

**Bangor University**

## **DOCTOR OF PHILOSOPHY**

### **Exploring Relationships Between Catchment Dissolved Organic Matter Characteristics and the Formation of Disinfection Byproducts.**

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## Abstract

Dissolved organic matter (DOM) is found in all freshwaters globally, by dissolving in rainwater during its path through soil and on to oceans *via* rivers and streams. To provide potable water fit for human consumption, selected streams and rivers are used by either direct abstraction, or by diversion into reservoirs prior to treatment. For *ca.* 100 years, chlorine and its compounds have been used by water treatment companies to disinfect water. However, research has shown that reactions between chlorine and DOM can produce compounds (disinfection by-products, or DBPs) which may be hazardous to human health. This thesis explores the relationship between catchment character, organic matter concentration, and the potential formation of DBPs. In particular, trihalomethanes (THMs) were measured as these are currently the only regulated DBPs in the UK. To achieve this, water samples were collected quarterly over one year from two contrasting catchments, to study seasonal variations in DOM concentration and character. A third catchment was also sampled, with similar catchment characters to the first two catchments, to determine whether geographical location and land use types affected the data.

Each catchment was studied to see if catchment characteristics (e.g. class of vegetation, soil type or bedrock) could be mapped using a Geographical Information Systems (GIS) approach), to observe any effects on DOM and/or the DBPs found in treated water, with the aim of producing a risk assessment map to aid the choice of future abstraction locations for drinking water. Hence, samples were chlorinated and chloraminated in the laboratory before being analysed for DBP formation and residual chlorine concentrations were measured. Catchment specific GIS derived data were statistically analysed with water chemistry data, and detected relationships were explored statistically.

Major findings include medium to strong positive correlations between the standardised THM4 (STHM4 – the concentration of THM4 formed from 1 mg L<sup>-1</sup> dissolved organic carbon (DOC)) concentration and geology, where an increase of the area of inland rock in a catchment increases STHM4 concentration. Medium strength positive correlations were found between STHM4 and vegetation classes, where, as the area of acid grassland, and heather increase, so does the concentration of STHM4. Negative relationships were discovered showing the obverse, where, as loamy and clayey floodplain soils with naturally high groundwater increased in area, STHM4 concentration dropped (at the Hampshire Avon

and Conwy catchments combined). The occurrence of coniferous woodland in a catchment was found to correlate with the  $\text{CHCl}_3$  formation potential of waters (Pearsons,  $f=0.530$ ,  $p<0.05$ ,  $n=20$ ), supporting findings in published literature.

Laboratory based chlorination and chloramination of sample waters, followed by gas chromatography provided DBP data, specifically THM4. These data show that more chloroform was formed after chlorination than chloramination, and that chloramination formed 3 times more  $\text{CHBr}_3$  (another THM4 compound) than chlorination, under laboratory formation potential conditions. Results showed that the chlorination of water prior to DOM removal could result in a THM4 concentrations 5 times greater than the current UK regulatory limit, per  $\text{mg L}^{-1}$  dissolved organic carbon (DOC), whereas chloramination forms *ca.* 5 times less than the current UK regulation per  $1 \text{ mg L}^{-1}$  DOC. However, chlorination of water prior to DOM removal is never done in practice, so this data provides information on the composition of the organic matter and whether DOM from a specific catchment contains specific components that are responsible for an increase in a specific DBP.

Data also show that increasing organic nitrogen or organic carbon does not necessarily increase nitrogenous or carbonaceous DBPs (N-DBPs or C-DBPs). However, importantly, data shows that an increase in the area of land use classed as 'urban', results in an increase in DON (likely due to human influences) in the water draining from them, posing potential issues for eutrophication in downstream water bodies and the formation of N-DBPs at water treatment works. Whilst N-DBP detection was explored from several different angles, the development of a definitive method was not possible due to very low N-DBP concentrations, time and financial constraints. However, various methods were adapted to aid in the detection of them, showing promising initial results, providing the background for future projects into the discovery of a suite of N-DBPs such as haloacetonitriles and halonitromethanes.

Finally, the data in this thesis have been inputted into maps for each major catchment to present data with a high visual impact, but also to illustrate land use types that have been found to correlate with increases in DBPs and specific nutrients in the water draining from them. However, the high variation in DOM concentration and character from site to site make extrapolation of these risk assessment data, to other catchments, unsafe. Nevertheless, collection of data from a catchment (similar to the work presented here) where a new water abstraction location is desired can prove advantageous in providing information to utility

companies of what difficulties they may encounter when treating the water. Though this can be done by grab sampling at each site of interest, this can prove costly and timely and involves both field and laboratory based work aspects, whereas the method presented here requires less cost and time, once the method is initialised, to derive data of similar value. Despite the fact that disinfection performance would always trump DBP minimalisation, this is likely to be a vital tool in ensuring the provision of safe and healthy water fit for the consumption of an ever increasing human population.

## **List of abbreviations**

AOM – Algogenic Organic Matter  
ANOVA – Analysis Of Variance  
APHA – American Public Health Association  
APS – Ammonium Persulphate  
ASL – Above Sea Level  
BCAN - Bromochloroacetonitrile  
BGS – British Geological Survey  
C – Carbon  
CAN – Chloroacetonitrile  
CCW – Countryside Council for Wales  
C-DBP – Carbonaceous Disinfection Byproduct  
CDC – Cell division Cycle  
CDK – Cyclin Dependent Kinase  
CDS1 – CDP-Diacylglycerone Synthase 1  
CHBr<sub>2</sub>Cl – Dibromochloromethane  
CHBr<sub>3</sub> – Tribromomethane/Bromoform  
CHCl<sub>3</sub> – Trichloromethane/Chloroform  
CHCl<sub>2</sub>Br – Dichlorobromomethane  
CHK1 – Checkpoint Kinase 1  
CEH – Centre of Ecology and Hydrology  
CH – Chloral Hydrates  
CHO – Chinese Hamster Ovary  
CPU – Central Processing Unit  
DA - Daltons  
DAF – Dissolved Air Flotation  
DBAN - Dibromoacetonitrile  
DBP – Disinfection Byproduct  
DBPFP – Disinfection Byproduct Formation Potential  
DCAN – Dichloroacetonitrile

DEFRA – Department for Environment, Food and Rural Affairs

DEM – Digital Elevation Model

DHAA – Dihalogenated Acetic Acids

DIN – Dissolved Inorganic Nitrogen

DMA – Dimethylamine

DMAB – Dimethylaminobenzene

DMAI – 3-(Dimethylaminomethyl)indole

DMAP – 4-Dimethylaminoantipyrine

DMDC - Dimethyldithiocarbonate

DMEA – N,Dimethylethanolamine

DMFA - Dimethylformamide

DNA – Deoxyribonucleic Acid

DOC – Dissolved Organic Carbon

DON – Dissolved Organic Nitrogen

DOM – Dissolved Organic Matter

DOMAINE – Dissolved Organic Matter in the Natural Environment

DPI – Dots Per Inch

DTM – Demographic Transition Model

DPD –N,N-diethyl-p-phenylenediamine

DWDS – Drinking Water Distribution System

DWI – Drinking Water Inspectorate

DXAA – Dihaloacetic Acid

EA – Environmental Agency

EAFRD – European Fund for Rural Development

EAGF – European Agricultural Guarantee Fund

ECD – Electron Capture Detector

EDTA – Ethylenediamine Tetraacetate Dehydrate

EfOM – Effluent Organic Matter

EOM – Extracellular Organic Matter

EPA - Environmental Protection Agency

FAA – Free amino Acids  
FID – Flame Ionisation Detector  
FTIR – Fourier Transform Infrared Spectroscopy  
GC – Gas Chromatography  
GC-MS – Gas Chromatography – Mass Spectrometry  
GIS – Geographical Information Systems  
GPS - Global Positioning System  
GPU – Graphical Processing Unit  
HAA – Haloacetic Acid  
HAN - Haloacetonitrile  
HCL – Hydrochloric Acid  
HK - Haloketones  
HOCL – Hypochlorous Acid  
HPIA – Hydrophilic Acid  
HPIB – Hydrophilic Bases  
HPIN (also X-Res) – Hydrophilic Neutral  
HPLC – High Pressure Liquid Chromatography  
HPOA – Hydrophobic Acid  
HPOB – Hydrophobic Bases  
HSD – Honest Significant Difference  
IHSS – International Humic Substances society  
IOM – Intracellular Organic Matter  
IRIS – Integrated Risk Information Service  
LCM – Land Cover Map  
MAP – Mitogen Activated Protein  
MCL – Maximum Contaminant Level  
MW – Molecular Weight  
N- Nitrogen  
NAC – N-acetylcysteine  
NDBA – N-nitrosodibutylamine

N-DBP – Nitrogenous Disinfection Byproduct  
NDEA – N-nitrosodiethylamine  
NDELA – N-nitrosodiethanolamine  
NDIR – non-dispersive infrared  
NDMA – N-nitrosodimethylamine  
NDPA – 2-nitrodiphenylamine  
NERC – Natural Environment Research Council  
NGO – Non-Governmental Organisation  
NMEA – N-methylethyl nitrosamine  
NMOR – N-nitrosomorpholine  
NOM – Natural Organic Matter  
NPOC – Non-Purgeable Organic Carbon  
NPYR – N-nitrosopyrrolidine  
NRW – Natural Resources Wales  
NSRI – National Soil Resources Institute  
NTP – National Toxicology Program  
NVZ – Nitrate Vulnerable Zone  
OPAME – *o*-phthalaldehyde methanol  
OS – Ordinance Survey  
PBS – Phosphate buffered saline  
PDMS – Polydimethylsiloxane  
PET – Polyethylene Terephthalate  
PPM – Parts Per Million  
PTFE – Polytetrafluoroethylene  
PVC – Polyvinyl Chloride  
RAM – Random Access Memory  
RPM – Revolutions per minute  
SDS – Sodium Dodecyl Sulphate  
SDS-PAGE – Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis  
SEC – Size Exclusion Chromatography

SEM – Standard Error of the Mean  
SPME – Solid Phase Micro Extraction  
STHM – Standardised Trihalomethane  
STHMFP – Standardised Trihalomethane Formation Potential  
STW – Sewage Treatment Works  
SUVA – Specific UV Absorbance  
TCAN – Trichloroacetonitrile  
TCC – Total Coliform Forming Units  
TDI – Tolerable Daily Intake  
TDN – Total Dissolved Nitrogen  
TEMED - Tetramethylethylenediamine  
THM – Trihalomethane  
THM4 – Trihalomethane 4  
TTHM4 – Total Trihalomethane  
TTHM4FP – Total Trihalomethane Formation Potential  
TMA – Trimethylamine  
TOC – Total Organic Carbon  
*TOC* – Table of Contents  
TPTZ – 2,4,6-Tris(2-pyridyl)-s-triazine  
TRIS – Tris(hydroxymethyl)aminomethane  
TTHM – Total Trihalomethane  
TTHM4FP – Total Trihalomethane 4 Formation Potential  
TN – Total Nitrogen  
US EPA – United States Environmental Protection Agency  
UV – Ultra Violet  
UTOX – Unknown Total Organic Halogens  
WHO – World Health Organisation  
WTW – Water Treatment Works

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Chapter 6: Effects of Chlorine Upon Natural Organic Matter During Water Disinfection

**Equation 6.a**

# Chapter 1a

The composition and  
characterisation of  
dissolved organic  
matter

## 1a.1: Introduction

The need to study and characterise dissolved organic matter (DOM) in waters destined for human consumption is increasing. For example, global climate change has been found to drive organic carbon loss from peatland catchments, due to elevated carbon dioxide concentrations and decreased sulphur deposition (Freeman *et al.*, 2004), and if this water is abstracted for human consumption, the increase of carbon can hinder the creation of safe drinking water. Findings in the 1970s of potentially carcinogenic and mutagenic compounds, known as disinfection by-products (DBPs), which were purported to be formed during a reaction between the DOM and halogens, such as chlorine, which are added to disinfect drinkingwater (Hua and Reckhow, 2007). The understanding of the individual components of DOM and how they react with these halogens can help water providers to more carefully select abstraction locations and provide a more targeted treatment for the water. There are many different constituents of DOM, and depending on the concentration of these constituents, freshwater DOM can have many different characters. Several characterisation techniques are widely used to identify DOM in freshwater, and these have been explored in the current works.

The concentrations of humic and fulvic matter (two major constituents of DOM), and their constituents, in freshwater, naturally differ depending upon the catchment from which the water drains. As the quantitative determination of humic substances is labourious, three different methods are commonly combined to describe humic matter concentration (dissolved organic carbon (DOC), colour, and chemical oxygen demand) (Kortelainen, 1993). The landscape, alongside anthropogenic sources of DOM (such as wastewater) influences its water bodies through multiple pathways and mechanisms (such as controls on chemistry, hydrology and sediment delivery), operating at different spatial scales (Allan and Johnson, 1997). Studies have found that high water colour (thus signifying high concentrations of humics and fulvics) originated from organic rich soil catchments, especially those subjected to moorland burning, ditching, and with south facing slopes (Mitchell and McDonald, 1995)

## 1a. 2: Humic substances

Humic substances are major components of the natural organic matter (NOM) in soil and water as well as in geological organic deposits (lake sediments, brown coals and shales and

peats). They are best described as refractory, dark coloured, heterogenous organic compounds produced as byproducts of microbial metabolism (Sutton and Sposito, 2005). They contribute strongly to the brown colour of decaying plant debris and therefore contribute to the brown and black colour of surface soils due to interaction between soils and water. These humic substances can therefore be found in surface water, and they are major components of NOM in these waters.

Being complex and heterogenous mixtures of polydispersed materials, humic substances are formed by chemical and biochemical reactions during the decay and transformation of plant and microbial remains, a process called humification. Important components involved in the humification process are plant lignin, polysaccharides, melanin, cutin, proteins, lipids and nucleic acids.

Whilst humic substances in soils can be broken down into three main fractions, only two of these are present in aquatic systems; humic acids and fulvic acids (with humin being the third, non aquatic fraction) (IHSS, 2007).

### 1a. 2.1: Humic acids

DOM in freshwater has been the focus of increasing numbers of scientific studies over the past two decades. Many of these studies have focussed on several aspects of organic matter, including the humic fraction of DOM. Humic DOM is dominated by aromatic compounds; molecules that are cyclic and planar, containing a ring of resonance bonds, thus making the molecule less susceptible to alteration by chemical reactions, and therefore more stable.

Humic substances compose between 50 – 80% of DOM in freshwater ecosystems, with both autochthonous and allochthonous sources. In oligotrophic waters, with DOC concentrations ranging from 1 to 100 mg L<sup>-1</sup>, humic substances have been found to exceed the organic carbon in all living organisms by approximately one order of magnitude (i.e. tenfold) (Mayhew, 2004). Humic acids can form complexes with commonly occurring ions in the environment creating humic colloids (MacCarthy, 2001). In freshwater ecosystems, the majority of humic substances originate from terrestrial plant debris, lignins (cumaryl alcohol, coniferyl alcohol and sinapyl alcohol) and terpenes (Steinberg *et al.*, 2006).

### 1a.2.2: Lignin

Lignin is a strengthening phenolic polymer that is found in water-containing cells in vascular plants, and is a result of the adaptation of plants to grow to taller heights than bryophytes; producing a stronger stem that can withstand wind and drooping, and which can transport water and mineral nutrients high above the ground (Campbell and Reece, 2005). Once a plant dies and decays, the lignin can enter the waterbody and then becomes a component of DOM. See Figure 1a.1.

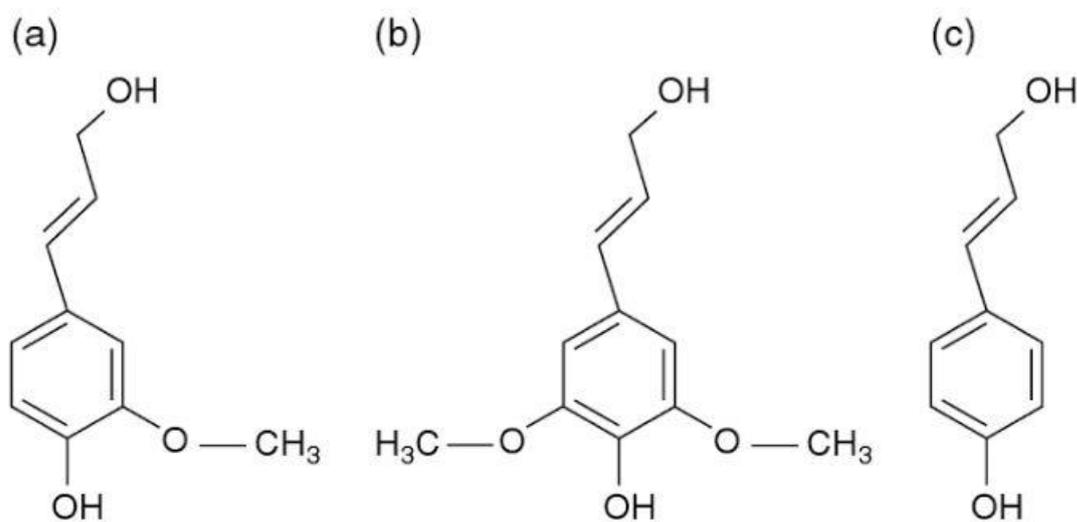


Figure 1a.1: Molecules from which components of lignin are obtained – a) trans-coniferyl alcohol, b) trans-sinapyl alcohol and c) trans-p-cumaryl alcohol (Clarke and Deswarte, 2015).

### 1a.2.3: Terpenes

Terpenes are amongst the thousands of different structures of low molecular weight organic compounds produced by living organisms, although many of these have no apparent function in the basic processes of growth and development. As an example of their use and function, a large group of terpenes, drimane sesquiterpenes, are common in plants, fungi and some marine organisms, and they have potent antibacterial and antifungal activity, are toxic to insects, nematodes, molluscs and fish

(thus deterring feeding on plants by insects and fish) (Gershenzon and Dudereva, 2007). Their structures differ considerably, as demonstrated in Figure 1a.2.

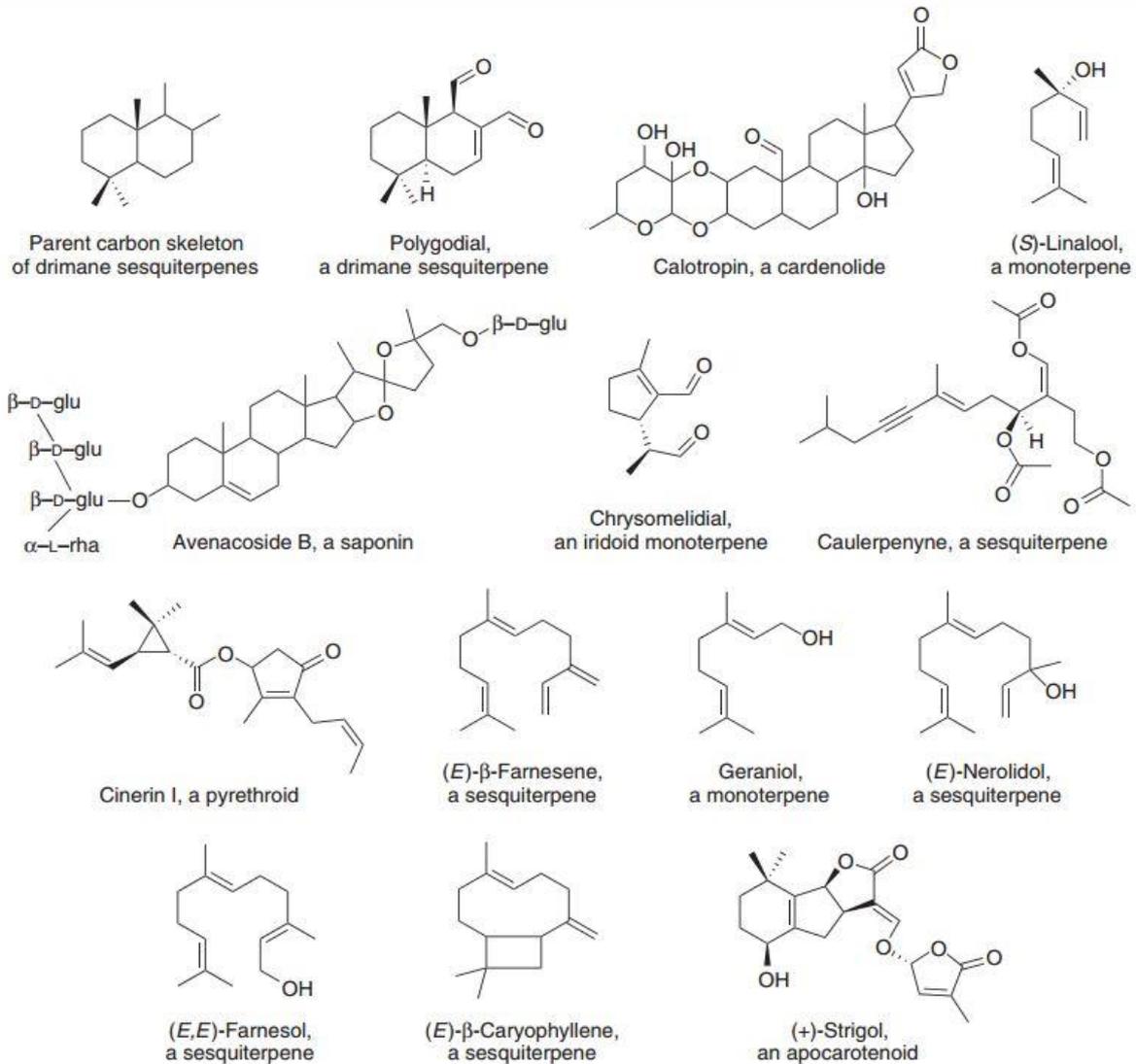


Figure 1a.2: Structure of common naturally occurring drimaine sesquiterpenes (Gershenzon and Dudereva, 2007).

#### 1a.2.4: Fulvic acids

Whilst humic acids are insoluble in waters in the acidic pH range, fulvic acids (also derived from humic substances) are water soluble in the whole pH range (MacCarthy, 2001). Derived from soils and plants of the surrounding watershed (and algae and bacteria in the water or sediments), the chemical properties of fulvic acids in temperate lakes and streams are heavily influenced by their origins in the

watershed. In comparison to humic acids, fulvic acids have a molecular weight of around 800 Da (with humics being a little larger at 1500-3000 Daltons (Da)), although some published work suggests fulvics can range from 400-2000 Da (Beckett, 1987 and McKnight *et al.*, 1994). In aquatic environments, fulvics are a class of biomolecules, available in some concentration in all natural waters. Fulvics can be defined as a heterogenous mixture of moderate molecular weight yellow organic acids, that function as part of the not readily degradable detrital organic matter in freshwater ecosystems. Due to their poorly degradable nature, fulvics are able to alter the properties of water in several ways, such as light absorption, contribution to pH buffering (due to their weak acidity) and their sorption onto mineral and oxide surfaces (thus giving the surfaces an overall negative charge) (McKnight *et al.*, 1994). Due to their low MW, fulvics are harder to remove at a water treatment works than other components of DOM, by techniques such as coagulation (Babcock and Singer, 1979).

### 1a.3: Biological monomers

Free and combined biological monomers such as neutral sugars and amino acids have been identified in groundwater, although they represent less than 10% of the total organic carbon (Longnecker and Kujawinski, 2011), and therefore, their identification as components of DOM should be considered.

#### 1a.3.1: Amino Acids

Amino acids constitute one group of the most prevalent nitrogenous organics in drinking water sources, and can be present in either free or combined moieties. They comprise an important fraction of hydrophilic NOM, which is not well removed during common water treatment processes (Shan *et al.*, 2012). Ubiquitous in surface waters, amino acids comprise a significant proportion of dissolved organic nitrogen (DON). They typically represent 2-5% of total NOM and have been identified as precursors for carbonaceous and nitrogenous disinfection by-products (C-DBPs and N-DBPs respectively, see chapter 5a and 5b) (Bond *et al.*, 2014a). Research by Thurman, 1985, shows that amino acid concentrations in surface waters range from 50-2000  $\mu\text{g L}^{-1}$ , accounting for 2-13% of DOC in natural waters (Thurman, 1985).

However, How *et al.* suggest that amino acids can contribute up to 75% of DON in surface waters (How *et al.*, 2014).

Free amino acids (FAA) have been found in highest concentrations in large rivers and estuaries subject to high organic loading from densely populated, or intensively farmed catchments. As a general rule, the farming intensity will increase with further distance from the source of the river (due to more accessible and fertile land), it can be expected that FAA concentrations will show an overall increase from source to sea. It has also been postulated that some other allochthonous sources of FAA could include groundwater and rainwater. Autochthonous sources of amino acids have been demonstrated in bacteria, algae, zooplankton, macrophytes, soft bodied worms or molluscs and vertebrates such as fish (Thomas, 1997). Figure 1a.3 shows the sources of free amino acids to water.

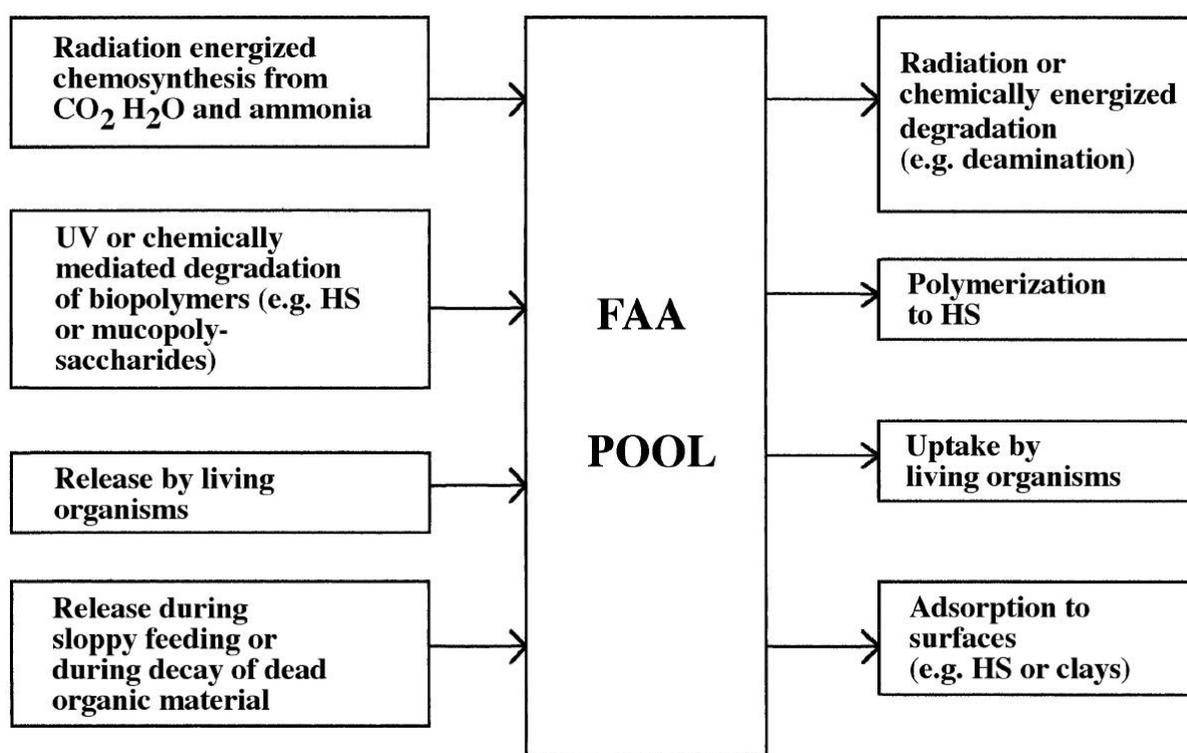


Figure 1a.3: Free amino acid contributions and uses to and from the FAA pool (Thomas, 1997).

The major amino acids present in treated water are alanine, glycine, valine, phenylalanine, serine, threonine, isoleucine, aspartic acid, tyrosine, proline, glutamic acid and leucine, and their concentrations can range from 0.33 - 1.05  $\mu\text{g L}^{-1}$  (Brosillon *et al.*, 2009). Amino acids contribute to the hydrophilic fraction of NOM, and have

been identified as the major precursors for halonitromethanes, a group of nitrogenous disinfection byproducts (N-DBPs) (Shan *et al.*, 2012) and haloacetonitriles (Bond *et al.*, 2014a). Disinfection byproducts (DBPs) are compounds formed in drinking water treatment where the halogen used to disinfect the water reacts with organic matter dissolved within the water, and are studied in depth in chapters 5a and 5b. Studies show that certain individual or groups of amino acids have been found to be precursors for several other classes of N-DBPs such as halonitriles (Yang *et al.*, 2010) and cyanogen halides (Hirose *et al.*, 1988), and some odorous DBPs such as *N*-chlorophenylacetalimine (Freuze *et al.*, 2005).

When chlorinated, such as in water destined for human consumption, amino acids demonstrate a breakpoint curve phenomena (see Figure 1b.2), similar to that of ammonia, resulting in a higher chlorine demand for the distributed waters, and if inorganic chloramine is used as the disinfectant, the presence of amino acids introduces a risk of overestimation of disinfection capabilities. Thus, understanding the occurrence of amino acids in source waters is important in ensuring sufficient disinfectant is added during water treatment (How *et al.*, 2014), and to be able to predict the formation potential of any DBPs associated with the halogenations of amino acids. therefore, detection of amino acids must be the first step in this process, before a chlorine demand test on the most prolific amino acids can determine the concentration of chlorine required, and analysis of the sample containing the amino acid can determine the concentration of any DBPs that have been formed.

Detection of amino acids can be difficult however. Many underivatized amino acids are very weak chromophores in the UV-Vis region and possess no native fluorescence (Chaimbault *et al.*, 1999), so detection of amino acids by fluorescence is not feasible. Size exclusion chromatography (SEC) is also not successful in the accurate detection of amino acids, as SEC provides a low response for NOM structures with low UV molecular absorbances (also including proteins, amino sugars and aliphatic acids) (Leenheer and Croué, 2003). High performance liquid chromatography (HPLC) has been utilised to identify and quantify both individual and total free amino acids, at low, naturally occurring concentrations (Thomas, 1997). Therefore, it is necessary to obtain pure model compounds of each amino acid, to create a library of detection characteristics that can then be compared to unknown samples, to analyse similarities.

The detection of aquatic amino acids is usually achieved by HPLC, which involves some form of pre-derivatisation step. However, some amino acids, such as the more hydrophobic tryptophan, are not captured by many HPLC methods due to a susceptibility to hydrolysis (Bond *et al.*, 2009 and Bond *et al.*, 2012), and therefore more accurate analytical techniques are required.

Studies show that a mixture of autochthonous and allochthonous compounds in water, such as amino acids and proteinaceous compounds are precursors of N-DBPs. These waters are typically characterised by low MW, low hydrophobicity and poor removal efficiencies in conventional coagulation/sedimentation/filtration methods (Chu *et al.*, 2012). Their poorly moderated movement through the treatment works means that they can react with disinfectants to form DBPs, therefore, research into their effective removal is paramount. Other studies have shown that amino and carboxyl groups are the main precursors for dichloroacetic acid and chlorinated trihalomethanes (THMs), and these can be found in the hydrophilic fraction of DOM (Li *et al.*, 2014). For example, Ueno *et al.* found that 15 out of 23 amino acids studied contributed towards the formation potential of two haloacetonitriles (dichloroacetonitrile and trichloroacetonitrile), forming between  $0.2 \mu\text{g L}^{-1}$  and  $15.4 \mu\text{g L}^{-1}$  of total haloacetonitriles (Hureiki *et al.*, 1994, Ueno *et al.*, 1996 and Navalon *et al.*, 2009), with further findings by Yang *et al.*, in 2012 replicating Ueno *et al.*'s findings (Yang *et al.*, 2012). Furthermore, Bond *et al.*, found chlorination of 7 amine model compounds ( $\beta$ -Alanine, L-Aspartic acid, L-Methionine, L-Cysteine, Ala-Ala, 3-Aminophenol and 2-Aminophenol) formed a range of 6 DBPs at between 1 and 23 % M/M (Bond *et al.*, 2014a)

Algal and effluent organic matter (AOM and EfOM respectively) have been shown to contain a greater amount of amino acids than NOM, therefore, the formation of DBPs associated with amino acids can be expected to be higher in waters containing high quantities of algae. The chlorination of these waters can result in the formation of aldehydes and nitriles (both DBP precursors/components), with certain amino acids (e.g. aspartic acid) forming chloral hydrate (trichloroacetaldehyde – from the haloaldehyde family) and dichloroacetonitrile (from the haloacetonitrile) (Krasner *et al.*, 2012). Therefore, at water treatment works (WTWs) treating water with high AOM, it would be expected that these N-DBPs would be abundant. AOM consists of cells, extracellular and intracellular organic matter (EOM and IOM respectively). In

raw water, AOM can elevate total organic carbon (TOC) levels, cause membrane fouling and lead to an increase in certain DBPs such as THMs. Algal cells are also problematic for drinking water treatment because they are responsible for poor settling, filter blocking, and the smaller algae can break through sand filters. Algae can also impact upon the taste and odour of the final water, and can be responsible for the release of harmful toxins, (Fang *et al.*, 2010). Studies show that EOM is mainly released during the exponential growth phase of algae, and is composed of lower MW compounds such as glycolic acid and amino acids. On the other hand, IOM, released mainly during the death phase, is composed of much higher MW products such as polysaccharides (more than 1 carbohydrate joined together). Several previous studies have examined the fractional character of AOM throughout various stages of growth, suggesting that algogenic DOC is predominantly comprised of hydrophilic neutrals (found to contain nitrogen rich constituents such as proteinaceous-type structures that represent an important class of this fraction (Leenheer and Croué, 2003)), thus showing that algae comprises of mainly low MW, un-charged particles – difficult to remove by coagulation, one of the most common steps in water treatment works (Leloup *et al.*, 2013 and Gough *et al.*, 2015). See Table 1a.1 below for examples of algae found in different types of water.

Peptides (combined amino acids) have not been commonly examined by research into DBP formation potential (FP), although they can pose a knock-on-effect risk; the electron-withdrawing effect of a carbonyl group renders the peptide (amide) linkage in these compounds relatively unreactive towards chlorine. In order to cleave the peptide linkage, high chlorine doses and/or long contact times are required, which increases contact time (and therefore occurrence) for other DBP precursors (Bond *et al.*, 2014b). Despite the very limited research into peptides, they are thought to be up to fivefold more common than free amino acid species in freshwater, and are considered to be of an intermediate MW (Bond *et al.*, 2011). Their more common occurrence ultimately means that studies into the removal of peptides could be more important than studies into the removal of free amino acids.

Further research into individual amino acids and peptides, and their role in N-DBP formation potential (N-DBPFP), could lead to the production of a more thorough precursor removal technique and a wider understanding of their potential to be hazardous to humans.

Proteins in water often originate from algae or phytoplankton and, based on pyrolysis data, can include phenol, pyridine, toluene and styrene groups (Bond *et al.*, 2011). Algal cells are known to be enriched in organic N in the forms of proteins, amino acids and amines; and although it is currently unclear, it is thought that the occurrence of algae can enhance N-DBP formation. In addition to this, AOM appears to contain more hydrophilic and less aromatic carbon content when compared to NOM.

It has been reported by Fang *et al.* that algae in different growth stages have different protein, carbohydrate and lipid compositions. Further findings from this work show that most DBP concentrations increased with the further progression of the algal cell growth period because the proportion of proteins in the AOM composition was decreased with increasing algal growth period. The study also shows that chlorination of algal cells, enriched in organic N, generated higher concentrations of N-DBPs such as haloacetonitriles, but much lower concentrations of most C-DBPs, such as THMs, comparatively (Fang *et al.*, 2010).

Table 1a.1: Characteristics of lakes with different trophic qualities and the algae species likely to be found within them.

Parameter	Oligotrophic	Eutrophic
<b>General levels of biomass in waterbody</b>	Low	high
<b>Occurrence of algal blooms</b>	Rare	frequent
<b>Relative quantity of green and blue-green algae</b>	low	high
<b>Vertical extent of algal distribution</b>	into hypolimnium (bottom waters) in thermally stratified waterbodies	usually only in surface waters
<b>Common algae species</b>	<u>Green algae</u> : Desmids, <i>Staurastrum</i>	<u>Blue-green algae</u> : <i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Microcystis</i> ,

	<u>Diatoms:</u> <i>Tabellaria, Cyclotella</i>	<i>Oscillatoria</i>
	<u>Golden-brown algae:</u> <i>Dinobryon</i>	<u>Diatoms:</u> <i>Melosira, Fragilaria, Stephanodiscus, Asterionella</i>

### 1a.3.2: $\beta$ -Dicarbonyl Acids

Carbonyl compounds play an important role in aquatic oxidation processes. In natural waters, these compounds can be created by the photodegradation of DOM, and may also be released as metabolites by microbiological processes. Low MW carbonyl compounds, such as formaldehyde, acetaldehyde and acetone, have been found to be major organic by-products in the ozonation of natural waters. Links have been made between these compounds, and poor taste and odour of final water, stomach tumours and mutagenic and carcinogenic properties (Bao *et al.*, 1998).  $\beta$ -dicarbonyl acids are often encompassed under the general term ‘carboxylic acid’, which is an organic compound that contains a carboxyl group (COOH). Carboxylic acids found in NOM are assumed to be smaller and more hydrophilic than humic species, commonly found within the NOM transphillic fraction known for high carboxylic acid functionality (Bond *et al.*, 2011).  $\beta$ -dicarbonyl acids have been identified as DBP precursors, so research into their contribution to NOM can provide deeper understanding of their role in DBP formation potential (DBPFP) and steps can be made to reduce this.

$\beta$ -dicarbonyl moieties consist of an  $\alpha$ -carbon, which is flanked by two adjacent carbonyl functional groups where one of the carbonyl groups can be associated with a carboxylic acid, a  $\beta$ -ketone acid. Aromatic and aliphatic  $\beta$ -carbonyl structures are likely moieties within NOM, and NOM structures could be potentially oxidised by chlorine to yield  $\beta$ -carbonyl moieties, however, the extent of this is unknown. Studies by Dickenson *et al.*, (2008) also show that aliphatic  $\beta$ -dicarbonyl acid moieties, particularly in the hydrophilic fraction of NOM, could contribute to the significant formation of THM and di-haloacetic acids observed after the chlorination of natural waters. Dickenson *et al.* shows that 5,7-dioxooctanoic, 4,6-dioxoheptanoic, 3-oxohexanedioic and 3-oxopentanedioic acids ( $\beta$ -dicarbonyl acids– see Figure 1a.4) are suspected to contribute towards the composition of hydrophilic DOM, and could

be responsible for some THM formation. There is evidence that 3-oxopentanedioic acid forms chloroform (trichloromethane) when chlorinated (Larson & Rockwell, 1979). However, 3-oxobutanedioic and 3-oxopentanedioic acids could be responsible for some DXAA formation. Dickenson *et al.* concludes that  $\beta$ -dicarbonyl structures, or structures that can be readily oxidised to  $\beta$ -dicarbonyl structures should receive more attention in the overall DBP precursor removal procedure (see Figure 1a.4 for examples of these compounds). It has also been proposed that  $\beta$ -dicarbonyl moieties within aromatic and aliphatic groupings are precursors for the formation of the haloacetic acids; dichloroacetic acid and dichloroacetic acid (Dickenson *et al.*, 2008).

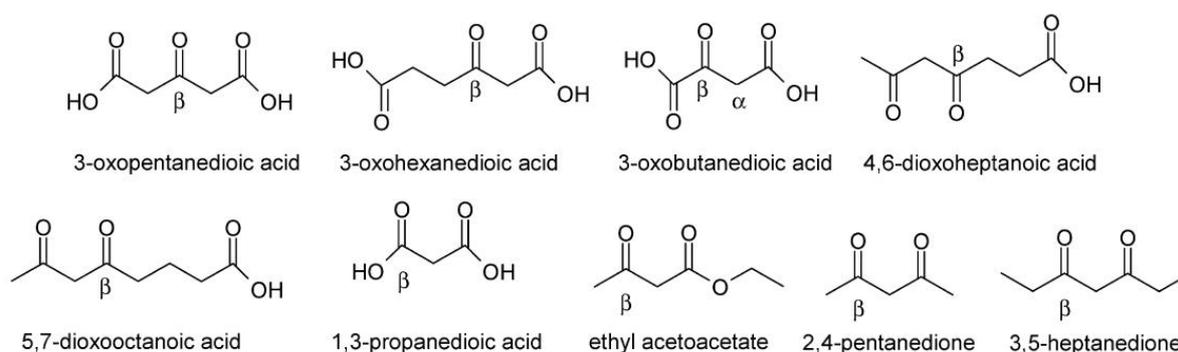


Figure 1a.4: Structures of aliphatic compounds with  $\beta$ -dicarbonyl moieties (adapted from Dickenson *et al.*, 2008).

### 1a.3.3: Carbohydrates

Carbohydrates are found in fresh and sea water, and are among the most abundant biopolymers to be found there. In the most simplest explanation, carbohydrates (hydrates of carbon) are generally molecules that are comprised of carbon, hydrogen and oxygen atoms, and all carbohydrates, including various sugars, starches and cellulose (the most prolific carbohydrate on Earth), are important for the maintenance of life in both plants and animals. Even the most basic viable organisms require carbohydrates in some guise to be able to sustain life, hence they are found in waters globally. Free and combined sugars constitute a large fraction of most organisms – up to 60% dry weight of some algae. They are released into natural waters as a result of excretion, inefficient grazing, death and lysis of cells. Carbohydrates are important food sources for heterotrophs (Mopper *et al.*, 1992). On even cursory consideration,

carbohydrates provide ribose derivatives (the 'glue' that cements genetic code), glucose (a major cellular fuel), cellulose (a glucose polymer and the most abundant substance on this planet) and oligosaccharides (key cell recognition antigens in the immune system) (Bubb, 2003).

In marine environments, polysaccharides are common structural and storage compounds, and represent the major form of photosynthetically assimilated carbon in the biosphere (Cowie and Hedges, 1984). In rivers and lakes, however, glucose, dominates the carbohydrate pool, although fructose and galactose have been reported at similar concentrations (Jørgensen and Jensen, 1994 and Gremm and Kaplan, 1997), although both of the waters studied are reported to have suffered from recent increases in pollution levels, and additional studies suggest that carbohydrates can comprise over 50% of the total weight of the DOM in a Spanish river, with some sources of water containing over 1 mg L<sup>-1</sup> of saccharides (Navalon *et al.*, 2008). In water draining bogs, it has been suggested that carbohydrates (greater than 100kD) contribute to the high MW fraction of DOC by up to 15% (Tranvik and Jørgensen, 1995). Other work has determined the dissolved concentrations of acid polysaccharides to correspond to 12% of total carbohydrate fraction in Gulf of Mexico sea water (Hung *et al.*, 2001).

The determination of carbohydrates in natural waters is helpful for their chemical characterisation and for studies related to the cycle of organic matter in the environment. Carbohydrates are often present as neutral carbohydrates, uronic acids and aminosugars (Mecozzi, 2005). The study of carbohydrates in aqueous solutions is fundamental in areas of environmental research and industrial applications (Albalasmeh *et al.*, 2013). Due to their obvious important role in organisms and organic matter, study of them and the role that they play in DBP formation can prove very advantageous for isolation of key carbohydrate-related DBP precursors. It is highly likely that waters high in carbohydrate content are sources of water for human consumption, and as such, these waters will be treated with chlorine to inactivate bacteria and viruses prior to consumption. Although chemical disinfection of potable water is essential, this disinfection can also form DBPs. On current knowledge, the predominant carbohydrates in water are small, neutral and relatively hydrophilic (thus coagulation and ion exchange removal techniques are not effective) (Bond *et al.*, 2011). This shows that carbohydrates are located within the hydrophilic fraction of

DOM, and research into reducing these precursors is fundamental in improving drinking water quality. Studies have discovered that disinfection of saccharides can form THMs in variable quantities depending on the presence of promoters and the water treatment conditions, such as pH and bromide concentration (which has previously been shown to affect THM formation). The results of these studies show that saccharides do form significant concentrations of THMs ( $0.1\text{ mg L}^{-1}$ ) when chlorinated under conditions found in drinking water treatment ( $2\text{ mg L}^{-1}$  DOC,  $10\text{ mg L}^{-1}$  Cl, pH 8.0), and that the pH strongly influences the THM formation potential and chlorine consumption (Navalon *et al.*, 2008).

However, carbohydrates have also been discovered in natural organic macromolecules (humic acids), and thus, a humic acid can be considered as being constituted by the binding of simple constituents including carbohydrates. In spite of their ubiquitous presence, there are very few studies on carbohydrates as THM precursors upon chlorination. As already established, carbohydrates are among the most abundant biopolymers in freshwater, and it has been shown that acid polysaccharides such as uronic acids can be excreted by algae and bacteria in response to low nutrient stress. Saccharides have been found to form THMs in variable water treatment conditions, including pH and chloride concentration, and studies have found that saccharides can contribute to over 50% of the DOM in certain freshwaters (Navalon *et al.*, 2008). Glucose, arabinose and mannose are thought to be widespread saccharides in drinking water – these carbohydrates are neutral with relatively hydrophilic and relatively low MW properties (Bond *et al.*, 2011). Kaplan and Newbold reported that the average stream contains  $690\text{ nM}$  carbohydrates, which contributes to approximately 1% of total DOC (Kaplan and Newbold, 2003). See Figure 1a.5 for structure of commonly occurring carbohydrates in freshwater.

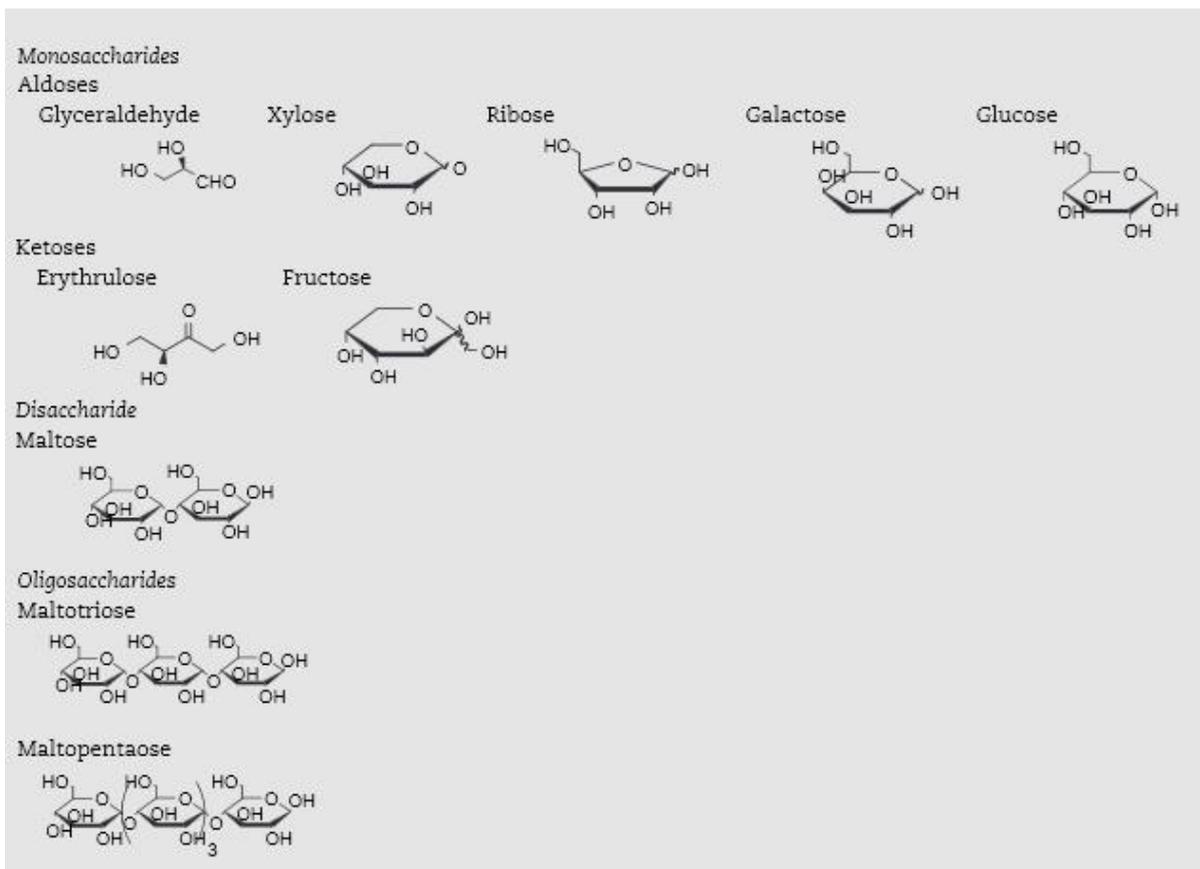


Figure 1a.5: Carbohydrates commonly found in water (adapted from Navalon *et al.*, 2008).

There are many methods developed for the determination of carbohydrates in environmental samples, such as ultraviolet (UV) absorbance, despite the fact that carbohydrates fluoresce very poorly. Due to this fact, various colouring reagents are used, that react in the presence of carbohydrate and produce a colour detectable in the visible range of the UV spectrum. These chemicals include phenol (including orcinol) (Dubois *et al.*, 1956, Irwin and Leaver, 1956, Monsigny *et al.*, 1988, Mecozzi, 2005, and Masuko *et al.*, 2005) alkaline ferricyanide (Englis and Becker, 1943 and Myklestad *et al.*, 1997) and anthrone (Dreywood, 1946 and Laurentin and Edwards, 2003).

The phenol-sulphuric acid method (Dubois *et al.*, 1951) is reported as being the quickest and most reliable (as well as being sensitive and simple) method for measuring neutral sugars in oligosaccharides, proteoglycans, glycoproteins and glycolipids (Masuko *et al.*, 2005). The method has various drawbacks too, including the use of phenol, which is corrosive to skin, eye and respiratory systems, causing

burns, dermatitis and edemas/long term exposure can have a serious impact on the central nervous system and other vital organs. The results produced by this method differ depending on whether the carbohydrates are neutral or anionic (Albalasmeh *et al.*, 2013).

The ferricyanide method proposed by Myklestad *et al.* in 1997 (a development on Englis and Becker's work in 1943) is published and tested on mono- and polysaccharides in marine samples, so takes into account the presence of salt in seawater and the low carbohydrate content typically found in marine samples. Although this may not seem the best suited method for freshwater samples, the precision needed to detect such low quantities of carbohydrates in marine samples will help provide an accurate and precise detection of carbohydrates in freshwater, in both nutrient poor and nutrient rich catchments.

#### 1a.4: Nitrogen and Nitrogenous DBPs

N contamination is a major problem in shallow aquifers in rural areas and poses a threat to groundwater supply. N compounds in groundwater mostly originate from surface or near-surface sources and enter ground water during, and after, high precipitation events (recharge events). Contamination of shallow ground water with nitrate from agricultural fertilizers, animal waste or septic systems is a widely documented problem (Landon *et al.*, 2000 and Jun *et al.*, 2005). Areas of land that drain into nitrate high waters have been deemed as 'Nitrate Vulnerable Zones' by the UK Environment Agency (EA). These zones are classified as areas of land that have been identified as exceeding the 50 mg NO<sub>3</sub> L<sup>-1</sup> maximum contaminant level (MCL) in drinking water. Research has studied the potential to use nitrogen isotopes found in nitrate and ammonium ions, as they can be utilised to identify nitrate sources. Each source has a typical range of isotopic values, and the change between these values and nitrate concentrations can provide information on the natural attenuation process (Jun *et al.*, 2005).

Dissolved organic nitrogen (DON) is an important precursor of N-DBPs such as haloacetonitriles and nitrosamines (such as N-Nitrosodimethylamine), and the formation of these potential carcinogens occurs during the chlorination and chloramination disinfection process. Chlorine (and its compounds) use is widespread as a disinfectant due to its effective control of THMs and haloacetic acids. However, links to its formation of toxic N-DBPs have

been made, especially in water with frequent algal blooms (which could result in higher DON concentrations) (Chuang *et al.*, 2013).

Although N-DBPs generally form at lower concentrations than THMs and HAAs, N-DBPs are believed to be more carcinogenic and mutagenic than the currently regulated DBPs.

Nitrosamines, a group of N-DBPs, have been identified as probable human carcinogens. The USEPA estimate a lifetime cancer risk of  $10^{-6}$  from the consumption of drinking water containing 0.2-20 ng L<sup>-1</sup> of nitrosamines. In England and Wales, only three of 41 surveyed water treatment plants had detectable nitrosodimethylamine concentrations, always under 6 ng L<sup>-1</sup> (Dillon *et al.*, 2008) and in another study, nitrosodimethylamine was barely above the detection limit of 0.9 ng L<sup>-1</sup> (Templeton and Chen, 2010). Furthermore, the United States Environmental Protection Agency (USEPA) has recently included 6 N-DBPs in the Unregulated Contaminant Monitoring Regulation 2 for future monitoring (USEPA 2007 and Miyashita *et al.*, 2009). Generally, the accepted formation mechanisms of N-Nitrosodimethylamine and related nitrosamines are nitrosation of organic nitrogen precursors by nitrous acid and/or nitrite (Lee *et al.*, 2007). Haloacetonitriles, a group of N-DBPs, have been reported to be more carcinogenic and mutagenic than currently regulated DBPs (Nihemaiti *et al.*, 2016 and Sfynia *et al.*, 2017). Although much attention has been paid to the formation of HANs, little is known about their environmental behaviour, and therefore their stability in drinking water supplies (Chen *et al.*, 2014).

### 1a.5: Analytical characterisation techniques

Many analytical characterisation techniques have been derived to understand the composition and character of many surrogates of organic matter; the most commonplace are discussed below.

#### 1a.5.1: Fractionation by molecular charge

A procedure for characterising DOC and DON into various fractions using HPLC coupled with Amberlite DAX-8 and XAD-4 resins is increasingly commonplace in the study of drinking water. This procedure splits the water into 5 fractions according to its molecular charge, thus creating fractions named: hydrophobic acids, hydrophobic bases, hydrophilic acids, hydrophilic bases and hydrophilic neutrals (Hughes *et al.*, 2016). These fractions are generated and contained as separate 'sub-samples' which can then be stored and analysed for DOC and DON content and

carbohydrate content, for example. Other published methods involve the use of methanol to form another fraction, hydrophobic neutrals (Lara and Thomas, 1994, Leenheer, 1981).

Characterisation of DON via XAD and DAX resin based fractionation has been achieved previously by Chang and Wang (2013), although their study appears to have several shortcomings. Focussing mainly on DON components in treated wastewater effluents, their method utilised three resins rather than the two commonly used for DOM fractionation (Chang and Wang, 2013), thus increasing experiment costs and time, although providing data for one extra fraction when compared to Hughes *et al.*, 2016. Their studies also focussed only on DON with a mass of more than 100 Da, although it has been suggested that 30-50% of DON recorded has a mass of <1000 Da (Xu *et al.*, 2011), showing that Chang and Wang's method may be excluding components of DON (that are of a very low MW) that could be potentially harmful when chlorinated. For example, aniline has a mass of 93.13 Da, is highly aromatic, and is widely used in the manufacture of pharmaceuticals, photographic developers, shoe polish and dyestuffs. It is a suspected carcinogen and is highly toxic to aquatic life (Yan and Jen, 2004), therefore the detection and removal of aniline in water abstracted for human consumption is paramount. Furthermore, analysis of the ammonium cation in Chang and Wang's work was completed using a Merck Millipore MColorTest 14423, which has detection levels of 0.2 – 8 mg L<sup>-1</sup> NH<sub>4</sub><sup>+</sup> (Merck, 2013), whereas more accurate equipment, such as an ion chromatogram (with a cation column), can provide concentrations as low as to 0.005 mg L<sup>-1</sup>. Finally, and arguably the biggest downside to Chang and Wang's method was that it required 1300 L of sample, whereas the method proposed here uses just one litre. Westerhoff *et al.* have also used similar resins to achieve DON fractionation into 8 fractions, including colloidal, amino acid and and amphiphilic acid fractions, concluding that the bulk of the dissolved organic nitrogen in an algal culture, a bacterial culture, a freshwater sample and a sample from a water treatment works was found in the colloidal fraction (Westerhoff *et al.*, 2007)

Once the individual fractions of water have been identified and separated, research into these fractions and their carbonaceous and nitrogenous (C and N, respectively) DBP formation potentials (DBPFP) can be undertaken. Whilst hydrophobic fractions (which can constitute up to ~75 % of NOM) are thought to be the major source of

DBP precursors, a range of species can be involved. Aliphatic and nitrogenous compounds, such as carbohydrates, amino acids, sugars and carboxylic acids comprise much of the hydrophilic fractions, whereas humic and fulvic substances dominate the hydrophobic fractions (rich in aromatic carbon) (Bond *et al.*, 2014b). Studies using model compounds have linked both hydrophobic and hydrophilic molecules as reactive DBP precursors, including activated aromatic species,  $\beta$ -dicarbonyl compounds and a small number of amino acids (Bond *et al.*, 2011). In general, though, hydrophobic NOM is found to be a more important source of the DBP groups known as trihalomethanes, haloacetic acids and total organic halogens than hydrophilic NOM. However, hydrophilic NOM also is found to contribute substantially to the formation of DBPs, especially for waters with low humic content, partially due to its tendency to be difficult to remove by common DOM removal techniques. Therefore, hydrophilic DOM should be considered as having an equal contribution towards C- and N-DBPFP as hydrophobic DOM, however, understanding the relationship between their occurrence and the dominant fraction of molecular charged DOM compounds is still vital in producing a cleaner, safer drinking water.

### 1a.5.2: Spectrophotometric analysis

Spectrophotometric analysis of surface water absorption of UV light is widely attributed to the aromatic chromophores present in the (primarily humic) DOM molecules dissolved in the water. Specific UV Absorbance (SUVA) data generated from this analysis can help determine whether the DOM consists of hydrophobic or hydrophilic contents, and absorbance at 280nm has been found to correlate well with aromatic content of DOC (Simonsson *et al.*, 2005). Other studies have suggested that absorbance at 272nm can act as a proxy to indicate activated aromatic groups important for DPB formation (Korshin *et al.*, 2009), absorbance at 350nm correlates with DOC concentration and the E2E4 ratio (254nm/410nm) acts as a proxy for molecular weight (Spencer *et al.*, 2007). The methods for obtaining this data are both time and cost effective, with possibilities of analysing up to 96 samples instantaneously, although individual sample analysis, using a cuvette instead of a microplate, is deemed more accurate. Data generated from this method shows absorbance of the sample between a range of 200-800nm, thus generating a lot of

useable data in a short time period, although only the first 50-100nm worth of data is typically studied.

#### 1a.5.2.1: UV detection of carbohydrates

The detection of mono and polysaccharides will help to provide 'total carbohydrate' concentration data as it encompasses both single and multi-chained carbohydrates. One of the limitations of this method is that it assumes total monosaccharides and polysaccharides available in the water are in the form of glucose, so it is unable to provide concentration data on individual carbohydrates. This reaction relies upon oxidation at an alkaline pH, during which  $\text{Fe}^{3+}$  is reduced to  $\text{Fe}^{2+}$ , which is then subjected to condensation with the chromogen 2,4,6-tripyridyl-*s*-triazine (TPTZ) to produce a violet colour. This method detects monosaccharides, non-reducing sugars and polysaccharides (made by reduction, by hydrolysis of the glycosidic bonds) (Myklestad *et al.*, 1997). Liu *et al.*, in 2009, report that the ferricyanide mediated technique has detrimental effects on microorganisms. Although the ferricyanide ion is usually considered nearly nontoxic, its toxicity is caused by 'free' cyanide anions that can block the electronic transfer and disrupt the respiratory chain when it is irradiated by UV light. The authors work shows that a 50.8% inhibition of growth of *E. coli* was achieved with a concentration of 50 mM ferricyanide (Liu *et al.*, 2009). Therefore, the safe disposal of UV-radiated samples produced throughout this method is paramount.

Although spectrophotometric analysis of water samples is rapid (1 sample per 2 minutes, using a cuvette) and accurate, there is currently no spectrophotometry method published for the determination of total or free carbohydrates in freshwater using ferricyanide, although methods for the determination of carbohydrates in seawater (Myklestad *et al.*, 1997), marine mucilages (Mecozzi, 2005) and soil samples (Grandy *et al.*, 2000) have been published. It is proposed that the Myklestad method can be adapted for freshwater samples. Data obtained from the adapted Myklestad method can provide information on the range of total monosaccharides and polysaccharides concentrations in freshwater samples.

### 1a.5.2.2: Size exclusion chromatography

Size exclusion chromatography is a method of determining the molecular mass distribution of the DOM in a sample. An aliquot of sample is injected into a column held at a set temperature, and the retention time of the molecules is directly related to their molecular mass – the shorter the retention time on the column, the higher the molecular mass of the compounds, and vice versa. The time these molecules exit the column is recorded by the UV absorbance of the sample at 254nm, as this wavelength is a proxy for molecular weight (Chowdhury, 2013). The understanding of the molecular mass of DOM present in water can provide an insight into the membrane fouling and DBP formation potential during water treatment (Her *et al.*, 2002).

### 1a.5.3: Pyrolysis

Another method for the fractionation of waters is *via* pyrolysis, achieved here by Gas Chromatography/Mass Spectrometry (GS/MS). Pyrolysis is the process of decomposition of compounds by high temperatures, in an inert atmosphere; in this case, up to 550 °C. Despite the high temperatures, the advantage of using this method is that natural polymers (thought to represent the bulk of NOM precursors) can clearly be identified because they yield very specific fragments with few interferences among the biopolymers (Leenheer and Croué, 2003).

### 1a.5.4: Ion Chromatography

Ion chromatography is a form of liquid chromatography, where the efficient chromatographic separation of ionic species is achieved. It is the identification technique of choice for the analysis of ionic/ionisable species in a solution. For effective chromatographic analysis, a mobile phase, made up primarily of water and an organic solvent and a column that shows affinity for the sample and a detection system. Within this column, the ions to be analysed are separated by the pellicular coating of the column, before being passed into a second ion exchange column, removing the ions present in the mobile phase. The remaining ions are then measured by electrical conductivity (Anderson, 1976). Data generated from ion chromatography can also be linked to DOC concentration in waters, for example. The reduction of atmospheric sulphate ( $\text{SO}_4^{2-}$ ) due to a decrease in fossil fuel burning has been

identified as a key driver of rising DOC concentrations in waters within the northern hemisphere (Evans *et al.*, 2012).

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# Chapter 1b

The history and  
mechanisms of potable  
water chlorination

## 1b.1: The History of Water Disinfection

Water is the fundamental source of life for all living organisms, from the largest fauna to the smallest flora, and access to clean water can determine the survival of a population of these organisms. Historical evidence of work by Hippocrates (460-354 B.C.) shows understanding of the importance of a clean water source, which is recorded in his hypothesis: *“the qualities of water differs from one site to another in both taste and weight. One should consider the source of the waters which they use, whether they be marshy and soft, or hard and running from elevated and rocky situations, and then if salty and unfit for cooking... for water contributes much to health”* (Baker, 1948, and LeChevallier and Au, 2004).

Progression in time has brought with it both advances in technology, water distribution and availability but also the use of water for washing, cooling and sorting, agriculture and manufacture, such as in the industrial age. In 1849, Snow published his hypothesis on the causation of the waterborne infection cholera, after studying data collected on a cholera outbreak in London, UK. He found that water supplied to the southern districts (which were worst affected by the outbreak) was abstracted from a point in the nearby River Thames, situated just downstream from a sewage discharge outlet. With the other districts of London (much less affected by the outbreak) abstracting from the cleaner, higher reaches of the Thames, or its tributaries, it was hypothesised that cholera was distributed through the water system (Snow, 1849, and Bingham *et al.*, 2004). It can be argued that this discovery has set the path for research into drinking water quality, and the need to remove pathogens to improve the health of its consumers. This research ultimately led to the decision to use chemical disinfectants to cleanse water for human consumption.

Chlorine was discovered in 1772 by Carl Wilhelm Scheele, a Swedish pharmacist, who reported chlorine's bleaching properties, but Berthollet recognised that chlorine could be used to remove colour from cloth in France, by using a potash aqueous solution of chlorine gas. In 1799, Tennant substituted limestone with the potash and made a bleaching powder called calcium hypochlorite, which was much safer to store and transport. Calcium hypochlorite use was commonplace in industrial applications throughout the 1920s, and demand markedly increased in the early 1900s as it was required for bleaching of wood-pulp newsprint.

Chlorine has also been used for military purposes, such as at the battle of Ypres on the 22<sup>nd</sup> April 1915, where 5730 cylinders containing a total of 180000 kg of chlorine gas, were dug into a 6000 m long front, causing thousands of casualties and fatalities. In modern day uses,

chlorine is used in plastic production (for example, polyvinyl chloride – PVC), paper production (as a bleaching agent and biocide), production of chlorinated solvents, water purification (including swimming pools and water parks) and for other chemical and pharmaceutical uses (Evans, 2005).

Chlorination of water supplies became increasingly widespread following 1912, likely due to the successful disinfection of water in New York, USA (Evans, 2005). The use of chlorine as a disinfectant for water supplies was demonstrated to the public after a typhoid outbreak in 1897 in Maidstone, UK, where the water main was disinfected with sodium hypochlorite to kill off the bacteria responsible. This method of disinfection was then adopted in the USA in 1908 in Chicago and Jersey City. The first recorded large scale water disinfection using chlorine (gas) was performed in Niagara Falls, New York, USA, the site of one of the first chlorine gas production facilities. Since then, the effect of chlorination on the death rate from typhoid fever dropped in USA from a pre-1908 level of 30/100,000 people to 8/100,000 people by 1920, and finally to 0/100,000 people by 1950 (Evans, 2005). Chlorine and its compounds are the most commonly used oxidants/disinfectants in the water industry to date. These water treatment reagents are able to disinfect microorganisms, partially degrade and oxidise the organic and inorganic impurities, and remove the colloidal particulate material and heavy metals (Jiang and Lloyd, 2002), thus creating a final product that is deemed safe for human consumption.

Further advances in technology, and therefore research, however, show that waters high in naturally occurring organic matter may cause health issues when they are disinfected, especially *via* by-products created during the disinfection process, called disinfection by-products (DBPs). Natural organic matter (NOM) occurs naturally in all fresh and marine water, derived from terrestrial and aquatic sources of degraded organic and microbial material. NOM can be derived from autochthonous (produced within the water) and allochthonous (external) sources and naturogenic and anthropogenic activities. Thus, NOM structure and characteristics are complex (Gondar *et al.*, 2008). Studies by Wang *et al.*, (2009), for example, have shown that hydrophobic materials with high molecular weight (MW) originate predominantly from an allochthonous source, whereas autochthonous compounds presented more hydrophilic character with low MW comparatively (Wang *et al.*, 2009 and Leloupet *et al.*, 2013). When NOM is dissolved in water, it is referred to as dissolved organic matter (DOM), and is the focus of many studies into DBPs (Leenheer and Croué, 2003). NOM is also purported to attribute to staining of porcelain sinks and baths, clothing

washed in it, it can block filters used in the water treatment works, and it can promote creation of a bio-film on the inside of distribution pipes acting as a substrate for bacterial growth (Matilainen *et al.*, 2010). Although NOM is not hazardous to humans, it produces an unsavoury colour, odour and taste.

Therefore, water treatment companies focus their energy on removing this organic matter, with various methods and degrees of success. No two waters are chemically identical, and therefore their treatments need to be targeted. For example, abstraction locations predominantly fed by waters from an allochthonous source are likely to contain a large portion of high MW hydrophobic compounds that are comparatively easy to remove at the water treatment works (WTW), whereas abstraction locations fed by water originating from an autochthonous source are likely to contain a large proportion of low molecular weight hydrophilic compounds, which are much harder to remove at the WTW.

A common DOM removal technique is coagulation, which takes place at the treatment works prior to treatment with chlorine. Coagulation is a process where the repulsive potential of electrical double layers of colloids are reduced so that micro-particles can be produced. These micro-particles can then collide with each other and form larger structures, called flocs, in the flocculation process. These flocs are then settled out and/or filtered from the water before disinfection. Coagulation doses vary according to many water parameters, including turbidity, pH, temperature and specific UV absorbance. There are many coagulants to choose from, each with their merits and drawbacks. For example, aluminium chloride is used as a coagulant as it releases trivalent aluminium ions into the water, where they are hydrolysed and form into soluble complexes with high positive charges. Aluminium chloride is stable and easy to use, and has high colour removal efficiency, however, if not properly dosed, it can leave high aluminium residuals in the water, which have been linked with Alzheimer's disease (Flaten, 2001). Another method is electrocoagulation, which is not commonly used. This method passes electricity through the water to stabilise the charge of the NOM particles, meaning that several particles can combine to form larger agglomerates that can then be easily removed. This method is very effective in all temperatures and removes the smallest of charged NOM particles, however, it has a high energy consumption (Matilainen *et al.*, 2010). In waters where reactive precursors are low in anionic charge, or neutral, coagulation will have little impact upon their removal. Such waters are likely to be of low specific UV absorbance (SUVA – measured at 254 nm/DOC), have hydrophilic properties and have a lower MW, and are likely to contain aqueous amino acids (such as glutamic acid, glycine,

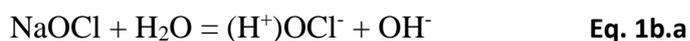
serine and aspartic acid, with MWs ranging from 75-147 g mol<sup>-1</sup>), proteins (originated from algae or phytoplankton, and can include phenol, pyridine, toluene and styrene groups) and carbohydrates (such as glucose, arabinose and mannose – MW ranging from 150-180 g mol<sup>-1</sup>).

Sludge formed from the removal of NOM can be added to agricultural fertiliser, downstream from the treatment works. Waters are often subjected to a sedimentary and filtration stage to remove final flocs before disinfection.

## 1b.2: Mechanisms of Potable Water Chlorination

Chlorination is the act of adding chlorine to water. Chlorine is commonly available in 3 forms – sodium hypochlorite, calcium hypochlorite and liquid chlorine. Sodium hypochlorite is the most commonly used form of chlorine in water treatment processes, and it can be added by gravity, a chemical metering pump, or by manual addition. Calcium hypochlorite is also known as ‘powder chlorine’, and this powder usually contains around 70% available chlorine. This can be added to the water in pellet form, or by mixing a strong solution with an aliquot of the water, then adding this to the larger water body to be treated. Finally, liquid chlorine (chlorine in its elemental form) is available as a compressed gas in 100% purity, and is normally added to the water by a vacuum operated solution feed system, or under pressure. The oxidising potential of all 3 of these types of chlorine are the same – all 3 produce hypochlorous acid, the oxidising agent that is needed for disinfection in water and waste water treatment.

When sodium hypochlorite is added to water, it reacts with the water to form a hypochlorite ion and a hydroxide, and although this produces a slight alkali pH, this usually makes no difference to the final water pH – the quantity of chlorine added is very small compared to the buffering power of most waters. See Equation 1b.a



Furthermore, the pH of the water to which chlorine is added is important relative to the varying proportions of the hypochlorous acid and hypochlorite ions. For example, in a water with a pH of over 6.0, the proportion of hypochlorous acid declines from virtually 100% down to almost 0% at pH 9. The disinfecting power of hypochlorous acid as a bactericide is approximately 80 times more powerful than hypochlorite. Therefore, in free residual

chlorination, the higher the pH value, the powerful active the residual is because of its lower proportion of hypochlorous acid. See Figure 1b.1.

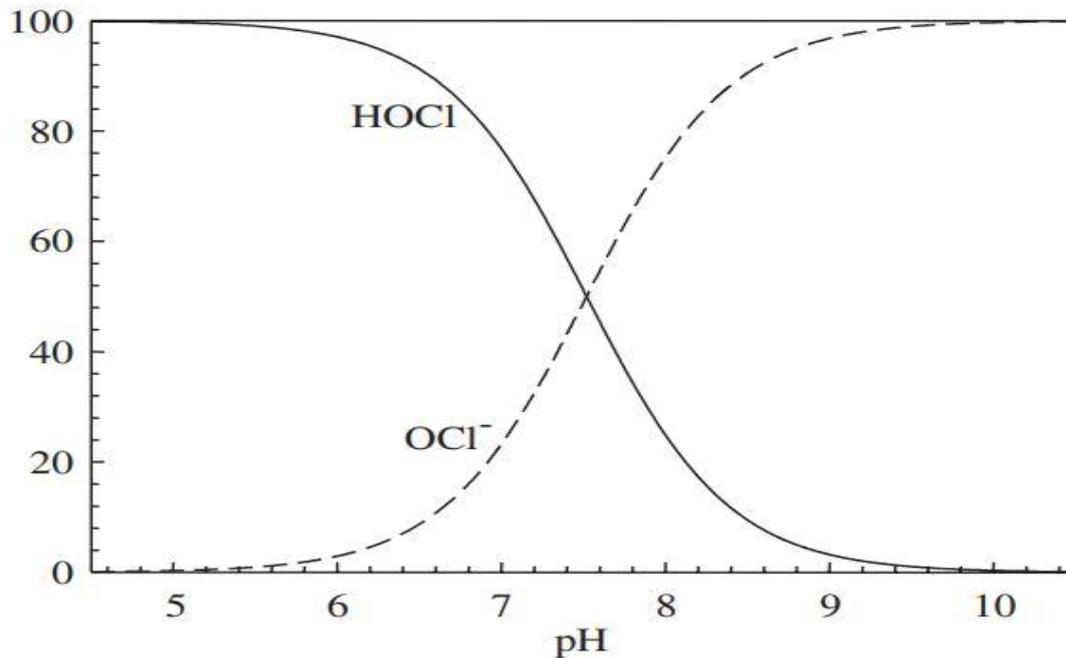
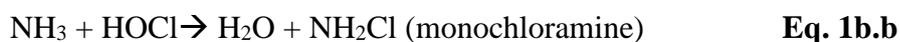


Figure 1b.1: Relationship between HOCl and OCl<sup>-</sup> at various pH levels, with pH displayed on the 'x' axis and percentage of available chlorine displayed on the 'y' axis (Feng, Smith and Bolton, 2007)

Often, when potable water is chlorinated, ammonia is added to maintain a residual for a long time. This forms chloramine *via* the following reactions. These reactions are immediate and are pH dependant – at a pH of 8.5 or higher, only monochloramine is formed, below this, mixtures of monochloramine and dichloramine are formed, and below pH 4.2 only nitrogen trichloride is formed (see equation 1b.b).



For drinking waters treated with both chlorine and ammonia, the most common species is monochloramine. Low doses of chlorine result in the formation of monochloramine and dichloramine when ammonia is present in the water naturally. When all of this ammonia is used up, in the formation of chloramines (typically when the dosage reaches 8-10 times the ammonia concentration), this is known as the breakpoint. After this is reached, the combined

chloramines are present as free chlorine which is a mixture, at normal pH levels, of hypochlorous acid and hypochlorite. This can be described as the destructible combined residual. See Figure 1b.2.

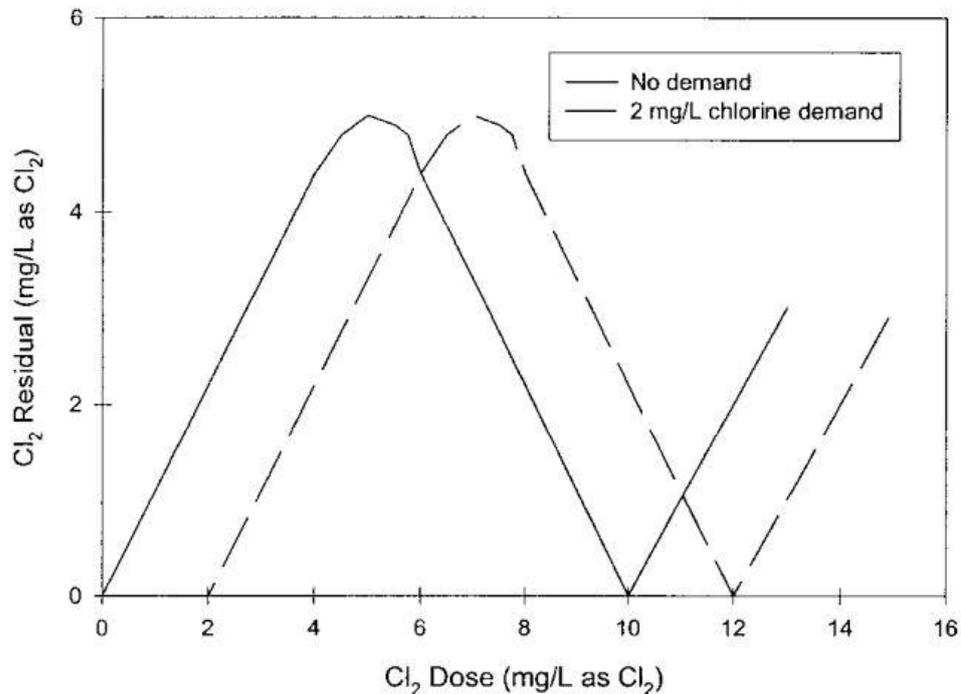


Figure 1b.2: The breakpoint curve of chlorine, with combined residual displayed under initial curve, breakpoint achieved as residual drops to 0 mg L<sup>-1</sup> and the free residual (or nondestructible combined chlorine residual) displayed as the re-increase from 0 mg L<sup>-1</sup> upwards (Source: Chen and Jensen, 2001)

Chloramines are much weaker disinfectants than hypochlorous acid, as the bactericidal and virucidal properties of free chlorine are vastly superior to residual chlorine (Hydro Instruments, 2017 and Brungs, 1973)

Chlorination treatment for:	Typical dosage rates (mg L <sup>-1</sup> )
Algae	3-5
Bacteria	3-5
Colour removal	1-500 depending on severity
Odour	1-3
Raw Sewage	15-20
Swimming Pool	1-5
Taste	1-3

Table 1b.1: Typical dosage rates of chlorine (in mg L<sup>-1</sup>) for the following unsavoury contaminants in an average water destined for human consumption (Source: Hydro Instruments, 2017).

Table 1b.1 displays average typical dosage rates for the successful treatment of various waste waters, recreational waters and typical conditions found in some freshwaters. It is important to note that these concentrations vary depending upon the target water and the concentrations of each target within the water.

### 1b.3: An Introduction to Disinfection Byproducts

There are two main constituents of DOM found in freshwater; dissolved organic carbon and dissolved organic nitrogen (DOC and DON respectively). As a general rule, the DOC fraction contains the predominantly carbonaceous compounds whereas DON fractions contain the predominantly nitrogenous compounds (although some heavily carbonaceous compounds may contain a nitrogen compound, and *vice versa*). Although DOM is present in all freshwaters, its concentrations can vary considerably depending on the water source. For example, DOM will be elevated in water draining peat-accumulating wetlands (where the rate of photosynthetic production of organic matter exceeds that of its decomposition) (Freeman *et al.*, 2004), such as bogs, fens and peatland. Due to the very wet nature of these wetlands, soil pore water drains into rivers and carries with it DOM, resulting in a very brown coloured water rich in DOM. Despite this fact though, water for human consumption is still sourced from these sparsely populated highlands (where peat accumulating wetlands predominantly occur), due to the lower potential for pollution and contamination by industry and mankind (when compared to the densely populated lowlands), but also due to necessity for freshwater to supply surrounding settlements where other sources are less favourable.

When water is treated to make it suitable for human consumption, chlorine is usually the disinfectant of choice because of its low cost, high availability and its high oxidising potential, which leaves a residual throughout the distribution system and thereby protects against microbial recontamination (Sadiq and Rodriguez, 2003). However, although the chlorine can reduce the risk of waterborne illnesses, it can react with the NOM in the water to create many (around 600 reported by Richardson *et al.*, 2007), potentially harmful compounds, collectively called disinfection byproducts (DBPs). The results of a handful of studies that examine these DBPs shows that exposure to carbonaceous and nitrogenous DBPs (C-DBPs and N-DBPs respectively) can be linked to an enhanced risk of bladder cancer, although the contribution

of each individual DBP is uncertain (Villanueva *et al.*, 2007). Other links to health risks from DBPs include adverse pregnancy outcomes, and some DBPs are mutagens, carcinogens (see Table 1b.1), teratogens or developmental toxicants (Muellner *et al.*, 2007). Table 1b.2 (below) shows concentrations of certain DBPs that are associated with a  $10^{-6}$  lifetime cancer risk.

Table 1b.2: Drinking water concentrations of DBPs associated with a  $10^{-6}$  lifetime cancer risk (Mitch *et al.*, 2009).

<b>Compound</b>	<b>Concentration (<math>\mu\text{g L}^{-1}</math>)</b>
Bromodichloromethane	0.6
N-nitrosodimethylamine	0.0007
N-nitrosomethylethylamine	0.002
N-nitrosodiethylamine	0.0002
N-nitrosodipropylamine	0.005
N-nitrosodibutylamine	0.006
N-nitrosodiethanolamine	0.01
N-nitrosopyrrolidine	0.02

However, despite these efforts to reduce the NOM concentration in drinking water, DBPs were still forming in the treated water at unfavourable levels (maximum concentrations of  $120 \mu\text{g L}^{-1}$  for chloroform found from one study (Savitz *et al.*, 2006). Chloroform, a tri-halogenated methane (THM) compound was first reported in drinking water in the early 1970s (Xie and Reckhow, 1994), and further examination of the water found other compounds such as bromoform, chlorodibromomethane and bromodichloromethane; these four compounds are collectively known as THM4. The initial research into DBPs by Rook in 1974, showed that the development of these THMs was linked to reactions between chlorine and carbon found in Dutch drinking water. As a result of this work, and other related studies including those linking THMs to cancer risks, THMs were regulated in drinking water in USA by the United States Environment Protection Agency (USEPA) (1979) to a maximum contaminant level (MCL – at the consumer’s tap) of  $100 \mu\text{g L}^{-1}$  (USEPA, 1979). Recent

studies have shown that bladder cancer in European males was 47% more prevalent in those consuming a final water containing greater than  $50 \mu\text{g L}^{-1}$  THM4, compared to those consuming water containing less than  $4 \mu\text{g L}^{-1}$  THM4 (with lifetime odds of an average male in the USA being around 4%) (Li and Mitch, 2018). (At a similar time, haloacetic acids (HAAs – another group of DBPs) had been identified in drinking water at comparable levels to THMs. The MCL level for THM4 was lowered to  $80 \mu\text{g L}^{-1}$  in 1998 (as an annual average from 4 locations within the distribution system for each plant (Westerhoff, 2006)), and the supporting regulations also set MCLs for five HAAs (called HAA<sub>5</sub>) at  $60 \mu\text{g L}^{-1}$ , bromate at  $10 \mu\text{g L}^{-1}$  and chlorite at  $1000 \mu\text{g L}^{-1}$  (based on monthly data averages) (USEPA, 1998, and Richardson *et al.*, 2007). In 2003, the UK updated its MCL from a rolling mean over 3 months to an absolute value, set at  $100 \mu\text{g L}^{-1}$  (although the European Union deemed  $150 \mu\text{g L}^{-1}$  to be a safe MCL in 2008) (Brown, 2009).

Due to these regulations, water treatment companies were frequently found to be exceeding these limits, and they therefore sought a different disinfection method to help them achieve the MCL. Chloramine was introduced as a replacement for chlorine in such locations, as studies found that chloramine generally helped the WTWs meet the MCL, when WTWs using chlorination found it harder to meet these levels (Goslan *et al.*, 2009).

However, although chloramination forms fewer of the regulated THMs, it has been widely reported that disinfection by chloramination generates much higher concentrations of N-DBPs than chlorination, such as nitrosodimethylamine (Richardson *et al.*, 2007). Although these compounds are found at concentrations much lower than C-DBPs, they are believed to be carcinogenic/mutagenic (Yang *et al.*, 2010), and therefore harmful to humans. These N-DBP compounds have been detected in chlorinated drinking water, and are thought to be much more hazardous than C-DBPs. As an example of the dangers of N-DBPs, research into haloacetonitriles (HANs), a group of N-DBPs that contain the compounds bromoacetonitrile (BAN), chloroacetonitrile (CAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN) and bromochloroacetonitrile (BCAN), has determined that all of the above HANs induce acute genomic DNA damage with cytotoxic potencies ranging from  $2.8 \mu\text{M}$  (for dibromoacetonitrile) to  $0.16 \text{ mM}$  (for trichloroacetonitrile) and are therefore classed as toxic (Muellner *et al.*, 2007). Studies have shown concentrations of haloacetonitriles in Scottish final distribution network samples of between  $0.01$  to  $0.35 \mu\text{g L}^{-1}$  (Goslan *et al.*, 2009).

Due to increasing research and findings in the area of NDBPs, the USEPA cited N-DBPs as a research priority in 2002. However, to date, no US or UK MCLs have been set, although the California Department of Health Services has established a  $0.01 \mu\text{g L}^{-1}$  notification level for N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and N-nitrosodiopropylamine (NDPA) in drinking water. At concentrations of  $0.2 \mu\text{g L}^{-1}$  for NDMA,  $0.1 \mu\text{g L}^{-1}$  for NDEA and  $0.5 \mu\text{g L}^{-1}$  for NDPA, the source is recommended to be removed from service (Mitch *et al.*, 2009). Furthermore, the World Health Organisation (WHO) has suggested guideline values of  $20 \mu\text{g L}^{-1}$  for DCAN and  $70 \mu\text{g L}^{-1}$  for DBAN (Goslan *et al.*, 2009 and WHO, 2006).

First world countries are generally taught that the healthiest drink they can consume is water, the source of this is normally the kitchen tap – many people would be shocked to hear that their drinking water contains chloroform, albeit at safe concentrations. Furthermore, it is very difficult to track the safety of the water once it leaves the treatment works, as distribution pipes are typically buried several meters underground, and are too narrow to navigate, therefore our understanding of the microbial ecology of drinking water distribution systems is limited. Treated water is delivered to consumers through a complex distribution infrastructure, and although most microbial contamination is removed at the treatment works, some microorganisms can persist after treatment, and enter and live in the distribution network. The installation of filters at the consumers taps could help reduce the need for residual chlorine, but increases consumer costs. Microorganisms will often have a better chance of survival in these networks attached to the distribution pipe surfaces, within a biofilm, rather than in the water. Studies by Flemming (1998) show that 95% of the microbial biomass in a drinking water distribution system (DWDS) is attached to the pipe walls, forming biofilms (Flemming, 1998 and Douereloet *et al.*, 2014), therefore, a constant disinfectant residual concentration is required to limit the regrowth of bacteria in the DWDS (Hwang *et al.*, 2012).

In order to gain a broader understanding of the formation of DBPs, the only logical way of reducing their formation potential, it would seem, is to identify the source of the DBP precursor. In identifying the source, steps can be taken to reduce the concentration of this precursor in the raw water, and therefore lesser doses of chemicals and disinfectants will be required to create a safe final drinking water. To do this, NOM must be broken down into smaller sub-categories, such as those outlined below. Knowledge of DBP formation as a function of precursor hydrophobicity or size may help drinking water utilities optimize

treatment systems to remove those fractions associated with high DBP yields (yielding N-DBPs such as those outlined in Table 1b.2) (Hua and Reckhow, 2007). Research into this area has shown that hydrophilic molecules are harder to treat than hydrophobic ones, however, removal *via* nanofiltration is suggested as a best suited method for hydrophilic DBP precursor removal (Bond *et al.*, 2011). Nanofiltration, originally introduced to the drinking water industry in the mid 1980s, was designed to ‘soften’ water, but was also found to be very effective at removing organics and inorganics, changing the original purpose of nanofiltration (Van der Bruggen and Vandecasteele, 2003). Due to the high number of detected DBPs in drinking water, and the fact that most total organic halogens are still unidentified, identifying the sources of each DBP precursor will need to be studied at the individual level, with most research efforts dedicated to the most hazardous compounds primarily, with a view to help reduce their occurrence in the final drinking water.

The majority of DBP precursor studies to date have focussed on THMs and HAAs, whereas the relationship between NOM and unidentified DBPs has, understandingly, not been well established (Hua and Reckhow, 2007). Therefore, data collected relating to the content and properties of the DOM measured in freshwater samples can contribute to a wider understanding of the precursors of the regulated and unregulated DBPs, and more accurate research into their formation potential and removal can be conducted.

### 1b.3.1 Health Implications of Disinfection Byproducts

Whilst DBPs have been shown to be carcinogenic, carcinogenicity can vary. Whilst the word ‘carcinogenic’ instantly instills thoughts of critical health conditions, carcinogenicity is measured on a scale, similar to poisoning (where a mild poison may cause stomach upset but a strong poison can ultimately cause organ failure and sometimes death).

Early studies into THM4 found chloroform to be carcinogenic in rodents, which led to the banning of the use of chloroform in a wide range of applications such as medicines and cosmetics (Hrudey *et al.*, 2015). The findings of the report, which dosed male rats at 90 and 180 mg/kg of USP grade chloroform from the age of 7.5 weeks to the age of 111 weeks (approx. 2 years of treatment), and female rats at 125/250 mg/kg, which reduced to 90/180 mg/kg after 22 weeks, averaging 100/200 mg/kg for the study. All rats were found to decrease in weight and overall survival rate, with a significant finding of tumors of the kidney in male rats with incidences of

0% in controls, 8% in the low dose, and 24% in the high dose groups, and female rats were found to have an increase in thyroid tumours (NCI, 1976).

More recent studies have calculated the human health risks of DBPs. Drinking and bathing water in Indian cities was studied, presenting data that shows that chloroform is a probable carcinogen, with a cancer slope factor (i.e. the increased cancer risk from a lifetime exposure to an agent by ingestion or inhalation at a 95% confidence interval) of  $6.1 \times 10^3$  (61,000). The lifetime cancer risk for bromodichloromethane was found to be 1 in 770,000 and 1 in 630,000 in females and males (respectively), with findings that the average lifetime cancer risk of THM4 were found to be higher than 1 in 100,000 (Mishra *et al.*, 2014). Further studies have examined the impact of DBPs (specifically haloacetic acids) upon bacteria as a proxy for human cells, finding that they were mutagenic in bacteria and induced DNA damage in mammalian cells (Richardson *et al.*, 2007), but again, these do not necessarily translate to human health impacts.

#### 1b.4: Conclusion

With an understanding that DOM is a precursor for DBPs, and that DOM can vary from site to site, it is apparent that characterising different DOM types is paramount in understanding the major triggers for DBP formation, and their sources. By identifying potentially problematic sites (i.e. high in regulated DBPs), DOM samples can be collected and analysed to determine the structure, properties and character of this specific type of DOM. A database can then be collated of different character DOM and its formation potential for THM4, attributing to a risk assessment outlining treatment issues at potential water abstraction locations. However, it is important to remember that ensuring effective disinfection is always the primary concern of a treatment works, before efforts to reduce DBPs, if the two ever conflict.

#### 1b.5: Thesis Aims

This study formed part of a larger NERC funded project, called DOMAINE, designed to study Dissolved Organic Matter In the Natural Environment with the aims being:

- To determine whether amino acids and carbohydrates in surface waters are significant precursors for CDBP and NDBP formation.
- To identify water supply areas where the risk of DBP formation is greatest.
- Whether the risk of DBP formation in public water supply increases along gradients of nutrient enrichment in relation to climatic gradients.
- To generate a risk assessment tool for water utilities and map the risk of population exposure to DBP formation.
- To collect water samples from three contrasting UK freshwater catchments to use as proxy waters for drinking water treatment works, and to determine their likelihood to form detectable DBPs once chlorinated and chloraminated to excess.
- To determine whether chlorination or chloramination treatment formed similar or different concentrations of DBPs.
- To achieve the detection of a suite of N-DBPs by gas chromatography/mass spectrometry and solid phase micro-extraction.
- To investigate relationships between DBP concentrations in water samples and the area of land use categories in the catchment.
- To produce a user friendly and relatively simplistic tool to assist land managers, owners and other end users/stakeholders in determining whether their management practices contribute towards elevated organic matter loadings in the water draining it.
- Use these sample waters to create a risk assessment map for the least problematic new abstraction location for water treatment companies in terms of DBPFP.

## 1b.6: Objectives

- To collect waters from 3 different catchment types across a nutrient gradient (*Chapter 2*)
- To create a risk assessment map to inform water abstraction companies of catchment specific DBP formation potential (*Chapters 3a and 3b*)
- To characterise the dissolved organic matter from the 3 selected catchments and explore relationships between the organic matter and the land use, and also the DBP formation potential (*Chapter 4*).
- To determine the C- and N-DBP formation potential of these waters (*Chapters 5a and 5b*)

- To determine whether a GC/MS using s SPME approach to detect N-DBP is suitable in terms of accuracy and detection limits (*Chapter 5b*)
- To analyse the bactericidal and disinfection activities of chlorine and chloramine based treatments and relationships with DOM (*Chapter 5a, 5b and 6*)

### 1b.7: Hypotheses

- **Chapter 2:** The dissolved organic carbon and nitrogen concentrations will vary along a nutrient gradient from the oligotrophic Conwy to the eutrophic Hampshire Avon catchments.
- **Chapter 3a and 3b:** That catchment specific risk assessment maps can be useful for a specific catchment, but due to an almost inexhaustible global variety in DOM, cannot be extrapolated to other catchments.
- **Chapter 4:** That the oligotrophic Conwy will have much lower concentrations of DOM than the eutrophic Hampshire Avon, and the varying Scottish catchments will span a range of DOM concentrations between those of the Conwy and Hampshire Avon catchments.
- **Chapter 5a:** That an increased DOM concentration does not always dictate an increased DBP concentration.
- **Chapter 5b:** That GC/MS using SPME will be suitable for the initial detection of N-DBPs but further sample preparation will be required to ensure optimum results.
- **Chapter 6:** That DOM concentrations will correlate positively with the use of chlorine and chloramine during disinfection activities.

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# Chapter 2

## Experimental Methods

## 2.1: Site Selection

In order to determine whether a link exists between catchment characteristics of dissolved organic matter (DOM) character with knock on effects for disinfection byproducts, geographical information systems (GIS) and water chemistry data were combined with a focus on carbonaceous and nitrogenous compounds.

Sampling sites were selected in the 3 mainland regions of the UK; England, Wales and Scotland, these are known to represent a nutrient and DOM gradient (i.e. DOM concentrations from high through to low) from previous studies namely, the Hampshire Avon, the river Conwy (Tier 1) and southern Scotland (Tier 2) (see Figure 2.1). Chosen under the larger NERC DOMAINE project, Tier 1 sites were selected as baseline catchments, and Tier 2 sites were then used to compare catchment data between, to determine whether any relationships discovered between Tier 1 catchment datasets, can be replicated in Tier 2 catchments, thus adding validity to any relationships discovered in Tier 1 catchments. Study sites were not only chosen for their aforementioned attributes, but also because they have been extensively studied before (in the case of the Hampshire Avon (Jarvie *et al.*, 2005, Yates and Johnes, 2013 and Yates *et al.*, 2016) and Conwy (Evans *et al.*, 2007, Ellis *et al.*, 2009 and Withers *et al.*, 2014)). The Hampshire Avon, Conwy and Scottish catchments were also chosen for study under the NERC Dissolved Organic Matter in the Natural Environment (DOMAINE) project (Ref: NE/K010689/1). The study site locations are outlined in Figure 2.2.

It is intended that the data generated from these sites within this study will be of use to drinking water companies managing the Welsh, Scottish and Hampshire Avon catchments studied here, in locating a hypothetical new location for future drinking water abstraction, with a focus upon the likelihood of DBP formation in the final water. However, it is important to note that abstraction locations will typically be selected upon logistical reasons such as volume of water available, access routes, and sufficient space for WTW development, rather than being selected for low DBP formation potential, which is easier to combat than the above issues.

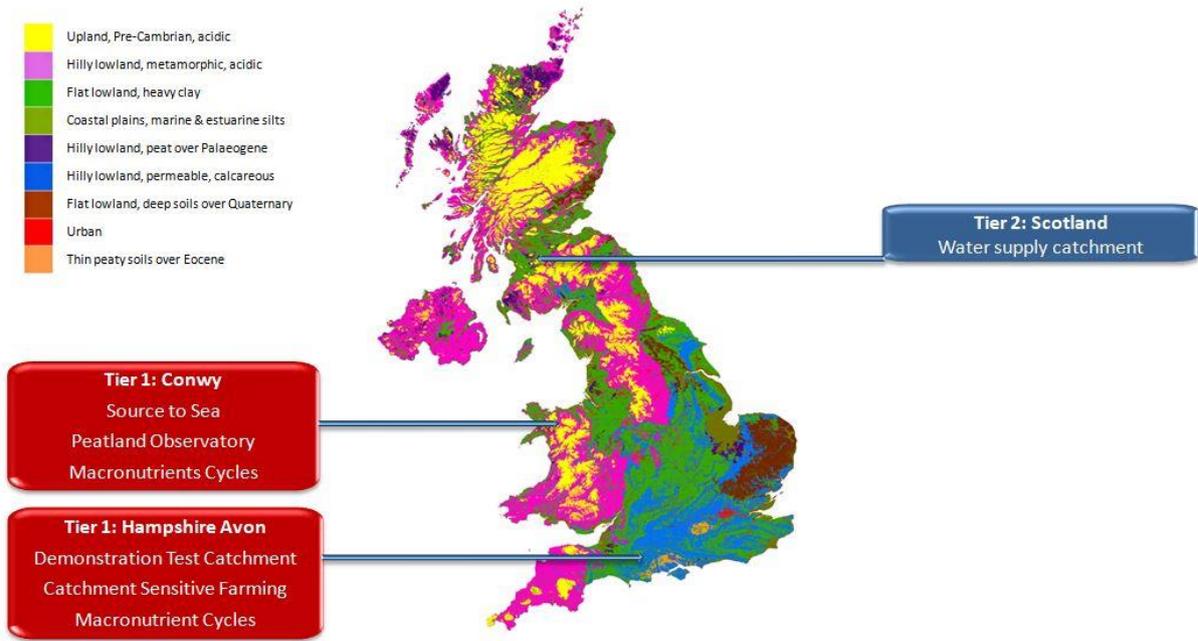


Figure 2.1: Basic GIS map of UK, outlining major soil characters, and identifying Tier 1 and Tier 2 catchments (Source: Greene *et al.*, 2015).



Figure 2.2: Approximate location of Avon, Conwy and Scottish sites within the England, Scotland and Wales. Map orientated vertically in a northerly direction.

The Hampshire Avon catchment was selected to represent a nutrient rich catchment, as studies have reported enhanced phosphorus, nitrate and sediment concentrations, likely originating from the predominantly 75% agriculture land use in the catchment (Zhang, Collins and Gooday, 2012). Studies have shown that the Hampshire Avon has been subject to a condition known as Chalk Stream Malaise, likely due to agricultural non-point source pollution (i.e. linked to nutrient enrichment from fertilisers and manures, coupled with reduction in river flow rates due to drought and abstraction (Jarvie *et al.*, 2005)). Within the Hampshire Avon catchment, two smaller order rivers were selected, the River Nadder and the River Wylye, which drain the higher reaches of the catchment and merge to form the River Avon, just west of the city of Salisbury. The section of the Hampshire Avon catchment studied here, 678.73 km<sup>2</sup>, is dominated by arable and horticultural land uses, with a mainly chalky bedrock resulting in shallow, lime rich soils (Yates *et al.*, 2016). Along the Nadder and Wylye, there are 19 individual sampling points, situated at important sites along the rivers, such as downstream from septic tank outflows or waste water treatment works. The Hampshire Avon was selected for study during this project as it drains a lowland catchment, with different geology and soils to the Conwy, and therefore many different sources of DOM that are not present in the Conwy catchment. See Figure 2.3 for a map of the Hampshire Avon catchment.

To compare data from the nutrient rich Hampshire Avon, the Conwy catchment (located in north Wales) sites were selected as they are representative of a nutrient poor system (after Nedwell, 2002). Twenty seven sites in the Conwy catchment were identified as part of the NERC DOMAINE project, and were within the Tier 1 catchment outlined by this project. . The source of the river is Llyn Conwy, located in the Migneint - an upland moor spanning almost 200 km<sup>2</sup>; the largest area of blanket bog in Wales. Along the reaches of the Conwy and its tributaries are 27 individual sampling sites, selected to represent different sub-catchments in terms of vegetation and land use (bog, montane, urban etc.), which will all have different impacts upon the DOC concentration. However, as the lower reaches of the Conwy are estuarine and tidal, the last sampling point of the main river was situated some 18 km directly, or 23 km by river, upstream to avoid tidal influences (although some sub catchments are still sampled from nearer the sea, but with no tidal influences). Therefore, the total area of all catchments studied was 2789 km<sup>2</sup>. These catchments have many varying land uses, although improved grassland is the most common, the soil is typically freely draining, acidic and loamy soils over predominantly mudstone, siltstone and sandstone geology. The

proportions of land use and soil type classes are close to the Welsh national average, hence, these sites provide a good representation of Welsh catchments and also, upland UK catchments. The upper and mid reaches (above the town of Betws-y-Coed) are predominantly rural, and contain a mixture of open moorland, mountainous terrain (maximum elevation of 872 m above sea level (ASL)), coniferous plantations and enclosed farmland. The south western section of the catchment drains an extensive area of blanket bog (Evans *et al.*, 2006). See Figure 2.4 for a map of the Conwy catchment.

Thirteen much smaller sub-catchments were also studied in south Scotland, under Tier 2 (Figure 2.1), which encompassed many of the land use practices that are found in the Hampshire Avon and Conwy catchments. These Scottish sites were selected to compare to the Avon and Conwy sites, to confirm whether or not the data obtained from these locations was site-specific, or whether the data represented a coherent pattern for a given land use type, nationwide. The sampling sites in the Scottish catchments were selected due to soil and vegetation similarities with the Hampshire Avon and Conwy catchments, to help prove or disprove whether any significant findings were site specific or likely to correlate with similar catchments nationwide. The process of Scottish site collection was influenced by information from Scottish Water that these sites could prove problematic for drinking water purposes as they are suspected to have high concentrations of nutrients, carbon and nitrogen and high DBP formation potential (i.e. concentrations higher than those detected from similar catchments) (Froggatt, 2015). These samples were collected between 2<sup>nd</sup> and 7<sup>th</sup> September 2016, to coincide with the final collection from Avon and Conwy catchments. These thirteen sites drain a total catchment area of 62.96 km<sup>2</sup>, and are located in southern Scotland, spanning the region from East to West. Fifty individual sample sites were located in these thirteen catchments to reflect streams feeding reservoirs from different catchment types that were hypothesised to match those from Conwy and Avon sites. See Figures 2.5 to 2.6 for a location map of Scottish catchments.

## 2.2: Catchment and site characteristics

To further characterise these sites, and to confirm the hypothesis that the catchments are different and representative of typical UK catchments, a Geographic Information Systems (GIS) based method was adapted (below) to obtain data on land use, bedrock and soil type for each sampling location in these three regions, which was then statistically analysed to the water chemistry data obtained from the sample taken from each catchment, to determine

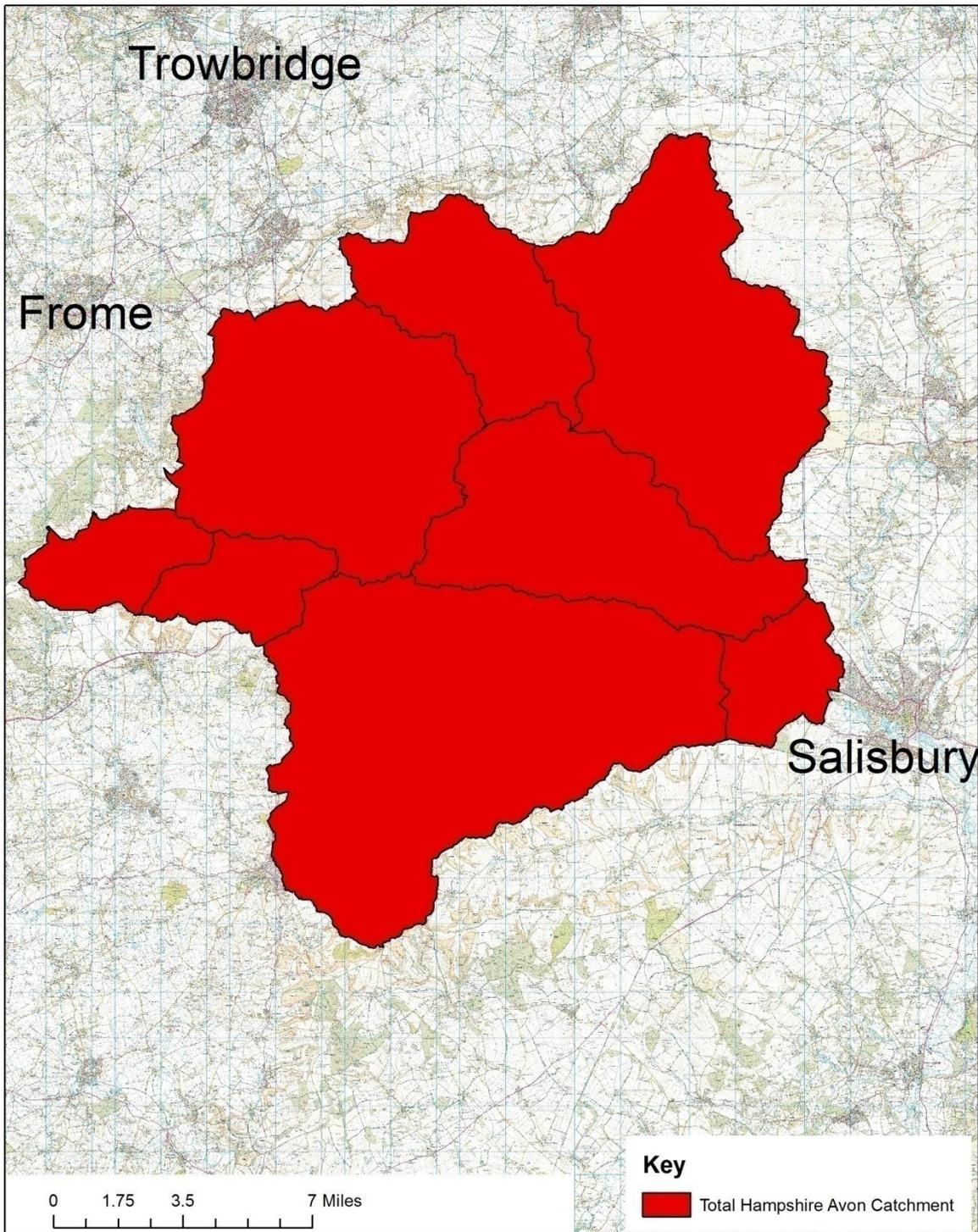
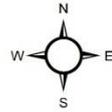
catchment specific relationships between water chemistry and catchment character, allowing for analysis of point sources of DOM, for example, septic tanks, sewage treatment works etc.

### 2.2.1 Geographical Information Systems data production

To obtain data relating to the physical properties of the catchment from which freshwater was sampled, a Geographical Information Systems (GIS) method was implemented. Using ArcGIS 10.5.1 software (ESRI, California, USA), with map data obtained from the EDINA Digimap website (Edinburgh University, UK). Briefly, sampling locations were inputted to the software and plotted on the map. Then a digital elevation model (DEM - acquired with the map data) was used to create a 3-dimensional copy of the map, taking into account elevation. This DEM enabled the calculation of catchments and sub-catchments from the exact sampling locations, giving total catchment area data and a visual representation of the catchment.

Further data was obtained relating to the land use (Land Cover Map 2007 – LCM2007) obtained from the Centre of Ecology and Hydrology (Wallingford, UK), soil type (SoilScape) obtained from National Soil Research Institute (NSRI) (Cranfield University, UK) and geology (BGS) obtained from the British Geological Survey (Keyworth, UK). The full method is covered in Appendix 1, examples of GIS visual outputs are given in Figures 2.2 to 2.6.

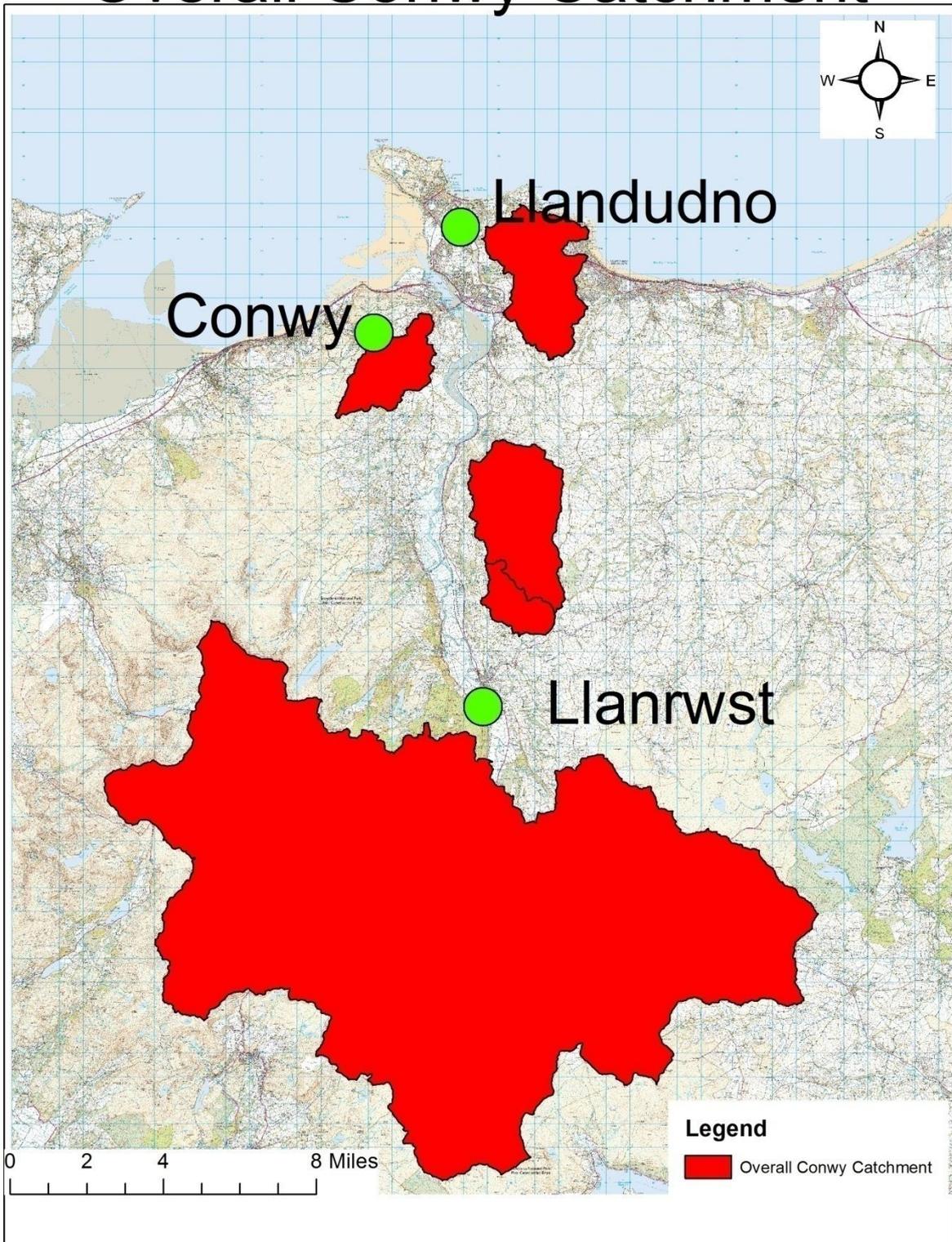
# Avon Catchment



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Figure 2.3: The Hampshire Avon catchment, extending west from the town of Salisbury, England. The general direction of flow is from West to East.

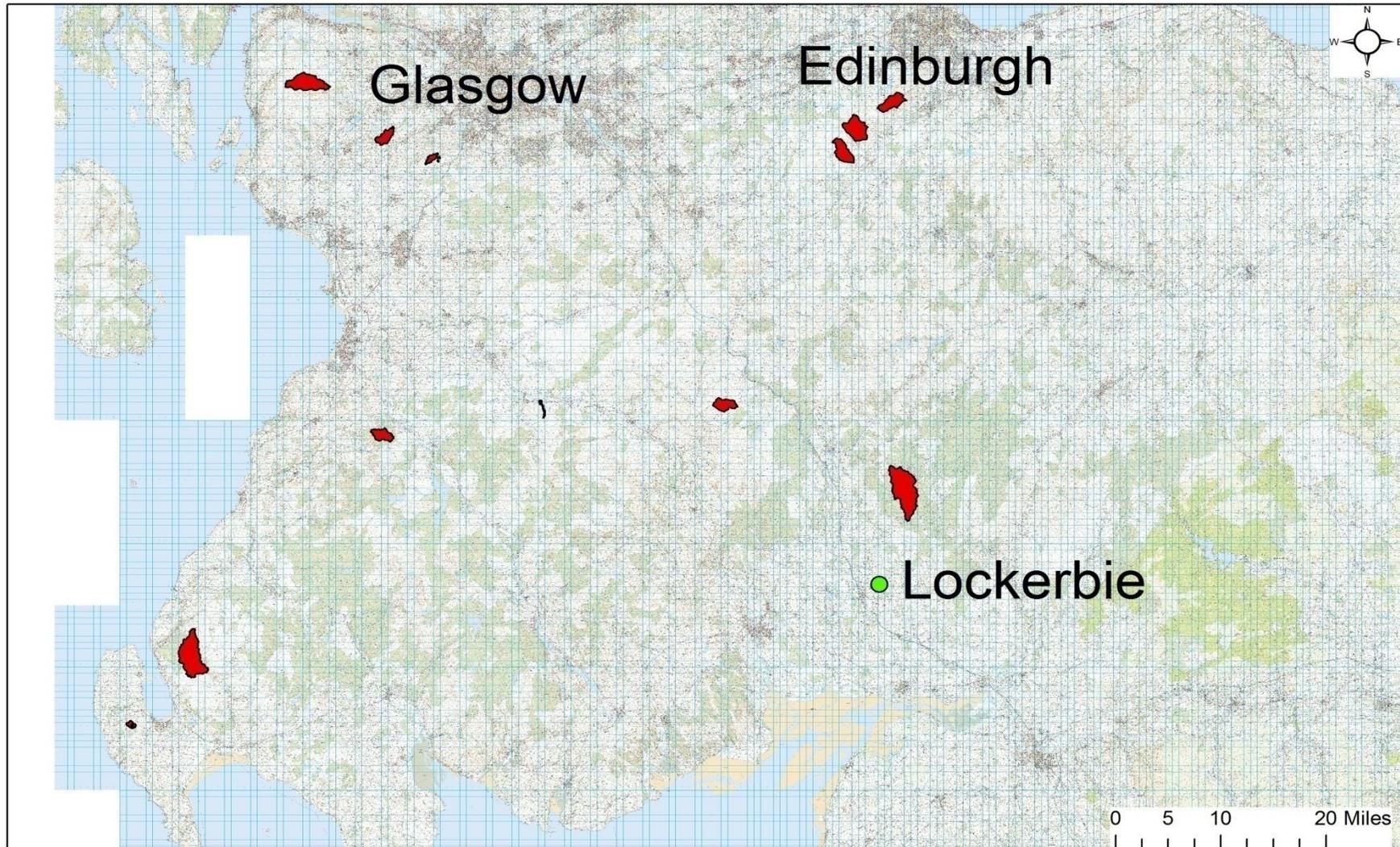
# Overall Conwy Catchment



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Figure 2.4: The Conwy catchment, which extends southerly from the town of Conwy, Wales. The general direction of flow is South to North.

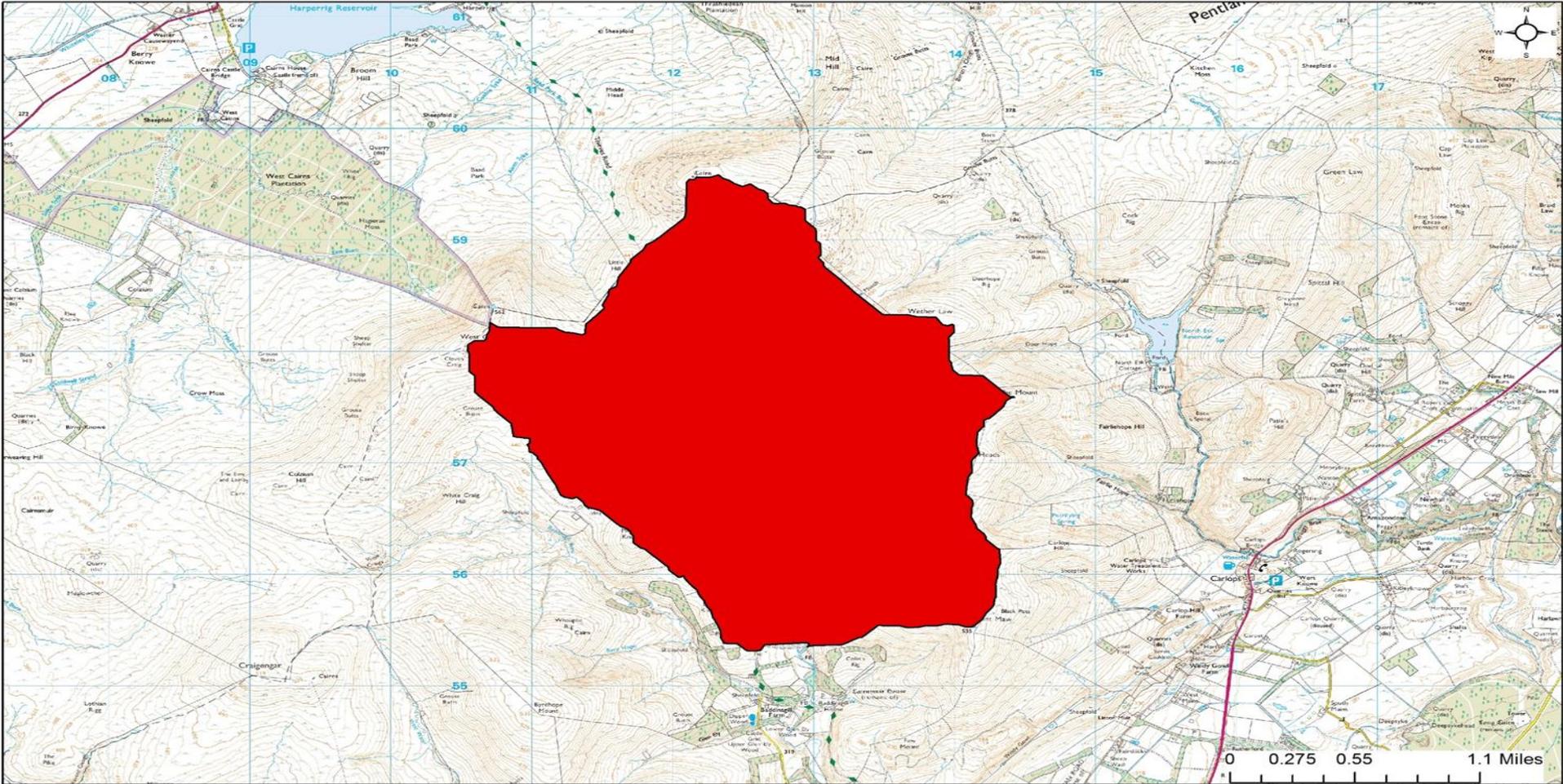
## Scottish Catchments



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Figure. 2.5: Map showing location of Scottish sites located in south westerly Scotland, no far further north than the towns of Glasgow and Edinburgh, and not far further south than the border with England. As an example of the level of detail at which these Scottish sites were analysed at, Figure. 2.6 is presented.

# Baddingsgill Reservoir Catchment



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Figure. 2.6: Location map of Baddingsgill Reservoir catchments, located north of the town of West Linton, in eastern central Scotland,

UK, as an example of the level of GIS representation for all Scottish sites.

## 2.3: Sampling Regime

All water samples were collected and stored in 500 mL PET plastic bottle (Patrico, UK, #PET00500MLJUICESQ) in a cooled, insulated box for transport to the laboratory. Once back at the laboratory, samples were vacuum filtered through 0.45µm Whatman membrane filters within 48 hours of collection, to remove suspended solids and minimise bacterial activity (unless otherwise stated), and stored in the dark at 4° C before analysis. These conditions were selected, following S·liwka-Kaszyńska, Kot-Wasik and Namiesnik, 2003. Samples were not collected in triplicates, however, machine replicates were run to minimise erroneous results.

### 2.3.1: Preliminary Sampling

Water samples from 10 different locations in the Conwy catchment were initially collected and analysed fortnightly, to gain a broad understanding of the different characters of the water and to become familiar with techniques and methods, as outlined below. A year-long quarterly sampling regime was then established, over the course of September 2015 to September 2016, to obtain a representative sample from each location in all 3 month increments to encompass any seasonal variations and to create a better representation of annual concentrations.

### 2.3.2: Quarterly Sampling

Samples from the Conwy catchment were collected quarterly alongside monthly sampling for the larger DOMAINE project, to ensure data was comparable. 28 (later reduced to 27) sampling sites were collected from manually, in the same order monthly to try and eliminate any changes in daily fluxes between the samples. Weather conditions at the time of sampling were not recorded as it was deemed that any significant changes these would present in the data would fall into insignificance once the data from each 3 monthly connection was combined for statistical analysis, From the Hampshire Avon, 19 sites in total were sampled from, alongside the larger DOMAINE project, and shipped to Bangor University quarterly in polystyrene boxes (with ice to keep samples cool), for analysis, also between September 2015 and September 2016.

Samples from Scotland were collected as a one off, over the course of a week in late summer 2016. These samples were stored in a polystyrene box which contained ice, and were filtered daily after sampling to reduce any bacterial/microbial activities altering the water properties. The samples were stored at 4° C for the duration of the week before they were transported to Bangor, by road, in a polystyrene box surrounded by ice, to keep the samples cool.

## 2.4: Analytical methods

### 2.4.1: Ion Chromatography

A Metrohm Professional IC, fitted with a Metrohm 858 Autosampler was utilised, using a method outlined by Hughes, 2013. Samples were loaded into the sample rack in 5 mL plastic vials. The columns fitted to the IC were a Metrosep A (150.0 x 4.0 mm ID) for anions and a Metrosep C4 (150.0 X 4.0 mm ID) for cations. The mobile phase/eluent for anion detection was an aqueous mixture of 0.168 g sodium carbonate (#222321, Lot# MKBQ8223V, Sigma Aldrich) (3.2 mM) and 0.678 g sodium bicarbonate (1.0 mM, Sigma Aldrich, #S6014, lot# BCBC3667), made up to 2.0 L with Milli-Q. The cation mobile phase/eluent was an aqueous mixture of 0.22 mL nitric acid (1.7 mM, P & R, UK, #2317142, lot # 11539/27515) and 0.57 g 2,4,-pyridinedicarboxylic acid (0.7 M) (Sigma Aldrich, # P63808-100G, lot#: BCBK6294V) made up to 2.0 L with Milli-Q. The ion suppressor unit was an MSM11 (Metrohm UK) and the re-generation solution was 18 g oxalic acid (0.1 M, Aldrich, UK, #19413-1, lot # S19352-016) 100 mL acetone and 16.4 mL sulphuric acid (100 mM), diluted and made up to 2.0 L with Milli-Q water. A check standard was inserted into the run in 10 sample intervals, and was made by mixing Multielement Ion Chromatography Cation Standard Solution, certified (Sigma Aldrich, #89316-50ML-F, Lot#: BCBR1826V) in a 1:9 ratio mix with Milli-Q water for cations and Multi Anion Standard 1 for IC (Sigma Aldrich, #69734-100 mL, Lot#: BCBQ1667V) for anions. Limit of detection for all anions and cations is 0.005 mg L<sup>-1</sup>.

Standards were created from individual compounds all acquired from Sigma Aldrich. For anions, sodium sulphate (S9627, Lot# BCBC8999V), sodium fluoride (#201154,

lot# MKBD1665), sodium nitrate (#221341, lot# MKBC5128V), and sodium nitrite (#S2252, lot# MKBG1647V) were utilised.

For cations, potassium chloride (#P4504, Lot# BCBF2693V), sodium chloride (#S9888, Lot# BCBD4494V), ammonium chloride (A4514, lot# 080M0117V), lithium chloride (#213233, lot# BCBC9714V), calcium chloride (#383147, lot#06531AJ), magnesium chloride hexahydrate (#M9272, lot# 100M0075V), sodium bicarbonate (#S6014, lot# BCBC3667) and sodium bromide (#S4547) were used.

Before each analysis, a calibration curve was created using a series of external standards. Dionex 7 Anion Standard was selected for fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphite (#56933 Thermo Fisher Scientific, Massachusetts, USA) whilst a Dionex 6 Cation Standard was selected for lithium, sodium, ammonium, potassium, magnesium and calcium (#40187, Thermo Fisher Scientific, Massachusetts, USA). Each curve was only accepted if it presented an  $R^2$  correlation coefficient of greater than 0.99.

#### 2.4.2: Spectrophotometric Analysis

Spectrophotometric analysis of surface water absorption of UV-visible light is widely attributed to the aromatic chromophores present in the (primarily humic) DOM molecules dissolved in the water. Therefore, when Specific UV Absorbance (SUVA – absorbance at 254 nm divided by the DOC concentration of the sample) data generated from this analysis is correlated with visible light absorbance, this can help determine whether the DOM consists of hydrophobic or hydrophilic contents (Carter *et al.*, 2012).

#### 2.4.3: Amino Acids

Detection of underivatized amino acids can be problematic; many underivatized amino acids are very weak chromophores in the UV-Vis region and possess no native fluorescence (Chaimbault *et al.*, 1999), so detection of amino acids by fluorescence without the addition of chemicals is often not feasible. Size exclusion chromatography (SEC) is also not successful in the accurate detection of amino acids, as SEC provides a low response for NOM structures with low UV absorbance (also including proteins,

amino sugars and aliphatic acids) (Leenheer and Croué, 2003). HPLC has been utilised to identify and quantify both individual and total free amino acids, at low, naturally occurring concentrations (Thomas, 1997). Therefore, it was necessary to obtain pure model compounds of each amino acid, to create a library of detection characteristics that can then be compared to unknown samples, to analyse similarities. The detection of aquatic amino acids is now usually achieved by HPLC methods, which involve some form of pre-derivatisation step. However, some amino acids, such as the more hydrophobic tryptophan, are not captured by many HPLC methods due to a susceptibility to hydrolysis (Bond *et al.*, 2012). Therefore more accurate analytical techniques were required.

## 2.5: Carbonaceous and Nitrogenous DOM Detection

### 2.5.1: Non purgeable organic carbon (NPOC) and total nitrogen (TN)

It is important to note that although TN encompasses organic and inorganic nitrogen, this dataset was recorded for the purpose of calculation of dissolved inorganic and dissolved organic nitrogen groups by combining data from this method and the  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  data obtained from section 2.4.1

NPOC and TN concentrations were detected using an Analytikjena multi N/C 2100s (Analytikjena, Germany) which contains a focus radiation non-dispersive infrared (NDIR) detector, for more precise detection of  $\text{CO}_2$  over other instruments. Standards were made using potassium hydrogen phthalate (Sigma Aldrich, UK #P1088, Lot# MKBW1364V) for NPOC and creatinine (Sigma Aldrich, UK, #C4255) for TN, and Milli-Q water was used as a blank. One mL of sample was added to a 2 mL vial in the sample rack, after which, 10  $\mu\text{L}$  of 2.0 M HCl was added to remove inorganic carbon (i.e. carbonates). The sample rack was positioned on the top of the machine and covered in Aluminium foil to help prevent atmospheric losses, before the sample injection was set up according to the equipment manual. For NPOC measurements, an aliquot of sample (250  $\mu\text{L}$ ) was removed by an auto-sampler needle and then injected into a column heated to 750° C, which vaporised the sample *via* combustion, allowing for  $\text{CO}_2$  detection, which was converted to provide the carbon concentration present in the sample. For TN detection, the process is very similar, but in this case, the oven

is set to 1000° C NO<sub>x</sub> gases are swept from the furnace through to the NO<sub>x</sub> detector, by the oxygen carrier gas.

### 2.5.2: Nitrogenous DOM Compounds

Total amino acid data were generated using a the *o*-phthaldialdehyde and β-mercaptoethanol ('OPAME') spectrophotometric method outlined by Jones *et al.*, 2002. A borate buffer was created by dissolving 3.05 g of potassium tetraborate (Aldrich, UK, P5754, lot# SLBJ1570V) in 500 mL Milli-Q, before adjusting to pH 9.5 using 10 M KOH (made using KOH pellets, Sigma Aldrich, #60377). This solution was stored at 4° C until required.

A concentrated OPAME stock solution was created by dissolving 50 mg of *o*-phthaldialdehyde (Sigma Aldrich, UK, #P0657, lot# BCBN6399V) in 5 mL HPLC grade methanol (VWR Chemicals, UK, #20837.320, batch# 15K160513). This takes approximately 1 minute with mild agitation, before 100 μL of β-mercaptoethanol (Sigma Aldrich, UK, #M6250, lot# STBD6281V) was extracted from the sample bottle by a syringe fitted with a needle, in a fume hood, added to the solution and, shaken to mix.

This mixture was then added to 200 mL of the potassium borate buffer, in an acid washed glass bottle, and left for a minimum of 2 hours to minimise background fluorescence. This working reagent remained stable for up to 48 hours.

To obtain the total amino acid data, a quartz cuvette was rinsed with Milli-Q water, methanol and working reagent 3 times to minimise contamination. The Spectromax M<sup>2</sup>E was 'zeroed' with a blank, containing 20 μL of Milli-Q in 2 mL potassium borate buffer. Samples were then analysed by adding a 20 μL aliquot to the cuvette before adding 2 mL of the OPAME reagent, leaving for 1 minute, and then analysing the fluorescence (at 340 nm excitation, 450 nm emission), with *o*-Phthalaldehyde being the chromophore responsible for the readings. Two different standards were used, ammonium chloride (Sigma Aldrich, UK, #A9434, Lot# BCBM8228V) and glycine (AnalaR, UK, #101193L, Lot# K26449766). A range of glycine standards were created from 5-15 μM, to represent concentrations typically found in water, and ammonium chloride standards were made at 142, 286, 429, 571, 714 and 1000 μM to represent concentrations of 2,3,4,5 and 10 μg mL<sup>-1</sup> NH<sub>4</sub>Cl.

Ammonium data was required for this equation, and was generated using ion chromatography (see section 2.4), before all data was processed using the equation in

$$\text{Amino Acids } (\mu\text{M}) = \frac{O_o - B_o - A_o}{(S_o / 10)}$$

Where  $O_o$  is the OPAME fluorescence reading of the sample

$B_o$  is the blank fluorescence reading with no OPAME reagent present

$S_o$  is the fluorescence reading of 10  $\mu\text{M}$  Amino acid standard

$A_o$  accounts for the interference of  $\text{NH}_4^+$  in the OPAME procedure

Unless the samples show a high degree of brown colouration,  $B_o$  is close to zero and can largely be ignored.

$A_o$  is defined as:

$$A_o = \frac{AC_o}{AS_o} \times AR_o$$

$$AS_o$$

Where  $AC_o$  is the  $\text{NH}_4^+$  concentration of the sample determined by ion chromatography ( $\mu\text{M}$ )

$AS_o$  is the  $\text{NH}_4^+$  standard concentration ( $\mu\text{M}$ )

$AR_o$  is the fluorescence reading of this  $\text{NH}_4^+$  standard using the OPAME procedure.

Figure 2.7: Equation to calculate total amino acid concentration in freshwater samples, adapted from Jones *et al.*, 2002, which achieved a recovery of  $99.2 \pm 0.5\%$  for 5 soilwater samples spiked with 20  $\mu\text{M}$  glycine).

### 2.5.2.1: Amino Acid Analysis by GC- MS FID

A suite of 33 physiological amino acids were detected by GC-MS FID using a Varian CP-3380 fitted with a Zebron ZB-AAA (10 mm x 0.25 mm) column and a CP8400 autosampler, with chloroform as a needle rinse.

The Phenomenex EZ:faast Physiological amino acid determination kit was utilised (#KGO-7166, Phenomenex, California, USA), the method of which is outlined here:

A Varian CP-3380 GC was fitted with a Zebron ZB-AAA column. A carrier gas of helium at 1.1 mL min<sup>-1</sup> constant flow was used. A split 1:15 injection method was used at 250° C, and 1.5 to 2 µL of sample were injected per sample. The oven was set to ramp at 30° C min<sup>-1</sup> from 110° C to 320° C. A FocusLiner (#AGO-4680, Phenomenex, California, USA) was fitted into the injection port. Finally, a Hamilton injection needle was used (Badawy, Morgan and Turner, 2008).

