-Chlorination samples) similar to the Finished Water also demonstrated an increase in the brominated compounds after chlorination with NaOCl of +15 % for the Cefni and + 6 % for Cwellyn. Some DOC characteristics may have influenced this change, for example, the coagulation processes may have reduced the more substituted aromatics, leaving behind un-saturated rings for which HOCl demonstrates a slow or negligible affinity (Deborde and von-Gunten, 2008), hence the HOBr oxidant becomes the principal halogenation species and reacts readily with unsaturated bonds. Another contributing factor may have been due the use of $\text{Cl}_{2(g)}$ by the water treatment plant effectively oxidizing the natural Br^- more efficiently than NaOCl.

Although the data presented cannot culminate in an unequivocal set of conclusions, there was evidence that THMFP increases during and after a dry period suggesting active modification by microbial processes, with the Conwy lake associated with the bog catchment having the highest THMFP and the Cefni with its predominant fen catchment

with the lowest. This different physiochemical characteristic may be directly related to the residence times of the DOC (Rosario-Oritz *et al.* 2007), with the DOC passaging through the bog at a slower rate, hence longer microbial contact. We found no direct relationship between phenolic concentrations and THMFP and it was considered necessary to have the hydroxyl functional groups in specific ring positions to have a positive THMFP. Infrared spectroscopy suggested β -diketones as the active functional groups related to THMFP in DOC. Again, as reported by White *et al.* (2003), the data established that SUVA and Aromaticity were not found to be THMFP surrogates.

9.3 Concluding points and implications

- The fen catchment demonstrated the greatest response to dry conditions whilst the blanket bog remained in a more stable state.
- DOC and temperature exhibited correlations in three of the four lakes/reservoirs with Conwy (blanket bog) demonstrating no response.
- A correlation between DOC and rainfall was only evident in the Conwy.
- Monitoring of DOC flushing during a rainfall event demonstrated that the topographical characteristics of the catchment influenced the DOC release. The DOC release and transfer to the stream for blanket bog peat was slower and prolonged whilst the mineral streams response was faster and shorter.
- The removal of DOC in treatment plants was effective but there was limited evidence of an unidentified characteristic reducing removal in the early spring period.
- DOC phenolic compound concentrations per mg were lowest in the Cefni.
- DOC concentrations reduced with an increase in phenolic compounds in the Cefni, Cwellyn and Teyrn DOC, but was independent of the DOC in the Conwy.
- Phenolic compounds demonstrated an increase in late summer-early autumn in all four lakes/reservoirs suggesting decreases in microbial activity.
- Phenolic compounds concentrations increased in the treated water (Pre-Chlorinated) of both Cefni and Cwellyn.
- Rainfall events caused phenolic compounds concentrations to increase relative to the stream DOC concentrations and their concentrations were highest in the peat stream samples. UV-vis spectroscopy demonstrated strong absorbance between 200 – 350 nm, indicating the presence of unsaturated/aromatic molecular structures.

- The DOC sample with the lowest absorbance at 254 nm was the Cefni, thus indicating lower concentrations of unsaturated/aromatic molecular structures than the Upland lakes/reservoirs.
- The HPOA and Neutral fractions demonstrated the highest absorbance at 254nm, suggesting high concentrations of unsaturated/aromatic molecular structures.
- SUVA values of the Upland lakes/reservoirs DOCs were $> 4 \text{ L/mg m}^{-1}$, suggesting high concentrations of aromatic structures. The SUVA value of the Lowland lake DOC was $< 4 \text{ L/mg m}^{-1}$, suggesting lower aromatic concentrations.
- SUVA values in the pre-Chlorination water DOC were reversed with Cefni $> 4 \text{ L/mg m}^{-1}$ and Cwellyn $< 3 \text{ L/mg m}^{-1}$. This suggested different responses by the DOC molecular structures to flocculation i.e., removal of non- absorbing DOC species in the Cefni and removal of absorbing species in the Cwellyn.
- SEC MM analysis of the Raw Surface Water and the fractioned DOC exhibited dominant ranges between 5,000 – 3,000 Da.
- All four lake/reservoir DOC demonstrated an increase in the 4,000 – 3,000 Da range during the dry period April – July 2010, with a drop recorded when rainfall returned and an increase was recorded for the larger MMs (10,000 – 5,000 Da)
- Pre-chlorination MM range distributions were dominated by $< 2,000 \text{ Da}$ with the 10,000 – 2000 Da ranges reduced.
- PARAFAC analysis of the fluorescence spectra identified four principal components in the Raw Surface Water and three in the Pre - Chlorination water.
- Cefni demonstrated the highest concentration of ions. Ca^{2+} and SO_4^{2-} were the dominant ions present in all four lake/reservoir waters.
- NO_3^- concentrations were highest in the Cefni but were not a constant. Seasonal variations were recorded with November 2010 – April 2011 demonstrating the highest concentrations.
- Ionic concentrations increased in the Pre-Chlorination waters, suggesting inputs from treatment reagents.
- Fractions retained trace concentrations of the ions identified in the Raw Surface Waters, suggesting a DOC form of physical or ionic trapping. The majority of the ion concentrations were recorded in the Neutral fraction.
- Cefni pH was > 7 due to high inferences of geological carbonates, Cwellyn, Teyrn and Conwy ≤ 7 due to organic degradation by- products and presence of geological acidic/neutral formations .

- IR spectroscopy identified common functional groups in both Conwy and Cefni freeze dried DOC, but Cefni demonstrated a bias to the Fulvic Acid spectra of the Nordic reference sample (IR 105 F) and Conwy to the Nordic Humic reference sample (IR 105 H).
- The principal THM formed by the four lake/reservoir DOC was CHCl_3 .
- The Upland DOC had the highest THMFP with the blanket bog (Conwy) of the group of 3 generating the highest concentration/mg of DOC.
- The THMFP of each of the four lake/reservoir DOC increased over the dry period April – July 2010, with Conwy demonstrating the greatest increase although it exhibited stable DOC concentrations.
- The formation of brominated products increased after coagulation and flocculation.
- SUVA and Aromaticity are not necessarily surrogates of the DOC THMFP.
- Although phenolic compounds are important THMFP precursors, their properties are the key factor to their ability to form THMs. That is, phenolic compounds may be present in high concentrations but not necessarily active in the presence of oxidizing agents.

The above points suggest that if meteorological events continue to change and demonstrate greater perturbation and extremes, treatment plants would require contingency plans to respond to the rapidly imposed concentration changes.

The increase in phenolics during autumn and reduction in DOC removal in spring during the coagulation and flocculation tends to indicate that the treatment process is not effectively disrupting the molecular charges of DOC the phenolic compounds, especially during late autumn. Hence the determination of the Zeta potential may require constant updating during periods of high phenolic and DOC concentration.

The increase in brominated products in the post treatment samples suggests that bromide contamination is being introduced via the treatment reagents, or the treatment coagulants are catalysing the reactions. With brominated products having greater health implications (Bull and Kopfler. 1991), the necessity to intensify the use of coagulants to flocculate greater DOC concentrations may increase them to dangerous levels.

Finally, the proposed hypothesis (Chapter 1, Section 1.8), stating that the DOC character and THMFP of each lake/catchment was unique, could not be established conclusively as the data collected for this work confirmed underlying DOC characteristic similarities with some subtle unique aspects.

