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Fine Scale Survey of Metals, Nutrients and Lipid Biomarkers in the Conwy Estuary

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Award date:
2018

Awarding institution:
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Fine Scale Survey of Metals, Nutrients and Lipid Biomarkers in the Conwy Estuary

Caroline Duce

July 2018



PRIFYSGOL
BANGOR
UNIVERSITY

A thesis submitted to Bangor University in candidature
for the degree

Philosophiae Doctor

School of Ocean Sciences

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Summary

The environmental quality of coastal ecosystems is an issue of growing international attention. Estuarine organic matter is derived from a multitude of natural and anthropogenic allochthonous and autochthonous sources that originate across a freshwater continuum. Various point and diffuse sources, such as discharges of treated and untreated sewage as well as urban and agricultural run-off, contribute significantly to the deterioration of the water and sediment quality of aquatic ecosystems. The EU Water Framework Directive requires all surface waters to fulfil the criterion of “good ecological status” by the end of 2021, this status is defined with reference to undisturbed conditions. Within heavily impacted coastal areas with no prior monitoring of baseline data assessing these reference conditions will be inherently difficult. Understanding near-pristine conditions within estuaries provides reference sites for assessing natural variability of organic and inorganic chemical parameters which in turn will provide baseline conditions for management to allow them to distinguish between changes caused by human and those that are natural.

A fine scale survey of metals, nutrients and lipid biomarkers was investigated within an estuary that was perceived to be of relatively pristine condition. The data obtained has been used to assess existing models that aimed to define elevated versus baseline concentrations. The majority of the data focused on the sediment fraction due to its importance as a historic sink of organic and inorganic compounds which in turn enables an understanding of the potential inputs from both natural and anthropogenic sources. Regional geochemical baseline models were constructed for metals and nutrients to enable an understanding whether the organic and inorganic compounds were from natural or anthropogenic origins. The lipid biomarkers were utilised to establish the origin of the organic matter. Within the Conwy estuary there were a number of locations that yielded higher concentrations that were deemed above background levels, these have been discussed to highlight potential sources of contamination. A proportion of locations sampled fell below those that were deemed enriched for metals, nutrients and lipids resulting in concentration ranges of pristine conditions which other contaminated estuaries can refer to.

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Dedication

This PhD is dedicated to my father for all his support over the years,

I wish he was still here to see me finish.

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Acknowledgements

The production of this thesis would not have been possible without the help and assistance of many different people and I would like to take this opportunity to thank everyone who has assisted and encouraged me in this endeavour.

Firstly I would like to thank everyone who was involved in the data collection for this thesis particularly the boat skippers Gwynne, Ben and Pete. I would also like to thank Susan and Katie for all their help and support in the field and the lab and constant support to get this finished. Katie

I would also like to thank Dave Barnes who funded this PhD through his business Hebog Environmental Ltd and my supervisors Shelagh, Vera and Jon, for their guidance and encouragement especially towards the end. Vera thank you for your continuous optimism to get me finished and amazing guidance and Shelagh for your support throughout this very long PhD. I would also like to thank my colleagues for their continued encouragement to get it finished.

I would also like to thank my friends and family for putting up with me, especially Kate for all her help and assistance in getting me writing and providing brilliant constructive criticism. I'd also like to thank my Dad who over the years has been so supportive, shame he didn't get to see me finish.

Lastly I would like to thank Paul for his support and understanding and always smiling even when I was at my lowest and just keep bringing me the tea.

Abbreviations

AA3	Analytical continuous-flow Auto Analyser 3
Ag	Silver
Al	Aluminium
As	Arsenic
B	Boron
Ba	Barium
Be	Beryllium
Bi	Bismuth
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
µm	Micro meter
C	Carbon
Cd	Cadmium
CEFAS	Centre for Environment Fisheries and Aquaculture Science
CEH	Centre of Ecology and Hydrology
CFU	Colony Forming Units
cm	Centimetre
Co	Cobalt
CO₂	Carbon dioxide
C_{org}	Organic carbon
Cr	Chromium
Cs	Caesium
CTD	Conductivity, temperature and depth
Cu	Copper
CV	Coefficient of variation
°C	Degrees Centigrade

DOC	Dissolved organic carbon
EC	Electrical Conductivity
ECS	Elemental Combustion System
EF	Enrichment factor
EU	European Union
Fe	Iron
g or g⁻¹	Gram
Hcl	Hydrochloric acid
GCMS	Gas chromatography –mass spectrometry
GF/F	Glass micro fibre filters
GIS	Geographical Information System
Hg	Mercury
HNO₃	Nitric acid
Ho	Holmium
H₂SO₄	Sulphuric acid
ICP-MS	Inductively coupled plasma mass spectrometry
In	Indium
Kg	Kilogram
KOH	Potassium hydroxide
L	Litre
La	Lanthanum
LCOH	Long chain alcohol
Li	Lithium
Lu	Lutetium
M	Molar
m	Meters
m³	Cubic meters

Mn	Manganese
Mo	Molybdenum
mg	Milligram
µg	Microgram
µg/L	Microgram per litre
mg/l	Milligram per litre
Mg/kg	Milligram per kilogram
MgSO₄	Magnesium sulfate
ml	Millilitre
ml/min⁻¹	Millilitre per minute
mm	Millimetre
µm	Micrometer
µmol/g⁻¹	µmol per gram
µmol/L	Micromol per litre
N	Nitrogen
NaOH	Sodium hydroxide
NERC	National Environment Research Council
NH₄⁺	Ammonium
Ni	Nickel
nm	nanometer
NO₂⁻	Nitrite
NO₃⁻	Nitrate
N_{org}	Organic nitrogen
NRW	National Resources Wales
OC	Organic Carbon
ON	Organic nitrogen
%	Percent

P	Phosphate
Pb	Lead
PC	Principal component
PCA	Principle component analysis
PO₄³⁻	Phosphate
ppm	Parts per million
PSU	Practical Salinity Unit
Rb	Rubidium
RGB	Regional geographical baseline
RSD	Relative standard deviation
s or s⁻¹	Second or per second
Sb	Antimony
Sc	Scandium
SCOH	Short chain alcohol
S.D	Standard Deviation
Se	Selenium
Sm	Samarium
Sn	Tin
SPSS	Statistical Package for Social Sciences
Sr	Strontium
STS	Septic tank systems
Tb	Terbium
TC	Total Carbon
Th	Thorium
Tl	Thallium
TN	Total Nitrogen
TOC	Total Organic Carbon

TP	Total Phosphate
UK	United Kingdom
UKTAG	United Kingdom Technical Advisory Group
U	Uranium
V	Vanadium
W	Tungsten
WFD	Water Framework Directive
WFD EQS	Water Framework Directive Environmental quality standard
Y	Yttrium
Yr⁻¹	per Year
Zn	Zinc

CHAPTER 1

Introduction

1.1 Estuaries

Estuaries are semi-enclosed bodies of water which receive natural inputs from both marine and terrestrial origins. Understanding the near-pristine conditions within estuaries provides reference sites for assessing natural variability of organic and inorganic chemical parameters, which in turn will provide baseline conditions for management to allow them to distinguish between changes caused by human activities and that which are natural (Deeley and Paling 1999). A pristine environment is one that is not subjected to anthropogenic influence and it is unlikely that any coastal waterway are in a pristine condition because global biogeochemical cycles have been altered over the last century by human processes (Galloway 2005). Near-pristine or estuarine systems with limited human influences would give more realistic baseline chemical concentrations for contaminated estuaries to refer to.

Estuarine inputs originate from multiple sources of organic matter from both marine and terrestrial environments. Allochthonous sources originate from rivers and groundwater from the terrestrial environment and marine materials brought in through tidal action from the open ocean. Autochthonous sources are from production of algal and intertidal vegetation (Goñi, Teixeira and Perkey 2003, Ruttenberg and Goni 1997). Of these, rivers represent the main pathway of natural weathered materials into the ocean through the estuaries. Since the beginning of the 20th century it has been estimated that the industrial revolution and therefore human influence has more than doubled the annual discharge of materials into oceans by rivers which has resulted in an extensive increase in nutrients, organic matter and metals from anthropogenic sources into the natural environment (Gaillardet, Viers and Dupré 2003). Typically only particles less than 5 µm in diameter are transported to the sea, as a consequence many substances leave estuaries only as a result of incorporation into mobile organisms (Carballeira et al. 2000). Therefore, estuarine sediments serve as an effective trap of these river-borne elements, of both natural and anthropogenic sources which accumulate simultaneously. Pathogens, nutrients, metals, and organic chemicals tend to sorb onto organic fine-grained particles which eventually settle in depositional areas (Burton 2002). This is promoted largely by the very high surface area of these particles and the tendency for higher concentrations of organic matter in the fine particles that adsorb organic contaminants. These accumulated contaminants within the sediments, can serve as sources of pollution to the ecosystem they are connected with. Once contaminants are bound to a particle surface they become less likely to be bio transformed and desorption is

usually very slow; therefore sorbed contaminants will reside for long periods in the sediment (Carballeira et al. 2000).

Estuaries which are the transitional zone between freshwater and seawater have limited defined legal limits and are therefore difficult to assess. Within the United Kingdom water quality standards are set in accordance with the Water Framework Directive (WFD), the precise values for standards have been set with advice from the UK Technical Advisory Group (UKTAG) however the majority of these legal limits define freshwater and seawater parameters. The Water Framework directive Environmental Quality Standards (WFD EQS) (Directive 2008/105/EC) includes 33 priority substances of which the priority metals four are addressed within this study. The WFD EQS standards are only applicable in the water column and there are no equivalent standards for sediments within the European Union and therefore no legislative monitoring is undertaken. Within the UK in the absence of formal standards, CEFAS cautiously recommends the guidance criteria of Canadian sediment quality guidelines (SQGs) for the protection of aquatic life devised by the Canadian council of Ministers of the Environment. This involves the derivation of Threshold Effects levels, where sediments which contain concentrations below the no effect level or at the lowest effect level constitute unpolluted or marginally polluted sediments. While sediments which range between the lowest effect level and severe effect level are considered as moderately polluted and concentrations above severe effect level indicate that the sediments are heavily polluted (Hubner, Astin and Herbert 2009, Persaud, Jaagumagi and Hayton 1996). However these SQGs are used within USA and Canada's governing bodies but the European Union has not supported the use of this criteria. One specific issue within estuarine sediments regarding the SQGs is that limits have been defined for only freshwater and seawater but nothing specifically for the transitional zone of estuaries. In addition this approach has a severe limitation of not distinguishing between areas impacted by anthropogenic sources and those that are naturally occurring. To identify the true contamination level the assessment of anthropogenic contributions to sediments must be accompanied primarily by the estimation of contributions by natural processes (Windom et al. 1989).

1.1.1 Metalloids

Metals are naturally occurring constituents in the environment from natural processes such as weathering and erosion of the geological matrix and atmospheric deposition and vary in concentrations across geographic regions (Linnik and Zubenko 2000, Lwanga et al. 2003, Gupta et al. 2014). They provide aquatic ecosystems with varying amounts of trace elements that are essential to life within certain limits (eg. Iron (Fe), Manganese (Mn), Copper (Cu), Cobalt (Co), Zinc (Zn) and Selenium for organisms only (Se)) (Allen, Garrison and Luther 1998). However anthropogenic sources also contribute metals to the estuarine system causing adverse effects if concentrations are above the requirement of the ecosystem.

The main sources of metals to estuarine systems are transported through riverine input and are a combination of point, diffuse, atmospheric deposition and groundwater enrichment (Kennish 1991, Nemerow 1991). Point sources include mining, (historic or present day); marina/harbour activities, and sewage and industrial effluents. Diffuse sources can be from natural or anthropogenic origins and include urban and agricultural runoff. These metals are transported in dissolved or particulate form, where sediments and suspended particles play important roles in metal adsorption, desorption and dissolution (Boyle et al. 1974). Concentration of metals between the particulate and dissolved fraction depends upon the availability of negatively charged sediment surfaces for the positively charged metal ions (Förstner and Wittmann 1981). Therefore the higher the concentration of suspended particles within an aquatic ecosystem, the lower the dissolved concentrations of metals. As a consequence, higher concentrations of total and particulate metal concentrations are found in the maximum turbidity zone where higher concentrations of suspended particles exist and dissolved metal concentrations are low (Gonzalez, Thouvenin and Dange 2007). In addition salinity also affects the partitioning of metals (Förstner and Wittmann 1981), where increased desorption from sediments occurs with increasing salinity (Mwanuzi and De Smedt 1999). There are two types of surface complexes made between metals and colloidal particle surfaces. The first are the outer-sphere complexes where a water molecule is present between the metal and particle surface (Begeal 2008). These bonds tend to be weak and are easily broken by increases in ionic strength and generally formed by alkali, and alkaline earth metals. Transitional metals form the second type of bonding, where inner-sphere complexes occur between the metal and the colloidal particle surface, the metal is bonded directly to the anion on the mineral surface and therefore produces a stronger bonds which are not easily broken by increases in ionic strength (Begeal 2008). Change in the colloidal particle pH has to decrease below the point of zero charge (pzc) and therefore the particle surface

has a neutral charge resulting in the metal being released into the dissolved phase (Sparks 2003). Within estuarine and saline waters where the pH is reduced compared to freshwater desorption of Cu, Cd, Zn and Pb into the dissolved phase has been shown to occur and chlorocomplexes observed to be particularly strong with increased chloride concentrations (high salinity) (Valenta et al. 1986, Golimowski, Merks and Valenta 1990, Warren and Zimmerman 1994, Thouvenin, Gonzalez and Boutier 1997).

Therefore metal mobility within an estuary is dependent on sediment type, salinity and the concentration of suspended sediments (Machado et al. 2016). Metal adsorbed to particulate material can settle out of suspension, accumulating in sediments and providing one of the main sinks of metals in the estuarine system, or they can be released from sediments, acting as a source back to the overlying water column via natural or anthropogenic disturbances (Pan and Wang 2012).

As coastal sediments are reservoirs of materials derived from both anthropogenic and natural sources, determining the natural levels of metals is essential to the accurate assessment of the degree to which a particular metal has been enriched in a given environment (de Paula Filho et al. 2015).

1.1.2 Nutrients

Estuaries play a key role in the processing and cycling of nutrients which depend on several interlinked processes. These nutrients exist in numerous particulate or dissolved organic and inorganic forms (Malham et al. 2014). Nitrogen, from allochthonous and autochthonous origins, and phosphorus originating from allochthonous sources enter estuarine systems from both natural and man-made sources. Naturally, phosphorus enters estuarine and coastal waters from the dissolution of geological formations rich in phosphate and nitrogen and phosphorous when decomposing organic matter is flushed into rivers and streams (Mann 2000). However the load of macro-nutrients carbon (C), nitrogen (N) and phosphorus (P) to aquatic systems has increased with time through human activity (Smith et al. 2005). Within some estuarine and coastal waters this increase in nutrient concentration has had detrimental effects on the health of ecosystems. The increase in nutrient loading can lead to an excess of phytoplankton biomass (Malone et al. 1986), which in turn can potentially cause episodes of noxious blooms, (Sand-Jensen and Borum 1991, Duarte 1995) and depletion of dissolved oxygen in bottom waters (Malone et al. 1986).

Within the aquatic environment, phosphates are generally bound to sediment particles where they are absorbed on iron oxides, aluminium hydroxides, clay surfaces and organic matter particles and becomes incorporated into the sediment (Borggaard et al. 1990). Natural phosphate weathers from rocks and minerals, the most common mineral being apatite (Nash 1984). Point sources of phosphorus are related to sewage input, however tertiary treatment with phosphate strippers resolves this problem and therefore more significant phosphorus fluxes to estuaries are dominated by agriculture (Carpenter et al. 1998). However nitrogen, is found within sediments but is more soluble and therefore found in higher concentrations within the dissolved form than phosphorus. Therefore significant exports can occur in ground water (Paerl 1997) or as dissolved nitrogen in surface waters. In addition, reactive nitrogen compounds can be quite mobile in the atmosphere (Bouwman et al. 1997, Holland et al. 1999). The most common forms of inorganic nitrogen within the aquatic environment are ammonium ions (NH_4^+), nitrite ions (NO_2^-) and nitrate ions (NO_3^-). Naturally occurring inorganic nitrogen in aquatic ecosystems is present as a result of atmospheric deposition, surface and groundwater runoff, dissolved components of nitrogen-rich geological formations, N_2 fixation by certain prokaryotes and degradation of organic matter (Kinne 1984, Gleick 1993, Wetzel 2001, Rabalais 2002). In addition to natural sources, anthropogenic sources of inorganic nitrogen enters aquatic ecosystems from urban and agricultural runoff, industrial waste, and sewage effluent (including effluents from sewage treatment plants that are not performing tertiary treatments) (Carpenter et al. 1998, Wetzel 2001, Rabalais 2002, Camargo, Alonso and Salamanca 2005). Within oxidised waters, which can occur for example within acidic mine waters (Drever 1997), ammonium is converted to nitrate in a two-step process ($\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) by aerobic chemoautotrophic bacteria, therefore nitrate concentrations in aquatic ecosystems are usually higher than ammonium and nitrite. (Kinne 1984, Rabalais 2002).

Organic matter content (Total Organic Carbon (TOC) & Nitrogen (N) distribution) is generally related to the amount of silt and clay that is present in the sediments. The ratio of organic carbon/total nitrogen allows the origin of organic matter to be distinguished between terrestrial and marine due to different types of organic matter with different TOC and TN contents (Stein 1991). C/N ratios higher than 20 indicate a terrestrial source due to the higher carbon content and between 5 and 7 a pelagic source (Saito et al. 1989). Stein (1991) also inferred that ratios lower than 10 were from a strict marine origin and around 10 the origin was both marine and continental. In semi-enclosed and shallow environments, it is common to find C/N ratio

indicative of a variable marine and continental contribution due to the mixed nature of organic matter (Bordovskiy 1965).

1.1.3 Lipid Biomarkers

Sewage contamination is a source of excess nutrients. It is traditionally identified from biodegradable organic matter, ammonia or faecal bacteria counts as tracers for effluents in the water column and sediments. Most of these methods lack specificity or sensitivity and therefore they are only useful as short-term tracers for wastewater deposition (Seguel et al. 2001). The need for more reliable and persistent markers of sewage resulted in the development of the lipid biomarkers approach. These are biological markers comprising chemical compounds which indicate particular biological sources. They must be specific to certain organisms or groups of organisms and persist in the marine environment in a form which resembles the compound from which they are derived, and they must be easily detectable (Lintern 1996).

Lipid biomarkers have the potential to identify the major sources within sediments. Due to their structural diversity, source specificity and relative stability, lipid biomarkers have proved to be useful tools for assessing the sources and fates of organic matter to marine systems (Zimmeman & Canuel 2001). Various researchers (Wakeman 1995, Mudge & Norris 1997, Mudge & Seguel 1999) have undertaken studies using sterols, fatty alcohols and fatty acids, as these lipids are present in large enough quantities in the marine environment to make analytical extraction relatively easy (Lintern 1996). The limitations of the 'biomarker approach' are related to the wide distribution of some compounds, the different reactivity of the components and the multiplicity of processes (biological, chemical, physical and geochemical) which transform the original source signals. In order to minimise these problems, relative and absolute concentrations are used instead of presence and absence and the quantification of several biomarkers in different lipid classes (Colombo et al. 1996).

Sterols have successfully been used as tracers of inputs from various species of marine and terrestrial plants and animals, and transformation processes such as oxidation and reduction (Leeming et al. 1996, Derrien et al. 2012, Martins, Fillmann and Montone 2007, Venkatesan and Mirsadeghi 1992, Green and Nichols 1995). They are better preserved in sedimentary environments than most other biological products e.g. amino acids and carbohydrates. The sterols of greatest importance as a biomarker have a four ring structure, which has a β -hydroxy

group at C3 and a side chain at C17. Saturated sterols are known as stanols, whilst structures with a double bond in the ring structure are known as stenols. These structures of side chains and double bonds can be attributed to specific organisms and therefore they provide a good tool for indicating the origin of the organic matter (Lintern 1996).

Fatty alcohol biomarkers are used to assess the marine and terrestrial contribution to aquatic sediments. These are primarily derived from wax esters which are known to be widely present in the biosphere, for example as external lipids in the cuticular wax of land plants, and as internally stored energy reserves in organisms (Mudge & Norris 1997). Marine sediments are likely to have wax esters originating from a variety of organisms. However, the primary sources are thought to be from marine zooplankton and terrestrial plants (Parameswaran et al. 1994). In comparison to sterols, knowledge of fatty alcohol occurrence is still quite limited and therefore it may be difficult to clearly define their origin (Seguel et al. 2001) from fatty alcohols alone.

The most common sterol biomarkers used for establishing organic matter sources are marine markers (cholesterol), phytoplankton (cholest, 5, 22 dien-ol, brassicasterol, dinosterol), terrestrial (β -sitosterol and Ergosterol) and sewage (coprostanol)

1.2 Characteristics of the Conwy Estuary

The Conwy estuary is the largest and most westerly of the major estuaries of the North Wales coast, draining an area of approximately 660 km² from its source at Llyn Conwy (Zhou, Liu and Abrahams 2003). It is a well-mixed macro-tidal estuary with shallow water depths, which is typical for the UK and therefore representative of other estuarine systems (Robins et al. 2014a). The estuary is tidally dominated with a spring tidal range of about 7m, and the tidal limit extends approximately 25 km upstream of the estuary mouth at Llanrwst (Mudge and Norris 1997). The morphology is such that the estuary almost entirely drains each tidal cycle, and resembles a meandering river channel at low slack water, flanked by muddy tidal flats in the upper estuary and sand in the lower estuary (Robins et al. 2014a). The Conwy Bay and inner estuary provide a significant environmental shelter and habitat for breeding birds and other fauna and flora. The catchment landscape is of exceptionally high aesthetic quality, and tourism is the main contributor to the local economy and to a lesser extent business enterprises. (Borough 2017).

1.2.1 Conwy Estuary sample location

From the previous research undertaken on the Conwy estuary (Table 1.1) it is apparent there is a paucity of information on the levels of nutrients, metals and lipids within the estuarine sediments to enable classification of whether the Conwy can be utilised as an example of natural variability within an estuarine system. A fine scale survey of nutrients, metals and lipid concentrations undertaken at the same point in time and location covering the entire estuary from the mouth up to the tidal limit would enable this classification to be made.

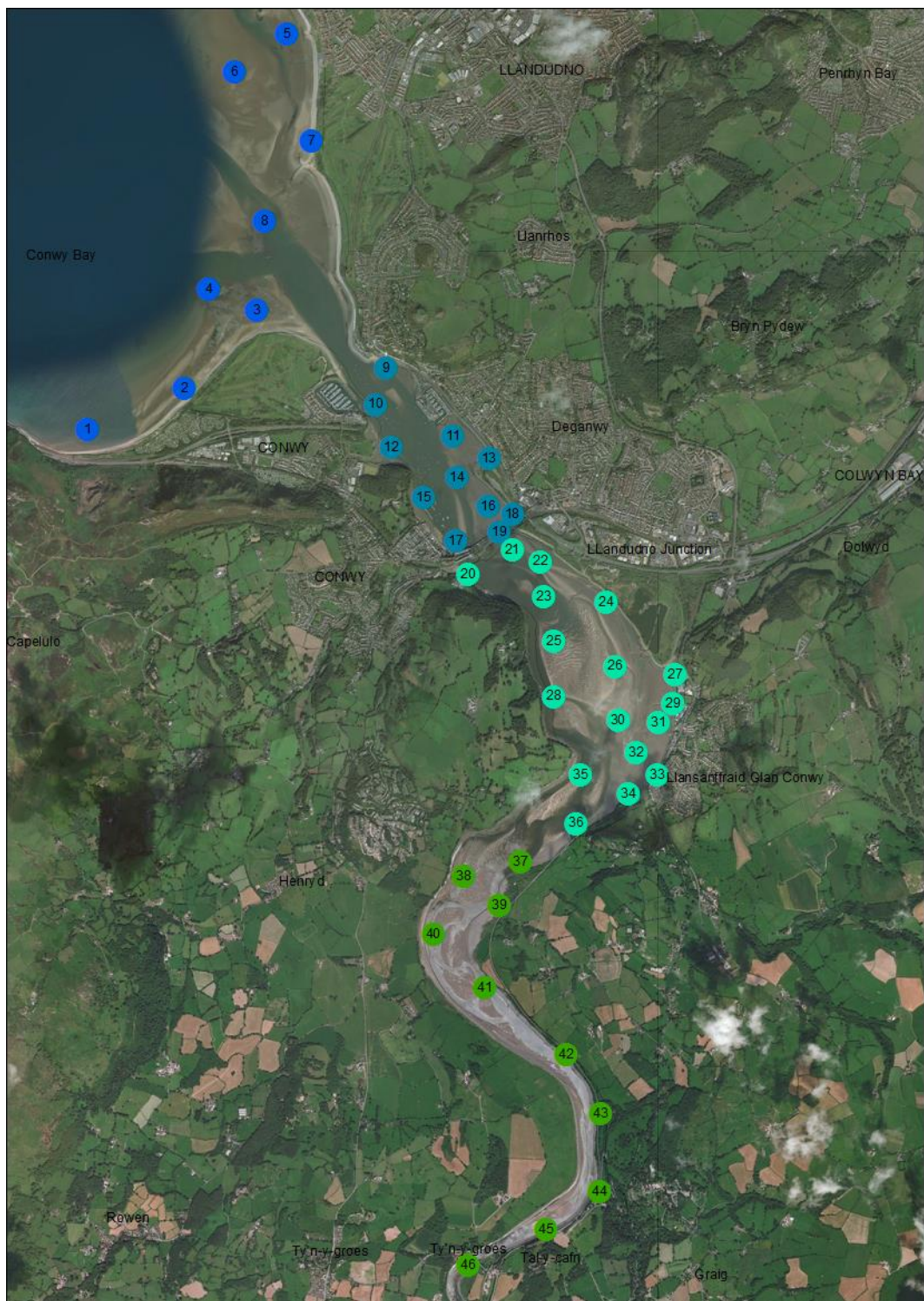
Table 1.1: Summary of previous research undertaken on the Conwy estuary and catchment tributaries to establish the extent to which this site has been studied.

Sample location	Type of sample	Analyte	Reference
Estuary mouth to Dolgarig	Water & Spm	Zn, Ni, Mn, Fe, Cu, Cd, Pb & Cr	(Zhou et al. 2003)
River catchment + Conwy bridge/Gyffin	Water	<i>E.coli</i> & hetrotrophic bacteria	(Williams et al. 2012)
Conwy marina to Tal-y-Cafn	Water	<i>E.coli</i>	(Quilliam et al. 2011)
Conwy marina to Tal-y-Cafn	sediment	<i>E.coli</i> , total coliforms, enterococci, <i>Vibrio</i> spp, <i>Campylobacter</i> spp, <i>Salmonella</i> spp, & total heterotrophs.	(Perkins et al. 2014)
Estuary mouth to Betws-y-Coed	sediment	Fatty alcohols & Sterols	(Mudge and Norris 1997a)
Nant Gwydyr (river leading to Afon crafnant)	Water	Zn, Pb, Cd & Fe	(Gao and Bradshaw 1995)
Conwy catchment tributaries leading to estuary	Water	DOC, N-NO ₃ ⁻ & total coliforms.	(Emmett et al. 2016)
Conwy catchment tributaries leading to estuary	Sediment & Water	Zn, Pb, Ca, Ni, Cr, V, Mn, Ti, Mo & Sn	(Elderfield, Thornton and Webb 1971)
Estuary mouth to Betws-y-Coed	Sediment cores, Water & Spm	Cu, Ni, Zn, Cd, Pb, Fe, Mn, Co, Cr & Ti	(Elderfield et al. 1979)
Conwy Bay and inlets to lower estuary	Water & Mussel flesh	<i>Ecoli</i>	(Cefas 2014)
Conwy catchment tributaries leading into estuary	Water & sediment	<i>E.coli</i> & total coliforms	(Bussi et al. 2017)
Estuary mouth to Betws-y-Coed + catchment tributaries.	Water & sediment	K, Na, Ca, Mg, Fe, Cl, SO ₄ , NO ₃ , Pb & Zn	(Brydie and Polya 2003)
Llanrwst & Trefriw	Water	As, Al, Cd, Cr, Cu, Fe, Pb, Mn, Ni, Zn, K, Na,	(AMEC 2011)

Figures 1.1 and 1.2 identify the locations of each site. The survey area was divided up into six sectors (Table 1.2). Sector 1 is within the Conwy Bay and purely marine influenced. Sector 2 is influenced by both semi-urban and rural, likely inputs will be from marinas/boating activity, urban and agricultural runoff. Sector 3 again will be influenced by semi-urban and rural contributions. Sectors 4 to 6 will mainly be from rural influence with sector 6 having an additional significant impact from the historic metal mines at Trefriw. All sectors have the potential of sewage effluents during storm events and occasionally at other times through consented discharges. Satellite image (Figure 1.1) illustrates the land use and highlights the main channel, sand banks and mudflats present within the estuary. The GIS map (Figure 1.2) identifies riverine and tributary inputs to the estuary and highlights the extensive drainage channels of sector 5 & 6. Within the text of this thesis, descriptions of the estuary have included lower and upper estuary. Lower estuary is classified as sectors 1-3 and upper estuary is classified as sector 5-6. Within the context of one survey (cross sectional transect) reference is made between the west and east side of the estuary. East refers to the Llandudno / Deganwy area (right side of the map, Figure 1.1 & 1.2) and west refers to the Conwy side of the estuary (left side of map, Figure 1.1 & 1.2)

Table 1.2: Sample sites separated into sectors and sites of interest within the thesis are highlighted.

Sector	Sample sites	Sites of interest within this thesis
Sector 1	1-4 5-8	Lower estuary, mussel beds Conwy Bay – Conwy Side Conwy Bay – West shore Llandudno side
Sector 2	9-19	Lower Estuary Conwy Marina (10), Deganwy Marina (11). Deganwy side (11, 13, 14, 26, 18, 19).
Sector 3	20-36	Lower estuary Gyffin river (20), storm runoff (22), Ganol river (27), Glan Conwy side (27, 29, 31, 33), Riverine input (33)
Sector 4	37-46	Eglwysbach river (44) and Tal-y-Cafn Bridge (45)
Sector 5	47-52	Upper estuary. Riverine inputs (49, 51, 52), Dolgarrog (52)
Sector 6	53-58	Upper estuary. Riverine input (53-57), Trefriw & Afon crafnant influence (57), Tidal limit at Llanrwst (58).



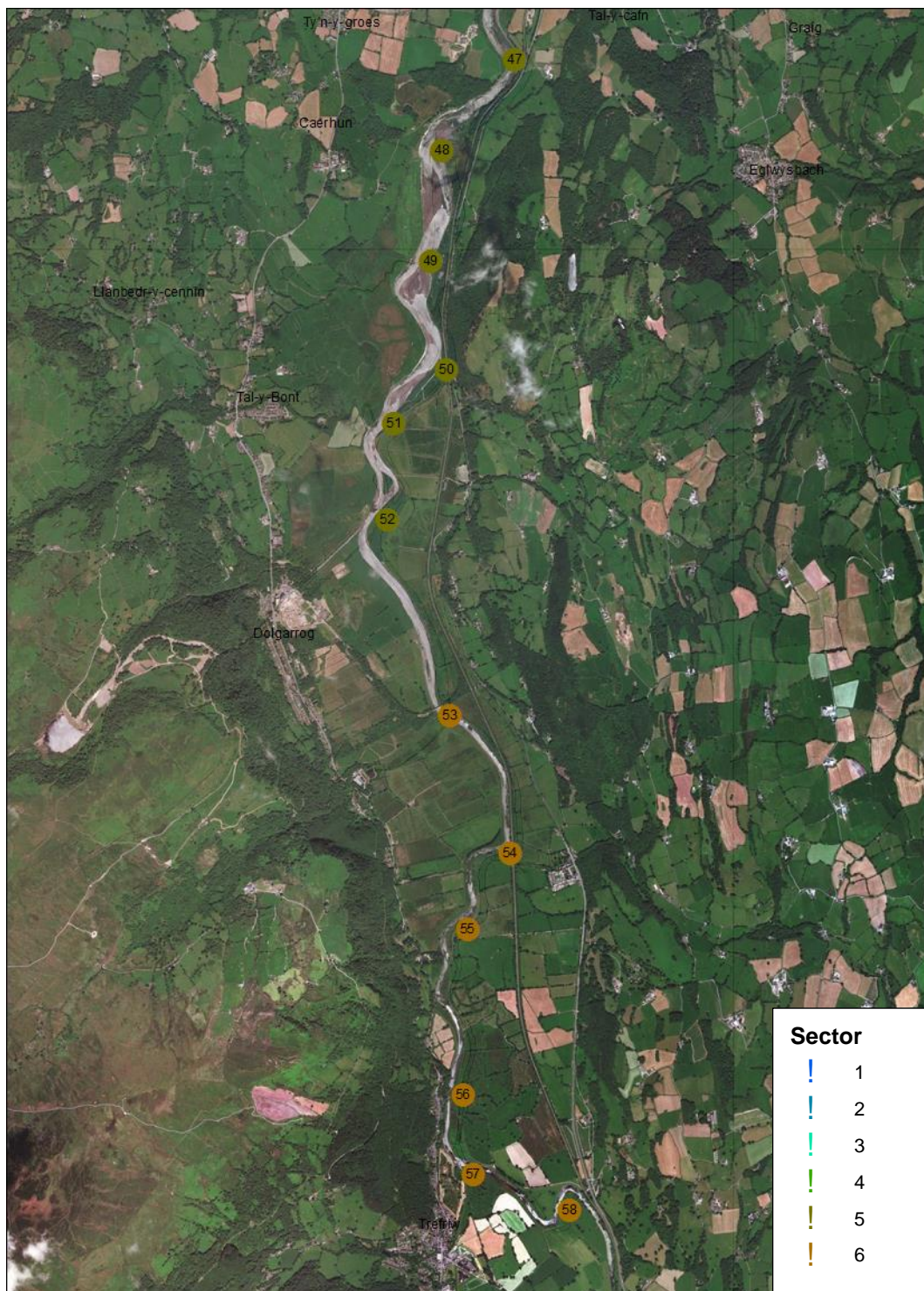
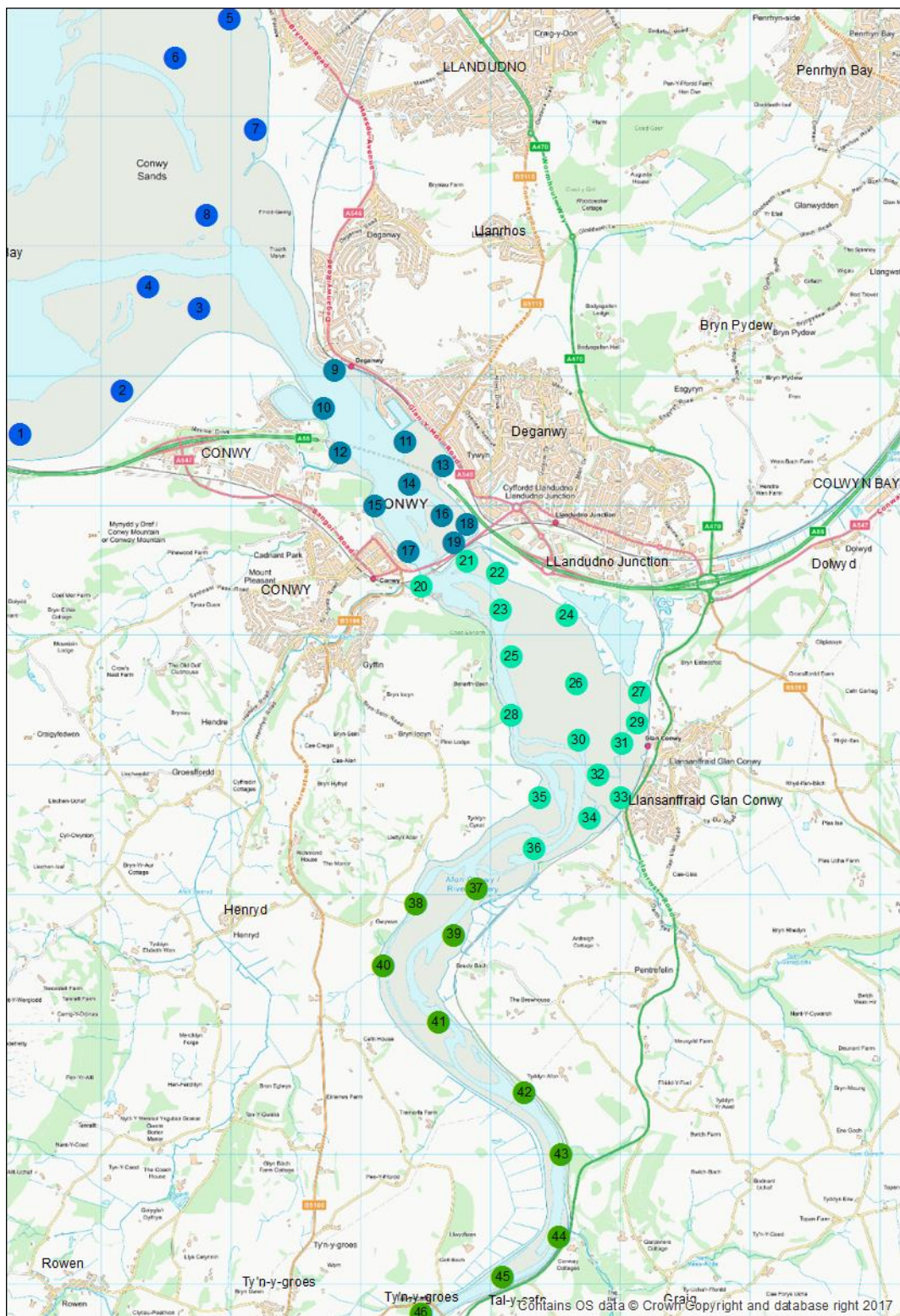


Figure 1.1: Satellite image of sediment sample locations for metals, nutrients and lipid biomarkers within the Conwy estuary. Survey area has been split into sectors 1-6 for ease of sample locations. Scale: 1:50,000.



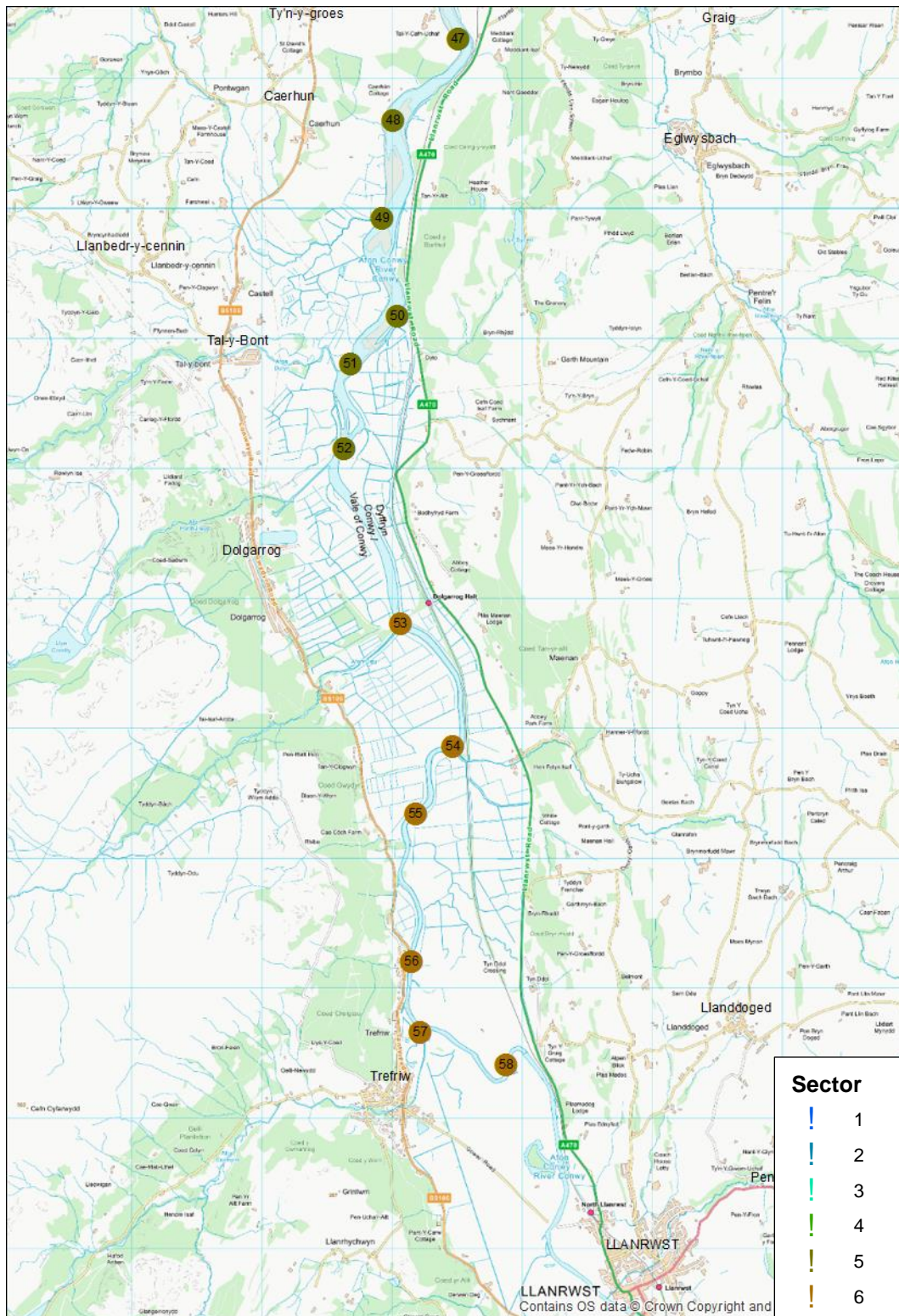


Figure 1.2: GIS map of sediment sample locations for metals, nutrients and lipid biomarkers within the Conwy estuary. Survey area has been split into sectors 1-6 for ease of sample locations. Scale 1:50,000.

1.2.2 Geology and land use

The geology of the area is mixed with rocks from Ordovician, Silurian and Cambrian periods; igneous and metamorphic rocks, from which baseline elements are more likely to originate, make up the western side giving rise to a steeper and more rugged landscape. Whereas the eastern side of the valley, characterised by siltstone and mudstone hills of the Migneint Moors, with more gentle slopes formed from rocks of sedimentary origin. The siltstone and mudstone bedrocks are generally covered in thin, often acidic soils and as a result the water entering the river tends to be high in humic acid. The impermeability of this landscape leads to flash flooding into the estuary. The mean flow in the river Conwy between 1964 and 2012 was approximately $19 \text{ m}^3 \text{ s}^{-1}$, measured from the flow gauge near Llanrwst (Cwm Llanerch; SH80258) and approximately 73% of the freshwater input into the estuary enters from this river which originates in the Migneint moors to the tidal limit near Llanrwst with the remaining input entering directly from slope runoff and smaller streams (Robins et al. 2014b). Historically this has caused frequent inundation of the floodplain and its villages and towns (Bussi et al. 2017). The catchment is typical of many in the UK, with land types ranging from relatively unproductive poorly drained peaty gleys and peat soil moorland in upland areas with relatively high rainfall to fertile lowland areas of freely-drained brown earth soils of relatively low rainfall that support livestock farming (Williams et al. 2012, Elderfield et al. 1979).

The Conwy catchment is predominantly rural and agricultural but with significant areas of woodland. High elevated areas support low intensity sheep rearing on moorland and blanket bog. Lower land supports mixed dairy, beef and sheep farming with little arable land present. In addition to coniferous plantation, there is scattered semi-natural woodlands in the lower lying part of the catchment including ecologically important areas of broad leaf woodland (Emmett et al. 2016)

1.2.3 Factors considered not natural but man-made.

Within the Conwy catchment there are both point and diffuse sources of contamination, however these are limited when compared to other UK urban and industrial estuarine catchments for example Liverpool Bay (Greenwood et al. 2011). Within the context of this thesis contamination refers to the increase of concentrations in organic and inorganic chemical parameters caused by human activity. Potentially the most significant point sources contributing to the Conwy Estuary

will be from sewage treatment works, intermittent discharges, marinas and the disused metal mines within the upper estuary (Figure 1.3). Diffused sources are likely to originate from freshwater inputs carrying agricultural and urban runoff from within the catchment.

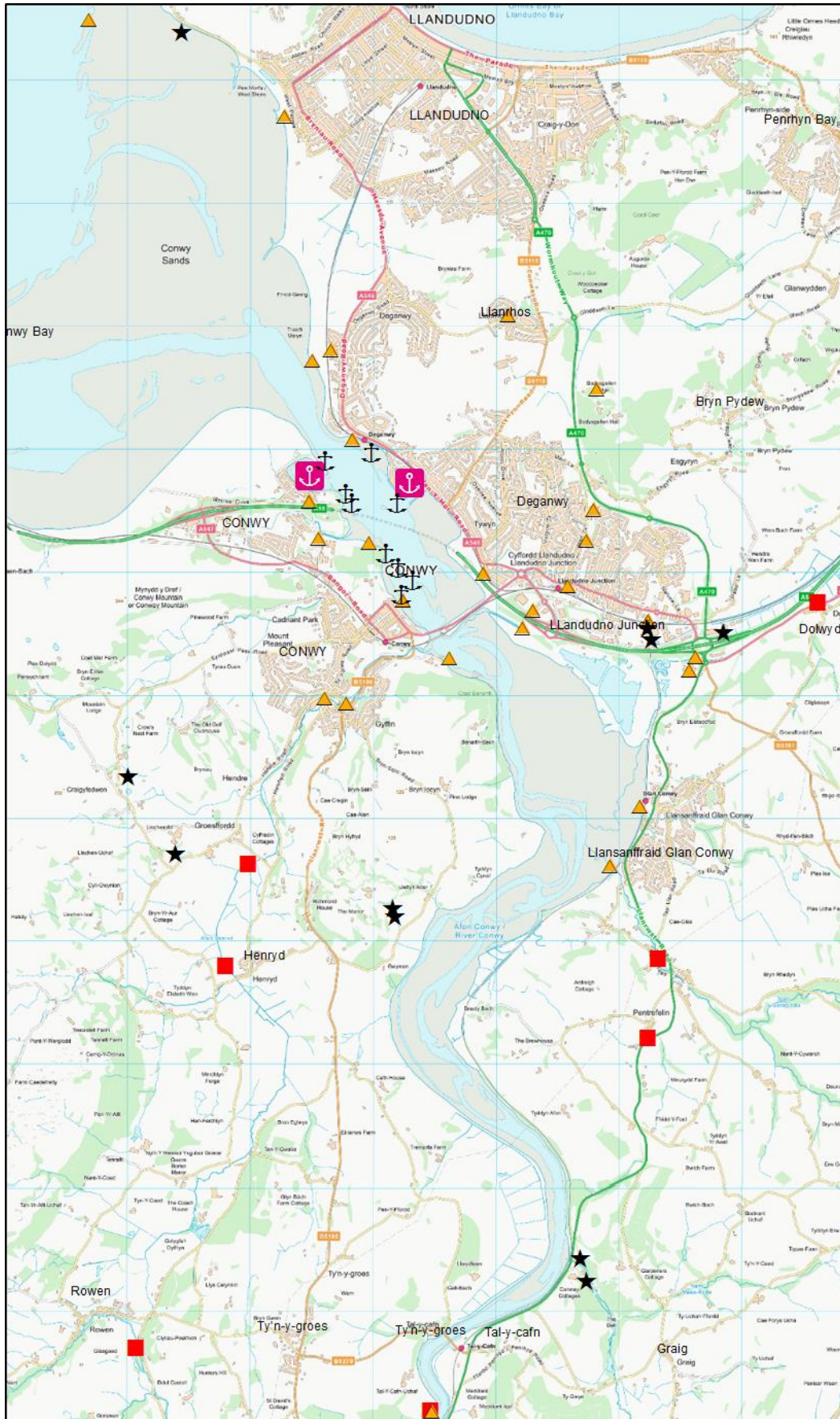
Although the majority of the area is rural and agricultural with both coniferous and deciduous woodland, the Conwy catchment has a population of approximately 112,000, 80% of which live in coastal resorts that present the main economic and tourist areas (Perkins et al. 2014). There are several towns within the catchment, with Llandudno having the highest population of 15,371 residents (population census 2011-03-27) at the mouth of the estuary. However the population substantially increases due to tourists by approximately 26,000 within the summer months, which will potentially put pressure on the wastewater treatment plants. There are 24 Welsh water sewage work discharges within the Conwy catchment. Four discharge to the upper reaches of the Conwy, at Tyn-Y-Groes, Tal-Y-Bont, Trefriw and Llanwrst and one discharges to soakaway (Dolwyd septic tank) the remainder discharge to water courses that drain to the Conwy estuary (Cefas 2014). In addition there are 49 intermittent discharges, the majority cluster around the lower estuary within semi-urban areas. Within the Conwy Estuary and at the west shore of Llandudno there are a number of storm and emergency overflows which operate during and following periods of heavy rainfall when sewage systems release excess sewage which could potentially result in reduced water quality. Although most properties are connected to main sewers, there are a further 1,269 private sewage discharges either permitted by or registered with National Resources Wales (NRW), providing treatment via septic tanks or package plant.

Contamination from boats is also a possibility within the lower estuary, although no records of tributyltin, where two large marinas are present providing 765 berths between them, in addition there are numerous other moorings and pontoon berths (Figure 1.3). However, there are no longer any commercial ports within Conwy Bay or Estuary and therefore impacts will purely be associated with the large number of private vessels, illustrating tourism and recreation as the main industrial activity within the estuary.

Within the Conwy catchment the Ordovician rocks are mineralized at some localities and there is a long history of mining of sphalerite and galena and associated industry which dates back to the 18th Century (Archer 1959). Elevated dissolved and active fluvial sediment bound Pb and Zn concentrations in the upper catchment are predominantly the result of the abandoned mine workings of the Llanwrst Mining District (Mines located in Figure 1.3) with evidence of enrichment within the River Crafnant which empties directly into the River Conwy (Brydie and Polya 2003). Sphalerite was a gangue mineral; therefore tips are very much enriched in zinc,

although closed since 1960, large spoil heaps still remain (Gao and Bradshaw 1995). Through gully erosion by streams, and leaching of the waste heaps, both the agricultural land of the flood plains and the river Conwy itself have been contaminated with lead (Pb) and zinc (Zn) (Gao and Bradshaw 1995). As a result, there are many potential sources of trace metals into the Conwy estuary and the adjacent coastal zone (Zhou et al. 2003).

With respect to the diffuse sources, the principle land use within the catchment is pasture where 348,766 sheep and 18,567 cattle (Agricultural consensus 2012) are within grazing lands. Higher elevations support sheep on rough grazing and lower elevations support a mix of sheep and cattle production on more productive land with higher stocking rates and some fertiliser application. In addition, manure is also added to land as fertilizer and sewage sludge occasionally applied to the lower catchment (Cefas 2014). Within the coast there is also limited built up areas where urban runoff will also enter watercourses during periods of high rainfall, both of these will contribute to contamination within rivers and streams feeding into the Conwy Estuary.



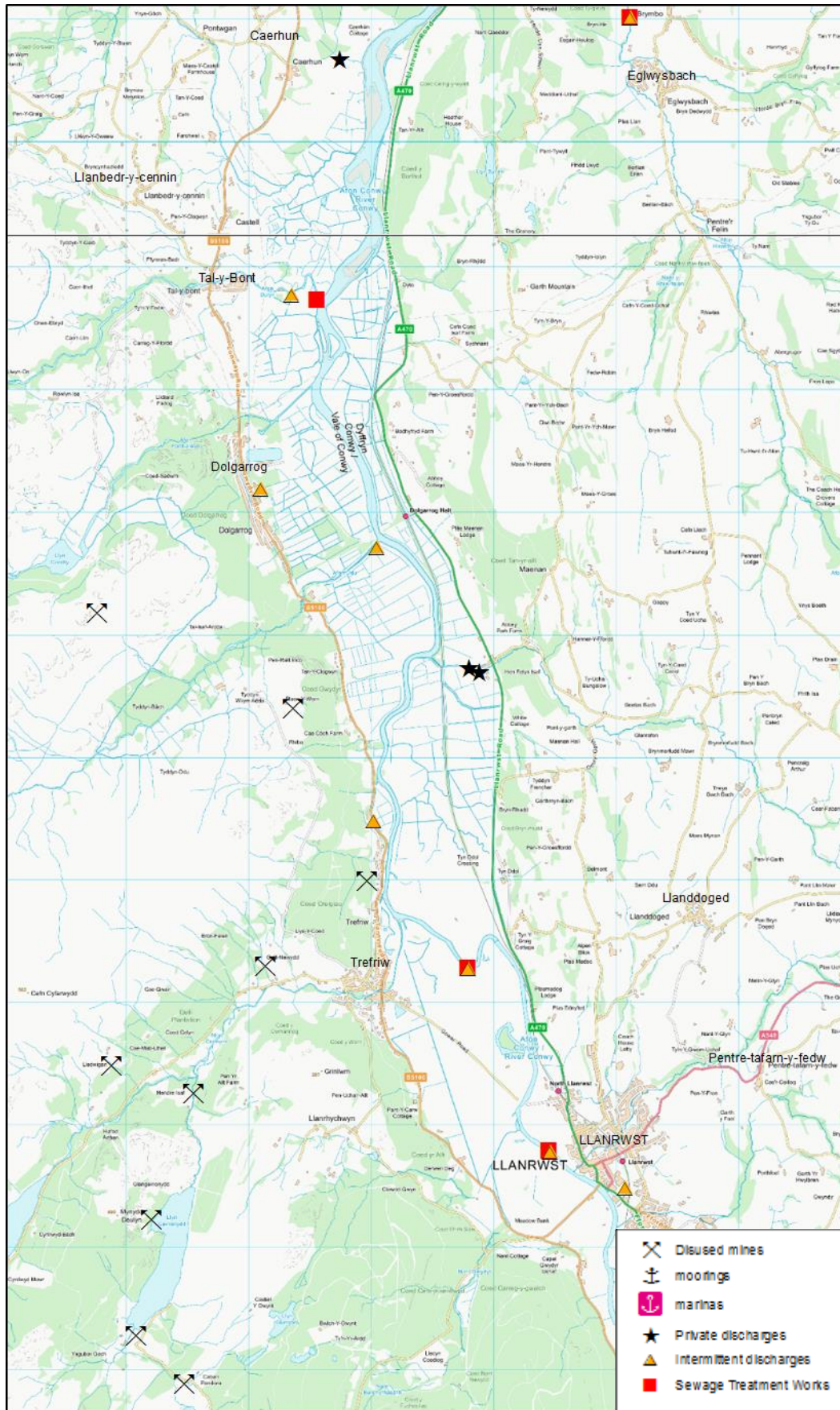


Figure 1.3: GIS map of superimposed locations of potential contaminants within the Conwy estuary. Scale: 1:50,000

1.3 Published previous research on contaminants within Conwy Catchment

1.3.1 Metals

Numerous studies within the Conwy catchment have established that the sources of heavy metal contamination in the upper catchment are predominately from the historic mines, primarily from zinc, arsenic and lead sulphide ores, sphalerite, arsenopyrite and galena respectively (Elderfield et al. 1971, Brydie and Polya 2003, AMEC 2011). Other sulphide minerals such as pyrite may also be present. Metals, other than zinc and lead, are likely to be present as impurities in ore bodies. These include silver, cadmium, copper and manganese. The present sources of contamination are likely to be remaining reserves of ore within the historic mines (either above or below the water table). Areas at most risk are those that were exposed during mining and deposited material in spoil heaps during mining and mineral processing (AMEC 2011). In oxidising waters (as found in the Conwy), cadmium, copper, lead and zinc sulphides are soluble in acid conditions (Drever 1997). Acidic waters are typically generated in mine waters by oxidation of pyrite and other iron sulphides.

Elderfield (1971) & (1979) established that the main contribution of dissolved and sediment-bound lead and zinc to the river Conwy was highest within the river Crafnant compared to any other tributaries in the upper catchment. Within this study, a baseline survey of dissolved and particulate lead (Pb) and zinc (Zn) concentrations within tributaries was undertaken within a limited number of estuarine sites, this revealed additional Pb and Zn concentrations, comparable to those in the upper catchment, occur within sediments of the River Gyffin in the lower estuary and are derived solely from sewage and domestic outfalls (Mudge and Norris 1997a). Brydie and Polya (2003) confirmed the findings of dissolved Pb and Zn concentrations, however, sediment bound Pb and Zn were found to be a factor of 20 less than that of the Elderfield (1979) study, which they felt was due to remediation effects of a series of water-treatment plants placed along the river in recent years. Zhou et al. (2003) studied the Conwy estuary and found that dissolved levels of Pb and Cd were generally close to or below the limit of detection. Zinc, Nickel (Ni), Manganese (Mn) and Iron (Fe) decreased with increasing salinity, suggesting river derived signals. Particulate metals fluctuated throughout the estuary. Zn, Mn and Fe tended to show a trend of decreasing concentrations with increasing salinity, suggesting a mixing of river-borne material of high metal content with marine or re-worked estuarine sediment of lower metal content. Amec (2011) concluded that the only instances of WFD EQS being exceeded other than for primary metals of concern (Cd, Cu, Pb Zn) of the Conwy catchment, were from Fe and

Ni which are entirely consistent with pyrite oxidation (B.Perkins, Aberyswyth University. *Per.comm*, 2015). The Amec (2011) study focused on establishing concentrations above the WFD environmental quality standards within the Llanrwst metal mines. The river Crafnant which feeds into the Conwy river as well as two locations within the estuary near Trefriw showed that dissolved cadmium exceeded the WFD environmental quality standard (0.09 µg/l). Whereas dissolved lead and total zinc both exceeded the WFD EQS (7.2 & 50 µg/l respectively) only within the river Crafnant and not the estuary itself. Dissolved copper concentrations were found not to exceed the WFD EQS at any of the monitoring points within the Conwy catchment.