The reagents were supplied ready mixed (see Table 2.1)

Table 2.1: List of reagents and ingredients contained within, supplied with EZ:Faast kit.

<b>Reagent Name</b>	<b>Ingredients</b>	<b>Volume (mL)</b>
Reagent 1 (internal standard solution)	Norvaline (0.2 mM) N-propanol (10%)	50
Reagent 2 (Working solution)	N-propanol	90
Reagent 3A (Eluting medium component I)	Sodium hydrochloride	60
Reagent 3B (Eluting medium compound II)	N-propanol	40
Reagent 4 (Organic solution I)	Chloroform	4 vials x 6
Reagent 5 (Organic solution II)	Iso-octane	50
Reagent 6 (Re-dissolution solvent)	Iso-octane (80%) Chloroform (20%)	50

SD 1, 2 and 3 (Amino acid standard mixtures)	Std 1 (23 amino acids) Std 2 (3 amino acids) Std 3 (6 amino acids)	2 vials of each, 200 nmoles mL <sup>-1</sup> ) of each compound
Eluting medium	Reagents 3a and 3b	3 parts reagent 3a to 2 parts reagent 3B, mixed

Sample preparation was conducted as follows.

A 100  $\mu$ L aliquot of sample followed by a 100  $\mu$ L aliquot of an internal standard solution (0.2 mM Norvaline in 10% N-propanol) was pipetted into a 4mL vial using a Gilson pipette. A sorbent tip (US patent# 6, 7000,246 2004) was fitted to a 1.5 mL plastic syringe and the contents of the vial were slowly passed through the sorbent material (a proprietary cation exchange resin). Keeping the tip and syringe in the vial, 200  $\mu$ L of N-propanol was pipetted into the vial, which was also slowly drained through the sorbent tip. The syringe was disconnected from the sorbent tip and the solution contained within was discharged into a waste container. 200  $\mu$ L of freshly prepared eluting medium (sodium hydroxide and a N-propanol and 3-picoline solution mixed in a 3:2 ratio allowing for 200  $\mu$ L per sample/standard/blank) was pipetted into the sample vial (without removing the sorbent tip fully from the vial to prevent any contamination), before a 0.5 mL plastic syringe was connected to the sorbent tip and a small volume of eluting medium was pulled through to wet the sorbent material, before the contents of tip were ejected into the 4 mL vial. The tip was disposed of (but the syringes were re-usable). A Drummond Dialamatic Microdispenser (fitted with a glass tip) was used to pipette 50  $\mu$ L of a solution containing chloroform and propyl chloroformate which was then added to the 4 mL vial, before this was emulsified using a vortex mixer for 5-8 seconds, before allowing a minute of settling and re-vortexing for a further 5-8 seconds. 100  $\mu$ L of iso-octane was then added using the Drummond microdispenser, before vortexing again for 5-8 seconds and allowing to sit for 1 minute. Finally, using a Gilson pipette, 100  $\mu$ L of 1 M

hydrochloric acid was added and the vial was vortexed again for 5-8 seconds. The resulting solution in the vial separated into two layers.

The top layer of the solution in the vial was then removed by pipette and placed in a 1.5 mL clear glass sample vial (Supelco# 854165, Lot# 2007156) fitted with 0.1 mL vial inserts (Supelco# SU860067, Lot# 2625840) and septum lids (Supelco# 854161, Lot#2583654). These samples were then loaded, alongside standards, into the CP-8400 autosampler for analysis.

Standards were made by mixing 25  $\mu\text{L}$  of SD1 solution with 25  $\mu\text{L}$  of SD2 and SD3, plus 100  $\mu\text{L}$  of reagent 1, to create a 50 nmoles  $\text{mL}^{-1}$  standard concentration. This was repeated using 50 and 100  $\mu\text{L}$  of SD 1,2 and 3, and still using 100  $\mu\text{L}$  of reagent 1, to create a 100 and a 200 nmoles  $\text{mL}^{-1}$  standard concentration, respectively. The internal standard, included in all samples, was norvaline, at a concentration of 200 nmoles  $\text{mL}^{-1}$ , and this was used to calibrate data.

Amino acid recovery using this method varies from 89% to 107% in 5 spiked samples at 200 nmols  $\text{mL}^{-1}$ ; alanine, asparagine, phenylalanine, lysine and tyrosine (Phenomenex, 2018), and other studies have found recoveries of 3 amino acids (valine, leucine and isoleucine) at 3 different concentrations (50, 200 and 400  $\mu\text{M}$ ) at an average of 102.44% (Badawy, Morgan and Turner, 2008)

## 2.5.3 Carbonaceous DOM Compounds

### 2.5.3.1: Carbohydrates

A method for detection of total carbohydrates present in freshwater was adapted from Myklestad *et al.*, 1997, outlined below.

A 0.7 mM ferricyanide solution was created by adding 20 mg sodium hydroxide (#S5881, lot# SZBF3240V, Sigma Aldrich, UK), 1.0 g sodium carbonate (#222321, Lot# MKBQ8223V, Sigma Aldrich) and 11.55 mg of potassium hexacyanoferrate (III) (#244023, Lot# WXBB4949V, Sigma Aldrich) to Milli-Q (18.2  $\text{M}\Omega$  water) to make a total volume of 50 mL.

A 2 mM buffer solution was made by combining 8.2 g of anhydrous sodium acetate (W302406, Lot# MKBR9565V, Sigma Aldrich), 2.1 g of citric acid (#C0759, Lot# SLBM0430V, Sigma Aldrich) and 12 mL acetic acid (#A/0360/PB17, Lot# 1153754, Fisher Chemical) to Milli-Q for a final volume of 50 mL.

A 2 mM ferric chloride solution was made by dissolving 16.20 mg of iron (III) chloride (#157740, lot# SZBF2500V, Sigma Aldrich) with 50 mL of the buffer and dissolving. This solution was stable for 2 days at 4° C in the dark, and so was refrigerated prior to use.

A 2.5 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (#T1253, Lot# BCBR8510V, Sigma Aldrich) solution was made by combining 8.5 mL glacial acetic acid (#A/0360/PB17, Lot# 1153754, Fisher Chemical) to Milli-Q for a total volume of 50 mL (and thus making a 3 M solution) before 39.0 mg of TPTZ was added and dissolved. This solution was stable for 1 week at 4° C in the dark, and so was refrigerated prior to use.

For the analysis, 100 µL of 0.7 mM ferricyanide solution was added to 100 µL aliquot of sample in a 1.50 mL centrifuge tube, which was vortexed to mix before being incubated at 105° C for 10 minutes in an aluminium heat block. After incubation, 100 µL of the 2 mM ferric chloride solution was added, alongside 200 µL of the TPTZ solution, which was vortexed and incubated at room temperature for 30 minutes. A 300 µL aliquot of each standard/sample was then added to a 96-cell clear polystyrene microplate (BIOFIL, #011096) and absorbance was measured at 595 nm using a Molecular Devices Spectromax M5e plate reader.

A standard curve was generated using D+ Glucose (AnalaR, lot# K32455814) in various concentrations from 1 to 100 µM and the absorbance of the samples at wavelengths between 200 and 800 nm were used to calculate a concentration from this using the equation  $Y=MX+C$  (Figure 2.8).

Recoveries of this method are reported as between 94 and 102%.

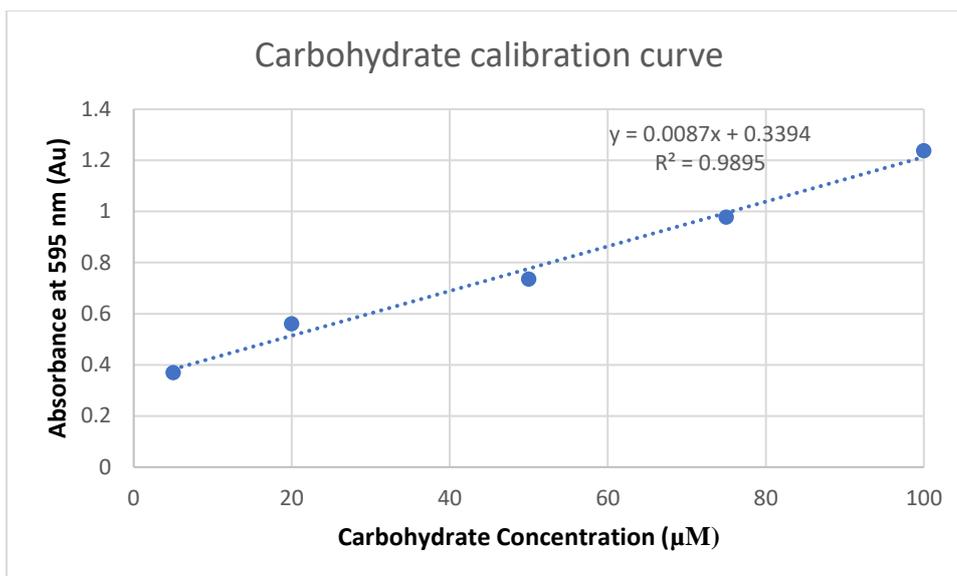


Figure 2.8: An example of calibration graph for carbohydrate concentration calculation, using absorbance and the ferric-cyanide method outlined above.

## 2.6: Disinfection of Water for Drinking Purposes

### 2.6.1: Chlorination

Water containing chlorine was stored in reusable 100 mL amber glass bottles with PTFE lids (#565625, Fisher Scientific), and baked for 3 hours in a furnace at 550° C to remove any organic matter.

The APHA standard method 4500-Cl-C was followed. The principle behind the method is that the chlorine will liberate free iodine from the potassium iodide at pH 8 or less. This free iodine was titrated with a sodium thiosulphate solution with starch as the indicator. Acetic acid was added to drop the pH to between 3 or 4, because the reaction is not stoichiometric at neutral pH due to the partial oxidation of the thiosulphate to sulphate. The minimum detectable concentration approximates 40 µg Cl as Cl<sub>2</sub>/L if 0.01N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is used with a 1000 mL sample. Concentrations below 1 mg L<sup>-1</sup> could not be determined accurately by the starch-iodine end point used in this method. (APHA, 1992a).

400 µL of sodium hypochlorite (NaOCl) (6-14% available chlorine, Sigma Aldrich, UK, #425044, lot# SZBF3010V) was added to a 25 mL volumetric flask and made up to the mark with Milli-Q. Meanwhile, in a 100 mL volumetric flask, approx. 1.58 g

sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) (#217263 (99%), *Sigma Aldrich*), was weighed, and filled to the mark with Milli-Q.

In a conical flask, 5 mL 99% glacial acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ) (#A/0360/PB17, *Fisher Chemical*) was added to 1 g potassium iodide (KI, *Sigma Aldrich*, UK, # 60399, lot # BCBM8375V) to create an excess, mixed well and the 25 mL diluted sodium hypochlorite solution was added. The sodium thiosulphate solution was then slowly titrated into the conical flask, whilst agitating, until the intensity of the yellow colour has almost disappeared. 1 mL of 1% starch solution (#319554, Lot MKBT3835V, *Sigma Aldrich*) was then added and the solution was agitated in a circular motion until the colour turned inky black, before continuing titration using the sodium thiosulphate solution, stopping when the black colour was fully discharged. The volume of titrant used was then recorded and applied to the formula in Figure 2.9.

It is important to note that the titration method should require at least 10 mL of titrant to present a 0.1% error. If this error was not sufficiently low enough for the purpose of experimentation, 0.8 mL of sodium hypochlorite solution was used instead of 0.4 mL to form a 0.05% error.

Each water sample to be analysed was diluted to contain only  $1 \text{ mg L}^{-1}$  DOC. This dilution was applied to all samples, and the data generated was referred to as standardised THM4 (or STHM4). In some cases, the STHM4 concentration was multiplied by the original DOC concentration, and is therefore referred to as total THM4 formation potential, or TTHM4FP.

The calculated volume of sample was added to a 100 mL measuring cylinder, and made up to 50 mL with Milli-Q. 2 mL of phosphate buffer (0.5M  $\text{KH}_2\text{PO}_4$ , *Sigma*, UK, #P9791, lot# SLBL2497V) was added to stabilise pH throughout the experiment and the solution was made up to 99.50 mL with Milli-Q.

The solution was then added to a 100 mL amber bottle, and 0.5 mL of  $1000 \text{ mg L}^{-1}$  chlorine solution was added (to give  $5 \text{ mg L}^{-1}$  free chlorine per  $1 \text{ mg L}^{-1}$  of DOC). This was added to the sample bottle rather than the measuring cylinder to prevent any additional exposure of further samples to residual chlorine remaining in the measuring cylinder, or the reaction of the chlorine with the plastic. The amber bottle was capped, labelled and stored in the dark at  $25 \text{ }^\circ\text{C}$  for 7 days. Chloraminated samples are

created using exactly the same method, but the correct dose of chloramine solution was first created (see section 2.6.2).

Hypochlorite concentration (mg mL<sup>-1</sup> Cl<sub>2</sub>) = (M x 35.45 x titrant volume)

hypochlorite added

(Where *M* is molarity of titrant (0.1 M), hypochlorite added is 0.4 mL (or 8 mL, see below). All volumes are in mL).

The volume of hypochlorite needed to make a 1000 mg L<sup>-1</sup> dosing solution was calculated using the following formula:

$$\text{Hypochlorite required (mL)} = \frac{1250}{\text{hypochlorite concentration (mg mL}^{-1}\text{)}} \quad \left. \vphantom{\frac{1250}{\text{hypochlorite concentration (mg mL}^{-1}\text{)}}} \right\} /5$$

Figure 2.9: Formula to show calculation of hypochlorite concentration

After 7 days (to ensure an excess of contact time was allowed) in the dark at 25° C in a temperature controlled oven, 15 mL aliquots of samples were transferred into 20 mL Amber GC vials (#SU860098, Supelco) with 18 mm screwcap silicone/PTFE septum lids (#SU8660101, Supelco), with 0.5 mL of sodium sulphite solution (Na<sub>2</sub>SO<sub>3</sub>) (98%, #SO505, Sigma Aldrich) to quench the chlorine (by creating an excess of sodium sulphite, which destroys the chlorine at an approximate ratio of 0.1 mL of sodium sulphite to 1 mg of Cl<sub>2</sub>, thus preventing any remaining free chlorine from continuing to react with organic molecules still present in the sample. The chlorine residual was not measured at this point for all samples, however, for specific experiments, the residual was calculated, see section 2.7 of this chapter. It is also important to understand that this procedure does not mimic real treatment processes in a water treatment works, but this method is best suited for laboratory experiments. The sodium sulphite solution was created by adding 10 g sodium sulphite to a 100 mL volumetric flask (to quench any remaining chlorine), and filling to the mark with Milli-Q, thus creating 100 g L<sup>-1</sup> (793.38 mM) sodium sulphite solution. The vial lid

was screwed on tightly, the vial was labelled and placed in the GC for analysis. 6 THM calibration solutions were used as standards and placed in vials for analysis before and after the unknown samples, along with two blanks (untreated Milli-Q).

### 2.6.2: Chloramination

The molarity of sodium hypochlorite solution (see Figure 2.9 above) was determined by dividing the concentration (in mg mL<sup>-1</sup>) by molar weight of sodium hypochlorite; 74.442 g mol<sup>-1</sup>.

This solution was diluted to 0.8 M in a 100 mL volume, before being labelled, and stored in the dark at 4° C. Meanwhile, 5.349 g of ammonium chloride (NH<sub>4</sub>Cl) (#A9434, Lot# BCBM8338V, >99%, Sigma Aldrich) was measured out and added to a 100 mL volumetric flask, before being made up to the mark with Milli-Q to form 1.0 M ammonium chloride. This was also labelled and stored in the dark.

Before use, 10 mL each of 1.0 M ammonium chloride solution and 0.8 M sodium hypochlorite solution were mixed into a 100 mL amber glass bottle in a fume cupboard (see Equation 2.a below)).



The solutions were allowed to mix before being capped and stored for 2.5 hours. It should be noted that the chloramine solution should be kept in an enclosed container, such as a GC vial with a septa in the lid, to ensure chlorine gas does not escape. This is partially due to its dangerous properties but also due to the rapid dissociation of the chloramine solution. To ensure a high strength of chloramine solution, using a septa capped vessel and removing the desired volume with a syringe would be the most effective method, but this was not necessary for the concentrations required in this experiment.

Performed chloramination was carried out following APHA Method 4500-CL-F (APHA, 1992a)

The theory behind this method involves the use of N,N-diethyl-p-phenylenediamine (DPD), as it is an indicator in the titrimetric procedure with ferrous ammonium sulphate. In the absence of iodide ion, free chlorine reacts instantly with the DPD indicator to produce a red colour, and the further addition of iodide ion to create an

excess evokes a rapid response from dichloramine, and as a result, in the presence of an iodine ion, part of a nitrogen trichloride is included with dichloramine and part with free chlorine (APHA, 1992a).

Following APHA Method 4500-Cl-F, the standard ferrous ammonium sulfate titrant was made by dissolving approx. 1.106 g ammonium iron (III) sulfate hexahydrate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ) (#215406, Lot MKBS7802V, Sigma Aldrich), accurately weighed, in Milli-Q containing 250  $\mu\text{L}$  sulphuric acid ( $\text{H}_2\text{SO}_4$ ) (95-98%, #320501, Sigma Aldrich) and was made up to 1 L with Milli-Q.

A phosphate buffer solution was made by dissolving approx. 24 g anhydrous disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) (#71640, Lot BCBN8058V, Sigma Aldrich) and approx. 46 g anhydrous monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) (#P9791, Lot SLBL2497V, Sigma Aldrich), both accurately weighed, in Milli-Q. These were mixed, before 800 mg disodium ethylenediamine tetraacetate dehydrate (EDTA) (#EDS, Lot BCBN4261V, Sigma Aldrich) was dissolved into 100 mL Milli-Q and the 2 solutions combined. This new solution was then diluted to 1 L with Milli-Q and 20 mg mercury (II) chloride ( $\text{HgCl}_2$ ) (#215465, Lot SLBG6022V, Sigma Aldrich) was added to prevent mould growth and interference in free chlorine test.

In a conical flask, 5 mL phosphate buffer and 500 mg of N,N-Diethyl-p-phenylenediamine sulphate salt (#07672, Lot BCBQ7288V, Sigma Aldrich) were mixed and agitated in a circular motion until fully dissolved. 100  $\mu\text{L}$  of chloramine stock solution, made 2.5 hours previously, was added to a 100 mL calibrated measuring cylinder containing 99.90 mL Milli-Q to make a 1000 times dilution of the original (raw) chloramine solution. This diluted sample was then added to the conical flask and agitated in a circular motion. A gradual colour change to bright pink was observed over the course of 1 minute.

The titrant was poured into a 50 mL burette ensuring that no bubbles were present in the burette, and that bottom of the concave meniscus was sitting exactly on 0.0 mL. The titrant was then titrated into the conical flask until the pink colour was discharged, with thorough mixing using a circular swirling motion by hand. The volume of titrant used was recorded (reading from the bottom of the meniscus) and used in equation 2.b:

$$\text{Volume of titrant used (mL)} = \text{free chlorine (mg L}^{-1}\text{)} \quad \text{Eq. 2.b}$$

Note, if more than 5 mL of titrant was required, the solution would not change colour from pink to clear as the chlorine concentration is too strong for this accurate method. A smaller volume of the raw chloramine solution was therefore required until the desired colour change was noticed between 0 mL and 5 mL titrant used.

The concentration of the chloramine dosing solution was then adjusted to 1000 mg L<sup>-1</sup> free chlorine. This high concentration of dosing solution will be diluted 200-fold when added to the 95.50 mL water sample thus creating a 5 mg L<sup>-1</sup> concentration in the sample. As chloramine concentration varies over time, it was important to measure chloramine concentration and then chloramine as quickly as possible after determining concentration to ensure that the desired concentration of chloramine was added to the sample to be treated.

It is important to remember though that the method detailed above does not match the chloramination practices that typically take place in a water treatment works, and therefore the data obtained from this method, whilst useful in determining the formation potential of DBPs, should never be compared to real water samples from a water treatment works nor its distribution network, and should not be compared to drinking water regulations as the DBP concentrations have been obtained through non-standard methods for the drinking water industry.

## 2.7: Chlorine Demand

Chlorine burden (the total quantity of chlorine used in the formation of THMs) was calculated by taking the 85 mL remaining sample (after 15 mL was taken for DBP analysis by GC), making up to 100 mL with Milli-Q, and then adding to 5 mL phosphate buffer and 500 mg N,N-Diethyl-p-phenylenediamine sulphate salt in a conical flask, and titrating the ferrous ammonium sulphate titrant, as above, to determine the residual. As the initial chlorination concentration was 5 mg mL<sup>-1</sup> free chlorine, it was possible to calculate how much chlorine was used in the reaction.

As a further step, it was possible to calculate how much of the chlorine was used to form THM4. A simple mass balance equation, using the remaining chlorine concentration and the concentration of the THM4 measured by GC, showed how much chlorine was used to form the individual THMs. This was given as a percentage, and the remaining chlorine that was

used but not accounted for, was assumed to have formed other un-detectable DBPs, of carbonaceous and nitrogenous character. See Table 2.2 for example of data generated.

Table 2.2: Example of data collected to be used in mass balance equation, in a 100 mL sample

<b>Cl<sub>2</sub> in CHCl<sub>3</sub> (mM)</b>	<b>Cl<sub>2</sub> in CHCl<sub>2</sub>Br (mM)</b>	<b>Cl<sub>2</sub> in CHBr<sub>2</sub>Cl (mM)</b>	<b>Mass of Cl<sub>2</sub> used to form THM4 (mg)</b>	<b>Total Cl<sub>2</sub> used (mg)</b>	<b>Cl<sub>2</sub> used to form non-THM4 DBPs (mg)</b>	<b>Residual Cl<sub>2</sub> (mg)</b>
0.0384	0.0004	0.000002	4.11	3.89	0.00	1.11
0.0287	0.0006	0.000006	3.10	3.37	0.28	1.63
0.0300	0.0001	0.000000	3.18	2.56	0.00	2.14
0.0308	0.0003	0.000002	3.30	3.83	0.53	1.17

## 2.8: Disinfection Potential of Freshwater

To test the disinfection potential of chlorine and chloramine, APHA method 9222B (APHA, 1992b) was followed to assess the efficiency of bacterial removal of the more commonly used chlorine (NaOCl) and also chloramine. This method is suited for the growth (and therefore the detection) of *Escherichia coli*, *Enterobacter aerogenes*, and *Proteus mirabilis*. These strains are found prolifically in intestinal tracts of mammals and are also found soil and water, thus good indicators of the bacterial concentration of water.

Agar medium (*m-Endo Agar LES*, #85766-500g-F, lot BCBM2741V, Sigma Aldrich), was created following instructions before 47 mm agar plates (#800100, lot 511160IT, Advantec MFS Inc, California, USA) were poured and allowed to cool. 10 mL of river water (collected in bulk from a north Wales farmland dominated catchment, was added to 90 mL Milli-Q, before being subjected to 0.1, 0.5, 1.0 and 5.0 mg L<sup>-1</sup> doses of chlorine and chloramine. The treated samples were stored in sterile amber glass 100 mL bottles at room temperature for 1.25 hours, to allow sufficient contact time with available chlorine, before the samples were passed through sterile 0.45 µm HA MF<sup>TM</sup> membrane filters (#HAWP04700, lot R6EA56474,

*Merck Millipore Ltd., Ireland*). The membranes were carefully transferred to an absorbent pad (#AP10047E0, lot R5AA60853, *Merck Millipore Ltd., Ireland*) soaked in 2 mL lauryl sulphate broth (#17349-500g, lot BCBP6927V, *Sigma Aldrich*) (in the lid of the agar plate), and left to incubate at 35° C in high humidity for 2 hours (to mimic ideal growth conditions for the growth of bacteria. The lauryl sulphate broth is used as a medium for the detection of coliform organisms in water). Then, the filters were carefully transferred to the agar surface using flat bladed forceps, the soaked membrane discarded, the lids put on top of the plate and the plates inverted. After 20 hours further at 35° C (without high humidity), the metallic looking colonies were counted and total colony forming units (TCC) was calculated using the equation in Figure 2.10, and averaged (as triplicates of each concentration under each treatment were conducted to reduce impact of any errant data. Controls were included, being subject to the same conditions as the samples, but with Milli-Q water being passed through the filter instead of sample water, and no colonies were counted on any of the controls, showing no external bacterial inputs.

$\text{Total Coliform Colonies/100 mL} = \frac{\text{coliform colonies counted} \times 10}{\text{mL Sample filtered (undiluted)}}$
--

Figure 2.10: Equation to calculate total coliform colony forming units.

## 2.9: Disinfection By-Products

A method adapted from Sarrion *et al.* (2000) was employed for the detection of trihalomethane compounds (THM), using a Varian 450 GC, equipped with a <sup>63</sup>Ni Electron Capture Detector (ECD) and utilising solid phase micro-extraction (SPME). A Zebron ZS CL Pesticides 1 column was used (Phenomenex, UK, #7AB-G000-00-GZ0) with a carrier gas (N<sub>2</sub>) flow of 10 mL min<sup>-1</sup>. The oven was programmed to start at an initial temperature of 35° C held for 9 minutes, before increasing to 140° C at 10° C min<sup>-1</sup> and held for 2 minutes, followed by an increase to 180° C at 10° C min<sup>-1</sup>, held for 3 minutes. A 0.75 mm ID Straight/SPME Inlet Liner (Restek, USA, #21113) was fitted into the injection port, the temperature set to 290° C with an applied split ratio of 35:1. The ECD temperature was set to 300° C with a make-up flow of 25 mL N<sub>2</sub> and cell contact potential at -540 ± 100 mV to

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

The samples were incubated at 40° C for 10 minutes, before a SPME fibre (Carboxen™, polydimethylsiloxane, 75 µm, 23 gauge (#57343-U, Sigma Aldrich) for THM4 analysis, PDMS Fused Silica (#57342-U, Sigma Aldrich), 23 gauge, for N-DBP analysis) was then immersed to a depth of 5 mm into the sample for 15 seconds before being desorbed in the injection port for 5 minutes at 295° C (Hughes, 2013).

Standard solutions were acquired from AccuStandard, as 2.0 mg mL<sup>-1</sup> in MeOH 1mL vials. Bromoform (#M-502-05-10X), bromodichloromethane (#M-502-04-10X), dibromochloromethane (#M-502-17-10X) and chloroform (#M-502-13-10X) were mixed in a 100 mL volumetric flask and filled to the mark using ultra pure Milli-Q water. This was used as a 20,000 µg L<sup>-1</sup> stock solution, and standards of 1, 5, 10, 20, 50, 100 and 200 µg L<sup>-1</sup> (to represent the large range of THM concentration in samples) were made by taking aliquots of this stock and adding to Milli-Q. The THM4 recovery achieved using this method is 102.4±0.62% (Hughes, 2013)

Figure 2.11 outlines the standardisation procedure. The THM4 standards were made up and run through the GC-MS to obtain peak area information, which was internally correlated with the previous calibration file. The reported concentration was then correlated with the actual concentration, a line of best fit added and the R<sup>2</sup> correlation coefficient examined. If an R<sup>2</sup> of greater than 0.95 was obtained, the line equation was calculated and all unknown sample data was then standardised to this equation.

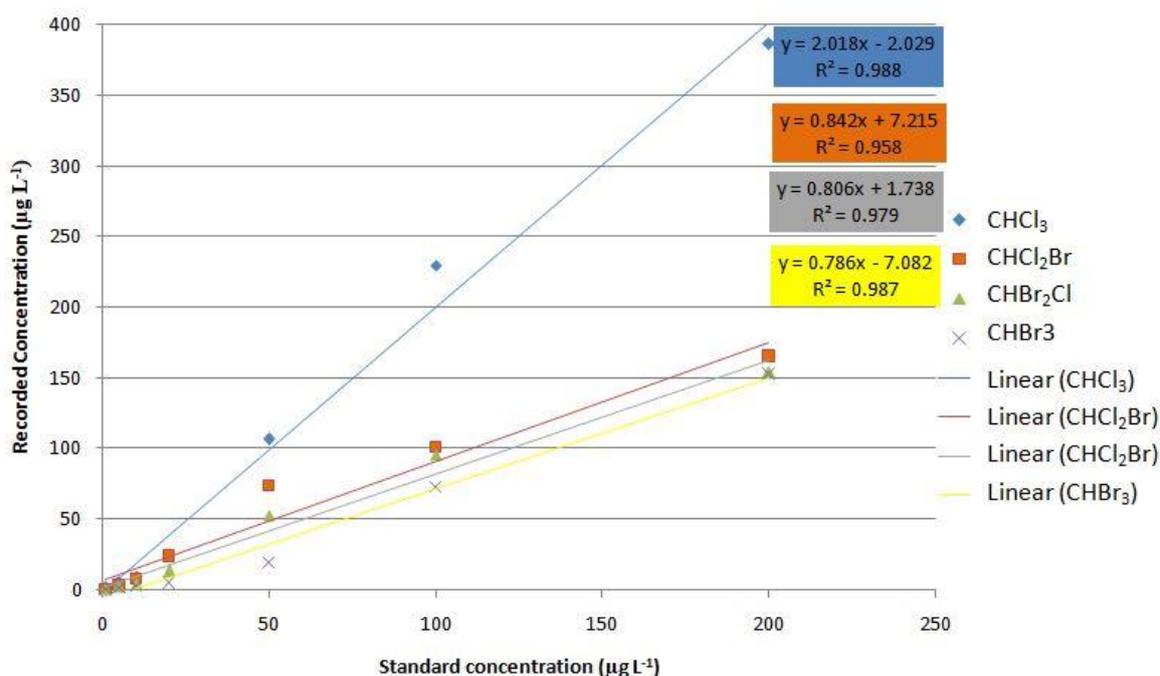


Figure 2.11: Calibration procedure of THM4 compounds for GC-MS analysis.

## 2.10 Dibromoacetonitrile Toxicity Detection Using Electrophoresis and a Western Blot

As a way of visualising the cell cycle, total protein extracts and a Western blot can be used to display the strength of cell growth. Western Blots are used to separate and identify proteins. Synthetic or animal derived antibodies (such as from mouse, goat or rabbit) that react with a specific protein are identified and isolated/synthesised. This antibody is mixed with the sample to be tested, and an aliquot is used in a gel electrophoresis experiment, where, if the protein sought for is present, a band will become stained upon the membrane paper used for the western blot (Mahmood and Yang, 2012). The Comet Assay (Single Cell Electrophoresis Assay), developed by Östling and Johanson (1984) but later modified by Singh *et al.*, (1998) is a simple and sensitive technique for the detection of DNA damage in an individual eukaryotic cell, where the pattern left by the DNA migration through the gel resembles a comet. This assay has been used to demonstrate that drinking water containing N-DBPs can cause DNA damage in yeast (Banerjee *et al.*, 2008). Whilst the actual cellular target of dibromoacetonitrile is unknown, it is known to block cell cycle progression and prevents

activation of the DNA checkpoint kinase Chk1 when DNA replication forks break (Caspari et al., 2017). Dibromoacetonitrile has been reported to increase rates of squamous cell adenomas (i.e. cancerous tumours) and carcinomas (i.e. skin cancer) in the mouth of rats, at high concentrations (100 to 200 mg L<sup>-1</sup>) (NTP, 2010).

A freshwater sample was collected from the River Conwy catchment, UK, and filtered through 0.45 µm poresize Whatman membrane filters (#WHA7404004, Sigma Aldrich, St. Louis, USA), to isolate suspended solids. The sample was then diluted to 1 mg L<sup>-1</sup> DOC using ultrapure water (Milli-Q) and chlorinated at 5 mg L<sup>-1</sup> free chlorine to 1 mg L<sup>-1</sup> DOC, and left at 25° C in the dark for 7 days to allow reactions to complete. The sample was then passed through a 0.2 µm pore size Whatman membrane filter (#WHA7402004, Sigma Aldrich, St. Louis, USA) to ensure the sample was sterile, and placed in a 50 mL Falcon tube (#10788561, Fisher Scientific, Loughborough, UK).

A rich yeast medium was made by mixing 3% glucose solution (30 g L<sup>-1</sup> glucose, 5 g L<sup>-1</sup> yeast extract and 200 mg L<sup>-1</sup> adenine). 10 mL of this medium was added to a 50 mL Falcon tube to provide sufficient nutrients for the growing cells. A pea sized amount of the analysed strain cells, grown on an agar plate, were collected on an inoculation loop, and were suspended into each sample. These samples were placed in a 30° C 180 RPM orbital shaker overnight to allow cell replication. The next day, the falcone tubes were centrifuged (3000 RPM, 3 minutes) to pellet the cells, before the supernatant was discarded.

1 mL PBS buffer (diluted from a 10 L stock solution of 10x phosphate buffered saline (PBS) made using 800 g NaCl, 20 g KCl, 144 g Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 24 g KH<sub>2</sub>PO<sub>4</sub> and 8 L distilled water) was added to the vial and the pellet was re-suspended by repeated pipetting of the solution and pellet, before the solution was placed in a 2 mL centrifuge tube. This new tube was labelled and placed in a desktop centrifuge (8,000 RPM, 1 minute), before the supernatant was, again, discarded and the centrifuge tube was dabbed dry on absorbent paper. It is important to note that samples could be frozen at this point at -20° C for future analysis.

Once sufficient samples were accumulated for a full analysis run, the samples were removed from freezing and brought back to room temperature. Approximately 7 pea sized spoons of silica beads were added to each thawed sample, along with 300 µL of a 20% trichloroacetic acid solution to denature the protein and to open the cells. These samples were subjected to vigorous agitation for 10 minutes. The supernatant in the samples was then transferred to a

new tube using a blue 1 mL pipette tip. The supernatant was then placed in a desktop centrifuge (12,000 RPM, 5 minutes) to pellet the protein.

Again, the supernatant was taken from the centrifuge tubes but this time it was discarded, leaving the protein pellet behind. Then, depending on pellet size, 150  $\mu$ L of blue 4x Sodium dodecyl sulfate (SDS) (200 mM TRIS-HCl pH 6.8, 400 mM Dithiothreitol, 8% sodium diosulphate, 0.4% bromophenol blue, 40% glycerol and 300  $\mu$ L of 1 M Tris-HCl pH 8) sample buffer was added to each sample, causing the sample to turn yellow (which indicates a low pH). To neutralise this effect, 150  $\mu$ L of TRIS pH 8.8 was added to neutralise the pH.

A fresh 1 mL pipette tip was used to break up the protein pellet to re-suspend the cells, before the vial was placed in a heat plate at 95° C for 3 minutes, taking sure not to get burnt on the hot gas vapours leaving the vial once re-opened.

A 10% resolving gel was made (as this is best suited for the Rad9 protein) by mixing 2 mL 40:1 acrylamide (#A3553, Sigma Aldrich, Dorset, UK), 1.2 mL 1.5 M Tris pH 8.8 (#T9568, Sigma Aldrich, Dorset, UK), 3.8 mL ultrapure water, 80  $\mu$ L 10% Sodium dodecyl sulfate (L3771, Sigma Aldrich, Dorset, UK), 8  $\mu$ L Tetramethylethylenediamine (TEMED, #T9281, Sigma Aldrich, Dorset, UK) and 80  $\mu$ L 10% ammonium persulphate (APS, #A3678, Sigma Aldrich, Dorset, UK), to give a total volume of 8 mL. This solution was transferred into a liquid-tight gel casting stand, ensuring that approx. 15 mm of space was remaining on top of the liquid. Isopropanol (approx. 100  $\mu$ L) was pipetted very slowly on top of the gel to ensure it set straight, before the gel was left to set (approx. 20 minutes). The isopropanol was discarded and the gel was washed with pure water to remove any alcohol that remained. The gel casing was then dabbed dry on absorbent paper before an upper gel was mixed up. This was created by mixing 200  $\mu$ L acrylamide:bisacrylamide (37.5:1), 500  $\mu$ L 0.5 M Tris pH 6.8, 1.25 mL pure water, 20  $\mu$ L 10% SDS, 2  $\mu$ L TEMED and 10  $\mu$ L APS to make a total volume of 2 mL. This solution was mixed and poured on top of the resolving gel, before a sample well comb was then added to the top of the gel casting stand to produce 10 channels for adding samples/standard solutions.

A horizontal gel tank was half filled with a 1% running buffer, diluted from a 10% running buffer stock solution (30.3 g Tris Base, 144 g glycine and 10 g SDS diluted in 1000 mL pure water plus 15% methanol, e.g. 150 mL) just prior to use. The glass casing containing the gel was then released from the clamp stand and fitted into the gel running tank. If only one gel was used then a blanking plate was used on the other side of the tank, otherwise, both gels

were loaded and the tank was filled to the mark with 1% running buffer. Once the gel had set, the green comb could be carefully removed, leaving 10 channels. A Pierce pre-stained protein molecular weight marker (#26612, Thermo Fisher Scientific, MA, USA) was thawed and 5  $\mu\text{L}$  was pipetted into the first channel of the gel, and 10  $\mu\text{L}$  of sample/standard was pipetted into the remaining channels, with sample identifications being recorded. The lid was affixed to the top of the horizontal gel tank (Bio Rad, Watford, UK) before it was plugged into a converter, set to 110 V for 1.5 hours, ensuring that the upwards movement of bubbles started once plugged into converter.

Once the 90 minutes had passed, the converter was turned off and a transfer box (Bio Rad, Watford, UK) was filled with transfer buffer (made by mixing 750 mL methanol, 3750 mL pure water and 5400 mL 10x transfer buffer). A Western Blot wet transfer sandwich was made, 2 pieces of nitrocellulose membrane slightly larger than the gel were cut to size, and a green spade tool was used to separate the gel from the glass casing, and to remove the stacking gel from the top of the resolving gel. Identification numbers were written on the top corner of the membrane filters, and, in a tray of transfer buffer, a sponge, filter paper, the gel, another filter paper and another sponge were stacked, ensuring that all air bubbles were expelled. The black half of the clamp was inserted under the stack and the red half of the clamp was placed on top of the stack, before being clamped together. This fixture was then placed into the transfer box, filled with transfer buffer earlier, with the red side of the clamp facing the positive terminal. The lid was then fitted and the gel was run at 55 V for 2 hours or 10 V for between 12 and 15 hours. This process is known as SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE), and created a 10% gel (Burnette, W. N., 2011).

Once the transfer gel had finished running, the gel was removed from the transfer buffer and the stack was disassembled. The coloured bands from the gel should now be visible on the membrane paper, and the gel was discarded. This paper was then immersed in Ponceau S solution (1 g L<sup>-1</sup> Ponceau S (#P3504, Sigma Aldrich, Dorset, UK), which is a dye stain, plus 50 mL acetic acid) briefly, before being washed off under warm water. This now pink membrane filter was submerged under milk buffer (3 g milk powder to 100 mL 1x phosphate buffered saline (PBS) plus 0.05% TWEEN) for 20 minutes, whilst being agitated. Whilst this was being agitated, Covance HA mouse antibody (#901501, BioLegend, California, USA) was selected and mixed in milk buffer in a 5  $\mu\text{L}$  to 40 mL ratio. This solution lasted for 2 weeks at 4° C in the dark. The Covance HA mouse antibody is derived from the human

influenza hemagglutinin surface glycoprotein, and is commonly used as a tag to facilitate detection, isolation and purification of proteins (BioLegend, 2017).

After the 20 minutes were up, the transfer placed in a plastic sleeve, and 3 sides were heat sealed. 2 to 3 mL of the HA Covance mouse antibody (Biosource, Covance MMS-101P-200) diluted 1:1000 in milk was added and the 4<sup>th</sup> side was heat sealed to produce a liquid and air tight package. The liquid was manually agitated to ensure it covered every section of the transfer, before this pouch was left on an agitator overnight at 4° C in the dark..

In the morning, the pouch was opened and the antibody was drained away. The transfer was removed and washed in wash solution for 30 minutes, refreshing the solution every 10 minutes. During this period, a secondary anti-mouse antibody (Dako, P0447)) was diluted 1 in 10,000 and before the transfer was again placed in a pouch with this antibody for a minimum of 45 minutes, overnight if possible. This transfer was then, again, washed for 30 minutes at 10 minute solution changing intervals.

After the last wash, 500 µL white and 500 µL brown developing solution (Western Lightning Plus-ECL Enhanced Chemiluminescence Substrate, #ORT2655 and #ORT2755) were mixed on a shallow tray and pipetted over the drip-dried membrane, ensuring that the whole membrane had been covered. A developing cassette was loaded with a plastic document wallet (masking tape was used to secure this in place) with the non-hinge long side cut. The membrane was placed inside this wallet, all air bubbles were removed, and the cassette was shut. Photosensitive sheets (#34091, CL-XPosure Film, Thermo Fisher Scientific, MA, USA) were taken along with the cassette to a dark room and the sheet was cut to size (in the dark), before being placed on top of the membrane containing wallet, inside the cassette, taking care not to move it once it has been positioned. The top left corner of the photosensitive paper was folded to ensure correct orientation when developed. The cassette was locked shut and was left for 10-25 minutes to develop, before being passed through a developer machine. Once this machine has produced the ‘photograph’, it had been ‘fixed’, so that light could not over expose the image.

## 2.11 Fractionation

To determine the hydrophobic/hydrophilic components contained within the DOM in samples, equipment was designed and built to split sample water into 5 different fractions

(hydrophobic acids and bases, and hydrophilic acids, bases and neutrals). The apparatus components were collected and the equipment was constructed (see Hughes *et al.*, 2016).

Briefly, 4 solenoid valves (Bio-Chem, Florida, USA), operated via bespoke software and relays (K.M. Tronic, Bulgaria) directed the flow of influents (HCl, NaOH, sample and ultrapure water), pumped by a peristaltic pump through two different resins. Amberlite XAD-4 (#XAD4, Sigma Aldrich, Missouri, USA), a highly hydrophobic styrene-divinylbenzene copolymer, was selected to retain fluvic and humic acids from samples, and Supelite DAX-8 (#21568, Sigma Aldrich, Missouri, USA), a hydrophilic acrylic ester, was selected to retain hydrophilics (Hughes *et al.*, 2016).

Samples were collected and pre-filtered through 0.45  $\mu\text{m}$  Whatman filter papers prior to analysis to isolate the dissolved organic matter in solution. Several iterations of the fractionation apparatus were built and tested (to develop upon errors and issues with the older versions) prior to the machine used to generate the data presented here. Several problems persisted throughout the course of the method development, including faulty pumps introducing air to the system. These air pockets would easily pass through the tubing and valves, but would get trapped within the resins, creating a dry portion of the resin. Dry resin does not retain optimal concentrations of organic matter, thus reducing the efficiency of the machine, and can also cause a solid block of resin within the column, blocking the system from working. This blocking would then create back-pressure in the system which forced open valves that should have been shut for the run, thus bypassing the resins and producing fractions that were highly diluted. These problems were counteracted by the introduction of a new peristaltic pump, and the flushing of the resins between samples to ensure that any small air bubbles that may have become introduced were flushed out before they could dry too much resin. Furthermore, valves weakened by high pressure were replaced and small modifications were made to the controlling software to account for different flow rates of the pump. Further issues were discovered with the 0.20  $\mu\text{m}$  pore size filters at either end of each column becoming blocked as the samples were only filtered through 0.45  $\mu\text{m}$  pore size filters prior to being passed through the column, and thus material that measured between 0.2 and 0.45  $\mu\text{g L}^{-1}$  would be blocking the filter. To rectify this issue, 0.50  $\mu\text{m}$  pore size filters were sourced and fitted which helped prevent column blocking further.

Once fractions were collected, they were stored and then analysed for DOC and total dissolved nitrogen (TDN) data. The raw water sample was run through an ion

chromatograph(Metrohm 850 IC, Metrohm, Herisau, Switzerland), which produced data on the concentrations of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the raw water samples. These concentrations allowed for the calculation of inorganic nitrogen, to be subtracted from the TN concentration to provide DON. Fractionation data was then corrected according to the initial volume of raw sample used (between 85% and 99% raw water, topped up to 1010 mL with ultrapure water to avoid addition of air to the system at the end of the sample run) before the data was presented as a percentage of the corrected raw water TN concentration.

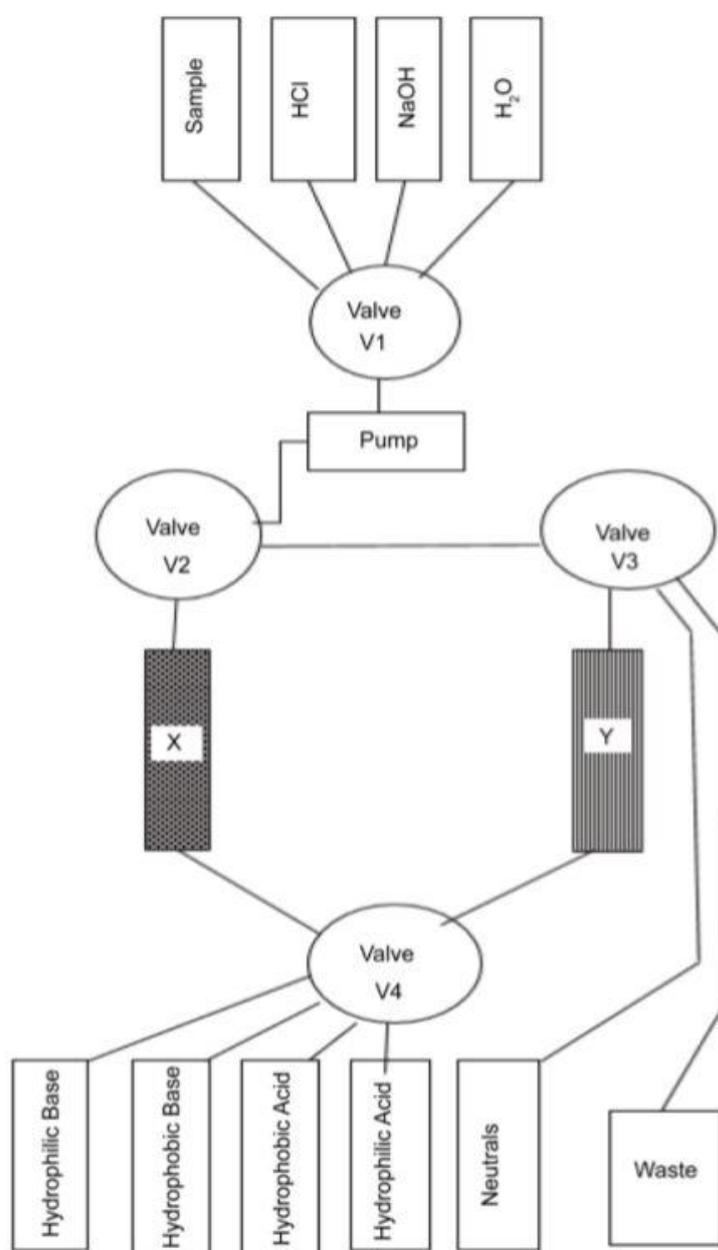


Figure 2.12: Schematic of fractionation equipment. V1, 2, 3 and 4 represent the 4 different valves, 'X' represents the XAD-4 resin and Y represents the DAX-8 resin (Hughes *et al.*,

2016). The 5 fractions that result from this work are displayed at the bottom, and abbreviated to HPOA/HPOB for hydrophobic acids and bases, and HPIA/HPIB/HPIN for hydrophilic acids, bases and neutrals respectively.

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# Chapter 3a

**A GIS based tool for  
catchment managers,  
using DOM as a case  
study**

### 3a.1: Introduction

Directives and policies increasingly call for more integrated management of land and water, and it is suggested that managing water and land as an integrated whole is a major challenge for the 21<sup>st</sup> century and beyond (Lerner *et al.*, 2010). The acceptance of this challenge is apparent through the UN's Millennium Development Goals, and on a European scale, *Integrated Catchment Management* is the philosophy behind the Water Framework Directive, which requires *River Basin Management Plans* to be prepared and acted upon every 6 years up until 2027 (Lerner *et al.*, 2010). Furthermore, in a recent publication, The Department for Environmental, Food and Rural Affairs (DEFRA) set out a vision for the future of water in 2030, where it is outlined that large bodies of water in England should have a good ecological and chemical status, because healthy rivers, lakes, estuaries, coasts and ground-waters provide maximum resilience to climate change and to sustain biodiversity. The report also envisages major improvements to nutrient pollution, chemical pollution and water resources, and a reduced impact of agriculture, through land being increasingly flexibly managed for flood reduction and water quality (DEFRA, 2008), thus showing that there is an inherent need for a better understanding of the complex relationship between catchment and water quality.

At the UK scale too, catchment management is increasingly embedded in policy, a fact that is likely to increase with time and a heightened global interest in the environment. The inputs of contaminants from urban land uses are often much easier to estimate due to a complex presence of regulated sewage systems and treatment works (Macleod, Scholefield and Haygarth, 2007), however, this is not the case for rural land uses as these are near impossible to effectively monitor and regulate. For this reason, projects such as the Natural England and DEFRA run 'catchment sensitive farming' aims to help improve water quality in England through free training, education (where required) and advising farmers in selected areas of England, called 'priority catchments' on the best practices in reducing agricultural water pollution. Funding was also recently available, via both the European Agricultural Guarantee Fund (EAGF) and the European Agricultural Fund for Rural Development (EAFRD), to farmers in priority catchments in England, to assist in mitigation and reduction of sediment and nutrient inputs to freshwater streams, such as the implementation of constructed wetlands, for example (GOV.UK, 2015). However, this funding has now expired meaning the expense of managing catchments comes down to the land owner again, highlighting the need for further analysis and understanding of the sources and impacts of these contaminants in

catchments to provide evidence to the land owners that their catchment management efforts are having a positive impact.

Increasing pressures on water companies to consistently provide a healthy drinking water, alongside increased personal health awareness, has led to a rise in the scientific study of drinking water and its treatment. Arguably, the most topical issue in water treatment to date is the formation of DBPs caused by a reaction between the halogen chlorine (and its compounds), and DOM during the disinfection of raw water destined for human consumption. These halogen compounds can react with DOM, which is present in all natural waters, to form various DBPs (Bond *et al.*, 2011). In 1998, around 500 DBPs had been reported in the literature (Krasner *et al.*, 2006), a number which increased to around 600 in 2007 (Richardson *et al.*, 2007) and will likely further increase due to the development of more sophisticated equipment and detection limits, with time. However, C-DBP formation is much better understood than N-DBP formation from organic compounds contained within DOM.

This study uses DOM data to demonstrate a relatively simple, yet highly effective method of generating spatial data relating to the catchment of a river/reservoir and its characteristics. In this example, the hypothetical end user is a water company examining the catchments that feed their reservoirs, to identify problematic inputs and to educate on the potential source of these issues in the catchment, whether these are agriculture related or land use based. DOM removal is important for water treatment companies as it can affect the flavour, colour and odour of final drinking water, it can promote bacterial re-growth in water distribution networks, and it can be responsible for carcinogenic DBPs that can be formed when the disinfectant (namely chlorine and its compounds) reacts with the organic matter. Removal of DOM, however, inflates treatment costs and chemical usage costs which are passed onto the end user (Prévost *et al.*, 1998, Davies *et al.*, 2004 and Gough *et al.*, 2016). Therefore, improved understanding of catchment characteristics and their influences upon the character of DOM can have many uses, such as informing the targeted removal of contaminants and DOM at water treatment works and can also provide a deeper understanding of which DBPs are likely to form and in what concentrations. Identifying which catchment characteristics (such as specific vegetation type) produce the highest level of DBP precursors, will allow water companies to either target their DBP precursor removal more efficiently (for example, targeting smaller, hydrophilic neutrally charged compounds which may be associated with a dominant vegetation type in the catchment, or adjusting pH which may be influenced by the

occurrence of a specific vegetation type), or will provide them with a deeper understanding of what specific treatment their potential abstraction point will require before the water is fit for human consumption, when high water demand and distribution costs result in water companies planning new reservoirs. Although the DBP formation potential of a potential site is not a high priority for drinking water companies seeking a new abstraction location, this method can also be used to help identify problematic subcatchments within a drinking water reservoir's catchment, and can also help to identify the DBP compounds that are likely to form if the DOM in the reservoir is not suitably removed prior to disinfection.

DOC is often used interchangeably with DOM, as DOC contributes most, in terms of quantity at least, to DOM. DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have attached amide, carboxyl, hydroxyl, ketone and various other functional groups (Leenheer & Croué, 2003). DOM is often split into just 2 main major groups, DOC and DON, and it is these hydrocarbon structures (and their associated functional groups) that differ, depending on their source, resulting in DOM with vast differences in character from catchment to catchment and also depending on season (Matilainen *et al.*, 2010). Each abstraction location and treatment works will have different treatment practices suited to the DOM type in the water. Most freshwater systems such as rivers and lakes, in temperate climates at least, are significantly influenced by terrestrial inputs; therefore allochthonous organic matter is often dominant in these ecosystems. However, when confined in lakes and reservoirs, the longer hydrologic residence times may be long enough to allow transformation of allochthonous carbon inputs but there is also potential for autochthonous production from algal and microbial communities (Mash *et al.*, 2004). DOM can be generated from both allochthonous and autochthonous sources although the importance of these differs depending on site location – reservoirs will likely be rich in allochthonous DOM whereas rivers will be rich in autochthonous DOM.

DOM has been profiled across land use types in previous studies. Mattsson, Kortelainen and Räike (2005) studied the export of organic forms of carbon, nitrogen and phosphorus from various land use types in a boreal catchment in Finland, finding a relationship between total organic carbon and the peatland area ( $r=0.39$ ,  $p<0.01$ ), TON increased with an increase in agricultural land use ( $r=0.60$ ,  $p<0.01$ ). Bhaduri *et al.*, (2006) assessed the long term hydrological impacts of land use change using historic data on water resource to calculate average annual runoff and non point source pollution at a watershed scale. Xiaohui *et al.*, (2010), proposed a general catchment delineation method using ArcGIS software suitable for

both watershed or regional scales, allowing for the inclusion of inlet points, and also allowing for the new consideration of a new location for hydraulic gauge stations and reservoirs, but not water treatment works, as is presented in this chapter. Gough *et al.*, 2016, studied the relationships between reservoir water quality and GIS derived catchment habitat data, finding significant correlations between the catchment and organic matter data (explored in further detail in Table 3a.1). No other relevant literature appears to have studied the impacts of land use upon DOM concentration in a) the United Kingdom, and b) combining GIS derived data with water chemistry data.

Many studies have, however, attempted to decipher the plethora of information that analysis of DOM can provide, such as separating DOC into fractions with similar characteristics (hydrophobicity/hydrophilicity, for example), molecular weight classes (Aiken *et al.*, 1992, Chin, Aiken and O'Loughlin, 1994 and Li *et al.*, 2014). and spectroscopy (such as Fourier Transform Infrared Spectroscopy (FTIR) (Kanokkantapong *et al.*, 2006), whilst others have focussed on individual groups of components of DOM, such as those contributed by algae (Gough *et al.*, 2015) and DON (Worrall *et al.*, 2012). However, given that the catchment is usually quantitatively the most important source of DOM, catchment characteristics would seem a logical place to start.

DOM typically enters the aquatic system when precipitation falls on land, leading to overland and through-flow, before drainage into a water body, eventually reaching the sea *via* streams and rivers – this is the case for every catchment worldwide. The types of land and the intensity of its use have a strong influence on freshwater that drains from it, whether the source is natural, or results from human activities; the impact of any land use practices on either the quantity or quality of water can be substantial (Gyawali *et al.*, 2013). For example, runoff from agricultural lands may be enriched with nutrients and sediments, whereas runoff from highly developed urban areas may contain sewage and heavy metals from fossil fuel combustion, as well as sodium and sulphate ions from road de-icers, all of which can contribute to contamination of freshwater, and in some cases, can elevate DOM concentrations. Moreover, via evapotranspiration, interception, infiltration, percolation and adsorption, different types and coverages of vegetation can modify the land surface characteristics, water balance and hydrological cycle, and as a result, the chemical and biological processes in water bodies can be affected (Tong and Chen, 2002).

The UK Environment Agency has examined the potential of using Geographical Information Science (GIS) to provide information for integrated catchment management, providing information to assist in the management of land use change and diffuse pollution in the Frome-Piddle Catchment in Southern UK. A survey of the local users of this GIS system showed that the majority of users did not want to spend the time learning how to use the ArcGIS software, and there was a demand for more analysis for their local issues (Hulme *et al.*, 2008). This outlines the two major issues with GIS systems – the time invested in learning how to use the software, and the access to relevant data. Therefore, the need for a simple to follow walk through guide to enable land users to (relatively) quickly assess catchments and extrapolate relevant data, without a vast knowledge of the software, is obvious.

Here, a simple method for obtaining spatial data for individual catchments and their characters using GIS software, is proposed. Whilst the primary target for this method is utility companies, specifically the water industry and their catchment managers, this method could be of use to a wide spectrum of end users such as farmers, land managers, non-governmental organisations (NGOs) *etc.* Water companies, searching for new abstraction locations, for example, could gain an invaluable insight into the land that drains into the abstraction location, to provide information on expected contaminants and DOM type and therefore targeted removal. This would contribute to cost-analysis of abstraction locations in terms of treatment costs due to differing DOC characters. Similarly, it would also be advantageous to a land manager, looking to improve the quality of water flowing through/from their land, providing information on likely contaminants (such as upstream septic tank effluent sources, sheep dips, campsites *etc.*) and therefore informing catchment improvement decisions, such as the creation of constructed wetlands or installation of treatment and settling tanks. It is important to remember that DBP formation potential of a potential abstraction location for a new WTW plays a very insignificant role in the final selection of the site. Factors such as accessibility, water demand from surrounding settlements, links to other treatment works and existing infrastructure play much more dominant roles in the selection of a new WTW than DBP formation potential, however, with new scientific focuses into the formation of DBPs, if an individual DBP, or a group of DBPs is found to be of high risk to human health, the DBP formation potential of a site may begin to play a more significant role in the selection of a new abstraction location. This method could, however, be useful in determining the efficiency of the WTW DOM removal and disinfection practices to determine whether the

treatment is successfully removing the precursors, or whether the addition of chemicals to the water is having a negative effect on the final water.

This method could also be of importance in the scientific industry, where statistical analysis of water and catchment characteristics could provide a greater insight into the relationship between catchment and raw water character. Data obtained from this method can contribute to the understanding of catchment characteristics, which can then be compared to site-specific water chemistry data (such as DOC content/character, DBP formation potential and pH) and allows statistical analysis to be undertaken to determine any significant determinands of catchment character and water chemistry data. Whilst this method uses DOM as an example, this method can be used to obtain data relating to a river/reservoirs catchment and how it is comprised (vegetation, bedrock, soils *etc.*) that can then be statistically analysed alongside other biogeochemical data such as phosphate, nitrate and nitrite (eutrophication determinands) concentrations, for example.

The CCW Phase 1 Habitat survey is the most comprehensive and widely used national level map of semi-natural habitats in Wales, UK (Lucas *et al.*, 2011). It has been suggested by Gough *et al.*, (2016) that predictive models for surface water characteristics could be improved by incorporating CCW Phase 1 Habitat data, outlining a rarely used dataset (in this field) that can provide useful data to the scientific community and to land managers alike (Gough *et al.*, 2016). There are a vast selection of free and subscription based GIS datasets available online, including habitat surveys such as the Biodiversity Action Plan Broad Habitats, EU Habitats Directive, Vegetation communities of British Lakes and also the LCM2007. The latter, LCM2007, is a land cover map for the whole of the UK and a conversion table between habitat survey classifications from different datasets (such as CCW Phase 1) is available (CEH, 2011). This will likely prove to be an important tool in comparing habitat classifications and identifying which is best suited for the desired application. See Figure 42, Appendix 1 for a basic comparison table of habitat classes between LCM2007 and the CCW Phase 1 habitat classifications recorded in this study. As well as habitat and vegetation surveys, there are also datasets containing detailed information on bedrock and underlying geology (Beamish, 2012), and also National Soil Resources Institute (NSRI) soilscape data (Farewell *et al.*, 2011).

This chapter outlines a relatively simple, cheap and highly effective tool for catchment delineation using ArcGIS software. Once a catchment is digitally created, external datasets,

such as Phase 1 Habitat Survey (Natural Resources Wales (NRW), formerly Countryside Council for Wales (CCW)), can be overlaid and then ‘clipped’ to the catchment, to provide information on naturogenic (such as vegetation cover) and anthropogenic (such as farmyard runoff) sources of nutrients and organic matter to the catchment, either for a river (or watercourse) as a whole, or specifically for the site of a sample collection point. It can also be used to calculate catchment area, and areas of specific land types (such as the area of all land in a catchment that drains mixed woodland, for example). Here, the GIS method is applied to samples from the Afon Conwy catchment and sub-catchments, North Wales, UK, in order to demonstrate the effectiveness and ease with which detailed information can be obtained from digitally available data and Ordnance Survey maps in a relatively short time period.

### 3a.1.2 Hypothesis

It is hypothesised that a GIS based risk assessment map will provide a high resolution visual representation of the land use types that are likely to be problematic for drinking water treatment works.

### 3a.2: Methodology

Water samples were collected from both the Hampshire Avon and Conwy catchments once every 3 months over the course of a year, and a one off collection from Scottish sites, as outlined in Chapter 2.

Map data was obtained from the EDiNA Digimap tool. The spatial area of which data was required was selected from the interactive map, before additional datasets (such as a digital elevation model) were selected and downloaded (see additional information in section 3a.5)

Land use data was obtained from four different sources. For initial analysis of the Conwy catchment, the CCW Phase 1 Habitat Survey dataset was used. However, this dataset was only available for Welsh catchments, and although it could be directly extrapolated to another dataset, for continuity purposes and ease of use, the CEH LCM2007 dataset was used to provide data on landuse (available by license from CEH), and was used to generate the data displayed in Chapter 3b only. Another potential shortfall of the CCW Phase 1 habitat survey

is that it is a vegetation survey rather than a land use survey, and therefore does not explicitly state how the land is used. This is not problematic when trying to link the DOM character to the vegetation of the catchment, however, may provide issues when attempting to link DOM to land use practices such as agriculture (although the type of vegetation that is typically used for agriculture can be assumed to encompass DOM from agricultural sources). Although there are slight issues with the dataset, it can still be used within this chapter as an example of how the GIS software can be used, but it is recommended that the LCM2007 dataset is used to examine the impact of landuse upon the DOM concentration draining from it.

Soil data was obtained from the National Soil Research Institute (NSRI) to provide data on the structure and distribution of different soils types.

Bedrock data was obtained from the British Geological Survey (BGS) to provide data on the bedrock in each catchment..

CCW Phase 1 data was acquired through Bangor University, Wales, UK (although available at <http://jncc.defra.gov.uk/page-4258>).

Access to these datasets can prove very costly both financially and in time. However, large organisations especially in the Environmental sector should have means of access to these datasets due to the plethora of information that is contained within them. However, if institutional access is not available, the initial financial outlay is far outweighed by the implications of findings that could arise from analysis of the data.

Site location and jurisdiction play a major role in dataset selection. For example, whilst the CCW Phase 1 habitat survey proved immensely important for the study of the Afon Conwy catchment, the dataset only covers Wales and not the rest of the UK. The CCW Phase 1 habitat survey was initially chosen for this project as it was already subscribed to and utilised in previous studies. As additional sites were added to the project, located outside of Wales, the LCM2007 dataset was selected due to its availability, full UK coverage, but mainly due to the fact that the CCW phase 1 habitat data can be converted directly to LCM2007 meaning that the data produced from analysis and subsequent findings would encompass a UK wide comparison of sites rather than just a constituent country-wide comparison, meaning data would have a much larger relevance. The LCM2007 dataset is available at [www.ceh.ac.uk/data](http://www.ceh.ac.uk/data) and documentation is available on converting CCW Phase 1 to this

dataset

<https://www.ceh.ac.uk/sites/default/files/LCM2007%20dataset%20documentation.pdf>

The resulting maps provide a high quality visual representation and interpretation of catchments and their management, allowing dissemination of results to non-specialists (e.g. farmers, land managers and other stakeholders and end users) as well as specialists, thus increasing the target audience and applications of this method. For example, sub-catchments can be highlighted to understand drainage patterns, the effect of proposed river alterations and dam building can have upon water quality, or for choosing abstraction locations for drinking water that require the least treatment. This latter point is used as an example in the case study in section 3a.16.b below. The findings of these uses, and many others, can be used to aid the understanding and mitigation of the major challenges such as acidification (e.g. high pH water draining coniferous woodland) and eutrophication (e.g. phosphate rich water draining agricultural land) of water bodies.

Although not essential, a basic understanding of the ArcGIS software is advantageous (ArcGIS 10.2.2 is used in this method but most procedures will be the same/similar between versions). As the software package is vast, and power consumption is high, a medium to high specification computer is advised. The specification of computer used in this example includes an Intel Core i5 3.1 GHz CPU and 8 GB RAM on a 64bit operating system, and although useable, the processes were still slow (this may be improved by a standalone GPU/graphics card and a solid state hard drive). Software can be acquired from ESRI (New York, USA) and a license for the ‘Spatial Analysis Tools’ add-on will also be required. Access to UK digital maps was achieved via EDiNA Digimap (Edinburgh, UK), which requires institutional subscription (and involves a registration delay of 12-48 hours before full access is granted). Finally, a spreadsheet containing grid references and/or coordinates of each sample site is required.

Images outlining each important stage are listed in-text and located in Appendix 1.

### 3a.3: Field Data Collection

Samples were collected from 28 sample sites over a range of seasons from Afon Conwy and its various sub-catchments. Samples were collected in acid washed bottles and stored in temperature controlled boxes before transportation to a cold room/fridge where samples were stored at 4° C in the dark before filtration in the laboratory.

### 3a.4: Laboratory Methodology

Samples were filtered through 0.45 µm pore size Whatman membrane filters to isolate DOM (defined as organic matter that can pass through 0.45 µm). These samples were then analysed for DOC (as a proxy for DOM) using a Thermalox TOC/TN analyser (Analytical Sciences, UK). See Chapter 2 for methodology.

Statistical analysis (in the form of a Pearson's correlation) of mean DOC, anion and cation concentration data and soil, bedrock and vegetation cover, was carried out using IBM SPSS 22.

### 3a.5: Obtaining Digital Map Data from EDiNA

Map data was downloaded from EDiNA Digimap via the *Ordnance Survey >Data Download* links (see Figure 1, Appendix 1). After agreeing to the terms and conditions by pressing the green button in the dialogue box, the sample site/catchment area was identified by zooming in on the map, and was selected using the *Draw Rectangle* feature. Map data was selected under the *Backdrop Mapping* dropdown in the left hand pane, and the correct resolution map was selected (this example used a 1:10000 resolution raster which is no longer available, but the *1:25000 Raster* can be used) (see Figure 2, in Appendix 1). Under *Land and Height Data*, *OS Terrain 5 DTM* was selected. It was important to only select the area of map required for the purpose of this study as additional data can drastically increase computing times. The *Add to Basket* button was clicked, the download was named appropriately, the most up to date version of the software selected, and finally *Request Download* was selected, resulting in the selected data being sent in a downloadable link to the registered email address (see Figure 3, Appendix 1). It was important to ensure that the *OS5DTM* data was downloaded in ASC (ASCII) format, and the *1:25000 Raster* data was downloaded in TIFF format. An initial email was delivered immediately to alert the user to the request made, and once ready to download, a link was sent in a second email. Data was downloaded from this link, uncompressed (as data was sent in a .zip format) and stored in a directory appropriately named *GIS* with >2gb of space available, and the *ArcMap* software was opened.

Throughout the course of this GIS method, no spaces were included in file names, instead, underscores ('\_') were used to denote spaces. ArcGIS software often struggles to recognise directories or files that aren't named with a continuous string of characters, so this was more a precautionary measure but still a potentially essential time and problem saving step.

If OS map tiles are not required and instead, satellite imagery would suffice, this can be added as a layer, free of charge, to your workspace by navigating to *File>ArcGIS Online*, typing ‘World Imagery’ into the *search* bar and pressing search. Once results are shown, click add under the result titled ‘World Imagery’.

### 3a.6: Opening downloaded data in ArcMap software

Once ArcMap was opened (the *Getting Started* window was dismissed), the *Spatial Analyst* extension was loaded by selecting from the *Customize* dropdown at the top of the window, and then by selecting *Extensions*, and, in the new window, ticking *Spatial Analyst* before closing the window (see Figure 4, Appendix 1). The *ArcToolbox* was then opened by clicking the *Geoprocessing* dropdown at the top of the window and selecting *ArcToolbox*, or alternatively by clicking the *ArcToolbox* button (see Figure 5, Appendix 1). This contains most of the tools required to delineate catchments.

The *Add Data* button on the *Standard* toolbar was selected (see Figure 5, Appendix 1). In the new window, *Connect to Folder* was clicked and directory containing downloaded map data (Raster-25k...) was selected, and all .tiff files opened (selecting *No* from the *Create pyramids...* dialogue box). If the data spanned two different 100km grids (such as SH and SJ), then both sets of .tiff files were opened. If the *Unknown Spatial Reference* dialog box appeared, *OK* was selected – spatial reference information is added later in the process. The map was now visible on the screen, and was navigable with the mouse by clicking and moving the map around, and also by using the mouse wheel (if applicable) to zoom in/out, plus full extent and pan buttons in the *Tools* toolbar (see Figure 6, Appendix 1).

The *Add Data* button was clicked again, and this time the digital terrain model (DTM) data was opened (in the *terrain-5-DTM...* folder downloaded from *Digimap*), resulting in many new DTM tiles added to the map displaying terrain data. Again, if there were two or more 100km grids contained in the selected area for download, all sets of tiles under the *terrain-5-DTM...* folder were opened. It should be possible to see various features such as large valleys and/or mountain ranges in the newly formed DTM (see Figure 7, Appendix 1).

Once the data was opened in the ArcMap software, it was necessary to convert the OS Terrain 5 DTM files from their original ASCII format to a grid format, to allow accurate analysis of the data contained within the files. This was completed by navigating to *Conversion Tools, To Raster, and ASCII to Raster* in the *ArcToolbox*. By right clicking on

*ASCII to Raster*, and selecting *Batch*, it was possible to batch convert the files to raster instead of individually.

In the new window, the first cell was selected, right clicked and *Browse* was selected. The folder containing the ASCII files was opened and all tiles in the folder were selected (using control and shift keys to multi-select). All selected tiles were then opened in the *ASCII to Raster* window. Any other files containing tiles which are ASCII files were also opened in the same way – but in a separate batch, to prevent potential system failure. Next, the files were named according to their original names (e.g. SH64SE) in the correct directory, and the *Output data type* selected changed to *Float* before *OK* was selected and the raster files were created. This process took a long time as each individual file had to be located individually, as this was the only way to get the software to recognise file locations. To speed the process up a little, the first output raster file location was navigated to by right clicking on the relevant *Output Raster* cell and selecting *Browse*, before navigating to the correct directory and naming the file and saving. Then, by re-right clicking on the cell and selecting *Fill*, this fills the remaining cells with the same info, and the filenames can then be edited in the *ASCII to Raster* dialogue box. The same *Fill* function can be used to change *Integer* to *Float* in the *Output Data Type* column (see Figure 8, Appendix 1)

Once complete, the function was run and the resulting layer added to the map. This also took a long time to complete due to the fact that over 100 tiles were being converted from ASCII to Raster. To speed up computer processing times, and to tidy up the display, the individual ASCII and raster tiles were removed from the *Table Of Contents (TOC)* by highlighting them, right clicking and selecting remove, once the process was complete. The .tiff map files were kept.

To reduce processing power and memory required, and to ensure all tiles are of the same scale, these many DTM tiles were joined together to make just one layer, by navigating to *ArcToolbox*, and expanding *Data Management Tools, Raster, Raster Dataset, Mosaic To New Raster*. The input rasters were all of the DTM files (selectable all at once by holding the shift and control keys and clicking on the title of each tile in the *TOC* (the side window at the left of the screen), before dragging to the *Input Rasters* section of the *Mosaic To New Raster* window), the file *Output Location* was selected (the directory where the file was to be saved), *Number of Bands* was changed to '1' but all other fields left unedited, and finally, the *Raster*

*Dataset Name with Extension* was named *OS5M* and *OK* was clicked (see Figure 9, Appendix 1).

Once completed (notified by a popup window at the bottom right of the ArcMap window), the *Add Data* button was selected, and the new file (*OS5M*) was selected and opened if it did not automatically appear at the top of the *TOC*.

Before proceeding, the *File* dropdown at the top of the screen was opened, *Map Document Properties* was selected and the *Store relative pathnames to data sources* tick box and then the *OK* button were selected. This ensures that if the data is moved to a new directory and/or drive, all of the datasets will remain linked to the map document (See Figure 10, Appendix 1).

### 3a.7: Processing the map data for catchment delineation

Due to a large area of ocean and the *Great Orme* Island at the estuary of *Afon Conwy*, the next step was completed to eliminate any potential source of confusion in the following steps for the software. Therefore, this step was not necessary but can save problem solving and repeating many steps down the line, should the selected map contain large areas of ocean/lakes, for example.

The *Draw* toolbar was selected from the *View* drop down at the top of the screen, followed by the *Toolbars* dropdown in this menu. On the newly added toolbar, the *Rectangle* shape was selected, and drawn over the desired catchment area to exclude any areas not required.

Once the rectangle was positioned correctly (see Figure 11, Appendix 1), clicking *Drawing* on the *Draw* toolbar and clicking *Convert Graphics to Features* opened a new dialogue box (see Figure 12, Appendix 1). All options were left as standard with only the *Output shapefile or feature class:* field changed to save the file as a .shp document in the desired directory, under the name *Clip*, and selecting *Ok* and then *Yes* in the following two windows. The yellow rectangle was then clicked on, so that the blue ‘handles’ appeared at the edges of it, the rectangle was dragged downwards to ensure that there were now two rectangles, before the *Delete* key was pressed to remove the initial rectangle but leave the .shp file just created.

In *ArcToolbox*, *Clip* was navigated to under *Data Management Tools>Raster>Raster Processing*, the *Input Raster* field was selected as the original DEM mosaic (*OS5M*) and the output extent was chosen as the *Clip.shp* layer just created. The *Output Raster Dataset* was named *OS5MClip* before *OK* was selected. If the coordinates for a hypothetical rectangle to

cover the catchment area were known, then these could have been entered in the previous window instead of creating a rectangle and converting it to a polygon, but this was completed for ease of use and for saving time. The Clip.shp and OS5M files were then selected and removed, using the right click function, resulting in a smaller DEM visible with less ocean and/or area in it. Finally, the OS5MClip layer was right clicked, *Properties* was selected and the *General* tab was navigated to. Under the *Layer Name* box, the layer was renamed to OS5M and *OK* was selected.

In *ArcToolbox*, *Spatial Analyst Tools* and then *Hydrology* were both expanded, and *Flow Direction* option was chosen (see Figure 13, Appendix 1).

The *Flow Direction* feature computes the direction in which rainfall would flow if it were to fall on the land, for every cell/pixel in the map, by using the DTM. In the *Flow Direction* dialog box, the *Input Surface Raster* was selected as the DTM (OS5M) (drag and drop from the *TOC* in ArcMap or find the file from the *GIS* directory that was made previously), and the *Output flow direction raster* was named *FlowDir\_OS5M* and saved in the *GIS* directory (see Figure 14, Appendix 1). No other changes were made, and *OK* was selected, resulting in *FlowDir\_OS5M* layer being computed, created and added to the *TOC* pane.

This new layer provides a 3 dimensional view but it is possible to see various cells that contain no data (as standard, they are shown as white, and reservoirs/rivers can be easily identified), and these are known as sinks (see Figure 15, Appendix 1). A sink is a cell that contains no data, likely because it is lower than all surrounding cells. This posed a problem; hypothetically, all rainfall would fall into this sink and the software would not recognise that it could not escape. Therefore, these sinks had to be identified, and then filled. Typically, the *FlowDir\_OS5M* layer should have had a range of 1 to 128 (this can be seen in *TOC* under the layer name – in this example, the range was from 1 to 255). A range of 1 – 128 signifies that there are no sinks, but it would be wise to run the next step regardless, to ensure that there are no sinks remaining, and even more so if the range displayed was higher than this.

In *ArcToolbox*, under *Spatial Analyst Tools* and *Hydrology*, *Sink* was selected. The *Input flow direction raster* was chosen as *Flow\_DirOS5M*, and the output was appropriately named *Sink\_flowdir1*, and located in the *GIS* directory. The *OK* button was clicked and the sinks were identified (see Figure 16, Appendix 1). Depending on the number of sinks, this process can be time consuming. In this example, 70,956 sinks were identified and the layer

*Sink\_flowdir1* was added to the *TOC*. The next step was to fill the identified sinks to ensure accurate drainage mapping.

In the *Hydrology* section of *ArcToolbox*, the *Fill* tool was selected, and the *Input surface raster* was chosen as *Flow\_dirOS5M* and the *Output surface raster* was named *FlowDir\_fill1*, all other options were left unedited and the *OK* button was clicked. This created a layer with all sinks filled in (see Figure 17, Appendix 1).

*FlowDir\_Fill1* was then used to create a flow direction map with the sinks filled in (therefore, a more accurate representation of actual rainfall/water flow compared to the flow direction map without sinks filled in). *Flow Direction* was selected from the *Hydrology* section of the *ArcToolbox*, with the *Input surface raster* being *FlowDir\_Fill1* and the output raster named *FlowdirOS5M*. *Ok* was selected and the flow direction was computed (see Figure 18, Appendix 1). Note that the new layer added to the *TOC* has 8 categories (1, 2, 4, 8, 16, 32, 64, and 128) – these represent the flow direction in terms of East, South East, South, South West, West, North West, North and North East (See Figure 19, Appendix 1).

The stream network was now able to be calculated using the *Flow Accumulation* function, which calculates the number of upslope cells flowing into a location. In *ArcToolbox*, under *Hydrology*, *Flow Accumulation* was selected, and the *Input flow direction raster* was selected as *FlowdirOS5m*, the *Output accumulation raster* called *FlowaccOS5m* and the *Output data type* was changed to *Integer*, before *OK* was clicked.

The resulting layer displayed calculated rivers and streams according to the DTM and the various modification techniques already undertaken (see Figure 20, Appendix 1). As standard, only major rivers were shown but it was possible to adjust this so that smaller rivers and even streams could be made visible. In the *TOC*, the *FlowaccOS5M* layer was right clicked and *Properties* was selected. After navigating to the *Symbology* tab in the newly opened dialog box, in the *Show:* section, *Classified* was clicked. Under *Classification*, the number of classes was changed to 20, and the *Classify* button was clicked (see Figure 21, Appendix 1). Experimenting with changing the classification method from *Natural Breaks (Jenks)* to other options to resulted in the determination of which classification best suited the data used (see Figure 22, Appendix 1). For the purpose of this study, *Natural Breaks (Jenks)* was chosen, and *OK* was clicked to return to the *Properties* window. Under the *Classification* section that has just been explored, the colour ramp was changed, to adjust the colours that each class of river is displayed as. A vibrant colour ramp was chosen, with individual colours

rather than a spectrum from colour to colour, before *Apply* and *Ok* were clicked. Note that it is possible to manually enter the range of each class if needed in the *Symbology* tab to gain greater control over which sized rivers are to be included in each class.

### 3a.8: Inputting and attributing sample locations as grid references

A basic table was populated with sample site location coordinates and/or 12 figure grid references, using Microsoft Excel, and saved under the name of *Sample Points* (see Figure 23, Appendix 1). The file was saved as an Excel 1997-2003 workbook as earlier/later versions can sometimes cause compatibility issues, although ArcGIS 10.2.2 seemed to work well with Excel 2013 in this particular case. It was ensured that there was only one worksheet in the Excel document that contained all data required, with this worksheet also named 'Sample Points'.

Within ArcMap, *Add Data* from the *Standard* toolbar was selected, and the Excel file containing grid references/coordinates was opened. In the *TOC*, right clicking on the new layer (named *Sample Points*) and selecting *Open* opened up a view of the table, allowing the user to ensure the data is all present. When all data was present, right clicking on the layer again, and selecting *Display XY Data* opened up a dialog box. The *X Field* was set to *X*, or *Easting* and the *Y Field* was set to *Y* or *Northing*, with *<none>* displayed in the *Z Field* (see Figure 24, Appendix 1). Under the *Coordinate System of Input Coordinates* heading, the *Edit* button was selected and the correct projection of points system was selected (*Projected Coordinate Systems > National Grids > Europe > British National Grid*) (see Figure 25, Appendix 1). The *OK* buttons were clicked to exit 3 windows, and the sample locations were seen plotted on the map.

The new layer *Sample Points* was right-clicked, and *Data > Export Data* was selected. Leaving *All Features* and 'this layer's source data' checked, the *Output feature class* was renamed to *Sample\_Points* and saved as a shapefile, before *Yes* was selected on the dialog box (see Figure 26, Appendix 1).

*If grid references were not obtainable for sample sites, another method is to select the Marker option from the dropdown in the Draw toolbar (under where rectangle was previously located) and positioning the marker at the site the watershed was required for. Then, by converting this graphic to a feature, like before, deleting the old graphic and naming the location point, a pour point and then watershed could be calculated from this one*

site by selecting the desired location to snap the pour point to. All of the above processes have been explained previously. Read on for more information on pour point creation.

### 3a.9: Tidying display before watershed calculation

To improve the user interface and experience, and to preserve computing power, unneeded layers were switched off or removed. The *Sample Points* layer inputted as an Excel spreadsheet was removed by simply right clicking on the layer and selecting 'Delete'. The *flow-direction* and *sink-flowdirection* layers were unchecked to remove them from view but not delete them. Finally, the folder containing the original OS map files was moved to the top of the *TOC*, by first changing the *TOC* view from *List by Source* to *List by Drawing Order* using the buttons at the top of the *TOC*. All of the map files were then highlighted and dragged to the top of the *TOC* so that they were visible over the top of all other layers made, before the *Sample\_Points* layer was dragged to the top of the list to display the sample points over the map. Finally the *FlowAccOS5M* layer was dragged to the top but made invisible, so that the *Sample\_Points* layer was beneath it, and the map tiles were beneath the *Sample\_Points* layer (See Figure 27, Appendix 1). Double clicking on the marker under the *Sample\_Points* layer opens the *Symbol Selector* dialog box. Changing the symbolshape and/or colour allows for a more obvious marker to be selected so that sample points stand out on the map (see Figure 28, Appendix 1).

### 3a.10: Creation of watershed from a predetermined sampling point

With the display tidied up, it was possible to start creating watersheds for each sample site. On the *Tools* toolbar, *Clear selected features* was clicked before *Select Features* was clicked, and a box was drawn around the first sample point. A colour change of the sample location pointer was noticed, indicating that only that one point was selected, when compared to the standard colour of all other sample point locations (see Figure 29, Appendix 1). By displaying the *FlowaccOS5M* layer, it was possible to determine how close to the projected river/stream that the sample point is located.

In *ArcToolbox*, *Spatial Analyst Tools>Hydrology>Snap Pour Point* were selected and opened. The pourpoint tells the software where the sample site is located, and is defined as the last cell that all cells above it drain into. The *Input raster or feature pour point data* was *Sample\_Points* (although this layer contains all sample points, only the one point previously selected will be used). The *Input accumulation raster* was *flowaccOS5M* and the *Pourpoint*

*field* was *FID*. The snap distance varied depending on how far from the projected river/stream the data point is. With each individual cell representing 5 x 5 m, some estimation was required to get the pour point as close to the projected river/stream as possible (figures are in meters). A distance of 5 m was chosen for this example, although estimated values may need to be used for pour points closer/further away to the appropriate watercourse (simply deleting the layer and creating a new pourpoint will enable the user to estimate correct distances. It was ensured that the new layer had a different name to the previously deleted ones). The *Output raster* needed to be named, and for the sake of ease of use, *prpt\_site1* was chosen, however, any name with 13 characters or fewer (and no spaces) can be used. After clicking *OK*, the pour point was calculated and shown on the map as a cell completely filled in a solid colour (see Figure 30, Appendix 1).

The watershed was then calculated from this pourpoint by selecting *Watershed* from the *Hydrology* section of *ArcToolbox*, and by using the *Input flow direction raster* as *flowdirOS5M* and the *Input raster or feature pour point data* as the pour point just created (*prpt\_site1*). The output raster was named *Watersh\_flow1* and the *Pour point field* option left as *Value*. After selecting the correct directory to save the file in, the *OK* button was selected and the watershed was created (see Figure 31, Appendix 1).

To allow the watershed to be used for visual aids, and to allow the abstraction of data, the watershed was converted into a polygon file. In *ArcToolbox*, *Conversion Tools>From Raster>Raster to Polygon* was selected. The input was the newly created *Watersh\_flow1* layer, and the output was named *Watersh\_Site1*. *Simplify polygons* was selected and *OK* was clicked, resulting in the layer *Watersh\_Site1* being added to the *TOC* and displayed on the map.

- The two previous layers *Watersh\_flow1* and *prpt\_site1* were made invisible, as was *flowaccOS5M* resulting in the *Watersh\_Site1* layer being displayed directly above the map layer.

To improve visual qualities of the map, the *Watersh\_Site1* properties were changed by right clicking on the layer name in the *TOC* and selecting *Properties*.

Firstly, under the *Display* tab, the transparency was set to 30% to allow the underlying map to be seen through the catchment (see Figure 32, Appendix 1), before, under the *Symbology* tab, the colour was changed to a more bold one, by double clicking on the coloured square under the *Symbol* heading and selecting an appropriate colour (a border can be added at this step too

to help the watershed stand out) (see Figure 33, Appendix 1). Clicking *OK* exited the *Properties* window, and then by right clicking on the *Sample\_Points* layer in the TOC, and selecting *Label Features*, each site was given its name from the initial Excel spreadsheet (See Figure 34, Appendix 1).

### 3a.11: Clipping external data to the watershed

External data, such as the previously mentioned CCW Phase 1 Habitat Survey, was available by contacting Natural Resources Wales. This data is only available for Wales, UK, but a GIS lecturer/technician at most institutions will have access to a wide range of data, and some is available for download from EDiNA Digimap.

The Phase 1 data was added as a shapefile, named *Phase 1*. To extract Phase 1 data only from a catchment, it was necessary to ‘clip’ the data to the catchment boundaries. This was completed by selecting *Analysis Tools>Extract>Clip* from the *ArcToolbox*, using the *Phase 1* layer as an *Input Feature* and the *Watersh\_Site1* shapefile as the *Clip Features*. The *Output Feature Class* was saved as *clip\_site1*, *OK* was clicked and the clip was completed, resulting in the layer *clip\_site1* being displayed over the top of *Watersh\_Site1* both on the map and in the *TOC*. There are many polygons visible in the new layer, each representing boundaries between different land use types. It is possible to colour each individual land use type, simply by right clicking on the *clip\_site1* in the *TOC* and selecting *Properties*, navigating to the *Symbology* tab and selecting ‘under the *Show:* box. Changing the ‘Value Field’ dropdown to *Habitat\_Ty* (which is the text definition of the habitat type) and clicking *Add all Values* displays all of the different habitat types in the catchment. After adjusting the colour ramp for clarity, *Apply* and *OK* were pressed, resulting in a ‘mosaic’ like design for the catchment (see Figures 35 and 36, Appendix 1).

### 3a.12: Calculating watershed area

Calculating the area of the watershed is a simple procedure, as is calculating the area of the individual land use types per catchment. By right clicking on *clip\_site1* layer and selecting *Open Attribute Table*, displays the data that the layer contains (see Figure 37, Appendix 1). After clicking *Table Options* and *Add Field*, a dialog box opens. The new field was named *Area\_M2* and *Float* was selected under the *Type* dropdown box, before clicking *OK*. Simply by right clicking on the new *Area\_M2* column heading, and selecting *Calculate Geometry*, a new dialog box opens. After ensuring ‘British National Grid’ projection is still being used,

and by setting the units to 'Meters Squared', and clicking OK, the area of each instance of each land-use type is calculated. However, to obtain total data for each land use type as a whole (so not every individual instance in the catchment), right clicking on the *clip\_site1* layer again, reselecting *Open Attribute Table* and then right clicking on the *Habitat\_Ty* column header and selecting *Summarize*, it is possible to consolidate the data to a more manageable size. In the new window, *Area\_M2* was expanded and *Sum* was checked, the output location selected and the file named *area\_site1clip*, saved as a text file, OK was clicked twice and the table was added to the bottom of the *TOC*, although the layer is not visible in ArcMap.

By navigating to this file's directory outside of ArcGIS software, and renaming the file with a .csv ending rather than a .txt ending, the document can be opened in Microsoft Excel, where the areas of each land use type were totalled up providing the total area per catchment. The total area was provided in meters squared, so dividing this figure by 1000000 provided kilometer<sup>2</sup> data and dividing this new figure by 2.58999 provided miles<sup>2</sup> data (see Figure 38, Appendix 1)

### 3a.13: Editing maps to improve visual impacts

Once the watersheds are created for each sample site, pictures of each individual watershed can be saved for use in literature and/or publications. Therefore, the maps will need more information added to them to make them suitable for these purposes, and this is again, simply done in ArcMap.

At the bottom left of the map window, the view was changed to *Layout View* by using the small buttons (see Figure 39, Appendix 1). Alternatively, the *View* dropdown at the top of the page can be clicked, and *Layout View* selected. By using the magnification and pan tools in the *Tools* toolbar, the catchment and map was moved to a desired location in the centre of the page. It was possible to change the page orientation between landscape/portrait by selecting *File > Page and Print Setup* and selecting the preferred orientation.

A north arrow and title were added and moved to their desired locations by clicking the *Insert* tab at the top of the window and selecting the required feature (as a white background and not a transparent background was required, right clicking on the item and selecting *Properties*, and then changing the background colour to white made the item stand out better). A legend was also added, the correct layer name selected and the background colour changed to white

(in the following dialog boxes) (see Figure 40, in Appendix 1). Add the legend by pressing the *Finish* button and drag the legend to its location. The name of the layer can be changed by right clicking on the layer, selecting *Properties*, and adjusting the name under the *General* tab to *Site 1*. A scale bar was also added, but before this was done, the correct grid reference system needed to be chosen to ensure that the scale bar represents the true scale of the map being displayed. This was achieved by navigating to the *View* drop down box at the top of the window, selecting *Data Frame Properties* and opening the *Coordinate System* tab, before selecting British National Grid from *Projected Coordinate Systems > National Grids > Europe > British National Grid* and click *OK*.

The final addition to the map is an acknowledgement of the data providers. This is added in the same way as the north arrow, for example, but by selecting 'text'. For example, for this project: © Crown Copyright / Database right 2018. An Ordnance Survey / EDINA supplied service, and positioned at the bottom of the map document.

Finally, the map is ready to be exported. This is simply achieved by selecting the *File* dropdown at the top of the window, selecting *Export Map*, and navigating to an appropriate directory. For the purpose of this study, the resolution was set to 300 dots per inch (DPI) to allow high clarity once the map was enlarged, before the map was saved as a .jpg file. See Figure 41 in in Appendix 1 for an example of the finished, saved map file.

### 3a.14: Troubleshooting

#### 'Spatial Analyst' function not visible in ArcToolbox

The Spatial Analyst feature is an add-on for the basic GIS software that contains all of the hydrological processes that are required for this method; and a license is required to access this section of the tool box. Sometimes, if a license is already held, the add-on needs to be activated by simply navigating to *Customise* on the top toolbar in ArcMap, and selecting *Extensions*, and ensure that 'Spatial Analyst' is checked.

#### Generated streams not displaying correctly

If a stream is not visible, it may be worth manually adjusting the classes of the flow accumulation layer so that smaller streams are identified. Some really small streams are not visible as fewer than 1000 cells drain into them. This can be rectified by changing the break values when reclassifying the flow accumulation layer, although

this may be a case of trial and error to get the correct intervals to see a particular watercourse at a particular location.

### File name issues

As a general rule, ArcGIS only allows 13 characters in a file name, and all file names should not contain any spaces. The use of an underscore is suggested to replace a space.

### Failure to create a layer

Although it may seem like a very basic solution, sometimes, saving the document, closing ArcGIS software and then reopening it can resolve many ‘failure to create layer’ errors. It could also be worth ensuring that there is enough space available on the directory that you are using and that the layer name has no spaces in it.

### Deleting a layer

To permanently delete a layer, the *Catalog* window needs to be opened. This can be done by selecting the *Windows* dropdown at the top of the window and choosing *Catalog*. By navigating to the directory containing the layer file, and right clicking on the file, it is possible to permanently delete this file.

### No notification of layer creation

For an unknown reason, in the first instance of running an *ArcToolbox* command once ArcMap is newly opened, a message to inform the user that their tool has finished working and whether creating a new layer has been successful/unsuccessful does not appear. The user had to study the TOC and the map screen to determine when the first layer was created. If the dataset was vast, this would take some time (2-5 minutes in this example). Each further instance of running tools from ArcToolbox, for example, under the Hydrology section, resulted in a blue scrolling message at the bottom of the window to inform the user that the software was working, followed by a pop-up box at the lower right hand side of the window to alert the user when the layer had been created.

### Issues snapping pourpoint correctly

Sometimes, gauging the distance required to snap the pourpoint to the digital representation of a stream/river can be very time consuming. Instead of trial and error, it is possible to manually move the pour point. To do this, select the layer that contains the point and navigate to the *Editor* toolbar. Click on the *Editor* drop down and select *Start Editing*, choosing the layer containing the point once again, and clicking ‘OK’ and ‘Continue’ in the following dialogue boxes. After ensuring that the *Editor Tool* is selected (located to the right of the *Editor* drop down), click on the point and then click and drag it to the relevant location on the flow accumulation layer. Once the position is correct, select the *Editor* dropdown once again, click ‘Stop Editing’ and save edits when prompted. It will then be possible to snap the pourpoint once again but this time with a distance of zero, and the pourpoint will be exactly where required.

### Converting M<sup>2</sup> to Km<sup>2</sup> and Miles<sup>2</sup>

This method provides output area data in M<sup>2</sup> but it could be more suitable to use larger units. Therefore, to convert area from M<sup>2</sup> to Km<sup>2</sup>, simply divide M<sup>2</sup> by 1,000,000. To convert Km<sup>2</sup> to Miles<sup>2</sup>, divide Km<sup>2</sup> by 2.58999.

For example:  $123,456,789\text{M}^2 / 1000000 = 123.46\text{Km}^2 / 2.58999 = 47.67 \text{ Miles}^2$

### 3a.15: Further help and assistance

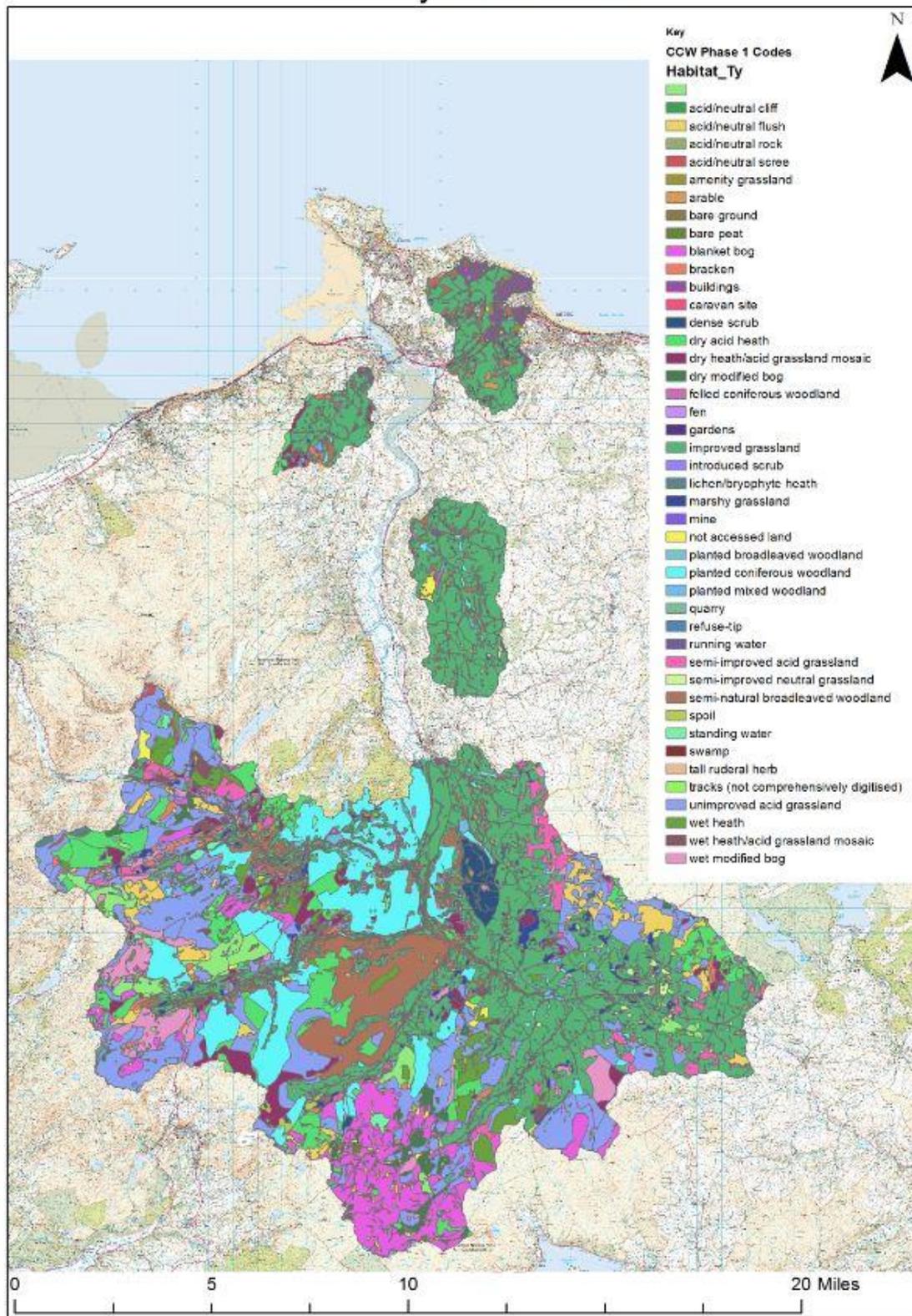
The ArcGIS website contains a good help section which will answer many queries a user may have. This can be located at <http://resources.arcgis.com/en/home/>

Data accuracy plays a vital role in GIS studies, as any outputs calculated from this data (such as catchment area) will carry over any error from the initial data input (for example, grid references).

### 3a.16 – Results

#### 3a.16.1 – The Conwy catchment – A case study

# Conwy Catchment



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Figure 3a.1: Image to show all sub-catchments of the River Conwy studied in the present study, and the corresponding land use. Image also serves as an example of a detailed and simple to use output from this method.

Figure 3a.1 shows CCW Phase 1 data overlaid onto the calculated catchment of all sample sites in the River Conwy Catchment. It can be seen that the catchments feeding these sampling locations contain a high proportion of ‘improved grassland’ land use. Table 3a.2 shows the data output generated from the CCW Phase 1 clip of the watershed, summarised and arranged in alphabetical order. It is interesting to note that there are relatively few landuse types that would indicate major human influences, such as quarries, tracks, buildings etc, and these contribute to an area between 0.27 km<sup>2</sup> and 2.9 km<sup>2</sup> depending on characteristics of ‘unclassified land’ and ‘not accessed land’ in this catchment, therefore the majority of the land in this catchment is comprised of natural or partially managed land, thus a good example to show the relationship between these land uses and the quality of water draining from them, without major human influences which can drastically alter the chemistry and composition of the water draining from them.

A Pearson correlation statistical analysis of average DOC data was undertaken, showing that there are strong positive correlations between several vegetation types and DOC. The strongest correlations were recorded between DOC and limestone pavement, non-ruderal herb (not a plant commonly described as a weed, that is first to colonise disturbed land/waste land) and fern, semi improved calcareous grassland and unimproved calcareous grassland (each at  $p=0.723$ ,  $s<0.01$ ,  $n=28$ ). All correlations recorded between vegetation type and DOC were positive and displayed a moderate to strong relationship, and were compared against a findings from a similar study by Gough *et al.*, 2016 (see Table 3a.1), where data was also obtained from Welsh catchments, but focussed on reservoir water quality and not stream water quality, which should provide a more indepth insight as to the role that the catchment land use plays in water quality, as the water will not have mixed with waters from other soruces. Interesting differences between the two datasets show that, for example, the occurrence of ‘non ruderal herb and fern’ in the present study correlates in a strong positive relationship with an increase of DOC, whereas, the study by Gough *et al.*, found a medium to strong negative correlation.

Table 3a.1: Table to show Pearson’s correlations between DOC and land use parameters from the present study and from Gough *et al.*, 2016, who studied the relationship between reservoir water quality and the CCW Phase 1 Habitat Survey in Welsh catchments. Data from the present study is examined in Table 3a.2.

	Present Study	Gough <i>et al.</i> , 2016 (whole catchment analysis)	
<b>Parameter</b>	<b>Quarterly Average</b>	<b>Autumn</b>	<b>Spring</b>
Amenity Grassland	.660**		
Arable	.497**	.734**	.651**
Bare Ground	.711**		
Buildings	.426*	.596**	
Caravan Site	.487**		
Gardens	.566**		
Limestone Pavement	.723**		
Non-Ruderal Herb and Fern	.723**	-.499*	
Semi-Improved Calcareous Grassland	.723**		
Unimproved Calcareous Grassland	.723**		
<b>** p&lt;0.01 * p&lt;0.05</b>			

Anion and cation data has also been analysed to show the correlation between these ions and land use type and it can clearly be seen that there are very few correlations between ammonium concentration and any land use type, and, it can be seen that the

sulfate ion concentration had the strongest positive correlations with 4 different land use types; limestone pavement, non ruderal herb and fern, semi improved calcareous grassland and unimproved calcareous grassland ( $p=0.813$ ,  $s<0.01$ ,  $n=28$  for each) – the same as for DOC. These 4 habitat types comprise only 0.05% of the total land covered in all 28 sites; at 0.000179%, 0.000668%, 0.026221% and 0.030795% respectively. Interestingly, land classed as gardens has the highest occurrence of strong positive correlations with ion data, with the lowest correlation being nitrate ( $p=0.525$ ,  $s<0.01$ ,  $n=28$ ) and the highest being sulfate ( $p=0.656$ ,  $s<0.01$ ,  $n=28$ ). As every single correlation recorded between each land use type and ion concentration is positive, the data suggests that as any specific land use area increases in size, ionic concentrations and also DOC concentrations increase (thus it is rational to expect that DOM would increase too) (see Table 3a.3), suggesting that the larger the area of any specific land use type, the higher the concentration of the specific ion(s) leaching from it.

Table 3a.2: Table to show example data output generated from ArcGIS software arranged in an easy to view format, and area conversions below.

<b>Land Use</b>	<b>Area M2</b>	<b>Land Use (cont..)</b>	<b>Area M2 (cont...)</b>
Unclassified	8,548,052.23	marshy grassland	6,206,485.70
acid/neutral cliff	2,174.96	mine	2,369.29
acid/neutral flush	13,393,348.35	non-ruderal herb and fern	2,750.78
acid/neutral rock	7,312.42	not accessed land	2,904,210.17
acid/neutral scree	616,995.18	planted broadleaved woodland	209,867.35
amenity grassland	307,981.12	planted coniferous woodland	39,637,295.51
arable	835,157.58	planted mixed woodland	1,596,898.28
bare ground	15,982.05	quarry	103,324.17
bare peat	381,361.72	refuse-tip	6,323.17
blanket bog	26,277,851.30	running water	1,491,739.35
bracken	7,630,386.48	semi-improved acid grassland	8,616,251.54
buildings	5,852,877.62	semi-improved calcareous grassland	107,986.66

caravan site	218,500.96	semi-improved neutral grassland	2,041,093.48
dense scrub	4,395,353.50	semi-natural broadleaved woodland	28,178,185.67
dry acid heath	26,848,287.29	spoil	683,171.25
dry heath/acid grassland mosaic	10,500,814.78	standing water	1,787,540.92
dry modified bog	4,959,627.28	swamp	62,915.88
felled coniferous woodland	337,409.60	tall ruderal herb	25,940.07
fen	161,379.74	tracks (not comprehensively digitised)	417,986.26
gardens	153,212.54	unimproved acid grassland	60,842,641.57
improved grassland	120,782,696.77	unimproved calcareous grassland	126,823.48
introduced scrub	2,315.96	wet heath	10,634,774.93
limestone pavement	738.68	wet heath/acid grassland mosaic	2,410,816.48
lichen/bryophyte heath	413,348.60	wet modified bog	11,097,214.71

<b>Total Area M2</b>	411837773.37	<b>Total Area Km2</b>	411.84
<b>Total Area Yards2</b>	492553877.6	<b>Total Area Miles2</b>	159.01

The data in Table 3a.2 represents one of the two main outputs desired from GIS based catchment delineation. Whilst a map may be a valuable visual resource, actual numerical data can be used for statistical analysis, for example. Basic calculations to convert M<sup>2</sup> to Km<sup>2</sup> and Miles<sup>2</sup> are the only additional input required to obtain data as shown in the figure.

Table 3a.3: Pearson's correlation data between anion and cation concentrations and area of each specific land use type (\*p=>0.05, \*\*p=>0.01) (Overleaf).

<b>Habitat</b> \ <b>Ion</b>	<u>Fluoride</u>	<u>Chloride</u>	<u>Nitrite</u>	<u>Bromide</u>	<u>Nitrate</u>	<u>Phosphate</u>	<u>Sulfate</u>	<u>Sodium</u>	<u>Ammonium</u>	<u>Potassium</u>	<u>Calcium</u>
<u>Acid Neutral Rock</u>									P=0.426* (S=<0.05)		
<u>Amenity Grassland</u>	P=0.782** (S=<0.01)	P=0.659** (S=<0.01)	P=0.692** (S=<0.01)	P=0.807** (S=<0.01)	P=0.437* (S=<0.05)	P=0.627** (S=<0.01)	P=0.762** (S=<0.01)	P=0.622** (S=<0.01)		P=0.638** (S=<0.01)	P=0.754* * (S=<0.01)
<u>Arable</u>	P=0.449* (S=<0.05)		P=0.443* (S=<0.05)	P=0.495** (S=<0.01)	P=0.507** (S=<0.01)	P=0.506** (S=<0.01)	P=0.527** (S=<0.01)	P=0.475* (S=<0.05)		P=0.502** (S=<0.01)	P=0.462* (S=<0.05)
<u>Bare Ground</u>	P=0.832** (S=<0.01)	P=0.677** (S=<0.01)	P=0.730** (S=<0.01)	P=0.852** (S=<0.01)	P=0.469* (S=<0.05)	P=0.681** (S=<0.01)	P=0.808** (S=<0.01)	P=0.663** (S=<0.01)		P=0.685** (S=<0.01)	P=0.8** (S=<0.01)
<u>Buildings</u>	P=0.475* (S=<0.05)	P=0.536** (S=<0.01)	P=0.434* (S=<0.05)	P=0.508** (S=<0.01)		P=0.421* (S=<0.05)	P=0.508** (S=<0.01)	P=0.486** (S=<0.01)		P=0.431* (S=<0.05)	P=0.540* * (S=<0.01)
<u>Caravan Site</u>		P=0.395* (S=<0.05)						P=0.521** (S=<0.01)			
<u>Gardens</u>	P=0.604** (S=<0.01)	P=0.617** (S=<0.01)	P=0.577** (S=<0.01)	P=0.649** (S=<0.01)	P=0.525** (S=<0.01)	P=0.582** (S=<0.01)	P=0.656** (S=<0.01)	P=0.551** (S=<0.01)		P=0.603** (S=<0.01)	P=0.610* * (S=<0.01)
<u>Limestone Pavement</u>	P=0.843** (S=<0.01)	P=0.673** (S=<0.01)	P=0.738** (S=<0.01)	P=0.861** (S=<0.01)	P=0.467* (S=<0.05)	P=0.684** (S=<0.01)	P=0.813** (S=<0.01)	P=0.660** (S=<0.01)		P=0.686** (S=<0.01)	P=0.797* * (S=<0.01)
<u>Non ruderal herb and fern</u>	P=0.843** (S=<0.01)	P=0.673** (S=<0.01)	P=0.738** (S=<0.01)	P=0.861** (S=<0.01)	P=0.467* (S=<0.05)	P=0.684** (S=<0.01)	P=0.813** (S=<0.01)	P=0.660** (S=<0.01)		P=0.686** (S=<0.01)	P=0.797* * (S=<0.01)
<u>Semi improved calcareous grassland</u>	P=0.843** (S=<0.01)	P=0.673** (S=<0.01)	P=0.738** (S=<0.01)	P=0.861** (S=<0.01)	P=0.467* (S=<0.05)	P=0.684** (S=<0.01)	P=0.813** (S=<0.01)	P=0.660** (S=<0.01)		P=0.686** (S=<0.01)	P=0.797* * (S=<0.01)
<u>Tall ruderal herb</u>		P=0.431* (S=<0.05)									P=0.374* (S=<0.05)
<u>Unimproved calcareous grassland</u>	P=0.843** (S=<0.01)	P=0.673** (S=<0.01)	P=0.738** (S=<0.01)	P=0.861** (S=<0.01)	P=0.467* (S=<0.05)	P=0.684** (S=<0.01)	P=0.813** (S=<0.01)	P=0.660** (S=<0.01)		P=0.686** (S=<0.01)	P=0.797* * (S=<0.01)

### 3a.17 - Discussion

As with all GIS and mapping applications, the accuracy of data used determines the output quality. The CCW Phase 1 Habitat Survey is the primary spatial dataset in Wales, showing the distribution of semi natural habitats. The CCW Phase 1 survey was initiated in 1979 and completed in 1997, and the mapping was based largely from vantage point observations, along with aerial photographs, to enable the mapping of 103 distinct habitats (Lucas *et al.*, 2011). The CCW Phase 1 survey was initiated to create a post war map containing areas of agricultural improvement and establishment of conifer plantations, for example, which had encroached upon semi-natural habitats throughout Wales, with the primary goal of understanding the extent and location of remaining semi-natural habitats in the uplands and lowlands of Wales (Blackstock *et al.*, 2006). Lucas *et al.* focussed upon updating the dataset using satellite sensor data, but the satellite sensor was not sufficiently accurate to determine the varied types of vegetation types, and the resolution was slightly coarser (at 5m pixel size), and therefore the original Habitat Survey data was still considered broadly accurate (J. Rothwell, NRW, 2015, pers. comm. 11<sup>th</sup> May 2015). However, the satellite imagery update did include hedgerow data, an advantage over the original, which only contained hedgerow data for the county of Powys (Lucas *et al.*, 2011). This corresponds with findings from Gough *et al.* (2016) who concluded that the Phase 1 Habitat survey data was still relevant despite the various land use changes that will have taken place since its creation. Gough *et al.* (2016) also found that the topographic watershed may not necessarily correspond with the groundwater influence of the catchment, showing that watershed creation issues are apparent for other users of the software/similar methods (Gough *et al.*, 2016).

Lucas *et al.*, (2007) argue that data obtained through field surveys, such as the CCW Phase 1 Habitat survey data have various advantages, such as comprehensive descriptions of plant species, presence/absence and diversity. However, there are also a number of limitations. These limitations can include individual assessors having different subjective views and varying degrees of knowledge and perception. There is also an argument for the time of year that the survey is completed, with vegetation appearing differently in spring than in autumn, for example (Lucas *et al.*, 2007). However, as the Phase 1 data was collected over a span of 18 years, it would be reasonable to assume that the seasonal variations and different knowledge and perceptions of assessors would play a relatively insignificant role in such a dataset covering nearly 21,000 km<sup>2</sup>. It can also be argued that the data obtained through this

medium is more useful for understanding of general catchment characteristics rather than precise data – if very high resolution data was required for a catchment then it could be more advantageous for one assessor to assess the entire catchment. However, this method would still require an accurate map of the specific catchment, which is made most accurately and simply using the GIS method mentioned above. With data extraction from the watershed taking between 5 and 10 minutes, it would seem sensible to use the Phase 1 data and work with any error that the data is assumed to contain.

### 3a.18: GPS and Grid References

It is important to remember that UK grid references are only as accurate to the number of digits that they contain. For example the type of coordinates that can be read from a 1:50,000 or 1:25,000 OS map are 6 figures (3 for X and 3 for Y) and are accurate to the nearest 100 metres. Grid references with 12 figures (6 for X and 6 for Y) are accurate to 1 meter. Some GPS receivers can even provide data to an accuracy of centimetres, but this precision is not required for such large areas of land, and therefore it is usually not worth recording GPS locations past the first decimal.

Unidentifiable land (such as the classes ‘not accessed land’ or ‘unclassified’) can provide anomalies in data interpretation measures such as *via* statistical analysis. However, as with any data collected by satellite, there will likely always be areas that cannot be characterised due to artificial covering, unclear data, and cloud cover. For the entire Afon Conwy catchment mapped in this work, only 2.8% of the overall area was classified as not accessed land/unclassified land, and therefore 97.2% of the area could be characterised, suggesting that any negative impact this missing data may have upon further analysis would likely be, in most cases, negligible.

### 3a.19 - Case Study

Table 3a.1 shows that DOC has strong positive correlations with numerous land use types, with over half of these differing land use types being anthropogenically managed. These data suggest that as the area of land used for these purposes increases, so does the DOC concentration (in line with intensification and eutrophication).

Pearson’s correlation statistical data is displayed in Table 3a.3. The strong positive relationships between the sulfate ion and limestone pavement, non ruderal herb and fern, semi improved calcareous grassland and unimproved calcareous grassland is surprising,

given that, combined, these land uses comprise only 0.05% of the overall catchment area. This data suggests that the 4 vegetation classes are major contributors of sulfate ions in the water that drains catchments containing them. Interestingly, combined, all of the vegetation land use types that correlated positively in Table 3a.3 comprises only 1.86% of the overall catchment area, showing that only small pockets of vegetation can impact upon water quality drastically. The correlations recorded between land use and DOC and ion data leads to the conclusion that, as the specific land use increases, as does the specific ion/DOC concentration, thus leading to the postulation that these land uses are the major contributors of this specific ion/DOC concentration in the catchment. This information is useful to water companies and land managers as they grow a deeper understanding of the relationship between land use and the quality of the water under their ownership. For water companies, this data will be vital in choosing future water abstraction locations, as DOC and ion removal costs are high, and also to gain an understanding of the propensity of a catchment to form DBPs before the organic matter is removed, to determine whether the treatment practices can be improved. Land managers looking to increase the quality of their water course (to encourage wildlife for example) can obtain the information required to make educated decisions on catchment management and what land use practices will need to be changed to help increase the water quality.

Similarly to work by Gough *et al.*, (2016) showing statistical correlations between whole catchment and percentage CCW Phase 1 vegetation cover, correlations were also found between the ion nitrate ( $\text{NO}_3^-$ ) and arable land ( $p=0.507$ ,  $s=<0.01$ ,  $n=28$ ), although this study is using total area of land use type rather than percentage of land use in overall catchment. Significant medium to strong positive Pearson correlations were also found between DOC and arable and buildings classes ( $p=0.497$ ,  $s=<0.01$ ,  $n=28$ , and  $p=0.426$ ,  $s=<0.05$ ,  $n=28$  respectively) also replicating similar trends displayed by Gough *et al.*, (Gough *et al.*, 2016). Comparison of results from this study and the present study are displayed in Table 3a.1. This data suggests to land managers that the strong correlations between DOC and arable and building dominated land use is one of the major inputs of DOC to the water bodies and therefore focussing on these land use types to reduce DOC concentration is advisable. The present study has many more positive correlations with DOC and land use and this can be explained simply by the fact that the data in Gough *et al.*, (2016) was studying reservoirs only, whereas this present study is focusing more on rivers and streams, but also encompassing reservoirs, thus gaining a broader idea of real world catchment influences.

### 3a.20 - Conclusion

The data produced from this method can be statistically analysed to determine whether a certain land use type, or catchment size, for example, has a significant impact on parameters measured at sampling sites, such as pH, conductivity, discharge or DOM concentration, for example, providing a quantitative dataset to be discussed alongside conventional water quality datasets. This can assist in providing a deeper understanding of intra- and inter-catchment influences upon the quality of the water yielded. This method could prove useful in many applications, from water researchers interested in catchment characteristics from which their sample site rivers drain or to water/utility companies wishing to choose the best site locate a new drinking water abstraction point, to target minimal DBP formation. Furthermore, the data generated from this method can be used in a vast array of applications, such as the mitigation of the previously mentioned eutrophication and acidification issues, and most importantly, these can be statistically analysed in combination with biogeochemical parameters.

With the increasing interest in integrated catchment management, this method proves timely in providing an accessible, relatively simple and highly versatile tool for land managers, owners and developers to generate numerical data and visual aids for use with any target audience. Further datasets, such as the aforementioned NSRI SoilScape and BGS Bedrock files, for example, can also be used to provide more qualitative data to suit the application, such as the role soil type has upon the humic content of DOM, for example. Finally, this method also allows for the creation of a professional visual representation of the catchment of an individual river, or a selection of sampling points along a whole river network, from source to sea, thus answering the hypothesis for this chapter. This method is one of the many thousands of applications for GIS, a tool that is currently arguably underutilised despite the quality and quantity of data it can provide.

### 3a.21 Future Work

Future data from the overall project will be added to this method and then statistically analysed. Whilst vegetation data can remain the same, further water quality parameters will be correlated with the area data of land use type to look for relationships. These water quality parameters will include total and individual concentrations of DBPs, chlorine demand, pH, various UV-Vis wavelengths, total carbohydrate and amino acid concentrations, percentage

hydrophobicity/hydrophilicity and percentage of various DOM molecular weight fractions. Confirmation of the findings generated from this study would help solidify its use, which could be achieved by the collection of grab samples from other catchments with the same land use types that have been found to correlate with DBPs in the present study, to ensure that the method can be extrapolated to other catchments.

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# Chapter 3b

## Studies of Relationships Between Physiochemical Characteristics and Standardised THMFP

### 3b.1 Introduction

DOM character can be influenced by a multitude of environmental factors, including erosion, wild fire, temperature and land use (Beggs and Summers, 2011). DOM is accumulated in water as precipitation moves through the atmosphere and vegetation, infiltrates soil horizons and percolates downwards through mineral soils. DOM, therefore, is typically the product of dissolved atmospheric dust and gases, through fall, root exudate, leaf and root litter, and the primary and secondary metabolites of microorganisms (Aitkenhead-Peterson *et al.*, 2003). All terrestrial freshwater is sourced from aquifers *via* boreholes, springs, or ultimately *via* precipitation in the case of lake and river abstractions. The pathway water takes after falling as precipitation within a catchment involves mixing, evaporation and transpiration, until the remaining water accumulates in a downstream lake or river (Jasechko, 2012). As precipitation moves through vegetation, it is enriched with DOC and DON, likely through the removal of dry deposits of organic materials, such as pollen, dust, aphid honeydew and insect exudate, or even from leachate from the plant itself (Aitkenhead-Peterson *et al.*, 2003). Depending on factors such as watershed slope, depth of water table, barriers to precipitation and through-fall infiltration, DOM from the forest floor, for example, and organic soils can contribute considerably to allochthonous DOM in surface waters, therefore, DOM differs greatly from catchment to catchment (Aitkenhead-Peterson *et al.*, 2003). DOM character differs vastly from site to site depending upon the characteristics of the catchment from which the DOM containing water drains (Singh *et al.*, 2015).

In water abstracted for human consumption, DOM character dictates overall water treatment practices to ensure they are best suited for the specific source water. For example, DON has been found to be inefficiently coagulated by aluminium and iron salts commonly used in conventional treatment (Lee and Westerhoff, 2006). DON removal is paramount, as hypochlorite, often added to disinfect the water, can react with this DON, creating N-DBPs, such as haloacetonitriles, halonitromethanes and N-nitroso-dimethylamine, some of which have been found to be carcinogens (Richardson, 2003) and are explored in more detail in Chapter 5b. For example, brominated nitromethanes have been shown to be extremely cytotoxic and genotoxic in mammalian cells. Bromonitromethane was found to have a genotoxic potency of up to 136  $\mu\text{M}$  (Richardson *et al.*, 2007).

DOC has been the focus of studies (Dunnick and Melnick, 1993) due to the suspected risk of carcinogenicity of C-DBPs after long exposures that are also formed when hypochlorite is

added to disinfect the water. DOC is better known than DON to contribute towards the formation of DBPs, and studies have determined concentrations of C-DBPs such as haloacetic acids (Heller-Grossman *et al.*, 1993, Guay *et al.*, 2015 and Malliarou *et al.*, 2005) and trihalomethanes (Goslan *et al.*, 2009, Gough *et al.*, 2014 and Whitaker *et al.*, 2003) in UK freshwaters.

Currently, in the UK, only 4 of over 700 identified DBPs (Richardson *et al.*, 2007) are under regulation, in the UK, at  $100 \mu\text{g L}^{-1}$  at the consumer's tap (DWI, 2010), for 4 compounds collectively known as trihalomethanes (THM4), comprised of the chemicals chloroform ( $\text{CHCl}_3$ ), dibromochloromethane ( $\text{CHBr}_2\text{Cl}$ ), bromodichloromethane ( $\text{CHBrCl}_2$ ) and bromoform ( $\text{CHBr}_3$ ) (WHO, 2005) (Figure 3b.1). These are regulated, as, in 1999, the USEPA reported that 3 of the THM4 compounds,  $\text{CHCl}_3$ ,  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_3$  were type B2 (human) carcinogens and  $\text{CHClBr}_2$  was a type C (probable human) carcinogen (Panyakapo *et al.*, 2008 and USEPA, 1999).

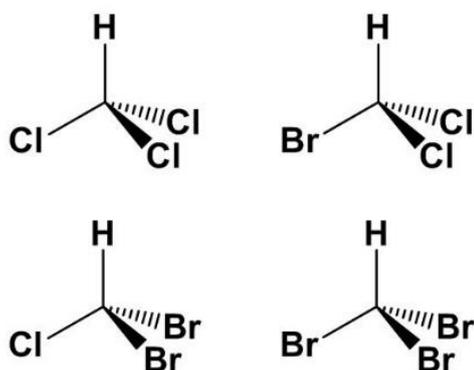


Figure 3b.1: The four regulated THM compounds (in the UK), clockwise from top left: chloroform, bromodichloromethane, chlorodibromomethane and bromoform, typically formed during a reaction between chlorine and DOC.

Geographic information systems (GIS) is a powerful tool in the study of environmental sciences. One of its many uses is to delineate the catchment area of any given point, and to provide data on the spatial area, and land uses that take place within this catchment. This GIS based approach of catchment delineation is well documented (Amaguci *et al.*, 2012, Gough *et al.*, 2016 and Wang and Yin, 1997), although only one instance of the exploration of the relationship between upstream land use and DOM and THM formation potential can be found in literature (Hur *et al.*, 2014). This work, though, was based over 2 different seasons but more importantly, only 4 broad land use classes were used: agriculture, residential, forest and

bare earth. Standardised THM formation potential was found to correlate significantly ( $p < 0.05$  for all instances) with residential ( $f = 0.563$ ), agricultural ( $f = 0.542$ ) and forested catchments ( $f = -0.542$ ) in the month of May, with no correlations with standardised THM formation potential and land use in the month of July (Hur *et al.*, 2014). Chow *et al.*, in 2009, found that standardised THM formation potential significantly differed in leachates derived from different habitats in California, USA (Chow *et al.*, 2009).

In order to better understand the link between DOM and catchment characteristics, and how they impact the formation potential of disinfection byproducts, here we use THM4 data from two contrasting catchments, including 46 sites, and quarterly sample data for the duration of a year, alongside data from nationwide land cover (LCM2007, CEH), soils (SoilScape, NSRI) and Bedrock (BGS) datasets. This data is used to compare to the THM4 concentrations from water draining different catchments to determine whether there are any correlations between the area of a specific land use type, and the concentration of a THM4 compound, upon statistical analysis of datasets collected. Furthermore, several Scottish catchments were selected, with similar characters and land use classes to those in the Hampshire Avon and Conwy catchments, to determine whether the data collected from each site was site specific or whether the catchment character played a deeper role in the data produced. It is important to remember, however, that many other factors can contribute to the overall formation potential of THM4, such as the age of the water, pH, chlorine residual and bromine concentrations, and thus, this present work is only examining the impact that a group of organic compounds dissolved in the water (which is arguably the largest contributor to THM4 formation).

### 3b.1.2: Hypothesis

It is hypothesised that some instances of land use, in terms of vegetation, soil type and bedrock type, will influence THM4 formation either positively or negatively, and that an increased land use area will not necessarily correlate to an increased DOM concentration and therefore an increased THM4 formation.

### 3b.2 Methodology

DOC data (as a proxy for DOM) was collected from sites. THM4 data was also generated from laboratory experimentation and analysis by GC-MS and SPME under chlorination and

chloramination. GIS data was generated using datasets detailed above. A full methodology for THM4 and DOC/DON detection is available in Chapter 2. It is known that pH, temperature and bromide concentration can influence THM4 formation (in terms of pH and temperature) and speciation (in terms of bromide). pH was controlled using buffered solutions at  $\text{pH } 6.8 \pm 0.4$ , temperature was controlled at  $25^\circ \text{C}$ , and bromide concentrations were measured using ion chromatography, and found to be negligible.

### 3b.3 Site Selection

It is important to note that the chlorination of raw water before any other treatments is never undertaken in water treatment works – the water undergoes many different particulate matter removal, and chemistry adjusting steps before a halogen is added to inactivate bacteria. Raw, freshwater samples were collected and analysed in the present study to determine the formation potential of disinfection byproducts, and to understand the chemistry of the water prior to treatment, as this would likely remove most of the organic matter that is required for the purposes of the studies in this thesis.

19 sites were selected along two tributaries of the River Avon, Hampshire, UK – the River Nadder and the River Wylde, and one point just downstream of where they converge near the town of Salisbury. These tributaries drain the headlands of the River Avon, and are dominated by chalky agricultural land, with nutrient rich waters draining from it, although both rivers differ in terms of catchment characteristics and DOM content.

In contrast, 27 sites were selected along the headwaters and various tributaries of the River Conwy, north Wales, UK. This catchment is typically nutrient poor and contains many variations of land use, including blanket bog, agricultural, urban and forestry. These sites were sampled quarterly in 2016 (January, April, July, October). Scottish sites with similar catchment characteristics were used as check sites to determine whether relationships between the DOM and the catchment character were site specific or replicatable across a nutrient and geographical gradient.

### 3b.4 Landuse

Areas (in M<sup>2</sup>) of different land use classes were calculated using ArcGIS Software (ESRI, California, USA), before being correlated with the mean concentration of the THM4 per site, formed from chlorination and chloramination (where available). Data was statistically analysed using IBM SPSS (IBM, New York, USA, Statistical analysis was carried out between datasets, including Avon and Conwy individually, and also combined, to explore broad scale patterns. A Cohen’s *d* statistical test was carried out to determine the effect size of selected variables.

Data was tested for normality before being correlated using Pearson’s correlation (if the data was normally distributed, or could be transformed by Log<sup>10</sup> to normality). If the data was not normal, and could not be transformed to normality, a Spearman’s correlation analysis test was used. Only non-normal variables needed to be transformed to attempt to achieve normality (Field, 2013). Cohen’s *d* statistic was used to examine effect size (Cohen, 1988, and Sawilowsky, 2009) (Table 3b.1, and Equation 3.a). Statistical analysis was not possible for Scottish sites as only one data point per site was collected.

Table 3b.1: Effect size of Cohen’s *d* statistic parameters (Cohen, 1988)

Effect Size	<i>d</i>
Very Small	0.01-
Small	0.2
Medium	0.5
Large	0.8
Very Large	1.2
Huge	2.0+

$$d = \frac{M_1 - M_2}{S}$$

Equation 3.a

Effect size is calculated as the difference between two means, divided by the standard deviation for the data (see Equation 3.a).

Where *d* = Cohen’s statistic, M<sub>1</sub> is mean of first sample, M<sub>2</sub> is mean of 2<sup>nd</sup> sample, and S is pooled standard deviation, see Equation 3.b:

$$(S_1^2 + S_2^2)/2 \quad \text{Equation 3.b}$$

Where  $S_1$  is standard deviation of first sample and  $S_2$  is standard deviation of 2<sup>nd</sup> sample.

Regression analysis of the significantly correlating datasets show how close to a line of best fit the data points are, and this closeness of fit is given an  $R^2$  value, with 0 being 0 % and 1 being 100 % fit.

### 3b.5 Results

Table 3b.2 summarises the physical characteristics of the Hampshire Avon and Conwy catchments studied in this chapter, obtained through GIS, including the total area, dominant vegetation, bedrock and soil, and the number of sites sampled from.

Table 3b.2: Total catchment area, individual sample sites, and dominant bedrock, vegetation and soil type per major site, obtained using GIS software method.

<b>Site</b>	<b>Total Area (Km<sup>2</sup>)</b>	<b>Dominant Vegetation</b>	<b>Dominant Bedrock</b>	<b>Dominant Soil</b>	<b>Number of individual sites</b>
Avon	678.81	Arable and Horticulture (42%)	Chalk (76%)	Shallow lime-rich soils over chalk or limestone (59%)	19
Conwy	416.08	Improved Grassland (23%)	Mudstone Siltstone and Sandstone (68%)	Freely draining acid loamy soils over rock (54%)	27

The data presented in Tables 3b.3 and 3b.4 represent the correlations (Pearson's correlation for normally distributed data, Spearman's correlation for non-normally distributed data)

between land use classes and formation of total standardised (to 1 mg L<sup>-1</sup> DOC) THM<sub>4</sub> formation potential. Further statistical analysis was then conducted in the form of a Cohen's *d* test, to determine the strength of the effect of one parameter upon the other. The tables include treatments of both chlorination and chloramination, and analysis was conducted on data from Avon and Conwy both separately and combined, for both individual and combined THM<sub>4</sub> compounds.

The most prominent relationship between catchment and treatment was observed between chlorination formed THM<sub>4</sub> and loamy and clayey floodplain soils at both Hampshire Avon and Conwy, where as the area of this land use increased, the THM<sub>4</sub> concentration decreased.

The samples were buffered to minimise pH influences on the concentrations of THM<sub>4</sub> compounds formed. Samples were chlorinated at 1 mg L<sup>-1</sup> concentration and all 4 THM<sub>4</sub> compound concentrations were added together to produce a total standardised THM<sub>4</sub> concentration. Bromide and chloride concentrations fluctuated between samples, but were deemed low enough to not create a major influence on the data.

Table 3b.3: Statistical analysis results showing correlation and effect size of standardised total THM<sub>4</sub> concentration for two different treatments (chlorination and chloramination) and the total area of land use class.

Site	Treatment	Land Use Type	Total land use Area (Km <sup>2</sup> )	Statistical Test	Statistic	Cohen's <i>d</i> effect
Avon and Conwy	Chlorination	Loamy and clayey floodplain soils with naturally high groundwater	66.06	Spearman's	f=-686, p<0.05, n=9	0.84

Avon and Conwy	Chlorination	Arable and Horticulture	946.44	Spearman's	f=-0.609, p=<0.01, n=35	0.70
Avon and Conwy	Chlorination	Acid Grassland	386.26	Spearman's	f=0.547, p=<0.01, n=39	0.67
Avon and Conwy	Chlorination	Heather	69.99	Spearman's	f=0.491, p=<0.05, n=26	0.82
Avon and Conwy	Chlorination	Inland Rock	27.19	Spearman's	f=0.423, p=<0.05, n=23	0.94
Conwy	Chlorination	Coniferous Woodland	176.93	Pearson's	f=0.500, p=<0.05, n=20	0.82

Individual standardised (to 1 mg L<sup>-1</sup> DOC) THM<sub>4</sub> compounds (Table 3b.4) and land use types were also statistically analysed. The most prominent relationship was between CHCl<sub>3</sub> formed from chlorination, and coniferous woodland land use at the Hampshire Avon.

Table 3b.4: Statistical analysis of individual THM<sub>4</sub> compounds and correlations and effect size of area of land use class upon concentration.