10.0 Further work.

10.1 Characterisation of DOC removed by coagulant

Although the Finished Water was within the limits of current legislation (total THMs 100 µg/L) the increase in brominated THMs after coagulation and chlorination in the water treatment works contact tank requires further investigation to establish if the current practice of injecting chlorine gas directly into the water flow influences the formation of brominated THMs. An increased formation of brominated THMs was also noted in the Pre-chlorination samples that were laboratory chlorinated using NaOCl also demonstrated an increase in brominated THMs, but to a lesser extent. This suggests that coagulation also influenced the final THMFP. It would therefore be of interest to characterise the DOC removed by the coagulant over a time series and establish its THMFP.

10.2 Laboratory manipulation of variables that affect DOC characteristics.

It was clear from the data that both Conwy and Cefni lake DOC exhibited different THMFP. Under natural conditions there were a combination of variables that could not be controlled. Hence it would be advantageous to set up a laboratory based experiment where all these could be controlled, i.e. pH, water flow, water table, temperature, ions/nutrients and residence time.

10.3 Extension of characterisation techniques

The limited freeze drying of samples during this project requires to be extended to generate a larger sample base of Raw Surface Water and fractionated samples of both Cefni and Conwy DOC. These would be used to further investigate the differences between the bog and fen DOCs and their THMFP. An introduction of different techniques such as NMR (solid phase), IR, Matrix-assisted Laser Desorption Ionization (MALDI) and Mass Spectroscopy could be considered to further the DOC's characterisation with a view to identifying which functional groups predominate. Additional knowledge of these different catchments could be acquired by characterising and monitoring the contribution of the root exudates of the vegetation to the DOC pool over a time period.

Appendices

Appendix 1

1.0 Validation of Fractionation of Freshwater Dissolved Organic Carbon using DAX 8 (XAD 8) and XAD 4 Resins in Tandem

1.1 Introduction

The objective of exercise is to validate the method and establish the resin carbon bleed of an in house designed and built automated fractionation method using both DAX 8 and XAD 4 resins in tandem to fractionate lake and reservoir freshwater DOC. The method uses High Performance Liquid Chromatography (HPLC), in-house packed columns and a standard HPLC quaternary pump connected to a series of Rheodyne® valves controlled by computer. The series of validation experiments will establish the DAX 8 and XAD 4 resin carbon bleed and will make a comparative study of a series of samples fractionated by the manual and automated system.

2.0 Experimental

2.1 Resins and Columns

The DAX 8 resin is an hydrophilic acrylic ester with low ion exchange capacity (10^{-2} Mequiv g^{-1}), average pore size of 225 Å and surface area of 160 $m^2 g^{-1}$. The XAD 4 resin (a styrene-divinylbenzene copolymer) is highly aromatic and hydrophobic and possess no ion exchange capacity. The average pore size is much smaller at 40 Å and the surface area much larger at 725 $m^2 g^{-1}$. XAD resins have previously been reported for the successful separation/removal of fulvic and humic acids from water (Aiken *et al.*, 1979; Malcolm, 1992).

To calculate the minimum resin quantity for the DAX 8 and XAD 4 fractionation columns, Equation (1) was re-arranged to give (2). V_0 was calculated where $V_{0.5r}$ is the sample volume and $k'_{0.5r}$ is the distribution coefficient of resin sorbed solute *versus* solute dissolved in water (Leenheer, 1981).

$$\text{Eqn (1)} \quad V_{0.5r} = 2V_0 (1 + k'_{0.5r}) \qquad \text{Eqn (2)} \quad V_0 = V_{0.5r} / 2 (1 + k'_{0.5r})$$

e.g., for a 1000 ml sample where $k'_{0.5r}$ is 50, $V_0 = 9.8$ ml. Given that the void volume of the resins is *ca.* 65% (Malcolm, 1992) the minimum volume of resin required is 15 ml (9.8/0.65).

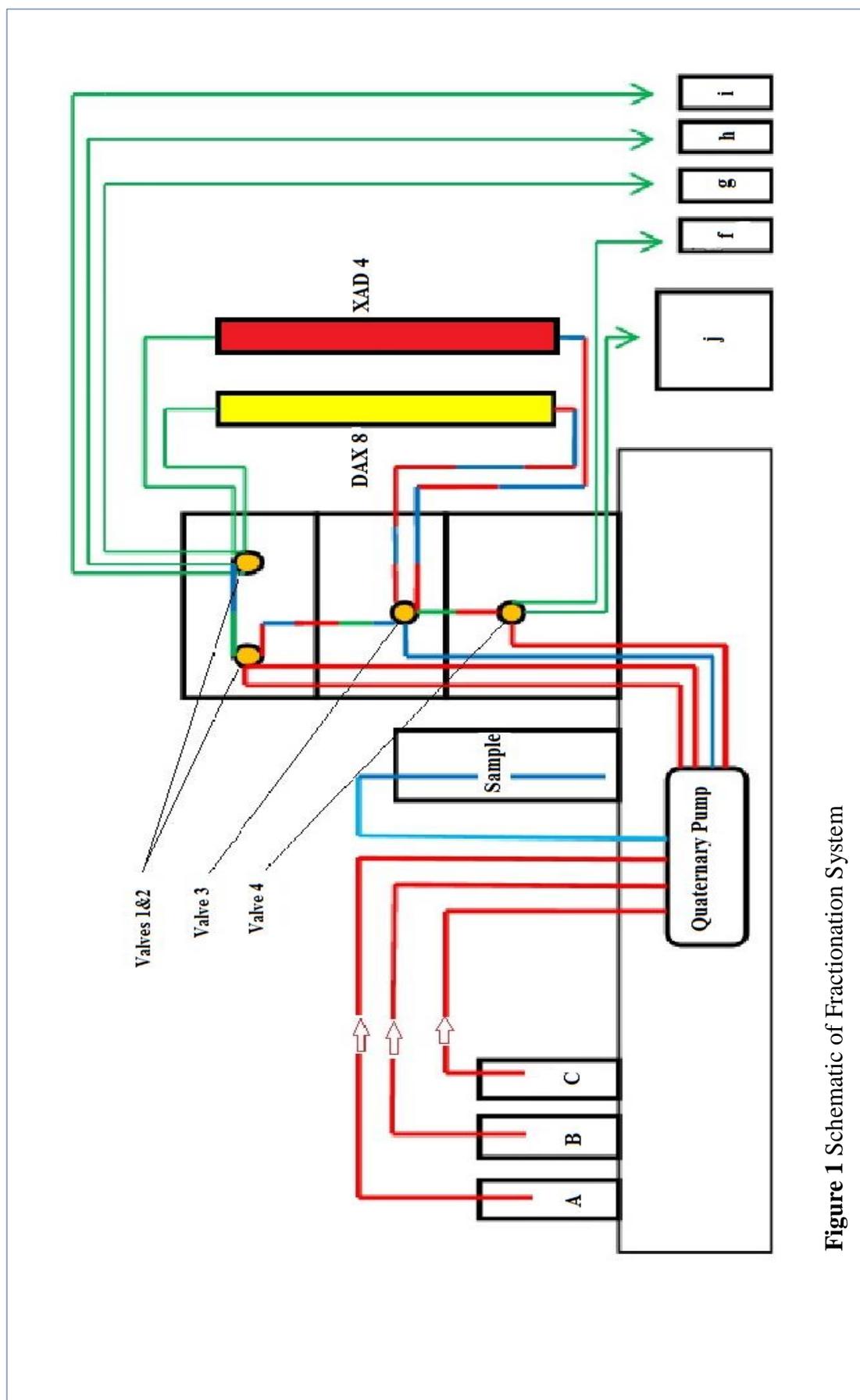


Figure 1 Schematic of Fractionation System

The tandem column arrangement was prepared using two pristine, blank HPLC columns (250 mm x 10 mm). Each column was packed with 25 cm³ of the appropriate resin. The resin volumes used represent 40% more than the calculated, minimum volume required for a 1 litre sample. Each column entrance and exit was then fitted with a 0.2 µm frit (Supelco Analytical) and sealed in place with HPLC end fittings.