1.3.2 Nutrients

By far the most research undertaken to date within the Conwy catchment refers to nutrient concentrations and to a lesser extent coliform data. Extensive research has been undertaken by both Bangor University and CEH within the terrestrial, estuarine and marine areas of the catchment. There is a direct relationship between nutrient concentrations and land use (Williams et al. 2012). Dissolved organic carbon is greater in waters draining from upland grassland and peat moorland than those from downstream. A significant higher concentration of phosphate and nitrates are found in water that was associated with agricultural land (119 ± 27.9 & 11 ± 3.5 µmol/l, respectively) compared to upland grasslands (3.0 ± 2.1 & 5.25 ± 1.03 , respectively) (Williams et al. 2012). Cooper et al.(2014) found that DOC concentrations were higher in summer when there is greater microbial activity, while nitrate is higher in winter when there is less uptake. The study of Emmett et al.(2016) which focuses on the whole of the Conwy catchment supports these findings, where DOC concentrations are noticeably high within drainage from the Migneint peat area in the south of the catchment and lowest at high elevations where peat is largely absent. Concentrations are also high in the south east part of the catchment with peaty gley soils, which are generating large amounts of DOC. NO₃⁻-N concentrations within the catchment did not approach the limit of 11.3mg/l for drinking water (Drinking Water Directive 98/83/EC). However, highest concentrations (>2.5 nitrate-N mg/l) can be seen indicative of losses from agricultural land within the lower catchment (Emmett et al. 2016). Coliform concentrations (both *Escherichia coli* (*E. coli*) & non *E. coli*) are highly localised, several sites exceed the revised Bathing Water Directive (2006/7/EC) classifications of excellent quality (250 *E.coli* CFU/100ml) and good or sufficient quality (500 CFU/100ml) in several areas of the catchment, with Tal-Y-Cafn shown as having a high number of coliforms (CFU/100ml,

Emmett et al. (2016)). Quilliam et al. (2011) also identified elevated coliform levels within the lower estuary establishing increased concentrations within the Deganwy side of the estuary which Perkins et al. (2014) identified as most likely due to resuspension of sediments. Localised distribution of coliform concentrations within the catchment is believed to be associated with scattered point sources such as septic tanks and sewage treatment works (Emmett et al. 2016).

1.3.3 Lipid Biomarkers

Mudge and Norris (1997a) undertook a lipid biomarker study within the sediments of the Conwy estuary to establish the origin of the organic matter. This study divided the estuary into three sections: (1) the offshore marine environment where marine biomarkers were dominant but also evidence of terrestrial organic matter probably originating in the Conwy river and transported to the marine environment within suspended sediments (Mudge and Norris 1997a); (2) The lower estuary was dominated by sewage -derived materials, of the remaining organic matter marine-derived material was present in greater amounts than terrestrially derived inputs, and exceptions were seen at sites of freshwater runoff; (3) The upper estuary which was characteristic of sporadic inputs of organic matter and an increasing dominance of terrestrially derived materials. Sewage inputs can be seen adjacent to local treatment discharge points. Freshwater sites above the tidal limit are dominated by terrestrial vegetation and sewage discharges of unknown origin.

1.4 Justification and aims of this thesis

The Conwy was selected within this study as a representative UK catchment where more than 90% of land cover consists of natural, semi natural and managed rural land use types (Sgouridis and Ullah 2014) and would potentially be an ideal candidate for a pristine example of an estuary with limited human impact. Moreover, this catchment has been identified as one of the priority catchments by the Natural Environment Research Council (NERC) Macronutrient Cycles Programme due to being a typical non-industrial area with few discrete agricultural and industrial sources. With the overarching aim to quantify the scales (magnitude and spatial/temporal variation) of nitrogen and phosphorus fluxes and the nature of transformations through the catchment to enable easier attribution of large scale drivers of change i.e. Climate and

atmospheric pollution (NERC 2015). Along with previously published studies within the Conwy catchment this supports the notion that the Conwy estuary is a good example within the UK of a relatively pristine environment to enable detailed research to be undertaken to establish natural variability within a catchment due to its limited human impacts.

By utilising a multi-tool approach where by different analytical techniques are utilised to identify concentrations of contaminants within an environment, a clearer picture of potential sources can be made and verification whether the Conwy estuary can be classified as a pristine example of a modern day estuary. This thesis focuses on assessing the concentration of metals, nutrients and lipids in water and sediment samples to define elevated versus baseline concentrations, to address whether the Conwy Estuary is able to full fill the paucity of information regarding concentrations within a pristine environment. The objectives of this thesis were:

Apply known models to sediment data and compare and contrast results of these in relation to whether an environment is pristine or affected by human activity (chapter 2)

Assess the variation in natural ecosystems of sampling different locations within the estuarine environment or influence of tides on the water chemistry. Application of known models and ratios to water and sediment data to identify whether sites can be deemed pristine or influenced by human activity (chapter 3).

Assess and identify the source of organic matter utilising known lipid biomarker ratios on sediment data and compare and contrast results from the Antarctic to establish whether from pristine or affected by human activity (chapter 4).

Utilising this multi tool approach elevated locations were scrutinised to highlight whether there were any potential contaminant sources which deviated from the pristine condition. A proportion of sample locations fell below modelled enriched concentrations and therefore deemed near-pristine locations which can be utilised as baseline conditions for further studies.

CHAPTER 2

Metals

2.1 Introduction

The environmental quality of coastal ecosystems is an issue of growing international attention. Trace metals are pollutants of concern due to their persistence in the environment with the potential to be bioavailable and toxic to aquatic biota at high concentrations (Fairbrother et al. 2007, Kennish 1991, Nemerow 1991). Metal pollution is a global problem in estuaries due to the legacy of historic contamination and currently increasing metal emissions (Machado et al. 2016).

Many elements are sourced naturally in the environment and provide aquatic ecosystems with trace elements that are essential to life within certain limits (eg. Iron (Fe), Manganese (Mn), Copper (Cu), Cobalt (Co), Zinc (Zn) and Selenium (Se)) and above required concentrations many of these elements (e.g. Zn, Cu and Se) can have adverse effects (Allen, Garrison and Luther 1998). Elements are introduced into the aquatic environment from both natural processes, such as weathering and erosion of the geological matrix and atmospheric decomposition, or through anthropogenic sources such as industrial waste water discharge, sewage effluent, fossil fuel combustion, urban runoff or mining (Linnik and Zubenko 2000, Lwanga et al. 2003, Gupta et al. 2014). Their ecological significance is related to accumulation and subsequent toxicity from bioavailable metals to benthic flora and fauna, which can bio accumulate through the food chain leading to detrimental impacts on a range of species as well as humans (Nurnberg 1984, Munksgaard and Parry 2002).

Contaminants are controlled by the physical and chemical parameters of the sediments with which they are associated, for example sediment characteristics such as grain-size, carbonate contents, mineral and organic matter strongly influence the adsorption and in turn the total concentrations of elements onto sediment type (Loring 1990, Salomons and Forstner 1984, Machado et al. 2016). It is commonly known that positively charged elements i.e. metals show a strong affinity with the negatively charged clay fraction and its coating formations (e.g. organic matter, iron and manganese oxides). Within sediments grain size distribution is rarely homogeneous and greater concentrations of elements do not necessarily reflect anthropogenic inputs but may be due to a greater clay content within the sample (Roussiez et al. 2005) or higher abundance of minerals that are naturally enriched within certain metals (Angelidis and Aloupi 2000). For these reasons metal concentrations alone are misleading and difficult to distinguish between anthropogenic and natural sources.

The procedure of normalisation, removes the effect of the above parameters and enables correct comparisons between sites to be made ensuring that the elemental concentrations are the only contributing factor. There are various techniques to normalise data involving utilising only one fraction for all sites (<62.5µm) or the total sediment concentration is divided by total organic carbon or a conservative element which reflects the clay fraction. (Birch and Taylor 2000), (Ackermann, Bergmann and Schleichert 1983). This study utilised the elemental normalisation approach, which involves the use of “conservative” trace elements, which are non-anthropogenic and act as a proxy for the fine fraction in the sediment, particularly clay minerals (Daskalakis and O'Connor 1995, Loring 1990, Matthai and Birch 2001). It avoids the time consuming separation of grain size fractions, which is a process with inherent imprecision and risk of cross contamination (Loring 1991). This technique, in addition, compensates for the mineralogical as well as the natural granular variability of elemental concentrations in sediments. Figure 2.1 illustrates 82% of the variability within the Cs concentration is due to the clay content, this high correlation is a good example of elemental normaliser.

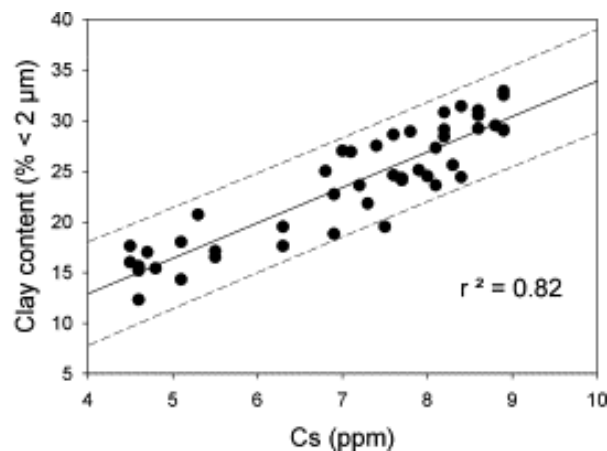


Figure 2.1: Source Roussiez et al. (2005): Relationship between clay content (% <2µm) and Cs (ppm) within the sediments of NW Mediterranean. The dotted lines represent prediction interval at the 95% confidence level.

Estuarine sediments serve as effective traps of river-borne elements, of both natural and anthropogenic sources to accumulate together. Traditional techniques to identify samples as contaminated are based on a concentration limit, a comparison of pollutant levels measured in sediments with legally determined limits. This approach has severe limitations as natural

background and anthropogenic sources are not differentiated (Renzi, Bigongiari and Focardi 2015).

The use of elemental background models is an important tool for environmental management of estuarine and coastal areas. The tool can aid in distinguishing between areas impacted by anthropogenic sources and those that are natural. To identify the true contamination level the assessment of anthropogenic contributions to sediments must be accompanied primarily by the estimation of heavy metal contributions by natural processes (Windom et al. 1989b). Regional geochemical baseline models have been used successfully by many researchers (Renzi et al. 2015, Mil-Homens et al. 2013, Rubio, Nombela and Vilas 2000, Roussiez et al. 2005, Song et al. 2014) to establish the background concentrations using regression analysis with conservative trace elements to calculate enrichment factors.

Luoma (1990) defined several quality criteria for the use of conservative elements for the geochemical baseline models; firstly the normalizer must co-vary in proportion with the natural concentrations of the metals of interest. Secondly it must be insensitive to anthropogenic sources and finally it should not be influenced by changes in the geochemical conditions such as redox potential (Roussiez et al. 2005).

Previous sedimentary studies to date have only concentrated either on heavy metal distribution or a select few elements within coastal, estuarine, and river catchments. Although Sarkar et al. (2004) have undertaken extensive geochemical analysis with 52 elements the study only sampled five sites, three in an estuary and two in the adjacent rivers, and did not take into account background levels. They also saw the need for a more detailed survey of surface sediments to determine the patterns of distribution of the elements, which will lead to source identification.

The purpose of this study was to undertake a geochemical survey of the Conwy estuary, North Wales, in order to establish naturally occurring baseline levels of an extensive suit of elements in a non-industrial area that other studies could refer to. In addition, to characterise the relationships among trace elements and normalisers suggested by the literature as useful tracers of natural background levels due to aluminosilicates. Where levels exceed the baseline, enrichment factors have been calculated to identify the areas of anthropogenic enrichment.

2.2 Materials and methods

2.2.1 Sediment sampling and geochemical analysis

Sampling was undertaken throughout the estuary, from Conwy bay at the mouth to the tidal limit at Llanrwst; in total, 69 locations were sampled, by a spatial survey to understand the distribution of concentrations across the entire estuary (Figure 1.1 & 1.2). Exact locations for all sampled sites were determined using a global positioning system and entered into a geographical information system (GIS). At each sample site, sediment was collected using a Peterson grab and the top 2-3 cm was scrapped into pre-labelled plastic bags and transported to the laboratory where it was frozen until analysis was conducted.

In the laboratory, each sample was homogenised to ensure all sediment sizes were represented in the sample. From this, 10g was extracted for grain size analysis, using the method based on Folk's (1974) standard dry and wet sieving technique. A second portion of each homogenised sample was air-dried to a constant weight, finely powdered using a ball mill and 1g was digested in 70 % nitric acid (HNO_3) at 100 °C for 1 hour and diluted based on the technique of Hudson-Edwards et al. (2001). The total concentrations of all elements were then analysed using ICP-MS processed by Aberystwyth University using Pearce (1991) method, range of metals analysed in Appendix 2.1. Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Samples were duplicated with results as mean values. Results are expressed as mg kg^{-1} dry sediment. Limit of detection and quantification was calculated using 3 times and 10 times, respectively, of the standard deviation of the mean of the blank data (Appendix, Table 2.2). Certified reference material of CAMET TILL river sediment were run with the sediment samples, precision and accuracy for selected metals shown in Appendix, Table 2.1.

For total carbon analysis 20mg of the homogenised and ball-milled sediment was weighed directly into a tin capsule. The weight was recorded and capsule folded to seal the sample prior to analysis. For organic carbon a similar quantity of sediment was weighed into a glass ampoule. Carbonate was removed following the adapted methodologies of (Verardo, Froelich and McIntyre 1990), whereby 1ml of sulphurous acid was added to the ampoule. Samples were left overnight to react and de-gas in a fume cupboard. Samples were then transferred to a vacuum desiccator and held under vacuum for 2-3 hours to ensure all CO_2 was removed, before being frozen for a minimum of 4 hours. After freezing the samples were placed in a freeze drier for 24 hours. The dry residue was transferred to tin capsules which were sealed by folding prior to

loading into the auto sampler. Standards of acetanilide (BDH chemicals – 71.09% Carbon) were utilised for the calibration standard ranging from 0.1 mg to 3.0mg. Empty capsules were used as blank correction for the standards and samples. Acetanilide were run as an unknown sample after every 12 samples to monitor for instrument drift. Certified reference material of medium organic content soil standard B2178 were used for the validation of the method for C & N. Results obtained were well within acceptable values, being 3.26 ± 0.02 % w/w (certified value 3.07 ± 0.07 % w/w) and 0.23 ± 0.01 % w/w (certified value 0.27 ± 0.02 % w/w) respectively.

All carbon analysis was performed on a Costech International Elemental Combustion System (ECS) 4010 using a pneumatic auto-sampler. Operational temperatures were set to 950 °C in the left combustion furnace, 630 °C in the right reaction furnace and 50 °C in the gas chromatographic separation oven. The helium (>99.9%) carrier gas pressures was set to 1.2 bar giving a flow rate ~ 100 ml/min. Oxygen (>99.9%) used for the combustion was set at 1.9 bar and air (water and oil free) 2.4 bar. A calibration coefficient of ≥ 0.999 was achieved for each analytical run. Limit of detection was calculated using 3 times the standard deviation of the mean of the blank data (empty tin capsules) which resulted in $0.08 \mu\text{mol g}^{-1}$ for carbon.

2.2.2 Definition of regional geochemical baselines for the Conwy estuary

For each of the studied metals, linear regression was performed, considering the whole dataset, with the different normalizer elements (Lithium (Li), Cobalt (Co), Caesium (Cs), Scandium (Sc), Fe & Rubidium (Rb)). The selection of the surface sediments which constituted the baseline was based on the identification of statistical outliers and influential cases from the studied sites through regression analysis using Cook's distance, leverage levels and residual plots as in (Mil-Homens et al. 2013). A Shapiro-Wilk test was used to test for normality of distributions. The dataset underwent log transformations to meet assumptions of normality and stability of variance. In addition when none of the transformations would result in a normal distribution the assumption of normality was based on that a variable is reasonably close to normal if its skewness and kurtosis have values between -1.0 - +1.0 (Mil-Homens et al. 2013). The process of testing for normality and selection of outliers was iterated until a normal distribution was achieved (Schiff and Weisberg 1999). A 95% prediction interval has been drawn for each regression using Statistical Package for the Social Sciences (SPSS) software. Then all samples that were standing outside the upper limit were eliminated and considered enhanced (Doherty, Brunskill and Ridd

2000). After each elimination step the regression was recalculated until no sample fell outside the upper limit (figure 2.1).

2.2.3 Enrichment Factors

After defining the baseline for each element, it is possible to estimate the level of elemental enrichment relative to the baseline through the determination of Enrichment Factors (EF) that were calculated by dividing its ratio to the normalizing element by the same ratio obtained from the baseline (Mil-Homens et al. 2013):

$$EF_{\text{metal}} = \frac{(\text{heavy metal/normalising element})_{\text{sample}}}{(\text{heavy metal/normalising element})_{\text{baseline}}}$$

Where heavy metal (or metalloid) and normalising element concentrations for (metal/normalising element)_{baseline} were obtained from the regression line. Enrichment factors higher than 1.5 indicate that an important fraction of the metals and metalloids is derived from non-crustal materials associated with biota and/or anthropogenic contributions (Zhang and Liu 2002). The main advantage of considering these baseline values is that they reflect natural variability of sediments in the study area when compared with total elemental concentrations of crustal abundances (Loring 1991), which can differ widely from the regional baseline values, leading to a misinterpretation of the EFs (Mil-Homens et al. 2013).

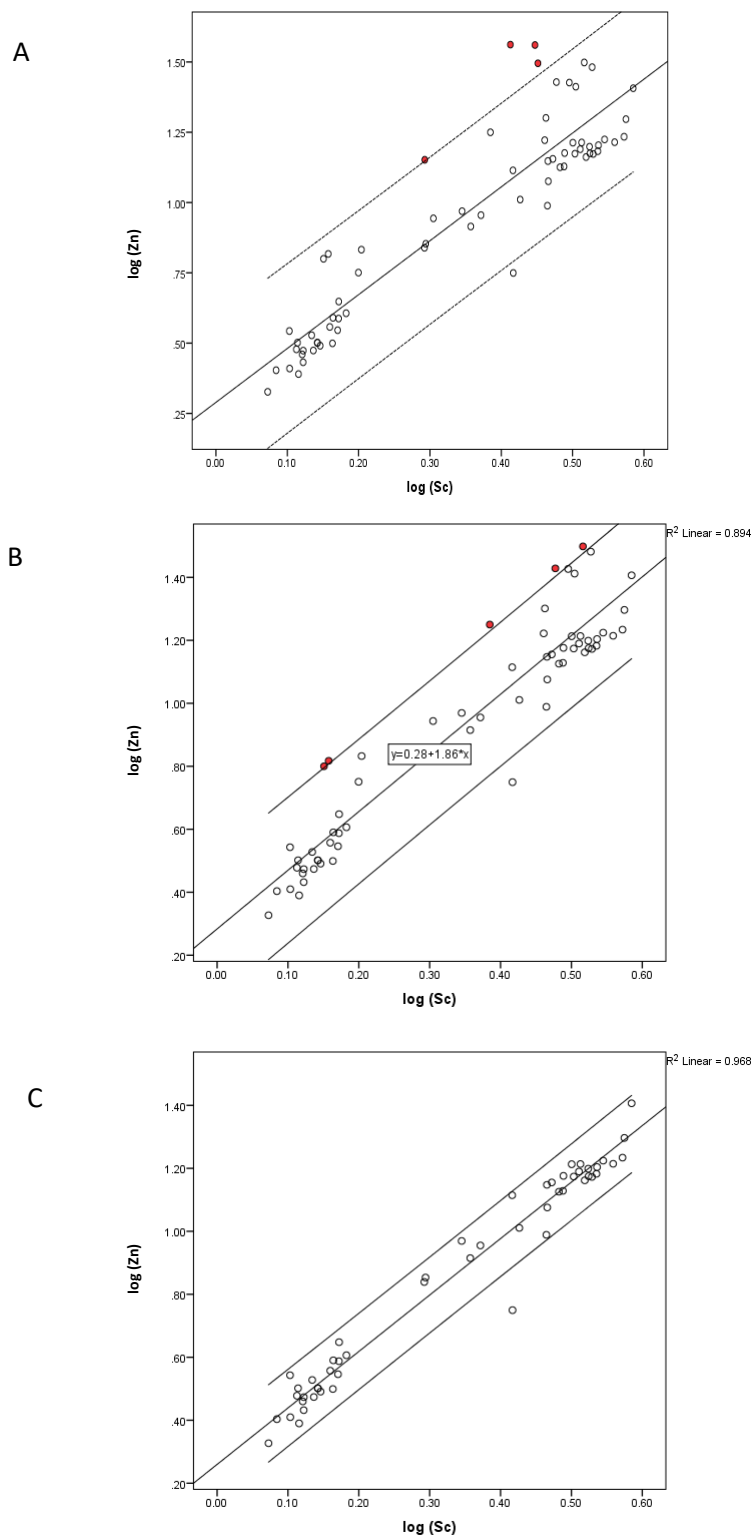


Figure 2.1: Example of regressions during the elimination of enhanced samples for Li using Sc as the normaliser. A is the original regression, B is the regression after elimination of A's outliers and C is the final regression following all eliminations. Red circles signify the samples that fall outside the 95% prediction interval and are therefore enhanced and removed. The slope and intercept of each regression and therefore each metal can then be used to predict baseline conditions.

2.3 Results

2.3.1 General sediment composition

The total composition of grain size and organic content of the investigated sediments show that there is a great range of variability between sample sites throughout the estuary. Sand present in sites ranges between 1.4 - 99.9 %, gravel between 0-81.2% and mud 0.1 - 98.6 %. Average grain-size composition is dominated by sand and mud contents of 55% and 43%, respectively (Figure 2.2).

Sites with the highest percentage of sand were located in Conwy Bay (sector 1) and through the middle of the widest section of the estuary (sector 2 & 3), where 94-99% of the >2mm fraction was found at each site, with the exception at the commercially used mussel beds, located at site 8 (sector1) which showed high fine content. The highest proportion of fines, (between 91-99%) were found in the sheltered area of the marinas (sector 2), Glan Conwy (sector 3) and upper part of the estuary (sector 5 & 6) which corresponds to more terrestrial influence (Figure 2.3).

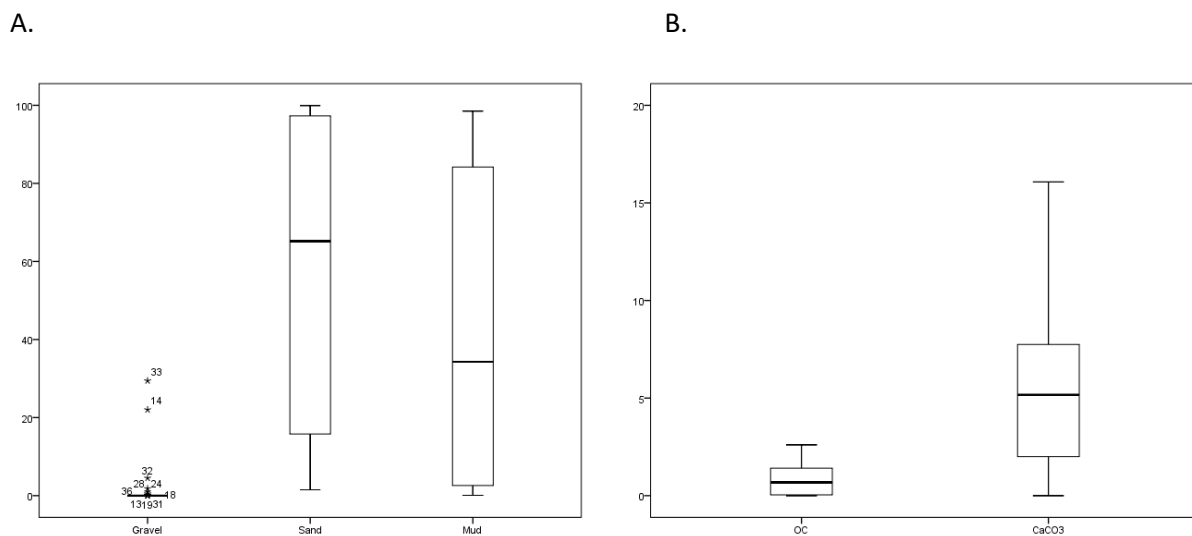


Figure 2.2: Box plot of the percentage distribution of the different grainsizes characterised as sand, mud and gravel of the total grainsize across all sites within the Conwy Estuary (A) and boxplot of the range of Organic carbon and calcium carbonate concentrations (wt%) for all sites in the Conwy Estuary (B). Horizontal bar refers to the median value of each physical parameter for all sites. Error bars show the extreme ranges. * indicates outliers, o indicates extreme outliers.

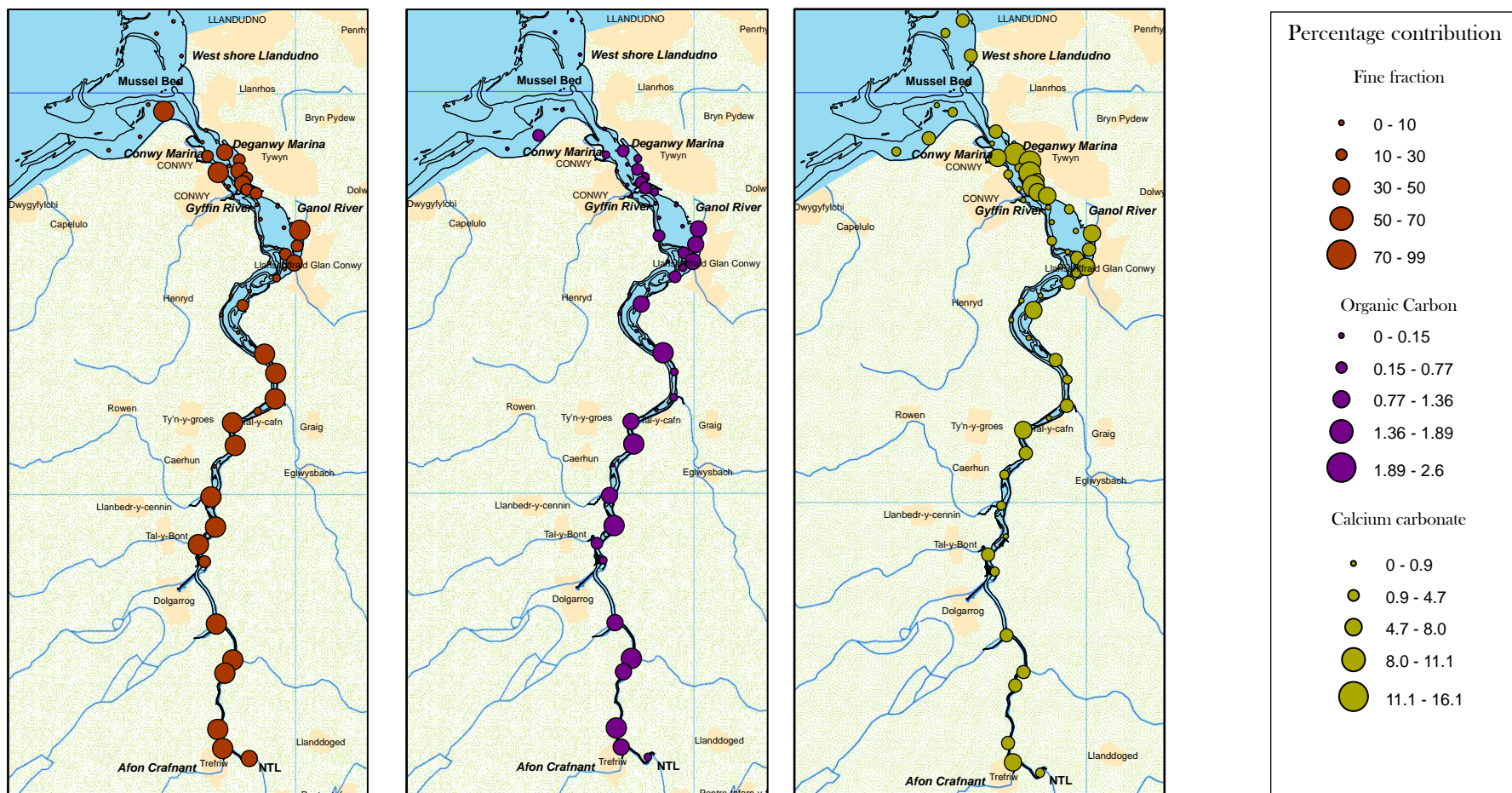


Figure 2.3: Percentage contribution of the distribution of fine grainsize fraction (<63 μm) and the concentrations of organic carbon and calcium carbonate in sediments within the Conwy Estuary, up to the tidal limit (Llanrwst). Detection limit of Carbon is 0.1%.

Organic carbon represents the concentration of organic matter in each sample in the study area, organic carbon ranged from 0.05% to 4.14%; the highest organic carbon content corresponds with the fine grained sediment where the highest concentrations can be seen in vicinity of the marinas, Glan Conwy and the upper part of the estuary (Figures 2.2 & 2.3). Calcium carbonate content ranged between 0.08% and 16.08% and contrary to organic carbon had higher concentrations in Conwy bay and the lower estuary (Figures 2.2 & 2.3).

2.3.2 Spatial distribution patterns of elemental concentrations and relationships

Total elemental concentrations in the surface sediments for each site of the Conwy estuary are presented in Appendix 1. Antimony (Sb) was omitted from the results as only 18% of samples had values above the detection limit. Descriptive statistics were undertaken on the raw data, Table 2.1 summarises these results, which includes mean concentrations, standard deviations, range and fold difference from minimum to maximum concentrations. Coefficient of variation (CV) is also included to indicate the precision of the analysis. Fe, Mn, Zn and Strontium (Sr) had the highest concentrations in the Conwy sediments. Fe and Mn are considerably higher in concentration than the other elements, highest concentration at 35.5 g/Kg and 3.08 g/Kg respectively with mean concentrations of 12.6 g/Kg & 0.44 g/Kg respectively. Zn and Sr maximum concentrations are 0.24 g/Kg and 0.21 g/Kg respectively with mean concentrations of 0.07 g/kg & 0.04 g/kg respectively. Sr and Uranium (U) had the highest variation from minimum to maximum values of 3.44 mg/kg to 206.34 mg/kg and 0.05mg/kg to 2.8mg/kg respectively. The minimum variability between concentration ranges is Indium (In) with values ranging from 0 – 0.05mg/kg.

Table 2.1: Range of concentrations of the investigated elements, Concentrations are mg/kg, N is the number of samples and times greater refers to the difference between minimum and maximum values. Mean reference refers to the mean concentration of 10 reference materials. Coefficient of variation (CofV) % is the S.D./mean ref * 100% which represents the precision of the analysis, 10% is the general expectable limit

Sample descriptive statistics								References	
	N	Min	Max	Diff	% <LOD	Mean	Std. Dev	Mean ref	C of V%
In	58	0.0005	0.05	0.05	8.6	0.02	0.01	0.19	9.63
Tl	58	0.002	0.11	0.11		0.04	0.03	0.32	8.54
W	58	0.020	0.2	0.2	62.1	0.03	0.04	0.13	31.47
Hg	58	0.040	0.36	0.32	51.7	0.08	0.09	2.48	14.21
Sn	58	0.014	0.52	0.51	8.6	0.09	0.07	7.12	44.85
Mo	58	0.0035	1.13	1	3.4	0.23	0.2	2.37	8.08
Ag	58	0.0005	1.42	1.42	46.5	0.07	0.17	0.57	70.2
Cd	58	0.0007	2.79	2.79	5.2	0.52	0.56	1.95	12.65
As	58	2.29	13.06	5		5.27	2.11	12.18	8.69
V	58	5.23	37.89	7		14.93	6.85	55.44	10.17
Sm	58	0.31	2.34	7		1.07	0.54	1.31	10.15
Gd	58	0.05	0.4	8		0.18	0.09	0.23	8.43
Y	58	1.14	10.14	9		4.42	2.36	6.33	11.52
La	58	1.32	12.38	9		4.46	2.31	5.28	9.57
Ho	58	0.04	0.37	9		0.16	0.09	0.22	9.36
Lu	58	0.01	0.11	11		0.05	0.03	0.07	10.76
Th	58	0.34	3.89	11		1.61	1	1.57	9.26
B	58	1.13	14.38	12	1.7	6.95	3.01	20.78	6.59
Cr	58	2.36	28.37	12		12.17	7.22	33.04	11.86
Cs	58	0.11	1.34	12		0.56	0.38	0.66	6.62
Se	58	0.15	2.08	13		0.87	0.46	1.22	13.78
Be	58	0.06	0.87	14		0.34	0.21	0.31	12.71
Sc	58	0.18	2.84	15		1.4	0.86	4.03	10.53
Fe	58	2362.8	35507.58	15		12610.22	8137.83	20931.99	15.49
Co	58	1.25	19.89	15		5.95	4.12	5.58	11.57
Ni	58	2.26	40.02	17		14.89	9.79	28.06	12.53
Rb	58	0.61	11.83	19		4.84	3.59	8.89	6.76
Cu	58	0.0005	25.64	25	10.3	8.14	6.86	285.69	12.11
Li	58	1.12	35.45	31		10.76	8.54	10.91	7.39
Pb	58	2.81	91.23	32		23.02	20.26	154.73	11.11
Zn	58	7.14	239.81	33		65.08	48.52	129.74	12.13
Mn	58	74	3087.79	41		444.13	475.4	196.9	8.34
Ba	58	0.94	42.94	45		15.17	12.4	49.65	16.6
Bi	58	0.01	0.47	47		0.14	0.11	0.24	13.03
U	58	0.05	2.8	56		0.39	0.38	1.09	8.44
Sr	58	3.44	206.34	59		43.89	40.03	52.7	14

The fold difference between minimum and maximum values for each element indicates if an element is potentially enriched above that of natural variation. The table has been ordered to show the elements which have the greatest difference (fold difference from minimum to maximum). Tungsten (W), Tin (Sn) & Silver (Ag) (31.5% at 0.1 ppm, 44.9% at 7ppm & 70% at 0.6ppm respectively) have very high discrepancies in the precision of this analysis. In, Thallium (Tl), Mercury (Hg) and Molybdenum (Mo) results were all considerably lower than the reference material and below accurate quantification of concentration. Hence W, Sn, Ag, In, Tl, Hg & Mo were omitted from the data set. Barium (Ba) also had a high RSD at 45% and therefore the precision of the concentrations in Table 2.1 may be inaccurate. However it has not been omitted due to its importance, being the third highest fold difference from minimum to maximum concentrations, but caution will be applied to this element. Elements above Cu in Table 2.1 indicate natural variation due to their limited fold difference but Cu, Li, Lead (Pb), Zn, Mn, Ba, Bismuth (Bi), U and Sr all appear to have evidence of enrichment.

2.3.3 Relationships between components

The remaining dataset was subjected to statistical analysis after being log transformed in order to explore the possible association existing between the different variables analysed for each site. Despite transformation the data did not show a normal distribution; therefore a non-parametric test was used. Table 2.2 shows the Spearman's rank-order correlation matrix for all the metals analysed to assess the relationship between all the elements. Preliminary analysis showed the relationship between all elements to be monotonic, as assessed by visual inspection of a scatterplot. Apart from Boron (B) and Sr (which showed a weak to moderate correlation to all other elements), there was a strong to very strong significant correlation (0.6-1.0) between all elements, as defined by Cohen(1988), $r_s(67) = .61-.99$, $p < .0005$ and therefore no significant difference between them. B and Sr showed a strong positive correlation, $r_s(67) = .61$, $p < 0.0005$ indicating a strong similarity to each other. All the elements, apart from B and Sr, which had a strong or very strong significant correlation with each other, were strongly correlated with grainsize; fine fraction: $r_s(67) = .719 - .830$, $p < 0.0005$ and sand fraction: $r_s(67) = -.713 - -.830$, $p < 0.0005$. This indicates that all these elements were positively correlated to fines and negatively correlated to sand which signifies that for the large group of elements the largest contributing factor is grainsize.

Table 2.2: Correlation matrix for all elemental (A) and physical (B&C) parameters in the Conwy estuary. Green signifies a strong to very strong correlation, yellow a moderate correlation and red a weak to very weak correlation as defined by Cohen (1988). ** refers to significant to 99% and * significant to 95%.

A.

	Li	Be	B	Sc	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Y	Cd	Cs	Ba	La	Sm	Tb	Ho	Lu	Pb	Bi	Th	U
Li	1																												
Be	.957**	1																											
B	.277*	.367**	1																										
Sc	.896**	.933**	.496**	1																									
V	.870**	.889**	.488**	.947**	1																								
Cr	.952**	.942**	.393**	.962**	.923**	1																							
Mn	.936**	.900**	0.2343	.845**	.834**	.909**	1																						
Fe	.966**	.953**	.307*	.893**	.880**	.937**	.943**	1																					
Co	.983**	.961**	.263*	.891**	.856**	.946**	.942**	.969**	1																				
Ni	.980**	.955**	.331*	.935**	.895**	.981**	.943**	.969**	.977**	1																			
Cu	.878**	.910**	.457**	.952**	.894**	.959**	.823**	.860**	.872**	.924**	1																		
Zn	.868**	.875**	0.1139	.741**	.699**	.804**	.805**	.848**	.909**	.840**	.725**	1																	
As	.904**	.890**	0.2457	.803**	.818**	.849**	.869**	.924**	.902**	.887**	.793**	.825**	1																
Se	.897**	.902**	.458**	.941**	.905**	.950**	.850**	.867**	.897**	.926**	.923**	.765**	.790**	1															
Rb	.859**	.905**	.558**	.951**	.872**	.926**	.789**	.847**	.856**	.890**	.936**	.747**	.768**	.913**	1														
Sr	.283*	.304*	.608**	.502**	.570**	.435**	.382**	.308*	0.2322	.356**	.459**	0.0158	0.2536	.463**	.489**	1													
Y	.935**	.948**	.416**	.964**	.931**	.974**	.899**	.927**	.934**	.958**	.949**	.819**	.850**	.947**	.938**	.461**	1												
Cd	.830**	.844**	0.2034	.753**	.660**	.808**	.808**	.822**	.874**	.847**	.759**	.887**	.725**	.775**	.749**	0.142	.804**	1											
Cs	.859**	.894**	.566**	.962**	.892**	.934**	.785**	.848**	.856**	.893**	.935**	.731**	.757**	.933**	.986**	.509**	.940**	.743**	1										
Ba	.919**	.913**	.390**	.921**	.866**	.954**	.895**	.910**	.907**	.945**	.947**	.784**	.827**	.903**	.925**	.480**	.952**	.802**	.922**	1									
La	.963**	.948**	.361**	.902**	.858**	.947**	.913**	.946**	.961**	.961**	.884**	.863**	.864**	.900**	.885**	.309*	.950**	.833**	.883**	.934**	1								
Sm	.955**	.960**	.387**	.949**	.907**	.981**	.912**	.940**	.954**	.972**	.952**	.847**	.862**	.932**	.930**	.407**	.984**	.825**	.927**	.958**	.976**	1							
Tb	.951**	.959**	.398**	.955**	.920**	.975**	.907**	.939**	.947**	.968**	.953**	.836**	.864**	.935**	.930**	.430**	.991**	.820**	.931**	.955**	.967**	.992**	1						
Ho	.952**	.959**	.395**	.958**	.921**	.979**	.904**	.939**	.947**	.968**	.952**	.832**	.860**	.938**	.932**	.426**	.991**	.812**	.936**	.957**	.964**	.991**	.997**	1					
Lu	.957**	.959**	.411**	.947**	.920**	.978**	.907**	.954**	.951**	.972**	.951**	.835**	.879**	.923**	.923**	.407**	.983**	.820**	.924**	.953**	.963**	.985**	.989**	.992**	1				
Pb	.937**	.943**	.370**	.909**	.844**	.943**	.873**	.910**	.936**	.954**	.935**	.865**	.852**	.913**	.901**	.307*	.932**	.874**	.895**	.935**	.925**	.946**	.944**	.943**	.948**	1			
Bi	.830**	.863**	.537**	.912**	.844**	.891**	.777**	.794**	.813**	.859**	.937**	.651**	.727**	.878**	.903**	.516**	.885**	.727**	.907**	.898**	.827**	.872**	.881**	.891**	.885**	.866**	1		
Th	.963**	.938**	.370**	.927**	.876**	.968**	.892**	.933**	.954**	.966**	.901**	.828**	.856**	.915**	.894**	.324*	.945**	.807**	.902**	.925**	.964**	.964**	.956**	.962**	.960**	.930**	.863**	1	
U	.893**	.893**	.491**	.926**	.893**	.921**	.824**	.872**	.876**	.900**	.866**	.726**	.788**	.899**	.909**	.475**	.919**	.690**	.919**	.894**	.900**	.915**	.914**	.920**	.919**	.888**	.857**	.909**	1

B

	Li	Be	B	Sc	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Y	Cd	Cs	Ba	La	Sm	Tb	Ho	Lu	Pb	Bi	Th	U
Salinity	-.496**	-.500**	0.1891	-.352**	-0.256	-.440**	-.454**	-.481**	-.551**	-.492**	-.407**	-.685**	-.403**	-.384**	-.322*	.344**	-.423**	-.635**	-.291*	-.391**	-.503**	-.469**	-.437**	-.434**	-.435**	-.523**	-.299*	-.474**	-.327*
% Gravel	0.0115	0.006	0.1604	0.066	0.0454	0.0281	-0.117	-0.044	-0.033	-0.02	0.0443	-0.038	-0.069	0.0255	0.0621	0.0124	0.0311	-0.09	0.1068	0.0639	0.0141	0.0212	0.0225	0.0359	0.0411	0.0491	0.1051	0.0695	0.1203
% Sand	-.808**	-.802**	-.331*	-.787**	-.713**	-.828**	-.821**	-.793**	-.822**	-.830**	-.799**	-.729**	-.713**	-.763**	-.821**	-.353**	-.819**	-.824**	-.792**	-.828**	-.794**	-.821**	-.823**	-.821**	-.820**	-.817**	-.801**	-.802**	-.755**
% Mud	.808**	.803**	.322*	.784**	.719**	.823**	.823**	.800**	.826**	.830**	.795**	.742**	.733**	.764**	.815**	.354**	.817**	.824**	.783**	.822**	.786**	.815**	.818**	.815**	.817**	.819**	.793**	.798**	.754**
%oC	.747**	.787**	0.2619	.752**	.740**	.777**	.700**	.708**	.763**	.765**	.797**	.747**	.668**	.751**	.727**	.358**	.762**	.862**	.723**	.776**	.735**	.775**	.773**	.773**	.775**	.835**	.784**	.752**	.694**
CaCo3	.441**	.490**	.502**	.641**	.617**	.590**	.479**	.434**	.424**	.502**	.594**	.295*	.357**	.635**	.664**	.781**	.604**	.370**	.679**	.668**	.513**	.576**	.578**	.586**	.566**	.547**	.715**	.531**	.603**

C

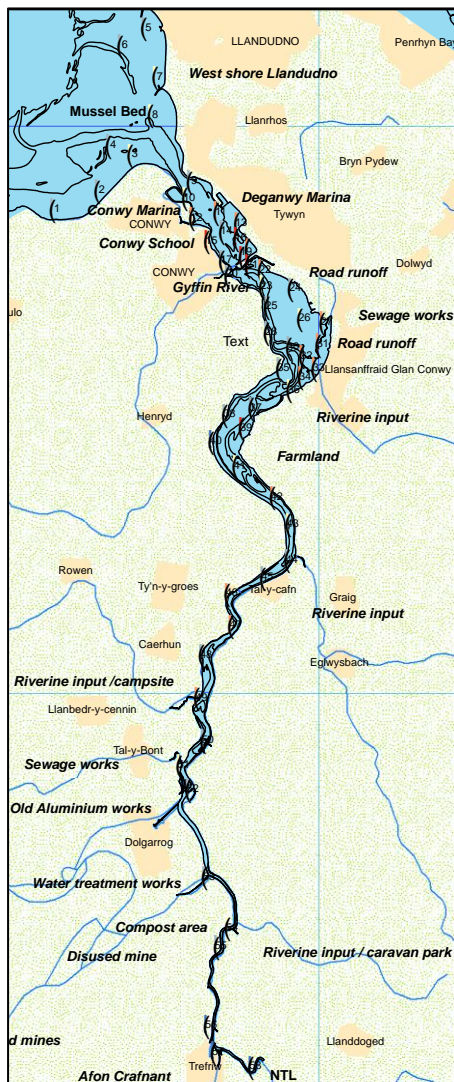
	Salinity	% Gravel	% Sand	% Mud	%oC	CaCo3
Salinity	1					
% Gravel	0.2096	1				
% Sand	.437**	0.0985	1			
% Mud	-.459**	-0.168	-.985**	1		
%oC	-.401**	0.0208	-.726**	.727**	1	
CaCo3	0.0838	0.0735	-.478**	.481**	.565**	1

Samples were divided in two clearly differentiated groups: (1) sandy sediments which comprise samples with less than 10% of mud and include sand and gravel rich samples; (2) muddy sediments corresponding to samples with high contents of mud (table 2.3). The average concentration of Cu and Cadmium (Cd), in the sediments with high amounts of the fine fraction was nine times their average concentration in the coarse, Ba, Pb, U and Bi had a five to six times difference. The majority of the remaining elements in Table 2.3 showed an increase in concentrations of between three to five times higher in the fine fraction. While Arsenic (As), Lanthanum (La), Vanadium (V) and Samarium (Sm) are doubled. Sr and B show similar concentrations in both again signifying the lack of relationship these elements have to grain size. The general trend of both B and Sr concentrations in the Conwy Estuary showed high concentrations in the marine end of the survey area (sector 1-3) reducing to low levels in the riverine/terrestrial environment (sector 5 & 6) (Figure 2.4). The highest concentrations of B at 11.7-14.3 mg/kg were found at sites 15, 16, 19 and 21 with a concentrations of 9.0-11.7 mg/kg at sites adjacent to these signifying a gradient towards the sheltered areas of Deganwy and Conwy. Site 39 also had a high concentration (11.7-14.3 mg/kg), however surrounding sites are at very low concentrations (1.1 – 6.4 mg/kg) and therefore no signs of a gradient. Between 9 and 11.7 mg/kg was found near Tal-y-Cafn (sites 46 & 47), site 49, 42 and a large cluster in the region of Glan Conwy, these sites all showed reducing gradients with surrounding samples.

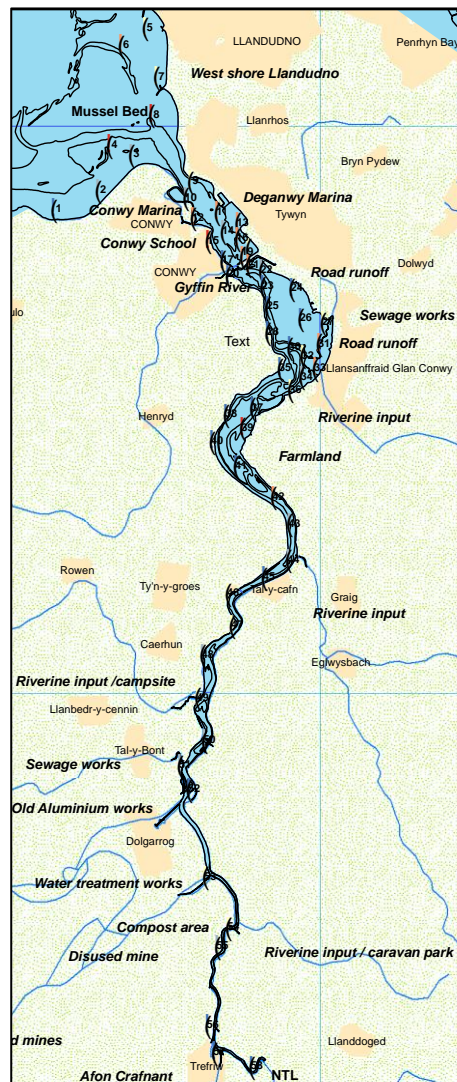
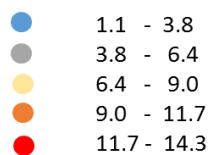
Table 2.3: Difference between elemental concentrations in sandy sites compared to sediments with high amounts of fine fraction sediments. Ranked by fold difference, greatest to least. Units mg/kg.

Sediment type	Cu	Cd	Ba	Pb	U	Bi	Li	Rb	Mn	Ni
Sandy sediments (n=29)	1.42 ±2.45	0.09 ±0.08	3.79 ±6.17	6.26 ±4.95	0.11 ±0.07	0.04 ±0.05	3.48 ± 3.37	1.54 ±1.99	151.51 ±81.19	5.16 ±2.92
Muddy sediments (n=40)	12.94 ±4.90	0.84 ±0.57	24.32 ±9.14	36.17 ±19.42	0.59 ±0.42	0.21 ±0.09	16.49 ±7.69	7.00 ±2.67	688.99 ±566.42	22.11 ±6.94
difference	9.11	9.3	6.42	5.7	5.36	5.25	4.7	4.5	4.5	4.28
Sediment type	Sc	Cs	Be	Th	Lu	Cr	Co	Fe	Ho	Y
Sandy sediments (n=29)	0.50 ±0.38	0.20 ±0.18	0.13 ±0.07	0.61 ±0.36	0.02 ±0.01	4.93 ±3.56	2.56 ±1.17	5464.60 ±2722.29	0.07 ±0.04	1.97 ±0.97
Muddy sediments (n=40)	2.00 ±0.49	0.80 ±0.27	0.50 ±0.14	2.32 ±0.69	0.07 ±0.02	17.41 ±4.33	8.61 ±3.94	18075.51 ±7247.92	0.22 ±0.05	6.15 ±1.44
difference	4	4	3.85	3.8	3.5	3.5	3.36	3.31	3.14	3.12
Sediment type	Gd	Se	Zn	Sm	La	V	As	Sr	B	
Sandy sediments (n=29)	0.08 ±0.04	0.40 ±0.16	30.64 ±25.57	0.53 ±0.27	2.27 ±1.01	8.64 ±4.64	3.62 ±1.38	37.40 ±50.33	6.01 ±1.53	
Muddy sediments (n=40)	0.25 ±0.06	1.20 ±0.30	92.87 ±46.09	1.47 ±0.33	6.10 ±1.74	19.47 ±4.45	6.48 ±1.87	50.82 ±36.49	7.59 ±3.75	
difference	3.1	3	3	2.7	2.7	2.25	1.79	1.35	1.26	

Highest concentrations of Sr (115.6 – 206.3 mg/kg) was found at sites 4, 8 and 15. At site 15 (near Conwy Marina, sector 2) there looks to be a general trend of a gradient with low levels in the centre of the channel. Site 4 has an isolated high value with low values of 16.9-45.8 mg/kg adjacent to it and therefore no gradient apparent. Site 8 which again has a very high concentration seems to be influencing the west shore in Llandudno. Site 31 has relatively high concentration and a gradient is apparent in that area. Site 39 and 42 have isolated high moderate concentrations of 75.8 – 115.6 mg/kg.



Concentrations of Boron mg/kg.



Concentrations of Strontium mg/kg.

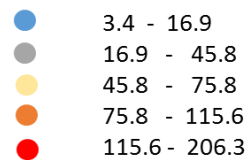


Figure 2.4 Concentration of Boron and Strontium in the Conwy Estuary (mg/kg). Site numbers superimposed on each position.

2.3.4 Normalization of heavy metal and metalloid concentrations and regional geochemical baseline models (background models)

B and Sr were removed from the normalisation and geochemical baseline model analysis because there was no relationship between elemental concentrations and grain size. Therefore it was not believed to be an influencing factor in the concentration of these elements. Calcium carbonate had a very strong correlation ($r_s(58) = .781$, $p < 0.0005$) with Sr (Table 2.2 B). Strontium is known to be strongly associated with calcium and is indicative of calcareous rock. In addition due to B and Sr having no positive relationship with the normalising elements there would be an inability to predict B and Sr from geochemical models. Based on the fact that the greatest influence over the remaining 27 elements is the sediment composition, elemental concentrations were normalised against a grain-size proxy (six normalisers highlighted in the literature: Li, Sc, Fe, Co, Rb & Cs) following the criteria of Lumoa (1990).

To establish the best normaliser the relationship between each of the elements and physical parameters was investigated (Table 2.2). Li, Sc, Fe, Co, Rb and Cs are all positively correlated with the fine fraction ($r_s(58) = .808, .784, .800, .826, .815$ and $.783$, $P < 0.0005$ respectively). All elements exhibit significant linear correlations with the six proxies, correlation coefficients ranged between $.830-.983$, $.741-.964$, $.794-.969$, $.813-.983$, $.747-.986$ and $.731-.962$ respectively, all $p < 0.0005$. However, a regression analysis of the six normalisers against the fine fraction (Figure 2.5) indicated that for Li, Fe and Co concentrations the percentage of the fine fraction could only explain 35%, 37% and 36% of the variance in the data. The intercepts for these three normalisers showed 3.89 ± 0.96 , 6028.77 ± 881.23 and 2.70 ± 0.41 , respectively and were significantly different from zero ($p = < 0.001$). This indicates other processes other than the fine fraction, are effecting the relationship. These processes may involve interference by carbonates or the coarse grain fraction and may involve incorporation not just adsorption. Sc, Rb and Cs exhibits a much higher relationship with 64%, 58% and 61% respectively, intercept values are much lower 0.58 ± 0.09 , 1.60 ± 0.39 and 0.22 ± 0.04 respectively and were significantly different from zero. This identifies that Sc and Cs have the closest relationship with the fine fraction without any additional processes influencing the regression. With reference to Figure 2.1, where Roussiez et al. (2005) identified that the interaction between Cs and the fine fraction explained 82% of the variance in the data, the current study showed a considerably lower relationship (61%) which was likely due to that the current study was within an estuary of tidal mixing whereas Roussiez et al (2005) study was within a bay receiving riverine inputs with a sediment platform which follows a granulometric gradient from the coastline to the outer shelf. The normaliser should also be able to predict the lower levels of an element and therefore should represent 0% at $< 63 \mu\text{m}$ (Figure 2.5 y-intercept).

The lowest values of the y-intercept/ averaged metal concentration ratio were found in Rb, Cs and Sc, 0.374, 0.42 and 0.46 respectively. This suggests that Sc, Rb and Cs might be candidates for the best geochemical normaliser, explaining the grain size effect of elements in surface sediments in this area.

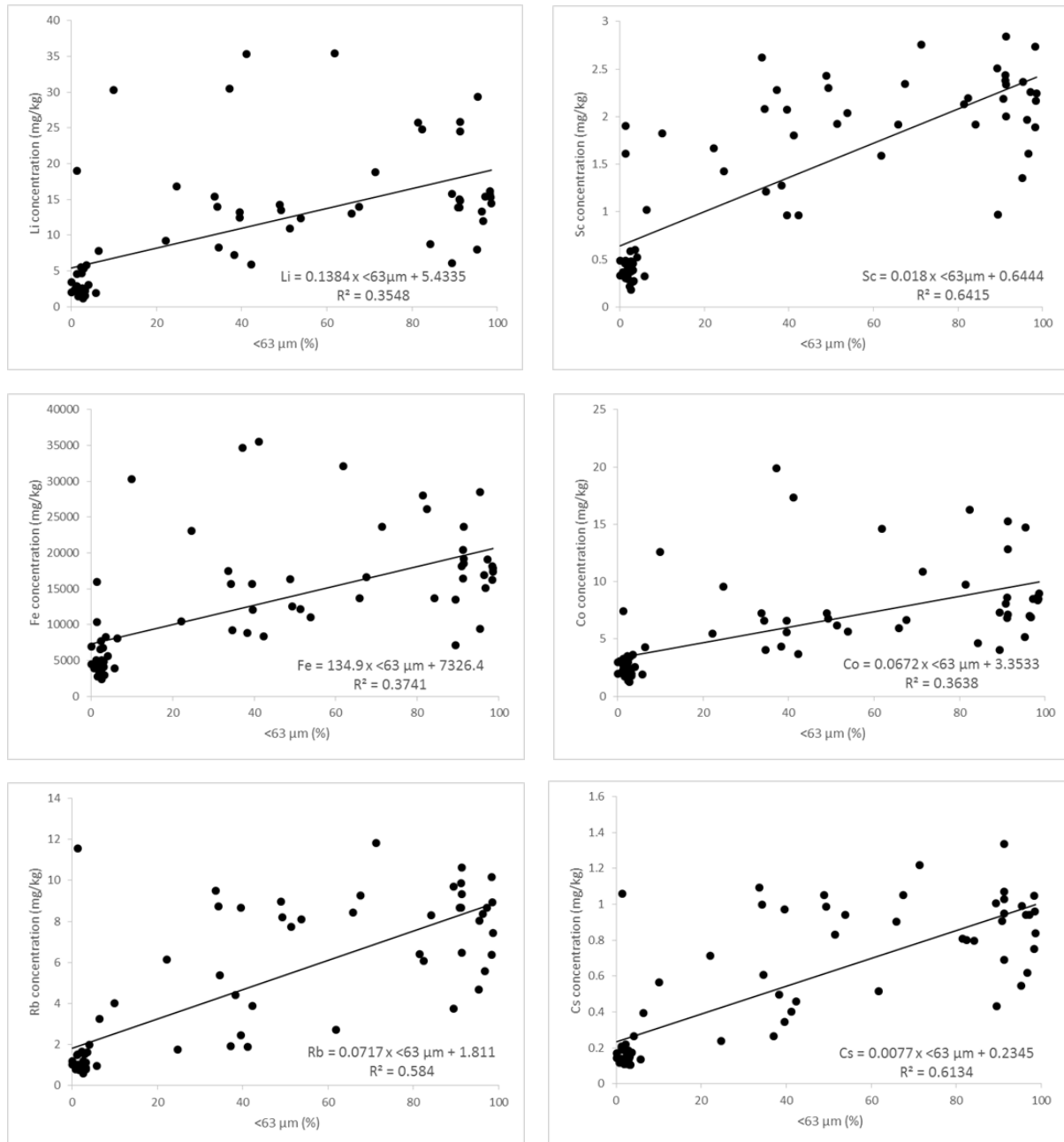


Figure 2.5. Relationship between the content of the < 63 μm fraction and conservative elements (Li, Sc, Fe, Co, Rb and Cs) in sediments of the Conwy Estuary.

To enable the geochemical baseline models to be constructed all elements were log transformed to meet the assumption of normality. Regression analysis was performed on each element with respective normaliser before and after the elimination of enhanced samples (Table 2.4). The regression analysis between each element and normalising element (Sc, Rb & Cs) after the elimination of enhanced samples, showed that the majority were highly correlating pairs ($R^2 > 0.7$) with only Zn and Cd with less significantly related pairs ($0.5 < R^2 < 0.7$). Therefore Sc, Rb and Cs are considered good proxy for natural variations. In addition, these normalisers are highly correlated to the fine fraction (Table 2.2) which supports that the high r^2 values are related to the textural and mineralogical changes in the sediment and therefore agrees with the association of these elements with the fine grained aluminosilicates (Table 2.3). However, the existence of intercept values greater than zero indicates that there are other sources potentially influencing the relationship (e.g. organic matter, Mn-Fe oxi-hydroxides) in addition to the aluminosilicate fraction. Amongst all the normalisers, elements V, Mn, Fe and Zn all had intercept values greater than zero.