Site	Treatment	THM <sub>4</sub> Compound	Land Use Type	Total land use area (km <sup>2</sup> )	Statistical Test	Statistic	Cohen <i>d</i>
Avon	Chlorination	CHCl <sub>3</sub>	Fen, Marsh and Swamp	0.10	Spearman's	f=0.812, p=<0.05, n=6	2.42
Conwy	Chlorination	CHCl <sub>3</sub>	Coniferous Woodland	176.93	Pearson's	f=0.530, p=<0.05, n=20	5.36
Conwy	Chlorination	CHBr <sub>2</sub> Cl	Freely draining slightly acid loamy soils	93.12	Spearman's	f=0.843, p=<0.01, n=9	1.71
Conwy	Chloramination	CHCl <sub>3</sub>	Shallow very acid peaty soils over rock	354.83	Pearson's	f=0.739, p=<0.05, n=10	1.98
Avon and Conwy	Chlorination	CHBr <sub>2</sub> Cl	Freely draining slightly acidic	674.50	Spearman's	f=0.533, p=<0.01,	1.18

Combined			loamy soils			n=25	
Avon and Conwy Combined	Chlorination	CHCl <sub>3</sub>	Loamy and clayey floodplain soils with a naturally high groundwater	66.06	Spearman's	f=-0.686, p=<0.05, N=9	0.84
Conwy	Chlorination	CHBr <sub>2</sub> Cl	Slowly permeable wet very acid upland soils with a peaty surface	560.14	Spearman's	f=-0.504, p=<0.05, n=17	1.37

### 3b.6 Conwy Catchment

In the Conwy catchment,  $\text{CHCl}_3$  increased with the area of shallow acidic peat land (Figure 3b.2) an  $R^2$  (coefficient of determination) of 0.5455, showing a medium strength fit to the line of best fit. It is possible to deduce from the graph that, as the area of the soil class increases, so does the  $\text{CHCl}_3$  concentration. A  $\text{CHCl}_3$  concentration of  $19.49 \mu\text{g L}^{-1}$  and a total area of  $81222301 \text{ M}^2$  at one site compares to a  $\text{CHCl}_3$  concentration of  $10.15 \mu\text{g L}^{-1}$  and a total area of  $46266749 \text{ M}^2$ , suggesting that as land area roughly doubles, so does the concentration of  $\text{CHCl}_3$ , likely due to a larger catchment area influencing DOC concentrations, which in turn, influence the THM4 formation potential.

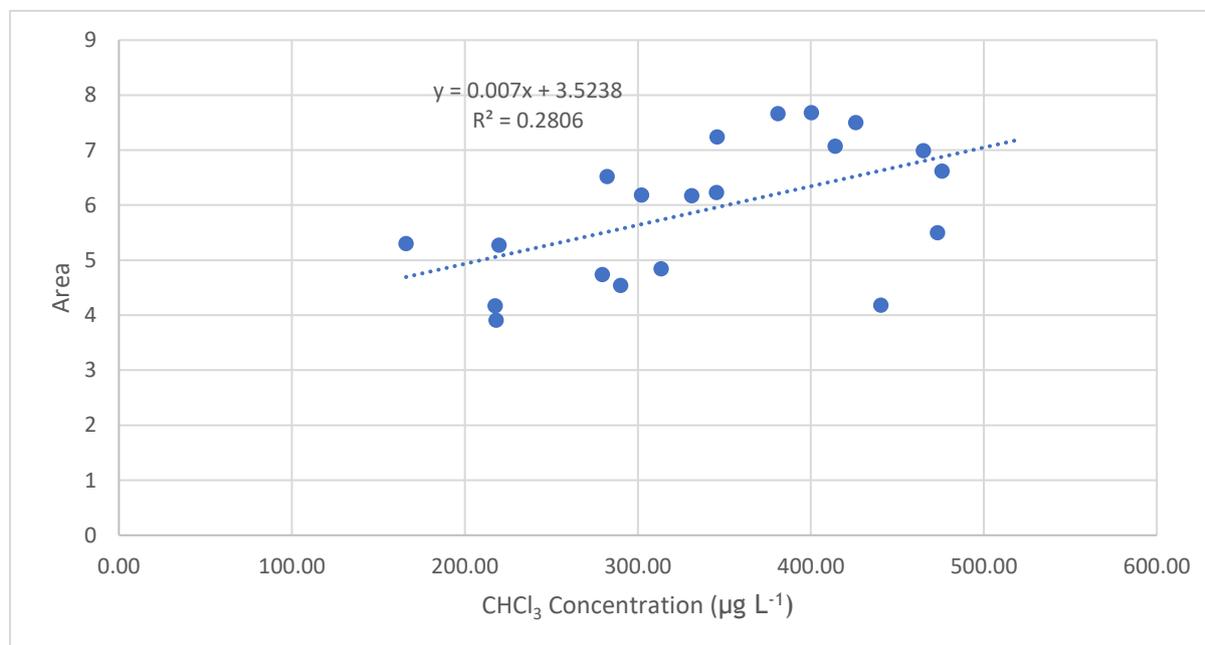


Figure 3b.2: Regression and line of best fit between the area ( $\text{M}^2 \cdot \text{Log}_{10}$ ) of shallow very acid peaty soil over rock vs.  $\text{CHCl}_3$  concentration formed from chloramination, at Conwy sites. Data was not normally distributed, so the area data is presented as  $\text{log}_{10}$  of the area in  $\text{m}^2$ .

Figure 3b.3 shows the line of best fit between data points of  $\text{CHCl}_3$  and coniferous woodland (logged to transform the data to normality). An  $R^2$  coefficient of determination of 0.28 shows a weak to medium fit to the line of best fit. Compared to the correlation data presented in Table 3b.4, a medium strength positive correlation is apparent ( $f=0.530$ ,  $p<0.05$ ), where, as the area of coniferous woodland increases, so does  $\text{CHCl}_3$  concentration.

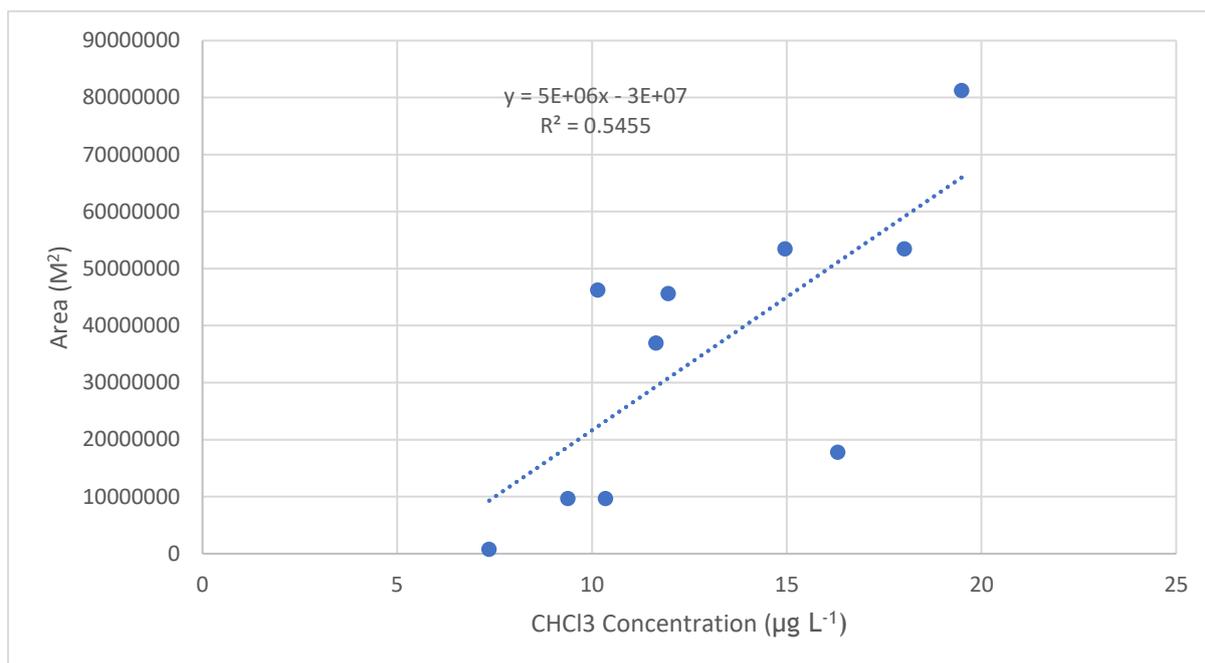


Figure 3b.3: Regression and line of best fit between the area (M<sup>2</sup>) of coniferous woodland (transformed by log<sub>10</sub>) vegetation class and standardised CHCl<sub>3</sub> formation (µg L<sup>-1</sup>) at the Conwy catchment.

Regression and line of best fit between CHCl<sub>2</sub>Br concentration (µg L<sup>-1</sup>) and slowly permeable very wet acid upland soils with a peaty surface area (M<sup>2</sup>) were analysed (Figure 3b.4), presenting an R<sup>2</sup> value of 0.12, which shows a poor fit of data points to the line of best fit. Coupled with data displayed in Table 3b.3, a medium strength negative correlation between the two datasets was found, where, as the area of slowly permeable very wet acid upland soils with a peaty surface decreases, the CHBr<sub>2</sub>Cl concentration increases.

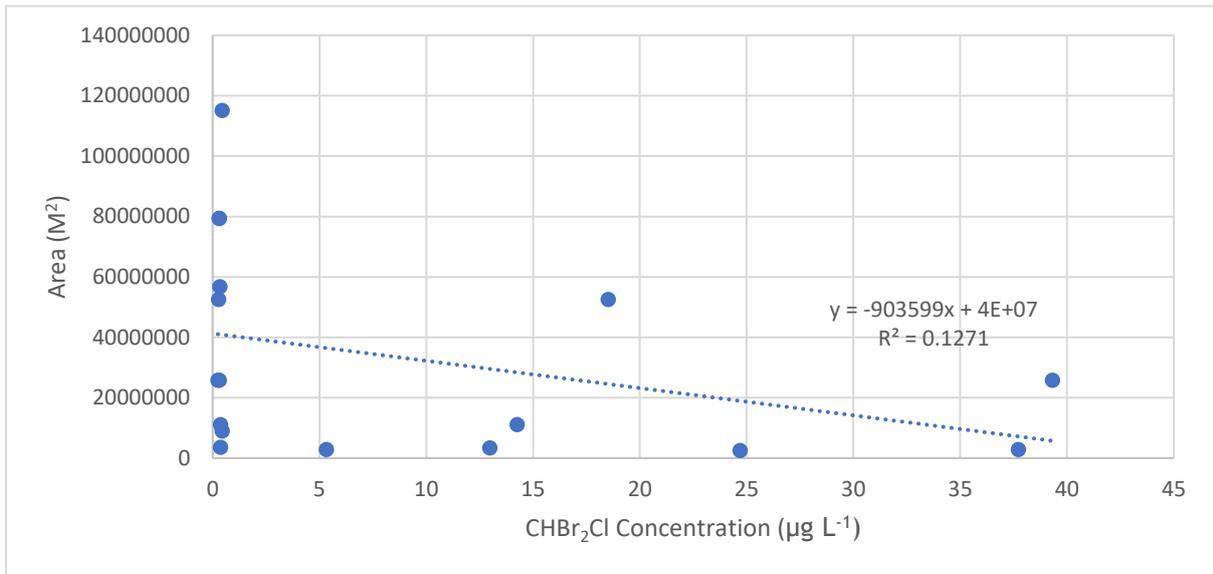


Figure 3b.4: Regression analysis between CHCl<sub>2</sub>Br concentration (µg L<sup>-1</sup>) and slowly permeable very wet acid upland soils with a peaty surface soil classification, with data taken from both Hampshire Avon and Conwy site samples, treated by chlorination.

### 3b.7 Hampshire Avon Catchment

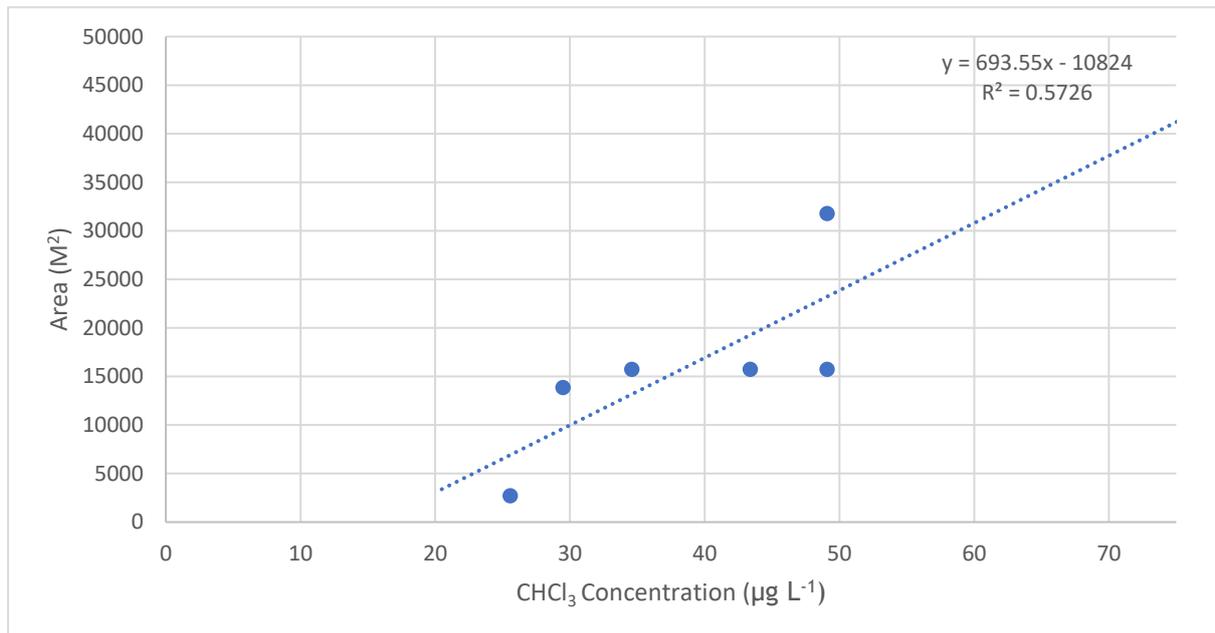


Figure 3b.5: Regression and line of best fit between CHCl<sub>3</sub> concentration (µg L<sup>-1</sup>) formed under chlorination, and fen marsh and swamp vegetation class, in the Hampshire Avon catchment.

Figure 3b.5 shows a regression and line of best fit between  $\text{CHCl}_3$  and fen, marsh and swamp vegetation class, in the Hampshire Avon catchment. An  $R^2$  of 0.57 shows a medium strength fit of data points to the line of best fit, which, coupled with correlation data in Table 3b.4, shows a strong positive correlation between the two data points ( $f=0.812$ ,  $p<0.05$ ), where, as area of fen marsh and swamp vegetation class increases, so does the  $\text{CHCl}_3$  concentration.

### 3b.8 Hampshire Avon and Conwy Catchment Data Combined

Figure 3b.6 shows a line of best fit through  $\text{CHBr}_2\text{Cl}$  concentration ( $\mu\text{g L}^{-1}$ ) and freely draining slightly acidic loamy soil area ( $\text{M}^2$ ) data, at the Hampshire Avon and Conwy site combined, under chlorination. An  $R^2$  of 0.74 shows a medium to strong fit of all data points to the line of best fit, which, compared to the correlation data in Table 3b.2, shows a medium strength relationship between the two factors, suggesting that, as freely draining slightly acidic loamy soil area increases, so does  $\text{CHBr}_2\text{Cl}$  concentration. Interestingly, the mean bromide concentration at all sites in the Hampshire Avon and Conwy catchment were recorded at  $0.79 \pm 0.35 \text{ mg L}^{-1}$ , which, when compared to other concentrations found between the two catchments (e.g. mid Wales, UK) are found at between 0 and  $55 \mu\text{g L}^{-1}$  (Neal *et al.*, 2007) appear very high, potentially suggesting why relationships are found between brominated THM compounds and the land use.

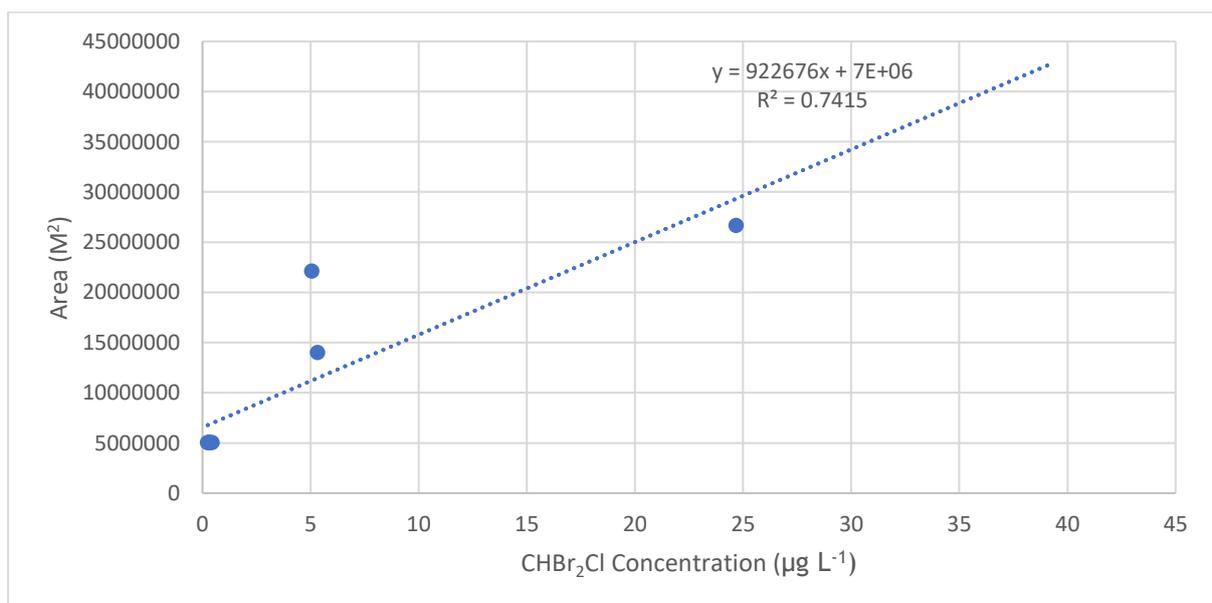


Figure 3b.6: Regression and line of best fit between  $\text{CHBr}_2\text{Cl}$  concentration ( $\mu\text{g L}^{-1}$ ) and freely draining acid loamy soil classification at the Hampshire Avon and Conwy sites under chlorination.

$\text{CHCl}_3$  concentration ( $\mu\text{g L}^{-1}$ ) and loamy and clayey floodplain soils with a naturally high groundwater soil classification area ( $\text{M}^2$ ), and a corresponding line of best fit are shown in Figure 3b.7. An  $R^2$  value of 0.2618 shows a weak to medium fit of data points to the line of best fit, which, coupled with the correlation data presented in Table 3b.4 ( $f=-0.686$ ,  $p<0.05$ ) shows a medium to strong negative correlation between the two datasets, where, as catchment area decreases,  $\text{CHCl}_3$  concentration increases.

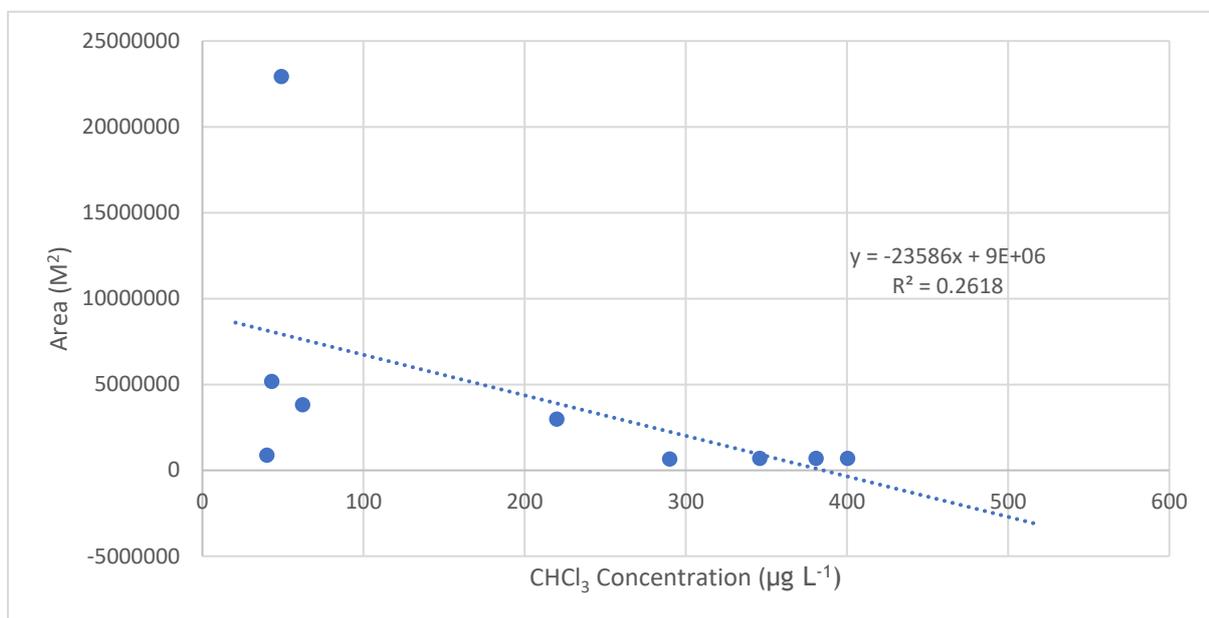


Figure 3b.7: Regression and line of best fit between  $\text{CHCl}_3$  (formed from chlorination, in  $\mu\text{g L}^{-1}$ ) and loamy and clayey floodplain soils with a naturally high groundwater, from data obtained at the Hampshire Avon and Conwy catchments, combined.

All data was explored in a correlation table, and all positive correlations were further explored. Figure 3b.8 shows regression between  $\text{CHBr}_2\text{Cl}$  and freely draining slightly acid loamy soils at both Hampshire Avon and Conwy sites, combined, under chlorination. An  $R^2$  of 0.05 shows a very poor fit between data points and the line of best fit, which, coupled with data displayed in Table 3b.4 shows a medium strength positive correlation between the two data points ( $f=0.533$ ,  $p<0.01$ ). The regression suggests that this is not an important correlation, as most of the data is clustered around approximately  $5 \mu\text{g L}^{-1}$ , regardless of area

of the land type. There are a couple of outliers at both low and high concentrations, which give rise to this positive correlation.

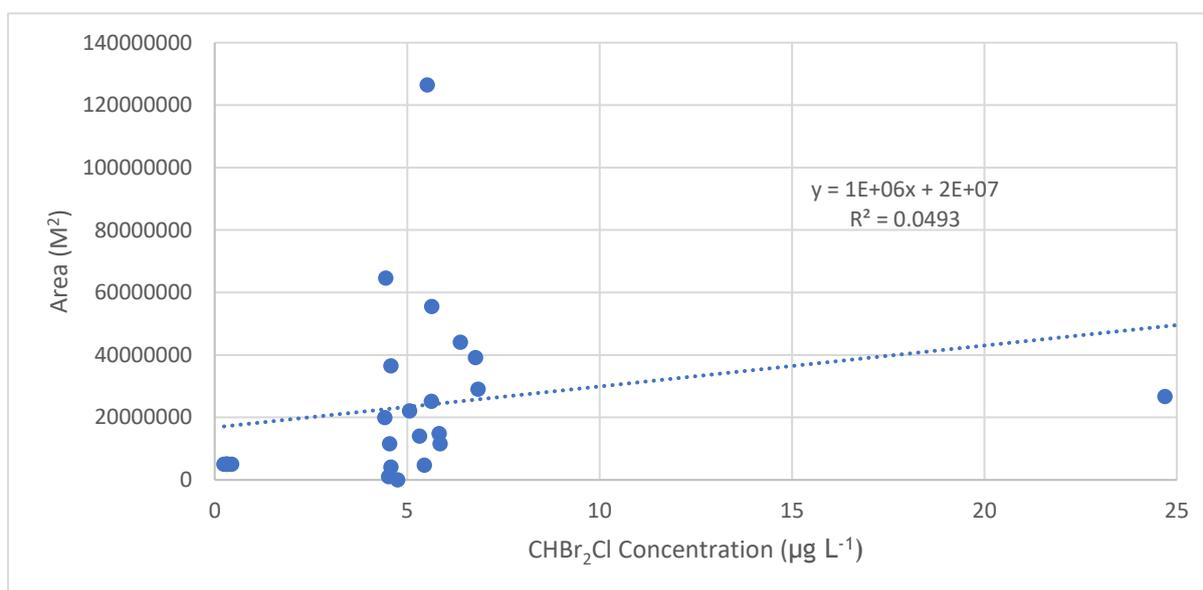


Figure 3b.8: Regression between CHBr<sub>2</sub>Cl concentration (µg L<sup>-1</sup>) and freely draining slightly acidic loamy soils soil classification, from data from both Hampshire Avon and Conwy sites.

### 3b.9 Visual Assessment of Risk

In order to further explain the relationships discovered, GIS was again utilised as a medium to display the areas of land use responsible for these relationships. A visual and geographical representation of the data provides a clearer image of what the issues are, and can be shown to a larger audience (e.g. not just scientists but also end users of the product) to help increase success of any mitigation measures that may be required to reduce the risk of the formation of these compounds in drinking water. The following maps display the major relationships determined from statistical analysis of the data presented in this chapter, highlighting the areas of land use that correlate with either a statistically significant increase or decrease in STHM<sub>4</sub> or individual THM<sub>4</sub> compound concentrations.

Farm Technologies Site - Positive Correlation with CHCl<sub>3</sub> concentration

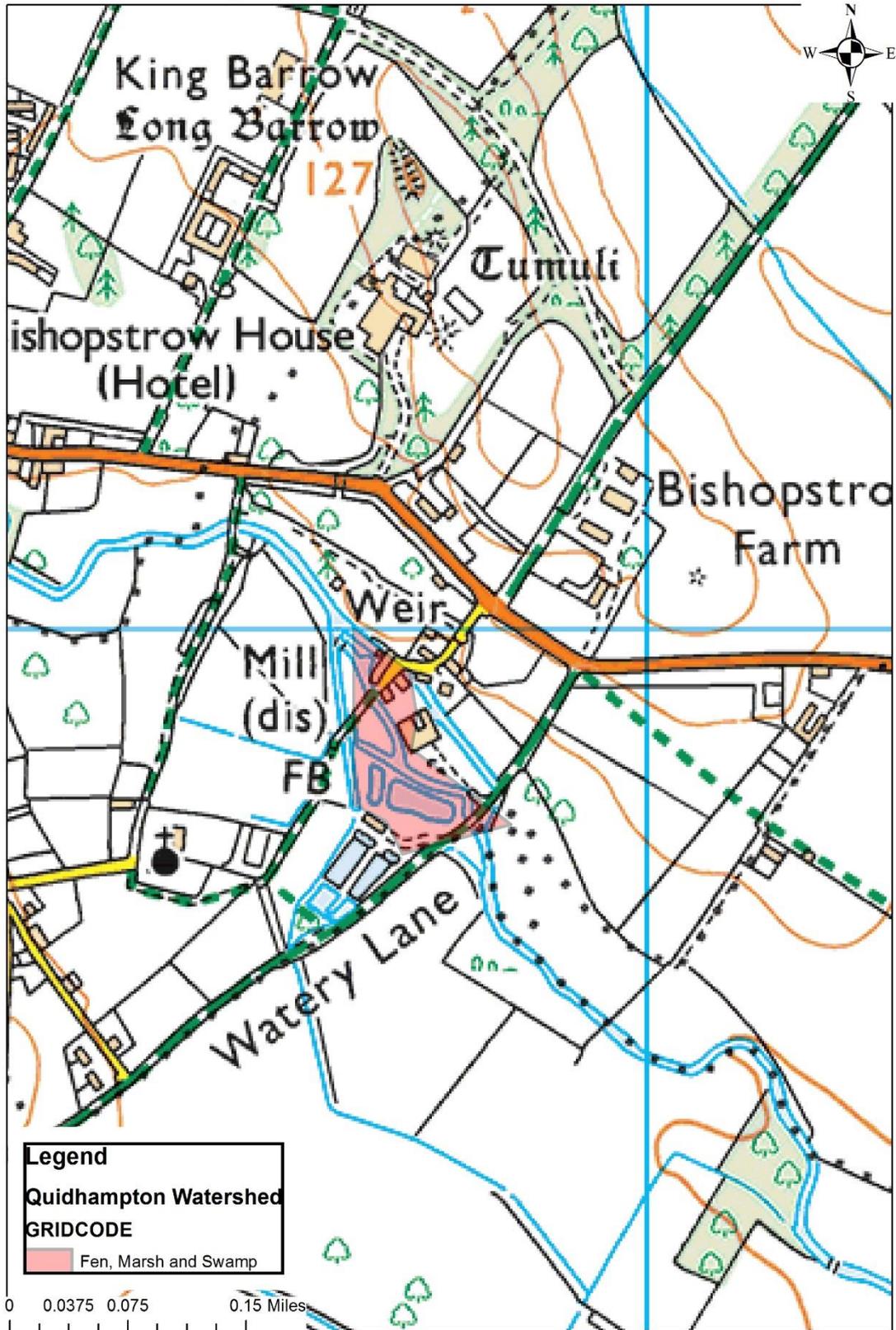
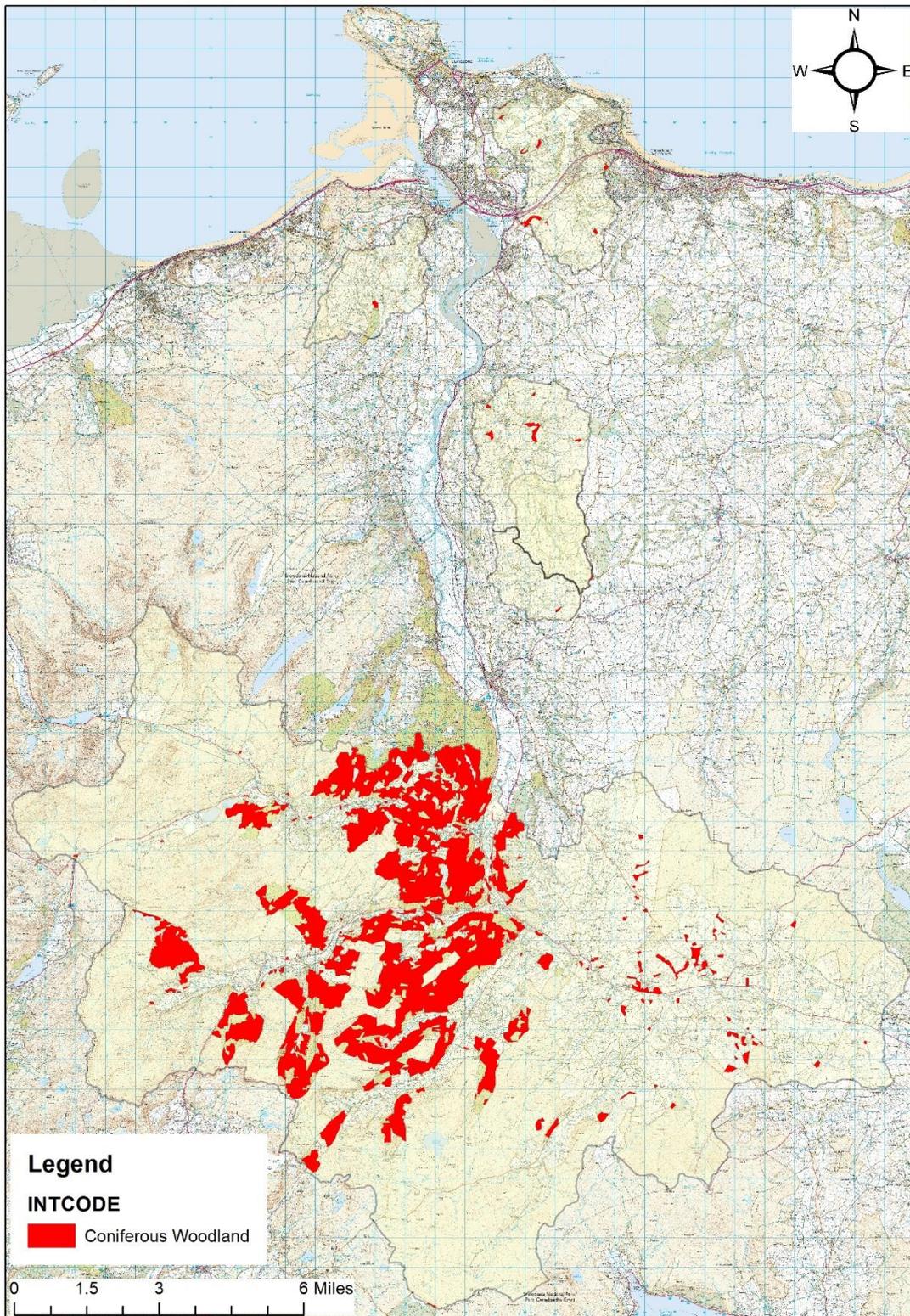


Figure 3b.9: The only occurrence of fen, marsh and swamp land use in the Hampshire Avon Catchment (in red), which correlates with chlorination formed CHCl<sub>3</sub> concentrations, in a laboratory experiment.

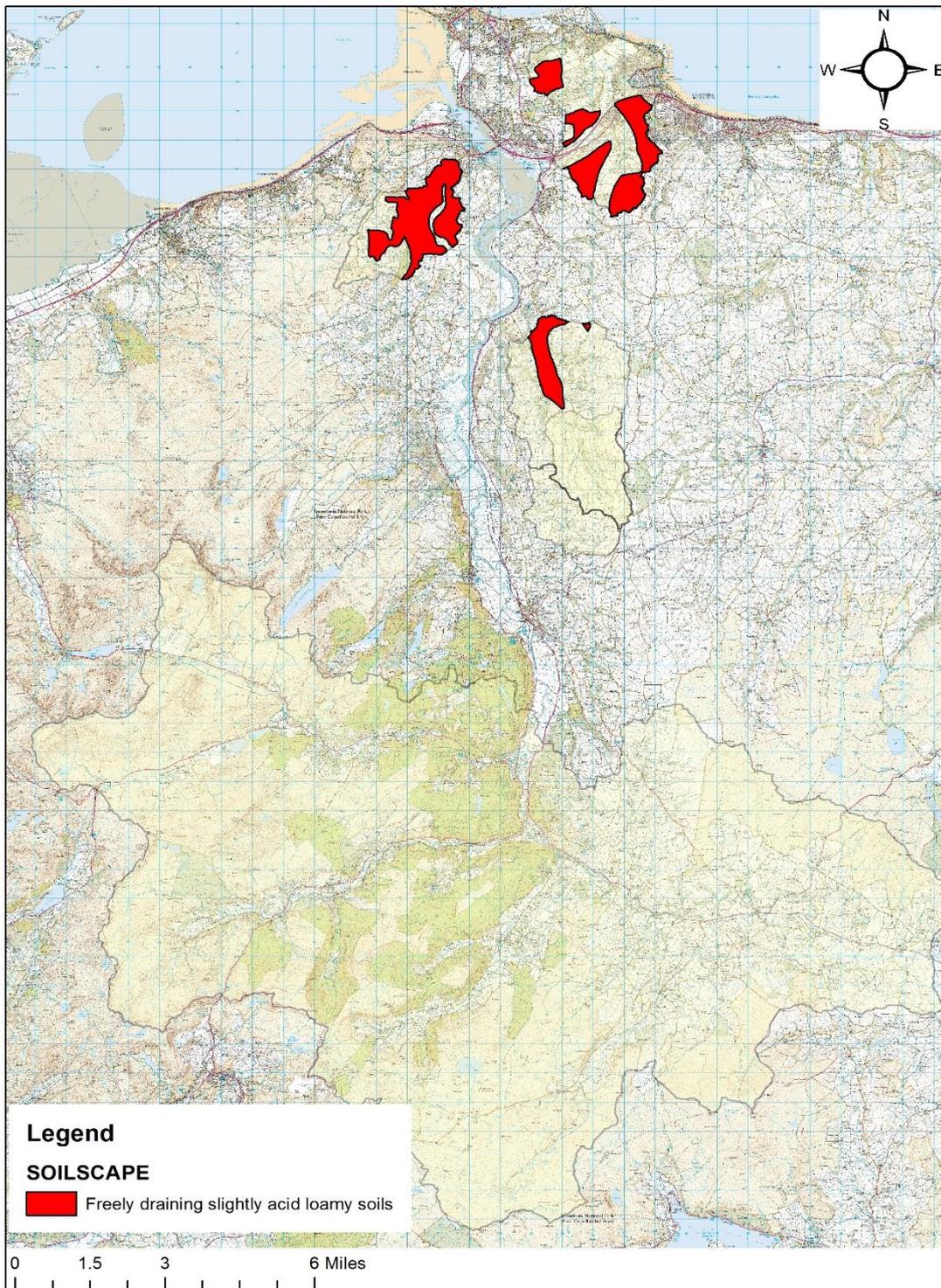
Conwy catchment land use which correlated significantly with CHCl<sub>3</sub> formation



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Figure 3b.10: Coniferous woodland occurrences in the Conwy catchment, which shows significant positive correlation with chlorination formed CHCl<sub>3</sub>, in a laboratory experiment (i.e. not actual CHCl<sub>3</sub> concentration at the catchment).

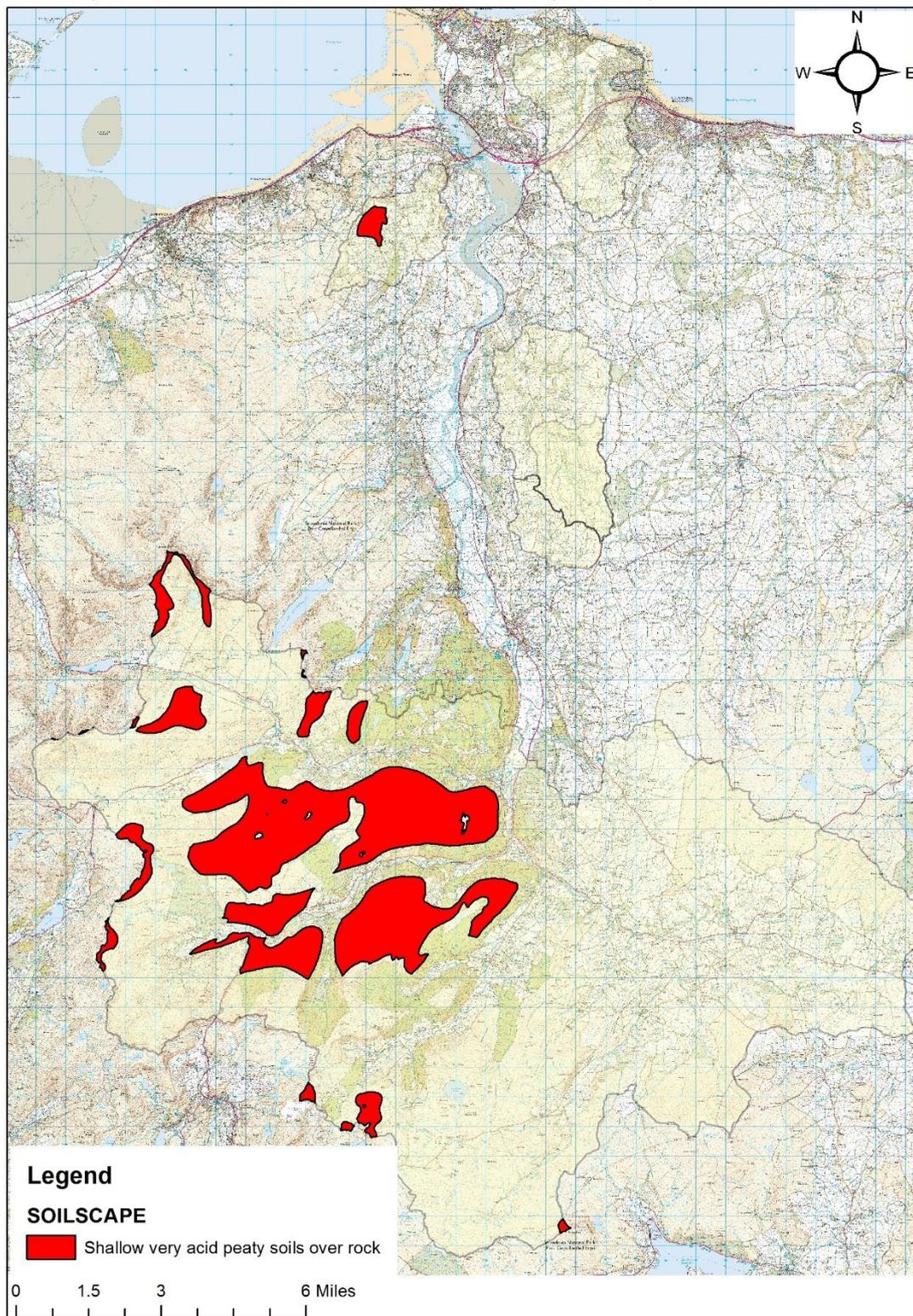
Conwy catchment land use which correlated significantly with  $\text{CHBr}_2\text{Cl}$  formation



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Figure 3b.11: Showing Conwy catchment soil type freely draining slightly acid loamy soils which correlates significantly with  $\text{CHBr}_2\text{Cl}$  formation from chlorination treatments, in a laboratory experiment.

Conwy catchment land use which correlated significantly with CHCl<sub>3</sub> formation



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Figure 3b.12: Conwy soil type (shallow very acid peaty soils over rock) which correlated significantly with chloramination formed CHCl<sub>3</sub> in the Conwy catchment, in a laboratory experiment.

Avon Catchment and Soil Types with Significant Correlations with  $\text{CHBr}_2\text{Cl}$  and  $\text{CHCl}_3$

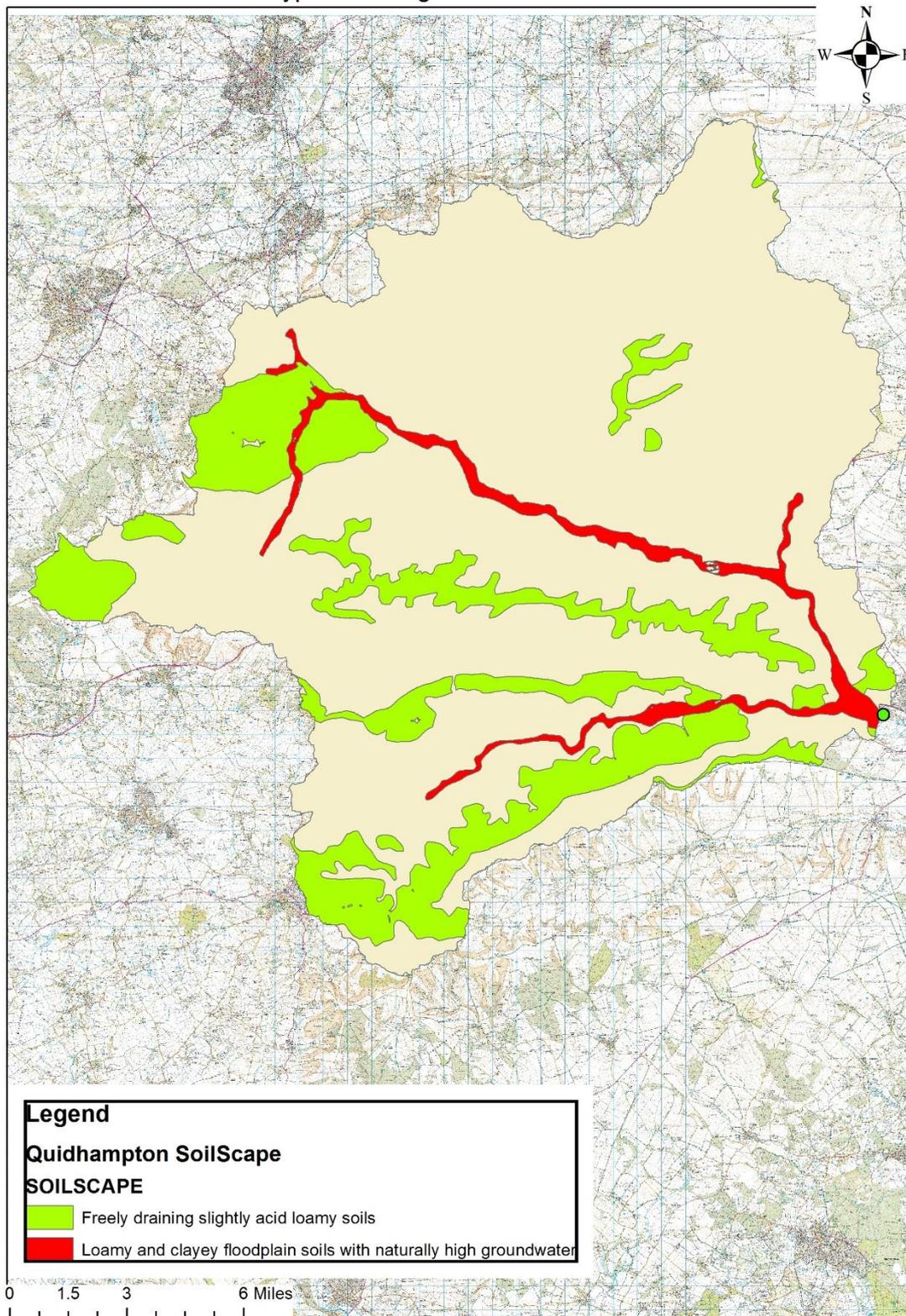


Figure 3b.13: Soil types which correlated significantly with  $\text{THM}_4$  data from both the Hampshire Avon and Conwy catchments under chlorination, presented here at the Avon catchment, in a laboratory experiment.

Conwy catchment land use which correlated significantly with CHBr<sub>2</sub>Cl formation

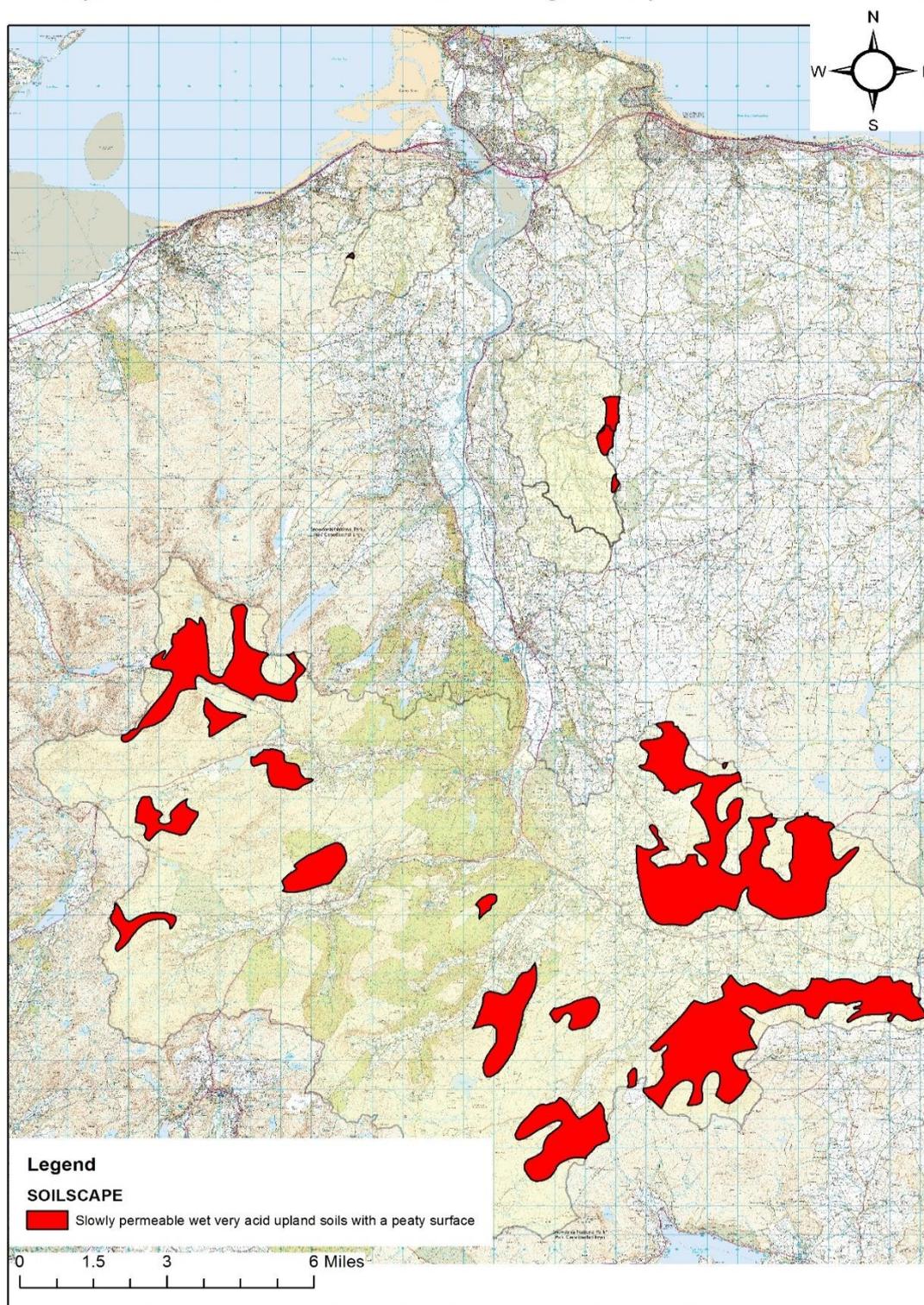


Figure 3b.14: Combined THM<sub>4</sub> data from Avon and Conwy and the soil type 'slowly permeable wet very acid upland soils with a peaty surface' which significantly correlated with CHBr<sub>2</sub>Cl, in a laboratory experiment.

Figure 3b.10 displays, in red, the land use in the Conwy catchment classed as coniferous woodland. It is apparent that the western half of the catchment headlands contains the vast majority of this land use class, although there are small instances in the east of the headlands, and also in the lower catchments, in much lower quantities. The correlation between freely draining slightly acidic loamy soils and  $\text{CHBr}_2\text{Cl}$  is displayed in Figure 3b.11. The occurrences of this land use are found in the lowland reaches of the Conwy catchment, below the tidal influence zone up the river. There are no instances of this land use type in the higher reaches of the Conwy catchment and therefore any abstraction activities that want to reduce the possibility of  $\text{CHBr}_2\text{Cl}$  in their final product would be best situated in the headwaters, although this is commonplace for many abstraction locations already due to pollution issues etc, however, in practice, the treatment company would just change their treatment and disinfection practices to help reduce the occurrence of these DBPs. The shallow very acid peaty soils over rock soil type was found to exist mainly in the western headwaters of the catchment, in the Migneint bog. These areas were found to correlate strongly and positively with the concentration of  $\text{CHCl}_3$  formed when the water was chloraminated.

Relationships between freely draining slightly acid loamy soils, and loamy and clayey floodplain soils with naturally high groundwater were found to correlate with  $\text{CHBr}_2\text{Cl}$  and  $\text{CHCl}_3$  in the Hampshire Avon catchment are displayed in Figure 3b.13. It is clear that the instance of loamy and clayey floodplain soils with naturally high groundwater follows the course of the two main rivers draining this catchment, the Wylde and the Nadder.

The land classed as slowly permeable wet very acid upland soils with a peaty surface was found to correlate significantly with  $\text{CHBr}_2\text{Cl}$  in the Conwy catchment. The instances of this class of land use are primarily in the headlands of the catchment above the tidal influence, and typically appear at the far edges of the catchment, and thus it is likely that low  $\text{CHBr}_2\text{Cl}$  forming abstraction locations in this catchment are sparse.

### 3b.10 Discussion

Spearman's and Pearson's correlation tests were deemed the most suitable tests for statistical analysis for this application as they are relatively robust and can be used to produce graphs to further aid the visual impact of the GIS maps.

The regression data between  $\text{CHCl}_3$  (formed from chloramination) and shallow very peaty acid soils over rock (as displayed in Figure 3b.2), displays a  $R^2$  value of 0.5545. This is a positive trend, with the trend line explaining 55.45 % of the variability of the response of the data around its mean. Under the chloramination treatment, the shallow very acid peaty soils over rock soil class significantly correlated positively with  $\text{CHCl}_3$  concentration ( $f=0.739$ ,  $p<0.05$ ), with a very strong effect ( $d=1.98$ ). This soil classification is known to have high carbon concentrations, and it is also known that overgrazing can damage the vegetation and lead to erosion of the peaty surface (LandIS, 2017). It is proposed that the high carbon content and propensity for damage of the peaty surface that lead this soil type to correlate with chloramination formed  $\text{CHCl}_3$ , it is proposed. Upland peat damage has been widely catalogued, and Clay *et al.*, (2012) state that gully erosion and water incision into the peat can damage the vegetation and hydrology, and can lead to increases in carbon loss and sediment transfer downstream, further suggesting that gullies represent a hotspot of carbon loss. It could also be possible that the pore water from the very wet peat is running between the peat and the bedrock, and thus eroding the peat from underneath, bringing organic matter out into the rivers and streams draining this land class.

The positive relationship between coniferous woodland and  $\text{CHCl}_3$  (formed during chlorination of freshwater draining from coniferous woodland, shown in Figure 3b.3) data can likely be attributed to the high DOC concentrations found to be associated with this land use type. For example, Fröberg *et al.*, (2007) found that fresh coniferous leaf litter contributed to  $176 \text{ mg L}^{-1}$  DOC, which dropped to  $5 \text{ mg L}^{-1}$  DOC at the end of a period of 5 months (Fröberg *et al.*, 2007), thus suggesting that DOC inputs from coniferous woodland can be expected to be highest in autumn, when leaf litter is falling, or in woodland where forestry activities are taking place. Statistical analysis, in these current works, of the effect of coniferous woodland dominated land has revealed that coniferous woodland has a large effect upon the concentration of  $\text{CHCl}_3$  in water draining from it ( $d=5.36$ ), whereas all THM4 compounds combined show a much smaller, yet still significant effect, with coniferous woodland ( $d=0.82$ ). Many UK reservoirs are surrounded by coniferous woodland and are owned in joint partnerships between the water company and local woodland management firms and therefore a joint approach to catchment management would be essential in helping to reduce this potential source of carcinogenic precursors to the drinking water. The map in Figure 3b.10 can therefore act as a risk assessment tool for water abstraction companies, identifying areas containing land uses that have been found to form higher  $\text{CHCl}_3$ , allowing

the water abstraction companies to make informed decisions as to where is most cost and health effective to abstract clean water for an ever increasing population. Other considerations would be taken into account, though, such as the pH of the water, the proximity to other treatment works and the population the water would be destined for, the organic matter content, and the access to/from the treatment works, for example.

Shallow very acid peaty soils over rock soil type correlates with  $\text{CHCl}_3$ , and this soil type is found mainly in the western half of the catchment, with only a couple of small instances in the north and south of the catchment. It would be interesting to examine the adjacent catchment (to the west) to determine whether this phenomenon is simply due to the fact that this soil type is found in this geographical location, or whether there is another factor influencing this relationship.

It would appear that switching treatments from chlorination to chloramination in this catchment would produce higher concentrations of  $\text{CHCl}_3$  than a conventional chlorination treatment (as no significant relationship between chlorination formed  $\text{CHCl}_3$  and this soil type was found), if the treatment was applied to raw waters. The data presented in Figure 3.13 shows that loamy and clayey floodplain soils with naturally high groundwater soil type appears to follow the main course of the two rivers in the Avon catchment, suggesting that rivers that flow over this soil type could all correlate negatively with  $\text{CHCl}_3$  concentration. Finally, Figure 3b.14 shows the land use class (slowly permeable wet very acid upland soils with a peaty surface), found to correlate with  $\text{CHBr}_2\text{Cl}$  formation. These land uses appear to be found in the higher reaches of the catchment, which typically contain more humic DOC. There are two possibilities for an increased  $\text{CHBr}_2\text{Cl}$  concentration: either increased total DOC, meaning that during competitive halogenation, the chlorine is used up preferentially, but the bromide is then left to react with the residual DOC, or, this humic DOC reacts preferentially with bromide. The distribution of this specific land use appears to be sporadic and therefore more difficult to isolate, if  $\text{CHBr}_2\text{Cl}$  was found to be problematic in this catchment, when the water was disinfected for drinking.

Figure 3b.4 shows the highest  $R^2$  value for all regression analysis, between  $\text{CHBr}_2\text{Cl}$  concentration ( $\mu\text{g L}^{-1}$ ) and freely draining acid loamy soil classification at the Hampshire Avon and Conwy sites under chlorination. There is a clear positive regression between the two data sets, and all data points are within close proximity of the trend line, resulting in an  $R^2$  value of 0.74. A Spearman's rank of  $f=0.843$  ( $p<0.01$ ) shows that there is a significant

relationship between these two parameters. These data can be used to predict  $\text{CHBr}_2\text{Cl}$  concentrations from larger or smaller areas of the land use class in other catchments with this soil type. This soil class, although having a low carbon concentration, is known to contribute to nutrient enrichment from soil erosion (LandIS, 2017), and it could be the accumulation of bromine flushed from these eroded soils that are contributing to the formation of  $\text{CHBr}_2\text{Cl}$ , both at Conwy sites, and also at the Hampshire Avon and Conwy sites combined, suggesting that this potential accumulation of bromine is possible across a geographic and nutrient gradient. Interestingly however, the mean bromide concentration from the entire Hampshire Avon catchment is  $0.05 \pm 0.003 \text{ mg L}^{-1}$ , thus seemingly eliminating any significant bromide inputs from the catchment which could be driving these relationships, however, bromide concentrations from nearby catchments have been recorded at much lower concentrations (Neal *et al.*, 2007).

In contrast to the above, Figure 3b.7 shows regression analysis of  $\text{CHCl}_3$  formed under chlorination, with loamy and clayey floodplain soils with a naturally high groundwater. The data points are quite varied in their spread, with an obvious outlier, however, further statistical analysis showed the correlation between these two groups to be statistically significant. A Spearman's rank test showed a medium-high strength negative relationship  $f = -0.686$  ( $p < 0.05$ ). Both regression and correlation analysis show a negative regression, suggesting that, as the area of this land class decreases,  $\text{CHCl}_3$  concentration increases. These soils are primarily located within close proximity to the river (see Figure 3b.13), and the negative correlation between  $\text{CHCl}_3$  and this land class could suggest that loamy and clayey floodplain soils with naturally high groundwater (which drain to local ground waters feeding into rivers) are acting as a buffer zone, where through-flow or overland-flow is intercepted and ions (such as chloride) or DOM is trapped and utilised or stored rather than passing into the water body (LandIs, 2017).

Figure 3b.5 shows a strong positive regression analysis between  $\text{CHCl}_3$  formed under chlorination at the Hampshire Avon, and the area of fen, marsh and swamp land class. There is a medium to high strength positive trend between these two datasets, and there is a relatively good fit to trend line, ( $R^2 = 0.57$ ) when compared to other data presented. A strong positive Spearman's correlation ( $f = 0.812$ ,  $p < 0.05$ ) show that there is a strong relationship between the two data points, suggesting that, as the area of fen, marsh and swamp land class increases, so will the concentration of  $\text{CHCl}_3$ . The Cohen's  $d$  effect size of these data was classed as 'huge' ( $d = 2.42$ ) (see Table 3b.1 and Table 3b.3). It was interesting, however, to

note that there was only one instance of this land classification within the catchment, and a closer inspection of this (see Figure 3b.9) shows that the land seems to consist of man-made holding tanks for the waste water/effluent from a nearby company, which supplies dairy hygiene chemical products, many of which contain chlorine and iodine. The statistical analysis presented here, therefore, could not be highlighting a relationship between fen, swamp and marsh classified land, but possibly instead an identification of the potential source of  $\text{CHCl}_3$  in river water – the 6 sites that drain land including these man-made storage tanks all had significantly higher  $\text{CHCl}_3$  than other sites. Furthermore, the mean DOC concentration at this site was recorded as  $5.23 \pm 0.21 \text{ mg L}^{-1}$ , which, when compared to the mean DOC concentration for the Hampshire Avon catchment which is  $7.69 \pm 0.89 \text{ mg L}^{-1}$ , suggesting that the DOC at this site is not excessive for the catchment, which could be driving the  $\text{CHCl}_3$  concentration measured here.

Figures 3b.4 and 3b.8 show regression analysis of  $\text{CHBr}_2\text{Cl}$  and slowly permeable very wet acid upland soils with a peaty surface, between both Hampshire Avon and Conwy data, and also  $\text{CHBr}_2\text{Cl}$  and freely draining slightly acid loamy soils, at Conwy and Hampshire Avon too. Despite both of these graphs displaying weak regressions ( $R^2 = 0.1271$  and  $R^2 = 0.0493$ , accordingly), the data was still found to correlate significantly with each other.  $\text{CHBr}_2\text{Cl}$  and slowly permeable very wet acid upland soils with a peaty surface showed a negative Spearman's correlation of  $f = -0.504$  ( $p < 0.05$ ), whereas  $\text{CHBr}_2\text{Cl}$  and freely draining slightly acid loamy soils correlated with a Spearman's correlation of  $f = 0.533$  ( $p < 0.01$ ). Other correlations were found between standardised  $\text{CHCl}_3$  and loamy and clayey floodplain soils with naturally high groundwater, with a Spearman's correlation of  $-0.686$ ,  $p < 0.05$  and a regression of  $R^2 = 0.26$ , showing that, as the area of this land use increases, the standardised  $\text{CHCl}_3$  concentration decreases. There were no significant correlations found between  $\text{CHBr}_3$  and  $\text{CHCl}_2\text{Br}$  and any land uses.

A data set containing a wider range of areas and concentrations of these measured parameters may help determine whether there is a discernible trend or whether the current data is only a small snapshot of an overall non-significant relationship. These soils are dominated by moorland and rough grazing, and forestry, with high carbon content. Gripping or over grazing, particularly in winter, can accelerate runoff and erosion, and it is likely that this is a driver for these relationships.

### 3b.11 Conclusions

The findings from the present work suggest land use classes, from both vegetation and soil types, can contribute towards the formation of both THM<sub>4</sub> as a group of compounds, but also individual compounds under two different treatments. This data will provide useful information for utility companies to identify potentially problematic areas for water abstraction, taking into account individual THM compounds as well as TTHM<sub>4</sub>, providing a deeper understanding of the propensity of the organic matter to form THM<sub>4</sub>, contained within the water draining these catchments. The knowledge of which land use has a higher propensity for the formation of these DBPs will help direct water companies to abstract from the best suited locations to create a cleaner, more cost effective product for consumers. However, an inherent problem exists – the majority of current UK drinking water reservoirs are surrounded by coniferous woodland plantations, a factor which this study has found to significantly increase the CHCl<sub>3</sub> concentration in waters draining it. It is difficult to suggest remediation strategies however, as other land uses can cause other potential issues for the water quality, and the act of deforestation and changing the land use will likely cause further and more immediate problems for the quality of the water. A larger sampling regime, from across the United Kingdom, which balances the number of samples against the seasonal variation, could help produce a definitive database of THM<sub>4</sub>. Concentrations and land use practices, and further statistical analysis of these data could help pinpoint land use practices that have the smallest impact upon the quality of water draining the catchments, in terms of its qualities as drinking water. However, cost implications for this sort of project will be significant. Data generated can also be useful to determine the implication of changing from a chlorination treatment to a chloramination treatment. Further analysis should take into account the impact that rainfall has upon the flow of organic matter from these catchments, as dilution from increased rainfall, or concentration, from reduced rainfall could considerably affect the results, although this effect was attempted to be reduced, by using mean data from 4 different times of the year.

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online at [http://www.who.int/water\\_sanitation\\_health/dwq/chemicals/THM200605.pdf](http://www.who.int/water_sanitation_health/dwq/chemicals/THM200605.pdf)  
on 18/05/17

# Chapter 4

## Characterisation of Dissolved Organic Matter from 3 UK catchments

## 4.1 Introduction

Three field sampling sites were selected in the three mainland constituents of the UK, England, Wales and Scotland, representing a nutrient, DOM and latitudinal gradient (see Figure 4.1 and Table 4.1), from the nutrient rich Hampshire Avon in the South of U.K. (England), the nutrient poor Conwy catchment in central U.K. (Wales), and a series of intermediate sites in terms of nutrient content in Scotland, northern U.K.

The Hampshire Avon catchment has a high nutrient load, due to intense agricultural activities within the catchments. Within the Hampshire Avon catchment, two lower order rivers were selected for analysis, the River Nadder and the River Wylye, which drain the higher reaches of the catchment and merge to form the River Avon, just west of the city of Salisbury (Figure 4.2). Both of these streams naturally produce water of different characters, but together, represent a lowland, nutrient rich catchment. The sub-catchment of the overall Hampshire Avon catchment studied here (679km<sup>2</sup>) is dominated by arable and horticultural land uses, with a mainly chalky bedrock resulting in shallow, lime rich soils. Along the Nadder and Wylye, there are 19 individual sampling points, situated at important sites along the rivers, such as downstream from septic tank outflows or waste water treatment works, or downstream from agricultural inputs.

Data collected and analysed in this chapter does not just include NPOC and TN, but also includes a suit of ionic compounds. These ion datasets, although not encompassed under the collection of dissolved organic material classed as 'DOM' as they are not organic, still play a role in the formation of DBPs. Calcium and magnesium are known to affect water hardness and alkalinity (Werner, Arnold and McNeill, 2006). However in a WTW, pH will be closely controlled during chlorination, so a significant influence of these ions upon DBPs is not anticipated. Chloride, bromide, fluoride (Chen, 2011) and the 3 nitrogenous ions have all been found to influence DBPFP (Han *et al.*, 2018). The advantage of ion chromatography is that it separates out all of the relevant ions to enable a wider suite to be measured in a single sample run.

The Conwy catchment sites were selected to represent a nutrient poor system. The source of the river Conwy, the Llyn Conwy reservoir is located in the Migneint, which is an upland moor spanning 200 km<sup>2</sup> (JNCC, 2018); the largest area of blanket bog in Wales. Along the reaches of the river Conwy are 28 individual sampling sites, selected to represent different catchments in terms of vegetation and land use (bog, montane, urban *etc.*). However, as the

lower reaches of the Conwy are estuarine and tidal (with spring tides reaching up to the town of Llanrwst) the last sampling point of the main river is situated some 23 km upstream from the sea to avoid tidal influences (although some sub catchments are still sampled from nearer the sea, but with no tidal influences). Therefore, the total area of all of catchments studied is 416 km<sup>2</sup> (Figure 4.3). Despite these catchments having many varying land uses, improved grassland is the most common, the soil is typically freely draining, acidic and loamy soils over predominantly mudstone, siltstone and sandstone geology.

Thirteen catchments were also selected for study in Scotland, chosen to represent intermediate nutrient status but also to compare to the Avon and Conwy sites, to confirm whether or not the data given from these locations was site-specific, or whether the data represented a coherent pattern for that land use type, nationwide. These 13 sites drain a total catchment area of 101 km<sup>2</sup> (although the majority are located in separate catchments to each other), and are located in south-western Scotland. 50 individual sample sites were located in these 13 catchments for basic analysis (Figure 4.4).

To further characterise these sites and to confirm the hypothesis that all three sites differ in terms of DOM character, all of the data obtained from these three catchments over a course of two years, was averaged. This was an important step in determining whether the data from these sites differed significantly from the other two sites.

A Geographic Information Systems (GIS) based method was adapted (detailed in Chapter 3) to obtain catchment area data about land use, bedrock and soil type for each sampling location in these 3 catchments; data that is related to the chemistry of the water that drains them. It also allowed analysis of point sources of DOM, for example, septic tanks, sewage treatment works etc. Table 4.1 contains a basic overview of catchment characteristics.

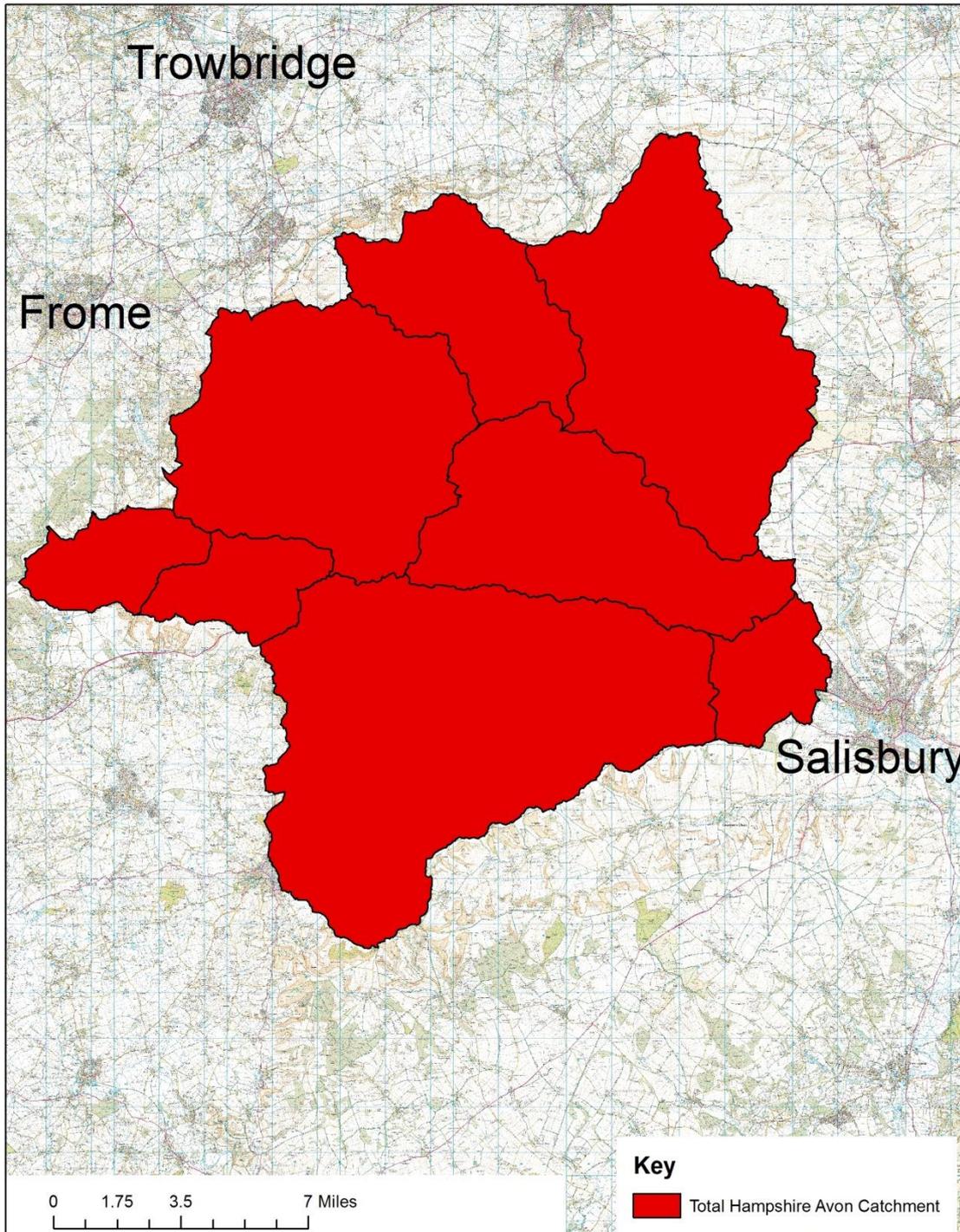
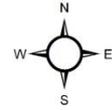
Table 4.1: Total catchment area, individual sample sites, and dominant bedrock, vegetation and soil type per major catchment.

<b>Site</b>	<b>Total Area (Km<sup>2</sup>)</b>	<b>Dominant Vegetation</b>	<b>Dominant Bedrock</b>	<b>Dominant Soil</b>	<b>Number of individual sites</b>
<b>Hampshire Avon</b>	678.81	Arable and Horticulture (42%)	Chalk (76%)	Shallow lime-rich soils over chalk or limestone (59%)	19
<b>Conwy</b>	416.48	Improved Grassland (23%)	Mudstone Siltstone and Sandstone (68%)	Freely draining acid loamy soils over rock (54%)	28
<b>Scotland</b>	101.32	Coniferous Woodland (31%)	Wacke (44%)	Blanket Peat (33%)	50



Figure 4.1: Approximate location of Avon, Conwy and Scottish sites within the U.K. Map orientated in a northerly direction.

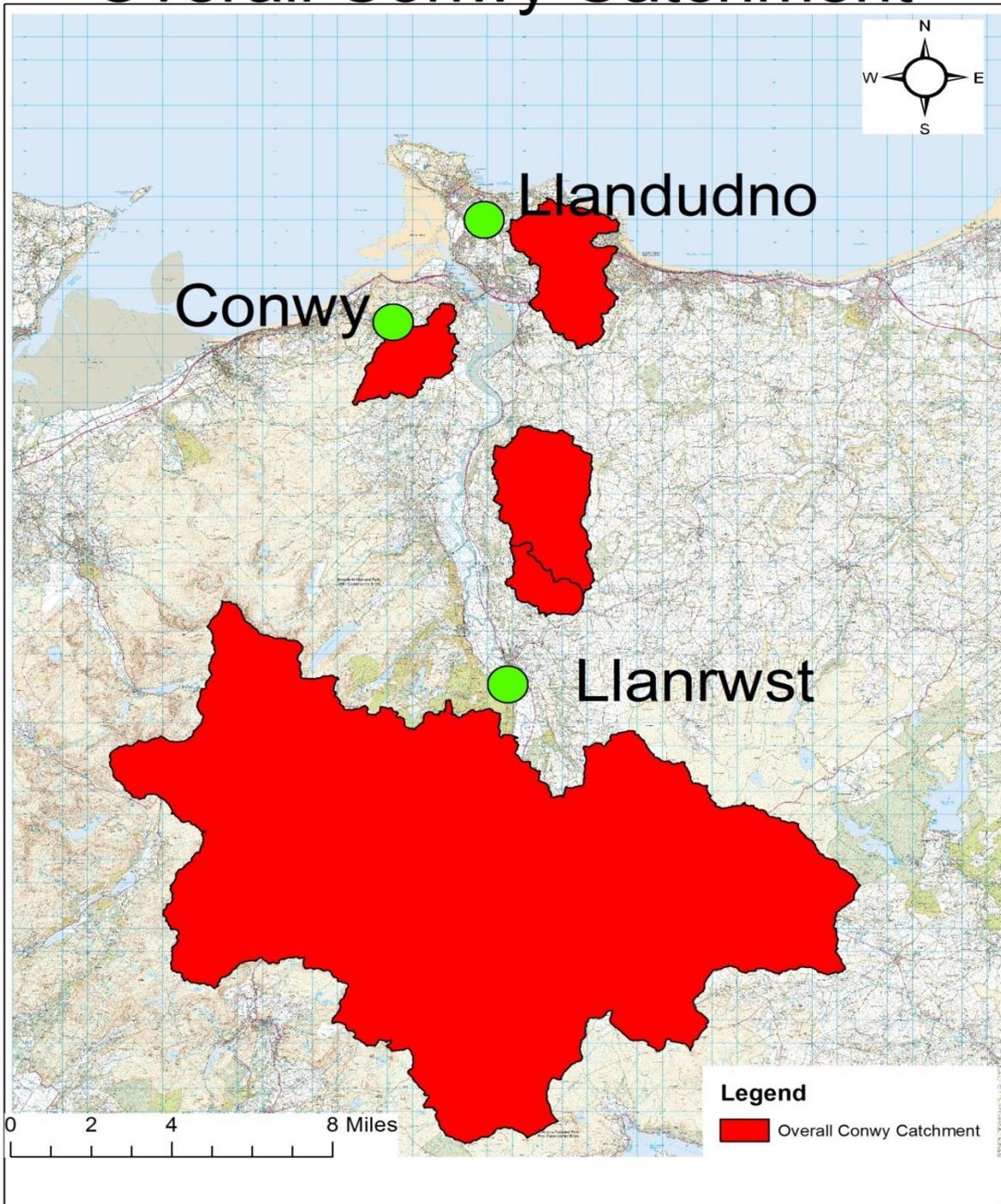
# Avon Catchment



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Figure 4.2: The Hampshire Avon catchment, extending west from the town of Salisbury, England.

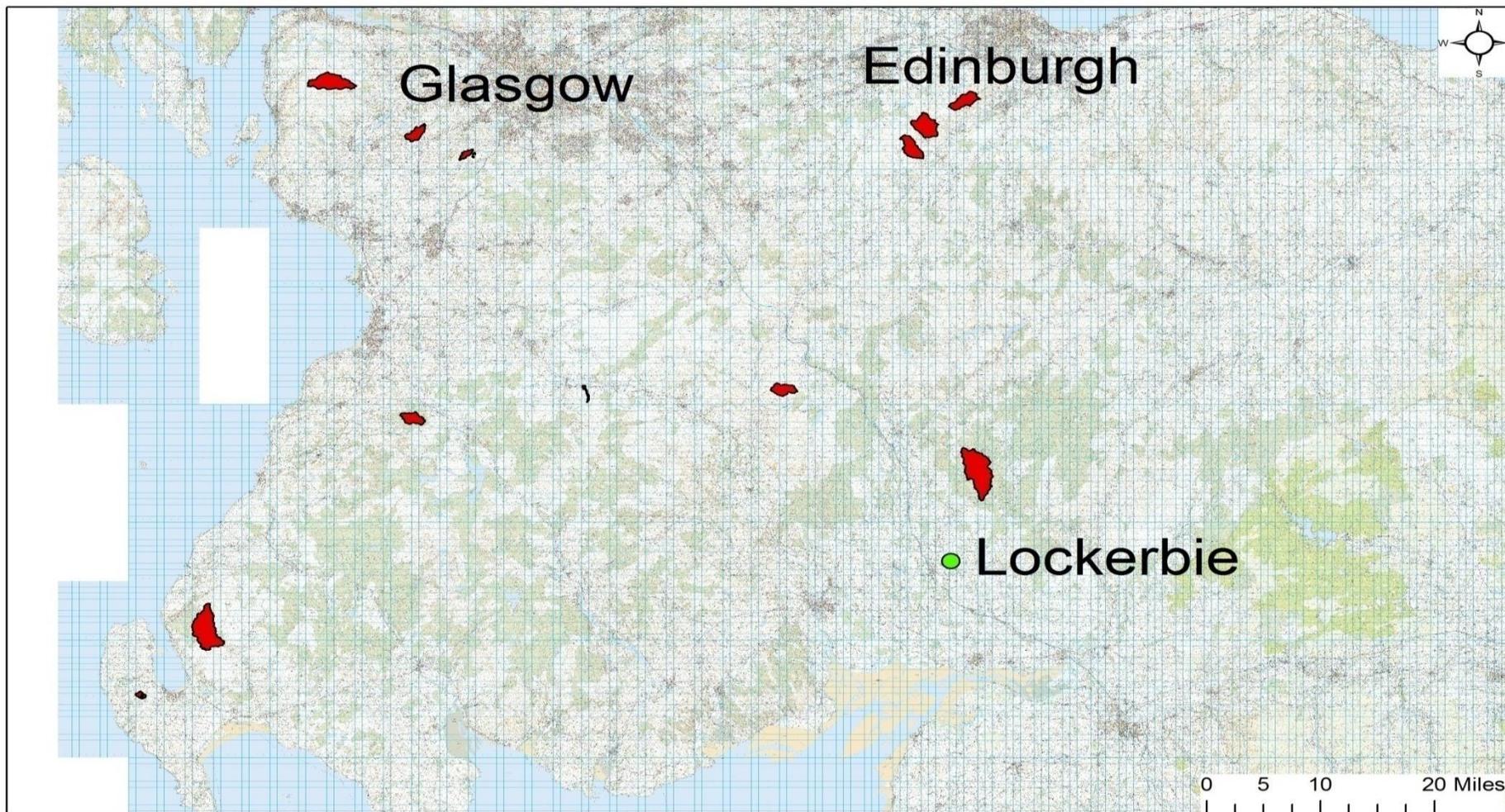
# Overall Conwy Catchment



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Figure 4.3: Conwy catchment, which extends southerly from the town of Conwy, Wales. Catchments are not contiguous to prevent tidal influence.

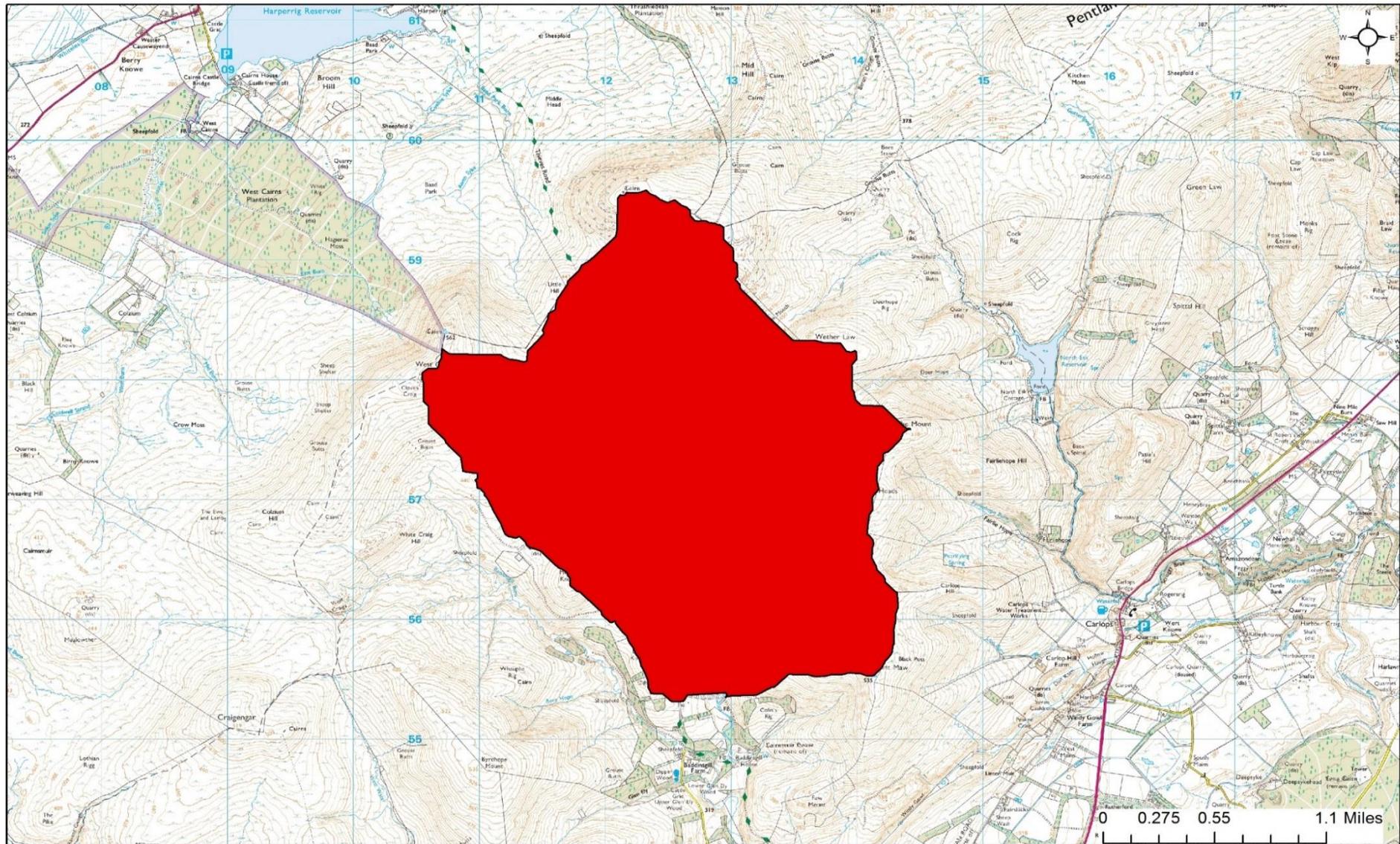
### Scottish Catchments



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Figure 4.4: Location of Scottish sites situated in south western Scotland, no far further north than the towns of Glasgow and Edinburgh, Scotland, and no far further south than the border with England – see Appendix 2, Table 1 for more detailed location information. Figure already displayed in Chapter 2, but shown again for clarity.

# Baddingsgill Reservoir Catchment



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Figure 4.5: Catchment overview of Baddingsgill Reservoir as an example of a Scottish site.

### 4.1.1: Hypothesis

It is hypothesised that the water draining from the three selected catchments will contain significantly different concentrations of organic matter despite any geophysical and vegetation similarities.

## 4.2: Methodology

Water samples were collected and prepared prior to storage, before ion data was generated by ion chromatography using a Metrohm 850 Professional IC and nitrogen and carbon data was achieved using an Analytik Jena Multi N/C, using the methods outlined in Chapter 2. Data was then statistically analysed using IBM SPSS software.

Sampling sites were chosen under the DOMAINE project, and their coordinate locations are displayed in Appendix 2.

## 4.3: Catchment Summary Data

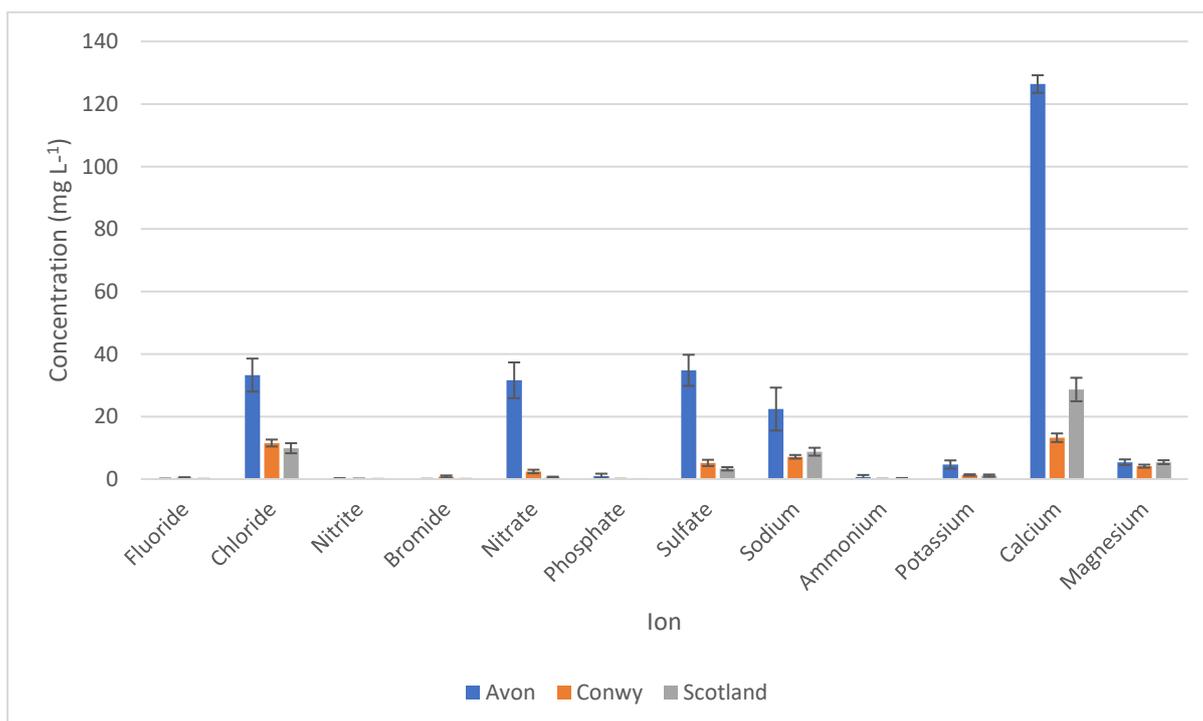


Figure 4.6: Mean ion concentrations from all sites in the Hampshire Avon, Conwy and Scottish catchments. Error bars represent standard error of mean (SEM)

Figure 4.6 shows that the Hampshire Avon catchment has a visibly higher concentration of chloride, nitrate, sulphate, sodium and calcium when compared to the Conwy and Scottish sites. The highest average ion concentration for all 3 catchments was calcium. The data was analysed statistically, and as data was not normally distributed, all datasets were transformed, which gave a skewness of between -0.004 to 1.763, except ammonium which gave a transformed skewness of 2.787. Due to the robustness of the test, an ANOVA statistical test was conducted which showed that there were significant differences between 7 ions between the sites at  $p < 0.01$ . A Tukey HSD post hoc test was therefore used to determine where these differences occurred. The results showed that there were no significant differences between the 3 sites in terms of fluoride, bromide, ammonium and magnesium. Chloride, sulphate, sodium, potassium, nitrate, phosphate and calcium were found to be significantly different at the Hampshire Avon when compared to the Conwy and Scottish sites ( $p < 0.01$ ). Furthermore, nitrite concentrations were significantly different between the Hampshire Avon and Scottish catchments ( $p < 0.05$ ). Further to these statistical analyses, Table 4.2 displays percentage differences of mean concentrations of each ion at each overall catchment, and it is quite clear that the Hampshire Avon has greater concentrations of 10 of the 12 ions detected when compared to the Conwy catchment and the mean concentrations of 12 of the 12 ions at the Hampshire Avon are greater than at the Scottish catchments.

Table 4.2: Percentage increase of ions between sites (brominated THMs were studied in Chapter 3 and links between concentration of bromine can be explored there).

	F <sup>-</sup>	Cl <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	Br <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Ca <sup>+</sup>	Mg <sub>2</sub> <sup>+</sup>
<b>% Increase at Hampshire Avon over Conwy catchments</b>		651	47		92	94	84	68	91	71	89	24
<b>% Increase at Hampshire Avon over Scottish catchments</b>	49	70	81	21	98	98	90	60	70	75	77	0
<b>% increase at Conwy over Hampshire Avon catchments</b>	80			94								

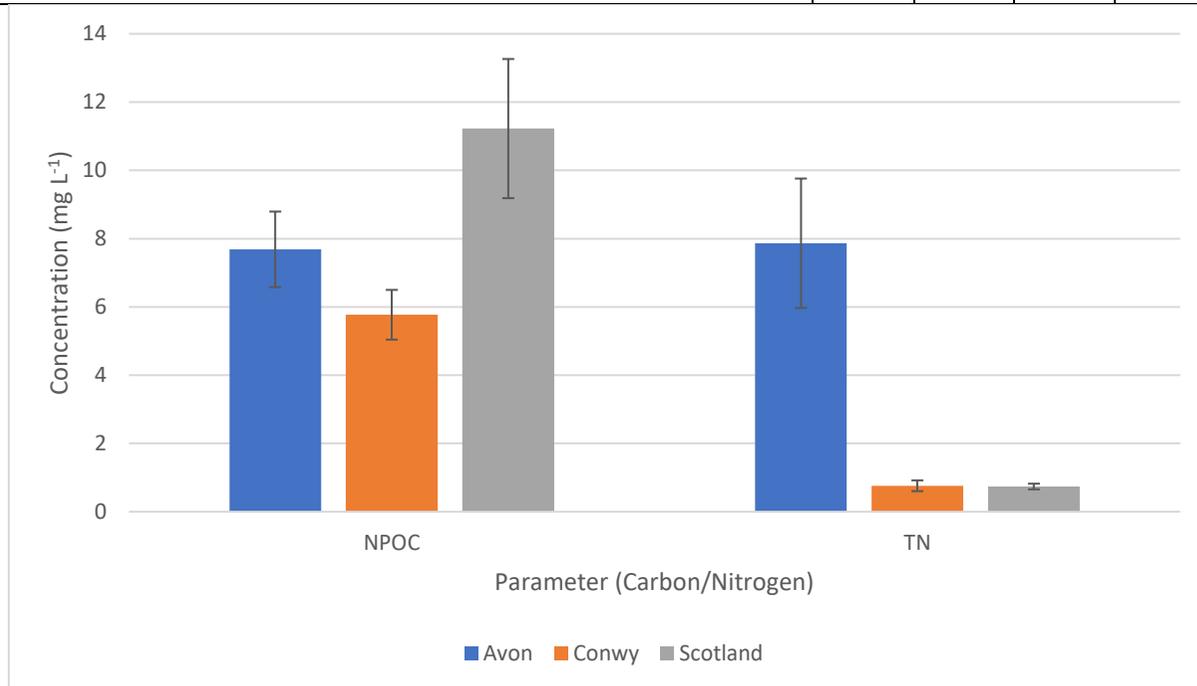


Figure 4.7: Mean non-purgeable organic carbon (NPOC) and total nitrogen (TN) concentrations for all Hampshire Avon, Conwy and Scottish sites. Error bars represent SEM (n= 38, 145 and 50 respectively for NPOC and 57, 108 and 50 respectively for TN).

Figure 4.7 shows that the Scottish catchments produced water significantly higher in NPOC than at Conwy and Hampshire Avon catchments. However, waters from the Hampshire Avon catchments produced a water significantly higher in TN than Conwy and Scotland sites. Statistical analysis showed that there were 38 NPOC data points for Hampshire Avon, 145 data points for Conwy catchment, and 50 data points for Scottish catchments, and 57 data points for Hampshire Avon, 108 for Conwy and 50 for Scottish catchments in terms of TN. The data was not normally distributed and so it was transformed to provide a skewness of -0.119 for NPOC and 0.394 for TN. A Levene's Statistic of 16.835 ( $p < 0.01$ ) for NPOC and 8.134 ( $p < 0.01$ ) for TN shows that the variability in the two transformed datasets are significantly different from each other. An ANOVA followed by a Tukey HSD posthoc test suggests that, for NPOC, Hampshire Avon sites are significantly different to Conwy and Scottish sites at  $p < 0.05$  and  $p < 0.01$  respectively. For TN, the Hampshire Avon catchment was found to be significantly different to Conwy and Scottish sites, again, both at  $p < 0.01$ .

A significance of  $p = 0.850$  between Hampshire Avon and Scotland sites shows that there is only a 15% chance that the difference in data between the two sites is statistically significant in terms of NPOC concentration, and  $p = 0.236$  for TN shows a 76.4% chance that the differences in TN concentrations between Conwy and Scottish sites are statistically significantly different. These large  $p$  values support the hypothesis that the Scottish sites were intermediate and thought to drain similar catchments to those studied at both the Conwy and Hampshire Avon catchments.

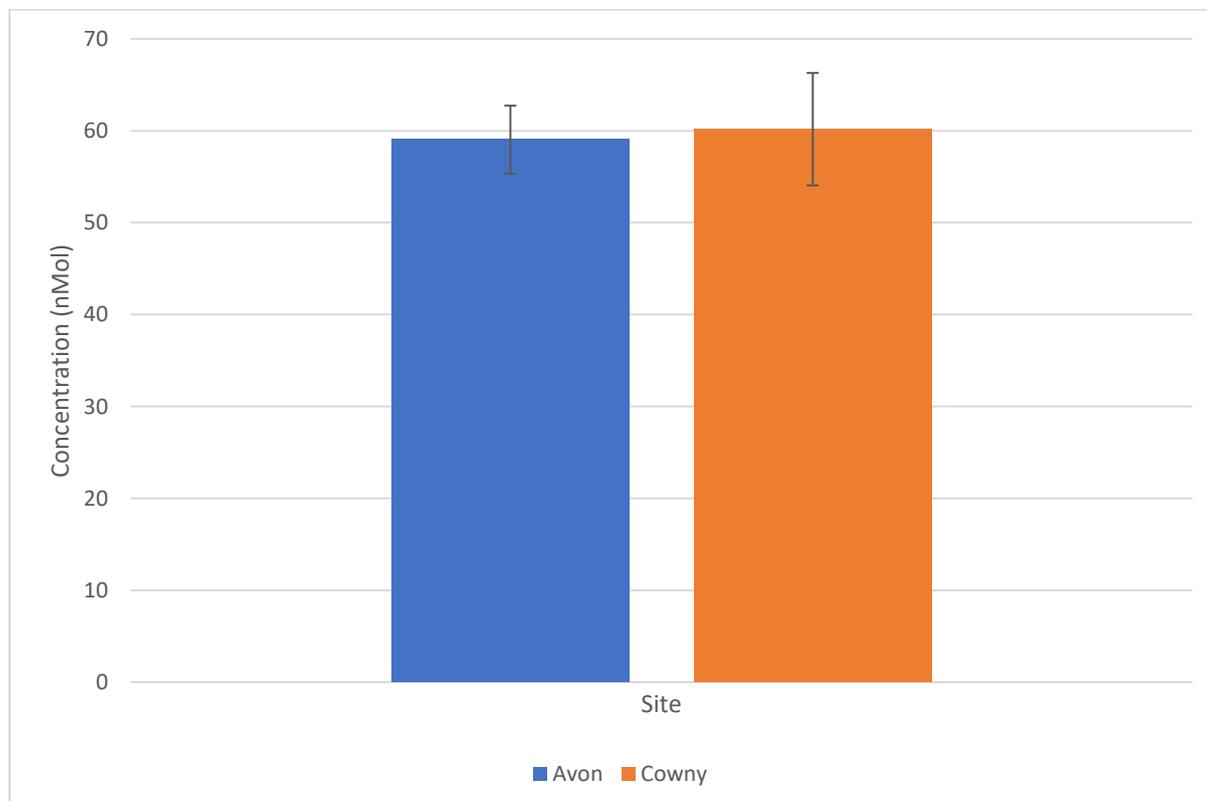


Figure 4.8: Total mean carbohydrate concentrations at all Hampshire Avon and Conwy sites. Error bars represent SEM (n= 38 at Avon and 136 at Conwy)

Figure 4.8 shows that the total mean carbohydrate concentration for the Hampshire Avon and Conwy catchments are very similar, although the Conwy catchment shows a slightly higher variance than the Hampshire Avon sites, as represented by the error bars. Scottish carbohydrate data was not determined, however, the dataset contained 38 data points from the Hampshire Avon (with a mean concentration of 59.04 nMol) and 136 data points from the Conwy catchment sites (with a mean concentration of 59.23 nMol).

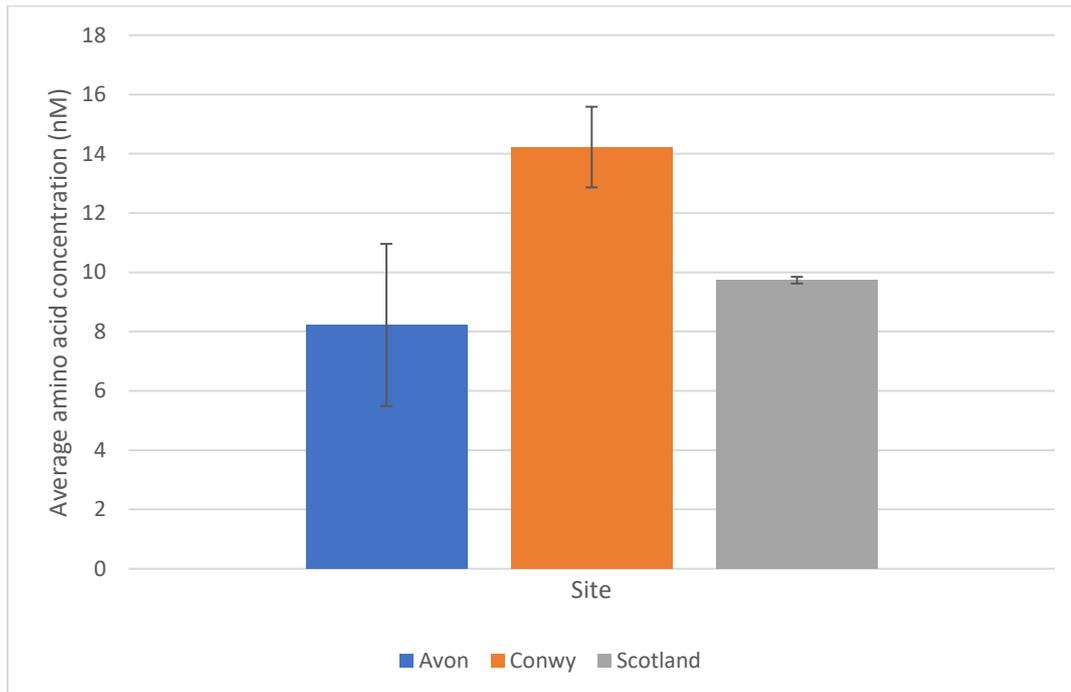


Figure 4.9: Total mean amino acid concentrations at the Hampshire Avon, Conwy and Scottish sites using OPAME method. Error bars represent SEM (n= 9, 27 and 48 respectively)

Figure 4.9 shows that Conwy has the highest total average amino acid concentration and Hampshire Avon has the lowest. However, the Hampshire Avon does present the highest variance which suggests that the sample sites in this catchment differ between each other more than the sites at the Hampshire Avon and Conwy. The Conwy catchment drains predominantly agricultural land which could explain the higher concentration here. The variation in land use at the Hampshire Avon could explain the variance in the mean concentration displayed in Figure 4.9 above. Overall however, concentrations are very low and the differences between catchments way not actually show anything apart from natural variation. The Hampshire Avon contained 9 data points with a mean concentration of 4.96 nM, the Conwy catchment contained 27 data points with a mean concentration of 0.0169 nM and the Scottish dataset was comprised of 47 data points with a mean concentration of 9.03 nM. The OPAME dataset was normally distributed (skewness of 0.373), and a Levene's statistic of <math><0.01</math> shows that there are considerable differences between

the variances of the 3 data sets, which violates the assumptions of the one way ANOVA test, and therefore a robust test of equality of means test (in this case, Welch and Brown Forsythe) was conducted, showing  $p < 0.01$  for both. Therefore, an ANOVA between the 3 sites showed  $p < 0.01$  signifying that there are significant differences between the 3 sites. A Tukey HSD post hoc test identified these differences, showing that all sites are significantly different to each other at the  $p < 0.01$  level, i.e. there is a 99% chance that the data recorded for all 3 sites are significantly different to each other in terms of OPAME concentration.

#### 4.4 Analysis of Sample Sites Within Catchments

##### 4.4.1 Hampshire Avon

##### 4.4.1.1 Ions

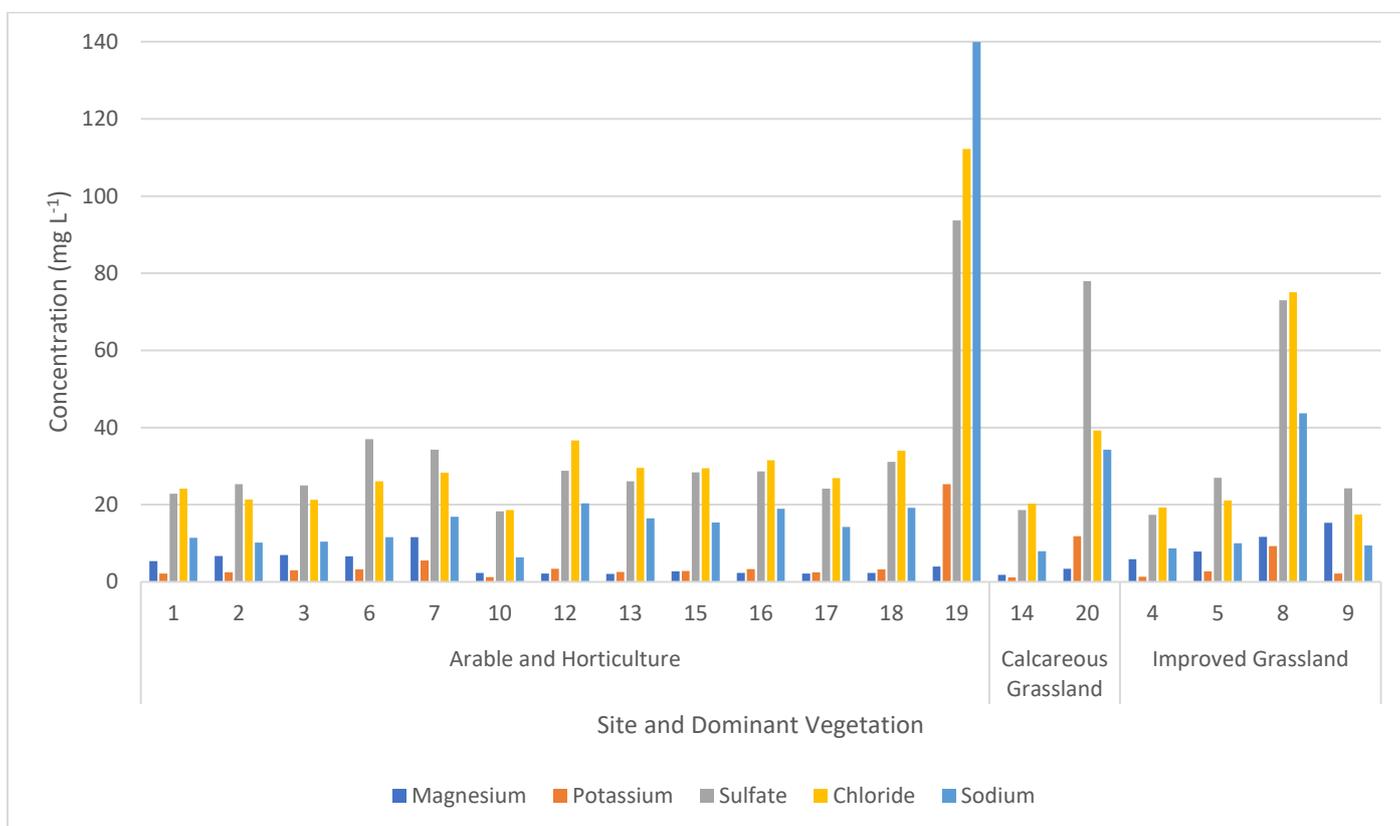


Figure 4.10 Part 1: Selected ions from sites at the Hampshire Avon catchment, classed into dominant land use types.

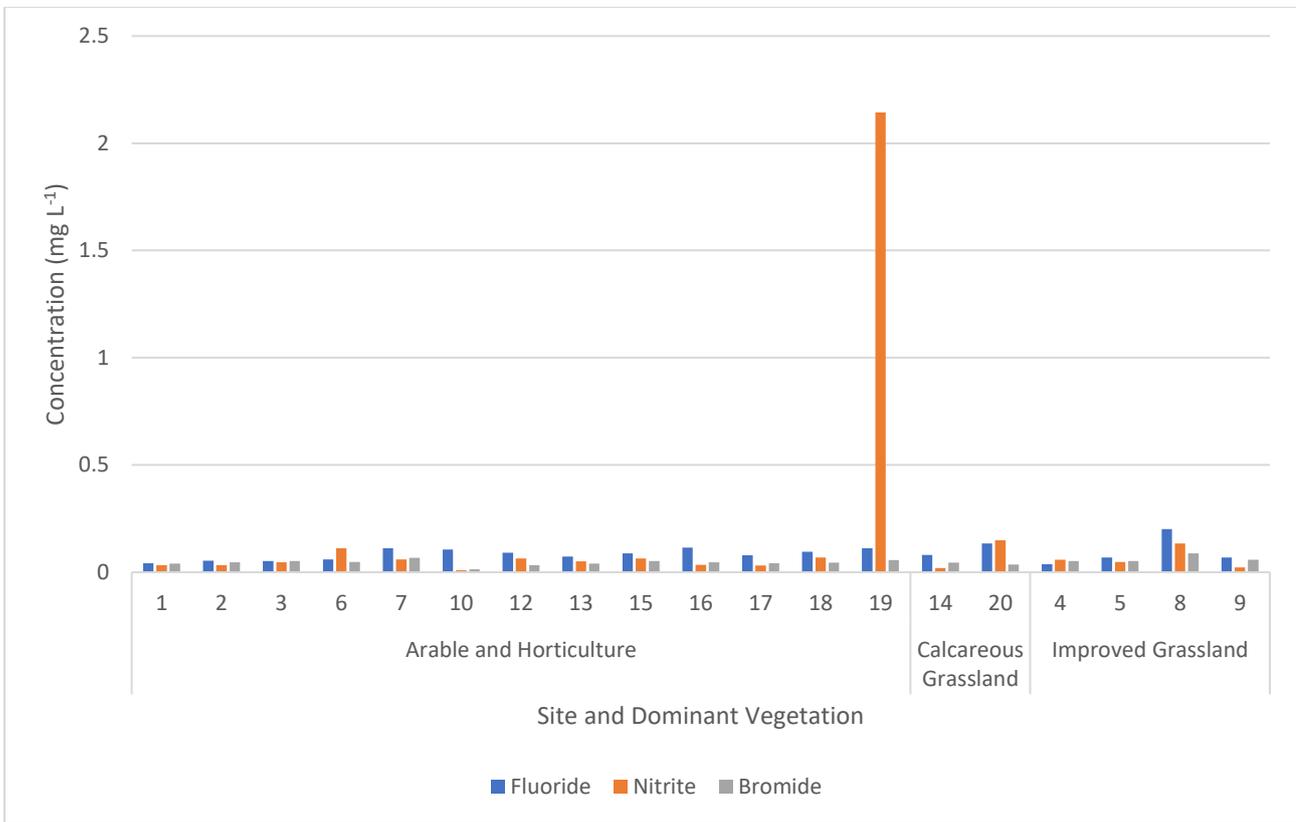


Figure 4.10 Part 2: Selected ion concentrations from sites at the Hampshire Avon catchment, classed into dominant land use types.

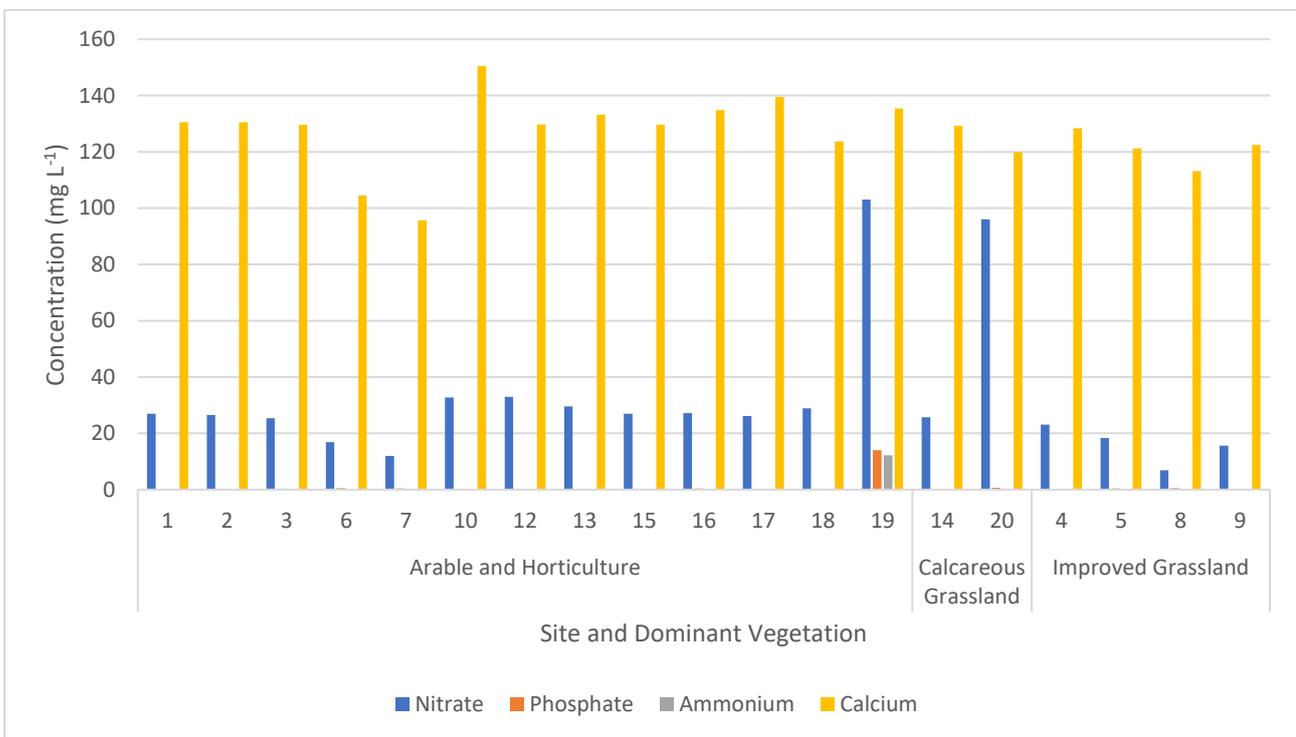


Figure 4.10 Part 3: Selected ion concentrations at sites in the Hampshire Avon catchment, classed by dominant land use type.

Ion data for the Hampshire Avon catchment (presented in Figures 4.10 parts 1-3) was analysed for skewness and all ions except calcium and bromide were found to exceed the +2.0 to -2.0 threshold for normal distribution. Therefore all data points were transformed by  $\text{Log}^{10}$  to create a dataset that contained a more acceptable mean skewness reading (with two outliers, ammonium and bromide). A Levene's test of homogeneity of variances showed that all data sets were significantly different except from nitrite (at  $p=0.06$ ), and an ANOVA showed that chloride, nitrite, nitrate, phosphate, sulphate, sodium, ammonium, potassium, calcium and magnesium had significant differences between the sites within the dataset. A Tukey post hoc test was selected, as equal variances could be assumed, and the significantly different sites were identified, see Table 4.3 and Appendix 2 (Tables 1 to 8).

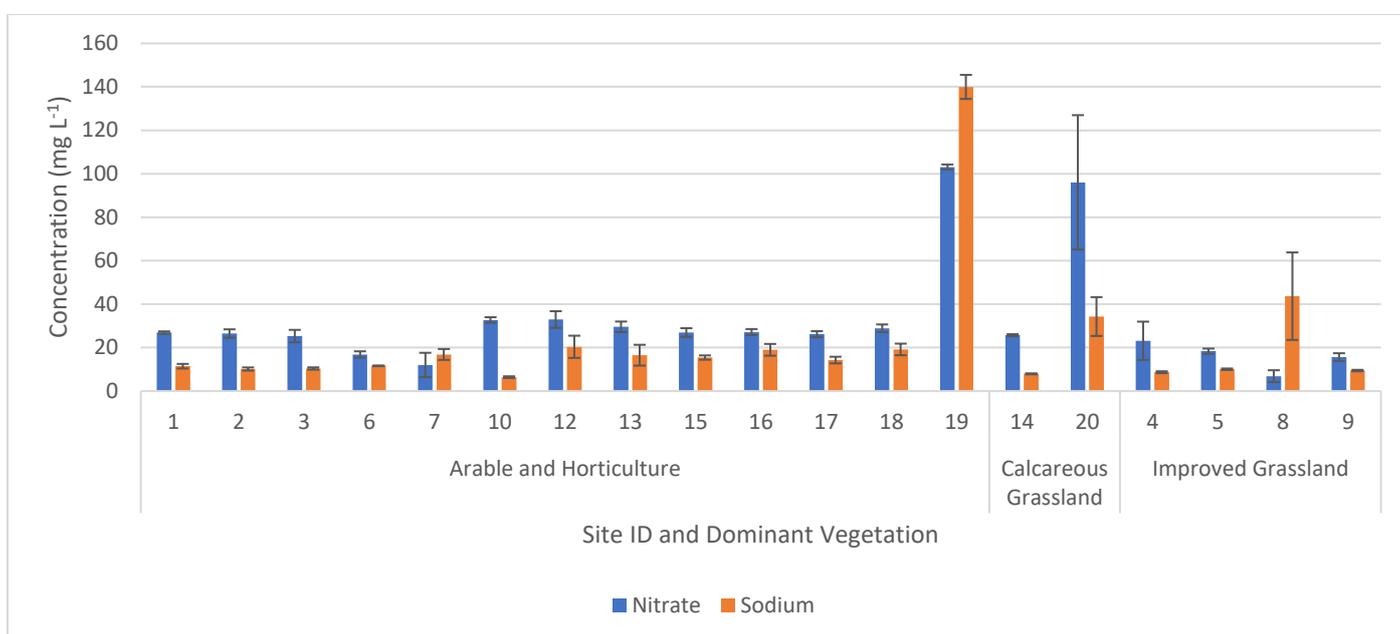


Figure 4.11: Total average nitrate and sodium concentration at Hampshire Avon sites. Error bars represent SEM.

Figure 4.11 shows total average nitrate and sodium concentrations at Hampshire Avon sites, sorted by dominant land use type. Data was not normally distributed, with many ions producing a skewness value of greater than +2 to -2, and was therefore transformed and the skewness was found to be much more acceptable. An ANOVA was conducted between the two ions and the land use types, and nitrate was the only ion found to be statistically significantly different between the land use types ( $f=4.024$ ,  $p<0.05$ ). A Tukey post hoc test was run which showed that the nitrate concentration in calcareous grassland and improved grassland are significantly different to each other (mean difference 0.532,  $p<0.05$ ).

Table 4.3: Tukey HSD results to show which sites significantly differ at the Hampshire Avon in terms of mean chloride concentration. Red represents  $p < 0.01$  green represents  $p < 0.05$ , values represent mean difference.

Chloride Hampshire Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1								0.383											0.660
2								0.438											0.716
3								0.439											0.716
4								0.480											0.757
5								0.443											0.720
6																			0.641
7																			0.605
8	- 0.383	- 0.438	- 0.439	- 0.480	- 0.442				- 0.527	- 0.498			- 0.462						
9								0.527											0.805
10								0.498											0.776

12																			0.484
13																			0.583
14								0.462											0.739
15																			0.576
16																			0.554
17																			0.614
18																			0.518
19	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	
	0.660	0.716	0.716	0.757	0.720	0.641	0.605		0.805	0.776	0.484	0.583	0.740	0.576	0.554	0.614	0.511		
20																			0.463

Bromide, Sulphate, Sodium, potassium, calcium and magnesium statistical data from the Hampshire Avon catchment can be seen in Appendix 2, Tables 1-8.

A Pearson’s correlation was conducted between ion and land use data from the Hampshire Avon, and very few significant correlations were found. In fact, nitrate was found to be the only ion that correlated with any land use type. These land use types were slightly acid loamy and clayey soils with impeded drainage at  $r=0.895$  ( $p<0.05$ ) and slowly permeable seasonally wet slightly acid but base rich loamy and clayey soils at  $r=0.796$ , ( $p<0.05$ ). For a regression plot of these two data points, see Figure 4.12.

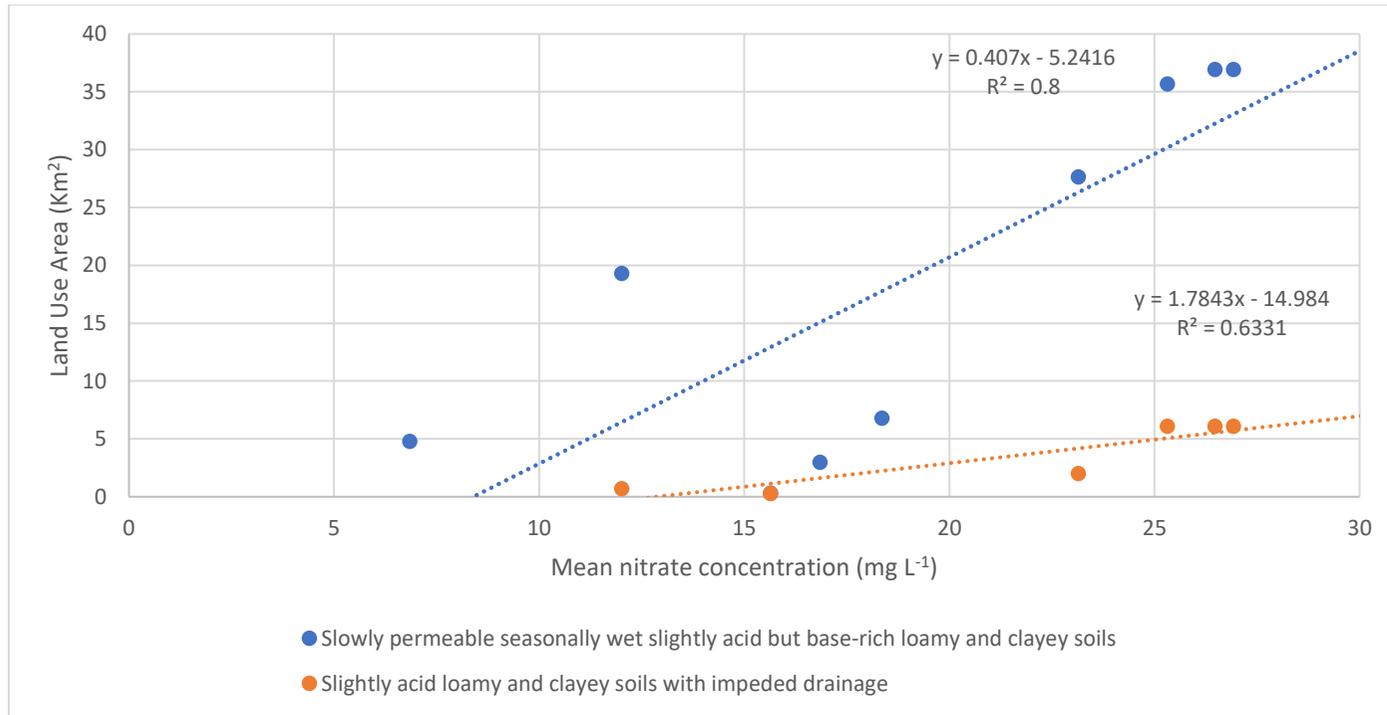


Figure 4.12: Relationship between nitrate concentration and slowly permeable seasonally wet slightly acid but base rich loamy and clays soils (blue) and slightly acid loamy and clayey soils with impeded drainage (orange) in the Hampshire Avon catchment.

An ANOVA test was run to compare the differences between ionic concentrations and dominant vegetation cover. Data was not normal so it was transformed ( $\log^{10}$ ) which gave much better normality but all ion concentrations were not between the recommended values of +2 and -2 for Skewness. The ANOVA test outlined which ions may have significant differences between dominant land use types, showing that nitrate ( $f=4.026$ ,  $p<0.05$ ) and magnesium ( $f=5.535$ ,  $p<0.05$ ) were likely to show significant differences. A Dunnett's T3 post hoc test was selected as it didn't assume equal variance. This Post Hoc test showed that magnesium was significantly different

between Arable and Horticulture dominated land and Improved Grassland at  $p < 0.05$  with a mean difference of 0.412, with Improved Grassland having a mean concentration of more than twice that of the Arable and Horticulture land cover magnesium concentration.

#### 4.4.1.2 Hampshire Avon TN and NPOC

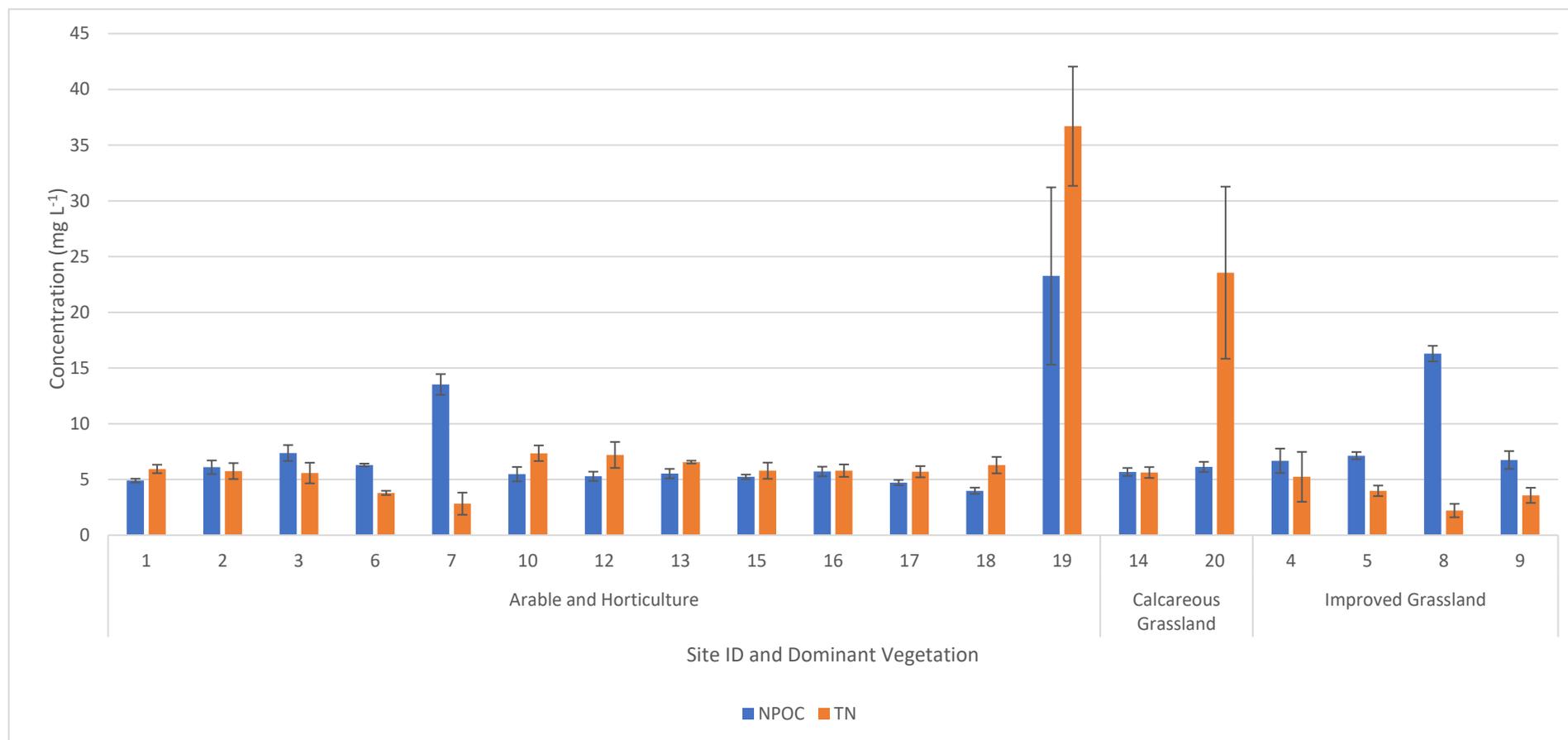


Figure 4.13: Mean concentration of TN and NPOC at Hampshire Avon sites, arranged by dominant land use type. Bars represent SEM.

TN and NPOC data for the Hampshire Avon (Figure 4.13) was found to be not normally distributed (Skewness of 2.850 for NPOC and 3.113 for TN) so the data was transformed by  $\log_{10}$ . The data was found to be much more normally distributed after transformation, showing a skewness of 1.237 for logged NPOC and 0.758 for logged TN. A Levene's statistic of  $>0.05$  was shown for log NPOC, therefore, robust tests of equality of means were carried out (Welch and Brown Forsythe) both showing  $p=<0.01$ . The Levene's statistic for log TN was  $<0.05$ . An ANOVA test was run, producing  $p=<0.01$  for both parameters showing that there are differences between the means of log NPOC and log TN data within the sites at the Hampshire Avon. As the Levene's test for log NPOC was  $p=>0.05$ , the assumption of homogeneity of variance had not been violated, although it had been violated for log TN. Therefore, a Tukey Post Hoc analysis on log NPOC was selected (as equal variances were assumed) and a Dunnett's T3 test was conducted, see Appendix 2 Table 9.

Table 4.4: Statistical analysis of NPOC data between sites at the Hampshire Avon catchment. Green represents  $p=<0.05$ , values represent mean difference.

TN	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
Avon																			
1	■																	0.782	
2		■																0.802	
3			■															0.821	
4				■															
5					■													0.961	
6						■						0.239						0.976	
7							■												
8								■											
9									■									1.017	
10										■								0.692	
12											■								
13							-0.239					■							
14													■					0.808	



#### 4.4.1.3 Hampshire Avon Landuse

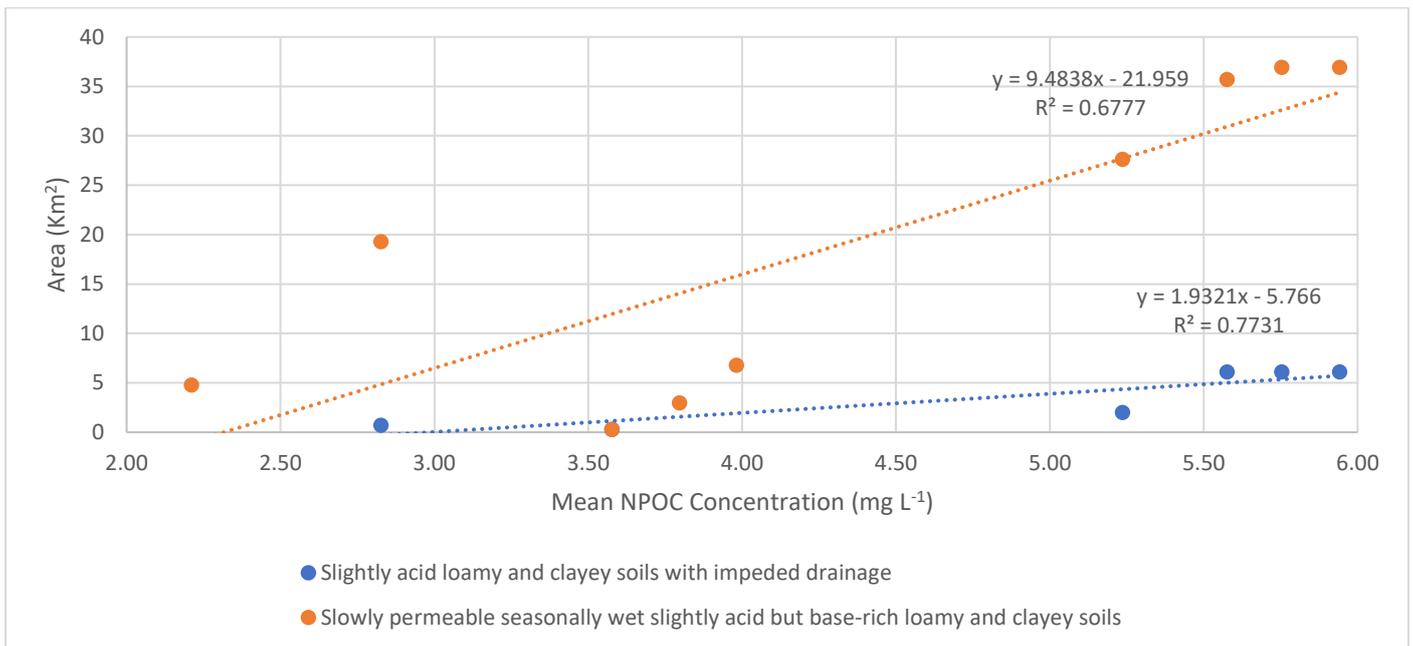


Figure 4.14: Positive correlations between mean NPOC concentration and the area of slightly acidic loamy and clayey soil with impeded drainage soil type data (blue) and slowly permeable seasonally wet slightly acidic but base rich loamy and clayey soils soil type data (orange) at the Hampshire Avon.

A Pearson's correlation statistical test was conducted between the catchment land use characteristics and the TN and NPOC concentration at the Hampshire Avon site. Whilst there were no correlations between TN and the land use, NPOC correlated strongly with slightly acidic loamy and clayey soil with impeded drainage at  $r=0.879$   $p<0.05$  and also slowly permeable seasonally wet slightly acidic but base rich loamy and clayey soils at  $r=0.823$   $p<0.01$  (Figure 4.14).

An ANOVA was run between the dominant land use types at the Hampshire Avon and TN and NPOC concentrations to see whether a specific dominant land use type produced a significantly different NPOC or TN concentration to other dominant land use types. However, no significant differences were found.