Prior to packing, each resins was first washed in NaOH_(aq) (0.1M) for 24 h. Floating debris was removed by careful decantation, the NaOH_(aq) was removed and the resin washed with de-ionised water until pH neutral. The resin beads were then transferred to a 50 ml burette followed by series of alternate washes with methanol (AnaLar) and acetonitrile (AnaLar). The resin was then rinsed with de-ionised water to remove residual methanol and acetonitrile before packing into the HPLC columns as a slurry. The DAX 8 and XAD 4 columns were then connected in tandem to a series of 4 Rheodyne® valves using PEEK fittings and tubing (0.030" ID) as shown in Figure 1.

The HPLC quaternary pump and valves are controlled by a pre-programmed method loaded onto an Agilent hand held controller. Eluent bottles and receivers are attached as; a) and b)

- a) Quaternary pump tube to the 1L '**Sample**' bottle, tube **A** to 'de-ionised water' bottle, tube **B** to the 'NaOH (0.1M)' bottle and tube **C** to the 'HCl (0.1M)' bottle.
- b) Four 100mL bottles (**f,g,h,i**) are connected to the configured outlet pipes of the valve systems, **1,2,3** and **4** to collect the hydrophobic acid, hydrophilic acid, hydrophilic base, and hydrophobic base fractions. A 1L bottle (**j**) is placed to receive the effluent (neutral fraction) during the column loading procedure (**Fig 1**)
- c) The tailored method program is initiated by the hand controller and the following actions proceed automatically for the sample loading of the columns and their subsequent desorption and fraction collection:-
 - 1) The 1L sample is pumped at 4mL/minute through the columns in tandem and the effluent is collected in (**j**)
 - 2) Column wash sequence: 50 mL of deionised water passes via both columns to a waste container.
 - 3) Valve switches to isolate the DAX 8 column and 60mL of NaOH (0.1M) at 2mL/minute desorbs the hydrophobic acid to bottle (**f**).
 - 4) DAX 8 column is then washed with 40mL of de-ionised water at 2mL/minute to bottle (**f**).

- 5) DAX 8 column is then desorbed with 60mL of HCl (0.1M) at 2mL/minute and the hydrophobic base is collected in bottle (g). The column is again washed with de-ionised water (40mL) into bottle (g).
- 6) Valve switches and then isolates DAX 8 column and opens to desorb the hydrophilic acid and base of XAD 4 column to bottles (h and i) as per steps 3,4,5.
- 7) The valves then re-configure and the DAX 8 and XAD 4 are washed in tandem with de-ionised water (50 mL) at 2mL/minute to waste.
- 8) The columns are then re-generated by pumping 50mL of HCl (0.1 M) at 2mL/minute via column A and B in tandem to waste.
- 9) The valves at the end of the regeneration step switch to start and the system is ready to fraction the next sample.

2.2 Resin Bleed

To establish and measure any resin bleed, five 1L aliquots of milli Q water were fractioned in a continuous sequence. Each fraction was collected as described above and the TOC was measured with an Analytical Services Ltd, Thermalox TOC/TN analyser. Standards (0 to 5 mgL⁻¹) were prepared using Potassium Hydrogen Phthalate, AR grade (Lot No F78575AR). The results demonstrated that there was evidence of column bleed but collectively was limited to < 1.0mg/L for each complete sequence of fractions.

Table 1 Five 1 L samples of acidified Milli Q water passed through the DAX 8 and XAD 4 resins in tandem, each fraction recovered was analysed for total organic carbon			
Sample	Fraction	Average Carbon (mg/L)	Standard Deviation
1,2,3,4,5	Hydrophobic acid	0.17	0.025
1,2,3,4,5	Hydrophilic acid	0.15	0.026
1,2,3,4,5	Hydrophobic base	0.04	0.008
1,2,3,4,5	Hydrophilic base	0.03	0.010
1,2,3,4,5	Neutrals	0.27	0.080
	<i>Sum of Averages</i>	0.66	

2.3 Natural DOC Samples

Two natural fresh water samples (February 2012) were selected for the exercise; Cefni and Conwy (Table1). Five 1L aliquots of each were collected and were vacuum filtered through pre-washed Nylon membrane filters (0.2 μm) within 3hours after sampling. The pH was then adjusted to 2.5 with $\text{HCl}_{(\text{aq})}$ (2 M) and stored in darkness at 4 $^{\circ}\text{C}$.

Table 2. Sample sources, descriptions and typical DOC concentration values.		
Sample Source	Source Description	DOC(mg l^{-1})
Cefni	Man-made reservoir with fen as principal catchment.	6.50 ± 1.2
Conwy	Shallow upland lake/reservoir within blanket peat bog	4.28 ± 0.4

Table 3. Eluents used to desorb Hydrophobic Acid and Base fractions from DAX 8 resin and Hydrophilic Acid and Base fractions from XAD 4 resin		
Fraction	Eluent and Volume (ml)	Deionised water (ml)
<u>DAX 8</u>		
Hydrophobic Acid	60 ml, 0.1M $\text{NaOH}_{(\text{aq})}$	40
Hydrophobic Base	60 ml, 0.1M $\text{HCl}_{(\text{aq})}$	40
<u>XAD 4</u>		
Hydrophilic Acid	60 ml, 0.1M $\text{NaOH}_{(\text{aq})}$	40
Hydrophilic Base	60 ml, 0.1M $\text{HCl}_{(\text{aq})}$	40

The recovered Hydrophobic and Hydrophilic Acid and Base fractions for the samples using the above method were each diluted to 1 litre with de-ionised milli Q water. The DOC in each fraction plus the non-retained Neutral was then measured and the DOC data from these samples is shown in Tables 3, 4.