With respect to Cs, Rb and Sc, these normalisers could have been used to calculate the background levels in the Conwy Estuary. However, Sc explained more of the relationship with the fine fraction and a higher regression for Zn and Cd, compared to the other normalisers. Rb has the lowest relationship with the fine fraction but represents the low concentrations the best (y-intercept/mean concentration ratio). With Cs having the second highest relationship with the fine fraction, regression for Zn and Cd and representation of the low concentrations. Due to there being no distinguishable normaliser the best procedure would be the application of a multi-element normalisation, and Cs and Sc were used in this study due to the additional reliability of limited influence from other processes for these two normalisers compared to the others.

2.3.5 Estimation of Enrichment Factors

Background values were predicted using the Sc and Cs regression values (Table 2.4), these were then compared to the metal concentration using the enrichment calculation to enable an understanding of whether the elemental concentrations in the Conwy estuary are at background levels and therefore naturally occurring or impacted by human sources. Figure 2.6 illustrates the level of enrichment for each element when normalising with Cs and Sc. Ratios of over 1.5 signifies an enrichment (black line), however up to 2 represents a minimal enrichment, 2 to <5 moderate (orange line) and above 5 represents a significant enrichment (red line). Comparing

both the normalisers it can be seen that they are calculating very similar EF values which supports their use as background models for the Conwy Estuary.

Table 2.4: Regression results of the selected normalisers for the surface sediments in the Conwy Estuary showing R^2 , slope and intercept values in order to predict background levels. First figure in the R^2 table shows the value before elimination steps, second figure shows after all elimination steps and therefore no outliers and bracket shows number of sites removed due to enhanced concentrations. Table A shows linear regression outputs for R^2 greater than 0.70. B shows outputs for R^2 greater than 0.50 but less than 0.70. Red shading indicates intercept values are indicative of other processes other than the fine fraction are influencing the relationship. Intercept is significantly different to zero ($p < 0.001$) and therefore suggests changes in these elemental concentrations are associated with changes in the normaliser element.

A.

	R2			Slope			Intercept		
	Sc	Rb	Cs	Sc	Rb	Cs	Sc	Rb	Cs
Li	0.833/0.968 (17)	0.623/0.989 (23)	0.640/0.98 (20)	1.793	1.002	2.894	0.26	0.174	0.326
Be	0.907/0.946 (6)	0.740/0.945 (10)	0.770/0.97 (15)	0.367	0.196	0.56	-0.009	-0.018	0.01
Sc	-	0.848/0.974 (8)	0.869/0.977 (8)	-	0.547	1.566	-	-0.035	0.049
V	0.931/0.98 (10)	0.688/0.972 (11)	0.697/0.956 (9)	1.169	0.644	1.814	0.738	0.695	0.802
Cr	0.961/0.975 (6)	0.796/0.98 (16)	0.808/0.962 (11)	1.649	0.899	2.595	0.456	0.39	0.532
Mn	0.672/0.888 (12)	0.398/0.897 (16)	0.410/0.858 (14)	1.592	0.851	2.319	1.856	1.801	1.959
Fe	0.827/0.948 (15)	0.575/0.941 (14)	0.592/0.935 (16)	1.575	0.854	2.468	3.406	3.357	3.478
Co	0.787/0.938 (14)	0.575/0.925 (14)	0.563/0.928 (16)	1.135	0.595	1.743	0.336	0.316	0.397
Ni	0.914/0.965 (11)	0.707/0.977 (19)	0.721/0.977 (20)	1.722	0.939	2.734	0.473	0.399	0.53
Cu	0.900/0.919 (1)	0.844/0.939 (8)	0.840/0.922 (7)	2.681	1.493	4.252	-0.171	-0.271	-0.037
As	0.669/0.877 (15)	0.461/0.727 (6)	0.475/0.908 (20)	0.726	0.354	1.132	0.487	0.516	0.518
Se	0.899/0.916 (2)	0.744/0.905 (7)	0.771/0.917 (6)	0.591	0.315	0.941	0.051	0.038	0.08
Rb	0.848/0.852 (1)	-	0.978/0.979 (1)	1.651	-	2.854	0.085	-	0.15
Y	0.954/0.966 (3)	0.806/0.957 (8)	0.812/0.953 (8)	1.228	0.663	1.909	0.256	0.222	0.321
Cs	0.869 (0)	0.978/0.985 (3)	-	0.583	0.34	-	-0.024	-0.048	-
Ba	0.887 (0)	0.812/0.954 (12)	0.810/0.936 (9)	2.492	1.369	3.973	0.177	0.063	0.272
La	0.865/0.923 (7)	0.684/0.956 (14)	0.706/0.962 (15)	1.036	0.551	1.648	0.324	0.294	0.365
Sm	0.924/0.956 (6)	0.760/0.963 (13)	0.780/0.975 (14)	0.67	0.356	1.056	0.064	0.046	0.093
Tb	0.929/0.952 (3)	0.751/0.949 (11)	0.779/0.967 (10)	0.192	0.101	0.304	0.002	-0.002	0.011
Ho	0.928/0.953 (3)	0.744/0.951 (11)	0.773/0.966 (9)	0.179	0.094	0.282	0	-0.004	0.009
Lu	0.913/0.943 (4)	0.713/0.943 (11)	0.749/0.964 (9)	0.063	0.033	0.097	-0.002	-0.003	0.002
Pb	0.840/0.87 (5)	0.711/0.906 (11)	0.736/0.894 (9)	2.019	1.1	3.282	0.501	0.426	0.581
Bi	0.734/0.738 (1)	0.785/0.797 (1)	0.810/0.806 (1)	0.205	0.117	0.345	-0.016	-0.022	-0.006
Th	0.869/0.949 (9)	0.660/0.97 (13)	0.690/0.976 (13)	0.892	0.487	1.427	0.055	0.023	0.091
U	0.665/0.936 (13)	0.520/0.904 (9)	0.553/0.931 (12)	0.366	0.21	0.598	-0.016	-0.029	0.002

B.

	R2			Slope			Intercept		
	Sc	Rb	Cs	Sc	Rb	Cs	Sc	Rb	Cs
Zn	0.610/0.677 (2)	0.491/0.644 (4)	0.481/0.642 (4)	1.767	0.906	2.646	1.078	1.07	1.196
Cd	0.538/0.654 (18)	0.441/0.562 (16)	0.477/0.599 (18)	0.353	0.187	0.539	-0.015	-0.015	0.009

Within the Conwy Estuary with the exception of Cd there seems to be a general trend of background concentrations within the marine section/mouth of the estuary (sector 1 & 2) with some isolated sporadic elevated enrichment and an increase in enrichment towards the last few stations in the terrestrial end of the study area (sector 6). However, the general trend of cadmium is a steady decline of enrichment from the highest site in the vicinity of the historic metal mined tributary, Afon Cefn, (site 57, sector 6) to background levels in the marine section of the study area.

Elements Lu, Ho, Sm, Be, Y, Bi, Se and Tb in this study area have been shown (Figure 2.6) to be at background levels apart from site 45 (Tal-y-Cafn) any other sites which have been enriched have only been at minimal level and therefore have presumed that it is due to local geological parameters and not human influence. Therefore, the concentrations in this study for these elements for this type of estuary can be referenced as background concentrations. The remaining elements Zn, Cu, Pb, Cd, Mn, Fe, Ni, V, Bi, Co, As, Cr, Li, U, Ba, La and Th all exhibit sites with moderate to significant enrichment. This enables us to understand which sites were experiencing enrichment from human sources instead of increased natural levels. Throughout the survey area there seems to be five areas of potential contamination.

Site 9 which is in the vicinity of a storm overflow and emergency pumping station (Cefas 2014) has significant enrichment of Cu and moderate of Li, Ni and Mn with site 8, a commercial mussel bed also moderately elevated in Cu but to a lesser extent. In the vicinity of this area there has been sightings of boat maintenance, stripping of paint from boats (Personal comm. L.Levay). Localised enrichment is found at site 24, with moderate enrichment of Zn, in the vicinity there is a road runoff outlet pipe which empties into the estuary. The most significant bend on the west side of the estuary exhibits moderate enrichment of Zn and Li at site 40 and Cd at site 39 with lower concentrations surrounding these sites indicating a potential source. This area is surrounded by farmland but also a potential sediment depositional site due to its location. Site 45 has significant enrichment in Zn, Mn, Ba and Li however these areas are all isolated with surrounding sites at background concentrations. Fe, Pb, La, Th, V, As, Co, Cr, Cu, U and Ni have all moderate levels of enrichment but again within this isolated site with background levels at surrounding sites.

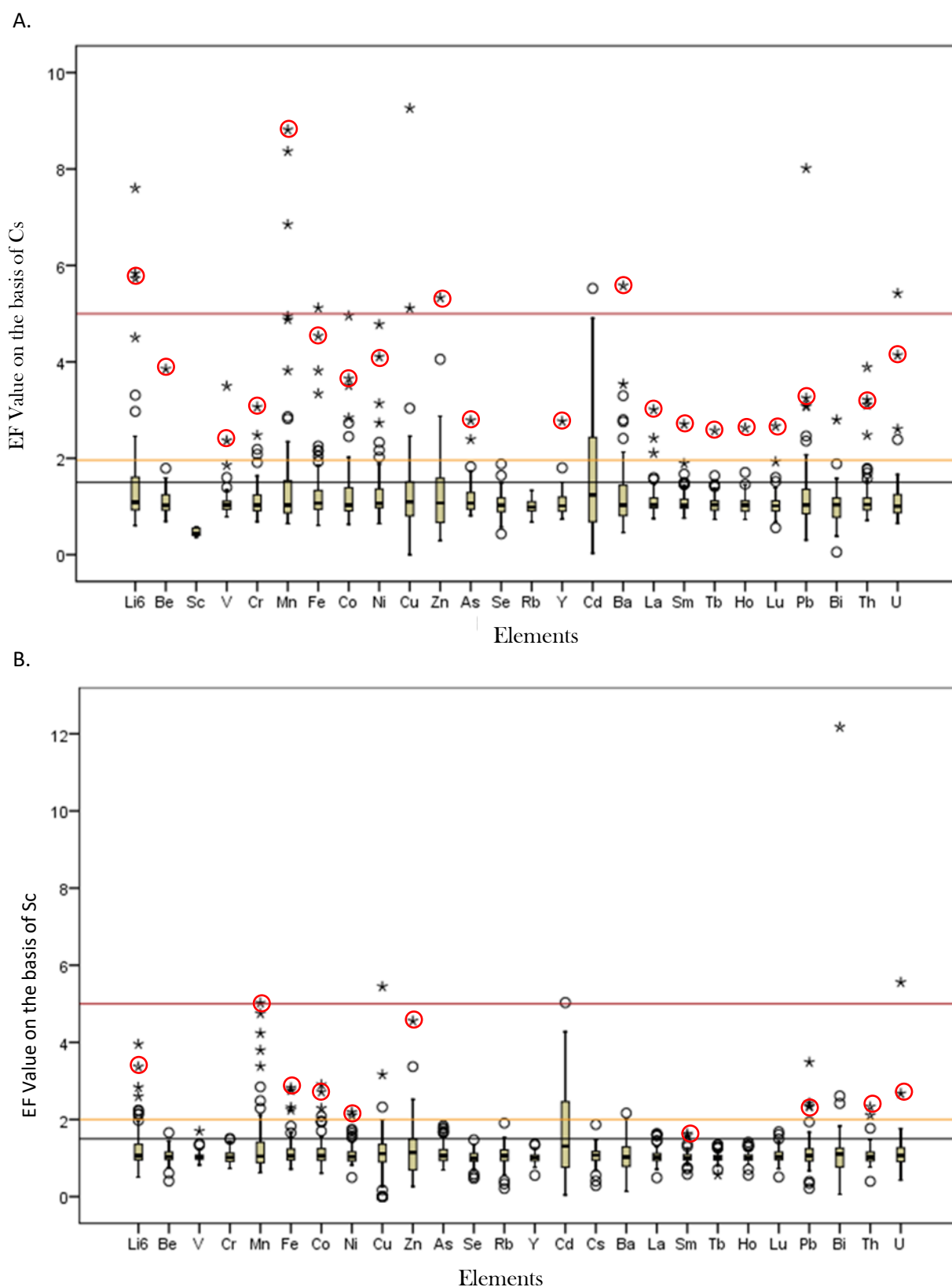


Figure 2.6: Box and whisker plot for the EF for each element using the background values established from the regression for A. Cs and B. Sc. Black line represents the minimal enrichment, Orange moderate enrichment and red significant enrichment. o represents suspected outliers and * refers to definite outlier. Red circles indicate site 45.

Within this location a riverine input which drains a catchment of livestock, mainly sheep and a horticultural site where potentially pesticides and herbicides are used. Road runoff could also be a potential input due to the small residential area of Egwylsbach. Cd is also moderately enriched at this site however this is a general trend throughout this section of the estuary, potentially coming from the mining area (site 57, sector 6). Site 58 again was enriched with the majority of elements, with 56 also enriched with a significant number, the Afon Crafnant inlet is in the vicinity of these sites which historic mining runoff has been documented within this area. Cd contamination is highest within these sites and likely to be originating from the metal mine effluent and zone of influence stretches further downstream than any other metal.

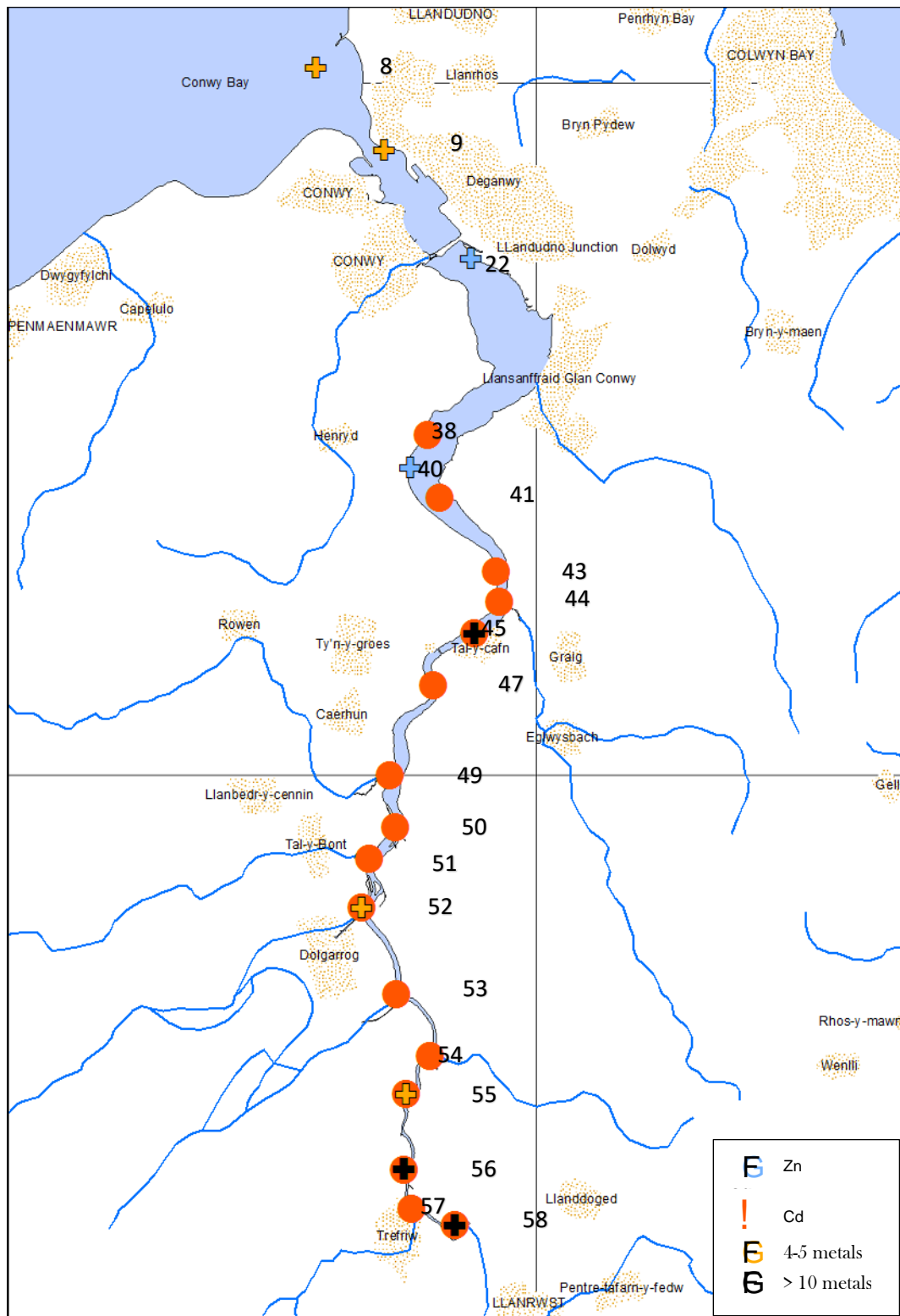


Figure 2.7: Visual representation of enriched samples within the Conwy estuary highlighting areas which are enrichment with more than one metal.

2.4 Discussion

It is well established that granulometry, carbonate and organic matter contents are important controlling factors in the abundance of elemental concentrations (Rubio et al. 2000). Fine grained and high organic carbon sediments tend to have high elemental contents, due in part to the high specific surface of the smaller particles. This enrichment is mainly due to surface adsorption and ionic attraction (McCave 1984, Horowitz and Elrick 1987). Also, coatings of organic matter are prevalent in fine-grained sediments, and these coatings bind a variety of trace elements (Wangersky 1986). On the other hand high quartz (sand) are associated with low concentrations of trace metals. These relationships are clearly evident in Table 2.2 and 2.3 where variations for absolute elemental concentrations are linked to variations in grain size or carbonate content with respect to Sr and B. Bivariate correlations between elements and sediment properties are positive and statistically significant with organic matter content and the fine fraction which has been previously reported by Rubio et al. (2000).

The large granulometric spectrum and variation in metal deposition with particle size makes it difficult to compare the metal deposits occurring in different regions of the study area. In geochemical studies, normalising metals relative to a Al, Fe, Sc, Cs or Rb (grain size proxy) is widely used to compensate for both the granulometric and mineralogical variability of metal concentrations in the sediment (Chapman and Wang 2001). Elemental concentrations will come from numerous sources, natural and anthropogenic, and therefore enable us to understand their environmental implications as localised background concentrations is very important.

B and Sr were unable to be normalised for grain size due to having no relationship with the <0.63 μm size fraction and therefore regression analysis and in turn background calculations were unable to be undertaken, therefore the concentrations within this study are total concentrations only and unable to comment whether these are background or human influenced. Although it was evident that Strontium was significantly correlated to the calcium carbonate content which reflects the known relationship between strontium and calcium which is indicative of calcareous rocks.

Within this study normalisers Sc and Cs, agreed with previous studies (Roussiez et al. 2005, Mil-Homens et al. 2013, Roussiez et al. 2006, Song et al. 2014, Rubio et al. 2000, Veinott, Perron-Cashman and Anderson 2001) that they are both highly correlated with the fine fraction and that the majority of elements (except Zn and Cd) being tested exhibited significant correlations before and after elimination of enhanced samples ($r^2 > 0.7$). This signifies that they are both good proxies

for natural variations as the r^2 is related to textural and mineralogical changes in the sediments and therefore agrees with the association of these elements with the fine grained aluminosilicates (Mil-Homens et al. 2013), hence these elements are good candidates for normalisation for the Conwy Estuary. Mil-Homens et al (2013) also acknowledged that intercept values greater than zero indicate that other sources are potentially influencing the relationship (e.g. organic matter Mn-Fe oxyhydroxides) in addition to aluminosilicate fraction. Within this study V, Mn, Fe and Zn all exhibited greater than zero intercepts for all normalisers tested and therefore it is evident that other interactions are present. In addition, the Fe concentrations resulted in a very low explanation of variance of the data when compared to the fine fraction. Even though Fe has been previously used as a grain size proxy by a number of authors working on marine and estuarine sediments (Ackermann 1980, Emmerson et al. 1997, Lee, Fang and Hsieh 1998) a potential difficulty is that in certain circumstances this element can be mobile during diagenesis (Finney and Huh 1989). Another real difficulty would be if the sediments were enriched by Fe contamination. McKenna et al. (2003) reported a natural mineral water spa located in Trefriw which is rich in Fe, which has been in use since Victorian times and is bottled as a tonic to date. To enable the normaliser to work effectively other authors have cited that the elemental constituent chosen as a normaliser must have concentrations that have not been anthropogenically altered (Ackermann 1980). It is evident that Fe is having an influence in the Conwy Estuary.

Sc and Cs elements have been previously used as a normalisers in estuarine sediments. Ackermann (1980) referenced both to be structurally combined in clay minerals and suitable for use in estuarine and coastal sediments. Loring (1991) demonstrated the high dependence of the Cs concentrations in the total sediment on the percentage of the fraction <20 μm , the phase highly associated with metals, indicating that Cs is a good fine-grained sediment indicator. Roussiez et al.(2005) study also emphasised that Cs was the best normaliser for their study due to the highest correlation both with the fine sands and with the heavy metals investigated compared to other candidates. Therefore both Cs and Sc are apparently well established as a suitable normaliser for marine and estuarine sediments. The background concentrations of all the elements of the Conwy estuary were calculated using these regressions.

Once the elemental background concentrations were defined through the geochemical baseline models, it is possible to assess human impacts by determining the elemental enrichment factor (EF) relative to baseline values. In agreement with Mil-Homens et al.(2013) surface samples with EF greater than 1.5 indicate that additionally to elemental concentrations associated with detritus

(natural component), sediment contains other elemental contributions delivered from non-crustal contributions interpreted as being associated with anthropogenic contributions derived from agricultural, urban and industrial activities that exist in the study area. Elements within the surficial sediments enable us to understand the historic concentrations at each location. Human influence of the Conwy catchment goes back to the bronze and roman times, and therefore contamination can be recent, a one-off event or persistent over time.

From our study samples considered as belonging to an enriched population are in general enriched in more than one element (Figure 2.7) suggesting that these locations (Deganwy, Tal-Y-Cafn, Dolgarrog and upper part of the survey area) are effected by diverse anthropogenic influences or reflecting favourable environmental conditions for supporting the accumulation of contaminated particles. In the upper part of the sample area, these elemental enrichments reflect where fine-grained “enriched” particles transported by the Crafnant River from both natural rock formations and tailings of abandoned mining areas tend to accumulate. This was especially the case for sites 56 and 58 which is adjacent to the input of the River Crafnant, and in addition in the locality of Trefriw natural spa which has very high concentrations of Fe. The proximity of Deganwy, Tal-Y-Cafn Bridge and Dolgarrog locations with riverine inputs influenced by urban areas and rural catchments, may explain their elevated enrichments due to combined sewage overflows, farmland and urban runoff. Within the proximity of the Tal-Y-Cafn Bridge (site 45) all enriched elements (Figure 2.7) tend to accumulate. Under usual summer conditions Robins et al.(2014) identified that the saline water does not penetrate further then the Tal-Y-Cafn area. Therefore at site 45 freshwater potentially combines with the saline water resulting in flocculation of colloids and suspended sediments which aggregate together (Ji 2017), encouraging metalloid laden suspended sediments to drop out of solution and deposit. Supporting this within this region sediment grainsize shows mainly fine grained sediments (Figure2.3, Tal-Y-Cafn).

Cadmium is characterised by showing the highest level of enrichment with 32% of the studied locations being spread from site 41 to 58 with the highest enrichment at the site in the vicinity of the historic mining (site 58) within the freshwater end of the estuary and the least concentration within site 41 with the highest salinity. Supporting this finding AMEC (2011a) found high concentrations of Cadmium within the River Crafnant where concentrations exceeded both the water framework directive environmental quality standards (WFD EQSs) and the freshwater Fish Directive standards. It is well established that transitional metals like Cadmium form inner-sphere complexes (bonds) between the metal and the anions on the particle surface producing a bond that is not easily broken by increases in ionic strength as with alkali and alkaline earth metals

(Begeal 2008). However with transitional metals pH has to decrease below the point of zero charge (pzc) and therefore the particle surface has a neutral charge resulting in the metal being released into the dissolved phase (Sparks 2003). Within estuarine and saline waters where the pH is reduced compared to freshwater desorption of Cu, Cd, Zn and Pb into the dissolved phase has been shown to occur and chlorocomplexes observed to be particularly strong with increased chloride concentration (high salinity) (Valenta et al. 1986, Golimowski, Merks and Valenta 1990, Warren and Zimmerman 1994, Thouvenin, Gonzalez and Boutier 1997). Therefore within the Conwy estuary the reduction in EF and concentration of cadmium from the source (site 58) to background levels with the marine section was likely to be due to desorption, supporting this finding the correlation between Cd and salinity identified as a very strong significant correlation. Cadmium is one of the known toxic metals found within estuaries with strong enrichment factors (Senthil Kumar et al. 2008, Lim et al. 2008, Weng et al. 2008). Contamination of sediments and water with cadmium causes its incorporation into the food chain, which may result in a wide variety of adverse effects in animals and humans (Berenger et al. 2014)

In comparison to the Enrichment Factor approach some studies have utilised the guidance criteria of the Canadian sediment quality guidelines (SQGs) for the protection of aquatic life devised by the Canadian council of Ministers of the Environment. This involves the derivation of Threshold Effects levels, where sediments which contain concentrations below the no effect level or at the lowest effect level (TEL) constitute unpolluted or marginally polluted sediments. While sediments which range between the lowest effect level (TEL) and severe effect level (PEL) are considered as moderately polluted and concentrations above severe effect level (PEL) indicate that the sediments are heavily polluted (Hubner, Astin and Herbert 2009, Persaud, Jaagumagi and Hayton 1996). However these SQGs are used within USA and Canada's governing bodies but the European Union has not supported the use of this criteria. One specific issue within estuarine sediments regarding the SQGs is that limits have been defined for only freshwater and seawater but nothing specifically for the transitional zone of estuaries. In addition this approach has a severe limitation of only having a fixed value for characterising whether a sample constitutes an effect level and does not take into account whether the element is naturally occurring. Table 2.5 illustrates how the SQGs can overestimate (for example Pb & As) and sometimes underestimate (example Cr & Cu) leading to an inconsistency and therefore an unreliable tool. To identify the true contamination level the assessment of anthropogenic contributions to sediments must be accompanied primarily by the estimation of contributions by natural processes (Windom et al. 1989a).

Table 2.5: Comparison of absolute elemental concentration (mg/kg) referenced to TEL and PEL and EF for each site within the Conwy Estuary up to the tidal limit (Llanrwst). Yellow represents concentrations as moderately contaminated and red as severely contaminated.

Site	Cr		Cu		Zn		As		Cd		Pb	
	mg/kg	EF	mg/kg	EF	mg/kg	EF	mg/kg	EF	mg/kg	EF	mg/kg	EF
1	3.02	0.81	0.50	0.87	12.29	0.59	3.37	1.20	0.003	0.03	3.50	0.73
2	2.66	0.76	0.43	0.97	7.79	0.39	2.80	1.03	0.012	0.15	2.81	0.64
3	12.69	0.87	5.34	0.53	21.22	0.29	6.15	1.14	0.033	0.08	10.51	0.42
4	3.64	1.00	0.63	0.58	11.71	1.10	4.63	1.44	0.013	0.15	4.43	0.70
5	5.14	1.30	0.16	0.23	11.94	0.54	2.49	0.86	0.028	0.27	5.09	0.99
6	3.45	0.94			9.68	0.47	3.05	1.10	0.019	0.21	3.53	0.76
7	4.51	1.10	1.32	1.69	10.00	0.44	2.77	0.95	0.032	0.29	4.42	0.83
8	9.46	2.19	2.03	2.23	16.90	0.71	3.56	1.18	0.029	0.24	4.40	0.77
9	5.87	1.42	7.49	9.26	25.23	1.10	4.88	1.66	0.051	0.45	6.77	1.25
10	5.41	1.03	2.16	1.45	24.56	0.87	3.32	1.01	0.150	0.95	6.04	0.84
11	15.97	1.04	13.76	1.25	57.05	0.74	6.34	1.14	0.406	0.98	27.26	1.02
12	10.85	1.24	6.38	1.55	34.54	0.77	4.62	1.10	0.227	0.84	17.49	1.31
13	20.28	0.92	16.77	0.83	61.44	0.56	6.43	0.97	0.347	0.67	36.93	0.88
14	5.02	1.13	1.46	1.49	20.15	0.83	2.90	0.95	0.096	0.77	4.77	0.81
15	18.79	0.87	16.42	0.85	67.75	0.64	6.77	1.04	0.559	1.09	32.75	0.81
16	15.82	0.93	13.59	1.03	51.81	0.61	6.00	1.03	0.342	0.77	30.83	1.01
17	2.49	0.73			20.41	1.05	2.96	1.10	0.083	1.07	5.41	1.26
18	10.52	0.98	6.94	1.17	35.70	0.66	3.82	0.82	0.203	0.63	19.75	1.15
19	15.05	0.83	12.68	0.88	54.62	0.61	5.30	0.89	0.448	0.97	27.43	0.84
20	3.79	0.92	0.62	0.78	28.33	1.24	2.56	0.87	0.138	1.24	4.64	0.86
21	17.69	0.91	14.26	0.87	63.06	0.65	5.58	0.90	0.397	0.82	33.78	0.94
22	8.86	1.10	5.25	1.46	40.43	0.97	4.05	1.00	0.265	1.05	13.49	1.11
23	3.06	0.88	0.59	1.34	22.90	1.16	3.80	1.40	0.056	0.70	4.44	1.01
24	4.78	1.02	1.18	1.05	73.19	2.87	5.34	1.71	0.037	0.27	6.64	1.06
25	3.05	0.86			24.02	1.19	3.78	1.38	0.051	0.60	4.83	1.07
26	3.13	0.76	0.49	0.63	23.48	1.03	2.98	1.02	0.088	0.79	3.68	0.69
27	19.69	1.00	16.89	1.01	73.61	0.75	5.47	0.88	0.502	1.03	36.40	1.00
28	3.74	0.99			29.62	1.40	3.00	1.07	0.128	1.35	4.71	0.97
30	3.92	1.03			27.62	1.30	2.70	0.96	0.157	1.64	4.20	0.86
31	19.85	0.95	17.07	0.93	72.60	0.70	6.03	0.94	0.518	1.03	38.46	0.98
32	17.90	0.93	15.03	0.94	68.07	0.71	5.52	0.89	0.597	1.25	33.02	0.94
33	18.23	0.87	16.08	0.87	69.74	0.67	5.86	0.91	0.638	1.27	37.51	0.96
34	9.46	1.34	5.63	2.03	44.69	1.21	3.24	0.85	0.286	1.29	17.24	1.67
35	4.94	1.23	1.11	1.50	39.54	1.77	4.71	1.62	0.140	1.31	7.71	1.47
36	14.21	1.12	10.87	1.35	60.36	0.94	4.95	0.98	0.533	1.47	24.44	1.15
37	4.90	1.29	1.33	2.15	41.78	1.96	4.81	1.70	0.170	1.76	9.99	2.04
38	4.12	0.91	0.82	0.79	44.78	1.80	3.28	1.07	0.308	2.37	7.19	1.19
39	17.35	0.92	15.05	0.97	75.73	0.81	5.44	0.89	0.686	1.45	49.48	1.44
40	5.82	1.35	1.01	1.12	96.13	4.05	4.99	1.66	0.137	1.15	6.25	1.10
41	3.57	0.91	0.81	1.20	40.80	1.87	3.09	1.08	0.254	2.50	5.90	1.17
42	21.47	1.03	19.46	1.06	102.28	0.99	7.65	1.19	0.838	1.67	38.88	0.99
43	8.91	1.16	5.19	1.61	61.30	1.55	3.19	0.81	0.597	2.50	14.07	1.23
44	11.83	1.24	7.46	1.54	70.81	1.45	3.90	0.89	0.652	2.24	19.42	1.30
45	12.20	2.48	3.88	3.04	141.96	5.33	8.90	2.78	0.360	2.48	20.51	3.07
46	17.82	0.88	14.34	0.82	90.98	0.90	5.75	0.91	0.822	1.66	30.18	0.80
47	18.17	1.06	15.87	1.19	126.31	1.47	7.46	1.28	1.207	2.71	35.09	1.14
48	3.49	0.97			10.22	0.50	2.73	0.99	0.021	0.24	3.55	0.78
49	19.82	1.10	15.57	1.08	116.08	1.29	7.77	1.30	1.100	2.40	38.22	1.17
50	17.19	1.27	12.89	1.45	116.16	1.71	6.90	1.32	1.007	2.65	33.37	1.46
51	14.91	1.38	10.03	1.65	101.47	1.85	4.98	1.07	1.146	3.55	27.27	1.56
52	9.60	1.52	4.11	1.84	67.11	2.01	3.82	1.06	0.823	4.17	16.32	1.80
53	19.34	1.06	16.29	1.11	123.53	1.36	7.33	1.22	1.380	2.98	34.44	1.04
54	19.18	1.03	16.72	1.11	130.84	1.42	7.00	1.15	1.544	3.30	39.29	1.16
55	19.38	1.31	13.64	1.32	118.13	1.59	6.55	1.20	1.233	3.05	42.39	1.66
56	20.11	1.63	16.90	2.23	150.71	2.43	7.94	1.60	1.743	4.91	66.17	3.25
57	28.37	0.95	25.64	0.78	207.11	1.41	8.31	1.09	2.788	4.55	91.23	1.50
58	18.82	2.08	10.83	2.46	77.52	1.67	7.82	1.82	0.778	2.80	34.38	2.46

2.5 Conclusion

The purpose of this study was to establish background concentrations for an extensive suit of elements and in turn test the most suitable normalizer element for the Conwy Estuary. Amongst the different candidates for normalization Sc and Cs appeared to be the most appropriate choice when compared to Li, Co, Fe and Rb, the most currently used normalizer elements within the literature. Both elements better reflected the fine grained fraction, range of concentrations and explained more variance in the elemental concentrations. The traditional techniques of comparing elemental concentrations to reference values by itself has been documented to be inadequate for the correct identification of possible anthropogenic enrichments. It does not account for the regional variability and therefore comparison of anthropogenic values between different geographical areas are inaccurate. The research in this study involved the regional geographical baseline (RGB) models allowing the estimation of naturally occurring components of the elements studied in the sediments. The identification of enriched locations in areas which are characterised by coarse-grained sediments (and consequently total low elemental concentrations) demonstrates the importance of considering normalised elemental concentrations along with the definition of RGB models.

The supply of slopes and intercepts for each elemental regional geographical baseline can be used by other researchers in future monitoring programs for evaluating the quality of the obtained results. The background concentrations can also be used as a baseline value to identify critically contaminated areas of which further monitoring can be concentrated.

CHAPTER 3

Nutrients

3.1 Introduction

Plants require many nutrients (e.g. carbon, nitrogen, phosphorus, oxygen, silica, magnesium, potassium, calcium, iron, zinc & copper) to grow and reproduce. Of these, nitrogen and phosphorus are the most essential for aquatic plants and algae (Mann 2000). Nitrogen, from allochthonous and autochthonous origins, and phosphorus originating from allochthonous sources enter estuarine systems from both natural and man-made sources. Naturally, phosphorus enters estuarine and coastal waters from the dissolution of geologic formations rich in phosphate and nitrogen and phosphorus when decomposing organic matter is flushed into rivers and streams. Whereas manmade sources of nutrients enter estuarine ecosystems from sewage and industrial wastewater, septic tanks, runoff from agricultural and urban areas and acid rain (Mann 2000). Human activity has greatly increased the load of nutrients to aquatic systems, as a consequence detrimental effects have been observed on the health of coastal ecosystems (Smith et al. 2005). Increased nutrients can lead to an excess of phytoplankton biomass (Malone et al. 1986), in turn potentially cause episodes of noxious blooms, (Sand-Jensen and Borum 1991, Duarte 1995) and the depletion of dissolved oxygen in bottom waters (Malone et al. 1986). Therefore nutrients, especially nitrogen and phosphorus, are key indicators of water quality in estuaries and other shallow coastal regions.

Within the aquatic environment, phosphorus is generally bound to sediment particles where it absorbs onto iron oxides, aluminium hydroxides, clay surfaces and organic matter particles and becomes incorporated within the sediment (Borggaard et al. 1990). Nitrogen species tends to be more mobile within the dissolved form and the most common forms of inorganic nitrogen are ammonium ions (NH_4^+), nitrite ions (NO_2^-) and nitrate ions (NO_3^-) (Rabalais 2002). Naturally occurring inorganic nitrogen in aquatic ecosystems is present as a result of atmospheric deposition, runoff from surface and ground water, dissolved components of nitrogen-rich geological formations, N_2 fixation by certain prokaryotes (cyanobacteria, particularly), and degradation of organic matter (Kinne 1984, Gleick 2002, Gleick 1993, Wetzel 2001, Rabalais 2002). Ammonium is oxidised to nitrate in a two-step process ($\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) by aerobic chemoautotrophic bacteria (Wetzel 2001). In consequence, concentrations of nitrate in marine ecosystems usually are higher than those of ammonium and nitrite (Kinne 1984, Gleick 2002, Gleick 1993, Wetzel 2001, Rabalais 2002).

Nutrient over-enrichment of estuaries has been well documented as a significant problem and the need to understand, predict and mitigate the impact of human activity on these waters (Nixon 1995, Jickells 1998). As estuaries receive both natural and anthropogenic sources it is important to understand how far a particular system has deviated from natural conditions. The EU Water

Framework Directive (WFD) requires all surface waters in Europe to fulfil the criterion of “good ecological status” by the end of 2021 (Priestley 2015). This status is defined with reference to undisturbed conditions. However within heavily impacted coastal areas with no prior monitoring of baseline data, assessing these reference conditions for the implantation of the WFD will be a challenge (Weckstrom, Juggins and Korhola 2004). In the absence of baseline data for many estuaries information from relatively uncontaminated catchments would act as a proxy measure of background conditions. In order to establish undisturbed conditions it is necessary to distinguish natural conditions from anthropogenic inputs within these relatively uncontaminated catchments.

Within the current evidence base, monitoring of nutrient concentrations for water quality assessment is within the dissolved, particulate or sediment phase (Krinvang 1992; Dorioz 1996; Foster et al 1996,). Dissolved and particulate nutrient concentrations reflect the conditions within the estuary at a single point in time whereas the nutrients absorbed onto sediments give a more historical record of nutrient fluxes over time. Analysing both the particulate and dissolved fraction is necessary as phosphate and nitrogen behave differently within the aquatic environment. Most export of inorganic phosphorus is bound to sediment particles whereas nitrogen export tends to be dissolved within ground and surface waters (Paerl 1997). Sediment analysis is widely used for evaluating the degree of contamination of aquatic environments most notably because their capacity to trap and concentrate contaminants and provide an integrated measure of the contamination of the water body in question over an extended period of time (Carballeira et al. 2000). Nutrients within the sediments are a combination of both natural and anthropogenic origins and it is important to distinguish between the two in order to establish the true contamination level (Windom et al. 1989). Regional geochemical baseline models were used within this study to establish whether areas were either background or enriched by anthropogenic sources.

This study has focused on the Conwy estuary because it is a relatively uncontaminated catchment, thus it represents an ideal location for the assessment of nutrient loading for baseline data collection. National resources wales (NRW) data supports this within their Conwy management catchment plan (2015 classification), which identifies the overall status of water bodies within Conwy as moderate to good status (NRW 2015) . In terms of contamination, the Conwy estuary is not influenced by heavy industry, arable farming or extensive urban areas; the primary contamination arises from wastewater effluent, agriculture, septic tank discharges and storm event runoff. This allows an assessment of nutrient loading within a catchment of limited influences to

establish background concentrations which can be used as a baseline for other contaminated estuarine environments.

The purpose of this study was not to undertake a detailed monitoring programme for the dissolved and particulate fraction but to establish fine scale nutrient distributions in near-pristine environments and assess whether there were any high carbon, nitrogen and phosphate concentrations. In addition, if parts of the estuary has been historically enriched in nutrients, the sediment and water samples were investigated to see whether there were any links between the sediment and water concentrations. Assessment of dissolved (nitrate, nitrite, ammonium and phosphate) and particulate nutrients (Carbon, nitrogen and phosphorus) on different states of the tide, position in the estuary, longitudinally from marine influence to terrestrially influenced and seasonal inflows to the system were undertaken to enable perspective for the deposited sediments (carbon, nitrogen and phosphorus) and in addition establish background concentrations within an relatively uncontaminated estuary which can be used as reference for contaminated study areas.

3.2 Materials and methods

3.2.1 Sampling Location

Sampling was conducted throughout the entire Conwy estuary up to the tidal limit. Five different surveys were undertaken to establish background and elevated concentrations of dissolved inorganic, particulate and settled sediment nutrients within the Conwy estuary. Sample sites for the sediment survey were identical to chapter 2 and can be found in Figure 1.1 & 1.2. Dissolved and particulate surveys were undertaken by four separate surveys, all sample sites can be seen in figure 3.1. Briefly these were

Tidal Transect survey. Five stations within the main channel of the Estuary, from the inlet of Conwy Bay to Tal-Y-Cafn were sampled on high water and low water to establish whether there is a difference in concentration between the two. The positions were chosen to include potential nutrient influences. Marine influence (site TS1), significant river (Gyffin) and mud flats (site TS2), Ganol river influence with known intermittent discharges within this river system (site TS3) and terrestrial and agricultural land influence (sites TS4 & TS5).

Horizontal Transect. Within four sites of the tidal transect horizontal transects (T1-T4) were undertaken to establish whether there was a difference in distribution and therefore monitoring implications between the banks of the Conwy side compared to Llandudno side (A to E).

Inflow survey. 24 freshwater inflows, (Figure 3.1) within the entire Conwy estuary up till the tidal limit, were located that may introduce elevated concentrations of nutrients or coliforms to the estuarine system. These were sampled on one occasion in the summer and winter.

Longitudinal transect. 20 sites from Llanrwst (tidal limit) to the inlet of Conwy Bay within the main channel on an ebb tide were sampled to understand the overall distribution of nutrients within the estuarine system. Summer inflow survey was undertaken during the same week to enable comparison to be made.

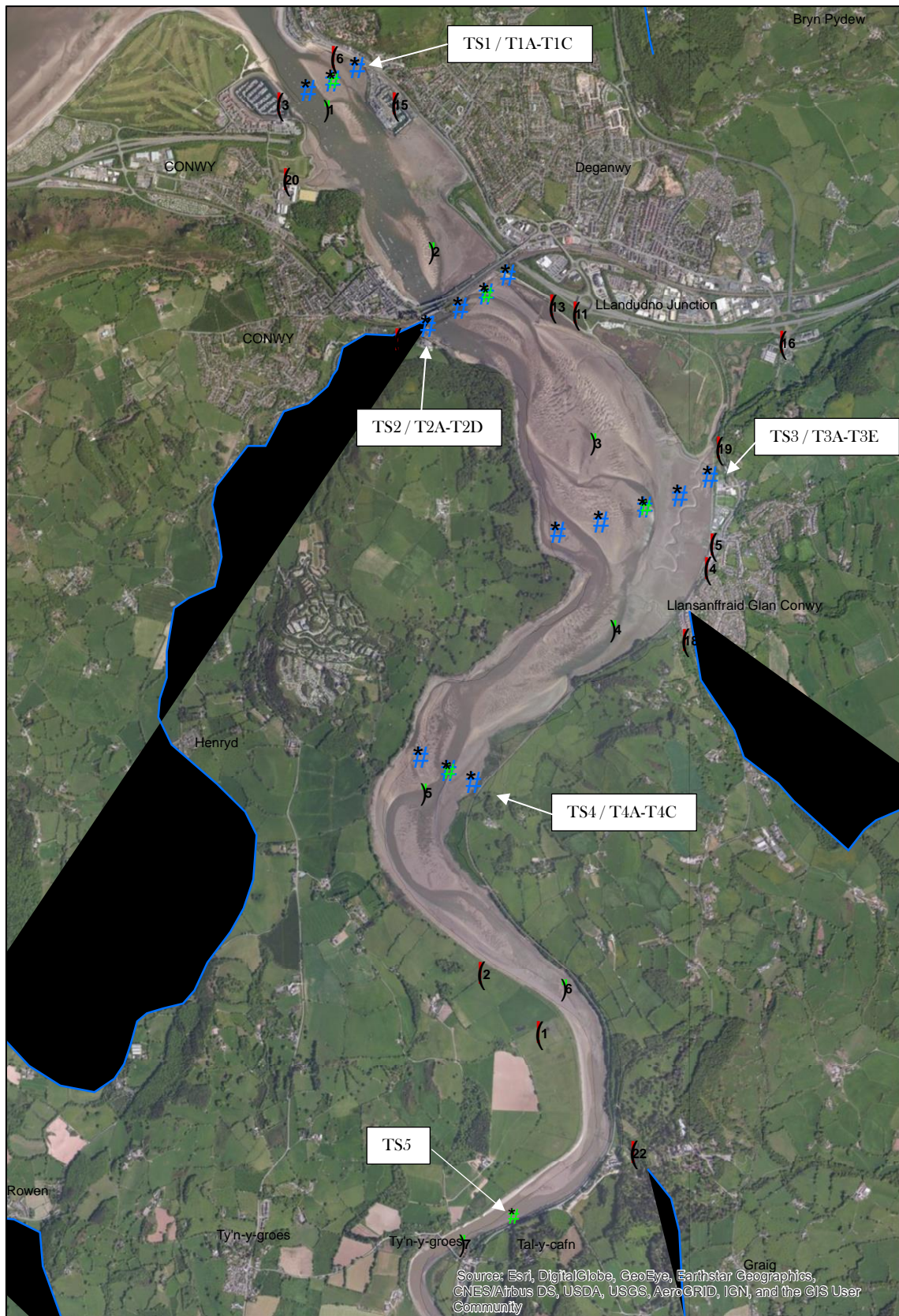




Figure 3.1: Sample locations for dissolved and particulate nutrient surveys within the Conwy Estuary: Longitudinal (survey 4); Tidal (survey 1); Cross section (survey 2) and freshwater inflows (survey 3) as described within section 3.2.1. TS 1-4 refers to Tidal Survey & T1-4 refers to cross sections transects. A-E represents locations from Conwy side to Llandudno side depending on width of estuary.

3.2.2 Sample collection

The data logger was deployed for the collection of both water and sediment samples, with water recorded 0.2m below the water surface whilst for sediment sites the location was immediately above the sediment. A Van Veen sediment grab was utilised to collect the sediment samples at each site. A scrape of the top 2-3cm of the sediment from three subsamples representing the survey site were collected and these were homogenised to obtain a representative sample. The well-mixed sample was separated into two; sample to be analysed for grain size was stored in plastic bags whilst the remainder of the sample used for elemental analysis and was stored in acid pre-cleaned plastic containers ensuring no cross contamination. The containers were double bagged and stored in an ice box immediately after collection. After transportation to the laboratory, the elemental samples were frozen at -20°C until analysis.

Water samples were collected from 0.2 m below the water surface using a pre-cleaned acid washed 1L polypropylene container, the dissolved fraction was filtered through a 0.45 µm membrane syringe filter on site and the remaining sample transported back to the laboratory for filtering through precombusted GF/F glass filters to capture the particulate fraction.

3.2.3 Elemental analysis

3.2.3.1 Sediment samples.

Prior to analysis the elemental sediment samples were dried at room temperature. The sediment samples were finely powered using a ball-mill to ensure the samples were fully homogenised. For total carbon and nitrogen analysis 20mg (\pm 2mg) of the ball milled sediment was weighed directly into a tin capsule. The weight was recorded and capsules folded prior to analysis. For organic carbon analysis a similar quantity of sediment was weighed into a 2ml glass ampoule. Carbonate was removed following the adapted methodologies of (Verardo, Froelich and McIntyre 1990). Briefly 1ml of sulphurous acid was added to the ampoule. Samples were left overnight to react and de-gas in a fume cupboard. Samples were then transferred to a vacuum desiccator and held under vacuum to ensure all CO₂ was removed, before being frozen for a minimum of 4 hours. Once frozen the samples were then freeze dried. The dry residue was agitated using a vortex and scrapped into tin capsules. These were sealed by folding prior to loading into the auto-sampler. Standards of acetanilide (BDH chemicals - 10.36% Nitrogen,

71.09% Carbon) were utilised for the calibration standard ranging from 0.1 mg to 3.0mg. Empty capsules were used as blank correction for the standards and samples. Acetanilide was run as an unknown sample after every 12 samples to monitor for instrument drift. Certified reference material of medium organic content soil standard B2178 was used for the validation of the method for C & N. Results obtained were well within acceptable values, being 3.10 ± 0.02 % w/w (certified value 3.07 ± 0.07 % w/w) and 0.24 ± 0.01 % w/w (certified value 0.27 ± 0.02 % w/w) respectively.

All carbon and nitrogen analysis was performed on a Costech International Elemental Combustion System (ECS) 4010 using a pneumatic auto-sampler. Operational temperatures were set to 950 °C in the left combustion furnace, 630 °C in the right reaction furnace and 50 °C in the gas chromatographic separation oven. The helium (>99.9%) carrier gas pressures were set to 1.2 bar giving a flow rate ~ 100 ml/min. Oxygen (>99.9%) used for combustion was set at 1.9 bar and air (water and oil free) 2.4 bar. A calibration coefficient of ≥ 0.999 was achieved for each analytical run. Limit of detection was calculated using 3 times the standard deviation of the mean of the blank data (empty tin capsules) which resulted in 0.07 and 0.08 $\mu\text{mol g}^{-1}$ for nitrogen and carbon respectively.

For total phosphate (P) analysis, 200mg of the ball milled sediment was ashed in a muffle furnace at 450 °C. Ash was transferred to 50ml centrifuge tubes along with 20ml of 3.5M HCL and placed onto an orbital shaker overnight for 16 hrs. Samples were then centrifuged for 15 minutes and 10ml of the supernatant placed into a 50ml volumetric flask. Five drops of 0.25% nitroresol was added and neutralised with either 5M HCL or 5M NaOH and made up to 50ml with distilled water. Calibration standards were prepared in the same way but using between 5 and 100 $\mu\text{g P}$. Neutralised samples or standards were mixed with distilled water and potassium antimony tartrate, ammonium molybdate, ascorbic acid and sulfuric acid, which act as a colour developing agent. The solution was held at room temperature for 15 minutes. Measurements were analysed on a spectrophotometer absorbance at 880nm in a 1cm cell using the calibration blank in the reference cell and converted to mg P/Kg. Standards of anhydrous potassium dihydrogen phosphate were utilised for the calibration standard and a calibration coefficient of 0.999 was achieved. Certified reference material of BCR-684 river sediment was used for the validation of the method for P. Results obtained were well within acceptable values, being 1159.97 ± 108.17 mg/kg (certified value 1113 ± 24 mg/kg). Limit of detection was calculated to be 0.08 $\mu\text{mol g}^{-1}$ and a coefficient of variation of 4.47%.

3.2.3.2 Suspended particulate matter

Total carbon and nitrogen analysis was based on Sharp (1974) where filters were dried in a desiccator and wrapped in tin disks. Samples for the determination of organic carbon and nitrogen were exposed to HCl fumes in the desiccator to remove inorganic carbonate, these were then dried and packed into tin capsules for analysis. The samples were processed on a Costech International Elemental Combustion System (ECS) 4010, operating programme explained earlier.

Total phosphorous analysis was based on Solorzano and Sharp (1980) method. In brief filter papers were dried to a constant weight then placed in an acid cleaned and pre-combusted (450 °C, 5h) glass vial with Magnesium sulfate (MgSO_4) and dried in an oven taking care of cross contamination. Samples were sealed and baked in a muffle furnace for 2h. After cooling, HCl was added and the supernatant heated for 30 minutes. Ammonium molybdate, H_2SO_4 , potassium antimonyl tartrate & ascorbic acid was added, as a colour developing agent, to the supernatant and centrifuged at room temperature for one minute. Samples were then processed in a Spectrometer at 885 nm.

3.2.3.3 Water samples

All water samples that had been filtered through a sealed GF/F syringe filter were analysed for nutrients (phosphate, nitrate+nitrite, nitrite, and ammonia) using a Seal Analytical continuous-flow AutoAnalyzer 3 (AA3) following the methodologies of Seal Analytical. After each run, the charts are reviewed for discrepancies and blanks subtracted, and the final concentrations ($\mu\text{mol/L}$) calculated. Phosphate was determined as ortho-phosphate based on the methods of Murphy and Riley (1962) and Drummond and Mahr (1995). This method was based on the colorimetric method where orthophosphate, molybdate ion and antimony ion were mixed and reduced with ascorbic acid. This reduced phosphor-molybdenum complex is read at 880nm. Nitrate + Nitrite was determined using a modified method of (Armstrong, Stearns and Strickland 1967) where the sample is passed through a cadmium column where nitrate is quantitatively reduced to nitrite. Sulphanilamide is introduced to the sample stream followed by N-(1-naphthyl) ethylene diamine dihydrochloride which mixes to form a red azo dye. The stream is then passed through a 10mm flow cell and the absorbance measured at 520nm. The same technique is employed for nitrite analysis, except the cadmium column is not present. Nitrate

concentration is calculated by subtracting nitrite from the combined Nitrate + Nitrite value. Ammonium was determined based on the method described in (Kerouel and Aminot 1997) where the sample is reacted with o-phthalaldehyde (OPA) at 75 °C in the presence of borate buffer and sodium sulphite to form a fluorescent species which is proportional to the ammonia concentration, the fluorescence is measured at 460nm following excitation at 370nm.

3.2.4 Sediment grain size analysis

A Malvern Hydro 2000MU particle size analyser was used with Mastersizer 2000 software to determine the particle size distribution of all samples. Particles were separated by sonication and grain size was determined by laser diffraction. All data represents the average of three independent replicate samples. Grain size statistics were then entered into the GRADISTAT software programme for analysis and subsequent classification of sediment type.

3.2.5 Coliform analysis

Samples were taken approximately 1m below the surface with pre sterilised plastic bottles. All samples were stored at 4°C and processed with 6 hours of collection following EU guidelines. Each sample was shaken and 25mL was extracted and vacuum-filtrated through a 0.2 µm cellulose acetate membrane (Sartorius Stedim Biotech, Gottingen, Germany). The membrane was aseptically transferred to the surface of a plate containing M-endo agar LES (Oxoid Ltd, Basingstoke, UK); the plate was inverted and incubated at 37°C and enumerated 24hr later.

3.2.6 Data transformation and Statistical Analysis

Within the suspended particulate matter content for carbon and nitrogen, comparison was made between the total and organic fraction to identify whether there was a significant difference between the two fractions. When total carbon was equal to organic carbon (1:1 gradient), inorganic carbon was insignificant and therefore could be ignored. The TC would then be reported as OC. The same principle was undertaken for nitrogen, if TN was equal to ON it was evident that no significant adsorption of NH_4^+ was present and TN was reported as ON.

A well-established technique is the simple mixing curve approach, the principle uses a very simple model where there are two end members; riverine (freshwater) and marine (saline water). A constituent whose concentration in an estuary is passively diluted by increasing proportions of either fresh or salt water which is referred to as conservative behaviour (Raine and Williams 2000). Any samples which deviates from the conservative line would constitute non-conservative behaviour (Figure 3.2) and therefore not due to simple mixing and sites are potentially elevated or due to other processes. This technique locates regions within the estuary which are sources or sinks (removal) of any particular dissolved compound which have deviated from the line.

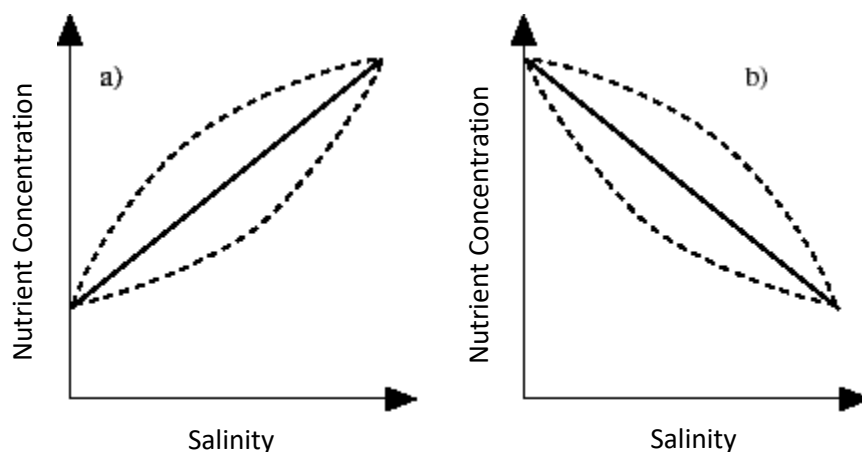


Figure 3.2: Example of conservative behaviour within an estuary (a) for a constituent whose concentrations is higher in seawater than freshwater and (b) for a constituent whose concentrations is higher in freshwater than seawater. Points will fall above or below these curves if there is a source (above) or a sink (below) for the constituent within the estuary (dashed line) (Raine and Williams 2000).

All dissolved and particulate data within this study used this technique to normalise data against salinity to identify whether nutrients were behaving conservatively due to simple mixing or non-conservatively due to potential elevated concentrations.

The data was not normally distributed with transformation unable to establish normality. Basic correlations were determined with non-parametric Spearman Rank Correlation Coefficient (r.) by using the statistical package for Social Sciences SPSS v22. In addition, the Mann-Whitney U test was utilised, due to the data not conforming to normal distribution, to establish whether significant differences between two groups existed in the data. Regression analysis was undertaken on the sediment data as the assumption of normality was based on the fact that all variables were reasonably close to normal as skewness and kurtosis values were between -1.0 & +1.0.

3.2.7 Definition of regional geochemical baselines for the Conwy Estuary

Using the same concept of regional geochemical baselines for elements in chapter 2, these models were constructed for TN and TP. For both the TN and TP linear regression was performed, considering the whole dataset utilising TOC as the normalising element because the Sc, Rb from chapter 2 is related to the clay fraction whereas TOC is related to the organic matter and therefore relevant for the nutrient data.

3.2.8 Estimation of sediment contamination

The same concept and analysis which was undertaken in chapter 2 has been applied to the nutrients TN and TP concentrations to calculate enrichment factors (EF) and establish whether an area is at background level or enriched by anthropogenic sources. The equation explained in chapter 2 is:

$$EF_{\text{nutrient}} = \frac{(\text{nutrient /normalising element})_{\text{sample}}}{(\text{nutrient /normalising element})_{\text{baseline}}}$$

Baseline nutrient and normalising elements were obtained using Total organic carbon (TOC) regression line. An enrichment factor greater than 1.5 constitutes an enrichment above natural background conditions.