## 4.4.2 Conwy

### 4.4.2.1 Conwy Ions

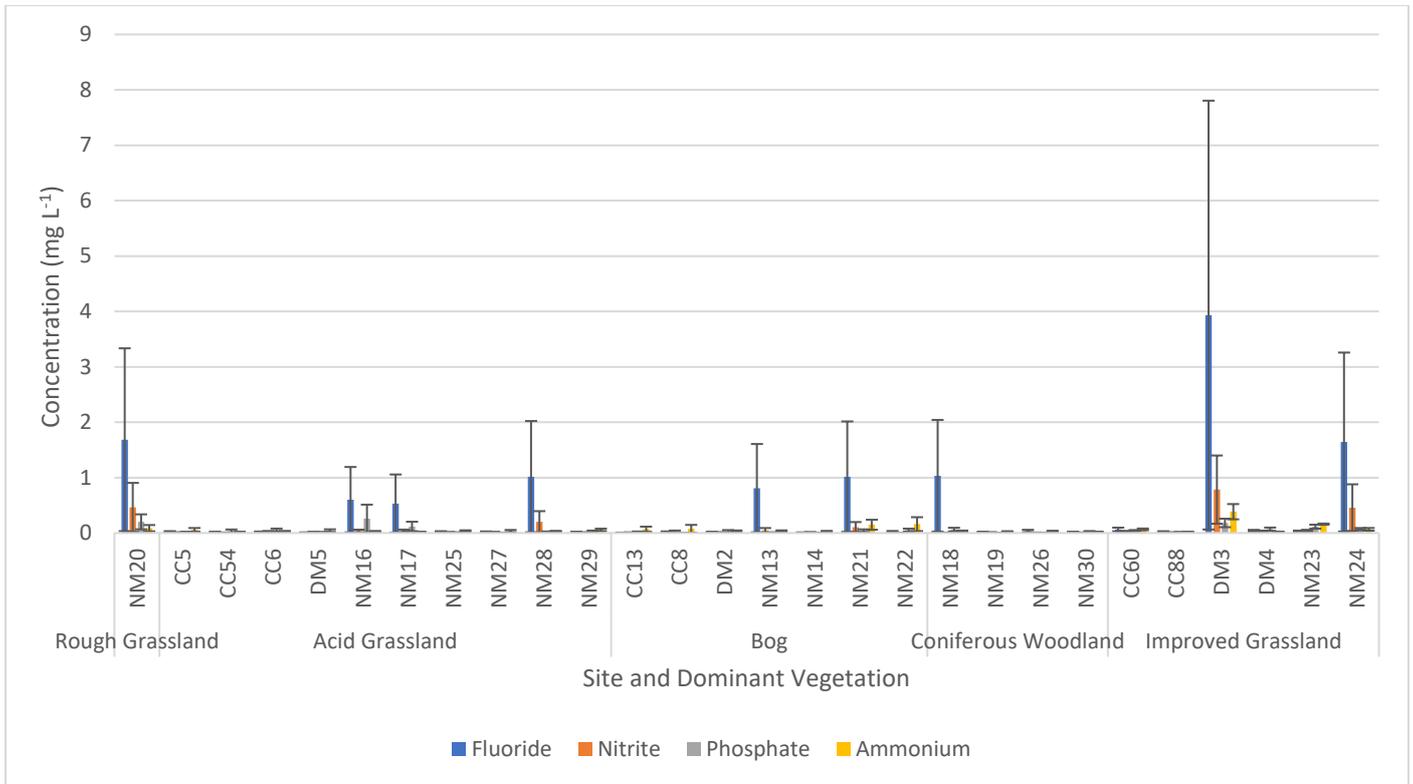


Figure 4.15 Part 1: Mean concentration of 4 ions at Conwy catchment organised by dominant land use type. Bars represent SEM.

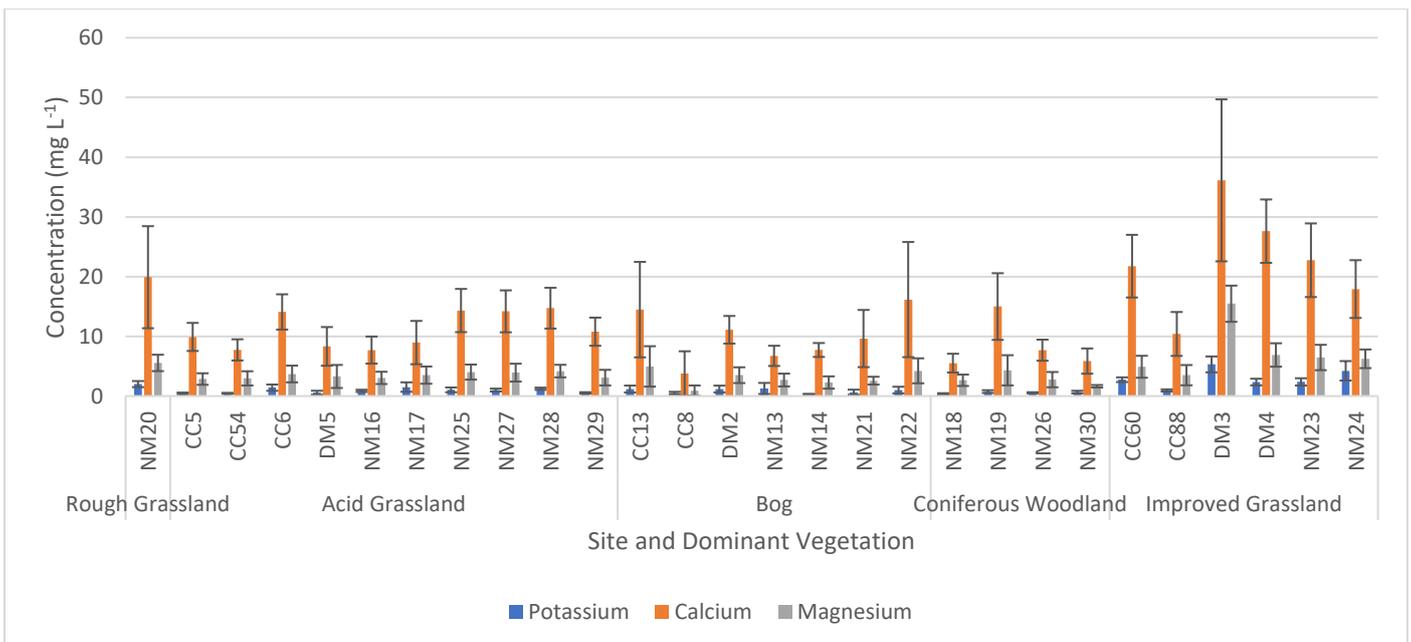


Figure 4.15 Part 2: Mean concentration of 3 ions in the Conwy catchment organised by dominant land use type. Bars represent SEM.

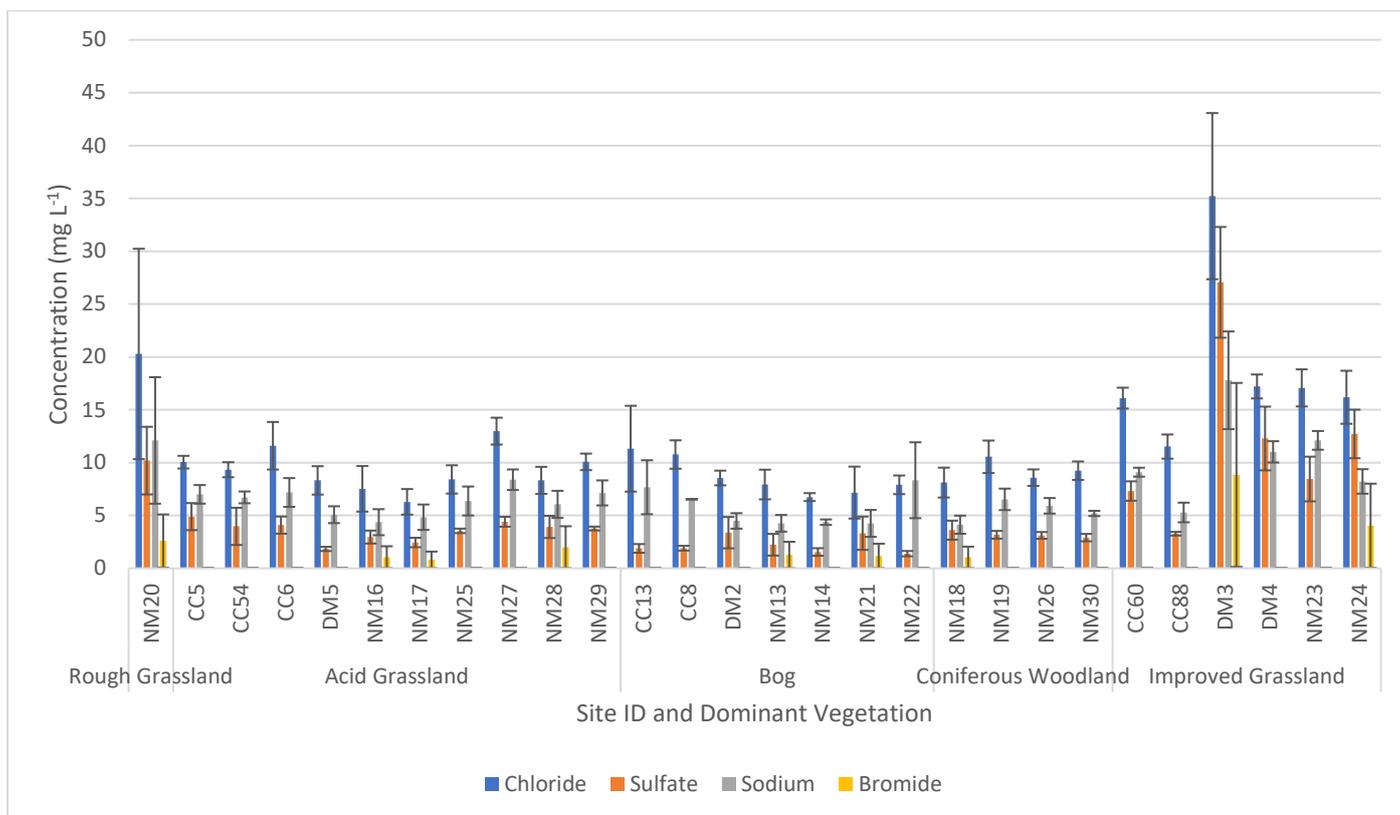


Figure 4.15 Part 3: Mean concentration of 4 ions in Conwy catchment organised by dominant land use type. Bars represent SEM.

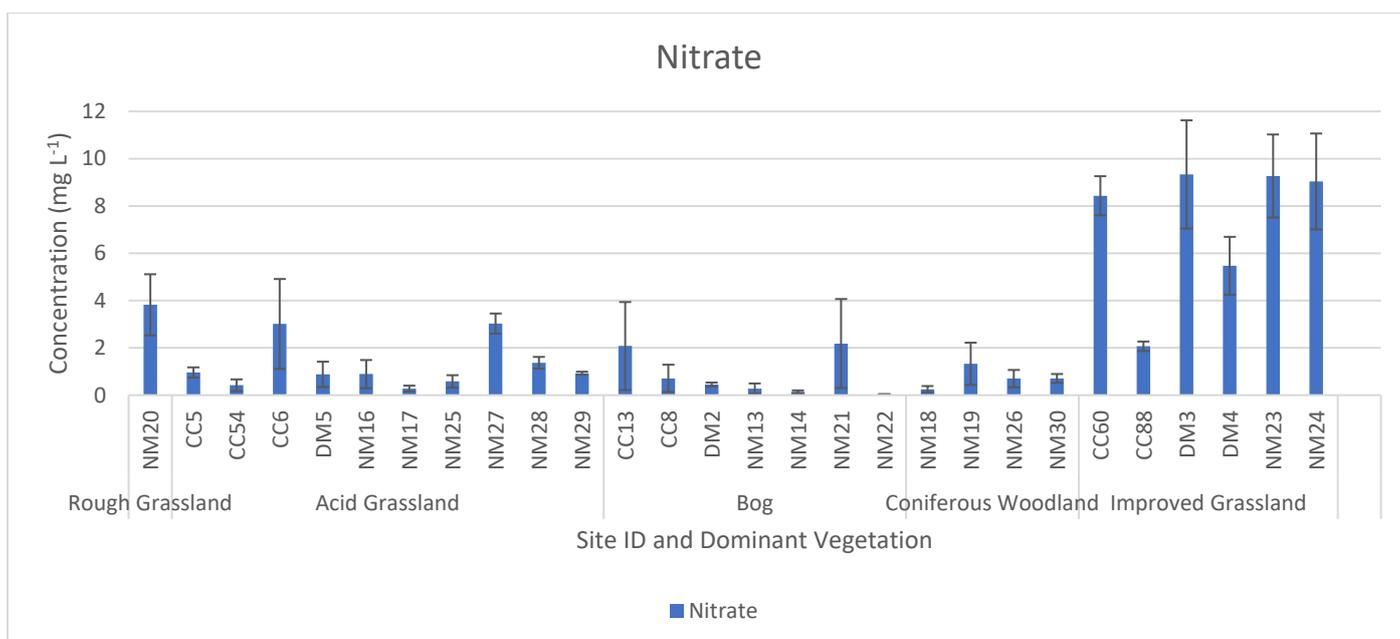


Figure 4.15 Part 4: Mean nitrate concentrations in the Conwy catchment organised by dominant land use. Bars represent SEM.

Ion data at Conwy sites (Figure 4.15 Parts 1-4) was found to be not normally distributed. The data was therefore transformed by  $\log_{10}$  to improve normality, providing skewness values of between -2.660 to 1.815, which, despite being higher than the recommended +2.0 –to -2.0 skewness values, were a marked improvement on the non-logged skewness readings and the robustness of the chosen test suggests that the data could still be used with a degree of confidence. An ANOVA test showed that there were some significant differences between means of some ions (nitrite, bromide, nitrate, phosphate, sulphate) within the datasets. A Tukey HSD post hoc test identified the following sites which were significantly different to each other. Table 4.5 shows the significant differences between sites in the Conwy Catchment in terms of nitrate concentration. See Appendix 2, Tables 10-13, for statistical data from other ions.

Table 4.5: Table to show the significant differences between sites of the Conwy catchment in terms of nitrate. Green represents a significance of  $p < 0.05$ , red represents a significant difference of  $p < 0.01$ . Values represent mean difference

Nitrate	CC5 4	CC6 0	CC6 8	CC8	DM3	DM4	NM1 3	NM1 4	NM1 6	NM1 7	NM1 8	NM2 0	NM2 1	NM2 2	NM2 3	NM2 4	NM2 5	NM2 7	NM 28
CC54			-2.02		-1.51	-1.77						-1.53			-2.01	-1.67		-1.56	
CC6													-1.64	-1.77					
CC60	2.02						2.05	2.04	1.54	1.99	1.78		-2.34	2.46			1.68		
CC88													1.733	-1.851					
DM3	1.54						1.58	1.57		-1.51			1.86	1.99					
DM4							-1.81	-1.80		-1.75	-1.54			-2.22					
NM13			-2.05		-1.58	1.81						1.57			2.05	1.70		1.59	
NM14			-2.04		-1.57	1.80						1.56			2.03	1.69		1.60	
NM16			-1.54												1.53				
NM17			-1.99		-1.51	1.75						1.50			1.98	1.64		1.53	
NM18			-1.78			1.54									1.77	1.43			
NM20	1.53						-1.57	-1.56		-1.50			-1.85	-1.98					
NM21		-1.64	-2.34	-1.73	1.86	2.09						1.851			2.328	1.985		1.875	
NM22		-1.77	-2.46	-1.86	-1.99	2.22						1.98			2.46	2.11		2.00	1.44

NM23	2.01						-2.05	-2.03	-1.53	-1.98	-1.77		-2.33	-2.46			-1.67		1.56
NM24	1.67						-1.70	-1.69		-1.64	-1.43		-1.99	-2.11					
NM25			-1.68												1.67				
NM27	1.56						-1.59	-1.58		-1.53			-1.88	-2.00					
NM28													-1.44	-1.56					

An ANOVA was carried out between the dominant land use type and the concentration of ions in the Conwy catchment. However, no significant differences were found, despite the visual differences seen in Figures 4.15 Parts 1-4.

#### 4.4.2.2 Conwy TN and NPOC

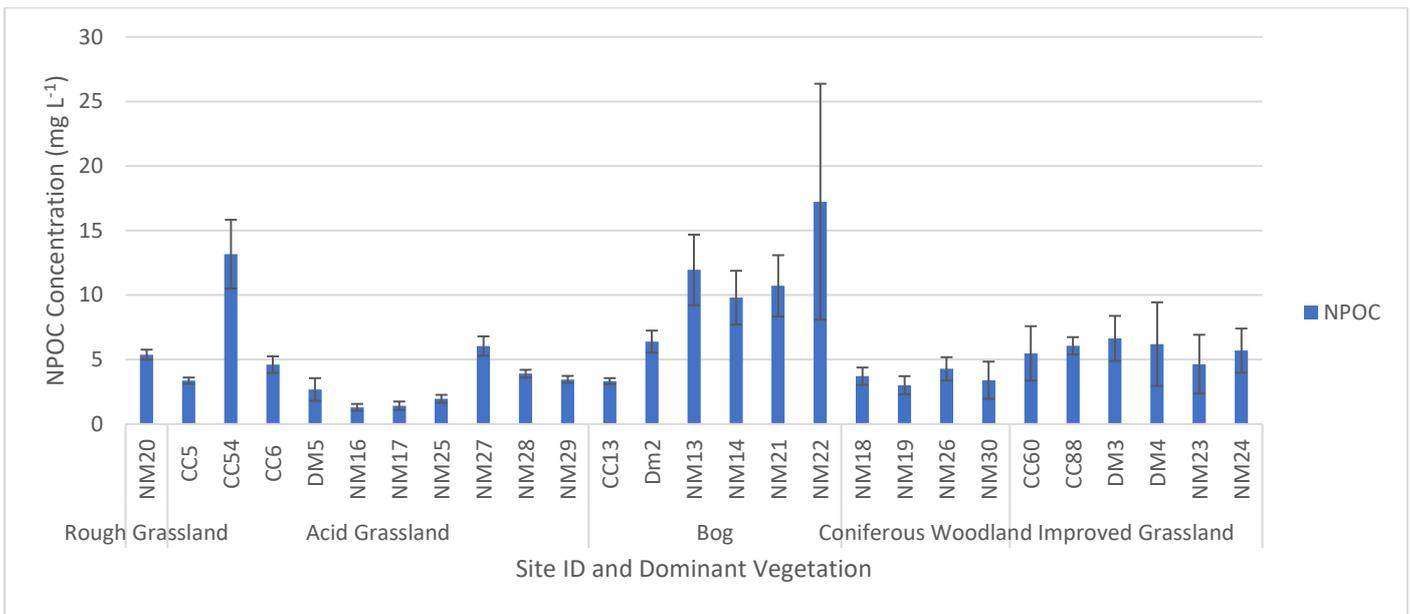


Figure 4.16: Mean NPOC concentration at Conwy sites arranged by dominant catchment vegetation type. Bars represent SEM

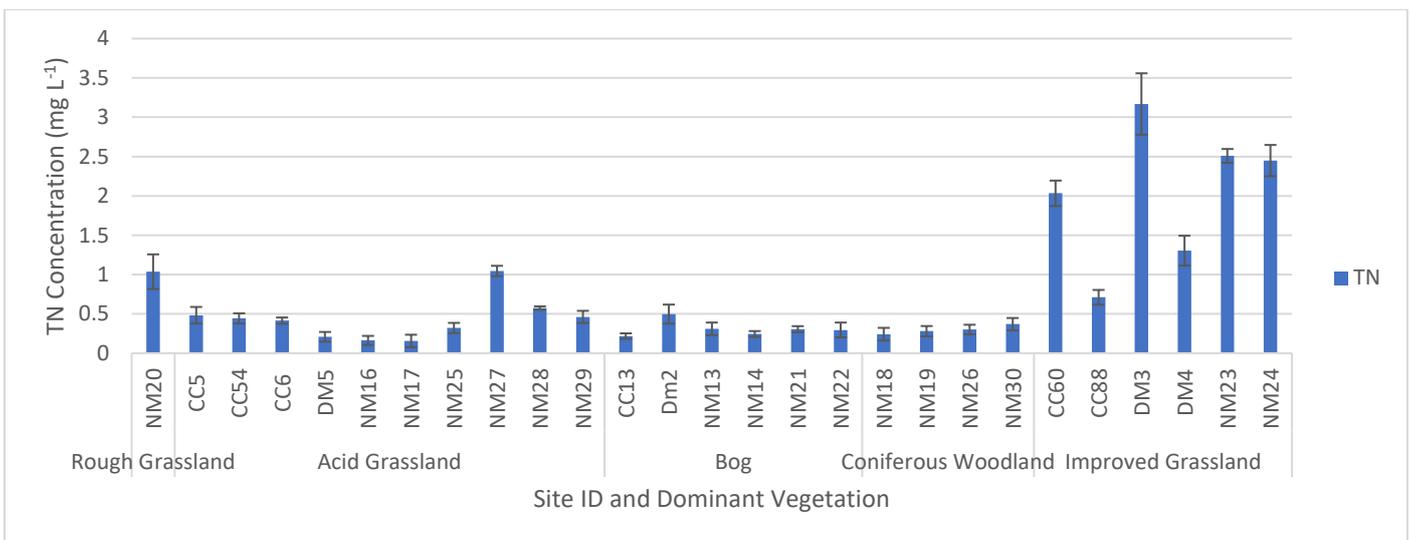


Figure 4.17: Mean TN concentration at Conwy sites organised by dominant catchment vegetation. Bars represent SEM.

TN and NPOC data for Conwy (Figures 4.16 and 4.17) was found to be not normally distributed (Skewness was not between -2.0 and +2.0) so the data was transformed ( $\log^{10}$ ). The data was found to be more normally distributed after transformation (skewness of 0.336 for NPOC and 0.126 for TN).

A Levene's tests showed  $p < 0.01$  for logged TN data but  $p = 0.190$  for logged NPOC data. As equal variances could be assumed for TN, a Tukey post hoc test was used. However, a Dunnett's T3 post hoc test was used for NPOC as this test does not assume equal variances.

Results from the Dunnett's T3 post hoc test for NPOC data are shown in Table 4.6

Table 4.6: Significantly different sites in the Conwy catchment in terms of NPOC concentration. Green represents  $p < 0.05$ , red represents  $p < 0.01$ , numbers represent the mean difference, statistical test used was ANOVA and Dunnett T3 post hoc.

NPOC	CC1	CC	CC5	CC8	DM2	NM1	NM1	NM1	NM1	NM1	NM2	NM2	NM2	NM2	NM28	NM29
<i>Dunnett T3</i>	3	5	4	8		3	4	6	7	8	0	1	5	7		
CC54	0.56 6	0.56 1						1.021	0.980	0.561			0.813		0.499	0.550
NM13	0.50 8	0.50 4						0.963	0.923				0.755			0.492
NM16			- 1.021	- 0.711	- 0.731	- 0.963	- 0.887				- 0.661	- 0.917		-0.707		
NM17			- 0.980	- 0.670	- 0.691	- 0.923	- 0.847				- 0.621	- 0.876		-0.666		
NM25			- 0.813			- 0.755	- 0.679					- 0.709				

Table 4.7: Tukey Post Hoc analysis of mean TN concentration between sites in the Conwy catchment. Green indicates  $p < 0.05$ , red indicates  $p < 0.01$ , numerical values represent mean difference result of post hoc test.

TN	C	CC	CC	CC6	CC8	DM2	DM5	NM1	NM1	NM1	NM17	NM1	NM1	NM2	NM2	NM2	NM2	NM	NM	NM
<i>Tukey</i>	C1	5	54		8			3	4	6		8	9	1	2	5	6	28	29	30
<i>HSD</i>	3																			
<b>CC60</b>	-0.99	-0.65	-0.67	-0.69		-0.64	-1.05	-0.85	-0.93	-1.18	-1.05	-1.07	-0.89	-0.83	-0.94	-0.83	-0.86		-0.66	-0.77
<b>DM3</b>	-1.18	-0.84	-0.86	-0.88	-0.65		-1.23	-1.04	-1.12	-1.37	-1.233	-1.26	-1.08	-1.02	-1.13	-1.01	-1.05	-0.73	-0.85	-0.96
<b>DM4</b>	-0.79						-0.84	-0.65	-0.73	-0.98	-0.84	-0.97	-0.69	-0.63	-0.74	-0.62	-0.65			-0.57
<b>NM20</b>	-0.675																			
<b>NM23</b>	-1.08	-0.75	-0.77	-0.79		-0.74	-1.14	-0.95	-0.10	-1.28	-1.14	-1.17	-0.99	-0.93	-0.10	-0.92	-0.98	-0.64	-0.75	-0.87

<b>NM24</b>	-	-	-	-0.77		-0.72	-1.13	-0.94	-1.02	-1.26	-1.13	-1.15	-0.97	-0.91	-1.02	-0.91	-0.94	-	-	-
	1.0	0.7	0.75															0.63	0.74	0.85
	7	3																		
<b>NM27</b>	-						-0.76	-0.57	-0.65	-0.89	-0.76	-0.79	-0.61		-0.66		-0.57			
	0.7																			
	0																			

TN concentrations at the Conwy catchment were found to significantly differ between many more sites than NPOC concentrations; the sites with most significant differences can be seen in Table 4.7.

#### 4.4.2.3 Conwy Landuse

Pearson’s correlations and regressions were explored between NPOC and TN concentrations and different land uses (see Table 4.10). For NPOC, the only correlation was a strong positive relationship between NPOC and freely draining slightly acidic but base rich soils ( $r=0.963$ ,  $p<0.05$ ). For TN, however, there are many more positive relationships.

Regression analysis shows a medium strength positive relationship with an  $R^2$  value of 0.49 (see Figure 4.18) between bog and TN concentration. There are also positive correlations with TN and urban land use ( $r=0.676$ ,  $p<0.05$  and  $n=12$ ), blanket bog peat soils ( $r=0.597$ ,  $p<0.05$ ,  $n=18$  - see Figure 4.19 for regression analysis), freely draining slightly acid but base rich soils ( $r=0.998$ ,  $p<0.01$ ,  $n=4$ ), freely draining slightly acidic loamy soils ( $r=0.707$ ,  $p<0.05$ ,  $n=9$ ), and loamy and clayey floodplain soils with naturally high ground water ( $r=0.939$ ,  $p<0.05$ ,  $n=5$ ). See Figures 4.18-4.19 for regression analysis between TN and blanket bog peat soils and bog vegetation class, regression analysis statistics shown alongside trend line data. Positive regression analysis data from between

land classed as neutral grassland and the concentration of 8 ions at the Conwy catchment is displayed in Figures 4.20 Parts 1 and 2 and Tables 4.8 and 4.9. Furthermore, regression analysis between suburban land use and 5 ions at the Conwy catchment was carried out, and the data displayed in Figure 4.21 and Tables 4.11 and 4.12.

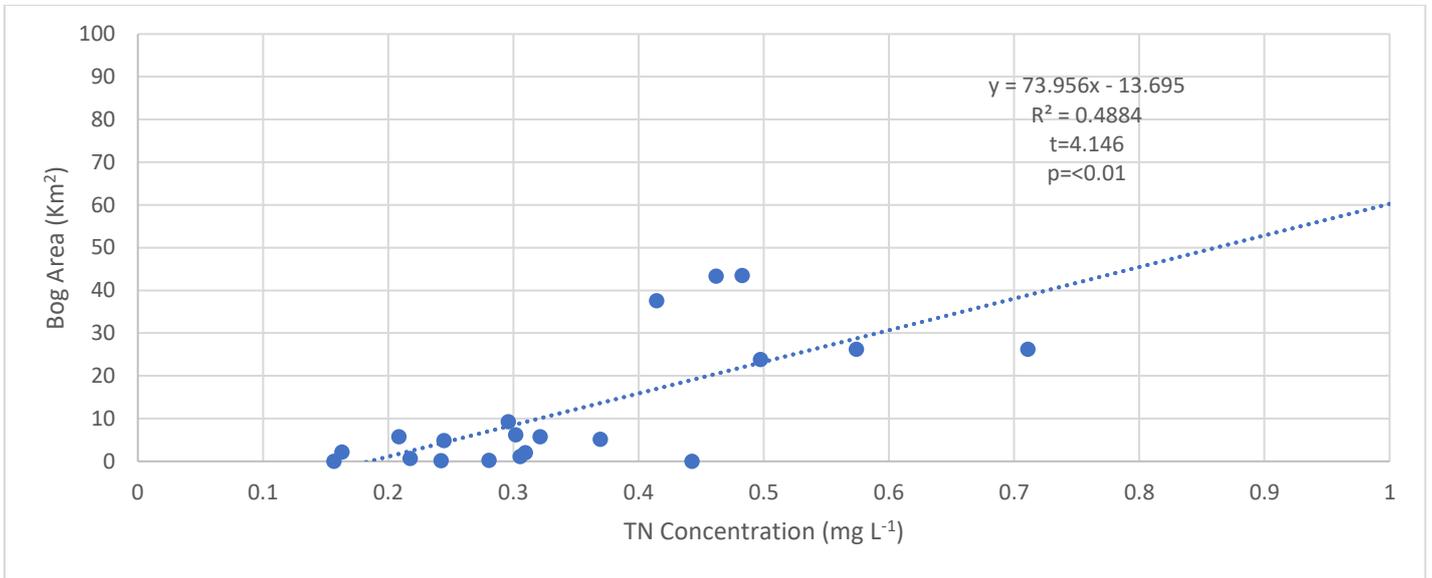


Figure 4.18: Regression between mean TN concentration and area of bog covered land.

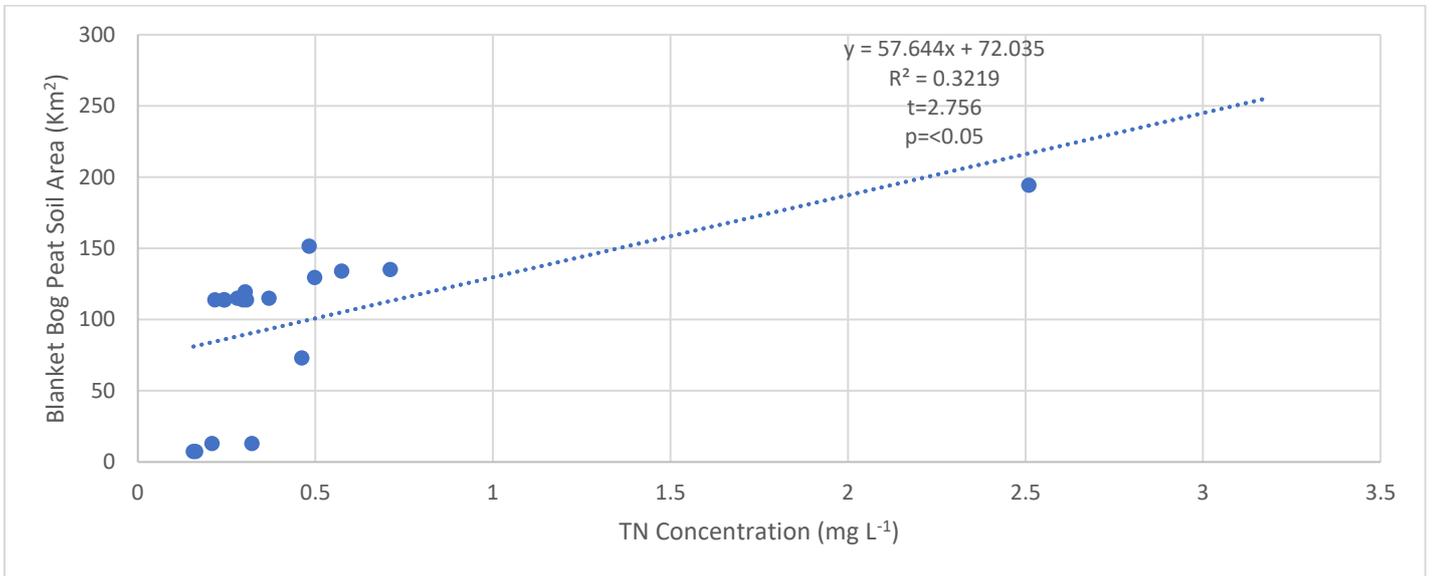


Figure 4.19: Regression between TN and blanket bog peat soil covered land.

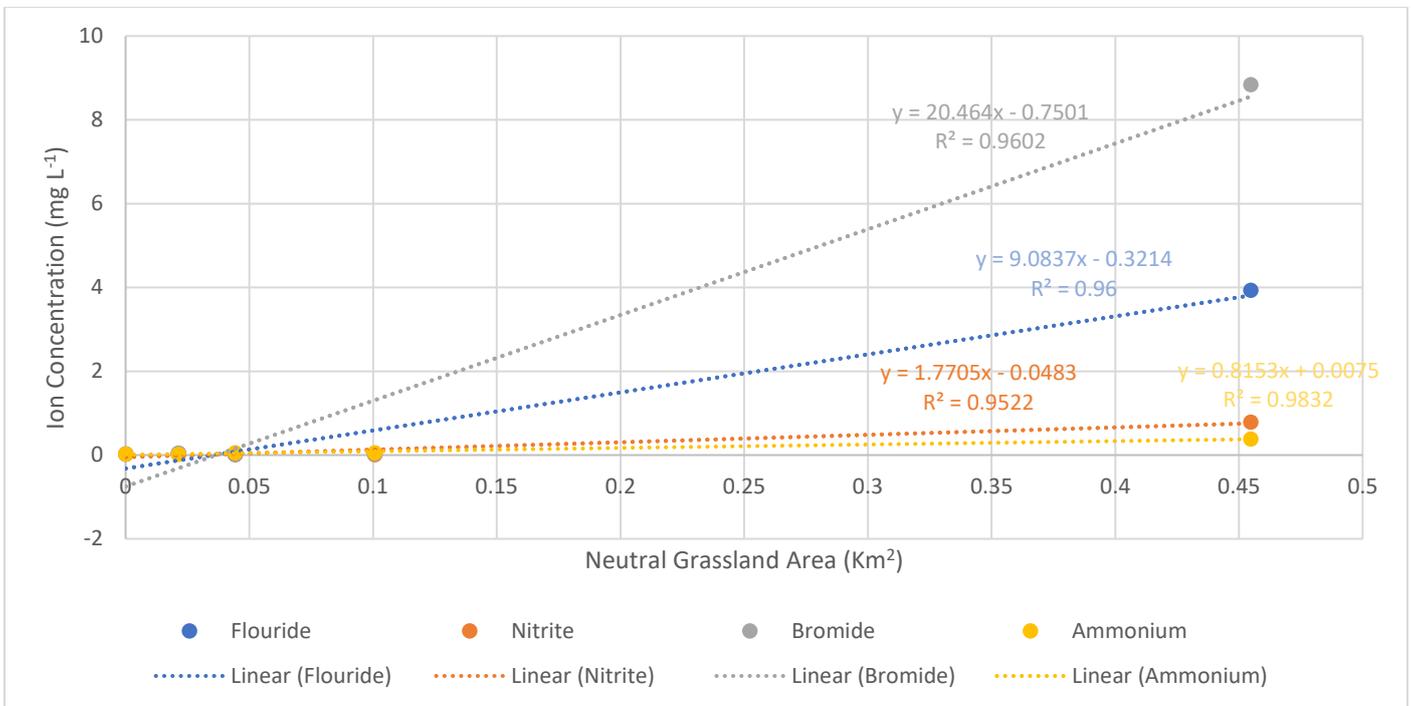


Figure 4.20 Part 1: Regression between neutral grassland and the concentration of 4 ions at Conwy catchment.

Table 4.8: Statistical analysis of regression analysis displayed in Figure 4.21

Ion	Equation	R <sup>2</sup>	T Test (t)	Significance (p)
<b>Flouride</b>	$Y=9E-06x - 0.3214$	0.9600	8.489	<0.01
<b>Nitrite</b>	$Y=2E-06x - 0.0483$	0.9522	7.852	<0.01
<b>Ammonium</b>	$Y=8E-07x + 0.0075$	0.9832	12.931	<0.01
<b>Bromide</b>	$Y=2E05x - 0.7501$	0.9602	8.498	<0.01

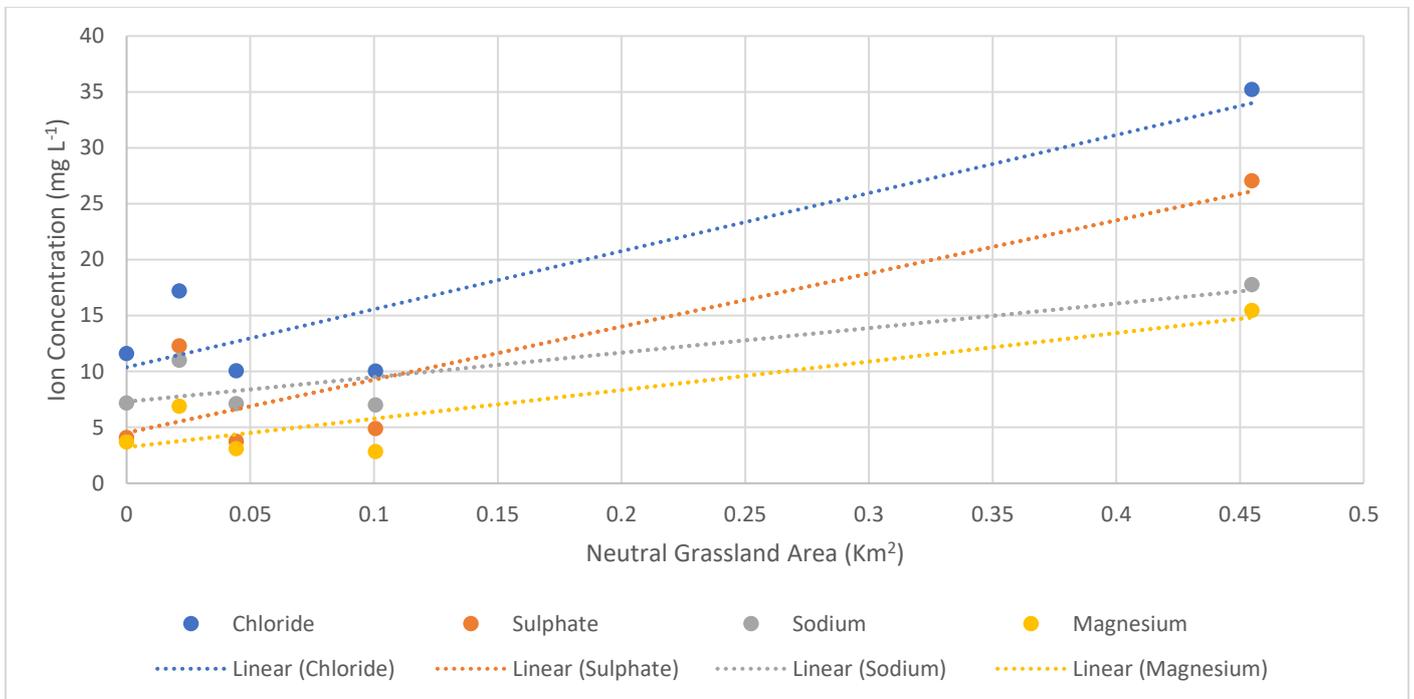


Figure 4.20 Part 2: Regression between neutral grassland and the concentration of a further 4 ions.

Table 4.9: Statistical analysis of regression shown in Figure 4.22

<b>Ion</b>	<b>Equation</b>	<b>R<sup>2</sup></b>	<b>T Test (t)</b>	<b>Significance (p)</b>
<b>Chloride</b>	$Y=51.944x + 10.372$	0.8395	3.962	<0.05
<b>Sulphate</b>	$Y=0.47.499x + 4.5149$	0.8122	3.602	<0.05
<b>Magnesium</b>	$Y=25.527x + 3.2271$	0.8184	3.677	<0.05
<b>Sodium</b>	$Y=21.942x + 7.2959$	0.788	3.339	<0.05

Table 4.10: Pearson's correlation between ions and land use types at the Conwy Catchment. Green represents  $p < 0.05$ , red represents  $P < 0.01$  significance levels.

	<b>Bog</b>	<b>Montane habitats</b>	<b>Inland Rock</b>	<b>Urban</b>	<b>Blanket Bog Peat Soils</b>	<b>Freely Draining Slightly Acidic Loamy Soils</b>	<b>Shallow Very Acid Peaty Soils Over Rock</b>	<b>Very Acid Loamy Upland Soils With A Wet Peaty Surface</b>
<b>Chloride</b>	R=0.53 N=20			R=0.71 N=12	R=0.58 N=19	R=0.76 N=10		
<b>Sulphate</b>	R=0.58 N=20			R=0.691 N=12	R=0.472 N=19	R=0.808 N=10		
<b>Ammonium</b>		R=0.77 N=7	R=0.89 N=11	R=0.82 N=12				
<b>Nitrate</b>					R=0.48 N=19	R=0.78 N=10		
<b>Potassium</b>				R=0.595 N=12		R=0.793 N=10	R=0.739 N=10	R=0.638 N=16
<b>Magnesium</b>				R=0.652		R=0.764		

				N=12		N=10		
<b>Phosphate</b>						R=0.775 N=10		
<b>Calcium</b>						R=0.894 N=10		
<b>Sodium</b>				R=0.731 N=12				
<b>Nitrite</b>				R=0.691 N=12				
<b>Flouride</b>				R=0.666 N=12				
<b>Bromide</b>				R=0.684 N=12				

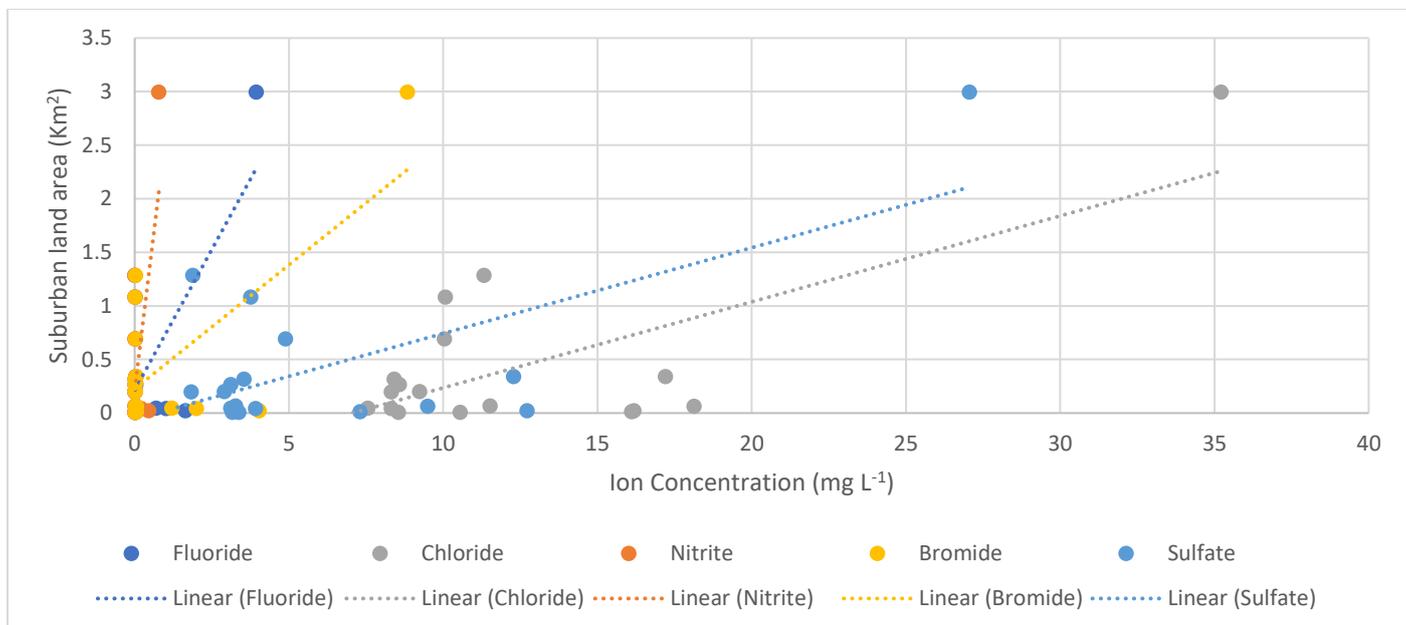


Figure 4.21: Regression analysis between chloride, nitrite, bromide, sulphate and fluoride concentrations and the area of suburban land use in the Conwy catchment.

Table 4.11: Statistics of regression analysis for data presented in Figure 4.23

Ion	Equation	R <sup>2</sup>	T test (t)	Significance (p)
Fluoride	$Y=0.525x + 0.124$	0.4872	3.775	<0.01
Chloride	$Y=0.0802x - 0.567$	0.5108	3.957	<0.01
Nitrite	$Y=2.3655x + 0.2131$	0.4257	3.335	<0.01
Bromide	$Y=0.2315x + 0.2251$	0.4875	3.777	<0.01
Sulphate	$Y=0.08x - 0.0575$	0.4441	3.461	<0.01

Table 4.12: Statistics of regression analysis for data presented in Figure 4.27 minus site DM3

Ion	Equation	R <sup>2</sup>	T test (t)	Significance (p)

<b>Fluoride</b>	Y=-0.2582x + 0.3493	0.0967	-0.897	0.391
<b>Chloride</b>	Y=-0.0119x + 0.4242	0.0117	0.287	0.780
<b>Nitrite</b>	Y=-0.9252x + 0.3427	0.0752	-0.463	0.653
<b>Bromide</b>	Y=-0.1034x + 0.3391	0.0828	0.751	0.470
<b>Sulphate</b>	Y=-0.0267x + 0.4243	0.0558	-0.442	0.668

Figure 4.21 (and data in Tables 4.11 and 4.12) show the positive relationships between fluoride, nitrite, chloride, sulphate and bromide and the area of land classed as suburban in the Conwy catchment. All ions exhibit positive relationships of medium strength, but all seem to be driven by one sample site, DM3. When this datapoint is removed, however, the relationships are negligible and some even turn negative.

Data was organised based upon dominant vegetation cover per catchment (see Figures 4.16 to 4.17) and it was obvious that improved grassland dominated catchments had much higher concentrations of many ions when compared to the other catchment types. Therefore, an ANOVA test was run between the dominant land use types and the ions to determine whether there were any significant differences between sites. The ANOVA results showed that 8 ions were significantly different between dominant land use types and therefore a Tukey HSD post hoc test was conducted to determine where these differences lay (Table 4.13).

Table 4.13: Results of Tukey HSD Post Hoc test, showing which dominant land use types were significantly different to Improved Grassland dominated catchments, also showing ANOVA values and mean differences. Red shows  $p < 0.01$  and green shows  $p < 0.05$ .

	<b>Chloride</b>	<b>Nitrite</b>	<b>Nitrate</b>	<b>Sulphate</b>	<b>Sodium</b>	<b>Potassium</b>	<b>Calcium</b>	<b>Magnesium</b>
	<i>ANOVA</i> <i>(f=12.804,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=3.353,</i> <i>p=&lt;0.05)</i>	<i>ANOVA</i> <i>(f=9.685,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=15.144,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=5.882,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=9.919,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=6.943,</i> <i>p=&lt;0.01)</i>	<i>ANOVA</i> <i>(f=6.202,</i> <i>p=&lt;0.01)</i>
Acid Grassland	0.289		0.835	0.450	0.203	0.503	0.297	0.090
Bog	0.320	0.968	1.194	0.662	0.257	0.529	0.371	0.097
Coniferous Woodland	0.291		1.006	0.485	0.226	0.666	0.434	0.113

The results displayed in Table 4.13 show that improved grassland dominated catchments are significantly different to acid grassland, bog and coniferous woodland dominated catchments in terms of specific ion concentrations. It can be seen that the highest difference is between the dominant land use types and nitrate, where the mean differences are much higher than at other sites, whereas magnesium has the lowest mean differences, which show that although the sites are significantly different, the mean differences are small.

Figures 4.16 and 4.17 show the mean TN and NPOC data collected at the Conwy catchment, organised by dominant catchment vegetation. It is apparent that TN concentrations are elevated in the improved grassland dominated catchments whereas NPOC concentrations are elevated in the bog catchments. An ANOVA test was run to determine whether there were any significant differences between these sites and the TN and NPOC concentrations. Data was transformed to give a normal skewness of between +2 and -2, and an ANOVA presented significant results ( $p < 0.01$ ) for both TN and NPOC, see Tables 4.14 and 4.15 for post hoc analysis.

Table 4.14: Significant differences identified in TN concentrations between improved grassland and the listed dominant vegetation classes, with ANOVA and mean difference values quoted. Red represents a significance of  $p < 0.01$ . Table 4.15 shows the same data but for NPOC and between bog and the listed dominant vegetation classes.

	<b>TN</b> <i>ANOVA</i> <i>(f=19.316,</i> <i>p=&lt;0.01)</i>		<b>NPOC</b> <i>ANOVA</i> <i>(f=4.928,</i> <i>p=&lt;0.01)</i>
Acid Grassland	0.698	Acid Grassland	-0.423
Bog	0.783	Coniferous Woodland	
Coniferous Woodland	0.791	Improved Grassland	

The results shown in Tables 4.14 and 4.15 suggest that improved grassland has a significantly higher TN concentration in the water draining from it, when compared to the other dominant vegetation classes in the Conwy catchment. The mean difference values are similar to each other suggesting that the TN concentration in improved grassland was the only area where TN concentrations were high in comparison to other sites. NPOC data shows that the bog dominated land is significantly different to acid grassland dominated land in terms of NPOC concentration.

### 4.4.3 Scottish Sites

#### 4.4.3.1 Scottish Ions

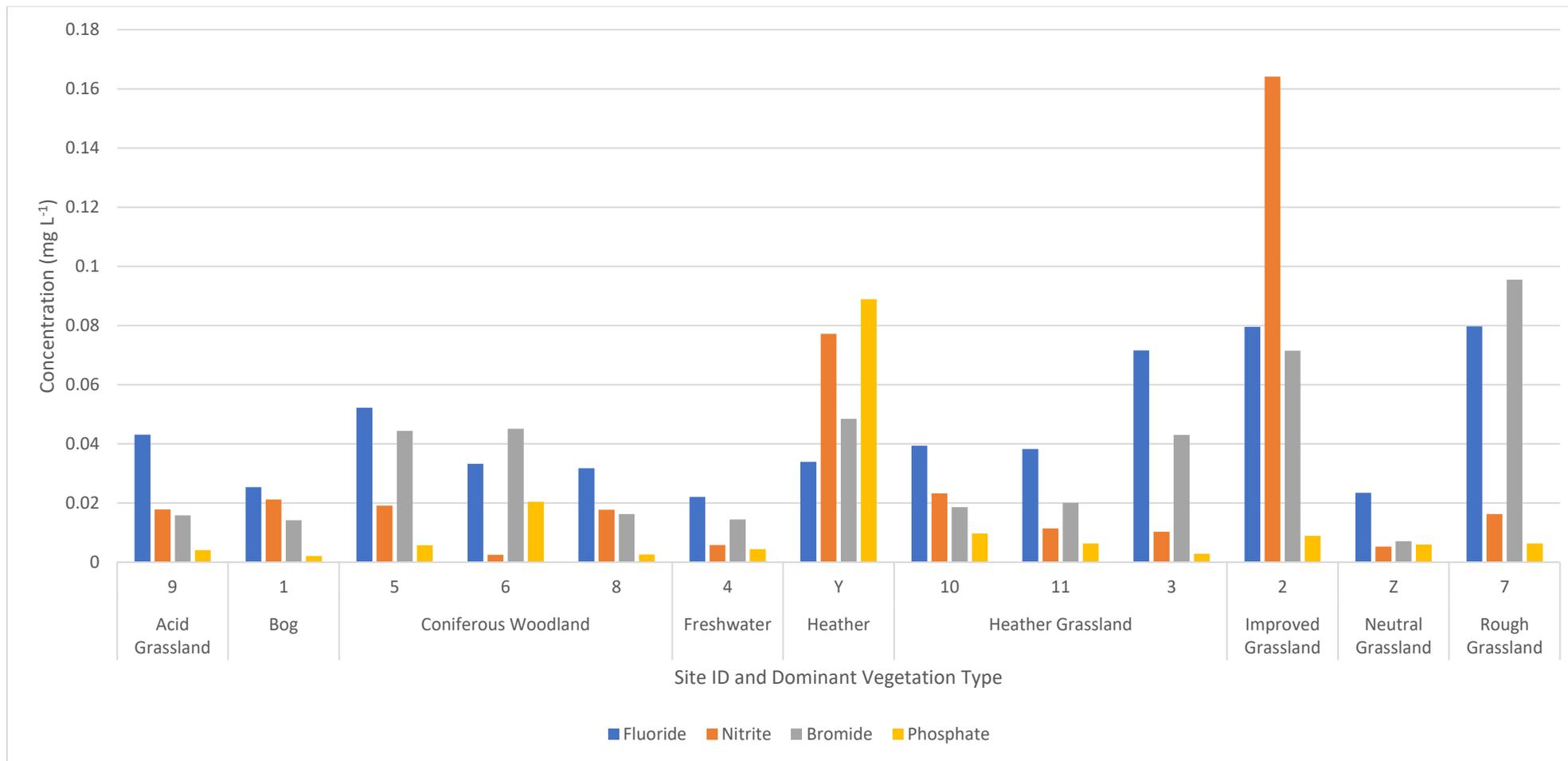


Figure 4.22 Part 1: Selected mean ion concentrations at Scottish catchments arranged by dominant vegetation type.

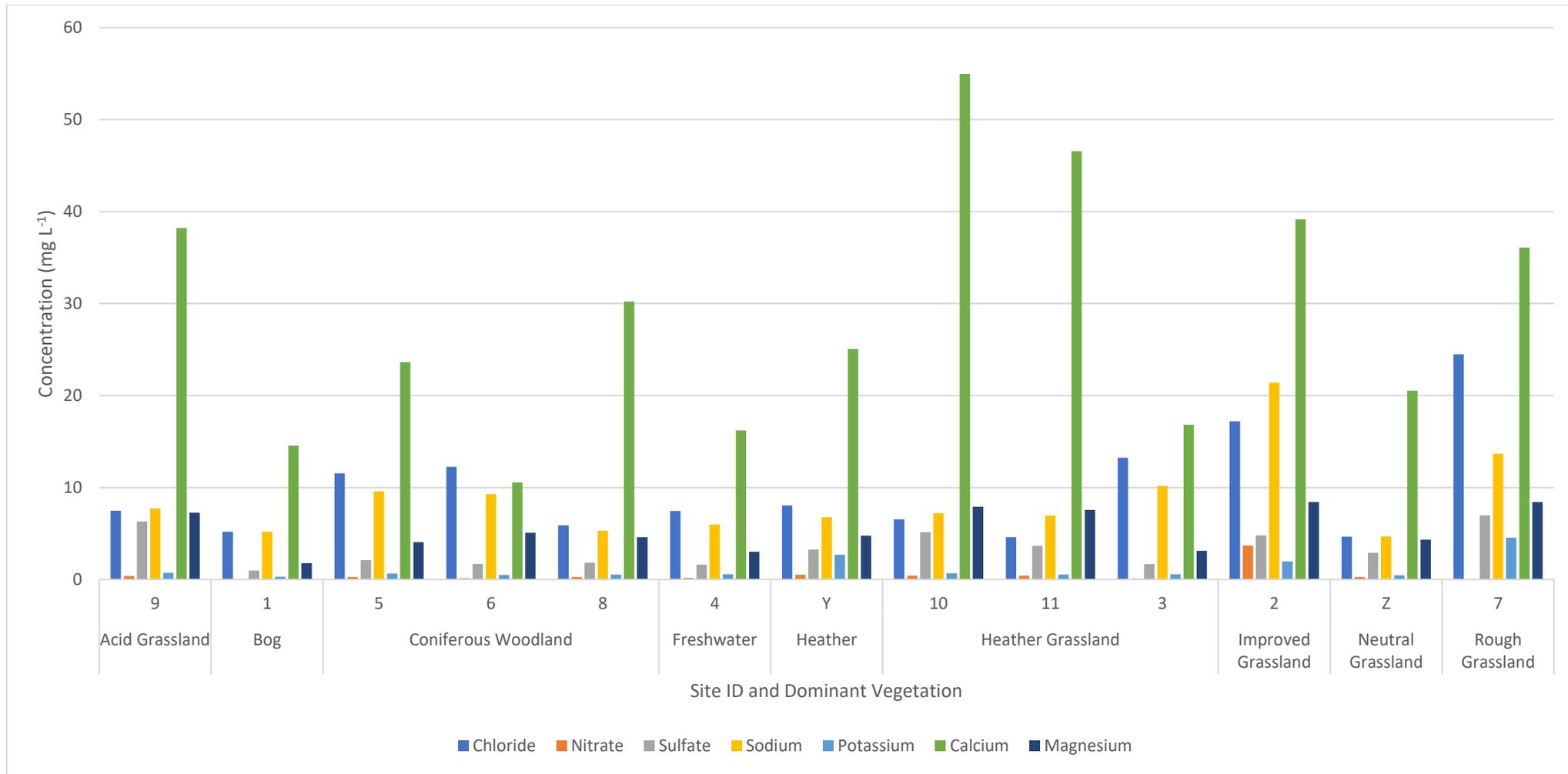


Figure 4.22 Part 2: Mean selected ion concentrations at sites in Scotland, arranged by dominant vegetation type

The data from Scottish sites with fewer than 3 sampling locations was pooled, hereafter named site 1, to determine whether there were significant differences between these sites collectively and other sites.

Ion data (Figures 4.22 Part 1 and Part 2) was analysed for normality, and due to skewnesses over 2.000, all data was transformed.

This logged data was tested for homogeneity of variance and 5 ions showed a significance greater than  $p < 0.05$ , thus showing that there were likely differences between these sites and others. 9 statistically significant ANOVA results ( $p < 0.05$ ) showed that there were differences between the sites, and a Tukey Post Hoc test was carried out to identify these specific differences, due to homogeneity of variances being assumed, results are displayed in Table 4.16.

Table 4.16: Results of ANOVA and Tukey HSD post hoc between ions at Scottish catchments. Red represents  $p < 0.01$ , green represents  $p < 0.05$ , value represents mean difference.

	1	2	3	4	5	6	7	8	9	10	11
1						NO <sub>2</sub> <sup>-</sup> 1.092 K <sup>+</sup> 0.522		K <sup>+</sup> 0.478			K <sup>+</sup> 0.501
2											
3											
4			F <sup>-</sup> 0.495								
5											
6	Ca <sup>+2</sup> 0.564							Ca <sup>+2</sup> -0.560	Ca <sup>+2</sup> -0.701	Ca <sup>+2</sup> -0.854	Ca <sup>+2</sup> -0.786
7											
8	SO <sub>4-2</sub> 0.246										
9			SO <sub>4-2</sub> -0.593	SO <sub>4-2</sub> -0.612		SO <sub>4-2</sub> -0.598		SO <sub>4-2</sub> -0.535			
10			Mg <sub>2</sub> <sup>+</sup> -0.388	Mg <sub>2</sub> <sup>+</sup> -0.401		SO <sub>4-2</sub> -0.458		SO <sub>4-2</sub> -0.419			
11	Cl <sup>-</sup> 0.445				Cl <sup>-</sup> 0.450	Cl <sup>-</sup> 0.478					

Ion concentrations at Scottish catchments were statistically analysed by dominant vegetation cover to determine whether any land use type produced a significantly different concentration of any ion when compared to others. An ANOVA test was run which showed that nitrite, bromide, ammonium and potassium ions may have differences within their data ( $p < 0.05$ ,  $f = 2.696$ ,  $f = 3.203$ ,  $f = 10.605$  and  $f = 4.241$  respectively). A Tukey posthoc test was conducted, which showed that acid grassland and bog classed land differs in terms of nitrite concentration ( $p < 0.05$ ) with a mean difference of -1.051. Bromide in water draining from rough grassland significantly differed to acid grassland ( $p < 0.01$ ), coniferous woodland ( $p < 0.05$ ), heather ( $p < 0.05$ ) and heather grassland ( $p < 0.05$ ) classed lands with mean differences of 0.817, 0.641, 0.822 and 0.665 respectively. Finally, calcium concentration significantly differed between heather grassland and rough grassland at  $p < 0.05$  with a mean difference of -0.900.

#### 4.4.3.2 Scottish TN and NPOC

For statistical analysis (and separate from the data displayed in Figure 4.23 below), data from the catchments containing less than 3 sites (i.e. sites 1, 2, Y1 and ZA), was amalgamated to determine whether these Scottish catchments differed from the Conwy and Hampshire Avon catchments. This data was encompassed under the name 'site 1' throughout further statistical analysis.

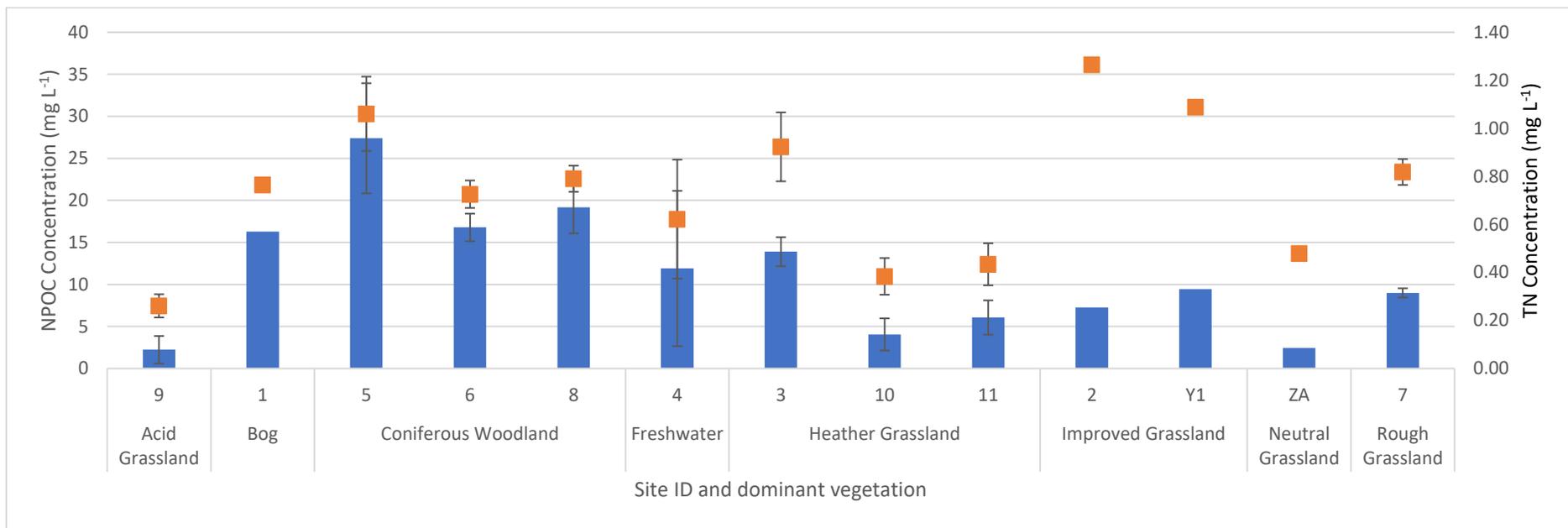


Figure 4.23: Mean NPOC and TN concentrations at Scottish catchment sites organised by dominant vegetation type in catchment. Error bars represent SEM. NPOC concentrations are displayed on the primary axis and represented by bars, TN concentrations are displayed on the secondary axis and are represented by the dots.

Figure 4.23 shows the mean NPOC and TN at Scottish sites. Site 5 has the highest mean NPOC concentration out of all catchments, and site 9 has the lowest. For TN data, again, site 9 had the lowest NPOC concentration whereas site 2 has the highest. NPOC and TN data was analysed for normality, showing a skewness of 1.271 and 0.358 respectively. A Levene's statistic of  $p < 0.01$  for both TN and NPOC, and an ANOVA score of  $p < 0.01$  for both parameters too, meant that a posthoc

analysis would be suitable for determining where the differences lie in this dataset. A Tukey HSD posthoc test was selected as this assumes equal variances. Results are displayed in Tables 4.17 and 4.18.

Table 4.17: Results of ANOVA and Tukey Posthoc between sites at Scottish catchments in terms of NPOC concentration. Green represents  $p < 0.05$ , red represents  $p < 0.01$ , numerical values represent mean difference.

NPOC	5	8
9	25.158	16.936
10	23.337	15.115
11	21.324	

Table 4.18: Results of ANOVA and Tukey Posthoc between sites at Scottish catchments in terms of TN concentration. Green represents  $p < 0.05$ , red represents  $p < 0.01$ , numerical values represent mean difference.

TN	9	10	11
1	0.635	0.514	
3	0.658		
5	0.796	0.675	0.624
8	0.527		

#### 4.4.3.3 Scottish Landuse

TN and NPOC data was correlated with landuse data, and the only significant relationship detected was between TN and urban land at  $p < 0.05$  and  $r = 0.842$ , suggesting that as land area classed as urban increases, TN concentrations also increase at this land type in the water draining the land (Figure 4.24 shows regression).

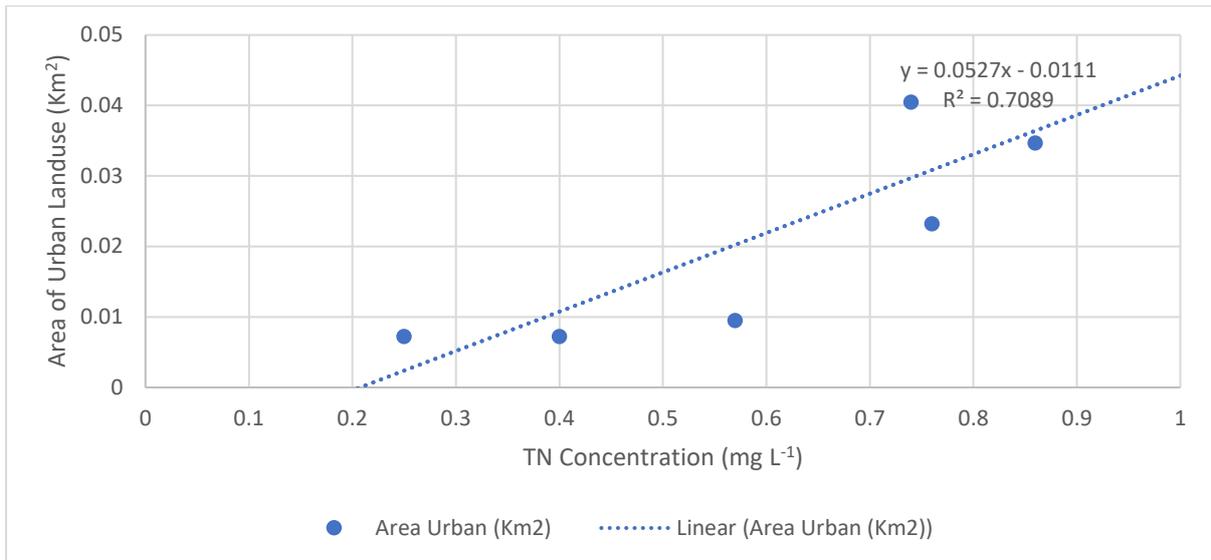


Figure 4.24: Relationship between TN concentration and area of urban land use in the catchments.

Whilst the relationship between data points in Figure 4.24 looks more exponential than linear, a curve fitting algorithm was run, analysing 11 different curve types in terms of its R value,  $R^2$  value and ANOVA score and significance. Although an exponential relationship produced  $r = 0.879$ ,  $R^2 = 0.761$ ,  $f = 15.910$  and  $p = 0.010$ , a linear relationship produced stronger stats ( $r = 0.943$ ,  $R^2 = 0.889$ ,  $f = 40.041$  and  $P = 0.001$ ), thus this curve was used.

Ion data collected from the Scottish catchments was correlated in a Pearson's correlation with the land use types in the catchment, producing the results displayed in Table 4.19.

Table 4.19: Significant Pearson's correlations between ions and land use types in Scottish catchments. Data displayed is r value, green represents significance of  $p < 0.05$ , red represents significance of  $p < 0.01$ .

	<b>Sandstone and conglomerate, interbedded</b>	<b>Improved Grassland</b>	<b>Acid Grassland</b>	<b>Humus iron podzols</b>	<b>Brown earths with gleying</b>	<b>Peaty gleys</b>	<b>Suburban</b>	<b>Water</b>
<b>Fluoride</b>	-0.753							
<b>Nitrite</b>		0.550						
<b>Nitrate</b>		0.511			0.999	0.527		
<b>Bromide</b>	-0.555		-0.433	0.903				
<b>Phosphate</b>				0.999			0.929	0.646

The negative relationship between fluoride and sandstone and conglomerate interbedded geology is a strong negative correlation, showing that, as sandstone and conglomerate interbedded land cover decreases, fluoride concentration increases.

Bromide was found to correlate negatively with acid grassland showing that, as acid grassland land cover decreases, bromide concentration increases.

Bromide also correlated negatively with sandstone and conglomerate interbedded geology, again, showing that as the area of this bedrock in the catchment decreases, the bromide concentration increases. All positive relationships are self-explanatory.

TN and NPOC were also analysed in an ANOVA with dominant land use data to determine whether a specific type of land use was producing a water with a significantly different NPOC or TN concentration in it. Data was normally distributed so an ANOVA was selected, however, no significant differences were found.

## 4.5 Discussion

### 4.5.1 Comparison Between Catchments

The Hampshire Avon drains a catchment that is 76.30% underlain by chalk, with 41.87% arable and horticulture classed land, and 58.90% shallow lime-rich soils over chalk or limestone soil, whereas the Conwy river drains a catchment that has 53.55% freely draining acid loamy soils over rock, situated on top of 67.62% mudstone, siltstone and sandstone bedrock, both supporting a vegetation of 42.96% improved and acidic grassland.

Scottish sites comprised of, in total, 30.82% coniferous woodland, growing in a 33.43% spread of blanket peat on a 44.28% coverage of wacke bedrock. This, alongside the DOM character data, shows that, although the Scottish sites were chosen as they were thought to be similar to the Conwy and Hampshire Avon catchments, they are in fact vastly different. This suggests that DOM character will be catchment specific.

The data presented suggests strong significant differences between the DOM at various sites in all 3 catchments, Hampshire Avon, Conwy and Scottish catchments. This data shows that the sites contain significantly higher or lower concentrations of the DOM parameters explored, than other sites in the catchment. It is these sites that are of particular interest to the study, as understanding the reasons behind the occurrence of these significant differences can lead to an explanation of why these

sites differ. This explanation, in turn, can be used to determine common factors across significantly different sites that are contributing to the difference (e.g. agricultural land use being dominant in all sites that are significantly higher in nitrate concentrations than other sites). Whilst the present study begins by examining data from 96 sites averaged into 3 major sites, the further analysis of the data on a more detailed level has led to a deeper understanding of the influences of individual parameters upon the water quality. In order to take account for unequal variance, all data was compared using statistically robust analysis methods (Pearson's Correlation, Spearman's Correlation, ANOVA and Dunnett's tests, for example).

Moreover, the findings of the present study show that, on a whole, the 3 sample sites studied are significantly different from each other on many levels, and therefore a 'demonstration catchment' cannot be used to provide data that relates to other catchments with very similar physical characteristics.

Further differences were noticed in DOM components, for example, a typical freshwater concentration of carbohydrates has been reported as 690 nM (0.69 mM) (Kaplan and Newbold, 2004), however, the mean carbohydrate concentration recorded at the Hampshire Avon catchments is 59.04 mM and at the Conwy catchment is 59.23 mM. This shows that, although the carbohydrate content from these sites is 85 times higher than the quoted typical freshwater concentration, there is no difference in the mean carbohydrate concentrations between the two sites.

Figure 4.9 shows mean amino acid concentrations for Hampshire Avon, Conwy and Scottish catchments. It is clear that the Hampshire Avon samples contain the lowest concentration of amino acids but also has the highest variance in data. The Scottish catchment has slightly higher mean concentration of amino acids with a very small variance; however, the Conwy catchment is shown to contain the highest concentration of amino acids with a relatively small variance. Statistical analysis found that all 3 sites contain amino acid concentrations that significantly differ to each other. Freshwater amino acids have been reported at between 7 and 2000 nM in rivers and between 20 and 3700 nM in lakes (Kaplan and Newbold, 2004). The concentrations detected in the 3 catchments studied here is therefore right at the lower end of the spectrum found in rivers, as Scottish rivers show a mean concentration of

9.73 ±1.03 nM, Conwy rivers show a mean concentration of 14.22 ±1.36 nM and Hampshire Avon rivers show a mean concentration of 8.22 ±2.74 nM.

Ionic concentrations at the 3 sites differed greatly. The Hampshire Avon was found to contain a much higher concentration of 10 out of 12 ions when compared to the Conwy and Scottish sites. This was expected as the intense agricultural activities that take place on the majority of the land draining into the streams in the Hampshire Avon is likely to have elevated ionic concentrations. Alongside these major inter-catchment differences, intra-catchment differences were explored too, detailed below.

## 4.5.2 Hampshire Avon Catchment

### 4.5.2.1 Hampshire Avon Ions

In the Hampshire Avon catchment, the most significantly different site from all other sites, in terms of ion concentrations, is site 19 (see data in Figure 4.10 Parts 1 to 3). This site is at the outlet to a sewage treatment works (STW), and thus concentrations of specific nutrients are expected to be significantly higher than at other sites. The two ions that are significantly different at this site compared to most other sites are sodium and nitrate along with other indicators of sewage, such as carbon *etc.* Wastewater is likely high in sodium due to human activities (water softeners, alkaline cleaning chemicals, road gritting *etc.*) and thus the concentration leaving a STW will be concentrated due to the accumulation of many human inputs. Whilst ammonium is the most common form of nitrogen found in waste water, nitrate concentrations are higher when oxidised forms of nitrogen are present in the water. The biggest contributor of nitrates in wastewater are artificial fertilisers, although septic tanks, erosion of natural deposits, and cattle manure are also thought to contribute highly to the nitrate concentration.

The lack of significant differences between most other ions and sites could show that there are no more significantly different inputs to the streams in the Hampshire Avon catchment, although the extra high concentrations at this STW could be dwarfing any other effects.

Furthermore, site 20 is a military barracks with its own STW built in, which would explain the higher nitrate concentrations at this site, site 4 has a large

rural catchment containing many small villages, which likely do not have access to mains drainage, thus, it is suggested that at this site, the nitrate concentration is higher due to smaller STW or septic tank leakage, combined with possible agricultural activity. Septic tanks can elevate nitrate, amongst other ion concentrations. Potential failures of a septic tank system, due to exceeded capacity, damaged tank, storm water connections, blocked biomat or lack of onsite drainage, runoff to near streams, from surface and subsurface pathways (Withers *et al.*, 2014).

#### 4.5.2.2 Hampshire Avon TN and NPOC

The mean NPOC concentration at the Hampshire Avon catchment (displayed in Figure 4.7) was  $7.69 \pm 0.89 \text{ mg L}^{-1}$ , which is higher than the Conwy and lower than the mean concentration at Scottish catchments. However, the mean TN concentration at the Hampshire Avon is much greater than at the Conwy and the Scottish catchments, at  $7.87 \pm 1.33 \text{ mg L}^{-1}$ . However, as the data used to generate these Figures contains the NPOC and TN concentrations from site 19 and 20 at the Hampshire Avon, it is important to note that the high concentrations of C and N in these samples could be driving these relationships. Despite this, with site 19 and 20 removed from the equation, a mean TN concentration of  $5.25 \pm 0.72 \text{ mg L}^{-1}$ , which is still 6.9 times greater than the TN concentration at Conwy and more than 7.7 times greater than the Scottish sites, thus showing that the TN concentration at the Hampshire Avon is greatly higher than at other catchments, despite the influence of sewage treatment works, and is likely attributed to the intensive agricultural practices which take place in the catchment.

#### 4.5.2.3 Hampshire Avon Land Use

A Pearson's correlation was conducted between ion data and land use data from the Hampshire Avon, and very few correlations were found. In fact, nitrate was found to be the only ion that correlated with any land use type, slightly acid loamy and clayey soils with impeded drainage and slowly

permeable seasonally wet slightly acid but base rich loamy and clayey soils at  $r=0.895$  and  $r=0.796$  respectively, both with a significance of  $p<0.05$ .

The data in Figure 4.12 shows strong positive regressions between mean NPOC concentration and slightly acid loamy and clayey soils with impeded drainage and slowly permeable seasonally wet slightly acid but base rich loamy and clayey soils, both suggesting that as the area of these land use types increase, so does the concentration of NPOC at these sites.

The statistical analyses for the data displayed in Figure 4.11 are calculated as a mean for each land use type, and therefore, the spike in nitrate and sodium concentrations at site 19 will be made much less significant when averaged with all other sites in arable and horticulture classed land. The same is true for site 20 in calcareous grassland, although the effect here will be lesser as there are only 2 data points to average.

### 4.5.3 Conwy Catchment

#### 4.5.3.1 Conwy Ions

Although there are many significant differences observed between nitrate concentrations of all Conwy sites, the three sites with the highest number of significant differences are NM21, NM22 and DM3. NM21 and NM22 are both located in the headwaters of the Conwy. These sites are likely significantly lower than many other sites as they are draining the nutrient poor Migneint blanket bog dominated catchments of the headwaters of the Conwy. The bulk of the significant differences observed here are between the aforementioned highlands and the lower catchments, likely to have high nutrient input due to agricultural and urban sources. Sites NM22 and NM23, catchments dominated by agriculture, are shown to be significantly different to NM13, NM14, NM17 and NM18, which are all very natural and relatively unadulterated catchments, in terms of human and agricultural interaction. They are also significantly different to sites NM21 and NM22, located in the Migneint, as these two sites are very low in nitrate (Figure 4.15 Part 4).

Site DM3 has a mean sulphate concentration of  $27.05 \pm 5.24 \text{ mg L}^{-1}$ , over 9 times greater than the mean sulphate concentration of all Conwy of  $2.9 \pm 0.35$

mg L<sup>-1</sup>. Sulphates and sulphuric acid products are used in the production of fertilisers (sulphate can also be found in runoff from agricultural fields), chemicals, dyes, glass, paper, soaps, textiles, fungicides, insecticides, astringents and emetics. They are also used in mining, wood pulp, metal and plating industries, and also in sewage treatment (WHO, 2004). Sulphate was commonly attributed to acid rain, but since the introduction of low sulphur petroleum (in 2000) and diesel (2001) the concentrations are now much lower as there is less sulphur to be collected within the rainfall (Likens *et al.*, 1996). Sea spray is also a known sulphur source to freshwater, and given that site DM3 is situated next to the coast, this could also be a reason for the higher concentrations detected here.

Site DM3 contains a sewage treatment plant (STP) which treats waste from approximately 88,000 people, and therefore the effluent from this treatment works is likely to increase the load of sulphate in the rivers draining the catchment. The WHO state that sulphate concentrations are elevated in municipal drinking water sources by the treatment methods used (WHO, 2004). It is possible that the common coagulant, aluminium sulphate, is used at this STW to bind the suspended solids in semi treated water, to aid removal, it is possible that excess coagulant added to drinking water could be another potential source of the high sulphate concentrations detected at DM3, where the coagulation of suspended solids in the STW could be entering the river, unregulated. Sulphide is undesirable in freshwaters as it promotes methylation of mercury to its most toxic and bio-accumulative form, methyl mercury, it is toxic to plants and animals, it promotes the release of nutrients from sediments and enhances the biodegradation of organic soils (Orem, 2011). Therefore, a biological study of flora and fauna in this stream could help to identify whether the high sulphate concentrations determined here are only relative to other sites in the Conwy catchment, or whether the sulphate concentration here is exceptionally high and damaging to the natural environment.

As their use in industry, and their purposes are vast, it is unlikely that sulphate concentrations in freshwater do not increase in catchments with high concentrations of human populations, such as DM3.

DM3 is also found to be a driver in the positive relationships discovered between land use and specific ion concentrations in the water that drains from them, as outlined in Figures 4.20 and 4.21. It would seem that this influence is because the catchment of DM3 is located within a suburban/urban location.

Ionic data presented further shows how the sites differ in terms of 12 ions detected in the samples. Site DM3 at the Conwy catchment was significantly different to 4 other sites in terms of nitrite concentration – CC54, NM18, NM22 and NM26. As previously mentioned, DM3 drains land with many different land use practices - the southern half of the catchment appears to be primarily agricultural grassland, whereas the more northern side of the catchment encompasses nature reserves, camp sites, sewage treatment works, alongside a town and the major A-road into north Wales (the A55).

Progressive increases in nitrite concentrations in freshwater environments have been attributed to an increase in the use of artificial fertilisers, wastes (especially from animals), and changing of land uses (WHO, 2016), and it is likely to be due to these reasons that site DM3 is significantly different to other sites.

Many sites statistically significantly differed from each other in terms of nitrate concentration, the highest 3 sites in terms of quantity of significant differences were CC60, NM21, NM22 and NM23 with 9 – 11 significant differences between sites. Sites CC54, NM13, NM14, NM17, and NM24 all had 7 significant differences between sites. These sites are all dominated by grassland and/or bog land use types, which in this region are both used for sheep grazing, and it is suspected that the waste products from these sheep are increasing the nitrate concentration. Indeed, Poor and McDonnell (2007) state that a significant portion of nitrogen export from catchments is due to non-point source fertiliser runoff, and that the proportion of agricultural land in a catchment is often correlated to stream nitrate export (Poor and McDonnell, 2007).

For phosphate, NM19 was the only site in the Conwy catchment found to significantly differ with any other sites; DM3 and NM23. NM19 drains primarily managed coniferous woodland. NM19 was found to have a mean

concentration of  $0.002 \pm 0.00064 \text{ mg L}^{-1}$  phosphate, some 11 times smaller than the mean phosphate concentration for all Conwy sites. There is evidence that woodlands can reduce phosphate pollution through nutrient uptake and the trapping of soil-bound material (Broadmeadow and Nisbet, 2010) by roots and leaf litter. It has been shown that sheep faeces represent a greater source of soluble reactive phosphorus when compared to that of cattle (McDowell, 2006), which goes some way to explaining the differences between NM19 and sites DM3 and NM23, which had phosphate concentrations of  $0.18 \pm 0.08 \text{ mg L}^{-1}$  and  $0.13 \pm 0.04 \text{ mg L}^{-1}$ , many times greater than the Conwy catchment mean of  $0.02 \pm 0.012 \text{ mg L}^{-1}$  phosphate

#### 4.5.3.2 Conwy TN and NPOC

Carbon concentrations in water draining the Conwy catchment has been the focus of many recent studies (Adams *et al.*, 2015 and Hughes *et al.*, 2016).

Site NM23 is dominated by sheep grazing and other agricultural activities. The catchment is steep sided with a history of flooding, and the animals have free access to the stream, potentially reasons for the high NPOC detected here. On the contrary, site NM17 is situated in the steep slopes of the Dyffryn Mymbyr valley. This stream is fed by springs high up in the mountains and the water flows over bedrock down to the Nantgwryd river, which is sampled from at site NM16. The mean concentration of NPOC at both of these sites is  $1.33 \pm 0.16 \text{ mg L}^{-1}$  (NM16) and  $1.42 \pm 0.16 \text{ mg L}^{-1}$  (NM17), over 4 times lower than the mean concentration for all sites in the Conwy catchment. This is likely due to the pristine condition of these catchments and the fact that they drain into primarily bedrock streams, with therefore little organic carbon inputs from erosion, leaching and grazing, thus explaining the strong significant differences recorded between the datasets here.

It is interesting to note, however, that although the bog and peat dominated catchments of NM21 and NM22 have a significantly lower TN concentration than the agriculturally dominated NM23 and NM24, strong positive

correlations were found between TN concentration and bog vegetation class and blanket bog peat soils soil class.

Mean TN concentrations detected at the 27 Conwy sites showed that many catchments were significantly different to other Conwy catchments. Whilst all sites significantly differed from a minimum of 4 other sites at both  $p < 0.01$  and  $p < 0.05$ , DM3 was the most significantly different site, with differences between 20 other sites at  $p < 0.01$ . CC60 was also significantly different to 18 different sites at  $p < 0.01$ , and NM23 and NM24 which were significantly different at  $p < 0.01$  to 19 other sites each (although one relationship between NM24 and NM28 has a significance of  $p < 0.05$ ). NM23, NM24 and CC60 are intensively sheep grazed grassland and/or have other agricultural practices taking place on them, and DM3 drains agricultural and urban/suburban land which includes a sewage treatment plant, and these sites have been found to contain high nitrate concentrations. Indeed, all 4 sites mentioned here produce a mean nitrate concentration of  $8.4 - 9.3 \text{ mg L}^{-1}$ ). It is interesting to note that CC60 TN is comprised of 95% inorganic N, DM3 is comprised of 72% inorganic N, and NM23 and NM23 are both comprised of 86% inorganic N, suggesting that the major drivers of these statistically significant findings are inorganic sources of N. Ammonium, nitrite and nitrate are the most common ionic forms of DIN in aquatic ecosystems, with sources ranging from atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen rich geological deposits, nitrogen fixation by certain prokaryotes and biological degradation of organic matter. However, human influences have increased inorganic nitrogen entering freshwater ecosystems *via* point sources and nonpoint sources (Camargo and Alonso, 2006). Therefore it is possible that the human activities that are taking place on the land that drains to these sites is a major source of inorganic N to the aquatic ecosystems downstream of these catchments.

A mean NPOC concentration of  $13.16 \pm 0.44 \text{ mg L}^{-1}$  at CC54 compared to the overall mean concentration (for the entire Conwy catchment) of  $5.77 \pm 0.73 \text{ mg L}^{-1}$  NPOC show this site to be over twice the mean concentration of NPOC. However, site NM22 has a concentration higher than this at  $17.23 \text{ mg L}^{-1}$ , although one data point recorded in July 2016 of  $62.75 \text{ mg L}^{-1}$  is likely

erroneous as it is more than 3.8 times greater than the mean. Despite this, the statistical analysis shows that site NM22 is not significantly different to any other sites, despite the inclusion of this potentially problematic data point. If the offending data point is removed, however, site NM22 is statistically significantly different to sites NM16 and NM17 at  $p < 0.01$  each, not shown in Table 4.6.

#### 4.5.3.3 Conwy Land Use

Site DM4 is significantly different to many other sites in the Conwy catchment, including many sites that are assumed to have less polluted or nutrient enriched water as they are in the headlands of the Conwy. DM4 is situated west of the Conwy estuary and contains campsites, fishing lakes and nature reserves, as well as hotels, residential and commercial properties; the reasons for a significantly different sulphide concentration here is likely due to the same reasons as site DM3. Out of all of the significantly different sites found, NM23 and NM24 are significantly different, in terms of ions and TN/NPOC etc., to noticeably more other sites than the remaining sites, and this is likely due to the agricultural activity in the area. NM23 and NM24 both comprise of over 70% improved grassland, which is used for both sheep and cattle farming. Waste products from these animals, along with artificial fertilisers that may be spread on the fields to improve fodder growth, contain sulphate alongside phosphates, nitrogen and potassium. With decreasing sulphur deposition due to the combustion of cleaner fuels (and therefore less acid rain, which contains sulphuric acid), agricultural land owners are applying more sulphur to their land as a fertiliser to boost plant productivity, and this will likely show in the water draining from the land. It has been suggested by Evans *et al.*, 2005, that the decline in sulphur and other acidic ion deposition since 1988 could be the reason for elevated DOC concentrations in bog draining water (see Figure 4.16), as pH has been shown to be positively related to DOM release.

This land in DM3 is highly developed and diverse, containing quarries, farms and golf courses, as well as residential and commercial properties and a nature reserve. With such a vast array of different land use practices found in a small

catchment, it is no surprise that there are significant differences determined between this site and others, especially in terms of sulphate concentration. Site DM3, which is located right next to the mouth of the river Conwy, comprised of 17% suburban land use, and less than 3% of the land is classed as urban. When this is compared to other sites, it is possible to determine that site DM3 contains over 19 times more suburban land use, as a percentage of total catchment area, than the mean percentage cover of all other sites (including data from DM3). Percentage land use classed as urban is over 22 times higher at DM3 than the mean percentage of urban land use in all other catchments, thus possibly explaining the higher number of significant differences noticed between DM3 and other sites.

Freely draining slightly acidic but base rich soils at the Conwy catchments were found to correlate with NPOC ( $r=0.963$   $p<0.05$ ), which shows that, as the area of land classed as freely draining slightly acidic but base rich soils increased, so did the NPOC concentration in the water draining it.

TN correlated with the area of neutral grassland, showing that, as the area of neutral grassland increases, as does TN concentration ( $r=0.915$ ,  $p<0.05$ ). Interestingly, a positive correlation is found between TN and the area of bog at  $r=0.699$ ,  $p<0.01$  ( $n=20$ ), again, showing that as the area of land classed as bog increases, as does the TN concentration. Furthermore, blanket bog peat soils, urban, freely draining slightly acidic but base rich soils, freely draining slightly acidic loamy soils and loamy and clayey floodplain soils with naturally high ground water were all found to correlate positively with TN, again showing that as the area of blanket bog peat soils increased, so did the TN concentration in the water draining it. Therefore, it is sensible to assume that in the Conwy catchment, it may be possible to predict TN concentration depending upon the area of these land uses in the catchment. However, due to the lack of significant relationships between TN and these land use classes in the Hampshire Avon and Scottish sites it will not be possible to extend these predictions to other catchments.

#### 4.5.3.4 Amino Acids and Carbohydrates

In terms of carbohydrate content, there are no significant differences between the sites at the Conwy and Hampshire Avon catchments individual sampling sites. This lack of significant differences suggests that the carbohydrate concentrations detected in this study are typical of a freshwater catchment, and that there are no major inputs of carbohydrates from any of the catchments studied.

Similarly to carbohydrates, there were no significant differences detected between the sites in terms of amino acid concentration. Individual amino acid data was collected for the Conwy sites, showing that the most abundant amino acids are aspartic acid (at a mean concentration of 213.79 nmol mL<sup>-1</sup>), phenylalanine (at a mean concentration of 115.1 nmol mL<sup>-1</sup>) and tyrosine (detected at a mean concentration of 31.38 nmol mL<sup>-1</sup>). A mean total amino acid concentration detected in freshwater environments has been quoted at 40 nM (Kaplan and Newbold, 2004). The mean total concentration of amino acids detected in the Conwy catchment is 14.51 ±7.88 nM, much lower than the typical concentration quoted above. Statistical analysis of individual amino acid concentration data per site at the Conwy catchment was not possible due to the low number of data points detected in analysis.

#### 4.5.5 Scottish Catchments

##### 4.5.5.1 Scottish Ions

Ion concentrations were compared on 3 levels: between sites, between catchments and between land use covers.

On a catchment level, Scottish ions were found to be overall, much lower than at the Hampshire Avon and Conwy catchments. In fact, the only 4 ions that are greater at the Scottish catchments than at the Conwy catchment are sodium (19.45% greater mean concentration than at Conwy), ammonium (71.02%), calcium (54.53%) and finally magnesium (24.28%). There were no ions at Scottish sites that were significantly greater than the Hampshire Avon (see Table 4.2).

Between sites at the Scottish catchments, although it is difficult to compare the different catchments due to the fact that they were selected to be different, sites 3, 4, and 5 appear to have lower mean concentrations of ions when compared to the other sites. Site 10 contains the highest concentration of calcium, site 2 contains the highest concentration of nitrate and site 7 contains the greatest concentration of potassium. Fluoride and bromide concentrations are dwarfed by all other ionic data. Sulphate and calcium concentrations differed the most between sites, as shown by the ANOVA (Table 4.16).

Finally, the comparison between land use types suggests that catchments dominated by rough grassland vegetation releases significantly higher concentrations of bromide than other land use types in the Scottish catchments. Despite other significant differences between other sites, no one land use type presented as many significant differences as rough grassland, suggesting that, in the Scottish catchments, rough grassland dominated catchments could prove problematic for treatment due to the natural formation of brominated disinfection byproducts.

#### 4.5.5.2 Scottish TN and NPOC

Although Figure 4.7 shows that the Hampshire Avon has a significantly greater mean NPOC concentration when compared to the mean NPOC concentrations of the Conwy and Hampshire Avon catchments, Figure 4.30, and the stats associated with it, show that the TN and NPOC concentrations between sites in Scotland differ significantly, similarly to the Hampshire Avon and Conwy catchments, thus furthering the consensus that DOM character and composition varies greatly from catchment to catchment.

The Scottish catchments were selected as intermediates in terms of nutrient concentrations and to determine if any relationships found were likely to be site specific or more universal as they were hypothesised to be similar to the Hampshire Avon and Conwy catchments in terms of vegetation and land use.

Figures 4.7 and 4.9 show that the Scottish sites were significantly higher than the Hampshire Avon and Conwy in terms of total amino acid concentration and total NPOC concentration. Inter-site analysis of ions showed that there

were significant differences between chloride, sulphate, sodium, potassium, nitrate and phosphate at  $p < 0.01$  and nitrite at  $p < 0.05$ . Perhaps the most obvious reasons that similarities were not found can be attributed to the land use data.

#### 4.5.5.3 Scottish Land Use

The statistics displayed in Table 4.19 show that as the area of suburban land increases, as does the concentration of phosphate in the streams draining it. This is likely due to the fact that many suburban properties in this area are likely not connected to mains drainage, and therefore rely on septic tanks and domestic waste water treatment plants. Problems with these plants, such as leakage, overflowing or improper bacteria balance could be responsible for this correlation.

Other strong correlations shown in Table 4.19 are found between fluoride and sandstone and conglomerate interbedded, where as the area of sandstone and conglomerate interbedded increases, the fluoride concentration decreases. Misra (2013) outlines studies that suggest that high fluoride in groundwater is generally expected in the areas where fluoride bearing minerals are abundant in the rocks, because fluoride leaches out and dissolves in groundwater during weathering and movement of water within the rocks and soils. As sandstone and conglomerate is typically made up of mineral particals such as quartz and feldspar, it makes sense that the fluoride concentration in water draining the catchment decreases as the area of a non-flouride containing bedrock increases. The other major relationship is between phosphate and humus iron podzols. Studies outlined in Giesler *et al.*, (2005) have shown that iron is likely to accumulate in the humus layer of groundwater discharge areas, and thus, potential sorption sites for phosphate are created in these humus soils, which explains the strong positive relationship between phosphate and humus iron podzols. Nitrate and nitrite concentrations are likely elevated in improved grassland as this will be used for agricultural grazing.

## 4.6 Conclusion

From the data presented in this chapter, it is apparent that the characters of DOM (in terms of TN and NPOC, and also ions) between the Hampshire Avon, Conwy and Scottish catchments used in this study are vastly different, and due to the fact that these sites were chosen to represent a nutrient gradient, this data can be extrapolated to other catchments of similar characters. Whilst differences are to be expected between the overall sites (as they were chosen due to the belief that they have different nutrient inputs), it is the differences between the individual sampling sites within these overall catchments that show how the organic matter composition can vary from one site to another, despite how similar their catchment characteristics are.

Some relationships between catchment characters were detected though. For example, the increase of TN concentration as the area of urban classed land increased in the Scottish sites, and the increase of the nitrite ion upon the increase of suburban land use in the Conwy catchment show that densely populated land is influencing the nitrogen concentration in the water draining from the land. With an ever increasing population, and therefore an ever increasing nitrogen content in the water draining from this land will lead to further instances of nitrate vulnerable zones (NVZ). The Hampshire Avon catchment already encompasses land classed as 'surface water NVZ' and also 'groundwater NVZ'. Although the Conwy catchment does not currently encompass land classed as NVZ, an increase in population in this region could lead to the designation of NVZs in this region. Some Scottish catchments also fall into NVZs, and again, an ever increasing population will lead to more issues.

Figures 4.20 Parts 1 and 2 show strong positive regressions between 8 ions and neutral grassland land use type at the Conwy catchment. This suggest that activities that take place upon neutral grassland, or the soil and bedrock that play host to this vegetation class, release many nutrients, and as the area of the land use increases, so does the concentration of the ions. Furthermore, the concentration of 5 ions was found to increase in a positive regression with the area of land classed as suburban, as shown in Figure 4.21. This data could be useful in estimating the nutrient flush from neutral grassland in the Conwy catchment, however, it is unlikely that this data could be extrapolated to other catchments as the studies here have shown that the DOM between 3 catchments vastly differs.

## 4.7: References

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**Chapter 5a:**  
**Formation of THM4**  
**From Dissolved Organic**  
**Carbon:**  
**The Organic Chemistry**  
**of the Formation of**  
**Carbonaceous**  
**Disinfection By-Products**

## 5a.1: Introduction

Water, in its purest and chemical form, is always a ratio of two hydrogen atoms to one oxygen atom, although it is very rarely found in this exact form outside of laboratories. As a universal solvent, and despite looking, smelling and even tasting clean, water carries with it many other chemicals (Calderon, 2000), and in the natural environment, these include naturally occurring organic matter. Natural organic matter (NOM), comprises of a complex and heterogeneous mixture of aromatic and aliphatic hydrocarbon structures that have attached amide, carboxyl, hydroxyl, ketone, and various minor functional groups, and therefore, exploring its functional character is challenging (Leenheer & Croue, 2003, and Świetlik & Sikorska, 2005). The quantity of NOM found in water differs with climate and hydrological regime, as well as a number of other environmental factors, therefore the character of NOM can vary greatly due to source and season (Matilainen and Sillanpää, 2010), thus posing further issues for WTW.

NOM removal in water destined for human consumption is important as its presence causes many problems in drinking water and drinking water treatment processes. These include negative effects on water quality (by causing colour, taste and odour problems), increased coagulant and disinfectant doses at the WTW (which results in greater waste sludge volumes and greater production of harmful DBPs), promotion of biological growth in distribution systems, and increased levels of complex heavy metals and adsorbed organic pollutants (Matilainen *et al.*, 2010). Although the steps in treating drinking water are all necessary in their contribution to maintaining the safety of drinking water, the most problematic is the addition of chemical coagulants and disinfectants.

The disinfection of drinking water is paramount to prevent the widespread dispersion of waterborne diseases, for example, cholera. This disease, which had swept through London, UK in 1849, was found to be caused by the drinking of unsanitary water, and hence the need for disinfection arose. The introduction of chlorine as a chemical disinfectant was an alternative for those communities that could not afford filtration plants, but it is now responsible for the rapid decline in cholera, dysentery and typhoid worldwide today (alongside better sanitation and wastewater treatment) (Calderon, 2000). Although chlorine is the most commonly used disinfectant, ozonation and chloramination are also popular, and are sometimes used in tandem. The numbers of utilities using free chlorine relative to those using chloramines for residual in the distribution system have fluctuated dramatically over time

based on the availability of ammonia (for example, during World War II) (Kirmeyer *et al.*, 2004). In recent years, in water treatment works where DBP regulations are often breached, chloramine has been used as it forms much lower concentrations of THM4. Chloramination usually involves the addition of ammonia and chlorine as individual chemicals, usually with the chlorine first. However, chloramination has been found to increase the formation of certain nitrogenous disinfection byproducts such as cyanogens halides, which have been shown to be considerably more cytotoxic and genotoxic than the regulated THMs (Goslan *et al.*, 2009). To date, only 11 DBPs are regulated by the USEPA under the Stage 2 Disinfectants/DBP Rule; THM4, HAA5, chlorite and bromate (Mash *et al.*, 2014). In the UK, only THM4 is currently limited, to 100 µg L<sup>-1</sup> (Tables 5a.1 and 5a.2).

Table 5a.1: Tolerable daily intake (TDI) (i.e. the concentration that produces no effect in the appropriate toxicological studies of the chemical) for the most relevant potentially toxic compounds (Adapted from Palacios *et al.*, 2000)

<b>Compound</b>	<b>TDI (µg kg<sup>-1</sup> body weight/day)</b>	<b>TDI (µg L<sup>-1</sup>)</b>	<b>Percentage of TDI allocated to drinking water</b>	<b>Excess lifetime cancer risk</b>
<b>CHCl<sub>3</sub></b>	10	200	50	10 <sup>5</sup>
<b>CHBrCl<sub>2</sub></b>		60		10 <sup>5</sup>
<b>CHBr<sub>2</sub>Cl</b>	21.4	100	20	N/A
<b>CHBr<sub>3</sub></b>	17.9	100	20	N/A

Table 5a.2: List of trihalomethane compounds that are currently regulated in UK drinking waters.

Group	Names	Concentration limited?	Precursors	Reference
<b>Trihalomethanes (THMs)</b>	Trichloromethane (Chloroform) Dibromochloromethane Bromodichloromethane Bromoform	<b>UK:</b> No single compound limit <b>USA:</b> 80 µg L <sup>-1</sup> (USEPA)	<b>Aromatic:</b> Aniline Resorcinol <b>Aliphatic:</b> 2,4-pentanedione 3-oxopentanedioic acid L-aspartic acid Ketones	<b>Bond et al., 2012</b> <b>Colman et al., 2011</b> <b>Heller-Grossman et al., 1993</b>

With several disinfection practices being utilised at treatment works, the understanding of the chemical pathways that lead to the formation of DBPs can provide a fundamental insight into the best reduction and removal methods of these DBPs and their precursors, which can ultimately lead to the complete removal of them, before and after the treatment stage (Figure 5a.1).

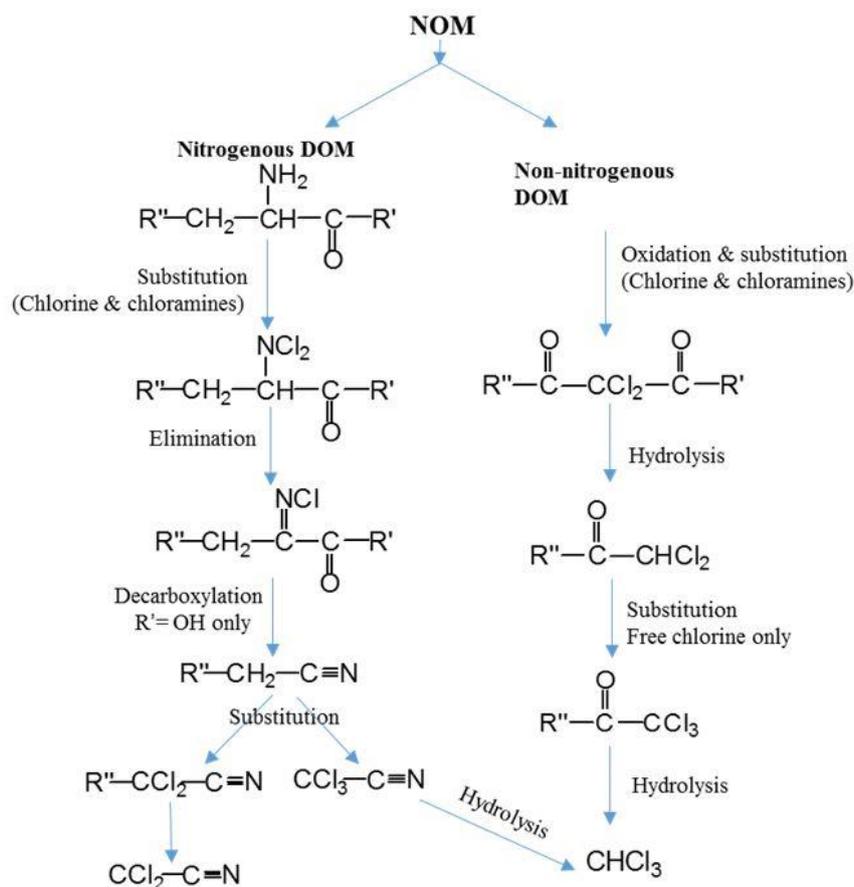


Figure 5a.1: The basic DBP formation pathways during chlorine and chloramine disinfection of natural water (Ma *et al.*, 2016).

An understanding of the merits and disadvantages of different disinfectants in reducing the formation of THM4 has been attempted by Hua and Reckhow (2009), who compared chlorine and chloramines efficiencies. They found that THM comprised 2.20% of total organic halogen (TOX) formed under chloramination and 23.10% of TOX under chlorination. Similarly, unknown total organic halogen (UTOX) formations were calculated between treatments of chlorine and chloramine, with chlorine forming 53.00% UTOX and chloramination forming 83.10% UTOX. This work highlights the fact that chloramines form a lower concentration of THM but do form significantly higher concentrations of UTOX, which could contain currently undiscovered compounds that could be many times more hazardous than the regulated THM (Hua and Reckhow, 2008).

The occurrence of DBPs in treated and distributed drinking water can vary depending on the quality of the original water source, and the treatment procedures at the treatment works. Overall, the influential factors are the nature and quantity of NOM (especially humic substances), along with pH, temperature and residence time of water in the distribution system (Rodriguez *et al.*, 2004). The formation of DBPs depends primarily on source water quality characteristics, and on the location in the treatment process where the disinfectant is added. As a general rule, less DBPs will be formed when the disinfectant is added later in the treatment process (Liang and Singer, 2003), due to the increased removal of organic matter by processes such as filtration, coagulation and dissolved air flotation. However, some DBPs can be formed from naturally occurring halogens in fresh water before the treatment stage, such as bromide and chloride, reacting with organic matter present in the water.

C-DBPs such as THMs, HAAs, haloketones and chloral hydrates can be formed from the chlorination of blue-green algae *Microcystis aeruginosa* (Fang *et al.*, 2010). THMs and HAAs are the most abundant DBPs currently detected in chlorinated water (Fang *et al.*, 2010), and thus, the majority of DBP studies focus on these.

Although a broad spectrum of compounds have been recognised as THM precursors, including humic substances, algal cells, polyelectrolytes, tannic acid and chlorophyll. Research has shown that aromatic compounds are the primary contributors to THM formation (White *et al.*, 2003). A study by Symons *et al.*, in 1975 found that concentrations of THM<sub>4</sub> at finished drinking water supplies were found at concentrations up to 311  $\mu\text{g L}^{-1}$  (Symons *et al.*, 1975 and Munson *et al.*, 1982), some 3 times greater than the MCL.

There are many reactions that can yield THMs, but the haloform reaction is likely the most widely studied. The first step involves the formation of an enol (an alkene with a hydroxyl group joined to one of the carbons in a double bond), known as enolisation. This is a slow procedure, but can be promoted by acid or base (therefore any constituent that is capable of promoting enolisation could be expected to increase THM formation). Once it is formed, the enol is subjected to rapid halogenation, with halogen substitution on the methyl group, which occurs until the methyl group is trihalogenated. This trihaloketone is then attacked by hydroxide to form a THM molecule, and the conjugate base of a carboxylic acid (Blatchley *et al.*, 2003). See Figure 5a.2 for an illustrated diagram of a haloform reaction to form chloroform, and Table 5a.2 for a list of the THM<sub>4</sub> compounds regulated in UK drinking water.

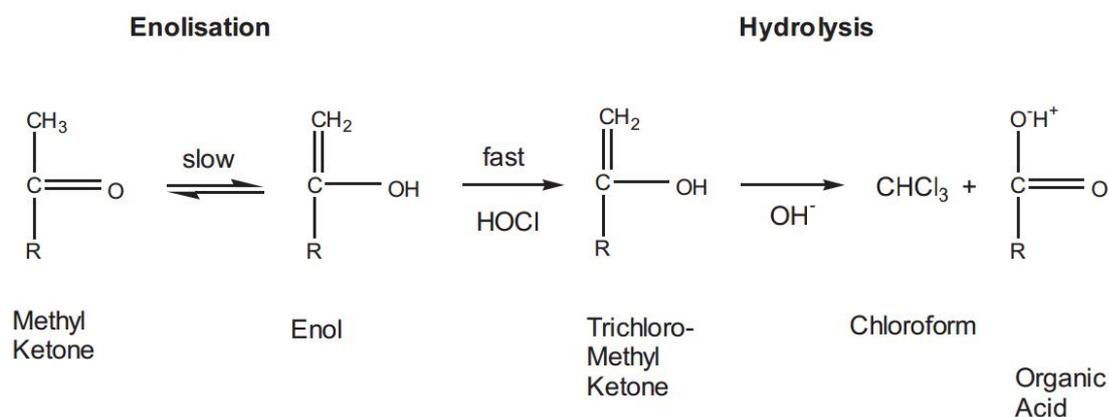


Figure 5a.2: Haloform reaction pathway for the enolisation of a methyl ketone to chloroform and organic acid (adapted from Blatchley *et al.*, 2003).

The current work aims to determine the formation of these THM4 compounds from two different disinfectants (chlorine and chloramine), to provide a greater understanding of the chemistry of DBP formation. These sites have not been studied in a comparison before, but have been studied individually. However, rising DOC concentrations and atmospheric issues such as the decline of sulphur deposition (due to cleaner combustion and fuels) mean that DOC characteristics from previous studies may not reflect current day samples. Samples from two different catchments will be chlorinated and chloraminated to compare the formation of both total THM4 formation potential (TTHM4FP), standardised THM4 (STHM4) and individual THM4 compound formation at both sites. Comparisons will provide information on the formation of THM4 between sites in the same catchment, and between catchments overall, and which disinfection method produces higher concentrations of the regulated THM4.

### 5a.1.1: Hypothesis

It is hypothesised that identical chlorination and chloramination treatments will form different concentrations of THM4, and that the THM4 formation may not be due to a relationship between DOC concentration.

## 5a.2: Methods

Samples were collected from two different catchments in the UK; the river Conwy catchment, situated in north Wales and the Hampshire Avon catchment, in south western UK (see Chapter 2 for full details on catchments). The Hampshire Avon contains 19 sample sites and the Conwy Catchment contains 27.

Samples were filtered through 0.45  $\mu\text{m}$  Whatman membrane filters prior to storage. Before analysis, DOC concentration data was collected, and samples were then diluted to 1  $\text{mg L}^{-1}$  DOC before being chlorinated/chloraminated to excess (5  $\text{mg L}^{-1}$  free chlorine to 1  $\text{mg L}^{-1}$  DOC), buffered, and stored in the dark at 25 °c for 7 days. If chloramine was the disinfectant of choice, a preformed solution of ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and sodium hypochlorite ( $\text{NaOCl}$ ) was added at an equivalent concentration of free chlorine to the chlorination method. Full method details are outlined in Chapter 2.

THM4 data was generated by gas chromatograph mass spectrometry (GC-MS) and solid phase micro extraction (SPME), with detection by an electron capture detector (ECD). Data generated shows the STHM4 concentration (i.e. THM4 formation potential standardised to 1  $\text{mg L}^{-1}$  DOC). The full method is also outlined in Chapter 2.

Statistical analysis was performed using SPSS software (IBM, New York, USA). Data was tested for normality, and transformed by  $\log_{10}$  if necessary.

Although not strictly classed as DOC, bromide concentrations were also generated to compare to the brominated THM4 species to determine whether a relationship was present. The data was generated *via* ion chromatography, following the method outlined in Chapter 2.

### 5a.3 Results

Whilst normalising the data by DOC (i.e. displaying STTHM4 data, the concentration of THM4 formed from disinfecting a sample containing 1  $\text{mg L}^{-1}$ ) ensures that the data is relevant to a DOC based examination, as this study is exploring the formation of carbonaceous and nitrogenous compounds, it is important to analyse the TTHM4FP (i.e. the THM4 concentration from 1  $\text{mg L}^{-1}$  multiplied by the DOC concentration of the sample) as the data generated takes into consideration other important components of the water such as ion and cation concentrations, which can impact the formation potential. The STHM4 and TTHM4FP are displayed in Table 5a.3 for clarity.

Table 5a.3 STTHM4 and TTHM4FP concentrations for Hampshire Avon and Conwy sites, under both chlorination and chloramination.

Site ID	DOC (mg L <sup>-1</sup> )	Treatment	STTHM4	TTHM4FP	Treatment	STHM4	TTHM4FP
<b>Avon</b>							
1	5.07	Chloramination	18.54	93.96	Chlorination	67.00	339.62
2	6.71	Chloramination	12.39	83.05	Chlorination	58.90	394.96
3	6.66	Chloramination	7.70	51.29	Chlorination	96.31	641.70
4	5.58	Chloramination	6.92	38.63	Chlorination	58.81	328.34
5	6.82	Chloramination	9.43	64.33	Chlorination	65.09	443.90
6	6.42	Chloramination	16.79	107.82	Chlorination	75.89	487.39
7	12.59	Chloramination	19.46	245.11	Chlorination	114.51	1441.95
8	15.59	Chloramination	8.47	132.11	Chlorination	344.24	5366.06
9	5.95	Chloramination	5.32	31.65	Chlorination	62.69	372.87
10	4.83	Chloramination	7.60	36.71	Chlorination	55.00	265.76
12	4.87	Chloramination	5.13	24.99	Chlorination	48.93	238.45
13	5.10	Chloramination	11.39	58.02	Chlorination	43.70	222.64
14	5.33	Chloramination	2.21	11.76	Chlorination	42.32	225.37
15	5.02	Chloramination	2.94	14.79	Chlorination	45.68	229.54
16	5.28	Chloramination	1.46	7.69	Chlorination	51.53	272.11
17	4.48	Chloramination	5.10	22.88	Chlorination	41.84	187.54
18	3.71	Chloramination	5.28	19.58	Chlorination	63.82	236.57
19	31.21	Chloramination	5.60	174.81	Chlorination	50.53	1576.77
20	6.58	Chloramination	14.48	95.30	Chlorination	52.39	344.86
<b>Conwy</b>							
CC13	3.329803	Chloramination	56.31	187.49	Chlorination	534.46	1779.66
CC5	3.362636	Chloramination	71.06	238.95	Chlorination	427.19	1436.48
CC54	13.16392	Chloramination	83.44	1098.37	Chlorination	370.21	4873.37

CC6	4.608022	Chloramination	80.70	371.86	Chlorination	451.43	2080.21
CC60	5.479666	Chloramination	82.89	454.21	Chlorination	259.92	1424.25
CC88	6.388525	Chloramination	23.53	142.57	Chlorination	351.64	2130.87
DM2	6.641915	Chloramination	72.31	461.94	Chlorination	483.68	3089.97
DM3	6.196134	Chloramination	101.29	672.77	Chlorination	113.70	755.20
DM4	2.680479	Chloramination	144.04	892.51	Chlorination	351.15	2175.80
DM5	11.9458	Chloramination	78.15	209.48	Chlorination	312.42	837.44
NM13	9.799625	Chloramination	362.21	4326.88	Chlorination	498.65	5956.77
NM14	1.296016	Chloramination	121.19	1187.60	Chlorination	465.32	4559.92
NM16	1.422813	Chloramination	81.22	105.26	Chlorination	447.82	580.38
NM17	3.709483	Chloramination	45.72	65.05	Chlorination	413.92	588.93
NM18	3.008912	Chloramination	99.05	367.42	Chlorination	371.18	1376.90
NM19	5.37865	Chloramination	79.17	238.21	Chlorination	499.23	1502.14
NM20	10.71477	Chloramination	47.09	253.30	Chlorination	291.23	1566.42
NM21	17.22995	Chloramination	73.59	788.48	Chlorination	434.07	4650.93
NM22	4.644868	Chloramination	158.13	2724.57	Chlorination	383.78	6612.49
NM23	5.687684	Chloramination	57.51	267.10	Chlorination	190.48	884.73
NM24	1.957453	Chloramination	110.95	631.04	Chlorination	298.55	1698.06
NM25	4.280028	Chloramination	50.12	98.11	Chlorination	430.16	842.03
NM26	6.046768	Chloramination	90.45	387.12	Chlorination	378.24	1618.87
NM27	3.905346	Chloramination	34.47	208.43	Chlorination	321.82	1945.98
NM28	3.458364	Chloramination	86.87	339.27	Chlorination	315.81	1233.35
NM29	3.396836	Chloramination	83.73	289.56	Chlorination	392.71	1358.13
NM30	3.329803	Chloramination	85.30	289.76	Chlorination	482.80	1640.01

The data presented in this section shows formation potentials of THM4 and individual THM compounds. These formation potentials are generated from raw, unprocessed water, and are chlorinated to excess in terms of chlorine dose and contact time, and are therefore not representative of real world drinking water conditions. It does, however, provide information on the succetability of the DOC to form THM4.

The TTHM4FP data represents the total THM4 that is able to be formed from the raw DOC concentration of the sample, given an excess of chlorine. This dataset shows which sites are most likely to contribute to the formation of THM4 at a treatment works if the DOC concentration is not reduced before disinfection.

Standardised THM4 data represents the total concentration of THM4 that could form from just 1 mg L<sup>-1</sup> DOC, thus producing a dataset which takes into account the characteristics of the DOC rather than just the concentration of DOC.

Finally, THM4 data is compared between treatments of chlorination and also chloramination.

The data presented below is split into individual catchments, and further split into comparisons between individual sites and comparisons between catchments.

### 5a.3.1 Hampshire Avon Catchment

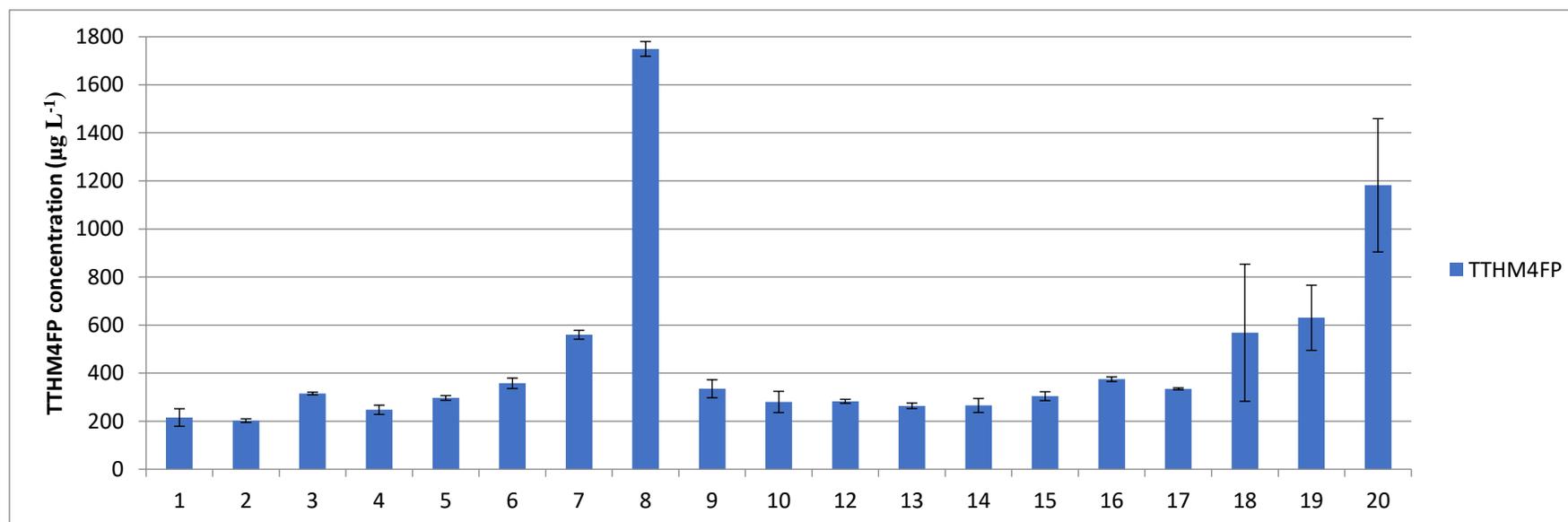


Figure 5a.3: Mean total THM4 formation potential under chlorination from the 19 Hampshire Avon sites. Error bars represent standard error, data averaged from 3 machine triplicates.

Figure 5a.3 shows that the mean TTHM4FP formed under chlorination between sites at the Hampshire Avon catchment differ vastly at some sites whilst remaining relatively stable at others. Specifically, sites 8 and 20 form the highest ( $1749 \pm 30.65$  and  $1181 \pm 277.39$  respectively) TTHM4FP whereas sites 2 and 13 form the lowest TTHM4FP ( $201 \pm 772$  and  $263 \pm 11.45$  respectively).

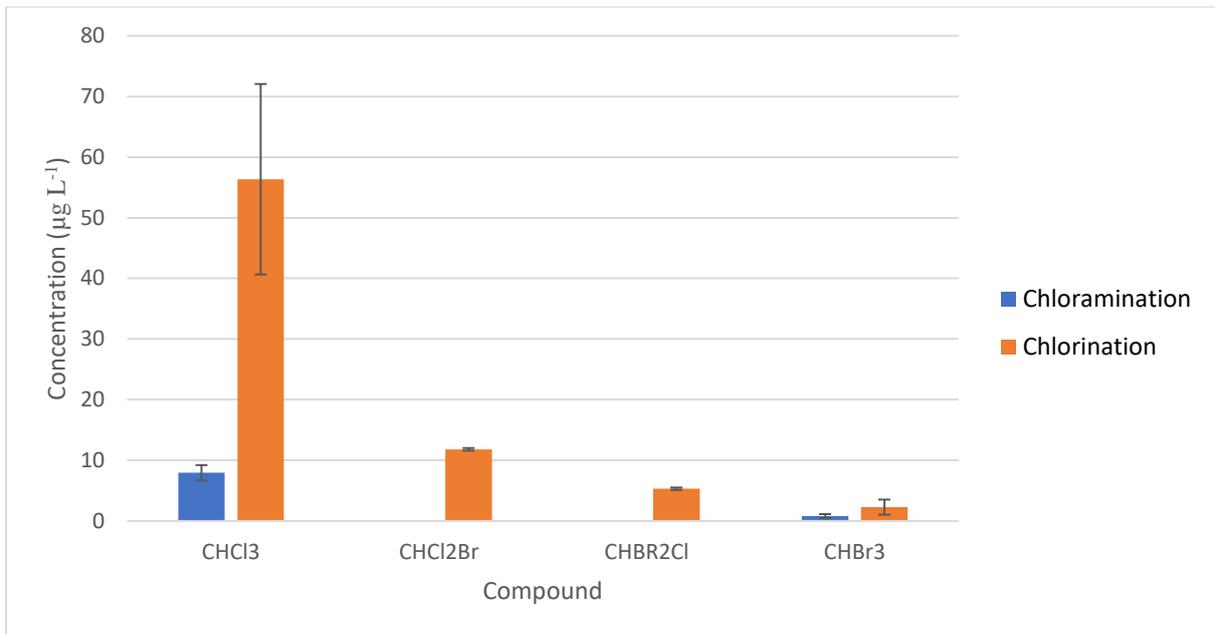


Figure 5a.4: Mean standardised THM4 concentration at Hampshire Avon per individual THM4 compound for chlorination and chloramination.

Figure 5a.4 shows that, under chloramination, CHCl<sub>3</sub> is the most dominant THM4 compound formed at the Hampshire Avon catchment, accounting for 91% of total THM4. No CHCl<sub>2</sub>Br and CHBr<sub>2</sub>Cl was formed under chloramination. Under chlorination, CHCl<sub>3</sub> was also by far the most abundant THM4 compound, however, both CHBr<sub>3</sub> and CHBr<sub>2</sub>Cl are also formed. The error bars show that the CHCl<sub>3</sub> formed under chlorination varies approximately 15 µg L<sup>-1</sup> above and below the mean concentration at 56 µg L<sup>-1</sup>, whereas the standard error is much lower for all other data points. The raw data used to generate this graph is displayed in Table 5a.4, below.

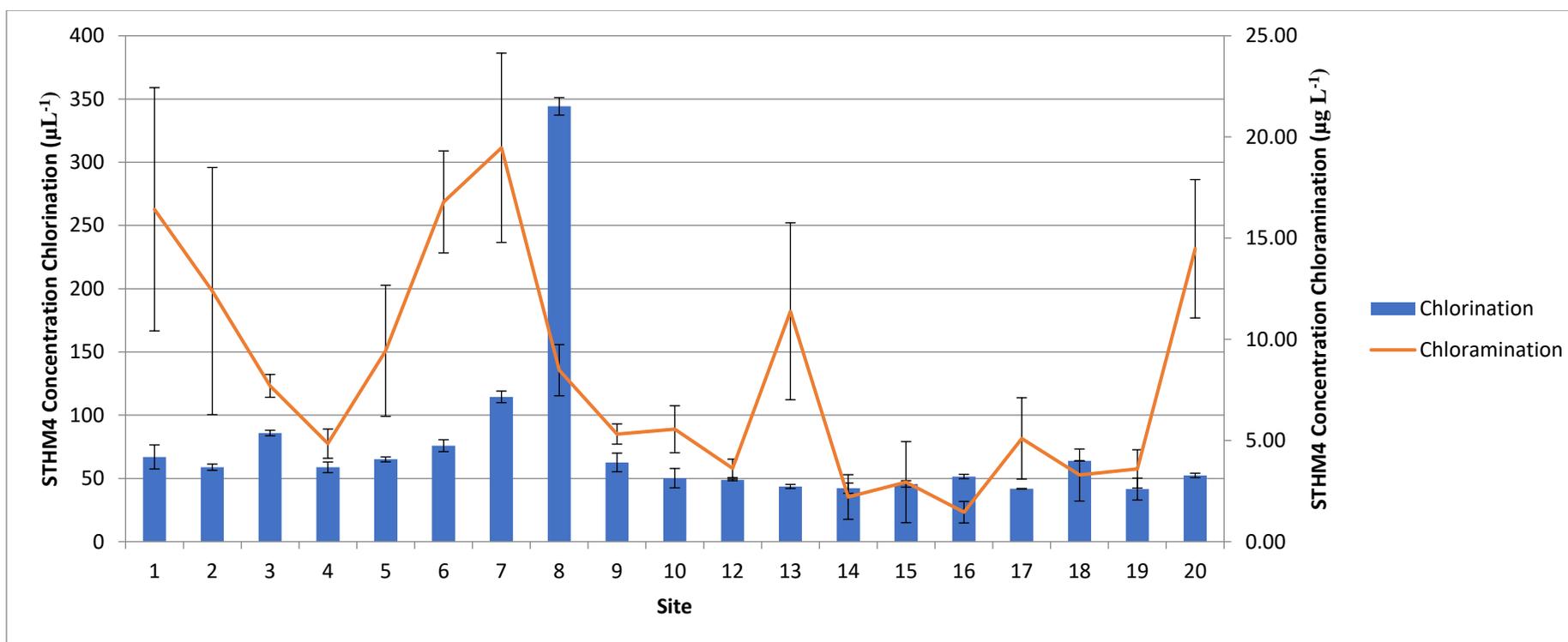


Figure 5a.5: Standardised THM4 chlorination *vs.* chloramination data formed from 1 mg L<sup>-1</sup> DOC at Hampshire Avon sites. Chlorination formed data is displayed on the primary Y axis (left), chloramination data displayed on secondary Y axis (right). Error bars represent variance in data (as standard deviation taken from 3 machine replicates).

The data displayed in Figure 5a.5 shows that site 8 forms by far the highest concentration of STHM4 under chlorination (at 344.24 µg L<sup>-1</sup>) when compared to other sites in the Hampshire Avon. All other sites, when treated by chlorination, form less than 114.51 µg L<sup>-1</sup> STHM4 (i.e. a minimum of 3 times lower than site 8). For STHM4 formed under chloramination, site 7 produces the highest concentration, at 19.46 µg L<sup>-1</sup> and the lowest concentration at 1.46 µg L<sup>-1</sup>.

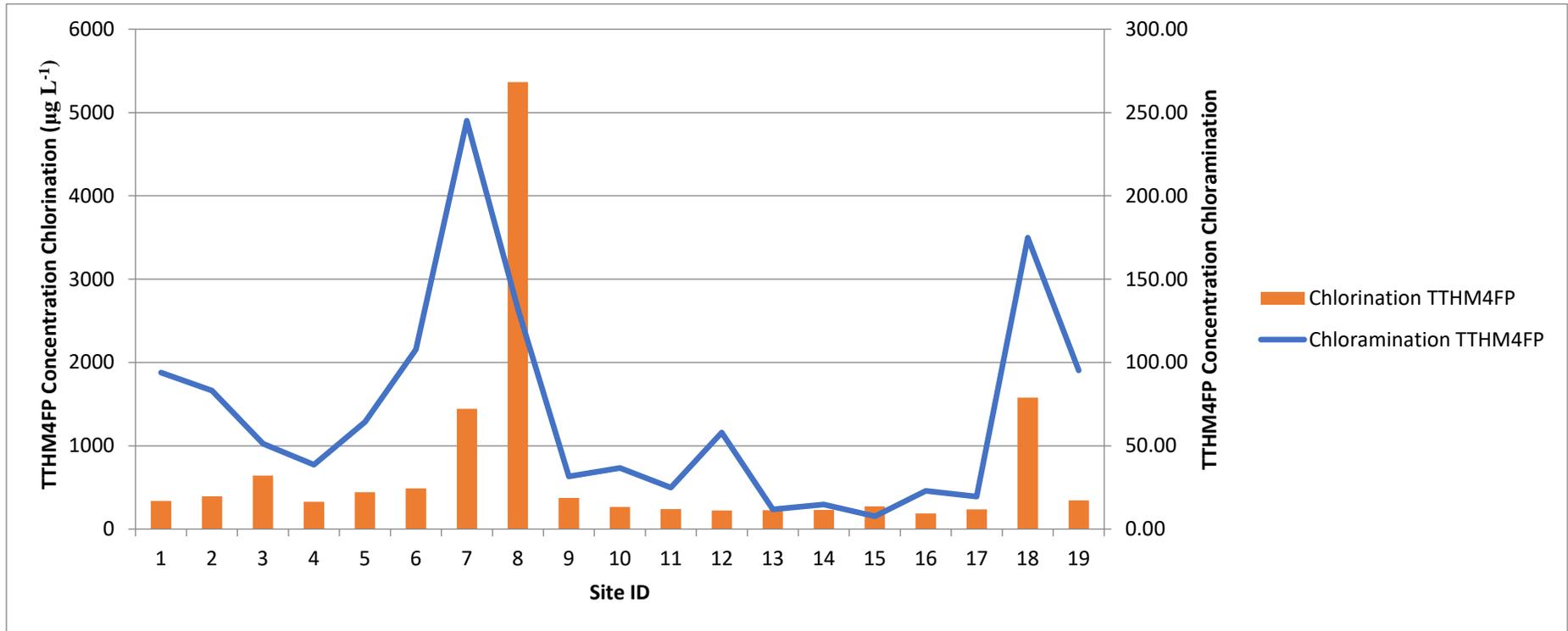


Figure 5a.6: Comparison of TTHM4 formation potential at all 19 Hampshire Avon sites under chlorination and chloramination.

Table 5a.4: Individual THM4 compounds formed from chlorination and chloramination from samples collected in the Hampshire Avon catchment, and the corresponding DOC and bromide concentration.

Site ID	Chlorination				Chloramination				DOC (mg L <sup>-1</sup> )	Bromide (mg L <sup>-1</sup> )
	CHCl <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>2</sub> Br (µg L <sup>-1</sup> )	CHBr <sub>2</sub> Cl (µg L <sup>-1</sup> )	CHBr <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>2</sub> Br (µg L <sup>-1</sup> )	CHBr <sub>2</sub> Cl (µg L <sup>-1</sup> )	CHBr <sub>3</sub> (µg L <sup>-1</sup> )		
1	49.10	12.37	5.52	0.00	15.37			3.16	5.07	0.057
2	43.06	11.40	4.44	0.00	12.39			0.00	6.71	0.028
3	62.24	12.83	5.64	15.60	7.70			0.00	6.66	0.030
4	40.02	13.16	5.63	0.00	3.81			3.11	5.58	0.070
5	49.11	11.57	4.42	0.00	9.43			0.00	6.82	0.024
6	58.66	11.79	5.45	0.00	16.79			0.00	6.42	0.071
7	99.22	11.00	4.29	0.00	19.46			0.00	12.59	0.069
8	328.72	10.76	4.75	0.00	8.47			0.00	15.59	0.097
9	45.79	12.38	4.52	0.00	5.32			0.00	5.95	0.086
10	22.57	12.18	5.86	14.38	4.54			3.06	4.83	0.002
12	29.90	12.18	6.84	0.00	5.13			0.00	4.87	0.046
13	25.58	12.29	5.83	0.00	11.39			0.00	5.10	0.036
14	26.09	11.65	4.58	0.00	2.21			0.00	5.33	0.051
15	29.49	11.61	4.58	0.00	2.94			0.00	5.02	0.060
16	34.61	10.54	6.38	0.00	1.46			0.00	5.28	0.045

<b>17</b>	26.03	11.27	4.54	0.00	5.10			0.00	4.48	0.042
<b>18</b>	43.39	13.65	6.78	0.00	2.30			2.98	3.71	0.041
<b>19</b>	20.48	12.11	4.54	13.40	2.59			3.01	1	0.070
<b>20</b>	36.53	9.51	6.34	0.00	14.48			0.00	6.58	0.061

Table 5a.5: Table to display Pearson's correlation and regression between the brominated THM4 species and the bromide concentration in the Hampshire Avon catchment.