Table 4. % DOC concentration per fraction of Cefni.							
	DOC (%)						
Fraction	Cefni S1	Cefni S2	Cefni S3	Cefni S4	Cefni S5	SD	RSD
Hydrophobic Acid	36.6	37.2	37.9	34.3	36.6	1.34	0.04
Hydrophobic Base	0.8	1.1	0.8	1.2	0.8	0.22	0.24
Hydrophilic Acid	26.8	27.2	27.1	26.1	27.2	0.45	0.02
Hydrophilic Base	1.1	0.9	1.1	1.1	0.9	0.11	0.08
Non- retained Neutrals	29.9	19.9	28.0	27.7	28.9	4.01	0.15
% Recovery	95.1	86.3	94.8	90.5	94.5	3.80	0.04

Table 4. % DOC concentration per fraction of Conwy							
	DOC (%)						
Fraction	Conwy S1	Conwy S2	Conwy S3	Conwy S4	Conwy S5	SD	RSD
Hydrophobic Acid	38.8	40.9	38.9	38.1	37.6	1.26	0.03
Hydrophobic Base	0.3	0.3	0.6	0.8	0.5	0.57	0.03
Hydrophilic Acid	21.3	21.1	20.3	21.3	20.1	0.21	0.42
Hydrophilic Base	0.9	1.1	0.9	1.2	0.9	0.14	0.14
Non- retained Neutrals	33.9	32.7	35.0	36.2	34.7	1.3	0.03
% Recovery	95.2	96.1	95.7	97.6	93.8	1.38	0.01

Table 5 Cefni and Conwy fractions using standard and automated methods.				
	DOC (%)			
Fraction	Cefni Oct 2010 (Standard Method)	Cefni Oct 2011 (Auto method)	Conwy Oct 2010 (Standard Method)	Conwy Oct 2011 (Auto method)
Hydrophobic Acid	39.7	38.6	38.5	41.5
Hydrophobic Base	1.5	0.3	0.4	0.1
Hydrophilic Acid	22.2	25.5	27.1	23.7
Hydrophilic Base	1.1	0.2	0.4	0.1
Non- retained Neutrals	28.6	29.6	28.4	30.0
% Recovery	93.1	94.2	94.9	95.1

The data show similar trends (Fig 2) between the two different sites with the Hydrophobic Acid and Neutral fractions showing the highest percentage DOC for all samples. For both sites, the Hydrophobic and Hydrophylic base fractions showed the smallest percentage DOC. The Relative Standard Deviation (RSD) and Standard Deviations (SD) of the data for both series highlights the small spread within the sets with acceptable recoveries that are comparable to ones reported in the literature (Leenheer, 1981; Peuravuori *et al*, 1997; Wei et al, 2007; Ratpukdi *et al*, 2009) The data also demonstrates that the resins operate selectively and that the subsequent % DOC differences between the Cefni and Conwy hydrophobic acids, hydrophilic acids and neutrals fractions reflect the variable molecular structures that exist within each individual DOC sample series. Table 5 includes the Cefni and Conwy fractionation results for October 2010/11 using the two methods the results are comparable considering that the ratio of the DOC components leaving the catchments may have been subjected to different meteorological conditions. The residual standard deviations of the collective sample fractions from Tables 3,4 and 5 was less than 1.

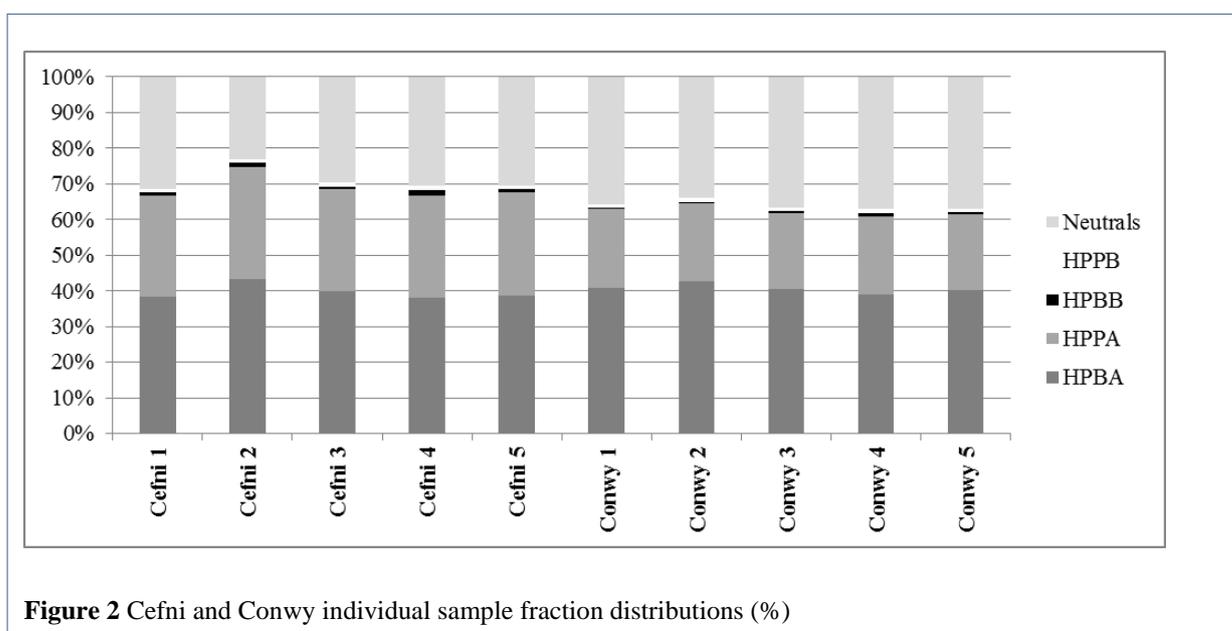


Figure 2 Cefni and Conwy individual sample fraction distributions (%)

2.4 Discussion

The combined resin procedure recovers natural DOC relatively simply without the use of contaminating organic solvents or reagents. The sequence of resins is important in part because of the significant difference in pore size and surface area between the resins. This combination of the DAX 8 and XAD 4 resins are believed to recover a large part of the DOC from natural water predominantly through physical processes (Malcolm *et al.*, 1992). The dimension of the pore sizes of the DAX 8 resin facilitate more rapid diffusion of fulvic and humic acid macromolecules (termed in the literature as the hydrophobic acids) in and out of the pores whilst interacting with the sorption sites held within them. When the hydrophobic acid macromolecules have been sorbed by the DAX 8, the sample then progresses through the XAD 4 resin. This resin consists of a highly cross-linked surface

with a much smaller pore size ($40 \text{ \AA} = 4 \text{ nm}$). These pores are believed to only allow the diffusion of lower molecular weight solutes, i.e., hydrophilic acids (Aitken *et al.*, 1979). The molecules which are described in this paper as the Neutrals are believed to exhibit characteristics that do not comply with the properties of the sorption sites of both resins, hence they are not retained.

Considering the DOC data for the fractions for the two sites in the above context, although there are differing relative concentrations, it can be seen that the responses are mirrored for both sites, although the subtle differences within the acid, base and neutral classifications can be determined. This supports the fact that rapid fractionation can be successfully achieved and the development of the method described here has significantly reduced the time and tedious work (McDonald *et al.*, 2004) required to fractionate samples. The classical method uses resins packed into glass columns and a peristaltic pump and is both labour intensive and time consuming. Here, using a combination of Rheodyne® valves, HPLC pump and HPLC packed columns, controlled with a tailored program, the time has been reduced to less than 8 hours for complete sample fractionation into its individual components without intervention at any point in the exercise. Although we have conducted this experiment to complete the fractionation within an 8 hour time period the columns and program control may be changed to accommodate different sample volumes, loading flow rates, desorption flow rates, resins and resin volumes.