3.2.9 Sediment quality guidelines.

Sediment quality guidelines (SQGs) published by the Ministry of Environment and Energy, Ontario, Canada are used widely to assess the sediment contamination. The guidelines specify three contamination levels that can be expected to result in different potential effects; the no effect level (TN <550 mg/kg & TP <600 mg/kg), the lowest effect level (TN 550 mg/kg & TP 600mg/kg) and the severe effect level (TN 4800 mg/kg & TP 2000 mg/kg) (Persaud, Jaagumagi and Hayton 1996). Nutrients below the no effect level or at the lowest effect level constitute unpolluted or marginally polluted sediments, while sediments, which range between the lowest effect level and the severe effect level, are considered as moderately polluted and concentrations above the severe effect level indicate that the sediments are heavily polluted. These SQGs were analysed for the Conwy dataset to enable a comparison between the enrichment factors and C/N ratios to be made.

3.3 Results and Discussion

3.3.1 Spatial distribution of dissolved and particulate nutrients within the Conwy Estuary.

3.3.1.1 Identified freshwater inflows to the estuarine system

Locations of the most evident inflows (Figure 3.1, survey 3) were investigated by sampling once in summer and once in winter in one calendar year to understand whether concentrations differed temporally or spatially and to identify the origins of the significant inflows to the estuary. Concentrations of dissolved inorganic (Table 3.1) and particulate nutrients (Table 3.2) were analysed and presented in Appendix 2.

Sample sites 7 (NO_2^- winter), 14, 18 (NH_4^+ summer & winter and winter respectively), 11 (PTN & PON summer and winter and TP winter), 9 (PON & PTN winter), 24 (TP winter) and 12 (all sites) were analytical outliers and therefore values were not calculated for these sites. Within the remaining sites during the summer survey PO_4^{3-} at sites 7,13,14 and 24, OC at site 21 and TP at sites 3,7,11 and 23 and during the winter PO_4^{3-} at sites 1,7,9,10,14,17 & 24, NO_2^- at site 9, OC, TN and ON at site 24 and TP at sites 7,15,18 & 23 had concentrations below the limit of quantification and therefore all these sites have been omitted from further analysis. Descriptive statistics were undertaken on the remaining raw data, Table 3.1 summarises these results, which includes mean concentrations, standard deviations and range.

Within the remaining dataset (Table 3.1) there was no statistically significant differences in concentrations of PO_4^{3-} , NH_4^+ , TC, OC, TN, ON and TP between the summer and winter surveys ($p = 0.895, 0.155, 0.247, 0.560, 0.916, 0.991, \& 0.579$ respectively). However NO_2^- , NO_3^- and coliforms show a significant difference between the two surveys ($P = 0.052, 0.036 \& 0.012$ respectively), this supports figure 3.1, which shows that there is a temporal difference between summer and winter for these nutrients.

Table 3.1: Range of concentrations of the investigated dissolved and particulate nutrients ($\mu\text{mol/L}$), N is the number of sites and LOQ is the limit of quantification.

Sample descriptive statistics						
	N	Min	Max	LOQ	Mean	St. Dev
PO4 summer	18	0.36	33.11	0.311	4	7.98
PO4 winter	15	0.58	18.26	0.311	2.6	4.5
NO2 summer	21	0.09	9.43	0.024	1.17	2.5
NO2 winter	20	0.12	11.88	0.024	1.36	2.61
NO3 summer	21	0.4	144.41	0.057	50.48	48.01
NO3 winter	21	6.44	293.42	0.057	96.04	84.61
NH4 summer	20	1.49	260.38	0.078	37.04	76.29
NH4 winter	19	0.76	490.8	0.078	58.95	124.86
TC summer	21	4.96	130.44	3.081	36.89	34.11
TC winter	20	0.99	573.19	3.081	70.31	141.45
OC summer	20	3.1	135.29	3.081	33.5	34.38
OC winter	19	3.65	530.5	3.081	55.78	120.45
TN summer	19	0.4	4.59	0.357	1.8	1.27
TN winter	17	0.45	19.12	0.357	3.92	5.62
ON summer	18	0.46	4.59	0.357	1.69	1.17
ON winter	17	0.45	10.2	0.357	2.19	2.55
TP summer	15	0.03	0.161	0.03	0.08	0.04
TP winter	15	0.03	0.36	0.03	0.11	0.1
Coliforms summer	21	0	191733		14717.14	41206.7
Coliforms winter	21	0	151900		8050.41	32964.7

Inflows to the Conwy estuary were distinguished between river, stream, intermittent discharge, land runoff and marinas to enable an understanding of volume from the inflows entering the estuary due to the lack of quantified flow data (Figure 3.1 & 3.2). Comparing nutrient concentrations at all sites only five show high concentrations in comparison to the other sites within riverine inputs and therefore potentially a higher volume entering the estuary (Figure 3.1 & 3.2). These sites included 17 (NH_4^+ , TC, TN, TP & coliforms), site 22 (NO_3^- & TN), 18, 21 (NO_3^-), 14 (TC) and 12 (TP). The majority of sites showed elevated concentrations for both seasons, with NO_2^- and NO_3^- showing higher concentrations within the winter survey compared to the summer and coliform concentrations showing higher concentrations within the summer survey. With the exception of TC, site 6 showed high concentration for all dissolved and particulate nutrients, however this site is classified as an intermittent discharge and therefore low

flow yielding potentially a lesser risk within the estuary due to dilution with estuarine water. Nitrate concentrations are relatively high within inflows entering the marine section of the estuary however with the exception of rivers previously discussed other inflows are from low flow categories and therefore potentially less impacting after dilution within estuarine waters.

Particulate concentrations are all relatively higher at inflows entering the freshwater section of the estuary compared to the marine end, with riverine inputs having the highest contributions compared to the marine section with the highest contributors evident from low flow categories and therefore less impacting.

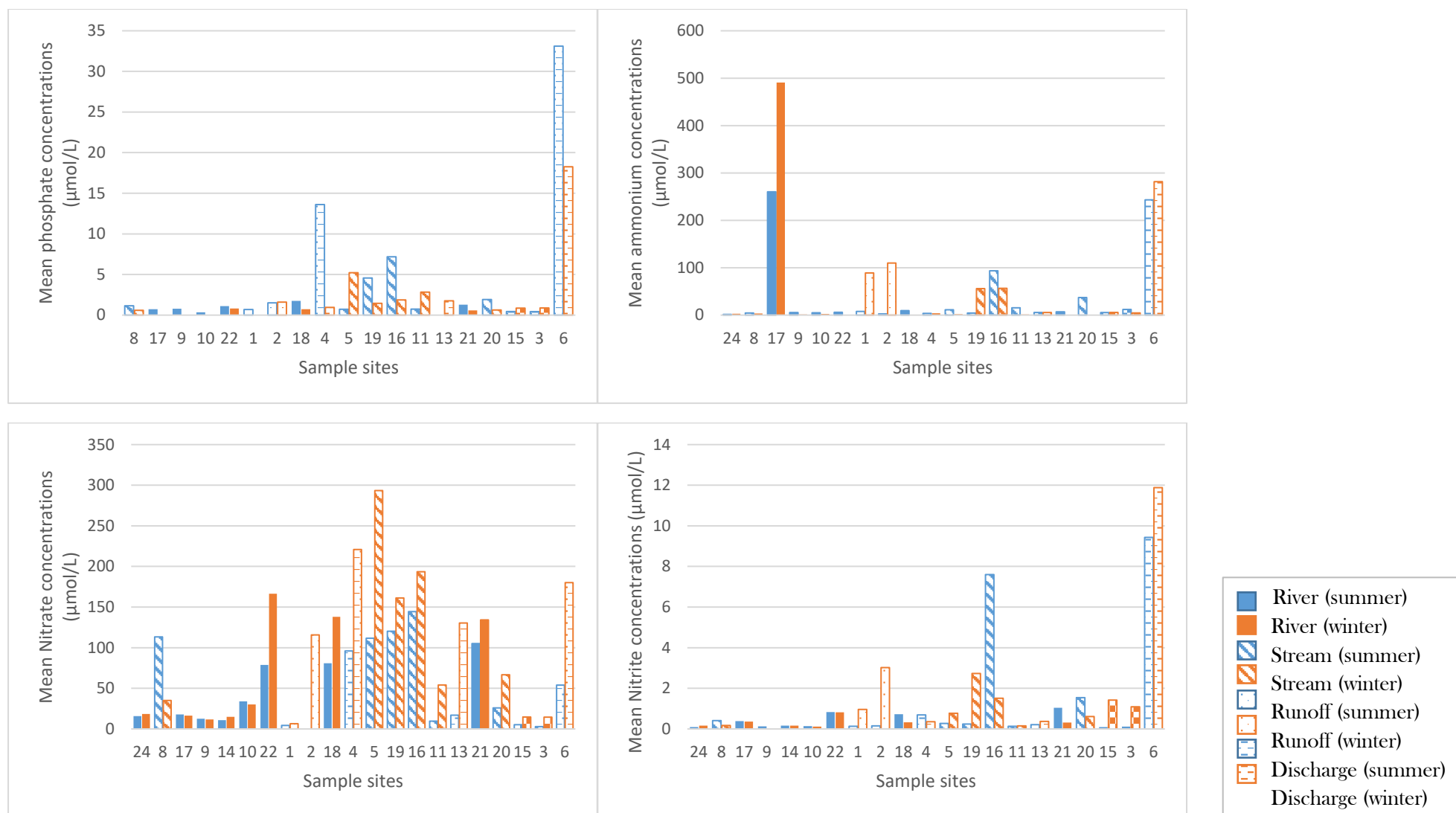


Figure 3.1: Concentrations of dissolved nutrients within the evident inflows to the Conwy estuary, categorized by type of inflow; river, stream, intermittent discharge, land runoff or marina. Sample sites ordered in relation to riverine (left) to marine (right) location.

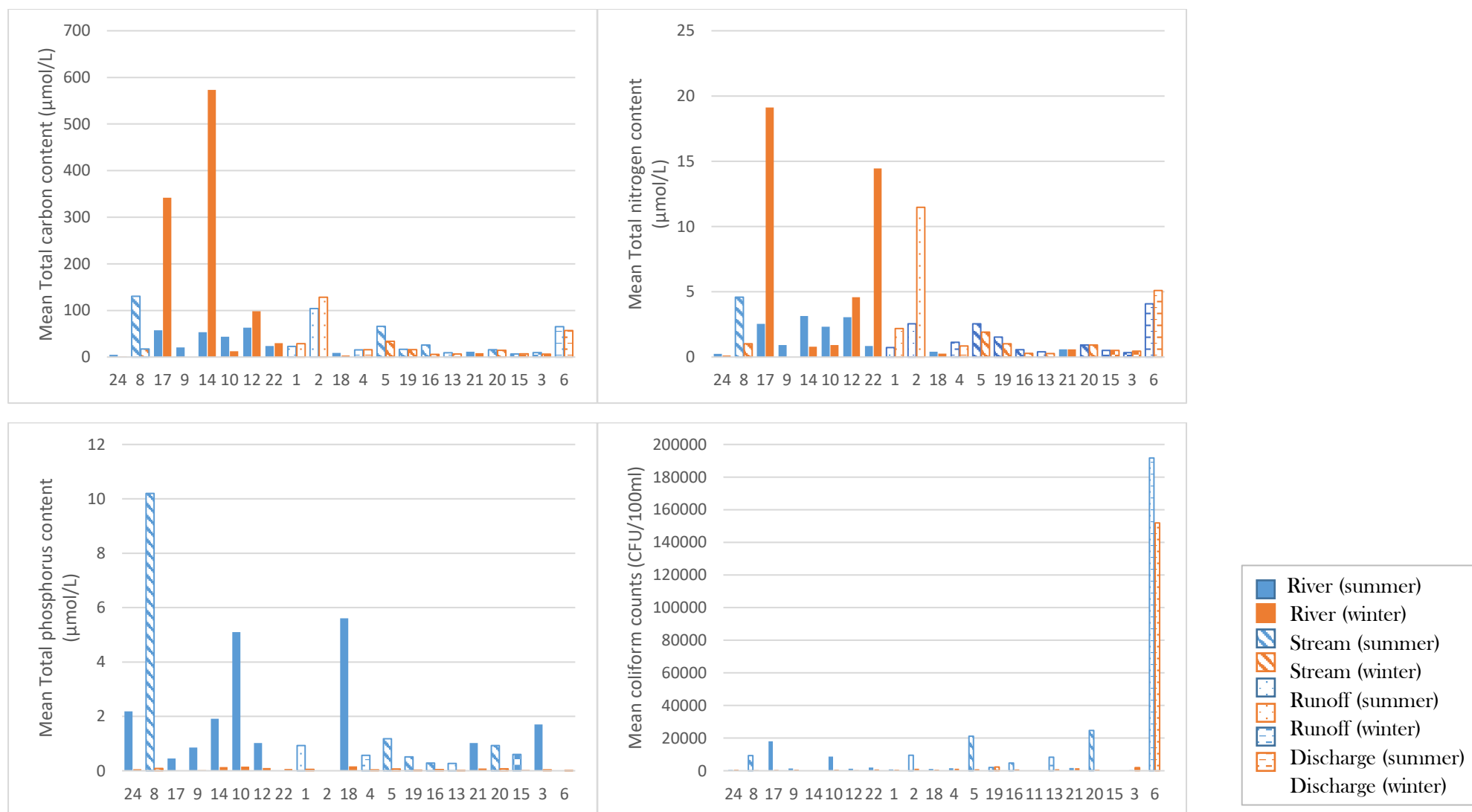
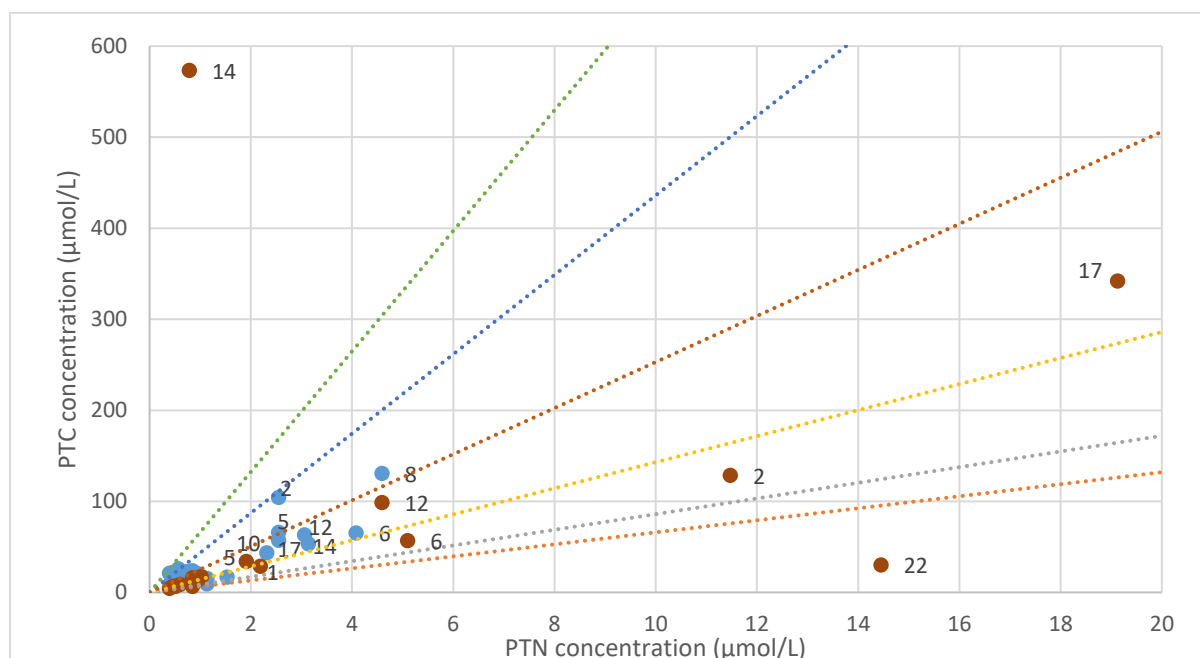
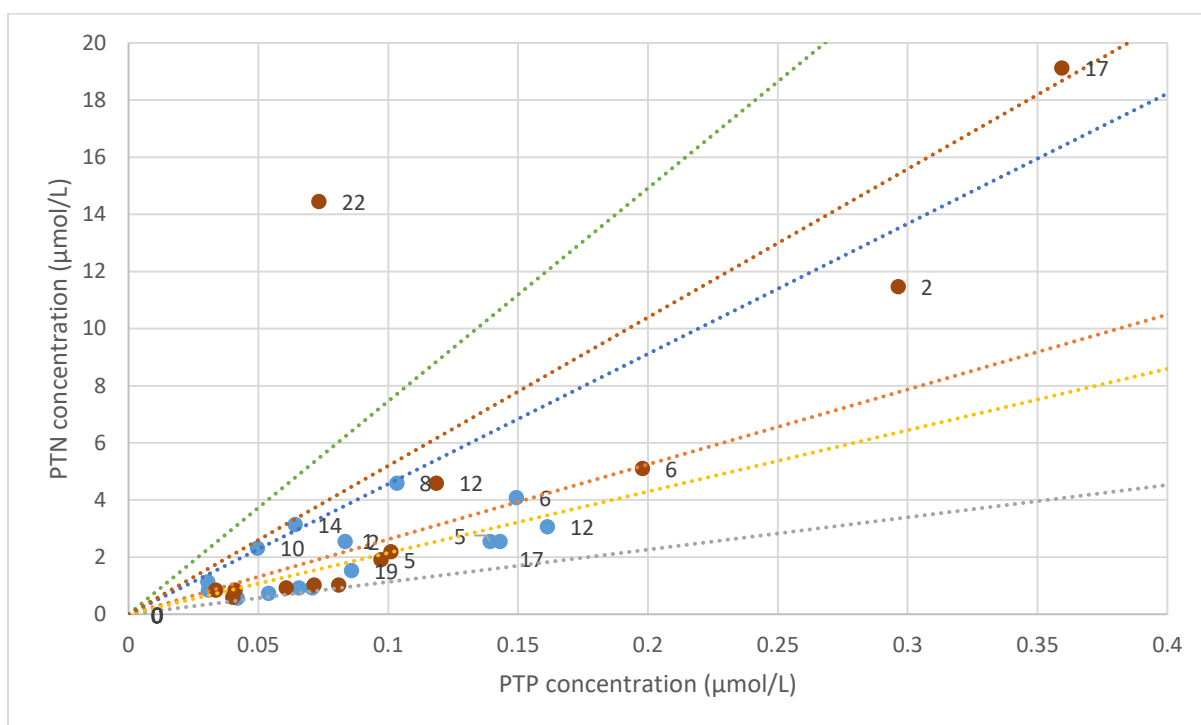
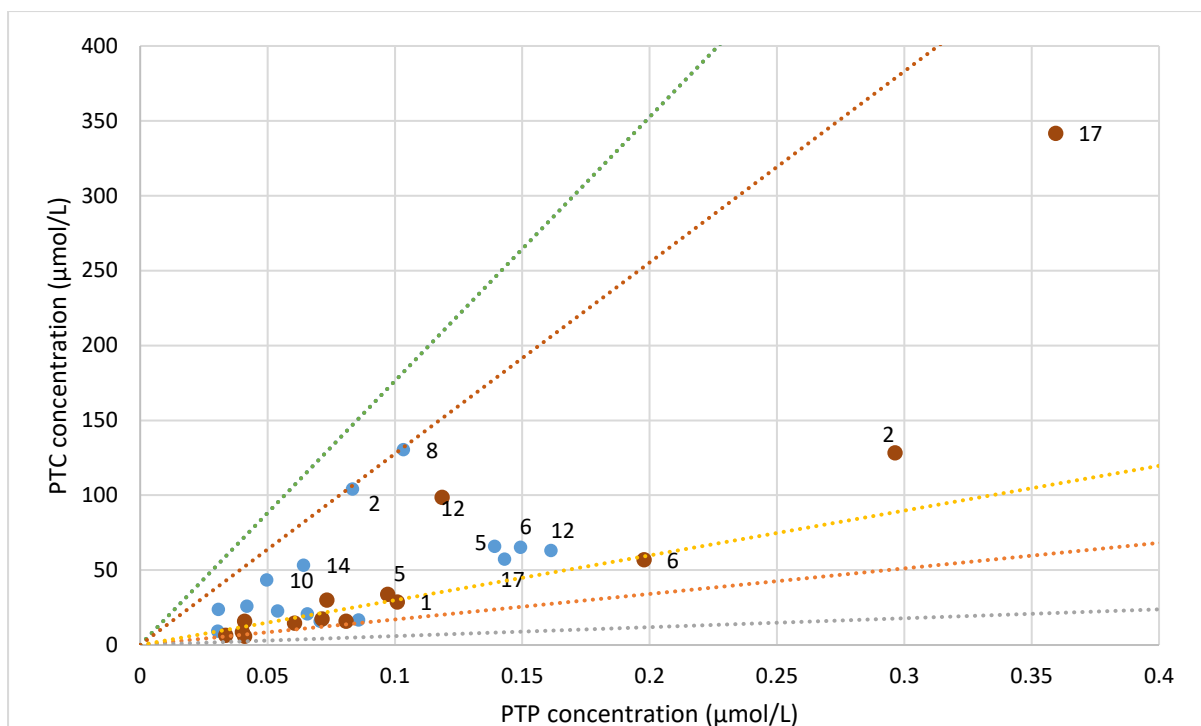


Figure 3.2: Concentrations of particulate nutrients and coliforms within the evident inflows to the Conwy estuary, categorised by type of inflow; river, stream, intermitent discharge, land runoff or marina. Sample sites ordered in relation to riverine (left) to marine (right) location.

C:N, C:P and N:P ratios were calculated using the particulate data for all the inflows (Figure 3.3 A-C) to the Conwy estuary. Known marine (Hecky, Campbell and Hendzel 1993) and terrestrial (Cleveland and Liptzin 2007) elemental ratios have been superimposed onto Figure 3.3 to identify the origins of the organic matter. It is evident that no single source is contributing to the observed C:N, C:P or N:P trends but samples represent different relative proportions of potential sources across the sites. A large proportion of the sample sites are indicating a mixture of peat and soil origins. Sites 14 (OC), 2 (TN & TP), 22 (TN & TP) and 17 are all exhibiting higher concentrations and maybe elevated in comparison to other sites within the Conwy inflows.





● Summer
 ● Winter
 — Phytoplankton
 — Microbial
 — Soil
 — Tree foliage
 — Leaf litter
 — Peat

Figure 3.3: Comparison of PTC, PTN and PTP concentrations against known biomass ratios to establish the origin of the organic matter within the inflow entering the Conwy estuary over a summer and winter survey.

3.3.1.2 High and low water survey

The tidal survey enabled an understanding of whether nutrient concentrations differed temporally or spatially within the marine influence (high water) or terrestrial influence (low water). Concentrations were evaluated to establish whether the trends were due to simple mixing within the estuary or potentially elevated due to other factors. The origin of sites which showed elevated concentrations above natural condition, were established to classify whether from natural or potentially human impacted sources. Concentrations of dissolved inorganic nutrients (Table 3.3) and particulate nutrients & coliforms (Table 3.4) for five sites over high and low water within the main channel of the Conwy Estuary are presented in Appendix 2. During the high water survey PO_4^{3-} at sites TS2 & TS5 and within the low water survey at TS2 & TS3 concentrations were lower than the limit of quantification and therefore omitted from further analysis. Descriptive statistics were undertaken on the remaining raw data, Table 3.2 summaries these results, which include mean concentration, standard deviation and range.

Table 3.2: Range of concentrations of the investigated dissolved and particulate nutrients ($\mu\text{mol/L}$), N is the number of samples and LOQ is the limit of quantification. Percentage of samples that were removed due to less than LOQ (%LOQ).

Sample descriptive statistics							
	N	Min	Max	LOQ	% <LOQ	Mean	Std.Dev
PO4 high water	4	0.33	0.52	0.311	40	0.39	0.09
PO4 low water	2	0.32	0.36	0.311	40	0.34	0.03
NO2 high water	5	0.16	0.29	0.024	0	0.24	0.05
No2 low water	5	0.23	0.5	0.024	0	0.36	0.12
NO3 high water	5	5.77	14.39	0.057	0	11.58	3.41
NO3 low water	5	4.07	19.85	0.057	0	12.42	5.92
NH4 high water	5	1.66	7.09	0.078	0	4.14	2.08
NH4 low water	5	2.26	8.94	0.078	0	5.58	2.57
TC high water	5	95	216.66	3.081	0	145.34	49.59
TC low water	5	78.13	180	3.081	0	129.08	42.84
OC high water	5	94.44	216.66	3.081	0	142.4	49.77
OC low water	5	67.71	175	3.081	0	100.05	42.89
TN high water	5	5.95	14.29	0.357	0	9.37	3.13
TN low water	5	6.49	23.69	0.357	0	11.57	6.88
ON high water	5	5.95	14.29	0.357	0	8.97	3.24
ON low water	5	4.2	21.43	0.357	0	10	6.69
TP high water	5	0.21	2.45	0.03	0	1.46	0.87
TP low water	5	0.21	1.63	0.03	0	1.08	0.63
Coliforms high water	5	262	6013			3469	2209.18
Coliforms low water	5	317	5698			3295	2120.94

Total carbon and nitrogen contents of the suspended particulate matter were found to consist of insignificant amounts of inorganic carbon and no significant amount of nitrogen absorbed as NH_4^+ (Figure 3.4) due to samples positioned along the 1:1 gradient and therefore ON and OC are omitted from further analysis and reported as TN and TC. Within the remaining dataset there was no significant difference in concentrations of PO_4^{3-} , NH_4^+ , NO_2^- , NO_3^- , TC, TN, TP and coliforms between high and low water ($p = 0.275, 0.465, 0.142, 0.917, 0.602, 0.602, 0.268$ & 0.917 respectively). This identifies that there is no temporal difference between high and low water for all nutrients.

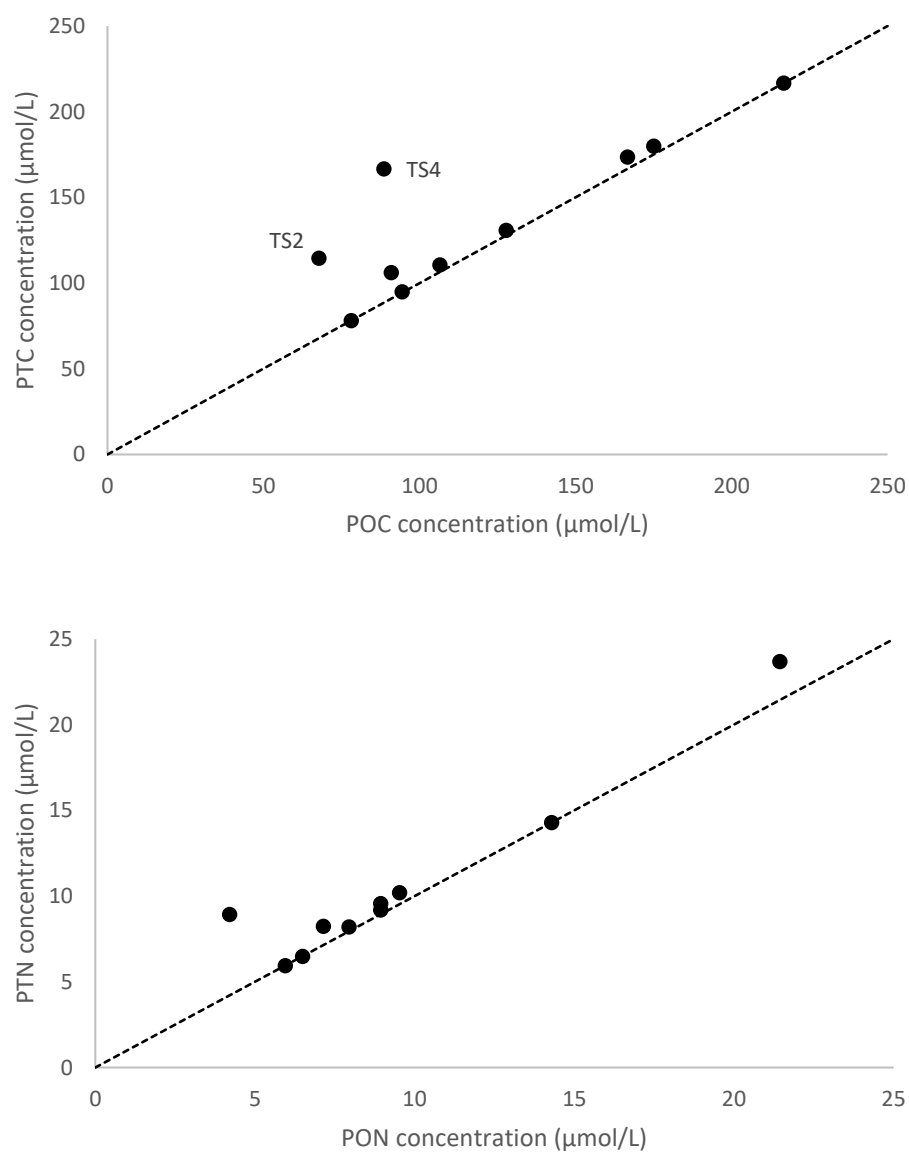
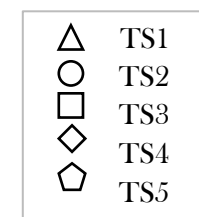
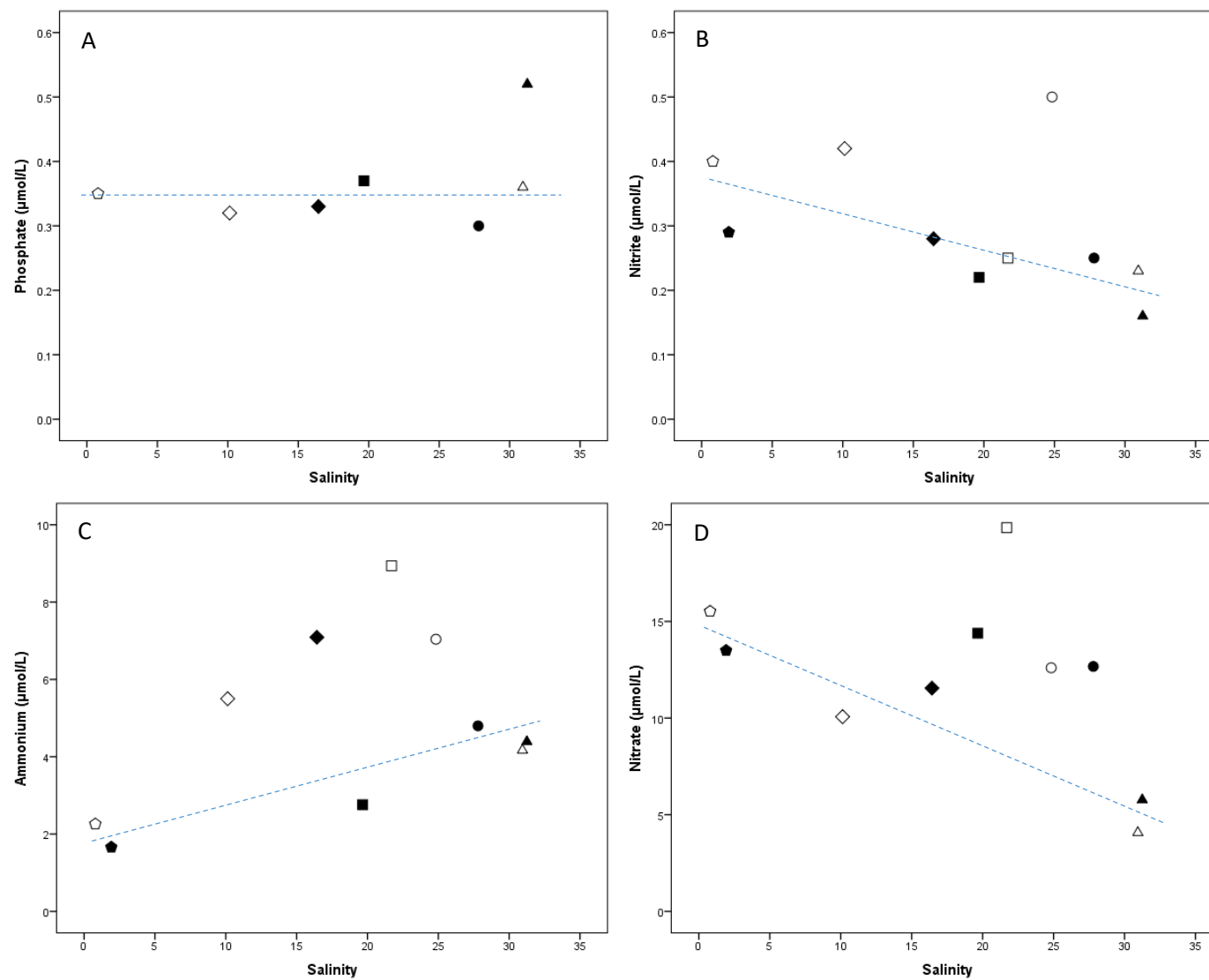


Figure 3.4: Comparison between organic and total species of particulate carbon and nitrogen to establish whether concentrations differ with the Conwy estuary. Dashed line represent a 1:1 gradient.

Phosphate concentrations, with the majority close to limit of quantification ($0.311 \mu\text{mol/L}$), behaved non-conservatively with no change of concentrations across the salinity gradient with the exception of TS1 on high water (Figure 3.4a) potentially constituting an elevated site. Ammonium, nitrite and nitrate are showing a general pattern of conservative mixing with ammonium increasing whilst nitrite and nitrate decrease towards higher salinities. However production of NH_4^+ and NO_3^- can be seen at sites represented by salinity 10-25 and 15-25 respectively (Figure 3.5 C&D) indicating elevated concentrations above general mixing. The general pattern for TC, TN, TP and coliforms show highest concentrations within the terrestrially influenced section of the estuary and reduces towards high salinity. All sites of TC content are behaving conservatively with no elevated sites, whereas TP exhibited non-conservative behaviour, however this was dominated by only one elevated site (site 2 high water) and the remaining sites were behaving conservatively. TN indicates a reduction between salinities 10 and 27 otherwise showing conservative mixing with no elevated sites. Coliform counts show to have non-conservative behaviour with salinities 10 to 27 positioned above the mixing line (Figure 3.5H).



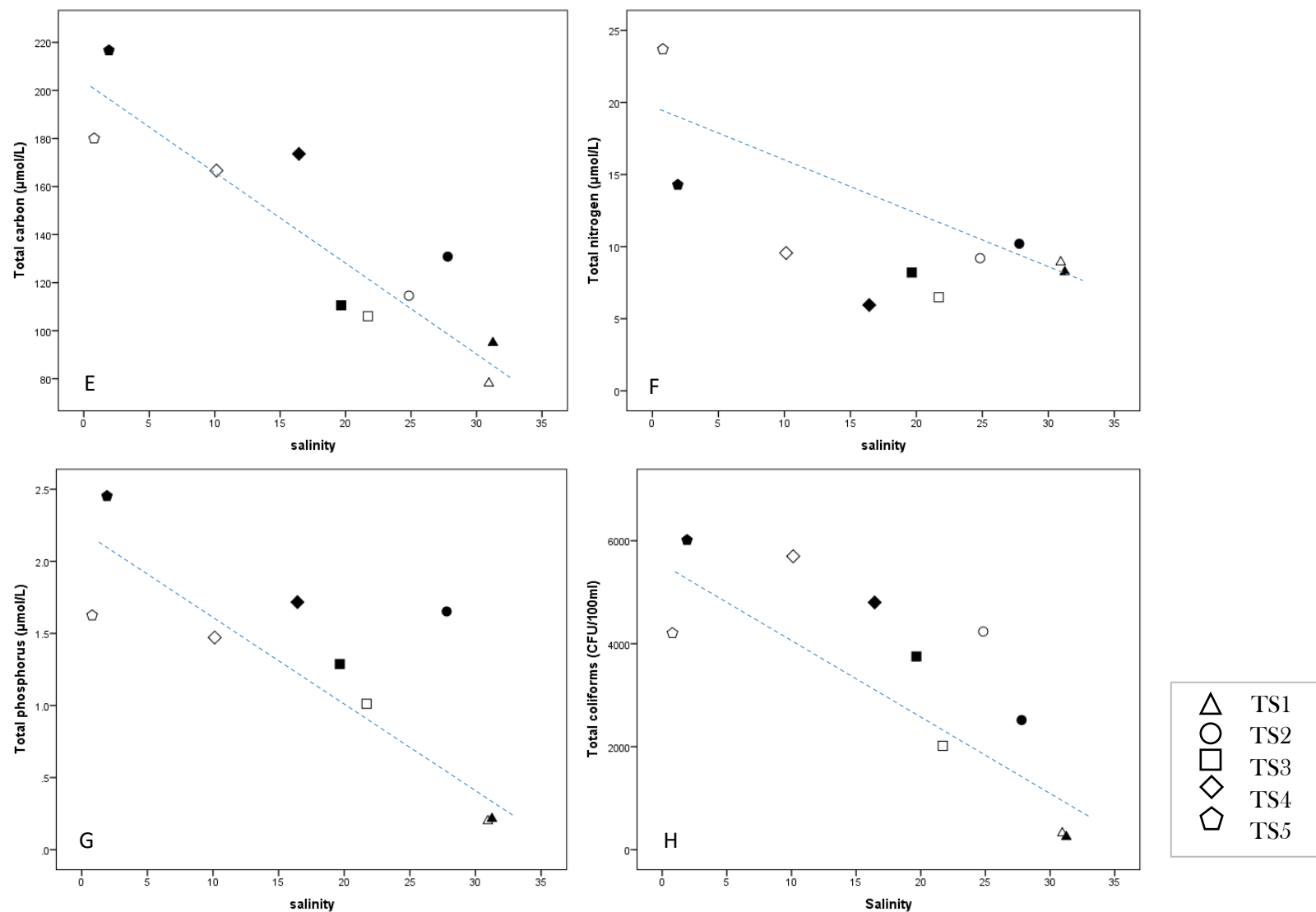
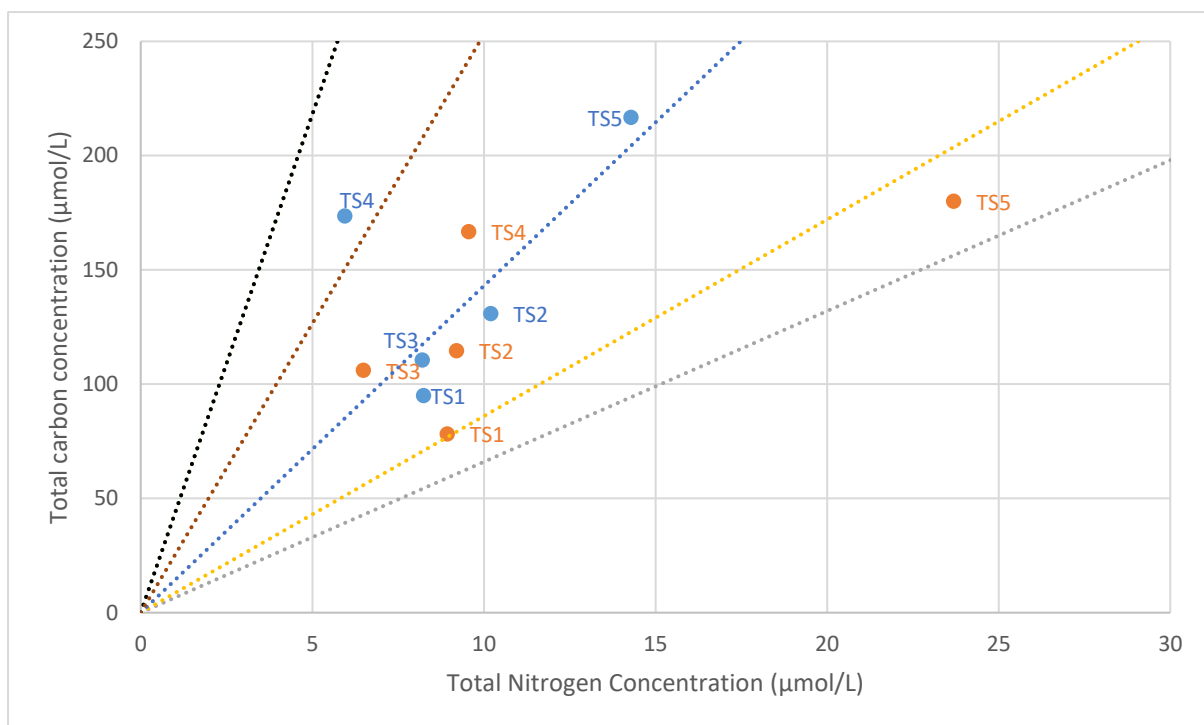
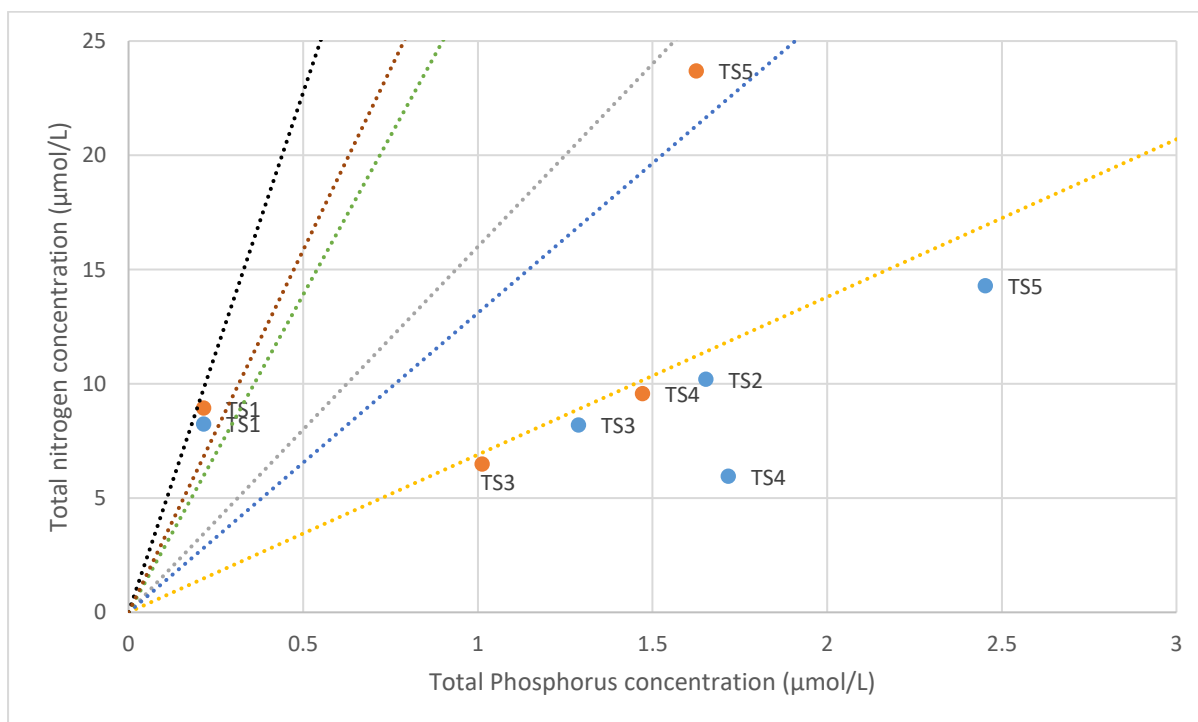
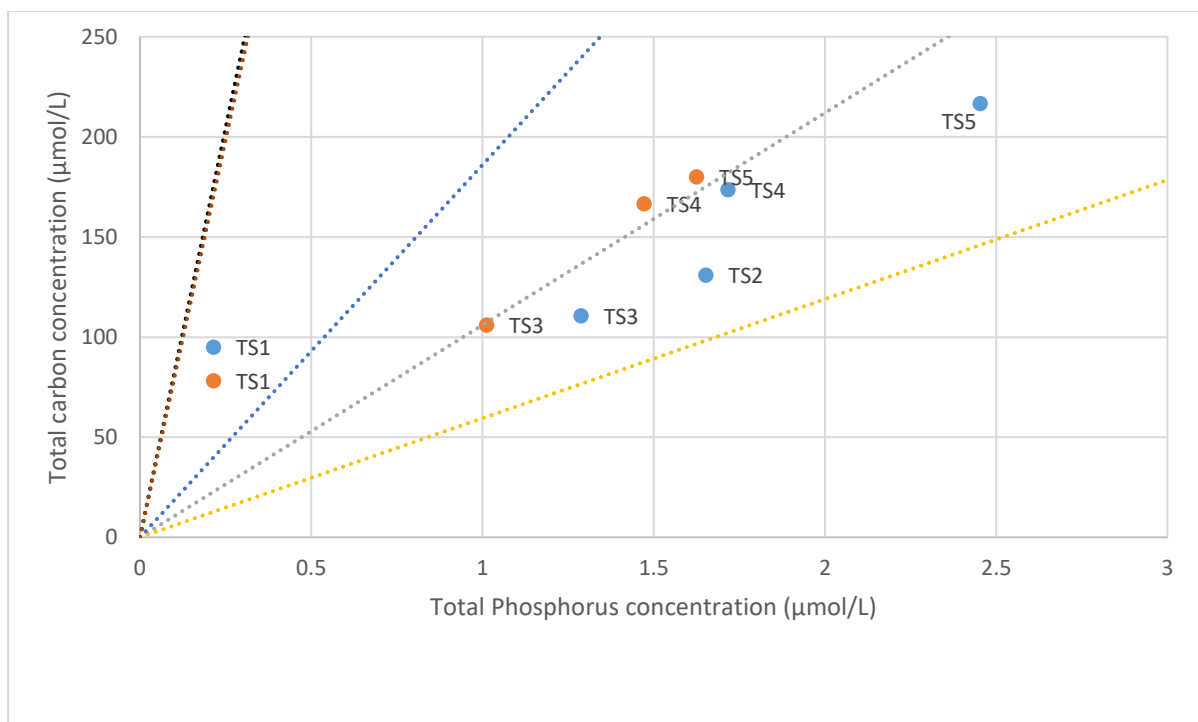


Figure 3.5: Nutrient concentrations (A-G) and coliform counts (H) normalised against salinity to establish whether simple mixing is responsible for the concentrations within the Conwy estuary. Closed symbols (high water), open symbols (low water) & blue dashed line (conservative mixing line).

CN, CP, and NP ratios were calculated using the particulate data (Figure 3.6 A-C) from the Conwy estuary. Known marine (Hecky et al. 1993) and terrestrial (Cleveland and Liptzin 2007) elemental ratios have been superimposed onto Figure 3.6 to identify the origins of the organic matter. It is evident that again no single source is contributing to the observed C:N, C:P or N:P trends but samples represent different relative proportions of potential sources across the sites. There are no sites which are exhibiting high concentrations in comparison to other sites.





● High water ● Low water — Phytoplankton — Microbial — Soil — Tree foliage — Leaf litter — Peat

Figure 3.6: Comparison of PTC, PTN and PTP concentrations against known biomass ratios to establish the origin of the organic matter within the Conwy estuary over a high and low water.

3.3.1.3 Cross section survey

The cross section survey established whether there was a spatial difference in nutrient concentrations across the estuary. Data was compared to the tidal survey as certain locations were identical to establish whether temporal differences existed between the surveys. This could have implications for water quality monitoring where one side of the estuary could have high concentrations of nutrients or coliforms compared to the other side (Quilliam et al. 2011) and whether time of sampling would affect the concentration. Different concentrations could be due to the various point and diffuse inputs into the estuary and physical processes associated with the estuary. The origins of the organic matter were investigated to establish whether from natural or potentially human impacted sources. Concentrations of dissolved inorganic (Table 3.5, Appendix 2) and particulate nutrients (Table 3.6, Appendix 2) were taken at four different horizontal transects across the Conwy estuary at the same sites of the tidal survey (Figure 3.1 T1-4). Sample sites T3A (TP) and T4A (TN, TC, ON & OC) were extreme outliers and therefore no values were calculated for these sites. Within the remaining dataset PO_4^{3-} at sites T3A, T3B, T3C, T4A and T4C all had concentrations below the limit of quantification and therefore were omitted from further analysis. Descriptive statistics were undertaken on remaining raw data, Table 3.3 summaries these results, which include mean concentration, standard deviation and range.

Table 3.3: Range of concentrations of the investigated dissolved and particulate nutrients ($\mu\text{mol/L}$), N is the number of samples and LOQ is the limit of quantification. Mean reference refers to the mean of 10 certified reference materials

Sample descriptive statistics							
	N	Min	Max	LOQ	% <LOQ	Mean	Std.Dev
PO4	10	0.316	0.702	0.311	33%	0.508	0.123
NO2	15	0.196	0.702	0.024	0	0.354	0.125
NO3	15	1.374	27.7	0.057	0	6.25	6.24
NH4	15	3.031	16.25	0.078	0	7.37	3.85
TC	14	46.299	229.16	3.081	0	164.65	50.14
OC	14	20.83	229.16	3.081	0	129.31	64.27
TN	14	3.97	31.75	0.357	0	12.61	7.19
ON	14	3.97	31.75	0.357	0	11.02	7.31
TP	14	1.502	5.76	0.03	0	3.11	1.2

As with the tidal survey, the particulate total carbon and nitrogen concentrations were found to consist of insignificant amounts of inorganic carbon and no significant amount of inorganic nitrogen absorbed as NH_4^+ (Figure 3.7), therefore ON and OC are reported as TN and TC.

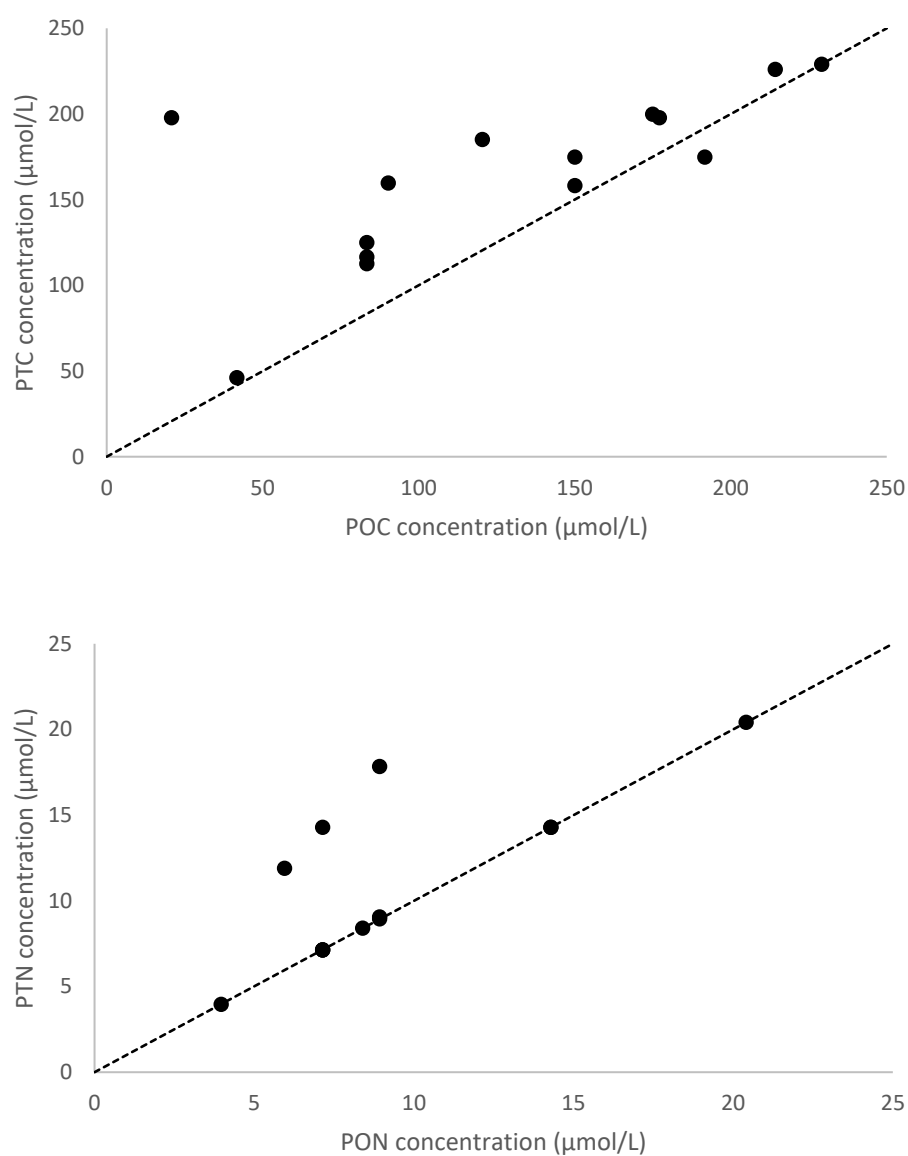
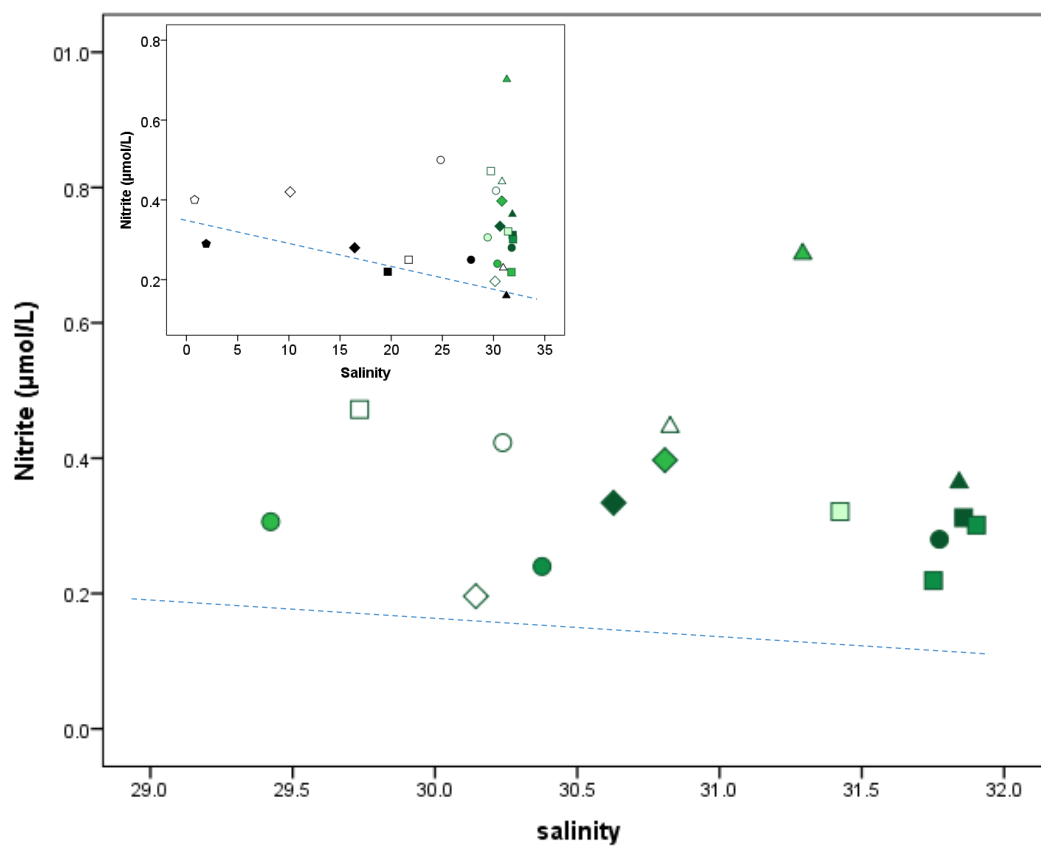
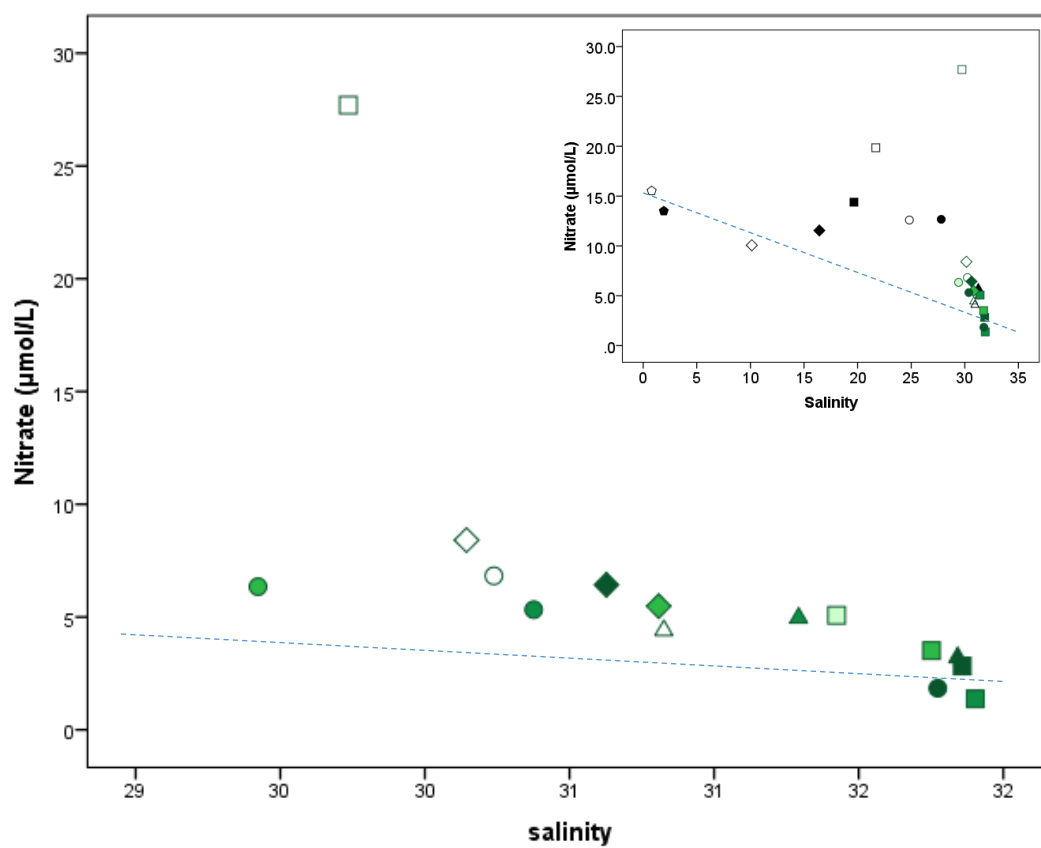


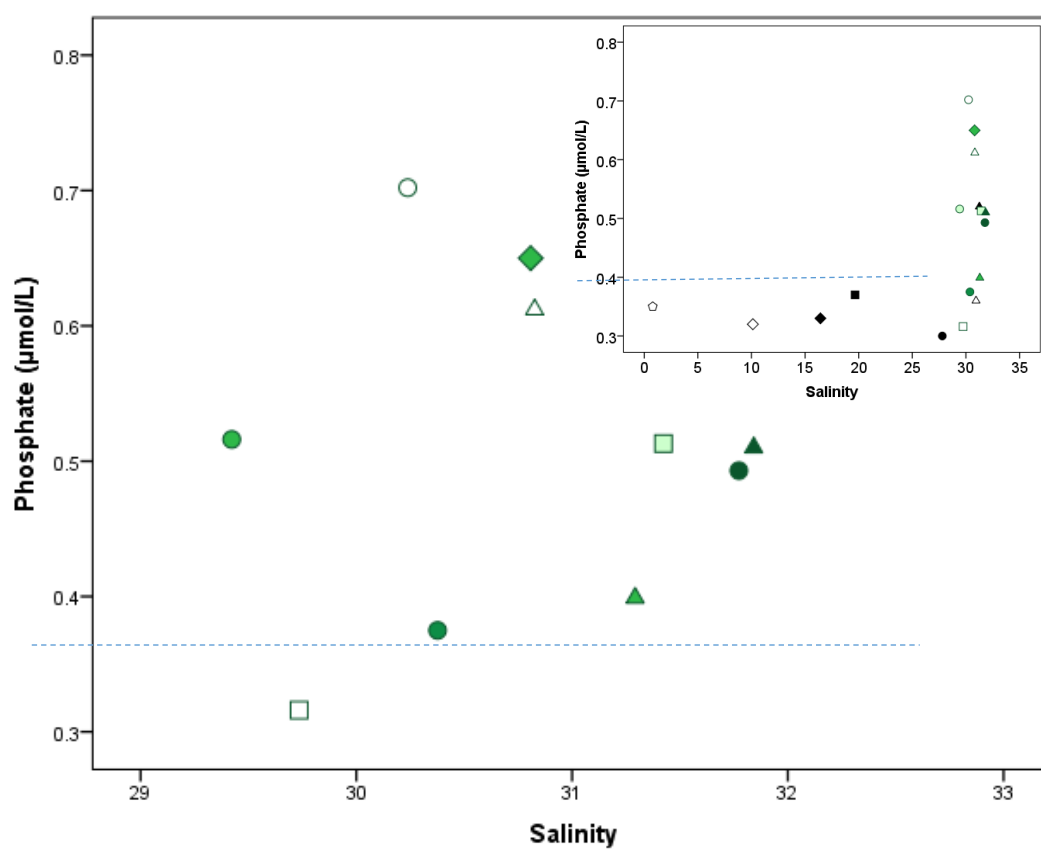
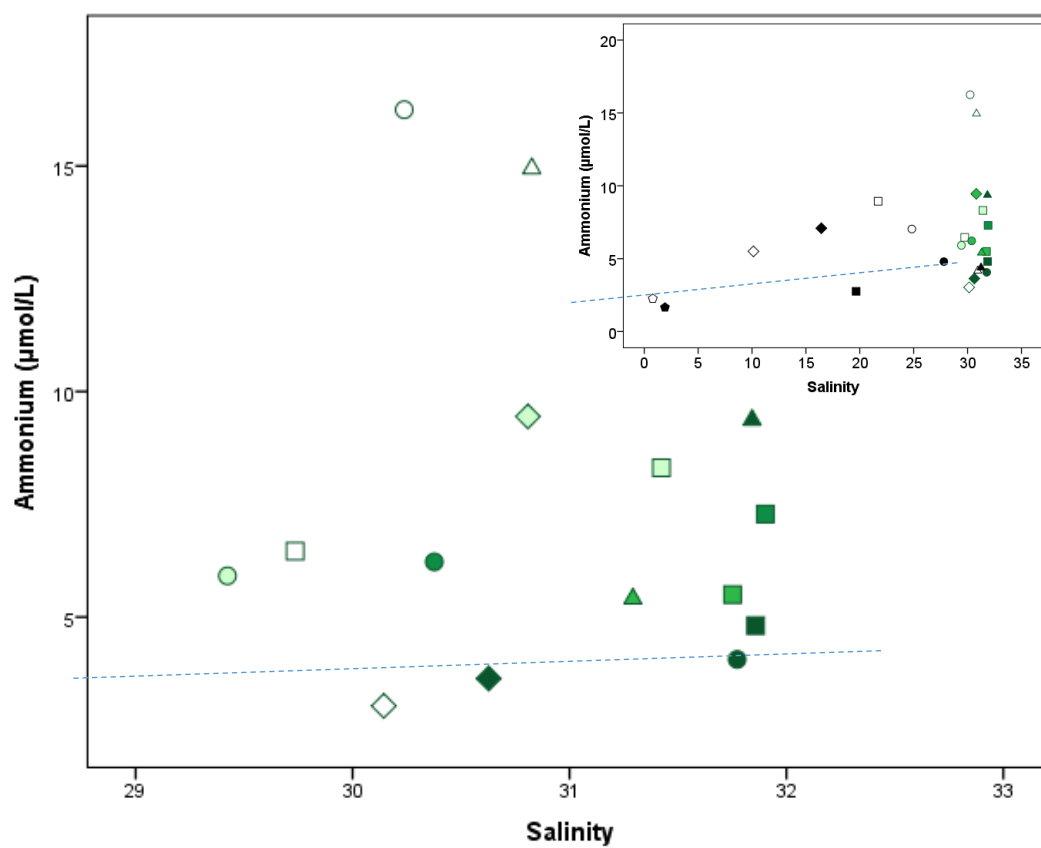
Figure 3.7: Comparison between organic and total species of particulate carbon and nitrogen to establish whether concentrations differ within the Conwy estuary. Dashed line represent a 1:1 gradient.