<b>THM4 Species</b>	<b>Treatment</b>	<b>Correlation</b>	<b>Regression Equation</b>	<b>Regression R<sub>2</sub></b>
CHCl <sub>2</sub> Br	Chlorination	-0.246 P=<0.05	Y=-0.005x + 0.115	0.06
CHBr <sub>2</sub> Cl	Chlorination	-0.411 p=<0.05	Y=-0.010x + 0.109	0.192
CHBr <sub>3</sub>	Chlorination	-0.279 P=<0.05	Y=0.001x + 0.058	0.076
CHBr <sub>3</sub>	Chloramination	-0.360 P=<0.05	Y=0.004x + 0.059	0.113

Table 5a.5 shows the correlation and regression analysis between the brominated THM4 species, averaged, and the bromide concentration detected in the Hampshire Avon catchment, using both chlorinated and chloraminated THM4 datasets. It is interesting to note that the bromide concentration does not correlate with any of the brominated THM4 species at the Hampshire Avon both under chlorination and chloramination treatments, thus suggesting that the brominated THM4 species that are being formed in these samples are either forming due to the extended contact time with the bromide in the water (as the contact time in the laboratory chlorination and chloramination is 7 days rather than a more typical 30 minutes in a water treatment works), or that the brominated compounds form regardless of the bromide

concentration in the water. Furthermore, it is important to remember that the sodium hypochlorite concentration used to create the chlorine and chloramine dosing solution originated as sea water, and therefore, will always contain a residual level of bromide which can influence the formation of brominated DBPs. Whilst these influences are eradicated by using the same dosing solution for the creation of check standards, using different batches of sodium hypochlorite over the course of the experimentation can lead to increased or decreased brominated DBP concentrations once chlorinated/chloramination.

### 5a.3.2 Conwy Catchment Sites

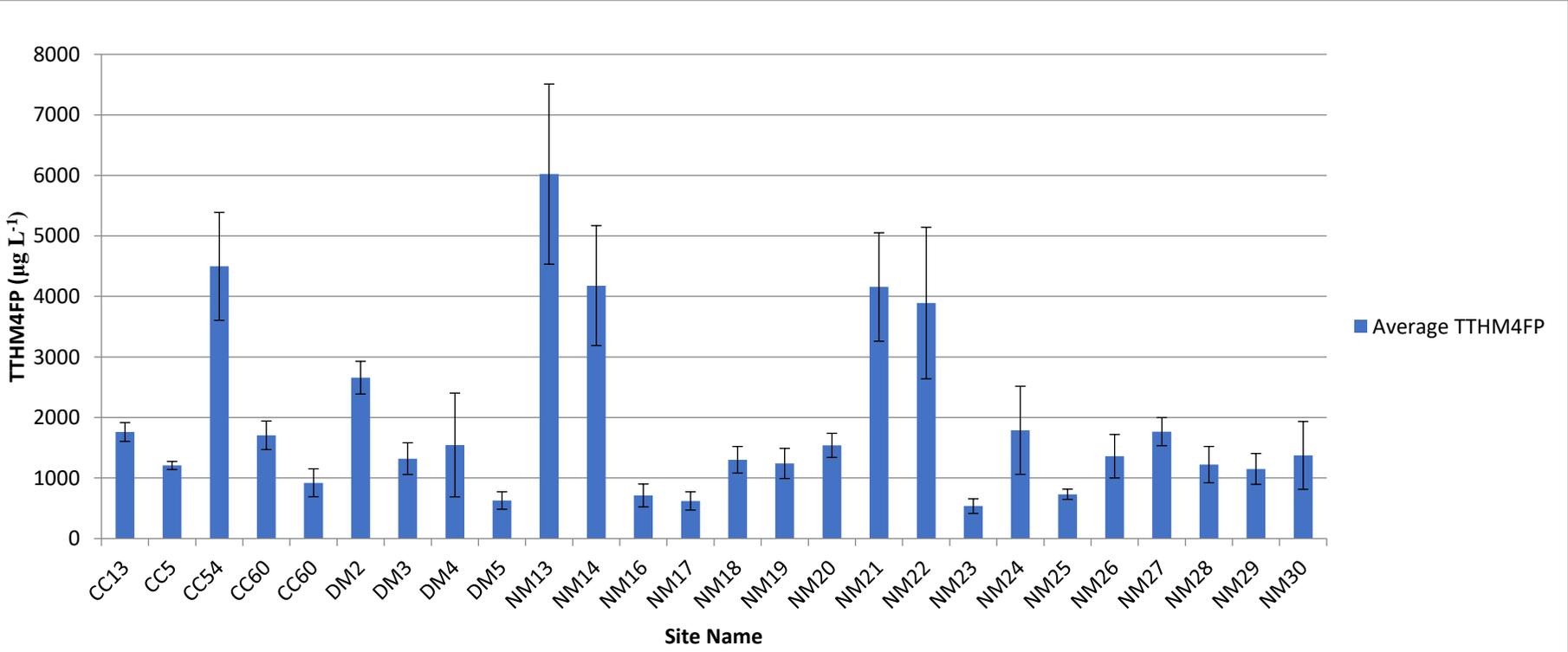


Figure 5a.7: Mean TTHM4FP from sites in the Conwy catchment under chlorination treatment, with variance displayed in error bars representing standard deviation.

Figure 5a.7 outlines the mean THM4FP of all sites in the Conwy catchment under chlorination. There is a large difference in maximum and minimum concentration with site NM13 forming by far the highest TTHM4FP and site NM23 forming the lowest concentration. It

can be seen that site NM23 has, on average, the lowest TTHM4FP at  $535.59 \mu\text{g L}^{-1}$  THM4, where as site NM13 has the highest average TTHM4FP of  $6021.46 \mu\text{g L}^{-1}$

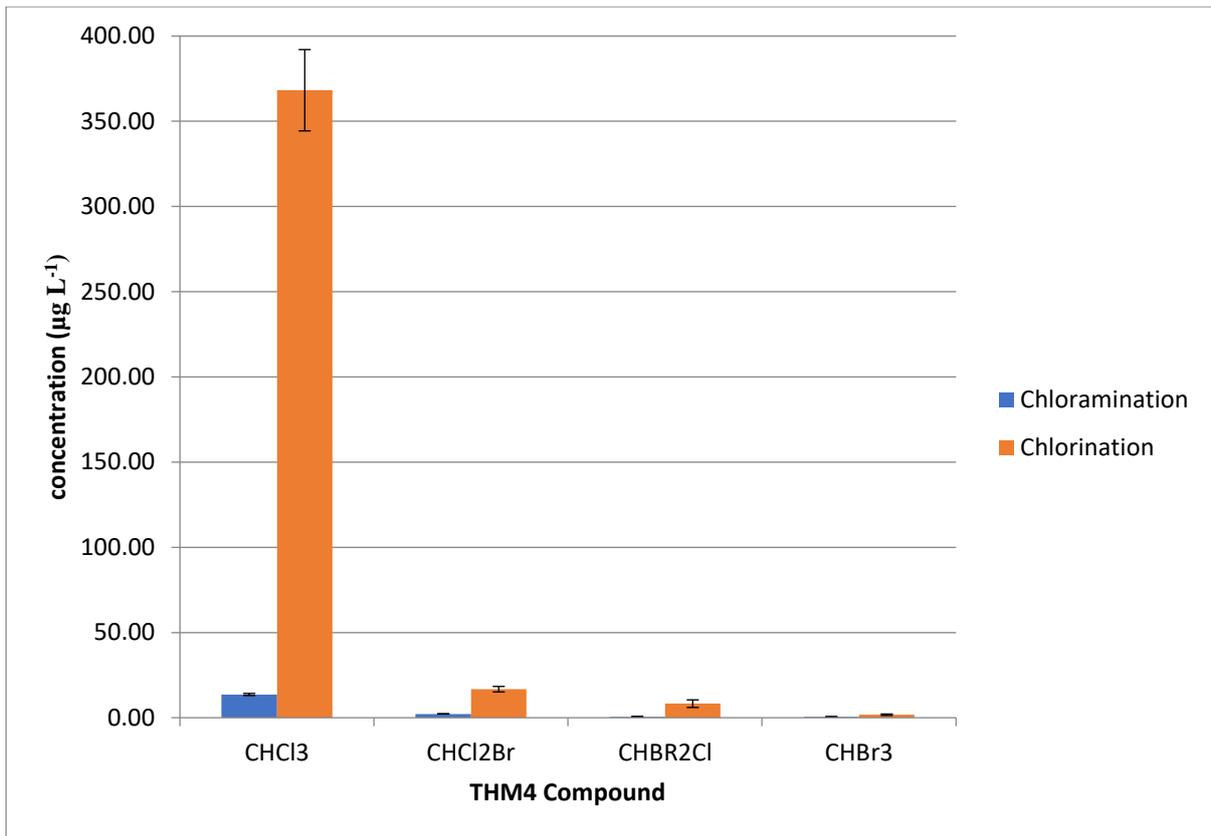


Figure 5a.8: Mean concentration of STHM formed from chloramination and chlorination of waters draining the Conwy catchment. Bars represent variance, calculated as standard deviation.

Figure 5a.8 shows the mean concentration of STHM4 compounds formed from chloramination and chlorination at the Conwy catchment, with CHCl<sub>3</sub> being found in the highest mean concentration at 368.23 (±23.85) µg L<sup>-1</sup> for chlorination of 1 mg L<sup>-1</sup> DOC and 13.63 (±0.63) µg L<sup>-1</sup> for chloramination, and CHBr<sub>3</sub> forming the lowest mean concentration at 1.74 ±0.44 µg L<sup>-1</sup> for chlorination and 0.51 ± 0.19 µg L<sup>-1</sup> for chloramination. The raw data used in this figure is outlined in Table 5a.6.

Table 5a.6: Individual THM4 compound concentrations formed from the chlorination and chloramination of samples from the Conwy catchment, and corresponding DOC concentration.

Site ID	Chlorination				Chloramination				DOC (mg L <sup>-1</sup> )
	CHCl <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>2</sub> Br (µg L <sup>-1</sup> )	CHBr <sub>2</sub> Cl (µg L <sup>-1</sup> )	CHBr <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>3</sub> (µg L <sup>-1</sup> )	CHCl <sub>2</sub> Br (µg L <sup>-1</sup> )	CHBr <sub>2</sub> Cl (µg L <sup>-1</sup> )	CHBr <sub>3</sub> (µg L <sup>-1</sup> )	
CC13	520.02	14.19	0.25	0.00	18.02	2.42	0.00	0.00	18.02
CC5	400.40	24.90	0.44	1.45	19.49	2.32	0.00	0.00	19.49
CC54	332.29	18.67	18.52	0.74	14.65	2.33	0.00	0.00	14.65
CC6	425.95	25.00	0.48	0.00	10.91	2.49	0.00	0.00	10.91
CC60	217.55	28.83	10.34	3.20	14.81	2.27	2.07	0.00	14.81
CC88	345.40	4.19	0.31	1.74	17.29	2.14	0.00	0.00	17.29
DM2	473.28	10.15	0.24	0.00	16.11	2.19	0.00	2.22	16.11
DM3	98.31	13.13	2.26	0.00	10.07	2.39	2.07	2.40	10.07
DM4	290.03	36.27	24.69	0.17	7.36	2.22	2.18	2.31	7.36
DM5	282.16	13.61	14.26	2.38	18.02	2.82	2.15	0.00	18.02
NM13	465.40	12.64	19.91	0.69	16.06	2.02	2.07	2.31	16.06
NM14	423.52	15.14	25.63	1.02	19.17	2.23	2.07	2.12	19.17
NM16	440.45	6.93	0.44	0.00	11.65	2.12	2.07	0.00	11.65
NM17	402.06	9.69	0.32	1.85	15.59	2.17	0.00	0.00	15.59
NM18	331.20	18.99	14.52	6.47	10.35	2.03	0.00	2.31	10.35
NM19	475.79	21.25	0.40	1.79	9.38	2.15	0.00	0.00	9.38

<b>NM20</b>	279.42	10.04	0.28	1.49	11.49	2.02	0.00	0.00	11.49
<b>NM21</b>	429.05	4.74	0.27	0.00	10.12	2.17	0.00	0.00	10.12
<b>NM22</b>	315.36	20.22	39.32	8.87	14.34	2.17	0.00	0.00	14.34
<b>NM23</b>	165.97	18.15	5.32	1.03	12.23	2.02	0.00	0.00	12.23
<b>NM24</b>	217.99	34.63	37.72	8.21	11.80	2.44	0.00	0.00	11.80
<b>NM25</b>	414.09	15.71	0.36	0.00	14.96	2.12	0.00	0.00	14.96
<b>NM26</b>	345.77	17.31	12.98	2.18	10.15	2.14	0.00	0.00	10.15
<b>NM27</b>	313.46	6.69	0.30	1.37	13.26	2.29	0.00	0.00	13.26
<b>NM28</b>	302.12	12.02	0.31	1.36	12.48	2.20	0.00	0.00	12.48
<b>NM29</b>	380.88	10.14	0.34	1.35	11.96	2.18	0.00	0.00	11.96
<b>NM30</b>	465.07	16.02	0.37	1.35	16.31	1.99	0.00	0.00	16.31

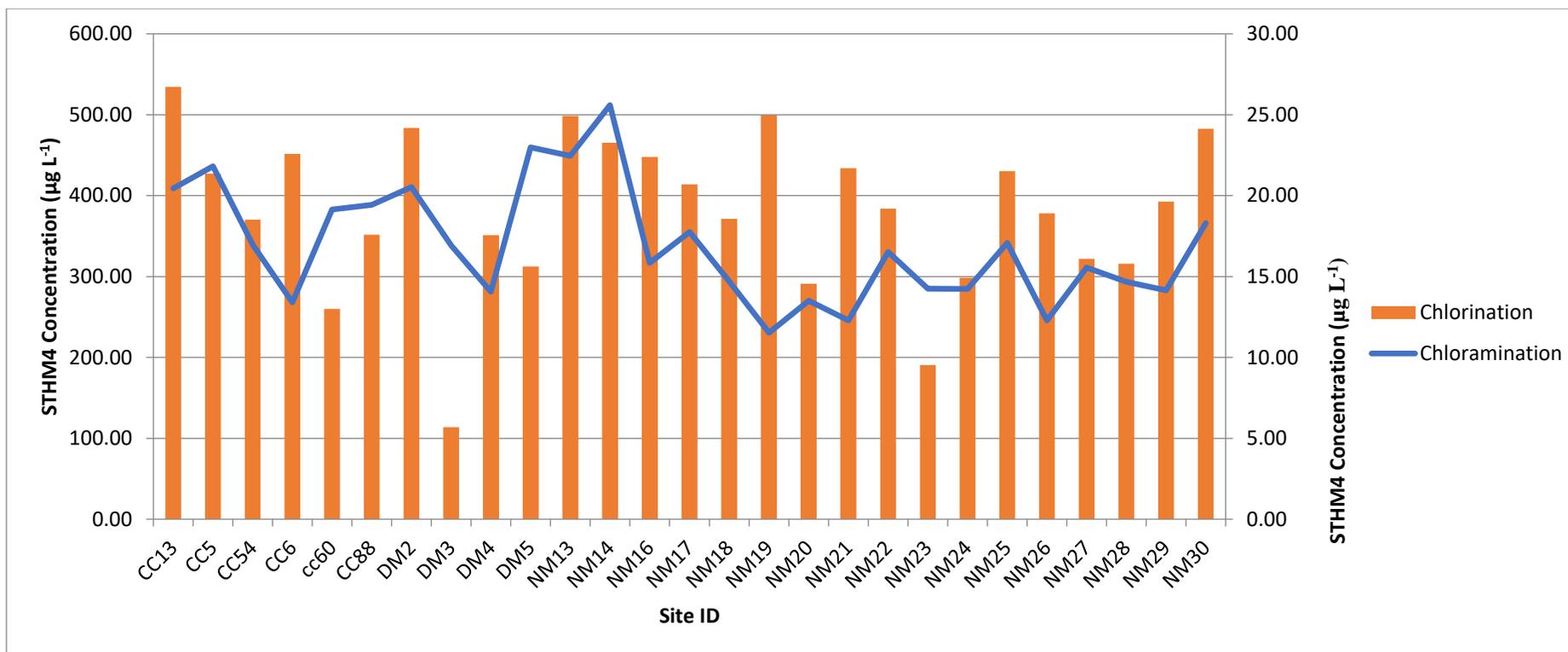


Figure 5a.9: Comparison of STHM4 concentration between chlorination and chloramination treatments at Conwy sites. Standard error data is not available due to only one dataset available.

These data displayed in Figure 5a.9 show that, under chlorination, site DM3 produced the lowest STHM4 at 113.70 µg L<sup>-1</sup> and site CC13 produced the highest STHM4 concentration at 534.46 µg L<sup>-1</sup>. Conversely, under chloramination, the highest STHM4 concentration out of all Conwy sites at 25.60 µg L<sup>-1</sup> at site NM14, and the lowest STHM4 concentration at 11.19 µg L<sup>-1</sup>.

Table 5a.7: Table to display Pearson's correlation and regression between the brominated THM4 species and the bromide concentration (logged) in the Conwy catchment.

<b>THM4 Species</b>	<b>Treatment</b>	<b>Correlation</b>	<b>Regression Equation</b>	<b>Regression R<sub>2</sub></b>
CHCl <sub>2</sub> Br	Chlorination	0.322 (p=<0.05)	Y=0.12x – 1.816	0.174
CHBr <sub>2</sub> Cl (logged)	Chlorination	0.117 (p=>0.05)	Y=0.538x – 1.409	0.425
CHBr <sub>3</sub>	Chlorination	-0.034 p=>0.05	Y=-0.019x – 1.597	0.028
CHCl <sub>2</sub> Br (logged)	Chloramination	0.322 P=>0.05	Y=2.495x – 2.485	0.096
CHBr <sub>2</sub> Cl	Chloramination	0.117 P=>0.05	Y-0.034x – 1.650	0.014
CHBr <sub>3</sub>	Chloramination	-0.034 P=>0.05	Y=-0.009x – 1.626	0.001

Table 5a.7 shows correlation and regression analysis between the brominated THM4 species detected from both chlorination and chloramination at the Conwy catchment sites, averaged, and bromide concentration. Interestingly here, the CHCl<sub>2</sub>Br compounds showed

a weak but positive correlation with bromide concentration under chlorination at the Conwy, suggesting that there is a link between the bromide concentration and the formation of the  $\text{CHCl}_2\text{Br}$ , which is contrary to the findings at the Hampshire Avon catchment. Interestingly, however,  $\text{CHCl}_2\text{Br}$  did not correlate with chloramination formed brominated THM4 species, suggesting that this relationship is more chlorine driven than chloramine driven. Brominated DBPs may also be more likely due to form due to the presence of bromide in the chlorine dosing solution. See section 5a.3.1.

## 5a.4 Statistical Analysis

Datasets were first analysed for normality using the skewness test, where skewness values between +2.0 and -2.0 were deemed normally distributed, and any skewness value outside these parameters was deemed not normally distributed, and thus, the dataset was transformed by  $\log_{10}$ . If the transformed dataset was also found to be not normally distributed, then a Spearman's Rank correlation test was used, however, if the data was normally distributed, then a Pearson's correlation test was used. Statistically significant results were identified as having a significance value of  $<0.05$ , and are reported below.

Correlations were also found with land use types, where every instance of a certain land use type was analysed in a correlation with chemical data such as NPOC, DON, and ion data.

The land use data was not standardised to the mean DOC concentration of the waters draining the catchment for several reasons. Typically, the land use type that was to be analysed would only make up a section of the overall catchment that the water was sampled from (and the DOC concentration was derived from), and thus, the DOC concentration of the water was not directly related to, or intrinsically linked to the area of the land use type in question. Whilst it would be practicable to assume that the DOC concentration would increase with an increase in the land area (purely because there is more DOM in a larger area of land), some datasets show that there are lower concentrations of DOC in waters draining larger areas of land (see Table 5a.6 and 5a.8).

Table 5a.8: Comparison of three catchments, the mean DOC concentration and the DOC concentration per  $\text{km}^2$  of Arable and Horticulture land use, as an example of how land use, STHM4 and DOC concentration do not appear to be linked.

<b>Catchments</b>	<b>Arable and Horticulture (Km<sup>2</sup>)</b>	<b>Mean DOC (mg L<sup>-1</sup>)</b>	<b>Mean DOC/Km<sup>2</sup> (mg L<sup>-1</sup>)</b>	<b>Mean STHM4 concentration (µg L<sup>-1</sup>)</b>
Hampshire Avon	51.84	7.69 ±0.83	0.13 – 0.16	75.75±15.50
Conwy	177.22	6.02±0.55	0.03 - 0.04	380.43±18.84
Scotland	63.10	12.72±1.49	0.18 - 0.23	No Data

### 5a.4.1 Mean TTHM4FP Conwy

Mean TTHM4FP data, from March 2015 to July 2016 data collections, are displayed in Figure 5a.7. The data was statistically analysed, and, despite a non-normal distribution, a Brown-Forsythe robust test of equality of means showed a significance of  $p < 0.05$ . Sites CC8 and NM23 were the only sites that significantly differed from each other ( $p = 0.01$ , Tukey HSD). Interestingly, Figure 5a.9 shows that DM3 has the lowest mean TTHM4 concentration, even with variance taken into account, but this was found to be not significantly different to other sites.

### 5a.4.2 Individual THM4 Compounds

Statistical analysis between the 4 THM compounds formed from samples collected from the Conwy catchment was carried out. Only the  $\text{CHCl}_3$  data was normally distributed (showed by a Levene's statistic of  $> 0.05$ ), so  $\text{CHCl}_2\text{Br}$ ,  $\text{CHBr}_2\text{Cl}$  and  $\text{CHBr}_3$  were transformed by  $\log_{10}$ . Levene's statistics which were greater than 0.05 were generated for  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_2\text{Cl}$  but for some sites,  $\text{CHBr}_3$  data was only present for 2 or fewer replicates, and therefore post hoc tests were not suitable. A Tukey post hoc test was chosen for  $\text{CHCl}_3$ ,  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_2\text{Cl}$  as the groups had equal variances, but no sites were shown to be significantly different to any other site in terms of individual THM4 compounds at the Conwy.

### 5a.4.3 Chlorination and Chloramination of Individual THM4 Compound Formation: Conwy vs. Hampshire Avon (Tables 5a.9 to 5a.12)

A statistical comparison of the individual THM4 compounds formed from chlorination and chloramination at the Conwy catchment sites was carried out to determine whether chlorination and chloramination treatments formed different concentrations of these compounds.  $\text{CHCl}_3$  was found to be normally distributed for both treatments (skewness of -0.499 and -0.100 for chlorination and chloramination respectively), and therefore, an independent samples t-test was conducted. However, a Levene's test showed that equal variances could not be assumed ( $p < 0.01$ ), and as a result, the appropriate statistic was read from the results table, showing that, with a t value of 14.788 at 28.538 degrees of freedom, the concentration of  $\text{CHCl}_3$  formed

from chlorination was significantly different from the concentration of  $\text{CHCl}_3$  formed from chloramination at  $p < 0.01$ .

$\text{CHCl}_2\text{Br}$  concentrations were also analysed, showing skewness values of 0.685 and 0.584 respectively showing that the data was normally distributed. A Levene's test of equality of variance showed that equal variances could be assumed, and therefore the independent samples t-test was conducted showing  $t = 4.536$  at 52 degrees of freedom, showing that the  $\text{CHCl}_2\text{Br}$  concentration formed from chlorination was significantly different to the concentration of  $\text{CHClBr}_2$  formed from chloramination at a significance of  $p < 0.01$ .

$\text{CHBr}_2\text{Cl}$  was normally distributed for chlorination and chloramination (skewness of 1.388 and 1.404 respectively), however a Levene's test for equality of variance showed a significance of  $p = > 0.05$ . A t statistic of 0.049 at 51.989 degrees of freedom and a significance of  $p = > 0.05$  show that there is no significant difference between the chlorination and chloramination formed  $\text{CHBr}_2\text{Cl}$ .

Finally,  $\text{CHBr}_3$  data was analysed. Chlorination and chloramination showed skewness of 2.111 and 2.409 respectively, so the data was transformed by  $\log_{10}$ , showing skewness of -0.168 and -0.047 respectively which were much more acceptable. This transformed data was then run through an independent T test. The Levene's test of equality of variances showed that equal variances could not be assumed, and a t statistic of 0.258 and 37.932 degrees of freedom were produced at a significance of  $p = 0.798$ , showing that there is no statistically significant difference between the  $\text{CHBr}_3$  concentrations under chlorination and chloramination.

#### 5a.4.4: Formation of Individual THM4 Compounds, Conwy vs. Hampshire Avon, Chlorination and Chloramination

Table 5a.11 shows the statistical comparison of individual THM4 compound concentrations, between the Hampshire Avon and the Conwy catchment, to identify whether the concentrations of each THM4 compounds formed at each catchment are significantly different between the Hampshire Avon and Conwy. The data was transformed if required, and each compound was compared between the two catchments.  $\text{CHBr}_2\text{Cl}$  was found to not be statistically significantly different between

the two catchments, however,  $\text{CHCl}_3$ ,  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_3$  were found to be statistically significantly different at  $p < 0.01$ , under chlorination (Table 5a.11).

Under chloramination, the Hampshire Avon sites only formed  $\text{CHCl}_3$  and  $\text{CHBr}_3$ , and therefore, statistical comparison between  $\text{CHBr}_2\text{Cl}$  and  $\text{CHCl}_2\text{Br}$  at the Hampshire Avon and the Conwy was not possible, so a comparison between  $\text{CHCl}_3$  and  $\text{CHBr}_3$  was conducted, showing that the  $\text{CHCl}_3$  concentrations between the two catchments were statistically significant ( $p < 0.01$ ), but  $\text{CHBr}_3$  was not ( $p > 0.05$ ) (Table 5a.12).

Table 5a.9: Statistical analysis to determine whether concentration of individual THM4 compound is significantly different under chlorination or chloramination at the Conwy catchment. Data comprises of all sites at Conwy and all sites at Hampshire Avon catchments, collected in the month of April 2016, as this was the only dataset that contained full data for both catchments and both treatments.

Compound	Transformed	Skewness		Levene's test	Equal Variances assumed?	T	DF	Significance P=
		Chlorination	Chloramination					
CHCl <sub>3</sub>	No	-0.499	-0.100	P=<0.01	No	14.788	28.538	<0.01
CHCl <sub>2</sub> Br	No	0.685	0.584	P=<0.05	Yes	4.536	52	<0.01
CHBr <sub>2</sub> Cl	No	1.388	1.404	P=>0.05	No	0.049	51.989	>0.05
CHBr <sub>3</sub>	Yes	-0.168	-0.047	P=>0.05	No	0.258	37.932	>0.05

Table 5a.10: Statistical analysis to determine whether concentration of individual THM4 compound is significantly different under chlorination and chloramination in the Hampshire Avon catchments.

Compound	Transformed	Skewness		Levene's test	Equal Variances assumed?	T	DF	Significance P=
		Chlorination	Chloramination					

<b>CHCl<sub>3</sub></b>	Yes	1.999	-0.177	P=>0.05	Yes	8.435	36	<0.01
<b>CHCl<sub>2</sub>Br</b>								
<b>CHBr<sub>2</sub>Cl</b>								
<b>CHBr<sub>3</sub></b>	Yes	0.213	0.220	P=>0.05	Yes	44.193	6	<0.01

Table 5a.11: Statistical analysis between the TTHM4 concentration formed under chlorination between Hampshire Avon and Conwy sites to determine whether the Hampshire Avon and Conwy sites form THM4 concentrations that are significantly different.

Compound	Transformed	Skewness - Chlorination		Levene's test	Equal Variances assumed?	T	DF	Significance P=
		Avon	Conwy					
<b>CHCl<sub>3</sub></b>	Yes	1.999	-1.189	P=>0.05	Yes	-13.758	44	<0.01
<b>CHCl<sub>2</sub>Br</b>	No	-0.377	0.707	P=<0.01	No	-2.961	26.913	<0.01
<b>CHBr<sub>2</sub>Cl</b>	No	0.447	1.391	P=<0.01	No	-1.388	26.380	>0.05
<b>CHBr<sub>3</sub></b>	Yes	0.213	-0.206	P=>0.05	Yes	9.983	19.277	<0.01

Table 5a.12: Statistical analysis between the TTHM4 concentration formed under chloramination between Hampshire Avon and Conwy sites to determine whether the Hampshire Avon and Conwy sites form THM4 concentrations that are significantly different.

Compound	Transformed	Skewness - Chloramination		Levene's test	Equal Variances assumed?	T	DF	Significance P=
		Avon	Conwy					
<b>CHCl<sub>3</sub></b>	No	0.708	0.131	P=<0.01	No	-4.008	26.677	<0.01
<b>CHBr<sub>3</sub></b>	No	1.172	1.423	P=<0.05	No	0.814	29.978	>0.05

Tables 5a.9 to 5a.12 explore the statistical relationships between the data. The differences between data points in Figure 5a.3 are likely due to the fact that the two most common THM compounds are CHCl<sub>3</sub> and CHCl<sub>2</sub>Br, and therefore with more likelihood of forming, these compounds are more likely to significantly differ between the two catchments. However, Table 5a.6 shows that both CHCl<sub>3</sub> and CHBr<sub>3</sub> concentrations significantly differ from each other at the Hampshire Avon, suggesting that a higher bromide concentration at the Hampshire Avon may be driving a higher concentration of CHBr<sub>3</sub> when compared to the Conwy catchment.

Table 5a.13: Percentage of each individual compound (of STHM4) at the Hampshire Avon and Conwy catchments, under chlorination and chloramination treatments

	Hampshire Avon		Conwy	
	Chlorination	Chloramination	Chlorination	Chloramination
<b>CHCl<sub>3</sub></b>	74.39%	90.78%	93.23%	80.63%
<b>CHCl<sub>2</sub>Br</b>	15.58%	0.00%	4.24%	13.16%
<b>CHBr<sub>2</sub>Cl</b>	7.01%	0.00%	2.09%	3.21%
<b>CHBr<sub>3</sub></b>	3.01%	9.22%	0.44%	3.00%

Table 5a.13 explores the composition of THM4 in terms of individual compound composition, at both the Hampshire Avon and the Conwy catchments, under both chlorination and chloramination. Interestingly, CHCl<sub>3</sub> (the most carcinogenic THM compound, see Table 5a.1) is formed in a higher concentration under chloramination at the Hampshire Avon, but lower under chloramination at the Conwy sites. Chloramination forms no CHCl<sub>2</sub>Br and CHBr<sub>2</sub>Cl at the Hampshire Avon, but both of these compounds are formed in higher concentrations under chloramination than under chlorination at the Conwy.

## 5a.5 Discussion

### 5a.5.1 Hampshire Avon Catchment

Figure 5a.3 examines the TTHM4FP under chlorination at the Hampshire Avon catchment. Site 8 produces by far the highest concentration whereas the lowest concentration recorded is at site 2. Both of these sites are located within the same subcatchment of the Hampshire Avon, the river Nadder. Site 2 drains a catchment of 205 km<sup>2</sup> where site 7 drains a catchment of 26 km<sup>2</sup>, and thus a dilution factor should be taken into account when comparing the two catchments. Conversely, however, the highest TTHM4FP concentration at the Hampshire Avon is found in water draining from a relatively small catchment (average catchment size of  $110 \pm 35$  km<sup>2</sup>), and thus it is practicable to assume that a component of the DOC in waters draining the catchment is driving this relationship.

Table 5a.4 shows statistical examinations of the individual THM4 compounds formed from chlorination and chloramination in the Hampshire Avon catchment, and although  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_2\text{Cl}$  are not formed from both chlorination and chloramination, both  $\text{CHCl}_3$  and  $\text{CHBr}_3$  are formed, and their concentration is significantly higher under chlorination when compared to chloramination at the Hampshire Avon catchment at  $p < 0.01$ .

Figure 5a.4 displays the relationship between STHM4 formed from chlorination and also chloramination at the Hampshire Avon catchment. It is apparent that there is no obvious relationship between the formation of THM4 from chlorination when compared to that formed from chloramination, suggesting that the two disinfectants target and react with the organic matter in different ways. In figure 5a.5, at a brief glance, there does appear to be two major spikes in both datasets, however, upon closer inspection, these do not appear to be linked. This is most apparent at site 7 where chloramination formed THM4 is at its highest concentration from all Hampshire Avon sites at  $19.46 \mu\text{g L}^{-1}$  and chlorination formed THM4 at  $114.51 \mu\text{g L}^{-1}$ , a concentration difference of  $95.05 \mu\text{g L}^{-1}$ . Whereas at site 8, chloramination formed THM4 is at  $8.47 \mu\text{g L}^{-1}$  and chlorination formed THM4 is  $344.24 \mu\text{g L}^{-1}$ , a concentration difference of  $335.77 \mu\text{g L}^{-1}$ . This suggests that a switch from chlorination to chloramination at water treatment works is not as straight forward as

simply swapping the two disinfectants, and that some THM4 compound concentrations may actually increase rather than decrease.

### 5a.5.2 Conwy Catchment

Figure 5a.7 displays the wide variety of TTHM4FP that has the potential to form at the Conwy catchment, with concentrations ranging from 535.59  $\mu\text{g L}^{-1}$  at site NM23 to 6021.46  $\mu\text{g L}^{-1}$  at site NM13, 11 times greater than the lowest concentration in the catchment. This vast difference in THM4 formation potential concentrations is likely due to the variations in catchment characteristics (especially vegetation cover) in this large catchment. Had two catchments draining very similar land uses (for example, two dairy farms on acid grassland) been tested for THM4FP, it is practicable to assume that the THM4FP from both catchments would be similar (on a broad level), as they would be subjected to very similar organic matter sources from both the vegetation and the land use practices that are carried out in the catchment. However, studies by Autio *et al.*, (2016) found that soil type was more important in determining the concentration and quality of riverine DOM, suggesting that vegetation is not the best suited predictor of DOM.

Table 5a.9 shows that  $\text{CHCl}_3$  and  $\text{CHCl}_2\text{Br}$  concentrations are significantly different in samples treated by chlorination and chloramination from the Conwy catchment at  $p < 0.01$ .  $\text{CHBr}_2\text{Cl}$  and  $\text{CHBr}_3$  concentrations do not significantly differ between the two treatments ( $p > 0.05$ ). This could be due to varying concentrations of nutrients found in the waters, such as bromide and chloride, which could be increasing the formation potential of brominated compounds and also attributing to the TTHM4 data due to a residual of  $\text{Cl}_2$  in the water prior to disinfection.

### 5a.5.3 Hampshire Avon and Conwy Catchments Combined

#### 5a.5.3.1 Chlorination

The difference between the maximum and minimum concentrations of TTHM4 is great within the Hampshire Avon catchment, with the maximum concentration being more than 33 times higher than the lowest concentration, the mean TTHM4FP concentration at the Hampshire Avon is  $716.65 \pm 273.23$

$\mu\text{g L}^{-1}$  compared to  $1916.89 \pm 279.93 \mu\text{g L}^{-1}$  at the Conwy catchment, as shown in Figure 5a.6 and 5a.7. Therefore, the mean TTHM4FP concentration at all of the sites in the Conwy catchment is 2.67 times greater than at the Hampshire Avon catchment (Figures 5a.6 and 5a.7). It appears that, despite the streams draining the Hampshire Avon catchment having a higher nutrient loading than the Conwy (see data in Chapter 4), the higher mean DOC ( $13.63 \text{ mg L}^{-1}$ ) concentration at the Conwy catchment, compared to the mean concentration of DOC at the Hampshire Avon ( $7.78 \text{ mg L}^{-1}$ ) is driving the relationships, although there is likely an influence from DOC character in these figures too, which differs from site to site. It is likely that the Conwy catchment samples contain a higher proportion of the hydrophobic, aromatic and humic compounds, which have been found to be responsible for a higher TTHM4FP when compared to waters high in hydrophilic, fulvic and non-aromatic compounds (Jung and Son, 2008).

Figures 5a.4 and 5a.8 display the mean standardised concentration of individual THM4 compounds formed from chlorination at the Hampshire Avon and Conwy catchments, showing that the Conwy forms a higher concentration of  $\text{CHCl}_2\text{Br}$  when compared to the Hampshire Avon catchment. This, coupled with the data presented in Table 5a.6, outlines the difference in individual THM4 compound formation under chlorination and chloramination at both the Hampshire Avon and also the Conwy catchment. The dominant THM4 compound under both treatments and at both catchments is  $\text{CHCl}_3$ , always forming approximately 75% or more of THM4, although at the Hampshire Avon,  $\text{CHCl}_3$  is formed in a higher concentration under chloramination than under chlorination. This could be due to  $\text{CHBr}_2\text{Cl}$  and  $\text{CHCl}_2\text{Br}$  not being formed under chloramination, and thus, more chlorine compounds were available to form  $\text{CHCl}_3$  (the THM4 compound reported to be most likely to cause cancer, see Table 5a.1). This is likely due to the theory of fast and slowly reactive DOC species. Hughes (2013) shows over the course of 7 days, THM concentration of  $\text{CHCl}_3$  increased over the course of day 3 to day 7, suggesting that some DOC is more slowly reactive than other types.  $\text{CHCl}_3$  formation dominated the THM4 compound group, with over 93.23% of chlorination formed THM4 detected in the Conwy catchment

comprising of  $\text{CHCl}_3$ , and 80.63% of the THM formed from chloramination in the Conwy catchment was  $\text{CHCl}_3$ . Small error values between the data for  $\text{CHCl}_2\text{Br}$ ,  $\text{CHBr}_2\text{Cl}$  and  $\text{CHBr}_3$  show that the trend displayed does not vary considerably between sites in the Conwy catchment (see Figure 5a.8).

Chloramination formed 3 times more  $\text{CHBr}_3$  than was formed under chlorination, again, likely due to the above mentioned factors. The data presented in Table 5a.9 shows that chlorination of samples from the Conwy catchment formed 93.23%  $\text{CHCl}_3$  under chlorination and 80.63% under chloramination, showing an approx. 13% decrease in formation of  $\text{CHCl}_3$ . However, this could be countered by the increased  $\text{CHCl}_2\text{Br}$  (at the Conwy catchment alone) which is approximately 3 times higher under chloramination when compared to chlorination. This data suggests that chlorine and chloramine are being favoured in different ways, and used for different mechanisms, such as those outlined in Figures 5a.1 and 5a.2. Further data relating to the organic matter in the water, such as molecular weight, nutrient concentrations and pH could help to identify possible reasons for the changing concentrations under each treatment and catchment. For example, increased bromide concentrations could be attributed to a greater formation of the brominated THM compounds, or, dissolved nitrogen species could be favourably treated before carbonaceous ones, thus decreasing the chlorine concentration available to form THM4 (although this is unlikely). The information generated from this further work would further help water companies to identify problematic waters and find treatment solutions best suited for it.

#### 5a.5.3.2 Chloramination

Figures 5a.6 and 5a.7 show the mean concentration of individual THM4 compounds formed from chloramination at the Conwy and Hampshire Avon catchments. Chloramination treatment formed no  $\text{CHBr}_2\text{Cl}$  and no  $\text{CHCl}_2\text{Br}$  at the Hampshire Avon, but did form mean concentrations of  $7.94 (\pm 1.27) \mu\text{g L}^{-1}$   $\text{CHCl}_3$  and  $0.81 (\pm 0.32) \mu\text{g L}^{-1}$  for  $\text{CHBr}_3$ .

At the Conwy catchment, a treatment of chloramination formed  $\text{CHCl}_3$  at a concentration of  $13.63 (\pm 0.63) \mu\text{g L}^{-1}$ ,  $2.22 (\pm 0.03) \mu\text{g L}^{-1}$  for  $\text{CHCl}_2\text{Br}$ ,  $0.54$

( $\pm 0.18$ )  $\mu\text{g L}^{-1}$  for  $\text{CHBr}_2\text{Cl}$  and finally,  $0.51 (\pm 0.19) \mu\text{g L}^{-1}$  for  $\text{CHBr}_3$ , showing that chloramination forms higher THM4 at the Conwy catchment when compared to the Hampshire Avon catchment.

Figure 5a.6 displays the TTHM4 at the Hampshire Avon under chlorination and chloramination treatments. The relationships between the two datasets appear to be very similar to the STHM4 data displayed in Figure 5a.8. Similarly, Figure 5a.7 shows the TTHM4FP from the Conwy catchment, showing a general trend in data between the two disinfection treatments (regression analysis from both data sets split into chlorination and chloramination treatments shows an  $R^2$  of 0.837 to a linear gradient of  $y=0.034x + 1.699$

Table 5a.11 explores the TTHM4 concentrations formed under chlorination at the Hampshire Avon and Conwy catchments. P values of  $<0.01$  for  $\text{CHCl}_3$ ,  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_3$  show that there is a significant difference in the concentration of these compounds between the two catchments, however,  $p=>0.05$  for  $\text{CHBr}_2\text{Cl}$  shows that there is no significant difference between these two sites, and again, this could be due to the bromide concentration present in the water prior to chlorination. Table 5a.8 compares the TTHM4 formation between the Hampshire Avon and the Conwy catchments under chloramination. Due to a lack of data for  $\text{CHCl}_2\text{Br}$  and  $\text{CHBr}_2\text{Cl}$  (due to no compounds present above minimum detection limit) for chloramination at the Hampshire Avon, data from only two THM4 compounds was able to be statistically analysed, with chloramination formed  $\text{CHCl}_3$  concentrations being statistically different between the two catchments (at  $p=<0.01$ ), however,  $\text{CHBr}_3$  concentrations were not significantly statistically different ( $p=>0.05$ ). Bromide removal prior to disinfection could be a procedure for drinking water treatments works to implement to reduce the brominated THM4 concentration found in the final water. Boyer and Singer (2005) found that bromide removal *via* a magnetic ion exchange resin was possible, but depended upon water alkalinity and initial bromide concentration. Bromide and THM4FP in 3 catchments that they studied showed a strong positive regression with an  $R^2$  value of 0.984 to a linear gradient of  $y=0.385x + 70.51$ , although a very similar regression between TTHM4FP concentration and DOC ( $R^2$  0.998,  $y=52.97x +$

13.58) at these sites was also found, possibly hinting to an intricate link between the two datapoints (Boyer and Singer, 2005).

All data explored in this chapter is used as a representation of the formation potential of THM4 compounds under water disinfection. However, it is important to note that the samples being disinfected have not been subjected to the same DOM removal practices that take place at water treatment works. The samples have also been in contact with an excess of chlorine/chloramines and left for 7 days, whereas in a real world scenario, the dose could be much lower and for a much shorter duration. Chloramination is typically applied to drinking water as an initial dose of chlorine, and then the addition of ammonia after approximately 30 minutes to form chloramine, however, the APHA standard method used in this thesis used preformed chloramines and contact times that vastly exceed the contact time used at water treatment works, and thus chloramination data generated from the present works should not be compared to water treatment works figures. The samples used in this project had only been filtered through 0.45 µm pore size filters to isolate any particulate organic matter (POM), whereas at a WTW, the water would pass through several filtration and chemical coagulation steps and also pH adjustment before treatment with a suitable dose of disinfectant to render the drinking water fit for human consumption once it reached the consumer's tap. This is without further filtration, coagulation and secondary disinfection which the water would undergo if it was passing through a WTW.

## 5a.6 Conclusions

The data presented within this chapter shows that chlorination and chloramination treatments do form different concentrations of THM4 (agreeing with work by Goslan *et al.*, 2009), however, these differences do not seem to be intricately linked to the DOC concentration of the water being disinfected, instead, there seem to be much more complex relationships between the organic matter and the disinfection process that need to be explored in further detail before a full understanding of the formation of THM4 compounds is possible. There is very little available literature on this subject and therefore, future studies should focus on the relationships between DOC and THM4 concentration from a wide variety of DOC types. Currently, water companies cannot rely on data generated simply from the relationship

between DOC and THM to determine the disinfection chemical and dose required for a specific water, and in fact, a much broader spectrum of information would help to provide a much more targeted response, which could ultimately reduce both disinfectant demand (and thus treatment costs), and also THM4 concentrations in final waters. However, ultimately, the removal of as many organic matter compounds as possible and nutrients in the raw water prior to chlorination is by far the best method to reduce THM4 formation

## 5a.7 References

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# Chapter 5b

## Characterisation of Dissolved Organic Nitrogen and Nitrogenous Disinfection By- Product Formation.

## 5b.1: Introduction

DOM in drinking water can produce unsavoury organoleptic (involving the use of sense organs) properties of water, such as taste, odour and colour (Ødegaard *et al.*, 2010), all of which are targeted during water treatment processes. However, any DOM that passes through these removal stages (such as low MW compounds) is then present when chlorine is added to disinfect, and is free to react with the disinfectant. The need for disinfection of water for drinking purposes has been recognised since a cholera outbreak in London, UK, in 1849, and thus, was linked to a source of unsafe drinking water, the River Thames, which contained waste water from settlements upstream, transporting cholera (Snow, 1849 and Bingham *et al.*, 2004). Chlorine is the disinfectant most widely utilised by water treatment companies, due to its higher disinfecting potential, ease of use and low cost. Chlorine residual is left throughout the distribution system protecting against microbial re-contamination (Gopal *et al.*, 2007), and is typically added after DOM removal practices have taken place. The quantity and characteristics of DOM in these waters depends upon climate, geology and topography, amongst other factors (Fabris *et al.*, 2008, Matilainen *et al.*, 2011). The compounds that surpass the DOM removal stages, however, are free to react with chlorine, which can lead to the formation of C-DBPs and N-DBPs.

DBPs have been studied extensively over the past 40 years, after the discovery of THMs in water by Rook in 1974 triggering growing interest into the health concerns associated with them. Studies have shown that DBPs can increase the risk of serious birth complications (Dodds *et al.*, 2004), and have been shown to have carcinogenic properties, especially in the colon and the bladder (Burgess, 1999 and Chow *et al.*, 2005). Ingestion of water, however, is not the only way in which a human can be exposed to DBPs. Studies by Dodds *et al.*, in 2004 suggested that a 5 minute shower or a 15 minute bath can expose you to equivalent concentrations of DBPs ingested from 1 litre of drinking water, as DBPs can be absorbed dermally, or inhaled through vapour (Dodds *et al.*, 2004). As a typical human requires 2 litres of water a day, a 10 minute shower or a 30 minute bath every day can expose someone to double that found in just water consumed. Therefore, research into their formation is important.

NOM (becoming DOM when dissolved in water) generally contains approximately 30-50% of carbon by weight, and this fraction is known as DOC. Total dissolved nitrogen (TDN) is a term that encompasses organic and inorganic nitrogen species dissolved in water, although

DON is generally thought to only encompass 5% of this fraction (Xu *et al.*, 2011), with typical DON concentrations ranging from  $<0.1 \text{ mg L}^{-1}$  to  $>10 \text{ mg L}^{-1}$  in surface waters, and a median value of  $\sim 0.3 \text{ mg L}^{-1}$  (Westerhoff and Mash, 2002). The primary sources of DON to freshwater include agricultural fertilisers, wastewater discharges, forest litter, and excretion of algal products in eutrophic water (Xu *et al.*, 2011). Ammonium ( $\text{NH}_4$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) are the most common ionic (reactive) forms of dissolved inorganic nitrogen (DIN) in aquatic ecosystems (Vandenbruwane *et al.*, 2007). These ions can be present naturally as a result of atmospheric deposition, surface and groundwater runoff, wastewater discharges and septic tank releases and algal activity, fostered by inorganic loadings such as agricultural and storm water runoff (Mitch *et al.*, 2009), dissolution of nitrogen-rich geological deposits, nitrogen fixation by certain prokaryotes (cyanobacteria with heterocysts, in particular), and biological degradation of organic matter (Camargo and Alonso, 2006).

Geological deposits can be a major source of nitrogen to an aquatic environment. With an estimated 20% of the Earth's global nitrogen pool (750,000,000 Mt N found in sedimentary and metasedimentary rocks) contained within geologic deposits (approx  $1.27 \text{ mg N kg}^{-1}$ ), elevated nitrogen concentrations (roughly  $2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Holloway *et al.*, 1998)) in water and soil have been attributed to weathering of bedrock, with sedimentary rock containing more nitrogen than igneous and metamorphic rocks. Nitrogen released through weathering of geological deposits may contribute to nitrogen saturation of an ecosystem, leading to elevated stream water nitrate concentrations (Holloway, 2002). Bebout *et al.*, found that mudstones and siltstones from the Bath District, UK, contained between 803 and 837  $\text{mg kg}^{-1}$  of nitrogen, whereas Rau *et al.*, found that North Atlantic limestone contained around 20-300  $\text{mg kg}^{-1}$  of nitrogen, showing that mudstone geology has the potential to release much more inorganic nitrogen than limestone geology, despite both being sedimentary (Bebout *et al.*, 1999, Holloway and Dahlgren, 2002, and Rau *et al.*, 1987).

Water sources polluted with algae and municipal waste water will contain key sources of DON (mainly ammonia, nitrate and nitrated organics in waste water (Adams, 1973)), and in turn, N-DBP precursors. With water demands increasing, the use of these waters is likely to increase (Bond *et al.*, 2012). Studies have shown that intracellular and extracellular algal organic matter are nitrogen-rich materials, and the higher organic nitrogen concentrations in algal materials will contribute to the formation of dichloroacetic acid, chloral hydrate, dichloroacetonitrile and cyanogen chloride in larger quantities after chlorination (Fang *et al.*, 2010). Furthermore, waste water has been shown to account for  $16,600 \text{ kg year}^{-1}$  (14% of

total) nitrogen loading to a catchment in the USA, a figure which is set to rise with further urbanisation (Valiela and Bowen, 2002). Thus, the disinfection of algae rich water will likely result in elevated concentrations of N-DBPs.

A variety of dissolved organic compounds (amino acids and urea), particulate nitrogen (Rabalais, 2002) and reactive nitrogen (N) that affect aquatic ecosystems include inorganic dissolved forms ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ). Lotic (i.e. contained within fast moving freshwater) loads of N have increased in many river basins due to intensified agriculture (estimated at  $25 \text{ kg N km}^{-1} \text{ yr}^{-1}$  (Howarth *et al.*, 1996)), urban development, industry and atmospheric deposition, with estimations that 5.5% N had leached from the catchment into the river up to 2003 in Belgian and Dutch test catchments (Pieterse *et al.*, 2003), and elevated concentrations of N in freshwater, especially that which is abstracted for human consumption, are of concern due to the financial implications and health risks posed by the formation potential of harmful N-DBPs when the water is disinfected to render it fit for consumption, typically by chlorination or chloramination.

It has been suggested that free chlorine (either as a dissolved gas, hypochlorous acid or hypochlorite ions) can react with DOM by 3 general pathways – oxidation, addition and substitution. Chlorine can undergo an addition reaction if the organic compound has a double bond, although this can be too slow to be of relevance in water treatment terms. Therefore most chlorinated DBPs are formed through oxidation and substitution (Johnson and Jensen, 1986, and Amy *et al.*, 2004). Oxidation and reduction are processes that take place in a redox reaction, where the loss or gain of electrons creates a change in oxidation state of the atom involved. An example of this reaction is when chlorine gas,  $\text{Cl}_2$ , is added to water, resulting in the formation of  $\text{HClO}$  and  $\text{HCl}$  (hypochlorous acid and hydrogen chloride respectively). The  $\text{Cl}_2$  becomes oxidised to form  $\text{HClO}$  and reduced to form  $\text{HCl}$  (see Equation 5b.a), known as a disproportionation reaction.

Half Equations:  $\text{Cl}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HClO} + \text{HCl}$

Chlorine oxidation:  $\frac{1}{2} \text{Cl}_2 \rightleftharpoons \text{H}^+\text{Cl}^+\text{O}^{2-} + \bar{e}$

Chlorine reduction:  $\text{H}^+ + \bar{e} + \frac{1}{2} \text{Cl}_2 \rightleftharpoons \text{H}^+ + \text{Cl}^-$

Equation 5b.a: Reaction of chlorine gas when added to water, and the formation of hypochlorous acid by chlorine oxidation and hydrogen chloride (by chlorine reduction) *via* a disproportionate redox reaction (adapted from Fair *et al.*, 1948)

Once the disinfectant has reacted with the DOM, C-DBPs and N-DBPs can be formed. Although THMs are currently regulated in UK (see Chapter 5a), recent research has shown that certain N-DBPs are more cytotoxic and genotoxic than these regulated DBPs, and furthermore, some have a higher propensity to form under chloramination, although it depends on how the chloramine is formed – preformed or sequential (in this instance, a preformed chloramine is used). Chloramine is a disinfectant that many water treatment works are switching from chlorination to, to help reduce the concentration of the regulated C-DBPs. The World Health Organisation (WHO), in 2006, has suggested guideline values for two N-DBPs (dichloroacetonitrile at 20 mg L<sup>-1</sup> and dibromoacetonitrile at 70 mg L<sup>-1</sup> – see Table 5b.1), however, they still remain unregulated in UK and USA drinking waters (Goslan *et al.*, 2009, Lee *et al.*, 2007, Muellner *et al.*, 2007, Plewa *et al.*, 2004). In 2007, Lee *et al.*, examined the impact of increased nitrogen content upon N-DBP formation, finding that the higher the proportion of organic nitrogen in DOM, the higher the rate of certain N-DBP formation (for example, *N*-Nitrosodimethylamine was formed at 4.5 nmol mg<sup>-1</sup> DON compared to 0.26 nmol mg<sup>-1</sup> DOC) (Lee *et al.*, 2007)). In the presence of organic nitrogen, it is possible for chloramination to produce organic chloramines. Several researchers have shown that chloramine readily transfers its chlorine at a comparatively rapid rate to organic amines to form organohalogen amines (Isaac & Morris, 1983; Bercz & Bawa, 1986, Amy *et al.*, 2004).

Other N-DBPs, such as those encompassed in the group nitrosamines, have been estimated to produce a lifetime cancer risk of 10<sup>-6</sup> (i.e. a lifetime risk of death from cancer from this source of 1 in 1,000,000) from the consumption of water containing 0.2 - 20 ng L<sup>-1</sup> of nitrosamines (Miyashita *et al.*, 2009 and EPA, 2002). It has also been shown that one nitrosamine, *N*-nitrosodimethylamine, is thought to produce cancer potencies much higher than those of THMs (Mitch *et al.*, 2003), thus suggesting that the efforts to reduce THM formation by changing from chlorination to another disinfection process/disinfectant, such as chloramination, can actually exacerbate overall DBP concentrations in drinking water. The story is the same for other groups of N-DBPs; recent research has shown that haloacetamides can be up to 12 times more toxic than the 5 regulated haloacetic acids (Chu *et al.*, 2010a), and therefore research into reducing its concentration in drinking water is important.

Table 5b.1 outlines two groups of N-DBPs, the concentration at which they are limited (if applicable), and their typical precursors.

Table 5b.1: List of current regulations and suggested regulations for two groups of N-DBP compounds.

Group	Names	Concentration limited?	Precursors	Reference
<b>Haloacetonitriles</b> (HANs – R <sub>3</sub> CCN)	Dichloroacetonitrile (DCAN) Bromochloroacetonitrile (BCAN) Dibromoacetonitrile Trichloroacetonitrile (TCAN) Chloroacetonitrile Bromodichloroacetonitrile Dibromochloroacetonitrile Tribromoacetonitrile Bromoacetonitrile Dibromoacetonitrile (DBAN)	<b>DBAN:</b> 70 µg L <sup>-1</sup> (Guideline value) <b>DCAN:</b> 20 µg L <sup>-1</sup> (provisional guideline value, WHO)	L-Aspartic Acid, L-Tryptophan (DCAN)	<b>Krasner et al., 2006</b> <b>Richardson, 2003</b> Sourced from: <b>Bond et al., 2012</b>
<b>Nitrosamines</b> (R <sub>2</sub> NNO)	N-nitrosodimethylamine (NDMA)	<b>USA:</b> 0.42 ng L <sup>-1</sup> for NDMA (EPA, 2014) in tap water	Dimethylamine (DMA), Trimethylamine (TMA),	<b>Bond et al., 2012</b>

	<p>N-nitrosopyrrolidine (NPYR)</p> <p>N-nitrosomorpholine (NMOR)</p> <p>N-Methylethyl nitrosamine (NMEA)</p> <p>N-nitrosodiethylamine (NDEA)</p> <p>2-nitrodiphenylamine (NDPA)</p> <p>N-nitrosopiperidine (NPIP)</p> <p>N-nitrosodibutylamine (NDBA)</p> <p>N-nitrosodiethanolamine (NDELA)</p>	<p><b>Canada:</b> 40 ng L<sup>-1</sup></p> <p><b>Japan:</b> 100 ng L<sup>-1</sup> (index value)</p>	<p>Diuron,</p> <p>Ranitidine, Nizatidine,</p> <p>N-Dimethylethanolamine (DMEA),</p> <p>Dimethylformamide (DMFA)</p> <p>Dimethyldithiocarbonate (DMDC)</p> <p>Dimethylaminobenzene (DMAB)</p> <p>3-(Dimethylaminomethyl) indole (DMAI)</p> <p>4-Dimethylaminoantipyrine (DMAP)</p>	<p><b>Plumlee et al., 2008</b></p> <p><b>Miyashita et al., 2009</b></p> <p><b>Lee et al., 2008</b></p> <p><b>Mitch et al., 2009</b></p> <p><b>Kosaka et al., 2015</b></p>
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### 5b.1.1 Nitrogen Character and Concentration and N-DBP Formation

Nitrogen rich colloidal, hydrophilic neutral and hydrophilic base fractions tend to dominate N-DBP formation. On the contrary, C-DBPs, such as THMs, are typically formed from the hydrophobic acid fraction, although this is susceptible to removal by coagulation during water treatment (Bond *et al.*, 2011). An understanding of these fractions, therefore, and their occurrence in freshwater destined for human consumption, could guide treatment practices to reduce the exposure of humans to these carcinogenic and potentially carcinogenic compounds.

A method for characterising DOC into fractions such as those mentioned above, was proposed by Hughes *et al.*, (2016), who fractionate a sample of freshwater into a minimum of 5 individual fractions: hydrophobic acids and bases, hydrophilic acids and bases, and hydrophilic neutrals. This fractionation is achieved by the selective absorptivities of two hydroscopic resins – DAX8 and XAD-4, which absorb the organic matter in the freshwater sample passed through it. This then desorbs into an acidic or basic eluent depending upon the organic matter's charge, thus separating the matter into the aforementioned classes. However, this method has not been widely utilised for DON fractionation.

DON has been fractionated previously by molecular weight (Lee and Westerhoff, 2006) and by ultrafiltration (Xu *et al.*, 2011), but there are fewer studies that focus upon the XAD and DAX resin based method of fractionation for DON samples. Chang and Wang, 2013, were able to recover hydrophobic acid/neutral and base fractions, amphiphilic acid/neutral and base fractions, hydrophilic acids/neutrals and base fractions and amino acid fractions from waste water plants in Taiwan and China. However, this method required between 52 and 132 L of sample water to generate data, compared to the 1 L of sample used by Hughes *et al.*, 2016, for DOC fractionation. Therefore, it is proposed in this thesis that the XAD/DAX resin based fractionation of DON will be carried out following Hughes *et al.*, to determine whether equivalent DON fractionation data is possible to collect alongside DOC data, and whether the DON data can provide an insight into the formation potential of N-DBPs.

Studies suggest that only a minor DON fraction is composed of labile low molecular weight substances, which include amino acids (Horňák *et al.*, 2016). In contrast to

THM and haloacetic acid precursors, (which are typically allochthonous and derived from terrestrial locations), amine precursors of N-nitrosodimethylamine, for example, are thought to be largely anthropogenic in origin (Sacher *et al.*, 2008).

The decomposition of organic chloramines can also lead to the formation of N-DBPs such as haloacetonitriles, cyanogen halides and halonitromethanes (Yang *et al.*, 2010). Decomposition of organic chloramines has been found to be related to the concentration and structure of reactants, where  $\alpha$ -N-chloroamino acids lead to the formation of corresponding aldehydes or ketones, ammonia and chloride ion, while  $\alpha$ -N,N-dichloroamino acids lead to the formation of corresponding chloraldimines (as intermediate products), and nitriles, aldehydes, ammonia, carbon dioxide and chloride ion (as end products) (Shang *et al.*, 2000). N-DBPs can feature nitrofunctional groups (NO<sub>2</sub>, e.g. halonitromethanes) and the nitrile group C $\equiv$ N (e.g. cyanogen chloride and haloacetonitriles), which are of concern due to their toxicity (Joo and Mitch, 2007).

There are 3 potential contributions to N-DBP formation – (i)DON, (ii)DOC reacting with the nitrogenous component of chloramine to form N-DBPs (this is least likely due to the use of preformed chloramine in this work), or (iii) a combination of (i) and (ii). Precursors of N-DBPs are normally dissolved in the water, such as amino acids, and can be further classified as organics/inorganics, and classified by their acidity/basicity and hydrophobicity/hydrophilicity. Amino acids typically represent 2-5 % of total NOM and have been identified as precursors for C-DBPs and N-DBPs (Bond *et al.*, 2014). Furthermore, the hydrophobic fractions of NOM, which can contribute up to ~75% of NOM, are thought to be a major source of DBP precursors (Bond *et al.*, 2011). Later research by Bond *et al.*, suggests that identified N-DBP precursors are typically low molecular weight and low electrostatic charge when compared to bulk NOM. Although amino acids have been found to be easier to remove during water treatment than the known molecular properties of the individual free amino acids would suggest (Bond *et al.*, 2012), a study of Californian and Canadian drinking waters found that coagulation removed between 34% and 75% of total amino acids, but the concentration of free amino acids was not affected. Other disinfection practices are not very effective at amino acid removal either. For example, prechlorination increased the free amino acid concentration because of the partial hydrolysis of proteins and peptides, forming aldehydes and nitriles, and

filtration has been found to both increase and decrease total amino acid concentration (Dotson and Westerhoff, 2009).

#### 5b.1.1.1 Amino Acids

The chlorine reactivity of amino acids depends on the side chain groups attached to the alpha carbon (for example, amino acids containing a thiol group such as methionine and cysteine, have high reactivities to chlorine) (Hu *et al.*, 2010), showing that each individual amino acid has differing properties and therefore formation potentials that differ between individual compounds. The chlorine reaction with amino acids and peptides (only terminal amines) is usually fast. For compounds containing no sulphur, the chlorination results in N-halo-(amino acids or peptides) formation. In addition, in the case of  $\alpha$ -amino acids, a decarboxylation and desamination follows an initial chloramination step which leads to a carbonyl compound, ammonia and a nitrile (Deborde and von Gunten, 2008).

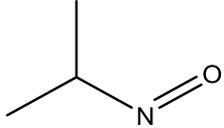
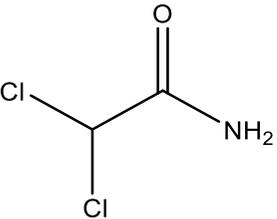
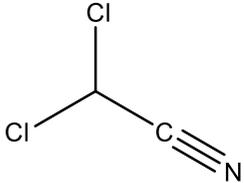
When chlorinated, such as in water destined for human consumption, amino acids demonstrate a breakpoint curve phenomena, similar to that of ammonia (where all ammonia is oxidised to nitrogen gas, nitrate or nitrogen trichloride at a ratio of 7.6 chlorine to ammonia N, before free chlorine residual can increase), resulting in a higher chlorine demand for the distributed waters, and if inorganic chloramine is used as the disinfectant, the presence of amino acids introduces a risk of overestimation of disinfection capabilities. Thus, understanding the occurrence of amino acids in source waters is important in ensuring sufficient disinfectant is added during water treatment (How *et al.*, 2014). Studies show that certain individual or groups of amino acids have been found to be precursors for several classes of N-DBPs such as halonitriles (Yang *et al.*, 2010) and cyanogen halides (Hirose *et al.*, 1989 (see Table 5b.2)), and some odorous DBPs such as *N*-chlorophenylacetaldimine (Freuze *et al.*, 2005).

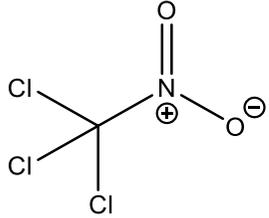
An amino acid undergoes chlorination via HOCl, which chlorinates the amine group, which in turn reacts releasing HOCl and CO<sub>2</sub>. This yields an aldehyde with chloramine attached, which can then react to form a nitrile and an

aldehyde (Morris *et al.*, 1980 and Isaac and Morris, 1983). This is the decarboxylation pathway, detailed in Figure 5b.3 (Joo and Mitch, 2007). Work by Ueno *et al.* studied 23 amino acid model compounds, showing that 15 amino acids formed haloacetonitriles (dichloroacetonitrile and trichloroacetonitrile) when chlorinated, with a mean concentration of 3.31  $\mu\text{g L}^{-1}$  total haloacetonitriles (range: 0.2 - 15.431  $\mu\text{g L}^{-1}$ ), where glutamic acid and histidine showed the highest concentration of haloacetonitriles (Ueno *et al.*, 1996). Chemical analyses of total amino acid concentrations in freshwater samples between sites is outlined in Chapter 2.

The most common amino acids found in freshwater have been examined, with a study of the Oosterschelde basin, located within The Netherlands, finding that alanine, aspartate, glycine, leucine and serine constituted a minimum of 60% of the dissolved free amino acid pool, although no total concentration was given (Laanbroek *et al.*, 1985, and Kaplan and Newbold, 2003). Table 5b.2 shows that glycine and serine can form cyanogen chloride when chloraminated, when found at concentrations of between 2 and 18  $\mu\text{g L}^{-1}$ .

Table 5b.2: Examples of precursors for N-DBPs, the N-DBPs formed, and the disinfectant responsible, in freshwaters (after Bond *et al.*, 2014).

N-DBP	Structure	Disinfectant	Precursors	Concentrations ( $\mu\text{g L}^{-1}$ )	Reference
N-Nitrosodimethylamine		Monochloramine	Dimethylamine (DMA) <sup>[1]</sup> Dithiocarbamates <sup>[2]</sup> Dithiocarbamate-based fungicides (e.g. thiram) <sup>[2]</sup>	0.31 (Dimethylamine)	<sup>[1]</sup> Gerecke and Sedlak, 2003 <sup>[2]</sup> Weissmahr and Sedlak, 2000
Dichloroacetamide		Chlorine	7 amino acids (asparagine, aspartic acid, glutamine, histidine, phenylalanine, tryptophan and tyrosine) <sup>[3]</sup>	2.2-5.3 (Tyrosine)	<sup>[3]</sup> Chu <i>et al.</i> , 2010a
Dichloroacetonitrile		Chlorine	Kynurenine (metabolite of tryptophan) <sup>[4]</sup> , Aspartic Acid <sup>[6]</sup>	270 (Aspartic Acid)	<sup>[6]</sup> Bond <i>et al.</i> , 2012

Cyanogen Chloride	$\text{N}\equiv\text{C}-\text{Cl}$	Monochloramine	Formaldehyde, glycine <sup>[5]</sup> , serine and threonine <sup>[6]</sup>	2 – 18 (Glycine)	<sup>[5]</sup> Mitch <i>et al.</i> , 2009 <sup>[6]</sup> Bond <i>et al.</i> , 2012
Trichloronitromethane/chloropicrin		Chlorine	Nitromethane and moieties <sup>[6]</sup> Nitrophenols <sup>[6]</sup> Resorcinol <sup>[6]</sup>		<sup>[6]</sup> Bond <i>et al.</i> , 2012

### 5b.1.2: N-DBP Precursors, Formation and Occurrence

The final step in understanding the N-DBP formation of waters is to be able to detect the concentrations of them in the final water (i.e. the disinfected water destined for human consumption). There are several groups of N-DBPs, and their formation is outlined below alongside an example compound of each group.

By far the most studied N-DBP group are the nitrosamines, and most attention seems to have been given to research into the nitrosamine N-nitrosodimethylamine, due to its high reported carcinogenicity, mutagenicity and teratogenicity (Andrzejewski *et al.*, 2005). The US Environmental Protection Agency (USEPA)'s integrated risk information service (IRIS) has classified this compound as a probable human carcinogen with a  $10^{-6}$  risk of contracting cancer from a  $0.7 \text{ ng L}^{-1}$  concentration (Mitch and Sedlak, 2002). N-Nitrosodimethylamine is produced as a by-product of industrial processes that use nitrates and/or nitrites and amines, under a range of different pH conditions, and is likely to be present in aqueous discharges of rubber, leather, pesticide, food processing, foundries and dye manufacturers. Whilst most of these industrial activities are often performed in large urban areas, and the release of waste water into freshwaters and oceans will be regulated, pesticides are likely to be found in waters draining agricultural land and thus in a more rural setting, such as the catchments studied in this thesis. Other potential sources of nitrogen rich organic matter could be damaged/overflowing septic tanks, caravan and campsites, and agricultural runoff.

N-Nitrosodimethylamine can also be present in drinking water through the degradation of dimethylhydrazine, a component of rocket fuel, and has been found in diesel engine exhausts (Mitch *et al.*, 2003, Goff *et al.*, 1980 and WHO, 2008). A study of Japanese drinking waters has found concentrations of N-nitrosodimethylamine in 10 out of 31 drinking water samples at concentrations as high as  $20 \text{ ng L}^{-1}$  (20% of the Japanese index value, see Table 5b.1). This study concluded that the concentration of N-nitrosodimethylamine was higher in raw water sources that drain catchments highly populated with humans (Asami *et al.*, 2009). Whilst chlorine is not required to form nitrosamines, chlorination of surface waters usually results in the formation of less than  $10 \text{ ng L}^{-1}$  N-nitrosodimethylamine, formed *via* the nitrosation of primary amines,

which decay almost instantly to release nitrogen gas and a carbocation (Shah and Mitch, 2012). For example, Mitch and Sedlak (2002) propose that the formation of N-nitrosodimethylamine in water and wastewater treatment involves chlorination reactions resulting in the formation and oxidation of 1,1-dimethylhydrazine (also known as unsymmetrical dimethylhydrazine,  $\text{H}_2\text{NN}(\text{CH}_3)_2$ ). In the presence of an oxidant (such as chlorine or chloramine), and at moderate pH levels, 1,1-dimethylhydrazine is oxidised into a variety of compounds including dimethylcyanamide, dimethylformamide, formaldehyde dimethylhydrazone, formaldehyde monomethylhydrazone and N-nitrosodimethylamine (which only counts for <5% overall product yield) (Mitch and Sedlak, 2002). Therefore, 1,1-dimethylhydrazine, found in high-energy fuel such as for rocket and military applications (EPA, 2016) is a precursor of N-nitrosodimethylamine.

During chloramination,  $\text{NH}_2\text{Cl}$  can be an additional source of nitrogen and produce halogenated N-DBPs, however, nitrogen containing organic compounds such as amino acids, pyrroles, and pyrimidines have also been associated with N-DBP formation (Nihemaiti *et al.*, 2016).

N-nitrosodimethylamine and related nitrosamines can also be formed *via* the nitrosation of secondary amines by nitrite (as hypothesised by Kimoto *et al.*, 1981 and Child *et al.*, 1996). Secondary amines have one hydrogen atom and two alkyl or aryl groups attached to the nitrogen. Examples of secondary amines are dimethylamine, dipropylamine and diphenylamine (Brown, 2000). The rate of nitrosation has been found to be extremely slow under conditions encountered in wastewater (i.e. low and neutral pH) (Mitch *et al.*, 2003).

Another group of commonly studied N-DBPs are known as haloacetonitriles. Haloacetonitriles are produced from the chlorination or chloramination from naturally occurring substances including algae, fluvic acid and proteinaceous material. A general trend shows that as temperature increases and/or as pH decreases, haloacetonitrile concentrations have been found to increase. Data generated from a study of USA derived data indicates that dichloroacetonitrile (a haloacetonitrile) is present in groundwater at  $0.87 \mu\text{g L}^{-1}$  and surface water at  $2.21 \mu\text{g L}^{-1}$  (WHO, 2004), whereas a further study of USA and Canadian water utilities found concentrations of haloacetonitriles up to  $40 \mu\text{g L}^{-1}$ , typically 12% of the concentration of the 4 regulated

THMs (Richardson *et al.*, 2007). Haloacetonitrile genotoxicities have been studied in Chinese hamster ovary cells, showing that concentrations of 37.1  $\mu\text{M}$  or 6.19  $\text{mg L}^{-1}$  (for idoacetonitrile) to 2.75  $\text{mM}$  or 302  $\text{mg L}^{-1}$  (for dichloroacetonitrile) are likely the highest genotoxic potency in these cells (Richardson *et al.*, 2007).

There are 2 different pathways which could be relevant to the formation of haloacetonitriles, proposed by Huang *et al.*, in 2012. The first is *via* a decarboxylation pathway, where the application of free chlorine or chloramines to free amino acids results in rapid di-chlorination of the alpha-terminal amine group. Joint (coupled) loss of  $\text{CO}_2$  and  $\text{HCl}$  forms a nitrile. In the instance of aspartic acid, the nitrile and carboxylic acid groups in the intermediate, render the methylene carbon acidic, promoting its chlorination. Hydrolysis released carbonic acid and dichloroacetonitrile, and further hydrolysis of dichloroacetonitrile forms dichloroacetamide and then dichloroacetic acid. The second method is *via* the aldehyde pathway, which typically requires formaldehyde. Formaldehyde is produced by the oxidation of methyl alcohol, which in turn, is produced by the oxidation of methane. Thus, formaldehyde is typically present in chlorinated water, and provides the aldehyde required for the aldehyde pathway which could lead to the formation of haloacetonitriles. The attachment of monochloramines electron lone pair upon an aldehyde (for example, formylacetic acid), incorporates the monochloramine N into the aldehyde. Removal of  $\text{H}_2\text{O}$  and  $\text{HCl}$  forms a nitrile, with all further steps the same as the decarboxylation pathway. It is important to note that the decarboxylation pathway can occur with both free chlorine and chloramines, though the aldehyde pathway predominantly applies to chloramines (Huang *et al.*, 2012). During chloramination, the aldehyde and decarboxylation pathways may operate simultaneously (Shah and Mitch, 2012). See Figure 5b.1 for a detailed example of the decarboxylation and aldehyde pathways from an amino acid to an N-DBP.

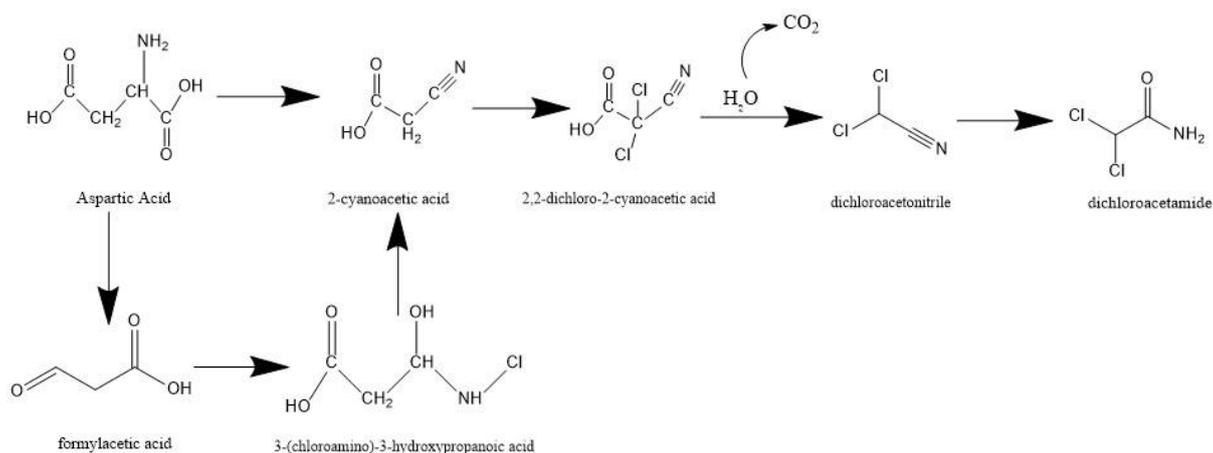


Figure 5b.1: Image to show decarboxylation (above) and aldehyde pathway (below) leading to the formation of dichloroacetonitrile (Huang *et al.*, 2012).

Cyanogen chloride is part of the cyanogen halide group of N-DBPs. Studies by Hirose *et al.*, (1988) show that aliphatic amino acids can react with hypochlorous acid and chloramine to form cyanogen chloride. 13 aliphatic amino acids were tested to determine their reactivity with hypochlorous acid and ammonium ions (i.e. chloramine) to form cyanogen chloride. Results show that all 13 aliphatic amino acids formed concentrations of cyanogen chloride, with threonine and serine forming the highest yields of cyanogen chloride from 1  $\mu\text{M}$  of amino acid and 20  $\mu\text{M}$  of  $\text{NH}_4\text{Cl}$  (13.7% and 11.2% respectively) (Hirose *et al.*, 1988). Work by Young and Uden in 1994, showed that cytosine and purine (both DNA nucleotide bases) created considerable amounts of  $\text{CNCl}$ , along with minor amounts of other products, such as chloroform and dichloroacetonitrile (Young and Uden, 1994), thus forming further DBPs.

Concentrations of dichloroacetamide have been detected in chlorinated drinking water in China at between 1.5 - 2.6  $\mu\text{g L}^{-1}$  (Yu *et al.*, 2015). Further studies by Chu *et al.*, (2010b) suggest that dichloroacetamide amino acid precursors are very similar to the ones that form dichloroacetonitrile (Figure 5b.2 shows that they are both formed during the decarboxylation and aldehyde pathways) during chlorination, proposing that dichloroacetamide and dichloroacetonitrile share very similar initial formation pathways, especially as hydrolysis of dichloroacetonitrile can form dichloroacetamide. Despite 20 amino acids analysed in this work, 7 were found to form dichloroacetamide, with histidine and aspartic acid producing the highest yields.

The authors noted that the amino acids that formed dichloroacetamide were very similar to those that formed dichloroacetonitrile during chlorination, such as histidine and glutamic acid (forming at concentrations of 15.2 and 13.2  $\mu\text{g L}^{-1}$  respectively), as outlined by Ueno *et al.*, (1996) (Chu *et al.*, 2010b). The formation of dichloroacetamide is outlined in Figure 5b.2.

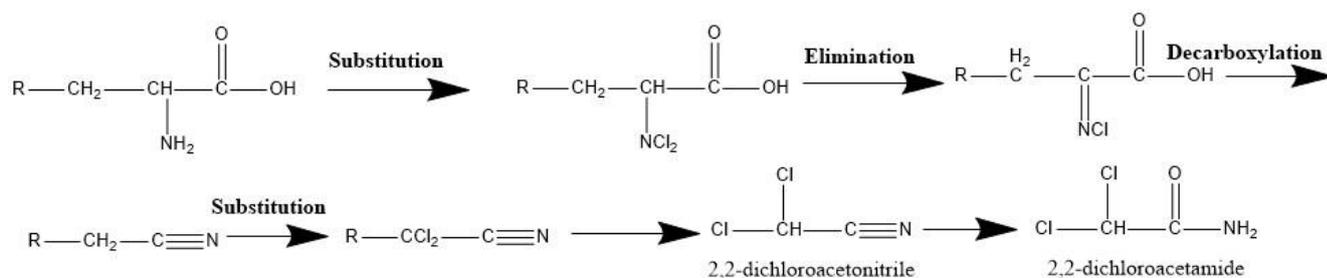


Figure 5b.2: Chemical pathway to show the formation for dichloroacetamide from amino acids (Chu *et al.*, 2010b).

Halonitromethanes are another group of N-DBP compounds, one of which is trichloronitromethane. Work by Yang *et al.*, in 2012 proposes a formation pathway for the formation of trichloronitromethane from the chlorination of an imine functional group (formed from the decomposition of organic chloramines in the presence of excessive chlorine or chloramines), where the C-N double bond is oxidised by HOCl. HCl was eliminated, followed by further oxidation of HOCl and elimination of  $\text{H}_2\text{O}$  and an aldehyde, forming  $\text{CHCl}_2-\text{N}(\text{OH})\text{Cl}$ , resulting in the formation of trichloronitromethane (after a series of elimination, oxidation and deprotonation). The authors also proposed a release of the RCHO group as being a step leading towards trichloronitromethane formation, with final concentrations of less than  $1.2 \mu\text{mol mmol}^{-1}$  trichloronitromethane formed from the chlorination of tryptophan (Yang *et al.*, 2012). See Figure 5b.3 for the proposed formation pathway of trichloronitromethane.

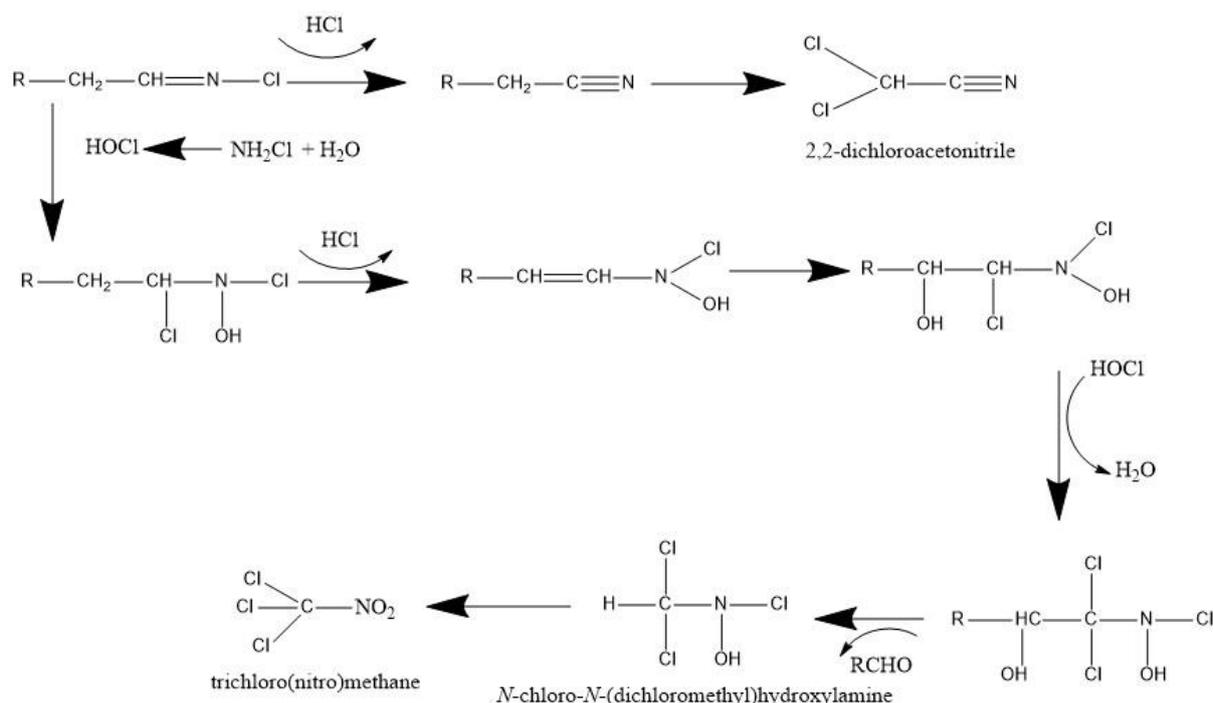


Figure 5b.3: Formation pathway of trichloronitromethane from chlorination of amino acids (adapted from Yang *et al.*, 2012). The R-CH<sub>2</sub> group at the beginning of the equation represents the side chain of an amino acid.

This work aims to detect the concentrations of a suite of amino acids in 3 reservoirs in north Wales, situated along a nutrient gradient, and to determine whether these amino acids occur at concentrations that can lead to the formation of substantial concentrations of N-DBPs.

## 5b.2 Detection of haloacetonitriles and halonitromethanes

The detection of haloacetonitriles and halonitromethanes can be achieved by gas chromatography – mass spectrometry (with recoveries between 80 and 120 %) (GC-MS). Bond *et al.*, 2014 were able to extract sample into methyl tertiary-butyl ether (MTBE), acidify it to pH 3.5 and add copper sulphate (to create a blue colour to facilitate the observation of the phase interface when the extract is transferred) and sodium sulphate (to eliminate free chlorine) (EPA, 1995). This sample was analysed by GC-ECD, and detection of chloroform, chloropicrin, dichloroacetonitrile, trichloroacetonitrile, 1,1-dichloropropanone and 1,1,1-trichloropropanone at minimum detection limits of 0.1, 0.5, 0.1, 0.5, 0.1 and 0.2 µg L<sup>-1</sup> respectively. Chu *et al.*, 2011, were able to detect haloacetonitriles and halonitromethanes using a purge and trap sample concentrator and GC-MS equipment. These authors were also

able to detect haloacetamides, after a liquid-liquid extraction method. The authors achieved detection limits of 0.091 – 0.26  $\mu\text{g L}^{-1}$  for haloacetonitriles and 0.15  $\mu\text{g L}^{-1}$  for halonitromethanes, far below the regulated limits some haloacetonitriles outlined in Table 5b.1.

Another way of detecting the presence of N-DBP compounds is to determine toxicological effects that can be isolated and attributed to a specific N-DBP compound. Although the use of human cells would prove problematic, yeast cells grow in a very similar way to human cells and therefore they can be used as a proxy for DNA damage and has been well studied particularly in the areas of cell cycle and DNA repair and replication (Costanzo *et al.*, 2001). Yeast cells have been used since the early 21<sup>st</sup> century discovery that the same genes that control the cell cycle in baker's yeast (*Saccharomyces cerevisiae*) exist in more or less the same capacity in human cells (Hartwell, 2002). Over 100 genes involved in cell cycle control were identified by Hartwell in yeast, and this strain of yeast has been selected for work in this thesis. These genes are known as cell division cycle (CDC) genes, and many of the same genes that work to regulate cell division in yeast were found to work similarly in humans. This regulation was found to be by either stimulating or inhibiting cell division, in response to the signals that the cells constantly receive from their environment, called proto-oncogenes. In cancer cells, however, mutated genes that normally stimulate cell division become stuck (oncogenes), and other mutated genes that normally inhibit excessive cell division (called tumour suppressor genes), stop working. This results in out of control cell growth and reproduction, resulting in the replication of cancerous cells, into a tumorous mass (Pray, 2008). Thus, this method can be used to detect carcinogenicity in certain compounds, which in turn can highlight the presence of this compound in drinking water, acting as a detection method too.

Haloacetonitriles have been shown to cause DNA damage in fission yeast, using the comet assay (Muller-Pillet *et al.*, 2000), and all 4 of the most prominent haloacetonitriles have been proven to induce sister chromatid exchange and DNA breaks (WHO, 2008 and Caspari *et al.*, 2017).

There are 4 main stages of the cell cycle in eukaryotic cell division, G1, S Phase, G2 and M phase. G1 phase is the first phase, called the interphase, and is where the cell synthesises mRNA and proteins, in preparation for later stages. Synthesis (S) phase is where DNA is replicated, before heading into G2 phase, which ends with the onset of prophase, which is the

first phase of mitosis where the cells chromatin condenses into chromosomes. Finally, the M phase is where cell division occurs. A 5<sup>th</sup> stage of the cell cycle is widely accepted (where the cell is neither dividing nor preparing to divide), called G<sub>0</sub>, where cells can stop cycling, after division, entering a state of quiescence (Collins *et al.*, 1997) (Figure 5b.4). In the figure, the black lines separating the different stages are labelled with CDK (cyclin dependant kinase). These kinases act as checkpoints to ensure that the activity of the previous stage of the cell cycle has been completed – when activated, they block the movement to the next stage of the cell cycle. For example, when CHK1 is activated after G<sub>2</sub>, mitosis is blocked, stopping the cell from replicating. There are several proteins that can block this pathway, such as RAD4, CRB2, Histone 2AX and HRC1.

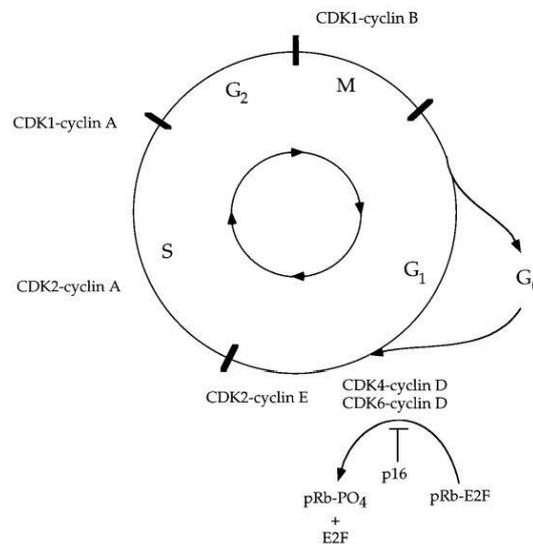


Figure 5b.4: A schematic representation of the mammalian cell cycle (Collins *et al.*, 1997). M represents mitosis phase, G<sub>1</sub> represents Gap 1 phase, G<sub>0</sub> represents a quiescent state in the cell cycle, and S phase represents synthesis phase.

The eukaryotic cell is well equipped for protection against DNA damage, allowing for damage detection, the halting of the cell cycle, before initiating repair or death. In order for cells to continue replicating, they must pass cell cycle checkpoints, which ensure that only healthy cells are able to replicate. Tel1<sup>ATM</sup> and Rad3<sup>ATR</sup> are phosphatidylinositol 3-kinase-related proteins that respond to double and single DNA strand breaks, respectively. When a double stranded break occurs, a checkpoint complex is formed via Cds1<sup>CDK2</sup> and the inactivation of Cdc2<sup>CDK1</sup>. However, in the event of a single stranded break, the exposed DNA is coated with single stranded DNA binding protein, leading to the recruitment of the

Rad3<sup>ATR</sup>-Rad26<sup>ATRIP</sup> complex and Rad17-RFC, which loads the Rad9-Rad1-Hus1 complex onto the chromatin at the site of the damage, which eventually leads to cell cycle arrest *via* the activation of another checkpoint (Takeishi *et al.*, 2015 and Dyer, 2015). This process can be visualised by the Western blot and electrophoresis, which, although seemingly ideal for determining the actions of haloacetonitriles upon DNA, it can also be used to determine the presence of a specific haloacetonitrile within a sample, such as freshwater.

As an example of a haloacetonitrile, Dibromoacetonitrile, and trichloroacetic (a haloacetic acid) acid have been found to block these CDK checkpoints, thus preventing cells from replicating correctly. Caspari *et al.*, 2017, have studied the proteins involved in activating CHK1, to check for phosphorylation of CHK1, finding that, with CHK1 deactivated, dibromoacetonitrile and trichloroacetic acid can still promote cell cycle arrest (i.e. stopping cells from replicating). Other studies show that, in response to DNA damage, cells can activate checkpoints at critical stages in the cell cycle, that prevent cells from progressing through the cell cycle, citing a human toxicogenomic analysis experiment of haloacetic acids, focussing on DNA damage signalling pathways. The damage caused by the haloacetic acids included genes related to double strand DNA break repair, cell cycle arrest and apoptosis (programmed cell death) regulation (Komaki, 2014, Attene-Ramos *et al.*, 2010), thus showing the different effects different DBPs can have upon cells.

Another kinase pathway, identified during G2 events, is called the MAP (mitogen activated protein) kinase pathway (equivalent to P38 MAP kinase in humans), which regulates the CDS1 (checking DNA synthesis) variant. MAP kinases can be affected by 3 major stresses – heat, osmotic and oxidative, thus preventing the cells from replicating by stopping them from reaching M phase.

In this thesis, an experiment was performed to detect the toxic properties of dibromoacetonitrile upon yeast cells, as a proxy for DNA. A range of dibromoacetonitrile concentrations would be injected into growing yeast cell media and the cell cycle arrest, thought to be triggered by this chemical, would be visible on a Western blot, thus not only showing what concentration of dibromoacetonitrile causes cell cycle arrest, but also, can be used purely to detect dibromoacetonitrile between the limits of detection. For the purposes of this experiment, dibromoacetonitrile was selected. Dibromoacetonitrile has been found to initiate tumours in ‘sensitive to carcinogenesis’ mouse skin when applied topically (Bull *et al.*, 1985), and research has found that 100µM of dibromoacetonitrile is toxic for HeLa S3

cells (an immortal cell) (Muller-Pillet *et al.*, 2000). It was hypothesised that the detection of cell damage caused by a range of concentrations of dibromoacetonitrile, 2 to 12  $\mu\text{M}$  (0.4 to 2.39  $\mu\text{g L}^{-1}$ ), by the western blot and electrophoresis, could be used to determine the presence of dibromoacetonitrile in chlorinated drinking waters either to understand the likelihood of dibromoacetonitrile to form in new potential abstraction locations, or as a detection method without the use of gas chromatography/mass spectrometry.

### 5b.2.1: Hypothesis

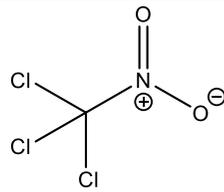
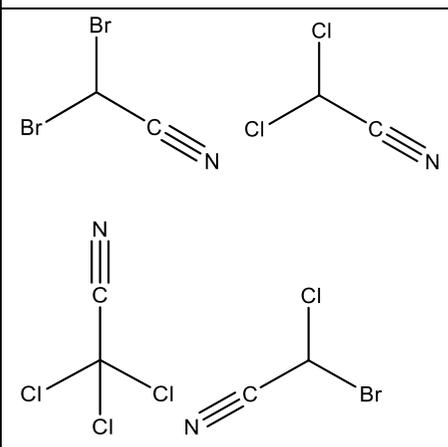
It is hypothesised that NDBP detection will be possible by GC-MS using SPME, however, this may not be the best suited method. Similarly, DBAN detection by electrophoresis is possible but limits of detection and the duration of the experiment may not make the method suitable for further use.

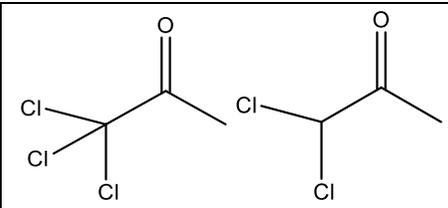
## 5b.3 Methodology

### 5b.3.1 Method Development

To explore and better understand the difficulties in N-DBP detection, and to attempt to be able to detect N-DBPs in freshwater samples without complicated derivitisation steps, 7 N-DBP standards were obtained from AccuStandard (Connecticut, USA) for analysis using a Varian 450 GC, equipped with a  $63^{\text{Ni}}$  ECD. See Table 5b.3 for a comprehensive list of these compounds.

Table 5b.3: 7 compounds selected for detection by GC-SPME, arranged by N-DBP compound group. T and B signifies top and bottom, L and R signifies left and right, in reference to the structure of each compound.

N-DBP Group	Compound	Product ID	Lot #	Structure	Molar Mass (g mol <sup>-1</sup> )
Halonitromethane	Chloropicrin	M-551B-3	215061279		164.366
Haloacetonitrile	Dibromoacetonitrile (TL)	M-551B-4	215071116		198.845 (TL)
	Dibromoacetonitrile (TR)	M-551B-5	215061407		109.937 (TR)
	Dichloroacetonitrile (TR)	M-551B-7	215061408		144.39 (BL)
	Trichloroacetonitrile (BL)				154.393 (BR)
	Bromochloroacetonitrile (BR)				

Haloacetone	1,1,1-Trichloro-2-propanone (L)	M-551B-8	215091334		163.43 (L)
	1,1-Dichloro-2-propanone (R)	M-551B-6	215111178		126.97 (R)

**Chloropicrin**, also known as nitrochloroform, has been known to be hazardous to human health for over 30 years. It is commonly used as a tear gas (due to its known eye irritation properties), a pesticide and to kill small mammals. Chloropicrin is not stable in water and it will be partially eliminated during treatment with activated carbon (Hoigné and Bader, 1988). As a member of the halonitromethane group, chloropicrin carcinogenicity has been studied in rats and mice, however, results were found to be inconclusive (NTP, 1978 and Richardson *et al.*, 2007), thus showing that further research is required to help provide conclusive evidence on the dangers of chloropicrin. However, Hoigné and Bader report that concentrations of less than 1  $\mu\text{g L}^{-1}$  are not thought to exhibit toxic effects on humans. Chloropicrin has been found to form from several precursors when chlorinated at 0.002M  $\text{Cl}_2 \text{L}^{-1}$  (including nitromethane (45% of nitromethane was converted to chloropicrin), nitrophenols (between 5.7 and 53% of nitrophenols were converted to chloropicrin), nitrobenzene (0.3 % was converted to chloropicrin) and glycine (0.01 % was converted to chloropicrin)). Perhaps most importantly, this work found a chloropicrin yield of 1.45  $\mu\text{g L}^{-1}$  per mg TOC in a 20 mg  $\text{L}^{-1}$  TOC sample under conditions found in WTWs (Merlet *et al.*, 1985). More recent studies have found that ozone, sometimes used in addition to chlorine in water treatment works (WTWs), promotes chloropicrin formation by oxidising amines to nitro-compounds (McCurry *et al.*, 2016). Therefore, chloropicrin may be found in drinking waters that do not pass through activated carbon, and may further form in the distribution network from other precursors not removed at the treatment stage.

**Dibromoacetonitrile** (as part of the haloacetonitrile group of N-DBPs) has been found in chlorinated drinking water for over 35 years. Detection of dibromoacetonitrile by SPME has been achieved before, in a range of 0.05 to 10  $\mu\text{g L}^{-1}$ , showing a limit of detection of 0.04  $\mu\text{g L}^{-1}$ , with an  $r^2$  value of 0.9822 and a recovery of 98.4% (Luo *et al.*, 2014). Water from a Florida WTW was found to contain concentrations of dibromoacetonitrile ranging from 0.01 - 3  $\mu\text{g L}^{-1}$  but have been reported as high as 42  $\mu\text{g L}^{-1}$  (Trehy and Bieber, 1981). They have been recognised as potentially harmful compounds, with a study by Bull *et al.*, (1985) finding that haloacetonitriles initiate skin tumors in mice, with dibromoacetonitrile being the most reactive. In 1999, Boorman *et al.*, argue that there was essentially no carcinogenicity information available for haloacetonitriles, leading their research into

dibromoacetonitrile in rodents. More recent research shows that haloacetonitriles have been found to promote genotoxic activity in Chinese hamster ovary (CHO) cells, with the brominated, di and tri halogenated species being more toxic (Muellner *et al.*, 2007). Dibromoacetonitrile is not mutagenic (Caspari *et al.*, 2017), but its removal from drinking waters is still vital.

**Dichloroacetonitrile**, also a haloacetonitrile, is formed during the chlorination of tryptophan, tyrosine and aspartic acid, and during the chloramination of glutamic acid, tryptophan and cytosine (Yang *et al.*, 2012). Other sources include heterocyclic nitrogen in nucleic acids, proteinaceous materials and combined amino acids bound to humic substances. Dichloroacetonitrile formation potential has been found to correlate positively with chloroform upon chloramination ( $r^2=0.75$ ) (Lee *et al.*, 2007). The toxicity of dichloroacetonitrile has been found to range from concentrations of 10  $\mu\text{M}$ , and concentrations of 57.30  $\mu\text{M}$  have been found to reduce 50% of cell density when compared to a negative control (Muellner *et al.*, 2007). Studies into the health concerns that may be posed by dichloroacetonitrile have found that it induces aneuploidy (the presence of an abnormal number of chromosomes in a cell) in a species of fly (Osgood and Sterling, 1991). In 1996, dichloroacetonitrile was formed in experiments chlorinating humic acids, at approx. 2.60 to 3.10  $\mu\text{g L}^{-1}$  (Ueno *et al.*, 1996). More recent research has found that dichloroacetonitrile caused much more serious cell density reduction and genomic DNA damage in CHO cells than haloacetic acids (Muellner *et al.*, 2007), and Esmat *et al.*, 2012, conclude that dichloroacetonitrile induced oxidative stress and developmental apoptotic imbalance in mouse foetal brains (Lin *et al.*, 2016).

**Trichloroacetonitrile** is used as an intermediate in insecticides, pesticides and dyes (Han *et al.*, 2015). In 2011, the WHO published their 4<sup>th</sup> edition of Guidelines for Drinking Water Quality, stating that current available data proved insufficient to serve as a basis for the derivation of a guideline value for this compound. A provisional guideline of 1  $\mu\text{g L}^{-1}$  was in place prior to this, but re-evaluation found the study that suggested this guideline was unreliable (WHO, 2011). However, the effects of trichloroacetonitrile have been studied upon pregnancy rates of Long-Evans rats, in 1987, showing a significant reduction in the pregnancy rates after exposure to 0.1 mL per 100  $\text{g}^{-1}$  body weight, and it has also been found that trichloroacetonitrile induces heart and kidney malformations (Smith *et al.*, 1987 and Smith *et al.*, 1988). Further

analysis of this compound and its occurrence and potential toxicity is therefore necessary in maintaining a healthy and safe drinking water for the public.

**Bromochloroacetonitrile** studies from 1991 suggest that there was no data available upon the degree of evidence of carcinogenicity of bromochloroacetonitrile in humans, and that there is inadequate evidence available for carcinogenicity in animals, with conclusions showing that the agent was not classifiable as to its carcinogenicity to humans (WHO, 2004). In 2011, the WHO published that bromochloroacetonitrile toxicity data was insufficient to serve as a basis for the derivatisation of a baseline value (WHO, 2011). However, other published works have outlined that bromochloroacetonitrile has been reported to have carcinogenic and mutagenic effects in mice, and damage the DNA of mice (Nikolaou *et al.*, 2000). Other compounds within the haloacetonitrile group, such as dichloro- and dibromoacetonitrile, have had a qualitative target of 90  $\mu\text{g L}^{-1}$  and 100  $\mu\text{g L}^{-1}$  in drinking water, respectively (WHO, 1995).

**1,1,1-trichloro-2-propanone** (a haloketone) formation has been studied in the distribution of a WTW over a 1 year cycle, finding that concentrations were highest in summer under ozone-chlorine treatments (approx. 6  $\mu\text{g L}^{-1}$ ) as opposed to summer under chlorine-chlorine treatments (5  $\mu\text{g L}^{-1}$ ) and summer chlorine-chloramine treatments (1.5  $\mu\text{g L}^{-1}$ ). 1,1,1-trichloro-2-propanone was measured at 3 stages in the WTW distribution, beginning, middle, and end, and it appears that concentrations of the compound do not increase only under the chlorine-chloramine treatment, the further through the system that measurements are taken (LeBel *et al.*, 1997). Bull and Robinson, 1986, found 1,1,1-trichloro-2-propanone to exert carcinogenic or mutagenic effects in mice, and its formation has been found to decrease with increasing pH of the water (Yang *et al.*, 2007).

**1,1-dichloro-2-propanone** was also included in studies by LeBel *et al.*, 1997, finding that concentrations over all 4 seasons were < 2  $\mu\text{g L}^{-1}$  with no visible changes in concentration at different stages of the water distribution system for chlorine-chloramine treatments, a subtle decrease for chlorine-chlorine treatments, and an increase at the midpoint of the distribution network before falling to below the initial concentration for ozone-chlorine treated water. Fang *et al.*, 2010, found that 1,1-

dichloro-2-propanone yields were higher under chloramination than under chlorination.

It is clear, therefore, that further research into the formation of these compounds is required to attempt to determine their source, their carcinogenicity and toxicity, and how to successfully remove or regulate them from occurring in final drinking water

In order to determine whether an existing method to detect THM4 compounds would be suitable for the detection of N-DBP compounds, 7 individual N-DBP compounds were acquired and mixed into aqueous solutions at known concentrations, to act as standards. These standards would be analysed in series using the existing THM4 method, and the data compared to determine whether this method was suitable for accurate and reliable detection of these nitrogenous compounds.

Each of the 7 standard compounds were supplied in as 1 ml vials of a  $5 \text{ mg mL}^{-1}$  concentration of the compound, suspended in acetone, equating to a concentration of  $5000 \text{ mg L}^{-1}$ . Each standard vial was emptied into a 100 mL volumetric flask and made to the mark with Milli-Q water, to form a working stock concentration of  $50,000 \text{ } \mu\text{g L}^{-1}$ . These 7 different  $50,000 \text{ } \mu\text{g L}^{-1}$  working stock standards were then diluted, using ultrapure water, to form concentrations of 200, 150, 100, 50, 20, 10 and  $1 \text{ } \mu\text{g L}^{-1}$ , thus resulting in 7 concentrations of each of the 7 compounds to be analysed by GC-MS.

Of the many consumable components and settings of the GC-MS that could be tailored to suit detection (carrier gas, detection method, temperature, cell contact potential, inlet liner, SPME fibre, syringe type, incubation period etc), fitting a more suitable SPME fibre (than the fibre currently used by the GC-MS for THM4 detection) was deemed to be the first step in adapting and developing a suitable detection method for the selected DBPs. Despite many SPME fibres available, the  $100 \text{ } \mu\text{m}$  PDMS Fused Silica fibre was selected, as it is best suited for the compounds to be analysed (i.e. volatiles in the MW 60-275 range). Luo *et al.*, 2012, used GC-MS with a mass selective detector to detect 13 haloacetonitriles and halonitromethanes in drinking water, using SPME and standards available from Accustandard (Newhaven, USA). The authors tested 7 different commercially available SPME fibres, as they argue that the fibre coating is generally considered to be the critical factor for achieving optimal extraction efficiencies, resolution and detection limits. A bipolar

divinylbenzene carboxen polydimethylsiloxane (able to detect a MW range of 40-275) was finally selected for the detection of haloacetonitriles, as it was best suited for the heavier compounds such as bromoacetonitrile, bromochloroacetonitrile and dibromoacetonitrile, and other studies (Boadas-Vaello *et al.*, 2008 and Kristiana *et al.*, 2012) have found that they were effective in extracting haloacetonitriles from aqueous solutions with limits of detection at less than 3 ng mL<sup>-1</sup> and between 0.009 and 0.8 ng mL<sup>-1</sup> respectively. Although this fibre was deemed suitable for the detection of these haloacetonitriles in other studies, the PDMS SPME was selected as it appeared to be suitable for the range of compounds that were to be detected, and was already fitted to the GC-MS from previous analysis.

### 5b.3.2. Methods

Dibromoacetonitrile detection by electrophoresis, N-DBP detection by gas chromatography/mass spectrometry and DON fractionation were all achieved following methods outlined in Chapter 2.

### 5b.4. Statistical Analysis

Data was tested for normality by examining the skewness values. Values between -2.0 and +2.0 were deemed acceptable (i.e normally distributed) and the data could be analysed without transformation, using a Pearson's correlation. If the data was not normally distributed (i.e. a skewness below -2.0 or greater than +2.0) then it was transformed by log<sup>10</sup> and analysed for skewness again. If the data was deemed normally distributed, it was analysed using a Pearson's correlation, otherwise, a Spearman's Rank test was used

ANOVA tests were then carried out between some parameters, and a Tukey HSD post hoc test was selected to show where any statistically significant differences occurred. Other data was analysed *via* regression analysis, and data relating to the gradient of the line of best fit and the R<sup>2</sup> value was presented alongside the graph.

## 5b.5 Results

Figure 5b.5: Graphs to show the detection of 7 nitrogenous compounds by GC-ECD from aqueous standards ranging in concentration from 1 to 200  $\mu\text{g L}^{-1}$ . Peak area is the signal response to the compound as it passes through the detector chamber, measured in  $\mu\text{V min}^{-1}$ . Line of best fit and equations show relationship between peak area and concentration of the different standards.

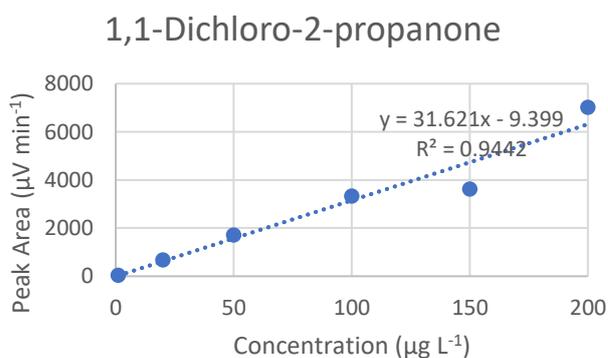
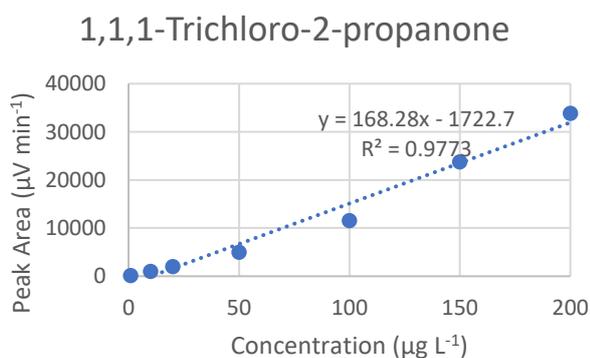
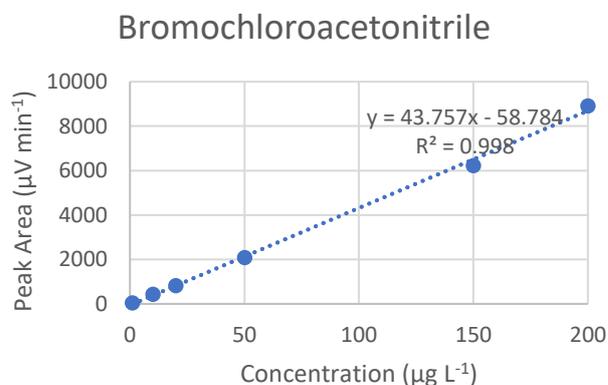
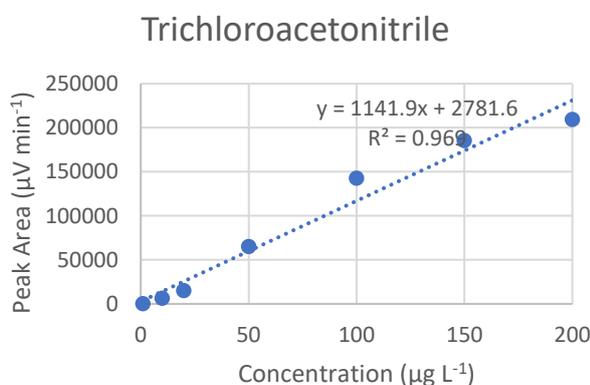
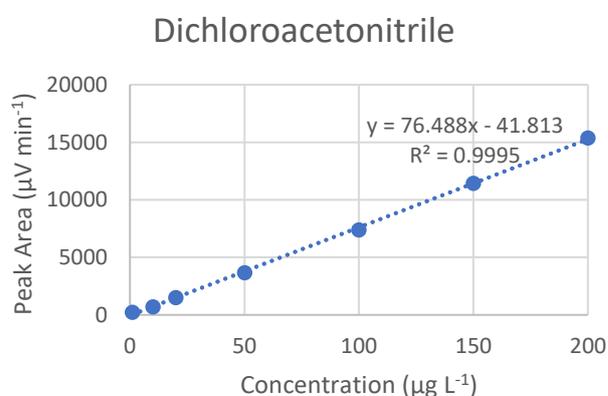
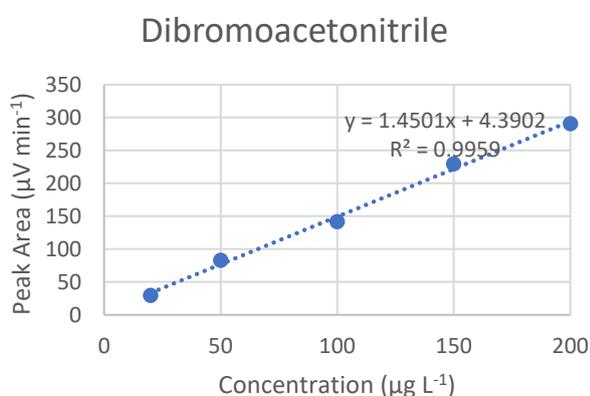
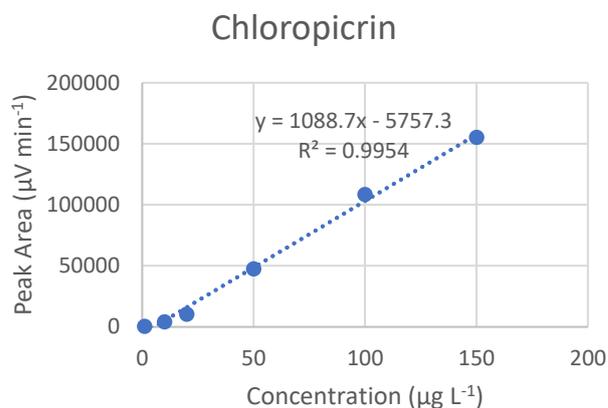


Table 5b.4: Compounds detected by GC-MS fitted with an ECD and SPME fibre, concentration range, retention time, other published detected concentration ranges and R<sup>2</sup> value of line of best fit.

Compound	Concentration Range ( $\mu\text{g L}^{-1}$ )	Retention time (Seconds)	Concentration Range ( $\mu\text{g L}^{-1}$ ) (Luo <i>et al.</i> , 2014).	R <sup>2</sup> value (Luo <i>et al.</i> , 2014)
Chloropicrin	1 to 150	456		
Dibromoacetonitrile	20 to 200	523.8 ( $\pm 0.684$ )	0.05 to 10	0.9822
Dichloroacetonitrile	1 to 200	408.6 ( $\pm 0.372$ )	0.05 to 10	0.9925
Trichloroacetonitrile	1 to 200	267.6	0.05 to 10	0.9756
Bromochloroacetonitrile	1 to 200	543 ( $\pm 12$ )	0.05 to 10	0.9819
1,1,1-Trichloro-2-propanone	1 to 200	615 ( $\pm 0.72$ )		
1,1-dichloro-2-propanone	1 to 100 150 to 200	471.6 ( $\pm 3.78$ ) 563.7 ( $\pm 2.1$ )		

The data presented in Figure 5b.5 and Table 5b.4 shows that the detection of these compounds from  $1 \mu\text{g L}^{-1}$  to  $200 \mu\text{g L}^{-1}$  is possible, confirming that the method selected and adapted could be suitable for the detection and quantification of these compounds in aqueous samples. The R<sup>2</sup> value is a statistical measure of how well the line of best fit approximates the real data points (which were diluted serially), with a score of one being classed as perfect, and a score of zero showing that the line of best fit does not approximate any of the real data points. Of the 7 compounds that were analysed, the lowest R<sup>2</sup> is at 0.9442, for 1,1-dichloro-2-propanone, which is likely skewed by the concentration of the  $150 \mu\text{g L}^{-1}$ , which it seems was incorrectly mixed.

Of the 7 compounds, trichloroacetonitrile produced the highest peak area at  $209,650.8 \mu\text{V min}^{-1}$ . On the contrary, dibromoacetonitrile produced the lowest response at  $30 \mu\text{V min}^{-1}$ , and also was only detected at concentrations as low as  $20 \mu\text{g L}^{-1}$ . All other compounds were

detectable at all concentrations except chloropicrin, which was not detected at  $200 \mu\text{g L}^{-1}$ , although this may be due to a mechanical or user error, as the trend line suggests that detection should continue. Dibromoacetonitrile was the only compound that was not detected at  $1 \mu\text{g L}^{-1}$ , and was first detected at a concentration of  $20 \mu\text{g L}^{-1}$ .

The distribution of data points for trichloroacetonitrile seems to better suit a polynomial relationship rather than a linear relationship, as it appears that the datapoints are reaching a plateau, possibly heading towards the limit of detection of the apparatus for this specific compound. Furthermore, 1,1,1-trichloro-2-propanone also appears to better suit a polynomial trend, but this time, showing a steeper increase in sensitivity as the concentration of the standard increases.

### 5b.5.1 Amino Acids

Amino acid data from 27 Conwy sites were averaged from 3 seasons of data collection (February, April and September 2015) to provide a good representation of an annual average.

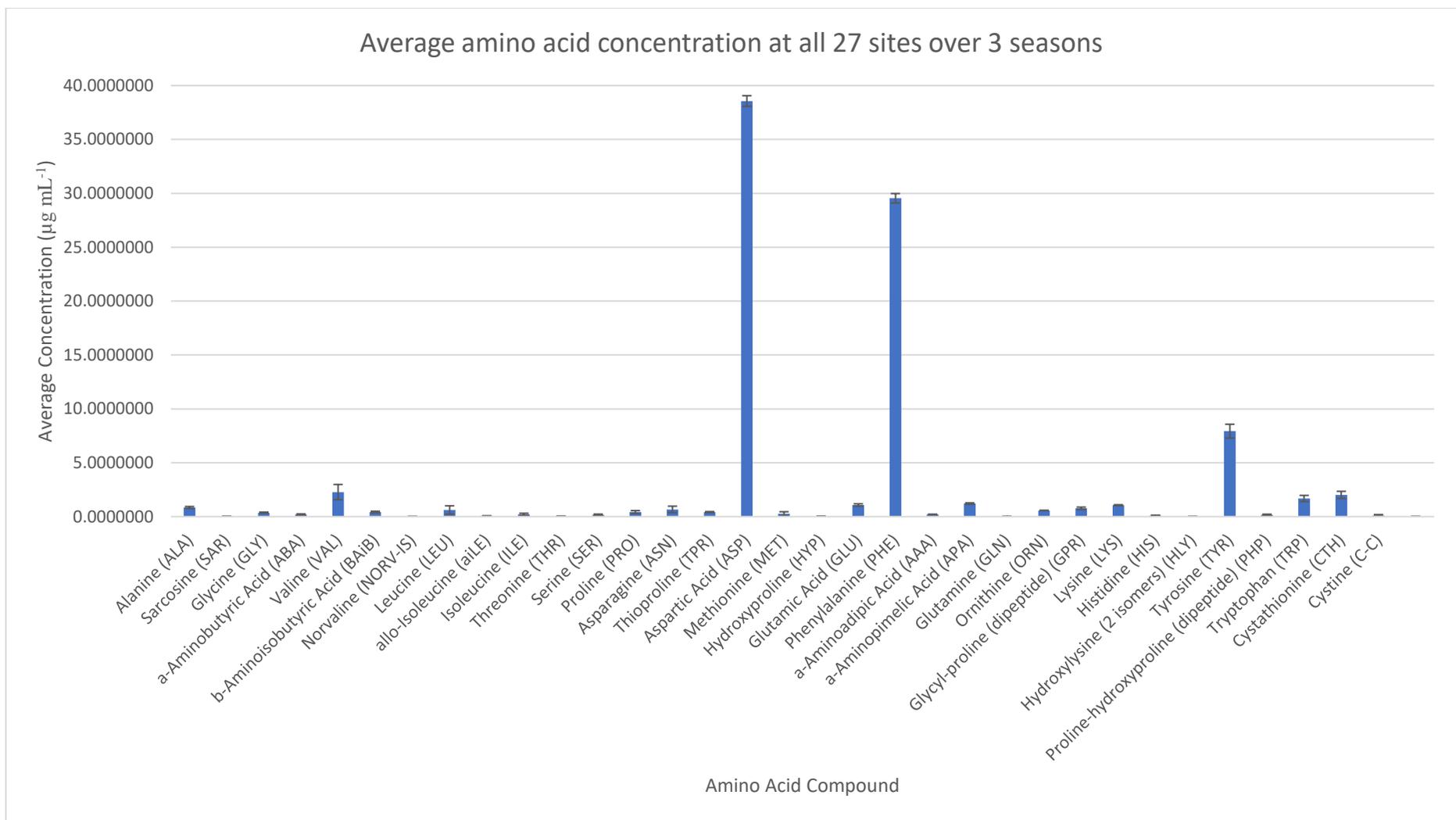


Figure 5b.6: Graph to show mean total amino acid concentration, averaged (to minimise seasonal variance) from all 27 Conwy catchment sites over 3 seasons. Error bars represent seasonal variance.

It is important to remember that norvaline is included as an internal standard and therefore data relating to this compound has been used to normalise all other amino acid data points (hence the exclusion of the detection of this compound from the data). By examining the data in Figure 5b.6, it is quite clear that there are 3 amino acids which appear in abundance between all sites; aspartic acid, phenylalanine and tyrosine, at concentrations of  $38.57 \pm 0.50$ ,  $29.54 \pm 0.44$  and  $7.93 \pm 0.64 \mu\text{g L}^{-1}$  respectively. Valine is also detected at a mean of  $2.28 \pm 0.71 \mu\text{g L}^{-1}$ . These amino acids may be driving concentrations of N-DBPs produced once the water is disinfected for human consumption, if the compounds are not effectively removed at the water treatment stage, and appear as precursors for some N-DBPs studied here. Bond *et al.*, 2009 find that chlorination of aspartic acid formed dichloroacetic acid at 0.26 mol/mol, trichloroacetaldehyde at 0.02 mol/mol and dichloroacetonitrile at 0.06 mol/mol. However, with an initial chlorine dosing solution of  $5 \text{ mg L}^{-1}$  ( $67.17 \mu\text{mol/L}$ ) and an aspartic acid concentration in this study of  $290 \mu\text{mol L}^{-1}$ , it can be expected that minute concentrations of DBPs are likely to form from these waters.

### 5b.5.2 Fractionation

5 different fractions of DOC and DON (determined by hydrophobicity/hydrophilicity and/or acidity/basicity) recovered from raw water samples collected in February totalled more than the initial concentration of DOC (i.e. higher than 100% recovery), whereas DOC and DON recovery from January shows a recovery of approx. 90%. It can be seen that the variance is greater in February data when compared to January data, although this is likely due to natural processes, it could be that the resins were not suitably cleared from previous runs thus retaining and releasing DON in different cycles. Jan and Feb DON data was found to be normally distributed (Kolmogorov-Smirnov  $p=0.200$ ) with skewness of 0.475 and 0.831 respectively. However, DOC concentrations in Jan and Feb were found to not be statistically normally distributed, despite skewness of 0.206 and 1.492, which are still thought to be normally distributed data (George and Mallery, 2010). A Levenes test for equal variance shows that the data has not violated the assumption of equal variance, showing that the data have similar variances. See Figure 5b.7.

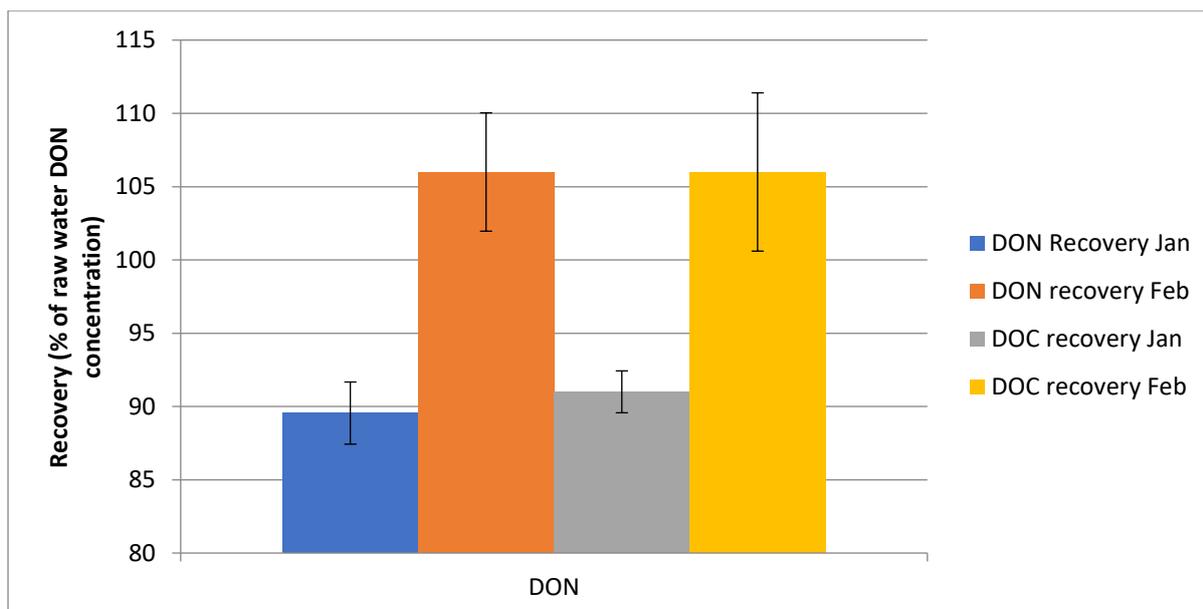


Figure 5b.7: Mean DOC and DON percentage recovered in fractions compared to original raw sample, across 15 sites at 3 reservoirs (Conwy, Cefni and Alwen, North Wales, UK) for the months of January and February, 2013. Error bars represent standard error of the mean (SEM).

Table 5b.5: Mean concentration data from DOC and DON fraction samples from 3 reservoirs in North Wales. Concentrations are in mg L<sup>-1</sup> and have been averaged from two consecutive months, January and February 2013.

	DOC (mg L <sup>-1</sup> )				(DON (mg L <sup>-1</sup> ) (displayed as TDN-TIN))		
	Alwen	Conwy	Cefni		Alwen	Conwy	Cefni
<b>Raw</b>	11.9	7.6	8.0		0.5	0.3	2.0
<b>HPOA</b>	33.9	23.0	17.3		0.7	0.4	0.3
<b>HPIA</b>	24.3	15.2	15.9		0.6	0.3	0.5
<b>HPOB</b>	2.0	2.2	2.3		0.1	0.1	0.5
<b>HPIB</b>	1.5	3.0	2.6		0.1	0.1	0.3
<b>HPIN</b>	4.7	3.5	5.6		0.3	0.2	1.7

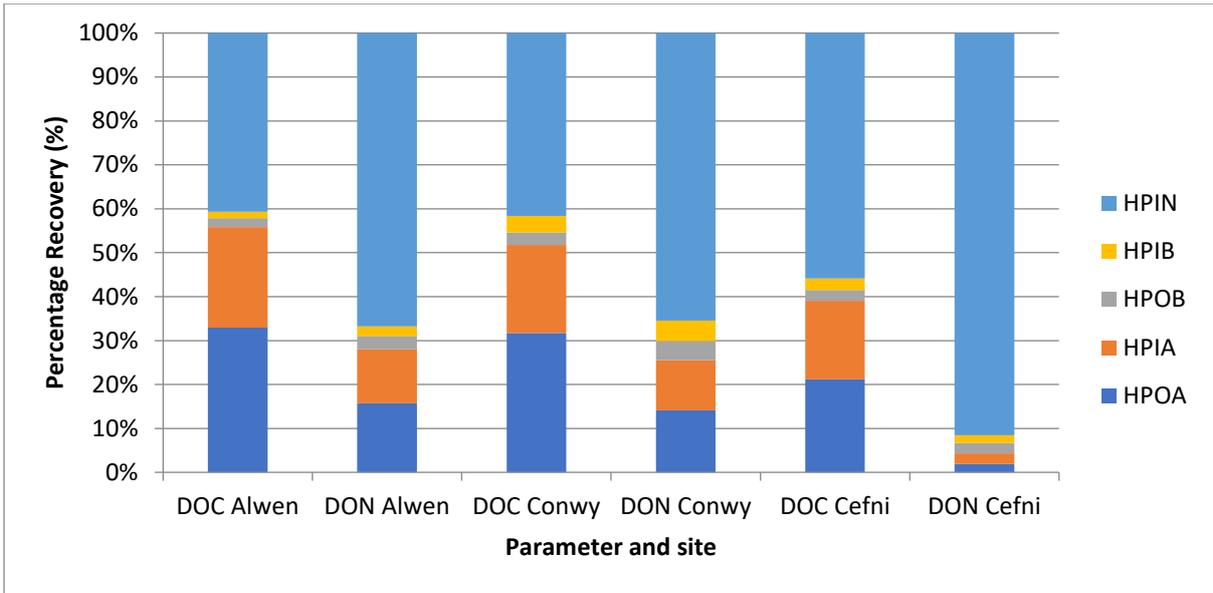


Figure 5b.8: Percentage fraction of recovered DOC/DON present in each fraction of raw filtered sample, where HPIN/HPIB/HPIA denote hydrophilic neutrals, bases and acids, and HPOA and HPOB denote hydrophobic acids and bases. Mean concentrations from January and February 2013.

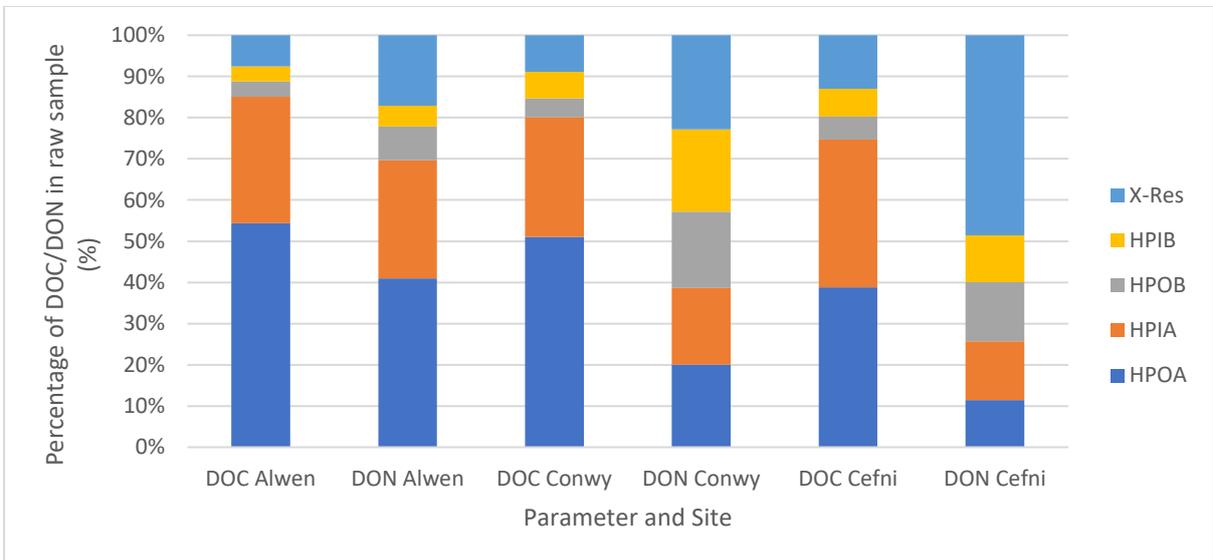


Figure 5b.9: Percentages of DOC and DON of the original raw water concentration of DOC and DON fractionation from mean Alwen, Conwy and Cefni reservoir data from Jan and Feb 2013. The water samples explored in Tables 5b.8 and 5b.9 were collected from the input to the water treatment works at each reservoir and thus representing the mean fraction composition of DOC and DON at each catchment.

The data presented in Figure 5b.9 shows that the DON at Cefni Reservoir is very different to the Alwen and Conwy reservoirs, with 91.50% of the DON being comprised of hydrophobic neutrals, as opposed to 66.75% at Conwy and 65.48% at Cefni reservoirs. The hydrophilic acid fraction is considerably smaller in the DOC samples than in the DON samples, with a mean of 20.31% for DOC and a mean of 8.71% for DON. The hydrophobic acid fraction in the DON samples is also considerably smaller than in the DOC samples at a mean of 28.57% for DOC and 10.58% for DON. It is interesting to see that DOC and DON concentrations from the same catchments do not correlate with each other, suggesting that the DON from a catchment is not composed of the same concentration of hydrophobic acid compounds, for example, than the equivalent DOC concentration.

The mean concentration of the raw fraction of DON at the Cefni reservoir is statistically different to the Alwen and the Conwy ( $p < 0.01$ ) ( $2.07 \text{ mg L}^{-1}$ , compared to  $0.52 \text{ mg L}^{-1}$  at the Alwen and  $0.29 \text{ mg L}^{-1}$  at the Conwy at Cefni. The HPOA fraction at the Cefni is statistically significantly different to the HPOA fraction at the Alwen reservoir but not at Conwy ( $0.34 \text{ mg L}^{-1}$  at the Cefni compared to  $0.71 \text{ mg L}^{-1}$  for the Alwen and  $0.35 \text{ mg L}^{-1}$  at the Conwy catchment). Although the significance of  $p = 0.068$  suggests that the chance of the HPOA fraction being statistically significantly different at the Conwy is 93.2%, the raw data shows that this is not the case.

The HPIA fraction did not statistically significantly differ between all 3 reservoirs, with the mean Alwen HPIA fraction being  $0.60 \text{ mg L}^{-1}$  the Conwy being  $0.30 \text{ mg L}^{-1}$  and the Cefni being  $0.45 \text{ mg L}^{-1}$ . The HPOB fraction was also statistically significantly different between the Cefni and the Alwen and the Conwy at  $p < 0.01$  for both ( $0.14 \text{ mg L}^{-1}$  at Alwen compared to  $0.13 \text{ mg L}^{-1}$  at Conwy and  $0.45 \text{ mg L}^{-1}$  at Cefni. The HPIB fraction at the Cefni reservoir was found to be statistically significantly different to the Alwen and Conwy reservoirs at  $p < 0.01$  (Cefni mean concentration was  $0.34 \text{ mg L}^{-1}$  compared to the Alwen at  $0.11 \text{ mg L}^{-1}$  and the Conwy at  $0.14 \text{ mg L}^{-1}$ ).