2.5 Conclusion.

The automated system developed to fraction Raw surface Water is fully validated and is suitable for the purpose with recoveries of $> 80 \%$ and $\% \text{ RSD}$ of $< 2\%$ for repeatability and a standard deviation of $< 2 \%$ between the manual and automated method.

Resin bleed was established at 0.66 mg/L of carbon/sample

Appendix 2

1.0 Validation of GC Analytical Method for the Determination of Tri- halomethanes in Chlorinated Raw and Fractioned Water samples.

1.2 Introduction

Evaluation of the instruments capabilities and the method's characteristics were established by carrying out the following validation exercises using the International Conference on Harmonization (ICH) guidelines (Chan *et al.* 2004).

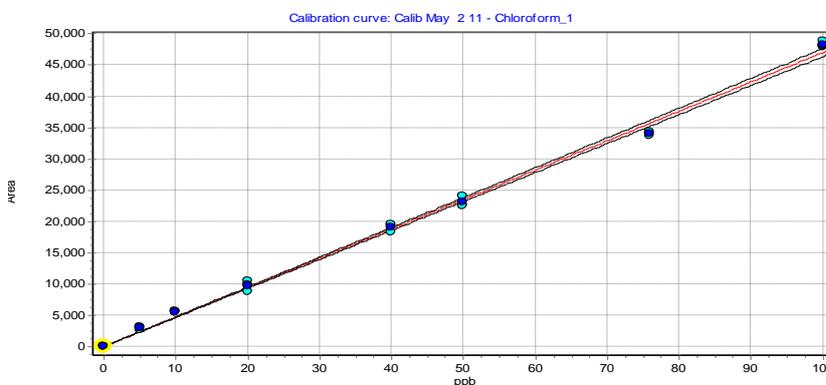
2.0 Results

2.1. Linearity/Standard Calibration Curves

A 20 µg/mL four component stock solution of the THMs Chloroform, Dichloromethane, Dibromochloromethane and Bromoform was prepared. Using the stock solution, a series of external calibration standards were made up in Milli Q water (18Ω) that included the following concentrations : 5, 10, 20, 40, 50, 76, 100µg/L. The concentrations were analysed as per method and instrument parameters stated. Standard calibration curves for each component were prepared using the pre-loaded 'Galaxy' program.

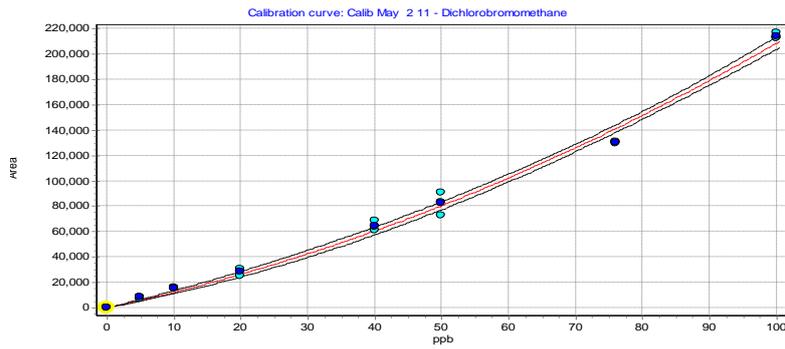
2.1.1 Results

Chloroform



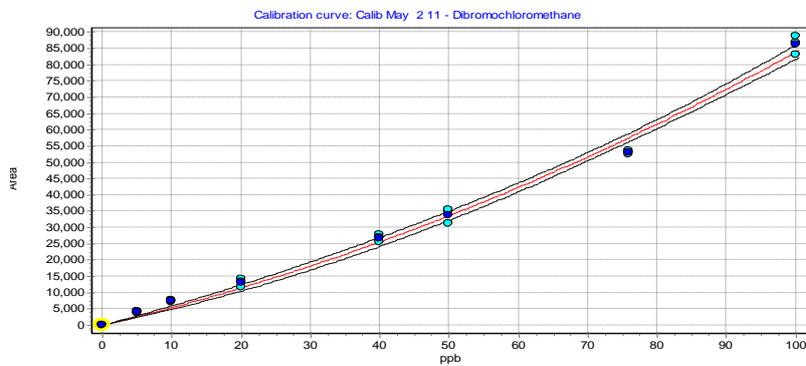
Regression coefficient: 0.9971

Dichlorobromomethane



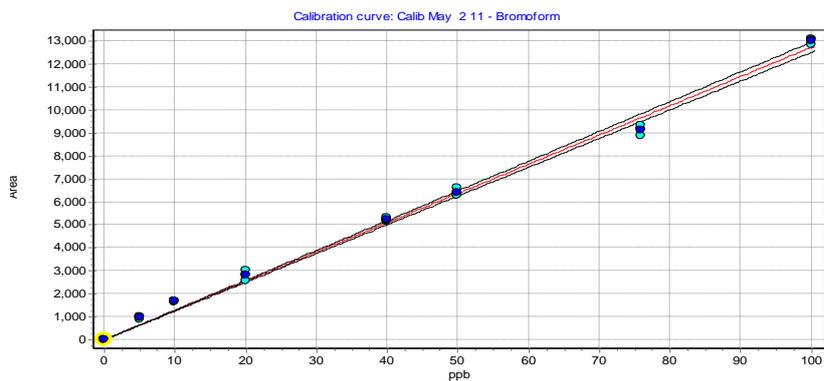
Regression coefficient: 0.9952

Dibromchloromethane



Regression coefficient: 0.9942

Bromofom



Regression coefficient: 0.9953

A non-linear response was achieved over the range of concentrations for both Dichlorobromomethane and Dibromochloromethane, hence the curves were generated using a 2nd order polynomial equation.

2.2. Accuracy

Recoveries were determined by preparing a series of concentrations of the four THM components Chloroform, Dichloromethane, Dibromochloromethane and Bromoform and analysing for them using the stated method, instrumental parameters and calibration curves.

2.2.1 Results

Chloroform

Component	Theoretical Concentration (ppb)	Analysed Concentration Mean (ppb)	% Recovery
Chloroform	1.0	1.11	111.0
Chloroform	10.0	10.4	104.0
Chloroform	50.0	50.07	100.1
Chloroform	100.0	99.68	99.7

Dichlorobromomethane

Component	Theoretical Concentration (ppb)	Analysed Concentration Mean (ppb)	% Recovery
Dichlorobromomethane	1.0	1.12	112.0
Dichlorobromomethane	10.0	10.59	105.9
Dichlorobromomethane	50.0	49.53	99.0
Dichlorobromomethane	100.0	93.94	93.9

Dibromochloromethane

Component	Theoretical Concentration (ppb)	Analysed Concentration Mean (ppb)	% Recovery
Dibromochloromethane	1.0	1.09	109.0
Dibromochloromethane	10.0	10.40	104.0
Dibromochloromethane	50.0	49.75	99.5
Dibromochloromethane	100.0	97.30	97.3

Bromoform

Component	Theoretical Concentration (ppb)	Analysed Concentration Mean (ppb)	% Recovery
Bromoform	1.0	1.02	102.0
Bromoform	10.0	10.06	100.6
Bromoform	50.0	50.35	100.7
Bromofrom	100.0	99.40	99.4

2.3 Precision/Repeatability.

Evaluation of the method's precision was achieved by repeated analysis (x 5) of the same sample, using the same instrument parameters as stated in the method. The samples were prepared by spiking Milli Q water with known concentrations (2.0, 40.0, 60.0 and 80.0 µg/L) of the four stated THMs.