To distinguish whether the dissolved and particulate nutrients were behaving conservatively the data was normalised against salinity (Figure 3.8). Within this survey, sites were reflective of marine influence (TS1) due to sampling time being on high water slack tide and therefore a very small difference in salinity from 29 to 32. To determine the conservative mixing line a salinity of zero to 35 is needed. The tidal survey data (Figure 3.5) has been used in addition with the current survey to enable the correct range of salinities, including a riverine end member, to produce the conservative mixing line which are reported in the inserts. The conservative mixing line is then reproduced for the current survey within the main figure. It is evident that generally the finer salinity scale is showing a strongly non-conservative behaviour for all constituents (Figure 3.8) and therefore concentrations are due to other processes or sources rather than simple mixing. T3E (NO_3^-) showed to be an extreme outlier compared to other sites and potentially constitutes an elevated site above natural conditions. It is evident that the highest concentrations are generally within the east side of the estuary (Deganwy), which is a higher depositional area, compared to the west (Conwy) (Figure 1.2 & 3.1). However, an exception is seen within sites one and four, where the highest concentrations are within this middle site (NO_2^- , PO_4^{3-} , NO_3^- and NO_2^- , NH_3 , PO_4^{3-} respectively). These cross sections have less tidal mud flats and the main channel runs through the middle site. In addition at sites one (TN & TC) and two (TN) the highest concentrations are within the east which may be due to inflows within these areas (inflow 21 and 6, Figure 3.2). However NO_3^- and PO_4^{3-} are the only nutrient which are significantly different between the east and west of the estuary ($p = 0.028$ & 0.055 , respectively) indicating that there is a spatial difference in concentration and an implication for monitoring.

Table 3.4: Mann-Whitney significance value comparing concentrations between different locations within the Conwy estuary

	No3	Po4	No2	NH3	TN	TC	TP
West v middle	0.142	0.564	1	0.327	0.309	1	0.149
West v East	0.028	0.245	0.361	0.361	0.915	1	0.055
East v middle	0.136	0.655	0.831	0.394	0.453	0.915	0.67





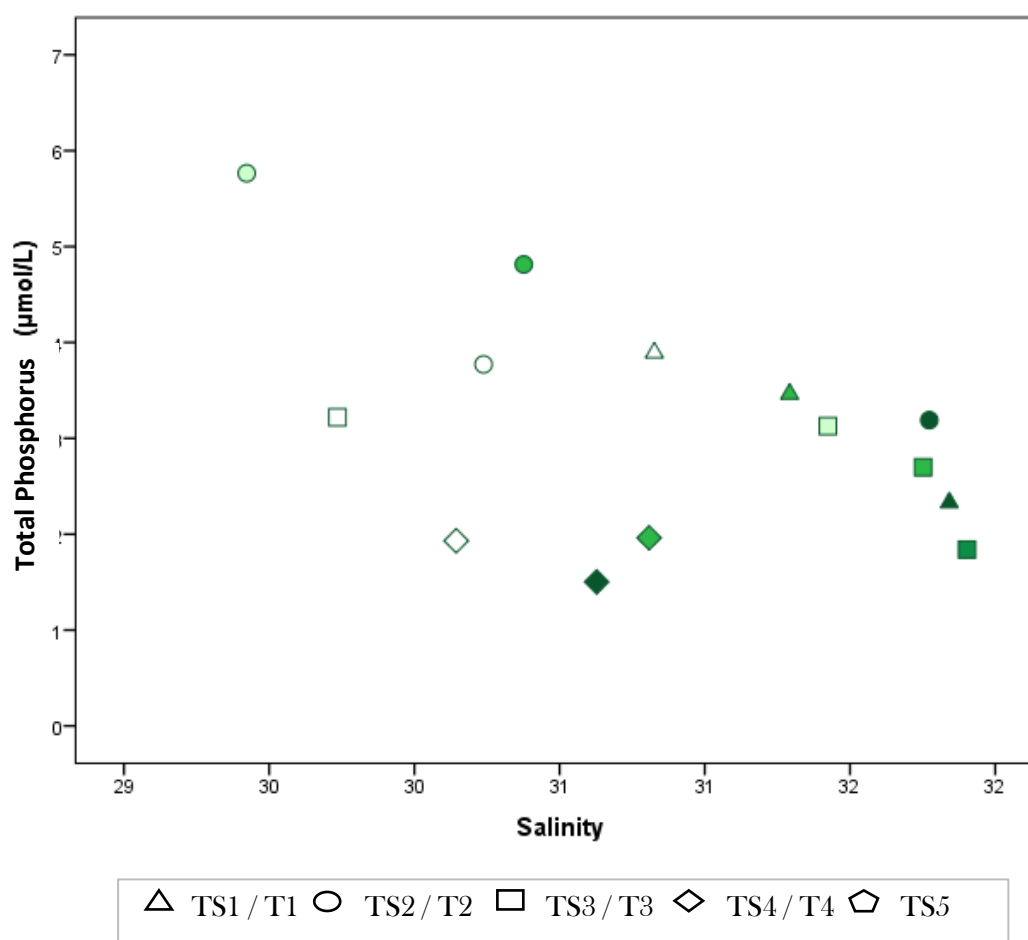
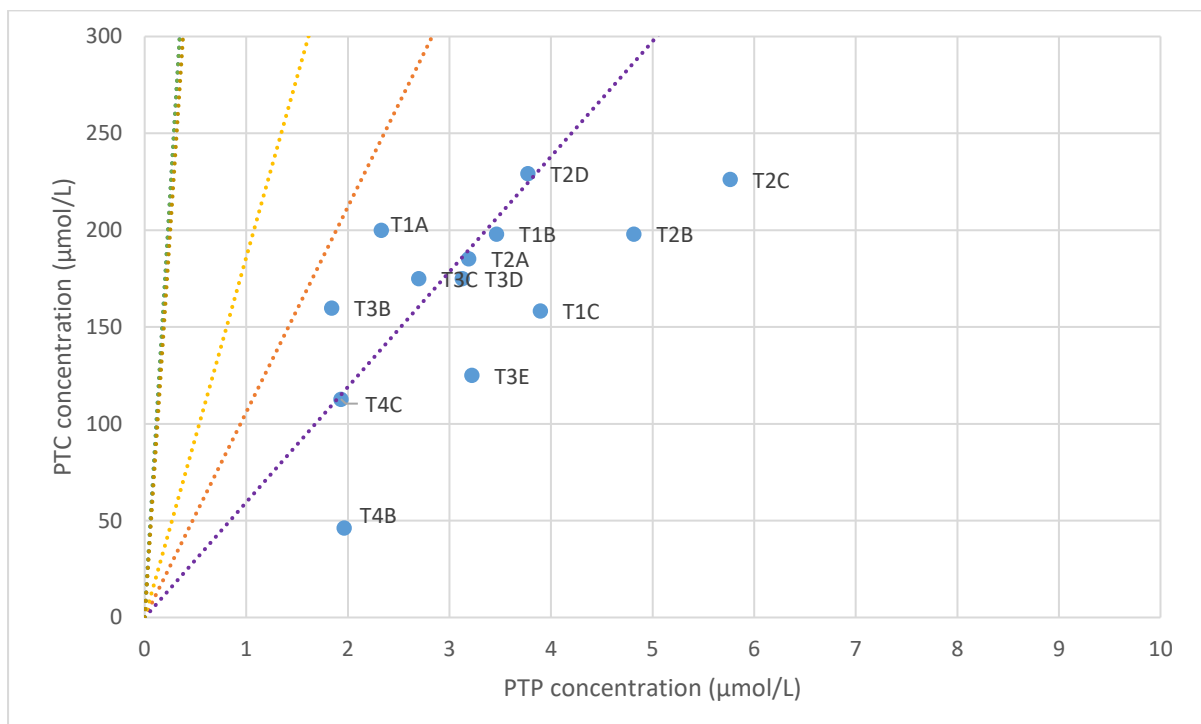
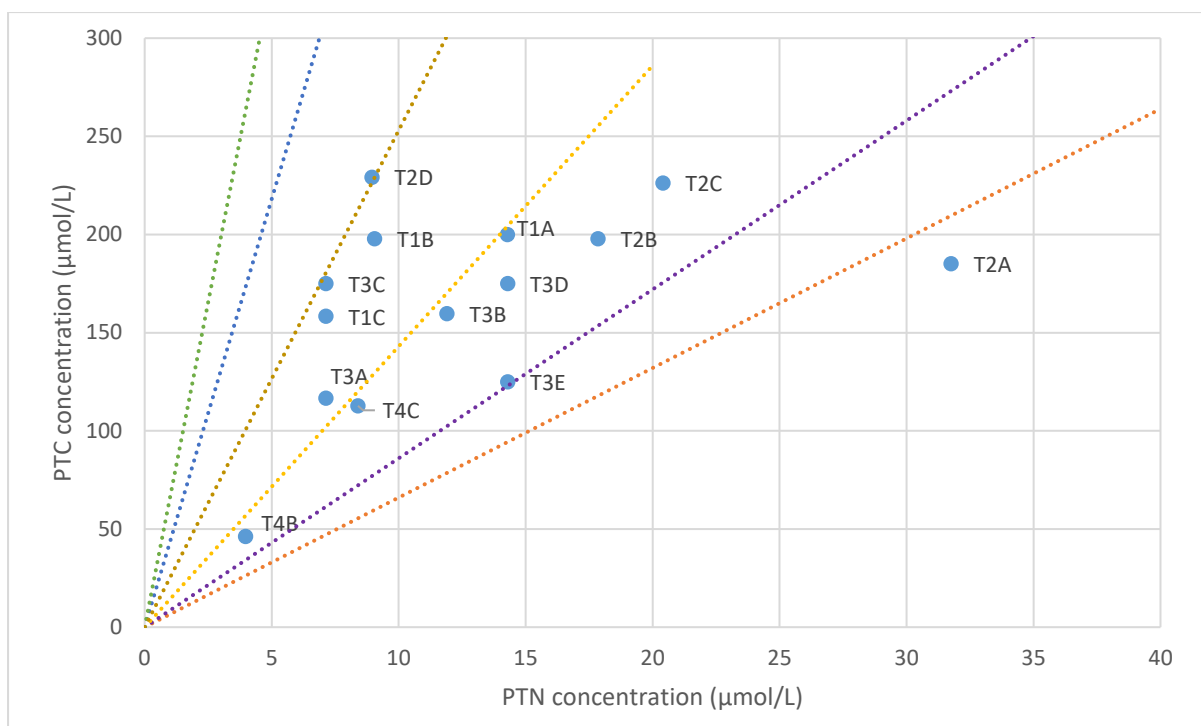


Figure 3.8: Nutrient concentrations normalised against salinity to establish whether simple mixing is responsible for the concentrations within the Conwy estuary. Green symbols represent the cross section survey, with open symbols reflecting east and closed symbols west of the estuary with colour gradient between the two end members. Black closed symbols = high water survey, Open symbols = low water survey. Blue dotted line represents conservative mixing line.

CN, CP, and NP ratios were calculated using the particulate data (Figure 3.9 A-C) to the Conwy estuary. Known marine (Hecky et al. 1993) and terrestrial (Cleveland and Liptzin 2007) elemental ratios have been superimposed onto Figure 3.9 to identify the origins of the organic matter. It is evident that as with the previous surveys no single source is contributing to the observed C:N, C:P or N:P trends but samples represent different relative proportions of potential sources across the sites.



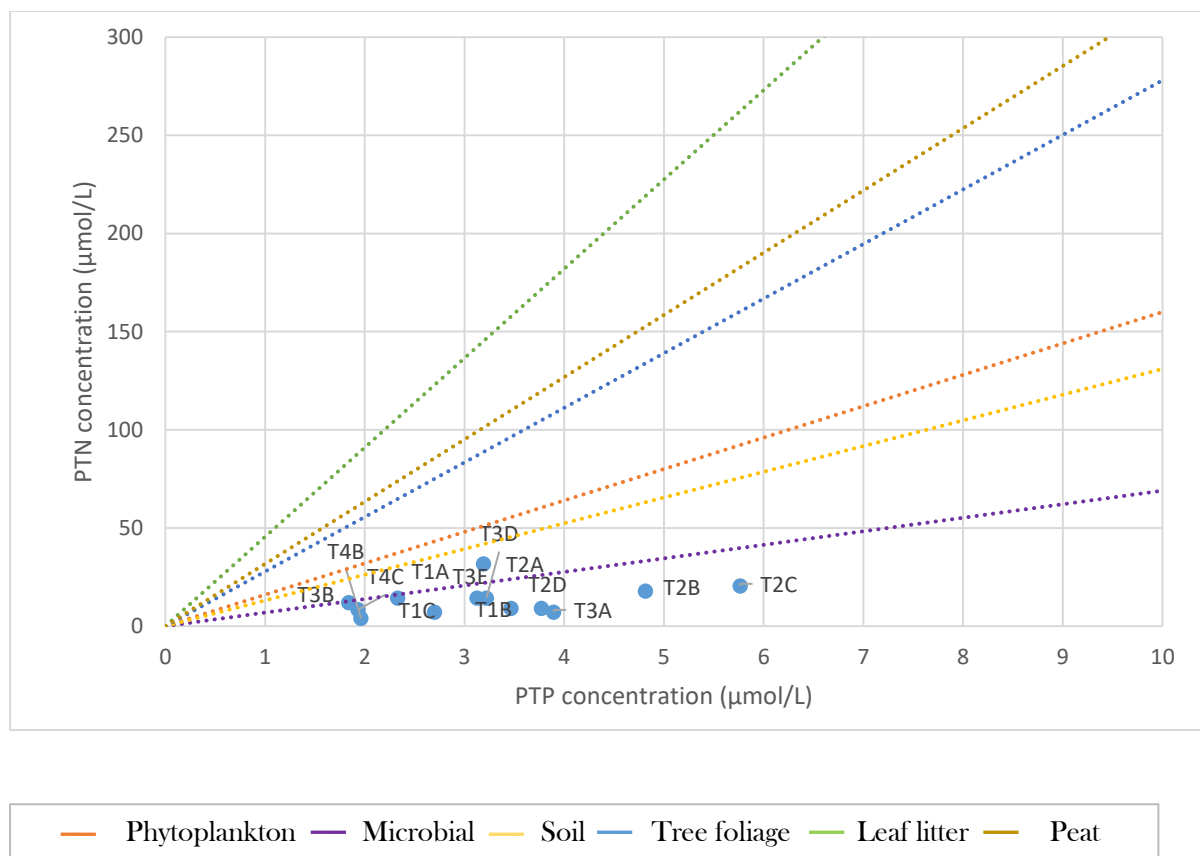


Figure 3.9: Comparison of PTC, PTN and PTP concentrations against known biomass ratios to establish the origin of the organic matter within the Conwy estuary from east side to west side.

3.3.1.4 Longitudinal Survey

A longitudinal survey enabled an understanding of whether nutrient concentrations differed spatially throughout the whole estuary, from the tidal limit to the sea inlet. Data was compared to the tidal and cross section surveys to understand whether temporal differences existed at the same sites. Concentrations were evaluated to establish whether due to simple mixing within the estuary or potentially elevated due to other factors. The origins of the organic matter were investigated to establish whether from natural or potentially human impacted sources. Concentrations of dissolved (Table 3.7) and particulate (Table 3.8) nutrients were taken at 20 sites within a longitudinal transect throughout the entire estuary up to the tidal limit, these are presented in Appendix 2. Sample sites 1 (TN, TC, ON & OC) and 2 (TP) were extreme outliers and therefore no values were calculated for these sites. Within the remaining data set PO_4^{3-} at sites 13, 15, 16 and 20 all had concentrations below the limit of quantification and therefore due

to the unreliability were removed from further analysis. Descriptive statistics were undertaken on the remaining raw data, Table 3.5 summaries these results, which include mean concentration, standard deviation and range.

Table 3.5: Range of concentrations of the investigated dissolved and particulate nutrients ($\mu\text{mol/L}$), N is the number of samples and LOQ is the limit of quantification.

Sample descriptive statistics							
	N	Min	Max	LOQ	% <LOQ	Mean	Std.Dev
PO ₄	16	0.32	0.52	0.311	20%	0.42	0.06
NO ₂	20	0.15	0.8	0.024	0	0.34	0.14
NO ₃	20	3.16	38.75	0.057	0	14.51	9.07
NH ₄	20	1.32	19.58	0.078	0	7.22	5.8
TC	19	55.55	1111.08	3.081	0	322.49	294.45
OC	19	55.55	1027.75	3.081	0	284.53	265.55
TN	19	2.38	47.62	0.357	0	13.87	12.7
ON	19	2.45	55.55	0.357	0	15.64	12.29
TP	19	0.42	19.87	0.03	0	4.97	5.02
Coliforms	20	3.33	24700			5558	7488.35

As with the previous surveys, TC and TN contents of the suspended particulate matter were found to consist of insignificant amounts of inorganic carbon and no significant amount of nitrogen absorbed as ammonium (Figure 3.10). Therefore ON and OC are omitted from further analysis and reported as TN and TC.

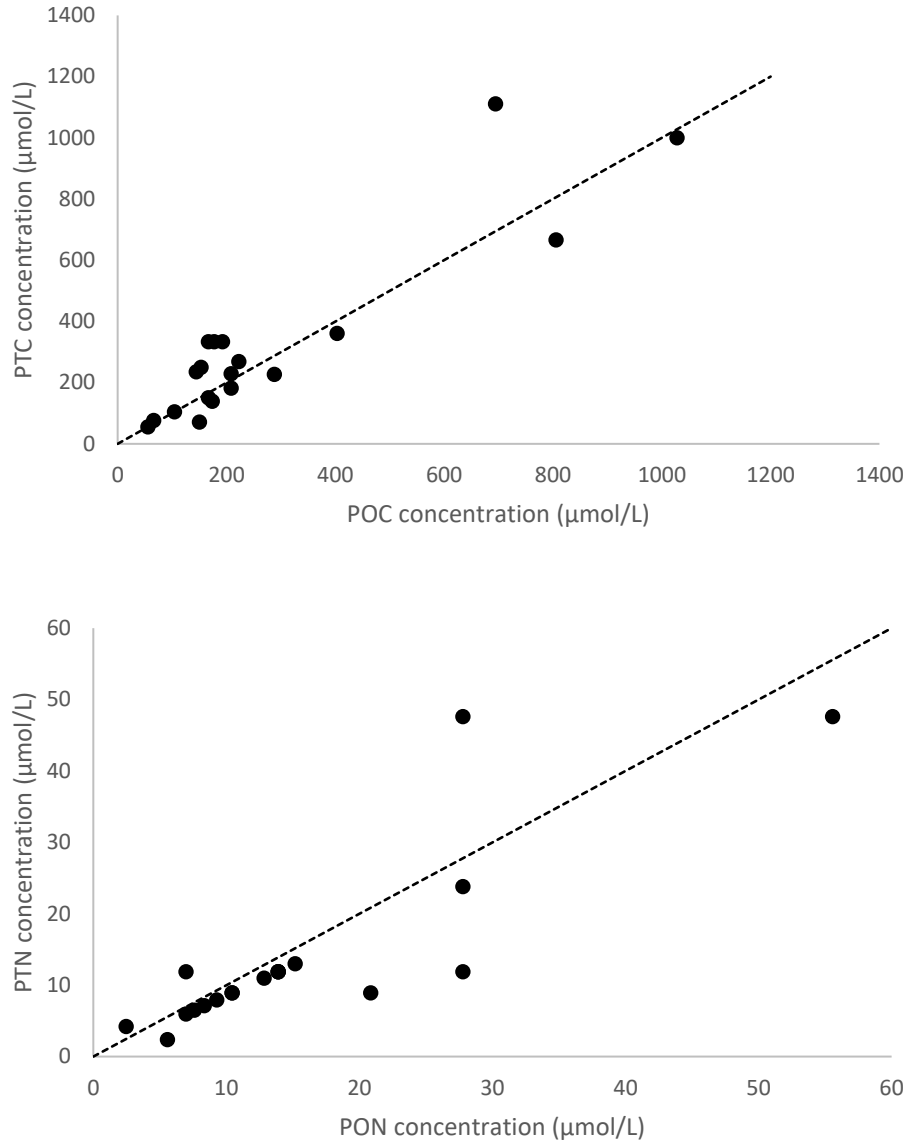
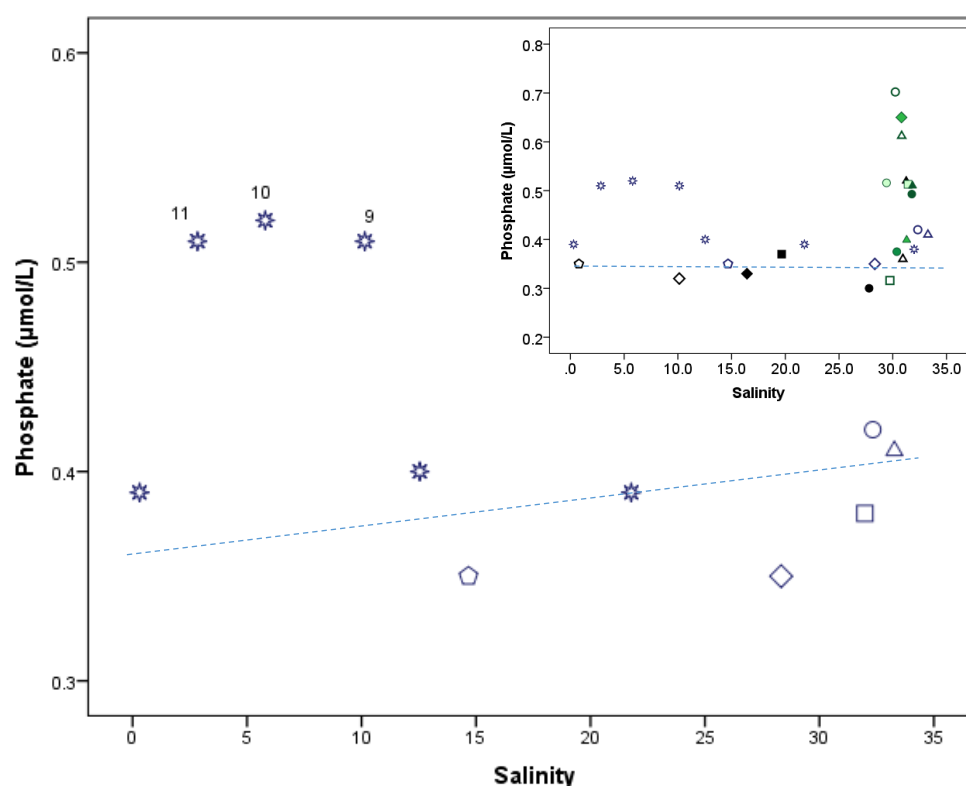
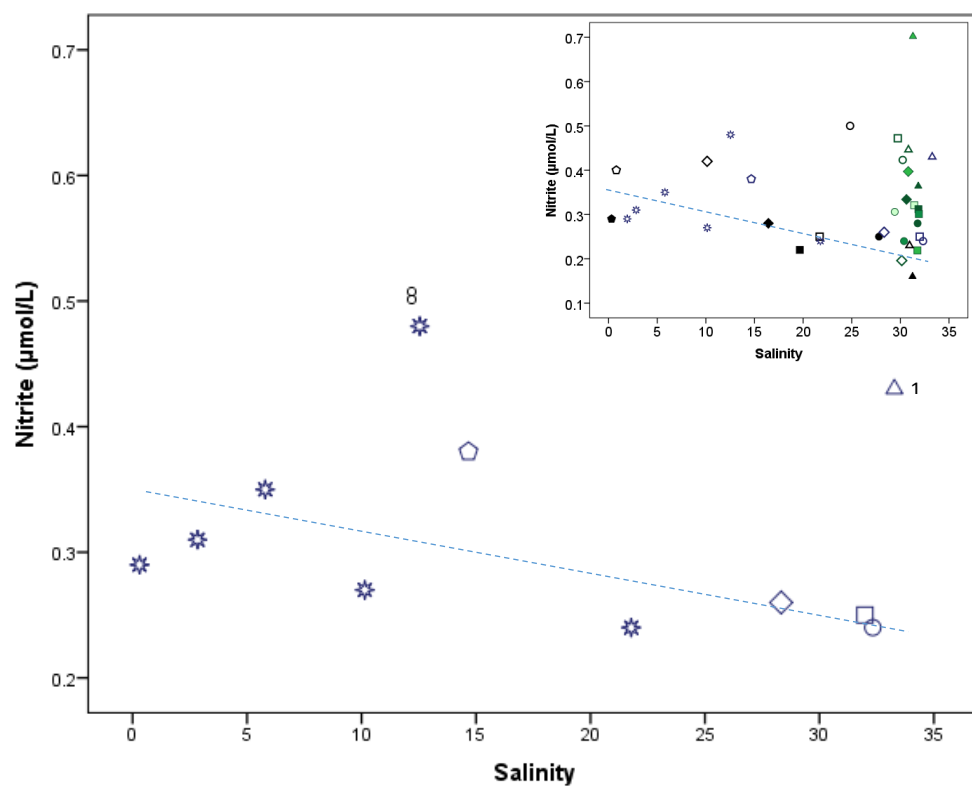
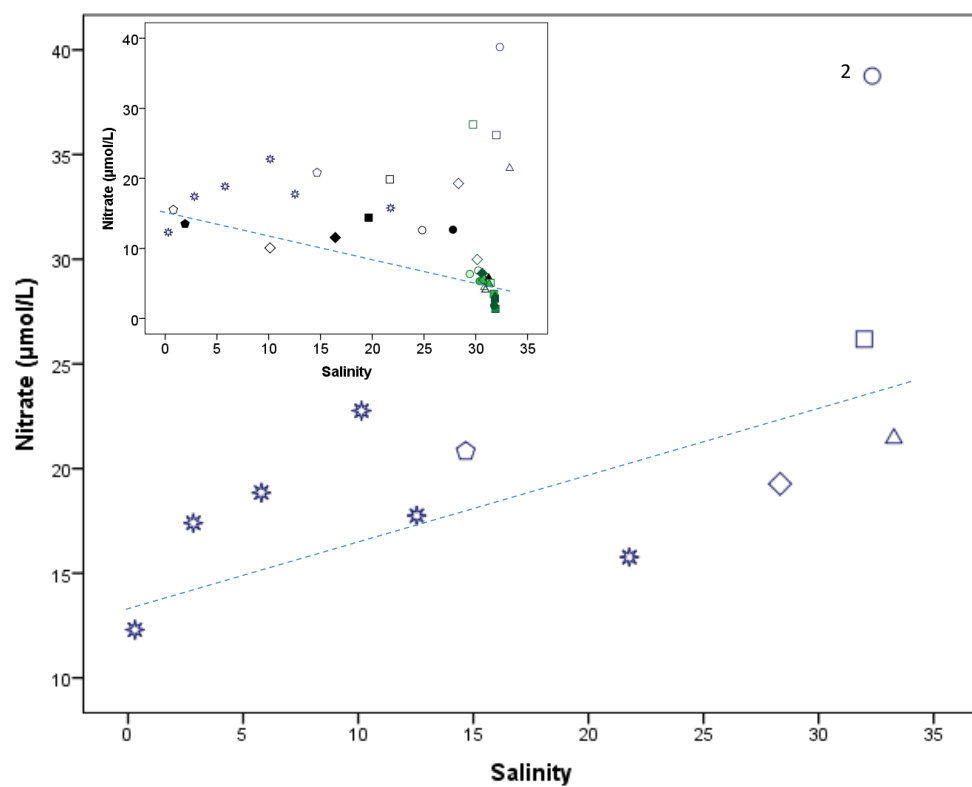


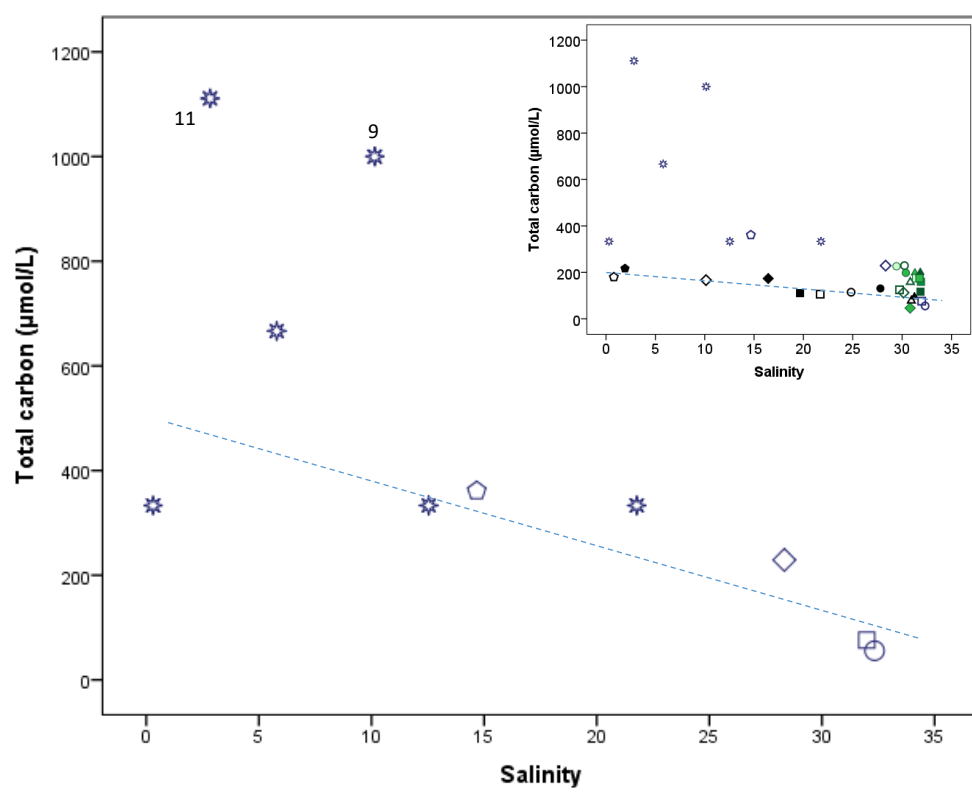
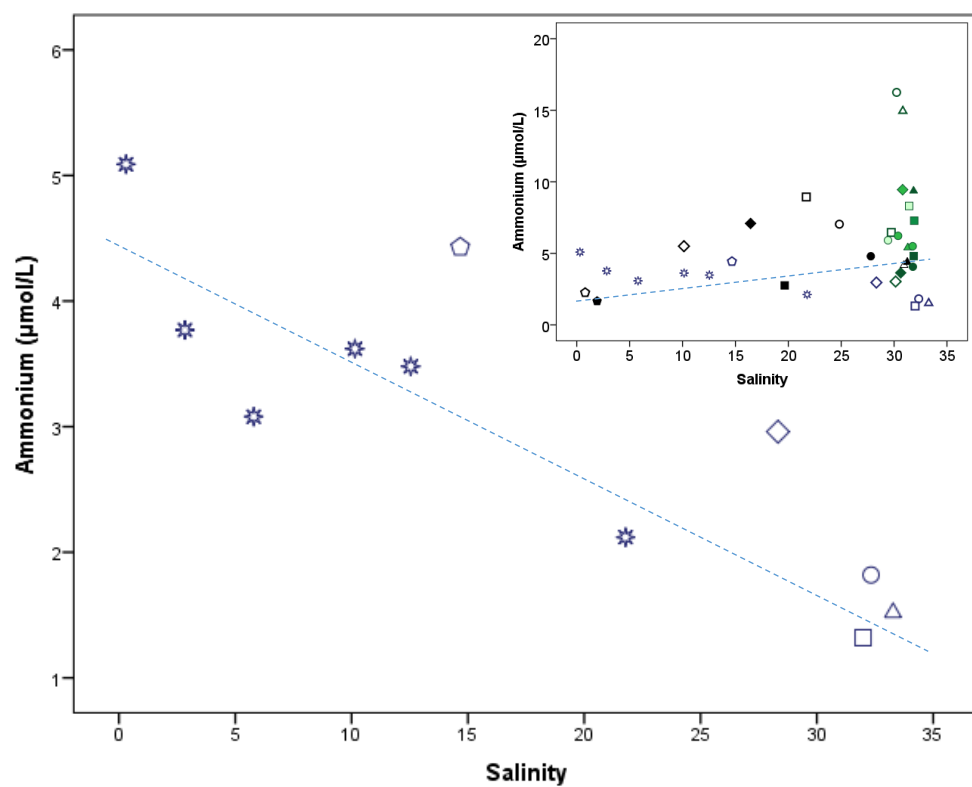
Figure 3.10: Comparison between organic and total species of particulate carbon and nitrogen to establish whether concentrations differ within the Conwy estuary. Black dotted line presents 1:1 gradient.

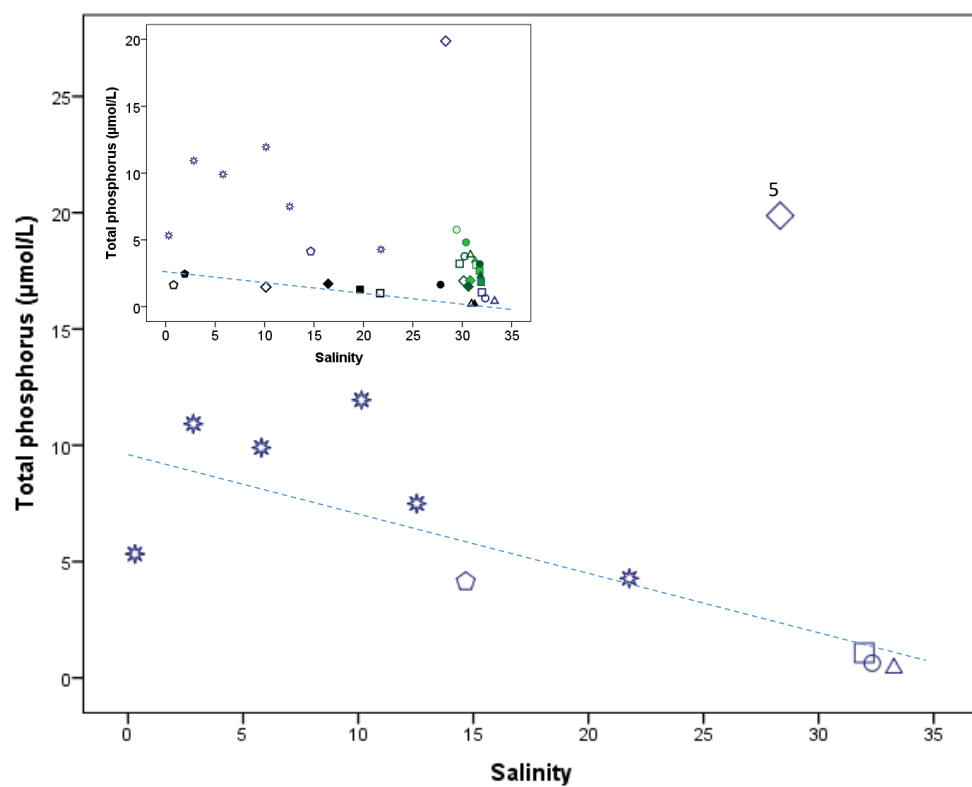
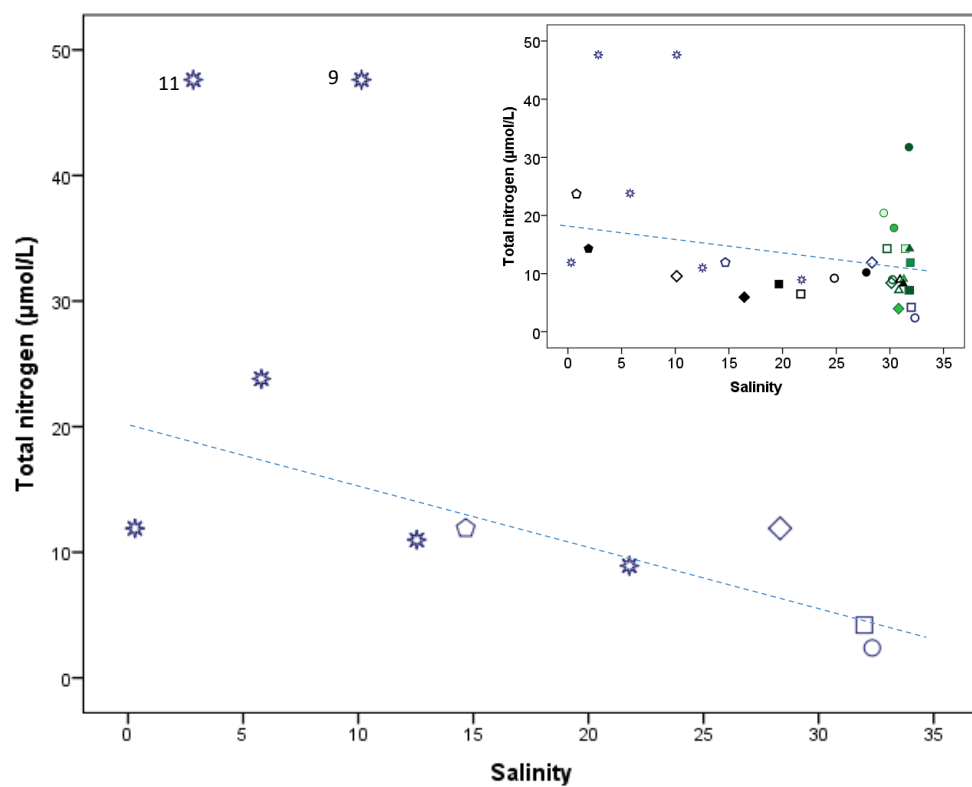
As with the previous surveys, the dissolved and particulate data was normalised against salinity to establish whether the concentrations were due to simple mixing or potentially elevated. PO_4^{3-} concentrations mainly behaved conservatively with an increase in concentration within the higher salinity. This differs from the non-conservative behaviour seen in the winter surveys (tidal and cross section survey Figure 3.11A insert). Sites 9-11 are evidently showing non conservative behaviour and potentially elevated compared to other surveys, although these salinities have not been represented previously. NO_3^- concentrations are behaving conservatively with lower

concentrations within the terrestrial end and increasing towards the higher salinity marine end. This behaviour is not reflective of the previous surveys (tidal and cross section survey figure 3.11b insert) where concentrations were highest within the terrestrial freshwater end member and reduced with increasing salinity. The majority of samples exhibit higher concentrations than previous surveys (Figure 3.11b insert) and sample 2 is showing non conservative behaviour and is potentially an elevated site. NO_2^- , NH_4^+ , TP, TC and TN are all showing conservative behaviour, with the exception NH_4^+ concentrations are higher in the freshwater end member and decreasing to lower concentrations with increasing salinity, which reflects the tidal survey (winter). Whereas NH_4^+ exhibits the opposite trend within the winter survey (tidal survey) with higher concentrations within the higher salinities. NH_4^+ , NO_2^- and TN have similar concentrations within the summer and winter surveys, whereas TC, TP and Coliforms show to have higher concentrations within the summer survey (Figure 3.11) compared to winter. Again the cross section data is not reflecting the concentrations from this survey. Samples which indicate an elevated concentration within this survey include 1 & 8 (NO_2^-), 5 (TP), 9 & 11 (TN & TC), 2 & 7 (coliforms).









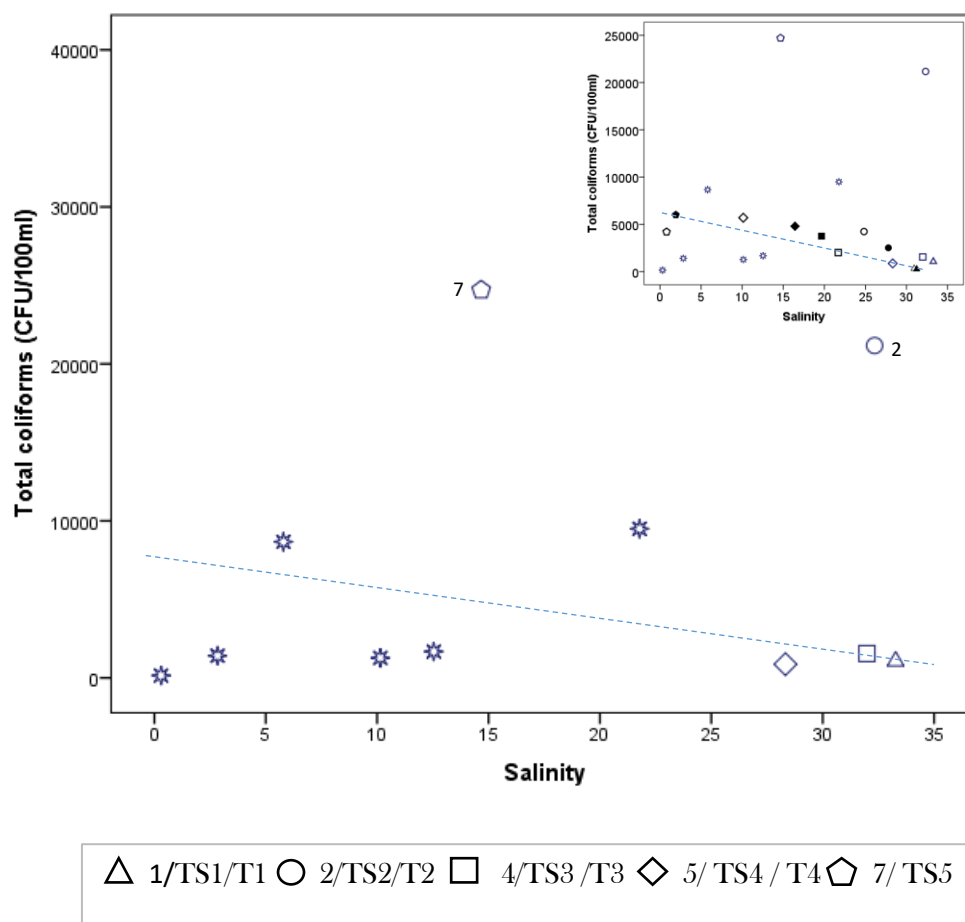
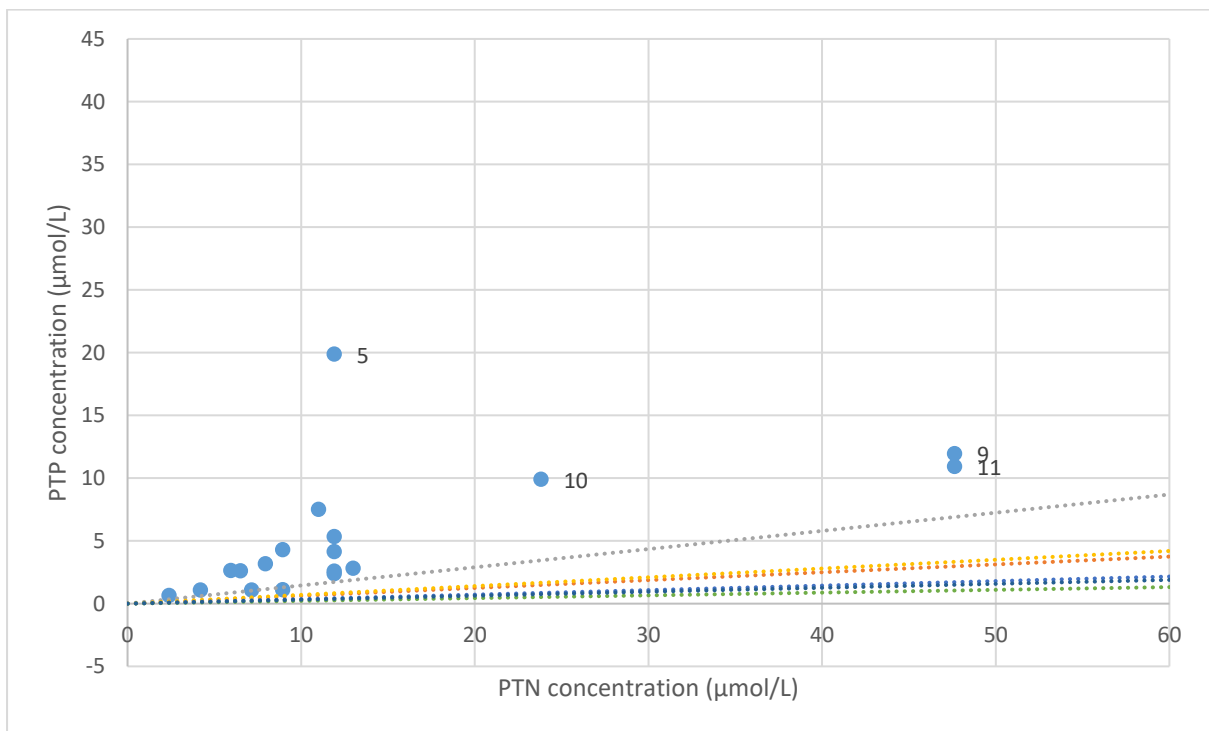
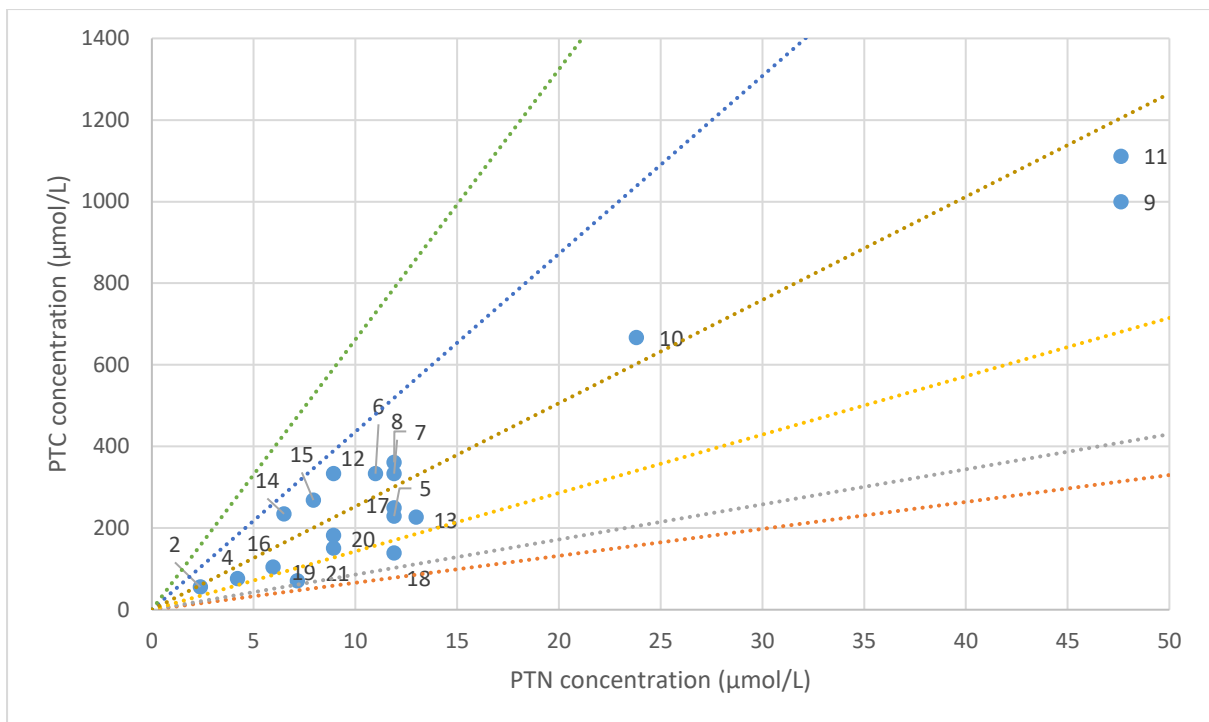


Figure 3.11: Nutrient concentrations normalised against salinity to establish whether simple mixing is responsible for the concentrations within the Conwy estuary. Blue symbols represent the transect survey (summer). Insert identifies all surveys together with green symbols representing the cross section survey (winter), with open symbols reflecting East and closed symbols west of the estuary with colour gradient between the two end members. Black closed symbols = high water survey (winter), Open symbols = low water survey (winter).

CN, CP, and NP ratios were calculated using the particulate data (Figure 3.12 A-C) to the Conwy estuary. Known marine (Hecky et al. 1993) and terrestrial (Cleveland and Liptzin 2007) elemental ratios have been again superimposed onto Figure 3.12 to identify the origins of the organic matter. It is evident that as with the previous surveys no single source is contributing to the observed C:N, C:P or N:P trends but samples represent different relative proportions of potential sources across the sites.



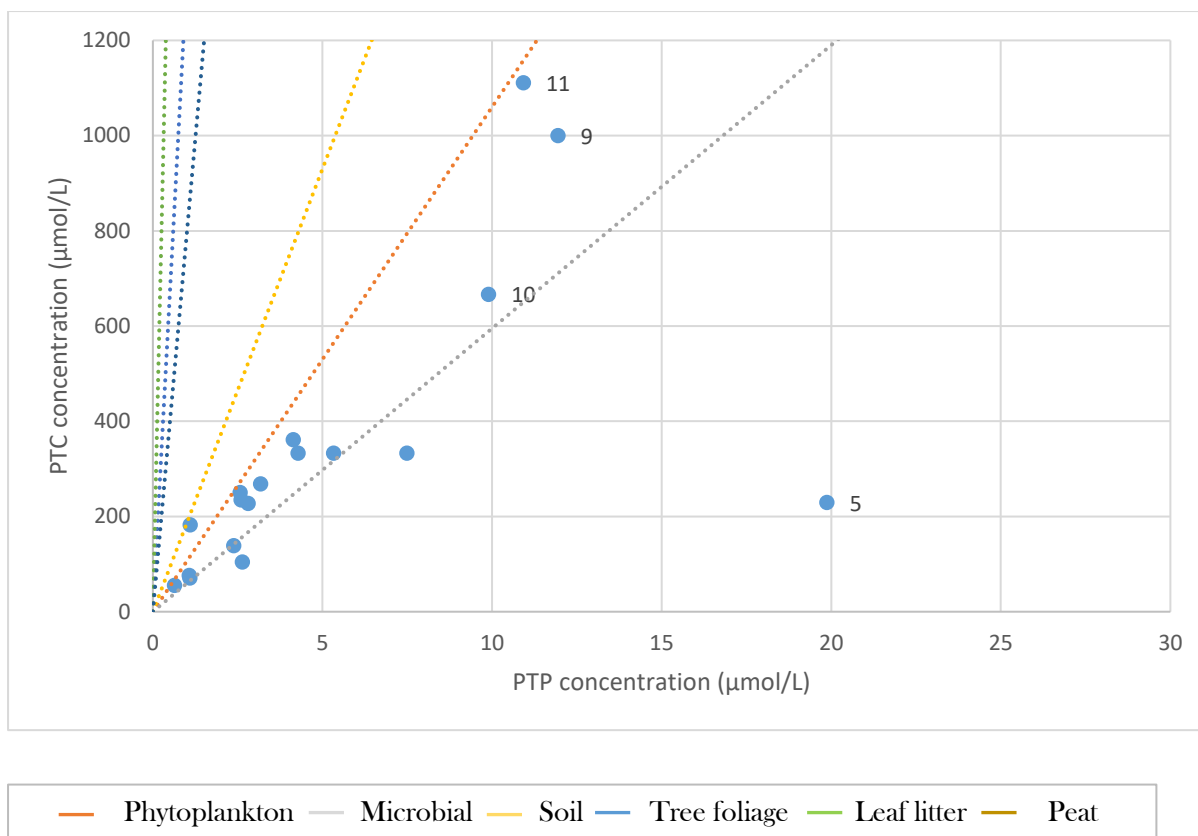


Figure 3.12: Comparison of PTC, PTN and PTP concentrations against known biomass ratios to establish the origin of the organic matter throughout the Conwy estuary from the tidal limit to the sea inlet.

3.3.2 Overall dissolved and particulate spatial patterns and identification of elevated levels within the Conwy Estuary.

Nutrient over-enrichment of estuaries has been well documented as a significant problem (Nixon 1995, Jickells 1998, Priestley 2015) and the importance of how far a particular system has deviated from natural conditions. Within heavily impacted coastal areas with no prior baseline data, relatively uncontaminated estuaries could be used as a proxy measure for these background conditions. Conwy estuary has been proposed as a potential candidate for a relatively uncontaminated estuary within the UK, in order to establish undisturbed conditions it is necessary to distinguish natural condition from anthropogenic inputs. It is well-established that the simple mixing curve approach enables a differentiation to be made between conservative natural mixing within the estuary and evidence of sources and sinks (Raine and Williams 2000). It is evident that there is a general trend within the Conwy estuary of conservative behaviour with some isolated elevated sites, for dissolved nitrate, nitrite, ammonium, particulate total carbon, total nitrogen, total phosphorus and coliforms. However, phosphate concentrations show non-conservative behaviour. This is in agreement with both Raine & Williams (2000) and Roach (2003) studies undertaken within the Conwy estuary and suggests that as was reported by Rehm (1985) and Shengquan et al (1993), inorganic phosphate behaviour is primarily controlled by non-linear desorption/adsorption particle-water processes rather than the simple dilution of river water. Concentrations of nitrate were shown to be higher and ammonium concentrations lower within the riverine (terrestrial) end member during the winter surveys (tidal and cross section) compared to higher nitrate concentrations with lower ammonium concentrations within the marine end member during the summer. In catchments of impervious bed rock as seen with the Conwy catchment, seasonal variations of nitrate concentrations are characteristic, with high subsurface flow during periods of high rainfall with high nitrate concentrations and active denitrification during low water periods explains the high stream water nitrate concentrations in winter than in summer (Martin et al. 2004). Supporting this Pomeroy & Deibel (1986), Pomeroy & Wiebe (2001) and Wilhelm et al. (2014) all established that within marine systems bacterial production during colder winter months is disconnected from primary production and that temperature was the primary cause. This behavioural mechanisms will explain the high ammonium in comparison to nitrates within the marine sector during the winter months. The source of the Conwy originates within the Migneint moors, which is an extensive area of blanket mire with high rainfall and high dissolved organic carbon (Emmett et al. 2016). Within these

acidic soils aerobic conditions are low and therefore could favour denitrification (Hutchin et al. 1996) instead of nitrification resulting in high concentrations of ammonium compared to nitrates. Due to the impermeable bedrock peat soils are saturated with rainwater which potentially increases the amount of land runoff. Robins et al. (2014) inferred that 73% of the freshwater input to the Conwy estuary came from the Conwy river, where Emmett et al. (2016) found high concentrations of dissolved organic carbon in drainage of the Migneint peat area and within the south east of the peaty gleys which feed into the Conwy river. This supports that the possible influence of ammonium within the upper estuary maybe influenced by the peat runoff within the river Conwy and is a natural phenomenon not anthropogenic. Within the summer, low concentrations of ammonium in the marine end member and high concentrations of nitrate are likely to be due to nitrification within the saline waters. Williams et al. (2012) found that land use had a significant effect on the number of heterotrophic bacteria with far greater numbers in the tidal saline waters compared to freshwater influences ($1,240 \pm 102$ & 605 ± 79 MPN ml⁻¹, respectively) within the Conwy estuary.