Finally, the X-Res/HPIN fraction was found to be statistically different at the Cefni reservoir when compared to the Alwen and the Conwy ( $p < 0.01$  each), at mean concentrations of  $0.33 \text{ mg L}^{-1}$  at the Alwen,  $0.19 \text{ mg L}^{-1}$  for Conwy and  $1.74 \text{ mg L}^{-1}$

for the Cefni Reservoir. No statistically significantly different relationships were detected between the Alwen Reservoir and the Conwy Reservoir. The mean concentration of DOC and DON, averaged per reservoir per month, is displayed in Table 5b.6.

Table 5b.6: Mean concentration of DOC and DON in the waters at 3 reservoirs for the months of January and February

	<b>Alwen</b>		<b>Conwy</b>		<b>Cefni</b>	
<b>Mean concentrations (mg L<sup>-1</sup>)</b>	<b>DOC</b>	<b>DON</b>	<b>DOC</b>	<b>DON</b>	<b>DOC</b>	<b>DON</b>
<b>Jan</b>	14.6	0.6	6.3	0.3	6.3	2.1
<b>Feb</b>	9.2	0.4	8.9	0.3	7.0	2.1

Table 5b.7: Mean concentration of DOC in fractions from the Alwen, Conwy and Cefni sites, and standard deviation. Data averaged from all sites in a catchment from January and February.

	<b>Alwen</b>		<b>Conwy</b>		<b>Cefni</b>	
	<b>Mean (mg L<sup>-1</sup>)</b>	<b>Std. Dev (mg L<sup>-1</sup>)</b>	<b>Mean (mg L<sup>-1</sup>)</b>	<b>Std. Dev (mg L<sup>-1</sup>)</b>	<b>Mean (mg L<sup>-1</sup>)</b>	<b>Std. Dev (mg L<sup>-1</sup>)</b>
<b>RAW</b>	11.9	1.0	7.6	1.0	6.7	1.6
<b>HPOA</b>	34.0	2.7	23.0	3.5	14.4	3.01
<b>HPIA</b>	24.3	1.3	15.2	1.9	13.3	2.9
<b>HPOB</b>	2.0	0.4	2.2	0.5	1.9	0.6
<b>HPIB</b>	1.5	0.2	3.0	1.0	2.2	0.8
<b>HPIN</b>	4.7	0.6	3.5	1.0	4.7	1.8

Table 5b.8: Mean concentration of DON in fractions from the Alwen, Conwy and Cefni reservoirs, and the standard deviation. Data average from all sites in a catchment from January and February.

	Alwen		Conwy		Cefni	
	Mean (mg L <sup>-1</sup> )	Std. Dev (mg L <sup>-1</sup> )	Mean (mg L <sup>-1</sup> )	Std. Dev (mg L <sup>-1</sup> )	Mean (mg L <sup>-1</sup> )	Std. Dev (mg L <sup>-1</sup> )
<b>RAW</b>	13.5	1.2	67.0	2.3	8.3	1.8
<b>HPOA</b>	16.78	0.8	9.2	5.8	2.5	1.8
<b>HPIA</b>	10.9	0.6	6.3	3.5	2.9	2.4
<b>HPOB</b>	3.3	1.4	6.0	2.8	2.5	2.1
<b>HPIB</b>	1.9	0.3	6.1	2.9	2.0	1.5
<b>HPIN</b>	63.4	1.4	77.5	6.9	83.1	1.3

The data in Tables 5b.7 and 5b.8 show that the Alwen reservoir contains the highest DOC and DON concentrations. This is most likely due to the fact that the Alwen reservoir data encompasses a whole catchment, spanning 14.03 Km<sup>2</sup>, compared to the Conwy combined sites catchment area of 1.34 Km<sup>2</sup> and Cefni at 31.20 Km<sup>2</sup>. Despite the Cefni reservoir catchments having a greater catchment area, the geomorphology of this catchment encompasses a high percentage of agricultural land rather than peat bog dominated land, such as at the Conwy and Alwen. It is this intensive agricultural land use that is thought to drive the medium strength DON concentrations recorded at the Cefni, whilst the Conwy catchment contains much lower concentrations.

Statistical analysis was carried on the DOC and the DON concentrations between the 3 catchments using a Pearson's correlation. There were no statistically significant differences detected between the Alwen and Cefni and Conwy reservoirs for the Raw, HPOB, HPIB and X-Res/HPIN fractions. However, HPOA at the Cefni reservoir is statistically different to the HPOA at the Alwen reservoir ( $p < 0.05$ ), but not at the Conwy reservoir (mean concentrations of 33.89 mg L<sup>-1</sup> for Alwen, 22.97 mg L<sup>-1</sup> for

the Conwy catchment and  $14.44 \text{ mg L}^{-1}$  for the Cefni reservoir). HPIA at the Alwen was statistically significantly different when compared to the Cefni and the Conwy ( $p < 0.01$  for each), but there were no significant differences between the HPIA concentrations at Cefni and Conwy reservoirs (mean concentrations of  $24.32 \text{ mg L}^{-1}$  at the Alwen reservoir, compared to  $15.23 \text{ mg L}^{-1}$  at the Conwy reservoir and  $13.29 \text{ mg L}^{-1}$  at the Cefni reservoir).

For the raw DON concentration of the sample, the Cefni reservoir is significantly different to the Conwy and the Alwen ( $p < 0.01$ ). For the concentration of the HPOA fraction, the Alwen reservoir is significantly different from the Conwy and the Cefni ( $p < 0.05$  and  $p < 0.01$  respectively). For HPIA, the Conwy and the Alwen significantly differ from each other ( $p < 0.05$ ), and for HPOB, the Cefni reservoir is significantly different to the Conwy and Alwen reservoirs ( $p < 0.05$  and  $p < 0.01$  respectively). The Alwen was found to be significantly different to the Cefni reservoir for the HPIB fraction at  $p < 0.01$ , and finally, the HPIN fraction at the Cefni was significantly different to the Conwy and Alwen reservoirs ( $p < 0.01$ ).

Fraction percentage data for DOC and DON between the 3 catchments were also statistically analysed using a Pearson's correlation. There were no significant differences between the DOC concentrations in the raw water at each reservoir. For the HPOA fraction, the Alwen and Cefni reservoirs were significantly different to each other ( $p < 0.05$ ), and for the HPIA fraction, the Alwen reservoir was significantly different to both the Conwy and Cefni reservoirs ( $p < 0.05$  and  $p < 0.01$  respectively). There were no significant differences between the HPOB fraction at each of the reservoirs, a trend which extends for HPIB and HPIN too.

## 5b.6 Discussion

The  $200 \text{ } \mu\text{g L}^{-1}$  concentration of trichloroacetonitrile presented the highest peak area when compared to equivalent concentrations of the other compounds analysed, showing that the ECD was more sensitive to this compound. This could possibly be because the equipment was already calibrated to detect tri-halogenated methane compounds (trihalomethanes/THMs), and thus, favoured the detection of another tri-halogenated compound. Conversely,  $1 \text{ } \mu\text{g L}^{-1}$  of dibromoacetonitrile presented the lowest peak area when compared to equivalent concentrations of the other compounds to be analysed, showing that the detector was least sensitive to this compound. Interestingly, the 3<sup>rd</sup> lowest response was

given by bromochloroacetonitrile, the only other bromo-containing compound in the suite of compounds analysed, possibly suggesting that this method is not well suited to the sensitive detection of bromine containing compounds.

Luo *et al.*, in 2014, were also able to detect several of these compounds using SPME and GC-MS. The authors managed to detect 4 haloacetonitrile compounds at ranges from 0.05 to 10  $\mu\text{g L}^{-1}$  with very similar  $R^2$  values from a trend line. Although the present study did not attempt to detect concentrations of these compounds below 1  $\mu\text{g L}^{-1}$ , it appears that a much lower concentration is achievable, however, this may involve amendments of methods and consumables. It does appear that the ECD would be able to detect most of these compounds at lower concentrations, as the lowest concentration (i.e. 1  $\mu\text{g L}^{-1}$ ) of the compounds measured appears as peaks above what would be determined as background noise on the chromatogram.

Further work was planned to determine whether any other volatile organic compounds would be retained on the column and pass the detector at the same retention time as the target compounds in this study, to be able to determine the authenticity of this method. However, prior to further analysis being carried out, the Varian 450 GC's ECD became unserviceable and irreplaceable. This machine was located in an unsupervised laboratory and was therefore accessible to under-qualified personnel. This resulted in the improper use of the equipment, either by not inserting the correct samples into the sample rack (and thus the SPME fibre becoming saturated constantly), by not ensuring that there was a sufficient volume of carrier gas (resulting in the ECD becoming oxidised, and the column becoming damaged), or by excessive heat in the injector port (causing septa to melt and/or defigure). The initial findings from this experiment, however, do suggest that detection of these N-DBP compounds by GC-MS and SPME is possible and this may prove advantageous when compared to other N-DBP detection methods which require sample derivatisation (for example, Andersson *et al.*, 2018 who used an acidic methanol derivitisation to determine HAA5).

When compared to the data presented in Table 5b.2 it is apparent to see that these waters contain concentrations of amino acids higher than concentrations found to form N-DBPs. If these raw waters were to be treated with chlorine with no other treatment practices (such as filtration, coagulation etc) the tyrosine concentration present in the water would form dichloroacetamide, and it is also likely to form from the concentrations of aspartic acid, phenylalanine and tyrosine, as suggested by Chu *et al.*, (2010a). Concentrations of aspartic

acid appear too low to form dichloroacetonitrile above the minimum detection limit under chlorination, according to Bond *et al.*, (2012).

Statistical analysis, using an Analysis Of Variance (ANOVA) of the amino acid data detected over the 3 seasons showed that no sites produced significantly different concentrations of any of the amino acids ( $p=1.000 - 0.059$ ), which was confirmed by a Tukey HSD post hoc test, which showed no significantly different concentrations between sites. This suggests that amino acid concentrations in freshwater samples taken from the Conwy catchment are relatively stable both in terms of seasonal variation and overall concentration. The concentrations detected in freshwater samples appear to be similar to the concentrations found by Horňák *et al.*, (2016) who found 19 amino acids at 0.05 – 35 nM concentrations, with the 3 most prevalent being alanine, serine and glutamine in the pre-alpine Lake Zurich, Switzerland. Dotson and Westerhoff (2009) reported amino acid concentrations of approx. 5-60 nmol L<sup>-1</sup> (approximately 0.685 to 8.00 µg L<sup>-1</sup>) at 4 stages throughout a WTW, which, when compared to published data, suggests that cyanogen chloride, dichloroacetamide and n-nitrosodimethylamine are able to form depending upon the disinfectant used.

Hughes *et al.*, (2016) published work that studied the Cefni and Conwy reservoirs and found DOC similar to the recoveries found here, with 95.68 ±0.62 % DOC recovered at the Conwy reservoir and 92.26 ±1.71% recovered at the Cefni reservoir compared to 102.50% for Conwy vs 118% for Cefni.

DON at the Cefni differs considerably when compared to the Alwen and the Conwy in terms of percentage of DON from original raw sample (Figure 5b.10). However, the statistical analysis between the 3 reservoirs in terms of concentration of DON in each fraction helps demonstrate that the assumption that these 3 reservoirs differ significantly in terms of their organic matter content, is true. This is most likely down to the catchments that these reservoirs drain. The Conwy reservoir is located in the Migneint, which is a large area of ombrotrophic and oligotrophic blanket bog dominated moorland in Snowdonia National Park, North Wales, UK (Fenner and Freeman, 2011). The reservoir is also described as a shallow natural upland reservoir within a blanket peat bog (Hughes *et al.*, 2016). The Alwen reservoir collects water that drains predominantly peat or acid organo-mineral upland soils (Peacock *et al.*, 2014). In contrast, the Cefni Reservoir is a man-made reservoir with fen as its principal catchment (Hughes *et al.*, 2016), and is also dominated by agricultural practices such as cattle and sheep farming, small settlements likely not connected to mains drainage, and leisure

facilities such as caravan parks – this is likely to increase nutrient loadings in the waters considerably. The vastly different catchment that drains into the Cefni reservoir is likely to be responsible for a DON fraction profile (much lower in hydrophobic acids and much higher in hydrophilic neutrals when compared to Alwen and Conwy) that is entirely different from the Alwen and the Conwy reservoirs, which, although differing in nutrient DOC/TN concentrations, are still relatively similar when compared to the Cefni.

DOC HPOA fractions were found to be significantly different at the Cefni reservoir as opposed to the Conwy and the Alwen reservoir, whereas the HPIA percentage was significantly higher at the Alwen than at the Conwy and Cefni reservoirs (see Table 5b.7). This is reflected in the concentration data too which also shows that the HPOA and HPIA fractions are the only significantly different fractions between sites. Chang *et al.*, (2013) find that the HPOA and HPIA fractions were the most important contributors to C-DBP formation potential in their studies, and therefore, it is sensible to assume that this is a trend that can be replicated at these sites too, as the methods utilised to generate data in this work and in the present work are very similar.

In terms of DBP formation potential when these waters reach treatment works, it would seem most likely that C-DBP compounds will be most prevalent in water from the Alwen, as the Alwen has the highest concentration of DOC compared to Conwy and Cefni. However, the DOC at the Alwen appears to be comprised of 40% HPIN, which are the smallest and least charged DOC particles, notoriously the most difficult to remove at conventional water treatment works. The composition of the water from the Conwy reservoir looks to be similar. However, the hydrophilic neutral fraction at the Cefni reservoir accounts for approximately 60% of the water in the Cefni, and thus, although the initial DOC concentration is almost equal to the Conwy, and just over half of the concentration at the Alwen, more of the HPIN DOC is likely to pass through the treatment stages and be able to react with the chlorine, thus potentially forming N-DBPs.

The story is similar for DON concentrations, where the Cefni has a much higher percentage of HPIN (91.50%) when compared to the Conwy (65.48%) and the Alwen (66.75%), however, the DON concentration at the Cefni reservoir is also much greater than the Conwy (approximately 7 times) and the Alwen reservoir (approx 3.5 times), therefore, N-DBPs are thought to be most likely to form at the Cefni reservoir. Had continued data collection been possible, it was proposed to analyse the individual fractions for specific N-DBP formation

potential in an attempt to attribute a specific fraction of organic nitrogen to an increased formation of N-DBPs.

The western blot was chosen as it is relatively simple to introduce shorter CDS1 bands, which are the products of internal translation in the response to environmental stress. There are 3 stresses known to affect these shorter CDS1 bands, and these are heat, low glucose concentration, and dibromoacetonitrile. By ensuring that the analytes were kept at an acceptable temperature and with enough glucose to maintain healthy cell growth, it is possible to deduce that, if the shorter CDS1 bands are visible, then cell cycle arrest has occurred. *Schizosaccharomyces pombe* fission yeast was selected, grown to mid logarithmic phase, as dibromoacetonitrile has been found to delay G1-S transition and specifically blocks the activation of the DNA damage checkpoint kinase (CHK1) at broken DNA replication forks in this strain, a proxy for human DNA (Caspari *et al.*, 2017). Previous studies have found that dibromoacetonitrile can induce this band at between 4 (0.80 mg L<sup>-1</sup>) and 12 (12.39 mg L<sup>-1</sup>) μM, and therefore, for the strongest signal, a solution of 12μM was used.

Figure 5b.10 shows the detection of 12 μm dibromoacetonitrile. This experiment examines the time taken for dibromoacetonitrile activity to show, from 0 to 90 minutes. As the yeast used for this experiment can suffer from 3 different types of stress, by blocking one of those types of stress, it is possible to see whether the CDS1 variants are still induced. The addition of n-acetylcysteine (NAC) inactivates free radicals, and therefore blocks the oxidative stress that would normally be present under the addition of dibromoacetonitrile. However, the band is still prevalent with the addition of NAC, suggesting that dibromoacetonitrile does not affect cells *via* this stress pathway. The less solid bands below the main band at 90 minutes are inducible Cds1 truncated protein variants (Fletcher, Griffiths and Caspari, 2018).

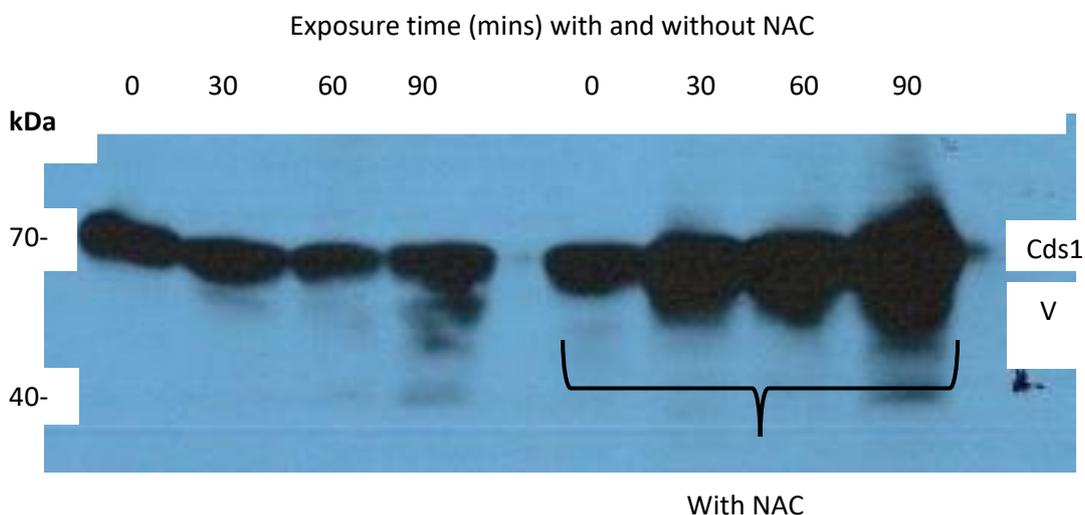


Figure 5b.10: Image to show dibromoacetonitrile activity at an exposure of 12  $\mu\text{M}$  from 0 to 1.5 hours, both with and without n-acetylcysteine added, showing high activity from 30 minutes to 90 minutes, exhibiting the detectability of dibromoacetonitrile from a minimum experiment duration of 30 minutes.

### 5b.7 Conclusion

Although only a very brief examination of the effects of dibromoacetonitrile upon cell cycle arrest has been conducted, this study shows an alternative (i.e. not by gas chromatography/mass spectrometry) method to detect dibromoacetonitrile concentrations, which can be extrapolated to water samples. However, downsides of this assay include the detection range (4-12  $\mu\text{M}$ ) of dibromoacetonitrile, where concentrations found in drinking water are less than 0.04  $\mu\text{M}$  (Caspari *et al.*, 2017), therefore, water samples would need to be concentrated hundredfold in order to reach the minimum detection limit. Furthermore, studies have shown a concentration of <250  $\mu\text{M}$  induce cancer in the stomach and liver of rats (NTP, 2010), a concentration that is far outside the detection limits of this assay.

Whilst the initial detection of N-DBPs using the GC-ECD SPME method appeared promising, there are several drawbacks meaning that the techniques used in the method have been superseded by newer technology. The largest issue appears to be with the SPME fibres, which only have a certain number of sorption sites on them in which the volatiles can be absorbed. Therefore, if a sample contains a very high concentration of a N-DBP compound (for example, 500  $\mu\text{g L}^{-1}$  upwards), the data produced by the GC will not be a true representation of the concentration in the sample, as the SPME fibre will become saturated, thus suggesting that this fibre may have a low working range and another SPME fibre should be considered for this application. This phenomenon may be beginning to appear in the trichloroacetonitrile detection in Figure 5b.5 where the peak area seems to be tapering out from 100  $\mu\text{g L}^{-1}$  onwards. The use of an ECD provides a much better resolution when compared to a flame ionisation detector (FID), however, a mass spectrometer detection of these compounds would provide better and more in depth detail. For these reasons, along with time and financial restraints, this method development ceased here.

The relevance of the individual amino acid detection experiment conducted here is outlined by Bond *et al.*, 2012 and Bond *et al.*, 2014, where it is stated that, essentially, many reactive

N-DBP precursors are amino acids or amines. Therefore, the detection and quantitative analysis of the concentrations of these amino acids can pinpoint specific land use practices, vegetation, or even soil types that can increase concentrations of N-DBPs in the water should it be chlorinated prior to human consumption. However, from the data explored in this chapter, it is possible to see that there are no specific sites located within the Conway catchment (from source to sea), that contain significantly different amino acid concentrations when compared to other sites in the catchment. This may be a trait of this individual catchment, or a limitation of the equipment used to detect these compounds, and cannot be extrapolated to other similar catchments. However, an understanding of the concentration of amino acids in water destined for drinking water, can inform different treatment techniques to help reduce N-DBP formation.

The significant difference in character of both DOC and DON fractionation data between different catchments shows that the DOC and DON structure differs vastly from site to site. It is therefore highly likely that these waters will form different concentrations of C- and N-DBPs. Chang *et al.*, (2013) found that the majority of the DON in their samples (source waters to a Chinese reservoir) were present in the HPIA fraction (77.00%) whereas their HPIN fraction contained only 54.50%, further concluding that most DON-enriched materials were too heterogeneous to be ascribed to the traditional surrogates of DOC and DON contents. The current work, however, shows a range of 65.50% to 91.50% DON present in the HPIN fraction, with a mean fraction percentage (averaged over the 3 reservoirs) at 75.00%. Furthermore, with HPIA fractions ranging from 2.36% to 12.34% (mean 8.71%), it is quite obvious that the characteristics of water, at least in terms of nitrogen, vary significantly from site to site.

The potential health risks posed by drinking water containing N-DBPs and their precursors has been the focus of many recent studies. It is generally understood that the concentrations of N-DBPs necessitate limitation to a safe concentration in drinking water, but more information on the occurrence, formation and concentration of these N-DBPs is required. Because the formation of different N-DBPs varies greatly from compound to compound, it is difficult to collate all information on each individual N-DBP and its precursors to help set regulatory standards. Indeed, it is unknown how to measure and quantify some precursors thought to be found in fresh water. Further research into the formation, occurrence and removal of N-DBPs will provide valuable information to help ultimately increase the safety of treated drinking water for consumers. A comprehensive dataset including data relating to

the formation, occurrence, potential precursors and their likely habitats, and best removal practices can certainly help to identify N-DBP formation in certain types of water (e.g. wastewater *vs.* freshwater), water draining certain types of soil (e.g. peaty *vs.* sandy) and vegetation (e.g. coniferous forest *vs.* arable grassland) and in certain locations (rural *vs.* urban). This dataset could assist creation of a risk assessment map, outlining areas where high formation of (or where a concentration of high-risk) N-DBPs are likely to occur. This risk assessment map can ultimately assist water treatment companies to choose the most easily treatable abstraction locations to meet the increasing demand on freshwater by an ever growing population in the safest and most cost effective way.

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# Chapter 6

## Effects of Chlorine Upon Natural Organic Matter During Water Disinfection

## 6.1 Introduction

It is widely known that disinfection of freshwater can lead to DBP formation (Hua and Reckhow, 2007) due to a reaction between a given disinfectant, for example, chlorine, chloramine or ozone, and NOM (Richardson *et al.*, 2007). It is widely reported that the organic matter differs depending upon its origin, for example, autochthonous (*in-situ*) vs. allochthonous (originating from external sources such as within the catchment) sources (Leenheer and Croué, 2003), and that different natural waters may vary significantly with respect to concentration and type of precursors for THM4 (Gallard and von Gunten, 2002), which are the only currently regulated DBPs in the UK (WHO, 2006). THM4 fall into a broader category of compounds, C-DBPs, which also contain other groups such as haloacetic acids (HAAs) (Muellner *et al.*, 2007), which are limited in the USA to  $60 \mu\text{g L}^{-1}$  for 5 of these HAAs (US EPA, 1998). The THM4 compounds are regulated due to their reported carcinogenicity and toxicity (Cowman and Singer, 1996). However, N-DBPs have been the focus of many recent studies because it is thought that, although they form at much lower concentrations, these N-DBP compounds may be much more dangerous to human health (Templeton *et al.*, 2012).

Since the discovery of THM4 (Rook, 1974), over 600 DBPs have been identified (Richardson *et al.*, 2007), and have been the focus of many studies linking them to health concerns such as stillbirths (Dodds *et al.*, 2004). THM4 are composed of halogenated methane either with or without the presence of bromine, and are regulated at  $100 \mu\text{g L}^{-1}$  at the consumer's tap. THM4 has been found to promote carcinogenic activity when administered to female mice (Coffin *et al.*, 2000).

WTWs employ organic matter removal procedures, as, despite it being harmless to humans, it causes unfavourable tastes, colours and odours in water (LeChevallier and Au, 2004 and Matilainen *et al.*, 2010), and can promote biofilm growth in the distribution network due to nutrients contained within it (such as nitrogen and carbon) (Gauthier *et al.*, 1999). These treatment practices differ slightly depending upon the source water, and upon the character of the organic matter, but the main principles are the same. Commonly, WTWs will employ the coagulative properties of specific chemicals to bind DOM particles together, before they are floated to the surface of the treatment tank, or, more commonly, settled and filtered and removed. Further steps include filtration and passing the water through granular activated carbon and a pH adjust before the water is treated with halogens.

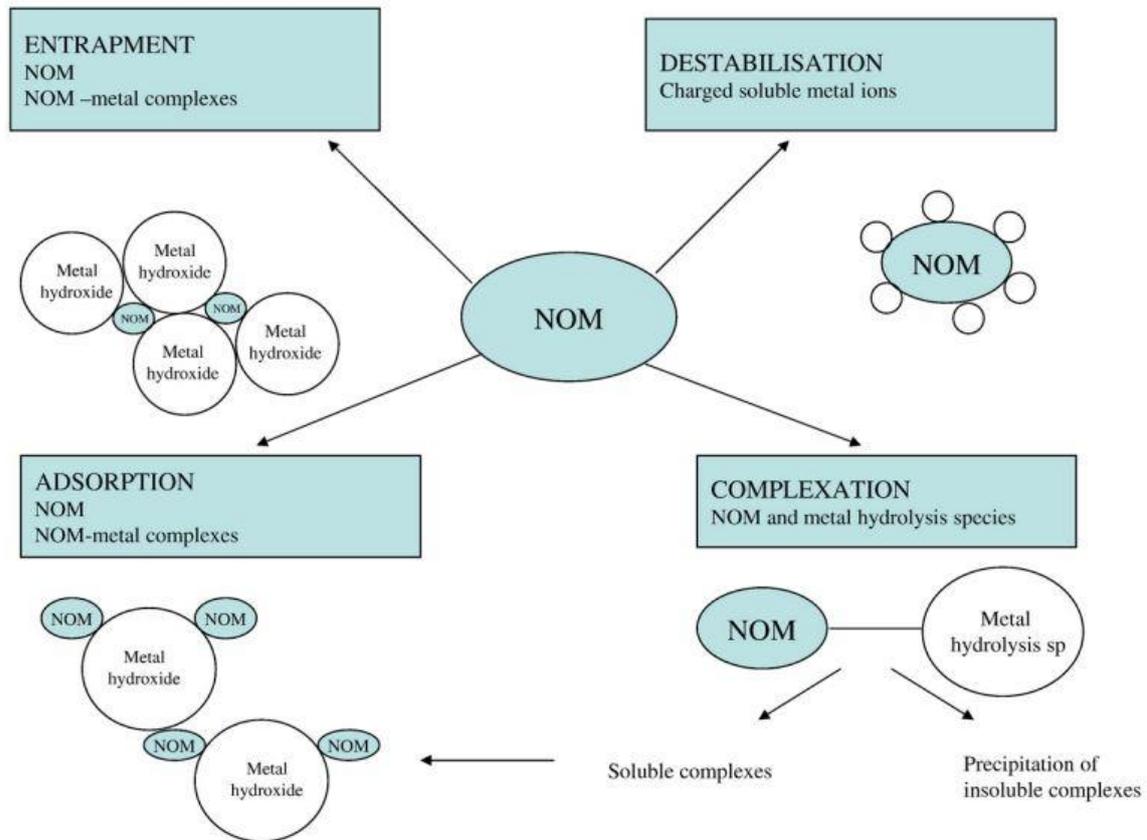


Figure 6.1: Diagram of the possible mechanisms of NOM during coagulation, showing entrapment, destabilisation, adsorption and complexation. Sourced from Jarvis *et al.*, 2004 and Matilainen *et al.*, 2010.

Many studies have focussed upon the characterisation of organic matter using methods such as fractionation by absorbent resins (Hughes *et al.*, 2016), spectrofluorometric properties (McKnight *et al.*, 2001), and size exclusion chromatography (Matilainen *et al.*, 2006). These have been used in an attempt to identify optimum treatments for specific waters at individual WTWs. The focus of these studies has been upon characterising the organic matter to inform better removal techniques, but there has been less research focusing on the organic matter that makes it through these treatment phases, and the relationship it has with the disinfectant.

Coagulation is a process where the repulsive potential of electrical double layers of colloids is reduced in such a way that micro-particles can be produced, which then collide with each other and form flocs (Jarvis *et al.*, 2004) (see Figure 6.1). Chemical coagulation is

commonplace, and involves the addition of inorganic coagulants, usually aluminium and iron salts, which are dissociated to their trivalent ions (e.g.  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ ) when added to water (Bishop, 1995). The flocs are generally removed by dissolved air floatation (DAF) where 10 to 100  $\mu\text{m}$  sized air bubbles are passed from the bottom to the top of the holding tank (Edzwald, 1993), transporting the flocs to the surface where they can be mechanically removed. Once the majority of DOM has been removed, a secondary treatment process can be used, such as removal by granular activated carbon (GAC), membrane filtration, reverse osmosis, or ultrafiltration (Jacangelo *et al.*, 1995). Whilst these processes are highly effective, some DOM can pass through these removal procedures. Edzwald and Tobiason (1999) found that waters with a specific UV absorbance (SUVA) of around 4 or greater had a DOC removal of >50% for aluminium based coagulants, and waters with a SUVA of less than 2 showed DOC removal of <25% under aluminium based coagulants. See Table 6.1 below.

The organic matter remaining in the water after these removal techniques is free to react with chlorine and its compounds, added to disinfect the water prior to human consumption, and can therefore form DBPs. The water is disinfected with an oxidising agent to remove bacteria; the most common disinfecting agent used is NaOCl, although chloramine, ozone and other halogens can be used. Information on the activities of these halogens can be found in Chapters 5a and 5b.

Table 6.1: Coagulant removal efficiencies depending upon SUVA of water (adapted from Edzwald and Tobiason, 1999).

SUVA	Composition	Coagulation	DOC Removals
~4 or greater	Mostly aquatic humics, high hydrophobicity, high MW	NOM controls, good DOC removals	>50% for alum, a little greater for ferric*
2-4	Mixture of aquatic humics and other NOM, mixture of hydrophobic and hydrophilic NOM, mixture of MWs	NOM influences, DOC removals should be fair to good	25-50% for alum, a little greater for ferric*

<2	Mostly non-humics, low hydrophobicity, low MW	NOM has little influence, poor DOC removals	<25% for alum, little greater for ferric*
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\*Where alum refers to aluminium sulphate, and ferric refers to ferric sulphate

The potential health implications of DBPs formed from organic matter is a serious concern for the water industry, thus, research has been focussed on reducing the concentration of organic matter in the raw water. Whilst many studies examine the link between C and N concentration and the concentration of DBPs formed, it would seem that little attention has been paid to the concentration difference of the dissolved C and N before and after disinfection. Whilst bacteria have been inactivated, the carbon and nitrogen that remains dissolved in the final water can provide nutrition for algae and bacteria growing as biofilm in the distribution network. Many different microbes have developed the ability to survive in the distribution system with some possessing the ability to grow and/or produce biofilms. These organisms can be primary pathogens (can cause disease in healthy individuals) or opportunistic pathogens (can cause disease in individuals with already-weakened immune systems) (EPA, 2002).

Here, a mass balance equation is used to calculate the Cl<sub>2</sub> consumed for every disinfection activity except THM4 formation (i.e. pathogenic and non-pathogenic bacteria deactivation, C- and N-DBP formation etc.), alongside the chlorine used to form THM4. This mass balance equation therefore accounts for the chlorine used to form THM4 and removes this from the equation.

This study also examines the fate of dissolved forms of nitrogen and carbon upon chlorination. This is achieved by using mass balance equations and concentration data derived from industry standard titration techniques.

### 6.1.2: Hypothesis

It is hypothesised that THM4 formation potential is not always dictated by the NPOC concentration of the water for both chlorination and chloramination treatments.

## 6.2 Site Introduction

Freshwater samples were collected manually from 2 different sites in the UK, the Hampshire Avon, Hampshire, UK, and the Afon Conwy, Gwynedd, North Wales (See Figure 6.2 and 6.3), described fully in Chapter 2. Briefly, these sites were chosen as they are both situated at opposite ends of a hypothetical nutrient gradient. The River Conwy drains the Migneint, an area of upland blanket peat bog which supports acid heathland vegetation and low intensity sheep production (Brailsford *et al.*, 2017). The river is fed from sub catchments of coniferous plantations, farmland dominated lower hillslopes, through urban settlements to the estuary in Conwy, North Wales; thus, this site is classed as nutrient poor. In contrast, the Hampshire Avon is fed by two smaller streams, the River Wylfe and the River Nadder, which are both chalk streams that drain land dominated by intense agricultural activities, with few organic soils in their catchments (Yates and Johnes, 2013), thus, this site is classed as nutrient rich. 27 sites were situated within the Conwy catchment and 19 sites were situated within the Hampshire Avon catchment, and the dissolved matter at these sites is expected to vary considerably from site to site due to the many different land use practices they drain. The locations of these sites are displayed in Figures 6.2 to 6.4.

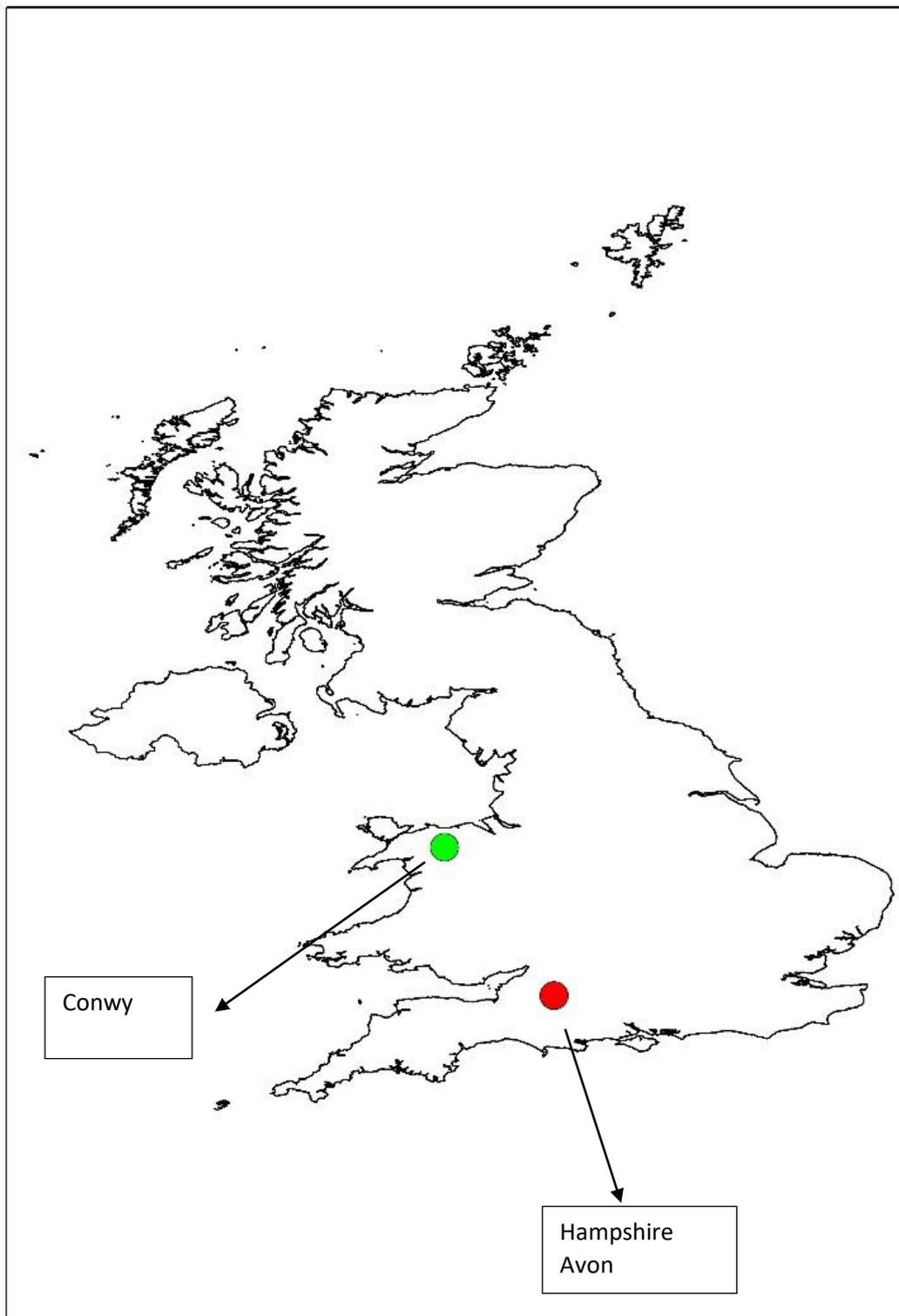


Figure 6.2: Location of River Conwy catchment (green) and the Hampshire Avon catchment (red) (left), located within the United Kingdom. Map is orientated in a northerly direction.

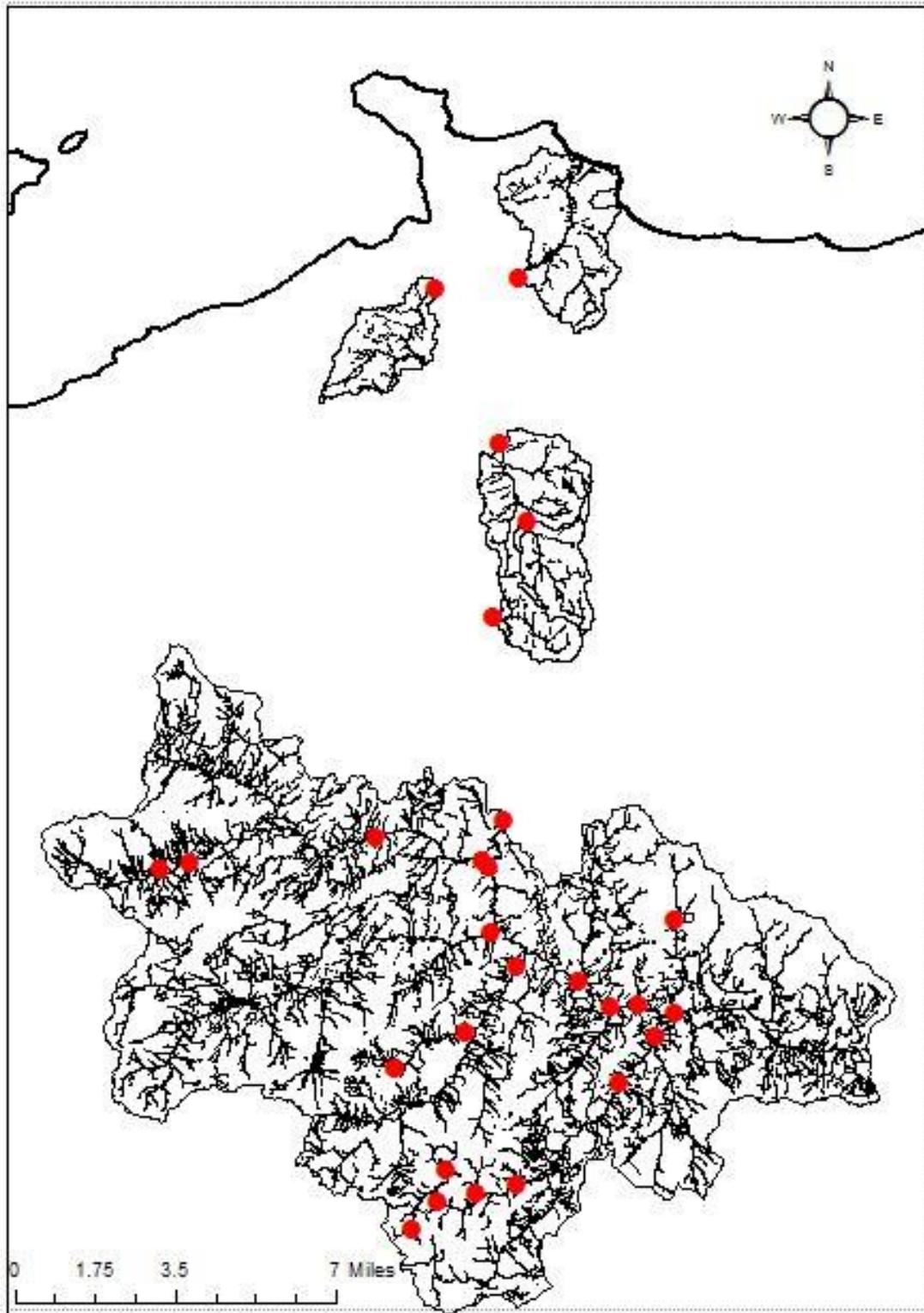


Figure 6.3: Sampling sites located within the River Conwy catchment, North Wales, UK, represented as red circles. The black lines within the catchment represent the major rivers that drain the catchments. Catchments are not continuous from source to sea due to tidal influences reaching high into the catchment.

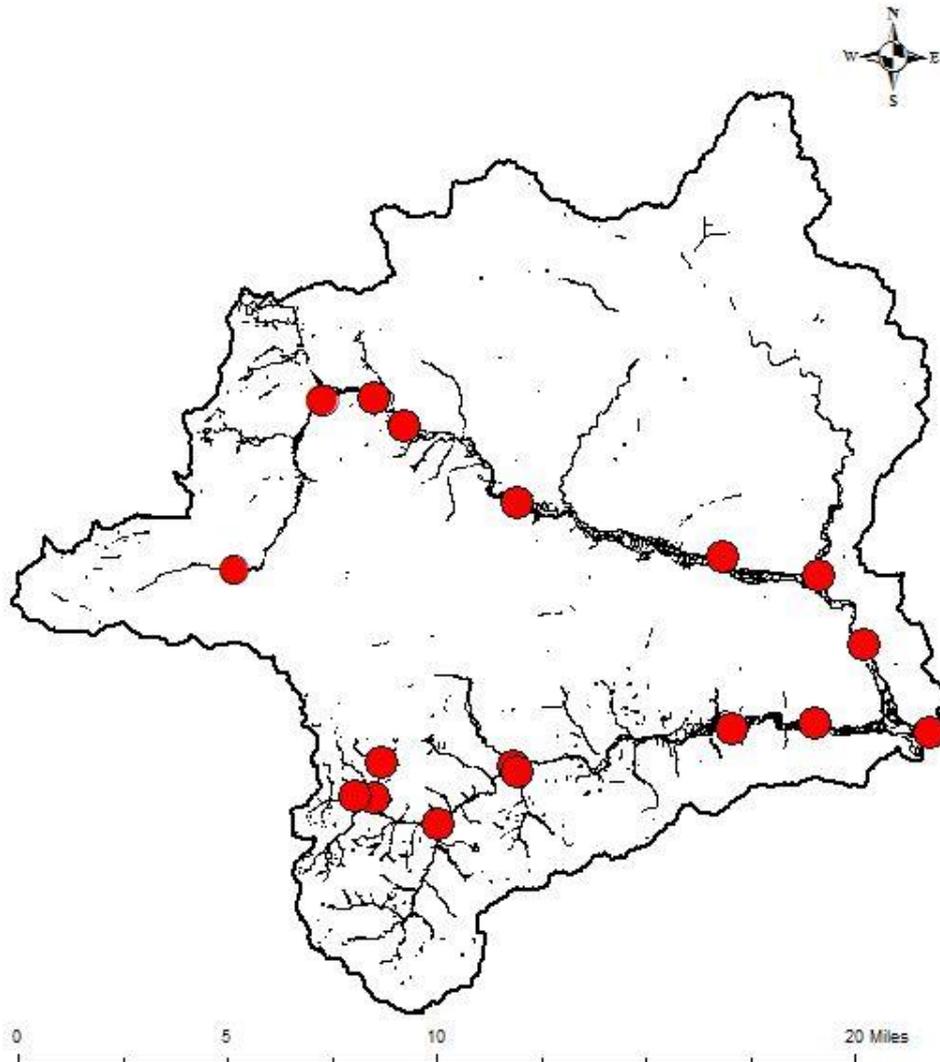


Figure 6.4: Map of the Hampshire Avon catchment showing two rivers, the Nadder and the Wylde, draining towards Salisbury, at the east of the catchment. The red circles represent sampling sites and the black lines within the catchment represent the major rivers draining these catchments.

### 6.3 Methodology

NPOC and TN data was obtained *via* thermal oxidation using an Analytik Jena Multi N/C (Germany), using the method outlined in Chapter 2, check standards of known N and C concentration and quality control standards, created from creatinine (#C4255, Lot SLBJ4862V, Sigma Aldrich, UK) for TN and potassium hydrogen phthalate (#P1088, Lot MKBW1364V, Sigma Aldrich, UK) for NPOC.

DIN was derived from the 3 inorganic nitrogen species ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) present in the samples, detected by ion chromatography using a Metrohm 850 Professional Ion Chromatogram (Metrohm UK LTD, Cheshire, UK). The ion chromatogram was equipped with a Dionex AS14A column (Thermo Scientific, Leicestershire, UK) to measure anions and a Metrosep C6 column (Metrohm UK LTD, Cheshire, UK) to measure cations. The anion eluent was a 4.5 mM sodium carbonate ( $\text{NaCO}_3$ ) /1.4 mM sodium bicarbonate ( $\text{NaHCO}_3$ ) solution made using ultrapure (Milli Q) water. The cation eluent was a 1.7 mmol  $\text{L}^{-1}$  nitric acid ( $\text{HNO}_3$ ) /1.7 mmol  $\text{L}^{-1}$  dipicolinic acid ( $\text{C}_7\text{H}_5\text{NO}_4$ ) solution made using Milli Q water. Concentrations were determined using a five point calibration with two sets of standards in the sample run (Fluka), compared to quality control standards to test for machine drift. Then, DON was calculated following Equation 6.a.

$$\text{DON (mg L}^{-1}\text{)} = \text{TN} - (\text{nitrate}/4.4) - (\text{ammonium}/18) - (\text{nitrite}/3.29). \quad \text{Equation 6.a}$$

\*Where all concentrations are in  $\text{mg L}^{-1}$ , this method will detect  $5 \mu\text{g L}^{-1}$ .

Data for this experiment was collected using two different methods.  $\text{Cl}_2$  concentration was measured using the APHA 4500-Cl F titration method, detailed in Chapter 2. After determining the concentration of the chlorine dosing solution, a  $5 \text{ mg L}^{-1}$  dose of chlorine was added to each 100 mL chemically buffered freshwater sample (prefiltered through  $0.45 \mu\text{m}$  filter papers). The cap to the sample bottle was securely fastened and the samples were incubated at  $25 \text{ }^\circ\text{C}$  for 7 days prior to analysis by GC-MS for detection of DBPs.

THM4 was detected using the GC-MS method adapted from Sarrion *et al.*, 2000, detailed in Chapter 2. Briefly, 15 mL of sample was pipetted into a GC vial containing 0.8 M sodium sulphite (to quench the chlorine) and analysed by Varian 450GC for THM4 compounds. The remaining 85 mL of sample was made up to 100 mL using Milli-Q water, before undergoing titration again to determine the concentration of chlorine remaining in the sample, allowing for a calculation of mass of chlorine used to form THM4 and other reactions.

THM4 concentration data obtained from the GC-MS was converted from  $\mu\text{g L}^{-1}$  to moles by multiplying the concentration by 0.00001, then dividing by the molar mass

of the THM4 compound, to determine the moles of chlorine in each of the 3 chlorine containing THM compounds ( $\text{CHCl}_3$ ,  $\text{CHBr}_2\text{Cl}$  and  $\text{CHBr}_2\text{Cl}$ ). The number of moles of chlorine present in the compound was then multiplied by the number of chlorine molecules in the compound, to give the mass of chlorine in the compounds, in grams, which was then converted to mg by multiplying by 1000. The sum of chlorine used to create the 3 containing THM compounds was calculated.

The total chlorine concentration used by the disinfection process was calculated by subtracting the concentration of chlorine shown by titration from the concentration of the dose of chlorine added ( $5\text{mg L}^{-1}$ ). If the total chlorine used to form THM compounds was less than the total chlorine concentration used in the disinfection process, then this is referred to as non-THM4 DBPs as although the compounds could not be measured, it was suspected that they were present in the sample. This fraction of unmeasurable DBPs could contain both C-DBPs, N-DBPs and could also account for the  $\text{Cl}_2$  used to deactivate bacteria. Chlorine residual was measured as the concentration of free chlorine remaining in the sample after incubation at  $25^\circ\text{C}$  for 7 days in the dark, measured by APHA standard method 4500-Cl-C (see Chapter 2 for full method).

### 6.3 Statistical Analysis

Data was analysed both collectively (i.e. both catchments together) and individually to determine overall relationships and site-specific relationships between sites respectively.

All data was tested for normality and equality of variance using IBM SPSS Statistics 22 (IBM, New York, USA). Normality was determined by assessing the skewness values of the datasets. A skewness value of between + 2 and -2 was deemed acceptable (George and Mallery, 2010), and if data was not between the acceptable values, it was transformed by  $\log^{10}$ . However, if the transformation did not produce a skewness value between +2 and -2, it was not used for tests that required normal data, unless the test was deemed robust enough to cope with minor skewness.

If data was found to be non-normal, a Pearson's correlation was carried out (as opposed to a Spearman's Rank test), as analysis of the linear relationship between two continuous variables was required. A Cohen's *d* test of effect size was also carried out to determine the

strength of the effect that one variable has upon the other. Cohen's *d* relates to the standardised difference between two means, and can be used to accompany the reportings of ANOVA tests. The test results range from values of 0 to 3 and above, with 0 representing a 50% chance that the treatment group will be above the mean of the control group. With a value of 3 or greater, there will be a greater than 99.8% chance that the treatment group will be greater than the mean of the control group, and thus, that a <98% chance that a data point picked at random from the treatment group will be greater than a data point picked from the control group. Cohen suggests that an effect size as low as 0.2 shows that there is a real effect present, but only something that likely can only be determined through numerical analysis, whereas a large effect size is something that is visibly different to the naked eye (Walker, 2008).

This effect test was chosen, because, although the test slightly over-estimates results from a sample size of >50, the alternative effect size test, a Hedges' *g*, is reported to only marginally differ, and thus, there are few differences between the two available tests.

## 6.4 Results

Chlorine use data was generated from both the Hampshire Avon (April 2016) and the River Conwy (July 2016) catchments, and compared to explore the concentration of chlorine used, per site. The mass of chlorine used to form THM4, the concentration of chlorine remaining after 7 days of incubation, and the mass of chlorine used but not accounted for (i.e. not used to form THM4 but used in other disinfection reactions). These data were analysed to determine whether the sample, diluted to a 1 mg L<sup>-1</sup> concentration of DOC from all sites, used a similar concentration of chlorine in its disinfection, or whether each site required different doses of chlorine. The data also indicates whether a catchment creates DOM that consumes a high concentration of chlorine, thus forming higher concentrations of THM4, or a DOM that requires less chlorine to form THM4 but uses a lot more chlorine to form non-measurable DBPs.

To better understand the relationships between the chlorine usages at each site, the data was statistically analysed alongside DOM data such as nitrogen and carbon fractions. This data has been analysed from 3 perspectives – the River Avon catchment, the River Conwy catchment, and both catchments combined to investigate overall patterns.

### 6.4.1 Hampshire Avon

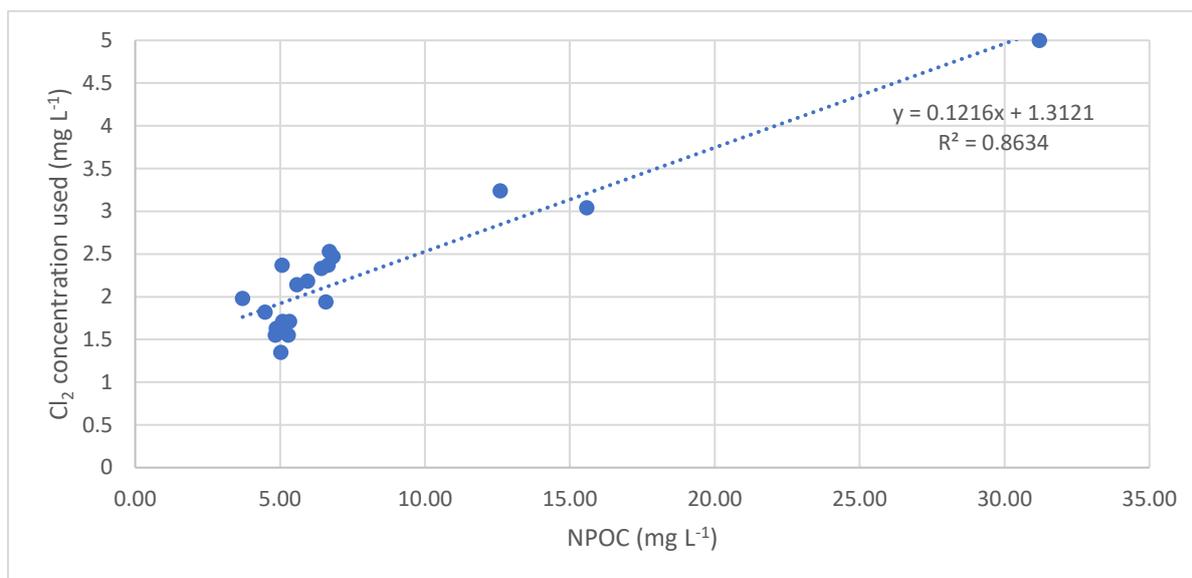


Figure 6.5: Regression analysis between NPOC concentration and the total concentration of Cl<sub>2</sub> used at the Hampshire Avon sites.

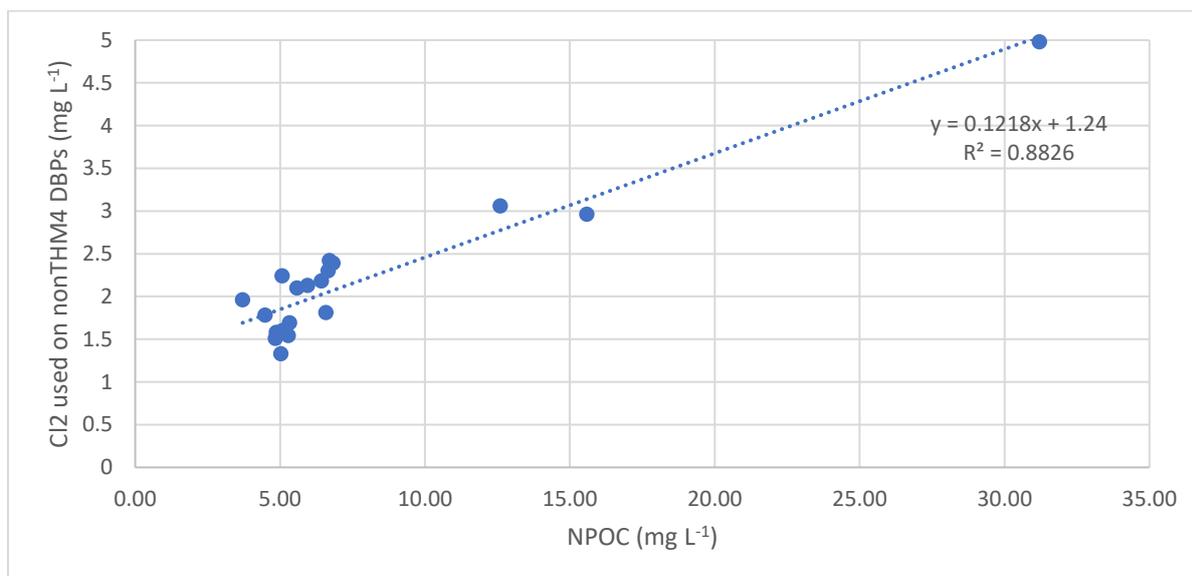


Figure 6.6: Regression analysis between non purgeable organic carbon (NPOC) and total Cl<sub>2</sub> used (top) and Cl<sub>2</sub> used to form non-THM4 (bottom). Data includes site 19, a sewage treatment works, and shows a positive regression between the two parameters.

The relationships between total chlorine used and chlorine used for other disinfection activities, and the concentration of NPOC in the water sample from the Hampshire Avon was explored to determine whether a sample higher in NPOC used more chlorine than a sample lower in NPOC.

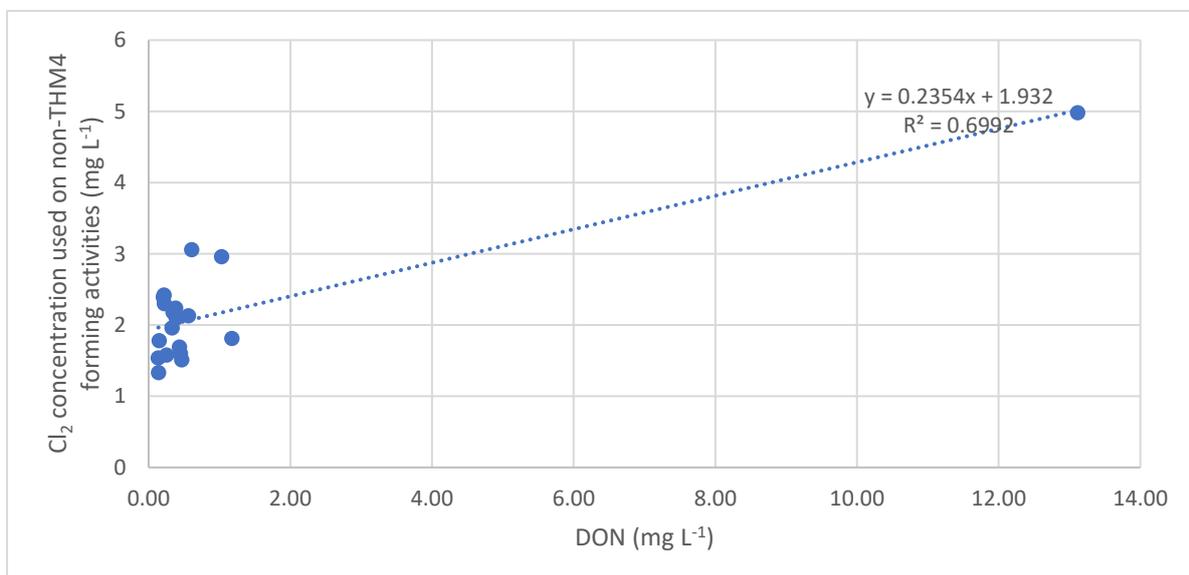
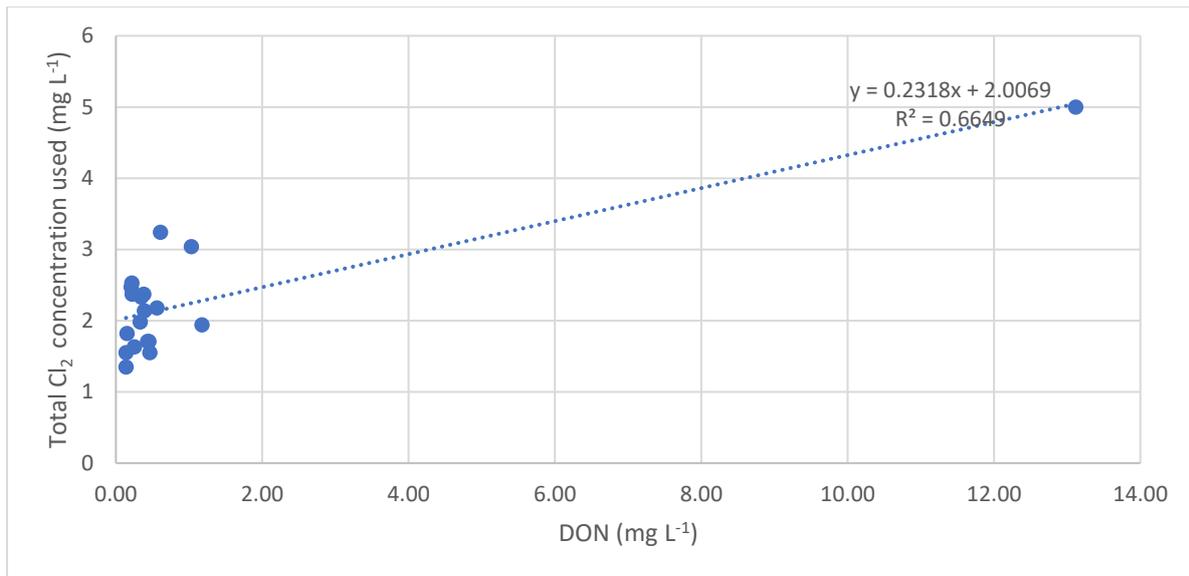


Figure 6.7: Regression analysis between DON and total Cl<sub>2</sub> used (top) and total Cl<sub>2</sub> used for other disinfection activities (bottom), showing positive relationships between DON and chlorine use for both (n=19) at the Hampshire Avon catchment.

Similarly, the relationship between total chlorine used, and chlorine used on non-THM4 formation disinfection activities were examined, to determine whether an increased DON concentration was a driver for chlorine use. Pearson's correlation coefficients of  $r=0.815$  and  $r=0.836$  respectively were found both with  $p < 0.01$  and  $n=19$ , showing that a strong positive relationship was prominent. Regression analysis between these variables shows an  $R^2$  of 0.665 to a positive linear gradient of  $y=0.2318x + 2.0069$  for DON vs. total chlorine used, and  $R^2$  of 0.70 to a positive linear gradient of  $y=0.2354x + 1.932$  for DON vs. chlorine used for other disinfection

activities (Figure 6.7). However, when site 19 (the site containing a STW) is removed from these analyses, the  $R^2$  values drop considerably ( $R^2$  of 0.13 and 0.12 respectively), which suggests that there is a very weak correlation between DON and the concentration of chlorine used, and the concentration of chlorine used for other disinfection activities in similar catchments with no STW. However, data covering a wider range of DON concentrations would be required to conclude whether a positive relationship is present or not as the current dataset contains a large data gap between DON concentrations of less than 2 mg L<sup>-1</sup> and site 19, the STW, at higher than 14 mg L<sup>-1</sup> DON.

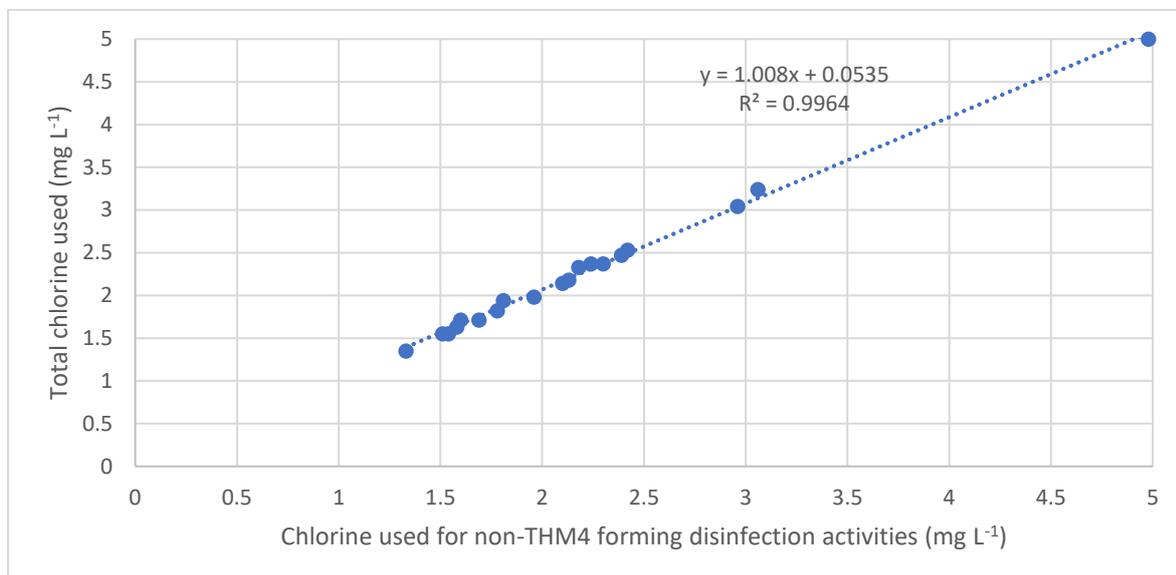


Figure 6.8: Linear regression between total Cl<sub>2</sub> used and Cl<sub>2</sub> used for other disinfection activities (not on THM4) in the Hampshire Avon catchment showing a very strong positive correlation.

A very strong relationship is also visible between total chlorine used in non-THM4 formation activities, and the total chlorine concentration used, at  $r=0.998$ . Regression analysis shows an  $R^2$  of 0.9964 to a positive linear gradient of  $y=1.008x + 0.0535$  (Figure 6.8), although a Cohen's  $d$  effect size of 0.08 suggests that this relationship is only identifiable through statistical analysis and is not noticeable by the naked eye.

A regression analysis between 3 inorganic nitrogen ions, and the total chlorine concentration used, can give a simple idea as to whether chloramines and/or N-DBPs are likely to have formed from the chlorination of these waters, and also to determine

whether inorganic nitrogen was driving use of chlorine in the sample. The total  $\text{Cl}_2$  concentration used during a 7 day contact time in a sample containing  $1.00 \text{ mg L}^{-1}$  NPOC correlates strongly with all 3 inorganic nitrogen species; nitrite, nitrate and ammonium in a Pearson's correlation at  $p < 0.01$ ,  $p < 0.05$  and  $p < 0.01$  respectively. A medium strength positive relationship was also detected between DIN (i.e. TN minus DON) and  $\text{Cl}_2$  used for non-THM4 activities, at  $p < 0.01$  and a Cohen's  $d$  effect size of 0.67 (medium to strong), supporting this. Regression analysis shows that total  $\text{Cl}_2$  used vs. nitrite gives an  $R^2$  of 0.65, to a positive linear gradient of  $y = 0.6136x - 1.2095$ , nitrate giving an  $R^2$  of 0.27 to a positive linear gradient of  $y = 12.187x + 2.7078$  and ammonium giving  $R^2$  0.64 to a positive linear gradient of  $y = 3.3777x - 6.7905$ , with all relationships heavily influenced by the concentrations recorded at site 19. These statistical outputs suggest that, as the 3 inorganic nitrogen ions increase in concentration, as does the use of  $\text{Cl}_2$  (Figure 6.9).

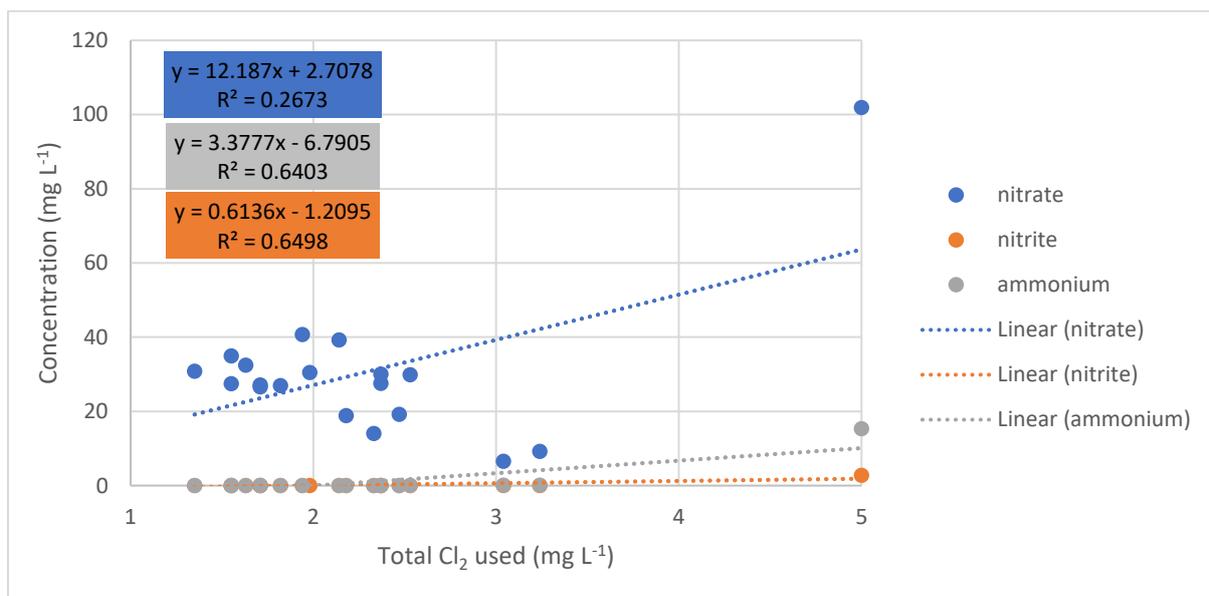


Figure 6.9: Regression analysis between total  $\text{Cl}_2$  used and nitrate, nitrite and ammonium ions detected in freshwater samples collected from the Hampshire Avon catchment.

#### 6.4.2 River Conwy

There were many fewer relationships between the parameters in the Afon Conwy data when compared to the Hampshire Avon and both catchments combined, which could

be a result of the lower nutrient concentration when compared to the Hampshire Avon.

A positive Pearson's correlation was found between total chlorine used, and chlorine used for non THM4 forming activities, at  $r=0.603$ ,  $p<0.05$  and  $n=11$ , which is a medium strength relationship. However, the Cohen's  $d$  effect that chlorine used for non-THM4 forming activities has upon the total  $\text{Cl}_2$  used, is extremely high, at 4.35, showing that the relationship between total chlorine used and chlorine used for non-THM4 forming activities is greatly noticeable without intricate data analysis, thus suggesting that the relationship represents an increase in total chlorine use for non-THM4 compounds. Regression analysis between  $\text{Cl}_2$  used for non-THM4 forming activities, and total  $\text{Cl}_2$  used, shows an  $R^2$  of 0.362 with a positive linear gradient of  $y=0.1703x + 4.1774$  which supports the existence of a relationship between the fate of total chlorine used and  $\text{Cl}_2$  used to form non-THM4.

Furthermore, TN and DIN also correlated with  $\text{Cl}_2$  used for non-THM4 forming disinfection activities is at  $r=0.668$  and  $r=0.633$  respectively, with both  $p<0.05$ ,  $n=11$ . The Cohen's  $d$  effect of these datasets is at 1.2 and 1.32 respectively, showing a high effect each parameter has upon the other, which will be easily determinable without the use of statistical analysis.

Relationships also exist between chlorine used to form THM4 and nitrogen containing groups in the Conwy catchment samples. There are medium to strong negative Pearson's correlations between the chlorine concentration used to form THM4 and TN, DIN, diluted TN and nitrate ( $r=-0.707$ ,  $-0.673$ ,  $-0.699$  and  $-0.690$  respectively, each with  $p<0.05$  and  $n=11$ ). Regression analysis also shows negative relationships between the parameters. Chlorine used to form THM4 vs. TN gives an  $R^2$  value of 0.37 with a negative linear gradient of  $y=-0.8636x + 3.0889$ . DIN gives a negative linear gradient of  $y=-0.8295x + 2.8706$  with an  $R^2$  of 0.32, diluted TN gives a negative linear gradient of  $y=-0.2293x + 0.8111$  with an  $R^2$  of 0.39 and finally, nitrate gives a negative linear gradient of  $y=-3.7072x + 12.784$  with an  $R^2$  of 0.32 (Figure 6.16). These data suggest, as the concentration of nitrogenous groups increases, the mass of chlorine used to form THM4 decreases.

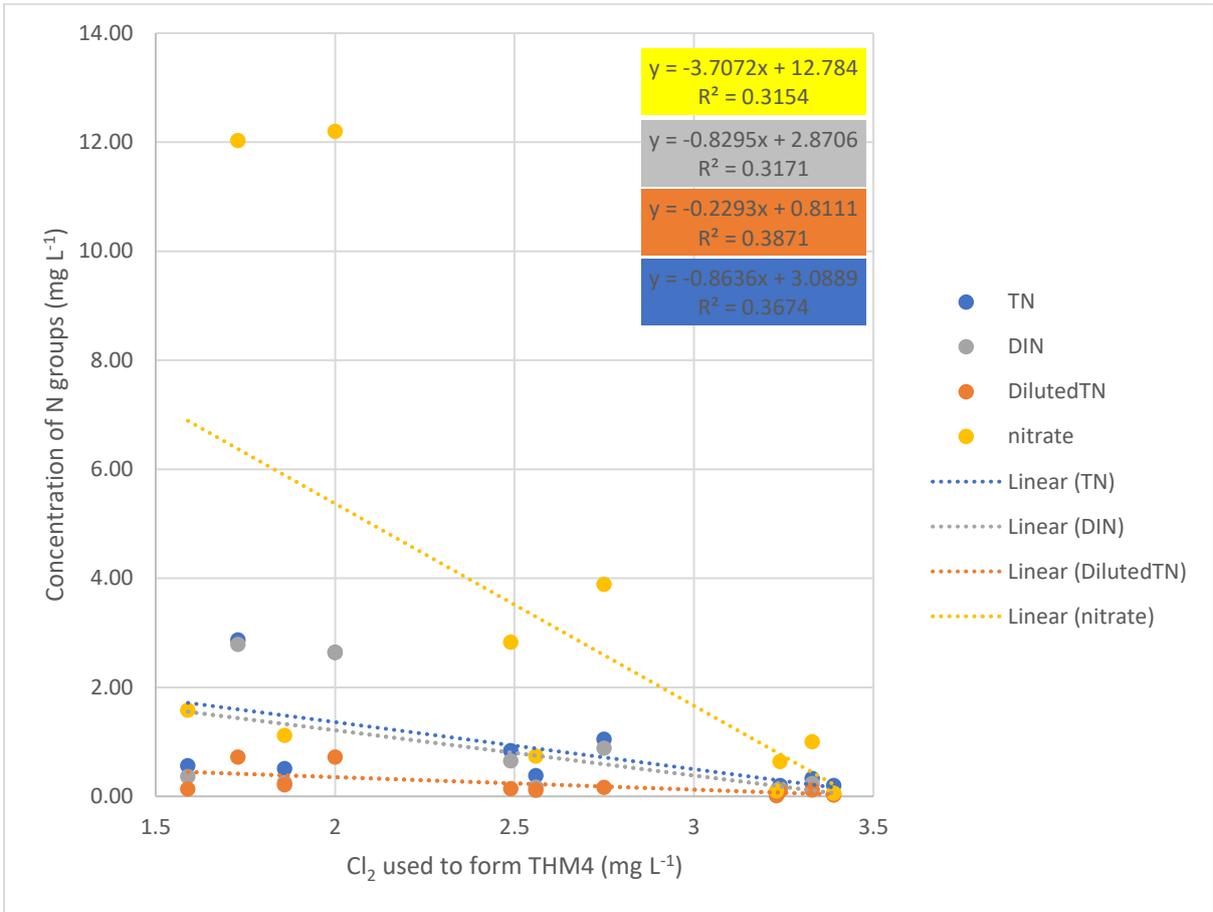


Figure 6.10: Negative linear regressions between total  $\text{Cl}_2$  used to form THM4 ( $\text{mg L}^{-1}$ ) and TN, DIN, diluted TN and nitrate concentrations (all in  $\text{mg L}^{-1}$ ) data from the Conwy catchment, showing the increase in  $\text{Cl}_2$  usage on THM4 formation as the concentration of the N groups decreases.

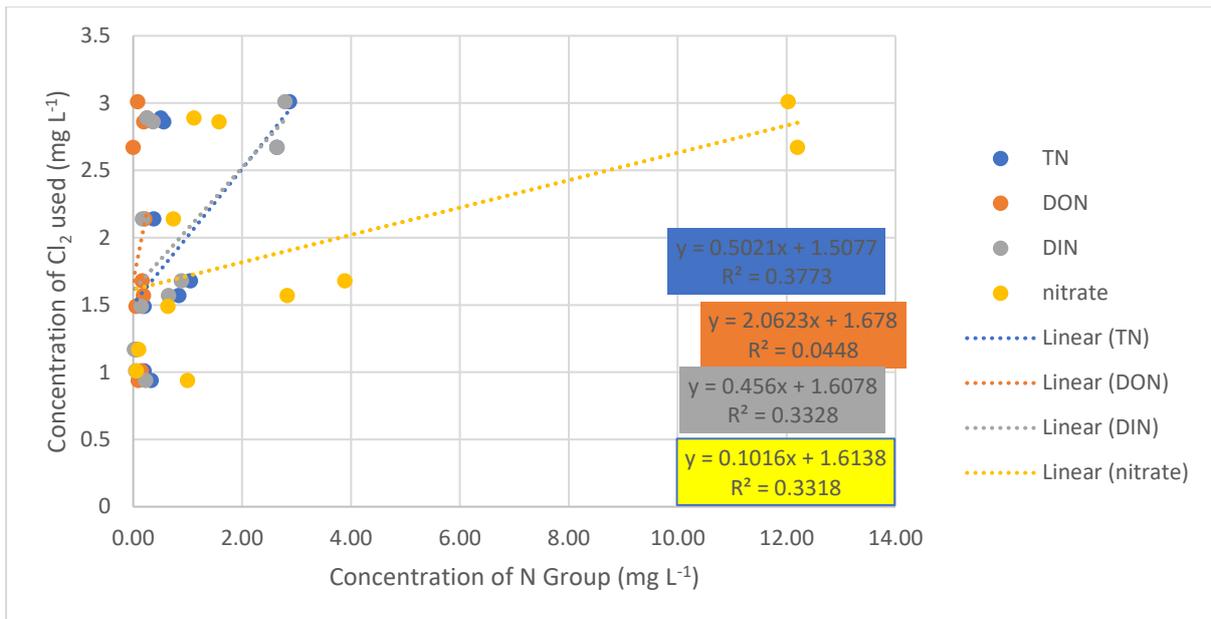


Figure 6.11: The relationships between the concentration of  $\text{Cl}_2$  used and the concentration of a nitrogenous group (TN, DON, DIN and nitrate).

The regressions in Figure 6.11 are all positive, with the regression for TN being the strongest and the regression for DON being the weakest. There are no significant Pearson's correlations between these compounds, with p values ranging from 0.060 ( $r = -0.583$ ,  $n = 11$ ) for total chlorine used to  $p = 0.533$  for TN ( $r = 0.533$  and  $n = 11$ ). This data could suggest that DIN is responsible for more  $\text{Cl}_2$  use than DON.

A relationship was discovered between the NPOC concentration of samples from the River Conway catchment and the total concentration of chlorine used over the 7 days incubation period. A Pearson's correlation of  $p < 0.05$  shows that, as NPOC concentration increases, the total chlorine used decreases. A regression analysis also shows this relationship, with an  $R^2$  of 0.34 to a linear gradient of  $y = -4.7232x + 25.687$ , see Figure 6.12.

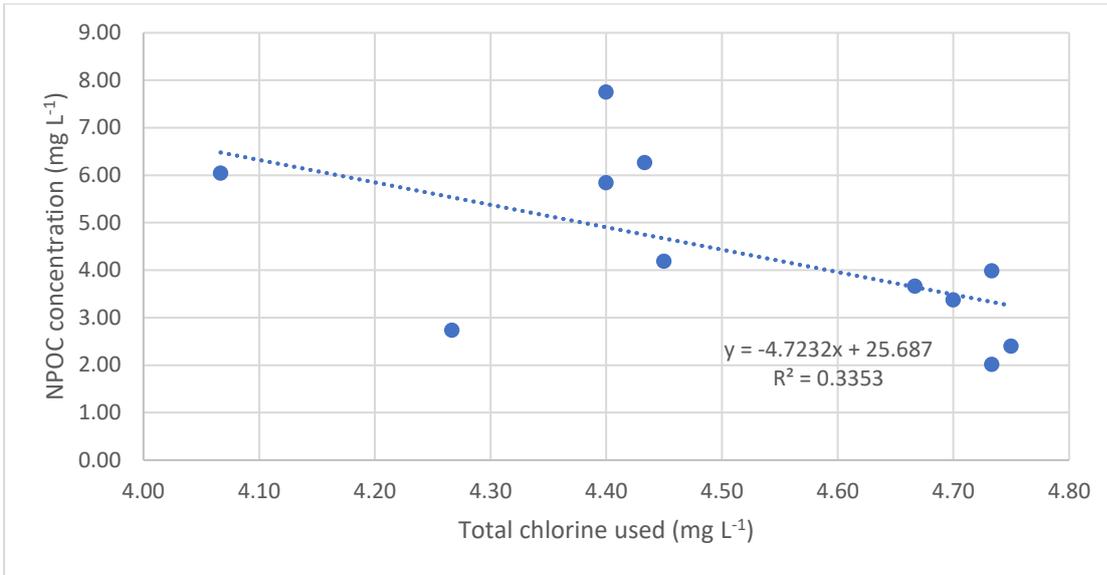


Figure 6.12: Regression analysis between total chlorine used over 7 days at 25 °C from the 5 mg L<sup>-1</sup> initial dose of chlorine, and the raw NPOC concentration of the sample prior to dilution to 1 mg L<sup>-1</sup>.

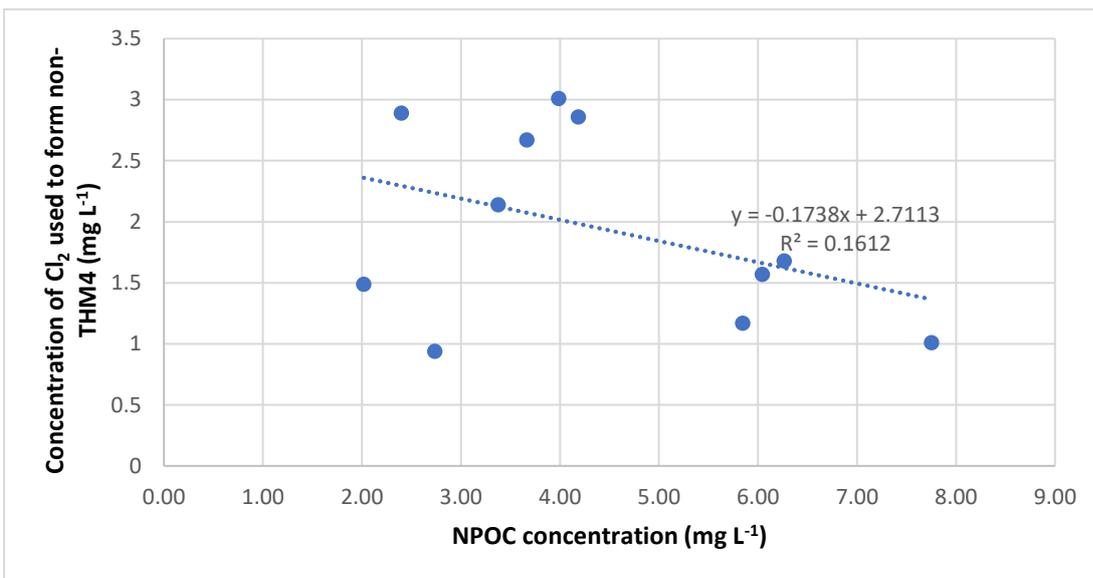


Figure 6.13: Chlorine used to form non-THM4 plotted against NPOC concentration at the Conwy catchment.

At the Conwy catchment, as the concentration of NPOC in the water decreases, the concentration of chlorine used in disinfecting the water increases, although the relationship is relatively weak (a linear gradient of  $y=0.173x + 2.711$ ) and the data points are sparsely distributed ( $R^2=0.161$ ).

#### 6.4.3 Data from Hampshire Avon and River Conwy Sites Combined

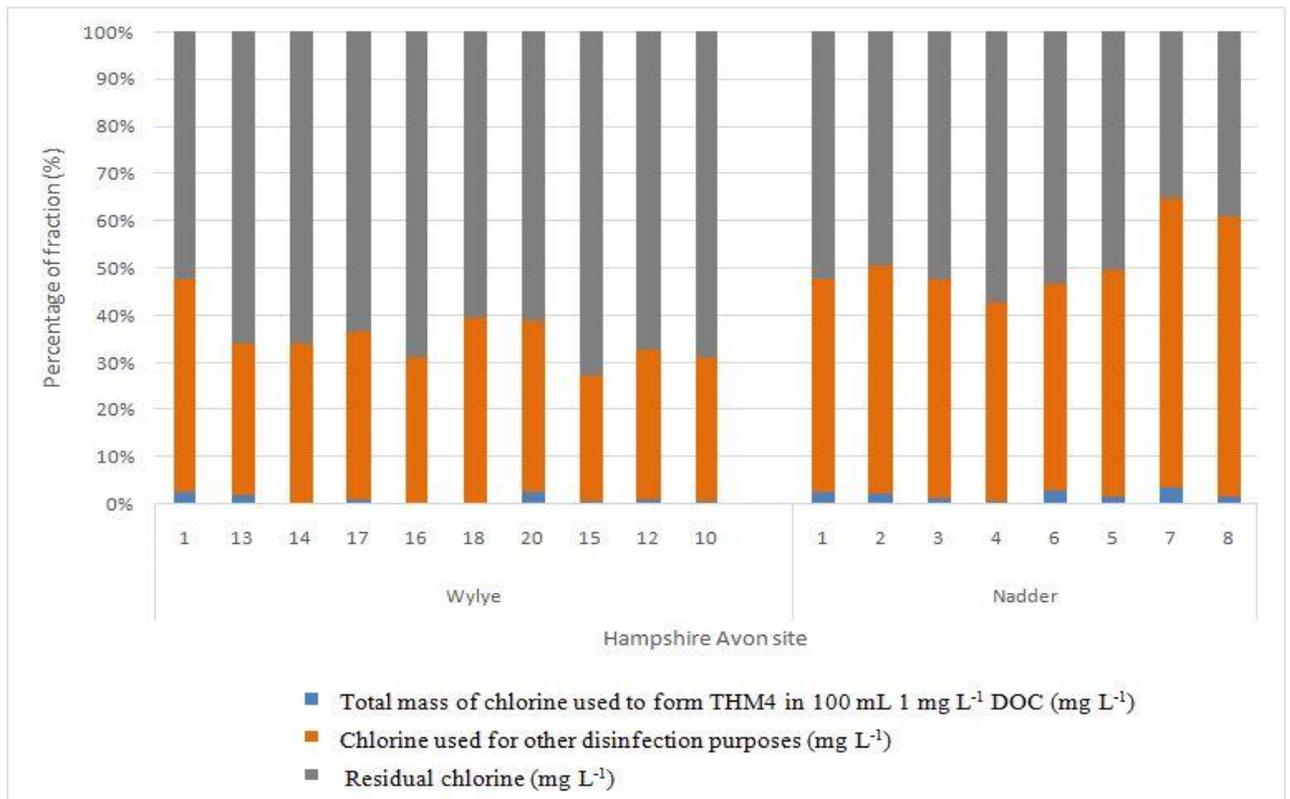


Figure 6.14: Chlorine mass balance data (formed from a standardised 7 day chlorination experiment) from Hampshire Avon catchment for April 2016, arranged in site order, from the confluence at Salisbury (site 1) towards the uplands of the catchment (i.e. site 10 for Wylde and 8 for Nadder). Salisbury data is included as a reference point for both rivers.

The data in Figure 6.14 shows the percentage breakdown of chlorine use in samples from the Hampshire Avon. The mean residual chlorine concentration (the chlorine concentration remaining in the sample after 7 days) along the River Nadder appears to be less than the sites along the River Wylde; mean residual chlorine concentration remaining in the River Nadder samples is  $2.48 \pm 0.13 \text{ mg L}^{-1}$  whereas that remaining in the River Wylde is  $2.98 \pm 0.34 \text{ mg L}^{-1}$ , showing a  $0.5 \text{ mg L}^{-1}$  decrease in chlorine demand from the River Wylde compared to the River Nadder. Furthermore, the mean total mass of chlorine used to form THM4 in the River Nadder is  $0.10 \pm 0.02 \text{ mg L}^{-1}$  per  $\text{mg L}^{-1}$  DOC, whereas the mean concentration of chlorine used to form THM4 in the River Wylde is half of that at  $0.05 \pm 0.01 \text{ mg L}^{-1}$  per  $\text{mg L}^{-1}$  DOC. Finally, the mean concentration of chlorine used for other disinfection purposes at the River Nadder is  $2.42 \pm 0.12 \text{ mg L}^{-1}$ , whereas the mean concentration of chlorine used for

other disinfection purposes at the River Wylde is  $1.98 \pm 0.34 \text{ mg L}^{-1}$ ,  $0.50 \text{ mg L}^{-1}$  less than at the River Nadder. When arranged in a confluence to headland orientation (Figure 6.14), an increase in residual chlorine concentration is seen. However, the River Nadder seems to have an increase in the percentage of chlorine used for other disinfection activities, the further into the headwaters the sample sites are situated.

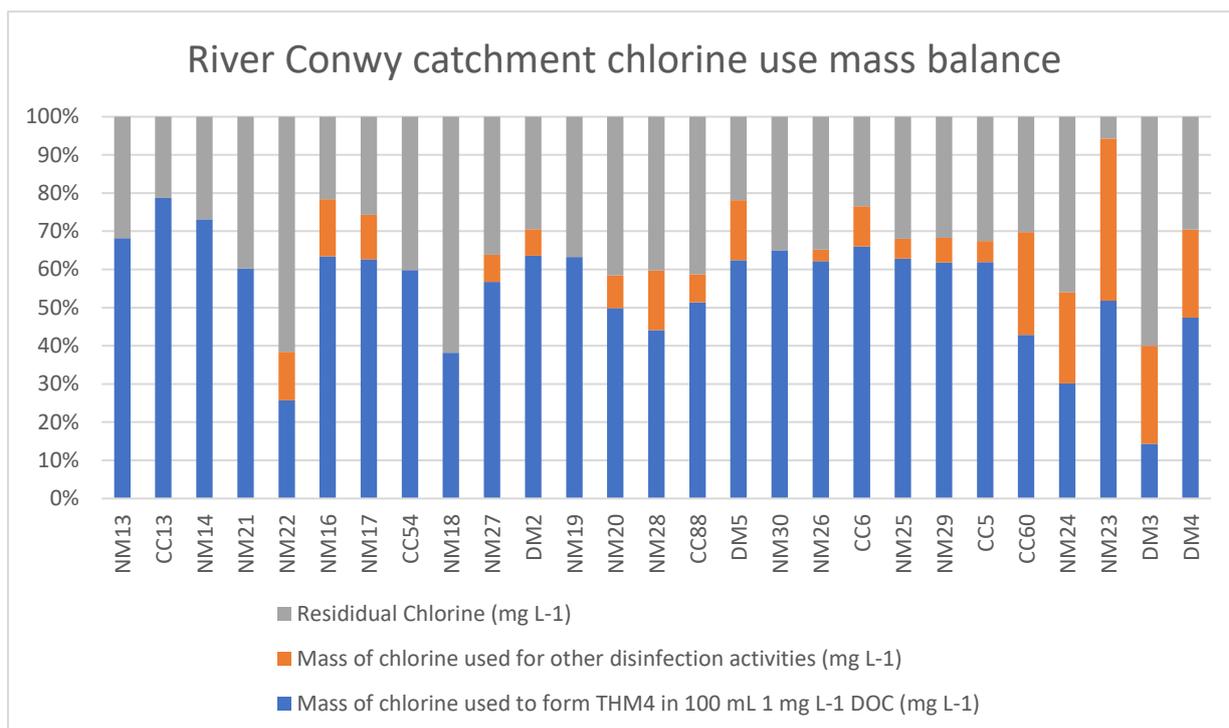


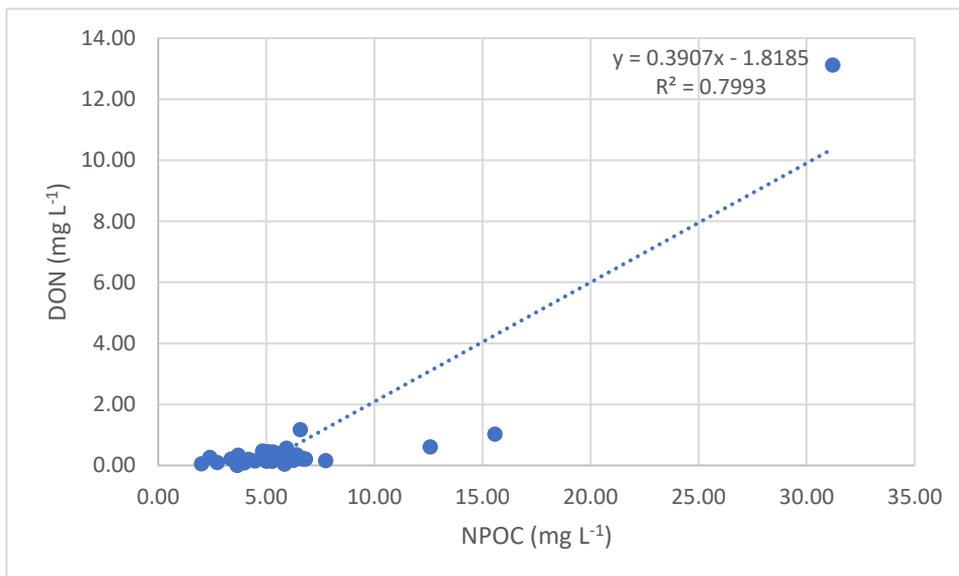
Figure 6.15: Chlorine mass balance data, showing residual chlorine concentration, total mass of chlorine used to form THM4 and the total mass of chlorine used for other disinfection activities from River Conwy catchment, organised by distance from source (NM13) to sea (DM4).

Any negative values were corrected to zero to compensate for differences in detections between the titration derived data which is more susceptible to human error, than other detection methods available, such as HPLC. Whilst no obvious trends are visible between the use of chlorine and the distance from source/sea, the variation of the mass of chlorine used for various purposes varies vastly from site to site.

Comparing the Hampshire Avon with the Conwy suggests that the catchments have different chlorine demand, with the Hampshire Avon sites using only  $45.16 \pm 3.82 \%$

of the chlorine added and  $3.20 \pm 0.46$  % of the chlorine used to form THM4. In contrast, Conwy sites used  $64.24 \pm 2.73$  % of the total chlorine added, and used  $90.20 \pm 6.06$  % chlorine used to form THM4. Overall, the total mean chlorine used by sites in the Conwy catchment is 1.99 times greater than at the Hampshire Avon catchment. Raw data is presented in the Table 1, Appendix 3.

Relationships between DON and NPOC with chlorine demand were examined to investigate potential interactions and to examine whether either acts as a driver. These examinations both included and excluded site 19, a site draining STW effluent which contains a much greater concentration of organic matter. Figure 6.16 shows a regression analysis between DON and NPOC, which generates an  $R^2$  value of 0.799 to a positive linear gradient of  $y=0.3907x - 1.8186$  with site 19 included, but when this sites data is removed, the regression reduces to an  $R^2$  of 0.37 to a positive linear gradient of  $y=0.05922 - 0.0279$ , thus showing that site 19 is still a driver for this relationship, however, an  $R^2$  value of 0.37 shows that 37% of the variability of the response data around the mean can be attributed to the model.



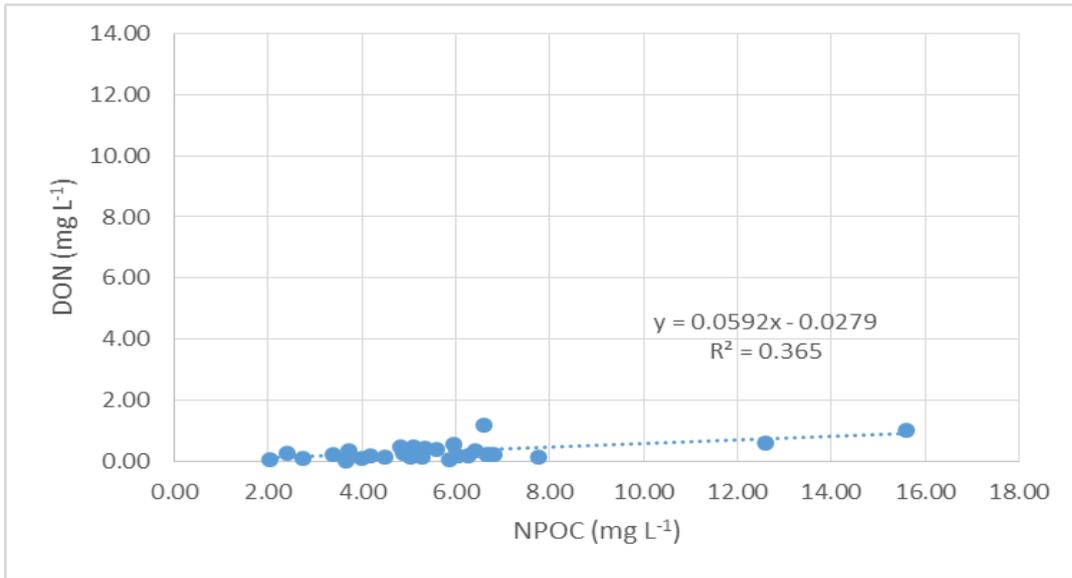


Figure 6.16: Graph to show a) relationship between DON and NPOC with the STW input at site 19 (top), Hampshire Avon, and b) relationship without site 19 included (bottom).

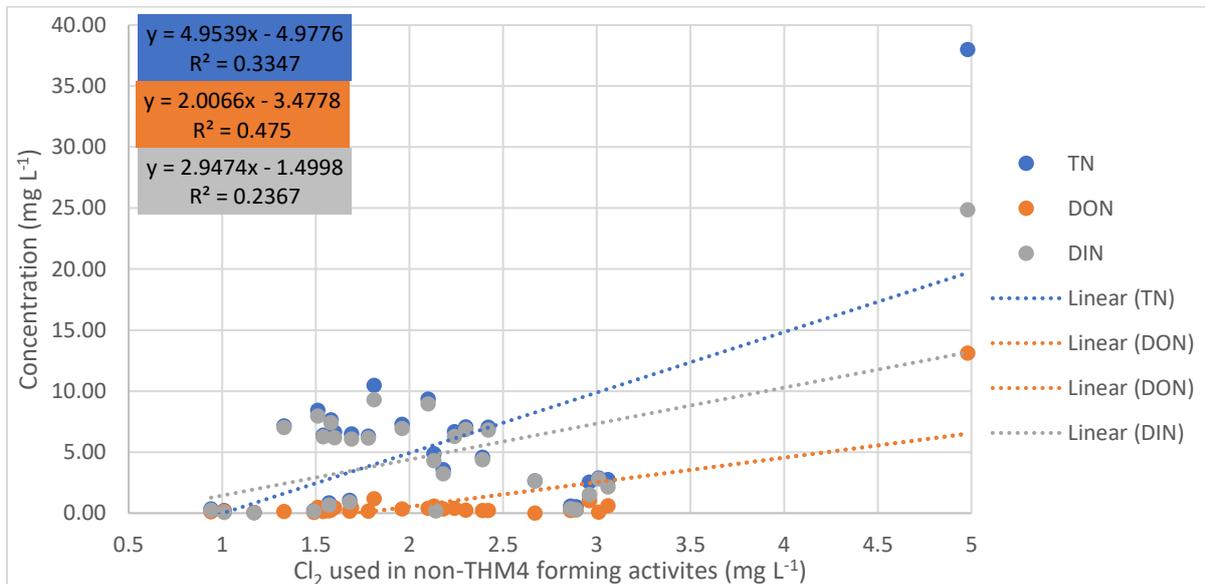


Figure 6.17: Regression analysis between TN, DON and DIN, and the concentration of Cl<sub>2</sub> used in non-THM4 forming activities, including site 19, a sewage treatment plant.

Further analysis between NPOC and nitrogenous group concentrations at the Hampshire Avon and Conwy catchments shows positive Pearson's correlations between NPOC concentration and the nitrogen concentration, either as TN ( $p < 0.01$ ),

DON ( $p < 0.01$ ) or DIN ( $p < 0.01$ ). The strongest relationship was found between NPOC and DON, which also had the highest Cohen's  $d$  effect, at 1.4. This is a very strong effect size and can be seen visually in the raw data, displayed in Table 2, Appendix 3.

Relationships were also found between TN, DON and DIN, and the concentration of chlorine used in non-THM4 forming activities ( $p < 0.01$ ,  $n=30$  for all). A Cohen's  $d$  effect size of 0.77 for DON, 0.67 for TN and 0.74 for DIN, and the concentration of chlorine used on non-THM4 DBPs and other disinfection activities shows medium to strong effects between the two parameters suggesting that the relationships are recognisable with the naked eye. Regression analysis of these 3 nitrogenous groups and their relationship with  $\text{Cl}_2$  concentration used to form non-THM4 DBPs (and other disinfection activities) show low to medium strength positive regressions with  $R^2$  ranging from 0.237 (DIN) through 0.335 (TN) up to 0.475 (DON) (Figure 6.17), suggesting that there are medium-weak to medium relationships between DIN, TN and DON and the  $\text{Cl}_2$  concentration used for non-THM4 formation, hinting at a relationship between the variables.

Interestingly, the chlorine used in non-THM4 forming activities correlates strongly with nitrite ( $r=0.697$ ,  $p < 0.01$ ,  $n=30$ ,  $d=2.93$ ) and ammonium ( $r=0.702$ ,  $p < 0.01$ ,  $n=30$ ,  $d = 0.63$ ), as well, showing a medium strength relationship with nitrate ( $r=0.467$ ,  $p < 0.01$ ,  $n=30$ ,  $d = 1.26$ ). Regression analysis of these relationships reflect the Pearson's correlation data (Figure 6.18).

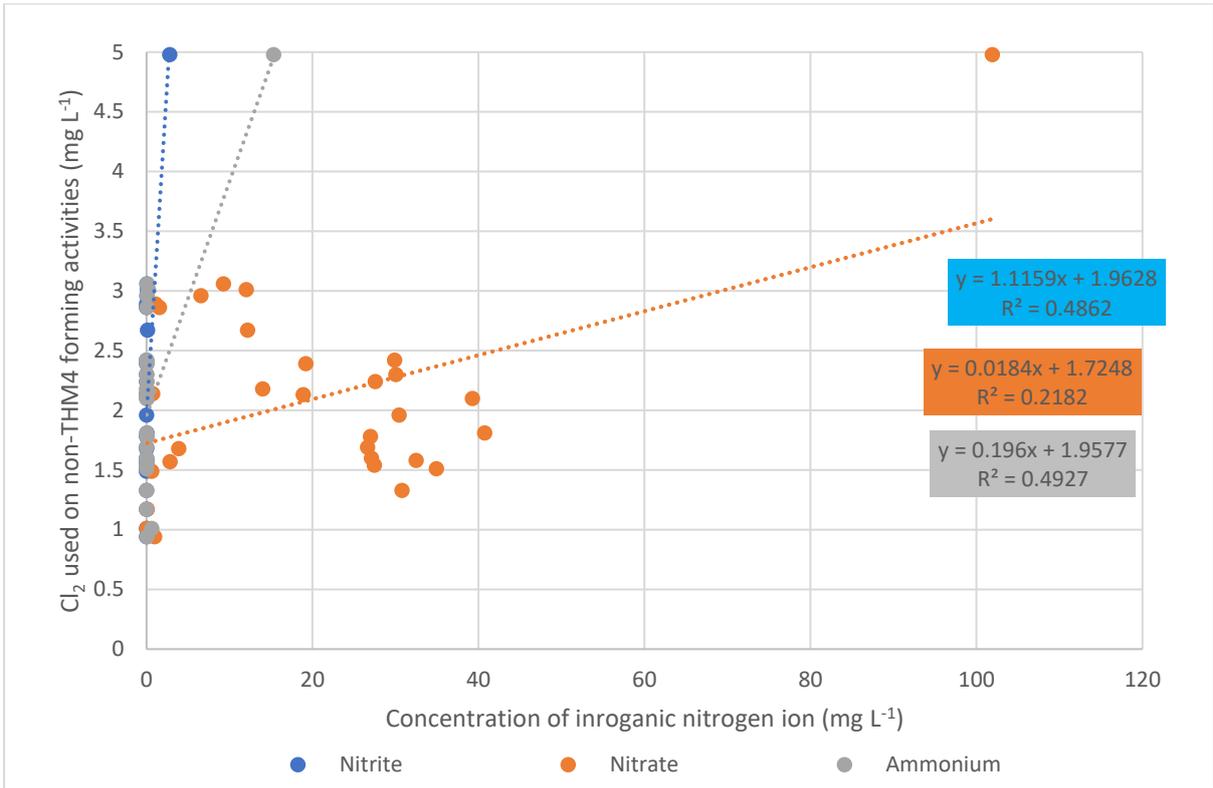


Figure 6.18: Relationship between the concentration of chlorine used on non-THM4 forming disinfection activities and the concentration of the 3 inorganic nitrogen ions in the River Conwy and Hampshire Avon catchment.

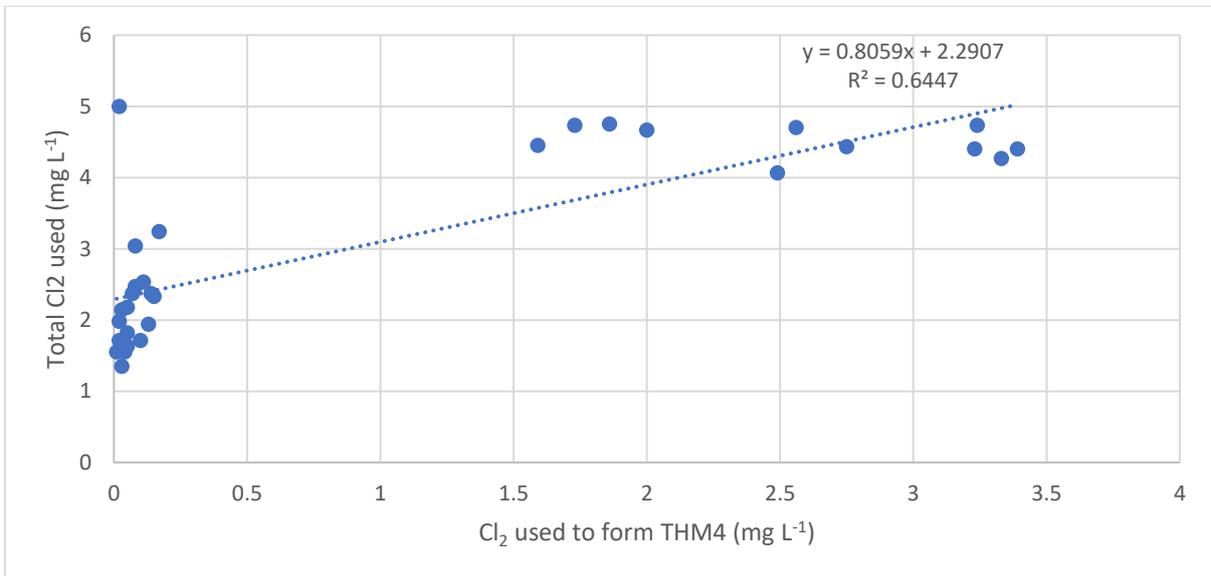


Figure 6.19: Regression between Cl<sub>2</sub> concentration used to form THM4 vs. total Cl<sub>2</sub> used at Hampshire Avon and Conwy catchments combined.

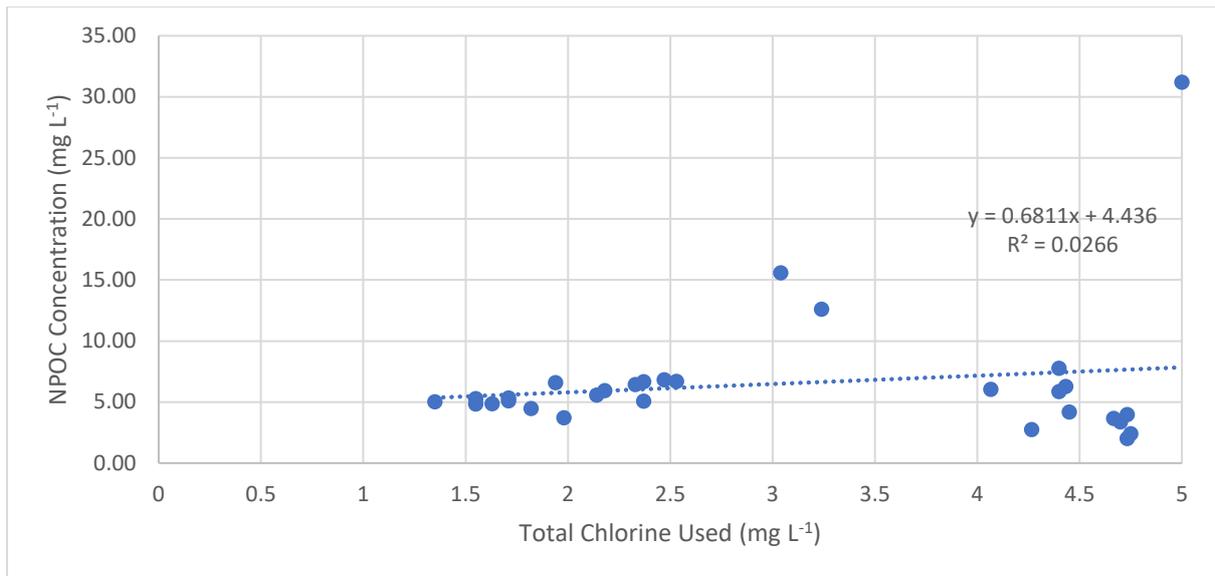


Figure 6.20: Regression analysis between total chlorine concentration used from the initial 5 mg L<sup>-1</sup> dose that was applied prior to 7 days incubation at 25 °C, and NPOC concentration at both Hampshire Avon and Conwy catchments.

The relationship between the total chlorine concentration used and the total concentration of chlorine used to form THM4 was analysed (Figure 6.19), showing that there is a medium to strong relationship between the two datasets. As the total chlorine concentration used in the sample increases, so does the chlorine concentration used to form THM4. The total chlorine used dataset was then statistically analysed to determine whether there was a relationship between this parameter, and the NPOC concentration of the sample (regression is displayed in Figure 6.20), prior to dilution to 1 mg L<sup>-1</sup> for chlorination. Due to the data being non-normal, a Spearman's Rank correlation test was used in place of a Pearson's correlation, showing that no relationship was present ( $p > 0.05$ ).

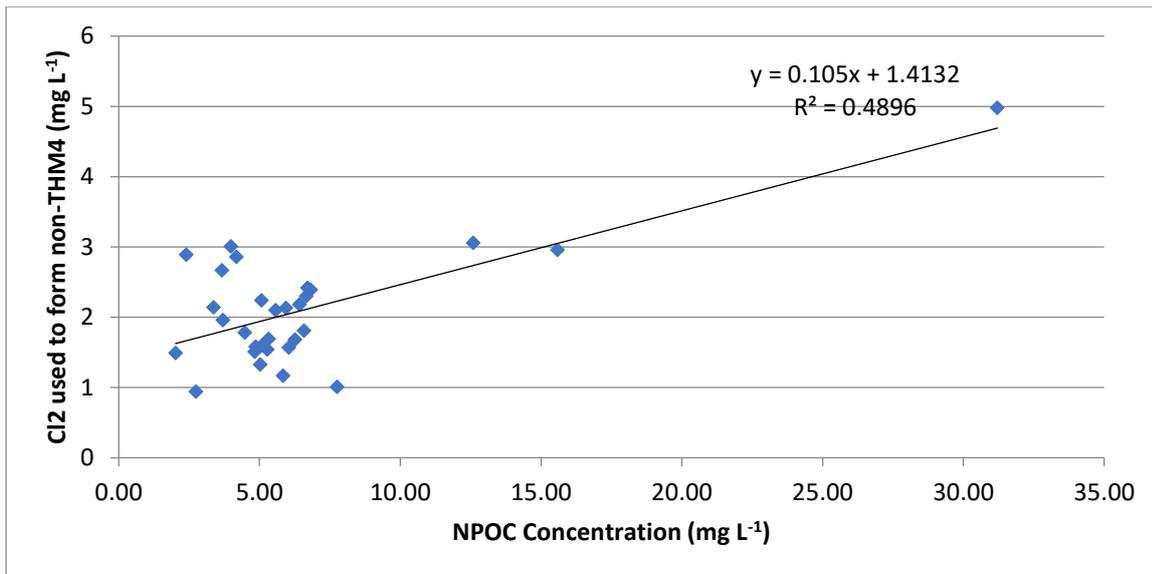


Figure 6.21: Regression analysis between NPOC concentration and total concentration of Cl<sub>2</sub> used to form non-THM4 in the Hampshire Avon and the Conwy catchments combined.

A regression between NPOC concentration and total concentration of chlorine used to form non-THM4 shows that, as the NPOC concentration increases, so does the concentration of chlorine used in non-THM4 forming activities. The line of best fit (Figure 6.21) shows a  $R^2$  value of 0.49, suggesting that the data points do not deviate far from their mean, indicating a medium strength robustness of the relationship.

When compared to identical datasets from the Hampshire Avon and Conwy catchments combined, it can be seen that the Hampshire Avon dataset is driving the relationship visible in Figure 6.21, due to the negative trend in the dataset from the Conwy catchment (Figure 6.14), which is seemingly dwarfed by the strong regression analysis between the datasets at the Hampshire Avon (Figure 6.7).

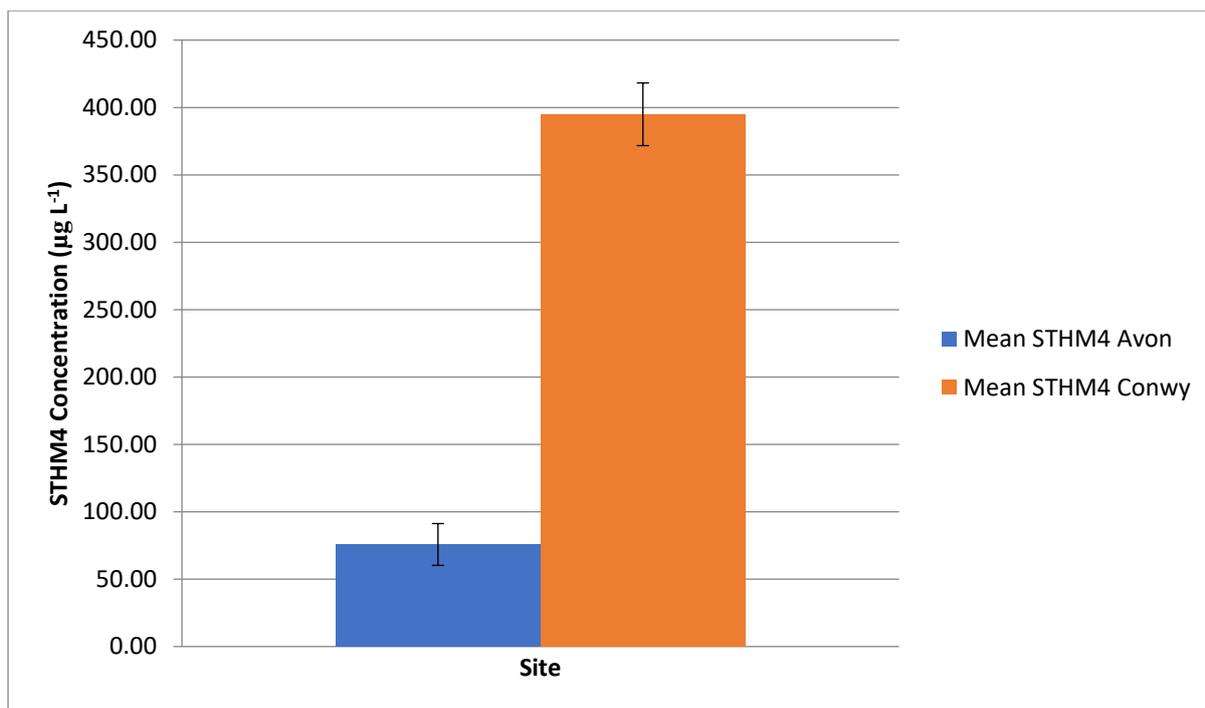


Figure 6.22: Comparison between mean standardised THM4 concentration at the Hampshire Avon and the Conwy catchments. Error bars show standard deviation.

The difference between the standardised THM4 formation (i.e. THM4 formed from a 1 mg L<sup>-1</sup> concentration of NPOC in an excess of chlorine) at the Hampshire Avon and the Conwy catchments is vast (Figure 6.22), with the Hampshire Avon forming, on average, 5.2 times fewer THM4 compounds than the Conwy catchment, thus suggesting that the NPOC character at the Conwy catchment is much more susceptible to chlorination than at the Hampshire Avon.

## 6.5 Discussion

### 6.5.1 Hampshire Avon

NPOC and total chlorine used for THM4 formation data, and NPOC and chlorine used for non-THM4 formation data was analysed statistically using a Pearson's correlation, which showed a strong positive correlation at  $r=0.929$  and  $r=0.939$  respectively. Both compared datasets have a Cohen's  $d$  effect size of 1.22-1.24, showing that these changes are not trivial and can be easily detected within the data with the naked eye, which suggests that the relationship between NPOC concentration in the sample and the concentration of chlorine used in other disinfection practices is

quite prominent. The relationship between NPOC and total chlorine used (Figure 6.6) shows a positive regression analysis presenting an  $R^2$  correlation coefficient of 0.86. This shows that, as the concentration of NPOC increases, so does the concentration of chlorine used to form THM4. However, the  $R^2$  only accounts for 86% of the variability of the response of the data around its mean, suggesting that not only NPOC is being attacked by the available chlorine, and that other compounds are being targeted. Furthermore, the data shows that between 4.07 and 4.75 mg L<sup>-1</sup> (e.g. 80 and 95%) of chlorine have been used to attack the NPOC (concentrations of between 2.02 and 7.75 mg L<sup>-1</sup>), compared to at the Hampshire Avon, where between 1.98 and 5 mg L<sup>-1</sup> chlorine was used (on NPOC concentrations of 3.71 - 31.21 mg L<sup>-1</sup>), showing that chlorine use at the Conwy is much higher than at the Hampshire Avon.

Figure 6.5 shows a regression analysis of the concentration of chlorine used for non-THM4 formation and NPOC data sets, showing an  $R^2$  value of 0.88 to a positive linear gradient of  $y=0.121x + 1.24$ , and for NPOC vs. total chlorine used,  $R^2$  0.863 to a positive linear gradient of  $y=0.121x + 1.312$  (Figure 6.5). The similar trends between the two datasets may suggest that, at the Hampshire Avon, chlorine is favouring the non-THM4 forming compounds over the THM4 forming compounds, which could have implications for the formation of N-DBPs. The regression analysis between NPOC and total chlorine concentration used shows that there is a strong relationship between the two parameters, and, as the NPOC concentration increases, so does the concentration of chlorine used, which is contrary to the data displayed in Figure 6.20 for both the Hampshire Avon and the River Conwy. This relationship may be due to two reasons – a higher concentration of NPOC in a sample means that the sample can host many more bacteria due to an increased food source for them (Raymond and Bauer, 2000), and in some cases, requiring more chlorine to deactivate them, such as antibiotic resistant bacteria (Yuan, Guo and Yang, 2015). See figure 8 in appendix 4 for bacterial studies in this work. The other reason could be that an increase of NPOC brings with it an increase of THM4 (and other C-DBP) precursors, thus consuming more chlorine. The relationship between increased NPOC and TTHM4 concentrations are very similar between the Hampshire Avon and the Conwy catchments (Figure 1 and Figure 2, Appendix 3). Figure 6.6, therefore, examines the relationship between NPOC and Cl<sub>2</sub> used in anything other than THM4 formation, and a similar relationship to the one displayed in Figure 6.12 is visible. The regression

shows a strong positive relationship between the NPOC concentration of the sample, pre-dilution, and the concentration of Cl<sub>2</sub> used in non-THM<sub>4</sub> forming activities. An average Cohen's *d* effect size of 1.23 between NPOC and total Cl<sub>2</sub> used, and total Cl<sub>2</sub> used on non-THM<sub>4</sub> disinfection activities shows that NPOC has a very strong effect upon the fate of chlorine when added to freshwater at the Hampshire Avon and this trend in the data is visible without the need for in-depth statistical analysis. Judd and Bullock (2003) found that a large proportion of the use of chlorine, nitrogen, and carbon was unaccounted for, however, this work was conducted in chlorinated swimming pool water. The authors further suggest that accumulation of chlorinated and unchlorinated organic matter was taking place within the water. Yee *et al.*, found that water samples with higher DOM concentration had a higher chlorine demand and also a higher formation of THM<sub>4</sub>, further claiming that hydrophilic neutral and hydrophobic acid fractions were found to play an important role on causing a high chlorine demand (Yee *et al.*, 2006). The data from Figures 6.5 and 6.6 confirms that NPOC concentration is intrinsically linked to chlorine use in samples from the Hampshire Avon, whether or not it is being used to form THM<sub>4</sub>.

Figure 6.7 displays regression analysis between DON and two chlorine containing groups – total chlorine used, and chlorine used on non-THM<sub>4</sub> forming activities. The relationships are heavily driven by one data point, each, and this is site 19, a STW. However, this data is included as the site is not anomalous and therefore contributes to the overall DON concentration of the river water. The data suggests that DON concentrations (as well as DOC) have a strong influence upon the fate of chlorine used in the Hampshire Avon, although this influence appears to be weaker than the influence that NPOC has (see Figures 3 to 8, Appendix 3, for a comparison between DOC, DON and the use of chlorine at the individual and combined catchments). The Cohen's *d* effect size for DON and total Cl<sub>2</sub> used and total Cl<sub>2</sub> used on non-THM<sub>4</sub> forming activities are 0.51 and 0.54 respectively, showing a medium strength effect which shows that the relationship is not very obvious but can be seen through simple statistical analysis. The data suggests that DON in these waters is being chlorinated to form N-DBPs. These relationships are strongly affected, again, by site 19 which is suspected to be draining effluent from an STW – when site 19 is removed from the dataset, the R<sup>2</sup> values drop considerably to 0.13 and 0.12 respectively. This, coupled with the vast time and experimental costs involved in N-DBP detection, make

conclusions regarding the relationship between DON and chlorine hard to draw. DON data is included in Appendix 3 Table 2. Further work would include a subset of samples with varying DON concentrations spanning 0 – 50 mg L<sup>-1</sup> to determine whether the relationship between DON and Cl<sub>2</sub> used can be extrapolated to other waters to predict Cl<sub>2</sub> use in them.

A regression analysis between total Cl<sub>2</sub> used and Cl<sub>2</sub> used in non-THM4 forming activities is shown in Figure 6.8, which shows an exceptionally strong relationship between the two variables. A Pearson's correlation of  $r=0.998$ ,  $p<0.01$  and  $n=19$  and a regression analysis of  $y=1.008x + 0.0535$ , with a  $R^2$  value of 0.9964 shows that a very significant relationship is present between the two variables, however, a Cohen's  $d$  effect size of 0.08 suggests that the relationship is not easily identifiable when initially examining the data (i.e. before statistical analysis). Nevertheless, the relationship between the two variables suggests that the chlorine used in non-THM4 forming activities drives the total concentration of chlorine used in the sample. The raw THM4 data from the Hampshire Avon (outlined in Table 4, Appendix 3) shows the mean THM4 concentration of these sites under a 7 day incubation at favourable temperatures and with an excess of chlorine (see method in Chapter 2) is minimal, even at site 19, when compared to other THM4 data such as that generated from the nutrient poor Conwy.

As the relationship between DON and total Cl<sub>2</sub> concentration used has already been explored (Figure 6.7), the individual constituents of DIN; nitrite, nitrate and ammonium, were explored in a regression and correlation analysis with the total concentration of Cl<sub>2</sub> use in the samples from the Hampshire Avon catchment (Figure 6.9). Again, site 19 is driving the relationships, but there are 3 distinct relationships between the 3 DIN constituent ions. All 3 ions produced strong Pearson's correlation coefficients at  $p<0.05$  and  $R=>0.517$ . The regression analysis shows that ammonium and nitrite have very similar relationships with the total concentration of Cl<sub>2</sub> used, with an  $R^2$  of 0.64 or greater. These strong relationships suggest that an increase in chlorine consumption in a sample is related to a higher initial concentration of inorganic nitrogen ions. The relationship that these ions have with the chlorine could be explained by several different factors – the ammonium could be binding to the chlorine to make natural chloramines (How *et al.*, 2016), or the ions could be forming monomers, acting as food sources for bacteria in the water (Kaplan and Newbold,

2004), thus requiring more chlorine to deactivate them. Of these 3 species, the mean Cohen's *d* effect size is 1.79, showing that inorganic species are being targeted by chlorine, and effect that the concentration of these inorganic nitrogen compounds has upon the chlorine use is high and likely noticeable by a simple examination of the raw data. The data presented in Figure 6.11 suggests that nitrate correlates the strongest with total Cl<sub>2</sub> used, suggesting that nitrate is responsible for the maximum usage and may be the most reactive form of inorganic nitrogen. Once again, however, site 19 is skewing results.

### 6.5.2 Data from River Conwy Only

The data sets explored in Figure 6.9 at the Hampshire Avon were analysed for samples from the River Conwy, and the data is presented in Figure 6.10. To determine whether DIN ions could be used to form disinfection by-products other than THM4, the concentration of Cl<sub>2</sub> used for non-THM4 forming activities were correlated with the concentration of 4 nitrogenous groups – TN, DIN, diluted TN and nitrate, with all 4 showing a negative Pearson's correlation TN ( $r=0.691$ ), DIN ( $r=0.574$ ), and nitrate ( $p=0.546$ ) at the River Conwy catchment. Regression analysis also shows negative trends between the 4 groups, showing that, as the concentration of these nitrogen containing groups decreases, the chlorine concentration used to form THM4 increases. Linear trends with an R<sup>2</sup> value of between 0.32 and 0.39 show that these relationships are not trivial. Pearson's correlation analysis between the concentration of Cl<sub>2</sub> used in non-THM4 forming activities were conducted, however, there is no correlation between the diluted DIN concentration and the concentration of chlorine used for non-THM4 forming activities. Total chlorine concentration used vs. the DIN ions and TN concentration were also tested in an regression analysis (Figure 6.11), showing that as the concentration of the DIN ions and TN concentration increased, so did the total concentration of chlorine used, again, with R<sup>2</sup> values of between 0.04 and 0.37). The data in Figure 6.9 could suggest that chlorine binds more readily to nitrogenous compounds over the carbonaceous THM4 forming compounds.

To confirm the above, Figure 6.12 shows a regression between NPOC concentration and the total chlorine used. Although the concentration of chlorine used was always above 80% of the total concentration added for disinfection purposes at each site in the Conwy catchment, there is a negative trend between the NPOC concentration and

the concentration of chlorine used, showing that as NPOC concentration increases, the concentration of chlorine used decreases. A negative Pearson's correlation shows that there is a significant relationship between the data, thus suggesting that the NPOC in the samples from the River Conwy catchment is not as favourable to the chlorine as other compounds present in the water. Figure 6.13 analyses the NPOC concentration vs. the total chlorine concentration used to form non-THM4 DBPs. Again, a negative relationship is present, showing that, as the NPOC concentration increases, the concentration of chlorine used to form non-THM4 DBPs decreases. This indicates that, the higher the concentration of NPOC, the more likely that the chlorine available will be used to form THM4.

### 6.5.3 Hampshire Avon and River Conwy Catchments Combined

The data showing the chlorine used for 3 different activities in the samples from the Conwy catchment and the Hampshire Avon catchments individually (Figures 6.14 and 6.15) show the differences between the chlorine demand at each site. The decrease in the percentage of chlorine used from the confluence at Salisbury back into the headlands can be attributed to the additional flow of the river at lower reaches due to more water draining into the river. This water will inevitably bring with it organic matter (mean NPOC is  $7.78 \pm 6.35 \text{ mg L}^{-1}$  and DON is  $1.09 \pm 2.93 \text{ mg L}^{-1}$ ) and bacteria which will all consume chlorine, and thus, the residual chlorine concentration remaining in the sample at site 1 will be lower than at site 8.

The percentage of chlorine used in disinfection activities at most sites in the Conwy catchment do not appear to differ significantly between sites (Figure 6.15), although there are several sites that appear different, namely NM22, NM23, NM24 and DM3. This is likely due to the fact that the catchment is large and draining many different land use types, but these produce a nutrient poor water when compared to the Hampshire Avon, thus having a lower demand for chlorine due to fewer bacteria in the water. It further suggests that as the river travels a further distance away from the source, there doesn't appear to be a greater chance of THM4 formation, suggesting that each site is creating specific precursors that take up chlorine use, however, these precursors do not appear to accumulate the further downstream a sample is taken. The data is presented in Table 3, Appendix 3.

The Hampshire Avon forms lower concentrations of THM4 compounds than the River Conwy, as shown by the data in Figure 6.22. The mean concentration of THM4 in samples collected from the Hampshire Avon during April 2015 (i.e. the data used in this study) is  $74.48 \pm 15.54 \mu\text{g L}^{-1}$  whereas the THM4 concentration from the Conwy catchment during this time is  $355.05 \pm 20.26 \mu\text{g L}^{-1}$ , and thus THM4 formation from  $1 \text{ mg L}^{-1}$  NPOC is more than 4.75 times greater at the Conwy than at the Hampshire Avon. It is also apparent that the Hampshire Avon has a much higher mean concentration of residual chlorine from the mass balance equation (i.e. chlorine concentration after sample has been dosed with  $5 \text{ mg L}^{-1}$  NaOCl and incubated at  $25^\circ\text{C}$  for 7 days) at  $2.74 \pm 0.19 \text{ mg L}^{-1}$  - a little over half of the initial dose of  $\text{Cl}_2$ . The River Conwy catchment has a mean concentration of residual chlorine at  $1.79 \pm 0.14 \text{ mg L}^{-1}$ . Comparisons between the two sites show that the Hampshire Avon uses 19.08% (approx.  $1 \text{ mg L}^{-1}$ ) less chlorine than the Conwy catchment sites, on average. It is also interesting to note that the Conwy sites use, on average,  $2.87 \pm 0.15 \text{ mg L}^{-1}$   $\text{Cl}_2$  to form THM4, whereas the Hampshire Avon sites use, on average,  $0.07 \pm 0.01 \text{ mg L}^{-1}$   $\text{Cl}_2$  to form THM4 – a greater than 4000 % increase of  $\text{Cl}_2$  usage to form THM4 at Conwy sites compared to Hampshire Avon sites. There does not appear to be any current or past literature outlining such a large variation between the THM4 concentrations formed from two sites of contrasting nutrients.

Figure 6.16 shows regression analysis between NPOC and DON concentrations in both Hampshire Avon and Conwy catchments combined. The relationship between NPOC and DON is an important factor in determining whether an increase in NPOC results in an increase in DON – whether sources of DOM to the water always contain similar ratios of NPOC to DON. These data include site 19, a site which has a STW effluent source in its catchment and therefore is more likely to contain much higher concentrations of organic matter when compared to other sites (Yates and Johnes, 2013). The regression analysis shows a strong positive relationship which is backed up by a strong Pearson's correlation. The relationship between NPOC and DON was further tested for effect size using Cohen's *d*, which an effect size of 1.4, i.e., 92% of the NPOC data points are greater than the mean of the DON data points, presenting an 84% chance that a data point picked at random from the NPOC dataset will be higher than a data point picked from the DON dataset (Magnusson, 2014).

This suggests that organic matter sources to these catchments are confined to a mean ratio of 2.79:0.07 showing that, on average, for every 2.79 ( $\pm 1.10$ ) mg L<sup>-1</sup> NPOC in a water sample from Hampshire Avon and the Conwy catchment, there is, on average, a concentration of 0.07 ( $\pm 0.01$ ) mg L<sup>-1</sup> DON, including site 19. Despite a decline of over 50% when site 19 data is removed, the regression still shows a positive trend, where, as the concentration of NPOC increases, so does the concentration of DON, thus showing that there are other forces driving the relationship rather than the STW, or that the same force is driving the relationship but at a lower magnitude. Site 19 seems to play a major role in the majority of the relationships examined in the present study.