2.3.1 Results

2.0 µg/L THMs

Component	Injection No	Result (ppb)
Chloroform	1	2.02
Chloroform	2	2.15
Chloroform	3	2.03
Chloroform	4	2.08
Chloroform	5	2.00
Dichlorobromomethane	1	1.99
Dichlorobromomethane	2	2.17
Dichlorobromomethane	3	2.15
Dichlorobromomethane	4	2.21
Dichlorobromomethane	5	2.07
Dibromochloromethane	1	1.98
Dibromochloromethane	2	2.19
Dibromochloromethane	3	2.07
Dibromochloromethane	4	2.11
Dibromochloromethane	5	2.06
Bromoform	1	2.05
Bromoform	2	2.18
Bromoform	3	2.02
Bromoform	4	2.16
Bromoform	5	2.00
	Mean	2.08
	SD	0.075
	%RSD	3.6

40.0µg/L THMs

Component	Injection No	Result (ppb)
Chloroform	1	40.96
Chloroform	2	40.97
Chloroform	3	40.26
Chloroform	4	39.66
Chloroform	5	40.02
Dichlorobromomethane	1	40.82
Dichlorobromomethane	2	40.70
Dichlorobromomethane	3	40.80
Dichlorobromomethane	4	40.53
Dichlorobromomethane	5	40.33
Dibromochloromethane	1	40.38
Dibromochloromethane	2	40.24
Dibromochloromethane	3	40.10
Dibromochloromethane	4	40.70
Dibromochloromethane	5	40.39
Bromoform	1	39.75
Bromoform	2	40.69
Bromoform	3	39.68
Bromoform	4	40.35
Bromoform	5	39.25
	Mean	40.32
	SD	0.473
	%RSD	1.2

60.0µg/L THMs

Component	Injection No	Result (ppb)
Chloroform	1	60.53
Chloroform	2	60.80
Chloroform	3	60.33
Chloroform	4	60.96
Chloroform	5	59.72
Dichlorobromomethane	1	59.45
Dichlorobromomethane	2	61.95
Dichlorobromomethane	3	60.79
Dichlorobromomethane	4	59.59
Dichlorobromomethane	5	60.80
Dibromochloromethane	1	60.51
Dibromochloromethane	2	61.95
Dibromochloromethane	3	60.03
Dibromochloromethane	4	59.90
Dibromochloromethane	5	60.91
Bromoform	1	59.67
Bromoform	2	60.23
Bromoform	3	60.20
Bromoform	4	60.85
Bromoform	5	60.73
	Mean	60.50
	SD	0.70
	%RSD	1.1

80.0µg/L THMs

Component	Injection No	Result (ppb)
Chloroform	1	79.87
Chloroform	2	79.20
Chloroform	3	80.27
Chloroform	4	80.50
Chloroform	5	80.04
Dichlorobromomethane	1	80.99
Dichlorobromomethane	2	79.12
Dichlorobromomethane	3	80.52
Dichlorobromomethane	4	80.64
Dichlorobromomethane	5	80.89
Dibromochloromethane	1	80.06
Dibromochloromethane	2	80.10
Dibromochloromethane	3	80.93
Dibromochloromethane	4	80.85
Dibromochloromethane	5	81.08
Bromoform	1	79.99
Bromoform	2	79.33
Bromoform	3	80.57
Bromoform	4	81.21
Bromoform	5	80.31
	Mean	80.32
	SD	0.62
	%RSD	0.8

2.4 Limit of Detection

The limit of detection (LOD) was determined using the signal to noise ratio approach (Chan *et al.* 2004). The noise of an area of a blank chromatogram, where peaks were absent was measured and recorded. The peak height of each THM component at a concentration of 1 ppb was measured and recorded. The signal to noise ratio was then calculated and used in the equation to determine the corresponding concentration that would give a signal to noise ratio of 3:1.

Component	Noise (μV)	Peak Height (μV)	S/N	LOD (ppb)
Chloroform	290	1900	6.5	0.46
Dichlorobromomethane	290	8000	27.6	0.11
Dibromochloromethane	290	6000	20.7	0.14
Bromoform	290	1600	5.5	0.54

2.5 Limit of Quantitation

The limit of quantification (LOQ) was determined using the signal to noise ratio approach (Chan *et al.* 2004). The limit is calculated using the same equation but the corresponding concentration is determined as one that would give a signal to noise ratio of 10:1

Component	Noise (μV)	Peak Height (μV)	S/N	LOQ (ppb)
Chloroform	290	1900	6.5	1.5
Dichlorobromomethane	290	8000	27.6	0.4
Dibromochloromethane	290	6000	20.7	0.5
Bromoform	290	1600	5.5	1.8

2.6 Conclusion

Evaluation of the results listed below generated by the GC 450 established its limits and capabilities for the analysis of the trihalomethanes; CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3

2.6.1 Linearity	
Component	Regression Coefficient
Chloroform	0.99
Dichlorobromomethane	0.99
Dibromochloromethane	0.99
Bromoform	0.99

2.6.2 Recovery	
Component (1 to 100 $\mu\text{g/L}$)	Mean Recoveries %
Chloroform	103.7
Dichlorobromomethane	102.7
Dibromochloromethane	102.5
Bromoform	100.7

2.6.3 Precision		
Concentration ($\mu\text{g/L}$)	SD	% RSD
2.0	0.075	3.6
40.0	0.473	1.2
60.0	0.70	1.1
80.0	0.62	0.8

2.6.4 Limit of Detection	
Component	Concentration ($\mu\text{g/L}$)
Chloroform	0.5
Dichlorobromomethane	0.1
Dibromochloromethane	0.1
Bromoform	0.5

2.6.5 Limit of Quantification	
Component	Concentration ($\mu\text{g/L}$)
Chloroform	1.5
Dichlorobromomethane	0.4
Dibromochloromethane	0.5
Bromoform	1.8