Concentrations of particulate carbon, nitrogen, phosphorous and coliforms all exhibited conservative behaviour with higher concentrations within the terrestrial end member and reduced in concentration with increasing salinity for both summer and winter surveys. This anticipated conservative behaviour is consistent with other estuaries where intermediate-salinity waters produced by the mixing of low nutrient, high salinity seawater with high nutrient river freshwaters (Neal et al. 1997, Robson and Neal 1997) and therefore constitutes a dilution affect. Significant strong correlations were found between particulate total carbon with both particulate phosphorous and nitrogen during the summer survey whereas within winter only particulate phosphorous and carbon had a strong significant relationship. This behaviour is likely to be due to within the summer survey the major influence will have been from land runoff due to a flood event after a long period of dry weather within the territorial end of the estuary. Supporting this, within the terrestrial end of the estuary, inflows exhibited higher nutrient concentrations within the rivers compared to the inflows entering the marine section. In comparison within the winter survey contributions from inflows within the marine sector were significantly higher for nitrates in comparison in summer and therefore the lack of relationship between total carbon and total nitrogen is likely to be due to total nitrogen originating from the dissolved fraction of the inflows rather than adsorbed within sediments from the terrestrial end of the estuary. The strong relationship with total carbon and total phosphorus for both summer and winter will be due to the preferential state of phosphorous absorbed on iron oxides, aluminium hydroxides, clay

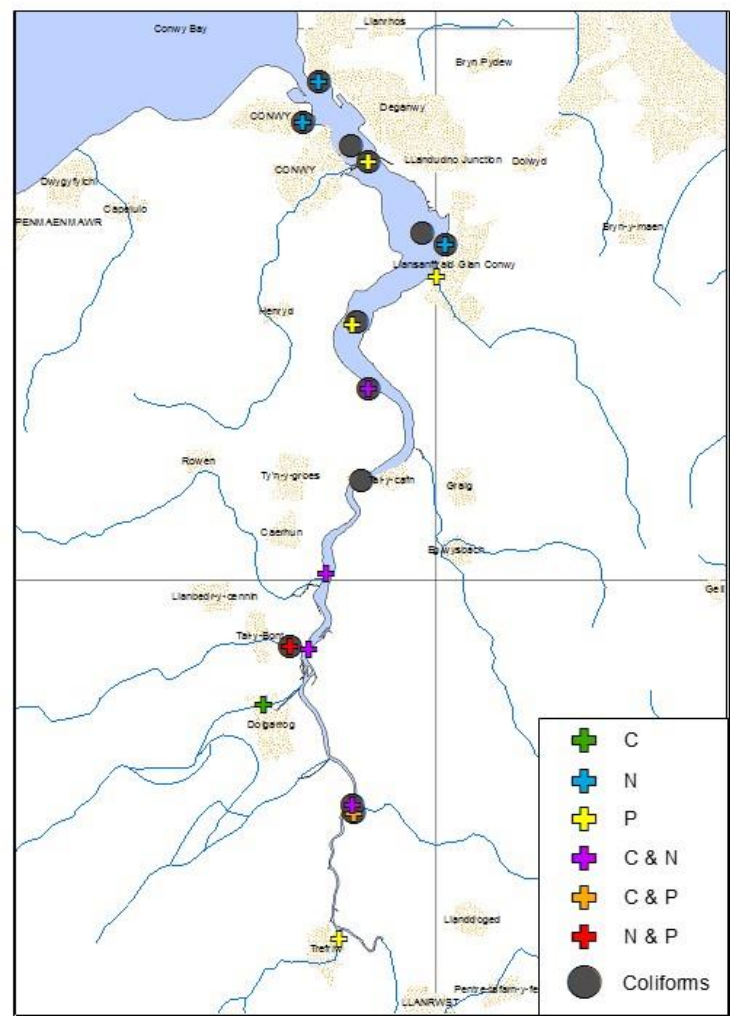
surfaces and organic matter particles and becomes incorporated within the suspended sediment (Borggaard et al. 1990). Within the elemental ratios it was evident that there was no single source contributing to all the observed ratio trends but samples presented different relative proportions of potential sources across the sites. The carbon nitrogen ratio highlighted peat and soil to be the dominant origin for the majority of sites. This is consistent with a river dominated estuary, with limited human impacts, with a significant input from blanket mire peat moorlands supporting the findings of Emmett et al. (2016).

Within this study it is evident that there is as expected spatial variation of nutrients within the marine section and terrestrial end member. Concentrations differed temporally at sites one to five, although no significant differences were shown between high and low water, differences were evident within sites over different seasons (winter and summer) due to different biological mechanisms working within summer and winter. These differences in concentrations within summer and winter were presumably due to negligible biological activity in the rivers and the release of stored terrestrial nutrients, following the previous growing season and autumn die-back (Uncles, Frickers and Harris 2003). In addition concentrations were not uniform across the estuary and concentrations were shown to be higher within areas of intertidal mudflats. Significant differences were established for nitrate and phosphates from the west bank compared to the east bank, with the later showing higher concentrations where intertidal mudflats were present (Figure 2.3). Reddy et al. (1996) found that fine sediment resuspension triggers release of nutrients trapped within the sediment pore water and that high organic matter laden sediments are more prone to resuspension (Lovstedt and Bengtsson 2008). The findings within this study confirms the study of Quilliam et al. (2011) who identified spatial variations in waterborne *E.coli* numbers on contrasting sides of the four transects taken at the same time and locations as this study. In addition Perkins et al. (2014) found higher concentrations of *E.coli* within the sediments adjacent to the east bank and waterborne *E.coli* increased within this area suggesting resuspension as a possible cause.

Sporadic elevated nutrient concentrations were found within the Conwy estuary and likely to be linked to either freshwater inflows or resuspension from intertidal mud flats. During the winter survey elevated dissolved concentrations within the estuary were related to the localised inflows, however the particulate concentrations are consistent with dilution from terrestrial to marine and high values are likely to be due to resuspension of sediments within intertidal mud flats. Within the summer survey which occurred directly after a flood event following a long period of dry weather, the particulate phase within the estuary is related to the inflows and likely runoff from

saturated peatlands and therefore sediment driven. Whereas the dissolved fraction within the elevated sites are not reflecting inflow concentrations.

Comparing the Conwy estuary to the Tweed estuary, which is located on the border of England and Scotland, and characterised as an estuary of high water quality with the same residence times as the Conwy estuary of typically less than one day (Uncles and Stephens 1996) allows us to establish whether the Conwy can be used as a good example of a high water quality estuary and used as proxy measure for baseline conditions for contaminated catchments. In comparison the maximum nitrate and phosphate concentrations within the Conwy estuary were 38.7 and 0.7 $\mu\text{mol/L}$, respectively, compared to 370 and 4 $\mu\text{mol/L}$, respectively, within the Tweed estuary. Indicating that within a very similar estuarine system the Conwy yielded considerably lower nutrient concentrations. In addition, Nedwell et al. (2002) study identified the annual loads of nutrients to all the 93 estuaries on the mainland of the united Kingdom and identified that Conwy estuary exhibited the 8th lowest concentration for Nitrate, Nitrite and Ammonium and the lowest phosphate concentrations. This supports the case for the Conwy estuary to be used as a proxy for baseline concentrations for estuaries which are highly contaminated with no baseline values. Within this study it was evident that there was temporal and spatial differences of nutrient concentrations within an estuary, locations on adjacent banks, within intertidal mudflats and seasonality will have implications for monitoring on an environmental management level and these variations need to be built into all monitoring programmes of estuaries.



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3.3.3 Spatial distribution and relationship of TP, TOC and TN in surface sediments

As discussed in chapter 2 sample sites throughout the Conwy Estuary, are composed of a variation of sediment types with sand and mud being the dominant grain size. Conwy Bay and the middle of the widest section of the estuary is dominated with sand whilst the highest proportion of fines are located near the marinas, Glan Conwy and the upper parts of the estuary whilst the remaining areas resulting in a combination of sandy muds.

Total concentrations of carbon (TC), organic carbon (OC), nitrogen (TN), organic nitrogen (ON) and Phosphorus in the surface sediments for each site of the Conwy Estuary are presented in Table 3.9, Appendix 2. Sample sites 1 (OC & ON), 4 (TC, OC, TN & ON), 6 (TN & ON) 39 (OC) and 3, 5, 6, 7, 9, 10, 14, 15, 17, 20, 23-26, 30, 35, 37, 38, 40, 48 (ON) were lower than the limit of quantification and therefore omitted from further analysis. The remaining data set showed distinct variation of all nutrients throughout the Conwy Estuary.

The TC content in the sediments ranged from 8.32 to 2730.83 $\mu\text{mol g}^{-1}$ (Figure 3.12) with a mean for all sites of 1203 $\mu\text{mol g}^{-1}$. The organic carbon content of these sites ranged from 8.32 to 2164 $\mu\text{mol g}^{-1}$ with an overall average of 684 $\mu\text{mol g}^{-1}$. All nutrient concentrations were converted to proportions to enable patterns to be seen within the dataset (Figure 3.13 & 3.14). The sites with the highest values were found within sectors 3 and 4 (Figure 1.1 & 1.2) and sectors 5 and 6 (Figure 1.1 & 1.2) showed consistently higher concentrations than other sectors. The general trend throughout the estuary was an increase of TOC from Conwy Bay to the upper reaches of the estuary near the tidal limit (Figure 3.13 & 3.14).

Total Nitrogen ranged from 14.28 to 164.20 $\mu\text{mol g}^{-1}$ with an average of 66.68 $\mu\text{mol g}^{-1}$. The lowest concentrations were evident within the Conwy Bay (sector 1) and the mid sections (figure x) which contained coarse sediment sites of sector 2 and 3 which also had the lowest TOC value. The highest concentrations of TN were found within the upper estuary of Trefew and Tal-Y-Cafn (sector 5&6) and sites with high fine-grained sediment content, similar to the pattern observed for TOC (Figure 3.13 & 3.14). Organic nitrogen ranged from 7.14 to 162.04 $\mu\text{mol g}^{-1}$ with an average of 86.21 $\mu\text{mol g}^{-1}$. It was evident that within the coarse grained (sand) sediment concentrations of ON were below the limit of quantification.

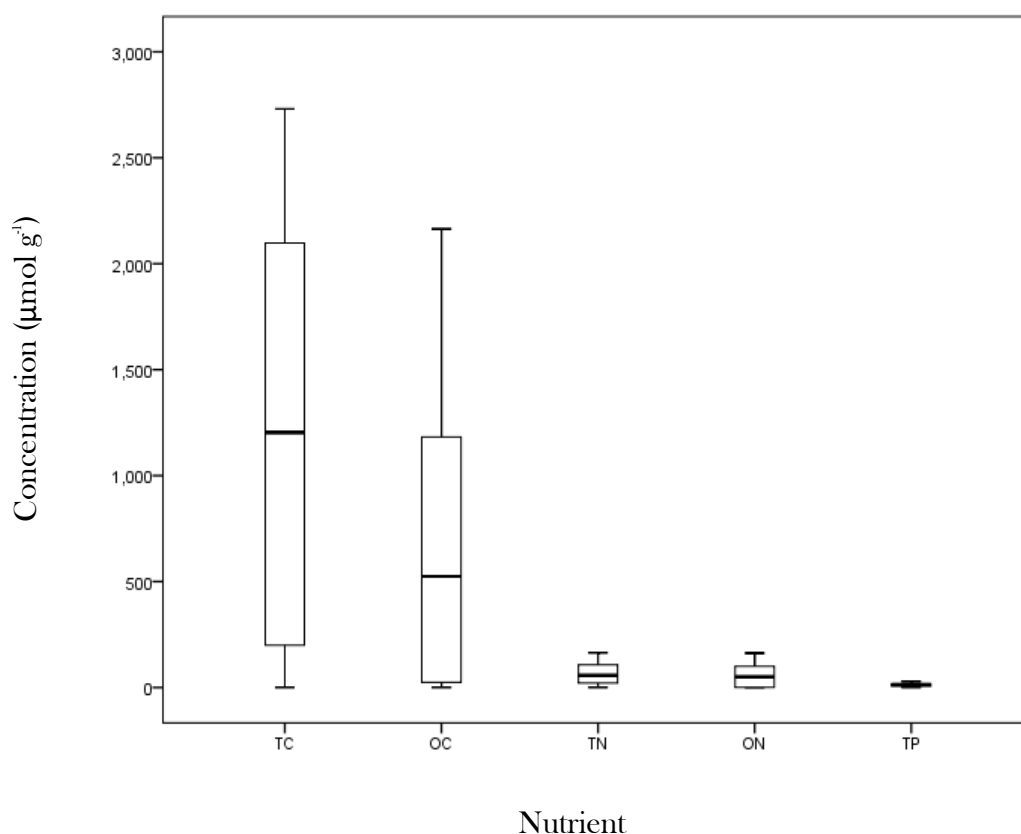


Figure 3.12: Box plot of the average concentration of total carbon (TC), organic carbon (OC), total nitrogen (TN), organic nitrogen (ON) and Phosphate (TP) for all sites in the Conwy Estuary. Horizontal bar refers to the median value for each element and error bars show the extreme ranges. Limit of detection and quantification for Carbon, Nitrogen and Phosphorus were 0.015 & 0.249, 0.071 & 0.238 and 0.077 & 0.255 $\mu\text{mol g}^{-1}$, respectively.

Total Phosphate concentrations showed the same pattern as described with TN. Higher concentrations of TP were associated with the same sites as were seen with high TOC. Low concentrations of TP were associated with coarse sediment as also demonstrated with low TOC sites (Figure 3.13 & 3.14). The concentrations of TP ranged from 3.78 to 27.75 $\mu\text{mol g}^{-1}$ with an average of 13.37 $\mu\text{mol g}^{-1}$.

C/N ratios were calculated and a difference was evident between samples with less than 10% mud concentration, which has been referred to as sand, (Figure 3.13), compared to samples with a high silt/clay content (Figure 3.14). The sand grouping which corresponds to Conwy Bay (sector 1) and the main channel within sectors 2 and 3 showed a very low C/N ratio ranging from 0.008 to 5.1 with an average of 1.8. Within all of these sites ON concentrations are below the level of

quantification and therefore the nitrogen is reflecting the inorganic species and likely to be ammonium absorption as this is the predominant inorganic nitrogen species within sediments (Simon and Kennedy 1987) . This results in an over estimation of TN and therefore unrealistic C/N ratios for the sand fraction of these samples. Whilst the silt/clay grouping corresponds to the mussel beds of sector 1, marina areas and mud flats of sector 2 and 3 and the majority of sector 4-6, C/N ratios were higher ranging from 8.0 to 38.9 with an average of 12.5. Higher plants organic matter have a lower nitrogen content and therefore higher C/N ratio. High C/N ratios within marine sediments indicate a terrigenous organic matter contribution (Burone et al. 2003). Sediment organic matter is mainly exogenous where ratios of C/N greater than 10, but mainly endogenous organic matter when ratios are less than 10. When the ratio is approximately 10 it reaches a balance between allochthonous sources and autochthonous sources (Zhang et al. 2015). The C/N ratios of this study indicates the majority of sites with exogenous origin and only a few of indogenous, therefore the Conwy estuary is a mix of both allochthonous and autochthonous sources within these sediments.

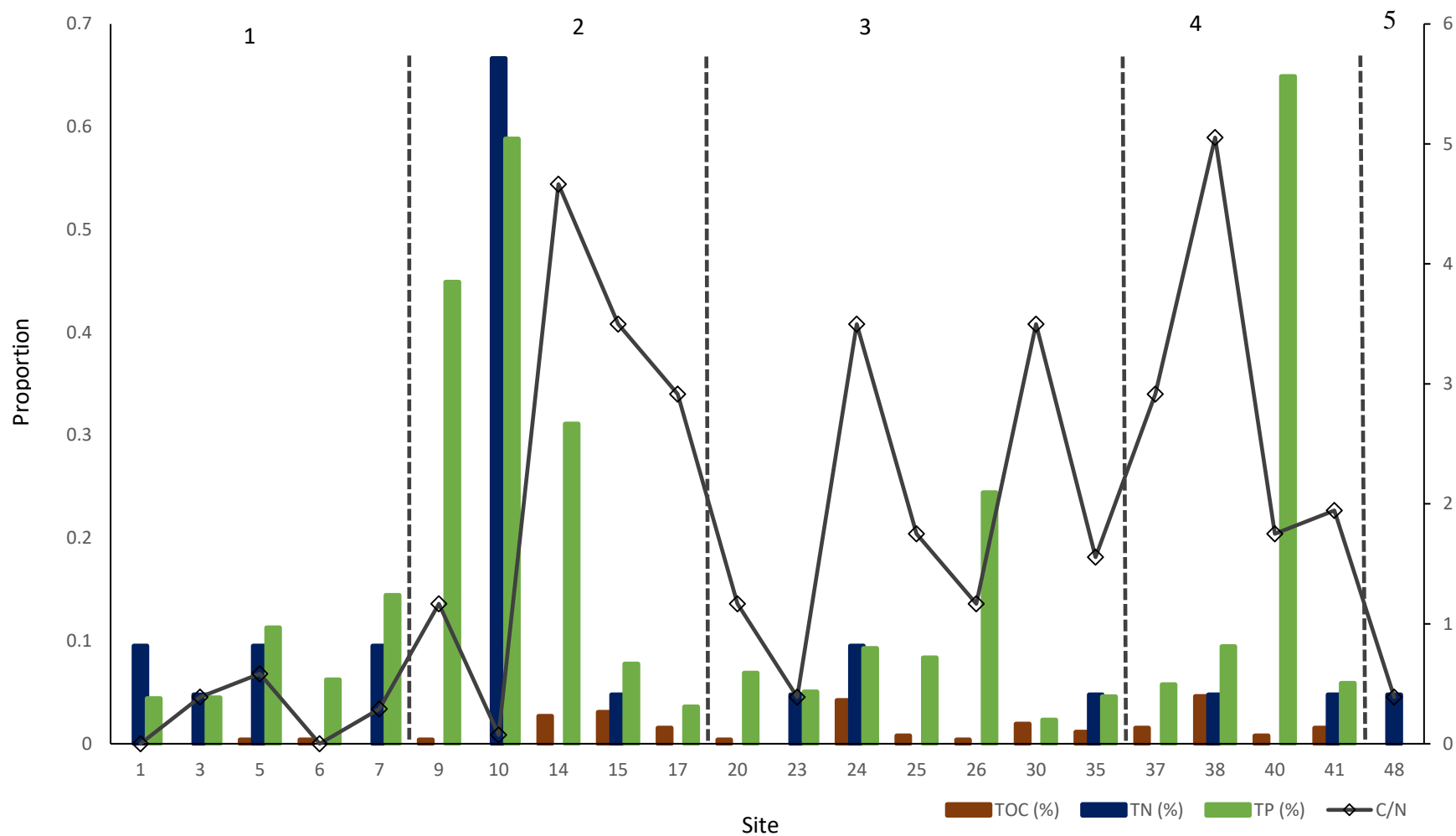


Figure 3.13: Proportions of particulate nutrients, total organic carbon (TOC), total nitrogen (TN), total phosphate (TP) and C/N ratio at sediment sites classified as sand from the mouth of the Conwy estuary to the tidal limit. Proportions represent 0 (lowest concentration) to 1 (highest concentration) for each nutrient. Sample sites refer to locations in Figure 1.1 & 1.2. 1-6 represent the sectors.

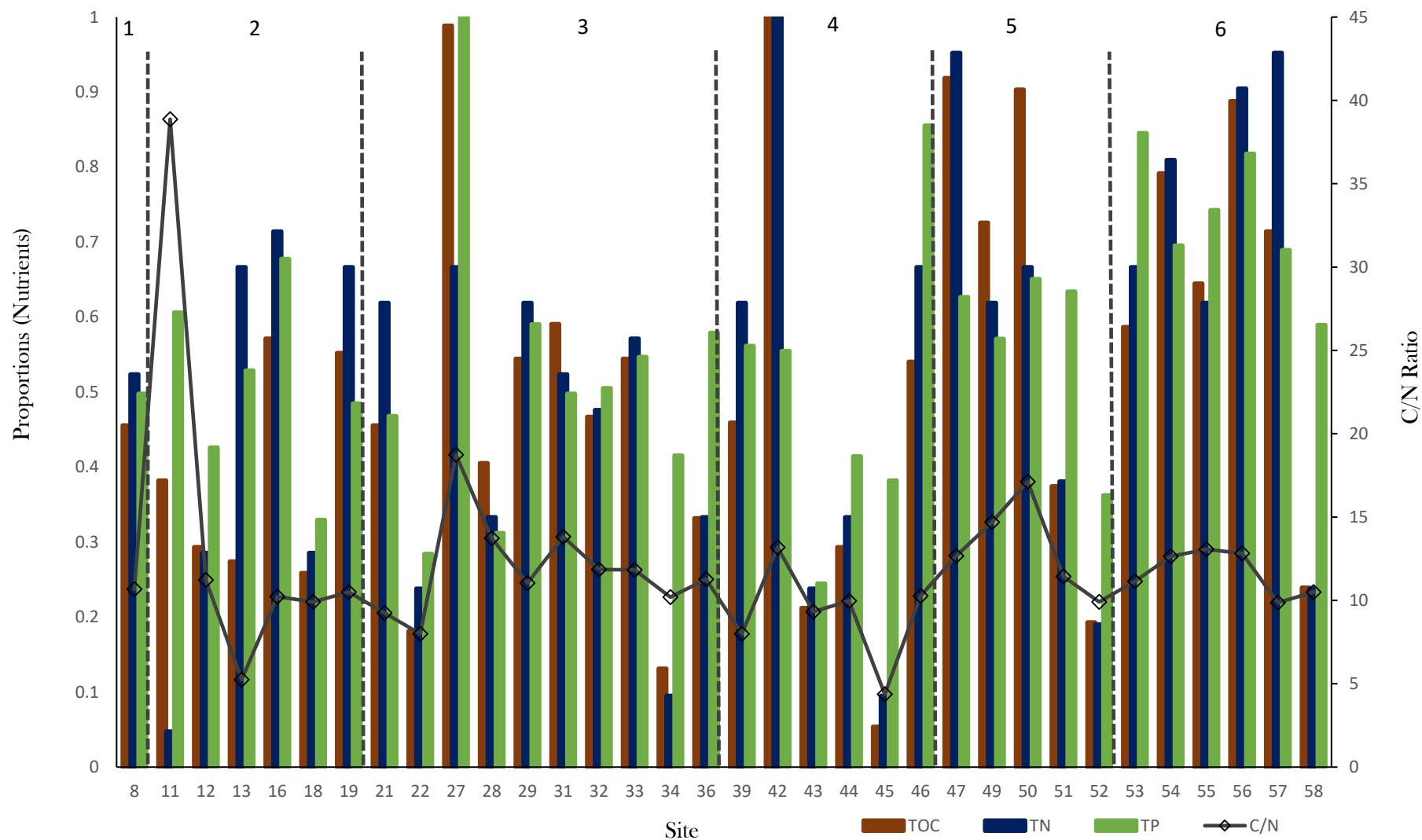


Figure 3.14: Proportions of particulate nutrients, total organic carbon (TOC), total nitrogen (TN), total phosphate (TP) and C/N ratio at sediment sites classified as silt/clay from the mouth of the Conwy estuary to the tidal limit. Proportions represent 0 (lowest concentration) to 1 (highest concentration) for each nutrient. Sample sites refer to locations in Figure 1.1 & 1.2.

TC content within the sediments were found to differ from the OC content (Figure 3.15a) and therefore significant amounts of inorganic carbon were present within the total fraction. Total carbon was omitted from further analysis and replaced by OC. After removal of sites which yielded the majority of the TN as inorganic nitrogen (1, 3-7, 9, 10, 14, 15, 17, 20, 23-26, 30, 35, 37, 38, 40, 41 & 48) TN content was found to have insignificant amounts of nitrogen absorbed as ammonium (Figure 3.13b) and therefore for the remaining sites ON was omitted and replaced by TN content.

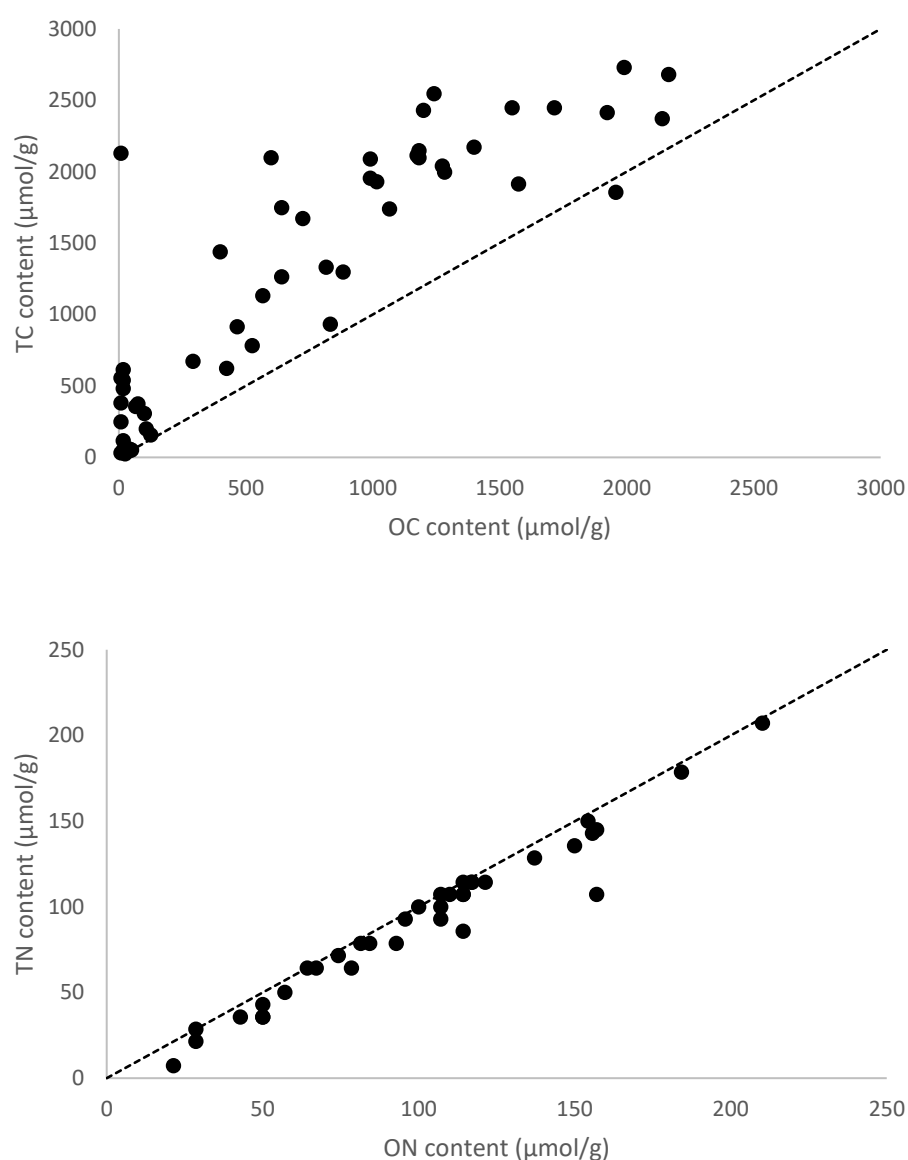
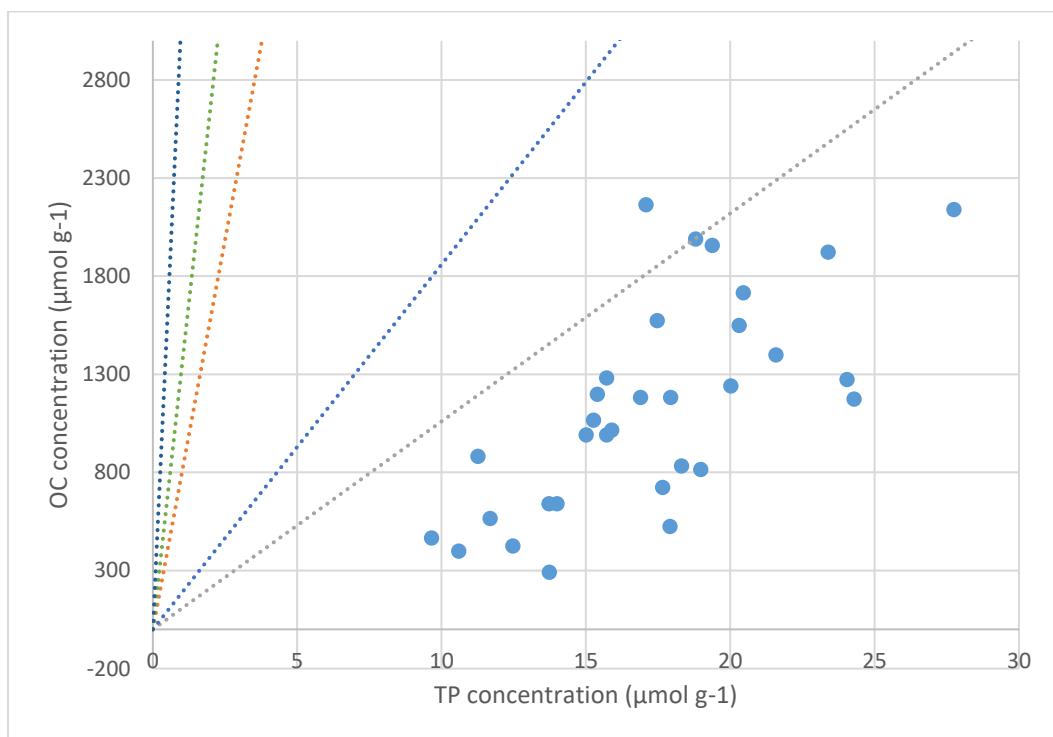
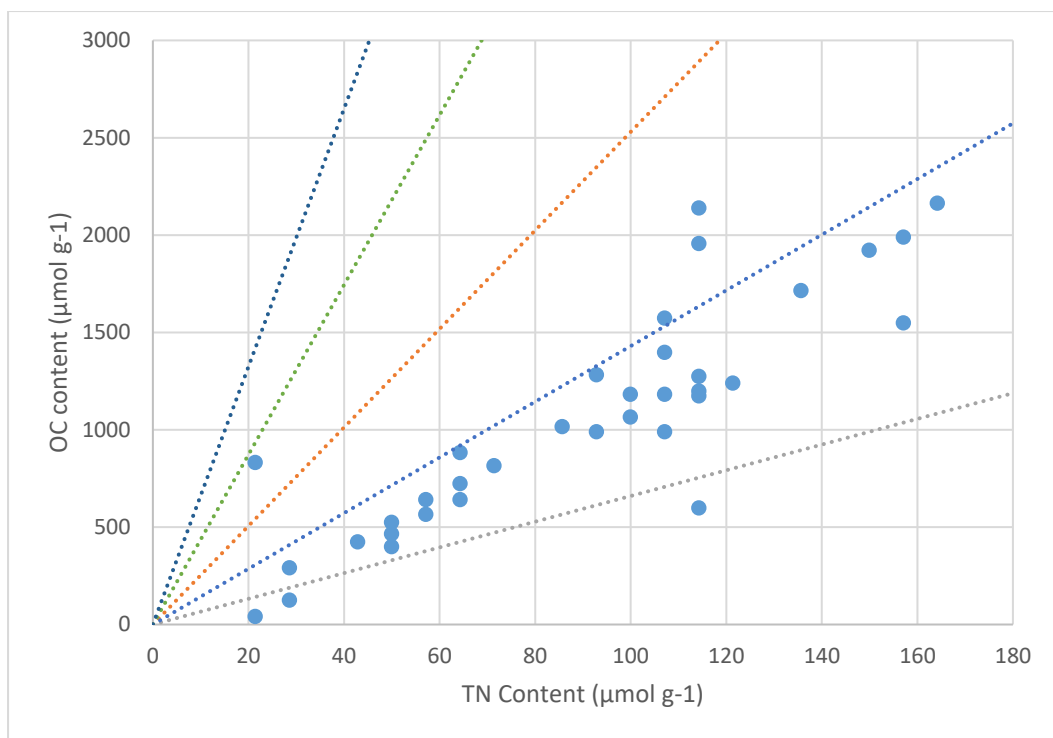


Figure 3.15: Comparison between organic and total species of sediment carbon and nitrogen to establish whether concentrations differ within the Conwy estuary.

For the remaining sites a general behavioural trend between the TOC, TN and TP was identified throughout the estuary with TN and TP demonstrating higher concentrations at the same sites as those shown to have higher TOC concentration. Linear relationships between these parameters were analysed and compared in Table 3.1. Significant correlations were found between all parameters with the highest correlation found between TOC and TN ($n = 57$, $R^2 = 0.926$). These correlations show that 80% of the variance in the TN data and 63% of the variance in the TP data can be explained by the TOC concentration and that the majority of both nitrogen and phosphorous are from the organic form. Within the literature, studies have also demonstrated that the nutrient sorption capacities of sediments are related to the contents of organic matter in the sediments (Wang et al. 2006, Liu and Lee 2007, Wang et al. 2009). Ye et al. (2014) and Yang et al. (2017) both support the notion that correlation between TN and TP and TOC suggest that the basins were dominated by organic nitrogen and phosphorous. Notably these correlations yielded zero intercepts, which suggest that the nitrogen and phosphate concentrations in the samples measured for this study were from the organic fraction. These findings have also been demonstrated by other researchers in other catchments (Andrews, Greenaway and Dennis 1998, Kao, Lin and Liu 2003, Owen and Lee 2004, Liu et al. 2007) which also indicated that the TOC/TN ratios reliably approximate the C_{org}/N_{org} (Rumolo et al. 2011). In addition 60% of the variance of TP data is due to TN concentrations.

Table 3.1: Linear relationship among TOC, TN and TP in sediments of the Conwy Estuary.

	n	Regression equation	significance	R^2
TOC/TN	57	$P_{TN} = 0.064P_{TOC} + 21.926$	<0.005	0.804
TOC/TP	57	$P_{TP} = 0.007P_{TOC} + 8.342$	<0.005	0.632
TN/TP	57	$P_{TP} = 0.070P_{TN} + 9.793$	<0.005	0.487



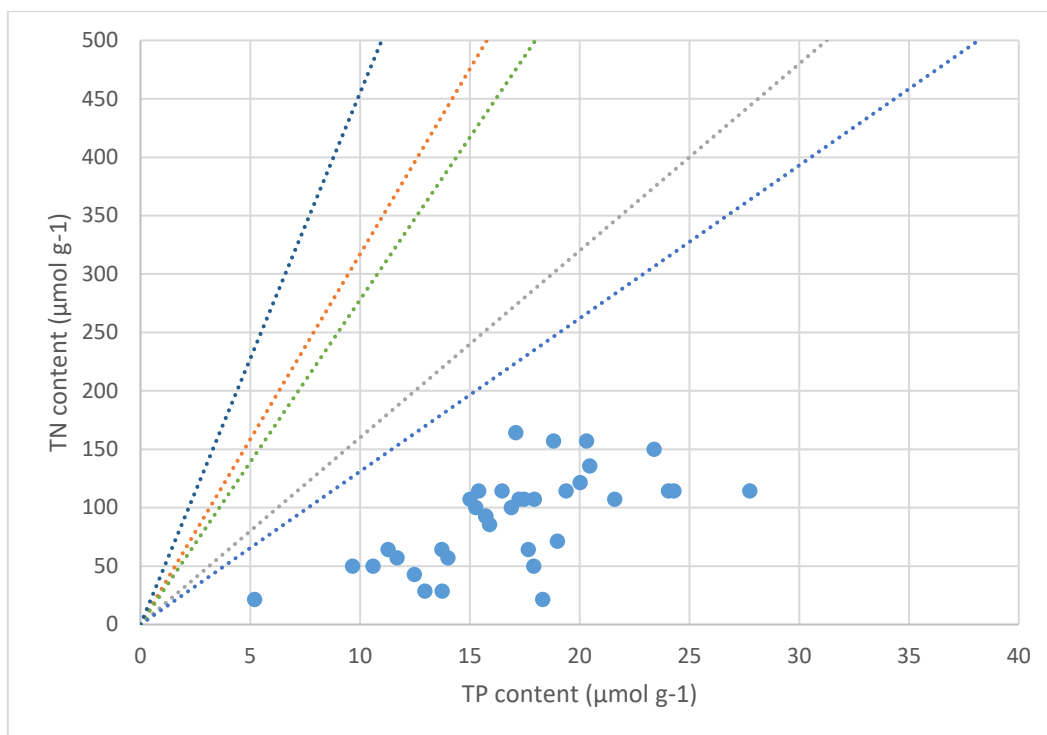


Figure 3.16: Comparison of TC, TN and TP concentrations against known biomass ratios to establish the origin of the organic matter within the Conwy estuary sediments.

CN, CP and NP ratios were calculated using the sediment data within the Conwy estuary (Figure 3.16). Known marine (Hecky et al. 1993) and terrestrial (Cleveland and Liptzin 2007) elemental ratios have been superimposed onto Figure 3.16 to identify the origins of the organic matter. As with the suspended particulate matter no single source is contributing to the observed C:N, C:P or N:P trends but samples represent different relative proportions of potential sources across the sites. C:N analysis is indicating that the majority of the organic matter within the estuary is from a soil origin and supports Emmett et al (2016) that the Conwy estuary is dominated by natural riverine inputs rather than human impacted.

3.3.4 Normalisation of nutrient concentrations and regional geochemical baseline (RGB) models

A Mann-Whitney U test was run to determine if there were differences in TOC, TN, TP and C/N ratio between sites dominated by sand and sites dominated by mud. Distributions of TOC, TN, TP and C/N were found to be statistically significantly different within the two grainsize fractions ($p = <0.001$) (Table 3.17, Appendix 2).

As reported previously the dataset showed significantly higher ($p = <0.001$) concentrations of TOC, TN and TP in fine grained sediments whilst coarse grainsize showed low concentrations. Statistical analysis was then performed on the data set after being log transformed to explore the relationships between the different variables analysed for each site and to identify whether grainsize was a contributing factor for the distribution of nutrients in the Conwy estuary.

Data would not transform to a normal distribution and therefore spearman's rank non-parametric test was used. There was a strong significant correlation between TOC, TN and TP, as defined by Cohen (1988), $r_s(58) = .774 - .870$, $p < .0005$ (Table 3.2) and therefore no difference between them. These nutrients were found to be strongly correlated to grainsize; fine fraction: $r_s(58) = .526 - .659$, $p < 0.0005$ and sand fraction: $r_s(58) = -.513 - -.656$, $p < 0.0005$. These results indicate that the nutrients in the Conwy estuary were positively correlated to the fine grain sediment size fraction and negatively correlated to sand which signified that grainsize was a contributing factor.

In order to remove the major influence of grainsize on the distribution of TN and TP within the estuary, the sediments are normalised against a grainsize proxy. Removal of the grain size effect allows a comparison to be made between the sites. Fewer published studies are available for the normalisation of organic compounds in comparison to elemental compounds (Borja and Collins 2004) discussed in chapter 2. There is a known strong affinity between organic matter and organic contaminants but there is no agreement on the representative parameter to be used (Borja and Collins 2004). TOC has been used previously as a normaliser by many researchers (Uncles and Mitchell 2017) and with the sediments of this study was shown to be significantly correlated to the fine fraction ($r_s(67) = .658$, $p = <0.0005$), and therefore was investigated as a proxy for the TN and TP of the Conwy sediments. Following the criteria used by Luoma (1990) for regional geochemical baseline (RGB) models to establish the best normaliser, the relationship between each element and physical parameters was investigated. TN and TP exhibit significant

linear correlations with TOC (.870 & .774, $p = <0.0005$, respectively) Table 3.2. and TOC as previously stated is significantly correlated with the fine grained sediment and negatively correlated with the course grained sediments.

To enable the geochemical baseline models to be constructed TN, TP and TOC concentrations were log transformed to meet assumption of normality. Regression analysis was performed on TN and TP with TOC as the normalising element before and after the elimination of enhanced samples (Tables 3.3). The regression analysis between TN and TP and the normalising element (TOC) after the elimination of all enhanced samples, showed that both nutrients were highly correlating with TOC ($R^2 > 0.7$) and therefore considered a good proxy for natural variation. In addition, TOC was highly correlated to the fine fraction (Table 3.2) which supports that the high r^2 values are related to the textural changes in the sediment and therefore agrees with the association of TN and TP with the fine grain sediments. However the intercept values were greater than zero which indicates that there are other sources potentially influencing the relationship in addition to the organic matter.

Table 3.2 Spearman's correlation coefficient (r) for the studied variables of the Conwy estuarine surface sediments (considering 58 samples).

	Gravel	Sand	<0.63	TOC	TN	TP
Gravel	1					
Sand	0.091	1				
<0.63	-0.160	-.986**	1			
TOC	0.027	-.656**	.658**	1		
TN	-0.165	-.513**	.526**	.870**	1	
TP	-0.061	-.628**	.629**	.774**	.687**	1

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

Table 3.3: Transformations and regression results of sedimentary nutrients to TOC relationships for the Conwy surface samples. N is the number of samples selected to define the regional geochemical baseline and r^2 represents percentage of total response variation explained by TOC (first figure shows value before elimination steps, second after all elimination steps and bracket shows number of sites removed due to enhanced concentrations. Slope and intercept values enable to predict background values.

Element	Transformation	N	r^2	slope	intercept
TN	log (TN), log (TOC)	36	0.712/0.894(4)	0.413 ± 0.05	0.705
TP	log (TP), log (TOC)	52	0.610/0.862(5)	0.279 ± 0.033	0.411

3.3.5 Estimation of TN and TP enrichment factors and Sediment quality guidelines.

Enrichment factors (EF), which were explained in chapter 2, can assess human impacts relative to the baseline which are defined through the RGB model above.

Background values were predicted using the TOC regression values (Table 3.3), these were then compared to the measured TN and TP concentrations using the enrichment calculation to enable an understanding of whether the nutrient concentrations in the Conwy estuary are at background levels and therefore naturally occurring or impacted by human sources.

The general pattern of enrichment for TN and TP from Figure 3.17 showed background concentrations (EF between 0-1.5) within the majority of the estuarine sediments (94% and 89% respectively). Surface sediment samples with EF greater than 1.5 indicate that additionally to concentrations associated with the natural component of the sediment (baseline), other nutrient contributions are delivered from non-crustal contributions, which are associated with anthropogenic inputs within the study area. There was no significant enrichment (>5) of TN or TP within the Conwy estuary. Moderate enrichment (between EF 2 and 5) of TN can be found at site 41 (sector 4) and TP at sites 9, 10, 26 and 40 (sector 2, 2, 3 & 4 respectively). Slightly elevated (EF between 1.5 - 2) TN was seen at site 13 (sector 2) and for TP at sites 7 and 14 (sector 1 & 2 respectively).

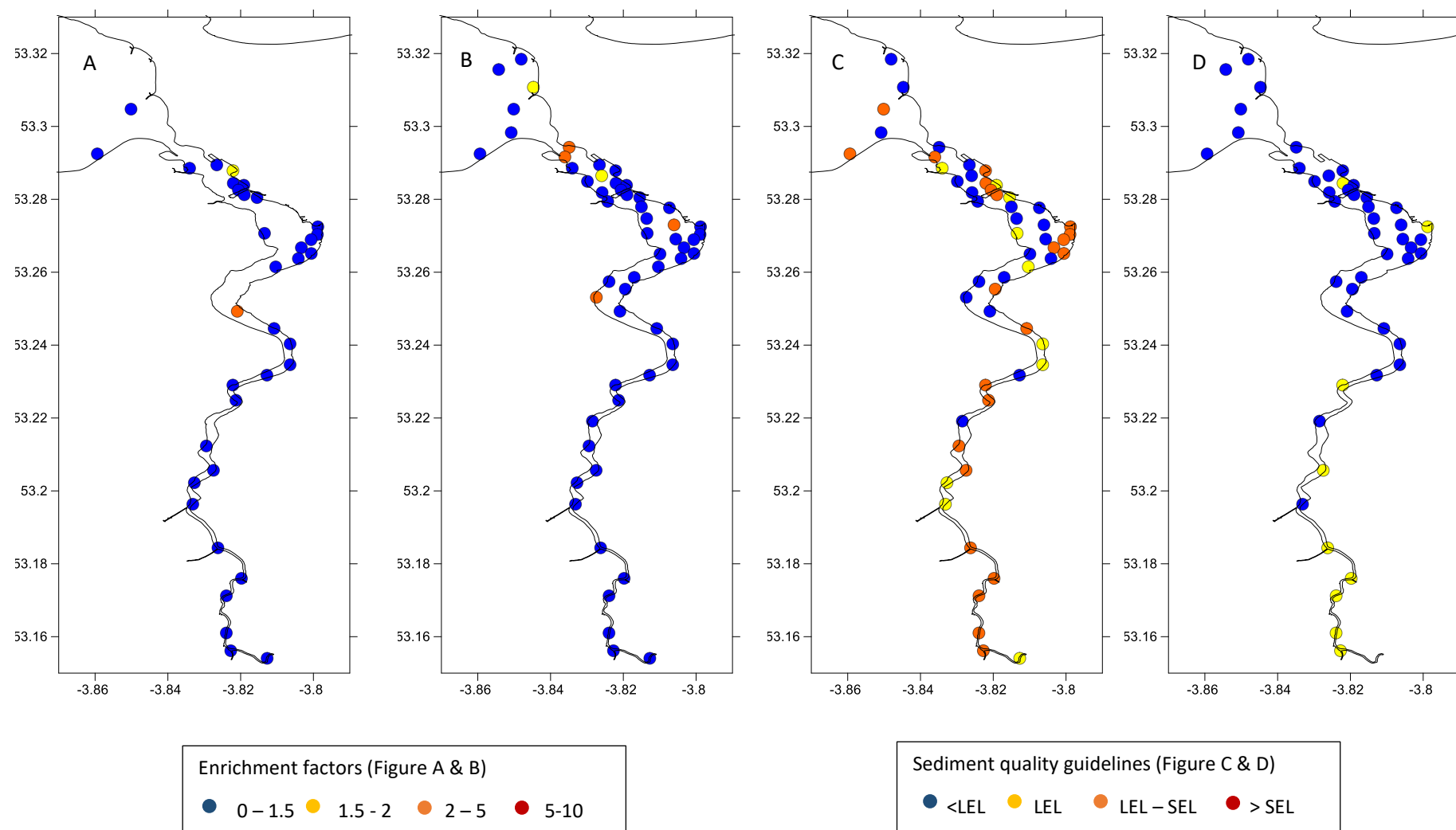


Figure 3.17: Spatial distribution of Enrichment factors and Pollution indices for TN (A & C respectively) and TP (B & D respectively) within sediments of the Conwy Estuary. LEL refers to lowest effect level and SEL to severe effect level.

Enrichment factors were compared against the widely used sediment quality guidelines (SQGs) published by Ministry of Environment and Energy, Ontario, Canada (Yang et al. 2017). According to the SQGs, bulk sediments that have TN content of 550mg/kg and TP content of 600mg/kg are indicative of below or at the lowest effect level can be regarded as sediments that are unpolluted (Yang et al. 2017). This threshold was compared to the present study and presented in Figure 3.16. These results show that a high proportion of the samples classified as fine grained are higher than the lowest effect level but below the moderately effected level. The disadvantage of this method is the lack of concentrations being normalised to grain size and bias may occur within high concentrations of fines. In addition the SQGs do not take into account the localised concentrations of TN and TP and rely on one value for all sediments to be referenced to, which can over or underestimate baseline conditions. However the enrichment factor approach does not take into account the source of the nutrient, the sediment content of carbon, nitrogen and phosphorus may vary naturally due to varying landscapes being eroded during high rainfall and not due to contamination. Therefore it is paramount to utilise a combination of elemental ratio along with the enrichment factor approach. Within this study it was apparent that sample sites with high percentage of course grain fraction yielded TN concentrations with significantly higher inorganic than organic nitrogen and therefore overestimated the TN in sediments due to inorganic ammonium absorption, which was identified by very low OC/N ratios.. This highlights that the combination of the biomarker approach with the EF approach is a much more accurate presentation of whether a site is experiencing enrichment and allows identification of whether naturally occurring i.e. Migneint moors or potentially from an anthropogenic origin.

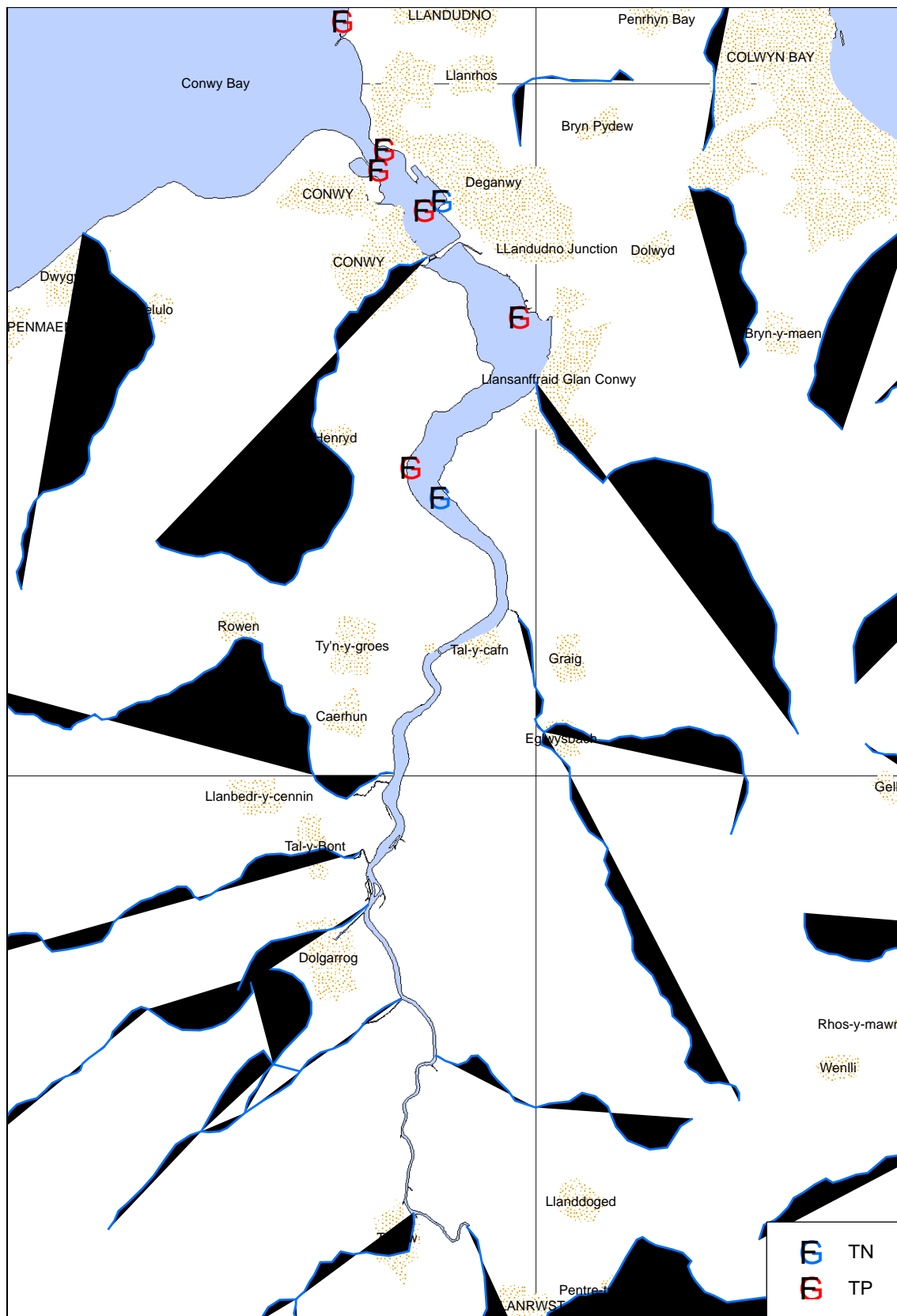


Figure 3.17: Visual presentation of all sites classified as enriched for particulate nutrients to highlight the locations where multiple species exist within the Conwy estuary.

3.4 Conclusion

The purpose of this study was to establish whether the Conwy estuary can be used as an example of a near-pristine estuary for the purpose of WFD reference conditions. The dissolved and particulate nutrients mainly showed natural and background concentrations within the entire estuary up to the tidal limit. No significant changes were evident between high and low water. Slight elevation of concentrations were evident within areas of mudflats and resuspension of nutrients were suspected. Therefore future monitoring procedures need to take into consideration underlying sediment type. Ammonia concentrations were slightly high near to the tidal limit, however these concentrations may be associated with leaching from the peatlands further up the catchment and could be a natural phenomenon for this type of mountainous watershed. Nitrates were found to be highest within the lower estuary reducing to the upper estuary. This could be explained as being consistent with leaching from agricultural and urban runoff and/or natural conditions from a semi-urban and agricultural watershed. Some elevated nutrient concentrations were found within inflows to the estuary however the majority of nutrients were still within the legal limits set out by the WFD (Drinking water directive 98/83/EC). Future monitoring of flow and continuous sampling though out all seasons including during storm and drought events would need to be undertaken to establish whether these inflows are influencing the estuarine concentrations. To date sediment quality guideline (SQGs) published by the Ministry of Environment and Energy, Ontario, Canada (Hubner et al. 2009) are used to assess whether deposited sediments are contaminated. However, these limits do not take into account natural conditions and can be very misleading. Regional geochemical baseline models were constructed for the first time within this study for TP and TN to gain an understanding of whether the concentrations within the sediments were from naturally occurring components or anthropogenic origins. It was evident that enrichment factors alone did not allow enough explanation of type of nitrogen absorbed onto the sediments and therefore in combination with elemental ratios, would produce the most useful tool appropriate for an environmental manager.

CHAPTER 4

Lipid Biomarkers

4.1 Introduction

Estuarine organic matter is derived from a multitude of natural and anthropogenic allochthonous and autochthonous sources that originate across a freshwater to seawater continuum. Various point and non-point pollution sources, such as discharges of treated and untreated sewage as well as urban and agricultural run-off, contribute significantly to the deterioration of the water and the sediment quality (Bujagic et al. 2016).

One of the most important causes of water quality degradation is faecal contamination from human and animal waste because it often leads to an increase in nutrient concentrations which are favourable to alga proliferation (Mainstone and Parr 2002) and conducive to eutrophication of the system (Hilton et al. 2006). Another problem related to faecal contamination is the serious threat to human health from exposure to pathogenic bacteria, viruses and protozoa (Sinton, Finlay and Hannah 1998, van Asperen et al. 1998, Derrien et al. 2012). In Europe, management plans are implemented in order to limit these risks and improve water quality. The revised Bathing Water European Directive (2006/7/EC) introduced more stringent microbial concentration limits together with the need to establish bathing water profiles in which the animal or human origin of the faecal contamination must be determined, a requirement that is also included in the revised European Shellfish Directive (Regulation (EC) 854/2004) (Derrien et al. 2012).

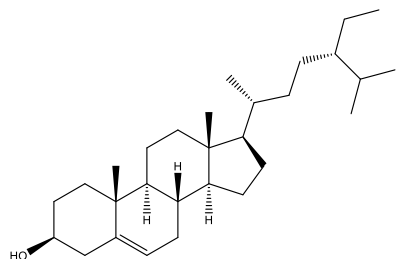
Escherichia coli and enterococci bacteria were conventionally used for the assessment of faecal contamination of the environment (Savichtcheva and Okabe 2006, Field and Samadpour 2007). However there are a number of short comings with bacteria indicators such as environmentally instability, susceptibility to disinfection and the lack of source specificity. Chemical (i.e. organic molecules) markers were proposed as an alternative for identification of faecal pollution sources and evaluation of sewage inputs (Bujagic et al. 2016). The rationale is the use of a molecule that can be related to a particular source and whose structure is preserved (or can be tracked back) after the death of an organism (Saliot 1994). Certain chemical markers have a relatively high resistance to bacterial degradation, compared to other classes of organic molecules and allows them to withstand degradation in the environment. These markers tend to be hydrophobic in nature and they can potentially be assigned to allochthonous (riverine, continental, anthropogenic) and/or autochthonous sources of organic matter. Lipid biomarkers have been extensively used proxies to assess the origin and distribution of organic matter in an aquatic system. (Bianchi and Canuel 2011, Volkman 2006). In addition compared to geochemical

proxies such as the relative abundance of total carbon to total nitrogen (TOC/TN), lipid biomarkers can provide detailed information about the origins of sediment organic matter (Hu, Peng and Chivas 2009, Xing et al. 2011).

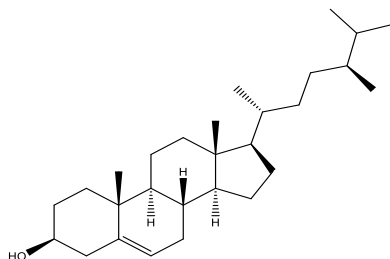
Numerous studies have successfully used sterols, as molecular markers, as they are specific to a source and retain their chemical structure throughout different processes within the environment. This enables characterisation of the sources of natural organic matter (e.g. marine and terrigenous) and the identification of anthropogenic inputs (e.g. faecal contamination) (Leeming et al. 1996, Derrien et al. 2012, de Abreu-Mota et al. 2014, Martins, Fillmann and Montone 2007, Venkatesan and Mirsadeghi 1992, Green and Nichols 1995, Carreira et al. 2016).