This reduction in percentage is to be expected, as NPOC and DON both enter the water as NOM, becoming DOM when dissolved. DOM will typically include nitrogenous and carbonaceous compounds (Leenheer and Croué, 2003) and therefore there will always be a slight positive relationship between the two groups. The mean NPOC/DON ratio at the Hampshire Avon catchment is 7.78 ( $\pm 6.35$ ):1.09 ( $\pm 2.93$ ) inclusive of site 19, and 6.48 ( $\pm 0.42$ ): 0.42 ( $\pm 0.29$ ) exclusive of site 19. Therefore, the mean data shows that, the NPOC to DON ratio is 7.14 mg L<sup>-1</sup> to 1 mg L<sup>-1</sup> inclusive of site 19, and 15.43 mg L<sup>-1</sup> to 1mg L<sup>-1</sup> DON exclusive of site 19.

This data therefore suggests that, as NPOC concentrations increase, so will DON concentrations, and thus, a water with a high NPOC will be likely to form a higher concentration of N-DBPs than a lower NPOC water.

Relationships were also found between TN and DIN, and NPOC, however the positive relationship between DON and NPOC was the strongest (Figure 6.17).

Further relationships between TN, DIN and DON concentration and the concentration of Cl<sub>2</sub> used in non-THM4 forming activities were explored (Figure 6.8), and each nitrogenous group was found to form a positive relationship with the Cl<sub>2</sub> concentration used in non-THM4 forming disinfection activities. The regression analysis shows that DON concentration correlated best with the concentration of chlorine used for non-THM4 forming activities, thus suggesting that DON is the nitrogen group most likely to be contributing to the use of chlorine on non-THM4 forming activities. Although the R<sup>2</sup> value of 0.475 is relatively low, there is still a clear relationship between the two parameters, suggesting that as the concentration of

TN in a sample from the Hampshire Avon and River Conwy catchments increase, so does the concentration of  $\text{Cl}_2$  used for non-THM4 forming activities. Whilst it was not possible to determine where and what concentration of chlorine had been used, the data suggests that the nitrogen was reacting with the chlorine added to the sample and not being used as a food source for bacteria as the bacteria would be inactivated upon contact with the chlorine. It is, however, possible that any ammonium present (i.e. encompassed under the TN and DIN groups) could be binding to the chlorine and forming an organic chloramine, which would account for the relationship. This has been shown in a study by Lee and Westerhoff, 2009, which shows that, as chlorine is added to NOM containing water, organic chloramines were detected after just 10 minutes contact time, and the formation of organic chloramines increased as the DON concentration decreased. Interestingly though, the regression analysis between DON and the total  $\text{Cl}_2$  used for non-THM4 forming activities shows the highest  $R^2$  at 0.475, compared to the  $R^2$  of 0.3347 for TN and 0.2367 for DIN. The fact that DON (containing no inorganic nitrogen species such as ammonium) has the strongest relationship suggests, however, that the nitrogen is not binding to the chlorine to form chloramines. A different titration method may be more suitable to account for chloramines, which could be included as further work.

Each of the relationships appear to be driven by just one point, which is site 19 in the Hampshire Avon. Overall, these relationships suggest that, as TN, DON, and DIN concentrations increase, so does the concentration of  $\text{Cl}_2$  used in non-THM4 forming disinfection activities. This could be either due to a reaction taking place between the chlorine and the nitrogen compounds, or the nitrogen could be providing a source of nutrition for bacterial growth, thus requiring more chlorine to inactivate the bacteria. However, Demoling *et al.*, (2007) found that increasing the nitrogen concentration in soil samples tended to decrease bacterial growth rates, although a freshwater equivalent of this experiment could not be found. Interestingly, there are medium to strong positive relationships between these three inorganic nitrogen species and the concentration of chlorine used in non-THM4 forming disinfection activities, suggesting that DIN is being targeted by the chlorine compounds. Whilst chlorine is a non-specific reactant, it does react faster with some DOM than others in these samples. Inorganic chloramines are common in waters that contain reduced nitrogen

in the form of ammonia, which can later be oxidised by chlorine to nitrogen gas, nitrite and nitrate, (Li and Blatchley, 2009) which can potentially form N-DBPs.

Figure 6.18 further explores the findings from above, by displaying a regression analysis between the 3 inorganic nitrogen ions and the concentration of Cl<sub>2</sub> used in non-THM4 forming disinfection activities. The 3 relationships are positive, with ammonium showing the highest R<sup>2</sup> fit at 0.4927, although nitrite is also very similar at R<sup>2</sup>=0.4862, with nitrate with the lowest fit of R<sup>2</sup>= 0.2182. A Pearson's correlation of these 3 relationships mirrors these regression findings, with each of the relationships significant at the p=<0.01 level. Interestingly, the Cohen's *d* effect size shows that the relationship between nitrite is much more noticeable than nitrate and ammonium. These relationships show that the 3 inorganic nitrogen species are responsible for some of the increase of concentration of Cl<sub>2</sub> used in non-THM4 forming activities. As mentioned previously, these inorganic nitrogen ions may be responsible for the formation of organic chloramines (Lee and Westerhoff, 2009) but may also be responsible for the formation of N-DBPs, which unfortunately cannot be detected in the current work.

For the total Cl<sub>2</sub> used over the 7 day incubation period and the total concentration of Cl<sub>2</sub> used to form THM4 data, a clear relationship can be seen from the regression analysis (Figure 6.19), displaying two distinct groups of data. This data suggests that, as the total Cl<sub>2</sub> concentration used increases, so does the concentration of Cl<sub>2</sub> used to form THM4, suggesting that THM4 formation is a major driver for chlorine use in both of these catchments, and also suggesting that THM4 is the most common DBP formed in the chlorination of these waters, by mass, although it is important to remember that other DBPs with higher toxicities than THM4 could also be forming. The distinct data groups represent the two catchments, with the Hampshire Avon sites using a much lower concentration of Cl<sub>2</sub> than the River Conwy sites.

A Pearson's correlation was run between the concentration of chlorine used to form THM4 data and the total chlorine concentration used in the 7 days incubation period. A positive correlation ( $r=0.803$ ) was reflected in a regression analysis, which presented an R<sup>2</sup> value of 0.645 to a positive linear gradient of  $y=0.8059x + 2.2907$  (Figure 6.19). This shows that, as the chlorine used to form THM4 increases, so does the total concentration of chlorine used during the 7 day incubation period. The R<sup>2</sup>

value shows that the model represents 64.50% of the variability of the response data around the mean.

Figure 6.20 displays the relationship between the total concentration of  $\text{Cl}_2$  used vs. the NPOC concentration of the pre-diluted (*i.e.* raw) sample. Regression analysis shows that there is no significant relationship between the two parameters. Whilst it is known that THM4 is formed from carbonaceous precursors (Liang and Singer, 2003), and therefore will be encompassed in the NPOC data category, it would appear that THM4 formation is not a major driver of chlorine use between these two catchments. However, this data does not explore the relationships between the actual concentration of NPOC in the chlorinated sample, as these samples were diluted to  $1 \text{ mg L}^{-1}$ , it does, however, explore the total NPOC concentration of the sample prior to dilution. The lack of a relationship between these data, therefore, show that it is not simply the NPOC concentration that determines the concentration of chlorine used in the disinfection of freshwater, and that there are many other factors playing a role in chlorine demand. This is backed up by the lack of a significant Spearman's correlation between the two data points.

The concentration of chlorine used vs. the total concentration of NPOC in the sample was analysed by a simple regression (Figure 6.20). The graph shows a near neutral relationship, with an  $R^2$  value of 0.026, ranging from NPOC concentrations of  $5.02 - 31.21 \text{ mg L}^{-1}$  and total chlorine concentrations used ranging from  $1.35 - 5.00 \text{ mg L}^{-1}$ . This shows that, with both the Hampshire Avon and the Conwy catchments combined, there does not appear to be a visible relationship between NPOC and chlorine use. This suggests that THM4 and other carbonaceous DBPs are not comprising a significant proportion of the total concentration of chlorine used, and hence, N-DBP formation may be prevalent, or the water may be ridden with bacteria.

The medium to strong positive Pearson's correlations found between DIN and the concentration of chlorine used on non-THM4 DBPs and other disinfection activities found at both the Hampshire Avon and the River Conwy, individually and combined, suggest that chlorine acts upon DIN, thus showing that the chlorine is preferentially reacting with DIN and therefore there is less chlorine available to react with other species (e.g. DON).

Schreiber and Mitch (2007) proposed that in the presence of nitrite, if breakpoint chlorination is conducted to achieve a significant free chlorine residual (as used here), nitrosamines and nitramines will form through a reaction with nitrite and hypochlorite. It seems likely then that further halogenation of these nitrosamines and nitramines would lead to halonitrosamines, an N-DBP (Lakhian, 2016) and halonitramines. Therefore, it is likely that these could form in the present waters studied.

## 6.6 Conclusions

Strong relationships between NPOC and chlorine used on THM4 and also the chlorine used to form non-THM4, at the Hampshire Avon catchment, suggests that NPOC comprises of a group of compounds that are precursors for the formation of both THM4 and other DBPs (undetectable in this work), as is already known in the scientific community (Chow, Tanji and Gao, 2003). However, these relationships are not present at the Conwy catchment, where as the NPOC concentration increased, the total chlorine use decreased. When the Hampshire Avon and Conwy data was combined, there was a very weak positive relationship between NPOC and total chlorine used, but a medium strength positive relationship between chlorine used to form non-THM4 and NPOC concentration, confirming that the Hampshire Avon and Conwy catchments vary significantly in their concentration and character of carbonaceous organic matter.

Nitrogenous organic matter at the Hampshire Avon was found to increase as NPOC concentration increased, shown by a strong positive regression. The removal of site 19 from this dataset seriously reduced the strength of the relationship. However, the concentration of chlorine used to form THM4, and also to form non-THM4, was found to increase as the DON concentration increased at the Hampshire Avon, both in medium to strong positive regressions. At the Conwy catchments, DON showed a very weak positive relationship with the total concentration of chlorine used in disinfecting the water, suggesting that DON was not reacting with the chlorine to form detectable levels of N-DBPs at this catchment. Interestingly though, nitrate, TN and DIN datasets were found to form a more positive regression with concentration of chlorine used, however, these are still weak to medium strength relationships. When the data of Hampshire Avon and the Conwy Catchment were combined, the regression between the chlorine used to form non-THM4 compounds and TN,

DON and DIN concentrations showed a low to medium strength relationship, suggesting that the organic and inorganic nitrogen data from the Hampshire Avon is driving this relationship.

Overall, the findings from this work suggest that the Hampshire Avon and the Conwy catchments differ vastly in their character and concentration of organic matter, alongside the concentration of chlorine required to disinfect these waters, and the concentration of chlorine that has been used to form THM4 and non-THM4. Further studies could involve collecting bacterial data from each site, to determine whether specific catchments produced higher bacteria concentrations than others, and to determine whether these concentrations are primarily responsible for the use of chlorine, with only the most reactive organic matter forming DBPs. A further experimentation between chlorine use and the organic matter surrogates could be conducted, disinfecting varying concentrations of organic matter with different concentrations of chlorine, with an aim to predict chlorine use and DBP formation for future drinking water abstraction locations. Whilst this has been achieved before, there appears to be no data relating to the catchments studied in this thesis. The relationship between chlorination and chloramination, and their disinfection efficiencies, has been explored and is available in Appendix 4.

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# Chapter 7

## Conclusions

## 7.1 Conclusions

### 7.1.1 Main Findings

The work presented here suggests the following novel contributions to the wider scientific community, stakeholders and end users involved in the treatment of potable water:

- The creation of a freely available, standardised and simple method for catchment delineation using ArcGIS software, which is intended for publication in an open access scientific journal, namely Remote Sensing of Environment.
- The area of acid grassland, and also loamey and clayey floodplain soils with naturally high groundwater, correlates positively with THM4. Therefore land managers should be advised that water draining land containing these vegetation and soil classes could be likely to have elevated THM4 concentrations, should the water be destined for a water treatment works or other industries that chlorinate water. An increase in the area of NSRI classed ‘slightly acid loamy soils’ correlates strongly with  $\text{CHBr}_2\text{Cl}$  in laboratory experiments, suggesting that this THM4 compound is more likely to form in water draining from such soil. Therefore, land managers should expect an increase of this THM4 concentration in the waters draining from this soil type, and necessary precautions should be implemented should the water be destined for human consumption.
- It is well documented that coniferous woodland contributes to  $\text{CHCl}_3$  concentration in water draining from it and that many drinking water reservoirs are surrounded by large conifer plantations. However, this study suggests that the removal of these trees and change to a different land use type will present other issues, which may be harder to control. Most notably, the act of deforestation can cause huge releases of DOM from the catchment into the reservoir, and then the land use that takes place after deforestation (such as ruminant grazing, for example), may cause other, less well identified and studied compounds, when the organic matter reacts with chlorine in a water treatment works..
- 60% of the NPOC from 3 diverse catchments was found to be composed of hydrophilic neutral compounds. These substances are notoriously difficult to remove at drinking water treatment works, and passing through to the disinfection stage of treatment and being available to react with chlorine to form DBPs. Thus,

it is recommended that future research is focussed on removal of this fraction to help prevent them forming. Recent studies have examined the removal of hydrophilic fractions using catalysts and ozonation, which has been found to increase the removal of hydrophilic components (Wang *et al.*, 2019)

### 7.1.2. Discussion of findings

All freshwater globally contains DOM, which can prove problematic in sources that are used for human consumption. DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have attached amide, carboxyl, hydroxyl, ketone and various minor functional groups and thus can range in molecular weight from a few hundred to 100,000 daltons (Leenheer and Croué, 2003). Organic matter is typically removed from drinking waters, as it is reported to be responsible for unsavoury tastes, odours and colours. At a WTW, the first step in improving the quality of the water for consumption is the removal of this organic matter, however, due to the vastly differing nature of organic matter compound characters, it is impossible to successfully remove all of it. The failure to remove all of the organic matter from the water prior to the addition of a halogen/halogens to disinfect the water, can result in the formation of compounds that are hazardous to human health, collectively known as DBPs.

As freshwater originates as rain, and passes through the environment, its chemistry should remain relatively similar across a catchment until it reaches land. However, the concentration and character of DOM collected from different catchments in a watershed can differ greatly. Studies by Mattsson *et al.*, (2005) suggest that sources of DOM to streams are usually dominated by inputs from soils and terrestrial leaf litters, with the concentrations and fluxes of this DOM being affected by soil properties, hydrological conditions, biotic factors and land use of the catchment, and thus, the interactions that this rainwater has with the vegetation, soil and geology in the catchment, drive the concentration and composition of organic matter. Indeed, the character and composition of organic matter found in freshwaters has been linked to the management and land use of the catchment from which the water drains (Mattsson *et al.*, 2005 and Ritson *et al.*, 2014). Upon this understanding, the experimental chapters in this thesis have

each used different approaches to link the character of organic matter in freshwaters draining 3 catchments (located across a nutrient gradient) to the formation of DBPs.

- a) The Hampshire Avon, 19 sites (draining two rivers), located in southern UK.
- b) The Conwy Catchment, 27 sites, located in North Wales, UK.
- c) Various Scottish sites located within southern Scotland, UK.

The differing nutrient concentrations in these catchments (from the oligotrophic Conwy to the eutrophic Hampshire Avon) were selected to determine whether DBPs were more, or less, likely to form from a nutrient poor or a nutrient rich catchment. A higher nutrient concentration encompasses more carbon and nitrogen species, and thus is practicable to assume that a nutrient rich water is likely to form a higher concentration of DBPs, and conversely, a lower nutrient concentration is therefore likely to form a lower concentration of DBPs. Scottish sampling sites were then selected due to their individual similarities to sub-catchments of the Conwy and Hampshire Avon catchments, to determine whether any relationships found were likely site specific or whether they could be extrapolated to other catchments with similar characters.

A geographical information system (GIS) approach was utilised, which provided data on the area of each specific land use in each catchment. THM4 (a group of DBPs – see Chapter 5a) data was then correlated with the area of each land use type, and chlorine use data, to determine if a specific land use practice was likely to be responsible for a significant increase in the formation of THM4.

Furthermore, links between the catchment and the character of organic matter were then explored to determine whether a nutrient rich or nutrient poor catchment is likely to be responsible for formation of specific DBPs and/or their precursors, or whether a specific land use type would generate more precursors for these DBPs.

The overall aim of this project was to provide informed advice to water abstraction companies, to help target either the least problematic new water abstraction locations, and/or to provide information on potentially problematic abstraction locations currently used.

Chapters 1 and 2 introduce the current organic matter characterisation techniques in the scientific community, the history and current use of halogens to disinfect drinking water, and finally, the catchments studied in this thesis. Whilst the importance of halogenation of water for human consumption is now globally recognised (after the chlorination of the River Thames water helped reduce cholera outbreaks in 1849 (Snow, 1849)), the reaction with these halogens was found to be causing carcinogens in the drinking water (Rook, 1974), and over the next four and a half decades, over 600 potentially carcinogenic, mutagenetic or cytotoxic DBPs have been identified (Richardson *et al.*, 2007), formed from compounds in the water, including dissolved organic matter (DOM).

Therefore, the characterisation of this DOM can help to identify individual known compounds which act as precursors for these DBPs. These data, some of which is generated from chlorination and chloramination, can be coupled with land use data, to help to provide information to water abstraction companies for potential new abstraction locations, or to help current water treatment works to target currently untargeted compounds.

The first step in order to address these targets, once study sites were determined, was to collect information relating to the land use in the catchment, and a GIS approach was deemed most suitable due to the high level of detail achievable, the ability to create visual aids for stakeholders and end users, the low costs involved in data generation and the quality of data produced. Information was generated on the vegetation cover, the soil type and the bedrock of each catchment, encompassing 19 vegetation categories, over 15 bedrock categories and 17 soil categories. The method used to determine these datasets is outlined in Chapter 3a, whilst the practical application of the method generated the data used in Chapter 3b. The main findings of this chapter were that, with data from the Hampshire Avon and Conwy sites combined, chlorination formed THM4 correlated with the area of loamy and clayey floodplain soils with naturally high groundwater, arable and horticulture, acid grassland, heather and inland rock land use types in each catchment. Whilst the relationships were found to be both positive and negative, ranging from  $f=-0.686$  (Spearman's) for loamy and clayey floodplain soils with naturally high groundwater versus STHM4 concentration at Hampshire Avon and Conwy catchments combined, to  $f=0.454$  when acid grassland catchment area was combined with STHM4

concentration. Each of these relationships was found to have a Cohen's *d* effect of between 0.67 and 0.94, showing that the effect of these relationships is classed as between medium large to between large and very large. This suggests that water draining from land cover classed as acid grassland, and soil types classed as loamy and clayey floodplain soils with naturally high groundwater would form a greater THM4 concentration when chlorinated (when normalised for DOC), and thus, it is recommended that future water abstraction locations are situated in catchments that drain as small an area of these soil and vegetation types as possible to help meet the THM4 regulations. Negative relationships were also found, with the strongest relationship between  $\text{CHCl}_3$  formed from chlorination and soils classed as loamy and clayey floodplain soils with a naturally high groundwater, and also, chlorination formed  $\text{CHBr}_2\text{Cl}$  with slowly permeable wet very acid upland soils with a peaty surface. Both of these negative relationships had a strong Cohen's effect, and both of the relationships were generated from data collected from the Hampshire Avon and the Conwy catchment datasets combined. Thus, it could be recommended that future water abstraction locations be situated in catchments draining high areas of these soil types to help produce water lower in  $\text{CHCl}_3$  and  $\text{CHBr}_2\text{Cl}$ .

THM4 compounds formed from both chlorination and chloramination treatments were also found to correlate with land use types at the Hampshire Avon and Conwy sites both combined and separate, with some correlations (for example chlorination formed  $\text{CHCl}_3$  from coniferous woodland) being higher than the correlation coefficients of the THM compounds combined (i.e. THM4), likely due to the strength of the relationship between the individual THM compound driving the relationship between THM4 compounds combined. The highest correlation coefficient was found between chlorination formed  $\text{CHBr}_2\text{Cl}$  and freely draining slightly acid loamy soils at  $f=0.843$ , with a Cohen's *d* effect size of 1.71, which is classed as 'huge'. It is therefore recommended that any future abstraction locations are carefully selected to include as little area of this soil type as possible/help keep both the  $\text{CHBr}_2\text{Cl}$  concentration, and the THM4 concentration in final water from this new abstraction location at a minimum. This soil class does not have the highest effect size found in this chapter though; chlorination formed  $\text{CHCl}_3$  at the Conwy catchment was found to correlate with the area of coniferous woodland at  $f=0.530$ , but with a Cohen's *d* score

of 5.36, which shows that the relationship is easily visible with the naked eye, when examining the dataset, as opposed to the requirement of statistical analysis.

Whilst the relationship between THM4 and coniferous woodland has been explored previously (see Gough *et al.*, 2016 and Albers *et al.*, 2010) interesting revelations in the scientific community suggest that microbial or fungal activity that takes place within the soil structure can be responsible for the formation of trichloromethane (chloroform), the most prevalent THM compound in this study (Haselmann *et al.*, 2000, and Albers, Laiter and Jacobsen, 2008), which brings to light another issue. Coniferous trees such as Sitka spruce (*Picea sitchensis*) hybrid larches (*Larix eurolepis*), Scots pine (*Pinus sylvestris*), Douglas fir (*Pseudotsuga menziesii*) and Corsican pine (*Pinus nigra*) are planted for their economic value – 10.7 million green tonnes (i.e. not dry final product weight) of softwood was harvested in the UK in 2016 (Forestry Commission, 2017). These coniferous trees are planted in their thousands in the catchments of reservoirs used for drinking water purposes, and thus these plantations could be increasing the concentration of chloroform in the drinking water before it is disinfected at the WTW. Furthermore, the continuous ground disturbance caused by the harvesting of this wood (which is designed to be continuous and sustainable to ensure a steady supply of timber over a number of years rather than once every approximately 30 years when the timber has grown to size) will increase the DOM loadings into the reservoir, also potentially impacting the DBP formation at the WTW. Sweeny *et al.*, 2004, found that riparian deforestation reduces the total amount of stream habitat and compromises in-stream processing of pollutants, which can then reach the WTW. Thus, it would seem a sensible decision to cease the planting and harvesting of these trees, however, it is not that simple. Firstly, the removal of these trees, and to return the land to a non-forestry purpose would create great DOM loadings into the reservoirs, but furthermore, alternative land use practices could present further problems for the WTW. For example, if the land was to change to agricultural use, nitrogen loadings to the reservoir would likely increase, which could act as precursors for N-DBPs. If the land was developed upon, the pollution from the building and then human activity would also increase nitrogen concentrations, and heavy metal contamination in the reservoir. It would therefore be practicable to focus research on eliminating the formation of these DBPs from the known causes rather than changing the land use in an attempt to reduce them, and to

optimise treatments by targeting the removal of compounds which are associated with water draining coniferous woodland, which can be linked to the increased formation of THM4.

Chapter 4 explores the differing characters of organic matter at the three catchments, concluding that the characters vastly differ, not only between catchments, but within catchments too, depending upon land use. Whilst the data from these studies show that the relationships discovered suggest that the research could be applicable to a wider range of catchments, it also suggests that the ability to characterise the organic matter from one catchment and then extrapolate this to other catchments with similar physical properties is not necessarily feasible due to the vast number of variations in DOM concentration and character, and the catchment management practices. However, extrapolation of data from one peaty catchment to another in a similar geographical location may be possible if the DOM is of a similar character too, although this would need to be confirmed by further work.

Some relationships between the organic matter concentration and the area of specific land use types were found. For example, as the area of land classed as urban increases, comprising dense urban areas such as towns and city centres, where there is typically little vegetation, but also including areas such as dock sides, car parks and industrial estates (CEH, 2011), so does the concentration of TN recorded. High concentrations of organic and inorganic nitrogen would be expected to increase in urban land use areas due to the human related activities that take place in the catchment (e.g. road runoff, STW effluent, garden fertiliser *etc.*). However, this increase was only noticeable in the Scottish catchments and not in the Conwy or Hampshire Avon catchments. Interestingly, the total area of urban land use in the Scottish catchments totals 88,491 M<sup>2</sup>, compared to the total urban land use of 1,539,620 M<sup>2</sup> in the Conwy catchment and 3,960,960 M<sup>2</sup> in the Hampshire Avon catchment, which did not correlate with TN concentration, suggesting that the Scottish sites contained a point source of TN which is very different to sources of TN to the Conwy and Hampshire Avon catchments, or possibly that this source of TN in Scottish catchments is also found in the Conwy and Hampshire Avon catchments, but the effect is 'diluted' by a much greater land use area and variety. These point sources are likely septic tank effluent systems, as the majority of the Scottish catchments are located in very rural surroundings and connection to a sewerage system is highly

unlikely. Other sources of precursors could be inputs from the farming and/or fishing industry which is widespread in these Scottish catchments.

A comparison of the 3 different catchments did not present an organic matter parameter that was found to correlate with land use at all 3 of the catchment locations, suggesting that land use type and area data can be used to identify problematic sub-catchments in a drinking water abstraction location, but this may not be suitable to extrapolate to a nationwide scale. This is likely due to the multitude of factors that can influence the DOM character draining from a catchment (i.e. not just vegetation, soil type and underlying geology), as well as temporal and climatic differences between the catchments (for example, a lowland catchment may be warmer and less windy than a highland catchment and therefore a much more suitable habitat for insects and plants which release exudates, known to contribute to DOM). Ultimately, it is the fraction of DOM which represents THM precursors which is the key. However, this is very difficult to analyse separately from total DOM, but should be focussed on in any further studies.

Chapters 5a and 5b study the formation of C-DBPs and N-DBPs respectively, using water samples collected from the Hampshire Avon and Conwy catchments.

Different disinfection procedures can form different DBPs and can react with the organic matter in different ways, so work was carried out to analyse how different disinfectants can affect the formation of DBPs and how the available chlorine is utilised in the sample. The formation of C-DBPs under treatments of chlorination and chloramination at both the Hampshire Avon and Conwy catchments confirms that different organic matters form different concentrations of THM<sub>4</sub>, where some catchments form high concentrations of THM<sub>4</sub> from low concentrations of organic matter, and *vice versa* (explored in Table 5a.6, Chapter 5). Furthermore, when samples are treated with an identical dose of chloramine, the THM<sub>4</sub> concentration formed drops considerably, supporting work by Bougeard *et al.*, 2010, Hua and Reckhow, 2007 and Goslan *et al.*, 2009. Whilst this present work has been unable to determine the presence and concentration of any DBP compounds other than THM<sub>4</sub>, it is practicable to assume that the free chlorine (in the chloramine) that is not reacting with organic matter to form THM<sub>4</sub>, is freely available to react with the organic matter to form other DBPs in the reported suite of 600+ other DBPs (Krasner *et al.*, 2006).

Contained within this suite of non-THM4 DBPs are N-DBPs, formed from the halogenation of nitrogenous compounds, as opposed to carbonaceous compounds.

Chapter 5b focuses on the formation of these N-DBPs and outlines experimental methods for the detection of a suite of these N-DBP compounds, alongside a novel approach to the detection of a specific N-DBP using cell growth cycles. A GC-MS method adapted from the detection of THM4 showed potential for the detection of a suite of 7 N-DBP compounds, with limits of detection ranging from 1 to 20  $\mu\text{g L}^{-1}$ . Whilst some N-DBP compound concentrations have been detected at lower concentrations than these in previous studies (for example Luo *et al.*, 2014), concentration of samples can result in the equivalent detection limits. Despite the promising initial findings, financial and time constraints resulted in the inability to pursue this detection method. Therefore, a different approach was taken to detect the presence of only one N-DBP compound, dibromoacetonitrile. Haloacetonitriles, a group of N-DBPs, have been found to cause DNA damage in fission yeast by inducing sister chromatid exchange, and DNA breaks (Caspari *et al.*, 2017). It is important to note that this method takes approximately 48 hours and does require access to biologically focussed facilities. In this study, one of these haloacetonitrile compounds, dibromoacetonitrile, was found induce DNA breaks at concentrations of between 4 and 12  $\mu\text{M}$  (0.8 – 2.39  $\mu\text{g mL}^{-1}$ ). This method, therefore, can be utilised to detect the presence and concentration of dibromoacetonitrile in sample waters, which can help WTWs to adapt removal practices to target this compound. Finally, the measurement of individual amino acids provides information on the propensity of N-DBPs, many of which are known to form from amino acids (Bond *et al.*, 2012). The three most abundant amino acids in Conwy catchment samples were aspartic acid, phenylalanine and tyrosine, all of which have been reported to form the N-DBP dichloroacetamide under chlorination (Bond *et al.*, 2012). WTWs can therefore determine the concentration of amino acid precursors in the water to be treated, to predict N-DBP concentrations in final water, and to put further research into precursor removal.

Due to the relatively low molecular weight of many nitrogenous compounds, their removal from water is more complex, when compared to carbonaceous compounds. In an attempt to understand the nature of the DON, water samples were fractionated to separate the organic matter by charge and molecular weight. Three catchments were

analysed, and the hydrophilic neutrals fraction was found to contain the highest percentage of DON (>60%) for each catchment. The resin used to retain and then desorb the DON into these fractions was a DAX-8 absorbent resin, which has moderate polarity for compounds up to 150,000 MW, and is commonly used for the adsorption of fulvic and humic acids. The DAX-8 resin is responsible for the fractionation of 3 different groups – hydrophilic acids, hydrophilic bases and hydrophilic neutrals, and it is the hydrophilic neutrals which are known to be difficult to remove by conventional water treatment mechanisms due to their low MW. Carroll *et al.*, (2000) state that aluminium and iron based coagulants preferentially remove hydrophobic rather than hydrophilic, charged rather than neutral, and larger sized rather than smaller sized substances. This selectivity thus allows hydrophilic neutrals to be capable of passing through the removal practices at the WTW and remaining in the water when it is disinfected, potentially forming DBPs (Carroll *et al.*, 2000). It would therefore be recommended that if WTWs are continuously struggling to produce a final water below the regulatory limit for THM4, instead of switching to chloramination, further efforts should be focussed on the identification and removal of these low MW hydrophilic neutral compounds to prevent the potential formation of N-DBPs. The development of selective sorption technology is required to remove these polar compounds from aqueous solutions.

Whilst N-DBP detection was not carried out due to timescale and financial constraints, a mass balance equation was used to determine how much chlorine was used upon the formation of the DBPs that could be detected (THM4) and how much chlorine remained in the sample after an excess of contact time and free chlorine (7 days, 5 mg L<sup>-1</sup> Cl<sub>2</sub> per 1 mg L<sup>-1</sup> DOC), and therefore, the concentration of chlorine used to disinfect but not used in the formation of THM4 was calculated, which is likely to have been used to form other C- and also N-DBPs, and this was explored in Chapter 6. The prediction and detection of the character of organic matter, at the catchment level, can provide an insight into the species of DBPs that are likely to form from disinfecting the waters in which they are contained, however, these concentrations would need to be correlated with the area of land use in the catchment, as shown in Chapter 3.

The concentration of free chlorine used in standardised disinfection reactions were calculated, to determine whether the NPOC character was responsible for the

differential use of chlorine. This was achieved by adding 5 mg L<sup>-1</sup> free chlorine to samples containing 1mg L<sup>-1</sup> NPOC, and incubating them at 25 °C for 7 days. The concentration of chlorine remaining was then measured before this chlorine was quenched, and samples were analysed for THM4. The strong relationships detected at the Hampshire Avon between the concentration of NPOC and both the concentration of chlorine used and the concentration of chlorine used in non-THM4 formation suggests that the NPOC in this catchment contains precursors that are responsible for the majority of the chlorine usage for both THM4 and non-THM4 forming compounds. However, in the Conwy catchment, the relationship was the opposite – as the NPOC concentration increased, the total use of chlorine decreased, suggesting that at the Conwy catchment, an increased use of chlorine is not directly related to an increased NPOC concentration, and that there are likely to be specific NPOC compounds in the water that do not necessarily increase in concentration as the overall NPOC concentration increases. Further work would involve the isolation and detection of these precursors, followed by the disinfection of these compounds by chlorination and chloramination, to determine whether the precursor is responsible for THM4 formation or not.

Interestingly though, at the Hampshire Avon, the concentration of chlorine used to form THM4 and non-THM4 compounds increases as the DON concentration increases. This relationship could be skewed, possibly, by an increase in DOM, and thus, although the DON concentration has increased, it is likely that the NPOC concentration will increase with it which could explain the increase in THM4 (formed from carbonaceous compounds) concentration. No positive correlations were found between DON and the total concentration of chlorine used at the Conwy catchment, however, inorganic nitrogen compounds did form weak to medium relationships with the total concentration of chlorine used, suggesting that inorganic nitrogen sources could be responsible for the formation of N-DBPs in waters draining the Conwy catchment. For example, Yang and Cheng (2007) state that nitrate can combine with amines to form nitrosamines (a group of N-DBPs, and nitrosamides, which can cause tumors in humans). When the Hampshire Avon and Conwy catchments data are combined, low to medium strength relationships were found between NPOC and the chlorine used to form non-THM4 compounds, suggesting that other C-DBPs (such as haloacetic acids (HAAs)) could be forming in this water. Further work should aim to

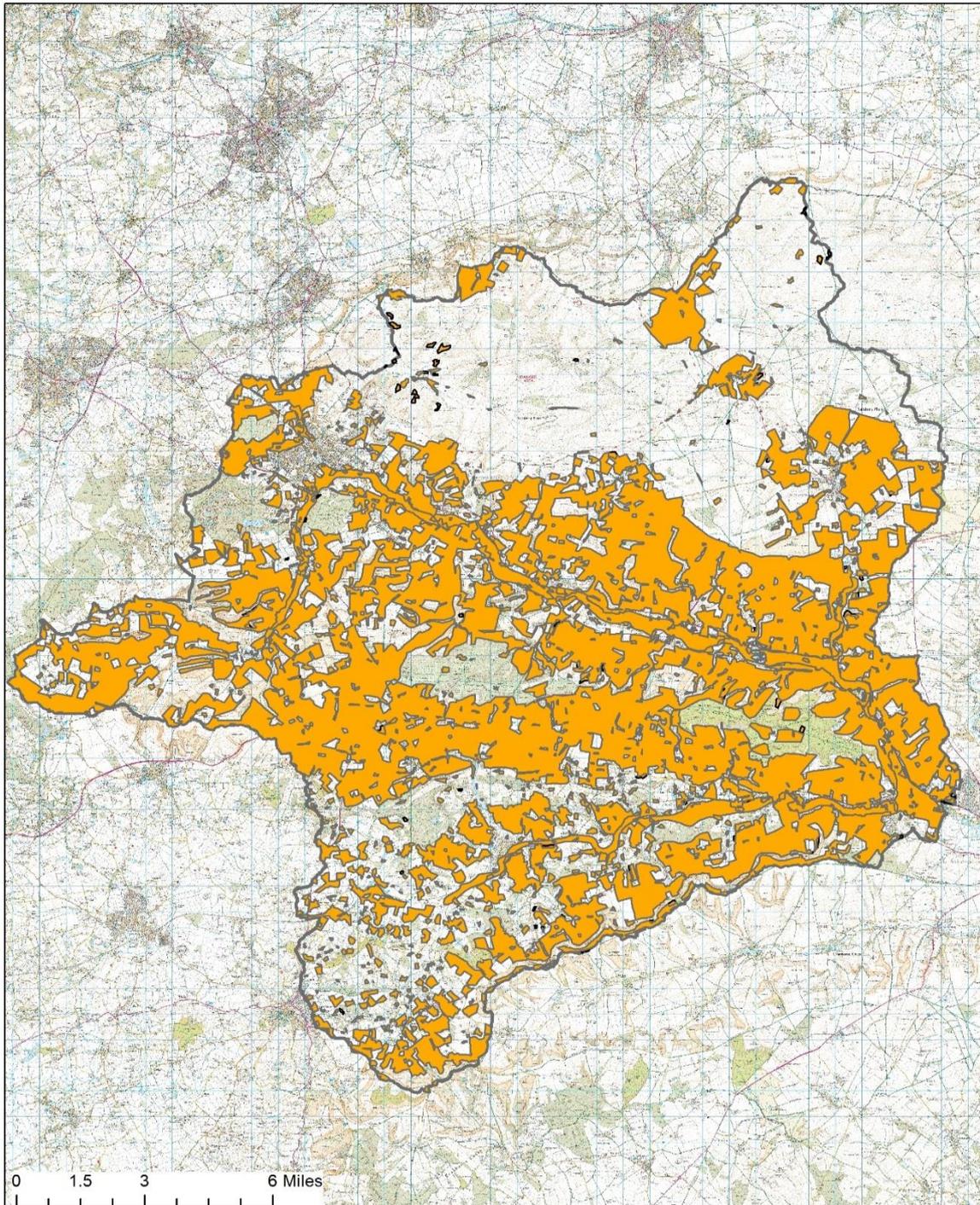
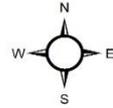
identify these HAAs and other C-DBPs within these catchments, because DBP formation is a competitive process, and to encompass them into a mass balance equation to determine the concentration of chlorine used to form C-DBPs, allowing for a more accurate estimation of

## 7.2 Risk Assessment Maps

In order to display the findings from this project (which involves a large quantity of information) in an easy to understand format, risk assessment maps were devised. These maps outline areas of a catchment that are likely to cause significantly higher concentrations of DBPs than others. Despite the fact that the creation of a nationwide risk assessment map for DBP formation potential was not feasible due to the vastly different nature of DOM and its interactions between chlorine, the creation of catchment specific maps was possible.

The findings from Chapter 3 show that chlorination formed STHM4 correlated positively with one soil type and 4 vegetation classes at medium to strong relationships. These land use classes were therefore highlighted and presented in a catchment map, Figure 7.1. It is clear from the map that all but the most northern reaches of the catchment are dominated by potentially problematic land use types. The majority of the STHM4FP forming land uses are focussed around the two main rivers, the Nadder and the Wylye. This may be due to fewer interactions with the natural environment before the water reaches the streams, but could also be due to increased urban land use near the water bodies. Importantly though, the STHM4 concentrations are not the actual concentrations found in drinking water, and no association between the freshwater data and any drinking water regulations should be made.

## Avon Catchment Risk Assessment Map STHM4 and Land Use

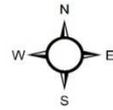


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Figure 7.1: Hampshire Avon risk assessment map. Orange colour signifies land use and soil types found to correlate with STHM4 concentration formation potential at a correlation coefficients of  $f=0.400$  to  $f=0.749$ .

In terms of individual THM4 compounds correlating with land use, only one instance was found, and this was between land classed as Fen, Marsh and Swamp, and the concentration of  $\text{CHCl}_3$ . This land area is very small, and is situated surrounding a dairy hygiene chemical production facility, whose products contain chlorine, and thus it is highly likely that this chlorine is leeching its way into the watercourse at this site, and that the instance of Fen, Marsh and Swamp land use is just coincidental. See Figure 7.2.

# Avon Catchment Risk Assessment Map

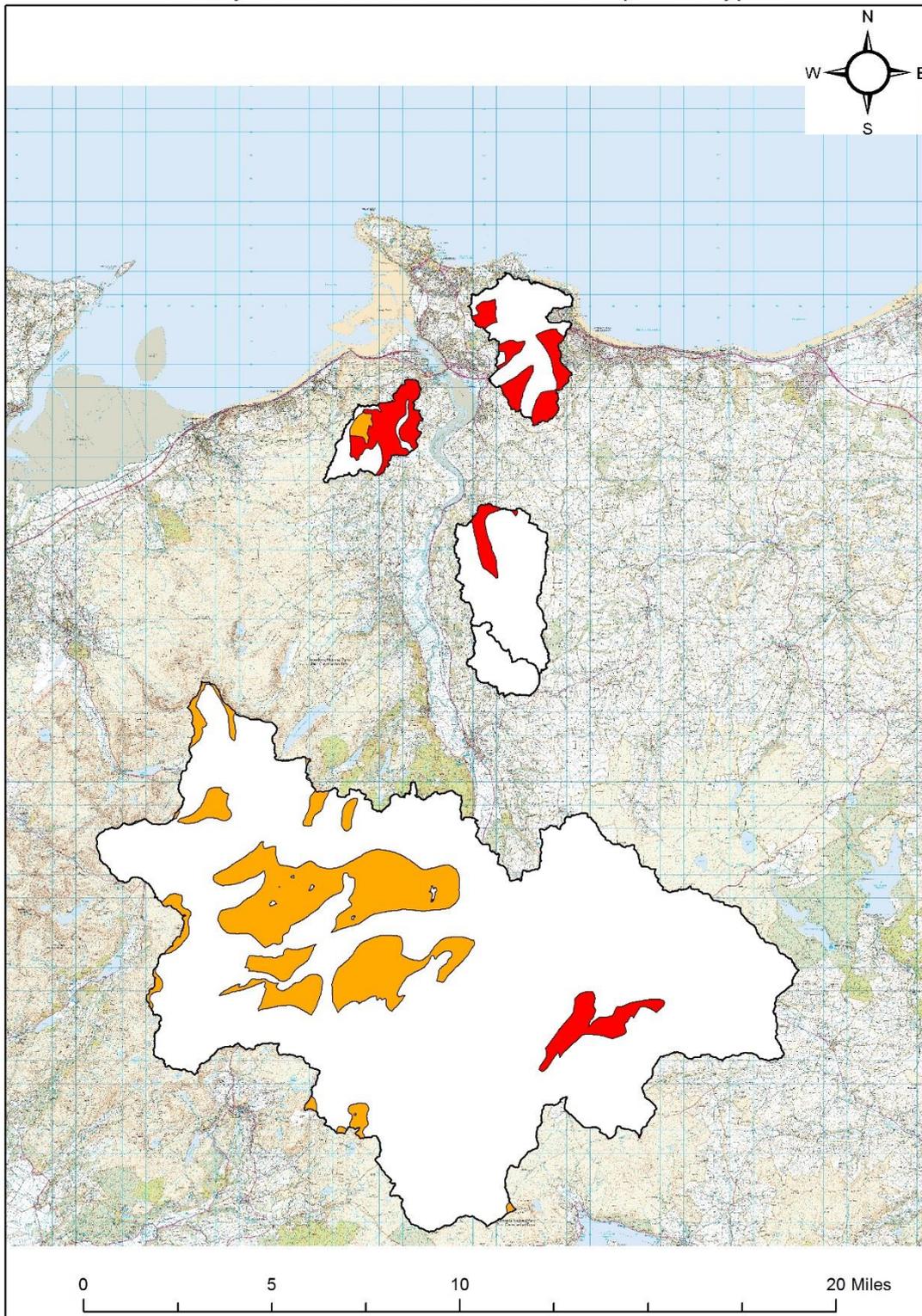


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Figure 7.2: Red colour showing area of Fen, Marsh and Swamp land use that correlates with  $\text{CHCl}_3$  concentration in sample waters in the Hampshire Avon catchment at a coefficient of  $f=0.750$  or higher.

At the Conwy catchment, two THM4 compounds ( $\text{CHBr}_2\text{Cl}$  and  $\text{CHCl}_3$ ) were found to correlate in medium to strong positive correlations with soil types (freely draining slightly acid loamy soils and shallow very acid peaty soils over rock). These soil types dominate the conwy catchment, and therefore, removal of these compounds should be prioritised in catcments containing these land use types for a WTW. See Figure 7.3 for the risk assessment map.

Conwy Catchment Risk Assessment Map - Soil Types



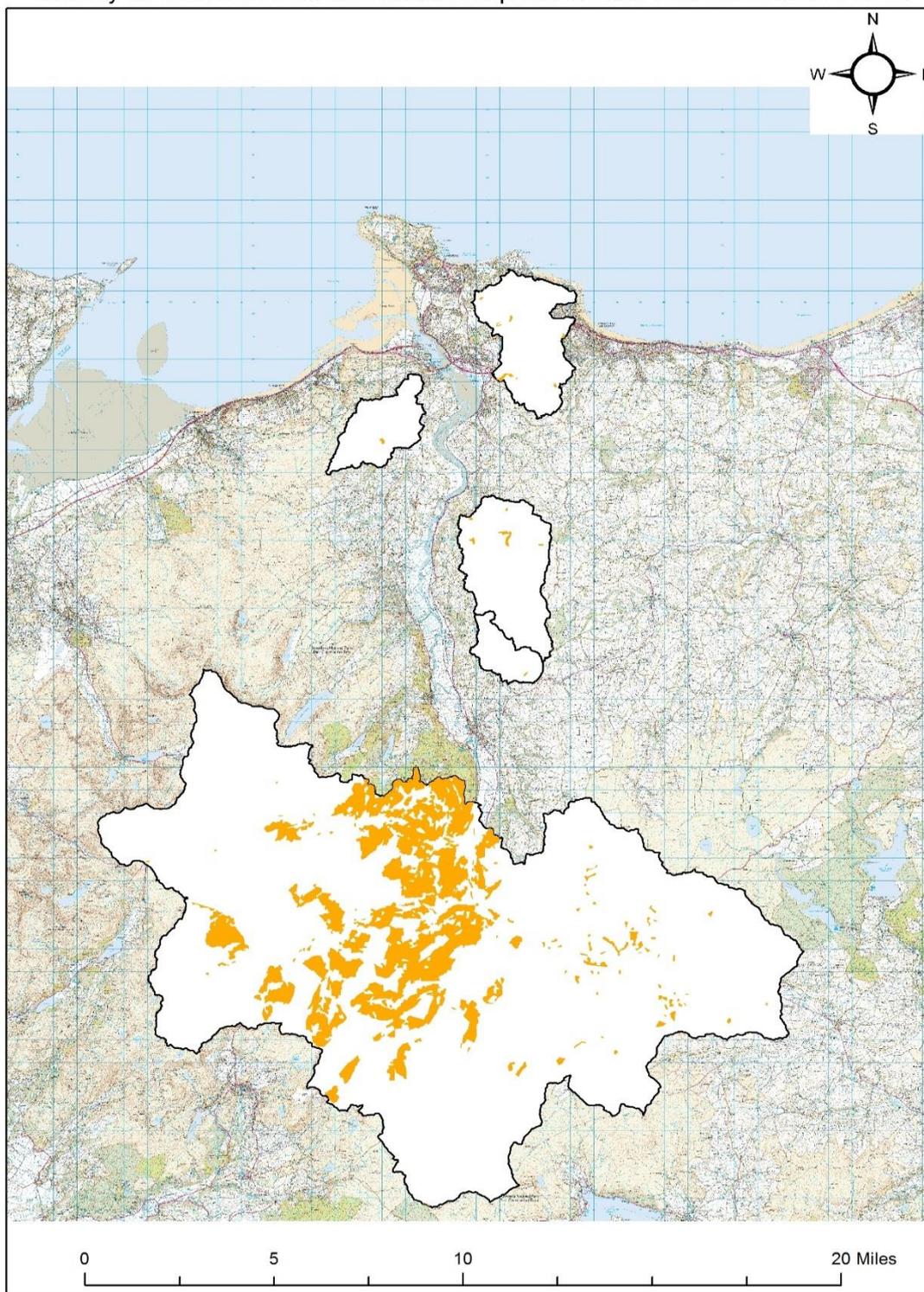
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Figure 7.3: Risk assessment map to show instances of the two soil types found to correlate with an increase in THM4 compounds at the Conwy catchment, where red represents a

correlation coefficient of greater than  $f=0.750$  and orange represents a correlation coefficient of between  $f=0.400$  to  $f=0.749$ .

Possibly the largest cause for concern, however, is the medium to strong relationship found between an increase in  $\text{CHCl}_3$  concentration and coniferous woodland at the Conwy catchment. As previously mentioned, many of the UK water storage reservoirs are surrounded by coniferous woodland, which are highly likely contributing to the  $\text{CHCl}_3$  concentration in the reservoir. Figure 7.4 shows the instances of coniferous woodland in the Conwy catchment, which shows that water abstraction locations from alternative local reservoirs in the catchment is also not advisable.

Conwy Catchment Risk Assessment Map Coniferous Woodland and CHCl<sub>3</sub>



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Figure 7.4: Risk assessment map showing the coniferous woodland land area in orange representing a correlation coefficient between coniferous woodland and CHCl<sub>3</sub> concentration

of between  $f=0.400$  and  $f=0.749$ , at the Conwy catchment, in laboratory experiments without DOM removal.

### 7.3 Overall Summary of Findings

DOM character rather than just DOC concentration is the key factor in understanding DBP formation potential. So, during chlorination, some DOC is more likely to react to produce DBPs, some will react to use up the chlorine source (i.e. NaOCl or chloramine) to form non-DBP chlorinated compounds and some DOC will not react at all. Thus, identifying the DOC precursors which give rise to DBPs is the key factor.

1. THM formation potential and NDBP formation is a competitive process where the chlorine source reacts with the most labile DOC functional groups first. As these reactions take place, the chlorine source is used up and so subsequent reactivity to form further DBPs will gradually slow down.
2. Points 1 and 2 drive all DBP formations, and this fact has been explored using 3 sites. The Conwy and Scottish sites are widely classified as 'peaty' whereas the Hampshire Avon is distinctly different, but data generated on DOM from all 3 sites vastly differs. This is believed to be due to different vegetation, hydrology and climate at the 3 different sites.
3. NDBPs are hard to analyse due to their very low concentrations (i.e.  $<5 \mu\text{g L}^{-1}$ ), and their detection requires long sample preparation and sophisticated and specialised equipment. Hence, to study these compounds in detail, simpler and more sensitive NDBP analytical methods are required
4. Specific methods for DBP precursor analysis will help guide specific DOM removal at WTWs. These treatments need to be identified to either stop the DBP formation (e.g. stopping run off, changing land use, selective filtration), or to treat the water post disinfection.
5. The data in this thesis suggest that, with higher population comes higher pressures on the water industry in terms of supply and demand, but with premium abstraction locations being few and far between, further research into effectively treating waters from catchments known to be problematic will be paramount in maintaining healthy drinking water for the increasing population. Increased population not only increases

water demand, but also puts stresses on current water sources, as increased pollution, urban runoff and other human influences mentioned previously in this thesis will provide further complications in the provision of clean drinking water. The increasing dissolved organic matter resulting from continued climate change needs to continue to be studied in depth, to ensure that the WTW processing is set to tackle the contaminants before they start negatively effecting the water quality. Specifically, an increase in pastoral farming practices (namely pork, beef and lamb farming, and the storage of silage feed), to produce enough food for the growing population, could result in the increase of specific amino acids, for example, tryptophan, known to form dichloroacetamide, an N-DBP (Baker, 2002, and Chu *et al.*, 2010).

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# Appendices

## Appendix 1:

### A GIS based tool for catchment managers, using DOM as a case study

The screenshot displays the Digimap website interface. At the top left is the Digimap logo, and at the top right is an information icon. Below the logo is a navigation bar with the text: "The most comprehensive maps and geospatial data available in UK Higher and Further Education".

The main content area is divided into two columns. The left column, titled "Map and Data Collections:", contains a vertical list of buttons: "Welcome", "Ordnance Survey" (highlighted in orange), "Historic", "Geology", "Marine", "Discover", and "Environment". Below this list is a message: "Your Institution does not subscribe to:".

The right column, titled "Digimap Ordnance Survey", contains several sections of links and icons:

- View, annotate and print OS maps**: Includes a link for "Roam" with the description "For maps at user defined scales, (A4 to A0)".
- Download data for use in GIS/CAD**: Includes a link for "Data Download" with the description "Download multiple OS products in various formats."
- Postcode & placename information**: Includes links for "Gazetteer Query" (description: "Search for placenames/view their locations."), "Gazetteer Plus" (description: "Download placename records."), and "Postcode Query" (description: "Look up postcodes by location attributes.").
- Help Resources**: Includes links for "Ordnance Survey Help" and "Frequently Asked Questions".

At the bottom of the page, there is a footer with "© University of Edinburgh" on the left and "Privacy & Cookies Policy" on the right. The EDINA logo is positioned at the bottom left of the page.

Figure 1: Image to show data download section location once logged in to ED/INA Digimap.



Figure 2: Image to show data download process for Afon Conwy, North Wales, UK, and datasets selected.

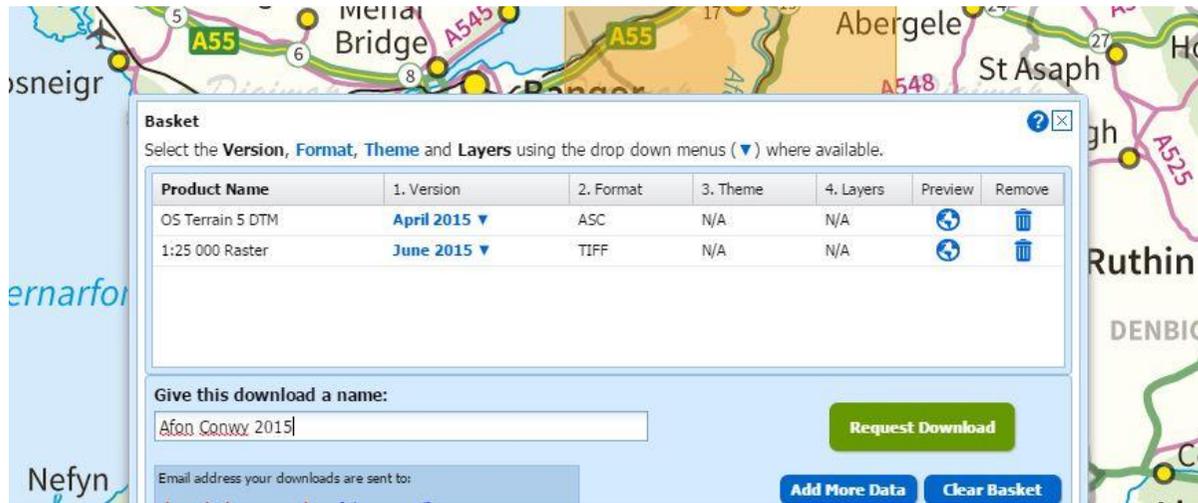


Figure 3: Image to show data download basket on ED/NA Digimap.

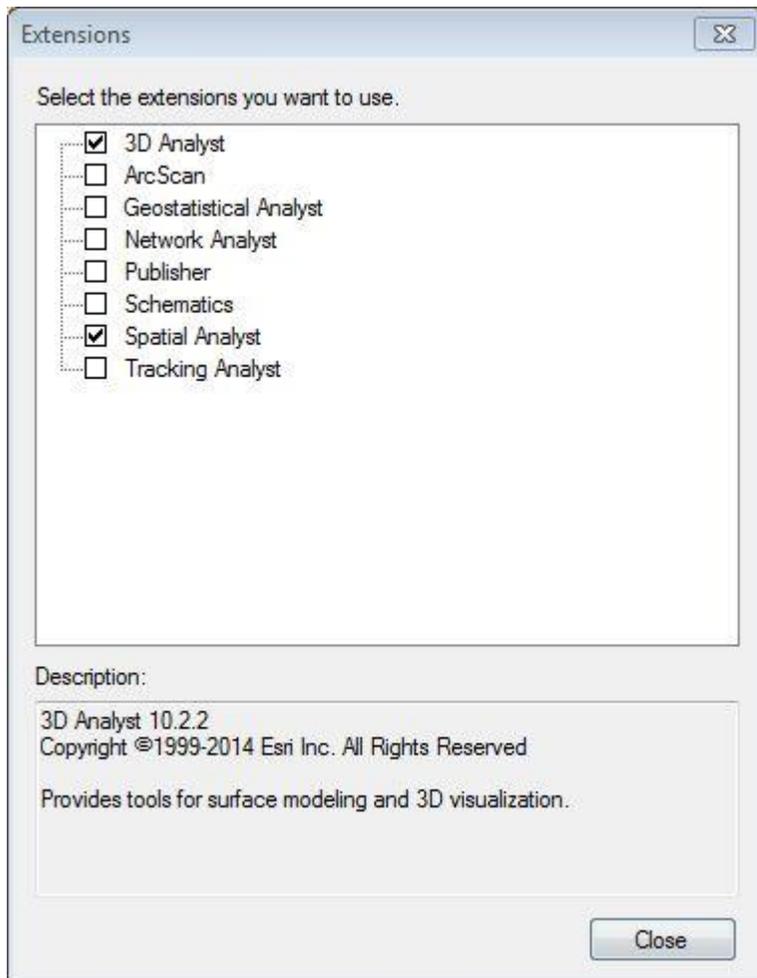


Figure 4: Image to show location of *Spatial Analyst* extension.



Figure 5: Image to show *Add Data* button (left) and *ArcToolbox* button (right).



Figure 6: Image to show Tools toolbar with zoom, unzoom and 'full extent' buttons circled.

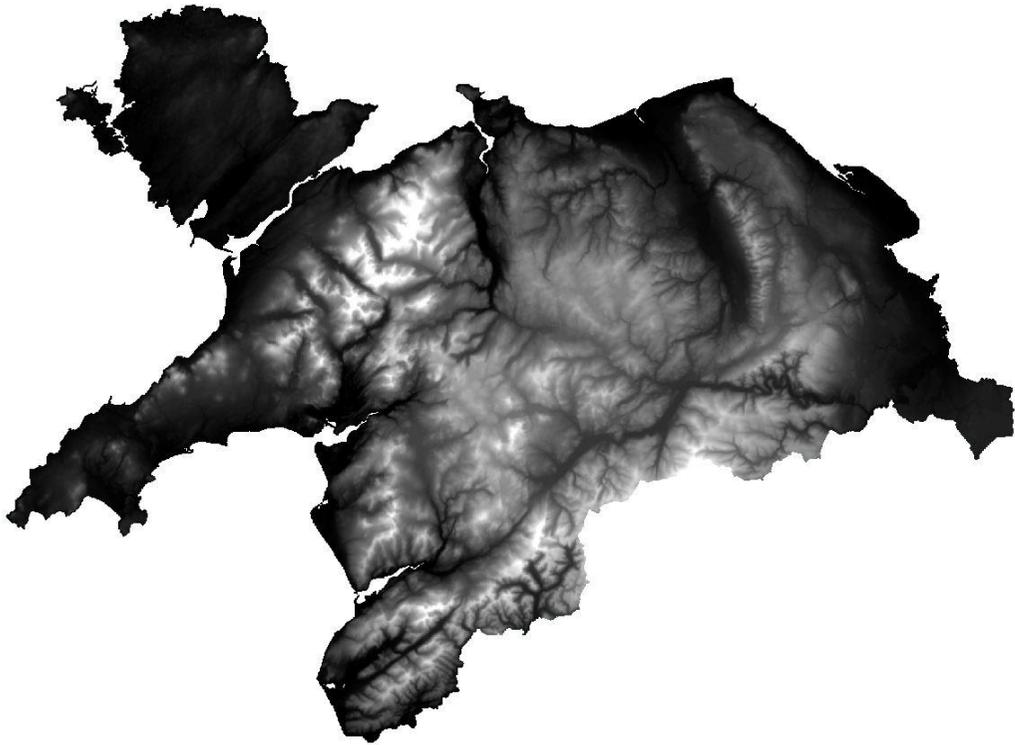


Figure 7: Image to show OS5M DTM layer and valley/mountain features visible

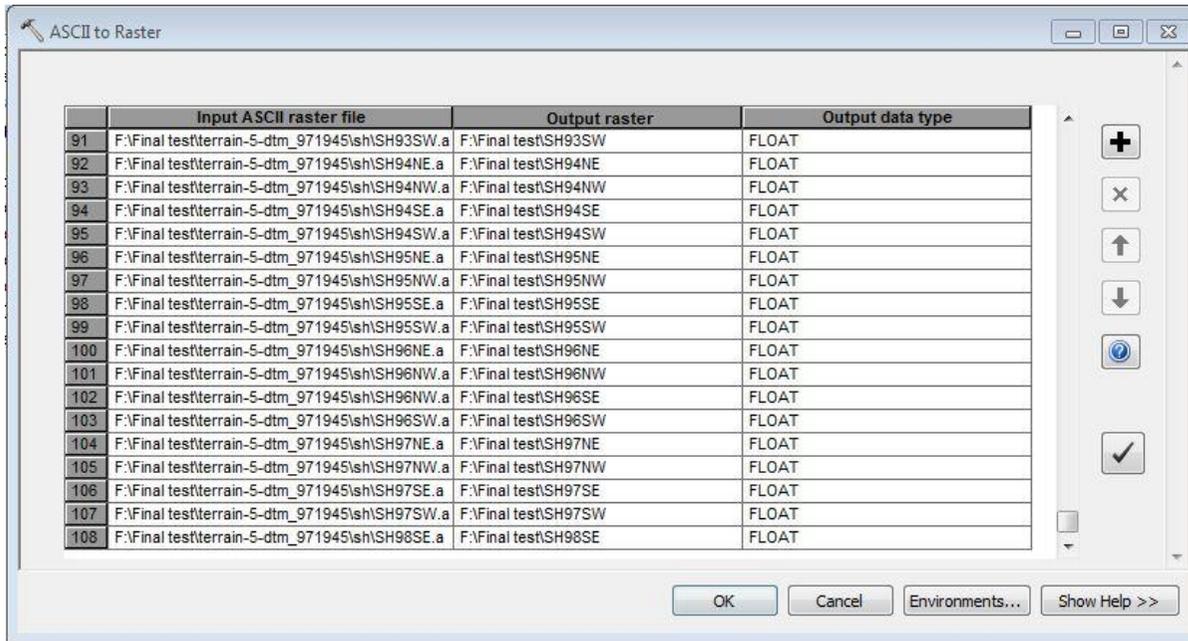


Figure 8: Image to show *ASCII to Raster* batch conversion tool

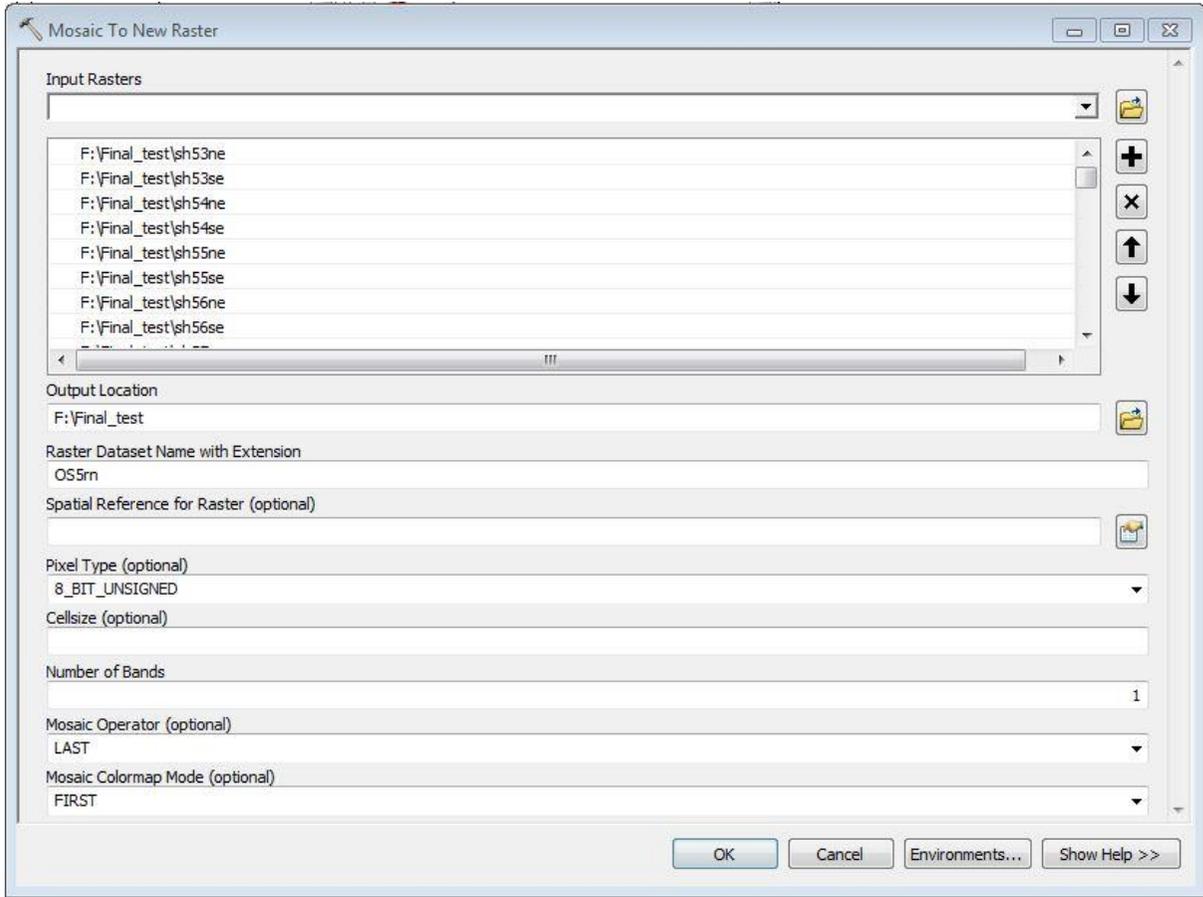


Figure 9: Image to show *Mosaic To New Raster* tool.

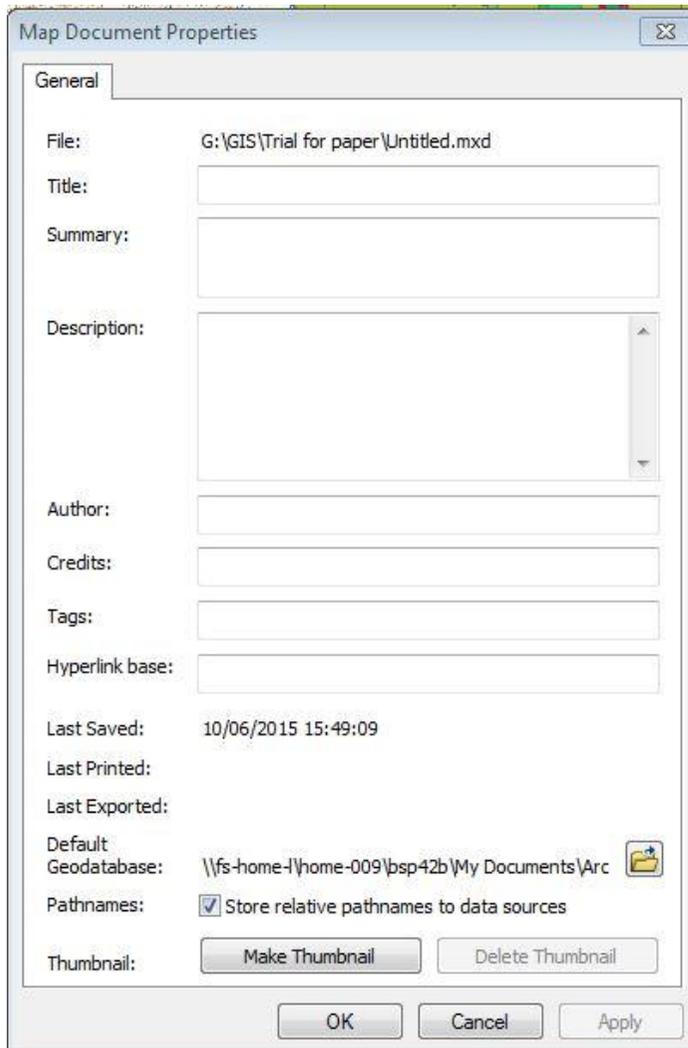


Figure 10: Image to show location of *store relative pathnames to data sources* checkbox.

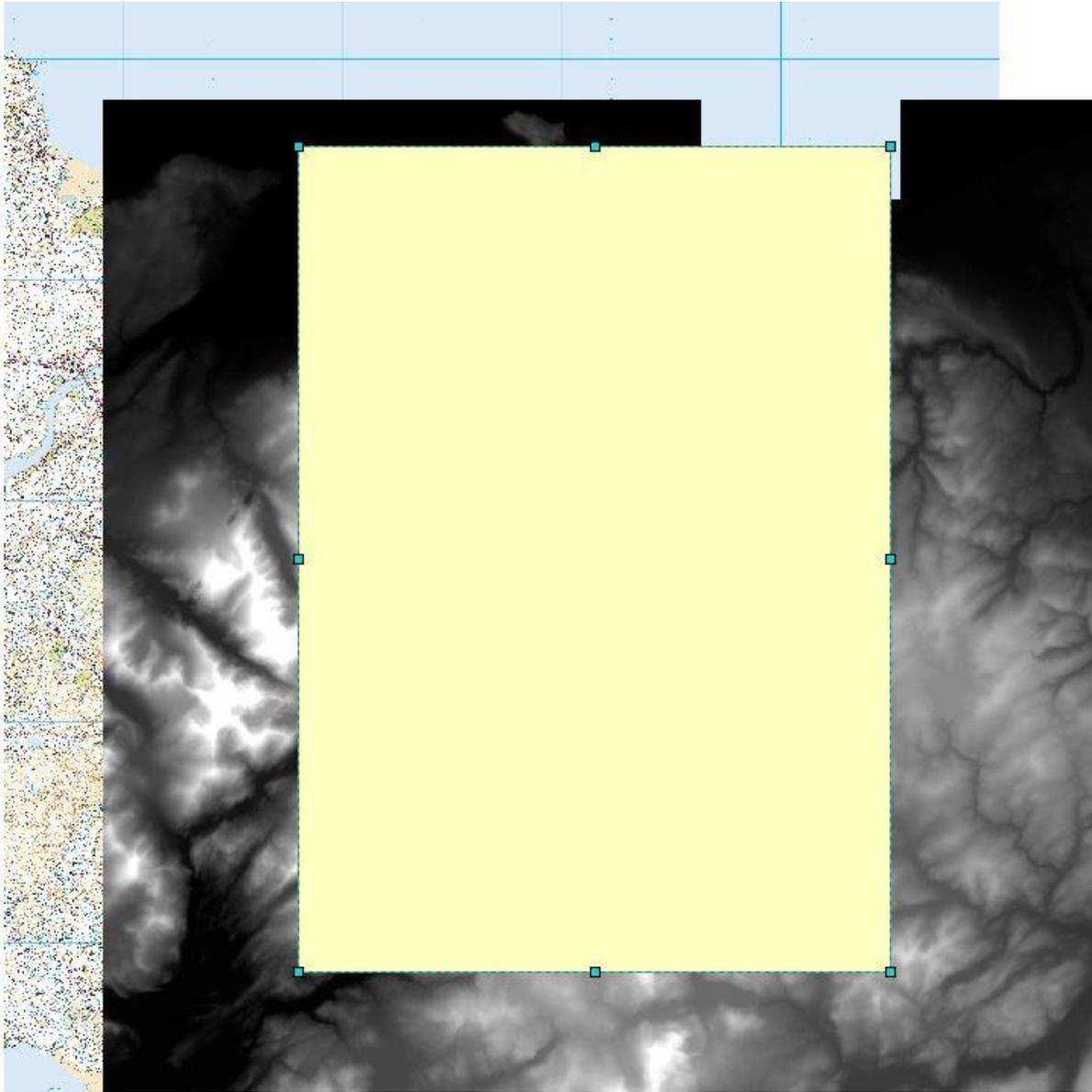


Figure 11: Image to show rectangle drawn to clip DTM from.

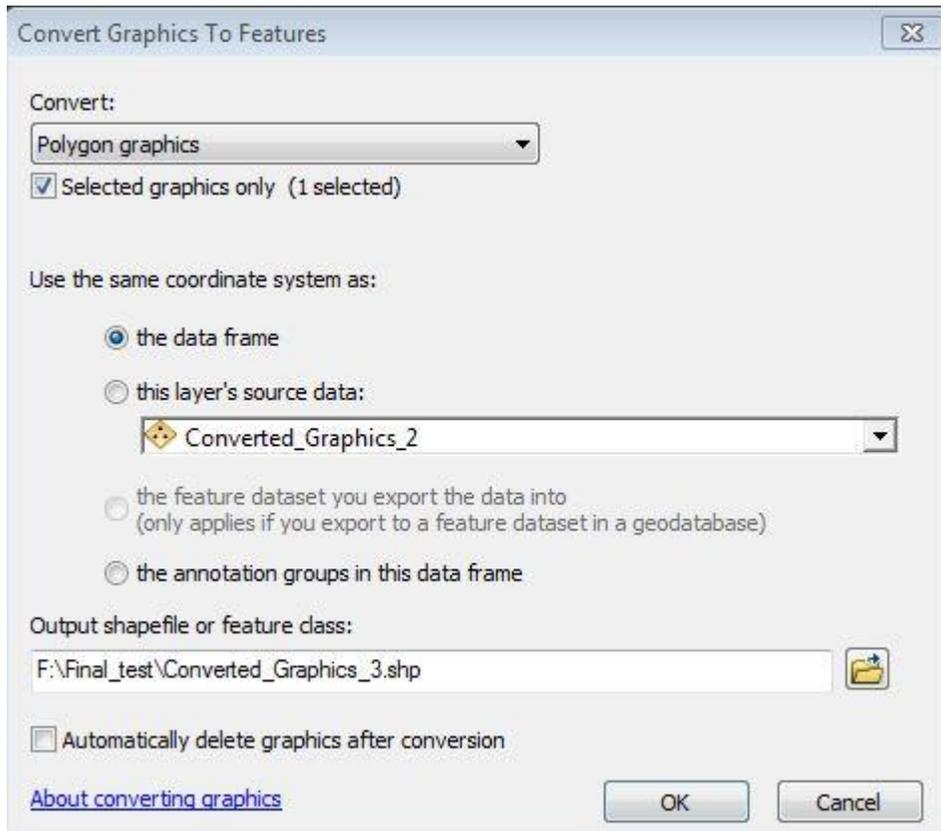


Figure 12: Image to show *Convert Graphics To Features* dialogue box.

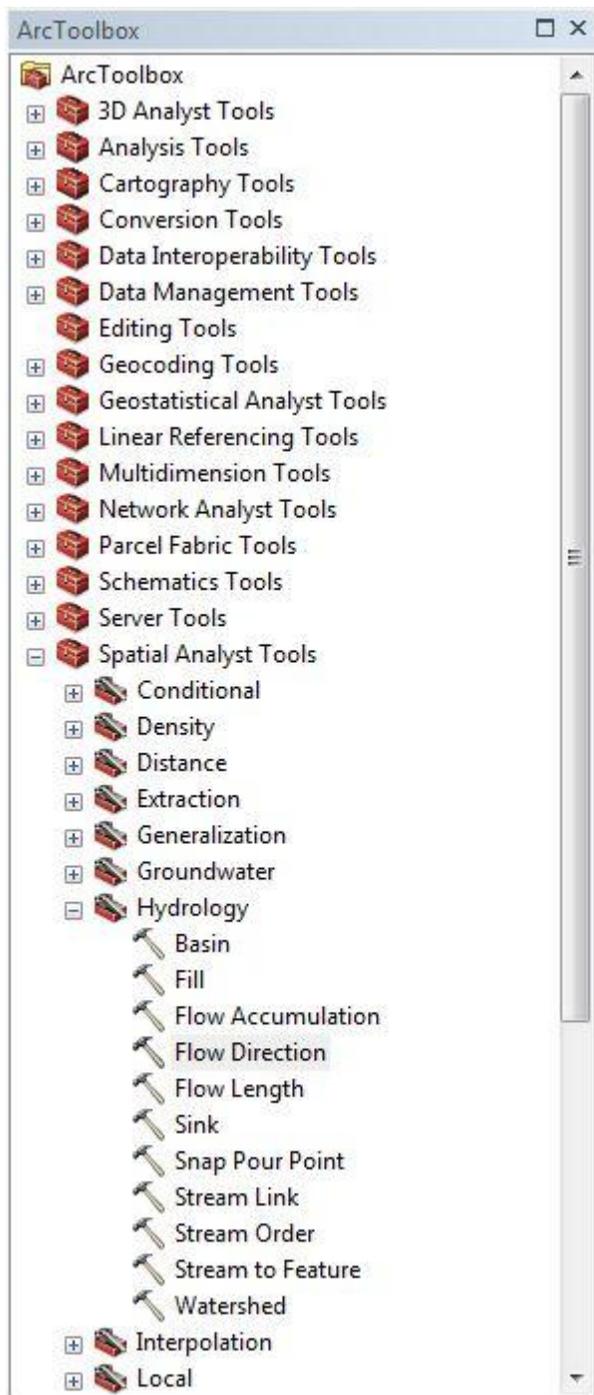


Figure 13: Image to show location of *ArcToolbox*, *Spatial Analyst* and the *Hydrology* features.

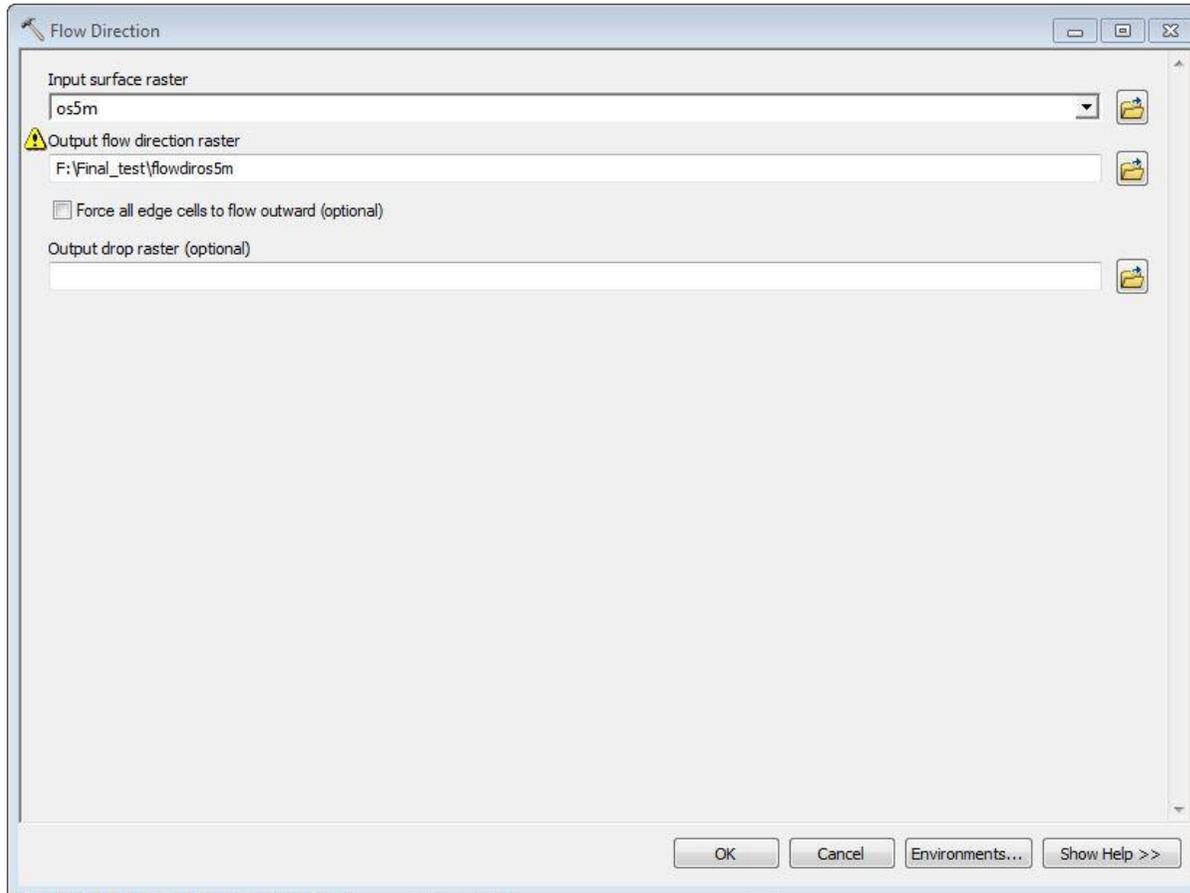


Figure 14: Image to show *Flow Direction* dialog box (note: Ignore the warning triangle, this is because the overwrite feature is turned on and flow\_diros5m has already been created before taking the screen capture shown above).



Figure 15: Image to show sinks (in white) when flow\_dirOS5M has been created.



Figure 16: Image to show sink\_flowdir1 layer after all sinks have been filled. Note, the largest sinks are likely lakes/reservoirs.

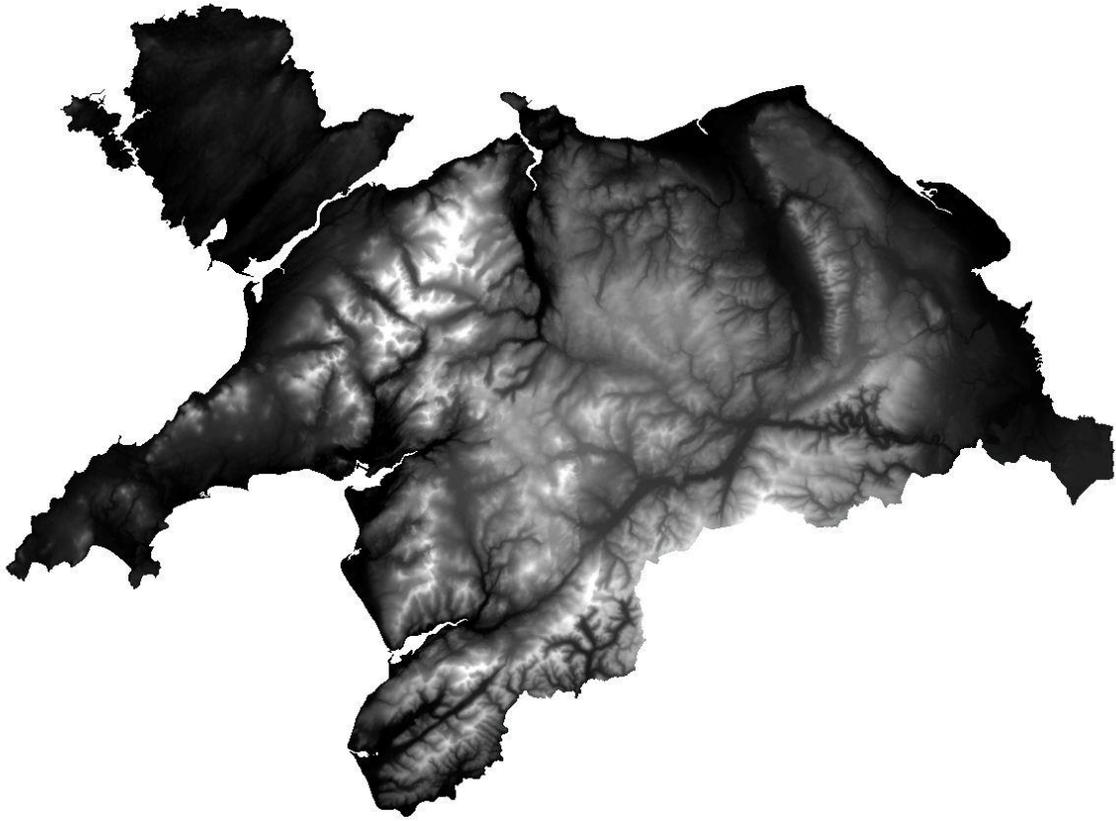


Figure 17: Image to show flowdir\_fill1 layer.

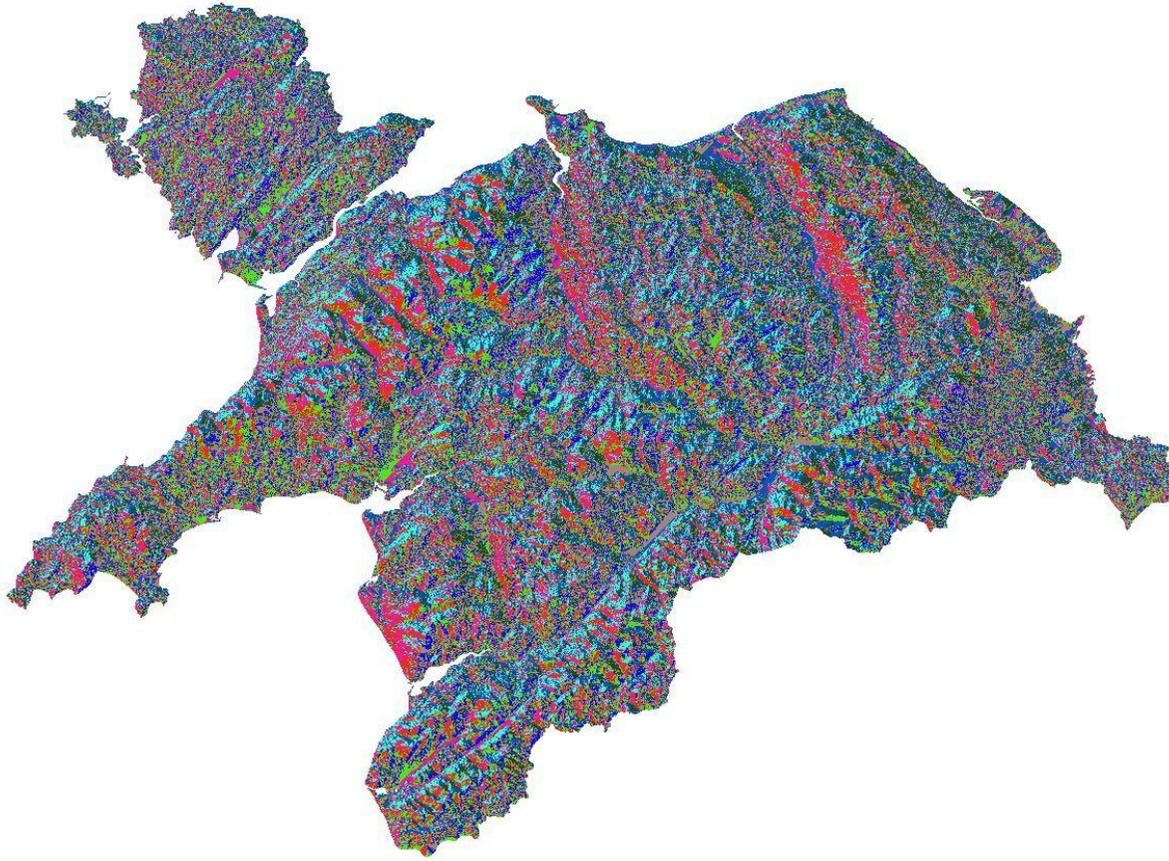
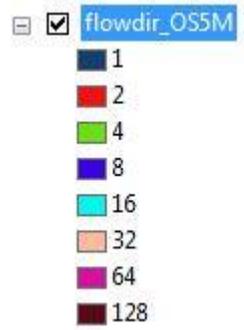


Figure 18: Image to show flowdirOS5m layer



32	64	128
16		1
8	4	2

Figure 19: Image to show 8 different directional classes in flowdirOS5m layer

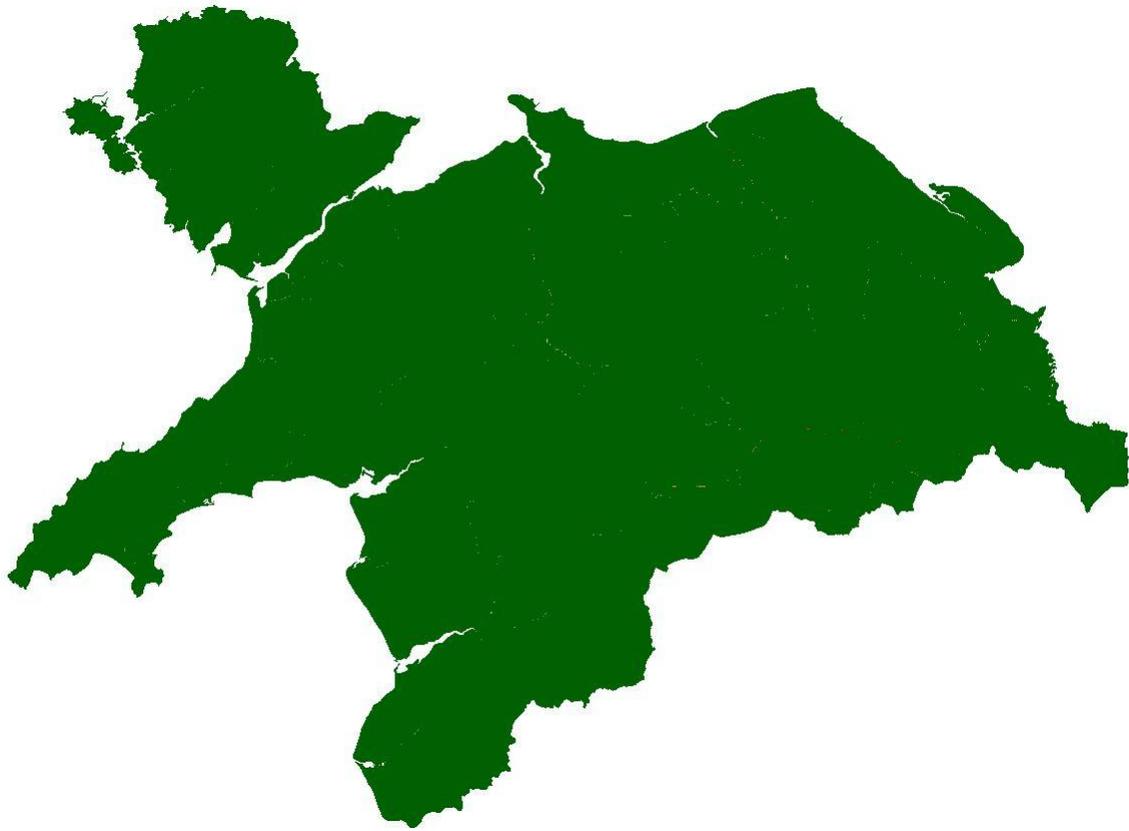


Figure 20: Image to show flowaccOS5M layer with 5 classes

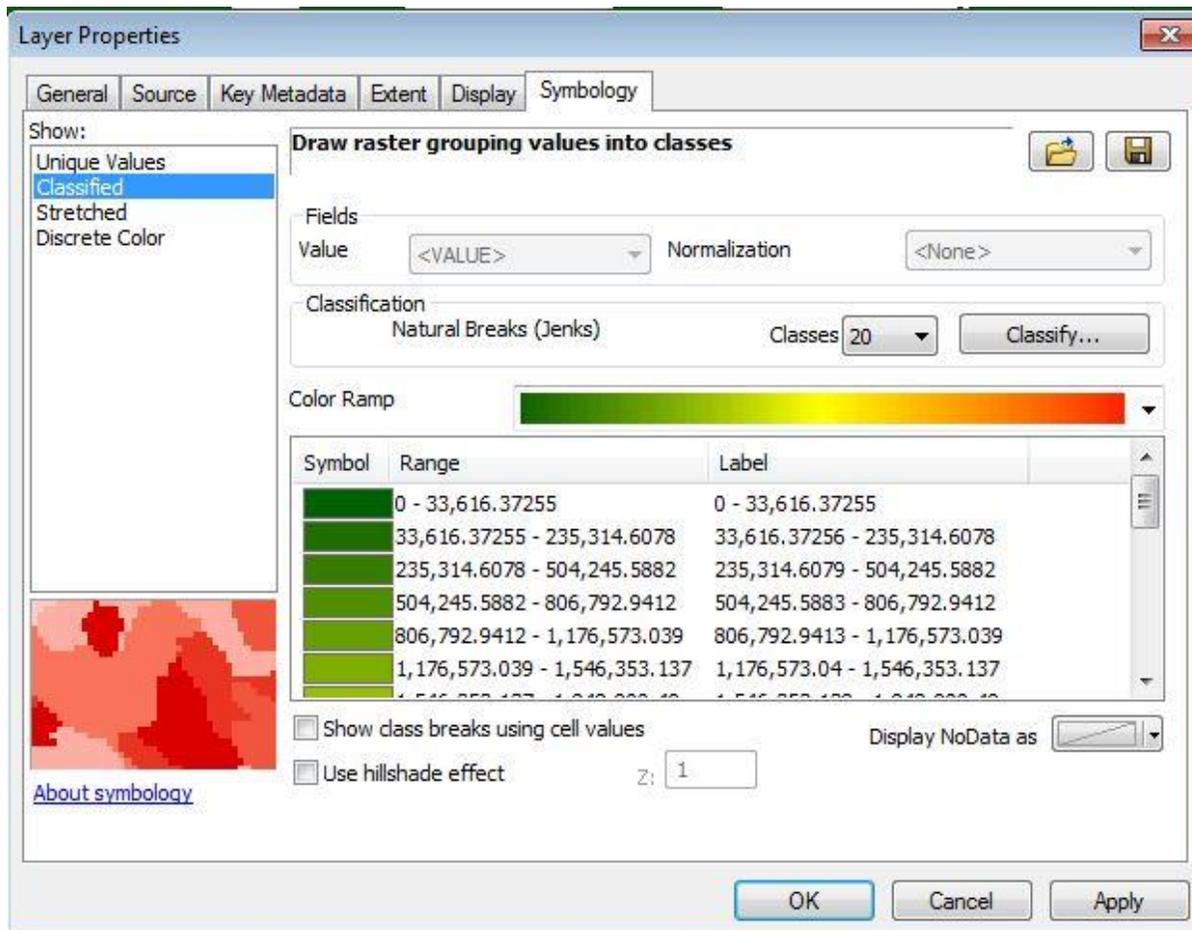


Figure 21: Image to show *Layer Properties* window with *Symbology* tab selected.

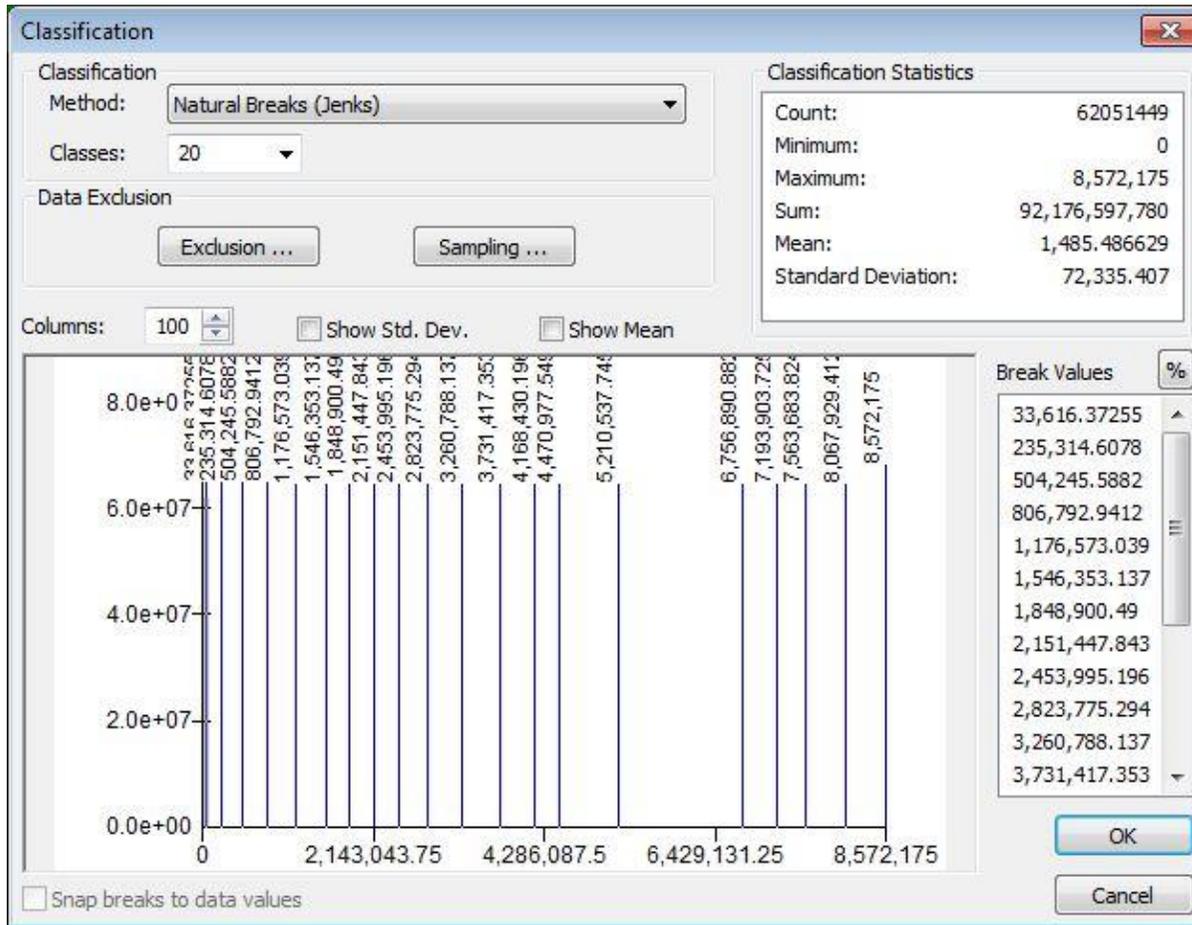


Figure 22: Image to show *Classification* window, accessed from the *Symbology* tab of *Layer Properties* window.

	A	B	C	D	E	F
1	Site Name	Labels	Easting	Northing	Latitude	Longitude
2	Site 1	Site 1	279400	356700	53.09383914	-3.80242883
3						

Figure 23: Image to show Microsoft Excel spreadsheet containing site grid references and coordinates.

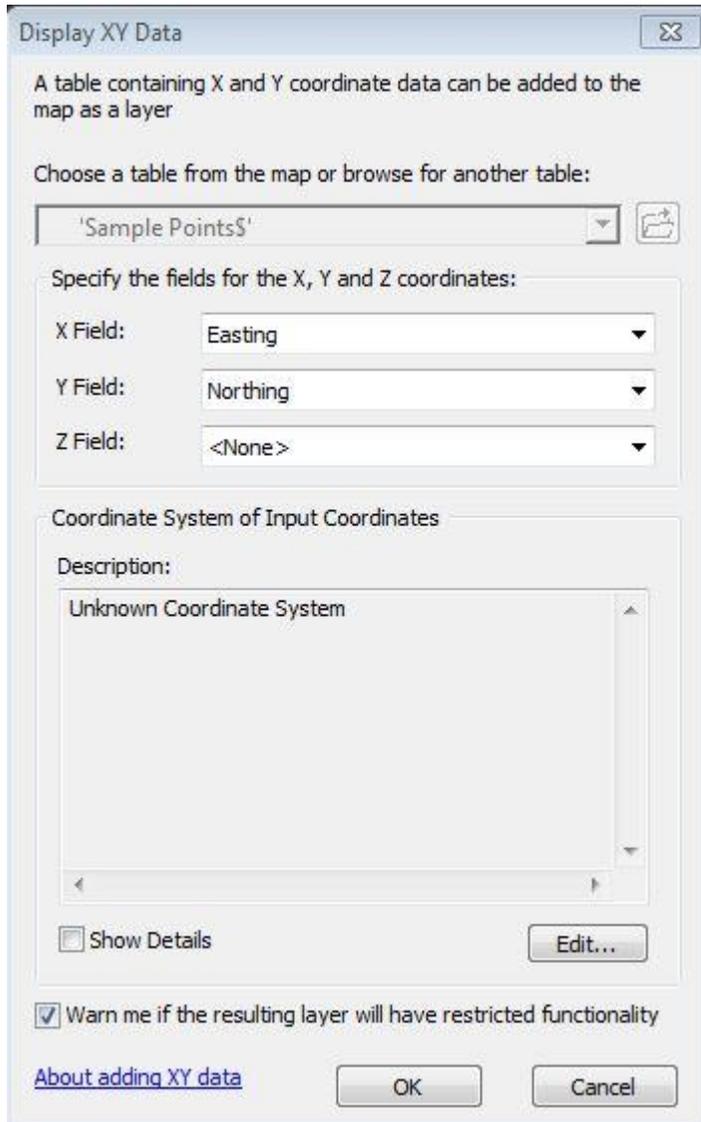


Figure 24: Image to show *Display XY Data* dialog box

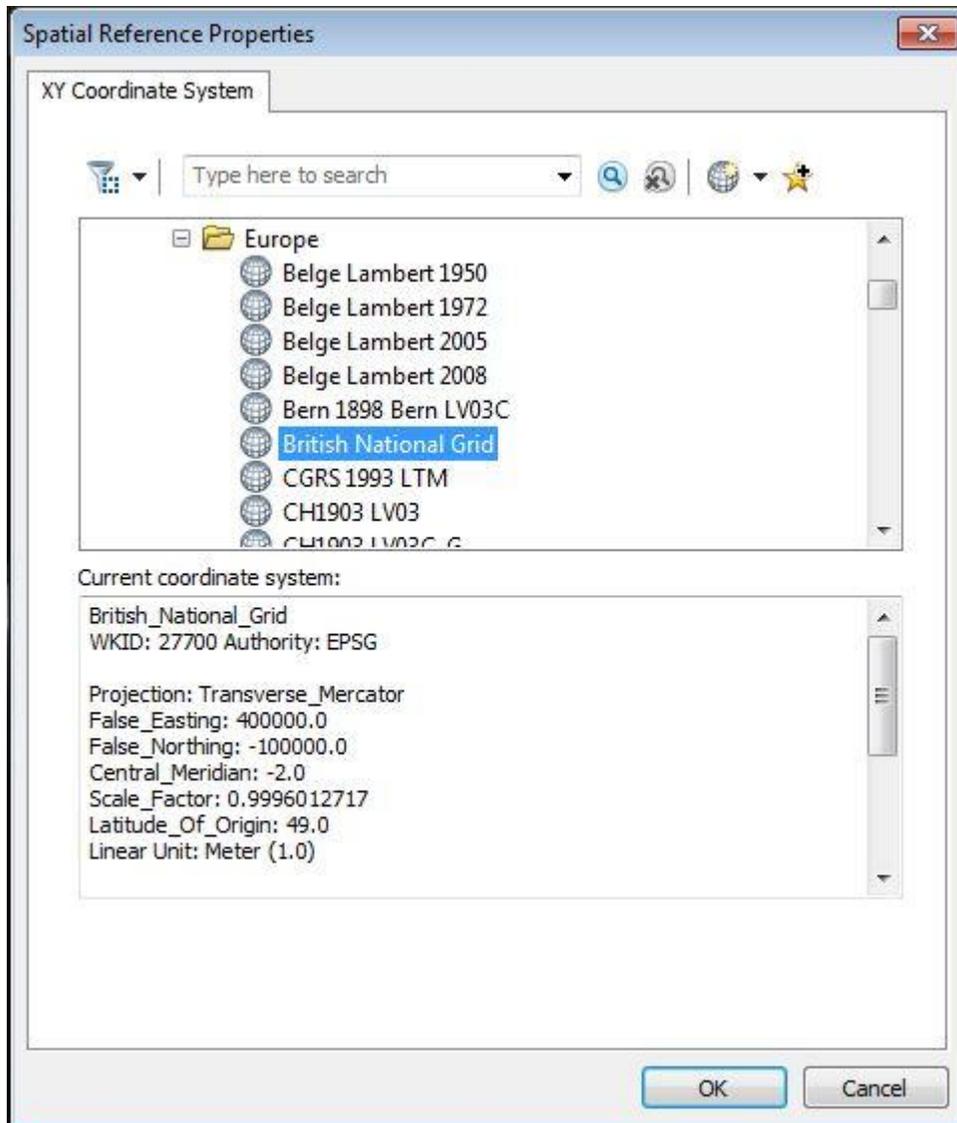


Figure 25: Image to show location of British National Grid projection system in the *Spatial Reference Properties* dialog box.

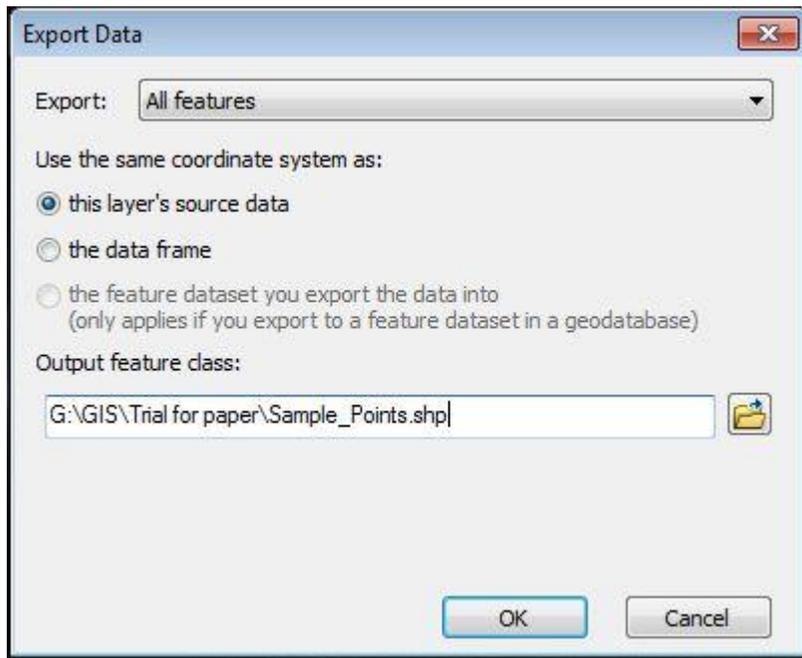


Figure 26: Image to show Export Data dialog box and settings.

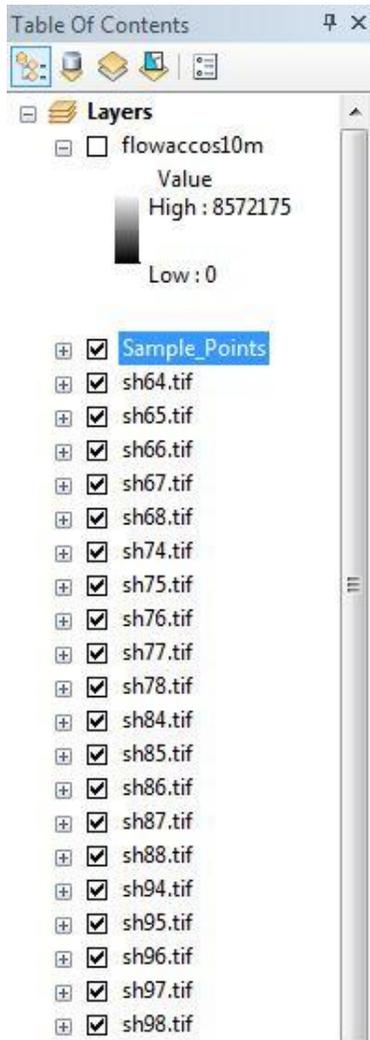


Figure 27: Image to show *Table of Contents* in 'List by Drawing Order' view, and correct arrangement of layers.

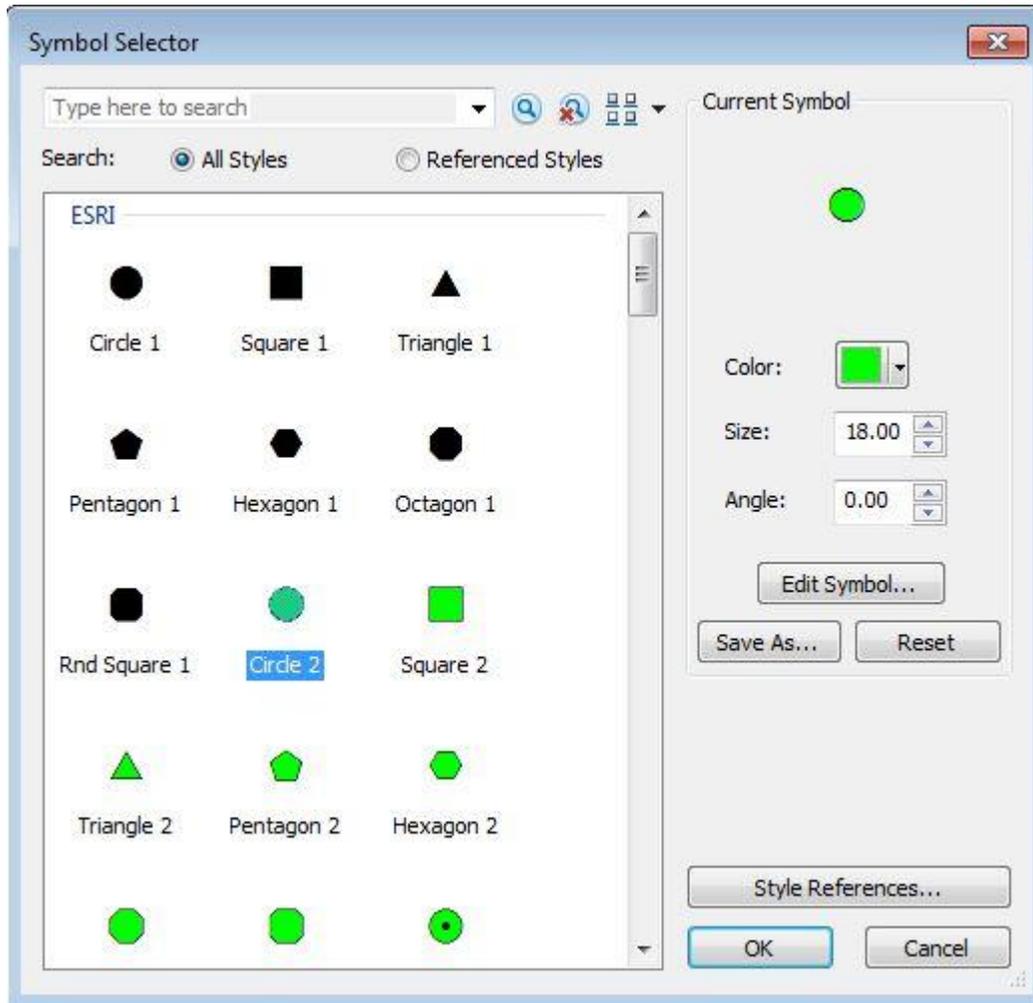


Figure 28: Image to show *Symbol Selector* dialog box.

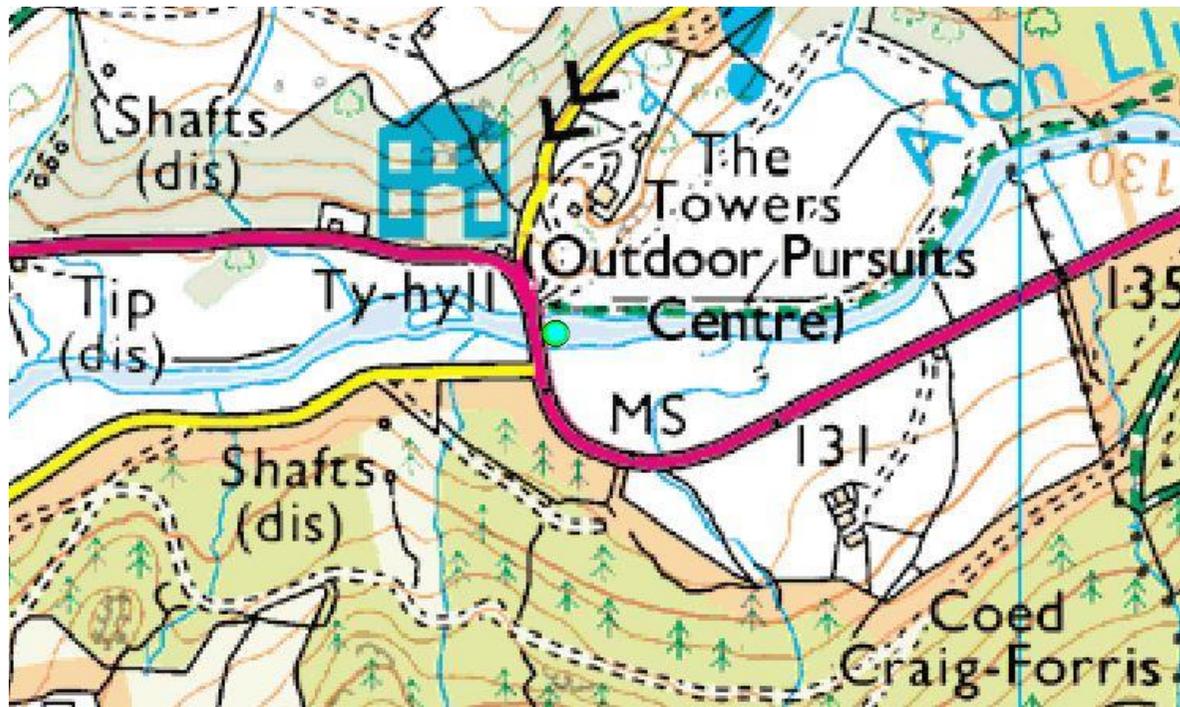


Figure 29: Image to show selected sample point

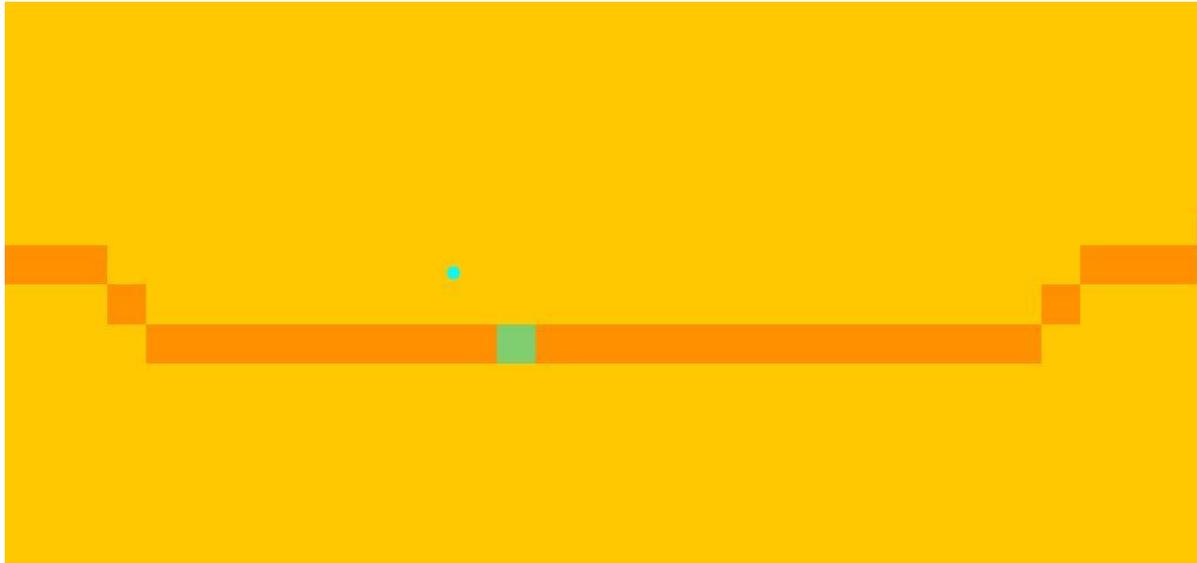


Figure 30: Image to show pourpoint location (square) along the projected river (orange) and the original site location (blue circle).

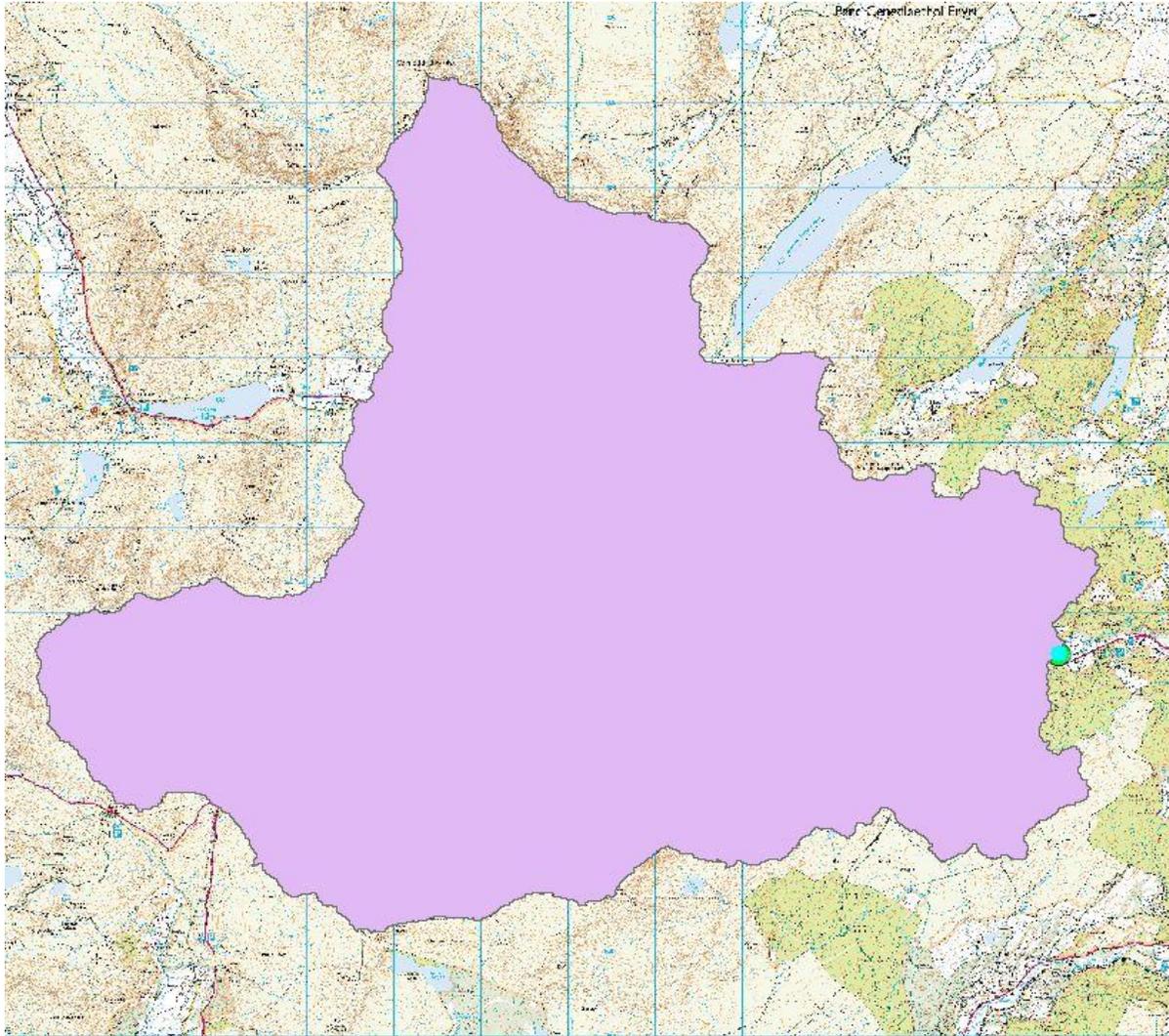


Figure 31: Image to show calculated watershed from sample point *Site 1*.

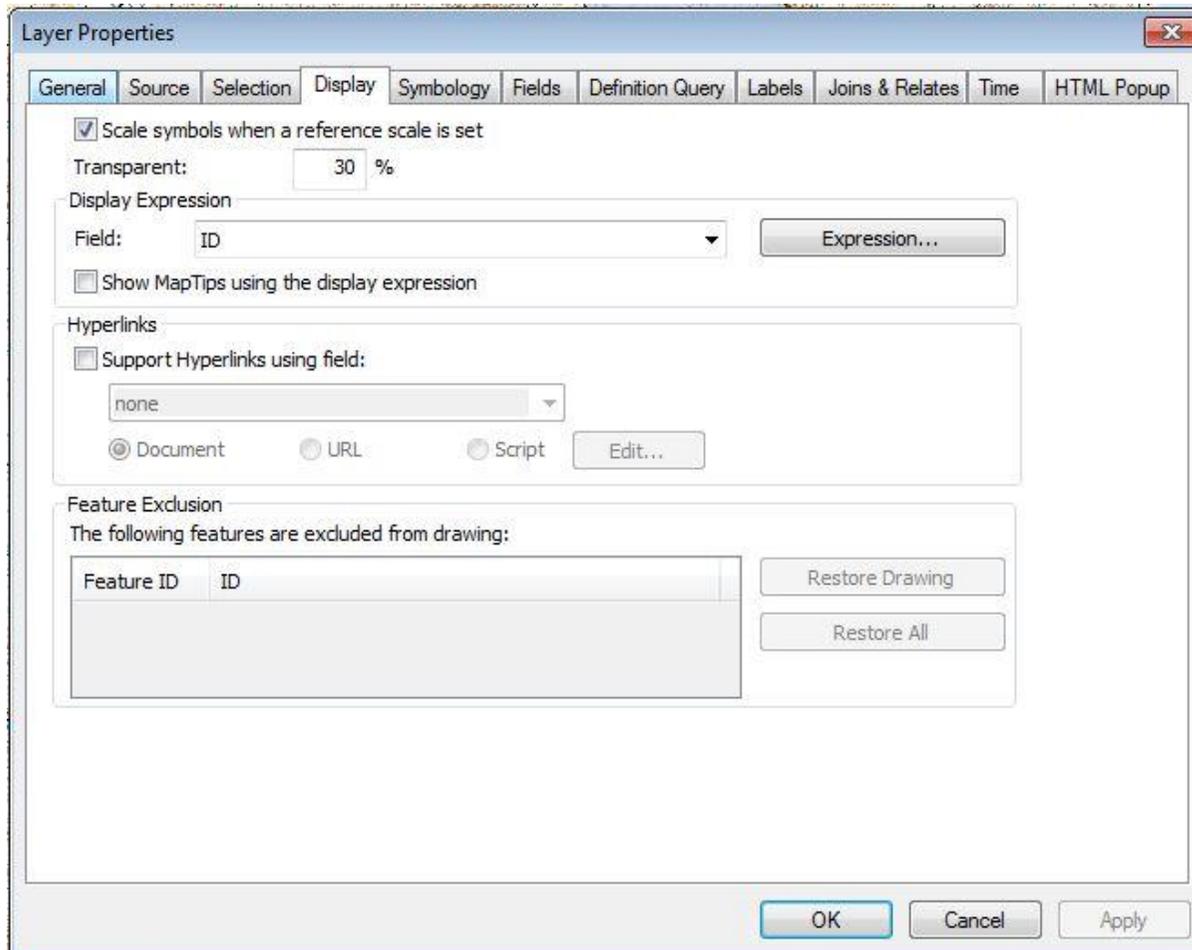


Figure 32: Image to show the *Display* tab selected in *Layer Properties* dialog box, with transparency set to 30%.

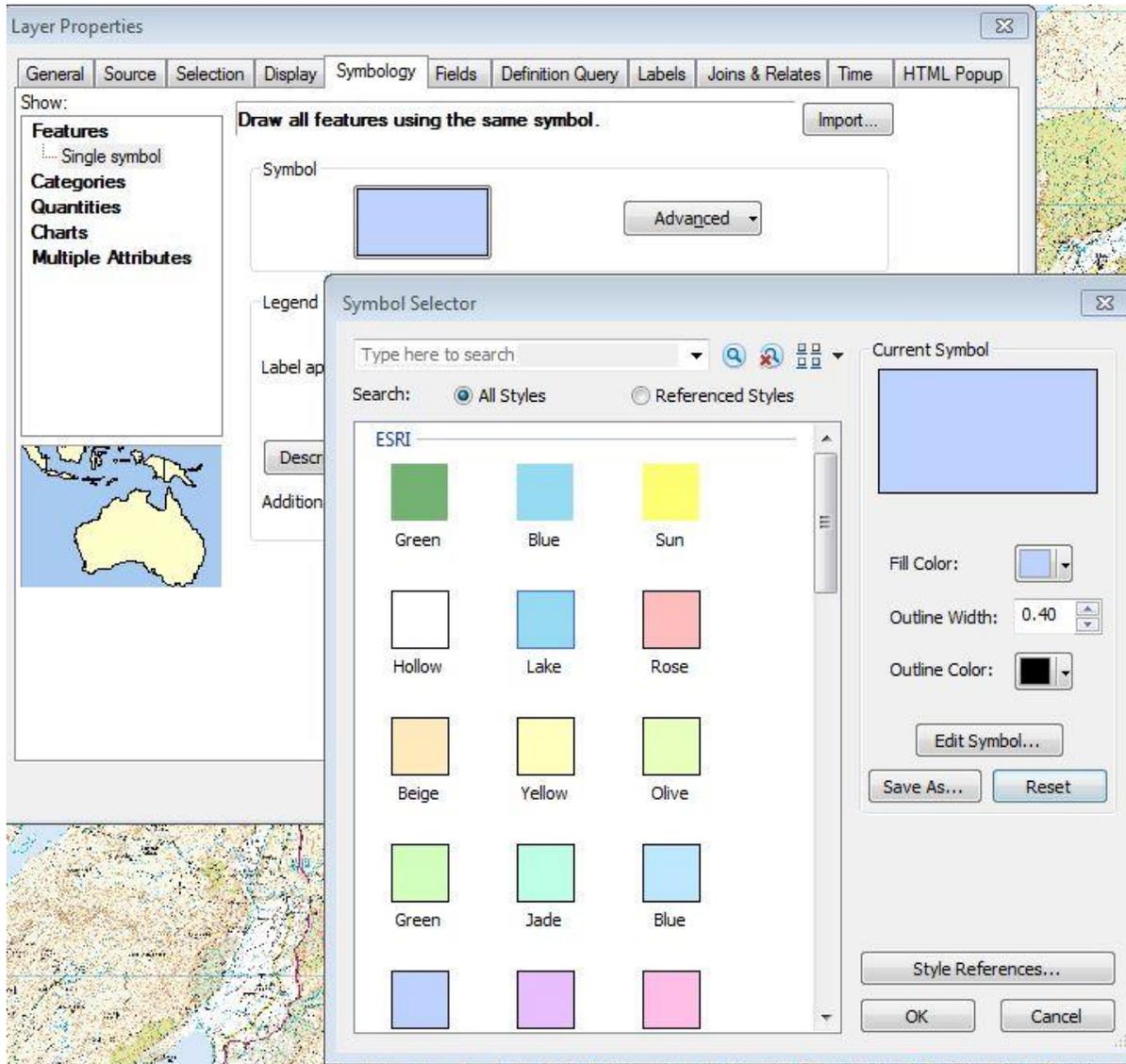


Figure 33: Image to show colour change section of *Symbology* tab of *Layer Properties* dialog box

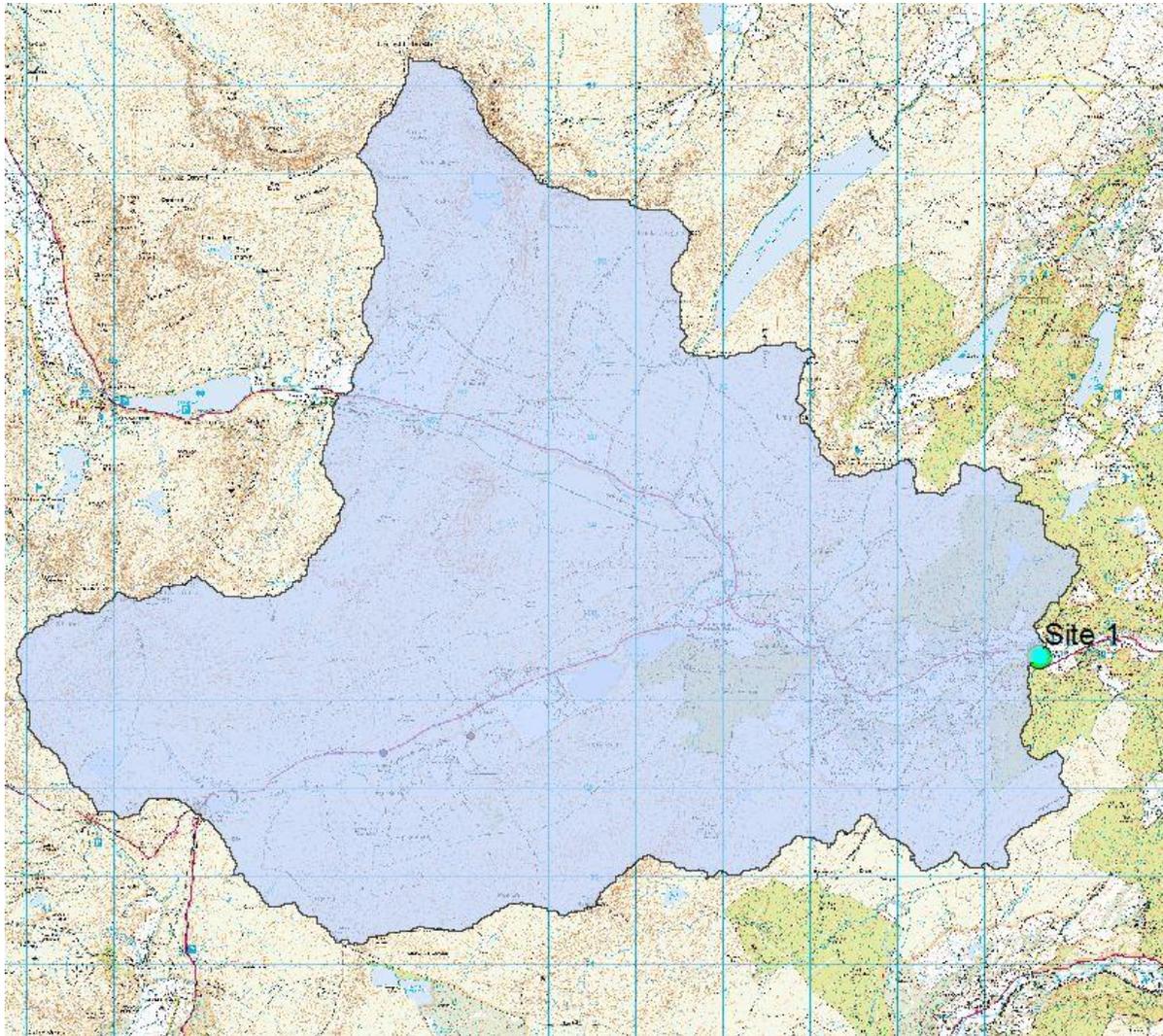


Figure 34: Image to show site labelling and overall watershed with transparency and colour changes implemented.

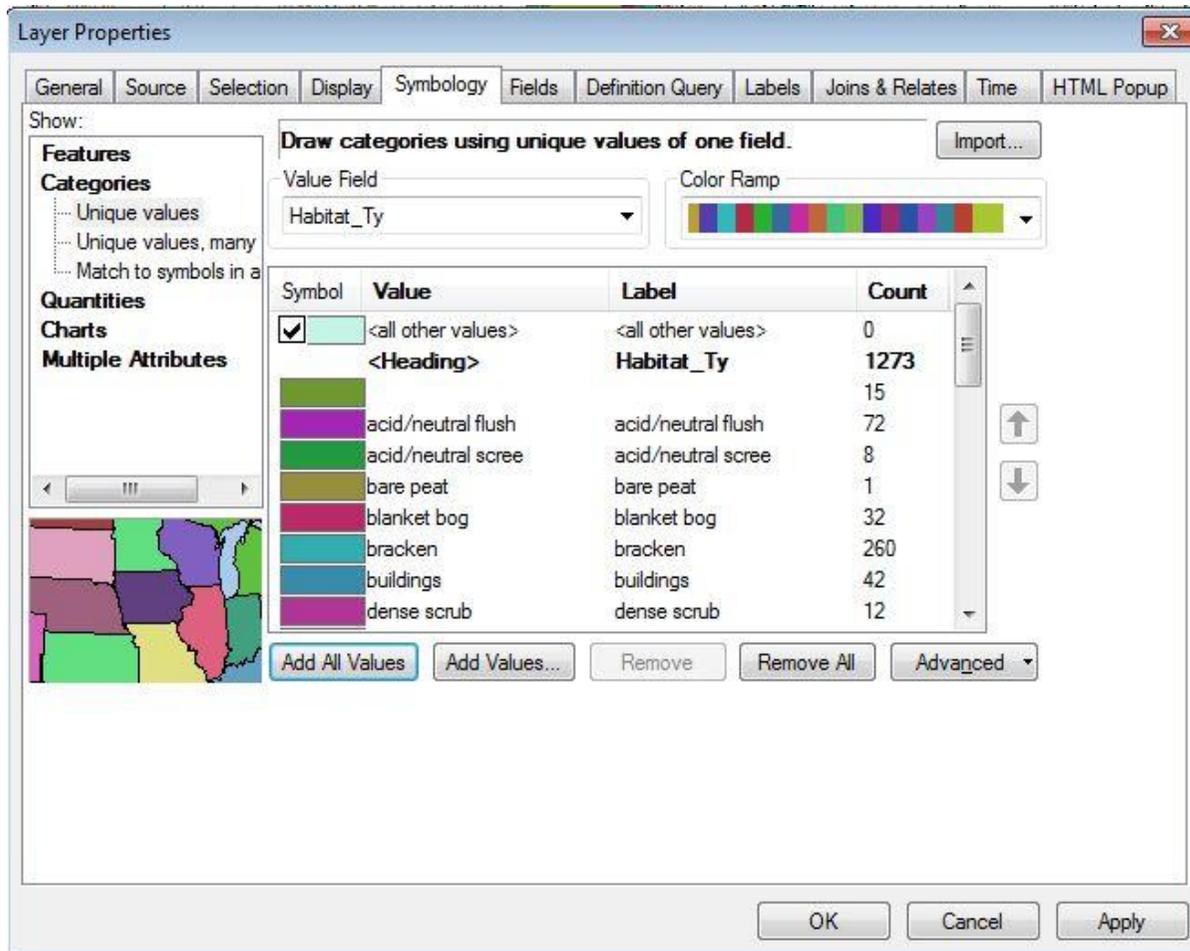


Figure 35: Image to show *Symbology* tab in *Layer Properties* dialog box, with colour ramp changed and all values from *Habitat\_Ty* added to the display.