Appendix 3

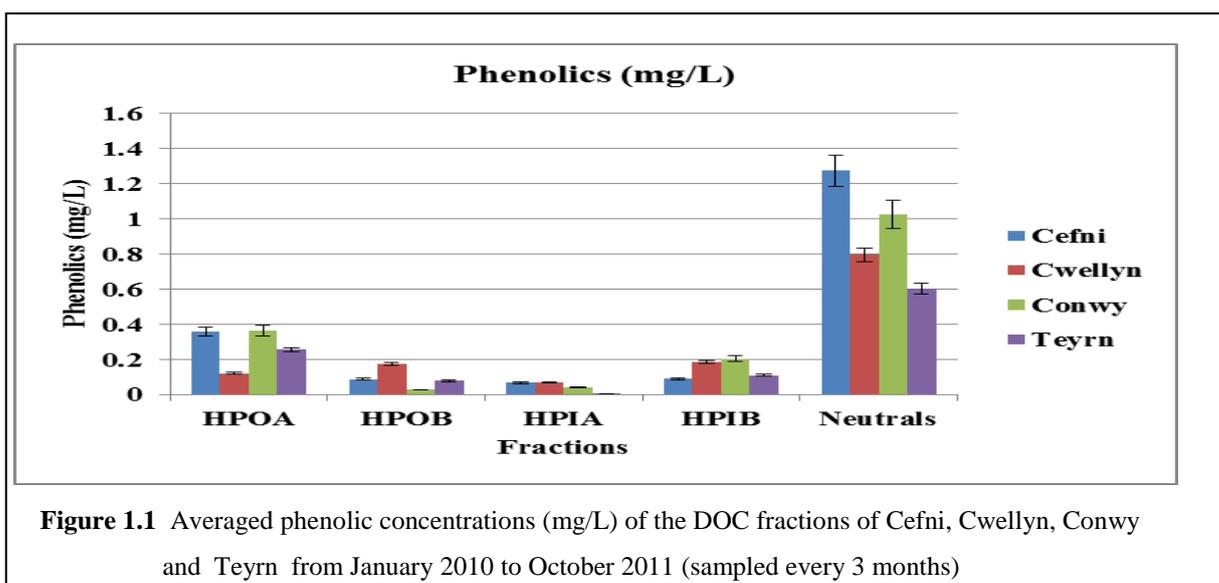
1.0 Determination of phenolic compounds in fractionated DOC samples.

1.1 Introduction

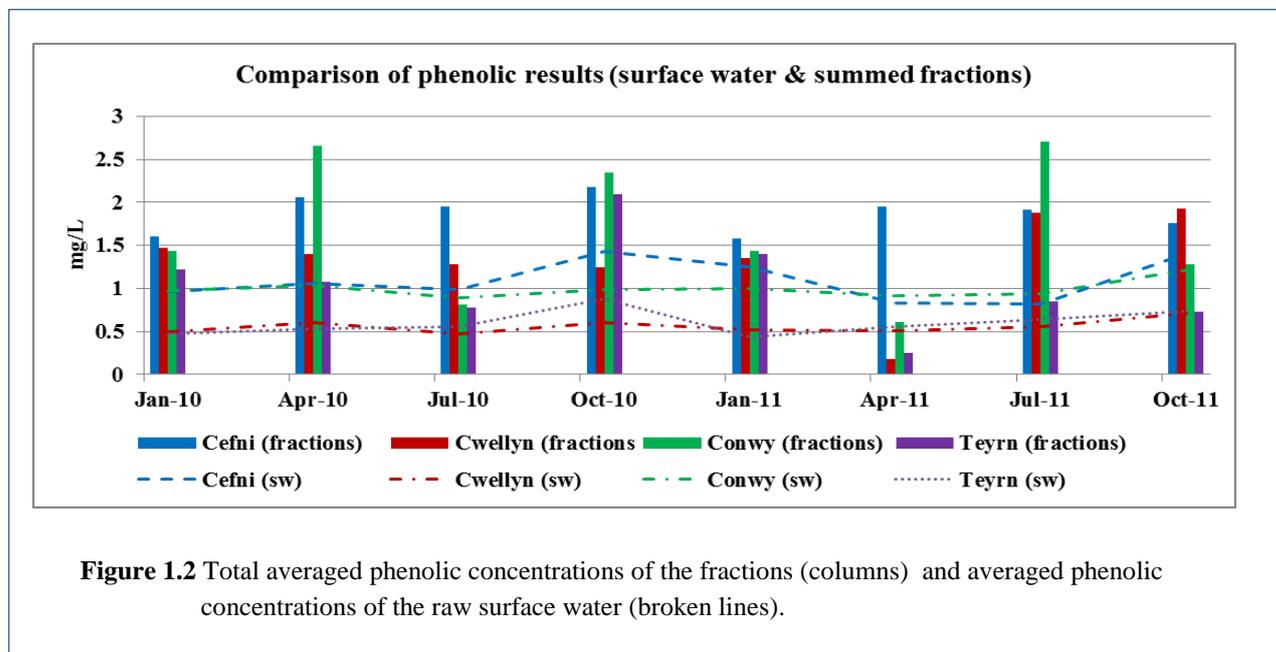
Phenolic concentration values ranged between 0.56 – 1.1 mg/L in the Raw Surface Water for the four lake samples Cefni, Cwellyn, Conwy and Teyrn for the period November 2009 – October 2011. Phenolic concentrations of the individual fractions during the exercise could not be accounted for neither did the fractionated Raw Surface Water samples analysed did not demonstrate a mass balance recovery within the acceptable limits of 80 – 120 % (ICH guidelines, Chan *et al.* 2004). A limited investigation into possibly influences such as resin bleed, matrix effects could not be identified as positive contributors, hence a conclusion could not be established. The phenolic compounds were analysed for, in the surface water DOC fractions by the Folin-Ciocalteu (Method 2.3.2).

1.2 Results

Fig 1 shows the averaged concentrations of phenolics determined in the five DOC fractions over the period January 2010 – October 2011. From the results it could be seen that the neutral fraction of each lake demonstrated the highest phenolic concentrations, with Cefni exhibiting the highest of the four at 1.3 mg/L.



The HPOA which is said to consist predominantly of fulvic acids (Aiken *et al.*, 1992; Lu *et al* 2009) averaged the second highest series of phenolic concentrations (> 0.4 mg/L) in three of the lakes (Cefni, Conwy and Teyrn), with Cwellyn demonstrating its second highest in the HPIB.



Comparing the phenolic analytical results for the fractions with those of the surface water DOC, the fraction sample results demonstrated higher phenolic concentrations (Fig 2). The averaged differences (mg/L) are shown in Table 1.1.

Lake/Reservoir	Phenolics summed fraction averages (mg/L)	Phenolics surface water averages (mg/L)	Differences (\pm mg/L)
Cefni	1.9	1.1	0.8
Cwellyn	1.3	0.6	0.7
Conwy	1.7	1.0	0.7
Teyrn	1.0	0.6	0.4

2.0 Experiment to establish performance of DAX 8 and XAD 4 phenolic selectivity.

2.1. Introduction

To establish that the DAX 8 and XAD 4 resins were selective relative to phenolics and would recover a known concentration of phenol a 2 L sample of Cefni surface water and 1L of Milli Q water (18Ω) was subjected to the standard laboratory sample preparation (see 2.2.1).

2.2 Experimental

2.2.1 Cefni Raw Surface Water Spiked with phenol

The 2 L Cefni surface water sample was split into 1 L aliquots and identified as A and B and the 1L Milli Q was identified as C. Sample A was fractioned as per (Method 2.1.4.6). Both samples B and C were spiked with 1.2 ± 0.03 and 1.2 ± 0.04 mgs of phenol (99 %) respectively. The spiked samples were then fractioned as per (Method 2.3.6). The recovered un-spiked and spiked fractions of Cefni surface water and Milli Q water samples were then analysed for phenolics as per Folin-Ciocalteu (Method 2.3.2).