Sterols are ubiquitous compounds and are important constituents of cells. Some sterols, i.e. cholesterol, are present in both vegetal and animal kingdoms where as phytosterols (such as Campesterol, stigmasterol and β -sitosterol) are important constituents of higher plants (Biache and Philp 2013). Campesterol, stigmasterol and sitosterol (C_{28} and C_{29}) (Figure 4.1) have been commonly used as markers of terrigenous organic matter because of their predominance in terrestrial higher plants (Huang and Meinschein 1976, Bouloubassi et al. 1997, Volkman 2005, Martins et al. 2011). The main marine sterol marker used is generally cholesterol, because it is the dominant sterol in invertebrates and marine zooplankton. Brassicasterol and dinosterol (Figure 4.1) can also be included in the marine marker group, as they are more specific for marine phytoplankton and dinoflagellates, respectively (Volkman 1986, Harvey 1994, Bouloubassi et al. 1997). Anthropogenic input can be characterised via the 5β -Stanol C_{27} sterols (e.g. coprostanol, Figure 4.1) (Grimalt et al. 1990, Leeming et al. 1996). These compounds are by-products of cholesterol, via intestinal anaerobic microbial reduction in the digestive system of higher animals (Leeming et al. 1996). They are commonly associated with domestic/animal sewage contamination (Bull et al. 2002, Isobe et al. 2002, Jarde et al. 2007, Shah et al. 2008, Derrien et al. 2012). In humans, cholesterol is preferentially reduced to coprostanol where it constitutes 60% of the total sterols found in human faeces (Leeming et al. 1996). This biomarker has been widely used as a human waste pollution indicator and thus can be used as an indication of human activities in adjacent areas ((Mudge and Seguel 1999, Mudge and Duce 2005), Peng et al 2005, Cordeiro et al 2008). By contrast, cholesterol is predominately reduced to cholestanol in the environment. Similarly, plant-derived 24-ethylcholesterol is reduced to 24-ethylcoprostanol in the gut of herbivores (Figure 4.1), whereas in the environment it is primarily reduced to 24-ethylcholestanol. As a consequence, analysis of the sterol composition of faeces

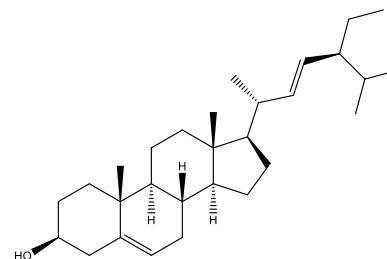
Plant origin sterols, indicators of terrestrial organic matter: β -Sitosterol, Campesterol, Stigmasterol.



β -Sitosterol
(24 Ethyl Cholest-5-en-3 β -ol)

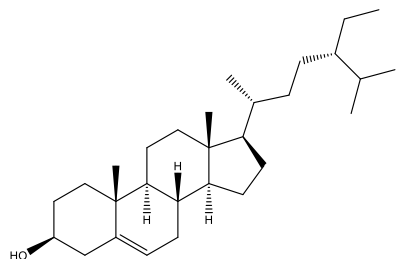


Campesterol
(24 Methyl Cholest-5-en-3 β -ol)

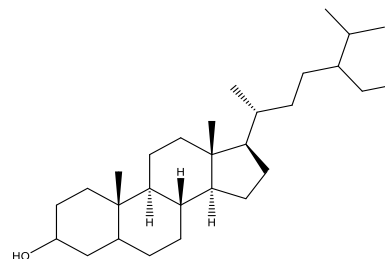


Stigmasterol
(24 Ethyl Cholestan- 5,22(E)-dien-3 β -ol)

Degraded plant sterols: β -Sitosterol to 24ethyl-Coprostanol via removal of double bond.

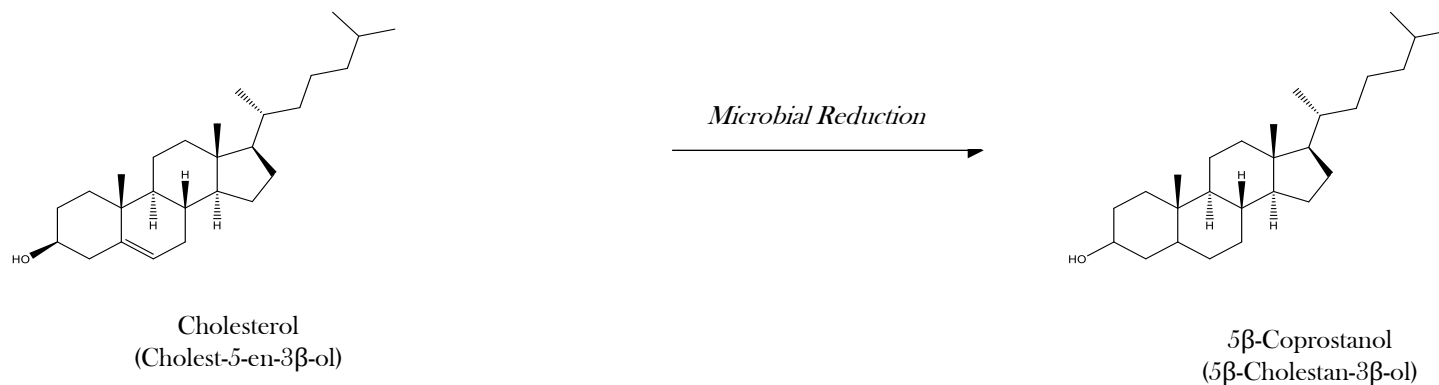


β -Sitosterol
(24 Ethyl Cholest-5-en-3 β -ol)



24 Ethyl-coprostanol
(24 Ethyl Cholestan-3 β -ol)

Human and animal origin sterols: cholesterol. Degraded human and animal sterol: Cholesterol removal of double bond to 5 β -Coprostanol



Algae, phytoplankton and fungi origin sterols: Brassicasterol & Dinosterol (algal & phytoplankton) and Ergosterol (fungi).

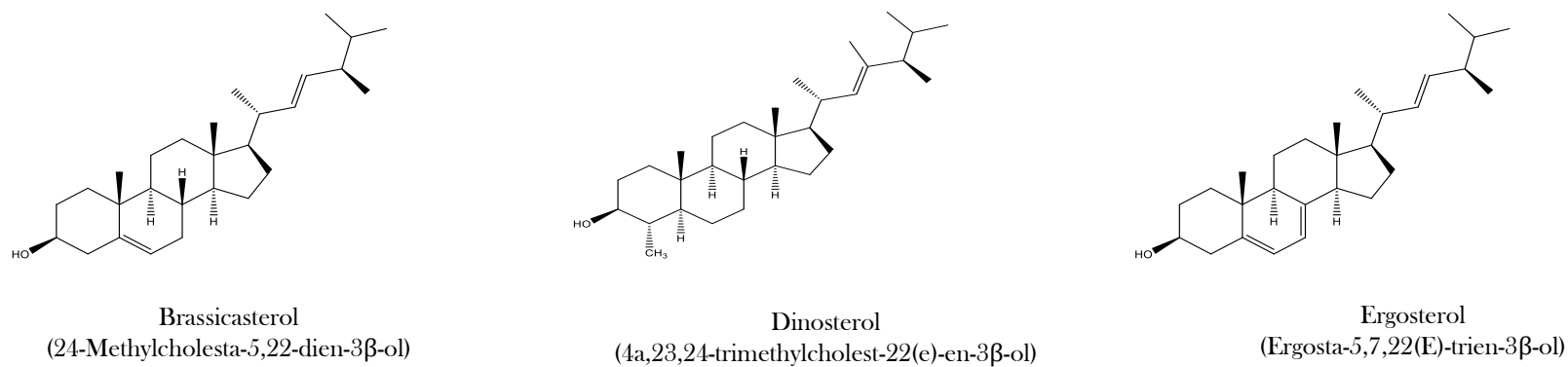


Figure 4.1: The structures of the key environmental sterols

can generate a sterol fingerprint, which can distinguish one species from another, particularly in the case of differentiating human from animal pollution.

According to numerous authors (Shah et al. 2008, Tolosa, Mesa and Alonso-Hernandez 2014, Martins et al. 2014b, Saeed et al. 2015) individual levels of sterols are not reliable enough as biomarkers of human faecal contamination, and therefore ratios of different selected sterols should be used to enhance reliability of the pollution assessment. From the literature seven main ratios are identified (Table 4.1). Sterol Ratio 1 and 2 are typically greater than 0.5 if faecal material is present (Leeming et al. 1996). Ratios 3 and 4 are all indicative of human faeces when thresholds are exceeded and are based around elevated relative levels of coprostanol (Grimalt et al. 1990, Reeves and Patton 2001). Sterol ratio 5 is indicative of herbivore faecal contamination. Ratios 6 and 7 differentiate between human and herbivorous source (Bull et al. 2002, Gilpin, Gregor and Savill 2002), and ratio 8 depicts whether herbivorous source is from plant decay or a diet of plant material such as ruminants.

Table 4.1: Sterol ratios used within the literature to identify sources of organic matter. References refer to 1:(Bull et al. 2002), 2: (Bethell et al. 1994), 3: (Leeming et al. 1997), 4: (Martins et al. 2014b), 5: (Grimalt et al. 1990), 6: (Shah et al. 2008), 7: (Isobe et al. 2002), 8: (Gilpin et al. 2002).

Sterol ratio	Interpretation	References
Ratio 1: Coprostanol/cholestanol	>0.5 Faecal	1
Ratio 2: 24-Ethylcoprostanol/24-Ethylcholestanol	>0.5 Faecal	1
Ratio 3: Coprostanol/(coprostanol + cholestanol)	>0.7 Human Faecal	1,4,5
Ratio 4: Percent coprostanol/total sterol	>5-6% Human Faecal	7
Ratio 5: Percent 24-ethylcoprostanol	>5-6% Herbivore	7
Ratio 6: Coprostanol / 24-ethylcoprostanol	<1.0 Herbivore; \geq 1.0 human	1,2,
Ratio 7: Coprostanol/(coprostanol+24-ethylcoprostanol)	<30% Herbivore; >75% human	1,3, 6
Ratio 8: 24-Ethylcholesterol/24-ethyl coprostanol	<1.0 Herbivore; >4.0 Plant decay	8

To a lesser extent within the literature, fatty alcohols have been used to also distinguish between sources of organic matter. These are primarily derived from wax esters which are known to be widely present in the biosphere, for example as external lipids in the cuticular wax of land plants and as internally stored energy reserves in organisms (Mudge and Norris 1997a). In coastal marine sediments it is likely that wax esters are supplied by a variety of organisms, but primary sources are thought to be marine zooplankton and terrestrial plants (Fukushima and Ishiwatari 1984, Parameswaran, Das and Kamat 1994). Long chain, C22-C30 alkanols, are generally considered to originate from terrestrial plants (Brassell et al. 1980) and short chain alkanols are derived from unspecified marine, terrestrial and bacterial origins (Sargent 1978). Therefore the long chain / short chain ratio is a good indication of terrestrial input into the system. In comparison to sterols, knowledge of fatty alcohol occurrence is still quite limited and therefore it may be difficult to clearly define their origin from fatty alcohols alone (Seguel et al. 2001).

The determination of baseline values of these organic markers within pristine estuaries is important to provide information about natural sources of faecal sterols and to enable assessment of anthropogenic impacts. Within the literature the vast majority of studies have concentrated on contaminated environments with limited research undertaken on baseline conditions to compare to.

The objective of this research was to undertake a lipid biomarker survey on the sediments of the Conwy Estuary by utilising sterol ratios in order to characterise the sources of organic matter and potential anthropogenic inputs to the sediments. This will enable us to evaluate whether the Conwy Estuary can be used as an example of a pristine environment and background concentrations established for further baseline studies.

4.2 Materials and methods

4.2.1 Sample Collection

The study area is situated in the Conwy Estuary. Fifty eight sample sites were chosen to give a wide spatial coverage of the entire system. Sample sites were the same location as the metals (Figure 1.1 & 1.2). Access to all the study sites was achieved from the shore line or via use of a boat. A scrape of the top 2-3cm of the sediment was collected in pre-cleaned 120ml glass jars. The samples were immediately covered with aluminium foil, capped and kept in an icebox before transporting back to the laboratory where they were frozen until analysis was undertaken.

4.2.2 Lipid analysis

A portion of the sample (approximately 30g) was oven dried at 70°C to enable the moisture content and therefore the dry weight of the samples to be calculated. This dry portion was then sonicated and wet sieved to provide a simple grainsize analysis for percentages of silt ($<0.63\ \mu\text{m}$), sand ($0.63\ \mu\text{m} - 2.0\text{mm}$) and gravel ($>2\text{mm}$).

From the remaining sediment sample, approximately 30g of the wet sediment, was analysed for lipid content using the method described in Mudge and Norris (1997) for extraction of the lipid content of the sediments. All samples were refluxed in 50 ml of 6% KOH in methanol for 4 h. The resulting liquid was centrifuged and rinsed with methanol; the sterols and fatty alcohols in the supernatant were extracted twice with liquid-liquid partitioning into hexane. Samples were rotary evaporated and reduced to dryness under nitrogen. All samples were derivatised with bis(trimethylsilyl) trifluoroacetamide (BSTFA) to produce the trimethyl silyl derivatives of the sterols and fatty alcohols. (Mudge and Norris 1997) spiked reference materials to confirm the quantitative yield of the method. The efficiency of the whole extraction process was also confirmed by repeat reflux of some sediments; no further fatty alcohol or sterols could be detected in these later injections. Mudge and Norris(1997) utilised spiked reference materials which have shown that this extraction method released greater than >90% of the sterols and fatty alcohols from similar sediments with in the Conwy Estuary and therefore the same recovery is assumed within this study. Internal and external standards were analysed

Instrumental analysis was carried out on an Agilent gas chromatography –mass spectrometer (GC-MS) to quantify the concentrations of sterols and fatty alcohols in the samples. Helium was used as the carrier gas, with a column flow rate of 1 ml min⁻¹. A non-polar capillary column, BPX-5 (SGE) with dimensions of 30m x 0.32 mm (internal diameter) and 0.25 µm (film thickness), was used. The temperature programme started at 60°C and increased by 15 °C per minute to 300°C, then reaching a maximum of 350 °C at 5 °C per min. The MS was configured for electron impact ionisation at 70 eV and a mass scan range of 45-585 *m/z* per second.

To enable quantification of the samples, an internal standard of known concentration of 2-dodecanol was added to each sample and an external calibration series of cholesterol (to represent the sterols) and 2-dodecanol (to represent the fatty alcohols) was achieved. Blanks were interspersed with samples and standards to ensure no carry over between samples. All results are expressed as µg g⁻¹ dry weight by using the following equations:

$$\text{Lipid Concentration} = \frac{\text{Area of lipid peak}}{\text{Area of IS peak}} \times \text{Concentration of added IS}$$

$$\text{Dry weight correction} = \left(\frac{\text{Lipid Concentration}}{\text{sample weight (g)} \times \text{moisture content (\%)}} \right) \times 1000$$

4.2.3 Statistical analysis

Raw data was log transformed, however normal distribution was still not obtained therefore basic correlations were determined by spearman rank correlation coefficient (r) using the statistical package for Social sciences SPSS v22.

Principal component analysis (PCA) was conducted only on the elevated samples to determine the origins of the organic matter. Raw data was converted to proportions of the total chemical class (relative sterol concentrations and relative fatty alcohol concentrations) prior to analysis, this removed any concentration effect. The data was then log-ratio transformed, so that it approximated a near normal distribution. The PCA models were developed using the statistical package SIMCA[™] from Umetrics, only on lipid compounds that constituted greater than 5% contribution to the total concentration.

4.3 Results and Discussion

4.3.1 Total Organic Carbon (TOC) and grainsize

From the data in chapter 2 within the Conwy estuary there is a large variability between grainsize and organic content and the average grainsize composition is dominated by sand and mud contents. A wide range of TOC content was observed in the sediments (0 - 25.7 mg g⁻¹) from this study. The highest concentrations (Figure 2.3) were observed at the stations with high percentage of fines, mainly the upper part of the estuary and in the mud flats adjacent to the River Ganol.

4.3.2 Overall distribution of lipid concentrations within the Conwy estuary

Lipid concentrations in the surface sediments for each site of the Conwy estuary are presented in Table 4.1, Appendix 3. Descriptive statistics were undertaken on the raw data. The results are summarised in Table 4.2, including concentration range (minimum (limit of detection) and maximum), mean and standard deviations. Limit of detection is presented in Table 4.2, Appendix 3.

Similar to the TOC and proportion of fines, there is a general increasing gradient of total quantified lipid concentrations towards the upper area of the estuary (Figure 4.2), which suggests retention of organic matter within the terrestrial regions. The total concentration of lipids ranged from 3.03E-3 to 1005.62 µg g⁻¹ (Table 4.2). Values below 0.335 µg g⁻¹ of total lipids were found at sites within the lower estuary and within the Conwy Bay area (Figure 4.2) which was associated with sandy sediments and low TOC. Higher values of total lipids (>0.772 µg g⁻¹) were found in the upper estuary and within depositional areas of fines within the marinas and Ganol river. However, despite the apparent similar distribution between TOC and total lipids, there is only a weak but significant correlation ($r_s(70) = 0.383, p < 0.005$) between the two parameters. This may highlight the existence of different sources of organic matter to the studied area.

Table 4.2: Range of surficial concentrations of the investigated lipids ($\mu\text{g g}^{-1}$) for the 58 sample sites within the Conwy estuary. % LOD refers to the percentage of samples that fell below the limit of detection.

Sample descriptive statistics				
	Minimum	Maximum	Mean	Std. Deviation
Mud (%)	0	91.30	33.82	30.50
TOC (mg/g)	0.007	25.70	8.06	7.63
13:OH	0.001	0.82	0.08	0.19
14:OH	0.021	6.49	0.91	1.72
15:OH	0.001	3.46	0.51	1.01
15:OH iso	0.006	4.49	0.62	1.25
15:OH anteiso	0.006	1.50	0.19	0.37
16:OH	0.004	17.09	3.05	5.77
17: OH iso	0.020	1.39	0.22	0.40
17:OH antiso	0.014	5.14	0.64	1.36
17:OH	0.006	2.53	0.39	0.76
18:OH	0.021	19.40	3.55	6.32
19:OH	0.023	7.30	0.80	1.81
20:OH	0.003	87.33	6.61	17.37
21:OH	0.005	7.19	0.74	1.66
22:OH	0.004	478.64	25.97	90.66
23:OH	0.001	10.41	0.77	2.09
24:OH	0.002	267.30	12.69	49.79
25:OH	0.001	13.98	0.79	2.71
26:OH	0.015	76.68	6.41	18.0
27:OH	0.009	2.58	0.18	0.60
28:OH	0.007	25.70	1.67	5.50
29:OH	0.014	2.20	0.12	0.42
30:OH	0.005	13.83	0.70	2.63
coprostanol	0.003	11.91	1.58	3.20
Cholesta-5,22(E) - dien-3(beta)	0.004	3.44	0.50	1.03
cholesterol	0.001	25.66	3.57	6.96
5 alpha cholestanol	0.009	8.23	1.13	2.20
brassicasterol	0.010	8.20	1.21	2.46
ergosterol	0.002	2.93	0.35	0.76
24- ethylcoprostanol	0.012	7.61	1.20	2.36
campesterol (right sided)	0.007	9.43	1.17	2.38
stigmasterol	0.004	5.17	0.70	1.40
beta sitosterol	0.008	32.86	5.00	9.82
Dinosterol	0.017	3.51	0.46	0.94

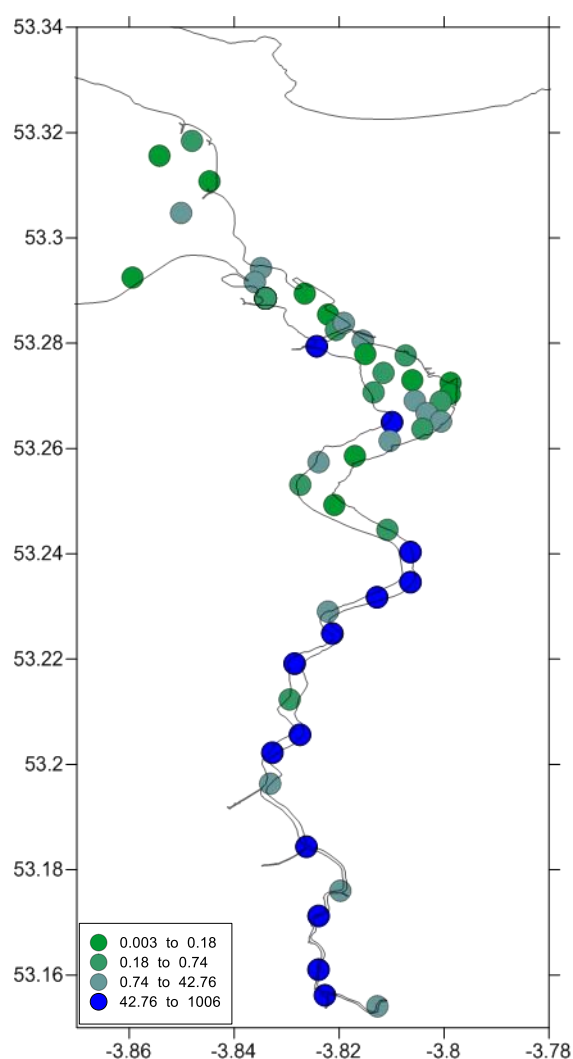


Figure 4.2: Distribution of total lipid content in $\mu\text{g g}^{-1}$ for each site within the Conwy Estuary.

4.3.3 Composition and distribution of lipid biomarkers

Eleven separate sterols were identified and quantified against standards throughout the data set; the sum of the sterol concentrations ranged from 0.077 to 114.80 $\mu\text{g g}^{-1}$, this supported Mudge and Norris (1997a) who found concentrations of 2.4 to 125 $\mu\text{g g}^{-1}$ also within the Conwy estuary. β -Sitosterol was the most abundant sterol throughout the estuary (Table 4.2) and was present in all samples. It was the principle sterol in 79.6% of the samples with concentrations ranging 0.003 to 32.86 $\mu\text{g g}^{-1}$. The overall mean concentration was 5.00 $\mu\text{g g}^{-1}$, which constituted 31.5% of the total sterol concentration. Cholesterol was the next principal sterol, with a much reduced

concentration range of 0.001 to 25.66 $\mu\text{g g}^{-1}$ and a mean of 3.57 $\mu\text{g g}^{-1}$. This constitutes 22.9% of the total sterols. Sitosterol is usually related to organic matter produced by higher plants, although algae can also produce this compound, whereas cholesterol is ubiquitous in plankton although it can also be derived from anthropogenic sources (Bianchi 2012, Volkman et al. 2007).

All the remaining sterols had a mean concentration less than 1.58 $\mu\text{g g}^{-1}$. 5 β -coprostanol, the principal indicator of mammalian sewage, and 24-ethyl coprostanol, an indicator of herbivorous faecal matter contributed up to 9.86% & 8.1% respectively of the total sterol concentration. Additional to sitosterol other terrestrial indicators campesterol and stigmasterol had much lower percentages (7.24 & 4.62% respectively). The algal indicator, brassicasterol and cholest 5, 22 dien-ol, also had low percentages of the total sterol concentration (8.17 & 3.39% respectively). Dinosterol, an indicator of dinoflagellates, also had a low concentration of 3.5 %.

A total of twenty two *n*-alcohols were identified throughout the data set, composed of C13 to C30, two of which are branched chain fatty alcohols. The sum of the entire dataset of fatty alcohol concentrations ranged from 3.08E-3 to 978.88 $\mu\text{g g}^{-1}$. The most prominent fatty alcohol was C22 which was present in 57 of the 59 samples tested. The concentrations ranged from 0.004 to 478.64 $\mu\text{g g}^{-1}$ with a mean of 25.97 $\mu\text{g g}^{-1}$, which constitutes a total of 36.85% of the fatty alcohols in the samples. C24 was the next principal alcohol with a concentration of 0.002 to 267.30 $\mu\text{g g}^{-1}$ and a mean of 12.69 $\mu\text{g g}^{-1}$. C24 constituted 16.28% of the total alcohol concentration. All the remaining alcohols had a mean concentration less than 6.61 $\mu\text{g g}^{-1}$. The long chain alcohols (LCOH) which accounted for 69.8% of the total alcohols were more abundant than the short chain alcohols (SCOH). Among the LCOH, the compounds with 22 and 24 carbon atoms are the most abundant *n*-alcohols. There is a predominance of LCOH over SCOH, which suggest a higher influence of terrestrial vegetation compared to marine sources in the distribution of alcohols in the Conwy Estuary sediments (Bianchi 2012, Volkman et al. 2007).

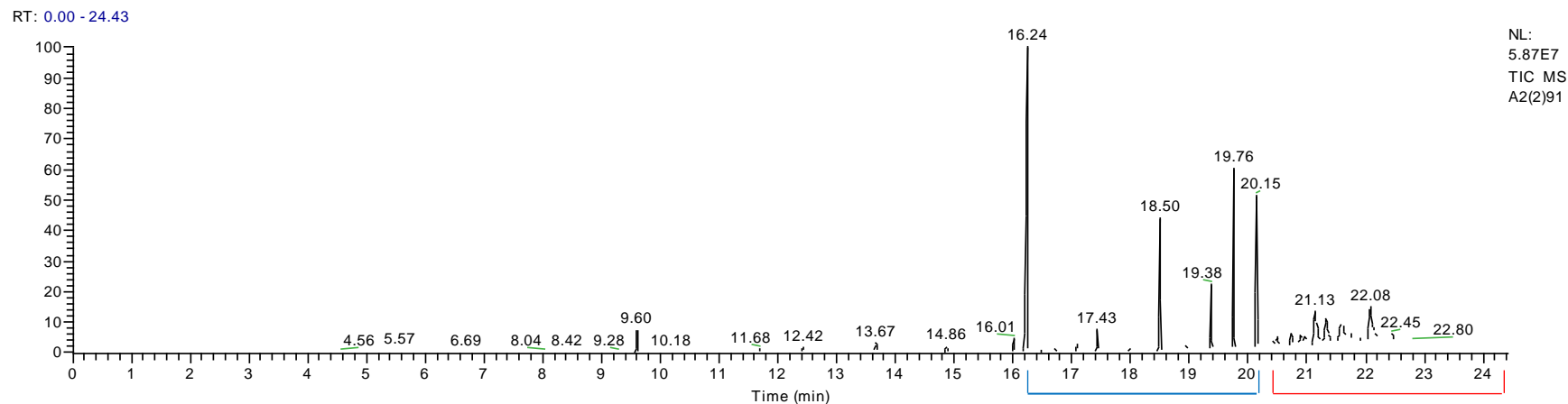
4.3.4 Evaluating organic matter sources within the Conwy Estuary

The dataset showed that for the majority of sites the sterol concentrations were substantially lower than studies from contaminated estuaries within the literature. GCMS chromatograms of the sterol compounds produced very small unresolved peaks compared to the fatty alcohols (Figure 4.3 a). The normal binomial pattern of sterol peaks seen with high concentrations was not

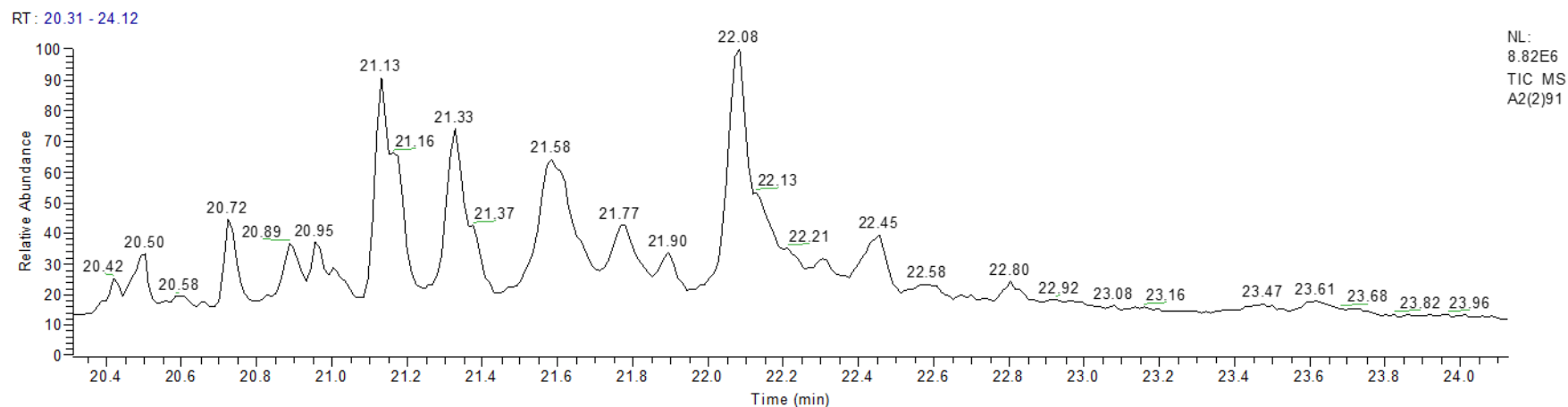
evident . It was difficult to establish the areas for each compound and therefore, difficult to establish accurate concentrations, as the start and finish points of the small unresolved peaks were difficult to identify (Figure 4.3b). These very low concentrations support the hypothesis that the Conwy estuary is a good candidate of a relatively pristine environment. Coprostanol, an indicator of mammalian contamination, was found to be less than $0.05 \mu\text{g g}^{-1}$ at 72% of all sites. There is no consensus of an absolute concentration threshold within the literature which establishes sewage contaminated sites. However Martins et al. (2014a) found baseline conditions within Antarctica and classified less than $0.05 \mu\text{g g}^{-1}$ from biogenic sources, therefore this study indicates that 72% of sites sampled in the Conwy were pristine. Within the dataset of the 72% of sites all compounds reflected similar low concentrations and therefore were omitted from further investigation. The remaining 28% of sites were investigated to establish the source of organic matter and whether from anthropogenic origin.

In order to establish relationships and clusters within the remaining dataset, sterol and fatty alcohols which contributed to more than 5% of the total lipid content were utilised for a Principal Component Analysis (PCA). Data was transformed into proportions of the total lipid content in order to remove grain size effect. In addition data was log transformed in order to approximate near normal distribution before conducting the PCA.

The loadings cluster compounds according to their biogeochemical source. Terrestrial plant biomarkers (campesterol and β -sitosterol) and 24-ethylcoprostanol, a degradation by product of sitosterol, along with the long chained fatty alcohols (C20, C22 & C24) are positively loading on PC2 and therefore reflect a close relationship (Figure 4.4). Marine biomarkers (Cholesterol, 5α cholestanol & brassicasterol) are loading negatively with the short chained fatty alcohols (C16 & C18), supporting a marine origin (Figure 4.4). C26 within the PCA is behaving out of character as it is representing marine rather than terrestrial. The scores plot locates samples dependant on the contribution of the geochemical compounds within each site. Sites loading positively on PC2 are conducive to terrestrial input whereas sites negatively loading originate from within the estuary. The majority of the sites showed terrestrial input (Figure 4.5) these sites were all positioned within the vicinity of riverine inputs.



A.



B.

Figure 4.3: Typical chromatogram of Conwy samples, (A) illustrates the full scan and (B) an enhancement of the sterol section of the chromatogram. Blue section are the fatty alcohols and red is the sterols within the chromatogram.

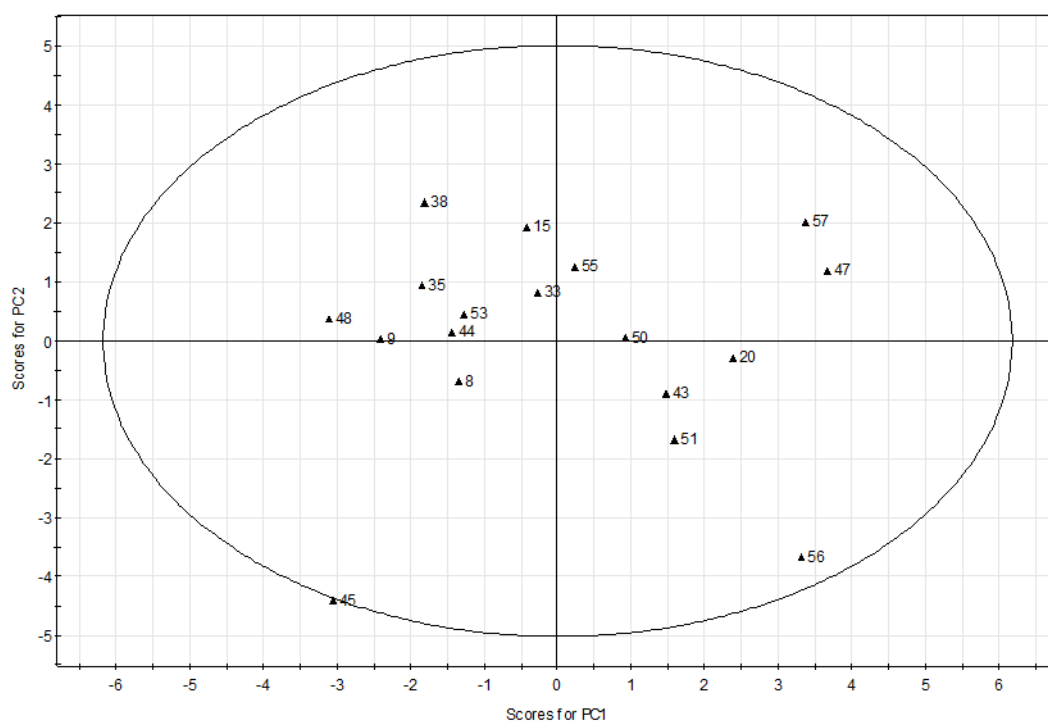
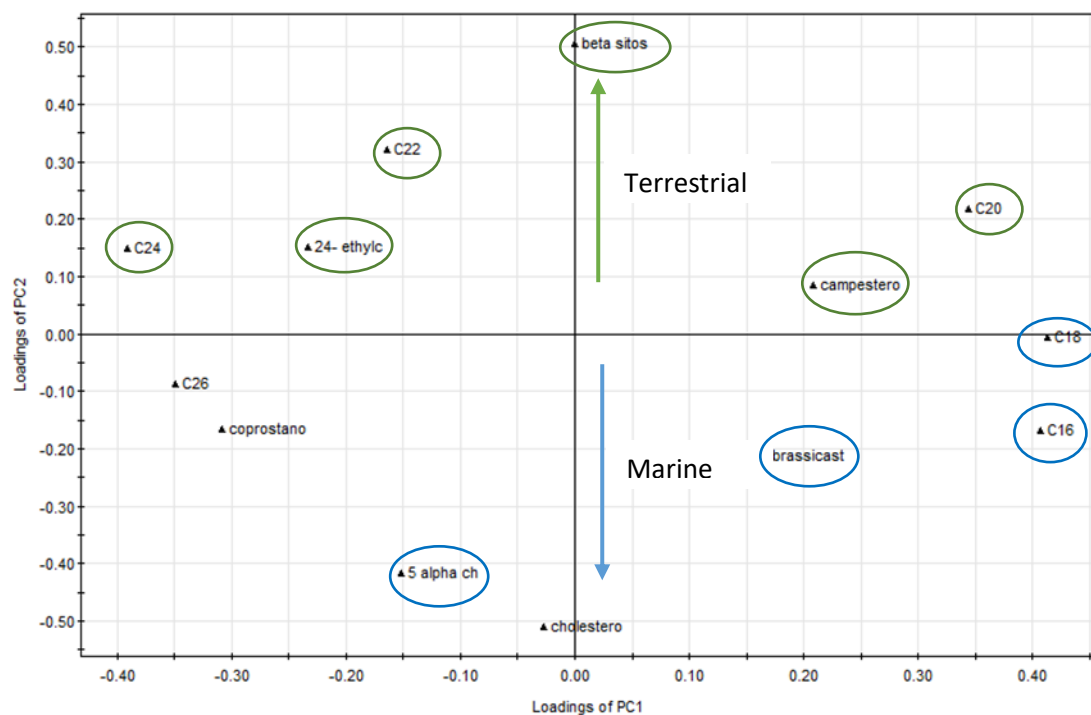


Figure 4.4: Principal Component Analysis of loading and scores for two principal components PC1 and PC2 for log tranformed sterols and fatty alcohol concentrations ($\mu\text{g g}^{-1}$) from sediment within the Conwy estuary. Blue represents sterols of marine origin and green terrestrial origin.

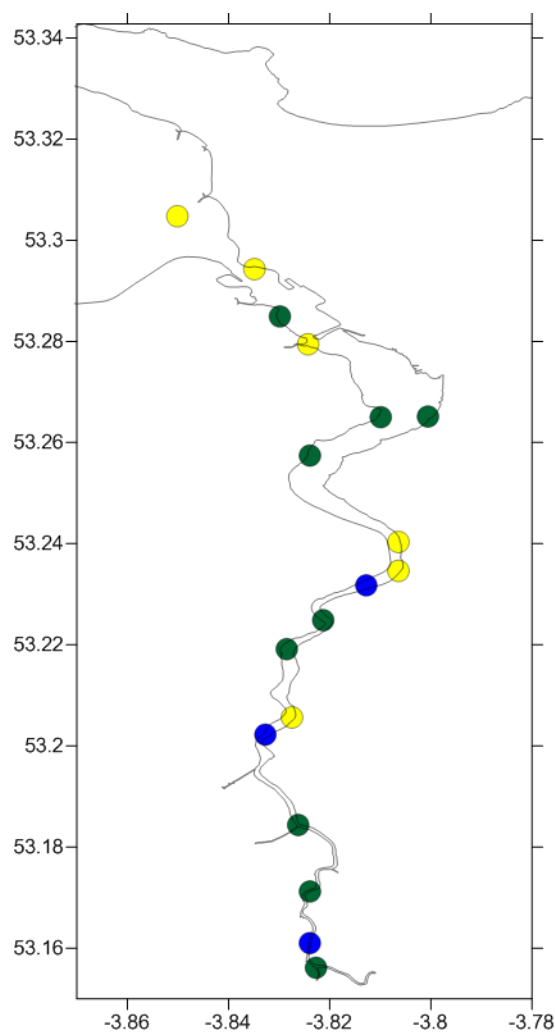


Figure 4.5: Origin of organic matter at each sampling site throughout the Conwy estuary from the PCA analysis. Green presents terrestrial, blue estuarine and yellow a combination of both. Y axis represents latitude and X presents longitude.

4.3.5 Lipid ratios

To minimise the ambiguity of sources from lipid compounds, the use of numerical ratios is an important tool in the differentiation of the sources of organic matter (Martins et al. 2014a). Ratios between selected sterols were used for identification of faecal contamination as well as for differentiation between the sources of the organic matter. The calculated ratios of sterols detected in the sediment samples are shown in Table 4.2, Appendix 3.

Cholesterol is present in many organisms and can be found in domestic sewage discharges, consequently, marine and estuarine sediments have cholesterol present in substantial quantities. Due to cholesterol having a variety of potential sources, its use as an independent biomarker is

limited (Mudge et al. 1999). However, it can be used in conjunction with other sterols in the form of ratios as a normaliser. There was a good correlation between cholesterol and total sterol concentration in the sediments throughout the estuary ($r = 0.760$, $p < 0.001$). These ratios have been used within the literature as a normaliser for total sterols and grain size (Mudge and Norris 1997a).

Terrestrial and marine sterol markers can be utilised to distinguish whether the contamination is within the marine system or is being influenced from a terrestrial source (Biache and Philp 2013). β -sitosterol, campesterol and stigmasterol are all indicative of terrestrial input. However as β -sitosterol was the most abundant sterol throughout the study, this was chosen to indicate terrestrial input. The ratio of β -sitosterol/cholesterol should indicate regions of either terrestrial runoff containing vascular plant matter or regions of phytoplankton production (Mudge et al. 1999). Any sites with a ratio of β -sitosterol/cholesterol above 1.0 demonstrates a significant input of material from a terrestrial origin. Conwy estuary has a large influence of terrestrial inputs throughout the whole of the estuarine system, this supports the finding in figure 4.5. The largest influences are at sites 38 and 57 (Figure 4.6). The estuary does not have an obvious marine and terrestrial end this is most likely due to the freshwater inputs along the whole length of the estuary.

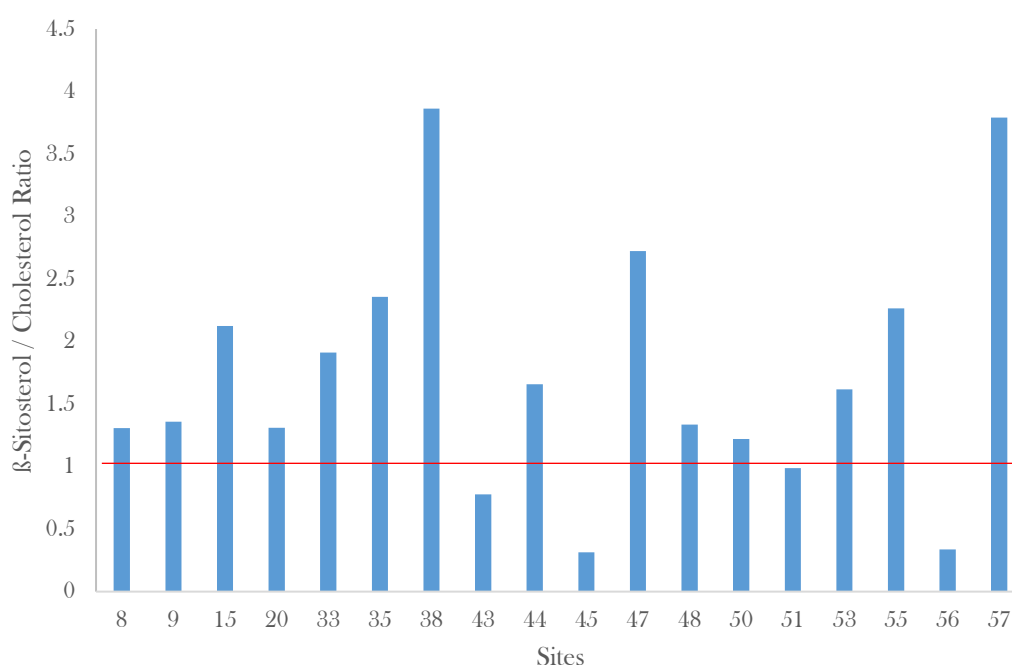


Figure 4.6: β -sitosterol/cholesterol ratio for all sites within the Conwy Estuary to identify terrestrial and marine sources. Red line presents the threshold between the two parameters.

Coprostanol is the principal human biomarker present in human faeces at concentrations ten times higher than those of cattle and other vertebrates (Leeming et al. 1996). Therefore high levels of coprostanol in sediments strongly indicate human contamination, whereas low levels are associated with higher vertebrates as natural, biogenic sources of this sterol (Bujagic et al. 2016).

Coprostanol/cholesterol ratio indicates whether the coprostanol present within the sediment sample is of faecal origin. Coprostanol is the preferential reduction of sterols by gut microbes which could be from human or animal source. The classic ratio between the faecal stanol and parent sterol (5β -coprostanol/cholesterol) also indicates faecal contamination, with raw sewage having a value of ~ 10 , although all values greater than 0.2 may be considered contaminated (Grimalt et al. 1990). These authors also suggest a further ratio 5β - coprostanol/ (5β -coprostanol + 5α - cholestanol) which is the most commonly used diagnostic ratio of human faecal contamination. Values higher than 0.7 (red line Figure 4.7) are indicative of sewage contamination, whereas values less than 0.3 (green line Figure 4.7) are typically from uncontaminated sites and biogenic inputs that originate from natural sources. Ratio values in the range of 0.3-0.7 suggest combined sewage and natural sterol inputs (Grimalt et al. 1990). Utilising these ratios indicates whether sites are contaminated or whether the inputs are from biogenic sources (Figure 4.7).

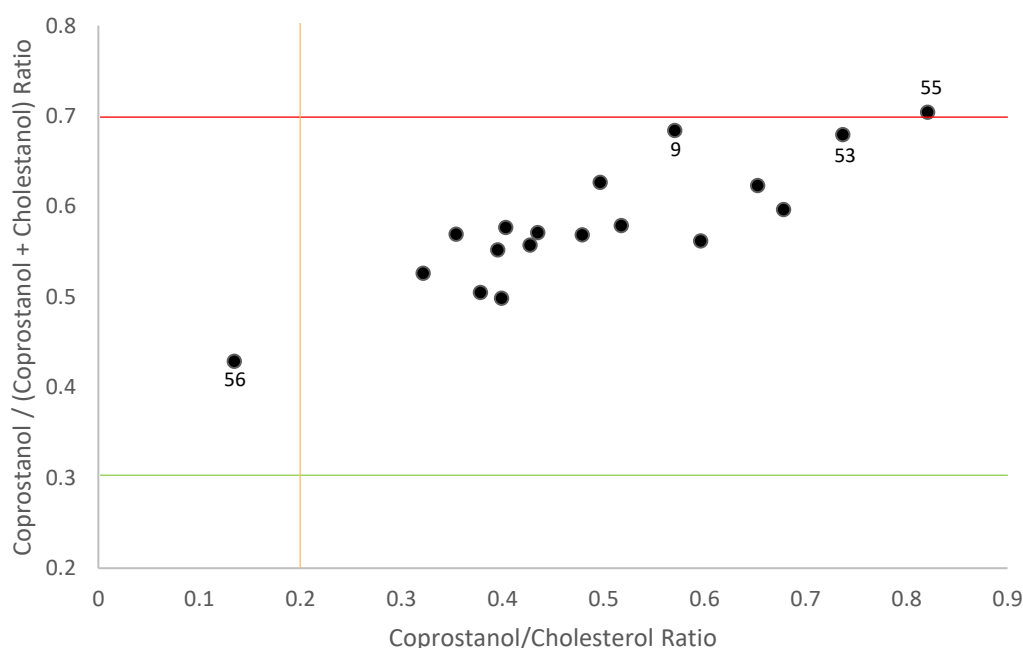


Figure 4.7: 5β -coprostanol/cholesterol and 5β -coprostanol/ (5β -coprostanol + 5α -cholestanol) ratios highlighting sites in which faecal contamination is present. Green line represents values below this are uncontaminated when using the $5\beta/5\beta+5\alpha$ ratio, whilst the red lines represent sewage contamination. Orange line represents contaminated samples using the 5β -coprostanol/cholesterol ratio.

The samples taken within the Conwy Estuary showed that site 56 was uncontaminated with the coprostanol/cholesterol ratio as it was positioned below 0.2 (Figure 4.7) however, the $5\beta/5\beta+5\alpha$ ratio categorised site 56 as possibly contaminated as the ratio was above 0.3 but below 0.7. Overall it was put down to contamination being of biogenic origins as the most likely explanation. Site 55 and potentially sites 53 and 9 were identified as contaminated by human sewage (Figure 4.7) as the ratios placed these sites close to or above the 0.7 marker on the $5\beta/5\beta+5\alpha$ ratio and classified as contaminated by coprostanol/cholesterol ratio. The remaining samples are classified as contaminated with faecal contamination by both the ratios (Figure 4.7). All these samples fall into the 0.3-0.7 category (Figure 4.7) suggesting combined sewage and natural sterol inputs. These natural inputs may also include ruminant faecal contamination from the terrestrial environment.

Further insights into the sewage contamination can be obtained by normalising coprostanol to the total sterol concentrations at each site where greater than 5-6% coprostanol suggests sewage as the source of pollution (Reeves and Patton 2001, Isobe et al. 2002, Hatcher and McGillivray

1979). This ratio can also be utilised for 24 ethyl coprostanol to suggest ruminant as the source of pollution. These ratios support the findings in Figure 4.7. Sites 56 shows no contamination from either human or ruminant (Figure 4.8) as they are positioned below the red line which signifies the 5-6 % indicator. The remaining sites showed a mixed concentration of contamination from both human and ruminant sources (Figure 4.8).

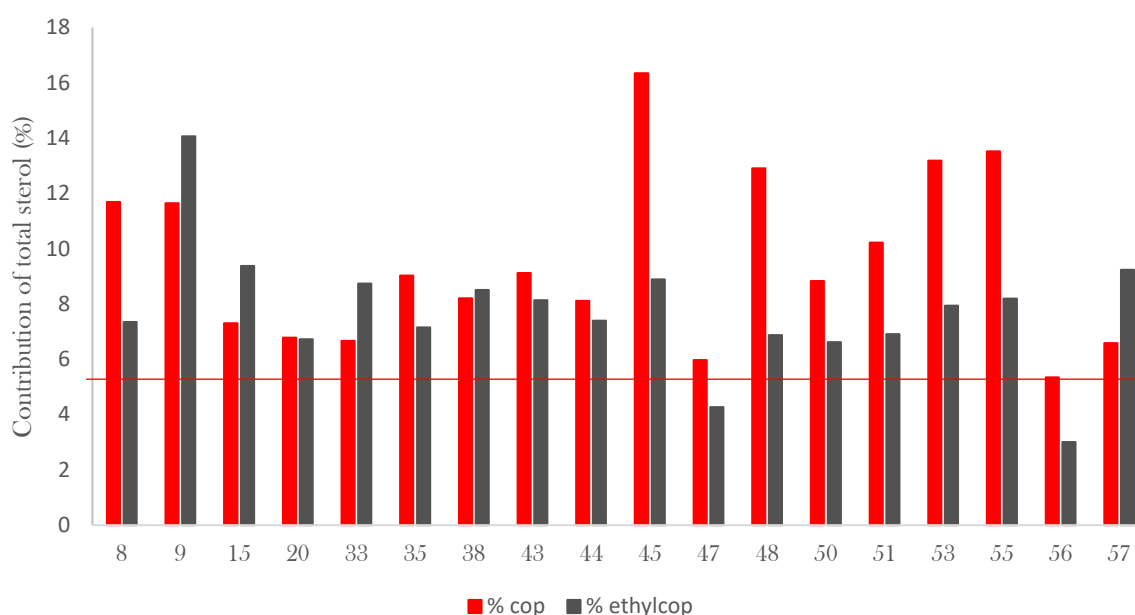


Figure 4.8: Percentage of coprostanol (red) and 24 ethylcoprostanol (green) of the total sterol concentrations. Red line represents faecal contamination.

Bethell et al. (1994) and Evershed and Bethell (1996) proposed the use of coprostanol: 5 β -stigmasterol ratio as a useful index by which human faecal deposition and that of ruminant (sheep and cows) might be differentiated. Leeming et al. (1997) extended this work by estimating the relative contribution of different faecal sources. Relative human and herbivore inputs were assessed using the index $[\text{coprostanol} / (\text{coprostanol} + 24\text{ethylcoprostanol})] \times 100$. Where values greater than 73% represented solely human origin, whereas values less than 38% were assigned as being solely from herbivores. Values between 38% and 73% were described as being from a mixture of sources. Due to a large proportion of the data potentially being of mixed source, the relative human and herbivore inputs were assessed by this index. There was no evident contamination coming from solely human or ruminant source and all sites show a mixed

signature of both human and ruminant. Bull et al. (2002) established that in the event of mixed sources, the proportion of herbivore and human derived input can be calculated by the following equations:

$$\% \text{ herbivore derived contribution} = (73 - Y) \times 2.86$$

$$\% \text{ human derived contribution} = (38 - Y) \times 2.86$$

Where Y is the mixed value given by the index $[(\text{coprostanol} / (\text{coprostanol} + 24\text{ethylcoprostanol})) \times 100]$ and the value of 2.86 is the percentile increment derived from the inverse of the difference between mean maximum percentages measured of the two scores divided by 100 [i.e. $100 / (73 - 38)$]. Such ratios whilst still used tentatively, represent a potentially powerful diagnostic tool in the assessment of faecal pollution through the analysis of 5β -stanols (Bull et al. 2002).

Utilising the percentage contribution of human and herbivorous contamination at each site (Figure 4.9 & 4.10) it is evident that there is a general pattern of increasing human contamination from the lower estuary to the more remote upper estuary, whereas herbivorous contamination is showing the reverse pattern. Sites 45 and 48 contribute greater than 73% of a human source (Figure 4.9) and therefore can be assigned to solely human sources of contamination. Sources which were assigned to solely human or herbivorous from herein are stated as 100% (Figure 4.10).

Sites contributing to 60-70% human source are mainly within the upper estuary apart from site 8 which is a mussel bed. Filter-feeding bivalves, such as mussels, have strong water-filtration ability. They are able to filter a large number of fine particulates and cause sedimentation of the suspended solids in form of faeces and pseudo faeces (Zhou et al. 2014) and therefore encourage concentration of contaminants.

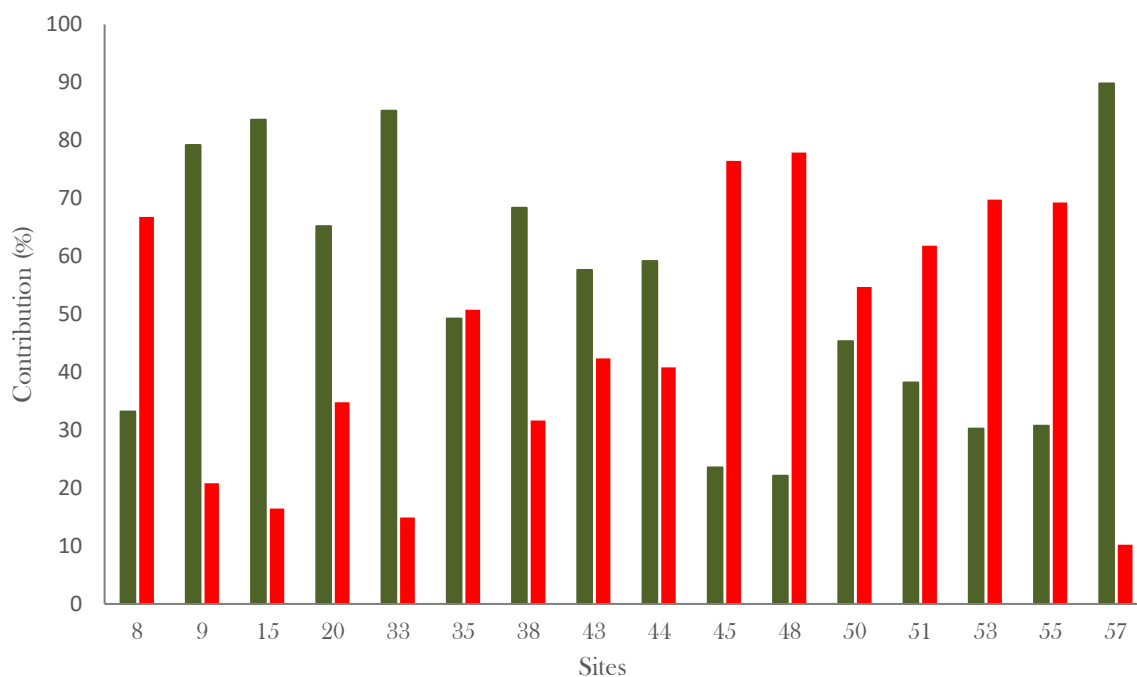


Figure 4.9 Contribution of human and herbivorous origin for all mixed source samples. Red represents human, green represents herbivorous.

Sites of human contamination (between 60-100%) are all within the vicinity of known private or intermittent discharges where the highest contributions are only within private discharges. (Withers et al. 2014) identified that septic tank systems (STS), which are widely distributed in rural areas and are numerous within the Conwy catchment, are one potential source of pollution which are usually not considered a sufficiently important source of water pollution for policy-driven regulatory controls at watershed scale. However, there is now a body of evidence linking STS discharges to water quality impairment (Carrara et al. 2008, Katz, Eberts and Kauffman 2011, Macintosh et al. 2011, Withers, Jarvie and Stoate 2011, Mallin and McIver 2012). Therefore septic tanks within these rural areas of Conwy have potential to effect water quality within the upper reaches of the estuary.

Sites 9, 15, 33 and 57 can be assigned to purely herbivorous contamination (Figure 4.9 green bar). To distinguish whether the herbivore source is truly from a ruminant source or from plant decay the ratio 24-ethylcholesterol (β -sitosterol)/24-ethylcoprostanol from (Nash et al. 2005) was utilised. Where values <1 and typically <0.25 originated from faeces, for most grasses this ratio was generally >4.

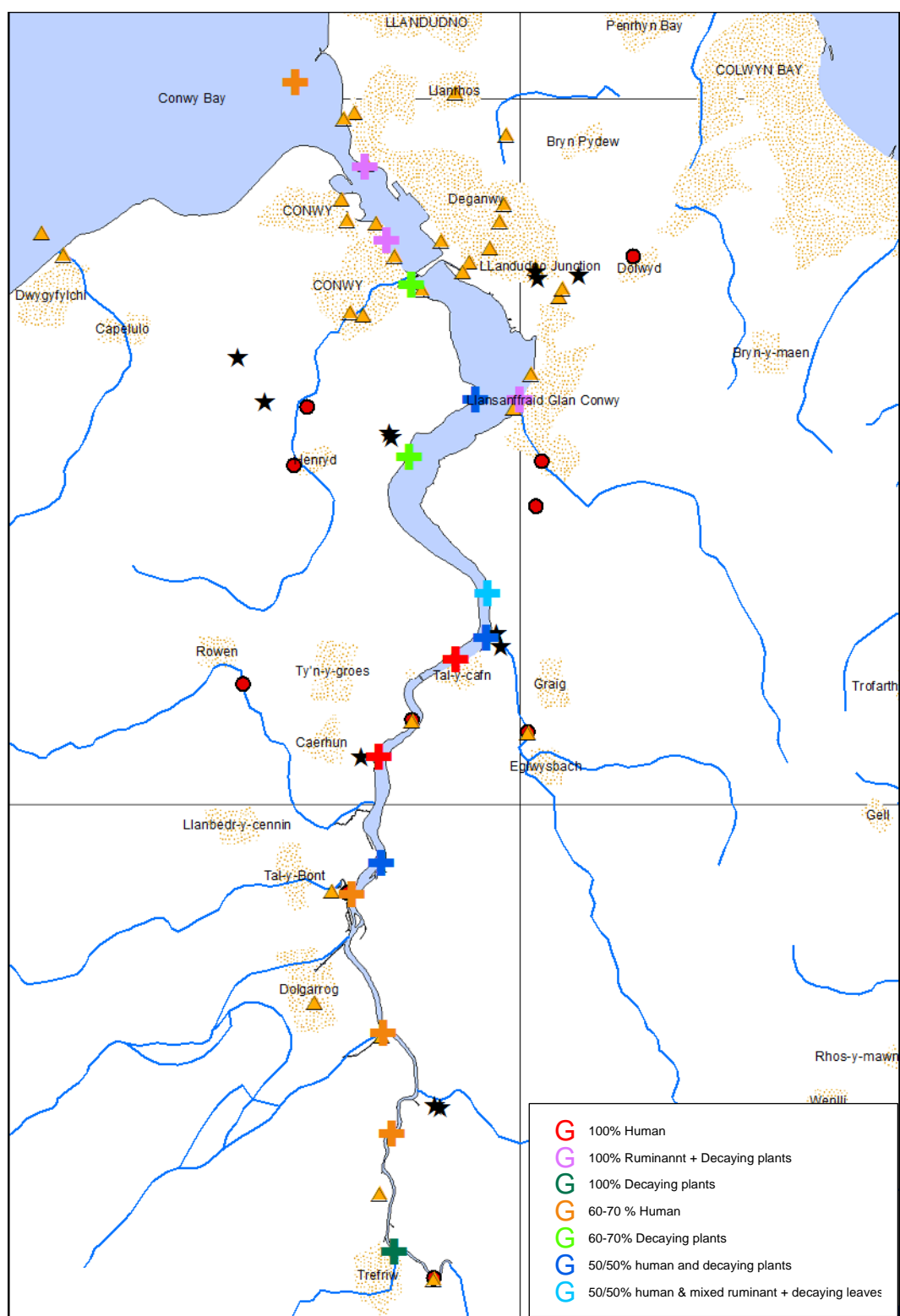


Figure 4.10: Visual presentation of the contribution of human or herbivorous origins of organic matter within the elevated lipid biomarker sites within the Conwy estuary. Triangles = intermittent discharges, Stars = septic tanks and circles = sewage treatment works to highlight potential contamination sources.