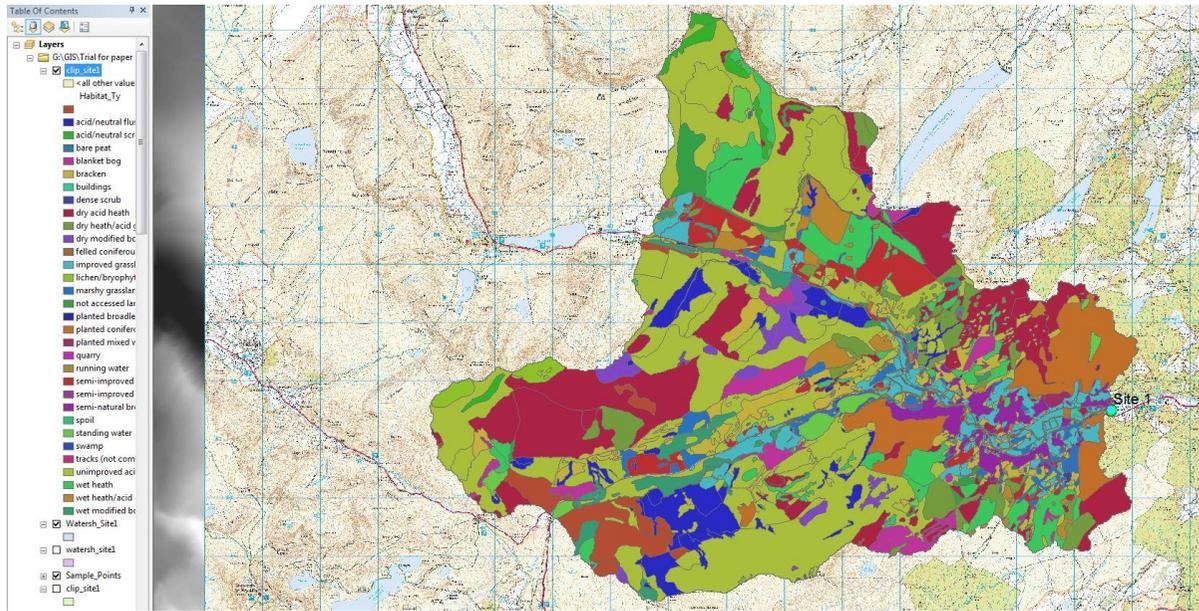


Figure 36: Image to show the CCW Phase 1 Habitat Survey data displayed over the *Watersh\_site1* layer.

Table

clip\_site1

FID	Shape *	CODE1	Habitat_Ty	Area_M2
0	Polygon	D.1.1	dry acid heath	711.621
1	Polygon	D.1.1	dry acid heath	1711.94
2	Polygon	D.1.1	dry acid heath	2583.92
3	Polygon	D.1.1	dry acid heath	4180
4	Polygon	D.6	wet heath/acid grassland mosaic	407727
5	Polygon	E.2.1	acid/neutral flush	38993.9
6	Polygon	D.5	dry heath/acid grassland mosaic	14247.5
7	Polygon	D.5	dry heath/acid grassland mosaic	1603.19
8	Polygon	D.6	wet heath/acid grassland mosaic	48290.3
9	Polygon	D.1.1	dry acid heath	4923.64
10	Polygon	E.1.6.1	blanket bog	11000.1
11	Polygon	B.1.1	unimproved acid grassland	1399.56
12	Polygon	B.5	marshy grassland	27374.1
13	Polygon	D.5	dry heath/acid grassland mosaic	15203.4
14	Polygon	C.1.1	bracken	972.623
15	Polygon	D.1.1	dry acid heath	1858.33
16	Polygon	D.1.1	dry acid heath	2441.1
17	Polygon	D.2	wet heath	14909.2
18	Polygon	D.1.1	dry acid heath	2217.2

Figure 37: Image to show attribute table of *clip\_site1* with area of each individual instance of isolated landuse types given in meters<sup>2</sup>.

	A	B	C	D
1	FID	Habitat_Ty	Cnt_Habitat_Ty	Sum_Area_M2
2	-1	Unclassified	15	1876312.97
3	-1	acid/neutral flush	72	3101891.95
4	-1	acid/neutral scree	8	578645.42
5	-1	bare peat	1	6477.29
6	-1	blanket bog	32	1748554.26
7	-1	bracken	260	2897087.25
8	-1	buildings	42	185623.66
9	-1	dense scrub	12	47791.82
10	-1	dry acid heath	112	9198588.43
11	-1	dry heath/acid grassland mosaic	56	2502050.81
12	-1	dry modified bog	20	1055897.48
13	-1	felled coniferous woodland	1	9643.27
14	-1	improved grassland	62	2542185.75
15	-1	lichen/bryophyte heath	4	413731.77
16	-1	marshy grassland	85	1208948.77
17	-1	not accessed land	42	747958.39
18	-1	planted broadleaved woodland	2	8384.32
19	-1	planted coniferous woodland	19	3584116.36
20	-1	planted mixed woodland	2	85551.65
21	-1	quarry	1	13499.60
22	-1	running water	20	255381.13
23	-1	semi-improved acid grassland	31	2084639.93
24	-1	semi-improved neutral grassland	3	11474.10
25	-1	semi-natural broadleaved woodland	81	1491911.44
26	-1	spoil	1	21252.90
27	-1	standing water	10	769847.30
28	-1	swamp	8	17447.80
29	-1	tracks (not comprehensively digitised)	16	74464.29
30	-1	unimproved acid grassland	151	19739406.28
31	-1	wet heath	50	3957152.35
32	-1	wet heath/acid grassland mosaic	21	1231480.63
33	-1	wet modified bog	33	1295195.27
34				
35		Total Area (M2)		62762594.62

Figure 38: Image to show edited output .csv file with total areas calculated.



Figure 39: Image to show the change view button location for creation of maps.

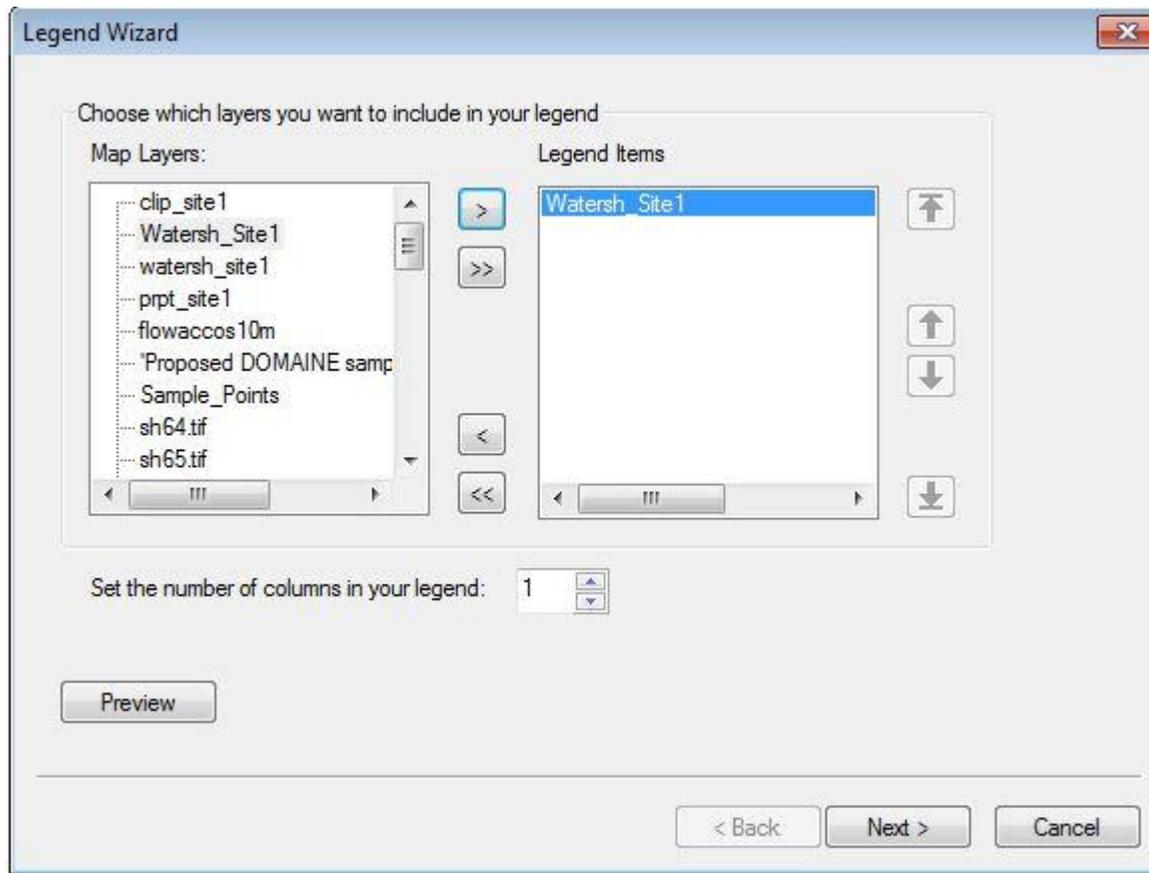
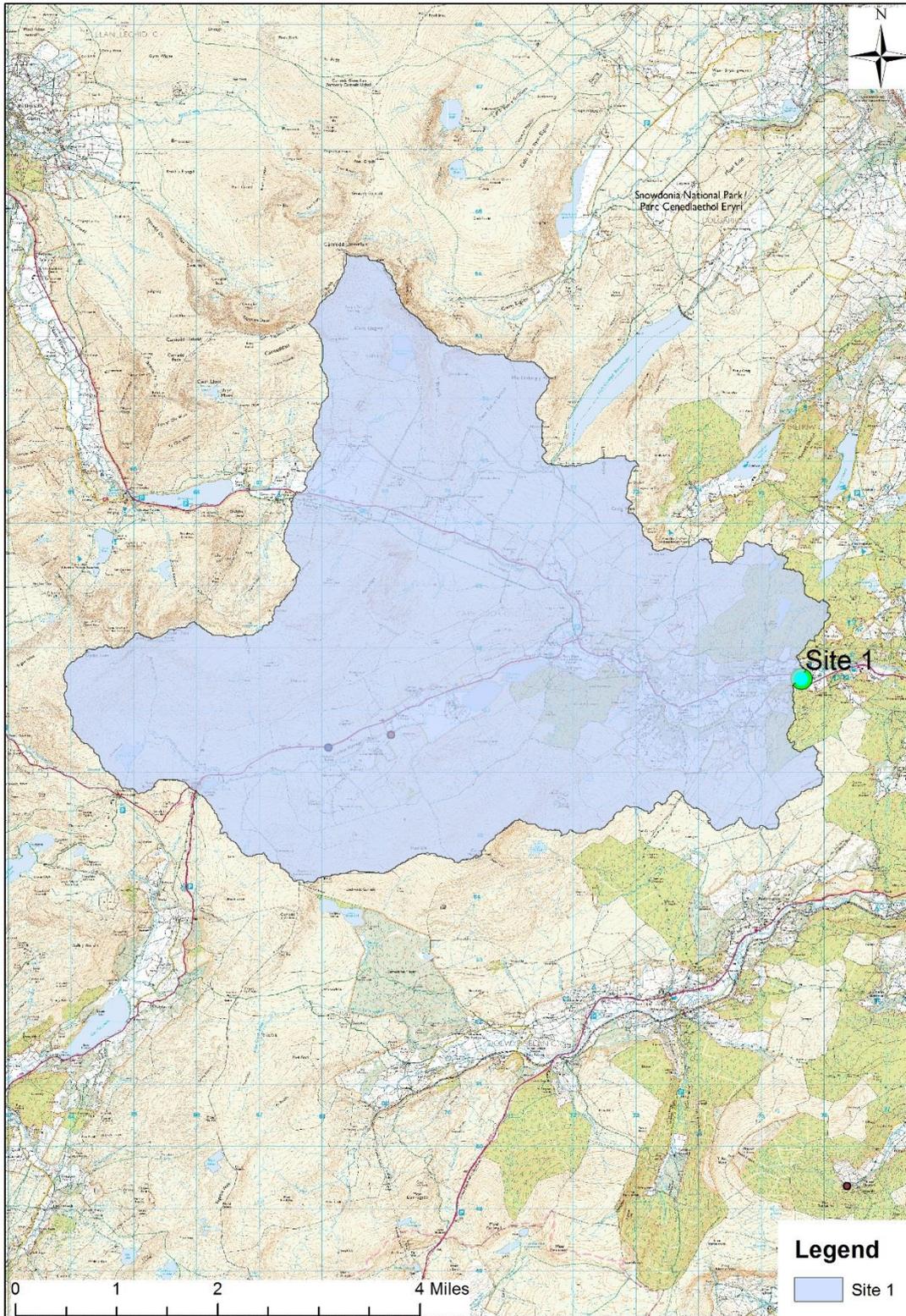


Figure 40: Image to show the *Legend Wizard* dialog box and selection of correct layer to be included in the legend.

# Site 1 Watershed



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Figure 41: Image to show final watershed of *Site 1* with legend, north arrow, scale bar and copyright text added.

A	B	C	D	E
	<b>CCW Phase 1</b>		<b>LCM2007</b>	
	unimproved acid grassland		Acid Grassland	
	bracken		Acid Grassland	
	wet heath/acid grassland mosaic		Acid Grassland	
	arable		Arable and Horticulture	
	blanket bog		Bog	
	wet modified bog		Bog	
	dry modified bog		Bog	
	bare peat		Bog	
	semi-natural broadleaved woodland		Broadleaved Woodland	
	dense scrub		Broadleaved Woodland	
	planted mixed woodland		Broadleaved Woodland	
	planted broadleaved woodland		Broadleaved Woodland	
	semi-natural mixed woodland		Broadleaved Woodland	
	felled broadleaved woodland		Broadleaved Woodland	
	introduced scrub		Broadleaved Woodland	
	planted coniferous woodland		Coniferous Woodland	
	felled coniferous woodland		Coniferous Woodland	
	acid/neutral flush		Fen Marsh and Swamp	
	marshy grassland		Fen Marsh and Swamp	
	salt marsh		Fen Marsh and Swamp	
	fen		Fen Marsh and Swamp	
	swamp		Fen Marsh and Swamp	
	inundation vegetation		Fen Marsh and Swamp	
	standing water		Freshwater	
	running water		Freshwater	
	dry acid heath		Heather	
	dry heath/acid grassland mosaic		Heather	
	wet heath		Heather	
	lichen/bryophyte heath		Heather	
	improved grassland		Improved Grassland	
	semi-improved acid grassland		Improved Grassland	
	semi-improved neutral grassland		Improved Grassland	
	acid/neutral scree		Inland Rock	
	spoil		Inland Rock	
	quarry		Inland Rock	
	acid/neutral cliff		Inland Rock	
	acid/neutral rock		Inland Rock	
	mine		Inland Rock	
	intertidal mud/sand		Littoral Sediment	
	tall ruderal herb		Rough Grassland	
	tracks (not comprehensively digitised)		Suburban	
	caravan site		Suburban	
	shingle/gravel above mhw		Supra Littoral Rock	
	intertidal cobbles/shingle		Supra Littoral Sediment	
	Unclassified		Unclassified	
	not accessed land		Unclassified	
	buildings		Urban	
	amenity grassland		Urban	
	gardens		Urban	
	refuse-tip		Urban	
	bare ground		Urban	

Sheet1



Figure 42: Image to show basic conversion from CCW Phase 1 classifications recorded in this example to CEH LCM2007 classifications (CEH, 2011).

# Appendix 2: Data Obtained through GIS Method

Table 1: Overview of individual sites with location, site ID, and dominant vegetation, soil type and geology at Hampshire Avon, Conwy and Scottish catchments

	Site Number	Site ID	Latitude	Longitude	Dominant Vegetation	Dominant Soil	Dominant Bedrock	Total Area (km <sup>2</sup> )
Conwy	CC13	Llyn Conwy	52.99472258	-3.817072017	Bog (51.19%)	Blanket bog peat soils (99.64%)	Felsic Tuff (67.84%)	1.25
Conwy	CC5	Llanwrst	53.1361817	-3.796577041	Acid Grassland (22 73%)	Freely draining acid loamy soils over rock (29.32%)	Mudstone, Siltstone and Sandstone (68.34%)	363.49
Conwy	CC54	AfonCadnant	53.0764648	-3.700187627	Acid Grassland (78.05%)	Slowly permeable wet very acid upland soils with a peaty surface (85.00%)	Sandstone and Conglomerate, Interbedded (100.00%)	3.42
Conwy	CC6	Conwy at Betws	53.09195941	-3.798915386	Acid Grassland (21.16%)	Freely draining acid loamy soils over rock (32.20%)	Mudstone, Siltstone and Sandstone (69.77%)	261.55
Conwy	CC60	Maenan	53.17119641	-3.79968573	Improved Grassland (87.36%)	Freely draining acid loamy soils over rock (95.69%)	Sandstone and Conglomerate, Interbedded (50.75%)	5.21
Conwy	CC8	Ysbytylfan	53.023877	-3.7275758	Bog (61.86%)	Blanket bog peat soils (75.98%)	Mudstone, Siltstone and Sandstone (78.20%)	36.85
Conwy	CC88	Conwy at Dylasau	53.05596226	-3.750113413	Improved Grassland (28.34%)	Freely draining acid loamy soils over rock (36.32%)	Mudstone, Siltstone and Sandstone (58.34%)	137.57
Conwy	DM2	BryniauDefaid	53.03876133	-3.708875828	Bog (45.24%)	Blanket bog peat soils (69.57%)	Mudstone, Siltstone and Sandstone (84.11%)	52.73
Conwy	DM3	Ganol	53.27531129	-3.835013825	Improved Grassland (39.51%)	Freely draining acid loamy soils over rock (81.93%)	Mudstone, Siltstone and Sandstone (57.34%)	17.61

Conwy	DM4	Gyffin	53.27952518	-3.790840695	Improved Grassland (43.30%)	Very acid loamy upland soils with a wet peaty surface (52.67%)	Mudstone, Siltstone and Sandstone (70.29%)	9.69
Conwy	DM5	Ugly House	53.10015395	-3.85887129	Acid Grassland (40.39%)	Freely draining acid loamy soils over rock (59.60%)	Mudstone, Siltstone and Sandstone (67.45%)	63.04
Conwy	NM13	AfonDdu Upper	52.97557126	-3.834736841	Bog (96.70%)	Blanket bog peat soils (100.00%)	Mudstone, Siltstone and Sandstone (93.22%)	2.06
Conwy	NM14	Pont arGonwy	52.98477339	-3.820974513	Bog (85.46%)	Blanket bog peat soils (99.64%)	Mudstone, Siltstone and Sandstone (59.83%)	5.65
Conwy	NM16	DyffrynMymbyr Outlet	53.0905126	-3.956108593	Acid Grassland (47.94%)	Freely draining acid loamy soils over rock (76.52%)	Mudstone, Siltstone and Sandstone (56.89%)	15.38
Conwy	NM17	Nant Cwm CasegFraith	53.08846955	-3.97094927	Acid Grassland (66.81%)	Freely draining acid loamy soils over rock (55.36%)	Felsic Tuff (39.71%)	0.31
Conwy	NM18	GlasgwmAutosampler	53.02719924	-3.84487593	Coniferous Woodland (56.96%)	Freely draining acid loamy soils over rock (61.06%)	Mudstone, Siltstone and Sandstone (100.00%)	2.64
Conwy	NM19	Glasgwm at Penmachno	53.03889725	-3.809085118	Coniferous Woodland (57.70%)	Freely draining acid loamy soils over rock (60.83%)	Mudstone, Siltstone and Sandstone (100.00%)	7.18
Conwy	NM20	Nant Y Coed	53.04912355	-3.71847606	Rough Grassland (50.75%)	Freely draining acid loamy soils over rock (78.73%)	Mudstone, Siltstone and Sandstone (100.00%)	1.43
Conwy	NM21	Nant y Brwyn	52.9877683	-3.801434868	Bog (80.54%)	Blanket bog peat soils (100.00%)	Mudstone, Siltstone and Sandstone (100.00%)	1.42
Conwy	NM22	Conwy above Serw	52.99079571	-3.779510049	Bog (77.92%)	Blanket bog peat soils (81.27%)	Mudstone, Siltstone and Sandstone (71.92%)	11.84
Conwy	NM23	Hiraethlyn at Pont	53.22649583	-3.799008288	Improved Grassland	Blanket bog peat	Mudstone, Siltstone	20.07

		Newedd			(71.61%)	soils(85.96%)	and Sandstone (67.15%)	
Conwy	NM24	Hiraethlyn Autosampler	53.20156285	-3.783096166	Improved Grassland (76.09%)	Freely draining acid loamy soils over rock (94.34%)	Mudstone, Siltstone and Sandstone (83.75%)	7.45
Conwy	NM25	Llugwy at Betws	53.09383914	-3.80242883	Acid Grassland (33.77%)	Freely draining acid loamy soils over rock (59.03%)	Mudstone, Siltstone and Sandstone (68.49%)	76.40
Conwy	NM26	Lledr at Pont Lledr	53.07099614	-3.796996399	Coniferous Woodland (24.29%)	Freely draining acid loamy soils over rock (52.47%)	Mudstone, Siltstone and Sandstone (80.67%)	71.37
Conwy	NM27	Merddwr	53.04636467	-3.699002451	Acid Grassland (43.28%)	Slowly permeable wet very acid upland soils with a peaty surface (49.48%)	Mudstone, Siltstone and Sandstone (58.99%)	3.05
Conwy	NM28	Eidda above Confluence	53.04811605	-3.733384653	Improved Grassland (23.24%)	Blanket bog peat soils (37.32%)	Mudstone, Siltstone and Sandstone (58.22%)	117.81
Conwy	NM29	Cwm Llanerch	53.10686294	-3.791320846	Acid Grassland (23.24%)	Freely draining acid loamy soils over rock (22.91%)	Mudstone, Siltstone and Sandstone (69.46%)	343.15
Conwy	NM30	Machno at Roman Bridge	53.0599785	-3.782360769	Coniferous Woodland (25.29%)	Freely draining acid loamy soils over rock (56.87%)	Mudstone, Siltstone and Sandstone (83.45%)	38.71
Avon	1	Quidhampton	51.076766	-1.8415242	Arable and Horticulture (41.88%)	Shallow lime-rich soils over chalk or limestone (58.90%)	Chalk (76.30%)	678.50
Avon	2	Burcombe	51.079966	-1.9048473	Arable and Horticulture (47.31%)	Shallow lime-rich soils over chalk or limestone (37.03%)	Mudstone, Sandstone and Limestone (57.16%)	205.27
Avon	3	Horse Shoe Bridge	51.078367	-1.9507441	Arable and Horticulture (45.55%)	Shallow lime-rich soils over chalk or limestone (36.29%)	Chalk (37.68%)	178.86
Avon	4	Tisbury Bridge	51.065299	-2.0708040	Improved Grassland	Slowly permeable	Mudstone,	71.57

					(49.35%)	seasonally wet slightly acid but base-rich loamy and clayey soils (38.61%)	Sandstone and Limestone (44.87%)	
Avon	5	R.Nadder at Share Farm	51.044720	-2.1114120	Improved Grassland (47.43%)	Freely draining slightly acid loamy soils (57.73%)	Mudstone, Sandstone and Limestone (72.96%)	34.60
Avon	6	Tisbury Row	51.063025	-2.0685030	Arable and Horticulture (48.33%)	Freely draining slightly acid loamy soils (37.93%)	Mudstone, Sandstone and Limestone (58.71%)	12.37
Avon	7	R.Sem at Share Farm	51.053834	-2.1473732	Improved Grassland (55.92%)	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (74.58%)	Mudstone, Siltstone and Sandstone (66.42%)	25.87
Avon	8	Priors Farm	51.054838	-2.1568929	Improved Grassland (60.07%)	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (99.95%)	Mudstone, Siltstone and Sandstone (98.85%)	4.78
Avon	9	Cools Farm	51.066546	-2.1426614	Improved Grassland (33.06%)	Freely draining slightly acid loamy soils (62.94%)	Limestone and Calcareous Sandstone (48.03%)	15.99
Avon	10	Kingston Deverill	51.133149	-2.2235151	Arable and Horticulture (53.26%)0	Freely draining slightly acid loamy soils (37.76%)	Chalk (60.35%)	30.58
Avon	12	Hensford Marsh	51.192767	-2.1758504	Arable and Horticulture (50.70%)	Shallow lime-rich soils over chalk or limestone (43.99%)	Chalk (65.58%)	82.45
Avon	13	South Newton	51.107412	-1.8782090	Arable and Horticulture (57.86%)	Shallow lime-rich soils over chalk or limestone (67.97%)	Chalk (97.11%)	168.24
Avon	14	The Pelican Pub	51.131812	-1.9025259	Calcareous Grassland (54.27%)	Shallow lime-rich soils over chalk or	Chalk (100.00%)	127.61

						limestone(88.96%)		
Avon	15	Farm Technologies	51.193454	-2.1470605	Arable and Horticulture (46.28%)	Shallow lime-rich soils over chalk or limestone (38.68%)	Chalk (63.97%)	103.67
Avon	16	Crop Research Station	51.156696	-2.0679987	Arable and Horticulture (43.40%)	Shallow lime-rich soils over chalk or limestone (46.67%)	Chalk (75.38%)	171.45
Avon	17	Steeple Langford	51.137914	-1.9550834	Arable and Horticulture (95.21%)	Shallow lime-rich soils over chalk or limestone (73.98)	Chalk (100.00%)	0.18
Avon	18	Norton Bavant	51.183708	-2.1310768	Arable and Horticulture (44.59%)	Shallow lime-rich soils over chalk or limestone (38.49%)	Mudstone, Sandstone and Limestone (100.00%)	115.39
Avon	19	Monkton Deverill SPTW	51.134758	-2.2093736	Arable and Horticulture (52.42%)	Freely draining slightly acid loamy soils (36.10%)	Chalk (62.10%)	31.99
Avon	20	Warminster Barracks	51.193862	-2.1370015	Calcareous Grassland (34.80%)	Shallow lime-rich soils over chalk or limestone (56.69%)	Chalk (100.00%)	7.62
Scotland	1a	River Calder	55.83756	-4.70072	Bog (58.37%)	Blanket Peat (69.02%)	Felsic Lava and Felsic Tuff (53.11%)	11.93
Scotland	2a	Dusk Water	55.763109	-4.56404	Bog (67.04%)	Blanket Peat (63.24%)	Mafic Lava and Mafic Tuff (88.57%)	4.08
Scotland	3a	White Loch	55.73881	-4.41015	Acid Grassland (77.73%)	Peaty Gleys (96.76%)	Felsic Lava and Felsic Tuff (90.07%)	0.04
Scotland	3b	White Loch	55.7422	-4.41055	Heather Grassland (99.50%)	Peaty Gleys (57.00%)	Felsic Lava and Felsic Tuff (99.17%)	0.09
Scotland	3c	White Loch	55.74233	-4.40948	Heather Grassland (99.67%)	Peaty GleyedPodzols (99.80%)	Felsic Lava and Felsic Tuff (56.54%)	0.04
Scotland	4a	Long Loch	55.74941	-4.41663	Freshwater (35.82%)	Peaty Gleys (52.44%)	Felsic Lava and Felsic Tuff (98.04%)	1.48
Scotland	4f	Long Loch	55.74546	-4.42701	Acid Grassland (57.29%)	Peaty Gleys (83.43%)	Felsic Lava and Felsic Tuff (100.00%)	0.04
Scotland	5a	Loch Spallander	55.34099	-4.54672	Coniferous	Peaty Gleys (91.63%)	Sandstone, Siltstone	4.71

					Woodland (91.03%)		and Mudstone (100.00%)	
Scotland	5b	Loch Spallander	55.33676	-4.53809	Coniferous Woodland (100.00%)	Peaty Gleys (100.00%)	Sandstone, Siltstone and Mudstone (100.00%)	0.94
Scotland	5c	Loch Spallander	55.33926	-4.53241	Coniferous Woodland (100.00%)	Peaty Gleys (100.00%)	Sandstone, Siltstone and Mudstone (100.00%)	0.27
Scotland	5d	Loch Spallander	55.34051	-4.52911	Coniferous Woodland (99.48%)	Peaty Gleys (99.48%)	Sandstone, Siltstone and Mudstone (100.00%)	2.43
Scotland	6b	Penwhirn Reservoir	54.98268	-4.94463	Heather Grassland (83.09%)	Blanket Peat (92.10%)	Wacke (100.00%)	1.03
Scotland	6b2	Penwhirn Reservoir	54.98458	-4.94629	Rough Grassland (69.12%)	Peaty Gleyed Podzols (100.00%)	Wacke (100.00%)	0.01
Scotland	6c	Penwhirn Reservoir	54.98584	-4.9482	Coniferous Woodland (58.36%)	Blanket Peat (59.33%)	Wacke (100.00%)	1.71
Scotland	6g	Penwhirn Reservoir	54.99296	-4.94865	Coniferous Woodland (86.85%)	Blanket Peat (100.00%)	Wacke (100.00%)	0.004
Scotland	6g2	Penwhirn Reservoir	54.9917	-4.94721	Coniferous Woodland (100.00%)	Blanket Peat (100.00%)	Wacke (100.00%)	0.004
Scotland	6i	Penwhirn Reservoir	54.98588	-4.9164	Coniferous Woodland (100.00%)	Blanket Peat (100.00%)	Wacke (100.00%)	18.89
Scotland	7a	Dindinnie Reservoir	54.90193	-5.08662	Improved Grassland (47.34%)	Brown Earths (75.09%)	Wacke (100.00%)	0.26
Scotland	7b	Dindinnie Reservoir	54.90276	-5.08944	Rough Grassland (48.67%)	Brown Earths (52.34%)	Wacke (100.00%)	0.64
Scotland	8a	Black Esk Reservoir	55.252842	-3.2533143	Coniferous Woodland (94.80%)	Blanket Peat (99.97%)	Wacke (100.00%)	0.33
Scotland	8b	Black Esk Reservoir	55.256529	-3.2564726	Coniferous Woodland (98.22%)	Blanket Peat (58.22%)	Wacke (100.00%)	0.53
Scotland	8c	Black Esk Reservoir	55.261831	-3.2549840	Coniferous Woodland (100.00%)	Peaty Gleys (84.73%)	Wacke (100.00%)	0.02
Scotland	8c2	Black Esk Reservoir	55.264303	-3.2556009	Coniferous	Peaty Gleys (51.62%)	Wacke (100.00%)	0.03

					Woodland (99.99%)			
Scotland	8c3	Black Esk Reservoir	55.264975	-3.2554775	Coniferous Woodland (100.00%)	NoncalcareousGleys (54.12%)	Wacke (100.00%)	0.08
Scotland	8d	Black Esk Reservoir	55.269260	-3.2602412	Coniferous Woodland (93.17%)	Peaty Gleys (61.31%)	Wacke (100.00%)	14.33
Scotland	8e	Black Esk Reservoir	55.265767	-3.2521382	Coniferous Woodland (85.10%)	Peaty Gleys (89.87%)	Wacke (100.00%)	1.62
Scotland	8e1	Black Esk Reservoir	55.260986	-3.2479888	Coniferous Woodland (100.00%)	Peaty Gleys (100.00%)	Wacke (100.00%)	0.11
Scotland	8e2	Black Esk Reservoir	55.259751	-3.2482249	Coniferous Woodland (100.00%)	Peaty Gleys(100.00%)	Wacke (100.00%)	0.12
Scotland	8f	Black Esk Reservoir	55.230823	-3.2513684	Coniferous Woodland (61.34%)	Peaty Gleys (62.14%)	Wacke (100.00%)	23.23
Scotland	9a	Loganlea Reservoir	55.84543	-3.29378	Acid Grassland (35.80%)	NoncalcareousGleys (25.92%)	Sandstone, Siltstone and Mudstone (35.44%)	4.96
Scotland	9b	Loganlea Reservoir	55.84868	-3.28866	Heather (39.42%)	Humo-Ferric Podzols (76.20%)	Felsic Lava and Felsic Tuff (78.81%)	0.64
Scotland	9c	Loganlea Reservoir	55.85009	-3.28209	Acid Grassland (35.12%)	NoncalcareousGleys (20.13%)	Mafic Lava and Mafic Tuff (33.75%)	6.39
Scotland	10a	Baddinsgill Reservoir	55.783444	-3.3897248	Heather Grassland (54.00%)	Humo-Ferric Podzols (25.40%)	Sandstone and Conglomerate, Interbedded (42.02%)	9.67
Scotland	10b	Baddinsgill Reservoir	55.792084	-3.3932009	Heather Grassland (88.01%)	Peaty GleyedPodzols (39.12%)	Sandstone and Conglomerate, Interbedded (85.27%)	0.78
Scotland	10c	Baddinsgill Reservoir	55.794635	-3.3925116	Heather Grassland (48.35%)	Humo-Ferric Podzols (39.25%)	Sandstone and Conglomerate, Interbedded (98.74%)	6.26
Scotland	10d	Baddinsgill Reservoir	55.792403	-3.3873403	Heather Grassland (73.43%)	Peaty GleyedPodzols (45.92%)	Sandstone and Conglomerate,	0.70

							Interbedded (58.46%)	
Scotland	10e	Baddinsgill Reservoir	55.791339	-3.3876193	Heather Grassland (86.40%)	Peaty GleyedPodzols (60.56%)	Sandstone and Conglomerate, Interbedded (98.74%)	0.75
Scotland	10f	Baddinsgill Reservoir	55.788893	-3.3881932	Heather Grassland (80.65%)	NoncalcaroeusGleys (79.30%)	Sandstone and Conglomerate, Interbedded (100.00%)	0.12
Scotland	11a	West Water Reservoir	55.757271	-3.4147525	Heather Grassland (99.26%)	Peaty GleyedPodzols (100.00%)	Sandstone and Conglomerate, Interbedded (82.39%)	0.24
Scotland	11b	West Water Reservoir	55.755771	-3.4150314	Heather Grassland (100.00%)	Peaty GleyedPodzols (99.99%)	Mafic Lava and Mafic Tuff (99.93%)	0.01
Scotland	11c	West Water Reservoir	55.755472	-3.4145433	Heather Grassland (88.92%)	Peaty GleyedPodzols (99.99%)	Mafic Lava and Mafic Tuff (94.76%)	0.16
Scotland	11d	West Water Reservoir	55.754591	-3.3993673	Heather Grassland (45.75%)	Peaty GleyedPodzols (52.16%)	Sandstone and Conglomerate, Interbedded (43.83%)	7.01
Scotland	11e	West Water Reservoir	55.758919	-3.4058583	Acid Grassland (68.76%)	Peaty GleyedPodzols (83.59%)	Sandstone and Conglomerate, Interbedded (94.75%).	0.13
Scotland	11e2	West Water Reservoir	55.759463	-3.4104073	Heather Grassland (56.49%)	Peaty GleyedPodzols (99.30%)	Sandstone and Conglomerate, Interbedded (100.00%)	0.05
Scotland	11f	West Water Reservoir	55.761497	-3.4180999	Heather Grassland (42.83%)	Peaty GleyedPodzols (47.20%)	Sandstone and Conglomerate, Interbedded (100.00%)	4.46

Scotland	11g	West Water Reservoir	55.760664	-3.4177566	Heather Grassland (73.45%)	Peaty GleyedPodzols (100.00%)	Mafic Lava and Mafic Tuff (39.37%)	0.75
Scotland	12	New Cumnock (y1)	55.393972	-4.1466200	Heather Grassland (49.56%)	Brown Earths (31.77%)	Wacke (56.29%)	0.49
Scotland	13	Elvanfoot (Z1)	55.392683	-3.6664402	Acid Grassland (73.00%)	Peaty GleyedPodzols (77.74%)	Wacke (100.00%)	5.07

Hampshire Avon ion post hoc results. Red represents  $p < 0.01$ , green represents  $p < 0.05$ , numerical values represent mean difference.

Table 1: Statistical analysis of sulphate concentration at the Hampshire Avon catchment.

Sulphate Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1								0.463										0.610	0.505
2								0.421										0.568	0.463
3								0.424										0.571	0.466
4						0.325		0.581										0.728	0.623
5								0.360										0.437	0.432
6				-0.325														0.403	
7																		0.440	0.335
8	-0.463	-0.421	-0.424	-0.581	-0.390				-0.442	-0.560	-0.369	-0.415	-0.549	-0.368	-0.367	-0.438	-0.330		
9								0.442										0.589	0.484
10								0.560										0.707	0.601
12								0.369										0.516	0.411
13								0.415										0.562	0.457
14								0.549										0.696	0.591
15								0.368										0.514	0.409
16								0.367										0.514	0.409
17								0.438										0.585	0.480
18								0.330										0.476	0.371
19	-0.610	-0.568	-0.571	-0.728	-0.537	-0.403	-0.440		-0.589	-0.707	-0.516	-0.512	-0.696	-0.514	-0.514	-0.585	-0.476		
20	-0.505	-0.463	-0.466	-0.623	-0.432		-0.335			-0.601	-0.411	-0.457	-0.591	-0.409	-0.409	-0.480	-0.371		

Table 2: Statistical analysis of sodium concentration at the Hampshire Avon catchment.

Sodium Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1								0.491										1.090	0.449
2								0.541										1.141	0.500
3								0.530										1.130	0.488
4								0.608										1.208	0.567
5								0.544										1.144	0.503
6								0.480										1.080	0.439
7										-0.410								0.930	
8	-0.491	-0.541	-0.530	-0.608	-0.544	-0.482			-0.571	-0.740			-0.646					0.600	
9								0.571										1.171	0.530
10							0.412	0.740			0.478				0.463		0.469	1.340	0.698
12										-0.478								0.862	
13																		0.963	
14								0.646										1.246	0.605
15																		0.959	
16										-0.463								0.877	
17																		0.996	
18										-0.469								0.871	
19	-1.090	-1.141	-1.130	-1.208	-1.144	-1.080	-0.930	-0.600	-1.171	-1.340	-0.862	-0.963	-1.246	-0.959	-0.877	-0.996	-0.871		-
20	-0.449	-0.500	-0.488	-0.567	-0.503	-0.439			-0.530	-0.698			-0.605					0.641	

Table 3: Statistical analysis of potassium concentration at the Hampshire Avon catchment.

Potassium Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1								0.630										1.089	0.693
2								0.565										1.023	0.628
3								0.486										0.944	0.549
4							0.610	0.801										1.280	0.884
5								0.518										0.977	0.581
6																		0.899	0.504
7				-0.610						-0.627			-0.666					0.670	
8	-0.630	-0.565	-0.486	-0.821	-0.518				-0.644	-0.838		-0.572	-0.877	-0.501		-0.567			
9								0.644										1.103	0.707
10							0.627	0.838										1.297	0.901
12																		0.897	0.502
13								0.572										1.030	0.635
14							0.666	0.877										1.335	0.940
15								0.501										0.959	0.564
16																		0.901	0.505
17								0.567										1.026	0.630
18																		0.900	0.504
19	-1.089	-1.023	-0.944	-1.279	-0.977	-0.899	-0.670		-1.103	-1.297	-0.897	-1.030	-1.335	-0.959	-0.901	-1.026	-0.900		
20	-0.693	-0.628	-0.549	-0.884	-0.581	-0.504			-0.707	-0.901	-0.502	-0.635	-0.940	-0.564	-0.505	-0.630	-0.504		

Figure 3: Potassium at the Hampshire Avon catchment

Table 4: Statistical analysis of calcium concentration at the Hampshire Avon catchment.

Calcium Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1																			
2																			
3																			
4																			
5																			
6										0.157									
7										0.198		0.146			0.151	0.165		0.151	
8										0.140									
9								-0.198											
10						-0.157		-0.140											
12																			
13								-0.146											

14																			
15																			
16							-0.151												
17							-0.165												
18																			
19							-0.151												
20																			

Table 5: Statistical analysis of magnesium concentration at the Hampshire Avon catchment.

Magnesium Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1																			
2																			
3																			
4																			
5																			
6																			
7										-0.682	-0.697	-0.722	-0.768		-0.670	-0.702	-0.673		
8										-0.685	-0.699	-0.725	-0.771		-0.672	-0.704	-0.676		
9										-0.815	-0.829	-0.854	-0.601	-0.743	-0.802	-0.834	-0.805		
10							0.682	0.685	0.815										
12							0.697	0.699	0.829										
13							0.722	0.725	0.854										
14							0.768	0.771	0.901										
15									0.743										
16							0.670	0.672	0.802										
17							0.702	0.704	0.834										
18							0.673	0.676	0.805										
19																			
20																			

Table 6: Statistical analysis of nitrite concentration at the Hampshire Avon catchment.

Nitrite Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1																		1.808	
2																		1.800	
3																		1.672	
4																		1.564	
5																		1.696	
6										-0.963								1.349	
7																		1.735	
8																		1.289	
9																		1.964	
10						0.963												2.312	1.064
12																		1.610	
13																		1.680	
14																		1.883	
15																		1.543	
16																		1.784	
17																		1.852	
18																		1.573	
19	-1.808	-1.799	-1.672	-1.564	-1.696	-1.349	-1.735	-1.289	-1.964	-2.312	-1.610	-1.680	-1.883	-1.543	-1.784	-1.852	-1.573		-1.248
20										-1.064								1.248	

Table 7: Statistical analysis of bromide concentration at the Hampshire Avon catchment.

Bromide Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1	█																		
2		█																	
3			█																
4				█															
5					█														
6						█													
7							█												
8								█											
9									█										
10										█									
12											█								
13												█							
14													█						
15														█					
16															█				
17																█			
18																	█		
19																		█	
20																			█

Table 8: Statistical analysis of nitrate concentration at the Hampshire Avon catchment.

NPOC Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
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Nitrate Avon	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20
1								-0.684										0.583	
2								-0.674										0.593	
3								-0.651										0.616	0.528
4								-0.545										0.722	0.635
5								-0.515										0.751	0.664
6																		0.790	0.703
7										0.539	0.537							1.038	0.951
8	0.684	0.674	0.651	0.545	0.515					0.768	0.765	0.722	0.664	0.682	0.686	0.671	0.713	1.267	1.180
9																		0.825	0.737
10								-0.539	-0.768										
12								-0.537	-0.765										
13								-0.722											
14								-0.664										0.603	0.516
15								-0.682										0.585	
16								-0.686										0.581	
17								-0.671										0.596	
18								-0.713											
19	-0.583	-0.593	-0.616	-0.722	-0.751	-0.790	-1.038	-1.267	-0.825				-0.603	-0.585	-0.581	-0.596			
20			-0.528	-0.635	-0.664	-0.703	-0.951	-1.180	-0.737				-0.516						

Table 9: Statistical analysis of NPOC concentration at the Hampshire Avon catchment.

1							0.413	0.504										0.632	
2							0.355	0.448										0.574	
3																		0.436	
4							0.328	0.421										0.547	
5							0.324	0.417										0.543	
6							0.275	0.468										0.594	
7	-0.413	-0.355		-0.328	-0.324	-0.375			-0.326	-0.430	-0.408	-0.434	-0.436	-0.440	-0.409	-0.474	-0.518		-0.324
8	-0.506	-0.448		-0.421	-0.417	-0.468			-0.420	-0.524	-0.502	-0.527	-0.529	-0.533	-0.502	-0.568	-0.611		-0.417
9							0.326	0.420										0.545	
10							0.430	0.524										0.640	
12							0.408	0.502										0.627	
13							0.434	0.527										0.653	
14							0.436	0.529										0.655	
15							0.440	0.533										0.659	
16							0.409	0.502										0.628	
17							0.474	0.568										0.693	
18							0.518	0.611										0.737	
19	-0.632	-0.574	-0.436	-0.547	-0.543	-0.594			-0.545	-0.650	-0.627	-0.653	-0.655	-0.659	-0.628	-0.693	-0.737		-0.543
20							0.324	0.417										0.543	

Table 10: Post Hoc analysis between Nitrite and individual sites at the Conwy catchment, green represents  $p < 0.05$ , red represents  $p < 0.01$ , numerical values represent mean difference

Phosphate	D M 3	N M 2 3
NM19		

Table 11: Post hoc analysis between Phosphate and individual sites at the Conwy catchment. Green represents  $p < 0.05$

Nitrite	CC54	NM18	NM22	NM26
DM3	1.290	1.351	1.317	1.205

Table 12: Post hoc analysis between Bromide and individual sites at the conwy Catchment. Green represents  $p < 0.05$

Bromide	NM14
DM3	1.418

Table 13: Post hoc analysis between Sulphate and sites at the Conwy catchment. Green represents  $p < 0.05$ , red represents  $p < 0.01$ .

Sulphate	CC 13	CC 5	CC 54	CC 6	CC 60	CC 8	CC 88	D M 2	D M 3	D M 4	D M 5	N M 13	N M 14	N M 16	N M 17	N M 18	N M 19	N M 20	N M 21	N M 22	N M 23	N M 24	N M 25	N M 26	N M 27	N M 28	N M 29	N M 30	
CC13	Black				Green				Red	Red									Red			Green	Red						
DM3	Red	Red	Red	Red	White	Red	Red	Red	Black	White	Red	Red	Red	Red	Red	Red	Red	Red	White	Red	Red	White	Red	Red	Red	Red	Red	Red	Red
DM4	Red								White	Black	Red	Red	Red	Red	Green	Red				Red	Red								
DM5					Green				Red	Red	Black								Red			Green	Red						
NM13					Red				Red	Red		Black							Red			Red	Red						
NM14					Red				Red	Red			Black						Red			Red	Red						
NM16														Black								Red	Red						
NM17															Black				Green			Red	Red						
NM18																Black						Green							
NM20																		Black	Red	Red									
NM21					Red														Black	White	Red	Red							
NM22					Red															Black	White	Red	Red						
NM24			Red					Red													Black			Green		Green			

# Appendix 3

## Chlorine use data

Table 1: Chlorine use by samples from 19 different sites within the Hampshire Avon catchment.

Catchment	Site ID	Initial chlorine dose (mg L <sup>-1</sup> )	Total chlorine used (mg L <sup>-1</sup> )	Chlorine used to form THM4 (mg L <sup>-1</sup> )	% chlorine used to form THM4	% total chlorine used
Avon	1	5.00	2.37	0.14	5.91	47.40
Avon	2	5.00	2.53	0.11	4.35	50.60
Avon	3	5.00	2.37	0.07	2.95	47.40
Avon	4	5.00	2.14	0.03	1.40	42.80
Avon	5	5.00	2.47	0.08	3.24	49.40
Avon	6	5.00	2.33	0.15	6.44	46.60
Avon	7	5.00	3.24	0.17	5.25	64.80
Avon	8	5.00	3.04	0.08	2.63	60.80
Avon	9	5.00	2.18	0.05	2.29	43.60
Avon	10	5.00	1.55	0.04	2.58	31.00
Avon	12	5.00	1.63	0.05	3.07	32.60
Avon	13	5.00	1.71	0.10	5.85	34.20
Avon	14	5.00	1.71	0.02	1.17	34.20
Avon	15	5.00	1.35	0.03	2.22	27.00
Avon	16	5.00	1.55	0.01	0.65	31.00
Avon	17	5.00	1.82	0.05	2.75	36.40
Avon	18	5.00	1.98	0.02	1.01	39.60
Avon	19	5.00	5.0	0.02	0.40	100.00
Avon	20	5.00	1.94	0.13	6.70	38.80
<b>Mean</b>		<b>5.00</b>	<b>2.26</b>	<b>0.07</b>	<b>3.20</b>	<b>45.17</b>
<b>Std Dev</b>		<b>0.00</b>	<b>0.20</b>	<b>0.01</b>	<b>0.46</b>	<b>3.82</b>
Conwy	NM19	5	4.27	3.33	66.60	85.33
Conwy	NM20	5	4.07	2.49	49.80	81.33
Conwy	NM21	5	4.40	3.39	67.80	88.00
Conwy	NM22	5	4.40	3.23	64.60	88.00
Conwy	NM23	5	4.67	2.00	40.00	93.33
Conwy	NM24	5	4.73	1.73	34.60	94.67
Conwy	NM25	5	4.73	3.24	64.80	94.67
Conwy	NM26	5	4.70	2.56	51.20	94.00
Conwy	NM27	5	4.43	2.75	55.00	88.67
Conwy	NM28	5	4.45	1.59	31.80	89.00
Conwy	NM29	5	4.75	1.86	37.20	95.00
Conwy	NM30	5	4.27	3.33	66.60	85.33
<b>Mean</b>		<b>5</b>	<b>4.51</b>	<b>2.56</b>	<b>2.56</b>	<b>51.22</b>
<b>Std Dev</b>		<b>0</b>	<b>0.68</b>	<b>0.68</b>	<b>0.21</b>	<b>4.12</b>

Table 2: Raw data of nitrogenous and carbonaceous containing groups from the Hampshire Avon.

Site	NPOC (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )	DON (mg L <sup>-1</sup> )	DIN (mg L <sup>-1</sup> )
1	5.07	6.66	0.38	6.28
2	6.71	7.03	0.22	6.81
3	6.66	7.07	0.22	6.85
4	5.58	9.34	0.39	8.95
5	6.82	4.58	0.21	4.37
6	6.42	3.55	0.35	3.20
7	12.59	2.74	0.61	2.13
8	15.59	2.54	1.03	1.51
9	5.95	4.86	0.56	4.30
10	4.83	8.41	0.46	7.95
12	4.87	7.65	0.25	7.40
13	5.10	6.62	0.45	6.17
14	5.33	6.49	0.43	6.06
15	5.02	7.15	0.14	7.01
16	5.28	6.39	0.14	6.25
17	4.48	6.29	0.15	6.14
18	3.71	7.26	0.33	6.93
19	31.21	37.98	13.11	24.87
20	6.58	10.46	1.17	9.29
<b>Mean</b>	4.39	0.88	0.13	0.75
<b>Std. Dev</b>	1.84	0.97	0.08	1.01

Table 3: Distribution of chlorine use at the sites from the Conwy catchment.

Site	Cl <sub>2</sub> used on THM4 (mg L <sup>-1</sup> )	Cl <sub>2</sub> used on non-THM4 formation (mg L <sup>-1</sup> )	Total Cl <sub>2</sub> used (mg L <sup>-1</sup> )	Total Cl <sub>2</sub> remaining (mg L <sup>-1</sup> )
NM19	3.33	0.94	4.27	0.73
NM20	2.49	1.57	4.07	0.93
NM21	3.39	1.01	4.40	0.60
NM22	3.23	1.17	4.40	0.60
NM23	2.00	2.67	4.67	0.33
NM24	1.73	3.01	4.73	0.27
NM25	3.24	1.49	4.73	0.27
NM26	2.56	2.14	4.70	0.30
NM27	2.75	1.68	4.43	0.57
NM28	1.59	2.86	4.45	0.55
NM30	1.86	2.89	4.75	0.25

Table 4: Mean concentration of THM compounds and total THM4 concentration formed from 1 mg L<sup>-1</sup> NPOC at the Hampshire Avon sites.

Site ID	CHCl <sub>3</sub> (µg/L)	CHCl <sub>2</sub> Br (µg/L)	CHBr <sub>2</sub> Cl(µg/L)	CHBr <sub>3</sub> (µg/L)	THM4 (µg/L)
1	49.10	12.37	5.52		67.00
2	43.06	11.40	4.44		58.90
3	62.24	12.83	5.64	15.60	96.31
4	40.02	13.16	5.63		58.81
5	49.11	11.57	4.42		65.09
6	58.66	11.79	5.45		75.89
7	99.22	11.00	4.29		114.51
8	328.72	10.76	4.75		344.24
9	45.79	12.38	4.52		62.69
10	22.57	12.18	5.86	14.38	55.00
12	29.90	12.18	6.84		48.93
13	25.58	12.29	5.83		43.70
14	26.09	11.65	4.58		42.32
15	29.49	11.61	4.58		45.68
16	34.61	10.54	6.38		51.53
17	26.03	11.27	4.54		41.84
18	43.39	13.65	6.78		63.82
19	20.48	12.11	4.54	13.40	50.53
20	36.53	9.51	6.34		52.39
<b>Mean</b>	<b>56.35</b>	<b>11.80</b>	<b>5.31</b>	<b>14.46</b>	<b>75.75</b>
<b>Std. Dev</b>	<b>15.71</b>	<b>0.22</b>	<b>0.20</b>	<b>0.64</b>	<b>15.50</b>

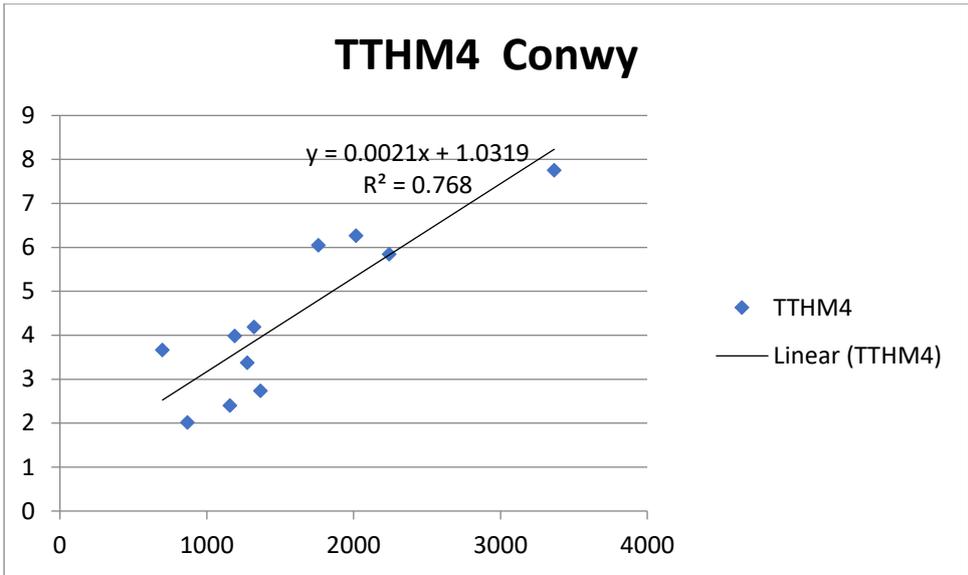


Figure 1: Regression analysis between NPOC concentration and the TTHM4 concentration at the Conwy catchment, showing a strong positive regression and an  $R^2$  of 0.77, showing that, as the NPOC concentration increases, so does the TTHM4 concentration.

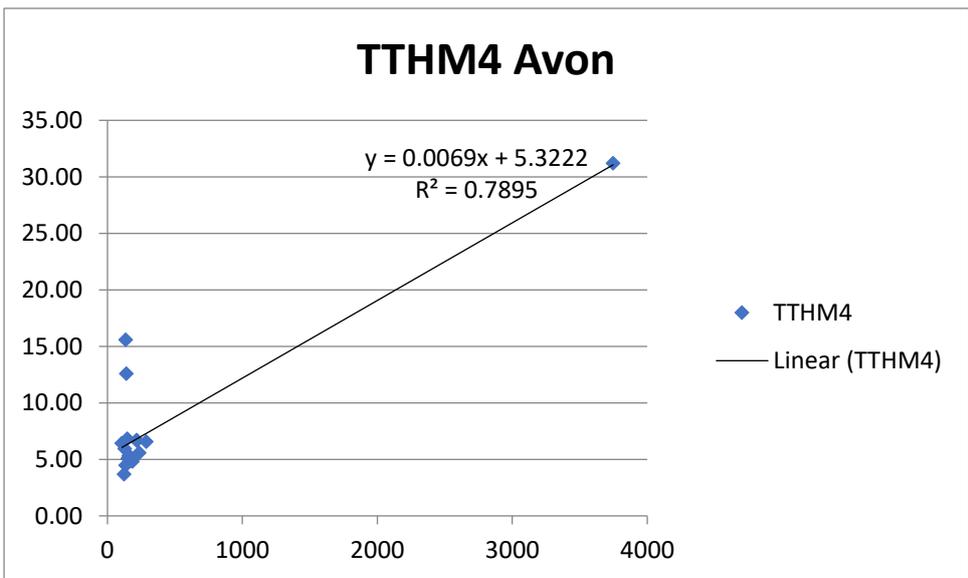


Figure 2: Regression analysis between NPOC concentration and the TTHM4 concentration at the Hampshire Avon catchment, showing a strong positive regression and an  $R^2$  value of 0.79, showing that, as NPOC concentration at the Avon catchment increases, so does the TTHM4 concentration

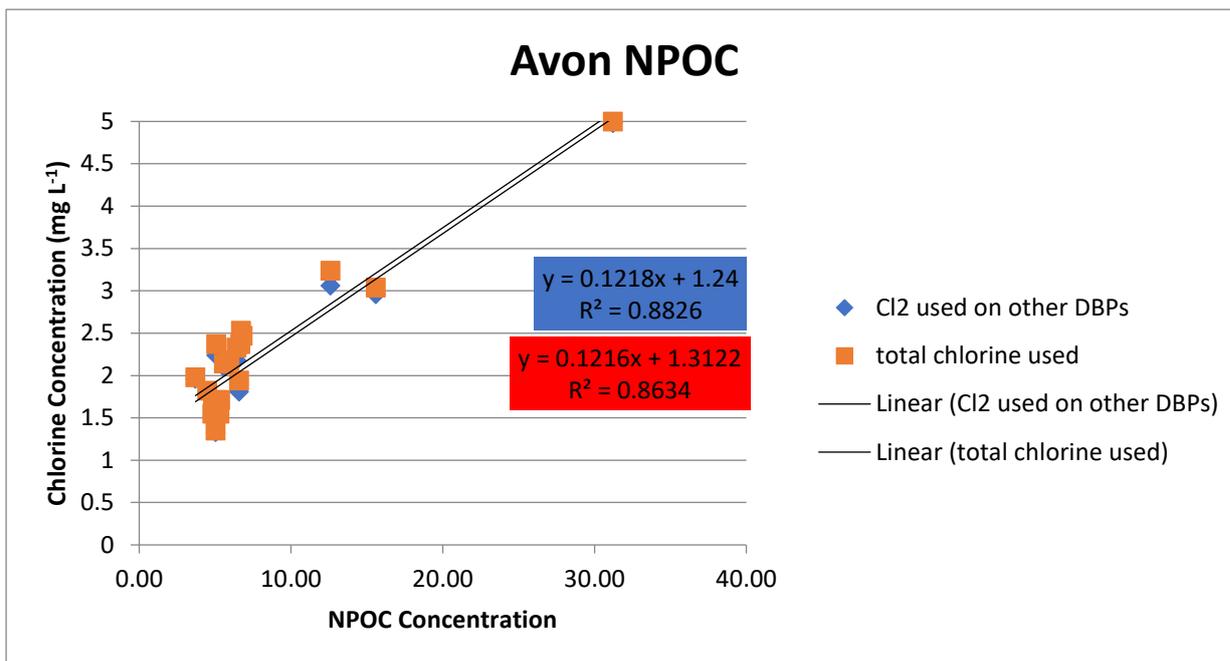


Figure 3: Regression analysis between NPOC concentration at the Hampshire Avon and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

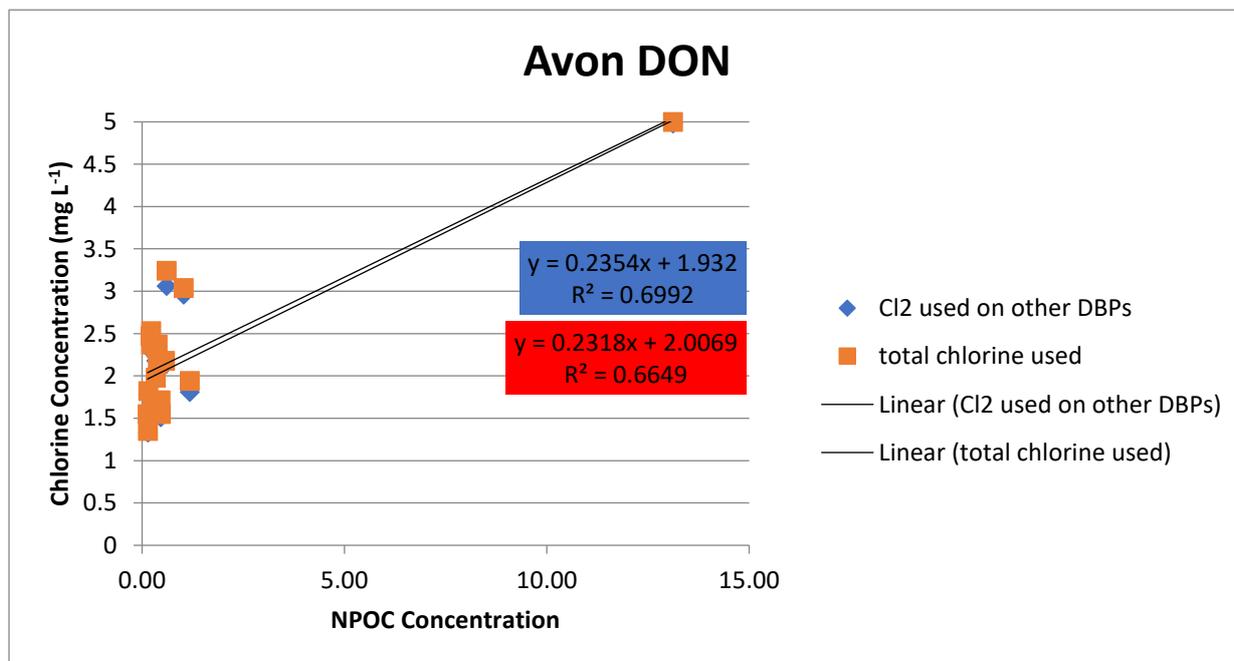


Figure 4: Regression analysis between DON concentration at the Hampshire Avon and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

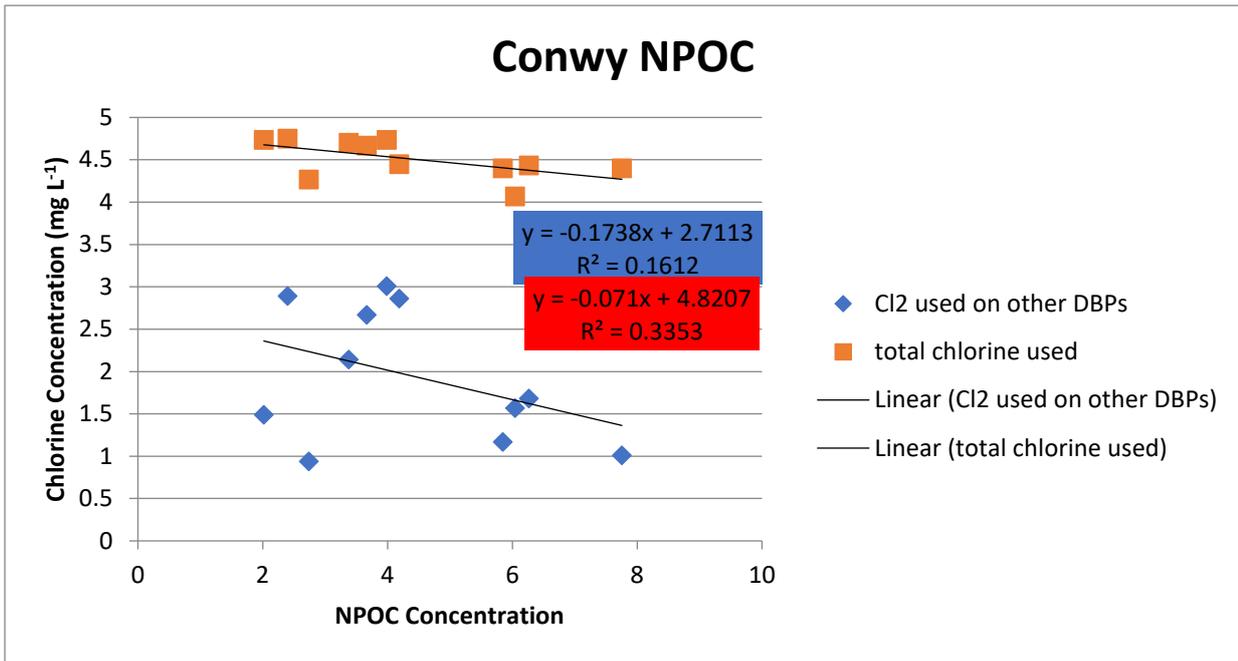


Figure 5: Regression analysis between NPOC concentration at the Conwy catchment and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

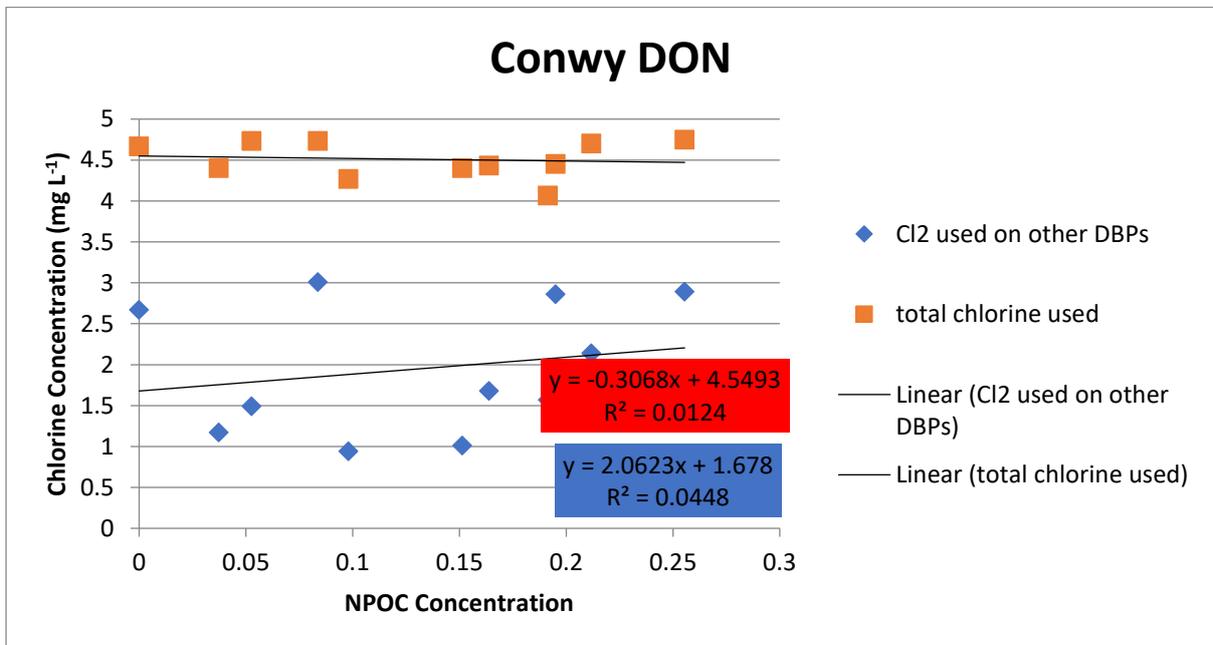


Figure 6: Regression analysis between DON concentration at the Conwy catchment and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

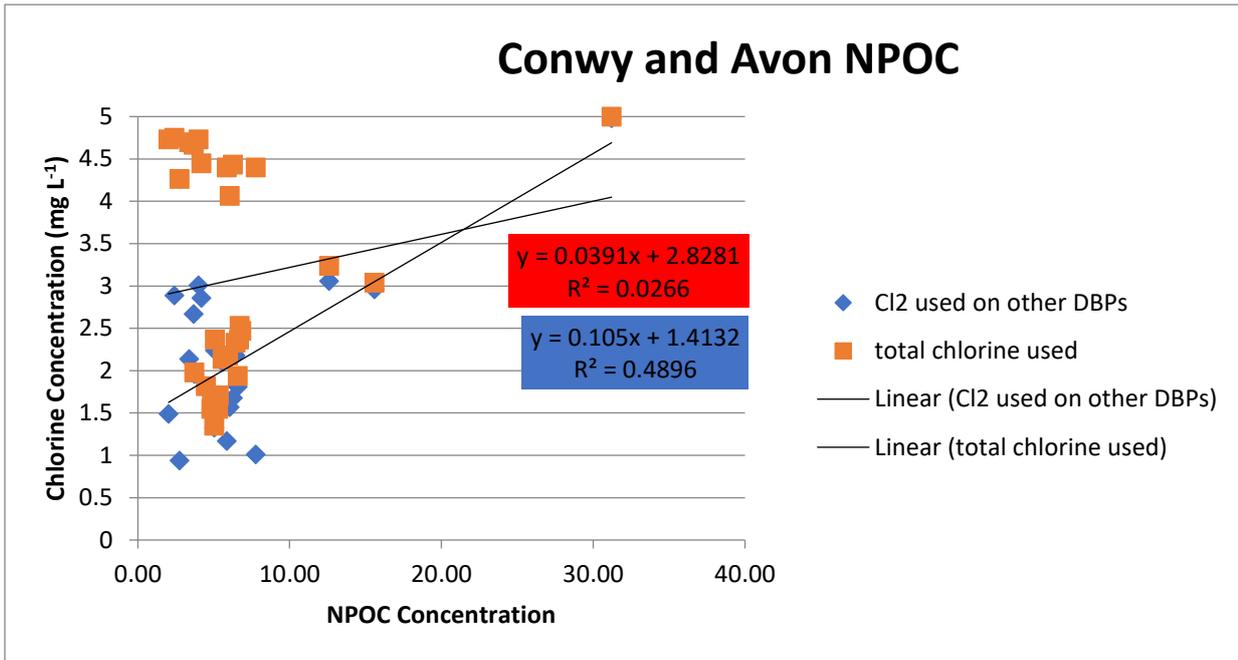


Figure 7: Regression analysis between NPOC concentration at the Hampshire Avon and the Conwy catchments and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

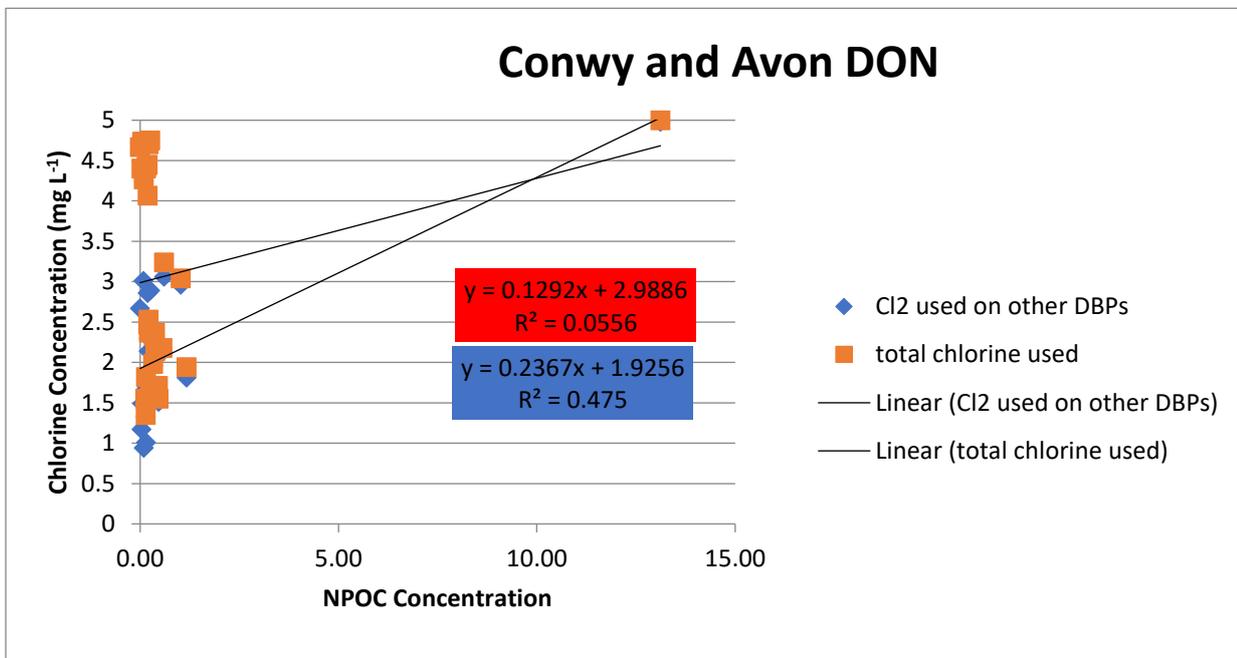


Figure 8: Regression analysis between DON concentration at the Hampshire Avon and the Conwy catchments and the concentration of chlorine used (from a total of 5 mg L<sup>-1</sup> free chlorine) and the concentration of chlorine used in anything but THM4 formation.

## **Appendix 4**

### **A comparison of chlorination vs. chloramination of natural waters: disinfection by-products and disinfection efficiency.**

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#### **Abstract**

Water destined for human consumption has historically been chlorinated for nearly 120 years, initiated by a cholera outbreak in London, UK in 1897. Increased detection of emergent compounds, and an increasingly health-conscious population has led to the investigation of the effects of chlorine upon domestic water supply. Studies have outlined 600 compounds, thought to pose a danger to humans due to their potential toxicity and carcinogenicity, coined disinfection by-products (DBPs). Human exposure can be through daily usage, such as consumption, bathing and cooking. However, only four of these compounds are currently regulated in the UK, called trihalomethanes (THMs), formed when organic matter is exposed to chlorine. An alternative disinfectant called chloramine is increasingly utilised to reduce THM concentrations, but this disinfectant can elevate concentrations of other unregulated compounds, such as nitrogenous DBPs (NDBPs) about which much less is known. Little literature is available comparing the efficiencies of chlorination vs. chloramination, leaving a

knowledge gap in understanding the advantages and disadvantages of both disinfectants. Here, a method to determine bacteria removal efficiencies and disinfection potential of both chlorine and chloramine treatments has been developed. Chlorination showed a two times greater bacteria removal when compared to chloramination, whereas chloramination appeared to favour the formation of DBPs compounds over destroying bacteria. Whilst chloramination formed on average 82% less  $\text{CHCl}_3$  than chlorination. Findings from this study can help to bridge the gap in knowledge regarding the advantages and disadvantages of both chlorination and chloramination, contributing towards a cleaner, safer drinking water for future generations.

## **Introduction**

Disinfection by-products (DBPs) are the focus of many studies due to their reported carcinogenicity (Dunnick and Melnick, 1993). To render it fit for drinking, all fresh water should be disinfected to remove any bacteria, viruses and pathogens, and treated to remove suspended solids, such as dissolved organic matter (DOM), which is present in all freshwater globally. Rudimentary systems (such as the utilisation of sand filtration) and chemical treatment (such as chlorine disinfection), have paved the way to ending waterborne virus and bacteria epidemics in the developed world for over a century, however, progressions in analytical biology and chemistry have led to more sophisticated cleaning and disinfection methods.

Although these methods can control microbial pathogens effectively, they can also contribute to elevated levels of DBPs (Li *et al.*, 2008). The majority of removable DOM is comprised of large, high molecular weight compounds, and as a result, conventional treatments (coagulation and flocculation, gravity filters and activated carbon, for example) are more favoured towards their removal. Therefore, the smaller DOM compounds can pass through these treatment systems and remain present in the water at the disinfection stage of treatment (Zhang *et al.*, 2012), and this is where the majority of DBPs are formed.

DBPs are formed when chlorine, or its compounds, reacts with organic matter and/or bromine/iodine found in the natural water, forming halogenated compounds, many of which have found to be potential carcinogens in relation to human health (Goslan *et al.*, 2009). The most commonly studied DBPs include tri-halogenated methane compounds, formed when

halogens (in this example, chlorine and bromine) bind to the carbon atom in the methane compound in place of two or three hydrogen atoms. These are classed as THMs, and are the four most widely recognised DBPs, containing the compounds dibromochloromethane, bromodichloromethane, trichloromethane and tribromomethane (see figure 1), known collectively as THM4. Despite over 600 DBPs being identified in chlorinated drinking water to date (Krasner *et al.*, 2006), only 4 DBPs, THM4, are regulated in UK and USA drinking water at  $100 \mu\text{g L}^{-1}$  by the World Health Organisation (WHO) (2006). Another group of halogenated compounds, named haloacetic acids (HAAs), are also regulated in USA drinking water at  $60 \mu\text{g L}^{-1}$ , and although their regulation in the UK is under consideration, more research into their formation and toxicity, and indeed the formation and toxicity of other non-regulated harmful DBPs, can only assist the regulatory boards responsible.

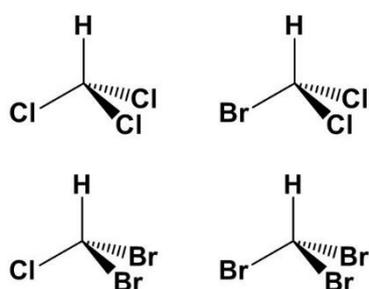


Figure 1: The chemical structure of the 4 regulated THM4 compounds. From left to right top to bottom: Trichloromethane/Chloroform, Bromodichloromethane, Chlorodibromomethane, Tribromomethane/Bromoform.

THM4 are known to form in high concentrations when DOM rich water is treated with chlorine, although their formation is also influenced by chlorine dose, concentration and nature of DOM, contact time, pH, temperature of water, bromide ion presence and occurrence (Gopal *et al.*, 2007). In an effort to reduce THM formation potential (THMFP), many water treatment companies have switched from chlorine to chloramine disinfection as this has been found to form fewer THMs than chlorine (Brodthmann and Russo, 1979). This change from

chlorine to chloramine is to ensure that the final water falls below the THM4 limit of  $100\mu\text{g L}^{-1}$  at the consumer's tap, over an average of 4 measurements per year, saving money on treatment costs and chemical usage.

However, further research has proved that chloramination, whilst forming less of the regulated THM4, formed many other potentially more toxic DBPs. These DBPs occur in much smaller concentrations but have much higher potency, and as a result, disinfection choice has primarily reverted to chlorination again. The DBPs that are favourably formed by chloramination are typically nitrogenous in nature (such as haloacetonitriles, haloacetic acids and haloacetamides) and are therefore referred to as N-DBPs. Few studies have systematically compared chlorination and chloramination efficiencies in terms of THM4 formation, despite increasing pressures on water companies due to an increase in DOC (Freeman *et al.*, 2004). A deeper understanding of the characteristics of chloramine, and the DBPs that it forms, is needed to provide information on its advantages and disadvantages for the drinking water industry. Therefore, a treatment regime, which controls pathogens effectively but also forms fewer DBPs, is required to continue to ensure the safety and cleanliness of drinking water.

This paper analyses the DBP formation potential from a  $5\text{mg mL}^{-1}$  dose of chlorine per  $1\text{mg mL}^{-1}$  DOC concentration to a  $0.025\text{mg mL}^{-1}$  dose of chloramine per  $1\text{mg mL}^{-1}$  DOC concentration, to determine whether a lower dose of chloramine (than chlorine) is sufficient to disinfect and protect drinking water and form fewer DBPs. This paper also provides a standardised parallel chloramination and chlorination method of disinfecting natural waters in an attempt to bridge the 'chloramine knowledge gap', assessing the different concentrations of DBPs formed from each treatment at the same temperature and pH, over the same timeframe, but more importantly, from the same water.

## Methodology

### Site Description

Sampling sites were located along the headwater reaches of the River Avon, Hampshire, UK, which drains a series of sub-catchments of relatively similar character (high nutrient waters draining typically agricultural land over chalky geology). The two rivers, the River Wyyle and the River Nadder were selected, which join to form the River Avon just south-west of Salisbury, UK (See figure 1 for location map). They are both chalk streams that drain land dominated by intense agricultural activities, thus containing high concentrations of DOC and DON, amongst other nutrients (Yates *et al.*, 2016 and Withers *et al.*, 2012). Analysis from between September 2015 to September 2016 showed a mean DOC concentration of 7.69 ( $\pm 1.11$ ) mg mL<sup>-1</sup> and a mean DON concentration of 7.87 ( $\pm 1.89$ ) mg mL<sup>-1</sup>. Grab samples were collected in clean 500mL PET plastic bottles and shipped (in a cool box) overnight to Bangor University for analysis, where the samples were promptly filtered through 0.45  $\mu$ m pore size papers (#HAWP04700, lot R6EA56474, Merck Millipore Ltd., Ireland) and stored in the dark at 4°C prior to analysis.

Water for bacterial analysis was collected from Afon Hiraethlyn, just outside the hamlet of Graig, north Wales, UK before it joins the river Conwy. This river drains predominantly agricultural land and was therefore thought to contain high concentrations of bacteria. The water was collected and left outside in an amber brown 5L bottle for 7 days to encourage bacterial growth, prior to analysis. See figure 2 for a catchment map of the Hiraethlyn catchment.

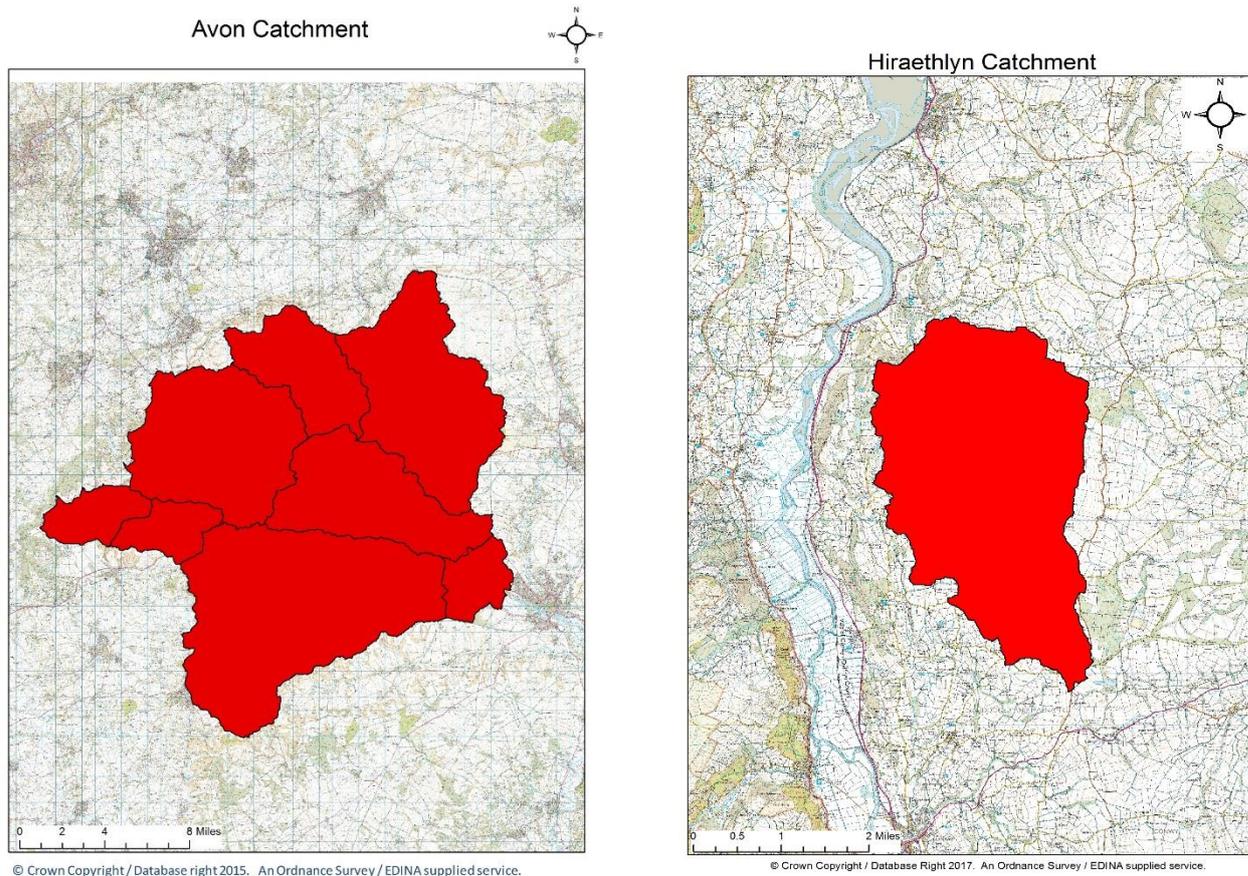


Figure 1 (left): Location of Avon catchment in south England, UK.

Figure 2 (right): Catchment map of the Hiraethlyn catchment in the lowland catchment of the River Conwy, north Wales, UK.

**Measuring concentration of sodium hypochlorite (NaOCl) solution(Following APHA Standard Method 4500-Cl C (AHPA, 1992) and Lee and Westerhoff (2009).**

*(see supplementary material for images)*

Following the APHA standard method 4500-Cl-C, 400 $\mu$ L of sodium hypochlorite (6-14% available chlorine, #425044, Sigma Aldrich) was added to a 25 mL volumetric flask with ultrapure water (henceforth referred to as Milli-Q). In a 100 mL volumetric flask, 1.58g

sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) (#217263 (99%), *Sigma Aldrich*), was dissolved in Milli-Q to 100 mL.

In a conical flask, 5 mL 99% glacial acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ) (#A/0360/PB17, *Fisher Chemical*) was added to 1 g potassium iodide (#P2963, *Sigma Aldrich*) to create an excess, mixed well and the 25 mL diluted sodium hypochlorite solution was added. The sodium thiosulphate solution was then slowly titrated into the conical flask, whilst agitating, until the intensity of the yellow colour had almost disappeared. 1 mL of 1% starch solution (#319554, *Lot MKBT3835V, Sigma Aldrich*) was then added and the solution was agitated in a circular motion until the colour turned inky black, before continuing titration using the sodium thiosulphate solution, stopping when the black colour was fully discharged. The volume of titrant used was then recorded and applied to the formula in equation 1.

$$\text{Hypochlorite concentration (mg mL}^{-1}\text{ Cl}_2) = \frac{(\text{M} \times 35.45 \times \text{titrant volume})}{\text{hypochlorite added}}$$

(Where *M* is molarity of titrant (0.1M), hypochlorite added is 0.4mL (or 8 mL, see below). All volumes are in mL. See supplementary material Equation 1 for an example of the above equation).

The volume of hypochlorite needed to make a 1000mg L<sup>-1</sup> dosing solution was calculated using the following formula:

$$\text{Hypochlorite required (mL)} = \frac{1250}{\text{hypochlorite concentration (mg mL}^{-1}\text{)}} \left. \right\} /5$$

(See supplementary material Equation 2 for an example of the above equation)

It is important to note that the titration method should require at least 10 mL of titrant to present a 0.1% error. If this error was not sufficiently low, 0.8 mL of sodium hypochlorite solution was used giving a 0.05% error.

Equation 1: Calculation of hypochlorite concentration.

### Adjusting molarity of chloramine components

The molarity of sodium hypochlorite solution was determined by dividing the concentration (in mg mL<sup>-1</sup>) by molar weight of sodium hypochlorite; 74.442 g mol<sup>-1</sup> (*see supplementary material equation 3 for an example of this calculation*).

This solution was diluted to 0.8 M in a 100 mL volume with Milli-Q, (see equation 4 in supplementary material for an example calculation), before being labelled, and stored in a dark fridge. Meanwhile, 5.349 g of ammonium chloride (NH<sub>4</sub>Cl) (#A9434, Lot# BCBM8338V, >99%, Sigma Aldrich) was added to a 100 mL volumetric flask, before Milli-Q was added to form 1.0 M ammonium chloride. This was also labelled and stored in the dark. This mixture of 0.8 M sodium hypochlorite to 1 M ammonium chloride provided a ratio of approximately 4:1 by mass, to minimise ammonia (which can cause nitrification in the distribution system), maximise chlorine and to avoid generating dichloramines (Goslan, 2016).

Before use, 10 mL each of 1.0 M ammonium chloride solution and 0.8 M sodium hypochlorite solution was mixed into a 100 mL amber brown bottle in a fume cupboard, before being capped and stored for 150 minutes at room temperature. A 100 mL amber septa-capped vessel is suggested due to the pressures generated.

### **Chloramine titration (following AHPA method 4500-Cl-F (APHA, 1992)).**

Following AHPA Method 4500-Cl-F, the standard ferrous ammonium sulfate titrant was made by dissolving 1.106 g ammonium iron (III)sulfate hexahydrate (Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O) (#215406, Lot MKBS7802V, Sigma Aldrich), in Milli-Q containing 250 µL sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) (95-98%, #320501, Sigma Aldrich) and was made up to 1 L with Milli-Q.

A phosphate buffer solution was made by dissolving 24 g anhydrous disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>) (#71640, Lot BCBN8058V, Sigma Aldrich) and 46 g anhydrous monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) (#P9791, Lot SLBL2497V, Sigma Aldrich), in Milli-Q. These were mixed, before 800 mg disodium ethylenediamine tetraacetate dehydrate (EDTA) (#EDS, Lot

*BCBN4261V, Sigma Aldrich*) was dissolved into 100 mL Milli-Q and the two solutions combined. This new solution was then diluted to 1 L with Milli-Q and 20mg mercury (II) chloride ( $\text{HgCl}_2$ ) (#215465, Lot *SLBG6022V, Sigma Aldrich*) was added to prevent microbial growth and interference.

In a conical flask, 5 mL phosphate buffer and 500 mg of N,N-Diethyl-p-phenylenediamine sulphatesalt (#07672, Lot *BCBQ7288V, Sigma Aldrich*) were mixed and agitated until fully dissolved. 100  $\mu\text{L}$  of chloramine stock solution, made 150 minutes previously, was added to a 100 mL graduated measuring cylinder containing 99.9 mL Milli-Q to make a 1000 times dilution of the original (stock) chloramine solution. This working solution was then added to the conical flask and mixed. A gradual colour change to bright pink was observed over the course of 1 minute (See image 3 in supplementary material).

The titrant was poured into a 50 mL burette ensuring that no bubbles were present. The titrant was then titrated into the conical flask until the pink colour was discharged (see image 4 in supplementary material), with thorough mixing using a circular swirling motion by hand. The volume of titrant used was recorded (reading from the bottom of the meniscus (see images 1 and 2 in supplementary material)) and used in the following equation:

$$\text{Free chlorine (mg L}^{-1}\text{)} = \text{volume of titrant used (mL)}$$

Note, if more than 5 mL of titrant was required, the solution would not change colour from pink to clear. A smaller volume of the stock chloramine solution was therefore required until the desired colour change was observed between 0 mL and 5 mL titrant used. Ensuring the stock solution is kept sealed in a 100 mL amber septa capped bottle would help reduce the rapid decline of the chloramine solution concentration (see figure 4).

### **Dosing solution**

The concentration of the chloramine dosing solution was adjusted to 1000 mg L<sup>-1</sup> free chlorine. This high concentration of dosing solution was diluted 200-fold when added to the 95.5 mL sample (created later in experiment) thus creating a 5 mg L<sup>-1</sup> concentration in the sample (*see equation 5 in supplementary material*). As chloramine concentration declines over time, it is important to measure chloramine concentration and then treat as quickly as possible after determining concentration to ensure that the desired concentration of chloramine is added to the sample to be treated (see figure 4).

### **Chloramination of samples**

Each water sample was diluted to contain only 1 mg L<sup>-1</sup> DOC (*see equation 6 in supplementary example for an example of this equation*). The calculated volume of sample was added to a 100 mL measuring cylinder, and made up to 50 mL with Milli-Q. 2 mL of phosphate buffer (0.5M KH<sub>2</sub>PO<sub>4</sub>) was added to stabilise pH throughout the experiment and the solution was made up to 99.5mL with Milli-Q.

The solution was then added to a 100 mL amber bottle with PTFE lid (#565625, Fisher Scientific), and 0.5 mL of 1000 mg L<sup>-1</sup> chloramine solution was added (to give 5 mg L<sup>-1</sup> free chlorine per 1 mg L<sup>-1</sup> of DOC). This was added to the sample bottle rather than the measuring cylinder to prevent any additional exposure of further samples to residual chloramine remaining in the measuring cylinder. The bottle was capped, labelled and stored in the dark at 25 °C for 7 days. Chlorinated samples were created using exactly the same method but the correct dose of chlorine solution (made from sodium hypochlorite) was used instead of chloramine.

After 7 days in the dark at 25 °C, 15 mL aliquots of samples were transferred into 20mL amber GC vials (#SU860098, Supelco) with 18 mm screwcap silicone/PTFE septum lids (#SU8660101, Supelco), with 0.5 mL of sodium sulphite solution to quench the chloramine, thus preventing any remaining free chlorine from continuing to react with organic molecules still present in the sample. The sodium sulphite (Na<sub>2</sub>SO<sub>3</sub>) (98%, #SO505, Sigma Aldrich) solution was created by adding 10g sodium sulphite to a 100 mL volumetric flask, and filling to the mark with Milli-Q, thus creating 100 g L<sup>-1</sup> (793.38mM) sodium sulphite solution.

0.5 mL of this solution was then added to the vial after the 15 mL of sample was first added, to produce an excess of sodium sulphite, which destroys the chlorine (0.1 mL of sodium sulphite destroys approx. 1 mg L<sup>-1</sup> of Cl<sub>2</sub>). These samples were analysed using a GC. 6 calibration solutions (1, 5, 20, 50, 100 and 200 µg L<sup>-1</sup>, made using Accustandard THM4 standards (See supplementary material for specific information) and placed in vials for analysis before and after the samples, along with two blanks (untreated Milli-Q).

### **Disinfection potential**

APHA method 9222B was followed to determine the bactericidal properties of chlorine (NaOCl) and chloramine. This method is suited for the growth (and therefore the detection) of *Escherichia coli*, *Enterobacter aerogenes*, and *Proteus mirabilis*. These strains are found prolifically in intestinal tracts of mammals and are also found soil and water, thus good indicators of the pathogenic bacteria concentration of water. It is well established that runoff from lowland UK pastoral catchments can be a significant source of faecal indicator organisms (Crowther *et al.*, 2002), and so by combining a carefully selected site with this APHA method, high colony numbers can be detected and the impact that free chlorine has upon them can be visualised in greater detail .

Agar medium (*m-Endo Agar LES*, #85766-500g-F, lot BCBM2741V, Sigma Aldrich), was created and poured into 47 mm agar plates (#800100, lot 5111601T, Advantec MFS Inc, California, USA) and allowed to cool. 10 mL of river water (collected from a stream draining agricultural land in north Wales, UK 1 week prior to experiment, to increase the possibility of detectable bacteria), was added to 90 mL Milli-Q, before being subjected to 0.1, 0.5, 1 and 5 mg L<sup>-1</sup> doses chlorine and chloramine. The treated samples were stored in sterile amber brown 100 mL bottles (baked at 550 °C for 3 hours after washing, to sterilise at room temperature for 75 minutes, to allow sufficient contact time with available chlorine, before the samples were passed through sterile 0.45 µm HA MF<sup>TM</sup> membrane filters (#HAWP04700, lot R6EA56474, Merck Millipore Ltd., Ireland). The membranes were carefully transferred to an absorbent pad (#AP10047E0, lot R5AA60853, Merck Millipore Ltd., Ireland) soaked in 2 mL lauryl sulphate broth (#17349-500g, lot BCBP6927V, Sigma Aldrich) (in the lid of the agar plate), and left to incubate at 35 °C in high humidity for 2 hours. Then, the filters were carefully transferred to the agar surface using flat bladed forceps, the soaked membrane

discarded, the lids put on top of the plate and the plates inverted. After 20 hours further at 35 °C (without high humidity), the colonies (metallic in appearance) were counted and total coliform forming units (TCC) was calculated using the following equation:

$$\text{Total Coliform Colonies/100 mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL Sample filtered (undiluted)}}$$

Due to the possibility of bacterial interference in the coliform colony growth stage, a blank plate was created that was subjected to the same environment as all sample plates.

## Analysis

Analysis was carried out using a Varian GC 450, equipped with a <sup>63</sup>Ni Electron Capture Device, using solid phase micro-extraction (SPME) to determine the concentration of CHCl<sub>3</sub>, using a method developed from Sarrion *et al.*, (2000). The column selected was a Zebron ZB-CLPesticides-1 (#7HM-G028-51, Phenomenex, UK) with a N<sub>2</sub> carrier gas flow of 10 mL min<sup>-1</sup>. The oven was programmed to start at an initial temperature of 35 °C held for 9 minutes, before increasing to 140 °C at 10 °C min<sup>-1</sup> and held for 2 minutes, followed by an increase to 180 °C at 10 °C min<sup>-1</sup>, and held for 3 minutes. A straight glass liner (Restek, USA, #21113) was fitted into the injection port, the temperature set to 290 °C with an applied split ratio of 35:1. The ECD temperature was set to 300 °C with a make-up flow of 25 mL N<sub>2</sub> and cell contact potential at -540 ± 100 mV to establish maximum sensitivity and to accommodate any changes of the detector foil surfaces due to oxidation products over time

Samples were incubated at 40 °C for 10 minutes, before a SPME fibre (Carboxen™, polydimethylsiloxane, 75 µm, 23 gauge (#57343-U, Sigma Aldrich)) was then immersed to a depth of 5 mm into the sample for 15 seconds before being desorbed in the injection port for 5 minutes at 295 °C (Hughes, 2013).

## Map creation

Location maps of catchment (see figures 1 and 2) and dominant land use data (see figure 5),

was created using ArcGIS 10.4.1 software (Environmental Systems Research Institute (ESRI), California, USA), using map data acquired from Digimap (University of Edinburgh, UK), and the LCM2007 land use dataset (Centre for Ecology and Hydrology (CEH), Wallingford, UK).

### **Statistical Analysis**

Statistical analysis was carried out using IBM SPSS Statistics 22 software (IBM, New York, USA). The following tests were used, unless otherwise stated.

A test of skewness determined normality (values between -2 and +2 were deemed acceptable after George and Mallery, 2010). If data was skewed, it was transformed by  $\text{Log}^{10}$  and skewness checked again. ANOVA tests were carried out and Levene's Statistic determined whether there were any significant differences between the groups. If Levene's statistic was not showing any significant differences (i.e.  $p < 0.05$ ), then a Welch robust test of equality of mean was used. Tukey HSD was selected as the posthoc test to determine which specific sites the differences were between.

Due to its high concentration in all samples ( $67.45\% \pm 2.98$  of total THM4 for chlorination,  $88.82\% \pm 4.72$  of total THM4 for chloramination), chloroform ( $\text{CHCl}_3$ ) was used to represent THM4. In order to determine whether the differences in concentration between sites were statistically significant to each other, an ANalysis Of VArience test was carried out on both river datasets.

Pearson's correlations were carried out to determine relationships between two quantitative and continuous datasets.

## Results

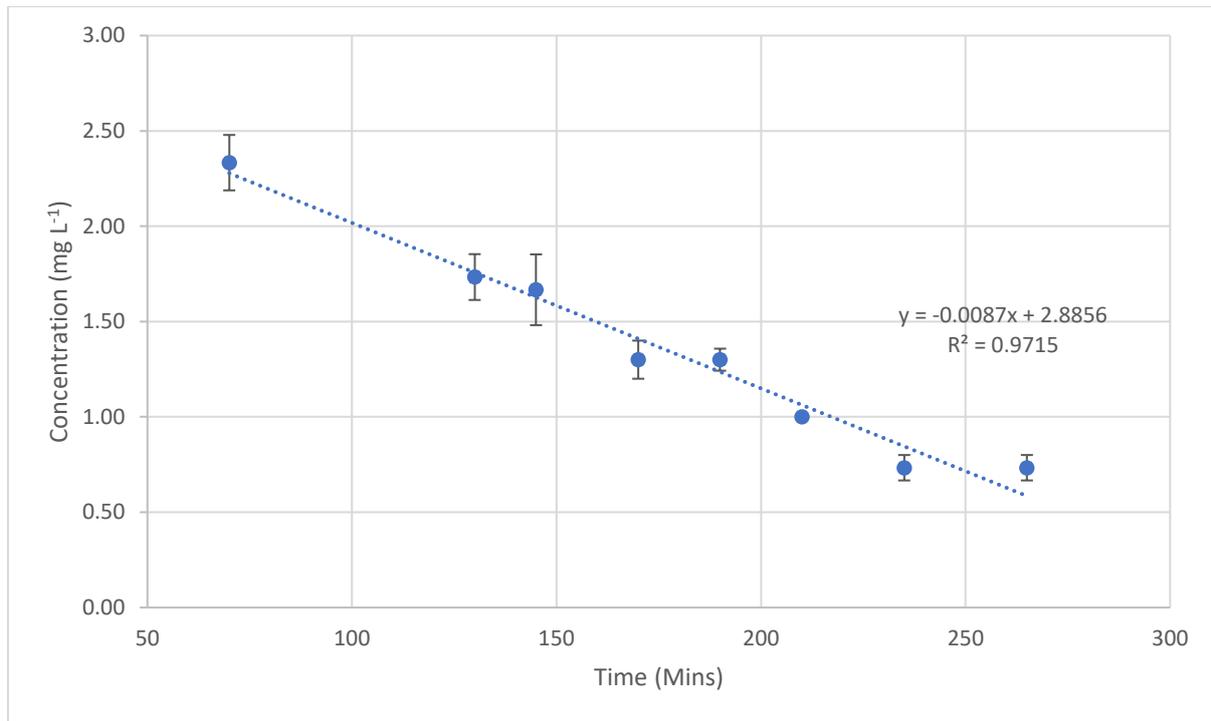


Figure 4: Chlormaine decline over time, with error.

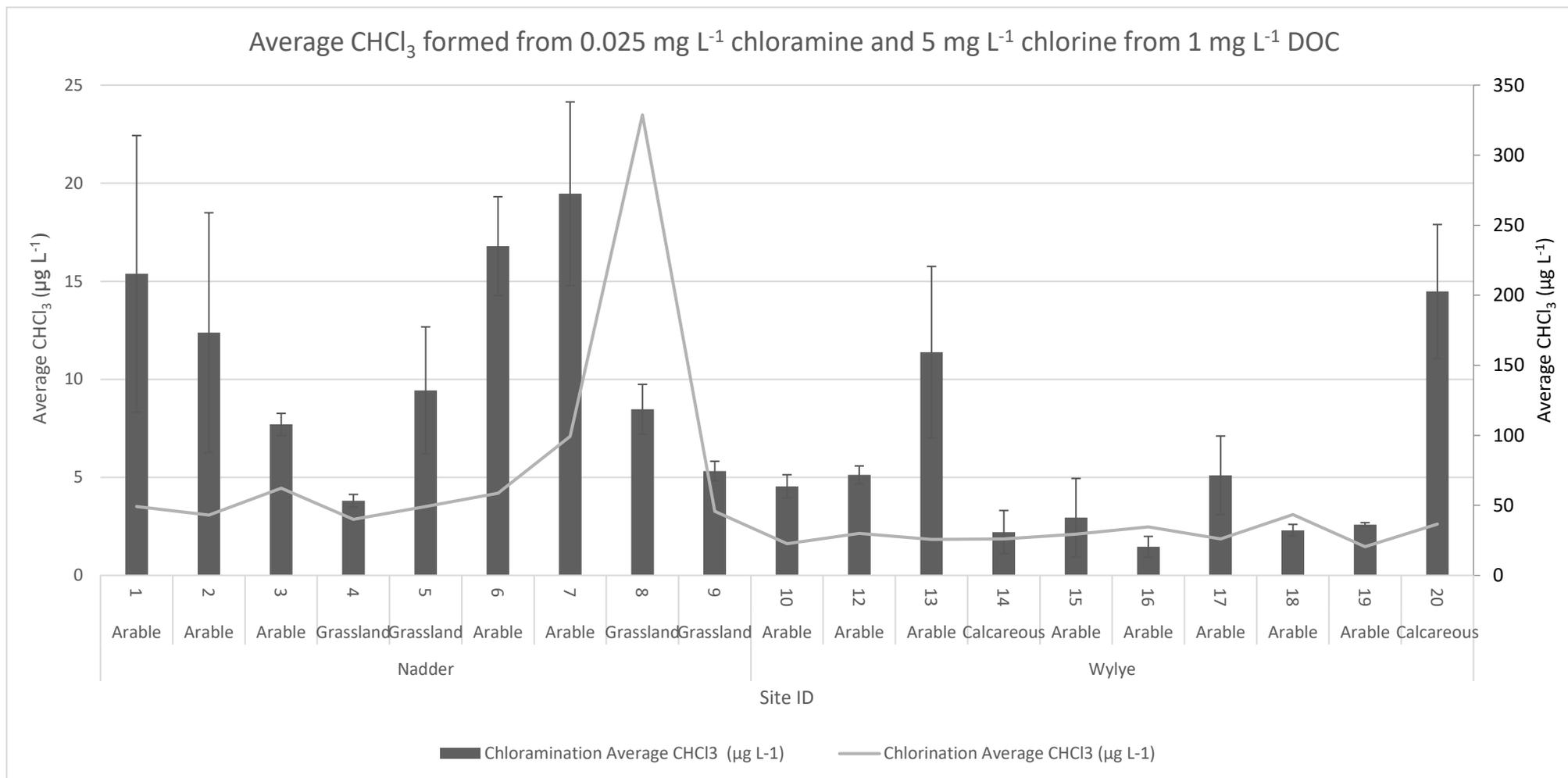


Figure 5: Average CHCl<sub>3</sub> concentration (µg L<sup>-1</sup>) formed between chlorination (at 5 mg L<sup>-1</sup> free chlorine) and chloramination (at 0.025 mg L<sup>-1</sup> free chlorine) in 19 samples with a DOC concentration of 1mg L<sup>-1</sup> (n=57 per treatment). Chlorination on right axis, chloramination on left axis.

Average CHCl<sub>3</sub> concentration was found to vary substantially between sites under the same treatment. Site 8 is the most clearly different site, with more than double the concentration of CHCl<sub>3</sub> than the next highest chlorinated site, site 7. Generally, the higher the concentration of CHCl<sub>3</sub> under chloramination, the higher the error becomes.

### Multiple Comparisons

Dependent Variable: CHCl<sub>3</sub>L\_Transformed

Tukey HSD

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
7	1	.31887*	.05858	.001	.1136	.5241
	2	.36290*	.05858	.000	.1577	.5681
	3	.20343	.05858	.053	-.0018	.4087
	4	.39733*	.05858	.000	.1921	.6026
	5	.30506*	.05858	.002	.0998	.5103
	6	.23042*	.05858	.021	.0252	.4357
	8	-.52103*	.05858	.000	-.7263	-.3158
	9	.34442*	.05858	.000	.1392	.5497
	8	1	.83990*	.05858	.000	.6347
2		.88394*	.05858	.000	.6787	1.0892
3		.72446*	.05858	.000	.5192	.9297
4		.91836*	.05858	.000	.7131	1.1236

5	.82609*	.05858	.000	.6209	1.0313
6	.75145*	.05858	.000	.5462	.9567
7	.52103*	.05858	.000	.3158	.7263
9	.86545*	.05858	.000	.6602	1.0707

Figure 6: ANOVA and Tukey HSD output showing differences between sites 7 and 8, and all other sites.

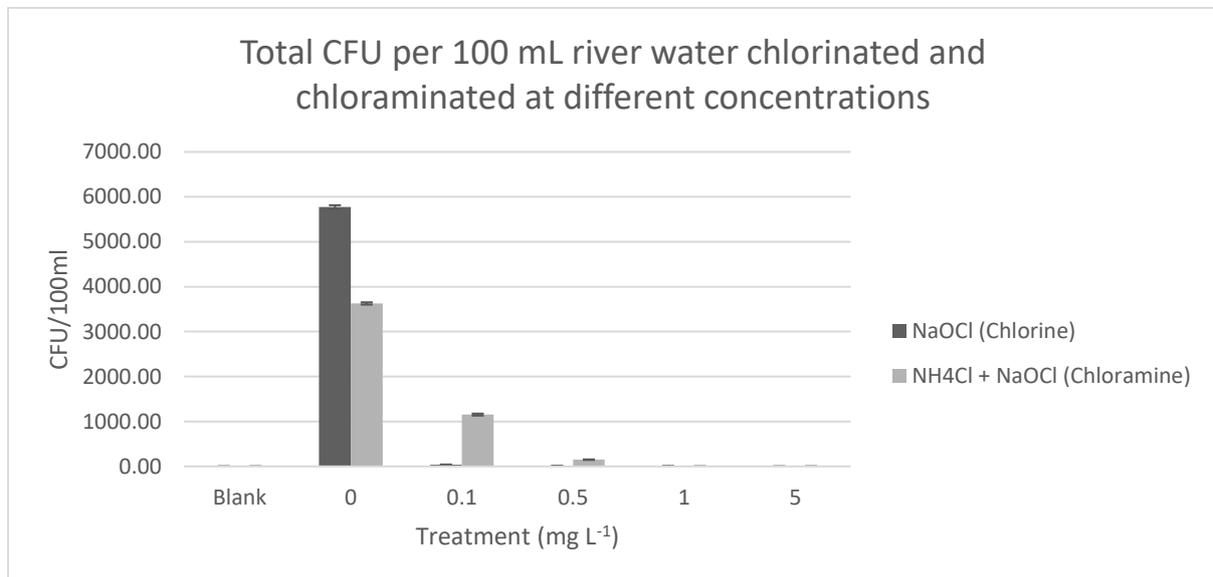
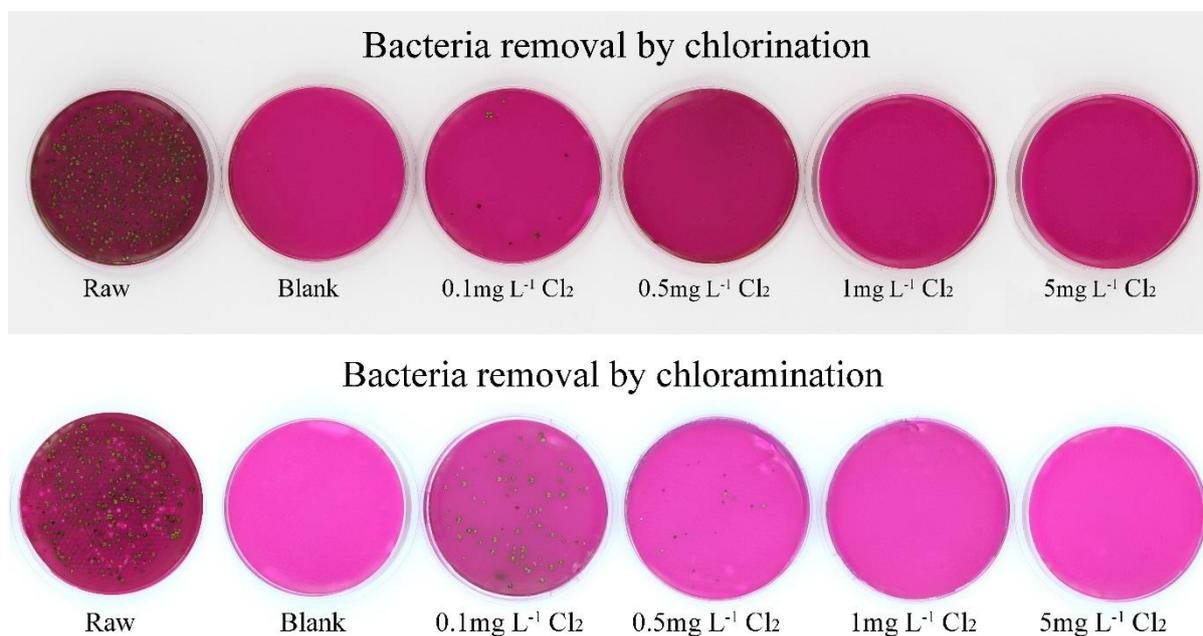


Figure 7: Average coliform forming unit (CFU) per 100 mL of river water before and after being treated to 0, 0.1, 0.5, 1 and 5 mg L<sup>-1</sup> chlorine and chloramine, with error bars representing standard error (n=18 chlorination treatment, n=17 chloramination treatment)

A total of zero colonies on both blank plates (for chlorination and chloramination treatments) show that the culture preparation and growth environment was clean and there were no external bacterial influences on the dataset (see figure 7 and 8).



*Figure 8: Bacteria removal by chlorination (top) and chloramination (bottom) under treatments with varying concentrations of free chlorine treatment solutions.*

The images in figure 8 show the bacteria removal efficiencies of chlorination vs chloramination. Whilst all bacteria has been removed between 0.1 mg L<sup>-1</sup> and 0.5 mg L<sup>-1</sup> free chlorine in chlorination treatments, it takes a concentration of between 0.5 mg L<sup>-1</sup> and 1 mg L<sup>-1</sup> free chlorine in chloramination to effectively remove all bacteria present, showing a two times decrease in bactericidal properties from chlorination to chloramination.

*Table 1: Mean concentrations of CHCl<sub>3</sub> formed from chlorination (5 mg L<sup>-1</sup> free chlorine) and chloramination (0.025 mg L<sup>-1</sup> free chlorine), with standard error, per sample site, plus percentage decrease of CHCl<sub>3</sub> concentrations between chlorination and chloramination treatments.*

	Chloramination			Chlorination		
Site	Mean CHCl <sub>3</sub> (µg L <sup>-1</sup> )	Std. Error		Mean CHCl <sub>3</sub> (µg L <sup>-1</sup> )	Std. Error	Percentage decrease (%)
1	15.37	7.05		49.10	8.85	68.69
2	12.39	6.11		43.06	2.27	71.23
3	7.70	0.56		62.24	4.04	87.63
4	3.81	0.32		40.02	3.89	90.48
5	9.43	3.24		49.11	1.64	80.79
6	16.79	2.52		58.66	4.79	71.38
7	19.46	4.68		99.22	4.38	80.38
8	8.47	1.27		328.72	7.25	97.42
9	5.32	0.50		45.79	6.49	88.38
10	4.54	0.59		22.57	1.62	79.89
12	5.13	0.46		29.90	2.29	82.85
13	11.39	4.37		25.58	0.89	55.50
14	2.21	1.10		26.09	3.34	91.54
15	2.94	2.00		29.49	2.28	90.02
16	1.46	0.53		34.61	1.48	95.79
17	5.10	2.01		26.03	0.26	80.39
18	2.30	0.30		43.39	0.22	94.70
19	2.59	0.10		20.48	1.79	87.33
20	14.48	3.42		36.53	1.67	60.37
Average	7.94			56.35		81.83

The data displayed in table 1 shows average CHCl<sub>3</sub> concentration, and the percentage decrease in CHCl<sub>3</sub> concentration from a chlorinated sample to a chloraminated sample. The total average percentage decrease in CHCl<sub>3</sub> concentration from chlorination to chloramination is 81.83%, with an average CHCl<sub>3</sub> concentration of 7.94 µg L<sup>-1</sup> for

chloraminated (at 0.025 mg L<sup>-1</sup> free chlorine) samples and 56.35 µg L<sup>-1</sup> for chlorinated (at 5 mg L<sup>-1</sup> free chlorine) samples.

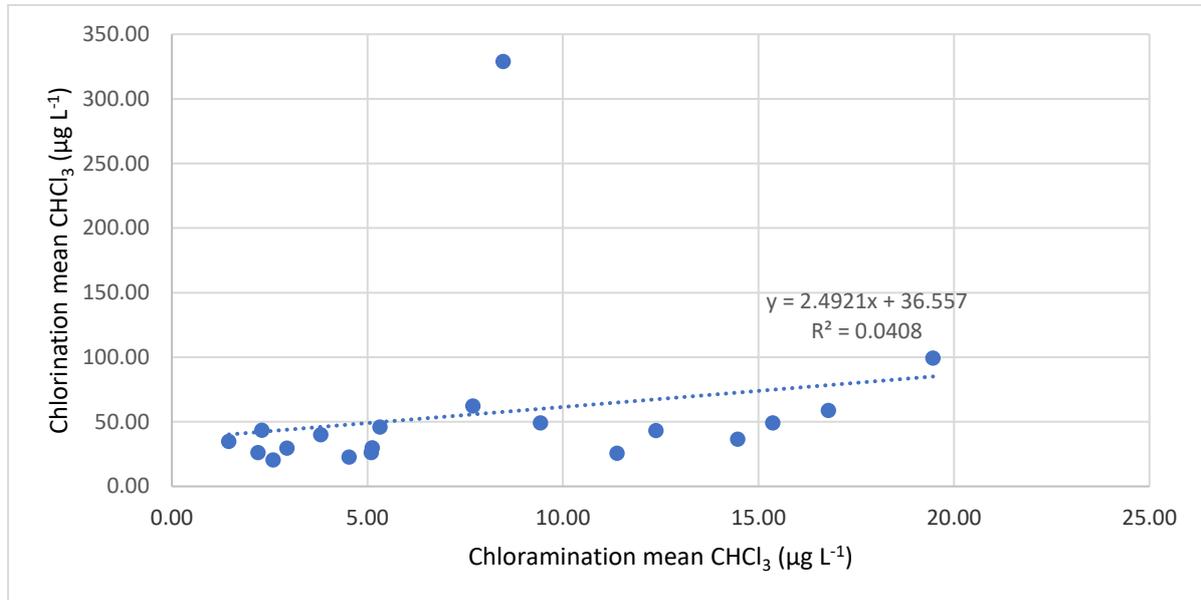


Figure 9: Correlation between CHCl<sub>3</sub> formed from chlorination and chloramination.

Figure 9 outlines the correlation between mean CHCl<sub>3</sub> concentrations formed from chlorination and chloramination. Obvious outlier is noticeable, representing site 8 under chlorination. R<sup>2</sup> value of very close to zero signifies no significant correlation between the two data groups

## Discussion

### Chloramination lifetime

The chloramination lifetime experiment (see figure 4) shows a strong correlation between time since creation of solution, and concentration of the solution. A Pearson's correlation was conducted showing a significant negative relationship between the two datasets ( $r = -0.986$ ,  $p < 0.00$ ). The line equation suggests that at a total of 328 minutes (or just under 5 and a half hours), the chloramine concentration will be at zero.

The mean  $\text{CHCl}_3$ , formed from only  $0.025 \text{ mg L}^{-1}$  free chlorine (figure 5) in a chloramine solution, data concurs with findings by Brodtmann and Russo (1979), who found that chloraminated drinking water contains between  $1/10^{\text{th}}$  to  $1/20^{\text{th}}$  of the proposed maximum contaminant level (MCL) for THM, even from raw river waters, whilst also killing 100% of pathogenic bacteria when applied at an effective dosage (Brodtmann and Russo, 1979). Whilst  $0.025 \text{ mg L}^{-1}$  chloramine appears not to be an effective dose for bacterial removal, the formation of this  $\text{CHCl}_3$  was found to be on average 14.1% the concentration that a 200 times stronger dose of chlorine created. Guay *et al.*, (2005), found that chloramine formed 98% less THM4 than chlorination in their laboratory studies, however, these experiments were carried out at a concentration of  $2.5 \text{ mg L}^{-1}$  for both chlorination and chloramination, possibly explaining the difference in findings with the present work.

For chlorinated samples from the river Nadder, the data was transformed to give normality (Skewness = 1.720, Kolmogorov-Smirnov  $f=0.236$ ,  $p=0.000$ ). An ANOVA was carried out and a Levene's Statistic of 2.149 ( $p=0.085$ ) was produced. A Tukey HSD posthoc test was selected as equal variances could be assumed. Data created for samples from the Nadder shows that the  $\text{CHCl}_3$  concentration from chlorinated water collected at site 7 is significantly different to sites 1, 2, 4, 5, 6, 8 and 9 at  $p<0.01$  except site 6 where  $p<0.05$ . These sites were found to be, on average,  $50\% \pm 3.08$  lower than the  $\text{CHCl}_3$  concentration at site 7. Site 8 was found to be significantly different to all other sites at  $p<0.01$  (See figure 6). These other sites are on average  $83\% \pm 0.22$  lower than the  $\text{CHCl}_3$  concentration at site 8. Sites 7 and 8 are located on the same stream, where cattle poaching and sewage inputs in the headwaters, and manure runoff in the lower reaches are prevalent. Site 7 drains a larger catchment than site 8, explaining the larger concentrations of  $\text{CHCl}_3$  formed during chloramination, however, under chlorination, site 8 has a higher concentration of  $\text{CHCl}_3$ , suggesting that the chlorine and chloramine are reacting to different organic matter moieties in the waters.

Chlorinated samples from the river Wylye's data was transformed (Skewness = 0.079, Kolmogorov-Smirnov  $f=0.127$ ,  $p=0.200$ ) and an ANOVA was carried out. However, a Levene's Statistic test ( $f=3.496$ ,  $p=0.009$ ) and a Welch test ( $f=180.932$ ,  $p=0.000$ ) showed that there were no significant differences between the groups of data for the Wylye catchment.

CHCl<sub>3</sub> data from chloraminated samples from the Nadder catchment was transformed (Skewness -1.461, Kolmogorov-Smirnov  $f=0.126$   $p=0.200$ ) and an ANOVA was carried out. A Levene's Statistic of  $f=9.751$  and  $p=0.000$  meant that a Welch or Brown Forsythe robust test of equality of means could be used. A Brown Forsythe test was used in this case, with  $f=0.868$  and  $p=0.599$ , meaning that equal variances could be assumed and the ANOVA was run again with Tukey HSD post hoc analysis. However, this showed no sites significantly differed from each other at  $p<0.05$  for all CHCl<sub>3</sub> concentrations formed under chloramination from the Nadder.

CHCl<sub>3</sub> data from chloraminated samples from the Wylye catchment was transformed (Skewness -0.016, Kolmogorov-Smirnov  $f=0.099$ ,  $p=0.200$ ) and an ANOVA was carried out. A Levene's Statistic ( $f=4.015$ ,  $p=0.005$ ) showed that there were no significant differences between the groups of data for the Wylye catchment (see figure 6)

Figures 7 and 8 show the bacteria removal efficiencies of chloramination and chloramination treatments of equal concentrations. Chlorination treatment killed all viable bacteria cells at a concentration between 0.1 mg L<sup>-1</sup> and 0.5 mg L<sup>-1</sup> free chlorine, whereas chloramination treatment killed all viable cells between 0.5 mg L<sup>-1</sup> and 1 mg L<sup>-1</sup> free chlorine.

This suggests that chloramines disinfection potential is approximately half that of chlorine.

No significant relationship between CHCl<sub>3</sub> concentrations formed from chlorination and chloramination treatments were found (Pearson's Correlation,  $f=0.202$ ,  $p=0.407$ ), and there are no obvious correlations between the data (see figure 9). The obvious outlier in the data is not anomalous, however, as identical samples were run in triplicate through the GC, in separate runs and rack positions, thus giving extra credibility to the legitimacy of the data point. It can be hypothesised that the water at this location contains a chemical, compound or organism that is a major precursor for CHCl<sub>3</sub> formation under chlorination. This suggests that chlorine and chloramine preferentially bind to different compounds present in the water, thus forming differing concentrations of CHCl<sub>3</sub>.

## **Conclusion**

The  $\text{CHCl}_3$  formed from chloramine agrees with literature stating that THM4 formed from chloramines are typically  $1/10^{\text{th}}$  to  $1/20^{\text{th}}$  that of chlorine (Brodtsmann and Russo, 1979), these results have been detected in samples at 200 times lower concentration of chloramine when compared to chlorine. Conversely, bactericidal properties of chloramine have been found to be up to five times less than chlorine. This could show that chloramine targets organic matter and the available free chlorine is ‘used up’ on these organic compounds before it can kill bacteria.

The data presented here, however, is generated from raw river waters, and is not representative of treated water, where high MW DOM removal is achieved, coagulation and filtration of the water creates a product with a much lower overall DOC concentration, and as a result the data presented in this study cannot be used in a treatment context; this is a potential next step in understanding the mechanisms of chloramination and its advantages and disadvantages to drinking water quality. This protocol can be of use to academic research and utility companies alike, though further iterations should include a matched  $5 \text{ mg L}^{-1}$  chlorination and chloramination treatment to determine whether the  $\text{CHCl}_3$  concentration formed by chloramination increases with dose or whether all of the present DOM had been reacted with before bacteria and other compounds could be disinfected.

Key findings show:

- Chloramination and chlorination target different organic moieties in the water.
- No significant correlation between  $\text{CHCl}_3$  concentrations formed from chlorination and chloramination treatments were found.
- Bactericidal efficiencies of chlorination have been found to be two times greater than identical chloramination treatments.
- Site 8 contains a chemical, compound, or DOM that is a strong precursor for  $\text{CHCl}_3$  formation under chlorination but not under chloramination.

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### Supplementary Material:

The following images are for reference for some of the steps outlined in the methods section.



Image 1: Image to show position of meniscus at zero before titration of ferrous ammonium sulphate



Image 2: Image to show position of meniscus at 3.50 mL after titration of ferrous ammonium sulphate



Image 3: Image to show colour of chloramine solution when added to 500 mg N,N-Diethyl-p-phenylenediamine sulphate and 5 mL of phosphate buffer



Image 4: Image to show colour change from bright pink to clear once 3.50 mL ferrous ammonium sulphate was titrated into chloramine solution, indication a concentration of 3.5 mg L<sup>-1</sup> free chlorine in chloramine solution.

### **Standard solution information**

A standard solution of THM4 was made using individual compounds. Briefly, chloroform (trichloromethane) (#M-502-13-10X, Lot 215061379, AccuStandard, Connecticut, USA), bromodichloromethane (#M-502-04-10X, Lot 216011332, AccuStandard, Connecticut, USA), dibromochloromethane (#M-502-17-10X, Lot 216021126, AccuStandard, Connecticut, USA) and bromoform (tribromomethane) (#M-502-05-10X, Lot B4020287-1A, AccuStandard, Connecticut, USA) were obtained at 2 mg mL<sup>-1</sup> in methanol. The solutions were added together and made up to 100 mL with Milli-Q water to make a 20,000 µg L<sup>-1</sup> THM4 stock solution, from which working 100 mL standards of 200, 150, 100, 80, 40, 20, 10, 5, 3, 1 and

0.5  $\mu\text{g L}^{-1}$  were made (by adding aliquots of stock solution to Milli-Q water up to 100 mL). Triplicates of each concentration of working standards were analysed by the above method to check for drift before a calibration file was created, using peak area and retention times as indicators of THM presence and concentration in unknown samples.

**Equation 1:**

For example: Hypochlorite concentration ( $\text{mg mL}^{-1}$ ) =  $(0.1 \times 35.45 \times 10.5) / 0.4 = 93.056 \text{ mg mL}^{-1}\text{Cl}_2$ .

**Equation 2:**

For example:  $(1250 / 93.056) / 5 = 2.71 \text{ mL}$  hypochlorite solution

**Equation 3:**

For example:  $93.056 \text{ mg mL}^{-1}$  (concentration of NaOCl measured in section 1) / 74.442 (MW of NaOCl in  $\text{g mol}^{-1}$ ) = 1.25005 M, therefore the solution has a molarity of 1.25 M.

**Equation 4:**

For example:  $0.8 \text{ M} / 1.25 \text{ M} \times 100 \text{ mL} = 64 \text{ mL}$ , so 64 mL sodium hypochlorite was added to 36mL Milli-Q to make a 0.8M solution of sodium hypochlorite.

**Equation 5:**

For example, 100  $\mu\text{L}$  chloramine in 100 mL of milli-Q water = 3.5  $\text{mg L}^{-1}$  chloramine (detected by titration). Simple multiplication shows that 1000  $\mu\text{L}$  chloramine in 100 mL Milli-Q water = 35 $\text{mg L}^{-1}$  chloramine, 10,000  $\mu\text{L}$  in 100 mL Milli-Q water = 350  $\text{mg L}^{-1}$  chloramine and hence forth. Therefore, 28,570  $\mu\text{L}$  of chloramine (28.57 mL) in 100 mL Milli-Q creates 1000  $\text{mg L}^{-1}$  chloramine dosing solution.

Equation 6:

For Example:  $100 / [\text{DOC}]$ , so if DOC is  $5.84 \text{ mg L}^{-1}$ ,  $100 / 5.84 = 17.12 \text{ mL}$  of sample per 100 mL Milli-Q

Table 1 shows the average  $\text{CHCl}_3$  concentration for each site along with a percentage measure of the decrease in  $\text{CHCl}_3$  concentration of a chlorinated compared to a chloraminated sample. The data show that the average drop in  $\text{CHCl}_3$  concentration taken across all the sites when comparing chlorination ( $56.35 \text{ } \mu\text{g L}^{-1}$  at  $5 \text{ mg L}^{-1}$  free chlorine) versus chloramination ( $7.9 \text{ } \mu\text{g L}^{-1}$  at  $0.025 \text{ mg L}^{-1}$  free chlorine) is 82%.

**Table 1** Mean concentrations of  $\text{CHCl}_3$  formed with standard error from chlorination ( $5 \text{ mg L}^{-1}$  free chlorine) and chloramination ( $0.025 \text{ mg L}^{-1}$  free chlorine) per sample site, plus percentage decrease of  $\text{CHCl}_3$  conc. between chlorination and chloramination treatments.

Site	Chloramination		Chlorination		% drop
	Mean $\text{CHCl}_3$ ( $\mu\text{g L}^{-1}$ )	Std. Error	Mean $\text{CHCl}_3$ ( $\mu\text{g L}^{-1}$ )	Std. Error	
1	15.4	7.1	49.1	8.9	68.7
2	12.4	6.1	43.1	2.3	71.2
3	7.7	0.6	62.2	4.0	87.6
4	3.8	0.3	40.0	3.9	90.5
5	9.4	3.2	49.1	1.6	80.8
6	16.8	2.5	58.7	4.8	71.4
7	19.5	4.7	99.2	4.4	80.4
8	8.5	1.3	328.7	7.3	97.4
9	5.3	0.5	45.8	6.5	88.4
10	4.5	0.6	22.6	1.6	79.9
12	5.1	0.5	29.9	2.3	82.9
13	11.4	4.4	25.6	0.9	55.5
14	2.2	1.1	26.1	3.3	91.5
15	2.9	2.0	29.5	2.3	90.0
16	1.5	0.5	34.6	1.5	95.8

17	5.1	2.0	26.0	0.3	80.4
18	2.3	0.3	43.4	0.2	94.7
19	2.6	0.1	20.5	1.8	87.3
20	14.5	3.4	36.5	1.7	60.4
<hr/>					
Mean	7.9		56.4		81.8
<hr/>					

Table 2: Mean concentrations of THM4 formed with standard error from chlorination (5mg L<sup>-1</sup> free chlorine) and chloramination (0.025mg L<sup>-1</sup> free chlorine) per sample site, plus percentage decrease of THM4 conc. between chlorination and chloramination treatments.

<u>Site</u>	<u>Chloramination</u>		<u>Chlorination</u>		<u>% drop</u>
	<u>Mean</u> <u>THM4</u> <u>(µg L<sup>-1</sup>)</u>	<u>Std.</u> <u>Error</u>	<u>Mean</u> <u>THM4 (µg</u> <u>L<sup>-1</sup>)</u>	<u>Std.</u> <u>Error</u>	
1	16.43	6.01	67.0	9.5	75.5
2	12.39	6.11	58.9	2.4	79.0
3	7.70	0.56	85.9	2.2	91.0
4	4.85	0.72	58.8	4.2	91.8
5	9.43	3.24	65.1	1.9	85.5
6	16.79	2.52	75.9	4.7	77.9
7	19.46	4.68	114.5	4.6	83.0
8	8.47	1.27	344.2	6.9	97.5
9	5.32	0.50	62.7	7.4	91.5
10	5.56	1.16	50.2	7.7	88.9
12	3.63	0.46	48.9	0.7	92.6
13	11.39	4.37	43.7	1.6	73.9
14	2.21	1.10	42.3	4.0	94.8
15	2.94	2.00	45.7	2.6	93.6
16	1.46	0.53	51.5	1.7	97.2
17	5.10	2.01	41.8	0.3	87.8

18	3.29	1.29	63.8	0.1	94.8
19	3.60	0.95	41.6	8.6	91.4
<u>20</u>	<u>14.48</u>	<u>3.42</u>	<u>52.4</u>	<u>1.8</u>	<u>72.4</u>
Mean	8.13		74.5		87.4