2.2.2 Desorption Matrix spiked with phenol

To verify the stability and influence of the desorption matrix (60 mL NaOH 0.1M + 40 mL MilliQ water) for the HPOA and HPIA fractions, a comparable volume and concentration was prepared and spiked with 1.10 ± 0.03 mg/L of phenol (99%), but were not passed through the resins. The pH of the solution was adjusted to 2.8 with HCl (3.0 M). The solution was then subjected to analysis for phenolic content by the Folin-Ciocalteu (Method 2.3.2)

2.2.3 Results of Cefni Raw Surface Water Spiked with phenol.

The results (Table 2.1) demonstrated a phenolic distribution in the suite of fractions A and B and C that were similar, i.e., the ratio of recovered phenolics to HPOA averaged 3:1 and recovered phenolics to Neutrals averaged 2:1, overall the recoveries were outside acceptable limits of 80 – 120 % for experimental use (Chan *et al* 2004).

Table 2.1 Results of Cefni Raw Surface Water samples A) no phenol spike B) phenol spiked C) Milli Q water phenol spike fractionated via DAX 8 and XAD 4 resins in tandem.

Fraction/Surface water	Sample (A) (no phenol spike)	Sample (B) (+ 1.2 mg/L phenol spike)	Milli Q water (C) (+ 1.2 mg/L phenol spike)
Surface water	0.892 ± 0.014	1.898 ± 0.026	1.574 ± 0.044
HPOA	0.797 ± 0.015	1.380 ± 0.013	1.411 ± 0.017
HPIA	0.471 ± 0.011	0.310 ± 0.007	0.042 ± 0.003
HPOB	ND	ND	0.013 ± 0.012
HPIB	0.103 ± 0.023	ND	0.057 ± 0.022
Neutrals	1.344 ± 0.073	2.975 ± 0.068	1.365 ± 0.033
Fractions (Total phenol recovered)	2.72	4.67	2.89
Recovery %	304	246	184

2.2.4 Results of Desorption Matrix spiked with phenol.

The results to establish possible influences on the Folin-Ciocalteu reagents by the desorption matrix are shown in Table 2.2. The recovery of the phenolics from the matrix averaged 104.7 %.

Table 2.2. Determination of desorption matrix solution effect on Folin-Ciocalteu phenolics method

Matrix	Phenolics (mg/L)
Milli Q water (18Ω)	0.195 ± 0.002
Matrix (HPOA, HPIA desorption)	1.347 ± 0.018

2.3 Conclusion

The recovery variability was not within acceptable experimental limits (80 – 120 %) therefore the data for phenolic concentrations of the recovered fractions could not be reported.

The desorption matrix experiment demonstrated that the solutions used did not affect the Folin-Ciocalteu (Method 2.3.2).

Appendix 4

1.0 Instrumentation

a) Analysis of THMs

Varian Gas Chromatography 450 with a ^{63}Ni Electron Capture Detector (ECD) and a Solid Phase Micro Extraction (SPME) facility and Galaxy software. Column: Restek MX 1, 30m, 0.53mm id, 0.7 μm stationary phase (phenyldimethyl polysiloxane) film.

b) Size Exclusion Chromatography of DOC samples

Varian (Polymer Laboratories) PL- GPC 50, Integrated GPC System.

Column: Phenomenex BioSep – SEC - s2000, S/N 597004-6.

c) Analysis of cations and anions in Raw and Fractioned Surface Water.

Metrohm 850 Professional Ion Chromatography with a Professional Sample Processor 858 and MagIC Net software. Columns: Metrosep Anion 150mm, 4.0mm, S/N 7906567. Metrosep Cation 150 mm, 4 mm, S/N 10312544.

e) Analysis of DOC for carbon content.

Thermalox 2000 (Analytical Science Ltd, Cambridge), TOC analyser.

f) Recovery of solid DOC from Raw and Fractioned Surface Water.

Edwards Freeze Dryer, Modulyo, Edwards RV 5 vacuum pump.

g) Fluorescence Spectroscopy.

Spectramax M2, UV-Vis spectrometer with Softmax pro software. (Molecular Devices, UK, Ltd).

h) Infrared Spectroscopy.

Perkin Elmer FTIR, Spectrum 100 with Spectrum Express software.

i) UV-vis Spectroscopy.

Hewlett Packard Lambda 35

2.0 Reagents/Standards

a) Analysis of DOC for carbon content.

- 1) Glucose $\geq 99.0\%$, BDH, lot K32455814-410.
- 2) Sodium hydrogen phthalate A.R. Philip Harris, Batch 31442.
- 3) Platinum catalyst 5%, Elemental Microanalysis, BN 169894.

b) Analysis of THMs

- 1) Sodium hypochlorite, Sigma Aldrich (active Cl_2 6 – 14%), Lot 91820.
- 2) Sodium sulphite, Sigma Aldrich, Lot 090M0096V.
- 3) Trihalomethane calibration mixture (2000 $\mu\text{g}/\text{mL}$ of each THM component), Supelco, Lot LB 65544. (Certificate of Analysis)
 - i. Trichloromethane 99.1% (analysed concentration Certificate of Analysis).
 - ii. Bromodichloromethane 99.0% (analysed concentration Certificate of Analysis).
 - iii. Dibromochloromethane 98.8% (analysed concentration Certificate of Analysis).
 - iv. Tribromomethane 98.5% (analysed concentration Certificate of Analysis).

c) Analysis for phenolics

- 1) Sodium carbonate $\geq 99.5\%$ Sigma Aldrich, Lot 058K0687.
- 2) Folin Ciocalteus reagent, 2M, Sigma Aldrich, Lot 52K3671
- 3) Phenol 99%: Sigma Aldrich, Lot 121K16R6.

d) Size Exclusion Chromatography of DOC samples

- 1) Poly(styrenesulfonic acid sodium salt) GPC, Fluka.
 - i. MM 150,000 Lot 0001391453.
 - ii. MM 77,000 Lot 81612
 - iii. MM 32,000 Lot 0001 409244
 - iv. MM 13,000 Lot 81608
 - v. MM 4,300 Lot 0001 409884.
- 2) Cyanobalamin (B12), MM 1350, Supelco, LB68564.

e) Raw Surface Water Fractioning

- 1) Supelite DAX 8, Supelco, Lot 164884E.
- 2) Amberlite XAD 4, Supelco, Lot 1648400.
- 3) Sodium Hydroxide, Lot 0001127117.
- 4) Hydrochloric acid, 37%, Fisher Scientific, Lot 1173850.

f) Determination of free chlorine.

- 1) Potassium iodide, Sigma Aldrich, Lot BCBD 2647V
- 2) Potassium Iodate, Sigma Aldrich, Lot 1445474V
- 3) Sodium thiosulphate, Sigma Aldrich, Lot S7026 – 250G.

g) Infrared Spectroscopy.

- 1) Potassium bromide, Lot 523611 Fluka Analytical.

h) Ion Chromatography.

- 1) Dionex 7 anion standard [F^- (20 mg/L), Cl^- (30 mg/L), NO_2^- (100 mg/L), Br^- (100 mg/L), NO_3^- (100 mg/L), PO_4^{3-} (150 mg/L), SO_4^{2-} (150 mg/L)].
- 2) Dionex 6 cation standard [Li^+ (50 mg/L), Na^+ (200 mg/L), NH_4^+ (400 mg/L), K^+ (200 mg/L), Mg^{2+} (200 mg/L), Ca^{2+} (100 mg/L)] .

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