Throughout the whole of the Conwy estuary there seems to be no pure ruminant source (Figure 4.10). The upper estuary showed limited sites from an herbivorous source apart from site 57 which identified 100% decaying plant matter (Figure 4.10), this is in the vicinity of the Trefriw River which receives runoff from the Migneint peat moors. Within the lower estuary it is evident that there is a higher contribution of the contamination from the herbivorous sources (Figure 4.9 and 4.10). Sites 9, 15 and 33 identify the source as from mixture of ruminant and decaying plants, these are in the vicinity of riverine inputs and an intermittent discharge, potentially receiving catchment runoff water. Sites 20 and 38 identified as 60-70% from decaying plants are from areas of woodland and rural catchment. Within this lower catchment it is evident that these contributions are from agricultural land which is supported by the finding of Emmett et al. (2016).

4.4 Conclusion

The purpose of this study was to undertake a detailed lipid biomarker survey to establish whether the Conwy Estuary can be used as an example of a pristine environment. The Estuary could be used as a comparison to studies of estuary's suffering from extensive faecal contamination to gain an understanding of background concentrations within a rural catchment, not influenced by high populations. The results showed that the sedimentary sterols consisted of a mixture of both natural and anthropogenic sources, with the largest influence of organic matter from terrestrial origins. Coprostanol levels at 72% of sites were comparable with samples within Antarctica and classified as uncontaminated, from biogenic sources. Areas found to be elevated were generally sporadic with no evidence of gradient. Sterol ratios were used to identify the origin of the faecal contamination for the purpose of legislative drivers for water quality management plans. Within the elevated sites the sterol ratios did not indicate extreme faecal contamination from a human sewage sources. The Conwy estuary is however influenced by a mixture of human and herbivorous sources. The human sources are more evident within the upper estuary and potentially linked to septic tank miss management. However this needs to be confirmed with further monitoring within these identified areas and identification of all septic tanks within the watershed to establish point sources. Within the lower estuary the only site identified as receiving human faecal contamination was within the mussel bed of Conwy Bay, this again needs further investigation to establish the cause as this is a commercial mussel bed which suffers from fluctuating water quality classifications. This area is also within an area classified as bathing waters and therefore could impact on the local economy. Herbivorous sources were more dominant within the lower catchment with a mixture of ruminant and decaying plant matter which were potentially from agricultural land runoff. Within the upper estuary the influence of decaying plants is the greatest and in relation to the location, the most probably cause will be the Migneint moors. This study is a good example of a pristine environment within an area of low population and relatively low influence from agricultural inputs and can be used as a baseline for further studies.

CHAPTER 5

General Discussion

5.1 Discussion

Overall this study aimed at assessing the concentrations of metals, nutrients and lipids in water and sediment samples of a relatively pristine environment judged to have good to moderate ecological status within the Water Framework Directive River Basin Management plans. With this assumption, the data obtained has been used to assess existing models that aimed to define elevated versus baseline concentrations. These approaches were detailed in chapters 2 to 4. To conclude, there were a number of locations within the estuary that yielded higher concentrations deemed above background levels. These locations are further scrutinised in this conclusion chapter to highlight potential sources of contamination in the estuary. The tracing of these contaminant sources was outside the scope of this study, however, they may pinpoint towards future recommended research in this area. In addition, concentrations in the natural environment fluctuate. As a proportion of locations sampled fell below those that are deemed enriched for metals, nutrients and lipids, summary data of these pristine locations is presented in this conclusion chapter.

Overall findings within all sediment samples showed higher concentrations of compounds within the fine sediment size fraction compared to the coarse sediment size fraction. This supports the well-established thought that due to physical and chemical characteristics, fine grained particles (clay minerals, organic and inorganic colloids) have greater capacity to adsorb heavy metals, nutrients and lipid compounds in their structures and transport them to the aquatic environment (Mil-Homens et al. 2013). The importance of utilising sediment, which acts as a historic sink of these compounds (Carballeira et al. 2000), within an estuary aids in understanding the potential inputs from both natural and anthropogenic sources to infer whether an area is pristine or contaminated.

Traditional techniques of nutrient and metal concentrations within sediments which utilise legal limits (sediment quality guidelines) to determine whether an area is influenced by anthropogenic inputs has severe limitations as the criteria is limited to freshwater and seawater with no limits specifically for the transitional zone of estuaries and the proportion of anthropogenic sources are not differentiated from natural backgrounds (Renzi, Bigongiari and Focardi 2015). High total elemental concentrations do not necessarily indicate the degree of contamination (Tam and Yao 1998). The assessment of anthropogenic contributions to sediments therefore must be accompanied primarily by the estimation of the contribution by natural processes (Windom et al. 1989). Within this study enrichment factors have been calculated for both metals and

nutrients in chapters 2 & 3 to establish the difference. However it is evident that for nutrients the C/N ratio needs to be utilised along with the enrichment factor to verify that the organic fraction is being calculated not the inorganic.

Within the Conwy sediments, some sites were found to be enriched over baseline concentrations (Table 5.1). Enrichments were only seen sporadically at a multitude of sites which were generally enriched in more than one element suggesting these locations are effected by diverse anthropogenic influences or reflecting favourable environmental conditions for supporting the accumulation of contaminated particles. Overall trends identified that metal contamination was highest within the upper estuary near the tidal limit compared to more marine influenced sites, which is consistent with conservative mixing and the desorption of positively charged elements from the change of sediment particle charge from negative to neutral due to decrease in pH within saline waters. Cadmium was the only element which showed an overall enrichment within a large section of the study site. Other elemental enrichments were only seen at a very few sites, nitrogen and phosphate enrichment was evident within the lower estuary which supports Williams et al. (2012) and Emmett et al. (2016) studies where they found that the highest concentrations of phosphate and nitrates in water were associated with agricultural land within the lower catchment. . The sterol contributions identified that the origin of the organic matter within the upper estuary near the tidal limit was dominated by human sewage whereas agricultural sources dominated the lower estuary, where phosphate enrichment was seen apart from one site within the commercial mussel beds where enrichment was found from a human source and greater than four metals with phosphate within the vicinity. Potentially due to mussels being filter feeders, which can filter a large number of fine particles, removing particulate matter from the water column, causing sedimentation of the suspended solids in form of faeces and pseudo faeces (Zhou et al. 2014).

Within the limited surveys of estuarine water the majority of the sites showed background conditions with a very limited number of samples which showed higher levels (Table 5.1). High coliform counts were more apparent within the lower estuary compared to the upper where elevated coliform counts were found in inlets rather than the estuary which reflects areas identified as originating from human sewage (Figure 5.1 & 5.2). Suspended particulate nutrients (C, N & P) showed higher enrichment of carbon within the upper estuary compared to the lower, which supports the findings of Emmet et al. (2016) and Williams et al. (2012) who concluded that high dissolved organic carbon was greater within drainage from the upland Migneint peat area and peaty gley soils within the southeast part of the catchment which drains into the upper

estuary section within this study area. Whereas nitrogen and phosphate enrichment was evident within the lower estuary.

Table 5.1: Enhanced samples identified within the fine scale survey of the Conwy Estuary. Sediment samples; metals and nutrients are in enrichment factors (EF), sterols in % contribution; water samples (estuary and inlets) for nutrients and coliforms in $\mu\text{mol/L}$ and CFU/100ml respectively. Shortened abbreviations not in the text are particulate phosphate (PP), particulate carbon (PC), particulate nitrogen (PN), human (HU), ruminant & decay (R&D), decay (DE) & coliforms (Col).

Site	Sector	Estuarine sediments																		Estuary water								Inlets (winter)								Inlets (summer)											
		Metals																Nutrient		Sterols		Nutrients & coliforms								Nutrients & coliforms								Nutrients & coliforms									
		Cu	Fe	Mn	Ni	Li	Ba	V	Zn	Pb	Cd	U	Co	As	Cr	La	Th	TN	TP	HU	R+D	DE	PP	PC	PN	NO3	NH4	PO4	Col	PP	PC	PN	NO3	NO2	NH4	PO4	Col	PP	PC	PN	NO3	NO2	NH4	PO4	Col		
7	1																	2																													
8	1	2.2	2.2					3.5						2.2					66.8																												
9	2	9.3		2.9	2.3	2.5	3.3											4		100							0.5				5	180	12	282	18	191733			4	54	9	243	33	151900			
10	2																	3																													
12	2																													1										1							
13	2																2																														
14	2																	2																													
15	2																			100																											
16	2																									39			21167																		
17	2																																														
20	3																		34.8		65.2										134									105							
21	3																					2				13	7		4237																		
22	3																															130															
24	3							2.9																																							
26	3																	4																													
27	3																										28					161								120				5			
28	3																																														
29	3																																2	293		5				3	112			14	21167		
30	3																									20	9		3752																		
33	3																			100												138										81					
34	3	2																																													
35	3					2														50.8		49.2																									
37	4	2.2								2																																					
38	4											2.4								31.6		68.4					7		5698																		
39	4											2.4											20																								
40	4					2.1			4										5																												
41	4										2.5							4																													
42	4																																														
43	4										2.5									42.4	57.6										128	12	116						104						9500		
44	4										2.2									40.8		59.2																									
45	4	3.1	4.5	8.4	4.1	5.7	5.6	2.3	5.3	3.1	2.5	4.2	3.7	2.8	2.5	3	3.2		1.8	100												166									79						
46	4																									</																					

Incorporating all the different survey techniques; metals, nutrients, sterols and coliforms a clearer understanding of potential sources can be identified within certain areas of the estuary. Combining Figure 5.1 and 5.2 it is evident there are 5 distinct different areas of contamination within the Conwy estuary. Two sites in the vicinity of Trefriw (sector 6) showed enrichment of greater than ten metals, these will be influenced by the River Crafnant which empties into the Conwy estuary at this location and has been identified by several authors as contaminated due to historic metal mining (Elderfield, Thornton and Webb 1971, Elderfield et al. 1979, Brydie and Polya 2003, Zhou, Liu and Abrahams 2003, AMEC 2011). In addition, contributions of metals to these sites are also likely to be from sewage effluents as identified by the sterols, nutrients and coliforms concentrations which confirm potential sewage contamination within this area. Site 45 (sector 4) at Tal-Y-Cafn shows an extensive enrichment of all metals analysed. Within this site coliforms were elevated, nitrogen and phosphate concentrations were enriched and sterols identified human sewage as the origin of the organic matter. Riverine input adjacent to the site was identified to have elevated nitrogen and phosphate concentrations likely to be contributed by sewage treatment works and agricultural runoff within the river catchment. Site 45 is therefore being influenced by the historic mines, sewage effluent and agricultural runoff. As discussed within chapter 3 the physical environment within this area is likely to be encouraging flocculation potentially encouraging contaminant locked sediments to aggregate (Ji 2007) resulting in high particulate concentrations and potentially encouraging deposition of sediments. Within sector 5 & 6 the sterols show an influence from human sewage, with high nitrogen, phosphate and coliforms. Nitrogen, phosphate and coliforms showed elevated concentrations within localised riverine inputs devoid of sewage treatment works with the suggestion that these levels are potentially from septic tank seepage. This would be consistent with Withers et al. (2014) notion that septic tanks which are widely distributed within rural and peri-urban areas are a potential source of nutrient pollution. Sector 2-4 up until Tal-Y-Cafn showed an influence from agricultural runoff with the sterols supporting the organic matter origin to be from an herbivorous source. The majority of riverine inputs in this section had high concentrations of nitrogen. This is consistent with the findings of Emmett et al. (2016) who found the highest concentrations of nitrates within the lower catchment and concluded losses were indicative of agricultural land. The mussel bed site, within sector 1, (Figure 1.1 & 1.2, site 8) appears to be a depositional zone potentially due in part to the physical characteristics of filter feeders (Zhou et al. 2014). The mussel bed area showed elevated concentrations of sewage effluent, nitrogen and phosphate. Due to the lack of these parameters within the lower estuary, the likely source is from intermittent discharges within the Conwy Bay (Figure 1.3).

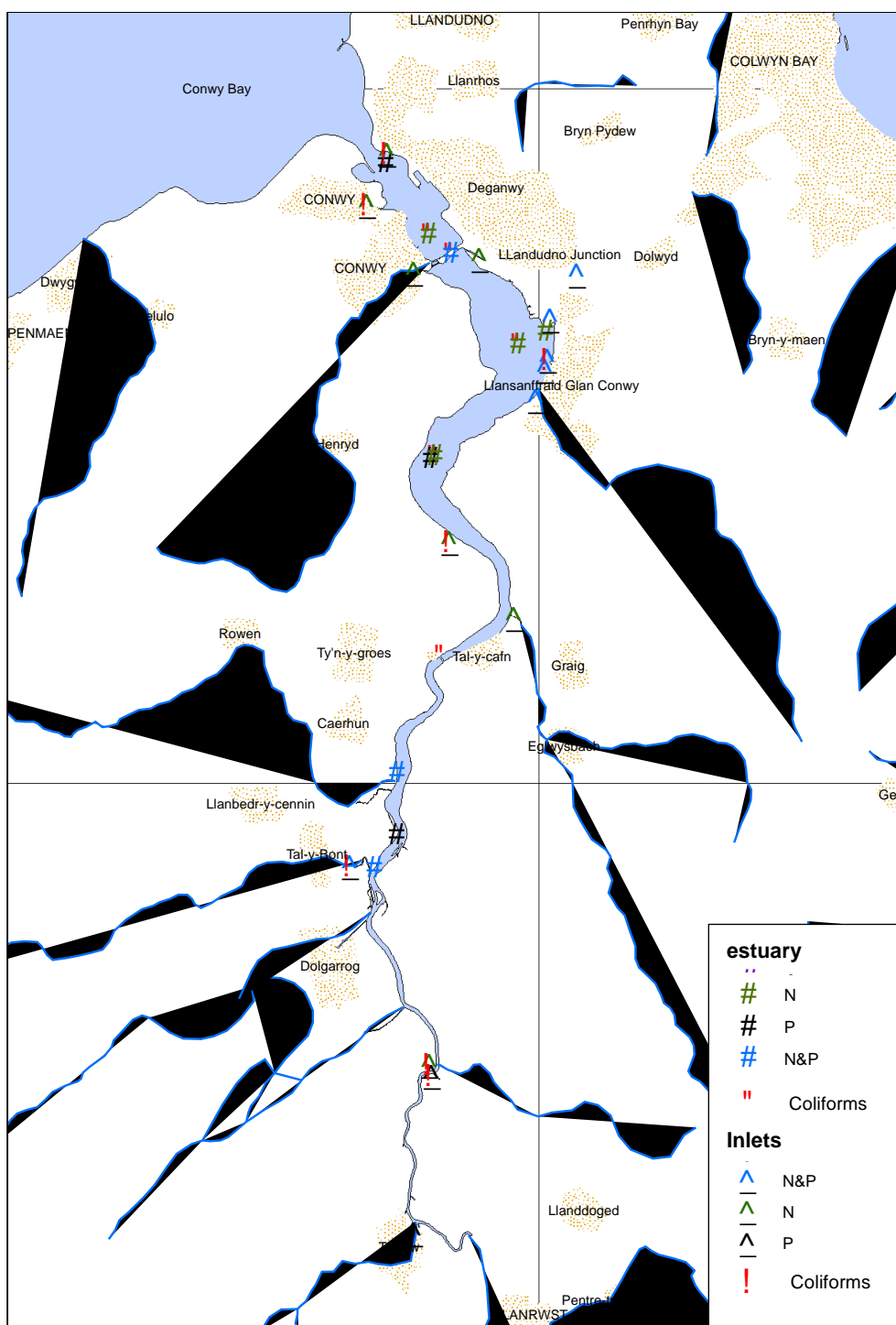


Figure 5.1: Identified elevated concentrations of dissolved and particulate nutrients from estuarine waters and freshwater inflows to the Conwy Estuary. This highlights the locations where multiple nutrients species exist which may assist with source identification.

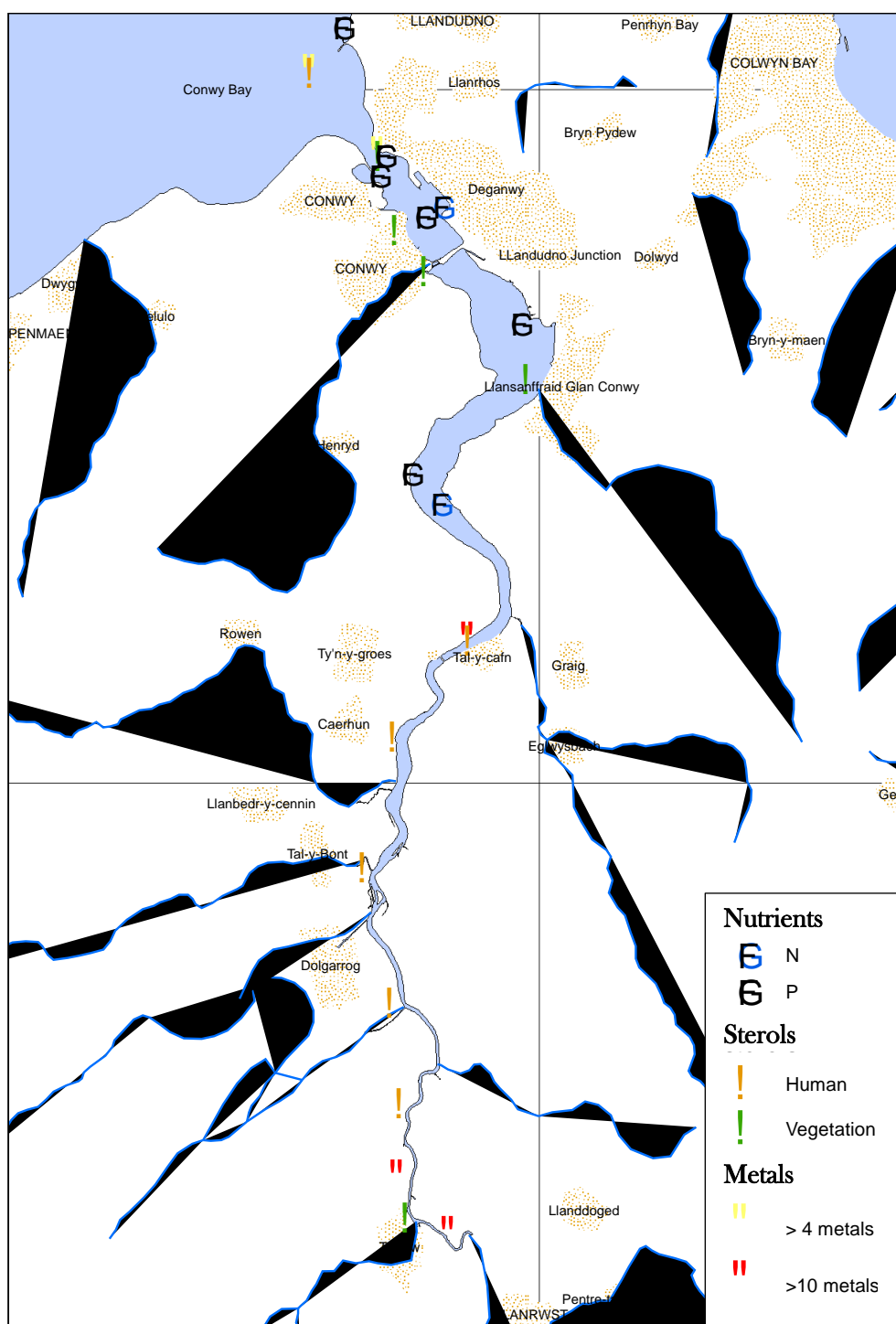


Figure 5.2: Identified enriched sediment samples for metals and nutrients and elevated concentrations for lipid biomarkers which assist in classifying the origins of organic matter to the Conwy estuary. This highlights the locations where multiple organic and inorganic chemicals are concentrating which may assist with source identification.

In relation to the samples taken for this study, a proportion of sites fell below enriched concentrations of metals, nutrients and lipids and therefore were deemed as baseline concentrations for UK estuaries. However, cadmium has been excluded due to enrichment at a large proportion of sites and therefore calculated natural conditions cannot be relied upon. Table 5.2 identifies concentration ranges of these baseline concentrations within coarse to fine grain sediments, due to the consistently high concentrations of either metal, nutrients or lipid biomarkers within sites in sector 5 & 6 these sites have been omitted from the baseline range calculations.

Table 5.2: Concentration range for TN ($\mu\text{mol g}^{-1}$), TP ($\mu\text{mol g}^{-1}$), metalloids ($\mu\text{g g}^{-1}$) and lipid biomarkers ($\mu\text{g g}^{-1}$) of baseline concentrations within the Conwy estuary.

Element	Minimum	Maximum		Lipid biomarker	Minimum	Maximum
TN	21.42	164.20		C13	0.001	0.19
TP	3.78	27.75		C14	0.021	3.80
Li	1.12	16.14		C15	0.001	1.92
Be	0.06	0.62		C16	0.004	10.32
V	5.23	26.83		C17	0.006	1.20
Cr	2.36	21.47		C18	0.021	15.25
Mn	74.00	470.80		C19	0.023	0.91
Fe	2362.80	18487.36		C20	0.003	11.88
Co	1.25	8.40		C21	0.005	0.93
Ni	2.26	25.27		C22	0.004	6.24
Cu	0.001	19.46		C23	0.001	0.59
Zn	7.14	102.28		C24	0.002	1.14
As	2.29	7.65		C25	0.001	0.18
Se	0.28	1.54		C26	0.015	0.57
Y	1.14	7.18		C27	0.009	0.01
Ba	0.94	30.95		C28	0.007	0.19
La	1.32	6.87		C30	0.005	0.01
Sm	0.31	1.72		Coprostanol	0.003	3.02
Tb	0.046	0.29		Cholest-5,22	0.004	1.42
Ho	0.04	0.25		Cholesterol	0.001	8.54
Lu	0.008	0.08		5 α Cholesterol	0.009	2.29
Pb	2.81	38.88		Brassicasterol	0.010	3.16
Bi	0.02	0.35		24-ethylcoprostanol	0.012	2.16
Th	0.34	2.49		Campesterol	0.007	2.61
U	0.05	0.75		Stigmasterol	0.004	1.54
				β -sitosterol	0.008	23.26
				Dinosterol	0.017	1.06

Taking into consideration the dissolved, suspended particulate matter concentrations and sediment contents of nutrients, metals and lipid biomarkers it is evident that different sampling regimes are needed for different mediums. To understand the behavioural trends within the dissolved inorganic and suspended particulate nutrient fraction intensive sampling needs to be undertaken incorporating the full salinity range from zero to 35 to ascertain whether conservative mixing behaviour is occurring within the estuary. This study found no statistical temporal difference between high and low water concentrations but did find spatial differences from different sides of the estuary which has an implication for monitoring. Considerations need to be taken with reference to the underlying sediments and sampling dissolved and particulate organic matter within areas with tidal mudflats in comparison to coarse grained sediments, rather than just location specific within an estuary transect. Although no difference with high and low water was evident within the tidal survey, differences were evident between the three dissolved and suspended particulate matter surveys due to different states of the tide and seasonality, therefore for management purposes sampling an estuary during different seasons (incorporating flood and drought events), on different states of the tide and possibly a full tidal cycle would be paramount to understand the full behavioural trends within the estuary. Initial surveys of only incorporating end member samples (freshwater and seawater) with limited samples between will allow an estimation of whether the system is conforming to conservative mixing and to identify whether an estuary is impacted by human activity to ensure if further investigation is needed resulting in a more economically viable approach to managers. Within the sediment fraction all constituent concentrations were significantly higher within samples with high amounts of fine grained sediments compared to coarse grained sediments. For management purposes the recommendation would be a less intensive survey, concentrating on fine grained depositional areas unless the survey interest was Strontium and Boron concentrations. This medium is useful for managers to understand the historic concentration within the estuary without intensive dissolved sampling, and allows us to understand the potential inputs from both natural and anthropogenic origins. The present method of utilising the sediment quality guidelines to establish an effect level is very misleading and has been shown within this study to overestimate impacts due to not normalising against grainsize and the natural concentrations. The known behavioural trend of constituents which was reflected within this survey is high concentrations within the terrestrial freshwater end member reducing to low concentrations with the high salinity marine end member. This is due to desorption of elements within the higher pH (saline conditions) where sediment charge becomes neutral and therefore constituents release into the dissolve phase which attach to chloride ions within saline waters. This mechanism enables a

simple prediction which is a helpful tool for managers to assess the general condition of an estuary with sampling within the two end members and limited samples between, with concentrations deviating from this highlighting a potential elevation above background. Techniques devised in this study can be utilised by managers to easily understand elevated levels over natural condition without expertise needed. Caution as described in chapter 3 needs to be taken with carbon and nitrogen ensuring the organic element is being calculated not the inorganic.

Concentrations given within this final discussion chapter (Table 5.2) can be utilised for background condition within areas of high humic (peat) sources, limited industry, sparse to moderate population with the main human influences of tourism i.e. marinas, water treatment works and limited agricultural runoff. However the techniques within this study can be utilised for all estuaries to establish background condition for nutrients and metals and identify organic matter origins via lipid biomarkers.

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Appendices

Appendix 1

Table 2.1

Site	Latitude	Longitude	Metalloid Concentration µg g ⁻¹																																Depth	Salinity	Sediment composition (%)											
			Li	Be	B	Sc	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Y	Mo	Ag	Cd	In	Sn	Sb	Cs	Ba	La	Sm	Tb	Ho	Lu	W	Hg			Tl	Pb	Bi	Th	U	% Gravel	% Sand	% Mud	TC	OC	inorg C	CaCo3
1	53.289280	-3.871030	1.70	0.08	6.15	0.32	7.10	3.02	97.20	3931.34	1.79	3.43	0.50	12.29	3.37	0.28	0.86	16.91	1.39	0.07	0.001	0.003	0.003	0.05	0.001	0.13	1.42	1.62	0.35	0.06	0.05	0.01	0.02	0.04	0.004	3.50	0.02	0.40	0.06	1.40	33.39	0.00	98.20	1.80	0.23		0.23	1.92
2	53.292470	-3.859390	1.53	0.06	4.16	0.21	5.87	2.66	101.44	3652.57	1.37	2.77	0.43	7.79	2.80	0.32	0.76	37.21	1.17	0.05	0.001	0.01	0.002	0.01	0.001	0.11	1.52	1.32	0.31	0.05	0.04	0.01	0.02	0.09	0.004	2.81	0.04	0.34	0.06	2.42	33.39	0.00	97.60	2.40	2.09	1.28	0.81	6.75
3	53.298310	-3.850810	8.74	0.35	7.87	1.92	16.33	12.69	244.72	13704.74	4.64	15.37	5.34	21.22	6.15	0.79	8.29	37.36	4.35	0.48	0.001	0.03	0.013	0.01	0.001	0.80	8.33	3.82	0.98	0.17	0.15	0.05	0.02	0.08	0.042	10.51	0.12	1.67	0.62	3.65	32.91	0.00	15.80	84.20	0.46	0.01	0.45	3.75
4	53.299690	-3.856690	2.49	0.08	5.32	0.27	8.28	3.64	184.86	4986.73	1.91	4.25	0.63	11.71	4.63	0.34	0.85	206.34	1.72	0.07	0.001	0.01	0.004	0.04	0.001	0.11	3.45	1.78	0.42	0.06	0.06	0.02	0.02	0.04	0.007	4.43	0.02	0.37	0.08	4.29	33.22	0.00	96.80	3.20				
5	53.318440	-3.848060	2.52	0.08	4.40	0.48	8.01	5.14	178.56	4189.26	2.20	4.80	0.16	11.94	2.49	0.52	1.06	53.10	2.11	0.08	0.001	0.03	0.001	0.06	0.001	0.16	3.25	2.13	0.51	0.08	0.07	0.02	0.02	0.04	0.009	5.09	0.03	0.79	0.13	4.18	16.67	0.00	97.30	2.70	0.63	0.02	0.63	5.25
6	53.315610	-3.854250	2.10	0.08	6.15	0.40	7.37	3.45	144.66	3483.45	1.83	3.92	0.001	9.68	3.05	0.35	0.94	111.32	1.66	0.06	0.001	0.02	0.001	0.07	0.001	0.13	2.17	1.89	0.42	0.07	0.06	0.02	0.02	0.04	0.006	3.53	0.08	0.51	0.10	3.69	33.12	0.00	98.10	1.90	0.58	0.02	0.56	4.66
7	53.310750	-3.844720	2.16	0.09	7.26	0.46	8.33	4.51	170.59	4876.90	1.94	4.37	1.32	10.00	2.77	0.35	1.13	73.49	2.31	0.08	0.001	0.03	0.004	0.04	0.001	0.17	3.64	2.35	0.53	0.08	0.08	0.02	0.02	0.04	0.007	4.42	0.03	0.50	0.14	2.21	32.15	0.00	96.80	3.20	0.67	0.01	0.66	5.50
8	53.304670	-3.845750	4.62	0.16	6.49	1.61	26.83	9.46	218.10	10376.08	3.29	7.22	2.03	16.90	3.56	0.51	1.04	135.49	2.68	0.13	1.418	0.03	0.015	0.07	0.001	0.19	2.16	2.31	0.70	0.12	0.11	0.03	0.20	0.19	0.013	4.40	0.03	0.66	0.16	3.78	33.04	0.00	98.60	1.40				
9	53.294280	-3.834890	5.80	0.16	4.68	0.60	10.43	5.87	374.11	8262.92	3.60	9.88	7.49	25.23	4.88	0.45	1.64	56.41	2.24	0.13	0.001	0.05	0.010	0.03	0.001	0.17	8.33	1.93	0.62	0.10	0.08	0.02	0.02	0.04	0.010	6.77	0.05	0.63	0.11	3.83	32.49	0.00	96.40	3.60	0.74	0.02	0.72	6.00
10	53.291610	-3.836060	3.04	0.13	7.25	0.52	8.41	5.41	170.20	5579.12	2.56	5.52	2.16	24.56	3.32	0.38	1.99	38.72	1.95	0.16	0.153	0.15	0.006	0.05	0.166	0.26	3.80	2.38	0.51	0.08	0.07	0.02	0.10	0.18	0.018	6.04	0.05	0.61	0.10	8.67	32.64	0.00	95.90	4.10	2.56		2.56	
11	53.289470	-3.827610	10.9	0.41	11.26	1.92	19.66	15.97	560.55	12193.01	6.18	18.22	13.76	57.05	6.34	1.33	7.75	106.05	5.76	0.25	0.016	0.41	0.024	0.10	0.001	0.83	19.23	5.33	1.33	0.22	0.20	0.06	0.02	0.04	0.064	27.26	0.23	2.03	0.38	5.09	33.01	0.00	48.62	51.38	2.93	1.00	1.93	16.08
12	53.288528	-3.834028	7.22	0.26	9.30	1.28	14.22	10.85	370.75	8808.71	4.35	12.37	6.38	34.54	4.62	0.85	4.42	107.42	4.41	0.18	0.001	0.23	0.012	0.08	0.001	0.50	12.96	4.07	1.00	0.17	0.15	0.04	0.02	0.04	0.039	17.49	0.14	1.42	0.26	1.01	32.89	0.00	61.70	38.30	2.1	0.77	1.33	11.08
13	53.287860	-3.822110	15.39	0.56	10.66	2.62	23.33	20.28	422.28	17472.59	7.26	23.05	16.77	61.44	6.43	1.27	9.49	86.45	7.30	0.39	0.005	0.35	0.039	0.12	0.001	1.09	35.11	6.41	1.66	0.28	0.25	0.08	0.05	0.14	0.074	36.93	0.33	2.58	0.65	0.84	30.43	0.50	65.89	33.61	2.52	0.72	1.80	14.99
14	53.286472	-3.825944	2.89	0.14	5.85	0.46	7.30	5.02	146.64	4774.52	2.21	4.51	1.46	20.15	2.90	0.38	1.48	25.89	1.98	0.09	0.001	0.10	0.006	0.10	0.107	0.20	3.73	2.68	0.57	0.09	0.08	0.02	0.02	0.04	0.010	4.77	0.05	0.64	0.11	4.53	32.57	22.02	76.74	1.24	0.43	0.08	0.35	2.92
15	53.284920	-3.829860	14.80	0.52	14.38	2.34	21.69	18.79	461.14	18487.36	7.15	22.19	16.42	67.75	6.77	1.20	9.34	135.44	7.06	0.22	0.090	0.56	0.034	0.12	0.055	1.07	27.38	6.50	1.61	0.29	0.25	0.08	0.04	0.36	0.077	32.75	0.26	2.47	0.46	1.08	32.46	0.00	8.70	91.30	0.45	0.09	0.36	3.00
16	53.285440	-3.822110	13.05	0.48	12.42	1.92	19.21	15.82	413.66	13716.55	5.93	18.41	13.59	51.81	6.00	1.03	8.42	112.22	5.75	0.18	0.024	0.34	0.030	0.12	0.001	0.90	26.87	5.49	1.40	0.24	0.22	0.07	0.05	0.09	0.063	30.83	0.27	2.12	0.43	0.99	32.60	0.00	34.17	65.83	3.06	1.13	1.93	16.08
17	53.281860	-3.825830	1.57	0.08	4.71	0.27	5.50	2.49	75.65	2954.06	1.77	2.85	0.00	20.41	2.96	0.28	0.78	5.15	1.14	0.06	0.001	0.08	0.001	0.08	0.001	0.11	0.94	1.42	0.32	0.05	0.04	0.01	0.02	0.04	0.003	5.41	0.02	0.34	0.05	2.17	28.15	0.00	96.70	3.30	0.06	0.04	0.02	0.17
18	53.283861	-3.819111	8.32	0.31	9.65	1.21	14.40	10.52	245.72	9194.87	4.05	11.83	6.94	35.70	3.82	0.70	5.39	45.86	3.94	0.20	0.024	0.20	0.020	0.07	0.001	0.61	19.87	3.85	0.99	0.16	0.15	0.05	0.06	0.14	0.044	19.75	0.18	1.39	0.35	1.69	31.85	0.15	65.20	34.65	1.36	0.68	0.68	5.66
19	53.282528	-3.820611	12.36	0.44	13.55	2.04	19.25	15.05	327.83	109893.																																						

Table 2.2

	LOD	LOQ	Mean CRM	Certified value
Li	0.012	0.041		
Be	0.002	0.006		
B	1.836	6.119		
Sc	0.027	0.091		
V	0.022	0.074		
Cr	0.042	0.141		
Mn	0.161	0.536	861.58 ± 256.28	1020
Fe	77.000	256.668	25293.28 ± 11596.87	34000
Co	0.003	0.011	9.87 ± 3.09	12
Ni	0.093	0.309	14.55 ± 3.25	17
Cu	0.001	0.005	41.00 ± 9.07	49
Zn	0.288	0.960	64.40 ± 11.87	71
As	0.012	0.041		
Se	0.009	0.029		
Rb	0.010	0.032		
Sr	0.040	0.133		
Y	0.002	0.007		
Mo	0.007	0.023	0.79 ± 0.27	1
Ag	0.001	0.005	0.29 ± 0.009	<0.2
Cd	0.014	0.047		
In	0.001	0.004		
Sn	0.028	0.009		
Cs	0.002	0.005		
Ba	0.030	0.100		
La	0.001	0.005		
Sm	0.003	0.009		
Tb	0.000	0.001		
Ho	0.000	0.000		
Lu	0.000	0.001		
W	0.040	0.134		
Hg	0.080	0.267		
Ti	0.001	0.003		
Pb	0.777	2.591	13.29 ± 0.91	14
Bi	0.013	0.043		
Th	0.015	0.049		
U	0.002	0.007		

Appendix 2

Table 3.1

Site	Nutrient concentration (µmol/L)							
	PO4		NO2		NO3		NH3	
	summer	Winter	summer	Winter	summer	Winter	summer	Winter
1	0.69	0.05	0.13	0.96	4.30	6.44	7.78	89.00
2	1.51	1.60	0.15	3.02	0.40	115.72	2.52	109.89
3	0.43	0.88	0.11	1.09	2.84	14.50	11.96	4.56
4	13.61	0.95	0.69	0.36	95.97	220.88	3.64	3.83
5	0.72	5.22	0.27	0.77	111.65	293.42	11.28	1.79
6	33.11	18.26	9.43	11.88	53.96	180.11	243.24	281.63
7	0.20	0.05	0.04		18.57	17.10	0.95	0.92
8	1.15	0.59	0.41	0.17	113.29	35.01	4.47	3.65
9	0.78	0.15	0.13	0.02	12.48	11.63	5.53	1.49
10	0.36	0.19	0.14	0.12	33.89	30.04	4.76	2.57
11	0.74	2.83	0.13	0.15	9.59	54.10	15.42	1.38
13	0.12	1.76	0.21	0.37	16.92	130.39	5.57	5.52
14	0.15	0.05	0.16	0.16	10.87	15.04		
15	0.44	0.86	0.09	1.42	5.21	14.66	5.42	5.65
16	7.18	1.87	7.60	1.51	144.41	193.50	93.55	56.55
17	0.72	0.05	0.38	0.36	17.69	16.32	260.38	490.80
18	1.75	0.72	0.72	0.33	80.80	138.08	9.37	
19	4.57	1.45	0.24	2.73	120.26	161.20	4.37	55.93
20	1.92	0.63	1.54	0.61	25.88	66.68	37.11	1.03
21	1.29	0.58	1.04	0.31	105.08	134.19	7.14	1.02
22	1.11	0.82	0.83	0.82	78.88	166.47	5.72	0.76
23	0.43	0.13	0.11	0.18	11.47	29.83	2.02	0.45
24	0.21	0.05	0.09	0.16	15.71	18.44	1.49	3.09

Table 3.2

Site	Nutrient concentration (µmol/L)										Coliforms (CFU/100ml)	
	PTC		POC		PTN		PON		PTP			
	summer	Winter	summer	Winter	summer	Winter	Summer	Winter	Summer	Winter	summer	Winter
1	22.80	28.74	11.89	25.77	0.73	2.19	0.73	2.19	0.05	0.10	870.00	701.52
2	104.07	128.35	43.36	124.89	2.55	11.47	2.55	10.20	0.08	0.30	9500.00	1278.72
3	9.56	6.32	6.32	4.63	0.34	0.45	0.23	0.45	0.01	0.02	416.67	1918.05
4	15.42	15.80	6.94	20.04	1.13	0.85	0.57	0.85	0.03	0.04	1550.00	1278.72
5	65.91	33.82	73.72	30.35	2.55	1.91	2.55	1.91	0.14	0.10	21166.67	976.80
6	65.22	56.89	65.22	55.50	4.08	5.10	3.06	5.10	0.15	0.20	191733.30	151900.00
7	20.81	4.27	11.21	4.27	0.39	0.39	0.78	0.39	0.01	0.00	3.33	0.00
8	130.44	17.35	135.29	11.79	4.59	1.02	4.59	1.02	0.10	0.07	9300.00	410.67
9	20.81		17.03		0.93		0.93		0.07	0.05	1406.67	719.28
10	43.52	12.61	40.37	13.88	2.32	0.93	1.85	0.93	0.05	0.02	8666.67	661.56
11									0.01		156.67	2.00
12	63.14	98.52	83.95	124.19	3.06	4.59	3.06	5.61	0.16	0.12	1276.67	350.76
13	9.25	6.55	35.85	3.85	1.13	0.85	1.13	0.57	0.03	0.03	8300.00	881.34
14	53.37	573.19	50.70	530.50	3.14	0.78	2.35	1.18	0.06	0.01	153.33	5.67
15	6.59	6.94	9.02	9.02	0.51	0.51	0.51	0.51	0.02	0.00	0.00	65.33
16	25.83	5.78	6.94	5.78	0.57	0.28	1.13	0.28	0.04	0.04	4733.33	843.60
17	57.24	341.70	36.42	27.75	2.55	19.12	2.55	2.55	0.14	0.36	18066.67	559.44
18	9.13	3.47	3.10	3.65	0.40	0.27	0.27	0.27	0.01	0.01	1066.67	630.48
19	16.65	15.96	13.88	17.35	1.53	1.02	1.02	1.02	0.09	0.08	2006.67	2273.28
20	15.77	14.51	11.98	11.98	0.93	0.93	0.46	0.93	0.07	0.06	24700.00	702.63
21	11.43	8.57	0.82	7.75	0.60	0.60	0.60	0.60	0.03	0.04	1680.00	1540.25
22	23.71	30.07	12.14	31.22	0.85	14.45	0.85	1.70	0.03	0.07	1913.33	740.15
23	6.73	2.45	7.55	2.65	0.15	0.30	0.30	0.15	0.01	0.01	2390.00	590.10
24	4.96	0.99	5.78	0.83	0.24	0.12	0.24	0.12	0.009		553.33	620.35

Table 3.3

	Nutrient concentration (μmol/L)								Coliforms (CFU/100ml)		Salinity	
	PO4		NO2		NH4		NO3					
Tide	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
TS1	0.52	0.36	0.16	0.23	4.39	4.17	5.77	4.07	262	317	31.25	30.94
TS2	0.30	0.16	0.25	0.50	4.80	7.04	12.67	12.60	2516	4237	27.80	24.83
TS3	0.37	0.16	0.22	0.25	2.76	8.94	14.39	19.85	3752	2017	19.66	21.70
TS4	0.33	0.32	0.28	0.42	7.09	5.50	11.55	10.07	4802	5698	16.43	10.13
TS5	0.16	0.35	0.29	0.40	1.66	2.26	13.50	15.52	6013	4206	1.92	0.79

Table 3.4

	Nutrient concentration (μmol/L)										Salinity	
	PTC		POC		PTN		PON		PTP			
Tide	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
TS1	95.00	78.13	94.44	78.13	8.24	8.93	7.14	4.20	0.21	0.21	31.25	30.94
TS2	130.87	114.58	127.78	67.71	10.20	9.20	9.52	8.93	1.65		27.80	24.83
TS3	110.58	106.06	106.48	90.91	8.20	6.49	7.94	6.49	1.29	1.01	19.66	21.70
TS4	173.61	166.67	166.67	88.54	5.95	9.56	5.95	8.93	1.72	1.47	16.43	10.13
TS5	216.66	180.00	216.66	175.00	14.29	23.69	14.29	21.43	2.45	1.63	1.92	0.79

Table 3.5

Site	Nutrient concentration (μmol/L)				salinity
	NO3	PO4	NO2	NH3	
T1A	3.20	0.51	0.36	9.37	31.84
T1B	4.96	0.40	0.70	5.41	31.29
T1C	4.41	0.61	0.45	14.94	30.83
T2A	1.84	0.49	0.28	4.06	31.77
T2B	5.33	0.38	0.24	6.22	30.38
T2C	6.35	0.52	0.31	5.92	29.42
T2D	6.83	0.70	0.42	16.25	30.24
T3A	2.83	0.16	0.31	4.81	31.86
T3B	1.37	0.16	0.30	7.28	31.90
T3C	3.51	0.16	0.22	5.50	31.75
T3D	5.08	0.51	0.32	8.31	31.42
T3E	27.70	0.32	0.47	6.46	29.74
T4A	6.43	0.16	0.33	3.64	30.63
T4B	5.49	0.65	0.40	9.45	30.81
T4C	8.41	0.16	0.20	3.03	30.14

Table 3.6

Site	Nutrient concentration (μmol/L)					salinity
	PTN	PTC	PTP	PON	POC	
T1A	14.29	200.00	2.33	7.14	175.00	31.84
T1B	9.05	197.92	3.46	8.93	177.08	31.29
T1C	7.14	158.33	3.89	7.14	150.00	30.83
T2A	31.75	185.19	3.19	31.75	120.37	31.77
T2B	17.85	197.92	4.81	8.93	20.83	30.38
T2C	20.41	226.18	5.76	20.41	214.28	29.42
T2D	8.95	229.16	3.77	8.93	229.16	30.24
T3A	7.14	116.67		7.14	83.33	31.86
T3B	11.90	159.72	1.84	5.95	90.28	31.90
T3C	7.14	175.00	2.70	7.14	150.00	31.75
T3D	14.29	175.00	3.13	14.29	191.67	31.42
T3E	14.29	125.00	3.22	14.29	83.33	29.74
T4A			1.50			30.63
T4B	3.97	46.30	1.96	3.97	41.67	30.81
T4C	8.40	112.75	1.93	8.40	83.33	30.14

Table 3.7

Site	Nutrient concentration (µmol/L)				salinity
	NO3	PO4	NO2	NH3	
1	21.45	0.41	0.43	1.52	33.27
2	38.75	0.42	0.24	1.82	32.33
4	26.18	0.38	0.25	1.32	31.99
5	19.27	0.35	0.26	2.96	28.33
6	15.77	0.39	0.24	2.12	21.78
7	20.82	0.35	0.38	4.43	14.67
8	17.75	0.40	0.48	3.48	12.54
9	22.76	0.51	0.27	3.62	10.14
10	18.85	0.52	0.35	3.08	5.79
11	17.40	0.51	0.31	3.77	2.83
12	12.30	0.39	0.29	5.09	0.30
13	7.29	0.30	0.30	9.52	0.16
14	10.23	0.37	0.46	13.21	0.12
15	4.19	0.31	0.33	19.58	0.06
16	3.16	0.28	0.32	8.89	0.04
17	8.54	0.48	0.26	8.84	0.04
18	8.13	0.44	0.80	19.04	0.04
19	7.51	0.41	0.27	8.35	0.03
20	4.20	0.30	0.15	16.72	0.03
21	5.68	0.32	0.35	7.06	0.03

Table 3.8

Site	Nutrient concentration (µmol/L)					Coliforms (CFU/100ml)	salinity
	PTN	PTC	PTP	PON	POC		
1			0.42			1066.67	33.27
2	2.38	55.55	0.64	5.56	55.55	21166.67	32.33
4	4.20	75.98	1.07	2.45	66.17	1550.00	31.99
5	11.90	229.16	19.88	13.89	208.33	870.00	28.33
6	8.93	333.33	4.28	20.83	177.08	9500.00	21.78
7	11.90	361.10	4.14	27.78	402.77	24700.00	14.67
8	10.99	333.33	7.49	12.82	192.30	1680.00	12.54
9	47.62	999.98	11.95	55.55	1027.75	1276.67	10.14
10	23.81	666.65	9.90	27.78	805.54	8666.67	5.79
11	47.62	1111.08	10.92	27.78	694.43	1406.67	2.83
12	11.90	333.33	5.33	13.89	166.66	153.30	0.30
13	12.99	227.27	2.81	15.15	287.87	18066.67	0.16
14	6.49	234.84	2.60	7.58	143.94	553.33	0.12
15	7.94	268.51	3.18	9.26	222.22	3.33	0.06
16	5.95	104.16	2.64	6.94	104.16	2390.00	0.04
17	11.90	249.99	2.57	6.94	152.77	9300.00	0.04
18	11.90	138.89	2.38	13.89	173.61	1913.33	0.04
19	7.14	70.83	1.09	8.33	150.00	4733.33	0.03
20	8.93	182.29	1.10	10.42	208.33	2006.67	0.03
21	8.93	151.04	0.00	10.42	166.66	156.67	0.03

Table 3.9

Site	Nutrient concentration (μmol/L)				
	ON	TN	OC	TC	TP
1	0.24	28.56	0.25	191.49	4.84
2	99.95	99.95	1065.69	1740.07	15.26
3	0.24	21.42	8.33	382.98	4.86
4	0.24	0.24	0.25	0.25	0.26
5	0.24	28.56	16.65	541.17	6.49
6	0.24	0.24	16.65	482.89	5.27
7	0.24	28.56	8.33	557.82	7.25
8	78.53	92.81	990.76	1956.54	15.72
9	0.24	14.28	16.65	616.10	14.54
10	0.24	114.23	8.33	2131.38	17.88
11	78.53	21.42	832.57	133.21	18.31
12	64.25	57.11	641.08	1748.40	14.00
13	85.67	114.23	599.45	2098.08	16.46
14	0.24	14.28	66.61	358.01	11.24
15	0.24	21.42	74.93	374.66	5.64
16	114.23	121.37	1240.53	2547.66	20.02
17	0.24	14.28	41.63	49.95	4.65
18	49.98	57.11	566.15	1132.30	11.68
19	107.09	114.23	1198.90	2431.10	15.40
20	0.24	14.28	16.65	116.56	5.43
21	99.95	107.09	990.76	2089.75	15.00
22	35.70	49.98	399.63	1440.35	10.59
23	0.24	21.42	8.33	33.30	5.00
24	0.24	28.56	99.91	308.05	6.00
25	0.24	14.28	24.98	41.63	5.79
26	0.24	14.28	16.65	49.95	9.63
27	178.48	114.23	2139.71	2372.82	27.75
28	78.53	64.25	882.52	1298.81	11.26
29	114.23	107.09	1182.25	2098.08	17.93
30	0.24	14.28	49.95	33.30	4.34
31	114.23	92.81	1282.16	1998.17	15.71
32	92.81	85.67	1015.74	1931.56	15.89
33	107.09	99.95	1182.25	2148.03	16.89
34	28.56	28.56	291.40	674.38	13.74
35	0.24	21.42	33.30	58.28	4.88
36	71.39	64.25	724.34	1673.47	17.66
37	0.24	14.28	41.63	58.28	5.16
38	0.24	21.42	108.23	199.82	6.05
39	107.09	107.09	0.25	2322.87	17.24
40	0.24	14.28	24.98	24.98	19.34
41	7.14	21.42	41.63	8.33	5.20
42	207.04	164.20	2164.68	2680.88	17.08
43	35.70	49.98	466.24	915.83	9.65
44	64.25	64.25	641.08	1265.51	13.72
45	21.42	28.56	124.89	158.19	12.95
46	107.09	114.23	1173.92	2114.73	24.29
47	144.93	157.06	1989.84	2730.83	18.80
48	0.24	21.42	8.33	249.77	3.78
49	128.51	107.09	1573.56	1914.91	17.46
50	149.93	114.23	1956.54	1856.63	19.38
51	64.25	71.39	815.92	1332.11	18.98
52	35.70	42.84	424.61	624.43	12.47
53	114.23	114.23	1273.83	2039.80	24.04
54	142.79	135.65	1715.09	2447.76	20.45
55	92.81	107.09	1398.72	2173.01	21.59
56	135.65	149.93	1923.24	2414.45	23.38
57	107.09	157.06	1548.58	2447.76	20.30
58	42.84	49.98	524.52	782.62	17.91

Appendix 3

Table 4.1

Site	Fatty alcohol concentration (µg g-1)																				Sterol concentrations (µg g-1)												
	C13	C14	c15 iso	c15 anteiso	C15	C16	c17 iso	c17 antiso	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	coprostanol	Cholesta-5,22(E) -dien-3(beta)	cholesterol	5 alpha cholesterol	brassi -casterol	ergosterol	24-ethylcoprostanol	camp-esterol	stig-masterol	beta sitosterol	Dinosterol
1	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.016	0.001	0.004	0.003	0.015	0.009	0.007	0.014	0.005	0.003	0.004	0.004	0.009	0.011	0.002	0.012	0.007	0.004	0.008	0.017
2	0.001	0.021	0.006	0.006	0.001	0.008	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.054	0.001	0.013	0.003	0.024	0.009	0.017	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
3	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.026	0.006	0.021	0.023	0.003	0.005	0.004	0.001	0.011	0.001	0.039	0.009	0.021	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
5	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.036	0.006	0.021	0.023	0.003	0.005	0.100	0.005	0.056	0.075	0.172	0.009	0.058	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
6	0.001	0.021	0.006	0.006	0.001	0.006	0.020	0.018	0.006	0.021	0.023	0.003	0.005	0.018	0.001	0.006	0.001	0.015	0.009	0.007	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
7	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.026	0.001	0.006	0.007	0.020	0.009	0.011	0.014	0.005	0.003	0.004	0.011	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
8	0.001	0.021	0.010	0.006	0.008	0.059	0.020	0.045	0.007	0.097	0.023	0.127	0.018	0.318	0.019	0.216	0.037	0.416	0.019	0.053	0.014	0.009	0.067	0.037	0.099	0.046	0.052	0.004	0.042	0.048	0.024	0.130	0.025
9	0.002	0.025	0.013	0.006	0.014	0.082	0.020	0.026	0.012	0.088	0.023	0.119	0.019	0.383	0.022	0.260	0.043	0.663	0.011	0.047	0.014	0.009	0.052	0.018	0.092	0.024	0.045	0.002	0.063	0.030	0.004	0.125	0.017
10	0.001	0.021	0.006	0.006	0.004	0.041	0.020	0.014	0.006	0.047	0.023	0.057	0.013	0.178	0.016	0.121	0.019	0.219	0.009	0.044	0.014	0.005	0.040	0.010	0.014	0.024	0.022	0.002	0.026	0.020	0.014	0.065	0.017
12	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.009	0.005	0.027	0.002	0.021	0.005	0.052	0.009	0.007	0.014	0.005	0.003	0.004	0.007	0.009	0.010	0.002	0.012	0.007	0.004	0.010	0.017
11	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.004	0.001	0.002	0.001	0.015	0.009	0.007	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
13	0.001	0.021	0.006	0.006	0.001	0.008	0.020	0.014	0.006	0.021	0.023	0.020	0.005	0.044	0.003	0.017	0.001	0.015	0.009	0.007	0.014	0.005	0.005	0.004	0.009	0.009	0.010	0.002	0.012	0.007	0.004	0.015	0.017
14	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.004	0.001	0.002	0.001	0.015	0.009	0.007	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
15	0.095	3.016	0.957	0.333	1.025	10.684	0.328	1.166	1.065	19.396	2.408	34.754	4.025	129.205	3.660	31.103	1.587	13.968	0.150	2.088	0.130	0.436	5.691	3.003	13.080	4.273	4.860	0.968	7.306	4.693	3.690	27.804	2.527
16	0.001	0.021	0.006	0.006	0.003	0.020	0.020	0.014	0.006	0.030	0.023	0.044	0.006	0.099	0.005	0.034	0.001	0.019	0.009	0.007	0.014	0.005	0.009	0.004	0.018	0.009	0.010	0.002	0.012	0.008	0.006	0.033	0.017
17	0.001	0.021	0.006	0.008	0.001	0.012	0.020	0.019	0.006	0.021	0.023	0.009	0.005	0.036	0.003	0.016	0.013	0.019	0.009	0.011	0.014	0.005	0.003	0.004	0.016	0.009	0.010	0.002	0.012	0.007	0.004	0.011	0.017
19	0.001	0.021	0.006	0.006	0.001	0.008	0.020	0.014	0.006	0.021	0.023	0.017	0.005	0.047	0.006	0.042	0.013	0.068	0.009	0.017	0.014	0.005	0.006	0.008	0.038	0.009	0.013	0.002	0.012	0.010	0.005	0.021	0.017
18	0.003	0.021	0.006	0.006	0.003	0.039	0.020	0.021	0.006	0.076	0.027	0.114	0.021	0.226	0.022	0.127	0.017	0.112	0.009	0.041	0.014	0.010	0.025	0.013	0.073	0.015	0.025	0.002	0.025	0.019	0.014	0.067	0.017
20	0.261	3.554	2.980	0.355	2.717	14.507	0.948	1.108	2.528	12.303	0.849	12.718	1.434	17.295	0.800	4.419	0.482	2.698	0.129	0.376	0.014	0.005	4.670	3.072	14.518	4.204	7.866	1.552	4.631	5.025	2.387	19.039	1.833
21	0.001	0.021	0.006	0.006	0.004	0.026	0.020	0.014	0.006	0.026	0.023	0.038	0.006	0.077	0.005	0.024	0.004	0.016	0.009	0.007	0.014	0.005	0.012	0.006	0.025	0.011	0.011	0.003	0.012	0.011	0.006	0.034	0.017
22	0.001	0.021	0.006	0.006	0.002	0.023	0.020	0.014	0.006	0.039	0.023	0.062	0.009	0.196	0.012	0.098	0.006	0.079	0.009	0.020	0.014	0.005	0.011	0.006	0.047	0.009	0.017	0.005	0.016	0.013	0.007	0.050	0.017
23	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.003	0.005	0.004	0.001	0.002	0.001	0.015	0.009	0.007	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
28	0.001	0.021	0.006	0.006	0.001	0.004	0.020	0.014	0.006	0.021	0.023	0.013	0.005	0.041	0.003	0.031	0.004	0.054	0.009	0.024	0.014	0.010	0.003	0.004	0.009	0.009	0.010	0.002	0.012	0.007	0.004	0.016	0.017
25	0.001	0.021	0.006	0.006	0.001	0.006	0.020	0.023	0.006	0.021	0.023	0.003	0.005	0.070	0.001	0.031	0.001	0.082	0.009	0.025	0.014	0.005	0.003	0.004	0.001	0.009	0.010	0.002	0.012	0.007	0.004	0.008	0.017
24	0.001	0.021	0.006	0.006	0.002	0.019	0.020	0.014	0.006	0.033	0.023	0.039	0.005	0.092	0.006	0.070	0.008	0.125	0.009	0.056	0.014	0.018	0.010	0.008	0.014	0.009	0.013	0.002	0.017	0.010	0.006	0.051	0.017
26	0.001	0.021	0.006	0.006	0.001	0.004																											